Exploring the phylogeographic patterns and genetic diversity of urban cockroaches (Dictyoptera:Blattodea) of Bogota, Colombia.

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ABSTRACT

Cockroaches are one of the most successful insects on the planet, due to morphological and physiological adaptations which allow them to have a wide distribution. Some species can inhabit human dwellings, spread diseases and microorganisms' reason why they are considered domiciliary pests. Here, I wanted to identify the species of urban cockroaches living in three localities of Bogotá D.C; establish their phylogenetic relationships to other populations across the world and explore the genetic diversity and genetic population structure among the three localities. To accomplish these goals, I used two mtDNA amplicons: the ribosomal 16S (~400bp) fragment and the barcode gene COI (~600bp). I collected a total of 44 individuals across the localities sampled: Bosa, Kennedy, and Chapinero. From those, I molecularly identify 1 individual of *Periplaneta americana*, 1 of *Periplaneta brunnea* and 42 individuals of *Blattella germanica*. The phylogenetic reconstructions didn't show a clear geographical pattern of relatedness for the three species, consistent with human-mediated dispersion. For the most common species, *Blattella germanica*, I was able to establish genetic diversity and population differentiation across the three localities within the city. Our results suggest, a high genetic diversity for the COI gene, however, this diversity was not explained by the geographical proximity of the populations. Finally, the population structure F_{ST} among these populations indicates an existent genetic flow across the evaluated localities in the city, which may indicate that cities do not represent a barrier and that human-mediated transport could be helping in the genetic exchange for these populations.

Keywords: Cockroach, Blattodea, pest, genetic diversity, urban areas, Neotropics

RESUMEN

Las cucarachas son uno de los insectos más exitosos del planeta debido a sus adaptaciones morfológicas y fisiológicas que les permiten tener una amplia distribución en el mundo. Algunas especies tiene la habilidad de habitar viviendas humanas y propagar enfermedades y microorganismos, razón por la cual son consideradas pestes domiciliarias. Aquí queremos identificar las especies de cucarachas urbanas habitando en tres localidades de Bogotá D.C, establecer sus relaciones filogenéticas con otras poblaciones de la misma especie a través del mundo y explorar la diversidad y estructuración genética entre las localidades evaluadas. Para lograr estos objetivos usamos dos amplicones de ADNmt: el fragmento ribosomal 16S (~400pb) y el gen Barcode COI (~600pb). Colectamos un total de 44 individuos en las localidades de Bosa, Kennedy y Chapinero. Molecularmente se identificó 1 individuo de *Periplaneta americana*, 1 de *Periplaneta brunnea* y 42 individuos de *Blattella germanica*. Las reconstrucciones filogenéticas no mostraron un patrón geográfico claro de parentesco para las tres especies, consistente con la dispersión mediada por humanos. Para la especie más común, *Blattella germanica*, se estableció la diversidad genética y la diferenciación poblacional en las tres localidades estudiadas. Nuestros resultados sugieren una alta diversidad genética para el gen COI, sin embargo esta no está explicada por una proximidad geográfica entre las poblaciones. Finalmente, la estructuración genética (F_{ST}) entre las poblaciones indica un flujo genético existente entre las localidades, sugiriendo que estas no representan una barrera y que el transporte mediado por humanos puede ayudar en el intercambio genético para estas poblaciones.

Palabras clave: Cucarachas, Blattodea, peste, diversidad genética, áreas urbanas, Neotrópico

INTRODUCTION

Population genetics and phylogenetic reconstructions can provide insights into adaptation and speciation processes (Slatkin, 1985). Urban areas are defined as human settlements with high population density and enough infrastructure that can create an environment. The process of urbanization has changed the natural environments into different categories like, cities, towns and suburbs. In Colombia, Bogota is the largest metropolitan area which more than 7.1 million inhabitants (DANE, 2018). As we now consider these urban areas as fast changing ecosystems (Alberti & Marzluff, 2004) the infrastructure (e.g. roads, buildings, parks) within these cities can be considered as possible drivers of isolation for populations, and therefore can be shaping the genetic structure of populations that inhabits the cities or highly urbanized places (Miles, Rivkin, Johnson, Munshi-South, & Verrelli, 2019). On the other hand, the migration of individuals in the cities can be human mediated considering the transit between places in the city and even between other cities; suggesting a panmictic population (Vargo et al., 2014).

Because of its high capacity to infest human structures and their fast life cycle along with its implication with the mechanical transmission of infectious and/or pathogenic organisms; cockroaches are considered pests (Gutiérrez et al., 2017). Nowadays, at least six species of cockroaches inhabit human structures (Fig. 1; Bell, Roth, & Nalepa, 2007) including *Blattella germanica* and *Periplaneta americana* that are the major species found in human dwellings around the world (Sriwichai, Nacapunchai, Pasuralertsakul, Rongsriyam, & Thavara, 2002; Vahhabi et al., 2011). Specifically, *B. germanica* is an obligate commensal species in human-built structures and is not known to inhabit rural outdoors and is a worldwide pest (Roth, 1985; Resh & Cardé, 2003). Contrary to *P. americana* that can be found in rural environments and human-built structures and is widely distributed around the world but does not extend into the temperate regions (Roth, 1982; Resh & Cardé, 2003; Cochran, 1999).

Another important urban cockroach is *Blatta orientalis* that have a restricted distribution to temperate regions and inhabit in basements and crawls spaces under buildings as well as outdoors (Resh & Cardé, 2003). On the other hand, other urban cockroaches like *P. brunnea* and *P. australasiae* tend to be outdoor cockroaches in tropical and subtropical regions (Cochran, 1999) while *Supella longipalpa,* a cosmopolitan pest, occupies homes and other buildings like *B.*

germanica (Resh & Cardé, 2003). Likewise, all urban cockroaches' species can disperse using human vectors (i.e., buses and trucks), hence, it is important to understand how the populations are changing along the city to incorporate this information for new public health and pest control strategies.

Recently, in Colombia, researchers explored the genetic structure among several populations of *P. americana* from different cities, including Santiago de Cali, Popayan and Buenaventura (Jaramillo-Ramirez et al., 2011). They used AFLPs markers, and establish that these evaluated populations where structures, meaning that there was some degree of genetic isolation among the population evaluated of *P. americana* (Jaramillo-Ramirez et al., 2011). Until now, there no studies that explore the diversity, at species and genetic level across the largest city in Colombia, Bogotá of these common human insects. Here, I wanted to answer the following questions: 1) Which species of urban cockroaches are in Bogota? 2) How are the Bogota individuals related to other populations of these species around the world? And 3) Is there high or low genetic structure across different localities within the city? To answer these questions, I estimated the genetic diversity of urban cockroaches' populations in three different districts within Bogotá using two mitochondrial gene fragments (Cytochrome oxidase I and ribosomal 16S). These data allow me to explore evolutionary patterns for multiple urban cockroaches in a high elevated city; and will contribute to generate new protocols of pest control in the city and possible new patterns of fast evolution within urban areas.

