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# Genomes of two indigenous clams *Anomalocardia flexuosa* (Linnaeus, 1767) and *Meretrix petechialis* (Lamarck, 1818)

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**Clam digging has a long history in Hong Kong, but unregulated clam digging activities depletes clam populations and threatens the ecosystem. Population genomics is useful to unravel the connectivity of clams at different geographical locations and to provide necessary conservation measures; and yet, only limited number of clams in Hong Kong have genomic resources. Here, we present chromosomal-level genome assemblies for two clams commonly found in Hong Kong, *Anomalocardia flexuosa* and *Meretrix petechialis*, using a combination of PacBio HiFi and Omni-C reads. For *A. flexuosa*, we assembled the genome into 19 pseudochromosomes with a genome size of 1.09 Gb (scaffold N50 = 58.5 Mb), and BUSCO scores of 94.4%. A total of 20,881 gene models were also predicted using the transcriptomes generated in this study. For *M. petechialis*, the genome was mainly assembled into 19 pseudochromosomes with a genome size of 1.04 Gb (scaffold N50 = 53.5 Mb), and BUSCO scores of 95.7%. A total of 20,084 gene models were also predicted using the transcriptomes generated in this study. The two new genomic resources established in this study will be useful for further study of biology, ecology, and evolution of clams, as well as setting up a foundation for evidence-informed decision making in conservation measures and implementation.**

## Background & Summary

Clams refer to the common name for several kinds of bivalve molluscs. The Veneridae family contains more than 700 described living species of bivalves or clams, and most of them are edible and exploited as food in different cultures around the world, including America, Asia and Europe (Huber, 2010)[1]. Clam digging activities, which refer to harvesting clams from below the surface of tidal sand or mud flats, also has long history in many places including Hong Kong. In the last century, clam digging in Hong Kong were mainly confined to villagers or recreational collection using hand tools on beaches during low tides for consumption or as a source of income. Nevertheless, clam digging activities have grown increasingly popular in recent years which threatens the clam populations and disturbs benthic biodiversity in some areas (Griffiths *et al.*; So *et al.*)[2,3]. Unlike many other places where sustainable clam digging practices, such as limiting the number of clams taken and/or temporary closure of clamming sites, Hong Kong does not have her own practices in the meantime due to the lack of information on the population structure of clams. As of to date, 12 genome assemblies in the Veneridae are available in NCBI (8 November 2024), including five chromosome-level genomes in the genera *Mercenaria*, *Mysia*, *Ruditapes* and *Venus*. Among the common clams that can be found in Hong Kong, such as that of *Anomalocardia* and *Meretrix* species which are the two frequently collected genera by local clam-diggers (So *et al.*)[3], genomic resources are currently lacking which hinders our understanding of their connectivity at different geographical locations.

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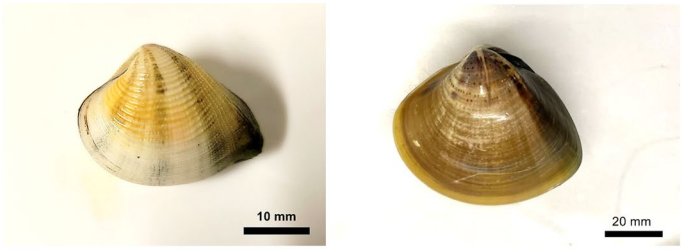
A)

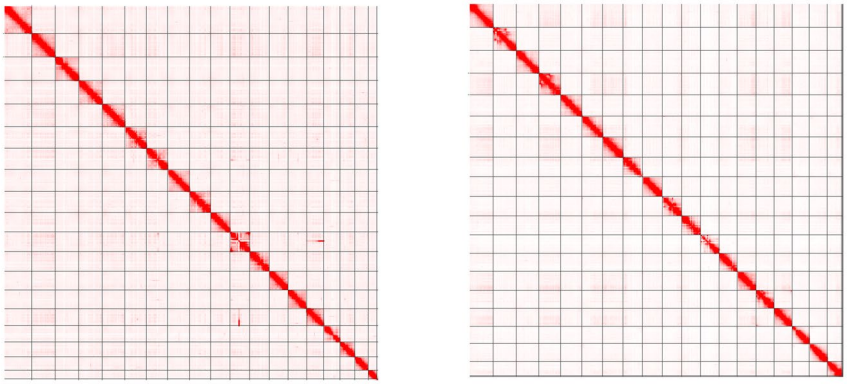



*A. flexuosa* *M. petechialis*

B)

| | *A. flexuosa* | *M. petechialis* |
|---|---|---|
| Total length (bp) | 1,090,751,803 | 1,044,279,863 |
| Number | 715 | 85 |
| Contig L/N50 | 79/4.555 Mb | 37/9.125 Mb |
| Scaffold L/N50 | 9/58.467 Mb | 9/53.513 Mb |
| GC content | 31.05% | 34.76% |
| BUSCOs (genome) | 94.40% | 95.70% |
| Gene model | 20,881 | 20,084 |
| No. of proteins | 26,853 | 25,196 |
| BUSCOs (proteome) | 96.20% | 98.30% |

C)



*A. flexuosa* *M. petechialis*

**Fig. 1** (**A**) Pictures of *A. flexuosa* (left) and *M. petechialis* (right); (**B**) Statistics of the genome assembly generated in this study; (**C**) Hi-C contact map of the assembly *A. flexuosa* (left) and *M. petechialis* (right); (**D**).

Here, utilizing PacBio HiFi long reads and Omni-C sequencing data, we present two chromosomal-level genomes of common clams in Hong Kong, *Anomalocardia flexuosa* and *Meretrix petechialis*. Together with transcriptome data from various tissues, we produce high-quality predicted gene models for the two clam species. These genome assemblies and transcriptome data provide valuable genomic resources for the understanding of genetic diversity and connectivity for future population genomics research in view of conserving local clam species and assessing the sustainability of clam digging activities.

