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Morphological and molecular identification of the American cockroaches (*Periplaneta americana*) in Jeddah province (Dictyoptera: Blattidae)

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Abstract
Cockroaches are household pests, and their presence causes psychological discomfort to humans, and their feeding on different foods pollutes them with various deadly pathogenic that cause dangerous diseases. The present work is designed to identify the morphological and molecular characterization of *Periplaneta americana*. Random samples of *P.americana* were collected from different regions of Jeddah Governorate, classified, and identified. The morphological study focused identifying the important characters of different stages of *P.americana* (adult's male & female, nymphs and oothecae) using standard taxonomic keys and dissected under a stereomicroscope at 10 X and/or 20 X magnification and many authors were agreed with previous results of this study In molecular identification DNA isolation, PCR and sequencing were used. Our results showed that using cytochrome CO1 confirmed the identification of this type of cockroach and the genotype with a high degree of accuracy, where the first two types were defined with a percentage of 96-100% and the second with a percentage of 92-96%. Further studies on the current topic are therefore recommended.

Keywords: American cockroaches, cockroaches, morphological, molecular, Co1, *P. americana*

Introduction
American cockroaches (*Periplaneta americana*) (Linnaeus), order: Dictyoptera, is an important insect in medical [1], they are the most notorious pests, found in kitchens [2]. They are the biggest species of cockroaches [3]. Dictyoptera comprises about 5000 species under 398 genera in 28 families [4]. Out of 500, 30 species are considered a household pest [5]. Several cockroaches' pests live in/or around homes, and they are omnivorous scavengers [1]. *P. americana* can survive in warm places with high moisture also in dirty environments such as sewers [6]. Since 1945, many studies have investigated the pathogenic transmission by cockroaches [7]. *P. americana* can hold and spread dangerous pathogens such as bacteria, fungi, parasites, protozoa [8], and cause allergies to humans [9]. They play important role in the transmission of different diseases by mechanical and biological ways [10]. *P.americana* spends most of its time in sewage, sewer pipe which usually contains a high density of pathogens [11]. Also, they feed on trash, and they have big chances to spread many pathogens [12]. Besides, their dirty habits of eating their feces make them ideal carriers of numerous pathogenic microbes [13]. Cockroaches spread pathogens through their cuticle [14] because their nymphal cuticles go through ecdysis [15]. Therefore, they transfer pathogens in different ways such as vertical transmission which occurs when an infected mother passes on the pathogen or disease to her progeny [16]. A cockroach can cause allergic reactions in sensitive people, which is the most important disease, and sometimes it causes a higher rate if anyone infected with allergy touching cockroaches. In addition, allergic reactions are not linked only with cockroach body parts but also their different secretions such as dry feces and exuviae [17]. For their morphological characteristics, many studies identified the *P.americana* according to the standard taxonomic keys [18]. These identification keys were provided for male, female nymphal, and egg stages for *P.americana.* In the adult stage, the body has an oval and flattens shape, with 35-50 mm length and reddish-brown color, excluding the pronotum which is yellowish. Both have two wings (front and hind), and the hind ones are used for flight. The cockroach body has three parts: head, thorax, and abdomen. The head is composed of mouthparts, antennae, three simple eyes, and one pair of compound eyes. The thorax is divided into three sections, and each segment has a pair of legs [19]. Both males and females have a pair of sense organs called cerci at the end of their abdomens. Males consist of another appendage called styli between the cerci used as a reproductive organ [20]. Female has the productive organs in the abdomen which gives their abdominal wider. They produce ootheca or egg case with dark brown color, and 5-7 mm long and each one has 14 to 16 embryos [21]. The female drops her egg capsule in wet and warm places [22]. After the egg hatches, the nymphal stage begins and it takes from 7-14 times of molting, new nymphs are white and then became radish brown when their exoskeleton is attached to the air. Nymphal stages resemble adults but without wings [23]. The present work is designed to collect different stages of *P. americana* from different places in Jeddah province and identify the morphological and molecular characterization of them.

