Supplementary Material

Table S1. Analysis of *Ct* values of seven candidate housekeeping genes in all samples.

Table S2. Pairwise comparison of candidate housekeeping genes based on the analysis by the comparative *ΔCt* method.

Table S3. Statistical analyses of seven selected candidate housekeeping genes based on the *BestKeeper* algorithm.

Table S4. Reference gene selection in arthropod species.

Figure S1. Validation of seven selected candidate housekeeping genes. (A) The PCR products of selected housekeeping genes amplified under conditions described in Materials and Methods were resolved on a 2% agarose gel, stained with SYBR® safe DNA gel stain (ThermoFisher Scientific, Massachusetts, USA). **(B)** Melting curves of seven selected candidate housekeeping genes including three technical replicates in a 96-well plate with 4 cDNA samples.

Figure S1

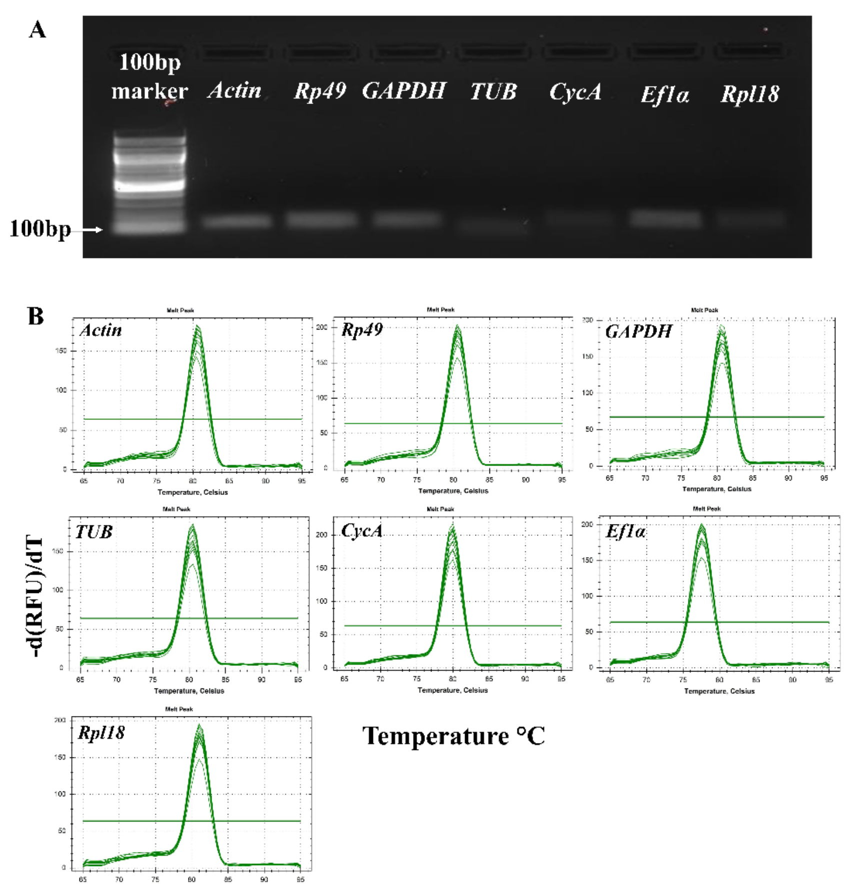

Table S1. Analysis of *Ct* values of seven candidate housekeeping genes in all samples.

Gene	N[a)]	Median	Lowest	Lower quartile	Higher quartile	Highest	Mean	SD[b)]
Actin	84	18.70	17.41	18.21	20.24	23.41	19.25	1.41
Rp49	84	16.41	15.17	16.14	17.98	21.14	17.19	1.66
GAPDH	84	16.67	15.40	16.27	18.29	21.31	17.46	1.75
TUB	84	17.40	16.28	16.18	18.94	24.44	18.38	2.06
CycA	84	16.96	16.22	16.78	18.56	22.32	17.88	1.79
Ef1α	84	15.42	14.59	15.12	17.40	23.48	16.55	2.31
Rpl18	84	16.65	15.73	16.43	18.09	21.30	17.44	1.58

a) N: number of *Ct*s; b) SD: standard deviation.

