



The NompC channel regulates *Nilaparvata lugens* proprioception and gentle-touch response

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ARTICLE INFO

Keywords:

Nilaparvata lugens
NompC
RNAi
Proprioception
Gentle-touch

ABSTRACT

NompC channel is a member of the transient receptor potential (TRP) ion channel superfamily. It can regulate gentle-touch, locomotion, hearing and food texture detection in *Drosophila*. We cloned the *NompC* gene of *Nilaparvata lugens* (*NilNompC*). The full length *NilNompC* possessed similar structure as *DmNompC*, which belongs to TRPN subfamily. The expression pattern analysis of different developmental stages and body parts showed that the transcription of *NilNompC* was more abundant in adult stage and in the abdomen. Injection of double-stranded RNA (dsRNA) of *NilNompC* in the third-instar nymphs successfully knocked down the target gene with 75% suppression. At nine days after injection, the survival rate of dsRNA injected nymphs was as low as 9.84%. Behavioral observation revealed that the locomotion of the dsRNA injected nymphs was defective with much less movement compared to the negative control. Feeding and honeydew excretion of the dsRNA injected insects also decreased significantly. These results suggested that *NilNompC* is a classical mechanotransduction channel that plays important roles in proprioception and locomotion, and is essential for the survival of *N. lugens*. The results also contribute to the understanding of how TRP channels regulate proprioception.

1. Introduction

Transient receptor potential (TRP) ion channels are a collection of non-voltage-gated cation channels that are conserved from nematodes to insects and to humans (Ramsey et al., 2006; Venkatachalam and Montell, 2007). This group of ion channels plays important roles in both vertebrate and invertebrate organisms as detectors of the environment. The channels can be activated by a wide variety of mechanisms and involved in virtually every sensory modality including vision, thermo-sensation, olfaction, hearing, and mechanosensation (Fowler and Montell, 2013). TRP channels are classified into seven subfamilies based on primary amino acid sequence homology (TRPC, TRPA, TRPN, TRPV, TRPM, TRPML, TRPP) (Montell, 2005a; Montell et al., 2002) containing six common transmembrane segments in the C-terminal that form sensor and pore domains. TRPC, TRPM and TRPN channels also contain a TRP domain after the sixth transmembrane segment. With the exception of TRPM, TRPML and TRPP channels, all other TRP channels have multiple N-terminal ankyrin repeats (ARs).

Among all known TRP channels, NompC channel, which belongs to

TRPN subfamily, has the largest number of ARs (Montell, 2005b) that are important for channel functions in locomotion of both larvae and adults of *Drosophila melanogaster* (Cheng et al., 2010). ARs are also essential for NompC mechanogating both *in vitro* and *in vivo* (Zhang et al., 2015). In *Drosophila*, NompC mutant adults are severely uncoordinated (Kernan et al., 1994; Walker et al., 2000), and mutant larvae displayed severe defects in behavioral response to gentle-touch (Yan et al., 2013). NompC also is required for hearing (Effertz et al., 2011; Sun et al., 2009). Loss of NompC abolishes active amplification in *Drosophila* antenna (Göpfert et al., 2006), and NompC is necessary for the active amplification of sound evoked motion by the auditory organ (Lee et al., 2010; Lehnert et al., 2013). Moreover, a sensory pathway of texture detection also requires NompC to avoid substrates that are either too hard or too soft (Sánchez-Alcañiz et al., 2017). All these observations suggest that NompC is a primary mechanotransducer channel.

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), (Hemiptera: Delphacidae), is widely distributed in south and east Asia, north Australia and west Oceania (Liu and Han, 2006). This insect is

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economically important because it is one of the most harmful insect pests of rice plants, causing damage by directly sucking the phloem sap, and by acting as vector of viruses that causes ragged stunt diseases on host plants (Cuong et al., 1997; Murakami et al., 2013). The BPH *NompC* has not been characterized. In this study, we cloned the *NlNompC* of *N. lugens*, analyzed the predicted protein sequence, and characterized its temporal and spatial expression profiles. Furthermore, gene knockdown was performed through *NlNompC*-dsRNA injection. The *NlNompC* knockdown effects on mortality, feeding, behavior and response to gentle touch were investigated.

2. Materials and methods

2.1. Insects

N. lugens were collected from a rice nursery located at the Plant Protection Station of Jiangpu County (Jiangsu, China) in 1993, and were reared on fresh rice seedlings (Taichung Native 1, TN1) in a growth chamber at $27 \pm 1^\circ\text{C}$, $70 \pm 10\%$ relative humidity and a 16 h:8 h (Light: Dark) photoperiod.

2.2. Gene identification and cloning

Total RNA was isolated from whole insects using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Single-stranded cDNA was synthesized from the total RNA with M-MLV reverse transcriptase and oligo (dT)₁₈ (BioTeke, Beijing, China).

Using the amino acid sequences of *NompC* proteins from *D. melanogaster*, which was obtained from GenBank, as queries, TBLASTN searched the *N. lugens* genomic (Genbank accession numbers: AOSB00000000) (Xue et al., 2014) and transcriptomic databases. The search identified three segments of *NlNompC*. Based on these sequences, the 5' and 3' regions of the corresponding cDNA were obtained using a SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) and 3'-Full RACE Core Set with PrimeScript™ Rtase (TaKaRa, Dalian, China), respectively, following the manufacturer's instructions. Nested PCR was performed for RACE cloning, and then six pairs of primers were designed to amplify the full length of *NlNompC*. LA taq with GC Buffer (TaKaRa) was used for the PCR amplification. The amplicon was cloned into pMD™19-T Vector (TaKaRa) and verified by Sanger sequencing. The sequences of the primers used in this study are listed in Table 1.

