

# Characteristic and Expression of Hsp70 and Hsp90 Genes from *Tyrophagus Putrescentiae* and Their Response to Thermal Stress

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## Research Article

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2 *Tyrophagus putrescentiae* and their response to thermal stress

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27 **Abstract**

28 *Tyrophagus putrescentiae* (*T. putrescentiae*) is a worldwide stored grain pest mite  
29 which has done great harm to human's economy and health. Temperature has a great  
30 influence on the population dynamics of *T. putrescentiae*. In this study, the cDNA of *T.*  
31 *putrescentiae* HSP70 and HSP90 (designated *TpHSP70-1*, *TpHSP70-2* and *TpHSP90*)  
32 were cloned, and their expression was determined by fluorescent real time  
33 quantitative PCR. Results indicate that *TpHSP70-1*, *TpHSP70-2* and *TpHSP90*  
34 contained an open reading frame of 1,998 bp, 1,984 bp and 2,157 bp that encoded 665,  
35 661 and 718 amino acid residues, respectively. Sequences and phylogenetic analyses  
36 suggested that *TpHSP70* and *TpHSP90* showed high homology with other species.  
37 The RT-qPCR results indicated the relative expression level of *TpHSP90* and  
38 *TpHSP70* gene at different stages were different. Also thermal stress could increase its  
39 expression significantly. This study indicates that *TpHSP70* and *TpHSP90* are very  
40 important genes for *T. putrescentiae* to defend against thermal stress.

41

42 **Key words:** *Tyrophagus putrescentiae*, *Hsp70*, *Hsp90*, gene cloning, RT-qPCR

43

44 **1. Introduction**

45 *Tyrophagus putrescentiae* (*T. putrescentiae*) can be found throughout buildings  
46 around the world such as farms, laboratories, urban environments, and food industries  
47 (Duek et al., 2001; Solarz et al., 2007), and have successfully been isolated from these  
48 places (Smrz and Jungova, 1989; Rozej et al., 2012). Recently, *T. putrescentiae* has  
49 been reported to be associated with host bacterial communities or symbionts in the gut,  
50 fat body or other tissues. (Hubert et al., 2012; Kopecky et al., 2014; Brown and Lloyd,  
51 2015). The storage mites is one of the aeroallergens, can caused asthma and rhinitis in  
52 frequently sensitising individuals (Liao et al., 2013a; Fernandez-Caldas et al., 2014).  
53 In some case, Storage mites may lead to anaphylaxis caused by the ingestion of  
54 contaminated foods (Liao et al., 2013b; Yu et al., 2014).

55 Temperature is usually the environmental factor with the greatest effect on  
56 developmental rate of immature mites and other poikilotherms, because of their weak  
57 ability to regulate body temperature (Southwood 1978). Their body temperature is  
58 close to their habitat temperature and they are vulnerable to extreme temperature  
59 (Rinehart et al., 2005). Insects can perform normal growth and development at the  
60 appropriate temperature. If the external temperature is too high or too low, their  
61 growth and development will stagnate and even lead to their death (Trullas et al.,  
62 2007). In addition, high temperatures can cause developmental abnormalities or  
63 defects in insects (Mironidis et al., 2005).

64 Heat shock proteins (*Hsps*) are molecular chaperones which play crucial roles in  
65 protein folding, unfolding, aggregation, degradation, and transport (Tiwari et al.,  
66 2015). It is an important vector protein molecule for temperature change in insect  
67 experimental environment. Under thermal stress, the participation of *Hsps* in the  
68 formation of cell tolerance is conducive to improving the viability of organisms in

69 adversity (Xu et al., 2011; Colinet et al., 2013). Bettencourt had treated drosophila at  
70 30-39 °C and detected the expression of *Hsp70* in different stage, found that heat  
71 resistance of flies is closely relative to the expression of *Hsp70* (Bettencourt et al.,  
72 2007). The expression of *Hsp70* gene in *Bemisia tabaci* female was inhibited by  
73 RNAi, and the survival number was found to be significantly lower than that in the  
74 control group (Lv et al., 2011). Those results suggested that *Hsps* played an important  
75 role in improving the heat resistance of insects.

76 To explore temperature tolerance of *T. putrescentiae* under thermal stress, we  
77 cloned the full-length of *Hsp70* and *Hsp90* cDNAs from *T. putrescentiae*, and  
78 measured the mRNA expression of *Hsp70* and *Hsp90* at different temperature using  
79 real-time quantitative polymerase chain reaction (RT-qPCR). The results will help us  
80 understand the adaptation of *T. putrescentiae* under temperature stress.

81

## 82 **2. Materials and methods**

### 83 **2.1 Mites**

84 The *T. putrescentiae* adults were collected from the storages at Nanchang suburb,  
85 Jiangxi Province, China. The mites were maintained on wheat bran (Shangdong,  
86 China) and the tailor-made plastic containers (18×11×8 cm), which was covered with  
87 a lid to prevent escape, was used to feeding mites. A 3cm diameter hole was shorn on  
88 the lid for ventilation (Sánchez-Ramos et al., 2007).

89 We use climate-controlled incubators (RXZ-260B) to keep the rearing units for  
90 several generations at 25±0.5°C, 75±5% relative humidity (controlled by YADU  
91 ultrasonic humidifier) and dark conditions.

### 92 **2.2 Total RNA extraction and first strand cDNA synthesis**

93 300 female adults of *T. putrescentiae* were separated from experimental

94 population. Total RNA was extracted using the TRIzol method (Invitrogen, San Diego,  
 95 CA, USA) and then treated with DNase I (Tiangen, Beijing, China). Concentration  
 96 and purity of the RNAs were detected by NanoDrop2000 (Thermo, USA) at 260 nm  
 97 and 280 nm, respectively. Finally, using 1% agarose gel electrophoresis to check the  
 98 integrity of total RNA. The first strand cDNAs were obtained using Reverse  
 99 Transcription M-MLV Kit (TaKaRa, Tokyo, Japan).

### 100 **2.3 Degenerate primers and amplification of cDNA**

101 To amplify partial cDNA fragments of *Hsp70* and *Hsp90*, degenerate primers  
 102 (Table 1) were designed and used in PCR according to previous study (Feng et al.,  
 103 2009, Yang et al., 2012). The PCR reactions use 0.1 µg cDNA as templates, 0.3 µM of  
 104 each primer, 12.5 µL 2×Taq polymerase Mix (Tiangen, Beijing, China) and add  
 105 ddH<sub>2</sub>O to 25µl.

106 The PCR programs were operated with following cycling conditions: initial  
 107 denaturation step of 3 min at 94°C, followed by 35 cycles of 94°C for 30 S, 49°C for  
 108 30 S and 72°C for 60s, 72°C for 10min. The PCR products were detected with 1%  
 109 agarose gel. Bands with expected size being purified by a universal DNA purification  
 110 kit, Purified DNA fragments were cloned into the pGEM-T Easy vector and  
 111 transfected into *Escherichia coli* DH5α cells (Promega, Madison, WI, USA).The  
 112 DNA inserts of the recombinant clones were confirmed by PCR with the same  
 113 degenerate primers used previous and by sequencing in both directions.

114 **Table 1.** Primers used in this study.

<b>Experiments</b>	<b>Primer name</b>	<b>Primer sequences(5'-3')</b>
Full length PCR amplification	TpHSP70-1F	CAAGCTACCAAACGCGACTAACC
	TpHSP70-1R	AAAGGAGCCGCAATGTTGAATG
	TpHSP70-2F	AAAGGAAGAGGTTGGCAAGGAA

	TpHSP70-2R	CAGTTGAAGGAAGGGTAGAGTGGA
	TpHSP90F	TACTCTACGCTTTACATCCGAACAC
	TpHSP90R	GAACAACCTTAATCAACCTCCTCCAT
Hsp70-3'-RACE	3TpHSP70-1F1	CACCAAGATGAAGGAGACCGCCGAG
	3TpHSP70-1F2	CAAGAAGACCCAGGGCGAGAAGAAC
	3TpHSP70-2F1	ACAAGAAGGAGGGCGAGAAGAAC
	3TpHSP70-2F2	GATCGACAATGAAGCCCGTGCAG
Hsp70-5'-RACE	5TpHSP70-1R1	GATCGACTTGTTGAGCTCCTTGCC
	5TpHSP70-1R2	GGCACGGGTGATCGAGGAGTAGAA
	5TpHSP70-2R1	GCTGCGAATGTCCTTGCCGGTCT
	5TpHSP70-2R2	CCACCGCCCAGATCGAACACCAG
Hsp903'-RACE	3TpHSP90F1	CGTCAAGAAGTGCCTGGAGCTGT
	3TpHSP90F2	TGAGGAGAAGAAGAAGCGCGAGG
Hsp90-5'-RACE	5TpHSP90R1	CATGTAGACGACCTCGAAGCCAC
	5TpHSP90R2	CGTCAAACAGCTCCAGGCACTTC
	qTpHSP90F	TTCCAGTGGCGACGAGATGT
	qTpHSP90R	TCCTCAGGCAGCTCCAGACC
	qTpHSP70-1F	CACTAACGACAGGCGATTCTACG
	qTpHSP70-1	TCAACCGAACAACCACTCAAGC
Real-time PCR	qTpHSP70-2F	GAACTGGTCCTGCTCGATGTGAAC
	qTpHSP70-2R	ACCCTCGAAGACTTGAATTGTGACG
	qTpTubulinF	TATCCTCGCATTCACCTTCTCTGG
	qTpTubulinR	CGAAGCAGGTGTTGGTAATCTCAGC

115

### 116 2.3 Rapid amplification of cDNA ends

117 The rapid amplification of cDNA ends (RACE) method was applied to obtain  
118 full-length cDNAs. Gene specific primers in Table 1 were designed by identified  
119 *Hsp70* and *Hsp90* cDNA fragments, and 5' and 3'-Full RACE Kits (TaKaRa, Tokyo,  
120 Japan) were used to amplify the 5' and 3'-ends of the two genes. The nested PCR

121 reactions were as following: The first-round PCR program was as follows:  
122 pre-denaturation for 180 S at 94°C, 35 cycles of 94°C for 30 S, 60°C for 30 S and  
123 72°C for 90 S with a final extension of 6 min at 72°C. The second-round PCR  
124 program was similar to that of the first-round.

125 PCR products from 5'- and 3'-RACE reaction were cloned into the pGEM-T Easy  
126 vector and transfected into *Escherichia coli* DH5α cells (Promega, Madison, WI,  
127 USA). Six recombinant clones were identified by PCR amplification and then  
128 sequenced (Sangon, Shanghai, China).

#### 129 **2.4 Confirmation of full-length cDNA sequences**

130 After the 5'-ends and 3'-ends sequences were obtained, the contigs were assembled  
131 to produce putative full length of HSP70 and HSP90 sequences by using the Seqman  
132 software. To verify the full length of putative HSP70 and HSP90 cDNAs, amplifying  
133 the ORF of HSP70 and HSP90 cDNAs using full length confirmation primer listed in  
134 table 1, and the PCR products cloned into pGEM-T Easy vector and sequenced. The  
135 PCR conditions and cloned methods were same with previously described.

#### 136 **2.5 Bioinformatics analysis**

137 Blast the sequence of *TpHSP70* and *TpHSP90* at the National Center for  
138 Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>) at both nucleotide  
139 and amino acid levels by performing the BLAST program. Using Expert Protein  
140 Analysis System (<http://www.expasy.org/>) to analyse the amino acid sequence.  
141 Executing multiple alignments of *TpHSP70* and *TpHSP90* in DNASTar software (7.1  
142 versions) to further analysis. Using ClustalX 2.0 and MEGA 5.0 constructing  
143 Neighbor-joining phylogenetic tree, based on the sequences of HSP70 and HSP90  
144 genes (Table 2 had list the sequence code). Estimate the confidence of branches  
145 produced by using 1000 replicates' Bootstrap analysis.

