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Heat shock genes in the stingless bee *Melipona interrupta* (Hymenoptera, Meliponini)

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ABSTRACT. Heat shock proteins (HSPs) are highly conserved molecules across all plant and animal species. In insects, HSPs are expressed in response to biotic and abiotic stressors and have a well characterized expression pattern in response to heat stress, especially heat shock genes Hsp60, 70 and 90. Temperature affects many aspects of eusocial Hymenoptera, including the stingless bees (Apidae, Meliponini). Consequently, these insects have developed thermal adaptation mechanisms, including thermoregulation. However, this ability decreases when there is deviation from the optimum temperature, compromising colony survival. The study of molecular responses to heat shock stress can be important for the preservation of these pollinizers. We identified and validated *in silico* the genes encoding HSP60, 70 and 90 in *Melipona interrupta*, one of the main stingless bees used for honey production in the Amazon region. cDNA fragments of males, workers and queen bees at the white-eyed pupal stage were amplified using degenerate primers. After sequencing, aligning and editing, the sequences were compared with public genomic databases for *in silico* validation. One fragment of Hsp60, three fragments of Hsp70 and two fragments of Hsp90 were obtained for *M. interrupta*. These fragments showed 100%

similarity with mitochondrial sequences of HSP60 and cytosolic sequences of HSP70 and HSP90 of bees of the genera *Apis* and *Bombus*. Therefore, the fragments obtained in this study correspond to parts of HSP60 (mitochondria), HSP70 and HSP90 (cytosol) in white-eyed pupae of *M. interrupta*. The nucleotide sequences of these fragments did not vary between genders and castes. Our validation *in silico* of the genes encoding HSPs will be useful for future investigations regarding differential expression of HSPs in response to environmental factors that affect the development, maintenance and survival of stingless bee colonies in the Amazon.

Key words: Chaperones; HSPs; *Melipona interrupta*

INTRODUCTION

Temperature is one of the most important abiotic factors regulating the activities of cold-blooded (pecilothermal) organisms, such as insects. Due to a large variety of behavioral and physiological strategies, insects have the ability to survive at different temperatures by synthetizing heat shock proteins HSPs, which are molecules that provide thermal protection (Rafael *et al.* 2012; Lu *et al*., 2016; Willot *et al.*, 2017). The HSPs are part of a highly conserved and universal group of proteins found in all organisms that have been studied. They are molecular chaperones synthetized under stress conditions with fundamental roles in maintaining homeostasis and cell integrity as well as avoiding protein aggregation by recovering damaged or denatured proteins. HSPs are classified into three categories according to their molecular weight and similarities in the amino acid sequences: high (90-110 kDa), medium (62-70 kDa) and low (15-30 kDa) molecular weight in the families HSP90, HSP70, HSP60, HSP40 and small HSPs (sHSPs) (Feder and Hofmann, 1999; Evgen'ev *et al*., 2014; Lu *et al*., 2016).

The phenomenon of thermal shock was first described in the fruit fly *Drosophila melanogaster* by Ritossa (1962); this led to the discovery of HSPs (Tissières *et al*., 1974), which are common and related to immunity (Wojda, 2017). In insects, these proteins are expressed after exposure to abiotic stressors, such as heavy metals, ultraviolet radiation, chemical compounds, hypoxia, dehydration and inanition, as well as biological stressors such as parasites, pathogens and hormones. In addition, HSPs are regulated during insect development, being up-regulated during diapause (Zhao and Jones, 2012; Shen *et al*., 2015). However, the most well characterized molecular responses in insects are those involving thermal shock, mainly heat stress (Lu *et al*., 2016; Zhang *et al*., 2016; Willot *et al*., 2017), because temperature can affect fecundity, development, growth, population abundance, survival and geographic distribution of these organisms (Paul and Keshan, 2016; Lu *et al*., 2016; Li *et al*., 2017). Temperature increase can be more harmful to tropical insects because they live close to their maximum thermal limit when compared to temperate zone insects (Evgen'ev *et al*., 2014; Paul and Keshan, 2016).