2.3. Sequence analysis and phylogenetic tree construction

Nucleotide sequences were assembled and the open reading frame (ORF) was identified by DNASTar software package (Version 5.02). Multiple alignments of the complete amino acid sequences were performed with Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo>). The ankyrin repeat domain organizations of *NlNompC* protein were predicted by the Simple Modular Architectural Research Tool (SMART) (<http://smart.embl-heidelberg.de/>) and the TMHMM v2.0 was used to assess the location and number of predicted transmembrane domains in the sequence. The exon and intron architectures of *NlNompC* were predicted based on alignments of mRNA sequences against their corresponding genomic sequences in Spidey (<http://www.ncbi.nlm.nih.gov/spidey/>), and then structured on the website of GSDS v2.0 (<http://gsds.cbi.pku.edu.cn/index.php>) (Hu et al., 2015).

The amino acid sequences corresponding to the six transmembrane ion transport segments were aligned by the Clustal Omega. Poor alignments were trimmed by the trimAl tool (<http://phylemon.bioinfo.cipf.es/>) to make the analyses more reliable (Capella-Gutiérrez et al., 2009). The alignments were checked manually in MEGA 5.2.2 (BioDesign Institute, Center for Evolutionary Functional Genomics, Tempe, AZ, USA) (Tamura et al., 2011). The phylogenetic tree was constructed using Markov chain Monte Carlo (MCMC) as implemented in MrBayes

ver. 3.2.4 (<http://nbsweden.github.io/MrBayes/download.html>) (Ronquist et al., 2012). The analyses of four simultaneous chains were run from random trees for 2000000 generations and sampled every 500 generations. The first 25% of generations were discarded as burn-in, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01. The accession numbers of the sequences used in the phylogenetic analysis are listed in Table 2.

2.4. Real-time qPCR analyses

Specimens of eggs (n = 40–50), first-instar (n = 40–50), second-instar (n = 40), third-instar (n = 15–20), fourth-instar (n = 15–20), fifth-instar nymphs (n = 10–15), and adults of both sexes and wing forms (n = 10–15) were obtained from the laboratory colony. Eggs were collected at 4 days after the oviposition began. Nymphs and adults were collected every 24 h from the beginning of each instar until molting or eclosion. BPH adults can develop into short-winged (brachypterous) or long-winged (macropterous) morphs in response to environmental cues. In the current study, we selected brachypterous female adults as the research object. Various tissues of brachypterous female adults (n = 60) were dissected and collected. The total RNA of these samples was isolated using TRIzol Reagent (Invitrogen). The first-strand cDNA was synthesized following the instructions of the HiScript® II Q RT SuperMix for qPCR (+gDNA wiper) kit (Vazyme, Nanjing, China).

Pairs of gene-specific primers designed using the Primer Premier 5 Software (Table 1) and the cDNA prepared as described above were used for real-time qPCR on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using the UltraSYBR Mixture (with ROX) Kit (CWBio, Beijing, China). Each PCR reaction included 4 µl of 10-fold diluted cDNA (500 ng), 1 µl of each primer (10 µM), 10 µl 2 × UltraSYBR Mixture, and RNase-free water added to a final volume of 20 µl. The *N. lugens* 18S ribosomal RNA (*NI18S*) housekeeping gene was used as an internal control (Xu et al., 2015). The standard two-step PCR cycle conditions were as follows: 95 °C for 10 min, followed by 40 cycles of amplification consisting of 95 °C for 15 s, 60 °C for 40 s. After the amplification phase, a dissociation curve was generated to ensure that there was only one product. Control without any template was included in all the qPCR assays. For all developmental stage, qRT-PCR was repeated six times with each replication performed based on an independent RNA sample preparation. Tissue samples had three biological replicates and two technical replicates. The relative quantitative method [$2^{-\Delta\Delta Ct}$: $2^{-(Ct_{\text{target}} - Ct_{NI18S})_{\text{timeX}} - (Ct_{\text{target}} - Ct_{NI18S})_{\text{time0}}}$], where timeX is any time point and time0 represents the 1 × expression of the target gene normalized to *NI18S*. Ct: cycle threshold (Livak and Schmittgen, 2001)] was used to evaluate expression quantities.

2.5. RNAi and real-time qPCR analysis after dsRNA injection

A 485 bp of *NlNompC* cDNA and a 657 bp green fluorescent protein (*gfp*) fragments were amplified by PCR using specific primers conjugated with the T7 RNA polymerase promoter (5'-taatacagactacta-taggg-3') (primers listed in Table 1). Amplification reactions were conducted in 50 µl using the 2 × Phanta MASTER Mix (Vazyme, Nanjing, China). The PCR products were purified with the E.Z.N.A Cycle-Pure Kit (OMEGA, Doraville, CA) and used as templates for dsRNA synthesis using the T7 MEGAscript kit (Ambion, Austin, TX) according to the manufacturer's instructions. The dsRNA was synthesized at 37 °C for as long as 16 h. The reactions were treated with the TURBO DNase at 37 °C for 15 min to remove the template DNAs and then terminated at 65 °C for 10 min. RNA concentration was measured using a spectrophotometer (Nanodrop) after a 1:10 dilution of the dsRNA product in water. The final concentration was adjusted at 5 µg/µl, then centrifuged at 12,000 rpm for at least 5 min at 4 °C and stored at –80 °C until use.

Table 1
The primers used in this study.

