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Identification, expression profiling and potential functional roles of nuclear receptors in the social aphid *Pseudoregma bambucicola*

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Abstract

Background Nuclear receptors (NRs) constitute a superfamily of transcription factors that regulate diverse biological processes. In insects, NRs not only govern essential physiological functions including metabolism, development, and reproduction, but also play pivotal roles in regulating caste differentiation and division of labor within social insect colonies. *Pseudoregma bambucicola* is a species of social aphid in which adults exhibit a specialized reproductive division of labor. This unique system produces first-instar nymphs and soldiers, which share an identical genetic background yet exhibit distinct morphological and behavioral traits. Although NRs exhibit pleiotropic regulatory capacities, their roles in the unique developmental patterns of *P. bambucicola* remain unclear.

Results This study identified 21 NR genes based on the genomic data of *P. bambucicola* and analyzed the duplication and loss events of these genes through phylogenetic analysis. Additionally, differential expression of NR genes was analyzed using transcriptomic data. The *TLL* exhibited significant differential expression in adults with distinct reproductive behaviors, suggesting its involvement in the regulation of reproductive division of labor. *E75* and *HNF4* were found to be important for the post-embryonic development of soldiers. Furthermore, quantitative real-time PCR confirmed caste-specific expression patterns of *HR4* and *HR39*, indicating their potential involvement in morphological differentiation and developmental regulation among castes.

Conclusions This study conducted bioinformatic identification of NR genes in the social aphid *P. bambucicola*, and investigated their potential roles in morphological differentiation and behavioral division through analysis of differential gene expression. The findings provide preliminary evidence for the functional significance of NR genes in social aphids, while offering novel insights for subsequent research exploration.

Keywords Nuclear receptor, Gene expression, Caste, Social aphid

Background

Nuclear Receptors (NRs) are a superfamily of ligand-dependent transcription factors widely distributed in Metazoans, which can interact with target genes in the form of monomers, homodimers or heterodimers [1, 2]. NRs generally regulate downstream gene transcription by stimulating activity through binding to small molecule ligands, in addition, there is a class of Orphan NRs that

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function without ligands or for which no ligands have been identified [3, 4].

NRs have highly conserved structural features throughout evolution, including the A/B region, the DNA-binding domain (DBD), the ligand-binding domain (LBD), and the hinge region that connects the DBD and LBD [5, 6]. Among these, the DBD and LBD are the most conserved domains of NRs. The DBD binds to specific DNA sequences to recognize and regulate the transcription of target genes. The LBD is the ligand-binding part of the NRs, capable of binding specific small-molecule ligands to regulate the receptor's activity and function [5, 7]. In summary, the DBD and LBD domains regulate gene transcription and expression through their interactions with DNA and ligands.

NRs are capable of adapting to different signals and ligands, thereby regulating diverse functions. Current research focuses on the roles of NRs in various physiological processes in insects, such as metabolism, development, and reproduction. For example, NRs facilitate the metabolism of toxic substances in the cotton bollworm, *Spodoptera litura*, enabling it to adapt to a wider range of hosts [8]. The NR genes *HR96*, *HR39*, *HR38*, *TLL*, *KNIL* and *DSF* are essential for embryonic development in the red flour beetle, *Tribolium castaneum* [9]. In *Bemisia tabaci*, the abnormal expression of NR genes results in developmental arrest and molting failure [10]. The NR gene *HR3* in *Aedes aegypti* can downregulate the expression of vitellogenin precursor genes, thereby controlling the reproductive cycle [11]. The functions of NRs extend beyond these examples; they also regulate the immune response in *T. castaneum* and control male sexual behavior in *Agrotis ipsilon* [12, 13].

The functions of NRs in social insects are also gradually being confirmed. For example, the NR genes *HR38* and *ECR* can regulate the differentiation of the feeding behavior in *Bombus ignitus* [14]. Meanwhile, studies have shown that the NRs can influence caste differentiation in Hymenoptera social insects [15, 16]. Thus, NRs play a crucial regulatory role in social insects. In addition to the common social insects, some aphids in Hemiptera have also evolved sociality. These social aphids can reproduce via parthenogenesis to produce two types of offspring with distinct morphologies and/or behaviors: normal nymphs and soldiers [17, 18]. Research on social aphids remains limited, and whether NRs play a role in their morphological and behavioral differentiation remains unclear.

Pseudoregma bambucicola is a social aphid that reproduces parthenogenetically throughout the year on bamboo of the genus Bambusa [19]. The maternal generation of *P. bambucicola* demonstrates reproductive division of labor, producing first-instar normal nymphs and soldiers, resulting in offspring with distinct morphological and behavioral characteristics [20, 21]. Normal first-instar nymphs proceed to develop into reproductive adults, while soldiers undergo permanent developmental arrest, remaining at the first-instar stage throughout their lifespan [20]. Morphologically, soldiers possess specialized traits such as enlarged forelegs, elongated and sharpened frontal horns, and increased sclerotization, all of which enhance their defensive capabilities against predators [22]. Previous studies have indicated that morphological differences between soldiers and normal nymphs emerge during the embryonic stage and become rapidly pronounced during the early post-embryonic development, particularly in the enlargement of the forelegs [19].

The potential involvement of NR genes in the morphological and behavioral differentiation of social aphids remains largely unexplored. Therefore, this study focuses on *P. bambucicola*, a social aphid species characterized by caste differentiation and division of labor. Based on genomic data, we systematically identified the members of the NR gene family in *P. bambucicola* and conducted bioinformatics analyses. Furthermore, transcriptomic data and RT-qPCR were employed to investigate NR genes involved in the morphological and behavioral differentiation of *P. bambucicola,* thereby revealing their potential functions. This study not only provides a theoretical basis for exploring the functions of NR genes in social aphids but also contributes to a deeper understanding of the developmental regulatory mechanisms in social aphids.