MATERIAL AND METHODS

Taxon Sampling

A total of forty-four individuals were collected in three localities (16 individuals of Bosa, 20 individuals of Chapinero and 8 individuals Kennedy, Fig. 2A) of Bogotá City from July to August of 2019 using sticky traps and manual capture during the night (Fig. 2B). We sampled in buildings, bakeries and restaurants within the three selected localities. After collection, individuals were kept on individual Eppendorf tubes with ethanol solution 90%-100% at 80°C to preserve the tissue and genetic material. (See supplementary material taxon sampling)

DNA extraction, amplification and alignment

I extracted DNA from the legs of the individuals using a *DNeasy Tissue Qiagen Kit* following the manufacturer's protocol, except for the following variations: incubation of samples in proteinase K at 56°C for 24h. I amplified two mitochondrial fragments, one protein encoding region: *Cytochrome Oxidase I* (COI) and one ribosomal subunit (16S) using the primers LepF1-CLeoFoIF & LepR1-CLeoFoiR and 16sF & 16sAR respectively (Brandon-Mong et al., 2015; Sanchez Herrera et al., 2018; Simon et al., 1994). The PCR conditions for COI were as follows: 94°C for 5 min, then 30 cycles of 94°C for 30s, 49.8°C for 30s, 72°C for 30s, and a final step of 72°C for 10 min. The PCR conditions for 16s were as follows: 94°C for 5 min, then 35 cycles of 94°C for 30s, 52°C for 30s, 72°C for 30s and a final step of 72°C for 7 min (Modified from Kang et al., 2015). I performed the purification protocol for the PCR products (ExoSap *BioLabs*) and MACROGEN Inc. (Seoul, Korea) performed the Sanger DNA sequencing. All fragments were aligned using MUSCLE (Edgar, 2004) and MAFFT (Katoh, Misawa, Kuma, & Miyata, 2002) with Geneious R 11 platform (Kearse et al., 2012), subsequently, the alignments were then checked and manually adjusted in Mesquite and Alliview (Larsson, 2014; Maddison & Maddison, 2018).

Phylogenetic analysis

I retrieved the sequences from GenBank corresponding to the COI and 16S fragments for the species of cockroaches found (See Accession Numbers in supplementary table S2). I performed an alignment with the sequences obtained experimentally and the sequences of GenBank following the specification previously mentioned. I made phylogenetic analysis according to the following two criteria of Maximum Likelihood (ML) and Bayesian inference (BI). For ML, we obtain models of DNA substitution with ModelFinder (Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermiin, 2017) , IQ-tree topologies and we made 10.000 pseudoreplicates of Ultra-Fast Bootstraps (Hoang, Chernomor, von Haeseler, Minh, & Le, 2007) and a single branch test of SH-aLRT in IQ-TREE software (Nguyen,Schmidt, Von Haeseler & Minh, 2015).

Prior to Bayesian Inference analysis, I used the Bayesian Information Criteria (BIC) in IQTREE ModelFinder (Kalyaanamoorthy et al., 2017) to select an appropriate model of evolution for the evaluated genes. For the BI, I implemented in Mrbayes (Huelsenbeck & Ronquist, 2001) four chains of MCMC, 10 million generations and sampling each 5000, after we made burning of 10% and we obtain a consensus of the best topologies obtained to calculate the posterior probability. In the case the model could not be implemented in MrBayes we use the GTR model for estimate the consensus topology and the posterior probability.

Population analysis

I made haplotypes networks for each gene using Median Joining Networks (MJN) method in POPArt (Leigh & Bryant, 2015) to establish the patterns of genetic diversity inside and between the populations of each species. Also, I calculated the populational statistics as nucleotide diversity (π), the number of segregating sites (S) and the Watterson estimator (θ) in Arlequin v3.5.2.2 (Excoffier & Lischer, 2010). To determine the degree of population structure between the populations F_{ST} values were calculated with a 95% statistical significance and 1000 randomizations to establish the patterns of population structure. Finally, we performed an AMOVA in ARLEQUIN (Excoffier & Lischer, 2010) with 1000 permutations for each species to know if the genetic variation present is stronger inside or between the populations.

RESULTS

I was able to recover three species of cockroaches from the 44 individuals collected in the three localities, *Periplaneta americana, P. brunnea* and *Blattella germanica* (Fig. 3). The most abundant species, *Blattella germanica*, was found in all three localities while *P. americana* and *P. brunnea* were found only in Kennedy location. Also, *B. germanica* individuals was found in different types of indoor constructions while *P. brunnea* and *P. americana* individuals were found only in houses.

Phylogenetic analysis

At a worldwide scale, we recovered two monophyletic clades, one for *P. americana* and the other *P. brunnea* in both COI and 16S genes (Figs. 4 & S1). Our sample of *P. brunnea* VA001 seems to be more closely related to individuals from Bangladesh than to other American members (Fig. 4). On the other hand, most of *P. americana* individuals in GenBank are coming from the USA, and our only sample MV002 was closely related to individuals from USA, Bahamas and South Korea (Fig. 4).

For *B. germanica,* at a worldwide scale there we recovered incongruence among the phylogenetic reconstructions for both genes COI and 16S. For COI, our Bogota populations were recovered as a monophyletic well- supported clade (Fig. 5) closely related to individuals from Asia, the Middle East and Europe. For 16S, there was no monophyletic clade for our Bogota samples they were scattered across the phylogenetic trees and seem to be equally related to other Asian and American individuals (Fig. S2). At a local scale, we recovered low supported clades with no clear locality association, however it seems that most of the individuals of Bosa and Chapinero are more closely related (Fig. S3).

Genetic diversity patterns

All genetic and populations statistics were obtained only for *Blattella germanica*. The haplotype network with COI gene shows a high genetic diversity pattern across the sampled populations (Fig. 6A). However, the diversity observed seem not to be explained by the district of sampling. All three localities, Bosa, Chapinero and Kennedy seem to have shared haplotypes and some unique haplotypes, that some are separated by significant number of mutational steps (Fig. 6A). Moreover, Bosa and Chapinero are showing a higher level of shared haplotypes, which is surprising because Bosa and Kennedy are contiguous districts, so we expected them to share more haplotypes among each other. For 16S, there is lower genetic diversity most likely because it is a more conserved gene for this species (Fig. 6B).

For all the population genetic statistics estimated we saw lower genetic diversity for the 16S gene than for the COI. However, Kennedy population has the highest genetic diversity followed by Chapinero. Bosa genetic diversity is almost is very low in comparison with the other populations (Table 1). Additionally, the analysis of molecular variance (AMOVA) for *B. germanica* individuals shows that most of the genetic variation is within each population (See Table S3). While among populations it was only 23,98 % (P= 0.00098) of the total variation for 16S and 12,44% (P=0.04594) for the COI gene (Chapinero, Kennedy and Bosa). Worth mentioning that all p-values were significant (P>0,05).

Population structure

Our estimates of population structure agree with the patterns of genetic diversity observed in the geographical zones. For the Kennedy location we found a high and significant F_{ST} values compared with the others location evaluated in the 16S gene (Table 2) indicating that this population has some genetic structure. Although, the Bosa and Chapinero populations show almost zero F_{ST} values between them indicating low or zero genetic structure with each other. The above correspond with the haplotype network where Bosa and Chapinero are grouped in the same haplotype and Kennedy is separated from them. Otherwise, in the COI gene we observed that the F_{ST} values are high but mostly are not significant. However, these values show that exist some sort of genetic diversity between the population in Bogota that supports the haplotype network where the samples form few shared haplotypes between the populations.

DISCUSSION

We were able to establish at least for the three localities evaluated that the most common urban cockroach in Bogota city was, *Blatella germanica.* One possible explanation could be a sampled biased, because most of my sampling was done in intra-domiciliary zones, especially houses, restaurants and apartments. It has been reported that *B. germanica* is geographically restricted to human environments and depends completely of them (Jeffery et al., 2012; Roth, 1985), contrary to other *Periplaneta sp.* species that can be found in natural and human environments (Bell et al., 2007; Jaramillo-Ramirez, Cárdenas-Henao, González-Obando, & Rosero-Galindo, 2011). Despite not evaluating individuals captured in the peri-domiciliary zones, I still was able to detect an environment sustainable for *P. americana* and *P. brunnea.* Moreover, it has been reported that multiple cockroach pest species can coexist and or have interspecific competition under the appropriate conditions of food and water, for example: Bujang & Lee (2010) report these behaviors for *B. germanica* and *Symploce pallens.* Similarly, Boyer & Rivault (2004) report interspecific competition between *P. americana*, *P. australasiae* and *Blatta orientlais* species when they share the same shelters resulting in special and/or temporal segregation according with their dominant/subordination relationships. Thus, *B. germanica*, *P. americana* and *P. brunnea* could being experimenting interspecific competition in localities (Chapinero Kennedy, and Bosa) where they are found giving and alternative explanation to the dominance of one species over the others in different localities.