Materials and Methods
Collecting different stages of *P.americana*
P.americana was collected from sewers from different areas (east, west, south, north, and the middle), in Jeddah province, the kingdom of Saudi Arabia. For the traps, we used glass jars covered with dark cloth [24]. They were

dropped into different sewers in Jeddah's Street. Samples were collected every three days and then placed in glass containers (30 × 60 × 30 cm), coated from the top with petroleum jelly 2 cm to keep the cockroaches from escape. Collected samples were supplied with dry cat food as a food source and wetted cotton as a water source, and cardboard as shelter. Samples were kept under the laboratory condition (12:12 photoperiod, 25 ± 3 °C, and 75 ± 5 % RH)

Morphological identification

The study was based upon male, female, and nymphs specimens of *P.americana*. They were identified according to their morphological characteristics using standard taxonomic keys [18]. Adult male, female, and seven nymphs were used external dissection. For morphological examination, cockroaches were fixed in 70% ethanol [25] and dissected also in 70% ethanol under a stereomicroscope at 10 X and/or 20 X magnification (OLYMPUS, TOKYO, JAPAN).

Molecular identification

All genomic DNA extraction experiments were conducted in molecular identification experimental unit, in the Dengue Mosquito Experimental Station (DMES), belonging to the Department of Biological Sciences, Faculty of Sciences, King Abdul-Aziz University, Jeddah, Saudi Arabia. Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721 was used for *P.americana* DNA extraction. For molecular identification of *P.americana*, LCO1490 and HCO2198 primers were used with the following conditions: Initial denaturation: 5 min at 94 °C, Denaturation: 1 min at 94 °C, Annealing: 1 min at 58 °C, Extension: 2 min at 72 °C, Number of cycles: 35, Final extension: 10 min at 72 °C, by a thermal cycler (Thermo Scientific). For gel electrophoresis, 1% of agarose powder was weighed and dissolved in 1X TAE buffer and it was run at 127 V for one hour, which was attached firmly and connected to the power supply (MOLECULE-ON PS-M-300V Electrophoresis Power Supply, India). DNA ladder was used to determine the molecular weights of DNA fragments.

Sequencing reaction

DNA sequencing was carried out in Macrogen Company, Korea. The nucleotide and amino acid sequence data were compared with the National Centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST).

Results

Morphological identification

General features

Observations of external morphological characters of male, female, nymphs, and oothecae of *P.americana* were described and illustrated using stereomicroscopes. The terminology of [18] was followed. *P.americana* male and female are oval and flattened shape, with 35-40 mm long and brown color as in (Figure 1). In our collected samples, we found a simple difference in adult's pronotum, some had a dark edge, and some have a light one as in (Figure 2). *P.americana* have long wings cover the abdomen. Forward wings type of tegmina (Figure 3a), and membranous hind wings (Figure 3b). The head consists of simple and compound eyes (Figure 4), antenna type of setaceous as in (Figure 5), mouthpart type of chewing mouthparts as in (Figure 6). The legs are modified for walking or running (Figure 7a) end with 5 segmented tarsi with five arolium's (Figure 7b). Middle and hind femora both with numerous strong spines along the ventral margin as in (Figure 8). The abdomen in adults bears some appendages at its posterior end (the style in males and ovipositor in females).

Fig 1: *P. americana* long and color.

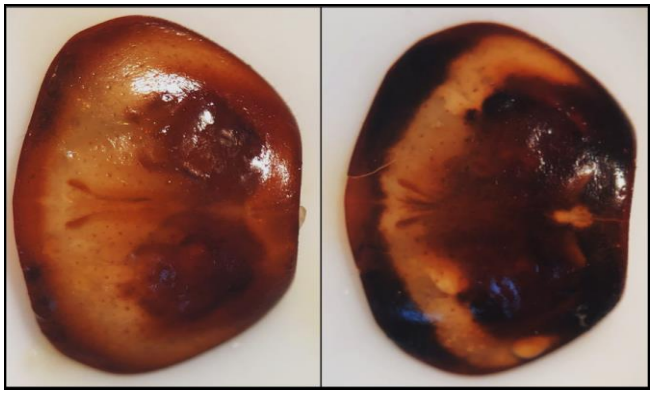

Fig 2: Difference in adult's pronotum.

