

**Revision of Diagnostic Morphological Characters in Three Puerto Rican Species of the  
Termite Genus *Heterotermes* (Dictyoptera: Rhinotermitidae)**

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## Abstract

Termites of the genus *Heterotermes* Froggatt (Rhinotermitidae: Heterotermitinae) are pantropical subterranean wood-feeders capable of causing significant structural damage. Despite their economic importance, the taxonomy of *Heterotermes* remains understudied due to a lack of robust morphological characteristics enabling reliable identification. The aim of this study was to investigate a range of morphometric attributes in three species of *Heterotermes* previously identified by sequencing of two mitochondrial genes. All samples were collected from the Puerto Rican archipelago (Puerto Rico and Culebra Island) and attributed to either *Heterotermes cardini* (Snyder), *H. convexinotatus* (Snyder), or *H. tenuis* (Hagen). Soldiers were the only caste examined. Soldiers (n=99) were imaged and measured using the image-stacking AutoMontage program. Seven characters were measured on each specimen: mandible length, head capsule width and length, pronotum width and length, and depths of the anterior and posterior pronotal notches. These latter two metrics were novel to this study. From a subset of these metrics, three indices were derived from the ratios of (1) head width to length, (2) pronotum width to length, and (3) head capsule length to mandible length. Discriminant and cluster analyses determined that none of these traits, or combination thereof, were useful in reliably identifying Puerto Rican *Heterotermes* soldiers to species level. Examination of cephalic setae counts demonstrated that these data also could not be reliably used to distinguish soldiers of these three species. However, previously described characters of the soldier tergal setae were confirmed to be useful in discriminating *H. tenuis* from its Puerto Rican congeners. Additionally, I found that *H. tenuis* soldiers possessed long bristles (>100  $\mu\text{m}$ ) along the posterior margins of posterior tergites, excluding the pygidium, thereby providing another character for distinguishing the species.

## Introduction

*Heterotermes* Froggatt, 1897 (Rhinotermitidae: Heterotermitinae) is a pantropical genus of subterranean wood-feeding termites (Constantino 2000). Thirty extant species have been described worldwide (Krishna et al. 2013), of which 17 have been reported as pests that damage human structures (Scheffrahn and Su 2000). Some of these species are economically noteworthy in tropical regions to a degree comparable to that of the closely related eastern subterranean termite (*Reticulitermes flavipes* Kollar [Rhinotermitidae: Heterotermitinae]) in the temperate zones of the Northern Hemisphere. In South America, *Heterotermes tenuis* Froggatt has been reported as not merely a structural pest, but as a pest of standing hardwood and softwood timber and a number of crops (Batista-Pereira et al. 2004). In Brazil, Arrigoni et al. (1989) reported that this species caused 10 tons/ha/year in damage to sugarcane in the state of São Paulo alone.

The *Heterotermes* fauna in the Caribbean Region (the Bahamas, Greater Antilles, and Lesser Antilles) is thought to consist exclusively of pest species that have been introduced from the South American mainland (Constantino 1998; Evans et al. 2013). Caribbean *Heterotermes* are consequently of interest in that they are both invasive and economically significant.

Our understanding of *Heterotermes* species' identity and their distribution in the Caribbean Region has fluctuated over time. Due to morphological ambiguity, Snyder's (1924) original description of *Heterotermes convexinotatus* Snyder and *Heterotermes cardini* Snyder (from the Bahamas) suggested that these two species might be synonymous with another Caribbean termite, *H. tenuis*. The survey by Szalanski et al. (2004) reported *H. tenuis* throughout the Lesser Antilles excepting Barbados and Martinique. Molecular analyses of the 16S mitochondrial gene have additionally suggested the presence of one or more undescribed *Heterotermes* species in the Caribbean Region (Szalanski et al. 2004), although Eaton et al.

(2016) found that these same samples were a genetically close match to *H. cardini* when a much larger dataset was examined.

The species composition of *Heterotermes* fauna in Puerto Rico and its associated islands has been especially ambiguous. This archipelago is of potential biogeographic significance in that it is the easternmost island of the Greater Antilles and hence is situated near the younger, actively volcanic Lesser Antilles, providing a biogeographic link to the South American mainland. Surveys of the Puerto Rican termite fauna have provided disparate results. Scheffrahn et al. (2003) left Puerto Rican *Heterotermes* specimens unidentified to the species level due to taxonomic uncertainty, whereas others have reported both *H. tenuis* and *H. convexinotatus* based on morphological data (Snyder 1956); only *H. convexinotatus*, based on the 16S gene from 3 Puerto Rican termite samples (Szalanski et al. 2004); or *H. tenuis*, *H. convexinotatus*, and *H. cardini* based on the 16S and COII genes from 76 samples (Eaton et al. 2016).