## Methods

**Sample collection and high molecular weight DNA extraction.** *A. flexuosa* and *M. petechialis* samples were collected in Shui Hau, Lantau Island, Hong Kong (22°13′14.2″N 113°55′09.0″E) on 6th July, 2023 and Yi O, Lantau Island, Hong Kong (22°13′58.4″N 113°51′02.0″E), on 28th August, 2022, respectively (Fig. 1A). Approximately 300 mg adductor muscle was used for high molecular weight (HMW) DNA extraction for both *A. flexuosa* and *M. petechialis*. For *A. flexuosa*, the tissue was first ground into powder with liquid nitrogen, from which HMW DNA was isolated by NucleoBond HMW DNA kit (Macherey-Nagel), following the manufacturer's protocol. For *M. petechialis*, HMW DNA was extracted using MagAttract HMW DNA Kit (Qiagen), following the manufacturer's instructions. The DNA samples were eluted with 120 µL of elution buffer (PacBio Cat. No. 101-633-500) and were subjected to quality check by the Qubit® Fluorometer, NanoDrop One Spectrophotometer, and overnight pulse-field gel electrophoresis.

**PacBio library preparation and long-read sequencing.** Prior to library preparation, approximately 5 µg of HMW DNA isolated from *A. flexuosa* and *M. petechialis* in 120 µL of elution buffer were transferred to a g-tube (Covaris Cat. No. 520079) for DNA shearing with 6 passes of centrifugation at 1,990 × *g* for 2 min. The fragment size of sheared DNA samples was assessed with overnight pulse-field gel electrophoresis. A SMRTbell library was constructed for both samples using the SMRTbell® prep kit 3.0 (PacBio Cat. No. 102-141-700), following the manufacturer's instructions. Qubit® Fluorometer and overnight pulse-field gel electrophoresis were used to examine the quantity and quality of the SMRTbell libraries. Subsequently, the Sequel®II binding kit 3.2 (PacBio Cat. No. 102-194-100) was used for the final library preparation. Briefly, 3 µL of the SMRTbell library was mixed with 1.5 µL annealing buffer and 1.5 µL Sequel II Primer 3.2, and further incubated for 15 minutes in room temperature. Subsequently, a dilution step of Sequel II DNA polymerase 2.2 was carried out according to the manufacturer's instructions, where 6 µL diluted polymerase was added to the SMRTbell mixture and incubated for 15 minutes in room temperature for polymerase binding, and followed by a purification step using SMRTbell® clean-up beads. The polymerase-bound complexes were eluted with 50 µL Sequel II Loading Buffer 3.2, which were then mixed with an addition of 67 µL Sequel II Loading Buffer 3.2 and 3 µL diluted internal DNA control to prepare a final loading library of 120 µL in volume. All mixing procedures during the SMRTbell library preparation and the final library preparation were performed with 200 µL wide bore tips (Rannin Cat No. 30389188) in 2 mL DNA LoBind® Tubes (Eppendorf Cat No. 022431048). 115 µL of the two final libraries were loaded at an

| Species | Samples | Reads | Bases | Accesion number |
|---|---|---|---|---|
| Genome sequencing data | | | | |
| *Anomalocardia_flexuosa* | Afle_HiFi | 2,666,215 | 21,375,799,631 | SRR28740828 |
| *Anomalocardia_flexuosa* | Afle_omnic | 402,870,354 | 60,430,553,100 | SRR28728919 |
| *Meretrix_petechialis* | Mpet_HiFi | 2,850,426 | 30,583,425,339 | SRR28712952 |
| *Meretrix_petechialis* | Mpet_omnic | 377,451,754 | 56,617,763,100 | SRR28712977 |
| Transcriptome sequencing data | | | | |
| *Anomalocardia_flexuosa* | Af_Dg (Digestive gland) | 44,264,938 | 6,639,733,793 | SAMN41013774 |
| *Anomalocardia_flexuosa* | Af_Gl (Gill) | 43,570,390 | 6,535,551,419 | SAMN41013775 |
| *Anomalocardia_flexuosa* | Af_Gn (Gonad) | 42,937,050 | 6,440,550,167 | SAMN41013776 |
| *Anomalocardia_flexuosa* | Af_Mc (foot and adductor muscle) | 39,583,428 | 5,937,506,340 | SAMN41013777 |
| *Anomalocardia_flexuosa* | Af_Mt (Mantle) | 41,021,228 | 6,153,176,405 | SAMN41013778 |
| *Meretrix_petechialis* | Mp9YO_Dg (Digestive gland) | 37,997,880 | 5,699,679,344 | SAMN41013738 |
| *Meretrix_petechialis* | Mp9YO_Ft (foot muscle) | 38,741,520 | 5,811,225,289 | SAMN41013739 |
| *Meretrix_petechialis* | Mp9YO_Gl (Gill) | 39,108,764 | 5,866,311,436 | SAMN41013740 |
| *Meretrix_petechialis* | Mp9YO_Gn (Gonad) | 38,134,110 | 5,720,113,520 | SAMN41013741 |

**Table 1.** Genome and transcriptome sequencing data.

| Species | *Anomalocardia flexuosa* | *Meretrix petechialis* |
|---|---|---|
| Total length (bp) | 1,090,751,803 | 1,044,147,259 |
| Number | 715 | 85 |
| Mean length (bp) | 1,525,527 | 12,284,085 |
| Longest | 76,979,603 | 65,874,028 |
| Shortest | 1,000 | 1,000 |
| N count | 78,200 | 39,200 |
| Gaps | 391 | 195 |
| N50 | 58,466,825 | 53,512,864 |
| N50n | 9 | 9 |
| N70 | 52,832,318 | 51,039,052 |
| N70n | 13 | 13 |
| N90 | 37,743,970 | 49,958,606 |
| N90n | 18 | 17 |
| HiFi reads coverage (X) | 20 | 29 |
| Reads | 2,666,215 | 2,850,426 |
| Bases | 21,375,799,631 | 30,583,425,339 |
| HiFi reads average length (bp) | 8,017 | 10,729 |
| BUSCOs (genome) | C:94.4%[S:92.8%,D:1.6%],F:3.7%,M:1.9%,n:954 | C:95.7%[S:93.2%,D:2.5%],F:2.7%,M:1.6%,n:954 |
| No. of proteins | 26,853 | 25,196 |
| BUSCOs (proteome) | C:96.2%[S:82.0%,D:14.2%],F:0.4%,M:3.4%,n:954 | C:98.3%[S:81.7%,D:16.6%],F:0.1%,M:1.6%,n:954 |

