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CDNA CLONING, CHARACTERIZATION AND EXPRESSION ANALYSIS OF RIBOSOMAL PROTEIN S23 GENE OF *PERIPLANETA AMERICANA* (BLATTODEA: BLATTIDAE)

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ABSTRACT

Ribosomal protein S23 (*RPS23*) constitutes the ribosomal binding site for mRNA in the 40S initiation complex, and appears to be involved in initiating the translation of messenger RNA into protein (translation step). In this study, a full-length of *RPS23* cDNA was cloned from the American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae) by using rapid amplification of cDNA ends (RACE) approaches. The full-length cDNA of the *P. americana RPS23* (*PaRPS23*) was of 615 bp, containing a 5′ untranslated region (UTR) of 116 bp, a 3′ UTR of 67 bp with a poly (A) tail, and an open reading frame of 432 bp encoding a polypeptide of 143 amino acids with the predicted molecular weight of 16.03 kDa and estimated isoelectric point of 10.47 (NCBI accession number: KJ472479). BLAST analysis revealed that amino acids of *PaRPS23* shared identity with *Graphocephala atropunctata* (Signoret) 99%, (Hemiptera: Cicadellidae)*, Tribolium castaneum* (Herbst) 99%, (Coleoptera: Tenebrionidae), *Pediculus humanus corporis* L. 98%, (Phthiraptera: Pediculidae), *Riptortus pedestris* (F.) 98%, (Hemiptera: Alydidae), *Biphyllus lunatus* (F.) 98%, (Coleoptera: Biphyllidae), *Maconellicoccus hirsutus* (Green) 97%, (Hemiptera: Pseudococcidae), and *Drosophila yakuba* Burla 97%, (Diptera: Drosophilidae) and so on. Quantitative RT-PCR analysis indicated that *PaRPS23* could be detected in all the tested tissues, in which the relative expression levels were 1.2-, 17.9-, 7.3-, 5.6- and 2.2-fold higher in the ovary, head, thorax, leg and gut than in the testis, respectively. The results provided some important base data for further functional studies in *P. americana*, and the information may also help to understand the evolution of ribosomes in insects.

Key Words: American cockroach, ribosomal protein S23, RACE; quantitative RT-PCR; cloning; characterization

RESUMEN

La proteína ribosomal S23 (*RPS23*) constituye el sitio de unión ribosomal para el ARNm en el complejo de iniciación 40S y parece estar implicada en la iniciación de la traducción de ARN mensajero en la proteína (paso de traducción). En este estudio, se clonó una longitud completa del ADNc de *RPS23* de la cucaracha americana, *Periplaneta americana* (L.) (Blattodea: Blattidae) por medio del uso de la amplificación rápida de los extremos de ADNc (AREC). El ADNc de longitud completa de la RPS23 (PaRPS23) de *P. americana* fue de 615 pb y contiene una región 5' no traducida (RNT) de 116 pb, una RNT 3' de 67 pb con una cola poli (A) y un marco de lectura abierto de 432 pb que codifica un polipéptido de 143 aminoácidos con el peso molecular predicho de 16.03 kDa y un punto isoeléctrico estimado de 10.47. El análisis de BLAST reveló que los aminoácidos de *PaRPS23* compartieron 99 %, 99 %, 98 %, 98 %, 98 %, 97 % y 97 % de identidad con los de *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Pediculus humanus corporis* L. (Phthiraptera : Pediculidae), *Riptortus pedestris* (F.) (Hemiptera: Alydidae), *Biphyllus lunatus* (F.) (Coleoptera: Biphyllidae), *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), y *Drosophila yakuba* Burla (Diptera: Drosophilidae), respectivamente. El análisis cuantitativo del tiempo real de PCR indicó que *PaRPS23* podría ser detectada en todos los tejidos analizados. Los niveles de expresión relativos fueron de 1.2, 17.9, 7.3, 5.6 y 2.2 veces más altos que en el ovario, cabeza, tórax, pierna e intestino que en el testículo, respectivamente. Los resultados proveen algunos datos básicos importantes para futuros estudios funcionales de *P. americana,* y la información también puede ayudar a entender la evolución de los ribosomas en los insectos.

