Nong et al. BMC Genomics (2020) 21:32
https://doi.org/10.1186/s12864-019-6433-3

BMC Genomics

DATABASE

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A crustacean annotated transcriptome (CAT) database

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Abstract

Background: Decapods are an order of crustaceans which includes shrimps, crabs, lobsters and crayfish. They occur worldwide and are of great scientific interest as well as being of ecological and economic importance in fisheries and aquaculture. However, our knowledge of their biology mainly comes from the group which is most closely related to crustaceans – insects. Here we produce a de novo transcriptome database, crustacean annotated transcriptome (CAT) database, spanning multiple tissues and the life stages of seven crustaceans.

Description: A total of 71 transcriptome assemblies from six decapod species and a stomatopod species, including the coral shrimp *Stenopus hispidus*, the cherry shrimp *Neocaridina davidi*, the redclaw crayfish *Cherax quadricarinatus*, the spiny lobster *Panulirus ornatus*, the red king crab *Paralithodes camtschaticus*, the coconut crab *Birgus latro*, and the zebra mantis shrimp *Lysiosquillina maculata*, were generated. Differential gene expression analyses within species were generated as a reference and included in a graphical user interface database at http://cat.sls.cuhk.edu.hk/. Users can carry out gene name searches and also access gene sequences based on a sequence query using the BLAST search function.

Conclusions: The data generated and deposited in this database offers a valuable resource for the further study of these crustaceans, as well as being of use in aquaculture development.

Background

The Arthropoda is a phylum containing the largest number (nearly 85%) of described living species in the world. For various historical reasons, most of our knowledge of their biology comes from insects, particularly fruit flies *Drosophila*. Crustacea (including shrimps, lobsters, crayfish, crabs) forms a large subphylum of arthropods now proven to be the closest relatives of Insecta. In the past decade, a substantial number of insect genomes have been sequenced across the different groups (e.g. beetle, wasp, bee, aphid, butterfly, and moth), especially in the course of the on-going 5000 insect genome project (i5k Consortium). By contrast, the genomic resources of crustaceans are relatively scarce, and are limited to a few species (e.g. [1–6]). Carcinology, or the study of crustaceans, benefits both basic science and the aquaculture industry, presently the fastest growing animal food-producing sector worldwide. Here, we generated a user-friendly database, the crustacean annotated transcriptome (CAT) database, which enables users to search for the annotated gene name as well as gene sequences based on sequence query. This database contains newly generated crustacean transcriptomic data from different developmental stages and the tissues of seven crustacean species, including a stomatopod mantis shrimp, two decapod shrimps, a crayfish, a lobster, and two anomuran crabs (Fig. 1).

Construction and content

Sample collection

Specimens of the seven crustacean species were acquired either from fish markets and aquarium shops in Hong Kong or from overseas sources (see details below). The creatures were then maintained in the laboratory before being dissected, as described below:

Coral shrimps (Decapoda: Stenopodidea: Stenopodidae: *Stenopus hispidus*) were sourced from an aquarium

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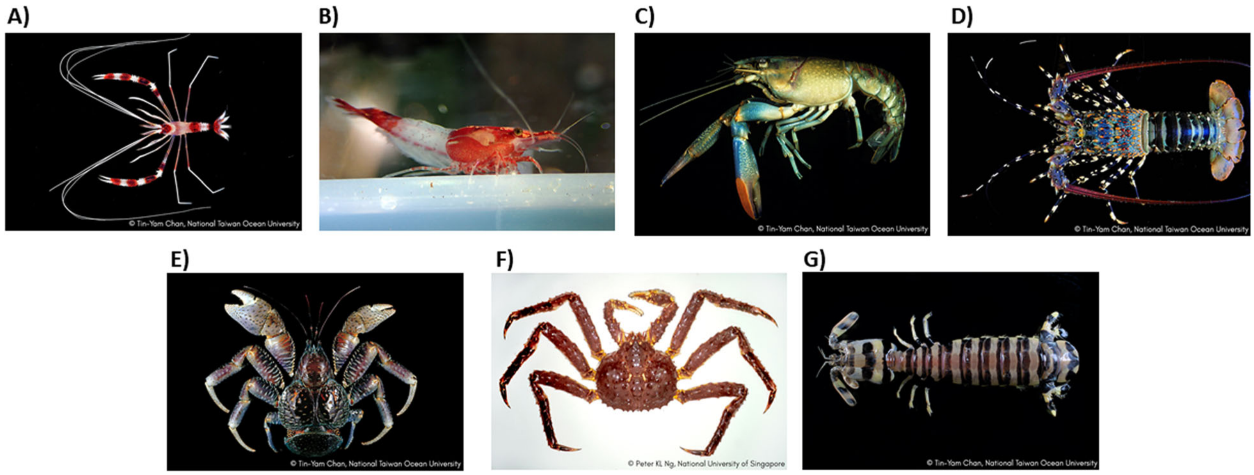

