Phylogenetic characterization of Cochroaches (Insecta: Blattaria) in Türkiye and determination of their vector potential for medically important parasites

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ARTICLE INFO

Article History
Received : 31.10.2023
Accepted : 22.02.2024
DOI: 10.33988/auvfd.1300194

Keywords
Cockroach
DNA barcoding
ITS-2
mt-COI and mt-COII
Vector

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How to cite this article: Cevahir F, Düzlü Ö, Atelge M, Yıldırım A (2024): Phylogenetic characterization of Cochroaches (Insecta: Blattaria) in Türkiye and determination of their vector potential for medically important parasites. Ankara Univ Vet Fak Derg, 71 (4), 417-426. DOI: 10.33988/auvfd.1300194.

ABSTRACT

This study was performed to investigate the phylogenetic characters of the cockroaches in the Kayseri region for mitochondrial cytochrome c oxidase subunit I (mt-COI), mt-COII, and internal transcribed spacer-2 (ITS-2) gene regions. It was also aimed to determine their mechanical transmission of medically important parasites. PCR-restriction fragment length polymorphism (RFLP) was performed by using mt-COI, mt-COII, and ITS-2 DNA gene regions to identify cockroach species (n=220) collected from different regions. Differentiation of cockroach species was based on RFLP models using two restriction enzymes: AvaI and EciI. For phylogenetic analysis, mt-COI, mt-COII, and ITS-2 DNA barcode regions were amplified with standard primers. The obtained amplicons were purified and sequenced using the PCR primers. According to PCR-RFLP, the cockroach species were identified as *Blattella germanica* (n=105), *Blatta orientalis* (n=86), and *Periplaneta americana* (n=29). A total of 13 haplotypes were detected and maximum likelihood (ML) analyses revealed that the sequences of all three species showed a monophyletic structure for the three gene regions. The cockroaches were examined for the presence of parasites. It was found that of the 58 parasitic forms identified, 46 (79.3%) belonged to helminth species and 12 (20.7%) to protozoan species. The results showed that *B. germanica* (58.6%) had the highest prevalence, followed by *Bl. orientalis* (32.8%) and *P. americana* (8.6%). The results of the study not only contribute to the molecular epidemiology of cockroaches but also confirm their important role as mechanical vectors of protozoan and helminth parasites.

Introduction

Cockroaches are one of the most important pests found in apartments, houses, restaurants, hospitals, and health care facilities. Especially German cockroaches show an exploitative effect in poor living conditions. Cockroaches feed on garbage, rotting food, and even the feces of other insects. They are important vectors because they carry pathogens to meals, dishes, kitchen surfaces and other areas around the house. They can cause food poisoning in humans by leaving pathogens such as fungi, viruses, and bacteria on the food (34).

Moreover, they cause allergic reactions in many people and can trigger asthma. 95% of cases of food poisoning are caused by humans consuming cockroach saliva, feces, and the nutrients left by their eggs. They mechanically transmit parasites, bacteria, and viruses by crawling on feces and other organic materials to obtain food. In this respect, they are of medical and economic importance (49).

It has been found that until today, molecular-based studies on cockroaches and their vector potentials are limited in the world, and they are not yet available in Türkiye. In this context, it is aimed to determine the molecular characters of cockroaches and to reveal the phylogenetic structures between cockroach populations in our study and in the world. It is also aimed to determine

the level of genetic differences and the current situation of the mechanical vectoring of the samples determined on the basis of species in terms of parasitic infections. The study yielded data, indicating the first molecular information on cockroaches in Türkiye. In addition, the results provided important scientific data on the zoonotic risks of the mechanical vectoring potential of cockroaches, which are widespread in the study area.

Materials and Methods

Sample Collection and DNA Extraction: A total of 220 adults and nymphs belonging to *Blattella germanica*, *Bl. orientalis*, and *P. americana* species were trapped from different locations such as hospitals, food companies and houses in Kayseri region of Türkiye. Cockroaches were individually placed in plastic containers, inactivated at -20°C, and then identified using morphological keys (22, 42). Genomic DNA extraction from the legs of cockroaches was performed using the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, USA). Total DNA was eluted to the elution tube and stored at -20 °C until use.

PCR-RFLP: Before the RFLP analyses, nested PCR protocol (43) was used to amplify the gene regions for small subunit ribosomal RNA (SSU rRNA). The first PCR step employed IMS-GR1-SSUF1 (5'-TAARGTGAAA CCGCGAATG-3') and IMS-GR1-SSUR1 (5'-ACCTTGTTACGACTTTTAC-3') primers to amplify the relevant gene region and produce 1.793 bp (43). The internal primers IMS-GR1-SSU-F2-(5'-ACCGC GAATGGCTCATTAAATC-3') and IMSGR1-SSU-R2-(5'-TACGACTTTTACTTCCT C-3') were utilized in the second step to amplify the 1.775 bp segment of the corresponding gene region (43). A 50 µl PCR reaction was comprised of 25 pl of HotStarTaq Master Mix (QIAGEN) and 25 pl of a solution containing 200 nM of each primer, 1.5 mM of additional $MgCl_2$, and template DNA (50 ng) that was diluted in PCR-grade water (43). The reactions were performed for 35 cycles, each consisting of 94°C for 45 s, 50°C for 45 s, and 72°C for 60 s, in a thermocycler, with an initial hot start at 94°C for 15 min and a final extension at 72°C for 10 min. For the second round of PCR, a 1.775 bp fragment was amplified from 2.5 ml of primary PCR reaction. The PCR conditions for this round were the same as in the primary PCR, except for a higher annealing temperature of 55°C. The resulting PCR products were analyzed using agarose gel electrophoresis and visualized following ethidium bromide staining.

For the RFLP analysis, AvaI and EciI (New England Biolabs, Beverly, MA) restriction enzymes were selected by the manufacturer. 15 µL of the PCR products were digested in a 25 µL reaction mixture containing the enzymes and 2.5 µL of the appropriate restriction buffer at 37°C by overnight according to the manufacturer's instructions. The digested products were fractionated on a 1.5% agarose gel and visualized by ethidium bromide staining under ultraviolet light.