Table S2. Pairwise comparison of candidate housekeeping genes based on the analysis by the comparative *ΔCt* method.

Gene	Pair Gene	Acaricide resistance selection		Host plant shift		Both conditions	
		Mean *ΔCt*	SD	Mean *ΔCt*	SD	Mean *ΔCt*	SD
Actin	*Rp49*	2.34	0.37	1.70	1.31	2.06	0.95
	GAPDH	2.16	0.31	1.30	1.09	1.79	0.86
	TUB	1.16	0.34	0.49	1.46	0.87	1.04
	CycA	1.62	0.32	1.03	1.38	1.37	0.97
	Ef1α	3.17	0.45	2.08	1.57	2.70	1.20
	Rpl18	1.98	0.44	1.60	1.35	1.82	0.96
	Mean SD	-	0.37	-	1.36	-	1.00
Rp49	*Actin*	-2.34	0.37	-1.70	1.31	-2.06	0.95
	GAPDH	-0.18	0.21	-0.40	0.46	-0.27	0.36
	TUB	-1.18	0.38	-1.21	1.38	-1.19	0.94
	CycA	-0.72	0.23	-0.67	0.47	-0.70	0.35
	Ef1α	0.83	0.37	0.38	1.51	0.64	1.04
	Rpl18	-0.36	0.20	-0.10	0.10	-0.25	0.21
	Mean SD	-	0.29	-	0.87	-	0.64
GAPDH	*Actin*	-2.16	0.31	-1.30	1.09	-1.79	0.86
	Rp49	0.18	0.21	0.40	0.46	0.27	0.36
	TUB	-1.00	0.33	-0.80	1.10	-0.92	0.76
	CycA	-0.54	0.22	-0.27	0.54	-0.42	0.41
	Ef1α	1.01	0.38	0.78	1.22	0.91	0.85
	Rpl18	-0.18	0.22	0.30	0.48	0.02	0.43
	Mean SD	-	0.28	-	0.82	-	0.61
TUB	*Actin*	-1.16	0.34	-0.49	1.46	-0.87	1.04
	Rp49	1.18	0.38	1.21	1.38	1.19	0.94
	GAPDH	1.00	0.33	0.80	1.10	0.92	0.76
	CycA	0.46	0.29	0.54	1.14	0.49	0.77
	Ef1α	2.01	0.34	1.58	0.51	1.83	0.47
	Rpl18	0.82	0.40	1.10	1.39	0.94	0.96
	Mean SD	-	0.34	-	1.16	-	0.82
CycA	*Actin*	-1.62	0.32	-1.03	1.38	-1.37	0.97
	Rp49	0.72	0.23	0.67	0.47	0.70	0.35
	GAPDH	0.54	0.22	0.27	0.54	0.42	0.41
	TUB	-0.46	0.29	-0.54	1.14	-0.49	0.77
	Ef1α	1.55	0.30	1.05	1.22	1.33	0.86
	Rpl18	0.36	0.23	0.57	0.46	0.45	0.36
	Mean SD	-	0.27	-	0.87	-	0.62
Ef1α	*Actin*	-3.17	0.45	-2.08	1.57	-2.70	1.20
	Rp49	-0.83	0.37	-0.38	1.51	-0.64	1.04
	GAPDH	-1.01	0.38	-0.78	1.22	-0.91	0.85
	TUB	-2.01	0.34	-1.58	0.51	-1.83	0.47

	CycA	-1.55	0.30	-1.05	1.22	-1.33	0.86
	Rpl18	-1.19	0.41	-0.48	1.52	-0.89	1.09
	Mean SD	-	0.37	-	1.26	-	0.92
Rpl18	*Actin*	-1.98	0.44	-1.60	1.35	-1.82	0.96
	Rp49	0.36	0.20	0.10	0.10	0.25	0.21
	GAPDH	0.18	0.22	-0.30	0.48	-0.02	0.43
	TUB	-0.82	0.40	-1.10	1.39	-0.94	0.96
	CycA	-0.36	0.23	-0.57	0.46	-0.45	0.36
	Ef1α	1.19	0.41	0.48	1.52	0.89	1.09
	Mean SD	-	0.32	-	0.88	-	0.67

Table S3. Statistical analyses of seven selected candidate housekeeping genes based on the *BestKeeper* algorithm.

