Molecular characterization and expression of six heat shock protein genes in relation to development and temperature in *Trichogramma chilonis*

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Abstract

*Trichogramma* is a kind of egg parasitoid wasp that is widely used to control lepidopterous pests. Temperature is one of the main factors that determines the various life activities of this species, including development, reproduction and parasitism efficiency. Heat shock proteins (HSPs) are highly conserved and ubiquitous proteins that are best known for their responsiveness to temperature and other stresses. To explore the potential role of HSPs in *Trichogramma* species, we obtained the full-length cDNAs of six HSP genes (*Tchsp10*, *Tchsp21*, *Tchsp60*, *Tchsp70*, *Tchsc70-3*, and *Tchsp90*) from *T. chilonis* and analyzed their expression patterns during development and exposure to temperature stress. The deduced amino acid sequences of these HSP genes contained the typical signatures of their corresponding protein family and showed high homology to their counterparts in other species. The expression levels of *Tchsp10*, *Tchsp21*, *Tchsp60*, *Tchsc70-3*, and *Tchsp90* increased from the pupal stage to the adult stage. *Tchsp70* and *Tchsp90* exhibited the highest expression levels in the adult stage. The expression of six *Tchsp* was dramatically upregulated after 1 h of exposure to 32 and 40˚C but did not significantly change after 1 h of exposure to 10 and 17˚C. This result indicated that heat stress, rather than cold stress, induced the expression of HSP genes. Furthermore, the expression of these genes was time dependent, and the expression of each gene reached its peak after 1 h of heat exposure (40˚C). *Tchsp10* and *Tchsp70* exhibited a low-intensity cold response after 4 and 8 h of exposure to 10˚C, respectively, but the other genes did not respond to cold at any time points. These results suggested that HSPs may play different roles in the development of this organism and in its response to temperature stress.
Introduction

Wasp species of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are tiny egg parasitoids of numerous insect species that are distributed around the world [1–3]. These wasps are easily mass reared and have a broad range of hosts [4]. Since 1975, several species of *Trichogramma* have been commonly used as biological control agents for various pests in agricultural and forest systems [5, 6]. Among these parasitoid wasps, *T. chilonis* is one of the most successful species in controlling lepidopterous pests, including *Chilo* spp. (Lepidoptera: Pyralidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) [2]. In China, *T. chilonis* is widely distributed and is employed in integrated pest management for rice, cotton, sugarcane and other crops [7].

Temperature is a vital factor that determines the distribution and abundance of animals [8]. It is also crucial for the successful introduction of *Trichogramma* species because it influences their development, survival, reproduction, and sex ratio, as well as their parasitism efficiency [3, 9]. Previous studies have claimed that *Trichogramma* species can live under a wide range of temperatures from 9 to 36°C [10]. The temperature range of 25–30°C is considered optimal for rearing *T. chilonis* in the laboratory [11]. Temperatures beyond the optimal conditions could cause detrimental effects on various biological aspects of the wasps. For instance, *T. chilonis* and three other *Trichogramma* species cannot parasitize the eggs of *Cnaphalocrocis medinalis* (Gueneé) (Lepidoptera: Pyralidae) at 36°C [12]. The emergence and host parasitization of *T. chilonis* are 98.0% and 95.6% at 28°C but decrease to 33.7% and 60.1%, respectively, at 35°C [9]. Moreover, a low temperature of 15°C leads to long developmental periods for *T. chilonis* (26.3 days) and *Trichogrammatoides bactrae* (25.6 days) (Hymenoptera: Trichogrammatidae) [4]. Although detrimental consequences caused by temperature stress have been well reported, little is known about the molecular response to temperature stress in *Trichogramma*.

