TAXONOMIC REVIEW AND BIOGEOGRAPHY OF *RETICULITERMES* (RHINOTERMITIDAE) TERMITES FOUND IN GEORGIA

by

SU YEE LIM

(Under the Direction of BRIAN T. FORSCHLER)

ABSTRACT

The importance of improving *Reticulitermes* taxonomy has been reiterated numerous times since the early 1960s. This work contains two different but complementary sections targeted at improving the systematics of *Reticulitermes* with a focus on the southeastern region of the United States. A formal description for a new species, *Reticulitermes nelsonae* is the provided as part of this dissertation. The first section is a morphological study of the alate and soldier castes to identify better supported and additional morphological characters to build an improved dichotomous and online, interactive matrix key for *Reticulitermes*. The second portion consists of generic-level phylogenetic analyses based on two different genomic DNA regions utilizing distance, parsimony, and likelihood software methodologies.

INDEX WORDS: Systematics, Taxonomy, Phylogenetic, Rhinotermitidae, *Reticulitermes*, *Reticulitermes nelsonae*, subterranean termites

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DEDICATION

This is dedicated to my beloved parents, Jin Wooi, my grandmother and my dog, Snowflakes. They are my pillars of strength. May they always be blessed, be well and happy.

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My sincere gratitude goes out to many people who have made this journey and adventure a reality. I have been very fortunate to have the support, encouragement and opportunities generously shared with me during my entire graduate program at UGA. This was a dream, not only for me but also for my parents, Jin Wooi, grandparents and ancestors that has come true.

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Universiti Sains Malaysia, I have always kept at a distanced from the creepy-crawlies. Although I was not afraid of them, I was definitely not comfortable with insects. Myrmecology was the field I was first introduced to. Soon, as any true teacher would, he encouraged me to further my studies in the US although I have started the Masters program with him. Dr. Lee did all he could to help me secure a place and fulfill my dream of studying abroad. He is truly an exemplary teacher, mentor, and good friend. My family and I thank you Professor Lee.

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From many hours of field sampling, rigorous laboratory sessions and computer logged-on days, long beautiful drives along the southern roads, breath-taking views of Sapelo Island, magnificent long-leaf pine trees at Greenwood Plantation, snow-capped mountains of Lake Tahoe, bright lights of Las Vegas, ice cold temperatures of Indianapolis, rainy lush-green Oregon waterfalls to the 'Gathering Place' of Oahu island Hawaii, every step and breathe of this journey has been very rewarding and humbling. These moments have changed my little world, and will forever be cherished with joy.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Termites, aptly described as "Dwellers in Darkness" by S.H. Skaife (1961), have been described as the oldest surviving group of social organisms, having evolved about 200 MYA (Skaife 1961, Pearce 1997, Thorne and Forschler 1999). All members of the Order Isoptera are eusocial, having overlapping generations, cooperation in care for the young, and differentiation of life forms based on role within the colony (Wilson 1971). Termites however, differ from other social insects in that both sexes can be found in all castes and developmental forms (Nutting 1990, Pearce 1997, Thorne and Forschler 1999).

The idea that termites are close relatives to cockroaches (Order: Blattodea) has been debated for at least 50 years (Snyder 1948, Nutting 1990, Pearce 1997), and recent molecular evidence supports the concept of close kinship (Eggleton et al. 2007, Inward et al. 2007a, Inward et al. 2007b, Lo et al. 2007). Various approaches to preserving the nominal hierarchies with appropriate ordinal and subordinal names are discussed in Inward et al. (2007a, 2007b) Lo et al. (2007) and Eggleton et al. (2007), but are beyond the scope of this study. Here, we focus on the taxonomy at the generic level for *Reticulitermes* to provide a start for reviewing this complicated genus.

Major revisions within the termite order have been made in the recent years and the latest revision recognizes thirteen families within the order Isoptera (Engel et al. 2009). There are six subfamilies within the Rhinotermitidae and *Reticulitermes* is under the subfamily Heterotermitinae Froggatt 1897 (Engel et al. 2009).

The diversity of *Reticulitermes* is highest in temperate zones (Pearce 1997). Colonies can build large populations exceeding 6000/m^2 and are typically found at elevations below 7000-8000 feet (Fig. 1.1) (Snyder 1948, Pearce 1997, Eggleton 2000). *Reticulitermes flavipes* is the most widely distributed species within the genus and commonly abundant around human structures (Abe et al. 2000, Pinzon and Houseman 2009). Although the genus is mostly known as an urban pest that causes billions of dollars in damages yearly, termites have a larger role than simply destroying wood in human structures (Kofoid 1934, Forschler and Jenkins 1999). These insects are ecologically important in certain food chains because they break down cellulose, and help recycle nutrient (La Fage and Nutting 1978, Pearce 1997).

The developmental biology of *Reticulitermes* is not completely known (Thorne and Forschler 1999, Laine and Wright 2003) in part because of its cryptic lifestyle which complicates field sampling and testing research assumptions (Haverty and Nutting 1975, Nutting 1990, Forschler and Jenkins 1999). Various developmental pathways have been hypothesized pointing to a plastic life cycle for these termites (Thompson 1916, 1917, Thompson and Snyder 1919, Buchli 1958, Noirot and Pasteels 1987, Noirot and Pasteels 1988, Thorne and Forschler 1999, Thompson et al. 2000, Laine and Wright 2003, Thompson et al. 2003). Methods for distinguishing male and female *Reticulitermes* were reported by Thompson (1916), Zimet and Stuart (1982) and Pearce (1997). Thorne and Forschler (1999) provided definitions for the different developmental forms and caste terminology of termites.

The most common castes or developmental forms encountered during sampling in the field are workers and soldiers (Miller 1964, Weesner 1965, Nutting 1990, Thorne and Forschler 1999). Workers are the most abundant caste within a colony and provide the labor required to keep the colony functioning (Thorne and Forschler 1999). Soldiers defend nestmates from

invaders while the reproductive caste propagates (Thorne and Forschler 1999). The reproductive caste consists of different forms (Thorne and Forschler 1999). Alates are ephemeral and seasonal depending on species, geographic region as well as temperature and weather conditions (Pearce 1997, Thorne and Forschler 1999). Alates are one of the reproductive forms that are responsible for dispersion and founding new colonies (Nutting 1990, Thorne and Forschler 1999). Male alates that eventually become the founding king of new colonies do not perish after mating; instead they live with the queen within the colony (Thorne and Forschler 1999). The founding king and queen are also postulated to be among of the longest lived of the insects (Snyder 1948), as shown by 15-year life spans from laboratory cultures.

The majority of termites found within a colony do not have functional eyes, thus communication amongst nestmates is achieved through chemical and mechanical signals (Thorne and Forschler 1999, Lenz et al. 2001, Evans et al. 2005, Evans et al. 2009).

Taxonomic Status of *Reticulitermes* spp. found in the southeastern USA

Mayr and Ashlock (1991) stated "Taxonomy is the oldest biological discipline". Winston (1999) defined taxonomy as a subset of the larger more encompassing field of systematics which is the "practical process of identifying, recognizing, researching, or redescribing a taxon for scientific publication according to the current rules of biological nomenclature" (Winston 1999). Morphology, physiology, genetic information, behavior, ecology and geography of a taxon can be investigated to provide taxonomic characters that allow distinction between different taxa (Mayr and Ashlock 1991). Yet, the majority of the taxonomic literature on *Reticulitermes* has relied on morphological characters that do not allow complete and precise distinction of species because of phenotypic plasticity (Banks and Snyder 1920, Miller 1949, Snyder 1954, Weesner 1965, Nutting 1990, Scheffrahn and Su 1994, Hostettler et al. 1995, Thorne and Forschler 1999,

King et al. 2007, Wang et al. 2009). Research in the past 10 years has gravitated to describing and identifying *Reticulitermes* using molecular data (Forschler and Jenkins 1999, Jenkins et al. 2000, Jenkins et al. 2001, Austin et al. 2005, Szalanski et al. 2006, Tripodi et al. 2006, Austin et al. 2007, Austin et *al.* 2008, Sillam-Dussès and Forschler 2010).

The latest review of the status of *Reticulitermes* taxonomy was by Vargo and Hussender (2009) that tabulated eight *Reticulitermes* spp. in North America, seven of which they designate as valid from the 75 described worldwide. They concurred with Austin et al. (2007) and delegated *R. arenincola* as *nomen dubium* because they found that species designation represents *R. flavipes,* however *R. arenincola* would be more fitting as a junior synonym instead of being delegated as *nomen dubium* (ICZN, 1999). Of the seven species they named valid, three, *R. hageni*, *R. tibialis* and *R. hesperus* were designated as a possible species complex (Vargo and Husseneder 2009).

Contradictions within the systematics literature of *Reticulitermes* from southeastern USA (1813-2011)

Current valid taxonomic hierarchy for *Reticulitermes*:

Family: Rhinotermitidae Froggatt, 1897

Subfamily: Heterotermitinae Froggatt, 1897

Genus: *Reticulitermes* Holmgren, 1913

Genus *Reticulitermes* Holmgren 1913

Type Species: *Reticulitermes* (*Termes*) flavipes (Kollar) 1837 as designated by Banks and Snyder (1920) (Holmgren 1913, Emerson 1971).

Hemerobites antiquus Germar 1813 and *Maresa plebeja* Giebel 1856 are two related species names referring to the same type specimen *Reticulitermes antiquus* (Germar) (Banks and

Snyder 1920, Emerson 1971, Engel and Krishna 2007, Roisin 2008). The ICZN approved a petition submitted by Engel and Krishna (2007) to conserve *Reticulitermes* Holmgren 1913 as the genus-group name, and give it precedence over *Hemerobites* and *Maresa* because of wide and current usage in the scientific literature (Engel and Krishna 2007, Roisin 2008, ICZN 2009).

Leucotermes Silvestri 1901 was once incorrectly used as a genus-group name in the scientific literature and in several patents of chemical compounds referring to *Reticulitermes* Holmgren 1913. However, Banks and Snyder (1920), Emerson (1971) and M. S. Engel (pers. comm.) clarified that subgenus *Leucotermes* Silvestri 1901 is actually a junior synonym of *Heterotermes* Froggatt 1896 and the type specimen for *Leucotermes* was different from *Reticulitermes (Termes)* Holmgren 1913. These scientific references were published between 1901-1920 (Osborn 1908, Snyder 1915, Marlatt 1916, Snyder 1916, Thompson 1916, 1917, Comstock 1918, Thompson and Snyder 1919, Banks and Snyder 1920, McDaniel 1920). A timeline of events for the history of *Reticulitermes* taxonomy is detailed in Table 1.1 and Figure 1.2. Table 1.2 lists the author, year of description and taxonomic status of each species of *Reticulitermes*.

***Reticulitermes* (*Termes*) *flavipes* (Kollar) 1837 and *Reticulitermes santonensis* Feytaud 1924**

Reticulitermes flavipes was originally described by Kollar in 1837 from specimens collected from greenhouses at Schönbrunn, Vienna, Austria (Banks and Snyder 1920). *Reticulitermes santonensis* was described in 1924 by Feytaud (Feytaud 1924). Bagnères et al. (1990) thought that *R. santonensis* is a sibling species of *R. flavipes* as they reported that the cuticular hydrocarbons of *R. flavipes* and *R. santonensis* are identical, but differ in proportions and quantities, while the soldier defense secretions produced were different (Bagnères et al.

1990). *Reticulitermes santonensis* was confirmed as a junior synonym of *R. flavipes* based on strong genetic data support (Jenkins et al. 2000, Jenkins et al. 2001, Austin et al. 2005b).

***Reticulitermes malletei* Clèment et al. 1986**

Reticulitermes malletei was described in 1986 as a new species from specimens collected in Athens, GA based on differences in chemical and behavior in alates and workers (Clément et al. 1986). Morphological characters, however, for distinguishing *R. malletei* from *R. flavipes* and *R. virginicus* were not provided, thus the species was not entirely accepted or embraced by the scientific community and its description was relegated *nomen nudum* by Scheffrahn et al. (2001) on grounds that Clèment et al. (1986) did not described *R. malletei* properly. However, *R. malletei* is valid because Clèment et al. (1986) did fulfill the requirements imposed by ICZN for new species described before 2000. Clèment et al. (1986) provided characters that uniquely distinguished the taxon from others. Austin et al. (2007) concurred that the species was valid and provided genetic data (16S rRNA), as well as morphological characters that further differentiate *R. malletei* from *R. flavipes*, *R. virginicus* and *R. hageni*. Wang et al. (2009) noted *R. malletei* was *nomen nudum* in the survey of termites in Indiana although they referenced Austin et al. (2007).

Additional contradictions that the author is aware of within the genus *Reticulitermes* in USA

***Reticulitermes arenincola* Goellner 1931**

Reticulitermes arenincola Goellner 1931 was described as a species found in sandy soils such as sand dunes in Indiana along the southern shore of Lake Michigan (Goellner 1931). Colonizing flights for *R. arenincola* were reported toward the end of May (Goellner 1931). Banks (1946) and Ye et al. (2004) acknowledge the validity of *R. arenincola*. Although, Ye et al.

(2004) found an "apparent synonymity between *R. flavipes* and *R. arenincola"* using DNA sequence data, they also reported "clear morphological differences in the soldier caste". Vargo and Hussenender (2009) contradict this view and delegate the *R. arenincola* as a *nomen dubium* based on the molecular data, citing Austin et al. (2004, 2005a, 2007). In the same year, Wang et al. (2009) contradicts Vargo and Hussenender (2009) by concluding that *R. arenincola* is a distinct species based on RFLP profiling and 16S rRNA sequence. Wang et al. (2009) also reported their morphometric measurements for *R. arenincola* were sometimes similar to those of *R. flavipes* and *R. virginicus*. The overlapping morphometric measures and phenotypic ambiguity reported by Wang et al. (2009) contradicts Ye et al. (2004) who reported a 'clear' morphometric difference. If genetic and morphological data shows that *R. arenincola* is similar to *R. flavipes, R. arenincola* should then be a junior synonym of *R. flavipes,* and not designated as *nomen dubium*. Lastly, we note that Austin et al. (2004) and Austin et al. (2005a) used an alternate spelling *R. arenincola – R. arenicola* (without 'n').

"Reticulitermes okanaganensis"

Szalanski et al. 2006 distinguished an undescribed species referred to as "*Reticulitermes okanaganensis"* to be a cryptic species, based on genetic differences observed in 428bp of the 16SrRNA region. The spelling of "*R. okanaganensis"* is slightly confusing as there are two versions of the name currently in the literature, "*R. okanaganensis"* (Szalanski et al. 2006, Tripodi et al. 2006, Austin et al. 2008) and "*R. okana**n**ganensis"* (Austin et al. 2008). According to Szalanski, "*Reticulitermes okanaganensis* has not been formally described as a taxonomic species; our paper on it only provided genetic evidence of it being a new species" (Szalanski, per. comm.). A species that has not been formally described does not have a formal scientific name and technically does not exist. It should also be noted that, McKern et al. (2007) cited the

Szalanski et al. (2006) article entitled "Genetic evidence for a new subterranean termite species (Isoptera: Rhinotermitidae) from Western United States and Canada" using a different title "*Reticulitermes okanaganensis*, a new subterranean termite (Isoptera: Rhinotermitidae) from western United States and Canada, based on morphology and mtDNA sequences".

Description and keys for *Reticulitermes*

Dichotomous keys have been for more than two centuries the primary tool for organizing character states and guiding users toward proper identification of biological specimens (Osborne 1963, Walter and Winterton 2007). Keys can also be classified as directed or undirected pathway keys (Walter and Winterton 2007). A dichotomous key would be an example of a directed pathway key while synoptic (filter) keys are undirected pathway keys (Walter and Winterton 2007). An in-depth review of the advantages and disadvantages of the methods available for taxonomic identification involving keys was done by Walter and Winterton (2007) and the reader is directed to that source for more information.

Morphological descriptions and keys are available for *R. flavipes* (Kollar) 1837, *R. virginicus* Banks 1907, *R. hageni* Banks 1920, as these species were described early in the 20th century (Banks and Snyder 1920, Snyder 1954, Weesner 1965, Nutting 1990, Scheffrahn and Su 1994, Wang et al. 2009). Nelson et al. (2008) wrote an excellent review of discrepancies within the termite literature concerning the use of keys for identifying *Reticulitermes* species within the USA. It should however be noted that Hosteller et al. (1995) did not provide a key in his review of the intraspecific variation within the Florida *Reticulitermes*.

Use of other behavioral and genetic characters in assisting taxonomic designations

Cuticular hydrocarbons (CHC) (Haverty et al. 1996, Haverty et al. 1999, Jenkins et al. 2000, Nelson et al. 2001, Page et al. 2002, Copren et al. 2005), soldier defense secretions (SDS)

(Nelson et al. 2001), alate pheromones (Clément et al. 1986), flight phenology (Banks and Snyder 1920, Snyder 1954, Weesner 1965, Scheffrahn and Su 1994) aggression studies on soldiers and workers (Clément et al. 1986, Polizzi and Forschler 1999), and examination of the protozoan community (Lewis 2003, Lewis and Forschler 2006) are some character states that have been evaluated to assist in taxonomic studies.

Swarm times or flight phenology is commonly thought to be an important behavioral identification of species differences indicative of reproductive isolation. Flight summaries of *R. flavipes*, *R. virginicus*, *R. hageni*, *R. malletei*, and *R. nelsonae* are tabulated in Figures 1.3-1.6. Literature that reported on the flight times of *Reticulitermes* are summarized in Table 1.3. Much of the literature reports are for flight times in Florida (Miller and Miller 1943, Miller 1949, 1964, Scheffrahn et al. 1988, Scheffrahn and Su 1994), one is for Louisiana (Messenger 2004), while the others have information on a national or regional scale (Light et al. 1934, Snyder 1954, Weesner 1965, Suiter et al. 2002) or are descriptions for species (Banks and Snyder 1920, Goellner 1931, Clément et al. 1986, Austin et al. 2007).

The phylogenetic relationships among families for the order Isoptera have been studied using molecular techniques by Engel and Krishna (2004), Legendre et al. (2008) and Engel et al (2009). The relationships at the generic level for four species of *Reticulitermes* (*R. flavipes*, *R. virginicus*, *R. hageni*, *R. malletei*) have been researched by Jenkins et al. (2000, 2001), and Austin et al. (2002, 2005b, 2007).

Advancement in molecular biology

There are a variety of molecular markers available for use in systematics allowing scientists the opportunity to examine relationships based on genetic or chemical evidence. The various molecular markers have different inheritance pathways, evolution (mutation) rates,

inherent characteristics that might be different among lineages, and are therefore useful for different research questions (Loxdale and Lushai 1998, Behura 2006). A variety of molecular markers and gene fragments can be used to examine taxonomic questions and the type chosen depends on the intention of the study. Common molecular markers include allozymes, mitochondrial DNA, nuclear coding and non-coding regions, microsatellites or short simple repeats (SSRs), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), expressed sequence tag (EST), single nucleotide polymorphism (SNP) (Loxdale and Lushai 1998, Jenkins et al. 1999b, Jenkins et al. 1999a, Jenkins et al. 2001, Behura 2006, Simon et al. 2006, Ware et al. 2010). These markers can be used for species identification, phylogenetics, evolutionary biology, phylogeography, molecular dating and population studies (Loxdale and Lushai 1998, Jenkins et al. 1999b, Jenkins et al. 1999a, Jenkins et al. 2001, Behura 2006, Simon et al. 2006, Ware et al. 2010).

Phylogenetic programs are used to infer phylogenetic relationships examples include PHYLIP (general-purpose package), PAUP v4.1b (general-purpose package), MEGA5 (general-purpose package), PHYML (ML inference), RaxML (ML inference), MrBayes (Bayesian inference) and many more. The reader is directed to http://evolution.genetics.washington.edu/phylip/software.html for a more comprehensive list of phylogeny programs and web servers that are available. A review of the importance of using proper parameters (i.e. choosing evolutionary models) to analyze genetic data was written by Simon et al. (2006) and the reader is directed to that work for more details. Baldauf (2003) and Harrison and Langdale (2006) wrote excellent reviews on “how-to” perform phylogenetic analyses. The ‘Phylogenetic Trees Made Easy’ series by Hall is another great resource in

addition to the manuals, tutorial and help forums available on the internet for each specific program (Hall 2004, Igic 2005, Drummond et al. 2010, Ronquist et al. 2011).

Organization of the dissertation

The objectives of this dissertation, presented in manuscript style, are to:

1. Provide a taxonomic description for *Reticulitermes nelsonae* a previously undescribed species of subterranean termite found in the southeastern United States.
2. Characterize morphological characters for accurate distinction of five *Reticulitermes* spp. in the Georgia, USA.
3. Develop an online interactive matrix key for *Reticulitermes* spp. found along the eastern seaboard of the USA.
4. Examine the phylogenetics of *Reticulitermes* from Georgia, USA using cytochrome oxidase II (COII) and internal transcribed spacer array (ITS).

Chapter 2 summarizes past literature published on the taxonomy of *Reticulitermes* spp. This chapter also reviews previous techniques used for species discrimination to improve the taxonomy for the genus. Key evolutionary studies and past literature on molecular techniques that were used for the genus were summarized. This chapter will be submitted to Entomological Review.

Chapter 3 addresses the first objective: a taxonomic description for a new species, *R. nelsonae* endemic to the southeastern USA. A dichotomous key for the five *Reticulitermes* spp. found in the state of Georgia was created. This key includes two species not found in the published literature: *R. malletei* described by Clement et al. (1986) and recently made recognized by Austin et al. (2007) and the new species *R. nelsonae*. This chapter will be submitted to Zootaxa.

Chapter 4 comprises the second and third objectives: determining and describing simple, distinct morphological characters for the *Reticulitermes* spp. in Georgia and development of an online interactive matrix key using LUCID 3.5. This section will be submitted to ZooKeys.

Chapter 5 is the result of examining Objective 4 that will be submitted to Molecular Phylogenetics and Evolution. This chapter is a phylogenetic study using mitochondrial genes (cytochrome oxidase II, COII and cytochrome oxidase I, COI) and the non-coding nuclear region (the ITS array) to decipher the relationship of *Reticulitermes* spp. found in Georgia, North Carolina and Florida. These locations represent different soil provinces: the Piedmont, South Coastal Plain and Atlantic Coast Flatwoods that delimit the endemic range of *Reticulitermes* across the southeastern United States. In the final chapter, I attempt to summarize the major highlights and draw an overall conclusion to this body of work. It should be noted that because all chapters are presented in the manuscript format, each chapter has its own respective set of acknowledgements, references, tables and figures.

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Table 1.1. Brief history of the genus *Reticulitermes* taxonomic name

Genus	Genus Author	Genus Year	Type species	Species Author	Status
Hemerobites	Germar	1813	*Hemerobites antiquus*	Germar	*Nomen oblitum*
Maresa	Giebel	1856	*Maresa plebeja*	Giebel	Suppressed by ICZN ruling to give *Reticulitermes* priority
Leucotermes	Silvestri	1901	*Leucotermes tenuis*	Hagen	Synonym of *Heterotermes*
Reticulitermes	Holmgren	1913	*Reticulitermes* (*Termes*) *flavipes*	(Kollar)	Valid

(Holmgren 1913; Banks and Snyder 1920; Emerson 1971; Engel and Krishna 2004)

Table 1.2. *Reticulitermes* in the southeastern United States.

Species name	Author	Year	Status
R. (*Termes*) *flavipes*	(Kollar)	1837	Valid
R. (Termes) virginicus	(Banks)	1907	Valid
R. hageni	Banks	1920	Valid
R. malletei	Clement et al.	1986	Valid

Table 1.3. Key for the flight summaries literature of *Reticulitermes* corresponding to numbers in Figures 3-6.

No.	References
1	Banks N and Snyder TE (1920) A revision of the nearctic termites. In: Smithsonian (Ed), 228 pp.
2	Light, SF, Horner, AC, Randall, M, Herms, WB & Bowe EE (1934) Termites and Termite Control. Berkeley: University of California Press
3	Snyder TE (1954) Order Isoptera: The termites of the United States and Canada. National Pest Control Association, New York, 64 pp.
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5	Suiter DR, Jones SC, Forschler BT (2002) Biology of Subterranean Termite in the Eastern United States. Bulletin: 6pp.
6	Messenger MT (2004) The Termites Species of Louisiana: An Identification Guide. New Orleans Mosquito & Termite Control Board Bulletin. 3rd ed. New Orleans Mosquito & Termite Control Board, New Orleans, LA, pp.
7	Austin JW, Bagnères A-G, Szalanski AL, Scheffrahn RH, Heintschel BP, Messenger MT, Clement J-L, Gold RE (2007) *Reticulitermes malletei* (Isoptera: Rhinotermitidae): a valid Nearctic subterranean termite from Eastern North America. Zootaxa 1554: 1-26.
8	Miller EM, Miller DB (1943) Preliminary study on the distribution and habits of South Florida termites. Proc Fla Acad of Sciences 6: 101-107.
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11	Scheffrahn RH, Mangold JR, Su N-Y (1988) A Survey of Structure-Infesting Termites of Peninsular Florida. The Florida Entomologist 71: 615-630.
12	Scheffrahn RH, Su N-Y (1994) Keys to soldier and winged adult termites (Isoptera) of Florida. Florida Entomologist 77: 460-474.
13	Clément JL, Howard R, Blum M, Lloyd H (1986) ÉCOLOGIE GÉNÉRALE (BIOGÉOGRAPHIE). - L'isolement spécifique des termites du genre *Reticulitermes* (Isoptera) du sud-est des États-Unis. Mise en evidence grace a la chimie et au portement d' une espéce jumelle de *R.virginicus = R. malleti* sp.nov. et d' une semi-species de R. flavipes. C R ACAD SC PARIS 302: 67-70.
14	Goellner EJ (1931) A new species of termite, *Reticulitermes arenincola*, from the sand dunes of Indiana and Michigan, along the shores of Lake Michigan. Proc Ent Soc Wash 33: 227-234.
15	Laboratory collections from Georgia.

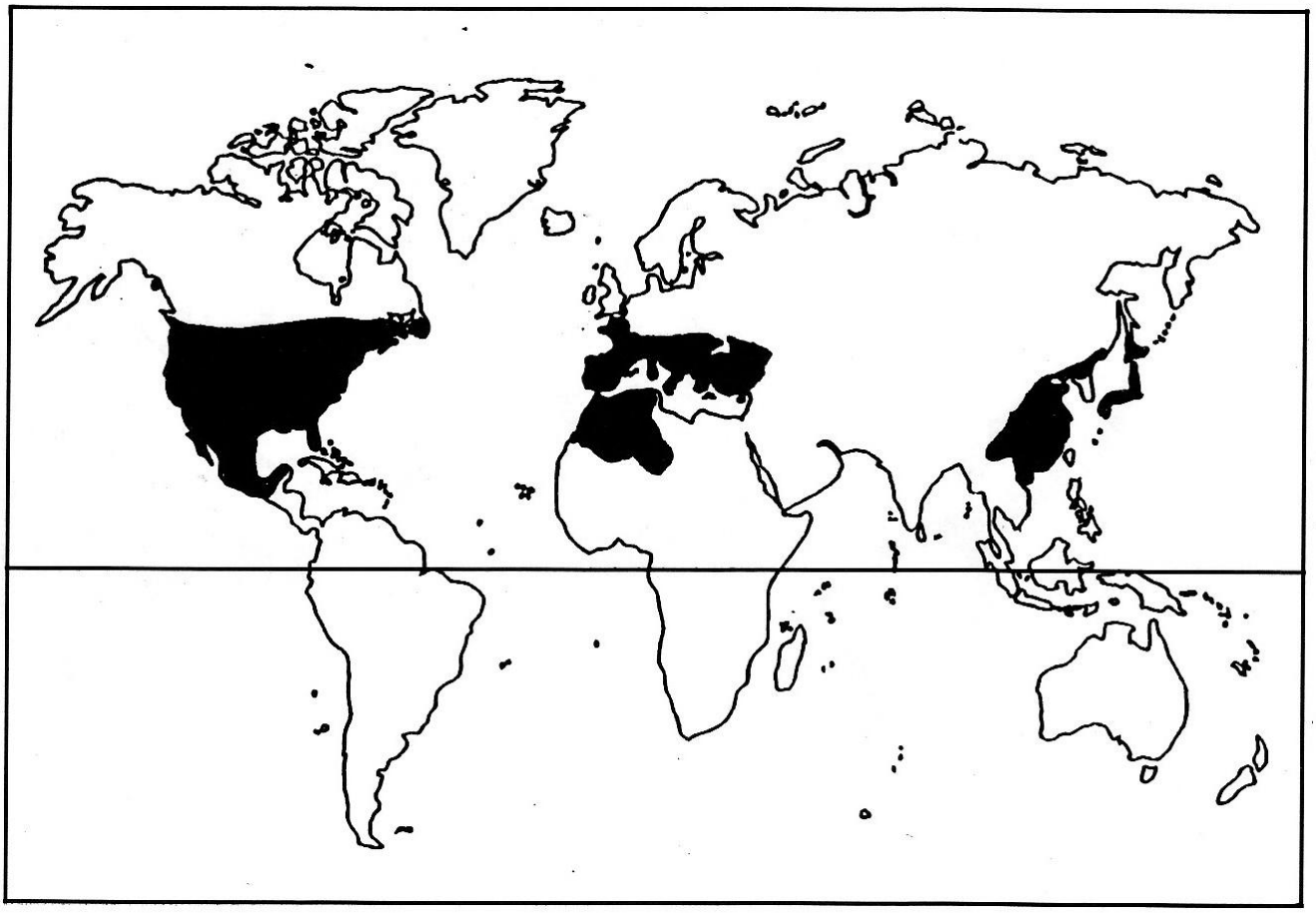


Figure 1.1. Worldwide distribution of *Reticulitermes* according to Pearce (1997).

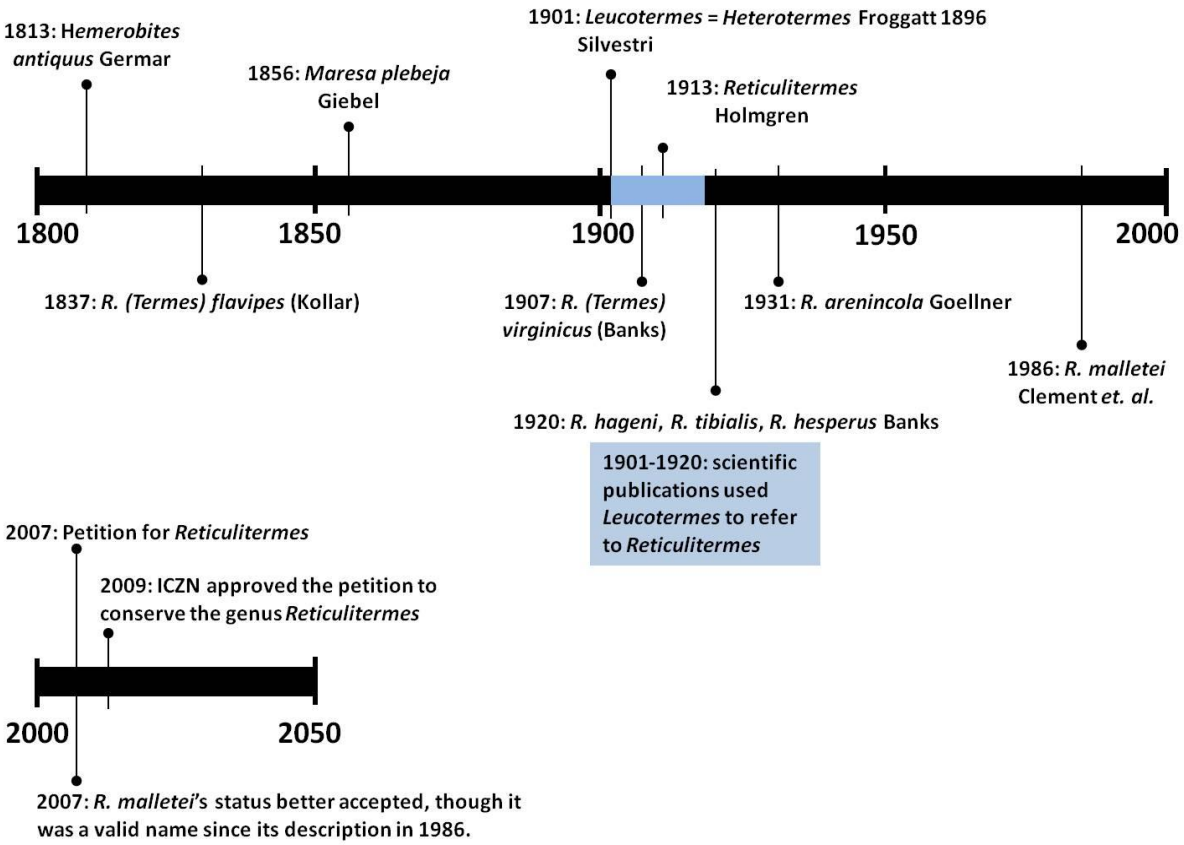


Figure 1.2. A timeline of significant events in the taxonomy of *Reticulitermes* species from the Nearctic region of the world.

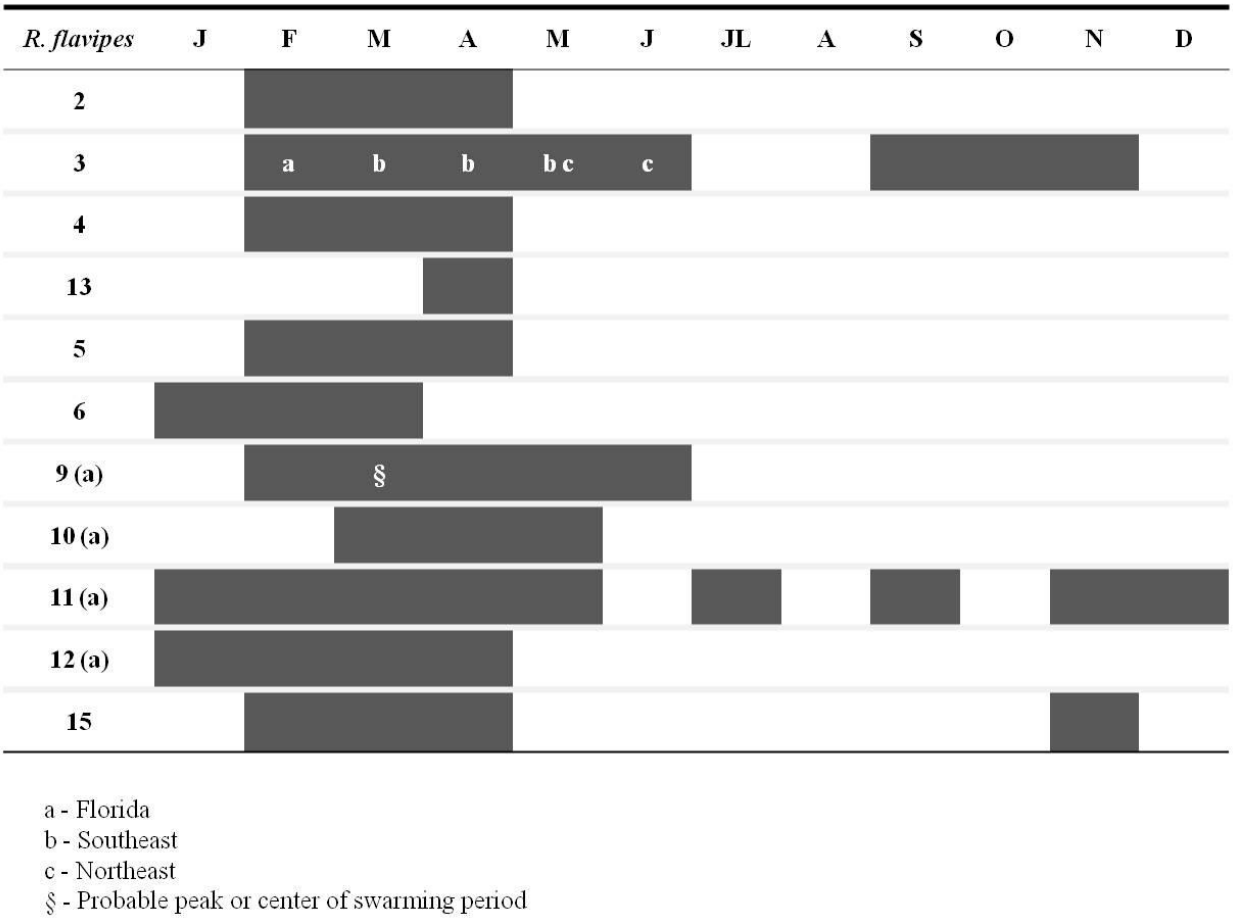


Figure 1.3. Flight summary for *R. flavipes*. Numbers refer to publications referenced in Table 3. Abbreviations J-D refer to months in a year.

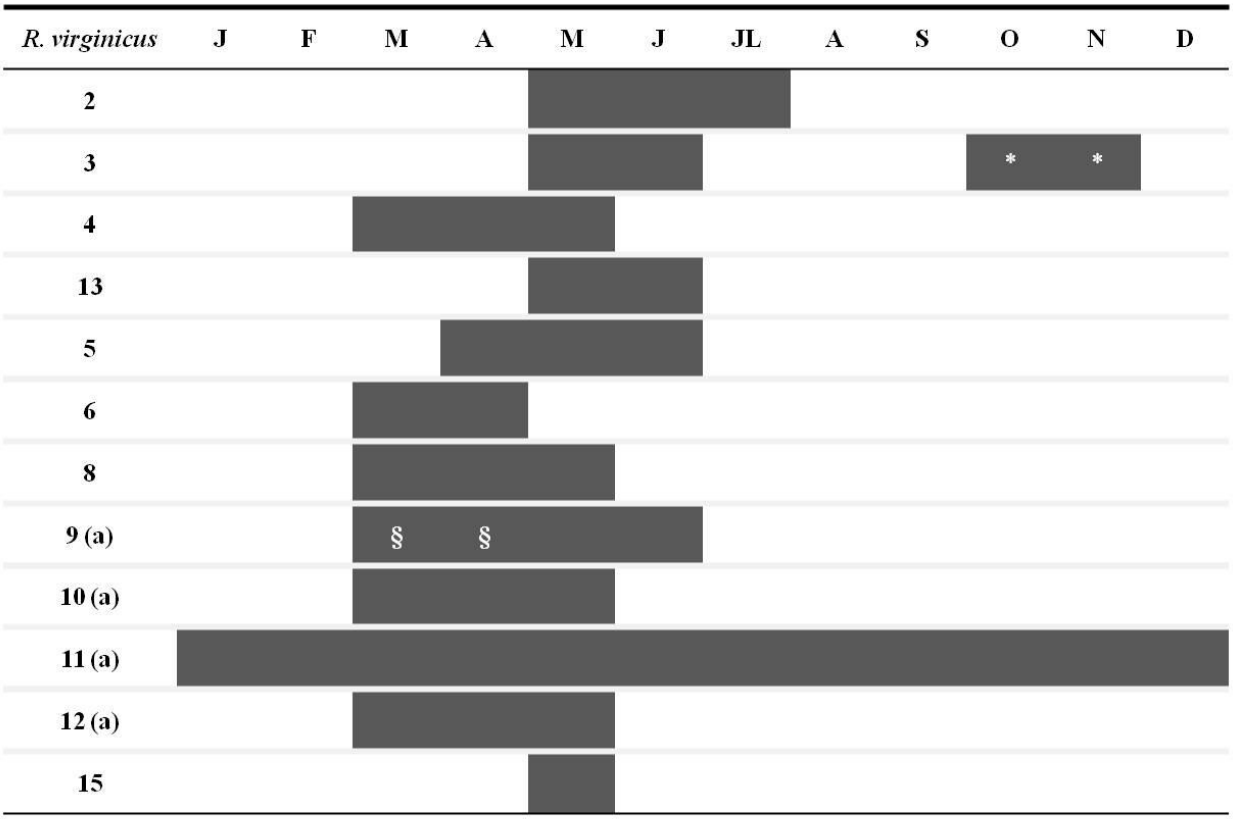
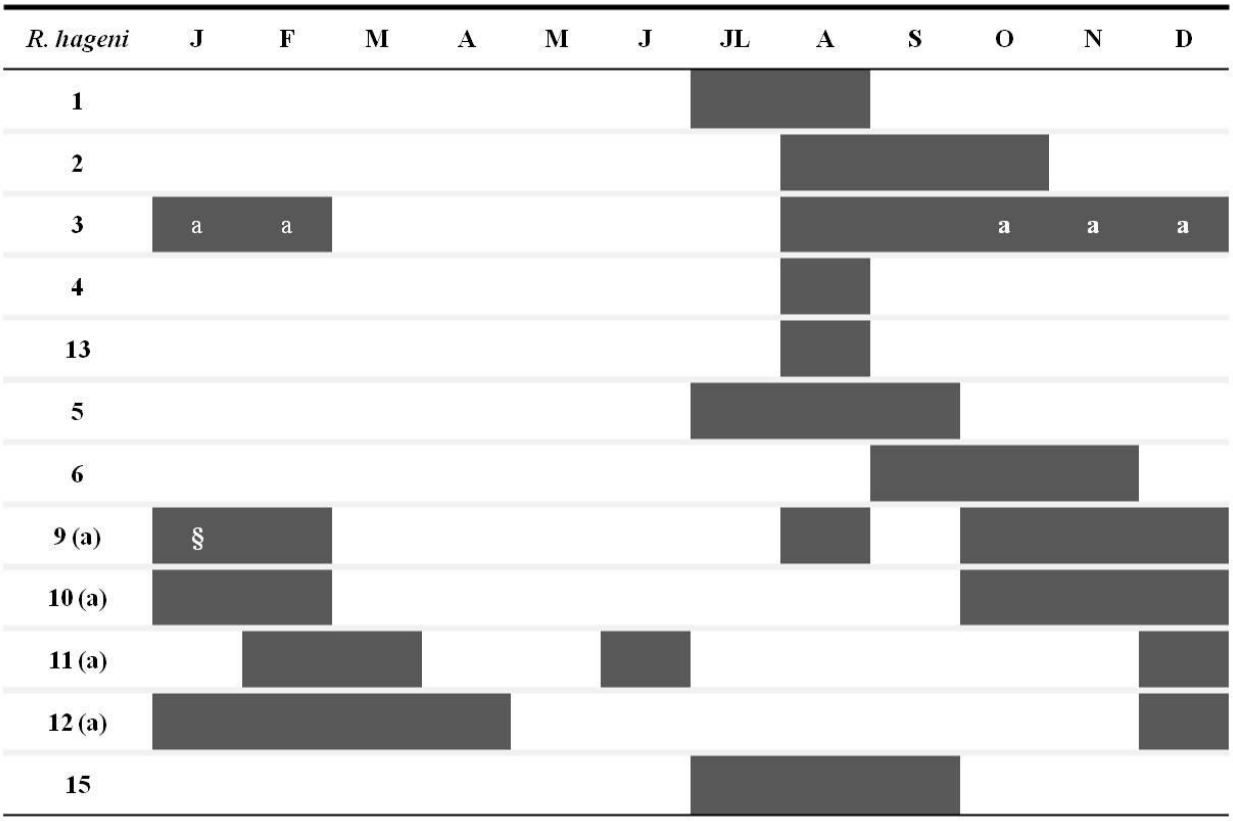


Figure 1.4. Flight summary for *R. virginicus*. Numbers refer to publications referenced in Table 3. Abbreviations J-D refer to months in a year.



a- Florida
§- Probable peak or center of swarming period

Figure 1.5. Flight summary for *R. hageni*. Numbers refer to publications referenced in Table 3. Abbreviations J-D refer to months in a year.

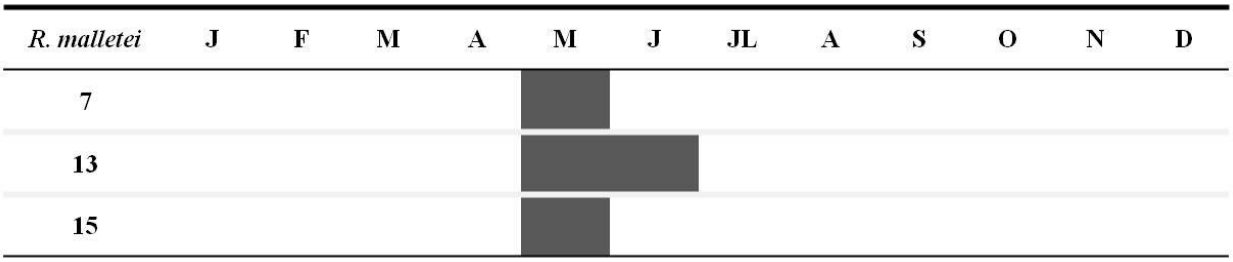


Figure 1.6. Flight summary for *R. malletei*. Numbers refer to publications referenced in Table 3. Abbreviations J-D refer to months in a year.

CHAPTER 2

RETICULITERMES NELSONAE, A NEW SPECIES FROM THE SOUTHEASTERN UNITED STATES. [1]

[1]Lim, S.Y. and B.T. Forschler. To be submitted to Zootaxa.

Abstract

Reticulitermes nelsonae, a new species of Rhinotermitidae (Isoptera) is described based on specimens from Sapelo Island, GA, Thomasville, GA, Havelock, NC, and Branford, FL. Adult (alate) and soldier forms are described. Diagnostic characters are provided and incorporated into a supplemental couplet of a dichotomous key to the known species of *Reticulitermes* found in Georgia, USA.

Keywords: taxonomy, phylogenetics, morphometrics, dichotomous key, Rhinotermitidae

Introduction

Members of the family Rhinotermitidae, commonly known as "subterranean termites," have a cryptic lifestyle making them difficult to study (Miller, 1964; Thorne et al., 1996). The genus, *Reticulitermes*, found in temperate regions of the Nearctic includes several economically notorious species that cause billions of dollars in structural damage every year (Mallis, 1990; Su & Scheffrahn, 1990; Thorne & Forschler, 1999; Abe et al., 2000; Su et al., 2001). There are four described species of *Reticulitermes* endemic to the southeastern United States (Banks & Snyder, 1920; Clément et al., 1986; Austin et al., 2007) and proper identification is critical to a better understanding of the economic and ecological importance of these insects (Logan et al., 1992; Pearce, 1997).

The genus *Reticulitermes* Holmgren 1913 was established to accommodate *Termes flavipes* (Kollar) 1837 (Engel & Krishna, 2007; ICZN, 2009). Three related generic names were proposed prior to *Reticulitermes*, namely *Hemerobites* Germar 1813, *Maresa* Giebel 1856 and *Leucotermes* Silvestri, 1901 (Banks & Snyder, 1920; Engel & Krishna, 2007; Roisin, 2008). Both *Hemerobites* and *Maresa* were not commonly used and considered congeneric with *Reticulitermes (Termes) flavipes* Kollar by Emerson (1971). *Hemerobites antiquus* Germar 1813 was deemed *nomen oblitum* because the name was not used after 1899 (Engel & Krishna, 2007), while *Maresa* Giebel 1856 (type: *Maresa plebeja* Giebel 1856) was used only once by Handlirsch (1906) (Engel & Krishna, 2007). *Leucotermes* Silvestri 1901 was used over a 5-year period in reference to *R. flavipes* (Snyder, 1915; Snyder, 1916a; Snyder, 1916b; Thompson, 1916; Thompson, 1917; McDaniel, 1920). "The type species of *Leucotermes* (*L. tenuis* Hagen) is really a species of *Heterotermes*" (Engel, pers. comm.), which is congruent with Banks & Snyder's (1920) comment that the type specimen of *Leucotermes* is not congeneric with the

Reticulitermes. This was also reflected in the generic name change when Thompson and Snyder (1919) mentioned *Leucotermes* as an old name (Thompson & Snyder, 1919; Banks & Snyder, 1920). A petition case (no. 3412) was submitted in 2007 by Engel and Krishna (2007), supported by Roisin (2008) and approved by the ICZN (2009) to give *Reticulitermes* precedence over *Maresa* thus maintaining the availability and validity of the genus *Reticulitermes*.

Reticulitermes (Termes) antiquus (Germar) is the earliest known member of the Rhinotermitidae dated at about 40 MYA (Engel et al., 2009). Kollar (1837) described the first extant member of the genus from specimens found in Vienna, Austria, emerging from wooden crates and infested plants that arrived from the USA. *Reticulitermes flavipes* was later found to be endemic to the eastern United States (Banks & Snyder, 1920). Three additional species were subsequently described from the eastern USA, including *R. virginicus* Banks 1907, *R. hageni* Banks 1920 and *R. malletei* Clément et al. 1986. Earlier species descriptions were based on morphological characters of alates; however *R. malletei* was originally described using chemical and behavioral characters (Clément et al., 1986). Scheffrahn et al. (2001) proposed that *R. malletei* was a *nomen nudum* because Clèment et al. (1986) did not describe morphological features of the species. Austin et al. (2007), however, showed that all nomenclatural requirements for the designation had been satisfied and provided diagnostic 16S mitochondrial ribosomal gene sequence data and morphometric characters in addition to previously published chemical and behavioral characters supporting the species status of *R. malletei.* Table 2.1 summarizes the literature on *Reticulitermes* descriptions.

A new species of *Reticulitermes* was discovered on Sapelo Island, Georgia, USA, based on characterization of cuticular hydrocarbons of workers (Haverty et al., 1996; Haverty et al., 1999) and soldier defense secretions (Nelson et al., 2001). Corroboration of a taxonomic

separation was provided by genetic analysis (Jenkins et al., 2000; Sillam-Dussès & Forschler, 2010). Herewith we provide a formal description for this new taxon, *Reticulitermes nelsonae* Lim and Forschler, with diagnostic morphological characters and additional genetic corroboration. We also include a dichotomous key to soldiers and alates of *Reticulitermes* spp. found in Georgia, USA.

Materials and Methods

Specimens

Termite species, number of specimens, and soil province data are listed in Table 2.2. All specimens were preserved in 70-100% ethanol. Ninety-six soldiers, 141 alates and 20 soldier mandible pairs of the new species were examined for morphological data, while worker, soldier and alate specimens were used to provide DNA data.

Specimens from this study were deposited in the following collections: American Museum of Natural History (AMNH), New York, New York; National Museum of Natural History (NMNH), Smithsonian Institution, Washington, DC; and University of Georgia Collection of Arthropods (UGCA), Georgia Museum of Natural History, Athens, Georgia, USA.

Morphometrics and imaging

Soldier and alate specimens, were examined using a binocular dissecting microscope (CIT-OVAL2, Carl Zeiss aus Jena, Jena, Germany and Leica WILD M10, Wetzlar, Germany). Images were taken with a Sony DKC-5000 camera attached to a Leica WILD M10 stereomicroscope (Wetzlar, Germany) using Adobe Photoshop v. 8.0 (Adobe Systems, Delaware, USA). All soldier and alate images were taken at 25x and 20x magnification respectively, and calibrated with a micrometer using the internal preset calibration setting in AutoMontage Pro, v. 5.0.1 (Cambridge, United Kingdom). Morphometric measurements were

recorded using the AutoMontage Pro, v. 5.0.1 and exported to Microsoft Office Excel (Redmond, Washington, USA). All statistical analyses of mean, standard deviation and simulation of sample size were performed using SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA) (Lim, 2011).

Soldier

Images were taken of all prepared specimens. Soldier head capsules were removed from the body and mounted by placing a minuten pin into the occipital foramen. The opposite end of the minuten was pinned into a cube of foam mounted on a standard size # 2 insect pin. Soldier head capsule length (*sl*) was measured from the clypeal sulcus to the posterior edge near the occipital foramen, and width (*sw*) was measured at a 90° angle from the mid-point of *sl* (Fig. 2.1). Soldier mandible pairs were dissected from head capsules and mounted on two-sided tape positioned inside a 2mm x 2mm grid box printed on standard copier paper. Mandibles were positioned with the dorsal side up and parallel to the bottom line of the grid box to establish a 90° vertical line for curvature measurement. The soldier right mandible angle of curvature (*sma*) was measured from two different positions: the dorsal condyle (*sma1*) and external curvature inflexion point (*sma2*) (Fig. 2.2).

Alate

Whole alates were mounted between a glass slide and cover slip in 100% ethanol (Lim, 2011). Qualitative characters included body color (*abc*) and wing pigmentation (*awp*). Quantitative characters included body length (*abl*), body length including wings (*ablw*), average forewing length (*afw*) and average hind wing length (*ahw*) (Fig. 2.3).

