Gnathostoma spinigerum
Mitochondrial Genome Sequence: a Novel Gene Arrangement and its Phylogenetic Position within the Class Chromadorea

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Human gnathostomiasis is an emerging food-borne parasitic disease caused by nematodes in the genus *Gnathostoma*. In spite of their significance as pathogens, these parasites remain poorly understood at the molecular level. In the present study, we sequenced the mitochondrial (mt) genome of *G. spinigerum*, which infects a range of definitive hosts including dogs, cats, tigers, leopards and humans. The mt genome of *G. spinigerum* is 14,079 bp in size and shows substantial changes in gene order compared to other nematodes studied to date. Phylogenetic analyses of mt genome sequences by Bayesian inference (BI) revealed that the infraorder Gnathostomatomorpha (represented by *G. spinigerum*) is closely related to the infraorder Ascaridomorpha. *G. spinigerum* is the first species from the infraorder Gnathostomatomorpha for which a complete mt genome has been sequenced. The new data will help understand the evolution, population genetics and systematics of this medically important group of parasites.

Human gnathostomiasis caused by *Gnathostoma* spp. is a highly endemic disease in some under-developed communities in Asia, particularly in China. Recently, it has become an emerging disease among travelers from Europe and other continents who are coming into contact with endemic areas. Nematodes of the genus *Gnathostoma* (Nematoda: Gnathostomatidae) are the etiological agents of human gnathostomiasis and may also infest dogs, cats, tigers and leopards. There are 12 species in this genus with four species recorded in humans: *G. spinigerum*, commonly found in India, China, Japan and southeast Asia; *G. hispidum*, found in Europe, Asia and Australia; *G. doloresi*, found in southeast Asia; and *G. nipponicum*, found in Japan. *G. spinigerum* is frequently reported in Asia as being responsible for human gnathostomiasis, but *G. hispidum*, *G. doloresi*, and *G. nipponicum* have also occasionally been reported. Humans are infected when consuming raw or uncooked infected meats of intermediate hosts (fish) or other paratenic hosts (snake, pig and poultry). The majority of gnathostomiasis patients present with cutaneous lesions; involvement of eyes and central nervous system has also been sporadically reported.

The genus *Gnathostoma* is in the infraorder Gnathostomatomorpha of the suborder Spirurina. There are four other infraorders in this suborder: Ascaridomorpha, Spiruromorpha, Rhigonematormorpha and...
Oxyuridomorpha\textsuperscript{10}. The phylogenetic relationships among the infraorders of the Spirurina have been assessed using nuclear small subunit (SSU) rRNA (five infraorders) gene and mitochondrial (mt) gene/genome sequences (four infraorders) and there are inconsistencies between the nuclear phylogeny and the mt phylogeny\textsuperscript{11–18}. In the phylogeny inferred from SSU rRNA gene sequences, the Ascaridomorpha is sister to the Rhigonematomorpha, and the Spiruromorpha is sister to the Ascaridomorpha + Rhigonematomorpha, the Oxyuridomorpha is sister to the Spiruromorpha + Ascaridomorpha + Rhigonematomorpha, and the Gnathostomatomorpha is sister to the Oxyuridomorpha + Spiruromorpha + Ascaridomorpha + Rhigonematomorpha\textsuperscript{12,19}. In the mt gene/genome phylogeny, however, the Ascaridomorpha is sister to the Rhabditomorpha + Diplogasteromorpha in most analyses, and the Spiruromorpha is sister to the Rhabditomorpha + Diplogasteromorpha + Ascaridomorpha + Rhigonematomorpha + Panagrolaimomorpha + Tylenchomorpha + Oxyuridomorpha\textsuperscript{15–17}. More recently, Kim \textit{et al.}\textsuperscript{20} inferred the phylogeny with mt genome sequences and showed that the Rhigonematomorpha is sister to the Ascaridomorpha. Taxon sampling was limited in both the nuclear SSU rRNA and the mt gene/genome phylogenetic analysis; furthermore, no species from the Gnathostomatomorpha was included in any of these mt analyses.

Animal mt genomes are typically a circular DNA, 15–20 kb in size, containing 36–37 genes: 12–13 protein-coding genes (PCGs), 22 transfer RNAs (tRNA) and two ribosomal RNA (rRNA) genes\textsuperscript{21,22}. Mt genome sequences are commonly used for phylogenetic, population genetic and taxonomic investigations of animals\textsuperscript{23,24}. To understand the phylogenetic relationship of the infraorder Gnathostomatomorpha with other infraorders of the class Chromadorea, we sequenced the mt genome of \textit{G. spinigerum}.

