Alabama Rhinotermitidae: Nomenclature, Identification, Survey, and Phenology

by

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Abstract

Nomenclature, identification, distribution, and phenology of the subterranean termites (Rhinotermitidae) of Alabama were studied. Arguments are proposed for suppressing the names of four *R. flavipes* subspecies with reported Alabama distributions: *R. f. 1, R. f. 2, R. f. i*, and *R. f. ii*. The first morphological identification key for Alabama Rhinotermitidae is presented, which was created using specimens verified by unequivocal morphology and DNA barcoding with the COII and 16S genes. Keys were developed for the imago, soldier, and worker castes. Data from a state-wide survey of Rhinotermitidae in 18 forests was combined with museum and Extension data from 1969-2012 to produce the first state checklist for Alabama, county checklists, and both updated and novel distribution maps. Data collected from 35 locations during 2010-2011 was combined with museum and Extension data from 1969-2011 to document the phenology of rhinotermitid swarming flights.

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Chapter 1

Literature Review

Introduction

This literature review will cover topics relevant to the presented research on Alabama Rhinotermitidae. Points reviewed include evolution, biogeography, taxonomy, nomenclature, distribution, and phenology.

Evolution of Termites from Roachoid Ancestors

Arthropoda is the most species-rich animal phylum known to have existed on Earth. Estimates of its modern species richness that include undescribed taxa have ranged up to 30 million species (Erwin 1982). Currently, phylogenetic hypotheses divide the arthropods amongst four major lineages: Pancrustacea, Myriapoda, Euchelicerata, and Pycnogonida (Giribet and Edgecombe 2012) (Fig. 1). Hexapoda, which is grouped under the Pancrustacea, first appeared at least 300 million years ago (MYA) in the fossil beds of the Rhynie Chert in Scotland (Scourfield 1940). This early hexapod is the springtail *Rhyniella praecursor*, a non-insect hexapod of the clade Collembola, which is now considered basal to the insects (Deharveng 2004) (Fig. 1). Insecta is believed to have derived from the Hexapoda concurrent with the origin and spread of vascular plants (Grimaldi and Engel 2005). Definite fossils of Pterygota, the winged insects, first appear in Devonian strata, while fragments that may be pterygotes are known as early as the Silurian (Giribet and Edgecombe 2012) (Fig. 1). The mutualism between plants and insects is reflected in the concurrent appearance in the Devonian fossil record of the first winged insects and the first woody plants large enough to form terrestrial forests (Bateman et al. 1998). Neoptera, insects which are able to fold their wings, appear in the late Devonian, around the time of the formation of Pangaea (Ishiwata et al. 2011) (Fig. 1). Several lineages derive from Neoptera. One of these, Polyneoptera, arose shortly after the formation of Pangaea, between the Devonian and the Carboniferous (Grimaldi and Engel 2005) (Fig. 1). Polyneoptera diversified in the early Carboniferous into two main lineages: one branch radiated into groups from which evolved modern stoneflies, webspinners, zorapterans, earwigs, icecrawlers, rockcrawlers, orthopterans *sensu stricto*, and stick insects; the second branch diversified into Paleozoic roachoids, and eventually into the modern Dictyoptera (Yoshizawa 2011) (Fig. 1). Lineages of roachoids begin disappearing from the fossil record during the Permian extinctions, and by the middle Jurassic (~225 MYA), these Paleozoic insects appear to have become entirely extinct (Grimaldi and Engel 2005). Survivors of the dictyopteran lineage radiated into two branches: mantids and cockroaches + termites (Hennig and Schlee 1981, Inward et al. 2007b) (Fig. 1). The cockroach + termite lineage split several times beginning with the rifting of Pangaea in the late Jurassic; one of these cockroach + termite lineages, the Euisoptera, arose shortly before the splitting of Pangaea into Gondwana and Laurasia (Engel et al. 2009). Descendents of Euisoptera radiated into the living cockroach family Cryptocercidae and the >3000 extant species of termites (Engel et al. 2009).

Biogeographic Explanations for the Modern Distributions of Termites

Termites likely diverged from modern cryptocercid-like roaches in the early Jurassic during the rifting of Gondwana from Pangaea, and underwent their first radiation parallel to that of the angiosperms (Rogers 1996, Engel et al. 2009). Following the Cretaceous-Tertiary extinctions, subterranean wood-feeders such as Rhinotermitidae arose during the Tertiary thermal maximum, while the mound-building soil- and fungus-feeding Termitidae arose in the Oligocene, simultaneously with the origin of grasslands (Engel et al. 2009). The radiation of Termitidae, the most derived family, does not appear to have ended (Grimaldi and Engel 2005). This family arose on Gondwana, and has a modern Afrotropical, Oriental, Australasian, and Neotropical distribution (Engel et al. 2009). More basal groups, such as Kalotermitidae and Rhinotermitidae, originated prior to the rifting of Pangaea, and have modern Pantropic and Holarctic distributions (Emerson 1955). The timing of these ancient events helps to explain the relatively weak diversity of Nearctic Isoptera. As Laurasia drifted north from its former equatorial position as part of Pangaea, it was covered in glaciers through several cycles of ice ages (Rogers 1996). Several temperature-sensitive groups were likely driven to extinction prior to the Cenozoic joining of the American continents, which were formerly parts of the supercontinents Laurasia and Gondwana (Rogers 1996). The Nearctic fauna is predominantly composed of the basal families Hodotermitidae, Kalotermitidae, and Rhinotermitidae (Weesner 1970). It is difficult to make many solid hypotheses of Laurasian diversity due to the subterranean and wood-feeding habits of termites: they only rarely become fossilized (Engel et al. 2007). Nonetheless, that the more derived

Termitidae are absent in Baltic ambers and present in Dominican ambers does suggest that the few species of this family with modern distributions in southwestern Nearctic deserts and peninsular Florida are the result of Cenozoic incursions from the Neotropical continent of South America, which has a Gondwanan history (Engel et al. 2009).

Termites: the Eusocial Cockroaches

Eusociality is the hallmark trait of termites. Eusociality is an uncommon subset of social behavior, formally defined on the basis of three criteria: cooperation in care for young; reproductive division of labor, with essentially sterile individuals commensally benefiting others engaged in reproduction; and overlap of at least two generations of life stages capable of performing tasks that benefit the society (Wilson 1971). Eusociality is only known in a few insect lineages and one vertebrate. Of these groups, termites were the first animals to develop this special case of social behavior (Engel et al. 2009). The singular example in vertebrates is in one rodent species of Mammalia, the naked mole rat *Heterocephalus glaber* (Bathyergidae) (Grimaldi and Engel 2005). In the insects, other than termites only a few Hymenoptera taxa exhibit eusociality: some wasps, some bees, and all of the ants (Wilson 1971). Unlike the eusocial Hymenoptera, termites do not exhibit haplodiploidy; non-reproducing males are retained across castes, and both males and females perform the tasks of the colony (Noirot 1989, Krishna 2005). Also unlike Hymenoptera, termites are hemimetabolous: development proceeds gradually through several intermittent stages, or instars (Noirot and Pasteels 1987).

In the Rhinotermitidae, after eclosion, development proceeds through two larval instars then branches into two pathways, which may be generalized as a worker + soldier line and a nymph line (Thorne 1996). Workers may remain as workers throughout their life cycle, remaining at the last developmental instar without further development (Hare 1934). Alternatively, workers may development along a defensive or reproductive path (Miyata et al. 2004). Mechanisms governing the switch in workers that dictate whether individuals remain as workers or develop along defensive or reproductive pathways are continuing to be elucidated (Hayashi et al. 2007).

Workers that develop along the defensive pathway become a soldier. This is to be distinguished from hymenopteran soldiers, which are merely workers with modified behavior (Oster and Wilson 1978). Developmental modifications of termite soldiers are primarily located in the head capsule. These anatomical changes enable individuals with physical or chemical means of defending the colony. Physical means include overall increased size and sclerotization of the head capsule; modification of the mandibles into grossly enlarged forms that may be scythed for spearing, strongly toothed for piercing, or crossed for dismemberment; and extreme sclerotization of the head into a phragmotic form used to plug breaches in tunnels (Prestwich 1984). Chemical means of defense include enlargement of the frontal gland and alteration of its opening, the fontanelle. Modifications of the fontanelle include change in placement on the head capsule and change in the direction of its opening, which together alter the rate of flow of secretions from the frontal gland to the outside of the body (Grassé 1982). Rhinotermitid soldiers are characterized by highly sclerotized head capsules with grossly enlarged mandibles attached to extensive musculature, and in some species, by the enlargement of the frontal gland and the fontanelle (Quennedey and Deligne 1975). In Nearctic rhinotermitids, soldier defensive behaviors include snapping with the powerful mandibles (Scholtz et al. 2008); phragmosis (Matsuura 2002); and chemical secretions from the frontal gland, such as latexes, that entrap attacking invertebrate predators (Mill 1983). Soldiers are generally present in low numbers in rhinotermitid colonies (Haverty 1977), and are believed to be incapable of feeding themselves without being aided by workers (Banks and Snyder 1920). Recently, it has been demonstrated that in some *Reticulitermes* species, workers also play an active role in defending the colony against invaders (Matsuura 2002).

If a worker develops along a reproductive line it will become an ergatoid secondary reproductive (Thorne 1996). Ergatoid secondary reproductives, which may be male or female, are characterized by enlarged abdomens with mature sex organs and the absence of thoracic wing pads (Miyata et al. 2004). Rhinotermitid colonies may include large numbers of this type of secondary reproductive (Myles 1999).

The second line of development from the second larval instar is into an entirely reproductive line (Weesner 1969). Individuals in this pathway pass through an intermittent stage with incompletely developed wing pads on their thorax. Reproductives may halt at this stage and remain in the colony, actively producing offspring (Thorne 1996). In this situation they are referred to as nymphoid secondary reproductives, which may be externally distinguished from ergatoid secondary reproductives by the presence of incompletely developed thoracic wing pads (Thompson 1917). Nymphoid secondary reproductives are common in natural Rhinotermitidae colonies in the Nearctic (King et al. 2009). At certain times of the year, depending on the species, massive numbers of individuals are produced that develop from the second larval instar along the entirely reproductive line (Banks and Snyder 1920). High proportions of these individuals will continue to gradually develop into a fully mature imago, or adult form. These are known as primary reproductives (Thorne 1996). Primary reproductives are the only caste with eyes, fully developed wings, and an entirely sclerotized body (Weesner 1969). When primed by cues relevant to that species, primary reproductives leave their colonies *en masse* in what is known as swarming flights (Nutting 1969). Hundreds of thousands of winged imagos may be released at a single event or over multiple occasions during a short window of time (Banks and Snyder 1920). Shortly after their flight, male and female imagos land on a substrate, break off their wings along a suture line at the wing base, and form pairs (Nutting 1969). These pairs, which are then referred to as a king and queen, burrow into the soil, construct a chamber, mate, and quickly begin producing offspring to establish a new colony (Thorne 1996). Once workers are produced they take over all tasks of the colony, including construction of new subterranean tunnels, chambers, aerial feeding tubes, and caring and feeding of developing larvae and soldiers (Hare 1934). The king and queen may live for decades (Nutting 1969)

Systematics: Death of an Order?

Substantial evidence supports the placement of all termites into the monophyletic unranked lineage Isoptera (Engel et al. 2009). Cryptocercidae, a family of social cockroaches that has a distribution including the southern Appalachians in the Nearctic, shares several characters with termites (Burnside et al. 1999). These include living entirely within deadwood substrates and mutualistic relationships with bacteria and protozoans they harbor in their gut, which enable these insects to digest cellulose without having to produce enzymes capable of cellulose digestion themselves (Aldrich et al. 2004). For these reasons, cryptocercids are widely regarded as the sister group to all termites (Kambhampati and Peterson 2007). Isoptera is nested within cockroaches, "Blattaria", a group that is paraphyletic without the inclusion of Isoptera (Inward et al. 2007a) (Fig. 1). If the extinct groups Paleozoic roachoids are to be included in the Blattodea phylogenetic tree, the clade becomes paraphyletic without including both Mantodea and Isoptera (Engel et al. 2009) (Fig. 1). The inclusion of Mantodea is necessary as extinct mantid taxa share several apomorphies with extinct cockroach taxa (Inward et al. 2007a). The name for this most inclusive clade has come to be Dictyoptera (Grimaldi and Engel 2005), although it was originally coined to include mantids and cockroaches alone (Hennig and Schlee 1981). The nesting of termites within cockroaches has invoked some authors to suggest the demotion of the order Isoptera to the new family "Termitidae", and the demotion of all existing termites families the subfamily rank (Inward et al. 2007a). However, the existing family Termitidae and subfamily Termitinae would have to be renamed if the order Isoptera were to be demoted and renamed as the family Termitidae. The current Termitidae contains most of the 3000 known species of termites and is composed of monophyletic subfamilies, with the exception of the subfamily Termitinae, which is paraphyletic (Inward et al. 2007b). An elegant solution may be to rename Isoptera as the family Termitidae, dismantle the existing family Termitidae into its constituent subfamilies, dismantle and redistribute the paraphyletic subfamily Termitinae amongst its closest relatives, and demote the remaining termite families to subfamily rank (Inward et al. 2007a). In this scenario, termites would be considered eusocial cockroaches, similar to the situation of the hyperdiverse Formicidae being nested within the Hymenoptera (Inward et al. 2007a). This change would be desirable, as a goal of systematics is to place taxa within monophyletic assemblages (Moritz and Hillis 1996). Similar shifts of highly diverse groups have occurred recently within the insects. Springtails (Collembola) was removed from Insecta and elevated to ordinal rank, with all of its families elevated to orders, and its subfamilies to families (Deharveng 2004). Wood-boring beetles, formerly the family Scolytidae, were found to be paraphyletic with some weevils (Curculionidae), and were moved into Curculionidae as a subfamily (Crowson 1960), thus demoting Scolytidae to the subfamily Scolytinae, and its former subfamilies to infrafamilies. Despite Blattodea having been considered paraphyletic without the inclusion of Isoptera for some time now (Hennig and Schlee 1981), the idea of termites as eusocial cockroaches has been met with much resistance (Lo et al. 2007).

Evolving Higher-Rank Nomenclature

The adoption of nomenclatural changes to the ranking and naming of termites has been sporadic. An ISI Web of Science search in March 2012 covering the years 2001-2012 retrieved 71 articles combining termites with Dictyoptera as an ordinal name (i.e., “Dictyoptera: Rhinotermitidae”), 3 as a superordinal name (i.e., “Dictyoptera: Isoptera”), 13 simply placing termites within Blattodea (i.e., “Blattodea: Rhinotermitidae”), and 830 continuing to use Isoptera as an order (i.e., “Isoptera: Rhinotermitidae”). Unfortunately, this confusion in the literature seems likely to continue for some time before a consensus is reached. This is particularly unfortunate as termites as a group have been plagued by taxonomic and systematic errors (Lo et al. 2007). There are internationally accepted rules in zoological nomenclature, established by the International Code of Zoological Nomenclature ("the Code"), but the Code has no provisions for taxa above the rank of family (ICZN 1999). One advantage of taking the suggestion of adopting the oldest available name, Termitidae (Inward et al. 2007a), is that after the initial chaos, a new stability would be created, thanks to the control of family-ranked names by the Code (ICZN 1999). A second advantage is that taxonomy would mirror a correct phylogeny, which is a goal of modern systematics (Eggleton et al. 2007). One compromise solution that has been proposed is to retain Isoptera as an unranked clade, with the wood roaches (Cryptocercidae) as sister group to all extant termite families, with intra-group relationships within termites being unaffected (Lo et al. 2007, Engel et al. 2009).

Monophyly of Isoptera and Rhinotermitidae

Regardless of its validity, the unranked clade Isoptera, when used in the sense of all extant and extinct termite species, has retained its monophyly after repeated tests with increasing depth of taxon sampling and character inclusion (Donovan et al. 2000, Engel et al. 2009). Further, even when all families of Mantodea, Blattaria, and Isoptera are initially placed on an equal plane, Isoptera continues to resolve itself as monophyletic, far removed from Mantodea families, sister to Cryptocercidae, and separate from other Blattaria (Davis et al. 2009). Thus it is appropriate for systematists to examine intragroup termite relationships, as they do not need to be concerned about the absence of potential outgroups biasing the results of phylogenetic hypotheses within termites themselves (Engel and Krishna 2004).

Rhinotermitidae is basal to the most derived group, the Termitidae (Engel and Krishna 2004). The monophyly of Rhinotermitidae is somewhat controversial (Legendre et al. 2008), but generally well-supported in studies incorporating multiple molecular techniques. These techniques have included mitochondrial DNA, nuclear DNA, cuticular hydrocarbons, morphology, caste structure, and interspecific antagonism (Davis et al. 2009).

Termites in Alabama

Two families of Isoptera have been reported in Alabama: Rhinotermitidae and Kalotermitidae (Weesner 1970). Kalotermitidae, the drywood termites, are rarely reported in Alabama, but are thought to occur in the coastal regions (Weesner 1970, Nutting 1990). As this family has been reported in Piedmont forests of South Carolina (Syren and Luykx 1981, Nalepa 1998) it is possible that these species may occur in natural situations of more northerly locations in Alabama. Rhinotermitidae, the subterranean termites, are of primary economic importance in the state, and are believed to be distributed throughout the state’s boundaries (Nutting 1990). A systematic survey of Alabama's termites has never been completed, nor has a catalog been produced with county checklists for all species occurring in the state.

Alabama Rhinotermitidae

Commonly known as subterranean termites, Rhinotermitidae was first described by Froggatt in 1897. The last comprehensive Nearctic catalog defined the family by the following features: the clypeus being divided medially, the forewing radial lacking superior branches, and the presence of a fontanelle (Banks and Snyder 1920). Five species of two subfamilies, Coptotermitinae Holmgren, 1910 and Heterotermitinae Froggatt, 1897 are known or thought to occur in Alabama.

One species of Coptotermitinae is known to occur in Alabama, the invasive *Coptotermes formosanus* Shiraki, 1909. This species was introduced to the southeastern Nearctic from the Oriental through as many as ten separate introduction events (Austin et al. 2006). Molecular evidence suggests the origin of the Nearctic populations being at least two sympatric lineages located in southeastern mainland China and Taiwan (Austin et al. 2006). *Coptotermes formosanus* was first recorded on the coast of Alabama in 1985 (Su and Scheffrahn 1986). Three years later it was noted in central-eastern Alabama (Sponsler et al. 1988), and has now been recorded from several counties across the state, typically in urban areas near interstate highways (Hu and Oi 2004). It is unclear whether it is strictly a tramp species in Alabama or whether it is establishing in forests, as has been suggested to be occurring in Louisiana (Brown et al. 2007) and Mississippi (Lax and Wiltz 2010). Laboratory studies suggest that *C. formosanus* may outcompete native subterranean termites in agonistic interactions (Hu and Zhu 2003).

Heterotermitinae Frogatt, 1897 contains the four native rhinotermitid species, all of which are within the genus *Reticulitermes*. This genus was described by the Swede Nils Holmgren in 1913, as a subgenus under *Leucotermes* Silvestri, which he created while revising *Termes flavipes* Kollar. Holmgren (1913) transferred *T. flavipes* to his subgenus and designated *R. flavipes* to be its type species. Recently, it was discovered that two fossil genera, *Hemerobites* Germar, 1813 and *Maresa* Giebel, 1856, were synonymized with *Termes flavipes* prior to Holmgren's (1913) designation of *T. flavipes* as the type for *Reticulitermes* (Engel and Krishna 2007). This created a problem, as by the Principle of Priority of the Code, all species in a genus belong to the first-named synonym of all associated synonymized taxa, provided there is evidence of use of that name (ICZN 1999). Thus, due to the use of *Maresa* after 1899 and the lack of use of *Hemerobites* after 1899, "*Maresa flavipes*" would become the new type species, and all of the species in *Reticulitermes* would be transferred to *Maresa*. However, due to the massive instability this would cause in the extensive body of literature on *Reticulitermes* species in the United States and Europe, Engel and Krishna (2007) petitioned to retain *Reticulitermes* by Article 23.9.2 of the Code. This motion to save *Reticulitermes* despite the priority and use of *Maresa* was affirmed (Roisin 2008), and subsequently the petition was granted (ICZN 2009). The last monograph of Nearctic *Reticulitermes* was produced by Banks and Snyder (1920). There have since been many nomenclatural changes, and numerous errors have crept into the literature through authors apparently not checking original references (Engel and Krishna 2004). Interestingly, despite the recent surge of systematic research into *Reticulitermes*, the genus continues to resolve as a monophyletic clade, although intra- and interspecies relationships continue to be problematic to discern (Austin et al. 2005). As noted by Austin et al. (2007), a revised *Reticulitermes* monograph is long overdue. Four *Reticulitermes* species are known or have been suggested to occur in Alabama.

Reticulitermes flavipes (Kollar, 1837) was initially described from a colony invading a greenhouse in Vienna, Austria (Kollar 1837). Kollar (1837), who named the species for its yellow legs, thought it was not native to Europe. His reasoning was based on its dissimilarity with its closest-appearing relatives in Austria, and its apparent intolerance to temperatures outside of greenhouses, as evidenced by it being found exclusively in hot, humid greenhouses, while not being known to occur outdoors in woodlands like the native European subterranean termites. It is now believed that the Vienna greenhouse population was transported through trade from the southeastern United States (Ghesini et al. 2010). The result of this is that a species native to the Nearctic has the nonsensical Palearctic type locality of Schoeubrunn, Austria (Kollar 1837). The location and castes of Kollar's types are unknown, although the American author Nathan Banks (Banks and Snyder 1920) claimed to have examined them. The Swedish author Nils Holmgren, noting the species' North American distribution, transferred it to his new subgenus *Reticulitermes* (Holmgren 1913). Shortly thereafter, Banks elevated Holmgren's subgenus to the genus rank, designated its type species as *R. flavipes*, and demoted his species *R. claripennis* Banks to a junior synonym of the new combination *R. flavipes* (Banks and Snyder 1920). This also had the effect of creating a new Nearctic type locality: Beaumont, Texas, although Banks did not designate new type specimens (Banks and Snyder 1920). In the Nearctic, *R. flavipes* is the most widely distributed rhinotermitid species, occurring across the eastern USA and as far north as southeastern Canada (Weesner 1970). With 47 mitochondrial DNA haplotypes, *R. flavipes* is the most genetically diverse termite species known in the Nearctic, with few haplotypes being widespread on several continents, and most endemic to the southeastern United States (Austin et al. 2005). *Reticulitermes flavipes* continues to establish and spread through Europe, where it is causing economic damage in France, Germany, and Italy (Ghesini et al. 2010).

Reticulitermes virginicus (Banks, 1907) was originally described as *Termes virginicus* from specimens collected in 1907 from Falls Church and Chain Bridge, Virginia, and from Washington, D.C. (Banks 1907). The name is derived from its type locality. A single type specimen of an imago with wings was deposited in the United States National Museum (now Smithsonian Institute National Museum of Natural History (NMNH)), Washington, DC, with the catalog number 21862 (Banks and Snyder 1920). The most notable characters Nathan Banks used to separate *Termes virginicus* from *R. flavipes* (which he incorrectly referred to as *Termes flavipes* Burrmeister) were in the winged imagoes: overall color being brown instead of black, length 7.5-8 mm instead of 9-10 mm, wings not darkened except on the costa instead of plainly darkened, and flights in June instead of April to early May (Banks 1907). Many of these characters continue to be used in keys written almost a century later (Scheffrahn and Su 1994). Holmgren

(1913) transferred *T. virginicus* to *Reticulitermes* (not in 1910, as reported in Banks and Snyder 1920). *Reticulitermes virginicus* occurs throughout the southeastern Nearctic, and like *R. flavipes*, is considered a pest species due to its proclivity for structural wood (Weesner 1970).

Reticulitermes hageni Banks, 1920 was initially described from specimens collected at the type locality of Falls Church, Virginia (Banks and Snyder 1920). Type specimens, consisting of imagos with wings and soldiers, were deposited in the NMNH with catalog number 21863 (Banks and Snyder 1920). The name is a patronym of Hermann A. Hagen, a prolific German entomologist (Banks and Snyder 1920). Additional distribution records are noted as far west as Illinois and the Gulf of Mexico coast of Texas, and southward into coastal Georgia and the Atlantic coast of Florida. It has since been found as far west as Oregon. It likely crossed the biogeographic barrier of the Rocky Mountains through anthropogenic means, as suggested by the finding of haplotypes in the eastern United States that are identical to those of the Oregon populations (McKern et al. 2006). Aside from this likely introduction, the natural western limit of *R. hageni* appears to be in Kansas and Texas, where the Great Plains may form a biogeographic barrier to its natural westward expansion (Weesner 1970, Austin et al. 2004). *Reticulitermes hageni* tends to be rarely reported, which is unsurprising given that it is of little economic importance (Su and Scheffrahn 1990), because its colonies are rarely reported infesting structural wood (Messenger et al. 2002). This species has the least amount of observed genetic variation of the four *Reticulitermes*, with only four observed haplotypes (Messenger et al. 2002, McKern et al. 2006). However, its low apparent genetic diversity may be an artifice of poor collection and subsequent haplotype sequencing effort.

Reticulitermes malletei Howard and Clément, 1985 (Clément et al. 1985) presents another example of the ubiquitous confusion in the rhinotermitid literature. It was initially described primarily on the basis of chemical characters of soldiers (Clément et al. 1985), then cuticular hydrocarbons of workers (Clément et al. 1986), and still later, morphological characters and DNA sequences (Austin et al. 2007). The name is a patronym of Howard Mallette, a USDA employee who suggested to the authors that an undescribed *Reticulitermes* species may occur in Georgia. Clément et al. (1985) did not designate type specimens. Austin et al. (2007) designated a series of types based on specimens collected by Clément et al. (1986), which were deposited in l'Institut de Recherche sur la Biologie de l'Insecte, Université de Tours, France; University of Arkansas Department of Entomology Museum, Fayetteville, AR; American Museum of Natural History, New York, NY; and the NMNH, with catalog number 2043708. The papers describing and then revising *R. malletei* were essentially ignored in North America, likely due to their being published in the French language in journals located in France that were inaccessible or ignored by American workers. In 1995, different-seeming *Reticulitermes* specimens that would not key to recognized species using morphological techniques alerted termite researchers that a "new" species may be present (Hostettler et al. 1995). In 2001, in a footnote of an otherwise unrelated American paper, *R. malletei* was considered an invalid name (Scheffrahn et al. 2001). In 2007, the case for *R. malletei* was exhaustively reexamined in an American journal, and deemed to be a valid name in accordance with the rules of the Code in effect at the time of its original description (Austin et al. 2007). As of March 2012, *R. malletei* has not been reported from any specific localities in Alabama, although the species has been suggested to occur in this state (Clément et al. 1985, Clément et al. 1986). Reported distributions with specific localities include Georgia, Maryland, and Delaware (Clément et al. 1985, King et al. 2007, King et al. 2009).

