Genome-wide identification and molecular evolution of elongation family of very long chain fatty acids proteins in *Cyrtotrachelus buqueti*

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Abstract

To reveal the molecular function of elongation family of very long chain fatty acids(ELO) protein in *Cyrtotrachelus buqueti*, we have identified 15 ELO proteins from *C.buqueti* genome. 15 CbuELO proteins were located on four chromosomes. Their isoelectric points ranged from 9.22 to 9.68, and they were alkaline. These CbuELO proteins were stable and hydrophobic. CbuELO proteins had transmembrane movement, and had multiple phosphorylation sites. The secondary structure of CbuELO proteins was mainly α-helix. A total of 10 conserved motifs were identified in CbuELO protein family. Phylogenetic analysis showed that molecular evolutionary relationships of ELO protein family between *C. buqueti* and *Tribolium castaneum* was the closest. Developmental transcriptome analysis indicated that *CbuELO10*, *CbuELO13* and *CbuELO02* genes were key enzyme genes that determine the synthesis of very long chain fatty acids in pupae and eggs, *CbuELO6* and *CbuELO7* were that in the male, and *CbuELO8* and *CbuELO11* were that in the larva. Transcriptome analysis under different temperature conditions indicated that *CbuELO1*, *CbuELO5*, *CbuELO12* and *CbuELO14* participated in regulating temperature stress responses. Transcriptome analysis at different feeding times showed *CbuELO12* gene expression level in all feeding time periods was significant downregulation. The qRT-PCR experiment verified expression level changes of *CbuELO* gene family under different temperature and feeding time conditions. Protein-protein interaction analysis showed that 9 CbuELO proteins were related to each other, CbuELO1, CbuELO4 and CbuELO12 had more than one interaction relationship. These results lay a theoretical foundation for further studying its molecular function during growth and development of *C. buqueti*.

Keywords *Cyrtotrachelus buqueti*, Elongation family of very long chain fatty acids proteins, Genome-wide identification, Molecular evolution, Evolutionary analysis

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Introduction

Cyrtotrachelus buqueti belongs to Coleoptera. This insect is mainly distributed in the southwest of China, coastal areas of Guangdong and Shanghai, and other countries in South east Asia, such as Vietnam and Thailand [1–3]. The insect is completely metamorphosed and needs to go through four stages: adult, egg, larva and pupa [4]. Adults supplement nutrition by feeding on bamboo shoots. The eggs of the insect are laid under the shells of the bamboo shoots. After hatching, the larvae feed on the bamboo shoots and bite through the bamboo shoots. They pupate in the soil and overwinter as adults. It only takes 15 days, and *C. buqueti* is an oligophagous insect, mainly feeding on the tips of young bamboo shoots that grow in clusters [5–8]. It is one of the main pests of bamboo forests, and it was listed as one of the main pests in my country's forestry by the State Forestry Administration in 2003 [9]. At present, the research on *C. buqueti* focuses on the digestive system structure studied by Luo et al [10], the respiratory system structure studied by Deng et al [11], the reproductive system structure studied by Li et al [12], the habits studied by Díaz-García et al [13], the morphological structure studied by Li et al [14], and the control methods studied by Yang et al [15], but there is a lack of genome-wide identification of elongation family of very long chain fatty acids protein of *C. buqueti.*

Elongation family of very long chain fatty acids protein is called FAE in plants [16], in insects and fungal yeast, called ELO [17], and in fish and mammals, named ELOVL [18], it is the rate-limiting condensing enzyme in the first step of the fatty acid extension reaction [17]. The condensation and extension process of the carbon chain during the synthesis of very long-chain fatty acids (VLCFA) is a cyclic process similar to the β-oxidative reverse reaction. The four-step cycle of condensation, hydrogenation, dehydration, and rehydrogenation completes the extension of carbon chain, among which the extension of fatty acid carbon chain mainly occurs in mitochondria, endoplasmic reticulum, and peroxisome [19]. One of the condensation reactions is catalyzed by ELO, where malonic acid monoacyl CoA provides two carbon atoms to extend the fatty acid carbon chain, and the distance between the active site and the lysine residue determines the length of the ELO fatty acid [20]. This shows that *ELO* plays a crucial role in the regulation of fatty acid metabolism and the biosynthesis of lipids. Among insect field studies, Chertemps et al. reported the *ELO* gene of insects for the first time and named it *elo68α* and an *ELO* specifically expressed in female Drosophila, named *eloF* [16–21]. Studies on *ELO* have focused on *Drosophila melanogaster*, which mainly include effects in *ELO* on fertility during the formation of fertilized eggs [22, 23], on epidermal functions [24], on locomotor functions, reducing their viability [25], on oenocyte [26], and

on the synthesis of pheromones such as esters, hydrocarbons and alcohols in pheromone synthesis. It was found that *ELO* also had an effect on epidermal transcriptional function in *Apis mellifera* [27]. Juárez studied the function of ELO in the epidermis of *Blatella germanica* [28]. It has also been slightly reported in *Tenebrio molitor*, among which *ELO* can interfere with the genes of Tenebrio larvae, affecting the growth of *T. molitor* and resulting in an increase in *T. molitor* mortality [29].

At present, the research on *C. buqueti* mainly focuses on the morphological structure, living habits, and control methods. However, there are relatively few studies on the genetics of *C. buqueti*, mainly the karyotype analysis of Wang et al [30]. So far, apart from our research team completing the whole genome sequencing of *C. buqueti* and publishing its genome sequence, there is no research report on elongation family of very long chain fatty acids protein(CbuELO) in *C. buqueti* genome. This study aims to systematically identify and investigate the molecular mechanisms of *CbuELO* gene family in its fatty acid synthesis process and its involvement in temperature stress response and energy conversion processes. In this study, we have three objectives. Firstly, we have completed the identification of *ELO* gene family in the entire genome of *C. buqueti.* Secondly, we systematically analyze the genetic characteristics, protein characteristics, and molecular evolutionary relationships of *CbuELO* gene family. Thirdly, transcriptome sequencing was performed under different temperature and feeding time treatments to reveal the molecular mechanisms by which the *CbuELO* gene family regulates its temperature response and energy conversion. In this study, the physicochemical properties, subcellular localization, transmembrane domain structure, protein hydrophilicity and hydrophobicity, phosphorylation site situation, protein secondary structure, protein tertiary structure, conserved structure, chromosomal location and molecular relationship of CbuELO proteins were systematically analyzed by using bioinformatics software and websites. These research results will lay a certain theoretical foundation for further studying the biological functions of *C. buqueti.*

Materials and methods

Data acquisition and experimental design

All the whole genome sequences, protein sequences, gene annotation files, transcriptome sequencing data of adults and larvae of *C. buqueti* under different temperature conditions, and transcriptome sequencing and proteome sequencing data of *C. buqueti* at different feeding times were downloaded from these data (NCBI genome and transcriptome accession number: PRJNA675312, PRJNA719467 and PRJNA718062) submitted to NCBI database by Chun Fu et al [31]. The adults and larval samples of *C. buqueti* used in this study were collected

Fu *et al. BMC Genomics*   (2024) 25:758

Page 3 of 22

in Danan Town, Muchuan County, Leshan City, Sichuan Province, and the adults and larvae of *C. buqueti* were used for genome sequencing, transcriptome sequencing and proteome sequencing, respectively. Adults (female and male) and larvae of *C. buqueti* were treated at different temperature (25 ℃ in the control group, 4 ℃, 42 ℃ and 50 ℃ in the treatment group) and different feeding times (no feeding in the control group (0 h), 0.5 h after feeding fresh bamboo shoots, 1 h after feeding, and 2 h after feeding in the treatment group) were all carried out in the Molecular Biology Laboratory of Key Laboratory of Sichuan Province for Bamboo Pests Control and Resource Development of Leshan Normal University from July 15 to August 15, 2020. The *ELO* gene sequences and protein sequences of all insect species used in this study were downloaded from the InsectBase2.0 database (http://v2.insect-genome.com/) by searching for them and used for bioinformatics analysis [32] (Supplementary Table S1). The expression data of *ELO* gene family in *C. buqueti* genome comes from the expression data in the developmental transcriptome of *C. buqueti* studied by Yang et.al [33]. The total RNA was extracted from muscle tissue samples of *C. buqueti* under different temperature treatments and intestinal contents samples of *C. buqueti* under different time conditions after feeding. Reverse transcription of purified RNA into cDNA using a reverse transcription kit, and reverse transcribed cDNA was used for qRT-PCR to verify the expression of *CbuP450* genes in *C. buqueti* under different temperature treatments and different time conditions after feeding. The qRT-PCR experiments in this study were all completed on fluorescence quantitative PCR instrument (qToWer3 G) of the Analytick Jena AG. The number of replicates of biological samples in each treatment group and control group is 3, and the number of machine replicates on fluorescence quantitative PCR is 3.

Identification, chromosomal localization and gene structure of *ELO* gene family in *C. buqueti*

The Pfam model of *ELO* gene family was used to retrieve all the protein sequences of *ELO* gene family in *C. buqueti* genome, and then combined with SMART tool to retrieve the conserved structural domains of all the protein member sequences identified by Pfam searching, and the sequences without ELO conserved structural domains were removed to be the *ELO* gene family. Only by searching for Pfam model of *ELO* gene and searching for its conserved domain, can the gene sequence of both be preliminarily identified as a member of the *ELO* gene family. At the same time, the preliminarily identified *ELO* gene sequence needs to be verified in conjunction with *ELO* gene members in the genome of the model insect *Drosophila melanogaster*, and the qualified sequence is the true member of *CbuELO* gene family.

The chromosomes and gene start positions of *ELO* genes were obtained from the gff information of *C. buqueti*, and then the chromosomal localization analysis of *CbuELO* gene family was performed using MapChart software, and the maps were saved in PDF format. Gene Structure Display Server 2.0 (http://gsds.gao-lab.org), an online website, was used to analyze the exons and introns in the gene structure.

Analysis of physicochemical properties and subcellular localization of CbuELO proteins

The EXPASY-ProtParam tool (https://web.expasy.org/protparam/) was used to predict physicochemical properties of CbuELO proteins. The molecular weight size, number of amino acids, instability index, aliphatic amino acid index, isoelectric point, total average hydrophilicity, and total number of positively and negatively charged residues of the protein can be analyzed. Subcellular localization was performed by using CELLO: Subcellular (http://cello.life.nctu.edu.tw/).

Transmembrane domain, hydrophilic analysis and phosphorylation site prediction of CbuELO proteins

Transmembrane domain analysis of CbuELO proteins was performed by using TMHMM-2.0-Services (https://services.healthtech.dtu.dk/service.), and EXPASY-Proscale (https://web.expasy.org/protscale/) was used for hydrophilic analysis of CbuELO proteins [34]. Phosphorylation sites were predicted using NetPhos-3.1-Services (https://services.healthtech.dtu.dk/service.) with input in protein sequence fasta format, default parameters, and output saved in PDF format [35].

Analysis of secondary structure, tertiary structure, and conserved structure of CbuELO proteins

The secondary structure of CbuELO proteins was predicted by using SOPMA oneline tool (https://npsa-prabi.ibcp.fr/), the tertiary structure of CbuELO proteins was predicted by using SWISS-MODEL (https://swissmodel.expasy.org/interactive) and MEME-tool (https://meme-suite.org/) was used to analyze the conserved structure of CbuELO proteins, with the initial number of motifs set to 10 and the input protein sequence in fasta format, and finally saved in PDF format [36, 37].

Phylogenetic tree construction of CbuELO proteins

In order to understand evolutionary relationship between *CbuELO* genes and those of other species, the protein sequences of *ELO* genes in the same target species were downloaded from the online website InsectBase, and the evolutionary tree was constructed by MEGA11 software, whose protein sequences were in fasta format. The phylogenetic tree of ELO protein sequences of multiple species

Fu *et al. BMC Genomics*   (2024) 25:758

Page 4 of 22

was constructed using the maximum likelihood method (Maximum Likelihood, ML) [38–40].