When I looked at the global phylogenetic relationships of the Bogota populations for the three species, I found an expected pattern for a highly invasive pest species, a panmictic population (Figs. 4 & 5). For *Blatella germanica,* I recovered a monophyletic clade for all the individuals collected in Bogota for the COI gene fragment, and it seems to be related to other individuals from Europe, Middle East and China (Fig. 5). Although, I found a high diversity of COI haplotypes for this species within the city, the 16s phylogenetic reconstructions show no particular pattern suggesting is the same species (Supplementary Fig. S2). Previously, Vargo et al. (2014) conclude that at least for, *Blatella germanica,* globally they show low genetic structure; most likely due to many centuries of human transport and a lack of migration-drift equilibrium. Particularly, for *Periplanata* *americana*, I had only one individual, but the barcode marker was clustered with individuals from New York City, suggesting that possibly the source population for this individual comes from North America (Fig. 4 & Supplementary Table S2). Von Beeren et al. (2015) reported that *Periplanata americana*, had three deeply divergent and widely distributed haplotypes for the COI. They suggest that this diversity genetic pattern might be due to multiple introductions from genetically divergent source populations, followed by interbreeding in the invasive range (Von Beeren et al, 2015). For *Periplanata brunnea* our phylogenetic tree suggest that it closely related to individuals from Bangladesh (Fig. 4), however there is not enough genetic information of this species at a global scale in Genbank to draw any conclusion.

When I looked at the population analyses of *Blatella germanica* within the three localities evaluated the city. I found a similar pattern of high divergent COI haplotypes (Fig. 6 & Supplementary Table S3) previously reported in other cities such as New York City for other species like *Periplanata americana* (Von Beeren et al, 2015). While, the 16S marker showed a more conservative pattern (Fig. S1 & Supplementary Table S3), however other nuclear genes or other genomic approaches will be better to establish the patterns of diversity. The presence of common haplotypes among the populations from Chapinero, Bosa and Kennedy from two mitochondrial genes suggest that the city not act as an important barrier to gene flow. Therefore, the urban cockroaches' species found can exchange genes between population through natural dispersion or human-assisted migration of individuals and ootheca in transports like cars, buses or trucks being the last one the most likely due to the limited active spread of individuals (Vargo et al., 2014). Also, the intra-domiciliary is a substantial location where *B. germanica* populations maintain large numbers with high genetic diversity, which can be observed in the AMOVA results, which showed a significant variance between individuals within the population for 16S (76.02%) and COI (87.56%) genes. According with Schal (1990) factors including environment, resources and environmental stability can guarantee reproductive success and therefore favor the establishment of large populations of dominant species. In addition, the genetic diversity seems to be not affected by intraspecific competition between individuals of the same population, Tsai & Lee (2001) indicate that *B. germanica* has low intraspecific competition in domicile habitats, therefore resident individuals and conspecific intruders could mix homogeneously favoring genetic diversity and reducing genetic structure between populations across the city. Further studies increasing the population sampling, and other genomic approaches (RADseq) will be necessary to explore the connectivity within the city and worldwide population of these effective pest species. These types of studies are important to understand how globalization and urbanization can change natural ecosystems, and how we can build effective management for invasive species that can affect our public health.

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TABLES

Table 1. Genetic diversity indexes for 2 mitochondrial genes (16S and COI) in *B. germanica* populations sampled in Bogota. **Theta (S)**: θs, **Theta (Pi)**: θπ and **ND:** nucleotide diversity (π).

16S			
POPULATION	**Theta(S)**	**Theta(pi)**	**ND**
Chapinero	3.61338	2.338095	0.006758
Kennedy	1.313869	4	0.011834
Bosa	0	0	0
COI			
POPULATION	**Theta(S)**	**Theta(pi)**	**ND**
Chapinero	20.576482	25.189474	0.063933
Kennedy	31.094891	67.8	0.160664
Bosa	1.506828	1.508333	0.004132

Table 2. F_{ST} values for two mtDNA genes (16S and COI) from three *B. germanica* populations in Bogota.

Populations	***16S***	***COI***
Chapinero /Bosa	0.0000^{NS}	0.03469^{NS}
Chapinero /Kennedy	0.30786*	0.08682^{NS}
Bosa /Kennedy	0.60166*	0.38534^{NS}

NS, Not significant
* Significant, P value < 0,05

FIGURES

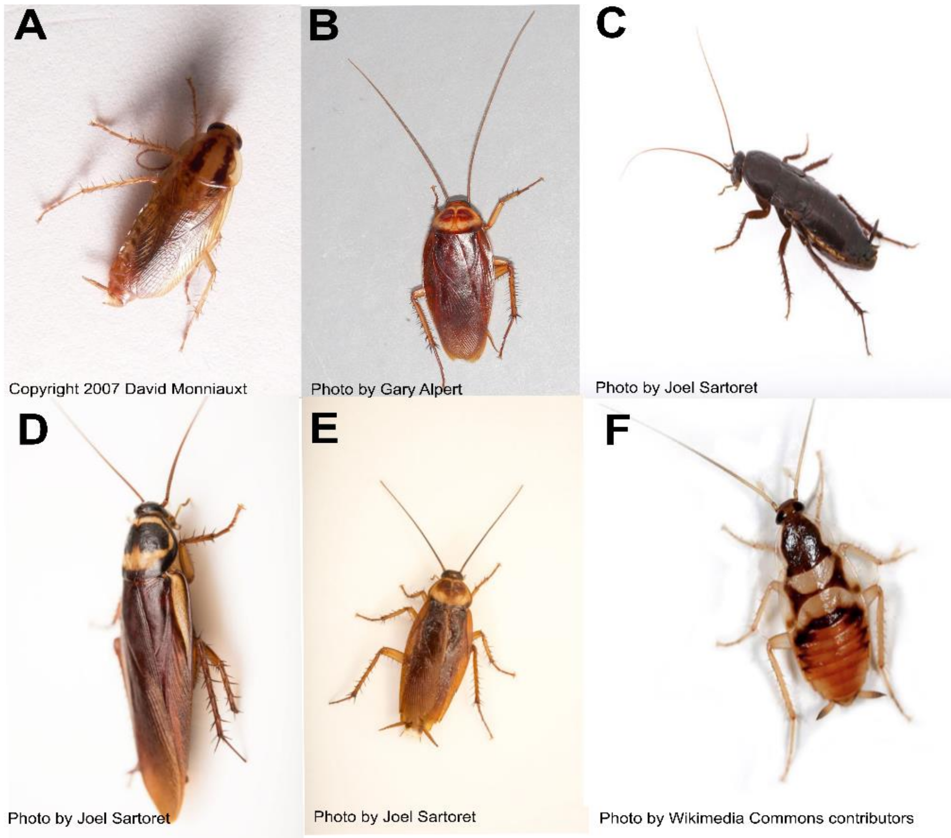

Figure 1. Major cockroach's species found in human dwellings around the world. A. *Blattella germanica* B. *Periplaneta americana,* C. *Blatta orientalis*, D. *Periplaneta australasiae,* E. *Periplaneta brunnea,* and F. *Supella longipalpa.*