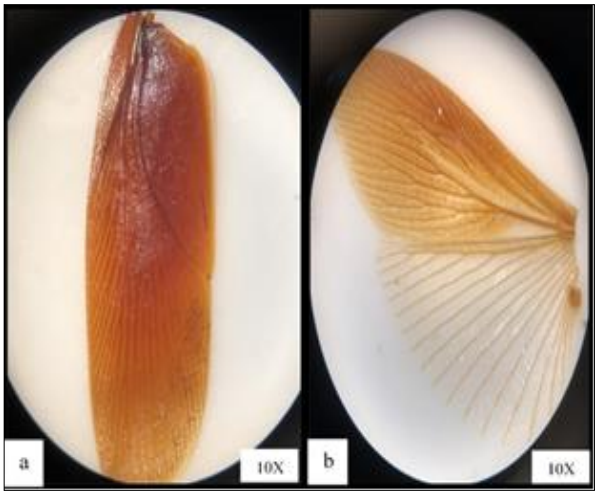

Fig 3: a-Forward wing, c-Hind wing.

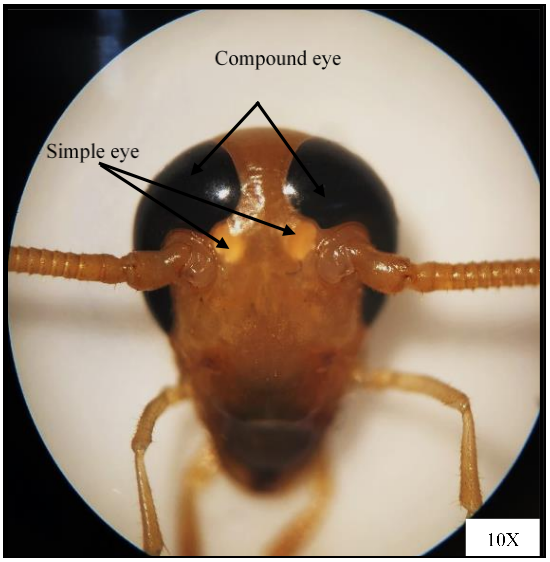

Fig 4: a- Simple, b- Compound eye.

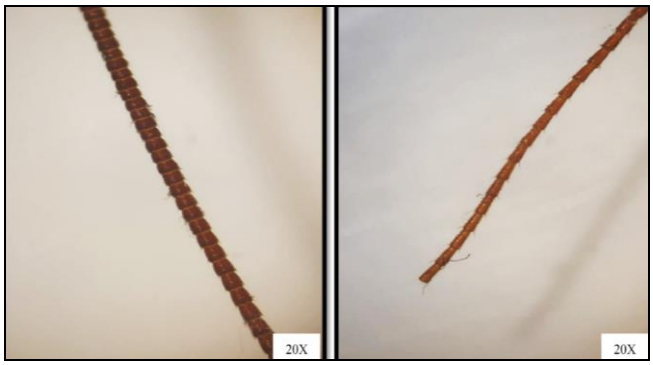

Fig 5: Antenna.

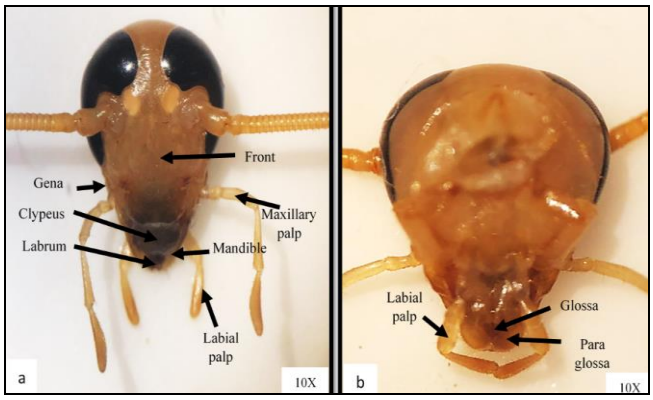

Fig 6: a-Dorsal view of mouth parts, b- Ventral view of mouth parts.