**Table 2.** Genome statistics.

on-plate concentration of 90 pM with diffusion loading mode, respectively. The sequencing was performed on the PacBio Sequel IIe system using circular consensus sequencing (CCS) sequencing mode for a 30-hour movie with 2-hour pre-extension to generate HiFi reads for each sample. One SMRT cell was used for sequencing for *A. flexuosa* and *M. petechialis*, respectively. Finally, 21.83 Gb and 30.58 Gb of Hifi reads were obtained for *A. flexuosa* and *M. petechialis* with average lengths of 8,017 bp and 10,729 bp and data coverages of 20X and 29X, respectively (Table 1).

**Omni-C library preparation and sequencing.** An Omni-C library was prepared for *A. flexuosa* and *M. petechialis*, respectively, using the Dovetail® Omni-C® Library Preparation Kit (Dovetail Cat. No. 21005), following the manufacturer's instructions. Approximately 50 mg of flash-freezing powered tissue was used for crosslinking with the addition of formaldehyde in 1 mL 1X PBS for each sample, followed by nuclease digestion. The lysate samples were assessed by Qubit® Fluorometer and TapeStation D5000 ScreenTape and were proceeded with the library preparation protocol. After the final quality check with Qubit® Fluorometer and TapeStation D5000 ScreenTape, the Omni-C libraries were sent to Novogene Co. Ltd for sequencing on an Illumina HiSeq-PE150 platform, from which 60.4 Gb and 56.6 Gb Omni-C data were generated for *A. flexuosa* and *M. petechialis*, respectively (Table 1).

| Chr no. | Scaffold id | Scaffold length (bp) | Cumulative % of the whole genome |
|---|---|---|---|
| *Anomalocardia flexuosa* | | | |
| 1 | scaffold_1 | 76,979,603 | 0.07 |
| 2 | scaffold_2 | 66,208,884 | 0.13 |
| 3 | scaffold_3 | 65,610,396 | 0.19 |
| 4 | scaffold_4 | 64,669,910 | 0.25 |
| 5 | scaffold_5 | 64,436,222 | 0.31 |
| 6 | scaffold_6 | 59,912,551 | 0.36 |
| 7 | scaffold_7 | 59,882,163 | 0.42 |
| 8 | scaffold_8 | 59,794,789 | 0.47 |
| 9 | scaffold_9 | 58,466,825 | 0.53 |
| 10 | scaffold_10 | 55,963,395 | 0.58 |
| 11 | scaffold_11 | 53,985,618 | 0.63 |
| 12 | scaffold_12 | 53,674,951 | 0.68 |
| 13 | scaffold_13 | 52,832,318 | 0.73 |
| 14 | scaffold_14 | 52,636,158 | 0.77 |
| 15 | scaffold_15 | 48,317,914 | 0.82 |
| 16 | scaffold_16 | 45,620,769 | 0.86 |
| 17 | scaffold_17 | 41,163,429 | 0.90 |
| 18 | scaffold_18 | 37,743,970 | 0.93 |
| 19 | scaffold_19 | 22,996,108 | 0.95 |
| *Meretrix petechialis* | | | |
| 1 | scaffold_1 | 65,874,028 | 0.06 |
| 2 | scaffold_2 | 64,475,212 | 0.12 |
| 3 | scaffold_3 | 62,842,314 | 0.19 |
| 4 | scaffold_4 | 60,700,655 | 0.24 |
| 5 | scaffold_5 | 59,338,163 | 0.30 |
| 6 | scaffold_6 | 58,351,309 | 0.36 |
| 7 | scaffold_7 | 56,358,383 | 0.41 |
| 8 | scaffold_8 | 55,055,675 | 0.46 |
| 9 | scaffold_9 | 53,512,864 | 0.51 |
| 10 | scaffold_10 | 53,341,073 | 0.56 |
| 11 | scaffold_11 | 53,188,068 | 0.62 |
| 12 | scaffold_12 | 51,880,032 | 0.67 |
| 13 | scaffold_13 | 51,039,052 | 0.71 |
| 14 | scaffold_14 | 50,896,477 | 0.76 |
| 15 | scaffold_15 | 50,371,808 | 0.81 |
| 16 | scaffold_16 | 49,989,395 | 0.86 |
| 17 | scaffold_17 | 49,958,606 | 0.91 |
| 18 | scaffold_18 | 49,380,020 | 0.95 |
| 19 | scaffold_19 | 39,964,719 | 0.99 |

**Table 3.** Pseudochromosome information.

**Transcriptome sequencing.** Total RNA was isolated from various tissues including foot and adductor muscle, mantle, digestive gland, gill and gonad for *A. flexuosa* and foot, digestive gland, gill and gonad for *M. petechialis*, using the TRIzol™ Reagent (Invitrogen Cat No. 15596018), following the manufacturer's protocol respectively. The RNA samples were subjected to quality control using NanoDrop One Spectrophotometer, and gel electrophoresis. The qualified samples were sent to Novogene Co. Ltd for polyA selected RNA sequencing library construction and 150 bp paired-end sequencing. A total of 31.7 Gb and 23.1 Gb transcriptome data were obtained from different tissue types of *A. flexuosa* and *M. petechialis*, respectively (Table 1).