Evidently, much of this confusion results from an inability to confidently identify *Heterotermes* beyond generic level when solely using morphological characters. Although the soldier caste alone is often sufficient for termite species identification (Scheffrahn and Su 1994), *Heterotermes* soldiers from the Caribbean are difficult to reliably distinguish due to non-robust diagnostic morphological characters. Soldiers are distinguished on the basis of relative pilosity of head capsules and pronota, along with such equally subjective criteria as overall bodily coloration and relative size (Snyder 1924). Consequently, alates (winged reproductives) are essential for reliable species identification in *Heterotermes* (Snyder 1924). Since alates are produced only seasonally, they are difficult to obtain and are seldom properly associated with their parent colonies.

The two most recent studies of Caribbean *Heterotermes* spp. (Szalanski et al. [2004] and Eaton et al. [2016]) utilized a phylogenomic approach conjunct with some morphometric data. Szalanski et al. (2004) proposed a phylogeny of Caribbean *Heterotermes* based upon analyses of 16S sequences of 59 samples spread across 30 islands throughout the Caribbean Region. Maximum parsimony analysis produced a tree consisting of five groups, three of which corresponded to *H. tenuis*, *H. convexinotatus*, and *H. cardini* on the basis of morphological evidence. The remaining two were sister clades of *H. cardini* and close to that species morphologically, but it was suggested that they might together “constitute a separate species or subspecies based on their genetic dissimilarities,” and these clades were reported as “*Heterotermes* sp. (Bonaire, FL [sic]; Grand Cayman; Grand Turk; Jamaica; and St. Barthelemy)” (Szalanski et al. 2004). By contrast, Eaton et al. (2016) focused on the Puerto Rican *Heterotermes* fauna and analyzed 16S and COII sequences from 76 samples (70 from the main island of Puerto Rico and 6 from Culebra Island) as well as available GenBank sequences from throughout the Americas. Eaton et al. (2016) found that both the highly conserved 16S gene and the less conserved COII gene displayed a congruent, robust (branch support values  $\geq 58\%$ ) phylogeny of three monophyletic species in Puerto Rico: *H. tenuis*, *H. convexinotatus*, and *H. cardini*. Physical characteristics of a small subset of these soldiers supported their respective species designations.

The purpose of this study was to measure morphometric parameters in the alate and soldier caste from a comprehensive sample of the Puerto Rican *Heterotermes* fauna, subsequently performing statistical analysis on these data to determine what metrics, if any, were most useful in identifying *Heterotermes* to species level. Additionally, I attempted to determine whether certain morphological characters used by Snyder (1926) in his description of *H.*

*convexinotatus* and *H. cardini* were robust for the purpose of species identification. The possibility of an additional, undescribed species of *Heterotermes* in the Caribbean Region (as per Szalanski et al. 2004) also was herein investigated with statistical analysis. This study is supplementary to that of Eaton et al. (2016) in that it uses a subset of the same Puerto Rican *Heterotermes* samples.

## **Materials and Methods**

**Samples.** A total of 38 samples of Puerto Rican *Heterotermes* from the 76 analyzed by Eaton et al. (2016) was included in this study, with samples assigned to 1 of 3 species (*H. tenuis*, *H. convexinotatus*, and *H. cardini*) on the basis of their mitochondrial phylogeny (Eaton et al. 2016). These samples were collected from different locales on the main island of Puerto Rico and adjacent Culebra Island in 2002, 2004, 2006, and 2010. Each sample consisted of termites collected from a single access point in a given colony and placed in individual vials filled with absolute alcohol. All were collected by Susan C. Jones.

In total, 99 individual soldiers were examined morphometrically (see definitions of metrics below), with 3 being drawn from each sample when available. Only 2 samples of *H. tenuis* were available, so all soldiers present ( $n=14$ ) were examined. A total of 21 soldiers of *H. convexinotatus* and 58 soldiers of *H. cardini* were examined. The cephalic setae of 3 soldiers from an additional sample of *H. cardini* were examined, without morphometric investigation.

**Methodology.** A stereomicroscope with Auto-Montage 3D imaging software (Synoptics Ltd., Cambridge, UK) was used to image and thereby measure the aforementioned subset of samples. The pronotum and head served as the source of investigated metrics in all specimens. I elected to investigate mandible length in combination with the same axis of the head capsule

along with head width, in order to provide additional commonality with the data presented in Constantino (2000) and Szalanski et al. (2004).

**Metrics and Indices.** Measured dorsally. The following is a comprehensive list of the metrics delineated for soldier specimens in this study, as defined by Roonwal (1969):

- 1) Maximum length of mandible (Fig. 1, line AA)—defined as the distance between two parallels marking the notch at the upper base of the outer mandibular condyle and the distalmost tip of the mandible;
- 2) Maximum width of head (Fig. 1, BB)—distance between two parallels marking the outermost lateral margins of the head capsule;
- 3) Length of head to lateral base of mandibles (Fig. 1, CC; effectively synonymous in the case of *Heterotermes* with “head-capsule length” as defined by Roonwal 1969)—defined as the distance between two parallels marking the hindmost margin of the head-capsule and the external articulations of the mandibles;
- 4) Maximum pronotal length (Fig. 2, AA)—distance between two parallels marking the foremost and hindmost margins of the pronotum at their widest separation;
- 5) Maximum pronotal width (Fig. 2, BB)—distance between two parallels marking the lateral margins of the pronotum at their widest separation.