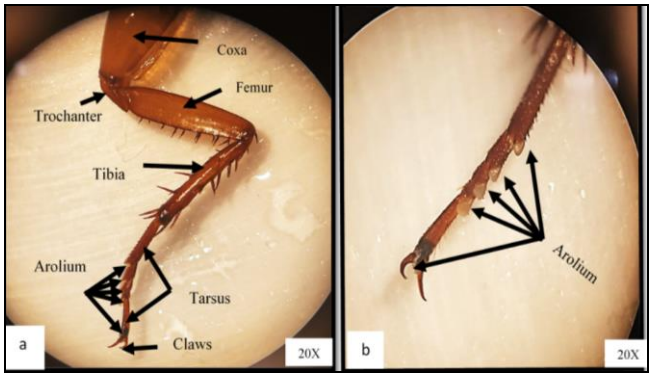

Fig 7: a- Walking leg, b- Adult's tarsus with aroliums.

Fig 8: *P. americana* strong spines in femur.

Description of *P.americana* male

The male wings expanded after the abdomen, and they have styli located between the cerci (Figure 9a). The male abdomen has 9 clear sternums, and the genital is located on the 9th segment which consists of cercus and slender, elongate, and straight style in-between as in (Figure 9b). Styli are very long and slender, longer than space between their bases, and cercus long and slender particularly, supra-anal plate deeply notched.

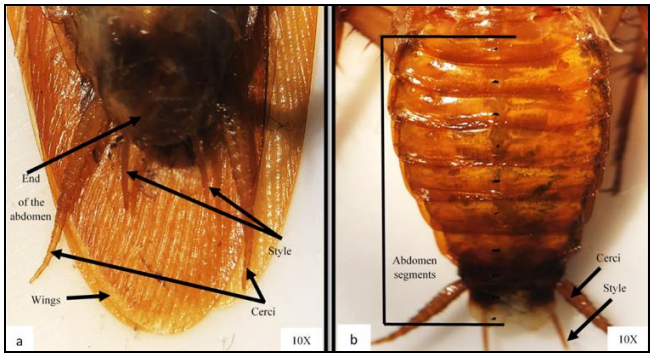

Fig 9: a- *P.americana* male. b- Male abdomen.

Description of *P.americana* female

The female wings in the same length as the abdomen. The abdomen of *P.americana* female has 6 clear sterna, and the sub-genital ovipositor is located on the 8th and 9th segment and it is divided longitudinally (Figure 10 a, b).

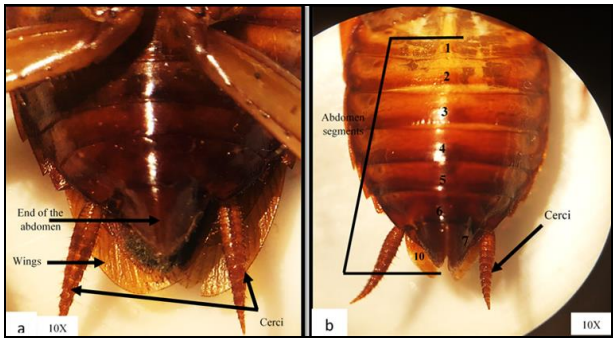

Fig 10: a- *P.americana* male. b- Male abdomen.

Description of *P.americana* nymphs

Nymphs are smaller and do not have wings with reddish-brown as in (Figure 11a). Their abdomen does not have appendages but, large nymphs have a genital appendage at their posterior ends, and wing pads appear in the 3rd or 4th instar as in (Figure 11 b).

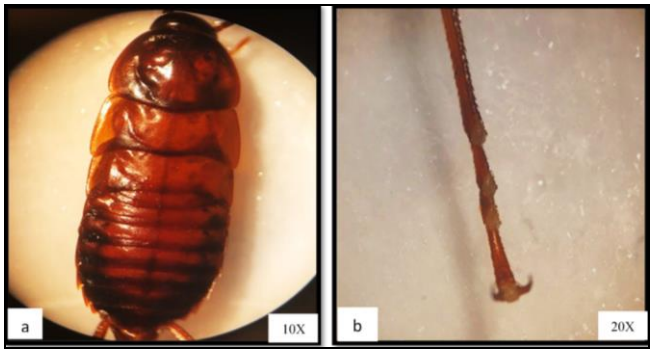

Fig 11: a- Nymphal stage, b- Arolium.