Nucleotide Sequencing and Phylogenetic Analysis: For the phylogenetic analysis of gDNAs obtained from individual cockroach samples, the mt-COI, mt-COII, and ITS2 gene regions were amplified by PCR using the primers C1J1718MF (5'-GGAGGATTTGGAAATT GATTAGT-3') and C1N2191BR (5'-CAGGTAAAATTA AAATATAAACTTCDGG-3') (17); COIIF (5'-AGAGCWTCACCTATTATAGAAC-3') and COIIR (5'-GTARWACRTCTGCTGCTGTTAC-3') (38); ITS2F (5'-CGATGAA GAACGCAGCAAA-3') and ITS2R (5'-TCCTCCGCTTATTGATATGC-3') (13), respectively. Recombinant plasmid DNAs containing Mt-COI, mt-COII and ITS2 target gene regions were bidirectionally sequenced using pJET1.2 forward and reverse primers. After careful analysis of the chromatograms of the plasmids whose bidirectional DNA sequence was determined, the final sequences of the isolates were obtained by determining the inserted target gene region in the vector nucleotide sequence and by performing pairwise alignments of the forward and reverse sequences using Geneious software (27). DnaSP 5.10.01 software (32) was used to determine DNA polymorphism and haplotype structure in the isolates characterized in the study. Intra- and inter-specific genetic differences were performed in MEGA 7 software (46) by using the Kimura two-parameter (K2P) distance model (28, 36). Bayesian (BA) inference and maximum likelihood (ML) analyses were used to determine the phylogenetic structures of cockroach species. jModelTest v.0.1.1 (40) was used to determine the most appropriate substitution model for sequence evolution in BA and ML analyses, and the models with the lowest AIC (Akaike Information, Criterion, Correction) value were used to construct the phylogenetic trees. BA and ML analyses were performed with the Geneious R10 software (27), using the MrBayes (25) and PhyML (21) plug-ins, respectively. A bootstrap test with 1000 replicates was used to determine the reliability of the trees generated by the ML analysis.

Investigation of Parasitic Forms of Medical Importance in Cockroaches: Cockroaches were transferred to appropriate sterile vials and 0.9% sterile physiological saline was added to them. They were then subjected to mechanical agitation in the Tissue Lyser LT (Qiagen) device for 2 minutes. After that, the obtained suspension was divided into two separate microcentrifuge tubes of 1 ml each. The first dividing tube was centrifuged at 2,000 rpm for 5 minutes, and after removing the supernatant, the sediment stained with %1 Lugol's iodine was examined under a light microscope for parasitic forms (10).

DOI: 10.33988/auvfd.1300194

Results

Identification of Cockroach Species: In the study, adult and nymph cockroaches were classified through morphological analysis as 128 (58.2%) *B. germanica*, 71 (32.3%) *Bl. orientalis* and 21 (9.5%) *P. americana*. The study identified that out of the 220 cockroaches examined, 105 (47.7%) (71 adults, 34 nymphs) were classified as *B. germanica*, 86 (39.1%) (54 adults, 32 nymphs) as *Bl. orientalis*, and 29 (13.2%) (21 adults, 8 nymphs) as *P. americana* species using PCR-RFLP results.

SSU rRNA Nested PCR and RFLP Analysis Results: Table 1 presents the band profiles of cockroach samples obtained through individual gDNA extraction via nested PCR and subsequent analysis of the partial SSU rRNA gene region using RFLP techniques. The results indicate that the *AvaI* and *EciI* enzymes consistently cleave these band profiles in all samples.

Table 1. Some band profiles determined after RFLP with *AvaI* and *EciI* restriction enzymes in positive samples.

Type of cockroach	*AvaI* (bp)	*EciI* (bp)
Blattella germanica	124, 865	1052
Blatta orientalis	613, 831	650, 1018
Periplaneta americana	834	1021

The band profile images obtained from the analysis of positive isolates using *AvaI* and *EciI* restriction enzymes and RFLP analysis, in a 1.5% agarose gel, are displayed in Figure 1 and Figure 2, respectively.

Phylogenetic Analysis Results: A total of 13 haplotypes, five, three and five, respectively, were determined for the mt-COI, mt-COII and ITS-2 gene regions of the related species. The mean haplotype diversities were 0.962±0.017, 0.842±0.047, and 0.810±0.080, respectively. The intraspecific nucleotide differences of *B. germanica*, *Bl. orientalis*, and *P. americana* species in the data sets were 1.0±0.2%, 0.4±0.1%, 2.4±0.5% for mt-COI, 0.1±0.1%, 0.1±0% for mt-COII, 0.3±0.1%, and 0.7%±0.3% and 0.8±0.3% for ITS-2, respectively. The interspecific nucleotide differences are for mt-COI, mt-COII and ITS-2: *B. germanica* and *Bl. orientalis* 22.7±2.5%, 26.0±2.9%, 45.7±6.1%; between *B. germanica* and *P. americana* 24.6±2.5%, 29.5±3.3%, 43.6%±5.8%, and between *Bl. orientalis* and *P. americana* 14.5±1.8%, 15.7%±2.1%, and 17.9±2.7%. According to ML analysis, the sequences of all three species showed monophyletic structure for three gene regions (Figure 3-5). The phylogenetic analysis of the isolates of all three-cockroach species showed similarity rates of 98.6-100% were determined with similar isolates in the world, although it varied depending on the species and gene region.

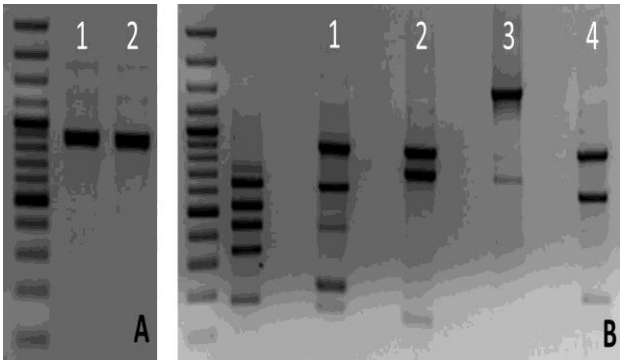

Figure 1. The gel electrophoresis of the band profiles obtained by RFLP analysis with AvaI restriction enzyme of the products obtained after the amplification of the partial SSU rRNA gene region in some cockroach isolates. Marker (100bp).
(A) 1-2: *P. americana*; (B) 1,4: *Bl. orientalis*; 2, 3: *B. Germanica*.

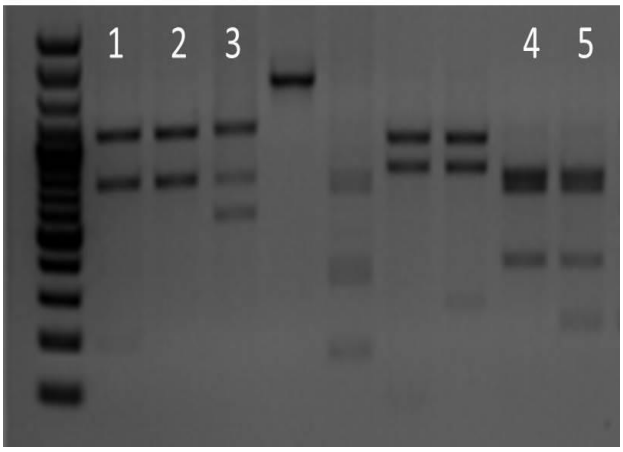

Figure 2. The gel electrophoresis of the band profiles obtained by RFLP analysis with Ecil restriction enzyme of the products obtained after the amplification of the partial SSU rRNA gene region in some cockroach isolates. Marker (100bp).
1-3: *B. germanica*; 4: *P. americana*; B. 5: *Bl. Orientalis.*

Table 2. Protozoan and helminth numbers detected in cockroach species.