Behavior

Fully developed, not-yet flown, winged alates of *R. nelsonae* were collected from infested wood on Sapelo Island.

Dichotomous Key

A dichotomous key for the *Reticulitermes* spp. of Georgia was constructed using morphological and behavioral (flight phenology) data. Morphological data for the four described *Reticulitermes* species were obtained as reported in the Morphometrics and Imaging section of this manuscript. The number of soldier and alate specimens examined to obtain the mean (±1 SD) values listed in Tables 2.3a-b ranged from 32 to 431 for each species, and details can be found in Lim (2011).

Molecular data

Sequence data from two mtDNA genes were employed to determine cohesive and coherent results for testing the robustness of the new species hypothesis. Genomic DNA was extracted from selected specimens using either Promega's Wizard Genomic DNA Purification Kit or Qiagen's DNeasy Extraction Kit, following a modified protocol (Sillam-Dussès & Forschler, 2010). Primers used for amplification of the mitochondrial entire length of the COII and, partial COI genes are listed in Table 2.4. Amplified PCR products were sequenced at Molecular Cloning Laboratories (MCLAB, California) or Eurofins MWG Operon (Huntsville, Alabama).

Sequences returned from the sequencing service provider were curated with Sequencher 4.5 (Gene Codes Corp., Ann Arbor, USA) and aligned with MUSCLE (MEGA 5, www.phylogeny.fr or http://phylogenomics.berkeley.edu/cgi-bin/muscle/input_muscle.py) using the default settings. Gaps were coded as missing. The sequence data were used to infer optimal

phylogenetic trees using the following tree estimation methods as implemented by the listed software package: Maximum Likelihood (PHYML), Distance Method (BIONJ), and Maximum Parsimony (MEGA 5), (Felsenstein, 1985; Nei & Kumar, 2000; Chevenet et al., 2006; Tamura et al., 2007; Dereeper et al., 2008; Kumar et al., 2008; Dereeper et al., 2010). ML analysis for COI and COII was performed with PHYML 3.0 on the www.phylogeny.fr web server using the GTR+G+I model. Phylogenetic trees inferred from COI and COII sequences were obtained using the distance method BIONJ performed on www.phylogeny.fr web server, utilizing the substitution model Kimura 2- parameter and bootstrap = 1000. The MP analysis performed for COI and COII used MEGA 5 with Close-Neighbor-Interchange (CNI) search with 1000 bootstrap replicates. Graphical representations of the resulting trees were improved using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Table 2.5 outlines the haplotype name, species, geographical region and GenBank accession no. for the 102 COII sequences analyzed and Table 2.6 for the 49 COI sequences. Primary molecular voucher specimens were deposited at the University of Georgia Collection of Arthropods (UGCA), Georgia Museum of Natural History, Athens, Georgia, USA. DNA extraction vouchers were deposited in HSERP Laboratory, University of Georgia, Athens, Georgia, USA.

Results

Morphological characters

Reticulitermes nelsonae had the smallest range of measurements for both alate and soldier samples in all morphological characters measured, except for: soldier head capsule ratio (*sl*:*sw*), and soldier right mandible angle of curvature (*sma1* and *sma2*) (Table 2.7a). The range

of *sl*:*sw* is similar to *R. virginicus*, and soldier right mandible angle of curvature (*sma1* and *sma2*) is similar to *R. flavipes* (Table 2.7a).

The range of soldier head capsule measurements for *R. nelsonae* was 1.14-1.72 mm for *sl* and 0.70-0.99 mm for *sw* (Table 2.7a). The ratio of the length to width for soldier head capsules ranged from 1.52 – 1.98 (Table 2.7a). The range for the soldier right mandible angle of curvature was 7.2-14.6° for *sma1* and 24.1-34.0° for *sma2* (Table 2.7a). Alate body length without wing (*abl*) ranged from 3.26-4.63 mm, and alate body length including wing (*ablw*) ranged from 6.53-7.88 mm (Table 2.7b). The length of fore wings (*afw*) was slightly longer than the hind wings (*ahw*), ranging from 4.94-5.98 mm, and 4.81-6.21 mm respectively (Table 2.7b).

Flight

Alate samples were collected from Sapelo Island on 6 February and 6 March 2007. It must be noted that these alates were collected directly from sampling devices prior to flight. We predict that *R. nelsonae* flights would occur in the same months because the alates were fully sclerotized and winged.

Dichotomous Key

Keys to the soldiers and alates of the *Reticulitermes* spp. of Georgia, USA were constructed (Keys 2.1-2.2, respectively). The values used for the dichotomous key are shown in Tables 2.3a-b. The values in the dichotomous key are given in the following format (mean ± 1 std. dev.). The minimum number of soldier specimens recommended to obtain a 95% confidence in correct species separation is 9 to identify *R. flavipes*, *R. virginicus*, and *R. nelsonae*, and 29 specimens for separating *R. malletei* from *R. hageni*. The minimum number of alate specimens recommended to obtain a 95% confidence in correct species separation when using alate

characters is 6. A more detailed discussion of the statistics and minimum specimen numbers can be found in Lim (2011).

Molecular Data

Similar clades and clusters were observed for all phylogenies obtained with the COII and COI sequence data as shown in Figures 2.4-2.6. The alignment of 102 sequences analyzed for COII included 665bp of the 685bp full length and for the *Reticulitermes* dataset, 216 variable sites were observed, of which 178 were parsimony-informative. There are five reference sequences (signified by α in Table 2.5) obtained from specimens that matched the described morphological criteria for the respective species.

Maximum likelihood (ML), distance method (BIONJ) and Maximum parsimony (MP) were used to construct phylogenetic trees for all the *Reticulitermes* taxa available in GenBank (as of 8 March 2011). The inferred phylogenetic trees were well resolved, showing consistent and strongly corroborated topologies across all three methods (Figs. 2.4-2.6). The inferred ML tree shows high branch support for most clades with Ln likelihood = -5075.935. The MP analysis resulted in 42 most parsimonious trees of 854 steps with a consistency index = 0.330, a retention index = 0.819, a composite index for all sites = 0.300, and a composite index for parsimony-informative sites = 0.270 that generated a bootstrap using the 50% majority-rule to generate the consensus tree (Fig. 2.6).

The ML phylogeny for COII provided three clusters. Clusters 1 and 2a-b contain *Reticulitermes* spp. found in the United States while Clusters 3a-b refer to *Reticulitermes* spp. found outside the United States (Table 2.8, Figs. 2.4-2.6). Cluster 1 consists of *Reticulitermes* spp. found in the southeastern USA and Clusters 2a-b *Reticulitermes* spp. found in the western region of the USA (Table 2.8, Figs. 2.4-2.6). The ML phylogeny contained groupings in Clades

1, 5, 9, and 12 that had mixed species designations (denoted by the dotted bars in Fig. 2.4). Clade 1: The *R. flavipes* clade, includes *R. santonensis* which has been synonymized as *R. flavipes*, and *R. arenincola* Goellner 1931 (AY168209, AY453589) (Goellner, 1931; Ye et al., 2004). Clade 5: The *R. nelsonae* clade has four GenBank sequences that were designated as *R. hageni* (NC009501, AY808088, AY808089, and AF525328). Clade 9 has a mixture of different species designations from Asia (Fig. 2.4). Clade 12: The *R. hesperus* clade, has an apparently undescribed species referred to as "*R. okanaganensis"* by Szalanski et al. (2006) (Banks & Snyder, 1920; Szalanski et al., 2006; Tripodi et al., 2006; Austin et al., 2008). The respective clades and clusters for each of the phylogenetic analyses are summarized in Table 2.8.

The alignment of 49 sequences analyzed for COI included 767bp of the 801bp partial length, the results showed 146 variable sites for the *Reticulitermes* dataset of which 113 were parsimony-informative. Detailed information for the COI sequences are in Table 2.6. Inferred ML tree shows high branch support for most clades with Ln likelihood = -2905.620 (Fig. 2.7). MP analysis was performed with MEGA5 resulting in 33 most parsimonious trees of 366 steps with a consistency index = 0.556, a retention index = (0.892), a composite index for all = 0.565, and a composite index for parsimony-informative sites = 0.496 that generated a bootstrap using the 50% majority-rule for the consensus tree which displayed similar tree topologies for the ML and BIONJ analyses (Figs. 2.7-2.9).

The COI analyses provided three clusters. Cluster 1 and 3 contain *Reticulitermes* spp. found in the United State while cluster 2 has *Reticulitermes* spp. found outside the United States (Table 2.9, Figs. 2.7-2.9). No COI sequences were found in the GenBank for *Reticulitermes* from the western USA. The ML phylogeny for the COI sequence (Fig. 2.7) has dotted bars referring to groupings with mixed species designations (Fig. 2.7: Clade 1, 4). Clade 1: The *R. flavipes* clade,

has *R. santonensis* which was synonymized with *R. flavipes*. Clade 4: The *R. nelsonae* clade, has one GenBank sequence that was designated as *R. hageni* (EF206320) (Figs. 2.7-2.9).

Systematics

Reticulitermes nelsonae Lim and Forschler, new species

Figures 2.10-2.12

The morphological diagnoses contain small differences between species therefore measurement must be made with attention to detail and accuracy according to the specific measurement points shown in Figures 2.1-2.3. Accurate species attributions are only to be determined with certainty by careful study of the characters described and preferably using both castes supported by genetic or behavioral information.

Diagnosis

Reticulitermes nelsonae (Figs. 2.10-2.12) can be distinguished from previously described, *R. flavipes, R. virginicus, R. hageni, R. malletei,* using a combination of morphometric measurements of alate and soldier specimens, genetic data and a behavioral character. *Reticulitermes nelsonae* is the smallest of the described *Reticulitermes* found in the southeastern United States whose adult stage flies in February-March.

SOLDIER (Fig. 2.10, Table 2.7a): The *sl* of *R. nelsonae* is typically more than 0.2 mm shorter than *R. flavipes* and *R. virginicus* (Table 2.7a). The *R. nelsonae sw* is typically more than 0.1 mm smaller than *R. flavipes* and *R. virginicus* (Table 2.7a). The *sl* and *sw* are typically more than 0.1 mm shorter than *R. malletei* (Table 2.7a). The *R. nelsonae sma2* is typically more than 24° (typically 24°-30°), while *R. hageni sma2* is typically less than 25° (typically 22°-25°) (Table 2.7a).

ALATE (Figs. 2.10-2.12, Table 2.7b): *R. nelsonae* has a light brown body color with 14 antennal segments (Figs. 2.10-2.12). Body color differs from that of *R. hageni's* yellowish to yellowish-brown body color (Fig. 2.12). *R. nelsonae* is expected to swarm from February to March, while *R. hageni* swarms from August to October. *R. flavipes* flights have been recorded from November through April thus overlapping with *R. nelsonae* (Lim, 2011). The *abl* and *ablw* measurement for *R. nelsonae* are typically shorter and do not overlap *R. flavipes* (Table 2.7b). In *R. nelsonae,* the *abl* and *ablw* are typically 3.7mm - 4.2mm and 6.8mm - 7.4mm, whereas in *R. flavipes* those same characters are typically more than 4.4mm and 8.6mm, respectively (Table 2.7b). The *R. nelsonae afw* and *ahw* are typically 1.0mm shorter than *R. flavipes* (Table 2.7b). *R. nelsonae* wings are non-pigmented, while *R. malletei* has pigmented wings (Clément et al., 1986; Austin et al., 2007). *Reticulitermes nelsonae* have *afw* measurements typically 0.4 mm shorter than *R. malletei*. *Reticulitermes nelsonae* and *R. virginicus* share similar morphometric ranges, but differ in flight phenology. *Reticulitermes nelsonae* is postulated to swarm earlier than the May-June dates recorded for *R. virginicus* (Banks & Snyder, 1920; Weesner, 1965; Krishna & Weesner, 1970). The *R. nelsonae* mean ±1 ratio of full body length including wings to forewing (*ablw*:*afw*) is typically 1.27-1.31, while for *R. virginicus* it is typically 1.32-1.37 (Table 2.3b). Genetically, the samples collected for the new species consistently form a clade, separate from the other described species using both COI and COII sequences (Figs. 2.4-2.9, Tables 2.8-2.9).

Distribution

The species is found in the southeastern region of the United States, in the Atlantic Coastal Flatwoods and South Coastal Plain soil provinces (Important to note that the species has not been genotyped in the Piedmont soil province although sampling has been carried out in that area). It was originally collected from Sapelo Island, GA, and has since been collected from the

Croatan National Forest near Havelock, NC, Greenwood Plantation near Thomasville, GA and Branford, FL.

Types

Holotype will be deposited at Smithsonian Institution, National Museum of Natural History (NMNH), Washington, DC). Paratypes will be deposited at, Museum of Natural History, Athens, Georgia, American Museum of Natural History (NMNH), New York, New York.

Description

There is little sexual dimorphism for this group of termites, males and females can however, be differentiated by the form of the 8th sternal plate (Zimet & Stuart, 1982).

SOLDIER: Head capsule rectangular in shape, typically longer than wide (Fig. 2.10). Head capsule color yellowish with dark brown to black mandibles (Fig. 2.10). Body color light yellowish to white. Mean head capsule length (*sl*) 1.41 mm ± 0.13 (Table 2.7a), mean width (*sw*) 0.78 mm ± 0.05 (Table 2.7a), mean ratio length-to-width (*sl:sw*) 1.793 ± 0.09 (Table 2.7a). Mean mandible curvature angles: *sma1*= 10.7° ± 2.21, *sma2*= 27.27° ± 2.65 (Table 2.7a).

ALATE: Body color light brown (Figs. 2.10, 2.12). Wings non-pigmented or clear (Figs. 2.10, 2.12). Legs light to dark brownish (Figs. 2.10, 2.12). Mean total body length without wing (*abl*) 3.93 mm ± 0.24 (Table 2.7b). Mean total body length with wings (*ablw*) 7.08 mm ± 0.29 (Table 2.7b). Mean forewing length (*afw*) 5.43 mm ± 0.21. Mean hindwing length (*ahw*) 5.32 mm ± 0.30 (Table 2.7b).

Genetics

Figures 2.4-2.9 show phylogenetic trees obtained from ML, BIONJ and MP analyses for *Reticulitermes* cytochrome oxidase II (COII) and cytochrome oxidase I (COI) sequences that were available from GenBank (as of Mar 8 2011). Haplotypes of *R. nelsonae* formed a clade

with the other *Reticulitermes* spp. from the southeastern USA as shown in Cluster 1 (Tables 2.8-2.9, Figs. 2.4-2.9). *Reticulitermes nelsonae* haplotypes were genetically isolated from *Reticulitermes* from other parts of the world (Tables 2.8-2.9, Figs. 2.4-2.9). Although there are four *R. hageni* GenBank sequences within the *R. nelsonae* clade, there was no mention of morphological confirmation for those three sequences in the GenBank database. Thus, we believe they might actually represent *R. nelsonae* haplotypes because the available taxonomic keys would have identified any *R. nelsonae* soldier as *R. hageni* (Weesner, 1965; Nutting, 1990; Scheffrahn & Su, 1994).

Cuticular hydrocarbon

Examining the work from Haverty et al. (1999) and Jenkins et al. (2000), it can be deduced that *R. nelsonae* corresponds with GA-L and GA-I hydrocarbon phenotypes.

Etymology

This patronym was established to honor Lori J. Nelson (USDA Forest Service, Buchanan, CA) who realized, 15 years ago, that specimens collected on Sapelo Island, a barrier island off the Atlantic coast of Georgia, were notably different from all previously described *Reticulitermes* species based on analysis of cuticular hydrocarbons research (Haverty et al., 1996; Haverty et al., 1999; Jenkins et al., 2000).

Discussion

Morphology

Morphological separation of species within the genus *Reticulitermes* is not straightforward and similar hurdles were encountered in this study. Our morphometric measurements provided a range of values that showed interspecific overlap consistent with past reports for the genus (Weesner, 1965; Nutting, 1990; Hostettler et al., 1995; Haverty et al., 1996;

Nelson et al., 2008; Wang et al., 2009). Dichotomous keys for soldiers and alates of *Reticulitermes* spp. collected in Georgia (Keys 2.1-2.2) were prepared to distinguish the five species endemic to the southeastern USA. The morphological characters used in the species keys require standardized technical preparation to obtain accurate measurements for species determination. The measurements used to build the key in this study can separate *R. nelsonae* soldiers from all previously described species with the exception of *R. hageni* which has a range overlapping all measures at the upper range for *R. nelsonae* (Table 2.3a, Key 2.1). Alates can be separated based on the combination of body color, morphometric measurements, and flight times (Table 2.3b, Key 2.2).

The authors have prepared a more extensive study of morphological variation in *Reticulitermes* spp. (Lim, 2011) that compares the range of measurements recorded across the literature for specimens collected from the southeastern US. We echo past recommendations that species discrimination based on morphology should include data from both castes for accurate identification (Weesner, 1965; Nutting, 1990). The number of specimens required is 6-29 to obtain a 95% confidence when using only morphological characters (Lim, 2011). We recommend that each state or geographic region be examined in detail to provide a more accurate determination of the morphological and haplotype diversity in *Reticulitermes*.

Genetics

Molecular phylogenies are an estimation of plausible species relationships and therefore detailed research and comparison is warranted to accurately identify species designations for gene sequence data. We provide reference sequences for COI and COI genes that were corroborated with morphological descriptions for *Reticulitermes* spp. from the southeastern United States (denoted by α in Tables 2.5-2.6 and Figs. 2.4-2.9). Cytochrome oxidase II (COII),

with a length of 685bp, has been a valuable marker for identification of *Reticulitermes* spp. (Jenkins et al., 2000; Copren et al., 2005; Cameron & Whiting, 2007; Sillam-Dussès & Forschler, 2010). Phylogenies inferred from ML, BIONJ, MP analyses of COII resulted in clusters congruent for *Reticulitermes* species found in the USA - Clusters 1 and 2a-b (Figs. 2.4-2.6). The evidence suggests that the haplotype range for *Reticulitermes* within the US is unique from those found outside of the country (Figs. 2.4-2.6). Haplotype diversity for *Reticulitermes* in the southeastern USA also is distinct from the western region, as Cluster 1 is separate from Clusters 2a-b (Figs. 2.4-2.6). A number of conflicting species designations seen in the COII phylogenies agrees with the suggestion that the taxonomy of the *Reticulitermes* needs additional attention (Figs. 2.4-2.6) (Nelson et al., 2008). Species designations within Clade 1, add weight to those that question the validity of the *R. arenincola* Goellner 1931 species designation, or at least call for proper identification of sequence data attributed to that species (Figs. 2.4-2.6) (Goellner, 1931; Austin et al., 2004; Ye et al., 2004; Austin et al., 2005; Austin et al., 2007). Clade 5 (Figs. 2.4-2.6) contains sequences (NC009501, AY808088, AY808089, AF525328) attributed to *R. hageni* but this can in part be explained because *R. nelsonae* soldiers would have been identified, using the available keys, as *R. hageni* (Scheffrahn & Su, 1994; Austin et al., 2002; Su et al., 2006; Cameron & Whiting, 2007). The *R. hesperus* clade (Clade 12-ML,7-DM,6-MP) includes the undescribed species referred to as "*R. okanaganensis*" by Szalanski et al. (2006) based on mitochondrial ribosomal 16S rRNA (Figs. 2.4-2.6, Table 2.8) (Szalanski et al., 2006; Tripodi et al., 2006). "*R. okanaganensis"* technically does not exist as it has not been formally described (Szalanski, pers. comm.).

The phylogenies obtained using 767bp sequence from the COI gene; also indicate haplotypes of *Reticulitermes* spp. from the southeastern USA are different from non-USA

Reticulitermes (Table 2.9, Figs. 2.7-2.9). Clusters 1 and 3 in Figures 2.7-2.9 represent the southeastern USA haplotypes, while Cluster 2 has haplotypes from Japan and Europe (Table 2.9). There are also mixed species designations within Clade 1 including *R. flavipes* with its synonym *R. santonensis*. Clade 4, *R. hageni* (EF 206320) with *R. nelsonae* forms a "basal", genetic species group (Baker & Bradley, 2006) with a distinct and traceable pattern of descent or phylogeny (Table 2.9, Figs. 2.7-2.9) (Cracraft, 1989). The latter mixed group is not surprising because the available keys would have identified soldier specimens as *R. hageni* (Weesner, 1965; Nutting, 1990; Scheffrahn & Su, 1994; Cameron & Whiting, 2007).

Cuticular hydrocarbon

Jenkins et al. (2000) observed that two collection groups were "different morphologically, chemically and genetically". We now believe these samples identified as haplotypes BH25 and HH11 to be *R. nelsonae* because these two sequences were recovered in the *R. nelsonae* clade. We, therefore, reason that the cuticular hydrocarbon phenotypes GA-L and GA-I reported by Haverty et al. (1996, 1999) belongs to *R. nelsonae*. Additional correlation between cuticular hydrocarbon phenotypes, genetics, and morphology should be explored for identifying *Reticulitermes* spp.

Conclusions

A prominent conclusion from this study is that *Reticulitermes* species discrimination should be attempted using morphometric characters from both castes accompanied by genetic and or other chemical evidence (Forschler & Jenkins, 1999; Lee, 2004; Cognato, 2006; Nelson et al., 2008). Based on the data obtained in this study, *R. nelsonae* is a true entity that satisfies the criteria of three species concepts: the morphological (Winston,1999), phylogenetic (Cracraft, 1989; Winston, 1999) and genetic species concept (Baker & Bradley 2006). We also present data

supporting that *R. nelsonae* meets the criteria of the ecological and reproductive isolation species concepts (Winston, 1999; Wilkins, 2009). Further examination will determine if the distribution of *R. nelsonae* is restricted, as described to the Atlantic Coastal Flatwoods and South Coastal Plain soil provinces across the southeastern United States.

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Table 2.1. Summary of taxonomic literature that use morphology and life histories to describe *Reticulitermes* spp. found in the southeastern United States, parenthetical letters are defined as follows: a = alate, s = soldier, species abbreviations are defined as follows: Rf=*R. flavipes*, Rv= *R. virginicus*, Rh=*R. hageni*, Rm=*R. malletei*, Ra=*R. arenincola*, Rhp=*R. hesperus*, Rt=*R. tibialis*.

Reference citation	Caste, notes	Pages	Species from USA
Banks, N. & Snyder, T.E. (1920)	(a, s) descriptions, illustrations, key, flight times	42-47, 148-164	Rf, Rv, Rh, Rhp, Rt
Goellner, E. J. (1931)	(a, s) descriptions, illustrations, ecology	227-234	Rf, Rt, Ra
Kofoid, C.A. (1934)	(a), descriptions, flight times	193-194	Rf, Rv, Rh, RaRhp, Rt
Miller, E.M. & Miller, D.B. (1943)	(a, s) descriptions, illustrations, flight times	101-107	Rf, Rv, Rh
Banks, F.A. (1946)	(a, s) descriptions, illustrations, key, flight times	1-29	Rf, Rv, Ra, Rt
Miller E.M. (1949)	(a, s) descriptions, illustrations, key, flight times	6-7, 14-15, 20-22, 26	Rf, Rv, Rh
Snyder, T.E. (1954)	(s, a) descriptions, illustrations, key, flight times	26, 51-56	Rf, Rv, Rh, Ra
Miller E. M. (1964)	(a) flight times	5, 16	Rf, Rv, Rh
Weesner, F.M. (1965)	(a) descriptions, illustrations, key, flight times	36-44, 51	Rf, Rv, Rh, Ra, Rhp, Rt
Clément, J.L., Howard, R., Blum, M. & Lloyd, H. (1986)	(a, s) descriptions, life history	67-70	Rf, Rv, Rh, Rm
Nutting, W.L. (1990)	(a, s) descriptions, illustrations, key, flight times	997-1030	Rf, Rv, Rh, Ra, Rhp, Rt
Scheffrahn RH, Su N-Y. 1994	(s, a) descriptions, illustrations, key, flight times	465-473	Rf, Rv, Rh
Hostettler, N.C., Hall, D.W. & Scheffrahn, R.H. (1995)	(s) descriptions, photographs	119-129	Rf, Rv, Rh
Ye, W., Lee, C.-Y., Scheffrahn, R.H., Aleong, J.M., Su, N.-Y., Bennett, G.W., Scharf, M.E., 2004	(s) descriptions, photographs	815-822	Rf, Rv, Rh, Ra
Brown, K., Kard, B. & Payton, M. (2005)	(s, a) descriptions, photograph	277-284	Rf, Rv, Rh
Austin, J.W., Bagnères, A.-G., Szalanski, A.L., Scheffrahn, R.H., Heintschel, B.P., Messenger, M.T., Clement, J.-L. & Gold, R.E. (2007)	(a, s) descriptions, illustrations, photographs, flight times	1-26	Rf, Rv, Rh, Rm, Rhp, Rt

Wang, C., Zhou, X., Li, S., Schwinghammer, M., Scharf, M., Buczkowski, G. & Bennett, G. (2009)	(a, s) descriptions, key, photographs	1029-1036	Rf, Rv, Rh, Ra, Rt

Table 2.2. Collection information for specimens by caste, species, soil province and number (N) used for the morphometric measurements reported in this study.

Caste	Species	Soil province	N
Soldier	*R. flavipes*	Piedmont	23
		Atlantic Coastal Flatwoods	34
		South Coastal Plain	69
	R. virginicus	Piedmont	26
		Atlantic Coastal Flatwoods	74
		South Coastal Plain	6
	R. hageni	Piedmont	77
	R. malletei	Piedmont	105
	R. nelsonae	Atlantic Coastal Flatwoods	58
		South Coastal Plain	38
Alate	*R. flavipes*	Piedmont	479
		Atlantic Coastal Flatwoods	245
	R. virginicus	Piedmont	30
		Atlantic Coastal Flatwoods	35
	R. hageni	Piedmont	96
	R. malletei	Piedmont	109
	R. nelsonae	Atlantic Coastal Flatwoods	141

Table 2.3a. Ranges of means ± 1 std. dev. for soldier characters by species for head capsule and right mandible angle of curvature used to build the dichotomous key.

Species/ Soldier Characters	Head capsule			Right mandible curvature	
	sl (mm)	*sw* (mm)	*sl*:*sw*	*sma1* (°)	*sma2* (°)
R. flavipes	1.57-1.81	0.97-1.12	1.54-1.71	8.0-13.0	25.0-30.0
R. virginicus	1.56-1.69	0.88-0.96	1.70-1.83	12.0-15.0	31.0-35.0
R. hageni	1.34-1.53	0.82-0.90	1.58-1.73	7.0-10.0	22.0-25.0
R. malletei	1.43-1.55	0.85-0.91	1.63-1.76	9.0-12.0	24.0-28.0
R. nelsonae	1.28-1.42	0.73-0.84	1.70-1.89	8.0-13.0	25.0-30.0

Table 2.3b. Ranges of means ± 1 std. dev. for alate characters by species for body, wing and ratio used to build the dichotomous key.

Species/ Alate Characters	Body		Wing		Ratio
	abl (mm)	*ablw* (mm)	*afw* (mm)	*ahw* (mm)	*ablw*:*afw*
R. flavipes	4.40-5.17	8.57-9.38	6.48-7.14	6.22-6.88	1.29-1.35
R. virginicus	3.81-4.24	7.20-7.63	5.34-5.73	5.23-5.61	1.32-1.37
R. hageni	3.76-4.41	7.49-8.13	5.70-6.23	5.48-6.00	1.28-1.34
R. malletei	3.72-4.33	7.84-8.63	6.04-6.70	5.76-6.44	1.27-1.32
R. nelsonae	3.69-4.16	6.79-7.37	5.22-5.64	5.02-5.61	1.27-1.31

Table 2.4. Primer sequences used for amplification and gene sequencing.

Gene of interest	Primer pair (forward and reverse)	Sequences (5'⟶ 3')
COII	TL2-J-3037	ATG GCA GAT TAG TGC AAT GG
	TK-N-3785	GTT TAA GAG ACC AGT ACT TG
COI	C1J2195	TTG ATT CTT TTG GTC ACT CCA TGA AGT
(partial sequence)	TL2N3014	TCC TAA TTG CAC TTA ATC TGC CAT ATT

Table 2.5. Sequence identification, species name, soil province, and GenBank accession number of ribosomal mitochondrial DNA sequences (COII) used for of the phylogenetic trees reported in this study.

No	Sequence names	Species	Geographical region[σ]	GenBank acc no. (COII gene)
1	RF_TJ[α]	*R. flavipes*	USA: P	JN207492
2	RF_B1 H04	*R. flavipes*	USA: P	JF796216
3	RF_GP002B	*R. flavipes*	USA: ACF	JF796217
4	RF_SI018 E05	*R. flavipes*	USA: SCP	JF796218
5	B15A	*R. flavipes*	USA: SCP	JF796219
6	RF_BIII001	*R. flavipes*	USA: SCP	JF796220
7	EU689003 *R. flavipes* hap Q	*R. flavipes*	USA: ACF	EU689003
8	EU689005 *R. flavipes* hap I	*R. flavipes*	USA: ACF	EU689005
9	EU689006 *R. flavipes* hap T	*R. flavipes*	USA: ACF	EU689006
10	EU689004 *R. flavipes* hap K	*R. flavipes*	USA: ACF	EU689004
11	EU689009 *R. flavipes* hap P	*R. flavipes*	USA: ACF	EU689009
12	EU689008 *R. flavipes* hap H	*R. flavipes*	USA: ACF	EU689008
13	EU689007 *R. flavipes* hap V	*R. flavipes*	USA: ACF	EU689007
14	RV_CHW1 C02	*R. virginicus*	USA: P	JF796221
15	RV_SI009	*R. virginicus*	USA: ACF	JF796222
16	RV_GP003D	*R. virginicus*	USA: SCP	JF796223
17	RV_LA05184a	*R. virginicus*	USA: SCP	JF796234
18	EU689027 *R. virginicus* RV1[α]	*R. virginicus*	USA: ACF	EU689027
19	RH_C10	*R. hageni*	USA: P	JF796224
20	RH_C13	*R. hageni*	USA: P	JF796225
21	EU689026 *R. hageni* hap RH1[α]	*R. hageni*	USA: P	EU689026
22	AF107486 *R. hageni*	*R. hageni*	USA: SCP	AF107486
23	AY027478 *R. hageni* USA3	*R. hageni*	USA:	AY027478
24	AY168208 *R. hageni*	*R. hageni*	USA: AR	AY536416
25	AY536416 *R. hageni* isolateRH	*R. hageni*	USA: AR	AY536416
26	DQ493729 *R. hageni* Rha1.2	*R. hageni*		DQ493729

27	GU550074 *R. malletei* hap1 C1[β]	*R. malletei*	USA: P	GU550074
28	RM_C4[β]	*R. malletei*	USA: P	JF796226
29	RM_AM015A	*R. malletei*	USA: P	JF796227
30	RM 1211as1505	*R. malletei*	USA: ACF	JF796228
31	FJ606690 *R. malletei* isolate DF.A	*R. malletei*		FJ606690
32	EU689013_RN_ hap A [γ]	*R. nelsonae*	USA: ACF	EU689013
33	RN_LA0618a	*R. nelsonae*	USA: SCP	JF796232
34	RN_LA061359a	*R. nelsonae*	USA: SCP	JF796233
35	RN_1011a1454	*R. nelsonae*	USA: ACF	JF796229
36	RN_GP002D	*R. nelsonae*	USA: SCP	JF796230
37	RN_B10A	*R. nelsonae*	USA: SCP	JF796231
38	BH25_TJ [δ]	*R. nelsonae*	USA: ACF	JF796235
39	HH11_TJ [δ]	*R. nelsonae*	USA: SCP	JF796236
40	NC009501_*R. hageni*	*R. nelsonae*	USA: FL	NC009501
41	AY808088_*R. hageni* voucher FL1888	*R. nelsonae*	USA: FL	AY808088
42	AY808089_*R. hageni* voucher FL1934	*R. nelsonae*	USA: FL	AY808089
43	AF525328_*R. hageni* USA	*R. nelsonae*	USA: GA	AF525328
44	AY168209_*R. arenincola*	*R. flavipes*	USA: IN	AY168209
45	AY453589_*R. arenincola*	*R. flavipes*	USA: IN	AY453589
46	DQ493730_*R. hesperus* isolate Rhe1.2	*R. hesperus*		DQ493730
47	GQ922442_*R. hesperus* isolate CHI0413066AR	*R. hesperus*	USA: CA	GQ922442
48	AY623446_*R. hesperus* isolate SCA5	*R. hesperus*	USA: CA	AY623446
49	AY808090_*R. hesperus* voucher US469	*R. hesperus*	USA	AY808090
50	AF525329_*R. okanaganensis* USA	*R. hesperus*	USA: CA	AF525329
51	DQ493741_*R. tibialis* isolate Rt1.1	*R. tibialis*		DQ493741
52	AY168207_*R. tibialis*	*R. tibialis*	USA: IN	AY168207
53	AY168206_*R. tibialis*	*R. tibialis*	USA: IN	AY168206
54	AF525355_*R. tibialis* USA	*R. tibialis*	USA: AZ	AF525355
55	AY808094_*R. tibialis* voucher US5	*R. tibialis*		AY808094
56	AF525320_*R. clypeatus*	*R. clypeatus*	Israel	AF525320
57	AB109534_*R. okinawanus* m123	*R. okinawanus*		AB109534
58	DQ493735_*R. okinawanus* isolate RONAGOKIO3CO2	*R. okinawanus*		DQ493735

59	DQ493733_*R. okinawanus* isolate Ro1.3	*R. okinawanus*		DQ493733
60	AB109535_*R. yaeyamanus* m125	*R. yaeyamanus*		AB109535
61	EU627782_*R. flaviceps*	*R. flaviceps*		EU627782
62	AB109532_*R. flaviceps*	*R. flaviceps*		AB109532
63	AB050708_*R. flaviceps*	*R. flaviceps*	Japan	AB050708
64	DQ493724_*R. flaviceps*	*R. flaviceps*		DQ493724
65	EF428207_*R. amamianus*	*R. amamianus*		EF428207
66	DQ493721_*R. amamianus*	*R. amamianus*		DQ493721
67	AB193241_*R. amamianus*	*R. amamianus*	Japan	AB193241
68	EF428208_*R. amamianus*	*R. amamianus*		EF428208
69	AB109531_*R. khaoyaiensis* t1710	*R. khaoyaiensis*		AB109531
70	EF016103_*R. kanmonensis* isolate Rk3.1	*R. kanmonensis*		EF016103
71	DQ493731_*R. kanmonensis* isolate Rk1.2	*R. kanmonensis*		DQ493731
72	FJ423460_*R. labralis*	*R. labralis*		FJ423460
73	AB050711_*R. labralis*	*R. labralis*	China	AB050711
74	AB050710_*R. perilabralis*	*R. perilabralis*	China	AB050710
75	AB050709_*R. guangzhouensis*	*R. guangzhouensis*	China	AB050709
76	AB109533_*R. miyatakei* m118	*R. miyatakei*		AB109533
77	EF016105_*R. miyatakei* isolate Rm2	*R. miyatakei*		EF016105
78	DQ493732_*R. miyatakei* isolate R.m1.1	*R. miyatakei*		DQ493732
79	EF016102_*R. aeyamanus* isolate Ry2	*R. aeyamanus*		EF016102
80	EF016099_*R. leptomandibularis* isolate R.1	*R. leptomandibularis*		EF016099
81	AB193237_*R. speratus* RsOg	*R. speratus*	Japan	AB193237
82	AB005584_*R. speratus*	*R. speratus*		AB005584
83	EF016101_*R. speratus* isolate Rs5.1	*R. speratus*		EF016101
84	AB109530_*R. speratus*	*R. speratus*		AB109530
85	AB050704_*R. ampliceps*	*R. ampliceps*	China	AB050704
86	HQ012033_*R. chinensis*	*R. chinensis*		HQ012033
87	AB050705_*R. chinensis*	*R. chinensis*	China	AB050705
88	FJ423455_*R. chinensis*	*R. chinensis*		FJ423455
89	FJ423454_*R. chinensis*	*R. chinensis*		FJ423454
90	AF525350_*R. urbis* France	*R. urbis*	France	AF525350

91	DQ487823_*R. urbis* hap c2	*R. urbis*	Croatia	DQ487823
92	AF525327_*R. grassei* France	*R. grassei*	France	AF525327
93	DQ442236_*R. grassei*	*R. grassei*		DQ442236
94	AY510582_*R. grassei* ARB	*R. grassei*	Spain	AY510582
95	AF291731_*R. grassei* Italy	*R. grassei*	Italy	AF291731
96	AF525331_*R. lucifugus corsicus*	*R. lucifugus corsicus*	France	AF525331
97	AY267858_*R. lucifugus corsicus*	*R. lucifugus corsicus*		AY267858
98	EF591535_*R. lucifugus corsicus*	*R. lucifugus corsicus*	France	EF591535
99	AF525343_*R. santonensis* France	*R. santonensis*	France	AF525343
100	AF291743_*R. santonensis* Italy	*R. santonensis*	Italy	AF291743
101	*Heterotermes* V1	*Heterotermes* sp.		
102	CoptoF_Jenkins 2004	*Coptotermes formosanus*		

[α] denotes a reference sequence was identified to the respective species using morphology of both soldier and alate specimens.
[β] denotes reference sequence for *R. malletei* that was correlated with the 16S rRNA gene for the original haplotype description in addition to morphology of soldier and alates (Austin et al., 2007).
[σ] P = Piedmont, ACF = Atlantic Coastal Flatwoods, SCP = South Coastal Plain geographical regions. Blanks in geographical region column indicate GenBank entries that did not have clear information on geographic origin.
[δ] these two sequences obtained from the primary author of Jenkins et al. (2000).

Table 2.6. Sequence identification, species name, soil province, and GenBank accession number of ribosomal mitochondrial DNA sequences (COI) used for the phylogenetic trees reported in this study.

No	Sequence names	Species	Geographical region[σ]	GenBank acc no. (COI gene)
1	A2 [α]	*R. flavipes*	USA: ACF	JN207470
2	A3	*R. flavipes*	USA: ACF	JN207471
3	A16	*R. flavipes*	USA: ACF	JN207472
4	A35	*R. flavipes*	USA: ACF	JN207473
5	A36	*R. flavipes*	USA: P	JN207474
6	A43	*R. flavipes*	USA: P	JN207475
7	A45	*R. flavipes*	USA: P	JN207476
8	A48	*R. flavipes*	USA: P	JN207477
9	AY027469_*R. flavipes*_strain_USA1	*R. flavipes*		AY027469
10	EF206314_*R. flavipes*_IS13	*R. flavipes*	USA: LA	EF206314
11	EF206316_*R. flavipes*_IS57	*R. flavipes*	USA: P	EF206316
12	EF206317_*R. flavipes*_IS58	*R. flavipes*	USA: P	EF206317
13	AY027465_*R. santonensis*_strain_F5	*R. santonensis*	France	AY027465
14	AY027466_*R. santonensis*_strain_F2	*R. santonensis*	France	AY027466
15	AY027467_*R. santonensis*_F1	*R. santonensis*	France	AY027467
16	AY027468_*R. santonensis*_F3	*R. santonensis*	France	AY027468
17	AY553156_*R. santonensis*	*R. santonensis*		AY553156
18	FJ802751_*R. santonensis*_isolate_IS054	*R. santonensis*		FJ802751
19	A20 [α]	*R. virginicus*	USA: ACF	JN207478
20	A21	*R. virginicus*	USA: ACF	JN207479
21	A34	*R. virginicus*	USA: P	JN207480
22	A38	*R. virginicus*	USA: P	JN207481
23	A39	*R. virginicus*	USA: P	JN207482
24	AY027471_*R. virginicus*	*R. virginicus*	USA: GA	AY027471
25	EF206318_*R. virginicus*_IS59	*R. virginicus*	USA: GA	EF206318
26	EF206319_*R. virginicus*_IS60	*R. virginicus*	USA: FL	EF206319

27	A41 [α]	*R. hageni*	USA: P	JN207483
28	AY027470_*R. hageni*_IS198	*R. hageni*	USA: GA	AY027470
29	A33 [β]	*R. malletei*	USA: P	JN207484
30	A42 [β]	*R. malletei*	USA: P	JN207485
31	EF206320 [α]	*R. hageni*	USA	EF206320
32	A14 [γ]	*R. nelsonae*	USA: ACF	JN207486
33	A17	*R. nelsonae*	USA: ACF	JN207487
34	A18	*R. nelsonae*	USA: ACF	JN207488
35	A24	*R. nelsonae*	USA: ACF	JN207489
36	A25	*R. nelsonae*	USA: ACF	JN207490
37	A26	*R. nelsonae*	USA: ACF	JN207491
38	AY553155	*R. speratus*		AY553155
39	AY027456	*R. lucifugus grassei*	UK	AY027456
40	AY027457	*R. lucifugus grassei*	UK	AY027457
41	AY027458	*R. lucifugus grassei*	UK	AY027458
42	AY027459	*R. lucifugus grassei*	UK	AY027459
43	AY027460	*R. lucifugus grassei*	UK	AY027460
44	AY027461	*R. lucifugus grassei*	UK	AY027461
45	AY027462	*R. lucifugus grassei*	UK	AY027462
46	AY027463	*R. lucifugus grassei*	UK	AY027463
47	AY027464	*R. lucifugus grassei*	France	AY027464
48	AY027472	*C. formosanus*	USA	AY027472
49	AY553154	*H. tenuior*		AY553154

[α] denotes reference sequence was identified to the respective species using morphology of both soldier and alate specimens.
[β] denotes reference sequence for *R. malletei* that was correlated with the 16S rRNA gene for the original haplotype description in addition to morphology of soldier and alates (Austin et al., 2007).
[σ] P = Piedmont, ACF = Atlantic Coastal Flatwoods, SCP = South Coastal Plain geographical regions. Blanks in geographical region column indicate GenBank entries that did not have clear information on geographic origin.

Table 2.7a. True mean, standard deviation and range of soldier characters measured by species for length (*sl*), width (*sw*), ratio (*sl:sw*) and soldier mandible angle of curvature (*sma1*, *sma2*).

Species	Length (*sl*)				Width (*sw*)				Ratio of length: width (*sl:sw*)			
	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm
R. flavipes	1.693	0.119	1.21	1.91	1.044	0.074	0.73	1.17	1.625	0.084	1.43	1.83
R. virginicus	1.625	0.068	1.37	1.84	0.920	0.039	0.76	1.01	1.767	0.063	1.56	1.95
R. hageni	1.434	0.092	1.16	1.59	0.862	0.030	0.75	0.91	1.656	0.073	1.44	1.78
R. malletei	1.490	0.058	1.33	1.64	0.879	0.029	0.78	0.95	1.695	0.066	1.52	1.87
R. nelsonae	1.407	0.127	1.14	1.72	0.784	0.054	0.70	0.99	1.793	0.092	1.52	1.98

Species	Soldier mandible angle of curvature (*sma1*)				Soldier mandible angle of curvature (*sma2*)			
	Mean, °	Std. dev.	Min.[a], °	Max.[b], °	Mean, °	Std. dev.	Min.[a], °	Max.[b], °
R. flavipes	10.61	2.089	7.3	15.1	27.29	2.730	22.1	31.4
R. virginicus	13.62	1.719	11.7	18.1	32.60	2.003	29.4	36.7
R. hageni	8.39	1.199	6.9	11.2	23.39	1.307	21.5	27.3
R. malletei	10.51	1.366	8.1	13.2	25.85	2.282	22.4	32.9
R. nelsonae	10.66	2.206	7.2	14.6	27.27	2.654	24.1	34.0

Std. dev. = Standard deviation values calculated for means
Min.[a] = Minimum value of measure found within the dataset
Max.[b] = Maximum value of measure found within the dataset

Table 2.7b. True mean, standard deviation and range of alate characters measured by species for body (*abl*), body-wing (*ablw*), average forewing (*afw*) and average hind wing (*ahw*).

Species	Body (*abl*)				Body-wing (*ablw*)			
	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm
R. flavipes	4.783	0.383	3.77	5.83	8.973	0.402	8.05	9.94
R. virginicus	4.021	0.214	3.56	4.44	7.414	0.213	6.89	7.90
R. hageni	4.083	0.323	3.41	5.35	7.810	0.318	7.25	8.64
R. malletei	4.023	0.302	3.53	4.99	8.238	0.394	6.91	9.28
R. nelsonae	3.928	0.236	3.26	4.63	7.080	0.291	6.53	7.88

Species	Forewing (*afw*)				Hind wing (*ahw*)			
	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm
R. flavipes	6.810	0.331	5.97	7.74	6.550	0.332	5.70	7.44
R. virginicus	5.532	0.193	5.15	6.05	5.418	0.193	4.78	5.78
R. hageni	5.965	0.263	5.47	6.52	5.739	0.257	5.24	6.19
R. malletei	6.375	0.328	5.13	7.31	6.100	0.339	4.92	6.95
R. nelsonae	5.430	0.212	4.94	5.98	5.315	0.297	4.81	6.21

Std. dev. = Standard deviation values calculated for means
Min.[a] = Minimum value of measure found within the dataset
Max.[b] = Maximum value of measure found within the dataset

Table 2.8. Table detailing clades and clusters that correspond to Figures 2.4-2.6 for phylogenies obtained from COII gene.

ML Clade	BIONJ Clade	MP Clade	Species found within respective clades	Cluster	Cluster designation
1	1	1	*R. flavipes, R. santonensis, R. arenincola*	1	Southeastern US species
2	3	2	*R. hageni*		
3	2	3	*R. virginicus*		
4	4	4	*R. malletei*		
5	5	5	*R. nelsonae, R. hageni*		
6	13	8	*R. tibialis*	2a	Western US species
12	7	6	*R. hesperus,* "*R. okanaganensis*"	2b	
7	10	11	*R. chinensis*	3a	Non US species
8	9	12	*R. speratus*		
9	8	13	Mixed species from Asia		
10	11	10	*R. lucifugus corsicus*		
11	12	9	*R. grassei*		
13	6	7	*R. urbis*	3b	
ML Indv.	BIONJ Indv.	MP Indv.			
1	3	1	*R. clypeatus*	3a	Non US species
2	2	2	*R. ampliceps*		
3	1	3	*R. leptomandibularis*		
Outgroup			*Coptotermes formosanus, Heterotermes* sp.	-	-

Table 2.9. Table detailing clades and clusters that correspond to Figures 2.7-2.9 for phylogenies obtained from COI gene.

ML Clade	BIONJ Clade	MP Clade	Species found within respective clades	Cluster	Cluster designation
1	1	2	*R. flavipes*	1	Southeastern US species
5	3	5	*R. hageni*	3	
3	6	3	*R. virginicus*		
6	5	6	*R. malletei*		
4	4	4	*R. nelsonae*		
2	2	1	*R. lucifugus grassei*	2	Non US species
Indv. 1	Indv. 1	Indv. 1	*R. speratus*		
	Outgroup		*Coptotermes formosanus, Heterotermes tenuior*	-	-

Figure Captions

Figure 2.1. Standard measurements for soldier head capsule length (*sl*) and width (*sw*). Note that the length (*sl*) measurement does not include mandibles. Scale bar = 0.5 mm.

Figure 2.2. Soldier mandible, dorsal, showing curvature angle *sma* character. Scale bar = 0.5mm.

Figure 2.3. *Reticulitermes hageni* is the species shown here. This alate is photographed with its dorsal side up. This species was chosen to clearly show the measurement points for alate characters: length of body only (*abl*) body-wing (*ablw*), wing (*afw* and *ahw*) measurememements. Scale bar = 1.0 mm.

Figure 2.4. Maximum likehood (ML) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene data (665bp). The ML analysis with GTR+G+I, gamma Pinv = 0.559, gamma shape parameter = 1.070 generated with a log likelihood of -5075.935. Branch support was calculated with Approximate Likelihood-Ratio Test (aLRT) SH-like. The scale bar represents 0.1 substitution/ site. The dotted bars refer to groupings that have mixed species designation within them.

Figure 2.5. BIONJ estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COII (665bp) with model of substitution was Kimura 2- parameter. Bootstrap support, calculate from 1000 replicates, is expressed as percentage. Scale bar represents 0.1

substitution/site. The dotted bars refer to groupings that have mixed species designations within them.

Figure 2.6. Maximum parsimony (MP) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COII (665bp) data. The MP analysis generated 42 most parsimonious trees (MPTs) of 854 steps, consistency index = (0.330), retention index = (0.819), composite index= 0.300 (0.270) for all sites and parsimony-informative sites (in parentheses). Bootstrap consensus tree inferred from 1000 replicates is expressed as percentage (are shown above to the branches) and taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The MP tree was obtained using the Close-*Neighbor*-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions (1st+2nd+3rd+Noncoding) were included. The dotted bars refer to groupings that have mixed species designations within them.

Figure 2.7. Maximum likelihood (ML) estimate of *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) data. The ML analysis with GTR+G+I, gamma Pinv = 0.578, gamma shape parameter = 0.878 generated with a log likelihood of -2905.620. The dotted bars refer to groupings that have mixed species designation within them. Branch support was calculated with Approximate Likelihood-Ratio Test (aLRT) SH-like. The scale bar represents 0.1 substitution/site. The dotted bars refer to groupings that have mixed species designation within them. The dotted bars refer to groupings that have mixed species designations within them.

Figure 2.8. BIONJ estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) with model of substitution was Kimura 2- parameter. Bootstrap support, calculate from 1000 replicates, is expressed as percentage. Scale bar represents 0.05 substitution/site. The dotted bars refer to groupings that have mixed species designation within them.

Figure 2.9. Maximum parsimony (MP) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) data. The MP analysis generated 33 most parsimonious trees (MPTs) of 366 steps, consistency index = (0.556), retention index = (0.892), composite index= 0.565 (0.496) for all sites and parsimony-informative sites (in parentheses). Bootstrap consensus tree inferred from 1000 replicates is expressed as percentage (are shown above to the branches) and taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions (1st+2nd+3rd+Noncoding) were included. The dotted bars refer to groupings that have mixed species designation within them.

Figure 2.10. *Reticulitermes nelsonae*, diagnostic morphological characters for species identification: **(a)** Alate, dorsal, scale bar = 1.0 mm, **(b)** Soldier head capsule, dorsal, scale bar = 0.5 mm, and **(c)** Soldier mandible pair, dorsal, scale bar = 1.0 mm.

Figure 2.11. Habitus drawing of *R. nelsonae* alate, showing the wing venation. Scale bar is 1.0 mm.

Figure 2.12. *Reticulitermes* spp. from the southeastern USA, alates. From left to right: *R. virginicus*, *R. nelsonae*, *R. hageni*, *R. malletei*, *R. flavipes*. Scale bar = 1.00 mm.