Termites in the Woods: Alabama's Ecoregions

Northern and central Alabama forests are largely composed of hardwoods, especially oaks, *Quercus* spp. (Griffith et al. 2001). Fire suppression has greatly changed the character of Alabama's forests from their pre-European colonial state (Abrams 1992). Central and nearly all of southern Alabama were previously covered with coniferous forests; the Longleaf Pine, *Pinus palustris* Miller, was the dominant tree species of pre-colonial forests (Abrams 1992). These woodlands were characterized by frequent and intense fires, and a grassy understory typically described as an open parkland savannah (Outcalt and Sheffield 1996). Fire suppression, logging, and agriculture over the past 200-300 years have largely extirpated this ecosystem; about 5% of it remains today in patches scattered across its former range (Outcalt and Sheffield 1996). Currently, they again cover much of central and southern Alabama, but are now largely composed of deciduous forests not substantially influenced by fire disturbance (Griffith et al. 2001). Endangerment, extirpation, and extinction of charismatic large animals such as birds and mammals are well known, but considerably less attention tends to be paid to invertebrate animals (Clark and May 2002). Little work has been done on the biodiversity and ecology of soil organisms such as subterranean termites outside of an economic context (Parker 2010). It is possible that fire suppression has favored the spread of some rhinotermitid species with higher moisture requirements over others that are more tolerant of droughts but tend to lose in agonistic interactions within their guilds. Rhinotermitids are subterranean and consume decaying and solid deadwood connected to the forest floor (Bignell and Eggleton 2000). The few distribution maps that have been made of southeastern termite species (Weesner 1970, Nutting 1990) must be interpreted with caution, as they are based on extrapolations from non-rigorous, non-replicated survey efforts. Invasions by *C. formosanus* may be expanding from urban areas into Alabama's forests, as they are believed to be doing in coastal Mississippi (Sun et al. 2007). The rapid spread of *C. formosanus* across Alabama is likely due to anthropogenic commercial transport (Hu and Oi 2004). It is probable that in Alabama *C. formosanus* colonies are present in suburban developments adjacent to forests. Forests represent virtually unlimited cellulosic resources and without the pressure from humans trying to control them, although the species would have to effectively deal with natural enemies. Many records of *C. formosanus* colonies appear to point to it only being found in structural wood (Hathorne et al. 2000, Li et al. 2008), which may indicate that it competes poorly with native termites, or is ineffective at repelling natural enemies such as ants. The ecological impact of this species in areas where it has been introduced by humans is not well understood (Cornelius and Osbrink 2000). If *C. formosanus* were to successfully establish in Alabama's woodlands, forests would become a permanent source of these injurious insects. The ability of the species to consume living trees of economic value (Ehrhorn 1946) may present an threat to the state's forestry industry. Due to the importance of termites in soils and as decomposers of deadwood on forest floors, successful replacement of native termites by *C. formosanus* has the potential to initiate both below- and above-ground trophic cascades (Lee and Wood 1971). It is imperative to explore baseline data of the ranges of the four native subterranean termites and the possibility of whether *C. formosanus* is establishing in natural woodlands of Alabama.

Gaps in Current Knowledge of Alabama Rhinotermitidae and Motivation for the Current Research

No systematic survey has ever been conducted in Alabama to document the distribution of the species known or suggested to occur in the state. As such there is no state catalog containing checklists for the state and its counties. The last national catalog was published in 1920 (Banks and Snyder 1920). The few data available that include records from Alabama are heavily anthropogenically biased from the near-exclusive employment of pest control records (Banks and Snyder 1920, Weesner 1970, NOMTCB 2003-2006) These systematic errors effectively prevent any statistical inferences regarding the true distribution of the subterranean termites in Alabama (Krebs 1999).

Termite distribution is naturally controlled through periodic release of massive swarming flights of primary reproductives (Nutting 1969). The control mechanism of the swarming flight signal is not entirely understood, but is believed to be correlated with seasonal shifts in humidity and temperature (Grassé 1982). Although southeastern Nearctic rhinotermitids are commonly reported to fly in the spring (Snyder 1954), bivoltine swarms are known to occur in California (Haverty et al. 2003). No systematic effort has been made in the southeastern United States to continuously monitor swarming phenology. Almost all available data come from specimens collected from structures. The temperature and humidity of the interiors of structures and the soil surrounding them are typically affected by interior climate control, both of which are known to influence rhinotermitid behavior (Hu and Appel 2004). There is a clear need to understand this basic biology of Alabama rhinotermitids.

Poor taxonomy and confused nomenclature have severely crippled any effort to understand the distribution or swarming flight phenology of subterranean termites in Alabama. No key has been developed for the state. Regional keys (Snyder 1954, Nutting 1990, Scheffrahn and Su 1994) are inappropriate for Alabama due to their excluding *R. malletei* and using characters that are misleading with the inclusion of *R. malletei* into the local fauna. Despite workers being the most commonly encountered and easily collected caste, no key in the United States has attempted to utilize this caste for morphological identification. A nomenclatural issue that remains unresolved is the existence of four subspecies of the widespread *R. flavipes* having been stated to occur in Alabama (Clément et al. 1985, Clément et al. 1986). Three of these subspecies were given names in violation of the provisions of the Code (ICZN 1999), leaving one subspecies of uncertain genetic and biogeographic origin that may or may not represent a discrete population of *R. flavipes*.

The current research first prevents further nomenclatural confusion by presenting arguments for the suppression of the four subspecies of *R. flavipes* with distributions in Alabama. With this resolved, the central focus of this thesis was to enable the utilization of termites in ecological research and increase the accuracy of control efforts through the development of a key to Alabama Rhinotermitidae. All castes were employed: imagoes, soldiers, and workers. With this basic tool developed, it became possible to investigate derived questions of distribution and phenology of Alabama's rhinotermitids. A state-wide survey was conducted to develop state and county checklists and distribution maps. Finally, to address the question of swarming phenology, a continuous monitoring program was implemented that simultaneously sampled at multiple locations distributed across Alabama.

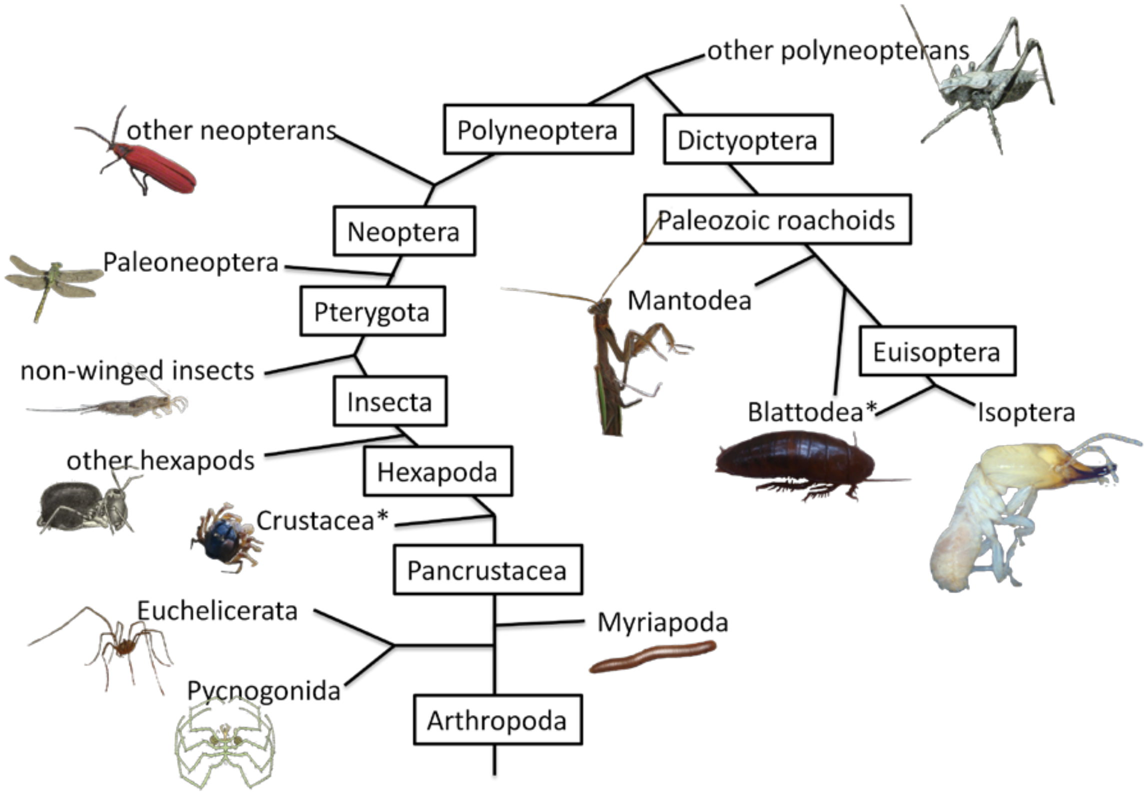

Figure 1. Phylogeny of termites. Adapted from Grimaldi and Engel (2005) and Giribet and Edgecombe (2012). Starred groups are paraphyletic; all others are monophyletic. All images original except for “other hexapods” and Pycnogonida, which are in the public domain.

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Chapter 2

The termite species-group and subspecies-group names *Reticulitermes flavipes 1* Clément *et al*., 1985 (= *Reticulitermes flavipes 2* (Kollar) sensu Clément et al., 1985), *Reticulitermes flavipes 2* Clément *et al*., 1985 (= *Reticulitermes flavipes 2* (Kollar) sensu Clément et al., 1985), *Reticulitermes flavipes I* Clément *et al*., 1986, and *Reticulitermes flavipes II* Clément *et al*., 1986 (Isoptera, RHINOTERMITIDAE): proposed suppression

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Abstract. The purpose of this application, under Articles 5 and 11 of the Code, is to avert continued nomenclatural confusion by suppression of the three incorrect trinomens *Reticulitermes flavipes 1* Clément *et al*., 1985 (= *Reticulitermes flavipes 1* (Kollar)), *Reticulitermes flavipes 2* Clément *et al*., 1985 (= *Reticulitermes flavipes 2* (Kollar)), and *Reticulitermes flavipes I* Clément *et al*., 1986; and the suppression of the incorrect and ambiguous trinomen *Reticulitermes flavipes II* Clément *et al*., 1986. *Reticulitermes flavipes* (Kollar, 1837) is an important destructive pest and xylophagous decomposer species that is known for high levels of genotypic and phenotypic variation across its geographic range.

Keywords. Nomenclature; taxonomy; RHINOTERMITIDAE; *Reticulitermes flavipes*; subspecies

1. Species of *Reticulitermes* Holmgren, 1913 (Isoptera: Rhinotermitidae) in North America have traditionally been described using morphology (Banks, 1907, pp. 392-393; Banks & Snyder, 1920, p. 44). More recently, two molecular methods have come to be used extensively to delineate systematic relationships and taxonomy: (1) polymerase chain reaction (PCR), applied to segments of the mitochondrial genome coding for cytochrome *c* oxidase (Jenkins et al., 1999, pp. 164-165), subunits of the ribosome (Kambhampati et al., 1996, pp. 230-231), and the AT-rich region (Foster et al., 2004, pp. 98-100); (2) gas chromatography (GC), applied to all castes for cuticular hydrocarbons (CHC) (Howard et al., 1978, pp. 235-244) and to the soldier caste for cephalic soldier defense secretions (SDS) (Zalkow et al., 1981, pp. 720-730). Genotypic haplotypes are generated by PCR and phenotypic chemical profiles by GC. These methods have demonstrated that, relative to its Nearctic congeners, *Reticulitermes flavipes* (Kollar, 1837) possesses high degrees of intraspecific variation in both its genotype (Cameron & Whiting, 2007, p. 192; Foster et al., 2004, p. 98; Jenkins et al., 2000, p. 1537) and phenotype (Haverty et al., 1996, p. 295). These molecular findings have been complemented by the demonstration of considerable morphological biometric variation over the range of this species (Hostettler et al., 1995, pp. 121-123). *R. flavipes* is common throughout its native Nearctic range, and is distributed beyond the isothermic limits of its physiological capability to survive (Esenther, 1969, p. 1283). This is due to its ability to infest structural wood that is kept by humans at higher than ambient temperatures and the species' capacity for behavioral avoidance of lethal low temperatures (Hu & Song, 2007, pp. 1452-1453). Although native to the Nearctic (Weesner, 1970, pp. 495-504), pest populations of *R. flavipes* have been established in the western Palearctic for at least a century (Ghesini et al., 2010, pp. 327-328), and recently, pest populations have been discovered in temperate regions of the Neotropic (Austin et al., 2005b, pp.

396-397). Continued mixing of synanthropic populations through human commerce may well contribute to the species' high degrees of intraspecific genotypic and phenotypic variation. PCR of the 16S ribosome subunit has generated 47 haplotypes of *R. flavipes* in North America (Austin et al., 2005a, pp. 981-982). No subspecies of Nearctic *Reticulitermes* have been proposed on the basis of haplotypes alone. Clément et al. (1985) and Clément et al. (1986) used phenotypic GC profiles and behavioral data to propose species-group and subspecies-group names associated with *R. flavipes* populations in the southeastern United States. It is possible that the specimens in their studies came from colonies representing more than one haplotype, as has been shown with *R. hesperus* Banks, 1920 in California (Copren et al., 2005, p. 696). To avoid further nomenclatural confusion with the cryptic genus *Reticulitermes,* we present here cases for suppressing names proposed by Clément et al. (1985) and Clément et al. (1986).

2. Clément *et al.* (1985) proposed "*Reticulitermes flavipes 1* (Kollar)" as a species-group name (p. 124), which they based on GC SDS profiles they cite from Zalkow et al. (1981). The putative species was separated from sympatric congeners by the GC SDS profile of the absence of geranyl-linalool, presence of monoterpenes, and presence of sesquiterpene aldehyde (Clément et al., 1985, p. 130). Zalkow et al. (1981, pp. 720-730) report GC SDS profiles for *R. flavipes,* but do not give an opinion on the taxonomic utility of GC SDS profiles. Equally important with respect to "soldier defense chemicals" in *R. flavipes,* Zalkow et al. (1981, pp. 727, 730) were unable to find evidence that these chemicals possess agonistic properties: all termite soldiers in their termite-ant arenas employed solely mechanical means to attack ants, and no trace of SDS compounds could be found on ants harmed or killed by termite soldiers. As a species-group name, *R. flavipes 1* is not valid because it violates the Principle of Binomial Nomenclature (Art.

5.1) of the Code: it is a trinomen. Additionally, the author attribution is incorrect and should have been "Clément *et al.* 1985", not "(Kollar)". Clément et al. (1985) do not propose *R. flavipes 1* as a subspecies-group name, but this name is equally invalid at this rank due to violating the Principle of Binomial Nomenclature (Arts. 5.2, 11.2) of the Code: *1* is less than two characters in length, and *1* is not in the Latin alphabet.

3. Clément et al. (1985, p. 130) proposed "*Reticulitermes flavipes 2* (Kollar)" as a species-group name in the same paper as *R. flavipes 1*. The justification for the new name was the GC SDS profile of the presence of geranyl-linalool, presence of sesquiterpenes, and absence of germacrene (Clément et al., 1985, p. 130). As a species-group name, *R. flavipes 2* is not valid because it is a trinomen and thus violates the Principle of Binomial Nomenclature (Art. 5.1) of the Code. As with *R. flavipes 1*, the author attribution is incorrect and should be "Clément *et al.* 1985", not "(Kollar)". Although not proposed as such, *R. flavipes 2* continues to be invalid as a subspecies-group name because it violates the Principle of Binomial Nomenclature (Arts. 5.2, 11.2) of the Code: *2* is less than two characters in length and is a non-Latin character.

4. Clément et al. (1986) proposed "*Reticulitermes flavipes I*" simultaneously as a species-group name (p. 67 (both French and English versions of abstract)) and a subspecies-group name (p. 67), and imply that the name is equivalent to *R. flavipes 1* Clément *et al*., 1985. The justifications for the new taxon were based on GC CHC profiles and aggression indices. The GC CHC profile unique to *R. flavipes I* was attributed to Howard et al. (1978). Howard et al. (1978, pp. 242-244) described GC CHC profiles in *R. flavipes* and discussed the potential functional significance of these compounds, but neither speculate on the taxonomic significance of their

findings nor propose nomenclatural changes. Clément et al. (1986, p. 69) reported aggression indices between *R. flavipes I*, *R. flavipes II* (first named in Clément et al. (1986, p. 67), see point 5 below), *R. malletei* Howard and Clément, 1985 (named as *sp. nov.* in Clément et al. (1986, p. 67), but actually first proposed in Clément et al. (1985, p. 124)), and *R. virginicus* Banks, 1907. Accounting for standard error, the mean aggression indices of both *R. flavipes I* - *R. flavipes I* pairs and *R. flavipes II* - *R. flavipes II* pairs overlapped with the mean aggression index of *R. flavipes I* - *R. flavipes II* pairs (Clément et al., 1986, p. 69). However, aggression indices were separated when *R. malletei* or *R. virginicus* were paired with the two proposed taxa (Clément et al., 1986, p. 69). Elsewhere, Clément (1986, p. 315) reported colonies of *R. santonensis* (Feytaud, 1924) to never exhibit intraspecific antagonism between different nests; *R. santonensis* is now considered a junior synonym to *R. flavipes* (Austin et al., 2005b, p. 398). Clément et al. (1986, p. 70) interpreted the aggression index between *R. flavipes I* and *R. flavipes II* as evidence that only conspecifics possess the ability to fuse colonies, and thus implied that the name *R. flavipes I* was intended for a subspecies and not a species. It appears that the explicit designation of *R. flavipes I* as a species-group name in the abstract of Clément et al. (1986, p. 67) was an error. If interpreted as a species-group name, *R. flavipes I* is not valid because it is a trinomen and thus violates the Principle of Binomial Nomenclature (Art. 5.1) of the Code. If taken to be a subspecies-group name, *I* is not valid because it violates the Principle of Binomial Nomenclature (Arts. 5.1, 5.2) of the Code: it is composed of less than two letters and contains uppercase characters.

5. Clément et al. (1986) also proposed *Reticulitermes flavipes II*, and again referred to the same name as both a species-group name (p. 67 (both French and English versions of abstract)) and a

subspecies-group name (p. 67), and implied (p. 67) that the name is equivalent to *R. flavipes 2* Clément *et al*., 1985. Four lines of argument were given for considering *R. flavipes II* a separate taxon: GC SDS profiles, sexual attraction of imagoes, temporal isolation of imago mating flights, and aggression indices. Implicitly referring to Clément et al. (1985), Clément et al. (1986, p. 67), attested that *R. flavipes II* had a distinct GC SDS profile characterized by the diterpene alcohol geranyl-linalool. Imagoes of *R. flavipes II* are reported to be more attracted to heterosex *R. flavipes II* than heterosex *R. malletei*, and to have a swarming period that is temporally separate from *R. malletei* (Clément et al., 1986, p. 68). As with *R. flavipes I,* it appears that the explicit designation of *R. flavipes II* as a species-group name was in error, and that Clément et al. (1986) intended to name a subspecies. If interpreted as a species-group name, *R. flavipes II* cannot be considered valid because it violates the Principle of Binomial Nomenclature (Art. 5.1) of the Code: it is a trinomen. However, if Clément et al. (1986)'s intention is interpreted as naming a subspecies-group, the name *II* only superficially violates the Principle of Binomial Nomenclature (Art. 5.2) of the Code: it is not composed of lowercase Latin characters. The subspecies-group name *II* ceases to violate the provisions of the Code if emended to *Reticulitermes flavipes ii* Clément *et al.*, 1986.

6. The International Commission on Zoological Nomenclature is accordingly asked to use its plenary power:

(1) to suppress the following species-group names for the purpose of upholding the Principle of Binomial Nomenclature:

(a) *Reticulitermes flavipes 1* Clément *et al*., 1985 (= *Reticulitermes flavipes 1* (Kollar) sensu Clément et al., 1985);

(b) *Reticulitermes flavipes 2* Clément *et al*., 1985 (= *Reticulitermes flavipes 2* (Kollar) sensu Clément et al., 1985);

(c) *Reticulitermes flavipes I* Clément *et al*., 1986;

(d) *Reticulitermes flavipes II* Clément *et al*., 1986

(2) to suppress the following subspecies-group names for the purpose of upholding the Principle of Binomial Nomenclature:

(a) *Reticulitermes flavipes 1* Clément *et al*., 1985 (= *Reticulitermes flavipes 1* (Kollar) sensu Clément et al., 1985);

(b) *Reticulitermes flavipes 2* Clément *et al*., 1985 (= *Reticulitermes flavipes 2* (Kollar) sensu Clément et al., 1985);

(c) *Reticulitermes flavipes I* Clément *et al*., 1986;

(3) to suppress the subspecies-group name *Reticulitermes flavipes II* Clément *et al*., 1986, for the purposes of upholding the Principle of Binomial Nomenclature and of retaining stability in the nomenclature of the cosmopolitan pest species *Reticulitermes flavipes* (Kollar, 1837).

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Chapter 3

Key to the species of Rhinotermitidae (Isoptera) of Alabama, using the worker, soldier, and imago castes

Abstract

Subterranean termites (Isoptera: Rhinotermitidae) are eusocial colonial insects that profoundly impact the economy of the southeastern USA. Species identification is a chronic problem with these cryptic insects. Few morphological keys have been attempted in the Nearctic, and no published keys exist for Alabama. Available regional keys do not include all species known to occur in the state, and employ only the imago and soldier castes. The imago caste is not available year-round; the soldier caste is always present only in low numbers. In contrast, the worker caste is found year-round and is the most numerous caste within foraging parties and colonies. Collections consisting solely of preserved specimens of the worker caste may only be unequivocally identified using molecular sequencing. To address this issue and expedite the processing of an ongoing state-wide survey, we developed a key that utilizes the worker caste in addition to the soldier and imago castes, and includes the five Rhinotermitidae species that have been reported to occur in Alabama. Our keys comprise the first morphological tool in the United States to utilize the worker caste, and the first keys using the imago and soldier castes for Alabama Rhinotermitidae.

Key words: morphology, *Coptotermes, Reticulitermes*

Introduction

Subterranean termites (Isoptera: Rhinotermitidae) are eusocial xylophagous insects whose habit of consuming and colonizing deadwood in forests transfers easily to infesting anthropogenic wooden structures. These insects profoundly impact the economy of the United States, particularly in the southeastern states (Su 2002). Through the course of using published regional keys (Emerson and Miller 1943a, Nutting 1990, Scheffrahn and Su 1994, Hostettler et al. 1995) to identify samples from a survey (Stephen et al., unpublished) of Rhinotermitidae in Alabama forests, it quickly became apparent that a local key was needed for Alabama. Available published keys rely heavily on winged imagos and soldiers, and employ morphometrics. In our survey we made every effort to collect the soldier caste but this was not always possible; we found no imagos. Most of the individuals encountered and collected were workers. These relative caste ratios are typical for rhinotermitid foraging parties (Brown et al. 2008). It has been shown in arthropods that the use of regional keys that employ morphometrics outside of the locale in which they were developed may lead to misidentifications, as clines may bias morphometrics (McGhee 1977). Clinal variation may occur in rhinotermitids (Banks 1946).

Traditional morphology-based species identification of Nearctic Rhinotermitidae has been cast in a derisive light as an unreliable, difficult, and subjective method (Austin et al. 2007, Haverty and Nelson 2007). These opinions have fueled an interest in developing new technologies to increase the accuracy of identifying termite species (Kirton 2005). Molecular applications to rhinotermitid identification include the use of polymerase chain reaction (PCR) and gas chromatography (GC). PCR has been used to analyze genotypic variation in mitochondrial DNA (mtDNA) coding for subunits I and II of cytochrome oxidase *c* (COI, COII) (Jenkins et al. 1999), ribosomal RNA coding for the 16S subunit (16S) (Kambhampati and Smith 1995), and for the mtDNA AT-rich region (Foster et al. 2004). Diagnostic phenotypic variation may be analyzed by GC analysis of cuticular hydrocarbons (CHC) (Howard et al. 1978) from all castes and from cephalic secretions (SDS) from the soldier caste (Moore 1968). PCR has gained wide acceptance as a method for delineating species and systematic relationships, but GC of CHC and SDS remains somewhat controversial (Tripodi et al. 2006). Other non-morphological diagnostic techniques used in the United States include comparative phenology of sympatric species' swarming flights (Banks 1907), interspecific aggression indices (Clément 1978a), and interspecific differences in symbiotic communities of gut protists (Ohkuma et al. 2000).

Non-morphological techniques are not without their problems. While PCR technology has become increasingly less costly due to rapid technological advances, the expense of large-scale molecular sequencing of samples from surveys and ecological work may remain beyond the reach of the many researchers who operate on shoestring budgets. Recent PCR-based revisions of Nearctic *Reticulitermes* species have been criticized for having chronically low sample sizes, overreliance on previous authors' GenBank sequences without any new sampling efforts, and simultaneously using and deriding inappropriate morphological taxonomic literature (Nelson et al. 2008). Basing a large sample's identification on sequencing the DNA from a few individuals may result in a misidentification, as *R. flavipes* and *R. hageni* are sometimes found together at bait stations (Foster et al. 2004). Similarly to PCR, GC of CHC and SDS requires expensive equipment and may be unrealistic for large-scale surveys that operate on limited budgets. The accuracy of GC analysis of CHC is controversial and may overestimate the

Nearctic *Reticulitermes* fauna that remains to be described (Austin et al. 2007). While GC analysis of SDS has been used as a source of diagnostic characters in *Reticulitermes* species (Clément 1978a), at least with respect to Nearctic *Reticulitermes*, no published papers have illustrated these "defensive secretions" as actually having a defensive function (Zalkow et al. 1981). It has been demonstrated that soldiers of the Palearctic *R. speratus* use phragmotic defense in conjunction with mandibular threats, and suggested that Nearctic *Reticulitermes* soldiers may employ similar defensive strategies (Matsuura 2002). In contrast, the defensive properties of the SDS from the frontal glands of *Coptotermes* soldiers, with their larger frontal gland pores, are well documented (Prestwich 1979). Calculating aggression indices requires extensive collection and maintenance of live cultures of putative different species prior to testing, which may prove difficult for researchers without appropriate insect rearing facilities. Establishing disparate swarming phonologies is not possible without time-intensive extended sampling throughout the known or suspected months of reproductive swarming flights, which, in the case of *R. flavipes*, may last through most of the year in southern localities (Weesner 1970). Further complicating this method is the danger of biased data from colonies stimulated to release swarming imagos due to anthropogenic climatic influences, such as climate-controlled buildings or buried plumbing carrying warm water (Hu and Song 2007). Although gut protists of *Reticulitermes* have been known for some time and some keys to rhinotermitids have been attempted with protists (Lewis and Forschler 2006), this method remains controversial due to the effects diet may have on gut fauna species composition (Hu et al. 2011).