The sequences of HSPs in insects are described and available in public genomic databases and show high homology (Lu *et al*., 2016; Wang *et al*., 2015). The number of

studies describing the expression profile of these genes has grown in the last years, aiming to clarify the effects and molecular mechanisms of resistance to heat shock in different insect orders: Coleoptera (Shen *et al*., 2015; Wang *et al*., 2015), Hemiptera (Qiao *et al*., 2015; Lu *et al*., 2016; Li *et al*., 2017), Lepidoptera (Hai-Hong *et al*., 2014; Zhang *et al*., 2016), Hymenoptera (Koo *et al*., 2015; Willot *et al*., 2017), among others.

Generally, expression analysis of heat tolerance genes in insects indicates increase of HSPs transcripts, mainly of the family HSP70 (Willot *et al*., 2017), but also from other HSPs families (Zhao and Jones, 2012). Despite presenting highly conserved sequences, the expression patterns of HSP families in insects are not the same (Zhang *et al*., 2016), varying between different tissues (Keshan *et al*., 2014; Koo *et al*., 2015; Wang *et al*., 2015; Zhang *et al*., 2016) and developmental stages (Wang *et al*., 2015; Li *et al*., 2017). In addition, HSPs expressions can vary between insect genders, occurring earlier or at different temperatures (Chen *et al*., 2014; Qiao *et al*., 2015; Lu *et al*., 2016). These differences in gene expression show that HSPs are important for the control of insect development.

Temperature has played a selective role in the evolution of eusocial Hymenoptera (ants, bees and wasps) and both individuals and colonies have developed strategies to reduce the effects of thermal stress (Nguyen *et al*., 2016).As in other groups of insects, the major HSPs, 60, 70 and 90 have been identified in the Hymenoptera (Elekonich, 2009; Xu et al., 2010; Kool et al., 2015, Nguyen et al., 2016). Among eusocial Hymenoptera, bees are highly tolerant to thermal stress (Koo et al., 2015).

Stingless bees (tribe Meliponini) are distributed throughout Brazil and are present in great diversity in the Amazon region (Pedro, 2014). These organisms have developed thermal adaptation mechanisms, among them thermoregulation, the ability to regulate the internal temperature of their nests (Roldão, 2011; Becker, 2014). However, species of the genus *Melipona* have decreased thermoregulatory capacity when temperature surpasses the optimal temperature of 30ºC (Becker, 2014). High temperatures can influence caste differentiation in *Melipona* species by decreasing the frequency of birth of bee queens, compromising the generation of fertile descendants (Brito *et al*., 2013; Becker, 2014).

In this context, it is assumed that climate change can affect bee survival and reproduction. The study of molecular mechanisms involving responses to heat shock in bees is important to elucidate questions related to the preservation of these pollinizing organisms. We identified and validated *in silico* the genes encoding HSP60, 70 and 90 in the stingless bee *M. interrupta* (Hymenoptera, Apidae, Meliponini), an autochtone species of the Amazon region. In addition, we characterized and compared the gene sequence between genders and castes.

MATERIAL AND METHODS

White-eyed pupae (three queens, three workers and three males per colony) were collected from five colonies of *M. interrupta* (N=45) kept in the Meliponary of the Bee Research Group of the National Institute for Amazonian Research (INPA), Manaus,

Brazil. The pupae were frozen in liquid nitrogen immediately after collection. The total RNA of each individual was extracted and purified using the *SV Total RNA Isolation System Kit* – Promega (Cat. no.Z3105), following the manufacturer's instructions. Total RNA concentration and quality were determined by UV analysis using a *Nanodrop*® spectrophotometer and electrophoresis on 1% agarose gels stained with *GelRed*™ 6,6X (Biotium). The first-strand cDNA was used as a template and partial cDNA sequences of HSP60, 70 and 90 were amplified using three pairs of degenerate primers (Table 1) designed in the computer program MEGA 5.0 (Tamura *et al*., 2011) and based on highly conserved regions of each gene derived from alignment of sequences of related species available in *GenBank* (NCBI).

Table 1. Degenerate primers designed from *GenBank* sequences to isolate the fragments of HSP60, 70 and 90 in *Melipona interrupta*.

