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Identification of ABC transporter genes in *Neoseiulus californicus* (Acari: Phytoseiidae) and functional analysis of key genes in the ABCA subfamily

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ABSTRACT

Neoseiulus californicus (McGregor) is an important predatory mite species characterized by its broad prey spectrum and strong adaptability. ATP-binding cassette (ABC) transporters represent a large protein superfamily present in both prokaryotic and eukaryotic organisms, primarily functioning in xenobiotic transport and maintenance of cellular homeostasis. However, research on this protein family in predatory mites remains largely unknown. In this study a total of 49 putative ABC transporter genes were identified, through a comprehensive analysis of the *N. californicus* transcriptome. Phylogenetic analysis classified these transporters into eight distinct subfamilies (ABCA-H). Expression profiling of ABCA subfamily genes across different developmental stages demonstrated ubiquitous expression patterns for all members except *NcABCA-07*. Functional characterization of two highly expressed genes in adult females, *NcABCA-02* and *NcABCA-05*, was performed through RNA interference. Silencing of *NcABCA-02* and *NcABCA-05* increased the mortality rate of adult female mites from 1.00% to 12.00% and 5.78%, respectively. Furthermore, predation efficiency on adult female *Tetranychus urticae* Koch significantly decreased from 3.87 prey individuals to 1.87 (51.7% reduction) and 1.70 (56.1% reduction) following silencing of *NcABCA-02* and *NcABCA-05*. Additionally, reduced egg hatching rates were observed in silenced individuals. These results indicate critical roles of *NcABCA-02* and *NcABCA-05* in maintaining survival, predatory capacity, and reproductive fitness in *N. californicus*. The findings provide valuable insights into the physiological mechanisms of predatory mites and establish a theoretical framework for enhancing their efficacy in biological control applications.

KEYWORDS

ABC transporter proteins, ABCA subfamily, Gene family identification, *Neoseiulus californicus*, RNAi

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INTRODUCTION

ABC transporters are a class of membrane-integrated transport proteins widely found in both eukaryotes and prokaryotes. Members of this family primarily function by utilizing ATP as an energy source to transport various substrates across cell membranes, with substrates ranging from small ions to relatively large peptides and polysaccharides. They are also involved in cellular physiological processes such as DNA repair, translation, or the regulation of gene expression (Sauvage *et al.* 2009; Wang *et al.* 2024). The arthropod ABC transporters consist of 8 subfamilies (ABCA-ABCH), each of which contains multiple members. All ABC transporters share a highly conserved ATP hydrolysis domain or protein

(nucleotide-binding domain [NBD]) (Davidson *et al.* 2008). Except for members of the ABCE and ABCF subfamilies, other family members also contain non-conserved transmembrane domains (TMDs) (Rees *et al.* 2009). The TMD and NBD can form half-transporters (one TMD and one NBD) or full transporters (two TMDs and two NBDs), with the latter being functional transport proteins (Locher 2009).

ABC transporters have been identified in various arthropods, and the number of ABC transporters varies among different species. *Helicoverpa armigera* (Hübner) (56 genes), *Nilaparvata lugens* (Stål) (32 genes), *Laodelphax striatellus* (Fallén) (40 genes), *Bemisia tabaci* (Gennadius) (55 genes), while only the ABC transporters of *Tetranychus urticae* Koch (103 genes) have been identified in mites (Dermauw *et al.* 2013; Sun *et al.* 2017; Tian *et al.* 2017; Jin *et al.* 2019; Li *et al.* 2020).

ABC transporter proteins in arthropods, especially the ABCA, ABCB, ABCC, and ABCG subfamilies, are extensively involved in the process of efflux to exogenous toxic substances (Merzendorfer 2014). Knockdown of *ABCA1* and *ABCD1* using RNAi significantly increases the susceptibility of *Aphis gossypii* Glover to sulfoxaflo (Wang *et al.* 2021). Inhibition of *A. gossypii ABCB5*, *ABCG4*, *ABCG7*, *ABCG16*, *ABCG17*, *ABCG26* and *MRP12* significantly increased the sensitivity of the cyantraniliprole-resistant strain (CyR) strain to cyantraniliprole (Li *et al.* 2022). In addition to this, some members of the ABC family are involved in maintaining normal life activities in arthropods. For example, knockdown of *DvvABCA_50718* in *Diabrotica virgifera virgifera* LeConte results in death of prepupae and defective wings that successfully develop into adults (Adedipe *et al.* 2019). *Drosophila melanogaster* Meigen ABC-H transporter protein *DmCG9990* is localized to the ectodermis and has been shown to transport epidermal hydrocarbons and participate in skin barrier formation (Zuber *et al.* 2018). The knocking down of the *DmCG9990* homologous gene in *Locusta migratoria* (Linnaeus) and *Plutella xylostella* (Linnaeus) resulted in larvae failing to molt and dying (Guo *et al.* 2015; Yu *et al.* 2017). Members of the ABCE and ABCF subfamilies lack the TMD domain and are composed only of two NBD domains; they are not involved in the transport of conventional substances. ABCE has been annotated as an RNase L inhibitor in eukaryotes, and ABCE is universally present and highly conserved across all eukaryotic species (Zhao *et al.* 2004).

Although significant progress has been made in the study of insect ABC transporters, research on ABC transporters in mites remains very limited. To date, only ABC transporter members have been identified in *T. urticae*, with preliminary expression analysis conducted, but functional analysis has not been performed (Dermauw *et al.* 2013). It is especially noteworthy that this type of research is still absent in predatory mites, which have important biological control functions.

Neoseiulus californicus (McGregor) is an important natural enemy, with a wide distribution range, capable of preying on *T. urticae*, *Panonychus ulmi* (Koch), and various small arthropods such as whiteflies and thrips (McMurtry *et al.* 2013; Jiang *et al.* 2020). Studies have shown that *N. californicus* exhibits superior predation and reproductive abilities compared to other predatory mites, and it has strong resistance to hypoxia, high temperatures, and various pesticides (Sato *et al.* 2002; Song *et al.* 2016; Wang *et al.* 2016; Hao *et al.* 2024; Siebert *et al.* 2025).

The ABC transporters, as crucial detoxification and physiological homeostasis regulators, likely play a pivotal role in the remarkable biological characteristics exhibited by *N. californicus*. This study systematically identified the ABC transporter gene family in *N. californicus* based on transcriptomic data. To elucidate the functional roles of ABCA subfamily members in maintaining vital biological processes, comprehensive mRNA expression profiling of ABCA genes was conducted. Two highly expressed genes in adult females (*NcABCA-02* and *NcABCA-05*) were selected for functional characterization using RNA interference technology to investigate their roles in predatory efficiency and reproductive capacity. The present research aims to uncover the fundamental physiological functions of ABC transporters in predatory mites, thereby providing novel theoretical foundations for understanding their biological mechanisms and promoting sustainable applications in biological control systems.

MATERIAL AND METHODS

Mites' samples

Neuseiulus californicus – provided by Agricultural Insect and Pest Control Laboratory of Sichuan Agricultural University.

T. urticae – collected from *Glycine max* (L.) Merr. leaves at Chongzhou Research and Development Base of Sichuan Agricultural University. *Arachis hypogaea* L. seedlings were used for long-term rearing at 25 ± 1 °C, 75 ± 5% RH and photoperiod of 16 L: 8D.

Identification of the ABC transporter protein gene of N. californicus

The transcriptome data of *N. californicus* were provided by the Laboratory of Agricultural Insects and Pest Control, Sichuan Agricultural University, Sichuan, China. The raw transcriptome sequencing data have been deposited in NCBI (https://www.ncbi.nlm.nih.gov/) under accession number PRJNA1308816).

