

Supplementary information

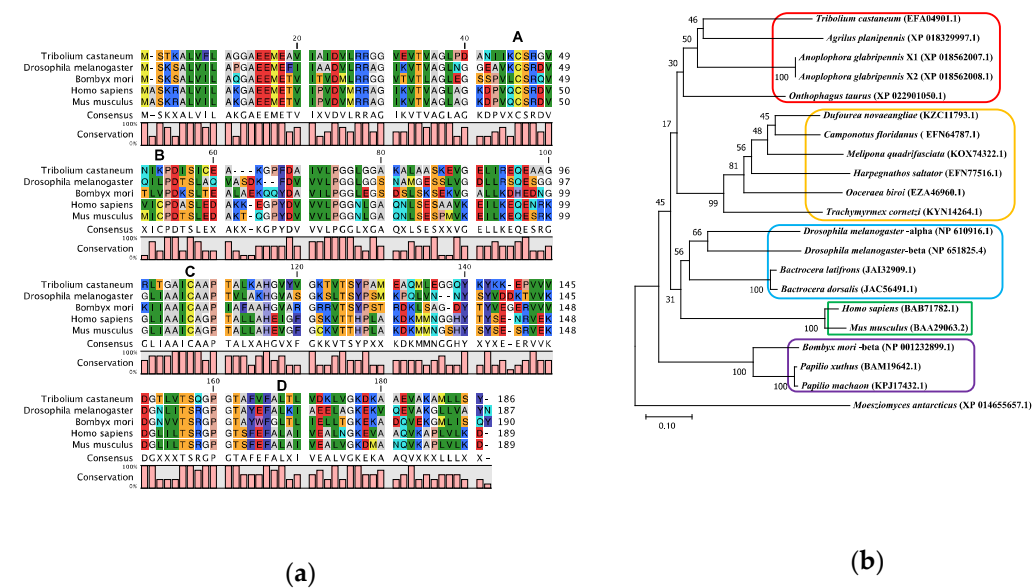


Figure S1. Alignment and the phylogenetic tree of *T. castaneum* DJ-1 and other DJ-1 amino acid sequences. (a) alignment of DJ-1 homologs. A, cysteine 46; B, cysteine 53; C, cysteine 106; D, leucine 166. Each amino acid position corresponds to human DJ-1. (b) Phylogenetic tree. The Coleoptera cluster is outlined in red, the Hymenoptera cluster is outlined in yellow, the Diptera cluster is outlined in light blue, and the Lepidoptera cluster is outlined in purple. The numbers above the branches represent the recall rate of each branch as a percentage when the bootstrap test was conducted 1000 times, following the neighbor-joining method.

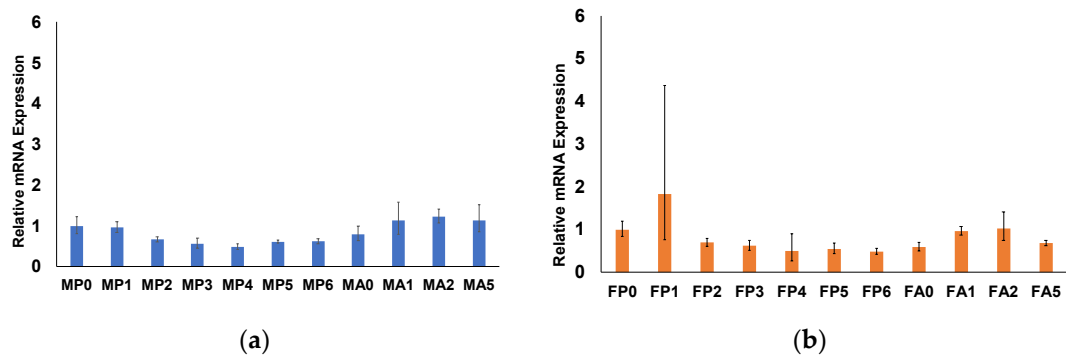


Figure S2. Expression of *TcDJ-1* in *T. castaneum* males (a) and females (b) at different developmental stages. Whole bodies of insects at each developmental stage were used for qRT-PCR. The expression level of *TcDJ-1* mRNA is shown compared to the expression level in day 0 pupae, which was set as 1. The expression levels of pupae, and adults are shown as RQ values. The RQ represents the relative expression level compared to the reference sample. The error bars represent the relative minimum/maximum expression levels relative to the mean RQ value. *TcRpS6* was used as an endogenous control.

Table S1. Accession numbers were used the alignment and phylogenic tree in this study.