Primer	Sequence (5'-3')	Expected Size
F60.1	ATG GGT CCA AAR GGR CGT AAT G	
R60.1	GC YGA RAT AGT TGC TAC YTG WGC	366 bp
F60.2	GCW CAR GTA GCA ACT ATY TCR GC	
R60.2	CT ATT RTC YCC AAA RCC RGG	421 bp
F60.3	GCY CCY GGY TTT GGR GAY AAT AG	
R60.3	GG AAC RAT BCC TTC TTC AAC VGC	412 bp
F70.1	C CCB AAC AAC ACY ATY TTY G	
R70.1	CG RTT RTC RAA RTC TTC RCC	521 bp
F70.2	ATC TTY GAY YTK GGW GGT GG	
R70.2	TA WGC HAC AGC YTC RTC AGG	523 bp
F70.3	ATY GTY YTR GTY GGT GGW TC	
R70.3	C WCC TTC RTA DAC TTG RAT C	330 bp
F90.1	ATY CGY TAT GAA TCT CTC AC	
R90.1	C AGT YTG ATC TTC YTT RAT GTG	411 bp
F90.2	GGT GTR GGT TTY TAY TCT GC	
R90.2	GG RTT TCT KGT CCA RAT TGG	490 bp
F90.3	CAC ATY AAR GAA GAT CAR ACT G	
R90.3	TTG TTG TAA CAT YTC ACG RG	641 bp

One specimen of each gender and caste was used in the PCR amplification of the gene fragments. PCR reactions were carried out with1µL cDNA, 0.4 pmol/µL of each primer (Genone), 0.24 mM dNTP's (Biotech Amazonia), 5 mM $MgCl_2$, (Uniscience), 1x *Taq* buffer (Uniscience), 1.5 unit of *Taq* DNA polimerase (Uniscience) and distilled water, giving a final volume of 25 µl. Cycle conditions were as follows: initial denaturation cycle at 94ºC for 2 min, 35 cycles of denaturation at 94ºC for 30 s, annealing of primers in a temperature range of 50 to 60ºC with 2ºC intervals for 30 s each, extension at 72ºC for 45 s and final extension at 72ºC for 10 min. Amplification products were purified from 1% agarose gels stained with *GelRed*™ 6,6X (Biotium).

The gel was then visualized and photographed using the LPIX (Loccus Biotecnologia) UV photo image documenter. The resulting molecular weight bands were excised from the gel, purified with the *Wizard® SV Gel and PCR Clean-Up System Kit* (Promega) and used as templates for subsequent sequencings using the *Big Dye® Terminator Cycle Sequencing Kit Standart Version 3.1* (Applied Biosystems), following the manufacturer's instructions. The DNA volume was adjusted according to the concentrations and size of fragments (330 to 530 bp). The concentration of the reactions varied from 14 to 25 ng/µL.

Sequencing reactions were performed in a Veriti 96 *Well Thermal Cycler* (Applied Biosystems) using the same primers applied in the PCR amplification. Cycle conditions were as follows: initial denaturation cycle at 96ºC for 1 min, 15 cycles of denaturation at 96ºC for 10 s, annealing at 50ºC for 15 s, extension at 60ºC for 75 s, 5 cycles of denaturation at 96ºC for 10 s, annealing at 50ºC for 15 s, extension at 60ºC for 90 s, 5 cycles of denaturation at 96ºC for 10 s, annealing at 50ºC for 15 s and extension at 60ºC for 120 s. DNA was precipitated following the EtOH/EDTA protocol, resuspended in 10 µL of Formamida *Hi Di*™ (Applied Biosystems), mixed by vortex, denatured at 95ºC for 1 min and injected electrokinetically in the ABI 3130XL *Genetic Analyzer* (Applied Biosystems) sequencer, following the manufacturer's instructions.