Description of *P.americana* oothecae

Limited research has been reported using light microscopy techniques to describe *P.americana* oothecae. The oothecae are brown on the first day and became black after they are exposed to the air as in (Figure 12). It is about 8 mm long and 5 mm high. The top of the two sides of the ootheca is attached and crimped on the edge called adjacent segmentations. Within the interface of each half of this keel, there are scooped-out depressions and grooves which meet to form a series of cells and tubes called convex on the external surfaces with squashed endings. There is a suture line in the contact zone of the valves and Zipper-like toothed rail structure of the crest as in (Figure 13).

Fig 12: Oothecae before (left) and after (right) depositing

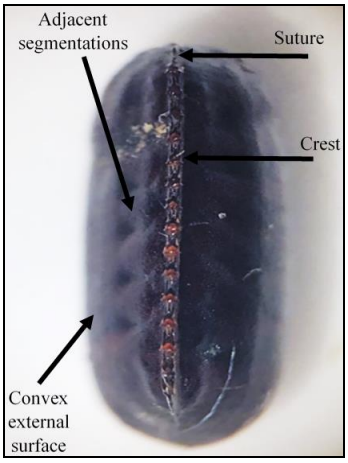

Fig 13: Oothecal structure.

Molecular identification

DNA extraction was prepared using Thermo Scientific Gene JET Genomic DNA Purification Kit #K0721 and examined on 1% agarose gel. PCR amplification was then carried out using universal primers, as shown in the materials and methods. The resulted from 700-800 bp was examined on 1% agarose gel electrophoresis as in (Figure 14). The PCR products were purified and sent to Macrogen for sequencing. The phylogenetic relationships of *P.americana* isolate and closely related species were analysed using the Multisequence Alignment Program (MEGA7) and the results are presented in the phylogenetic tree as in (Figure 15 and Table 1).

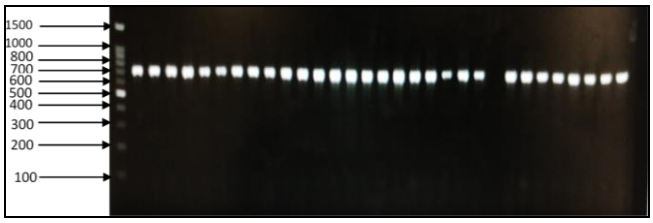

Fig 14: PCR products.

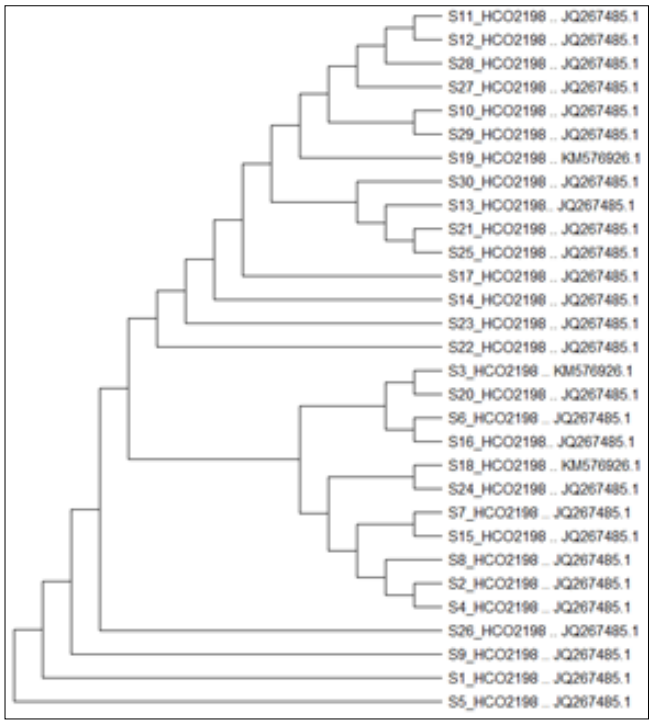

Fig 15: Phylogenetic relationship among *P.americana* DNA isolate and related species. The tree was generated by MEGA 7 software.

Table 1: Similarity percentages to the nearest neighbour (s) of the isolate.