SSR and codon bias analysis of *CbuELO* genes

SSR sites of *CbuELO* genes were identified by using MISA (https://webblast.ipk-gatersleben.de/misa/) and default parameters (SSR modes: 1–10, 2–6, 3–5, 4–5, 5–5, and 6−5; The maximum sequence length that can be registered as a composite SSR between two SSRs is 100 bp). CodonW software was used to analyze codon preference parameters of *CbuELO* genes, including the GC ratio of codon, the proportion of ATGC (A3s, T3s, G3s, T3s) on the third position of the codon, the GC (GC3S) ratio of the 3rd position of the synonymous codon, the codon bias index (CBI), the codon adaption index (CAI), The results of frequency of optimal codon (FOP), effective number of codon (ENC, NC) and relative synonymous codon usage (RSCU), which were analyzed by Origin and ggplot2 softwave.

Analysis of *ELO* gene expression in *C.buqueti*

The first experimental design was: a series of temperatures (25 °C, 4 °C, 42 °C, 50 °C) were set to treat the larvae, females and males of *C.buqueti* for 1 h, and set up 3 biological replicates respectively. The second experimental design was: both adults and larvae of *C.buqueti* were fed fresh bamboo shoots, while the control group was not fed (0 h), and the treatment group was fed at 0.5 h, 1 h, and 2 h after feeding, with three biological replicates set up. All samples from the treatment and control groups of two experiments were used for transcriptome sequencing and qRT-PCR analysis. Transcriptome sequencing data

and gene expression data validated by qRT-PCR experiment of all muscle tissue and intestinal contents samples of *C.buqueti* were plotted by OmicShare Tools (https://www.omicshare.com/tools/Home/Task). All *CbuELO* gene primers designed by TBtools Batch qRT-PCR primer design tool used in qRT-PCR validation experiment in this study are shown in Supplementary Table S2. The qRT-PCR primers used in this study were synthesized by Sangon Biotech (Shanghai) Co., Ltd on commission. The relative gene expression levels of *CbuELO* gene family obtained through qRT-PCR under different temperatures and feeding conditions were plotted in Excel and presented in a bar chart to visualize the relative gene expression levels.

### Protein-protein interaction analysis of CbuELO proteins

The transcription and translation of genes into proteins to exercise biological functions requires further research on whether the proteins encoded within individual gene family members will regulate or interact with each other. Moreover, studying protein-protein interactions within individual gene family members can help understand how protein members directly or indirectly interact with each other and what role they play in regulating gene expression. The ELO protein sequences of *C.buqueti* were imported into the string website (https://cn.string-db.org/), and the protein interaction diagram was obtained by selecting the reference species of *Tribolium castaneum* with the default parameters.

Results

Identification and chromosomal localization of *ELO* genes in *C. buqueti*

Through the identification of *ELO* genes in *C. buqueti*, it was known that there were 15 *ELO* genes in *C. buqueti*, which were named as *CbuELO01-CbuELO15* according to the gene order (Supplementary Table S3). Chromosomal localization analysis of *CbuELO* gene family by using MapChart software showed that *CbuELO* genes were located on chr0, chr5, chr8 and chr9, among which chr0 accounted for the most, with 11 *CbuELO* genes, whose genes were *CbuELO01-CbuELO11*, respectively. *CbuELO12* was located on chr5, *CbuELO13* and *CbuELO14* were located on chr8, and *CbuELO15* was located on Chr9, respectively (Fig. 1) [41].

The analysis rusults of *CbuELO* gene structure showed that the sequence length and coding length of each *CbuELO* gene were not identical to each other, with the sequence length of 15 *CbuELO* genes ranging from 1958 to 31756 bp, and the coding length ranging from 863 to 29913 bp. From Fig. 2, it can be seen that all *CbuELO* genes contained exons (CDS); all *CbuELO* genes had introns except *CbuELO12* which had no introns; all *CbuELO* genes had non-coding regions except
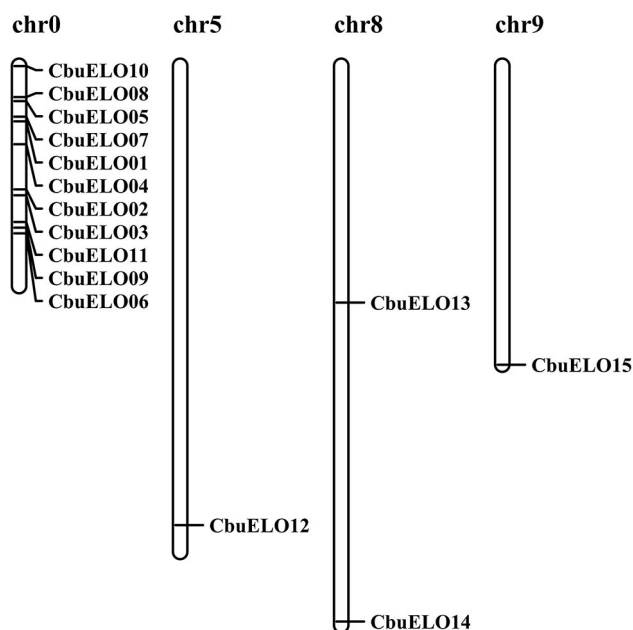
Fig. 1 Chromosome mapping of *ELO* gene family in *C. buqueti*
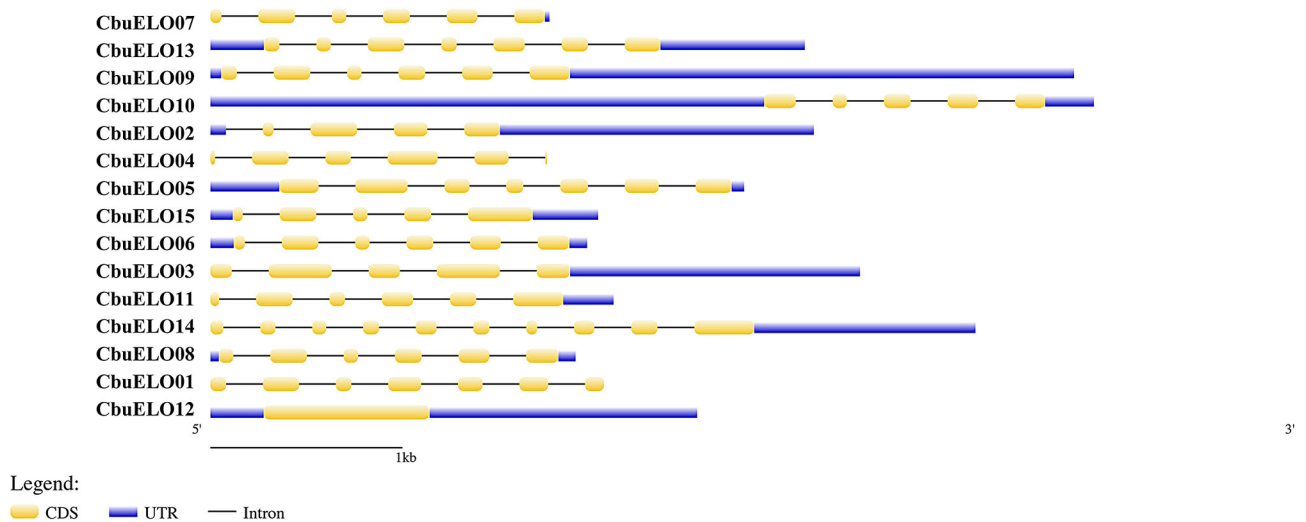
Fig. 2 Gene structure of *ELO* gene family in *C. buqueti*

CbuELO04 which had no non-coding region (UTR). Each *CbuELO* gene contained 1 to 10 exons, 0 to 9 introns and 0 to 2 non-coding regions. Among them, *CbuELO14* contained the largest number of exons and introns, 10 and 9, respectively (Fig. 2).

Physicochemical properties analysis of CbuELO proteins
Based on the amino acid sequence of CbuELO, the physicochemical properties (molecular weight, isoelectric point, instability, etc.) of CbuELO proteins were analyzed by using EXPASY-ProtParam tool. The results showed that the number of amino acids in CbuELO proteins ranged from 219 to 368 aa, CbuELO protein containing the highest number of amino acids was CbuELO03 with 368 aa. CbuELO protein containing the lowest number of amino acids was CbuELO02 with 219 aa, while the average amino acid content of CbuELO protein family was 285.4 aa. the molecular weights of CbuELO protein family ranged from 25.99 to 43.33 kD. The largest molecular weight was CbuELO03 with 43.33 kD. the smallest molecular weight is CbuELO with 25.99 kD. while the average molecular weight of the whole CbuELO protein family was 33.67 kD. the theoretical isoelectric point of CbuELO protein family was between 9.22 and 9.68. It can be concluded that the whole CbuELO protein family was basic, and this result was consistent with *ELO* gene family of *T.olitor* [29]. Based on the theoretical instability index greater than 40 were unstable proteins and less than 40 are stable proteins. From this, six of the unstable proteins were CbuELO03, CbuELO04, CbuELO06, CbuELO07, CbuELO10 and CbuELO12. The rest were all stable proteins, and overall the number of stable proteins was greater than that of unstable proteins. The overall content of aliphatic amino acid index ranged from 72.28 to 120.22, indicating that the thermal stability of CbuELO

protein family varied widely among them. Analyzed in terms of the total average hydrophilicity, negative values represent hydrophilicity, positive values represent hydrophobicity, and absolute values represent their hydrophilic size [42]. Except for CbuELO03 protein, which was hydrophilic, all other CbuELO proteins were hydrophobic, and the overall average coefficient of hydrophilicity of CbuELO protein family was 0.433, indicating that it was hydrophobic [43]. From the analysis of the number of positively and negatively charged residues, the total number of positively charged residues (Arg+Lys) of the overall CbuELO protein family was much larger than the total number of negatively charged residues (Asp+Glu), indicating that the overall CbuELO protein family was positively charged. The subcellular localization analysis results showed that CbuELO protein family was localized on plasma membrane, indicating that CbuELO protein family played a biological role on the cell membrane(Table 1).

Transmembrane domain, hydrophobicity, and phosphorylation site prediction of CbuELO proteins
The transmembrane domain analysis of CbuELO protein family showed that each member of CbuELO protein family had some number of transmembrane structures, with proteins CbuELO01, CbuELO003, CbuELO05 ~ CbuELO08, and CbuELO15 contained the largest number of transmembrane structures. Therefore, it can be inferred that CbuELO protein family was transmembrane proteins(Table 2; Fig. 3).

The hydrophilicity and hydrophobicity analysis results of CbuELO protein family showed that the maximum hydrophobicity of this protein family ranged from 2.311 to 3.378, and CbuELO05 had the maximum value of 3.378. While the maximum hydrophilic range was

Fu *et al. BMC Genomics* (2024) 25:758

Page 6 of 22

Table 1 Physicochemical properties of CbuELO proteins from *C. buqueti*

Protein	Amino acid number/aa	MW/kD	pI	Instability index	Aliphatic index	Hydropathicity (GEAVY)	Asp + Glu	Arg + Lys	Subcellular localization
CbuELO01	299	35.21	9.29	36.22	96.15	0.235	15	27	Plasma Membrane
CbuELO02	219	25.99	9.68	25.46	75.62	0.016	10	23	Plasma Membrane
CbuELO03	368	43.33	9.30	46.29	72.28	0.018	19	35	Plasma Membrane
CbuELO04	264	31.65	9.48	44.43	106.70	0.352	15	29	Plasma Membrane
CbuELO05	309	36.30	9.36	33.61	94.27	0.177	19	31	Plasma Membrane
CbuELO06	262	31.19	9.41	40.40	103.02	0.367	10	21	Plasma Membrane
CbuELO07	260	30.78	9.38	42.64	103.38	0.259	10	22	Plasma Membrane
CbuELO08	269	31.90	9.57	31.93	100.67	0.213	8	23	Plasma Membrane
CbuELO09	285	33.76	9.32	21.80	102.56	0.207	14	26	Plasma Membrane
CbuELO10	231	27.27	9.52	45.10	120.22	0.523	6	16	Plasma Membrane
CbuELO11	292	34.00	9.50	36.09	99.42	0.199	15	32	Plasma Membrane
CbuELO12	287	33.74	9.31	40.79	85.19	0.161	13	24	Plasma Membrane
CbuELO13	304	36.20	9.22	30.22	90.39	0.130	20	29	Plasma Membrane
CbuELO14	368	42.74	9.49	28.70	78.07	0.006	17	34	Plasma Membrane
CbuELO15	264	30.98	9.37	29.47	111.44	0.405	11	22	Plasma Membrane