Five Rhinotermitidae species are known to occur in Alabama (Clément et al. 1985, Su and Scheffrahn 1986): *Coptotermes formosanus* Shiraki, 1909; *Reticulitermes flavipes* (Kollar, 1837); *R. hageni* Banks, 1920; *R. malletei* Howard and Clément, 1985; and *R. virginicus* (Banks, 1907). *Coptotermes formosanus* is native to the southeastern Oriental, and became established in the southeastern Nearctic through repeated introductions to coastal urban areas along the Gulf of Mexico of the United States in the 1960s (Austin et al. 2006). The first report in Alabama was in 1984, in Mobile, Mobile Co. (Su and Scheffrahn 1986). In 1986, *C. formosanus* was observed in Lee Co. in central Alabama (Sponsler et al. 1988), and has since been reported in an additional 12 counties, including northern montane counties bordering Tennessee (Hu and Oi 2004). The four *Reticulitermes* species are considered native to Alabama (Nutting 1990, Austin et al. 2007). *Reticulitermes flavipes*, *R. hageni*, and *R. virginicus* have long been accepted as valid species (Banks and Snyder 1920). *Reticulitermes malletei* was first named in 1985 in a French journal (Clément et al. 1985) where it received little attention from North American workers, despite being the first *Reticulitermes* species named from the southeastern United States in 65 years. It was ignored by American authors until an otherwise unrelated paper by Scheffrahn et al. (2001) declared it a *nomun nudum* due to not following the provisions of the International Code of Zoological Nomenclature ("the Code") (ICZN 1985). However, the reasons for this rejection were based on an incorrect interpretation of the Code (ICZN 1985, 1999); the species has since been reaffirmed with additional diagnostic criteria. These include PCR of 16S rRNA and morphometrics (Austin et al. 2007) and PCR of COI and COII genes (Lim and Forschler 2012). However, American workers continue to misattribute *R. malletei* to Clément et al. (1986), despite the explicit attribution of its authorship to "Howard and Clément" in Clément et al. (1985). Additionally, although *R. malletei* was named from samples collected in Alabama, Georgia, and Mississippi, with no specific type locality given (Clément et al. 1985), Austin et al. (2007) incorrectly state the species' type locality as Athens, GA, and incorrectly designate holotypes. In imagos, *R. malletei* was described as having black colored wings and reproductive isolation from sympatric *Reticulitermes* species based on temporal separation of reproductive flights and from responses to sexual pheromones; in soldiers, by producing a unique chemical profile of defensive secretions consisting of only sesquiterpenes; and in workers, from differences in volatiles emitted from the cuticle as inferred through aggression index experiments (Clément et al. 1985, Clément et al. 1986). The Code, as applied to new species named from 1930-1999, does not stipulate which characters an author must use for a species name to be made available (ICZN 1999). We affirm that Austin et al. (2007) were correct in their argument that *R. malletei* is a valid, available name, and is compliant with the Code. As covered in the Code (Art. 16.4), types are required only for names designated after 1999 (ICZN 1999). Austin et al. (2007) performed a further service to the community through their designation and deposition of types for *R. malletei*. As of January 2012, no petition denouncing the availability of the species name *R. malletei* has been made to the Commission on Zoological Nomenclature. We thus accept *Reticulitermes malletei* Howard and Clément, 1985 as a valid, available species-group name, and include this species in our key. A sixth species, *R. nelsonae* Lim and Forschler, 2012, was recently described with a distribution including a locality within approximately 100 km of the southeastern Alabama border (Lim and Forschler 2012). It is possible that *R. nelsonae* is distributed in Alabama. However, we have not included this species in our keys, as our efforts to detect its presence within the state's borders have proven unsuccessful.

In order to arrive at a species determination for a rhinotermitid in Alabama, the best available keys are Nutting (1990), Scheffrahn and Su (1994), Hostettler et al. (1995), Messenger (2002), and quite recently, Lim and Forschler (2012). Except for Lim and Forschler (2012), none of these include *R. malletei*, most are devoid of species descriptions, and most are essentially repeats of older keys by N. Banks (1920), A. E. Emerson and E. M. Miller (1943a, b), T. E. Snyder (1954), and F. M. Weesner (1965). All keys applicable to known Alabama Rhinotermitidae rely exclusively on winged imagos (also known as alates or alate imagos) or soldiers. Without these diagnostic castes, available keys do not allow a species determination. Collecting these castes can be challenging. Winged imagos appear only briefly each year during seasonal mating swarms, are rarely encountered in foraging groups, and are often found without their wings (Weesner 1970). Imago forewings contain useful characters; without them, species identification may be difficult (Scheffrahn and Su 1994). Although present throughout the year, soldiers may be difficult to collect due to their low proportions in colonies (Haverty and Howard 1981). In *R. flavipes*, it has been reported that soldiers typically comprise about 2% of colonies in log nests when imagos or neotenics are not present (Howard and Haverty 1980), and approximately 5% in foraging groups in grassland habitat (Brown et al. 2008). *Coptotermes formosanus* tends to have higher proportions of soldiers than species of *Reticulitermes* (Haverty 1977).

Although it is generally believed that the only diagnostic castes of rhinotermitids are winged imagos and soldiers, it has nonetheless been demonstrated that, using workers alone, it is possible to make not only a family determination (Weesner 1987), but also a generic determination (Fontes 1987). Morphological characters of workers have been shown to be useful for generic determinations in rhinotermitids collected outside of the Nearctic (Clément 1978b, Sands 1998). Both *Coptotermes* and *Reticulitermes* occur outside of the Nearctic as well as within it; dentition of the left mandible readily separates this genera (Ahmad 1950). In Europe, it has been demonstrated that the clypeofrontal suture and postclypeus shape may separate *R. flavipes* from sympatric congeners (Austin et al. 2005b).

The primary goal of the present study is to develop a key for the five Rhinotermitidae species that have been demonstrated to occur in Alabama. To be useful, such a key must be able to be reliably employed by entomologists and pest control professionals alike. Our first objective is to utilize workers, the most easily collected caste due to its abundance in both foraging parties and within the colony. Our second objective is to develop keys using soldiers and imagos (including alates and dealates), to supplement or confirm identifications made with workers.

Materials and Methods

Specimens of the five species considered in this manuscript were collected from multiple counties in Alabama from 2004-2011. All samples included at least one of the traditional diagnostic castes (soldiers or alate imagos), and most of these samples contained associated workers. All samples were collected during a single collection event from the same point location (e.g., the same small log). We assumed that each sample came from the same colony. Species identifications were made using morphological characters aided by DNA barcoding.

Established morphological characters of imagos and soldiers were used to distinguish *Coptotermes* from *Reticulitermes*. Winged imagos were separated on the basis of wings having heavy setation and no reticulation in *Coptotermes* or with minimal setation and reticulation in

Reticulitermes; soldiers were separated based on the head capsule shape in dorsal or ventral view being teardrop-shaped *Coptotermes* or rectangular in *Reticulitermes* (Scheffrahn and Su 1994). All *Coptotermes* were assumed to be *C. formosanus*. We collected specimens from 10 colonies during the months of May-Jun. in 2004-2011, from Baldwin, Calhoun, Chilton, Covington, Lee, and Tuscaloosa counties, Alabama.

The four *Reticulitermes* species were identified first using morphological characters and then verified with DNA barcoding. We employed two widely used barcoding genes of the mitochondrion: large subunit of ribosomal RNA (16S) and cytochrome oxidase II (COII) (Jenkins et al. 2001, Austin et al. 2005a). Different methods were used for the two genes. Samples used for 16S included imagos, soldiers, and workers, and came from individuals associated with seven colonies, each sampled on different occasions in 2008 from Lee and Macon counties, Alabama. Workers from these samples had their DNA extracted and 16S gene sequenced according to the protocol of Hu and Scharf ((unpublished)unpublished) . The BLAST® search algorithm was then used to match resulting sequences to their closest GenBank® accessions. Three colonies were identified as *R. flavipes* (DQ001959, DQ001963, EU259768), one colony as *R. malletei* (DQ422137), and one colony as *R. virginicus* (AY257245). All samples used in 16S analysis included alate imagos, soldiers, and workers, except for the *R. malletei* colony sample, which did not contain soldiers. Alate imagos in these samples generally matched morphological species descriptions and keys (Scheffrahn and Su 1994, Austin et al. 2007), although some interspecific variation not reported in Austin et al. (2007) or Lim and Forschler (2012) was observed between *R. flavipes* and *R. malletei* in wing coloration. For DNA barcoding with COII, we used samples from 13 colonies collected 2008-

2010 from Baldwin, Blount, Clarke, Cleburne, Houston, Lee, Limestone, Marshal, Tallapoosa, and Tuscaloosa counties, Alabama, and a sample collected from one colony in East Baton Rouge parish, Louisiana. The Alabama samples were preserved in 70-95% ethyl alcohol and included all castes; the Louisiana sample was preserved in isopropyl alcohol of an unknown concentration and consisted of alate imagos. A single whole termite worker, imago, or soldier was selected from each sample for DNA extraction (Jenkins et al. 2002). A termite was crushed into a solution of 140 µL distilled water, 16 µL 10X Tris base, acetic acid, and ethylenediaminetetraacetic acid (TAE) buffer solution, and 4 µL ZyGEM *prep*GEM® solution. The solution was incubated in a MJ Research PTC-100® thermal cycler for 20 min at 75 °C, 5 min at 95 °C, and then cooled to 4 °C. Samples were transferred to a -20 °C freezer. PCR reaction volumes of each sample consisted of 100 µL extracted DNA supernatant, 100 µL 10X TAE buffer, 0.5 µL dNTP, 1 µL *Taq* polymerase, 100 µL of the forward primer ATG GCA GAT TAG TGC AAT GG (alias TL2-J-3037 and A-tLeu), and 100 µL of the reverse primer GTT TAA GAG ACC AGT ACT TG (alias TK-N-3785 and B-tLys). These primers are well-established as useful for many orders of insects (Liu and Beckenbach 1992), may be used in conjunction to sequence the entire COII gene of insects (Simon et al. 1994), and have been shown to be effective with species of Rhinotermitidae (Jenkins et al. 2002). Reaction volumes were transferred to the thermal cycler for PCR, using the following protocol: denaturing step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 45 s denaturing, 50 °C for 1 min annealing, and 70 °C for 9 min extension. Following PCR the reaction volumes were cooled to 4 °C. Agarose gels were prepared with a 1.5% agarose solution of 0.6 g agarose powder in 25 mL 1X TAE buffer and 4 µL ethidium bromide. Gel electrophoresis was run with a BIO-RAD Mini-Sub Cell GT for 20 min. Gels were viewed under ultraviolet light. Strong bands in the 650-750 bp range were extracted from the gel and prepared for DNA sequencing with a QIAGEN QIAQuick® Gel Extraction Kit, according to the manufacturer's instructions. Nucleic acid content of a 0.5 µL sample of resulting purified DNA from the gel extracts was measured with a Thermo Scientific NanoDrop® 1000 spectrophotometer. Samples of purified DNA with ≥15 ng/µL concentrations of nucleic acids were selected for sequencing. Purified DNA was sequenced with an ABI PRISM® 3100 Genetic Analyzer at the Auburn University Genomics and Sequencing Laboratory. Resulting sequences were searched on NCBI GenBank®, using the BLAST® nucleotide search algorithm. Sequences with ≥98% MaxIdent matches to several GenBank® depositions of the same species were considered to belong to that species. The DNA barcoding procedure was repeated until we obtained multiple collection samples representing all castes of the four *Reticulitermes* species. Vouchers of samples examined in the construction of the keys have been deposited into the Auburn University Entomology Museum in vials of EtOH or mounted on slides. Vouchers of specimens with DNA extracted in solution are stored at -20 °C at the Auburn University Genomics and Sequencing Laboratory.

From this combination of morphological and DNA barcoding methods we created a sample pool comprising all castes of the five species from collections across the geographical range of Alabama and one from eastern Louisiana (Table 1). Specimens were prepared for examination in one of three states: in alcohol, on temporary slides, or on permanent slides. Temporary slide specimens were neither cleared nor stained prior to slide-mounting. Ethyl alcohol or lactic acid was used as a slide medium. After examination those specimens observed in EtOH and on temporary slides were placed in a new vial with identical preservative. Printed locality, coding, and determination labels were included with a pencil-written label, as recommended by Wheeler et al. (2001) for long-term storage. Specimens designated for permanent slides were cleared and stained prior to slide-mounting, utilizing a method commonly used for coccoid scale insects (Williams and Kosztarab 1972, Williams 2012): 2 d in 5 mL 10% aq. KOH to macerate soft tissue, 3 d in an approx. 1 mL solution of 1 mL Essig's Aphid Fluid and 2 drops of Triple Stain to stain, 10-20 min in 5 mL 95-100% EtOH to rinse excess stain, and then 15 min in 1 mL clove oil to remove the alcohol and increase miscibility in mounting medium. Specimens were placed on slides with a mounting medium solution of Canada Balsam liquefied with xylene and then covered with a glass cover slip. Typically three specimens were placed on a single slide; a numbering system for each slide was employed to prevent duplicate measurements. Slides were kept at 50 °C for 4-5 wk to evaporate the xylene out of the mounting medium from under the cover slips. Heating also had the effect of removing any unwanted condensation or effervescence in the mounting medium that made it difficult to measure characters under high magnification. Slides were affixed with locality and determination labels in accordance with the recommendations of Wheeler et al. (2001). In some cases dissections were made of specimens. Dissected parts were affixed to slides or returned to vials with their associated individuals. All vials and slides were kept at 20-25 °C in climate-controlled rooms.

Iterative searches for taxonomically useful characters were performed on prepared specimens associated with samples identified through morphology or DNA barcoding. Characters were observed using a stereo dissecting microscope (Olympus® SZX12) and two different stereo compound microscopes (Olympus® BX51 and Laborlux 12 POL). Character evaluations were made under magnifications up to 90X on the dissecting microscope, and at

100X or 400X on the compound microscopes. Phase contrast was found to be useful for some characters, such as chaetotaxy of abdomen styli. Measurements were made with graticules mounted in the Olympus® SZX12 and Olympus® BXS51 microscopes. For workers, we preferentially used later developmental stages of workers that had recently molted, as evidenced by little wear on their mandibles. Characters examined in constructing the keys included 108 from workers, 24 from soldiers, and 14 from imagos. Shape-based characters were coded numerically as binary (present/absent) or categorical (if more than one character state was possible). A character was deemed useful if, when N ≥10, its $\bar{x} \pm 1$ SD did not overlap between species separated by the couplet.

Photographs of useful characters were taken with a ProgRes™C12Plus digital camera operated by ProgRes™C12Plus software. This camera was integrated with the Olympus® microscopes. To compensate for the shallow depth of field inherent to microscope photography, we used the program CombineZP (Hadley 2010) or ZereneStacker (Zerene Systems) to digitally stitch together images taken at different focal lengths. Features of resulting composite images were highlighted for illustrative purposes using the program GIMP (Kimball and Mattis 1995-2008) or Photoshop® (Adobe® Systems Incorporated).

Voucher specimens, preserved in 70-100% EtOH or on permanent slides, are stored in the Auburn University Entomology Museum.

Results

A. Key to Rhinotermitidae species of Alabama: imago caste

1. Wings setose (Fig. 2); left mandible first marginal tooth too short to meet a line drawn between apical tooth and second marginal tooth (Fig. 3); color of head, sternites, and tergites yellow-brown; often found flying at night and often attracted to lights; often found associated with structural wood and urbanized areas, rarely found in forests .... ***Coptotermes formosanus*** **Shiraki, 1909**

Wings reticulated (Fig. 2); left mandible first marginal tooth long enough that it will meet a line drawn between apical tooth and second marginal tooth (Fig. 3); color of head, sternites, and tergites yellow-brown to black; rarely found flying at night and rarely attracted to lights, often found flying during the day; found associated with structural wood, urbanized areas, or in forests **2**

2. Color of head, sternites and tergites yellow-brown (Fig. 4); wing membrane color when two wings overlap hyaline (Fig. 4); forewing does not widen on sagittal plane half-way along length (Fig. 5); rarely found in structural wood and urbanized areas, often found in forests .... ***Reticulitermes hageni*** **Banks, 1920**

Color of head, sternites and tergites brown to black (Fig. 4); wing membrane color when two wings overlap hyaline, light brown, or light grey (Fig. 4); forewing widens on sagittal plane half-way along length (Fig. 5) **3**

3. Wing membrane color when two wings overlap hyaline (Fig. 4); forewing veins in costal field light brown basally and whitish or hyaline distally; color of head, sternites, and tergites dark brown to black (Fig. 4)) .... ***Reticulitermes virginicus*** **(Banks, 1907)**

Wing membrane color when two wings overlap light brown to grey (Fig. 4); forewing veins in costal field light brown throughout; color of head, sternites, and tergites light brown to dark brown (Fig. 4) **4**

4. Forewing media vein terminal branch aligned with cubitus vein terminal branch (Fig. 6); forewing cell M_1 elongate, weakly curved, without large crossveins; distance of gap between eye and ocellus less than the widest diameter of ocellus ....
.... ***Reticulitermes malletei*** **Howard and Clément, 1985**

Forewing media vein terminal branch not aligned with cubitus vein terminal branch, media terminal branch distinctly basal to cubitus terminal branch; forewing cell M_1 squat, strongly curved, with several large crossveins (Fig. 6); distance of gap between eye and ocellus at least equal to the width of the widest diameter of the ocellus ....
.... ***Reticulitermes flavipes*** **(Kollar, 1837)**

B. Key to Rhinotermitidae species of Alabama: soldier caste

1. Head in dorsal view broadly triangular, narrowing anteriorad (Fig. 7); head anterodorsal region in lateral view smooth, abruptly curves ventrad ~90°; head in dorsal view with fontanelle located anteriorly, fontanelle in anterior view broadly triangular in shape; each abdomen stylus with 7-10 setae (Fig. 8); soldier proportion in colonies and foraging groups typically ≥10%; often found associated with structural wood and urbanized areas, rarely found in forests ....

.... ***Coptotermes formosanus* Shiraki, 1909**

Head in dorsal view broadly rectangular, does not narrow anteriorly (Fig. 7); head anterodorsal region in lateral view humped, gradually curves ventrad ≤60°; each abdomen stylus with 4-5 setae (Fig. 8); soldier proportion in colonies and foraging groups typically ≤5%; found associated with structural wood, urbanized areas, or in forests .. **2**

2. Pronotum in dorsal view with width 0.90-1.04 mm (Fig. 10); head in lateral view with height at approximate center 0.99-1.07 mm (Fig. 10); head in lateral view with length measured from mandible insertion to vertex 1.73-1.93 mm (Fig. 10); labrum in dorsal view rounded basally, with width 0.360-0.418 mm (Fig. 9) .... ***Reticulitermes flavipes* (Kollar, 1837)**

Pronotum in dorsal view with width 0.68-0.78 mm (Fig. 10); head in lateral view with height at approximate center 0.77-0.89 mm (Fig. 10); head in lateral view with length measured from mandible insertion to vertex 1.34-1.75 mm (Fig. 10); labrum in dorsal view rounded or angular basally, with width 0.261-0.365 mm (Fig. 9) **3**

3. Labrum in dorsal view rounded basally, with width 0.343-0.365 mm (Fig. 9); head in lateral view with height at approximate center 0.79-0.83 mm (Fig. 10); head in lateral view with length from mandible insertion to vertex 1.74-1.89 mm; pronotum in dorsal view with width 0.75-0.78 mm (Fig. 10) .... ***Reticulitermes virginicus* (Banks, 1907)**

Labrum in dorsal view angular basally, with width 0.261-0.343 mm (Fig. 9); head in lateral view with height at approximate center 0.77-0.89 mm (Fig. 10); head in lateral view with length from mandible insertion to vertex 1.34-1.71 mm; pronotum in dorsal view with width 0.68-0.76 mm (Fig. 10) **4**

4. Head in lateral view with length measured from mandible insertion to vertex 1.34-1.50 mm (Fig. 10); head in lateral view with height at approximate center 0.77-0.81 mm (Fig. 10); head capsule in dorsal view with width measured immediately posterior to mandibles 0.89-0.94 mm; labrum width in dorsal view 0.264-0.296 mm .... ***Reticulitermes malletei*** **Howard and Clément, 1985**

Head in lateral view with length measured from mandible insertion to vertex 1.49-1.71 mm (Fig. 10); head in lateral view with height at approximate center 0.79-0.89 mm (Fig. 10); head capsule in dorsal view with width measured immediately posterior to mandibles 0.93-0.99 mm; labrum width in dorsal view 0.261-0.343 mm .... ***Reticulitermes hageni*** **Banks, 1920**

C. Key to Rhinotermitidae species of Alabama: worker caste

1. Left mandible first marginal tooth shorter than apical tooth and second marginal tooth (Fig. 3); each abdomen stylus with 7-10 setae (Fig. 8); left maxilla lacinia ctenidium with 12-14 setae (Fig. 11); proventriculus width viewed through cuticle at basal widest width 0.38-0.50 mm (Fig. 12); leg I tibia length 0.80-0.91 mm; soldier proportion in colonies and foraging groups typically ≥10%; often found associated with structural wood and urbanized areas, rarely found in forests .... ***Coptotermes formosanus*** **Shiraki, 1909**

Left mandible first marginal tooth as long as apical tooth and second marginal tooth (Fig. 3); each abdomen stylus with 4-5 setae (Fig. 8); left maxilla lacinia ctenidium with 8-11 setae (Fig. 11); proventriculus width viewed through cuticle at basal widest width 0.24-0.40 mm (Fig. 12); leg I tibia length 0.50-0.77 mm; soldier proportion in colonies and foraging groups typically ≤5%; found associated with structural wood, urbanized areas, or in forests **2**

2. Pronotum width 0.75-0.91 mm; head width measured between mesal condyles of mandibles 0.65-0.73 mm (Fig. 13); head width measured between lateral edges of sockets where mandible lateral condyles attach 0.81-0.92 mm .... ***Reticulitermes flavipes*** **(Kollar, 1837)**

Pronotum width 0.56-0.71 mm; head width measured between mesal condyles of mandibles 0.50-0.60 mm (Fig. 13); head width measured between lateral edges of sockets where mandible lateral condyles attach 0.60-0.81 mm **3**

3. Head width measured between lateral edges of sockets where mandible lateral condyles attach 0.68-0.81 mm .... ***Reticulitermes malletei*** **Howard and Clément, 1985**

Head width measured between lateral edges of sockets where mandible lateral condyles attach 0.60-0.66 mm **4**

4. Each abdomen stylus, especially left stylus, usually with a mesal-pointing seta; each abdomen stylus usually with 5 setae .... ***Reticulitermes virginicus*** **(Banks, 1907)**

Each abdomen stylus, especially left stylus, usually without a mesal-pointing seta; each abdomen stylus usually with 4 setae .... ***Reticulitermes hageni*** **Banks, 1920**

Discussion

The precise biometric measurements required for separating soldiers, intraspecific variation, and slight interspecific overlap have motivated many authors to look for non-morphological characters (Haverty et al. 1996, Szalanski et al. 2003). It has been claimed that "subtle clinal variations" exist as justification for not trusting morphological-based identifications of Rhinotermitidae, particularly in *Reticulitermes* (Szalanski et al. 2003). Should clinal variations exist in *Reticulitermes,* it is expected that species would exhibit intraspecific variation according to some version of Bergmann's or Allan's Rule (Ray 1960), as has been demonstrated to occur in the leg length of harvestmen (McGhee 1977) between the southern and northern extents of the Appalachian Mountains. A cline in leg length of *R. virginicus* has been suggested to occur between Illinois and Florida (Banks 1946), but this was based on few samples and these results do not appear to have been replicated within other termite taxa (Blanckenhorn and Demont 2004). Szalanski et al. (2003) did not provide evidence for clinal variations: if they do occur in *Reticulitermes*, the scale at which their effect begins to show is not clear. The case for clines has been a prime motivator for developing non-morphological methods (Szalanski et al. 2003). However, all valid species of Rhinotermitidae to which these new, non-morphological techniques are being applied were originally described based at least in part on differences in their morphology, including morphometric data (Banks 1907, Banks and Snyder 1920, Austin et al. 2007).

The low species richness of Alabama's Rhinotermitidae has made it possible to create a key based on workers, the caste most readily available and most often collected by nonspecialists. Re-examination of established characters for imagos and soldiers was combined with novel characters in constructing keys for soldiers and imagos. A quality microscope with a graticule is required for determining many of the characters we employ, particularly those involving biometrics. In devising our keys we have found that 5-10 specimens will give the most reliable identification, particularly if only workers are available. These numbers are similar to what Hostettler et al. (1995) reported necessary for using labrum shape to make species determinations with soldiers. It is typically necessary to make permanent, stained slides to see characters of the abdomen styli used in separating *Reticulitermes*, but a temporary slide mount will do for separating *C. formosanus* workers from *Reticulitermes* workers on the basis of the abdomen styli. Whenever possible, recently molted, later-instar specimens should be used for mandibulate characters, as the mandibles of workers are subject to wear over time (Fontes 1987). Due to the continuous stationary molting of termite workers, and the ease of collecting this caste, it is not difficult to obtain enough appropriate specimens.

Soldier proportions we report are based on literature (Haverty 1977, Haverty and Howard 1981) and our own observations. Although we have not found reports of soldier proportions of *R. malletei,* the ratio appears to be similar as in the other three *Reticulitermes* species in Alabama. In one sample of *R. malletei* collected on 22 Sep. 2008 in Lee County, Alabama, we counted 1500 workers, 41 soldiers, and 298 nymphs with wing pads. In this sample the proportion of soldiers to workers was 0.03, and the proportion of soldiers to all castes was 0.02. In the portions of soldier key splitting *Reticulitermes* we made use of head height measurements, which do not appear to be reported in either keys or re-descriptions of the four *Reticulitermes* species occurring in Alabama. Our motivation for investigating this character was the colony-level stability of head height found in soldiers of Palearctic *Reticulitermes* (Matsuura 2002).

For imagos, wings are often detached from the body; sometimes one is faced with specimens consisting of only wings. The overall shape and venation pattern of wings in termites is highly distinctive; generic-level identification may be easily done on the basis of a single wing. Specimens captured on sticky traps, for example, may consist of only wings or badly preserved bodies. In separating *Reticulitermes* imagos, we made use of the shape and venation of the forewing. As with all isopterans, rhinotermitids have highly similar forewings and hindwings. This problem may be overcome by examining wing venation of detached wings. A forewing may be distinguished from a hindwing by the media vein: in the forewing the media vein branches terminally to create the M1 wing cell, while in the hindwing the media vein does not branch terminally and there is no M1 wing cell. Although useful for our specimens, it is unknown at present whether this method for separating the forewing from the hindwing may be employed with collections that are made outside of Alabama.

