TAXONOMY OF THE SOLDIERLESS TERMITES (ISOPTERA: TERMITIDAE: APICOTERMITINAE) OF THE DOMINICAN REPUBLIC BASED ON MORPHOLOGICAL CHARACTERS AND GENETIC ANALYSIS OF mtDNA AND NUCLEAR DNA

By
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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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I dedicate this to my mother and father who have always encouraged my love for entomology, making it possible for me to pursue my passion, and ultimately turn a dream into reality.

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LIST OF ABBREVIATIONS

AN	Anterior
PS	Posterior
VN	Ventral
DR	Dorsal
BL	Body length
HL	Head length
HW	Head width
CH	Clypeus height
CL	Clypeus length
CW	Clypeus width
PW	Pronotum width
FTW	Fore tibia width
MTW	Mid-tibia width
HTW	Hind tibia width
G1	Group one enteric valve morphology
G2	Group two enteric valve morphology
G3	Group three enteric valve morphology
G4	Group four enteric valve morphology
NP	No enteric pad
EP	Enteric pad
LR	Longitudinal ridge
US	Upturned scale
P1	First proctodeal segment
P2	Second proctodeal segment
P3	Third proctodeal segment
CR	Crop

MS	Mesenteron
EV	Enteric valve
MT	Malphigian tubules
CO	Colon
RE	Rectum
SS	Sensu stricto (strict sense; versus sensu lato, broad sense)
NG	New genus

Abstract of Thesis Presented to the Graduate School
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Chair: Rudolf Scheffrahn
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The Apicotermitinae pose a problem to traditional taxonomy by not having the solider caste for analysis or typological comparisons. The morphological character that has recently shown promise in identifying species within this group is the enteric valve. In this study, samples taken from the Dominican Republic were dissected to observe variation in the worker enteric valve, P1 segment of the gut, and external morphometrics. This variation was compared with inferred trees produced from gene sequences of the COII region. Four different enteric valve morphological groups were identified in this study. There was a distinct correlation between enteric valve morphology and the structure of the P1 segment. All samples with a constricted segment had group-one and group-two enteric valve morphology and all samples without a constriction in the P1 had group-three and group-four morphology. Genetic variation within each group with COII was about 4.1% and the distance between the two groups was 5.1%. A replicate was performed with a subset of samples from the initial dataset and some samples from the Bahamas that had analogous morphology as samples from the Dominican Republic. The COII locus produced

the same branching pattern and relatedeness as before with no congruence between morphotype and genotype. If the COII data for this study were accurate, the incongruency between morphology and genotype could be due to polymorphic species, recent divergence, or human error in record keeping of samples.

It is apparent that there are two different genera, *Anoplotermes* ss and *Anoplotermes* ng. Between these genera, there are two different species and potentially four different species. The separation of genera is due to a P1 constriction and lack of upturned scales on enteric pads for the *Anoplotermes* ss and an unconstructed P1 with enteric pads that have upturned scales for *Anoplotermes* ng. More work is needed on other morphological characters, such as worker mandibles and legs, to say with confidence that there are four species of soliderless termites in the Dominican Republic.

CHAPTER 1
INTRODUCTION

Global Termite Taxonomy and Importance

The order Isoptera consists of over 2600 species in about 280 genera (Inward et al. 2007). In the Neotropics alone, there are more than 500 species in about 83 genera (Constantino 1998). The Neotropical region has the second highest number of described termite species, being surpassed only by the Ethiopian region in Africa (Constantino 1992). These numbers and estimates have and will continue to fluctuate as previously unexplored regions are being surveyed and new molecular techniques and data emerge.

Termites are recognized to be the most important decomposer animals in lowland tropical ecosystems, where they can make up to 95% of the soil insect biomass (Eggleton et al. 1996). Roughly 70% of termite species are in the family Termitidae. In closed-canopy tropical rainforests, where species diversity is at its greatest, the Termitidae contribute over 90% of the species (Eggleton 2000). The Termitidae not only constitute the majority of species, but also exhibit the widest range of ecological and behavioral diversity, including many traits that are fundamental to the evolution of the Isoptera, such as soil-feeding and fungus-growing (Inward et al. 2007) that allow this group to utilize other sources of cellulose in addition to wood. Due to their ecological and economic importance, termite identification is essential in urban and agricultural pest management, study of soil fauna, analysis of the diet of vertebrates, and for any ecological or biological study involving termites (Constantino 2002). Documenting the global and regional diversity of termites is also an important area to pursue in order to fully understand their ecological and economic roles. Not only is termite species identification imperative in these studies, but is required for a clear understanding of termite phylogeny and evolution.

Previous Work and Issues in Termite Taxonomy

Traditional phlyogenetic studies of termites that have used morphological characters have suffered from one or more fundamental problems, making them problematic (Inward et al. 2007). Some of these problems include a bias towards certain groups, only analyzing certain biogeographic regions, lack of a rigorous cladistic analysis, or the use of a limited number of characters (Eggleton 2001). Other shortcomings in phylogenetic analyses, in a classical sense, are due to factors such as outdated descriptions, inconsistent surveys and collections, inaccessibility to certain regions for collecting, and difficulty in describing species of more ambiguous groups such as the soldierless termites of the subfamily Apicotermitinae in the Termitidae where soldiers are completely absent, some Old-World genera do have soldiers. Even in groups where soldiers are present, certain classical characters that traditionally have been used are greatly influenced by feeding or defense functions, so homoplasious convergence in form is frequent (Donovan et al. 2000). Another character that produces inconsistencies in phylogenetic studies are the worker mandibles. This is most apparent in the Termitidae, as the family has the widest range of trophic specializations (Eggleton 2001). Similar mandibular forms appear to have evolved numerous times. Eggleton and Davies (2003) reported that the *Nasutitermes*-group Nasutitermitinae in Madagascar and Southeast Asia show strikingly similar parallel diversification in feeding strategies and associated mandible structure. These incongruencies with traditional methods illustrate the necessity for new methods that compliment morphology and morphometrics and may lead to the inference of more strongly defended phylogenies.

As molecular phylogenetics and DNA barcoding evolve, more attempts are being made at analyzing termite phylogeny. Molecular phylogenetics and barcoding have many advantages but, if not done rigorously, can suffer the same shortcomings as phylogenetic studies based on morphological studies, such as poor taxon sampling or an insufficient number of gene loci. To date, the most comprehensive termite phylogenetic studies have been conducted by Donovan et al. (2000), Bitsch and Noirot (2002), and Noirot (1995; 2001). These studies have used morphological characters that suffer from lack of independence both from one another and their functional roles (Inward et al. 2007). Current approaches were a combination of morphological characters and variation in gene loci to infer a more well-rounded and robust phylogenetic hypothesis for termites as an order. Molecular phylogeny can be useful in inferring phylogeny where morphological characters available for analysis are highly conserved or more ambiguous.

Soldierless Termites (Isoptera: Termitidae: Apicotermitinae)

Phlyogenetic studies of termites have primarily focused on the position of Isoptera among the Dictyoptera and the family group relationships within the Isoptera, with current studies focusing on the relationship of subfamilies within the Termitidae. Past evidence that still holds, suggests that the termites are monophyletic with the Blattaria and Inward et al. (2007b) suggested that the order Isoptera be subsumed under the Blattaria. The relationship of families within the Isoptera is currently well understood. It is generally accepted that Mastotermitidae is the most basal family and the Termopsidae, Hodotermitidae, and Kalotermitidae are basal to the Termitidae and the Rhinotermitidae, which now contains what used to be the Serritermitidae (Miura et al. 1998, Lo et al. 2004). While the Termitidae are established as the most derived, monophyletic group in relation

to the other families, the relationship of subfamilies within the Termitidae is still unresolved. The only subfamily forming a clear monophyletic clade is the Syntermitinae, the mandibulate nasutes. This monophyly was first inferred by Inward et al. (2007a).

The subfamily, Apicotermitinae, was typologically proposed by Grassé and Noirot (1954) partly because of the anatomy of the digestive tube (Noirot and Noirot-Timothée 1969). Globally, there are about 208 species within 42 genera in the Apicotermitinae (Constantino 1998) and in the New World, there are only about 41 species in five different genera (Constantino 1998). This is a rough estimate due to a lack of investigation in the group, disagreement among taxonomists, and difficulty in describing species for lack of reliable morphological characters. Typically, termites are described based on solider or alate morphology. In a soldierless group, soldier morphology is irrelevant and alates can only be found at certain times of the year or are collected without workers, making both of these features deterrents for elucidating phlyogenetic relationships and taxonomic identity within the Apicotermitinae. Another problem is that one of the best characters for identification, the worker gut, needs to be dissected and this task can be so tedious that some researchers have not been willing to do it. The features of the gut that allow for recognition of the subfamily Apicotermitinae, are a voluminous and asymmetrical crop, a very reduced gizzard, lack of chitinous armor, no mixed segment, and a highly variable enteric valve armature (Noirot and Noirot-Timothee 1969) that ranges from a complete absence of pads to very ornate and complex, sclerotized structures (Figures 1-1 and 1-2). Gut morphology is also used typologically in generic identification (Fontes 1992). One of the key characters of the digestive tube that is used in identifying soldierless termites to species is the enteric valve, a structure located withing the P2, which lies between the P1

and P3 segments of the digestive tube (Figure 1-3). In some cases, the enteric valve is located on the inner surface of the P2 (Figure 1-3A) and is unsclerotized with very little structure and in other instances, it is everted into the P3 segment (Figure 1-3B) and is highly sclerotized with very ornate armature. These are two extremes in structure with many intermediate types. The exact function of the enteric valve is unknown but various hypotheses have been proposed ranging from serving as a specialized substrate for symbiotic bacteria to being used to direct the flow of food particles as they pass through the junction (Donovan 2002). Many soldierless termites are subterranean foragers that can often be found under rocks and underneath ungulate feces. In areas that are seasonally flooded, particularly in lowland Amazonian rainforest, above-ground nests can be observed at the base of trees (Bahder unpublished data). A biological characteristic of the Apicotermitinae, which is by no means limited to this group, is that they are "soil-feeders". The term soil-feeding is a misnomer because, upon careful observation, it is apparent that there are little to no inorganic soil particulates in the guts of specimens from this group, but mostly organic material.

DNA Taxonomy and DNA Barcoding

There are four major limitations in traditional species identification listed by Hebert (2003). The first being that both phenotypic plasticity and genetic variability in characters employed for species recognition can lead to incorrect identifications (Hebert et al. 2003). Second, the traditional approach overlooks morphologically cryptic taxa that are common in many groups (Knowlton 1993; Jarman et al. 2000). Third, since morphological keys are often effective only for a particular life stage or gender, many individuals cannot be identified (Hebert et al. 2003). Finally, the use of keys often demands such a high level of

expertise that misdiagnoses are common (Hebert et al. 2003). Not only does DNA taxonomy and barcoding help solve these problems, but it also strengthens phylogenetic analyses of organisms where morphological characters can be used in identification. As techniques in this field improve, it has become possible to analyze DNA variation from amber fossils of termites (DeSalle et al. 1992) although it still remains rather limited when compared to morphological analysis of fossil termites. This approach to phylogenetic analysis is by no means a tool to end all debate on relationships and evolution of a given taxa. Despite any shortcomings with either approach, both have useful properties in inferring reasonable hypotheses for phylogeny. The most accurate analyses, however, will most likely be produced as a result of combining both techniques, as well as more complete biological, geographical, and developmental data.

DNA barcoding was proposed by Hebert et al. (2003) as a method for identifying and cataloging unknown specimens (Monaghan et al. 2006). This method uses short mitochondrial DNA (mtDNA) sequences to group unknown individuals with *a priori* defined taxonomic entities based on nucleotide sequence similarity, deriving a species identification from DNA rather than morphological characters (Monaghan et al. 2006). One of the limitations of DNA barcoding is that it requires a near complete database of vouchers against which individuals can be placed (Moritz and Cicero 2004). Other pitfalls associated with DNA barcoding are universality of primers and intraspecific variation of haplotypes. Priming regions are not always conserved in groups so when isolates cannot be sequenced by "universal" primers, sequence data from sister taxa can be used to identify more suitable priming sites. Once an isolate is sequenced, the exact nucleotide sequence can be analyzed and the exact priming region can be identified. The amount of genetic variation between an

isolate and other sister groups or species is also an important parameter in defining what a species is at the molecular level. There is no defined level of genetic variation that is required to distinguish species from each other. This is problematic for DNA barcoding and poses the question, where do you draw the line between species with regard to genetic variation? This is where having many isolates to analyze with accompanying morphological data can guide decisions on species-specific character states.

In contrast, DNA taxonomy is the concept that DNA sequences themselves serve as the taxonomic reference system, from which, clades have to be derived (Vogler and Monaghan 2006). Other benefits of DNA taxonomy are its use as a database for standard DNA barcoding and that it can be based on more than one region of mtDNA or nuclear DNA (Vogler and Monaghan 2006). This is a useful quality for fitting unknown or unrepresented species into a database, which is why DNA taxonomy can be an invaluable tool in providing molecular characters for inferring the phylogeny of the soldierless termites, where traditional methods based upon soldier or reproductive castes cannot be applied.

These molecular techniques have been applied to taxonomic studies of a wide variety of organisms that include marine bacteria (Klein et al. 1998), parasite and disease vectors (Besnasky et al. 2003), planktonic copepods (Bucklin et al 1999), nematodes (Ye et al. 2007), sea turtles (Karl and Bowen 1999), and treefrogs (Barber 1999). Due to their abundance, diversity, and importance, insects have become a popular group for DNA taxonomy and barcoding studies. Of particular interest are the studies by Maekawa and Matsumoto (2000) on cockroach molecular phylogeny and by Inward et al. (2007a) on termite phylogenetics, combining both molecular and morphological data.

One of the more daunting aspects of DNA taxonomy and DNA barcoding is the analysis of the sequence data, both tree production and statistical analyses. There are currently a wide variety of computer programs that are available for statistical analyses on DNA sequences that produce phylogenetic trees based on variations (Table 1). There are basic components of these analyses that are needed for every study that incorporates DNA taxonomy or DNA barcoding. Programs are needed that will first combine the forward and reverse sequence of the successfully amplified, sequenced gene based on overlapping regions and match percentages. Once the sequence is cleaned and assembled into a contig, a sequence file that consists of both the forward and reverse sequences of the gene, file in Sequencher, it can be exported to a program that can align the sequence data. After all sequences have been aligned, they can be used in a BLAST (Basic Local Alignment Search Tool) search which allows matching with DNA sequences for identifying putative sisters or a candidate outgroup for the study. A X^2 test is then performed to check for homogeneity in base frequencies. Once all sequences have been uploaded, a tree is produced, typically the neighbor joining x, y, z methods. This non-cladistic (phenetic) method is accomplished by finding pairs of operational taxonomic units (OTUs) that minimize the total branch length at each stage of clustering of OTUs starting with starlike trees (Saitou and Nei 1987). This method is applied to neighbors, two taxa that are connected by a single node, and clustered based on similarity (Nei and Kumar 2000). A cladistic method, such as Bayesian analysis is then performed to confirm tree topology for each gene separately and then combined if deemed appropriate. In order to test the reliability of the tree topology, a bootstrap test is used. This is accomplished by testing the reliability of each interior node in the tree. When determining whether or not a node is reliable, probability of confidence (1 – Type I error)

is computed. If the resulting value is higher than the desired confidence interval, the node is considered reliable or statistically significant (Felsenstein 1985; Efron et al. 1996; Nei and Kumar 2008). Once all of these statistical analyses have been performed and considered, a tree is produced and discussed relative to the rate of nucleotide substitution, relatedness of neighbors, accuracy of each node, and maximum parsimony.

Samples and Objectives

As noted, one of the major flaws in termite taxonomy is the uneven analysis of given groups, being most evident in the soldierless termites due to their cryptic habits and the difficulty of using adequate features for their taxonomy. The region of interest for this study was the Dominican Republic on the island of Hispaniola in the West Indies. There are no records of described species of termites within the Apicotermitinae from the Dominican Republic. The only records of this group of termites are from collections that were focused on surveying diversity of the Isoptera without a focus on any particular group. There are only 140 samples of soldierless termites that have been collected from the island (Figure 1-4). Of these samples, none have been identified beyond genus or described as new. The main problem with identifying these specimens to species is that only workers were available for the study and virtually every species description within this group was based on alate morphology. All samples were identified as *Anoplotermes* sl based on a constriction in the P1 segment of the worker gut, or were placed in a morpho-group labeled *Anoplotermes* ng (new genus) based on an absense of a constriction of the P1 segment before it enters the P3 (Figure 2-2). The group *Anoplotermes* ng is used to label specimens that possessed the tubular P1 segment and do not correspond with the true *Anoplotermes* genus. The suggestion of a new genus is based on this feature and that this group did not

fall into any other genus of New World, soldierless termites and was proposed by Scheffrahn et al. (2006). Many samples of Apicotermitinae are lumped into *Anoplotermes* due to the lack of diagnostic characters of this group. However, many of these samples could very well be separated into different genera based on gut and enteric valve morphology or genetic differences. The samples that are shown in Figure 1-2 most likely belong to different genera but are labeled as *Anoplotermes* because a proper taxonomic revision has not been attempted for this group. The samples used in this study were collected from June 16, 1991 to November 8, 2004, with the majority being collected by J. Chase, J. Krecek, and R.H. Scheffrahn. All samples were stored in the University of Florida Termite Collection at the Fort Lauderdale Research and Education Center (FLREC) in Davie, FL.

Having an understanding of the diversity of this group is important due to their economic importance. This subfamily tends to forage in the soil for organic matter, they play a crucial role in soil ecology and health, which can directly affect the stability of climax communities or agriculture. The primary objective of this study was to supplement molecular data with morphological characters to infer a testable hypothesis concerning the taxonomy of the Apicotermitinae in the Dominican Republic. My hypothesis was that the morphology of the enteric valve from random samples of Apicotermitinae from the Dominican Republic could be mapped upon molecular phylogenetic trees generated separately from two different genetic loci, one ribosomal and one mitochondrial. Any monophyletic clades inferred during this project would serve as a starting point for further systematic and biogeographical work expanding the “morphotype” or “genotype” designation.

A B

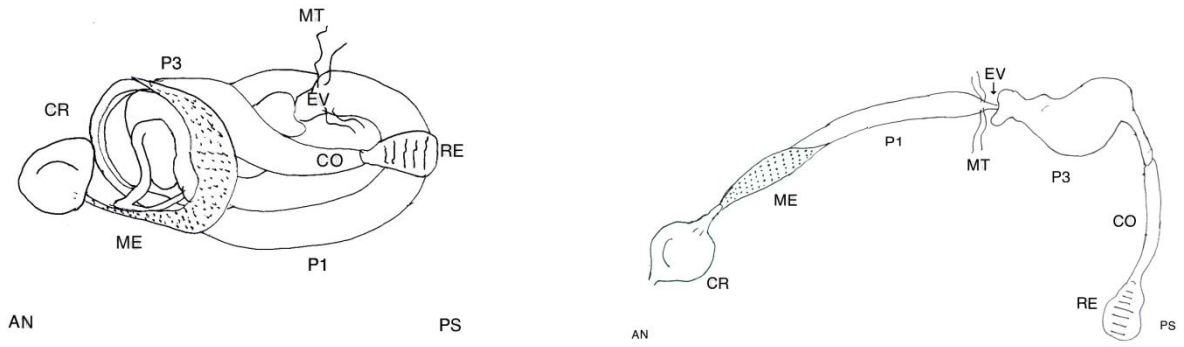


Figure 1-1. *Anoplotermes* sp. gut structure; A) coiled. B) uncoiled after dissection: CR = crop, ME = mesenteron, P1 = first proctodeal segment, EV = enteric valve, MT = malphigian tubules, P3 = third proctodeal segment, CO = colon, RE = rectum, AN = anterior, PS = posterior: figures are not drawn to scale and viewed dorso-ventrally.

A B

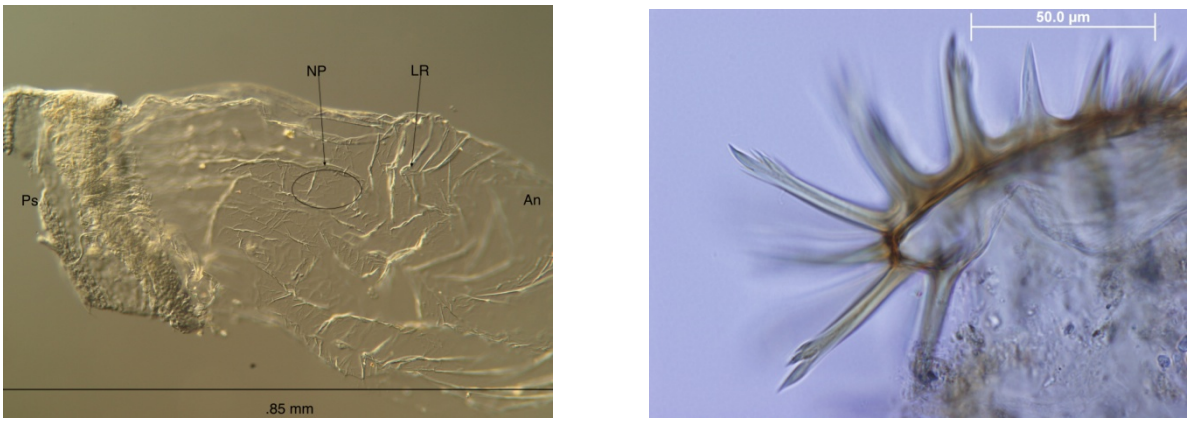
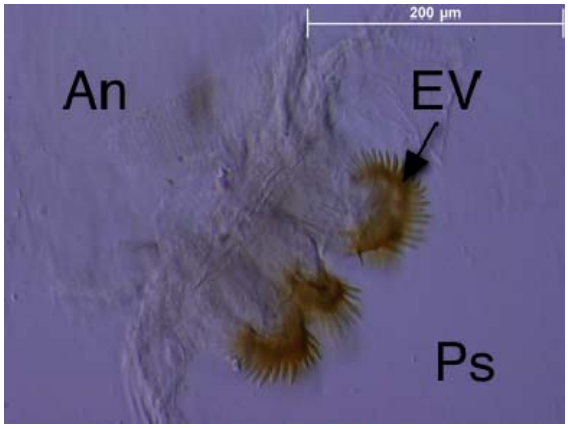


Figure 1-2. Enteric valve morphology: A) *Anoplotermes* sp. B) *Anoplotermes parvus*. C) *Anoplotermes* sp.2. D) *Anoplotermes* sp.3. E) *Anoplotermes* sp.4. F) *Anoplotermes* sp.5. (Photos courtesy of Jan Krecek).