In addition, two novel metrics were used: the depth of the anterior pronotal notch (Fig. 2, CC), defined as the distance between the hindmost extent of the anterior pronotal margin and a parallel tangential to both vertices of the anterior pronotal margin; and the depth of the posterior pronotal notch (Fig. 2, DD), defined as the distance between the foremost extent of the posterior pronotal margin and a parallel tangential to both vertices of the posterior pronotal margin. All

metrics were delineated *in situ*. From these metrics, the following indices (as defined by Roonwal 1969) were derived for statistical analysis in addition to the above-iterated metrics:

- 1) Mandible-head index I—defined as  $\frac{\text{Maximum length of mandible}}{\text{Length of head to lateral base of mandibles}}$ ;
- 2) Head index I—defined as  $\frac{\text{Maximum width of head with eyes (when present)}}{\text{Length of head to lateral base of mandibles}}$ ;
- 3) Pronotum index I—defined as  $\frac{\text{Maximum length of pronotum}}{\text{Maximum width of pronotum}}$ .

Analyses of variance (ANOVA), discriminant analyses, and cluster analyses were then performed on these data using SPSS (International Business Machines, Armonk, USA). The level of significance for all analyses was set at  $\alpha = 0.05$ .

## **Results and Discussion**

Only three samples included alate specimens, all of which were assigned to a single species, *H. convexinotatus*. Forewings of alate specimens ( $n=16$ ) were measured, in keeping with Szalanski et al. (2004) and Lim and Forschler (2012), and the width of the head (including eyes) and pronotum were examined in accordance with Snyder (1924). Since all alates were conspecific, there was no basis for comparison across species. Thus, alates were disregarded in my statistical analyses.

ANOVA concerning all indices and metrics I used demonstrated that all but two of the metrics surveyed—the respective depths of the anterior and posterior pronotal notches, which were novel to this study—displayed statistically significant variation across all three species (Table 1). Based on this information, a discriminant analysis was performed using all statistically significant metrics. This method determines the efficacy of any array of variables in determining group membership (Green et al. 2008), and here correctly classified 87.1% of specimens. Thus, while the majority of Puerto Rican *Heterotermes* could be accurately identified to species level



using these four metrics, these morphometric parameters were not invariably reliable for that purpose; nor, given the fact that there were four metrics in total, was this mode of identification concise.

In addition, multiple cluster analyses were performed using these statistically significant metrics. This statistical method does not assume *a priori* assignments of specimens to groups or the quantity of said groups, instead attempting to iteratively delineate groups *de novo* from the data given according to the number of groups provided by an *a priori* hypothesis. These analyses respectively specified 3, 4 and 2 morphometric-delineated groups within the sampled *Heterotermes* fauna. The former two analyses were premised according to the hypotheses of Eaton et al. (2016) and Szalanski et al. (2004), respectively. The latter 2-taxon analysis attempted to determine if cluster analysis would support the phylogenetic arrangement of *H. cardini* and *H. convexinotatus* as more closely related to one another than to *H. tenuis*, a hypothesis supported by molecular data (Szalanski et al. 2004, Eaton et al. 2016). The results of these morphometric cluster analyses did not consistently conform to the phylogenetic hypotheses of either Szalanski et al. (2004) or Eaton et al. (2016), nor did they support placement of *H. tenuis* as sister-group to the remaining two species of *Heterotermes* putatively present in the Puerto Rican fauna. Some consensus was observed in the form of clusters with compositions that did not change between analyses (Table 2), indicating relatively strong morphometric support, but none of these clusters were derived from all three analyses. Only one cluster, evident in the 3- and 4-cluster analyses (Table 2), consisted primarily of a single species (38% of all *H. cardini* and a single *H. convexinotatus*). No morphometric consensus, novel or otherwise, could be made across all three cluster analyses, demonstrating that this analytical approach to these data did not offer robust

species delimitation (Tables 3-5). These results were likely influenced by the fact that many specimens provided insufficient data to be included in cluster analyses (Table 2).

In an attempt to determine if morphology could be reliably used to distinguish species of Puerto Rican *Heterotermes*, I next quantified head capsule pilosity (Table 6) as a character for use in soldier-based species identification. Snyder (1924) noted the presence of “but few short hairs” on the soldier head capsules of *H. convexinotatus* when compared to *H. tenuis*. I also found short (uniformly <30  $\mu\text{m}$ ) setae on the head capsules of all *H. convexinotatus* specimens examined, but pilosity of these two species was comparable, with *H. convexinotatus* averaging  $19 \pm 0.25$  setae ( $n=14$  soldiers from 7 samples—that is, groups of specimens collected from a single colony) and *H. tenuis* averaging  $19 \pm 0.57$  setae ( $n=13$  soldiers from 2 samples). Snyder (1924) asserted that soldiers of *H. cardini* possessed “a few more” cephalic setae than *H. convexinotatus*, but I found that *H. cardini* soldiers usually had fewer cephalic setae ( $\bar{x} = 14 \pm 0.24$ ;  $n=38$  soldiers from 14 samples) than *H. convexinotatus*. However, I note that there is considerable overlap between the two species with reference to this trait. Hence, head capsule pilosity cannot be used as a diagnostic character to differentiate these three species.