Key 2.1. Key to soldiers of *Reticulitermes* spp. of Georgia

Key 2.2. Key to alates of *Reticulitermes* spp. of Georgia

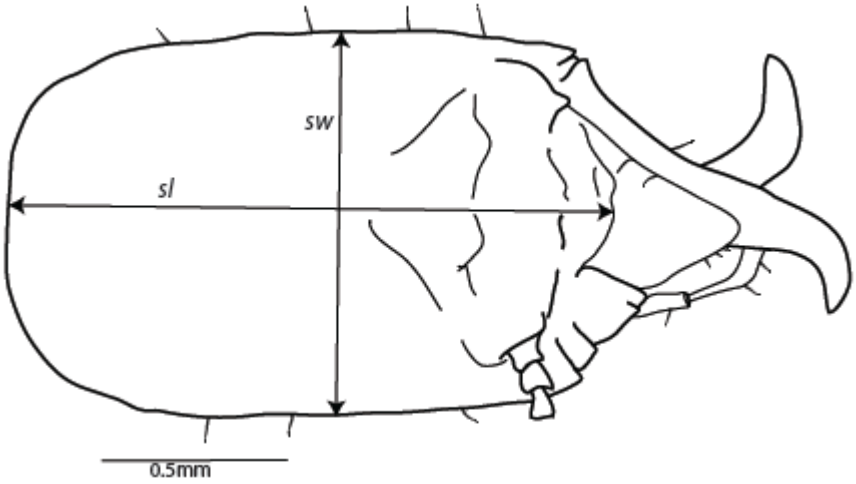


Figure 2.1

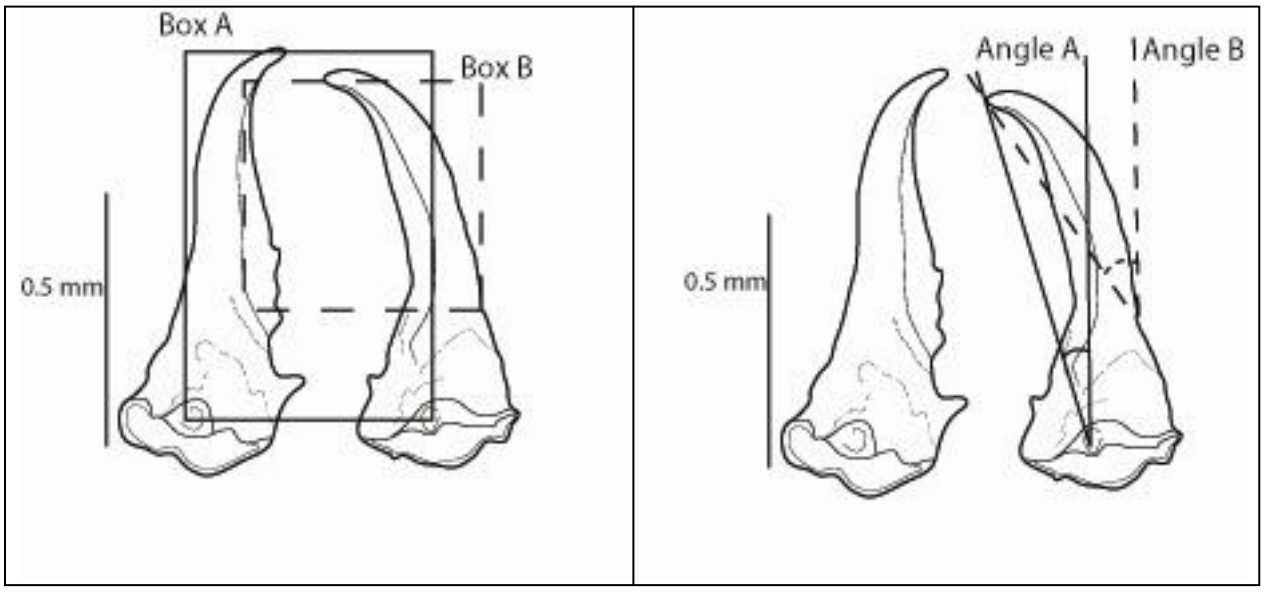


Figure 2.2

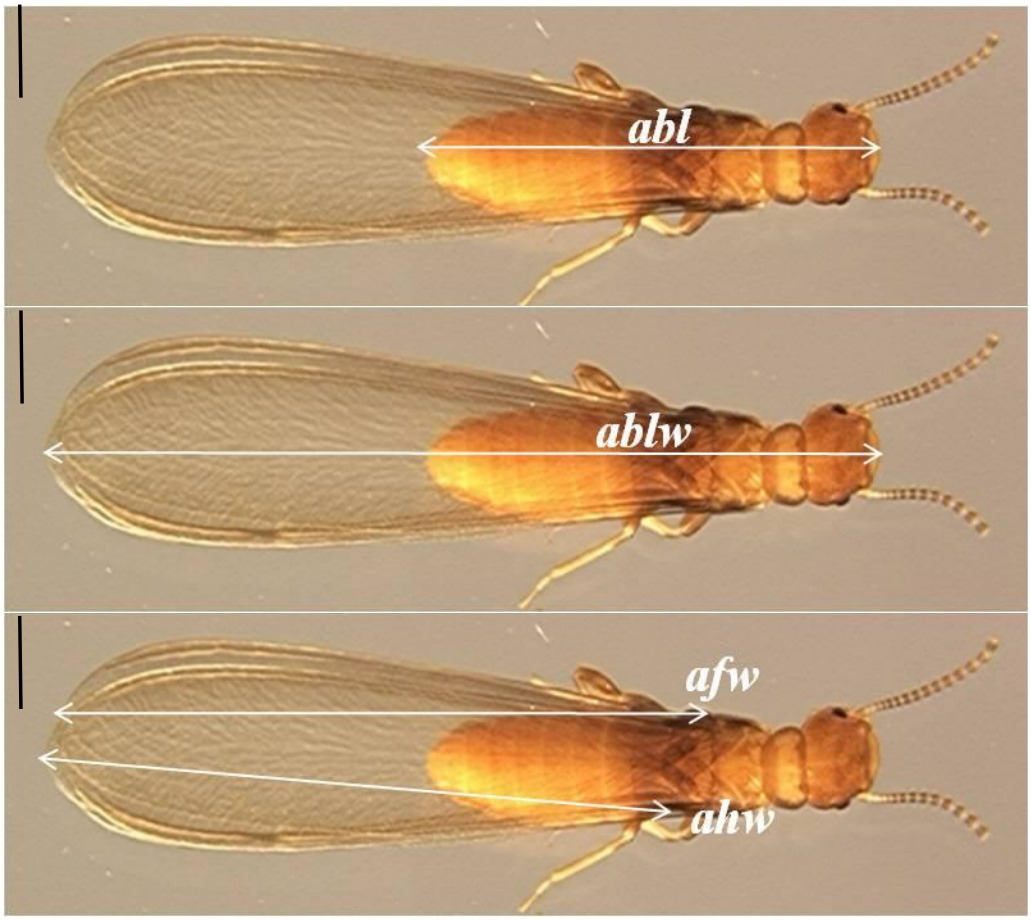
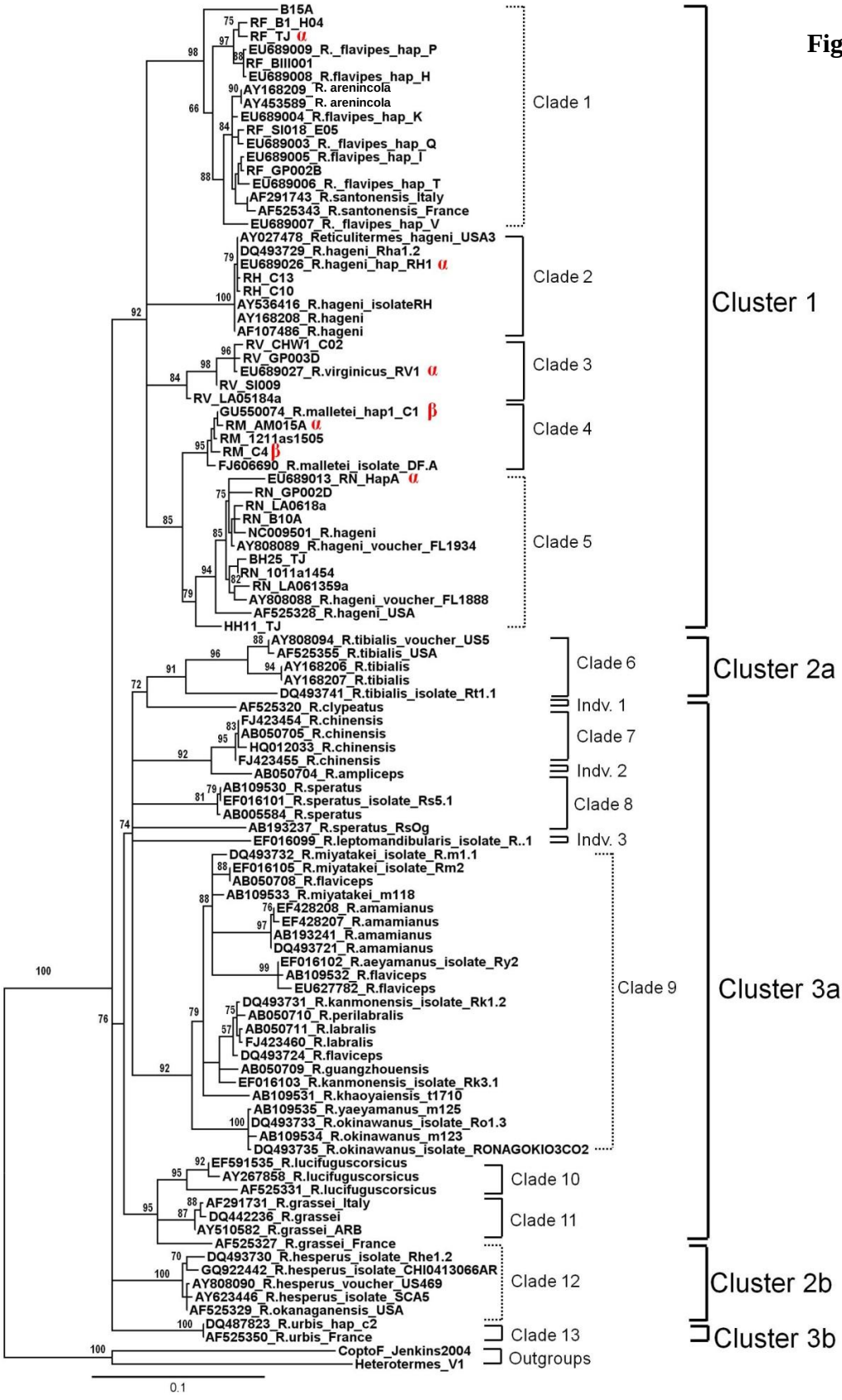


Figure 2.3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RF_B1_H04
RF_TJ α
EU689009_R._flavipes_hap_P
RF_BIII001
EU689008_R.flavipes_hap_H
AY168209_R. arenincola
AY453589_R. arenincola
EU689004_R.flavipes_hap_K
RF_SI018_E05
EU689003_R._flavipes_hap_Q
EU689005_R.flavipes_hap_I
RF_GP002B
EU689006_R._flavipes_hap_T
AF291743_R.santonensis_Italy
AF525343_R.santonensis_France
EU689007_R._flavipes_hap_V
Clade 1
AY027478_Reticulitermes_hageni_USA3
DQ493729_R.hageni_Rha1.2
EU689026_R.hageni_hap_RH1 α
RH_C13
RH_C10
AY536416_R.hageni_isolateRH
AY168208_R.hageni
AF107486_R.hageni
Clade 2
RV_CHW1_C02
RV_GP003D
EU689027_R.virginicus_RV1 α
RV_SI009
RV_LA05184a
Clade 3
GU550074_R.malletei_hap1_C1 β
RM_AM015A α
RM_1211as1505
RM_C4 β
FJ606690_R.malletei_isolate_DF.A
Clade 4
EU689013_RN_HapA α
RN_GP002D
RN_LA0618a
RN_B10A
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BH25_TJ
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RN_LA061359a
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AF525328_R.hageni_USA
HH11_TJ
Clade 5
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AF525355_R.tibialis_USA
AY168206_R.tibialis
AY168207_R.tibialis
DQ493741_R.tibialis_isolate_Rt1.1
Clade 6
AF525320_R.clypeatus
Indv. 1
FJ423454_R.chinensis
AB050705_R.chinensis
HQ012033_R.chinensis
FJ423455_R.chinensis
Clade 7
AB050704_R.ampliceps
Indv. 2
AB109530_R.speratus
EF016101_R.speratus_isolate_Rs5.1
AB005584_R.speratus
Clade 8
AB193237_R.speratus_RsOg
EF016099_R.leptomandibularis_isolate_R..1
Indv. 3
Cluster 2a
DQ493732_R.miyatakei_isolate_R.m1.1
EF016105_R.miyatakei_isolate_Rm2
AB050708_R.flaviceps
AB109533_R.miyatakei_m118
EF428208_R.amamianus
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Clade 9
Cluster 3a
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Clade 11
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Clade 12
Cluster 2b
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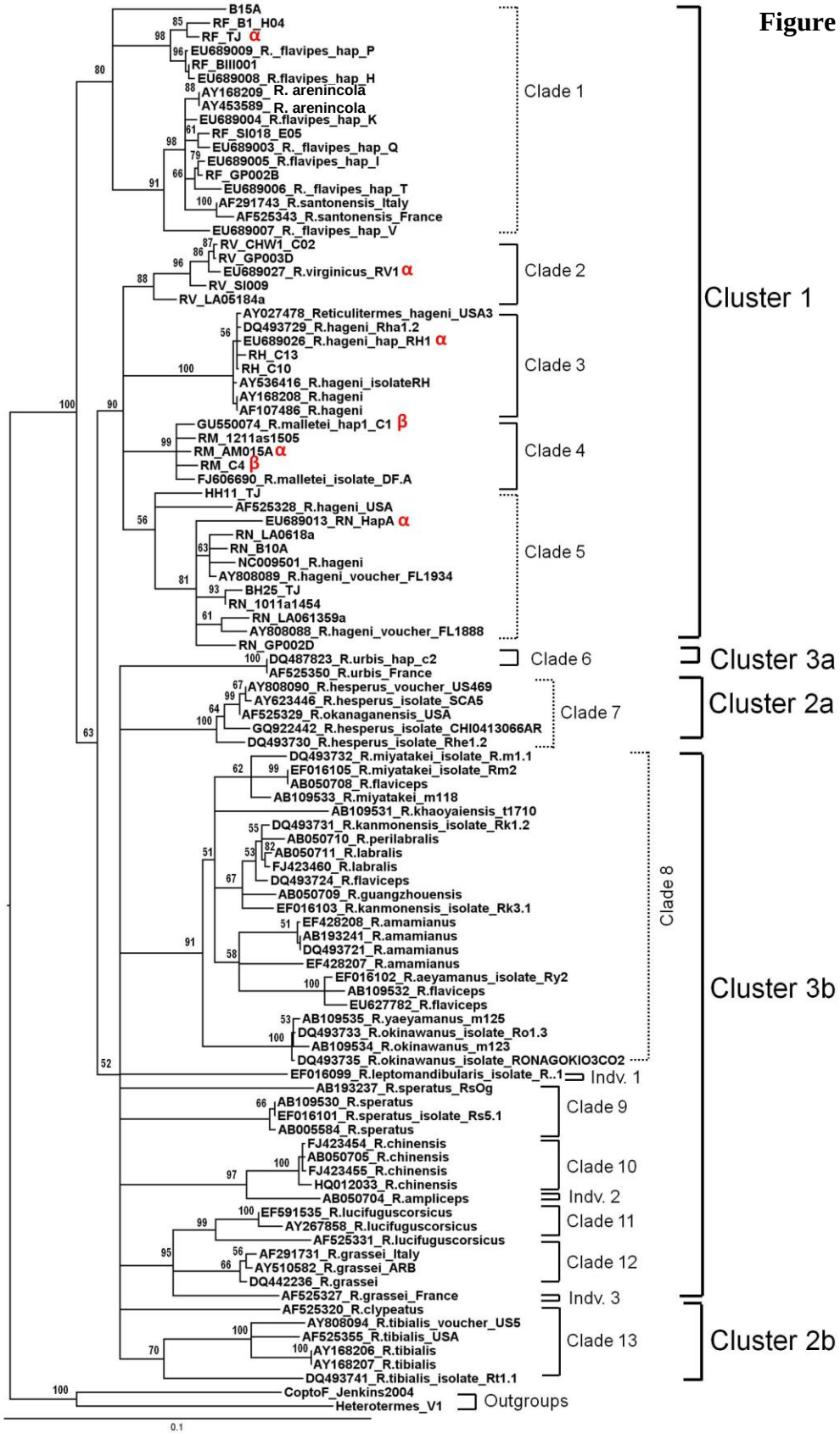
AF525350_R.urbis_France
Clade 13
Cluster 3b
CoptoF_Jenkins2004
Heterotermes_V1
Outgroups
0.1

Figure 2.4

Figure 2.5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AY453589_ R. arenincola
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RF_SI018_E05
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RF_GP002B
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AF525343_R.santonensis_France
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RV_SI009
RV_LA05184a
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EU689026_R.hageni_hap_RH1 α
RH_C13
RH_C10
AY536416_R.hageni_isolateRH
AY168208_R.hageni
AF107486_R.hageni
Clade 3
GU550074_R.malletei_hap1_C1 β
RM_1211as1505
RM_AM015A α
RM_C4 β
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RN_B10A
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AY808088_R.hageni_voucher_FL1888
RN_GP002D
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Cluster 1
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AF525350_R.urbis_France
Clade 6
Cluster 3a
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Clade 7
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FJ423460_R.labralis
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EF016103_R.kanmonensis_isolate_Rk3.1
EF428208_R.amamianus
AB193241_R.amamianus
DQ493721_R.amamianus
EF428207_R.amamianus
EF016102_R.aeyamanus_isolate_Ry2
AB109532_R.flaviceps
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AB109535_R.yaeyamanus_m125
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AB109534_R.okinawanus_m123
DQ493735_R.okinawanus_isolate_RONAGOKIO3CO2
Clade 8
Cluster 3b
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Indv. 1
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AB109530_R.speratus
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HQ012033_R.chinensis
Clade 10
AB050704_R.ampliceps
Indv. 2
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AY267858_R.lucifuguscorsicus
Clade 11
AF525331_R.lucifuguscorsicus
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AY510582_R.grassei_ARB
DQ442236_R.grassei
Clade 12
AF525327_R.grassei_France
Indv. 3
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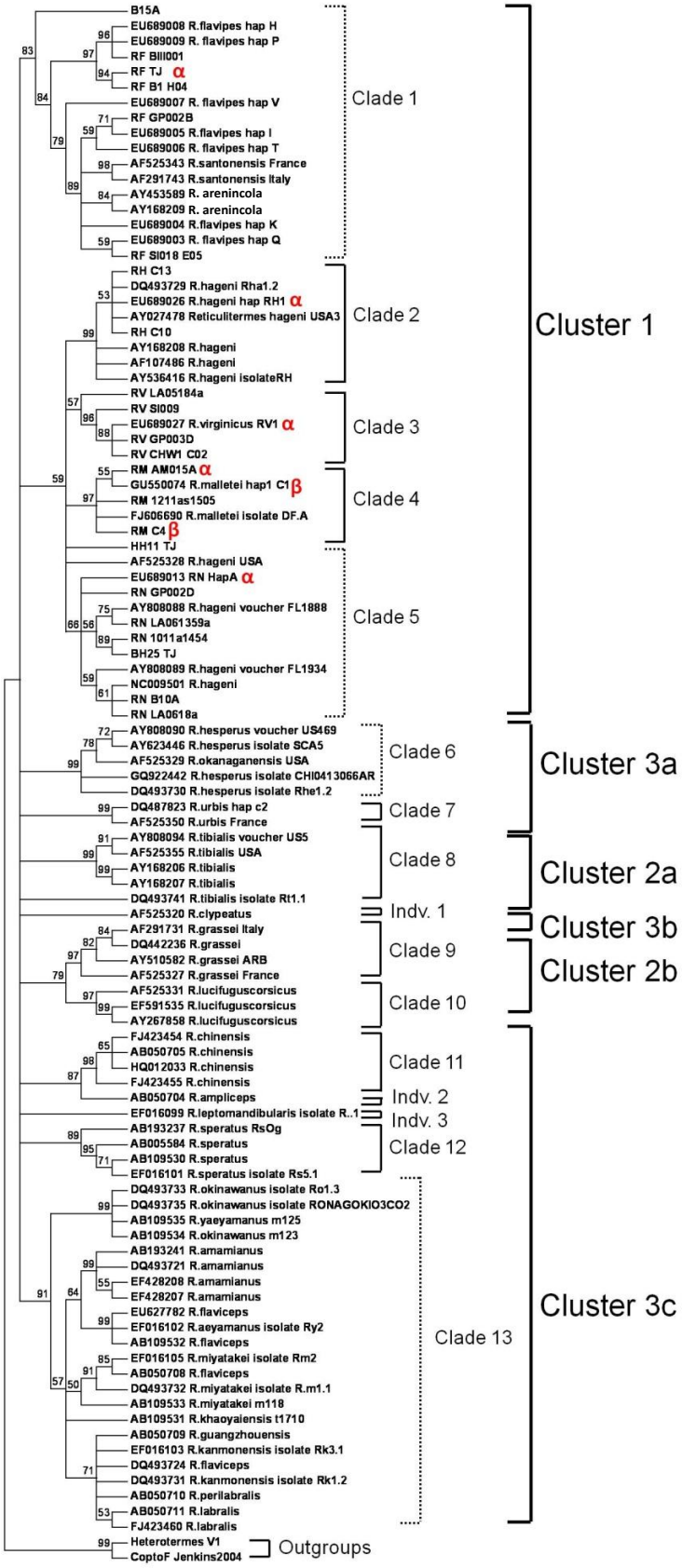
AY808094_R.tibialis_voucher_US5
AF525355_R.tibialis_USA
AY168206_R.tibialis
AY168207_R.tibialis
DQ493741_R.tibialis_isolate_Rt1.1
Clade 13
Cluster 2b
CoptoF_Jenkins2004
Heterotermes_V1
Outgroups
0.1

Figure 2.6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RF GP002B
EU689005 R.flavipes hap I
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AF291743 R.santonensis Italy
AY453589 R. arenincola
AY168209 R. arenincola
EU689004 R.flavipes hap K
EU689003 R. flavipes hap Q
RF SI018 E05
Clade 1
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RH C10
AY168208 R.hageni
AF107486 R.hageni
AY536416 R.hageni isolateRH
Clade 2
RV LA05184a
RV SI009
EU689027 R.virginicus RV1 α
RV GP003D
RV CHW1 C02
Clade 3
RM AM015A α
GU550074 R.malletei hap1 C1 β
RM 1211as1505
FJ606690 R.malletei isolate DF.A
RM C4 β
Clade 4
HH11 TJ
AF525328 R.hageni USA
EU689013 RN HapA α
RN GP002D
AY808088 R.hageni voucher FL1888
RN LA061359a
RN 1011a1454
BH25 TJ
AY808089 R.hageni voucher FL1934
NC009501 R.hageni
RN B10A
RN LA0618a
Clade 5
Cluster 1
AY808090 R.hesperus voucher US469
AY623446 R.hesperus isolate SCA5
AF525329 R.okanaganensis USA
GQ922442 R.hesperus isolate CHI0413066AR
DQ493730 R.hesperus isolate Rhe1.2
Clade 6
Cluster 3a
DQ487823 R.urbis hap c2
AF525350 R.urbis France
Clade 7
AY808094 R.tibialis voucher US5
AF525355 R.tibialis USA
AY168206 R.tibialis
AY168207 R.tibialis
Clade 8
Cluster 2a
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AF525320 R.clypeatus
Indv. 1
Cluster 3b
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DQ442236 R.grassei
AY510582 R.grassei ARB
AF525327 R.grassei France
Clade 9
Cluster 2b
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HQ012033 R.chinensis
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Clade 11
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Indv. 2
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Indv. 3
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AB005584 R.speratus
AB109530 R.speratus
EF016101 R.speratus isolate Rs5.1
Clade 12
DQ493733 R.okinawanus isolate Ro1.3
DQ493735 R.okinawanus isolate RONAGOKIO3CO2
AB109535 R.yaeyamanus m125
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EF428208 R.amamianus
EF428207 R.amamianus
EU627782 R.flaviceps
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AB050709 R.guangzhouensis
EF016103 R.kanmonensis isolate Rk3.1
DQ493724 R.flaviceps
DQ493731 R.kanmonensis isolate Rk1.2
AB050710 R.perilabralis
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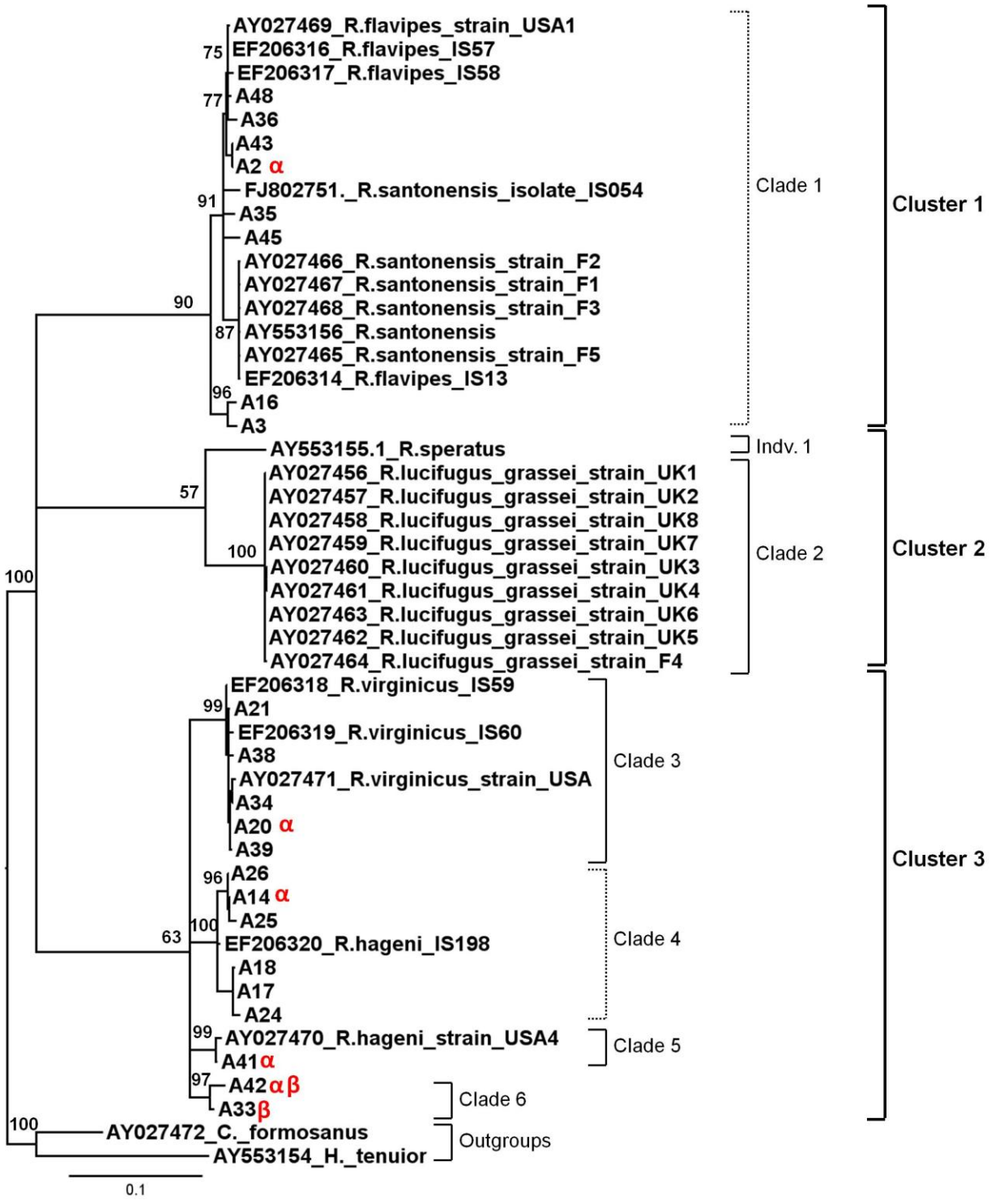
FJ423460 R.labralis
Clade 13
Cluster 3c
Heterotermes V1
CoptoF Jenkins2004
Outgroups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A35
A45
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87
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96
A16
A3
Clade 1
Cluster 1
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Indv. 1
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57
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AY027464_R.lucifugus_grassei_strain_F4
Clade 2
Cluster 2
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A21
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A38
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A34
A20 α
A39
Clade 3
96
A26
A14 α
A25
100
63
EF206320_R.hageni_IS198
A18
A17
A24
Clade 4
99
AY027470_R.hageni_strain_USA4
A41 α
Clade 5
97
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Cluster 3
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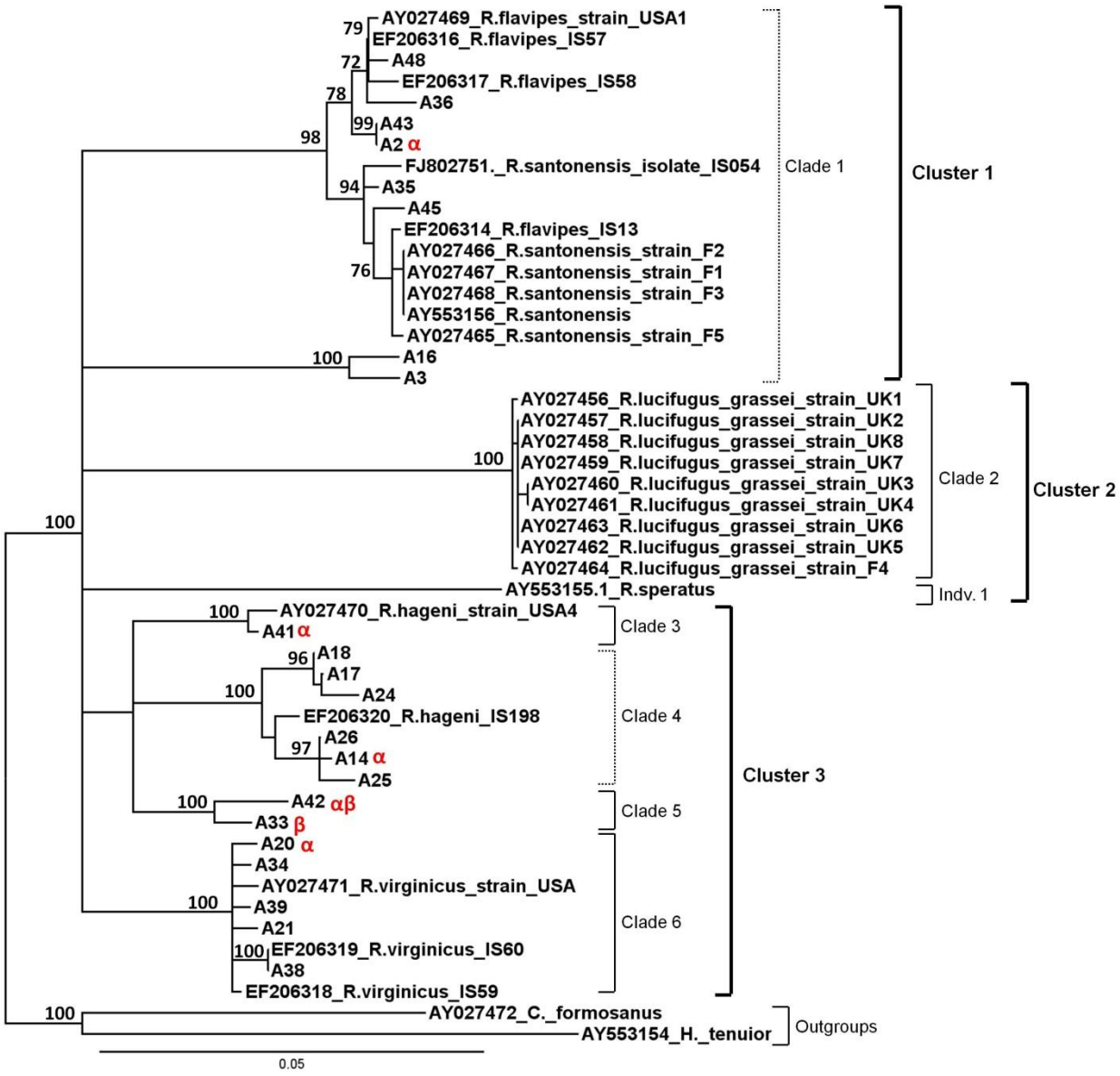
Outgroups
0.1

Figure 2.7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AY027465_R.santonensis_strain_F5
A16
A3
Clade 1
Cluster 1
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AY027464_R.lucifugus_grassei_strain_F4
Clade 2
Cluster 2
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Indv. 1
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A41 α
Clade 3
A18
A17
A24
EF206320_R.hageni_IS198
A26
A14 α
A25
Clade 4
Cluster 3
A42 αβ
A33 β
Clade 5
A20 α
A34
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A39
A21
EF206319_R.virginicus_IS60
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Outgroups
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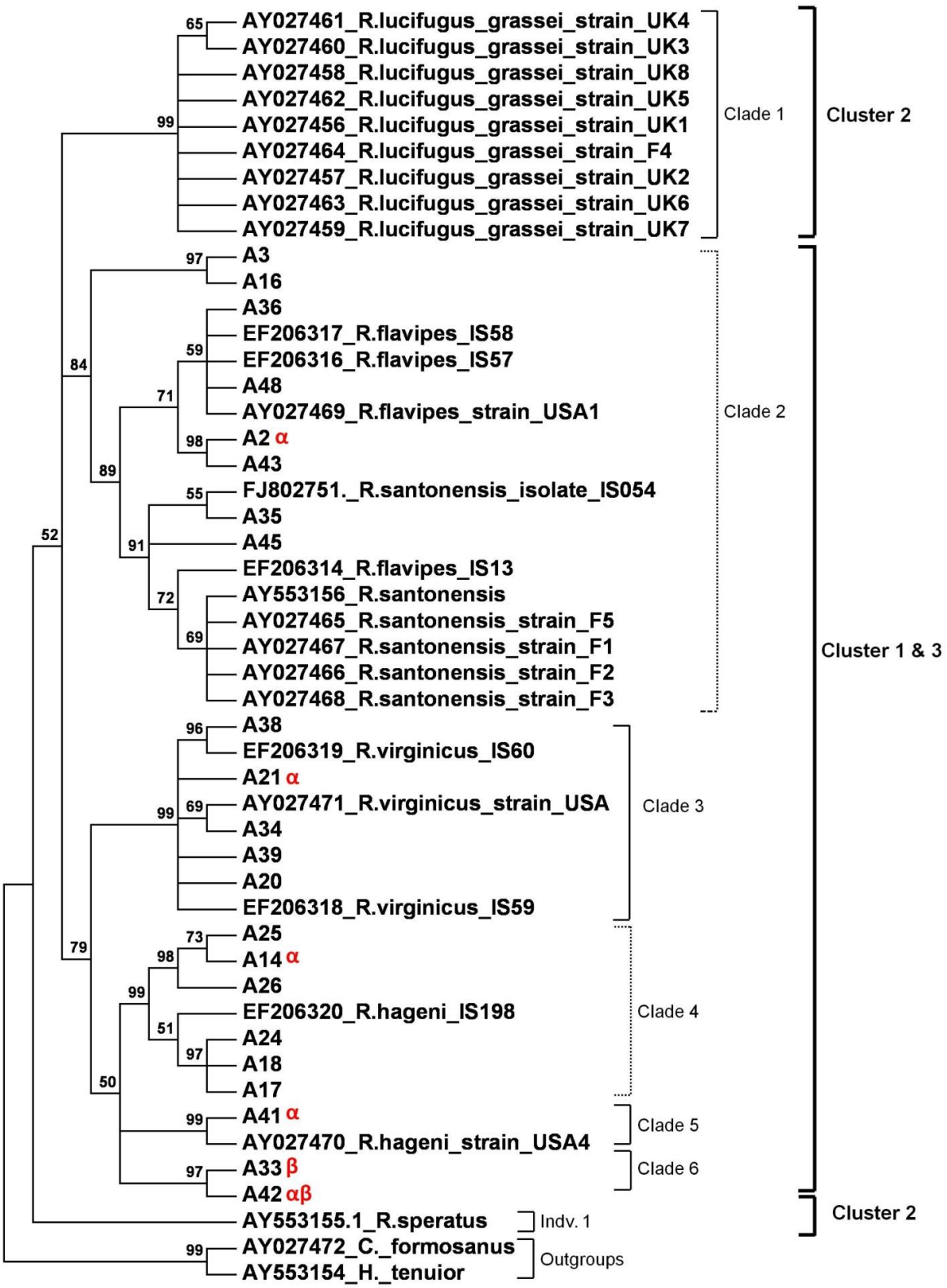
Figure 2.8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Clade 1
Cluster 2
A3
A16
A36
EF206317_R.flavipes_IS58
EF206316_R.flavipes_IS57
A48
AY027469_R.flavipes_strain_USA1
A2 α
A43
FJ802751._R.santonensis_isolate_IS054
A35
A45
EF206314_R.flavipes_IS13
AY553156_R.santonensis
AY027465_R.santonensis_strain_F5
AY027467_R.santonensis_strain_F1
AY027466_R.santonensis_strain_F2
AY027468_R.santonensis_strain_F3
Clade 2
Cluster 1 & 3
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AY027471_R.virginicus_strain_USA
A34
A39
A20
EF206318_R.virginicus_IS59
Clade 3
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EF206320_R.hageni_IS198
A24
A18
A17
Clade 4
A41 α
AY027470_R.hageni_strain_USA4
Clade 5
A33 β
A42 αβ
Clade 6
Cluster 2
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Indv. 1
AY027472_C._formosanus
AY553154_H._tenuior
Outgroups

Figure 2.9

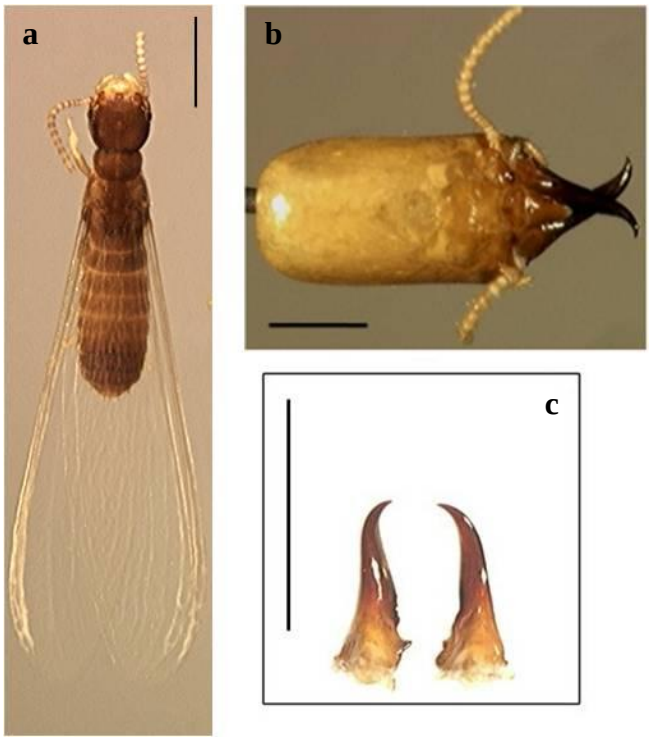

Figure 2.10

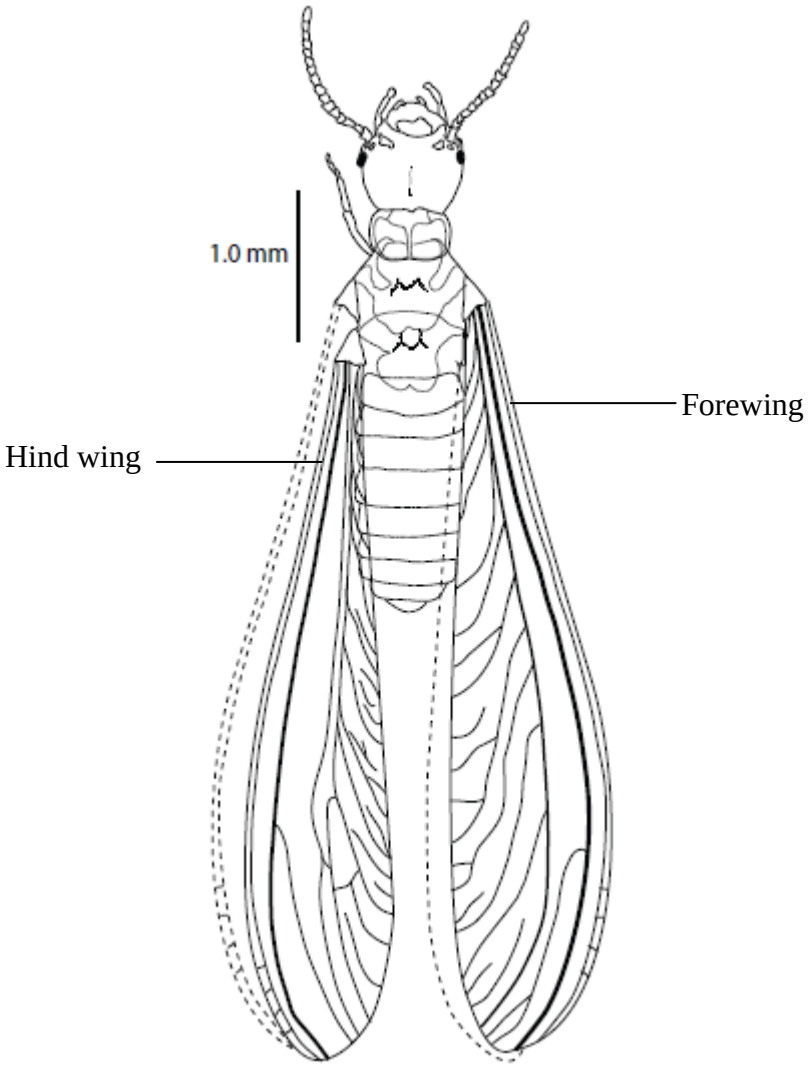

Figure 2.11


1.00mm
R. virginicus
R. nelsonae
R. hageni
R. malletei
R. flavipes

Figure 2.12

Key 2.1

1 Head capsule length without mandible (*sl*) typically ≥ 1.56mm (Figs. 3.1-3.2)...…….. 2

- Head capsule length without mandible (*sl*) typically ≤ 1.55mm (Figs. 3.1-3.2)…….. 3

2 Head capsule width (*sw*) (Figs. 3.1-3.2) typically 0.88-0.96mm. Mandible curvature angle (*sma*) (Figs. 3.3-3.4): AngleBII typically ≥ 31° (31°-35°). ..……..... ***R. virginicus***

- Head capsule width (*sw*) (Figs. 3.1-3.2) typically 0.97-1.12mm. Mandible curvature angle (*sma*) (Figs. 3.3-3.4): AngleBII typically ≤ 25°(25°-30°). ……..…..... ***R. flavipes***

3 Mandible curvature (*sma*): AngleBII typically ≤ 25° (22°-25°) (Figs. 3.3-3.4).
Head capsule length without mandible (*sl*) (Figs. 3.1-3.2) typically 1.34-1.53mm
……..…***R. hageni***

- Mandible curvature (*sma*): AngleBII typically ≥ 25° (25°-30°) (Figs. 3.3-3.4).
Head capsule length without mandible (*sl*) (Figs.3.1-3.2) typically ≥1.43mm (1.43-1.55mm)…….…… ***R. malletei***

- Mandible curvature (*sma*): AngleBII typically 24-28° (Figs. 3.3-3.4).
Head capsule length without mandible (sl) (Figs.3.1-3.2) typically ≤1.42mm (1.28-1.42mm)……..…. ***R. nelsonae***

Key 2.2

1 Body color light brown yellowish brown (Fig. 3.7). Swarm dates August - October. ……..***R. hageni***

- Body color dark brown blackish (Fig. 3.7). Swarm dates vary but does not occur in August - October …….. 2

2 Body length including wings (*ablw*) (Figs. 3.6-3.7) typically ≥ 8.57mm (8.57 -9.38mm). Body length without wings (*abl*) (Figs. 3.6-3.7) typically ≥ 4.40mm (4.40 -5.17mm). ……..... ***R. flavipes***

- Body length including wings (*ablw*) (Figs. 3.6-3.7) typically ≤ 8.57mm (6.79-8.57mm). Body length without wings (*abl*) (Figs. 3.6-3.7) typically ≤ 4.41mm (3.69-4.41mm)…...3

3 Average forewing length (*afw*) (Figs. 3.6-3.7) ≥ 6.04mm (6.04-6.70mm). Average hind wing length (*ahw*) (Figs. 3.6-3.7) typically ≥ 5.76mm (5.76-6.44mm). …….. ***R. malletei***

- Average forewing length (*afw*) (Figs. 3.6-3.7) ≤ 5.70mm (5.22-5.73mm). Average hind wing length (*ahw*) (Figs. 3.6-3.7) typically ≤ 5.61mm (5.02-5.61mm) …. 4

4 Ratio of body length with wing (*ablw*) to average forewing (*afw*) typically 1.32-1.37. Swarm dates April - May……...……..…… ***R. virginicus***

- Ratio of body length with wing (*ablw*) to average forewing (*afw*) typically 1.27-1.31. Swarm season probably February - March. …….. ***R. nelsonae***

CHAPTER 3

ONLINE INTERACTIVE MATRIX IDENTIFICATION KEY FOR *RETICULITERMES* SPECIES (RHINOTERMITIDAE) IN GEORGIA.[2]

[2]Lim, S.Y., McHugh, J. V. and B. T. Forschler. To be submitted to ZooKeys.

Abstract

Subterranean termites from the genus *Reticulitermes* have a complicated taxonomy, in part, because the most abundant caste, the worker, is an undifferentiated immature stage and the diagnostic castes; soldiers and adult reproductives (alates), exhibit overlapping ranges on measurable morphological characters. We quantified a set of simple morphological measurements for soldiers and additional character states for alates to resolve the identification process for the five *Reticulitermes* species found in Georgia, USA. Traditional dichotomous keys offer a means for identifying and distinguishing organisms, however, matrix-based computer interactive keys offer advantages over dichotomous keys. Multiple pathway identification as well as the ability to link images and supporting material to couplets provides the matrix key with more options and adaptability. We present a matrix key for soldier and alate castes to resolve the identification process for five *Reticulitermes* species found in Georgia. The efficacy and predictive power of the matrix key (developed in LUCID 3.5) for proper identification of *Reticulitermes* is compared to previous taxonomic studies.

Keywords: Taxonomy, morphometric, LUCID, matrix key, southeastern USA

Introduction

Subterranean termites from the genus *Reticulitermes* Holmgren 1913, (Rhinotermitidae) are represented in the southeastern United States by five species that are both economically important pests, and valued inhabitants of the soil ecosystem (Gentry and Whitford 1982; Su and Scheffrahn 1990; Su 1993; Pearce 1997; Su 2002). Kollar described the first species in 1837 as *Reticulitermes* (*Termes*) *flavipes* from specimens collected in Vienna that had emerged from crates that were imported from the USA (Kollar 1837; Banks and Snyder 1920). Seventy years later, *R. virginicus* (Banks, 1907) was described from collections made in Falls Church, Virginia, USA (Banks and Snyder 1920). *Reticulitermes hageni* Banks (1920) was later described from the same area (Banks and Snyder 1920). Sixty-six years passed before a fourth species, *R. malletei* was described by Clement et al. (1986). The validity of this species was supported by Austin et al. (2007) based on specimens collected in Clarke County, Georgia, USA. In 2011, a fifth species, *R. nelsonae* was described by Lim and Forschler from specimens collected on Sapelo Island, Georgia (Lim 2011).

Publications and identification keys for *Reticulitermes* have been published for specific regions of the USA (e.g., Scheffrahn and Su 1994; Hostettler et al. 1995; Messenger 2004; Brown et al. 2005; Wang et al. 2009) and nationwide (e.g., Banks and Snyder 1920; Snyder 1954; Weesner 1965; Nutting 1990). These resources have fulfilled the needs of most biologists, pest management professionals and other parties interested in identification of subterranean termites. All published keys are in the traditional dichotomous format with separate sections for soldiers and alates using morphological characters, with occasional information on alate flight phenology and/or species distribution (Banks and Snyder 1920; Miller 1949; Snyder 1954; Weesner 1965; Nutting 1990; Scheffrahn and Su 1994; Wang et al. 2009). However, none of the

published keys lists all, currently, known southeastern species (*R. flavipes*, *R. virginicus*, *R. hageni*, *R. malletei* and *R. nelsonae*).

Morphological characters reported in the literature for species determination of the soldier caste include body size (length), head capsule size (length and ratio), mandible shape, pronotum size and/or shape, gula size, ratio and/or shape, postmentum size and/or shape, and labrum size and/ or shape (Banks and Snyder 1920; Banks 1946; Miller 1949; Snyder 1954; Miller 1964; Weesner 1965; Nutting 1990; Scheffrahn and Su 1994; Hostettler et al. 1995; Messenger 2004; Brown et al. 2005; Wang et al. 2009). Alate characters reported in the literature include body size (length including, and excluding wings), pronotum size and/or shape, pronotum to head ratio, tibia color, shape of ocelli, distance between the compound eye and ocelli, and wing characters (length, wingspan and color) (Snyder 1954; Weesner 1965; Nutting 1990; Scheffrahn and Su 1994; Messenger 2004; Brown et al. 2005; Wang et al. 2009). Additional character states have been used to identify *Reticulitermes* species and they include chemical components (cuticular hydrocarbon "CHC", and soldier defense secretion "SDS"), genetic markers (mitochondrial DNA, nuclear genes, microsatellites), behavioral information (flight phenology for alates), behavioral data for soldiers (soldier aggression studies), geographical boundaries (species distribution) and protozoan symbionts (Polizzi and Forschler 1998; Forschler and Jenkins 1999; Haverty et al. 1999a; Haverty et al. 1999b; Polizzi and Forschler 1999; Jenkins et al. 2000; Vargo 2000; Page et al. 2002; Haverty 2003; Uva et al. 2004; Lewis and Forschler 2006; Cameron and Whiting 2007; Nelson et al. 2008).

Various authors have mentioned the need for a taxonomic revision of *Reticulitermes* in the United States (Weesner 1965; Collins 1988; Nutting 1990; Forschler and Jenkins 1999; Haverty et al. 1999b; Nelson et al. 2008). Such a revision is complicated by the range of

intraspecific and interspecific variation reported for morphological characters of soldiers or alates (Banks 1946; Weesner 1965; Nutting 1990; Hostettler et al. 1995; Haverty et al. 1996; Brown et al. 2005; Nelson et al. 2008; Wang et al. 2009; Lim 2011). This phenotypic plasticity has frustrated users of dichotomous keys which usually report measures as a single value within couplets (Nutting 1990, Scheffrahn and Su 1994, Nelson et al. 2008). Specialized techniques and equipment is required which compounds the lack of confidence users have in attempting to distinguish *Reticulitermes* species (Light 1927; Scheffrahn and Su 1994; Hostettler et al. 1995; Haverty et al. 1996; Forschler and Jenkins 1999).

This manuscript is the first to synthesize information on five species of *Reticulitermes* endemic to the southeastern United States using over 1300 termites collected from more than 150 localities representing three soil provinces in the state of Georgia. We present data on easily obtained morphometric measurements for soldiers and alates, specific qualitative characters and an additional behavioral character (flight phenology) to build an online interactive matrix key capable of distinguishing the five species of *Reticulitermes* found in Georgia, USA.

Materials and Methods

Sample Collection

Termite specimens from the soldier and imago caste of all five *Reticulitermes* are represented from collection localities in Clarke County, McIntosh County and Thomas County, Georgia (Tables 3.1-3.2). Termites in this study were collected over a 14 year period and stored in 70-100% ethanol. The specimens were collected from infested logs, (L), inspection ports, (IP - described in Forschler and Townsend, 1996), termite detectors, (TD - described in Silliam-Dussès and Forschler, 2010), five gallon plastic buckets half filled with wood, compressed cellulose and cardboard (B), and/or directed collections from swarm events (DC) (Tables 3.1-

3.2). Data on the soldier caste were obtained from 510 specimens representing 120 collection localities (Table 3.1). The soldier data includes the following morphometric measurements: head capsule length (*sl*), width (*sw*) and right mandible angle of curvature measurements *sma1* and *sma2* (Table 3.3). The alate data was obtained from 851 specimens representing 49 collection localities from which body length (*abl*), body length including wings (*ablw*), and wing lengths (*afw*, *ahw*) were measured for this study (Tables 3.2-3.3). Eleven morphological characters were scored: (i) soldier head capsule length (s*l*), (ii) soldier head capsule width (s*w*), (iii) ratio of soldier head capsule length: width (s*l:sw*), (iv) soldier mandible angle of curvature (*sma1*) ,(v) soldier mandible angle of curvature (*sma2*), (vi) alate body length (a*bl*), (vii) alate body-wing length (a*blw*), (iix) alate fore wing (*afw*), (ix) alate hind wing (*ahw*), (x) alate body color (*abc*), and (xi) alate wing pigmentation (*awp*) (Table 3.3). Alate flight phenology was recorded as behavioral data to further support species designations (collection dates in Table 3.2) (Table 3.3). All species designations in this study were cross-referenced with molecular data [full cytochrome oxidase II (COII), partial cytochrome oxidase I (COI)] to provide additional support for the species identifications used in developing the matrix key (Lim 2011).