Table 2 The transmembrane domain analysis of CbuELO proteins from *C. buqueti*

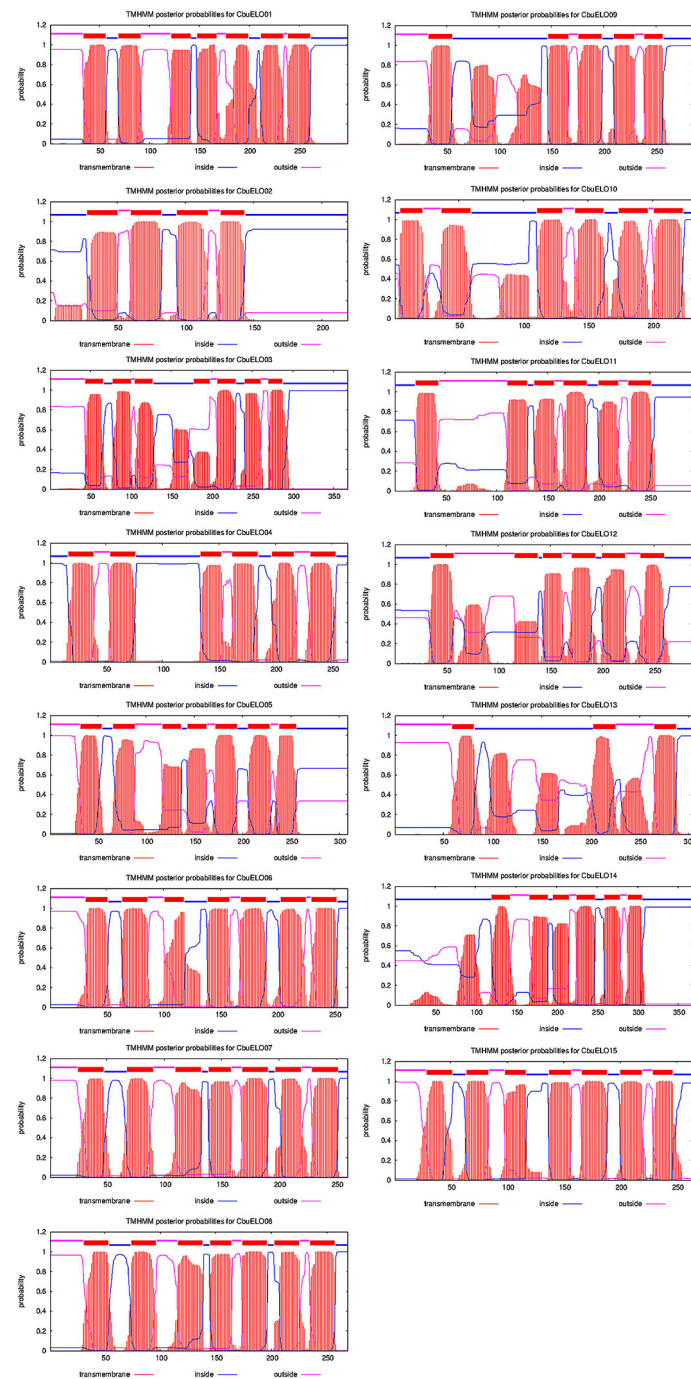| Protein	Transmembrane number	Transmembrane interval
CbuELO01	7	W_{34}-I_{56}, K_{69}-A_{91}, V_{122}-L_{141}, L_{148}-I_{167}, L_{177}-G_{199}, L_{212}-G_{234}, L_{239}-V_{261}
CbuELO02	4	F_{28}-V_{50}, F_{60}-G_{82}, Y_{94}-I_{116}, W_{126}-Y_{143}
CbuELO03	7	W_{44}-V_{66}, F_{78}-Y_{100}, Q_{105}-W_{127}, V_{178}-L_{197}, F_{207}-G_{229}, Y_{241}-L_{260}, T_{270}-L_{287}
CbuELO04	6	L_{17}-G_{39}, I_{54}-A_{76}, I_{134}-G_{152}, Y_{162}-Y_{184}, L_{197}-V_{216}, F_{231}-L_{253}
CbuELO05	7	S_{32}-W_{54}, A_{66}-V_{88}, T_{117}-L_{136}, V_{143}-L_{162}, A_{172}-G_{194}, Y_{206}-V_{228}, Y_{238}-Y_{255}
CbuELO06	7	P_{32}-P_{51}, Y_{64}-Y_{86}, S_{101}-L_{118}, V_{139}-T_{158}, F_{168}-G_{190}, L_{203}-S_{225}, S_{230}-V_{252}
CbuELO07	7	P_{25}-L_{47}, L_{68}-S_{90}, V_{110}-L_{132}, V_{139}-V_{158}, F_{168}-G_{190}, L_{197}-L_{219}, P_{229}-Y_{251}
CbuELO08	7	P_{31}-L_{53}, L_{74}-S_{96}, V_{116}-L_{138}, V_{145}-V_{164}, F_{174}-G_{196}, L_{203}-L_{225}, P_{235}-Y_{257}
CbuELO09	5	D_{33}-L_{55}, I_{147}-L_{166}, S_{176}-G_{198}, Y_{210}-I_{229}, W_{239}-V_{256}
CbuELO10	6	I_5-G_{22}, I_{37}-L_{59}, I_{111}-I_{130}, F_{140}-G_{162}, Y_{174}-P_{196}, P_{201}-Y_{223}
CbuELO11	6	D_{21}-T_{43}, S_{111}-L_{130}, V_{137}-V_{156}, L_{166}-G_{188}, Y_{200}-V_{219}, I_{229}-R_{251}
CbuELO12	6	W_{35}-Q_{57}, G_{116}-L_{138}, L_{143}-S_{161}, W_{171}-G_{193}, V_{200}-A_{222}, F_{237}-K_{259}
CbuELO13	3	W_{59}-W_{81}, F_{203}-G_{225}, Q_{265}-Y_{287}
CbuELO14	6	L_{120}-G_{142}, V_{167}-L_{189}, I_{196}-V_{215}, F_{225}-M_{247}, Y_{259}-L_{278}, V_{288}-R_{305}
CbuELO15	7	S_{29}-Y_{51}, I_{64}-V_{83}, A_{98}-L_{116}, I_{137}-L_{156}, L_{166}-G_{188}, Y_{200}-L_{219}, W_{229}-L_{246}

between −3.344 and −2.067, CbuELO01 had the smallest value of -3.344 (Supplementary Table 4). From the absolute value of the score representing the hydrophilicity and hydrophobicity, it can be seen that the number of hydrophobic amino acid residues was much larger than that of hydrophilic amino acid residues (Supplementary Fig. S1). It can be seen that CbuELO proteins were hydrophobic proteins, so it can be inferred as an insoluble protein.

The prediction of phosphorylation sites showed that CbuELO protein family had several different phosphorylation sites, and overall the protein family had 247 serine phosphorylation sites, 125 threonine phosphorylation sites, and 102 tyrosine phosphorylation sites. CbuELO03 protein contained the most phosphorylation sites, 51, while CbuELO10 contained the least phosphorylation sites with 15. The most serine phosphorylation site was CbuELO12 with 29, while the least number was member CbuELO13 with 9. The most likely phosphorylation site was located on 27 and 276 with a value of 0.996 (much larger than the threshold value of 0.500); the most threonine phosphorylation sites was CbuELO13 and CbuELO14 with 13, and the least abundant was CbuELO10 with only 1, of which the most likely phosphorylation site was located on 85 with a value of 0.964; the most abundant tyrosine phosphorylation site was CbuELO03 with 16, while the least abundant was CbuELO10 with only 1, of which the most likely phosphorylation site was located on 147 with a value of 0.973 (Table 3).

Secondary structure, tertiary structure and conserved structure of CbuELO proteins

The secondary structure analysis results of CbuELO proteins showed that overall secondary structure of CbuELO protein family was dominated by alpha helix, consisting of 2064 amino acids, accounting for 48.51% of the whole; the extended strand consists of 802 amino acid residues, accounting for 18.72% of the whole; beta turn consists of 126 amino acid residues, accounting for 2.98% of the whole; and the random coil consists of 1289 amino acid

Fu *et al. BMC Genomics*   (2024) 25:758

Page 7 of 22

Fig. 3 The transmembrane domain analysis of CbuELO proteins from *C. buqueti*

residues, accounting for 29.79% of the whole. Among α-helix, CbuELO10 protein accounted for the highest proportion, with 55.84% being α-helix, composed of 129 amino acid residues, while the least was CbuELO14 protein, 42.39% was α-helix, composed of 156 amino acid residues; In the extended strand structure, CbuELO04 protein accounted for the highest proportion, with 24.64% of the extended strand structure composed of 65 amino acid residues, while CbuELO13 with the smallest

proportion was only 15.13% of extended strand structure, composed of 46 amino acid residues Among β-turns, CbuELO12 had the highest proportion, with 4.53% being β-turn, consisting of 13 amino acid residues; while the least proportion was CbuELO01, with only 1.67% being β-turn, consisting of 5 amino acids residue composition; in random coils, CbuELO05 had the highest proportion, with a content of 35.92%, consisting of 111 amino acid residues, while the smallest proportion was CbuELO10,

Fu *et al. BMC Genomics*   (2024) 25:758

Page 8 of 22

Table 3 Prediction of phosphorylation sites in amino acid sequence of CbuELO proteins in *C. buqueti*

Protein	Phosphorylation site			Serine (S)		Threonine (T)		Tyrosine (Y)	
	Serine (S)	Threonine (T)	Tyrosine (Y)	Max	Position	Max	Position	Max	Position
CbuELO01	14	3	9	0.996	27	0.926	233	0.819	285
CbuELO02	11	9	4	0.960	173	0.920	153	0.973	147
CbuELO03	23	12	16	0.977	342	0.876	92	0.902	316
CbuELO04	12	10	5	0.940	254	0.964	85	0.947	247
CbuELO05	17	10	8	0.997	280	0939	281	0.893	111
CbuELO06	15	3	8	0.971	8	0.780	204	0.910	64
CbuELO07	18	9	6	0.979	21	0.936	102	0.920	64
CbuELO08	15	8	5	0.993	30	0.947	108	0.944	19
CbuELO09	21	4	8	0.996	276	0.953	175	0.948	21
CbuELO10	13	1	1	0.917	73	0.677	208	0.515	223
CbuELO11	10	13	6	0.978	272	0.962	267	0.699	282
CbuELO12	29	10	6	0.992	275	0.778	67	0.931	165
CbuELO13	9	13	4	0.788	127	0.920	296	0.904	40
CbuELO14	24	13	12	0.917	326	0.926	311	0.955	351
CbuELO15	16	7	4	0.917	252	0.612	257	0.811	5

with a content of 22.08%, consisting of 51 amino acid residues composition (Supplementary Table S5, Supplementary Fig. S2).

The tertiary structures analysis results showed that the tertiary structures of CbuELO proteins were divided into four categories according to the degree of structural similarity, and each graph was represented by A, B, C, D, E, F, G, H, I, G, K, L, M, N, and O in order for easy observation. Firstly, the first category was I, J, K, L, M, N, O, with the largest number of seven, corresponding to proteins CbuELO04, CbuELO06-CbuELO08, CbuELO10, CbuELO13, CbuELO15; the second category was E, F, G, H, corresponding to proteins CbuELO09, CbuELO11, CbuELO12, CbuELO14; the third category was B, C and D, corresponding to proteins CbuELO01, CbuELO03 and CbuELO05, respectively; the fourth category A has only one corresponding to protein CbuELO02. From the tertiary structure diagram, it can be seen that the α-helix and random coil were the main structural elements in CbuELO protein family, while β-turn was scattered sporadically, which point of structural prediction was consistent with the secondary structure analysis [44] (Supplementary Fig. S3).