Results and Discussion

General features of the mt genome of \textit{G. spinigerum}. The complete mt genome of \textit{G. spinigerum} (GenBank accession no. KP410547) was 14,079 bp in size (Fig. 1). This genome contains 12 PCGs (\textit{cox1-3}, \textit{nad1-6}, \textit{atp6} and \textit{cytb}), 22 tRNA genes, two rRNA genes (\textit{rrnL} and \textit{rrnS}) and two non-coding (AT-rich) regions. All genes are transcribed in the same direction. As in most other nematodes of the class Chromadorea, \textit{atp8} gene is not present in the mt genome of \textit{G. spinigerum} (Table 1). The mt genome sequence of \textit{G. spinigerum} is biased toward A and T (71.1%), similar to that of other nematodes in the subclass Spirurina\textsuperscript{25–28}. This nucleotide composition of the 12 PCGs of \textit{G. spinigerum} was strongly skewed away from A, in favour of T (AT skew between $-0.58$ and $-0.27$), and the GC skew was between 0.41 and 0.77 (Table 2). Codons composed of A and T were more frequently used in PCGs, reflecting the high A + T content in the mt genome of \textit{G. spinigerum}. The most frequently used amino acid was TTT (Phe; 13.14%), followed by TTG (Leu; 8.79%), ATT (Ile; 5.96%) and GTT (Val; 5.46%) (Table 3). ATA, TTG and ATT were used as initiation codons and TAA and TAG as termination codons; incomplete
termination codons (T or TA) were also identified (Table 1), which is consistent with the arrangement in the mt genomes of other nematodes. Twenty-two tRNA genes were identified in the mt genome of *G. spinigerum*, which range from 54 to 68 bp in size. The secondary structures inferred for the 22 tRNAs of *G. spinigerum* are similar to those of other nematodes. *rrnL* is located between *trnH* and *nad3* in the *G. spinigerum* mt genome; *rrnS* is between *trnE* and *trnS* (Table 1). The two non-coding regions in the mt genome of *G. spinigerum* were located between *trnI* and *trnN* (designated NCL, 750 bp), and between *nad1* and *atp6* (designated NCR, 77 bp) respectively. No repetitive sequences were detected in the non-coding regions of *G. spinigerum*, as in other Spirurina nematodes.

**Gene arrangement in the mt genome of *G. spinigerum***. The mt genome of *G. spinigerum* shows a different gene arrangement pattern from the other 29 patterns seen in nematodes revealed by previous