The distribution of setae on *Heterotermes* soldiers’ abdominal tergites was used by Constantino (2000) as a distinguishing character in his key to soldiers of South American *Heterotermes*, a synopsis which excluded *H. cardini*. Whereas both *H. convexinotatus* and *H. tenuis* had tergites bearing a line of bristles (setae that are significantly more robust than surrounding ones) on the posterior margin, the pilosity of their tergal surfaces differed (Constantino 2000). Likewise, I found that sparsely distributed short setae (<10  $\mu\text{m}$  long) predominated on the tergal surfaces of *H. convexinotatus* soldiers—perhaps equating to the “numerous microscopic hairs” referred to by Constantino (2000), whereas *H. tenuis* soldiers

possessed numerous long hairs on the tergal surfaces, forming a distinct row (circled in Figure 3A). Furthermore, the tergal surfaces of *H. convexinotatus* (Figure 3B) and *H. cardini* (Figure 3C) were similar to each other, but lacked long setae on their interior tergal surfaces, distinguishing them from *H. tenuis* (Figure 3A). Snyder (1924) asserted that the abdomen of *H. cardini* was “as in” the “pubescent” *H. convexinotatus*, and this study accordingly found that *H. cardini* was indistinguishable from *H. convexinotatus* on the basis of soldier tergite pilosity.

My examination of the posterior margins of *Heterotermes* soldiers’ posterior abdominal tergites, excluding the pygidium, revealed that all three Puerto Rican species of *Heterotermes* bore a distinct line of bristles. In keeping with morphological and genetic data (Eaton et al. 2016; SCJ and Tyler D. Eaton, unpublished data on internal transcribed spacer), the distribution of posterior marginal bristles in *H. cardini* was more variable at an intraspecific level than either of its examined congeners: tergites of some specimens of *H. cardini* possessed 2-3 short (~10 µm) setae flush with posterior marginal bristles (50-70 µm), while other specimens lacked these intervening short setae. All *H. convexinotatus* soldiers examined lacked intervening short setae along the posterior tergite margins. Thus, *H. cardini* and *H. convexinotatus* could not be reliably distinguished using this character. However, the posterior marginal bristles of *H. tenuis* tergites always exceeded 100 µm in length, whereas those in the remaining two species never exceeded this length, providing a novel identifying characteristic for *H. tenuis* in the Puerto Rican archipelago.

Congruent ambiguity in identification based upon morphological characters alone is common throughout the Termitoidae, and, as a result, molecular analysis has been helpful and necessary in clarifying termite phylogeny at multiple taxonomic levels (Husen et al. 2006). Morphological analyses complemented with phylogenomic data have proven useful at an

interspecific level. In contrast to Chiu's et al. (2015) approach for *Sinocapritermes*, Liang and Li (2016) used principal component analysis (PCA) and analysis of similarities (ANOSIM) of genetic and morphometric characters to confirm significant differences between species in the re-description of three Taiwanese *Nasutitermes* spp. (Termitidae: Nasutitermitinae). Austin et al. (2007) resurrected *Reticulitermes mallei* Clément from synonymy on the basis of conjunct genetic and morphological data from soldiers as well as alates, albeit without any form of statistical inquiry.

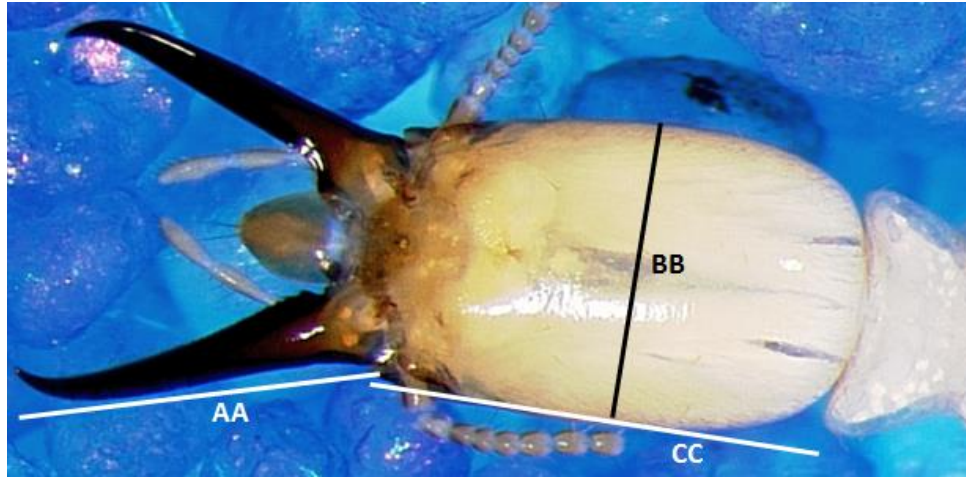
Conversely, my statistical results indicated that morphometric data from the soldier head and pronotum did not provide a robust means of discriminating Puerto Rican *Heterotermes* soldiers to species level, even in the cases of data comprised of those metrics (i.e., maximum pronotal width and length, maximum head width) found to be useful for species delimitation in termites from other regions of the world (Roonwal 1953; Roonwal and Sen-Sarma 1956; King et al. 2007; Lim and Forschler 2012; Liang and Li 2016).