Type of cockroach	Protozoa n (%)	Helminths n (%)	Total n (%)
Blattella germanica	26 (56.5)	8 (66.7)	34 (58.6)
Blatta orientalis	16 (34.8)	3 (25.0)	19 (32.8)
Periplaneta americana	4 (8.7)	1 (8.3)	5 (8.6)
Total	46 (100.0)	12 (100.0)	58 (100.0)

Parasitic Forms Detected in Cockroaches: It was found that 47 (21.4%) of the 220 cockroaches examined were infective with at least one parasitic form, and some cockroach samples were found to be infective with several parasitic forms (Table 2). Table 2 shows that *B. germanica* (58.6%) was the most parasitized cockroach. This was

followed by *Bl. orientalis* (32.8%) and *P. americana* (8.6%). It was determined that 46 (79.3%) of the total 58 parasitic forms identified belonged to protozoan species and 12 (20.7%) to helminth species. *Toxocara* spp. (4 eggs, 8.5%), Trichostrongylid type eggs (3 eggs, 6.4%), *Trichuris* spp. (3 eggs, 6.4%), *Ascaris lumbricoides* (2 eggs, 4.3%); *Blastocystis* sp. (12 vacuolar form, 25.5%), isosporoid type oocyst (10 oocyst, 21.3%), *Eimeria* spp. (7 oocysts, 14.9%), *Cryptosporidium* spp. (17 oocysts, 36.2%) were identified among protozoa (Figure 6-7).

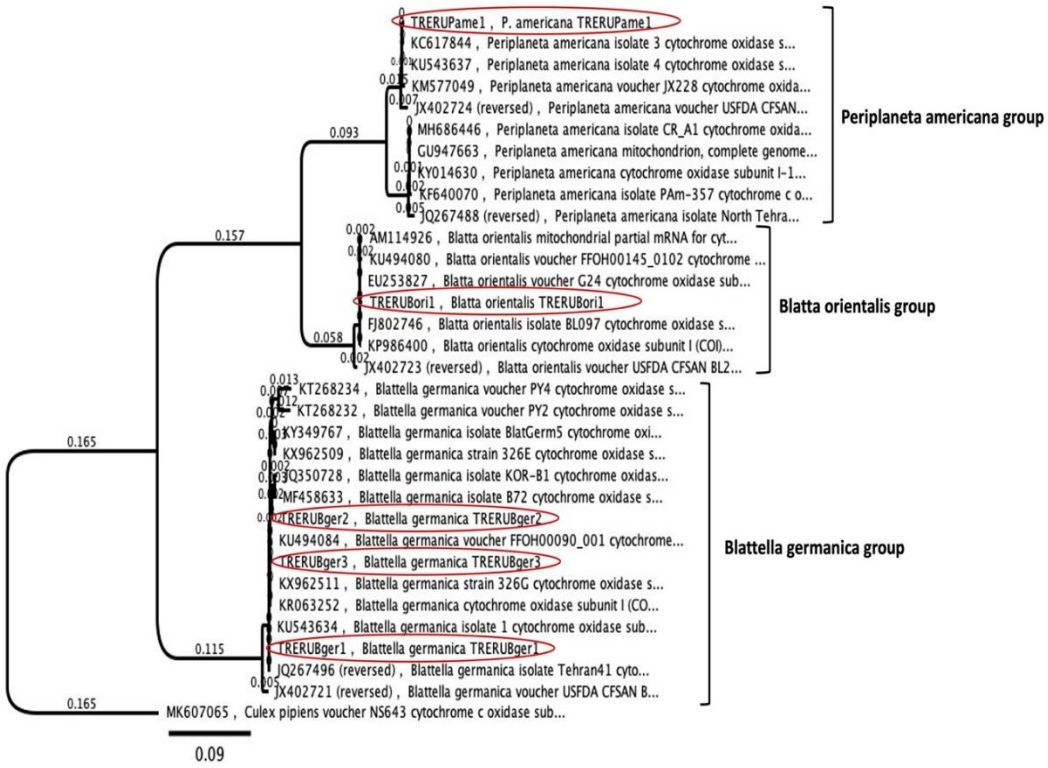

Figure 3. Phylogenetic relationships of cockroach isolates isolated from Kayseri region and other cockroach isolates registered in GenBank according to mt-COI gene region.

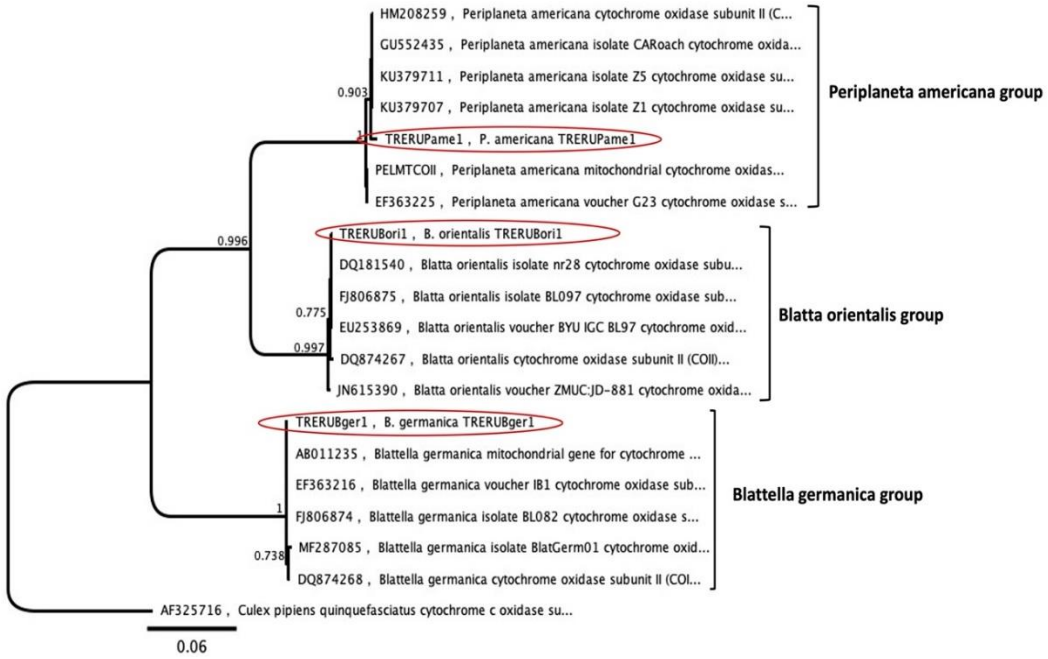

Figure 4. Phylogenetic relationships of cockroach isolates isolated from Kayseri region and other cockroach isolates registered in GenBank according to mt-COII gene region.

