



Molecular cloning and expression profile of an ATP-binding cassette (ABC) transporter gene from the hemipteran insect *Nilaparvata lugens*

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ABSTRACT. The ATP-binding cassette (ABC) transporters belong to a large superfamily of proteins that have important physiological functions in all living organisms. In insects, ABC transporters have important functions in the transport of molecules, and are also involved in insecticide resistance, metabolism, and development. In this study, the *Nilaparvata lugens* Stal (Hemiptera: Delphacidae) ABCG (*NIABCG*) gene was identified and characterized. The complete mRNA sequence of *NIABCG* was 2608-bp long, with an open reading frame of 2064 bp encoding a protein comprised of 687 amino acids. The conserved regions include three *N*-glycosylation and 34 phosphorylation sites, as well as seven transmembrane domains. The amino acid identity with the closely related species *Acyrtosiphon pisum* was 42.8%. Developmental expression analysis using quantitative real-time reverse

transcriptase PCR suggested that the *NLABCG* transcript was expressed at all developmental stages of *N. lugens*. The lowest expression of *NLABCG* was in the 1st instar, and levels increased with larval growth. The transcript profiles of *NLABCG* were analyzed in various tissues from a 5th instar nymph, and the highest expression was observed in the midgut. These results suggest that the sequence, characteristics, and expression of *NLABCG* are highly conserved, and basic information is provided for its functional analysis.

Key words: *Nilaparvata lugens*; *NLABCG*; Gene expression; Gene structure; Tissue distribution

INTRODUCTION

The ATP-binding cassette (ABC) transporters form one of the largest protein super-families of membrane proteins and are present in all organisms from bacteria to human (Dassa and Bouige, 2001; Dean et al., 2001a,b; Dean and Annilo, 2005). Coupling ATP hydrolysis to ADP to generate energy, ABC transporters move a wide variety of substrates across biological membranes, including inorganic ions, sugars, amino acids, lipids, lipopolysaccharides, peptides, metals, xenobiotics, and chemotherapeutic drugs (Dawson and Locher, 2006; Hollenstein et al., 2007a).

ABC transporters share a highly conserved structure consisting of four functional units: two nucleotide-binding domains (NBDs), which bind and hydrolyze ATP, and two transmembrane domains (TMDs), which are involved in substrate translocation (Rees et al., 2009). Many ABC proteins comprise all four domains in one polypeptide (two NBDs and two TMDs) and are so-called “full transporters”. Other ABC proteins are “half transporters”, consisting of one TMD and one NBD domain, and constitute a functional pump by forming homo- or heterodimers (Hollenstein et al., 2007b). According to their primary sequence, domain structures, and organization, ABC transporters can be divided into seven (A-G) or eight (A-H) subfamilies.

Based on their functions, the ABC transporters can be classified as exporters, importers, and non-transport proteins (Saurin et al., 1999). In insects, it has been shown that ABC transporters have functions that affect metabolism, development, and possibly also insecticide resistance (Dow and Davies, 2006; Vache et al., 2007; Borycz et al., 2008; Ricardo and Lehmann, 2009).

Due to the importance of ABC transporters, the completion of insect genome sequencing projects has generated complete inventories of ABC transporters for *Anopheles gambiae* (Roth et al., 2003), *Bombyx mori* (Liu et al., 2011), *Tribolium castaneum* (Broehan et al., 2013), and *Tetranychus urticae* (Dermauw et al., 2013). Some ABC transporters have specific functions that are well documented in arthropods. *Drosophila melanogaster* white is a member of the ABCG subfamily and is involved in the uptake of pigment precursors in the developing eye (Mackenzie et al., 1999). Its orthologs in *B. mori* (Bmwh3) and *T. castaneum* (TcABCG-9B) have similar functions, and *w-3^{oe}* *B. mori* mutants and adult beetles injected with *TcABCG-9B* dsRNA have white eyes (Komoto et al., 2009; Broehan et al., 2013). However, a functional analysis of genes encoding ABC transporters has not yet been performed for a hemipteran insect species. The brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae), is the most destructive insect pest of rice crops and a well-established UNKA (BPH) EST database (<http://bphest.dna.affrc.go.jp/>) provides the opportunity for gene func-

tion to be analyzed.