My findings demonstrated that comprehensive morphometric examination of the soldier pronotum and head could not be reliably used to differentiate *Heterotermes* species present in the Puerto Rican archipelago. These data were not found to provide any well-resolved distinction between the three putative species in question, such as is supported by rigorous phylogenomic investigation (Eaton et al. 2016). I also found that *H. convexinotatus* and *H. cardini* could not be unequivocally discriminated on the basis of qualitative characters of the soldier caste that were used in the species descriptions (Snyder 1924). However, *H. tenuis* could be readily identified by means of tergal setal distribution, as reported by Constantino (2000). I also described a novel qualitative character of *H. tenuis* soldiers (some tergal bristles >100 µm), further supporting its specific validity.

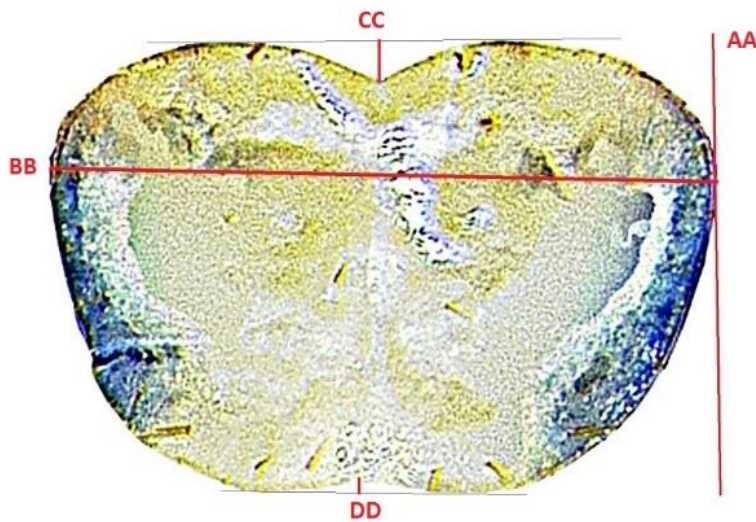
## ACKNOWLEDGEMENTS

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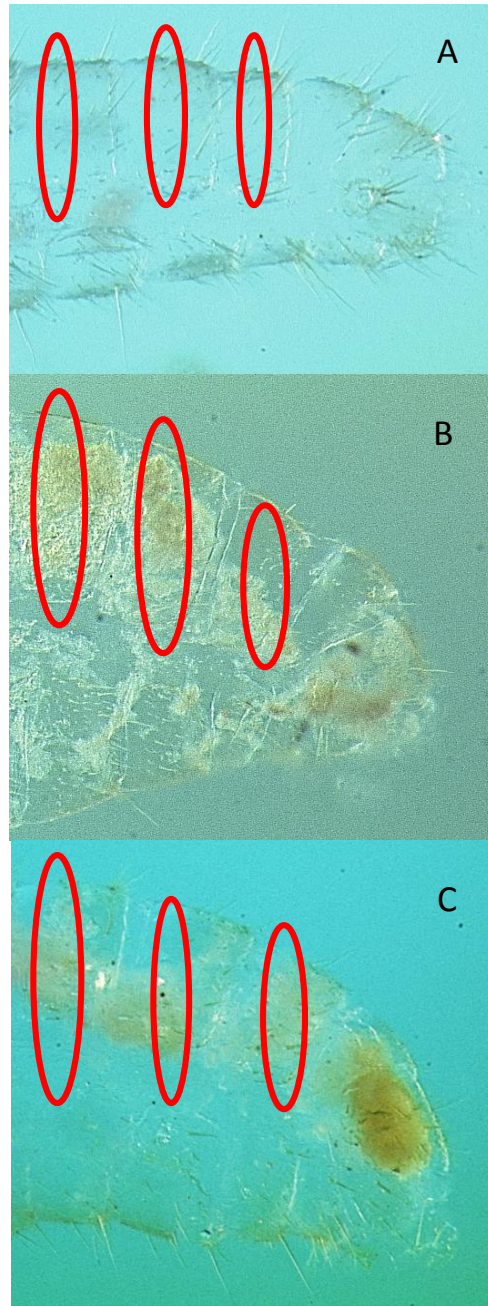
## FIGURES



**Figure 1.** Cephalic metrics used in this study. AA=maximum length of mandible; BB=maximum width of head; CC=length of head to lateral base of mandibles.