Imaging

Images of specimens were captured using a binocular dissecting microscope (Leica WILD M10, Wetzlar, Germany) attached to a camera (Sony DKC-500, Tokyo, Japan) that fed image data into a computer. Images were taken using Adobe Photoshop v. 8.0 (Adobe Systems, Delaware, USA), while calibration and measurements were implemented with Auto Montage Pro v. 5.0.1 (Cambridge, United Kingdom). All images for soldiers were captured at 25x and images for alates were captured at 20x and not magnified in the Auto Montage Pro program when taking

measurements. All images are archived at the Household and Structural Entomology Research Program, Department of Entomology, University of Georgia.

Soldier

Dissections were carried out under a binocular dissecting microscope (CIT-OVAL2, Carl Zeiss aus Jena, Jena Germany) using a pair of fine forceps. Morphometric measurements were taken after removing head capsules from specimens. Head capsules were mounted on a minuten pin, tipped with a drop of diluted Elmers® glue, inserted into the occipital foramen. Minuten-mounted soldier head capsules were pinned into a foam pad on a standard size #2 insect pin for easy manipulation. The plain of the head capsule was manipulated until specific structures were parallel and consistent during imaging. This was accomplished by having the following three features in focus; clypeal sulcus, longitudinal edge of the head capsule and the leading edge at the end of the occipital foramen. Soldier head capsule length (*sl*) was measured from the clypeal sulcus to the occipital foramen and head capsule width (*sw*) taken from the mid-point of the *sl* (Fig. 3.1). The width measure was taken at a 90° angle from the mid-point of *sl* (Fig. 3.1).

Twenty pairs of soldier mandibles, per species, were dissected from the head capsule to characterize the angle of curvature of the right mandible (*sma1* and *sma2*). Soldier mandibles were excised from randomly selected specimens using a pair of insect pins under a binocular dissecting microscope (CIT-OVAL2, Carl Zeiss aus Jena, Jena Germany) and mounted on clear two-sided tape applied to a piece of standard copier paper pre-printed with a 2 mm x 2mm box (Fig. 3.2). Mandibles were positioned dorsal side up and the pre-printed boxes were used to align the base of the mandibles to ensure the bases were parallel to the lower horizontal edge of the box (Fig. 3.3). Two boxes were drawn using Microsoft PowerPoint 2007 (Redmond, Washington, USA) or Adobe Photoshop to establish a 90° vertical line (Fig. 3.4). Box A was

drawn to originate from the dorsal condyle, and Box B is drawn to originate from the external curvature inflexion point (Fig. 3.4). The right mandible angle of curvature as measured using a program known as ImageJ (Research Services Branch, NIH, Bethesda, Maryland, USA) from a 90° line from Box A and Box B to the dorsal condyle, and external curvature inflexion point to a line at the tip of the right mandible (Fig. 3.4).

Alate

Alates used for measurements were stored in ethanol for less than 10 years. Specimens were held in position for imaging using a cover slip and glass slide with the ventral surface on a glass slide and wings (dorsal) against a cover slip (Fig. 3.5). Minor adjustments of the body and head were made to ensure that the entire body was on the same plain. The void between the glass slide and cover slip was filled with 100% ethanol using a pipette.

Classification of body (*abc*) and wing pigmentation (*awp*) were determined from specimens photographed as a group to control for exposure and lighting affects (Table 3.4, Fig. 3.6) There were two categories for body color (*abc*): light to dark brown, and yellowish brown, and two wing pigmentation categories (*awp*): pigmented (wp) and non-pigmented (wnp) (Table 3.4, Fig. 3.6). Alate body length (*abl*), body-wing length (*ablw*), forewing (*afw*) and hind wing (*ahw*) measurements were taken as shown in Fig.3.7. All wing measurements were made on wings that were attached to the body to allow distinction between the fore- and hind wing(s).

Key

Lucid v.3.5 (Queensland, Australia) was used to build a matrix key incorporating quantifiable and qualitative morphological characters of soldier and alate castes in addition to an alate behavioral character, flight phenology. The matrix key was developed by incorporating 12 character states for soldier and alate specimens (Table 3.3) with the five species of

Reticulitermes designated as entities. The key was build to reflect the entire range of measurements we recorded and was accomplished by placing emphasis on the mean $\pm$ 1 standard deviation (Tables 3.5a-b). The matrix format provides users the flexibility to choose and combine any of the 12 character states and includes images detailing measurement points as well as qualitative character comparisons in full-size images. The key systematically eliminates taxa that do not match the characteristics of a chosen criterion allowing users to focus on obtaining data for only those characters deemed useful for discrimination of the remaining species.

Statistics

Statistical analyses were conducted using SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA) with one-way analyses of variance (ANOVA) performed on each character state to determine if it contributed significantly to species separation. A sequential t-test with LSD (protected least square deviation) pair-wise comparison was used to evaluate species differences using all qualitative data from both castes, separately. We conducted additional t-tests and ANOVAs on the alate data to determine i) if the average hind wing (*ahw*) is shorter than the average forewing (*afw*), and ii) if the intraspecific variation in hind- and forewing measurements was greater within or between individuals.

Characters most important for classifying the five species were determined using the Step-Wise Discriminant Analysis (SWDA) procedure. The Pr > F values and error rates acquired from multiple Discriminant Function Analyses (DFA) with both proportional prior and cross-validation procedures were employed to determine accuracy and reliability of various character state combinations. SWDA was conducted on the soldier data using *sl*, and *sw* from the five species. The posterior probability values generated from DFA allowed predictive classification and became a measure of the diagnostic value for a particular set of characters (in the case of

soldiers *sl*, *sw* and alates *ablw* and *afw*). An independent DFA was conducted for *sma1* and *sma2* separate from the other soldier characters. Analysis of alate data was conducted using *ablw* and *afw* to separate *R. flavipes*, *R. virginicus* and *R. nelsonae*. This was possible because two, unique, qualitative characters used in the matrix key eliminate two species from the need for quantitative measurements; *abc* differentiates *R. hageni* and *awp* separates *R. malletei* from the list of five species.

Simulations of random populations from each species (1 million termite averages per trial) were conducted to determine the minimum sample size (N) needed to accurately identify a species at 95% confidence. DFA was used to determine the probability of incorrectly classifying the mean value given a certain sample size (e.g., N = 1, N = 2, N = 3, N = 4 termites, etc.). Simulations were run for both the soldier and alate data sets for all five species, except for the *R. hageni* alate data because the qualitative character body color (*abc*) separates *R. hageni* alates from the other species.

Results

Tables 3.5a-b list the number of specimens, means, standard deviation, and range for each of the quantitative morphometric characters by species. The LSD column in Tables 3.5a-b indicates the statistical separation of means for each measured character by species provided by ANOVA and LSD analysis. All five soldier characters (*sl*, *sw*, *sl*: *sw*, *sma1* and *sma2*) and four alate characters (*abl*, *ablw*, *afw*, *ahw*) provided significant contribution for classifying the species as indicated by the value of $Pr < F = < 0.0001$ from one-way ANOVA (Table 3.6).

Soldier

Mean values for *sl* and *sw* indicated that *R. flavipes* is the largest and *R. nelsonae* the smallest species (Table 3.5a, Figs. 3.8, 3.9a-b). Means for *sl*:*sw* showed an inverse relationship

with *R. nelsonae* having the largest and *R. flavipes* the smallest ratio (Table 3.5a, Fig. 3.9c). Average values for *sma1* and *sma2* indicated that *R. virginicus* has the largest and *R. hageni* the smallest values distinguishing them from *R. flavipes, R. malletei* and *R. nelsonae* (Table 3.5a; Figs. 3.9d-e). Results from the multiple t-test analysis with LSD are shown as designated lower case letters (a-e) above the modified boxplots to reflect mean separation indicated for the respective pair-wise comparison (Tables 3.5a-b, Figs. 3.9a-e). The range of values could not provide a clear separation of *R. malletei* and *R. nelsonae* with any characters although mean ± 1 std. dev. allowed separation using LSD for *sl*, *sw* and *sl:sw*. Using the mean ± 1 std. dev. with LSD, the *sl* character distinguished all species except *R. hageni* and *R. nelsonae* while *sw* distinguished all species except *R. hageni* and *R. malletei* and *sl:sw* separated all five species (Table 3.5a; Fig. 3.9c). Pearson correlation coefficient analyses for *sl*, *sw*, *sl*:*sw* determined that length and width are significantly positively correlated, while *sw* and *sl:sw* are significantly negatively correlated. A less significant positive correlation was also noted between *sl* and *sl:sw* (Table 3.7).

SWDA concluded that all three soldier head capsule measurements (*sl*, *sw, sl:sw*) were important (Pr>F = <0.0001) in discriminating species (Table 3.8). SWDA determined that the most useful soldier head capsule characters for species discrimination was *sw*, followed by *sl:sw* and *sl* (Table 3.8). The DFA error rate was lowest when both *sl* and *sw* are used together. Table 3.9a summarizes results from a randomized reclassification analysis using DFA that showed *R. virginicus* was correctly classified 85% of the time, *R. flavipes* 82%, and *R. nelsonae* 77% using *sl* and *sw* data from all species. Results were calculated with proportional priors (dependent on sample size) and crossvalidation to confirm the confidence values in Table 3.9b. The total error rates were low and similar for both resubstituted and crossvalidated data, 0.2725 and 0.2882

respectively (Table 3.9b). An independent DFA which evaluated the value of using *sma*1 and *sma2* and determined that *R. virginicus* could be correctly differentiated 90-95% and *R. hageni* 85-90% of the time from the other five species (Tables 3.10a-b, 3.11a-b).

DFA with equal prior (Fig. 3.10a) calculated that a minimum of 29 soldiers is required to identify *R. hageni* and *R. malletei* within 95% confidence. The minimum N required, based on proportional and equal prior analysis, for identifying *R. flavipes*, *R. virginicus* and *R. nelsonae* with a 95% confidence is 6 soldiers (Figs. 3.10a-b).

Alate

Quantitative morphometric alate characters included *abl*, *ablw*, *afw* and *ahw* (Table 3.3) because differences between the right and left forewing and right and left hindwings were on average 0.01254, they were considered not justifiably significant, (70% of P-values from comparisons within species were > 0.05) (Table 3.12a). The data demonstrate that despite the derivation of the term Isoptera (equal-wing), the hind and forewings on *Reticulitermes* are significantly different in size (Pr >|t| = <0.0001) for all the species we examined (Table 3.12b).

Average values for *abl*, *ablw*, *afw* and *ahw* agreed with soldier data that *R. flavipes* is the largest and *R. nelsonae* the smallest (Table 3.5b). Results from the multiple t-tests with LSD are shown as designated lower case letters (a-e) in Table 3.5b. The same data are presented as modified boxplots for the respective alate characters (Figs. 3.11a-d). The *ablw* character differentiated all five species (Table 3.5b; Fig. 3.11b) while wing lengths (*afw* and *ahw*) were useful in separating three species *R. flavipes*, *R. hageni*, and *R. malletei* but not *R. virginicus* and *R. nelsonae* (Table 3.5b; Figs.3.11c-d). LSD also indicated that *abl* mean values clearly separated *R. flavipes* and that *R. hageni* and *R. malletei* could be distinguished from *R. nelsonae*;

however, *R. virginicus* could not be separated from *R. hageni*, *R. malletei* or *R. nelsonae* (Table 3.5b; Fig.3.11a).

Pearson correlation coefficient analyses determined that all of the alate characters we measured (*abl*, *ablw*, *afw*, *ahw*) had significant positive relationships (P values < 0.0001) (Table 3.13). The most important alate character for discriminating species as determined by SWDA was *ablw*, and all variables measured were significantly useful for species separation (Pr>F <0.001) (Table 3.14).

The Classification and Regression Trees (CART) method was employed to provide justification for separating the five *Reticulitermes* species; first into two groups using *abc*, and later into two more groups based on *awp* (Table 3.4, Figs. 3.6, 3.12). Utilizing *abc*, *R. hageni* was identified from the other species and *awp* separates *R. malletei* from the remaining four species (Fig.3.12). DFA determined that *ablw* and *afw* best separates the remaining three species (*R. flavipes, R. virginicus* and *R. malletei*) (Table 3.15a). *Reticulitermes flavipes* could be correctly classified almost 100%, *R. nelsonae* 98 % and *R. virginicus* (78%) using the two characters (*ablw* and *afw*) together (Table 3.15a). This is supported by the low error rates obtained from the resubstituted (0.0275) and crossvalidated (0.0275) analyses (Table 3.15b). The high confidence level observed for identifying *R. flavipes* and *R. nelsonae* using *ablw* and *afw* was expected because *R. flavipes* is the largest species and *R. nelsonae* is the smallest species of *Reticulitermes* found in Georgia.

The identity of *R. virginicus* could be further confirmed based on alate flight times. *Reticulitermes virginicus* had only been collected in the month of May in Georgia, while *R. flavipes* were collected from November to March and *R. nelsonae* from February and March (Lim 2011) (Table 3.2).

The simulation analyses on the alate data were done without *R. hageni* because this species can be identified with 100 % confidence using *abc* (Fig. 3.12). DFA with equal prior (Fig. 3.13a) determined that a minimum of 5 alates are needed to have 95% confidence using all the quantitative morphometric measurements (Table 3.3) for *R. flavipes* and *R. virginicus*, 4 alates for *R. malletei* and 2 alates for *R. nelsonae*. The minimum N required, based on proportional prior analysis, for identifying *R. flavipes, R. virginicus, R. malletei and R. nelsonae* with a 95% confidence is 9 soldiers (Fig. 3.13b).

Models: Route of identification

We present two models (Figs. 3.12, 3.14) describing routes of identification based on lowest error rates obtained from DFA predictions and CART for each caste (Tables 3.9a-b, 3.10a-b, 3.11a-b, 3.15a-b, Figs. 3.9a-e, 3.11a-d). The route for soldiers (Fig. 3.14a) uses *sma1* and *sma2*, followed by *sl* and *sw* for discriminating the five *Reticulitermes* species. Alates would be most efficiently identified by first using *abc* to identify *R. hageni.* The next character *awp* would separate *R. malletei* while the remaining three species (*R. flavipes, R. virginicus* and *R. nelsonae*) could then be discriminated by *ablw* and *afw* (Fig. 3.12b). *Reticulitermes flavipes* is the largest species, *R. nelsonae* the smallest and *R. virginicus* intermediate (Table 3.5a-b). The flight time would also assist in validating species identity. *Reticulitermes virginicus* alates typically flies in May, *R. flavipes* in the months of November to March and *R. nelsonae* were in collected in February to March (Lim 2011) (Table 3.2).

Matrix Key

The online interactive matrix key will be available on the internet and can be accessed on UGA Department of Entomology's webpage. The key incorporates the entire range of values measured from this study and includes detailed images identifying measurement points for each

quantitative character. Colored photographs for comparing qualitative characters are also embedded in the matrix key and can be viewed in full size.

Discussion

This manuscript details the first key to the species of subterranean termites found in Georgia, USA, that includes all species endemic to the SE USA using qualitative and quantitative phenotypic characters in addition to behavioral characters in a matrix key format (Clément et al. 1986; Austin et al. 2007; Sillam-Dussès and Forschler 2010; Lim 2011). The efficiency of the matrix key was tested using data from more than 1,300 specimens from over 150 collection locals to provide an estimate of the number of soldier or alate specimens needed for reliable species determination. The data generated in producing these matrix key highlights the need to examine multiple character and more than one specimen, preferably examples of soldiers and alates, for reliable species determination. The importance of using of multiple characters must not be underestimated given the current research emphasis on using molecular tools for species discrimination (Vargo and Husseneder 2009). Lee (2004) described the need for caution when using one character for species discrimination and recommended the combination of genetic, anatomical and ethological information for delineating species boundaries. Cognato (2006) also showed that DNA sequence data, used in isolation, cannot accurately predict species boundaries. The species designations for all specimens used in this study were corroborated with gene sequence (COII, COI) based on phylogenetic analysis using reference sequence obtained from specimens that were identified using both soldier and alate (from the same collection local/date) morphometric characters (Lim 2011).

Miller (1949) was the first to mention, over 60 years ago, the difficulty in determining *Reticulitermes* species and recommended that both soldier and alate specimens, from the same

collection locality, be used for proper identification. It would be ideal if both soldier and alate specimens could be obtained but when only one caste is collected, the matrix key becomes an especially useful tool in the identification process. The five species of *Reticulitermes* spp. found in Georgia were easily distinguished using morphological characters from both soldiers and alates. *Reticulitermes flavipes* has been reported as the largest *Reticulitermes* species endemic to the southeastern USA and this study supports that designation (Tables 3.5a-b). *Reticulitermes nelsonae* is now the smallest species replacing *R. hageni* (Tables 3.5a-b) (Weesner 1965; Scheffrahn and Su 1994; Lim 2011). Our data indicate that at least 6 soldiers are needed to obtain a mean for accurate separation of three species (*R. flavipes, R. virginicus, and R. nelsonae*) while *R. hageni* and *R. malletei* require at least 29 (Figs. 3.10a-b). It is also recommended that at least 6 alates are measured for mean separation of Georgia *Reticulitermes* (Figs. 3.13a-b).

Statistical analyses indicated that despite differences observed in mean values the range of measurements overlapped among species (Tables 3.5a-b). This concurs with previously published data that mention intra- and inter- specific similarity within the genus *Reticulitermes* (Weesner 1965; Nutting 1990; Hostettler et al. 1995). In particular, soldier phenotypic plasticity requires precise measurements because of the small differences and overlapping values between species (Tables 3.5a-b) (Weesner 1965; Nutting 1990; Hostettler et al. 1995). We made an effort to describe detailed measurements in this study that are illustrated with explanation in the matrix key.

The range of values obtained for each quantifiable character state used in this study generally agrees with the literature. Disagreement between measurements could be the result of regional differences in species, sample size, and/or instrumentation yet requisite details

describing the instruments used and, in some cases, character features were not properly referenced with measurement points in most of the literature (Light 1927; Gay 1967; Hostettler et al. 1995; Wang et al. 2009). Wang et al. (2009) provide data were, on average, larger for both soldiers and alates in *R. flavipes*, *R. virginicus* and *R. hageni* (Snyder 1954; Weesner 1965; Nutting 1990; Scheffrahn and Su 1994). The soldier head capsule lengths we recorded were similar to those reported by Banks (1946) for *R. flavipes, R. virginicus* and *R. hageni*. Measurements by Austin et al. (2007) for *R. malletei* soldiers also were comparable to those obtained in our study (Table 3.5a). Miller (1949) was the first to identify the distinctive curvature of the right mandible in *R. virginicus* soldiers and despite being used in the Scheffrahn and Su (1994) key, details of the requisite measurement points were never characterized, until this study (Figs. 3.4, 3.9d-e).

We recorded smaller *ablw* than reported by Messenger (2004) while our wing measurements concur with values reported by Weesner (1965), Gay (1967) and Nutting (1990) (Table 3.5b, Fig. 3.11b). Two qualitative alate characters; *abc*, *awp*, provide a clear distinction and allow for separation of the five species into two groups. The character body coloration (yellowish brown) used for identifying *R. hageni* is in the original description and widely recognized (Banks and Snyder 1920; Banks 1946; Weesner 1965; Krishna and Weesner 1970; Nutting 1990; Scheffrahn and Su 1994). A caveat to using alate body color is that colors fade with time in ethanol and we recommend not using this character with specimens stored for greater than 10 years. We also identified the qualitative character, wing pigmentation that also is useful in alate specimen separation. The character ‘pigmented wings’ has been used in descriptions of *R. flavipes* and *R. malletei* (Banks & Snyder, 1920; Clément et al., 1986, Austin et al 2007). The quality of observed pigmentation in *Reticulitermes* wings is dependent on angle

and intensity of light in addition to requiring comparative examination. Based on our photographic evidence (Fig. 3.6) there are degrees of pigmentation attributable to the various species but certainly corroborates descriptions that *R. virginicus* has colorless (not pigmented) wings (Krishna & Weesner, 1970; Scheffrahn & Su, 1994). Our description of the gradation of wing pigmentation reveals that *R. malletei* is the most obviously pigmented with *R. hageni* next followed by *R. flavipes* (Fig. 3.6). We chose to describe two species with pigmented wings (Table 3.4, Fig. 3.6), in the absence of quantitative analysis, to facilitate separation of *R. malletei* (clearly darker) from all specimens that display a dark brown to black body color, especially *R. virginicus* which shares a similar flight phenology (Table 3.2, Fig. 3.6).

The order Isoptera, meaning equal wing in Greek, have always been considered to have wings equal in length and thus measurement values provided in past keys have simply denoted wing length/ wing span with no indication of fore - or hind wing (Weesner 1965; Nutting 1990; Messenger 2004; Wang et al. 2009). Statistical analyses with t-tests and ANOVAs determined that the average *Reticulitermes* hind wing was significantly shorter than the average forewing for all the five species ($Pr > |t| = < 0.0001$) (Table 3.12b). It is also recommended that future measurements for termite wings take note of the differences in length between the fore- and hind wings.

The matrix key presented in this study reflects a broad range of values based on the number of specimens examined (soldier, N=519, alate, N= 851) and we believe captures the diversity seen in the genus *Reticulitermes* in Georgia, USA. The structure of the matrix key allows the user to start with any one of several character states independently and progress on a chosen path. This should avoid the blocked path encountered with dichotomous key when a couplet requires a character that cannot be determined from a specimen in-hand. Soldier and

alate data are incorporated within the matrix key and users are encouraged to have specimens of both castes for definitive determination of specimens. The suggested routes were designed with statistical support to increase user confidence in proper species determination when using the recommended number of specimens (Figs. 3.9a-e, 3.11a-d, 3.12, 3.14).

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Table 3.1. Collection data for soldier termite specimen head capsule.

Species	Soil province	County	Collection localities	[a]Collection Type	[b]N	Collection date
R. flavipes	Piedmont	Clarke Co.	BOT 4	L	2	Oct 2007
R. flavipes	Piedmont	Clarke Co.	BOT 6	L	9	Oct 2007
R. flavipes	Piedmont	Clarke Co.	Bio Sci2	DC	12	Apr 2009
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	BH 22	IP	10	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	NINA 2	IP	11	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	SI001	L	3	Jul 2009
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	SI004	L	8	Jul 2009
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	SI020	L	1	Jul 2009
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	SI021	L	1	Jul 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP001A	L	1	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP002A	L	7	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP002B	L	1	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP002F	L	1	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP003A	L	7	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP003B	L	2	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP004A	L	8	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	GP004C	L	3	Aug 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P1.45	TD	1	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.181	TD	2	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.183	TD	1	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.186	TD	3	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.188	TD	2	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.199	TD	4	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.202	TD	1	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.209	TD	1	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.211	TD	1	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.216	TD	3	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.234	TD	4	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P4.317	TD	1	Nov 2009

R. flavipes	Southern Coastal Plain	Thomas Co.	P3.173	TD	2	Nov 2009
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.195	TD	5	Mar 2010
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.204	TD	4	Mar 2010
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.212	TD	2	Mar 2010
R. flavipes	Southern Coastal Plain	Thomas Co.	P3.223	TD	2	Mar 2010
			Total # col. localities = 35		N = 126	
R. virginicus	Piedmont	Clarke Co.	BOT 5	L	7	Oct 2007
R. virginicus	Piedmont	Clarke Co.	BOT 10	L	12	Oct2007
R. virginicus	Piedmont	Clarke Co.	BOT 3	L	2	Oct 2007
R. virginicus	Piedmont	Clarke Co.	Cobb1	B	2	Nov 2007
R. virginicus	Piedmont	Clarke Co.	FT 3	B	2	Nov 2007
R. virginicus	Piedmont	Clarke Co.	MAN 1	L	1	Nov 2007
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	BRZ 3	IP	17	Nov 2007
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI002	L	6	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI005	L	5	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI007	L	1	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI009	L	2	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI010	L	4	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI013	L	14	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI014	L	4	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI015	L	3	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI016	L	3	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	SI017	L	1	Jul 2009
R. virginicus	Atlantic Coastal Flatwoods	Camden Co.	CI001	L	16	May 2009
R. virginicus	Southern Coastal Plain	Thomas Co.	GP003D	L	6	Aug 2009
			Total # col. localities = 19		N = 108	
R. hageni	Piedmont	Clarke Co.	FT 8	B	8	Nov 2007
R. hageni	Piedmont	Clarke Co.	FT 27	B	19	Aug 2007
R. hageni	Piedmont	Lamar Co.	Barn Barnsville	L	15	Jul 1996
R. hageni	Piedmont	Lamar Co.	Tyler 6	?	7	? 1998

R. hageni	Piedmont	Lamar Co.	Barn Barnsville (Cultured)	L	8	Jul 1996
R. hageni	Piedmont	Lamar Co.	Barn Barnsville N = 5	L	20 N = 77	May 2009
R. malletei	Piedmont	Clarke Co.	BOT 1	L	4	Oct 2007
R. malletei	Piedmont	Clarke Co.	BOT 7	L	3	Oct 2007
R. malletei	Piedmont	Clarke Co.	BOT 9	L	45	Oct 2007
R. malletei	Piedmont	Clarke Co.	BOT 12	L	11	Oct 2007
R. malletei	Piedmont	Clarke Co.	BOT 13	L	40	Oct 2007
R. malletei	Piedmont	Clarke Co.	BioS4	B	2	Nov 2007
			Total # col. localities = 6		N = 105	
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BH 25,	IP	7	Nov2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-N_frm D3	TD	8	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF OP-D	TD	9	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-G	TD	2	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-H	TD	3	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-I	TD	4	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-J	TD	11	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BASF BP-N_frm H13	TD	8	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	SI003	L	2	Jul 2009
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	SI006	L	1	Jul 2009
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	SI008	L	2	Jul 2009
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	SI012	L	2	Jul 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.10	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.21	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.25	TD	4	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.26	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.30	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.51	TD	2	Nov 2009

R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.54	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.66	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.74	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.80	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.81	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P2.104	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P2.140	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.163	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.173	TD	3	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.201	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.235	TD	2	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P4.270	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P4.315	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.1	TD	1	Jan 2010
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.34	TD	1	Nov 2009
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.210	TD	1	Jan 2010
R. nelsonae	Southern Coastal Plain	Thomas Co.	P3.235	TD	2	Mar 2010
R. nelsonae	Southern Coastal Plain	Thomas Co.	P1.52	TD	1	Nov 2009
			Total # col. localities = 49		N = 96	
			Total # col. localities for 5 species = 120		Total N for all 5 species = 510	

[a] Collection type:
IP = inspection port,
TD = termite detector,
B= 5-gal. plastic bucket,
DC = directed collection from a swarm event,
L = infested timber (log)

[b]N= number of soldier head capsules

Table 3.2. Collection data for alate termite specimen.

Species	Soil province	County	Collection localities	[a]Collection Type	[b]N	Collection date
R. flavipes	Piedmont	Clarke Co.	UGA Bookstore (back stairwell)	DC	4	Mar 2002
R. flavipes	Piedmont	Clarke Co.	Clark Howell Hall (Lumpkin St. Entrance)	DC	27	Mar 2002
R. flavipes	Piedmont	Clarke Co.	Academic 104A	DC	4	Mar 2002
R. flavipes	Piedmont	Clarke Co.	Whitehall Log 5	L	23	Jan 2003
R. flavipes	Piedmont	Clarke Co.	Whitehall	L	10	Mar 2003
R. flavipes	Piedmont	Clarke Co.	Whitehall Site 4	L	10	Apr 2003
R. flavipes	Piedmont	Clarke Co.	Site 5 Whitehall	L	40	Apr 2003
R. flavipes	Piedmont	Clarke Co.	Steve Martin log A	L	14	Feb 2005
R. flavipes	Piedmont	Clarke Co.	Steve Martin log B	L	14	Feb 2005
R. flavipes	Piedmont	Clarke Co.	Rudolph log	L	18	Apr 2005
R. flavipes	Piedmont	Clarke Co.	Total Package A log	L	51	Jan 2006
R. flavipes	Piedmont	Clarke Co.	Mr. Wizard log, AM049	L	16	Apr 2006
R. flavipes	Piedmont	Clarke Co.	LeConte 135	DC	27	Mar 2008
R. flavipes	Piedmont	Clarke Co.	Ramsey Center	DC	4	Mar 2008
R. flavipes	Piedmont	Clarke Co.	Alates Log 93	L	50	Mar 2008
R. flavipes	Piedmont	Clarke Co.	GMOA Lobby	DC	9	Mar 2008
R. flavipes	Piedmont	Clarke Co.	186 Ecology	DC	36	Mar 2009
R. flavipes	Piedmont	Clarke Co.	111A LeConte Hall	DC	14	Mar 2009
R. flavipes	Piedmont	Clarke Co.	Holmes-Hunter 106A	DC	11	Mar 2009
R. flavipes	Piedmont	Clarke Co.	Academic Building (north crawl entrance)	DC	23	Apr 2009
R. flavipes	Piedmont	Clarke Co.	BioSci2	DC	27	Apr 2009
R. flavipes	Piedmont	Clarke Co.	Dean Rusk Hall	DC	7	Apr 2009
R. flavipes	Piedmont	Clarke Co.	UGA Chapel	DC	5	April 2010
R. flavipes	Piedmont	Clarke Co.	UGA Demosthenian	DC	10	April 2010
R. flavipes	Piedmont	Clarke Co.	Belle log	L	25	Oct 2005

R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	Yvonne's house	DC	16	Mar 2002
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	BH 13	DC	24	Mar 2000
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	BH 17	DC	21	Mar 2005
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	BH 3	DC	9	Jan 2005
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	OP-H	DC	50	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	OP-I	DC	50	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	Pine14 block	DC	50	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	PPI	DC	12	Nov 2007
R. flavipes	Atlantic Coastal Flatwoods	McIntosh Co.	Nina 4	DC	13	Nov 2007
			Total # col. localities = 27		N =649	
R. virginicus	Piedmont	Clarke Co.	Brian's Stump	DC	15	May 2006
R. virginicus	Piedmont	Clarke Co.	Chicopee Hallway	DC	15	May 2008
R. virginicus	Atlantic Coastal Flatwoods	McIntosh Co.	Alates Stump at Mary's	DC	35	May 2008
			Total # col. localities =3		N = 65	
R. hageni	Piedmont	Clarke Co.	FT9 Station 4	DC	81	July 2007
R. hageni	Piedmont	Commerce Co.	AM002	DC	15	Aug 2008
			Total # col. localities = 2		N = 96	
R. malletei	Piedmont	Clarke Co.	Dellwood Site 1	DC	50	May 2008
R. malletei	Piedmont	Clarke Co.	Dellwood Site 1	DC	13	May 2010
R. malletei	Piedmont	Clarke Co.	Dellwood Site 2	DC	8	May 2010
R. malletei	Piedmont	Clarke Co.	Dellwood Site 3	DC	3	May 2010
R. malletei	Piedmont	Clarke Co.	Ramsey Center	DC	1	May 2010
R. malletei	Piedmont	Clarke Co.	AM012	DC	1	May 2010
R. malletei	Piedmont	Clarke Co.	AM013	DC	1	May 2010
R. malletei	Piedmont	Clarke Co.	4 Towers	DC	20	May 2008
R. malletei	Piedmont	Oconee Co.	Whippoorwill	DC	12	May 2010
			Total # col. localities = 9		N = 109	
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-G	DC	14	Feb 2007

R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-I	DC	6	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-G	DC	19	Mar 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-N	DC	19	Mar 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	OP-D	DC	5	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-N	DC	14	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-H	DC	1	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	BP-J	DC	13	Feb 2007
R. nelsonae	Atlantic Coastal Flatwoods	McIntosh Co.	PPF	DC	50	May 2005
			Total # col. localities = 9		N = 141	
			Total # col. Localities for 5 species = 49		Total N for all 5 species = 851	

[a] Collection type:
IP = inspection port,
TD = termite detector,
B= 5-gal. plastic bucket,
DC = directed collection from a swarm event,
L = infested timber (log)

[b]N= number of soldier head capsules

Table 3.3. Description of soldier and alate morphological and biological characters measured and utilized in matrix key.

Caste		Type of characters	Characters
Soldier	Morphometric	Quantitative	1. Head capsule length (*sl*), mm: measured from the clypeal sulcus to the border of the occipital foramen (Fig. 3.1). 2. Head capsule width (*sw*), mm: measured at the mid-point of the head length measured (Fig. 3.1). 3. Ratio (*sl:sw*) of Length (*sl*): Width (*sw*): calculated by dividing the length (*sl*) by width (*sw*) of the head capsule. 4. Right mandible curvature angle : *sma1* (Fig 3.4) 5. Right mandible curvature angle : *sma2* (Fig 3.4)
Alate		Qualitative	6. Alate body length (*abl*), mm: measured from the tip of the head to the end of abdomen (Fig. 3.7). 7. Alate body-wing (s) (*ablw*), mm: measured from the tip of the head to the tip of wing(s) (Fig. 3.7) 8. Alate forewing (*afw*), mm: measured from the end-tip of scale to the tip of the front wing(s) (Fig. 3.7) 9. Alate hind wing (*ahw*), mm: measured from the end-tip of scale to the tip of the hind wing(s) (Fig. 3.7) 10. Alate body color (*abc*) Two categories i) light brown to dark brown, ii) yellowish brown (Fig. 3.6) 11. Alate wing pigmentation (*awp*) Two categories, i) pigmented (wp), ii) non-pigmented (wnp) (Fig. 3.6)
	Behavioral		12. Flight time of collected alate specimens in this study (Table 3.2).

Table 3.4. Categorization of alate qualitative characters.

Qualitative characters	Body color (*abc*)		Wing pigmentation (*awp*)	
Categories	Light to dark brown	Yellowish brown	Pigmented (wp)	Non-pigmented (wnp)
Species classification	*R. flavipes* *R. virginicus* *R. malletei* *R. nelsonae*	*R. hageni*	*R. malletei* *R. hageni*	*R. flavipes* *R. virginicus* *R. nelsonae*

Table 3.5a. Descriptive statistics showing the means, standard deviation, minimum, maximum values for the five characters measured from soldier head capsules.

Species	N	Length (*sl*)					Width (*sw*)				
		Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]
R. flavipes	126	1.69	0.119	1.21	1.91	a	1.04	0.074	0.73	1.17	a
R. virginicus	106	1.63	0.068	1.37	1.84	b	0.92	0.039	0.76	1.01	b
R. hageni	77	1.43	0.092	1.16	1.59	c	0.86	0.030	0.75	0.91	c
R. malletei	105	1.49	0.058	1.33	1.64	d	0.88	0.029	0.78	0.95	c
R. nelsonae	96	1.41	0.127	1.14	1.72	c	0.78	0.054	0.70	0.99	d

Species	N	Ratio (*sl*:*sw*)				
		Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]
R. flavipes	126	1.63	0.084	1.43	1.83	a
R. virginicus	106	1.77	0.063	1.56	1.95	b
R. hageni	77	1.66	0.073	1.44	1.78	c
R. malletei	105	1.70	0.066	1.52	1.87	d
R. nelsonae	96	1.80	0.092	1.52	1.98	e

Species	N	Soldier mandible angle of curvature (*sma*1)					Soldier mandible angle of curvature (*sma*2)				
		Mean,°	Std. dev.	Min.[a],°	Max.[b],°	LSD[c]	Mean,°	Std. dev.	Min.[a],°	Max.[b],°	LSD[c]
R. flavipes	20	10.61	2.09	7.32	15.09	a	27.29	2.730	22.09	31.37	a
R. virginicus	20	13.62	1.72	11.71	18.12	b	32.60	2.003	29.45	36.70	b
R. hageni	20	8.34	1.20	6.95	11.17	c	23.39	1.307	21.54	27.26	c
R. malletei	20	10.51	1.37	8.08	13.22	a	25.85	2.282	22.41	32.88	d
R. nelsonae	20	10.66	2.21	7.20	14.56	a	27.27	2.654	24.06	33.95	a d

Std. dev. = Standard deviation values calculated for means
Min.[a]= Minimum value of measure found within the dataset
Max.[b]= Maximum value of measure found within the dataset
LSD[c] = LSD means separation

Table 3.5b. Descriptive statistics showing the means, standard deviation, minimum, maximum values for the four characters measured from alates.

Species	Body (*abl*)						Body-wing (*ablw*)					
	N	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]	N	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]
R. flavipes	431	4.783	0.383	3.77	5.83	a	255	8.973	0.402	8.05	9.94	a
R. virginicus	65	4.021	0.214	3.56	4.44	b c	64	7.414	0.213	6.89	7.90	b
R. hageni	96	4.083	0.323	3.41	5.35	b	57	7.810	0.318	7.25	8.64	c
R. malletei	118	4.023	0.302	3.53	4.99	b	94	8.238	0.394	6.91	9.28	d
R. nelsonae	141	3.928	0.236	3.26	4.63	c	62	7.080	0.291	6.53	7.88	e

Species	Forewing (*afw*)						Hind wing (*ahw*)					
	N	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]	N	Mean, mm	Std. dev.	Min.[a], mm	Max.[b], mm	LSD[c]
R. flavipes	245	6.810	0.331	5.97	7.74	a	244	6.550	0.332	5.70	7.44	a
R. virginicus	32	5.532	0.193	5.15	6.05	b	64	5.418	0.193	4.78	5.78	b
R. hageni	58	5.965	0.263	5.47	6.52	c	56	5.739	0.257	5.24	6.19	c
R. malletei	91	6.375	0.328	5.13	7.31	d	90	6.100	0.339	4.92	6.95	d
R. nelsonae	52	5.430	0.212	4.94	5.98	b	61	5.315	0.297	4.81	6.21	b

Abbreviation as in Table 4.5a.

Table 3.6. One-way analysis of variance (ANOVA) of *Reticulitermes* spp. soldier and alate characters in relation to species.

Characters	Sum of Square	Mean Square	F Value	Pr > F
Length	6.619	1.655	173.54	<0.0001
Width	4.050	1.013	399.85	<0.0001
Ratio	2.180	0.545	93.33	<0.0001
sma1	408.327	102.082	32.95	<0.0001
sma2	909.44	227.36	44.71	<0.0001
Body	130.46	32.614	292.46	<0.0001
Body-wing	272.03	68.007	517.23	<0.0001
Hind wing	124.02	31.005	327.59	<0.0001
Forewing	124.78	31.196	337.09	<0.0001

Df = 4

Table 3.7. Pearson Correlation Coefficients between the three soldier characters measured, N=516 with prob > |r| under H0: Rho = 0.

	Length	**Width**	**Ratio**
Length	1	0.84834	0.05285
		<0.0001	0.2334
Width	0.84834	1	-0.47920
	<0.0001		<0.0001
Ratio	0.05285	-0.47920	1
	0.2257	<0.0001	

Table 3.8. Summary of Step-Wise Discriminant Analysis (SWDA) for soldier characters.

Step	Variables entered	Partial R-Square	F Value	Pr >F	Wilks' Lambda	Pr< Lambda	Avg. Sq. Canonical Correlation	Pr> ASCC
1	Width (*sw*)	0.7605	400.97	<0.0001	0.23946172	<0.0001	0.19013457	<0.0001
2	Ratio (*sl:sw*)	0.2848	50.81	<0.0001	0.17125755	<0.0001	0.25908429	<0.0001
3	Length (*sl*)	0.2332	38.25	<0.0001	0.13131542	<0.0001	0.31722434	<0.0001

Table 3.9a. Number of observations and percent classified into species from Discriminant Function Analysis (DFA) utilizing *sl* and *sw* of soldier head capsule.

	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Resubsititution	*R. flavipes*	**103 (81.75)**	16 (12.70)	4 (3.17)	2 (1.59)	1 (0.79)	126 (100)
	R. virginicus	5 (4.72)	**92 (85.79)**	0 (0)	7 (6.60)	2 (1.89)	106 (100)
	R. hageni	0 (0)	1 (1.30)	**29 (37.66)**	45 (58.44)	2 (2.60)	77 (100)
	R. malletei	0 (0)	14 (13.33)	14 (13.33)	**70 (66.67)**	7 (6.67)	105 (100)
	R. nelsonae	1 (1.04)	13 (13.54)	3 (3.13)	2 (2.08)	**77 (80.21)**	96 (100)
	Total	109 (21.37)	136 (26.67)	50 (9.80)	126 (24.71)	89 (17.45)	**510 (100)**
	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Crossvalidation	*R. flavipes*	**103 (81.75)**	16 (12.70)	4 (3.17)	2 (1.59)	1 (0.79)	126 (100)
	R. virginicus	5 (4.72)	**91 (85.85)**	0 (0)	8 (7.55)	2 (1.89)	106 (100)
	R. hageni	0 (0)	1 (1.30)	**28 (36.36)**	46 (59.74)	2 (2.60)	77 (100)
	R. malletei	0 (0)	14 (13.33)	14 (13.33)	**69 (65.71)**	8 (7.62)	105 (100)
	R. nelsonae	1 (1.04)	13 (13.54)	3 (3.13)	2 (2.08)	**77 (80.21)**	96 (100)
	Total	109 (21.37)	135 (26.47)	49 (9.61)	127 (24.90)	90 (17.65)	**510 (100)**

Table 3.9b. Error count estimates from Discriminant Function Analysis with proportional prior for *sl* and *sw* of soldier head capsule.

	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*	Total
Resubstitution	Rate	0.3333	0.6234	0.1321	0.1825	0.1979	0.2725[a]
	Priors	0.2059	0.1510	0.2078	0.2471	0.1882	
	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*	Total
Crossvalidation	Rate	0.3429	0.6104	0.1509	0.1905	0.2500	0.2882[a]
	Priors	0.2059	0.1510	0.2078	0.2471	0.1882	

[a]The total error rates were similar for both resubstituted and crossvalidated data.

Table 3.10a. Number of observations and percent classified into species from Discriminant Function Analysis (DFA) utilizing soldier mandible angle of curvature *sma1*.

	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Resubsititution	*R. flavipes*	**1 (5.00)**	3 (15.00)	5 (25.00)	3 (15.00)	8 (40.00)	20 (100)
	R. virginicus	0 (0)	**19 (95.00)**	0 (0)	0 (0)	1 (5.00)	20 (100)
	R. hageni	0 (0)	0 (0)	**17 (85.00)**	1 (5.00)	2 (10.00)	20 (100)
	R. malletei	1 (5.00)	2 (10.00)	4 (20.00)	**6 (30.00)**	7 (35.00)	20 (100)
	R. nelsonae	0 (0)	4 (20.00)	9 (45.00)	1 (5.00)	**6 (30.00)**	20 (100)
	Total	2 (2.00)	28 (28.00)	35 (35.00)	11 (11.00)	24 (24.00)	**100 (100)**
	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Crossvalidation	*R. flavipes*	**1 (5.00)**	3 (15.00)	5 (25.00)	3 (15.00)	8 (40.00)	20 (100)
	R. virginicus	0 (0)	**19 (95.00)**	0 (0)	0 (0)	1 (5.00)	20 (100)
	R. hageni	0 (0)	0 (0)	**17 (85.00)**	1 (5.00)	2 (10.00)	20 (100)
	R. malletei	1 (5.00)	2 (10.00)	5 (25.00)	**5 (25.00)**	7 (35.00)	20 (100)
	R. nelsonae	4 (20.00)	4 (20.00)	9 (45.00)	1 (5.00)	**2 (20.00)**	20 (100)
	Total	6 (6.00)	28 (28.00)	36 (36.00)	10 (10.00)	20 (20.00)	**100 (100)**

Table 3.10b. Error count estimates from Discriminant Function Analysis with proportional prior for soldier mandible angle of curvature *sma1*.

Resubstitution	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*	Total
	Rate	0.7000	0.1500	0.0500	0.9500	0.7000	0.5100 [a]
	Priors	0.2000	0.2000	0.2000	0.2000	0.2000	
Crossvalidation	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R.nelsonae*	Total
	Rate	0.7500	0.1500	0.0500	0.9500	0.9000	0.5600 [a]
	Priors	0.2000	0.2000	0.2000	0.2000	0.2000	

[a]The total error rates were similar for both resubstituted and crossvalidated data.

Table 3.11a. Number of observations and percent classified into species as determined from Discriminant Function Analysis (DFA) utilizing soldier mandible angle of curvature *sma2*.

	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Resubsititution	*R. flavipes*	**7 (35.00)**	4 (20.00)	3 (15.00)	5 (25.00)	1 (5.00)	20 (100)
	R. virginicus	2 (10.00)	**18 (90.00)**	0 (0)	0 (0)	0 (0)	20 (100)
	R. hageni	0 (0)	0 (0)	**18 (90.00)**	1 (5.00)	1 (5.00)	20 (100)
	R. malletei	2 (10.00)	1 (5.00)	3 (15.00)	**13 (65.00)**	1 (5.00)	20 (100)
	R. nelsonae	3 (15.00)	4 (20.00)	3 (15.00)	8 (40.00)	**2 (10.00)**	20 (100)
	Total	14 (14.00)	27 (27.00)	27 (27.00)	27 (27.00)	5 (5.00)	**100 (100)**
	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. hageni* N (%)	*R. malletei* N (%)	*R. nelsonae* N (%)	Total N (%)
Crossvalidation	*R. flavipes*	**6 (30.00)**	4 (20.00)	3 (15.00)	5 (25.00)	2 (10.00)	20 (100)
	R. virginicus	2 (10.00)	**18 (90.00)**	0 (0)	0 (0)	0 (0)	20 (100)
	R. hageni	0 (0)	0 (0)	**18 (90.00)**	1 (5.00)	1 (5.00)	20 (100)
	R. malletei	2 (10.00)	1 (5.00)	3 (15.00)	**13 (65.00)**	1 (5.00)	20 (100)
	R. nelsonae	3 (15.00)	4 (20.00)	3 (15.00)	8 (40.00)	**2 (10.00)**	20 (100)
	Total	13 (13.00)	27 (27.00)	27 (27.00)	27 (27.00)	6 (6.00)	**100 (100)**

Table 3.11b. Error count estimates from Discriminant Function Analysis with proportional prior for soldier mandible angle of curvature *sma2*.

	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*	Total
Resubstitution	Rate	0.3500	0.1000	0.1000	0.6500	0.9000	0.4200 [a]
	Priors	0.2000	0.2000	0.2000	0.2000	0.2000	
	Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*	Total
Crossvalidation	Rate	0.3500	0.1000	0.1000	0.7000	0.9000	0.4300 [a]
	Priors	0.2000	0.2000	0.2000	0.2000	0.2000	

[a]The total error rates were similar for both resubstituted and crossvalidated data.

Table 3.12a. T-test table showing the significance of difference between the right-left forewings and right-left hind wings.

Combination	Species	Mean difference	P- value
Difference: Forewing right-forewing left	*R. flavipes*	0.0108	0.0478
Difference: Forewing right-forewing left	*R. virginicus*	0.0552	0.0005
Difference: Forewing right-forewing left	*R. hageni*	- 0.0157	0.2451
Difference: Forewing right-forewing left	*R. malletei*	- 0.0652	0.513
Difference: Forewing right-forewing left	*R. nelsonae*	0.0212	0.2179
Difference: Hind wing right-hind wing left	*R. flavipes*	0.0223	0.0002
Difference: Hind wing right-hind wing left	*R. virginicus*	0.0315	0.0978
Difference: Hind wing right-hind wing left	*R. hageni*	0.0057	0.7033
Difference: Hind wing right-hind wing left	*R. malletei*	0.0449	<0.0001
Difference: Hind wing right-hind wing left	*R. nelsonae*	0.0147	0.2692

Table 3.12b. T-test of difference determining if average forewing and hind wing lengths were significantly different.

Species name	N	Mean difference	Pr > |t|
R. flavipes	430	0.2617	<0.0001
R. virginicus	32	0.1873	<0.0001
R. hageni	54	0.2490	<0.0001
R. malletei	88	0.2658	<0.0001
R. nelsonae	47	0.2290	<0.0001

Table 3.13. Pearson Correlation Coefficients between the four alates characters measured, body (*abl*) (n=851), body-wing (*ablw*) (n=532), average forewing (*afw*) (n=478) and average hind wing (*ahw*) (n=515) with prob > |r| under H0: Rho =0.

	Body (*abl*)	**Body-Wing (*ablw*)**	**Forewing (*afw*)**	**Hind wing (*ahw*)**
Body (*abl*)	1	0.79158	0.70465	0.71015
		<0.0001	<0.0001	<0.0001
Body-wing (*ablw*)	0.79158	1	0.97093	0.97030
	<0.0001		<0.0001	<0.0001
Forewing (*afw*)	0.70465	0.97093	1	0.98991
	<0.0001	<0.0001		<0.0001
Hind wing (*ahw*)	0.71015	0.97030	0.98991	1
	<0.0001	<0.0001	<0.0001	

Table 3.14. Summary of stepwise discriminant analyses for alate characters.

Step	Variables entered	Partial R-Square	F Value	Pr >F	Wilks' Lambda	Pr< Lambda	Avg. Sq. Canonical Correlation	Pr> ASCC
1	Body-wing (*ablw*)	0.7782	391.25	<0.0001	0.22177807	<0.0001	0.19455548	<0.0001
2	Body (*abl*)	0.2640	29.90	<0.0001	0.16322974	<0.0001	0.26054540	<0.0001
3	Average forewing (*afw*)	0.1671	22.27	<0.0001	0.13595398	<0.0001	0.30157531	<0.0001
4	Average hind wing (*ahw*)	0.0338	3.88	0.0041	0.13135469	<0.0001	0.309597489	<0.0001

Table 3.15a. Number of observations and percent classified as determined from Discriminant Function Analysis (DFA) utilizing *afw* and ablw of alates to differentiate *R. flavipes*, *R. virginicus* and *R. nelsonae*.

Resubstitution					Crossvalidation				
From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. nelsonae* N (%)	Total N (%)	From Species/ Into species	*R. flavipes* N (%)	*R. virginicus* N (%)	*R. nelsonae* N (%)	Total N (%)
R. flavipes	**243 (99.59)**	**1 (0.41)**	0 (0)	244 (100)	*R. flavipes*	**243 (99.59)**	**1 (0.41)**	0 (0)	244 (100)
R. virginicus	**0 (0)**	**25 (78.13)**	7 (21.88)	32 (100)	*R. virginicus*	**0 (0)**	**25 (78.13)**	7 (21.88)	32 (100)
R. nelsonae	0 (0)	1 (1.96)	**50 (98.04)**	51 (100)	*R. nelsonae*	0 (0)	1 (1.96)	**50 (98.04)**	51 (100)
Total	243 (74.31)	27 (8.26)	57 (17.43)	**327 (100)**	Total	243 (74.31)	27 (8.26)	57 (17.43)	**327 (100)**

Table 3.15b. Error count estimates from Discriminant Function Analysis with proportional prior of *afw* and *ablw* length of alates to differentiate *R. flavipes*, *R. virginicus* and *R. nelsonae*

	Species	*R. flavipes*	*R. virginicus*	*R.nelsonae*	Total
Resubstitution	Rate	0.0041	0.2188	0.0196	0.0275 [a]
	Priors	0.7462	0.0979	0.1560	
Crossvalidation	Rate	0.0041	0.2188	0.0196	0.0275[a]
	Priors	0.7462	0.0979	0.1560	

[a]The total error rates for both resubstituted and crossvalidated data.

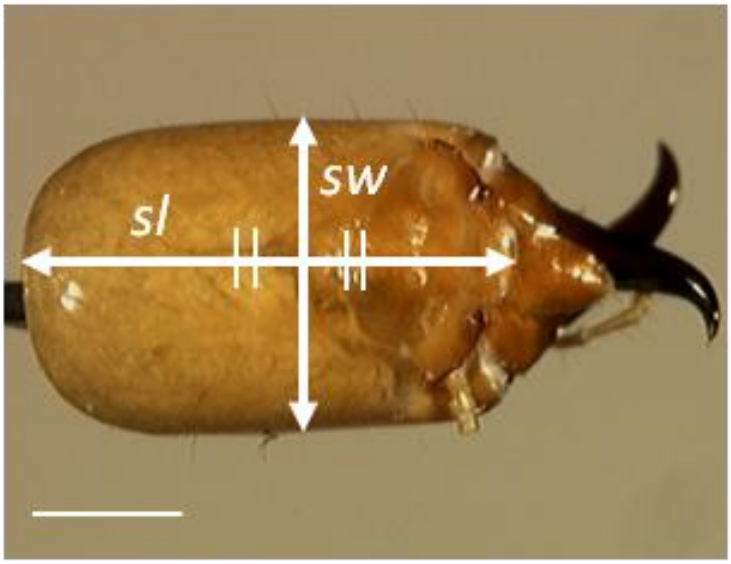

Figure 3.1. Standard measurements for soldier head capsule length (*sl*) and width (*sw*). Note that the length (*sl*) measurement does not include mandibles. Scale bar = 0.50 mm

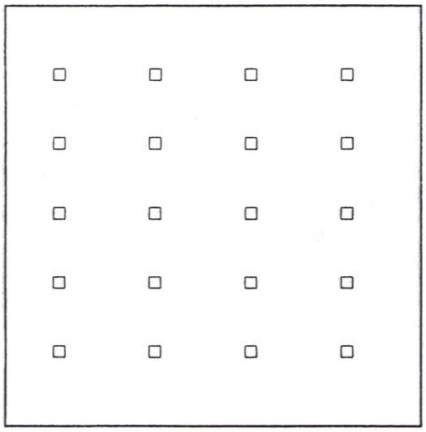

Figure 3.2. Printed ‘2mm x 2mm’ box used to position extracted soldier mandibles. This image contains 20 individual ‘2mm x 2mm’ grid.