Palabras Clave: cucaracha americana, proteína ribosomal S23, AREC; PCR cuantitativa en tiempo-real; clonación; caracterización

The eukaryotic ribosome is a complex macromolecular structure composed of 2 subunits: a large (60S) and small (40S) subunit; the first of these subunits consists of 3 RNAs and 46 proteins, and the second subunit consists of one RNA and 33 proteins (Wool et al. 1995). The exact ribosomal function of specific ribosomal proteins is largely unknown (Wool et al. 1995; Wool 1996).

Ribosomal proteins are highly conserved proteins encoded by the housekeeping genes, as their activity is required for the growth and maintenance of all cell types (Wool 1979); and, further, their expression levels are correlated with the proliferative status of the cells: a high level in rapidly proliferating cells and a lower level in quiescent cells (Bévort & Leffers 2000). In *Xenopus* spp. (Anura: Pipidae) frogs, and most likely other vertebrates, the synthesis of ribosomal proteins is coordinated with both the rRNA synthesis and the expression of some translational factors, and the expression levels of all are adjusted to the different need for protein synthesis under different conditions (Mager 1988; Pierandrei-Amaldi & Amaldi 1994). The expression levels of ribosomal protein mRNAs in higher eukaryotes are probably mainly determined by the growth rate of the cells, similar to observations in yeast and prokaryotes (Mager 1988; Pierandrei-Amaldi & Amaldi 1994; Planta 1997). This is supported by many reports describing growth-related increases in the expression levels of many ribosomal protein mRNAs and by the observation that at least some ribosomal protein mRNAs are up regulated when cells enter G1 (Wu et al. 1993).

The eukaryotic ribosomal protein S23 (*RPS23*) belongs to the ribosomal protein family, known as S12 in bacteria and as either S12 or S23 in *Archaea* (Kitaoka et al. 1994), a domain of single celled prokaryotes. *RPS23* is highly conserved among species (Hori et al. 1993), identified in several organisms such as mammals, for example *Homo sapiens* (Andreev et al. 2011), *Rattus norvegicus* (Kitaoka et al. 1994), *Chinchilla lanigera* (GenBank accession No. AAS55902), *Sus scrofa* (accession No. AAS59430), amphibians (Klein et al. 2002), teleosts (Karsi et al. 2002), insects [e.g., *Bombyx mori* (Traut et al. 2007) and *Plutella xylostella* (accession No. BAD26702)], nematodes (Gregory et al. 1997; Kamath et al. 2003), and the earthworm, *Lumbricus rubellus* (accession No. CAC14789). *RPS23* appears to be involved in the translation initiation step of protein synthesis from the messenger RNA template (McMahon et al. 1982). *RPS23* was identified as the protein component cross-linked to the globin mRNA protein complex, shown to attach directly to the mRNA (Takahashi et al. 2002), and constituted the ribosomal binding site for mRNA in the 40S initiation complex (Takahashi et al. 2002). In addition, the most frequently expressed genes in human megakaryocytes were identified as platelet factor 1 followed by annexin A1, *RPS23* (Kim 2003). Therefore it was demonstrated that *RPS23* play a control role in the megakaryocytes of human.

There are more than 4,000 cockroach species but only about 30 are household pests (Titlow et al. 2013). Perhaps the most recognized is the misnamed American cockroach *Periplaneta americana* (L.), which originated in Africa, and is now found nearly everywhere on the planet. In addition to its rapid running speed (Full & Tu 1991) and evasive behavior, *P. americana* in the tropics is capable of flight (Ritzmann et al. 1980; Libersat & Camhi 1988). *Periplaneta americana* is resistant to all major groups of insecticides, and has also been regarded as model for studying insect physiology (Irles et al. 2009; Yoon et al. 2009). However, no information has been available for *RPS23* in this evolutionarily important organism so far. The aims of the present study were to characterize *RPS23* cDNAs from the cockroach, *P. americana*.

Materials and Methods

Insect Sampling

Specimens of *P. americana* were obtained from our laboratory. The colony was maintained at 60-70% RH, 12:12 h L:D and 25 °C. The cockroaches, which molted to adults within 20 to 30 days were selected. We collected heads, thoraxes, legs and guts from 10 females and 10 males, and also ovaries from 10 females and testes from 10 males. All dissection and tissue sampling was carried out on carbon dioxide-anaesthetized specimens (Irles et al. 2009).