Sequencing data was analyzed using the *Phred* software (available from http://www.biomol.unb.br/phph/) and electropherograms were visualized using the *Chromas*© software 2.0. Files were converted into FASTA format (Notepad - Windows Operating System), named and organized. Sequences were manually edited and aligned with the *Clustal W* tool in the *MEGA* software *5.0* (Tamura *et al*., 2011) and *Clustal W2* (available from http://www.ebi.ac.uk/Tools/msa/clustalw2/). The consensus sequences (*contig*) were obtained using the *CAP3* software (available from http://mobyle.pasteur.fr/cgi-bin/portal). The sequences were later submitted to *GenBank* (NCBI) using the BLASTn tool (available from http://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparisons with existing sequences in the database in order to validate, *in silico*, the sequences corresponding to the fragments of HSP60, 70 and 90. The sequences were also qualitatively analyzed (presence or absence of sequences) and compared between genders and castes. In addition, the polypeptide sequences that were generated were compared with the protein sequences from the PROSITE database (http://www.expasy.org/prosite/), *Conserved Domains Database and Resources* (CDD-NCBI) and *GenBank* (NCBI) database using the BLASTp tool (available from http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Among the nine primer pairs designed in this study, six primer pairs were successfully amplified by PCR. The amplified primers were able to yield one gene fragment of HSP60, three fragments of HSP70 and two overlapping fragments of HSP90. The sequenced gene fragments did not differ between genders and castes (Fig. 1).

Figure 1. Alignment of nucleotide sequences and HSP60 fragment in samples of males (60_1_M_SEQ), workers (60_1_O_SEQ) and queens (60_1_R_SEQ) of *Melipona interrupta* showing 100% identity. These sequences were used to obtain the consensus sequence and the same procedure was also done with the fragments of HSP70 and HSP90.

A consensus sequence of 283 bp was obtained from a 300 bpHSP60 fragment of the corresponding region in *M. interrupta*. This sequence had 90% identity with the *GenBank – National Center for Biotechnology Information* (NCBI) sequences of *Bombus terrestris*, 89% with *Bombus impatiens* and 86% with *Megachile rotundata.* On the basis of the bioinformatics prediction, the nucleotide sequence of *M. interrupta* encodes a polypeptide fragment of 94 amino acids, showing 100% similarity with *Apis mellifera*, *Apis dorsata* and *Apis florea* at position 60-153, when aligned (BLASTp) with complete sequences of HSP60 (Fig. 2).The classic molecular signature of the HSP60 family (ATRAAVEEGIVPGGG) was not observed in *M. interrupta* because the gene fragment was small and did not cover this region. However, this signature sequence was present in the compared sequences at position 422-436, a common characteristic of mitochondrial HSP60 (available with the code PS00296 at http://prosite.expasy.org/).

A comparison with the data from CDD-NCBI showed similarity between the nucleotide fragment of Hsp60 and the conserved domains of the chaperonin superfamily, which also includes proteins analogous to eukaryote HSP60 in bacteria (GroEl) and chloroplasts (Cnp60). Therefore, it is possible that HSP60 in *M. interrupta* is a group I chaperonin present in the mitochondria of eukaryotic cells.

Figure 2. Aligning of the amino acid sequence of HSP60 in *Melipona interrupta* with the sequences from other bee species. Detail in: A = classic signature of mitochondrial HSP60 and B = (GGM)n motif.

Three fragments of Hsp70 containing 357, 284 and 330 bp each were obtained respectively. However, it was not possible to generate a *contig* sequence because the second and last fragment did not overlap. These fragments had 86-92% identity with the *GenBank* (NCBI) sequences for the HSP70 of *A. dorsata*, *A. mellifera*, *B. terrestris* and *B. impatiens.* On the basis of the bioinformatics prediction, these three nucleotide sequences (357, 284 and 330 bp) encode three polypeptide fragments: the first fragment of 151 amino acids at position 69-219, the second of 94 amino acids at position 232-325 and the third of 109 amino acids at position 337- 443. When aligned (BLASTp) with HSP70 of 651 amino acids, these three fragments present > 90% similarity with *A. mellifera*, *A. florea*, *A. dorsata*, *B. terrestris*, *B. impatiens* and *M. rotundata*. Two patterns of signatures were observed in fragments 1 and 3, respectively: IFDLGGGTFDVSIL (available with the code PDOC00329 at http://prosite.expasy.org/) at position 197-210 and IVLVGGSTRIPKIQK (available with the code PS01036 at http://prosite.expasy.org/) at position 334-348 (Fig. 3). Of the three signature patterns sequences of HSP70, two were observed in the fragments of *M. interrupta* obtained in this study. Therefore we validate, *in silico*, these sequences as part of the gene HSP70 in this species.