The ABC transporter sequences for *D. melanogaster*, *Homo sapiens* L., and *Daphnia pulex* Leydig were downloaded from NCBI, while the protein sequence for *T. urticae* was retrieved from UniProt (https://www.uniprot.org/). These files were integrated as template sequences for BLAST comparison against *Neusiulus californicus* protein files. BLAST alignment was performed using TBtools-II (version 2.096) analysis software, yielding potential ABC transporter gene amino acid sequences (threshold set at 1e-6). Next, the Simple HMM search tool in TBtools-II was used to query *N. californicus* protein files with the ABC transporter Hidden Markov Model (HMM) file (pfam 00005) as a template. To prevent missing potential ABC transporters, amino acid sequence information from both searches was merged as candidate genes. The InterPro (https://www.ebi.ac.uk/interpro/) tool was then used to scan the candidate genes for conserved domains, further identifying ABC transporter genes in *N. californicus*. TBtools-II software was used to analyze the amino acid count, relative molecular mass, and theoretical isoelectric point of the proteins. Finally, CELLO (v2.5) software was used to analyze the subcellular localization of the ABC transporter proteins.

Conservation motifs and domain analysis of the ABC transporter gene of N. californicus

The identified ABC transporter protein sequences in FASTA format were submitted to MEME (https://meme-suite.org/meme/) for the prediction of conserved motifs. Additionally, these sequences were submitted to the InterPro (https://www.ebi.ac.uk/interpro/) tool for the prediction of the transmembrane domain (TMD) and nucleotide-binding domain (NBD) of *N. californicus* ABC transporters.

Phylogenetic analysis of the ABC transporter system in N. californicus

The phylogenetic analysis was performed using ABC transporter protein sequences obtained from *D. melanogaster*, *H. sapiens*, *D. pulex*, *T. urticae*, and *N. californicus*. The evolutionary tree was constructed with TBtools-II software through its "One Step Build a ML Tree" function, which integrates multiple bioinformatics tools including Muscle for sequence alignment, trimAI for alignment trimming, and IQ-tree for maximum likelihood tree construction (bootstrap replicates = 1000). The resulting phylogenetic tree was subsequently visualized and annotated using the iTOL online platform for enhanced graphical representation. Based on the classification and grouping of ABC transporters from model species (*T. urticae*, *D. melanogaster*, *H. sapiens*, and *D. pulex*), the ABC transporter genes of *N. californicus* were classified.

Detection of gene expression levels through RT-qPCR

A total of 500 larvae, 500 nymphs, 300 female adults, and 500 male adults of *N. californicus* were collected. Total RNA was extracted from each sample using the MolpureCell/Tissue Total RNA kit. The purity and concentration of the RNA were measured using the NanoDrop 2000 spectrophotometer. The extracted RNA was then reverse-transcribed into cDNA using the Hieff UNICON Universal Blue RT-qPCR SYBR Green Master Mix reverse transcription kit. After the reaction was complete, the cDNA concentration was determined using the NanoDrop 2000 spectrophotometer, and the cDNA was diluted to 500 ng/μL for subsequent RT-qPCR experiments. Using Primer5, RT-qPCR primers for all sequences

in the ABCA subfamily of *N. californicus* ABC transporter were designed, and the primers were synthesized by Sangon Biotech (Table 1). The internal reference gene was synthesized from the *β-actin* gene of *Neoseiulus barkeri* Hughes and detected by RT-qPCR (Wang *et al.* 2019) (Table 1). The iCycler iQ Real-Time PCR Detection System and Taq Pro Universal SYBR qPCR MasterMix kit were used for RT-qPCR. The specificity of the PCR was confirmed by the dissociation curve. Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method, where the CT values of each gene at different developmental stages were standardized with respect to the CT value of the reference gene (*β-actin*) (Livak and Schmittgen 2001). The statistical significance of gene expression was calculated using one-way ANOVA, with $p < 0.05$ considered statistically significant. Each developmental stage included three biological replicates and three technical replicates.

Table 1. Primer sequences used for RT-qPCR.

Gene name	5，-3， sense	5，-3， antisense
NcABCA-01	GCCACTGTGGAAGGAGTAGGAA	CGTTTGGTTTATGCGGACTTT
NcABCA-02	AATATCATGCAGGATAAGGAGACG	ATTGTGACAAGGGCACAGAGG
NcABCA-03	CCAGGTGGAGACCATGATTGA	GCGAGCGATAGTTTGCGTTT
NcABCA-04	AGCTATTGGACCCCGAGACC	CGAGCCTTAGATCGTTGCGTT
NcABCA-05	TTCTTCTACGCAATGGATCAAGTC	CGGCTAAACCCATCATACGC
NcABCA-06	TCCCGTCTTGCTGACTCTGTT	CTTATGAAGCCGTTCGTGGG
NcABCA-08	TGATGAGGTCCTACTGGTTCCC	CCTGAGTTTGGAGGAGGCTTT
β-actin	TACGACCAGAAGCGTACAGC	CCAACCGTGAAAAGATGACC

RNAi treatment and interference efficiency detection

The cDNA sequences of *NcABCA-02* and *NcABCA-05* were submitted to Sangon Biotech for the synthesis of siRNA, and the siRNA sequence information is shown in Table 1. The RNAi experiment was conducted as follows: 300 female *N. californicus* that had completed molting on the same day were selected for RNAi treatment through feeding. A solution was prepared by mixing 0.125 g sucrose and 0.075 g cochineal red dye with 500 μL of distilled water to create a 25% sucrose dye solution. Then, 18 μL of 1300 ng/μL siRNA solution and 2 μL of the 25% sucrose dye solution were mixed to create an approximately 1300 ng/μL siRNA feeding solution. For feeding, female *N. californicus* that had undergone starvation treatment were placed on a feeding device (a Petri dish lined with absorbent cotton, on which a plastic film was placed and moistened with water). The siRNA feeding solution was dropped onto the plastic film for the mites to consume. Each treatment was set up with an NC negative control, prepared by mixing 18 μL of *Aequorea victoria* (Murbach) *GFP* gene solution (1300 ng/μL) with 2 μL of the sucrose staining solution (Zhu *et al.* 2022). Each treatment was repeated three times. *N. californicus* that ingested siRNA exhibited a red coloration, serving as a visual marker for successful siRNA uptake. The mites were maintained at 25 ±1 °C, 60% RH, and a 16L:8D photoperiod for feeding before subsequent experiments. After 48 hours, total RNA was extracted from siRNA-fed adult female *N. californicus* and subjected to qPCR to assess gene silencing.

Effect of RNAi treatment on the predatory capacity of adult female N. californicus

Newly molted adult females of *N. californicus* were fed with synthesized *siNcABCA-02* and *siNcABCA-05* to evaluate RNAi effects on their predatory behavior. The experiment was conducted in 6-well plates containing absorbent cotton pads, each overlaid with a 2–3 cm *Canavalia gladiata* (Jacq.) DC. leaf disc infested with 10 adult female *T. urticae*. *Neoseiulus californicus* females that had been siRNA-fed for 48 hours followed by 24-hour starvation were introduced into each well. Prey consumption was recorded after 24 hours. For each type of treatment, two controls were set up. Controls included: (1) blank control (CK) - 18 μL distilled water + 2 μL sucrose-dye solution; (2) negative control (NC) - 18 μL of 1300 ng/μL NC siRNA + 2 μL sucrose-dye solution. Each treatment used 10 mites with three biological replicates.