RNA Isolation and cDNA Preparation

Total RNA was isolated from female or male adult *P. americana* using RNeasy® Plant Mini Kit (Qiagen), following the manufacturer›s protocols (Irles et al. 2009). Considerable care was taken to ensure that all total RNA samples used for RACE were of high quality (A260/A280 >1.8 in nuclease-free water) with minimal degradation (Geng et al. 2009). To obtain a complete cDNA sequence, 5′ and 3′ RACE were conducted according to the manufacturer's instructions, using the SMARTer™ RACE cDNA Amplification Kit (Clontech, USA), following the manufacturer's protocols.

Cloning the Full-Length cDNA of *P. americana RPS23* (*PaRPS23*)

A putative *RPS23* expressed sequence tag (EST) sequence in *P. americana* was obtained from our previous study (Chen et al. 2013). Based

on the partial sequence obtained, specific primers, *RPS23*-F: 5′-CAACATGACAATACACAGA-3′ and *RPS23*-NF: 5′-CTCCCACAGCATGACCTTTAC-GACC-3′ for 5′ RACE, and *RPS23*-R: 5′-CGCC-GTGAACAGAGATGGGCTGATA-3′ and *RPS23*-NR: 5′-TGGTGGCAGGCTTTGGTCGTAAAGG-3′ for 3′ RACE, were designed. The PCR fragments were subjected to electrophoresis on 1.5% aga-rose gel to determine length differences, and the target band was purified by PCR purification kit (Promega, USA). The amplified fragments were cloned into pMD® 19-T Simple Vector (Takara) and sequenced.

Sequence Analysis

Open Reading Frame Finder in NCBI (http:// www.ncbi.nlm.nih.gov/gorf/ gorf.html) was used for distinguishing initiation codon, coding re-gion, termination codon, and 5′ and 3′ UTR of these *RPS23* transcript sequences. The nucleo-tide and deduced amino acid sequences of *RPS23* cDNA were analyzed and compared using Basic Local Alignment Search Tool (BLAST) (http:// www.blast.ncbi.nlm.nih.gov/ blast.cgi). Molecu-lar weight and isoelectric point of these proteins were predicted by using ProtParam (http://web. expasy.org/protparam/). The signal peptide was predicted by SignalP 4.1 (Bendtsen et al. 2004). N-linked glycosylation site was predicted by us-ing ProtParam (http://www.cbs.dtu.dk/services/ NetNGlyc/). Transmembrane domains were pre-dicted by the TMHMM program (http://www.cbs. dtu.dk/services/ TMHMM/).

Phylogenetic Analysis

Phylogenetic analysis of the *PaRPS23* gene was performed via multiple steps. Putative ortho-logues were identified by a PSI-BLAST (Schäffer et al. 2001) search of the NCBI nr database and compiled within a FASTA file. Sequences were aligned using stand-alone ClustalX software (Thompson et al. 1997). Alignments of amino acid sequences were performed using BioEdit 7.0.5 (Hall 1999). Phylogenetic trees were performed using MEGA 6.0 program (Tamura et al. 2013) in which the robustness of the nodes was assessed by bootstrap proportion analysis computed from 1,000 replicates.