**Genome assembly and Gene model prediction.** *De novo* genome assemblies of *A. flexuosa* and *M. petechialis* were first proceeded with Hifiasm (Cheng *et al.*)[4] and then were processed with searching against the NT database with BLAST to remove possible contaminations using BlobTools (v1.1.1) (Laetsch & Blaxter)[5]. Subsequently, haplotypic duplications were removed according to the depth of HiFi reads using "purge_dups" (Guan *et al.*)[6]. Proximity ligation data from Omni-C were used to scaffold the assembly using YaHS (Zhou *et al.*)[7] and manual checking using Juicebox (v1.1)[8]. The genomes were soft-masked by redmask (v0.0.2) (https://github.com/nextgenusfs/redmask) (Girgis *et al.*)[9]. The final genome assemblies of *A. flexuosa* and *M. petechialis* were 1.09 Gb and 1.04 Gb in size with 95.43% and 99.27% of the sequenced anchored into 19 chromosomes, and 391 and 195 gaps, respectively, which correspond to the kartotype (2n = 38) of *Anomalocardia* and *Meretrix* species

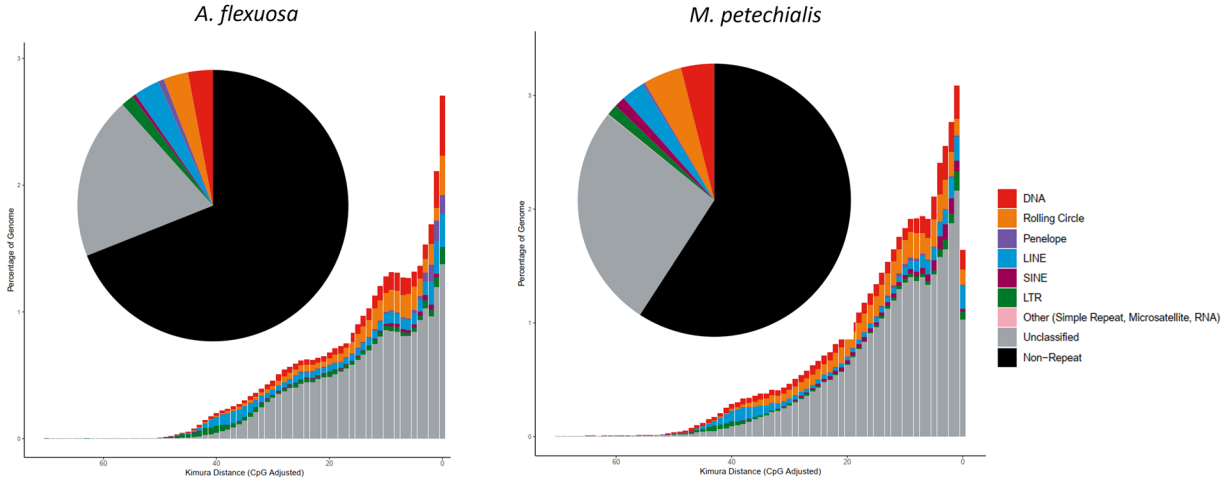



**Fig. 2** Pie chart and repeat landscape plot of repetitive elements of *A. flexuosa* (left) and *M. petechialis* (right).

| Classification | Coverage (bp) | Count | Proportion (%) | No. of distinct classifications |
|---|---|---|---|---|
| *A. flexuosa* | | | | |
| DNA | 32,355,348 | 49,773 | 2.97 | 6,979 |
| LINE | 33,557,383 | 42,257 | 3.08 | 6,509 |
| LTR | 16,669,352 | 16,758 | 1.53 | 4,072 |
| Other (Simple Repeat, Microsatellite, RNA) | 162,593 | 439 | 0.01 | 329 |
| Penelope | 7,635,347 | 5,280 | 0.70 | 2,121 |
| Rolling Circle | 32,188,869 | 35,849 | 2.95 | 3,391 |
| SINE | 4,543,005 | 11,316 | 0.42 | 1,466 |
| Unclassified | 211,213,474 | 381,513 | 19.36 | 9,250 |
| SUM | 338,325,371 | 543,185 | 31.02 | 34,117 |
| *M. petechialis* | | | | |
| DNA | 41,261,575 | 67,500 | 3.95 | 7,216 |
| LINE | 29,088,046 | 35,521 | 2.79 | 5,620 |
| LTR | 13,628,534 | 12,061 | 1.31 | 4,252 |
| Other (Simple Repeat, Microsatellite, RNA) | 975,819 | 1,065 | 0.09 | 728 |
| Penelope | 2,806,372 | 4,369 | 0.27 | 2,208 |
| Rolling Circle | 47,821,213 | 64,168 | 4.58 | 3,373 |
| SINE | 13,352,869 | 53,759 | 1.28 | 1,228 |
| Unclassified | 278,284,523 | 493,996 | 26.65 | 8,896 |
| SUM | 427,218,951 | 732,439 | 40.92 | 33,521 |

**Table 4.** EarlGrey repeat content summary.

(Fig. 1B-C; Tables 2, 3) (Lavander *et al.*; Park *et al.*)[10,11]. Both *A. flexuosa* and *M. petechialis* genomes were not only of high continuity, with scaffold N50 of 58.5 Mb and 53.5 Mb in 9 scaffolds, but also of high completeness after being assessed with Benchmarking Universal Single-Copy Orthologs (BUSCO, v5.5.0) using the "metazo_odb10" dataset (Manni *et al.*)[12], which resulted in BUSCO scores of 94.4% (Complete and single-copy BUSCOs (S): 92.8%, Complete and duplicated BUSCOs (D): 1.6%, Fragmented BUSCOs (F): 3.7%, Missing BUSCOs (M): 1.9%) and 95.7% (S:93.2%, D:2.5%, F:2.7%, M:1.6%), respectively (Fig. 1B; Table 2).

