Species Diversity of Puerto Rican *Heterotermes* (Dictyoptera: Rhinotermitidae) Revealed by Phylogenetic Analyses of Two Mitochondrial Genes

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Abstract

The goal of this study was to infer *Heterotermes* (Froggatt) (Dictyoptera: Rhinotermitidae) species diversity on the island of Puerto Rico from phylogenetic analyses of DNA sequence data from two mitochondrial genes, 16S rRNA and cytochrome oxidase II (COII). This termite genus is a structural pest known to be well adapted to arid environments in subtropical and tropical regions worldwide including Puerto Rico and many other Caribbean islands. Extensive sampling was accomplished across Puerto Rico, and phylogenetic analyses of individual gene sequences from these samples indicated robust datasets of congruent gene tree topologies showing three monophyletic groups: *H. cardini* (Snyder), *H. convexinotatus* (Snyder), and *H. tenuis* (Hagen). We found that *H. cardini* and *H. convexinotatus* were widespread in the arid coastal regions of Puerto Rico, whereas *H. tenuis* was uncommon and may represent a relatively new introduction. We found only *H. convexinotatus* on Culebra Island. We provide strong evidence that Puerto Rico may be linked to the *Heterotermes* in southern Florida, USA, since its GenBank 16S sequence was identical to that of seven Puerto Rican *H. cardini* sequences. Our study represents the first records of *H. cardini* from Puerto Rico and Grand Bahama.

Key words: Caribbean, *Heterotermes cardini*, *Heterotermes convexinotatus*, *Heterotermes tenuis*, molecular phylogenetics

*Heterotermes* (Froggatt) (Dictyoptera: Rhinotermitidae) is a genus of subterranean termites that is found in tropical and subtropical regions worldwide, particularly in arid climates (Constantino 2000). It tolerates drier environments than most other subterranean termites. *Heterotermes* species are important structural pests that can cause severe wood damage that is characterized by a dry, shredded appearance (Scheffrahn and Su 1995). Some species also are known to attack tropical agricultural crops (Sands 1973, Constantino 2002). Termites in the genus *Heterotermes* are well known invasive pests (Bourguignon et al. 2016).

Nine described species of *Heterotermes* occur in the New World in arid regions of the Caribbean, South America, Central America, Mexico, and southwestern United States (Constantino 2011). The majority of species have been described from South America: *Heterotermes asass* (Constantino), *H. convexinotatus* (Snyder), *H. crinitus* (Emerson), *H. longiceps* (Snyder), *H. sulcatus* (Mathews), and *H. tenuis* (Hagen). *H. cardini* (Snyder) was described from the Bahamas (Snyder 1924) in the northern Caribbean (see map in Fig. 1A). *H. maculatus* (Light) is native to Mexico, and *H. aureus* (Snyder) is native to the Sonoran Desert in the southwestern United States and northwestern Mexico. Two of these species, *H. convexinotatus* and *H. tenuis*, have been introduced into the Caribbean from the South American mainland (Evans et al. 2013).

A number of studies have focused on the distribution of termite species in the Caribbean or on specific islands (Snyder 1956, reviewed by Scheffrahn et al. 1994). The genus *Heterotermes* is reported throughout the Caribbean. However, species determinations often are not made because soldiers of most species are difficult to reliably distinguish due to non-robust morphological characters, and winged imagoes are seasonally produced and often are not concurrently collected with soldiers from the same colony. In fact, the earliest researchers noted the difficulty of species determinations. Snyder’s (1924) original descriptions of *H. convexinotatus* and *H. cardini* indicated that it was “very difficult to separate these two species from the soldier caste alone,” with the former having relatively fewer hairs (setae) on the soldier’s head. Snyder (1924) proposed two alternative hypotheses—there was a single but extremely variable *Heterotermes* species or a complex series of very closely related, intergrading species between *H. tenuis* and *H. convexinotatus*.