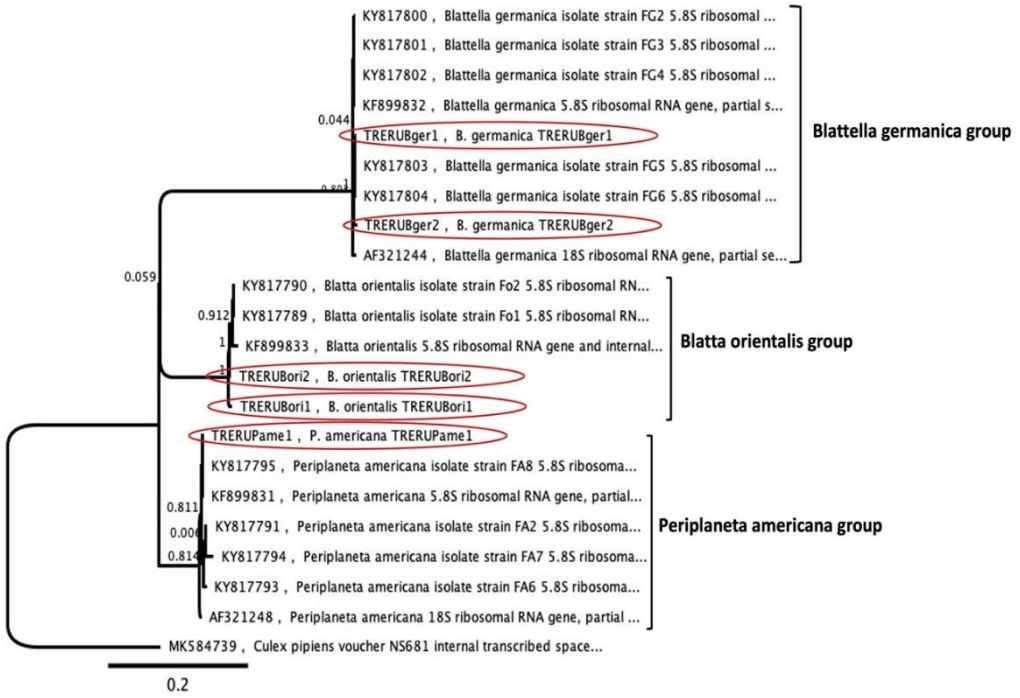

Figure 5. Phylogenetic relationships of cockroach isolates isolated from Kayseri region and other cockroach isolates registered in GenBank according to ITS-2 gene region.

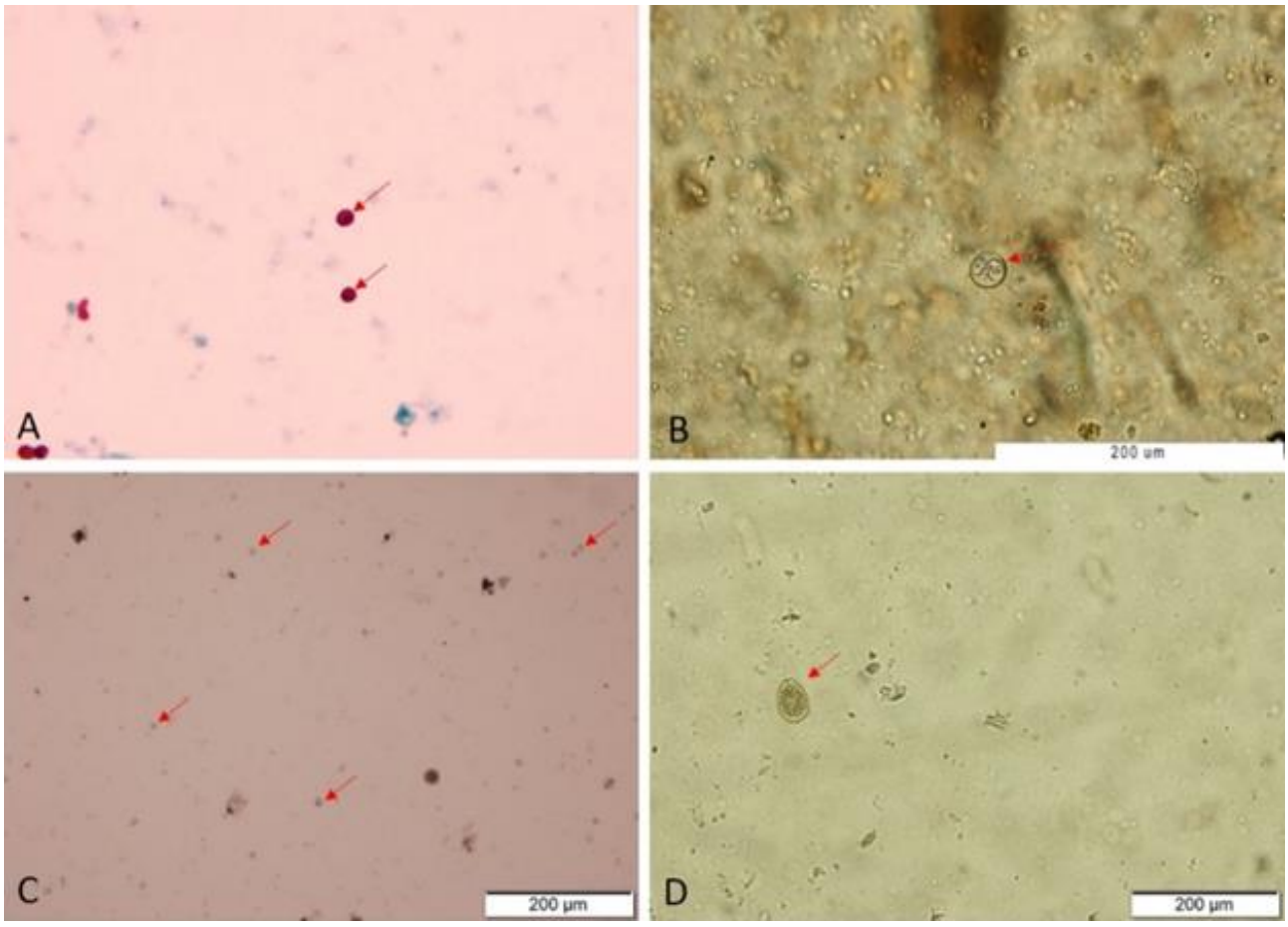

Figure 6. Protozoan species detected in cockroaches.