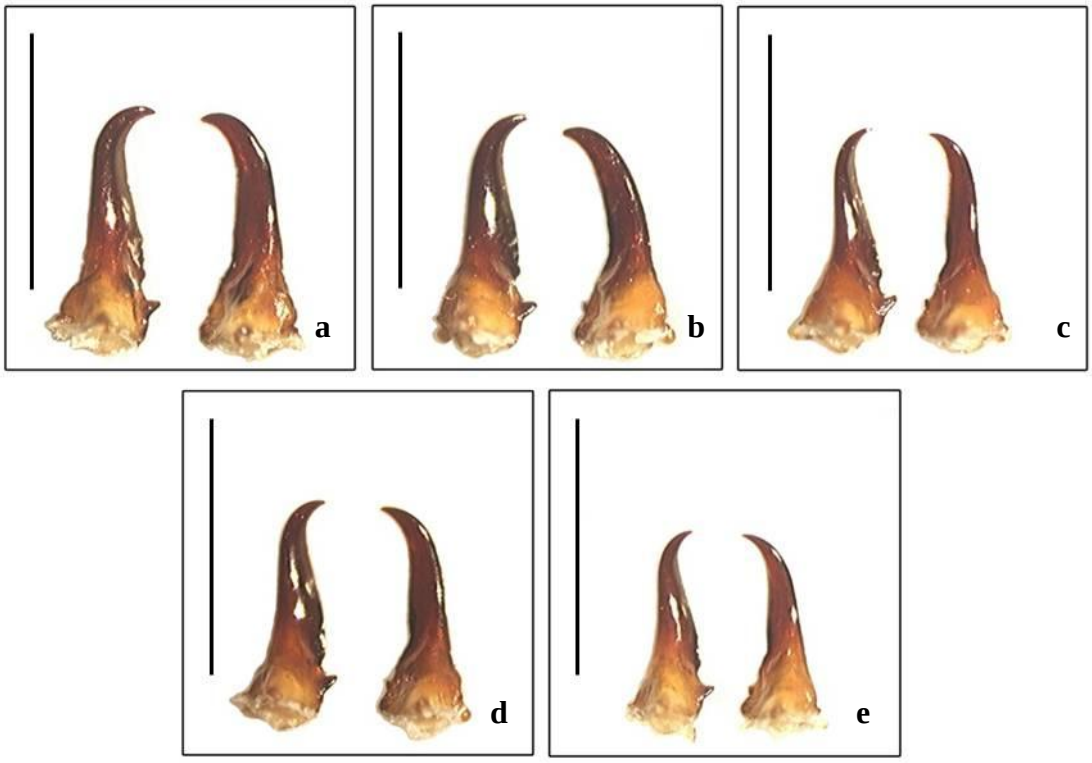

Figure 3.3. *Reticulitermes* spp., soldier mandible pairs, dorsal: **(a)** *R. flavipes*, **(b)** *R. virginicus*, **(c)** *R. hageni*, **(d)** *R. malletei*, **(e)** *R. nelsonae*. Scale bar = 1.00 mm.

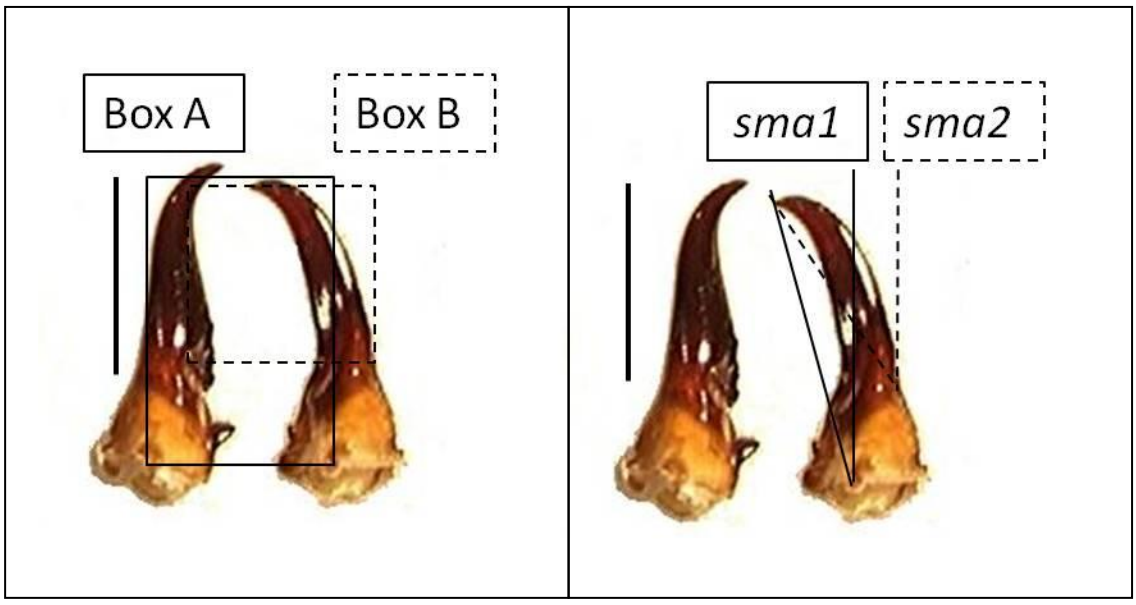

Figure 3.4. Soldier mandibles, dorsal, showing right soldier mandible curvature angle, *sma1* and *sma2* character. Scale bar = 0.50 mm.

Figure 3.5. Alate mounting preparation for imaging.

Figure 3.6. *Reticulitermes* spp. from the southeastern USA. From left to right: *R. virginicus*, *R. nelsonae*, *R. hageni*, *R. malletei*, *R. flavipes*. Scale bar = 1.00 mm.

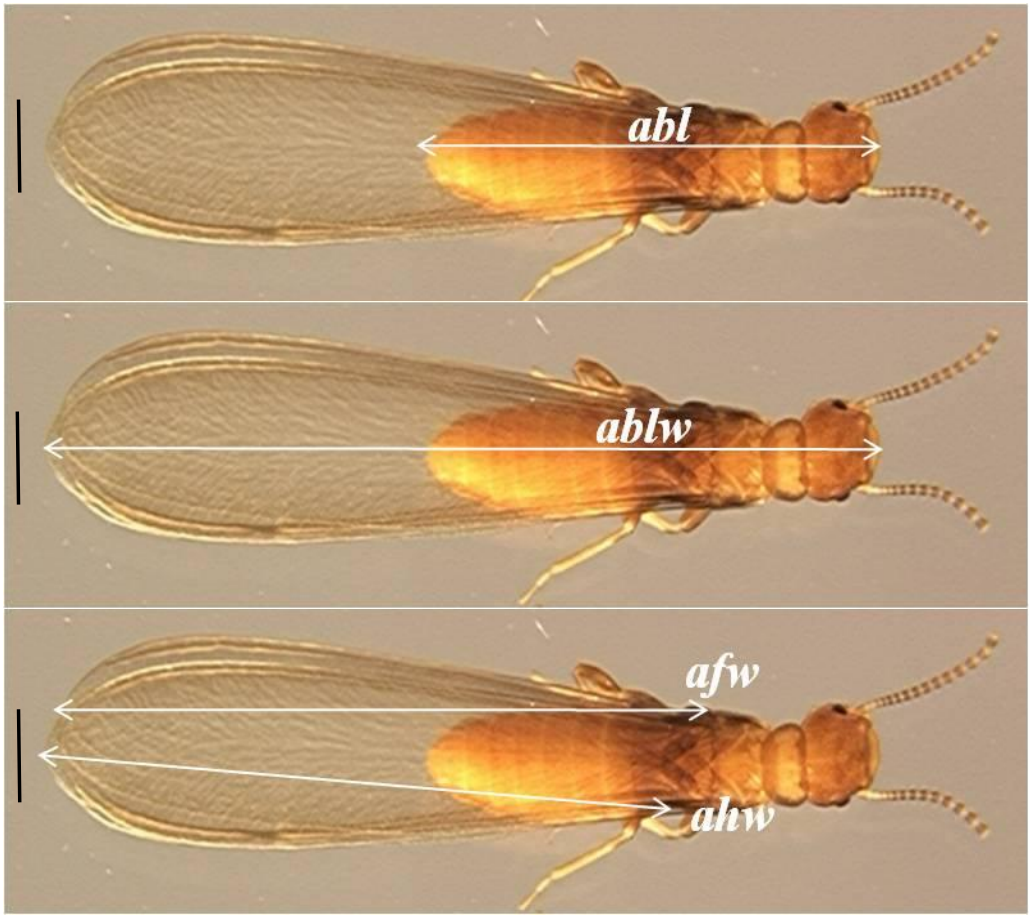

Figure 3.7. Specific measurement points for alate characters: length of body only (*abl*) body-wing (*ablw*), wing (*afw* and *ahw*) measurememements. Scale bar = 1.00 mm.

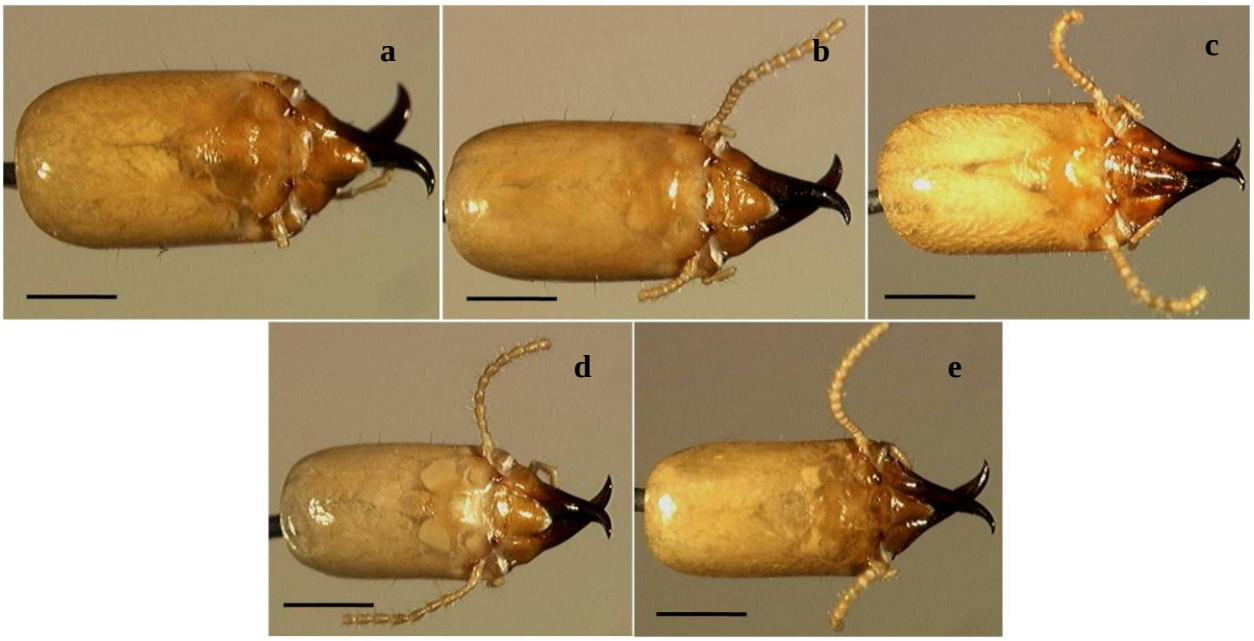

Figure 3.8. *Reticulitermes* spp., soldier head capsules, dorsal: **(a)** *R. flavipes*, **(b)** *R. virginicus*, **(c)** *R. hageni*, **(d)** *R. malletei*, and **(e)** *R. nelsonae*. Scale bar = 0.50 mm.

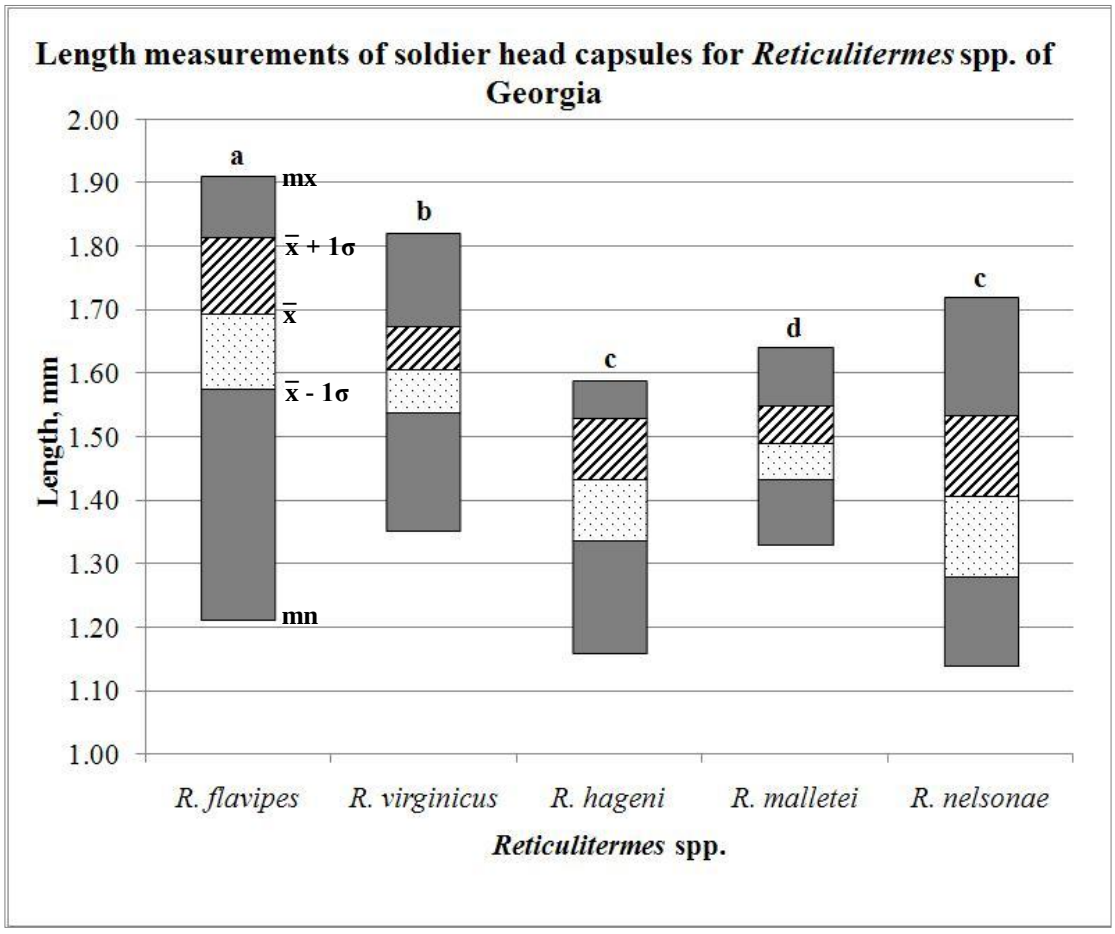

Figure 3.9a. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for length (*sl*) measurement of soldier head capsule of *Reticulitermes* spp.

Symbols placed corresponding to the boxplots lines that denotes its values on the y-axis. All other boxplots have similar relationships.
mn: minimum value
$\bar{x}$ - 1σ: (mean - 1 standard deviation)
$\bar{x}$: mean value
$\bar{x}$ + 1σ: (mean + 1 standard deviation)
mx: maximum value
Alphabets above stacked boxplots (a, b, c, d) reflect t-test (LSD) means separation.

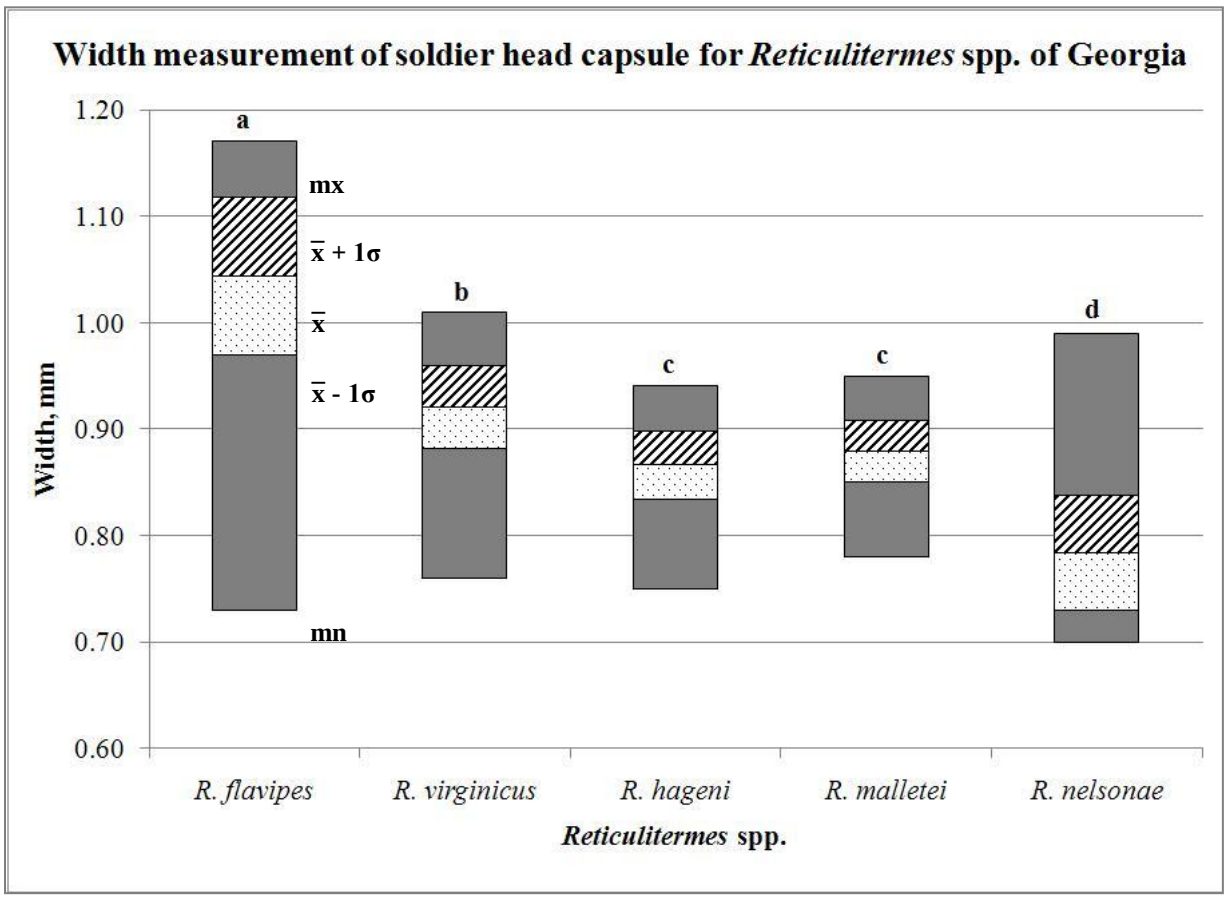

Figure 3.9b. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for width (*sw*) measurement of soldier head capsule of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

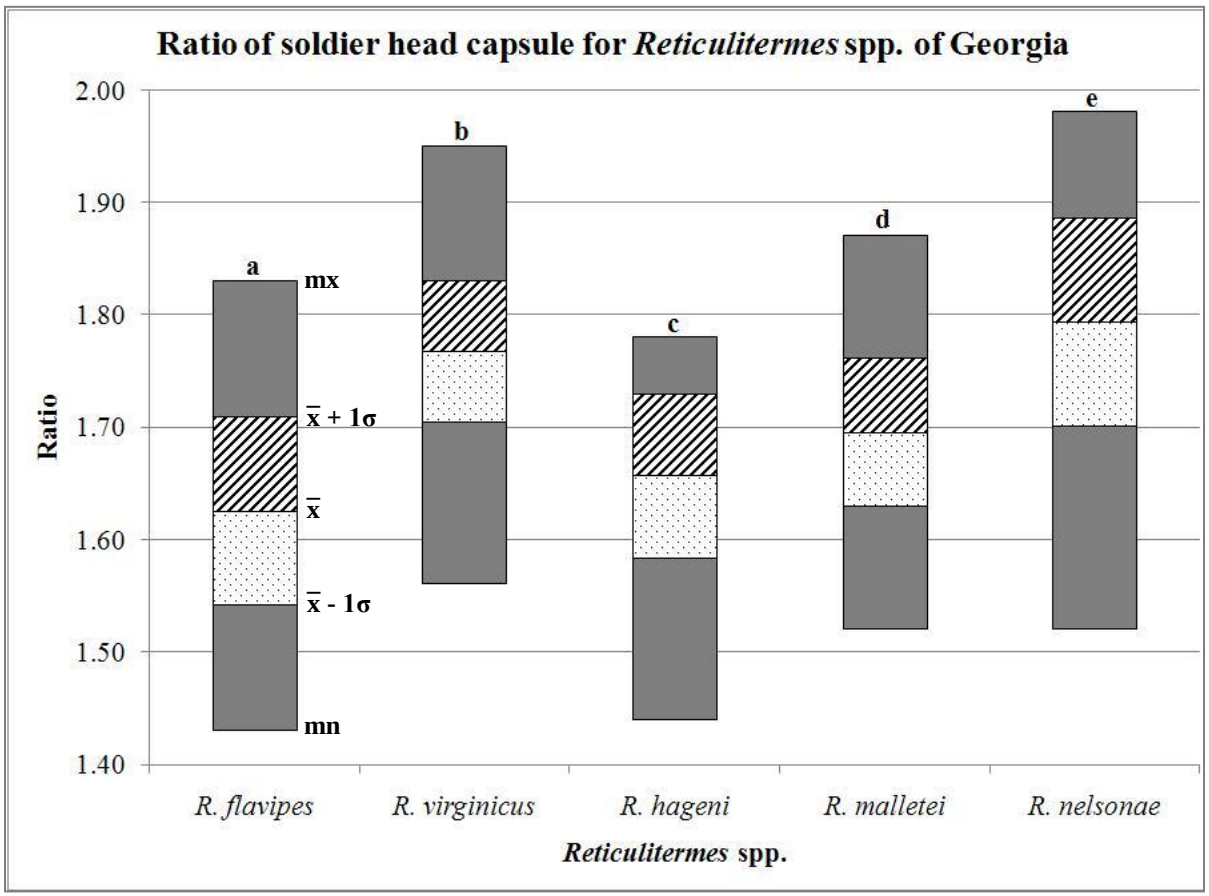

Figure 3.9c. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for ratio (*sl:sw*) measurement of soldier head capsule of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

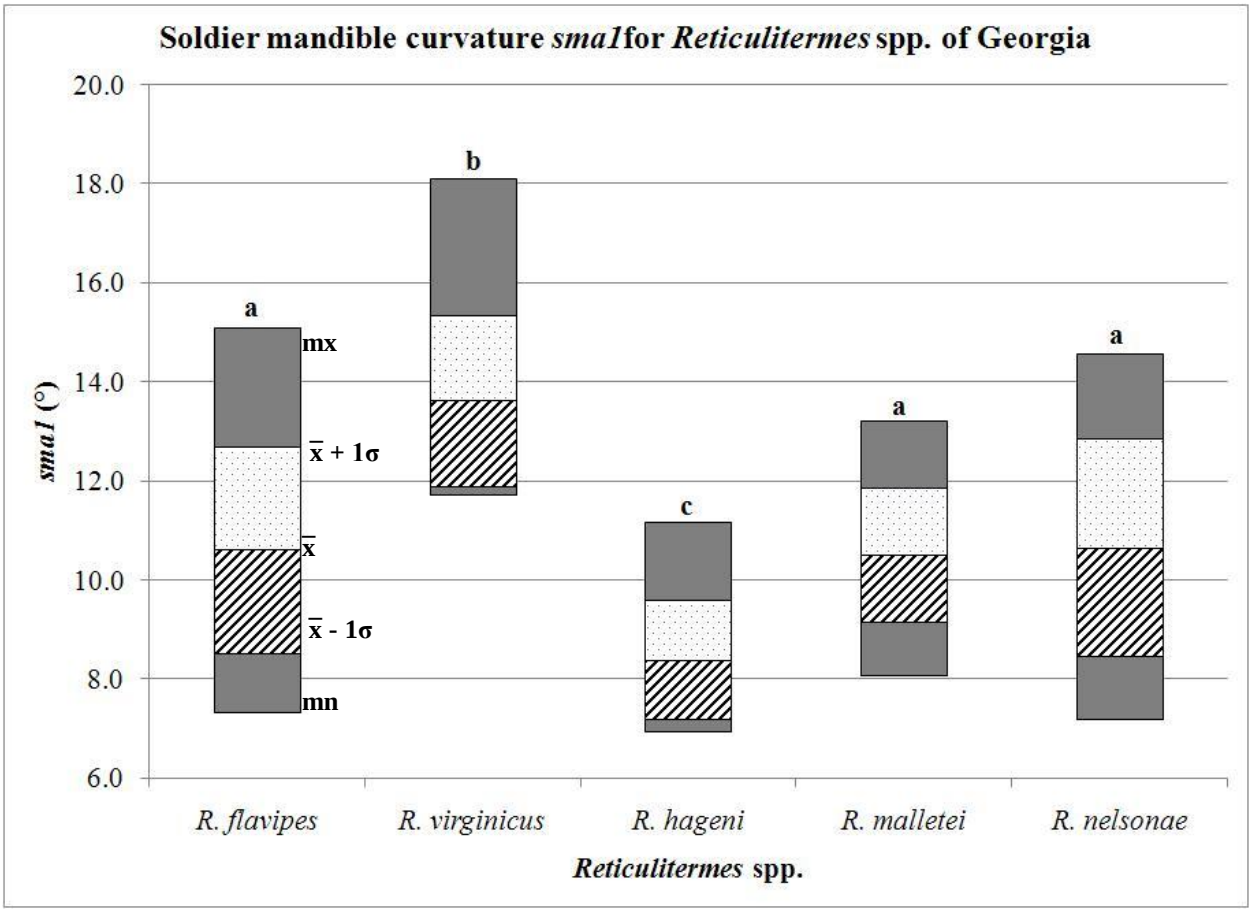

Figure 3.9d. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for *sma1* measurement of soldier head capsule of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

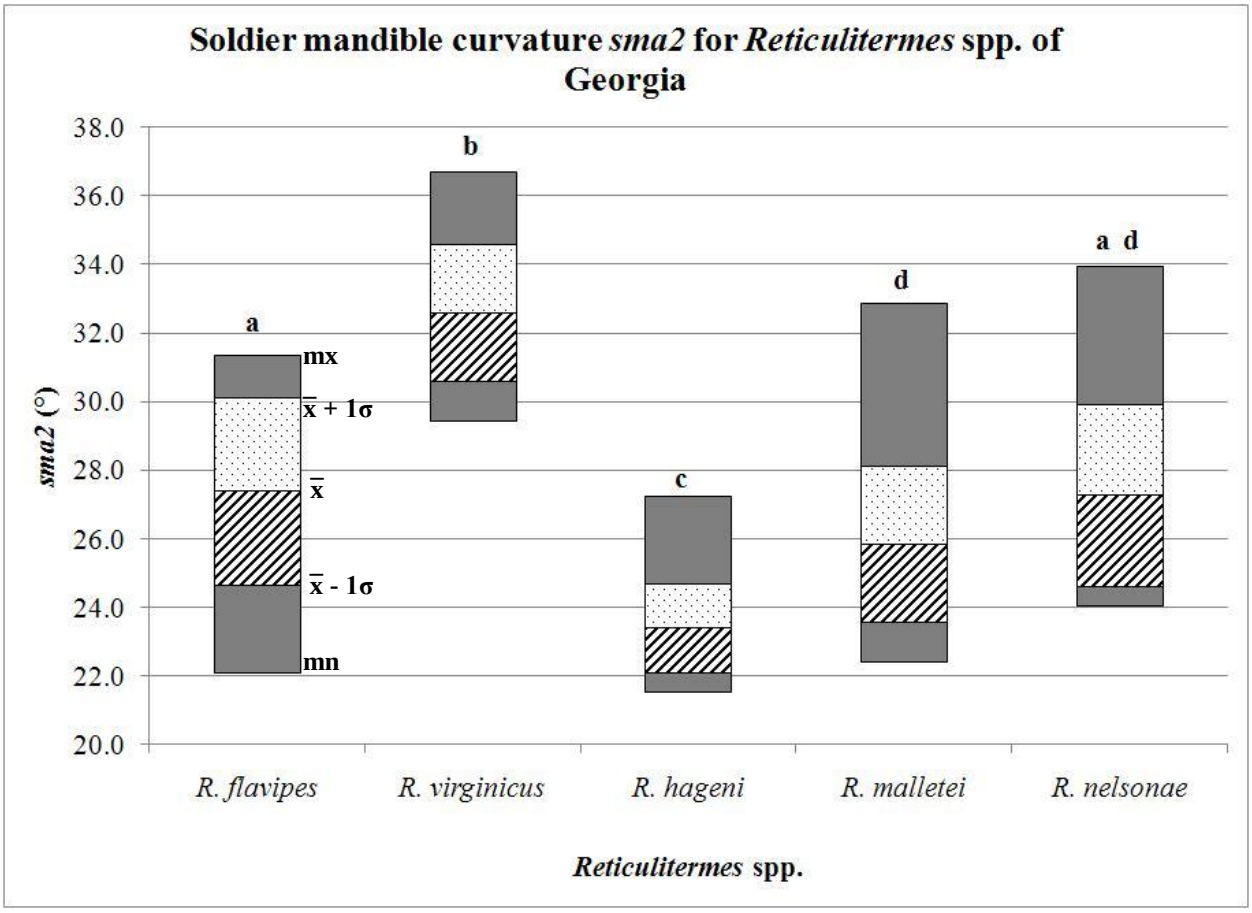

Figure 3.9e. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for *sma2* measurement of soldier head capsule of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

Figure 3.10a. Graph shows the probability of incorrect classification discriminant function analysis (using equal prior) from simulation of 1000000 termite averages for soldiers.

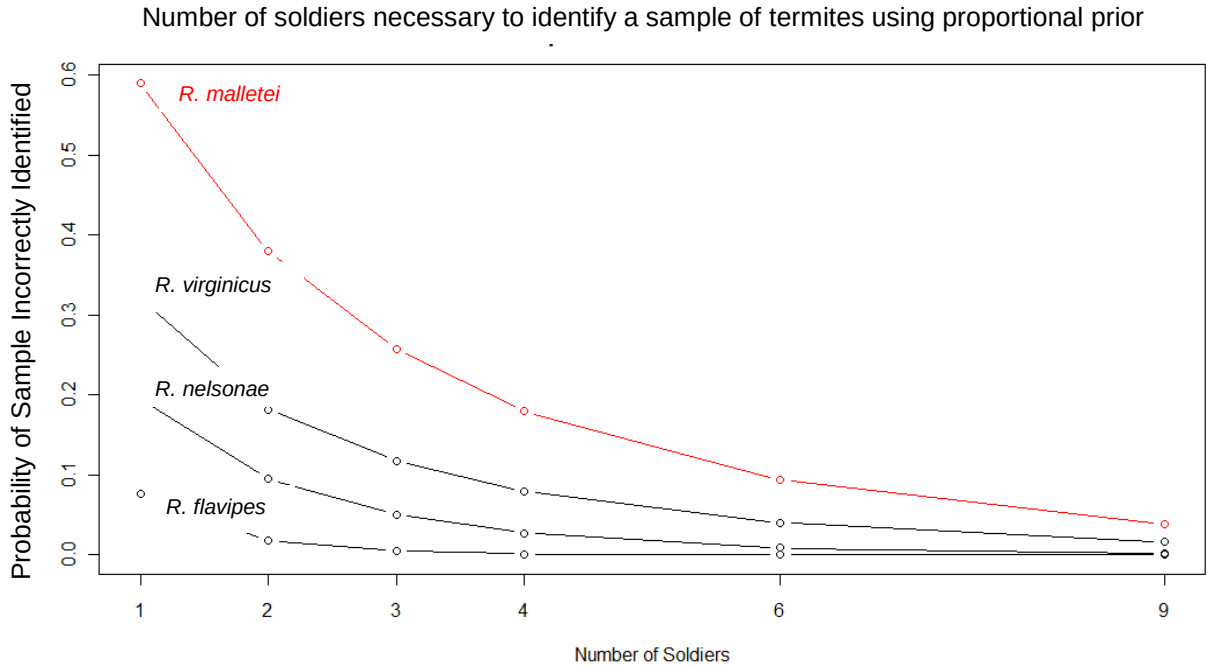

Figure 3.10b. Graph shows the probability of incorrect classification discriminant function analysis (using proportional prior) from simulation of 1000000 termite averages for soldiers.

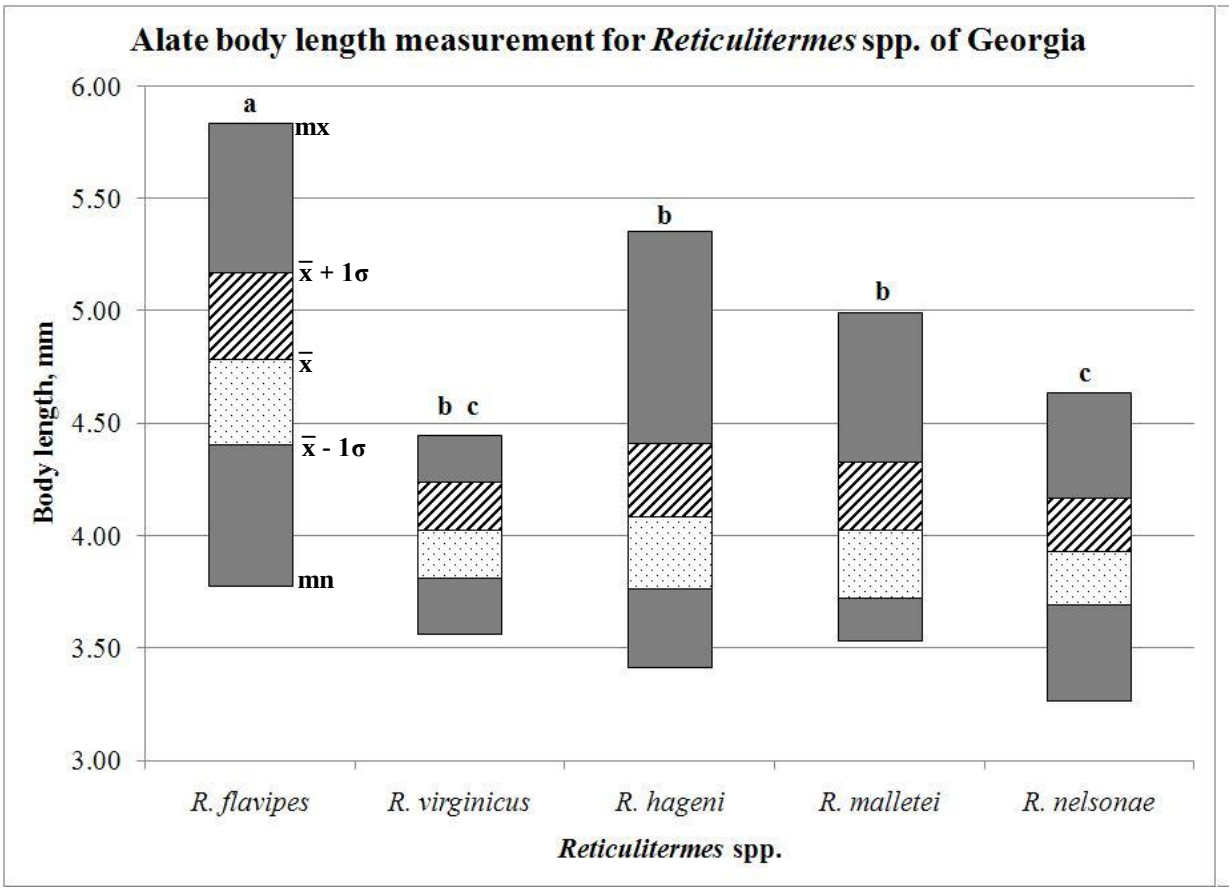

Figure 3.11a. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for alate body length (*abl*) measurement of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

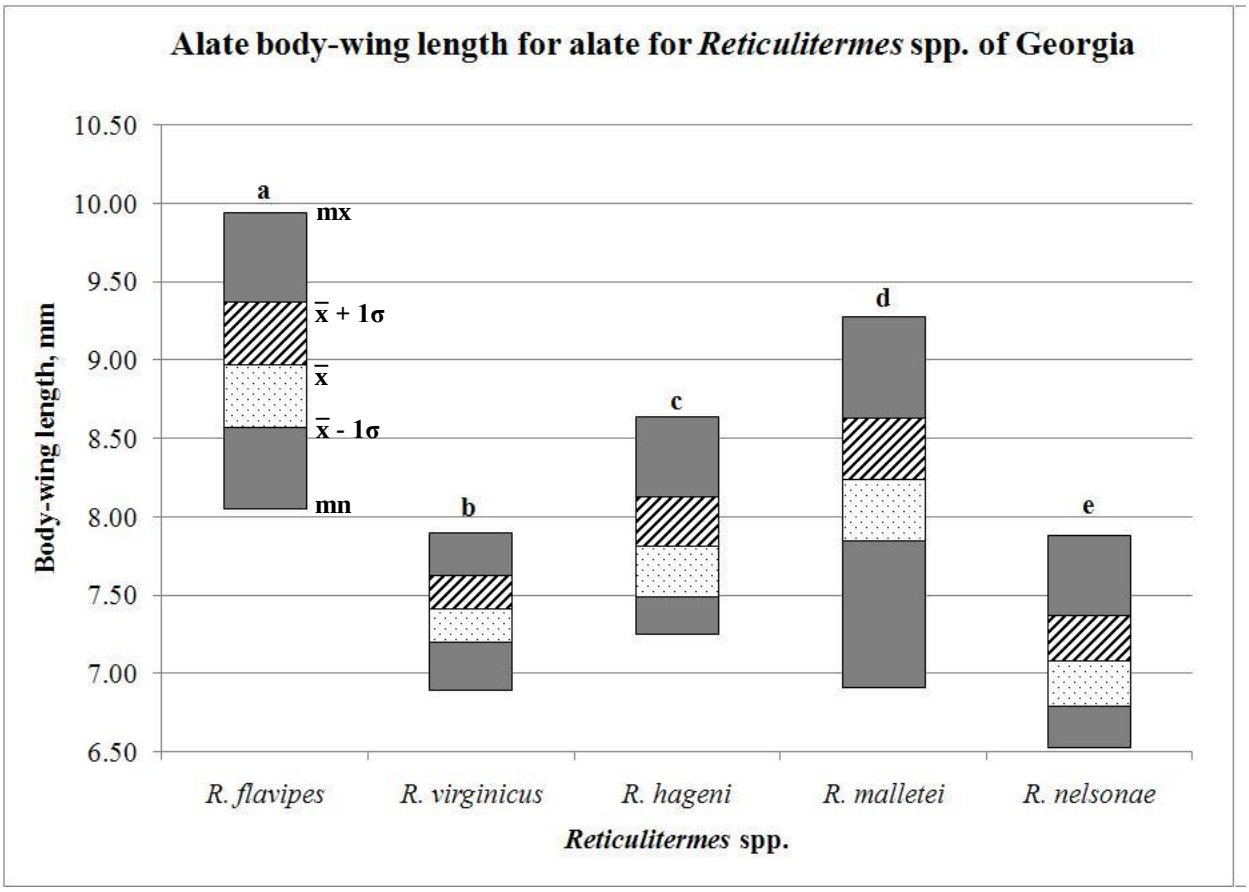

Figure 3.11b. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for alate body-wing length (*ablw*) measurement of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

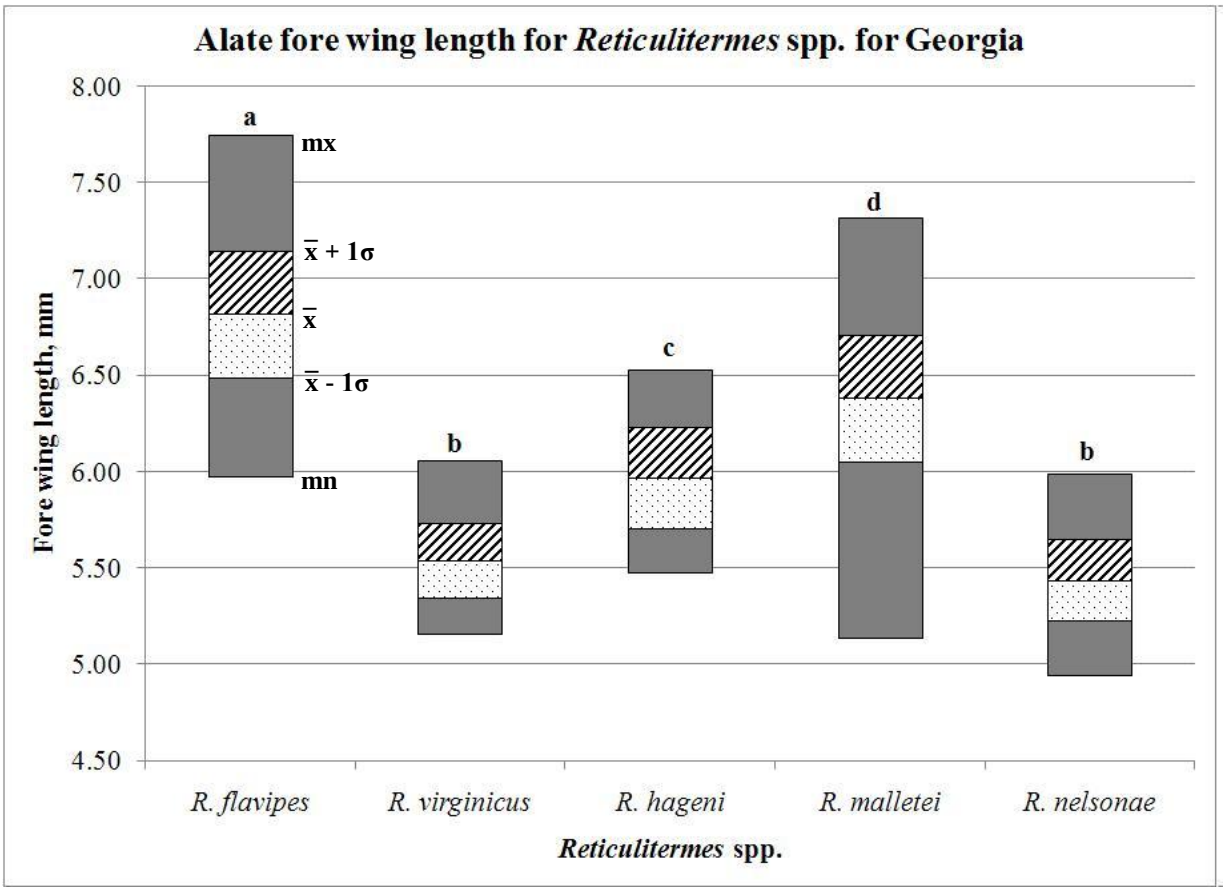

Figure 3.11c. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for alate forewing length (*afw*) measurement of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

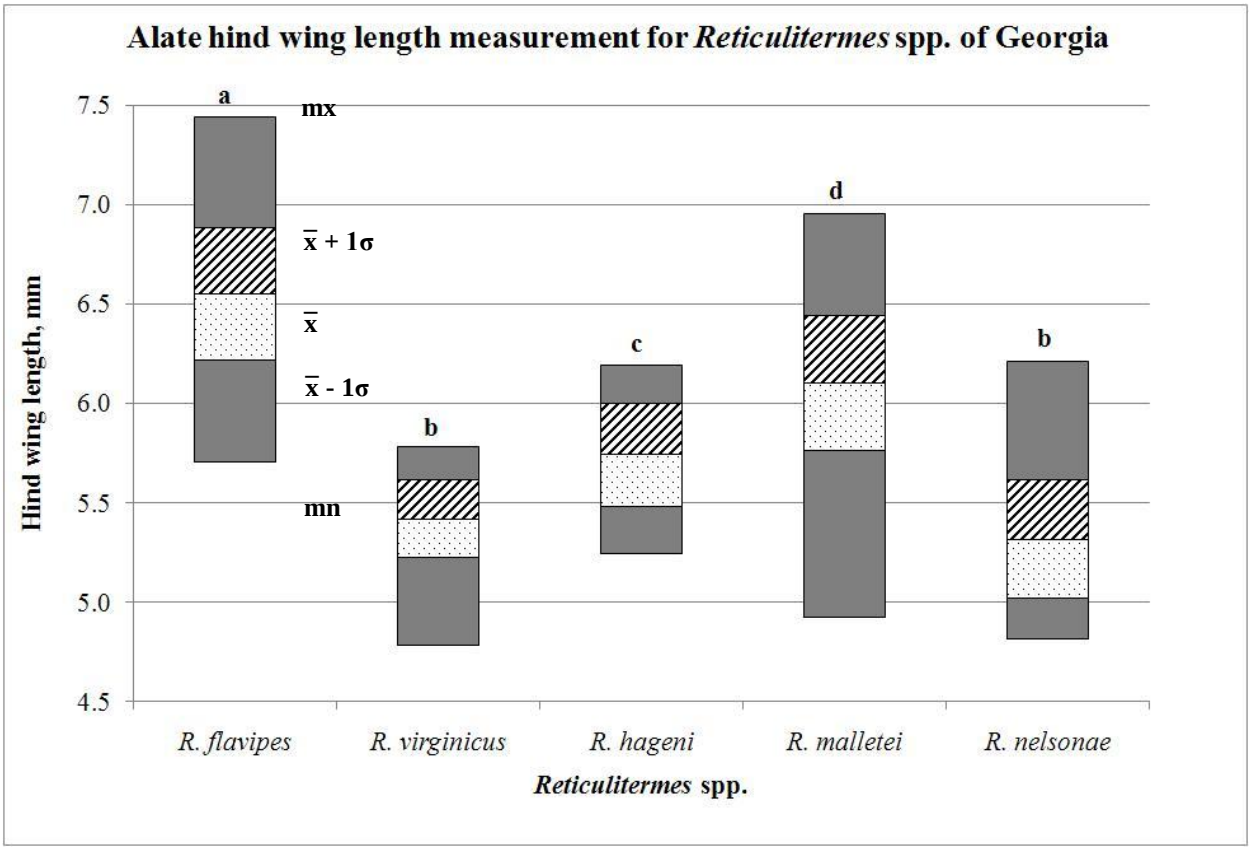

Figure 3.11d. Modified boxplot shows minimum (mn), mean - 1 standard deviation ($\bar{x}$ - 1σ), mean ($\bar{x}$), mean + 1 standard deviation ($\bar{x}$ + 1σ), and maximum (mx) values for alate hind wing length (*ahw*) measurement of *Reticulitermes* spp.

Abbreviation as in Figure 3.9a.

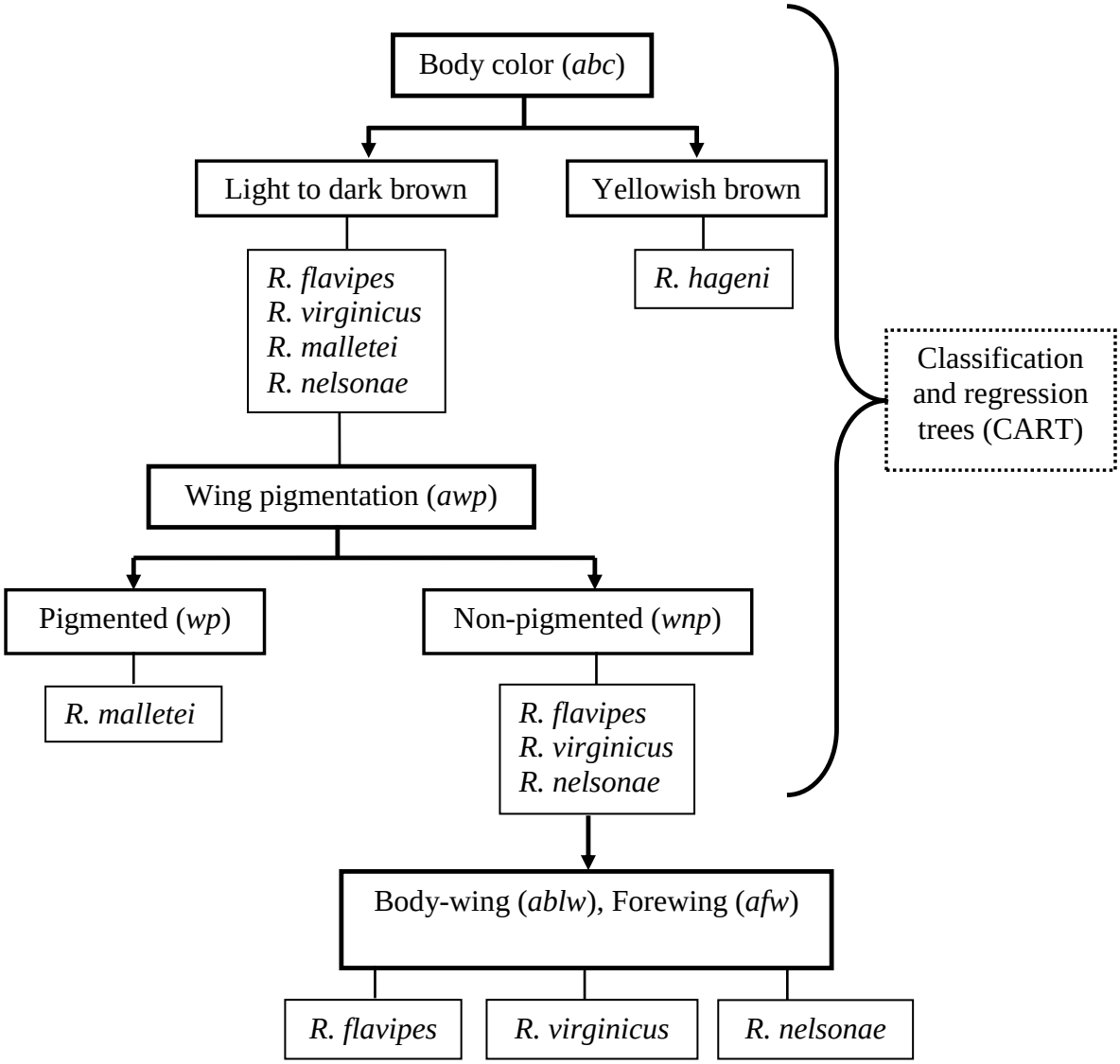

Figure 3.12. Proposed route for species identification for alates of *Reticulitermes* spp. in the Georgia.

