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A cuticular protein, BgCPLCP1, contributes to insecticide resistance by thickening the cockroach endocuticle

Tong Cai^{a,b,1}, Xuejun Wang^{c,1}, Baorui Liu^{a,b,1}, Haizheng Zhao^{a,b}, Caixia Liu^{a,b}, Xiancui Zhang^d, Yuting Zhang^{a,b}, Huiyuan Gao^{a,b}, Coby Schal^{e,*}, Fan Zhang^{a,b,**}

^a Dongying Key Laboratory of Salt Tolerance Mechanism and Application of Halophytes, Dongying Institute, Shandong Normal University, Dongying 257000, China

^b Key Laboratory of Animal Resistance Biology of Shandong Province, College of Life Science, Shandong Normal University, Jinan 250014, China

^c Shandong Center for Disease Control and Prevention, Jinan 250013, China

^d School of Life Science, Huzhou University, Huzhou 313000, China

e Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

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ABSTRACT

Overuse of insecticides has led to severe environmental problems. Insect cuticle, which consists mainly of chitin, proteins and a thin outer lipid layer, serves multiple functions. Its prominent role is as a physical barrier that impedes the penetration of xenobiotics, including insecticides. *Blattella germanica* (L.) is a major worldwide indoor pest that causes allergic disease and asthma. Extensive use of pyrethroid insecticides, including β -cypermethrin, has selected for the rapid and independent evolution of resistance in cockroach populations on a global scale. We demonstrated that BgCPLCP1, the first CPLCP (cuticular proteins of low complexity with a highly repetitive proline-rich region) family cuticular protein in order Blattodea, contributes to insecticide penetration resistance. Silencing *BgCPLCP1* resulted in 85.0 %–85.7 % and 81.0 %–82.0 % thinner cuticle (and especially thinner and more permeable cuticles resulted in 14.4 % and 20.0 % lower survival of β -cypermethrin-treated S- and R-strain cockroaches, respectively. This study advances our understanding of cuticular penetration resistance in insects and opens opportunities for the development of new efficiently and environmentally friendly insecticides targeting the CPLCP family of cuticular proteins.

1. Introduction

Pyrethroids are the third most applied group of insecticides worldwide while have several side effects, including environmental pollution, inhibition of crop growth, and chronic toxicity (mainly neurotoxicity and reproductive system toxicity) in animals and humans [1–3]. Their use in indoor environments undoubtedly greatly increases the likelihood of long-term human exposure. *Blattella germanica* (L.) (order Blattodea, family Ectobiidae) is a serious public health pest. It is an obligatory parasite in the indoor residential and food handling environment, and extremely difficult to control. *B. germanica* mechanically transmits a variety of pathogenic microorganisms and parasites, and their secretions and carcasses trigger severe allergic disease and asthma in humans [4,5]. Chemical insecticides remain the mainstay of cockroach control. Pyrethroid insecticides are extensively used due to their broad-spectrum and high efficacy [6–8]. Unfortunately, the overuse of pyrethroids has been accompanied by adverse effects on humans and the environment as well as rapid development of resistance in the German cockroach.

Insecticide resistance is a complex phenotype, often caused by multiple co-occurring mechanisms such as point mutations that lower binding affinity at the target site, heightened metabolic detoxification and sequestration, and reduced cuticular penetration [9,10]. Lower insecticide penetration of the cuticle can result from thickening and/or toughening of the cuticle [11–14], but these physical changes have been considerably less investigated than the other two mechanisms. The cuticle is a complex extracellular biocomposite that covers the entire

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^{*} Corresponding author.

^{**} Correspondence to: F. Zhang, Dongying Key Laboratory of Salt Tolerance Mechanism and Application of Halophytes, Dongying Institute, Shandong Normal University, Dongying 257000, China.

E-mail addresses: coby@ncsu.edu (C. Schal), zhangfan0531@163.com (F. Zhang).

¹ These authors contributed equally to this work.

insect body as well as the foregut, hindgut and tracheae, and is the first barrier against xenobiotics [15,16]. The role of the cuticle in impeding insecticide penetration has been demonstrated in *Cimex lectularius* (bed bug) and *Myzus persicae* (aphid), where lower mortality was observed after topical application of insecticides than after their injection or ingestion [17,18]. Cuticular penetration of radioactive insecticides was 30–50 % lower in insecticide-resistant *Drosophila melanogaster* (fruit fly) and *Anopheles gambiae* (mosquito) than in insecticide-susceptible strains of these insects [19,20]. This mechanism was thought to be conferred by alterations in the cuticular composition and physical structure, hindering insecticide molecules from reaching their receptor targets [21,22].

The insect cuticle is generally composed of an epicuticle, procuticle (exocuticle and endocuticle) and cellular epidermis [23]. Along with chitin and cuticular lipids, cuticular proteins (CPs) are major structural components of the cuticle, and their qualitative and quantitative features contribute significantly to variation in the function of the cuticle in different insects and tissues [24,25]. Thousands of CPs have been identified, and categorized into >10 families based on conserved motifs [26]. In resistant An. gambiae and Culex pipiens pallens mosquito populations, overexpression of a suite of CPR family proteins (CPs with Rebers and Riddiford (R&R) consensus, the largest CP family in insects; CPR63, CPR124, CPR127, CPR129) and CPLCG family CPs (CPF-like low complexity CPs with two invariant glycine residues in a conserved domain separated by eight amino acids; CPLCG3, CPLCG5) was found to be accompanied by reinforcement of the cuticle [11,27-29]. These studies have advanced our understanding of CPs, but they focused on holometabolous insect species that experience relatively slow evolution of resistance (largely due to few generations per year and stage-specific exposure to insecticides), and concentrated on large and well-studied CP families. CPLCP family members (CPs of low complexity with a highly repetitive proline-rich region) are characterized by a high density of PV and PY conserved sequence motifs [30-32]. They were first identified in An. gambiae [32], and were subsequently reported in 16 insect species from 6 orders (Lepidoptera: Plutella xylostella [33], Bombyx mori [34], Dendrolimus punctatus [25]; Diptera: Bactrocera dorsalis [24], D. melanogaster [26], Aedes aegypti [35], Anopheles sinensis [30], Culex quinquefasciatus [30], Cx. pipiens [32]; Hymenoptera: Microplitis mediator [36], Nasonia vitripennis [37], Apis mellifera [38]; Coleoptera: Tribolium castaneum [39]; Hemiptera: Nilaparvata lugens [31], Triatoma infestans [40]; Thysanoptera: Frankliniella occidentalis [41]). However, to our best knowledge, no member of the CPLCP family has been identified in order Blattodea.

In a previous study, a putative cuticular protein (GenBank accession No.: CAR94548.1) was significantly overexpressed at least 2-fold in a β -cypermethrin-resistant (R) strain of *B. germanica* compared to an insecticide-susceptible (S) strain [42]. Assuming that it might play a role in insecticide resistance, we cloned the complete sequence of this gene and characterized the protein as the first member of the CPLCP family in the Blattodea. Here, we demonstrate a significant contribution of BgCPLCP1 to cuticular penetration resistance to β -cypermethrin by quantifying its expression and contribution to cuticular thickening. This study focuses on insecticide use for environmental safety and public health.

2. Materials and methods

2.1. Cockroach strains

Two strains of *B. germanica* were used in this study. The S-strain was a standard insecticide-susceptible strain, provided by the Key Laboratory of Animal Resistance Biology of Shandong Province, which was collected from Shanghai city in 1995 and maintained in the laboratory without exposure to any insecticides. The R-strain was selected from the S-strain by exposure to β -cypermethrin residues that killed 50–70 % of the insects at each selection event [43,44]. The F25 generation of the R-strain was used in this study. Both the S- and R-strains were reared in a

growth chamber at 27 ± 10 °C and 60 ± 5 % relative humidity (RH) with a 12:12 h light/dark photoperiod, and were maintained on rat chow and water ad libitum [45].

2.2. LD₃₀ determination and insecticide bioassay

To determine the LD_{30} (dose that killed approximately 30 %) of β -cypermethrin to the S- and R-strains, a dose-response study was conducted. We topically applied various doses of β -cypermethrin in 1 μ L acetone to the mesosternum of each adult male [46–48]. Each S-strain male was treated with 0.5, 1, 1.5, or 2 ng β -cypermethrin, whereas each R-strain male received 15, 20, 25, or 30 ng β -cypermethrin. Acetone application was used as the control. Forty cockroaches were tested in each group and three independent biological replicates were performed. Mortality was recorded 48 h after treatment and the LD_{30} value was estimated from probit analysis.

To infer the role of the cuticle in insecticide resistance, adult males of the S- and R-strains were treated with 1 μ L β -cypermethrin at LD₃₀ of 0.83 ng/male and 17.16 ng/male using three methods: topical application, intraperitoneal injection and ingestion [17,18]. Acetone was used as control. Thirty cockroaches were tested in each group and three independent biological replicates were performed. The time-course of mortality was recorded and compared across the four groups using survival analysis.

2.3. Eosin penetration assay

We developed an in situ staining method with Eosin Y to compare the cuticular permeability of the S- and R-strains. Eosin Y is an inert dye widely used as a nonspecific marker. Twelve 7-day-old adult (AD7) males from each strain were anesthetized for 1 min with anhydrous ether, soaked for 3 min in 1 mL *n*-hexane to remove their cuticular lipids, and air-dried at room temperature before staining. The cockroaches were evenly divided into four test groups (n = 3 per group), soaked in Eosin Y solution for 15, 30, 45 and 60 min at 55 °C (a temperature at which the staining signal was mild but not excessive [49]), and then washed three times with tap water to remove excessive dye. The midleg, forewing, hindwing and abdomen were dissected, and the area and density of staining were quantified. The extent of staining was expressed as the average optical density, using ImageJ 1.48v (National Institutes of Health, USA) for image analysis.

2.4. Transmission electron microscopy (TEM)

Three AD7 males from each strain were randomly selected as three independent biological replicates, and their tergum, tibia and first tarsus (tarsus I) of the midleg were dissected for semi-thin and ultrathin sections. At least three sections were prepared from each sample. Tissue samples were fixed with 2.5 % glutaraldehyde in phosphate buffer (PBS, 0.1 M, pH 7.4) at 4 °C overnight, then washed for 15 min three times with PBS. Samples were then dehydrated by a graded series of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, 95 %, 100 %) for 20 min at each step, and transferred to fresh acetone for 20 min three times. Finally, the samples were embedded in epoxide resin. Using a Leica EM UC7 ultramicrotome (Leica, Germany), the samples in hardened epoxide resin were semi-thin and ultrathin sectioned. The semi-thin sections were stained with toluidine blue for 5-10 min; the ultrathin sections were stained with 2 % uranyl acetate for 20-30 min and lead citrate for 10-15 min. The semi-thin sections were observed and photographed on a 3DHISTECH digital slice scanner and analyzed using CaseViewer 2.1.2 software (3DHISTECH, Hungary), and the ultrathin sections were observed and photographed on a Hitachi HT-7800 transmission electron microscope (Hitachi, Japan).

2.5. Cloning and sequencing of BgCPLCP1

Total RNA was extracted from S-strain adult males using TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, Japan) according to the manufacturer's instructions. Poly A⁺ RNA was prepared, first-strand cDNA was synthesized, RACE (rapid amplification of cDNA ends) and In-Fusion cloning of RACE products was performed using SMARTer® RACE 5'/3' Kit (TaKaRa, Japan) according to the user manual. The 3' and 5' RACE products were sequenced at Shanghai Meji Biological Medicine Technology Co., LTD (Shanghai, China). Overlay of the middle, 3' and 5' sequences were performed using SnapGene 2.3.2 (Insightful Science, USA). Gene-specific primers 3'GSP and 5'GSP were designed using SnapGene 2.3.2, corrected manually and they are listed in Table S2.

2.6. Sequence analysis and phylogenetic analysis

Analysis and prediction of amino acid sequence, basic physicochemical properties, signal peptides, transmembrane structure, secondary structure, protein domains, tertiary structure, and phylogenetic relationships of BgCPLCP1 were performed with software and online tools shown in Table S3.

A phylogenetic analysis was conducted of BgCPLCP1 and 129 other CPLCP family proteins from 15 insect species (*P. xylostella, B. mori, B. dorsalis, D. melanogaster, Ae. aegypti, An. gambiae, An. sinensis, Cx. pipiens, N. vitripennis, A. mellifera, M. mediator, T. castaneum, N. lugens, T. infestans,* and *F. occidentalis*). The amino acid sequences of these proteins are listed in Table S4. Multiple alignments of the 130 proteins were carried out using ClustalW in the MEGA 11 (Mega, New Zealand) [50]; Phylogenetic analysis was conducted by the Neighbor-Joining method with the Poisson correction model and pairwise deletion for gaps [51]. A bootstrap analysis with 1000 repetitions was used to test the confidence of the various phylogenetic lineages.

2.7. Polyclonal antibody preparation

The *BgCPLCP1* gene was ligated into pET-28a (Merck, Germany) vector between the restriction endonuclease cutting site *Nde* I and *Xho* I using T4 DNA Ligase (TaKaRa, Japan). The ligation product was named pET-28a-*BgCPLCP1*. The pET-28a-*BgCPLCP1* was transformed into BL21 (DE3) competent cells (Angyubio, China) and cultured overnight in LB agar medium. Monoclonal white plaques were picked for expanding culture. 1 mM/L IPTG was added to induce protein expression. The fusion expression product was identified by SDS-PAGE. BgCPLCP1 protein was purified using Ni-TED column (Roche, Switzerland), and the purified protein was detected by SDS-PAGE.

Six-week-old New Zealand white rabbits were immunized four times in one month using a mixture of purified BgCPLCP1 protein and Freund's complete/incomplete adjuvant by subcutaneous multi-point injection. Rabbit blood was collected, antiserum titers were tested by an indirect ELISA method, and then antibodies were purified using an antigen-specific affinity purification method. Indirect ELISA showed that the antibody was successfully prepared with a titer of 128,000.

2.8. Western blotting

To study the tissue-specific expression patterns of BgCPLCP1 protein, midlegs, abdominal cuticle, wings and internal tissues were dissected from 20 adult males from each strain. These samples were ground to powders in liquid nitrogen and 50 mg was used for total protein extraction using Rapid lyse protein Assay (Beyotime, China) containing 1 % phenylmethanesulfonyl fluoride (Beyotime, China). Protein concentrations were detected by BCA Protein Assay Kit (Beyotime, China). The target protein was separated by 12.5 % SDS-PAGE electrophoresis, transferred to a PVDF membrane (Sangon Biotech, China), and blocked with 8 % milk at room temperature for 1.5 h. The BgCPLCP1 polyclonal antibody was used as the primary antibody, and the Goat Anti-Rabbit

IgG (H + L) HRP (Abways, China) was used as the secondary antibody. The primary antibody was incubated at room temperature for 2 h, and the secondary antibody was incubated at room temperature for 1.5 h. Finally, the PVDF membrane was imaged with a ECL luminescent reagent (Absin, China) in an automatic chemiluminescence imaging analysis system Tanon5200 (Tanon, China). Gray scales were calculated using ImageJ 1.48v to enable a comparison of the relative expression of BgCPLCP1 protein in the S- and R- strains. Three independent biological replicates were performed in this experiment.

