Complete mitochondrial genome of *Clistocoeloma sinensis* (Brachyura: Grapsoidea): Gene rearrangements and higher-level phylogeny of the Brachyura

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Deciphering the animal mitochondrial genome (mitogenome) is very important to understand their molecular evolution and phylogenetic relationships. In this study, the complete mitogenome of *Clistocoeloma sinensis* was determined. The mitogenome of *C. sinensis* was 15,706 bp long, and its A+T content was 75.7%. The A+T skew of the mitogenome of *C. sinensis* was slightly negative (−0.020). All the transfer RNA genes had the typical cloverleaf structure, except for the *trnS1* gene, which lacked a dihydroxyuridine arm. The two ribosomal RNA genes had 80.2% A+T content. The A+T-rich region spanned 684 bp. The gene order within the complete mitogenome of *C. sinensis* was identical to the pancrustacean ground pattern except for the translocation of *trnH*. Additionally, the gene order of *trnI-trnQ-trnM* in the pancrustacean ground pattern becomes *trnQ-trnI-trnM* in *C. sinensis*. Our phylogenetic analysis showed that *C. sinensis* and *Sesarmops sinensis* cluster together with high nodal support values, indicating that *C. sinensis* and *S. sinensis* have a sister group relationship. The results support that *C. sinensis* belongs to Grapsoidea, Sesarmidae. Our findings also indicate that Varunidae and Sesarmidae species share close relationships. Thus, mitogenomes are likely to be valuable tools for systematics in other groups of Crustacea.
its evolutionary status and rearrangement information by comparing it with complete Brachyuran mitogenomes available to date. This information may provide insights into phylogenetic rearrangement and enable phylogenetic analysis.
Methods
Sample and DNA Extraction. Adult specimens of *C. sinensis* were captured from Yancheng, Jiangsu province, China. Total genomic DNA was isolated from individual specimens using the Aidlab Genomic DNA Extraction Kit (Beijing, China). All procedures were completed following the manufacturer's instructions. The complete mitogenome was amplified from the DNA from one *C. sinensis* crab.

PCR Amplification and Sequencing. The complete mitogenome was obtained using a combination of conventional PCR and long PCR to amplify overlapping fragments spanning the whole mitogenome. Universal and specific primers were designed based on the conserved nucleotide sequences of known mitochondrial sequences in Brachyura (Table 1) and synthesized by Beijing Sunbiotech. The fragments were amplified using Aidlab Red Taq (Beijing, China) according to the manufacturer's instructions. All amplifications were performed on an Eppendorf Mastercycler and Mastercycler gradient in 50 µl reaction volumes with 5 µl 10 × Taq Buffer (Mg⁺²⁺) (Aidlab), 4 µl of dNTPs (2.5 mM, Aidlab), 2 µl of each primer (10 µM), 2 µl of DNA template (~30 ng), 34.5 µl ddH₂O, and 0.5 µl Red Taq DNA polymerase (5 U, Aidlab). PCR was performed using the following procedure: 94 °C for 3 min; followed by 40 cycles of 30 s at 94 °C, annealing for 35 s at 48–56 °C (depending on primer combination), and elongation at 72 °C for 10 min. The PCR products were separated by agarose gel electrophoresis (1% w/v) and purified using a DNA

Table 3. Summary of *Clistocoeloma sinensis* mitogenome.