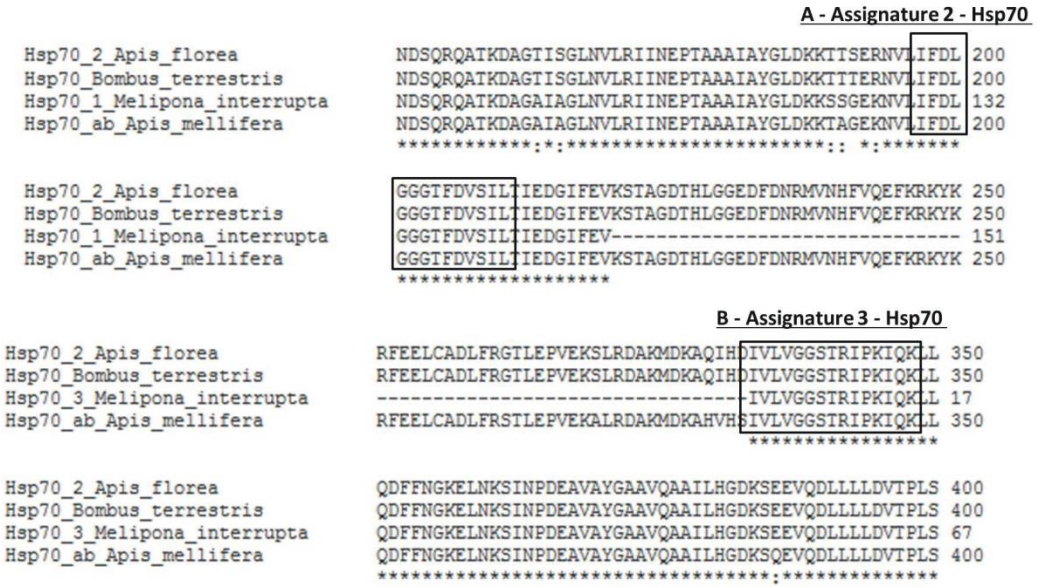

Figure 3. Aligning of the fragments with 151 and 109 amino acids in *Melipona interrupta*with the protein HSP70 of *Apis florea*, *Bombus terrestris* and *Apis mellifera*. Detail in: A = classic signature 2 of HSP70 and B = classic signature 3 of HSP70.

The amino acid sequences with which the *M. interrupta* fragments obtained 100% similarity to HSP70 from other bees present the C-terminal motif 648-651: MEEVD. This motif classifies this HSP70 as being of cytosolic origin. Thus, it can be inferred that the HSP fragment sequenced from *M. interrupta* is from a cytosolic HSP70.

The two overlapping fragments of HSP90 presented 307 and 325 bp. Both fragments were used to generate a *contig* sequence of 708 bp. Aligning of the *contig* with sequences from *GenBank* (NCBI) showed 89% identity with *B. terrestris*, 88% with *B. impatiens* and 84% with *M. rotundata.*

The nucleotide sequence of the HSP90 *contig* in *M. interrupta* encodes a polypeptide fragment of 235 amino acids located at position 55-289. When aligned with HSP90 (BLASTp) of 725 bp, this fragment presents 100% similarity with *A. mellifera*, 99% with *A. dorsata* and 97% with *B. terrestris.* This *contig* sequence in *M. interrupta* presented the signatures LGTIAKSGT located at position 103-111 and IGQFGVGFYSAYLVAD located at position 127-142 as well as a conserved domain GxxGxG (GQFGVG) located at position 128-133 (Fig. 4). Comparisons with the data from CDD-NCBI showed identity between the *contig* sequence with an ATP-binding domain (at position 55-190) and a HSP90 domain (at position 193-289). These results indicate that the *contig* obtained is a fragment of the HSP90 of cytoplasmic origin in *M. interrupta.*

Figure 4. Aligning of the fragments with 235 amino acids in *Melipona interrupta* with the protein HSP90 of *Bombus terrestris, B. impatiens* and *Apis mellifera.* Classic signatures 2 and 3 of HSP90 in the boxes and conserved motif GxxGxG underlined.