2.9. Total RNA extraction and RT-qPCR

To study the temporal expression patterns of *BgCPLCP1* mRNA at different instars, oothecae, second-instar nymphs, fourth-instar nymphs, male and female sixth (last)-instar nymphs, and male and female adults of both strains were sampled (10 individuals each) on the fourth day of each stage. To investigate the temporal expression patterns of *BgCPLCP1* mRNA in different developmental days of the same instar, 0 to 8-day-old last-instar male nymphs of both strains were sampled (10 individuals each day). To explore the tissue-specific expression patterns of *BgCPLCP1* mRNA, midlegs, abdominal cuticles, wings and internal tissues were dissected from 20 male adults from each of the two strains. All these samples were ground to powders in liquid nitrogen.

Total RNA was extracted from 15 mg of the sample powder and was reverse transcribed into cDNA using TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa) and PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. Real-time quantitative PCR (RT-qPCR) was carried out using SYBR® Premix Ex Taq[™] II kit (TaKaRa). *β*-Actin (GenBank accession No.: ACA79909.1) was used as internal control gene [52]. Primers of BgCPLCP1 and β -actin genes were designed using Prime Premier 5.0 (Premier, Canada) and are listed in Table S2 [53]. The following reagents were used for RT-qPCR reactions: 10 µL of SYBR Premix Ex Taq II, 0.8 µL of 10-fold diluted forward primer, 0.8 µL of 10-fold diluted reverse primer, 2 µL of cDNA, and 6.4 μL of deionized water to yield a 20 μL final reaction volume. Amplification reactions were performed in a LightCycler96 System (Roche, Switzerland) programmed as follows: a preincubation for 30 s at 95 °C, followed by 45 cycles of 2 step amplification for 5 s at 95 °C and 30 s at 60 °C, followed by melting for 5 s at 95 °C, 60 s at 60 °C and 5 s at 95 °C, and followed by cooling for 30 s at 50 °C. Relative expression levels of *BgCPLCP1* were calculated with the $2^{-\Delta\Delta Ct}$ method [54], and the gene expression level was normalized to the expression of the internal control β -actin gene that was stably expressed at all stages and in all tissues. Three independent biological replicates were performed in this experiment.

2.10. Immunofluorescence analysis

Paraffin sections were made from the tarsus I, tibia, thorax, abdomen of adult cockroaches. Tissues were dewaxed and rinsed with PBS three times for 5 min each, then blocked with 3 % bovine serum albumin (BSA, Servicebio, China) in PBS for 30 min at room temperature. Sections were incubated with BgCPLCP1 polyclonal antibody (1:100 in 2 % BSA in PBS) at 4 °C overnight. After washing sections with PBS, fluorescence anti-rabbit IgG (Servicebio, China) secondary antibody was added and incubated for 50 min at room temperature. Sections were washed with PBS, and nuclei were stained with DAPI (Servicebio) for 10 min in the dark. After washing sections were sealed. Sections were visualized and imaged using a fluorescence microscope (Nikon, Japan) and 3DHISTECH digital slice scanner.

2.11. RNA interference (RNAi)

The biological function of *BgCPLCP1* in cuticular development and penetration resistance in *B. germanica* was investigated by RNAi. The

design and syntheses of siBgCPLCP1 and siNC (as negative control) were conducted by Shenzhen BGI Co., LTD (Shenzhen, China). The sequences of siNC and siBgCPLCP1 are shown in Table S2. siBgCPLCP1 and siNC were diluted to $1 \mu g/\mu L$. Sixth-instar male nymphs on day 1 (N6D1) were divided into siBgCPLCP1 group and NC group. Each N6D2 nymph was fed 1 μL of siRNA using a micropipette (Eppendorf, Germany). These cockroaches were sampled 2, 4 and 6 days post-feeding and BgCPLCP1 mRNA quantified by RT-qPCR. Based on RNAi efficiency, to ensure low expression of BgCPLCP1 throughout the last instar, nymphs were repeatedly fed 1 μL siRNA every 48 h up to N6D8 for a total of 4 times, after which the last-instar nymphs emerged into adults. After treatment, cockroaches were kept under standard conditions with rat chow and water ad libitum. The RNAi efficiency was quantified in 2-day-old adults (AD2, 48 h after the last feeding).

2.12. Changes in wing phenotype, cuticle thickness and permeability after RNAi

Several AD2 males from each of the four groups (S-strain siBgCPLCP1 group, S-strain NC group, R-strain siBgCPLCP1 group, R-strain NC group) were randomly selected and observed for wing morphology (normal or defective wings, n = 20 per biological replicate), cuticle thickness (n = 1 per biological replicate) and cuticle permeability (Eosin penetration assay conducted at 55 °C for 30 min, n = 1 per biological replicate). Three independent biological replicates were performed in this experiment.

2.13. Changes in survival rate after RNAi

One hundred and eighty AD2 males from each group were equally divided into test and control groups for three independent biological replicates (n = 30 per biological replicate). Using the topical application method, all the test cockroaches were treated with 1 µL strain-specific LD₃₀ β -cypermethrin, while all the control cockroaches were treated with 1 µL acetone. The survival of siBgCPLCP1 and NC cockroaches was compared after 48 h exposure.

2.14. Statistical analysis

GraphPad Prism 8.0 (GraphPad Software, USA) was used for data processing and visualization. SPSS 20.0 software (SPSS, Inc., USA) was used for statistical analysis. Strain-specific LD_{30} value was estimated from probit analysis of the dose-response study. Independent sample *t*tests were used to compare the differences between two groups. ANOVA followed by Tukey's honestly significant difference (HSD) test was used to compare the difference among multiple groups. Mantel-Cox test was used to compare the survival of the S- and R-strains. All data were expressed as the mean \pm standard error (SEM). P < 0.05 was considered statistically significant.

3. Results

3.1. The role of cuticle in β -cypermethrin resistance

We compared the toxicity of β -cypermethrin delivered to cockroaches by topical application, intraperitoneal (hemocoel) injection and ingestion using a dose that kills 30 % of the insects by topical application LD₃₀ (S-strain: 0.83 ng/male; R-strain: 17.16 ng/male) (Table S1). After 48 h of exposure to the insecticide, 71.1 \pm 1.1 % of the S-strain males survived the topical application of cypermethrin; 58.9 \pm 1.1 % survived injection ($F_{[1, 4]}$ = 60.500, P = 0.001) and 16.7 \pm 3.8 % survived ingestion of cypermethrin ($F_{[1, 4]} = 184.692$, P < 0.001) (Fig. 1A). A similar result was evident in the R-strain, which had significantly higher survival of 67.8 \pm 2.2 % after topical application compared to 41.1 \pm 1.1 % ($F_{[1, 4]} = 115.200, P < 0.001$) after injection and 21.1 \pm 2.2 % $(F_{[1, 4]} = 220.500, P < 0.001)$ after ingestion of cypermethrin (Fig. 1B). Notably, the difference in survival after topical application and injection was significantly higher in the R-strain than the S-strain (26.7 \pm 1.9 % vs 12.2 ± 1.1 %, $F_{[1, 4]} = 42.250$, P = 0.003), suggesting that the R-strain has a more impermeable cuticle than the S-strain.

3.2. Less permeable cuticle of the R-strain cockroaches

We used Eosin Y staining as a measure of cuticle permeability of the S- and R-strains. The staining of cockroaches of both strains increased with longer treatment with Eosin Y (Fig. 2). Compared with the S-strain, R-strain males had significantly smaller and lighter areas stained by Eosin Y at all sampling sites for all treatment times. For example, at 60 min, the average optical density of coloration in the S-strain was significantly higher than in the R-strain by >2-fold in the midleg (0.086 ± 0.006 vs 0.041 ± 0.001, $F_{[1, 4]} = 46.944$, P = 0.002) and forewing (0.08 ± 0.004 vs 0.023 ± 0.002, $F_{[1, 4]} = 134.133$, P < 0.001) (Fig. 2).

3.3. Thicker endocuticle of the R-strain cockroaches

The cuticle of the R-strain males was significantly thicker than that of the S-strain at the tarsus ($15.5 \pm 0.6 \text{ vs} 12.3 \pm 0.3$, $F_{[1, 48]} = 22.126$, P < 0.001) and tibia ($12.0 \pm 0.6 \text{ vs} 10.1 \pm 0.4$, $F_{[1, 48]} = 7.495$, P = 0.009), and slightly thicker at the tergum ($12.1 \pm 0.3 \text{ vs} 11.9 \pm 0.4$, $F_{[1, 48]} = 0.372$, P = 0.545) (Fig. 3A and B). Further, the thickening of the cuticle in the R-strain was mainly caused by a significantly thicker endocuticle, whereas the thickness of the exocuticle was not significantly different



Fig. 1. Percentage survival of adult males of the S-strain (A) and R-strain (B) of *B. germanica* after topical application, injection, and ingestion of a strain-specific LD_{30} dose of β -cypermethrin. "S" represents the susceptible strain and "R" represents the β -cypermethrin-resistant strain. The LD_{30} for the S-strain was 0.83 ng/male, and 17.16 ng/male for the R-strain. P < 0.05 was considered a significant difference according to Log-rank (Mantel-Cox) test.



Fig. 2. Cuticle permeability of the S- and R-strains of *B. germanica* characterized by Eosin Y staining. Males were exposed to Eosin Y for 15 to 60 min. "S" represents the susceptible strain and "R" represents the β -cypermethrin-resistant strain. (A) Scale bars are 2 mm. (B) Data are shown as mean \pm SEM calculated from three biological replicates. *: P < 0.05; **: P < 0.01; ***: P < 0.01 (independent sample *t*-tests).

between the two strains (Fig. 3C and D). These results suggested that a thicker endocuticle may be an important mechanism that underlies less cuticular penetration in the R-strain.

3.4. Cloning and bioinformatics analysis of BgCPLCP1

The complete sequence of the putative cuticular protein (GenBank accession No.: CAR94548.1) identified in our previous study [42] was successfully cloned, named *BgCPLCP1* and submitted to GenBank database (GenBank accession No.: OP629555.1). The *BgCPLCP1* gene was 1568 bp and comprised of 999 bp open reading frame (ORF), encoding 332 amino acids residues (Fig. S1A and B). The secondary structure of BgCPLCP1 protein was mainly a random coil, accounting for 65.96 % of the sequence (Fig. S1C). The BgCPLCP1 protein had no transmembrane helix and contained a signal peptide of 20 amino acids at the N-terminal, which was predicted to be a secreted protein. This protein contained six low-complexity regions (106-144aa, 150-161aa, 176-201aa, 207-230aa, 232-250aa, 255-288aa), which were proline-rich region and valine-rich region (Fig. S1D), consistent with the characteristics of the CPLCP family. The motif analysis of BgCPLCP1 with other proteins of the CPLCP

family from other insects also suggested a high number of proline residues with the sequence feature of PV and PY (Fig. 4C). The tertiary structure of BgCPLCP1 protein was predicted (Fig. 4B). Phylogenetic analysis revealed that BgCPLCP1 clustered in a clade with AAEL008752 and CpBDAT14316 from the mosquitoes *Ae. aegypti* and *Cx. pipiens* (order Diptera, family Culicidae) (Fig. 4A).

3.5. Spatiotemporal expression and localization of BgCPLCP1

At different stages, including oothecae, nymphs and adults, the expression of *BgCPLCP1* in the R-strain was significantly higher than that in the S-strain (P < 0.05), with the most significant differences in fourth-instar nymphs ($F_{[1,4]} = 197.552$, P < 0.001), where expression in the R-strain was 2.72 \pm 0.12-fold higher than in the S-strain (Fig. 5A). During the last instar, the expression of *BgCPLCP1* was significantly higher in the R-strain from day-3 (P < 0.001) through day-8, when the expression of *BgCPLCP1* in the R-strain was 9.06 \pm 0.54-fold higher than in the S-strain ($F_{[1,4]} = 221.355$, P < 0.001) (Fig. 5B). In different body parts, the expression of *BgCPLCP1* in the R-strain was higher than in the S-strain at both mRNA and protein levels (midleg: mRNA level 2.02 \pm



Fig. 3. Cuticle thickness of the tarsus, tibia and tergum of adult male *B. germanica* of the S- and R-strains. "S" represents the susceptible strain and "R" represents the β -cypermethrin-resistant strain. (A) Left: Representative images of cross-sections of the cuticle observed with light microscopy; Right: Higher-magnification images observed with TEM. The red lines denote the exocuticle and the yellow lines denote the endocuticle. (B) Cuticle thickness quantified with light microscopy. Each dot represents an individual cuticle location, and means are shown as dashed lines with SEM (n = 25). (C, D) Endocuticle and exocuticle thickness was quantified from TEM images. Data are shown as mean \pm SEM calculated from three biological replicates. ns: not significant; **: P < 0.001; ***: P < 0.001 (independent sample *t*-tests). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.04-fold, $F_{[1,4]} = 598.062$, P < 0.001; protein level 1.77 \pm 0.09-fold, $F_{[1,4]} = 80.275$, P = 0.001; abdominal cuticle: mRNA level 1.98 \pm 0.02-fold, $F_{[1,4]} = 3328.836$, P < 0.001; protein level 1.42 \pm 0.02-fold, $F_{[1,4]} = 521.687$, P < 0.001; Wings: no significant difference; internal tissues: no significant difference) (Fig. 5C, D and E).

To determine the localization of BgCPLCP1 protein in the cockroach body, the midleg (tarsus and tibia), thorax and abdomen were subjected to paraffin sectioning and immunofluorescence analysis. In the tarsus and tibia of the midleg, BgCPLCP1 localized to the epidermis and procuticle; in the thorax and abdomen, BgCPLCP1 was detected in the cuticle, foregut, hindgut, trachea, and ventral nerve cord (Fig. 5F). Notably, all the structures mentioned above originate from the

embryonic ectoderm.

3.6. RNAi-mediated silencing of BgCPLCP1

A single feeding of siRNA to N6D2 cockroaches maximally suppressed *BgCPLCP1* expression on day 2; however, expression rebounded on days 4 and 6 (Fig. S2A and C). Based on this observation, a multiple-feeding RNAi strategy was used to maintain long-term inhibition of *BgCPLCP1* expression throughout the last instar. In AD2 males that emerged from RNAi-treated nymphs, *BgCPLCP1* in the S-strain was expressed at only 19.6 \pm 2.9 % of the NC control (*F*_[1, 4] = 711.336, *P* < 0.001) and at 13.8 \pm 3.5 % in the R-strain (*F*_[1, 4] = 534.162, *P* < 0.001)



Fig. 4. Bioinformatics analysis of BgCPLCP1. (A) Phylogenetic relationships of known CPLCP family proteins from *B. germanica* (red circle) and other insect species. (B) Tertiary structure of BgCPLCP1 protein predicted by THREADER. (C) Sequence logo of CPLCP family proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. S2B and D).