Two more recent studies have surveyed *Heterotermes* in the Puerto Rican archipelago, the easternmost islands of the Greater
Antilles. Scheffrahn et al. (2003) conducted a comprehensive survey of termites from Puerto Rico and two associated islands to the east, Culebra and Vieques. Note that *Heterotermes* had not been found on Mona Island (Jones et al. 1995), which is to the west of Puerto Rico and the third-largest island in the Puerto Rican archipelago. Scheffrahn et al. (2003) used a morphometric approach and chose not to classify *Heterotermes* to species level given the need for an extensive taxonomic investigation of the genus. Although Snyder’s latter hypothesis was favored based on *Heterotermes* collections from the West Indies (Scheffrahn et al. 1994) and Florida (Scheffrahn and Su 1995), S.C.J. became more convinced of multiple *Heterotermes* species during ongoing research activities in Puerto Rico over a period of decades (1992–2010) including participating in the Puerto Rican termite surveys.

![Fig. 1. (A) The Caribbean Basin, also known as the West Indies, includes the Greater Antilles and the Lesser Antilles along the border of the Caribbean Sea as well as the Bahama Archipelago in the North Atlantic Ocean. The Greater Antilles is comprised of the larger islands including Cuba, Hispaniola (Haiti and the Dominican Republic), Puerto Rico (encircled with dashed line), Jamaica, and the Cayman Islands. The Lesser Antilles is comprised of numerous small islands that form a long, partly volcanic island arc as well as several islands (i.e. Aruba, Curacao, Bonaire, etc.) near the northern coast of South America. The Bahama Archipelago consists of the Bahamas and the Turks and Caicos Islands. (B) Map of the Puerto Rican archipelago showing the locations of samples of *H. cardini*, *H. convexinotatus*, and *H. tenuis* on the main island of Puerto Rico and Culebra Island. Each dot represents a single collection site. Sequence data for the *H. tenuis* sample collected at Dorado, PR, were included in only the COII dataset.](image-url)
Szalanski et al. (2004) used the mitochondrial 16S rRNA gene to study the phylogeny and biogeography of *Heterotermes* in the Caribbean. Based on 59 samples from the Caribbean and 5 from nearby countries, *Heterotermes* species haplotypes were shown to be largely confined to separate regions: *H. cardini* was found in the Bahamas and Cuba, *H. convexinotatus* was rather widely distributed throughout the Greater Antilles, *H. tenuis* was found in the Lesser Antilles, and an undescribed *Heterotermes* sp. was found in the Cayman Islands and Jamaica. The latter haplotype was noted as confounding geographically due to its alignment with a sample from Bonaire, and the species was suggested to readily infest boats. Because a single haplotype was used for each island or cay in an archipelago, the Caribbean samples were spread over 30 islands, and the regions of the Caribbean were disproportionally represented, with the Greater Antilles accounting for 17% (*n* = 10) of Caribbean haplotypes compared with 36% (*n* = 21) for the Bahama Archipelago and 47% (*n* = 28) for the Lesser Antilles. Three samples came from the Puerto Rican archipelago, one each from Puerto Rico (the main island, ~9,104 sq km), Vieques Island (~348 sq km) and Culebra Island (~30 sq km); *H. convexinotatus* was the sole species reported. Given the orders of magnitude size difference of these islands, it seems possible that greater *Heterotermes* diversity may be present on Puerto Rico.

Szalanski et al. (2004) used a single gene in their study, which now is known to often provide insufficient genetic diversity to resolve species identity for termites (e.g. Gentz et al. 2008). This problem is exacerbated for species-level phylogenies as opposed to deeper phylogenies (i.e. comparisons at the genus or family level or higher) due to coamplification of pseudogenes, introgression events, incomplete lineage sorting, and linkage disequilibrium when endosymbionts are present (Funk and Omland 2003; Hurst and Jiggins 2005, Song et al. 2008). The cytochrome oxidase I (COI) gene, a proposed universal barcode for species identification (Hebert et al. 2003) that has been useful for many insects (Cameron 2014), is not widely used for termites because of polymerase chain reaction (PCR) amplification problems and suspected pseudogenes (Hausberger et al. 2011; Roy et al. 2014). We found similar problems when using COI for Puerto Rican *Heterotermes* (T.D.E. and S.C.J., unpublished data).

Consequently, termite genealogies have been further resolved by concordant evidence from multiple mitochondrial genes including 16S, COI, and COII (Lo et al. 2004), or even complete mitochondrial genomes (Bourguignon et al. 2015). The rapid sequence divergence of mitochondrial DNA can provide the resolution required to distinguish among source populations despite relatively short histories of isolation (Avise 2000). Hence, in this study, we used a phylogenetic approach with two mitochondrial genes, 16S and COII, to examine Puerto Rican *Heterotermes* species diversity.