| Genes        | Positions | Lengths (bp) | Start codons | Stop codons | Anticodons |
|--------------|-----------|--------------|--------------|-------------|------------|
| cox1         | 1–1572    | 1572         | ATA          | TAG         |            |
| trRNA-Cys (C)  | 1573–1629  | 57           |              |             | GCA        |
| trRNA-Lys (K)  | 1629–1691  | 63           |              |             | TTT        |
| trRNA-Met (M)  | 1699–1752  | 53           |              |             | CAT        |
| trRNA-Asp (D)  | 1753–1808  | 56           |              |             | GTC        |
| trRNA-Gly (G)  | 1813–1868  | 56           |              |             | TCC        |
| cox2         | 1869–2556  | 688          | TTG          | T           |            |
| trRNA-His (H)  | 2557–2612  | 56           |              |             | GTG        |
| rrnL         | 2613–3554  | 942          |              |             |            |
| nad3         | 3555–3890  | 336          | TTG          | TAA         |            |
| nad5         | 3894–5478  | 1585         | TTG          | T           |            |
| trRNA-Ala (A) | 5479–5533  | 55           |              |             | TGC        |
| trRNA-Pro (P) | 5535–5592  | 58           |              |             | TGG        |
| trRNA-Leu UUR (L) | 5598–5652  | 55           | TAA          |             |            |
| trRNA-Ser UCN (S1) | 5651–5710  | 60           |              |             | TCT        |
| nad2         | 5721–6553  | 833          | ATA          | TA          |            |
| trRNA-Ile (I) | 6554–6609  | 56           |              |             | GAT        |
| Non-coding   | 6610–7359  | 750          |              |             |            |
| trRNA-Assn (N) | 7360–7416  | 57           |              |             | GTT        |
| trRNA-Arg (R) | 7448–7504  | 57           | TCG          |             |            |
| trRNA-Gln (Q) | 7504–7557  | 54           | TTG          |             |            |
| trRNA-Phe (F) | 7557–7624  | 68           |              |             | GAA        |
| cytb         | 7648–8721  | 1074         | ATA          | TAG         |            |
| trRNA-Leu UCN (L1) | 8722–8776  | 55           | TAG          |             |            |
| cox3         | 8777–9544  | 768          | TTG          | T           |            |
| trRNA-Thr (T) | 9547–9601  | 55           | TGT          |             |            |
| nad4         | 9602–10826 | 1225         | TTG          | T           |            |
| trRNA-Tyr (Y) | 10827–10880 | 54          | GAT          |             |            |
| nad1         | 10881–11753 | 873         | TTG          | TAA         |            |
| Non-coding   | 11754–11830 | 77          |             |             |            |
| atp6         | 11831–12409 | 579         | ATT          | TAG         |            |
| trRNA-Val (V) | 12463–12517 | 55          | TAC          |             |            |
| nad6         | 12518–12955 | 438         | TTG          | TAG         |            |
| nad4L        | 12963–13190 | 228         | TTG          | TAG         |            |
| trRNA-Trp (W) | 13191–13247 | 57          | TCA          |             |            |
| trRNA-Glu (E) | 13255–13311 | 57          | TTC          |             |            |
| rrnS         | 13312–13985 | 674         |              |             |            |
| trRNA-Ser UCN (S2) | 13986–14039 | 54          | TGA          |             |            |

Table 1. The organization of the mitochondrial genome of *Gnathostoma spinigerum*.
Additionally, previous studies have suggested that several major lineages including the infraorder Gnathostomatomorpha were not monophyletic in phylogenetic analyses. Our Bayesian analysis showed that the infraorder Rhigonematomorpha was paraphyletic with respect to the infraorder Ascaridomorpha, which was most closely related to G. spinigerum (infraorder Gnathostomatomorpha) than they were to other six species from the infraorders Ascaridomorpha, Rhabditomorpha, Spiruromorpha, Oxyuridomorpha, and Gnathostomatomorpha. These controversial findings, along with the results from previous studies (pattern GA26 hereafter; see 17 for GA1-25; GA27 for Rhigonema thysanophora20; GA28-30 for Meloidogyne chitwoodi, M. graminicola and M. incognita, respectively22,33,35–37), suggest that the evolution of mt gene order in nematodes was mostly driven by transposition and TDRL events, with reverse-transposition playing a minor role relatively. Phylogenetic analyses. We inferred the phylogenetic relationship between G. spinigerum and other 57 species of Chromadorea nematodes with concatenated amino acid sequences of the 12 mt PCGs (Fig. 4). Phylogenies of the Chromadorea nematodes were inferred with mt genome sequences in previous studies14–18; however, several major lineages including the infraorder Gnathostomatomorpha were not represented.

Our Bayesian analysis showed that G. spinigerum was most closely related to Cucullanus robustus with moderate support [Bayesian posterior probabilities (Bpp) = 0.88, Fig. 4]. Our maximum likelihood (ML) and maximum parsimony (MP) analyses also recovered this relationship but the bootstrapping frequency (Bf) was weak (not shown). This grouping was inconsistent with those from morphological studies14–18, although the infraorder Ascaridomorpha was monophyletic in the present study (Bpp = 0.67, Fig. 4). The two species from the two families, Cucullanidae and Ascaridiidae, of the infraorder Ascaridomorpha were more closely related to G. spinigerum (infraorder Gnathostomatomorpha) and R. thysanophora (infraorder Rhigonematomorpha) than they were to other six species from the infraorder Ascaridomorpha. R. thysanophora was most closely related to Ascaridia galli (Bpp = 0.67). A recent study based on mt genome sequences also indicated that R. thysanophora was most closely related to an Ascaridia species20. Using nuclear SSU rRNA gene sequences, Meldal et al.12 showed that the infraorder Ascaridomorpha was sister to the group that included the infraorders Ascaridomorpha, Rhabditomorpha, Spiruromorpha, Oxyuridomorpha, and Gnathostomatomorpha. These controversial results surrounding phylogenetic placement of members in Spirurina may reflect the different evolutionary rates of the nuclear and mt genomes38,39.