Heat shock proteins (HSPs) are highly conserved and ubiquitous proteins that are best known for their responsiveness to multiple stresses such as extreme temperatures, desiccation, anoxia, hypertonic stress, ultraviolet radiation, heavy metals, ethanol, and other contaminants [13, 14]. In general, HSPs act as molecular chaperones that promote correct refolding of proteins and prevent the misfolding or aggregation of proteins [15]. According to their molecular weight and homology, HSPs are classified into several families, including HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs (sHSPs) [16]. To date, certain groups of HSP genes (hsps) have been identified and cloned from various insects such as *Spodoptera litura* (Lepidoptera: Noctuidae), *Thitarodes pui* (Lepidoptera: Hepialidae), *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and *C. suppressalis* [17–20]. An increasing number of hsps have been shown to respond to temperature stress [17, 21, 22]. In an endoparasitoid wasp, *Pteromalus puparum* (Hymenoptera: Pteromalidae), six hsps are induced by 1 h of exposure to −3 and 36°C, including hsp20, hsp40, hsp60, hsp70, hsc70, and hsp90 [23]. The expression patterns of five hsps vary but are indeed induced by heat or cold stress in *Cotesia chilonis* (Hymenoptera: Braconidae) [24]. In addition, hsps have also been reported to be involved in the developmental processes of endoparasitoid wasps such as *Venturia canescens* (Hymenoptera: Ichneumonidae), *Macrocentrus cingulum* (Hymenoptera: Braconidae) and *C. vestalis* [25–27].

*Trichogramma* wasps are often released in the fields during pupal stage [5]. Various biological parameters of the released parasitoids are influenced by ambient temperature [3, 9]. To date, the molecular mechanism of thermal tolerance remains unclear. In present study, six hsps of *T. chilonis* (*Tchsp10, Tchsp21.6, Tchsp60, Tchsp70, Tchsc70-3, and Tchsp90*) were cloned and characterized, and their expression profiles during development were explored. In addition, individuals at the pupal stage were collected to explore the expression patterns of these six hsps in response to various levels of temperature stress (10, 17, 32 and 40°C for 1 h).
temporal expression patterns of six Tchsp were also investigated during cold (10˚C) and heat (40˚C) exposure. To our knowledge, this is the first report on the isolation and analysis of hsps from *T. chilonis*. Our results are expected to help elucidate the potential contribution of these HSPs to thermal tolerance and development.

**Materials and methods**

**Insects**

Prepupae of *T. chilonis* (parasitized eggs) and eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) were obtained from the Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, People’s Republic of China. *T. chilonis* cultures were maintained on irradiated *C. cephalonica* eggs for several generations at 25 ± 1˚C with 75 ± 5% relative humidity and a 14 L:10 D photoperiod.

**Sampling at different developmental stages**

The irradiated eggs of *C. cephalonica* were glued on 4 paper cards (2 × 1 cm) and exposed to freshly emerged *T. chilonis* for 30 min. These egg cards were transferred to different glass cylinders and maintained at 25 ± 1˚C with 75 ± 5% relative humidity and a 14 L:10 D photoperiod. Parasitized eggs on different cards were dissected to collect the larvae, prepupae, pupae and adults of *T. chilonis*. The developmental stages of *T. chilonis* were confirmed under a stereo-scope as described in previous studies [28, 29]. Every 6 h, a small number of parasitized eggs were dissected to determine the developmental stage of *T. chilonis*. At the larval stage of *T. chilonis*, the colors of individuals and parasitized eggs were both white. Larvae with oval shapes were collected. At the prepupal stage, the color of parasitized eggs was black, and pulm spots were visible on the body. Prepupae were collected when pulm spots disappeared from the head and tail. At the pupal stage, the color of parasitized eggs turned deep, red compound eyes appeared on the body, and pulm spots disappeared. Pupae with small black spots on their bodies were collected. The adults were collected once they emerged from the eggs. To collect corresponding individuals, parasitized eggs were immediately placed on a filter-paper soaked with Sample Protector for RNA/DNA (TaKaRa, Dalian, China) and dissected under a stereoscope. The specimens were immediately transferred to TRIzol (Invitrogen, Darmstadt, Germany) and stored in a -80˚C refrigerator. Fifty wasps from each developmental stage were collected. The experiment was repeated three times.

**Temperature exposure**

Considering that *T. chilonis* wasps are often released as pupae inside the host eggs, these parasitized eggs were chosen for temperature exposure experiments. The parasitized eggs were exposed to temperatures of 10, 17, 32 and 40˚C for 1 h, and parasitized eggs kept at 25˚C were collected as controls. These parasitized eggs were then dissected to collect wasps under a stereoscope. In addition, wasps were collected at different time points (1, 2, 4 and 8 h) during cold (10˚C) and heat (40˚C) exposure. The sampling method was the same as described above.