In this study, we report that the full-length cDNA sequence of the *NIABCG* gene has been isolated from *N. lugens*. We report the structures of the *NIABCG* gene and its mRNA expression profile in different *N. lugens* instars. This provides basic information for the further study of the ABC transporter genes and their functions in *N. lugens*.

MATERIAL AND METHODS

Insect material

The rice brown planthopper (BPH; *N. lugens*) was obtained from rice fields in Zhejiang Province, China, and was reared on 2-3-month old rice plants of the susceptible variety TN1, under controlled environmental conditions (70-80% relative humidity, $25^{\circ} \pm 2^{\circ}\text{C}$, 16 h light/8 h dark).

Cloning of *NIABCG*

For the ABC Transporter gene, a TBLASTN search of the BPH expressed sequence tags (ESTs) database, using the *T. castaneum* ABC transporter protein (GenBank: XP_971735.1) as a query, revealed an *ABC Transporter* EST (C_NLEA0656). To obtain the full-length sequence of each truncated sequence from *N. lugens*, 5' and 3' RACE amplifications were performed using a 5' -Full RACE Kit and 3'-Full RACE Core Set Ver. 2.0 (TaKaRa) following the manufacturer protocol. For 5' -RACE, gene-specific primers were designed based on sequencing data, and an external reverse and nested primer (Table 1) were used. For 3'-RACE, the cDNA was then amplified by nested PCR with the external forward primer and nested forward primer (Table 1) with ExTaq DNA polymerase (TaKaRa). For the first round of PCR, the following cycling conditions were used: 94°C for 3 min, followed by 20 cycles of 94°C for 30 s, annealing was performed at 55°C for 30 s, and 72°C for 2 min, followed by a final elongation step of 72°C for 10 min. Nested PCR amplification was performed under the same conditions for 25 cycles. Purified PCR products were ligated to pGEM[®]-T Easy Vector Systems (Promega) and 3-5 positive colonies were sequenced.

Table 1. Primer sequences used in the present study.

Gene name	Primer name	Sequence 5'-3'
NIABCG	5' race_gsp	5' RACE
	5' race_ngsp	CCTCGGAATACTTCCACAT
	Outer Primer	TTCTTGAGGTGCTCGAAGT
	Inner Primer	CATGGCTACATGCTGACAGCCTA
NIABCG	3' race_gsp	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
	3' race_ngsp	3' RACE
	Outer Primer	TTGATGAGAGGCGGAGTTCT
	Inner Primer	ACTTCGAGCACCTCAAGGAA
NIABCG	Forward	TACCGTCGTTCCACTAGTGATTT
	Reverse	CGCGGATCCTCCACTAGTGATTCACTATAGG
β-Actin	Forward	Real-time PCR
	Reverse	CCACAGGAGGTAGCACTGAA
β-Actin	Forward	TCACCAATGAAGCCCTCTGT
	Reverse	TGGACTTCGAGCAGGAAATGG
β-Actin	Forward	ACGTCGCACTTC-ATGATCGAG
	Reverse	

Analysis of sequence structure and amino acids of *NIABCG*

Sequence similarity and analysis of conserved domains were performed using BLAST programs on NCBI. Signal peptide was analyzed by the online software SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP/>). Prediction of transmembrane domains was performed with the online software TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). *N*-glycosylation and phosphorylation sites were analyzed by NetNGlyc1.0 Server (<http://www.cbs.dtu.dk/services/NetNGlyc/>) and NetPhos 2.0 Server (<http://www.cbs.dtu.dk/services/NetPhos/>), respectively.

Sequence alignment and phylogenetic analysis of *NIABCG*

Multiple amino acid sequence alignments and phylogenetic tree construction using the neighbor-joining method were performed using CLUSTALW (Altschul et al., 1990). The bootstrap value and the phylogenetic tree were calculated using Mega 4.0 (Tamura et al., 2007). The bootstrap support was evaluated based on 1000 replicates. Conserved protein motifs were identified using regular expressions in a Perl script. Accession numbers of ABCG used for sequence alignment and phylogenetic analyses were as follows: *N. lugens*: KJ939366; *Acyrtosiphon pisum*: XP_001942931, *Harpegnathos saltator*: EFN84917, *Pediculus humanus corporis*: XP_002422992, *Ceratitis capitata*: XP_004531656, *Bombus terrestris*: XP_003401420, *Nasonia vitripennis*: XP_003426604, *Apis mellifera*: XP_001120768, *Musca domestica*: XP_005175158, *T. castaneum*: EFA09632