The purpose of our study was to assess the number and distribution of *Heterotermes* species on the main island of Puerto Rico based on phylogenetic analyses using maximum likelihood (ML) and Bayesian inference. We used greater sampling compared with previous studies, with 70 samples representative of the range of this genus on Puerto Rico. We sought to determine whether the 16S and COII datasets separated species of *Heterotermes* into monophyletic groups as this is a useful and practicable measure of species differentiation.

**Materials and Methods**

**Termite Samples**

We collected 70 samples of *Heterotermes* from the main island of Puerto Rico during 5 years (1992, 2002, 2004, 2006, and 2010) and 6 samples from Culebra Island in 2004 (see Fig. 1B). Each sample consisted of a collection of termites from a single access point into a termite colony preserved in 100% ethanol. We collected additional samples from locales where only a single species of *Heterotermes* has been documented including *H. tenuis* from Saint Lucia; *H. cardini* from Grand Bahama; and *H. aureus* from Maricopa, AZ, USA. S.C.J. collected all samples except those in the Bahamas.

We used these preserved *Heterotermes* spp. samples for genetic analyses described in the Materials and Methods section, and we also assessed physical characters of soldiers and winged imagos, when available, using Snyder’s (1924) and Constantinou’s (2000) taxonomic keys. We examined a subset of samples of each species from various collection sites throughout Puerto Rico. We used a stereomicroscope with Auto-Montage 3D imaging software (Synoptics Ltd., Cambridge, UK) to measure specimens. Voucher specimens preserved in ethanol are maintained at S.C.J.’s Entomology lab at The Ohio State University.

**DNA Extraction, PCR, and Sequencing**

We extracted DNA from individual termites (mean = 3; range 1–5) from each of the 76 samples using the DNeasy Blood & Tissue Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer’s protocol. We used PCR to amplify portions of two loci: 16S and COII. We amplified and sequenced the 16S rRNA gene with the forward primer LRJ-13007 (Kambhampati and Smith 1995) and the reverse primer LRN-13398 (Simon et al. 1994). We amplified and sequenced the COII gene in both directions using universal primers modified from Liu and Beckenbach (1992): forward primer A-tLEU (5′-TGGCAGATWAGTGCAMTG-3′) and reverse primer B-tLYS (5′-GTITAAAGAGACACKACTIG-3′).

We performed PCR with a standard 25 μl reaction containing 5–20 ng of total genomic DNA. The reactions for each of the three amplifications had 1 pmol of each primer, 2.5 mM MgCl₂, 1.0 mM dNTPs, and 0.06 U/μl Taq DNA polymerase. We amplified DNA in an Eppendorf Mastercycler Nexus thermocycler (Eppendorf AG, Hamburg, Germany). The procedure included a pre-cycle denaturation at 94°C for 2 min, a post-cycle extension at 72°C for 7 min, and 35 cycles of a standard three-step PCR. The first and third steps were the same (94°C for 1 min followed by 72°C for 2 min) for each primer pair. We ran the second step for 1 min for each primer pair, but at different temperatures: 48°C for 16S rRNA and 52°C for COII. We further purified PCR products using the QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA) in accordance with the manufacturer’s protocol. We then sent the purified PCR products to Eurofins MWG Operon LLC (1044 East Chestnut Street, Louisville, KY 40204) for direct sequencing in both directions. We deposited sequence data into GenBank; the accession numbers are in Supplementary Tables S1 and S2.

For each dataset, we grouped sequences that were identical, but from different termite samples, into a single haplotype. Although individuals from a single sample theoretically could separate into multiple haplotypes, this was not the case and each sample represented an individual colony.

**Phylogenetic Analyses**

We used unique sequences from our *Heterotermes* samples and from GenBank (as of 6-IV-2016) to generate two datasets: 1) 16S rRNA and 2) COII. Note that the COII dataset included a sample from Dorado, PR, which was not in the 16S analysis because PCR was unsuccessful in amplifying the gene. GenBank provided sequences for the 16S and COII genes of *Heterotermes*, but most New World species were poorly represented. For 16S and COII, we truncated all of our sequences at both the 5′ and 3′ ends in order to generate an
alignment that included the shortest GenBank sequence. (Refer to Supplementary Materials S1 for analyses showing that the branching pattern of our Heterotermes sequences was unaffected by the size of the 16S fragment.)

