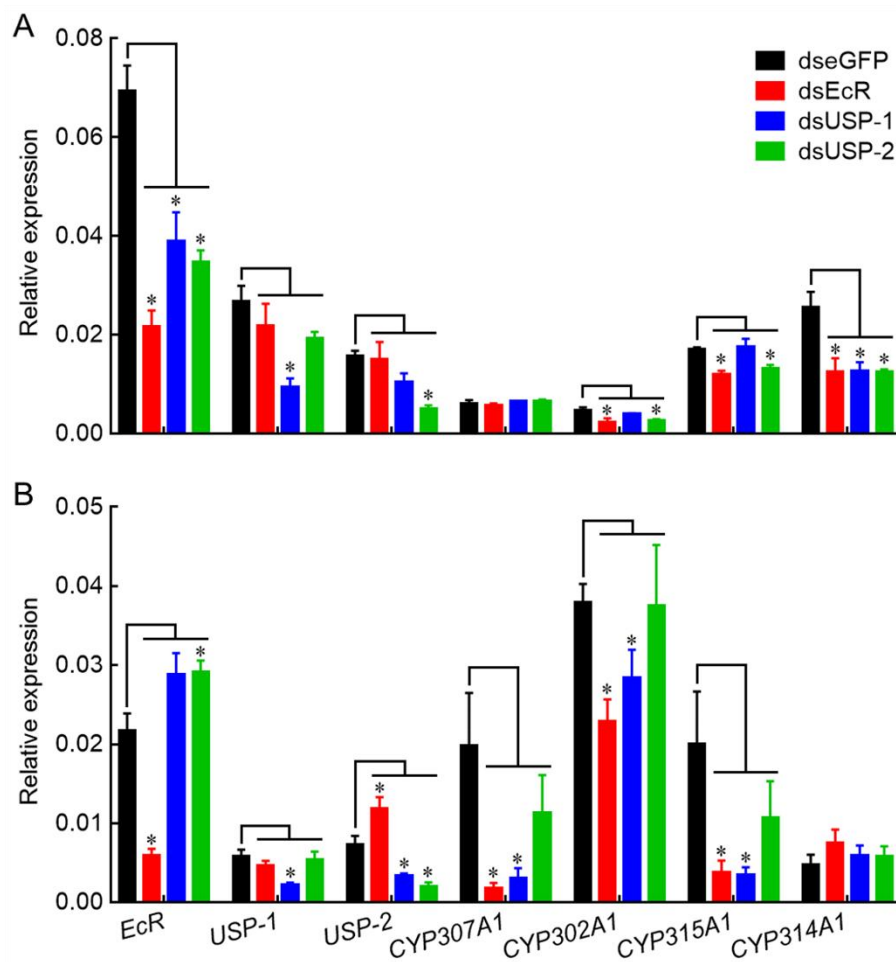


## Supplementary Materials



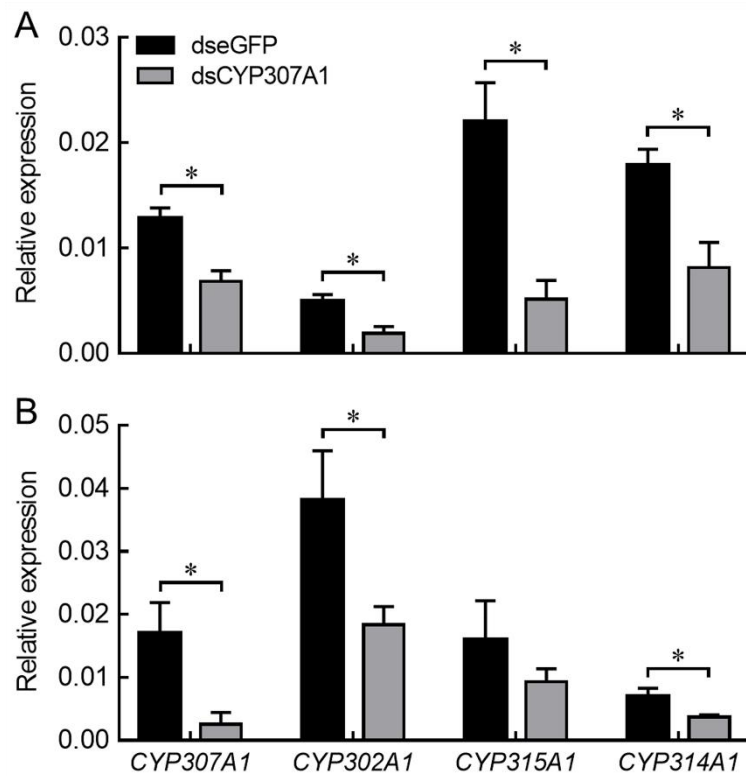
**Supplementary Figure S1** Developmental processes of *P. pseudoannulata* in egg sac  
Scales are 200  $\mu\text{m}$ .