3.7. Thinner cuticle after siBgCPLCP1-RNAi

Silencing of *BgCPLCP1* resulted in significantly thinner tarsal and tibial cuticles (P < 0.01) and slightly thinner tergal cuticle compared with their corresponding control groups in both the S- and R-strains (Fig. 6A-C and F). Ultrastructure analysis revealed that the decrease in cuticle thickness was mainly due to significant decreases in endocuticle thickness (P < 0.01; Fig. 6D and G), although the thickness of the

exocuticle also decreased significantly in some tissues (Fig. 6E and H). For example, in leg cuticle, endocuticle thickness in the tarsus and tibia decreased after RNAi by 85.0 % and 85.7 % in the S-strain and by 82.0 % and 81.0 % in the R-strain, respectively (P < 0.001). The respective decreases in the exocuticle were only 11.2 % (P < 0.05) and 20.6 % (P < 0.001) in the S-strain and 1.6 % (P > 0.05) and 27 % (P < 0.001) in the R-strain.

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Fig. 5. Spatiotemporal expression and localization of BgCPLCP1 in the S- and R-strains of *B. germanica*. "S" represents the susceptible strain and "R" represents the β -cypermethrin-resistant strain. (A) Relative expression of *BgCPLCP1* in different stages. OO: oothecae; N2, 4, 6: second-, fourth-, sixth (last)-instar nymphs; A: adults; φ : female; β : male. (B) Relative expression of *BgCPLCP1* during the course of the sixth (last) instar. (C–E) Relative expression of *BgCPLCP1* in different body parts of adult males (ML: midleg, AC: abdominal cuticle, WI: wings, IT: internal tissues) in the two strains (S: S-strain, R: R-strain). (F) Representative images for BgCPLCP1 localized in different body parts of adult males. Scale bars are 40 µm in tarsal and tibial images and 400 µm in thoracic and abdominal images. Data are shown as mean \pm SEM calculated from three biological replicates. ns: not significant; *: P < 0.05; **: P < 0.01; ***: P < 0.001 (independent sample *t*-tests).

3.8. Greater cuticle permeability and defective wings after siBgCPLCP1-RNAi

siBgCPLCP1-RNAi treated cockroaches showed an impaired surface barrier against Eosin Y, especially at the joints of legs, anterior region (remigium) of wings, and intersegmental membranes (segmacoria) of the abdomen (Fig. 7A). In the intact ventral view, *BgCPLCP1*-silenced cockroaches showed significantly higher staining area than cockroaches of the control group (S-strain: $133.8 \pm 4.7 \text{ mm}^2 \text{ vs } 85.8 \pm 9.3 \text{ mm}^2, F_{[1,$ $4]} = 21.137,$ *P* $= 0.01; R-strain: <math>105.3 \pm 6.8 \text{ mm}^2 \text{ vs } 83.2 \pm 4.2 \text{ mm}^2, F_{[1,$ $4]} = 8.030,$ *P*= 0.047) and significantly greater average optical density(S-strain: 0.16 ± 0.011 vs 0.09 ± 0.008,*F*_[1, 4] = 28.857,*P*= 0.006; Rstrain: 0.12 ± 0.010 vs 0.07 ± 0.009,*F*_[1, 4] = 13.117,*P*= 0.022)(Fig. 7B-E). Subsequently, the midlegs, forewings, hindwings, andabdomen of cockroaches were dissected and observed separately. Theaverage optical density of all parts increased significantly after RNAi (*P* < 0.05), and the stained area increased to varying degrees (Fig. 7B-E).

The impaired cuticle was also reflected in the defective wings. Normal wings are shaped "boat-like" and fit snugly over the abdomen, whereas defective wings may be short, narrow, wrinkled, and warped, and thus do not cover the entire dorsal abdomen (Fig. 7F). After RNAi, a significantly higher percentage of the wings of adult cockroaches were defective compared with control-RNAi cockroaches in both S-strain (3.3 \pm 1.7 % vs 21.7 \pm 1.7 %, $F_{[1,~4]}$ = 60.500, P = 0.001) and R-strain (6.7 \pm 1.7 % vs 16.7 \pm 1.7 %, $F_{[1,~4]}$ = 18.000, P = 0.013) (Fig. 7G).

3.9. Reduced resistance to β -cypermethrin after siBgCPLCP1-RNAi

Survival analysis revealed that RNAi with siBgCPLCP1 elevated the mortality of adult males treated with LD₃₀ of β -cypermethrin. At 48 h, low expression of BgCPLCP1 resulted in 14.4 % lower survival of S-strain males (64.4 \pm 2.2 % vs 50.0 \pm 1.9 %, $F_{[1, 4]} = 24.143$, P = 0.008) and 20 % lower survival of R-strain males (56.7 \pm 1.9 % vs 36.7 \pm 1.9 %, $F_{[1, 4]} = 54.000$, P = 0.002) compared to their respective control group (Fig. 7H and I). These results confirmed that the BgCPLCP1 gene conferred β -cypermethrin resistance to German cockroaches.

4. Discussion

In this study, we demonstrated that the cuticle of *B. germanica* is a significant barrier that protects the insect from the harsh environment, including insecticides. The cuticular protein BgCPLCP1, a member of the CPLCP family, plays a prominent role in thickening the cuticle and thus contributes to insecticide resistance by impeding the penetration of a common pyrethroid insecticide, β -cypermethrin. First, we showed that



Fig. 6. Cuticle thickness decreased after RNAi-based silencing of BgCPLCP1 in both S-strain (A, C–E) and R-strain (B, F–H) cockroaches. "S" represents the susceptible strain and "R" represents the β -cypermethrinresistant strain. (A, B) Left: Representative images of cross-sections of the cuticle observed with light microscopy; Right: Higher-magnification images observed with TEM. The red lines denote the exocuticle and the yellow lines denote the endocuticle. (C, F) Cuticle thickness quantified with light microscopy. Each dot represents an individual cuticle location, and each mean is presented by a dashed line with SEM of n = 25. (D, E, G, H) Endocuticle and exocuticle thickness was quantified from TEM images. Data are shown as mean \pm SEM calculated from three biological replicates. ns: not significant; *: P < 0.05; **: P < 0.01; ***: P < 0.001(independent sample *t*-tests). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 7. Impaired cuticle barriers mediated by siBgCPLCP1-RNAi resulted in a diminished resistance to β-cypermethrin in the S- and R-strains of *B. germanica.* "S" represents the susceptible strain and "R" represents the β-cypermethrin-resistant strain. (A–E) Cuticle permeability increased after RNAi. In (A), scale bars are 2 mm. In (B–E), data are shown as mean \pm SEM calculated from three biological replicates. ns: not significant; *: P < 0.05; **: P < 0.01 (independent sample *t*-tests). (F, G) The incidence of defective wings increased after RNAi. In (F), the morphology of normal and defective wings are compared and the scale bars are 2 mm. In (G), data are shown as mean \pm SEM calculated from three biological replicates. *: P < 0.05; **: P < 0.01 (independent sample *t*-tests). (H) Resistance to β-cypermethrin decreased after siBgCPLCP1-RNAi. Survival analyses were conducted on S- and R-strain males treated with the respective LD₃₀ of β-cypermethrin. Each strain is compared to its respective control-RNAi (NC) group. P < 0.05 was considered a significant difference according to Log-rank (Mantel-Cox) test.

more cockroaches survived cypermethrin applications to their cuticular surface than delivery of the insecticide by injection or ingestion, suggesting that the cuticle is an important barrier to insecticide penetration. Second, comparative studies of an insecticide-susceptible (S) strain and a resistant (R) strain showed that a thicker cuticle in the R-strain, mainly due to thickening of the endocuticle, made the cuticle less permeable. Cuticular thickening conferring resistance to insecticides was demonstrated in *Cx. pipiens pallens, Anopheles funestus* and *C. lectularius,* whose time-to-knockdown by deltamethrin, permethrin and λ -cyhalothrin were positively correlated with thickness of their cuticle [27,55,56]. Finally, we identified a unique cuticular protein, BgCPLCP1, as an important factor in cuticular thickening, characterized its spatiotemporal distribution in the various developmental stages, and confirmed its role in cuticular penetration resistance with RNAi studies.

Motif analysis showed that BgCPLCP1 contains a large number of proline residues displayed as PV or PY sequences, which is typical of the CPLCP family. Although >100 CPLCP family members have been identified in various holometabolous insects [24–26,30,32–39], only 9 CPLCP proteins were identified in hemimetabolous insects, from the order Hemiptera (*Nilaparvata lugens* and *Triatoma infestans*) and order Thysanoptera (*Frankliniella occidentalis*) [31,40,41]. BgCPLCP1 is the first annotated CPLCP in the order Blattodea, which is important for understanding the diversity of CPLCP family in hemimetabolous insects. Phylogenetic analysis showed that all other proteins in the BgCPLCP1 clade belonged to the order Diptera family Culicidae, including *Ae. aegypti* and *Cx. pipiens*, suggesting that BgCPLCP1 is relatively conserved in insect evolution.

BgCPLCP1 was mainly distributed in structures that developed from

the embryonic ectoderm, such as cuticle, foregut, hindgut, trachea, and ventral nerve cord, and it was expressed at significantly higher levels in the R-strain than in the S-strain in leg and abdominal cuticle. Adult cockroaches derived from BgCPLCP1 gene-silenced last-instar nymphs displayed significantly thinner leg procuticle with particularly thinner endocuticle, greater cuticular permeability and lower insecticide resistance. Thus, the overexpression of BgCPLCP1 in R-strain cockroaches contributes to cuticle thickening and insecticide resistance. Based on this observation, we propose that BgCPLCP1 may confer insecticide resistance in three ways. First, by specifically thickening the procuticle layer. The contribution of various cuticular layers to insecticide resistance depends on their composition and affinity to various pesticides [57]. The outermost epicuticle, which is mainly composed of proteins and lipids, is highly hydrophobic and does not effectively block the penetration of non-polar insecticides such as β -cypermethrin, which are mostly active by contact [58]. The procuticle, which is mainly composed of proteins and chitin, has strong polarity and is the main layer that blocks the penetration of non-polar insecticides [59]. Therefore, high expression of BgCPLCP1 in the thicker polar procuticle of the R-strain retards insecticide penetration into the hemocoel and target sites in the central nervous system. Second, the procuticle is traversed by wax canals, pore canals, and various transport channels that also provide important routes for insecticide penetration [57,60]. The high expression of BgCPLCP1 may obstruct these canals, making it difficult for insecticides to traverse the procuticle. Third, CPLCPs are important crosslinked structural proteins with an integral role in embedding of chitin microfibrils in proteins to form a rigid matrix [27,61,62]. Overexpression of BgCPLCP1 in the R-strain may generate a dense cuticle,

which is less conducive to insecticide penetration. Nevertheless, the exact mechanisms that underlie the contribution of BgCPLCP1 to insecticide resistance need to be further explored.

BgCPLCP1 also contributes to wing morphogenesis. As a pivotal adaptation in insect evolution, wings not only allow the insect to move quickly but they also function in thermoregulation and as a protective barrier to shield the abdomen from direct damage [63,64]. Studies in *T. castaneum* showed that cuticular protein genes, such as *TcCPR27* and *TcCPR18*, are involved in wing development [65]. Our study provides the first evidence for the involvement of CPLCP family proteins in wing morphogenesis. Silencing of *BgCPLCP1* resulted in adults with significantly more defective wings. Short and crinkled wings leave regions of the abdomen exposed and may allow insecticides to embed in the exposed waxy epicuticular layer, resulting in greater mortality and a decline in ecological fitness.

In summary, we documented that BgCPLCP1, an important member of the under-studied CPLCP proteins in hemimetabolous insects, affects insecticide resistance in the cockroach mainly by regulating the thickness of the procuticle which alters its permeability to insecticides. With the widespread use of chemical insecticides, resistance rapidly evolves, and both metabolic and physical resistance mechanisms often confer cross-resistance to related and other classes of insecticides. A vicious cycle comes into play as cockroach populations escape control efforts and new insecticides with novel modes of action need to be innovated. As the first barrier to contact insecticides, cuticular penetration resistance is a ubiquitous mechanism in pest insects. Importantly, structural changes in the cuticle, such as hardening and thickening, are expected to confer resistance to a broad array of insecticides, not only the insecticide that selected for these adaptive traits. The identification of cuticular proteins that contribute to insecticide resistance provides not only new biomarkers for resistance monitoring, but also new targets that may be exploited for the development of efficient RNAi-based insecticides.

5. Conclusions

Extensive use of synthetic insecticides has selected for resistance in global pest populations and has various adverse effects on the environment, crops, animals and humans. The insect cuticle is the first barrier to insecticides, and although insecticide penetration resistance is ubiquitous in pest insects, the diverse mechanisms that impede cuticular penetration are poorly understood. We demonstrate that the cuticular protein BgCPLCP1 plays a prominent role in thickening the procuticle and thus contributes to insecticide resistance in the German cockroach, *B. germanica*. Members of the CPLCP protein family have been scantly studied in hemimetabolous insects, and by linking BgCPLCP1 to cuticular penetration resistance, this study opens opportunities to develop new and/or hyperosmolar insecticides that target the CPLCP proteins, having the advantages of reducing chemical insecticide use, slowing the evolution of resistance and being environmentally friendly.

CRediT authorship contribution statement

Tong Cai: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. Xuejun Wang: Methodology, Investigation. Baorui Liu: Methodology, Investigation. Haizheng Zhao: Investigation. Caixia Liu: Investigation. Xiancui Zhang: Investigation. Yuting Zhang: Investigation. Huiyuan Gao: Investigation. Coby Schal: Conceptualization, Writing – review & editing. Fan Zhang: Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Supplementary Materials for

A Cuticular Protein, BgCPLCP1, Contributes to Insecticide Resistance by Thickening the Cockroach Endocuticle

Tong Cai^{a,b,1}, Xuejun Wang^{c,1}, Baorui Liu^{a,b,1}, Haizheng Zhao^{a,b}, Caixia Liu^{a,b}, Xiancui Zhang^d, Yuting Zhang^{a,b}, Huiyuan Gao^{a,b}, Coby Schal^{e,*}, Fan Zhang^{a,b,*}

^a Dongying Key Laboratory of Salt Tolerance Mechanism and Application of Halophytes, Dongying Institute, Shandong Normal University, Dongying, China, 257000.