We aligned sequences using the MUSCLE algorithm (Edgar 2004a,b) in the MEGA 5.0 software package (Tamura et al. 2011). The 16S rRNA sequence alignment had a total of 371 nucleotide sites, and COII had 647. We used the Bayesian information criterion metric in the IQTree software package (Minh et al. 2013) for model selection in the 16S and COII datasets, which resulted in the substitution models HKY + G and TIM2 + G, respectively.

We analyzed each dataset using both ML and Bayesian inference, with Reticulitermes flavipes (Kollar) as the outgroup. We used the IQTree software package (Minh et al. 2013) for ML and performed a standard nonparametric bootstrap analysis with 1,000 replicates. We generated a majority-rule consensus tree using a 50% support threshold. We generated the posterior probability or Bayesian inference for each dataset using MRBAYES (Ronquist et al. 2012).

We selected the COII dataset to test species delimitation by the Bayesian Poisson tree process (bPTP) model (Zhang et al. 2013). These authors suggest using a single locus with at least two sequences of each putative species, and only the COII dataset met these criteria as it included haplotypes for four species. Hence, we performed a second ML analysis of the COII dataset for only these four species, and the resulting phylogenetic tree was used as input for the bPTP analysis. The analysis was performed on the bPTP web server at http://species.h-its.org/ptp/.

**Results and Discussion**

ML and Bayesian inference analyses of mitochondrial 16S and COII genes generated trees (Figs. 2–3) indicating the same three monophyletic groups for Puerto Rican samples: H. cardini, H. convexinotatus, and H. tenuis. This diversity was corroborated by the bPTP analysis of the COII gene (Fig. 3). Hence, these two congruent gene trees show a robust picture of gene evolution in these species.

The highly conserved 16S gene differentiated Puerto Rican Heterotermes into three species. The 16S tree (Fig. 2) indicated that H. cardini formed a monophyletic group comprised of 16 haplotypes, including 6 haplotypes consisting of 39 samples from Puerto Rico and another haplotype with the Freeport, Bahamas sample. The haplotypes in this tree included not only H. cardini from Cuba and numerous islands in the Bahamas (Szalanski et al. 2004), but also the undescribed *Heterotermes* sp. from the Turks and Caicos Islands, Cayman Islands, Jamaica, Bonaire, St. Barthélemy, Guadeloupe, and Dade County, FL (Szalanski et al. 2004). Furthermore, the Dade County, FL sample (GenBank AY380254) shared an identical 16S sequence with seven of our H. cardini samples from Puerto Rico. This, as shown in other studies (Bourguignon et al. 2016), argues for human introduction into Florida particularly since these termites can nest undetected in wood and produce replacement reproductives.

16S sequences of *H. convexinotatus* formed a monophyletic group and included Puerto Rico sequences and others in GenBank (Fig. 2). All of our Puerto Rican *H. convexinotatus* sequences (29 samples from Puerto Rico and 6 from Culebra Island) formed a single haplotype. Such low levels of diversity are typical of invasive populations. Our 16S tree also included nine haplotypes representing *H. convexinotatus* GenBank sequences (Szalanski et al. 2004) ranging from as far south as Venezuela, South America, to as far north as the Dominican Republic in the Greater Antilles. Our analyses are in agreement with Szalanski’s et al. (2004) findings that their Puerto Rican *H. convexinotatus* fell into two haplotypes—their sample from Humacao, PR, fell in one haplotype whereas another haplotype contained samples from Culebra and Vieques Islands, the Dominican Republic, and eight states/territories in the Lesser Antilles.

On the 16S tree, *H. aureus* was a sister taxon of *H. convexinotatus* (Fig. 2). *H. aureus* was a monophyletic group represented by two haplotypes, both from Arizona.

Samples of *H. tenuis* also formed a monophyletic group on the 16S tree (Fig. 2); the eight haplotypes included our *H. tenuis* samples collected from Guayanabo, PR, and Anse La Raye, St. Lucia, and GenBank sequences of samples from Central America, South America, and the Lesser Antilles (Trinidad and Tobago, St Vincent, and St Lucia).