**Cloning the full-length cDNA of hsps**

Total RNA from adults was isolated with a TRIzol Reagent Kit according to the supplier’s instructions. Assessment of the quality and quantity of total RNA was performed by electrophoresis and with a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). First-strand cDNA was generated with a PrimeScript™ RT Reagent Kit (TaKaRa, Dalian, China). Templates for 5’ and 3’ RACE were constructed using a SMART™ RACE cDNA
Amplification Kit (Clontech, California, USA). Primers (S1 Table) were designed based on the nucleotide sequences from the transcriptome data of *T. chilonis* (SRA accession number: SRP119024). PCR products were cloned and then sequenced by Sangon (Shanghai).

**Bioinformatics analysis**

Using the DNASTAR software package, full-length cDNAs of *hsps* were obtained based on the sequenced fragments. The BLAST search was performed to find homologous sequences in GenBank. Multiple sequence alignment and identity analysis were performed using DNA-MAN software. The open reading frame (ORF) and deduced amino acid sequence of each *hsp* were identified and obtained using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The predicted molecular weight and theoretical isoelectric point (pI) of the deduced proteins were predicted with the ExPASy (http://www.expasy.org/). Domains were predicted by SMART tool (http://smart.embl-heidelberg.de/). Phylogenetic analysis was performed using MEGA software (version 6.0) with the 1000 bootstrap replicates. Five neighbor-joining (NJ) phylogenetic trees were constructed using members of HSP10, sHSPs, HSP60, HSP70 and HSP90 family.

**Quantitative real-time PCR**

Total RNA of the samples from each treatment was extracted and reverse transcribed as described above. Primers were designed based on the conserved regions of the *hsps*, and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as the control (S1 Table). The real-time PCR reaction was performed in a 10 μL reaction volume following the manufacturer’s protocol for SYBR® Premix Ex Taq™ (TaKaRa, Dalian, China). The expression profiles of *hsps* were determined on a Roche 480 Real-Time PCR System (Roche, Switzerland) under the following conditions: 95˚C for 3 min, 40 cycles of 95˚C for 10 s, 60˚C for 20 s and 72˚C for 20 s. The melting curve analysis was applied to ensure the specificity of primers at the end of the program. The relative abundance of each *hsp* was calculated according to the 2^−ΔΔCt method [30].

**Statistics**

The expression values of the *hsps* are presented as the means ± SEM. Statistical analysis was performed by SPSS v.16.0 software (SPSS, Chicago, IL, USA) with one-way analysis of variance (ANOVA) and Duncan’s post hoc tests.

**Results**

**Characterization of *hsp* genes**

*Tchsp10.* The full-length cDNA of *Tchsp10* was 705 bp, including an ORF of 315 bp, a 5'-untranslated region (UTR) of 205 bp and a 3'-UTR of 185 bp (GenBank accession number MH490973). The ORF of *Tchsp10* encoded a polypeptide of 104 amino acids with a predicted molecular weight of 11.26 kDa and a pI of 8.93. TcHSP10 showed topical Cpn10 superfamily characteristics with a conserved domain (aa 9–102) and a mobile loop (aa 25–38) (Fig 1).

*Tchsp21.6.* The full-length cDNA of *Tchsp21.6* was 2119 bp, including an ORF of 576 bp, a 5'-UTR of 218 bp and a 3'-UTR of 1325 bp (GenBank accession number MH490974). The ORF of *Tchsp21.6* encoded a polypeptide of 191 amino acids with a predicted molecular weight of 21.68 kDa and a pI of 5.6. TcHSP21.6 was a typical small HSP, containing a metazoan α-crystalline domain (ACD) (Fig 2).
The full-length cDNA of Tchsp60 was 2222 bp, including an ORF of 1716 bp, a 5'-UTR of 197 bp and a 3'-UTR of 309 bp (GenBank accession number MH490975). The ORF of Tchsp60 encoded a polypeptide of 571 amino acids with a predicted molecular weight of 60.48 kDa and a pI of 5.18. TcHSP60 contained a classical mitochondrial HSP60 signature motif (AAVEEGIVPGGG), a C-terminal Gly-Gly-Met repeat (GGM repeat motif) and ATP/ADP binding sites (Fig 3).

Two TcHSP70 genes. The full-length cDNA of Tchsp70 was 2573 bp, including an ORF of 1992 bp, a 5'-UTR of 13 bp and a 3'-UTR of 568 bp (GenBank accession number MH490976). The ORF of Tchsp70 encoded a polypeptide of 663 amino acids with a predicted molecular weight of 73.26 kDa and a pI of 5.62.