Fig. 1 Crustaceans used in this study, including (**a**) coral shrimp *Stenopus hispidus*, (**b**) cherry shrimp *Neocaridina davidi*, (**c**) redclaw crayfish *Cherax quadricarinatus*, (**d**) spiny lobster *Panulirus ornatus*, (**e**) red king crab *Paralithodes camtschaticus*, (**f**) coconut crab *Birgus latro*, and (**g**) stomatopod zebra mantis shrimp *Lysiosquillina maculata*

shop and maintained for over 2 weeks as mating pairs in separate 10-L seawater tanks at an ambient indoor temperature (20–26 °C) with diurnal lighting and environmental enrichments of moss and wood, and were fed with aquarist shrimp feed. Tissue samples were collected from a single adult female at the intermolt stage, while "whole body" samples were obtained from 50 to 100 early (no eye spot) and late (with eye spot) stage eggs obtained from two females separately.

Cherry shrimp (Decapoda: Caridea: Atyidae: *Neocaridina davidi*) were purchased from an aquarium shop in Hong Kong. Again, they were kept in 10-L freshwater tanks at an ambient indoor temperature with diurnal lighting, and fed with aquarist shrimp feed. Tissue samples were collected from a single female adult at the intermolt stage, while "whole-body" samples were obtained from a 15-day-old juvenile, as well as from ~ 20 early (no eye spot) and late (with eye spot) stage eggs (~ 6 eggs per replicate) from two females separately.

Red claw crayfish (Decapoda: Astacidea: Parastacidae: *Cherax quadricarinatus*) at different life history stages were sourced from a breeder in Queensland, Australia. The juvenile (~ 7–10 cm in length) and adult (15–18 cm in length) crayfish were acclimated for over 2 weeks in 100-L freshwater tanks at an ambient indoor temperature with diurnal lighting and enriched with hiding nets, and were fed aquarist shrimp feed. Tissue samples were collected from a single adult female at the intermolt stage, from a single juvenile, from 4 newborn larvae (less than 10 days old, 2 individuals per replicate) and from 6 early (orange) and 6 late (brown) stage egg (3 eggs per replicate).

Spiny lobsters (Decapoda: Achelata: Palinuridae: *Panulirus ornatus*) were purchased from a fish market in Hong Kong, and acclimated for 2 weeks in 500-L tanks in an outdoor enclosure at 25–30 °C and fed with live clams. Tissue samples were collected from a single adult female at the intermolt stage.

Adult male coconut crabs (Decapoda: Anomura: Coenobitidae: *Birgus latro*) were purchased and imported from a fish market in Okinawa, Japan.. The crabs were fed a diet of coconut meat and boiled root vegetables while acclimating for 2 weeks in a controlled environment in a large outdoor enclosure at 25–30 °C. The enclosure was enriched with damp straw, reptile lights on diurnal control and a pool of running fresh water, and a humidifier maintained a relative humidity of 70–80%. Tissue samples were collected from a single individual.

Adult male king crabs (Decapoda: Anomura: Lithodidae: *Paralithodes camtschaticus*) were imported from Alaska and fed with live clams while acclimating for 2 weeks in 100-L seawater tanks kept at 4 °C in a dark room. Tissue samples were collected from a single individual.

