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Genes acting in longevity-related pathways in the endoparasitoid, *Pteromalus puparum*

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Abstract

Among insects, lifespans vary over a broad range, from the short-lived mayflies to the 17-year periodical cicadas. Generally, lifespans are determined by a phase in life, the reproductive lifespan, which varies among species. Numerous pathways, such as the insulin/insulin-like growth factor signaling pathway, the target of rapamycin pathway and the mitogen-activated protein kinase/extracellular signal-regulated kinases pathways, influence aging and lifespan. Components of these pathways were identified as lifespan-related genes, including genes mediating growth, metabolism, development, resistance, and other processes. Many age-related genes have been discovered in fruit flies, honeybees, and ants among other insect species. Studies of insect aging and longevity can help understand insect biology and develop new pest management technologies. In this paper, we interrogated the new *Pteromalus puparum* genome, from which we predicted 133 putative lifespan-related genes based on their homology with known lifespan-related genes of *Drosophila melanogaster*. These genes function in five signaling pathways and three physiological processes. The conserved domain structures of these genes were predicted and their expression patterns were analyzed. Amino acid sequence alignments and domain structure analysis indicate that most components remain conserved across at least six insect orders.

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The data in this paper will facilitate future work on parasitoid lifespans, which may have economic value in biocontrol programs.

KEYWORDS
lifespan-related genes, longevity, *Pteromalus puparum*, signaling pathways

1 | INTRODUCTION

Aging can be taken as a progressive failure of maintaining homeostasis at the cellular level and it is influenced by environmental and genetic factors. Long-term exposure to environmental stressors, for example, lead to the accumulation of damage that goes beyond the capacities of homeostatic repair mechanisms (López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). Several signaling pathways are thought to influence lifespan, including the insulin/insulin-like growth factor (IGF)-1 (IIS; Altintas, Park, & Lee, 2016), the sirtuins (Dang, 2014), the adenosine monophosphate-activated protein kinase (AMPK; Burkewitz, Zhang, & Mair, 2014), and the target of rapamycin (TOR) pathways (Johnson, Rabinovitch, & Kaeberlein, 2013). There is substantial cross-talk among intracellular pathways and their aggregate response to events promotes cellular fitness and influences lifespan. Aging is a complex process and other signaling systems, such as the phosphatidylinositol 3-kinase (PI3K)/Akt, and Forkhead Box Class O (FOXO) signaling pathways also influence lifespan.

Dietary restriction or caloric restriction (CR) seems to extend the life of many organisms, including yeast, worms, flies, mice, monkeys, and possibly humans (Fontana, Partridge, & Longo, 2010), although the idea is not without serious critics (Sohal & Forster, 2014). CR reduces activities in nutrient-sensing pathways, IIS and, in *Caenorhabditis elegans*, FOXO signaling, which can increase resistance to misexpressed toxic proteins (Fontana et al., 2010). CR regulates lifespan through nutrient-sensing signaling pathways: the IIS, mechanistic target of rapamycin (mTOR), AMPK, and sirtuins (Kenyon, 2005). The mTOR and IIS pathways act together as an integrated nutrient-sensing network in mediating the lifespan extension following CR in laboratory animals (Partridge, Alic, Bjedov, & Piper, 2011). The IIS pathway connects energy metabolism, cell growth, and behavior to nutritional status. Inhibiting the IIS pathway systemically or increasing the activity of a transcription factor (TF) FOXO in the *Drosophila melanogaster* fat body led to lifespan increases. Mutations in the insulin receptor and in IIS pathway genes influence mice's lifespan (Kenyon, 2010).

mTOR is a kinase that regulates a wide variety of cellular functions, including growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription (Weichhart, 2018). mTOR serves as a core component in two complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Although the mechanism of rapamycin action is not fully understood, inhibiting mTORC1 may delay age-related diseases rather than aging *per se*. Cellular senescence follows leaving the cell cycle, while the cell remains viable. Senescent cells secrete proinflammatory and pro-oxidant signals that accumulate with age, leading to the termed senescence-associated secretory phenotype (SASP). mTORC1 promotes SASP (Herranz, Gallage, & Gil, 2015). mTORC1 inhibitors block the formation of the senescence-associated phenotype, which may, at least in part, target the life-extending mechanisms of rapamycin (Weichhart, 2018).

Currently, researchers always utilize four biomedical model organisms, including budding yeast, fruit flies, nematode worms, and laboratory mice, for studying the molecular mechanisms of aging and lifespan. Some genetic and environmental factors that modulate longevity are broadly conserved from yeast to humans (Fontana et al., 2010). Specifically, the manipulation of several orthologous genes in model organisms is sufficient to alter lifespan, suggesting that some fundamental patterns of aging are shared across taxa.

Many invertebrates are excellent genetic model systems. Among insects, *D. melanogaster* has emerged as a particularly useful model. Several other species, such as the silkworm, *Bombyx mori* and the red flour beetle, *Tribolium castaneum* are similarly useful models. Our research program uses the endoparasitoid, *Pteromalus puparum* and its host, *Pieris rapae*, as a model system for research into host-parasitoid interactions. In this paper, we interrogated the *P. puparum* genome to identify lifespan-related genes.

2 | MATERIALS AND METHODS

2.1 | Insect rearing and sample collections

The laboratory *P. puparum* and its host, *Pieris rapae*, cultures were reared at 25°C with a 14L:10D photoperiod (Yang et al., 2017). The duration of *P. puparum* life cycle is about 40 days. After parasitism, the parasitoid embryos hatch into larvae in 48 hr, and the larval stage costs 8 days. The pupal stage of *P. puparum* is around 10 days, and then the adults emerge. The lifespan of *P. puparum* adults is ~20 days. For sample collection, *P. puparum* embryos were sampled from newly parasitized *P. rapae* pupae within 12 hr to keep all samples at the early embryo stage. Wasp larvae were isolated at 2, 4, and 6 days after parasitism to prepare 1st, 2nd, and 3rd instars. Each sex of the early stage pupae and 2-day mated wasps were then collected separately. For sample collection of adult wasps under standard and high temperatures, newly emerged adults were placed in incubators at 25°C and 35°C. Adult females were obtained at 5, 10, and 15 days after eclosion, representing youth, middle, and old ages. All samples were collected, washed, and ground with TRIzol reagent (Invitrogen), following the manufacturer's instructions.

2.2 | Identification of lifespan-associated genes in *P. puparum* genome

We extracted lifespan-associated genes from the *D. melanogaster* and other insect genomes and retrieved each gene's protein sequence from the NCBI GenBank. We interrogated the *P. puparum* genome using BLASTP to acquire homologous candidate protein sequences with an E-value of E^{-5}. All lifespan-related gene candidates were analyzed by HMMER with the Pfam database to ensure that each sequence contains the iconic domain structures (Prakash, Jeffryes, Bateman, & Finn, 2017). After manually selecting candidate sequences with domain signatures, we submitted data onto BlastKOALA (http://www.kegg.jp/blastkoala/) for verification. Gene Ontology (GO) analysis was carried out using GeneMerge (Castillo-Davis & Hartl, 2003).

2.3 | Analysis of RNA-seq data

The acquisition and processing of RNA-seq data were performed following the protocol previously described by Yang et al. (2017). The fragments per kilobase of exon model per million reads mapped (FPKM) values of *P. puparum* lifespan-related genes are listed in Table S1. The transcript profiles of *P. puparum* lifespan-related genes in each development stage were visualized using TBtools (https://github.com/CJ-Chen/TBtools).

2.4 | qPCR analysis

Total RNA of adult wasps of indicated ages were extracted with TRIzol and quantified using a NanoDrop (Thermo Fisher Scientific) spectrophotometer. cDNA was synthesized using a PrimeScript™ One-Step RT-PCR Kit (Takara, Japan). One nanogram cDNA was used as a template in 25 μl reaction volume. Primers (Table S2) were designed with Primer3 web version 4.1.0 and Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). At first, we used reverse transcription polymerase chain reaction (RT-PCR) and the sequencing of its product to correct the nucleotide sequences of the selected genes for qPCR verification. At least two pairs of specific primers were designed for each tested gene. To verify the specificity of the qPCR primers, a dissociation curve was included from

60 to 95°C at the end of each qPCR reaction. To determine the efficiency of the primers, we performed qPCR for the templates with serial dilutions from 10 to 100,000, respectively, and calculated their efficiency values. Based on the results from the specificity and efficiency verifications, suitable primers for the gene expression profile determinations were selected. The qPCR was performed with SYBR Premix Ex Taq^{TM} II (Tli RNase H Plus) (Takara). Each sample was analyzed in three biologically independent replicates with three technical replicates each. Messenger RNA (mRNA) accumulations in the heat-stressed group were compared with the control group using the stably expressed 18S rRNA as reference gene. Statistical analysis was carried out in GraphPad Prism 6. The $2^{-\Delta\Delta C_t}$ method was utilized on the quantification of mRNA expression levels (Livak & Schmittgen, 2001). One-way analysis of variance was performed on data representing expression profiles of different development stages and multiple *t* test was used in expression profiles of thermal response.