Effect of RNAi treatment on the reproductive capacity of adult female N. californicus

To investigate the function of individual ABC transporters in *N. californicus*, newly molted adult females were fed with synthesized *siNcABCA-02* and *siNcABCA-05* to examine the effect of RNAi on their reproductive capacity. The experimental setup consisted of Petri dishes lined with absorbent cotton and a 2–3 cm plastic film floating on water. Approximately 300 adult female *N. californicus* were allowed to oviposit for 12 hours in these dishes. The eggs were then transferred to clean Petri dishes for hatching, and the hatched individuals were reared separately until reaching adulthood. Synchronously developed adult females were selected, starved for 24 hours, and then fed with siRNA. After 48 hours, these females were paired with untreated, synchronously developed adult males. The time from pairing to first oviposition was recorded at 12-hour intervals. Following the initial oviposition recording, all adult females and males were transferred to new Petri dishes. Daily total egg production and mortality were documented. All eggs laid daily in each Petri dish were carefully transferred to clean dishes for a 5-day incubation period, after which the egg hatching rate was recorded. Each treatment was set with a blank control (CK) and a negative control (NC). Each treatment group consisted of 20 individuals with three biological replicates.

Statistical analysis

Gene RT-qPCR data were plotted using GraphPad Prism 10, and their significance was evaluated using one-way analysis of variance. The significance of RNAi results was evaluated using the independent sample t-test. When analyzing the effects of RNAi treatment on the predation quantity, pre-oviposition, oviposition quantity, lifespan and egg hatching rate of female mites, the normality test was first conducted using GraphPad Prism 10 One-way analysis of variance is used for data that follow a normal distribution; otherwise, non-parametric tests are used. Plot using GraphPad Prism 10.

RESULTS

Identification of the ABC transporter protein gene of N. californicus

A total of 49 ABC transporter genes were identified from the transcriptome data of *N. californicus* (Table 2). These genes were classified into eight subfamilies: ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, ABCG, and ABCH. The amino acid lengths of the identified ABC transporters ranged from 574 to 3770, with molecular weights ranging from 63,721.57 to 421,016.86. The isoelectric points (pI) varied between 5.51 and 9.55, with most of the proteins having pI values greater than 7, indicating that the majority of these proteins are basic in nature. Additionally, the subcellular localization of the ABC transporters was predicted. Except for *NcABCD-01*, *NcABCD-02*, and *NcABCE-02*, which were located in the mitochondria, *NcABCF-01*, *NcABCF-02*, and *NcABCF-02* were found in the nucleus, and *NcABCE-01* was located in the cytoplasm. The remaining ABC transporters were predicted to be localized in the cell membrane.

Table 2. Members of the ABC transporter protein family identified from transcriptome data of *Neoseiulus californicus* and their physicochemical properties.

Gene ID	Name	Amino acid number	Isoelectric point	Subcellular structural localization
TRINITY_DN6319_c0_g1	*NcABCA-01*	1727	7.44	Plas
TRINITY_DN12099_c0_g1	*NcABCA-02*	1674	7.51	Plas
TRINITY_DN7855_c1_g1	*NcABCA-03*	1675	6.25	Plas
TRINITY_DN12612_c1_g3	*NcABCA-04*	2286	8.03	Plas
TRINITY_DN1004_c0_g1	*NcABCA-05*	1077	7.98	Plas
TRINITY_DN1900_c0_g3	*NcABCA-06*	1720	5.70	Plas
TRINITY_DN1959_c0_g1	*NcABCA-07*	3770	5.76	Plas
TRINITY_DN2228_c0_g1	*NcABCA-08*	1776	7.48	Plas
TRINITY_DN9111_c1_g1	*NcABCB-01*	1516	8.62	Plas
TRINITY_DN13872_c0_g1	*NcABCB-02*	630	9.54	Plas

Table 2. Continued.

Gene ID	Name	Amino acid number	Isoelectric point	Subcellular structural localization
TRINITY_DN1708_c0_g3	*NcABCB-03*	836	8.36	Plas
TRINITY_DN10766_c0_g1	*NcABCB-04*	647	7.23	Plas
TRINITY_DN2632_c0_g1	*NcABCB-05*	684	9.55	Plas
TRINITY_DN280_c0_g1	*NcABCB-06*	685	8.98	Plas
TRINITY_DN5902_c0_g1	*NcABCC-01*	1044	6.81	Plas
TRINITY_DN6080_c0_g2	*NcABCC-02*	1263	8.87	Plas
TRINITY_DN6356_c2_g1	*NcABCC-03*	1483	6.64	Plas
TRINITY_DN6608_c0_g2	*NcABCC-04*	582	8.37	Plas
TRINITY_DN729_c0_g1	*NcABCC-05*	1646	7.39	Plas
TRINITY_DN8618_c0_g3	*NcABCC-06*	1335	5.89	Plas
TRINITY_DN1299_c0_g1	*NcABCC-07*	1483	7.17	Plas
TRINITY_DN129_c1_g1	*NcABCC-08*	1455	8.53	Plas
TRINITY_DN10682_c0_g1	*NcABCC-09*	596	6.61	Plas
TRINITY_DN2090_c0_g1	*NcABCC-10*	1453	6.41	Plas
TRINITY_DN220_c1_g1	*NcABCC-11*	1510	6.90	Plas
TRINITY_DN2425_c0_g1	*NcABCC-12*	1563	6.76	Plas
TRINITY_DN2448_c0_g1	*NcABCC-13*	1335	5.85	Plas
TRINITY_DN245_c0_g1	*NcABCC-14*	1320	6.03	Plas
TRINITY_DN2555_c0_g2	*NcABCC-15*	1471	8.82	Plas
TRINITY_DN2845_c0_g1	*NcABCC-16*	661	6.39	Plas
TRINITY_DN3754_c0_g1	*NcABCC-17*	1453	6.52	Plas
TRINITY_DN4854_c0_g1	*NcABCC-18*	1299	8.37	Plas
TRINITY_DN49_c0_g1	*NcABCC-19*	808	5.82	Plas
TRINITY_DN49_c0_g2	*NcABCC-20*	1519	8.63	Plas
TRINITY_DN7922_c0_g1	*NcABCD-01*	661	9.30	Mito
TRINITY_DN9928_c0_g1	*NcABCD-02*	758	9.18	Mito
TRINITY_DN12907_c1_g1	*NcABCD-03*	574	7.82	Plas
TRINITY_DN6204_c0_g3	*NcABCE-01*	608	8.64	Cyto
TRINITY_DN6410_c0_g3	*NcABCE-02*	597	9.04	Mito
TRINITY_DN6923_c0_g2	*NcABCF-01*	704	5.51	Cyto
TRINITY_DN220_c1_g4	*NcABCF-02*	680	7.61	Nucl
TRINITY_DN4876_c0_g1	*NcABCF-03*	770	5.91	Nucl
TRINITY_DN12792_c0_g5	*NcABCG-01*	580	9.22	Plas
TRINITY_DN10633_c0_g2	*NcABCG-02*	667	8.21	Plas
TRINITY_DN14882_c0_g1	*NcABCH-01*	830	6.11	Plas
TRINITY_DN15421_c0_g1	*NcABCH-02*	637	5.96	Plas
TRINITY_DN25_c0_g1	*NcABCH-03*	793	6.91	Plas
TRINITY_DN25_c0_g2	*NcABCH-04*	771	8.77	Plas
TRINITY_DN25_c0_g3	*NcABCH-05*	766	5.95	Plas

Conservation motifs and domain analysis of the ABC transporter gene of N. californicus

Conserved motifs of ABC transporters in *N. californicus* were identified (Fig. 1), revealing 10 distinct

conserved motifs. Genes within the same family exhibited similar conserved motifs, with the majority of the ABCC family containing the highest number of conserved motifs. Motifs 1 and 9 were present in most ABC transporter family members, while motifs 6, 8, and 10 were predominantly distributed in the ABCC family.