Quantitative real-time PCR analysis

To investigate the expression of ABC transporter family members during development tissue was taken from various developmental stages of *N. lugens* insects, which included nymphs from 1st instar to 5th instar, male adults and female adults. The tissue expression pattern of the *N. lugens* ABC transporter family member was surveyed at the 3rd day of the 5th instar larvae of *N. lugens* strains. Midgut, Malpighian tubule, salivary gland, fat body, leg, cuticle, and head were collected from brown planthopper at the 3rd day of the 5th instar. Total RNA was isolated from dissected tissues using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized at 42°C from total RNA using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Fermentas). Expression of selected *N. lugens* genes as well as β -Actin (EU179846) (Liu et al., 2008) was quantified by quantitative RT-PCR, using an RG-6000 rotary analyzer (Corbett Research) and appropriate primers (Table 1) together with 1 mg of cDNA per reaction. β -actin was used as the internal housekeeping gene.

RESULTS

Cloning and characterization of the *NIABCG* gene in *N. lugens*

An ABC transporter gene in *N. lugens* was identified in the UNKA (BPH) EST database (C_NLEA0656). However, it lacked both a 5'UTR and a 3'UTR. To better understand ABC transporter genes in the brown planthopper, we amplified a full-length ABC transporter gene using the RACE strategy. Based on its similarity with other known ABC transporter

genes, the *ABC* transporter gene here was named *NLABCG*.

The full-length cDNA of *NLABCG* is 2608 bp long and contains an open reading frame (ORF) of 2064 bp (GenBank accession no. KJ939366), encoding a protein of 687 amino acids with a calculated molecular mass of 77.1 kDa and an isoelectric point (pI) of 5.67 (Figure 1). The amino acid sequence had a predicted N-terminal signal peptide consisting of 38 amino acids (Figure 2A), three *N*-glycosylation sites (N244, N348, N402) (Figure 2B), 34 phosphorylation sites (Figure 2C) and seven transmembrane domains (TM1, 295V-317G; TM2, 491L-513L; TM3, 533I-555T; TM4, 568L-590S; TM5, 600S-622G; TM6, 629A-647A, and TM7, 657V-679I in *NLABCG*) (Figure 2D).