**Table 2** HSP70 and HSP90 sequences used in phylogenetic analysis

Accession Number	Organism	Hsp70 or Hsp90
NP_001036837	<i>Bombyx mori</i>	<i>BmHSP70-1</i>
NP_727563	<i>Drosophila melanogaster</i>	<i>DmHSP70-3</i>
AIS39468	<i>Haemaphysalis lava</i>	<i>HlHsp70</i>
NP_005338	<i>Homo sapiens</i>	<i>HsHSP70</i>
XP_002407132	<i>Ixodes scapularis</i>	<i>IsHsp70-2</i>
XP_002433656	<i>Ixodes scapularis</i>	<i>IsHsp70-1</i>
ABK76338	<i>Marsupenaeus japonicus</i>	<i>MjHsp70</i>
BAE39187	<i>Mus musculus</i>	<i>MmHsp70-1</i>
BAE41082	<i>Mus musculus</i>	<i>MmHsp70-2</i>
AGQ50609	<i>Neoseiulus cucumeri</i>	<i>NcHsp70</i>
ADE34170	<i>Nilaparvata lugens</i>	<i>NlHsp70</i>
XP_001510947	<i>Ornithorhynchus anatinus</i>	<i>OaHsp70</i>
ADM83423.1	<i>Panonychus citri</i>	<i>PcHsp70-1</i>
ADM83424.1	<i>Panonychus citri</i>	<i>PcHsp70-2</i>
ADM83425.1	<i>Panonychus citri</i>	<i>PcHsp70-3</i>
NP_077327	<i>Rattus norvegicus</i>	<i>RnHsp70</i>
ACG60423	<i>Tetranychus cinnabarinus</i>	<i>TcHsp70-2</i>
ACG60424	<i>Tetranychus cinnabarinus</i>	<i>TcHsp70-3</i>
ABC33921	<i>Tetranychus urticae</i>	<i>TuHSP70</i>
AAB97092	<i>Xenopus laevis</i>	<i>XlHsp70</i>
KR479867	<i>Tyrophagus putrescentiae</i>	<i>TpHSP70-1</i>
KR479868	<i>Tyrophagus putrescentiae</i>	<i>TpHSP70-2</i>
AJQ31840.1	<i>Tyrophagus putrescentiae</i>	<i>TpHSP90</i>
NP_001036876.1	<i>Bombyx mori</i>	<i>BmHSP90</i>
ADP37710.1	<i>Helicoverpa armigera</i>	<i>HaHSP90</i>
BAE44307.1	<i>Chilo suppressalis</i>	<i>CsHSP90</i>

AAA96259.1	<i>Xenopus laevis</i>	<i>XlHSP90</i>
CAA30276.1	<i>Drosophila melanogaster</i>	<i>DmHSP90</i>
ACO83357.1	<i>Penaeus monodon</i>	<i>PmHSP90</i>
EEC05121.1	<i>Ixodes scapularis</i>	<i>IsHSP90</i>
XP_003745497.1	<i>Metaseiulus occidentalis</i>	<i>MoHSP90</i>
AAB23369.1	<i>Rattus sp.</i>	<i>RaHSP90</i>
AAI21063.1	<i>Homo sapiens</i>	<i>HsHSP90</i>
BAK08840.1	<i>Chara braunii</i>	<i>CbHSP90</i>
AGQ50610.1	<i>Neoseiulus cucumeris</i>	<i>NcHSP90</i>
ACF75907.1	<i>Tetranychus cinnabarinus</i>	<i>TcHSP90</i>
ADM83426.1	<i>Panonychus citri</i>	<i>PcHSP90</i>

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147

## 148 **2.6 mRNA expression of *TpHSP70* and *TpHSP90* under thermal stress**

### 149 2.6.1 Sample collection at different stages

150 Separating the eggs of *T. putrescentiae* with 140 mesh sieve. These eggs were  
 151 continued to grow and develop, and observed every 12 h. After proliferation for many  
 152 generations, about 400 eggs, 400 larvae, 200 nymphs and 200 female adults were  
 153 picked out for the extraction of total RNA, respectively. Each experiments group was  
 154 repeated for 3 times.

### 155 2.6.2 Treatments of themal stress

156 Transferred two hundred female adult mites into a 1.5 ml centrifuge tube in brush  
 157 (Jiangxi, China). The tubes were sealed by 0.45 um filter membrane (BBI, China) for  
 158 ventilation. Then ten tubes, each with 200 mites, were treated at a target temperature  
 159 including 0, 5, 10, 15, 20, 30, 33, 36, 39, and 42 for 1 h, respectively. Each groups  
 160 repeat for 3 biology treatment. Mites kept at 25°C were served as the control.

## 161 **2.7 qPCR for the *TpHSPs***

162 The qPCR assays were performed on Fx960 Real-time Quantitative PCR  
163 (BIO-RAD, USA) with alpha-tubulin gene from *T. putrescentiae* (GenBank accession  
164 number: AY986760) as endogenous reference. For each gene specific primer, there are  
165 three biological duplication and three technical repetition. Finally, the expression  
166 levels of *TpHSP70* and *TpHSP90* were calculated with the  $2^{-\Delta\Delta C_t}$  method (Livak and  
167 Schmittgen, 2001).

## 168 **2.8 Data analysis**

169 Statistical analysis was carried out using SPSS 22.0 software (Chicago, IL, USA)  
170 for ANOVA followed by Duncan's multiple comparison tests to detect statistical  
171 difference. Significance was set at a value of  $P < 0.05$ .

172

## 173 **3. Results**

### 174 3.1 Sequence analysis of *TpHSP70* and *TpHSP90* gene

175 Complete cDNA sequence of *TpHSP70-1* (GenBank accession number:  
176 KR479867) and *TpHSP70-2* (GenBank accession number: KR479868) were 2,494  
177 and 2354 bp in length with ORF of 1,998 and 1,984 bp. As shown in Figure 1A and  
178 1B, respectively. *TpHSP70-1* encoded a 665 amino acid protein. The *TpHSP70-1*  
179 cDNA included a 186 bp 5'untranslated region (*UTR*) and a 310 bp 3' *UTR*. The  
180 mature protein had a calculated molecular weight of 72.72 kDa with an isoelectric  
181 point of 5.21. A possible consensus signal sequence for polyadenylation (AATAAA)  
182 was located 45 bp upstream of the poly (A) tail. *TpHSP70-2* encoded a 661 amino  
183 acid protein. The *TpHSP70-2* cDNA included a 168 bp 5'untranslated region (*UTR*)  
184 and a 173 bp 3' *UTR*. The mature protein had a calculated molecular weight of 72.75  
185 kDa with an isoelectric point of 5.29. A possible consensus signal sequence for  
186 polyadenylation (AATAAA) was located 45 bp upstream of the poly (A) tail. The

187 *Hsp70-1* and *Hsp70-2* were both having three typical motifs which were observed in  
188 *Hsp70* proteins family.

189 Complete cDNA of *TpHSP90* gene was deposited in GenBank (accession  
190 number: KJ820823). As shown in Figure 1C, the full length of the *TpHSP90* cDNA  
191 consisted of 2,538 bp with an ORF of 2,157 bp, which encoded a 718 amino acid  
192 protein. The *TpHSP90* cDNA included a 165 bp 5' untranslated region (*UTR*) located  
193 upstream of the putative start codon (ATG) and a 216 bp 3' *UTR* located downstream  
194 of the stop codon. The mature protein had a calculated molecular weight of 82.79 kDa  
195 with an isoelectric point of 4.92. A possible consensus signal sequence for  
196 polyadenylation (AATTA) was located 15 bp upstream of the poly (A) tail. The  
197 typical histidine kinase-like ATPases domain, which is ubiquitous in all *Hsp90* family  
198 members, was located at the position of 37-181. The five typical motifs were observed  
199 in *Hsp90* proteins family. *Hsp90* was found to be highly conserved in eukaryotic  
200 organisms. The amino acid sequence of *TpHSP90* has five typical motifs of *Hsp90*  
201 family: NKEIFLRELISNASDALDKIR, LGTIAKSGT, IGVFGVGFYSAYLIAD,  
202 IKLYVRRVFI and GVVDSIDLPLNISRE. C-terminal "MEEVD" motif, which is  
203 specific to the cytoplasmic type of *Hsp90* family. Comparative analysis showed that  
204 the amino acids of *TpHSP90* had a high similarity of 79-81% with other species.

A

1 CREATEDSUNDAYAPRILPMGAGAAAATACTCACACAGCAAAAATTGCAAAAGTGAACAT  
61 AGCTAACAAAGCTAACAAAGCTACCAAACGGGACTAACCGCTAACTTTACTGCGAAAAATC  
121 TTTTCTCTGTGAATCTTTTCACTTCAGAATTTCAACTTCAACAAAATTTGATCCCTCAT  
181 CAGAAAAATCACCAACCACCAACAATGTCTCCTAAGCAGTCAAAACGTTGCCATCGGCA  
M S P K Q S N V A I G  
241 TCGACCTGTGCACGACTACTCTGCGTGGTGTCTTCCAGCACGGCCAGGTGGAGATCA  
L D L C T T Y S C V G V F Q H G Q V E I  
301 TCGTCAATGACCAGGGCAACCGAACGACGCCCTCATTGTCAGCTTCCAGCACCGGAGC  
I V N D Q G N R T T P S F V S F T D T E  
361 GACTGATTGGCGATGCCGCAAGAACCAGGTGGCACTGAACCGACGAACACCGTCTTTG  
R L I G D A A K N Q V A L N P T N T V F  
421 ACGCGAAGCGACTGATTGGCCGCGACTCGACGACGCCCTGTCCAGTCCGACATGAAGC  
D A K R L I G R R L D D A S V Q S D M K  
481 ACTGGTCTTTGAGTGGTGAACGACAGTGGCAAGCGAAGATCCAGGTGGAGTGAAGG  
H W S F A V V N D S G K P K I Q V E C K  
541 GCGAGACGAAACGCTTCGCCCTGAGGAGATCTCTCGATGGTGGCTACCAGGATGAAGG  
G E T K R F A P E E I S S M V L T R M K  
601 AGACCGCGAGGGCGTACCTGGGAACACGGTTACCAACGCGCTCATTACCGTACCTGCTT  
E T A E A Y L G T T V T N A V I T V P A  
661 ACTTTAACGATAGCCAACGCCAGGCGACCAAGGACGCGGAGTGATCGCCGGCTCAACG  
Y F N D S Q R Q A T K D A G V I A G L N  
721 TGCTGCGATCATCAACGAGCCACTGCTGCGGCCATCGCTACGGCCTGGACAAGAAGA  
V L R I I N E P T A A A I A Y G L D K K  
781 CCCAGGGCGAGAAGAAGCTGCTCATCTTCGACCTGGGCGGCGCACCTTCGATGTCTCCA  
T Q G E K N V L I F D L G G G T F D V S  
841 TTCTACCATCGAGGACGGCATCTTCGAGGTGAAGTCCACCGCGGTGACACCCACCTCG  
L L T I E D G I F E V K S T A G D T H L  
901 GTGGCGAGGACTTTGACAACCGCTGGTGAACCACTTTGTGAGGAGTCAAGCGCAAGC  
G G E D F D N R L V N H F V Q E F K R K  
961 ACAAGAAGGACCGCTCGGACAACAAGCGCGCCCTGCGCGCTGCGCACCGCTGCGAGC  
H K K D P S D N K R A L R R L R T A C E  
1021 GAGCCAAGCGAAGCTCTCCTCCTCCACCCAGGCTCCATCGAAATCGACTCGCTCTACG  
R A K R T L S S S S T Q A S I E I D S L Y  
1081 AGGCGATCGACTTCTACTCCTCGATCACCCGTCGCGCTTTGAGGAGCTGCGCGCGG  
E G I D F Y S S I T E A R F E E L C A D  
1141 TCTTCGCTCGACGCTGGACCCGGTGGAGAAGTCGCTGCGGACGCCAAGCTCGACAAGA  
L F R S T L D P V E K S L R D A K L D K  
1201 ACCAGATCCAGAGATTGCTCCTCGTGGTGGCTCCACTCGCATCCCAAAGATCCAGAAGC  
N Q I H E I V L V G G S T R I P K I O K  
1261 TGCTGACGACTTCTCAACGGCAAGGAGCTCAACAAGTCGATCAACCCGATGAGGGCG  
L L Q D F F N G K E L N K S I N P D E A  
1321 TCGCCTACGGAAGCGCGCTCCAGGCTGCCATTCTCAACGGGACGAGTCCGAGGCTGCC  
V A Y G A A V Q A A I L N G D E S E A V  
1381 AGGACCTGCTGCTCGACGTGGCGCCCTTTTCGATGGGATTGAAACTGCCGGGGCG  
Q D L L L L D V A P L S M G I E T A G G  
1441 TCATGACCACGCTGATCAAGCGCAACACCACCTCCCAACGAGACGACGACCTTCT  
V M T T L I K R N T T I P T K Q T Q T F  
1501 CCACCTACTCGGACAATCAGCCCGGTGTGCTGATTAGGTCTTTGAGGGGAGCGAGCAA  
S T Y S D N Q P G V L I Q V F E G E R A  
1561 TGACCAAGGACAACAACCTGCTGGGAAAGTTGAGCTGACGGGCATTCCGCCGGCACCTC  
M T K D N N L L G K F E L T G I P P A P  
1621 GCGGCGTCCCCAGATTGAGGTACCTTTGACATTGACGCCAACGGCATTCTCAACGTCT  
R G V P Q I E V T F D I D A N G I L N V  
1681 CTGCCGTGGACAAGAGCACCGGCAAGCAGAACAAGATCACCATCAACGACAAAGGGC  
S A V D K S T G K Q N K I T I T N D K G  
1741 GTCTGACAAAGGAGGACATTGAGCGCATGGTCAAGGAGGCGAGTCTACCGGACGAGG  
R L T K E D I E R M V K E A E S Y R D E  
1801 ACGAGAAGCAGCGCACCGCATCGCCCGCGCAATGCCCTCGAGGGTACGCCTTCCGA  
D E K Q R D R I A A R N A L E G Y A F Q  
1861 TCAAGTCGACGATTGAGGAGGAGTTATCAAGTCGAAGATCTCAGAGGAGGACCGCAAGA  
I K S T I E E V I K S K I S E E D R K  
1921 AGATTAGCGAGAAGGTGGAGGAGACGGTCAAGTGGCTGGACGCGAATGCCCTCGCCGAGA  
K I S E K V E E T V K W L D A N A L A E  
1981 AGGAGGAGTTTGGACACCAGCGGAAGGAGTGGAGGGCATCTGCAACCCCATCATCACCA  
K E E F E H Q R K E L E G I C N P I I T  
2041 AGCTGTACCAGCAGGCGGGCGTGGCCCTGGTGGATGCCCGGTGGAATGCCCGAAGGCT  
K L Y Q Q A G G A P G G M P G G M P E G  
2101 TCCCCGGCGGATTCCCCGGTGTGCCGGCGGAGCTCCCCGTGCCGGCGGCCAGCTCTG  
F P G G F P G A A G G A P G A G G A S S  
2161 GCGACAAGTCCGGCTCTGGCCCCACCATCGAGGAGGTGACFAAGTCTTCGCTCGCTCTC  
G D K S G S G P T I E E V D \*  
2221 GCCCTCTCTCCACTCTCATTCAACATTGGCGGCTCCTTAATCTTCTGTACACACAGC  
2281 ACACACCACACCCGAGCCACCATTTCTCTCTCTCCCTCTTTAATCTCTCTCAC  
2341 ACACACCACACACACCCCTCCCTTTCTGTTATTATAATTTCACTCATTCTTTAG  
2401 CATTCTCTTTATTCTTATTATAATACTAGCAATTATAAATTATAACACTGTATGC  
2461 GAAAATAAATCGGTTTGAATTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