Our analysis also showed that the infraorder Rhabditomorpha was paraphyletic with respect to the Diplogasteromorpha. Two species from the families Rhabditidae and Heterorhabditidae of the Rhabditomorpha were more closely related to Pristionchus pacificus (Neodiplogasteridae) than they were to other species from the same family. These results suggest that the Rhabditomorpha has been highly divergent over evolutionary time.
| Amino acid | Codon | Number | Frequency (%) | Amino acid | Codon | Number | Frequency (%) |
|------------|-------|--------|---------------|------------|-------|--------|---------------|
| Phe        | TTT   | 447    | 13.14         | Met        | ATA   | 92     | 2.70          |
| Phe        | TTC   | 13     | 0.38          | Met        | ATG   | 114    | 3.35          |
| Leu        | TTA   | 183    | 5.38          | Thr        | ACT   | 78     | 2.29          |
| Leu        | TCG   | 299    | 8.79          | Thr        | ACC   | 2      | 0.05          |
| Ser        | TCT   | 114    | 3.35          | Thr        | ACA   | 7      | 0.20          |
| Ser        | TCC   | 6      | 0.17          | Thr        | ACG   | 11     | 0.32          |
| Ser        | TCA   | 16     | 0.47          | Asn        | AAT   | 92     | 2.70          |
| Ser        | TCG   | 9      | 0.26          | Asn        | AAC   | 4      | 0.11          |
| Tyr        | TAT   | 177    | 5.20          | Lys        | AAA   | 43     | 1.26          |
| Tyr        | TAC   | 4      | 0.11          | Lys        | AAG   | 48     | 1.41          |
| Stop       | TAA   | 2      | 0.05          | Ser        | AGT   | 98     | 2.88          |
| Stop       | TAG   | 6      | 0.17          | Ser        | AGC   | 7      | 0.20          |
| Cys        | TGT   | 59     | 1.73          | Ser        | AGA   | 60     | 1.76          |
| Cys        | TGC   | 2      | 0.05          | Ser        | AGG   | 57     | 1.67          |
| Trp        | TGA   | 27     | 0.79          | Val        | GTT   | 186    | 5.46          |
| Trp        | TGG   | 46     | 1.35          | Val        | GTC   | 9      | 0.26          |
| Leu        | CTT   | 32     | 0.94          | Val        | GTA   | 62     | 1.82          |
| Leu        | CTC   | 1      | 0.02          | Val        | GTG   | 80     | 2.35          |
| Leu        | CTA   | 7      | 0.20          | Ala        | GCT   | 81     | 2.38          |
| Leu        | CTG   | 18     | 0.52          | Ala        | GCC   | 5      | 0.14          |
| Pro        | CCT   | 66     | 1.94          | Ala        | GCA   | 13     | 0.38          |
| Pro        | CCC   | 1      | 0.02          | Ala        | GCG   | 5      | 0.14          |
| Pro        | CCA   | 3      | 0.08          | Asp        | GAT   | 74     | 2.17          |
| Pro        | CCG   | 8      | 0.23          | Asp        | GAC   | 4      | 0.11          |
| His        | CAT   | 50     | 1.47          | Glu        | GAA   | 37     | 1.08          |
| His        | CAC   | 3      | 0.08          | Glu        | GAG   | 48     | 1.41          |
| Gln        | CAA   | 16     | 0.47          | Gly        | GGT   | 103    | 3.02          |
| Gln        | CAG   | 23     | 0.67          | Gly        | GGC   | 9      | 0.26          |
| Arg        | CGT   | 23     | 0.67          | Gly        | GGA   | 29     | 0.85          |
| Arg        | CGC   | 3      | 0.08          | Gly        | GGG   | 63     | 1.85          |
| Arg        | CGA   | 3      | 0.08          | Ile        | ATT   | 203    | 5.96          |
| Arg        | CGG   | 5      | 0.14          | Ile        | ATC   | 5      | 0.14          |

Table 3. Codon usage of *Gnathostoma spinigerum* mitochondrial protein-coding genes.