The full-length cDNA of Tchsc70-3 was 2668 bp, including an ORF of 1992 bp, a 5'-UTR of 186 bp and a 3'-UTR of 490 bp (GenBank accession number MH490977). The ORF of Tchsc70-3 encoded a polypeptide of 663 amino acids with a predicted molecular weight of 73.34 kDa and a pI of 5.12.

The two TcHSP70 sequences contained three conserved signatures, an ATP-GTP binding site and a non-organellar consensus motif (Fig 4). In addition, the KDEL motif was identified in the deduced amino acid sequence of Tchsc70-3. The EEVD motif was found at the C-terminus of TcHSP70.

Tchsp90. The full-length cDNA of Tchsp90 was 2643 bp, including an ORF of 2181 bp, a 5'-UTR of 145 bp and a 3'-UTR of 317 bp (GenBank accession number MH490980). The ORF of Tchsp90 encoded a polypeptide of 726 amino acids with a predicted molecular weight of 83.48 kDa and a pI of 4.88.

Five highly conserved signature sequences of the HSP90 family were found, including NKEIFLRELISNSSDALDKIR (aa 41–61), LGTIAKSGT (aa 108–116), IGQFGVGFYSAYL-VAD (aa 132–147), IKLYVRRVFI (aa 357–366) and GVVDSEDLPLNISRE (aa 383–397) (Fig 5). The MEEVD motif was identified at the C-terminus of the deduced amino acid sequence.

Phylogenetic analysis of Tchsps. Phylogenetic trees were constructed based on the deduced amino acid sequences of Tchps and their homologous sequences by the neighboring method. The results revealed that HSP10 sequences from T. chilonis and T. pretiosum
Fig 3. Nucleotide sequence and deduced amino acid sequence of \textit{Tchsp60}. The initiation and stop codons are marked with boxes. The conserved domain is shaded in light gray. The ATP binding sites are underlined. The GGM repeat motif is marked with a double line. The classical mitochondrial HSP60 signature motif is shown in bold.

https://doi.org/10.1371/journal.pone.0203904.g003

Fig 4. Multiple amino acid sequence alignments of two genes in the HSP70 family. Three signature motifs of the HSP70 family are shown in light gray. The non-organellar consensus motif is boxed, the localization motif is underlined, and the ATP/GTP binding site is double underlined.

https://doi.org/10.1371/journal.pone.0203904.g004
were clustered together into a single branch (Fig 6(A)). Two HSP60 sequences of endoparasitoid wasps (*T. chilonis* and *P. puparum*) were clustered within a branch (Fig 6(C)). A similar result was also found in the phylogenetic tree constructed with HSP90 sequences (Fig 6(E)). TcHSP21.6 showed a high similarity with HSP21.5 and HSP21.4 from other insects (Fig 6(B)). These sHSPs were clustered together and were separated from HSP beta-1 sequences. The sequences of the HSP70 family from insects presented two clusters, one with HSP70 sequences and another with HSC70-3 sequences (Fig 6(D)). The two TcHSP70 sequences showed a close relationship with their homologous sequences from the hymenopteran species.

**Expression of Tchsps during development.** Real-time PCR was used to measure the expression levels of Tchsps during development. The expression level in the larval stage was used as the control value. The developmental expression profiles of six Tchsps varied significantly in *T. chilonis* (Tchsp10: $F_{3,8} = 304.50, p < 0.001$; Tchsp21.6: $F_{3,8} = 98.80, p < 0.001$; Tchsp60: $F_{3,8} = 332.64, p < 0.001$; Tchsp70: $F_{3,8} = 183.91, p < 0.001$; Tchsc70-3: $F_{3,8} = 11.67, p = 0.003$; Tchsp90: $F_{3,8} = 115.27, p < 0.001$). The expression of Tchsp10, Tchsp21.6 and Tchsp60 decreased from the larval stage to the pupal stage and was maintained at a low level during the pupal and adult stages (Fig 7). Similarly, Tchsc70-3 had the highest expression level during the larval and adult stages, while it was upregulated from the pupal stage to the adult stage. In contrast, the expression of Tchsp70 and Tchsp90 peaked in the adult stage, with 5.63- and 3.27-fold increases, respectively compared to the levels in the larval stage.