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1   GAAAAGTAGTCCTTTCTGCTCACTCAATGTGCGGCGTATAGTTACTAGTAAGTCGCCGGCTGCAGTTAGTCTCGCTTGTGACTAGCTA
91  GAGCGATAGTAGACGGGACATTGAGTTAGAGTAGAGTAGACTTGACTAGAGTAGACATTCACTTTGGACTAGAGTAGAGTAGAGTAGAG
181 ATTAGCGAGTGCAATCAAGAAGCTGGTCAACAGATTTCGGAGTACGCCCGCTGCATTATTGATCATTFTTCTAGTGGAGACCATTC
271 ACTCACTGAAAGGGAGACATGGAGCTGAATTGTCCCCAGCCCATGGTGGCTGAGTGGAGCATTCAAGAGATACACTCCAAAAGTCCA
1   M E L N C P Q P M V A V S G A F K R Y T S K S P
361 ATCTTGCTGCGAGGATTTAGTAGACTGTACTACTGGAGTATATACGGACTGCTGGGACCGAGTGGCTGTGGCAAACTACTCTACTC
25  I L L R G F S M T V P T G A I Y G L L G P S G C G K T T L L
451 AACTGTATTGTTGGCAACAGCGGCTGGATAGCGGGCAGTCAAGTTGGGCATCACCACCAAGGAGTGGGATACATGCCACAGGAG
55  N C I V G N R R L D S G Q V T L G I T K R K E L G Y M P Q E
541 GTAGCACTGAACGAGGAATTCAGATAGAGGAGACTTCTTCTTATGGGAACTTTTCGACATGAGCAGAGCGCAAGTAAAAATGCCG
85  V A L N E E F T I E E T F F F Y G K L F D M S R A Q V K M R
631 ATGAAGGAATTTGAGCATTTCGATCTGCCAACAGAGGGCTTCATTGGTGGTAAAGTGGTGGTCAACAGAGAAGATTTCAATCGCA
115 M K E L L T F F D L P T E G F I G E L S G G Q Q R R V S F A
721 GTAGCTTATTACACAATCTCAACTCTCATCTGGATGAACCAACGGTGGTGGTACCCTGCTGCTAGTAGGATATGGGATGGC
145 V A L L H N P Q L L I L D E P T V G V D P V L C S R I W D A
811 CTGTAAAAATGGCTTTAGAAGAAAAGAAAATATTATTACACAACATTACATTGAGGAGGCTCGGCAATGCCAACTAATGGATTG
175 L V K M A L E E K K T I I I T T H Y I E E A R Q C Q L I G L
901 ATGAGAGGGGAGTTCTCTTGTCAAGAGCTCCCTTGACTGATGGCATCTCATAATTGTAACACACTGGAGCAGTGGTCTCGAG
205 M R G G V L L A E E P P L T L M A S H N C N T L E Q V F L E
991 CTAAGTCAAAACAGCAGCAAGCTCAACAAATCAACGAAGATTAAACAGAGAGTATACTGATTAAAAAGATGGCAATCAACAT
235 L S Q K Q H E A S N K S N E D L T E S D T D Y K K M A N Q H
1081 TTTCAACCTCTCTAATATCTAGTCAAATGGAAGAAATCCAGATCTCAGCTCAGTTATTTAAAAATGTCGTATGGATGAAACGAAAT
265 F Q P P L I S S Q M W K K S R F S A Q L F K N V V W M K R N
1171 GTGCCAATAATGATGTTCTCTGCTGTTCTGCTGCTATTCACAAATACTGCTCAGTGTTCGGCAAGACACCGACTGGCTGTGCG
295 V P I M M F L L P F L L F T I Y C S V F G K T P T G L S
1261 CTGGGATGTTAGCGAGGAATGGGCAAGTTGGCTGACTGTCAAGCGAAGTCTACAGAGCAGGCTTCAACTACACCGACTCAACTGCG
325 L G I V S E E L G S L A D C Q A N S T E Q A F N Y T D F N C
1351 TATTTCGACAAACCACTGAGTTGTCTCTACTTCGAGCACTCAAGGAAAACGATTTCAATCTGATAGAGTACAACAGCTGAAACCTGG
355 Y F D K P L S C L Y F E H L K E N D F N L I E Y N K L E T G
1441 CAAACGGCTCTGGAGGGAATAAGGTATGGGCTCTCTGCTTTCACAGAACTACAGATAGCCGCTTGGAGAGAAATGATTACGGC
385 Q T A L E R N K V W G L L H F T R N Y T I A V L E R I D Y G
1531 TTCGACACCGCTGAATCAGTTGTCAGAGATGGCACTGTCGATGTTAGACTOGATATGTCAGATCATGTCATTGGAACCGCTGCAAGG
415 F D T A E S V V R D G T V D V R L D M S D H V I G T S L Q R
1621 ATGCTGCTGACCACCTATAACAACCTCATTGGAGAAGTTTGAAGCATGGAAGTATTGGCAGGCAAGGAAATTCGAATCAAGTAT
445 M L L T T Y N N F I G E V Y E A C G S I A E A G K I P I K Y
1711 AATGAACCAATCTACGGTGAAGTATGCTCTTTTTTATACACCACTGACCTGATGATGTCATTGATGCTTTTATCTGCTATG
475 N E P I Y G G S D P L F S Y T T L P G Y L M S F S F Y L P M
1801 CTCTTCAATCAGGCGCTAATGTTGGAGAACTGAGTGGTCTTATGGAGAGGAAATGATTGCAGGAATGACAATGCTGGAAATGATT
505 L F T S G A I M L E K L S G L M E R K M I A G M T M L E I V
1891 ACCGCTCATGCAATGTCAGATCTTCACTCTCTGTCAACTGGAATCATATTCTACACAGCTTATGGCATTTCGATAATCCAGTT
535 T A H A V V Q I F I L F C Q T G I I F Y T A Y G I F D N P V
1981 GAAGGGACCTACTCTGATTCTCTCACATTCATCAATGAAATGCTGGAATGTTATGGTTTCATGCTCAGTGAATTCATGAC
565 E G D L Y L L I L L T F I N E I A G M C Y G F M L S E I H D
2071 TCAGATACAAATGCTCTTATGCTGGAACGGGAACGATATTAGCACTGTTCACTGACTACAGGCACTCTGTTGGCAATGGAAGGGCACAC
595 S D T N C S Y A G T G T V L A L F M T T G T L W P M E G A H
2161 CCAATCATGCTGACGCAATGGGTTCTTCCAGTGAACGGGCACTCACCAGCTACACAGATAGCACTGCGAGGCTACAGATCTCA
625 P I M R A A M W V L P V N A A V T S Y H S I A L R G Y S I S
2251 AACCCAGTGTCTACAGAGGATTCCTGTCAACACTGGCTGGTGGCCTTCTTTGCTATCGGTGGCCTCTCATCAACAGAGTCAGAAAA
655 N P V V Y R G F L S T L A W C A F F A I G A I L I N R V R K
2341 GCGCTCAGATAGTCAATCGAGACAATTCACATTCACATTTAAACAAGCTGTGATTAGAAAAGCCATGTGACCCCGTACTCAAGTGTG
685 G V R *
2431 AATCATGGAATGTGATGGTTAGTTAGGTTAGGTTCAATGGCAGTCTCCGGAAGGAGTATCTTGAAMAAATGGATCTCTTTGGCACAAGA
2521 AATGAGGTCCTGTTCTCACTGATTAAAGTCTGTAGAGGATATAAATCACTACCTCCATAAAAAAAGCTACAAGTCAAAAAA