DISCUSSION

Although they are mainly located in the mitochondria of eukaryotic cells, HSP60 proteins are also found in the cytosol and endoplasm (Li *et al*; 2017). The gene expression profile of HSP60 in insects is not as commonly studied as those of HSP70 and 90 because the expression of this protein cannot be induced by heat stress in certain species (Hai-Hong *et al*., 2014; Li *et al*., 2017). In *D. menalogaster*, HSP60 is a well-studied protein presenting four isoforms with different sizes and functions. This includes a paper on embryonic stages, transportation, signaling and development (Arya and Lakhotia, 2008). HSP60 family has eight conserved signature sequences (TFGPKG, DGVTVAKEI, AGDGTTTATVL, EGMQFDRGYISPY, PLLIIAED, AVKAPGFGDRRK, EKLQERLAKLAGGVAVIKVG, ATRAAVEEGIVPGGG (Brocchieri and Karlin, 2000) that mostly correspond to binding and transition sites between structural domains. The signature sequence ATRAAVEEGIVPGGG has three glycines that bind with an ATP molecule and a conserved GGM repeat domain, indicating mitochondrial localization of HSP60 (Hai-Hong *et al*., 2014; Li *et al*., 2017).

Signature sequences and motifs were not observed in the sequence fragment of Hsp60 in *M. interrupta*. However, alignment of this fragment with sequences of *A. mellifera, A. florea* and *A. dorsata* presented the classic signature of ATRAAVEEGIVPGGG at position 422-436.The nucleotide fragment of HSP60 showed similarity with the conserved domains of the chaperonin superfamily, which includes proteins analogous to the eukaryote HSP60 in bacteria (GroEl) and chloroplasts (Cnp60) (CDD-NCBI). Therefore, although it does not present the signature sequence, HSP60 in *M. interrupta* may be a group I chaperonin located in the mitochondria of eukaryote cells. Alignment of the HSP60 fragment with the sequences in public genomic databases presented 100% similarity with *A. florea*, *A. dorsata* and *A. mellifera*. Cui *et al*. (2010) found similarity in the complete amino acid sequence of *Chilo supressalis*, which was93% with HSP60A and 88% withHSP60C of *D. melanogaster*.

We also found 80% similarity with *A. mellifera* and *A. aegypti*. After sequencing the gene that encodes HSP60 in *Rhopalosiphum padi* (Hemiptera), Li *et al*. (2017) observed 73-81% homology of this species with other insects. Hai-Hong *et al*. (2014) also observed significant homology and identity greater than 80% between the sequences of *Frankliniella occidentalis* (Thysanoptera) and other insects, including *A. mellifera* and *D. melanogaster*. The HSP70 family provides primary protection during exposure to heat shock (Evgen'ev *et al*., 2014). This family includes HSP70 inducible proteins that are highly expressed during stress and HSC70 cognate proteins expressed in normal conditions and weakly or not expressed during stress (Luo *et al*., 2015; Wang *et al*., 2015; Li *et al*., 2017). Many studies have found high homology and identity over 90% in the sequences of HSP70 among different groups of insects (Luo *et al*., 2015; Li *et al*., 2017). Wang *et al*. (2015) showed that the sequences of Hsc70 and HSP70 in *Xestia c-nigrum* (Lepidoptera) were more closely related to the sequences of the corresponding genes of other insect species than the genes of another individual of *X. c-nigrum*. Our results confirm this high homology since the polypeptide fragments of HSP70 in *M. interrupta* showed 90-100% similarity with HSP70 from *A. mellifera, B. terrestris, B. impatiens, A. florea, A. dorsata* and *M. rotundata*. HSP70 has a well-conserved ATPase domain in the aminoterminal part and a less conserved domain that binds to the substrate in the carboxi-terminal part (Zhang *et al*., 2016; Li *et al*., 2017).