**Supplementary Figure S2** Expression of *EcR*, *USPs*, and Halloween genes in dsEcR- and dsUSP-treated *P. pseudoannulata* spiderlings (A) and females (B)

“\*” indicates significant difference in expression of *EcR*, *USPs*, and Halloween genes between

dsEcR and dsUSP treatment and control groups, analyzed by one-way ANOVA with Tukey test at  $P < 0.05$ .



**Supplementary Figure S3 Expression of Halloween genes in dsCYP307A1-treated *P. pseudoannulata* spiderlings (A) and females (B).**

“\*” indicates significant difference in expression of Halloween genes between dsCYP307A1 treatment and control groups analyzed by *t*-test at  $P < 0.05$ .

**Supplementary Table S1 Primers used for gene quantification and dsRNA synthesis**

Gene	Purpose	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')
<i>Ecr</i>	qPCR	ACTTGAGTCCTCCCAGTAGTGT	TGGTATCCTGACGCCCTATCC
	RNAi	GGATCCTAATACGACTCACTATAGG CCTATCGGAAGTCCAAGTGTTG	GGATCCTAATACGACTCACTATAGGT GGCAGTGAGGAGAGCATAAC
<i>USP-1</i>	qPCR	TGGCAGATGTCGATAGGATAGC	GAGAGTGATGATGCTCCATTGC
	RNAi	GGATCCTAATACGACTCACTATAGG GATTTGAGCATGTGCCAGGT	GGATCCTAATACGACTCACTATAGG CAGTCCTATACTCCTTAGAGCAGG
<i>USP-2</i>	qPCR	CCACTGTGCCAAGTCCAAGT	GCCGTTTGAGAAAGACTACTGCTG
	RNAi	GGATCCTAATACGACTCACTATAGG AGAAGAAAGGCAGCGGACTA	GGATCCTAATACGACTCACTATAGG GCAAACACCCAAGCTCAGTT
<i>CYP307A1</i>	qPCR	CAGTACGGTGACATCTACGGAATG	TTGGCGAAGTCTTGGCTCTTG
	RNAi	GGATCCTAATACGACTCACTATAGG ACGAAATCCCTCTCCAAGGT	GGATCCTAATACGACTCACTATAGG GCAGCCAGCAGATAAAGACC
<i>CYP302A1</i>	qPCR	GCGAAGTGTGCTCAAGTTCCATAG	GCCCATCTGAAGAGAAGTGGTTGA
<i>CYP315A1</i>	qPCR	TTGGAACAGGTGCGAGGTCAT	GGGTTTGTGAGGCGTCGTTATC
<i>CYP314A1</i>	qPCR	CGGGTCTGTGTCAAAGGAGGAA	CTGTCCGGTAGTGCTTGAGGAAGT
<i>EF-1<math>\alpha</math></i>	qPCR	AACGCAAGAGTAACAAGACTGACG	GGCACTGTTCCAATACCACCAAT
<i>GAPDH</i>	qPCR	AAGATGTGGCGAGATGGCAGAG	ATCAGGAGTAGGAACACGGAAAGC
<i>eGFP</i>	RNAi	GGATCCTAATACGACTCACTATAGG AAGTTCAGCGTGTCCG	GGATCCTAATACGACTCACTATAGG CACCTTGATGCCGTTT