Primers	Primer sequence (5'-3')	cDNA position in the coding area (bp)
<i>For cDNA fragment cloning</i>		
NI <i>NompC</i> -1F	TGGTGTGGTGTAATCCGT	–2487 – (–2468)
NI <i>NompC</i> -1R	AGGATGCGTGCCGAGATGTCTC	164–185
NI <i>NompC</i> -2F	ACATCAGGGAGCAACGTGAG	–570 – (–551)
NI <i>NompC</i> -2R	GCAGTGCCGGTTTGTCTACT	544–563
NI <i>NompC</i> -3F	GGGGATGACACCTCTGATGTATGC	312–335
NI <i>NompC</i> -3R	AAGTCTATCAGCTTCTCACGATG	1581–1604
NI <i>NompC</i> -4F	CCATCCACATATCAGCCATGCA	1448–1469
NI <i>NompC</i> -4R	TGGGCTAACAGTTCTCTCACCGT	2764–2786
NI <i>NompC</i> -5F	GGCTTTCACCTAAGGACGGCAATA	2397–2419
NI <i>NompC</i> -5R	ACCGTGTGCGAGATGACCTCCTC	3663–3686
NI <i>NompC</i> -6F	CCAATCTCGGAGTTCATCTGGTGTCT	3412–3438
NI <i>NompC</i> -6R	CTTCTTCTTTCAGTACCCTCC	5142–5165
<i>For RACE-PCR cloning</i>		
5'RACE outer long primer	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	
5'RACE outer short primer	CTAATACGACTCACTATAGGGC	
5'RACE inner primer	AAGCAGTGGTATCAACGCAGAGT	
3'RACE outer primer	TACCGTCGTTCCACTAGTGATT	
3'RACE inner primer	CGGGATCCTCCACTAGTATTCTACTATAGG	
5'RACE-A	TGGGTTGACATCCTCGCTCCACTGG	254–279
5'RACE-B	AGGATGCGTGCCGAGATGTCTC	164–185
3'RACE-A	CCAATCTCGGAGTTCATCTGGTGTCT	2764–2786
3'RACE-B	GAGGTGAAAAAGGAGTCAAGCCGAAC	4348–4374
<i>For qPCR</i>		
QNI <i>NompC</i> -F	ATCAGATGGTCGAGGTCTCT	3104–3123
QNI <i>NompC</i> -R	TCATTGTGACCTTCGCTGAC	3286–3305
QNI18S-F	CGCTACTACCGATTGAA	
QNI18S-R	GGAACCTTGTACGACTT	
<i>For dsRNA synthesis</i>		
T7-NI <i>NompC</i> -F	TAATACGACTCACTATAGGGCGTGAAAAACAATGCTACTCTT	3737–3758
T7-NI <i>NompC</i> -R	TAATACGACTCACTATAGGGCAGGGTCCGAACAGGTAATGG	4200–4220
T7-GFP-F	TAATACGACTCACTATAGGGAAGGGCGAGGAGCTGTTACCGC	
T7-GFP-R	TAATACGACTCACTATAGGGCAGCAGGACCATGTGATCGCGC	

Table 2
Accession numbers of TRPs' amino acid sequences used in this study.

Protein name	Accession number	Protein name	Accession number
Dm <i>NompC</i>	ADK73985.1	DmTRPA1	NP_648263.5
Bm <i>NompC</i>	XP_012546363.1	DmPain	NP_611979.1
Ph <i>NompC</i>	XP_002424810.1	DmPyx	NP_612015.1
Tc <i>NompC</i>	XP_008197616.1	DmWtrw	NP_731193.1
Am <i>NompC</i>	XP_006567193.1	DmTRPM	NP_001036548.1
Ap <i>NompC</i>	XP_008186116.1	DmTRPML	NP_649145.1
Nv <i>NompC</i>	XP_008204414.1	DmPkd2	AAR24077.1
DmTRP	AAA28976.1	Dmlav	NP_572353.1
DmTRPgamma	CAB96204.1	DmNan	NP_648696.2
DmTRPL	NP_476895.1		

Third-instar nymphs were anesthetized by CO₂ for about 30s and placed ventral side up on an agarose (2%) plate groove for injections of dsRNA. Each individual received 150 ng (about 30 nl) dsRNA of each gene using an UltraMicro Pump II (UMP2) microinjection device (World Precision Instruments, Sarasota, FL). The microscopic needle was made from capillary glass tube (outer diameter of 1.2 mm, inner diameter of 0.69 mm) using the Sutter Instruments P-97 micropipette puller (Sutter Instrument, Novato, CA) at setting of heat = 470, pull = 150, velocity = 30 and time = 90. The detail of the microinjection was described in Xu et al (Xu et al., 2015). A total of 140 nymphs were injected with the dsRNA of *NI*NompC**, and about 120 nymphs were injected with the dsRNA of *dsgfp* as negative controls for measuring nonspecific effects of the dsRNA treatment. The injected nymphs were recovered for 24 h and healthy nymphs were moved to rearing arenas (Containing 30 rice seedlings in plastic cups with 1.5% agar). The transcript levels of *NI*NompC** after injection were quantified by real-time qPCR using a set of five insects at 4 days after injection. Three biological replications were performed.

2.6. Morphology and behavioral assays

The morphological changes by the dsRNA injection were examined. The angle of femur and tibia was measured by Photoshop CS5 version (San Jose, CA, <http://www.adobe.com/products/photoshop.html>) based on images of each insects injected with *dsgfp* or *dsNI*NompC**.

In a movement assay, nymphs were placed at the center of a circle paper with concentric circles drawn at 2 cm apart (a total of 11 circles were drawn). The center circle radius was 2 cm. The setup was filmed for 30 min using a camera (SONY FDR-AX30, Japan) after injected nymphs were released at the center. Movement scores (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) were determined by the distance traveled from the center at 2 min intervals. The score of 10 was assessed when the nymphs exceeded the outermost circle.

For nymphal dropping tests, vials of 19 cm long and 2 cm in diameter containing one leafless rice seedling with roots anchored in agar (3 cm thick) were used (Fig. 4E). Nymphs that were injected with dsRNA for 5 d were transferred to the apparatus using an aspirator, and the nymphs were allowed to settle on the rice seedling at an upward position for 1 h. Then the apparatus was gently inverted 180°. Nymphs that were dropped from the rice stem were counted after 1 h.

A slightly modified gentle touch assay of *Drosophila* was used (Kernan et al., 1994). One side of the nymph prothorax notum was gently touched with a soft brush and the immediate behavioral responses were scored. Nymphs that showed no response to the touch were scored as 0'; a score of 1' was given to nymphs with response of leg swaying but no movement; movement of less than three steps was scored as 2' (body move); movement of more than three steps was scored as 3' (climb); and a jumping response was scored as 4' (jump) (Movie S1). During the assay, the nymphs were allowed to crawl freely on a piece of paper at room temperature (~25 °C). This assay was performed using the nymphs 5 d after receiving dsRNA injections.