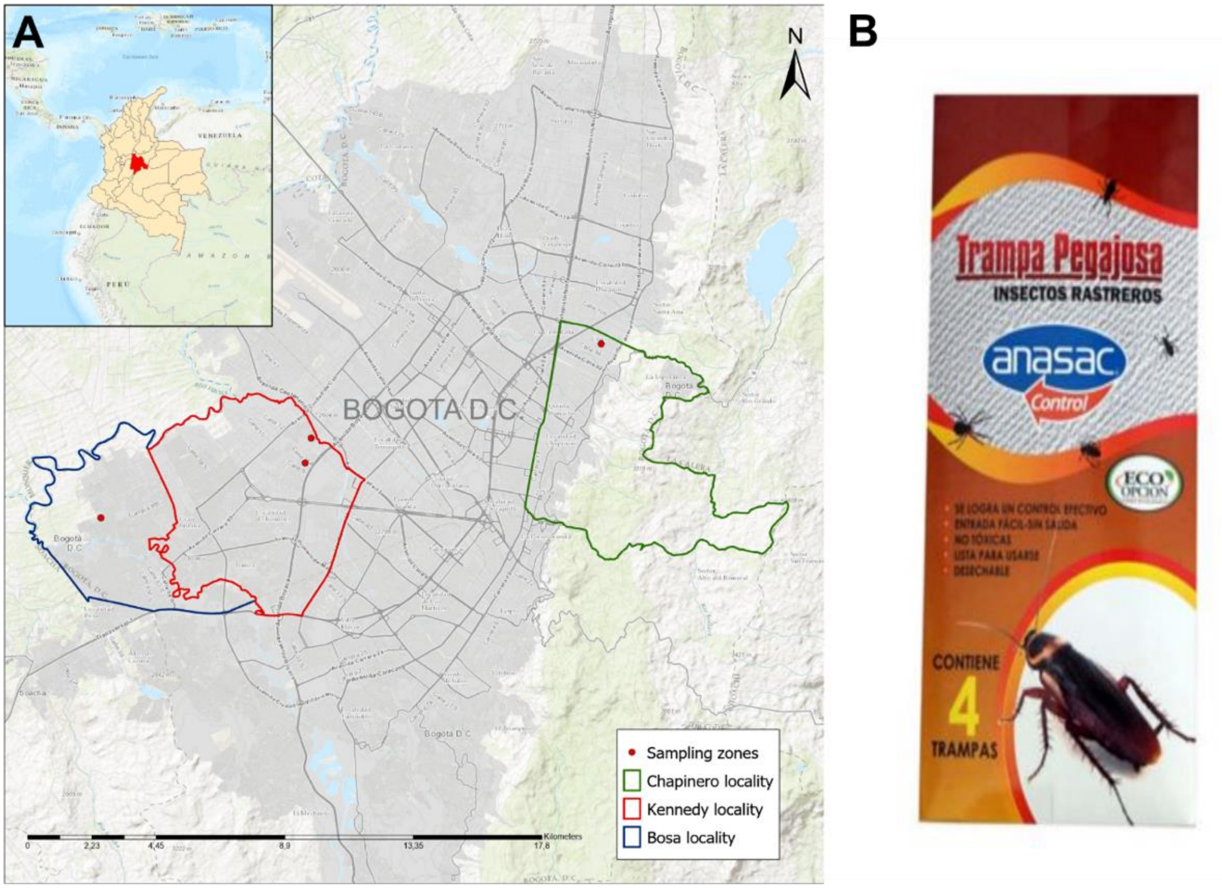

Figure 2. Sampling loculations and methods for cockroaches capturing. A. Geographical distribution of 43 individuals collected in Chapinero, Kennedy and Bosa localities of Bogotá, Colombia. **B.** Capturing methods in the field.

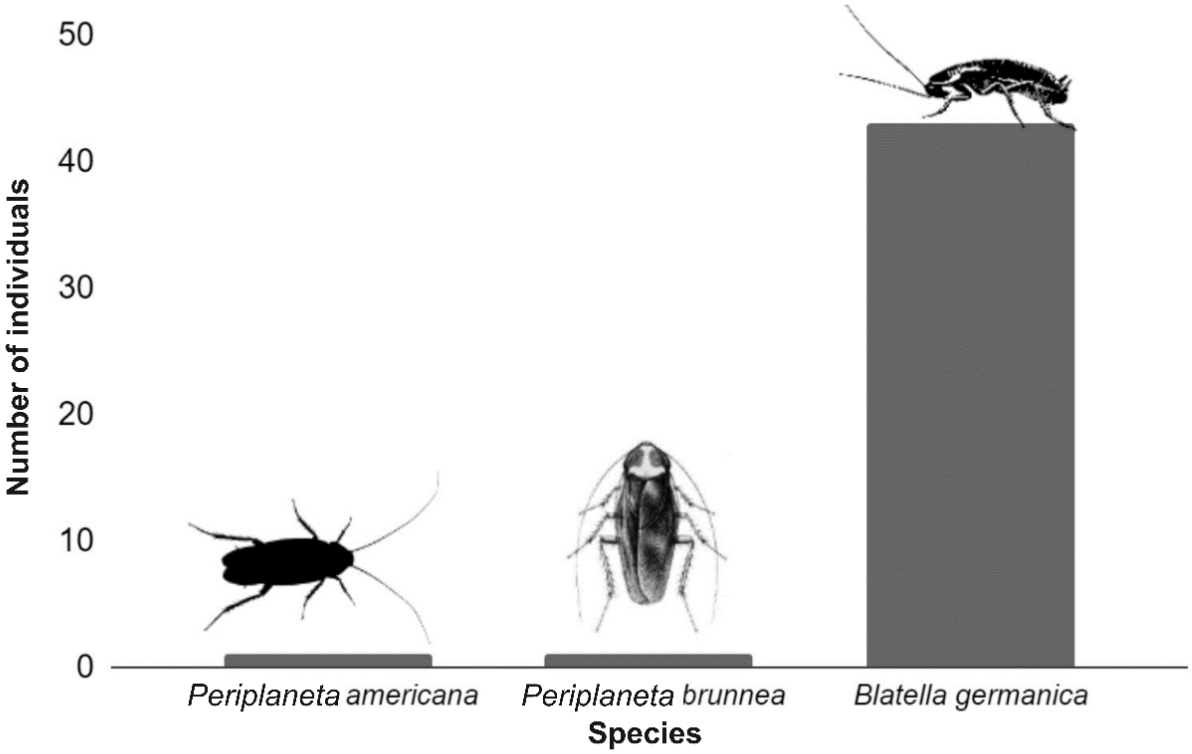

Figure 3. Cockroach abundance in the three localities of Bogota. In the Y-axis in the number of individuals.