The less conserved COII gene also differentiated Puerto Rican Heterotermes into three species. The tree generated from the COII dataset is represented in Figure 3. Our Puerto Rican samples consisted of eight haplotypes of *H. cardini* that formed a monophyletic group. Two of these eight haplotypes were abundant on the main island of Puerto Rico, with one representing 13 samples from 7 different locations and another representing 20 samples from 13 different locations. Rather low levels of diversity are typical of invasive populations. The COII tree also included two haplotypes of *H. cardini* from the Bahamas.

On the COII tree, two haplotypes of Puerto Rican *H. convexinotatus* also formed a monophyletic group, with the sole *H. aureus* haplotype as its sister taxon (Fig. 3). ML analysis indicated that *H. aureus* was basal to *H. convexinotatus* whereas Bayesian analysis showed the opposite trend. One of the *H. convexinotatus* haplotypes represented 2 samples from Ceiba, PR, and the other haplotype represented a total of 33 samples, including those from Puerto Rico (17 locations) and Culebra Island (6 locations).

*H. tenuis* haplotypes also formed a monophyletic group on the COII tree (Fig. 3). Two haplotypes were represented by our samples from Puerto Rico (Dorado and Guayanabo) and St. Lucia, and three haplotypes were GenBank sequences identified as *H. tenuis* from samples collected in three South American countries.

The bPTP analysis was able to differentiate four putative species (*H. cardini, H. convexinotatus, H. tenuis, and H. tenuior*) with high probability based on the phylogenetic tree of the COII dataset (Fig. 3). Therefore, the COII gene separated the three putative species on Puerto Rico into monophyletic groups on the phylogenetic tree and suggested their status as species based on species delimitation.

Both the 16S and COII genes were useful for differentiating *Heterotermes* species, and branch support values on both trees (≥58%) suggested that *H. cardini, H. convexinotatus, and H. tenuis* were monophyletic groups. Although the COII gene fragment was larger and more variable than 16S, it was not better at differentiating species. This indicates that using a single gene, 16S, can be a feasible molecular approach for Caribbean *Heterotermes*.

Both the COII and the 16S genes were congruent for the same three species groups in Puerto Rico, but the COII gene tree appeared to be more useful than 16S for determining ancestry. The COII phylogeny estimate provided a tree that suggested a lineage for *Heterotermes* species with multiple speciation events. This tree suggested that *H. aureus* was ancestral to *H. convexinotatus*. In contrast, the 16S dataset did not effectively determine ancestry of *Heterotermes* species as evidenced by the large polytomy (a section of a phylogeny wherein relationships cannot be fully resolved to dichotomies or monophyletic clades) at the basal node of the tree. Nonetheless, since GenBank does not include all *Heterotermes* species and these phylogenies are incomplete, sequence data from additional species are needed to further resolve ancestry.
Fig. 2. Fifty percent majority rule consensus tree of *Heterotermes* obtained from the mitochondrial 16S rRNA gene using 75 sequences from our study and all 73 unique sequences on GenBank as of 6-IV-2016. Haplotypes representing our Puerto Rican samples are in bold, with each haplotype including only one sample from a single locale unless indicated by a footnote. A species designation is shown for each monophyletic group in the Caribbean Basin. Lines are colored to depict geographic areas in the Caribbean Basin and nearby (refer to map in Fig. 1A): orange indicates the Bahama Archipelago, green indicates the Greater Antilles, yellow indicates the Lesser Antilles, pink indicates South America, and blue indicates the U.S. Support values are indicated at each node for ML, based on 1,000 bootstrap replicates, and Bayesian inference. Missing Bayesian values indicate differences in topology between ML and Bayesian analyses.
Fig. 3. Fifty percent majority rule consensus tree of *Heterotermes* obtained from the COII gene using 76 sequences from our study and all 22 sequences in GenBank as of 6-IV-2016. Haplotypes representing our Puerto Rican samples are in bold, with each haplotype including only one sample from a single locale unless indicated by a footnote. A species designation and its probability value (based on bPTP analysis) are shown for each monophyletic group in the Caribbean Basin. Support values are indicated at each node for ML, based on 1,000 bootstrap replicates, and Bayesian inference. Missing Bayesian values indicate differences in topology between ML and Bayesian analyses.