A. *Cryptosporidium* spp. oocysts, B. Sporulated isosporoid type oocyst, C. *Blastocystis* sp. vacuolar forms, D. Unsporulated *Eimeria* spp. oocyst.

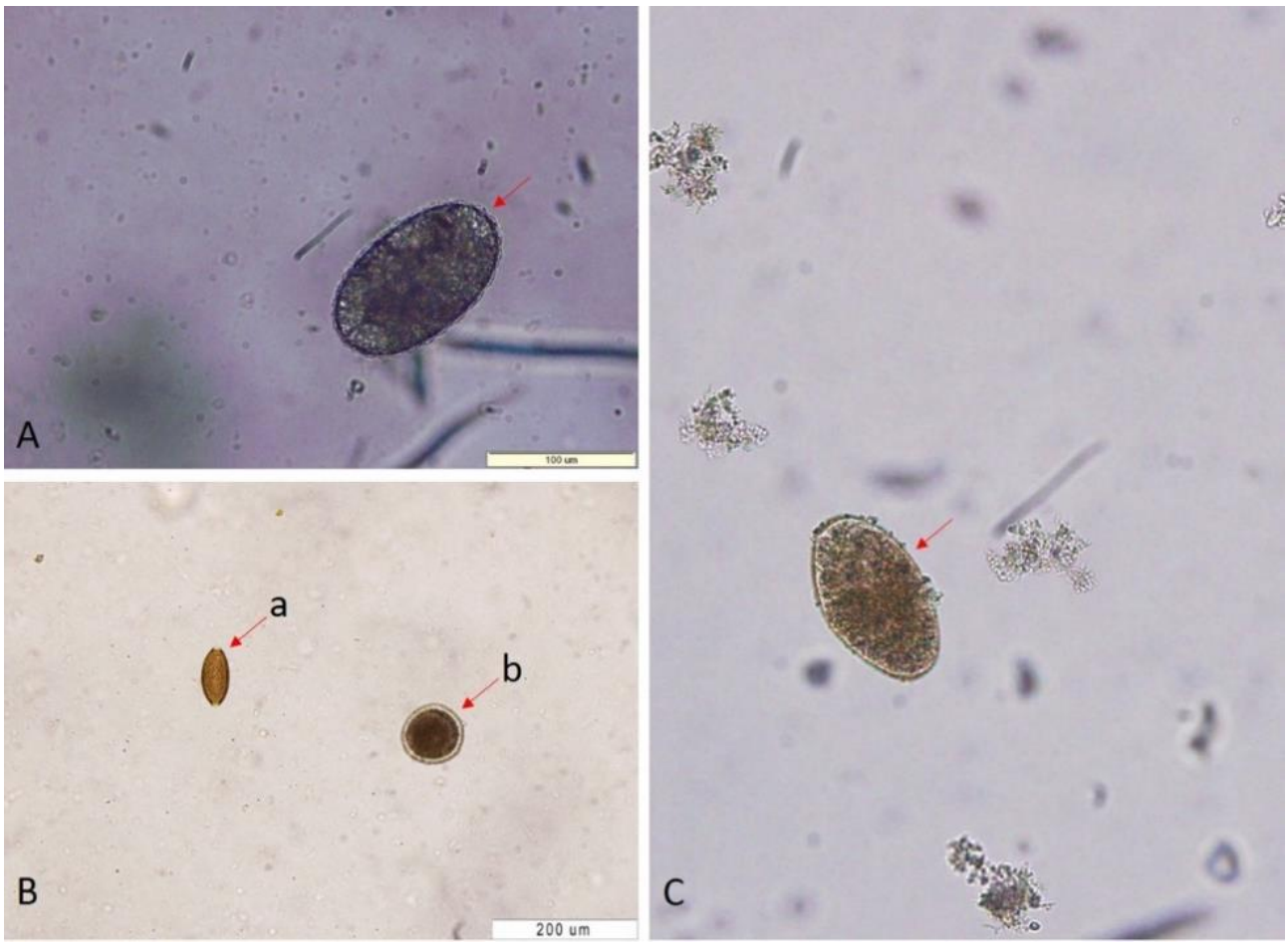

Figure 7. Helminth species detected in cockroaches.
A. Infertile *Ascaris lumbricoides* egg, B. a. *Trichuris* sp. egg, b. *Toxocara* sp. egg, C. *Trichostrongylid* type egg.

Discussion and Conclusion

In Türkiye, the existence of *Bl. orientalis*, *P. americana*, *P. australasiae* belonging to Blattidae family and *B. germanica* species belonging to Blattellidae family have been reported. In comparison with other studies conducted in Türkiye (30, 37), *B. germanica* was found to be the most dominant species in our current study in line with these studies. However, the prevalence of *B. orientalis* and *P. americana* in our study was quite high compared to other studies. Two other studies (30, 37) collected cockroach samples from houses and hospitals. However, in our current study, samples were obtained from hospitals and houses, as well as from food establishments. While *Bl. orientalis* and *P. americana* species tend to live in wet and humid areas due to their high moisture requirements, *B. germanica* is mostly adapted to living in areas such as kitchens, basements, and hospitals (1). Therefore, it is hypothesised that the variance in prevalence rates could be attributed to the fact that the cockroaches grouped by habitat originate from varying environments and regions.

Furthermore, it is worth mentioning that both studies relied solely on morphological criteria for the identification of cockroaches. According to the literature (6, 12, 16, 48, 51), it has been reported that it is very difficult to determine the species based on the morphological characteristics of adult individuals and young nymphs, especially in cockroaches. The diagnosis of all cockroaches (146 adults, 74 nymphs) sampled in our study was confirmed by RFLP and sequence analysis. In our study, we confirmed this situation through the varying rates of molecular and morphological prevalence we obtained.

It is usually very difficult to distinguish between adult and nymphal stages of cockroaches. Close-knit species often have very similar morphological characteristics. Cockroaches vary greatly in their developmental stages. Especially externally, differences in morphological criteria such as spination, setation and coloration make it very difficult to distinguish between species (6, 12, 48). Therefore, to overcome this situation, simple, accurate and easily applicable methods that can distinguish all developmental stages of cockroaches are needed (48, 51).