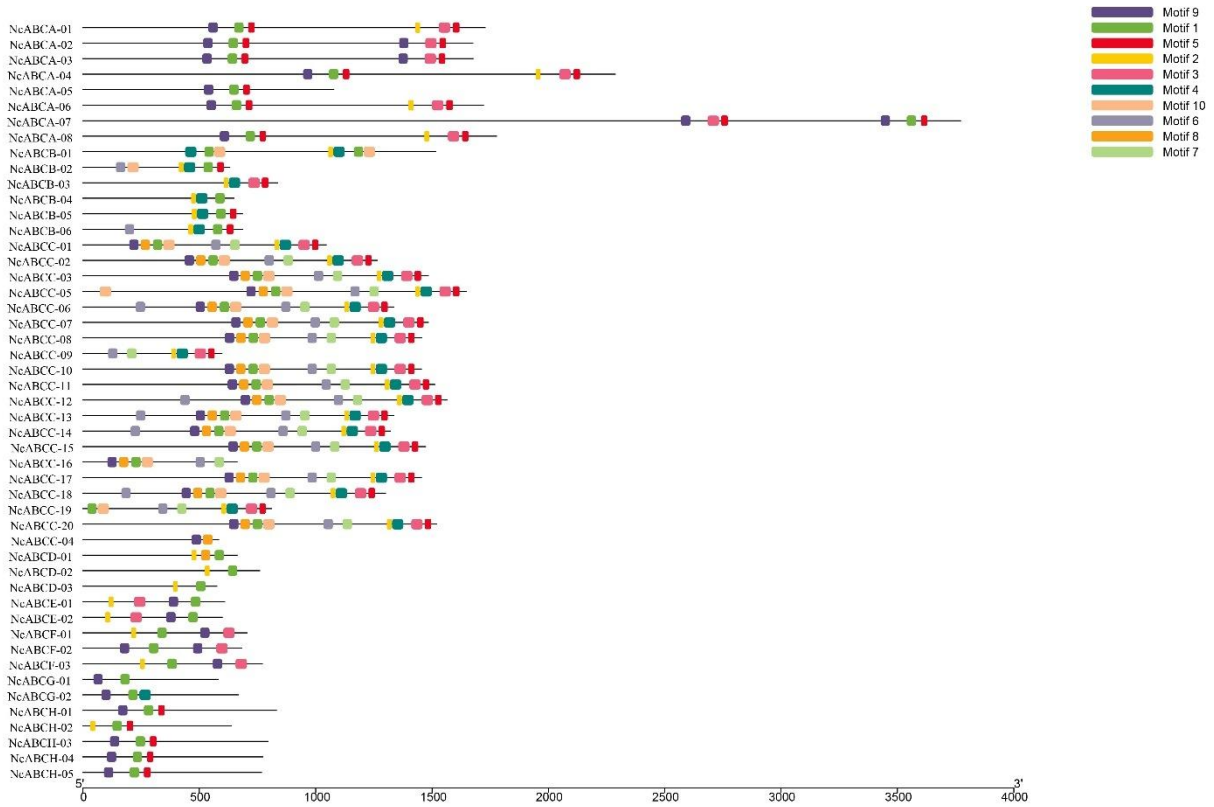


Figure 1. Conserved motifs of ABC transporter genes in *N. californicus*. The letters and numbers on the left indicate gene names, while the right side shows amino acid motifs (numbered 1–10) within the ABC transporter proteins, displayed in 10 colored boxes. The black lines represent the lengths of the amino acid sequences.

The structural domains of ABC transporters were examined, with results shown in Figure 2. All identified ABC transporters contain at least one conserved NBD. The A family includes 7 full transporter genes and 1 half-transporter gene. The B family contains 1 full transporter gene and 5 half-transporter genes. The C family, in addition to 16 full transporter genes and 2 half-transporter genes, also includes genes with 1 TMD and 2 NBDs, as well as genes with 2 TMDs and 1 NBD. The D family consists of 3 half-transporters. Both the G and H families each contain 1 TMD and 1 NBD, with genes in these families exhibiting a typical inverted domain arrangement. The E and F families only contain 2 NBD domains without TMD domains.

Phylogenetic analysis of the ABC transporter system in N. californicus

An evolutionary tree was constructed based on the 49 identified ABC transporter protein genes of *N. californicus*, as well as downloaded protein files of *D. melanogaster*, *H. sapiens*, *D. pulex*, and downloaded *T. urticae*. The results are shown in Figure 3. The 49 ABC transporter genes were ultimately classified into eight subfamilies (ABCA–H). Among them, subfamily C contained the largest number of members, with 20 genes, while subfamily G had relatively fewer members compared to other species, with only 2 genes. The remaining subfamilies (A, B, D, E, F, and H) contained 8, 6, 3, 2, 3, and 5 members, respectively. The phylogenetic analysis indicated a high degree of homology between the ABC transporter families of *N. californicus* and *T. urticae*. Notably, all ABCA subfamily genes from both species formed distinct clusters with strong bootstrap support, suggesting that these genes are orthologous.

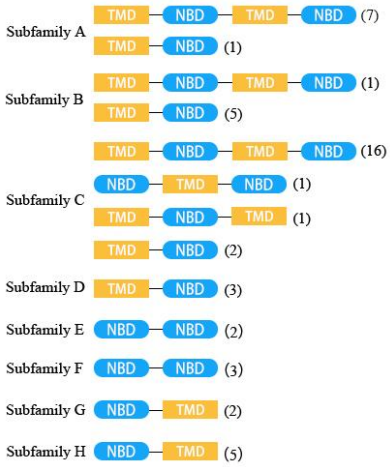


Figure 2. Conserved domains of ABC transporter genes in *Neoseiulus californicus*. The letters on the left represent the names of ABC subfamilies, and the numbers in parentheses on the right indicate the number of members in each subfamily sharing the same domain architecture. Each ABC transporter amino acid sequence was submitted to the InterPro program for the prediction of transmembrane domains (TMDs) and nucleotide-binding domains (NBDs). Yellow rectangles represent TMDs, and blue ellipses represent NBDs.