| Gene     | Direction | Location       | Size  | Intergenic nucleotides | Anticodon | Start codon | Stop codon |
|----------|-----------|----------------|-------|------------------------|-----------|-------------|------------|
| cox1     | F         | 1–1535         | 1535  | 0                      | ATG       | TA          |
| trnL2    | F         | 1536–1601      | 66    | 0                      | TAA       |             |
| cox2     | F         | 1608–2295      | 688   | 0                      | ATG       | T           |
| trnK     | F         | 2296–2365      | 70    | 0                      | TTT       |             |
| trnD     | F         | 2366–2433      | 68    | 0                      | GTC       |             |
| atp8     | F         | 2434–2592      | 159   | –7                     | ATG       | TAA         |
| atp6     | F         | 2586–3259      | 674   | 0                      | ATT       | TA          |
| cox3     | F         | 3260–4050      | 791   | 0                      | ATG       | TA          |
| trnG     | F         | 4051–4115      | 65    | 0                      | TCC       |             |
| nad3     | F         | 4116–4466      | 351   | 2                      | ATT       | TAA         |
| trnA     | F         | 4469–4532      | 64    | 5                      | TGC       |             |
| trnR     | F         | 4538–4601      | 64    | 2                      | TCG       |             |
| trnN     | F         | 4604–4674      | 71    | 1                      | GGT       |             |
| trnS1    | F         | 4676–4743      | 68    | –1                     | TCT       |             |
| trnE     | F         | 4743–4810      | 68    | 9                      | TTC       |             |
| trnH     | R         | 4820–4886      | 67    | 0                      | GTG       |             |
| trnF     | R         | 4887–4951      | 65    | 4                      | GAA       |             |
| nad5     | R         | 4956–6686      | 1731  | 0                      | ATG       | TAA         |
| nad4     | R         | 6687–8065      | 1379  | 0                      | ATG       | TA          |
| nad4L    | R         | 8066–8361      | 296   | 7                      | ATG       | A           |
| trnT     | F         | 8369–8434      | 66    | 0                      | TGT       |             |
| trnP     | R         | 8435–8502      | 68    | 2                      | TGG       |             |
| nad6     | F         | 8505–9008      | 504   | 0                      | ATT       | TAA         |
| cob      | F         | 9009–10,143    | 1135  | 0                      | ATT       | A           |
| trnS2    | F         | 10,144–10,212  | 69    | 18                     | TGA       |             |
| nad1     | R         | 10,231–11,169  | 939   | 59                     | ATA       | TAA         |
| trnL1    | R         | 11,209–11,276  | 68    | 0                      | TAG       |             |
| trnL     | R         | 11,277–12,612  | 1336  | 0                      | TAC       |             |
| trnY     | R         | 12,613–12,685  | 73    | 0                      | TAC       |             |
| trnS5    | R         | 12,686–13,517  | 832   | 0                      |          |             |
| CR       | —         | 13,518–14,201  | 684   | 0                      |          |             |
| trnQ     | R         | 14,202–14,269  | 68    | 70                     | TTG       |             |
| trnI     | F         | 14,304–14,405  | 66    | 12                     | GAT       |             |
| trnM     | F         | 14,418–14,487  | 70    | 0                      | CAT       |             |
| nad2     | F         | 14,488–15,493  | 1006  | 0                      | ATG       | T           |
| trnW     | F         | 15,494–15,562  | 69    | 11                     | TCA       |             |
| trnC     | R         | 15,574–15,637  | 64    | 0                      | GCA       |             |
| trnY     | R         | 15,638–15,706  | 69    | —                      | GTA       |             |
Figure 1. Graphical map of the mitogenome of *Clistocoeloma sinensis*. Protein-coding and ribosomal RNA genes are shown using standard abbreviations. Genes for transfer RNAs are abbreviated using a single letter. S1 = AGN, S2 = UCN, L1 = CUN, L2 = UUR. CR = control region. The 13 protein-coding genes are yellow, tRNAs are green, rRNAs are red, and CRs are dark red.