C



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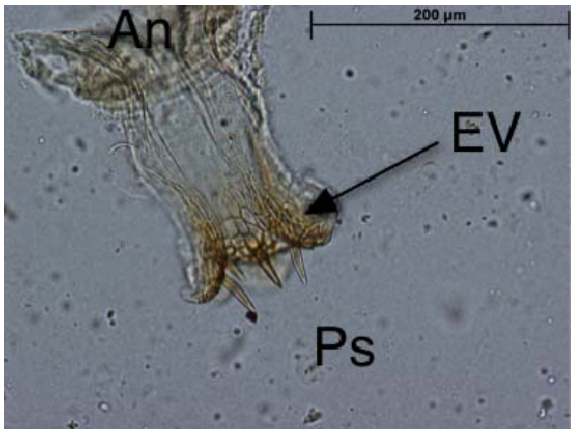

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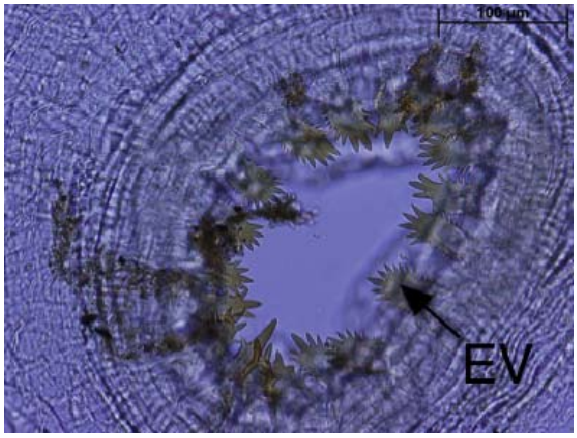


F

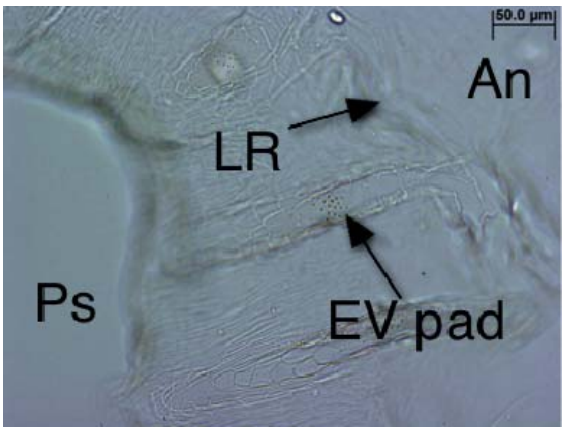
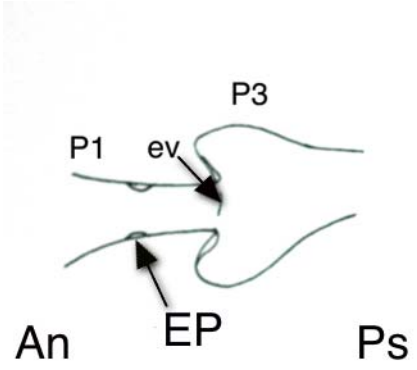


Figure 1-2. Continued

A



B

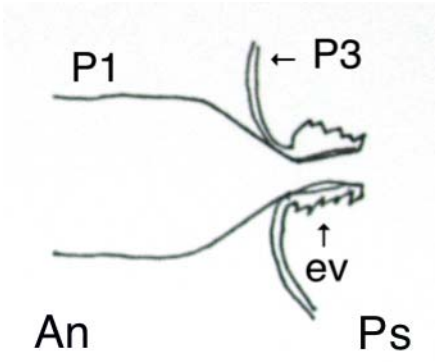


Figure 1-3. Location of the enteric valve: A) *Anoplotermes* sp. B) *Eburnietermes* sp. (Donovan 2002): P1 = first proctodeal segment, EV = enteric valve, P3 = third proctodeal segment.

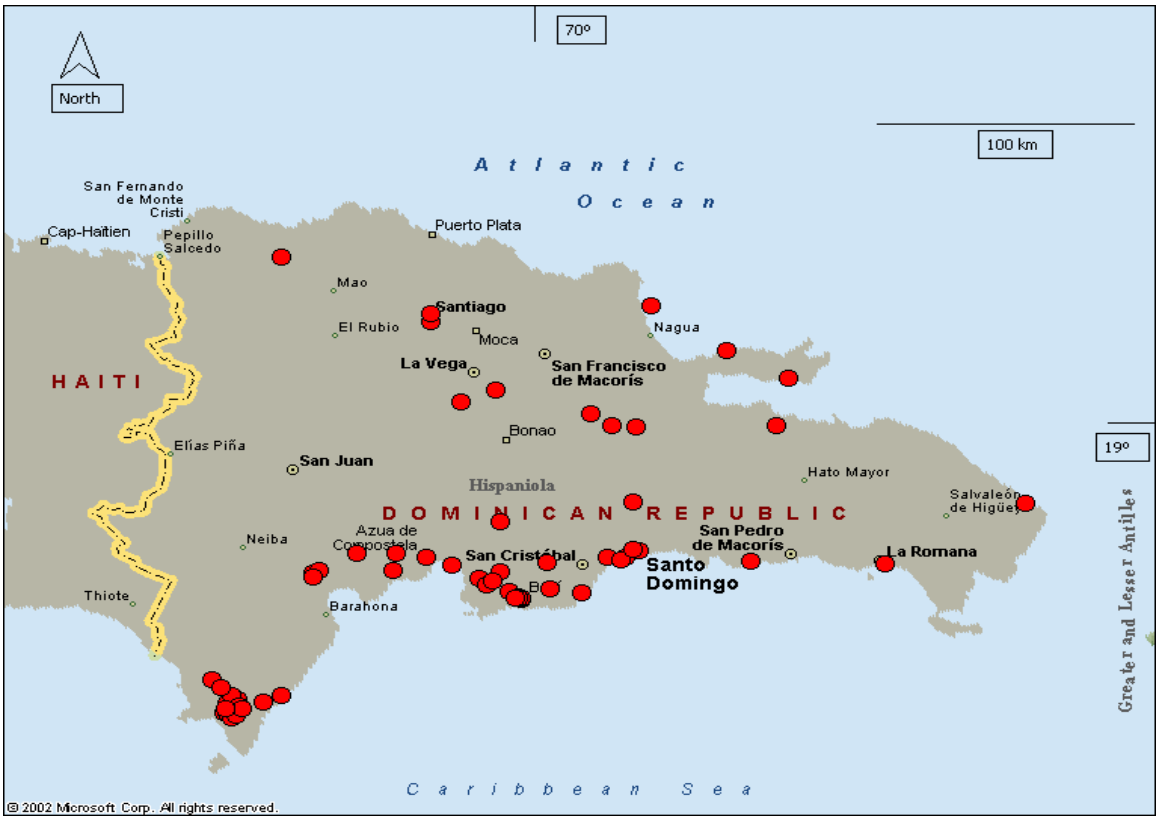


Figure 1-4. Map of the Dominican Republic with Apicotermitinae sample locations.

Table 1-1. Some common computer software used in analyzing DNA sequence data

Program	Function	Developed
MEGA version 4.0	Base compositional analyses	Kumar et al. 2008
CLUSTALW	DNA sequence alignment	Thompson et al. 1994
PAUP 4.0	X^2 test for homogeneity in base sequences	Swofford 1999
MODELTEST 3.7	Model base substitution in genes and combined sets	Posada et al. 2006
MrBayes	Conformation of tree topology	Huelsenbeck and Ronquist 2001
Sequencher 4.7	Edit sequence data and produce consensus sequences for tree construction	Gene Codes Corporation 2007

CHAPTER 2
TYPOLOGICAL DIFFERENCES IN MORPHOLOGY AMONG SOLDIERLESS TERMITES (ISOPTERA: TERMITIDAE: APICOTERMITINAE) IN THE DOMINICAN REPUBLIC

Introduction

Because the identification of termites to species is based primarily on the soldier caste, the Apicotermitinae pose a major problem to termite taxonomists. Species descriptions within the Apicotermitinae are based largely on typological differences in worker gut and alate morphology. The work needed for descriptions is necessarily tedious and time consuming. However, because of conservation of morphology and the danger of homoplasy in the worker caste, species delineation can be difficult. Modern descriptions of species within this subfamily have been based largely on morphometrics, dentition, gut morphology, and enteric valve structure in the worker caste while older (pre 1960) descriptions are based almost solely on the external imago characters such as in *Anoplotermes gracilis* and *Anoplotermes manni* by Snyder (1922). When alates are present, morphometrics, dentition, and wing venation are commonly used. The morphological character of interest in this study was the worker enteric valve because of preliminary, empirical observations suggesting discrete species-specific character sets.

The enteric valve is relatively insignificant in many wood-feeding termites, but is often well-developed and variable in some of the phylogenetically derived termites, the Termitidae, and is perhaps most developed in the sub-family Apicotermitinae (Donovan et al. 2000). Despite its value to termite taxonomy, practically nothing is known about the function of the enteric valve. Multiple hypotheses have been proposed that include; an aid in autothysis – a defense mechanism whereby the workers split open their abdomen to release its sticky contents, a mechanism for the inoculation of the gut contents with

symbiotic bacteria, or a means by which soil particles are physically sorted, or fractionated (Donovan et al. 2000; Donovan 2002). The enteric valve is a reduced proctodeal segment (P2 homolog) that is located between the first proctodeal segment (P1) and the third proctodeal segment (P3) (Figure 1-1). All of the more basal groups of termites and a few genera within the Apicotermitinae have the enteric valve located on the interior face of the P2 just before the junction with the P3 (Figure 1-3). It is here that this structure exhibits hexaradial symmetry in that there are six pads located around the interior of the gut and are evenly spaced and separated by longitudinal ridges (Figure 2-8) However, the Apicotermitinae is the only group with some members that have the enteric valve everted into the P3 (Donovan 2002) (Figure 1-3; Figure 2-1). In this instance, part of the P1 tissue lining the alimentary canal is pushed into the P3 segment and any pads or sclerotizations located on P1 tissue present on the external face of the actual tissue junction (Figure 2-1). This movement of structures has probably occurred over evolutionary time, rather than contemporaneously. African members within the Apicotermitinae that are classified as true soil-feeders by Donovan et al. (2001) all have highly structured and variable enteric valves that are everted into the P3 (e.g. *Ebunitermes, Coxotermes, Labidotermes,* and *Ateuchotermes*). Some of the members of the Apicotermitinae, however, feed mostly on organic material in the soil and do not have elaborate enteric valve armatures, or are not as extreme. The most diverse group in the Apicotermitinae is the genus *Anoplotermes* with 32 species that range throughout the Neotropical region. Within this group, there is a high level of variation in the structure of the enteric valve, based on preliminary dissections done by J. Krecek. The other four genera within the Apicotermitinae that are recorded from the neotropics are *Aparatermes, Grigiotermes, Ruptitermes,* and *Tetimatermes*

(Constantino 1998). These genera, however, only represent a few species from sparse collections from isolated regions. In the Dominican Republic, the only genus recorded to date is *Anoplotermes* (Snyder 1956, Scheffrahn et al. 1994). Some samples were recorded as *Anoplotermes* ng, an undescribed genus that differs from true *Anoplotermes* by having uniform diameter in the P1 segment of the gut where *Anoplotermes* has a constriction of the P1 right before it reaches the P3 segment (Figure 2-2). This distinction was first proposed by Scheffrahn et al. (2006) and was analogous to the samples represented in the Dominican Republic. The presence of these two discrete morphological differences in the Bahamas provides a good basis to compare to Dominican samples and supports the presence of two discrete groups that could be separated into two different genera. None of the specimens collected from the Dominican Republic have been identified to species because most of the original species descriptions are based on alate morphology or worker dentition and morphometrics, making them unusable for species identification of survey material consisting of workers only.

In this study, the enteric valve structure of the worker caste was examined to differentiate between different specimens of the Apicotermitinae from the Dominican Republic and to elucidate morphological patterns from this group in this region. Based on the variation of the enteric valve seen in different putative species of *Anoplotermes* that were dissected by J. Krecek, I hypothesized that there would be discrete variation in the enteric valves of the specimens that were collected from the Dominican Republic to place them in putative "species" groups that could be mapped on trees inferred from molecular phylogeny. Other morphological characters that were analyzed to elucidate typological differences included the constriction or lack thereof in the P1 segment, morphometrics of

the head, clypeus, pronotum, tibia width, and body length. The data were combined with enteric valve morphology to determine if there were any discernable patterns among samples indicating distinct groups that could represent different species or taxa.

Materials and Methods

Termite Samples

For this study, 140 samples of Apicotermitinae were used for analysis. Samples were collected from various sites in the Dominican Republic between June 16, 1991 and November 8, 2004. Samples were stored in 85% ethanol at room temperature (23˚C) in the University of Florida Termite Collection at the Fort Lauderdale Research and Education Center (FLREC), Davie, FL.

Gut Constriction and Morphometrics

All samples were measured and placed in the *Anoplotermes* ss or *Anoplotermes* ng group based on P1 constriction by using a dissecting microscope (Figure 2-2). When available, ten individual termites were measured per sample. Samples that contained fewer than five termites were not included. Worker characters measured for analysis of morphometrics were BL, HL, HW, CH, CL, CW, PW, FTW, MTW, and HTW.

Dissection and Slide Preparation

After measurement, all specimens were dissected on black wax in 85% ethanol. The abdominal integument was removed from each specimen with a 000 insect pin from BioQuip (Rancho Dominguez, CA) that was inserted into a 2 mm in diameter wooden rod. A scalpel (Fisher Scientific, Pittsburgh, PA) was used to cut out a section of the gut that included part of the P1, the enteric valve, and part of the P3 while P1 morphology was recorded as being either constricted or tubular. The gut section was placed in a glass vial

with 85% ethanol and immersed in a Branson ultrasonic cleaner (B-22-4 Fisher Scientific, Pittsburgh, PA) for a few seconds to remove organic matter from inside the gut section and muscle tissue from the outer wall of the gut. The inner lining of the gut is transparent and muscle tissue was removed because under a compound microscope, the tissue distorts the image of the hyaline enteric valve structures located on the cutucular gut lining. Once the tissue was removed, or as much of it that could be removed without destruction of the specimen, the gut section was cut in half, and splayed out on a glass microscope slide (Fisher Scientific, Pittsburgh, PA). After re-orientation on the slide so enteric pads and longitudinal ridges were facing upwards, PVA mounting medium (BioQuip Rancho Dominguez, CA) was added and a cover slip was added. Slides were allowed to dry for two days.

Contrast Settings and Slide Microphotography

All preparations were observed under an Olympus BH2-DIC (Differential Interference Contrast) photomicroscope with Nomarski optics. Samples where magnified 312x for a clearer picture of the pad structure. The 312x magnification was used because it was the highest magnification that could show the enteric pad structure with a resolution that made recognition of various features possible. The Nomarski optics were adjusted until the desired contrast was achieved. All preparations were photographed using an Olympus E 410 digital camera and images were optimized and prepared using Aperture (Apple) software. White balance and saturation level were altered to produce a uniform grayscale between all plates. White balance was increased to lighten the pictures while the saturation level was decreased to make the photo gray.

Results and Discussion

Enteric Valve Morphology

After qualitative assessment of photographs of all specimen mounts individually, there emerged four discrete morphological groups among the 140 samples (Table 2-1). The morphology of group one (G1) was characterized as having six longitudinal ridges along the alimentary canal with no enteric pads or structure of any kind in between them (Figure 2-3). The morphology of group two (G2) was characterized as having six longitudinal ridges with a pad located in between each ridge (total of six pads). These pads lacked any structure or armature other than their globular shape (Figure 2-4). The morphology of group three (G3) was characterized as having six longitudinal ridges with a pad in between each ridge. In contrast to G2, the pads had small "beads" at the apical point of each scale (Figure 2-5). These beads were the upturned points of each scale. The morphology of group four (G4) was characterized the same as G3, except that the pads in G4 were flatter, wider, and longer with many more beads, more than 20 while G3 never had more than 19 (Figure 2-6). G2, G3 and G4 all had pads located in the same location, on the interior surface of the P2 segment, just before its junction with P3.

In G1, there were no pads and there was no evidence of pads or armature being everted into the P3 segment. All scales in every specimen that covered the lining of the gut around the enteric valve were the same. They were roughly diamond-shaped with fringes on the anterior face, with respect to the direction of the gut (Figure 2-7). Due to the difficulty of seeing unsclerotized enteric valves in their natural orientation, the exact orientation was unknown. Based on the ridge structure between pads and the fact that all pads were the same size, it was evident that their orientation produced a symmetrical ring

around the interior of the P2 segment. Samples that were used to compare morphology were taken from the Bahamas and all samples fit into G1, G2, and G3 enteric valve morphology. They also exhibited the same constriction, or lack of, in the P2 segment. All photos of each enteric valve morphological group for samples from the Dominican Republic and the Bahamas are represented in figure 2-10.

The phylogeny of the Apicotermitinae is clearly resolved by Inward et al. (2007a) but lacked a morphological analysis of enteric valves to infer phylogenetic relationships. Thus far, where this structure has been observed and studied, it appears to be a potentially diagnostic character with discrete states between closely related species in different geographical regions. More observational work is needed on the Apicotermitinae before phylogenetic analysis can be attempted to infer potential relationships. In order to infer an accurate estimate of the phylogeny of this group, samples must be analyzed from all geographical regions, especially mainland sites, and from every habitat where these termites are found. Another critical variable is having a better understanding of the function of the enteric valve so homologies can be assigned with more confidence. Currently, without knowledge of their true function and developmental pathways, the wide range of their variation makes assigning homologies difficult (Donovan 2002).

The structure of the enteric valve in G3 was very similar to the structure of enteric valves seen in specimens from Trinidad and Central America. The features of G4 have also been observed in specimens from this region. The morphology in G1 and G2 seemed distinct and novel, not having been observed anywhere else before. When plotted on a map, the *Anoplotermes* ng was predominantly located in the western and southern arid parts of the Dominican Republic and the *Anoplotermes* ss was predominantly located in the

wetter, eastern parts of the country. (Figure 2-9). Based on the presence of the same enteric valve morphology in samples collected from the mainland, the Apicotermitinae in the Dominican Republic probably evolved in Central and South America before vicarial events led to their present day location on Hispaniola. The intermediate morphologies between the various groups could indicate hybridization or that some of the groups on the island represent sub-specific groupings. Based on the morphological variation observed among the specimens from the Dominican Republic, the groups with type one and type two enteric valve morphologies were typologically distinct from the *Anoplotermes* ng group that possessed type three and type four enteric valve morphology.

Morphometrics

All morphometric data is presented in Table 2-2. The mean size for each character in *Anoplotermes* ss is slightly smaller than that of *Anoplotermes* ng with the exception of clypeus height and pronotal width where *Anoplotermes* ss is slightly bigger than *Anoplotermes* ng and all tibial widths where *Anoplotermes* ss and *Anoplotermes* ng have the same mean measurement size. The range present for each group, for each character overlaps with that of the other group, i.e, there is no apparent difference in size between the two groups. Within the *Anoplotermes* ss there is a distinct difference in head width range. All samples with G1 enteric valve morphology ranged from 0.80 to 1.03 mm for head width and all samples with G2 enteric valve morphology ranged from 0.69 to 0.79 mm for head width. Within the *Anoplotermes* ng group, there was no difference in size for any character measured. The only discernable pattern within this group was the range in number of upturned scales on the enteric pads. In samples with G3 morphology, there

were between 5 and 19 upturned scales and in samples with G4 morphology, there were between 20 and 33 upturned scales.

Comparison of Gut Morphology, Enteric Valve Morphology, and Morphometrics

When looking at both the types of enteric valve morphology, the presence or lack of constriction of the P1 segment, and the morphometric data there was a distinct pattern that manifested itself as two major morphological groups defined by P1 morphology and type of enteric valve within each of these groups. Also, it showed that there were two typologically distinct morphotypes within each of the two major morphological groups. All samples identified as *Anoplotermes* ss had a distinct constriction in the P1 segment and had G1 or G2 morphology. All samples labeled *Anoplotermes* ng had G3 or G4 morphology. Within the *Anoplotermes* ss, the two morpho-types were identified as G1 and G2. The G1 possessed type-one enteric valve morphology and had a head width range of 0.80 to 1.03 mm and G2 possessed type-two enteric valve morphology and a head width range of 0.69 to 0.79 mm. Within the *Anoplotermes* ng, two morpho groups were also identified as G3 for samples that had an ovoid shaped enteric pad with between 5 and 19 upturned scales and G4 for samples that had more of a polygonally shaped enteric pad with between 20 and 33 upturned scales. This congruency makes it clear that there are two distinct morphological groups present and two morpho types within each of these major groups, and provides good support for characters for describing these as two genera with two species in each. I propose that the combination of characters found in this study be used to place all samples into one group, *Anoplotermes* ss, or the other, *Anoplotermes* ng and then into the appropriate enteric valve morphological groups, G1, G2, G3, or G4, due to the consistency of

the combination of *Anoplotermes* ss with G1 and G2 enteric valve morphology and of *Anoplotermes* ng with G3 and G4 enteric valve morphology (Table 2-3).

Conclusion

Based on these data, it is apparent that there are two typologically distinct morphological types, each with two typologically distinct morphotypes within each of them, within the Dominican Republic Apicotermitinae that have been collected to date. For complete descriptions of each of these groups as separate species or genera, more morphological work will be needed for the alates of each group after a more comprehensive survey of the island. Other characterisitics that could be used for identifying workers of this group that were used by Scheffrahn et al. (2006) are worker dentition and gut arrangement. These characters, along with P1 morphology and alate morphology were used in identifying and describing *Anoplotermes bahamensis* and *A. inopinatus* in the Bahamas. Other characters that could be analyzed are fore tibia size and clypeus size. In the Apicotermitinae, where soldiers are usually lacking, workers have developed enlargements of these structures, probably, as a means of defense and may be useful in distinguishing species.

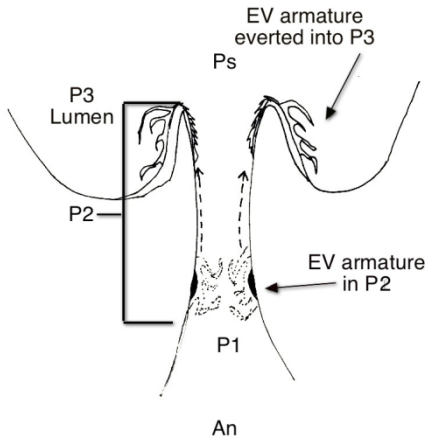


Figure 2-1. Eversion of enteric valve into P3 segment.

A B

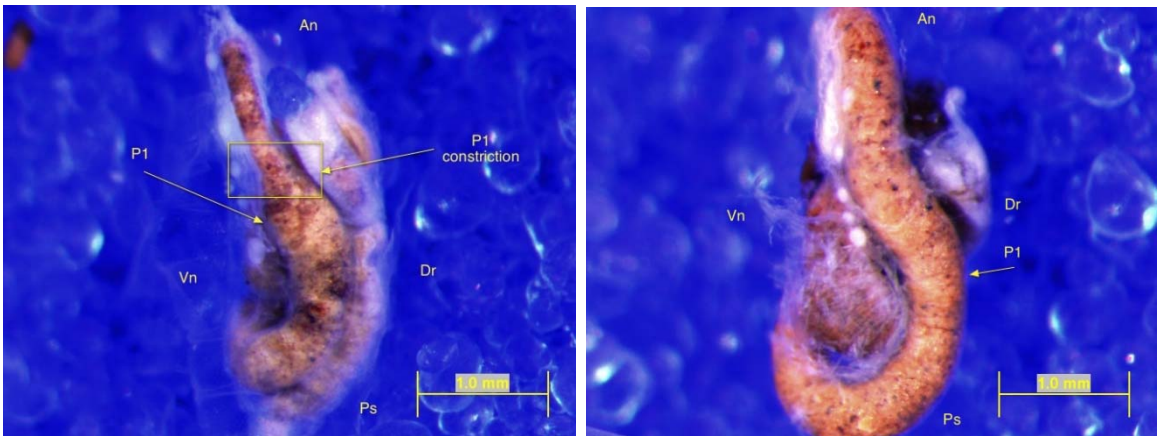


Figure 2-2. Morphology of P1 segment in *Anoplotermes* ss and *Anoplotermes* ng*:* A) *Anoplotermes* ss (constricted). B) *Anoplotermes* ng (Tubular).

Figure 2-3. G1 enteric valve morphology: An = anterior, Ps = Posterior, LR = longitudinal ridge, viewed dorso-ventrally, not drawn to scale.