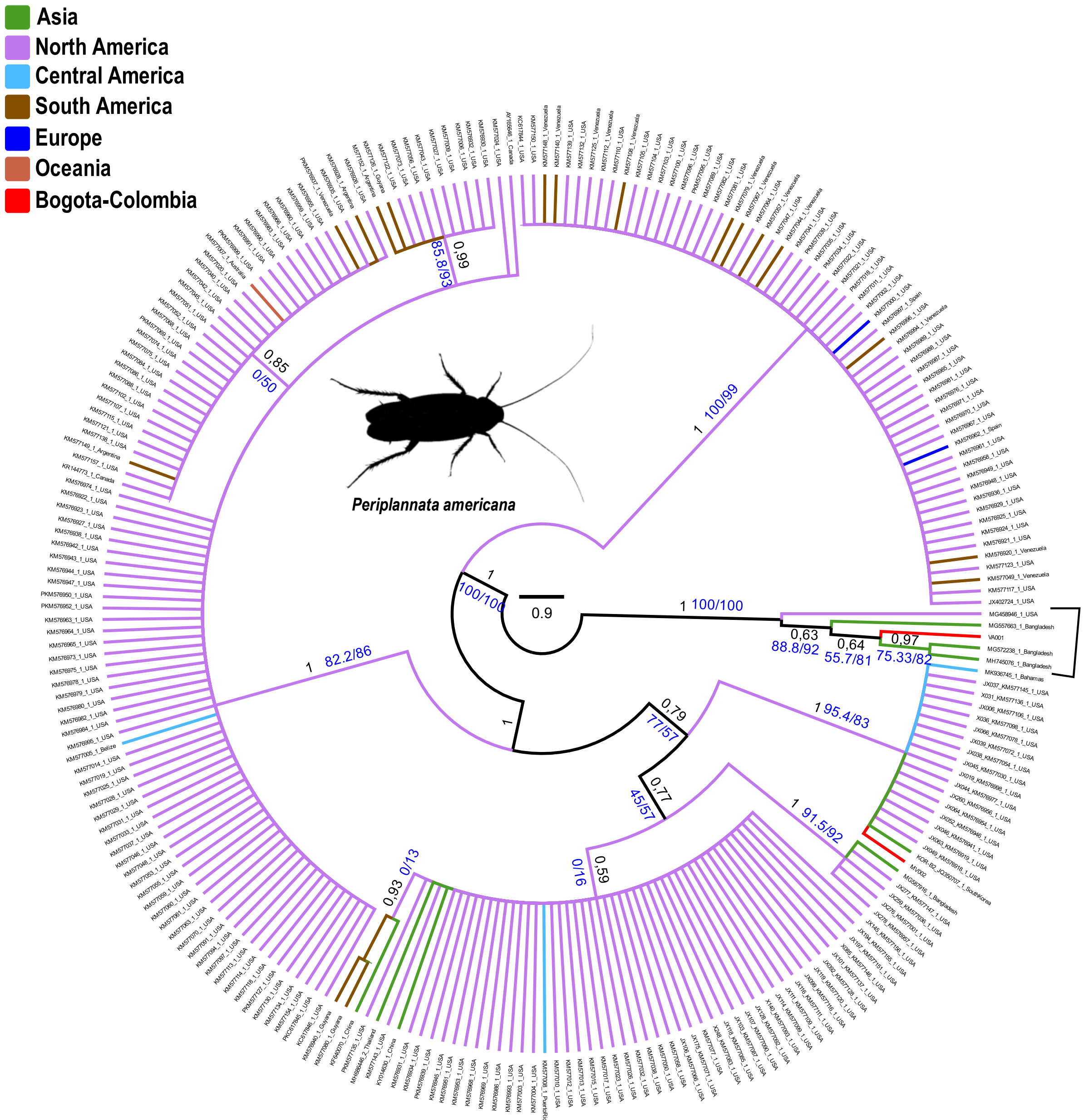

Figure 4. Phylogenetic tree of *Periplaneta sp.* with COI gene. Black values correspond to IB posterior probability and blue values ML UFboot over SH-aLRT test values.

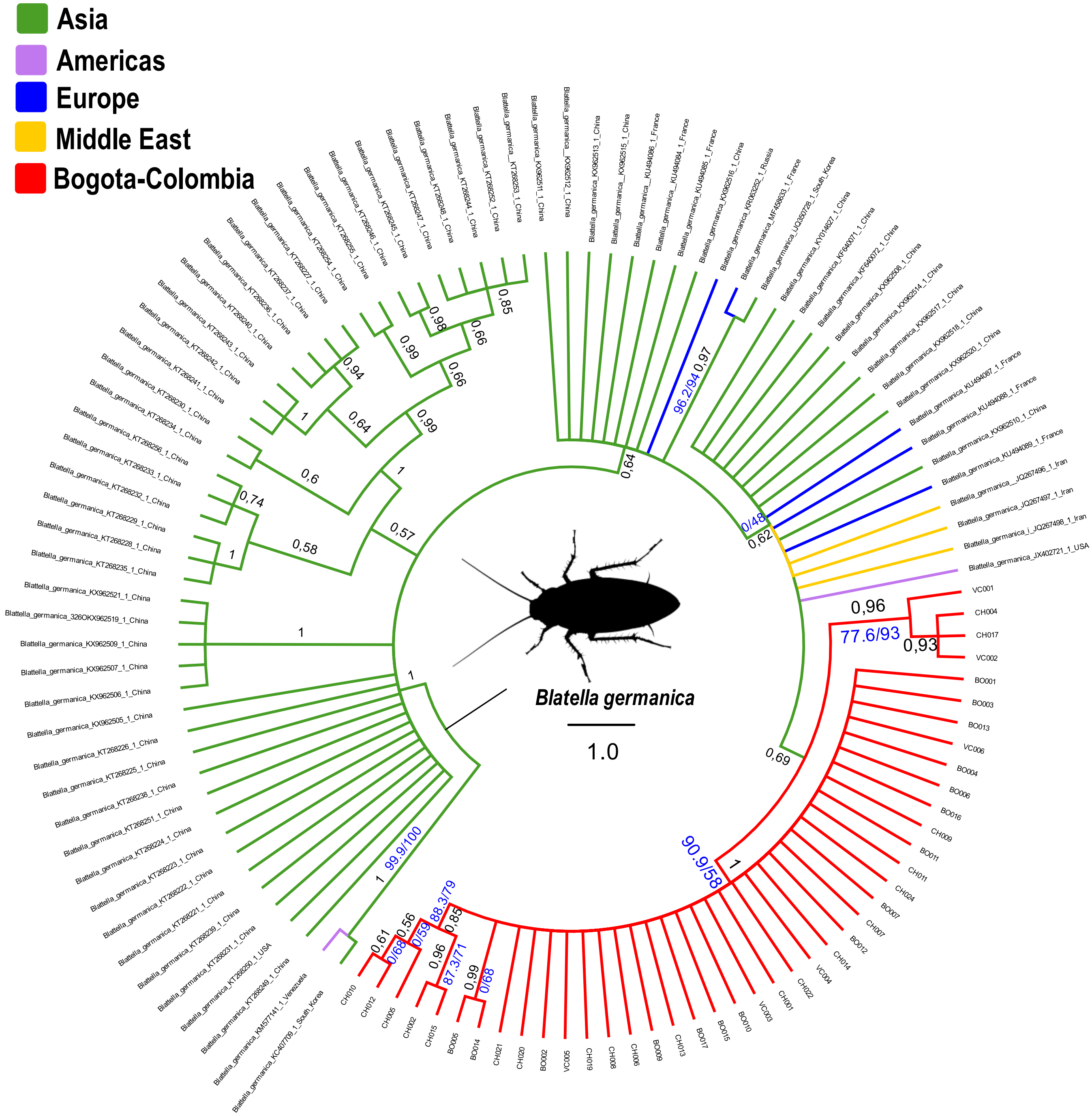

Figure 5. Phylogenetics tree of *Blattella germanica* with COI gene. Black values correspond to IB posterior probability and blue values ML UFboot over SH-aLRT test values.

