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# Transcriptional Landscape of Small Non-coding RNAs in Somatic and Gonadal Tissues of Brachyuran Crabs (Genus *Eriocheir* and *Portunus*)

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## INTRODUCTION

Small non-coding RNAs (sncRNAs) with a length <200 nucleotides (nt) play a crucial role in gene regulation at the transcriptional and post-transcriptional levels, and are mainly classified as miRNAs, piRNAs and siRNAs based on their size and Argonaute partner in biogenesis (Li and Liu, 2011). miRNAs and siRNAs are generated from double-stranded precursors by DICER and are of 20–23 nt in length, whereas piRNAs are generated from single-stranded precursors by PIWI and have a length of 24–31 nt (Huang et al., 2021). Among these, only miRNAs have been extensively investigated in animals, while both piRNA and siRNA have not been explored sufficiently. The well-known function of miRNAs it the regulation of gene expression, which is involved in immune system, differentiation and proliferation, growth and development, tumorigenesis, and cell death (O'Connell et al., 2010). To date, miRNAs have been identified and characterized in several crab species including Chinese mitten crab *Eriocheir sinensis* (Song et al., 2014; He et al., 2015; Chen et al., 2020; Fu et al., 2021; Luo et al., 2021), mud crab *Scylla paramamosain* (Li et al., 2013; Jia et al., 2018; Wang et al., 2018; Waiho et al., 2019; Jin et al., 2020; Lai et al., 2020), swimming crab *Portunus trituberculatus* (Ren et al., 2016; Meng et al., 2018, 2020), and freshwater crab *Sinopotamon henanense* (Xu et al., 2019). However, most of the studies often evaluated specific tissue expressed miRNAs in crabs.

piRNAs are a widespread strategy to effectively suppress transposable element (TE) activity to safeguard the genome from detrimental insertion mutagenesis (Tóth et al., 2016), a common issue in most animal germlines. Ubiquitously expressed piRNAs were discovered in the soma and germ lines of Annelida, Cnidaria, Echinodermata, Arthropoda, and Mollusca, but underwent tremendous changes in the Chordata (Huang et al., 2021). Interestingly, abundant piRNA-sized reads with a length of 24–31 nt were detected in the gonadal tissues of crabs (He et al., 2015; Meng et al., 2018; Luo et al., 2021), but very few studies have noticed these piRNAs in crabs (Waiho et al., 2020). Crabs, as important aquaculture species, have high commercial value as a food source. Their genetic mechanisms involved in growth, reproduction, and immune response are currently

active research areas for aquaculture species. With the development of next-generation sequencing (NGS), it is possible to identify high-throughput miRNAs and piRNAs in non-model organisms by small RNA sequencing (sRNA-seq).

In this data report, we performed a small RNA sequencing analysis and detected abundant miRNAs and piRNAs in three Brachyuran crabs (*E. sinensis*, *E. japonica*, and *P. trituberculatus*). miRNAs were found in all investigated tissues, whereas piRNAs were specifically detected in gonadal tissues in Brachyuran crabs. The quality control of sequencing data was conducted to present a high-quality dataset. This data descriptor provides sufficient information for small RNAs in Brachyuran crabs, which is useful for future miRNAs and piRNAs studies in crabs and related species, and serves as an important reference for studies on genomics and genetic studies in crabs.

## METHODS

### Sample Collections

Three Brachyuran crabs (*E. sinensis*, *E. japonica*, and *P. trituberculatus*) were collected from Tokyo Bay (35°60′N, 140°07′E) and the nearby fishery market (Ameyoko Market, Tokyo) in October 2020. Eight individuals of each species were collected and dissected the hepatopancreas, gill, leg muscle, and gonad (ovary or testis). The harvested tissues were stored in 2 ml tubes with NucleoProtect® RNA solution (TaKaRa, Shiga, Japan) for 12 h at 4°C and then stored at −80°C until further analysis.

### RNA Extraction, Library Construction, and Sequencing

Total RNA was extracted from the tissues by using the ReliaPrep$^{TM}$ miRNA Cell and Tissue Miniprep System (Promega, WI, USA) according to the protocols. The Qubit RNA Assay Kit in Qubit 3.0 Fluorometer (Life Technologies, CA, USA) and RNA ScreenTape Assay Kit in Agilent 2200 TapeStation (Agilent Technologies, Waldbronn, Germany) were used to assess the total RNA quantity and quality. For each Brachyuran crabs, two biological replicates were made for each tissue, and each replicate mixed with three equal total RNA from the same tissues. The libraries were constructed by using the SMARTer smRNA-Seq Kit for Illumina (TaKaRa, CA, USA) following the manufacturer's instructions and sequenced on an Illumina HiSeq 2500 platform with a 50-bp single-end module (Macrogen, Tokyo).