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Figure 1. Nucleotide and deduced amino acid sequences of *NLABCG*. Nucleotide numbering begins at the first bp at the 5' end. Amino acid numbering begins at the first methionine. The stop codon is marked by an asterisk. The sequence has been deposited into GenBank.

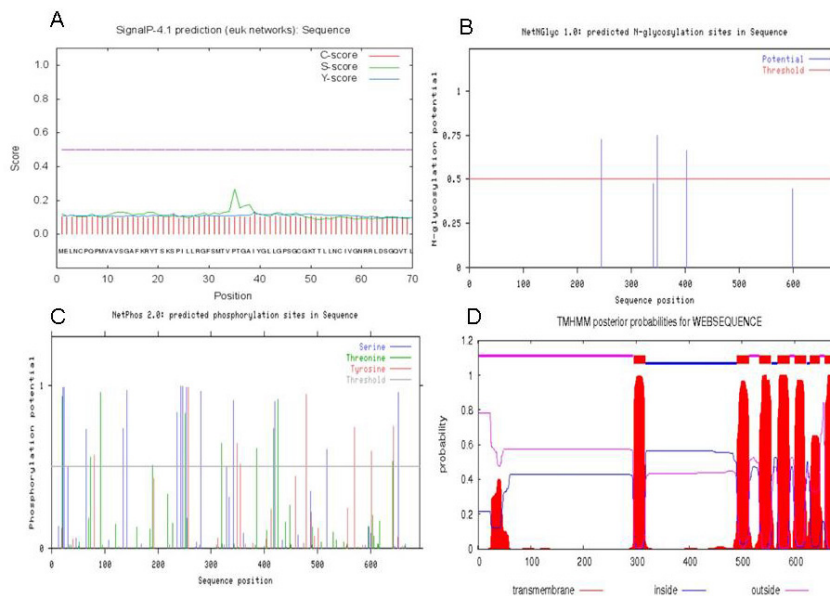


Figure 2. Analysis of the amino acid sequence of *NIABCG*. **A.** Signal P prediction. **B.** N-glycosylation site prediction. **C.** Phosphorylation site prediction. **D.** Transmembrane domains prediction.

Protein motif analysis of *NIABCG*

Prediction of the secondary structure of *NIABCG* demonstrated that the amino acid sequence contained 44.69% α -helices, 11.35% extended strand, and 40.90% random coil (Figure 3A).