| species                  | Size (bp) | A %  | G %  | T %  | C %  | A+T % | A+T skew | G+C skew |
|--------------------------|-----------|------|------|------|------|--------|----------|----------|
| *C. sinensis*            | 15,706    | 37.1 | 9.4  | 38.6 | 14.9 | 75.7   | −0.020   | −0.228   |
| *S. sinensis*            | 15,905    | 37.4 | 9.4  | 38.3 | 14.9 | 75.7   | −0.012   | −0.228   |
| *H. lattimena*           | 16,246    | 34.0 | 11.0 | 35.1 | 19.9 | 69.1   | −0.017   | −0.290   |
| *G. paia*                | 15,548    | 35.1 | 10.3 | 34.8 | 19.9 | 69.9   | 0.006    | −0.313   |
| *P. sanguinolentus*      | 16,024    | 31.6 | 12.9 | 34.0 | 21.5 | 65.6   | −0.037   | −0.243   |
| *E. j. sinensis*         | 16,378    | 35.2 | 10.8 | 36.4 | 17.6 | 71.6   | −0.016   | −0.243   |
| *E. j. hepuensis*        | 16,335    | 35.1 | 10.8 | 36.4 | 17.7 | 71.5   | −0.018   | −0.245   |
| *E. j. japonica*         | 16,352    | 35.2 | 10.7 | 36.5 | 17.7 | 71.7   | −0.018   | −0.245   |
| *X. testudinatus*        | 15,798    | 36.7 | 9.3  | 37.2 | 16.8 | 73.9   | −0.007   | −0.286   |
| *P. gigas*               | 15,515    | 35.0 | 10.8 | 35.5 | 18.7 | 70.5   | −0.006   | −0.268   |
| *G. dehaami*             | 18,197    | 36.9 | 8.3  | 38.0 | 16.8 | 74.9   | −0.014   | −0.341   |
| *L. brevifrons*          | 16,112    | 34.2 | 11.3 | 36.4 | 18.1 | 70.6   | −0.031   | −0.231   |
| *C. rapidus*             | 16,263    | 34.2 | 11.1 | 34.9 | 19.8 | 69.1   | −0.011   | −0.279   |
| *P. trituberculatus*     | 16,026    | 33.3 | 11.3 | 36.9 | 18.5 | 70.2   | −0.051   | −0.241   |
| *H. malayensis*          | 15,793    | 37.3 | 10.0 | 34.4 | 18.3 | 71.7   | 0.040    | −0.292   |
| *C. japonica*            | 15,738    | 33.8 | 11.9 | 35.4 | 18.9 | 69.2   | −0.024   | −0.228   |
| *S. paramamosain*        | 15,824    | 34.9 | 10.1 | 38.2 | 16.8 | 73.1   | −0.045   | −0.247   |
| *U. orientalis*          | 15,466    | 33.1 | 11.8 | 34.9 | 20.2 | 68.0   | −0.027   | −0.262   |
| *S. olivacea*            | 15,723    | 33.5 | 11.2 | 35.9 | 19.4 | 69.4   | −0.035   | −0.267   |
| *S. tranquebarica*       | 15,833    | 35.0 | 9.8  | 38.7 | 16.5 | 73.7   | −0.050   | −0.258   |
| *S. serrata*             | 15,775    | 34.5 | 10.4 | 38.0 | 17.1 | 72.5   | −0.047   | −0.242   |
| *D. spinosisimus*        | 15,817    | 33.3 | 10.5 | 36.9 | 19.4 | 70.1   | −0.050   | −0.294   |
| *C. ferdia*              | 15,660    | 34.1 | 11.2 | 36.1 | 18.6 | 70.2   | −0.028   | −0.246   |
| *G. yunohana*            | 15,567    | 34.3 | 10.8 | 35.6 | 19.3 | 69.9   | −0.019   | −0.281   |
| *P. pelagicus*           | 16,157    | 33.7 | 12.2 | 35.0 | 19.1 | 68.8   | −0.019   | −0.219   |
| *A. alveae*              | 15,620    | 34.4 | 11.4 | 32.4 | 21.8 | 66.8   | 0.029    | −0.316   |
| *A. rodriguezensis*      | 15,611    | 35.3 | 10.3 | 33.5 | 20.9 | 68.8   | 0.025    | −0.341   |
| *P. crassipes*           | 15,652    | 30.5 | 12.7 | 35.8 | 21.0 | 66.3   | −0.080   | −0.245   |
| *I. deschampsi*          | 15,460    | 34.1 | 10.7 | 35.5 | 19.7 | 69.6   | −0.019   | −0.294   |

Table 4. Composition and skewness of mitogenome in 29 Brachyura species.
gel extraction kit (Transgen, Beijing, China). The purified products were then ligated into the T-vector (Sangon, Shanghai, China) and sequenced.

**Complete Mitogenome Analysis.** The graphical map of the complete mitogenome was drawn using the online mitochondrial visualization tool mtviz. The secondary cloverleaf structure and anticodon of transfer RNAs were identified using the tRNA-scan SE webserver. Codon usage and the nucleotide composition of the mitogenome were determined using MEGA6. The sequences of 29 Brachyura species and *Alpheus distinguendus* were aligned using MAFFT.

**Phylogenetic Analysis.** Twenty-eight complete Brachyura mitogenomes were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). In addition, the mitogenome of *A. distinguendus* was downloaded from GenBank and used as an outgroup taxon. GenBank sequence information is shown in Table 2.