P.americana isolates	**Nearest neighbour (s)**	**Accession number**	**Similarities**
	P. americana	JQ267485.1	96-100 %
	P. americana	KM576926.1	92-96 %

Discussion

The focus of this part of the study was to identify the important characters of different stages of *P.americana* (adult male, female, nymph, and oothecae). Anyway, still there is a need for morphological characterization for *P.americana* oothecae with the help of clear photographs and keys for the identification in this region. The

morphological identification of *P.americana* nymph and adult stages is similar to the characters reported by many authors [22, 26, 23, 27, 28, 19, 20]. For oothecae identification, many authors were agreed with the previous results of this study [22]. DNA barcoding is a useful tool to identify pest species assuming adequate representation of genetic variants in a reference library [29]. In our finding, we used the barcode region of the mt-DNA (LCO 1490 and HCO2198) gene of DNA extracted from 30 cockroach specimens, along with the development of a PCR method. The PCR generates a single band between 700-800 bp-sized in all cockroach specimens, followed by direct sequencing. Two accession numbers were found from DNA sequencing (JQ267485.1 and KM576926.1) with 96-100% and 92-96%, respectively of the similarities were related to *P.americana*. Anyway, the molecular identification studies of *P. americana* are not much studied in Saudi Arabia in general and Jeddah governorate in particular. Many researchers have studied the molecular identifications of different cockroaches. [29] found that three deeply divergent, widely distributed *P.americana* COI haplogroups [30]. sequenced the complete mitogenomes of two cockroaches, reconstructed the molecular phylogeny, and attempted to infer the phylogenetic position of termites in Blattaria more reliably and he found that complete mt-DNA nucleotide sequences of *P.americana* is 15,025 bp in size and they found that *P.americana* shares only 75% sequence identity with *B.germanica*, which is lower than that with the two genome-sequenced termites, i.e., 79% to *Z.nevadensis* and 80% to *M.natalensis* [31]. determined the complete mitochondrial genomes of two cockroach species, *Periplaneta australasiae* and *Neostylopyga rhombifolia*, 15,605 bp and 15,711 bp in length, respectively. So, this study first time confirm the molecular characters of *P. americana* from Jeddah region of Saudi Arabia.