Accession number	Scientific name
LC628081	<i>Tribolium castaneum</i>
XP_018329997.1	<i>Agrilus planipennis</i>
XP_018562007.1	<i>Anoplophora glabripennis</i> X1
XP_018562008.1	<i>Anoplophora glabripennis</i> X2
XP_022901050.1	<i>Onthophagus taurus</i>
KZC11793.1	<i>Dufourea novaeangliae</i>
EFN64787.1	<i>Camponotus floridas</i>
KOX74322.1	<i>Melipona quadrifasciata</i>
EFN77516.1	<i>Harpegnathos saltator</i>
EZA46960.1	<i>Ooceraea biroi</i>
KYN14264.1	<i>Trachymyrmex cornetzi</i>
NP_610916.1	<i>Drosophila melanogaster</i> alpha
NP_651825.4	<i>Drosophila melanogaster</i> beta
JAI32909.1	<i>Bactrocera latifrons</i>
JAC56491.1	<i>Bactrocera dorsalis</i>

BAM19642.1	<i>Papilio xuthus</i>
KPJ17432.1	<i>Papilio machaon</i>
NP_001232899.1	<i>Bombix mori</i>
NP_001116849.1	<i>Homo sapiens</i>
NP_065594.2	<i>Mus Musculus</i>
XP_014655657.1	<i>Moesziomyces antarcticus</i>

Table S2 Primers used in cDNA cloning and dsRNA synthesis

<i>TcVer</i> for cDNA clonning	Forward	5'-TGATCCTCAAGGTATTGGTG-3'
	Reverse	5'-TGATCAGCGAGTCTATGTCC-3'
<i>TcVer</i> +T7	Forward	5'-TAATACGACTCACTATAGGTGATCCTCAAGGTCTTGGTG-3'
	Reverse	5'-TAATACGACTCACTATAGGTGATCAGCGAGTCTATGTCC-3'
<i>TcDJ-1</i> for cDNA clonning	Forward	5'-ATGAGCACAAAAGCCCTTGT-3'
	Reverse	5'-TTAATACGACAAAAGCATGGCTT-3'
<i>TcDJ-1</i> +T7	Forward	5'-TAATACGACTCACTATAGGGAAGTAGGGGAGTTGAT-3'
	Reverse	5'-TAATACGACTCACTATAGGGTTAATACGACAAAAGCATG-3'

Table S3. Primers used for qRT-PCR experiments

<i>TcRpS6</i>	Forward	5'-GGACCCAAAAGAGCATCAAA-3'
	Reverse	5'-CCTCAAGCAACGCTTCTTCT-3'
<i>TcVer</i>	Forward	5'-CGTAATGAGTTGCCCCACTGAGAC-3'
	Reverse	5'-CGACTCTTCTAAAACGTCGCTGA-3'
<i>TcDJ-1</i>	Forward	5'-GCAGAGGCGGTGTGGAAGTA-3'
	Reverse	5'-GGGCCTTTGGCCTCACAGAT-3'
<i>TcSOD1</i>	Forward	5'-GGTGATGGAGTTGCCAAAGT-3'
	Reverse	5'-GATTCCAACAACACCACACG-3'
<i>TcSOD2</i>	Forward	5'-CAAGTCCGGGAAATTGAAAA-3'
	Reverse	5'-CTCGAAAATGGCCTTGACAT-3'
<i>TcSOD3</i>	Forward	5'-CTTCCACATCCACGAGAAGG-3'
	Reverse	5'-CACCACAGCCCTCCCTATAA-3'

Table S4. Homology to the other species

Species	Identity %	Similarity %
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<i>Agrilus planipennis</i>	60	91
<i>Anoplophora glabripennis</i> X1	64	92
<i>Anoplophora glabripennis</i> X2	64	92
<i>Onthophagus taurus</i>	61	89
<i>Dufourea novaeangliae</i>	51	89
<i>Camponotus floridanus</i>	53	88
<i>Melipona quadrifasciata</i>	52	89
<i>Harpegnathos saltator</i>	47	87
<i>Ooceraea biroii</i>	51	91
<i>Trachymyrmex cornetzi</i>	52	89
<i>Drosophila melanogaster alpha</i>	54	82
<i>Drosophila melanogaster beta</i>	54	88
<i>Bactrocera latifrons</i>	60	86
<i>Bactrocera dorsalis</i>	59	87
<i>Papilio xuthus</i>	50	86
<i>Papilio machaon</i>	50	86
<i>Bombyx mori</i>	52	87
<i>Homo sapiens</i>	49	83
<i>Mus musculus</i>	50	84