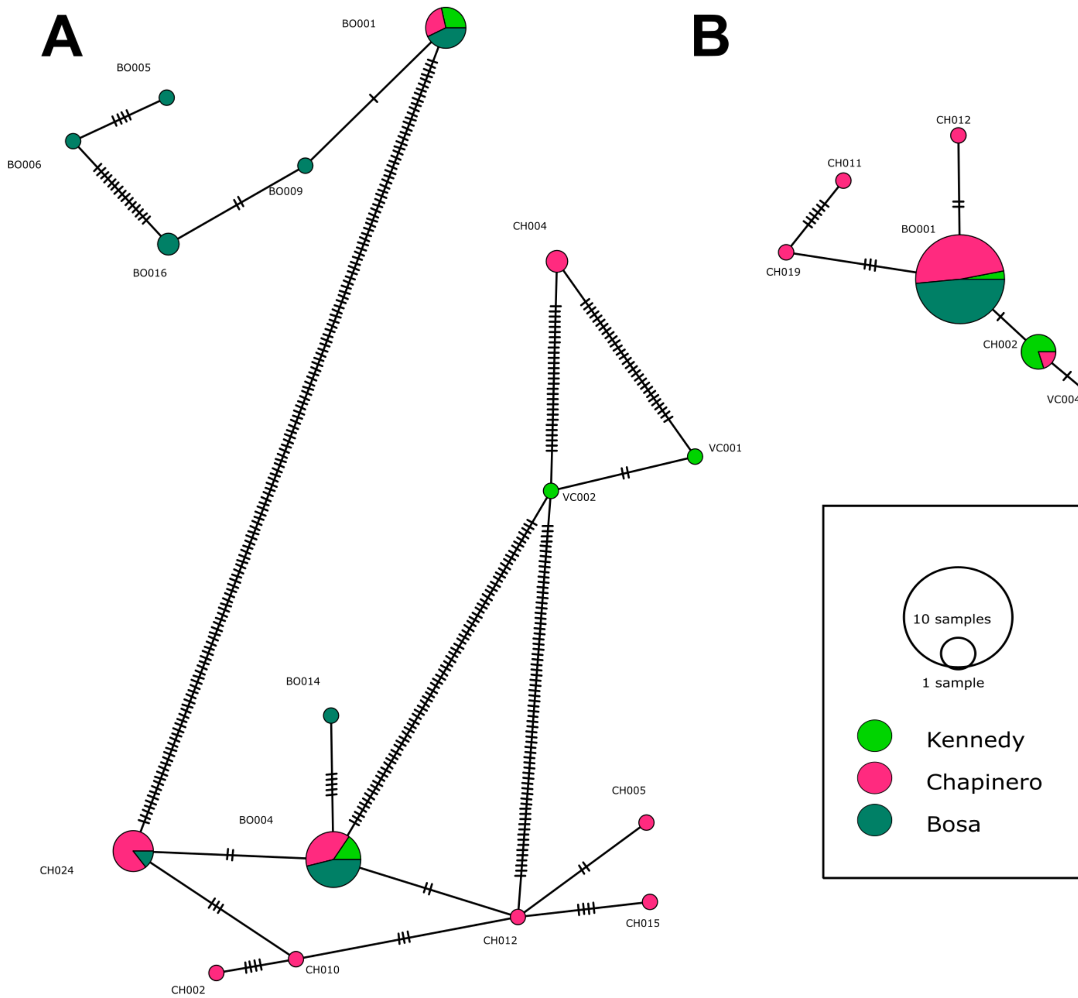

Figure 6. Median joining network for *B. germanica* individuals collected in Bogota, Colombia. In A we can see the network corresponding to COI gene. In B we can see the network corresponding to mtDNA fragment 16S.

SUPPLEMENTARY MATERIAL

Figure S1. Phylogenetic tree of *Periplaneta sp.* with 16S gene. Black values correspond to IB posterior probability and blue values ML UFboot over SH-aLRT test values.

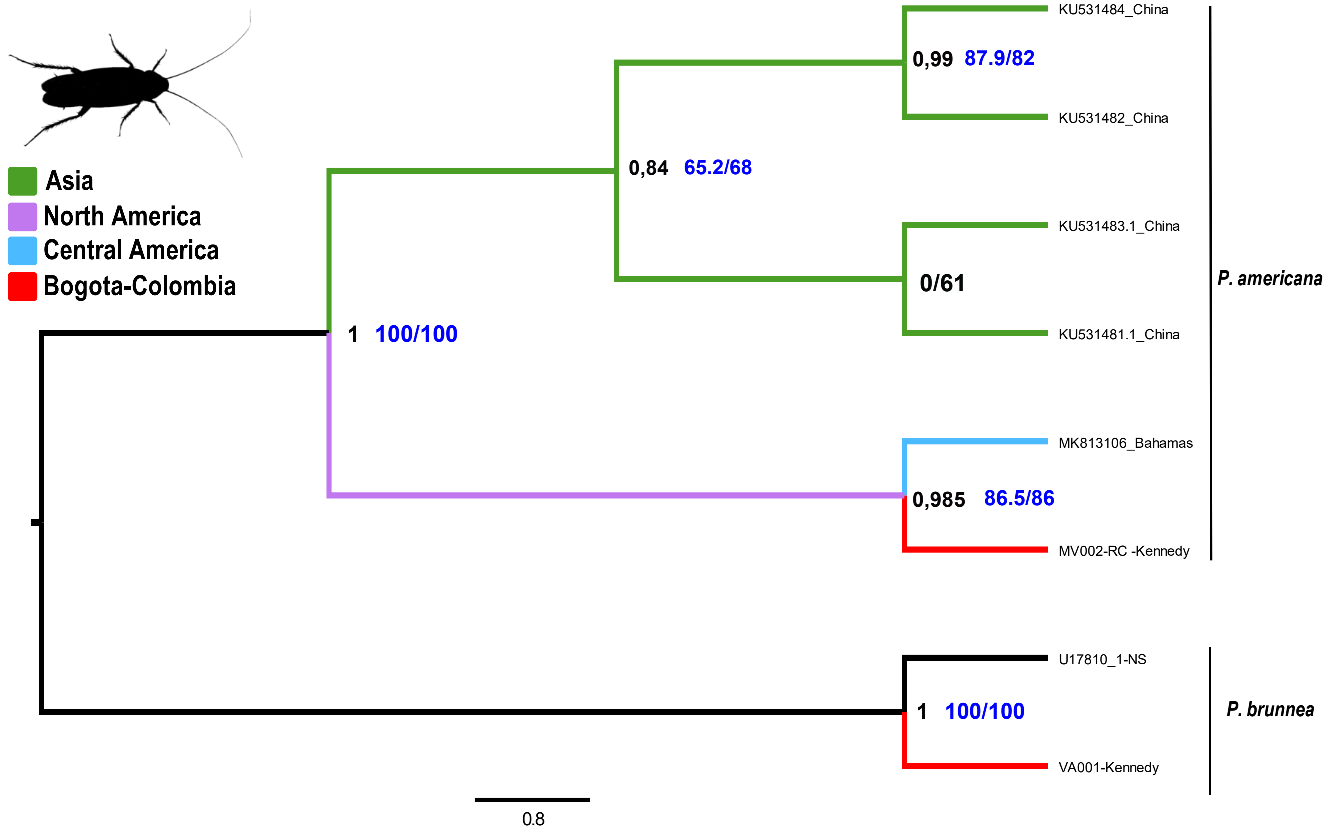

Figure S2. Phylogenetics tree of *Blattella germanica* with 16S gene. Black values correspond to IB posterior probability and blue values ML UFboot over SH-aLRT test values.