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**Figure 2.** The predicted secondary structures of representing tRNAs of the *Gnathostoma spinigerum* mitochondrial DNA determined in this study.
were to the other 22 species from the Rhabditomorpha. The close relationship between the species of the families Rhabditidae and Heterorhabditidae and *P. pacificus* was strongly supported in BI (Bpp = 1, Fig. 4). The results were consistent with that of a previous study using nuclear SSU rRNA gene dataset. The Oxyuridomorpha (2 species) and the Spiruromorpha (12 species) were both monophyletic with strong support in the present analysis (Bpp ≥ 0.67, Fig. 4). The nine species of the suborder Tylenchina included in this study were from two infraorders: Panagrolaimomorpha (2 species), and Tylenchomorpha (7 species). Both of these infraorders were paraphyletic in the present analysis (Bpp ≥ 0.67, Fig. 4). The Oxyuridomorpha and the Spiruromorpha were both monophyletic with strong support in the current analyses (Bpp = 1 for Oxyuridomorpha and Bpp ≥ 0.79 for Spiruromorpha, Fig. 4).

For decades, there have been controversies surrounding the systematics of the suborder Spirurina (infraorders Ascaridomorpha, Spiruromorpha, Rhigonematomorpha, Gnathostomatomorpha and Oxyuridomorpha). Given the demonstrated utility of mt datasets, there is now an opportunity to test the phylogenetic relationships of a wide range of Spirurina nematodes using expanded mt datasets. Analyses of mt genome sequences in the current study and several previous studies have provided insights into the phylogenetic relationships among major lineages of the Spirurina nematodes. However, some lineages of the suborder Spirurina are underrepresented or not represented in these analyses. So, more mt genome data from the suborder Spirurina would be required in future analyses to understand the phylogeny of the suborder Spirurina.

In summary, this is the first determination of a complete mt genome of a parasite belonging to the infraorder Gnathostomatomorpha. Although the length, gene and AT content are similar to other nematode mt genomes, the mt genome of *G. spinigerum* exhibits some interesting features. The gene order of *G. spinigerum* is distinct from that of other nematodes. Phylogenetic analysis shows that *G. spinigerum* was most closely related to *Cucullanus robustus* with moderate support, which is inconsistent with that from morphological and molecular studies. Our results provided insights into the phylogenetic relationships among several major lineages of nematodes.

**Methods**

**Ethics statement.** Specimens of *G. spinigerum* were collected from an Asian swamp eel, in accordance with the animal ethics procedures and guidelines China. All experimental protocols were approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

**Collection of *G. spinigerum* and DNA isolation.** Larval specimens of *G. spinigerum* were collected from an infected Asian swamp eel, *Monopterus albus*, imported from Indonesia, and were identified to species morphologically. The specimens were fixed in ethanol and stored at –20 °C until use. Total genomic DNA was isolated from individual worms using small-scale sodium dodecyl-sulphate (SDS)/proteinase K digestion and spin-column purification (Wizard® SV Genomic DNA Purification System, Promega). The identity of *G. spinigerum* specimens (coded GS5) was also verified by sequencing regions of ITS-2 and cox1 genes; both regions had 100% similarity with those of *G. spinigerum* from Thailand and Indonesia (GenBank accession Nos. AB181155 and JN408304).

**Long-PCR amplification and sequencing.** Fragments of *cox1* and *nad1* genes of *G. spinigerum* were amplified by PCR with primer pairs JB3-JB4 and JB11-JB12 (Table 4). After we obtained partial *cox1* and *nad1* sequences for *G. spinigerum*, we designed specific primers from these fragments for long PCR amplification. The complete mt genome of *G. spinigerum* (coded GS5) was amplified by long-PCR as two segments (~10 kb and ~4 kb) using genomic DNA extracted from a single specimen; the gaps between the two segments were filled by the short *cox1* and *nad1* fragments amplified initially. PCR was conducted in 25 μl using 2 mM MgCl₂, 0.2 mM each of dNTPs, 2.5 μl 10 × Taq buffer, 2.5 μl/M of each primer and 0.5 μl LA Taq DNA polymerase (5 U/μl, Takara) in a thermocycler (Biometra). The cycling conditions were: 92 °C for 2 min (initial denaturation), then 92 °C for 10 s (denaturation), 56 °C (10 kb) or 54 °C (4 kb) for 30 s (annealing), 72 °C for 1 min (extension).
30 s (annealing) and 60 °C for 10 min (extension) for 10 cycles, followed by 92 °C for 10 s, 56 °C (~10 kb) or 54 °C (~4 kb) for 30 s (annealing), and 60 °C for 10 min for 20 cycles, with a cycle elongation of 10 s for each cycle and a final extension at 60 °C for 10 min. PCR products were sequenced at Sangon Company (Shanghai, China) using a primer walking strategy.