Zebra mantis shrimp (Stomapoda: Lysiosquillidae: *Lysiosquillina maculata*) were purchased from a fish market in Hong Kong and acclimated for 2 weeks in 100-L seawater tanks at ambient indoor temperature with diurnal lighting and 20 cm of sand, and were fed with live fish. Tissue samples were collected from a single adult female at the intermolt stage.

Tissue samples of gill, eye stalk, ovary (female only), hepatopancreas, and muscle were obtained from adults of all target species and juveniles of crayfish. Gill tissues were dissected, pooled and homogenised. Tissue from eyestalks were dissected, avoiding the pigmented retina and discarding the exoskeleton. Ovary tissues were collected from mature females. Hepatopancreas tissues

Table 1 Transcriptomes generated in this study

Species	Samples	Samples ID	No. of Raw Reads	No. of Raw Bases	No. of Clean Reads	No. of Clean Bases	Accession No.
Coral shrimp *Stenopus hispidus*	Early Eggs	Early_Eggs_rep1	147,512,128	22,274,331,328	101,288,146	15,293,716,963	SAMN12640677
	Early Eggs	Early_Eggs_rep2	117,676,158	17,769,099,858	72,694,974	10,976,329,454	SAMN12640676
	Eye Stalk	Eye_Stalk_rep1	135,906,276	20,521,847,676	98,352,868	14,850,269,616	SAMN12640679
	Eye Stalk	Eye_Stalk_rep2	127,896,134	19,312,316,234	76,751,610	11,588,833,508	SAMN12640678
	Gill	Gill_rep1	100,628,810	15,194,950,310	67,364,496	10,171,330,448	SAMN12640681
	Gill	Gill_rep2	186,921,040	28,225,077,040	132,921,498	20,070,121,889	SAMN12640680
	Hepatopancreas	Hepatopancreas_rep1	115,393,260	17,424,382,260	80,776,662	12,196,447,379	SAMN12640683
	Hepatopancreas	Hepatopancreas_rep2	113,380,674	17,120,481,774	74,484,474	11,246,558,920	SAMN12640682
	Larvae	Larvae_rep1	122,870,566	18,553,455,466	83,149,430	12,554,935,209	SAMN12640684
	Late Eggs	Late_Eggs_rep1	140,726,264	21,249,665,864	88,090,514	13,300,967,462	SAMN12640686
	Late Eggs	Late_Eggs_rep2	100,688,840	15,204,014,840	71,842,070	10,847,454,859	SAMN12640685
	Muscle	Muscle_rep1	119,891,122	18,103,559,422	82,797,100	12,501,497,698	SAMN12640688
	Muscle	Muscle_rep2	118,371,384	17,874,078,984	79,667,912	12,029,237,295	SAMN12640687
	Ovary	Ovary_rep1	95,065,662	14,354,914,962	66,459,684	10,034,756,324	SAMN12640690
	Ovary	Ovary_rep2	104,613,968	15,796,709,168	72,830,790	10,996,915,202	SAMN12640689
Cherry shrimp *Neocaridina davidi*	Early Eggs	Early_Eggs_rep2	113,024,220	17,066,657,220	111,779,862	16,876,338,041	SAMN12640666
	Eye Stalk	Eye_Stalk_rep2	170,784,308	25,788,430,508	170,325,378	25,716,234,579	SAMN12640665
	Gill	Gill_rep2	124,909,566	18,861,344,466	124,168,480	18,747,387,245	SAMN12640667