^b Key Laboratory of Animal Resistance Biology of Shandong Province, College of Life Science, Shandong Normal University, Jinan, China, 250014.

^c Shandong Center for Disease Control and Prevention, Jinan, China, 250013.

^d School of Life Science, Huzhou University, Huzhou, China, 313000.

^e Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA.

¹ These authors contributed equally to this work.

* Corresponding author. Email: coby@ncsu.edu (Coby Schal) and zhangfan0531@163.com (Fan Zhang).

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Figs. S1 to S2

Tables S1 to S4



Fig. S1. Full-length cDNA and sequence analysis of BgCPLCP1. (A) Full-length cDNA of

BgCPLCP1 gene. The black font represents ORF, the red font 3' UTR and 5' UTR and the green font start codon and stop codon. (**B**) Amino acid sequence of BgCPLCP1 protein. The blue font represents signal peptide. (**C**) Prediction of secondary structure of BgCPLCP1 protein. The abscissa represents the position of the amino acid sequence. (**D**) Prediction **of** structural domains of BgCPLCP1. The abscissa represents the position in the amino acid sequence.



Fig. S2. RNAi-mediated silencing of *BgCPLCP1***.** (**A**, **B**) Schematic diagram of the experimental time-course for single and multiple siRNA feeding strategies. (**C**) RNAi efficiency of *BgCPLCP1* at 2, 4, and 6 days after a single siRNA feeding in the S- and R-strains of *B. germanica*. N6D4, 6, 8: 4, 6, 8-day-old sixth-instar male nymphs. (**D**) RNAi efficiency of *BgCPLCP1* using multiple-feedings of siRNA during the last instar of *B. germanica*. Relative expression of *BgCPLCP1* was quantified in 2-day-old adult (AD2) males from the S- and R-strains. All data are shown as mean \pm SEM calculated from three biological replicates. ns: not significant; ***: *P* < 0.001 (independent sample *t*-tests).

Strains	Treatment (ng/male)	Mortality (%)	Corrected Mortality (%)
S	0 (control)	$0.0{\pm}0.0^{ m D}$	
	0.5	21.7 ± 1.7^{aC}	21.7±1.7 ^{aC}
	1	37.5 ± 2.9^{aB}	37.5±2.9 ^{aB}
	1.5	45.0±0.0 ^{aB}	$45.0{\pm}0.0^{aB}$
	2	66.7 ± 1.7^{aA}	66.7±1.7 ^{aA}
R	0 (control)	$5.0 \pm 0.0^{\circ}$	
	15	33.3 ± 1.7^{aB}	$29.8{\pm}1.8^{aB}$
	20	38.3 ± 1.7^{aB}	$35.1{\pm}1.8^{aB}$
	25	$43.3{\pm}1.7^{aB}$	$40.4{\pm}1.8^{aB}$
	30	93.3±6.7ªA	93.0±7.0 ^{aA}

Table S1. Insecticide dose-response bioassay of the S- and R-strains of *B. germanica* by topical application method.

The data represent the mean \pm SEM mortality at 48 hrs post treatment.

"S" represents the susceptible strain; "R" represents the β -cypermethrin-resistant strain. Cumulative mortality (%) = (number of cumulative deaths) / (number of cumulative deaths + number of survival) × 100%; Corrected mortality (%) = (test group cumulative mortality – control group cumulative mortality) / (1 – control group cumulative mortality) × 100%. The different lowercase letters indicate significant difference between the means (± SEs) within the same row according to the independent sample *t*-test (*P* < 0.05). The different uppercase letters indicate significant difference between the same group within the same column according to the Tukey's honestly significant difference (HSD) test (*P* < 0.05). The probit analysis for the S and R strains was performed, and the LD_{30} was further calculated as 0.83 ng/male and 17.16 ng/male, respectively.

Primer		Sequence $(5' \rightarrow 3')$	Comment
5'GSP		GATTACGCCAAGCTTCCCTACAAAGTT	5' RACE
		CGGTGGATACCTCCC	
3'GSP		GATTACGCCAAGCTTAATGGTTTGGGGG	3' RACE
		ATTGGCACTGCGTACGG	
<i>BgCPLCP1</i> - F		AGAGCCTTATCCAGTACAGGTTCC	RT-qPCR
<i>BgCPLCP1</i> - R		TCTGGTACAGCTACTGTCTTCTCG	
β -actin - F		AGGTATCCTGACTTTGAAATATCCC	
β -actin - R		CAAACATGATCTGTGTCATCTTTTC	
siBgCPLCP1	Sense	GACCGGUAUCUACAAACAAdTdT	RNAi
	Antisense	UUGUUUGUAGAUACCGGUCdTdT	
siNC	Sense	UUCUCCGAACGUGUCACGUdTdT	
	Antisense	ACGUGACACGUUCGGAGAAdTdT	

Table S2. Primers and siRNA used in this study.

Software/online tool	Function	Website
ORF Finder	Prediction of open reading frame	https://www.ncbi.nlm.nih.
	(ORF);	gov/orffinder/
	Protein translation	
ProtParam	Prediction of basic physicochemical	http://web.expasy.org/prot
	properties	param/
ProtScale	Prediction of hydrophilicity and	http://web.expasy.org/prot
	hydrophobicity	scale/
SignalP-5.0 Server	Prediction of signal peptide	http://www.cbs.dtu.dk/serv
		ices/SignalP/
TargetP 1.1 Server	Prediction of cell localization	http://www.cbs.dtu.dk/serv
		ices/TargetP/
SOPMA	Prediction of secondary structure	https://npsa-prabi.ibcp.fr/
TMHMM Server	Prediction of transmembrane domain	http://www.cbs.dtu.dk/serv
V.2.0		ices/TMHMM/
SMART	Prediction of domains	http://smart.embl-
		heidelberg.de/
THREADER	Prediction of tertiary structure	http://bioinf.cs.ucl.ac.uk/in
		troduction/
MEGA 6.0	Phylogenetic analysis	
R 4.0	Motif analysis	

 Table S3. Software and online tools used in this study.

Table S4. Amino acid sequences of 130 CPLCP family proteins used for phylogenetic analysis, including one in *B. germanica* and 129 from 15 other species.

Blattella germanica (Order Blattodea)

>BgCPLCP1

MRSLVWTCALLVALVVYTDGTPTKREIAEPHEEGAPEQDSSQAKRSLPSQETEGAPEQD TSQSKRSLPFLGFEGFGKYADFPTKFGGYLPNVISETIKYPVPEPYPVPIDHPVPVEVPVPV PQPQPYPVKVPVRVPEPYPVEVQIPVENPVPIIVDRPVPVYERVPIEIPVHIDRPYPVEVKV PIPEPIPVEIPVERPYPIYIDRNVPVPEPYPVQVPVYIPVEVPVRIPSPRPFPVEVKVPFPEPY PVYLEKTVAVPEPYPVEVPVGVPRPIPVEVPVAVETPYAVPIPKPFPVEVEVDKLIPVPVK GGYLPVGFPYQGNSLHPFKGIYGL

Plutella xylostella (Order Lepidoptera)

>PxCPLCP-1

MRILRSSLLLAVVIGLATAEEKAEKDAKAEETKPVEDKKQDKRGLSEWYGDHGGYGHG GHDFGEHHHVDHHVDHHVEHHKTITTIKKVPVPYPVEKHIPVPVEKHVPYPVKVHVPQ PYPVVKTVHYPVKEIVKVPVHIPKPYPVTKHVPYPVKVPVDNPVPVKVYVPEPYPVEKK VHVPVKVHVPAPYPVEKTVHYPVKVPVKVNVPYPVEKVVHYPVKVPVDRPVPVHIEKP VPVHVEKPVPYPVEKEVPYPVKVHVDRPVPVHVEKPVPYPVKVPVPAPYPVEKLVPYP VEKKVPYPVKVPVERPYPVEIEKHVPYHVEKKVPHPVKVGVPVFVHEHKHEIKHEHHE LGGEEHGHGSLGGEGSYGGEGSFGGEGSYGGESSYGSEGSYGGEGYESHGHHEYQH

>PxCPLCP-2

MRLMFVAASLVALLATAHAEEAKKAEKEVAVTDNKDSKGAEEKKHEKRGLFDIGLGY GHGGFEGGFGGSYGGSYGGGYGGGWDHGHDTHKTITVVKNVAVPYPVEKHIPVPVEK HVPYPVKVHVPAPYPVVKHVPYPVKEVIKVPYHVPHPYPVEKKVPYPVHVPVDRPYPV KVYVPQPYPVEKHIPVPVKVPVPAPYPVEKKVPYPVKVPVHVPAPYPVEKIVHYPVKVP VDRPYPVHVPKPVPYPVEKPVPYPVEKPVPYPVKVPVDRPVPVHVEKPVPYPVKVPYPA PYPVEKLVPYPVEKHVPYPVKVPVDRPYPVHIEKHVPVHIEKPVPYPVKVPYPVHVSHG HGHEYGHHDSY

>PxCPLCP-3

MKKTVCLVFLLVAAVAASEKKSTEKKAEPLDKKLDKRGLLNLGYGYGISGLDLGYLGS GHSLGGAYNFVDEASLAHSSYGPILGGHTDVTKTITLVKGYPVPYPVEKHIPYPVEKHVP YPVKVPVPQPYEVVKHVPVHVKEYVKVPVHVPAPYPVEKKVPYPVHVPVDRPYPVKV LVPQPYPVEKHVPYPVKVPVPQPYPVEKHVPYPVEVKVPVPQPYPVYKHVGYPVKVPV DRPYPVHVPAPYPVEKPVPYPVPVEKPVAVPVHVPVDRPYPVHVEKPVAYPVKVPVPEP YPVYKHVPYAVEKPVPYPVKVPVDRPYPVHVEKPYPVAVEKPVPYPVKVPVLVDDHH YGHGGPDYAQEYSHLSLGGNHYSH

>PxCPLCP-4

MKYLVVLAASLAVVAFAAEEKGESKAAAEDKKQEKRGIHDFGSYGGGSSYGDFGSHG GHEFGHVSSDWKPISSGESYGHGHEHHHHTEHVKHVEVIKKVPVPYTVEKHVPYTVEK KVPYEVKVPVPQPYTVEKKVPYTVKEYVKYPVYVPEPYTVEKKVPYEVKYPVDKPYEV KVKVPTPYTVEKKIPYEVKVPVPQPYTVEKKVPYPVKYEVKVPQPYEVIKKVPYEVKVP VDKPYNVYVPKPYPVHVEKPYPVTVHKPVPYEVKVPVDKPYKVEVEKPYPVHVKVPV

PQPYDVYKKVPYTVEKKVPYEVKVPIDKPYPVYKDVHFPLVKEVPYPVKVHMPVYFKK EEEHHGHHEEHQEHQPQEHHGWH

>PxCPLCP-5

MAFVVRALFVSALVCAARAGVLSGAHSHHETYAPSAAYGAPAAQYGPPAAQYGAPAI ESHHDHHHIDHHHSDHHTHAELGSAGGSYIADGAGASYSSLSGPGASGPGPVIGGGSYS GASFGGSLQGAPLDGSLSGAALPAGAQLGGAIASGPGGIIDGSGLAGPSGAGFAGPAGG FSGPAGGFGPGPADGGFSGPGALAAGPGAPGFPAPAVLPGGVTTTVGQPRVLGQTVTV GRPRVSETRFELASVVQNVVRRVPVEVTRHVQVGVPQPYPVPVRQEVRVNVPQPYPVQ VDVPRQVPYPVYKTQHVEVERPVPYEVVKHVPFEVIRKVPVPVDRPYEVIRKVHFPVEK HVEVPVAVWKPYPIHIIKHVTHYEKKKCWHCGW

>PxCPLCP-6

MATFTKLALLAILVGSSQAGLLHTHEHHDHLHGDHIHHHDTVSSSSLGASSLGGAGLAG PAYAPAYSSSFSPSSFPAGSLPSASYSGAGLAGPSFSGASYSSGSALPGFPASGASLSSSYA PGIGASYASGAGLAGPASFGGGLSGPLGPSGLAGPLPGAALSGEPAPPGPAAPVVNTIHT LANTNGAPIRYNVREEPYNVYREVTHQVPQPYPVPVPQPVQVPVPQPYPVQVPVVRNIP VPVVQIQRVNVDKPVPYPVEKIVKVPVERVVSVPVDRPVPVERVVKVPVVHVVKVPVH VVKNYPVPVVKTVHHKAVSHGWHAHHDHHHGHHGW

>PxCPLCP-7

MINVICILALAVATSKAGYSSSYDAGHGGQEASSGYDYTPPAQGHDLTQGHQTYTSAIT GQGNTHDFGHGFSGSAGNLQAYDHGEQQGHGVQAFDLSGHGGQGYDLSGHGGSQGH EAQSFSGHSLGHGGQAFDLSGGSSHGQGFDLSGHRGSSHGVQSFDLSGHGASQGQSGG HGLDLSNHGGQGHGGQSFVLQSHGGEAGQARGYDLSGHDDATAFGGDIGSDSYLQAA HDFGHSGGSNDGQGHQSFPLQDGGHEFGGHGGDQNVVVEDGGHGQGHQDSFAVGEH TDVEHPVGIPHYKHVTVPIHKPLHIHVPKPILIGVPQPYPVKVPVNKPVAVPVETEVSIPFE KVVPYPVVKHVPYPVEKHVPIKVEKTVTVHVPQPYPVKIPVYKTIHHYHKSHHKW

MIAFKATTLLMLAAVTAGAPSGGGWEPENHSHSEHSRPYHYTVVKQIGVPIPHPVAVSV

PQYVKVPIPQPYPVHVTVEQPIHVPVYKVVPQIVEKPVPYTVEKPVPYEVEKPYPVEVEK

>PxCPLCP-8

>PxCPLCP-9

KVEVPIPKPYPVHVPVYKHIYHHKGKY

MCALKRLYFVVLISLLLHGCANGKKTKKIKRPTVDDEETTPQPNVVTYSSFGFNDVGNA YDGFVPSSPDYANYLSNSNQESSTRLYAPAFPTAMDHSPYGQQEAGFPGSAPDAREHSL LQYNPGTYNFGNSPNYGSSHLDMPSQVGREDNGDGNEDEYNSPVYGTKLGSKGNKMR PVNNFNSSIVYGSSPYNPVEEKAPSYHEMQAAEEQNKHQSQQGESGASSYNYFNNYAPI FPSSGNSNDGEDTKQNSNPGPNSVKYNKVVDFTKYKPSYPLELDNKYATGTVRPTNMN MNNMHMYNSMTNGQNDNSFSNQYYPSDNENQQTQKYKQEQYEPDEPEQVASKPIGTN YMEFSVNHVQNPNNYKEAKPNAEYKDKFKTKPWNNYNAEVNNYKNSFKNNEYNDTK SNNFRRPFRANNDETAASSNTNVVDFTNYRYPETDFSNFKNMNDYKSEGSENYPSVQSF KYQDFQGESDYYSQLKGLFTTTPSSTSHWGSMFKNGDRPRTKKPQSTDDEVVHIPKRLK LHKQQTGKHSEYQSPLKHGLYKGKPLDEWNKEVSNRFKNEEDLLGLRNHDTSHPQYLP