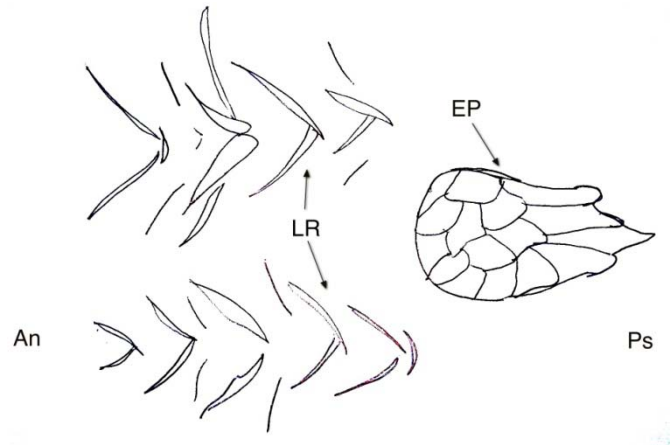


Figure 2-4. G2 enteric valve morphology: An = anterior, Ps = Posterior, LR = longitudinal ridge, EP = enteric pad, viewed dorso-ventrally, not drawn to scale.

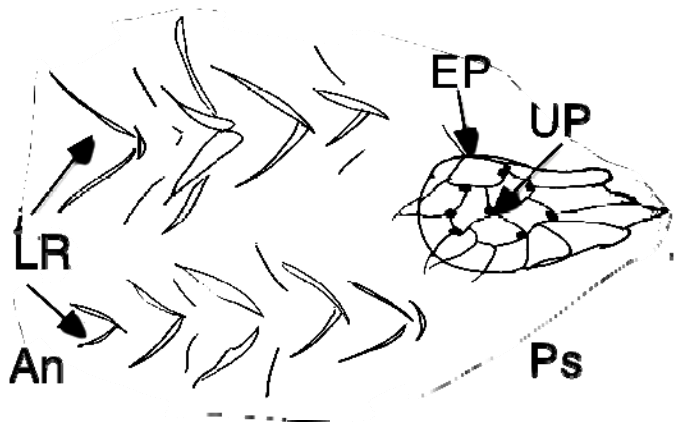


Figure 2-5. G3 enteric valve morphology: An = anterior, Ps = Posterior, LR = longitudinal ridge, EP = enteric pad, UP = upturned scales, viewed dorso-ventrally, not drawn to scale.

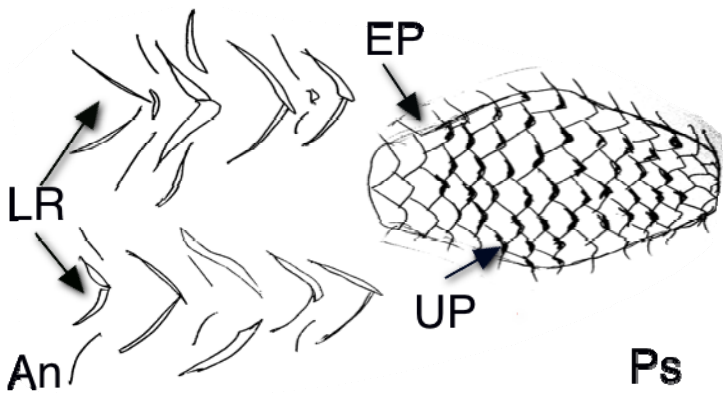


Figure 2-6. G4 enteric valve morphology: An = anterior, Ps = Posterior, LR = longitudinal ridge, EP = enteric pad, UP = upturned scales, viewed dorso-ventrally, not drawn to scale.

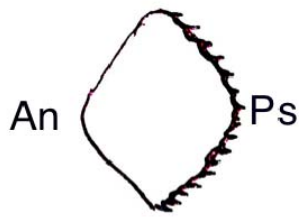
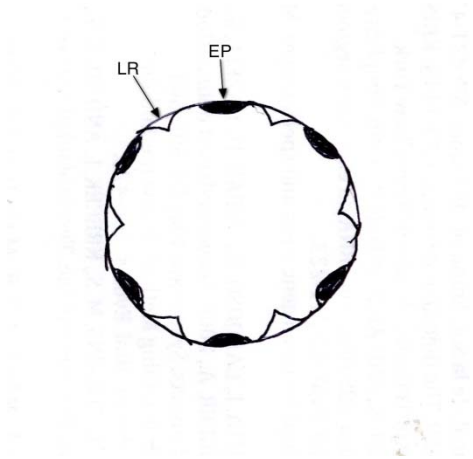


Figure 2-7. Morphology of scales in the enteric valve: An = anterior, Ps = Posterior.

A



B

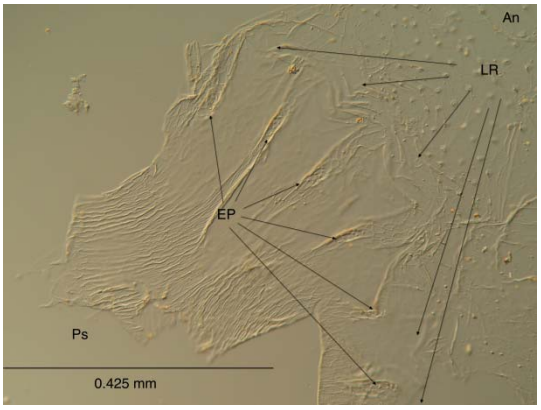


Figure 2-8. Enteric valve situation: A) Cross-section of P2 showing hexaradial symmetry of enteric pads and longitudinal ridges. B) Complete enteric valve of *Anoplotermes* ng.

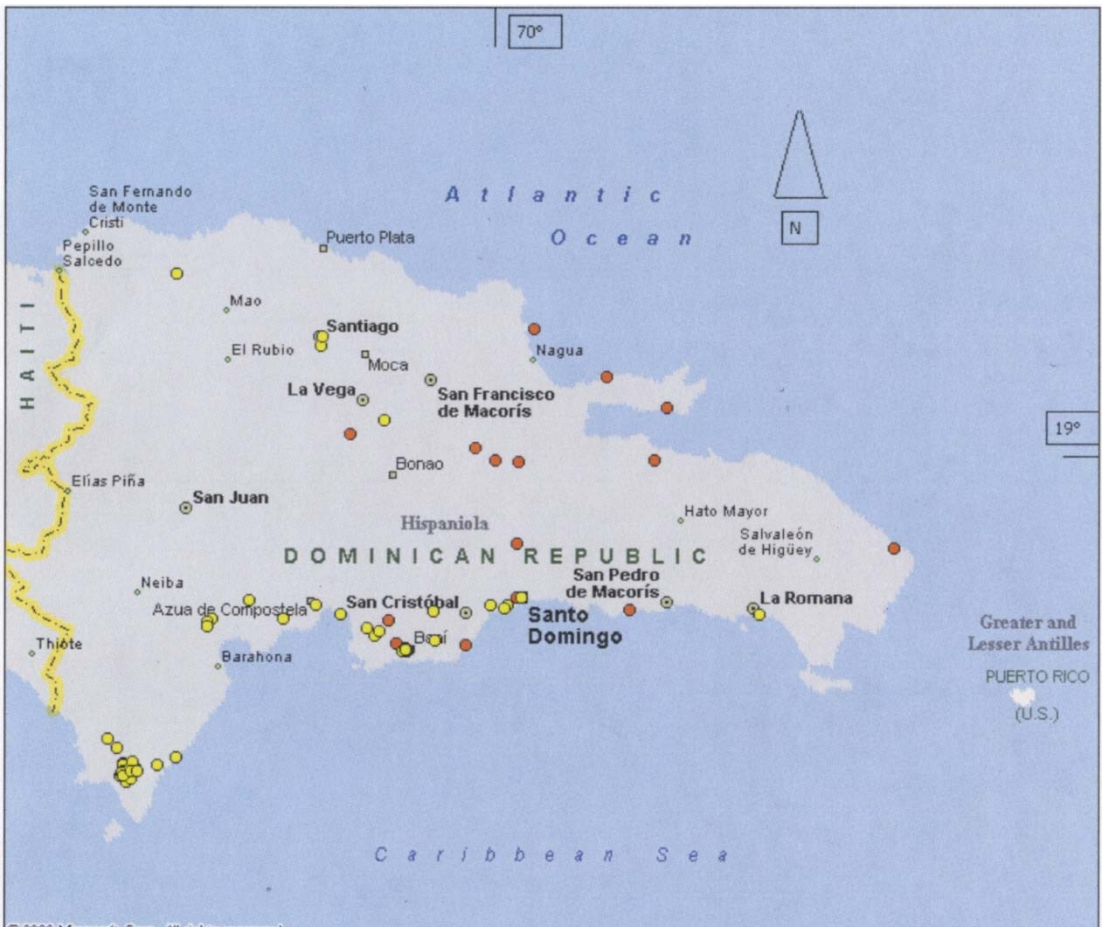


Figure 2-9. Distribution map: *Anoplotermes* ss (red circle) and *Anoplotermes* ng (yellow circle).

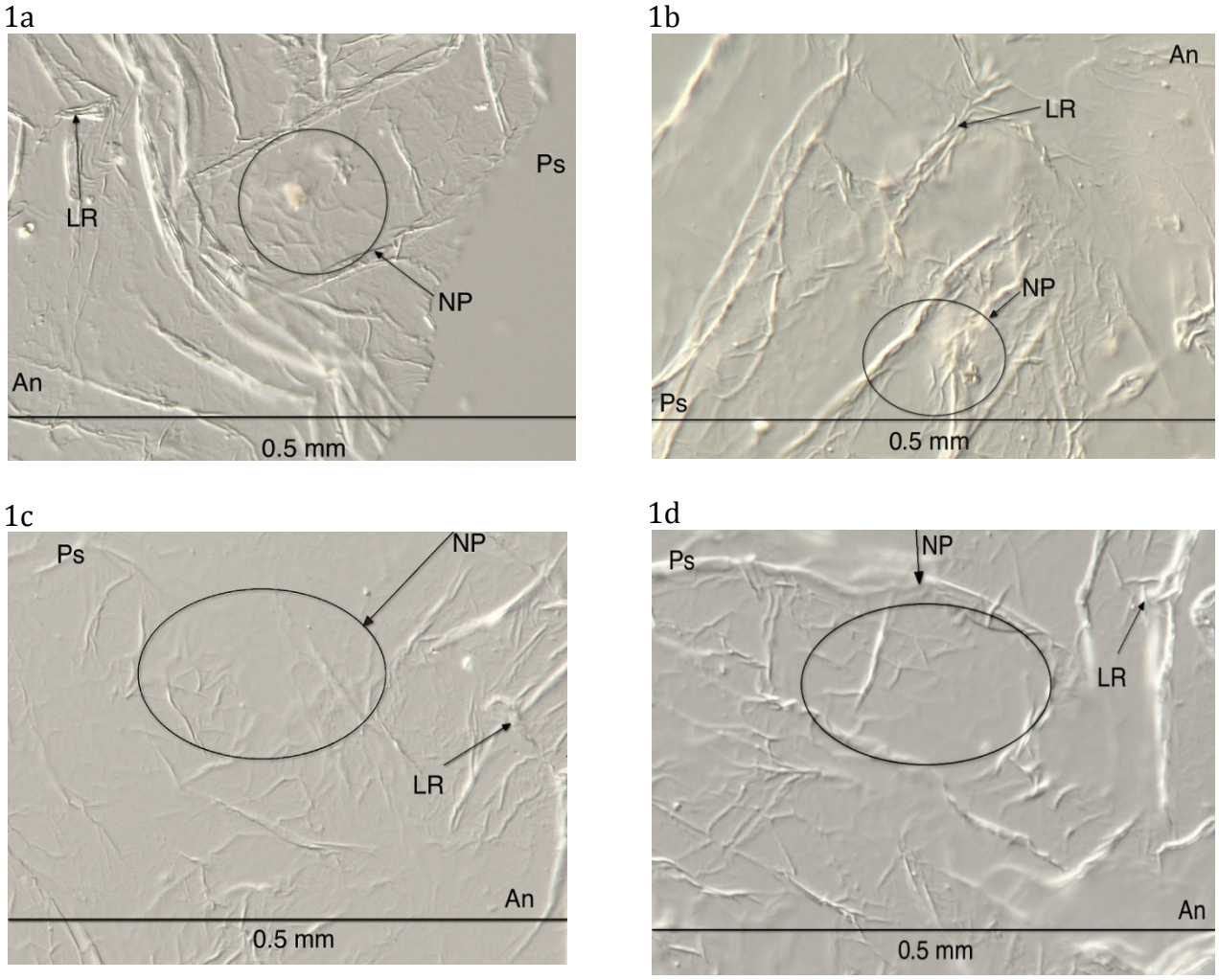
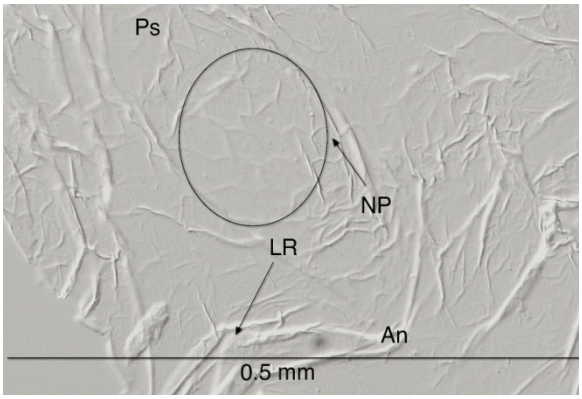
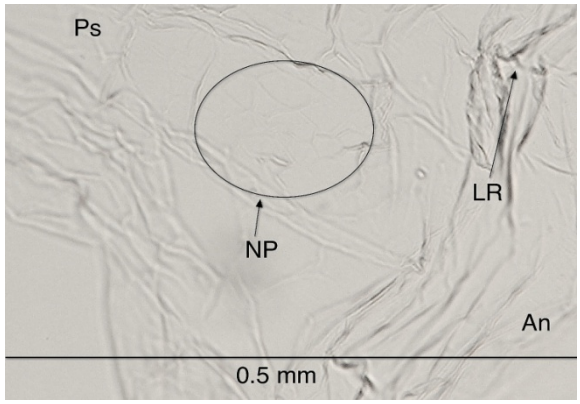


Figure 2-10. Photographs of enteric valves of each specimen used in COII sequence analysis, representing all morphological groups: Figure numbers correspond to EV morphological group, 1a) DR 1222. 1b) DR 1227. 1c) DR 1598. 1d) DR 1256. 1e) DR 1214. 1f) DR 1223. 1g) DR 1219. 1h) DR 420. 1i) DR 1339. 1j) DR 128. 1k) DR 1541. 1l) DR 970. 1m) DR 1316. 1n) DR 1317. 1o) DR 1355. 1p) BA 1916. 2a) DR 1173. 2b) DR 971. 2c) DR 1979. 2d) DR 1979. 2e) BA 2437. 3a) DR 1318. 3b) DR 1354. 3c) DR 1922. 3d) DR 1827. 3e) DR 1853. 3f) DR 1784. 3g) 1790. 3h) DR 456. 3i) DR 1288. 3j) DR 1803. 3k) DR 1226. 3l) BA 1136. 4a) DR 1533. 4b) DR 1333. 4c) DR 1319. 4d) DR 1286. 4e) DR 1260.

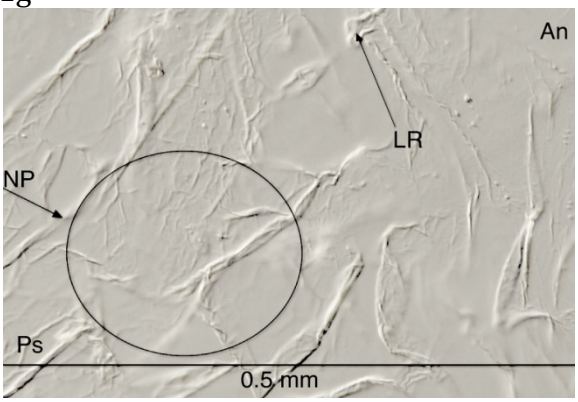
1e



1f



1g



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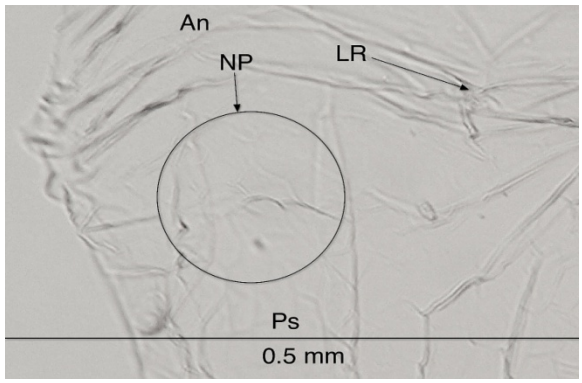

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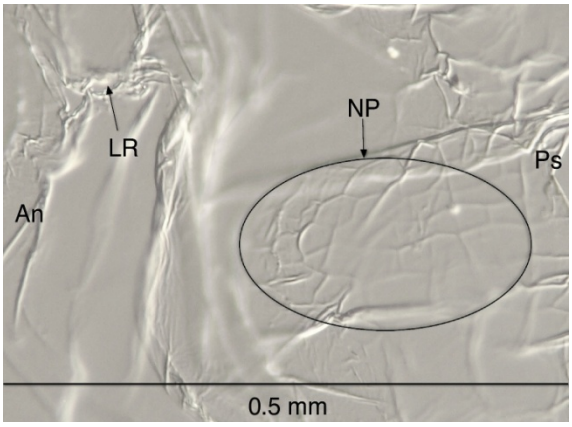

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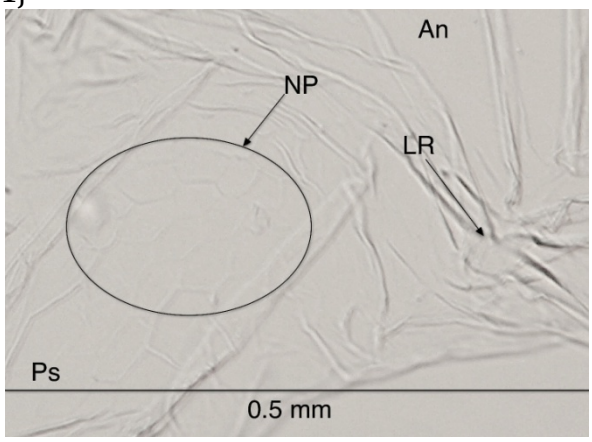
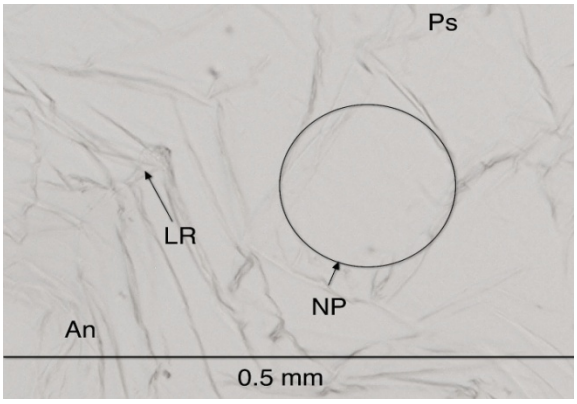
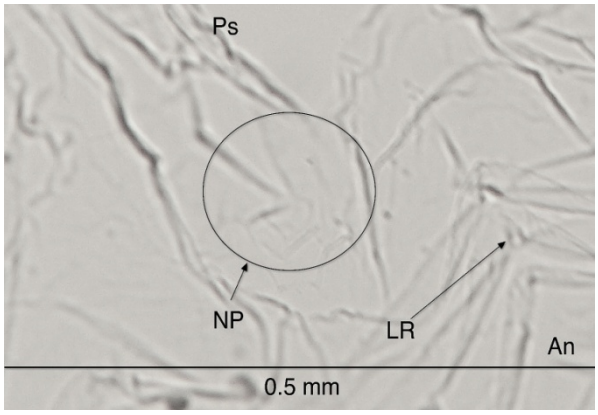


Figure 2-9. Continued.

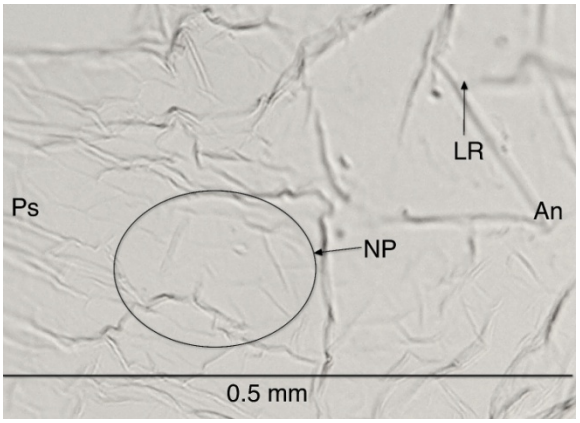
1k



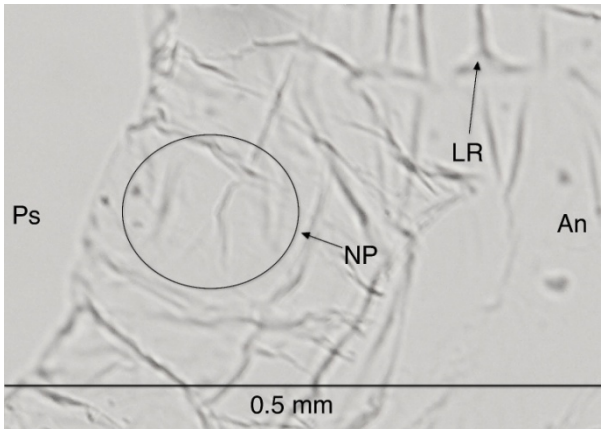
1l



1m



1n



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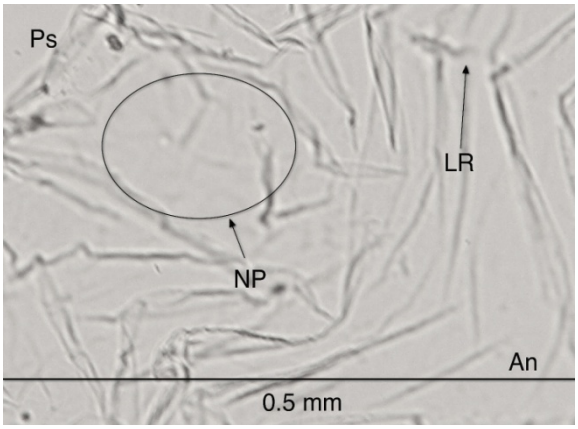


1p

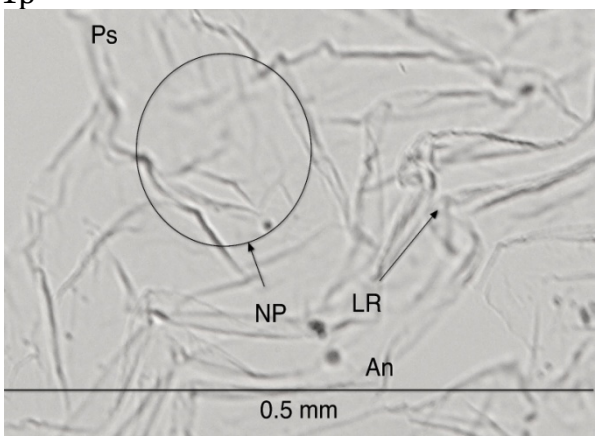
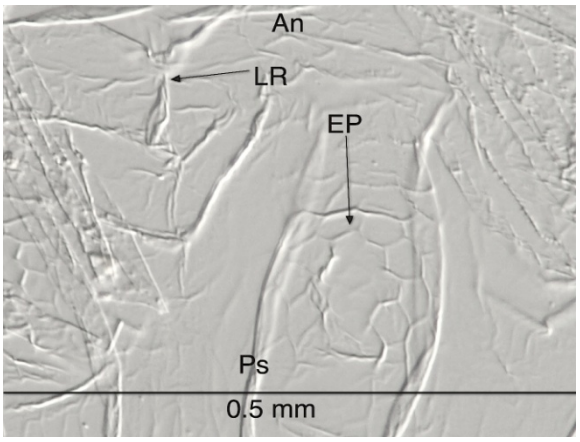
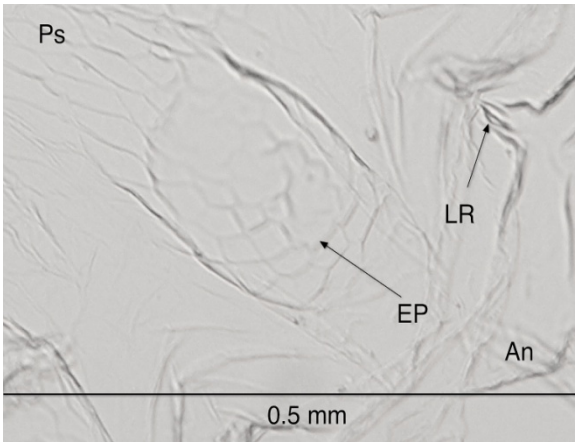


Figure 2-9. Continued.