3 | RESULTS AND DISCUSSION

3.1 | Genes in lifespan-related pathways

We identified 133 lifespan-related genes in *P. puparum* (Table 1), along with their sequences and functional domains (Table S3). A concept map of the longevity regulating pathway is represented in Figure 1. We predicted the possible function of *P. puparum* lifespan-related genes based on their homology to *D. melanogaster* and other model species. Based on the reported functions of the homologues of these genes involved in the lifespan regulation of the different model organisms, we speculate that they may also be related to the lifespan regulation of *P. puparum*. GO analysis indicates these genes fall into seven functional groups. The binding functional group has the most members (68 genes). Followed by a catalytic activity group, it contains 46 genes. In addition, 16 genes have TF activity (Tables 2 and S4). Our heat map based on RNA-seq data (Figure 2) shows that genes are more highly expressed in embryos, with high expression of a few genes in adults. The homologues of lifespan-related genes identified in *P. puparum* also possess functions in cellular processes involved in growth, metabolism, and development. Thus, the highly transcript levels of these genes at the embryo stage may be related to *P. puparum* embryogenesis. Cluster analysis roughly divides gene expression patterns into three types. Most of the genes with high expression in various developmental stages are related to stress resistance. In the following sections, we discuss genes acting in specific pathways.

3.1.1 | Insulin/IGF-1 signaling pathway

Insulin regulates some aspects of carbohydrate, lipid, and protein metabolism (Nassel & Vanden Broeck, 2016). IIS influences longevity, reproduction, and multiple diseases in humans (Mazucanti et al., 2015). We identified homologs of the *Drosophila* insulin receptor (InR), insulin receptor substrate 1 (IRS-1), and insulin-like peptide (ILP) in *P. puparum* (Table 1). There is one *P. puparum* insulin-like peptide (PpILP), compared to eight in *D. melanogaster*. The ILPs share a conserved family signature, from which we infer they are evolutionarily related. We did not record the IGF-1 in *P. puparum*. ILP and IGF-1 bind to InR, a tyrosine kinase receptor, encoded by a single gene *InR*. Subsequently, IRS-1 is activated via phosphorylation. *P. puparum* insulin receptor substrate 1 (PpIRS-1) is homologous to the *Drosophila* protein Chico. Reduced expression of *Chico* extends the median fly lifespan up to 48% in homozygotes and 36% in heterozygotes (Clancy, 2001). The mRNA expression level of PpILP is peaked at the embryo stage and become higher after larval period. It suggests that PpILP is involved into the processes of the nutrient uptake and growth regulation. The transcript level of *P. puparum* InR (PpInR) gene is higher at the embryo than that at the stages after larval period, which may indicate that PpInR plays roles in *P. puparum* embryo development. The expression level of the gene encoding PpIRS-1 is relatively stable during various development stages with the highest FPKM value near to 53 (Figure 2 and Table S1). We speculate that the functions of *P. puparum* IIS may be also related to nutrient uptake and the growth and metabolism regulations.

TABLE 1 Gene ID and annotation of *P. puparum* lifespan-related genes

Gene ID	Gene name	Gene description	Shown in Figure 1/Figure S2
IIS			
PPU07299-RA	PpInR	Insulin receptor	InR/IGF-1R
PPU08276-RA	PpIRS-1	Insulin receptor substrate 1	IRS-1
PPU01146-RA	PpILP	Insulin	INS/IGF-1
PPU11776-RA	PpHCF1	Host cell factor	HCF1
PPU04680-RA	PpSMEK	Protein phosphatase 4 regulatory subunit 3	SMEK
PI3K/Akt			
PPU14715-RA	PpAkt	RAC serine/threonine-protein kinase	Akt
PPU02657-RA	PpPDK1	3-Phosphoinositide dependent protein kinase 1	PDK1
PPU00499-RA	PpPI3K92E	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha/beta/delta	PI3K
PPU12539-RA	PpPI3K21B	Phosphoinositide-3-kinase regulatory subunit alpha/ beta/ delta	PI3K
PPU14545-RA	Ppwdb-1	Serine/threonine-protein phosphatase 2A regulatory subunit B′	wdb
PPU01704-RA	Ppwdb-2	Serine/threonine-protein phosphatase 2A regulatory subunit B′	wdb
PPU05718-RA	PpPTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	PTEN
PPU13406-RA	PpFOXO	Forkhead box protein O	FOXO
PPU05162-RA	PpFOXA2	Forkhead box protein A2	FOXA2
PPU15278-RA	Pp14-3-3ε	14-3-3 protein epsilon	14-3-3
PPU02576-RA	Pp14-3-3ζ	14-3-3 protein zeta	14-3-3
MAPK/ERK			
PPU04462-RA	PpJNK	C-Jun N-terminal kinase	JNK
PPU10958-RA	PpRas	GTPase K-Ras	Ras
PPU16623-RA	PpMEK	Mitogen-activated protein kinase kinase 1	MEK
PPU05765-RA	PpERK	Mitogen-activated protein kinase 1/3	ERK
PPU02803-RA	Ppp38	P38 MAP kinase	p38
PPU01990-RA	PpAop	ETS translocation variant 6/7	Aop
PPU09895-RA	PpRaf	B-Raf proto-oncogene serine/threonine-protein kinase	Raf
mTOR			
PPU04166-RA	PpEIF4E-1	Translation initiation factor 4E	EIF4E
PPU13693-RA	PpEIF4E-2	Translation initiation factor 4E	EIF4E
PPU04123-RA	PpEIF4E-3	Translation initiation factor 4E	EIF4E
PPU00322-RA	Pp4E-BP	Eukaryotic translation initiation factor 4E binding protein	4E-BP
PPU11457-RA	PpmTOR	Serine/threonine-protein kinase mtor	mTOR
PPU03798-RA	PpRaptor	Regulatory associated protein of mtor	Raptor
PPU06343-RA	PpPRAS40	Proline-rich Akt substrate 1	PRAS40

(Continues)

TABLE 1 (Continued)

Gene ID	Gene name	Gene description	Shown in Figure 1/Figure S2
PPU16116-RA	PpmLST8-1	Target of rapamycin complex subunit LST8	mLST8
PPU10534-RA	PpmLST8-2	Target of rapamycin complex subunit LST8	mLST8
PPU04711-RA	PpS6KA-1	Ribosomal protein S6 kinase alpha-1/2/3/6	S6K
PPU05283-RA	PpS6KA-2	Ribosomal protein S6 kinase alpha-1/2/3/6	S6K
PPU15964-RA	PpS6KB-1	Ribosomal protein S6 kinase beta	S6K
PPU05285-RA	PpS6KB-2	Ribosomal protein S6 kinase beta	S6K
PPU05284-RA	PpS6KB-3	Ribosomal protein S6 kinase beta	S6K
PPU10327-RA	PpS6KB-4	Ribosomal protein S6 kinase beta	S6K
PPU06188-RA	PpTSC1	Tuberous sclerosis 1	TSC1
PPU08526-RA	PpTSC2-1	Tuberous sclerosis 2	TSC2
PPU00208-RA	PpTSC2-2	Tuberous sclerosis 2	TSC2
PPU00209-RA	PpTSC2-3	Tuberous sclerosis 2	TSC2
PPU03684-RA	PpRheb	Ras homolog enriched in brain	Rheb
AMPK			
PPU00059-RA	PpPRKAA	5′-AMP-activated protein kinase, catalytic alpha subunit	AMPK
PPU10800-RA	PpPRKAB	5′-AMP-activated protein kinase, regulatory beta subunit	AMPK
PPU02727-RA	PpPRKAG	5′-AMP-activated protein kinase, regulatory gamma subunit	AMPK
PPU06179-RA	PpNF-κB	Nuclear factor NF-kappa-B p105 subunit	NF-κB
PPU09281-RA	PpLkb1	Serine/threonine-protein kinase 11	Lkb1
PPU12340-RA	PpPKA-1	Protein kinase A	PKA
PPU07580-RA	PpPKA-2	Protein kinase A	PKA
PPU03651-RA	PpDorsal-1	C-Rel proto-oncogene protein	NF-κB
PPU14444-RA	PpDorsal-2	C-Rel proto-oncogene protein	NF-κB
PPU14463-RA	PpDorsal-3	C-Rel proto-oncogene protein	NF-κB
PPU03121-RA	PpDorsal-4	C-Rel proto-oncogene protein	NF-κB
PPU08282-RA	PpDorsal-5	C-Rel proto-oncogene protein	NF-κB
PPU07824-RA	PpAC2	Adenylate cyclase 2	AC
PPU11296-RA	PpAC3	Adenylate cyclase 3	AC
PPU10905-RA	PpAC8	Adenylate cyclase 8	AC
PPU11536-RA	PpAC9	Adenylate cyclase 9	AC
PPU06056-RA	PpCyr1	Adenylate cyclase	AC
PPU02268-RA	Ppp53-1	Cellular tumor antigen p53 isoform X1	p53
PPU12298-RA	Ppp53-2	Cellular tumor antigen p53 isoform X2	p53
PPU13750-RA	PpSestrin	Sestrin-3	Sestrin
PPU11611-RA	PpCREB	Cyclic AMP-responsive element-binding protein 3	CREB
PPU13552-RA	PpCRTC-1	CREB-regulated transcription coactivator-1	CRTC-1