### Data Pre-processing

To obtain high-quality sRNA reads, the raw data were filtered by removing poly (A) and poly (N) sequences, and low-quality reads with an average quality score ≤20. The adapter sequences were trimmed by using the fastx_toolkit, followed by size filtration with a length range of 15–35 nt. The clean reads of these crabs were then filtered by removing genome no-mapping reads or known small non-coding RNAs from Rfam by using bowtie (v1.2.1, -f -k 3 -v2). The genome of *E. sinensis* (https://www.ncbi.nlm.nih.gov/genome/?term=Eriocheir$+$sinensis$+$) was used as reference genome for mitten crabs (*E. sinensis* and *E. japonica*), and the genome of *P. trituberculatus* (https://www.ncbi.nlm.

nih.gov/genome/?term=Portunus$+$trituberculatus) was used as reference genome for swimming crab.

## miRNA and piRNA Identification and Annotation

The sRNA reads were pooled from different replicates and tissues for each species, respectively, to perform miRNA prediction with miRBase 22.0 (Kozomara et al., 2019) and miRDeep2 (v.2.0.0.8) (Friedländer et al., 2012) using default parameters. After miRNA profiling, the miRNA reads were removed from the clean reads of gonadal tissues using seqkit (v0.7.3, common module) (Shen et al., 2016), which were considered as putative piRNAs for subsequent analysis. The ping-pong amplification signatures of piRNA reads were calculated by PPmeter (Jehn et al., 2018) and unitas (Gebert et al., 2017), while proTRAC (Rosenkranz and Zischler, 2018) were used to identify the piRNA clusters based on genomic mapping result. The reads have a length of 24–31 nt and significant ping-pong signal were considered as canonical piRNAs in these species.
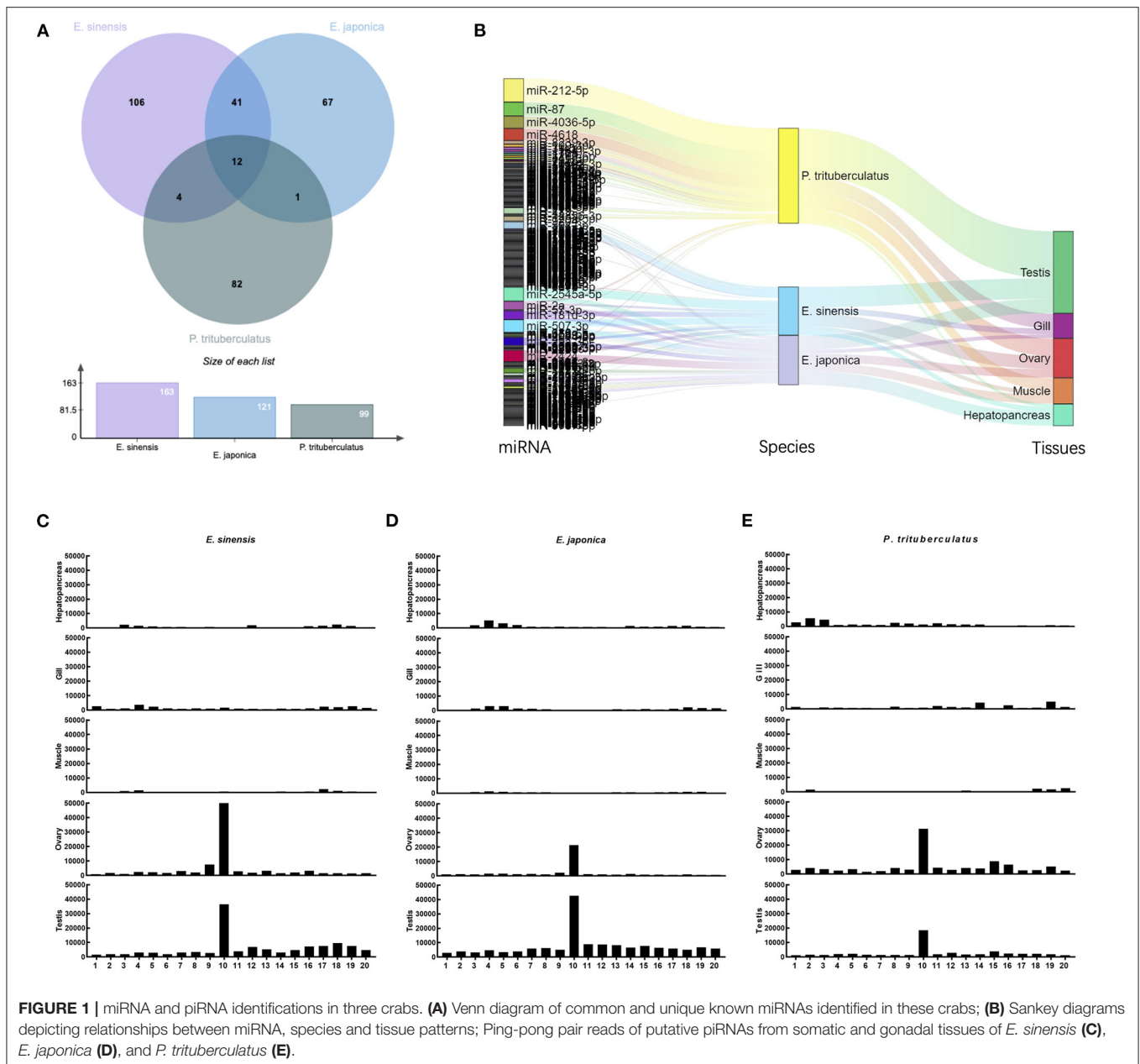
## Downstream Analysis