Methods

Data source

Genomic data for *P. bambucicola* was obtained from the database constructed by the Insect Systematics and Diversity Laboratory at Fujian Agriculture and Forestry University (GenBank BioProject ID: PRJNA913551) [21].

To systematically analyze the dynamic changes of NR genes in the development and differentiation of *P. bambucicola*, we detailed the developmental stages of normal nymphs and soldiers. The developmental process of normal nymphs was divided into first-instar nymphs born within 24 h (N1), first-instar normal aphids after a period of growth (N2), mid-instar aphids (M), and adults producing nymphs (An). The developmental process of soldiers was classified into soldiers born within 24 h (S1) and soldiers that had grown for a period of time (S2). Since the morphological characteristics of soldiers develop rapidly within a short time after birth, S1 was further divided into soldiers born at 2 h (2 hS) and soldiers born at 24 h (24 hS).

Furthermore, to determine the effect of the NR gene family on the reproductive differentiation of *P.*

bambucicola, we classified the ovaries of adults according to the caste of offspring they produce: ovaries that produce normal nymphs (On) and those that produce soldiers (Os). The corresponding maternal tissues, excluding the ovaries, were designated as An0 and As0, respectively.

Transcriptomic sequencing of the above samples was performed by Biomarker Technologies Corporation, which provided transcriptomic data for various stages of *P. bambucicola* to compare the expression levels of NR genes. Due to issues with sample collection, the samples N1, N2, S1, S2, M, and An had only two biological replicates, while all other samples had three biological replicates.

Identification of NR gene family members in *Pseudoregma bambucicola*

To identify NR genes in *P. bambucicola*, structural information files PF00104 and PF00105 were first downloaded from the Pfam database [23]. The hmmsearch program of HMMER3 [24] was then used to compare the amino acid sequences of *P. bambucicola* against these model files (E-value $< 10^{-12}$), selecting sequences containing the DBD and LBD domains, respectively.

Next, we downloaded the amino acid sequences of NR genes from *Drosophila melanogaster*, *Bombyx mori*, *B. tabaci*, and *Acyrthosiphon pisum* from NCBI and AphidBase (https://bipaa.genouest.org/is/aphidbase/). Using these sequences as queries, we performed BLAST comparison with TBtools [25] (E-value $< 10^{-5}$) to identify NR genes in *P. bambucicola*.

We predicted conserved domains for the NR genes identified by both methods using Batch CD-search. The sequences of these genes were then refined with the FGENESH online tool (http://linux1.softberry.com/berry.phtml) to ensure their completeness. The corrected sequences were then used as the final sequence files for the NR genes.

Bioinformatics analysis of NR genes in *Pseudoregma bambucicola*

The average molecular weight and theoretical isoelectric point of each NR gene were predicted by submitting sequences to ExPASy (http://web.expasy.org/protparam/). TBtools software was utilized to determine the chromosomal locations of the NR genes in *P. bambucicola*, and a localization map of these genes on the chromosomes was generated. Additionally, the conserved domains of each gene were visualized using Chiplot (https://www.chiplot.online/).

Phylogenetic analysis of NR genes in *Pseudoregma bambucicola*

The amino acid sequences of NR genes in *P. bambucicola* were aligned using MUSCLE in MAGA 5.2 software [26]. The sequences were trimmed with trimAl in PhyloSuite 1.2.1 software [27], and the phylogenetic tree was constructed using the Maximum Likelihood method. ModelFinder [28] was used to select the optimal model, with bootstrapping set to 1000. The tree was output in Newick format and visualized with iTOL [29]. The VT +G4 model was chosen for constructing the phylogenetic tree of the 21 NR genes in *P. bambucicola* to explore their evolutionary development.

Additionally, we constructed a phylogenetic tree that included NR genes from *D. melanogaster*, *B. mori*, *B. tabaci*, *Apis mellifera*, and *A. pisum* alongside *P. bambucicola*. Sequences were aligned using MAFFT, with all other steps following the same protocol described above. Finally, the Q.insect + R6 model was employed for multispecies phylogenetic tree construction to observe the evolution of NR genes across different species.

Transcriptomic data analysis

We filtered out adapter sequences, low-quality reads, and ambiguous nucleotides from the raw sequencing data to obtain clean reads. We then used Trinity [30] software to assemble these clean reads into Unigene sequences. For the transcriptomic data of S1 and S2, we employed HISAT2 [31] software to align the clean reads against the reference genome for accurate positioning and used StringTie [32] for assembly to prepare the data for further analysis.

We used Bowtie [33] software to map the reads obtained from sequencing to the Unigene library of *P. bambucicola*, obtaining the gene read counts. Gene expression levels were quantified using fragments per kilobase of transcript per million mapped reads (FPKM). We performed differential gene expression analysis using the DESeq2 package [34], with the false discovery rate (FDR) threshold set to less than 0.01 and the fold change (FC) threshold set to greater than or equal to 2. Based on these methods, we identified NR genes that were differentially expressed during the growth, reproductive differentiation, and caste differentiation of *P. bambucicola*.

Quantitative analysis

To further explore the expression differences of NR genes across different castes, we selected the *HR4* based on transcriptomic data, as well as *HR39*, which had previously been identified as under positive selection [21]. Given the significant temporal specificity of NR expression, this study selected first-instar normal

nymphs and soldiers at 2 h, 24 h, 72 h, and 90 h after birth. Quantitative real-time PCR (RT-qPCR) was subsequently performed to validate the dynamic changes in the expression levels of these target genes.

Total RNA was extracted from samples using TRIzol Reagent (Invitrogen, USA), followed by reverse transcription with the FastKing gDNA Dispelling RT SuperMix kit (Tiangen Biotech, China). *GAPDH* and *HSP70* were selected as reference genes, and RT-qPCR was performed using the ChamQ Universal SYBR qPCR Master Mix kit (Vazyme, China). Each sample group included at least three biological replicates and three technical replicates to ensure the reliability of the experimental results. The primer sequences are provided in Supplementary Table S1.