# B

1 GAAAAGTCAACGTTGTCTCGTGGACAGTTCTCTCAC  
61 TCAATCTTCAGACTTTCTTCAGTTTTACTCTTCAGTGTTCCTCACTTTCTCTCTCTCC  
121 CCTCCCTCTCTCTCTGAGGTGATTTACCTCTTTAATAGCGGATACATTGCGCGATCTGA  
181 GCCTCAGTATTATCACCTGATCGACTGAATTCCTCCACCATGAAGACACTGTTCCCGGGG  
M K T L F P A  
241 TTCTCCTGCTGACGACGTTGCTGGTGACGATCGTCTACTAAAGGAAGAGTTGGCAAGG  
V L L L T T L L V T I V Y S K E E V G K  
301 AAAAGGCTGACGTCGGCACTGTGATTGGCATTGATTAGGAACCACTTACTCTTGTGTGG  
E K A D V G T V I G I D L G T T Y S C V  
361 GCGTTTTCAAGAATGGTCGAGTGGAGATCATGCCAACGATCAAGGTAACCGAATTACGC  
G V F K N G R V E I I A N D Q G N R I T  
421 CCTCCTACGTGGCCTTTACCGCCGAGGGTGAGCGTCTGATTGGTGACGCGGCAAGAAGC  
P S Y V A F T A E G E R L I G D A A K N  
481 AGTCCACCTCGAACCCGAGAACACCGTCTTTGACGCTAAGCGACTCATTGGACGCGAGT  
Q L T S N P E N T V F D A K R L I G R E  
541 GGACTGACAAAAATGTCCAACATGATCTCAAGTTCTTCCCTTCAAAAATGATTGAAAAA  
W T D K N V Q H D L K F F P F K M I E K  
601 ACTCAAAACCATATCAAAGTAAAGACGAGCACCGGTGATAAGGTGTTGCCCCGAGG  
N S K P H I K V K T S T G D K V F A P E  
661 AGATCAGTGCCATGGTGTCTGCTGAAGATGAAGGAGACCGCGAGGCGTACCTGGGCAAGA  
E I S A M V L L K M K E T A E A Y L G K  
721 AGGTCACCCACGCGTGGTACAGTCCCTGCATATTTAACGATGCCAACGCCAGGCGA  
K V T H A V V T V P A Y F N D A Q R Q A  
781 CGAAGGACGCGGAGCCATTGCCGGTCTGAATGTGATGCGAATCATCAACGAGCCCACTG  
T K D A G A I A G L N V M R I N E P T  
841 CCGCCGCTTGCCTACGGTCTGGACAAGAAGGAGGGGCGAGAAGAACATTCTGGTGTTCG  
A A A I A Y G L D K K E G E K N I L V F  
901 ATCTGGGCGGTGGCACCTTTGATGTCTCCCTGCTGACCATCGACAACGGCGTCTTTGAGG  
D L G G G T F D V S L L T I D N G V F E  
961 TGGTGGCCACCAACGGTGACACTCACTTGGGCGGTGAGGACTTTGACCAAGCGCGTATGG  
V V A T N G D T H L G G E D F D Q R V M  
1021 AGCACTTTATCAAGCTGTACAAGAAAAAGACCGGCAAGGACATTGCGAGCGACAACCGAG  
E H F I K L Y K K K T G K D I R S D N R  
1081 CTGTGCAAGAAGCTCGCTCGAGAGGTGGAGAAGGCCAAGCGAAGCGTGTCTCCCGCCACC  
A V Q K L R R E V E K A K R T L S S A H  
1141 AGGCTCGCATTGAAATCGAGTCCCTCTTTGACGGCGAGGACTTCAGCGAAGTGTCTACCC  
Q A R I E I E S L F D G E D F S E V L T  
1201 GAGCCAAATTCGAGGAGCTGAACATGGACCTTTCAGATCGACAATGAAGCCCGTCGAGA  
R A K F E E L N M D L F R S T M K P V Q  
1261 AGGTGCTCGAGGACGCGGACCTGCAGAAGAAGGACATCGATGAGATCGTCTCGTGGTGG  
K V L E D A D L Q K K D I D E I V L V G  
1321 GCTCCACTCGCATTCCCAAGGTGCAGCAGTGGTGAAGGAGTTCTTCAACGGAAAGGAGC  
G S T R I P K V Q Q L V K E F F N G K E  
1381 CCACTCGAGGCATCAACCTGACGAGGCCGTGCGCTTGGTGGCCGCTGTCCAGGCTGGG  
P T R G I N P D E A V A F G A A V Q A G  
1441 TCCTCTCGGGCAGGAGAACACCGGTGAACCTGGTCTGCTCGATGTGAACCCCTCACCA  
V L S G E E N T G E L V L L D V N P L T  
1501 TGGGCATTGAAACCGTCCGGGTGTGATGACCAAACTGATCACCGAAAACCGTCATCC  
M G I E T V G G V M T K L I T R N T V I  
1561 CCACCAAAAAGTCGAGATCTTCAGTACCGCTGCTGACAACCAGAACACCGTCACAATTC  
P T K K S Q I F S T A A D N Q N T V T I  
1621 AAGTCTTCGAGGGTGAACGTCCTCAATGACCAAAAGACAACCATCAACTGGGCAATTGATC  
Q V F E G E R P M T K D N H Q L G K F D  
1681 TCACCGGCATCCCACTGCCCTCGTGGTGTCCCCAGATTGAGGTACCTTTGAAATTG  
L T G I P P A P R G V P Q I E V T F E I  
1741 ACGTCAACGGTATCTCAAGTTACCGCTGAGGACAAGGGCACCGGCAACAAGGAAAAAGA  
D V N G I L K V T A E D K G T G N K E K  
1801 TTGTATCACCAATGACCACAATCGTCTCTCACCCGAGGACATTGAACGATGATCAAGG  
I V I T N D H N R L S P E D I E R M I K  
1861 ATGCGGAAAAGTTCTCCGACGAGGACAAGAAGGTGAAGGAGAAGGTTGAGGCGAAGAAGC  
D A E K F S D E D K K V K E K V E A K N  
1921 AGCTCGAGTCGATGCGTACTCACTGAAAAACAGATCGGCGACAAGGAGAAGCTCGGGT  
E L E S Y A Y S L K N Q I G D K E K L G  
1981 GCAAACTGTCGACGATGACAAGAAGACTATCGAGAGCTCCGTTGATGAGACCATCAAGT  
G K L S D D D K K T I E S S V D E T I K  
2041 GGCTCGACGCCAACGCTGATGCCGACTCTGAGGAGCTAAAGGCCGCAAGAAGAACTCG  
W L D A N A D A D S E E L K A R K K K K L  
2101 AGGAGACCGTACGCCCATCATCTCGAAGCTGTACGCGGGTCCCGGGGCGGACGCGGGAC  
E E T V T P I I S K L Y A G A G G D A G  
2161 AGCCGCGCTGAGGAGACGCTCGTCCAAAGGACGAACCTAGAAAAAGGTTTCCCTT  
Q P P A E E D A S S K D E L \*  
2221 TCCGTTCTCTTTCCTACTTACCTTCTTCAACTGGATGTACATACGCACTACTAACA  
2281 ATTAACCAATTGGCTAGCATAGTAATCTCTTCTTTCTCTCTT AATAAA AATCTCAT  
2341 ATTTTACAGATCAAAAAAAAAAAAAAAAAAAAAAAAAA

**C**

1 GAAAAATTTCGCTTCTGCTCGCGTCCGGCAACGTTGCTCGCTCAACGCTTGGCAACTCTCCCATTCATCCCTTT  
76 GTCTCGGTTTCGACTTGTGCGTTTAAAGTTCAACGTTGCTTTTACTCTACGCTTTACATCCGAACACCACCACACA  
151 CCATCATCCACGACC[ATG]CTGAAGAAGCAGCAAGAGGTCGAGACCTTCGCGCTCCAGGCCGAGATCGCCCGAGTTG  
1 M P E E Q Q E V E T F A F Q A E I A Q L

226 ATGAGCCTGATCATCAACACTTTTCTACTCGAACAAGGAGATCTTCTCGGTGAGTTGATCTCTAACCGCTCTGAC  
21 M S L I I N T F Y S N K E I F L R E L I S N A S D

301 GCCTCGACAAAAATCCGCTACGAATCTCTACTGACCCGACCAAGATCTCCGAGGTGAAGGACTTTCACATTCCG  
46 A L D K I R Y E S L T D P T K I S E V K D F H I R

376 ATCATCCCGAACAAGGATGAGAAAACGCTGACCATCATCGATACCGGCATCGGCATGACCAAGGCCGACCTGATC  
71 I I P N K D E K T L T I I D T G I G M T K A D L I

451 AACAACTCTCGGCACCATTGCCAAGTCCGGCACGAAGGCGTTTATGGAGGCCCTCCAGGCCGGGCTGACATTTCG  
96 N N L G T I A K S G T K A F M E A L Q A G A D I S

526 ATGATTGGCCAGTTGCGGTGTGGGCTTCTACTCGGCCTACCTCATTGCGGACAAGGTGACGGTGCCTCGAAGCAC  
121 M I G Q F G V G F Y S A Y L I A D K V T V H S K H

601 AATGATGACGAGCAGTACATCTGGGAGTCTCTGCGCGGCTCTTTACCATCCGCGCTGACCCCGCGAACC  
146 N D D E Q Y I W E S S A G G S F T I R P D P G E P

676 CTCGGCCGCGGCACCAAGATTGTGCTGCACCTGAAGGAGGACAGATCGAGTACTCCGAGGAGCGCCGCATCAAG  
171 L G R G T K I V L H L K E D Q I E Y S E E R K I K

751 GAGATCGTGAAGAAGCACTCGCAGTTCATTGGCTACCCCATCAAGCTGCTGGTGCAGAAGGAGCGGAAAAGGAG  
196 E I V K K H S Q F I G Y P I K L L V Q K E R E K E

826 ATCTCCGACGACGAGGAGGAGGCCAAGGAGGGCGAGGAGAAGAAAAGAAAAGACCAGGAGTCTGGC  
221 I S D D E E E E P K E G E E K E K K E K T E E S G

901 GACGAGCCCAAGGTGGAGGACGTCGAGGATGAGGCCGAGTGAAGGAGAAAAGAAAAGACCATCAAGGAGAG  
246 D E P K V E D V E D E A E S K E K K K K T I K E K

976 TACATCGAGGACGAGGAGCTGAACAAGACGAAGCCCATCTGGATGCGCAACCCCGATGAGATCACCCAGGACGAG  
271 Y I E D E E L N K T K P I W M R N P D E I T Q D E

1051 TACGGCGAGTTCTACAAGTCGCTCACCAATGACTGGGATGACCACCTGGCGGTGAAGCATTTCGTTGAGGGC  
296 Y G E F Y K S L T N D W D D H L A V K H F S V E G

1126 CAGCTCGAGTTCCGCGCCCTGCTGTTTCCCAAGCGCGCCCTTTGATCTCTTTGAGAATAAGAAGCAGAG  
321 Q L L F R A L L F I P K R A P F D L F E N K K Q K

1201 AACAACTTAAGCTGTACGTCGCGGAGTCTTCATCATGGAGAACTCGGAGGAGCTCATTCCCGAGTACCTGAAC  
346 N N I K L Y V R R V F I M E N C E E L I P E Y L N  
Deg-hsp90F →

1276 TTCGTCAGGGCGTGGTTCGACAGCGAGGACCTGCCGCTGAACATCTCCCGAGAGACCCCTCCAGCAGAACAAAATC  
371 F V K G V V D S E D L P L N I S R E T L Q Q N K I  
← 3GSP1 5GSP2 →

1351 CTGAAGGTGATCCGCAAGAACCCTCGTCAAGAACTGCGCTGGAGCTGTTTACGAACTGGCCGAGGACAGGAGATG  
396 L K V I R K N L V K K C L E L F D E L A E D K E M

1426 TACAAGAAGTTCTACGAGAATTCAGCAAGAACATGAAGCTCGGCATCCACGAGGACAGCCAGAACCAGCAAGAG  
421 Y K K F Y E N F S K N M K L G I H E D S Q N R K K

1501 CTCGCTGAGCTGCTGCGCTACCACACCTCTTCCAGTGGCGACGAGATGTGCTCCCTGAAGGACTACGTGTCCCGC  
446 L A E L L R Y H T S S S G D E M C S L K D Y V S R

1576 ATGAAGGAGAACCAGAAGGACATCTACTTCATCACCGGCGAGTGAAGGAGATCGTTGCCAATCCGCGCTTCGTC  
471 M K E N Q K D I Y F I T G E S K E I V A N S A F V

1651 GAGCGCTCCGCAAGCGTGGCTTCGAGGTCGTTACATGGTGGAGCCCATGACGAGTACTGCGTCCAGCAGCTG  
496 E R V R K R G F E V Y M V E P I D E Y C V Q L  
← 5GSP1 →

1726 AAGGAGTTCGACGGCAAGACCCTGGTCTCGGTACCAAGGAGGGTCTGGAGCTGCCTGAGGACGATGAGGAGAAG  
521 K E F D G K T L V S V T K E G L E L P E D D E E K  
← 3GSP2 →

1801 AAGAAGCGGAGGACGACAAGAAGAAGTTCGAGAACCCTGTGCAAGGTGATGAAGGACGCTCTCGAGAAGAAGTGC  
546 K K R E D D K K K F E N L C K V M K D V L E K K V