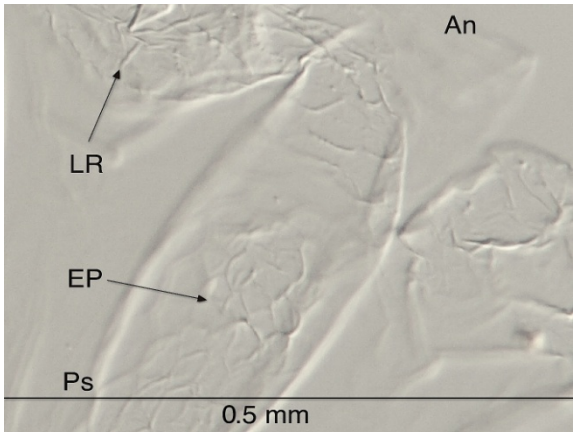
2a



2b



2c



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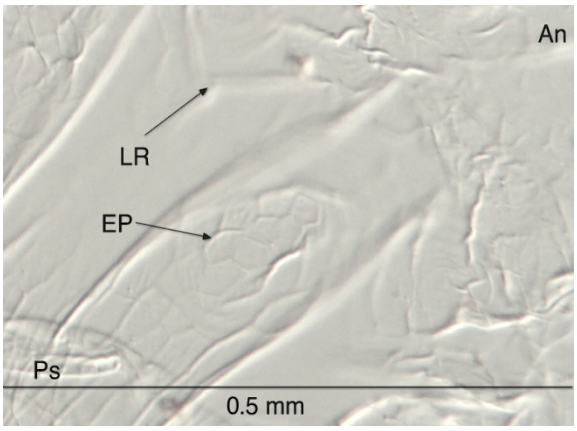

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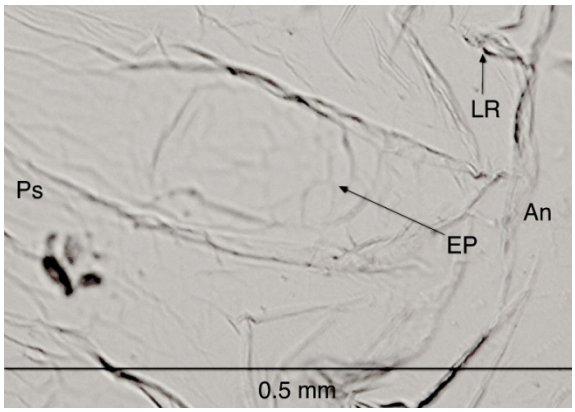

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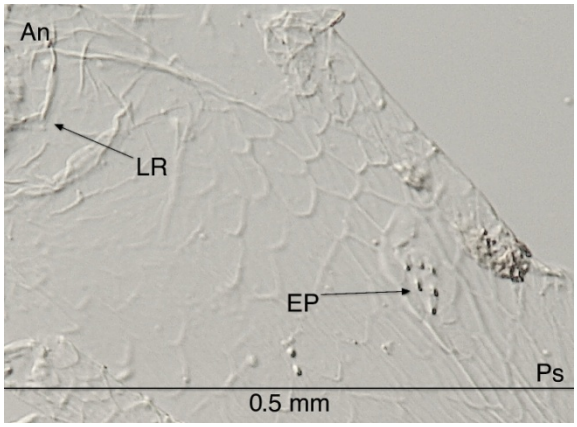
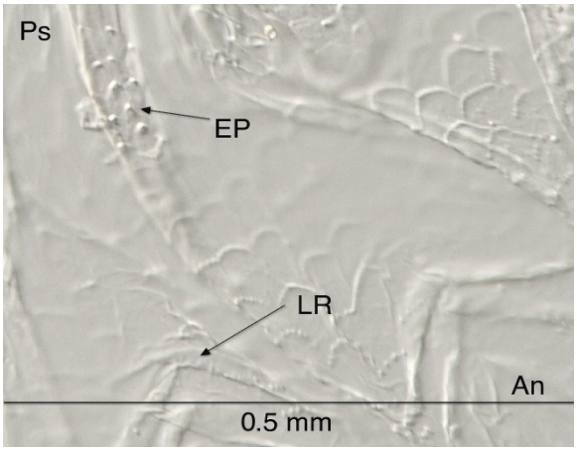


Figure 2-9. Continued.

3b



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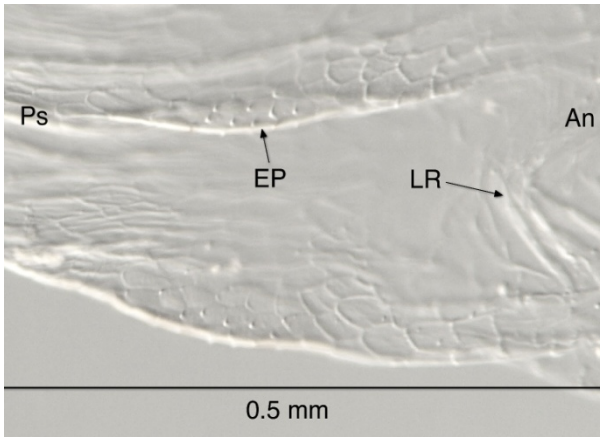

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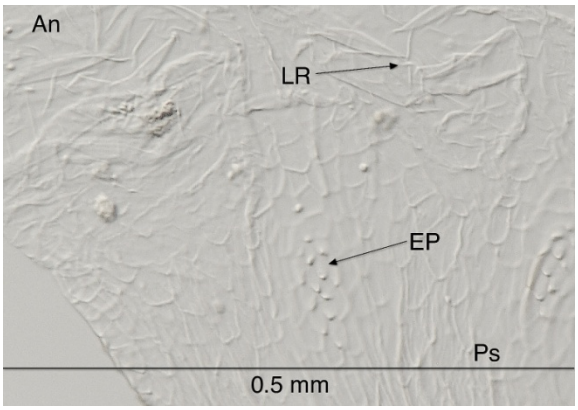

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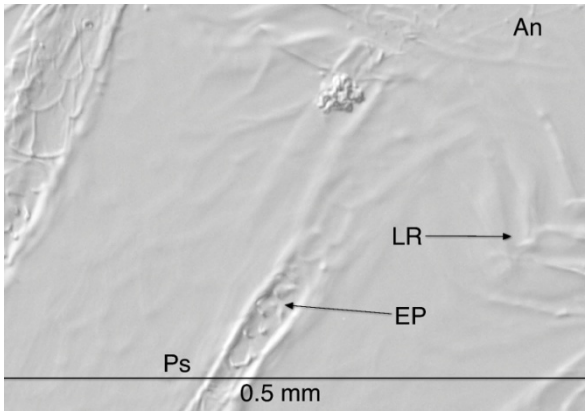

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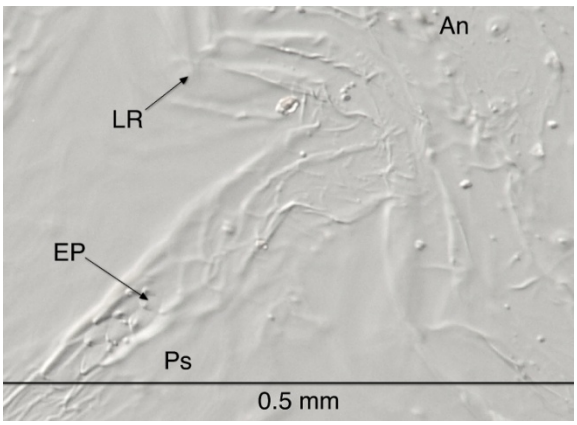


3g

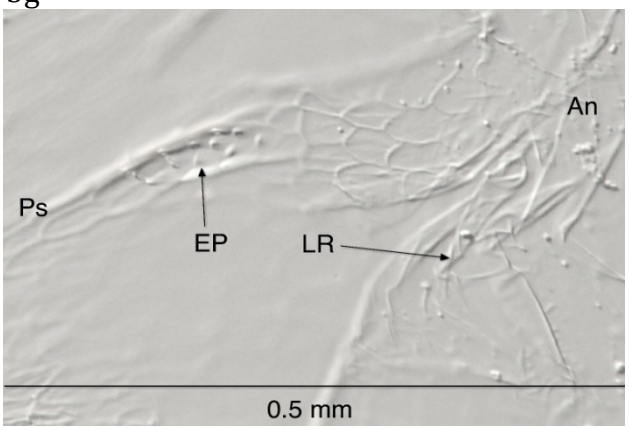
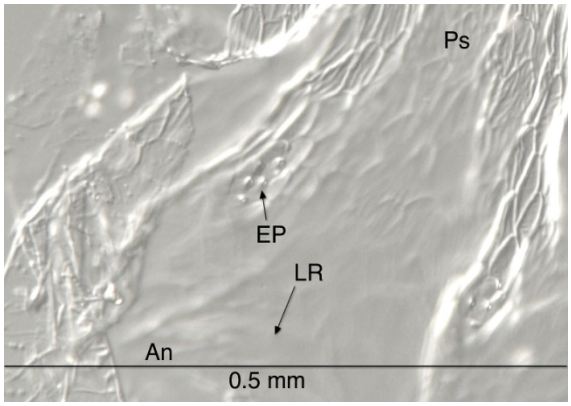
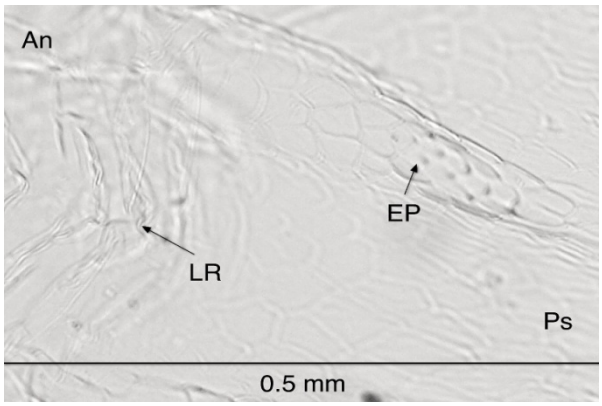


Figure 2-9. Continued.

3h



3i



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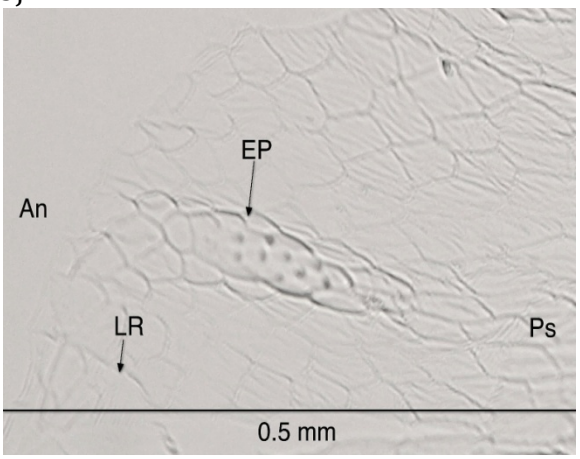

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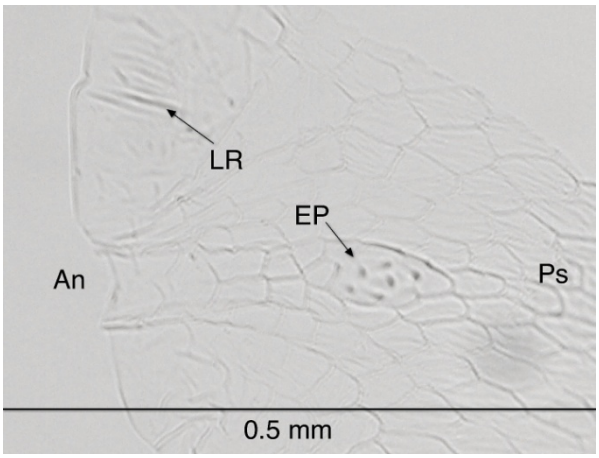
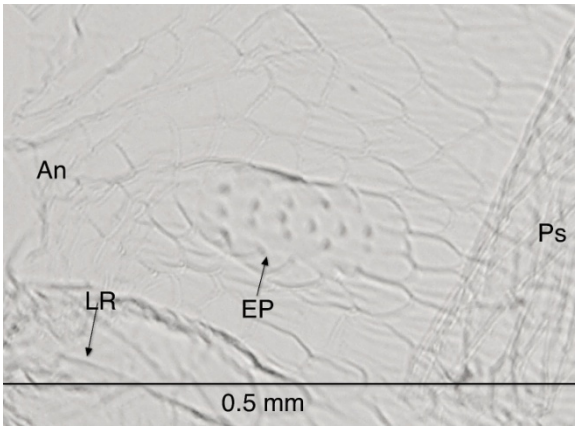


3l



4a

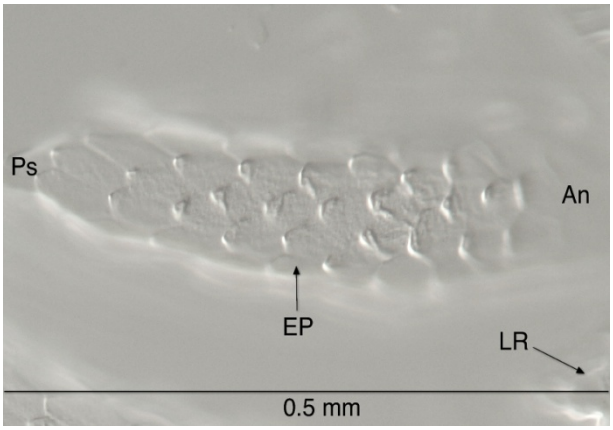
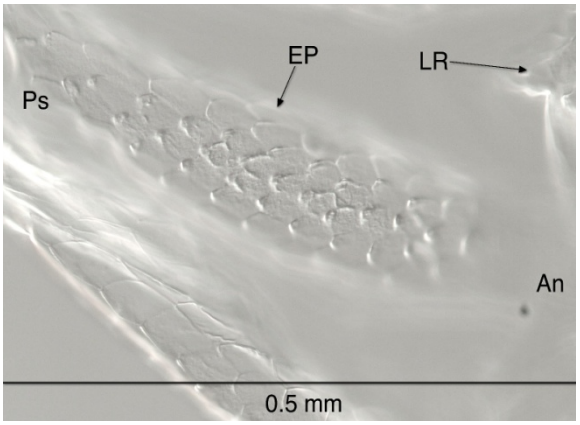


Figure 2-9. Continued.

4b



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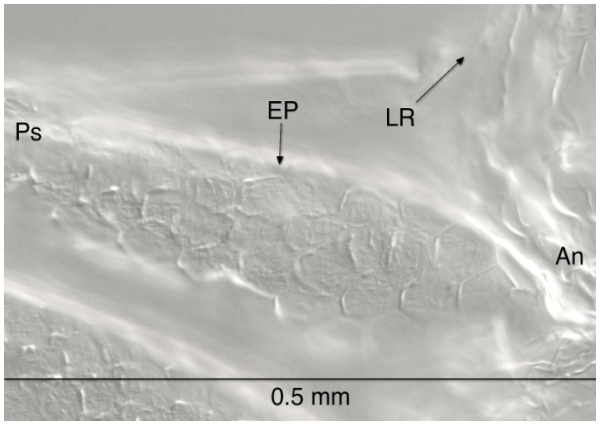

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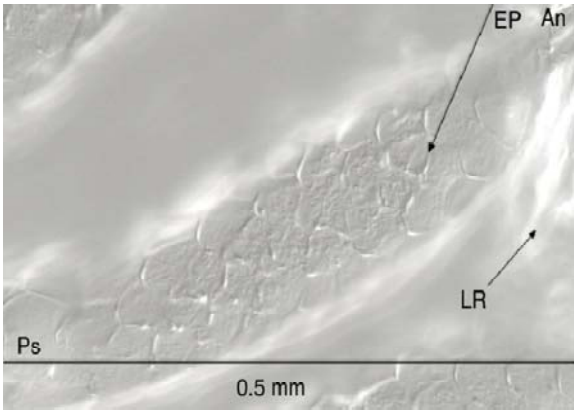

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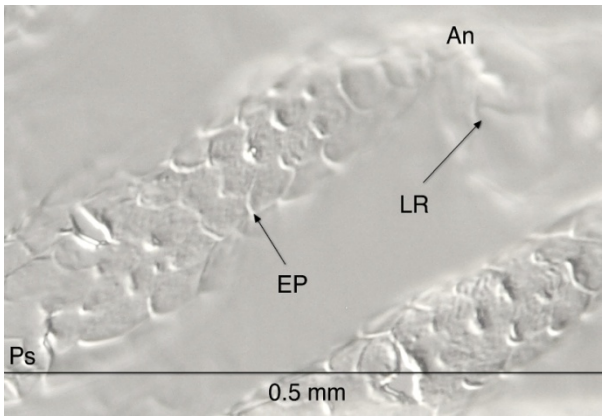

Figure 2-9. Continued.

Table 2-1. Termite sample codes and their corresponding enteric valve morphological characterization, morphology of P1 segment, locality data, and mean and range of head width mean: Ano = *Anoplotermes* ss, Ang = *Anoplotermes* ng, EV = enteric valve morphology, UP = upturned scales

Sample	**No. of Termites [1] and P1 type**	**Locality**	**Head Width Mean ± SD(mm)**	**Head Width Range (mm) : UP[3]**
G1 EV_Ano				
DR 127	10: Constricted	Hoyo de Pun	0.81 ± 0.01	0.80 - 0.83 ; n/a
DR 374	10:Constricted	Santa Domingo, C	0.82 ± 0.01	0.80 - 0.83 : n/a
DR 476	10: Constricted	3 km S Saban del Mar	0.82 ± 0.02	0.80 - 0.85 : n/a
DR 1214	8: Contstricted	2 km W Bani	0.83 ± 0.01	0.83 - 0.86 : n/a
DR 1219	8: Constricted	2 km E Cañafistol	0.86 ± 0.02	0.83 - 0.90 : n/a
DR 1222	10: Constricted	2 km E Cañafistol	0.88 ± 0.01	0.83 - 0.86 : n/a
DR 1223	9: Constricted	2 km E Cañafistol	0.83 ± 0.01	0.82 - 0.84 : n/a
DR 1227	10:Constricted	2 km E Cañafistol	0.89 ± 0.05	0.86 - 1.03 : n/a
DR 1228	3: Constricted	2 km E Cañafistol	0.86 ± 0.01	0.86 - 0.88 : n/a
DR 1256	1: Constricted	1 km E Galeon, DF	0.84 ± 0.00	0.84 : n/a
DR 1257	9: Constricted	1 km E Galeon, DF	0.86 ± 0.02	0.83 - 0.86 : n/a
DR 1258	9: Constricted	1 km E Galeon, DF	0.81 ± 0.01	0.81 - 0.83 : n/a
DR 1315	1: Constricted	2 km E Cañafistol	0.82 ± 0.00	0.82 : n/a
DR 1316	10: Constricted	2 km E Cañafistol	0.88 ± 0.02	0.83 - 0.91 : n/a
DR 1317	10: Constricted	2 km E Cañafistol	0.86 ± 0.01	.086 - 0.88 : n/a
DR 1339	9: Constricted	2 km E Cañafistol	0.86 ± 0.02	0.83 - 0.88 : n/a
DR 1355	10: Constricted	La Barilla, farm	0.80 ± 0.00	0.80 - 0.81 : n/a
DR 1516	7: Constricted	Bani	0.80 ± 0.00	0.80 - 0.81 : n/a
DR 1532	10: Constricted	2 km E Cañafistol	0.86 ± 0.01	0.83 - 0.88 : n/a
DR 1541	10: Constricted	1 km S Matadero	0.85 ± 0.02	0.81 - 0.86 : n/a
DR 1542	5: Constricted	1km S Matadero	0.84 ± 0.02	0.80 - 0.85 : n/a
DR 1598	10: Constricted	1 km W Bani	0.85 ± 0.02	0.86 - 0.88 : n/a
DR 1934	6: Constricted	E Bani	0.86 ± 0.02	0.83 - 0.86 : n/a
G2 EV_Ano				
DR 126	10: Constricted	Bomba de Gaunuma	0.72 ± 0.03	0.68 - 0.76 : n/a
DR 128	10: Constricted	9 km W Embassy, BE.	0.78 ± 0.01	.076 - 0.79 : n/a
DR 400	10: Constricted	Samana City Park	0.76 ± 0.02	0.76 - 0.79 : n/a
DR 420	10: Constricted	Playa Grande	0.74 ± 0.02	0.70 - 0.78 : n/a
DR 970	10: Constricted	Jimenoa nr. Jarabocoa	0.75 ± 0.02	0.73 - 0.78 : n/a
DR 971	10: Constricted	Jimenoa nr. Jarabocoa	0.78 ± 0.01	0.76 - 0.79 : n/a
DR 1166	10: Constricted	Guineal	0.78 ± 0.03	0.76 - 0.79 : n/a
DR 1167	10: Constricted	Guineal	0.76 ± 0.02	0.73 - 0.79 : n/a
DR 1168	10: Constricted	Guineal	0.76 ± 0.03	0.69 - 0.79 : n/a
DR 1173	10: Constricted	Guineal	0.75 ± 0.02	0.72 - 0.78 : n/a
DR 1505	9: Constricted	Batey, Los Guineos	0.77 ± 0.01	0.76 - 0.79 : n/a
DR 1979	10: Constricted	Las Terrenas	0.74 ± 0.02	0.70 - 0.78 : n/a
G3 EV_Ang				
DR 129	10: Tubular	La Guajaca	0.89 ± 0.02	0.88 - 0.91 : 19
DR 456	10: Tubular	Santiago, Inst. Sup. Ag.	0.85 ± 0.02	0.83 - 0.86 : 11
DR 459	10: Tubular	Santiago, Inst. Sup. Ag.	0.84 ± 0.02	0.81 - 0.86 : 18
DR 460	10: Tubular	Santiago, Inst. Sup. Ag.	0.84 ± 0.02	0.81 - 0.86 : 11
DR 469	10: Tubular	El Pino	0.88 ± 0.02	0.86 - 0.91 : 15