Data analysis

We calculated all RT-qPCR data using the $2^{-\triangle\triangle CT}$ relative quantification method, by taking the geometric mean of the Ct values from the two reference genes. The statistical significance of data differences was evaluated with SPSS 19.0, using a significance level of $P < 0.05$. In the figures, asterisks denote statistical significance as follows: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Non-significant results are denoted as $P \geq 0.05$ (ns). GraphPad Prism 8 software [35] was used to generate the plots.

Results

Identification and characterization of NR genes in *Pseudoregma bambucicola*

Based on the genomic data of *P. bambucicola*, we identified a total of 21 NR genes, which were named according to standard nomenclature [36, 37]. The amino acid sequences of the identified NR genes are provided in Supplementary Table S2. The molecular weights of these NR genes vary significantly, ranging from 8.03 kDa to 140.14 kDa, with isoelectric points ranging from 5.86 to 10.88 (Table 1). Domain analysis indicated that most NR genes possess both DBD and LBD domains, while *HR83* has lost the LBD domain. The NR0 subfamily, consistent with previous studies, includes members with only the DBD domain. In the NR1 subfamily, *HR3* appears to have undergone a gene duplication event, and we have provisionally named these duplicates *HR3*-paralog1 (*HR3P1*) and *HR3*-paralog2 (*HR3P2*), which each possess only the LBD domain (Fig. 1). Additionally, three NR genes previously identified in other insect species appear to be absent in the genome of *P. bambucicola*: *KNI* (*NR0 subfamily*), *HR96* (NR1 subfamily), and *NR2E6* (NR2 subfamily).

Table 1 Family and subfamily of NR genes in *Pseudoregma bambucicola*

Subfamily	Nomenclature	Name	ORF (aa)	Mw (kDa)	PI
NR0	NR0A2	*KNRL*	82	8.03	6.22
	NR0A3	*EAGLE*	501	53.30	10.35
NR1	NR1D3	*E75*	943	103.24	8.78
	NR1E1	*E78*	498	54.64	8.15
	NR1F4	*HR3*	773	82.92	8.57
		HR3P1	232	25.99	9.72
		HR3P2	412	44.60	10.88
	NR1H1	*ECR*	324	37.48	5.86
NR2	NR2A4	*HNF4*	416	46.80	7.15
	NR2B4	*USP*	647	73.14	9.43
	NR2D1	*HR78*	475	54.79	6.08
	NR2E2	*TLL*	405	45.48	9.17
	NR2E3	*HR51*	482	53.03	8.14
	NR2E4	*DSF*	1233	140.14	8.05
	NR2E5	*HR83*	284	31.80	9.30
	NR2F3	*SVP*	530	55.79	9.79
NR3	NR3B4	*ERR*	977	106.60	6.69
NR4	NR4A4	*HR38*	902	100.21	8.97
NR5	NR5A3	*FTZ-F1*	563	61.52	7.90
	NR5B1	*HR39*	958	107.78	8.76
NR6	NR6A2	*HR4*	1222	130.90	8.35

Chromosomal distribution of NR genes in *Pseudoregma bambucicola*

The chromosomal distribution of NR genes was visualized based on the genomic data of *P. bambucicola* (Fig. 2). The NR genes were found to be widely dispersed across six linkage groups (LG01–LG06), with most occurring as single genes located distantly from one another. Only *HR3* is clustered with *HR3P1* and *HR3P2*, and this clustering phenomenon is related to gene duplication events, which may imply that *HR3* has a unique function in *P. bambucicola*.

Phylogenetic analysis of NR genes in *Pseudoregma bambucicola*

We constructed a phylogenetic tree of the NR genes of *P. bambucicola*. As shown in Fig. 3, the NR genes of *P. bambucicola* are divided into seven subfamilies: NR0, NR1, NR2, NR3, NR4, NR5, and NR6. Within the NR1 subfamily, *HR3* can be clustered into a clade with *HR3P1* and *HR3P2*, which provides strong evidence for gene duplication events involving *HR3*. In addition, we constructed a phylogenetic tree by integrating NRs from *P. bambucicola* with those from five other insect species (Fig. 3B). This analysis revealed that NR evolution is highly conserved across species, suggesting that these genes are