1876 GAGAAGGTGCTCGTCTCGAACCCGCTGGTTCAGCTCGCCCTGCTGCATCGTCACTAGCCAGTACGGCTGGAGCGCC  
571 E K V L V S N R L V S S P C C I V T S Q Y G W S A  
← Deg-hsp90R →

1951 AACATGGAGCGCATCATGAAGGCCAGGCTCTTCTGCTGACTCCTCTGCCCTCGGTTACATGGCCGCGAAAAGAAC  
596 N M E R I M K A Q A L R D S S A L G Y M A A K K N

2026 CTCGAGATCAACCCGACCCCATCATCGAGAACCCTTCGCGCTCAAGGTGGAGGCCGACAAGAACGACAAGGCC  
621 L E I N P D H P I I E E N L R L K V E A D K N D K A

2101 GTCAAGGACCTGGTGAACCTGCTGCTGGAGACCTCGCTGCTGCTGCTGTTTGGCCCTCGAGGACCCCGAGACC  
646 V K D L V N L L L E T S L L C S G F G L E D P Q T

2176 CACGCTCTCGCATCTACCGCATGATCAAGCTTGGTCTCGGCATCGATGAGGATGATCTCCCGCTCGTGGAGGAA  
671 H A S R I Y R M I K L G L G I D E D D L P V V E E

2251 GCTCCCATTCGAGGAGCCCATTCGCGACCGCAAGCTGCGGATGCTTCTCGCATGGAGGAGTTGAT[TA]STT  
696 A P I A E E P I P D A E A D A S R M E E V D \*  
← 3GSP1 →

2326 GTTCTCCACCCACCCATTAGAATTAGTATTCGCTCTCCCTTTGTGTGTGAGATGCTTCTCTCATTACTGTA

2401 ATTAATATCATTTTATTTTACTTTTAACTCCACTCAACACTGCTATTTTTCATTTGAATAGTTGATCTGGAAT  
2476 CTATCTCACTG[ATTA]AGTGTAGAAGTCTTTAAAAA

207

208 **Figure 1** Nucleotide and deduced amino acid encoding region of *TpHSP70-1(A)*,

209 *TpHSP70-2* (B) and *TpHSP90*(C). Signature sequences of *TpHSP* genes are marked  
210 with underline.

### 211 **3.2 Homology analysis of *TpHSP70* and *TpHSP90***

212 A BLASTP (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) search of GenBank revealed  
213 that *TpHSP70-1* and *TpHSP70-2* were belong to *HSP70* family. At the same time,  
214 *TpHSP90* was belong to *Hsp90* family. Furthermore, multiple sequence alignment  
215 showed that the deduced amino acid sequence of *TpHSP70-1* and *TpHSP70-2* shared  
216 high similarity with three HSP70s from *Tetranychus cinnabarinus*, *Ixodes scapularis*,  
217 *Bombyx mori*, *Drosophila melanogaster* and *Homo sapiens* (Figure 2A). Additionally,  
218 phylogenetic analysis showed that the two *TpHSP70s* from *T. putrescentiae* belong to  
219 two branches of phylogenetic tree respectively, in which protein *TpHSP70-1* belongs  
220 to cytoplasmic type and protein *TpHSP70-2* belongs to endoplasmic reticulum type  
221 (Figure 3A). Meanwhile, the deduced amino acid sequence of *TpHSP90* shared high  
222 similarity with five HSP90s from *T. cinnabarinus*, *I. scapularis*, *B. mori*, *D.*  
223 *melanogaster* and *H. sapiens* (Figure 2B). Among the compared *Hsp90* proteins,  
224 *TpHSP90* showed the highest similarity to *Hsp90* from *I. scapularis* (81% identity),  
225 and the lowest similarity to *HSP90* from *D.melanogaster* (77% identity). *HSP90*  
226 homology was high within the arthropods, especially in the signature regions of the  
227 *Hsp90* family. The relationships of *HSP90* displayed in the phylogenic tree were  
228 consistent with the traditional taxonomy of these species (Figure 3B). *HSP90* from *T.*  
229 *putrescentiae* and *T. cinnabarinus* get together before come together with *I. scapularis*,  
230 *M. occidentalis* and *N. cucumeris* (Figure 3B).

A	Tyrophagus putrescentiae_HSP70-1	MKTLFP AVL L L T L L V T I V Y S K E E V G K E A D V G T V I G I D L G T T Y S C V G V F K N G R V E I I A N D Q G N R I T P S Y	70
	Tyrophagus putrescentiae_HSP70-2	MKTLFP AVL L L T L L V T I V Y S K E E V G K E A D V G T V I G I D L G T T Y S C V G V F K N G R V E I I A N D Q G N R I T P S Y	70
	Panonychus citri_HSP70	..... MS GK A P A I G I D L G T T Y S C V G V F Q H G K V E I I A N D Q G N R I T P S Y	42
	Ixodes scapularis_HSP70	..... M A K V P A I G I D L G T T Y S C V G V F Q H G K V E I I A N D Q G N R I T P S Y	41
	Bombyx_mori_HSP70	..... M V K M R W S F A L V L V P A V C A D D K K E K D K D I G T V I G I D L G T T Y S C V G V F K N G R V E I I A N D Q G N R I T P S Y	68
	Homo_sapiens_HSP70	..... M S V G I D L G F Q S C Y L A V A R A G G I E T I A N E Y S D R C T P A C	38
	Tyrophagus putrescentiae_HSP70-1	V A F T A E G B R L I G D A A R N Q L T S N P E N T V F D A K R L I G R E W T D K N V Q H D L K F F P F K M I E K N S K P H I K V K T S T	139
	Tyrophagus putrescentiae_HSP70-2	V A F T A E G B R L I G D A A R N Q L T S N P E N T V F D A K R L I G R E W T D K N V Q H D L K F F P F K M I E K N S K P H I K V K T S T	139
	Panonychus citri_HSP70	V A F T D T . E R L I G D A A R D Q V A M N P S N S V F D A K R L N G R R E D D P M Q S D M K H W P F D V S V D G K P K I Q M D F K G	110
	Ixodes scapularis_HSP70	V A F T D T . E R L I G D A A R D Q V A M N P S N S V F D A K R L I G R R E D D P A V Q S D M K H W P F D V S D G K P K I Q M E Y K G	109
	Bombyx_mori_HSP70	V A F T Q D G B R L I G D A A R N Q L T I N P E N T V F D A K R L I G R E W S D T V Q H D V K F F P F K V V E K N S K P H I V Q V Q T S Q	137
	Homo_sapiens_HSP70	I S E G P K . N R S I G A A A K S Q M I S N A K N T V Q G F R E F E R A S S D F F V E A E K S N L A Y D I V Q W P T G L T G I K V T Y M E	107
	Tyrophagus putrescentiae_HSP70-1	G D K V F A P E E I S A N V L L K K K E I A E A V L G K S V T H A V V T V P A Y F N D A Q R Q A T K D A G A I A G L N V M R I I N E P T A A	209
	Tyrophagus putrescentiae_HSP70-2	G D K V F A P E E I S A N V L L K K K E I A E A V L G K S V T H A V V T V P A Y F N D A Q R Q A T K D A G A I A G L N V M R I I N E P T A A	209
	Panonychus citri_HSP70	E T K T F P E E I S A N V L L K K K E I A E A C L G R P V S N A V I T V P A Y F N D S Q R Q A T K D A G I I A G L N V M R I I N E P T A T	180
	Ixodes scapularis_HSP70	E T K T F P E E I S A N V L L K K K E I A E A V L G R I V T N A V V T V P A Y F N D S Q R Q A T K D A G I I A G L N V M R I I N E P T A A	179
	Bombyx_mori_HSP70	G D K V F A P E E I S A N V L L K K K E I A E A V L G K S V T H A V V T V P A Y F N D A Q R Q A T K D A G I I A G L N V M R I I N E P T A A	207
	Homo_sapiens_HSP70	E E R N E T T E Q V T A M L S K K K E I A B S V L K K P V D C V V S V P C E Y T D A E R R S V M A D T Q I A G L N C L R L N E I T A V	177
	Tyrophagus putrescentiae_HSP70-1	A I A V G L D K K E . G E . . . . . K N L V F D L G G T F D V S L T I D N G V F E V A T N G D T H L G G E D F D R V M E H I I K L	273
	Tyrophagus putrescentiae_HSP70-2	A I A V G L D K K E . G E . . . . . K N L V F D L G G T F D V S L T I D N G V F E V A T N G D T H L G G E D F D R V M E H I I K L	273
	Panonychus citri_HSP70	A I A D G L D K K G C E . . . . . Q N L I F D L G G T F D V S L T I E D G I F E K S I A G D T H L G G E D F D N R M V N H I E E	245
	Ixodes scapularis_HSP70	A I A V G L D K R G T G E . . . . . R N V L I F D L G G T F D V S L T I E D G I F E K S I A G D T H L G G E D F D N R M V N H I V Q E	244
	Bombyx_mori_HSP70	A I A V G L D K K E . G E . . . . . K N L V F D L G G T F D V S L T I D N G V F E V A T N G D T H L G G E D F D R V M E H I I K L	271
	Homo_sapiens_HSP70	A I A V G I Y S Q D L P R L E E K P R N V F V D A G H S A Y O V S V C A F N R G K L K L A I A F D T I L G G K F E D V L N V H C E E	247
	Tyrophagus putrescentiae_HSP70-1	Y K K R T G K D I R S D N R A V Q L R R E V E K A R T I S S A H Q A R I E . E S L F D G E D F S E V L T B A K S E E L N N D I F R S T	342
	Tyrophagus putrescentiae_HSP70-2	Y K K R T G K D I R S D N R A V Q L R R E V E K A R T I S S A H Q A R I E . E S L F D G E D F S E V L T B A K S E E L N N D I F R S T	342
	Panonychus citri_HSP70	F K R S H K K D I I A N K R A V R E R T A E F R A K R T I S S S Q A S I E . D S L H E G V D Y S T I T B A R S E E L C S D F F R S T	314
	Ixodes scapularis_HSP70	F K R S H K K D I T V N K R A R R E R T A E F R A K R T I S S S Q A S I E . D S L H E G V D Y S T I T B A R S E E L N A D I F R S T	313
	Bombyx_mori_HSP70	Y K K R K K D I R K D N R A V Q L R R E V E K A R A I S S S H Q V K I E . E S F E E G D S E T T L T B A K S E E L N N D I F R S T	340
	Homo_sapiens_HSP70	F G K R Y K L D I K S K I R A I L R L S Q E E K L S K L S A N A S D L P L S I E C F N N D V S G T M N G K L L E M C N D I L A R V	317
	Tyrophagus putrescentiae_HSP70-1	M K P V Q K Y L E D A D L Q K D I D E I V I Y G G S E R I P K V Q O L V K E F F N G K E P T R G I N P D E A A F G A A Y Q A G V S G E	412
	Tyrophagus putrescentiae_HSP70-2	M K P V Q K Y L E D A D L Q K D I D E I V I Y G G S E R I P K V Q O L V K E F F N G K E P T R G I N P D E A A F G A A Y Q A G V S G E	412
	Panonychus citri_HSP70	L E P V E R A L R D A K L D S Q I K E I V I Y G G S E R I P K I Q K L O D F F N G K E L N K S I N P D E A A Y G A A Y Q A A I I S G N	384
	Ixodes scapularis_HSP70	L E P V E R A L R D A K L D S Q V H D I V I Y G G S E R I P K I Q K L O D F F N G K E L N K S I N P D E A A Y G A A Y Q A A I I I G D	383
	Bombyx_mori_HSP70	L K P V Q K Y L E D A D M N K D V D E I V I Y G G S E R I P K V Q O L V K E F F N G K E P S R G I N P D E A A Y G A A Y Q A G V S G E	410
	Homo_sapiens_HSP70	E P P L R S V L E Q T K L K S E D I Y A V E I Y G G A E R I P A M . K E K I S K E F G K E L S T T L N A D E A A T R C A L Q C A I I S P A	386
	Tyrophagus putrescentiae_HSP70-1	... E N T G E L V L L D V N P E T M G I E T V G G V M K L I T R N T V I P T K K S Q I F S T A A D N Q N T V T I Q V F E G E R P M T K D N	480
	Tyrophagus putrescentiae_HSP70-2	... E N T G E L V L L D V N P E T M G I E T V G G V M K L I T R N T V I P T K K S Q I F S T A A D N Q N T V T I Q V F E G E R P M T K D N	480
	Panonychus citri_HSP70	K D E S V Q D L L L L D V N P E S L G I E T A G G V M P L I A G S T T I P T K Q S Q I F T T Y S D N Q G V L I Q V F E G E R A M T K D N	454
	Ixodes scapularis_HSP70	K S E V Q D L L L L D V N P E S L G I E T A G G V M V L I K R N T I P T R Q T Q I T T Y S D N Q G V L I Q V F E G E R A M T K D N	453
	Bombyx_mori_HSP70	... Q D T D A I V L L D V N P E T M G I E T V G G V M K L I P R N T V I P T K K S Q I F S T A S D N Q H T V T I Q V F E G E R P M T K D N	478
	Homo_sapiens_HSP70	F K V R E F S I T D V V P Y D I S R W M S P A E E G S S D C E V F S K N H A A P F S K V L I F Y R K E F F T L E A Y S S P Q D L P Y P D	456
	Tyrophagus putrescentiae_HSP70-1	H Q L G K S D L T G I P P A P R G V P Q I E . V T F E I D V N G I L R V T A E D K G T G N K E K I V I T N D H N R L S B E D I E R M K D A	549
	Tyrophagus putrescentiae_HSP70-2	H Q L G K S D L T G I P P A P R G V P Q I E . V T F E I D V N G I L R V T A E D K G T G N K E K I V I T N D H N R L S B E D I E R M K D A	549
	Panonychus citri_HSP70	N L L G K S E L T G I P P A P R G V P Q I E . V T F D I N A N G I L N N S A V D K S T G R E N K I T I T N D K G R L S K E E I E R M V Q E A	523
	Ixodes scapularis_HSP70	N L L G K S E L T G I P P A P R G V P Q I E . V T F D I D A N G I L N N S A V D K S T G R E N K I T I T N D K G R L S K E E I E R M V K D A	522
	Bombyx_mori_HSP70	Y L L G K S D L T G I P P A P R G V P Q I E . V T F E I D A N G I L O V S A E D K G T G N R E K I V I T N D O N R L T P E D I E R M K D A	547
	Homo_sapiens_HSP70	P A I A Q S V Q K V T P Q S D S S K V R V K R V N V H G I F S V S A S L V E V H S E E N E P M E T D Q N A K E E E K M Q V D Q	526
	Tyrophagus putrescentiae_HSP70-1	E K F S D E D K K V K E V E A K N E L E S Y A Y S L K N Q I G D K E K L G G K L S D D K K T I E S S V D E T I K W L D A N A D A D S E E	619
	Tyrophagus putrescentiae_HSP70-2	E K F S D E D K K V K E V E A K N E L E S Y A Y S L K N Q I G D K E K L G G K L S D D K K T I E S S V D E T I K W L D A N A D A D S E E	619
	Panonychus citri_HSP70	E K Y K D D D E K V K D R V S A K N A L E S Y C F S M K S M E E . S S A G G K I S E D D K K I M D R V D E V I K W L D A N Q L A E K E E	592
	Ixodes scapularis_HSP70	E K Y K D E D D K Q K T R I T A K N A L E S Y S F N I K S T V E D E K I K D K L S E D D R K K I L E K V E E T I K W L D T N Q L A D K E E	591
	Bombyx_mori_HSP70	E K E A D D D K K I K E R V E S R N E L E S Y A Y S I K N Q L Q D K E K L G A K V T D D K A K M E E A L D A A I K W L E D N Q D A E S E E	617
	Homo_sapiens_HSP70	E P H V E E Q Q Q T P A E N K A E S E . . . . . E M E T S Q A S K L K K M D Q P P Q C Q E G S E D Q Y C G P A N R E	583
	Tyrophagus putrescentiae_HSP70-1	L K A R K K K L E E T V T P I . S K L Y . . . . . A G A G D A G O P P A E E D A S S K D E L . . . . .	661
	Tyrophagus putrescentiae_HSP70-2	L K A R K K K L E E T V T P I . S K L Y . . . . . A G A G D A G O P P A E E D A S S K D E L . . . . .	661
	Panonychus citri_HSP70	F E H R Q K E L E Q V C N P I . T K M Y . . . . . Q G A G G A P G G M P D F G A A A G A G A G A G A G A G A G T G S G P T I E E V D	658
	Ixodes scapularis_HSP70	Y E H R Q K E L E Q V C N P I . T K L Y . . . . . Q D G G M P A G G F E . . . . . G A G A P G G A G A P G A G A G S G P T I E E V D	648
	Bombyx_mori_HSP70	Y K K O K K T L E D V Q P I . A K L Y . . . . . Q G O G G V E P . P G A P E D D D F K D E L . . . . .	658
	Homo_sapiens_HSP70	S A I W I D R E M N L Y I E N E G K M M O D K L E K E R N D A . . . . . K N A V E V Y V E M R D K L S G E Y E K F V S E E D	644
	Tyrophagus putrescentiae_HSP70-1	.....	661
	Tyrophagus putrescentiae_HSP70-2	.....	661
	Panonychus citri_HSP70	.....	658
	Ixodes scapularis_HSP70	.....	648
	Bombyx_mori_HSP70	.....	658
	Homo_sapiens_HSP70	R N S F T L K L E D T E N W L Y E D G E D Q K Q V Y V D K L A E L K N L G Q P I K I R F Q E S E E R P N Y L K	700