For gene model prediction, RNA sequencing data were first processed using Trimmomatic (v0.39) (Bolger, Lohse & Usadel)[13] with parameters "TruSeq. 3-PE.fa:2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25" and kraken2 (v2. 0.8 with kraken2 database k2_standard_20210517)[14] to remove the low quality and contaminated reads, and then aligned to the repeat soft-masked genome using Hisat2[15] to generate the bam file. A total of 389,399 Mollusca reference protein sequences were downloaded from NCBI on 25 March 2024 as protein hits, along with the RNA bam file, to perform genome annotation using Braker (v3.0.8)[16] with default parameters. Briefly, RNA-Seq and protein hints were used to train GeneMark-ETP, from which the genes with high extrinsic evidence support were then used to train on AUGUSTUS. The predictions from AUGUSTUS and GeneMark-ETP were combined using TSEBRA to generate the final annotation files.

| Class | Sub class | *A. flexuosa* | | | *M. petechialis* | | |
|---|---|---|---|---|---|---|---|
| | | Count | Coverage (bp) | Proportion (%) | Count | Coverage (bp) | Proportion (%) |
| LINE | CR1 | 3,705 | 1,587,891 | 0.15 | 8,602 | 2,786,199 | 0.27 |
| | I | 1,661 | 1,280,085 | 0.12 | 9,909 | 4,497,473 | 0.43 |
| | Jockey | 353 | 226,456 | 0.02 | / | / | / |
| | L1 | 5,894 | 3,520,180 | 0.32 | 5,348 | 2,976,001 | 0.29 |
| | L2 | 7,109 | 3,632,478 | 0.33 | 5,551 | 2,659,894 | 0.25 |
| | Proto2 | 2,001 | 718,443 | 0.07 | 735 | 557,396 | 0.05 |
| | R2 | 83 | 41,096 | 0.00 | 415 | 261,746 | 0.03 |
| | RTE | 16,403 | 9,137,736 | 0.84 | 16,934 | 8,020,547 | 0.77 |
| | unknown | 3,347 | 746,822 | 0.07 | 515 | 358,762 | 0.03 |
| LTR | Copia | 75 | 50,987 | 0.00 | 206 | 141,833 | 0.01 |
| | Gypsy | 35,955 | 22,111,187 | 2.03 | 70,827 | 39,102,451 | 3.75 |
| | unknown | 145,153 | 46,830,883 | 4.29 | 180,620 | 47,254,644 | 4.53 |
| SINE | 5S | 2,179 | 423,609 | 0.04 | 215 | 62,099 | 0.01 |
| | B2 | / | / | / | 1,147 | 194,260 | 0.02 |
| | B4 | / | / | / | 580 | 88,098 | 0.01 |
| | MIR | / | / | / | 2,996 | 421,895 | 0.04 |
| | tRNA | 40 | 3,846 | 0.00 | 473 | 95,039 | 0.01 |
| TIR | CACTA | 121,235 | 32,582,612 | 2.99 | 199,577 | 47,915,739 | 4.59 |
| | Mutator | 106,489 | 31,931,868 | 2.93 | 184,499 | 48,139,374 | 4.61 |
| | PIF_Harbinger | 49,470 | 16,811,316 | 1.54 | 115,421 | 32,726,416 | 3.13 |
| | Tc1_Mariner | 6,802 | 5,909,616 | 0.54 | 10,153 | 3,082,456 | 0.30 |
| | hAT | 48,588 | 17,270,407 | 1.58 | 107,465 | 31,909,218 | 3.06 |
| nonLTR | Penelope | 4,175 | 4,629,802 | 0.42 | 5,522 | 2,074,138 | 0.20 |
| nonTIR | helitron | 91,445 | 23,765,704 | 2.18 | 154,749 | 35,312,100 | 3.38 |
| repeat_region | | 64,118 | 16,752,765 | 1.54 | 58,890 | 14,389,456 | 1.38 |
| Total | | 716,280 | 239,965,789 | 22.00 | 1,141,349 | 325,027,234 | 31.15 |

**Table 5.** EDTA repeat content summary.

| Classification | *A. flexuosa* | | *M. petechialis* | |
|---|---|---|---|---|
| | earlGrey Proportion (%) | EDTA Proportion (%) | earlGrey Proportion (%) | EDTA Proportion (%) |
| DNA | 2.97% | / | 3.95% | / |
| LINE | 3.08% | 1.92% | 2.79% | 2.12% |
| LTR | 1.53% | 6.32% | 1.31% | 8.29% |
| Other | 0.01% | 1.54% | 0.09% | 1.38% |
| Penelope | 0.70% | 0.42% | 0.27% | 0.20% |
| Rolling Circle | 2.95% | / | 4.58% | / |
| SINE | 0.42% | 0.04% | 1.28% | 0.09% |
| Unclassified | 19.36% | / | 26.65% | / |
| TIR | / | 9.58% | / | 15.69% |
| helitron | / | 2.18% | / | 3.38% |
| SUM | 31.02% | 22.00% | 40.92% | 31.15% |

**Table 6.** Comparison of repeat annotation from EarlGrey and EDTA.

For *A. flexuosa*, the transcriptome assembled by stringtie (v2.2.1)[17] contained 53,191 transcripts in a total of 131,817,025 bp with an average length of 2,478 bp and an N50 length of 4,030 bp. The completeness of the transcriptome was also assessed using the BUSCO "metazo_odb10" dataset (Manni *et al.*)[12], reporting a BUSCO score of 98.1%. The assembled transcriptome was then used for gene model prediction. These data collectively generated 20,881 gene models, comprising 26,853 predicted protein-coding genes with average lengths of 580 amino acids (Fig. 1B; Table 2). The completeness of proteomes were also evaluated with BUSCO "metazo_odb10" dataset (Manni *et al.*)[12], reporting BUSCO scores of 96.2% (Fig. 1B; Table 2). Protein-coding genes were mapped to the nr and swissprot databases using Diamond (v2.0.7)[18] with the parameter '--evalue 1e-3--outfmt 6' for functional annotation, and 97.56% of the protein-coding genes could be mapped to the database. The transcriptome data was mapped to gene modes using hisat2[15] with default parameters, 96.82% of genes are expressed in the transcriptome samples.