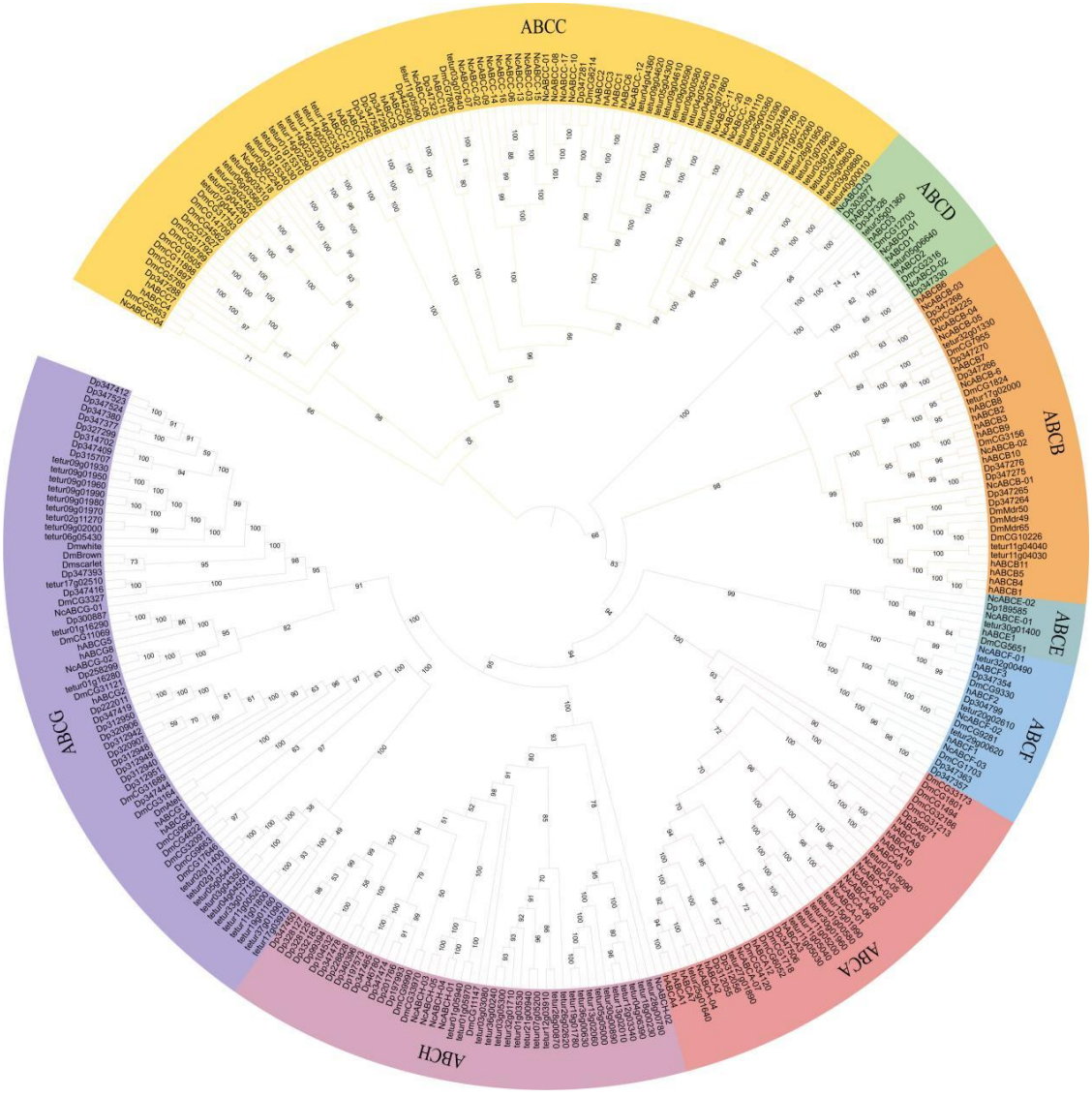


Figure 3. Phylogenetic analysis of ABC subfamilies in *N. californicus* and other species. The numbers at the branch nodes indicate posterior probability support values. The species and their abbreviations are as follows: tetur represents *T. urticae*; h represents *H. sapiens*; Nc represents *N. californicus*; Dm represents *D. melanogaster*; Dp represents *D. pulex*.

Expression analysis of genes in the ABCA subfamily of N. californicus

Expression levels of the ABCA subfamily genes in *N. californicus* were experimentally analyzed across four developmental stages: larva, nymph, adult female, and adult male (Fig. 4). Except for *NcABCA-07*, all other ABCA subfamily genes were broadly expressed across the four stages. *NcABCA-01*, *NcABCA-03*, and *NcABCA-08* exhibited higher expression levels in larvae, while the remaining genes showed relatively higher expression in nymphs. Compared to other genes, *NcABCA-02* and *NcABCA-05* had elevated expression in adult females, and both genes shared similar expression patterns across the different life stages. Notably, the expression of all ABCA genes was relatively low in adult males.

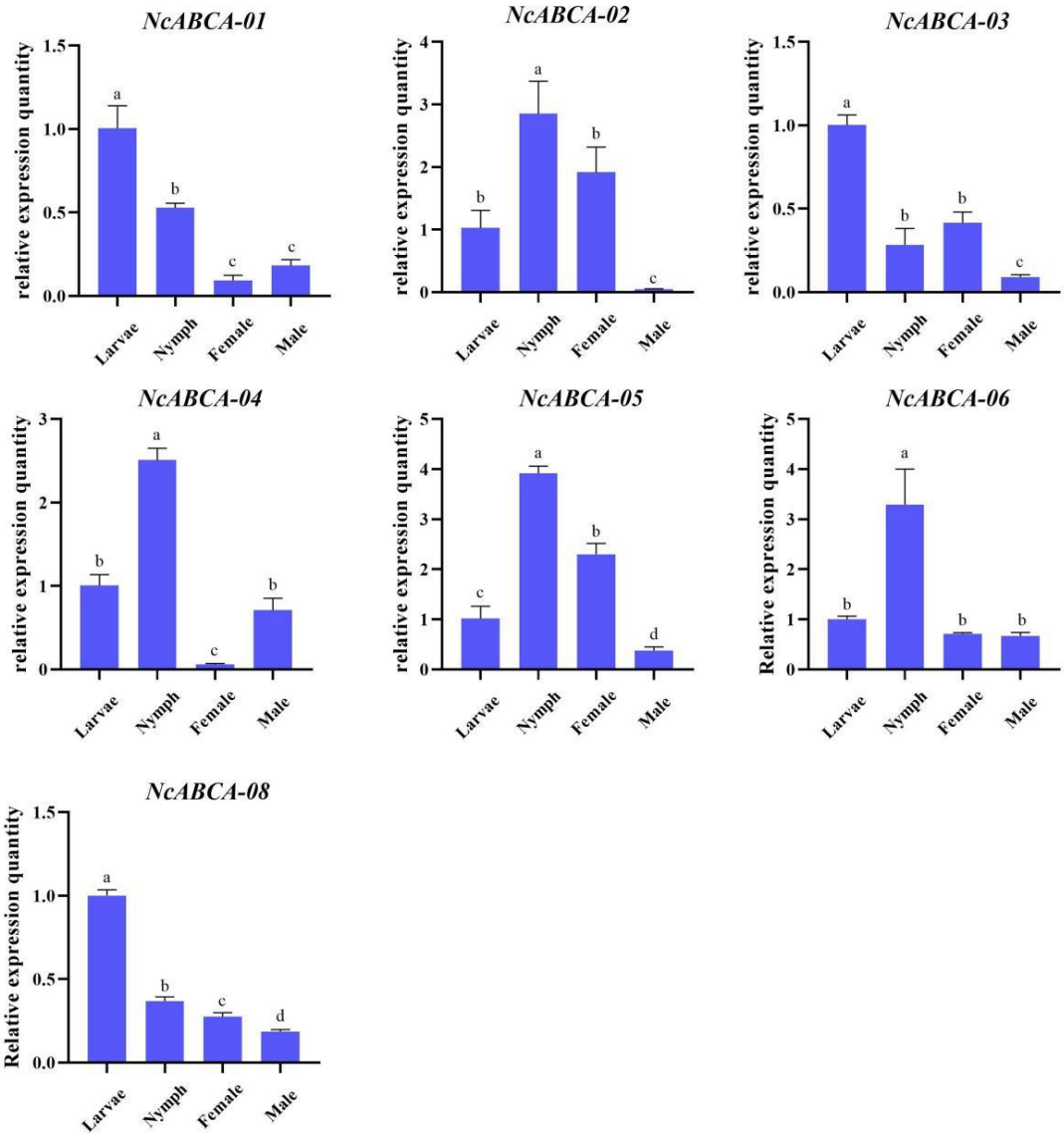


Figure 4. Expression levels of the ABCA subfamily genes in larval, nymphy, adult female, and adult male stages of *Neoseiulus californicus* were analyzed by RT-qPCR, using β-actin as the internal reference gene. Statistical significance was assessed using one-way ANOVA in GraphPad Prism 10, and the results were visualized accordingly. Bars sharing the same letter indicate no significant difference in expression levels ($p < 0.05$).