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a Capitanejo, Guanica (n=2 samples), Laguna Tortuguero (n=3), Las Mayas (n=3), Patillas, Puerto Nuevo, and San Juan (n=2).

b Aguadilla, Barceloneta (n=2), Cabo Rojo, Catano, Coamo, Dorado (n=2), Juncos Beach, Manati, Mayaguez, Sabana Seca (n=6), Toa Baja, and Tres Hermanos Beach.

c Two samples collected in this town.

d Carolina, Ceiba (n=2), Coamo (n=2), Combate Beach, Dorado, Fajardo, Humacao (n=2), Maunabo, Naguabo (n=2), Patillas, Patillas (n=4), Puerto Nuevo, San Juan (n=2), Toa Baja, Tres Hermanos Beach, Vega Alta, and Vega Baja (n=3), Culebra Island (n=6).
Physical characters of Heterotermes soldiers from Puerto Rico supported the designation of three species. Relative pilosity of the head capsule is a primary character presented by Snyder (1924), and consistent with that taxonomic key, samples morphologically determined to be H. tenuis had numerous hairs, followed by H. cardini with few hairs, and H. convexinotatus with very few hairs. One sample had dimorphic soldiers whose tergites had bristles along the posterior margins and one-third length bristles on the surface—characters indicative of H. tenuis (Constantino 2000). Puerto Rican soldiers classified as H. convexinotatus had tergites with bristles along the posterior margins and microscopic hairs on the surface—characters that are consistent with Constantino’s (2000) key. H. convexinotatus soldier head width averaged 0.77 ± 0.03 mm (mean ± SE) (n = 10), approaching the lower range of 0.79–0.84 mm reported by Constantino (2000). H. cardini soldiers were quite variable in size; our measurements of H. cardini soldier head width (0.75 ± 0.03 mm; n = 14) were smaller than 0.78–0.85 mm reported by Snyder (1924) and 0.80 mm (n = 20) for H. cardini from the Bahamas (Szalanski et al. 2004). Since non-robust morphological characters of Heterotermes soldiers predominate in current taxonomic keys, future research from the Jones’ lab will report on morphological characters that can better distinguish these three Heterotermes species on Puerto Rico.

We found winged imagos (alates) at only three sites (two on Culebra Island and one in Coamo, PR [Fig. 1B]), all during mid-July 2004. The physical characteristics of this caste were consistent with H. convexinotatus (Snyder 1924) as specimens (n = 17) had convex posterior margins of the meso- and metanota (hence the species epithet), wing scales covering the mesonotum—unlike H. cardini, tergites with posterior bristles and interior microscopic hairs, compound eyes less than their long diameter from lateral margins of the head, head width 0.88 ± 0.01 mm, pronotum width 0.79 ± 0.01 mm, and forewing length 7.89 ± 0.08 mm. Our genetic data likewise supported this species’ designation. Our alate measurements generally correspond with, but are slightly larger than those reported for nine H. convexinotatus alates from unspecified locations in Groups V and VI (Szalanski et al. 2004), representing coastal Venezuela, Hispaniola, Puerto Rico, and numerous neighboring islands in the Greater and Lesser Antilles.

Our study revealed that H. cardini and H. convexinotatus were widespread in the arid coastal regions of Puerto Rico (Fig. 1B). H. cardini and H. convexinotatus were represented by 39 and 29 samples, respectively, suggesting that H. cardini may be somewhat more prevalent. Our genetic data indicate no obvious species groupings based on geographic location. This is indicative of gene flow. Colonies that were more distantly separated on the island of Puerto Rico were sometimes more closely related genetically than colonies that were geographically closer together. H. convexinotatus was the only species that we collected on Culebra Island.

H. tenuis appeared to be uncommon on Puerto Rico and may be a more recent introduction. We collected only two H. tenuis samples in Puerto Rico (Guaynabo [March 2006] and Dorado [June 2010]; Fig. 1B) and none was reported in other recent studies (Scheffrahn et al. 2003, Szalanski et al. 2004). Guaynabo is one of three municipalities that compose the south side of the Port of San Juan, one of the busiest ports in the Caribbean. This sample was collected from the grounds of an industrial site, whereas the sample from Dorado was from the landscape near a highway. Since H. tenuis is suspected to have originated on the mainland of South America and already has invaded the islands of the Lesser Antilles, Puerto Rico would be a likely target given its proximity as the easternmost island of the Greater Antilles (Fig. 1A). Snyder’s (1956) report of H. tenuis as a common species in Puerto Rico appears to be in error.