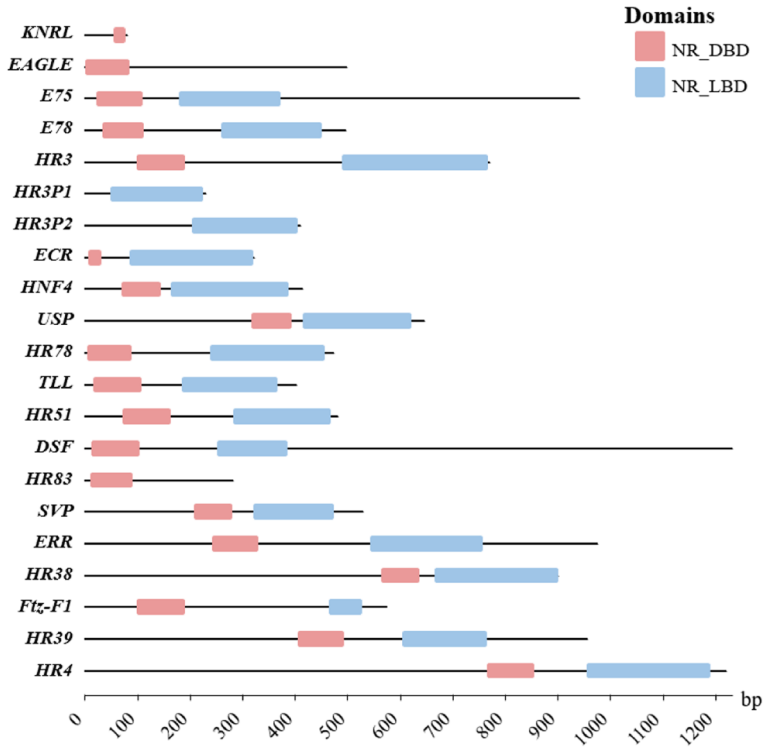

Fig. 1 Domains of NR genes in *Pseudoregma bambucicola*

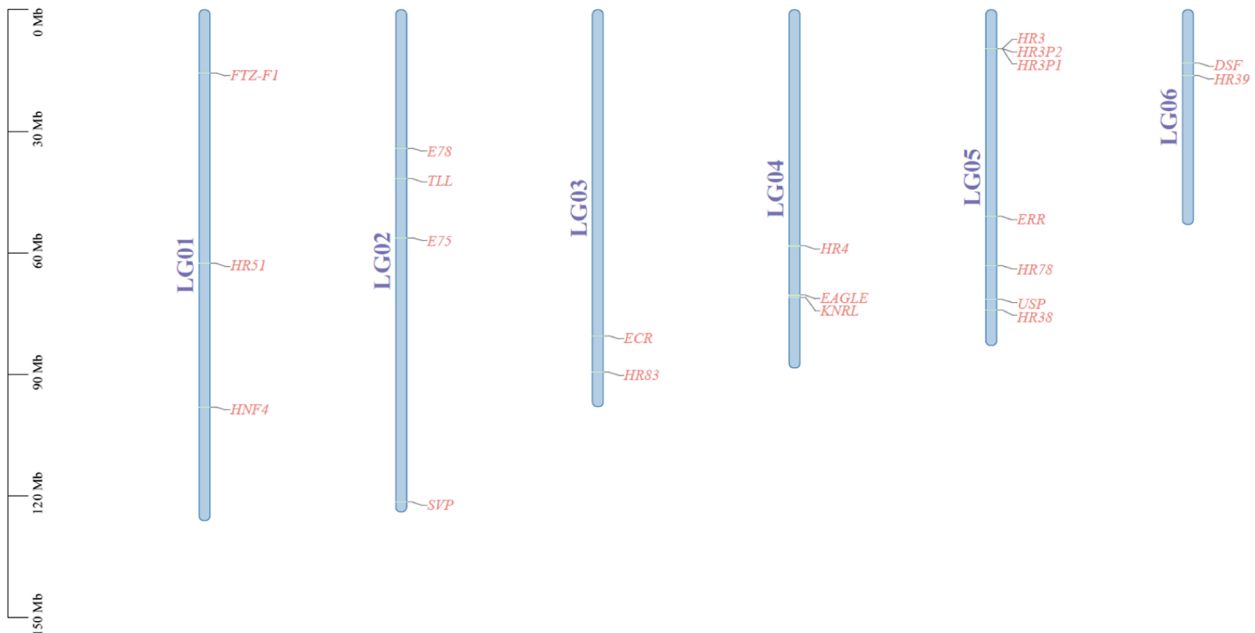

Fig. 2 The chromosomal distribution of predicted NR genes in the genome of *Pseudoregma bambucicola*. The scale on the left represents genomic position in megabases (Mb)

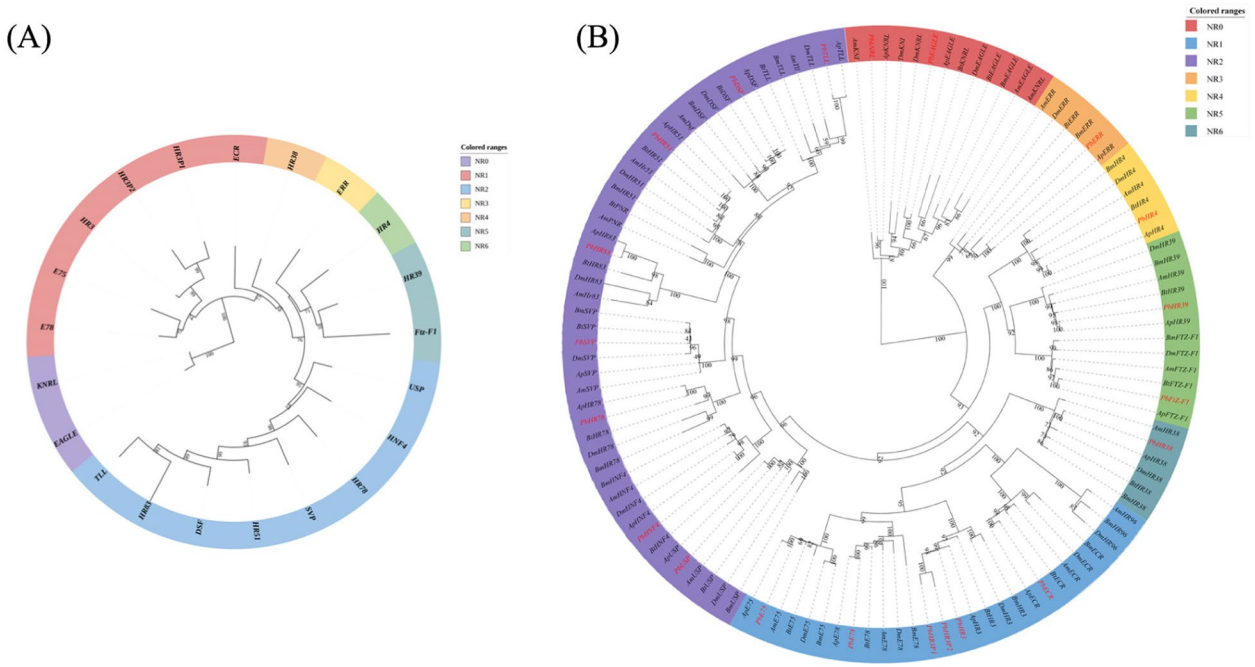

Fig. 3 Phylogenetic tree of NR genes. **A** Phylogenetic analysis of NR genes in *Pseudoregma bambucicola*. **B** Phylogenetic relationship of NR genes from *Pseudoregma bambucicola* (Pb), *Acyrthosiphon pisum* (Ap), *Bemisia tabaci* (Bt), *Drosophila melanogaster* (Dm), *Bombyx mori* (Bm), and *Apis mellifera* (Am). The NR genes of *Pseudoregma bambucicola* are marked in red in the figure. The amino acid sequences of NR genes are provided in Table S2

likely to perform the same or similar biological functions in different species.