**Figure 2.** Pronotal metrics used in this study. AA=maximum length of pronotum; BB=maximum width of head; CC=depth of anterior pronotal notch; DD=depth of posterior pronotal notch.



**Figure 3.** Pilosity of posterior tergites in *H. tenuis* (A), *H. convexinotatus* (B), and *H. cardini* (C). Rows of long setae on the interiors of the abdominal tergites of *H. tenuis* are circled, with their absence on homologous tergal regions of *H. convexinotatus* and *H. cardini* circled for comparison.

## TABLES

**Table 1.** Results of ANOVA using all metrics and indices. Parameters exhibited statistically significant differentiation if  $P < 0.05$ .

Parameter	df	F value	P value
Head capsule length	70	4.985	0.010
Mandible length	67	11.222	0.000
Head width	69	7.345	0.001
Pronotum width	92	5.362	0.006
Pronotum length	92	3.834	0.025
Depth of anterior pronotal notch	88	3.064	0.052
Depth of posterior pronotal notch	82	2.413	0.096

**Table 2.** Cluster membership under respective phylogenetic hypotheses. Robust morphometric clusters are indicated by repeated values between hypotheses.

Species <sup>a</sup>	2-Cluster Hypothesis		3-Cluster Hypothesis		4-Cluster Hypothesis	
	Cluster <sup>b</sup>	Distance <sup>c</sup>	Cluster <sup>b</sup>	Distance <sup>c</sup>	Cluster <sup>b</sup>	Distance <sup>c</sup>
<i>H. cardini</i>	1	50.107	1	96.529	2	96.913
<i>H. cardini</i>	1	47.518	1	125.714	2	124.566
<i>H. cardini</i>	1	63.105	1	113.849	2	114.142
<i>H. cardini</i>	1	100.778	1	30.497	2	30.599
<i>H. cardini</i>	1	104.829	1	150.702	2	152.38
<i>H. cardini</i>	1	220.225	1	172.082	2	166.081
<i>H. cardini</i>	1	277.275	1	262.24	2	253.441
<i>H. convexinotatus</i>	1	112.945	1	65.089	2	64.499
<i>H. convexinotatus</i>	1	62.373	1	88.839	2	92.044
<i>H. convexinotatus</i>	1	78.309	1	42.908	2	43.837
<i>H. convexinotatus</i>	1	204.647	1	118.778	2	123.353
<i>H. convexinotatus</i>	1	137.324	1	48.648	2	56.106
<i>H. convexinotatus</i>	1	165.039	1	88.301	2	86.993
<i>H. convexinotatus</i>	1	68.009	1	40.474	2	41.519
<i>H. convexinotatus</i>	1	167.615	1	79.637	2	85.539
<i>H. convexinotatus</i>	1	181.322	1	98.185	2	101.89
<i>H. convexinotatus</i>	1	69.1	1	61.49	2	57.654



<i>H. convexinotatus</i>	1	205.997	1	119.446	2	122.693
<i>H. cardini</i>	1	226.317	1	181.146	4	115.079
<i>H. cardini</i>	1	150.162	2	27.607	1	27.607
<i>H. cardini</i>	1	149.354	2	41.737	1	41.737
<i>H. cardini</i>	1	125.672	2	55.031	1	55.031
<i>H. cardini</i>	1	115.286	2	60.736	1	60.736
<i>H. cardini</i>	1	89.191	2	65.847	1	65.847
<i>H. cardini</i>	1	192.836	2	145.37	1	145.37
<i>H. cardini</i>	1	409.002	2	330.698	1	330.698
<i>H. cardini</i>	1	186.158	2	73.81	1	73.81
<i>H. cardini</i>	1	164.217	2	37.683	1	37.683
<i>H. cardini</i>	1	200.216	2	70.278	1	70.278
<i>H. cardini</i>	1	157.168	2	79.074	1	79.074
<i>H. convexinotatus</i>	1	164.84	2	139.254	1	139.254
<i>H. cardini</i>	2	53.114	3	53.114	3	45.491
<i>H. cardini</i>	2	78.737	3	78.737	3	24.912
<i>H. cardini</i>	2	63.919	3	63.919	3	33.454
<i>H. cardini</i>	2	77.874	3	77.874	3	49.824
<i>H. cardini</i>	2	63.955	3	63.955	3	46.14
<i>H. cardini</i>	2	93.725	3	93.725	3	75.667
<i>H. cardini</i>	2	146.294	3	146.294	3	161.99
<i>H. cardini</i>	2	89.101	3	89.101	3	46.952
<i>H. cardini</i>	2	45.247	3	45.247	3	83.308
<i>H. cardini</i>	2	79.632	3	79.632	3	64.777
<i>H. cardini</i>	2	102.095	3	102.095	3	60.43
<i>H. cardini</i>	2	168.28	3	168.28	3	115.188
<i>H. convexinotatus</i>	2	211.804	3	211.804	3	163.36
<i>H. convexinotatus</i>	2	100.201	3	100.201	3	50.513
<i>H. convexinotatus</i>	2	122.929	3	122.929	3	79.882
<i>H. convexinotatus</i>	2	78.186	3	78.186	3	96.718
<i>H. convexinotatus</i>	2	80.108	3	80.108	3	113.8
<i>H. convexinotatus</i>	2	118.639	3	118.639	3	72.615
<i>H. convexinotatus</i>	2	117.099	3	117.099	3	143.651
<i>H. tenuis</i>	2	47.493	3	47.493	3	39.694
<i>H. tenuis</i>	2	56.792	3	56.792	3	107.853
<i>H. tenuis</i>	2	217.787	3	217.787	3	173.789
<i>H. cardini</i>	2	201.513	3	201.513	4	70.572