Table 2-1. Continued

Sample	No. of Termites [1]and P1 type	Locality	Head Width Mean ± SD(mm)	Head Width Range (mm) : UP
DR 596	10: Tubular	La Romana	0.82 ± 0.02	0.81 - 0.86 : 13
DR 601	10: Tubular	La Romana	0.86 ± 0.02	0.83 - 0.88 : 16
DR 604	10: Tubular	La Romana	0.84 ± 0.02	0.80 - 0.87 : 13
DR 617	10: Tubular	La Romana	0.84 ± 0.02	0.80 - 0.86 : 12
DR 619	10: Tubular	La Romana	0.87 ± 0.02	0.83 - 0.88 : 16
DR 721	6: Tubular	La Colonia nr. Jauncho	0.75 ± 0.02	0.73 - 0.78 : 6
DR 722	4: Tubular	La Colonia nr. Jauncho	0.86 ± 0.00	n/a : 13
DR 754	10: Tubular	10 km W Oviedo	0.84 ± 0.01	0.83 - 0.86 : 10
DR 911	10: Tubular	Finca Ochoa	0.88 ± 0.02	0.86 - 0.91 : 13
DR 991	4: Tubular	Santo Domingo	0.76	n/a : 10
DR 1082	3: Tubular	El Carril	0.86 ± 0.00	n/a : 7
DR 1180	10: Tubular	13 km N San Jose Oco.	0.89 ± 0.04	0.86 - 0.98 : 11
DR 1191	10: Tubular	13 km N San Jose Oco.	0.87 ± 0.03	0.83 - 0.91 : 12
DR 1220	10: Tubular	2 km E Cañafistol	0.83 ± 0.02	0.81 - 0.85 : 12
DR 1226	10: Tubular	2 km E Cañafistol	0.83 ± 0.02	0.80 - 0.84 : 13
DR 1276	7: Tubular	Tres Chacos	0.82 ± 0.01	0.81 - 0.83 : 12
DR 1283	10: Tubular	3 km NW Tres Chacos	0.81 ± 0.01	0.78 - 0.83 : 7
DR 1286	10: Tubular	NW Tres Chacos	0.83 ± 0.02	0.81 - 0.86 : 12
DR 1287	10: Tubular	NW Tres Chacos	0.83 ± 0.02	0.81 - 0.86 : 12
DR 1288	10: Tubular	NW Tres Chacos	0.85 ± 0.02	0.81 - 0.86 : 10
DR 1291	10: Tubular	2 km S Canada de P.V.	0.84 ± 0.02	0.78 - 0.86 : 11
DR 1313	10: Tubular	E Cañafistol	0.85 ± 0.01	0.83 - 0.86 : 10
DR 1314	7: Tubular	E Cañafistol	0.86 ± 0.04	0.81 - 0.91 : 14
DR 1318	10: Tubular	2 km E Cañafistol	0.83 ± 0.02	0.81 - 0.86 : 10
DR 1320	10: Tubular	2 km E Cañafistol	0.82 ± 0.02	0.81 - 0.86 : 15
DR 1338	10: Tubular	2 km E Cañafistol	0.84 ± 0.02	0.83 - 0.86 : 13
DR 1353	10: Tubular	La Barilla, farm	0.76 ± 0.02	0.73 - 0.78 : 9
DR 1354	10: Tubular	La Barilla, farm	0.82 ± 0.03	0.76 - 0.86 : 10
DR 1517	10: Tubular	Bani	0.85 ± 0.02	0.83 - 0.86 : 15
DR 1527	10: Tubular	2 km E Cañafistol	0.84 ± 0.02	0.81 - 0.86 : 13
DR 1528	5: Tubular	2 km E Cañafistol	0.84 ± 0.01	0.83 - 0.86 : 11
DR 1559	10: Tubular	W Bani	0.85 ± 0.02	0.83 - 0.86 : 15
DR 1562	10: Tubular	W Bani	0.85 ± 0.02	0.83 - 0.86 : 15
DR 1663	10: Tubular	Botoncillo	0.79 ± 0.02	0.76 - 0.81 : 9
DR 1743	4: Tubular	Tres Chacos	0.80 ± 0.02	0.78 - 0.83 : 14
DR 1775	10: Tubular	Jaragua Natl. Park	0.81 ± 0.02	0.78 - 0.81 : 13
DR 1784	10: Tubular	Jaragua Ntl. Park	0.79 ± 0.02	0.78 - 0.81 : 12
DR 1790	10: Tubular	Jaragua Ntl. Park	0.89 ± 0.02	0.86 - 0.91 : 12
DR 1796	10: Tubular	Jaragua Natl. Park	0.83 ± 0.02	0.81 - 0.86 : 9
DR 1803	10: Tubular	Jaragua Natl. Park	0.82 ± 0.02	0.81 - 0.86 : 12
DR 1827	10: Tubular	Oviedo	0.83 ± 0.03	0.78 - 0.86 : 9
DR 1853	10: Tubular	13 km SW Oviedo	0.83 ± 0.04	0.78 - 0.93 : 11
DR 1854	10: Tubular	13 km SW Oviedo	0.86 ± 0.01	0.86 - 0.88 : 8
DR 1866	10: Tubular	12 km SW Oviedo	0.87 ± 0.03	0.83 - 0.93 : 8
DR 1867	9: Tubular	12 km SW Oviedo	0.86 ± 0.02	0.83 - 0.88 : 14
DR 1884	Tubular	W Tres Chacos	0.85 ± 0.02	0.82 - 0.88 : 11
DR 1885	10: Tubular	W Tres Chacos	0.85 ± 0.02	0.81 - 0.88 : 9

Table 2-1. Continued

Sample	No. of Termites [1]and P1 type	Locality	Head Width Mean ± SD(mm)	Head Width Range (mm) : UP
DR 1886	10: Tubular	W Tres Chacos	0.80 ± 0.01	0.78 - 0.83 : 12
DR 1887	5: Tubular	W Tres Chacos	0.80 ± 0.03	0.76 - 0.83 : 11
DR 1904	4: Tubular	Oviedo	0.84 ± 0.01	0.83 - 0.86 : 18
DR 1905	10: Tubular	Oviedo	0.80 ± 0.02	0.78 - 0.83 : 14
DR 1922	10: Tubular	N of site # 77	0.82 ± 0.02	0.78 - 0.86 : 6
G4 EV_Ang				
DR 125	10: Tubular	Santo Domingo	0.88 ± 0.06	0.78 - 0.93 : 26
DR 321	10: Tubular	9 km E Quita Coraza	0.82 ± 0.02	0.81 - 0.86 : 28
DR 325	10: Tubular	9 km E Quita Coraza	0.81 ± 0.02	0.78 - 0.81 : 30
DR 328	10: Tubular	2 km N Canoa	0.80 ± 0.01	0.78 - 0.83 : 27
DR 700	10: Tubular	Caracoles	0.83 ± 0.01	0.81 - 0.86 : 21
DR 1036	10: Tubular	11 km W of Azua	0.79 ± 0.00	0.78 - 0.79 : 26
DR 1037	10: Tubular	11 km W of Azua	0.79 ± 0.00	0.78 - 0.79 : 32
DR 1046	10: Tubular	1 km E Azua	0.80 ± 0.03	0.76 - 0.83 : 31
DR 1047	6: Tubular	1k E Azua	0.82 ± 0.01	0.81 - 0.83 : 26
DR 1048	10: Tubular	1k E Azua	0.81 ± 0.02	0.78 - 0.83 : 20
DR 1049	6: Tubular	1k E Azua	0.81 ± 0.02	0.78 - 0.83 : 22
DR 1050	10: Tubular	1k E Azua	0.81 ± 0.01	0.81 - 0.83 : 23
DR 1051	10: Tubular	1k E Azua	0.82 ± 0.03	0.78 - 0.86 : 33
DR 1062	9: Tubular	11 km W of Azua	0.82 ± 0.02	0.78 - 0.83 : 28
DR 1073	4: Tubular	11 km W of Azua	0.78 ± 0.01	0.78 - 0.79 : 20
DR 1221	8: Tubular	2 km E Cañafistol	0.78 ± 0.01	0.76 - 0.78 : 20
DR 1224	10: Tubular	2 km E Cañafistol	0.78 ± 0.01	0.76 - 0.78 : 24
DR 1225	5: Tubular	2 km E Cañafistol	0.80 ± 0.03	0.79 - 0.83 : 26
DR 1260	10: Tubular	E Las Carreras	0.78 ± 0.01	0.76 - 0.79 : 25
DR 1261	10: Tubular	E Las Carreras	0.78 ± 0.01	0.76 - 0.81 : 27
DR 1262	10: Tubular	E Las Carreras	0.78 ± 0.01	0.76 - 0.79 : 21
DR 1264	10: Tubular	11 km W of Azua	0.83 ± 0.01	0.81 - 0.83 : 21
DR 1295	10: Tubular	2 km S Canada de P.V.	0.84 ± 0.02	0.81 - 0.86 : 26
DR 1319	10: Tubular	E Cañafistol	0.83 ± 0.02	0.81 - 0.86 : 24
DR 1324	10: Tubular	2 km E Cañafistol	0.82 ± 0.01	0.81 - 0.83 : 36
DR 1333	10: Tubular	2 km E Cañafistol	0.82 ± 0.02	0.81 - 0.86 : 37
DR 1335	10: Tubular	2 km E Cañafistol	0.86 ± 0.02	0.83 - 0.88 : 29
DR 1345	10: Tubular	E Cañafistol	0.85 ± 0.01	0.82 - 0.89 : 31
DR 1533	10: Tubular	2 km E Cañafistol	0.81 ± 0.02	0.78 - 0.81 : 25
DR 1929	10: Tubular	E Azua	0.81 ± 0.02	0.78 - 0.83 : 23
DR 1960	10: Tubular	Santo Domingo	0.91 ± 0.02	0.88 - 0.91 : 22
No EV info				
DR 338[2]	n/a	Yayas de Viajama	n/a	n/a
DR 362	1: Constricted	Santa Domingo, C	0.88 ± 0.00	n/a
DR 697	1: Tubular	Caracoles	0.81	n/a
DR 1028[2]	n/a	11 km W Azua	n/a	n/a
DR 1038	1: Tubular	11 km W of Azua	0.80	n/a
DR 1052	1: Tubular	1k E Azua	0.79	n/a
DR 1072	1: Tubular	11 km W of Azua	0.82	n/a
DR 1086	1: Tubular	La Canoa	0.81	n/a

Table 2-1. Continued

Sample	No. of Termites [1]and P1 type	Locality	Head Width Mean ± SD(mm)	Head Width Range (mm) : UP
DR 1263	1: Tubular	11 km W of Azua	0.78	n/a
DR 1269	1: Tubular	4 km E Vicente Noble	0.81	n/a
DR 1277	1: Tubular	Tres Chacos	0.83	n/a
DR 1323	1: Tubular	2 km E Cañafistol	0.82	n/a
DR 1392[2]	n/a	1 km NW Playa Bavar	n/a	n/a
DR 1774	2: Tubular	Jaragua Natl. Park	0.79 ± 0.02	0.78 - 0.81 : n/a
DR 1783	2: Tubular	Jaragua Natl. Park	0.79 ± 0.02	0.78 - 0.81 : n/a
DR 1932	1: Constricted	E Bani	0.88	n/a
DR 1933	1: Constricted	E Bani	0.91	n/a

[1] Number of termites measured in each sample

[2] Alates only present caste

[3] Number represents one pad per sample

Table 2-2. Termite sample codes and mean measurements for each character: Ano = *Anoplotermes* ss, Ang = *Anoplotermes* ng

Sample	BL	HL	HW	CH	CL	CW	PW	FTW	MTW	HTW
Ano_G1										
DR 126	2.62	0.93	0.72	0.12	0.19	0.34	0.46	0.14	0.10	0.09
DR 127	3.20	1.02	0.80	0.11	0.18	0.35	0.50	0.15	0.10	0.09
DR 128	3.04	0.99	0.78	0.13	0.22	0.37	0.49	0.14	0.10	0.10
DR 400	3.03	1.02	0.77	0.12	0.19	0.35	0.49	0.15	0.11	0.09
DR 420	3.00	0.91	0.74	0.11	0.18	0.33	0.48	0.13	0.10	0.09
DR 476	2.44	0.95	0.77	0.14	0.20	0.36	0.49	0.14	0.10	0.09
DR 970	2.95	0.98	0.75	0.12	0.21	0.36	0.48	0.14	0.10	0.09
DR 1214	2.73	1.04	0.83	0.16	0.24	0.28	0.52	0.16	0.11	0.10
DR 1219	3.21	1.03	0.86	0.16	0.23	0.39	0.54	0.16	0.11	0.09
DR 1222	3.08	1.08	0.85	0.16	0.23	0.39	0.54	0.15	0.10	0.09
DR 1223	3.01	1.06	0.83	0.15	0.23	0.40	0.52	0.15	0.10	0.09
DR 1227	3.18	1.10	0.89	0.16	0.23	0.42	0.55	0.15	0.12	0.10
DR 1257	3.01	1.07	0.86	0.14	0.24	0.39	0.53	0.15	0.11	0.10
DR 1258	2.82	1.07	0.81	0.13	0.23	0.42	0.50	0.15	0.11	0.09
DR 1316	3.31	1.12	0.88	0.13	0.23	0.41	0.54	0.15	0.11	0.09
DR 1317	3.29	1.13	0.86	0.12	0.23	0.40	0.53	0.15	0.10	0.09
DR 1339	3.24	1.09	0.86	0.13	0.23	0.39	0.54	0.15	0.12	0.10
DR 1355	2.85	0.98	0.80	0.12	0.22	0.37	0.48	0.15	0.10	0.09
DR 1516	3.04	1.00	0.80	0.15	0.23	0.37	0.50	0.15	0.10	0.08
DR 1532	3.28	1.13	0.86	0.12	0.23	0.40	0.53	0.15	0.10	0.09
DR 1541	3.07	1.08	0.85	0.12	0.22	0.39	0.52	0.14	0.10	0.09
DR 1598	3.43	1.07	0.85	0.16	0.23	0.40	0.52	0.15	0.11	0.08
DR 1934	2.92	1.08	0.86	0.13	0.24	0.39	0.53	0.14	0.11	0.09
Ano_G2										
DR 971	3.03	0.99	0.78	0.12	0.21	0.36	0.51	0.14	0.10	0.09
DR 1166	2.90	1.01	0.78	0.12	0.18	0.35	0.50	0.15	0.10	0.10
DR 1167	3.03	0.98	0.76	0.13	0.18	0.35	0.49	0.14	0.10	0.09
DR 1168	3.16	0.99	0.76	0.11	0.18	0.35	0.50	0.13	0.10	0.09
DR 1173	2.85	0.97	0.75	0.12	0.18	0.34	0.49	0.14	0.10	0.10
DR 1505	2.90	0.99	0.78	0.12	0.18	0.35	0.50	0.14	0.10	0.09
DR 1979	2.64	0.92	0.74	0.12	0.19	0.34	0.47	0.14	0.10	0.09
Ang_G3										
DR 129	3.45	1.09	0.89	0.14	0.26	0.41	0.53	0.17	0.11	0.09
DR 456	3.31	1.08	0.85	0.12	0.26	0.40	0.51	0.16	0.10	0.09
DR 459	3.37	1.07	0.84	0.13	0.25	0.39	0.52	0.15	0.10	0.09
DR 460	3.30	1.06	0.84	0.14	0.25	0.38	0.50	0.15	0.10	0.09
DR 469	3.56	1.11	0.88	0.14	0.26	0.41	0.53	0.15	0.10	0.09
DR 596	3.35	1.07	0.82	0.15	0.23	0.38	0.51	0.15	0.10	0.08
DR 601	3.24	1.13	0.86	0.14	0.27	0.39	0.52	0.15	0.10	0.10
DR 619	3.30	1.10	0.87	0.14	0.28	0.40	0.53	0.15	0.11	0.09
DR 721	3.17	0.95	0.75	0.15	0.25	0.36	0.47	0.15	0.11	0.08

Table 2-2. Continued

Sample	BL	HL	HW	CH	CL	CW	PW	FTW	MTW	HTW
DR 754	3.02	1.09	0.84	0.15	0.26	0.39	0.50	0.15	0.10	0.09
DR 911	3.43	1.10	0.88	0.13	0.26	0.41	0.53	0.15	0.11	0.09
DR 1180	3.48	1.15	0.89	0.12	0.23	0.40	0.54	0.15	0.10	0.10
DR 1191	3.44	1.15	0.87	0.12	0.23	0.39	0.53	0.15	0.10	0.10
DR 1283	3.39	1.01	0.81	0.10	0.24	0.38	0.49	0.15	0.10	0.08
DR 1287	3.39	1.06	0.83	0.14	0.27	0.38	0.52	0.15	0.10	0.09
DR 1288	3.40	1.06	0.85	0.13	0.27	0.39	0.51	0.15	0.10	0.09
DR 1291	3.35	1.05	0.84	0.14	0.27	0.39	0.50	0.16	0.12	0.10
DR 1313	3.21	1.07	0.85	0.13	0.25	0.38	0.52	0.15	0.10	0.09
DR 1320	3.32	1.11	0.82	0.13	0.24	0.38	0.52	0.16	0.11	0.09
DR 1338	3.49	1.07	0.84	0.12	0.23	0.38	0.53	0.15	0.10	0.08
DR 1354	3.20	1.11	0.82	0.12	0.22	0.36	0.51	0.15	0.10	0.09
DR 1527	3.13	1.08	0.84	0.13	0.23	0.38	0.52	0.15	0.11	0.10
DR 1528	2.98	1.12	0.84	0.13	0.23	0.38	0.52	0.16	0.11	0.08
DR 1663	3.17	0.94	0.79	0.10	0.20	0.36	0.50	0.15	0.10	0.09
DR 1775	3.05	1.03	0.81	0.10	0.24	0.37	0.49	0.15	0.10	0.08
DR 1784	3.54	1.10	0.89	0.15	0.28	0.42	0.53	0.15	0.10	0.09
DR 1790	3.38	1.03	0.83	0.13	0.24	0.38	0.50	0.15	0.11	0.09
DR 1796	3.07	1.04	0.82	0.12	0.25	0.37	0.48	0.15	0.10	0.08
DR 1803	3.48	1.03	0.83	0.11	0.25	0.37	0.52	0.15	0.10	0.08
DR 1827	3.23	1.04	0.83	0.13	0.23	0.37	0.51	0.15	0.10	0.09
DR 1853	3.43	1.08	0.86	0.13	0.26	0.39	0.52	0.15	0.10	0.09
DR 1854	3.51	1.05	0.87	0.12	0.26	0.39	0.52	0.15	0.10	0.10
DR 1866	3.13	1.06	0.86	0.13	0.26	0.41	0.52	0.15	0.10	0.09
DR 1867	2.98	1.07	0.85	0.10	0.23	0.38	0.49	0.15	0.10	0.09
DR 1885	3.25	1.06	0.85	0.13	0.24	0.38	0.52	0.15	0.10	0.09
DR 1886	2.86	1.01	0.80	0.13	0.24	0.37	0.49	0.16	0.11	0.10
DR 1887	2.95	1.03	0.80	0.13	0.24	0.37	0.50	0.15	0.11	0.10
DR 1905	2.98	1.03	0.80	0.13	0.23	0.37	0.49	0.15	0.10	0.08
DR 1922	3.15	1.03	0.82	0.12	0.23	0.37	0.50	0.15	0.10	0.09
Ang_G4										
DR 125	3.41	1.13	0.88	0.12	0.12	0.40	0.54	0.15	0.11	0.10
DR 321	2.68	1.02	0.82	0.11	0.25	0.37	0.47	0.15	0.10	0.08
DR 328	2.74	0.99	0.80	0.12	0.25	0.37	0.47	0.14	0.10	0.08
DR 700	3.14	1.08	0.83	0.12	0.23	0.38	0.49	0.15	0.10	0.08
DR 1037	2.48	1.01	0.79	0.10	0.22	0.36	0.47	0.15	0.10	0.08
DR 1046	3.25	1.05	0.80	0.11	0.22	0.36	0.48	0.14	0.10	0.08
DR 1047	2.52	1.03	0.82	0.10	0.21	0.36	0.49	0.16	0.10	0.08
DR 1048	3.09	1.00	0.81	0.08	0.22	0.36	0.48	0.15	0.10	0.08
DR 1049	2.70	0.99	0.81	0.11	0.22	0.35	0.48	0.15	0.10	0.08
DR 1050	2.50	1.01	0.81	0.10	0.21	0.36	0.47	0.15	0.10	0.09
DR 1051	3.01	1.05	0.82	0.10	0.22	0.36	0.49	0.15	0.10	0.08
DR 1062	2.51	1.03	0.82	0.10	0.21	0.36	0.48	0.15	0.10	0.09
DR 1221	2.52	1.00	0.77	0.09	0.19	0.34	0.47	0.14	0.10	0.09

Table 2-2. Continued

Sample	BL	HL	HW	CH	CL	CW	PW	FTW	MTW	HTW
DR 1225	2.86	1.02	0.80	0.10	0.20	0.36	0.47	0.14	0.10	0.08
DR 1262	2.60	0.95	0.78	0.09	0.21	0.35	0.44	0.15	0.10	0.09
DR 1264	2.50	1.09	0.83	0.08	0.21	0.36	0.48	0.15	0.10	0.09
DR 1295	3.28	1.09	0.84	0.12	0.24	0.39	0.50	0.15	0.11	0.08
DR 1319	3.20	1.08	0.83	0.12	0.22	0.37	0.50	0.15	0.10	0.09
DR 1324	3.39	1.09	0.82	0.11	0.21	0.36	0.50	0.15	0.10	0.08
DR 1333	2.86	1.04	0.82	0.11	0.20	0.36	0.49	0.15	0.10	0.08
DR 1533	3.05	1.06	0.82	0.11	0.21	0.37	0.49	0.14	0.09	0.08
DR 1960	3.10	1.19	0.91	0.13	0.23	0.40	0.55	0.15	0.10	0.10

Table 2-3. Morphological characteristics used to identify *Anoplotermes* ss and *Anoplotermes* ng (measurements are in mm)

Morphological Character	*Anoplotermes* ss		*Anoplotermes* ng	
BL range & Mean ± SD	2.44 - 3.45	3.01±0.24	2.40 - 3.56	3.12±0.32
HL range & Mean ± SD	0.90 - 1.13	1.02±0.06	0.94 - 1.16	1.06±0.05
HW range & Mean ± SD	0.69 - 0.83	0.81±0.05	0.75 - 0.89	0.83±0.03
CH range & Mean ± SD	0.11 - 0.20	0.13±0.02	0.08 - 0.15	0.12±0.02
CL range & Mean ± SD	0.18 - 0.25	0.21±0.02	0.12 - 0.29	0.23±0.03
CW range & Mean ± SD	0.28 - 0.42	0.37±0.03	0.33 - 0.42	0.38±0.02
PW range & Mean ± SD	0.46 - 0.55	0.51±0.02	0.45 - 0.55	0.50±0.02
FLW range & Mean ± SD	0.13 - 0.16	0.15±0.01	0.14 - 0.17	0.15±0.01
MLW range & Mean ± SD	0.10 - 0.12	0.10±0.01	0.09 - 0.12	0.10±0.01
FLW range& Mean ± SD	0.08 - 0.10	0.09±0.01	0.08 - 0.10	0.09±0.01
Enteric Valve Morphology	Group-one or Group-two		Group-three or Group-four	
P1 morphology	Constricted before P3		Not constricted before P3	

CHAPTER 3
MOLECULAR ANALYSIS OF THE SOLDIERLESS TERMITES (ISOPTERA: TERMITIDAE: APICOTERMITINAE) IN THE DOMINICAN REPUBLIC BASED ON COII AND D3 GENE SEQUENCES AND ITS RELATIONSHIP WITH WORKER MORPHOLOGY.

Introduction

Currently, the only study that has resolved the phylogeny of the Apicotermitinae was that of Inward et al. (2007a). The study shows that the Apicotermitinae is a monophyletic clade and that there are multiple genera currently within the *Anoplotermes* but these genera are described as such due to the broad designation of the genus *Anoplotermes*. Many studies that focus on gene sequencing have been done on the economically important group, *Reticulitermes* sp. (Austin et al. 2002, Austin et al. 2005), mostly because *Reticulitermes* is the dominant structural pest in regions that have the resources to study genetic variation. This is changing as techniques become cheaper and more accessible.