In this context, diagnostic methods based on DNA barcoding have been developed in recent years to determine the species of cockroaches and other insects

with higher accuracy. To date, the number of studies using the mt-COI or ITS gene region to differentiate cockroach species is quite limited worldwide. Knebelsberger and Miller (29) used COI sequences to distinguish three conspecific morphotypes of *Phyllodromica iberica*, and to identify phylogenetic relationships among species in the subaptera-group. Evangelista et al. (11) used the COI gene region to confirm the existence of *P. japonica*, a new invasive cockroach species they found in New York. Similarly, Yue et al. (49) used a DNA barcoding system to determine that both macropterous and brachypterous females and males of *Hebardina concinna* belonged to this species. Evangelista et al. (12) used both morphological and genetic barcode information to reveal the species richness of the Blattodea family. Che et al. (5) determined the phylogenetic affinities of cockroaches of the family Ectobiidae collected in China by amplification of the COI gene region. Hashemi-Aghdam et al. (24) amplified the mt-COI gene regions of *B. germanica*, *Bl. orientalis*, *P. americana*, *Shelfordella lateralis* and *Supella longipalpa* species for DNA barcoding of cockroaches and developed the PCR-RFLP method for rapid identification of these species in their study in Iran. Similarly, Sulaiman et al. (43), developed the PCR-RFLP technique based on the SSU rRNA gene region for differentiation of *B. germanica*, *Bl. orientalis*, *P. americana* and *S. longipalpa* species. The same researchers (44) performed DNA barcoding of these four species according to the mt-COI gene region in 2016. Cheng et al. (7) extracted the complete mitochondrial genomes of the cockroach species *Gromphadorhina portentosa*, *Panchlora nivea*, *Blaptica dubia* in the family Blaberidae and *S. lateralis* in the family Blattidae. Mukha et al. (35) reported that the 28S rDNA gene region, together with the ITS-1 and ITS-2 gene regions, can be used to differentiate cockroaches in the *Blattella* and *Periplaneta* lineages. Similarly, Everaerts et al. (13) have reported that complex species of *Cryptocercus punctulatus* in the family Cryptocercidae utilized the 16S, mt-COII and ITS-2 gene regions for DNA barcoding. Park et al. (38) performed DNA barcoding of the mt-COII, 16S and 18S rRNA gene regions of *Cryptocercus* cockroach species native to North Asia. Farmani et al. (14) used the ITS-2 gene region for DNA barcoding of seven cockroach species (*P. americana*, *S. lateralis*, *Bl. orientalis*, *B. germanica*, *S. longipalpa*, *Polyphaga aegyptiaca*, *P. saussurei*) after morphological examination in Iran. They pointed out the importance of confirming the species by molecular techniques.

All studies on cockroaches in Türkiye have been carried out according to morphological criteria and no study has been revealed the molecular characters of cockroaches. In this sense, our present study has the feature of the first qualification in which the molecular characters of cockroaches in the *B. germanica*, *Bl. orientalis* and *P. americana* strains in Türkiye were revealed and their phylogenetic affinities with similar isolates in the world were determined. In our study, mt-COI, mt-COII and ITS-2 gene regions were used for DNA barcoding of three cockroach species in parallel with studies in the world. As a result of the study, it was confirmed that all three genes can be used as molecular markers to differentiate *B. germanica*, *Bl. orientalis* and *P. americana* species.

Cockroaches are mechanical vectors of many pathogens that infect both humans and animals by carrying them from one place to another with their bodies. In studies on this subject, it has been reported that cockroaches have reached dimensions that threaten human health by infecting humans with saprophytic and pathogenic microorganisms with this role (1, 4). Besides, many areas such as especially kitchens, rooms, basements of houses, sewer systems, manholes, storerooms, patient rooms, examination rooms, study rooms, warehouses, kitchens, laundries, warehouses, meeting rooms, canteens, tea stoves, toilets, and bathrooms in hospitals. They create ideal environments for insects to breed. The abundance of cockroach species captured in hospital environments via varied trapping methods suggests that hospitals represent vital zones for control purposes since these species serve as vectors of pathogenic microorganisms (2, 8, 18-20, 41). In addition to the many bacterial pathogens that cockroaches carry as mechanical vectors, one of the most important issues is the parasitic pathogens that they carry. Protozoa such as *Toxoplasma gondii*, *Blastocystis hominis*, *Cryptosporidium* spp., *Balantidium coli*, *Entamoeba histolytica*, *Giardia intestinalis*, and infective forms of some helminth species such as *Ascaris lumbricoides*, *Enterobius vermicularis*, *Ancylostoma duodenale*, *Necator americanus*, *Hymenolepis diminuta*, *Trichuris trichuria*, *Gongylonema pulchrum* have been detected in cockroaches. Although the possibility of cockroaches being biological vectors for these species is worth considering, recent reports suggest that this may be linked to cockroaches' feeding habits (1, 15, 31, 33, 39, 40, 47). A study by Hamu et al. (23) reported that *A. lumbricoides*, *T. trichiura*, *Taenia* spp., *Strongyloides* spp., *E. histolytica/dispar/moshkovski*, *G. duodenalis* and *B. coli* parasites were detected on the external surface of approximately 11% of 2,010 *B. germanica* cockroaches. El-Sherbini and El-Sherbini (9) found parasitic pathogens such as *E. histolytica*, *C. parvum*, *Cyclospora cayetenensis*, *Isospora belli*, *B. coli*, *A. lumbricoides*, *A. duodenale*, *E. vermicularis*, *T. trichura*, and *S. stercoralis* on the external surfaces of various species of cockroaches collected from toilets, kitchens, and bedrooms in their study in Egypt. Chamavit et al. (3) found parasitic forms on the external surfaces of about 54% of a total of 920