Acaricide resistance selection	***Actin***	***Rp49***	***GAPDH***	***TUB***	***CycA***	***Ef1α***	***Rpl18***
N	48	48	48	48	48	48	48
GM [C_t]	18.47	16.13	16.31	17.31	16.85	15.30	16.49
AM [C_t]	18.47	16.13	16.31	17.31	16.85	15.30	16.49
Min [C_t]	17.41	15.17	15.40	16.68	16.22	14.85	15.73
Max [C_t]	19.36	16.77	17.01	17.87	17.47	15.88	17.15
SD [± C_t]	0.35	0.25	0.24	0.18	0.20	0.19	0.26
CV [% C_t]	1.92	1.56	1.47	1.05	1.21	1.25	1.59
[*r*]	0.828	0.858	0.911	0.504	0.894	0.332	0.790
P-value	0.001	0.001	0.001	0.001	0.001	0.021	0.001
Host plant shift	***Actin***	***Rp49***	***GAPDH***	***TUB***	***CycA***	***Ef1α***	***Rpl18***
N	36	36	36	36	36	36	36
GM [C_t]	20.23	18.52	18.92	19.64	19.16	18.01	18.62
AM [C_t]	20.29	18.59	18.99	19.80	19.26	18.21	18.69
Min [C_t]	18.06	16.34	16.62	16.28	16.54	14.59	16.43
Max [C_t]	23.41	21.14	21.31	24.44	22.32	23.48	21.30
SD [± C_t]	1.34	1.45	1.48	2.09	1.69	2.27	1.46
CV [% C_t]	6.59	7.78	7.77	10.55	8.76	12.49	7.79
[*r*]	0.838	0.953	0.989	0.963	0.973	0.980	0.949
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Both conditions	***Actin***	***Rp49***	***GAPDH***	***TUB***	***CycA***	***Ef1α***	***Rpl18***
N	84	84	84	84	84	84	84
GM [C_t]	19.20	17.11	17.38	18.27	17.80	16.41	17.37
AM [C_t]	19.25	17.19	17.46	18.38	17.88	16.55	17.44
Min [C_t]	17.41	15.17	15.40	16.28	16.22	14.59	15.73
Max [C_t]	23.41	21.14	21.31	24.44	22.32	23.48	21.30
SD [± C_t]	1.14	1.36	1.42	1.61	1.41	1.82	1.26
CV [% C_t]	5.94	7.89	8.16	8.77	7.88	10.97	7.24
[*r*]	0.901	0.973	0.987	0.967	0.984	0.979	0.969
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

N: number of *Ct*s; GM [C_t]: geometric means of the threshold cycle (C_t); AM [C_t]: the arithmetic mean of *Ct*; Min [C_t] and Max [C_t]: the extreme values of C_t; SD [± C_t]: the standard deviation of the *Ct*; CV [% *Ct*]: the coefficient of variance expressed as a percentage at the C_t level; [*r*]: Pearson correlation coefficient.

Table S4. Reference gene selection in arthropod species.