Expression of NR genes in distinct reproductive patterns of *Pseudoregma bambucicola*

In the social aphid *P. bambucicola*, there is a phenomenon of reproductive division of labor, with soldiers and normal nymphs produced via parthenogenetic reproduction. The caste determination of soldiers occurs during embryonic development within the adult. To investigate the impact of NR genes on the reproductive division of labor, we screened for differentially expressed NR genes between An0 and As0. We compared the expression levels of NR genes in On and Os to determine their influence on soldier differentiation. The results showed that only *TLL* met the differential expression criteria in An0 and As0, while no significantly different NR genes were found between On and Os (Fig. 4).

Developmental stage-specific expression of NR genes

To investigate the expression levels of NR genes at different developmental stages of *P. bambucicola*, we compared the expression of these genes across four stages: N1, N2, M, and An. As shown in Fig. 5A, although NR genes did not exhibit significant differential expression across these four developmental stages, most genes displayed variable expression patterns. The *E75* gene consistently maintained a high expression level, whereas *HR83* and *HR51* exhibited very low or no expression across all developmental stages. Several genes showed increased expression levels at specific stages; for example, *SVP* and *HR4* showed increased expression in the An stage, and *FTZ-F1* displayed elevated expression in the N2 stage. *HR3* exhibited relatively higher expression in the N2 and M, but was expressed at lower levels in the N1 and An. In contrast, the expression of *DSF* and *E74* remained relatively stable, with moderate levels and minimal fluctuations across all developmental stages.

Although the soldier caste is determined during the embryonic stage, morphological differences become more pronounced after birth. As NR genes are essential transcription factors for growth and development, it is important to determine whether they influence the development of soldiers during the post-embryonic stage. Therefore, we analyzed NR gene expression in the 2 hS and 24 hS to explore the role of NRs in the post-embryonic stage of soldiers (Fig. 5B). The results showed that the expression levels of *E75* and *HNF4* decreased significantly in the 24 hS compared to the 2 hS.

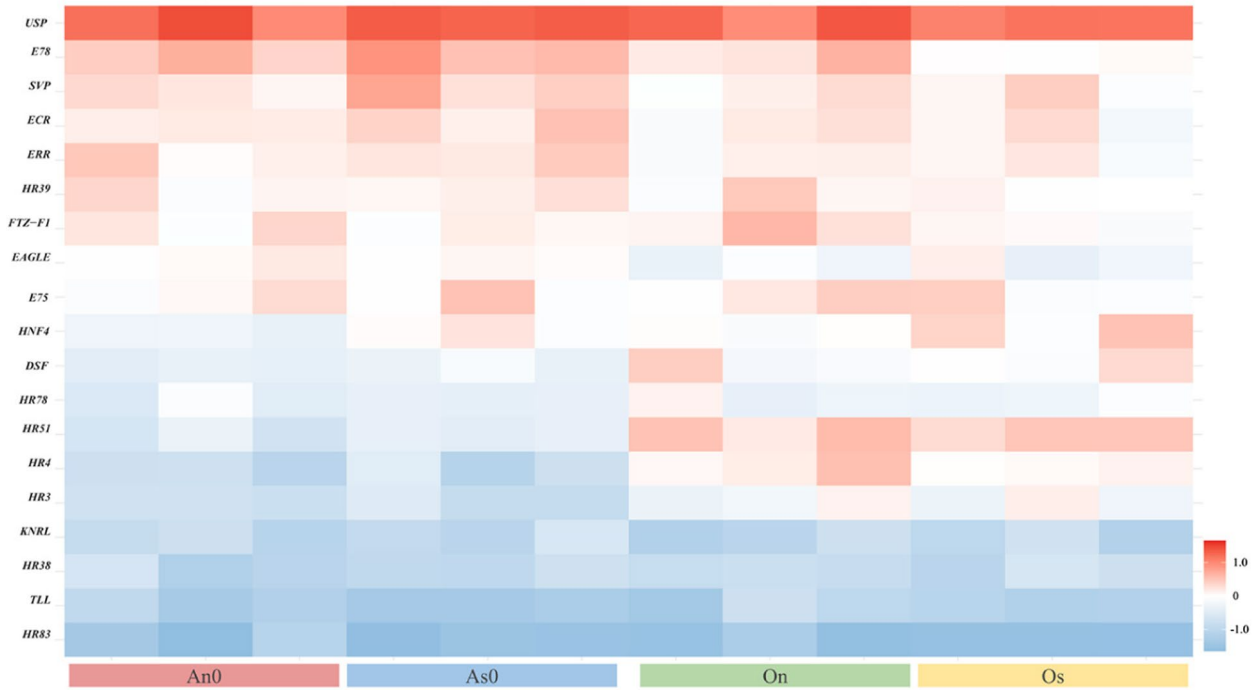

Fig. 4 Expression patterns of NR genes in An0, As0, On, and Os. Color intensity represents normalized expression levels, with red indicating higher expression and blue indicating lower expression