The conserved structures analysis result of CbuELO proteins showed that 10 conserved motifs were identified in CbuELO protein family, with motif lengths ranging from 10 to 50 aa. The four most important conserved motifs in CbuELO protein family were motif1, motif2, motif3 and motif4, among which motif4 was present in each CbuELO protein. CbuELO06-CbuELO09 contain the most conserved motifs, all with nine, while the least conserved motif is protein CbuELO12 with only one conserved motif [45]. This suggests that *CbuELO12*, a member of *CbuELO* gene family, may be a pseudogene as it has lost an important conserved motif module. The

molecular function of *CbuELO12* gene may have lost its structural gene function during its evolution, and may play a regulatory role in the gene. In the previous literature, histidine-box HXXHH was reported as important motif, the study found that only CbuELO4 and CbuELO12 do not contain histidine-box HXXHH.(Supplementary Table S6, Supplementary Fig. S4).

Phylogenetic evolution and expression analysis of *CbuELO* genes

The CbuELO protein family evolutionary tree was constructed by MEGA11 software with the calibration parameter Bootstrap repeated 1000 times. The results showed that the 15 CbuELO proteins were divided into 3 subfamilies (labeled as Group1, Group2 and Group3 accordingly) according to their aggregation degree in the evolutionary tree. Among them, Group1 had the most family members with six CbuELO proteins, accounting for 40% of the whole; while Group2 had the least family members with four CbuELO proteins, accounting for 26% of the whole. The homology of the genes can be obtained according to the bootstrap values of the evolutionary tree, and it can be seen that CbuELO02 and CbuELO10, CbuELO06 and CbuELO07, CbuELO11 and CbuELO15 had 100% bootstrap values. In the whole family Group2 was the most primitive, while Group3 was the fastest evolving. In *CbuELO* gene family of *C. buqueti* genome, CbuELO06 and CbuELO07 had the fastest evolutionary rate and were the least conserved. CbuELO02 and CbuELO10 had the slowest evolutionary rate and were the most conserved(Fig. 4).

Phylogenetic trees were constructed from ELO protein sequences of *C. buqueti* and *Dendroctonus ponderosae*. The evolutionary relationships between the two were divided into four groups (labeled Group1, Group2,
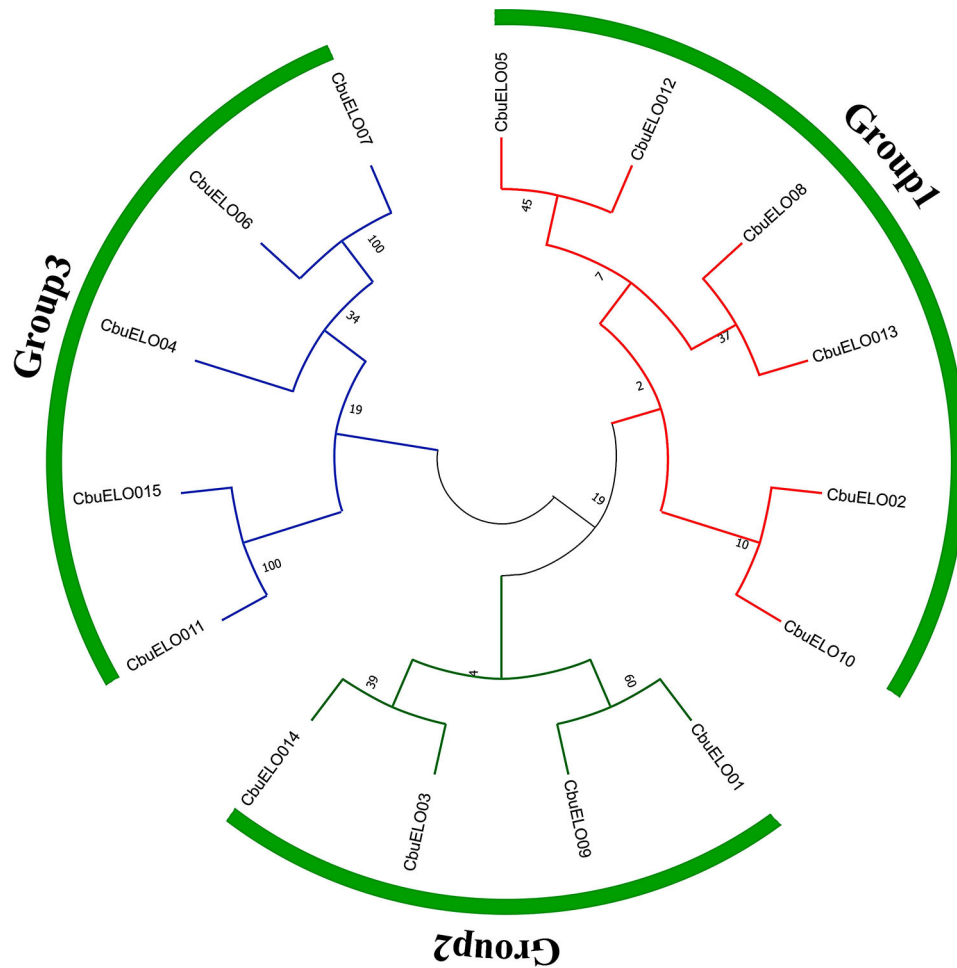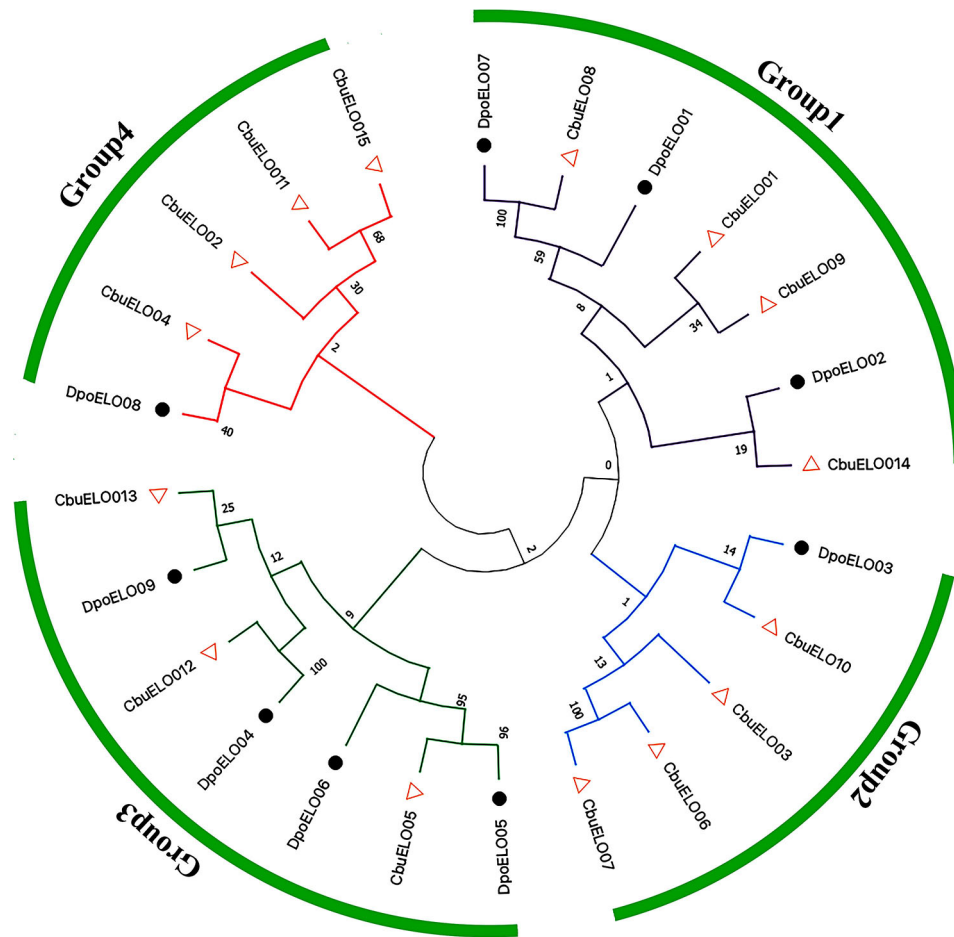
Fig. 4 Phylogenetic tree of *ELO* genes in *C. buqueti*

Group3, and Group4). There were four CbuELO members in Group 1 as CbuELO01, CbuELO08, CbuELO09 and CbuELO14; four CbuELO members in Group 2 as well as CbuELO03, CbuELO06, CbuELO07 and CbuELO10; and three in Group 3 as CbuELO05, CbuELO09 and CbuELO14. There were 4 CbuELO proteins in Group 4, namely CbuELO02, CbuELO04, CbuELO11 and CbuELO15. while the number of ELO proteins in *Dendroctonus ponderosae* was 3, 1, 4, and 1 in Groups 1 to 4, respectively. Homologous gene pairs, such as CbuELO08 with DpoELO07, CbuELO05 with DpoELO05 and DpoELO06, and CbuELO12 with DpoELO04, were found based on evolutionary tree bootstrap values, and the bootstrap values of these gene pairs were greater than 95%. Moreover, CbuELO08 and DpoELO07 had the fastest evolutionary rate, CbuELO04 and DpoELO08 had the slowest evolutionary rate and were the most conserved(Fig. 5).

Phylogenetic trees were constructed from the ELO protein sequences of *C. buqueti* and *Rhynchophorus ferrugineus*. Based on the evolutionary analysis, the

evolutionary relationships between the two were divided into four groups (labeled Group1, Group2, Group3, Group4). There were five CbuELO members in Group 1 as CbuELO01, CbuELO08, CbuELO09, CbuELO12, CbuELO13; two CbuELO members in Group 2 also as CbuELO02, CbuELO05; two CbuELO members in Group 3 as CbuELO03, CbuELO10, and the number of ELO proteins in *R. ferrugineus* was 5, 3, 1 and 4 in Groups 1 to 4, respectively. CbuELO12 and RfeELO03, CbuELO13 and RfeELO04, CbuELO09 and RfeELO10, RfeELO12, CbuELO05 and RfeELO01, RfeELO07, RfeELO08 had bootstrap values greater than 99%, Moreover, CbuELO12 and RfeELO03 had the fastest evolutionary rate, CbuELO06 and RfeELO11 had the slowest evolutionary rate and were the most conserved (Fig. 6).

Phylogenetic tree was constructed from ELO protein sequences of *C. buqueti* and *Tribolium castaneum*, and their evolutionary relationships were studied. According to the evolutionary analysis, the evolutionary relationship of ELO protein sequences of *C. buqueti* and *T.castaneum* was divided into 5 groups (marked as
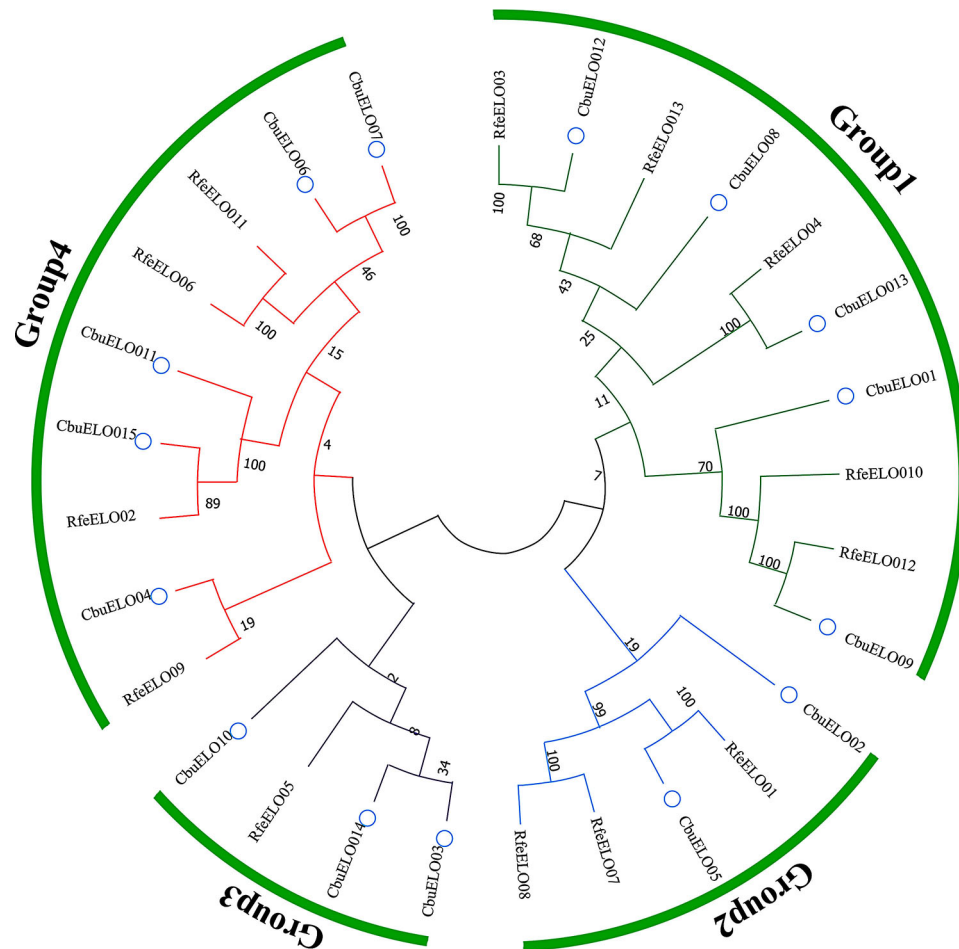
Fig. 5 Phylogenetic tree of *ELO* gene family in *C. buqueti* and *Dendroctonus ponderosae*