The HSP70 family presents three signature pattern sequences: IDLGTTYSCVGV, IFDLGGGTFDVSIL and IVLVGGSTRIPKIQ (Gupta, 1995; Shen *et al*., 2015). Two of these sequences were also found in the fragments of *M. interrupta*: IFDLGGGTFDVSIL (available with the code PDOC00329 at http://prosite.expasy.org/) in the first fragment at position 197-21 and IVLVGGSTRIPKIQ (available with the code PS01036 at http://prosite.expasy.org/) in the third fragment at position 334-348. The latter sequence represents part of the conserved peptide binding domain - NBD (available at https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). This data validates the fragments obtained in this study as the gene HSP70 in *M. interrupta*. HSPs have cell localization signals and the MEEVD motif that is located in the C-terminal domain is a HSP signal of cytosolic origin. Possibly, this motif binds to other co-chaperone proteins, helping protein folding (Gupta, 1995; Wang *et al.*, 2015; Qiao *et al*., 2015). The MEEVD motif is also present in HSC70 and HSP70 and conserved in most of the sequences in insects (Wang *et al*., 2015). The amino acid sequences of HSP70 fragments in *M. interrupta* with 100% similarity showed the MEEVD motif in the C-terminal part at position 648-651. Therefore, this fragment may correspond to the HSP70 from the cytosol of *M. interrupta.*

HSP90 proteins are among the most abundant and well-conserved proteins, corresponding to 1-2% of the total proteins in a cell under normal conditions (Sun *et al*., 2015; Qiao *et al*., 2015; Lu *et al*., 2016). HSP90 has an important role in cell homeostasis, acting in fundamental cell activities, such as protein folding and refolding and thermal shock stress as well as other physiological functions, such as cell signaling and protein degradation (Sun *et al*., 2015; Lu *et al*., 2016).HSP90 presents two isoforms in the cell: an inducible form (HSP90A) and a constitutive form (HSP90Beta) (Sun *et al*., 2015). However, only one isoform is present in many insect species (Lu *et al*., 2016). Xu *et al*. (2010) observed that *A. mellifera* showed two homologous copies of the gene HSP90 located in different chromosomes. The first gene encodes the transcript A, a transcript that is similar and conserved among other insect species. The other gene is less conserved and

specific to *A. mellifera,* encoding transcripts B and C. In addition, this gene may also encode another eight transcripts from the same loci by alternative splicing. We believe that this event originates from gene duplication and might have an important role in behavioral and morphological differentiation between castes, considering that gene expression is caste and age specific in the adult bee.

Many studies about multiple aligning of amino acids sequences in insects have shown identity over 80% and high homology between sequences of HSP90, demonstrating the conservation of this protein, especially in its signature region (Hai-Hong *et al*., 2014; Qiao *et al*., 2015). The HSP90 fragment in *M. interrupta* aligned with other sequences of bee species from *GenBank* (NCBI) presented an identity interval of 97-100% and 100% similarity with *A. mellifera,* 99% with *A. dorsata* and 97% with *B. terrestris*. The *contig* sequence in *M. interrupta* had an identity of 75% with transcript A and86-87% with transcripts B and C from *A. mellifera*. Thus, *M. interrupta* may present two isoforms of HSP90. HSP90 has three conserved domains: an ATPase domain in the N-terminal part, a central domain with catalytic sites and another binding domain in the C-terminal part (Qiao *et al*., 2015; Lu *et al*., 2016).

Gupta (1995) described five conserved amino acid sequences in HSP90: NKEIFLRELISN(S/A)SDALDKIR, LGTIA(K/R)SGT, IGQFGVGFYS(A/C)(Y/F)LVA(E/D), IKLYVRRVFI and GVVDS(E/D)DLPLN(I/V)SER. Many other studies have also confirmed these signature sequences in insects (Hai-Hong *et al*., 2014; Chen *et al*., 2014; Sun *et al*., 2015; Qiao *et al*., 2015). Moreover, the two conserved motifs MEEVD in the C-terminal part and the central domain GXXGXG in all HSP90 are also common in HSP70 (Gupta, 1995). Both motifs represent location signals of the cytosol with MEEVD binding to co-chaperones with tetrapeptide domains (TRP) (Chen *et al*., 2014; Hai-Hong *et al*., 2014; Qiao *et al*., 2015; Lu *et al*., 2016). We observed two signature sequences LGTIAKSGT at position 103-111 and IGQFGVGFYSAYLVAD at position 127-142 and the conserved motif GXXGXG (GQFGVG) at position 128-133 in the fragment of HSP90 in *M. interrupta.* Based on the CDD-NCBI database of conserved domains, the *contig* sequence presents identity with an ATP binding domain (at position 55-190) and an HSP90 domain (193-289). These results indicate that the *contig* obtained is a fragment of the HSP90 of cytoplasmic origin in *M. interrupta*.