Supplementary video related to this article can be found at <https://>

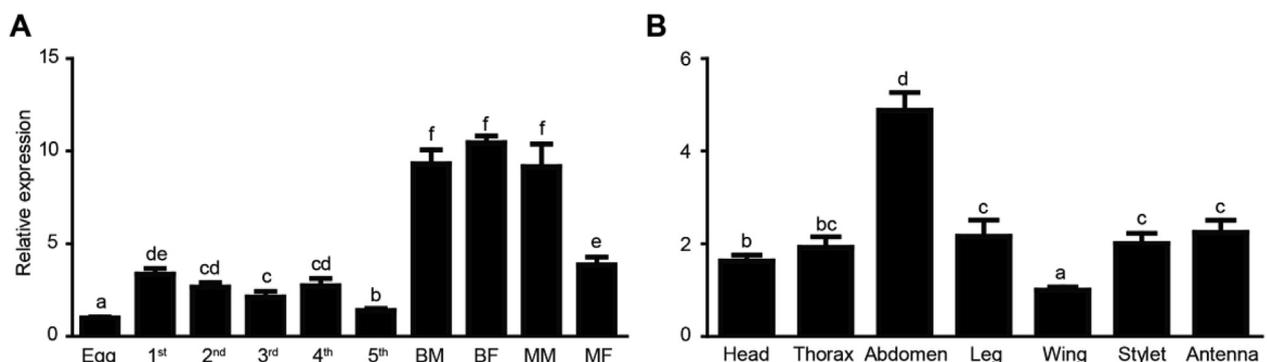
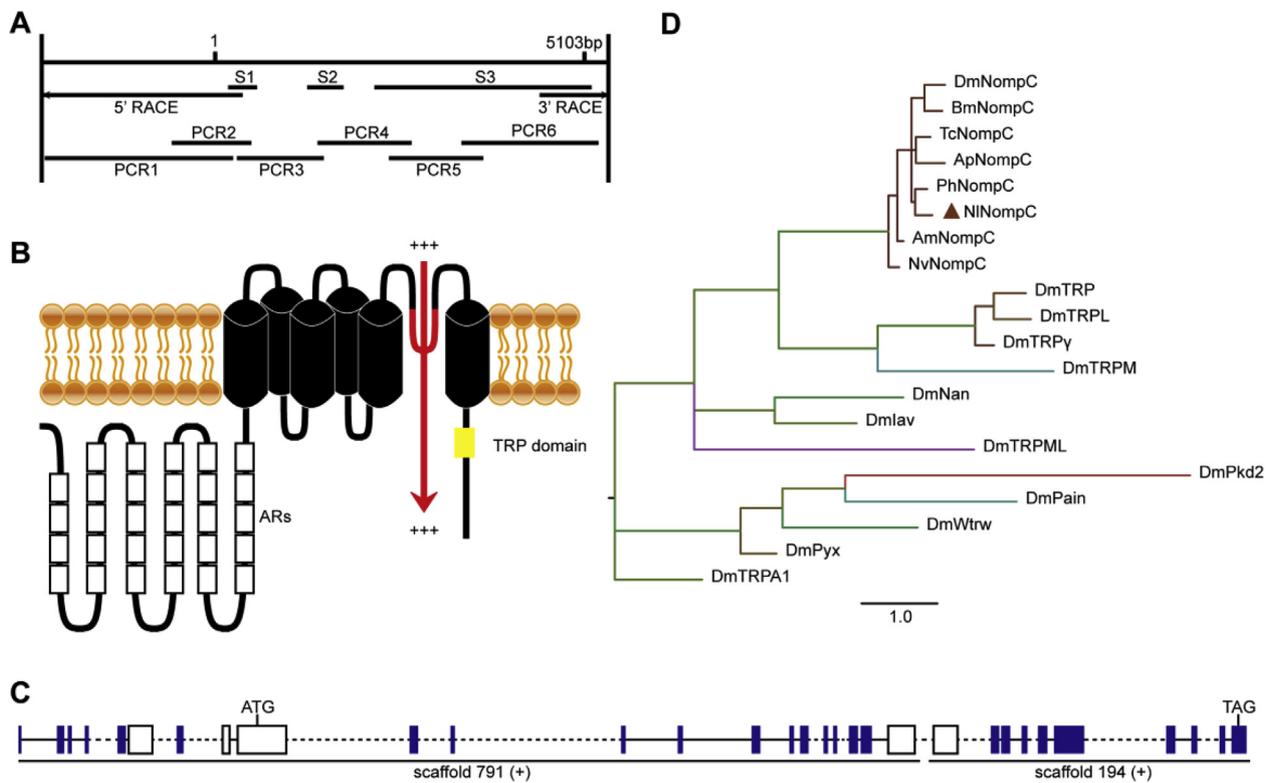


Fig. 2. The expression patterns of *NINompC* in different developmental stages and tissues.

(A) Expression patterns of *NINompC* in different developmental stages including egg, 1st to 5th instar nymphs, and adults of MF (macropterous female), BF (brachypterous female), MM (macropterous male), and BM (brachypterous male). (B) Expression patterns of *NINompC* in various tissues of BF including head, thorax, abdomen, legs, wing, stylet, and antenna. Data are presented as the mean \pm s.e.m. Different lower-case letters above the bars indicate significant differences (One-way ANOVA with Duncan's Multiple Range Test, $P < 0.05$).

doi.org/10.1016/j.ibmb.2018.11.005.

2.7. Feeding and honeydew excretion

A feeding assay was performed using a feeding chamber containing chemically defined liquid diet according to a method reported

previously (Fu et al., 2001). Each chamber received fifteen nymphs after being starved for 2 h. After 24 h of feeding, the remaining diet was weighed with an electronic balance (Sartorius, BS214D, Beijing, China) with 0.1 mg accuracy. There were six replicates.

Honeydew excretion was measured using a parafilm sachet (3.5 cm \times 4 cm) positioned on a healthy rice stem (Pathak et al., 1982).