Case No.	Scientific name	Common name	No. of housekeeping gene	No. of algorithm	Experimental condition	Best reference genes	Reference
1	*Apis mellifera*	Western honey bee#	11	3	Head gene expression following bacterial challenge	*RPS18, GAPDH*	Scharlaken *et al.* 2008*
2	*Apis mellifera*		4	3	Larva and pupa stages, tissues, and after juvenile hormone exposure	*Actin, Tbp-af, Rp49; Actin, Ef1α, Rp49; Ef1α, Tbp-af, Actin*	Lourenco *et al.* 2008*
3	*Apis mellifera*		3	3	Tissues in workers	*GAPDH, Rpl32, Ef1α-f1*	Reim *et al.* 2013*
4	*Bombyx mori*	Silkworm#	12	2	Developmental stages	*TF3, Proteasome β; TF4*	Wang *et al.* 2008*
5	*Schistocera gregaria*	Desert locust	7	2	Brains of nymphs and adults	*Rp49, Ef1α, Actin; GAPDH, Ubiquitin, Ef1α*	van Hiel *et al.* 2009*
6	*Rhipicephalus appendiculatus*	Brown ear-tick	9	2	All developmental stages	*Elf1α, GAPDH, H3F3A, PPIA, RPL4, TBP*	Nijhof *et al.* 2009*
7	*Rhipicephalus microplus*	Southern cattle tick	9	2	All developmental stages	*Elf1α, GAPDH, H3F3A, PPIA, RPL4, TBP*	Nijhof *et al.* 2009*
8	*Folsomia candida*	White springtail	10	2	PH, temperature, desiccation, cadmium phenanthrene treatments	*SDHA, ETIf, Ef1α; YWHAZ, SDHA, GAPDH; UBC, ETIf, ACTb*	de Boer *et al.* 2009 [1]
9	*Orchesella cincta*	Hairy-back girdled springtail	10	2	Cadmium, temperature, desiccation, starvation treatments	*28s, ACTb, YWHAZ; ACTb, GAPDH, Ef1α; TBa, SDHA, YWHAZ*	de Boer *et al.* 2009 [1]
10	*Tribolium castaneum*	Red flour beetle#	8	2	Four time points after fungal challenge in larva	*RPS3, RPS18, RPL13a, Syntaxin1&6, E-cadherin*	Lord *et al.* 2010 [2]
11	*Tribolium castaneum*		5	1	All developmental stages	*Rp49, RPS3, Actin*	Bai *et al.* 2011*
12	*Bombus terrestris*	Large earth bumblebee#	7	2	The labial gland and fat body in different ages	*Ef1α, GAPDH, H3F3a, PPIA, RPL4, TBP*	Hornáková *et al.* 2010*
13	*Bombus lucorum*	White-tailed bumblebee	7	2	The labial gland and fat body in different ages	*Ef1α, GAPDH, H3F3a, PPIA, RPL4, TBP*	Hornáková *et al.* 2010*
14	*Lucilia cuprina*	Australian sheep blowfly#	11	2	All developmental stages	*18S rRNA*, 28S *rRNA*, *GST1*, *β-tubulin*, *RPLP0*	Bagnall and Kotze, 2010 [3]
15	*Bactrocera dorsalis*	Oriental fruit fly	10	2	Tissues from both females and males	*α-TUB* with *ACT5* or *ACT3* or *ACT2* or *ACT1*	Shen *et al.* 2010 [4]
16	*Liposcelis bostsrychophila*	Booklouse	4	1	All developmental stages, deltamethrin induction	*β-actin1, GAPDH, Tubulin*	Jiang et al. 2010 [5]
17	*Tetranychus cinnabarinus*	Carmine spider mite	6	2	All developmental stages, susceptible and three acaricide resistant populations	*RPS18*, *5.8S rRNA*, *α-Tubulin*	Sun et al. 2010*
18	*Rhodnius prolixus*	Kissing bug#	7	2	Tissues in adult females 4 days after a blood meal	*RPS18*, *Ef1α*, *MIP, Actin*	Majerowicz *et al.* 2011*
19	*Rhodnius prolixus*		5	3	Tissues before and after feeding blood, infection with vectors	*18S, TUB; ACT, TUB*	Paim *et al.* 2012 [6]
20	*Daphnia pulex*	Water flea#	6	3	Predator exposure	*Xbp1, Tbp, CAPON, Stx16*	Spanier *et al.* 2010 [7]
21	*Drosophila melanogaster*	Fruit fly#	7	3	Injury, heat-shock stress, diet variations	*Ef1α, Actin, Tubulin, RPL32*	Ponton *et al.* 2011 [8]
22	*Drosophila melanogaster*		20	1	Ageing related head samples	*GAPDH2, RPL13A, L(3)02640*	Ling and Salvaterra 2011 [9]
23	*Delphacodes kuscheli*	Delphacodes planthopper	7	3	Virus-infected and naïve planthoppers	*UBI, 18S, Actin*	Maroniche *et al.* 2011 [10]
24	*Cimex lectularius*	Common bed bug#	8	3	Developmental stages, tissues, pesticide exposure	*RPL18, Ef1α*	Mamidala *et al.* 2011 [11]