The total miRNA reads of each library were used for normalization to analyze differentially expressed miRNAs (DEMs) among different tissues. The miRNA counts were normalized according to the reads per million reads (RPM) method. The differential expression analysis of miRNAs was conducted by the DESeq2 package in R software (v4.0.2), and the miRNAs with a |$\log_2$ (Fold change) | ≥ 1) and adjusted $p$ < 0.05 were considered as DEMs.

## Technical Validation

The quantity and quality of extracted total RNA were validated using Agilent 2200 TapeStation with an RNA ScreenTape Assay Kit (**Supplementary Figure 1A**). The RIN value > 8.5 of samples can be used for total RNA mixture. The cDNA libraries were validated by using a High Sensitivity D1000 ScreenTape Kit in Agilent 2200 TapeStation (**Supplementary Figure 1B**). The cleanup cDNA products were purified, and their sizes were selected using AMPure XP beads (Beckman Coulter, CA, USA) in order to obtain the appropriate size (<150 bp). The post-size-selection library was also validated by Agilent 2200 TapeStation (Agilent Technologies) with a High Sensitivity D5000 ScreenTape Kit (**Supplementary Figure 1C**). In total, we constructed thirty small RNA libraries for the three crabs in which two biological replicates for each tissue.

## RESULTS

A total of 132.6 million raw reads were generated by Illumina sRNA-seq with a range of 1.7–8.7 million reads for each library (**Supplementary Table 1**). After removing the low-quality, genomic no-mapping or outside-of-size range reads, the clean reads were remained for the statistical analysis of sequence length (**Supplementary Figure 2**), and

FIGURE 1 | miRNA and piRNA identifications in three crabs. (A) Venn diagram of common and unique known miRNAs identified in these crabs; (B) Sankey diagrams depicting relationships between miRNA, species and tissue patterns; Ping-pong pair reads of putative piRNAs from somatic and gonadal tissues of *E. sinensis* (C), *E. japonica* (D), and *P. trituberculatus* (E).

subsequently mixed for miRNA identification for each species. A total of 200, 161, and 129 miRNAs were identified in *E. sinensis*, *E. japonica*, and *P. trituberculatus*, respectively (**Supplementary Table 2**). Among these miRNAs, 12 miRNAs were detected in all crabs, namely miR-2a, miR-29a-3p, miR-34-3p, miR-87, miR-125a-5p, miR-181d-3p, miR-184-5p, miR-279, miR-375-5p, miR-680, miR-2424, and miR-7171-5p, whereas another 41 miRNAs were detected in both of *E. sinensis* and *E. japonica* (**Figure 1A**). The expression patterns of these identified miRNAs differed even among tissues of the same species (**Figure 1B**, **Supplementary Figure 3**). After identifying and removing the known and novel miRNAs, the remaining reads were considered as putative reads for

piRNA processing in gonadal tissues. Abundant piRNA-sized reads with a length of 24–27 nt were both detected in the ovary and testis of these crabs. A significant 10-nt overlap between sense and antisense strands was detected in the ovary and testis samples of these three crabs (**Figures 1C–E**), which was consistent with piRNAs generated by the ping-pong amplification mechanism.

In conclusion, our research provides a high-quality landscape of small non-coding RNAs in Brachyuran crabs, especially about the novel piRNAs detected in the gonadal tissues. The dataset provides a valuable resource for screening small RNAs in Brachyuran crabs and the related species, which will contribute to improve our understanding

of the molecular mechanisms of these small RNAs in Brachyuran crabs.

## CODE AVAILABILITY

The software utilized in this study were described in the part of methods and the following list:

1. FastQC version0.11.9 was used for the quality assessment of the FASTQ data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
2. Cutadapt version1.9.1 was used to trim the adapter sequence (http://code.google.com/p/cutadapt/).
3. Fastq_to_fasta.py from fastx_toolkit was used to transfer the sequencing data to fastq format (http://hannonlab.~cshl.edu/fastx_toolkit/index.html).
4. filter_fasta_by_size.pl was used to filter clean read by length (https://github.com/jorvis/biocode/tree/master/fasta).
5. Seqkit version0.7.3 common module was used to remove known RNAs (http://bioinf.shenwei.me/seqkit).

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: DDBJ DRA [accession: DRA012855].

## ETHICS STATEMENT

Ethics review and approval/written informed consent was not required as per local legislation and institutional requirements.

## AUTHOR CONTRIBUTIONS

SH designed and performed the experiments, analyzed the data, and drafted the manuscript. LZ collected the samples and assisted with the data analysis. XC conceived the study, supervised the work, and co-wrote the manuscript. All authors reviewed the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.795118/full#supplementary-material

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