**Supplementary Table S2 NRs in *P. pseudoannulata***

Subfamily	Nomenclature	Name	Abbreviation	Chromosomal location	No. of exons	ORF (bp)	Protein (aa)	GenBank accession no.
NR1	NR1D3	Ecdysone-induced protein 75	<i>E75</i>	Chr. 11	8	2724	907	ON528121
	NR1E1	Ecdysone-induced protein 78	<i>E78-1</i>	Chr. 9	5	912	303	ON528122
			<i>E78-2</i>	Chr. 9	3	1728	575	ON528123
	NR1F4	Hormone receptor-like in 46	<i>HR3-1</i>	Chr. 6	10	1488	495	ON528124
			<i>HR3-2</i>	Chr. 2	10	1467	488	ON528125
	NR1H1	Ecdysone receptor	<i>EcR</i>	Chr. 8	8	1560	519	ON528126
	NR1J1	Hormone receptor-like in 96	<i>HR96</i>	Chr. 12	2	1548	515	ON528127
NR2	NR2A4	Hepatocyte nuclear factor 4	<i>HNF4</i>	Chr. 10	7	1332	443	ON528128
	NR2B4	Ultraspiracle	<i>USP-1</i>	Chr. 11	9	1221	406	ON528129
			<i>USP-2</i>	Chr. 6	8	1287	428	ON528130
	NR2D1	Hormone receptor-like in 78	<i>HR78</i>	Chr. 7	14	1752	583	ON528131
	NR2E2	Tailless	<i>Tll</i>	Chr. 14	8	1215	404	ON528132
	NR2E3	Hormone receptor-like in 51	<i>HR51-1</i>	Chr. 2	7	1269	422	ON528133
			<i>HR51-2</i>	Chr. 9	9	1584	527	ON528134
	NR2E4	Dissatisfaction	<i>Dsf</i>	Chr. 14	7	1494	497	ON528135
	NR2F3	Seven up	<i>SVP-1</i>	Chr. 8	3	951	316	ON528136
			<i>SVP-2</i>	Chr. 14	3	864	287	ON528137
NR3	NR3B4	Estrogen-related receptor	<i>ERR</i>	Chr. 14	9	1410	469	ON528138
NR4	NR4A4	Hormone receptor-like in 38	<i>HR38</i>	Chr. 10	11	2040	679	ON528139
NR5	NR5A3	Fushi tarazu transcription factor 1	<i>FTZ-F1</i>	Chr. 2	6	1539	512	ON528140
	NR5B1	Hormone receptor-like in 39	<i>HR39</i>	Chr. 12	9	2589	862	ON528141
NR6	NR6A2	Hormone receptor-like in 4	<i>HR4-1</i>	Chr. 2	8	2085	694	ON528142

**Supplementary Table S3 FPKM values of *EcR* and two *USPs* in 11 developmental stages of *P. pseudoannulata***

Sample	<i>EcR</i>			<i>USP-1</i>			<i>USP-2</i>		
	Biological replicate_1	Biological replicate_2	Biological replicate_3	Biological replicate_1	Biological replicate_2	Biological replicate_3	Biological replicate_1	Biological replicate_2	Biological replicate_3
E	7.77	7.97	8.32	1.15	0.46	0.19	0.00	0.00	0.00
ES	19.69	21.31	23.36	0.36	0.25	0.62	1.15	1.23	0.73
AS	20.40	21.36	19.70	0.00	0.00	0.11	0.51	1.66	1.11
DS	19.64	19.48	18.15	0.11	0.00	0.31	0.76	0.82	1.10
VM	5.47	4.12	4.33	0.14	0.39	0.19	0.17	0.05	0.23
VF	3.53	5.12	4.61	0.00	0.80	0.00	0.40	0.22	0.37
MF	2.11	2.22	11.60	1.99	0.88	0.00	0.00	0.00	0.00
EESF	2.69	4.85	2.77	0.63	1.00	0.65	0.12	0.16	0.06
LESF	6.23	8.36	7.36	0.05	0.00	0.00	0.33	0.39	0.27
SCF	2.96	12.49	1.98	1.98	0.00	1.18	0.13	0.33	0.06
NSCF	1.62	1.96	1.49	0.46	1.13	1.05	0.00	0.11	0.00

E, egg; ES, spiderling in egg sac; AS, aggregated spiderling; DS, dispersed spiderling; VM, virgin male; VF, virgin female; MF, mated female; EESF, early-egg sac-carrying female; LESF, late-egg sac-carrying female; SCF, spiderling-carrying female; NSCF, non-spiderling-carrying female.