cockroaches collected from 18 public supermarkets in Thailand and reported that 56% of them were protozoans [*Cyclospora* spp. (1.3%), *Endolimax nana* (1.3%), *B. hominis* (1.2%), *I. belli* (9.6%), *E. histolytica/dispar* (4.6%), *Cryptosporidium* spp. (28.1%), *Chilomastix mesnilli* (0.3%), *E. coli* (4.0%), *B. coli* (5.8%), *Iodamoeba butschlii* (0.1%)], 1.5% pathogenic helminth species [*S. stercoralis* (0.8%), *A. lumbricoides* (0.3%), *T. trichiura* (0.3%), *Taenia* spp. (0.1%)] and 42.5% of them were non-pathogenic helminth species. Similarly, Jarujareet et al. (26) reported in their study on *P. americana* cockroaches that these insects carry sporulated *E. tenella* oocysts on their external surfaces. In the first study in Türkiye to detect parasitic infections in cockroaches (37), it was reported that 48% of 138 cockroaches collected in the Van region and diagnosed as *B. germanica* were infected with parasitic forms. In the same study (19), about 97% of the parasitic forms detected were protozoa and the rest were helminth species [*Toxocara* sp. (3%), *A. lumbricoides* (3%), *Trichostrongylus* sp. (1.5%), *T. trichiura* (1.5%), *E. nana* (7.6%), *B. hominis* (41%), *E. histolytica/E. dispar* (16.7%), unsporulated coccidial oocysts (7.6%), *C. mesnilli* (4.5%), *E. coli* (35%), *Giardia* spp. (13.6%), *I. butschlii* (7.6%)] were detected. In our current study, 47 (21.4%) of the 220 cockroaches we collected were found to be infective with at least one parasitic form, and some cockroach samples were found to be infective with several parasitic forms. In our study, *B. germanica* (58.6%) was found to be the most parasitized cockroach, followed by *Bl. orientalis* (32.8%) and *P. americana* (8.6%). It was found that 79.3% of the identified parasites belonged to protozoan species [*Blastocystis* sp. (25.5%), isosporoid type oocysts (21.3%), *Eimeria* spp. (14.9%), *Cryptosporidium* spp. (36.2%)] and 20.7% to helminth species [*Toxocara* spp. (8.5%), *Trichostrongylid* type eggs (6.4%), *Trichuris* spp. (6.4%), *A. lumbricoides* (4.3%)]. It has been concluded that the obtained results are similar to the results of studies on parasitic cockroaches both in the world and in Türkiye in terms of parasite species and prevalence rates.

In conclusion, this study presents the first molecular characterization of the mt-COI, mt-COII, and ITS-2 gene regions of cockroaches in Türkiye. Furthermore, the study establishes the phylogenetic relationship of these isolates with similar ones from around the world. It was concluded that the obtained results could contribute to the limited knowledge of the molecular epidemiology of cockroaches. Moreover, cockroaches were found to play a role as mechanical vectors of parasite-related diseases. In this regard, further studies should be conducted, such as the prevalence and status of cockroach-related parasitic diseases affecting health risks in different habitats and their appropriate control measures in our environment.

Acknowledgements

This study was derived from the the first author's doctoral dissertation.

Financial Support

This study was supported by the Erciyes University Scientific Research Projects Unit with project number TDK-2017-7760.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

Consept: FC, OD, AY, Design: FC, OD, AY, Sample Collection: FC, Processing: FC, MA, Analysis and Interpretation: FC, OD, AY, Literature Search: FC, OD, AY, Writing: FC, OD.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

References

1. **Altay K, Dumanlı N** (2015): Blattaria. 133-136. In: Karaer Z, Dumanlı N (Eds), Arthropodoloji. Medisan Press, Ankara.
2. **Arakere G, Nadig S, Swedberg G, et al** (2005): *Genotyping of methicillin-resistant Staphylococcus aureus strains from two hospitals in Bangalore, South India*. J Clin Microbiol, **43**, 3198-3202.
3. **Chamavit P, Sahaisook P, Niamnuy N** (2011): *The majority of cockroaches from the Samutprakarn province of Thailand are carriers of parasitic organisms*. EXCLI Journal, **10**, 218-222.
4. **Chan OT, Lee EK, Hardman JM, et al** (2004): *The cockroach as a host for Trichinella and Enterobius vermicularis: implications for public health*. Hawaii Med J, **63**, 74-77.
5. **Che Y, Gui S, Lo N, et al** (2017): *Species delimitation and phylogenetic relationships in ectobiid cockroaches (Dictyoptera, Blattodea) from China*. PLoS One, **12**, e0169006.
6. **Che Y, Wang Z** (2013): *Three new species of cockroach genus Symploce Hebard, 1916 (Blattodea, Ectobiidae, Blattellinae) with redescriptions of two known species based on types from Mainland China*. ZooKeys, **337**, 1.
7. **Cheng XF, Zhang LP, Yu DN, et al** (2016): *The complete mitochondrial genomes of four cockroaches (Insecta: Blattodea) and phylogenetic analyses within cockroaches*. Gene, **586**, 115-122.
8. **Cetin H** (2015): *The importance of vector management for prevention of hospital infections*. Turkiye Parazitol Derg, **39**, 227-230.