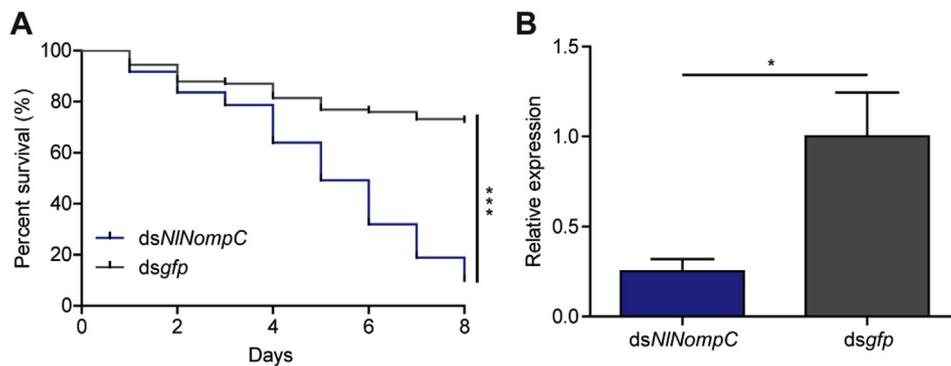


Fig. 3. *NINompC* is essential for the survival of *Nilaparvata lugens*.

(A) Down-regulation of *NINompC* gene using *dsNINompC* leads to a reduction in mRNA expression level. All data are presented as mean \pm s.e.m. * $P < 0.05$ (Student's *t*-test). (B) The survival rate (%) of *Nilaparvata lugens* after *dsNINompC* and *dsGFP* injection (*dsNINompC*: $n = 122$; *dsGFP*: $n = 108$). Survival analyses with log-rank test ($\chi^2 = 78.00$, *** $P < 0.001$).

Each sachet contained three nymphs starved for 2 h. After 24 h of feeding in the sachet, the honeydew excretion was measured by weighing the sachet with the same electronic balance (Sartorius). There were at least 15 replicates.

3. Results

3.1. Identification of *NompC* gene in *N. lugens*

Based on the amino acid sequence of *D. melanogaster* *NompC* protein, three segments (S1-S3) of the *NINompC* were identified by TBLASTN searches of the *N. lugens* genomic and transcriptomic databases. The full-length sequence of *NINompC* was obtained by rapid amplification of both 5'- and 3'- cDNA ends (RACE) using specific primers designed from the fragments and six pairs of designed primers (PCR1-PCR6) (Table 1). The *NINompC* cDNA of 7765 bp contains a putative coding region of 5103 bp, a 2495 bp 5'-untranslated region (5'-UTR) and a 167 bp 3'-UTR, with a putative polyadenylation signal upstream of the poly(A) tail (Fig. 1A). The complete ORF encodes 1701 amino acids, a predicted 185 kDa protein. *NINompC* is a member of TRPN subfamily based on the amino acid sequence (Fowler and Montell, 2013).

Alignment of *NINompC* protein with other insect *NompC* proteins revealed that the *NINompC* gene encodes a mechanotransduction ion channel with similar features: the N-terminal region until residue 1150 contains 29 predicted ankyrin repeats, six transmembrane domains in COOH-terminal, and TRP domain (EWKFAR and LPPPFN) (Fig. 1B and Fig. S1).

The full length of *NINompC* and the corresponding genomic sequence were aligned and analyzed for exon–intron organization. The analysis results showed that *NINompC* was located in scaffold 791 and scaffold 194 on the opposite orientations (Fig. 1C). This gene is split into 31 exons, spanning ~ 18 kb of genomic DNA (Fig. 1C).

To investigate the evolutionary relationship of *NINompC*, a phylogenetic analysis was performed with orthologs from 7 other insect species (Fig. 1D). BLASTP analyses of protein sequence alignment showed that *NINompC* had 76%, 74%, 74%, 74%, 73%, 72%, 71% sequence identity with the *NompC* proteins of *Pediculus humanus*, *D. melanogaster*, *Nasonia vitripennis*, *Apis mellifera*, *Acyrtosiphon pisum*, *Tribolium castaneum*, and *Bombyx mori*, respectively. *NINompC* is most closely related to the *NompC* genes of *P. humanus* and *A. pisum*. The alignments of *NINompC* protein with orthologous sequences showed high degree of conservation (Fig. S1).

3.2. Developmental and tissue-specific expression patterns of *NINompC*

The qPCRs of different developmental stage and tissue samples showed that *NINompC* was expressed in all developmental stages including egg, 1st to 5th instar nymph, and 4 d old adults of different sex and wing forms (BM, BF, MM and MF) with significantly different expression levels. *NINompC* was expressed at the lowest level in eggs,

followed by 5th instar nymphs. The expression was the highest in the BM, BF, and MM (Fig. 2A). *NINompC* was expressed at the highest level in abdomen, followed by leg, stylet, antenna, thorax and head. The lowest expression was observed in wing (Fig. 2B).

3.3. Down regulation of *NINompC* gene reduces *N. lugens* survival

Injections of dsRNA for *NINompC* gene were performed on the third-instar nymphs. Real-time qPCR analysis of RNA isolated at 4 d after the injection showed that *dsNINompC* effectively suppressed transcript levels of *NINompC* gene (Fig. 3A). The injection of *NINompC* dsRNA also led to a lethal phenotype with about 10% survival at 8 d, which is significantly lower than that of the control group (72%) (Fig. 3B).

3.4. *NINompC* affects the proprioception and locomotion of *N. lugens*

To investigate the reasons of lethal phenotype by *NINompC* knock-down, morphological observation and behavioral assays were performed since *NompC* mediates proprioception and locomotion in *Drosophila* (Cheng et al., 2010; Walker et al., 2000). Firstly, no developmental defects of *N. lugens* were found after injection of *dsNINompC* (data not shown). However, the angle of femur-tibia joint of hindleg during stationary of the *NINompC* silenced BPH was as great as 140° (Fig. S2). On average, this angle was 76° for *dsNINompC*-injected insects (Fig. 4A), significantly greater than that of *dsGFP*-injected insects (56°) (Fig. 4B). *dsNINompC*-injections significantly increased uncoordinated leg twisting movements, and dramatically reduced the walking speed.