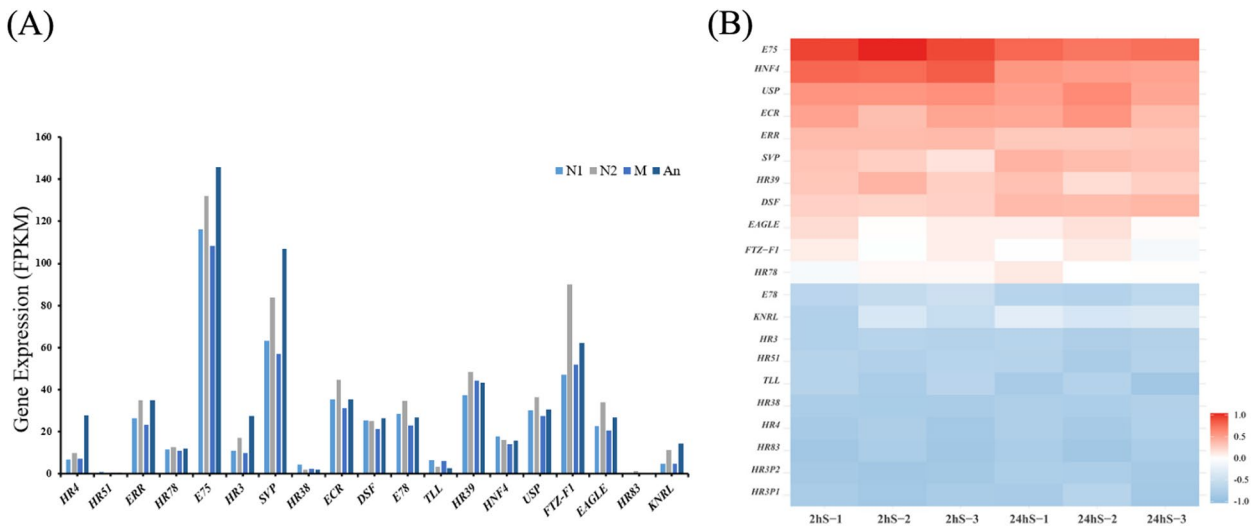

Fig. 5 Expression patterns of NR genes across different developmental stages. **A** Expression patterns of NR genes in N1, N2, M, and An. **B** Expression patterns of NR genes in 2 hS and 24 hS. Color intensity represents normalized expression levels, with red indicating higher expression and blue indicating lower expression

Expression levels of NR genes in different castes of *Pseudoregma bambucicola*

Normal nymphs and soldiers of *P. bambucicola* have distinct morphologies and behaviors. To investigate the role of NR genes in caste differentiation, we compared the changes in expression levels of NR genes in first-instar normal nymphs and soldiers at birth for some time (Fig. 6A). Among these, *HR4* met the criteria for differentially expressed genes, being significantly downregulated in soldiers.

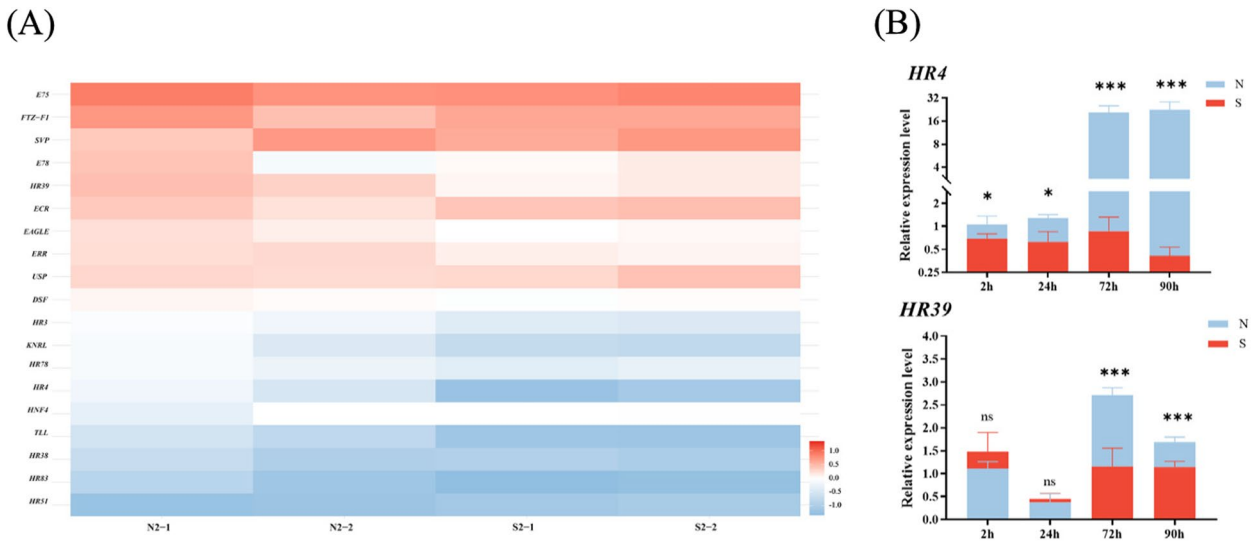

Fig. 6 Expression of NR genes in different castes of *Pseudoregma bambucicola*. (A) Heatmap of the expression of NR genes in N2 and S2. (B) Expression patterns of *HR4* and *HR39* in first-instar normal nymphs and soldiers at the same time points. Error bars represent the standard error of the mean from three biological replicates. Asterisks indicate significance levels: $P < 0.05$ (*) and $P < 0.001$ (***). Non-significant differences are shown as $P \geq 0.05$ (ns)

NR genes, serving as transcription factors, demonstrate pronounced temporal expression specificity [38]. To further investigate the expression differences of NR genes across different castes, this study selected *HR4* and *HR39* as research targets. The expression levels of these genes were analyzed in first-instar normal nymphs and soldiers at 2 h, 24 h, 72 h and 90 h after birth using RT-qPCR (Fig. 6B). The results showed that *HR4* expression was significantly downregulated in soldiers compared to first-instar normal nymphs at corresponding time points ($P < 0.05$). The expression levels of *HR39* in the early developmental stages of soldiers showed no significant difference compared to normal nymphs ($P > 0.05$). However, as *HR39* expression significantly increased in first-instar normal nymphs during late development, the disparity between the two groups gradually widened ($P < 0.05$). Additionally, both genes exhibited significant upregulation in 72 h first-instar normal nymphs, whereas their expression levels showed relatively minimal changes in soldiers.