<i>H. cardini</i>	2	177.057	3	177.057	4	67.194
<i>H. tenuis</i>	2	147.55	3	147.55	4	39.865
<i>H. tenuis</i>	2	116.095	3	116.095	4	46.25
<i>H. tenuis</i>	2	89.571	3	89.571	4	94.974
<i>H. tenuis</i>	2	173.703	3	173.703	4	37.751
<i>H. tenuis</i>	2	129.377	3	129.377	4	60.859
<i>H. tenuis</i>	2	169.478	3	169.478	4	28.956
<i>H. tenuis</i>	2	149.752	3	149.752	4	77.337

<sup>a</sup>Insufficient morphometric data precluded analysis of numerous specimens, including 25 *H. cardini*, 5 *H. tenuis*, and 7 *H. convexinotatus*.

<sup>b</sup>Arbitrary numeration.

<sup>c</sup>Distance from computed cluster center.

**Table 3.** Counts of cluster members under 2-taxon hypothesis.

Category	Cluster number	Membership counts
Cluster	1	31
	2	31
Missing <sup>a</sup>		37

<sup>a</sup>Specimens with insufficient data to incorporate in analysis.

**Table 4.** Counts of cluster members under 3-taxon hypothesis.

Category	Cluster number	Membership counts
Cluster	1	19
	2	12
	3	31
Missing <sup>a</sup>		37

<sup>a</sup>Specimens with insufficient data to incorporate in analysis.

**Table 5.** Counts of cluster members under 4-taxon hypothesis.

<b>Category</b>	<b>Cluster number</b>	<b>Membership counts</b>
Cluster	1	12
	2	18
	3	22
	4	10
Missing <sup>a</sup>		37

<sup>a</sup>Specimens with insufficient data to incorporate in analysis.

**Table 6.** Cephalic setae counts.

<b>Identifier<sup>a</sup></b>	<b>Number of cephalic microsetae</b>
10_02_01_cardini	18
10_02_02_cardini	13
218_04_01_cardini	9
218_04_02_cardini	11
218_04_03_cardini	6
22_02_01_cardini	15
22_02_02_cardini	19
22_02_03_cardini	19
222_04_01_cardini	15
222_04_02_cardini	14
222_04_03_cardini	15
25_06_01_cardini	13
25_06_02_cardini	13
25_06_03_cardini	8
31_06_01_cardini	4
32_02_01_cardini	16
32_02_02_cardini	20
32_02_03_cardini	18
414_04_01_cardini	3
44_02_01_cardini	6
44_02_02_cardini	14
44_02_03_cardini	5
65_02_01_cardini	10
65_02_02_cardini	20
65_02_03_cardini	20

66_06_01_cardini	14
66_06_02_cardini	8
66_06_03_cardini	11
67_02_01_cardini	16
67_02_02_cardini	21
67_02_03_cardini	14
78_02_01_cardini	17
78_02_02_cardini	18
78_02_03_cardini	9
79_02_01_cardini	9
79_02_02_cardini	20
79_02_03_cardini	26
79_02_04_cardini	18
235_04_01_convexinotatus	19
34_06_01_convexinotatus	13
66_06_01_convexinotatus	15
226_06_01_convexinotatus	20
226_06_02_convexinotatus	13
226_06_03_convexinotatus	21
233_04_01_convexinotatus	27
233_04_02_convexinotatus	17
233_04_03_convexinotatus	26
74_02_01_convexinotatus	18
74_02_02_convexinotatus	17
74_02_03_convexinotatus	21
64_02_01_convexinotatus	12
64_02_02_convexinotatus	19
64_02_03_convexinotatus	15
67_10_01_tennis	27
84_06_tennis_putative_major	26
84_06_01_tennis_putative_minor	25
84_06_02_tennis_putative_minor	25
84_06_03_tennis_putative_minor	29
84_06_04_tennis_putative_minor	2
84_06_05_tennis_putative_minor	10
84_06_06_tennis_putative_minor	16
84_06_07_tennis_putative_minor	22
84_06_08_tennis_putative_minor	19
84_06_09_tennis_putative_minor	26
84_06_10_tennis_putative_minor	8
84_06_11_tennis_putative_minor	8

"Sample #\_year of collection\_species.

## References

Arrigoni, E. B., L. C. Almeida, P. Kasten Jr., and A. A. C. M. Precetti. 1989. Distribuição de espécies de cupins, em cana-de-açúcar, em unidades cooperadas das regiões de Jaú e Sertãozinho. Bol. Tec. Copersucar 48: 38-47.

