MOLECULAR CHARACTERIZATION OF THE GENE ENCODING CHECKPOINT HOMOLOG 1 (CHK1) IN DAPHNIOPSIS TIBETANA SARS (CRUSTacea: CLADocera) AND ITS EXPRESSION AT DIFFERENT REPRODUCTIVE STAGES

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Abstract. Checkpoint homolog 1 (encoded by Chk1) plays an important role in the growth and development of water flea. In this study, the full-length cDNA of Chk1 encoding an aquaporin from Daphniopsis tibetana was isolated by rapid amplification of cDNA ends-PCR (GenBank ID. MW561428.1). The DtChk1 cDNA was 2025 bp long with a 318-bp 5'- untranslated region (UTR) and a 216-bp 3'-UTR containing a stop codon (TGA). DtChk1 cDNA was 1494 bp in open reading frame and encoded a 497-aa polypeptide. At the amino acid sequence level, DtChk1 showed high homology with homologs in Daphnia magna (78%), Daphnia carinata (77%), and Daphnia pulex (73%). Its homology with Chk1 sequences from other species ranged from 26% to 45%. Comparison of the deduced amino acid sequence of DtChk1 with homologs in other species revealed a high degree of conservation of the residues and domains that are essential for biological evolution. Using real-time quantitative PCR, we investigated DtChk1 gene transcript levels in male and female D. tibetana at different reproductive phases. The transcript levels of DtChk1 were significantly higher in sexual males than in parthenogenetic and sexual females (P<0.05), and significantly higher in sexual females than in parthenogenetic females (P<0.05). We will discuss the potential uses of DtChk1 in ecotoxicological studies in the future.

Keywords: Saline cladoceran, Daphniopsis tibetana, checkpoint homolog 1 (Chk1), reproductive status, ecotoxicology

Abbreviations: DtChk1, gene encoding checkpoint homolog 1 from Daphniopsis tibetana; ATM, the ataxia telangiectasia-mutated gene; ATR, ataxia telangiectasia-mutated gene; RT-PCR, reverse transcription polymerase chain reaction; qPCR, quantitative PCR; BLAST, basic local alignment search tool; RACE-PCR, rapid amplification of cDNA ends polymerase chain reaction; ORF Finder, open reading frame finder; pl, isoelectric point; N-J method, Neighbor-Joining method; Q-PCR, quantitative real time polymerase chain reaction; S-TKc, serine/threonine-protein kinase; SQ/TQ, Ser-Gln/Thr-Gln

Introduction

Checkpoint homolog 1, encoded by Chk1, is a type of serine/threonine-protein kinase with a highly conserved structure and function. When DNA is damaged, the ataxia telangiectasia-mutated gene (ATM) and ataxia telangiectasia-mutated gene (ATR) are activated immediately and phosphorylates Ser317 (S317) and Ser345 (S345) of Chk1 to activate the kinase (Furnari et al., 1997; Xiao et al., 2003). After activation, Chk1 can further act on its downstream protein Cdc25A/B/C, which can end the cell cycle in S and G2/M phase arrest for repairing damaged DNA, thereby regulating the progression of the cell cycle checkpoint (Chen and Sanchez, 2004; Merry et al., 2010). Genes encoding Chk1
proteins are present in the genomes of budding yeast, crustacea, insects, birds, amphibians, and mammals (Chen and Sanchez, 2004; Hajime et al., 2005; Kong et al., 2016). Moreover, other studies about Chk1 have been used in the treatment of cancer therapies (Chen et al., 2009; Merry et al., 2010).

Cladocera, or water fleas, are an important group of aquatic microcrustaceans in the Crustacea (Xu et al., 2009; Fang et al., 2015; Kong et al., 2016). Previous studies have detected Chk1 genes in members of the Cladocera, including Daphnia magna, Daphnia pulex, and Daphnia carinata at different reproductive stages. Studies on the function of Chk1 suggest that it plays a vital role in both the growth and development of these three freshwater fleas, especially in their reproductive plasticity (Hajime et al., 2005; Fang et al., 2015; Kong et al., 2016). Daphniopsis tibetana, a saline cladoceran crustacean water flea, inhabits nutrient-poor salt lakes in elevated or alpine regions of Tibet, Xinjiang, and Qinghai Province in China (Zhao et al., 2016; Wang et al., 2019). It is the dominant species of zooplankton in the food web of Chinese salt lake ecosystems (Zhao et al., 2005).