**Supplementary Table S4 Number of NRs in investigated species**

Subfamily	Nomenclature	Name	Abbreviation	<i>Drosophila melanogaster</i>	<i>Anopheles gambiae</i>	<i>Apis mellifera</i>	<i>Tribolium castaneum</i>	<i>Bombyx mori</i>	<i>Aedes aegypti</i>	<i>Acyrtosiphon pisum</i>	<i>Nilaparvata lugens</i>	<i>Bactrocera dorsalis</i>	<i>Tetranychus urticae</i>	<i>Pardosa pseudoannulata</i>
NR0	NR0A1	Knirps	<i>Knr</i>	1	1	1	–	–	–	–	1	1	–	–
	NR0A2	Knirps-like	<i>Knrl</i>	1	–	1	1	–	1	1	1	1	1	–
	NR0A3	Eagle	<i>EG</i>	1	1	1	1	1	–	1	1	1	–	–
NR1	NR1D3	Ecdysone-induced protein 75	<i>E75</i>	1	1	1	1	1	1	1	1	1	1	1
	NR1E1	Ecdysone-induced protein 78	<i>E78</i>	1	1	1	1	1	1	1	1	1	1	2
	NR1F4	Hormone receptor-like in 46	<i>HR3</i>	1	1	1	1	1	1	1	1	1	1	2
	NR1H1	Ecdysone receptor	<i>EcR</i>	1	1	1	1	1	1	1	1	1	1	1
NR1J1	Hormone	<i>HR96</i>	1	1	1	1	1	1	–	–	1	8	1	

		receptor-like in 96													
	NR1M	Hormone receptor-like in 10	<i>HR10</i>	-	-	-	-	-	-	-	-	-	-	1	-
NR2	NR2A4	Hepatocyte nuclear factor 4	<i>HNF4</i>	1	1	1	1	1	1	1	1	1	1	1	1
	NR2B4	Ultraspiracle (retinoid X receptor)	<i>USP (RXR)</i>	1	1	1	1	1	1	1	1	1	1	2	2
	NR2D1	Hormone receptor-like in 78	<i>HR78</i>	1	1	1	1	1	1	1	1	-	1	1	1
	NR2E2	Tailless	<i>Tll</i>	1	2	1	1	1	1	1	1	1	1	1	1
	NR2E3	Hormone receptor-like in 51	<i>HR51</i>	1	-	1	1	1	1	1	1	1	1	1	2
	NR2E4	Dissatisfaction	<i>Dsf</i>	1	1	1	1	1	1	1	1	1	1	1	1
	NR2E5	Hormone receptor-like in 83	<i>HR83</i>	1	1	1	1	-	1	1	1	1	1	1	-

	NR2E6	Photoreceptor specific NR	<i>PNR</i>	-	1	1	1	1	1	-	1	-	1	-
	NR2F3	Seven up	<i>SVP</i>	1	1	1	1	1	1	1	1	1	1	2
NR3	NR3B4	Estrogen-related receptor	<i>ERR</i>	1	1	1	1	1	1	1	1	1	1	1
NR4	NR4A4	Hormone receptor-like in 38	<i>HR38</i>	1	1	1	1	1	1	1	1	1	2	1
NR5	NR5A3	Fushi tarazu transcription factor 1	<i>FTZ-F1</i>	1	1	1	1	1	1	1	1	1	1	1
	NR5B1	Hormone receptor-like in 39	<i>HR39</i>	1	1	1	1	1	1	1	1	1	1	1
NR6	NR6A2	Hormone receptor-like in 4	<i>HR4</i>	1	1	1	1	1	1	1	1	1	1	2
Total				21	21	22	21	19	20	19	20	21	30	23



Refer ence	Adams et al., 2000; King-Jones & Thummel, 2005	Bertrand et al., 2004	Velarde et al., 2006	Bonneton et al., 2008; Tan & Palli, 2008	Cheng et al., 2008	Cruz et al., 2009	Christiaens et al., 2010	Xu et al., 2017	Yang et al., 2020	Grbic et al., 2011	This study
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“-” represents the absence of corresponding gene.

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