	Hepatopancreas	Hepatopancreas_rep2	138,609,020	20,929,962,020	138,331,074	20,885,684,808	SAMN12640668
	Juveniles	Juveniles_rep2	134,272,614	20,275,164,714	99,747,180	15,061,094,623	SAMN12640663
	Larvae	Larvae_rep2	100,436,246	15,165,873,146	72,861,546	11,001,351,157	SAMN12640664
	Late Eggs	Late_Eggs_rep2	109,565,698	16,544,420,398	109,240,904	16,493,316,999	SAMN12640669
	Muscle	Muscle_rep2	128,462,460	19,397,831,460	127,700,680	19,280,692,827	SAMN12640670
	Ovary	Ovary_rep2	160,480,332	24,232,530,132	159,595,528	24,096,017,598	SAMN12640671
Redclaw crayfish *Cherax quadricarinatus*	Brown Eggs	Brown_Eggs_rep1	117,111,332	17,683,811,132	115,820,338	17,486,985,986	SAMN12640702
	Brown Eggs	Brown_Eggs_rep2	176,933,368	26,716,938,568	118,628,494	17,911,929,304	SAMN12640691
	Eye Stalk	Eye_Stalk_rep1	111,581,360	16,848,785,360	110,935,184	16,749,377,279	SAMN12640703
	Eye Stalk	Eye_Stalk_rep2	126,534,188	19,106,662,388	80,263,766	12,119,128,408	SAMN12640692
	Gill	Gill_rep1	124,577,594	18,811,216,694	123,093,702	18,584,519,542	SAMN12640704
	Gill	Gill_rep2	159,247,046	24,046,303,946	84,175,868	12,709,786,121	SAMN12640693
	Hepatopancreas	Hepatopancreas_rep1	110,338,732	16,661,148,532	109,573,732	16,543,843,997	SAMN12640705
	Hepatopancreas	Hepatopancreas_rep2	99,741,906	15,061,027,806	68,186,392	10,295,575,098	SAMN12640694
	Juvenile Eye Stalk	Juvenile_Eye_Stalk_rep1	127,200,182	19,207,227,482	125,731,804	18,983,113,530	SAMN12640706
	Juvenile Eye Stalk	Juvenile_Eye_Stalk_rep2	117,482,430	17,739,846,930	85,517,580	12,912,288,884	SAMN12640695
	Juvenile Gill	Juvenile_Gill_rep1	114,896,974	17,349,443,074	113,497,714	17,136,277,633	SAMN12640707
	Juvenile Gill	Juvenile_Gill_rep2	116,513,728	17,593,572,928	73,106,766	11,038,515,397	SAMN12640696
	Juvenile Hepatopancreas	Juvenile_Hepatopancreas_rep1	121,738,036	18,382,443,436	120,085,356	18,130,904,762	SAMN12640712
	Juvenile Hepatopancreas	Juvenile_Hepatopancreas_rep2	100,906,200	15,236,836,200	75,709,538	11,431,284,772	SAMN12640697
	Juvenile Muscle	Juvenile_Muscle_rep1	104,072,454	15,714,940,554	103,357,890	15,605,125,325	SAMN12640708
	Juvenile Muscle	Juvenile_Muscle_rep2	144,025,464	21,747,845,064	105,450,768	15,922,196,458	SAMN12640698
	Larvae	Larvae_rep1	137,834,652	20,813,032,452	134,710,800	20,338,355,433	SAMN12640701
	Larvae	Larvae_rep2	131,545,402	19,863,355,702	91,717,902	13,848,639,715	SAMN12640699
	Muscle	Muscle_rep1	110,411,178	16,672,087,878	109,469,024	16,527,755,100	SAMN12640709
	Muscle	Muscle_rep2	102,282,720	15,444,690,720	75,616,522	11,417,419,932	SAMN12640700