We have not been able to replicate some characters reported in the literature. Banks and Snyder (1920) report Rhinotermitidae as a whole to not have tibia spines, but all species we examined possess spines on their tibiae at the point where the tarsi are inserted. Ahmad (1950) reported the ratio of tibia spines between fore-, mid-, and hindlegs to be 3:3:3 in Coptotermitinae and 3:2:2 in Heterotermitinae, but we found the tibia spine ratio to be universally 3:2:2 in the five species we examined. When viewed laterally, the postclypeus shape was reported to appear different between *R. flavipes* and native Palearctic *Reticulitermes* (Clément 1978b). We found no difference amongst the native Nearctic *Reticulitermes* we examined, or between these species and the introduced *C. formosanus*. However, we did find characters reported in the relative dentition of the left and right mandibles of *Coptotermes* and *Reticulitermes* (Ahmad 1950, Sands 1998) useful for separating these genera in Alabama. The discrepancies between reportedly useful characters and those actually apparent in our specimens may be due to peculiarities of the five species we have separated, or to variation between populations of these species in Alabama and the collection localities of the specimens from which these characters were derived. Several aspects of *R. malletei* reported by Austin et al. (2007) are mistaken. For example, they reported measurements of the gula of soldiers to be 2.897 mm for width at widest point, 6.995 mm for width at narrowest point, and 0.896 mm for length (Austin et al. 2007). We calculated the mean length of the head of *R. malletei* soldiers, measured from the clypeus to the vertex, to be 1.47-1.65 mm ($\bar{x}$ ±1 SD, N=7). The relative characters reported of the length of the soldier gula divided by the soldier gula width at widest and at narrowest points are both incorrect. As Austin et al. (2007) are the first to report biometrics of *R. malletei*, it is important to note that the measurements we provide comprise the first correct morphometrics for the soldier caste of this species.

Morphological-based identification should not be abandoned, even in the cryptic Nearctic *Reticulitermes*. We have demonstrated that morphology remains a powerful tool for identifying Rhinotermitidae species in our species. Nonetheless, whenever possible morphological identifications should be aided and verified by the many emerging alternative methods. The differing morphology and ease of collecting the different castes in Rhinotermitidae requires that any determination be made from as many methods as possible. This is particularly true if our key is applied to specimens captured outside the geographic range of our collections, a practice that we strongly discourage. Termites are easily collected in large numbers, and given patience at least soldiers and workers can be collected in most instances. As such, we recommend that many individuals of as many castes as possible should be collected. To take full advantage of all methods available, it is advisable to store some in the highest alcohol concentration available. Simultaneously, additional individuals should be kept alive in a darkened container with a moist cellulose substrate so that methods requiring live specimens may be used. Both morphological and molecular methods are sensitive to the quality of preservation: if preserving fluids are not available in the field, providing live specimens will ensure a better chance of an accurate identification.

If live specimens are available, work on gut protists of *R. flavipes*, *R. hageni*, and *R. virginicus* collected in the United States (Lewis and Forschler 2006) and of *C. formosanus* collected in Japan (Ohkuma et al. 2000) is showing some promise as an alternative identification method. In comparing these four species there is at least one protist unique to each. Unfortunately, recent work on *R. malletei* has neglected to include its gut protist fauna (Austin et al. 2007, King et al. 2009, Lim and Forschler 2012). It is possible that some specimens identified by Lewis and Forschler (2006) were *R. malletei*. If this were clarified, gut protists may provide another source of inexpensive identification of rhinotermitid species in Alabama. However, if only workers are available, combining morphological characters from preserved specimens with gut protists from live specimens should produce a more accurate identification. If gut protists are to be used, they should be extracted from workers as soon as possible, as the composition of the fauna may change in laboratory colonies depending on what cellulose substrate is used (Hu et al. 2011). A further underappreciated value of gut protists, particularly in *Reticulitermes*, lies in their potential value in systematics studies (Saldarriaga et al. 2011).

The keys we have presented comprise the first regional keys for Alabama's termite species, the first morphological key in the United States to use the worker caste, and the second published morphological tool to separate *R. malletei* from sympatric congeners. Morphological identification continues to hold advantages over other methods. This long-established technique can still be used where others will fail: specifically with specimens that are dead or preserved with methods that have degraded their DNA, or those consisting of just a wing, which may not contain enough nucleotides of the gene of interest for DNA barcoding with the current limits of technology. The time commitment continues to be far less compared to non-morphological techniques, and in most cases the only tool needed to employ the method is nothing more than the microscope already present in an arthropod-oriented laboratory. Morphological-based identification remains the least expensive and most time-effective method to determine termite species for scientists and pest control professionals, even with problematic genera such as *Reticulitermes*. However, it must be stressed that employing multiple castes including multiple specimens will give the most accurate identification; when necessary, further confirmation may be given employing non-morphological methods. We suggest that future research be directed at developing additional regional morphological keys, including gut fauna, that non-morphological techniques begin to incorporate gut fauna, and strongly discourage the utilization of regional keys for research that is geographically broad in scope.

Table 1. Numbers of voucher specimens used in constructing the keys.

Species	Imagos	Soldiers	Workers	Total
C. formosanus	131	225	157	513
R. flavipes	62	23	270	355
R. hageni	52	15	20	87
R. malletei	129	42	1570	1741
R. virginicus	292	13	175	480
				3176

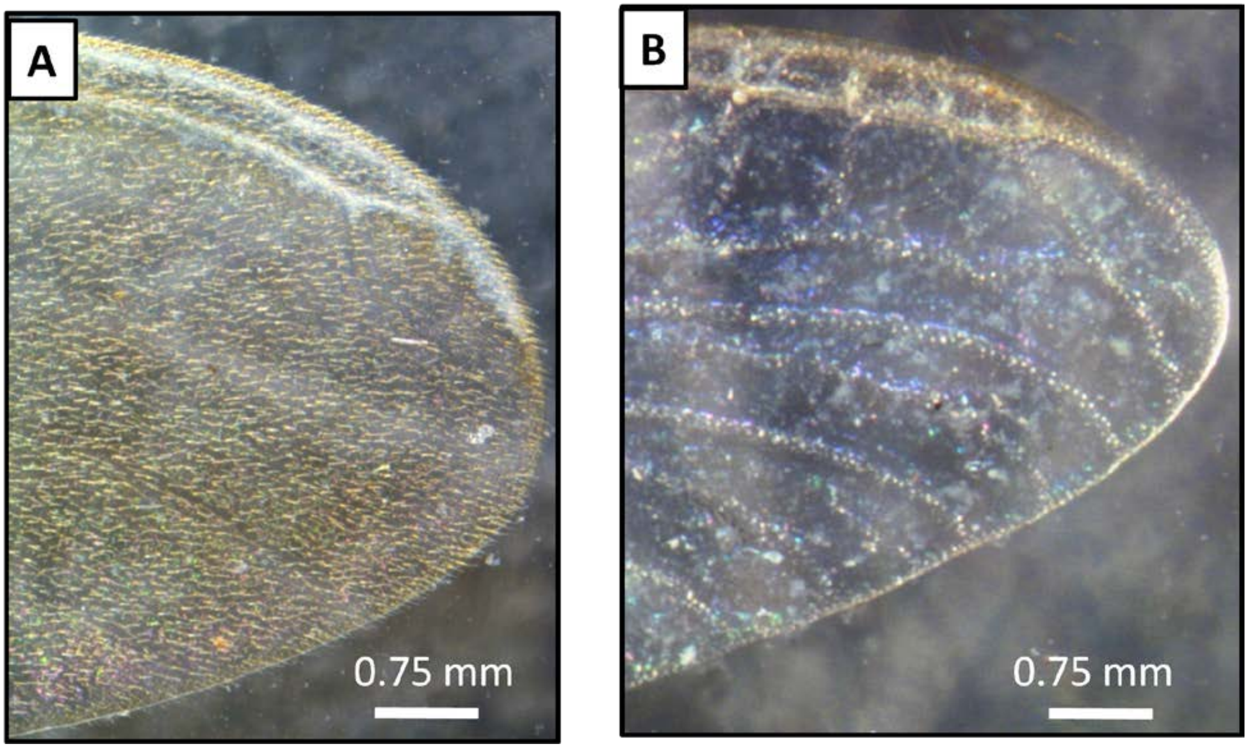

Figure 2. Apex of forewing. **(A)** *C. formosanus*; **(B)** *Reticulitermes*. Each image is a composite of photographs taken at 100X of specimens in EtOH that have been focally stacked with ZereneStacker using the PMax algorithm (A, 4 photographs; B, 5 photographs); resulting images were touched up for illustrative purposes with Photoshop.

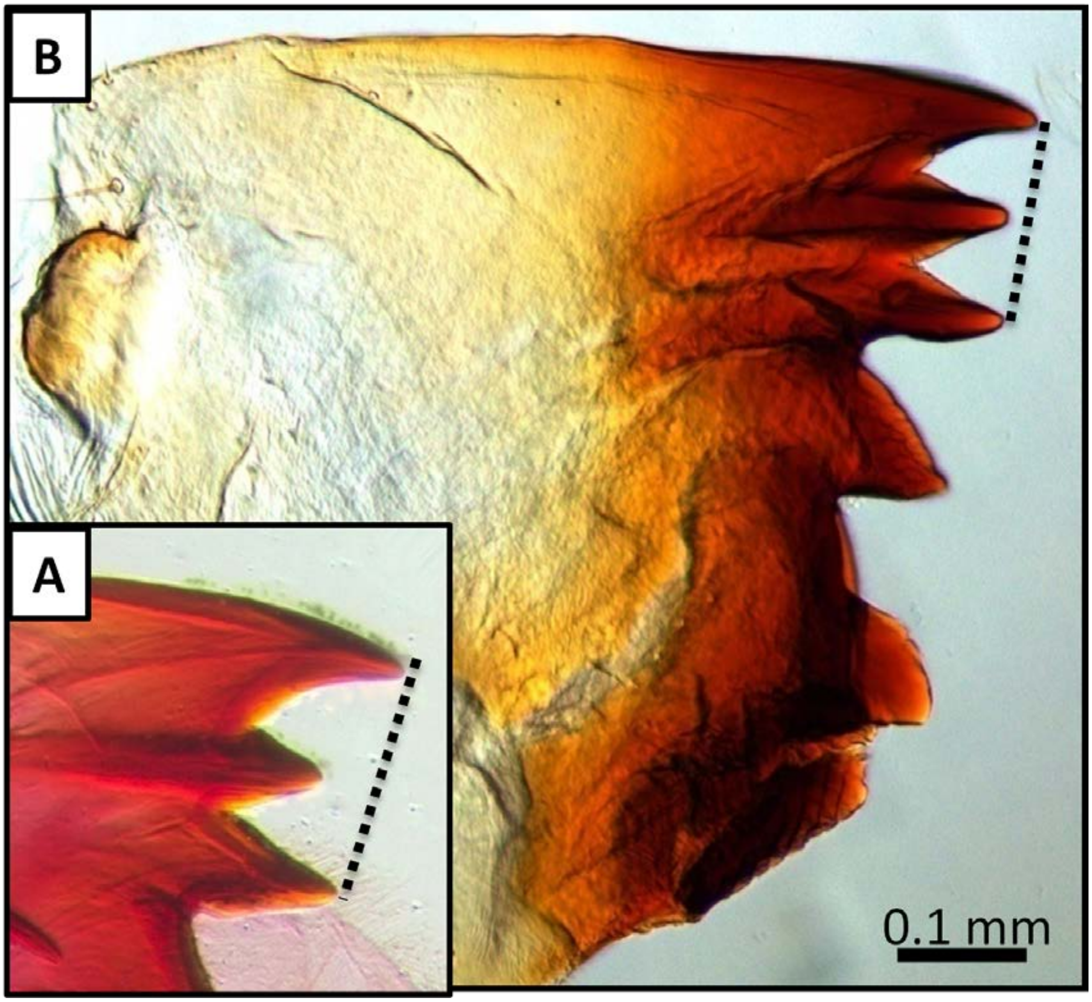

Figure 3. Imago and worker, left mandible. (**A)** *C. formosanus*; (**B)** *Reticulitermes.* Each image is a composite of photographs taken at 100X (A, 11 photographs of specimen on permanent slide; B, 2 photographs of specimen in lactic acid) that have been focally stacked using CombineZP, that was then touched up for illustrative purposes with Photoshop.

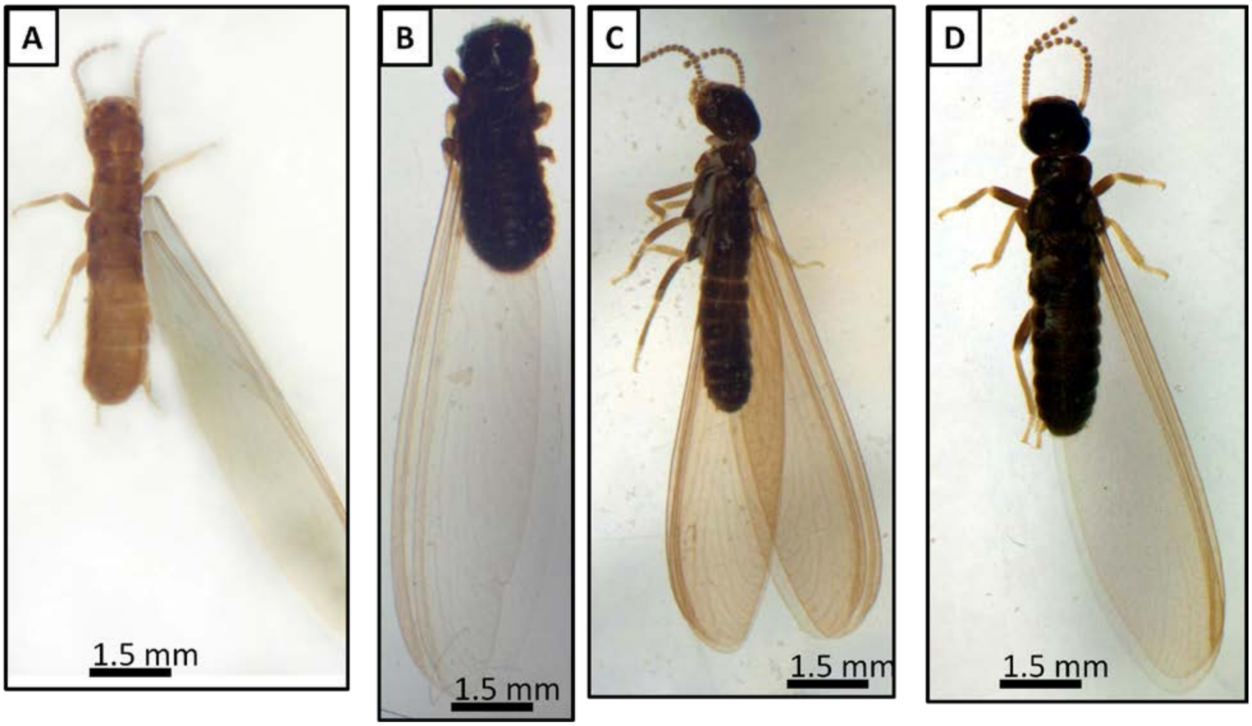

Figure 4. Imago, habitus. **(A)** *R. hageni*; **(B)** *R. virginicus*; **(C)** *R. malletei*; **(D)** *R. flavipes.* Each image is a composite of photographs taken at 125X of specimens in EtOH (A, 6 photographs; B-D, 7 photographs) that have been focally stacked using ZereneStacker with the PMax algorithm, that was then touched up for illustrative purposes with Photoshop.

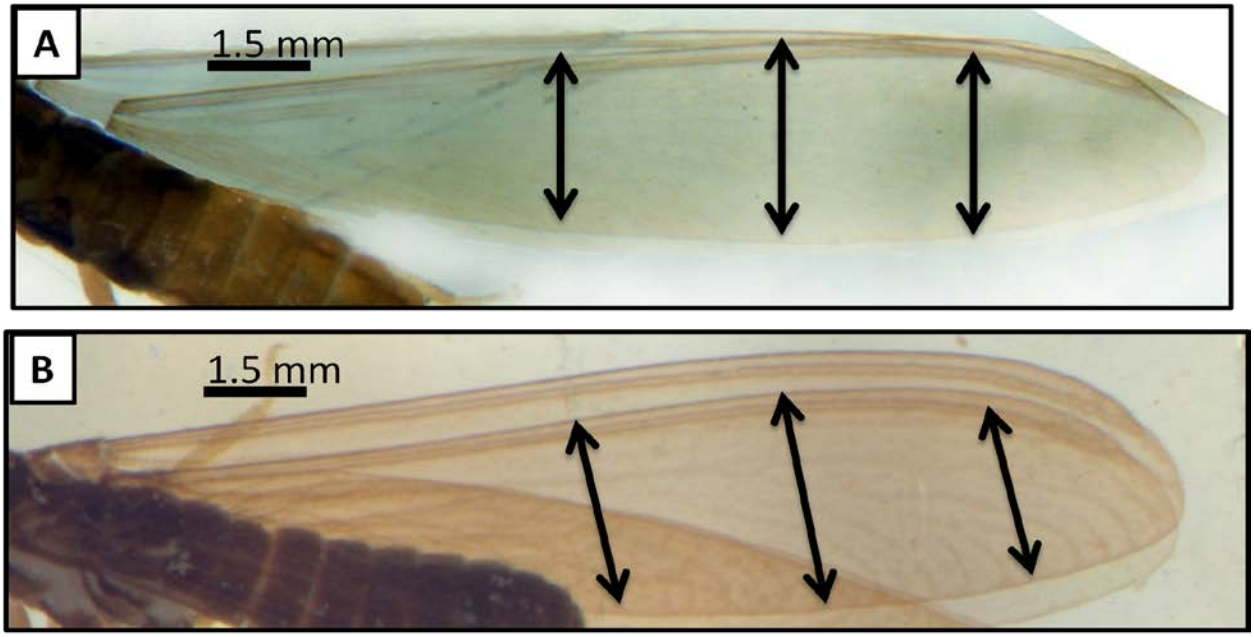

Figure 5. Imago, forewing. (**A)** Forewing does not widen on sagittal plane half-way along length (*R. hageni*); (**B)** forewing widens on sagittal plane half-way along length (*R. malletei*). Each image is a composite of photographs taken of specimens in EtOH at 125X (A, 6 photographs; B, 7 photographs) that have been focally stacked with ZereneStacker using the PMax algorithm, that was then touched up for illustrative purposes with Photoshop.

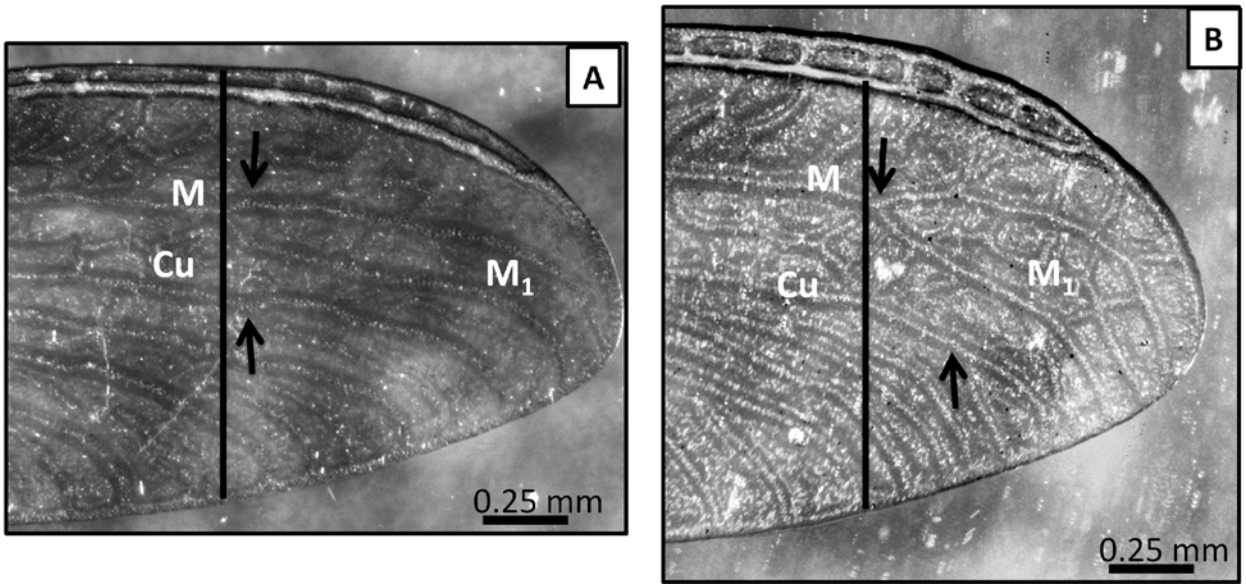

Figure 6. Imago, forewing. **(A)** Forewing media vein terminal branch aligned with cubitus vein terminal branch, forewing cell M_1 elongate, weakly curved, without large crossveins (*R. malletei*); **(B)** forewing media vein terminal branch not aligned with cubitus vein terminal branch, media terminal branch distinctly basal to cubitus terminal branch, forewing cell M_1 squat, strongly curved, with several large crossveins (*R. flavipes*). Each image is a composite of photographs taken at 100X of specimens in EtOH (A, 3 photographs; B, 4 photographs) that have been focally stacked with ZereneStacker using the PMax algorithm, that was then touched up for illustrative purposes with Photoshop.

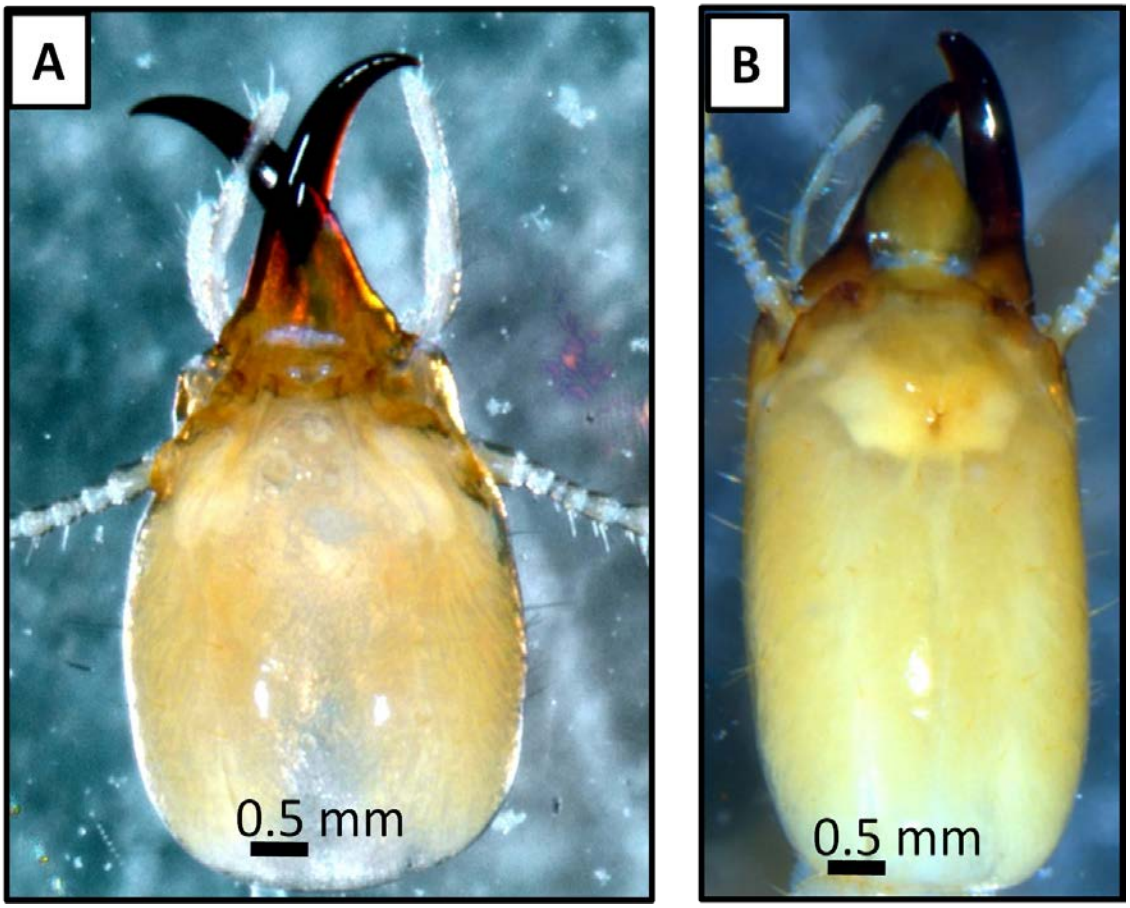

Figure 7. Soldier, head. (**A)** Head in dorsal view teardrop-shaped or broadly triangular, narrowing anteriorad (*C. formosanus*); (**B)** head in dorsal view broadly rectangular, does not narrow anteriorly (*Reticulitermes*). Each image is a composite of photographs taken at 100X of specimens in EtOH (A, 10 photographs; B, 9 photographs) that have been focally stacked with ZereneStacker using the PMax algorithm, that was then touched up for illustrative purposes with Photoshop.

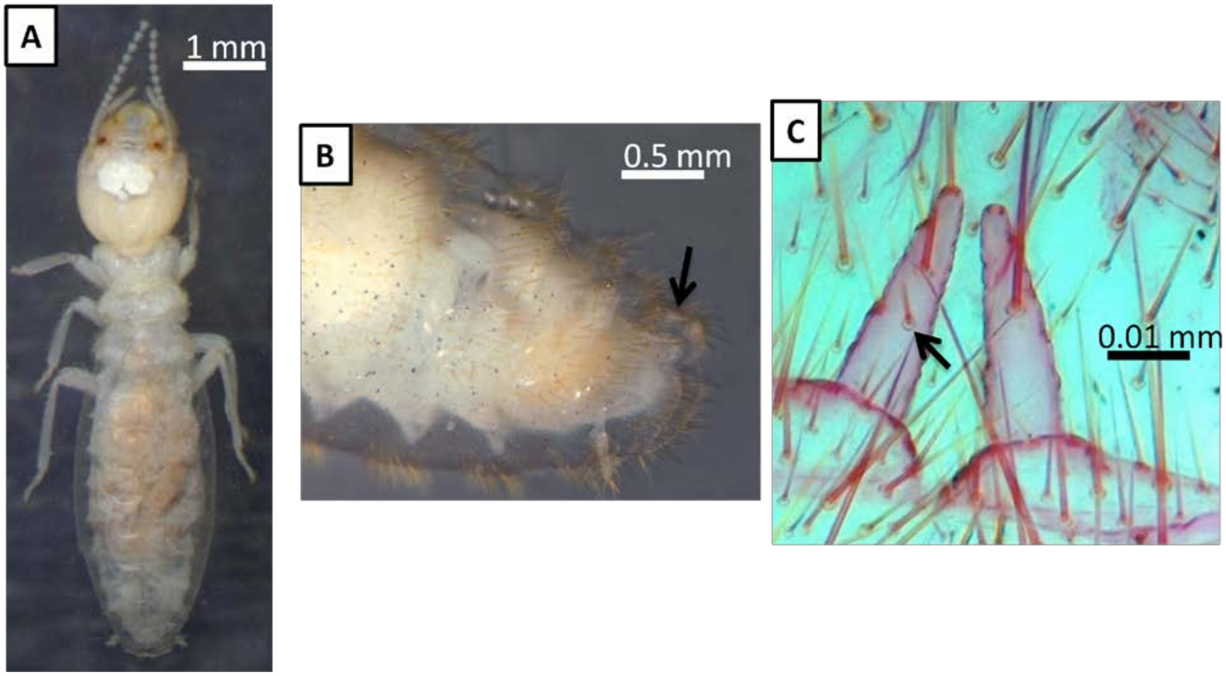

Figure 8. Worker, abdomen stylus. (**A)** Habitus of worker, dorsal view (*R. flavipes*); **(B)** posterior sternites of abdomen, ventral view, arrow points to location of styli *(R. flavipes*); **(C)** abdomen styli, ventral view, arrow points to a seta and its insertion socket (*R. flavipes*). Each image is a composite of photographs that have been focally stacked with ZereneStacker using the PMax algorithm (A, 8 photographs of specimen in EtOH at 200X; B, 10 photographs of specimen in EtOH at 100X; C, 5 photographs of slide-mounted specimen at 100X). Resulting composite images were touched up for illustrative purposes with Photoshop.