RNA interference efficiency detection

After 48 hours of siRNA interference (Fig. 5). RT-qPCR was performed to assess gene interference efficiency. The expression levels of *NcABCA-02* and *NcABCA-05* were reduced by 63.69% ($p = 0.0003$) and 70.55% ($p = 0.0011$), respectively, compared to the control group.

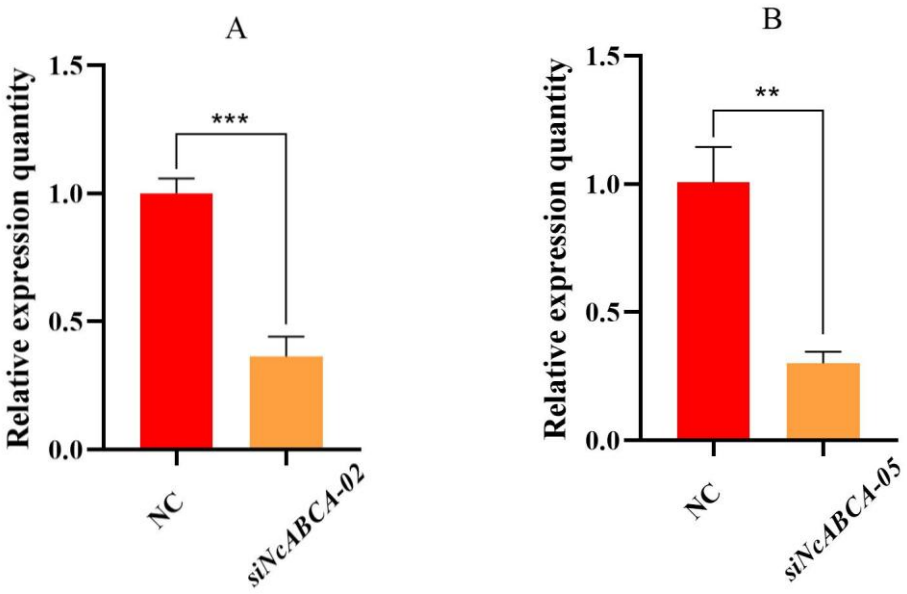


Figure 5. Gene *interference* efficiency of *siNcABCA-02* (**A**, $p = 0.0003$) and *siNcABCA-05* (**B**, $p = 0.0011$) in *Neoseiulus californicus*. Statistical significance was analyzed and plotted using t-test in GraphPad Prism 10. Statistically significant differences were evaluated by t-test (**$p < 0.01$; ***$p < 0.001$).

Effects of NcABCA-02 and NcABCA-05 silencing on survival, predation, and reproduction in adult female mites

Compared to the blank control (CK) and negative control (NC), the mortality of *N. californicus* significantly increased after 48 hours of RNAi treatment, rising from 1.00% and 0.33% to 12.00% and 5.78%, respectively. No obvious morphological abnormalities were observed in any treatment group. In addition, the lifespan of adult females treated with *siNcABCA-02* and *siNcABCA-05* showed no significant change (Fig. 6).

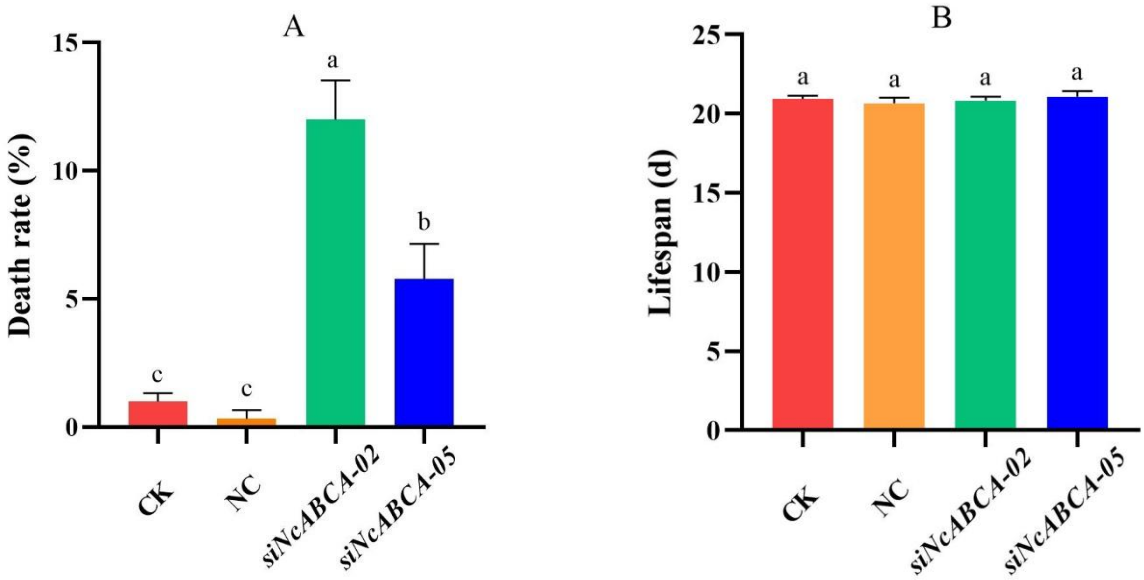
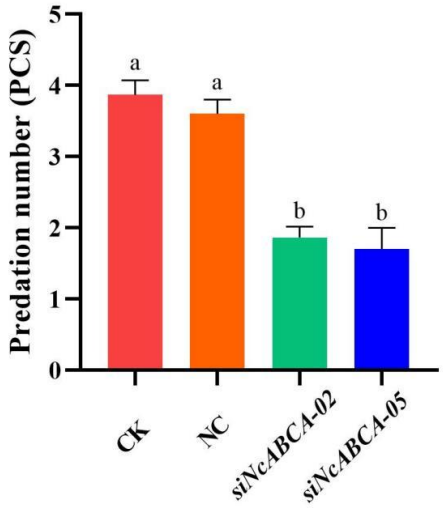


Figure 6. Mortality rate (**A**) and lifespan (**B**) of *Neoseiulus californicus* after feeding on *siNcABCA-02* and *siNcABCA-05*. CK represents the blank control, and NC is the negative control. Statistical significance was evaluated using one-way ANOVA in GraphPad Prism 10, and data were visualized using GraphPad Prism 10. Bars sharing the same letter indicate no significant difference in mortality rate or lifespan ($p < 0.05$).

The average predation rates of female adult mites after silencing *NcABCA-02* and *NcABCA-05* on female adult mites of *T. urticae* were 1.87 and 1.70, respectively. These values were significantly lower than those in the blank control (3.87) and negative control (3.60) groups, with no significant difference between CK and NC groups (Fig. 7).

Figure 7. Average number of adult female *Tetranychus urticae* preyed upon by adult female *Neoseiulus californicus* after *NcABCA-02* and *NcABCA-05* silencing. CK represents the blank control, and NC is the negative control. Statistical significance was assessed using one-way ANOVA in GraphPad Prism 10, and the figure was generated with GraphPad Prism 10. Bars sharing the same letter indicate no significant difference in predation quantity ($p < 0.05$).