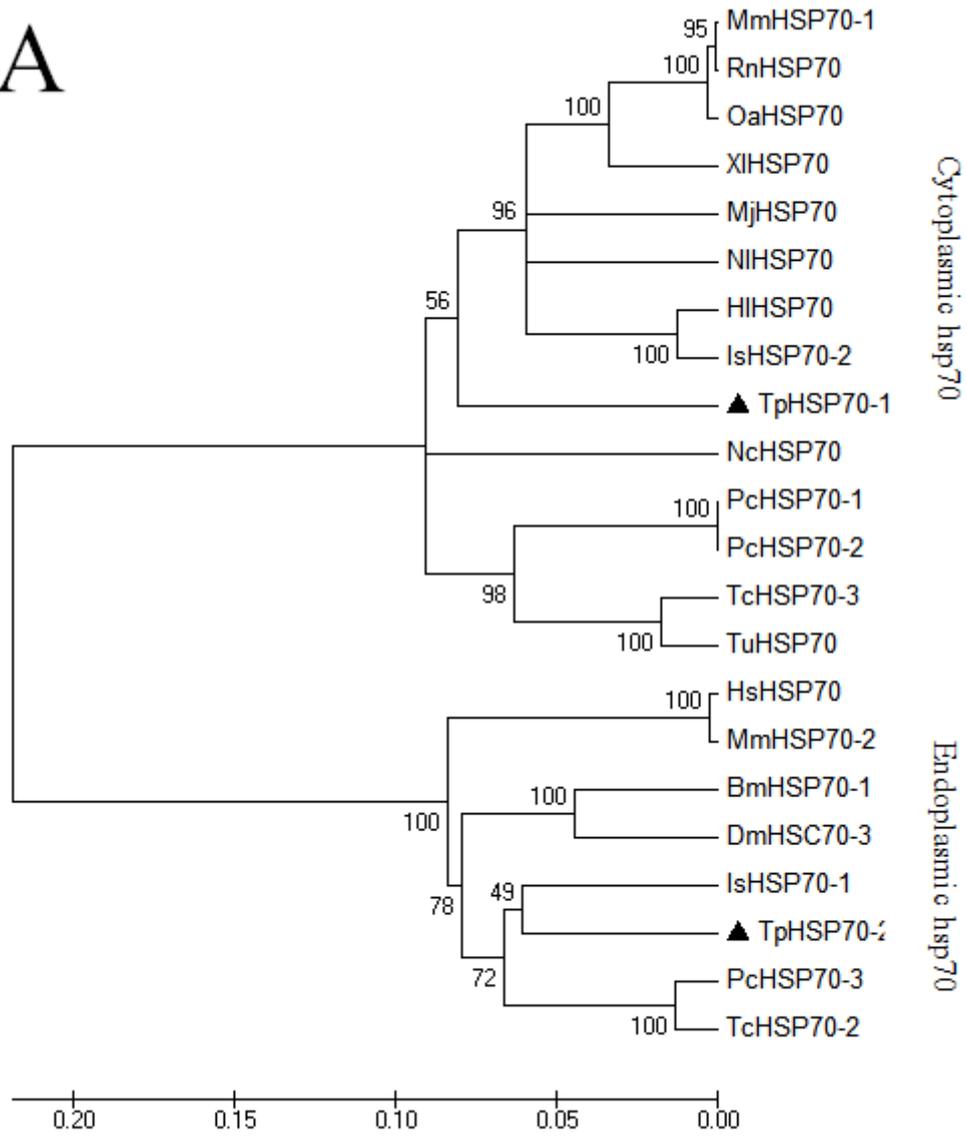
B

Tyrophagus putrescentiae_HSP90	.....	0
Panonychus citri_HSP90	.....	0
Ixodes scapularis_HSP90	MAS KLVSRLLSLCSRYGGCSRPLWVPRQSRPTLVVGGDSRRRLTQWKPTSVLFRPAGLWRPYS TPQS E P D	70
Bombyx mori_HSP90	.....	0
Homo sapiens_HSP90	.....	0
Tyrophagus putrescentiae_HSP90	..... MPEEQEVE T T F AQAE I AQLNS L I I NTFYSNKEI F I REL I SNAS DALDKI RYES ..... L	55
Panonychus citri_HSP90	..... MPEEPMTATNDAE T F L F AQAE I AQLM L I I NTFYSNKEI F I REL I SNSS DALDKI RYES ..... L	60
Ixodes scapularis_HSP90	SKDELHNI KNTKEGSGPSEKREF QAE TRMLLDI VAKS I YSEKEV F VREL VSNAS DAL E K I RYLRLS AQL	140
Bombyx mori_HSP90	..... MPEE M T Q P A E V E T T F AQAE I AQLNS L I I NTFYSNKEI F I REL I SNSS DALDKI RYES ..... L	59
Homo sapiens_HSP90	.....	0
Tyrophagus putrescentiae_HSP90	T D P T K I S E V K D F H I R I I P N K D E K T L I I D T G I G M T K A D L I N N L G T I A K S G T K A F M E A L Q A G A D I ... S M I	122
Panonychus citri_HSP90	I D A S L D S G R D L Y I K I V N K E E R T L I I D T G I G M T K A D L I N N L G T I A R S G T K A F M E A L Q A G A D I ... S M I	127
Ixodes scapularis_HSP90	E H E A P D A A A A P L E I H I A T D K L A N T I T I Q D T G I G M T H D E V V E S L G T I A R S G S K E F L R K L T E A G D T P A S I I	210
Bombyx mori_HSP90	T D P S K L D S G R E L Y I K I I P N K N E G T L I I D T G I G M T K A D L V N N L G T I A K S G T K A F M E A L Q A G A D I ... S M I	126
Homo sapiens_HSP90	.....	9
Tyrophagus putrescentiae_HSP90	Q F G V G F Y S A Y L I A D K V T V H S K H N D D E Q V I W E S S A G G S F T I R P D P G E P L G R G T K I V L H L K E D Q I E Y S E E R	192
Panonychus citri_HSP90	Q F G V G F Y S A Y L V A D R V V T S K H N D D C Y T W E S S A G G S F T I K K T T D P D C V R G T K I V L W L K D D Q N D Y L D E K	197
Ixodes scapularis_HSP90	Q F G V G F Y S C F M V A D R V E V F T R S R L P D A R A V R W S S D G S G V Y E V S E A E G V E H G T K I V L H L K P D C A D F A D E E	280
Bombyx mori_HSP90	Q F G V G F Y S X L V A D R V T V H S K H N D D E Q V W E S S A G G S F T V R P D S G E P L G R G T K I V L H V K D L A E F M E H E	196
Homo sapiens_HSP90	Q I D L G T T Y S C V G V F Q H G K V E I A N D Q G N R T T P S Y V A F T D T E R L I G D A A K N Q V A M P Q N T V F D A K R L I G R K	79
Tyrophagus putrescentiae_HSP90	R I K E I V K K H S ..... Q F I G Y P I K L L V O K E R E K E I S D D E E E E P . K E G E K E K K E K T E S G D E P K V D E V D E	256
Panonychus citri_HSP90	K I K E V V K K H S ..... Q F I G Y P I K L V V O K E R E K E V S D D E G E E E K K E E D E E K K K E N D E K K D D E E P K V E D V	262
Ixodes scapularis_HSP90	A V S K V V O K Y S ..... N F V G S P V F L N G .	301
Bombyx mori_HSP90	K I K E I V K K H S ..... Q F I G Y P I K L M V E K E R E K E L S D D E A E E E . K K E E D E K ..... P K I E D V D E	250
Homo sapiens_HSP90	F N D P V V Q A D M K L W P F Q V I N E G G K P K V L V S Y K G E N K A F Y P E E I . S S M V L T K L K T A E A F L G H P V T N A V I T V	148
Tyrophagus putrescentiae_HSP90	A . E S K E . . . . . K K K K T . . . . . I K E K Y I E D E E L N K T K P I W R N P D E I T Q D E V G F E Y K S L T N D W D H	310
Panonychus citri_HSP90	E D D K K D K . K K K K K . . . . . V T E K Y I E D E E L N R T K P I W R N P D D I T Q S E N G E Y K S L T N D W E E H	319
Ixodes scapularis_HSP90	..... R R A N T L Q V R L G C C V T N T L L I R L L A S R S T Q S	333
Bombyx mori_HSP90	E D D K K D T K K K K K T . . . . . I K E K Y T E D E E L N K T K P I W R N A D D I T Q D E V G F E Y K S L T N D W D H	308
Homo sapiens_HSP90	P A Y F N D S Q R Q A T K D A G V I A G L N V L R I I N E P T A A A I A Y G L D R G G G E R H V L I F D L G G T I D V S I L T I D D G I	218
Tyrophagus putrescentiae_HSP90	L A V K H F S V E G Q L E F R A L L F I F K R A P F D I F E N K K Q K N N I K L Y V R R V E I M E N C E E L I P E Y L N F V K G V D S E D	380
Panonychus citri_HSP90	L A V K H F S V E G Q L E F R A L L F V E K R A P F D I F E N R K Q K N N I K L Y V R R V E I M D N C E D L I P E Y L N E I K G V D F E D	389
Ixodes scapularis_HSP90	G F L P H K C F A G C A L F R D E K M C G W R D G F D . F P Q I S S A I Q S S L T H K F C E L F R T S L D F I H Q F L A S F L G I V I P S V	402
Bombyx mori_HSP90	L A V K H F S V E G Q L E F R A L L F V R R A P F D I F E N K R K N N I K L Y V R R V E I M D N C E D L I P E Y L N E I R G V D S E D	378
Homo sapiens_HSP90	F E V K A T A G D T H L G G E D E D N R L V S H F V E E K R K H K B I S Q N K R A V R R L R T A C E R A K R T L S S T Q A N L E I D S	288
Tyrophagus putrescentiae_HSP90	L P L N I S R E T L Q O N . . . . . K I L K V I R K N I V K K C L E L F D E L A E D K E M Y K K F Y E N F S K N M K L G I H E D S Q N R K K L	446
Panonychus citri_HSP90	L P L N I S R E M L Q O N . . . . . K I L K V I R K S L V K R C I E L S E E V A E G K E A Y K K F Y E Q F S K N M K L G I H E D T G N R K K I	455
Ixodes scapularis_HSP90	F C A F N N R E L L Q D S A L I R K I R S V I T S R L V K H L S K S A E K E P E S Y A R F Y R D Y G V F L K E G I L A S H E Q A E K E E . I	471
Bombyx mori_HSP90	L P L N I S R E M L Q O N . . . . . K I L K V I R K N I V K K C L E L F E E L A E D K E N Y K K Y Y E Q F S K N L K L G I H E D S Q N R A K L	444
Homo sapiens_HSP90	L Y E G I D F Y T S I T R . . . . . A R F E E L C A D L F R G T L E P V E K A L R D A K M D K A . . . . . K I H	334
Tyrophagus putrescentiae_HSP90	A E L R Y H T S S S G D . E M C S . K D Y V S R M K E N Q K D I Y F I T G E S K E I V A N S A F V E R V R K R G F . . . . . E V V M V E P I D	513
Panonychus citri_HSP90	A D L R F Y T S A S G D . E M C S . K D Y V S R M K D N Q K S I Y S I T G E S K E Q V A A S A F V E R V R S R G F . . . . . E V V M V E P I D	522
Ixodes scapularis_HSP90	A Q L R F E S S A R P A G E T V T I A Q Y C A G R E G O R D I Y Y L A A P S R O L A E S S P Y F E A V R S R D V . . . . . E V L F C T E P Y D	539
Bombyx mori_HSP90	S E L R Y H T S A S G D . E A C S . K E Y S R M K E N Q K H I Y Y I T G E N R D Q V A N S S F V E R V K R G Y . . . . . E V V M T E P I D	511
Homo sapiens_HSP90	D I V I V G G S T R I P K . V Q R L . Q D Y F N G R D L N K S I N P D . . . . . E A V A Y G A A V Q A A I L M G D K S . . . . . K V Q D L L L L D	397
Tyrophagus putrescentiae_HSP90	E Y C V Q O L K E F D G K T L V S V T K E G L E L P E D D E E K K K R E D D K K F E N L C K V M K D V L E K K V E K V L S N R I V S S .	582
Panonychus citri_HSP90	E Y C V Q O L K E Y E G K P L V S V T K E G L E L P E T D E E K K K R E D V K K F E T L C K V M K D V L E K R V E K V T I S N R I V S S .	591
Ixodes scapularis_HSP90	E L V I V Q L R Q F N R R N I T S V E K E M R R D A E A A S E E S . E D V K A . . . . . L S E W R S E L A P A M H K V K V T O R I Q S H L	602
Bombyx mori_HSP90	E Y V V Q Q R E Y D G K T L V S V T K E G L E L P E D E E E K K K R E D K V K F E G L C K V M K N I L D N K V E K V V S N R I V S S .	580
Homo sapiens_HSP90	V A P E S L G L E T A G G V M T A L I S R N S T I P T . . . . . K Q T Q I F T T Y S D N Q P G V L I Q V Y E G E R A M T D N N L L G R F D	462
Tyrophagus putrescentiae_HSP90	..... P C C I V T S Q Y G W S A N M E R I M K A Q A L R D S S A L G Y M A A K K N L E I N P D H P I I E N L R L K V E A D K N D K A	645
Panonychus citri_HSP90	..... P C C I V T S Q Y G W S A N M E R I M K A Q A L R D T T T M G Y M A A K K H L E I N P D H P I V E N L R Q R I E A D K S D K A	654
Ixodes scapularis_HSP90	..... P C L V S V E M A A .	614
Bombyx mori_HSP90	..... P C C I V T A Q Y G W S A N M E R I M K A Q A L R D T S T M G Y M A A K K H L E I N P D H S I V E T L R Q K A E A D R N D K A	643
Homo sapiens_HSP90	L T G I P P A R G W P Q I E V T F D I B A N G I L N V T A M D K S T G K V N K I T I T N D K G R L S K E E I E R V L D A E Y K A E D E	532
Tyrophagus putrescentiae_HSP90	V K D L V N L L . . . . . L E T S L L C S G E G L E D P O T H A S R I Y R M K L G L G I D E D D L P V V E E A P I . . . . . A E E P I P D A E A A	709
Panonychus citri_HSP90	V K D L V M L L . . . . . F E T A L L C S G E S L E D P Q N H S R I Y R M K L G L G I D E D L V E V G G G D K V A E A E M A L E G D A E	721
Ixodes scapularis_HSP90	R H F V K T S L . . . . . Q S F S E E Y R L L E P T L E I N P D H P I M A K L K T L R S S D P A L A K K A R Q V F G N A M A A G L V D D	681
Bombyx mori_HSP90	V K D L V I L L . . . . . Y E T A L L S S G E T L D E P Q V H A S R I Y R M K L G L G I D E D E P I Q V E E P A S . . . . . G D V P P L E G A D	707
Homo sapiens_HSP90	V Q R E K I A A K N A L E S Y A F N M K S V V S D E G L K G K I S E S D K N K I L D K C N E L L S W L E V N Q L . . . . . A E K D E F D H K R K	599
Tyrophagus putrescentiae_HSP90	D A S R M E E V D . . . . .	718
Panonychus citri_HSP90	D A S R M E E V D . . . . .	730
Ixodes scapularis_HSP90	P R K V T S E L N D L L A S L L E K H . . . . .	700
Bombyx mori_HSP90	D A S R M E E V D . . . . .	716
Homo sapiens_HSP90	E L E Q M C N P I I T K L Y Q G G C T G P A C G T G Y V P G R P A T G P T I E E V	640