Over the last 20 years, D. tibetana has been used as a test organism in marine ecological studies (ecotoxicology), in evolutionary and developmental biology research, and as a food source for marine fish and shrimp larvae (Zhao et al., 2005, 2017; Wei et al., 2018). This species can produce offspring by parthenogenesis or by sexual reproduction. One of the important characteristics of D. tibetana is its cyclical reproduction system. It can reproduce by parthenogenesis or by sexual reproduction depending on stimuli from the external environment. While several studies have focused on its cyclical parthenogenesis (Zhao et al., 2017; Wei et al., 2018; Wang et al., 2019), few have focused on the molecular mechanism of its reproductive plasticity.

In this study, we used molecular tools to explore the potential reproductive plasticity of Chk1 in D. tibetana during different reproductive phases. The full-length Chk1 cDNA from D. tibetana was cloned and characterized, and the transcript levels of Chk1 in sexual individuals (males and females) and parthenogenetic females were determined by quantitative reverse transcription polymerase chain reaction (RT-PCR) and compared. The results of this study provide new information about the molecular mechanisms of the reproductive plasticity of D. tibetana, and indicate that the Chk1 gene has potential applications in ecotoxicological research.

Materials and methods

Animal preparation

Daphniopsis tibetana was collected from Lake Namukacuo in September 2018 (Bange County, Tibet, China) and cultured in a closed laboratory at a controlled temperature of 16°C.

Domestication and acclimation of D. tibetana were achieved as described by Zhao et al. (2017). First, the salinity level of lake water was decreased by adding boiled pure water. The lake water was slowly replaced with boiled seawater (obtained off shore of Heishijiao, Dalian, China). After 6 months, D. tibetana was successfully acclimated and cultured in diluted seawater with a salinity of 20‰. Under these conditions, it was able to reproduce and develop normally.

During domestication and cultivation, D. tibetana was fed with four species of algae mixed with a specific volume ratio, 1 (Chlorella pyrenoidosa) : 1 (Chaetoceros muelleri) : 1 (Dunaliella salina) : 2 (Isochrysis zhanjiangensis) (Wei et al., 2018).
The main difference between female and male *D. tibetana* is the first pair of antennae. The first antennae of mature males are up to 6 mm long and have long bristles at the end. The long bristles appear as the length of newborn males increases (Fig. 1A and Fig. 1i). In contrast, the first antennae of females are undeveloped, very short (<0.5 mm), and have nine olfactory cilia (Fig. 1Bii and Fig. 1Cii). The main difference between parthenogenetic and sexual females are that the former produce parthenogenetic eggs (Fig. 1b) and the latter produce sexual eggs (Fig. 1c).

To avoid potential inaccuracies arising from using different individual animals in these experiments, we obtained synchronous female offspring from the same mother. The offspring were continuously cultured and the parents were removed. This allowed us to obtain a population of synchronous pregnant females for subsequent experiments (Wang et al., 2019).

Forty females were selected from the synchronized *D. tibetana* population, and each individual was incubated in a separate 100-mL beaker with an adequate amount of mixed algae. After the first larvae were produced, the newborn larvae were carefully removed and examined under a microscope (Germany) to confirm that they were all female. The mother *D. tibetana* that produced all-female larvae were identified as parthenogenetic females.

1. Parthenogenetic female group: Ten mature parthenogenetic females were aspirated into cell culture plates containing 1 mL culture medium to ensure 10 replicates. They were cultured under the following conditions: culture solution, boiled diluted seawater with salinity of 20‰; temperature, 16°C; photoperiod, 12 h light:12 h dark, with natural light at 700–900 lx.

2. Sexual female group: Ten mature parthenogenetic females were aspirated into cell culture plates containing 1 mL culture medium to ensure 10 replicates. This group of females was kept inside at 16°C under natural light during the daytime, at then kept overnight at a constant temperature in the dark at 8°C in an incubator (Thermo Scientific, Rockford, IL, USA). The photoperiod was 12-h light and 12-h dark, like that in the parthenogenetic females group.

3. Sexual male group: Because sexual males and females co-occurred, the sexual male group was treated in the same way as the sexual female group.

To avoid the influence of metabolites on the results, the culture medium was changed every 2 days. After 3 weeks, 10 mature individuals (body length 1.1±0.3 mm) were randomly selected from each of the three groups for molecular analyses.
**Total RNA extraction and first-strand cDNA synthesis**

Before extracting the total RNA from parthenogenetic females, the medium was replaced with algae-free medium, and the females were starved for 36 h. This step was necessary to reduce the impact of flea contents on total RNA. Total RNA was extracted using a SteadyPure RNA kit (Accurate Biology, Changsha, China) in accordance with the manufacturer’s instructions. The RNA concentration and its degree of integrity were determined by agarose gel electrophoresis and by a nucleic acid protein detector.