PIPKPYPIEVPHPVIVPVPQPYPVRVPVPKPVAVPVIREITVPIEKPVPYPVIKKVPYPVEKP VPVHVEKEVQVPVMKPYAVPVPHVRPVFHHSRPYDEVEGERELDHEELDYMPRPEASR GKKYIRSSYKKTRRCYHYVSSLATERVWLFT

Bombyx mori (Order Lepidoptera)

>BmorCPG12

MRPMLVAASLVALLALAYAEEAKKAEKEVAVTDKEPAADDKKHEKRGLLDIGWHGGF DGGYGGGGYGGGGYGGGGHYGGHEEVHKTVTVVKKVPVPYPVEKHIPYPVEKKIPYP VKVHVPQPYPVVKHVPYPVKEIVKVPVHVPQPYPVEKKVPYPVHVPVDRPVPVKVYVP EPYPVEKKVHVPVEVHVARSLPSREESTYPVKVPVHVPAPYPVYKEVQVPVKVHVDRP YPVHIPKPVPYPVEKPVPYPVKVHVDRPVPVHVEKPVPYPVKVPVPAPYPV EKHIPYPVEKAVPFPVNIPVDRPYPVHIEKHVPVHIEKPVPYPVKVPVPIVVSHEHGHEHG HDFGHHGGY

>BmorCPG13

MKTALCLVFLLVAAAAASEKKTTESKAEPLEKKLDKRGLLNLGYGYGIDGLDVGYIGH GQGLGGAYNYVDGGYSSGYGLNFGGHTDVTKTITLVKGVPVPYAVDRPVPYVEKHV PYPVKVAVPQPYEVVKHVPYHVKEYVKVPVHVPAPYPVEKKVPYPVHVPVDRPYPVK VLVPQPYPVEKHVPYPVKVPVPQPYPVEKHVPYPVEVKVPVPQPYPVVKHVGVPVNVP VDRPYPVHVPAPYPVEKPVPFAVPVEKPVAYPVHVPVDRPYAVHVEKPVAVPVKVPVP QPYPVYKHVPYAVDRPVAVPVKVPVDRPYPVTVERHVPITVEKPVPVPVKVPYLVSDH HDHHDHHDHYSQLSYGGSYYGH

13

>BmorCPG7

MATITKVMYLVVLLSSTYAKPGFDQHDHHATLEHHDDHHIANIETAPIGLSAPSAGGSSF APSSFPAGSLPSATFSGAGLIGGGHGGGFGFGGFNGGAGGGFGGAIGGPQGIFPGGDGPL AGLKSPVIDEIHGLANNHGAPTQYRIREEPYEVYREVTHRVPQPVPVPVPHPVQVPVPHP YPVQVPVIRKIPVPVVKIQHVKVDRPVPYPVEKIIKVPVEKVVEVPVDHPVPVEKIVEVPI VKLIKVPVHIVKSYPVPVVKTVHHKAHVSHHHSHGWSGW

>BmorCPG24

MKYTVILVASLAVVAFAKEEKGTPKAVEEKKQDKRGIYDIGSYGGHNFGGSDEGYGGH ESYGGHEGISFGHQEGHDFGGESYNLGGHGGGEYGSSGGDWKPIASEGDYHHGHHEHH EHIKTVEVVKKVPVPYTVEKHVPYTVEKKVPYEVKVPVPQPYTVEKKVPFTVKEYVKY PVYVPEPYTVEKKVPYEVKVHVDKPYEVKVKVPTPYTVEKKIPYEVKVPVPHPYTVEK KVPVPVKYEVKVPQPYEVIKKVPYEVKVPVDRPYNVYVPKPYPVHVEKPYPVTVHKPV PYEVKVPVDKPYKVEVEKPYPVPVKVPVPKPYEVIKKIPYTVEKKVPYEVKVPIDKPYP VYKEVQVPLVKEVPYPVKYHVPIYFKKEEEHHQHHQHHGWH

>BmorCPG8

MAFVMKAFFIFALAYGAQAGLLQDQHDSYVSSALGASSASFGPPSNTYGPPHINGITEA HVSGPTVLEESLIEQHSHGGHAFDAGHIDDHAHFEGHGHIDVHEHHDDHGHIGNGPLGG GAVESFGAADIASGPGLSGPDAGGFGAGFEGGAGIEGGFGGVEGGAEGGFFAGAGGAIG GGIGASGSFGEPRVIGTSVSVGRPDPGSTRYELQSVVQNVVRRIPIEVTRHIKVAFPQPVP VPVPHKVKVPVPQPYPVHVDVVKKVPYPVYKTEHVEVERPVPVEVVKHIPVEVIRKVPI PVERPYEVIKKVHVPVEKHVEVPVPVWKPYPLHIIKHVTHYKKKSCCW

>BmorCPG11

MRLMRSSLLLAVVIGLAAAEEKAAKAEEQLEPKKQDKRGLSEYYGSYDEHGGGHEEH VKTITVVKKVPVPYPVEKHIPVPVEKNVPYPVKVPVPHPYPVIKTVHFPVKEYIKVPEYIP KPYPVTKHVPVPVKVHVDNPVPVRVYEHVPVPVEKAVPVPVKVPVPHPYPVEKKVPFP VKVPVKVHVPYPVEKIIHYPVKVPVDNPIPVHVDKPVPVHIEKPVPYPVEKPVPYPVKVP VDRPVPVHVEKPVPYPVKVPGTRTIPCRKSNTVSR

Bactrocera dorsalis (Order Diptera)

> BdCPLCP-2

MRLFVLPCLALCVAIANCAPAAEKADAAAAETAKDAVEAKDDSKNVEKRGLHLGDYG HHHHEHIKTITIEKKVPVPYTVTKHVPYTVEKKVPYEVKVPVPEPYYVEKKVPVHVKEY VKVPVHVPKPYIVEKKVPYEVKVPVDKPYEVKVPVPQPYEVIKKIPYEVKVPVPQPYEVI KKIPYEVKVEVPVPKPYEVIKKVPYEVKVPVEKPYPVEVEKPYPVEVEKPYPVVVEKKV PYEVKVPVDKPYKVEVEKPYPVHVKVPVPQPYTVEKKVPYTVEKPVPYEVKVPIEKPYP VYSKVKVPVHKEIPIPEKYHVEVPIFHKEEHHDYHHGY

>BdCPLCP-4

MKSLFIFGLLALSAFTYAEEAQKSAEPKSAEPAAAASEVKPSSTAEKKQEKRGIIHGTGY GYGGGHGGAVLLSGGHGGGYGGGYGGGAIIAGTGLGGGLGGGLGGGLGGGISVPTNV QTSYVERQVAVPYQVERAVPYPVEQIVQVPVQVPVPQPYPVEKTVHVPVKEIVKVPVHI PQPYPVEKTVHVQVPVHVDRPIPVKVPVPAPYPVEKIVQVPVKVPVPQPYPVEKIVQVP VKVPVHVPQPYPVEKIVQVPVKVPVDRPYPVPVEKPYPVPVEKPVPYPVEKRITIPVQVP VDNPVPVHVDRPVAVPVKVAVPRPYPVIKEIPVPVERKVPYPVKVPIDVPRPVHVEQHV PVAVEQHVPYKVPVPVPVHVQSHVAPAAAIISGGYGGIGGYGGHSIGALGGHSLGGYG GHSISYGHGHIHKKK

>BdCPLCP-3

MKLFICFAALLVATAYAGDTAKKATVESSAASGKKVPLEKKLDKRGLLDLGYGYGHSG LDVGYIGHGSVDYGHGYGLVAAHSAPAAVSYGHTGLASAGIAHSPFLISKTADVHKTIT VTKGIPVPVTVDRPYPVVHQKQVPVEVKVPVPQPYEVIRKVPVSVKEYVKVPIHVPQPY TVEKRVEVKVPVPDRPYPVKVPVQPYEVIKHVQVPVKVPVPQPYEVIKHVQVPYKV QVPVPQPYEVIKQVQVPVHVPVDRPVPVAVPKPYPVPVEKPYPVVVEKQVKVEVPVRV DRPYPVPVDRPYTVKVPVDVPQPYTVEKHVPYTVDRPVPVVKVAIDRPYAVHVTRPV PVAVQKPVAVPVPVPVVAGHTVVEHGPAIAISGGGYDGGYGGHGFSSSGYGYSSLGHG YAHKKK

>BdCPLCP-5

MKLLRSASLLALVLLLAISTRAEDEKAVESKSAEPAQEGAKESSASAADDSDSTKTKRG LHHFEDYHHSYHHPIHEEKTLTIIKKVPEPYPVEKVVHVPVEKHIPVPIKVKVPKPYPVV KHVPYEVKEIVKVPHEVPAPYPVEKKIPVPVHVHYDRPVPVKVYVPAPYPVEKKVHVP VKVHVPAPYPVEKKVHYPVKVHVPVDKPYPVEKVVHYPVKVHVDKPVPYHVEKPVPY HVEKPVPVPVIKKVPVPVHVPYDNPVPVHVEKPVPYEVKVPVPAPYPVIKEIPVKVEKH VPYPVKVPVEKPVPVHIEKHVPEYHEKHISYKEPVFVHKEIHEPVHHEEYHHEESHDFGG HEH >BdCPLCP-1

MDPQPSIHQHRQRRLRPLILLFFLCYFASSQAKRTTRRLPRDEVHEYAIHHYNYAERDVE EPSGSVADLHADESRRVEVQVPSPKGSNNGGTTRDKRGTKKIRHIVHLVKTAQQKRKK PMTTPRNNNQLYRQQNAVDGDTKPYHTYEEVHEHIMEPAEEDESAQKSTYVPSSAHAQ TSGSKTKVHTQKEPLSEASSNSYATHALLKPHSVKMAQQKKKIPLPTTRTNNRHQNAYE **GDTKPYHNYEEMHEHVMEAAEEQESAQKPTYVASSAHAHTTFGSKAKAHAQNEPLPE** ATSDSYATHAFLQPPEEPQDEPHMSSTEHHEHEIHEEEEHHHVEKIKVKHHHHHHHHNH VKEIIKKVPEPYPVEKIVHVPVEKIVEKLVHVPKPYPVEKIVEKKVPYPVEKIVEKVVEKK **IPYPVEKIVEKIVHVPIEKIVHVPKPYPVEKIVEKKVPYPVEKIVEKIVEKKVPYPVEKVVE KVVHVPKPYPVEKVVEKVVEKKIHVPVEKIVEKVIHIPKPFPVEKIVEKIVHIPKPFPVIKH IPYPVEVKVPVHIEKPVPYPVEKKVHVPYRVEVEKKVPVPYKVEVEKKVPVFIHTPHVS** HEPYKFEHHEHEHKDDAHEHHEEKHDYEEHKHFEHHDLSGYPTNQNANYAKLQQQP RHITNGAKAVLAKAHTPAAAAAIRAELLHQQVQNFGYKIATSTTPTDLNQSSSENKPQM VRFEPTPENKFGVKQSTPATPSDLEPSASENKPIVAHFEPTALPFRIHVDDGATAGDGGS ALGEPSTANDMQAQASTHSFRMLARLQPIAMPLQVYQLHSLPFQQPLGFSLPAIRAVVP SSTS

Drosophila melanogaster (Order Diptera)

>DmCPLCP-CG13138

MNPMLTLIPGYLFITLIIIGSTAHSNSYAVNKTNSVESAEWSYPEYGKGSTHNTGRRSEKL DLQHYNRETERNVTLKRGVKNYQKQGDKMTRNKLKYKPSGARRKNVSPNYADESTES RHYHTFEEIHEHIDEDDGQYQASEQVGTQAILHEHVEHSSKKDAKRMKVKIKHHHHHH HHNHIKELIKTVPQPYPVEKVVHVPIEKIVEKIVHVPKLVNVTVEKIVHVPIEKIVEKVIHI PKPVQVPKPYVVEKIIEKIVHVPKPYPVLRTVPYPVEIKVPVHLEKKVPVPYKVEVERKV PVYIRSSEPYKFESSSLYESYPRGEEFKFNMEMDHPPPREHEPSSLPSSNYYNRIYKSREL DHPPRMEDYQSSVPTQLKHDYATKTNLDQQFKNEYVTKSSIDPQFKHDYVTKSSIDPQF KHDYVTKSSIDPQFKHDYVTKSSFDSQYNQEYVPKTSIESTSPGRDGVSLKLVGPPTPFNI TGKDLETANSAPDFVNSSNVDLSPQESANAYRGMPFSIPFQFVQLQPMAFQSPIHVELPI QSASAEGAQK

>DmCPLCP-CG30101

MRMFVLPCLAVCVALAHCGGAVEDKKAEGDGKTVEKRGLHLGDYHHYQPHHEHIKT VTIEKKIPVPYTVTKHVPYTVEKKIPYEVKVDVPQPYIVEKKVPVHVKEYVKVPVHVPK PYEVIKKIPYEVKVPVDKPYEVKVPVPQPYEVIKKIPYEVKVPVQPYEVIKKVPHEVKV EVPVPKPYEVIKKVPYEVKYEVEKPYDVEVPKPYDVEVEKPYTVVVEKKVPYEVKVPV DKPYKVEVEKPYPVHVKVPVPQPYTVEKKVPYTVEKPVPYEVKVPIEKPIPVYTEVKVPI HKEIPVPEKYHVEVPIFKHHQEDHHDYHSHGHGHY

>DmCPLCP-CG16886

MKSMRTATSLALVLLLATSYVRAEDEKAAAPAEEKKAEPEAKAAEAAASEDTTKSKRG LHHYEDYHHHHVPHFPVHEEKTLTVIKKVPVPVPIEKIVHVPVEKHIHVPVKVKVPKPYP VIKHIPYEVKEIVKVPYEVPAPYPVEKQVHVPVHVHYDRPVPVKVHVPAPYPVEKKVH VPVKVHVPAPYPVEKIVHYNVEKHVHVDKPYPVEKVVHYPVKVPVDKPVPHYIDKPVP HYVDKPVPVPVIKKVPVPVHVPYDRPVPVHVEKPVPYEVKVHVPAPYPVIKEVPVKVE KHVPYPVKIPVEKPVHVHIEKHVPEYHEKHVTYKEPEFHHKHIEEDHHHAPIHHHSHPIV EHEHEVEHEFASHDYHSYYGY >DmCPLCP-CG16885