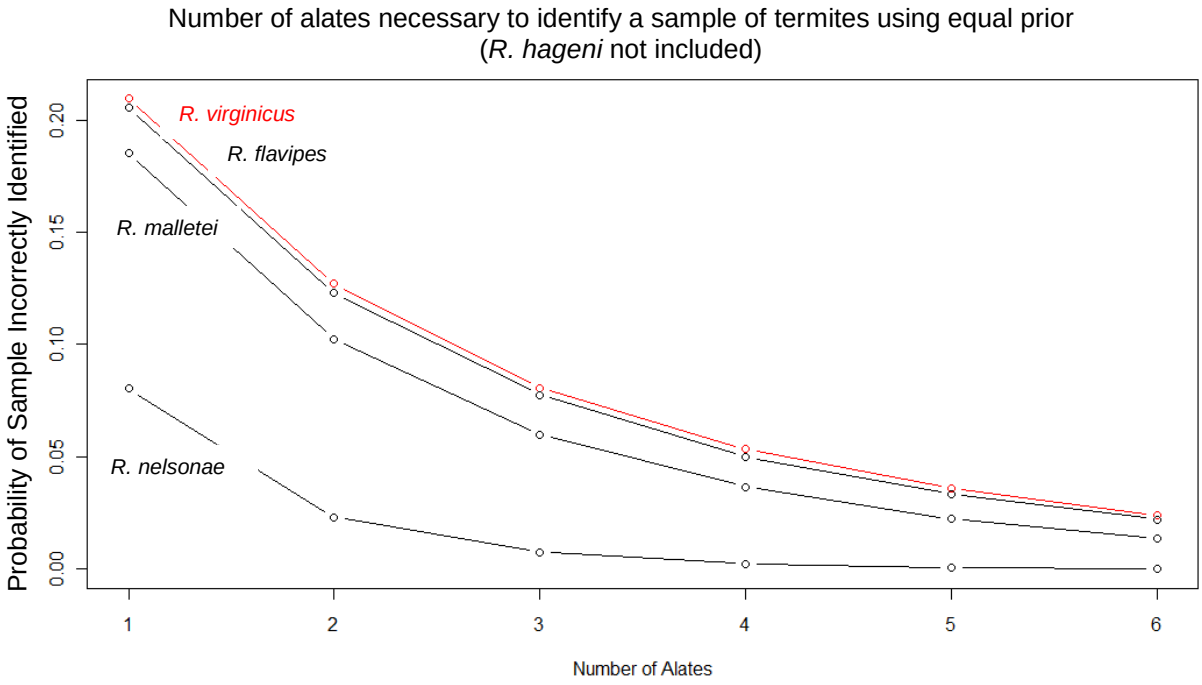

Figure 3.13a. Graph shows the probability of incorrect classification discriminant function analysis (using equal prior) from simulation of 1000000 termite averages for alates.

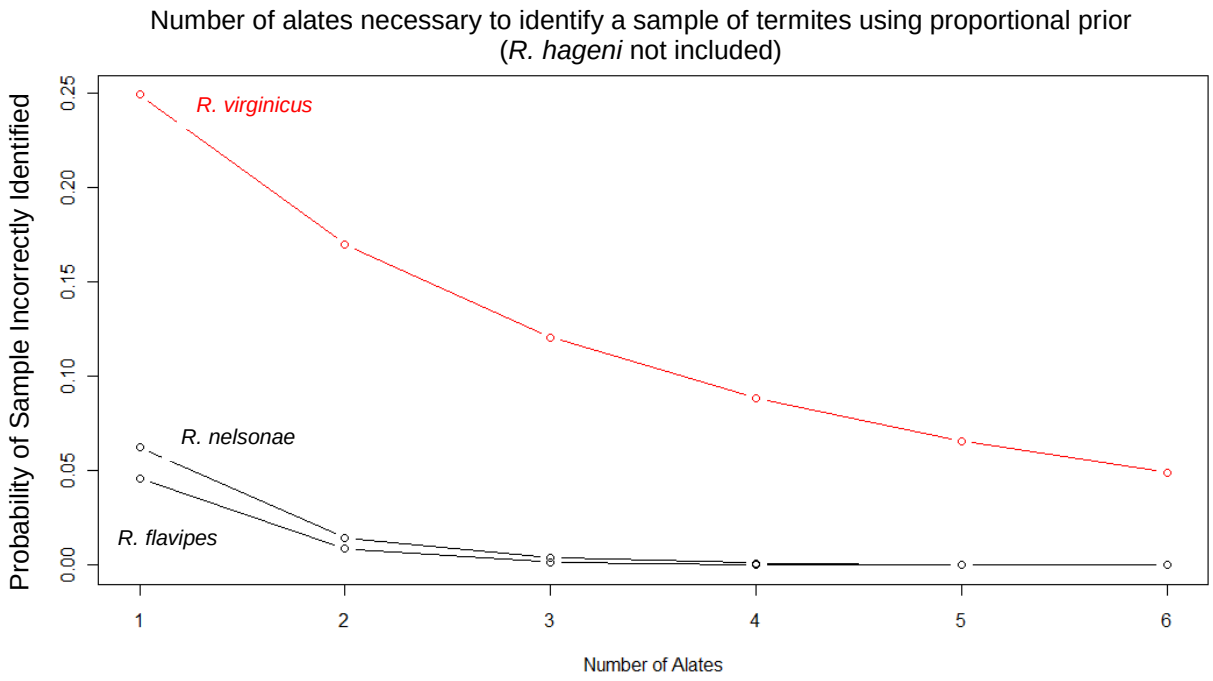

Figure 3.13b. Graph shows the probability of incorrect classification discriminant function analysis (using proportional prior) from simulation of 1000000 termite averages for alates.

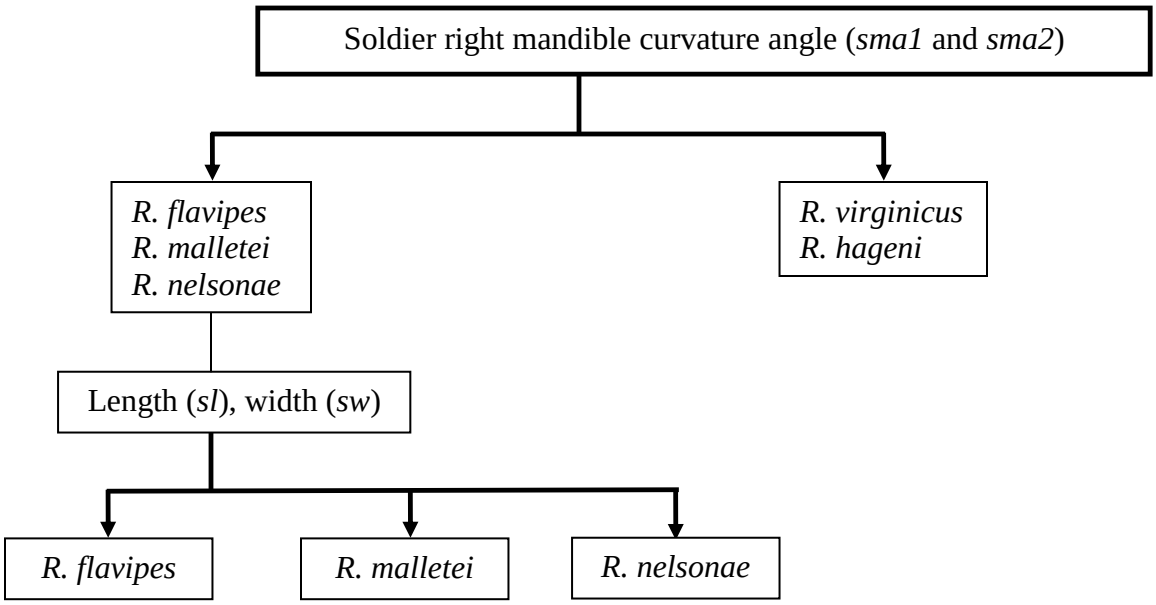

Figure 3.14. Proposed route of species identification for soldiers of *Reticulitermes* spp. of Georgia.

CHAPTER 4

MOLECULAR PHYLOGENETICS OF *RETICULITERMES* (RHINOTERMITIDAE) IN THE SOUTHEASTERN USA.[3]

[3]Lim, S.Y., Jenkins, T.M., McHugh J.V., and Forschler B.T. To be submitted to Molecular Phylogenetics and Evolution

Abstract

Relationships among the five species of *Reticulitermes* found in the three major soil provinces of the southeastern USA were investigated using the mitochondrial COII and COI genes, and the non-coding nuclear ITS region. Different phylogenetic estimation methods were utilized to determine the interspecific relationships. Both COII and COI genes were more informative for species discrimination when compared to the ITS nuclear array. *Reticulitermes malletei* and *R. nelsonae* were indistinguishable using ITS but formed a monophyletic clade in trees inferred from COII gene. We therefore recommend COII be used as a reliable molecular marker for species discrimination within the *Reticulitermes* of southeastern United States.

Keywords: Systematics, southeastern, phylogenetics, COII, ITS array.

Introduction

Subterranean termites in the genus *Reticulitermes* are mainly found in the temperate regions of the world (Pearce, 1997). Some species of this genus are native to the Nearctic ecozone in North America (Forschler and Jenkins, 1999; Jenkins et al., 2000; Jenkins et al., 2001; Szalanski et al., 2003; Ye et al., 2004; Austin et al., 2005; McKern et al., 2006; Szalanski et al., 2006; Tripodi et al., 2006; Austin et al., 2007; King et al., 2007; McKern et al., 2007; Engel et al., 2009; Sillam-Dussès and Forschler, 2010), while other species are indigenous to the Palearctic regions of Asia (mainly Japan, China, Korea, and Taiwan) (Park et al., 2006), Europe and the Mediterranean (Italy, France, Portugal, Greece, Israel) (Clément et al., 2001; Uva et al., 2004; Luchetti et al., 2005; Nobre et al., 2006; Luchetti et al., 2007).

Systematics of *Reticulitermes* at the species level is complicated due to the cryptic species biology and the lack of robust morphological, behavioral and chemical characters that definitively distinguish the species (Weesner, 1965; Nutting, 1990; Hostettler et al., 1995; Forschler and Jenkins, 1999; Nelson et al., 2008). Genetic markers such as mitochondrial DNA sequences have proven to be a valuable tool in assisting systematic studies especially when used with correct reference sequences (Forschler and Jenkins, 1999; Jenkins et al., 2000; Sillam-Dussès and Forschler, 2010).

Mitochondrial DNA sequences have been used successfully for inferring phylogenetic relationships and assisting systematic studies among closely related insect species (Liu and Beckenbach, 1992; Simon et al., 1994; Simon et al., 2006; Jenkins et al., 2009; Vargo and Husseneder, 2009). The rate of substitution for mitochondrial DNA is thought to be higher, however, than nuclear protein-coding genes by about ten times (Li, 1997), and perhaps even higher in *Reticulitermes* as hypothesized by Luchetti et al. (2009) for the COII gene from the

Mediterranean subterranean termites. The substitution rates observed in non-coding nuclear regions are slightly lower than in mitochondrial DNA but higher than protein-coding regions of the nuclear DNA (Li, 1997).

The ITS array has also been used in many phylogenetic and systematic studies of insects (Jenkins et al., 2001; Jenkins et al., 2007; Young and Coleman, 2004; Szalanski et al., 2008; Ruhl et al., 2010; Ullrich et al., 2010). This region of the nuclear genome was chosen to determine if the commonly used mitochondrial gene (COII) might be less informative due to possible higher rates of substitution and probable saturation of sites as hypothesized by Liu and Beckenbach (1992) and Simon et al. (1994).

The purpose of this study is to provide insights into the equivocal relationships among five *Reticulitermes* from the three major soil provinces of the southeastern USA and reconstruct a robust phylogeny for the five *Reticulitermes* species occurring there (*R. flavipes, R. virginicus, R. hageni, R. malletei* and *R. nelsonae*) using multiple molecular markers analyzed with different tree-inferring methods. Molecular markers with different rates and models of substitution were chosen to determine their usefulness for species discrimination, in an attempt to provide guidance for future taxonomic studies using genetic data with this genus.

Material and Methods

Sampling

Termites were collected extensively in Georgia from three major soil provinces of the southeastern USA, namely, the Piedmont (Athens, GA), South Coastal Plain (Thomasville, GA) and Atlantic Coastal Flatwoods (Sapelo Island, GA) (Fig. 4.1). Two additional sites in North Carolina and Florida were sampled for haplotype comparisons between these three states in the southeastern USA (Fig. 4.1). The collection specimen data are given in Tables 4.1-4.3. All

specimens were preserved in 70-100% ethanol. A total of 335 samples were studied using COII (Table 4.1). A subset of 37 samples was amplified for COI gene, (Table 4.2) and 62 samples was amplified for the ITS region, which includes regions of ITS1, 5.8S, and ITS2) (Table 4.3). GenBank sequences were also included in the analyses for COI gene (Table 4.2). The samples studied using COII, COI and ITS region are summarized respectively in Tables 4.4-4.6 by soil province and collection sites.

PCR Amplification and Sequencing

Entire termite body tissue were used for DNA isolation and prepared according to a modified protocol of the Promega's Wizard Genomic DNA Purification Kit or Qiagen's DNeasy Extraction Kit, following a modified protocol (Sillam-Dussès and Forschler, 2010). The complete region of mitochondrial ribosomal gene COII (685bp), COI (~801bp) and ITS region (~850bp) were amplified with the polymerase chain reaction. PCR was carried out in standard 25µl reaction with 2µl of extracted DNA. All amplification reactions had 1µM of primers, 2.0 mM $MgCl_2$, 0.1U/µl Taq DNA polymerase (Promega, Madison, Wisconsin). PCR amplifications were accomplished in a Eppendorf Mastercycler or Bio-Rad Minicycler. Each PCR amplification protocol for the specific DNA fragments is listed in Table 4.7. Primers for PCR amplification and internal primers used for DNA sequencing are listed in Table 4.8 (Jenkins et al., 2009). Purified PCR samples were sent for sequencing at Molecular Cloning Laboratories (MCLAB, California) or Eurofins MWG Operon (Huntsville, Alabama).

Sequence Alignment and Phylogenetic Analyses

Sequences were curated with Sequencher 4.5 (Genes Codes Corp., Ann Arbor, USA). The curated sequences were then aligned using MUSCLE v.3.7 and curated with Gblocks v.0.91b. Gaps were treated as missing data. These sequences were used for phylogenetic

analyses using the following tree estimation methods: Maximum Likelihood, ML (PHYML), Distance Method, DM (BIONJ), Maximum Parsimony, MP (MEGA 5), Bayesian Inference, BI (MrBayes) (Felsenstein, 1985; Nei and Kumar, 2000; Huelsenbeck and Ronquist, 2001; Guindon and Gascuel, 2003; Ronquist and Huelsenbeck, 2003; Tamura et al., 2004; Chevenet et al., 2006; Tamura et al., 2007; Dereeper et al., 2008; Kumar et al., 2008; Dereeper et al., 2010).

ML analyses were performed on the www.phylogeny.fr web server with PHYML 3.0 using the GTR+G+I model. The nucleotide sequences from different genes evolve at different rates, thus several methods can be applied for treatment of partitioned data: the combined data, separate analysis, and conditional combination approaches (Huelsenbeck et al., 1996). DM analyses were also performed on the www.phylogeny.fr web server with BIONJ using the Kimura 2-parameter substitution model and bootstrap=1000. MP analyses for the sequences were performed with MEGA5 using Close-Neighbor-Interchange (CNI) search and bootstrap=1000. Phylogenetic trees presentations were graphically improved using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Results

A total of 120 haplotypes for COII was observed from 335 samples, 20 haplotypes for COI from 37 samples, and 32 haplotypes for ITS from 62 samples originating from three different sites (Athens, Sapelo Island and Thomasville) in Georgia, Havelock in North Carolina and Branford in Florida (Tables 4.1-4.6). After alignment and curation with Gblocks, the COII (684bp), COI (767bp) and ITS (599bp) alignment data were analyzed using ML, (PHYML), DM (BIONJ), and MP (MEGA) resulting in inferred trees as shown in Figures 4.2a-4.10 (Gascuel, 1997; Guindon and Gascuel, 2003; Kumar et al., 2004; Anisimova and Gascuel, 2006; Tamura et al., 2007; Dereeper et al., 2008; Kumar et al., 2008). The nucleotide frequencies are as

follows: A =0.39, C=0.23, G=0.14, T=0.23 for COII, A= 0.34, C= 0.23, G=0.16, T=0.27m for COI, and A=0.20, C=0.30, G=0.33, T=0.18 for ITS (Table 4.9). From the curated alignment data, there were 577 variable sites and 194 parsimony-informative sites for COII gene, 146 variable sites and 133 parsimony-informative sites for COI gene, and 88 variable sites and 27 parsimony-informative sites for ITS. Table 4.10 summarized the number of haplotypes recovered for each species from COII, COI and ITS regions. Estimates of net evolutionary divergence among the five species of *Reticulitermes* for COII, COI and ITS are shown in Tables 4.11- 4.13(Tamura et al., 2004; Tamura et al., 2011).

Phylogenetic relationships inferred from COII, COI and ITS

The substitution model chosen for COII gene within ML was GTR+G+I and resulted in a tree with the Ln likelihood value = -4085.206, gamma shape parameter =0.681, and proportion of invariant = 0.394 (Figs. 4.2a-c). Branch support values for each species clades are high and clear separation of the clades are observed for the tree inferred from COII (Figs. 4.2a-c). The DM inferred tree was constructed using Kimura-2-parameter with bootstrap value = 1000 resulting in the tree shown in Figures 4.3a-c. Bootstrap support values for each of the species clades were high (>50%) and the tree topology was similar to ML inferred tree in Figure 4.2, with some minor rearrangements (Figs. 4.3a-c). The bootstrap consensus tree for MP was similar in tree topology with high branch support values for each species clade to ML and DM analyses (Figs. 4.4a-c). MP analysis generated 93 most parsimonious trees with length = 549, consistency index = 0.462, retention index = 0.910, composite index = 0.498 (0.421) for all sites and parsimony-informative sites (in parentheses) (Felsenstein, 1985; Nei and Kumar, 2000; Tamura et al., 2011).

Therefore, results from ML, DM and MP analyses for COII were highly concordant in tree topologies and showed consistent and corroborated branch support values across the different methods of phylogenetic inference (Figs. 4.2a-c). Species clades for phylogenies inferred from COII also had high branch support values (>50%) for all the analyses (Figs. 4.2a-4.4c). In ML and MP trees, all five species clades were monophyletic but BIONJ analysis did not fully resolve the position of two samples: P1.26 and AM010A (Figs. 4.2a-4.4c).

The phylogenetic tree obtained for COI that included GenBank sequences from ML analysis with GTR+G+I generated a log likelihood of -2905.620, pinv= 0.578, gamma shape parameter = 0.878 (Fig. 4.5). The phylogenetic tree recovered monophyletic species clades for *R. flavipes*, *R. virginicus*, *R. hageni*, *R. malletei* and *R. nelsonae* (Fig. 4.5). DM analysis that was inferred using bootstrap =1000 and Kimura 2-parameter model showed similar tree topology with the ML tree (Fig. 4.6). Branch support values for ML and DM trees were high (>50%) in both trees recovered (Figs. 4.5-4.6). MP analysis recovered 33 most parsimonious trees with length =366, consistency index= 0.556, retention index= 0.892, composite index= 0.565 (0.496) for all sites and parsimony-informative sites (in parentheses) (Fig. 4.7) (Felsenstein, 1985; Nei and Kumar, 2000; Tamura et al., 2011).Similar species clades in ML and DM analyses were also recovered in MP tree (Figs. 4.5-4.7). Overall, haplotypes of *R. lucifugus grassei* and *R. speratus* (from GenBank) for COI did not intermixed with haplotypes of *Reticulitermes* from southeastern USA (Figs. 4.5-4.7). *Reticulitermes santonensis* which is now recognized as a junior synonym of *R. flavipes* was recovered within the *R. flavipes* clade (Figs.4.5-4.7).

ML analysis for ITS region with GTR+G+I, pinv = 0.503 and gamma shape parameter = 0.823 resulted in a tree with the Ln likelihood = -1437.612 (Fig. 4.8). Monophyletic groups were recovered for three of the five species studied (*R. flavipes*, *R. virginicus*, *R. hageni*), while

haplotypes of *R. malletei* and *R. nelsonae* were intermixed (Fig. 4.8).One clade of intermixed *R. malletei* and *R. nelsonae* were observed. The mixed clade had haplotypes of *R. malletei* (ITSA5, DRMW1, and BOT2) mixed with haplotypes of *R. nelsonae* (SI008, ITSA7, B20, and LA06-18) (Fig. 4.8). The DM inferred tree for the ITS region that was constructed with Kimura-2-parameter with bootstrap value =1000 exhibits similar tree topology and branch support values to the ML tree (Fig. 4.9). The MP analysis for the ITS region including outgroup taxon found 387 most parsimonious trees with length = 475, consistency index = 0.904, retention index = 0.927 and composite index = 0.912 (0.838), for all sites and all parsimony-informative sites (in parentheses) (Felsenstein, 1985; Nei and Kumar, 2000; Tamura et al., 2011). The bootstrap consensus tree presented in Figure 4.10 has a similar tree topology and branch support values to the ML and DM trees with only minor rearrangements (Figs. 4.8-4.10). In addition to the intermixing of haplotypes observed in the phylogenies, samples of *R. malletei* (BOT11, 4TA1, AM013A, AM015A, AM019A, AM020A, AM021A, BIOS4, BOT 8, NC004) and *R. nelsonae* (P1.21, SI003, SI006, 1211P0047) shared a similar haplotype: ITSA5 (Table 4.3).

Discussion

Phylogenies inferred using COII and COI for samples from the three regions of southeastern USA reveals monophyletic clades supporting all five *Reticulitermes* species known to occur in that area (Figs. 4.2a-4.4c, 4.5-4.7). The estimate of net evolutionary divergence for COII and COI determined that *R. malletei* are probably most closely related to *R. hageni* because of the low number of base substitutions per site (0.031, SE=0.006, 0.027, SE=0.006) between them (Tables 4.11-4.12). The most recently described species; *R. nelsonae* is seen to be most closely related to *R. malletei* but has diverged considerably from *R. flavipes* (Tables 4.11-4.12). The number of haplotypes obtained for *R. flavipes* (56) was the highest, followed by *R. nelsonae*

(34), *R. virginicus* (20), *R. malletei* (7) and *R. hageni* (4) for COII (Table 4.10). The same trend was seen with COI gene with *R. flavipes* (8), *R. nelsonae* (6), *R. virginicus*, *R. malletei* (2) and *R. hageni* (1) (Table 4.10). The dominant presence of *R. flavipes* agrees with past literature and concurs with the invasive character of this species (Table 4.10) (Vargo et al., 2006; Pinzon and Houseman, 2009; Wang et al., 2009; Perdereau et al., 2011). The high number of haplotypes observed for the recently described *R. nelsonae* suggests that this species might be more commonly encountered than previously known (Sillam-Dussès and Forschler, 2010; Lim, 2011). The COII and COI haplotypes did not always segregate according collection localities or soil provinces (Tables 4.1-4.2). Two such examples from COII gene of these haplotypes include AM004 which was a consensus sequence for a sample from Whitehall Forest in Athens, GA, and 13 samples from Greenwood Plantation in Thomasville, GA, and GroupD which encompassed 8 samples from Sapelo Island, GA, and two samples from Havelock, NC (Table 4.1).

Some interesting distribution patterns for *Reticulitermes* can be observed from this study. *Reticulitermes flavipes* and *R. virginicus* were found in all the three soil provinces (Tables 4.4-4.5). *Reticulitermes hageni* haplotypes have only been observed from samples obtained in the Piedmont. *Reticulitermes malletei* haplotypes were found in samples obtained in the Piedmont and Atlantic Coast Flatwoods (Tables 4.4-4.5). *R. nelsonae* haplotypes were found in the Atlantic Coast Flatwoods and South Coast Plain but not the Piedmont (Tables 4.4-4.5). The distribution patterns could be an artifact of sampling or could be related to the different ecological attributes that might have limited the invasion of a certain termite species to a specific area, especially if the species was not invasive. Four of the five species studied are considered to be non-invasive. Further investigation on the distribution and possible dispersion routes for

Reticulitermes in the southeastern might provide valuable insights to the ecology and behavior of these native subterranean termites.

The ITS region that was amplified did not provide as much phylogenetic signal compared to COII and COI for discriminating the *Reticulitermes* species, due to the low number of variable (88 sites) and parsimony-informative sites (27 sites), compared to COII (variable sites = 577, parsimony-informative sites = 194) and COI (variable sites= 146, parsimony-informative sites= 133). Three of the five *Reticulitermes* were successfully inferred as monophyletic clades from ITS region but *R. malletei* and *R. nelsonae* were intermixed in phylogenies obtained from all phylogenetic analyses conducted (Figs. 4.8-4.10). The ITSA5 haplotype is also interesting because it is a consensus sequence for samples that was identified from both *R. malletei* and *R. nelsonae* samples (Table 4.3). This lends additional support to the hypothesis that *R. malletei* and *R. nelsonae* are most closely related to each other among the *Reticulitermes* evaluated.

We hypothesize that the ITS region in *Reticulitermes* probably has not evolved fast enough compared to the mitochondrial COII and COI genes for the region to be informative. It is known that ITS region usually has a lower rate of nucleotide substitution compared to mitochondrial genes (Li, 1997), therefore this region did not provide adequate phylogenetic signal for species discrimination. Although the ITS region has been used successfully in other orders of insects for phylogenetic and species discrimination studies (Young and Coleman, 2004; Szalanski et al., 2008; Jenkins et al., 2009; Ruhl et al., 2010; Ullrich et al., 2010), it seems less valuable for discriminating species of the genus *Reticulitermes.* Therefore, from the molecular analyses and phylogenies inferred, we could conclude that COII and COI were more useful as molecular marker for discriminating species compared to the ITS region.

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Table 4.1. Sample localities, collection date, soil province and COII haplotypes designation for *Reticulitermes* termite species.

No.	Species	Sample name	Soil province	Collection site	Collection date	Haplotypes for COII	No. of haplotypes
1	*R. flavipes*	BOT6	P	Botanical Gardens, Athens, GA	23 Oct 2007	C5	1
2	*R. flavipes*	4T1	P	Four towers, Athens, GA	21 Apr 2009		
3	*R. flavipes*	4T2	P	Four towers, Athens, GA	21 Apr 2009		
4	*R. flavipes*	AM001A	P	Whitehall Forest, Athens, GA	5Apr 2005		
5	*R. flavipes*	AM043	P	Whitehall Forest, Athens, GA	3 Apr 2003		
6	*R. flavipes*	AM004	P	Whitehall Forest, Athens, GA	26 Oct 2005	AM004	2
7	*R. flavipes*	P3.179	SCP	Greenwood Plantation, GA	9 Nov 2009		
8	*R. flavipes*	P3.181	SCP	Greenwood Plantation, GA	9 Nov 2009		
9	*R. flavipes*	P3.183	SCP	Greenwood Plantation, GA	9 Nov 2009		
10	*R. flavipes*	P3.186	SCP	Greenwood Plantation, GA	9 Nov 2009		
11	*R. flavipes*	P3.188	SCP	Greenwood Plantation, GA	9 Nov 2009		
12	*R. flavipes*	P3.189	SCP	Greenwood Plantation, GA	9 Nov 2009		
13	*R. flavipes*	P3.197	SCP	Greenwood Plantation, GA	9 Nov 2009		
14	*R. flavipes*	P3.202	SCP	Greenwood Plantation, GA	9 Nov 2009		
15	*R. flavipes*	P3.204	SCP	Greenwood Plantation, GA	9 Nov 2009		
16	*R. flavipes*	P3.173	SCP	Greenwood Plantation, GA	30 Mar 2010		
17	*R. flavipes*	P3.195	SCP	Greenwood Plantation, GA	30 Mar 2010		
18	*R. flavipes*	P3.197	SCP	Greenwood Plantation, GA	30 Mar 2010		
19	*R. flavipes*	P3.204	SCP	Greenwood Plantation, GA	30 Mar 2010		
20	*R. flavipes*	B1	P	Biosciences Building, Athens, GA	21 Apr 2009	BI_H04	3
21	*R. flavipes*	AM003	P	Biosciences Building, Athens, GA	22 Apr 2009		
22	*R. flavipes*	AM028	P	LeConte Hall, Athens, GA	20 Mar 2009		
23	*R. flavipes*	AM017	P	UGA Chapel, Athens, GA	5 Apr 2010	Group C	4

24	*R. flavipes*	AM018	P	Demosthenian, Athens, GA	5 Apr 2010		
25	*R. flavipes*	SI020	ACF	Sapelo Island, GA	5 Jul 2009		
26	*R. flavipes*	Y109	ACF	Sapelo Island, GA	14 Jul 2008		
27	*R. flavipes*	YM123	ACF	Sapelo Island, GA	15 Jul 2008		
28	*R. flavipes*	YM126	ACF	Sapelo Island, GA	14 Jul 2008		
29	*R. flavipes*	YM127	ACF	Sapelo Island, GA	15 Jul 2008		
30	*R. flavipes*	Y165	ACF	Sapelo Island, GA	17 Jul 2008		
31	*R. flavipes*	Y166	ACF	Sapelo Island, GA	18 Jul 2008		
32	*R. flavipes*	A1	P	Academic Building, Athens, GA	13 Apr 2009	A1	5
33	*R. flavipes*	A2	P	Academic Building, Athens, GA	13 Apr 2009	A2	6
34	*R. flavipes*	L2	P	Whitehall Forest, Athens, GA	25 Apr 2009	L2	7
35	*R. flavipes*	L3	P	Whitehall Forest, Athens, GA	25 Apr 2009	L3	8
36	*R. flavipes*	E3	P	East Campus, Athens, GA	21 Apr 2009	E3	9
37	*R. flavipes*	AM026	P	Holmes-Hunter Building , Athens, GA	13 Mar 2009	AM026A	10
38	*R. flavipes*	ClarkHowell	P	Clark Howell, Athens, GA	11 Mar 2002		
39	*R. flavipes*	AM029	P	186 Ecology Building, Athens, GA	18 Mar 2009	AM029A	11
40	*R. flavipes*	WM027	P	Dean Rusk Hall, Athens, GA	1 Apr 2009		
41	*R. flavipes*	SA001	P	Ecology Building, Athens, GA	16 Mar 2010	SA001	12
42	*R. flavipes*	AM032	P	Le Conte Hall, Athens, GA	2 Mar 2005	AM032	13
43	*R. flavipes*	AM014	P	Athens, GA	22 May 2007	AM014	14
44	*R. flavipes*	LCH93	P	Athens, GA	3 Sep 2000	LCH93	15
45	*R. flavipes*	E1	P	East Campus, Athens, GA	21 Apr 2009		
46	*R. flavipes*	BOT4	P	Botanical Gardens, Athens, GA	23 Oct 2007	C3	16
47	*R. flavipes*	DawHll2003	P	Dawson Hall, Athens, GA	10 Mar 2003		
48	*R. flavipes*	AM011	P	Athens, GA	25 May 2008		
49	*R. flavipes*	AM038	ACF	Sapelo Island, GA	28 Jan 2005	AM038	1
50	*R. flavipes*	AM039	ACF	Sapelo Island, GA	28 Jan 2005		

51	*R. flavipes*	AM040	ACF	Sapelo Island, GA	10 Nov 2004		
52	*R. flavipes*	NINA2	ACF	Sapelo Island, GA	23 Nov 2007	C11	18
53	*R. flavipes*	AM037	ACF	Sapelo Island, GA	11 Nov 2004		
54	*R. flavipes*	BH22	ACF	Sapelo Island, GA	23 Nov 2007	C12	19
55	*R. flavipes*	GP001A	SCP	Greenwood Plantation, GA	25 Aug 2009	GP001A	20
56	*R. flavipes*	GP002A	SCP	Greenwood Plantation, GA	25Aug 2009	GP002A	21
57	*R. flavipes*	GP002B	SCP	Greenwood Plantation, GA	25 Aug 2009		
58	*R. flavipes*	GP002E	SCP	Greenwood Plantation, GA	25 Aug 2009	GP002B	22
59	*R. flavipes*	GP002F	SCP	Greenwood Plantation, GA	25 Aug 2009		
60	*R. flavipes*	P4.317	SCP	Greenwood Plantation, GA	9 Nov 2009		
61	*R. flavipes*	GP003A	SCP	Greenwood Plantation, GA	25 Aug 2009		
62	*R. flavipes*	GP003B	SCP	Greenwood Plantation, GA	25 Aug 2009		
63	*R. flavipes*	GP003C	SCP	Greenwood Plantation, GA	25 Aug 2009	GP003A	23
64	*R. flavipes*	GP004C	SCP	Greenwood Plantation, GA	25 Aug 2009		
65	*R. flavipes*	P1.16	SCP	Greenwood Plantation, GA	26 Jan 2010		
66	*R. flavipes*	P4.247	SCP	Greenwood Plantation, GA	30 Mar 2010		
67	*R. flavipes*	GP004A	SCP	Greenwood Plantation, GA	25 Aug 2009	GP004A	24
68	*R. flavipes*	GP004D	SCP	Greenwood Plantation, GA	25 Aug 2009	GP004D	25
69	*R. flavipes*	SI001	ACF	Sapelo Island, GA	5 Jul 2009	SI001	26
70	*R. flavipes*	SI004	ACF	Sapelo Island, GA	5 Jul 2009	SI004	27
71	*R. flavipes*	SI011	ACF	Sapelo Island, GA	5 Jul 2009	SI011	28
72	*R. flavipes*	SI018	ACF	Sapelo Island, GA	5 Jul 2009	SI018_E05	29
73	*R. flavipes*	SI019	ACF	Sapelo Island, GA	5 Jul 2009		
74	*R. flavipes*	OPHT50	ACF	Sapelo Island, GA	13 Nov 2007		
75	*R. flavipes*	OPI-T4	ACF	Sapelo Island, GA	13 Nov 2007		
76	*R. flavipes*	Y24	ACF	Sapelo Island, GA	-		
77	*R. flavipes*	Y28	ACF	Sapelo Island, GA	17 Jan 2008	GroupD	30
78	*R. flavipes*	Y71	ACF	Sapelo Island, GA	14 Jul 2008		
79	*R. flavipes*	Y132	ACF	Sapelo Island, GA	15 Jul 2008		
80	*R. flavipes*	AM007	ACF	Sapelo Island, GA	13 Nov 2007		
81	*R. flavipes*	1211as1820	ACF	Havelock, NC	5 Nov 2009		

82	*R. flavipes*	2211e3241	ACF	Havelock, NC	5 Nov 2009		
83	*R. flavipes*	GP004B	SCP	Greenwood Plantation, GA	25 Aug 2009		
84	*R. flavipes*	SI021	ACF	Sapelo Island, GA	6 Jul 2009	GroupG	31
85	*R. flavipes*	AM006	ACF	Sapelo Island, GA	13 Nov 2007		
86	*R. flavipes*	P1011PS2754	ACF	Havelock, NC	May 2010		
87	*R. flavipes*	2211P0044	ACF	Havelock, NC	5 Nov 2009		
88	*R. flavipes*	P4.281	SCP	Greenwood Plantation, GA	9 Nov 2009		
89	*R. flavipes*	BH13	ACF	Sapelo Island, GA	7 Mar 2000	BH13A2	32
90	*R. flavipes*	3331S12027	ACF	Havelock, NC	5 Nov 2009	3331S12027	33
91	*R. flavipes*	P3.181	SCP	Greenwood Plantation, GA	9 Nov 2009	P3.181	34
92	*R. flavipes*	P3.199	SCP	Greenwood Plantation, GA	9 Nov 2009		
93	*R. flavipes*	P3.209	SCP	Greenwood Plantation, GA	9 Nov 2009		
94	*R. flavipes*	P3.211	SCP	Greenwood Plantation, GA	9 Nov 2009		
95	*R. flavipes*	P3.199	SCP	Greenwood Plantation, GA	9 Nov 2009		
96	*R. flavipes*	P3.211	SCP	Greenwood Plantation, GA	26 Jan 2010		
97	*R. flavipes*	P3.211	SCP	Greenwood Plantation, GA	9 Nov 2009		
98	*R. flavipes*	P3.216	SCP	Greenwood Plantation, GA	9 Nov 2009	P3.216	35
99	*R. flavipes*	P3.234	SCP	Greenwood Plantation, GA	9 Nov 2009		
100	*R. flavipes*	P3.223	SCP	Greenwood Plantation, GA	30 Mar 2010		
101	*R. flavipes*	P3.224	SCP	Greenwood Plantation, GA	9 Nov 2009		
102	*R. flavipes*	B36A	SCP	Branford, FL	31 Mar 2010	B36A	36
103	*R. flavipes*	B1A	SCP	Branford, FL	31 Mar 2010		
104	*R. flavipes*	B18A	SCP	Branford, FL	31 Mar 2010		
105	*R. flavipes*	B29A	SCP	Branford, FL	31 Mar 2010		
106	*R. flavipes*	BR001	SCP	Branford, FL	31 Mar 2010		
107	*R. flavipes*	1011P0304	ACF	Havelock, NC	5 Nov 2009	1011P0304	37
108	*R. flavipes*	3331E2614	ACF	Havelock, NC	5 Nov 2009	3331E2614	38
109	*R. flavipes*	NC001	ACF	Havelock, NC	5 Nov 2009	NC001	39
110	*R. flavipes*	NC002	ACF	Havelock, NC	5 Nov 2009		
111	*R. flavipes*	NC004	ACF	Havelock, NC	5 Nov 2009		

112	*R. flavipes*	NC006	ACF	Havelock, NC	5 Nov 2009		
113	*R. flavipes*	NC009	ACF	Havelock, NC	5 Nov 2009		
114	*R. flavipes*	NC008	ACF	Havelock, NC	5 Nov 2009	NC008	40
115	*R. flavipes*	NC015	ACF	Havelock, NC	5 Nov 2009		
116	*R. flavipes*	NC012	ACF	Havelock, NC	5 Nov 2009		
117	*R. flavipes*	NC017	ACF	Havelock, NC	5 Nov 2009	NC012	41
118	*R. flavipes*	NC021	ACF	Havelock, NC	5 Nov 2009		
119	*R. flavipes*	NC014	ACF	Havelock, NC	5 Nov 2009		
120	*R. flavipes*	NC018	ACF	Havelock, NC	5 Nov 2009	NC014	42
121	*R. flavipes*	NC020	ACF	Havelock, NC	5 Nov 2009		
122	*R. flavipes*	NC003	ACF	Havelock, NC	5 Nov 2009	NC003	43
123	*R. flavipes*	NC005	ACF	Havelock, NC	5 Nov 2009	NC005	44
124	*R. flavipes*	NC007	ACF	Havelock, NC	5 Nov 2009	NC007	45
125	*R. flavipes*	NC010	ACF	Havelock, NC	5 Nov 2009	NC010	46
126	*R. flavipes*	NC011	ACF	Havelock, NC	5 Nov 2009	NC011	47
127	*R. flavipes*	NC019	ACF	Havelock, NC	5 Nov 2009		
128	*R. flavipes*	NC013	ACF	Havelock, NC	5 Nov 2009	NC013	48
129	*R. flavipes*	NC016	ACF	Havelock, NC	5 Nov 2009	NC016	49
130	*R. flavipes*	NC022	ACF	Havelock, NC	5 Nov 2009	NC022	50
131	*R. flavipes*	B15A	SCP	Branford, FL	31 Mar 2010		
132	*R. flavipes*	B27A	SCP	Branford, FL	31 Mar 2010		
133	*R. flavipes*	B19A	SCP	Branford, FL	31 Mar 2010		
134	*R. flavipes*	B28A	SCP	Branford, FL	31 Mar 2010	B15A	51
135	*R. flavipes*	B6A	SCP	Branford, FL	31 Mar 2010		
136	*R. flavipes*	B8A	SCP	Branford, FL	31 Mar 2010		
137	*R. flavipes*	BIII002	SCP	Branford, FL	5 Nov 2010		
138	*R. flavipes*	B19A	SCP	Branford, FL	31 Mar 2010		
139	*R. flavipes*	BIII001	SCP	Branford, FL	5 Nov 2010	BIII001	52
140	*R. flavipes*	LA06-1231	SCP	Jacksonville, FL	8 Nov 2006	LA061231	53
141	*R. flavipes*	P4.289	SCP	Greenwood Plantation, GA	9 Nov 2009	P4.289	54
142	*R. flavipes*	P1.45	SCP	Greenwood Plantation, GA	9 Nov 2009	P1.45	55

143	*R. flavipes*	RF_TJ	P	Georgia	-	RF_TJ	56
144	*R. flavipes*	LA06-1	SCP	Lake Park, GA	23 Feb 2006	LA06-1	57
145	*R. virginicus*	BOT3	P	Botanical Gardens, Athens, GA	23 Oct 2007	RV1(EU689027)	58
146	*R. virginicus*	BOT5	P	Botanical Gardens, Athens, GA	23 Oct 2007		
147	*R. virginicus*	MAN1	P	Whitehall Forest, Athens, GA	7 Nov 2007		
148	*R. virginicus*	BOT10	P	Botanical Gardens, Athens, GA	23 Oct 2007	C6	59
149	*R. virginicus*	BSW1	P	Dellwood, Athens, GA	8 May 2006	C8	60
150	*R. virginicus*	Cobb1	P	Cobb house, Athens, GA	23 Nov 2007		
151	*R. virginicus*	WM024A	P	Cobb house, Athens, GA	28 May 2010		
152	*R. virginicus*	FT3	P	Four towers, Athens, GA	-	C9	61
153	*R. virginicus*	CHW1	P	Chicopee Building, Athens, GA	30 May 2008	CHW1_C02	62
154	*R. virginicus*	CHW2	P	Chicopee Building, Athens, GA	30 May 2008	CHW2	63
155	*R. virginicus*	BRZ3	ACF	Breezeway, Sapelo Island, GA	13 Nov 2007	C7	64
156	*R. virginicus*	ROW1	ACF	Red Oak, GA	3 Aug 2002		
157	*R. virginicus*	ROW2	ACF	Red Oak, GA	3 Aug 2002		
158	*R. virginicus*	ROW3	ACF	Red Oak, GA	3 Aug 2002		
159	*R. virginicus*	ROW4	ACF	Red Oak, GA	3 Aug 2002		
160	*R. virginicus*	ROW5	ACF	Red Oak, GA	3 Aug 2002		
161	*R. virginicus*	GP002C	SCP	Greenwood Plantation, GA	25 Aug 2009		
162	*R. virginicus*	SI010	ACF	Sapelo Island, GA	5 Jul 2009		
163	*R. virginicus*	GP003D	SCP	Greenwood Plantation, GA	25 Aug 2009	GP003D	65
164	*R. virginicus*	SI002	ACF	Sapelo Island, GA	5 Jul 2009		
165	*R. virginicus*	SI007	ACF	Sapelo Island, GA	5 Jul 2009		
166	*R. virginicus*	SI016	ACF	Sapelo Island, GA	5 Jul 2009		
167	*R. virginicus*	SI017	ACF	Sapelo Island, GA	5 Jul 2009		
168	*R. virginicus*	SI009	ACF	Sapelo Island, GA	5 Jul 2009	SI009	66
169	*R. virginicus*	SI013	ACF	Sapelo Island, GA	5 Jul 2009	SI013	67
170	*R. virginicus*	SI014	ACF	Sapelo Island, GA	5 Jul 2009		
171	*R. virginicus*	SI015	ACF	Sapelo Island, GA	5 Jul 2009		
172	*R. virginicus*	WS15	ACF	Sapelo Island, GA	30 Jan 2008	GroupW	68

173	*R. virginicus*	ASMSPA1	ACF	Sapelo Island, GA	12 May 2008		
174	*R. virginicus*	WSMSPW1	ACF	Sapelo Island, GA	12 May 2005		
175	*R. virginicus*	SI005	ACF	Sapelo Island, GA	5 Jul 2009		
176	*R. virginicus*	Y145	ACF	Sapelo Island, GA	16 Jul 2008		
177	*R. virginicus*	Y150	ACF	Sapelo Island, GA	16 Jul 2008		
178	*R. virginicus*	YN150	ACF	Sapelo Island, GA	16 Jul 2008		
179	*R. virginicus*	Y151	ACF	Sapelo Island, GA	16 Jul 2008		
180	*R. virginicus*	Y152	ACF	Sapelo Island, GA	17 Jul 2008		
181	*R. virginicus*	Y154	ACF	Sapelo Island, GA	17 Jul 2008		
182	*R. virginicus*	ASMP	ACF	Sapelo Island, GA	12 May 2005		
183	*R. virginicus*	LA0517	SCP	Thomasville, GA	16 Jun 2005	LA0517	69
184	*R. virginicus*	LA06-1071	SCP	Jasper, FL	26 Jul 2006	LA061071	70
185	*R. virginicus*	LA05184	SCP	Madison, FL	26 May 2005	LA05184a	71
186	*R. virginicus*	LA06-1087	SCP	Southport, FL	14 Aug 2006	LA061087	72
187	*R. virginicus*	WM022A	P	Biosciences Building, Athens, GA	28 May 2010	WM022A	73
188	*R. virginicus*	CI001	ACF	Carragio House, Cumberland Island, GA	28 May 2009	CI001	74
189	*R. virginicus*	BFPS0001	SCP	Bamboo Farm, GA	8 Oct 2009	BFPS0001	75
190	*R. hageni*	FT2710	P	Four Towers, Athens, GA	14 Aug 2007		
191	*R. hageni*	FTT1	P	Four Towers, Athens, GA	23 Nov 2007		
192	*R. hageni*	DawHll2005	P	Four Towers, Athens, GA	6 May 2005	C10	76
193	*R. hageni*	FT8	P	Four Towers, Athens, GA	23 Nov 2007		
194	*R. hageni*	WB27	P	Four Towers, Athens, GA	18 Dec 2007		
195	*R. hageni*	FT9A1H	P	Four Towers, Athens, GA	13 Jul 2007	C13	77
196	*R. hageni*	BB1	P	Barn Barnsville, GA	22 June 2007		
197	*R. hageni*	BBC1	P	Barn Barnsville, GA	22 June 2007		
198	*R. hageni*	T2N	P	Tyler 6, GA	98?	RH1	78
199	*R. hageni*	AM002A	P	Commerce, GA	13 Aug 2008		
200	*R. hageni*	BL001	P	Barn Lamar	21 May 2009		
201	*R. hageni*	FTT15	P	Four Towers, Athens, GA	27 May 2008	FTT15	79

202	*R. malletei*	4TA1	P	Four Towers, Athens, GA	27 May 2008		
203	*R. malletei*	4TW1	P	Four Towers, Athens, GA	27 May 2008		
204	*R. malletei*	DRMW1	P	Four Towers, Athens, GA	24 May 2008		
205	*R. malletei*	BOT1	P	Botanical Gardens, Athens, GA	23 Oct 2007		
206	*R. malletei*	BOT2	P	Botanical Gardens, Athens, GA	23 Oct 2007		
207	*R. malletei*	BOT7	P	Botanical Gardens, Athens, GA	23 Oct 2007		
208	*R. malletei*	BOT8	P	Botanical Gardens, Athens, GA	23 Oct 2007		
209	*R. malletei*	BOT9	P	Botanical Gardens, Athens, GA	23 Oct 2007	C1	80
210	*R. malletei*	BOT12	P	Botanical Gardens, Athens, GA	23 Oct 2007		
211	*R. malletei*	BOT13	P	Botanical Gardens, Athens, GA	23 Oct 2007		
212	*R. malletei*	BIOS4	P	Bioscience Building, Athens, GA	23 Nov 2007		
213	*R. malletei*	AM012A	P	Athens, GA	15 May 2007		
214	*R. malletei*	AM021A	P	Whippoorwill Circle, Athens, GA	29 May 2010		
215	*R. malletei*	BOT11	P	Botanical Gardens, Athens, GA	23 Oct 2007	C4	81
216	*R. malletei*	1211as1505	ACF	Havelock, NC	5 Nov 2009	1211AS1505	82
217	*R. malletei*	P1011P0022	ACF	Havelock, NC	May 2010		
218	*R. malletei*	1211AS1652	ACF	Havelock, NC	5 Nov 2009		
219	*R. malletei*	AM015A	P	East Campus, Athens, GA	21 May 2010	AM015A	83
220	*R. malletei*	NC004	ACF	Havelock, NC	May 2010	NC004	84
221	*R. malletei*	AM020	P	Athens, GA	29 May 2010	AM020A	85
222	*R. malletei*	AM013A	P	Athens, GA	22 May 2007	AM013A	86
223	*R. malletei*	AM019A	P	Athens, GA	29 May 2010		
224	*R. nelsonae*	GP002D	SCP	Greenwood Plantation, GA	25 Aug 2009	GP002D	87
225	*R. nelsonae*	SI006	ACF	Sapelo Island, GA	5 Jul 2009		
226	*R. nelsonae*	BPG-F2736	ACF	Sapelo Island, GA	6 Feb 2007		
227	*R. nelsonae*	BPG-F2747	ACF	Sapelo Island, GA	6 Feb 2007	GroupA	88
228	*R. nelsonae*	BPI	ACF	Sapelo Island, GA	6 Feb 2007		
229	*R. nelsonae*	Y47	ACF	Sapelo Island, GA	13 Jul 2008		

230	*R. nelsonae*	Y52	ACF	Sapelo Island, GA	14 Jul 2008		
231	*R. nelsonae*	SI008	ACF	Sapelo Island, GA	5 Jul 2009	GroupB	89
232	*R. nelsonae*	OP-D	ACF	Sapelo Island, GA	6 Feb 2007		
233	*R. nelsonae*	BP-N	ACF	Sapelo Island, GA	6 Feb 2007		
234	*R. nelsonae*	PP-M	ACF	Sapelo Island, GA	6 Feb 2007		
235	*R. nelsonae*	YP-J	ACF	Sapelo Island, GA	6 Feb 2007		
236	*R. nelsonae*	WS2	ACF	Sapelo Island, GA	6 Feb 2007		
237	*R. nelsonae*	BP-H	ACF	Sapelo Island, GA	6 Feb 2007	GroupE	90
238	*R. nelsonae*	SI012	ACF	Sapelo Island, GA	5 Jul 2009	GroupF	91
239	*R. nelsonae*	BP-J	ACF	Sapelo Island, GA	6 Feb 2007		
240	*R. nelsonae*	SI003	ACF	Sapelo Island, GA	5 Jul 2009	GroupN	92
241	*R. nelsonae*	Y37	ACF	Sapelo Island, GA	17 Jan 2008		
242	*R. nelsonae*	Y41	ACF	Sapelo Island, GA	13 Jul 2008		
243	*R. nelsonae*	Y44	ACF	Sapelo Island, GA	13 Jul 2008		
244	*R. nelsonae*	Y45	ACF	Sapelo Island, GA	13 Jul 2008		
245	*R. nelsonae*	Y74	ACF	Sapelo Island, GA	14 Jul 2008		
246	*R. nelsonae*	AM034A	ACF	Sapelo Island, GA	12 May 2005		
247	*R. nelsonae*	LA06-18	SCP	Hahira, GA	18 Jun 2009	LA0618a	93
248	*R. nelsonae*	P1.21	SCP	Greenwood Plantation, GA	9 Nov 2009		
249	*R. nelsonae*	P1.30S	SCP	Greenwood Plantation, GA	9 Nov 2009		
250	*R. nelsonae*	P1.30W	SCP	Greenwood Plantation, GA	9 Nov 2009		
251	*R. nelsonae*	P2.104	SCP	Greenwood Plantation, GA	9 Nov 2009		
252	*R. nelsonae*	P1.54	SCP	Greenwood Plantation, GA	9 Nov 2009		
253	*R. nelsonae*	P2.113	SCP	Greenwood Plantation, GA	26 Jan 2010		
254	*R. nelsonae*	P2.129	SCP	Greenwood Plantation, GA	9 Nov 2009		
255	*R. nelsonae*	P3.201	SCP	Greenwood Plantation, GA	30 Mar 2010		
256	*R. nelsonae*	P3.201	SCP	Greenwood Plantation, GA	9 Nov 2009		
257	*R. nelsonae*	P3.226	SCP	Greenwood Plantation, GA	9 Nov 2009		
258	*R. nelsonae*	P3.235	SCP	Greenwood Plantation, GA	30 Mar 2010		
259	*R. nelsonae*	P3.235	SCP	Greenwood Plantation, GA	9 Nov 2009		
260	*R. nelsonae*	P3.235	SCP	Greenwood Plantation, GA	26 Jan 2010		

261	*R. nelsonae*	P4.306	SCP	Greenwood Plantation, GA	26 Jan 2010		
262	*R. nelsonae*	P3.226	SCP	Greenwood Plantation, GA	26 Jan 2010		
263	*R. nelsonae*	P1.10	SCP	Greenwood Plantation, GA	9 Nov 2009		
264	*R. nelsonae*	P1.51	SCP	Greenwood Plantation, GA	9 Nov 2009		
265	*R. nelsonae*	P1.52	SCP	Greenwood Plantation, GA	9 Nov 2009		
266	*R. nelsonae*	P1.66	SCP	Greenwood Plantation, GA	9 Nov 2009		
267	*R. nelsonae*	P1.74	SCP	Greenwood Plantation, GA	9 Nov 2009		
268	*R. nelsonae*	P1.1	SCP	Greenwood Plantation, GA	26 Jan 2010		
269	*R. nelsonae*	P1.10	SCP	Greenwood Plantation, GA	30 Mar 2010	AM010A	94
270	*R. nelsonae*	P1.1	SCP	Greenwood Plantation, GA	30 Mar 2010		
271	*R. nelsonae*	P1.64	SCP	Greenwood Plantation, GA	9 Nov 2009		
272	*R. nelsonae*	P1.65	SCP	Greenwood Plantation, GA	9 Nov 2009		
273	*R. nelsonae*	P1.66	SCP	Greenwood Plantation, GA	9 Nov 2009		
274	*R. nelsonae*	P2.162	SCP	Greenwood Plantation, GA	26 Jan 2010		
275	*R. nelsonae*	B24A	SCP	Branford, FL	31 Mar 2010	B24A	95
276	*R. nelsonae*	B26A	SCP	Branford, FL	31 Mar 2010		
277	*R. nelsonae*	GP1GA	SCP	Greenwood Plantation, GA	26 Jan 2010		
278	*R. nelsonae*	P3.163	SCP	Greenwood Plantation, GA	9 Nov 2009		
279	*R. nelsonae*	P3.173	SCP	Greenwood Plantation, GA	9 Nov 2009		
280	*R. nelsonae*	P4.270	SCP	Greenwood Plantation, GA	9 Nov 2009		
281	*R. nelsonae*	P2.117	SCP	Greenwood Plantation, GA	26 Jan 2010		
282	*R. nelsonae*	P2.126	SCP	Greenwood Plantation, GA	30 Mar 2010		
283	*R. nelsonae*	P3.228	SCP	Greenwood Plantation, GA	30 Mar 2010		
284	*R. nelsonae*	P3.229	SCP	Greenwood Plantation, GA	9 Nov 2009	GP1GA	96
285	*R. nelsonae*	P3.239	SCP	Greenwood Plantation, GA	26 Jan 2010		
286	*R. nelsonae*	P3.239	SCP	Greenwood Plantation, GA	30 Mar 2010		
287	*R. nelsonae*	P4.252	SCP	Greenwood Plantation, GA	26 Jan 2010		
288	*R. nelsonae*	P4.252	SCP	Greenwood Plantation, GA	30 Mar 2010		
289	*R. nelsonae*	P4.261	SCP	Greenwood Plantation, GA	26 Jan 2010		
290	*R. nelsonae*	P4.269	SCP	Greenwood Plantation, GA	30 Mar 2010		
291	*R. nelsonae*	P4.270	SCP	Greenwood Plantation, GA	26 Jan 2010		

292	*R. nelsonae*	P1.26	SCP	Greenwood Plantation, GA	9 Nov 2009	P1.26	97
293	*R. nelsonae*	P1.80	SCP	Greenwood Plantation, GA	9 Nov 2009		
294	*R. nelsonae*	P1.81	SCP	Greenwood Plantation, GA	9 Nov 2009		
295	*R. nelsonae*	P1.34	SCP	Greenwood Plantation, GA	9 Nov 2009		
296	*R. nelsonae*	P1.26	SCP	Greenwood Plantation, GA	9 Nov 2009		
297	*R. nelsonae*	P1.34	SCP	Greenwood Plantation, GA	30 Mar 2010		
298	*R. nelsonae*	P1.71	SCP	Greenwood Plantation, GA	9 Nov 2009		
299	*R. nelsonae*	P1.81	SCP	Greenwood Plantation, GA	9 Nov 2009		
300	*R. nelsonae*	P2.140	SCP	Greenwood Plantation, GA	9 Nov 2009	P2.140	98
301	*R. nelsonae*	P2.84	SCP	Greenwood Plantation, GA	26 Jan2010		
302	*R. nelsonae*	P4.315	SCP	Greenwood Plantation, GA	9 Nov 2009	P4.315	99
303	*R. nelsonae*	P4.315	SCP	Greenwood Plantation, GA	26 Jan2010		
304	*R. nelsonae*	1011A1454	ACF	Havelock, NC	5 Nov 2009	1011a1454	100
305	*R. nelsonae*	1211A1528	ACF	Havelock, NC	5 Nov 2009		
306	*R. nelsonae*	1311A1416	ACF	Havelock, NC	5 Nov 2009		
307	*R. nelsonae*	2331S1741	ACF	Havelock, NC	5 Nov 2009		
308	*R. nelsonae*	P1211A1547	ACF	Havelock, NC	May 2010		
309	*R. nelsonae*	1211P0047	ACF	Havelock, NC	5 Nov 2009	1211P0047	101
310	*R. nelsonae*	P1011A1447	ACF	Havelock, NC	May 2010		
311	*R. nelsonae*	2331A1469	ACF	Havelock, NC	5 Nov 2009	2331A1469	102
312	*R. nelsonae*	2331P1734	ACF	Havelock, NC	5 Nov 2009		
313	*R. nelsonae*	LA06-1359	SCP	Apopka, FL	20 Dec 2006	LA061359	103
314	*R. nelsonae*	P1011AS1591	ACF	Havelock, NC	May 2010	P1011AS1591	104
315	*R. nelsonae*	2331AS1811	ACF	Havelock, NC	5 Nov 2009	2331AS1811	105
316	*R. nelsonae*	2331A1480	ACF	Havelock, NC	5 Nov 2009	2331A1480	106
317	*R. nelsonae*	P2.153	SCP	Greenwood Plantation, GA	9 Nov 2009	P2.153	107
318	*R. nelsonae*	P3.225	SCP	Greenwood Plantation, GA	9 Nov 2009	P3.225	108
319	*R. nelsonae*	B25A	SCP	Branford, FL	31 Mar 2010	B25A	109
320	*R. nelsonae*	B35A	SCP	Branford, FL	31 Mar 2010	B35A	110

321	*R. nelsonae*	B16A	SCP	Branford, FL	31 Mar 2010	B16A	111
322	*R. nelsonae*	B17A	SCP	Branford, FL	31 Mar 2010	B17A	112
323	*R. nelsonae*	B34A	SCP	Branford, FL	31 Mar 2010	B34A	113
324	*R. nelsonae*	B11A	SCP	Branford, FL	31 Mar 2010		
325	*R. nelsonae*	B23A	SCP	Branford, FL	31 Mar 2010	B23A	114
326	*R. nelsonae*	B21A	SCP	Branford, FL	31 Mar 2010	B21A	115
327	*R. nelsonae*	B30A	SCP	Branford, FL	31 Mar 2010		
328	*R. nelsonae*	B3A	SCP	Branford, FL	31 Mar 2010		
329	*R. nelsonae*	B5A	SCP	Branford, FL	31 Mar 2010		
330	*R. nelsonae*	BIII003	SCP	Branford, FL	5 Nov 2010		
331	*R. nelsonae*	B20A	SCP	Branford, FL	31 Mar 2010	B20A	116
332	*R. nelsonae*	B10A	SCP	Branford, FL	31 Mar 2010	B10A	117
333	*R. nelsonae*	B22A	SCP	Branford, FL	31 Mar 2010	B22A	118
334	*R. nelsonae*	B4A	SCP	Branford, FL	31 Mar 2010	B4A	119
335	*R. nelsonae*	P1.25	SCP	Greenwood Plantation, GA	9 Nov 2009	P1.25	120
336	*Heterotermes* sp.	*Heterotermes*_ V1				V1	121
337	*C. formosanus*	CF				CF_J2004	122

Table 4.2. Sample localities, collection date, soil province and COI haplotypes designation for *Reticulitermes* termite species.