(Continues)

TABLE 1 (Continued)

Gene ID	Gene name	Gene description	Shown in Figure 1/Figure S2
PPU08093-RA	PpATF4	Cyclic AMP-dependent transcription factor ATF-4	CREB
PPU07049-RA	PpATF2	Cyclic AMP-dependent transcription factor ATF-2	ATF-2
PPU03775-RA	PpADIPOR	Adiponectin receptor	ADIPOR
PPU04412-RA	PpEHMT	Euchromatic histone-lysine N-methyltransferase	EHMT
Autophagy			
PPU05400-RA	PpULK	Serine/threonine-protein kinase ULK2	ULK
PPU01680-RA	PpATG101	Autophagy-related protein 101	ATG101
PPU12664-RA	PpATG10L	Ubiquitin-like-conjugating enzyme ATG10	ATG10L
PPU10539-RA	PpATG12	Ubiquitin-like protein ATG12	ATG12
PPU04252-RA	PpATG13	Autophagy-related protein 13	ATG13
PPU05117-RA	PpATG14L	Beclin-1-associated autophagy-related key regulator	ATG14L
PPU10657-RA	PpATG16L1	Autophagy-related protein 16-1	ATG16L1
PPU12583-RA	PpATG18	Autophagy-related protein 18	ATG18
PPU05135-RA	PpATG2	Autophagy-related protein 2	ATG2
PPU04374-RA	PpATG3	Ubiquitin-like-conjugating enzyme ATG3	ATG3
PPU14597-RA	PpATG4	Cysteine protease ATG4	ATG4
PPU04431-RA	PpATG5	Autophagy-related protein 5	ATG5
PPU12802-RA	PpBeclin-1	Beclin	Beclin-1
PPU02491-RA	PpATG7	Ubiquitin-like modifier-activating enzyme ATG7	ATG7
PPU12140-RA	PpATG9-1	Autophagy-related protein 9	ATG9-1
PPU05842-RA	PpATG9-2	Autophagy-related protein 9	ATG9-2
PPU02365-RA	PpATG9-3	Autophagy-related protein 9	ATG9-3
PPU05508-RA	PpGABARAP	GABA(A) receptor-associated protein	GABARAP
PPU07497-RA	PpMAP1LC	Microtubule-associated protein 1 light chain	MAP1LC
PPU06071-RA	PpFIP200-1	RB1-inducible coiled-coil protein 1	FIP200-1
PPU07715-RA	PpFIP200-2	RB1-inducible coiled-coil protein 1	FIP200-2
PPU12417-RA	PpEPG5	Ectopic P-Granules Autophagy Protein 5	EPG5
PPU17080-RA	PpVps34	Phosphatidylinositol 3-kinase	Vps34
PPU15090-RA	PpVps15	Phosphoinositide-3-kinase, regulatory subunit 4	Vps15
Lipophilic			
PPU09234-RA	PpJHAMT-1	Juvenile hormone acid methyltransferase	
PPU03176-RA	PpJHAMT-2	Juvenile hormone acid methyltransferase	
PPU16032-RA	PpJHAMT-3	Juvenile hormone acid methyltransferase	
PPU06147-RA	PpJHEH-1	Juvenile hormone epoxide hydrolase	
PPU06149-RA	PpJHEH-2	Juvenile hormone epoxide hydrolase	
PPU04342-RA	PpEcR	Ecdysone receptor	
PPU14600-RA	PpUSP	Ultraspiracle	
Stress resistance			
PPU04133-RA	PpCRYAB-1	Alpha-crystallin B chain	HSPs

(Continues)

TABLE 1 (Continued)

Gene ID	Gene name	Gene description	Shown in Figure 1/Figure S2
PPU15439-RA	PpCRYAB-2	Alpha-crystallin B chain	HSPs
PPU04132-RA	PpCRYAB-3	Alpha-crystallin B chain	HSPs
PPU04131-RA	PpCRYAB-4	Alpha-crystallin B chain	HSPs
PPU04128-RA	PpCRYAB-5	Alpha-crystallin B chain	HSPs
PPU04129-RA	PpCRYAB-6	Alpha-crystallin B chain	HSPs
PPU04130-RA	PpCRYAB-7	Alpha-crystallin B chain	HSPs
PPU09172-RA	PpHDAC1_2	Histone deacetylase 1/2	HDACs
PPU03371-RA	PpHDAC3	Histone deacetylase 3	HDACs
PPU08145-RA	PpHDAC4_5	Histone deacetylase 4/5	HDACs
PPU04851-RA	PpHDAC6	Histone deacetylase 6	HDACs
PPU12453-RA	PpHSP110	Heat shock protein 110 kda	HSPs
PPU05744-RA	PpHSPA1s-1	Heat shock 70 kda protein 1/2/6/8	HSPs
PPU09869-RA	PpHSPA1s-2	Heat shock 70 kda protein 1/2/6/8	HSPs
PPU09871-RA	PpHSPA1s-3	Heat shock 70 kda protein 1/2/6/8	HSPs
PPU04479-RA	PpHSPA1s-4	Heat shock 70 kda protein 1/2/6/8	HSPs
PPU03831-RA	PpHSPA1s-5	Heat shock 70 kda protein 1/2/6/8	HSPs
PPU09930-RA	PpHSPA5	Heat shock 70 kda protein 5	HSPs
PPU12454-RA	PpClpB	ATP-dependent Clp protease ATP-binding subunit ClpB	HSPs
PPU13369-RA	PpDnak	Molecular chaperone Dnak	HSPs
PPU04710-RA	PpCAT	Catalase	CAT
PPU13433-RA	PpSOD1-1	Superoxide dismutase, Cu-Zn family	SODs
PPU08377-RA	PpSOD1-2	Superoxide dismutase, Cu-Zn family	SODs
PPU08376-RA	PpSOD1-3	Superoxide dismutase, Cu-Zn family	SODs
PPU08194-RA	PpSOD2	Superoxide dismutase, Fe-Mn family	SODs
PPU04683-RA	PpHSF1	Heat shock transcription factor 1	HSF-1
PPU05088-RA	PpGPX4-1	Phospholipid-hydroperoxide glutathione peroxidase	GPX
PPU05089-RA	PpGPX4-2	Phospholipid-hydroperoxide glutathione peroxidase	GPX
PPU13728-RA	PpSIRT1	NAD-dependent deacetylase sirtuin 1	SIRT1
PPU11183-RA	PpSIRT2	NAD-dependent deacetylase sirtuin 2	
PPU15351-RA	PpSIRT4	NAD-dependent deacetylase sirtuin 4	
PPU05545-RA	PpSIRT5	NAD-dependent deacetylase sirtuin 5	
PPU03042-RA	PpSIRT6	Mono-ADP-ribosyltransferase sirtuin 6	
PPU00054-RA	PpSIRT7	NAD-dependent deacetylase sirtuin 7	

Abbreviations: ETS, E-twenty-six transcription factor; HSP, heat shock protein; IIS, Insulin/insulin-like growth factor (IGF)-1 signaling; mTOR, mechanistic target of rapamycin.