The sequences were aligned with the mitochondrial sequences of closely related species. In order to remove the gaps in sequences, poorly aligned positions and divergent regions were removed using Gblocks. Then, fasta sequences were converted to nex format sequences and phylip format sequences for Bayesian inference (BI) and Maximum likelihood (ML) analyses using online software (http://sequenceconversion.bugaco.com/converter/biology/sequences/fasta_to_phylip.php). We used DAMBE to detect the saturation status of the sequences.

We determined the taxonomic status of *C. sinensis* within Brachyura by reconstructing the phylogenetic tree. Nucleotide sequences from 30 mitogenome PCGs were combined. The dataset was run using two inference methods: BI and ML analyses. The former was performed using Mrbayes v3.2.1, while ML analysis was performed using raxmlGUI. The nucleotide substitution model was selected using Akaike information criterion implemented in Mrmodeltest v2.3. The GTR+I+G model was the best model to examine nucleotide phylogenetic
analysis and molecular evolution. BI and ML analyses were performed under the GTRCAT model with nucleotide alignment (NT dataset) of the 13 mitochondrial PCGs. ML analyses were performed on 1000 bootstrapped datasets. The BI analysis ran as 4 simultaneous MCMC chains for 10,000,000 generations, sampled every 100 generations, and a burn-in of 5000 generations was used. The average standard deviation of split frequencies was less than 0.01, and the effective sample size determined using tracer v1.6 exceeded 200. These two findings indicate that our data was convergent. The resulting phylogenetic trees were visualized using FigTree v1.4.2.

Figure 3. Secondary structures of the 22 transfer RNA genes of Clistocoeloma sinensis. The tRNAs are labelled with the abbreviations of their corresponding amino acids. Dashes (−) indicate Watson-Crick pairing.
Results and Discussion

Genome Structure and Organization. The mitogenome of *C. sinensis* is 15,706 bp long, and its gene content and arrangement are similar to that of other known Brachyura: 13 PCGs, 2 rRNA genes, and 22 tRNA genes plus CR (Table 3 and Fig. 1). Twenty-three genes are coded on the J strand and the remaining 14 genes are transcribed on the N strand. It has been deposited in GenBank under accession number KU589292. The genome composition (A: 37.1%, T: 38.6%, C: 14.9%, G: 9.4%) shows a strong A+T bias, which accounts for 75.7% of the bases, and exhibits a negative AT skew (−0.080) and GC skew (−0.020) (Table 5). The A+T skews of other previously sequenced Brachyura mitogenomes ranged from −0.020 (Pachygrapsus crassipes) to 0.040 (Homologenus malayensis), while the G+C skew ranged from −0.341 (Austinsogastra rodriquezii, Geothelphusa dehaani) to 0.219 (Portunus pelagicus) (Table 4). However, different regions have different A+T contents. The CR had the highest A+T content (82.9%), whereas the PCG region had the lowest A+T content (74.2%) (Table 5).

Protein-Coding Genes. Among the 13 PCGs, 9 (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6*, and *cob*) were coded on the J strand, while the rest (*nad4*, *nad4L*, *nad5L*, and *nad1*) were on the N strand. The 13 PCGs ranged in size from 159 to 1731 bp (Table 3). Their A+T content was 74.2% and AT skew was −0.026 (Table 5). The relative synonymous codon usage for *C. sinensis* at the third position is shown in Fig. 2. The usage of the two- and four-fold degenerate codons was biased toward the use of codons abundant in A or T (Table 6), which is consistent with other Brachyura species35–37.

Transfer RNAs, Ribosomal RNAs, and A+T-Rich Region. Like most Brachyura mtDNA, the *C. sinensis* mitogenome contains a set of 22 tRNAs (Fig. 3), although this feature is not very well conserved in animal mtDNA. The tRNAs ranged in size from 64 to 73 bp and showed a strong A+T bias, as these bases accounted for 76.2% of the DNA. Further, they exhibited a negative AT skew (−0.010) (Table 5). Fourteen tRNA genes were present on the J strand and eight were on the N strand. All the tRNA genes had the typical cloverleaf structure, except for the *trnH* gene, whose dihydroxyuridine arm was instead just a simple loop (Fig. 3). These features are common in most Brachyura mitogenomes35–37. The secondary cloverleaf structure of 18 tRNAs was examined using tRNA-scan SE; 4 tRNAs not detected by tRNA-scan-SE were found in the unannotated regions by sequence analysis. The 2 rRNA genes with 80.2% total A+T content and positive AT skew were located between the *rrn15* and *rrn18* regions (Table 5).