9. **El-Sherbini GT, El-Sherbini ET** (2011): *The role of cockroaches and flies in mechanical transmission of medical important parasites*. J Entomol Nematol, **3**, 98-104.
10. **El-Sherbini GT, Gneidy MR** (2012): *Cockroaches and flies in mechanical transmission of medical important parasites in Khaldyia Village, El-Fayoum, Governorate, Egypt*. J Egypt Soc Parasitol, **240**, 1-10.
11. **Evangelista D, Buss L, Ware JL** (2013): *Using DNA barcodes to confirm the presence of a new invasive cockroach pest in New York City*. J Econ Entomol, **106**, 2275-2279.
12. **Evangelista DA, Bourne G, Ware JL** (2014): *Species richness estimates of B lattodea ss (Insecta: Dictyoptera) from northern G uyana vary depending upon methods of species delimitation*. Syst Entomol, **39**, 150-158.
13. **Everaerts C, Maekawa K, Farine JP, et al** (2008): *The Cryptocercus punctulatus species complex (Dictyoptera: Cryptocercidae) in the eastern United States: comparison of cuticular hydrocarbons, chromosome number, and DNA sequences*. Mol Phylogenet Evol, **47**, 950-959.
14. **Farmani M, Basseri H, Norouzi B, et al** (2019): *Ribosomal DNA internal transcribed spacer 2 sequence analysis and phylogenetic comparison of seven cockroach species in northwestern Iran*. BMC Res Notes, **12**, 1-5.
15. **Fathpour H, Emtiazi G, Ghasemi E** (2003): *Cockroaches as reservoirs and vectors of drug resistant Salmonella* spp. Iran Biomed J, **7**, 35-38.
16. **Fisk FW** (1982): *Abundance and diversity of arboreal Blattaria in moist tropical forests of the Panama canal area and Costa Rica*. T Am Entomol, 479-489.
17. **Folmer O, Black M, Hoeh W, et al** (1994): *DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.* Mol Mar Biol Biotechnol, **3**, 294-299.
18. **Galdiero E, Villari P, Onofrio V, et al** (2005): *Characterization of glycopeptide resistant enterococci isolated from a hospital in Naples (Italy).* New Microbiol, **28**, 171-176.
19. **Gazeta GS, Freire ML, Ezequiel OS, et al** (2007): *Artrópodes capturados em ambiente hospitalar do Rio de Janeiro, Brasil*. Rev Patol Trop, **36**, 254-264.
20. **Gliniewicz A, Sawicka B, Czajka E** (2003): *Occurrence of insect pests in hospitals in Poland*. Przegl Epidemiol, **57**, 329-334.
21. **Guindon S, Gascuel O** (2003): *A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood*. Syst Biol, **52**, 696-704.
22. **Hamu H, Debalke S, Zemene E, et al** (2014): *Isolation of intestinal parasites of public health importance from cockroaches (Blattella germanica) in Jimma Town, Southwestern Ethiopia*. J Parasitol Res, **186240**, 1-5.
23. **Hristov GH, Chobanov DP** (2016). *An annotated checklist and key to the Bulgarian cockroaches (Dictyoptera: Blattodea).* Zootaxa, **4154**, 351-388.
24. **Hashemi-Aghdam SS, Rafie G, Akbari S, et al** (2017): *Utility of mtDNA-COI barcode region for phylogenetic relationship and diagnosis of five common pest cockroaches*. J Arthropod-Borne Dis, **11**, 182-193.
25. **Huelsenbeck JP, Ronquist F** (2001): *MRBAYES: Bayesian inference of phylogenetic trees*. Bioinformatics, **17**, 754-755.
26. **Jarujareet W, Kobayashi M, Taira K, et al** (2019): *The role of the American cockroach (Periplaneta americana) as transport host of Eimeria tenella to chickens*. Parasitol Res, **118**, 2311-2315.
27. **Kearse M, Moir R, Wilson A, et al** (2012): *Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data*. Bioinformatics, **28**, 1647-1649.
28. **Kimura M** (1980): *A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences*. J Mol Evol, **16**, 111-120.
29. **Knebelsberger T, Miller MA** (2007): *Revision and phylogeny of the subaptera-group of Phyllodromica (Blattoptera: Blattellidae: Ectobiinae), including a parthenogenetic species and the evaluation of COI sequences for species identification (DNA barcoding).* Zootaxa, **1522**, 1-68.
30. **Kutrup B** (2003): *Cockroach infestation in some hospitals in Trabzon, Turkey*. Turk J Zool, **27**, 73-77.
31. **Lamiaa B, Lebbadi M, Ahmed A** (2007): *Bacteriological analysis of Periplaneta americana L. (Dictyoptera; Blattidae) and Musca domestica L. (Diptera; Muscidae) in ten districts of Tangier, Morocco*. Afr J Biotechnol, **6**, 2038-2042.
32. **Librado P, Rozas J** (2009): *DnaSP v5: a software for comprehensive analysis of DNA polymorphism data*. Bioinformatics, **25**, 1451-1452.
33. **Miranda RA, Silva JP** (2008): *Enterobactérias isoladas de Periplaneta americana capturadas em um ambiente hospitalar*. Ciência et Praxis, **1**, 21-24.
34. **Moges F, Eshetie S, Endris M, et al** (2016): *Cockroaches as a source of high bacterial pathogens with multidrug resistant strains in Gondar town, Ethiopia*. Biomed Res Int, 1-6.
35. **Mukha D, Wiegmann BM, Schal C** (2002): *Evolution and phylogenetic information content of the ribosomal DNA repeat unit in the Blattodea (Insecta)*. Ins Biochem Mol Biol, **32**, 951-960.
36. **Nei M, Kumar S** (2000): Molecular evolution and phylogenetics: Oxford University Press, USA.
37. **Oguz B, Ozdal N, Orunc Kilinc O, et al** (2017): *First investigation on vectorial potential of Blattella germanica in Turkey*. Ankara Univ Vet Fak Derg, **64**, 141-144.
38. **Park YC, Maekawa K, Matsumoto T, et al** (2004): *Molecular phylogeny and biogeography of the Korean woodroaches Cryptocercus spp*. Mol Phylogenet Evol, **30**, 450-464.
39. **Pongsiri MJ, Roman J** (2007): *Examining the links between biodiversity and human health: an interdisciplinary research initiative at the US Environmental Protection Agency*. EcoHealth, **4**, 82-85.
40. **Posada D** (2008): *jModelTest: phylogenetic model averaging*. Mol Biol Evol, **25**, 1253-1256.
41. **Salehzadeh A, Tavacol P, Mahjub H** (2007): *Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran*. J Vect Borne Dis, **44**, 105-110.
42. **Sharawi ES, Mahyoub JA, Assagaf AI** (2021): *Morphological and molecular identification of the American cockroaches (Periplaneta americana)in Jeddah*

province (Dictyoptera: Blattidae). Int J Entomol Res, **6**, 31-36.

43. **Sulaiman IM, Anderson M, Khristova M, et al** (2011): *Development of a PCR–Restriction Fragment Length Polymorphism Protocol for Rapid Detection and Differentiation of Four Cockroach Vectors (Group I "Dirty 22" Species) Responsible for Food Contamination and Spreading of Foodborne Pathogens: Public Health Importance*. J Food Prot, **74**, 1883-1890.
44. **Sulaiman IM, Jacobs E, Simpson S, et al** (2017): *Identification of 18 vector species belonging to Group I, Group II, and Group III 'Dirty 22'species known to contaminate food and spread foodborne pathogens: DNA barcoding study of public health importance*. Int J Trop Insect Sci, **37**, 1-10.
45. **Tachbele E, Erku W, Gebre-Michael T, et al** (2006): *Cockroach-associated food-borne bacterial pathogens from some hospitals and restaurants in Addis Ababa, Ethiopia: Distribution and antibiograms*. JRTPH, **5**, 34-41.
46. **Tamura K, Stecher G, Peterson D, et al** (2013): *MEGA6: Molecular evolutionary genetics analysis version 6.0*. Mol Biol Evol, **30**, 2725-2729.
47. **Tatfeng YM, Usuanlele MU, Orukpe A, et al** (2005): *Mechanical transmission of pathogenic organisms: the role of cockroaches*. J Vect Borne Dis, **42**, 129-134.
48. **Wang ZQ, Li Y, Che YL, et al** (2015): *The wood-feeding genus Cryptocercus (Blattodea: Cryptocercidae), with description of two new species based on female genitalia*. Fla Entomol, **98**, 260-271.
49. **Yılmaz YB, Tunaz H** (2013): *Bazı bitki uçucu yağlarının ve monoterpenoid bileşenlerinin Amerikan hamambceği, Periplaneta americana (Dictyoptera: Blattidae), erginlerine karşı fumigant toksisitesi*. Turk Entomol Derg, **37**, 319-328.
50. **Yue Q, Wu K, Qiu D, et al** (2014): *A formal re-description of the cockroach Hebardina concinna anchored on DNA barcodes confirms wing polymorphism and identifies morphological characters for field identification*. PLoS One, **9**, e106789.
51. **Zheng Y, Wang C, Che Y, et al** (2016): *The species of Symplocodes Hebard (Blattodea: Ectobiidae: Blattellinae) with description of a new species from China*. Ann Mag Nat Hist, **50**, 339-361.