Tissue Expression of *PaRPS23*

Ovary, testis, head, thorax, leg and gut were dissected from *P. americana*. The mRNA expres-sions of *RPS23* in different tissues were deter-mined by quantitative RT-PCR. Total RNA was extracted as described above. The cDNA syn-thesis was performed with 500 ng total RNA in a 10 µL reverse transcription reaction system, using PrimeScript® RT reagent Kit (Takara) for the genes, following the manufacturer›s proto-cols. The RT-PCR reactions were performed with Rotor-Gene Q RT-PCR system. The SYBR Pre-mix Ex Taq kit (Takara) was used according to the manufacturer's protocol. In each PCR reac-tion, the gene-specific primers, 5′-TGGATTGC-GTACTGCTCGTA-3′ and 5′-TGTTTGGCTGCT-TAGCTTCT-3′, were designed to amplify a product of 176 bp, each primer set has its own optimal Tm for the RT-PCR assays. The housekeeping gene, β-actin rRNA gene of *P. americana* was used as internal control for normalizing the expression level of *PaRPS23*. Two β-actin rRNA gene-specif-ic primers, 5′-TTACCACCACTGCCGAACGA-3′ and 5′-CCTCTGGACAACG GAACCTC-3′, were designed to amplify a product of 180 bp. RT-PCR was performed in a total volume of 15 µL containing 7.5 µL of 2 × SYBR Green™ Realtime PCR MasterMix, 1.5 µL of cDNA, 0.3 µL of each primer and 5.4 µL of double-distilled water. The reaction conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 53 °C for 30 s. The melt curve has to be checked to prove that the primers are amplifying the ampli-con, so the values reflect the increase in amplicon, not primer dimers or other unrelated nucleotides. The calculated efficiency values for *PaRPS23* and β-actin rRNA gene amplicons were always within the range of 95% to 100%; therefore, no correction for efficiency was used in further calculations. The relative expression values were calculated from 3 biological replicates using a modified $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001). Data from the quantitative RT-PCR were subjected to LSD test in analysis of variance (ANOVA) (SPSS 17.0 for Windows).

Results

Sequence Characterization of *PaRPS23*

Based on the 264 bp fragment obtained from our previous study, a 431 bp and a 198 bp frag-ment were amplified by 5′-RACE and 3′-RACE, respectively. These 3 fragments (i.e., 264 bp, 431 bp and 198 bp fragment) were assembled by overlapping to be a 615 bp nucleotide sequence representing the complete *PaRPS23* cDNA. The complete *PaRPS23* cDNA was further verified by end to end PCR. The *PaRPS23* cDNA consisted of an open reading frame (ORF) of 432 nucleotides encoding 143 amino acids, and included a 5′-UTR located 116 bp upstream of the start codon (ATG) and 3′-UTR of 67 nucleotides that ended in a poly (A) tail (Fig. 1). A polypyrimidine sequence, CTTTC, was at the 5′ noncoding sequence. A pos-sible consensus polyadenylation signal, AATAA, was at position 568-572, 14 bases upstream of the poly(A) site. The polyadenylation signal is required for post-translational cleavage-polyade-

Fig. 1. Nucleotide and deduced amino acid sequence of *RPS23* cDNA from *Periplaneta americana*. The start codon is indicated with bold and the stop codon is indicated both with bold and an asterisk. The polypyrimidine sequence is boxed. The polyadenylation signal is underlined. Sequence analyses using ExPASy translation software.

nylation of the 3′ end of the pre-mRNA (Proudfoot & Brownlee 1976). The calculated molecular mass of *PaRPS23* was 16.03 kDa, and the estimated isoelectric point was 10.47. The *PaRPS23* cDNA sequence has been submitted to GenBank (accession number: KJ472479).

PaRPS23 is a rather hydrophobic protein with 50 hydrophobic amino acids out of 143 residues. It has a high percentage of basic amino acids (18 lysine acids, 14 arginine acids and 5 histidine acids) mostly located in the N-terminal half of the deduced amino acid sequence, and a low percentage of acidic amino acids (5 aspartic acids and 8 glutamic acids) mostly situated in the C-terminal half. The strong basic character of S23 including *PaRPS23* may be instrumental for its binding to rRNA in the 40S subunit of eukaryotic ribosomes (Dudov & Perry 1984; Wiedemann & Perry 1984; Ulbrich et al. 1979). A signal peptide scan using the SignalP 4.1 program (http://www.cbs.dtu.dk/services/SignalP) was performed, but no signal peptide was identified. and *PaRPS23* had no potential N-linked glycosylation site detected by using ProtParam. Also the protein did not have any transmembrane domain predicted by the TMHMM program.