Sequence analyses. Sequences obtained from the PCR amplicons of *G. spinigerum* were assembled manually and aligned with the mt genome sequences of roundworm and pinworm (GenBank accession numbers: NC_016128 and NC_011300) using the program MAFFT 7.122 to identify gene boundaries. The sequence of each protein-coding gene was translated into amino acid sequence using the invertebrate mt genetic code in MEGA 5; the amino acid sequences were aligned using default settings. tRNAscan-SE and ARWEN were used to identify all of the tRNA genes except *trnS*, which was identified manually by sequence comparison with *trnS* of other nematodes reported previously. The two

Figure 4. Phylogenetic relationships among 58 species of Chromadorea nematodes inferred from Bayesian inference of deduced amino acid sequences of 12 mitochondrial protein-coding genes. *Trichuris suis* (GenBank accession number GU070737) was used as the outgroup. Bayesian posterior probabilities (Bpp) values were indicated at nodes.
rRNA genes were identified by BLAST searches and were verified by sequence comparison with these two genes of other nematodes reported previously. Tandem repeats in the non-coding regions were found using Tandem Repeat Finder program (http://tandem.bu.edu/trf/trf.html)\(^5\). The rearrangement events in the mt genomes were modelled with CREx (http://pacosy.informatik.uni-leipzig.de/crex)\(^34\).

Phylogenetic analyses. We combined the mt genome sequence of *G. spinigerum* with those of selected 57 other Chromadorea nematodes (Table S1) retrieved from the GenBank for phylogenetic analysis; *Trichuris suis* (GenBank accession number GU070737) was used as the outgroup\(^51\). Amino acid sequences inferred from the sequences of 12 mt PCGs were aligned individually first using MAFFT 7.122 and were then concatenated to form a single dataset; ambiguously aligned regions were excluded using Gblocks 0.91b (doc)\(^52\) with the default parameters (allow smaller final blocks, allow gap positions within the final blocks and allow less strict flanking positions). Phylogenetic analyses were conducted using Bayesian inference (BI). The MtArt \(+I+G+F\) model of amino acid evolution was selected as the most suitable model of evolution by ProtTest 2.4\(^53\) based on the Akaike information criterion (AIC). As MtArt model is not implemented in the current version of MrBayes, an alternative model, CpREV, was used in BI and four chains (three heated and one cold) were run simultaneously for the Monte Carlo Markov Chain. Two independent runs for 2,000,000 metropolis-coupled MCMC generations, sampling a tree every 100 generations in MrBayes 3.1.154; the first 5,000 trees represented burn-in and the remaining trees were used to calculate Bpp. Bayesian analysis was run until the potential scale reduction factor approached 1 and the average standard deviation of split frequencies was less than 0.01. All sites were coded as unordered and equally weighted characters. The topology was reconstructed using the 50% majority rule and the support values were assessed by 1000 bootstrap replicates. Phylogenograms were drawn using the program FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree).

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### Table 4. Sequences of primers used to amplify PCR fragments from *Gnathostoma spinigerum*.

| Name of primer | Sequence (5’ to 3’) | Reference |
|----------------|---------------------|-----------|
| Short PCR      |                     |           |
| JB3            | TTTTTTGGGCCATCCCTGAGTTTAT | 42        |
| JB4.5          | GAATTCGTAAGGGGGCTATAA  | 43        |
| JB12           | ACCACTAATCTTCAATTCCTTC |           |
| Long PCR       |                     |           |
| GSCF           | GGTTTTCTCGATGATGTTTTCCTT |           |
| GSNR           | CCACCATCCTATCCAGCTTTCCCT |           |
| GSUF           | CTTGAGTCCGGTTTTGTAACATGT |           |
| GSCR           | GTACGCAACCATCTAAAAACCCTCA |          |

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**Author Contributions**
X.Q.Z., R.S. and G.H.L. designed the research. G.H.L. performed the research. X.Q.C. and W.W.L. contributed reagents/materials/analyses. G.H.L., R.S. and X.Q.Z. analyzed the data. G.H.L., R.S. and X.Q.Z. wrote the manuscript.

**Additional Information**

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