Gene Arrangement. Gene order within the complete mitogenome of *C. sinensis* is similar to the pancrustacean ground pattern38–40 (Fig. 4A), except for the translocation of *trnH*. Typically, the *trnH* gene is located between the *nad4* and *nad5* genes in the pancrustacean ground pattern, but in *C. sinensis*, it is between the *trnE* and *trnF* genes (Fig. 4B). This translocation was also observed in the mitogenomes of Brachyura crabs available in GenBank that were compared with the *C. sinensis* mitogenome. In addition, in the pancrustacean ground pattern, the tRNA gene order between the CR and *nad2* is *rrnL-trnL-trnQ-trnM*. However, in *C. sinensis*, it is *trnQ-trnL-trnM* (Fig. 4B). The tRNA rearrangements are generally considered to be a consequence of tandem duplication of part of the mitogenome41. Similar non-coding sequences are present at the position of *trnL* originally occupied by the transposed *trnQ* in *C. sinensis*. Because these intergenic sequences have similar lengths to those of typical tRNA genes, they were presumed to be remnants of the *trnQ* gene and its boundary sequences42. The gene order

| Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU |
|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|------|
| UUU(F) | 291 | 1.75 | UCU(S) | 111 | 2.44 | UAU(Y) | 140 | 1.76 | UGU(C) | 27 | 1.8 |
| UUC(F) | 42 | 0.25 | UCC(S) | 14 | 0.31 | UAC(Y) | 19 | 0.24 | UGC(C) | 3 | 0.2 |
| UUA(L) | 401 | 4.24 | UCA(S) | 96 | 2.11 | UAA(*) | 8 | 2 | UGA(W) | 89 | 1.82 |
| UUG(L) | 45 | 0.48 | UCG(S) | 3 | 0.07 | UAG(*) | 0 | 0 | UGG(W) | 9 | 0.18 |
| CUC(L) | 10 | 0.11 | CCC(P) | 90 | 2.61 | CAU(H) | 16 | 1.68 | CGU(R) | 16 | 1.25 |
| CU(A) | 49 | 0.52 | CCA(Q) | 40 | 1.16 | CAA(Q) | 70 | 1.94 | CGA(R) | 32 | 2.51 |
| CUG(L) | 2 | 0.02 | CCG(P) | 2 | 0.06 | CAG(Q) | 2 | 0.06 | CGG(R) | 3 | 0.24 |
| AU(U) | 312 | 1.85 | ACU(T) | 86 | 2.21 | AUA(N) | 128 | 1.74 | AGU(S) | 47 | 1.03 |
| AUC(U) | 26 | 0.15 | ACC(T) | 13 | 0.33 | AAG(K) | 19 | 0.26 | AGC(S) | 1 | 0.02 |
| AUA(M) | 203 | 1.76 | ACA(T) | 55 | 1.41 | AAA(K) | 82 | 1.71 | AGA(S) | 77 | 1.69 |
| AUG(M) | 28 | 0.24 | ACG(T) | 2 | 0.05 | AAG(K) | 14 | 0.29 | AGG(S) | 15 | 0.33 |
| GUU(V) | 85 | 1.6 | GCU(A) | 113 | 2.46 | GAU(D) | 50 | 1.64 | GUG(G) | 72 | 1.34 |
| GUC(V) | 5 | 0.09 | GCC(A) | 11 | 0.24 | GAC(D) | 11 | 0.36 | GGC(G) | 9 | 0.17 |
| GUA(V) | 115 | 2.17 | GCA(A) | 54 | 1.17 | GAA(E) | 66 | 1.74 | GGA(G) | 117 | 2.18 |
| GUG(V) | 7 | 0.13 | GCC(A) | 6 | 0.13 | GAG(E) | 10 | 0.26 | GGG(G) | 17 | 0.32 |

Table 6. The codon number and relative synonymous codon usage in *Clistocoeloma sinensis* mitochondrial protein coding genes.
of *C. sinensis* is identical to that of *S. sinensis* (Fig. 4B), which indicates that *C. sinensis* may belong to the group Sesarmidae of the superfamily Grapsoidea and that *C. sinensis* and *S. sinensis* probably belong to sister groups.