25	*Cimex lectularius*		4	1	All developmental stages in susceptible and resistant strains	*RPL8, RPS16, HSP70, RPL11*	Zhu *et al.* 2012*
26	*Chortoicetes terminifera*	Australian plague locust	8	2	Tissues in 5th instar nymphs under three rearing densities	*Ef1α*	Chapuis *et al.* 2011 [12]
27	*Aphis glycines*	Soybean aphid	7	2	Tissues, developmental stages, fed on susceptible and host plant-resistant soybean	*TBP, RPS9, Ef1α*	Bansal *et al.* 2012
28	*Agrilus planipennis*	Emerald ash borer	6	3	Larval tissues, developmental stages, diet variations	*TEF1α, RPL7*	Rajarapu *et al.* 2012 [13]
29	*Panonychus citri*	Citrus red mite	7	3	Developmental stages, abiotic stresses (thermo stress, UV irradiation, acid rain stress, acaricide stress), and both	*GAPDH; Ef1α, GAPDH*	Niu *et al.* 2012*
30	*Amblyomma maculatum*	Gulf coast tick	5	3	All developmental stages, tissues	*β-actin; β-actin, GAPDH*	Browning *et al.* 2012*
31	*Leptinotarsa decemlineata*	Colorado potato beetle	3	1	Larva fed *in vivo* and *in vitro* expressed double-stranded RNA (dsRNA) of 5 target genes	*RP4, β-actin, RP18*	Zhu *et al.* 2011*
32	*Leptinotarsa decemlineata*		5	1	Susceptible and resistant adults, tissues, developmental stages, control and induction beetles	*RPL4, Ef1α, RpL18, HSP70, NADH*	Zhu et al. 2016*
33	*Leptinotarsa decemlineata*		9	3	All developmental stages, larval tissues, and after insecticide treatments	*ARF1, RP18; RP4, RP18*	Shi *et al.* 2013 [14]
34	*Ixodes scapularis*	Blacklegged tick#	6	2	Two tissues in female ticks in multiple blood-feeding phases	*RPS4, RPL13a*	Koci *et al.* 2013 [15]
35	*Bemisia tabaci*	Sweetpotato whitefly	9	2	Developmental stages, tissues, bacterium and insecticide treatments	*18S/SDHA; GST, TAF, aTUB; TAF, GST, ACT*	Su *et al.* 2013*
36	*Bemisia tabaci*		15	2	Developmental stages, host plants, nonviruliferous and viruliferous flies, tissues, biotypes, photoperiods, temperature variations, susceptible and thiamethoxam resistant	*HSP90, RPL29, Ef1α; HSP90, RPL29; NADH, HSP90, RPL29; RPL29, Ef1α; NADH, HSP90, Ef1α; HSP40, HSP90, PPIA; Ef1α, NADH, SDHA; PPIA, Ef1α, HSP20*	Li *et al.* 2013*
37	*Bemisia tabaci*		8	4	Insecticide treatments	*Ef1α, Tubulin, GAPDH*	Liang *et al.* 2014*
38	*Solenopsis invicta*	Red imported fire ant#	5	3	Developmental stages, castes, tissues	*RPL18, Ef1β*	Cheng *et al.* 2013 [16]
39	*Spodoptera litura*	Oriental leafworm moth	8	4	All developmental stages, tissues, geographic populations, temperature stresses, pesticide induction, diets induction and starvation	*GAPDH, UCCR; RPL10, AK, Ef1; RPL10, Ef1; GAPDH, Ef1; AK, β-Actin*	Lu *et al.* 2013 [17]
40	*Plutella xylostella*	Diamondback moth#	10	3	All developmental stages, tissues, strains, temperature variations, photoperiods, insecticide treatment, mechanical injury	*RPS13, RPS32, Ef1α; Ef1α,RPL32, RPS23; Ef1α,Actin, GAPDH; Actin, GAPDH, RPL32; RPS13, Ef1α,RPL32; Ef1α,RPS13, RPL32; GAPDH, RPL32, Ef1α,*	Fu *et al.* 2013*
41	*Spodoptera exigua*	Beet armyworm	9	3	All developmental stages, sexes, tissues in three larval physiological stages	*L10, Ef2, L17A; SOD, ACT2, GAPDH, Ef1, ACT1; ACT2, ACT1, L10; GAPDH, ACT1, ACT2; SOD, L17A; Ef2, SOD*	Zhu *et al.* 2014 [18]
42	*Nilaparvata lugens*	Brown planthopper#	8	4	All developmental stages, body parts, geographic populations, temperature stresses, pesticides induction, diets induction	*RPS15, RPS11, TUB; RPS15, RPS11*	Yuan *et al.* 2014 [19]
43	*Diabrotica virgifera virgifera*	Western corn rootworm	5	4	Developmental stages, tissues, dsRNAs exposure, *Bt* toxins exposure	*β-Actin; RPS9; Ef1α*	Rodrigues *et al.* 2014*