Group1, Group2, Group3, Group4, Group5). In Group 1, there was 1 CbuELO member, CbuELO05; in Group 2, there were also 3 CbuELO members, namely CbuELO08, CbuELO12, and CbuELO13; in Group 3, there were 5 CbuELO members, namely CbuELO04, CbuELO06, CbuELO07, CbuELO011, and CbuELO15; in Group 4, there was 1 with CbuELO02; in Group 5, there were also 5 CbuELO members, namely CbuELO01, CbuELO03, CbuELO09, CbuELO10 and CbuELO12. The number of ELO proteins of *T. castaneum* in Groups 1 to 5 were 8, 1, 4, 1, and 4, respectively. Among them, Group2 was the most primitive, and Group1 evolves the fastest. According to the bootstrap value of the phylogenetic tree, the orthologous gene pairs of *C. buqueti* and *Tribolium castaneum*, such as CbuELO05 and TcaELO14, CbuELO12 and TcaELO02, and CbuELO09 and TcaELO01. The bootstrap values of these gene pairs were all 100%, Moreover, CbuELO05 and TcaELO14 had the fastest evolutionary rate, CbuELO09 and TcaELO01 had the slowest evolutionary rate and were the most conserved (Fig. 7).

Construction of phylogenetic tree between ELO family proteins of *C. buqueti* and that of *D. ponderosae, R.*

ferrugineus, Hypothenemus hampei, Elaeidobius kamerunicus, Ips nitidus, Ips typographus, and *Sitophilus oryzae*. According to the evolutionary analysis, the evolutionary relationship between them was divided into 5 groups marked as (Group1, Group2, Group3, Group4, Group5). In Group 1, there were 2 CbuELO members, CbuELO05 and CbuELO12; in Group 2, there were also 5 CbuELO members, namely CbuELO06, CbuELO07, CbuELO11, CbuELO13, and CbuELO15; in Group 4, there were 5 CbuELO members, CbuELO01CbuELO4, CbuELO09; in Group 5, there are 3 CbuELO proteins namely CbuELO10, CbuELO08, and CbuELO14. From the whole phylogenetic tree, the ELO family proteins of *C. buqueti* had the closest relation ship with *T. castaneum*, in addition to *D. ponderosae* and *S. oryzae* [46] (Fig. 8).

The phylogenetic tree was constructed from ELO family proteins of *C. buqueti* and all the ELO sequences of Coleoptera, and a total of eight subfamilies (tagged with Group1, Group2, Group3, Group4, Group5, Group6, Group7 and Group8, respectively) were classified according to the evolutionary relationships between CbuELO and all the ELO family proteins of Coleoptera. Among

Fig. 6 Phylogenetic tree of *ELO* gene family in *C. buqueti* and *Rhynchophorus ferrugineus*

them, CbuELO family proteins had 3 members in Group1; 1 member in Group3; 2 members in Group5, 3 members in Group7; Group8 contained the most members with 6. As can be seen from Supplementary Fig. S5, Group1 evolved the fastest, while Group8 was the most primitive. Moreover, these results showed that *CbuELO* gene family was closely related to *ELO* gene family of the red palm weevil.

SSR analysis of *CbuELO* gene family
A total of 40 SSRs were found in 15 unigenes, distributed in 11 unigenes, and the total length of the sequences was less than 174906 bp, including 10 sequences containing more than 1 SSR. There were only three types of transcriptome SSRs, and the frequency of each type was also different, with the largest number of single nucleotide repeats, with a total of 34, all of which were A/T (85.00%), followed by dinucleotides and trinucleotides, with 3 respectively, 2 (5.00%) AT/AT, 1 (2.50%) AG/CT, and 3 (7.50%) AAT/ATT. In addition, it was found that there was also a certain variation in the number of repeats of SSRs, with the highest frequency of 10 repetitions (27),

7 repetitions of 11 repetitions, followed by 5 repetitions and 6 repetitions, both of which were 3, and the frequency of repetitions of 7, 8, and 9 was the lowest (Supplementary Table S7,Supplementary Table S8).

Codon bias analysis of *CbuELO* gene family
CodonW software was used to analyze the codon bias of *ELO* genes in *C. buqueti*, and the content of codon at the third position of different genes was different, the total GC ratio was 31.20%~37.50%, the mean value was 34.75%, the proportion of GC3s was 28.00%~36.40%, the mean value was 32.33%, and the proportions of T3s, C3s, A3s and G3s were 36.67%~46.14%, 18.65%~24.99% and 36.84%~48.48%, respectively, 18.42%~ 26.10%, the mean values were 42.50%, 21.55%, 43.43% and 22.27%, respectively, A3s>T3s>G3s>C3s, indicating that the content of the 3rd base AT of the synonymous codon in the genome of *C. buqueti* was high. The CAI value was 0.152~0.185, the mean value was 0.169, the CBI was −0.153~-0.088, the mean value was −0.111, the Fop value was 0.315~0.370, the mean value was 0.347, the Nc value was 49.47~56.57, and the mean value was 53.87, all of
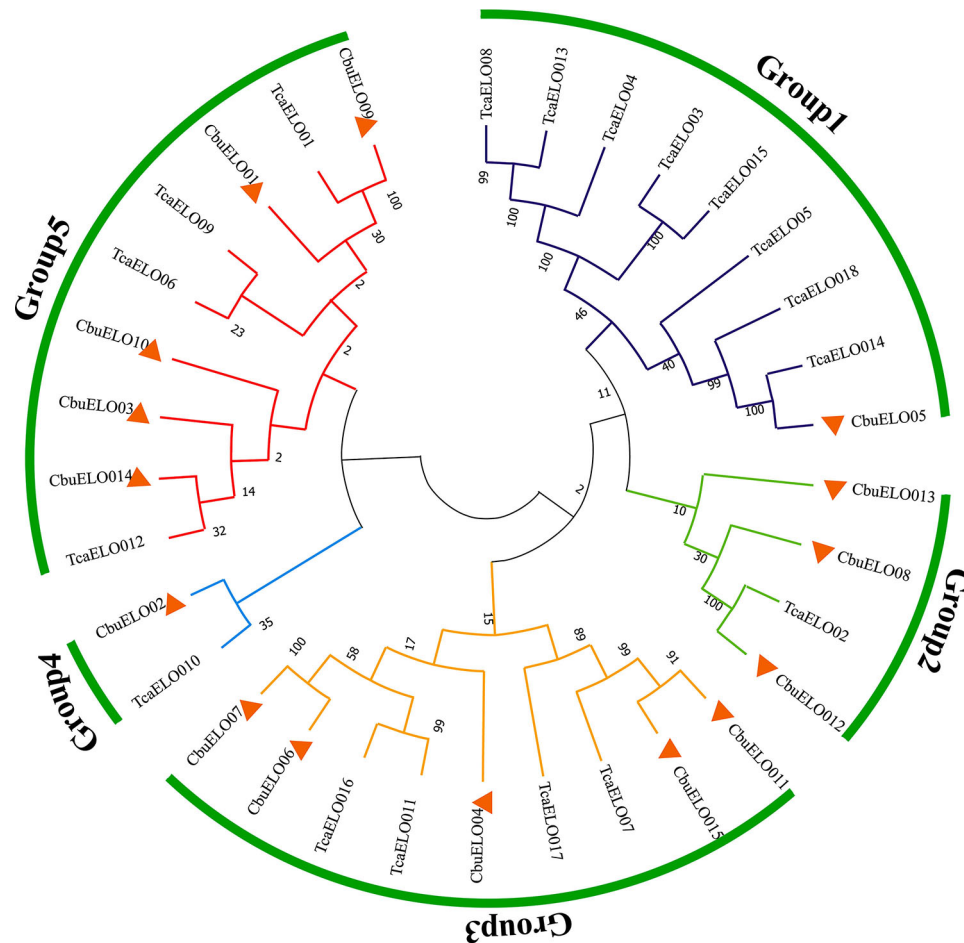
Fig. 7 Phylogenetic tree of *ELO* gene family in *C. buqueti* and *Tribolium castaneum*

which were greater than 35, indicating that the codon preference in the genome of *C. buqueti* was weak (Fig. 9).

There were 33 codons with RSCU values≥1.00, which were UUU, UUA, UUG, CUU, AUU, AUA, AUG, GUU, GUA, UCU, UCA, CCU, CCA, ACU, ACA, GCU, GCA, UAU, UAA, CAU, CAA, AAU, AAA, GAU, GAA, UGU, UGG, CGU, CGU, AGU, AGA, GGU and GGA, 14 of which ended in A, 12 ended in U, and only 3 ended in G, UUA had the highest RSCU value, was 1.92, these codons were the preferred codons in *C. buqueti* genome, indicating that the high-frequency codons in *C. buqueti* genome prefer to end in U or A. At the same time, the RSCU values of 31 codons<1, and most of the low-frequency codons end in C (16) or G (13) (Fig. 10).

Gene expression analysis of *CbuELO* genes under developmental transcriptome, different temperature and feeding time conditions

The developmental transcriptome analysis results of tissues and organs in different developmental stages of *C. buqueti*, including male, female, larva, pupae and egg, showed that among 15 *ELO* genes of *C. buqueti*, the gene expression contents of *CbuELO10*, *CbuELO13* and *CbuELO02* genes in pupa and egg were high, that of *CbuELO6* and *CbuELO7* in male were high, The expression contents of *CbuELO01*, *CbuELO03*, *CbuELO04*, *CbuELO05*, *CbuELO09*, *CbuELO12*, *CbuELO14* and *CbuELO15* in females were high, and the expression contents of *CbuELO8* and *CbuELO11* in larvae were high. It can be seen that *CbuELO10*, *CbuELO13* and *CbuELO02* genes were the key enzyme genes that determine the synthesis of very long chain fatty acids in pupae and eggs, *CbuELO6* and *CbuELO7* were the key enzyme genes that determine the synthesis of very long chain fatty acids in the male of *C. buqueti*, and *CbuELO8* and *CbuELO11* were the key enzyme genes that determine the synthesis of very long chain fatty acids in the larva of *C. buqueti*, The remaining eight *ELO* genes were the key enzyme genes that determine the synthesis of very long chain fatty acids in the female of *C. buqueti*(Supplementary Fig. S6).