Issues with DNA taxonomy and undescribed species

When using molecular approaches in taxonomy, sequence data is only valuable if it can be unambiguously associated with a higher taxon (Pons et al. 2006). Establishing this association is complicated due to intraspecific variation and the possible incongruence of gene and species histories that might prevent the easy recognition of these groups, in particular where only a single fragment of mtDNA is used (Lipscomb et al. 2003; Mallet and Willmott 2003; Pons et al. 2006; Will and Rubinoff 2004). Another major problem encountered in DNA taxonomy is gene polymorphism. This phenomenon is simply the presence of two or more alleles in a given population or nucleotide in a given position. Gene polymorphism is problematic for DNA taxonomy in that the analysis of genetic sequences looks at similarity percentage between segments and not at homology, so if two

different alleles are analyzed, they will appear to be more distantly related than they truly are. In current taxonomic DNA sequencing, species-level entities are known *a priori*, and their DNA signature is based on genotypes from a representative sample of well-identified individuals (Hajibabaei et al. 2006; Hebert et al. 2003; Meyer and Paulay 2005; Pons et al. 2006). A phylogenetic inference based on DNA-sequence variation can be erroneous even though the gene tree has been correctly resolved (Moore 1995). Such an error occurs when an ancestral species is polymorphic for the gene and sorting of lineages derived from alternate alleles (haplotypes in the case of mitochondrial genes) results in a gene tree that is incongruent with the species tree (Neigel and Avise 1986; Nei 1987; Pamilo and Nei 1988; Avise 1989; Avise and Ball 1990; Wu 1991; Hudson 1992; Moore 1995) or when a pseudogene is present. Pseudogenes resemble regular genes but are nonfunctional due, typically, to mutations that disrupt the coding regions. Species trees are determined by events that impact a population and the bifurcations within the tree result from these events, most commonly, geographic isolations. Gene trees are determined by DNA replication and marked by mutation (Moore 1995). Mitochondrial DNA (mtDNA) has been widely used in phylogenetic studies of recently evolved taxa, because it evolves rapidly and provides an abundance of genotypic characters, either by restriction fragment analysis or amplification by PCR and subsequent nucleotide sequencing (Moore 1995). However, its utility is limited by the phenomenon of lineage sorting, because the mitochondrial genes are inherited as a single linkage group and thus do not provide independent estimates of the species tree (Moore 1995). Nuclear genes can, however, be selected from distinct chromosomes, such that each gene tree provides an independent estimate of the species tree. It is because of these reasons that the idea was proposed by Pamilo and Nei (1988)

and Wu (1991) that a species tree is best inferred from several independent gene trees, preferably using both mtDNA and nuclear DNA or single and multiple copy nuclear genes.

Genes used in DNA taxonomy studies

There are many types of genes to choose from when inferring phylogenies. When dealing with mtDNA, there are ribosomal RNA (rRNA), transfer RNA (tRNA), and protein coding genes. These types of genes are also present in nuclear DNA along with other types that are not as useful in inferring phylogenies. The benefits of rRNA and protein coding genes will be discussed below due to their use in this study.

Ribosomal RNA genes are popular because of their universal occurrence, sequence and structural conservation, and abundance as multiple copy genes that evolve through a process known as concerted evolution where all copies basically incorporate any changes into all copies quickly (Simon et al. 1994). Mitochondrial rRNA genes are much simpler than nuclear rRNA genes (Simon et al. 1994). Nuclear rRNA genes typically have more subunits that can be separated by spacer regions. The gene and specific region used in this study for nuclear rRNA was the D3 expansion segment of the 28S gene. The 28S rRNA molecule of insects is composed of a series of highly conserved core elements and 13 expansion segments (Tautz et al. 1988). While the core regions of the large subunit (LSU) rRNA are highly conserved structurally across all domains of life (Clark et al. 1984), the expansion segments can vary greatly, even across recently diverged lineages (Gillespie 2005). The D3 expansion segment (~350 bp) is one of the more conserved expansion segments and is typically used as supporting evidence for analysis of the D2 expansion segment, also part of the 28S gene (Nunn et al. 1996). The use of the D3 segment of the 28S gene as a phylogenetic marker can be problematic in closely related taxa (Gillespie 2005).

In this study, where the level of divergence was unknown, it was hypothesized that D3 might be suitable for inferring the phylogeny of this group of termites with conserved morphology but potentially robust phylogenetic differences.

Carmen and Whiting (2007) sequenced and compared the entire mitochondrial genome of several species of *Reticulitermes* to identify regions of high interspecies variability and those with sufficient phylogenetic signal for assessing species or generic relationships. Their conclusion was that the protein coding genes did not vary much from each other in interspecies genetic distances or the proportion of variable sites. Simon et al. (1994) came to a similar conclusion, i.e. that one protein coding gene should be just as phlylogenetically useful as another per length of DNA sequenced. For this study, the cytochrome oxidase II (COII) gene was chosen empirically because it was the easiest to amplify and sequence in preliminary attempts with Apicotermitinae from the Dominican Republic. In addition, there were 87/685 (12.7%) variable sites and intraspecies distances of 3.07% for *R. flavipes* versus 1.31% for *R. virginicus* compared with 8.32% interspecies differences (Cameron and Whiting 2007). COII has been used by several researchers (Austin et al. 2002, Szalanski et al. 2003, Ye et al. 2007) for inferring termite molecular phylogenies, however it has been known to show incongruencies with species trees and is usually combined with other loci to infer a more accurate phylogeny. The 12S and 16S are common loci used with COII for inferring phylogenies.

The objective of this study was to infer a molecular phylogeny of the soldierless termites in the Dominican Republic by analyzing both mtDNA and nuclear gene sequences. The original goal was to combine both the phylogenies inferred from the gene sequences and overlay them with the morphological data from the previous chapter to see if the gene

trees correlate with the variation seen in the structure of the enteric valve, P1, and morphometric values.

Materials and Methods

Termite Samples and DNA Extraction

Termite samples were stored in 85% ethanol at room temperature and were prepared for DNA extraction by removing the abdomen of each specimen used. Gut contents have been shown to cause inhibition of PCR in some instances (Inward et al. 2007a). Total genomic DNA was extracted using DNeasy tissue kits (Qiagen, Valencia, CA). Five termites were used for each sample of DNA extracted to ensure there was sufficient tissue from which to extract DNA. Samples were dried and then ground up in 180 µl of buffer ATL in a 1.5 ml microcentrifuge tube. Next, 20 µl of proteinase K was added and samples were vortexed. Samples were then placed in a precision water bath for 90 minutes at 55˚ C and were removed and vortexed every 30 minutes. Samples were then vortexed for 15 seconds each, after which, 200 µl of buffer AL was added. Samples were then incubated at 70˚ C for 10 minutes. Next, 200 µl of 100% ethanol was added to each sample and vortexed. This mixture was transferred into a DNeasy Mini Spin Column in a 2 ml collection tube. Samples were centrifuged at 8,000 rpm for one minute. DNeasy Mini Spin Columns were transferred to a new 2 ml collection tube and 500 µl of buffer AW1 was added to the sample. Samples were centrifuged at 8,000 rpm for one minute. DNeasy Mini Spin Columns were transferred to a new 2 ml collection tube and 500 µl of buffer AW2 was added to the sample. Samples were centrifuged at 14,000 rpm for four minutes. DNeasy Mini Spin Columns were then transferred into a 1.5 ml microcentrifuge tube and 100 µl of buffer AE was added to the sample. Samples were centrifuged at 8,000 rpm for one minute.

Flow-through template was stored at -20° C before polymerase chain reaction (PCR) attempts.

A replicate was performed using a subset of samples from the original dataset and four samples from the Bahamas. Samples used for the second analysis were picked based on quality of DNA sequences produced in the first replicate and Bahamian samples were placed into the morpho groups that were determined for all of the samples from the Dominican Republic. The Bahamian samples used in this study have been described as *Anoplotermes inopinatus* and *Anoplotermes bahamensis*, where *A. bahamensis* is probably a new genus based on a lack of constriction in the P1 and differences in alate morphology (Scheffrahn et al. 2006). The four samples used from the Bahamas were BA 1916, BA 1291, BA 1136, and BA 2437 as recorded in the Uinversity of Florida database. When compared to samples from the Dominican Republic, *A. inopinatus* had the same worker morphology as *Anoplotermes* ss and *A. bahamensis* had the same worker morphology as *Anoplotermes* ng. Samples BA 1916 and BA 2437 were designated *Anoplotermes inopinatus* while BA 1291 and BA 1136 were designated *A. bahamensis.* All procedures were the same as the original dataset except that forceps and needles were heated with a flame to glowing in between each sample to reduce the possibility of cross contamination.

PCR and Purification

Primers used for mitochondrial COII were modified A-tLeu (Miura et al. 1998) for the forward direction, 5'-CAG ATA AGT GCA TTG GAT TT-3', and B-tLys (Miura et al. 1998) for the reverse direction, 5'-GTT TAA GAG ACC AGT ACT TG-3'. Primers for the D3 expansion region of 28S were D3a for the forward direction, 5'-GAC CCG TCT TGA AAC ACG GA-3', and D3b for the reverse direction, 5'-TGC GAA GGA ACC AGC TAC TA-3'. The 50 µl

PCR contained Amplitaq Gold, 10x reaction buffer, 0.1 mM of each dNTP, 0.1 mM of forward and reverse primers, and autoclaved water. The thermal cycling program was as follows: denaturation at 95˚C for five minutes, denaturation at 95˚C for one minute, annealing at 55˚C for one minute, and extension at 72˚C for two minutes. This was repeated 34 times, followed by 10 minutes of extension at 72˚C. The PCR products were electrophoresed on 1% Agarose Low EEO electrophoresis grade agar (Fisher Scientific, Pittsburg, PA) in a FB-SB-710 Electrophoresis Systems mini-Horizontal unit (BioRad Model 200/2.0). Samples that produced a band were cleaned using a Montage PCR kit (Millipore, Burlington, MA) by placing a Millipore filter into a 1.5 ml microcentrifuge tube. In each filter, 45 µl of PCR product and 360 µl of DNase free water were centrifuged at 3,000 rpm for 15 minutes. After being centrifuged, 17µl of elution buffer was added to the filter. The Millipore Filters were then turned upside down and transferred into a new microcentrifuge tube and centrifuged for two minutes at 3,500 rpm. The cleaned product was sent to ICBR labs (University of Florida, Gainesville, FL) for sequencing.

Sequence Analysis

Forward and reverse sequence data was imported into Sequencher 4.7 (Table 1-1) and noisy end sequences were trimmed using default settings separately for each gene locus. Using 100% minimum match percentage and 67% minimum overlap, all sequences were assembled. Only contigs that had forward and reverse sequences for each sample and contained samples from one enteric valve morpho group were kept. When a contig was produced from more than one sample, each sequence from all samples was observed next to a chromatograph. If a nucleotide in one sequence did not match the nucleotide present in all other sequences, the position was observed on the chromatograph to confirm whether

the mismatched nucleotide was real or artifactual. If the nucleotide that was present in all sequences was present at the mismatched location on the chromatograph, the mismatched nucleotide was replaced with the correct nucleotide. If the site was confirmed to be polymorphic, it was marked as such. All contigs were edited in this manner to assure that contigs contained identical sequences. When bonafide differences were confirmed in the chromatograph of the forward and reverse sequence, it was pulled out to make a separate contig. Once a consensus sequence was produced from a contig, all remaining sequences were assembled again using the same parameters. After all contigs and subsequent consensus sequences were produced at 100% minimum match percentage, all remaining sequences were assembled using 99% minimum match percentage and 67% minimum overlap. The same process was done by removing minority morpho groups from each contig. Enteric valve morphological groups were represented as G1, G2, G3, G4, and G3/G4, a contig that contained both G3 and G4 samples, for groups one, two, three, four, and three and four, respectively. Once all sequences that were of 90% quality or higher were placed in a contig and consensus sequence, they were exported in FASTA format. Sequence alignment, gap deletions, tree production, sequence variation analysis, and amino acid variation analysis were all performed using MEGA version 4.0 (Table 1-1). Trees were produced using the neighbor joining method (Saitou and Nei 1987) under a bootstrap test of phylogeny. All 36 samples used in this study sequenced but some were removed from molecular analysis, for both loci, due to poor sequence quality (<90%). In the analysis of the second dataset, sequences were first edited the same way as the first dataset in Sequencher 4.7. An analysis was then run using the SDSC (San Diego Supercomputer

Center) Biology Workbench. Trees were produced and all statistical analyses were performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2005).

Results and Discussion

D3 Sequence Variation

For the analysis of variation in the D3 expansion segment of 28S, seven contigs were produced in Sequencher 4.7 and imported into MEGA4.0. The first contig, G1_D3, contained sequence data for 14 samples (Table 3-1) that all had group-one morphology and identical sequences. Contig number two, G2_D3, contained sequence data for two samples (Table 3-1) that had group-two morphology and were identical to each other except at position 180 where the forward sequence of both samples had a thymine (T) and the reverse sequence of both samples had a guanine (G). The third contig, G2_DR1979, consisted of one sample (Table 3-1) that had group-two morphology. The fourth contig, G3/G4 (2)_D3, contained sequence data for five samples (Table 3-1) and contained both group three and group-four morphology. Despite the presence of two morpho groups, all five sequences were identical. The fifth contig, G3/G4 (3)_D3, contained sequence data for two samples (Table 3-1), one having group-3 morphology and one having group-four morphology. Both of these sequences were identical along the entire length of the sequence. In the sixth contig, G3/G4_D3, there were data for four samples (Table 3-1), two having group-three morphology and two having group-four morphology. All samples were identical along the entire length of the segment. The last contig, G3_DR 1784, consisted of one sample (Table 3-1) that had group-three morphology.

After being aligned in MEGA4.0, the uneven priming ends of each contig were trimmed so that each fragment was the same length, 375 bp. Sequence variation among

contigs (Figure 3-1) occurred at position 177, 222, 277, 327, and 328. At position 177, all contigs had a G except for G2_D3 and G2_DR1979. G2_D3 had a K because, as noted earlier, forward sequences had a T and reverse sequences had a G and there was too much ambiguity in chromatographs to determine which base was correct. The other variation in G2_DR1979 was the presence of a T instead of G. Because both variations at this position occured in contigs that possessed samples with a group-two morphology, it was unclear what G2_D3 had at this position because G2_DR1979 was the only sample that varied at position 222 by having a T instead of G, whereas G2_D3 had a G like all other contigs. The variation at position 277 was present in G3_DR 1784 and it contained a G instead of an A, a transition. At positions 327 and 328, contigs G3/G4 (2)_D3, G3/G4(3)_D3, and G3_DR 1784 had an insertion of two T's, whereas the other four contigs had a possible deletion gap. When looking at the amino acid sequence of each contig, the variation at position 59 and 177 in the nucleotide sequence, showed that even though G2_DR1979 varied in having a T where all others had a G, a transversion, it still coded for leucine (L) and even though G2_D3 still remained ambiguous, it seemed plausible that L was also coded here and the presence of a G or T was irrelevant because both coded for L. At position 93 in the amino acid sequence, there was an alanine (A) present instead of a threonine (T). All other variations in the nucleotide sequences did not produce any differences in the amino acid sequence except at positions 327 and 328 where the gap shows a leucine (L) and a cysteine (C) as an insertion in G3/G4(2)_D3, G3/G4(3)_D3, and G3_DR1784 and a deletion for all other consensus sequences.

No tree was included for D3, only actual DNA sequences because bootstrap tests of phylogeny for minimum evolution trees showed a bootstrap value of zero at each node.

For the neighbor-joining method, bootstrap values ranged from 6 to 17 and was thus regarded as unreliable in inferring relationships. Based on these results, D3 was too conserved to provide a clear picture of phylogeny for this group. This is consistent with the reports from a number of limited studies that have been done on termites using nuclear ribosomal genes (Jenkins et al. 2001, Uva et al. 2004).

COII Sequence Variation for first replicate

For the analysis of the COII gene, 14 contigs (Table 3-2) were produced in Sequencher 4.7 from 33 samples and imported into MEGA4 as consensus sequences. All contigs for COII sequences were produced the same way as was done for D3. The majority of contigs for the COII gene consisted of single samples; DR 1173(G2)_COII, DR 1260(G4)_COII, DR 1318(G3)_COII, DR 1354(G3)_COII, DR 1355(G1)_COII, DR 1784(G3)_COII, DR 1790(G3)_COII, DR 1853(G3)_COII, DR 1979(G2)_COII, and DR 971(G2)_COII. The first contig that contained multiple sequences was G1 (1)_COII which had 14 sequences. All of these samples had group-one morphology and were identical across the aligned sequence. The second contig contained two identical sequences that had group-one morphology. The next contig, G3/G4 (2)_COII, contained seven identical sequences from isolates that had either group three and group-four morphology. The final contig, G3/G4_COII, contained two samples, one with group-three morphology and one with group-four morphology. Both sequences were identical across the entire length of the sequence. Consensus sequences of each of these contigs were then uploaded into MEGA4 for analysis.

After being aligned, priming ends were trimmed off to produce fragment lengths of 578 base pairs long, which represented 84% of the full COII gene. The COII fragments

analyzed in this study were shorter than that of Cameron and Whiting (2007) due to varying quality in each sequence. The minimum amount of sequence information was trimmed off each end so that all sequences would be of equal length, making alignment and analysis easier. The variation among contigs in the analysis of COII for the first replicate (Figure 3-2) was much higher than that of D3. The majority of variation occurred in contigs DR 1784(G3)_COII, DR 1318(G3)_COII, DR 1354(G3)_COII, DR 1790(G3)_COII, and DR 1853(G3)_COII. When the amino acid sequences were observed, much of the variation was located at the third position codon and did not affect the resulting amino acid sequence. After all COII consensus sequences were aligned, out-group sequences were added from GenBank. Two sequences were used, one was from an unidentified *Anoplotermes* species that was sequenced by Inward et al. (2007a) to compare *Anoplotermes* ss and *Anoplotermes* ng from the Dominican Republic to another *Anoplotermes* species from French Guiana and the other was a *Microcerotermes* sp. (Legendre et al. 2008). The sequence from *Microcerotermes* was incorporated to show the relationship and level of variation between *Anoplotermes* and a closely related group, in this case, the subfamily Termitinae. As expected, the *Anoplotermes* from the study formed a monophyletic clade with the *Anoplotermes* sequence from GeneBank and the *Microcerotermes* appearing as sister and outgroup, respectively (Figure 3-3; Figure 3-4).

Patterns between genes and morpho groups in first replicate

The only locations in the D3 nucleotide sequence that varied and produced a difference in amino acid composition were 277, 327, and 328. The G present at position 277 translated for alanine in the G3_DR1784_D3 contig and there was a leucine and cysteine in positions 327 and 328, respectively, in contigs G3/G4(2)_D3, G3/G4(3)_D3, and

G3_DR1784_D3. Despite the lack of variability in the D3 region, the most variable sites were at 327 and 328 and the presence of two thymines occurred in contigs that had samples with group three or G3/G4 morphology. However, contig G3/G4 (1) lacked the presence of these nucleotides and was identical to samples with group-one morphology. Aside from these differences, there was no evidence from the D3 expansion segment supporting the hypothesis that any of these samples were different enough to be classified as different taxa. This region of DNA is known to be conserved and inferring phylogenies of cryptic species or closely related populations should be based on a more rapidly evolving gene. Thus, the justification for using COII in this study.

As expected, there were many more parsimony informative sites in the COII sequences than there were in D3. Cameron and Whiting (2007) sequenced the entire mitochondrial genome and observed sequence variation within the genus *Reticulitermes.* The same primers used for the COII gene in *Reticulitermes* (Forward: A-tLeu and Reverse: B-tLys) were used for *Anoplotermes* ss and *Anoplotermes* ng in this study. The amount of intraspecific genetic variation for COII for *R. flavipes* and *R. viriginicus* was 3.07% and 1.31%, respectively. The variation for intraspecific distance in *R. flavipes* also included sequence variation from *R. santonensis* because they are now considered to be synonymous. The variation for interspecific distance between *R. flavipes* and *R. viriginicus* was observed at 8.32%. These data are useful in providing a framework from which to compare both intra and interspecific variation of other groups of termites for COII. The average genetic variation was calculated for all consensus sequences from the Dominican Republic for the first replicate and showed 4.5% average genetic distance. Distance matrices are provided for all consensus sequences and for each morpho group (Figure 3-4).

When all consensus sequences of a corresponding morpho group were grouped together, the group that contained samples with only group-three morphology was the most genetically distant, having 5.6% difference from samples with group-two morphology, 5.4% difference from samples with group-three and group-four morphology mixed, and 5.2% difference from samples with group-one morphology. As was expected, the average genetic distance between the *Anoplotermes* outgroup and the study samples (7.5%) was much higher than the variation between Dominican Republic samples. The average genetic distance between the *Microcerotermes* outgroup and the study samples was even greater than that of the *Anoplotermes* outgroup and the study samples, as expected (≈ 11%).

Intragroup variation, intergroup variation, and interspecies variation for the first replicate of COII sequences from the Dominican Republic were calculated and compared. Intragroup variation was calculated as the average genetic distance within a particular enteric valve morphological group, i.e. the average genetic distance between samples with G1 (group-one) morphology. Intergroup variation was calculated as the average genetic distance between different enteric valve morphological groups, i.e. the average genetic distance between consensus sequences with G1 enteric valve morphology and consensus sequences with G3 enteric valves morphology. Interspecies variation was calculated as the distance between the outgroups and average genetic distance between all consensus sequences from the study. The intragroup variations were as follows, G1: 3.2%, G2: 5.2%, G3: 2.4%, G3/G4: 5.2%, and G4 only had one sample, so no intragroup variation was possible. The highest amount of intergroup distance was between G3 and both G2 and G4, 5.1%. Curiously, the same amount of distance occurs between G3/G4 and G3. The lowest amount of genetic distance occurred between G4 and G2, 0.0%. The next lowest distance

between groups was that between G1 and both G2 and G4, 1.5% (Figure 3-4). When the sequence data here was applied to the two morphological groups, *Anoplotermes* ss and *Anoplotermes* ng, there was 4.1% genetic variability within the *Anoplotermes* ss and 4.2% genetic variability within the *Anoplotermes* ng. Between the *Anoplotermes* ss and *Anoplotermes* ng, there was 5.1% genetic variability. The distance between these two groups represented the distance between G1 and G2 combined and G3, G4, and G3/G4 combined. This showed that the intragroup genetic variation for *Anoplotermes* ss was almost the same as the COII intragroup variation for *Anoplotermes* ng. Also, the intergroup variation between *Anoplotermes* ss and *Anoplotermes* ng was 5.1%, about 1% higher than that of the intragroup variation. The similar levels of intra and intergroup variation here suggests that these groups are closely related and cannot be separated by the COII locus.

When compared to the findings by Cameron and Whiting (2007), the intragroup variation in the first dataset for the morphological groupings of *Anoplotermes* ss, 4.1%, and *Anoplotermes* ng, 4.2%, was higher than the intraspecies variation for *R. viriginicus*, 1.31% and *R. flavipes*, 3.07%. The intergroup variation between *Anoplotermes* ss and *Anoplotermes* ng at 5.1%, was much lower than the interspecies variation between *R. flavipes* and *R. virginicus,* 8.32%. This analysis of variation was done by analyzing the molecular data for the morphological groupings as different species, not the genotypes presented on the tree (data not shown).

COII sequence variation for second replicate

Twelve samples were used in the second replicate dataset. Two samples represented each enteric valve morphological group from the Dominican Republic and two samples each represented *Anoplotermes* ss and *Anoplotermes* ng from the Bahamas.

Samples DR 1223 and DR 1256 were used for G1 enteric valve morphology, DR 1173 and DR 971 for G2 enteric valve morphology, DR 1790 and DR 1288 for G3 enteric valve morphology, and DR 1522 and DR 1319 for G4 enteric valve morphology. For the Bahamas samples, BA 1916 and BA 2437, *A. inopinatus*, were used to represent *Anoplotermes* ss (= group 1 & 2) and samples BA 1291 and BA 1136, *A. bahamensis*, were used to represent *Anoplotermes* ng (= group 3 & 4).