No	Species	Sample name	Soil province	Collection site	Collection date	Haplotypes for COI	No. of haplotypes
1	*R. flavipes*	BASF WS-12	ACF	Sapelo Island, GA	6 Feb 2007		
2	*R. flavipes*	BASF WS-9	ACF	Sapelo Island, GA	6 Feb 2007		
3	*R. flavipes*	A1	ACF	Academic Building , Athens, GA	13 Apr 2009	A2$^{\alpha}$	1
4	*R. flavipes*	A2	ACF	Academic Building , Athens, GA	13 Apr 2009		
5	*R. flavipes*	BH22	ACF	Sapelo Island, GA	23 Nov 2007	A3	2
6	*R. flavipes*	BASF WS-3	ACF	Sapelo Island, GA	30 Jan 2008		
7	*R. flavipes*	Pine16	ACF	Sapelo Island, GA	13 Nov 2007	A16	3
8	*R. flavipes*	Nina2	ACF	Sapelo Island, GA	23 Nov 2007	A35	4
9	*R. flavipes*	BOT4	P	Botanical Gardens, Athens, GA	23 Oct 2007	A36	5
10	*R. flavipes*	4T1	P	Four Towers, Athens, GA	21 Apr 2009	A44	6
11	*R. flavipes*	L1	P	Whitehall Forest, Athens, GA	25 Apr 2009		
12	*R. flavipes*	L2	P	Whitehall Forest, Athens, GA	25 Apr 2009	A45	7
13	*R. flavipes*	L3	P	Whitehall Forest, Athens, GA	25 Apr 2009		
14	*R. flavipes*	B3	P	Biosciences Building, Athens, GA	21 Apr 2009	A48	8
15	*R. flavipes*	AY027469		GA, USA		AY027469_*R. flavipes*_strain_USA1	9
16	*R. flavipes*	EF206314		LA, USA		EF206314_*R.*	10

						*flavipes*_IS13	
17	*R. flavipes*	EF206316	P	Oconee Co., GA, USA		EF206316_*R. flavipes*_IS57	11
18	*R. flavipes*	EF206317	P	Clarke Co., GA, USA		EF206317_*R. flavipes*_IS58	12
19	*R. santonensis*	AY027465		France		AY027465_*R. santonensis*_strain_F5	13
20	*R. santonensis*	AY027466		France		AY027466_*R. santonensis*_strain_F2	14
21	*R. santonensis*	AY027467		France		AY027467_*R. santonensis*_F1	15
22	*R. santonensis*	AY027468		France		AY027468_*R. santonensis*_F3	16
23	*R. santonensis*	AY553156				AY553156_*R. santonensis*	17
24	*R. santonensis*	FJ802751				FJ802751_*R. santonensis*_isolate_IS054	18
25	*R. virginicus*	BRZ3 [α]	ACF	Sapelo Island, GA	13 Nov 2007	A20 [α]	19
26	*R. virginicus*	BASF WS-15	ACF	Sapelo Island, GA	30 Jan 2008	A21	20
27	*R. virginicus*	BOT3	P	Botanical Gardens, Athens, GA	23 Oct 2007	A34	21
28	*R. virginicus*	BOT5	P	Botanical Gardens, Athens, GA	23 Oct 2007		
29	*R. virginicus*	MAN1	P	Whitehall Forest, Athens, GA	7 Nov 2007		
30	*R. virginicus*	BOT10	P	Botanical Gardens, Athens, GA	23 Oct 2007	A38	23
31	*R. virginicus*	COBB1	P	Cobb House, Athens, GA	23 Oct 2007	A39	24

32	*R. virginicus*	A40	P	Four Towers, Athens, GA			
33	*R. virginicus*	AY027471		GA, USA		AY027471_*R. virginicus*	25
34	*R. virginicus*	EF206318		Oconee Co., GA, USA	Sep 2002	EF206318_*R. virginicus*_IS59	26
35	*R. virginicus*	EF206319		Wakulla Co., FL, USA	July 2001	EF206319_*R. virginicus*_IS60	27
36	*R. hageni*	FT8	P	Four Towers, Athens, GA	23 Oct 2007	A41 [α]	28
37	*R. hageni*	BB1	P	Barn Barnsville, GA	22 Jun 2007		
38	*R. hageni*	BB3	P	Barn Barnsville, GA	22 Jun 2007		
39	*R. hageni*	BBC8	P	Barn Barnsville, GA	22 Jun 2007		
40	*R. hageni*	FTT1	P	Four Towers, Athens, GA	23 Oct 2007		
41	*R. hageni*	FTT2	P	Four Towers. Athens, GA	23 Oct 2007		
42	*R. hageni*	AY027470		GA, USA		AY027470_*R. hageni*_IS198	29
43	*R. malletei*	BOT1 [β]	P	Botanical Gardens, Athens, GA	23 Oct 2007	A33 [β]	30
44	*R. malletei*	BOT2	P	Botanical Gardens, Athens, GA	23 Oct 2007		
45	*R. malletei*	BOT7	P	Botanical Gardens, Athens, GA	23 Oct 2007		
46	*R. malletei*	BOT8	P	Botanical Gardens, Athens, GA	23 Oct 2007		
47	*R. malletei*	BOT9	P	Botanical Gardens, Athens, GA	23 Oct 2007		
48	*R. malletei*	BOT12	P	Botanical Gardens, Athens, GA	23 Oct 2007		
49	*R. malletei*	BOT13	P	Botanical Gardens, Athens, GA	23 Oct 2007		
50	*R. malletei*	BIOS4	P	Biosciences Building, Athens, GA	23 Nov 2007		
51	*R. malletei*	BOT11 [β]	P	Four Towers, Athens, GA	23 Oct 2007	A42 [β]	31

52	*R. hageni*	EF206320 [α]		Hamilton Co., FL, USA	Apr 2006	EF206320 [α]	32
53	*R. nelsonae*	BASF OP-D [γ]	ACF	Sapelo Island, GA	6 Feb 2007	A14 [γ]	33
54	*R. nelsonae*	BASF BP-N	ACF	Sapelo Island, GA	6 Feb 2007		
55	*R. nelsonae*	BASF BP-N	ACF	Sapelo Island, GA	6 Feb 2007		
56	*R. nelsonae*	BASF BP-J	ACF	Sapelo Island, GA	6 Feb 2007		
57	*R. nelsonae*	BASF PP-M	ACF	Sapelo Island, GA	6 Feb 2007		
58	*R. nelsonae*	BASF BP-J	ACF	Sapelo Island, GA	6 Feb 2007	A17	34
59	*R. nelsonae*	BASF WS-6	ACF	Sapelo Island, GA	30 Jan 2008		
60	*R. nelsonae*	BASF WS-8	ACF	Sapelo Island, GA	30 Jan 2008	A18	35
61	*R. nelsonae*	BASF BP-H	ACF	Sapelo Island, GA	6 Feb 2007	A24	36
62	*R. nelsonae*	BASF BP-G	ACF	Sapelo Island, GA	6 Feb 2007	A25	37
63	*R. nelsonae*	BASF WS-1	ACF	Sapelo Island, GA	6 Feb 2007		
64	*R. nelsonae*	BASF YPP-J	ACF	Sapelo Island, GA	6 Feb 2007	A26	38
65	*R. nelsonae*	BASF WS-2	ACF	Sapelo Island, GA	6 Feb 2007		
66	*R. speratus*	AY553155				AY553155	39
67	*R. lucifugus grassei*	AY027456		UK		AY027456	40
68	*R. lucifugus grassei*	AY027457		UK		AY027457	41
69	*R. lucifugus grassei*	AY027458		UK		AY027458	42
70	*R. lucifugus grassei*	AY027459		UK		AY027459	43
71	*R. lucifugus grassei*	AY027460		UK		AY027460	44
72	*R. lucifugus grassei*	AY027461		UK		AY027461	45
73	*R. lucifugus grassei*	AY027462		UK		AY027462	46
74	*R. lucifugus*	AY027463		UK		AY027463	47

	grassei				
75	*R. lucifugus grassei*	AY027464	France	AY027464	48
76	*C. formosanus*	AY027472	USA	AY027472	49
77	*H. tenuior*	AY553154		AY553154	50

Table 4.3. Collection localities, date of collection and soil province information for samples that were amplified for ITS array (ITS).

No.	Species	Sample name	Soil province	Collection site	Collection date	Haplotypes for ITS region	No. of Haplotypes
1	*R. flavipes*	AM026	P	Holmes-Hunter Building , Athens, GA	13 Mar 2009	ITSA1	1
2	*R. flavipes*	P3.181	SCP	Greenwood Plantation, GA	9 Nov 2009		
3	*R. flavipes*	P3.216	SCP	Greenwood Plantation, GA	9 Nov 2009		
4	*R. flavipes*	SI001	ACF	Sapelo Island, GA	5 Jul 2009		
5	*R. flavipes*	SI020	ACF	Sapelo Island, GA	5 Jul 2009		
6	*R. flavipes*	GP002A	SCP	Greenwood Plantation, GA	25Aug 2009	ITSA2	2
7	*R. flavipes*	SI021	ACF	Sapelo Island, GA	6 Jul 2009		
8	*R. flavipes*	AM017	P	UGA Chapel, Athens, GA	5 Apr 2010	AM017	3
9	*R. flavipes*	E1	P	East Campus, Athens, GA	21 Apr 2009	E1	4
10	*R. flavipes*	B2	P	Biosciences Building, Athens, GA	21 Apr 2009	B2	5
11	*R. flavipes*	BOT6	P	Botanical Gardens, Athens, GA	23 Oct 2007	BOT6	6
12	*R. flavipes*	SI004	ACF	Sapelo Island, GA	5 Jul 2009	SI004	7
13	*R. flavipes*	B36A	SCP	Branford, FL	31 Mar 2010	B36A	8
14	*R. flavipes*	GP004B	SCP	Greenwood Plantation, GA	25 Aug 2009	GP004B	9
15	*R. virginicus*	BOT10	P	Botanical Gardens, Athens, GA	23 Oct 2007	ITSA3	10
16	*R. virginicus*	Cobb1	P	Cobb house, Athens, GA	23 Nov 2007		
17	*R. virginicus*	WM024A	P	Cobb house, Athens, GA	28 May 2010		
18	*R. virginicus*	ASMSPA1	ACF	Sapelo Island, GA	12 May 2008		
19	*R. virginicus*	ROW1	ACF	Red Oak, GA	3 Aug 2002		
20	*R. virginicus*	SI005	ACF	Sapelo Island, GA	5 Jul 2009		
21	*R. virginicus*	SI009	ACF	Sapelo Island, GA	5 Jul 2009		
22	*R. virginicus*	SI013	ACF	Sapelo Island, GA	5 Jul 2009		
23	*R. virginicus*	SI017	ACF	Sapelo Island, GA	5 Jul 2009		

24	*R. virginicus*	GP003D	SCP	Greenwood Plantation, GA	25 Aug 2009		
25	*R. virginicus*	BOT3	P	Botanical Gardens, Athens, GA	23 Oct 2007	BOT3	11
26	*R. virginicus*	BSW1	P	Dellwood, Athens, GA	8 May 2006	BSW1	12
27	*R. virginicus*	CHW1	P	Chicopee Building, Athens, GA	30 May 2008	CHW1	13
28	*R. virginicus*	WM022A	P	Biosciences Building, Athens, GA	28 May 2010	WM022A	14
29	*R. virginicus*	ROW1	ACF	Red Oak, GA	3 Aug 2002	ROW1	15
30	*R. virginicus*	SI009	ACF	Sapelo Island, GA	5 Jul 2009	SI009	16
31	*R. hageni*	AM002A	P	Commerce, GA	13 Aug 2008		
32	*R. hageni*	FT8	P	Four Towers, Athens, GA	23 Nov 2007	ITSA4	17
33	*R. hageni*	FT9A4H	P	Four Towers, Athens, GA	13 Jul 2007		
34	*R. hageni*	BB10	P	Barn Barnsville, GA	22 June 2007	BB10	18
35	*R. hageni*	BB3	P	Barn Barnsville, GA	22 June 2007	BB3	19
36	*R. hageni*	DawHll2005	P	Four Towers, Athens, GA	6 May 2005	DawHll2005	20
37	*R. hageni*	FT2710	P	Four Towers, Athens, GA	14 Aug 2007	FT2710	21
38	*R. hageni*	FTT1	P	Four Towers, Athens, GA	23 Nov 2007	FTT1	22
39	*R. malletei*	BOT11	P	Botanical Gardens, Athens, GA	23 Oct 2007		
40	*R. malletei*	4TA1	P	Four Towers, Athens, GA	27 May 2008		
41	*R. malletei*	AM013A	P	Athens, GA	22 May 2007		
42	*R. malletei*	AM015A	P	East Campus, Athens, GA	21 May 2010		
43	*R. malletei*	AM019A	P	Athens, GA	29 May 2010		
44	*R. malletei*	AM020A	P	Athens, GA	29 May 2010	ITSA5	23
45	*R. malletei*	AM021A	P	Whippoorwill Circle, Athens, GA	29 May 2010		
46	*R. malletei*	BIOS4	P	Bioscience Building, Athens, GA	23 Nov 2007		
47	*R. malletei*	BOT8	P	Botanical Gardens, Athens, GA	23 Oct 2007		

48	*R. malletei*	NC004	ACF	Havelock, NC	May 2010		
49	*R. nelsonae*	P1.21	SCP	Greenwood Plantation, GA	9 Nov 2009		
50	*R. nelsonae*	SI003	ACF	Sapelo Island, GA	5 Jul 2009		
51	*R. nelsonae*	SI006	ACF	Sapelo Island, GA	5 Jul 2009		
52	*R. nelsonae*	1211P0047	ACF	Havelock, NC	5 Nov 2009		
53	*R. nelsonae*	GP002D	SCP	Greenwood Plantation, GA	25 Aug 2009	ITSA6	24
54	*R. nelsonae*	P1.10	SCP	Greenwood Plantation, GA	9 Nov 2009		
55	*R. nelsonae*	P2.140	SCP	Greenwood Plantation, GA	9 Nov 2009	ITSA7	25
56	*R. malletei*	BOT2	P	Botanical Gardens, Athens, GA	23 Oct 2007	BOT2	26
57	*R. malletei*	DRMW1	P	Four Towers, Athens, GA	24 May 2008	DRMW1	27
58	*R. nelsonae*	B17A	SCP	Branford, FL	31 Mar 2010	B17A	28
59	*R. nelsonae*	B20A	SCP	Branford, FL	31 Mar 2010	B20A	29
60	*R. nelsonae*	LA06-18	SCP	Hahira, GA	18 Jun 2009	LA06-18	30
61	*R. nelsonae*	P1.26	SCP	Greenwood Plantation, GA	9 Nov 2009	P1.26	31
62	*R. nelsonae*	SI008	ACF	Sapelo Island, GA	5 Jul 2009	SI008	32
63	*Heterotermes convexinotatus*	DQ923415		Puerto Rico		DQ923415	33
64	*Coptotermes gestroi*	EF092288				EF092288	34

Table 4.4. Summary on the number of samples studied using COII gene by soil province and collection sites.

Soil province	Piedmont	Atlantic Coast Flatwoods			South Coast Plain		
Collection sites/ Species	Athens, GA	Red Oak, Sapelo and Cumberland Island, GA	Havelock, NC	Total	Thomasville and South Georgia, GA	Branford and South Florida, FL	Total
R. flavipes	39	28	29	57	43	15	58
R.virginicus	11	27		27	4	3	7
R. hageni	12						
R. malletei	18		4	4			
R. nelsonae		22	12	34	59	19	78

Table 4.5. Summary on the number of samples studied using COI gene by soil province and collection sites for samples collected by authors.

Soil province	Piedmont	Atlantic Coast Flatwoods	South Coast Plain
Collection sites/ Species	Athens, GA	Sapelo Island, GA	Thomasville, GA
R. flavipes	8	6	
R.virginicus	6	2	
R. hageni	6		
R. malletei	9		
R. nelsonae		13	

Table 4.6. Summary of the number of samples studied using ITS region by soil province.

Soil province	Piedmont	Atlantic Coast Flatwoods	South Coastal Plain
R. flavipes	5	4	5
R. virginicus	7	8	1
R. hageni	8		
R. malletei	11	3	
R. nelsonae		3	7

Table 4.7. PCR amplification protocol for the specific DNA fragments.

DNA fragment of interest	PCR Protocols		
	Temperature (°C)	Duration (mins)	
COII	94	1	
	94	1	
	51	1	Repeat 29 x
	72	2	
	72	5	
COI	94	1	
	94	1	
	50	1	Repeat 29 x
	72	2	
	72	5	
ITS	94	2	
	94	30	
	46	45	Repeat 35x
	72	45	
	72	7	

Table 4.8. Primers for the PCR amplification and internal primers for sequencing of the specific DNA fragments.

DNA fragment of interest	Primer pair [9] (forward and reverse)	Sequences (5'⟶ 3')
COII	TL2-J-3037	ATG GCA GAT TAG TGC AAT GG
	TK-N-3785	GTT TAA GAG ACC AGT ACT TG
COI	C1J2195	TTG ATT CTT TTG GTC ACT CCA TGA AGT
	TL2N3014	TCC TAA TTG CAC TTA ATC TGC CAT ATT
ITS	CS249	TCG TAA CAA GGT TTC CG
	CS250	GTT (A/T)GT TTC TTT TCC TC

[9] Primer used for PCR and sequencing

Table 4.9. Average nucleotide frequency for COII, COI and ITS.

DNA region	Nucleotide			
	T(U)	C	A	G
COII	23.5	23.5	39.1	13.9
COI	26.8	23.3	34.4	15.6
ITS	17.6	29.9	19.9	32.6

Table 4.10. The number of haplotypes for COII, and COI gene and ITS region.

Species	Number of haplotypes (COII)	Number of haplotypes (COI)	Number of haplotypes (ITS)
R. flavipes	56	8	9
R. virginicus	20	5	7
R. hageni	4	1	6
R. malletei	7	2	2.5 *
R. nelsonae	34	6	7.5 *
Total	120	22	32

* These were noted in decimals because there was one haplotype that was shared between the two taxa.

Table 4.11. Estimate of net evolutionary divergence for COII showing the numbers of base substitutions per site obtained from net average between species.

Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*
R. flavipes		0.008	0.008	0.007	0.009
R. virginicus	0.052		0.006	0.006	0.008
R. hageni	0.047	0.033		0.006	0.008
R. malletei	0.046	0.036	0.031		0.007
R. nelsonae	0.059	0.049	0.044	0.039	

Note:

- Unshaded boxes are numbers of base substitutions per site.
- Shaded boxes are standard error estimates diagonal to the number of base substitutions per site.

Table 4.12. Estimate of net evolutionary divergence for COI showing the numbers of base substitutions per site obtained from net average between species.

Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*
R. flavipes		0.009	0.009	0.008	0.009
R. virginicus	0.051		0.008	0.007	0.008
R. hageni	0.049	0.040		0.006	0.007
R. malletei	0.042	0.038	0.027		0.006
R. nelsonae	0.050	0.045	0.037	0.030	

Note:

- Unshaded boxes are numbers of base substitutions per site.
- Shaded boxes are standard error estimates diagonal to the number of base substitutions per site.

Table 4.13. Estimate of net evolutionary divergence for ITS showing the numbers of base substitutions per site obtained from net average between species.

Species	*R. flavipes*	*R. virginicus*	*R. hageni*	*R. malletei*	*R. nelsonae*
R. flavipes		0.01036	0.01278	0.01060	0.09995
R. virginicus	0.010649		0.00287	0.00139	0.00139
R. hageni	0.013411	0.006957		0.00260	0.00260
R. malletei	0.008085	0.001458	0.005499		0.00005
R. nelsonae	0.008316	0.001509	0.005547	0.00005	

Note:

- Unshaded boxes are numbers of base substitutions per site.
- Shaded boxes are standard error estimates diagonal to the number of base substitutions per site.

Figure Captions

Figure 4.1. Map of the southeast region of USA showing main collection points Georgia, North Carolina and Florida.

Figure 4.2a-4.2c. Maximum likehood (ML) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene data (684bp). The ML analysis with GTR+G+I, gamma Pinv = 0.559, gamma shape parameter = 1.070 generated with a log likelihood of -5075.935. Branch support was calculated with Approximate Likelihood-Ratio Test (aLRT) SH-like. The scale bar represents 0.05 substitution/ site.

Figure 4.3a-4.3c. Distance Method (BIONJ) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COII (684bp) with model of substitution was Kimura 2-parameter. Bootstrap support, calculated from 1000 replicates, is expressed as percentage. Scale bar represents 0.01 substitution/site.

Figure 4.4a-4.4c. Maximum parsimony (MP) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COII (681bp) data. The MP analysis generated 93 most parsimonious trees (MPTs) of 549 steps, consistency index = (0.462), retention index = (0.910), composite index= 0.498 (0.421) for all sites and parsimony-informative sites (in parentheses). Bootstrap consensus tree inferred from 1000 replicates is expressed as percentage (are shown above to the branches) and taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are

collapsed. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions (1st+2nd+3rd+Noncoding) were included.

Figure 4.5. Maximum likelihood (ML) estimate of *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) data. The ML analysis with GTR+G+I, gamma Pinv = 0.578, gamma shape parameter = 0.878 generated with a log likelihood of -2905.620. The dotted bars refer to groupings that have mixed species designation within them. Branch support was calculated with Approximate Likelihood-Ratio Test (aLRT) SH-like. The scale bar represents 0.1 substitution/site. The dotted bars refer to groupings that have mixed species designation within them. The dotted bars refer to groupings that have mixed species designations within them.

Figure 4.6. BIONJ estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) with model of substitution was Kimura 2- parameter. Bootstrap support, calculate from 1000 replicates, is expressed as percentage. Scale bar represents 0.05 substitution/site. The dotted bars refer to groupings that have mixed species designation within them.

Figure 4.7. Maximum parsimony (MP) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene COI (767 bp) data. The MP analysis generated 33 most parsimonious trees (MPTs) of 366 steps, consistency index = (0.556), retention index = (0.892), composite index= 0.565 (0.496) for all sites and parsimony-informative sites (in parentheses).

Bootstrap consensus tree inferred from 1000 replicates is expressed as percentage (are shown above to the branches) and taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions (1st+2nd+3rd+Noncoding) were included. The dotted bars refer to groupings that have mixed species designation within them.

Figure 4.8. Maximum likelihood (ML) estimate of *Reticulitermes* phylogeny based on ITS region (599bp) data. The ML analysis with GTR+G+I, gamma Pinv = 0.503, gamma shape parameter = 0.823 generated with a log likelihood of -1437.612. Branch support was calculated with Approximate Likelihood-Ratio Test (aLRT) SH-like. The scale bar represents 0.01 substitution/ site. The dotted bars refer to groupings that have mixed species designation within them.

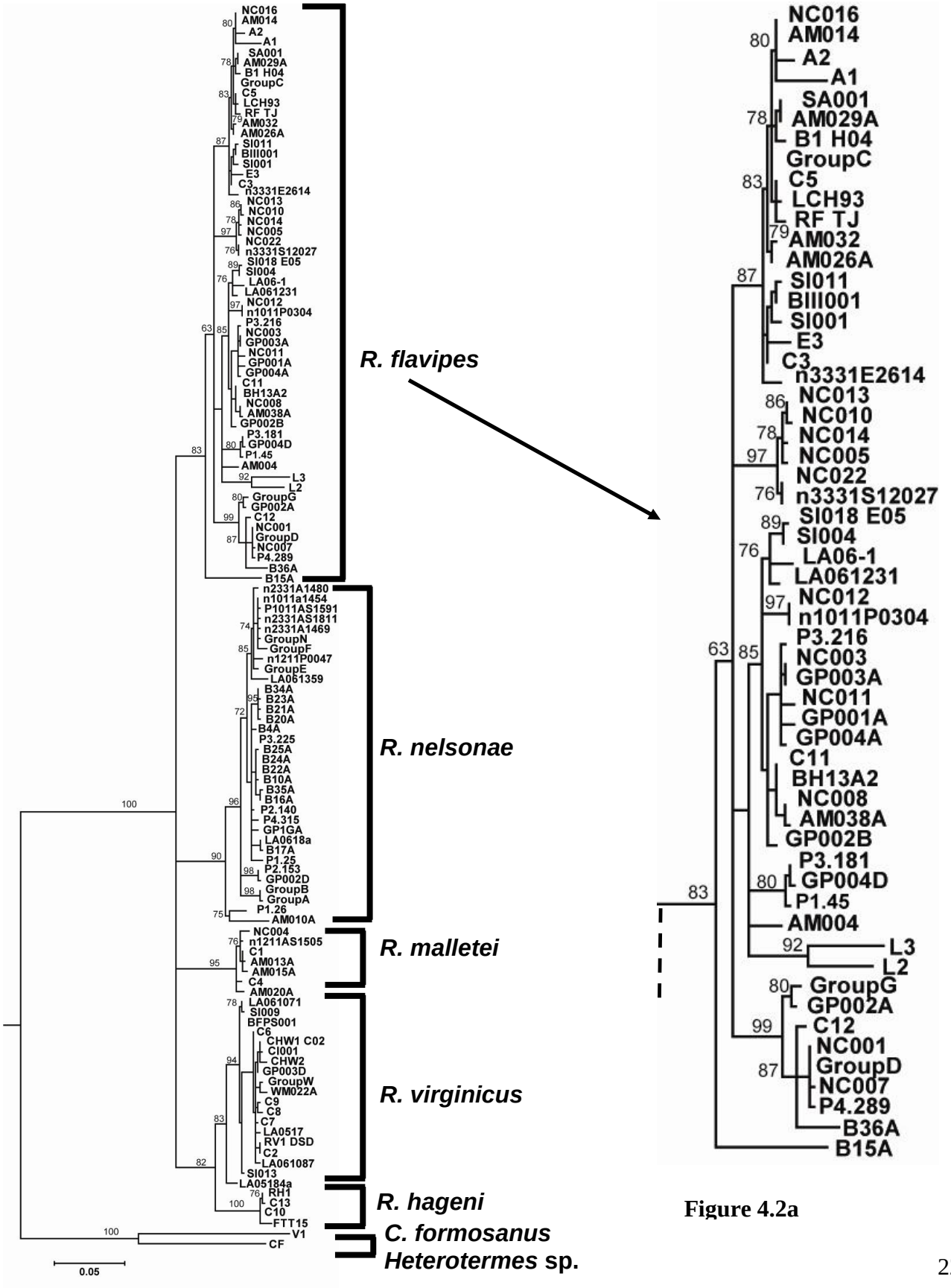
Figure 4.9. Distance Method (BIONJ) estimate of *Reticulitermes* phylogeny based on ITS region (599bp) data with model of substitution was Kimura 2- parameter. Bootstrap support, calculated from 1000 replicates, is expressed as percentage. Scale bar represents 0.01 substitution/site.

Figure 4.10. Maximum parsimony (MP) estimate of the *Reticulitermes* phylogeny based on mitochondrial ribosomal gene ITS (542bp) data. The MP analysis generated 387 most parsimonious trees (MPTs) of 475 steps, consistency index = (0.904), retention index = (0.927), composite index= 0.912 (0.838) for all sites and parsimony-informative sites (in parentheses).

Bootstrap consensus tree inferred from 1000 replicates is expressed as percentage (are shown above to the branches) and taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions (1st+2nd+3rd+Noncoding) were included.


Athens, GA
Havelock, NC
Thomasville, GA
Sapelo Island, GA
Branford, FL

Figure 4.1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SI001
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L2
GroupG
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GroupD
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CI001
CHW2
GP003D
GroupW
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C9
C8
C7
LA0517
RV1 DSD
C2
LA061087
SI013
LA05184a
RH1
C13
C10
FTT15
V1
CF
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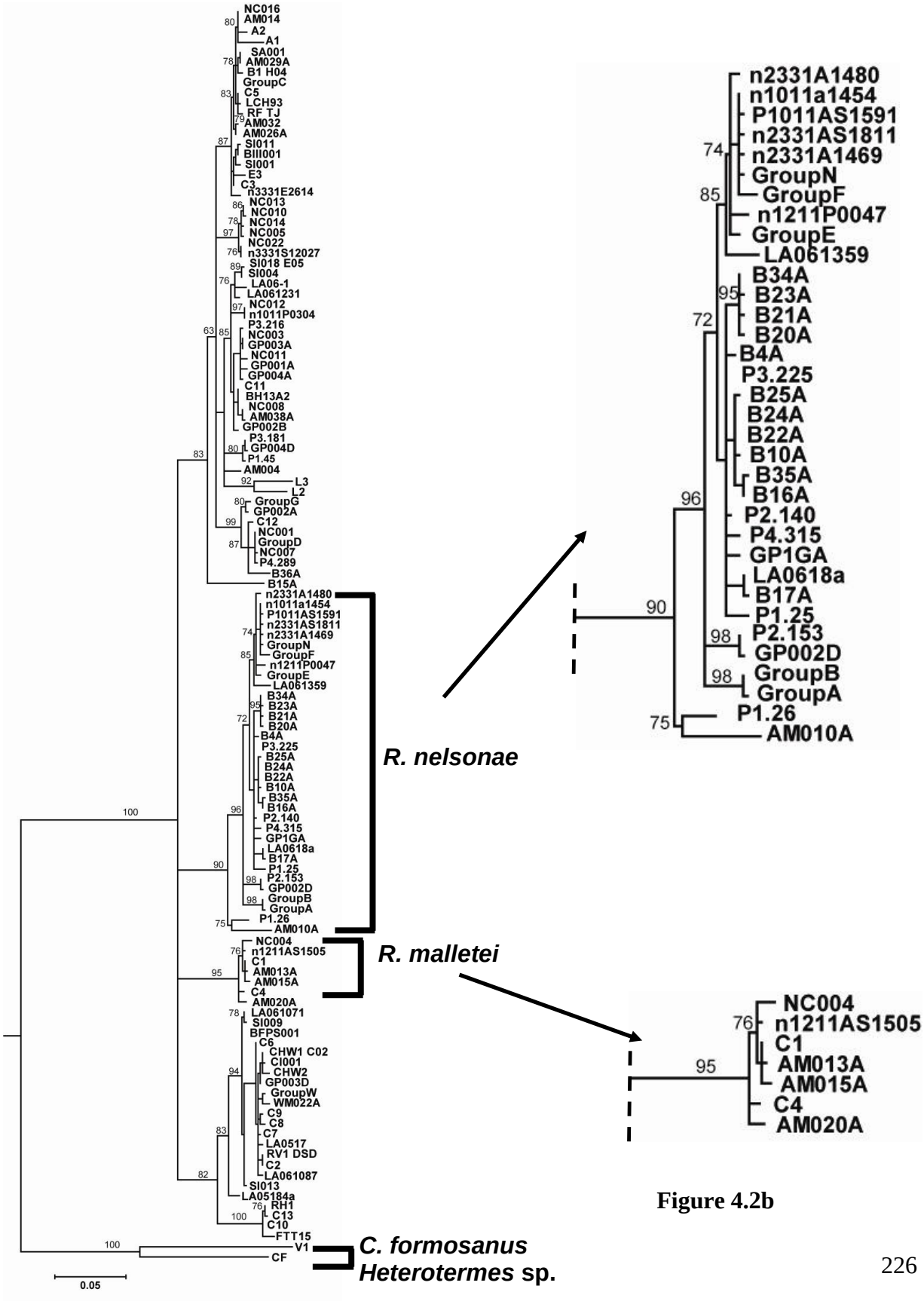
R. malletei
R. virginicus
R. hageni
C. formosanus
Heterotermes sp.

Figure 4.2a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AM015A
C4
AM020A
R. malletei
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BFPS001
C6
CHW1 C02
CI001
CHW2
GP003D
GroupW
WM022A
C9
C8
C7
LA0517
RV1 DSD
C2
LA061087
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C10
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V1
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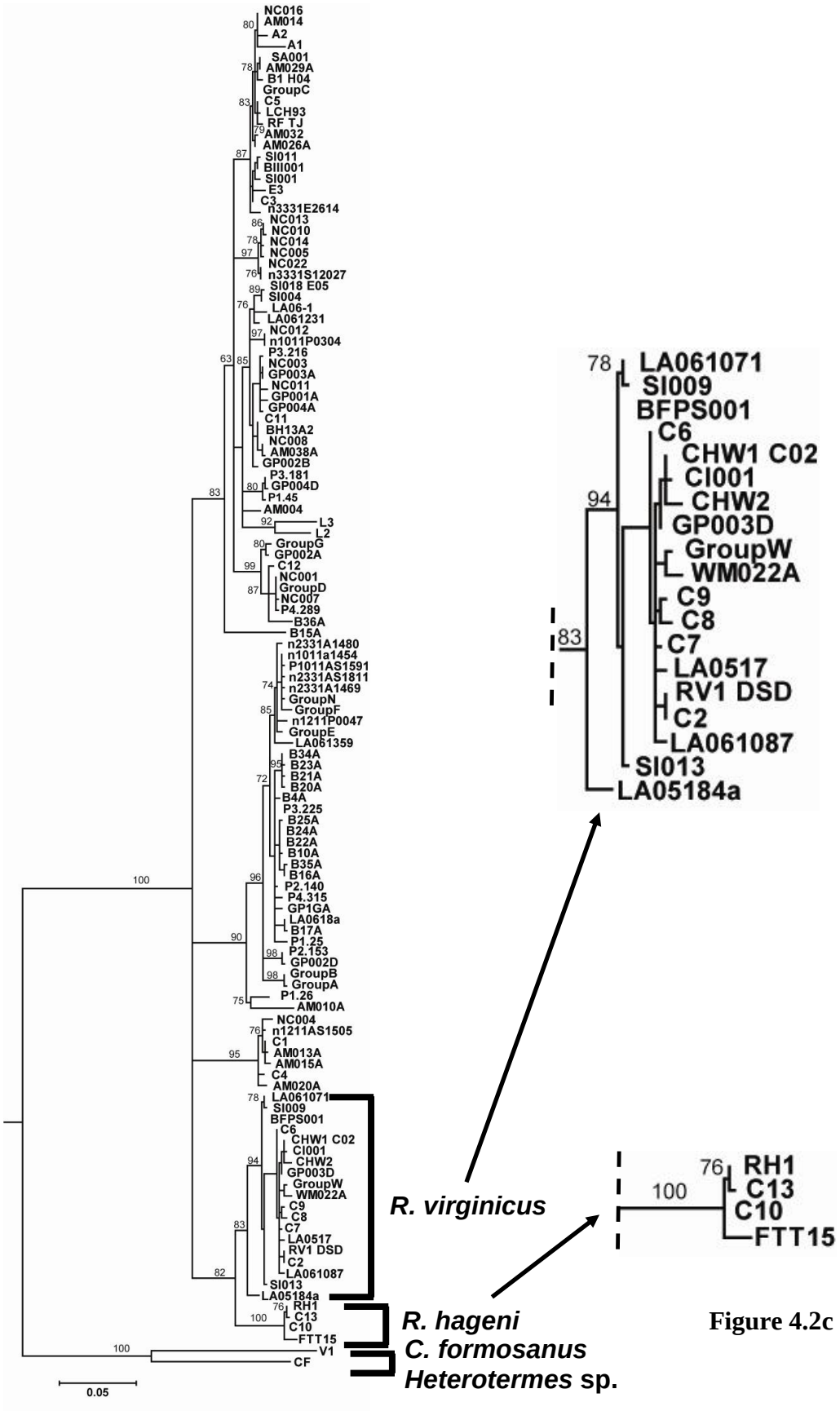
Heterotermes sp.
0.05

Figure 4.2b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GroupG
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C6
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CI001
CHW2
GP003D
GroupW
WM022A
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C8
C7
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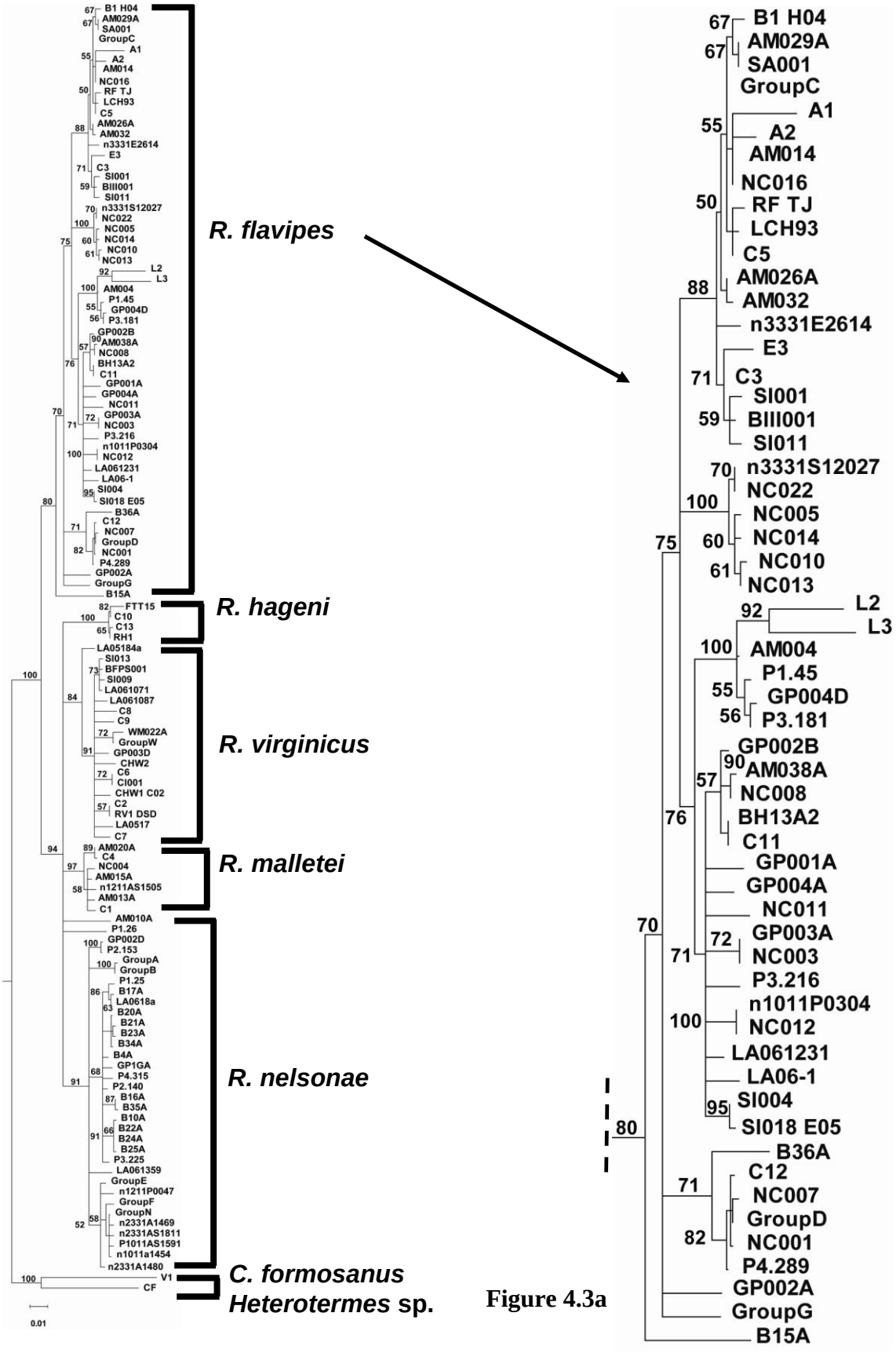
C2
LA061087
SI013
LA05184a
RH1
C13
C10
FTT15
V1
CF
0.05
R. virginicus
R. hageni
C. formosanus
Heterotermes sp.