The larvae, females and males of *C. buqueti* were treated at different temperatures (4 °C, 25 °C, 42 °C, 50 °C), and their muscle tissues were taken for
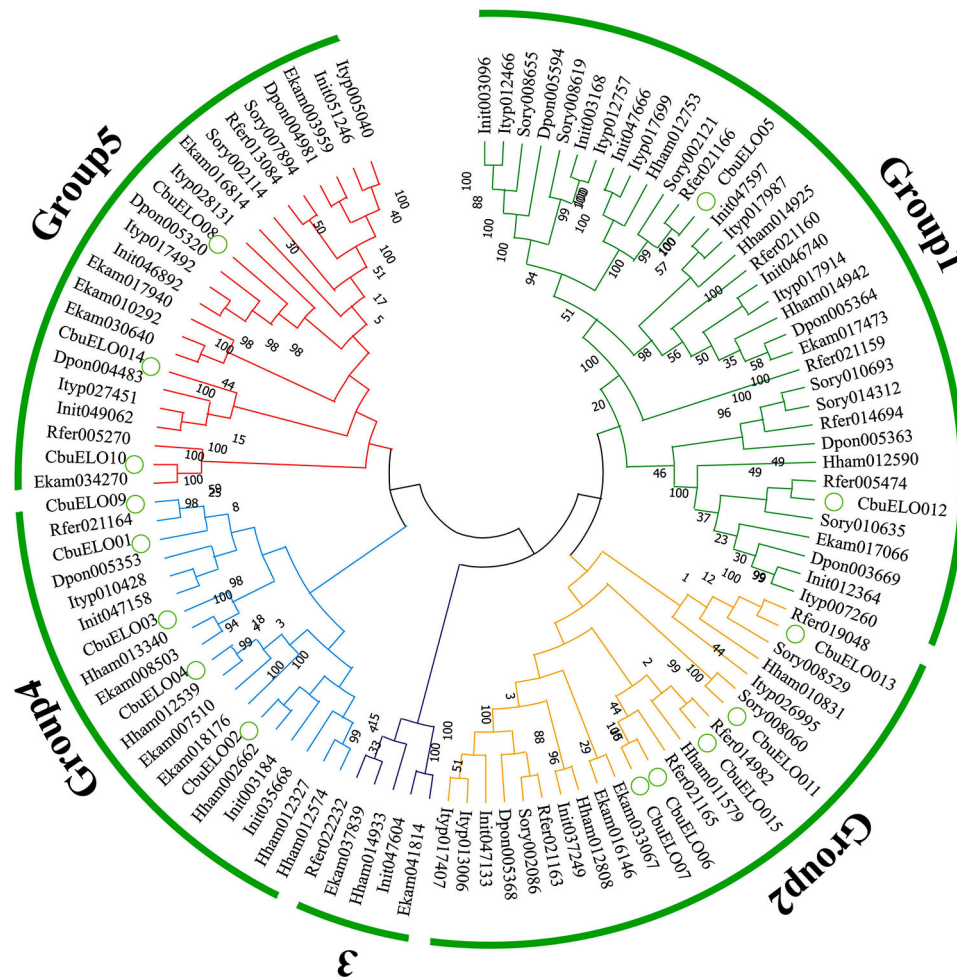
**Fig. 8** Phylogenetic tree of *ELO* gene family in *C. buqueti* and all species of *Curculionidea*

transcriptome sequencing after 1 h, and the obtained data were used as heat maps. The results showed that the average value of larvae was 15.11 at 25 °C, and changed to 14.49 at 4 °C, and the expression of larvae for temperature reduction was down-regulated, with *CbuELO12* being the most significant down-regulation, from 86.61 at 25 °C to 81.47 at 4 °C, followed by *CbuELO14*, from 30.60 at 25 °C to 25.59 at 4 °C, and *CbuELO5* and *CbuELO1*, which were significantly down-regulated, with 3.46 and 3.21 respectively.

For adults, the temperature increased to 42 °C, the temperature continued to increase, and the temperature decreased to 4 °C. For females, the average value at 25 °C was 10.51 and 42 °C was 14.72, with the most significant upward adjustment being *CbuELO14* (25.99) and *CbuELO12* (32.58). The gene expression of males changed more significantly with temperature, with an average value of 0.27 at 4 °C, and the most significant down-regulation was *CbuELO12* (65.02), followed by *CbuELO14* (14.71). The average value at 42 °C was 32.58, with the most significant upward revision being

CbuELO13 with an upward revision of 165.03, followed by *CbuELO11* with an upward revision of 125.29. The average value at 50 °C was 5.75, with the most significant downward revision being *CbuELO12* with a downward revision of 54.22, followed by *CbuELO14* with a downward revision of 16.03.

In terms of individual genes, the expression of *CbuELO12* was the highest at different temperatures compared to other genes, and although there was a large change in temperature increase, it was still greater than that of other genes. The female and male worms were 15.11 at 25 °C, and the female and male worms at 42 °C were 14.72 and 32.58, respectively, compared with 25 °C, the low temperature had little effect on the overall gene expression of larvae, and the male worms were more tolerant to high temperature than females, and the gene expression of males showed an upward trend with the increase of temperature, and the gene expression of males had a higher expression for the increase of temperature (Fig. 11).
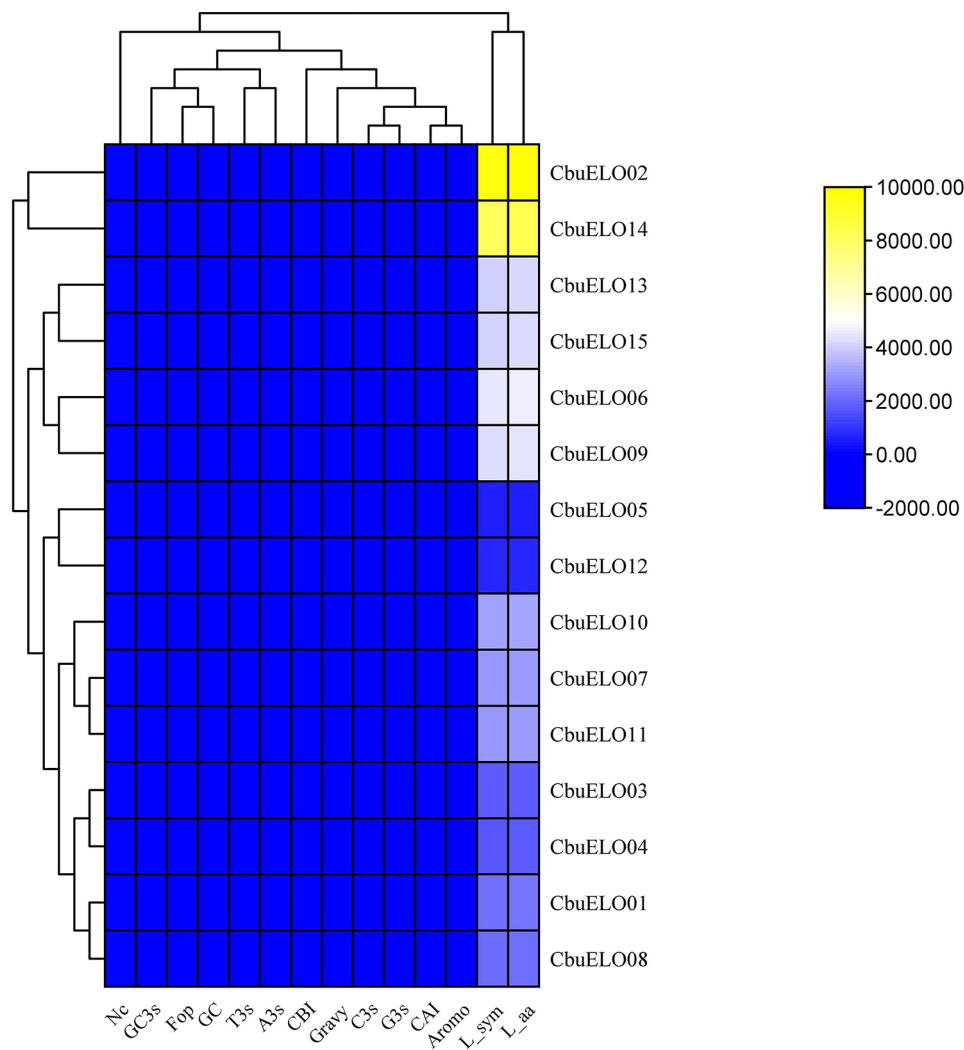
**Fig. 9** Codon bias-related parameters of *CbuELO* genes

Taking the time interval (0 h, 0.5 h, 1 h, 2 h) after feeding (0 h, 0.5 h, 1 h, 2 h) of female and male elephants as variables, three parallel experiments were performed in each group, and the intestinal contents were taken for transcriptome sequencing to obtain their transcriptional expression, and the data were used as heat maps.

In males, the gene with high expression was *CbuELO14*, with a maximum value of 9118.33 in 2 h after eating, and the gene was up-regulated with the increase of feeding time interval, followed by *CbuELO12*, with a maximum value of 8491.67 after 2 h of feeding. Compared with other genes, the gene expression of *CbuELO7* gene was the weakest, indicating that it may have little effect on the degradation of bamboo fiber. Compared with the genes that had just eaten for 0 h, the down-regulated genes accounted for the majority, and there were three genes that had the largest transcriptome gene expression at 0.5 h after eating, including *CbuELO1*, *CbuELO6 and CbuELO7*, among which the

gene *CbuELO1* was the most significantly up-regulated. It was up-regulated by 319.00, *CbuELO6* by 93.00 and *CbuELO7* by 5.33, and 12 genes were down-regulated, including *CbuELO13* by 1899.00, *CbuELO14* by 1515.00 and *CbuELO11* by 1311.67. There were 7 genes with the largest transcriptome gene expression 1 h after eating, including *CbuELO1*, *CbuELO3*, *CbuELO6*, *CbuELO7*, *CbuELO8*, *CbuELO12*, *and CbuELO14*, among which *CbuELO12* was the most significantly up-regulated. It was revised up by 834.33, followed by *CbuELO1* with 440.33, and *CbuELO13* by 681.67 and *CbuELO11* by 714.00. There were 11 genes that were up-regulated 2 h after eating, including *CbuELO1*, *CbuELO2*, *CbuELO3*, *CbuELO4*, *CbuELO5*, *CbuELO6*, *CbuELO7*, *CbuELO8*, *CbuELO12*, and *CbuELO14*, and *CbuELO12* was significantly up-regulated. It was raised by 4809.33, *CbuELO14* by 4399.00, *CbuELO3* by 747.33, *CbuELO12* by 529.00, and *CbuELO11* by 675.00.
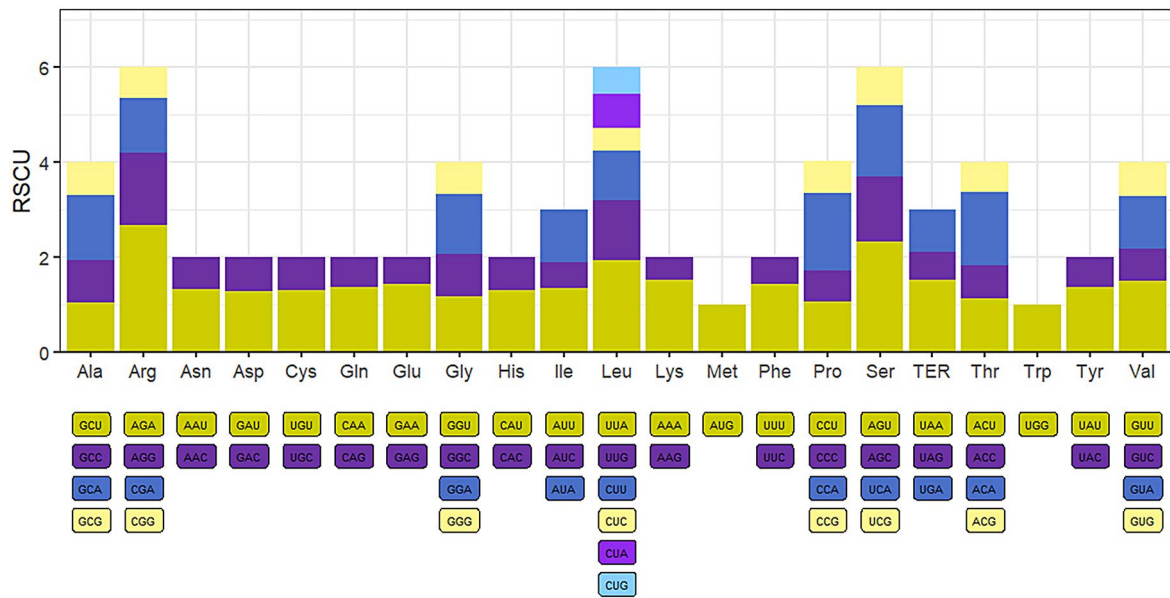
Fig. 10 RSCU values of *CbuELO* genes
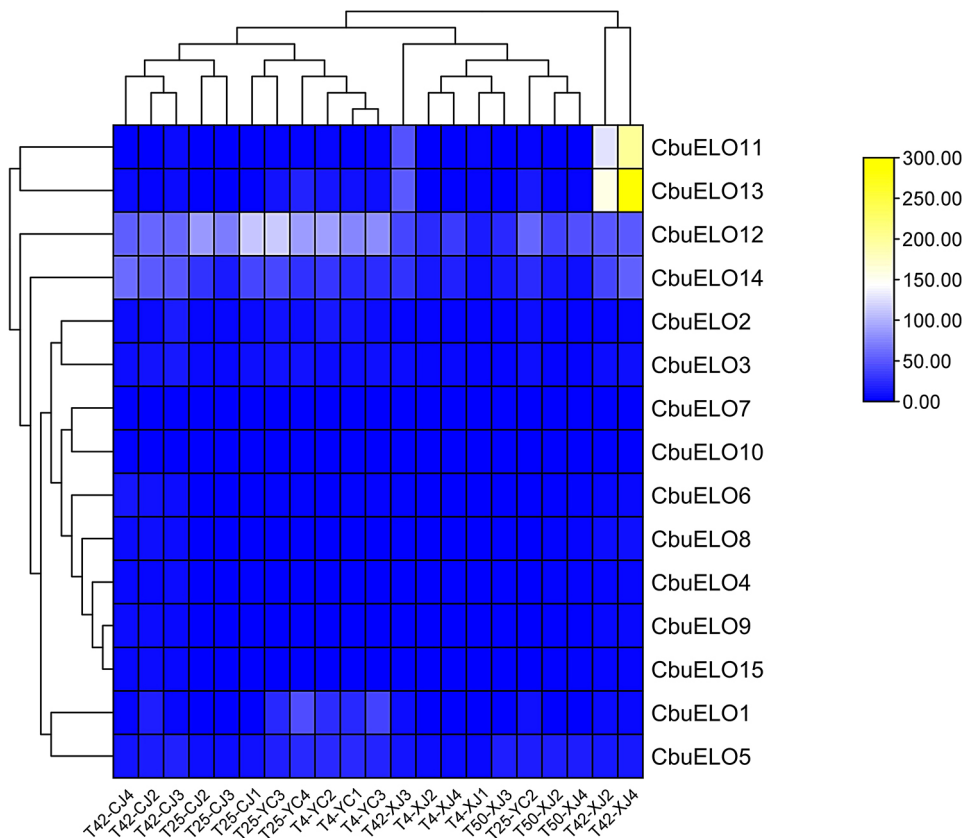
Fig. 11 Heat map of gene expression analysis of *CbuELO* genes under different temperature