MKSMLIFGLVAMCVLVANASEEAPKKAVETAEPAEKKQEKRGIGHGLGYGYGPSAGG AILGSGIGVGVPVAPAVAELPTQVHTNTVVRTVQVPYQVERHVPYPVEKTVTYPVKVP VPQPYPVEKIVHVPVKQIVKVPVEVPQPYPVEKVIRVPVKIPVDRPYTVHVDKPYPVPVE KPVPYTVEKRVIHKVPVHVERPVPYKVAVPVPVHVESHVKPAVAVTHTVAAAPAIISHG YSGHGISGYGISGHGISSYGSSSYGGSAHGGYLHKK

>DmCPLCP-CG16884

MKVFICLAALLVASACASKTEGEKVPLEKKLDKRGLLDLGYGYGHAGLDTGYLGHGSI SGHGSYGHGYGLTGYSAPAAVAVGHSGPAIAVGHTAPAVAVHHAPAPYVISKQADVH KTITITKGIPVPVHVDRPYPVVHEKRVPVEVKVPVPQPYEVIRKVPVTVKEYVKVPVPVP QPYEVIRHEKVPVHVPVDRPVPVEVPRPYPVPVAKPYPVYVEKAVNVQVPVHVDRPYP VYVKVPVVSHSVVKHAPTVAVSSYPVSAIGHDATVYSDHHGYHK

Aedes aegypti (Order Diptera)

>AAEL013370

MLVLLVFAVGWNSGDAGKSVSQKKRELPSGTSETVESSHIFDTQSPEVNHWGNKQFKE VTITKNVPVPFPVKVEKHVAVPVKIPFPVAIQNKIPIVVERKVPIYVEKPVPVQVDRPVPY PLPIEVPVFHRVAVEVPKPYPVHVPAPYPVYIQKPLFVEQSKKSKRVKKNTVKVYRYK

>Aa-gi|157116080

MIFLEYCNTHNQAKGSKPAGHAGVDGQLEQTPAEDRQWKVEKRHTGKKIHIFLGKGSG KSKKSSKKHADSDGAETQTRKTPELTTRSDKAEDLAASATSDAPDAAVGESQHYEVPE HQIVKEKIKIKHHHHHHHHNHVKTVVKKEPYPVEKVVQVPVEKIVHVPKPYPVEKIVEK VVHVPVEKIVHVPKPYPVEKIVEKVVHVPKPYPVEKIVEKPVHIAKPYPVEVKVPYPVE KIVHVDKPYPVEKIVEKVVHVPKPYPVYKQVPVPYEVKVPYEVPKPVPYPVEKKVPYPV EVKVPFEVERKVPVPVKVEVEKKVPVPVKVYVPVEKKVPYPVPVKSQFPFDKDIGYAST KLHPFESQQHEVRHYGDEYSNGEEGQQPKYGPSTFPQQNDYQSQATSQESHYHHNDHY GSGTTQQEVIVTPPANTAHLTFDMSQQSMPQAFALTAPSNPGTTLQLESQPQPFQLLQLS PFQFQTPTGFSLPTAR

>AAEL008752

MLTTIALLLVTSVLGTATQQSPSPNDPPAKPVRTILLPTVPPDELFTVIHAQSAPPTVGPPQ SDGGVRYEHSISLGPHSNYYVYYSDNIQQHAQEAQQHEQQHQQQLPSPIAYQPLVGYSN EQSSQIMFNPNAVPVQLVTLVQPTIDDAIRQENATAEQQFLPTPVPPPSPYLDINEEGIQK LVTSTQNLVTNEDVVSINNAEEGEGKPTEVTEEKIETKTSDSEPSESSKKESRYLDNVAV PSFDNPIVVGEMAETAPRNHRSGNKIDNLLKQINVEILGESDDHNKGQFLKETSYRANQL TSEPCDDDGKSVPVEIKHEVTTIRPITLSTKARGSTASRLSTRFRTTTASLPTATARYTAK EPTTVTPKPVSQKYLAPIQAGLRLSNSGKNIEDCVDEKGSKTVVEVQKSINLKNLVVGPQ PAQQVKQPEFGARTTVVHQPIIVEKPVERIVKQNVYIEKPVERLVHQPVYIEKPVPQPVD RIVEKKIPVPYPVEKIVEKPVPTPVHVPYHVEKQVPVHHYIDRPVPHHVPVPVTVEKIVE KPITVEKVITKEVQAPYPVTQIVEKIVDRPVPVEKVVTKEVQVPYPVTQFVNRPYPVEVP VEKVVEKIVDRPVETVVEKHVEVPVPVTVEKVVEKFIDRPVPPVQVPVEVPVQVPVHY

>Aa-gi|157120578 MLFKKSIRLRSFIGFGFSTVAVGSKGYSNEVLSMVLAIAFGAAVEKPVKEVDTENESKAE TGVKVQGKRGLYDFGYGSTPELQGGFKPSFGFDFSEPHQYEVKEDHHTIITKNIPVPYPV EVEKHVFIEKKVPVHIDRPVPYPVTVEKKVPYIVEKHIPVHVDRPVPYPVKVPYPVEVEK KVPVYIEKKVHVDRPVPYPVHVEKKVPVYVEKKVPVVVEKKVPVPYEVKVPVVQKVE VPVPKPYPVHVPKPYPVYIEKEVIKHVDRPIHVEVEKKVPVPVVQKVEVPQPYPVYIEKP VYIEKHEAQHNEEHQQVEQQDEDYNEQPIHVAEDRQHREESDHNEQEPEQHQQEEHQE HVQQDYVETSKEEEKSSNSDEKTAESAESRHHEQHHHMPLHHANNDE

>Aa-gi|157116511 MRMFIIPCCLALLVALATAEGKGKPAAADQNVAASERSVEAEQKPDRELERRGITLGD WNHVQDQHEQLQLQQPQFEAQESHAASDFQAHFAQQQQQPQQYHQQPQQLLQTAPQS FYQHQTQPQAHFQHQAQTQSFYQAHPQPHETQHYHMVPQHMHLVHLPSQHMVHYQP EHHQYEDIKTITVEKKVPVPYTVEKHVPYTVHKKVPYEVKVPIPQPYVVEKKVPYTVKE YVKVKEYVPEPYTVYTKVPYEVKVPVDKPYKVEVPVPKPYVVEKKVPVEVKVPVPVP YTVEKKVPYEVKYEVPVPKPYTVIKKVPYEVKVPVDRPYKVEVEKPYPVEVKKPYPVV VEKKVPYEVKYPVDKPYHVEVPKPYKVEVKVPVDRPYKVEVEKIPYTVEKAVPYTVKVE IDRPVPVYKEVKFAVTKEVPHPVKEKQYVPVPVQHETVHHYGGHHHGQESHY

LNKEQNAN

PPLPKPPVIEVPGKHYYFHNQFPKNDLHFGASIPAPSYVDVGHINVLPHHFPEKTKSIYGV PLTGEIFNGKSSYHFNPYQHYPLIDQSHFVGKFTYQAAPLPPPPPQHHHHFQTSTAQLLP GVYKDDYVGPTPLLEDHWAVKSDVKFRRSPAYGKSLRIEYGGFKPPLVPSLEIDENGVP >Aa-AAEL005941

MKAFAIVIVASFALSTVSAGVVRTEKRSIGEHGAQLASYNNHVLQQQQFDYQKNQPNQ EKWSQPDDQLNKAEEYYQQVQQQQLQWESQKERSPMHHGIDNHLYKHAEHQHQYQN QAAQPVQYHYAQQQNNHQQQYLHQPQYQQQEQYQHYEQHHEQDQTHYHQQPQNHY QHYDHQHQQDQEQHQHQHGWEQQKQVVSVSSVTQHVPYPVHVQKNVAVPVNVAYP VPVEKSVPVVVEKKVPVYVEKQIPYRVERPVPYPIKVPVQSLHKDIHVVHVPKPIAVHV DKPYPVYVNHPVYVEKPVPLQVVIMEHKKKSFWG

>Aa-supercont1.486-1

MKAFVVLSMALAVASCAAVDDSSSKKDKRGLWELGYGDHDLHGFDDHHHDHHEVKH LQTTITKKVPVPYPVEVEKHVPVHVKVPYPVEVEKKVPIYVEKKVPVYVEKKFPVHVD RPVPYPVEVKVPVVQKEYVEVPKPYAVHVEKPVPVYVHKPVYVEKHVPVTVTIKKHEK KHWF

>Aa-supercont1.486-2

MKAFVVLSMALAVASCAAVDDSSSKKDKRGLWELGYGDHDLHGFDDHHHDHHEVKH LHTTITKKVPVPYPVEVEKHVPVHVKVPYPVEVEKKVPVYVEKKVPVYVEKKVPVHVD RPVPYPVEVKVPVVQKEYVEVPKPYAVHVEKPVPVYVHKPVYVEKHVPVTVTIKKHEK KHWW

>Aa-supercont1.486-4

MKAFVVLSMALAVASCAAVDDSSSKKDKRGLWELGYGDHDLHGFDDHHHDHHEVKH LQTTITKKVPVPYPVEVEKHVPVHVKVPYPVEVEKKVSVYVEKKVPVYVEKKVPVHVD RPVPYPVEVKVPVVQKEYVEVPKPYAVHVEKPVPVYVHKPVYVEKHVPATVTIKKHEK KHWW

>Aa-supercont1.486-5

MSEGDATQNNGGKLHQKKVLSRCREACFVRTGRKWELGQQWRLPHQNADQCGEGGG IDVALIMFHCRPWSPTASTPVHAGVYRLVERHGRECIIKYSPVRRQDIIRRSPSRRTSSVR QQLERSSFKLGISNMKAFVVLSMALAIASCTVVDSSSKKDKRGLFDGGHGGSYDFGGH AEQEVKHVTTITQKVPVPYPVEVEKKVPVEVKVPFKVEIEKKVPVVVEKKVPIYVEKKIP VHVDRPVPYKVEVKVPVVEKEYIEVPQPYKVYVEKKVPVYVPKPVYIEKPLPVTLLVK KTYKKGW

>Aa-supercont1.486-6

MKAFVVLPMVLAIASCAAVDDSPKKEKRDLWDEGFGHGGDSGGTDKGYGGGLSSYGG DHGHVTVSFSKGHEEGSSGGGYGHEESSKGSSGGGDSGKGGEHGLSSYGGGHEHITVSF SKGHEEESSGHGGDHKESSGGKGHEISFGLFDAGHGHSSGGSSGGHGHSFGGSGGGGHG HSSGHSFGSIGGGHKESSGGSSGGHGHSFGDIGGGHGHSSGHSFGSIGGGHKESSGGGGHG ISFGSIGGGHKESSGGDHGISFGSIGGGHKESSGGGHGISFGGGHGHGSGGSSGGHGHGY GFIGGSDKGSSGGGHGHSFDSSGGDDKGSSGGHGHSFGSIGGGHGHSSGHSFGSIGGGH KESSGGGHGISFGSIGGGHKESSGGFGGGHGISFGSIGGGHKESSGGSDGGHGISFGGDH GHISGGSSGGHSYGFIGGTDKGSSGGGHGHSFGSIGGGHSHGSGGGGGHGISFESFGGGHGHS SEGHGSGGHSSGGGGHEISFGSFGGGHGHGSGGSSGGHGHSSGGSGGGHGHS

>Aa-supercont1.368 MGLAIAASAAVDDSKKSKRDLWDDLGVADDISLEGLAERHEVEDFPEDLGEWSPSVPE PEVKHVPVPYPVEVEKHIPVEVKVPVPVEVEKKVPVIVEKKVPVIVEKRVPVHIDRPVPY RVEVKVPVVHKEYVEVPRPFPVHVEKRIPVFLDRPVIVEKTIPLTIHIQEKKKKKTGFPLW G

MEFRESMKIHPDVSTPRFDSIQFNFVPRIHELPQYITLTRQTEFRNMGFKSRHCHNLFLNT CSPSSKATLPNFEKLTFSRLVDHTETYRPSCNSSVAWPKNVLTAGILLVCKSLRLFRSNE KHVGHLLSRRRTLVFIILSVAISLVVAAPSVDPEDQGKSVEKRGLHDSHYALDDHHHLV DKKVIITKKVPVPYPVEVEKHVKVPVPYPVPVEKKVPVYVEKSVPVYIEKKVHVPVDRP VPVPYKVPVEVPVVHKEYVEVPKPYPVHIEKPYPVYVKKPVYIEKPVPVSIKIKHHKH

>Aa-supercont1.1097

>Aa-gi|157126500 MRFDLVSLQAFVVLSMALAIASCAAVDDSSKKDKRGLWEEGHGSFDFDDHHVDEHEIK HVTITKKVPVPYPVEVEKHVPVEVKVPYPVEVEKKVPVYVEKKVPVYVEKKVPVHVDR PVPYPVEVKVPVVQKEYVEVPKPYAVHVEKHVPVYIQKPVYVEKPVPVTVIVKKHEKK GWGLF

SGGGHEPIFASSNGNHKESSGGSSGGGEKGSSGGSGGGHGKSSGTVDVVHEHGSGGLSG GYGSVGGGDGKSSGGDGGHGKSSGGGGGGGGGSSGSDKIPQPETKITIHKTVPAPYPVQIQ KDVHVPYKVPYPVKVEKTIPYVVEKTIPFVVEKTIPYKVDNPVPYPVEVKVPVVHKEYI KVPKPYPVHVEKIVPEYIEKPVYITKHVPVTYHIKEKKKASSKWF >Aa-supercont1.842-1

MKAFVVLSMALAIASSAAVDDSAKKEKRGLWELGYGHDNLNLDHHYNNHHLDFDHH KEVKHISTTITKNVPVPYPVEVEKHVPVEVKVPYPVHVEKKVPVYVEKQVPVIVEKKVQ YHVDRPVPYPVEVKVPVVHKEYVEVPKPYAVHVEKPVPVVVQKPVYVEKHVPVTVHV KEHHHKNKHWGLF

> Aa-supercont1.842-2

MKAFVVLSMALAIASSAAVDDSAKKEKRGLWELGYGHDNLNLDHHYNNHHLDFDHH KEVKHISTTITKNVPVPYPVEVEKHVPVEVKVPYPVHVEKKVPVYVEKQVPVVVEKKV PYHVDRPVPYPVEVKVPVVHKEYVEVPKPYAVHVEKPVPVVVQKPVYVEKHVPVTVH VKEHHHKNKHWGLF

> Aa-supercont1.842-3

XTLFQNRRVRTKRVFPGKRSHGFRVWVADIRVLLACCHLVCACTEGLFTRSDATVTVV KAFVVLSMALAIASSAAVDDSAKKEKRGLWELGYGHDNLNLDQNYNNHHLDFDHHKE VKHISTTITKNVPVPYPVEVEKHVPVEVKVPYPVHVEKKVPVYVEKQVPVVVEKKVPY HVDRPVPYPVEVKVPVVHKEYVEVPKPYAVHVEKPVPVVVHKPVYVEKHVPVTVHVK EHHHKNKHWGLF