Discussion

In this research, we identified 21 NR genes in the social aphid *P. bambucicola* based on genomic data, and this number is not much different from the number of NR genes of other insects. For example, 22 NR genes have been found in *A. mellifera* [39], 21 genes have been identified in *D. melanogaster* [40] and *T. castaneum* [9], 20 genes in *B. tabaci* [10] and *A. aegypti* [41], and 19 genes in *A. pisum* [42] and *B. mori* [43].

The NR members in *P. bambucicola* are similar to those in *A. pisum*, as they both lack *KNI*, *HR96*, and *NR2E6*. This may be due to evolutionary divergence within Aphidoidea, although further evidence is needed to support this hypothesis. *KNI* and *KNRL* result from lineage-specific duplications in NR genes [44], and studies in *D. melanogaster* have shown that *KNI* and *KNRL* share similar expression patterns and functions [45]. The absence of *KNI* in both *A. pisum* and *B. tabaci* suggests that the loss of *KNI* in *P. bambucicola* further supports the idea that this duplication occurred independently in different insect lineages [10, 42]. *HR96* is involved in detoxification metabolism [46–48] and the ecdysteroid hormone (20E) signaling pathway [49]. The loss of *HR96* in aphids may be due to functional redundancy or its replacement by other genes [42]. *NR2E6* was originally identified in *A. mellifera* as a homolog of the photoreceptor-specific nuclear receptor (*PNR*), which is highly expressed in the compound eyes of honeybees and may be involved in regulating insect eye development [39]. The retention or loss of the *PNR* across different species may contribute to the regulation of lineage-specific differences in compound eye structure [42].

During the identification of NR genes in *P. bambucicola*, it appears that the *HR3* gene in the NR1 group may have undergone a gene duplication event. This is supported by both the gene's arrangement and the phylogenetic tree analysis (Figs. 2 and 3). Future research should employ gene amplification techniques to confirm this duplication. Gene duplication is considered a driving

force for the evolution of genes with new functions and plays a crucial role in biological evolution [50, 51]. As a member of the 20E signaling cascade, *HR3* exhibits multiple functions: it can inhibit the production of 20E, while also activating the expression of downstream genes to support insect growth and development [52]. Additionally, *HR3* has been shown to be involved in embryonic development and reproductive processes in insects [11, 53]. Future studies should explore whether this duplication event has endowed *HR3* with novel biological functions.

The functions of NRs are relatively conserved across different species [54]. Therefore, this study utilizes transcriptomic data and RT-qPCR to identify differentially expressed genes in *P. bambucicola*, aiming to provide an initial understanding of the roles of NR genes in *P. bambucicola*.

First, we compared the expression levels of An0 and As0 to identify NR genes that may influence the reproductive division of labor in *P. bambucicola*. According to the analysis, the expression of *TLL* in adults of different castes differed significantly (Fig. 4). The gene not only participates in regional differentiation at both ends of the *D. melanogaster* embryo [55] but also regulates neuropeptide signaling in the adult *D. melanogaster* brain to control aggressive behavior [56]. Notably, no differential expression of *TLL* was observed between the On and Os samples. Therefore, the differential expression of *TLL* in maternal tissues may suggest its role in modulating the embryonic developmental environment or influencing the maternal nervous system, thus indirectly contributing to the determination of offspring caste fate.

The caste of soldiers is determined during embryonic development. Therefore, we compared the expression levels of NRs in the ovaries of normal nymph-producing and soldier-producing aphids but found no differentially expressed NR genes. This could be due to two reasons: firstly, NRs might play a conserved role during embryonic development of both castes, supporting normal embryonic development without directly participating in caste differentiation during embryogenesis [57]; Then, as members of a transcription factor family, NRs may exhibit spatiotemporal differences in their function [38]. Therefore, NRs might contribute to embryonic differentiation by regulating specific developmental time points or by directing the formation of particular tissues. Verification of this hypothesis will require more precise embryonic staging and comprehensive functional analyses.

Additionally, we compared the expression levels of NR genes in different developmental stages of *P. bambucicola*. No differentially expressed NR genes were found in the four developmental stages of normal nymphs. However, most NR genes were expressed at each stage, with varying levels of expression at different stages (Fig. 5A). Particularly, NRs involved in the 20E signaling cascade, such as *E75*, *FTZ-F1*, and *HR4* [58]. This indirectly suggests that NRs respond to 20E regulation to facilitate normal growth and development in *P. bambucicola*. Moreover, genes such as *DSF* and *E74* exhibited moderate expression across all stages, implying a potential role for these NRs in regulating fundamental developmental processes in *P. bambucicola*.

We also observed that the expression levels of *HR83* and *HR51* are either very low or absent (Fig. 5A). Moreover, the LBD domain of *HR83* was not found (Fig. 1). Current research indicates that *HR83* and *HR51* have diverse functions. For example, they may be involved in neuronal development and may have functions similar to *PNR* [59]. Furthermore, *HR83* has been shown to enhance resistance to chlorpyrifos in *Nilaparvata lugens* [60], and *HR51* is associated with wing development and reproductive capacity in *D. melanogaster* [61]. Thus, we propose two hypotheses: First, these two genes may be functionally redundant in *P. bambucicola*. For instance, since *P. bambucicola* feeds exclusively on bamboo stems, it may not require the metabolic functions provided by *HR83*, and normal nymphs may not need *HR51* to regulate wing development when developing into wingless parthenogenetic aphids. Second, *HR83* and *HR51* may be expressed in a highly restricted manner [38, 58]. For example, the homologous gene *FAX-1* in *Caenorhabditis elegans* is expressed in only a subset of neurons but is essential for axon pathfinding [62].