Homology Analysis of *PaRPS23*

The deduced protein sequence of *PaRPS23* was compared with those of the other known *RPS23* proteins from various animals in the GenBank nr database (Table 1). The comparisons that yielded the closest identities were with insect *RPS23*. *PaRPS23* is related to *Graphocephala atropunctata* (ABD98760.1) and *Tribolium castaneum* (XP_973351.1), both with a total score of 288 and in an alignment of the amino acid sequences with 141 identities in 143 possible matches (99% identity); to *Pediculus humanus corporis* (XP_002433088.1) with a total score of 287 and in an alignment with 140 identities in 143 possible matches (98% identity); to *Riptortus pedestris* (BAN20179.1) with a total score of 286 and in an alignment with 140 identities in 143 possible matches (98% identity); to *Biphyllus lunatus* (CAH04343.1) with a total score is 285 and in an alignment with 140 identities in 143 possible matches (98% identity); to *Maconellicoccus hirsutus* (ABM55585.1) with a total score of 286 and in an alignment with 139 identities in 143 possible matches (97% identity); to *Acyrthosiphon pisum* (NP_001156067.1) with a total score of 284 and in an alignment with 139 identities in 143 possible matches (97% identity); to *Diaphorina citri* (ABG81976.1) with a total score of 283 and in an alignment with 139 identities in 143 possible matches (97% identity) (Table 1).

The sequence alignment of *PaRPS23* with known insect *RPS23* amino acids was shown in Fig. 2. It was suggested that *RPS23* is highly conserved among various holometabolous and hemimetabolous insect species.

TABLE 1. HOMOLOGY ANALYSIS OF *PARPS23*. COMPARISON OF THE DEDUCED PROTEIN SEQUENCE OF *PARPS23* THE SEQUENCES IN THE GENBANK DATABASE OF OTHER KNOWN *RPS23* PROTEINS FROM VARIOUS INSECT SPECIES.

Protein	Organism	Accession No.	Amino acids	Total score	Identity
GaRPS23	*Graphocephala atropunctata*	ABD98760.1	143	288	99%
PhcRPS23	*Pediculus humanus corporis*	XP_002433088.1	143	287	98%
TcRPS23	*Tribolium castaneum*	XP_973351.1	143	287	99%
RpRPS23	*Riptortus pedestris*	BAN20179.1	143	286	98%
MhRPS23	*Maconellicoccus hirsutus*	ABM55585.1	143	286	97%
DyRPS23	*Drosophila yakub*a	AAR10268.1	143	285	98%
DmRPS23	*Drosophila melanogast*er	NP_610939.2	143	285	98%
BlRPS23	*Biphyllus lunatu*s	CAH04343.1	143	285	98%
EaRPS23	*Euphydryas aurin*ia	Q6EV23.1	143	285	97%
ApRPS23	*Acyrthosiphon pisum*	NP_001156067.1	143	284	97%
HmRPS23	*Heliconius melpome*ne	AEL28841.1	143	283	96%
DcRPS23	*Diaphorina citri*	ABG81976.1	143	283	97%
NvRPS23	*Nasonia vitripenn*is	XP_001600581.1	143	281	97%
MsRPS23	*Manduca sexta*	AAV91403.1	143	281	95%
BmRPS23	*Bombyx mo*ri	NP_001037273.1	143	280	94%

Phylogenetic Tree

A phylogenetic tree using the neighbor-joining algorithm was inferred from the amino acid sequences of different *RPS23* proteins using MEGA 6.0 program. The bootstrap values from 1,000 resamplings were given at each node and the branch lengths were drawn to scale (Fig. 3). Additionally, tree topologies assessed by the unweighted pair-group method with an arithmetic average (UPGMA), minimum evolution (ME) and maximum parsimony (MP) methods were substantially similar to the neighbor joining tree (data not shown). These observations suggested that the deduced amino acid sequence of *RPS23* from *P. americana* was in the subgroup of Hemimetabola, and this subgroup was significantly different from the subgroup of Holometabola (Fig. 3). These findings suggest that the *PaRPS23* has closer genetic relationships with the *RPS23* of other Hemimetabola than with Holometabolous species as revealed by the difference of genetic distance.

Tissue Expression of *PaRPS23*

In order to examine whether *PaRPS23* was ubiquitously expressed, quantitative RT-PCR was employed to investigate the distribution of *RPS23* mRNA in different tissues (Fig. 4), The *RPS23* transcript was expressed in all tissues, indicating that it has a role in various tissue development and insect life cycle. The lowest mRNA level was found in the testis, and the relative expression levels of *RPS23* were 1.2-, 17.9-, 7.3-, 5.6- and 2.2-fold higher in the ovary, head, thorax, leg and gut than in the testis, respectively.