Our study indicates the need for some revisions to the current phylogeography of Heterotermes species in the Caribbean Basin (i.e. West Indies [Fig. 1A]) (Szalanski et al. 2004). As indicated in Figure 2, wherein colored lines depict geographic areas in the Caribbean Basin and nearby, the geographic distributions of H. cardini, H. convexinotatus, and H. tenuis are not mutually exclusive, but rather overlapping. Furthermore, the proposed Heterotermes sp. (Szalanski et al. 2004) from Grand Cayman and Jamaica is consistent with H. cardini—our 16S tree revealed that these samples are a genetically close match to H. cardini (Fig. 2), and they also are morphologically closest to H. cardini (Szalanski et al. 2004). However, we are not excluding the possibility that undescribed Heterotermes sp. occur in the Caribbean Basin. The current geographic distribution of H. cardini extends from the Bahamas only into Cuba, but our phylogenetic analyses suggest that H. cardini also occurs in the Turks and Caicos in the Bahama Archipelago; it has a much larger distribution in the Greater Antilles as it also occurs in Puerto Rico, Jamaica, and Grand Cayman; and it extends into the Lesser Antilles as far south as Bonaire. This results in overlapping geographic distributions of H. cardini and H. convexinotatus. Our data also suggest that the current geographic distribution of H. convexinotatus extends into the Lesser Antilles. Whereas H. tenuis currently is thought to be restricted to the Lesser Antilles in the Caribbean, our study revealed that it was present in 2006 in Puerto Rico, and although scarce as of 2010, it may be a relatively recent invader in the Greater Antilles. Additional samples, particularly from the other large islands in the Greater Antilles, may reveal its presence elsewhere in the Caribbean. Our study suggests that large sample sizes from the numerous island nations in the Caribbean Basin are needed to better delineate the phylogeography of Heterotermes species. We recognize that Heterotermes are invasive species (Evans 2011) and their distributions are expected to change over time; strong baseline data can help to document their spread in the Caribbean Basin.

Our phylogenetic data also provide strong evidence that Puerto Rico, rather than Grand Cayman or Jamaica (Szalanski et al. 2004), is linked to the introduction of a Heterotermes sp. into southern Florida, USA, given that it shares an identical 16S sequence with seven of our H. cardini samples from Puerto Rico (Fig. 2). Based on our data, the Florida specimen is likely H. cardini rather than an undescribed Heterotermes sp. (Szalanski et al. 2004).

Our phylogenetic analyses indicate that termites in the genus Heterotermes are invasive to Puerto Rico. Human-mediated introductions undoubtedly play a large role in the introduction of exotic species. Islands act as sinks for invasive species because islands typically have to import so many goods from other areas. Furthermore, termites can raft on wood to new locations throughout the Caribbean. On small islands, one also can expect unimpeded movement of materials containing termites such as wood and soil.

Prior to the current study, there were 21 known species of termites on Puerto Rico (Scheffrahn et al. 2003), with one designated as Heterotermes sp. Our study raises the total count to 23 species and suggests that 3 Heterotermes species occur on the main island: H. cardini, H. convexinotatus, and H. tenuis.

New records reported herein include H. cardini from Puerto Rico and Freeport, Grand Bahama. Another rhinotermitid, R. flavipes, predominates on islands in the Bahamas, but our new record does not lend support to the hypothesis that Heterotermes species have been displaced by R. flavipes on Grand Bahama (Szalanski
et al. 2004, Scheffrahn et al. 2006). Perhaps *H. cardini* is a relatively new introduction to the island of Grand Bahama or it simply may be scarce.

Our study provides the most comprehensive phylogeny, to date, of *Heterotermes* in Puerto Rico, revealing the presence of *H. cardini*, *H. convexusnotatus*, and *H. tenuis*. With the exception of the latter species, these termites were widespread in the arid coastal regions of Puerto Rico. Using large sample sizes, the mitochondrial 16S and COII genes both provided high resolution trees that differentiated the three species into monophyletic groups.

**Supplementary Data**

Supplementary data are available at *Journal of Insect Science* online.

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