The *Drosophila* ILPs (DILPs) display distinct temporal expression patterns. Three of the eight DILPs are thought to modulate lifespan (Broughton et al., 2008). Grönke, Clarke, Broughton, Andrews, and Partridge (2010) reported that loss of DILP2, 3, and 5 greatly extend the median and maximum lifespan of females by 29% and 22%, but only in the presence of the bacterium *Wolbachia*. Mutation of *Drosophila* InR yields dwarf females with up to 85%

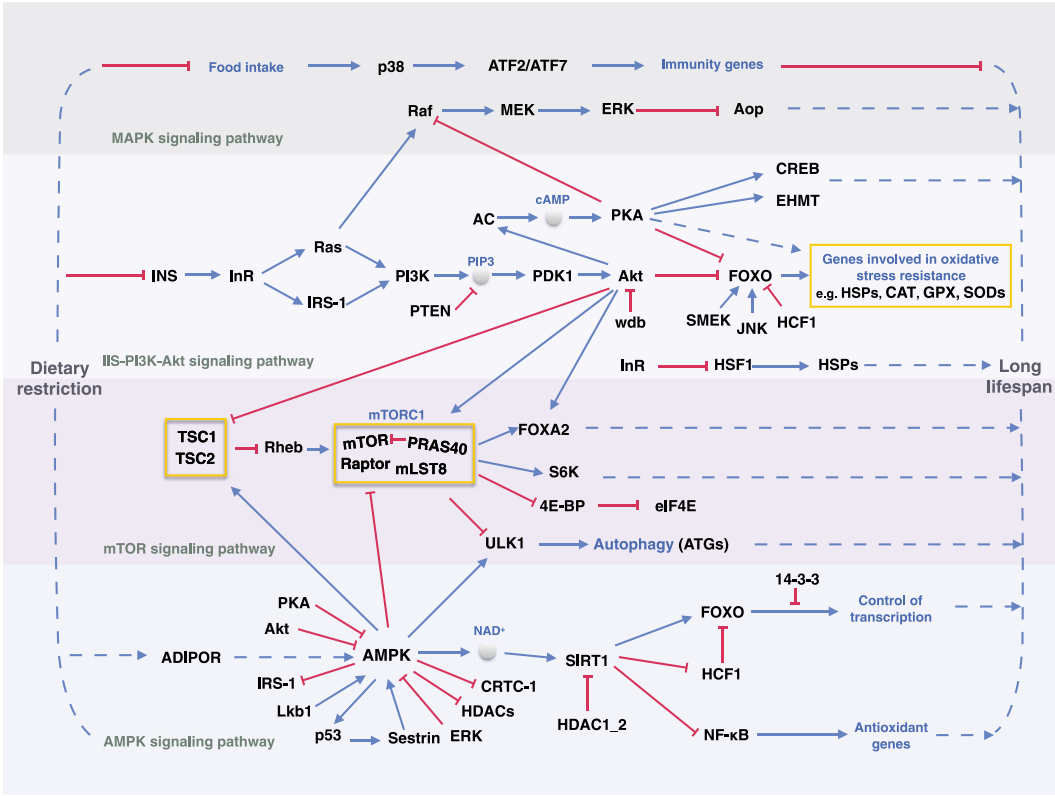

FIGURE 1 Putative signaling pathways and regulators/mediators/effectors for *P. puparum* longevity regulation. This schematic map compiled regulators, mediators, and effectors that were reported to be involved in longevity regulation in multiple species. We used four different colored backgrounds to indicate which signaling pathway they roughly belong to. Multiple pathways may converge on some key regulators. We use lines to show their relationships. Red line with a stop sign means inhibition, while blue line with an arrow means activation

lifespan extension and dwarf males also lived longer (Tatar et al., 2001). Overall, reduced IIS through genetic inhibition of IIS components including ILPs, InR, and IRS-1 can prolong lifespan in *Drosophila*. Similar results have been reported in *C. elegans* (Altintas et al., 2016).

3.1.2 | PI3K-Akt signaling pathway

We identified a set of genes encoding proteins in the PI3K-Akt signaling pathway, including two PI3Ks, one Akt, two wdb, a FOXO, a Forkhead Box protein A2 (FOXA2), a phosphatase and tensin homolog (PTEN), and a 14-3-3ε in *P. puparum* (Table 1). Akt can be activated via PI3K-dependent and -independent mechanisms (Mahajan et al., 2010). It acts in a cellular survival pathway by inhibiting apoptosis and is also involved in protein synthesis, stress response, and aging (Moskalev, Aliper, Smit-McBride, Buzdin, & Zhavoronkov, 2014). Akt features a PH domain, which binds either to PIP_3 or PIP_2. In *Drosophila*, ILPs bind to InR, activating the PI3Ks. Upon activation by InR, PI3Ks phosphorylate PIP_2 to PIP_3, which activates 3-phosphoinositide-dependent kinase 1 (PDK1), the latter in turn activates Akt by phosphorylating threonine (Thr308) and serine (Ser473) residues on Akt proteins to enable its activation (Moskalev et al., 2014; O' Neill, 2013; Figure S1a). wdb is a Ser/Thr phosphatase PP2A regulatory subunit. Overexpression of *wdb* in *Drosophila* inactivates Akt and prolongs lifespan (Funakoshi et al., 2011). PTEN is a tyrosine phosphatase known as a target of many cancer drugs. It contains a tension-like and catalytic domain for

TABLE 2 Gene ontology annotation of *P. puparum* lifespan-related genes

GO term (Level1)	GO term (Level2)	Count	Gene list
Biological process	Metabolic process	43	PpDorsal-1;PpCAT;PpGPX4-1;PpGPX4-2;PpSOD2;PpSOD1-3;PpSOD1-2;PpSOD1-1;PpPTEN;PpPI3K92E;PpVps34;PpPRKAA;PpPDK1;Ppp38;PpJNK;PpS6KA-1;PpS6KA-2;PpS6KB-3;PpS6KB-2;PpULK;PpERK;PpInR;PpPKA-2;PpLkb1;PpRaf;PpS6KB-4;PpPKA-1;PpAkt;PpVps15;PpS6KB-1;PpMEK;PpEHMT;PpHDAC3;PpHDAC1_2;PpEIF4E-3;PpEIF4E-1;PpEIF4E-2;PpCyr1;PpAC2;PpAC8;PpAC3;PpAC9;PpTSC2-3;
	Cellular process	55	PpATG12; PpEcR; PpPI3K92E; PpVps34; Ppwdb-2; PpRheb; PpRaf; PpRas; PpPI3K21B; Ppwdb-1; PpCyr1; PpAC2; PpAC8; PpAC3; PpAC9; PpPTEN; PpUSP; PpCRTC-1; PpATG7; PpATG5; PpATG2; PpBeclin-1; PpRaptor; PpPRAS40; PpSOD2; PpSOD1-3; PpSOD1-2; PpSOD1-1; PpPRKAA; PpPDK1; Ppp38; PpJNK; PpS6KA-1; PpS6KA-2; PpS6KB-3; PpS6KB-2; PpULK; PpERK; PpInR; PpPKA-2; PpLkb1; PpS6KB-4; PpPKA-1; PpAkt; PpVps15; PpS6KB-1; PpMEK; PpEHMT; PpHDAC3; PpHDAC1_2; Ppp53-1; Ppp53-2; PpEIF4E-3; PpEIF4E-1; PpEIF4E-2;
	Signaling	18	PpEcR; PpPI3K92E; PpVps34; Ppwdb-2; PpRheb; PpRaf; PpRas; PpPI3K21B; Ppwdb-1; PpCyr1; PpAC2; PpAC8; PpAC3; PpAC9; PpUSP; PpRaptor; PpPRAS40; PpInR;
	Single-organism process	31	PpEcR; PpPI3K92E; PpVps34; Ppwdb-2; PpRheb; PpRaf; PpRas; PpPI3K21B; Ppwdb-1; PpDorsal-1; PpCAT; PpGPX4-1; PpGPX4-2; PpSOD2; PpSOD1-3; PpSOD1-2; PpSOD1-1; PpCyr1; PpAC2; PpAC8; PpAC3; PpAC9; PpUSP; PpRaptor; PpPRAS40; PpEHMT; PpInR; PpHDAC3; PpHDAC1_2; Ppp53-1; Ppp53-2;
	Response to stimulus	21	PpEcR; PpPI3K92E; PpVps34; Ppwdb-2; PpRheb; PpRaf; PpRas; PpPI3K21B; Ppwdb-1; PpCyr1; PpAC2; PpAC8; PpAC3; PpAC9; PpUSP; PpRaptor; PpPRAS40; PpInR; PpCAT; PpGPX4-1; PpGPX4-2;
	Localization	2	PpRas; PpRheb
	Biological regulation	38	PpEcR; PpPI3K92E; PpVps34; Ppwdb-2; PpRheb; PpRaf; PpRas; PpPI3K21B; Ppwdb-1; PpCyr1; PpAC2; PpAC8; PpAC3; PpAC9; PpUSP; PpSestrin; PpRaptor; PpPRAS40; PpAop; Ppp53-1; PpDorsal-4; PpDorsal-1; PpHSF1; PpNF-κB; PpATF2; PpATF4; PpDorsal-5; PpCREB; Ppp53-2; PpFOXO; PpDorsal-2; PpDorsal-3; PpCRTC-1; Pp4E-BP; PpInR; PpTSC2-2; PpTSC2-1; PpATG14L;
	Cellular component organization or biogenesis	5	PpATG12; PpCRTC-1; PpEHMT; PpHDAC3; PpHDAC1_2;
Molecular function	Nucleic acid binding transcription factor activity	16	PpAop; Ppp53-1; PpDorsal-4; PpDorsal-1; PpEcR; PpHSF1; PpNF-κB; PpATF2; PpATF4; PpDorsal-5; PpCREB; Ppp53-2; PpFOXO; PpDorsal-2; PpDorsal-3; PpUSP;
	Catalytic activity	46	PpTSC2-3; PpGPX4-1; PpGPX4-2; PpDorsal-1; PpRas; PpClpB; PpCAT; PpATG7; PpJHEH-1; PpJHEH-2; PpInR; PpAC8; PpPRKAA; PpPDK1; PpJNK; PpS6KA-1; PpS6KB-2; PpULK; PpERK; PpPKA-2; PpLkb1; PpRaf; PpS6KB-4; PpPKA-1; PpAkt; PpVps15; PpS6KB-1; PpMEK; PpHDAC3; PpHDAC1_2; PpPTEN; Ppp38; PpS6KA-2;