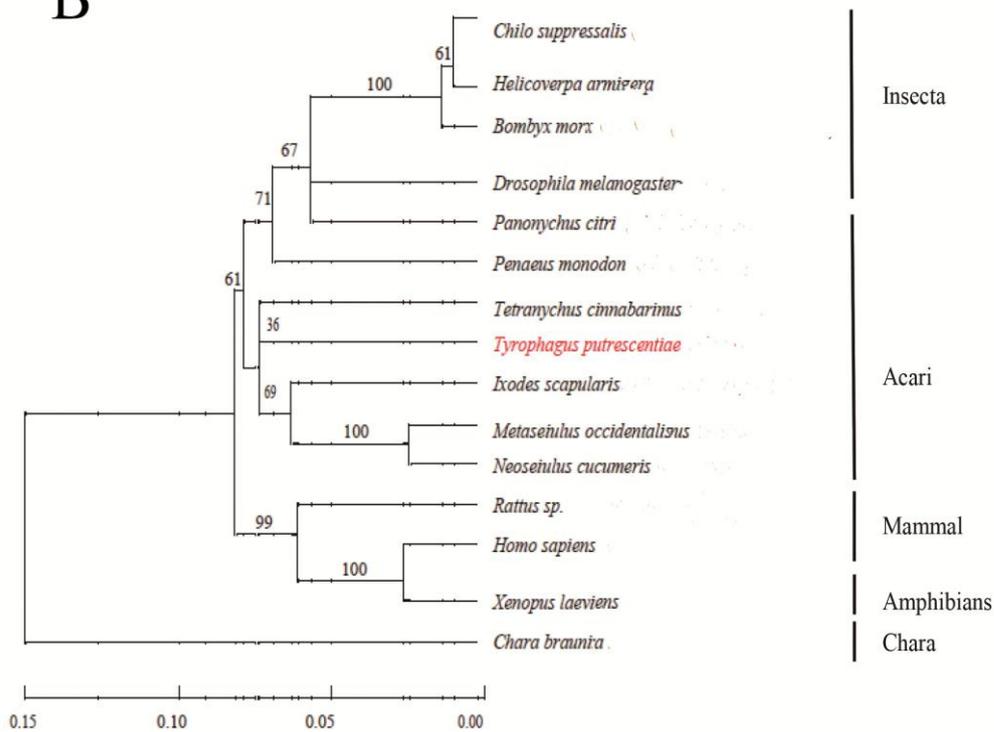
232

233 **Figure 2** Comparison of the amino acid sequences of *HSP70* protein family (A) and  
234 *HSP90* protein family (B).

A



B



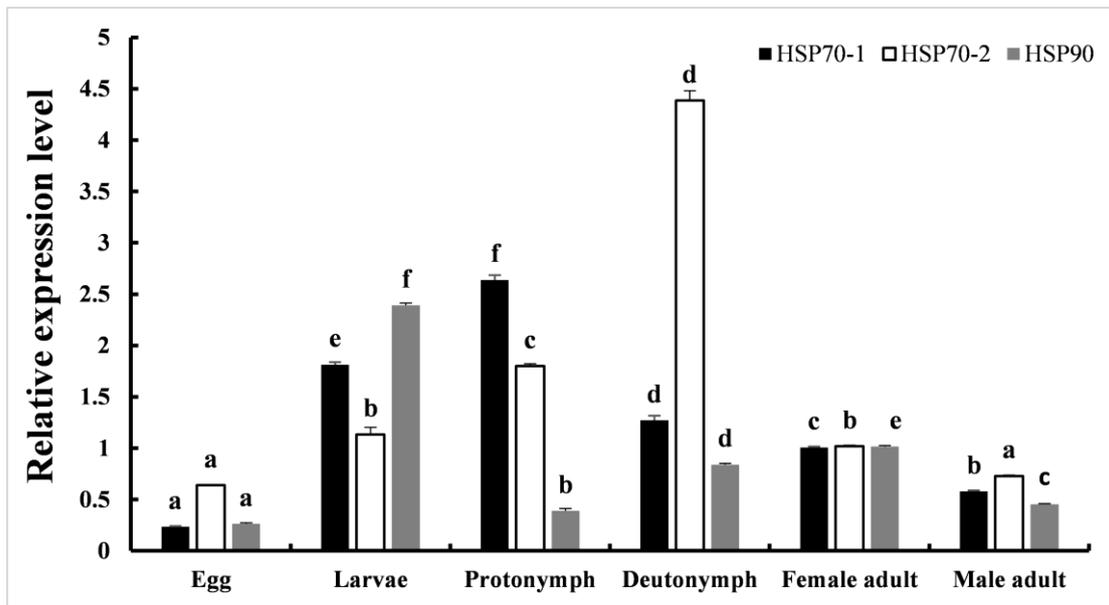
236

237 **Figure 3** Phylogenetic tree of HSP70 (A) and HSP90 (B) from *T. putrescentiae* and  
 238 other species. Constructed by the neighbor joining method based on amino acid  
 239 sequences. Numbers at each branch indicate the percentage of times, and a node is  
 240 supported in 1,000 bootstraps pseudo-replication by neighbor joining.

### 241 3.3 Expression of *TpHSP70* and *TpHSP90* in different stages of *T. putrescentiae*

242 Tublin was a reference gene used to measure the mRNA expression level of  
 243 *TpHSP90*, *TpHSP70-1* and *TpHSP70-2* in different developmental stages of *T.*  
 244 *putrescentiae* (egg, larva, protonymph, tritonymph and adult). As demonstrated in  
 245 Figure 4, the mRNA expression level of *TpHSP90*, *TpHSP70-1* and *TpHSP70-2*  
 246 showed rising with the development of the mites, but the expression level of three  
 247 *HSPs* in the life stages of *T. putrescentiae* were significantly different. The results  
 248 showed that there were significant differences in the expression levels of *TpHSP70-2*  
 249 gene ( $F_{(5,12)}=4.45$ ,  $P<0.05$ ) in different developmental stages of *T. putrescentiae*. But  
 250 there were no significant differences in the expression levels of *TpHSP70-1* gene

251 ( $F_{(5,12)}=2.75$ ,  $P>0.05$ ) and *TpHSP90* gene ( $F_{(5,12)}=1.76$ ,  $P>0.05$ ). The expression  
 252 level of *TpHSP70-1* gene in the protonymph was higher than that in other  
 253 developmental stages. Meanwhile, there was the lowest expression level in the egg.  
 254 The expression level of *TpHSP70-2* genes in the tritonymph was higher than that in  
 255 other stages. The expression level of *TpHSP90* genes in the larva was higher than that  
 256 in other developmental stages. Interestingly, the expression of heat shock protein gene  
 257 in female mites was significantly higher than that in male mites. In another word,  
 258 three *TpHSPs* expression were decreased in the adult stage compared to the immature  
 259 stages while they were still higher than those of the egg stage.