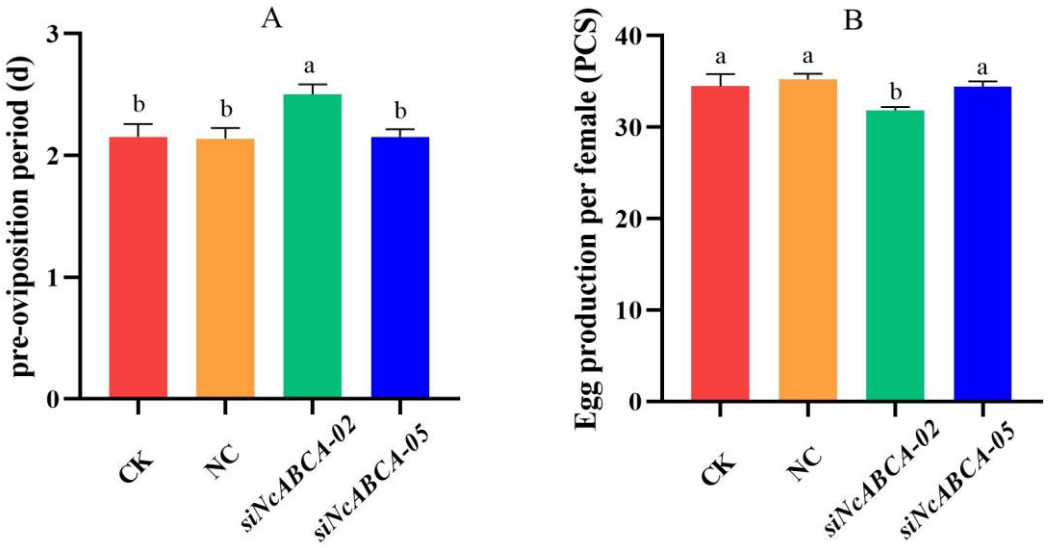


Figure 8. Effects of *NcABCA-02* and *NcABCA-05* silencing on the pre-oviposition period (**A**) and number of eggs laid per female (**B**) in *Neoseiulus californicus*. CK represents the blank control, and NC is the negative control. Statistical analysis was performed using one-way ANOVA in GraphPad Prism 10, and visualization was done with GraphPad Prism 10. Bars with the same letter indicate no significant difference in pre-oviposition period or egg production ($p < 0.05$).

To evaluate the effects of gene silencing on reproductive capacity, the pre-oviposition period (time from mating to the first egg), number of eggs laid per female, and egg hatching rate were measured. The results showed no significant differences between the CK and NC groups in any of these parameters. In the *siNcABCA-02* group, the pre-oviposition period was significantly prolonged, while the other parameters remained unchanged. In the *siNcABCA-05* group, the number of eggs laid per female was significantly reduced, with no changes observed in the pre-oviposition period or lifespan (Fig. 8).

To further examine the effects on egg viability, daily oviposition and egg hatching failure rates over

the first 15 days (beyond which accuracy declines due to a rapid drop in egg production) were analyzed using 20 individuals per group. The hatching failure rate curves (Fig. 9) showed little variation from day 1 to 15 in the CK and NC groups. After treatment with siNcABCA-02 and siNcABCA-05, the unhatched rates were relatively high during the initial 1–3 days, particularly on the first day, reaching 7.71% and 9.85%, respectively, which were significantly higher than those in the CK and NC groups. After this period, the unhatched rates returned to a lower range (Fig. 9).

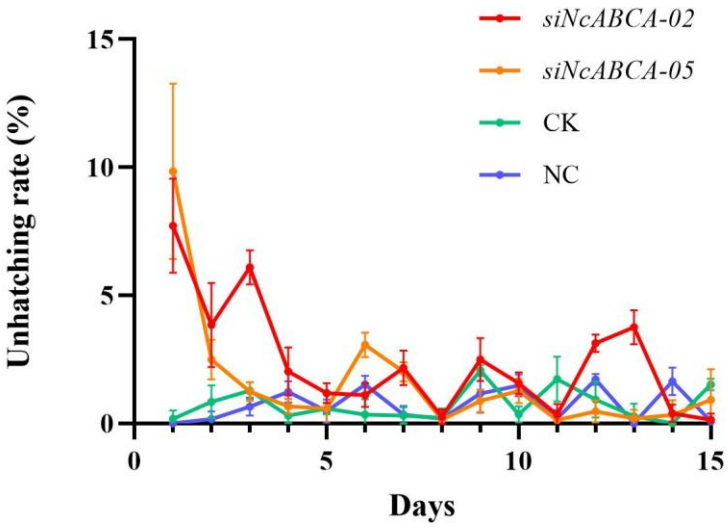


Figure 9. Line graph of daily unhatched egg rates in *Neoseiulus californicus* following RNAi treatment. The x-axis indicates time (days) and the y-axis shows the unhatched rate (individuals). Data are presented as mean ± SEM of three replicates, with the graph generated using GraphPad Prism 10.

DISCUSSION

This study identified 49 ABC transporter protein members from the transcriptome data of *N. californicus*. The nomenclature of each gene was referenced from previous studies on *T. urticae* as well as the conserved motifs and phylogenetic analyses of this study (Dermauw *et al.* 2013). Among these, *NcABCA-07* was the longest ABC transporter gene, encoding 3770 amino acids, while *NcABCD-03* was the shortest, encoding only 574 amino acids. The ABCA, ABCB, and ABCC subfamilies contained both full and half transporters, whereas other subfamilies lacked full transporter genes. These results align with findings in *Anopheles sinensis* Wiedemann and *Tigriopus japonicus* Mori (Jeong *et al.* 2014; Wu *et al.* 2019). In contrast, *T. urticae* was found to possess 103 ABC transporter genes, with all members of the ABCA and ABCC subfamilies being full transporters, while other subfamily structures remained consistent with *N. californicus* (Dermauw *et al.* 2013). Phylogenetic analysis confirmed that the ABC transporters of *N. californicus* could be classified into eight subfamilies (ABCA-H), containing 8, 6, 20, 3, 2, 3, 2, and 5 members, respectively. In many organisms, the ABCG subfamily is notably large—for example, *T. urticae* (23 genes), *Diaphorina citri* Kuwayama (15 genes), *Bactrocera dorsalis* (Hendel) (15 genes), and *Anopheles gambiae* Giles (21 genes) (Dermauw *et al.* 2013; Liu *et al.* 2011; Jeong *et al.* 2014; Xiao *et al.* 2018; Liu *et al.* 2019). However, this study identified only two ABCG members in *N. californicus*.

Homology analysis revealed that *N. californicus* ABC transporter genes are highly similar to those of *T. urticae*, especially in the ABCA subfamily, where all genes from both species clustered into a well-supported branch, suggesting a common ancestral origin. Phylogenetic results for *NcABCA-02* and *NcABCA-05* suggest they may have originated from a recent gene duplication event. Homologous genes across species often retain conserved physiological functions. Although ABC transporters vary in function among organisms, they frequently exhibit functional similarities (Deng *et al.* 2024; Wang *et al.* 2024). For instance, *ABCC2* mediates resistance to Cry1Ac in *P. xylostella* and *Spodoptera exigua* (Hübner) (Park *et al.* 2014; Guo *et al.* 2019), and knockout of *ABCB1* increases sensitivity to emamectin benzoate

in *S. exigua* and *Spodoptera frugiperda* (Smith) (Zuo *et al.* 2018; Li *et al.* 2022). In mammals, *D. melanogaster*, and other arthropods, ABC transporters are widely studied and are involved in organ development (Akiyama *et al.* 2005; Akasaka *et al.* 2006; Wenzel *et al.* 2007; Fahrenbach *et al.* 2014; De Franco *et al.* 2020), nutrient transport (Ewart and Howells, 1998; Mackenzie *et al.* 1999; Lu *et al.* 2001; Choi *et al.* 2019), and xenobiotic transport (Tarnay *et al.* 2004; Vache *et al.* 2007; Castanys-Muñoz *et al.* 2008; Sun *et al.* 2017; Chen *et al.* 2024), with functionally similar genes found across different subfamilies. Therefore, the known functions of homologous genes provide important clues for functional inference in target organisms.