	Orange Eggs	Orange_Eggs_rep1	129,459,428	19,548,373,628	128,222,938	19,359,518,492	SAMN12640710
	Orange Eggs	Orange_Eggs_rep2	151,646,640	22,898,642,640	80,163,194	12,103,966,602	SAMN12640714
	Ovary	Ovary_rep1	109,484,020	16,532,087,020	108,803,256	16,426,893,327	SAMN12640711
	Ovary	Ovary_rep2	117,795,878	17,787,177,578	68,721,586	10,376,361,317	SAMN12640713
Spiny lobster *Panulirus ornatus*	Eye Stalk	Eye_Stalk_rep2	108,244,990	16,344,993,490	77,762,572	11,741,253,661	SAMN12640719
	Gill	Gill_rep2	134,047,316	20,241,144,716	87,055,544	13,144,336,119	SAMN12640720
	Hepatopancreas	Hepatopancreas_rep2	123,016,922	18,575,555,222	65,184,592	9,842,042,208	SAMN12640721
	Muscle	Muscle_rep2	166,369,450	25,121,786,950	116,462,426	17,584,456,125	SAMN12640722
	Ovary	Ovary_rep2	147,034,728	22,202,243,928	85,355,520	12,887,639,607	SAMN12640723
Coconut crab *Birgus latro*	Eye Stalk	Eye_Stalk_rep1	127,480,250	19,249,517,750	126,653,790	19,122,629,140	SAMN12640675
	Gill	Gill_rep1	118,680,720	17,920,788,720	117,861,470	17,795,132,532	SAMN12640674
	Hepatopancreas	Hepatopancreas_rep1	116,366,940	17,571,407,940	114,479,626	17,283,923,830	SAMN12640673
	Muscle	Muscle_rep1	120,576,266	18,207,016,166	119,519,192	18,045,428,779	SAMN12640672
Red king crab *Paralithodes camtschaticus*	Eye Stalk	Eye_Stalk_rep1	121,406,996	18,332,456,396	118,402,398	17,876,510,688	SAMN12640718
	Gill	Gill_rep1	135,100,744	20,400,212,344	133,658,544	20,180,138,938	SAMN12640717
	Hepatopancreas	Hepatopancreas_rep1	134,693,082	20,338,655,382	132,142,302	19,950,589,220	SAMN12640716
	Muscle	Muscle_rep1	115,823,126	17,489,292,026	114,706,282	17,318,478,458	SAMN12640715
Zebra mantis shrimp *Lysiosquillina maculata*	Eye Stalk	Eye_Stalk_rep1	123,669,922	18,674,158,222	88,546,256	13,369,809,915	SAMN12640725
	Eye Stalk	Eye_Stalk_rep2	135,173,070	20,411,133,570	87,469,510	13,206,780,918	SAMN12640724
	Gill	Gill_rep1	153,621,642	23,196,867,942	83,229,070	12,566,924,697	SAMN12640727
	Gill	Gill_rep2	79,664,470	12,029,334,970	51,309,276	7,747,041,631	SAMN12640726
	Hepatopancreas	Hepatopancreas_rep1	128,606,686	19,419,609,586	78,190,890	11,806,208,600	SAMN12640729
	Hepatopancreas	Hepatopancreas_rep2	128,116,562	19,345,600,862	86,065,240	12,994,759,663	SAMN12640728
	Muscle	Muscle_rep1	102,523,380	15,481,030,380	60,386,638	9,117,844,856	SAMN12640731
	Muscle	Muscle_rep2	112,033,718	16,917,091,418	75,100,358	11,339,223,796	SAMN12640730
	Ovary	Ovary_rep1	104,974,818	15,851,197,518	70,045,014	10,576,184,867	SAMN12640733
	Ovary	Ovary_rep2	114,660,456	17,313,728,856	80,831,326	12,204,526,842	SAMN12640732