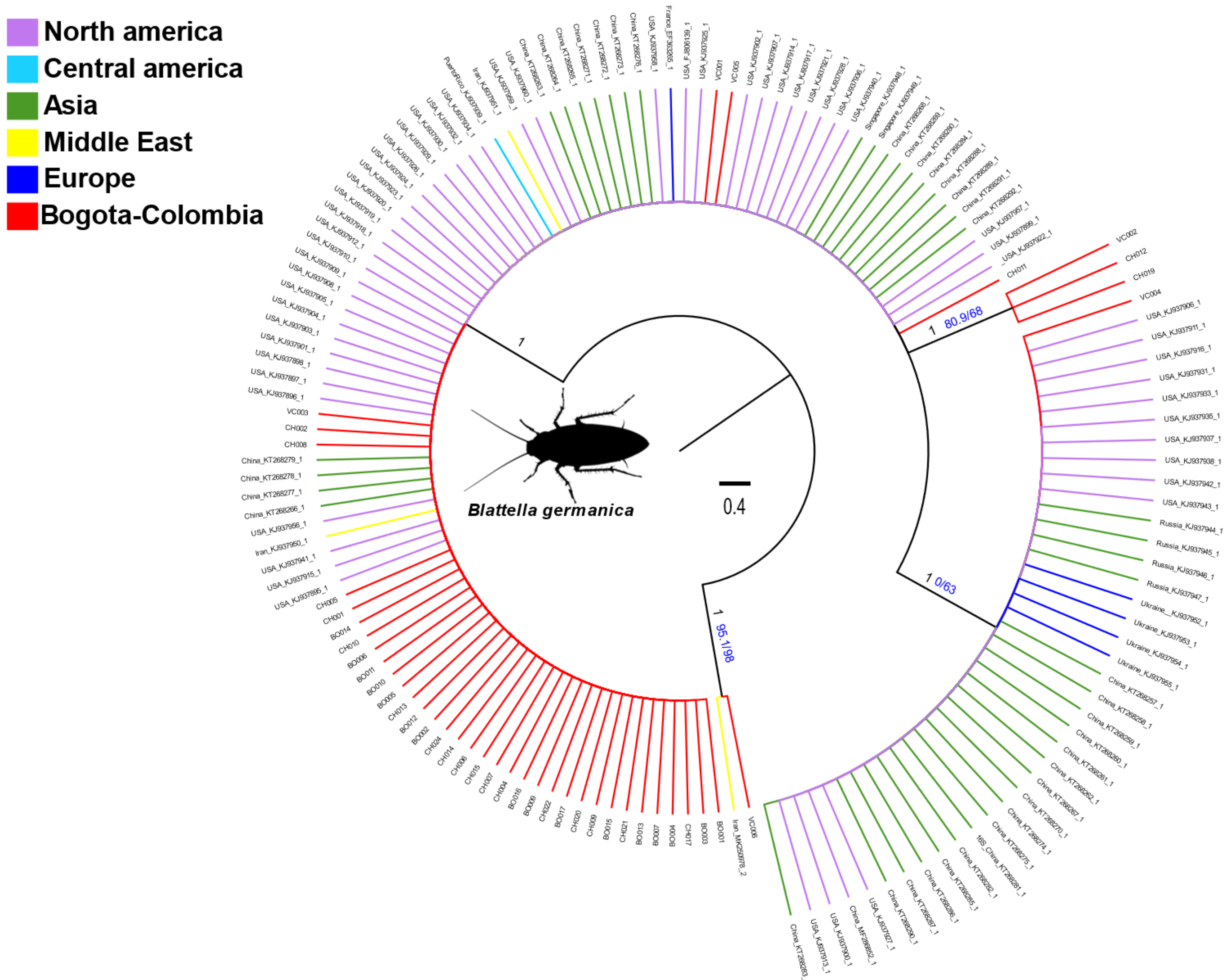

Figure S3. Phylogenetic tree from Bogota populations with both mtDNA. Black values correspond to IB posterior probability and blue values ML UFboot over SH-aLRT test values.

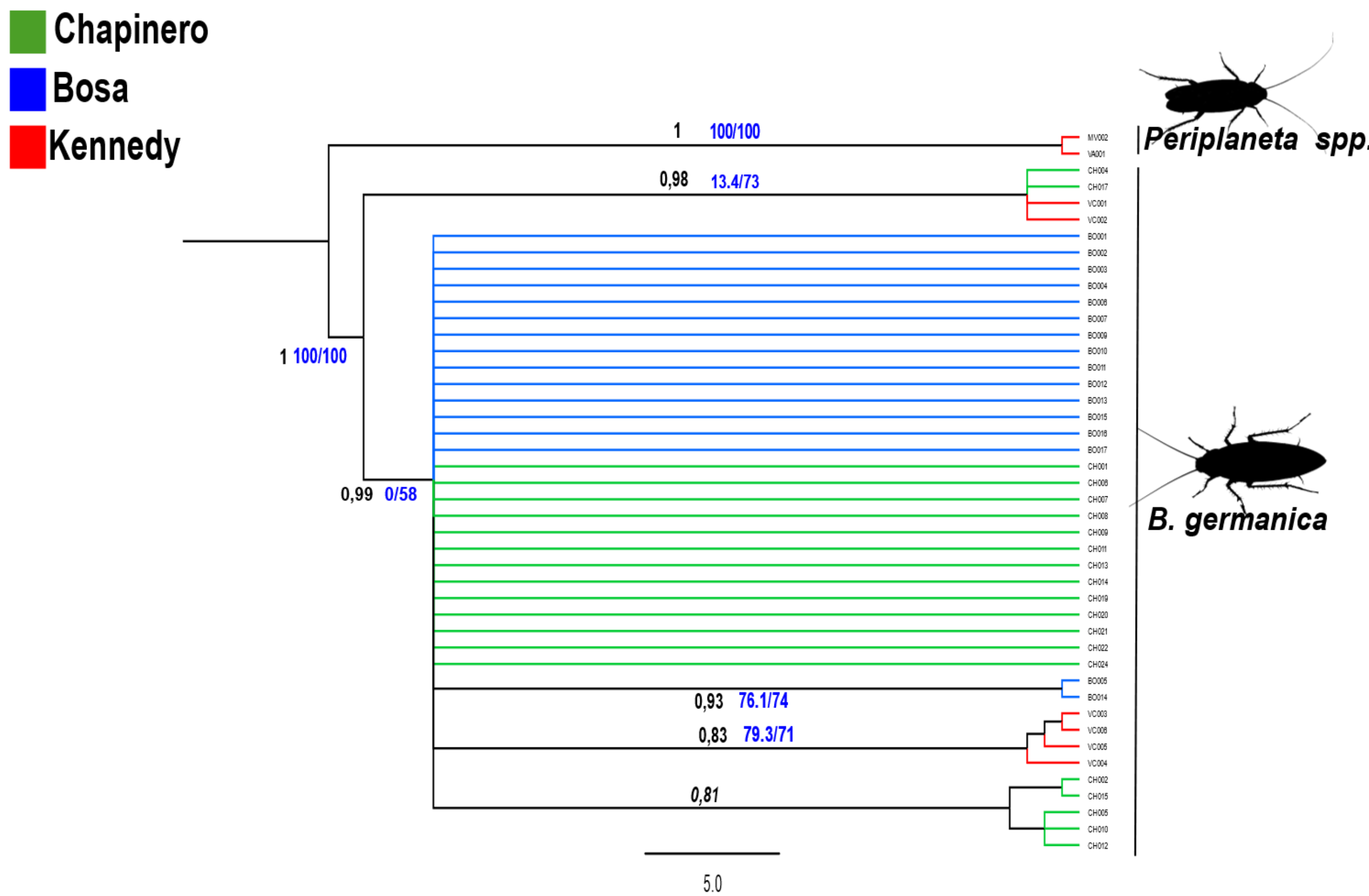

Table S1. Taxon sampling information for Bogota populations.

SAMPLE ID	SPECIES	PLACE	COORDINATES	LATITUDE	LONGITUDE
VA001	*Periplaneta brunnea*	Villa Alsacia, Kennedy, Bogotá	N 4° 38' 33.464'' O 74° 8' 12.368''	4,642629	-74.136769
BO001	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO002	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO003	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO004	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO005	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO006	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO007	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO009	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO010	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO011	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028

BO012	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO013	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO014	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO015	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO016	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
BO017	*Blattella germanica*	Bosa, Bogotá	N 4°37'31.771'' O 74°12'1.008''	4,625492	-74.20028
VC001	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
VC002	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
VC003	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
VC004	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
VC005	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
VC006	*Blattella germanica*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905
MV002	*Periplaneta americana*	Vision colombia, Kennedy, Bogotá	N 4°39'1.303'' O 74°8'5.658''	4,650362	-74.134905

CH001	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH002	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH004	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH005	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH006	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH007	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH008	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH009	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH010	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH011	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH012	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH013	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH014	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663

CH015	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH017	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH019	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH020	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH021	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH022	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663
CH024	*Blattella germanica*	Chico, chapinero, Bogotá	N 4° 40' 47.143'' O 74° 2' 40.789''	4,679762	-74.044663

Table S2. GenBank sequence accession numbers.