To evaluate the involvement of *NINompC* in locomotion and proprioception control, the movement and climbing ability of *dsNINompC*- and *dsGFP*-injected insects were examined and compared. The movement score of *dsNINompC*-injected nymphs was significantly lower than that of the *dsGFP*-injected nymphs (negative control) (Fig. 4C and D). The dropping assay also showed that *dsNINompC*-injected insects were easier to drop off from rice stem than that of the negative control insects (Fig. 4E and F).

3.5. Silencing of *NINompC* impaired the gentle touch sensation of *N. lugens*

The gentle touch behavioral response scores (Fig. 5A) indicated that *dsNINompC* injection reduced the response (Fig. 5B and C). Most *dsGFP*-injected insects were distributed at 2' (37.8%) and 3' (34.15%), even at 4' (7.32%), similar to that of un-injected insects (data not shown). However, the nymphs of *NINompC* silenced were mainly distributed at 0' (40.51%) with no individuals jumping upon contact (4' score) (Fig. 5B), exhibiting locomotor defects (Movie S1).

3.6. Effects of *NINompC* gene silencing on food intake and honeydew excretion of *N. lugens*

The feeding assay showed that 5 days after *dsNINompC* injection the insects consumed very little diet (2.67 ± 1.21 mg), significantly less

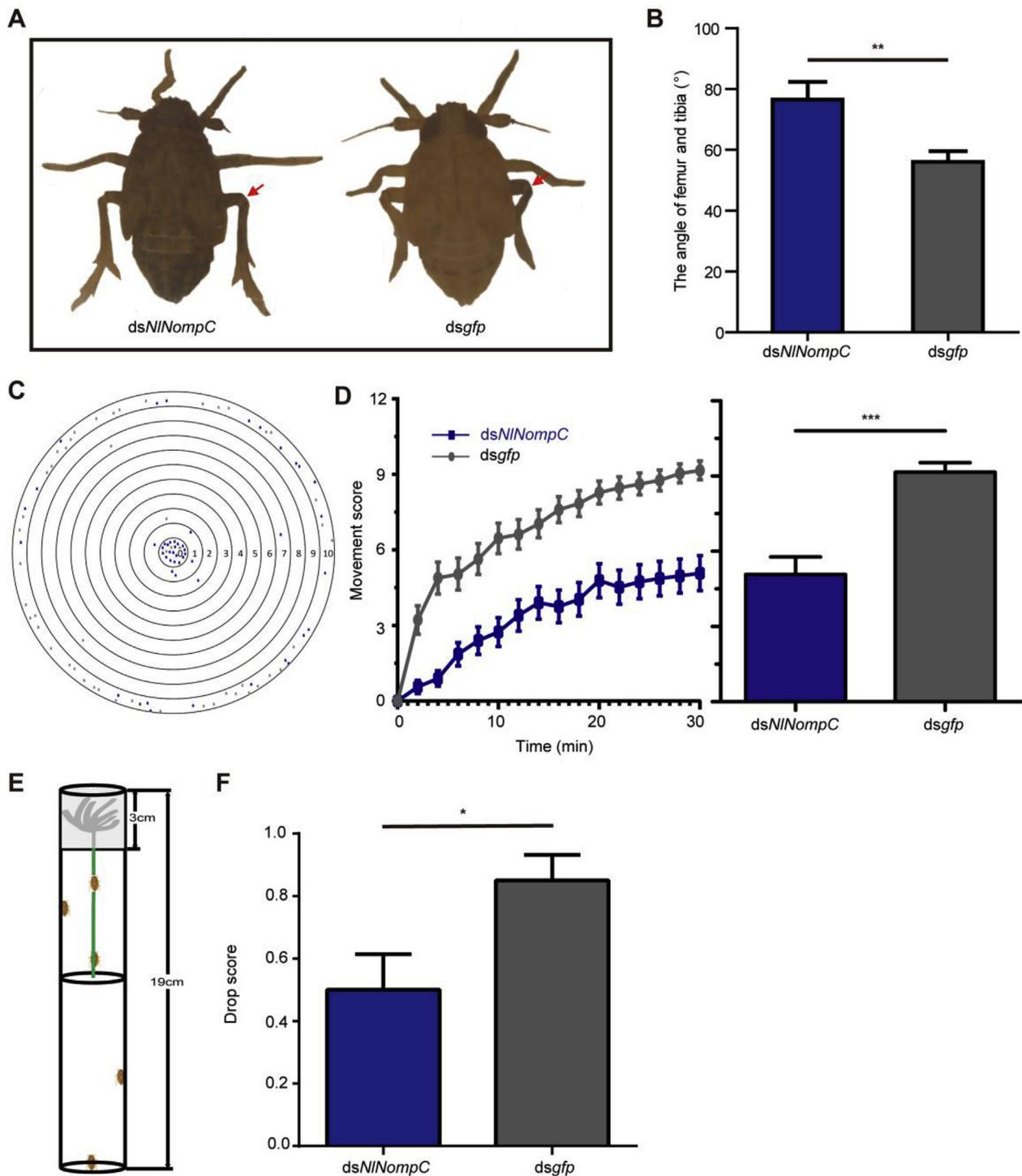


Fig. 4. Knockdown of *NINompC* gene in *N. lugens* exhibited severely uncoordinated movement.

(A) The phenotype of nymphs which were injected ds*NINompC* or ds*GFP*. The red arrow shows the femur-tibia joints of *N. lugens*. The average body length of the insects was 3.5 mm. (B) The average angle of femur-tibia joints of *N. lugens* injected with ds*NINompC* or ds*GFP*, $n = 24$, mean \pm s.e.m. $^{***}P < 0.01$ (Student's t-test). (C) The circular paper with concentric circles drawn 2 cm apart used in the movement assay. The center circle radius is 2 cm, where insects were released. Movement scores were recorded every 2 min up to 30 min based on the locations of the nymphs (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10). (D) Left: Movement score of nymphs that were injected ds*NINompC* or ds*GFP* ($n = 50$), mean \pm s.e.m. Right: Corresponding movement scores for ds*NINompC*- or ds*GFP*-injected nymphs at the 30 min point (Mann-Whitney test). (E) The apparatus of the dropping test. (F) The dropping score of nymphs after ds*NINompC* or ds*GFP* injection, $n = 20$, mean \pm s.e.m. $^{*}P < 0.05$ (Mann-Whitney test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

than that of the ds*GFP*-injected controls (7.10 ± 1.56 mg) (Fig. 6A). Similarly, the ds*NINompC*-injected insects excreted significantly less honeydew (0.19 ± 0.05 mg) compared with the ds*GFP*-injected ones (2.62 ± 0.35 mg) (Fig. 6B).