The HSPs genes in insects are induced and modulated in response to environmental factors (biotic and abiotic), being fundamental to an insect's survival in its habitat (Zhao and Jones, 2012). The identification and characterization of HSPs genes in Hymenoptera are advanced with the description of 36 Hsps genes in the genome of *A. mellifera.* However, the expression profile of HSPs in response to environmental stressors, such as heat shock, still needs to be investigated considering its importance to monitor insect susceptibility to environmental impacts (Elsik *et al*., 2014; Koo *et al*., 2015). In addition, the molecular characterization of Hsps in Hymenoptera shows functional divergences in the identity, functional proprieties, amino acid sequences and regulation of HSPs related to heat tolerance (Nguyen *et al*., 2016). The HSP60, 70 and 90 families play an important role in resistance to thermal stress in insects. HSP70 and 90 are the most inducible families during heat shock while the HSP60 induction may or not occur in certain insect species (Hai-Hong *et al*., 2014). The expression profile of these proteins also varies according to developmental stage, gender, age and tissue (Chen *et al*., 2014; Hai-Hong *et al*., 2014; Wang *et al*., 2015; Lu *et al*., 2016; Qiao *et al*., 2015; Zhang *et al*., 2016; Li *etal*., 2017).

The increased expression of HSPs in eusocial Hymenoptera such as bees is a physiological strategy to reduce the negative effects of heat stress (Nguyen *et al*., 2016). Many studies have focused on the influence of climate changes over insects, since high temperatures can harm the survival and reproduction of these animals. In addition, temperature increase can be more noxious to tropical insects, such as the bees of the genus *Melipona*, because these animals live close to their optimum temperature. Therefore, HSPs activity is important in adapting to climate changes (Evgen'ev *et al*., 2014; Becker, 2014; Paul and Keshan, 2016; Lu *et al*., 2016). The ability of *Melipona* bees to thermoregulate the internal nest temperature becomes limited in temperatures over 30ºC (Nunes-Silva *et al*.,2006; Roldão, 2011; Becker, 2014). The growth and development of the colony as well as it is function and survival is directly compromised when the temperature surpasses 30ºC (Becker, 2014). Therefore, environmental factors, such as high temperatures, can generate phenotypic changes that alter gene expression, caste segregation and morphological, physiological and behavioral characteristics of colonies (Carvalho, 2000; Nunes-Silva *et al*.,2006; Silva *et al*., 2009).

Here, we described the nucleotide sequences of HSP60, 70 and 90 in *M. interrupta.* No difference was observed between the sequences from different genders and castes. Despite highly conserved sequences in HSPs families (Evgen'ev*et al*., 2014) our results could not be extrapolated to generate complete sequences because we only sequenced gene fragments. Most organisms have multiple genes encoding HSPs and each family of these proteins also presents paralogous genes located in many cell compartments (Zhang *et al*., 2016; Nguyen *et al*., 2016). The expression profiles of HSPs are less conserved than the nucleotide sequences and vary between different tissues, genders and developmental stages (Qiao *et al*., 2015; Lu *et al*., 2016; Zhang *et al*., 2016). Studies have shown that there are differences in the expression profiles of HSP70 and HSP90 in *A. mellifera* that are caste and age specific; that is their level of expression is dependent on the behavioral function within the colony (Willians *et al*., 2008; Aamadot, 2008; Elekonich, 2009). In addition to differential expression, there are specific regulatory mechanisms that are dependent on alternative splicing and which may be related to caste differentiation (Aamadot, 2008; Xu *et al*., 2010; Hai-Hong *et al*., 2014).

It has been proven that environmental factors such as heat shock can influence the developmental trajectories of bees, including stingless bees of the genus *Melipona* (Kerr, 1974; Becker, 1974). Therefore, *in silico* validation of heat shock protein coding genes performed here opens up avenues for further studies that can elucidate questions regarding the differential expression of these genes in response to potentially influential environmental factors that may influence development, maintenance and survival of stingless bee colonies.

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