RT-qPCR results showed that, except for *NcABCA-07*, all ABCA subfamily genes were broadly expressed in larvae, nymphs, adult females, and adult males of *N. californicus*. The failure to detect *NcABCA-07* may be due to low expression levels or tissue-specific expression. Gene expression varied by developmental stage, suggesting that different genes are involved in distinct life processes. The low or specific expression of *NcABCA-07* warrants further investigation in different tissues or under various physiological conditions. Notably, *NcABCA-02* and *NcABCA-05* exhibited similar expression patterns, with highest levels in nymphs, followed by adult females, larvae, and adult males. Other ABCA genes showed peak expression in larvae, implying roles in early development. In contrast, some species exhibit higher ABC transporter expression in pupae and adults than in larvae (Bretschneider *et al.* 2016; Jin *et al.* 2019; Wang *et al.* 2024).

This study represents the first systematic identification of the ABC transporter family in predatory mites and the validation of their physiological functions through RNAi. By using RNA interference to knockdown the expression of two genes, *NcABCA-02* and *NcABCA-05*, the study found that silencing these genes led to increased mortality in adult mites. This result is consistent with the reported functions of ABCA family genes in *Tribolium castaneum* (Herbst), although the mortality rate observed in this study was lower than the 30% reported by Broehan *et al* (2013). The study also revealed that silencing both genes significantly reduced the daily predation rate of adult female *N. californicus* on adult female *T. urticae* and led to a short-term decrease in egg hatching rate. Additionally, knockdown of *NcABCA-02* expression prolonged the pre-oviposition period in adult female *N. californicus*. These findings are the first reported in the study of the ABCA subfamily. The functional similarities between the two genes align with their high homology and consistent gene expression patterns. Since siRNA can degrade and become ineffective in the diet, gut, or insect tissues (Kumar *et al.* 2009), the transient reduction in hatching rate observed in this study is understandable.

In this study, silencing *NcABCA-02* and *NcABCA-05* affected survival, predation, and reproduction in *N. californicus*, though with moderate lethality and a recovery in egg hatching rate over time. Functional effects also differed between the two genes. This study relied on transcriptomic data for gene identification; genomic data may yield more comprehensive results. Only two ABCA genes were functionally validated here, and other subfamilies remain unexplored. Future research should aim to conduct detailed functional analyses of these and other ABC genes—such as investigating their tissue-specific expression, localization, and roles in pesticide resistance and environmental stress tolerance, including high temperatures, in *N. californicus*.

CONCLUSIONS

This study successfully identified all 49 members of the ABC gene family and their subfamily classifications in *N. californicus*. Notably, it represents the first application of RNAi technology in predatory mites to functionally validate the critical roles of two ABCA subfamily genes (*NcABCA-02* and *NcABCA-05*) in regulating adult female survival, predatory efficiency, and reproductive performance (including egg hatchability and pre-oviposition period). Furthermore, the research reveals novel functions of ABC subfamily genes in modulating both predatory capacity and reproductive fitness. These findings significantly advance our understanding of fundamental physiological processes and adaptive mechanisms in beneficial arthropods. More importantly, the study provides crucial new insights for molecular-level investigation of key biological characteristics in predatory mites, particularly their exceptional predation efficiency and environmental stress resistance.

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Author contributions: Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing; J.X.L.: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft; Z.H.Z.: Investigation, Methodology, Writing – review & editing; W.Q.X.: Methodology, Resources, Supervision, Writing – review & editing; Q.Y.L.: Investigation, Methodology, Writing – review & editing; H.J.W.: Methodology, Resources, Supervision, Writing – review & editing; Q.L.: Methodology, Resources, Supervision, Writing – review & editing; S.R.J.: Methodology, Resources, Supervision, Writing – review & editing; C.X.J.: Conceptualization, Data curation, Methodology, Resources, Supervision, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Data availability: Data will be made available upon request from the authors.

Ethics approval: This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

Conflict of interest: The authors declare no conflict of interest.

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شناسایی ژن‌های ناقل ABC در *Neoseiulus californicus* (Acari: Phytoseiidae) و تجزیه و تحلیل عملکردی ژن‌های کلیدی در زیرخانوادهٔ ABCA

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چکیده

هرنای (McGregor) *Neoseiulus californicus* گونه مهم شکارگر است که با طیف گستردهٔ طعمه و سازگاری قوی خود مشخص می‌شود. ناقل‌های کاست اتصال ATP (ABC) یک ابرخانواده پروتئینی بزرگ‌اند که در موجودات پروکاریوتی و یوکاریوتی وجود دارند و بیشتر در انتقال مواد بیگانه‌زیست و حفظ هموستاز سلولی نقش دارند. با این حال، پژوهش‌ها در مورد این خانواده پروتئینی در کنه‌های شکارگرتا حد زیادی ناشناخته مانده است. در این مطالعه، در مجموع ۴۹ ژن ناقل ABC از طریق تجزیه و تحلیل جامع رونوشت *N. californicus* شناسایی شدند. تجزیه و تحلیل تبارشناختی این ناقل‌ها را در هشت زیرخانوادهٔ مجزا (ABCA-H) طبقه‌بندی کرد. پروفایل بیان ژن‌های زیرخانوادهٔ ABCA در مراحل مختلف رشد، الگوهای بیان فراگیر را برای همه اعضا به جز NcABCA-07 نشان داد. توصیف عملکردی دو ژن با بیان بالا در ماده‌های بالغ، NcABCA-02 و NcABCA-05، از طریق تداخل RNA انجام شد. خاموش کردن NcABCA-02 و NcABCA-05 میزان مرگ و میر کنه‌های ماده بالغ را به ترتیب از ۱/۰۰٪ به ۱۲/۰۰٪ و ۵/۷۸٪ افزایش داد. افزون بر این، پس از خاموش کردن NcABCA-02 و NcABCA-05، راندمان شکار کردن کنه‌های ماده بالغ *Tetranychus urticae* Koch به مقدار زیادی از ۳/۸۷ طعمه به ۱/۸۷ (کاهش ۵۱/۷٪) و ۱/۷۰ (کاهش ۵۶/۱٪) کاهش یافت. همچنین، کاهش میزان تفریخ تخم در افراد خاموش مشاهده شد. این نتایج نشان دهنده نقش حیاتی NcABCA-02 و NcABCA-05 در حفظ زندمانی، ظرفیت شکارگری و تناسب اندام تولید مثلی در *N. californicus* است. این یافته‌ها بینش‌های ارزشمندی در مورد مکانیسم‌های فیزیولوژیکی کنه‌های شکارگر ارایه می‌دهند و یک چارچوب نظری برای افزایش اثربخشی آنها در کاربرد مهار زیستی ایجاد می‌کنند.

واژگان کلیدی: پروتئین‌های ناقل ABC، زیرخانواده ABCA، شناسایی خانواده ژن، *Neoseiulus californicus*، RNAi

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