(Continues)

TABLE 2 (Continued)

GO term (Level1)	GO term (Level2)	Count	Gene list
			PpS6KB-3; PpCyr1; PpAC2; PpAC3; PpAC9; PpPI3K92E; PpmTOR; PpVps34; PpSOD2; PpSOD1-3; PpSOD1-2; PpSOD1-1; PpEHMT;
	Signal transducer activity	7	PpEcR; PpUSP; PpInR; Ppp38; PpJNK; PpERK; PpRaf;
	Binding	68	Ppp53-1; PpEcR; PpHSF1; PpCREB; Ppp53-2; PpUSP; PpIRS-1; PpDorsal-1; Pp14-3-3z; Pp14-3-3e; PpEIF4E-3; PpEIF4E-1; PpEIF4E-2; PpAop; PpATF2; PpATF4; PpFOXO; PpDorsal-4; PpRaptor; PpEHMT; PpNF-κB; PpInR; PpmLST8-2; PpATG16L1; PpPRKAB; PpmTOR; PpClpB; PpPI3K21B; PpATG18; PpDorsal-3; PpVps15; PpmLST8-1; PpCRTC-1; PpPRKAG; PpRheb; PpRas; Pp4E-BP; PpSIRT7; PpSIRT6; PpSIRT5; PpSIRT2; PpSIRT1; PpSIRT4; PpSOD2; PpSOD1-3; PpSOD1-2; PpSOD1-1; PpCAT; PpHDAC6; PpILP; PpPRKAA; PpPDK1; Ppp38; PpJNK; PpS6KA-1; PpS6KA-2; PpS6KB-3; PpS6KB-2; PpULK; PpERK; PpPKA-2; PpLkb1; PpRaf; PpS6KB-4; PpPKA-1; PpAkt; PpS6KB-1; PpMEK;
	Antioxidant activity	7	PpGPX4-1; PpGPX4-2; PpCAT; PpSOD2; PpSOD1-3; PpSOD1-2; PpSOD1-1;
	Molecular transducer activity	3	PpEcR; PpUSP; PpInR;
	Molecular function regulator	5	PpPI3K21B; PpTSC2-2; PpTSC2-1; Ppwdb-2; Ppwdb-1;
Cellular component	Extracellular region	1	PpILP
	Cell	29	PpATG7; PpEIF4E-3; PpEIF4E-1; PpATG5; PpATG12; PpCRTC-1; PpEIF4E-2; PpTSC2-2; PpTSC2-1; PpRaptor; Ppwdb-2; Ppwdb-1; PpAop; Ppp53-1; PpDorsal-4; PpDorsal-1; PpEcR; PpEHMT; PpHSF1; PpNF-κB; PpDorsal-5; PpCREB; Ppp53-2; PpSestrin; PpDorsal-2; PpDorsal-3; PpUSP; PpRheb; PpRas;
	Membrane	6	PpADIPOR; PpAC8; PpPI3K21B; PpRheb; PpInR; PpRas;
	Macromolecular complex	6	PpTSC2-2; PpTSC2-1; PpRaptor; PpPI3K21B; Ppwdb-2; Ppwdb-1;
	Organelle	16	PpAop; Ppp53-1; PpDorsal-4; PpDorsal-1; PpEcR; PpEHMT; PpHSF1; PpNF-κB; PpDorsal-5; PpCREB; Ppp53-2; PpCRTC-1; PpSestrin; PpDorsal-2; PpDorsal-3; PpUSP;
	Membrane part	3	PpADIPOR; PpAC8; PpPI3K21B
	Cell part	29	PpATG7; PpEIF4E-3; PpEIF4E-1; PpATG5; PpATG12; PpCRTC-1; PpEIF4E-2; PpTSC2-2; PpTSC2-1; PpRaptor; Ppwdb-2; Ppwdb-1; PpAop; Ppp53-1; PpDorsal-4; PpDorsal-1; PpEcR; PpEHMT; PpHSF1; PpNF-κB; PpDorsal-5; PpCREB; Ppp53-2; PpSestrin; PpDorsal-2; PpDorsal-3; PpUSP; PpRheb; PpRas;

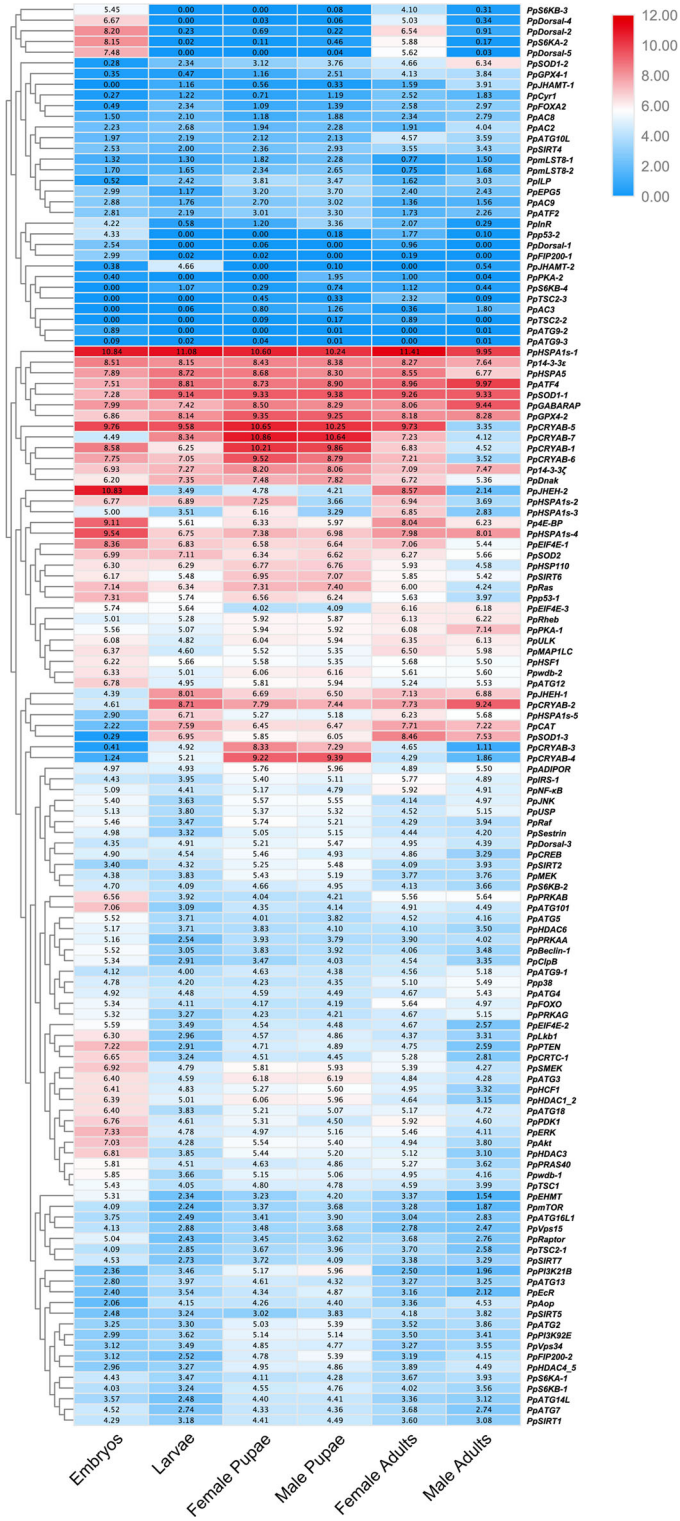

FIGURE 2 Continued.

the dephosphorylation of phosphoinositide substrates. It negatively regulates the PI3K/Akt pathway by dephosphorylating PIP_3 into PIP_2 (Shi et al., 2014).

Drosophila FOXO (dFOXO) has been identified as a key TF that regulates the lifespan downstream of Akt. Webb and Brunet (2014) reported that FOXO factors participate in lifespan through the regulation of genes in two functions, autophagy and cellular quality control. These two clearance processes are associated with aging (Kenyon, 2010). Overexpression of *dFOXO* in the fat body from the onset of adulthood prolongs female lifespans by 20–50% (Giannakou et al., 2004; Hwangbo et al., 2004). Loss of antagonistic dFOXO regulator 14-3-3ε results in increased stress-induced apoptosis, growth repression, and prolonged lifespan, phenotypes associated with upregulated FOXO function (Nielsen, Luo, Biteau, Syverson, & Jasper, 2008). The Forkhead Box (FOX) family shares a conserved DNA-binding domain, which differentiates them into Forkhead Box A (FOXA) to Forkhead Box S (FOXS; Lam, Brosens, Gomes, & Koo, 2013). A second Forkhead Box family member, *Drosophila* homolog of FOXA2 namely FKH has been revealed as a key TF regulating intestinal aging. FKH interacts with both the Akt and mTOR branches to extend lifespan with an up to 18% extension of median survival (Bolukbasi et al., 2017). Based on the functions of these homologous genes, the PI3K-Akt pathway identified in *P. puparum* may perform the fundamentally cellular functions like the roles in transcription, translation, growth, and survival of the parasitoid wasp.