The gene sequences of Varunidae species (*Eriocheir japonica sinensis*, *E. japonica hepuensis*, *E. japonica japonica*, and *Helice latimera*) are identical (Fig. 4C). As shown in Fig. 4D, the order and orientation of genes in 7 families are uniform. The order of genes in *C. sinensis* sequences is different from that in the sequences of the mitogenomes of these 7 families because of the rearrangement of two tRNA genes between CR and trnM: the placement of genes between CR and trnM in *C. sinensis* is CR-trnQ-trnI-trnM, while that in the 7 families is CR-trnI-trnQ-trnM. In this case, tandem duplication of gene regions may be the most likely mechanism for mitochondrial gene rearrangement, which includes trnI and trnQ, followed by loss of supernumerary genes. Slipped-strand mispairing occurred first, followed by gene deletion. Partial PCGs, tRNAs, and rRNAs of *Damithrax spinosissimus*, *G. dehaami*, and *Xenograpsus testudinatus* appear to be rearranged compared to *C. sinensis* (Fig. 4E–G).

**Phylogenetic analysis.** Our analyses were based on the NT dataset in mitogenomes derived from 29 Brachyura species belonging to 12 families (Varunidae, Xenograpsidae, Homolidae, Menippidae, Mithracidae, Potamidae, Portunidae, Raninidae, Bythograeidae, Sesarmidae, Grapsidae, and Dotillidae). The data matrix (15,706 bp in all) was analysed using the model-based evolutionary methods of BI and ML analyses (Fig. 5). The ML and BI analyses of the dataset gave the same tree topology. It is obvious that *C. sinensis* and *S. sinensis* clustered in one branch in the phylogenetic tree with high nodal support values (Fig. 5), indicating that *C. sinensis*
and *S. sinensis* have a sister group relationship. This result supported that *C. sinensis* belongs to Grapsoidae, Sesarmidae. From the phylogenetic tree, we found that *X. testudinatus* and two Sesarmidae species formed a group and showed close relationships. *X. testudinatus*, which was originally placed in Varunidae, has been transferred to its own family (Xenograpsidae)21–30. Analysis of the 124 13 mitochondrial PCGs using BI and ML showed that *E. j. sinensis*, *E. j. hepensis*, *E. j. japonica*, and *H. latimera* clustered together with high statistical support, showing that these species have a sister group relationship and belong to Grapsoidae, Varunidae. Our phylogenetic analysis indicated that Sesarmidae species, Xenograpsidae species and Varunidae species have close relationships31. In addition, *P. crassipes* belongs to Grapsidae, Grapsidae58.

The phylogenetic position of *Ilyoplax deschampsi* is always within Grapsidae31, 47, 49, 50. *I. deschampsi* belongs to the family Dotillidae, Ocyopodoidea. The real phylogenetic position of *I. deschampsi* should be closer to the Grapsoidae species that shown in Fig. 5. Recent studies on the genus *Ucides* have also shown similar classification31, 52. *G. dehaani* belongs to Potamidae, Potamidae53. However, the phylogenetic tree showed that Potamidae are associated closely with Varunidae, Grapsidae, Sesarmidae, Dotillidae, and Xenograpsidae. This result is in agreement to that inferred from 23 Brachyuran crabs, in which the author use the two mitogenomes24. Phylogenetic relationships between *I. deschampsi*, *G. dehaani* and Grapsidae species need to be reconsidered by integrating more mitogenomic data. More mitogenomic data will also lead to a better overall understanding the phylogenetic relationships among Brachyuran crabs.

### Availability of data and materials

The data set supporting the results of this article is available at NCBI (KU589292).

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Author Contributions
Q.N.L. and B.P.T. conceived and designed the experiments. Q.N.L., Z.Z.X., and X.Y.C. performed the experiments. Q.N.L., Z.F.W., H.B.Z. and Z.Z.X. analyzed the data. D.Z.Z., C.L.Z. and B.P.T. contributed reagents and materials. Q.N.L. and Z.Z.X. wrote the paper. Z.Z.X., and Q.N.L. revised the paper.

Additional Information
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