In the second dataset, 680 base pairs were used for analysis, about 99% of the full COII gene as recorded by Cameron and Whiting (2007), 53/680 were parsimony informative. Sequence data from the second experiment was compared to the orginal sequence data and all redone samples had identical sequences. Variation occurred in DR 1288 G3, DR 1319 G4, DR 1790 G3, and DR 971 G2 (Figure 3-5). The variation that was present produced four distinct genotypes where DR 1319 G4 and DR 1288 G3 represented one clade, DR 1790 G3 represented another lineage, DR 971 G2 represented another lineage, and the remaining samples (DR 1173 G2, DR 1223 G1, DR 1256 G1, DR 1533 G4, BA 1916 G1, BA 2437 G2, BA 1291 G3, and BA 1136 G3) were identical with each other and represented another lineage (Figure 3-6). The distance matrix for variation among lineages showed that between each clade there was from 7.4% variation to 8.3% variation (Figure 3-7). A BLAST search was performed on each sequence and all showed that the closest match as an unidentified *Anoplotermes* sp. from Inward (2007) that was collected from French Guiana with about 10% variation from all samples taken from the Dominican Republic and the Bahamas. This was the same result as the BLAST search comparison in the first dataset. An individual contig and consensus was produced for each sample using 100% minimum match percentage and 100% percent overlap in Sequencher 4.7.

Patterns between genes and morphogroups in second replicate

In the second dataset, four genotypes were resolved by the tree topology. Samples from the first dataset that were used to generate new sequences in the second replicate, exhibited the same branching pattern as the second dataset. Since there were fewer samples in the second dataset, genotypes were easier to discern.

The morphological groups that were assigned to both the Dominican Republic samples and the Bahamian samples did not match the tree topology, as was seen in the first replicate. The first genotype contained all morphological types and contained samples from the Dominican Republic and the Bahamas. This genotype contained DR 1173 G2, DR 1223 G1, DR 1256 G1, DR 1533 G4, and all of the *A inopinatus* and *A. bahamensis* samples. The second and third genotypes contained only one sample each, DR 971 G2 and DR 1790 G3, respectively. The fourth genotype contained samples DR 1319 G4 and DR 1288 G3. The distances between each genotype are presented in Figure 3-7. The analysis of intragroup variation revealed that in the first clade, all samples were identical with each other across the entire sequence. Clades two and three consisted of only single sample so variation withing these clades could not be determined and clade four had two samples but also had 0% variation.

In the second dataset, where intragroup variation was calculated on the genotypes represented in the tree and not based on the morphological groupings, it showed 0% variation for the two clades that had more than one sample, clade one and clade four. Between genotypes, there was about 7.6% variation in the second dataset (Figure 3-7). The degree of intergroup variation in the second dataset is closer to the interspecies variation, 8.32%, between *R. flavipes* and *R. virginicus* as reported by Cameron and Whiting

(2007). The genetic distance for *Reticulitermes* by Cameron and Whiting (2007) was the only framework to compare the genetic distance of the *Anoplotermes* ss and *Anoplotermes* ng in the Dominican Republic. This framework does not dictate that the same relative genetic distance is needed within the Apicotermitinae of the Dominican Republic to merit species status of these groups but should provide a good starting point to gauge the relationship between samples of Apicotermitinae.

Based on the data from both replicates, there is no relationship between morphology, gut structure and enteric valve structure, and genotype based on sequence data from the COII mitochondrial gene, leading to the rejection of the original hypothesis that the morphological variation observed would show the same groupings with regard to genotype. However, this does not prove that these characters are unreliable in distinguishing species. Phylogeny should not be based solely on sequence data from one locus but from multiple loci that include both mitochondrial genes and nuclear genes. Once the trees for each locus are produced, they can sometimes be combined to show the most accurate phylogeny based on DNA sequence variation.

Incongruency with COII and morphology in previous studies

In a study by Maekawa and Matsumoto (2000) the phylogeny of cockroaches was determined using COII sequence variation. Their data showed different relationships among cockroach families than derived from phylogeny based on morphological work by McKittrick (1964). The phylogeny inferred in their study was nearly identical to that inferred from other mitochondrial genes, 12S and 16S, for cockroaches by Kambhampti (1995) but was drastically different from the phylogeny inferred by the 12S gene by Kambhampti (1996) for the same cockroach families. These incongruent phylogenies

probably are a result of low bootstrap support (<95%) at certain internodes in the gene trees. The differences between the work of Maekawa and Matsumoto (2000) and Kambhampti (1996) could indicate that mitochondrial genes are not as reliable at inferring phylogenies, for both cockroaches and termites, as once suspected. Since cockroaches and termites are so closesly related (Inward et al. 2007), it is plausible that there might be inconsistencies between trees derived from 12S and COII in the soldierless termites from this study that are similar to those observed between Maekawa and Matsumoto (2000) and Kambhampti (1996). It has been noted by Szalanski and Scheffrahn (unpublished data) that in *Incisitermes* species 18S only seven variable sites between *Incisitermes* and *Mastotermes*. The 16S region was shown to have around 100 mucleotides difference, thus indicating that 16S is a much more useful gene for termites than is 18S and based on this study, more useful than the D3 segment of 28S and COII genes.

Sources of incongruency

Multiple possibilities exist that could explain the incongruency between the selected morphological characters and genotypes. It must also be noted that it is more difficult to explain the variation of different characters within and between species when the true function of a given structure is not known. Given the location of the enteric valve and the shape of the hindgut, it is almost certain that these characters play a role in food processing. That being said, the first hypothesis is that COII is an unreliable locus to infer phylogenetic relationships in this group. As previously described, COII inferred trees can be incongruent with well-accepted phylogenies based on morphology and other loci. This is not to say that COII is not valuable but that it may have to be combined with inferences from other loci to produce an accurate phylogeny. The second hypothesis is that each clade

represented on the tree is a polymorphic species that contains all or most of the morphological groupings. The first clade contained each morphological group, while the remaining clades only had one or two morphologies present. A larger sample size is needed from each of these clades to see if they each contain all four morphological types. When alate morphology was analyzed by Scheffrahn et al. (2006) the *A. inopinatus* alates were significantly smaller and had a concavity behind the fontanelle whereas the *Anoplotermes* ng were larger and had an evenly rounded head capsule behind the fontanelle. Scheffrahn et al. (2006) also showed that the *A. inopinatus* worker mandibles were significantly smaller than those of *A. bahamenesis.* These morphological characteristics were present in the Bahamian samples analyzed in the second dataset. Yet, the sequences from COII were identical.

Another potential possibility for the incongruence is that of convergence. Even though there were no patterns in distribution for the morphogroups, there could be minute environmental conditions, such as soil type, pH, moisture content, food availability, and exposure to light that would cause the convergence of form in the enteric valve and gut shape. To determine if convergence was the source of incongruencey between COII phylogeny and morphological groupings, all of the above-mentioned variables would have to be recorded relative to termite genotype and morphotype and then statistically analyzed to determine if there were any patterns. Another possible explanation is that of ancestral polymorphism where, in this case, a common ancestor possessed multiple forms of the COII gene and as speciation occurred, different alleles were inherited by different species giving the appearance that some are more closely related to individuals with a similar allele than they actually are. This question could be answered by sequencing nuclear loci that are not

part of a single, non-recombining linkage group. The most unexpected part of the second dataset is that samples from the Bahamas had identical COII sequences to each other and some of those from the Dominican Republic and that some Dominican samples were more closely related to Bahamian samples than they were to other Dominican samples. Due to their low dispersal distances, it is unlikely that they have been able to migrate from island to island and due to their widespread distribution in the region it seems likely that COII is not useful in inferring phylogenies for this group.

To determine if these termites are polymorphic species, origins of founder populations and what directions they moved relative to the identical sequences in the Bahamas and the Dominican Republic, it will be necessary to sequence samples for multiple loci from all surrounding islands in the Carribbean basin and from localities on the mainland. This method for determining point introductions was used by Su et al. (2006) in the identification of Chilean *Reticulitermes* species, which unlike *Anoplotermes* sp. are much more likely to be introduced and transported by humans due to their closer association with humans and their activities.

Another possible explanation is that of switch genes. This could cause different colonies of the same species to activate a certain gene or group of genes when exposed to certain environmental conditions that causes the production of gut morphology seen in *Anoplotermes* ss or, conversely, that which is seen in *Anoplotermes* ng. To determine if this was the cause it would also require data collection of a variety of environmental factors that impact a given colony and comparing varying environmental conditions to varying morphology to elucidate a pattern.

Other potential explanations as to the incongruency between COII sequence variation and morphology could be due to human error in record keeping or contamination in DNA extraction. Both, however, were highly unlikely due strenuous record keeping in the field and precautions taken in record keeping and in DNA extractions.

Conclusion

Based on the sequence variation of the D3 expansion segment and COII locus, analyses of molecular data showed that there was no resolution to the relationship of the Apicotermitinae of the Dominican Republic with regards to the morphological patterns that were observed. The morphological differences observed allowed for the categorization of all samples into two major groups, *Anoplotermes* ss and *Anoplotermes* ng based on a constriction of the P1 in *Anoplotermes* ss and a lack of constriction in *Anoplotermes* ng and by *Anoplotermes* ss having G1 or G2 enteric valve morphology and *Anoplotermes* ng having G3 or G4 enteric valve morphology. Within the *Anoplotermes* ss there were two morpho types, G1 and G2 and within the *Anoplotermes* ng there were two morpho types, G3 and G4. The group G1 was classified by having a head width range of 0.80 to 1.03 mm and by having G1 enteric valve morphology. The group G2 was classified by having a head width range of 0.69 to 0.79 mm and by having G2 enteric valve morphology. The group G3 was classified by having G3 enteric valve morphology and a range of upturned scales of 5 to 19. The group G4 was classified as having G4 enteric valve morphology and a range of upturned scales of 20 to 33. Samples that were analyzed from the Bahamas had morphological characters that were consistent with the morphological groupings that were used for samples from the Dominican Republic. There was also no resolution of morphometric data on the COII gene tree.

These four morpho types and their corresponding morpho groups, *Anoplotermes* ss and *Anoplotermes* ng, were not resolved on the neighbor-joining tree that was produced from COII sequence data in the first or second replicates. Since all four morphological groups are represented in one genotype and have identical sequences, it appears that the morphological characters used in this study are not reliable in indentifying species, especially since samples from the Bahamas have identical sequences to samples in the Dominican Republic and that some samples from the Dominican Republic appear more distantly related to each other than to samples from the Bahamas. These results, however, are not enough to negate the use of these characters in identifying species within the Apicotermitinae, and is more likely that COII is not reliable in resolving phylogeny in this group due to the consistency of characters. In a study by Maeakawa and Matsumoto (2000), the molecular phylogeny of cockroaches based on mitochondrial COII gene sequences did not completely match the currently accepted morphological phylogeny proposed by McKittrick (1964). Sequencing multiple other loci from nuclear DNA combined with the mitochondrial gene sequences of COII would provide the most accurate phylogeny of this group. Based on previous studies using COII and common incongruencies, we are reluctant to determine the relationship of soldierless termites in the Dominican Republic based only on this locus. Along with a more comprehensive look at multiple loci, with 16S being a good starting place, a wider sample size is needed along with an analysis of morphological characters in both alates and workers to have a clearer understanding of what morphological characters are best for identifying species.

Thus far, there is not enough data to make an accurate prediction as to the incongrunecy of COII sequence data and morphological characters in the Apicotermitinae

of the Dominican Republic. If multiple other loci of both mitochondrial and nuclear genes are sequenced and are identical and produce the same tree topology as the COII, it would indicate that these morphological characters are not indicative of species level variation. Multiple hypotheses were proposed to explain this incongruence between genotype and distinct morphological groups; these included unreliability of COII as the sole indicator of phylogeny, contamination during DNA extraction, polymorphic species, convergence, ancestral polymorphism, or improper record keeping. Polymorphisms could be solved by a more rigorous collecting effort that included alates and workers in one sample, sequencing of other genetic loci, and the collection of specimens from nearby geographical localities. If the same incongruencies are observed between COII phylogenies and other loci that have been seen previously (Maekawa and Matsumoto 2000; Inward et al. 2007), this would indicate that COII is not a reliable locus in determining phylogenies for these particular taxa. Much more work is needed on this group, from all regions of the neotropics, if a clear understanding of this group's phylogeny is to occur.

Based on these results, it is apparent that there are two different genera, *Anoplotermes* ss and *Anoplotermes* ng. Between these genera, there are two different species and potentially four different species. The separation of genera is due to a P1 constriction and lack of upturned scales on enteric pads for the *Anoplotermes* ss and an unconstructed P1 with enteric pads that have upturned scales for *Anoplotermes* ng. Within these genera, there are nonoverlapping characteristics that potentially constitute for the description of two species within each genus. More work is needed on other morphological characters, such as worker mandibles and legs, to say with confidence that there are four species of soliderless termites in the Dominican Republic.

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G1_D3       CGAAGGAACCAGCTACTAGATGGTTCGATTAGTCTTTCGCCCCTATACCTAGCTCTGACGATCGATTTGCACGTCAGA  [ 78]
G2_D3       ..................................................................................  [ 78]
G3/G4       ..................................................................................  [ 78]
G2_DR1979   ..................................................................................  [ 78]
G3/G4(2)    ..................................................................................  [ 78]
G3/G4(3)    ..................................................................................  [ 78]
G3_DR_1784  ..................................................................................  [ 78]

G1_D3       ATCGCTGCGGACCTCCATCAGGGTTTCCCCTGACTTCGTCCTGGCCGGGCATAGTTCACCATCTTTCGGGTCCCAACG  [156]
G2_D3       ..................................................................................  [156]
G3/G4       ..................................................................................  [156]
G2_DR1979   ..................................................................................  [156]
G3/G4(2)    ..................................................................................  [156]
G3/G4(3)    ..................................................................................  [156]
G3_DR_1784  ..................................................................................  [156]

G1_D3       TGTGCGCTCAGGGTGCGCCTGCGGCCGAAGCCGAGGCGCCCCGGGAGTGCTGACCCGCCGCACCGGTCGGGTACGACG  [234]
G2_D3       ....................K.........................................................  [234]
G3/G4       ..................................................................................  [234]
G2_DR1979   ....................T.........................................T...........  [234]
G3/G4(2)    ..................................................................................  [234]
G3/G4(3)    ..................................................................................  [234]
G3_DR_1784  ..................................................................................  [234]

G1_D3       GGCCGTCTTCCCTTCACTAGGGCTCGCAAGCGGCAATAAATTACCGTCGATGCGAGCCCCCAACGCTTTCACTGCGCC  [312]
G2_D3       ..................................................................................  [312]
G3/G4       ..................................................................................  [312]
G2_DR1979   ..................................................................................  [312]
G3/G4(2)    ..................................................................................  [312]
G3/G4(3)    ..................................................................................  [312]
G3_DR_1784  ........................................G.........................................  [312]

G1_D3       TCTGGGTTTAGTCT--GTCCCAGTGACTCGCGCACATGTTAGACTCCTTGGTCCGTGTTTCAA  [375]
G2_D3       ..............--............................................  [375]
G3/G4       ..............--............................................  [375]
G2_DR1979   ..............--............................................  [375]
G3/G4(2)    ..............TT............................................  [375]
G3/G4(3)    ..............TT............................................  [375]
G3_DR_1784  ..............TT............................................  [375]
```

Figure 3-1. Sequence data for the D3 extension segment of the 28S nuclear rRNA gene.

```
DR_1173(G2)_COII      GAACAATTAATCTACTTCCACGACCACGCACTAATAATTATATTGATAATTATTACAACCGTATTCTATACAATAATT  [ 78]
DR_1260(G4)_COII      ..............................................................................  [ 78]
DR_1318(G3)_COII      ....................T.................C...C.A..............T........C.........  [ 78]
DR_1354(G3)_COII      ....................T.................C...C.A..............T........C.........  [ 78]
DR_1355(G1)_COII      ........................T.............................T.....T...........  [ 78]
DR_1784(G3)_COII      ....................T..T..T...........C...C...............T.....T...........  [ 78]
DR_1790(G3)_COII      ..G................T................C...C.A..............T................  [ 78]
DR_1853(G3)_COII      ..G................-T................C...C.A..............T................  [ 78]
DR_1979(G2)_COII      .......................T..T..............C.A..............T.....T...........  [ 78]
DR_971(G2)_COII       ...........T...........T....................A..............T.....T...........  [ 78]
G1(2)_COII            ..............................................................................  [ 78]
G1_COII               ..............................................................................  [ 78]
G3/G4(2)_COII         ...........T..............T...............C.A..............T.....T...........  [ 78]
G3/G4_COII            ..............................................................................  [ 78]
A._cf._K_COII         ...........T........T........CT...........C.A.........................T........  [ 78]
M._sp._IS178_COII     .....G.......TT.....T.....T.................A...........G..A.....T..C.........  [ 78]

DR_1173(G2)_COII      AGAATTATCCAAAACAAACAAACAAGACGATTCATACTAGAAGGACAAATAATCGAAACCGTTTGAACAATTGCGCCA  [156]
DR_1260(G4)_COII      ..............................................................................  [156]
DR_1318(G3)_COII      ..............T................T...........G...............................  [156]
DR_1354(G3)_COII      ..............T................T...........G...............................  [156]
DR_1355(G1)_COII      ...................................G...............................G.....T...  [156]
DR_1784(G3)_COII      ...............................T...........G..............T...............A...  [156]
DR_1790(G3)_COII      ..............T................T...........G...............................  [156]
DR_1853(G3)_COII      ..............T................T...........G...............................  [156]
DR_1979(G2)_COII      ..............................................T........G.....C...  [156]
DR_971(G2)_COII       .....C...........G..............T...............................G.....T...  [156]
G1(2)_COII            ..............................................................................  [156]
G1_COII               ..............................................................................  [156]
G3/G4(2)_COII         .....C..T.....................................................................C......  [156]
G3/G4_COII            ..............................................................................  [156]
A._cf._K_COII         ........T.....T........C........T....................T.....A..............T...  [156]
M._sp._IS178_COII     ..............T........C..C........C.................T......A.............A..C  [156]

DR_1173(G2)_COII      GCAGTCATCCTAGTATTCATTGCAATGCCATCGCTACGACTACTATACTTAATAGACGAAATCCACAATCCAGTATTA  [234]
DR_1260(G4)_COII      ..............................................................................  [234]
DR_1318(G3)_COII      ...A....T.................A.....A...............C..............................  [234]
DR_1354(G3)_COII      ...A....T.................A.....A...............C..............................  [234]
DR_1355(G1)_COII      ...A....T.................A.....A.......................T........T..C......C..  [234]
DR_1784(G3)_COII      .....T..T..C..............A.....A.........T....T.................T..C.........  [234]
DR_1790(G3)_COII      ...A....T.................A.....AT..............C..............................  [234]
DR_1853(G3)_COII      ...A....T.................A.....AT..............C..............................  [234]
DR_1979(G2)_COII      ...A......................A.....A......T.G.....T.................T.........C..  [234]
DR_971(G2)_COII       ...A....T.................A...............T....T.................T..C......C..  [234]
G1(2)_COII            ..............................................................................  [234]
G1_COII               ..............................................................................  [234]
G3/G4(2)_COII         ........T.................A.....A.....................................C..C...C..  [234]
G3/G4_COII            ..............................................................................  [234]
A._cf._K_COII         ...A......................A.....A...............C......................C.....G  [234]
M._sp._IS178_COII     ...A......................C.....C.....G........C.............T............A..  [234]

DR_1173(G2)_COII      ACACTAAAAACAGTGGGACACCAATGATACTGAAGCTATGAATACTCAGACTTCACTAAGCTTGAATTTGACTCATAC  [312]
DR_1260(G4)_COII      ..............................................................................  [312]
DR_1318(G3)_COII      ...........G..A..............T.....T...............T.....A...........C..T......  [312]
DR_1354(G3)_COII      ...........G..A..............T.....T...............T.....A...........C..T......  [312]
DR_1355(G1)_COII      ...............................T...........G.......TA...........C..T......  [312]
DR_1784(G3)_COII      .....G........T..............T.....T.....................A........G.....T......  [312]
DR_1790(G3)_COII      ..............A..............T.....T...............T.....A...........C..T......  [312]
DR_1853(G3)_COII      ..............A..............T.....T...............T.....A...........C..T......  [312]
DR_1979(G2)_COII      ...........T......................T.....G..............A......................  [312]
DR_971(G2)_COII       ..C...........T..............T.....T...............T.....A......................  [312]
G1(2)_COII            ..............................................................................  [312]
G1_COII               ..............................................................................  [312]
G3/G4(2)_COII         .....T...........................T..............T.....A..A........C.........  [312]
G3/G4_COII            ..............................................................................  [312]
A._cf._K_COII         ..CT..........T..G..T.................................A.................G..T  [312]
M._sp._IS178_COII     ..C.....G..G..T.....T................C.....T.....T..T..A...........C.........  [312]
```

Figure 3-2. COII sequence data for first replicate.

```
DR_1173(G2)_COII      ATGGTACAACAAGAAGACATACAAATAGACACATTCCGACTACTAGACACAGACAACCGAGTAGTATTACCAATAAAT  [390]
DR_1260(G4)_COII      ........................................................................  [390]
DR_1318(G3)_COII      ..A..............T..G....C.....................T.....T..T..G..G..............C  [390]
DR_1354(G3)_COII      ..A..............T..G....C.....................T.....T..T..G..G..............C  [390]
DR_1355(G1)_COII      ..A.....G........T.....G.C.....................T.....T..T...................C  [390]
DR_1784(G3)_COII      ................T.......C...T.........................T..G..................  [390]
DR_1790(G3)_COII      ..A..............T..G....C.....................T.....T..T.....G..............C  [390]
DR_1853(G3)_COII      ..A..............T..G....C.....................T.....T..T.....G..............C  [390]
DR_1979(G2)_COII      ................T.....G.......................T.....T..T.........C....C......  [390]
DR_971(G2)_COII       ....................G..G.C..................C..T........T.....G...............  [390]
G1(2)_COII            ........................................................................  [390]
G1_COII               ........................................................................  [390]
G3/G4(2)_COII         .....G...........T.......C.............T.......T.....T..T.....................  [390]
G3/G4_COII            ........................................................................  [390]
A._cf._K_COII         ..A...............C....GGCG.....G........GT............T.....................  [390]
M._sp._IS178_COII     ..A...............CA..T...CA...........T.......T........T..T..T...C...........  [390]

DR_1173(G2)_COII      TCACCCATTCGAATAATCGTAACAGCAGCAGACGTACTACACTCATGAACAGTACCAAGACTCGGGGTAAAGTCAGAT  [468]
DR_1260(G4)_COII      ........................................................................  [468]
DR_1318(G3)_COII      ....................................TT....T.....................T...........T...  [468]
DR_1354(G3)_COII      ....................................TT....T.....................T...........T...  [468]
DR_1355(G1)_COII      ..............................................G........G........T..A............  [468]
DR_1784(G3)_COII      ................................T...T................G........T...........T...  [468]
DR_1790(G3)_COII      ....................................TT....T.....................T..A........C...  [468]
DR_1853(G3)_COII      ....................................TT....T.....................T..A........C...  [468]
DR_1979(G2)_COII      .....A..C...C.......................................................A..A........T...  [468]
DR_971(G2)_COII       ............T.........................T...............G........T..A........T...  [468]
G1(2)_COII            ........................................................................  [468]
G1_COII               ........................................................................  [468]
G3/G4(2)_COII         ................T..............T..............................T...........T...  [468]
G3/G4_COII            ........................................................................  [468]
A._cf._K_COII         ..C..A..C........T..............T...T.......T..................T..A............  [468]
M._sp._IS178_COII     .....A.....G.....T..................T.......G...........G.....T..A.....AA.....  [468]

DR_1173(G2)_COII      GCTACACCAGGACGCCTGAACCAAGTAAGATTCTCAATTAACCGACCAGGAATCCTATATGGACAATGCTCAGAAATC  [546]
DR_1260(G4)_COII      ........................................................................  [546]
DR_1318(G3)_COII      ...........G..A..A.......................T.....................................  [546]
DR_1354(G3)_COII      ...........G..A..A........G..............T.....................................  [546]
DR_1355(G1)_COII      ..C...........A..A.......................T.....................................  [546]
DR_1784(G3)_COII      ..C........G..A..A..............T........T.....................................  [546]
DR_1790(G3)_COII      ..............A..A.......................T.....................................  [546]
DR_1853(G3)_COII      ..............A..A.......................T.....................................  [546]
DR_1979(G2)_COII      .GA...........A..A.......................T.....................................  [546]
DR_971(G2)_COII       .....C........A..A.......................T.....................................T  [546]
G1(2)_COII            ........................................................................  [546]
G1_COII               ........................................................................  [546]
G3/G4(2)_COII         ..............G..A.....G................T.........................T.........  [546]
G3/G4_COII            ........................................................................  [546]
A._cf._K_COII         ..C...........A..A.......................T.....................................  [546]
M._sp._IS178_COII     ..A.....T.....A..A..T...........TA.......T..T......C.A..T..C................T  [546]

DR_1173(G2)_COII      TGCGGAGCAAACCACAGATTCATGCCAATCAC [578]
DR_1260(G4)_COII      ................................ [578]
DR_1318(G3)_COII      .......................A........ [578]
DR_1354(G3)_COII      .......................A........ [578]
DR_1355(G1)_COII      .......................A........ [578]
DR_1784(G3)_COII      ................................ [578]
DR_1790(G3)_COII      .......................A........ [578]
DR_1853(G3)_COII      .......................A........ [578]
DR_1979(G2)_COII      ................................ [578]
DR_971(G2)_COII       ................................ [578]
G1(2)_COII            ................................ [578]
G1_COII               ................................ [578]
G3/G4(2)_COII         ..T.................T..A........ [578]
G3/G4_COII            ................................ [578]
A._cf._K_COII         ....................T..A........ [578]
M._sp._IS178_COII     ..T........T..T.....T..A......GT [578]
```

Figure 3-2. Continued.