3.1.3 | MAPK/ERK signaling pathway

This pathway, also known as Ras-Raf-MEK-ERK, signals downstream of the IIS pathway and is an evolutionarily conserved regulator of animal lifespan (Slack et al., 2015; Figure S1c). The MAPK related genes identified in *P. puparum* may be involved in cellular processes, such as the stress responses, apoptosis, and survival of the wasp. *Drosophila* Ras is encoded by *Ras85D*, a proto-oncogene usually tethered to cell membranes, where it functions as a molecular on/off switch in several signaling pathways. There is one Ras in *P. puparum*, and it has been recorded in 19 other insect species (Tables 1 and S5). In *Drosophila*, reduced IIS extends lifespan through the PI3K/Akt branch and its TF FOXO, and through MAPK/ERK signaling and its TF Anterior Open (Aop; Slack et al., 2015). Each *Drosophila* MAPK/ERK component has one homolog in *P. puparum*. During signal transduction, activated Ras activates Raf kinase, which leads to phosphorylation and activation of MEK kinase. MEK phosphates and activates ERK. Raf and ERK are serine/threonine-selective protein kinases and MEK is a serine/tyrosine/threonine kinase. Raf kinases are a family of three serine/threonine-specific protein kinases, A-Raf, B-Raf, and C-Raf , that are related to retroviral oncogenes (Roskoski, 2010). As in *Drosophila*, *P. puparum* raf (PpRaf) is a B-Raf. Among 20 insect genomes, the dRaf homolog in *Diaphorina citri* is the only A-Raf. There are three *Drosophila* Raf homologs in *Trichogramma pretiosum* and two in *Dendroctonus ponderosae*. Seventeen other insects have a single *Raf* (Table S5). There are two MEKs in *D. citri* and one in other insects. There are two ERKs in *Ceratosolen solmsi* and *Aedes aegypti*, and one in other species. The ERKs act as an integration node of multiple signal pathways. These act in several physiological process such as signal transduction and transcription regulation (Slack, 2017). Aop is a E-twenty-six (ETS) transcription factor, which functions downstream of Chico in adult *D. melanogaster* to influence lifespan like FOXO and is essential for lifespan extension in addition to *Chico* mutation (Slack et al., 2015). P38 mitogen-activated protein kinases are MAPKs involved with stress responses and other biological processes such as

FIGURE 2 Expression profiles of *P. puparum* putative lifespan-related genes in different development stages. The expression levels of all putative lifespan-related genes, as represented by FPKM values, are shown in the gradient heat map from light pink to red. Panel B–I. The mRNA levels of the putative signaling genes are represented by $\log_2$(FPKM + 1) values and rounded. The processed values are shown in the gradient heat map from blue (0) to red (≥10). The cDNA libraries are constructed from the six stages: embryos, larvae, female pupae, male pupae, female adults, and male adults. FPKM, fragments per kilobase of exon model per million reads mapped; mRNA, messenger RNA

apoptosis and autophagy. In *Drosophila*, a p38K/Mef2/MnSOD signaling influences lifespan and stress, distinct from the insulin/JNK/FOXO pathway, which manages stress-induced aging (Vrailas-Mortimer et al., 2011).

Several signaling pathways, such as PI3K/Akt and MAPK converge on nuclear factor kappa B (NF-κB), another regulator of the stress response, autophagy, and apoptosis. Hormesis is understood by toxicologists to refer to any biological process with a biphasic response to an environmental agent. NF-κB may operate through a hormesis manner (Chirumbolo, 2012). Hormesis leads to favorable biological responses to a low dose of stressors or toxins, which can prolong lifespan (Calabrese, Dhawan, Kapoor, Iavicoli, & Calabrese, 2015). In mice, early animal aging due to the loss of the NF-κB encoding gene *Nfkb1* is connected with increased cellular senescence and reduced apoptosis (Bernal et al., 2014). A *P. puparum* NF-κB (PpNF-κB) protein (109-kDa) is homologous to *Drosophila* Relish. Five PpRELs are homologs of two other *Drosophila* NF-κBs, Dorsal, and Dif. Pharmacological inhibition of NF-κB in male flies leads to lifespan extensions up to 20% and extended the time when 90% of individuals died up to 14%. In females, pharmacological inhibition of NF-κB causes an increase in median lifespan (by 13%) and maximum lifespan (by 11%). The time when 90% of individuals died in the female population was prolonged up to 6% (Moskalev et al., 2014).

3.1.4 | The mTOR signaling pathway

mTOR is a highly conserved nutrient-responsive regulator that integrates the inputs from upstream pathways such as insulin and IGF-1 and phosphorylates multiple downstream targets (Antikainen, Driscoll, Haspel, & Dobrowolski, 2017; Figure S1b). Regulation of downstream processes like autophagy, stress response, mRNA translation, and amino acid transport may mediate its effects on lifespan. Modulation of various genes that encode components of the mTOR signaling pathway extend lifespan in *Drosophila* (Kapahi et al., 2004). Genes identified in *P. puparum* mTOR signaling pathway have peaked expression levels at the embryo stages while lower at other stages. This may indicate that they probably play key roles in *P. puparum* growth and development. We identified 20 components of the mTOR pathway, including three translation initiation factors 4E (eIF4E), a eukaryotic translation initiation factor 4E binding protein (4E-BP), a mTOR, a regulatory associated protein of mTOR (Raptor), a proline-rich Akt substrate 1 (PRAS40), two target of rapamycin complex subunit LST8s (mLST8), six ribosomal protein S6 kinases (S6K), a tuberous sclerosis complex 1 (TSC1), three tuberous sclerosis complex 2 (TSC2), and a Ras homolog enriched in brain (Rheb; Chalhoub & Baker, 2009).

The mTORC1 is composed of four subunits: mTOR, mLST8, PRAS40, and Raptor (Sabatini, 2006). Raptor functions as a scaffold to recruit downstream substrates to the mTORC1, such as 4E-BP and S6K (Hay & Sonenberg, 2004). Muscle-specific knockdown of *Drosophila Raptor* leads to reduced lifespans (Saxton & Sabatini, 2017). Studies in mammal cells and *Drosophila* identified two genes *TSC1* and *TSC2* as critical upstream inhibitors of mTORC1. TSC2 inactivates a GTP-binding protein Rheb, which is associated with and activates mTORC1 in vitro (Sabatini, 2006). Different expression patterns of three *P. puparum TSC2* (*PpTSC2*) at differently developmental stages, respectively, suggest that these genes are involved in different functions of *P. puparum*. Rheb also serves as an activator of AMPK and acts in other cellular processes (Antikainen et al., 2017). Overexpression of *Drosophila TSC1* (*dTSC1*) and *Drosophila TSC2* (*dTSC2*) in transgenic flies carrying UAS constructs led to extended mean lifespans at 29°C by 14% for *dTSC1* and 12% for *dTSC2* (Kapahi et al., 2004). 4E-BP in *Drosophila* is a translation repressor that inhibits multisubunit complex assembly and mRNA translation. Overexpression of *Drosophila 4E-BP* (*d4E-BP*) extends male lifespan by 11% and female lifespan by 22% on rich food, whereas no lifespan extension occurred under CR (0.25% yeast extract). These findings showed that extended lifespan during CR is mediated by an increase in 4E-BP activity (Zid et al., 2009). 4E-BP modulates the effect of temperature on lifespan. Lifespan extension under reduced temperature was enhanced in flies in which *4E-BP* expression was reduced via RNAi treatments. Increased *4E-BP* transcript levels negated lifespan extension under low temperature, 18°C, compared to 25°C. (Carvalho et al., 2017).

3.1.5 | The AMP-activated protein kinase signaling pathway

AMPK is an energy sensor that acts in cellular energy homeostasis and controls the antiaging signaling network (Figures 1 and S1b). The activation capacity of AMPK declines during aging (Salminen & Kaarniranta, 2012). Unlike mTOR, AMPK is activated in nutrient-rich conditions (Gwinn et al., 2008). AMPK consists of three subunits, a catalytic α (PRKAA), a scaffolding β (PRKAB), and a regulatory γ (PRKAG) that together makes a functional enzyme (Hardie, 2015). We predicted 26 proteins that function through the AMPK pathway, including three AMPK subunits, two PKAs, a NF-κB, five Dorsals, five adenylate cyclases (AC), two p53 proteins, a Sestrin, a Lkb1, a CRTC-1, and a cyclic AMP-responsive element-binding protein (CREB; Table 1). We speculate that genes identified in *P. puparum* AMPK pathway participate in the parasitoid energy metabolism and maintenance of homeostasis.