260

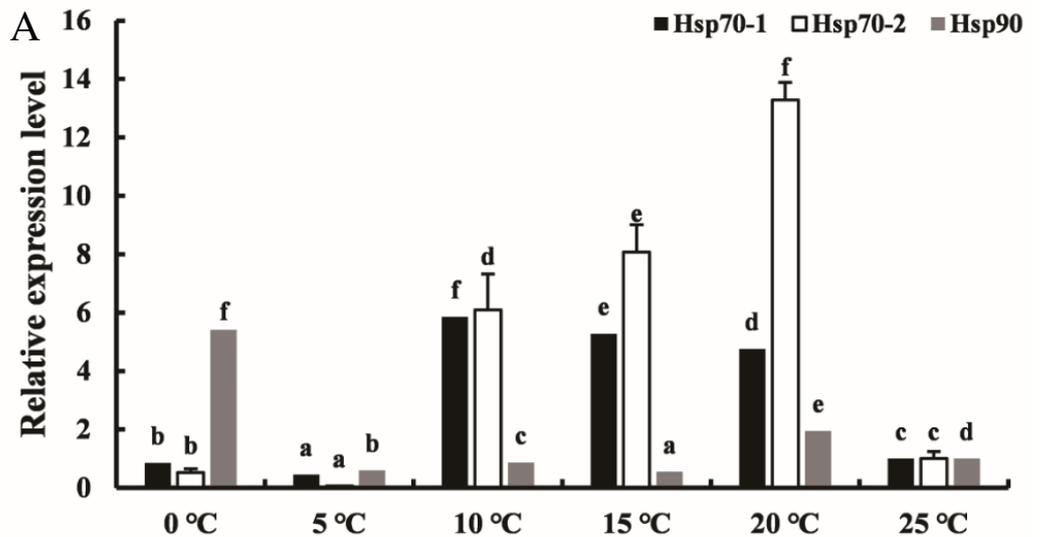
261 **Figure 4** Stage-specific *TpHSP70-1*, *TpHSP70-2* and *TpHSP90* expression in  
 262 *T.putrescentiae*. The mRNA expression level of *TpHSP70-1*, *TpHSP70-2* and  
 263 *TpHSP90* in different developmental stages including egg, protonymph, deutonymph,  
 264 tritonymph and adult stages of *T.putrescentiae* was measured by fluorescent real-time  
 265 quantitative PCR. Values are the mean±SD (n=200). The different letters above the  
 266 bars (a-f) indicate a significant difference in the means as assessed using Duncan's  
 267 multiple comparison tests ( $P<0.05$ ).

### 268 **3.4 Changes of *TpHSP90* and *TpHSP70* expression at different temperatures**

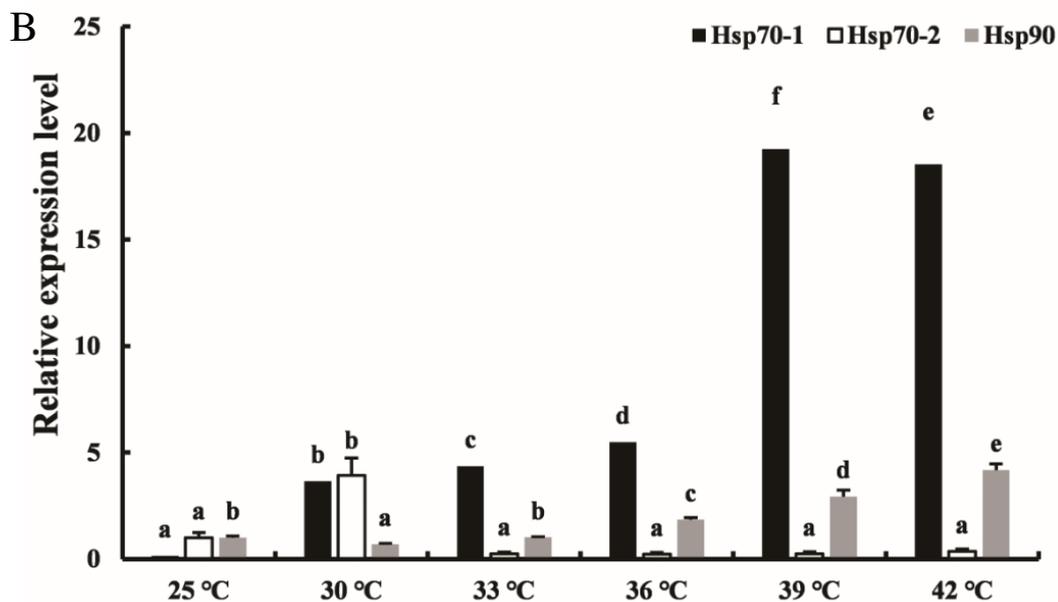
269 The expression pattern of *TpHSP90* and *TpHSP70* mRNA were examined using  
270 qPCR under different temperature conditions while the tubulin as a reference gene in  
271 low-temperature stress. Our results indicated that there were significant differences in  
272 the expression levels of three heat shock protein genes in different low-temperature  
273 stress. The expression of *TpHSP70-1* gene ( $F_{(5,12)}=35.67$ ,  $P<0.05$ ) increased firstly  
274 and decreased later. The expression of *TpHSP70-1* gene at 10°C was the 6.05 times  
275 than that in the control group. The expression of *TpHSP70-2* gene ( $F_{(5,12)}=179.99$ ,  $P<$   
276  $0.05$ ) showed an increasing trend when the increase of temperature, and reaching the  
277 highest level at 20°C. The expression level of *TpHSP90* gene ( $F_{(5,12)}=148.64$ ,  $P<$   
278  $0.05$ ) was the highest under 0°C stress. However, there was no difference between 5,  
279 10, 15 and 25°C (Figure 5A).

280 The mRNA expression level of *TpHSP90*, *TpHSP70-1* and *TpHSP70-2* in  
281 high-temperature stress were determined. The results showed that there were  
282 significant differences in the expression levels of three heat shock protein genes in  
283 different high-temperature stress (Figure 5B). The expression of *TpHSP70-1* gene  
284 ( $F_{(6,14)}=39.33$ ,  $P<0.05$ ) increased firstly and decreased later. The expression level of  
285 *TpHSP70-1* reached the maximum at 39°C and the expression was 19.73 times than  
286 the control's expression. The expression of *TpHSP70-2* gene ( $F_{(6,14)}=39.27$ ,  $P<0.05$ )  
287 was the largest at 30°C and there was no significant difference between the control  
288 group and other temperature stresses. The expression of *TpHSP90* gene ( $F_{(6,14)}=54.13$ ,  
289  $P<0.05$ ) showed its trend as similar to previous two genes. The expression level of  
290 *TpHSP90* gene was 4.17 times at 42°C than that of the control group.

291



292



293

294 **Figure 5** (A) Comparative quantitative RT-PCR analyzed of the relative expression of  
 295 *TpHSP 70-1*, *TpHSP 70-2* and *TpHSP 90* at low temperatures. Control: 25°C; Low  
 296 heat shock temperature: 0°C, 5°C, 10°C, 15°C, 20°C. (B) Comparative quantitative  
 297 RT-PCR analyzed of the relative expression of *TpHSP 70-1*, *TpHSP 70-2* and *TpHSP*  
 298 *90* at high temperatures. Control: 25°C; High heat shock temperature: 30°C, 33°C,  
 299 36°C, 39°C, 42°C. Each temperature treatment was three replicates. Data were  
 300 represented as the mean±SD (n=200). Letters above columns indicate levels of  
 301 difference significance at  $P<0.05$ . The same letters are not significantly different,

302  $P > 0.05$ .

#### 303 **4. Discussion**

304 *T. putrescentiae*, a storage mite, which colonises different human-related  
305 habitats and feeds on various post-harvest foods, was a major cause of allergic  
306 diseases (Liao et al., 2013a, 2013b; Yu et al., 2014). Administration with a crude  
307 extract of *T. putrescentiae* induced airway allergic inflammation in BALB/c mice  
308 (Liao et al., 2013a). One recent experimental study has shown that intranasal exposure  
309 to *T. Putrescentiae* induces an allergic inflammation in the mice. (Nuñez et al., 2016).  
310 All these call for urgent research into the prevention of the mite. In the present study,  
311 we cloned the three full-length cDNAs of *HSP70-1*, *HSP70-2* and *HSP90* genes and  
312 evaluated their expression in response to thermal stress, hoping to understand how  
313 they withstand extreme environmental temperature.

314 Temperature is one of the important environmental factors. A large number of  
315 studies have found that heat stress can induce the expression of HSPs genes in *D.*  
316 *melanogaster* (Colinet et al., 2013), *S. exigua* (Xu et al., 2011), *T. cinnabarinus*  
317 (Feng et al., 2010; Li et al., 2009; Feng et al., 2009) and *P. citri* (Tian et al., 2015;  
318 Yang et al., 2012). The heat resistance of organisms is closely related to heat shock  
319 protein synthesis. The result shows that the synthesized HSPs in organisms can help  
320 them to obtain the adaptability of high temperature environment, thus improving the  
321 thermal tolerance (Zhang et al., 2008). The results showed that rapid cold training  
322 increased the survival rate of *Locusta migratoria*. (Wang et al., 2003).

323 Tubulin was selected as an internal reference to detect the mRNA expression of  
324 *TpHSP70-1*, *TpHSP70-2* and *TpHSP90* gene of female adult mite of *T. putrescentiae*  
325 under heat stress. At the same time, the expression of three genes at different  
326 development stages in *T. putrescentiae* were also detected. In this study, it was found

327 that under cold excitation, *TpHSP70-1* and *TpHSP70-2* failed to express, which is  
328 similar to *Hsp70* expression of *T. cinnabarinus* (Li et al., 2009; Feng et al., 2009), *P.*  
329 *citri* (Yang et al., 2012) and *D. melanogaster* (Colinet et al., 2013). Our results  
330 suggested that there may be other mechanisms in *T. putrescentiae* to resist low  
331 temperature stress. Such as synthesis of trehalose, polyols and other small molecules,  
332 antioxidant reaction and production of other heat shock proteins (Liu et al., 2014;  
333 Yang et al., 2011)

334 The expression of *T. putrescentiae* *TpHSP70-1* and *TpHSP90* gene was  
335 up-regulated with the increase of temperature, reaching significant level. In particular,  
336 the relative mRNA expression level of *TpHSP70-1* gene at 39°C was 19.03 times than  
337 that of the control group. This result indicates that *TpHSP70-1* and *TpHSP90* plays a  
338 certain role in improving the heat resistance of *T. putrescentiae*. Studies have shown  
339 that the death time of *Bactrocera umbrosa*'s eggs and *Pandemis heparana*'s larvae at  
340 46°C is significantly higher than the control (Zhang et al, 2008; Beckett 1997). Jang  
341 et al. suggested that exposure of the *Ceratitidis capitata* to sublethal temperatures of  
342 42°C for 1h enhanced heat resistance (Jang 1992). *Anastrepha suspensa* raised at  
343 30°C had higher heat resistance than those raised at 20°C (Hallman 1992). After a  
344 short period of high temperature stress, the survival time of *Cydia pomonella* at lethal  
345 temperature was prolonged (Wang et al., 20012).

346 Studies have shown that the synthesis of heat shock protein began to decline after  
347 reaching a certain threshold (Didomenico et al., 1982). In this study, it was found that  
348 the expression level of *TpHSP70-1* was the highest at 39°C and decreased at 42°C,  
349 which may be due to its proximity to the threshold range of stress (Liu et al., 2014;  
350 Yang et al., 2011). The result indicates that heat shock protein can protect biological  
351 cells within a certain limit (Liu et al., 2014). On the one hand, induced high

352 expression of heat shock protein can improve the heat resistance of insects and  
353 prevent direct damage to insects under high temperature stress. On the other hand,  
354 synthesis of heat shock protein affects the synthesis of other proteins in the insect  
355 body, forcing insects to pay corresponding costs such as shortened life span and  
356 reduced fertility (Huang et al., 2007; Chen et al., 2014). Our research shows that it  
357 was also found that the number of eggs laid by *T. putrescentiae* decreased  
358 significantly after being treated at temperatures above 39°C.

359 In addition to responding to heat stress, heat shock protein genes are also  
360 involved in normal physiological activities and fertility, such as folding new peptide  
361 chains to form mature proteins in time, formation of gametes, and cell differentiation  
362 (Huot et al., 1996; Vrijenhoek 1994). Our study showed that the transcription level of  
363 The *HSP90* gene of *T. putrescentiae* was significantly different in each developmental  
364 stages, and its expression level varied from the different developmental stages. At the  
365 same time, the expression level was the highest in the larvae's stage. This result  
366 suggests that this gene may be involved in the regulation of the growth and  
367 development of *T. putrescentiae*. The expression of *Hsp70* gene in *T. putrescentiae*  
368 fluctuates with the development period, indicating that *Hsp70* in *T. putrescentiae* also  
369 involved in normal physiological activities and reproductive development.  
370 Interestingly, the mRNA expression level of heat shock protein genes of female adult  
371 *T. putrescentiae* was higher than that of male. Our results were similar to the  
372 *Grapholita molesta*, reflecting the different ability of *T. putrescentiae* of different  
373 genders to cope with environmental temperature stress (Chen et al., 2014).

374 In conclusion, the new *TpHSP90*, *TpHSP70-1* and *TpHSP70-2* genes sequences  
375 were isolated from *T. putrescentiae*, and their phylogeny with other mites were  
376 inferred. These three *TpHSPs* gene were differentially expressed in the developmental

377 stages of *T. putrescentiae*, with a decreased expression in the adult stage. Our results  
378 also confirmed that these three *TpHSPs* are the important genes for *T. putrescentiae* to  
379 defend against temperature. Thus, this study will help to better understand the  
380 resistant fitness of mites and other insects under environmental stress, and guide *T.*  
381 *putrescentiae* management using different temperatures in crops.

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392 No potential conflict of interest was reported by the authors.