```
DR_1173(G2)_COII      EQLIYFHDHALIIILIIITTVFYTIIRIIQNKQTRRFILEGQIIETV*TIAPAVILVFIAMPSLRLLYLIDEIHNPVLTL  [ 80]
DR_1260(G4)_COII      ..................................................................................  [ 80]
DR_1318(G3)_COII      ...................................................I..........................  [ 80]
DR_1354(G3)_COII      ...................................................I......I...................  [ 80]
DR_1355(G1)_COII      ......................................M..............I......I...................  [ 80]
DR_1784(G3)_COII      ...................................................I......I...................  [ 80]
DR_1790(G3)_COII      ...................................................I......I...................  [ 80]
DR_1853(G3)_COII      ......-............................................I......I...................  [ 80]
DR_1979(G2)_COII      ...................................................I......I...................  [ 80]
DR_971(G2)_COII       ...................................................I......I...................  [ 80]
G1(2)_COII            ..................................................................................  [ 80]
G1_COII               ..................................................................................  [ 80]
G3/G4(2)_COII         ...................................................I......I...................  [ 80]
G3/G4_COII            ..................................................................................  [ 80]
A._cf._K_COII         ........................S..........................I......I...................  [ 80]
M._sp._IS178_COII     ....F...........................S...........I......I......I................I..  [ 80]

DR_1173(G2)_COII      KTVGHQ*Y*SYEYSDFTKLEFDSYMVQQEDIQIDTFRLLDTDNRVVLPINSPIRIIVTAADVLHS*TVPRLGVKSDATPG  [160]
DR_1260(G4)_COII      ..................................................................................  [160]
DR_1318(G3)_COII      ........................I.....M.T.................................................  [160]
DR_1354(G3)_COII      ........................I.....M.T.................................................  [160]
DR_1355(G1)_COII      ................I.......I.......T.................................................  [160]
DR_1784(G3)_COII      ................................T.................................................  [160]
DR_1790(G3)_COII      ........................I.....M.T.................................................  [160]
DR_1853(G3)_COII      ........................I.....M.T.................................................  [160]
DR_1979(G2)_COII      ...................................................L......................G...  [160]
DR_971(G2)_COII       ...........................M.T.....................L.........................  [160]
G1(2)_COII            ..................................................................................  [160]
G1_COII               ..................................................................................  [160]
G3/G4(2)_COII         ................................T.................................................  [160]
G3/G4_COII            ..................................................................................  [160]
A._cf._K_COII         ........................I.....L.A.................................................  [160]
M._sp._IS178_COII     ........................I.....QL.N.......................................T.....  [160]

DR_1173(G2)_COII      RLNQVRFSINRPGILYGQCSEICGANHRFMPI [192]
DR_1260(G4)_COII      ............................... [192]
DR_1318(G3)_COII      ............................I.. [192]
DR_1354(G3)_COII      ............................I.. [192]
DR_1355(G1)_COII      ............................I.. [192]
DR_1784(G3)_COII      ............................... [192]
DR_1790(G3)_COII      ............................I.. [192]
DR_1853(G3)_COII      ............................I.. [192]
DR_1979(G2)_COII      ............................... [192]
DR_971(G2)_COII       ............................... [192]
G1(2)_COII            ............................... [192]
G1_COII               ............................... [192]
G3/G4(2)_COII         ............................I.. [192]
G3/G4_COII            ............................... [192]
A._cf._K_COII         ............................I.. [192]
M._sp._IS178_COII     .......T.....L...............I.. [192]
```

Figure 3-2. Continued.

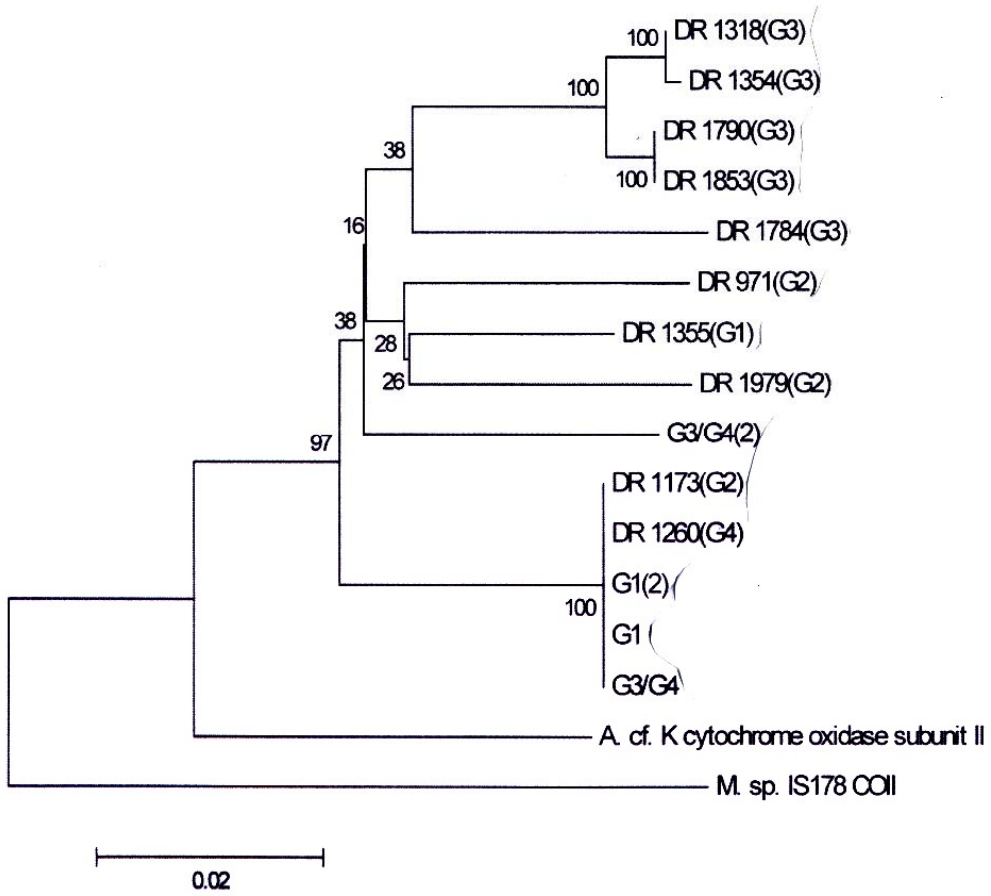

Figure 3-3. Neighbor-joining tree for COII sequences for first replicate using bootstrap method: DR #(G#) = consensus sequence containing one sample, G# = consensus sequence that contains multiple samples.; sum of branch lengths = 0.24645426; replicates = 500; outgroups: A. cf = *Anoplotermes* (Inward et al. 2007a), M. sp. = *Microcerotermes* sp. (Legendre et al. 2008).

	G1	G2	G3	G3/G4	G4	Ano OG
G1						
G2	0.015					
G3	0.049	0.051				
G3/G4	0.031	0.024	0.051			
G4	0.015	0.000	0.051	0.024		
An OG	0.070	0.067	0.072	0.071	0.067	
Mi OG	0.111	0.11	0.114	0.113	0.110	0.109

Figure 3-4. Distance matrix for COII sequence data for first replicate for each enteric valve morphological group: sister group, *Anoplotermes* (An OG) and an outgroup, *Microcerotermes* (Mi OG): Shows variation between morpho types.

```
BA_1136_Abr            TTTAACACTACAAGATAGAGCCTCCCCTATTATAGAACAATTAATCTACTTCCACGACCACGCACTAATAATTATATT  [ 78]
BA_1291_Abr            ......................................................................  [ 78]
BA_1916_Ano            ......................................................................  [ 78]
BA_2437_Ano            ......................................................................  [ 78]
DR_1173_G2             ......................................................................  [ 78]
DR_1223_G1             ......................................................................  [ 78]
DR_1256_G1             ......................................................................  [ 78]
DR_1288_G3             C.....G........C.............................T...............T...............C.  [ 78]
DR_1319_G4             C.....G........C.............................T...............T...............C.  [ 78]
DR_1533_G4             ......................................................................  [ 78]
DR_1790_G3             C...................................G................T................C...C.  [ 78]
DR_971_G2              CC.....................................T...........T....................  [ 78]
Anoplotermes DQ442076  .......T....................................T........T........CT...........C.  [ 78]

BA_1136_Abr            GATAATTATTACAACCGTATTCTATACAATAATTAGAATTATCCAAAACAAACAAACAAGACGATTCATACTAGAAGG  [156]
BA_1291_Abr            ......................................................................  [156]
BA_1916_Ano            ......................................................................  [156]
BA_2437_Ano            ......................................................................  [156]
DR_1173_G2             ......................................................................  [156]
DR_1223_G1             ......................................................................  [156]
DR_1256_G1             ......................................................................  [156]
DR_1288_G3             A...................T................C..T.............................  [156]
DR_1319_G4             A.............T.....T................C..T.............................  [156]
DR_1533_G4             ......................................................................  [156]
DR_1790_G3             A.............T.............................T................T...........  [156]
DR_971_G2              A.............T.....T................C...........G..............T...........  [156]
Anoplotermes DQ442076  A.......................T................T.....T........C........T...........  [156]

BA_1136_Abr            ACAAATAATCGAAACCGTTTGAACAATTGCGCCAGCAGTCATCCTAGTATTCATTGCAATGCCATCGCTACGACTACT  [234]
BA_1291_Abr            ......................................................................  [234]
BA_1916_Ano            ......................................................................  [234]
BA_2437_Ano            ......................................................................  [234]
DR_1173_G2             ......................................................................  [234]
DR_1223_G1             ......................................................................  [234]
DR_1256_G1             ......................................................................  [234]
DR_1288_G3             ........................C..............T................A.....A...........  [234]
DR_1319_G4             ........................C..............T................A.....A...........  [234]
DR_1533_G4             ......................................................................  [234]
DR_1790_G3             G...................................A....T................A.....AT..........  [234]
DR_971_G2              ......................G.....T......A....T................A...............T.  [234]
Anoplotermes DQ442076  .........T.....A..............T......A....................A.....A...........  [234]

BA_1136_Abr            ATACTTAATAGACGAAATCCACAATCCAGTATTAACACTAAAAACAGTGGGACACCAATGATACTGAAGCTATGAATA  [312]
BA_1291_Abr            ......................................................................  [312]
BA_1916_Ano            ......................................................................  [312]
BA_2437_Ano            ......................................................................  [312]
DR_1173_G2             ......................................................................  [312]
DR_1223_G1             ......................................................................  [312]
DR_1256_G1             ......................................................................  [312]
DR_1288_G3             .......................C..C...C.......T..............................T........  [312]
DR_1319_G4             .......................C..C...C.......T..............................T........  [312]
DR_1533_G4             ......................................................................  [312]
DR_1790_G3             ....C.....................................A..............T.....T........  [312]
DR_971_G2              ...T................T..C......C....C...........T..............T.....T........  [312]
Anoplotermes DQ442076  ....C......................C.....G..CT..........T..G..T.....................  [312]

BA_1136_Abr            CTCAGACTTCACTAAGCTTGAATTTGACTCATACATGGTACAACAAGAAGACATACAAATAGACACATTCCGACTACT  [390]
BA_1291_Abr            ......................................................................  [390]
BA_1916_Ano            ......................................................................  [390]
BA_2437_Ano            ......................................................................  [390]
DR_1173_G2             ......................................................................  [390]
DR_1223_G1             ......................................................................  [390]
DR_1256_G1             ......................................................................  [390]
DR_1288_G3             ......T.....A..A........C..............G...........T.......C.............T....  [390]
DR_1319_G4             ......T.....A..A........C..............G...........T.......C.............T....  [390]
DR_1533_G4             ......................................................................  [390]
DR_1790_G3             ......T.....A...........C..T........A..............T..G....C..................  [390]
DR_971_G2              ......T.....A.......................................G..G.C..................  [390]
Anoplotermes DQ442076  ............A................G..T..A...............C....GGCG.....G........GT.  [390]
```

Figure 3-5. COII sequence data for Apicotermitinae from the Dominican Republic for second replicate.

```
BA_1136_Abr            AGACACAGACAACCGAGTAGTATTACCAATAAATTCACCCATTCGAATAATCGTAACAGCAGCAGACGTACTACACTC  [468]
BA_1291_Abr            ......................................................................................  [468]
BA_1916_Ano            ......................................................................................  [468]
BA_2437_Ano            ......................................................................................  [468]
DR_1173_G2             ......................................................................................  [468]
DR_1223_G1             ......................................................................................  [468]
DR_1256_G1             ......................................................................................  [468]
DR_1288_G3             ...T.....T..T.......................................T..............T...........  [468]
DR_1319_G4             ...T.....T..T.......................................T..............T...........  [468]
DR_1533_G4             ......................................................................................  [468]
DR_1790_G3             ...T.....T..T.....G.............C.................................TT....T..  [468]
DR_971_G2              C..T........T.....G...........................T.......................T.....  [468]
Anoplotermes DQ442076  ............T......................C..A..C........T..............T...T.......  [468]

BA_1136_Abr            ATGAACAGTACCAAGACTCGGGGTAAAGTCAGATGCTACACCAGGACGCCTGAACCAAGTAAGATTCTCAATTAACCG  [546]
BA_1291_Abr            ......................................................................................  [546]
BA_1916_Ano            ......................................................................................  [546]
BA_2437_Ano            ......................................................................................  [546]
DR_1173_G2             ......................................................................................  [546]
DR_1223_G1             ......................................................................................  [546]
DR_1256_G1             ......................................................................................  [546]
DR_1288_G3             ..................T...........T..................G..A.....G.................T..  [546]
DR_1319_G4             ..................T...........T..................G..A.....G.................T..  [546]
DR_1533_G4             ......................................................................................  [546]
DR_1790_G3             ..................T..A........C..................A..A......................T..  [546]
DR_971_G2              .........G........T..A........T........C........A..A......................T..  [546]
Anoplotermes DQ442076  T.................T..A.............C...........A..A......................T..  [546]

BA_1136_Abr            ACCAGGAATCCTATATGGACAATGCTCAGAAATCTGCGGAGCAAACCACAGATTCATGCCAATCACTAT  [624]
BA_1291_Abr            ........................................................................  [624]
BA_1916_Ano            ........................................................................  [624]
BA_2437_Ano            ........................................................................  [624]
DR_1173_G2             ........................................................................  [624]
DR_1223_G1             ........................................................................  [624]
DR_1256_G1             ........................................................................  [624]
DR_1288_G3             .......................T...........T..................T..A...........  [624]
DR_1319_G4             .......................T...........T..................T..A...........  [624]
DR_1533_G4             ........................................................................  [624]
DR_1790_G3             ...........................................................A............  [624]
DR_971_G2              ...............................T........................................  [624]
Anoplotermes DQ442076  ...................................................T..A........A..  [624]
```

Figure 3-5. Continued.

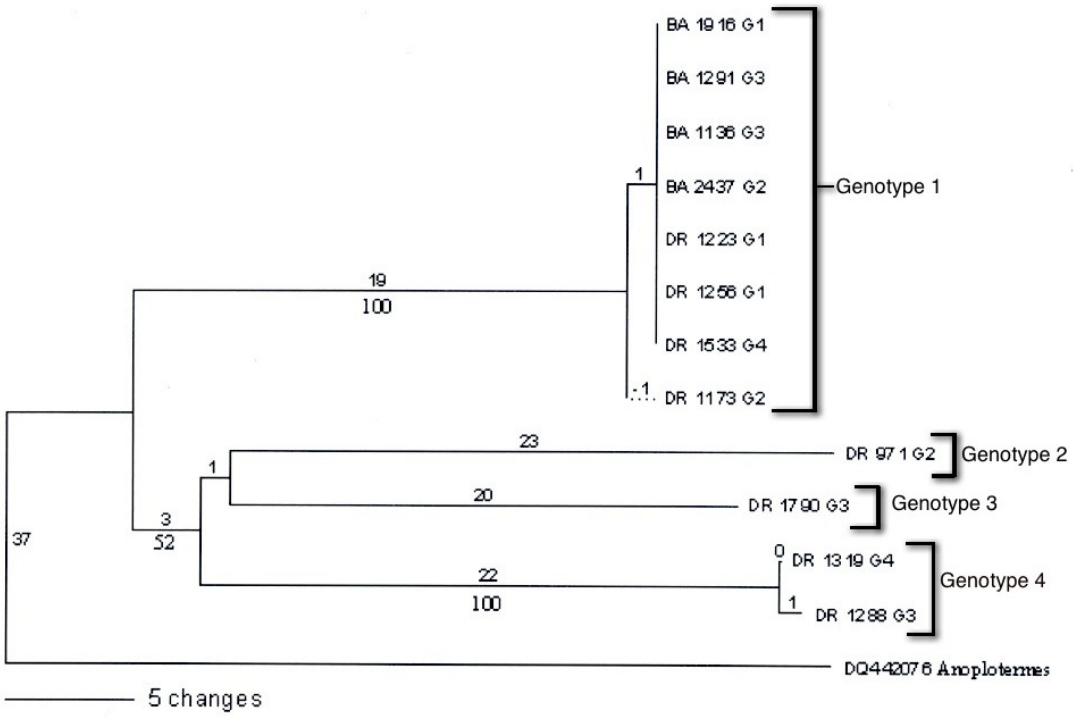

Figure 3-6. Neighbor-joining tree for COII gene for second replicate using bootstrap method: 1,000 replicates; #/# = base pair subsitutions/bootstrap values: DQ442076 = Accession number in GeneBank for *Anoplotermes* outgroup, BA = Bahamas, DR = Dominican Republic.

	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Genotype 1				
Genotype 2	0.079			
Genotype 3	0.074	0.076		
Genotype 4	0.079	0.083	0.074	
Outgroup	0.100	0.120	0.102	0.116

Figure 3-7. Distance matrix for COII for second replicate for each genotype: outgroup, *Anoplotermes* sp. DQ442076: Shows variation between genotypes presented in Figure 3-4.

Table 3-1. List of samples used in sequence analysis for D3 and complmentary data: An = *Anoplotermes* ss, Ang = *Anoplotermes* ng, EV= enteric valve, P1 = P1 morphology, C = constricted, T = tubular, os = single sample (not reflected in tree)

Sample_Sp_Date	**EV**	**Contig**	**P1**
DR1214_An_96	1	G1(1)	C
DR1219_An_96	1	G1(1)	C
DR1222_An_96	1	G1(1)	C
DR1223_An_96	1	G1(1)	C
DR1227_An_96	1	G1(1)	C
DR1256_An_96	1	G1(1)	C
DR1339_An_96	1	G1(1)	C
DR1541_An_96	1	G1(1)	C
DR1598_An_00	1	G1(1)	C
DR476_An_92	1	G1(1)	C
DR1934_An_04	1	G1(1)	C
DR400_An_92	1	G1(1)	C
DR1316_An_96	1	G1(2)	C
DR1317_An_96	1	G1(2)	C
DR1355_An_96	1	os	C
DR1173_An_96	2	os	C
DR1979_An_04	2	os	C
DR971_An_94	2	os	C
DR1884_Ang_04	3	G3/G4(1)	T
DR1287_Ang_96	3	G3/G4(1)	T
DR1288_Ang_96	3	G3/G4(2)	T
DR1905_Ang_04	3	G3/G4(2)	T
DR1291_Ang_96	3	G3/G4(2)	T
DR1318_Ang_96	3	os	T
DR1354_Ang_96	3	os	T
DR1226_Ang_96	3	G3/G4(1)	T
DR1784_Ang_04	3	os	T
DR1790_Ang_04	3	os	T
DR1853_Ang_04	3	os	T
DR1276_Ang_96	4	G3/G4(2)	T
DR325_Ang_92	4	G3/G4(2)	T
DR1319_Ang_96	4	G3/G4(2)	T
DR1333_Ang_96	4	G3/G4(2)	T
DR1533_Ang_96	4	G3/G4(1)	T
DR1260_Ang_96	4	os	T

Table 3-2. List of contigs used in sequence analysis for COII and each sample used to assemble the consensus sequence: Ano = *Anoplotermes*, Ang = *Anoplotermes* ng

	Consensus Sequences					
Sample	**G1**	**G2**	**G3/G4(1)**	**G3/G4(2)**	**G3/G4(3)**	**Single Sample**
DR970_Ano	X					
DR1339_Ano	X					
DR420_Ano	X					
DR1227_Ano	X					
DR1598_Ano	X					
DR1316_Ano	X					
DR1223_Ano	X					
DR1219_Ano	X					
DR1214_Ano	X					
DR1256_Ano	X					
DR128_Ano	X					
DR1541_Ano	X					
DR1317_Ano	X					
DR1355_Ano	X					
DR1173_Ano		X				
DR971_Ano		X				
DR1226_Ano			X (G3)			
DR1260_Ang			X (G4)			
DR1286_Ang			X (G3)			
DR1803_Ang			X (G3)			
DR1318_Ang				X (G3)		
DR1319_Ang				X (G4)		
DR1333_Ang				X (G4)		
DR1853_Ang				X (G3)		
DR1922_Ang				X (G3)		
DR1533_Ang					X (G4)	
DR1827_Ang					X (G3)	
DR1979_Ano						X (G2)
DR1784_Ang						X (G3)

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BIOGRAPHICAL SKETCH

Brian was born in Milford, Delaware on August 7, 1984 to Belinda and James Bahder. From an early age he loved the outdoors and collecting insects and fungi. At about the age of ten, he decided that he wanted to be an entomologist and turn his passion into a career. Brian started his education at the University of Delaware by doing research on termite biology and graduating with a Bachelor of Science in entomology in May of 2006. While at the University of Delaware, Brian was able to spend a semester abroad in Ecuador, doing research in the Amazon Rainforest. After receiving his bachelor's degree, Brian continued his research with termites at the University of Florida and received his master's degree in May of 2009. Aside from his education, Brian has had the opportunity to do research abroad in the Galapagos Islands, Costa Rica, Guatemala, Colombia, and Honduras. Brian's current research interests involve DNA taxonomy and molecular genetics and he hopes to receive a doctorate degree in this area of entomology.