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Conflicts of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References
1. Akbari S, Mohammad AO, Saedeh SH, Sara H, Ghazaleh O, Mohammad HS. Aerobic Bacterial Community of American Cockroach Periplaneta americana,a Step toward Finding Suitable Paratransgenesis Candidates. J Arthropod Borne Dis,2015:9(1):35-48.
2. El-Sherbini G, Gneidy MR. Cockroaches and Flies in Mechanical Transmission of Medical Important Parasites in Khaldyia Village, El-Fayoum, Governorate, Egypt.
3. Gary A. Museum of Comparative Zoology, Harvard University. Integrated Pest Management Program, Cornell University. 2012, 2015. www.nysipm.cornell.edu/factsheets/buildings/german_cockroach.pdf
4. Hanitsch R. Malayan Blattidae. J. Straits. Br.R.Asiat.Soc,1917:69:1.
5. Chung HS, Yu TH, Kim BJ, Kim SM, Kim JY, Yu HS. Expressed sequence tags analysis of Blattella germanica. Kor J Parasitiol,2005:43:149-156.
6. Jaramillo-Ramirez GI, Cárdenas-Henao H, González-Obando R, Rosero-Galindo CY. Genetic variability of five Periplaneta americana L. (Dyctioptera: Blattidae) populations in southwestern Colombia using the AFLP molecular marker technique. Neotrop Entomol,2010: 39:371-378.
7. Roh JY, Choi JY, Li MS, Jin BR, Je YH. Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. J. Mol. Biol,2007:17:547-559.
8. Czajka E, Pancer K, Kochman M. Characteristics of bacteria isolated from body surface of German cockroaches caught in hospitals. Przegl. Epidemiol,2003:57:655-662.
9. Kinfu A, Erko B. Cockroaches as carriers of human intestinal parasites in two localities in Ethiopia. Trans R Soc Trop Med. Hyg2008:102:1143-1147.
10. Kim I, Lee JH, Subramaniyam S, Yun E, Kim I. De Novo Transcriptome Analysis and Detection of Antimicrobial Peptides of the American Cockroach Periplaneta americana (Linnaeus) PLoS One,2016:11:5.
11. Basseri HR, Amir DK, Ronak B, Mandan A, Reza HB. Isolation and Purification of an Antibacterial Protein from Immune Induced Haemolymph of American Cockroach, Periplaneta americana. Journal of arthropod-borne diseases,2016:10:519-27.
12. Pai HH, Chen WC, Peng CF. Isolation of bacteria with antibiotic resistance from household cockroaches (Periplaneta americana and Blatella germanica). Acta Tropica,2005:93:259-265.
13. Allen BW. Excretion of viable tubercle bacilli by Blatta orientalis (the oriental cockroach) following ingestion of heat-fixed sputum smears: a laboratory investigation. Trans. R. Soc. Trop. Med. Hyg,1987:81:98-99.
14. Mpuchane S, Matsheka IM, Gashe BA, Allotey J, Murindamombe G, Mrema N. Microbiological studies of cockroaches from three localities in Gaborone, Botswana. Afr. J. Food Nutr, Sci, 2006b, 6.
15. Mpuchane S, Allotey J, Matsheka I. Carriage of micro-organisms by domestic cockroaches and implications on food safety. Int. J. Trop. Insect Sci,2006a:26:166-175.
16. Jennifer LP. intraspecific gene flow and vector competence among Periplaneta americana cockroaches (Blattodea: Blattidae) in central Texas. Texas A&M University,2008:218:3524927.
17. O'Connor GT, Gold DR. Cockroach Allergy and Asthma in a 30-Year-Old Man. Environmental Health Perspectives,1999:107(3):243-247.
18. Al-Hussaini AH, Demian ES. Practical animal biology. Dar elmaaref, 1962, 11(14).
19. Perrott J, Murphy NG, Zed PJ. L-carnitine for acute valproic acid overdose: a systematic review of published cases'. Ann Pharmacother,2010:44:1287-93.

20. Rachael CP, Dini MM. American Cockroach. Virginia Polytechnic Institute and State University, 2010.
21. Robert JW, Aqeel A. Cockroaches. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, 2007.
22. Bell WJ, Adiyodi KG. The American cockroach. Chapman and Hall London. Springer, 1981, 1(4).
23. Steven BJ. American Cockroaches (Periplaneta americana (L). The Pennsylvania State University, 2002.
24. Wang C, Bennett GW. Comparative study of integrated pest management and baiting for German cockroach management in public housing. J Econ Entomol,2006:99:879-85.
25. Wipfler B, Pohl H. Yavorskaya M, Beutel R. A review of methods for analysing insect structures–the role of morphology in the age of phylogenomics. Current Opinion in Insect Science, 2016, 18.
26. Jacobs DE, Clickner RP, Zhou JY, Viet SM, Marker DA, Rogers JW. The prevalence of lead-based paint hazards in U.S. housing. Environ Health Perspect,2002:10:599-606.
27. Barbara KA. American Cockroach, Periplaneta americana (Linnaeus) (Insecta: Blattodea: Blattidae). Entomology and Nematology Department, UF/IFAS Extension, Gainesville. http://entomology.ifas.ufl.edu/creatures. 2014.
28. Susan CJ. American Cockroach. Agriculture and Natural Resources. The Ohio State University, 2008, 1-3.
29. Beeren CV, Stoeckle MY, Xia J, Burke G, Kronauer DJC. Interbreeding among deeply divergent mitochondrial lineages in the American cockroach (Periplaneta americana). Scientific reports,2015:5:8297.
30. Xiao B, Chen AH, Zhang YY, Jiang GF, Hu CC, Zhu CD. Complete mitochondrial genomes of two cockroaches, Blattella germanica and Periplaneta americana, and the phylogenetic position of termites. Curr Genet,2012:58(2):65-77.
31. Ma J, Du C, Zhou C. Complete mitochondrial genomes of two blattid cockroaches, Periplaneta australasiae and Neostylopyga rhombifolia, and phylogenetic relationships within the Blattaria. PLoS One,2017:12(5):e0177162.