First-strand cDNA was synthesized using the PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China). Rapid amplification of cDNA ends (RACE)-PCR was carried out using a SMART Ter® RACE 5'/3' kit (TaKaRa). The 5'-RACE-ready cDNA templates and 3'-RACE-ready cDNA templates were synthesized in accordance with the manufacturer’s protocol. The DNA isolated from parthenogenetic females was stored at −20°C.

**Primer design**

Because there is a high degree of genetic homology between freshwater and saltwater fleas, we chose the *D. magna* sequence (GenBank accession number: XP_032794190.1) to design forward (F1) and reverse (R1) primers to amplify the conserved region of *DtChk1* (Table 1). Next, primers were designed for RACE-PCR (5'F, 3'R; Table 1) to amplify the whole gene sequence. Based on the full-length sequence and open reading frame (ORF) sequences of *DtChk1*, the gene-specific primers *DtChk1*-F3 and *DtChk1*-R3 (Table 1) were designed for quantitative PCR (qPCR). The primers q18SF and q18SR were used to amplify the reference gene (18SrRNA). All primers were synthesized by Sangon Biotech (Shanghai, China). The sequences of primers used for gene isolation, sequencing (Chk1-S and Chk1-V), and determination of transcript levels are shown in Table 1. All primers used in this study were designed using Primer 5.0 software.

**Table 1. Sequence and purpose of all primers used in this study**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Purpose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>CAGAGGGATGGCTCACAGG</td>
<td>Unigene sequence verification</td>
<td>Fang et al., 2015</td>
</tr>
<tr>
<td>R1</td>
<td>AGAAGCTAAACATCGCGCCT</td>
<td>Unigene sequence verification</td>
<td></td>
</tr>
<tr>
<td>5'R</td>
<td>GCAACAAGAAGACGCCACAGACC</td>
<td>5'-RACE reverse primer</td>
<td></td>
</tr>
<tr>
<td>3'F</td>
<td>CATCGCACTGAGTCTCTTGCG</td>
<td>3'-RACE forward primer</td>
<td></td>
</tr>
<tr>
<td>Chk1-S</td>
<td>GGTCGATGATGATCCAGG</td>
<td>Sequencing primer</td>
<td></td>
</tr>
<tr>
<td>Chk1-V</td>
<td>GTGGAATTGAGCAAGTAAAC</td>
<td>Sequencing primer</td>
<td></td>
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<tr>
<td><em>DtChk1</em>-F1</td>
<td>ACAGCGTCAGAACAAATGCC</td>
<td>Gene-specific forward primer</td>
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<tr>
<td><em>DtChk1</em>-R1</td>
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<td>Gene-specific reverse primer</td>
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<tr>
<td>q18SF</td>
<td>ACGATGCCAACCAGAATCCG</td>
<td>Internal primer</td>
<td></td>
</tr>
<tr>
<td>q18SR</td>
<td>TCTGTCAATCTCCAGTCCGG</td>
<td>Internal primer</td>
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**Cloning of cDNA fragment and full-length sequence of *DtChk1***

Using first-strand cDNA generated from parthenogenetic females as the template, the partial sequence of *DtChk1* was amplified using the PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa) with the following thermal cycling conditions: 42°C for 2 min followed by 4°C, 37°C for 15 min, 85°C for 5 s. The amplified product was stored at
−20°C. The PCR products were separated by electrophoresis in a 1% agarose gel, then cut out from the gel and purified using a MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa, Dalian, China).

The purified PCR products were either directly sequenced or ligated into the pMD™ 19-T vector (TaKaRa), transformed into Escherichia coli DH5α competent cells (TaKaRa), and then sequenced (Sangon).

Basic local alignment search tool (BLAST) sequencing results from 3′ and 5′ RACE-PCR were compared at the NCBI database (https://www.ncbi.nlm.nih.gov/), and confirmed the successful cloning of the full-length DtChk1. Next, to verify the accuracy of the obtained full-length DtChk1 sequence, we used the redesigned primers F2 and R2 to reamplify the full-length gene (Table 1). There was a high degree of homology between the obtained results and the splicing results, confirming that we had isolated the full-length DtChk1 cDNA.