**Expression profiles of Tchsps at different temperatures.** All six Tchsps showed the same expression pattern after exposure to different temperatures (10, 17, 25, 32 and 40˚C) for 1 h (Fig 8). They were all significantly upregulated at high temperatures (32 and 40˚C) compared with 25˚C (Tchsp10: $F_{4,10} = 20.71, p < 0.001$; Tchsp21.6: $F_{4,10} = 6.04, p = 0.01$; Tchsp60: $F_{4,10} = 5.56, p = 0.013$; Tchsp70: $F_{4,10} = 64.07, p < 0.001$; Tchsc70-3: $F_{4,10} = 24.07, p < 0.001$; Tchsp90: $F_{4,10} = 22.91, p < 0.001$). Although the expression of Tchsp90 at 32˚C was higher than the levels at 10, 17, and 25˚C, there were no significant differences. The expression levels of Tchsp70, Tchsc70-3 and Tchsp90 were significantly increased from 32 to 40˚C. Among these genes, Tchsp70 had the greatest heat response, with a 7.41-fold increase at 32˚C and a 13.74-fold increase at 40˚C. On the
other hand, the expression levels of six Tchsp s slightly increased after exposure to 10 and 17°C but were not significantly different from the expression levels at 25°C.

The temporal expression patterns of six Tchsp s were also investigated during cold (10°C) and heat (40°C) exposure. The results indicated that the expression levels of Tchsp10 and Tchsp70 were significantly increased after 4 and 8 h of cold exposure (10°C), respectively (F\textsubscript{4, 10} = 3.48, p ≤ 0.05; F\textsubscript{4, 10} = 3.69, p = 0.043). Although the expression levels of other Tchsp s were also slightly increased after cold exposure for different periods of time, they showed no significant differences compared with the control group (Tchsp10: F\textsubscript{4, 10} = 3.48, p ≤ 0.05; Tchsp60: F\textsubscript{4, 10} = 0.49, p = 0.74; Tchsc70-3: F\textsubscript{4, 10} = 1.37, p = 0.31; Tchsp90: F\textsubscript{4, 10} = 1.20, p = 0.37) (Fig 9). On the other hand, the six Tchsp s were strongly expressed after 1 h of heat exposure (40°C) (Tchsp10: F\textsubscript{4, 10} = 10.37, p = 0.001; Tchsp21.6: F\textsubscript{4, 10} = 5.08, p = 0.017; Tchsp60:...
$F_{4, 10} = 11.19, p = 0.001$; $Tchsp70: F_{4, 10} = 9.98, p = 0.002$; $Tchsc70-3: F_{4, 10} = 23.38, p < 0.001$; $Tchsp90: F_{4, 10} = 12.25, p = 0.001$. The expression of $Tchsp10$, $Tchsp60$, $Tchsp70$, $Tchsp70-3$ and $Tchsp90$ decreased from 1 h to 8 h. However, $Tchsp21.6$ exhibited the highest levels at 1 h and 8 h.
Discussion

In this study, full-length cDNAs of six HSP genes were obtained from *T. chilonis*, including *Tchsp10, Tchsp21.6, Tchsp60, Tchsp70, Tchsc70-3* and *Tchsp90*. TcHSP10 contained a mobile
Fig 9. Temporal expression patterns of Tchsp during cold (10°C) and heat (40°C) exposure. Data are presented as the means ± SE (n = 3). Different lowercase letters indicate significant differences. (A) Tchsp10, (B) Tchsp21, (C) Tchsp60, (D) Tchsp70, (E) Tchsp70-3 and (F) Tchsp90.

https://doi.org/10.1371/journal.pone.0203904.g009
loop, which is consistent with the characteristic of other HSP10 sequences described in many studies [31–33]. Through the mobile loop, HSP10 interacts with the HSP60, which helps the folding of protein [34, 35]. Tchsp21.6 encoded a polypeptide of 191 amino acids with a predicted molecular weight of 21.68 kDa. The deduced amino acid sequence of Tchsp21.6 contained an α-crystallin domain (ACD), which is a characteristic feature of the sHSPs family [36, 37]. This family is composed of many members containing variable N- and C-terminal extensions [32, 38]. TcHSP60 belongs to the mitochondrial HSP60 family, containing a conserved signature motif (AAVEEGIVPGGG) and a GGM motif [23, 39]. The GGM motif at the C-terminus has been suggested to provide a suitable physical environment for protein folding [40]. The ATP/ADP binding sites were found in TcHSP60, which have also been identified in HSP60 sequences from Rhopalosiphum padi (L.) (Homoptera: Aphididae) and Lucilia cuprina (Diptera: Calliphoridae) [39, 41]. The highly conserved motif among HSP60 sequences may indicate that a similar mechanism of coupling ATP hydrolysis to the substrate-refolding process exists [14, 39, 42]. The two TcHSP70 sequences had three conserved HSP70 family signatures and a non-organellar consensus motif, in accordance with the structures of the HSP70 sequences described in Nilaparvata lugens (Homoptera: Delphacidae), Sitodiplosis mosellana (Diptera: Cecidomyiidae) and Habrobracon hebetor (Hymenoptera: Braconidae) [43–45]. The two TcHSP70 sequences showed high similarity with their homologous sequences from other insects. These results indicated that the members of HSP70 family are highly conserved [46].