We used the NCBI CDS-Conserved-Domains-prediction-server to analyze *NIABCG*. The results indicated that *NIABCG* gene was structurally featured with essential ABC_ATPase and ABC2_membrane domains (Figure 3B). The predicted ABC_ATPase domain of *NIABCG* was 201 amino acids long, positioned between 10 and 210 of *NIABCG*. The ABC2_membrane domain of *NIABCG* was 171 amino acids long.

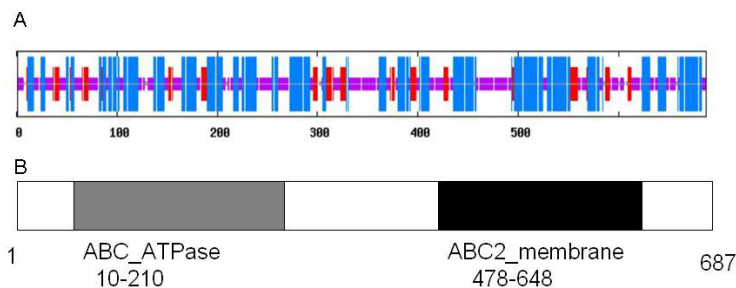


Figure 3. Structure of the *Nilaparvata lugens* ABCG protein. (A) Secondary structure prediction of the amino acid sequence of *NIABCG*. Random coils are shown in purple, alpha helices are shown in blue, extended strands are shown in red. (B) Conserved domains of *NIABCG*. It has the ABC_ATPase and ABC2_membrane domains that are typical motifs of ABC family proteins.

Analysis of multiple sequence alignments and the phylogenetic tree

Multiple alignments of known amino acid sequences of ABC transporter genes were analyzed, and the results indicated that the sequences were highly conserved among different species of insects (Figure 4). The amino acid sequence of NIABCG has the highest degree of conservation among Hemiptera, sharing 42.8% sequence identity with *A. pisum*. It has a relatively low identity to Hymenoptera, with 31.9% to *A. mellifera*. After comparing homology between amino acid sequences of ABC transporter genes among different insects, we found that the most highly conserved positions were located at three *N*-glycosylation sites (N244, N348, N402), 34 phosphorylation sites, and seven transmembrane domains (TM1-TM7) in *NIABCG*. The phylogenetic tree showed that the amino acid sequences of ABC transporter genes were the most closely related in species of the same order (Figure 5). Hymenoptera, Diptera, Coleoptera, and Hemiptera were well segregated from each other. The amino acid sequence of *NIABCG* had the nearest genetic distance to ABCG of *A. pisum* and the farthest genetic distance to ABCG of *H. saltator*. The genetic distances of the insects between Hemiptera and Coleoptera were generally close.