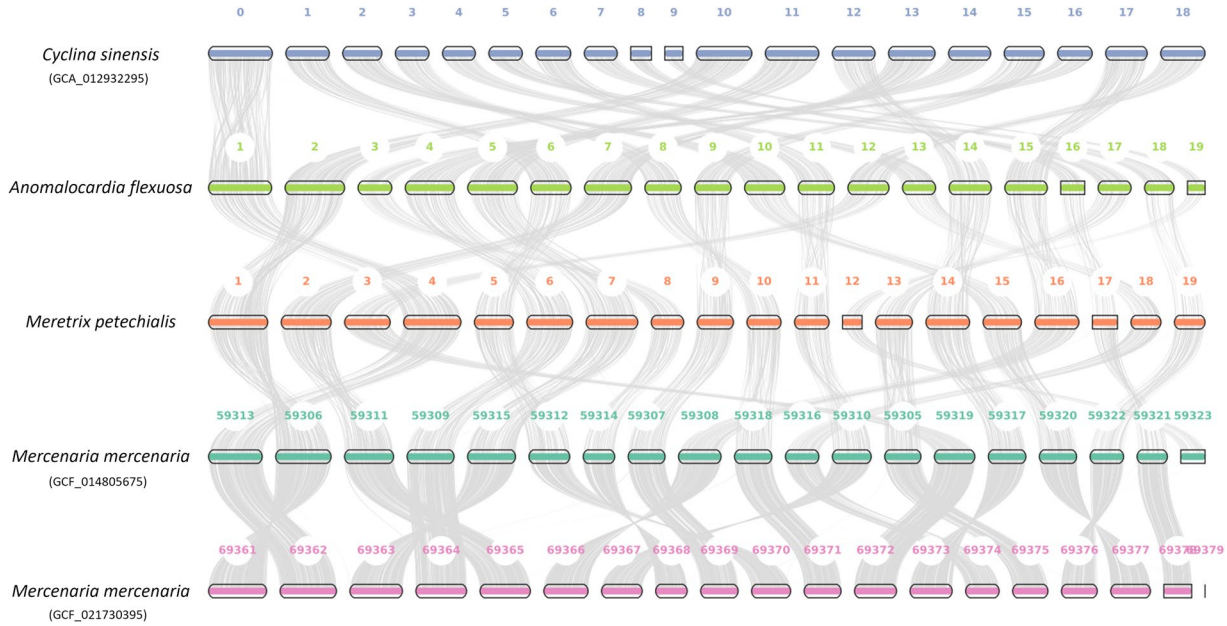



**Fig. 3** Macrosynteny plot of the 19 pseudochromosomes between *Cyclina sinensis*, *A. flexuosa*, *M. petechialis* and *Mercenaria mercenaria* (Mmer).

| Assembly Accession | Organism Name | Family | Genus | Organism Taxonomic ID | Assembly Stats Total Sequence Length | Assembly Stats Total Number of Chromosomes | Assembly Level | Assembly Stats Scaffold N50 | Annotation Count Gene Total |
|---|---|---|---|---|---|---|---|---|---|
| **JBCAUM000000000** | ***Anomalocardia flexuosa*** | **Veneridae** | **Anomalocardia** | **3139943** | **1,090,751,803** | **19** | **Chromosome** | **58,466,825** | **20,881** |
| GCF_021730395.1 | *Mercenaria mercenaria* | Veneridae | Mercenaria | 6596 | 1,858,199,728 | 19 | Chromosome | 82,914,371 | 45,375 |
| GCF_014805675.1 | *Mercenaria mercenaria* | Veneridae | Mercenaria | 6596 | 1,777,616,429 | 19 | Chromosome | 91,379,220 | 43,960 |
| **JBCJFF000000000** | ***Meretrix petechialis*** | **Veneridae** | **Meretrix** | **311198** | **1,044,147,259** | **19** | **Chromosome** | **53,512,864** | **20,084** |
| GCA_964106805.1 | *Mysia undata* | Veneridae | Mysia | 1920014 | 1,613,701,415 | 19 | Chromosome | 85,061,451 | / |
| GCA_009026015.1 | *Ruditapes philippinarum* | Veneridae | Ruditapes | 129788 | 1,123,164,463 | 19 | Chromosome | 345,005 | / |
| GCA_964200665.1 | *Venus verrucosa* | Veneridae | Venus | 55715 | 2,087,848,406 | 19 | Chromosome | 108,501,084 | / |
| GCF_026571515.1 | *Ruditapes philippinarum* | Veneridae | Ruditapes | 129788 | 1,408,116,862 | / | Contig | 183,074 | 48,037 |
| GCA_012932295.1 | *Cyclina sinensis* | Veneridae | Cyclina | 120566 | 903,119,975 | / | Scaffold | 46,470,132 | / |
| GCA_964106865.1 | *Mysia undata* | Veneridae | Mysia | 1920014 | 1,617,548,918 | / | Scaffold | 86,720,194 | / |
| GCA_022818135.1 | *Saxidomus purpurata* | Veneridae | Saxidomus | 311201 | 1,161,000,147 | / | Scaffold | 52,225,674 | / |
| GCA_032359765.1 | *Saxidomus gigantea* | Veneridae | Saxidomus | 410349 | 921,158,569 | / | Scaffold | 3,721 | / |
| GCA_041429985.1 | *Tivela stultorum* | Veneridae | Tivela | 345375 | 763,638,067 | / | Scaffold | 38,630,951 | / |
| GCA_041429975.1 | *Tivela stultorum* | Veneridae | Tivela | 345375 | 738,096,130 | / | Scaffold | 40,863,990 | / |