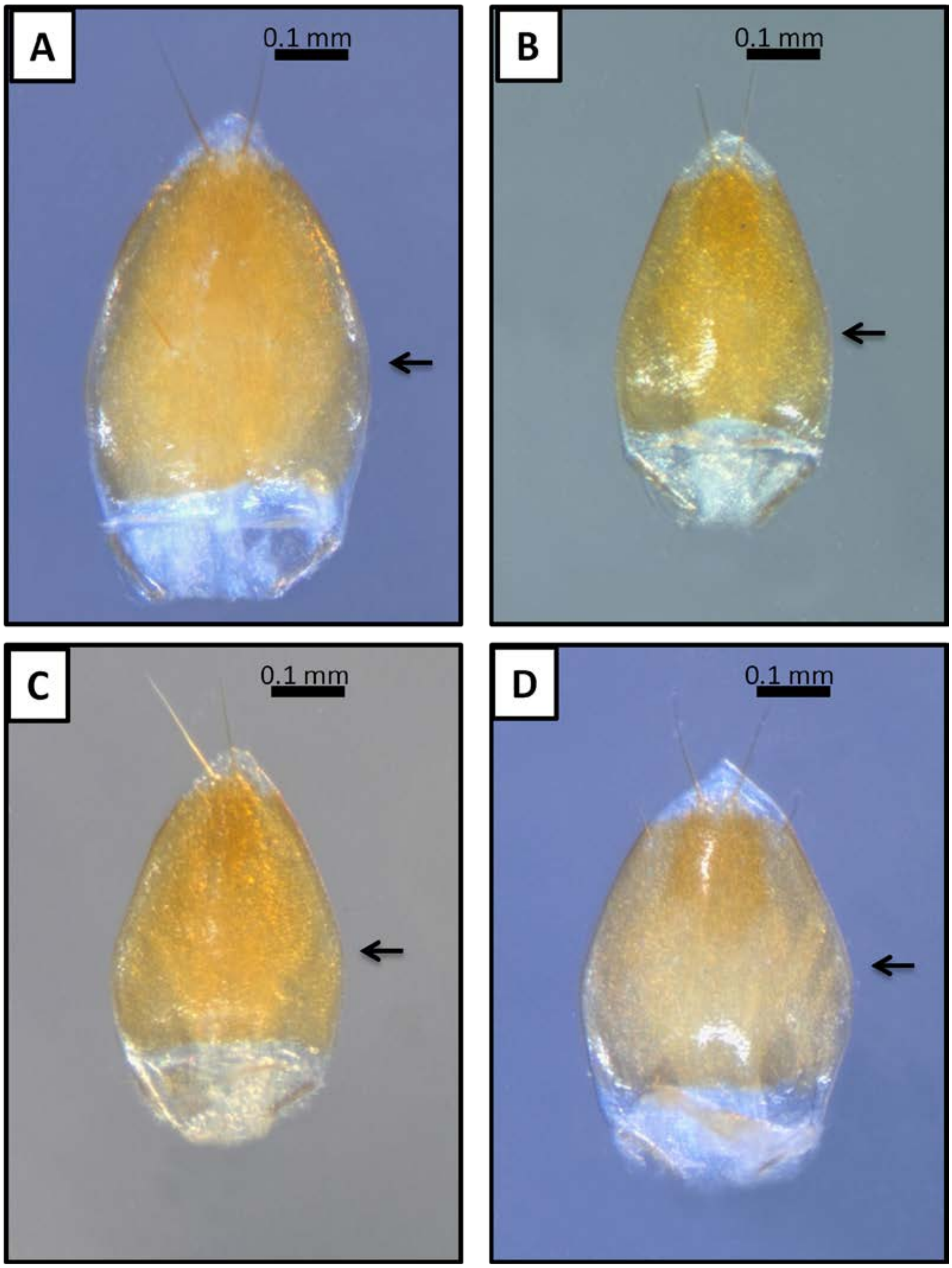

Figure 9. Soldier, labrum, in dorsal view. **(A)** *R. flavipes*, labrum rounded; **(B)** *R. hageni*, labrum angular; **(C)** *R. malletei*, labrum angular; **(D)** *R. virginicus*, labrum rounded. Arrow points to location where labrum is rounded or angular. Each image is a composite of 7 photographs taken at 900X that were focally stacked with ZereneStacker using the PMax algorithm; resulting composite images were touched up for illustrative purposes with Photoshop.

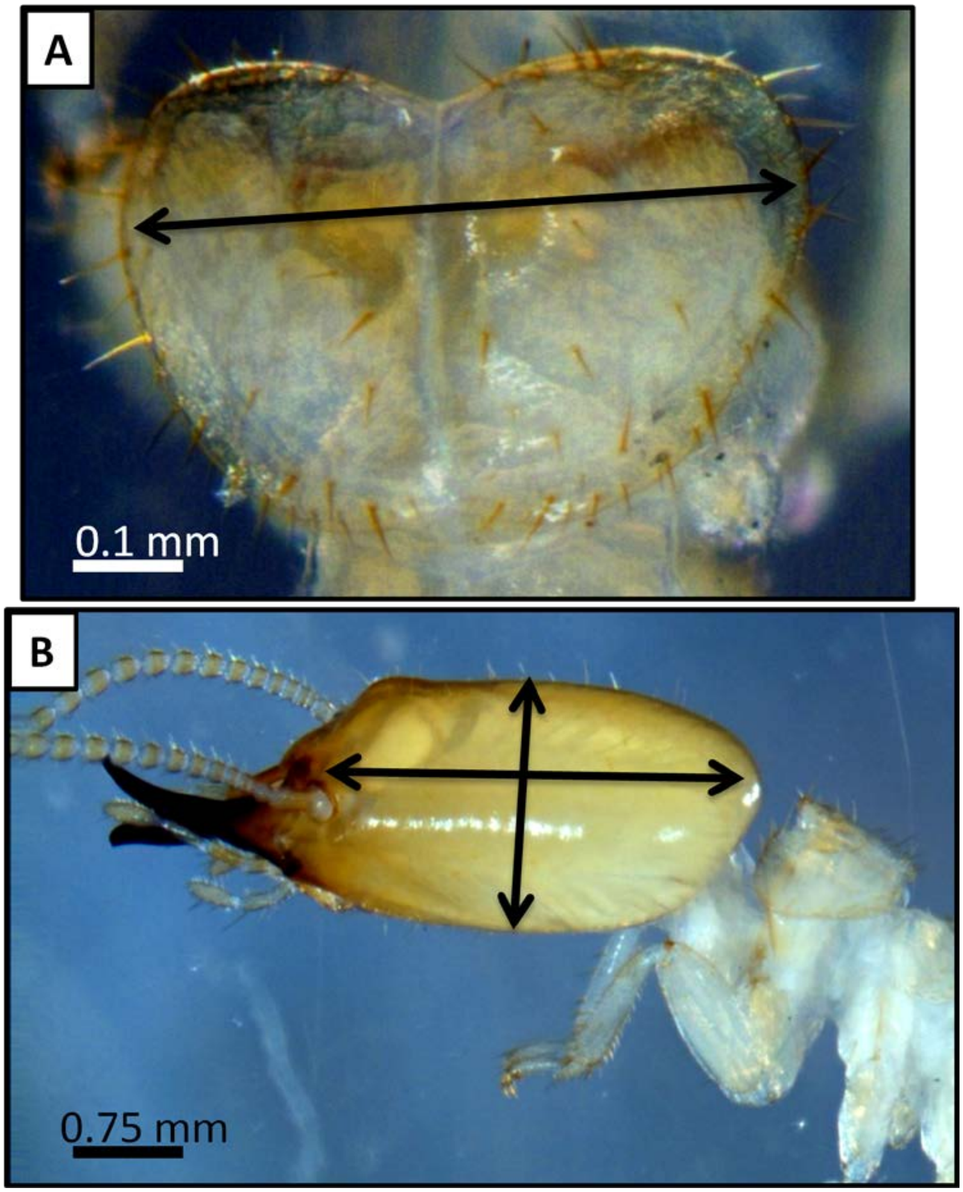

Figure 10. Soldier, pronotum and head. **(A)** Pronotum, in dorsal view (*R. flavipes*); **(B)** head, in lateral view (*R. hageni*). Arrows indicate where measurements were made. Each image is a composite of 5 photographs of specimens in EtOH that were focally stacked with ZereneStacker using the PMax algorithm (A, photographs taken at 400X; B, photographs taken at 250X); resulting images were touched up for illustrative purposes with Photoshop.

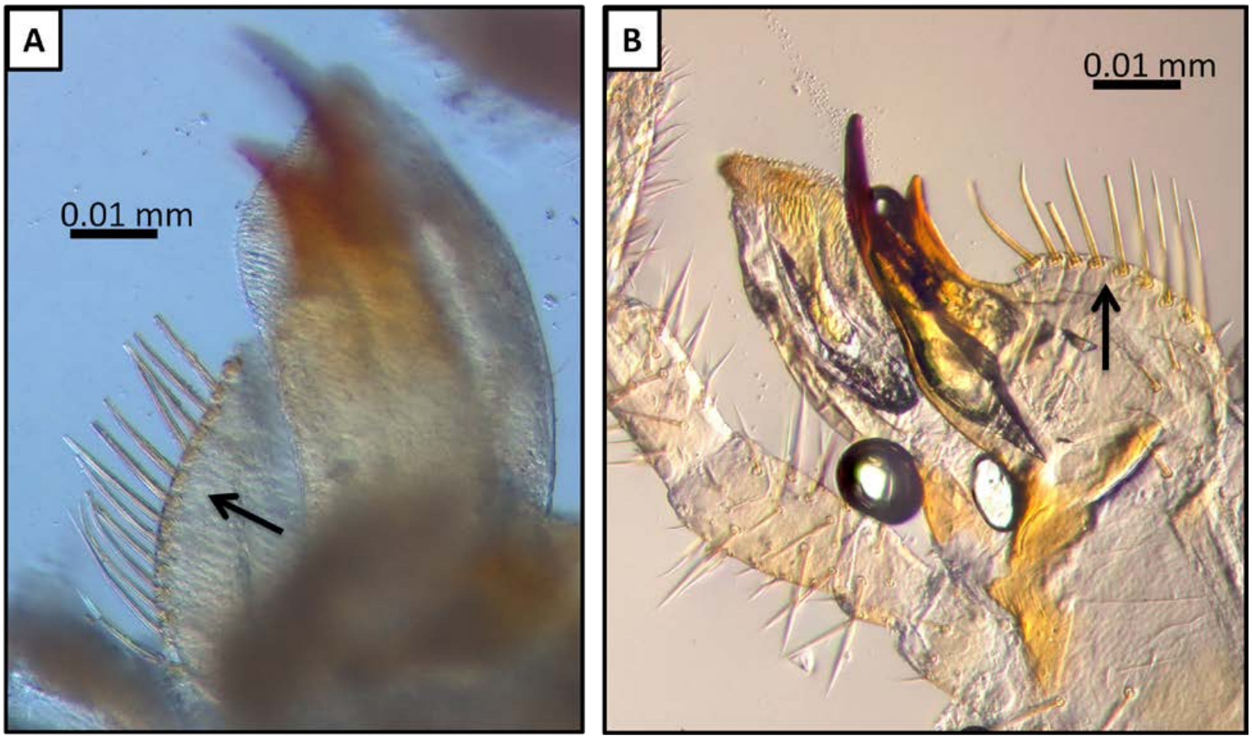

Figure 11. Worker, maxilla. **(A)** Maxilla ctenidium, ventral view (*C. formosanus*); **(B)** maxilla ctenidium, dorsal view (*R. flavipes*). Fig. 10A is a composite of 10 photographs of a specimen in EtOH at 100X, focally stacked with CombineZP; fig. 10B is a composite of 5 photographs of a specimen on a lactic acid slide-mount at 100X, focally stacked using ZereneStacker with the PMax algorithm. Resulting composite images were touched up for illustrative purposes with Photoshop.

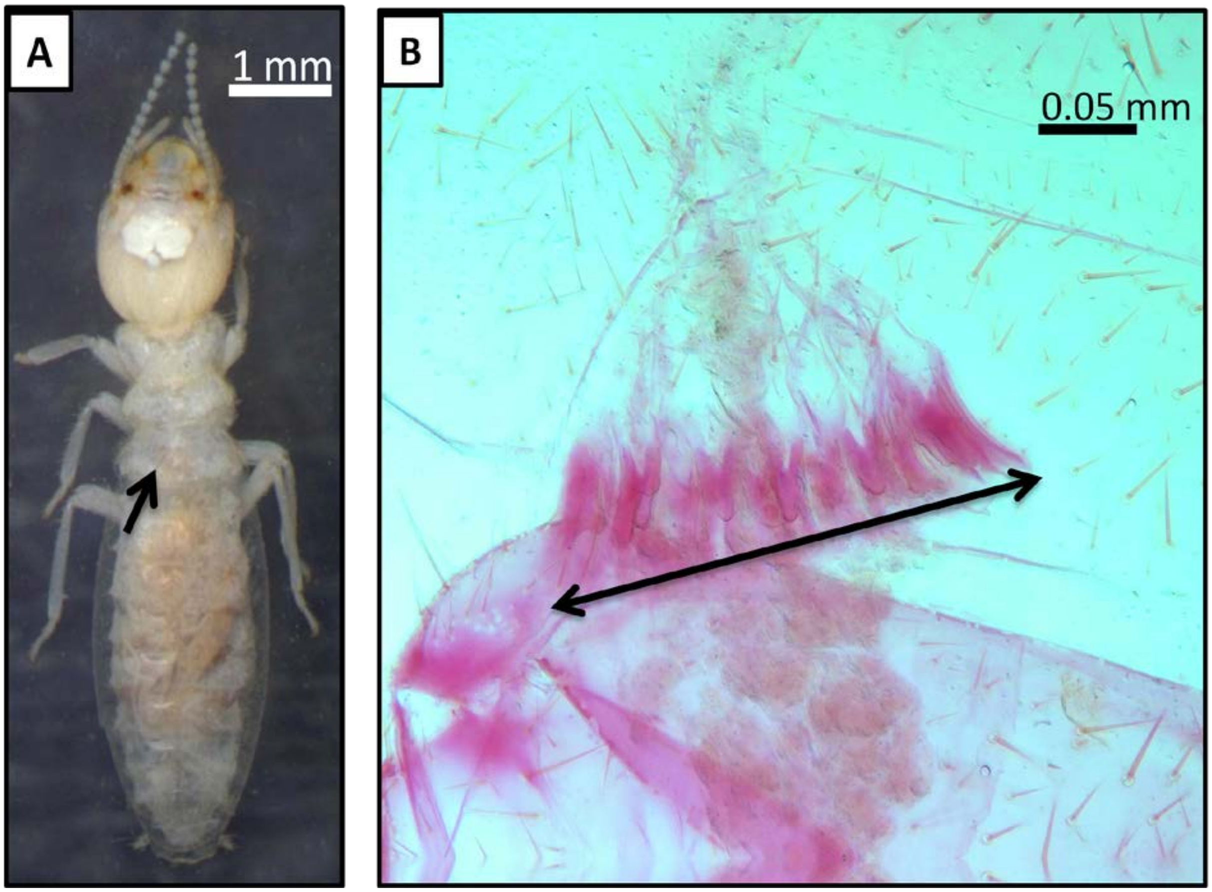

Figure 12. Worker, proventriculus. **(A)** Habitus, dorsal view, arrow pointing to approximate location of proventriculus (*R. flavipes*); **(B)** proventriculus viewed through cuticle, ventral view (*R. malletei*). Fig. 11A is a composite of 8 photographs of a specimen in EtOH at 200X, focally stacked using ZereneStacker with the PMax algorithm; Fig. 11B is a composite of 7 photographs of a specimen on a permanent slide that were focally stacked with CombineZP. Resulting composite images were touched up for illustrative purposes with Photoshop.

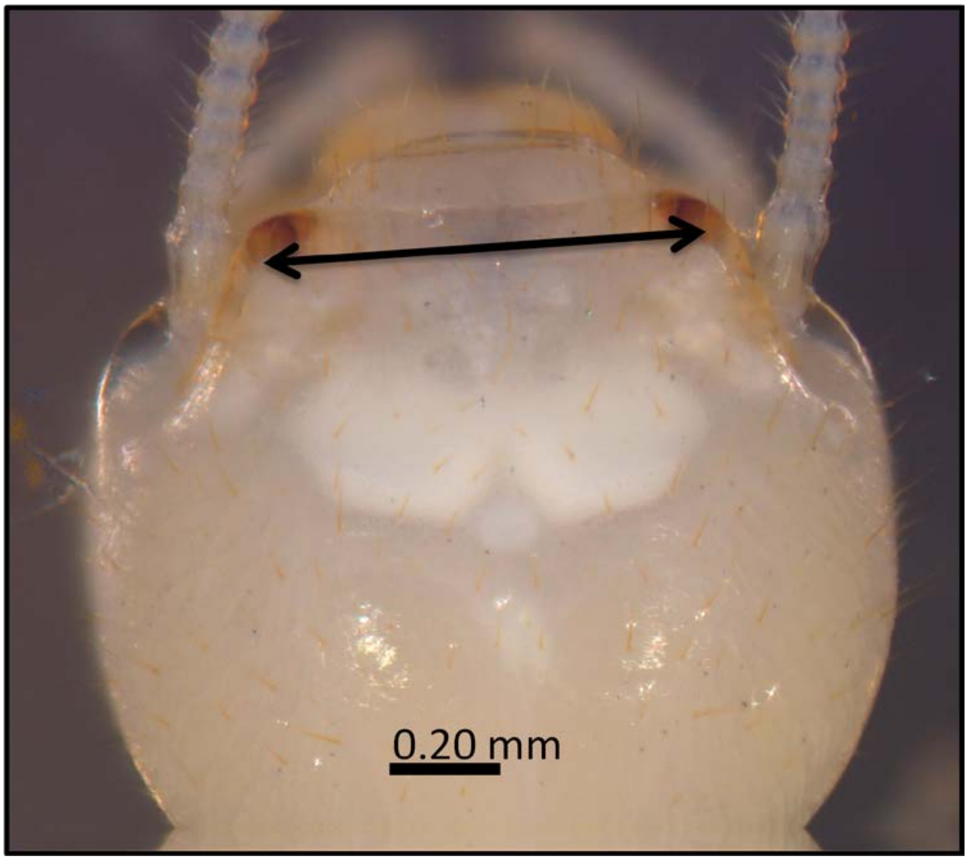

Figure 13. Worker, head. Arrows point to mesal condyles used in measuring head width (*R. flavipes*). This image is a composite of 6 photographs taken at 100X of a specimen in EtOH that have been focally stacked using CombineZP, that was then touched up for illustrative purposes with Photoshop.

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Chapter 4

An Ecoregion-based Survey of the Subterranean Termites (Isoptera: Rhinotermitidae) of Alabama, Using a Direct Sampling Technique

Abstract

A state-wide survey was conducted from Jul.-Oct., 2010 in 18 protected forests in Alabama. *Coptotermes formosanus* was not collected, suggesting that it has not established in the state outside of heavily anthropogenically disturbed areas. These data were combined with museum and extension specimens that were collected across Alabama from 1969-2012. The first state checklist is reported for Alabama Rhinotermitidae, along with the first county checklists and first distribution maps for the four *Reticulitermes* species occurring in the state.

Introduction

Termite species diversity decreases by orders of magnitude with increasing latitude: the entire Nearctic fauna contains approximately 40 species (Nutting 1990). Less than 20 species in two families are thought to be distributed in the southeastern Nearctic, with most concentrated in southern Florida (Eggleton 2000). The best sources of Nearctic termite diversity data are two major surveys (Weesner 1970, NOMTCB 2003-2006). Unfortunately these surveys suffer from several flaws that prevent inferring the distribution of Alabama's rhinotermitid fauna: near-exclusive sampling from anthropogenic habitat, lack of true replication, and the wide use of non-standardized techniques (Krebs 1999). Although posted online, the NOMTCB (2003-2006) data pertaining to Alabama have never been published in a peer-reviewed source. The website on which these data are available has not been updated in six years, and is largely defunct as of March, 2012. Compounding poor collection effort is a taxonomic impediment: *Reticulitermes*, in particular, is badly in need of a monographic revision (Austin et al. 2007). Existing collection data may have been misidentified: no Alabama rhinotermitid key has been published, and regional keys may be affected by clines that prevent accurate identification (Szalanski et al. 2003). Clines have been reported between populations of *R. virginicus* in Illinois and Florida (Banks 1946); similar clines are well-documented in other arthropods (McGhee 1977). Little effort has been made in Alabama to holistically survey subterranean termites. The distribution data of highly economically important species, in contrast, have been comparatively well-developed (Hu and Oi 2004).

Specific Alabama collection localities have been reported in peer-reviewed sources for three species: *Coptotermes formosanus* Shiraki, 1909 (Su and Scheffrahn 1986), *Reticulitermes flavipes* (Kollar, 1837) (Banks and Snyder 1920), and *R. virginicus* (Banks, 1907) (Banks and Snyder 1920). It has been suggested that *R. hageni* Banks, 1920 (Weesner 1970) and *R. malletei* Howard and Clément, 1985 (Clément et al. 1985) occur in the state, but to date no specific localities appear to have been published. Although only available online and not peer-reviewed, the NOMTCB (2003-2006) survey of pest control reports included specific location data for all of these species except *R. malletei*.

With respect to the damage they inflict upon structural wood, the most economically important of these five species are the invasive *C. formosanus* and the native *R. flavipes* (Su and Scheffrahn 1990). *R. virginicus* tends to be reported less often in structural wood, although this may be due to it being misidentified as *R. flavipes* (Su and Scheffrahn 1990). *R. hageni* is of little economic importance and has been rarely reported (Weesner 1970). The economic impact of *R. malletei* remains largely unknown. All of these species are believed to be sympatric across much of their ranges, and may be competing with one another (Nutting 1990). There is enormous potential for economic damage from *C. formosanus* invading woodlands (Woodson et al. 2001). If it establishes, *C. formosanus* may affect agricultural income from forestry (Delaplane and La Fage 1990), or these forests may act as a permanent source of future infestations of structures located near heavily treed areas. The ecological impact of *C. formosanus* remains largely unknown (Cornelius and Osbrink 2000). While no published reports have documented *C. formosanus* successfully invading Alabama’s woodlands, the species has been reported in forests of Mississippi and Louisiana (Brown et al. 2007, Sun et al. 2007, Lax and Wiltz 2010). Light traps of winged adults led some authors to suggest that *C. formosanus* is displacing native species in forests of southern Mississippi (Sun et al. 2007), although collection efforts made from directly sampling wood from forests recovered only *Reticulitermes* species and no *C. formosanus* (Wang et al. 2003). In Louisiana, Messenger et al. (2002) concluded that *C. formosanus* appeared to be localized around anthropogenic habitat, occurring only outside developed areas in parks when transported by infested wood such as rail ties brought from coastal areas. In contrast, Brown et al. (2007) collected *C. formosanus* in rural areas, and suggested the species was actively displacing native *Reticulitermes* species. Competitive exclusion occurs when one species completely displaces another through winning direct agonistic interactions or through being a better exploiter of shared resources (Gotelli 2008). Four competitive advantages possessed by *C. formosanus* that may allow them to outcompete native *Reticulitermes* in forests include: larger colony sizes (Bess 1970), superior wood consumption rate (Delaplane and La Fage 1990), tendency to win agonistic encounters in laboratory agonistic assays when forced to compete for resources (Cornelius and Osbrink 2000), and ethological ability to persist beyond their natural isothermic limits by avoiding sub-optimum or lethal cold temperatures (Hu and Song 2007).

No catalog for the state of Alabama appears to have been published in a peer-reviewed source. Similarly, it appears that neither checklists for the state nor detailed records for Alabama's 67 counties have been published. The distribution of the five species putatively occurring in the state must be inferred from several sources. *Coptotermes formosanus* has been tracked by several authors (Su and Scheffrahn 1986, Sponsler et al. 1988, Hu and Oi 2004), but no comparative efforts have been made with the four native *Reticulitermes* species. No attempts to compile these disparate data appear to have been published. What distribution data are available for Alabama are biased from near-total collection from highly anthropogenically disturbed or influenced habitat, such as the interior and immediate exterior of buildings in developed regions.

The two objectives of the present study are to: (1) assess the species diversity and distribution of Rhinotermitidae in Alabama through conducting a state-wide survey; and

(2) assess whether *C. formosanus* is establishing outside of highly anthropogenically disturbed habitat. To avoid anthropogenic bias, we will combine methods from field sampling using snapshot and continuous sampling techniques, museum holdings, and data from specimens submitted by the public to the Alabama Cooperative Extension Service (ACES).

Materials and Methods

Alabama has tremendous diversity in its soil provinces and forests. Six Level III ecoregions have been designated: Southern Coastal Plain, Southeastern Plains, Piedmont, Ridge and Valley, Southwestern Appalachians, and Interior Plateau (Griffith et al. 2001) (Fig. 14). Ecoregions may be broadly defined as geographic regions containing unique assemblages of soil types, topography, forest cover, moisture regimes, flora, and fauna that grade into adjacent unique assemblages (Loveland and Merchant 2004). Level III Ecoregions were used as a guide to effectively sample the beta diversity of subterranean termites (Rhinotermitidae) in Alabama. All sampled forests were protected natural areas, predominantly on lands managed by the Alabama Department of Conservation and Natural Resources. Permission was obtained from the State Chief Forester to access sites and sample termites from deadwood. A snapshot field sampling was used in three forests located in each of the six ecoregions, for a total of 18 sampling events. Continuous field sampling was done using sticky traps placed in rural and suburban areas in 26 sites distributed from the coast to the northern border with Tennessee. Museum and ACES specimens came from throughout the state.