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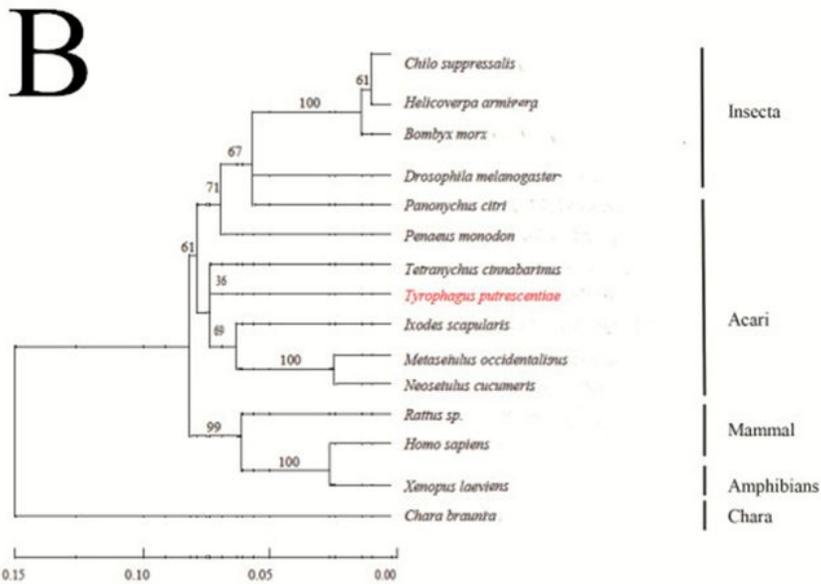
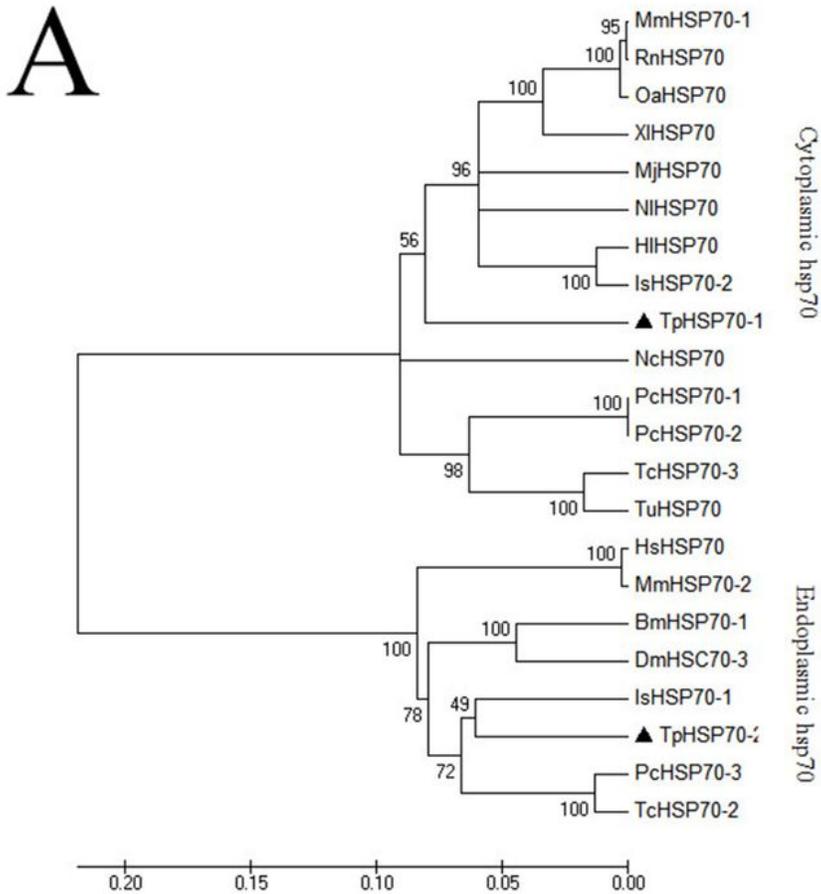
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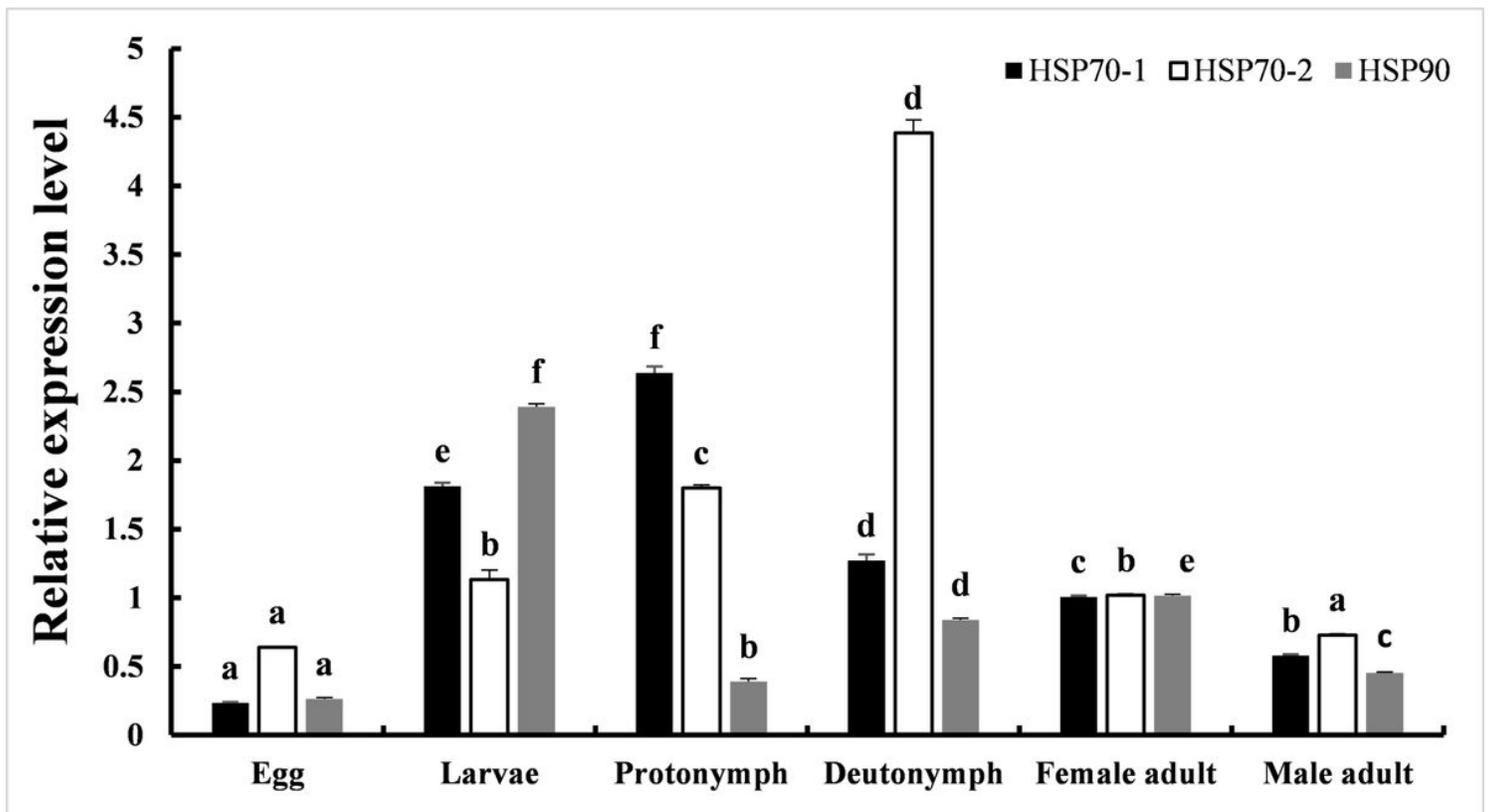






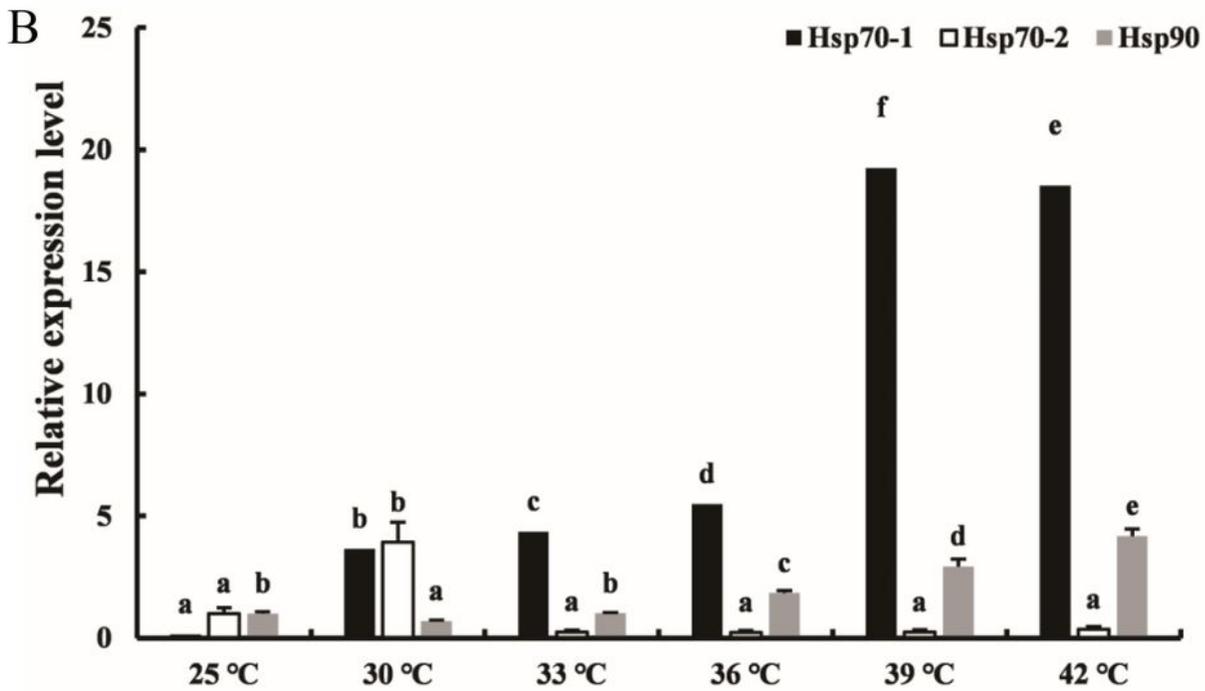
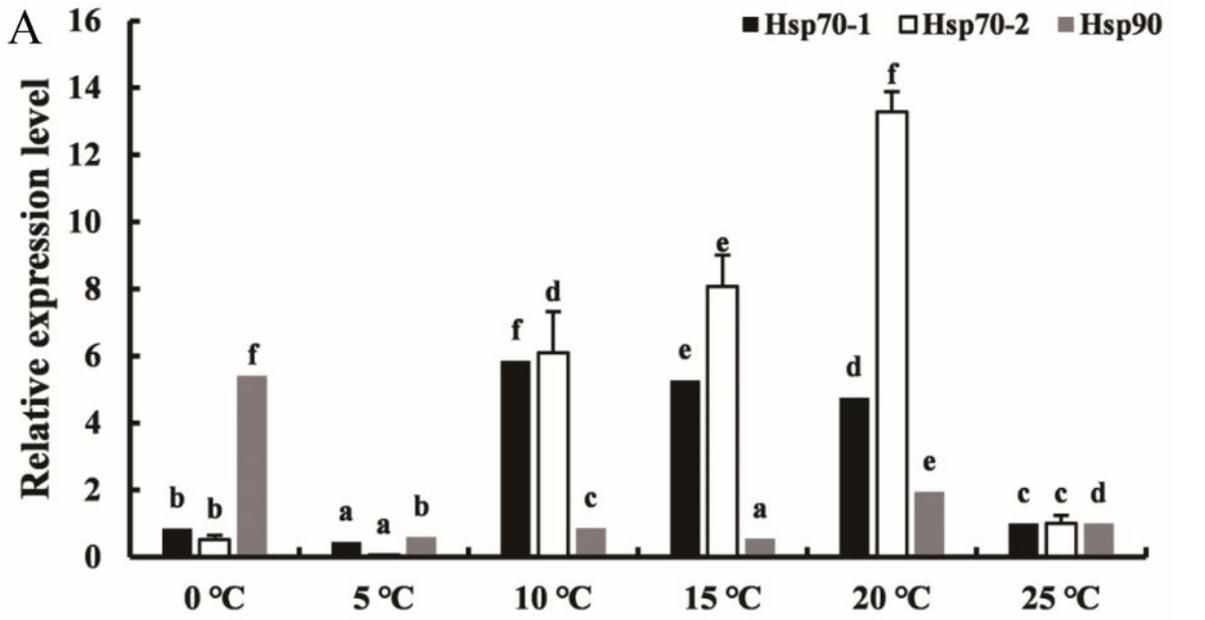
**Figure 3**

Phylogenetic tree of HSP70 (A) and HSP90 (B) from *T. putrescentiae* and other species. Constructed by the neighbor joining method based on amino acid sequences. Numbers at each branch indicate the percentage of times, and a node is supported in 1,000 bootstraps pseudo-replication by neighbor joining.



**Figure 4**

Stage-specific TpHSP70-1, TpHSP70-2 and TpHSP90 expression in *T. putrescentiae*. The mRNA expression level of TpHSP70-1, TpHSP70-2 and TpHSP90 in different developmental stages including egg, protonymph, deutonymph, tritonymph and adult stages of *T. putrescentiae* was measured by fluorescent real-time quantitative PCR. Values are the mean $\pm$ SD (n=200). The different letters above the bars (a-f) indicate a significant difference in the means as assessed using Duncan's multiple comparison tests ( $P < 0.05$ ).



**Figure 5**

(A) Comparative quantitative RT-PCR analyzed of the relative expression of TpHSP 70-1, TpHSP 70-2 and TpHSP 90 at low temperatures. Control: 25°C; Low heat shock temperature: 0°C, 5°C, 10°C, 15°C, 20°C. (B) Comparative quantitative RT-PCR analyzed of the relative expression of TpHSP 70-1, TpHSP 70-2 and TpHSP 90 at high temperatures. Control: 25°C; High heat shock temperature: 30°C, 33°C, 36°C, 39°C, 42°C. Each temperature treatment was three replicates. Data were represented as the mean±SD (n=200). Letters

above columns indicate levels of difference significance at  $P < 0.05$ . The same letters are not significantly different, B A302  $P > 0.05$ . 4. Discussio