Figure 4.2c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E3
C3
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BIII001
SI011
n3331S12027
NC022
NC005
NC014
NC010
NC013
L2
L3
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P1.45
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P3.181
GP002B
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NC008
BH13A2
C11
GP001A
GP004A
NC011
GP003A
NC003
P3.216
n1011P0304
NC012
LA061231
LA06-1
SI004
SI018 E05
B36A
C12
NC007
GroupD
NC001
P4.289
GP002A
GroupG
B15A
R. flavipes
FTT15
C10
C13
RH1
R. hageni
LA05184a
SI013
BFPS001
SI009
LA061071
LA061087
C8
C9
WM022A
GroupW
GP003D
CHW2
C6
CI001
CHW1 C02
C2
RV1 DSD
LA0517
C7
R. virginicus
AM020A
C4
NC004
AM015A
n1211AS1505
AM013A
C1
R. malletei
AM010A
P1.26
GP002D
P2.153
GroupA
GroupB
P1.25
B17A
LA0618a
B20A
B21A
B23A
B34A
B4A
GP1GA
P4.315
P2.140
B16A
B35A
B10A
B22A
B24A
B25A
P3.225
LA061359
GroupE
n1211P0047
GroupF
GroupN
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n2331AS1811
P1011AS1591
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R. nelsonae
V1
CF
C. formosanus
Heterotermes sp.
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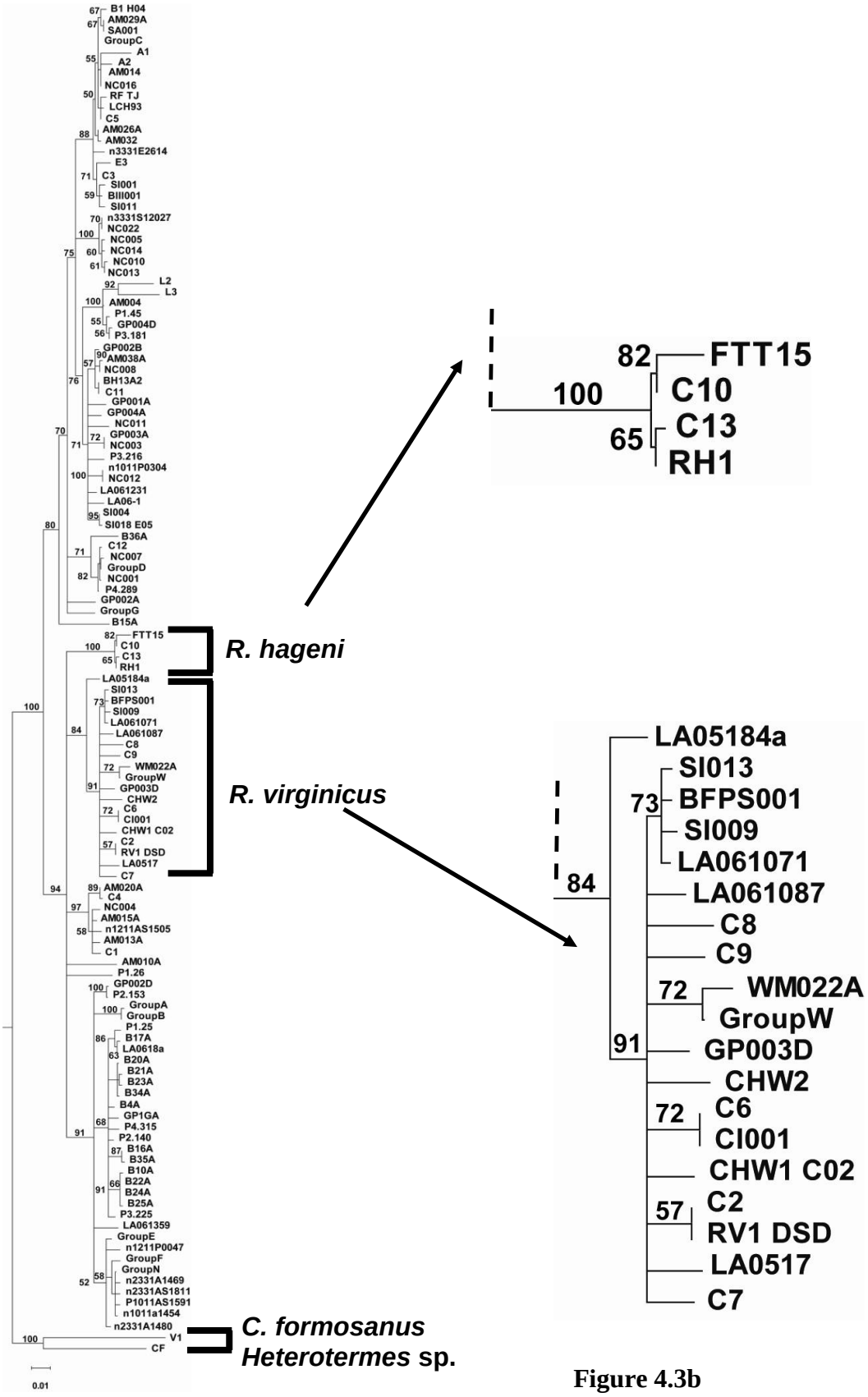
Figure 4.3a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E3
C3
SI001
BIII001
SI011
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NC005
NC014
NC010
NC013
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GP004A
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SI018 E05
B36A
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GroupD
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P4.289
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GroupG
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FTT15
C10
C13
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LA05184a
SI013
BFPS001
SI009
LA061071
LA061087
C8
C9
WM022A
GroupW
GP003D
CHW2
C6
CI001
CHW1 C02
C2
RV1 DSD
LA0517
C7
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C4
NC004
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C1
AM010A
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P2.153
GroupA
GroupB
P1.25
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LA0618a
B20A
B21A
B23A
B34A
B4A
GP1GA
P4.315
P2.140
B16A
B35A
B10A
B22A
B24A
B25A
P3.225
LA061359
GroupE
n1211P0047
GroupF
GroupN
n2331A1469
n2331AS1811
P1011AS1591
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n2331A1480
V1
CF
0.01
R. hageni
R. virginicus
C. formosanus
Heterotermes sp.
82
FTT15
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C10
65
C13
RH1
LA05184a
SI013
73
BFPS001
SI009
LA061071
84
LA061087
C8
C9
72
WM022A
GroupW
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CHW2
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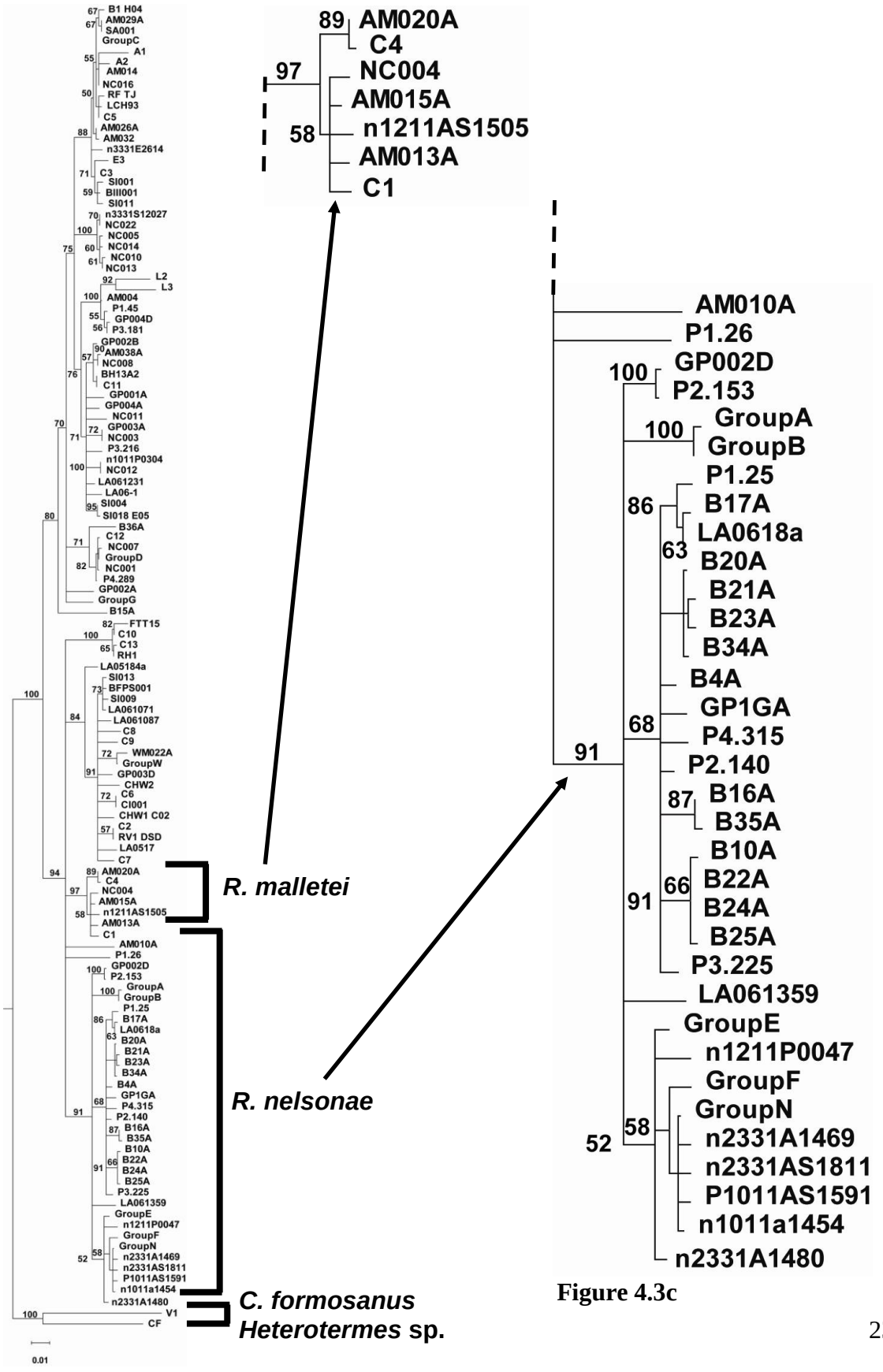
C6
CI001
CHW1 C02
57
C2
RV1 DSD
LA0517
C7

Figure 4.3b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E3
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SI001
BIII001
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NC005
NC014
NC010
NC013
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P1.45
GP004D
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GP001A
GP004A
NC011
GP003A
NC003
P3.216
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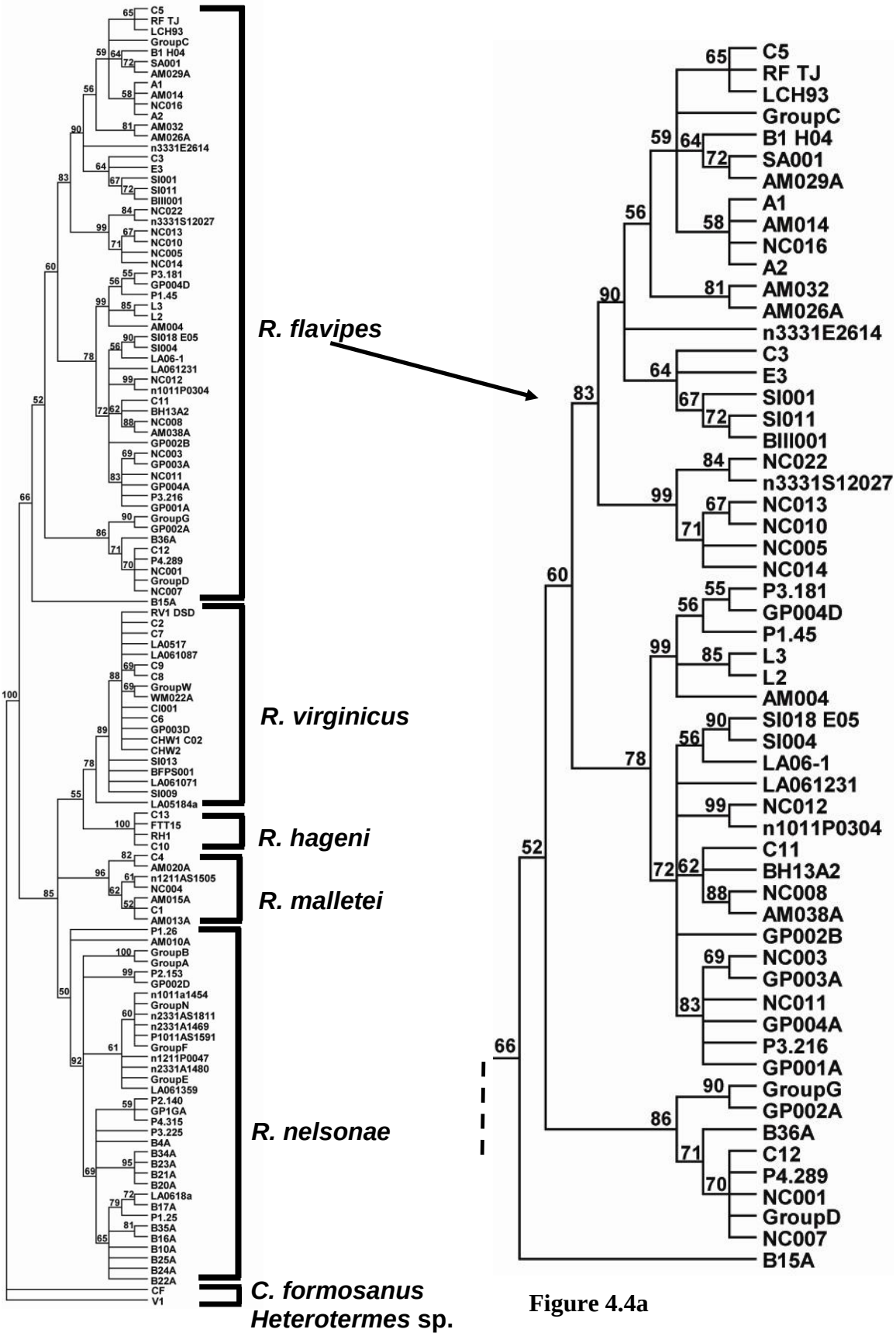
LA06-1
SI004
SI018 E05
B36A
C12
NC007
GroupD
NC001
P4.289
GP002A
GroupG
B15A
FTT15
C10
C13
RH1
LA05184a
SI013
BFPS001
SI009
LA061071
LA061087
C8
C9
WM022A
GroupW
GP003D
CHW2
C6
CI001
CHW1 C02
C2
RV1 DSD
LA0517
C7
AM020A
C4
NC004
AM015A
n1211AS1505
AM013A
C1
AM010A
P1.26
GP002D
P2.153
GroupA
GroupB
P1.25
B17A
LA0618a
B20A
B21A
B23A
B34A
B4A
GP1GA
P4.315
P2.140
B16A
B35A
B10A
B22A
B24A
B25A
P3.225
LA061359
GroupE
n1211P0047
GroupF
GroupN
n2331A1469
n2331AS1811
P1011AS1591
n1011a1454
n2331A1480
V1
CF
0.01
R. malletei
R. nelsonae
C. formosanus
Heterotermes sp.
89 AM020A
C4
97 NC004
AM015A
58 n1211AS1505
AM013A
C1
AM010A
P1.26
100 GP002D
P2.153
100 GroupA
GroupB
P1.25
86 B17A
LA0618a
63 B20A
B21A
B23A
B34A
B4A
68 GP1GA
P4.315
91 P2.140
87 B16A
B35A
B10A
91 66 B22A
B24A
B25A
P3.225
LA061359
GroupE
n1211P0047
GroupF
GroupN
52 58 n2331A1469
n2331AS1811
P1011AS1591
n1011a1454
n2331A1480

Figure 4.3c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E3
SI001
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NC010
NC005
NC014
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GP004D
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L2
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SI018 E05
SI004
LA06-1
LA061231
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NC008
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NC003
GP003A
NC011
GP004A
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GP001A
GroupG
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RV1 DSD
C2
C7
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LA061087
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GroupW
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CHW1 C02
CHW2
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BFPS001
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SI009
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C13
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RH1
C10
C4
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n1211AS1505
NC004
AM015A
C1
AM013A
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AM010A
GroupB
GroupA
P2.153
GP002D
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GroupN
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n2331A1469
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GroupF
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GroupE
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GP1GA
P4.315
P3.225
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B21A
B20A
LA0618a
B17A
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B10A
B25A
B24A
B22A
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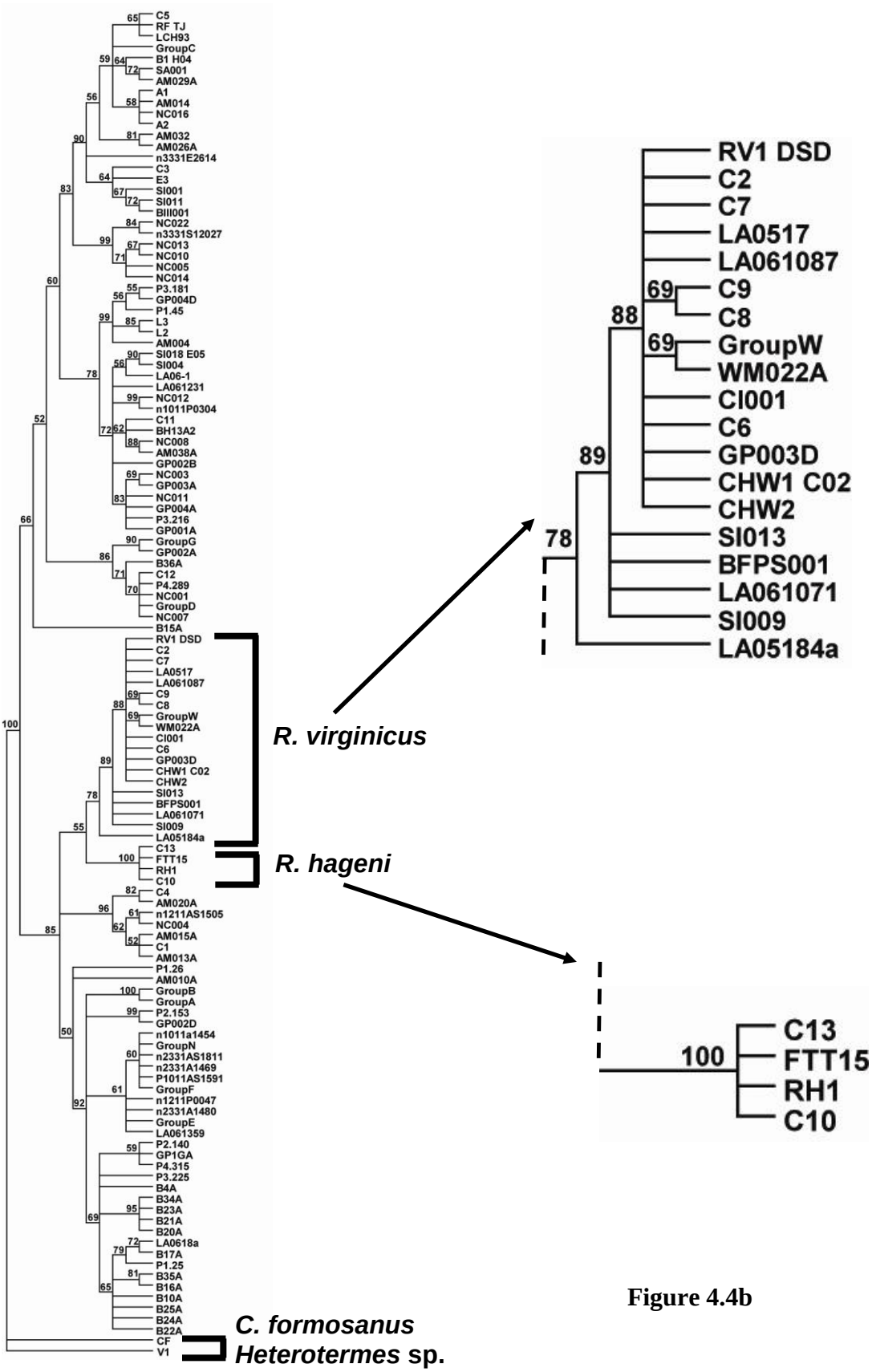
R. virginicus
R. hageni
R. malletei
R. nelsonae
C. formosanus
Heterotermes sp.

Figure 4.4a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C3
E3
SI001
SI011
BIII001
NC022
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NC013
NC010
NC005
NC014
P3.181
GP004D
P1.45
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L2
AM004
SI018 E05
SI004
LA06-1
LA061231
NC012
n1011P0304
C11
BH13A2
NC008
AM038A
GP002B
NC003
GP003A
NC011
GP004A
P3.216
GP001A
GroupG
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B36A
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P4.289
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GroupD
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B15A
RV1 DSD
C2
C7
LA0517
LA061087
C9
C8
GroupW
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CI001
C6
GP003D
CHW1 C02
CHW2
SI013
BFPS001
LA061071
SI009
LA05184a
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FTT15
RH1
C10
C4
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NC004
AM015A
C1
AM013A
P1.26
AM010A
GroupB
GroupA
P2.153
GP002D
n1011a1454
GroupN
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n2331A1469
P1011AS1591
GroupF
n1211P0047
n2331A1480
GroupE
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P2.140
GP1GA
P4.315
P3.225
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B34A
B23A
B21A
B20A
LA0618a
B17A
P1.25
B35A
B16A
B10A
B25A
B24A
B22A
CF
V1
R. virginicus
R. hageni
C. formosanus
Heterotermes sp.
RV1 DSD
C2
C7
LA0517
LA061087
C9
C8
GroupW
WM022A
CI001
C6
GP003D
CHW1 C02
CHW2
SI013
BFPS001
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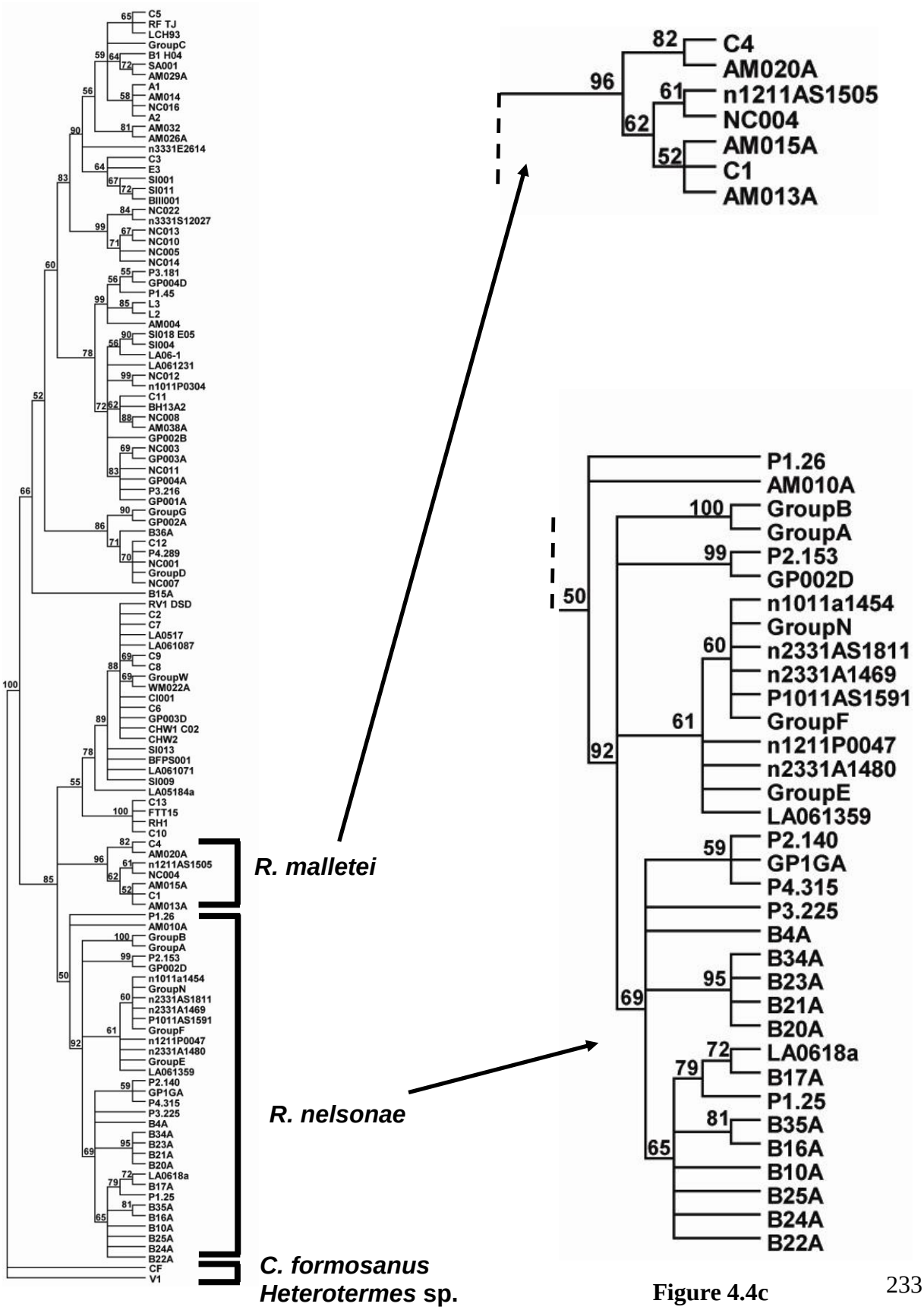
SI009
LA05184a
C13
FTT15
RH1
C10

Figure 4.4b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LCH93
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AM032
AM026A
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C3
E3
SI001
SI011
BIII001
NC022
n3331S12027
NC013
NC010
NC005
NC014
P3.181
GP004D
P1.45
L3
L2
AM004
SI018 E05
SI004
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LA061231
NC012
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BH13A2
NC008
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GP002B
NC003
GP003A
NC011
GP004A
P3.216
GP001A
GroupG
GP002A
B36A
C12
P4.289
NC001
GroupD
NC007
B15A
RV1 DSD
C2
C7
LA0517
LA061087
C9
C8
GroupW
WM022A
CI001
C6
GP003D
CHW1 C02
CHW2
SI013
BFPS001
LA061071
SI009
LA05184a
C13
FTT15
RH1
C10
C4
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NC004
AM015A
C1
AM013A
P1.26
AM010A
GroupB
GroupA
P2.153
GP002D
n1011a1454
GroupN
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n2331A1469
P1011AS1591
GroupF
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n2331A1480
GroupE
LA061359
P2.140
GP1GA
P4.315
P3.225
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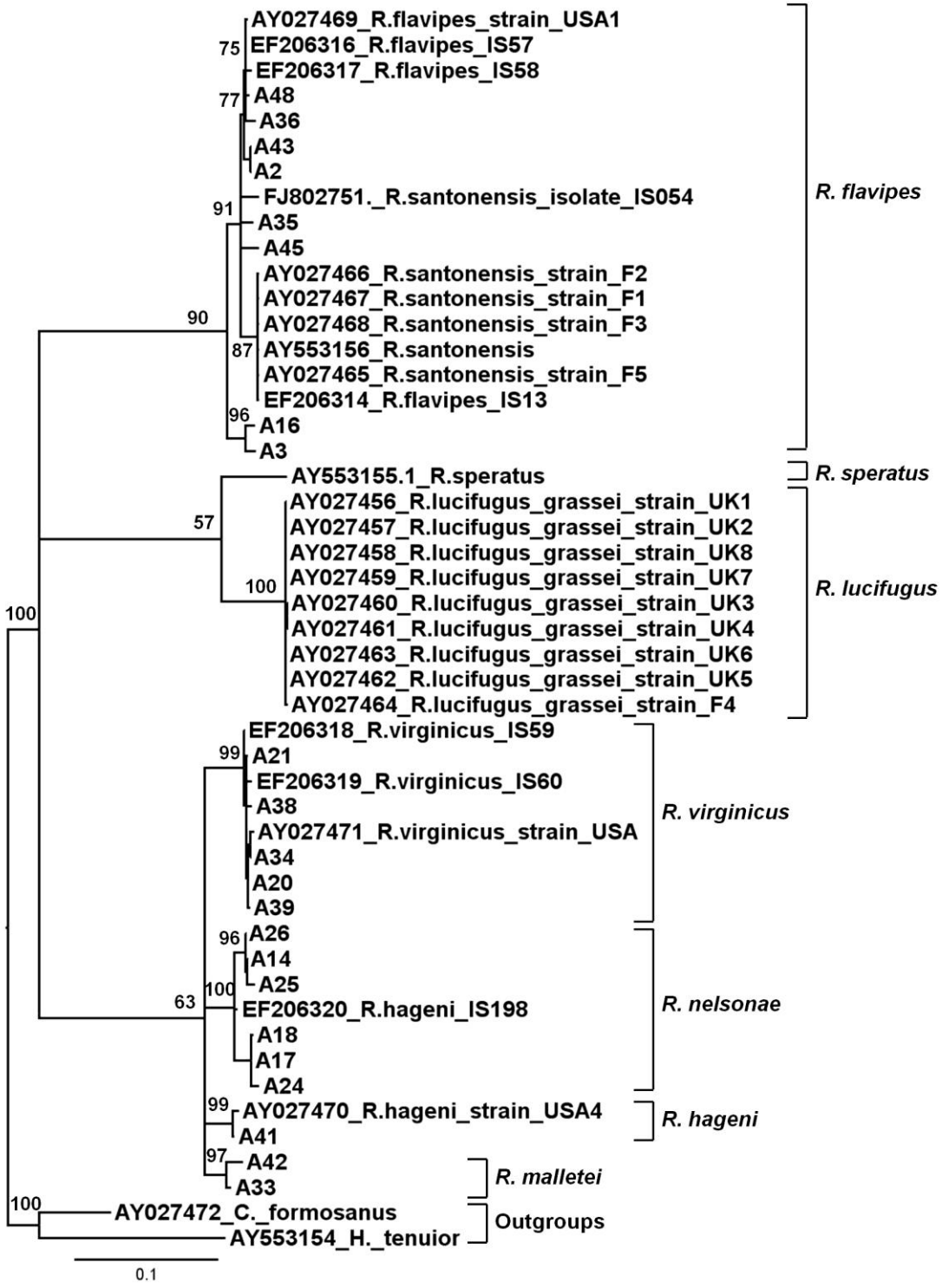
B34A
B23A
B21A
B20A
LA0618a
B17A
P1.25
B35A
B16A
B10A
B25A
B24A
B22A
CF
V1
R. malletei
R. nelsonae
C. formosanus
Heterotermes sp.
C4
AM020A
n1211AS1505
NC004
AM015A
C1
AM013A
P1.26
AM010A
GroupB
GroupA
P2.153
GP002D
n1011a1454
GroupN
n2331AS1811
n2331A1469
P1011AS1591
GroupF
n1211P0047
n2331A1480
GroupE
LA061359
P2.140
GP1GA
P4.315
P3.225
B4A
B34A
B23A
B21A
B20A
LA0618a
B17A
P1.25
B35A
B16A
B10A
B25A
B24A
B22A

Figure 4.4c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EF206317_R.flavipes_IS58
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A43
A2
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A35
A45
AY027466_R.santonensis_strain_F2
AY027467_R.santonensis_strain_F1
AY027468_R.santonensis_strain_F3
AY553156_R.santonensis
AY027465_R.santonensis_strain_F5
EF206314_R.flavipes_IS13
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A3
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R. speratus
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AY027458_R.lucifugus_grassei_strain_UK8
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AY027461_R.lucifugus_grassei_strain_UK4
AY027463_R.lucifugus_grassei_strain_UK6
AY027462_R.lucifugus_grassei_strain_UK5
AY027464_R.lucifugus_grassei_strain_F4
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EF206319_R.virginicus_IS60
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AY027471_R.virginicus_strain_USA
A34
A20
A39
R. virginicus
A26
A14
A25
EF206320_R.hageni_IS198
A18
A17
A24
R. nelsonae
AY027470_R.hageni_strain_USA4
A41
R. hageni
A42
A33
R. malletei
AY027472_C._formosanus
AY553154_H._tenuior
Outgroups
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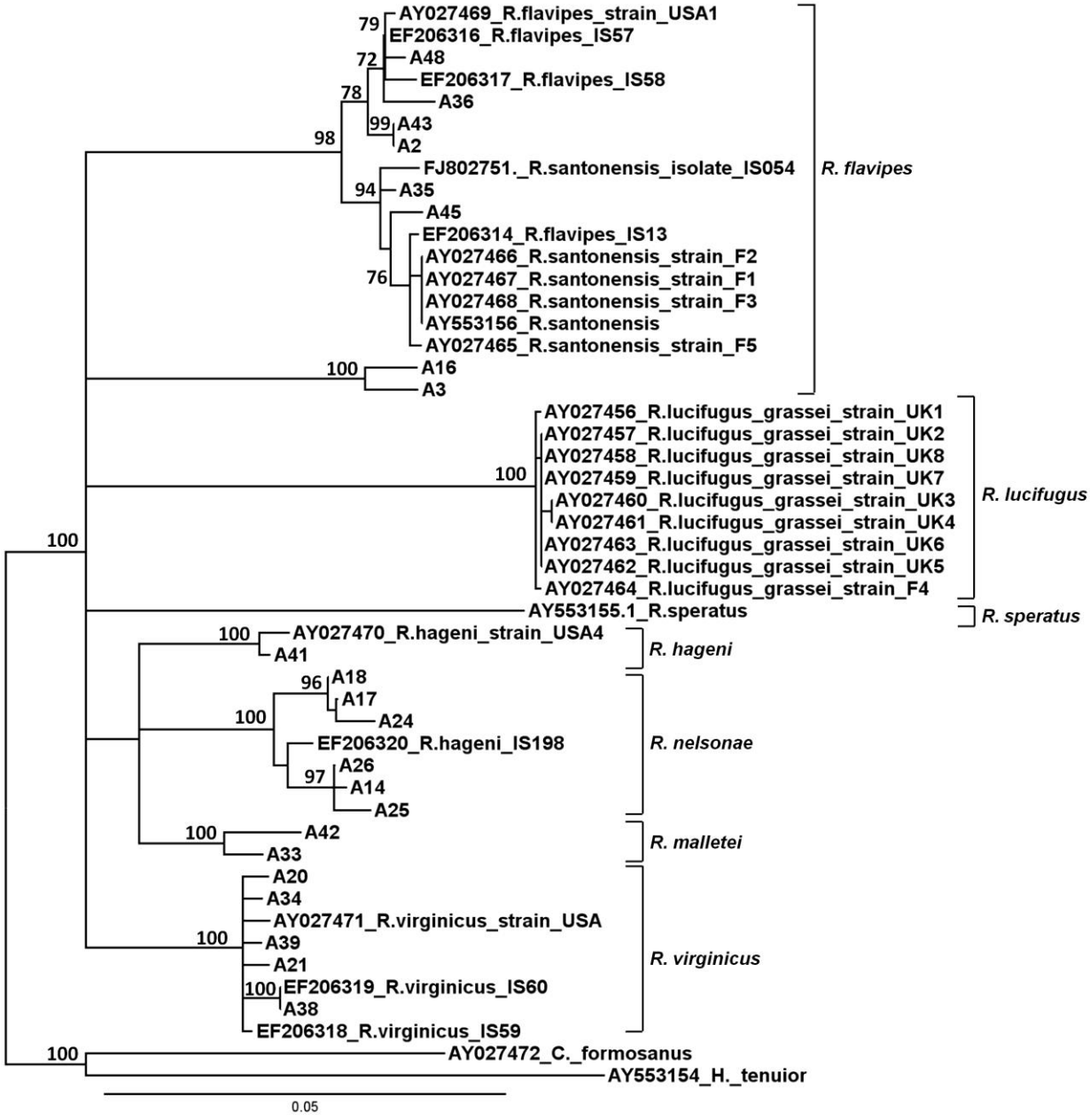
Figure 4.5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A45
EF206314_R.flavipes_IS13
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AY027467_R.santonensis_strain_F1
AY027468_R.santonensis_strain_F3
AY553156_R.santonensis
AY027465_R.santonensis_strain_F5
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A3
R. flavipes
AY027456_R.lucifugus_grassei_strain_UK1
AY027457_R.lucifugus_grassei_strain_UK2
AY027458_R.lucifugus_grassei_strain_UK8
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AY027461_R.lucifugus_grassei_strain_UK4
AY027463_R.lucifugus_grassei_strain_UK6
AY027462_R.lucifugus_grassei_strain_UK5
AY027464_R.lucifugus_grassei_strain_F4
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R. speratus
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R. nelsonae
A42
A33
R. malletei
A20
A34
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A21
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AY553154_H._tenuior
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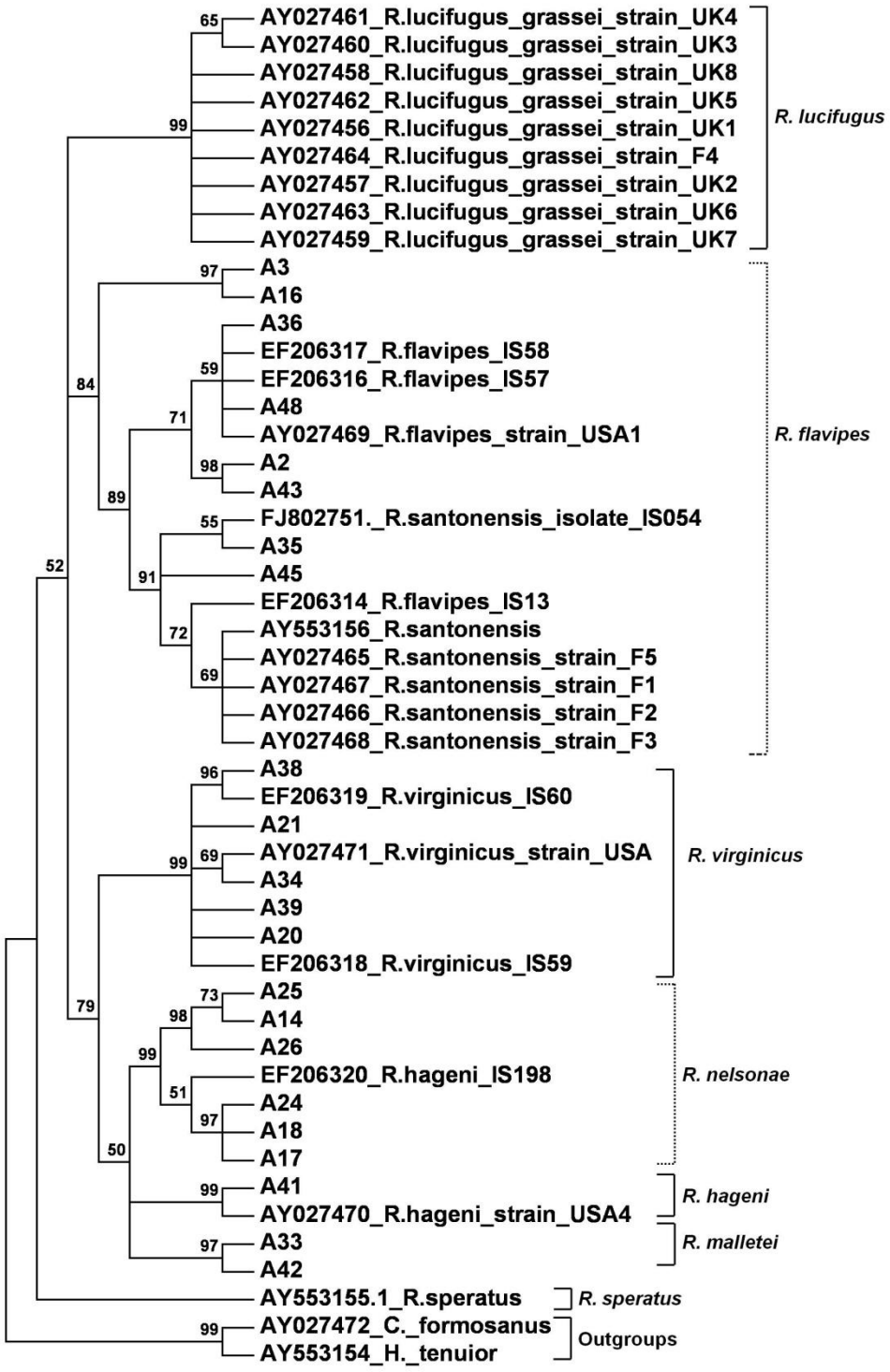
Figure 4.6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AY027462_R.lucifugus_grassei_strain_UK5
AY027456_R.lucifugus_grassei_strain_UK1
AY027464_R.lucifugus_grassei_strain_F4
AY027457_R.lucifugus_grassei_strain_UK2
AY027463_R.lucifugus_grassei_strain_UK6
AY027459_R.lucifugus_grassei_strain_UK7
R. lucifugus
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EF206317_R.flavipes_IS58
EF206316_R.flavipes_IS57
A48
AY027469_R.flavipes_strain_USA1
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A43
FJ802751._R.santonensis_isolate_IS054
A35
A45
EF206314_R.flavipes_IS13
AY553156_R.santonensis
AY027465_R.santonensis_strain_F5
AY027467_R.santonensis_strain_F1
AY027466_R.santonensis_strain_F2
AY027468_R.santonensis_strain_F3
R. flavipes
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EF206319_R.virginicus_IS60
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AY027471_R.virginicus_strain_USA
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A39
A20
EF206318_R.virginicus_IS59
R. virginicus
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A14
A26
EF206320_R.hageni_IS198
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R. nelsonae
A41
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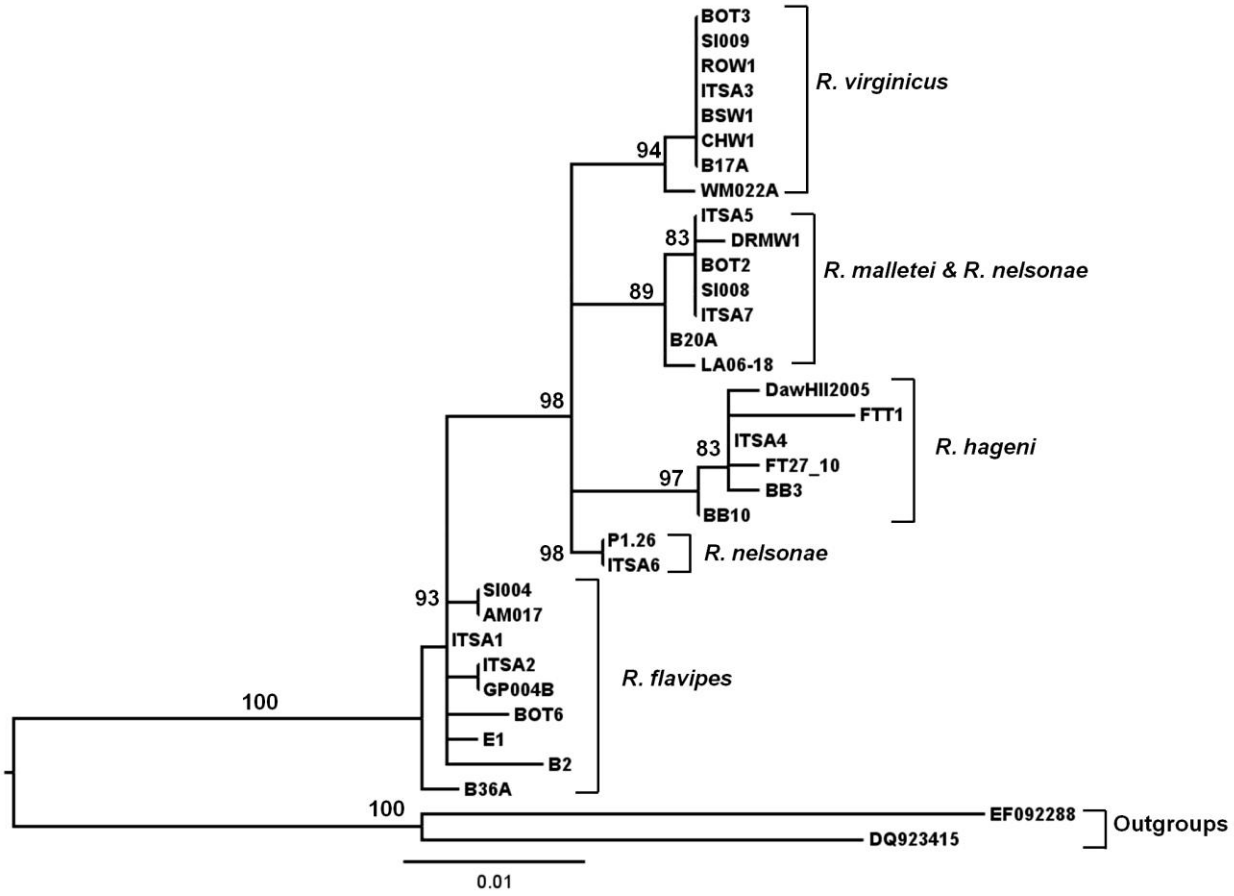
R. speratus
AY027472_C._formosanus
AY553154_H._tenuior
Outgroups

Figure 4.7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SI008
89
ITSA7
B20A
LA06-18
R. malletei & R. nelsonae
98
DawHll2005
FTT1
ITSA4
83
FT27_10
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BB3
BB10
R. hageni
98
P1.26
ITSA6
R. nelsonae
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ITSA1
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GP004B
R. flavipes
100
BOT6
E1
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B36A
100
EF092288
DQ923415
Outgroups
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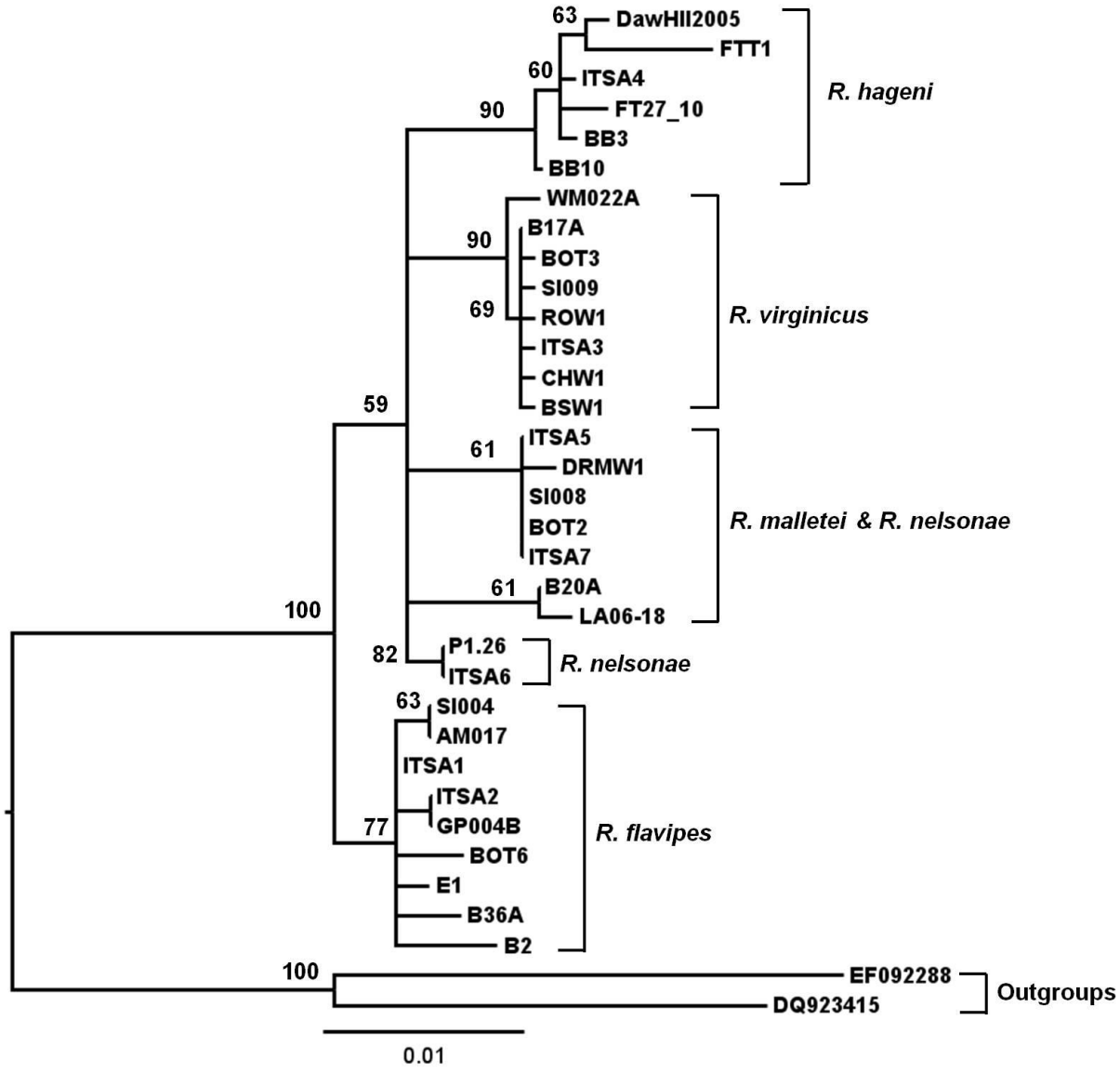
Figure 4.8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FT27_10
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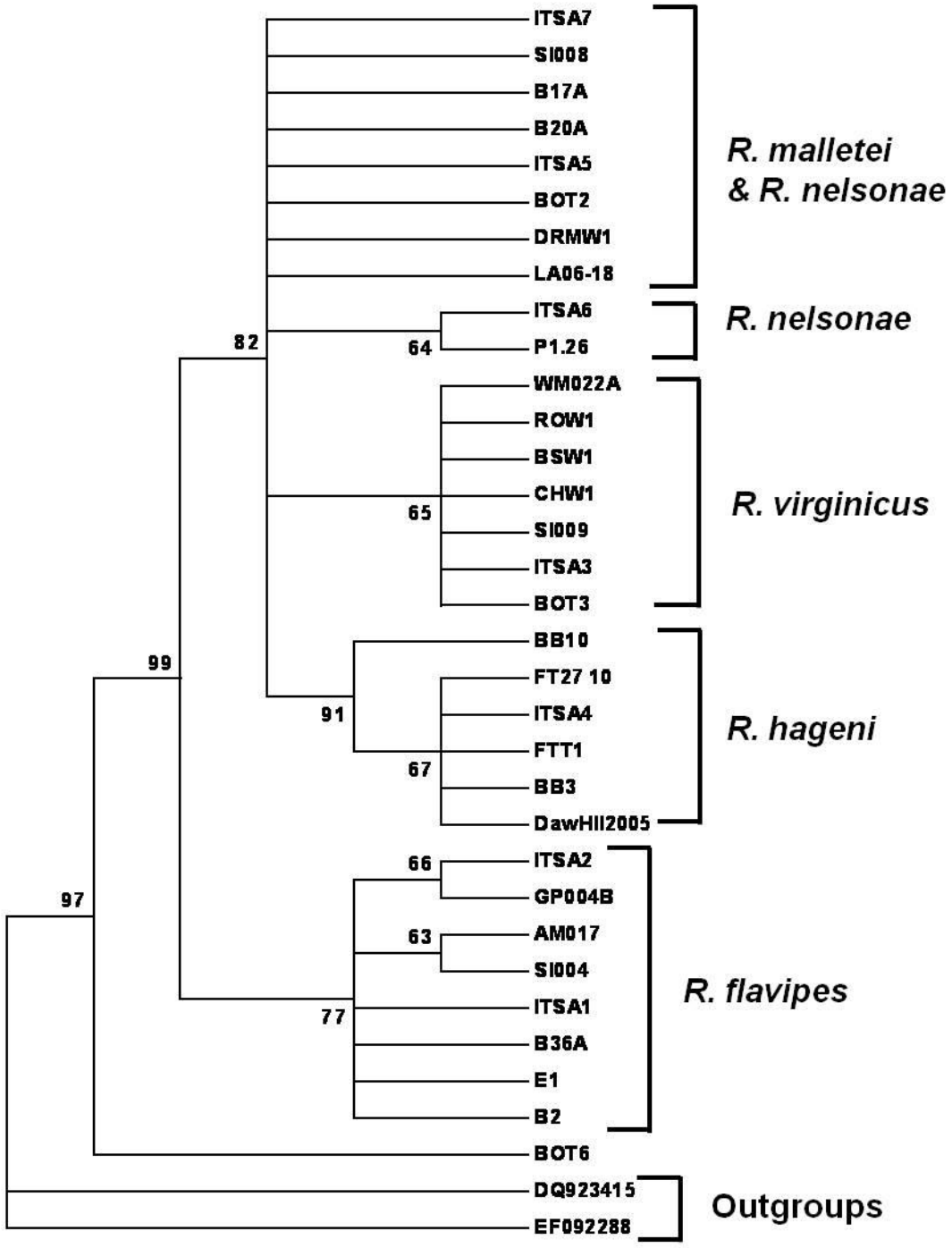
BB10
R. hageni
WM022A
B17A
90
BOT3
SI009
69
ROW1
ITSA3
CHW1
BSW1
R. virginicus
59
ITSA5
61
DRMW1
SI008
BOT2
ITSA7
R. malletei & R. nelsonae
61
B20A
100
LA06-18
82
P1.26
ITSA6
R. nelsonae
63
SI004
AM017
ITSA1
ITSA2
77
GP004B
BOT6
E1
B36A
B2
R. flavipes
100
EF092288
DQ923415
Outgroups
0.01

Figure 4.9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SI008
B17A
B20A
ITSA5
BOT2
DRMW1
LA06-18
R. malletei
& R. nelsonae
ITSA6
P1.26
R. nelsonae
82
64
WM022A
ROW1
BSW1
CHW1
SI009
ITSA3
BOT3
R. virginicus
65
BB10
FT27 10
ITSA4
FTT1
BB3
DawHII2005
R. hageni
99
91
67
ITSA2
GP004B
AM017
SI004
ITSA1
B36A
E1
B2
R. flavipes
66
63
77
97
BOT6
DQ923415
EF092288
Outgroups

Figure 4.10

CHAPTER 5

CONCLUSIONS

Economically one of the most important termites in the world, a taxonomic revision of *Reticulitermes* is extremely important to ensure all future work on the genus is accurate and cohesive. Numerous researchers have suggested the revision since the early 1960s.

The work began with a literature search for the genus, and references pertaining to *Reticulitermes* were scattered across different scientific literature, spanning more than 190 years. The literature was surprisingly confusing and incoherent with inaccuracies and numerous errors that were perpetuated by subsequent authors who probably did not refer to the original reference and nor adhering to the rules of nomenclature. Therefore, Chapter 2 represents the attempt to gather all this information together making it accessible for this project and all future researchers. This chapter includes a timeline which details the important historical references and provides a summary on the flight times and taxonomic status for each *Reticulitermes* species found in the southeastern USA.

The limited morphological diversity observed in *Reticulitermes* spp. presents major challenges to the discovery of taxonomically informative characters that can be used for accurate species discrimination across all species. Difficulties in standardizing morphometric measurements also complicated the process. Despite the challenges, we have successfully compiled a comprehensive morphological character matrix for *Reticulitermes* that yields support and resolution from statistics and phylogenetic analyses. In the process of compiling the

morphological character matrix, we discovered the need to describe a new species that has been eluded in previous research. Thus, Chapter 3 is a species description manuscript for *Reticulitermes nelsonae* which is erected with support from morphological characters derived from soldiers and alates, and phylogenetic inferences. A revised dichotomous key that includes two additional *Reticulitermes* (*R. malletei* and *R. nelsonae*) was also compiled.

An extensive morphometric study on the five *Reticulitermes* spp. from the southeastern USA which includes 851 alates and 510 soldiers was conducted and reported in Chapter 4. 11 morphological characters and 1 behavioral character were described, with 7 characters for alates and 5 characters for soldiers. These taxonomic characters were analyzed with various statistical analyses and compiled into an online, interactive matrix key that was developed using LUCID 3.5. This matrix key will revolutionize the identification process of the future by providing easy access for the user and detailed figures and guidelines for each of the morphological characters specified. From the statistical analyses that were conducted for wing measurements of *Reticulitermes*, the data revealed that wings of Isoptera were not exactly equal in length. Forewings were significantly longer than hindwings (with mean of differences ranging 0.19 mm – 0.27 mm, $Pr>|t| = < 0.0001$). This is an interesting discovery as all past research only reported wing length without identifying them as fore or hind- wings.

The updated molecular dataset for *Reticulitermes* that was collected from the three major soil provinces of the southeastern USA is investigated in Chapter 5. Cytochrome oxidase II (COII), cytochrome oxidase I (COI) and internal transcribed spacer region (ITS) sequences were chosen to determine if one of these two molecular markers (which have different evolutionary pattern) is much more accurate in deciphering the phylogenetic relationships of *Reticulitermes*. From the phylogenies inferred, our analyses suggest all five *Reticulitermes* spp. formed

monophyletic groups when tested using mitochondrial COII gene. The molecular phylogenetic analyses based on ITS region did not recover monophyletic groups for *R. malletei* and *R. nelsonae*. The haplotypes for *R. malletei* and *R. nelsonae* were intermixed and one haplotype (ITSA5) was a consensus sequence for samples from both species, suggesting that these two species might be the most closely related to each other and represent a more recent divergence for *Reticulitermes*. However, additional research will need to be conducted to test these hypotheses.

The region studied in this research represents the endemic range for *R. flavipes*, *R. virginicus*, *R. hageni*, *R. malletei* and *R. nelsonae* and is an attempt to start the revision of *Reticulitermes*. We tried to resolve many of the issues within the genus that have arisen throughout its history. Future researchers on the genus should attempt to identify these species with the collection of both soldier and alate with the recommended sample size, and ideally supported by genetic data. It is the hope that this work will serve as a valuable resource and platform for further improvement on the taxonomy of *Reticulitermes.*