4. Discussion

In this paper, the *NompC* gene of *N. lugens* was identified and the cDNA was cloned. Based on the full length cDNA, the *NINompC* belongs to TRPN subfamily and shares high homology with other insects (Li

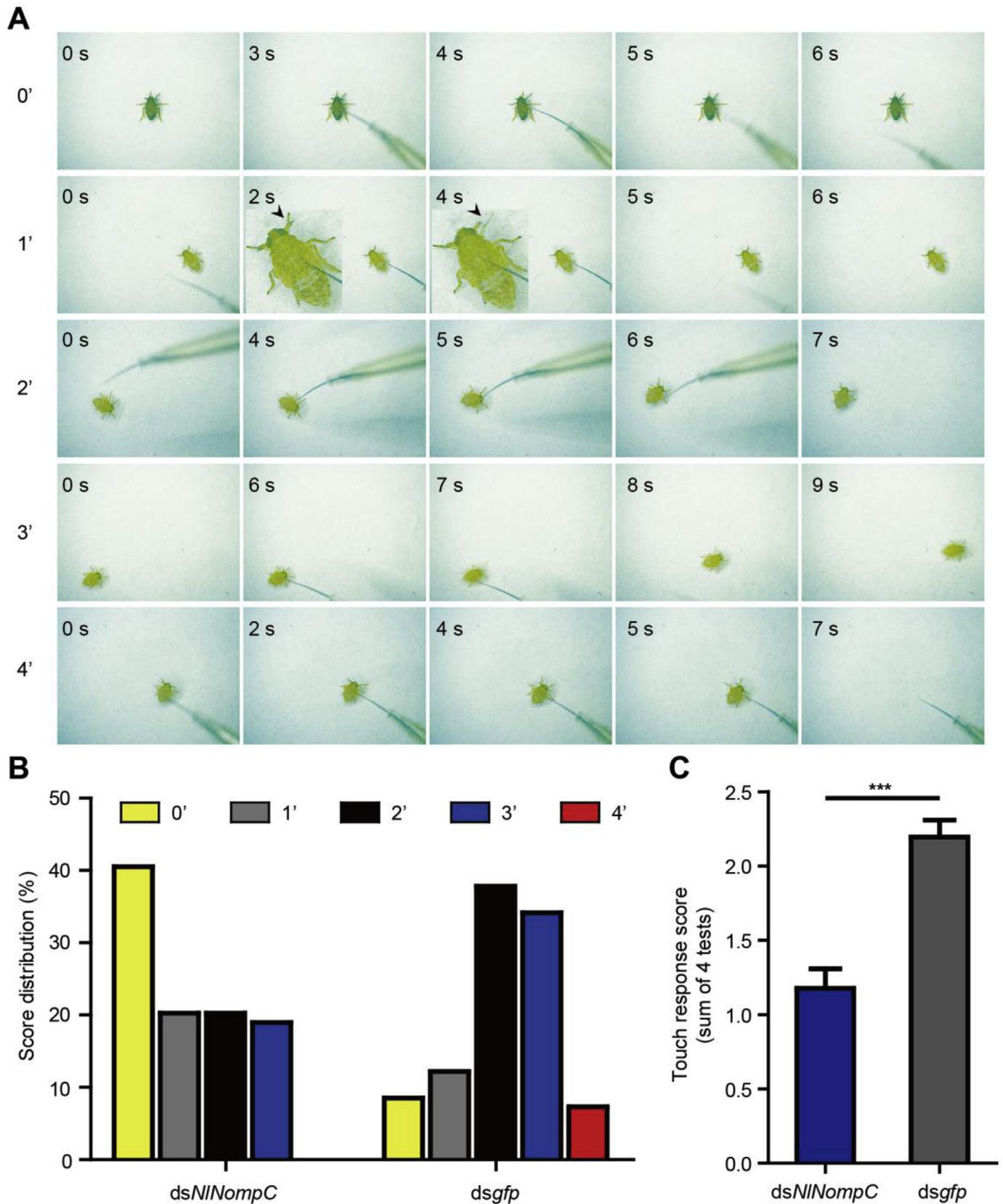


Fig. 5. Knockdown of *NINompC* gene in *N. lugens* impaired nymph behavioral responses to gentle-touch. (A) Detail scores of the behavioral test. 0, no response; 1, body sway; 2, body move; 3, climb; 4, jump. (B) Score distribution of *dsGFP*- ($n = 82$) or *dsNINompC*- ($n = 79$) injected nymphs. (C) Touch response score (sum of 4 tests) of *dsGFP*- or *dsNINompC*-injected nymphs. Data represent mean \pm s.e.m. *** $P < 0.001$ (Mann-Whitney test).