**Bioinformatics analysis of DtChk1**

The NCBI BLASTX and BLASTP tools (https://www.ncbi.nlm.nih.gov/) were used for comparative analyses of gene and protein sequences. The NCBI Open Reading Frame (ORF) Finder (http://www.ncbi.nlm.nih.gov/gorf/orf.cgi) was used to select and predict ORFs and predict amino acid (aa) sequences.

DNAMAN V6 software was used to predict the amino acid composition and isoelectric point (pl) of the putative DtChk1 protein. The structural domains of the protein encoded by DtChk1 were analyzed using tools at the SMART website (http://smart.embl-heidelberg.de/). The protein secondary structure analysis and construction of the relevant figures were conducted using tools at the GORIV website (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html). The protein tertiary structure analysis and construction of the relevant figures were conducted using tools at the SWISS-MODEL website (https://swissmodel.expasy.org/). The amino acid sequences of other proteins encoded by Chk1 genes in other species were obtained from the NCBI ate control was included to assess reagent contamination or primer dimer generation.

To validate the specificity of the products, a melting curve analysis and gel electrophoresis were performed. The Chk1 gene transcript levels were quantified using the \( \Delta \Delta C_T \) method, and were normalized against that of 18S rRNA.

One-way ANOVA and multiple comparisons (Duncan’s test) were conducted using SPSS18.0 software to detect the statistical significance of the differences among groups.

**Results and analysis**

**Cloning of full-length DtChk1**

Using the cDNA of parthenogenetic females from *D. tibetana* as the template and gene-specific primers, a 700-bp fragment was obtained by PCR amplification. BLASTX and BLASTP searches at the NCBI database revealed that the amplified Chk1 sequence showed the highest homology with homologs in *D. magna* and *D. pulex*. On the basis of these results, we designed RACE primers. The 5′-RACE and 3′-RACE products were sequenced and used to generate the full-length DpChk1 cDNA (2025 bp) (GenBank ID. MW561428.1; Fig. 2).
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- 5268 -

1  AAA ATA ATT TTT GAC CCG ACG TGC GTC CAA CAC ATT TTC CGA GCG  45
46  CAC CGT AAA ACG CGG CAA CGA CGG CAA ACA ACA GAA GTT TGA CAA  90
91  CAA GCT GAC GAA ATA TTT TGA GTC AAA ATG ACA CCT GTC TTG TTA 135
136  ATG AAA TCA ATT GCG TGA TTT CTG TAA TTA CAG CTC AAC CTG TGG 180
181  GTG AAA ATT CAT GAA ATT TTA TCA ACT AGC CAC AGG AAA GAC GGG 225
226  GAA GGC GAT AAA TAG CTG TCA TTC TGT CGT TTA TAC GGT CTT TGT 270
271  GTA CTG GCT GTG GTC TGG GTG GTG TGG GAT GAT ATT TAT CCT 315
316  ACC ATG GAA GAA AAG AGT AAA AAG TAT GAA GGA AAG AAC AGT GTT 360
0  MEE K S K K Y EG K N S V  14
361  ATT GTA TTT GAT GGA GTT TGG GAT ATG ATA CAG ACC CTG GTT GAA 405
15  LF V EG WD MI Q TL GE  29
406  GGA GCT TTT GGA GAG GTG AAA CTC CTA GTC AAT GCA AAA ACT GGA 450
30  GAFG EV KL LLV NAK TG  44
451  GAA GCG GTG GCA ATG AAA GAA ATT GAT TTA AAG AAG CAT ATC AAC 495
45  EAVAMKVIDLKKHIN  59
496  GCT GCC GAA ACA GTG AAA AAA GAA GTT TGT CTT CAC CGA ATG TTG 540
50  AAS EVK KEV C VHRML  74
541  AAT GAC CCT CAT GTC ATT CGG TTT TAT GGC AGA AGG GAA AAT GGC 585
75  NDPHVIFRYGYGRREN  89
586  AAC TTT GAA TTC ATT TTT TGT GAG TAT GCA AGT GGT GGA GAG TTG 630
90  NF F EF I FLE Y A S G GE L  104
631  TTT GAC AGA ATA GAA CCT GAT GTG GAA ATG CCC CA A ATG GAA GCT 675
105 FDRIEVPDVMGPQMEA  119
676  CAG CTG TAT TTT AAA CAA CTG ATT GCT GGT GTA AAG TAC TTT CAT 720
120 QR YFKQQLIAAGVNYLH  134
721  AGC AGA GGA GTT GCT CAT AGA GAC ATA AAA CCC GAA AAC TTG CTT 765
135 S R G V A H R D I K P E N L L  149
766  TTG GAT GCT CAT ATT GAT AAA TTA AAA ATA TCC GAT TTT GGA ATG GGC 810
150 LDANSDNLKISDFGMA  164
811  ACA ATC TTT AGA TTT CAA GCC GCA GAA AGA CAT CTG CAT AAA CGC 855
165 TIFRFQGRERHLDKR  179
856  TGT GGA ACT TTG CCT TAC ATA GCT CTT GGA GTG CTT TGT GGC AAG 900
180 CG TLP YIAP E V L CRK  194
901  TAC GCA GCA GAG CGG GCT GAT ATT TGG TCT GTC GGC GTC GTG CTT 945
195 YAAEPADIW S C G V V  209
946  GTT GCC ATG TTA GCT GGA AAA TTA CCT TTG GAC GTA CCT TCC AAT 990
210 VAMLAGELP WD VPSN  224
991  GAT TGG CCC CTG TAT ACA TCA TGG AAA GAA TGT CAC ATC AGC CAA 1035
225 DCP LTYT SW KE QCQITR  239
1036 TTG CCA TGG ACA AAG ATT GAT GCG CCT GCT TTG TCT TTA CTG AAG 1080
240 LPW T K I DTLALS LLR  254
1081 AAA GTC CTG ATG CCT CTA CCT GGG AAG CTG ACT ATC CAA CAA 1125
255 KVLP GLPKRYT IQQ  269
1126 ATA ACC AAT CAT CAG TGG TTT CAA AAG AAA TTC AAA GTT TCA AGT 1170
Figure 2. Full-length nucleotide sequence of DtChk1 and deduced amino acid sequence of DtChk1, protein kinase C phosphorylation site (blue with underline), casein kinase II phosphorylation site (red with underline), amidation site (black with underline), N-myristoylation site (green with underline), cAMP-and cGMP-dependent protein kinase phosphorylation site (purple with underline), GxGxxG field (yellow background), the SQ/TQ domain had several conserved Ser-Gln (SQ) or Thr-Gln (TQ) motifs (grey background), start codon (ATG with box), stop codon (TGA with box and the asterisk).
**Analysis of DtChk1 sequence and the encoding protein structure**