Although soldiers of *P. bambucicola* cannot progress to the next instar, growth continues during the early post-embryonic stage, further increasing the morphological differences compared to normal nymphs. To investigate the NR genes that influence soldier growth and development, we conducted a comparative analysis of soldiers at 2 h and 24 h (Fig. 5B). The results showed that the expression levels of *E75* and *HNF4* were significantly reduced at the 24 hS compared to the 2 hS. *E75* is a multifunctional gene that serves as a primary response gene to 20E, playing a crucial role in metabolic regulation in insects, particularly during molting and metamorphosis [63]. Additionally, *HNF4* is closely associated with the formation of insect cuticles. Studies have shown that in *D. melanogaster*, *HNF4* promotes the elongation of fatty acids and the synthesis of hydrocarbons [64]. The high expression levels of *E75* and *HNF4* at 2 h suggest that these genes may collectively contribute to the cuticle hardening process in soldiers.

The first-instar normal nymphs and soldiers of *P. bambucicola* exhibit distinct morphological and behavioral differences. We selected first-instar nymphs and soldiers after a period of growth for comparison. Given

that nuclear receptor gene expression is temporally specific [38], we used RT-qPCR to examine the expression changes of *HR4* and *HR39* at different time points, aiming to identify NR genes involved in caste development. The results show that, compared to first-instar nymphs, *HR4* expression is significantly downregulated in soldiers at the same time points (Fig. 6B). *HR4* can also regulate chitin synthesis and degradation to form the cuticle by mediating the 20E signaling pathway in hemimetabolous insects like *Locusta migratoria* [65]. In the early post-embryonic development of *P. bambucicola*, *HR4* expression already shows significant differences, suggesting its potential involvement in the morphological specialization of soldiers. Both *HR4* and *HR39* are essential components of the ecdysone signaling cascade and play key roles in normal insect growth and development [38]. For instance, abnormal *HR4* expression in *D. melanogaster* leads to irregular larval development [66] and inhibition of *HR4* in hemimetabolous insect *Blattella germanica* results in developmental arrest, preventing the molting process [67]. *HR39* in *L. migratoria* regulates chitinase genes, thereby affecting the degradation of cuticular chitin during the molting process, resulting in delayed molting and wing defects [68]. Consequently, the marked changes in the expression levels of *HR4* and *HR39* at the late first instar stage may be implicated in the developmental arrest of soldiers, preventing their progression to the next instar, in contrast to normal nymphs.

Additionally, soldiers exhibit sterility, and it has been confirmed in the social aphid *Ceratovacuna japonica* that their ovaries undergo degeneration, accompanied by abnormal embryonic development [69]. *HR4* is a key gene required for oocyte maturation and oogenesis [9], and *HR39* is also essential for insect reproduction [70]. Therefore, changes in the expression of these two genes may disrupt critical processes in ovarian development, preventing soldiers from reproducing normally.

It is possible that the expression patterns of other NR genes also exhibit dynamic changes at different time points, which requires further investigation. Additionally, NRs may play critical roles in the development and behavioral differentiation of social insects [71, 72]. However, it remains unclear whether NR genes regulate the defensive behavior of soldiers toward predators. Therefore, future studies could employ RNA interference or Clustered Regularly Interspaced Short Palindromic Repeats techniques to investigate the specific functions of NR genes in *P. bambucicola*, revealing their potential roles in behavioral and developmental regulation. Meanwhile, previous studies have shown that, compared to normal nymphs, the expression of genes related to the synthesis and degradation of 20E is altered in soldiers of *P. bambucicola* [73]. As core components of the 20E signaling cascade, the regulatory mechanisms of NR genes require further investigation and represent an important direction for future research.

Conclusions

In summary, this study provides the first comprehensive identification of the NR gene family in the social aphid *P. bambucicola* based on genomic data. A total of 21 NR genes were identified, and their phylogenetic relationships were analyzed. Transcriptomic profiling revealed the *TLL* associated with reproductive behavioral differentiation in *P. bambucicola*, as well as the *E75* and *HNF4* involved in post-embryonic soldier development. Additionally, RT-qPCR validation demonstrated soldier-specific expression patterns of *HR4* and *HR39*, suggesting potential functional specialization of NR genes across different castes. Furthermore, the study outlines future research directions for NR genes in *P. bambucicola*. This research establishes the foundation for future research on the functions of NR genes in *P. bambucicola* and contributes valuable insights to the broader field of social aphid research.

Abbreviations

NR	Nuclear receptor
DBD	DNA-binding domain
LBD	Ligand-binding domain
FPKM	Fragments per kilobase of transcript per million mapped reads
FDR	False discovery rate
FC	Fold change
RT-qPCR	Quantitative real-time PCR
20E	Ecdysteroid hormone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11724-5.

Supplementary Material 1. Supplementary Table S1. Primers used for RT-qPCR.

Supplementary Material 2. Supplementary Table S2. Amino acid sequences of nuclear receptor genes from *Pseudoregma bambucicola* and five other insect species.

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Authors' contributions

J.J.L. and X.L.H. conceptualized and designed the project. X.L.H. contributed to the data collection. J.J.L. performed the experiments and analyzed the data. J.J.L. wrote the draft manuscript and X.L.H. reviewed the manuscript. All authors have read and approved the final manuscript.

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Data availability
The datasets generated and analysed during the current study are available in the NCBI repository [PRJNA913551 and PRJNA843133] or from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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