> Aa-gi|157127909

MKVFIVLSVAIALVIGAPADDSNNAENGKKDKRGLVEIGHNFGYEEPLLVDPLHTTHVA KHVTVTKNVPVPYPVEVEKHVPVAVKVPYPVHYEKHVPVVVEKKVPVYVEKKVHVPV

DRPVPYPVKVKVPVVHKEYVEVAKPYPVHVEKPYPVYVKEPVYYEQAPVYVKDHYYQ HKKPYYG

>AAEL012828

MKAFVVFCVALAVTSSSAVDESSKKDKRGVWDLGYGHHDSYAYDSHHHDIHHYDSSQ WTPIKHYSDDHHHAVEIEKKVLVEKHVPVVVEKKIPVHIDRPVPYPVEVKVPVVHKEYV EVPKPYAVHVEKHIPVYIHKPVYVEKPVPVSIYIKQKKKHWGWF

>Aa-supercont1.366-1

RVVDDKRVTVKGSLTRTFCPPNSVPEWLHASAECISEGGLKIQHFCDDISVRSRAFVVFA MAIAIVASAAVEKKEAAAEGEKKQDKRGLWDLGYGYEPHGWDHSSLKLSHGWEDPH HITTVIKKEHIAVPVEVEKHVPYPVKVPYPVTVEKHVPVVVEKKVPVYVEKPVAVPVKV PYKVKEYVEVPKPYPVHVEKPYPVYVKKPVYVEKHVPIVVKSHGWEPHHSHSYSEVHS WH

>Aa-supercont1.292-1

XPWRATDQYPVSFRTLVFPVHRSPNQTSTSITMKTLVLLGLATLLVLSSAAEDKKAEEV KPVEQAEKKQEKRGLFDHDFGGHDFHEYEHHDHHDHHIDFHPHHEKTLTVIKKVPVPY PVEKHIPVPVEKHIPVPVKVGVPKPYPVYKTVHYPVKEIVKVPYHVPAPYPVEKKVPYP VHVPVDRPVPVKVYVPAPYPVEKKVPVPVKVHVPAPYPVEKKVLVPVKVPVHVEKPYP VEKVVHYPVHVPVDRPVPVHVAKPVPVPVEKPVPYPVYKKVPYPVHVPVDRPVPVHVE KPVPVVKVPVPQPYPVVKHIPYPVEKHVPYPVKVPVERPVPYTVEKHIPVEVEKPVPYP VKVPVHVPVHHEEHHHEEYHHFDDHHDFHHEH >Aa-supercont1.63

GVVCLFALVLLASAEDEKKPVAEAKVDDTGAAESANLEDKKQDKRGLHGWTSFGGFE EKHDVPHFETHEEKTLTVVKKVPVPYPVEKHIPVAVEKHVPVPVKVGVPKPYPVYKTV HYPVKEIVKVPYHVPAPYPVEKKVPYPVHVPVDNPVPVKVYVPAPYPVEKKVPVPVKV HVPAPYPVEKKVLVPVKVPVHVDKPYPVEKVVHYPVHVPVDRPVPVHVEKPVPVPVEK PVPYTVIKKVPYPVHVPVDRPVPVHVEKPVPVPVKVPVPQPYPVEKHIPYPVEKHVPYP VKVPVERPVPVEIEKHVPVTVEKPVPYPVKVPVHVPVHHEEHHHEEQHSFGSSDGHHEF HEHH

>Aa-supercont1.292-2

CSVSRCRAVRSIVNRNLTAAKDTAAKQPFHLFAVSLGRERKTVARFFQPQRVCVKRRIC LSALLLVSAKAESDQKKSDANAVPLEKKLDKRGLLNLGYGYGINGLDVGYIGGHGHLG GAYHAAPVHGYGHGHGFLLGGHTDVTKTVTLLKGVPVPYTVEKHVPYTVEKHVPYPV KVPIPQPYEVVKHVPVHYKEYVKVPVHVPAPYPVEKKVPYPVHVPVDRPYPVKVLVPQ PYTVEKHVPVPVKVPVPQPYEVTKHVPVPVKVEVPVPVPYTVEKKVPVEVKVPVDRPY PVHVPAPYPVPVEKPVPYTVEKPVPVPVQVPVDRPYPVPVEKPVPYPVKVPVPQPYYVE KHQQSTFSFTRLLGYIKTHRENKRATREGKQLTTARAFLTHCPGASVNVERSKRTYRTR HFFTLLDRCATCLILPSSPAPAGASVKVEGSNTTRFFRCYKISLYFIHYTKNLTKYDGLLR ITEKSNPKVFFDVYLRFDIQRLPMTRVGKNLEIHSVDVINYWHCRILVVVEIYVKDKING RIERYKIPRSAFDLRCLIRPLHRREASASPSQGALRSPLPSPRREAHPGREARPSSGEGPGG HPVSPPRTNPPSSPRAPAAPLRHQLHELQRLRTRLQLPPLSTF >Aa-supercont1.366-2

Anopheles gambiae (Order Diptera)

>AgCPLCP8

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>AgCPLCP12

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KPYYVEKHIPYTVEKPVPVPVKVPIDRPYPVTVEKHIPVEKPVPVPVKVPVAVPVPVHHD HHHHHLEHLHEPHHHHHHDVSYTSFSGYGQDYSYHH

>AgCPLCP1

MKIFIVLAVALAVAAGAAVEGGKAKRGVWDFGSGHESSFSSGGDFGGHDFGGLHKEEE HVKHVTIVKKVPVPYPVEVTKHVPVEVKVPYPVEVEKKVPVYVEKKVPVVVEKKVHV DRPVPYPVKVPVKVPVIHKEYVEVPKPYPVHVEKPVPVYIKKPVYIEKTVPVSIHIKKKS HWH

>AgCPLCP3

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>AgCPLCP5

GRAMFDGNCTLLPSQLPPSPEDLQEEIADSLHDDDDDDVRHALRTLFPQIRYHRAGWQH RDGSSHLFR

> AgCPLCP11

MKSLVLLGLATLLVLSSAAEEKQQSAAPEAKPAAEGEKRHDKRGLFEHDFGGHDFSGH HFESYAHGHHYDLHPHHEKTLTVVKKVPVPYPVEKHIPVPVEKHVPVPVKVGVPKPYP VYKTVHYPVKEIVKVPVHVPAPYPVEKKVPYPVHVPYDRPVPVKVYVPAPYPVEKKVH

VPVKVHVPAPYPVEKKVPYPVKVPVHVEKPYPVEKIVHYPVHVPVDRPVPVHVEKPVP VPVEKPVPYEVIKKVPYPVHVPYDRPVPVHVEKPVPVPVKVPVPQPYPVYKHIPVPVEK HVPYPVKVPVERPVPYTIEKHIPYEVEKPVPYPVKVPVHVPVHHHHEHEHVEYEHHHH

> AgCPLCP27

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> AgCPLCP28

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Anopheles sinensis (Order Diptera)

>AsCPLCP1

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MKAFIVFFVALAITSVLANDEPKKEKRGLYELGQESYESYGYESQLNNGYYGNDHVQK QVKQVITKKVPVPYPVEVEKHVPVEVKVPYPVEVEKKVPVYVEKKVPVYVEKKVPVH VDRPYPVEVKVPVHVPVYKKEYVEVPKPYAVHVDKPYPVYVKEPVYVEKQVPVTVHI KEHHKKPFWG

>AsCPLCP3

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>AsCPLCP4

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>AsCPLCP5

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MQHTASCPHKRDGRVGKRERERERDTASRTXXXXXKEKRGLFELGSSQQESFETYG YETQHNQGYYGNDYVQKEVKQVITKKVPVPYPVEVEKHVPVEVKVPYPVEVEKKVPV YVEKKVPVYVEKKVPVHVDRPYPVEVKVPVHVPVYKKEYVEVPKPYAVHVDKPYPVY VKQPVYVEKQVPVTVHIKEHHKKPFWG

>AsCPLCP7

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>AsCPLCP8

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>AsCPLCP9

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32

MRKCVSWRDRDNKTQEFSSEFLSVPITDNGHYKCIAAQTEKHHSITIVRSNSKQTVDAR TLNFGKANMKAFIVFTVALAIACAADVDESKKEKRGLWEESYDNYGYDNYGLGYEKE VKHVITKKVPVPYPVEVEKHVAVPVKVPYPVKVEKEVPYVVEKKVPVYVEKKVPVHV DRPYPVEVKVPVHVPVYKKEYVEVPKPYAVHVEKPYPVYVKQPVYVEKQVPVTVQIK EHHKKPFWG

>AsCPLCP11

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>AsCPLCP12

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>AsCPLCP13

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KKVPVYVEKKVPVHVDRPYPVEVKVPVEYTVYQKEYVEVPKPYAVHVDKPYPVYVKE PVYVEKPVPFTVLLKKEHKKPFWG

>AsCPLCP14

MKAFIVIFVALAIANAAPADEKTKEKRGLYEVESPQKESYESYGYENQHSHGYYGNENF ENHGFYGNDHVQKEVKQVITEKVPVPYPVEVKKHVPVEVKVPYTVEVEKKVPVYVEK KVPVYVEKKVPVHVDRPYPVEVKVPVEYTVYQKEYVEVPKPYAVHVDKPYPVYVKEP VYVEKPVPFTVLLKKEHKKPFWG

>AsCPLCP15

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>AsCPLCP16

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>AsCPLCP17

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>AsCPLCP18

>AsCPLCP19

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35

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>AsCPLCP20

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>AsCPLCP21

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>AsCPLCP22

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MFGEYDLVHSLGLVRGGGGGGERSAQLYLVTFIALSVLVAVVAGAAIESGEEKTVEKRG LAGQLQDRSDHHHEHHEHVKYVTITKKVPVPYPVEVEKHVPVPVKIPYPVHVEKKVPF VIEKKVPVYVEKKVPVHVDRPVPVEVKVPIEVPVVHKEYVEVPKPYPVHVEKPYPVYV KKPVYIEKPVPVSVHIKKVKKHH

>AsCPLCP24

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>AsCPLCP25

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MFVTSYAPCSYVGFRLQVVFFAVSGGVLGLLLLTVGSCHANPVPAKILGKYVLSYQPPG PRLELNPHGDPEADELDELSNGLESDEGDGEDSDSQPSANHYRYRPHYQDSVRQQYDE QEQDQDVDYVEDSAPASANHQHKYMTTYKTVTRHVGGPGHGHSSYSDSGSEEEEEDG TSASEEAHAYDAYRAYGGKGKKHQGHHTSYHPQLIQAYKILHGTPNRLPTSHEYHDED SSEEEDEDFRSGAAIGAAISPSGYGGYGSPQVIHTKGRAVPISQHVEIETPVPVPYVKKIH VPIPQEVRVKIPHPVLVPVPRPYPVHIPVSQPIAVPDIKEITVPIEKVVPYPVEKKIPVPIEKP VPYPIEKHVPVYLPQPIPVKVPIVKTIIHKVKQQTASGPPLPPGSGSFW

>AsCPLCP27

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Culex pipiens (Order Diptera)

>CpBDAT_01969

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>CpBDAT_02323

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>CpBDAT_05623

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>CpBDAT_08492

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>CpBDAT_08573

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>CpBDAT_10819

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>CpBDAT_10820

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>CpBDAT_10884

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>CpBDAT_11879

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41

>CpBDAT_11881

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>CpBDAT_11882

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>CpBDAT_11887

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42

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>CpBDAT_11942

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>CpBDAT_14316

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>CpBDAT_01043

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>CpBDAT_01049

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44

TKNVHVPYPVEVEKHVPYPVNVPYPVEVVKKVPVVVEKKVPVYVEKKVPVHVDRPVP YPVEVKVPVVQKEYVEVPKPYAVHVEKPVPVYVKKPVYIEKHVPVTVHIKEVHQKKH WGLF

>CpBDAT_14382

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>CpBDAT_14383

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>CpBDAT_15431

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>CpBDAT_18469

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Nasonia vitripennis (Order Hymenoptera)

>Nv-gi|156546318

MKFKICIVLALVALARCEEAAEKKTEKRGIGHAYGDYASHEHHEHVKTVEVVKKVPVP VEHIKHVPYEVIKHVPYEVKVPVPQPYTVEKHVPYPVKVFVKVPVHVPQPYTVEKKIPY EVKVPVDKPYEVKVHVPQPYEVVKHVPVEVKVPVPQPYTVEKHVPYPVKVKVPVPQP YEVIKHVPYEVKVPVDKPYPVPVPKPYPVTVEKPYPVTVEKHVPYEVKVPVDRPYPVEV QKPYPVPVKVPVPKPYEVIKHYPVEVQKPVPVVKVPVDRPYYVEKEVPYPVEKEVPYP VKVPYPVPVHVKEQHHHYEGGFEPSGHYGHH

>Nv-gi|156547895

MKSLMHVALIALVVSATWAEEAEKPAAQEAAAPEAKKQEKRGLLGLGYGYAYDGGY DIGAAAHGAPGLALAHGYGGGYDHGHVATHHEKVITVTKNVPVPYPVEKHIPYPVEKH VPYPVKVPVPQPYPVEKEVPVPYKVYVKVPVHVPQPYPVEKRIPVPVHVPVDRPYPVKV YVPQPYPVEKHIPYPVKVPVPQPYPVEKQVPYPVKVPVHVPQPYPVEKIVQVPVKVPVD RPYPVPVPKPYPVEVEKPVPYPVEKHIPYPVKVPVDRPYPVHVDRPVPVVKVPVPHPYP VEKQVPYPVERPVPYPVKVPVDRPYPVHVDRPVPVAVEKPVPYPVKVPVGVPVVHKQH YGHEAYNNYNNYNDYGQYGH

46

>Nv-gi|156548756

MRFSTAIILLSLLALGLCEEAKKEASATAQQESKADESKVEKRQEKRGLWGQEGDFGHS WGGSHGFGAGSHSWGGGGGGHEELGASEHYEHHGHQQEHGHGHEHEHAKIKQITIEK TVKVPYPVEKEVPYPVEKKVPYPVKVHVPHPYPVEKKIPVPVKVPVHIPAPYPVE KKVYYPVHVPVERPVPHKVYVPAPYPVEKKVHYPVKVPVPQPYPVVKHIPYPVKVPVH VAHPYPVIKKVPVAVKVPVEKPVPYPVEKKVPYPVEKKVPYPVEKLVHYPVKVHVDKP RPYPVEKHVPYPVKVPVPAPYPVEKKVPYTVEKEVPYPVKVPVDNPIKIEVEKKVPYTV HKPVPYPVKVPYPVHIHHQEEQHGQEEQHGQEEYQHGQEEYQHGAGDSGHGGWEAES SYGSGGDWEGDRR