The full-length cDNA was 2025 bp long, with a 318-bp 5′-untranslated region (UTR) and a 216-bp 3′-UTR containing a stop codon (TGA). The theoretical pI of the encoded protein was 8.66. The 1494 bp ORF encoded a polypeptide of 497-aa (Fig. 2).

Structural analyses of DtChk1 protein sequence revealed a serine/threonine-protein kinase (S-TKc) structural domain (aa positions 21–275, Fig. 3). The theoretical secondary structure is shown in Fig. 4, and included α-helices consisting of 177 amino acids (34.14%), extended strands consisting of 87 amino acids (17.51%), and random coils consisting of 239 amino acids (48.09%). β-Pleated sheets were rare.

The tertiary structure analysis revealed that α-helices occupied a larger space than did β-pleated sheets, consistent with the predicted secondary structure (Fig. 5).
Analysis of DtChk1 amino acid homology

The predicted amino acid sequences encoded by Chk1 genes from eight species, including D. tibetana, were compared in a homology analysis (GenBank information is shown in Table 2, Fig. 6). The predicted DtChk1 polypeptide had a typical Ser-Gln (SQ)/Thr-Gln (TG) structural domain, as well as conserved SQ and TQ sequences.

Table 2. Accession numbers of Chk1 genes from various species

<table>
<thead>
<tr>
<th>Species name</th>
<th>GenBank No.</th>
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<tr>
<td>Daphniopsis tibetana</td>
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<tr>
<td>Limulus polyphemus</td>
<td>XP_013782750.1</td>
</tr>
<tr>
<td>Culex quinquefasciatus</td>
<td>XP_038113736.1</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>XP_037775034.1</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>NP_001370816.1</td>
</tr>
<tr>
<td>Daphnia carinata</td>
<td>AJP08956.1</td>
</tr>
<tr>
<td>Daphnia pulex</td>
<td>AGN95867.1</td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>XP_032794190.1</td>
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<td>Homo sapiens</td>
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<tr>
<td>Mus musculus</td>
<td>NP_031717.2</td>
</tr>
</tbody>
</table>

Figure 6. Comparison of amino acid sequence of DtChk1 with Chk1 proteins in other species
DtChk1 showed the highest homology with its homolog in *D. magna* (78%), followed by *D. carinata* (77%) and *D. pulex* (73%), and homology ranging from 26% to 45% to its homologs in the other species (*Limulus polyphemus*, *Penaeus monodon*, *Culex quinquefasciatus*, and *Caenorhabditis elegans*) (Fig. 6).