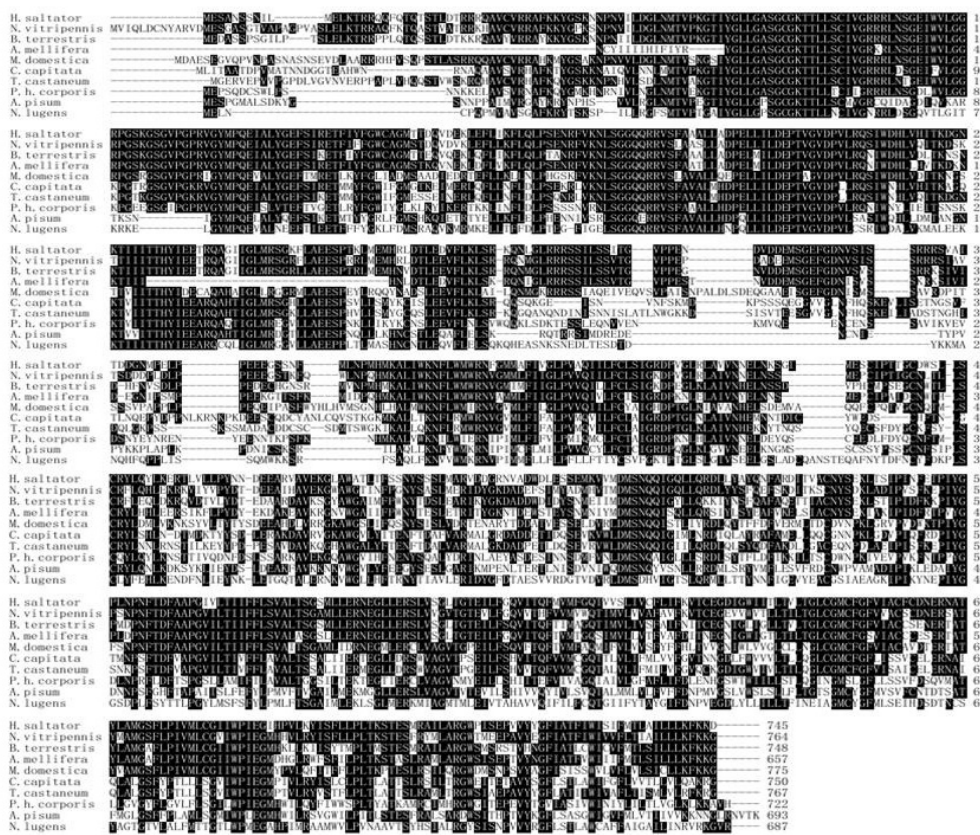


Figure 4. Multiple alignment of the amino acid sequence deduced from *Nilaparvata lugens* *NIABCG* with other ABC transporter genes in insects. Identical residues are shaded in black. Dashed lines indicate the gaps.

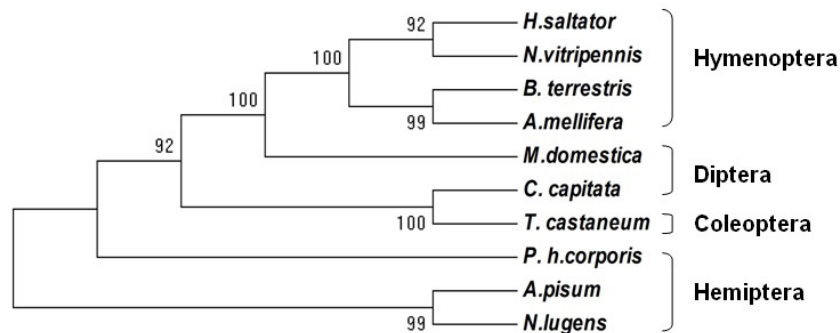


Figure 5. Phylogenetic relationships of the ABCG proteins from *Nilaparvata lugens* and other species.

Development- and tissue -specific expression patterns of *NIABCG*

To probe the functions of the *NIABCG* gene product, its mRNA level was analyzed, using qRT-PCR, at various developmental stages of *N. lugens* insect, including nymphs from 1st to 5th instars, female adults, and male adults. The developmental expression pattern revealed that *NIABCG* transcript was present at all development stages. The relative expression level of *NIABCG* in the 1st instar was the lowest among the various development stages and increased with larval growth. The relative expression levels in the 2nd to 5th instars and in male adults were 1.10, 1.24, 1.26, 1.34, and 1.57-fold higher than the expression in the 1st instar, respectively. The highest relative expression was found in female adults, which was 1.59-fold higher than that in the 1st instar (Figure 6A). To determine the tissue distribution,

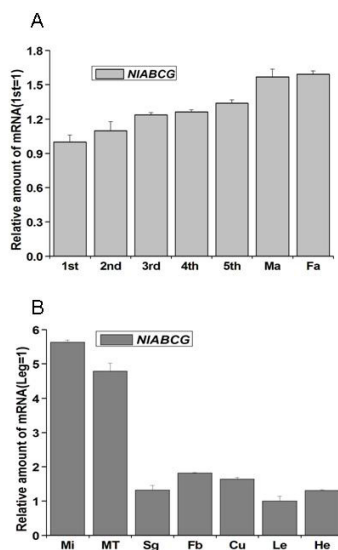


Figure 6. Expression of *NIABCG* in *Nilaparvata lugens*. **A.** Developmental expression of *NIABCG* in *N. lugens* from 1st nymph to male adult (Ma) and female adult (Fa). **B.** Tissue distribution of *NIABCG* in *N. lugens* 5th instar nymph. qRT-PCR analyses were performed using total RNA from midgut (Mi), Malpighian tubule (MT), salivary gland (Sg), fat body (Fb), cuticle (Cu), leg (Le), and head (He). Data shown are means \pm 6 standard errors (N = 3).

qRT-PCR was used to amplify *NIABCG* from cDNA made from RNA extracted from midgut, Malpighian tubule, salivary gland, fat body, cuticle, leg, and head tissues. The results indicated that *NIABCG* was expressed in all seven tissues tested. As shown in Figure 6B, the expression levels were normalized against that in the leg. The relative expression level in the midgut, Malpighian tube, salivary gland, fat body, cuticle, and head was 5.63, 4.79, 1.32, 1.82, 1.64, and 1.31-fold higher than the expression in the leg, respectively.