**Table 7.** Comparison of the Venerida genomes.

For *M. petechialis*, the transcriptome assembled by stringtie (v2.2.1)[17] contained 28,098 transcripts in a total of 83,316,136 bp with an average length of 2,965 bp and an N50 length of 4,271 bp. The completeness of the transcriptome was also assessed using the BUSCO "metazo_odb10" dataset (Manni *et al.*)[12], reporting a BUSCO score of 97.6%. The assembled transcriptome was then used for gene model prediction. These data collectively generated 20,084 gene models, comprising 25,196 predicted protein-coding genes with average lengths of 607 amino acids (Fig. 1B; Table 2). The completeness of proteomes were also evaluated with BUSCO "metazo_odb10" dataset (Manni *et al.*)[12], reporting BUSCO scores of 98.3% (Fig. 1B; Table 2). Protein-coding genes were mapped to the nr and swissprot databases using Diamond (v2.0.7)[18] with the parameter '--evalue 1e-3--outfmt 6' for functional annotation, and 96.72% of the protein-coding genes could be mapped to the database. The transcriptome data was mapped to gene modes using hisat2[15] with default parameters, 95.96% of genes are expressed in the transcriptome samples.

**Repetitive elements annotation.** Transposable elements (TEs) of the two genome assemblies were annotated as previously described (Baril *et al.*)[19] using the automated Earl Grey TE annotation pipeline (version 1.2, https://github.com/TobyBaril/EarlGrey) with "-r eukarya" to search the initial mask of known elements and other default parameters. Briefly, this pipeline first identified known TEs from Dfam with RBRM (release 3.2) and

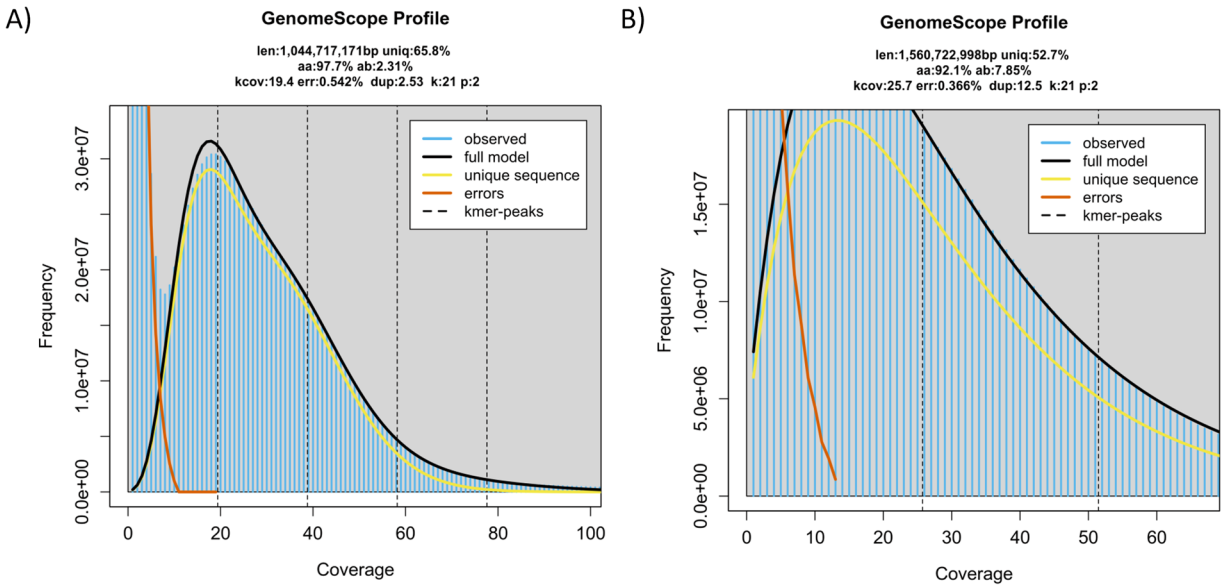



**Fig. 4** GenomeScope plots with the estimated genome size (*K*-mer = 21) of *A. flexuosa* (**A**) and *M. petechialis* (**B**).

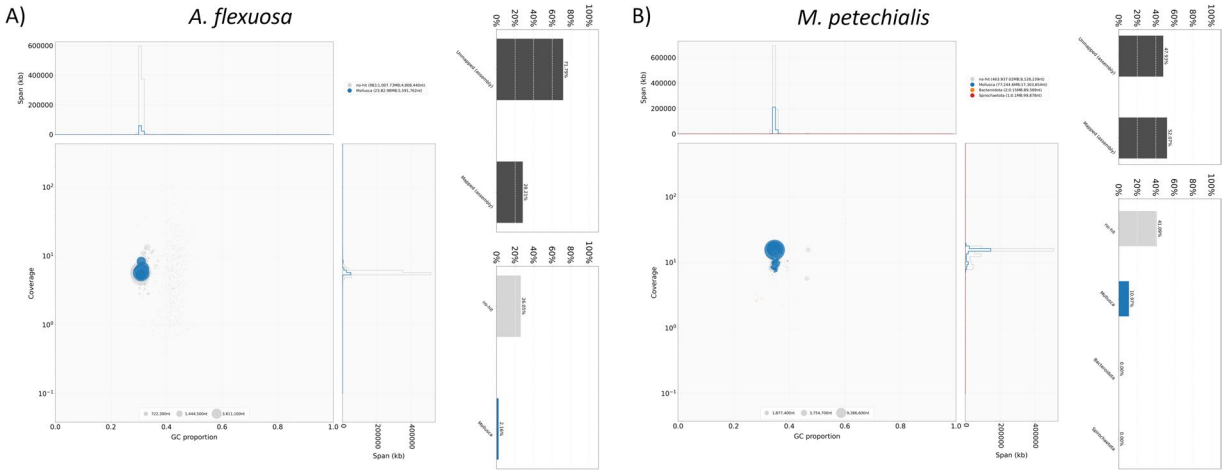



**Fig. 5** GC-coverage plots from BlobTools for genome assembly quality control and contaminants detection for *A. flexuosa* (**A**) and *M. petechialis* (**B**). The size of circles are proportional to the scaffold length. The upper bar plot shows the proportion of mapped and unmapped assembly to the NT database whereas the lower bar plot illustrates the distribution of the mapped assembly that are assigned to specific taxa.