44	*Frankliniella occidentalis*	Western flower thrip	7	4	Developmental stages, high temperature induction, low temperature induction	*Ef1α, RPL32; RPL32, GAPDH; 18S, Ef1α*	Zheng *et al.* 2014 [20]
45	*Frankliniella occidentalis*		11	4	Nonviruliferous and viruliferous thrips	*HSP60, HSP70, RPL32*	Yang *et al.* 2015*
46	*Locusta migratoria*	Migratory locust#	12	4	All developmental stages, tissues, insecticide-induced stresses, temperature-induced stresses, starvation treatment	*Ef1α, HSP70, RPL32; Ef1α, RPL32; SDH, RP49; RPL32, HSP70, RP49; ACT, SDH; RPL32, ACH; ACT, ACH*	Yang *et al.* 2014 [21]
47	*Acyrthosiphon pisum*	Pea aphid#	11	4	Developmental stages under different temperature conditions	*SDHB, 16S, NADH*	Yang et al. 2014 [22]
48	*Diuraphis noxia*	Russian wheat aphid#	5	3	Biotypes, feeding on host plants containing resistant genes or susceptible	*Actin, RPL27*	Sinha and Smith, 2014 [23]
49	*Phenacoccus solenopsis*	Mealybug	6	3	Thermo stresses	*α-TUB, RPL32*	Chen & Lu 2014 [24]
50	*Aphis craccivora*	Cowpea aphid	10	4	Developmental stages, temperature variations	*RPS8, RPL14, RPL11*	Yang et al. 2015 [25]
51	*Helicoverpa armigera*	Cotton bollworm	12	4	All developmental stages, larval tissues, adult tissues, virus challenge, insecticide induction, temperature variations	*28S, RPS15; RPS15, RPL13; Ef1α,RPL27; GAPDH, RPL27, β-Tubulin; RPS15, RPL32; RPS15, RPL27; RPL32, RPS15, RPL27*	Zhang *et al.* 2015*
52	*Danaus plexippus*	Monarch butterfly#	9	4	All developmental stages, larval tissues, temperature variations, photoperiod variations, larva feeding on dsRNA of different genes	*Ef1α, RP49*	Pan *et al.* 2015 [26]
53	*Hippodamia convergens*	Convergent lady beetle	7	4	All developmental stages, larval tissues, temperature variations, photoperiod variations, larva fed on dsRNA	28S, *Ef1α, CYPA; GAPDH, 28S, CYPA; GAPDH, CYPA; 28S, Ef1α; Actin, CYPA*	Pan *et al.* 2015*
54	*Coleomegilla maculata*	Spotted lady beetle	16	4	All developmental stages, larval tissues, sexes, larva fed on dsRNA	*GAPDH et al.*	Yang *et al.* 2015*
55	*Tetranychus urticae*	Two-spotted spider mite#	10	4	Developmental stages	*RPL13, v-ATPase*	Yang *et al.* 2015*
56	*Tetranychus urticae*		7	4	Xenobiotic adaptation including susceptible and three acracide resistant populations, host plant shifting, and both	*CycA, RP49; GAPDH, RP49, RPL18; GAPDH, RP49*	This study
57	*Aphis gossypii*	Cotton aphid	8	4	Developmental stages, geographical populations, temperature treatments, diet variations, and all samples	*Ef1α, β-Actin, RPL7; Ef1α, β-Actin; GAPDH, RFL7; 18S, β-Actin*	Ma *et al.* 2016 [27]

These species have genome sequence published; *These studies were cited in the main text.

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