AMPK can regulate the function of all prolongevity pathway mediators, such as FOXO, mTOR, tumor antigen p53, sirtuin 1 (SIRT1), and cyclic AMP-regulated transcriptional coactivator-1 (CRTC-1; Salminen & Kaarniranta, 2012). AMPK phosphorylates cyclic AMP (cAMP), which activates protein kinase A (PKA). PKA belongs to a group of serine/threonine kinases that respond to changes in local concentrations of cytoplasmic second messengers, for example, cAMP (Turnham & Scott, 2016). PKA acts in many cellular processes, including regulation of metabolism. AMPK can be activated by serine/threonine-protein kinase 11 (Lkb1). Overexpression of *Drosophila Lkb1* inactivates S6K and extends lifespan (Funakoshi et al., 2011). AMPK activation leads to a decrease in mTOR activity via direct phosphorylation of Raptor and indirectly through phosphorylation of TSC2 (Gwinn et al., 2008).

Overexpression of AMPK and its activation by pharmaceutical agents, for example, metformin, extend lifespan in *C. elegans* and *Drosophila* (Funakoshi et al., 2011; Onken & Driscoll, 2010). Neuronal and gut-specific activation of AMPK inhibits TOR and induces autophagy due to autophagy-related gene 1 (ATG1) activation, and prolongs *Drosophila* lifespan. The non-cell-autonomous effects of localized AMPK/ATG1 activation are linked to reduced ILP levels in the brain and a systemic increase in *4E-BP* expression (Ulgherait, Rana, Rera, Graniel, & Walker, 2014).

3.1.6 | The autophagy-related genes

Autophagy is implemented by autophagy-related genes (ATG; Vellai, 2009). We identified orthologs of all known longevity-related genes and predicted 24 ATG proteins in *P. puparum* (Table 1). These ATG proteins may participate in the autophagy processes at different stages of *P. puparum*. It is indicated that they are able to play a role in some multiply physiological processes, like aging, development, cellular homeostasis, cell death, and survival of the parasitoid wasp.

Some of the ATGs actions are depicted in Figure S2 (Hansen, 2016). One regulator of autophagy initiation is Unc-51 like autophagy activating kinase (ULK; Madeo, Zimmermann, Maiuri, & Kroemer, 2015). AMPK and mTOR regulate autophagy through inhibiting the homolog of mammalian ATG1, ULK (Chan, 2012). One *Drosophila* ATG1 ortholog *P. puparum* ULK (PpULK) was identified. ULK belongs to a protein complex containing ATG13, ATG101, and FIP200. Autophagy begins with phosphorylation of ULK by upstream kinases, for example, AMPK and mTOR, leading to activation of ATG6 homolog Beclin-1, a part of PI3KIII nucleation complex which contains ATG6, ATG14, Vsp15, and Vsp34 (Russell et al., 2013). Activated ULK and Beclin-1 contribute to the phosphorylation of downstream effectors. In *C. elegans*, downregulation of *ATG18* with RNAi leads to shorter worm lifespans, from which we infer ATGs act in aging regulation (Hashimoto, Ookuma, & Nishida, 2009).

The gene expression levels of *Drosophila ATG8* homolog (*PpGABARAP* and *PpMAP1LC*) are higher compared to other *P. puparum* ATG genes in all development stages, with the highest FPKM value of 692.

3.1.7 | Lipophilic signaling

Studies in *C. elegans* and several insects indicate lipophilic signaling is related to lifespan (Russell & Kahn, 2007). *Drosophila* has two primary lipophilic hormones, ecdysteroids and juvenile hormone (JH). We infer that ecdysone and JH are related to the adult lifespan (Herman & Tatar, 2001; Toivonen & Partridge, 2009).

JH acts in lifespan and in physiological trade-offs between longevity and fecundity in *D. melanogaster* and other insects (Flatt & Kawecki, 2007; Yamamoto, Bai, Dolezal, Amdam, & Tatar, 2013). Treatment of long-lived *InR Drosophila* with the JH analog methoprene reduced their lifespan. Our interpretation is JH deficiency is sufficient to prolong lifespan and there is a connection between IIS and JH (Tatar et al., 2001). We predict the JH nuclear receptor, ultraspiracle (USP), two JH acid O-methyltransferases (JHAMT), and two JH epoxide hydrolases (JHEH) in *P. puparum*. JHAMT is a JH-III synthase which transfers a methyl group from S-adenosyl-L-methionine to the carboxyl group of JH acids to produce active JH. JHEH catalyzes hydrolysis of the JH epoxide (Niwa et al., 2008).

Ecdysone is a prohormone of the major insect molting hormone 20-hydroxyecdysone (20E). Flies with the ecdysone receptor (EcR) heterozygous mutant showed an extension of lifespan from 20% to 50%. Lifespan increased in both sexes and in several lines with different genetic backgrounds. They exhibited increased resistance to three stresses: oxidative stress, heat, and dry starvation (Simon, Shih, Mack, & Benzer, 2003). We predicted an EcR in *P. puparum*. The EcR is a nuclear hormone receptor that heterodimerizes with USP (Schwedes & Carney, 2012). Similar to JH, ecdysone seems to function downstream of IIS in relation to lifespan (Tu, Yin, & Tatar, 2005). The mechanism may be through regulating the transcriptional level of nuclear hormone receptors (Russell & Kahn, 2007).

3.1.8 | Stress resistance

Organisms experience many stressors, such as inappropriate temperatures, UV radiation, toxins or extreme oxygen content (Miller, 2009). Thermal stress induces the formation of reactive oxygen species (ROS). Since the 1950s, the free-radical theory of aging holds that aging follows from the accumulation of cellular damage to the extent that the damage goes beyond the capacity of cellular repair mechanisms. In many species, including insects, superoxide dismutase (SOD), Glutathione peroxidase (GPX), and catalase (CAT) detoxify ROS (Mair & Dillin, 2008). We identified genes encoding three Cu-Zn superoxide dismutases (CuZnSOD), one Fe-Mn superoxide dismutase (FeMnSOD), a CAT, and two GPXs (Table 1). The loss-of-function mutant of the *Drosophila SOD* led to shortened lifespans and to high sensitivities to pro-oxidants, copper ions, paraquat, ionizing radiation, and hyperoxia (Boulianne, 2001). Parker, Parker, Sohal, Sohal, and Keller (2004) reported that long-lived black ant *Lasius niger* queens had slightly lower or approximately similar CuZnSOD activities compared to the relatively short-lived worker ants and males (Parker et al., 2004). Corona, Hughes, Weaver, and Robinson (2005) reached similar conclusions in bees (Corona et al., 2005). Our interpretations of these findings in ant and bee queens is that reducing ROS production is more important than antioxidant activity in the longevity mechanism of queens (Keller & Jemielity, 2006). The free-radical theory should not be accepted uncritically, however, because reports questioning the theory are increasing (Stuart, Maddalena, Merilovich, & Robb, 2014). We infer that these antioxidative stress response genes identified in *P. puparum* function as protecting its cells from the oxidative stresses and damages and antiaging.

Thermal or oxidative stress leads to the expression of heat shock proteins (HSPs). HSPs maintain protein integrity and inhibit apoptosis (Beere et al., 2000). We identified seven crystallins, five HSP70 protein 1/8, a HSP70 protein 5, a HSP70 homolog molecular chaperone DnaK (DnaK), and a heat shock transcription factor 1 (HSF-1) that mediates transcriptional response in *P. puparum*. Histone deacetylases (HDACs) act in the transcription of HSP genes. We predicted four HDACs and six sirtuins, NAD-dependent deacetylase Sirtuin 1, 2, 4 up to 7 (SIRT1, 2, 4 up to 7). Sirtuins are highly conserved NAD^+-dependent deacetylases that regulate various cellular process including metabolism and aging (Grabowska, Sikora, & Bielak-Zmijewska, 2017). Yeast and flies have five sirtuin proteins, *C. elegans* has four and mice and human have seven (Frye, 2000; Giblin, Skinner, & Lombard, 2014). Sirtuins act in lifespan in organisms from yeast to humans (Bitto, Wang, Bennett, & Kaeberlein, 2015). SIRT1, or mammalian Sir2 homolog, is the best-characterized sirtuin. SIRT1 responds to CR and mediates IIS signaling. It acts in stress resistance through several TFs including FOXO, p53, and NF-κB (Haigis & Sinclair, 2010). SIRT6 acts in genome DNA stability and DNA repair. Overexpression of *SIRT6* can significantly increase lifespan in male mice. The *SIRT6*-transgenic mice displayed reduced gene expression of IGF-1 (Kanfi et al., 2012). SIRT7 may act as an oncogene as it

is highly expressed in several human cancers (Roth & Chen, 2014). SIRT7 acts in DNA double-strand break repairs and the maintenance of genome integrity (Vazquez et al., 2016). The FPKM values of genes involved in stress resistance range from 0.2 to 2726, which are generally higher than that of other lifespan-related genes.