Apis mellifera (Order Hymenoptera)

>Am-gi|66541422

MDCDQIVVDHHAIRGGLAQFPVRYPGTGLNTFLQMQNETYKYNTDDELAIVLVALATQ VSCTEKSKAAEESVSPKDSVSESTEKKTEKRGLHGSFGDLGGGGGDGDLGGGGGGYDHH EEVKAVTVVKKVPVPYEVTKHVPYLVEKHVPYEVKVGVPQPYTVEKHVPYPVKVFVK VPVHVPQPYTVEKKVPYEVKVPVDKPYEVKVLVPQPYTVEKHIPVPVKVPVPQPYTVE KHVPFPVKVKVPVPQPYPVEKPVPYEVKVPVDKPYPVTVPKPYPVTVEKPYPVPVDKPV PYEVKVPVDKPYPVPVEKPYPVPVKVPVPQPYPVQKPVPVPVPKPVPYPVKVPVDKPYI VEKEVPVPVEKEVPVPVKVPVPIHEGHGGGGYGGGGYEGISGYGDGGGYHHR

>Am-gi|110759640

MAAVPSLAGELELSGHGLGDHGLTLGEIAVGHEDGDIGLEEIGGHEIGEYGGHGGHYVP IVKSIGVPVPKKVPVLIPKLEVESVPQNYPVPVIVPKPVPYQVEKQVFKKVEKKVPTPIEK IIPVKIEKPVPFHVVKHVPVPVVKPIPIKIPIYKTVIAFLALALASGIVSAGYIDEDHGSSTY EEKTKPVEIPIYKKYAIPIPHPVPVEIPQKIEIPIPQPQKVPVEIPHPYPVEVVKHVEVPIEKP EPVIVEKHVPFVVEKPYPVYVEKKFPIPVAKPYPVHVPVYKHVFHYTSKGKGWH

Microplitis mediator (Order Hymenoptera)

>MmCPLCP7

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>MmCPLCP6

MKTIVLAAFVAITSAFEKESYETESKEPVINKFDLPISYPVFVRVPEHIGVPIPQPYPVQLAI PHPVPFEIVKHVEIAVEKPEPVVVEKQVPFIVEKPYAVTIEKRFPVTIPKPYPVHVPVYKY VFHHQFKSKGRGH

>MmCPLCP5

MGHVIHVTLTLIIYCVAVVVSATIRYNYKKPILDCGYLRITPEEKYSWERPNFYVNYRTI DPDDIRDPSIGVLKKEDSKNYISKKQSMDLSVLTDRMSEDTDSTGTLSDQPLIRPTDNIAQ SSDSEPRFYDEDQKFTEEYSEHEKVITVVKNVAVPYPVEKQIPYPVIKKVPYPIHTPVAQP YPVEKEIPYPVKVVIKVPTKVPYPVPVYKEVPYAVHVPVERPVPYRTYIPDPYPVEKKVY YESKIPVPEPYPIEKTIAVPVKIPVSVPQPFPVEKPVHYPVEVKVDRPVAVPVEKPYPVTV FKHVPYPVFKPKPVPVEVKVERPVPYPVVKHVPYPVKVEVPQYYPVEKEVPYPVVNHR PYPVPVSVERPVPVPVEKPVPVPVEHEQVEHEPEHGYDDVVKSEIYSGYKHESCCGDEF RPSAIDTHETEYKYGDRNDWDHDKLIHSDEVVANAGVKAETQDSDVRPVVEDSKEDDT NSAEVRNNVAANASADGAKEATDLKEVNSEGVVVKNDNETINEIDNNNNI

>MmCPLCP4

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>MmCPLCP3

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>MmCPLCP2

MIFIVWIGTLALITIARASDYHESHTYEEKIKPVEVPVYKKYAIPIPHPVAVKVPQEIRIPIP QPYHVPIQIPQPYPVEVVKHVDVPVEKHEPYVVEKKVPFVVEKPYPVYVDKKYPITVNK PYPVYVPIYKHVFHHKH

>MmCPLCP1

MKMKMQLALVAVLAVCQLAQAESKDNKSIQESSADSQTEKKTEKRGLHHSYGDFGGY GGSYGGSWDHPHEEHVKTVTIEKKIPVPYEVTKHVPYLVEKPVPYEVKVGVPQPYTVE KHVPYPVKVFVKVPVHVPQPYTVEKKIPYEVKVPVDKPYEVKVYVPQPYTVEKHVPVH VKVPVPQPYTVEKHVPYPVKVKIPVPAPYPVEKPVPYEVKVPVDRPYPVHVPKPYPVTV EKPYPVPVDKPVPEVKVPVDKPYPVPVEKPYPVPYKVHVPQPYTVHKHVPVPVEKPV PYPVKVPVDKPYFVEKHVPVPVEKEVPVPVKVPVPVPVHVHHQHHTHHDHHTHHEHT HSHSHEGGFEPSSGYGGDEGGSYGEHSYH

Tribolium castaneum (Order Coleoptera)

>Tc-gi|91087633

MKALIVLSAVCLCVALAAEPEKKEATQETKKQDKRGLIGLGDHGGFGGFEGHGGFEGG IGGGGGGEHEHHHHEHVKTIVVEKKVPVPYTVTKHVPYTVEKKVPYEVKVPVPQPYTV EKKIPVPVKEYIKVPYIVPQPYEVIKKIPYEVKVPVPRPYEVKVPVPQPYTVEKKIPVPVK VPVPQPYTVEKKVPYPVKVEIPVPQPYTVEKKVPYEVKVPVDRPYPVHVPKPYPVTVEK HIPYPVEKPVPYEVKVPVDKPYPVEVPKPVPYHVKVPVPQPYTVEKKVPVEIEKPVPVPV KVPVDKPYPVYKEIPYPVEKPVPYPVKIPVHIPVKVHHHHHEESGGGFEGSFGGGYH

>Tc-gi|91093827

MKFLGYSALCALLVLASADNSKDTADQPDKKQEKRGLELGYGGGGGGGGGGGGGGGGEHEHH HHVKTVTVVKNVPEPYPVVKEIPVPVEKKVPYPITVHVPQPYPVEKKIAVPVKVPVKVP VPIHKPYPVEKPVHVPVKVHVDKPYPVKVLVPQPYPVEKTIHVPYHVHVPQPYPVEKKI PYPVKIPVHVPKPYPVEKIVPYPVEVKVDRPYPVHIPQPYPVHIEKKIPYPVHKPVPYPVK VPVERHIPYPVEKPEPVPVKVPYPVPYPVERNVPVPVEKPVPYPVKIPVFNPVPFEVHKK VPVEVEKPVPVPIKVPVPVPVHEEHHETGGGGQEFGQGFEGHHGY

>Tc-gi|91093831

MKPLLLLAILVLAVTAEEKIKEEKKQTKRGLLSLGYGYGSDIDHGYLVGAGGHGLGLSG LSGLSYGYGPIDLGHSTAIEKTITLVKGVPVPYPVEKHIPYPVEKHIPYPVKVPVPQPYPV EKHVPYPVKVPVKVPVPQPYPVEKIVHVPVKVPVDRPYPVKVLVPQPYPVEKHVPYP VKVPVPQPYPVEKHVPYPVKVPVHVPQPYPVVKHVPYPVKVPVDRPYPVHVPQPYPVH IEKKIAVPVEKLVPYPVKVPVDRPVAVPVPKPYPVEVKVPVPAPYPVEKPVPYPVEKPVP YPVKIAVERPVPYPVVKPVAYEVERPVPVVKVPVAVPVHGHYHHHVDHY

>Tc-gi|91076758

MKIFVCFVLVATIALCAAENKDKTEKSAKKEDKPKRHIFGTGYNNYDFSGFDTVGSLGG VTYGSGLGSVYSTGSGYGSGLASVYSHGSGYGSGLGSAYSYGSGYGSGYGSGYGSGYG YGSGYGYGTGYGSGLAAGVVTTRASTGLRAGSGYAAIEGLPKVSNVRSHEIHTVTQHV PVAVPQPYPVHITKTVPVPKPYPVAVEKPVPVPYKVNVPVEVPKPYPVKVPQPVAVPYE VKVPVEVPKPYPVHITKTVNVPVEKPVYVKVAHPVPVKVREPVPVAVPHPVPVKVPTP VVVKVPEVVGVNTVTHVDSGAGHIIGNDFGSEIVHGAGYGYGAGYGYGSGYGTGAEIIS

51

GSGFGSGAVLSTGSAGFGTGAVLSTGSAGFGTGAVLSSGYETSGLAGEAISSGLSSGAEII AAKSAHIVSGSNHGLVGSYVPSVYGGISGHTVHNTHWKHL

Nilaparvata lugens (Order Hemiptera)

>NlugCPLCP1

MKLVLVFLSVLGLIFVEGTNIGGLKSDEKGGENDKNSGLKLDIVGGTNSEVEKSEKTVG GDDKTQFKRGLVDEGQYGTSFHGQYGGKTVVGDDKTQFKRGLFEDGLYGGGFHQQSG GFGGYAVGEQVSEHKTISVTKEIPVPYPVTVEKKIPYPVDRPYTVHIPKPYTVHVEKPIPY PVKVPVPQPYTVLKPVPYTVHVPVDRPYTVHVPQPYKVYVDKPYPVEVIKKYAVPVEY PVDRPYTVPVPVDKPVPYTVEKPVAYPVKVPVDRPFTVDVPQPYPVEVEKKVPEPYQVP IKVDDGHVGKHH

>NlugCPLCP2

MKTKLFVLALCAVLALARAEEEKKEAAESAEKKVEVDAGEEKKQEKRGLFSFGYGGG YGGGYGGGYGGGYEGYESYGGEHHYEPHSHIKSIVINKEVAVPYPVHIEKKVPY PVKVPVDRPYPVHVPKPYPVHVEKPVPYPVKVHVPQPYPVHVEKHVPYPVKVHVDRPY PVPVPKPYPVYVEKKVPYPVEKHIPYPVKVPVDRPYPVHVPVEKPVPYPVEKHIPYPVK VPVDRPYPVPVPKPYPVPVEKPVPYPVEKPVPYPVKVPVKVPVKVPYPVEVKVPVHVPV HVPVKEHHHHEDYSSYEGASYEGSYEGSYGHSYH

>NlugCPLCP3

MRLLVSLVLCAGFTLGEEPVKKTDNELTGNQLNNVDNDSKKQGKRELYDLGNYGHGF GYDSFGLDHHFGGFDHGFGHHFPHHEVHKTVTLTKEVPVPYPVHIEKKIPVPVDRPYPV HVPKPYPVHVEKHVPVPVKVPVPAPYPVEKPVPYPVKVPVDRPYPVHVPKPYPVYVDK PYPVPVEKHVPVPVKVPVDRPYPVHVPVEKPVPYPVEKPVPYPVKVPVDRPYPVHVPKP YPVHVEKQVAVPYPVPYAVKEHHGHHGHGFFDHGFALGEHL

>NlugCPLCP4

MYYQITLCLLLATASFVRSAPADADESSPKQKRGIYGFGYGLDLSHGWDLGYGHGFGL DHGLSLDHGHVKTVTVTKEVAVPYPVHVPYEKHVPYPVKVPVAVPVDRPVPVAVPKP YPVAVEKPVLYPVDKLVPYPVKVPVKVPVAHPVPVPYPKPFPVPVEKPVAVPVPHPVLI EKPVPFAVKHVEHFEPHEALGYHHHF

>NlugCPLCP5

MKSKYCLAVFVALLALSEAAEQKTENADQPDKKQEKRGLHGSFGGDFGGGDIGGHSG GYDFGGSDFSSSSFDGGHYGGGFDQHHDEHHVKTVTVTKQVPVPYPVHVEKKVPYPV KVPVDRPYPVHVPKPYPVTVEKPVPVPVKVHVPQPYPVTVEKKIPYPVKVPVDRPYPVH VPKPYPVIVEKKVPYTVEKHIPYPVKVPVDKPYPVPYTVEKPYPVTVEKKVPYPVKVPV DRPYPVPVPKPYPVTVEKHVPYTVEKPVPYPVKVPIKVPVKVPYPVEVKVPVHVPIHVP VHHHDHHESHHGSSYSYHH

>NlugCPLCP6

MASVVFGAEDVTTTTTEQSSTDDPAKKQEKRGLSEYADFGTDFGGEHLSLGEHQEGIGE DEHYTPEHYPRSYPHHHEHVKTITVEKKVPVPYKVEVEKKVPVPVKVPVDRPYTVFVP KPYPVTVEKPVPYPVKVPYPQPYHVEKKVPVPVHVHVDRPYPVHVIKPYPVYVTKKVP VPVEKHIPVPYKVHVDRPVPVRVPVTKHVPYPVEKHIPVPYKVHVDRPVPVHVPKPYPV HVEKKVPVPVEKVVPYPVKVPVKVPYEVPVPYKVYKPYPVHVPVHEKEHHYDHQQQY HQL

Triatoma infestans (Order Hemiptera)

>TiCPLCP

KKQEKRQLQDFGYDGREQSNYGGIGVEGGAVGTTQIQDHGNIKAITITKKVPVPYEVTIE KKVPYKVEVPVDRPYPVYVPKPYPVFIEKKIPYEVKVPVPQPYTVEKRIPVAVKVPVDK PYPVHIPKPFPVYIEKKFHIPYLKKYHFPLKYQWINHIQFVYLWRNRYHIQ

Frankliniella occidentalis (Order Thysanoptera)

>FoccCPLCP1

MKFLAITIMCLALAAAESGKKATEGKKSEKRGIHDLGYGYGGSELSLGLGHTYSSGYGD YLSGHEYGHHEPITKVITKHVPYSIPQPVPVEITKHVPYIVKVPVHVPVDRPYEVAVPKP YTVHVDRPYPVTVEKPYPVHIKVPVKVAVPHPVPYDVPKPYPVHVEVPKPYKVPVEVP VYVKEHHHEHYPSLDLGYHGSSHYSSGHYDHY

> FoccCPLCP2

MKIAAVLLLGVTLVAGGGLQAPAPVYGPPAFVASPPSSPIAENIQYDSTGSSYGPPPPPPA LGGGYGGSGGFIASGGAGGFSGPACAGAGCAGTVQEYRSHHQVPTRTVTHHVTVPIPQ PYPVTVETRVPYEVKVPVHVPVDRPVEVPVPKPYHVHVDRPVPVHVEKRVPYPVHVKV PVKIPVHVPVDVPKPYPVKVWVPKPYPVPVVQYIHKKYIHKTYKVKSHDDEGWW