DISCUSSION

The *RPS23* belongs to the ribosomal protein family, known as S12 in bacteria and as either S12 or S23 in Archaea (Kitaoka et al. 1994). *RPS23* located near S6 and S4, and was cross-linked to both EF-lα and EF-2. *RPS23* appears to be involved in the translation initiation step of protein synthesis (McMahon & Landau 1982), constitutes the ribosomal binding site for mRNA in the 40S initiation complex (Takahashi et al. 2002), plays a control role in the megakaryocytes of humans (Kim 2003), and is functional during retinoic acid induced neuronal differentiation of human NTERA2 cells (Kitaoka et al. 1994). Also, *RPS23* participated in the process of tRNA transfer in the translocation reaction (Spahn et al. 2004). In addition, in mammals, *RPS23* can be also used as a novel candidate biomarkers of graft rejection reactions (Andreev et al. 2011).

In the present study, a novel *RPS23* gene was cloned from the Americana cockroach, *P. americana*. Before this study, no full-length cDNA of *RPS23* has been isolated in *P. americana*, and our study is the first report on cloning of the full-length cDNA of cockroach *RPS23*. The full length of *PaRPS23* was 615 bp, which consisted of an ORF of 432 bp, a 5′-UTR of 116 bp and a 3′-UTR of 67 bp. The ORF of *PaRPS23* encoded a polypeptide of 143 amino acids with a calculated molecular weight of about 16.04 kDa and an isoelectric point of 10.47. This result is similar to that of Cornish-Bowden (1980) in which S23 and S24 had similar compositions with the same molecular weight of 16 KD. The difference index *pI* calculated for this pair is 10.4, which indicates a close relationship between the 2 proteins. A polypyrim-

Fig. 2. Comparative analysis of the deduced amino acid sequence of *PaRPS23* ORF with other known insects. All the other known insects are listed in Table 1. Sequences were aligned using ClustalX software.

idine sequence, CTTTC, was at the 5' noncoding sequence. The 5' end of most, if not all, eukaryotic ribosomal protein mRNAs has a pyrimidine sequence (Wool et al. 1996), which is presumed to have a role in the regulation of their translation (Levy et al. 1991).

Homology analysis revealed that the deduced amino acid sequence of *PaRPS23* had more than 90% similarity with other insect *RPS23* (99 % with *T. castaneum*, 98% with *R. pedestris*, 98% with *B. lunatus*, 97% with *M. hirsutus*, 97% with *A. pisum*, 97% with *D. citri*, and so on). The high homology of *RPS23* in various animals indicates that *RPS23* is highly conserved among species (Hori et al. 1993).

In animals, ribosomal proteins have been widely used for phylogenetic analysis as they are highly conserved and offer useful comparisons between distant lineages. However, because of lacking sequence information for *P. americana*, a phylogenetic analysis between this species and other insects based on the *RPS23* protein sequences was never performed before. In this study, the results showed that the *PaRPS23* has closer genetic relationship with other Hemimetabola (Hemiptera and Anoplura) *RPS23* than with Holometabola (Lepidoptera, Hymenoptera, Diptera and Coleoptera) species, as revealed by the differences in genetic distance. Apparently, *RPS23* proteins may be quite suitable for the estimation of evolutionary relationships among different insects.

Quantitative RT-PCR revealed that *RPS23* was expressed in all the tested tissues, and the highest expression level occurred in the head and the lowest mRNA level was found in the testis. The relative expression levels were 1.2-, 17.9-, 7.3-, 5.6- and 2.2-fold higher in the ovary, head, thorax, leg and intestine than in the testis, respectively. The differential expression of *RPS23* in various *P. americana* tissues showed that it is an important gene that could effectively be involved in a number of physiological activities, such as regulating protein synthesis (McMahon et al. 1982) and functioning during retinoic acid induced neuronal differentiation (Bévort & Leffers 2000). However, the significance of the relative high expression level of *PaRPS23* in the head is unknown, need further investigation.