The snapshot field sampling method (Fig. 15) used a modification of a generalized protocol suggested by Krebs (1999) (Fig. 16). In each of the six ecoregions, three replicate sites were selected. Each 100m X 100 m (1 km^2) site was chosen to maximize sampling appropriate to the dominant flora and fauna of that ecoregion. Forest types (as determined by dominant tree species and understory vegetation), altitude, and the visual layout were recorded. Sites with large streams running through them or located on steep cliffs were avoided. Altitude and position were determined using a Garmin® GPS device. At each site, the four corners were flagged, and three 20 m X 20 m (400 m^2) transects were randomly selected. Randomization was obtained by using a stopwatch to choose X and Y coordinates within the site on the basis of thousandths of a second. New sets of X and Y coordinates were randomly generated whenever duplicate coordinates occurred. Adjacent transects were sampled. Transects with >67% of their area covered by water were not sampled. New transects were randomly selected in the event of an unsuitable transect. At the center of each transect the following variables were recorded: time, date, latitude and longitude, elevation, canopy cover (total, partial, or no cover), and dominant forest stand type (deciduous, coniferous, or mixedwood). Three 2 m X 2 m (4 m^2) quadrats were then randomly selected. Quadrats were selected by standing at northwestern corner of the transect being sampled, randomly generating X and Y coordinates using the same method for transects, and proceeding that many meters into the transect. Duplicate quadrats and adjacent quadrats were not sampled in the same transect to avoid pseudoreplication. Quadrats were deemed unsuitable for sampling if >25% of their area was covered with water, if they did not contain coarse woody debris (CWD), or if they were on a grade of >45%. Steep grades were avoided to prevent losing data from CWD and stones rolling off the quadrat during sampling. In the event of a duplicate, adjacent, or unsuitable quadrat, a new quadrat was randomly selected until a suitable quadrat was randomly selected. In each quadrat sampled, three haphazardly selected soil temperature points, forest floor relative humidity, and forest floor air temperature were recorded. Subterranean termites were then sampled destructively. All stones were overturned and removed from the quadrat, all CWD on the quadrat was opened with a hatchet and thoroughly searched, and all forest floor litter was overturned. This continued until 30 min of sampling effort passed or at least 5 soldiers and 10 workers were collected. All specimens were placed immediately into vials of 80% ethyl alcohol (EtOH). Other arthropods found in close association with foraging parties or colonies of rhinotermitids were also collected.

Continuous field sampling came from a two-year phenology monitoring program. This program used sticky traps that were placed at 26 locations distributed from the southern to northern parts of Alabama. The protocol for this collection method is covered in Chapter 5.

Museum specimens came from the Auburn University Entomology Museum and Mississippi Entomology Museum. Museum and ACES specimens comprised collections made 1967-2012 in locations distributed throughout the state.

Species were identified using the keys developed in Chapter 2. Voucher specimens have been deposited in the Auburn University Entomology Museum.

Results

From our collection efforts and from specific citations in peer-reviewed literature, five species of Rhinotermitidae are distributed in Alabama: *C. formosanus*, *R. flavipes*, *R. hageni*, *R. malletei*, and *R. virginicus*.

Coptotermes formosanus was found only in highly urbanized areas, and was generally distributed across the state's developed regions (Fig. 17). No collections from the snapshot sampling technique in protected forests yielded *C. formosanus*. Four new county records were recorded. Six counties with previous records of *C. formosanus* were confirmed, and six counties with previous distribution records were not confirmed by our sampling efforts (Su and Scheffrahn 1986, Sponsler et al. 1988, Hu and Oi 2004). *Coptotermes formosanus* occurs in 16 Alabama counties: Baldwin, Calhoun, Chilton*, Coffee*, Covington*, Cullman, Jefferson, Lee, and Mobile. Asterisks after a county name indicate a new record from this study.

Reticulitermes flavipes was collected from both developed and undeveloped areas (Fig. 18), including 26 counties from which it had not been reported in peer-reviewed literature (Banks and Snyder 1920). This species was found in forests of all six ecoregions (Table 2). It was the only species collected during sampling of Southwestern Appalachian and Interior Plateau forests that occurred during rainstorms. *Reticulitermes. flavipes* occurs in 33 Alabama counties: Autauga*[1], Baldwin*[1], Blount*, Calhoun*, Chambers*, Cleburne*, Colbert[1], Covington*, Cullman*, Dallas*[1], Elmore*[1], Etowah[1], Geneva*, Hale*, Houston*, Jefferson, Lee, Limestone[1], Macon*, Madison[1], Marengo*, Marion*[1], Marshall*, Mobile*[1], Monroe*, Montgomery, Morgan*, Pike*, Randolph*, Shelby*, Tallapoosa*, Tuscaloosa*, and Washington*. Asterisks after a county name indicate a new record from this study. Counties with the superscript "1" were reported by NOMTCB (2003-2006) but not found in peer-reviewed literature. Our collection efforts were unable to confirm the occurrence of *R. flavipes* in Colbert, Etowah Limestone, or Madison counties, as reported by NOMTCB (2003-2006) alone.

Reticulitermes hageni was collected from both developed and undeveloped areas (Fig. 19). Seven new county records were recorded. Although suggested to occur in the state (Nutting 1990), no specific locality information was found in peer-reviewed literature for this species. All county records we report comprise new records for the state. This species was collected from forests of all six ecoregions sampled (Table 2). *Reticulitermes hageni* occurs in 16 Alabama counties: Autauga[1], Baldwin, Blount, Clarke, Conecuh, DeKalb, Escambia, Etowah, Lee, Limestone, Macon, Marengo, Shelby, Talladega, Tallapoosa, and Tuscaloosa. Autauga, marked with the superscript "1", was reported by NOMTCB (2003-2006), and confirmed by our collection efforts.

Reticulitermes malletei was collected from both developed and undeveloped areas (Fig. 20). Although suggested to occur in the state (Clément et al. 1985, Clément et al. 1986), no specific locality information was found in the literature for this species. All county records we report comprise new records. This species occurred in forests of all ecoregions except the Interior Plateau (Table 2). *Reticulitermes malletei* occurs in 8

Alabama counties: Baldwin, Cleburne, Lee, Macon, Marengo, Marshall, Tallapoosa, and Tuscaloosa. The distribution of this species was not reported by NOMTCB (2003-2006).

Reticulitermes virginicus was collected from both developed and undeveloped areas (Fig. 21). Twenty-four new county records were recorded; the two county records in the literature were confirmed by our sampling efforts (Weesner 1970). This species was collected from forests in four of the six ecoregions, and was absent from the highest elevation forests with transects on the steepest grades with the least relative humidity (Table 2). *R. virginicus* occurs in 26 Alabama counties: Autauga*, Baldwin*, Butler*, Calhoun*, Choctaw*, Clarke*, Cleburne*, Conecuh*, Coosa*, Dale*, Dallas^1, Elmore*, Houston*, Jefferson*, Lauderdale^1, Lee*, Limestone*, Macon*, Marshall*, Mobile*^{1}, Monroe*, Montgomery*, Perry*, Randolph*, Shelby*, St. Clair*, Tallapoosa*, and Walker*^{1}. Counties marked with an asterisk indicate new records generated from this study. Counties with the superscript “1” were reported by NOMTCB (2003-2006), but not found in the scientific literature. Of the three counties reported by NOMTCB (2003-2006), our collection efforts were unable to confirm the occurrence of *R. virginicus* in Dallas and Lauderdale counties.

Discussion

That the combined collection methods were able to generate 48 new county records is indicative of the poor sampling effort of the past in surveying subterranean termites outside of pest control situations. The widespread distribution, ease with which they may be collected, and importance as decomposers in woodlands make rhinotermitids ideal candidates for ecological studies. It is hoped that the baseline data we provide may be used for future ecological work in Alabama's forests.

Landscape ecology appears to have little effect on the beta diversity of the four native *Reticulitermes* species. All species, including the poorly studied *R. malletei* and *R. hageni*, were sympatric through most of their range. Except for the absence of *R. virginicus* from forests with steep grades and lower relative humidity, no biogeographical pattern was apparent from the snapshot collection method in protected forests.

Coptotermes formosanus was not found in any of the 18 forests sampled across the state. This includes three forests in coastal forests of Mobile and Baldwin counties. Based on our collection data, *C. formosanus* appears to be neither invading natural protected forests nor presenting a credible threat to native rhinotermitid fauna. It is possible that we sampled before the effects of *C. formosanus* have begun to affect native termites. Should *C. formosanus* affect native rhinotermitid distributions in the future, the data generated from the snapshot collection method will provide a baseline against which future incursions and displacements may be compared.

Table 2. Species occurrence and recorded environmental variables at sites sampled. Temperatures and relative humidity (RH) measurements are means of 9 readings taken in each quadrat; latitudes, longitudes, and elevations are means of 3 measurements taken in the center of each transect. *C. f.*, *C. formosanus*; *R. f.*, *R. flavipes*; *R. h.*, *R. hageni*; *R. m.*, *R. malletei*; *R. v.*, *R. virginicus*.

Ecoregion	County	Site	Sampling Date	Lat. (°N)	Long. (°W)	Elev. (m)	Soil Temp. (° C)	Floor Temp. (° C)	RH (%)	*C. f.*	*R. f.*	*R. h.*	*R. m.*	*R. v.*
IP	Limestone	Elk River Lodge St. Pk. (ELKR)	8/28/2010	34.81	87.23	192	24.5	31.2	54	0	0	1	0	1
IP	Lauderdale	Joe Wheeler St. Pk. (JOEW)	8/29/2010	34.81	87.34	223	24.1	23.9	94	0	0	0	0	0
IP	Morgan	Wheeler Nat. Wildlife Ref., S. section (WHEE)	9/11/2010	34.51	86.96	193	24.3	24.5	100	0	1	0	0	0
SA	DeKalb	De Soto St. Pk., S. section (DESO)	7/26/2010	34.50	85.54	512	25	32.3	52	0	0	1	0	0
SA	Marshall	La. Guntersville St. Pk. (GUNT)	8/13/2010	34.37	86.21	246	26.1	28.8	83	0	0	0	1	0
SA	Blount	Rickwood Caverns St. Pk. (RICK)	9/4/2010	33.87	86.86	224	22.7	27.3	35	0	0	1	1	0
RV	Jefferson	Mtn. Longleaf Nat. Wildlife Ref. (LONG)	8/12/2010	33.71	-86.86	552	26.1	32.4	49	0	0	0	0	0
P	Cleburne	Talladega Nat. For., E. section (TALL)	7/28/2010	33.71	85.58	392	25.9	29.8	67	0	1	0	1	1
RV	Jefferson	Oak Mtn. St. Pk. (OAKM)	7/16/2010	33.35	87.08	196	22.4	24.0	56	0	0	1	0	0
RV	Tuscaloosa	Tannehill Hist. St. Pk. (TANN)	9/5/2010	33.24	87.08	146	21.9	nr	44	0	1	1	1	0
P	Tallapoosa	Horseshoe Bend Nat. Mil. Pk. (HORS)	8/17/2010	32.98	85.74	173	26.1	30.0	72	0	1	1	1	1
P	Tallapoosa	Wind Creek St. Pk. (WIND)	7/29/2010	32.87	85.92	209	26.4	32.0	64	0	1	0	1	1
SP	Macon	Tuskegee Nat. For. (TUSK)	10/6/2010	32.49	85.58	126	21	25.7	43	0	0	1	1	0
SP	Marengo	Roland Cooper St. Pk. (ROLA)	10/27/2010	32.05	87.83	30	nr	31.9	51	0	1	1	1	0
SP	Houston	Chatahoochee St. Pk. (CHAT)	8/10/2010	31.00	85.03	42	28.5	34.6	48	0	1	0	0	1
SCP	Baldwin	Blakeley Hist. St. Pk. Ala. Forever Wild (BLAK)	8/15/2010	30.73	87.92	35	27.2	32.2	76	0	1	1	0	1
SCP	Baldwin	Weeks Ba. Nat. Estuarine Research Res. (WEEK)	7/10/2010	30.42	87.83	13	26.2	29.0	nr	0	1	1	0	1
SCP	Baldwin	Bon Secour Nat. Wildlife Ref. (BONS)	9/18/2010	30.25	87.83	9	27.5	33.2	nr	0	1	1	1	1

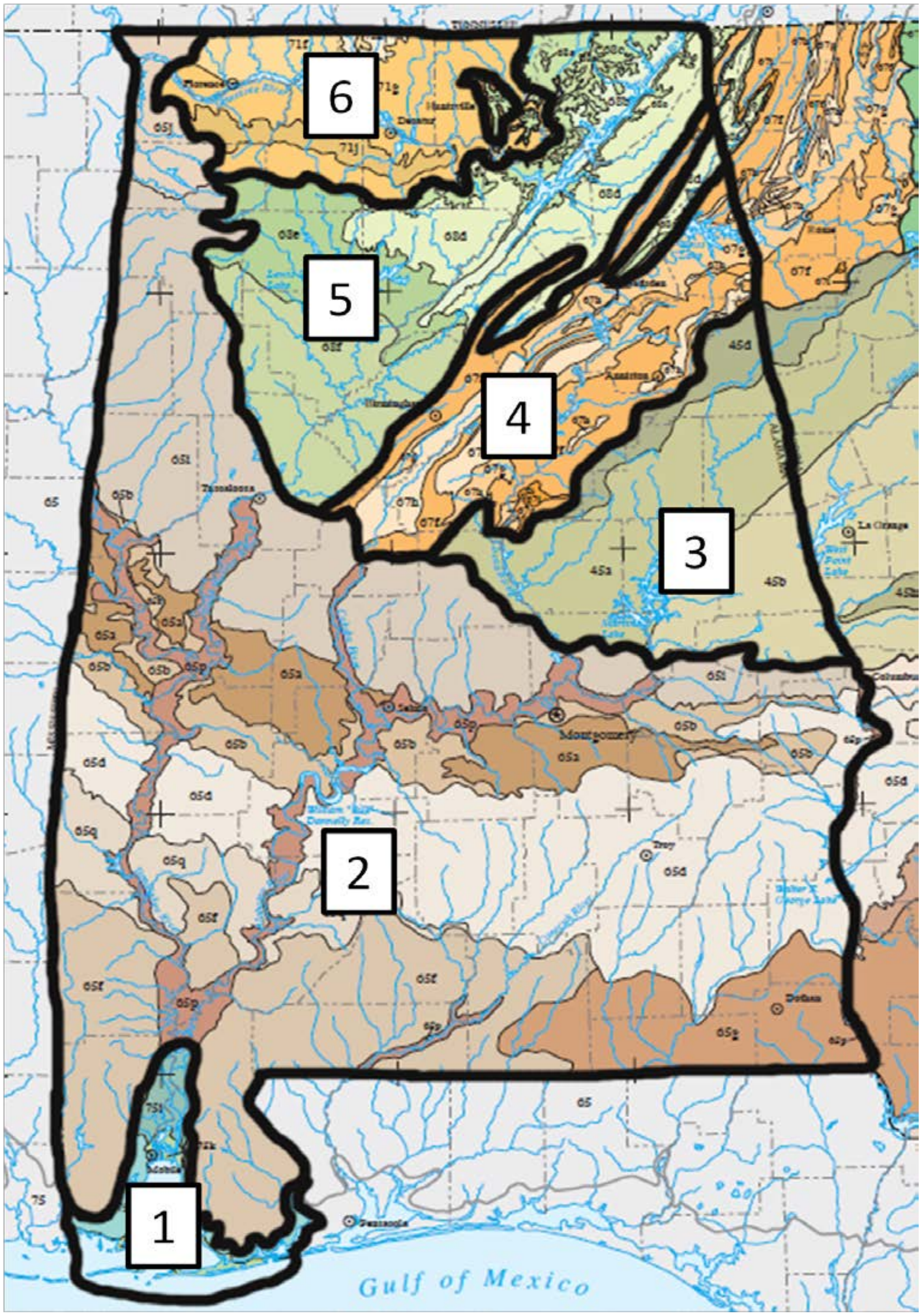

Figure 14. Level III ecoregions of Alabama. Adapted from Griffith et al. (2001). 1, Southern Coastal Plain; 2, Southeastern Plains; 3, Piedmont; 4, Ridge and Valley; 5, Southwestern Appalachians; 6, Interior Plateau.

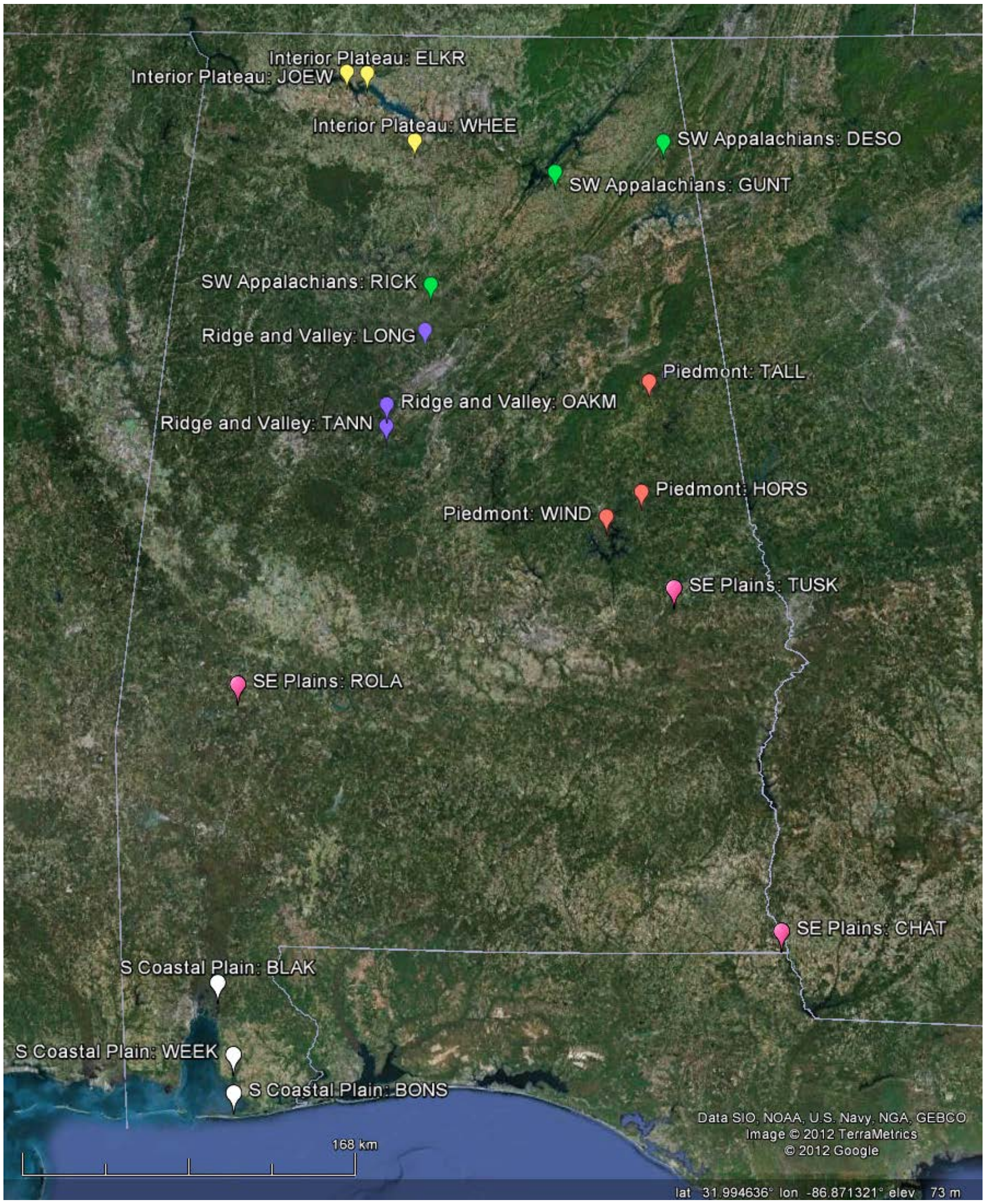

Figure 15. Map of field sampling sites. Locations in blue were sampled with the sticky traps as part of a two-year monitoring program. Locations in other colors were sampled directly from forests of the 6 ecoregions: Southern Coastal Plain (white), Southeastern Plains (pink), Piedmont (red), Ridge and Valley (purple), Southwest Appalachians (green), and Interior Plateau (yellow).

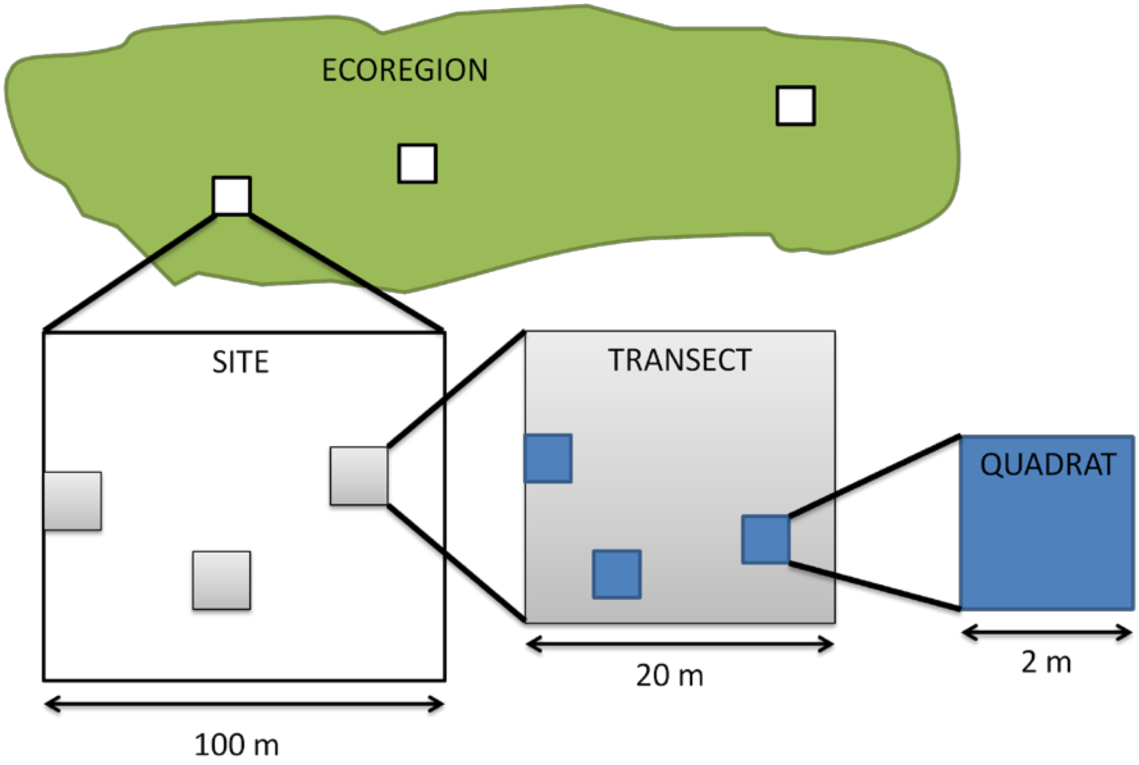

Figure 16. Schematic of selection process for direct field sampling protocol. Sites (white) were selected from ecoregions (green) on basis of suitability; transects (grey) and quadrats (blue) were randomly selected.

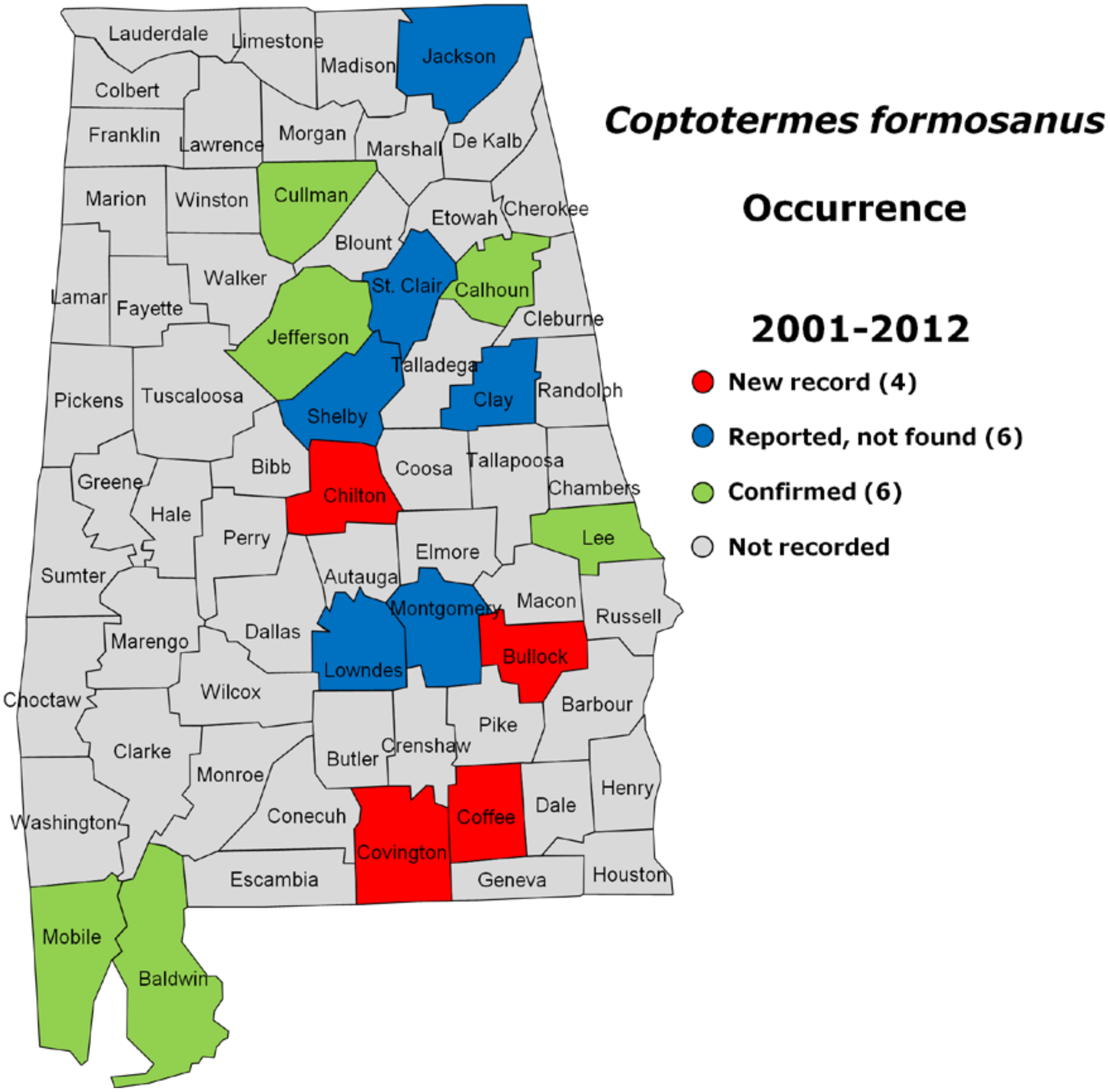

Figure 17. Distribution map of *C. formosanus*.