It has been well reported that HSPs are involved in the development of insects [47, 48]. However, the expression patterns of hsps during development vary in insects [49, 50]. For example, the expression of hsp60 increases from the larval stage to the adult stage in Liriomyza sativa (Diptera: Agromyzidae), while it decreases from nymph to adult in R. padi [39, 47]. In this study, Tchsp10 and Tchsp60 levels decreased during development, which is consistent with the findings of report on Galeruca daurica (Coleoptera: Chrysomelidae) [50]. Moreover, Tchsp21.6 showed the same expression pattern as Tchsp10 and Tchsp60. The high expression of three Tchsp in the larval stage indicated that these genes may be related to larval development. In T. chilonis, Tchsp70 and Tchsp90 levels peaked at the adult stage, and Tchsp70-3 expression was upregulated from the pupal stage to the adult stage. It is different from the findings in S. exigua and Frankliniella occidentalis (Thysanoptera: Thripidae) [48, 49]. In contrast, in the endoparasitoid wasp M. cingulum, hsp70 and hsp90 are highly expressed in the pupal and adult stages [26]. The high expression of hsp70 begins at the third-instar larval stage, when C. vestalis comes out of the host [27]. As an egg endoparasitoid, adults of T. chilonis emerge from host, thus facing very different environmental stresses. These HSP genes, i.e., Tchsp70, Tchsp90 and Tchsp70-3, might be needed to overcome these challenges.

HSPs also play important roles in the response to temperature stress [51]. As described in the introduction, HSPs are molecular chaperones that help to prevent potential damage to cellular and molecular structures under temperature and other stresses [15, 52]. Altered expression patterns of hsps have been widely reported under temperature stresses, although these responses seem to be species-specific among insects [39, 43]. In T. pui, the expression of hsp90, rather than hsp70, changes in response to temperature [22]. However, hsp90 and hsp70 in Empoasca onukii (Hemiptera: Cicadellidae) are both highly expressed under cold and heat treatments [53]. Cchsp60 in C. cichorialis responds to cold stress but is insensitive to heat stress [24]. In contrast, the highest expression level of hsp60 in P. picipes appears at 36°C [23]. In this study, Tchsp were sensitive to high temperatures (32 and 40°C), which is consistent with the expression patterns of hsps observed in other species [23, 48, 50]. The expression of Tchsp10, Tchsp21.6 and Tchsp60 showed the same expression pattern in response to high temperatures, each being significantly upregulated under high temperatures but with no significant differences at 32 and 40°C. The upregulation of hsp10 and hsp60 has also been reported in
**Conclusions**

In summary, six Tchsps were cloned and characterized from *T. chilonis*, namely, Tchsp10, Tchsp21.6, Tchsp60, Tchsp70, Tchsc70-3 and Tchsp90. These Tchsps exhibited different expression profiles at different developmental stages, suggesting they may be involved in the development of *T. chilonis*. In pupae of *T. chilonis*, the expression profiles of these genes could be induced by heat shocks (32 and 40°C for 1 h) but did not change in response to cold shocks (10 and 17°C for 1 h). In addition, their expression levels showed time-dependent responses to heat exposure. Tchsp10 and Tchsp70 exhibited a low-intensity cold response at 4 and 8 h. However, Tchsp21.6, Tchsp60, Tchsc70-3 and Tchsp90 did not respond to cold exposure. Due to the difficulty of sampling caused by the tiny size and parasitic characteristics of *T. chilonis*, our study initially explored the expression patterns of hsps during development and temperature stresses. Our study may aid in a better understanding of the roles of hsps at different development stages and in response to temperature stresses.
Supporting information

S1 Table. Primers used for different PCRs.

(DOCX)

Acknowledgments

We thank Ms. Xinxia Feng for the technical assistance and everyone who contributed to the development of this work at the Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences.

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