Gene	Sample ID	Accession numbers
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16S	Blattella_germanica_voucher_TIR4.16S_Iran	KJ937950.1
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16S	P_americana-voucher-201411200014-China	KU531481.1
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COI	Blattella_germanica_strain_326G_China	KX962511.1

COI	Blattella_germanica_strain_326H_China	KX962512.1
COI	Blattella_germanica_strain_326I_China	KX962513.1
COI	Blattella_germanica_strain_326K_China	KX962515.1
COI	Blattella_germanica_voucher_FFOH00089_001_France	KU494086.1
COI	Blattella_germanica_voucher_FFOH00090_001_France	KU494084.1
COI	Blattella_germanica_voucher_FFOH00158_0104_France	KU494085.1
COI	Blattella_germanica_strain_326L_China	KX962516.1
COI	Blattella_germanica_voucher_NY4_Chin	KT268249.1
COI	Blattella_germanica_voucher_NY5_USA	KT268250.1
COI	Blattella_germanica_voucher_PY1_China	KT268231.1
COI	Blattella_germanica_voucher_SMX1_China	KT268239.1
COI	Blattella_germanica_voucher_SQ1_China	KT268221.1
COI	Blattella_germanica_voucher_SQ2_China	KT268222.1
COI	Blattella_germanica_voucher_SQ3_China	KT268223.1
COI	Blattella_germanica_voucher_SQ4_China	KT268224.1
COI	Blattella_germanica_voucher_XY1_China	KT268251.1
COI	Blattella_germanica_voucher_ZK4_China	KT268238.1
COI	Blattella_germanica_voucher_ZZ1_China	KT268225.1
COI	Blattella_germanica_voucher_ZZ2_China	KT268226.1
COI	Blattella_germanica_strain_326B_China	KX962506.1
COI	Blattella_germanica_strain_326C_China	KX962507.1
COI	Blattella_germanica_strain_326E_China	KX962509.1
COI	Blattella_germanica_strain_326O_China	KX962519.1
COI	Blattella_germanica_strain_326Q_China	KX962521.1
COI	Blattella_germanica_isolate_B72_France	MF458633.1
COI	Blattella_germanica_isolate_KOR-B1_South_Korea	JQ350728.1
COI	Blattella_germanica_strain_326A_China	KX962505.1
COI	Blattella_germanica_isolate_Tehran41__Iran	JQ267496.1
COI	Blattella_germanica_isolate_Tehran42_Iran	JQ267497.1
COI	Blattella_germanica_isolate_Tehran_Iran	JQ267498.1
COI	Blattella_germanica_voucher_ZK1_China	KT268235.1
COI	Blattella_germanica_voucher_ZZ4_China	KT268228.1
COI	Blattella_germanica_voucher_ZZ5_China	KT268229.1
COI	Blattella_germanica_voucher_PY2_China	KT268232.1
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COI	Blattella_germanica_voucher_XY6_China	KT268256.1
COI	Blattella_germanica_voucher_NY1_China	KT268246.1
COI	Blattella_germanica_voucher_SMX7_China	KT268245.1
COI	Blattella_germanica_voucher_NY2_China	KT268247.1
COI	Blattella_germanica_voucher_NY3_China	KT268248.1
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COI	Blattella_germanica_voucher_XY2_China	KT268252.1
COI	Blattella_germanica_voucher_XY3_China	KT268253.1

COI	Blattella_germanica_voucher_XY4_China	KT268254.1
COI	Blattella_germanica_voucher_XY5_China	KT268255.1
COI	Blattella_germanica_voucher_PY4_China	KT268234.1
COI	Blattella_germanica_voucher_ZZ6_China	KT268230.1
COI	Blattella_germanica_voucher_SMX2_China	KT268240.1
COI	Blattella_germanica_voucher_ZK2_China	KT268236.1
COI	Blattella_germanica_voucher_ZK3_China	KT268237.1
COI	Blattella_germanica_voucher_ZZ3_China	KT268227.1
COI	Blattella_germanica_cytochrome_oxidase_subunit_I_Russia	KR063252.1
COI	Blattella_germanica_voucher_USFDA_CFSAN_BL20-007_USA	JX402721.1
COI	Blattella_germanica_voucher_SMX3_China	KT268241.1
COI	Blattella_germanica_voucher_SMX4_China	KT268242.1
COI	Blattella_germanica_voucher_SMX5_China	KT268243.1
COI	Blattella_germanica_voucher_KOR-B1-002_South_Korea	KC407709.1
COI	Blattella_germanica_voucher_JX240_Venezuela	KM577141.1
COI	Periplaneta_brunnea_cytochrome_oxidase_subunit_I_COI_Bangladesh	MG557663.1
COI	Periplaneta_brunnea_isolate_abc2_Bangladesh	MG572238.1
COI	Periplaneta_brunnea_voucher_CK0O3_Bangladesh	MH745076.1
COI	Periplaneta_brunnea_voucher_FDA_CFSAN_BL20-003_USA	MG458946.1
COI	Periplaneta_americana_voucher_BAH_28362_A05_Bahamas	MK936745.1
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COI	Periplaneta_americana_voucher_CK02525092017_Bangladesh	MG587916.1
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COI	Periplaneta_americana_voucher_JX269_Guyana	KM576940.1
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COI	Periplaneta_americana_voucher_JX139_USA	KM576976.1
COI	Periplaneta_americana_voucher_JX214_USA	KM576971.1
COI	Periplaneta_americana_voucher_JX165_USA	KM576970.1
COI	Periplaneta_americana_voucher_JX173_USA	KM576967.1
COI	Periplaneta_americana_voucher_JX081_Spain	KM576962.1
COI	Periplaneta_americana_voucher_JX090_USA	KM576961.1
COI	Periplaneta_americana_voucher_JX178_USA	KM576958.1
COI	Periplaneta_americana_voucher_JX199_USA	KM576949.1
COI	Periplaneta_americana_voucher_JX148_USA	KM576948.1
COI	Periplaneta_americana_voucher_JX067_USA	KM576936.1
COI	Periplaneta_americana_voucher_JX097_USA	KM576929.1
COI	Periplaneta_americana_voucher_JX032_USA	KM576925.1
COI	Periplaneta_americana_voucher_JX053_USA	KM576924.1
COI	Periplaneta_americana_voucher_JX008_USA	KM576921.1
COI	Periplaneta_americana_voucher_JX234_Venezuela	KM576920.1
COI	Periplaneta_americana_voucher_JX001_USA	KM577123.1
COI	Periplaneta_americana_voucher_JX228_Venezuela	KM577049.1
COI	Periplaneta_americana_voucher_JX010_USA	KM577117.1
COI	Periplaneta_americana_voucher_USFDA_CFSAN_BL20-002_USA	JX402724.1

Table S3. AMOVA analysis for COI and 16S in *Blattella germanica* population.

16S				
Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	2	8.503	0.26331 Va	23.98
Within populations	40	33.381	0.83452 Vb	76.02
Total	42	41.884	1.09783	
COI				
Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	2	58.416	1.47494 Va	12.44
Within populations	39	405.012	10.38494 Vb	87.56
Total	42	463.429	11.85988	

16S p-value, 0.00000+-0.00000
COI p-value, 0.04594+-0.00715