In females, the genes with high expression were *CbuELO12* and *CbuELO14*, the maximum value of *CbuELO12* was 12963.33 after 0 h of feeding, the maximum value of *CbuELO14* was 5975.00 after 0.5 h of feeding, and the gene with low expression was *CbuELO7*. Compared with the 0 h after eating, there were 11 genes with the largest transcriptome gene expression at 0.5 h after eating, including *CbuELO3*, *CbuELO4*, *CbuELO5*,

Fu *et al. BMC Genomics* (2024) 25:758

Page 16 of 22

CbuELO6, CbuELO7, CbuELO8, CbuELO9, CbuELO10, CbuELO11, CbuELO14 and CbuELO15, among which *CbuELO14* was the most significant up-regulated. It was up-regulated by 934.00, followed by *CbuELO9* with 186.33, and the other genes were slightly up-regulated, with *CbuELO12* down-regulated by 3156.67 and *CbuELO13* down-regulated by 1139.33. There were 10 genes with the largest transcriptome gene expression 1 h after eating, including *CbuELO1, CbuELO2, CbuELO6, CbuELO7, CbuELO8, CbuELO9, CbuELO10, CbuELO11, CbuELO14, and CbuELO15*, among which *CbuELO* was the most significantly up-regulated 14, 843.67 was up-regulated, followed by *CbuELO9*, 324.33, and the other genes were very small, and *CbuELO12* was down-regulated by 5849.67. There were 12 genes up-regulated 2 h after eating, including *CbuELO1, CbuELO2, CbuELO3, CbuELO4, CbuELO6, CbuELO7, CbuELO8, CbuELO9, CbuELO10, CbuELO11, CbuELO14, and CbuELO15*, among which the most significant up-regulation was *CbuELO9*, which was 363.00, followed by *CbuELO2*, It was raised by 284.00, *CbuELO6* by 261.33 and *CbuELO* 14 by 204.67, while *CbuELO12* was lowered by 4823.33 and *CbuELO13* by 315.00.

In summary, the CbuELO gene was the most up-regulated in females and males at 2 h after feeding, and *CbuELO12, CbuELO14 and CbuELO13* were highly expressed in females, *CbuELO14* was up-regulated, *CbuELO12* and CbuELO13 were down-regulated, *CbuELO12* was the highest in males, *CbuELO12, CbuELO14 and CbuELO13* were high-expression genes, and *CbuELO12*, The expression of *CbuELO14* was up-regulated, and *CbuELO13* was down-regulated. *CbuELO14* was up-regulated in both females and males, suggesting that this gene may be involved in the degradation of bamboo fibers (Fig. 12).

Taking the time interval (0 h, 0.5 h, 1 h, 2 h) after feeding (0 h, 0.5 h, 1 h, 2 h) of female and male adult as variables, three parallel experiments were performed in each group, and the intestinal contents of the insects were taken for protein sequencing, and the obtained data were used as heat maps. The results showed that most of the results were 0, only CbuELO14 protein had results, and the protein expression of males was slightly higher than that of females, in males, the protein expression was the highest at 2 h after eating, with an average value of 1.788, followed by 1 h after eating, with a mean value of 1.629, and in females, the protein expression was the highest at 2 h after eating, with a mean value of 0.809, followed by 0.5 h after eating, with a mean value of 0.461 (Supplementary Fig. S7).
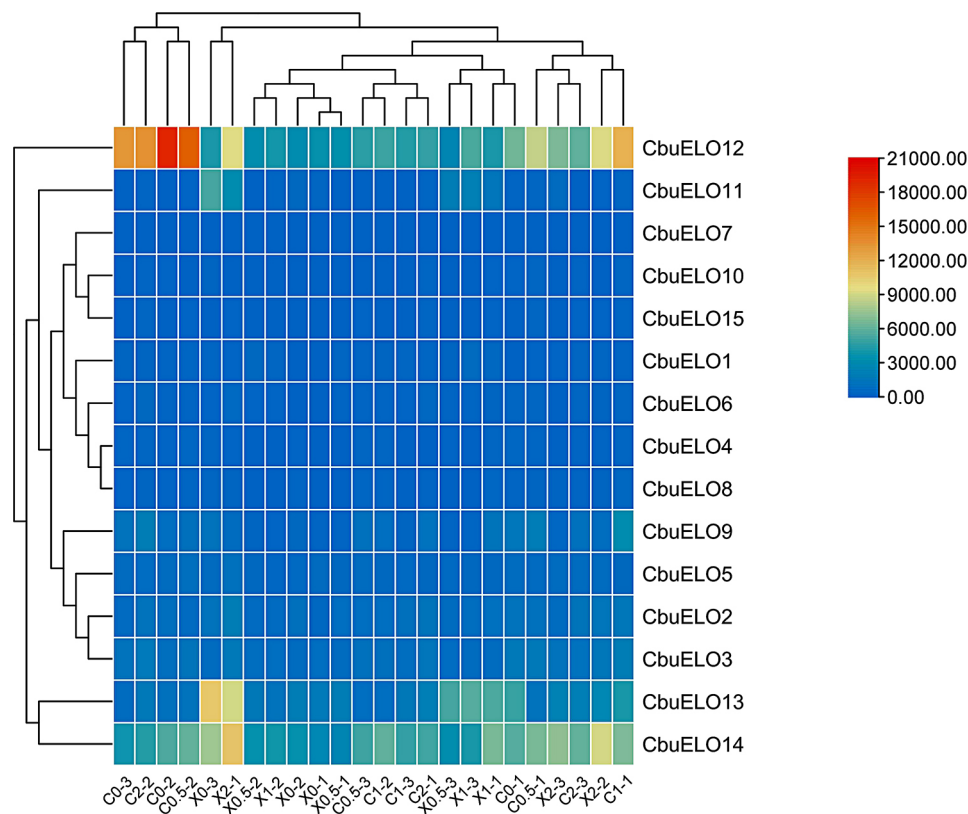
Fig. 12 Gene expression of *CbuELO* genes under different feeding time conditions
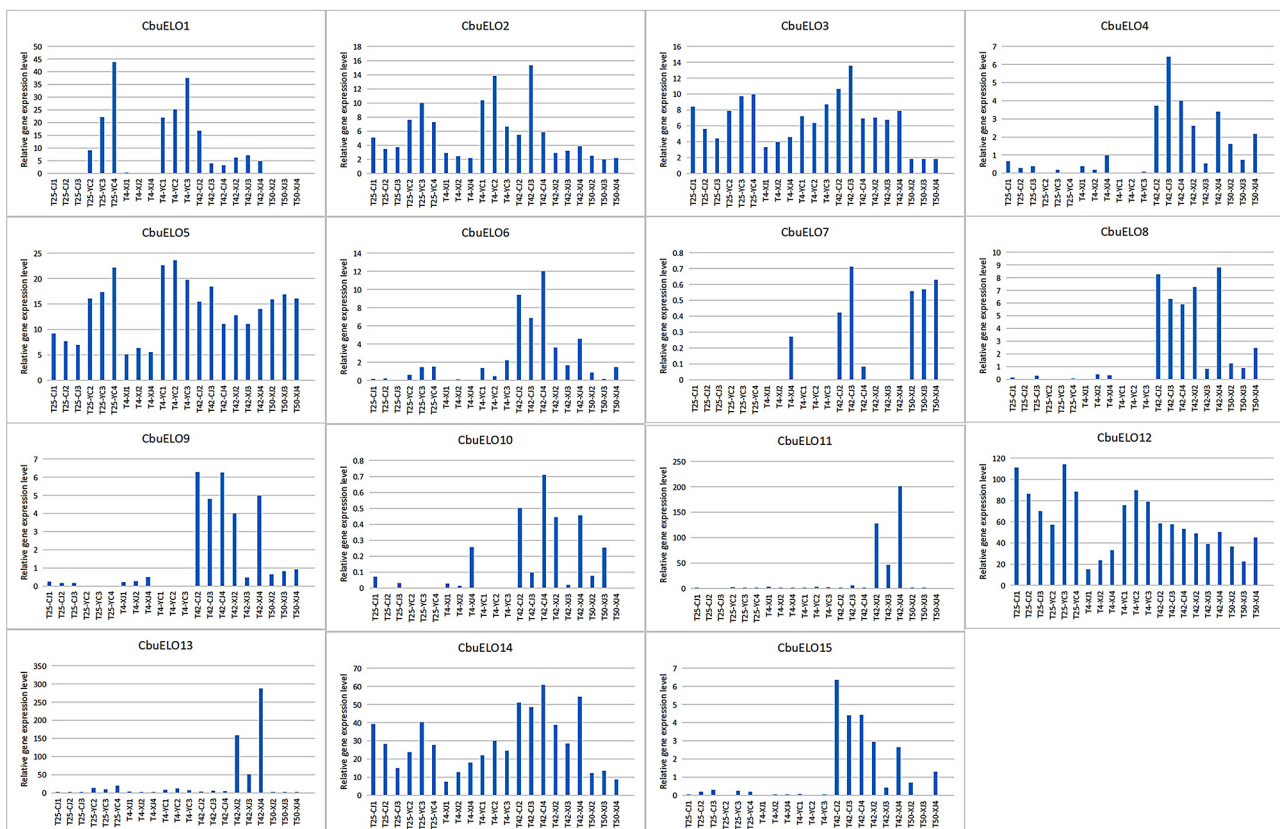
qRT-PCR validation analysis of *CbuELO* gene expression under different temperature and feeding time

The larvae, females and males of *C. buqueti* were treated at different temperatures (4 °C, 25 °C, 42 °C, 50 °C), and their muscle tissues were extracted from RNA after 1 h, reverse transcribed into cDNA, and cDNA was used as a template for qRT-PCR to detect the expression level of each gene. The results showed that with the increase of temperature, the general trend was as follows: 4 °C ~ 25 °C increased first, 25 °C ~ 50 °C increased and then decreased, the maximum was at 42 °C, compared with the control group (25 °C), the largest change was in the male of CbuELO13 at 42 °C, the smallest change was in the male of *CbuELO1* at 50 °C, the larvae of *CbuELO7*, *CbuELO8* and *CbuELO9* at 4 °C, and the male of *CbuELO7* at 42 °C. Compared with the control group (25 °C), the largest up-regulated genes were males with *CbuELO13* at 42 °C, the smallest up-regulated genes were males with *CbuELO10* at 50 °C, the most down-regulated genes were males with *CbuELO12* at 4 °C, and the smallest down-regulated genes were *CbuELO4* larvae at 4 °C. In summary, the validation results are basically consistent with the heat map results, *CbuELO12* was the highest expression at different temperatures compared with other genes, although there is a large change in

temperature increase, compared with 25 °C, low temperature has little effect on the overall expression of larvae genes, males are more tolerant to high temperature than females, and the gene expression of males increases with the increase of temperature, in summary, the gene has higher expression for temperature increase(Fig. 13).