3.2 | Validation of the RNA-seq data using qPCR analysis

Post RNA-seq and FPKM value calculation, we performed a correlation analysis between the data obtained from qPCR and the FPKM values from the RNA-seq. The correlations are represented by the R values of the spearman's rank correlation coefficient calculation (Figure 3). The correlation of two genes is extremely strong (R > 0.80), including *PpAkt* (R = 0.89) and *PpCRYAB-4* (R = 0.94), the other four genes are moderately strong (the range of R values from 0.60 to 0.79). At some developmental stages, there are visible discrepancies between the qPCR data and FPKM values. In our views, the producing of these discrepancies may be related to methodological difference between RNA-seq and qPCR, as well as the different batch of the samples used in RNA-seq and qPCR, respectively. Recently, this discrepancy is common and regular in the transcript level analyses. However, the variation tendencies of the RNA-seq data curve and qPCR histogram are almost similar. These results showed that our RNA-seq and qPCR data are both reliable.

3.3 | Expression of *P. puparum* lifespan-related genes under 25°C and 35°C conditions

Under harsh conditions, cellular processess are impaired, lifespans are reduced, and the stress-resistance proteins are likely to be activated (Moskalev et al., 2014). Therefore, we selected female adults for analysis of gene expression under normal and heat-stress treatments. Among nine selected genes, expression levels of three HSPs were significantly increased under heat stress, as expected from studies of many organisms (Figure 4a–c). Especially *PpCRYAB-4*, there was 10–20-fold increases of its expression compared to control group. In humans,

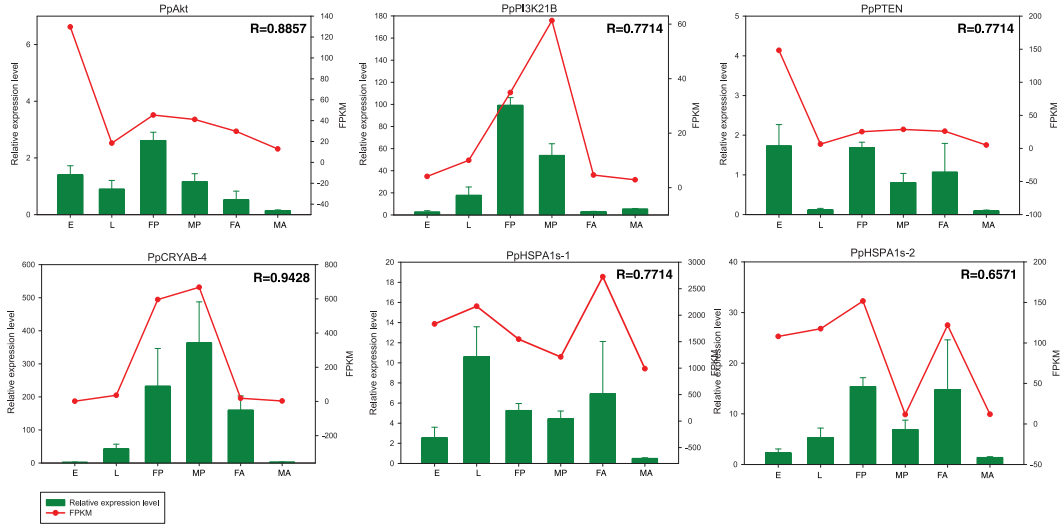

FIGURE 3 Transcript profile and qPCR results of selected genes in RNA-seq Data. FPKM values are indicated by red lines. Green bars represent relative expression levels. R value represents the correlations between the qPCR and RNA-seq results, calculated from spearman's rank correlation coefficient. E, embryo; FA, female adult; FP, female pupa; FPKM, fragments per kilobase of exon model per million reads mapped; L, larva; MA, male adult; MP, male pupa; qPCR, quantitative polymerase chain reaction

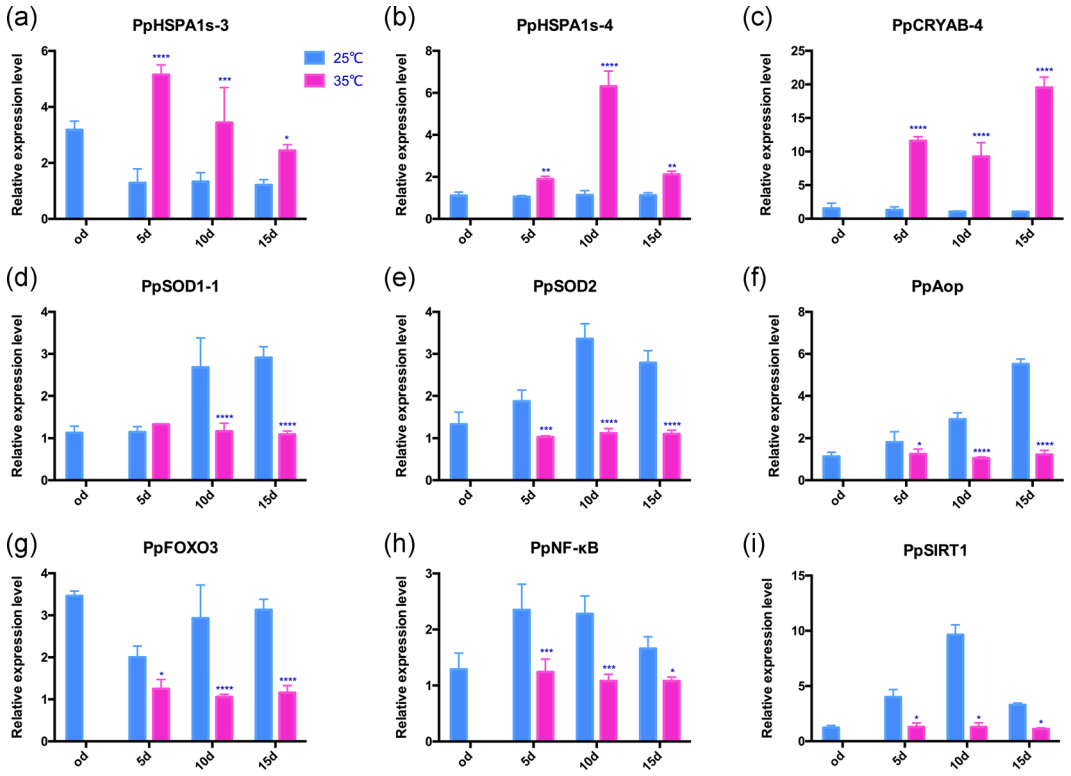

FIGURE 4 Expression patterns of selected genes under 25°C and 35°C conditions. Values represent the mean ± *SEM* of three assays. $^{*}p < .05$ when compared to its corresponding control. $^{**}p < .01$, $^{***}p < .001$, and $^{****}p < .0001$. *SEM*, standard error of the mean

Crystallin Alpha B (CRYAB) is a member of the small HSP family and, again, increased accumulations of mRNAs encoding CRYAB is expected under heat stress. This result suggests that CRYAB may react to heat stress response.

However, expression of *P. puparum* CuZnSOD-1 (*PpSOD1-1*) and *P. puparum* FeMnSOD (*PpSOD2*), *P. puparum* FOXO (*PpFOXO*), *P. puparum* Aop (*PpAop*), *PpNF-κB*, and *PpSIRT1* were significantly lower at 35°C compared to 25°C. The scales of the Y-axes in Figure 4d–h indicate relatively small differences in expression of the five genes, compared to Figure 4c. *PpSIRT1* was significantly reduced at 35°C (Figure 4i). The expression level of *PpSIRT1* at Day 10 was nearly 1/10 of that of the control group. *SIRT1* was reported to mediate IIS through TFs like FOXO. Since the expression pattern of *PpFOXO* and *PpSIRT1* are similar under heat stress, it may suggest that *PpFOXO* and *PpSIRT1* play a role in heat stress response and they interact in some way.

4 | CONCLUSION

In this manuscript, based on the genomic and transcriptomic data, we totally identified 133 of the lifespan-related genes of *P. puparum* in silico. These genes were classified into five signaling pathways and three physiological processes. GO analysis of these genes revealed that they were enriched in eight biological processes, mainly in cellular and metabolic processes. There were seven groups enriched in molecular function, and the most genes belonging to the binding activity group. We analyzed the relative expression levels of the identified genes of *P. puparum*, at six differently developmental stages by RNA-seq. This showed the developmentally dynamical and differentially sexual expression patterns of these genes in *P. puparum*. Generally, our results provide a data foundation for further research in *P. puparum*, especially for the gene

functional studies. Furthermore, these results also give some new insights into the molecular level of the parasitoid lifespan and may have economical values in parasitoid applications on pest biological control.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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