RepBase (v20181026). *De novo* TEs were then identified, and consensus boundaries were extended using an automated "BLAST, Extract, Extend' process with 5 iterations and 1,000 flanking bases added in each round. Redundant sequences were removed from the consensus library before the genome assembly was annotated with the combined known and *de novo* TE libraries. Overlap and defragment annotations were removed prior to final TE quantification. A total of 338.3 Mb and 427.2 Mb of repeat contents were annotated from the genomes of *A. flexuosa* and *M. petechialis*, which account for 31.02% and 40.92% of the assembly, respectively (Fig. 2; Table 4). Of the classified TEs, LINE, DNA, and Rolling Circle contribute to the major proportions (Fig. 2), which are listed in Table 4. Repeat landscape plots of both *A. flexuosa* and *M. petechialis* revealed substantial increase of activities of LINE, DNA, and Rolling Circle and LTR, although a sharp recent decrease in repetitive element activities was observed in *M. petechialis* (Fig. 2). 19.36% and 26.65% of the repeat content were marked as unclassified for *A. flexuosa* and *M. petechialis* respectively. Therefore, we also run the Extensive de novo TE Annotator (EDTA)[20] with parameters "--species others--threads 32--cds cds.fa--force 1--anno 1 " for comparison. Despite fewer repetitive content was annotated by EDTA than earlGrey method in *A. flexuosa* (22.00%) and *M. petechialis* (31.15%), TIR still accounts for nearly half of the annotated repetitive elements annotated by either method (Tables 5, 6). Previous studies also identified the expansion and diversification of transposon elements in bivalve genomes (Farhat *et al.*; Martelossi *et al.*)[21,22]. Therefore, the genomes of *A. flexuosa* and *M. petechialis* may serve as valuable resources for further studies of genome evolution in Veneridae.

**Syntenic analyses.** Macrosynteny analysis revealed a 1-to-1 pair relationship between the 19 pseudochromosomes of *Cyclina sinensis*[23], *A. flexuosa*, *M. petechialis* and *Mercenaria mercenaria*[21] using JCVI[24] (Fig. 3), showing a conserved chromosome architecture between the four species, and the same chromosome number as in other Veneridae genomes. The full genome details downloaded from NCBI on 24 October 2024 are shown in Table 7.

## Data Records

The genome assemblies are in GenBank under accessions JBCAUM000000000[25] (*A. flexuosa*) and JBCJFF000000000[26] (*M. petechialis*). The raw reads generated in this study, including Transcriptome, Omni-C and PacBio HiFi data, have been deposited in the NCBI database under the SRA accession number SRP502500[27] and SRP502172[28] for *A. flexuosa* and *M. petechialis*, respectively. The genome, genome and repeat annotation files have been deposited and are publicly available in Figshare[29] and CUHK Research Data Repository[30].

## Technical Validation

The pseudochromosomes of the final assemblies were validated by inspecting the Omni-C contact maps using Juicer tools (version 1.22.01) (Durand *et al.*)[8]. Briefly, Omni-C reads were mapped and aligned by BWA with parameters "mem -5SP -T0", the parsing module of the pairtools pipeline was used to find ligation junctions with parameters "--min-mapq 40--walks-policy 5unique--max-inter-align-gap 30--nproc-in 8--nproc-out 8". The parsed pairs were then sorted using pairtools sort with default parameters, PCR duplicate pairs were removed using pairtools dedup with parameters "--nproc-in 8 --nproc-out 8 --mark-dups", the pairs file was split using pairtools split with default parameters and used to generate the contact matrix using juicertools and Juicebox (v1.11.08)[8]. Regarding the genome characteristics of the assembly, the k-mer count and histogram were generated at k = 21 from Omni-C reads using Jellyfish (v2.3.0) (Marçais & Kingsford)[31] with the parameters "count -C -m 21 -s 1000000000 -t 10", and the reads.histo was uploaded to GenomeScope to estimate genome heterozygosity, repeat content and size using default parameters (v2.0) (http://qb.cshl.edu/genomescope/genomescope2.0/) (Ranallo-Benavidez *et al.*)[32]. The resulting GenomeScope plots can be found in Fig. 4. Omni-C reads and PacBio HiFi reads were used to measure assembly completeness and consensus quality (QV) using Merqury (v1.3)[33] with kmer 20, resulting in 75.3568% and 96.1486% kmer completeness for the Omni-C data and 57.149 and 62.1256 QV scores for the HiFi reads, corresponding to 99.999% and 99.9999% accuracy for *A. flexuosa* and *M. petechialis*, respectively. In addition, BlobTools (v1.1.1) assigned most of the assembled scaffolds that mapped to the NT database to the taxon Mollusca and scaffolds that assigned to other taxa such as Bacteroidota were removed (Laetsch & Blaxter)[5] (Fig. 5).

## Code availability

No specific script was used in this work.

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## Author contributions

S.Y.L., S.G.C. and J.H.L.H. conceived and supervised the study; S.T.S.L., M.F.F.A., L.H.T.C. and C.W.Y.S. carried out sample collection; S.T.S.L. and W.N. performed data curation on the analysis; J.H.L.H., S.T.S.L. and W.N. wrote the initial manuscript; all authors revised and contributed to the final version of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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