The time interval (0 h, 0.5 h, 1 h, 2 h) after feeding of female and male adult was taken as variables, and 0 h was used as the control group, and three parallel experiments were performed in each group, and the intestinal contents were extracted from RNA, reverse transcribed into cDNA, and cDNA was used as a template for qRT-PCR to detect the expression level of each gene. The results showed that although the expression of *CbuELO7* increased with the interval between meals, its expression level was lower than that of other genes, and the highest expression genes were *CbuELO12, CbuELO13 and CbuELO14*. For females, 0 h ~ 2 h decreases first and then increases, and reaches the maximum value in 2 h, but the increased value is always lower than the expression level of the control group (0 h). The expression of males decreased first at 0 h ~ 0.5 h, increased at 0.5 h ~ 2 h, and reached the maximum value at 2 h, which was higher than that of the control group. Among the genes up-regulated in females, *CbuELO14* was the largest, *CbuELO7* was the

Fig. 13 Gene expression level of *CbuELO* gene verified by qRT-PCR at different temperatures

smallest, *CbuELO12* was the most down-regulated, and *CbuELO4* was the smallest. Among the genes upregulated in males, *CbuELO12* was the largest, *CbuELO7* was the smallest, *CbuELO13* was the most down-regulated, and *CbuELO15* was the smallest. In summary, the validation results were basically consistent with the heat map results, and the *CbuELO14* was the most up-regulated gene at 2 h after feeding in females and males, *CbuELO12*, *CbuELO14 and CbuELO13* were highly expressed in both females and males, and *CbuELO14* was up-regulated in both females and males, indicating that this gene may be involved in the degradation of bamboo fiber. For males, with the increase of feeding intervals, the gene expression level of males decreased first and then increased, reaching the maximum value at 2 h after feeding, while the female increased first and then decreased, and then increased, reaching the maximum value at 2 h after feeding. The overall trend of *CbuELO14* was not large, and the expression level of *CbuELO14* was also small(Fig. 14).

Protein interaction analysis of CbuELO proteins

By constructing the interaction diagram of 15 CbuELO proteins in *C.buqueti*, the results showed that 9 of 15 CbuELO proteins were related to each other, and most of the members had more than one interaction relationship, such as CbuELO01, CbuELO04 and CbuELO12, indicating that there was a direct or indirect regulatory effect between them, and one protein was independent, which was CbuELO05. The results showed that CbuELO06, CbuELO07, CbuELO08, CbuELO09 and CbuELO10 had no interaction with other proteins of CbuELO protein family. According to the following types of proteins involved in the interaction: CbuELO01 and CbuELO12 were involved in the biosynthesis of long-chain fatty acids. CbuELO01, CbuELO02, CbuELO03, CbuELO11, CbuELO12, CbuELO13 and CbuELO15 were involved in the elongation of fatty acids. CbuELO1 and CbuELO04 were involved in pheromone metabolism. CbuELO05 was involved in the metabolism of organic matter (Fig. 15).

Discussion

Bioinformatics is a discipline that intersects with medicine, statistics, mathematics, physics, and computer science based on the development of both information science and molecular biology [47, 48]. Compared with traditional studies, bioinformatics methods are cost-effective and efficient, and the methods have been widely used to predict and analyze the structure, function, and other biological properties of proteins [49]. *C. buqueti*

Fig. 14 Gene expression level of *CbuELO* gene verified by qRT-PCR at different feeding intervals

Fig. 15 Protein interaction analysis of CbuELO proteins

occurs once a year in Sichuan and overwinters as an adult in a pupal chamber in the soil [50]. Adults insect are less active in the early morning but become more active and more capable of flight as the temperature rises [51]. Both larvae and adults feed on bamboo shoots, resulting in a large number of severed and deformed bamboos, and the adults have a pseudomortality and generally live from 50 to 70 d. The adults become extinct in bamboo forests in early October [52, 53]. *C. buqueti* is a major forestry pest, with damage rates of up to 50-80% and up to 100% in severe cases [54]. It mainly affects the bamboo shoots of *Bambusa textilis*, *Neosino Calamusaffinis* and other clumped bamboos [55].

Elongation family of very long chain fatty acids protein(ELO) are involved in the production of long-chain fatty acids (C16, C18 and C20) [56], which are formed in the presence of ELO and this reaction requires energy from NADPH [57].ELO is widely found in plants, insects and animals. Only in recent years has the specific role of ELO in fatty acids gained widespread attention. In mammalian studies, ELOVL family proteins not only control the length of fatty acid carbon chains, but also ELOVL2 has an effect on sperm maturation in male rats [58–60]. In addition to this study, ELOVL family proteins were found to be associated with diseases such as diabetes [61], obesity [62], and cancer [63]. In *Saccharomyces cerevisiae*, three genes encoding fatty acid elongases were identified and their functions were investigated and they were found to catalyze C14 into long-chain fatty acids of different lengths [64, 65]. In insects, ELOs are involved in life activity processes such as mating, reproduction, pheromone biosynthesis, and epidermal formation [66]. Different ELOs have different substrate preferences and tissue-specific expression in the same insect. Today, studies on ELO in insects have focused on Drosophilid, where 20 ELO family proteins have been identified and *ELO* is

a key gene in Drosophilid development [67, 68]. Studies on *Aedes albopictus* ELO revealed that the ELO family proteins are important for egg retention and resistance to desiccation [59]. In *Nilaparvata lugens*, 20 ELO family proteins were identified, with different express ion sites and expression profiles, thus performing different biological functions [62]. Although ELO genes have been intensively studied in some insects, there is still a gap in the study of *ELO* genes in *C. buqueti* [69].

In this study, comprehensive analysis of *CbuELO* gene family in *C. buqueti* was conducted using bioinformatics. The identification of its gene family revealed that there were 15 CbuELO proteins, and 15 CbuELO proteins were located on four chromosomes. Gene structure analysis results revealed that all CbuELO proteins contained exons (CDS); all family members had introns except for *CbuELO12*, which had no introns; all family members had non-coding regions (UTR) except for *CbuELO04*. The physical and chemical properties of CbuELO protein family were predicted by EXPASY-ProtParam, and the theoretical isoelectric points of CbuELO protein family were found to be between 9.22 and 9.68. The overall number of stable proteins was greater than that of unstable proteins, and the lipid coefficients were between 72.28 and 120.22. The average coefficient of hydrophilicity of CbuELO protein family was 0.433, indicating that they are hydrophobic proteins, which is consistent with the prediction of Chertemps et al [70]. The total number of Arg+Lys of overall CbuELO protein family was much larger than that of Asp+Glu, indicating that the overall CbuELO protein family is positively charged. Subcellular localization analysis showed that CbuELO protein family was fully localized to plasma membrane. The transmembrane domain analysis of CbuELO protein family showed that each member of the protein family had a transmembrane structure, so it can be inferred that the CbuELO protein family is a transmembrane protein, which is consistent with the prediction of Uttaro et al. study [71]. The prediction of phosphorylation sites using the online software NetPhos revealed that the protein family has multiple phosphorylation sites. The tertiary structure of the proteins was predicted using SWISS-MODEL and found that the α-helix and random coil was the main structural element in CbuELO protein family. The conserved structures of the proteins were analyzed using MEME, and the results showed that 10 conserved motifs were identified in the CbuELO protein family, among which motif4 was present in each member, which was consistent with the prediction of Zheng et al. study [72]. The phylogenetic tree of CbuELO protein family was constructed by MEGA11 software, and the homology of ELO family proteins of *C. buqueti*. to several gene sequences of *T. castaneum* reached 100%. In addition, it is closest to the *D. ponderosae* and *Elaeidobius kamerunicus*. The expression

analysis of *ELO* gene family in *C. buqueti* genome in different developmental organs showed that different *ELO* genes determined the synthesis of very long chain fatty acids in different tissues and organs of *C. buqueti.*

To sum up, this study carried out a systematic bioinformatics study on the elongation family of very long chain fatty acids proteins in *C. buqueti.* The results laid a foundation for revealing the molecular mechanism of *C. buqueti*, and also provided a new target for the green control of pests [73].

Conclusion

In this study, we have identified 15 ELO proteins in *C.buqueti* genome. 15 CbuELO proteins were located on four chromosomes. Phylogenetic analysis showed that molecular evolutionary relationships of ELO protein family between *C. buqueti* and *Tribolium castaneum* was the closest. Developmental transcriptome analysis indicated that *CbuELO10, CbuELO13* and *CbuELO02* genes were the key enzyme genes that determine the synthesis of very long chain fatty acids in pupae and eggs, *CbuELO6* and *CbuELO7* were that in the male, and *CbuELO8* and *CbuELO11* were that in the larva. Transcriptome analysis under different temperature conditions indicated that *CbuELO1, CbuELO5, CbuELO12, and CbuELO14* participated in regulating temperature stress responses. Transcriptome analysis at different feeding times showed *CbuELO12* gene expression level in all feeding time periods was significant downregulation. The qRT-PCR experiment verified the expression level changes of *CbuELO* gene family under different temperature and feeding time conditions. Protein-protein interaction analysis showed that 9 CbuELO proteins were related to each other, and CbuELO1, CbuELO4 and CbuELO12 had more than one interaction relationship. These results lay a theoretical foundation for further studying its molecular function during growth and development of *C. buqueti.*

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions
C.F. participated in the formal data analysis, data interpretation and scientifc paper writing. C.F., T.Y. participated in formal data analysis, data paper writing, omics data management and scientifc paper writing. H.L., W.C.L. and H.Y.W. participated in the conceptualization and investigation methodology. Y.L.H. and N.J. participated in the conceptualization of the project. C.F. and Y.J.Y. participated in the conceptualization, supervision, investigation, data analysis and interpretation, funding acquisition, project administration and scientifc paper writing. All authors read and approved the fnal manuscript.

Data availability
The genome assembly data and RNA-seq data used in this study were retrieved from the NCBI Sequence Read Archive (SRA) database (https://www. ncbi.nlm.nih.gov/sra) under the accession code PRJNA675312, PRJNA719467 and PRJNA718062.

Declarations

Ethics approval and consent to participate
The adults and larval samples of *C. buqueti* used in this study were collected in Danan Town, Muchuan County, Leshan City, Sichuan Province. All experiments were conducted within ethical limits and were conducted safely in the Molecular Biology Laboratory of Key Laboratory of Sichuan Province for Bamboo Pests Control and Resource Development of Leshan Normal University from July 15 to August 15, 2020. All experiments do not involve humans.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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