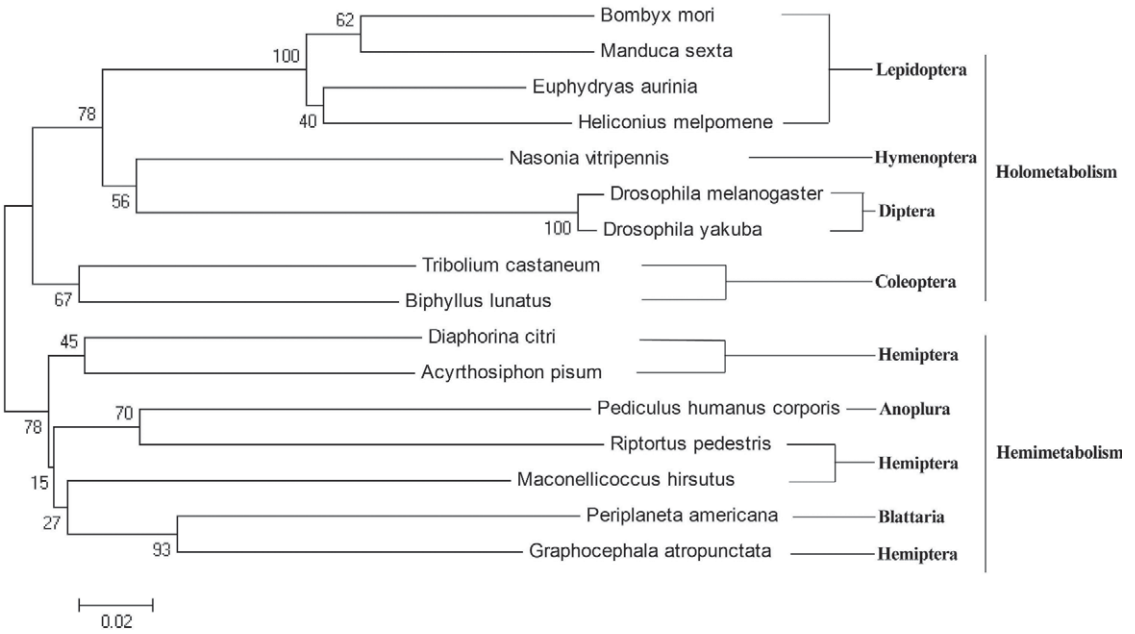

Fig. 3. Evolutionary relationships of deduced amino acid sequence of *PaRPS23* with other insects constructed using the neighbor-joining method. Bootstrap values with 1000 trials are indicated on branches. Phylogenetic trees were performed using MEGA 6.0 program.

In summary, we first cloned and characterized the cDNA of *PaRPS23*, and analyzed its gene expression in different tissues of adult *P. americana*. The determination of the amino acid sequence of cockroach *RPS23* is a contribution to a data set, which we hope will eventually include the structure of all proteins in the ribosomes of this species. However, the information may also aid understanding the evolution of ribosomes, unraveling the function of the protein, defining the rules that govern the interaction of these proteins, rRNAs, and unraveling the amino acid sequences that direct the proteins to the nucleolus for assembly on nascent rRNA. Future work should focus on the understanding of the roles of *RPS23* on regulating the organelles of *P. americana*.

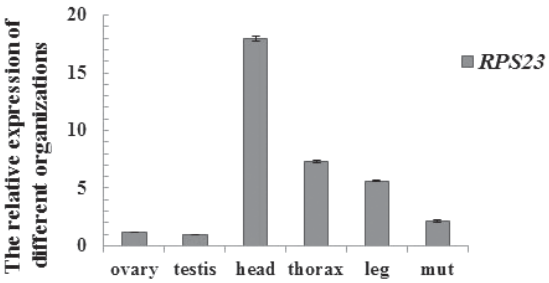

Fig. 4. The relative expression levels of the *PaRPS23* in different tagmata of adult *Periplaneta americana* (bar graph represents Mean ± SE; $P < 0.05$, LSD in ANOVA). β-actin rRNA gene of *P. americana* was used as internal control for normalizing the expression level of *PaRPS23*. As determined using quantitative RT-PCR.

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