DISCUSSION

ABC transporters possess comprehensive and interdependent functions. They are involved in the transportation of eye pigments (Mackenzie et al., 1999), lipid-modified peptide chemoattractants (Ricardo and Lehmann, 2009), glutathione-conjugated organic anions (Tarnay et al., 2004), and possibly 20-hydroxyecdysone to orchestrate circadian transcription of clock genes (Itoh et al., 2011). The current study reports the identification and characterization of an ATP-binding cassette (ABC) transporter gene in *N. lugens* for the first time.

The first eukaryotic ABC transporter identified was the P-glycoprotein on the cell surface of cancer cells (Kartner et al., 1983), which acts as a multidrug resistance (MDR) efflux transporter that prevents the accumulation of chemotherapeutic drugs. Since then, detailed studies of ABC families have been published for members of several different insect orders (Roth et al., 2003; Liu et al., 2011; Labbe et al., 2011; Broehan et al., 2013) and the crustacean *Daphnia pulex* (Sturm et al., 2009). In the current study, *NIABCG*, a putative ABC transporter gene, was identified from the *N. lugens* genome. The *NIABCG* protein exhibited similar characteristics as other ABCG orthologs. For instance, it comprised a predicted 7-TM domain structure and shared a high sequence identity with other ABCG genes. The reported ABCG transporters are half transporters. Importantly, each ABCG transporter has a TMD at the C-terminal region of NBD, showing a distinct structure (Holland et al., 2003). Moreover, *NIABCG* contained the remarkably conserved ABC_ATPase and ABC2_membrane of motif domains. Thus, we deduce that *NIABCG* belongs to the ABCG subfamily.

The expression of *NIABCG* showed a distinct developmental- and tissue-specific pattern in *N. lugens*. The expression level of *NIABCG* was the lowest 1st instar insects, which was probably due to the incomplete development of the transport system at this stage. It increased rapidly with larval growth, which is correlated with the rapid growth and complete development of tissues and the transport system in 2nd to 5th instars and adults. In the silkworm, multiple ABC genes are highly expressed during molting and pupation (Liu et al., 2011). The tissue-specific pattern of *NIABCG* indicated that it was mainly expressed in the midgut of the larvae, and was expressed at low levels in the Malpighian tubule, salivary gland, fat body, cuticle, leg, and head compared to the midgut. These results are in accordance with the circulatory system being mainly concentrated in the midgut, and the midgut region is the only part of the gut that contains surfaces of exposed cells. Liu et al (2011) demonstrated that midgut-specific ABCG genes in *B. mori* are upregulated by 20E during molting and pupation. Furthermore, the Malpighian tubule is the second tissue enriched with *NIABCG* gene expressions in *N. lugens*. The Malpighian tubule serves as the excretory and osmoregulatory organ for insects, in which the urine is produced and transported to the hindgut for the selective absorption of water and ions. Thus, the Malpighian tubule plays an important role in excretion and it is also involved in xenobiotic detoxification (Neira Oviedo et al., 2008). In particular, TcABCG-9A and TcABCG-9B genes in *T. castaneum* were also expressed in the intestinal/excretory tissues

including Malpighian tubules, where these ABC transporters function in the concentration of tryptophan and purines such as guanosine (Sullivan et al., 1979, 1980). Taken together, these observations suggest that *NLABCG* may be associated with the regulation of tissue-specific gene expression. The characteristics of expression of the *NLABCG* in *N. lugens* suggest that the best period for controlling *N. lugens* using pesticides targeting the *NLABCG* is in the early instars. These results may aid the development of novel, efficient, and safe insecticides that target the *NLABCG* gene. Furthermore, these data may help to create new methods that inhibit ABC transporters in pests, such as the development of RNA interference and pest-resistant transgenic plants.

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