Austin, J. W., A.-G. Bagnères, A. L. Szalanski, and R. E. Gold. 2007. *Reticulitermes mallei* (Isoptera: Rhinotermitidae): a valid Nearctic subterranean termite from eastern North America. Zootaxa 1554: 1-26.

Batista-Pereira, L. G., M. G. Dos Santos, A. Correa, J. F. Fernandes, A. Arab, A. M. Costa-Leonardo, C. R. R. C. Dietrich, D. A. Pereira, and O. Bueno. 2004. Cuticular hydrocarbons of *Heterotermes tenuis* (Isoptera: Rhinotermitidae): analyses and electrophysiological studies. Z. Naturforsch. 59(1-2): 135-139.

Chiu, C.-I., M.-M. Yang, and H.-F. Li. 2015. Redescription of the soil-feeding termite *Sinocapritermes mushae* (Isoptera: Termitidae: Termitinae): the first step of genus revision. Ann. Entomol. Soc. Am. 1-10.

Constantino, R. 1998. Catalog of the living termites of the New World (Insecta: Isoptera). Arq. Zool. 35: 135–231.

Constantino, R. 2000. Key to the soldiers of South American *Heterotermes* with a new species from Brazil (Isoptera: Rhinotermitidae). Insect Syst. Evol. 31: 463–472.

Eaton, T. D., S. C. Jones, and T. M. Jenkins. 2016. Genetic diversity of Puerto Rican *Heterotermes* (Isoptera: Rhinotermitidae) revealed by phylogenetic analysis of two mitochondrial genes. J. Insect Sci. 16(1): 111. DOI: <https://doi.org/10.1093/jisesa/iew099>

Evans, T. A., B. T. Forschler, and J. K. Grace. 2013. Biology of invasive termites: a worldwide review. Annu. Rev. Entomol. 58: 455–474.

Green, S. B., N. J. Salkind, and T. M. Akey. 2008. Using SPSS for Windows and Macintosh: Analyzing and understanding data. Prentice Hall, Upper Saddle River, New Jersey.

Husen, T. J., S. T. Kamble, and J. M. Stone. 2006. A characterization of subterranean termites in Nebraska using micro-morphological and molecular techniques. Sociobiology 48: 247-265.

Krishna, K., D. A. Grimaldi, V. Krishna, and M. S. Engel. 2013. Treatise on the Isoptera of the World. Bull. Am. Mus. Nat. Hist. Number 377.

Liang, W.-R. and H.-F. Li. 2016. Redescriptions of three *Nasutitermes* species (Isoptera: Termitidae: Nasutitermitinae) occurring in Taiwan. Ann. Entomol. Soc. Am. 1-17.

Lim, S. Y. and B. T. Forschler. 2012. *Reticulitermes nelsonae*, a new species of subterranean termite (Rhinotermitidae) from the southeastern United States. *Insects* 3: 62-90.

Roonwal, M. L. 1953. Systematics of Oriental termites. No. 1. A new species of termite, *Heterotermes gertrudae*, sp. nov., from North India (Isoptera, family Rhinotermitidae). *Indian J. Ent.* 15: 115-118.

Roonwal, M. L. 1969. Measurement of termites (Isoptera) for taxonomic purposes. *J. Zool. Soc. India* 21: 9-66.

Roonwal, M. L. and Sen-Sarma, P. K. 1956. Systematics of Oriental termites. No. 3. Zoological Survey of India Collections from India and Burma, with new descriptions of the genera *Parrhinotermes*, *Macrotermes*, *Hypotermes* and *Hospitalitermes*. *J. Agric. Sci.*, 26: 1-22.

Scheffrahn, R. H. and Su, N.-Y. 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. *Fla. Entom.* 77(4): 460-474.

Scheffrahn, R. H., and N.-Y. Su. 2000. Featured creatures—West Indian subterranean termite. University of Florida – IFAS.

<http://entnemdept.ufl.edu/creatures/urban/termites/heterotermes.htm>

Scheffrahn, R. H., S. C. Jones, J. Krecek, J. A. Chase, J. R. Mangold, and N.-Y. Su. 2003. Taxonomy, distribution, and notes on the termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of Puerto Rico and the U.S. Virgin Islands. *Ann. Entomol. Soc. Am.* 96: 181-201.

Snyder, T. E. 1924. Descriptions of new species and hitherto unknown castes of termites from America and Hawaii. *Proc. U. S. Nat. Mus.* 64: 1-40+5 plates.

Snyder, T. E. 1956. Termites of the West Indies, the Bahamas and Bermuda. *J. Agric. Univ. PR.*

Szalanski, A. L., R. H. Scheffrahn, J. W. Austin, J. Krecek, and N.-Y. Su. 2004. Molecular phylogeny and biogeography of *Heterotermes* (Isoptera: Rhinotermitidae) in the West Indies. *Ann. Entomol. Soc. Am.* 97: 556-566.