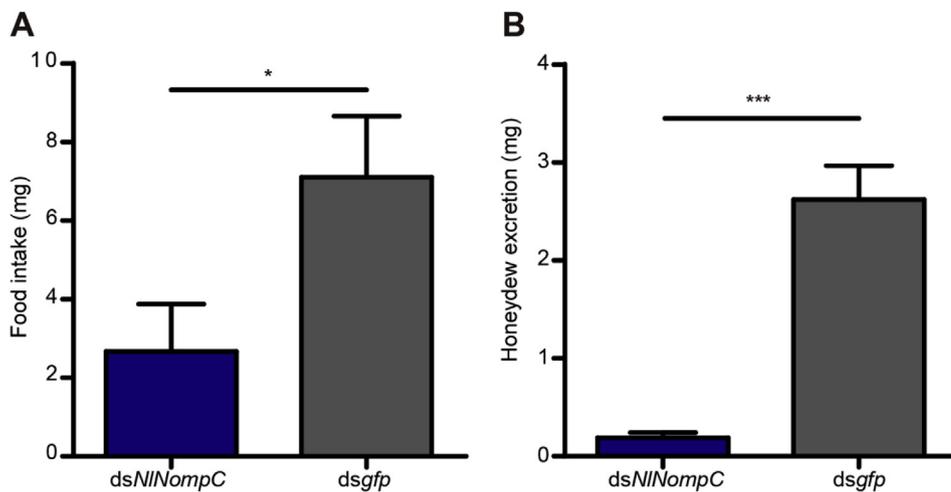


Fig. 6. Knockdown of *NILNompC* reduced food intake and honeydew excretion of *N. lugens*. (A) Food intake by *N. lugens* nymphs at 5 days after injection of ds*NILNompC* compared to the ds*GFP* control. Mean \pm s.e.m. * P < 0.05 (Student's *t*-test). Weight (mg) of liquid diet consumed in 24 h by fifteen nymphs (n = 6 repetitions). (B) The honeydew excretion of *N. lugens* after injection of ds*NILNompC* compared to the ds*GFP* control. Weight (mg) of honeydew excreted in 24 h by three nymphs. ds*NILNompC* (n = 25 repetitions), ds*GFP* (n = 18 repetitions). Mean \pm s.e.m. *** P < 0.001 (Student's *t*-test).

et al., 2006; Walker et al., 2000). Similar to the homologous gene of NompC in fruit fly, *NILNompC* has a large number (29) of ankyrin repeats (ARs) in the N-terminal region. The ARs of DmNompC form a tether between the channel and the microtubules that convey a force exerted via cell deformation to gate the channel and activate touch-sensitive neurons (Zhang et al., 2015). In *Drosophila*, NompC-dependent leg mechanosensory neuron activity is essential for encountering responses (Ramdya et al., 2015). DmNompC has been reported as a mechanotransduction channel for touch sensation, locomotion and sound response (Gong et al., 2013; Yan et al., 2013; Zhang et al., 2013). The DmNompC also is required for discriminating food texture, thus adjusting food consumption (Sánchez-Alcañiz et al., 2017). The functional research of *NILNompC* (RNAi) in the current study supports the sequence-based characterization. The knockdown of *NILNompC* adversely affected normal touch sensation, locomotion (movement and dropping behaviors) and feeding of the insects. All these results strongly suggest that *NILNompC* is a classical mechanotransduction channel playing important roles in proprioception and locomotion. In addition, since NompC plays a role in discriminating food texture, the adverse effect of *NILNompC* silencing on food consumption observed in the current study would be due to lacking the ability of detecting food structures causing failure in recognizing rice as preferred food. Further studies are required to prove this hypothesis.

NILNompC showed expression in all developmental stages and the examined tissues, suggesting that the gene has essential physiological functions. The lowest expression was found in eggs and 5th instar nymphs, the two developmental stages when locomotion is less important. High expression was found mainly in the adult stage. We speculate that the *NILNompC* may function as a mechanosensory receptor. Adults are more in need of the *NILNompC* than nymphs in order to find mates and oviposition sites, and escape from harmful stimulations (Turner et al., 2016). Although the tissue-specific expression profiling showed that *NILNompC* was ubiquitously expressed, and the highest relative expression was in abdomen and antenna. DmNompC also is mainly expressed in the body wall of *Drosophila* larvae and in the mechanosensory organs of adult (Cheng et al., 2010; Yan et al., 2013). Further studies of DmNompC indicated that it is expressed in a cluster of 60–70 medial chordotonal neurons in Johnston's organ, which is known as a detector of near-field sound (Sun et al., 2009; Yack, 2004). In addition, the dsRNA injected BPH had larger angle of femur-tibia joint in the hindlegs. Injection of afidopyropen or pymetrozine was shown to produce similar symptom (hindleg extension) in American grasshoppers (*Schistocerca americana*), and similar symptom was produced by silencing mechanosensory neurons in femoral chordotonal organs (Kandasamy et al., 2017).

Touch sensation is essential for behaviors ranging from

environmental exploration to social interaction (Yan et al., 2013). Knowing the difference between innocuous and noxious mechanical stimuli is critical for survival of insects. The proteins responsible for detecting mechanical stimuli have been identified in *Caenorhabditis elegans* and *Drosophila* (Geffeney and Goodman, 2012; Yan et al., 2013). Previous studies have shown that touch sensing requires the activity of distal leg mechanosensory sensilla neurons and the mechanosensory channel NompC (Walker et al., 2000; Yan et al., 2013). Touching response belongs to proprioception, which refers to the sensory input and feedback by which animals keep track of and control the different parts of their bodies for balance and locomotion (Cheng et al., 2010). When thoracic segments of BPH were touched by a brush, the planthopper would escape from the stimulus, or responded by shaking their body, crawling away or even jumping away (Fig. 5A). The BPH injected with ds*GFP* exhibited normal behavioral response to the gentle-touch (Fig. 5C). However, when the brown planthoppers injected with ds*NILNompC* were touched by the brush, we observed that the touching response and locomotion were impaired (Movie S1). This suggests that *NILNompC* may play an essential role for the gentle touch sensation and proprioception. The absence of *NILNompC* resulted in difficult of adapting the living in the environment and more likely to die. *NompC* mutant larvae of *Drosophila* exhibited a dramatical reduction in crawling speed, and the majority of the homozygous larvae (*NompC*¹, *NompC*² and *NompC*³) died at the first or third instar stage (Cheng et al., 2010). Our RNAi assay proved that *NILNompC* absence caused high mortality of *N. lugens*.

In conclusion, the results of the current study imply that *NILNompC* is an essential part of mechanotransduction channel required for the survival of brown planthopper.

Acknowledgements

This research was supported by the National Key R&D Program of China (2018YFD0200300) and the National Natural Science Foundation of China (No. 31471804, 31672068 & 31772205).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ibmb.2018.11.005>.

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