In the phylogenetic tree, *Homo sapiens* and *Mus musculus* were clustered as a single group. Invertebrates were clustered into a single group (*D. tibetana*, *D. magna*, *D. carinata*, and *D. pulex*). The closest relative of *D. tibetana* was *D. magna*. The remaining species (*L. polyphemus*, *P. monodon*, *C. quinquefasciatus*, and *C. elegans*) were also clustered into a single group (Fig. 7).

**Figure 7.** Neighbor-joining tree showing relationships among DtChk1 and homologs from other species, *indicates invertebrates and ** indicates vertebrates

**Analysis of DtChk1 expression**

The transcript levels of *DtChk1* were significantly higher in sexual males than in parthenogenetic and sexual females (*P*<0.05), and significantly higher in sexual females than in parthenogenetic females (*P*<0.05; Fig. 8).

**Figure 8.** Transcript levels of DtChk1 in males and females at different reproductive stages
Discussion

*Daphniopsis tibetana*, a salt water flea found in parts of China, is a food source for marine fish and shrimp larvae. Furthermore, it has become a popular test organism for monitoring in marine ecotoxicology research after its domestication. Thus, molecular studies on its reproductive state are of great ecological and technological significance.

In this study, BLAST \textsubscript{X} and BLAST \textsubscript{P} analyses and multiple sequence comparisons revealed a domain with catalytic activity typical of a serine/threonine-protein kinase in the putative *DtChk1* protein. These results confirmed that *DtChk1* is indeed a serine/threonine-protein kinase.

The amino acid sequence of *DtChk1* was compared with those encoded by homologous genes in related species, and showed the highest homology with those in *D. magna*, *D. carinata*, and *D. pulex*. This finding suggests that *Chk1* plays a vital role in the development and growth of both salt water and fresh water fleas (Hajime et al., 2005; Fang et al., 2015; Kong et al., 2016). As well as showing homology with Chk1 proteins of other crustaceans (four species of water fleas), *DtChk1* also showed homology with related proteins in insects, supporting the theory that crustaceans and insects share a common ancestor, i.e., the “Pancrustacea” theory (Song, 2006).

The transcript levels of *DtChk1* were higher in sexual males than in parthenogenetic and sexual females, and higher in sexual females than in parthenogenetic females. This suggests that sexual males and females have dramatic changes in their genetic information (DNA damage) along with environmental degradation, because *Chk1* and its cooperating proteins are involved in repairing damaged DNA and regulating the cell cycle to ensure the stability and integrity of the cellular genome (Chen and Sanchez, 2004; Merry et al., 2010; Kong et al., 2016).

Our findings are consistent with those reported for *Chk1* genes in *D. magna*, *D. carinata*, and *D. pulex*, indicating that there are certain similarities between salt water fleas and freshwater fleas (Hajime et al., 2005; Fang et al., 2015; Kong et al., 2016). Because *DtChk1* is involved in the process of DNA damage and repair during potential reproduction, its expression level may be a useful indicator of the toxic effects of environmental pollutants such as heavy metals, persistent organic pollutants, and ultraviolet radiation. Further studies are underway to determine the suitability of other potential reproductive genes (Transformer gene, *Doublesex1* gene, *Hsp90* gene) (Kong et al., 2015; Kato et al., 2018; Telli et al., 2020) from *D. tibetana* for use in ecotoxicological studies.

Conclusion

We obtained the full-length sequence of the reproduction-related gene *Chk1* of *D. tibetana*, and analyzed the gene sequence, predicted protein sequence and structure, amino acid sequence homology, and the transcript levels of *DtChk1* in male and female *D. tibetana* in different reproductive states. In the future, the results can be used for ecotoxicological studies.
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Author contributions. YIN, D. P: Data curation, Writing-Original draft preparation, Writing-Reviewing and Editing. ZHAO, W: Conceptualization, Methodology, Writing - Reviewing and Editing. ZHANG, Y: Test animals cultivation, Data curation. WEI, J: Visualization, Investigation. WANG, S: Software, Validation. BAO, X. B: Methodology, Software, Supervision.

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Declaration of competing interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES