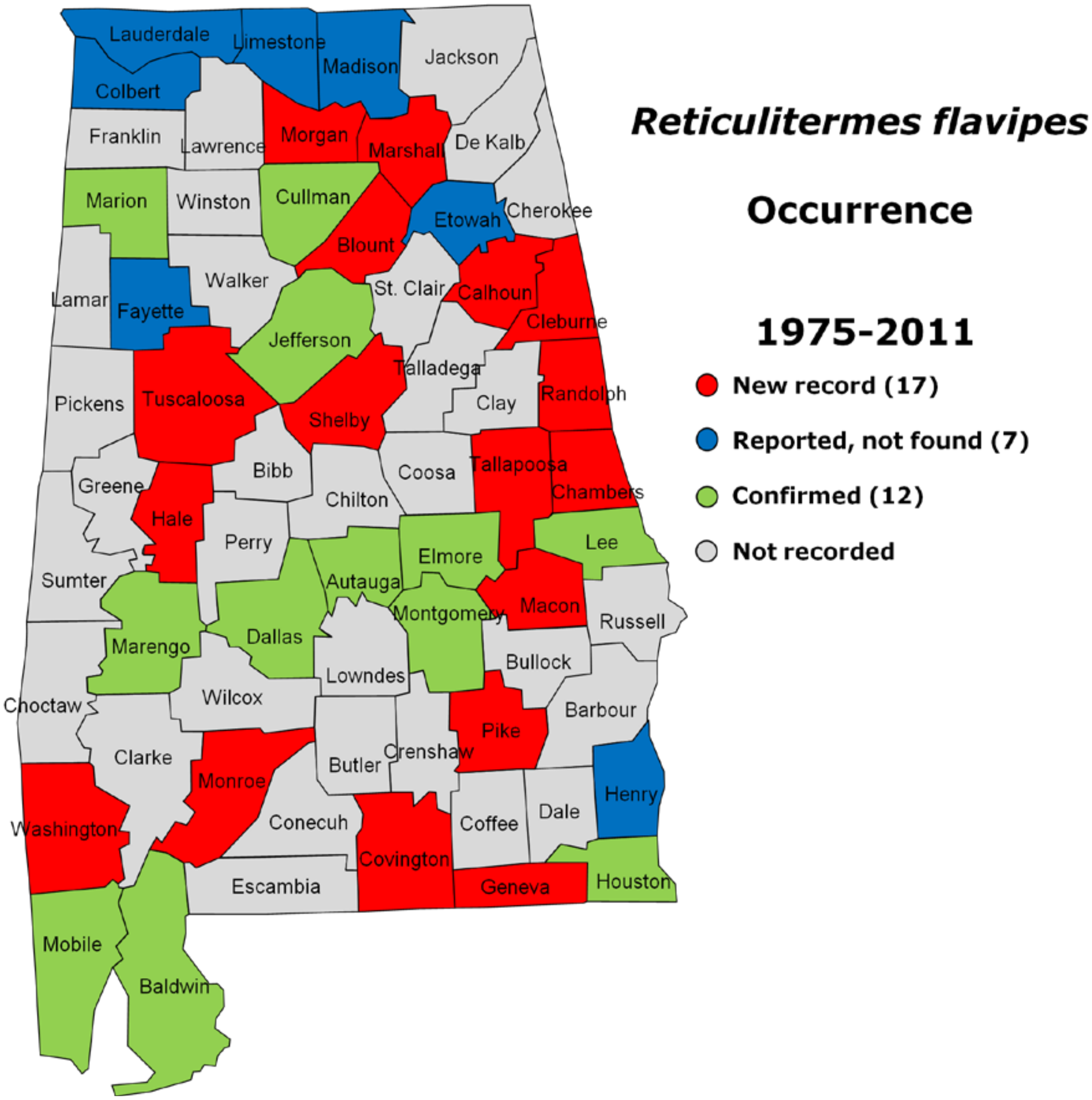

Figure 18. Distribution map of *R. flavipes*.

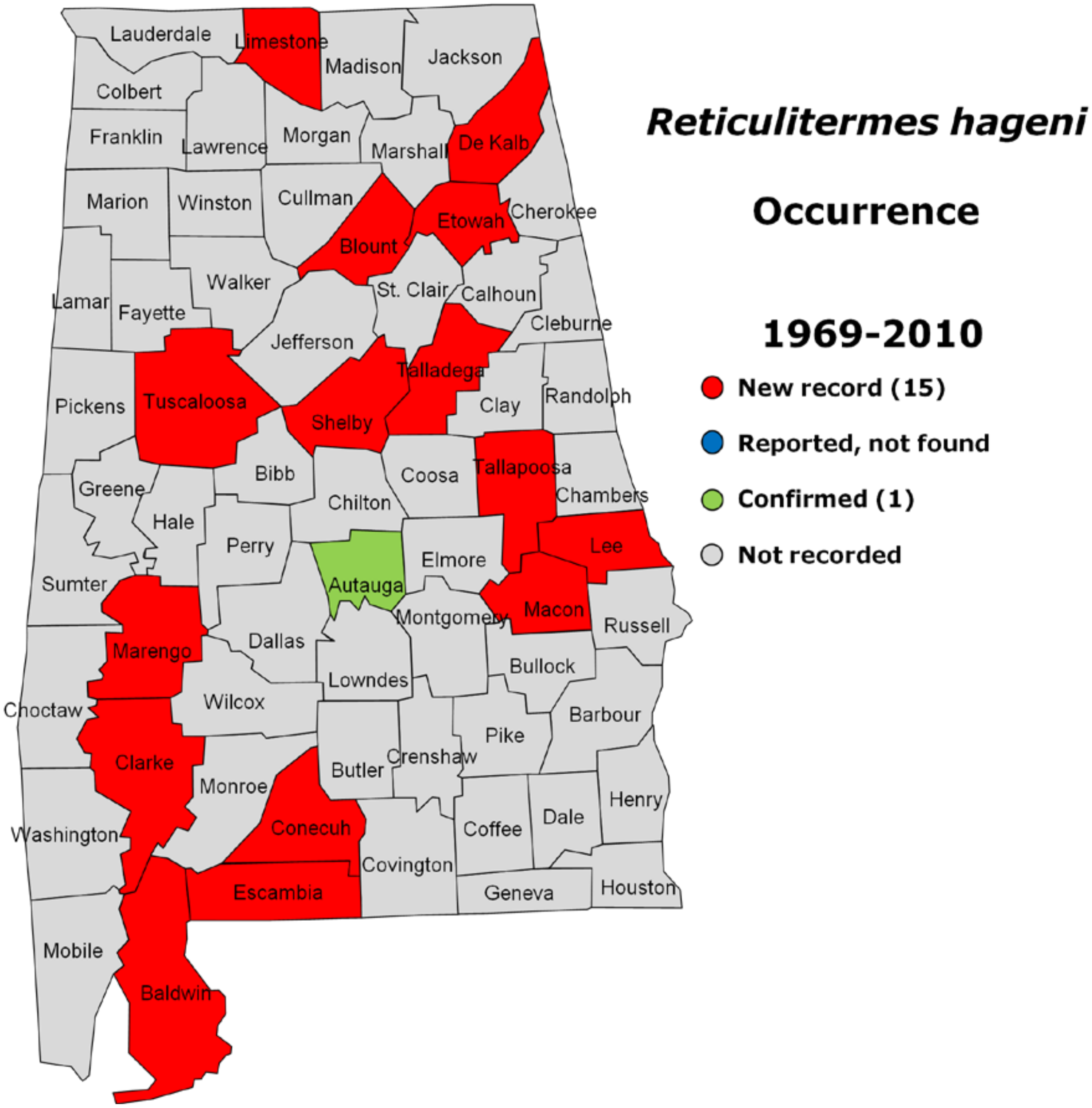

Figure 19. Distribution map of *R. hageni*.

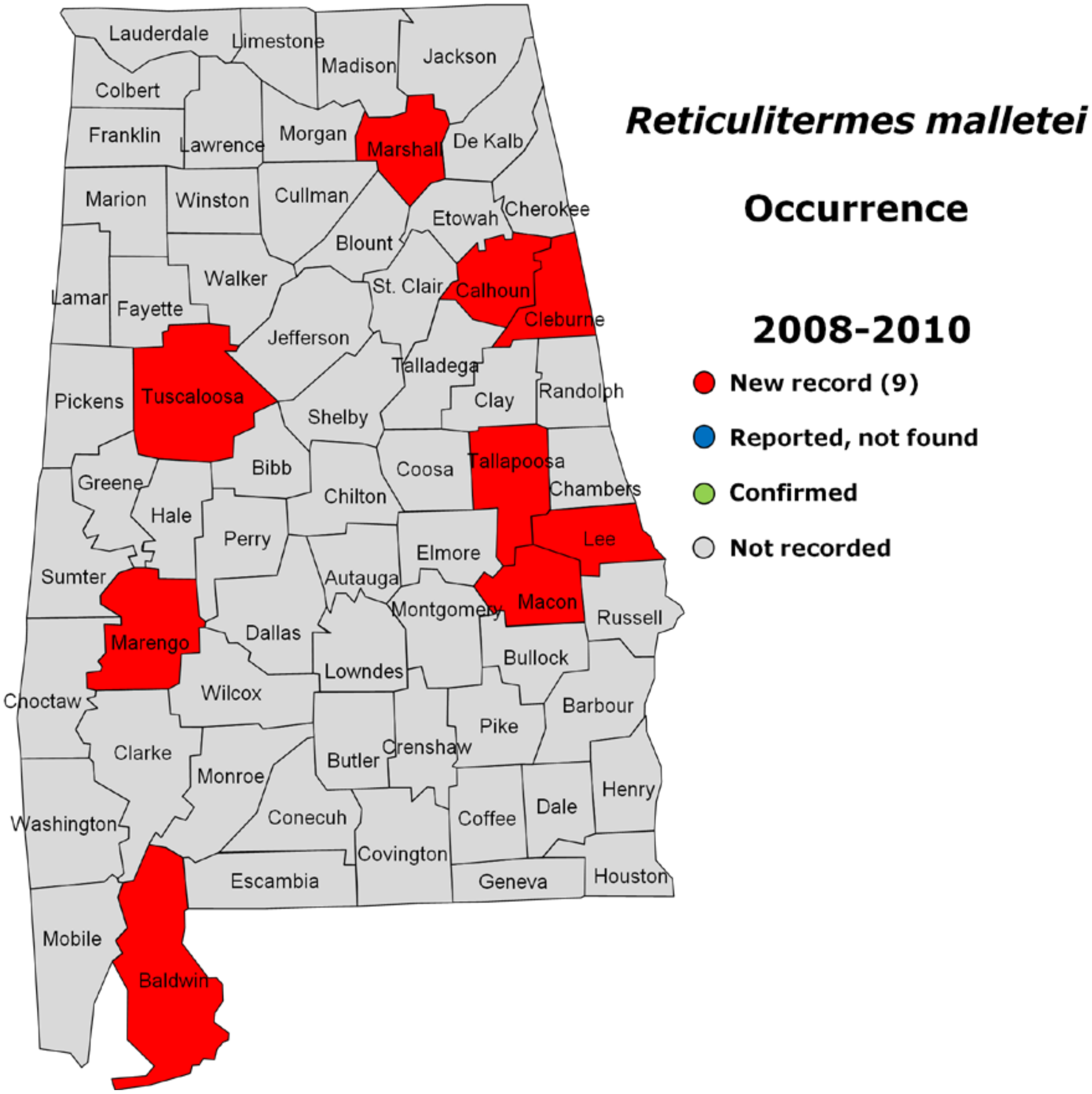

Figure 20. Distribution map of *R. malletei*.

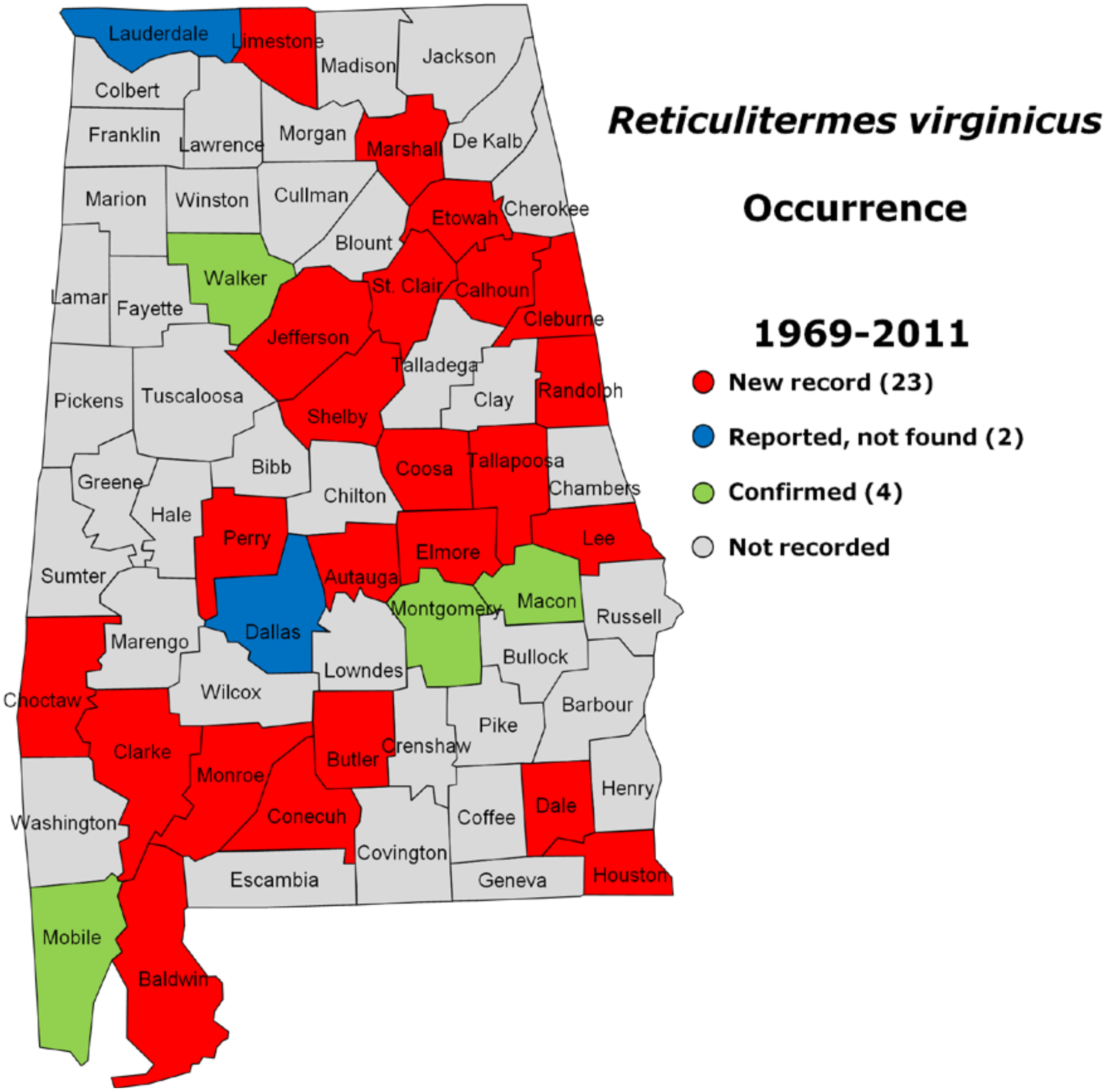

Figure 21. Distribution map of *R. virginicus*.

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Chapter 5

Phenology of Alabama Rhinotermitidae Swarming Flights

C. D. R. Stephen, C. H. Ray, and X. P. Hu

Abstract

A state-wide monitoring program using sticky traps was conducted at 35 locations in Alabama continuously from Mar. 2010 – May 2011 to record the swarming flights of Rhinotermitidae imagos. These data were combined with museum and extension specimens that were collected across Alabama from 1969-2011. Monthly occurrences of detected swarming flights are reported for *Coptotermes formosanus*, *Reticulitermes flavipes*, *R. hageni*, *R. malletei*, and *R. virginicus*. *Coptotermes formosanus* flights were detected two coastal and one central county, in or near developed areas, with peaks in May and June. Swarming flights not reported previously in Alabama and nearby states include *C. formosanus* flights in February and *R. virginicus* flights in October and November.

Introduction

Subterranean termite colonies are essentially sessile superorganisms that release their gametes in the form of reproductive individuals to be dispersed by the wind (Wilson 1971). The sexually reproductive individuals of colonies comprise the winged imago caste, which is only present in colonies in large numbers immediately prior to swarming periods, and then released en masse (Harris 1961). Factors thought to be related to swarming include the first rains of spring

(Snyder 1954) and the blooming phenology of the dogwood, *Cornus florida* (Banks and Snyder 1920).

Five species of Rhinotermitidae are known in Alabama: *Coptotermes formosanus* Shiraki, 1909, *Reticulitermes flavipes* (Kollar, 1837), *R. hageni* Banks, 1920, *R. malletei* Howard and Clément, 1985, and *R. virginicus* (Banks, 1907). *Coptotermes formosanus* swarms have been reported in June from across Alabama (Hu and Oi 2004), and from May, June, and October from light traps in South Carolina (Hathorne et al. 2000). Outdoor reports for Alabama swarms of *R. flavipes* appear to be unpublished in peer-reviewed literature; however, in northeastern Georgia, flights have been reported for this species in late April (Clément et al. 1986). In central Alabama, flights of *R. hageni* were reported in late June from a structural building setting (Weesner 1970), and in late May from light traps in South Carolina (Hathorne et al. 2000). Sympatric flights of *R. malletei* and *R. virginicus* in June have been observed in northeastern Georgia (Clément et al. 1986), while allopatric flights of *R. virginicus* in April and May have been observed in central Alabama (Weesner 1970). Essentially all of these disparate reports have come from pest control situations, either inside or immediately adjacent to buildings. Buildings are typically climate controlled, which will affect the temperature and humidity of their structural walls and surrounding soils. Irrigating soils surrounding buildings to maintain aesthetically placed plants will also contribute to elevated soil humidity. Subterranean termites are infamous for their utilization of habitat humans create for them; control efforts in the United States alone represent billions of dollars of annual expenditure (Su 2002). Colonies of rhinotermitids in anthropogenically altered climatic situations are able to persist far outside their expected natural isothermic ranges (Grace et al. 1989). Synthetic alteration of the temperature and humidity of the physical environment may artificially induce swarming at sub-optimal or lethal times, exposing swarming individuals to environmental regimes outside of their thermal tolerance limits (Hu and Appel 2004). Natural selection may be causing rhinotermitids to increase the production of secondary reproductives, as seen in highly pestiferous species such as *R. flavipes*, or conversely, species already possessing these strategies may be pre-adapted to invade anthropogenic habitat (Dronnet et al. 2005).

There is a great need to establish the basic biology of swarming phenology of these economically important insects (Houseman et al. 2001). No standardized, continuous collection effort has been made from habitat with minimal anthropic influence in Alabama. The objective of the present study is to create baseline data of the swarming flight phenology of the five species of Rhinotermitidae occurring in Alabama.

Materials and Methods

A network of 26 volunteers was established in 14 counties, in localities representing the breadth of Alabama's Level III ecoregions (Griffith et al. 2001), latitude, elevation, and range of urban development. This was supplemented by six sites in two counties maintained by C.D.R.S. and three sites in two counties maintained by C.H.R., for a total of 35 sites in 18 counties (Fig. 22).

Volunteers were mailed sticky traps (Scentry Multigard® Green Sticky Trap) with instructions. Sticky traps were placed by volunteers at 26 sites from April 2010 through October

2010 and seven sites from March 2011 through June 2011; C.D.R.S. and C.H.R. sampled nine sites from April 2010 through June 2011 (Fig. 22). Each site had three replicate locations where sticky traps were placed for the entire sampling period at that site. Allowed locations included vertical wooden surfaces, such as trees, fences, poles, and walls of unheated structures (Fig. 23). Traps were placed 1-2 m above the ground. This height was chosen for the convenience of the volunteers and to maximize the probability of traps being placed correctly and to reduce the number of volunteers dropping out of the program due from the effort involved in maintaining their trap locations. Volunteers were reminded biweekly to change traps and place used traps in a cool, shaded, preferably refrigerated location until a batch of traps was mailed back to Auburn University (AU). Once traps arrived at AU they were immediately placed in a walk-in refrigerator to keep them at 10-15°C until processed. The purpose of keeping the traps cool and out of direct sunlight was to reduce the amount the traps were compressed, heated, and specimens damaged prior to traps being examined. Using this protocol, a total of 1448 sticky traps were used.

Sticky traps were visually scanned for termites, using a dissecting microscope when necessary. The cardboard backing surrounding specimens were cut out from the traps, and the specimen with its cardboard backing was placed in a citrus oil solvent (Histo-Clear2®, National Diagnostics, Atlanta, GA) for 3-15 d to remove the glue on the sticky traps from the specimens (Miller et al. 1993). Citrus oil solvents possess the advantage of being minimally toxic and potentially not degrading DNA (Marshall et al. 2010). The range of days varied according to the amount of glue that had immersed the specimen. Specimens included whole termites and detached wings. After the glue was removed, all specimens were transferred to a labeled vial filled with 70% ethyl alcohol (EtOH).

Samples from field monitoring were combined with winged imago specimens in the holdings of the AU Entomology Museum and received by ACES from the public. This added 127 samples of to the sticky trap data, increased the period of sampling to 1969-2011, and added 26 counties from which sticky trap was not collected (Fig. 22).

Morphological identifications were made using the key for Alabama imagos developed in Chapter 2. Species identifications that could not be made unequivocally were placed into species-groups.

Results

The majority of detected flights across all species occurred Mar. through Aug. (Fig. 24). There was no apparent effect of latitude on the months of flights across in the five species detected (Figs. 25-28). Flights of *C. formosanus* occurred primarily in May and Jun. (Fig. 24); a singular flight was detected in Jul. from Baldwin County (Fig. 25). Flights of the species-group *R. flavipes*/*malletei* were detected in Feb. through Jun. and in Oct., with peak flight times occurring Mar. through May (Fig. 24). Early spring flights occurred in coastal and northern montane regions, while late autumn flights occurred only in central regions (Fig. 26). Flights of *R.hageni* were detected in Mar. through Aug., with peak flight times occurring Jun. through Aug., and less flights detected in Apr. and May, when peaks of other species occurred (Fig. 24).

Spring flights of *R. hageni* occurred in central regions, while summer flights occurred throughout the state (Fig. 27). Flights of *R.virginicus* were detected Feb. through Jul. and in Oct. through Nov., with the majority occurring in May (Fig. 24). The early spring flights occurred only in central and northeastern montane regions (Fig. 28).

Across all counties, sympatric peak flights occurred in May for *C. formosanus, R. flavipes/malletei*, and *R. virginicus*. The only months with allopatric flights were Feb. and and Oct., in which *R. flavipes/malletei* was detected (Fig. 24). All other species flew sympatrically with at least one other species in most counties sampled (Figs. 25-28).

Discussion

Our data show a peak for *C. formosanus* in May and June, with few detected flights in February and July, and no data coming from other months. This differs slightly from reported *C. formosanus* swarms in June coming from structures in locations across Alabama (Hu and Oi 2004), and from light traps in South Carolina detecting flights in October (Hathorne et al. 2000). The monitoring program only detected *C. formosanus* flights in Baldwin, Mobile, and Lee counties; records from other counties came primarily from pest control companies and homeowners that submitted samples to ACES (see Chapter 4). All detected swarms in Lee County were on a roadside bordering a forested area on AU campus. This corresponds closely with the first Alabama locality in which *C. formosanus* was detected away from coastal areas (Sponsler et al. 1988). Additional trap locations in Lee County and adjacent Macon County were at least 100 m within a completely forested area and did not detect *C. formosanus*. Two explanations for these findings include that this introduced species is uncommon in situations where colonies do not have access to climate-controlled structures and that it had not yet established in the forests sampled during the period in which they were sampled. Although detected flights for this species were sympatric with *Reticulitermes* species in most locality samples, the only truly sympatric flights of *C. formosanus* occur with *R. hageni*. While *C. formosanus* flies exclusively at night, *R. hageni* is reported as flying both during the day and at night (Weesner 1970, Austin et al. 2007, Lim and Forschler 2012).

We report peak swarms for *R. virginicus* in May, with minor flights in October (Fig. 25). To our knowledge *R. virginicus* is not known to swarm naturally at this latitude so late in the year. It was not always possible to separate *R. flavipes* and *R. malletei* collected with sticky traps on the basis of morphology using the key we developed or through regional available keys (Clément et al. 1985, Clément et al. 1986, Austin et al. 2007, Lim and Forschler 2012). For this reason we grouped *R. flavipes* and *R. malletei*. *Reticulitermes malletei* is reported was reported to fly in May and June in northeastern Georgia (Clément et al. 1986). Given the difficulty with separating these species, and both the recent description (Clément et al. 1985) and acceptance (Austin et al. 2007) of *R. malletei* by American researchers, it is possible that *R. flavipes* and *R. malletei* flights are allopatric in localities where both of these species are distributed. In agreement with Clément et al. (1986), we found sympatric flights of *R. virginicus* and *R. flavipes/malletei* (Figs. 24, 26, 28). Unexpectedly, Oct. flights of *R. flavipes/malletei* sympatric with *R. virginicus* (Fig. 25). Hathorne et al. (2000) report *R. flavipes* flights in Sep. from South Carolina, but as they did not use DNA barcoding to verify their determinations, it can not be known for certain whether they were describing flights of *R. flavipes* or *R. malletei*.

Sticky traps present some challenges. Specimens are often damaged or only consist of wings. Specimens that consist solely of a damaged wing are difficult or not possible to identify to resolutions higher than the generic level. Relatively undamaged specimens of *R. malletei* and *R. flavipes* pose challenges to unequivocally separate due to the poorly developed taxonomy of *R. malletei* and the similarity of *R. malletei* imagos with those of *R. flavipes* (Scheffrahn and Su 1994, Austin et al. 2007). Citrus oil solvent and sticky trap glue will not necessarily damage the DNA of genes of interest for DNA barcoding techniques in termites, although these limits have not been established in this group of insects (Marshall et al. 2010). For sticky traps to be useful for monitoring species swarming phenology, future research should focus on increasing the ease of morphological identification of degraded samples and establishing the maximum concentrations and duration of immersion in citrus oil solvent and sticky trap glue on the genes of interest for DNA barcoding.