were taken at distant tubules from the midgut caecae to avoid heavy bacterial contamination. Muscle was isolated from the abdomen from all the shrimp and crayfish species (including stomatopod) and from the large chela of crabs. Duplicate biological samples were collected. Tissue samples from adult and the "whole body" of juvenile animals, larvae, and eggs were frozen in liquid nitrogen and then stored at − 80 °C before total RNA extraction.

RNA extraction and sequencing

Total RNAs were isolated using the miRVana microRNA Isolation Kit (Thermo Fisher Scientific). RNA concentration and quality were assessed by a NanoDrop Flourospectrometer (Thermo Scientific). At least 5 μg of total RNA for each sample were enriched by ribo-reduction using Ribo-Zero rRNA removal kits (Epicentre). Transcriptome libraries were created using TruSeq Stranded RNA Library Prep Kit v2 (Illumina) by Theragen Bio Institute in Korea, followed by 150 bp paired-end sequencing on an Illumina HiSeq 4000 platform to obtain at least 51 million clean reads (after filtering and trimming).

Transcriptome assembly and annotation

Raw sequencing reads from 71 transcriptomes were preprocessed with quality trimmed by trimmomatic (v0.33 with parameters "ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25", [7]), followed by de novo transcriptome assembly using Trinity (v2.4.0, [8, 9]) with the options "--SS_lib_type RF --normalize_reads" and other default parameters. All biological duplicates were combined to carry out the de novo assembly and estimation of transcript abundance using the script "align_and_estimate_abundance.pl" of the Trinity software with "--est_method RSEM --aln_method bowtie" (v1.1.2, [10]). Coding regions within transcripts were annotated using TransDecoder (v5.0.2 [11];), and functional annotation and analyses were carried out using Trinotate (v3.1.1, [12]). A summary of the assembled transcriptomes is shown in Table 1.

Utility and discussion

Website construction

The Crustacean Annotated Transcriptome (CAT) database is available at http://cat.sls.cuhk.edu.hk/. It was built using CodeIgniter Web Framework. CodeIgniter (https://www.codeigniter.com/) is a powerful PHP framework with a tiny footprint. The website provides researchers with several tools for transcriptome visualization, gene search, and gene blast.

Transcriptome visualisation Gene expression data of various samples in each species can be visualised through the Degust (https://github.com/Victorian-Bioinformatics-Consortium/degust) toolset [13]. It allows the comparison of gene expression between different tissues of the same species. The users can browse differentially expressed genes (DEGs) between samples within the same species, perform their own DEG analysis, or analyse expression profiles using the inbuilt server.

Gene sequence search The database contains 462,877 pieces of gene annotation information (coral shrimp: 57240, cherry shrimp: 92956, red claw crayfish: 99100, spiny lobster: 28805, coconut crab: 72729, red king crab: 73144, zebra mantis shrimp: 38903). The users can search a gene of a certain species by querying the "gene id" or "gene name" and selecting the species in the gene search section. After users submit their request, the results will be displayed in a table. The number of results will be shown at the top of the table. The table will list the general information of all matched genes, including the gene id, gene name and species information. Clicking on the "gene id" or "gene name" will bring users to a detailed information page of the gene. The nucleic acid sequence derived from de novo assembly, protein sequence deduced from assembled transcripts, and the expression of the gene in each sample can be viewed on the page.

Gene blast The user can input or upload query sequence(s) in fasta format, select the corresponding species database and the blast type to perform the gene blast. Hits will be listed in the result table. The users can browse the detailed information of the hit genes by clicking on the hit IDs.

Conclusions

Carcinology benefits both the basic science and the aquaculture industry. We have here generated a platform (CAT) in hosting 71 new transcriptomes generated for seven species of decapod crustaceans and a stomatopod. CAT is constructed in a way aiming to facilitate research on this important branch of life, and will continue to be updated, to host more crustacean genomic resources in the future.

Abbreviations

BLAST: Basic Local Alignment Search Tool; CAT: Crustacean annotated transcriptome database; DEGs: Differentially expressed genes; IDs: Identifications; RNA: Ribonucleic acid; rRNA: Ribosomal ribonucleic acid

Acknowledgements

The authors would like to thank David Wilmshurst, former academic editor at CUHK, for editing the English of the manuscript. The authors would also thank Clive Jones of James Cook University, Australia for his kind assistance in procuring the crayfish samples; Henry So of The Chinese University of Hong Kong for providing the cherry shrimp picture; and Tin-Yam Chan of National Taiwan Ocean University and Peter Ng of National University of Singapore for providing other pictures in Fig. 1.

Authors' contributions
WN performed transcriptome assemblies and functional annotation. ZYHC and KYM extracted RNA. WN, ZYHC, KYM, XJ, JQ designed and constructed the website. KMC, TFC, BKC, HSK, CKCW, JWQ, JHLH, KHC conceived the study design. WN, JHLH, KHC wrote the manuscript. All authors critically reviewed and approved the manuscript.

Funding
The work presented in this paper was supported by a grant from the Collaborative Research Fund (project no. C4042-14G), Research Grants Council, Hong Kong SAR, China. The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
The transcriptome data were deposited in NCBI under BioProjects PRJNA562428.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 3 October 2019 Accepted: 26 December 2019
Published online: 09 January 2020

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