This study presents the first standardized, continuous monitoring data of the swarming flight phenology of Alabama rhinotermitids, and the first of its kind in the southeastern United States. Despite the difficulties inherent with specimen extraction from sticky traps, this collection method presents a viable, inexpensive method for long-term, continuous monitoring of these economically important insects.

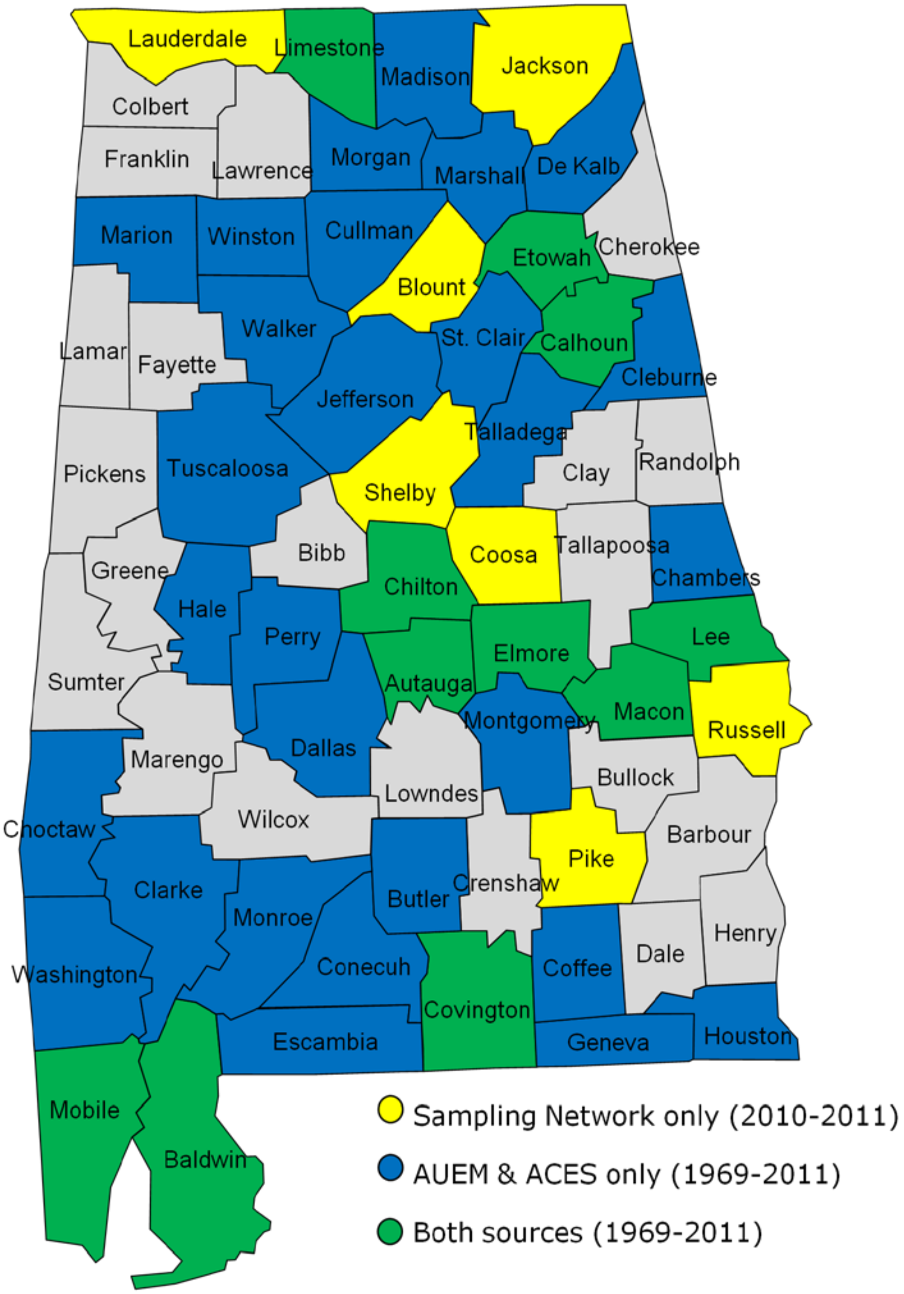

Figure 22. Map of counties monitored for swarming flight phenology. The 7 counties in yellow were continuously sampled by the Sampling Network from 2010-2011; the 28 counties in blue had at least one record of a flying imago deposited into the AUEM or received by ACES from 1969-2012; and the 11 counties in green had data from both sources.

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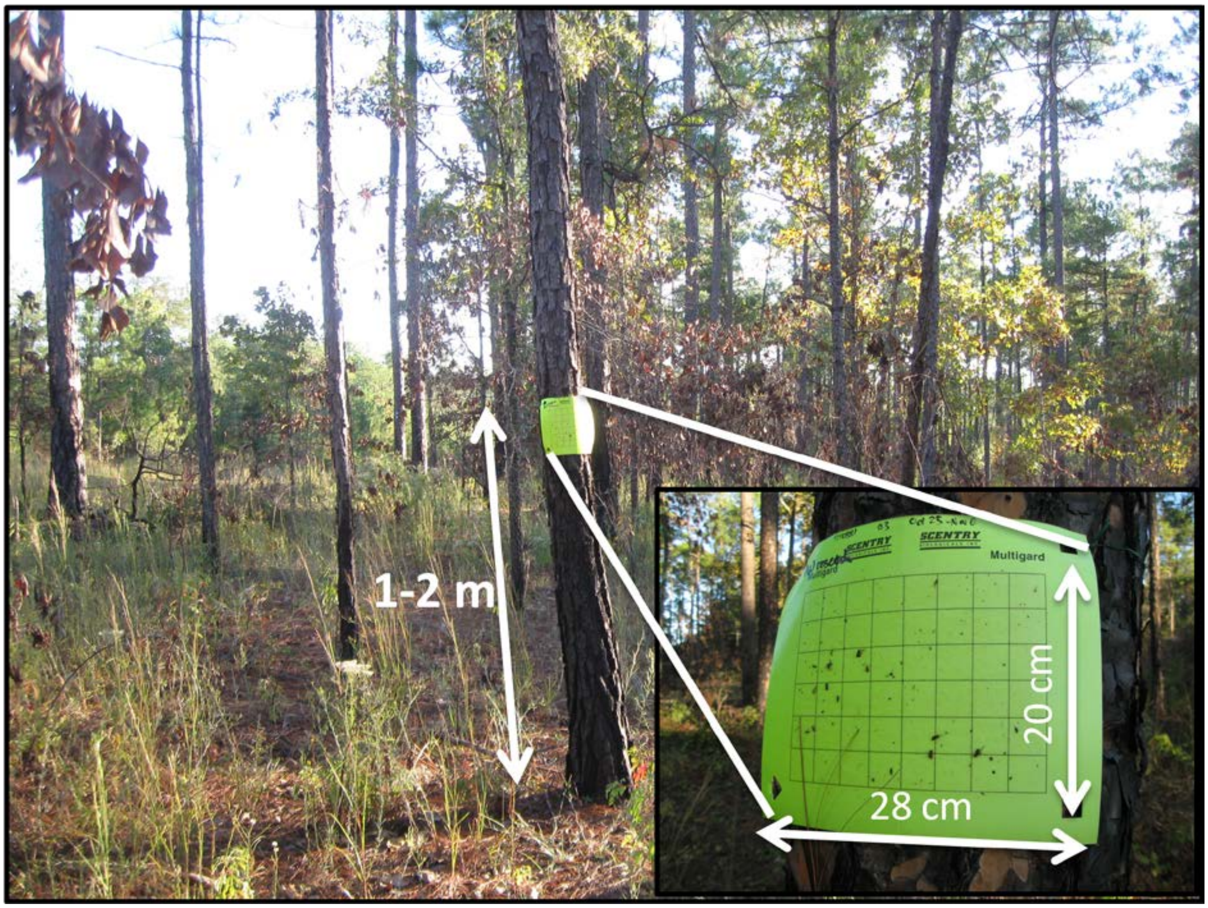

Figure 23. Sticky trap replicate location in Tuskegee National Forest. Three locations were chosen at each site. Sticky traps with a 20 cm X 28 cm sticky surface were placed 1-2 m above ground level on vertical wooden surfaces, such as trees. Traps were affixed with nails.

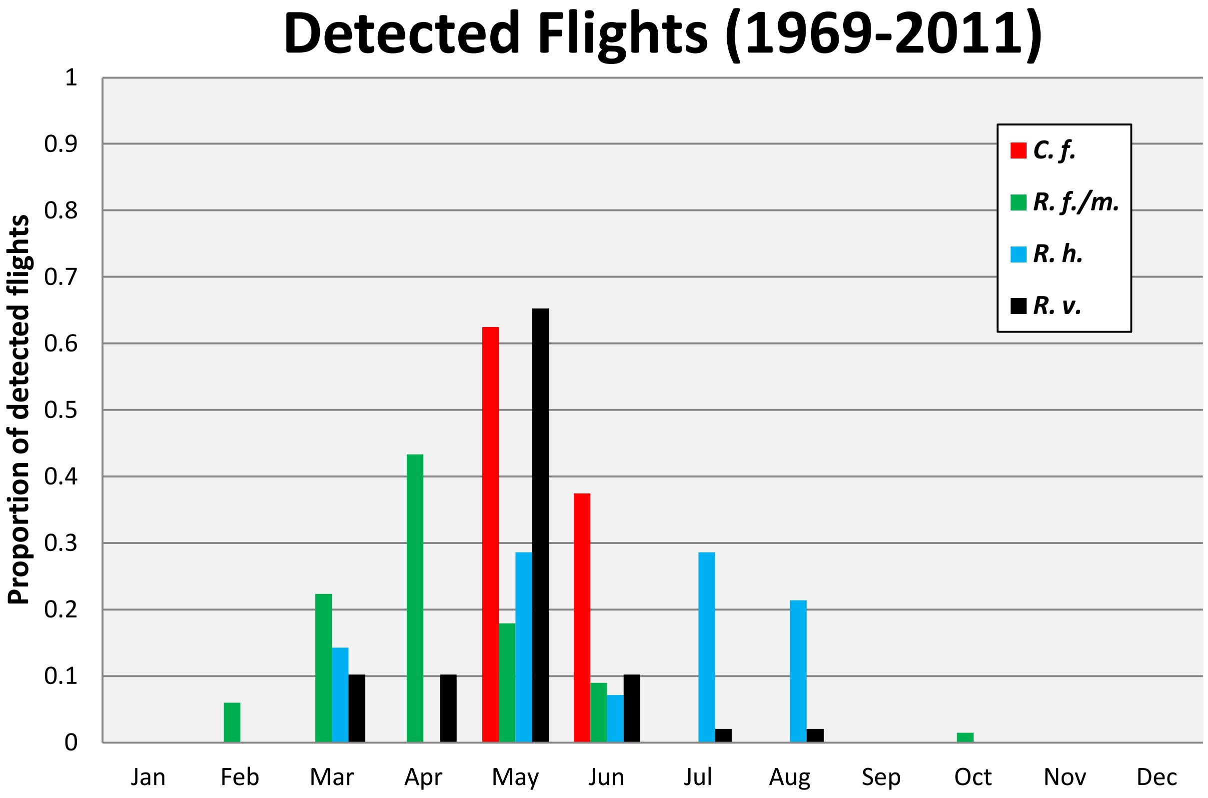

Figure 24. Detected Rhinotermitidae swarming flights in Alabama (1969-2011). Bars represent the proportion of detected flights for each species in each month over all years, from all data sources available for that species. Abbreviations: *C. f.*, *C. formosanus*; *R. f./m.*, pooled *R. flavipes* and *R. malletei*; *R. h.*, *R. hageni*; *R. v.*, *R. virginicus*.

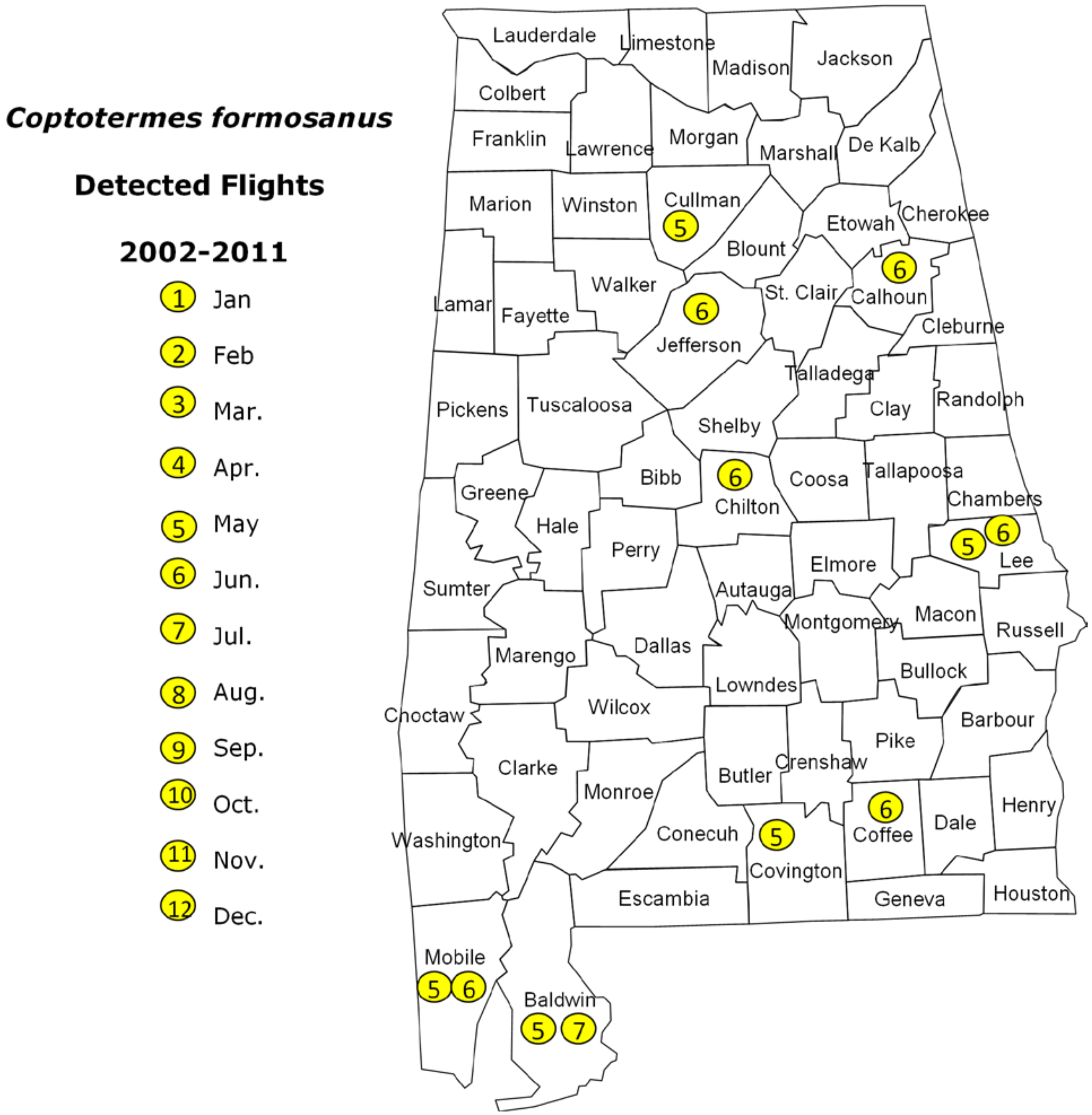

Figure 25. Map of detected flight localities and months for *C. formosanus*.

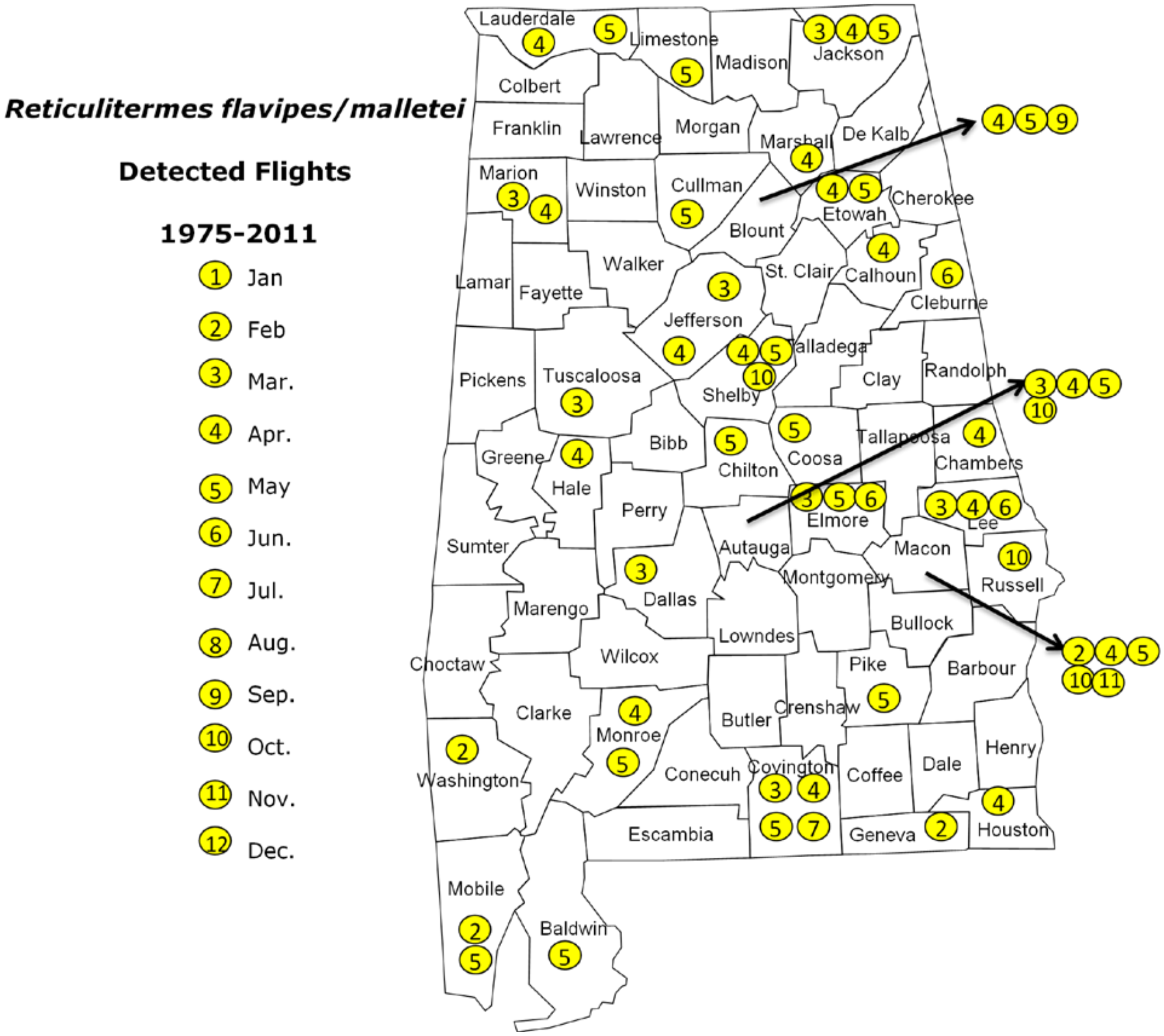

Figure 26. Map of detected flight localities and months for *R. flavipes/malletei*.

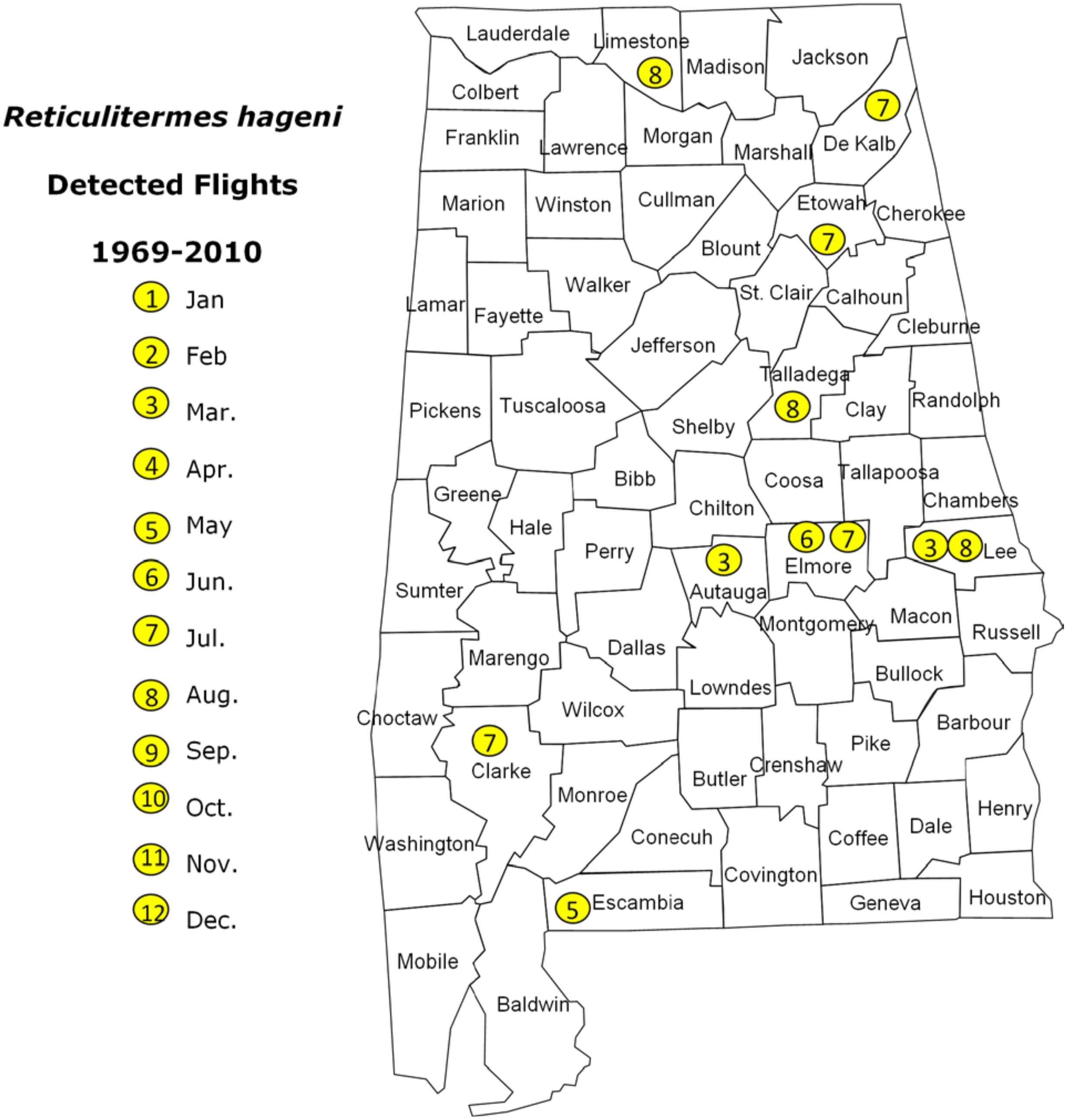

Figure 27. Map of detected flight localities and months for *R. hageni*.

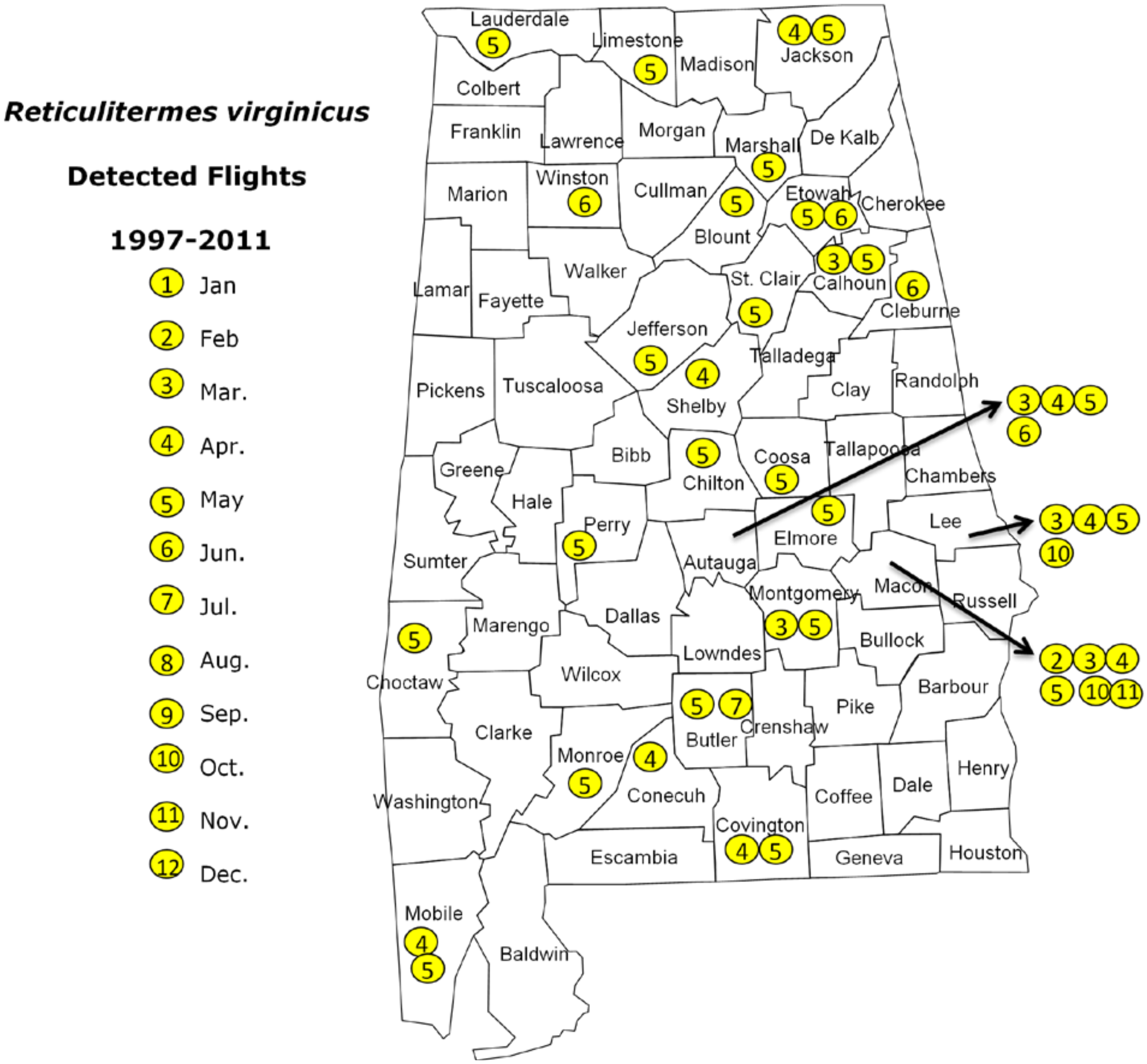

Figure 28. Map of detected flight localities and months for *R. virginicus*.

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