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Chromosome-level genome assembly defines female-biased genes associated with sex determination and differentiation in the human blood fluke *Schistosoma japonicum*

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Abstract

Schistosomiasis is a neglected tropical disease of humans caused by blood flukes of the genus *Schistosoma*, the only dioecious parasitic flatworms. Although aspects of sex determination, differentiation and reproduction have been studied in some *Schistosoma* species, almost nothing is known for *Schistosoma japonicum*, the causative agent of schistosomiasis japonica. This mainly reflects the lack of high-quality genomic and transcriptomic resources for this species. As current genomes for *S. japonicum* are highly fragmented, we assembled and report a chromosome-level reference genome (seven autosomes, the Z-chromosome and partial W-chromosome), achieving a substantially enhanced gene annotation. Utilising this genome, we discovered that the sex chromosomes of *S. japonicum* and its congener *S. mansoni* independently suppressed recombination during evolution, forming five and two evolutionary strata, respectively. By exploring the W-chromosome and sex-specific transcriptomes, we identified 35 W-linked genes and 257 female-preferentially transcribed genes (FTGs) from our chromosomal assembly and uncovered a signature for sex determination and differentiation in *S. japonicum*. These FTGs clustering within autosomes or the Z-chromosome exhibit a highly dynamic transcription profile during the pairing of female and male schistosomula, thereby representing a critical phase for the maturation of the female worms and suggesting distinct layers of regulatory control of gene transcription at this development stage. Collectively, these data provide a valuable resource for further functional genomic characterisation of *S. japonicum*, shed light on the evolution of sex chromosomes in this highly virulent human blood fluke, and provide a pathway to identify novel targets for development of intervention tools against schistosomiasis.

Keywords: Schistosomiasis, *Schistosoma japonicum*, Genome assembly, Sex chromosomes, Sex differentiation

1 Introduction

Schistosomiasis is a disease caused by blood flukes of the genus *Schistosoma* (Phylum Platyhelminthes; family Schistosomatidae). Schistosomiasis is a highly significant, neglected tropical disease (NTD) that remains a serious public health problem mainly in tropical and sub-tropical areas (World Health Organization, 2010). Currently, > 250 million people in 78 countries are affected (McManus et al., 2018). The People's Republic of China has achieved considerable success in preventing human infections by *Schistosoma japonicum* (the Asian schistosome) (Cao et al., 2020). In some parts of sub-Saharan Africa, efforts to control the two other most common disease-causing species, *S. mansoni* and *S. haematobium*, through large-scale preventive chemotherapy with praziquantel, have also reduced morbidity and improved human health in underprivileged communities (Webster et al., 2014). However, there is evidence that schistosomiasis is more prevalent than previously recognized (Colley et al., 2017). For instance, schistosomiasis has re-emerged in some endemic areas where control efforts had been successful, or has emerged due to the transmission of zoonotic or hybrid variants of schistosomes in non-endemic areas (Rudge et al., 2013; Noel et al., 2018). Moreover, reduced praziquantel efficacy observed in human populations under high preventive chemotherapy pressure warrants attention in relation to control (Crellen et al., 2016). These issues emphasize the complexities associated with the epidemiology and control of schistosomiasis, and the need to improve understanding of fundamental aspects of the biology (e.g. hybridization) and reproduction of schistosomes, in order to achieve improved prevention, treatment and effective control.

In contrast to most trematodes (flukes), which are hermaphrodites, schistosomes are dioecious with dimorphic adults (Mone & Boissier, 2004). Schistosomes have seven pairs of autosomes and one pair of sex chromosomes (ZZ and ZW); the female worms are heterogametic (ZW) and males are homogametic (ZZ) (Popiel et al., 1984; Basch, 1990; Kunz, 2001; Loker & Brant, 2006). Although dimorphic at the adult stage, the sexes of the larval stages (eggs, miracidia, sporocysts, cercariae, and schistosomula) are morphologically indistinguishable.

The acquisition of separate sexes in the schistosomes during evolution seems to be concomitant with their invasion of warm-blooded animals (Loker & Brant,

2006). However, the separation and establishment of sex chromosomes appear to be insufficient for complete female development. A unique feature of schistosome biology is that the female worm needs to be permanently coupled with a male for gonad differentiation, sexual maturation and effective reproduction (Kunz, 2001; Beckmann et al., 2010). In the paired adult female, the ovary produces oocytes, and the vitellarium provides the vitelline cells and egg-shell proteins needed for embryogenesis and egg production (Kunz, 2001; Beckmann et al., 2010). The eggs are critical for maintaining the life-cycle and, in the human or other mammalian host, cause granulomatous disease (schistosomiasis) usually in enterohepatic or urogenital tissues (Ross et al., 2002).

Due to the medical importance and unique sexual biology of schistosomes, there has been extensive investigation of their genomes and transcriptomes. Reference genomes have been published for eight distinct species of *Schistosoma*, including the three main human-infecting schistosome species (*S. japonicum*, *S. mansoni* and *S. haematobium*) (Berriman et al., 2009; Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium, 2009; Young et al., 2012; International Helminth Genomes Consortium, 2019; Luo et al., 2019; Oey et al., 2019). Assemblies with chromosomal or near-chromosomal contiguity have been achieved for the genomes of *S. mansoni* and *S. haematobium* (Protasio et al., 2012; Stroehlein et al., 2019), but not for other species. Recently, the assembly of the genome of *S. japonicum* was improved by combining PacBio long sequencing with Illumina paired-end reads (Luo et al., 2019). This new assembly (V2, BioProject PRJNA520774) reflects a genome size of ~ 370.5 Mb, with contig and scaffold N50 lengths of 871.9 kb and 1.09 Mb, respectively, representing 142.4-fold and 6.2-fold improvements over the earlier-released assembly (V1, BioProject PRJEA34885) (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009); however, the V2 genome assembly still lacks considerable chromosome information.

Here, we utilized PacBio, 10× Genomics and Hi-C as well as Illumina sequencing data from a clonal line of *S. japonicum* to generate a chromosome-level genome for female *S. japonicum*. The improved genome allowed us to comprehensively probe the evolutionary strata on the Z-chromosome, identify genes on the W-chromosome, and accurately map the genes preferentially

transcribed in female worms to autosomes. Our improved assembly and annotation provide a valuable genomic resource for future functional genomic analysis of *S. japonicum* and for studies of the emergence of sex chromosomes in schistosomes.

2 Materials and Methods

2.1 Procurement of *S. japonicum*

Eggs of *S. japonicum* were isolated from the livers of infected mice and then hatched in mineral water under light (Xu & Dresden, 1990). To establish clonal *S. japonicum*, individual *Oncomelania hupensis hupensis* snails were exposed to individual miracidia in 24-well cell culture plates (2 ml mineral water per well) overnight at room temperature. After cultivation for 3 months at 25 °C on moist straw paper in enamel-coated dishes, each snail was examined individually for cercarial release under a bright white light (Theron et al., 1997). Clonal cercariae were collected from each snail, and the sex of the cloned cercariae determined by PCR using female-specific primers (Figure S1) (Zhao et al., 2010). Female BALB/c mice (8-10 weeks of age) were each infected with both 10 clonal female cercariae and 10 clonal male cercariae. Adult worms of *S. japonicum* were collected by perfusion from the mesenteric veins surrounding the gut at 56 dpi (days post infection), and eggs from female worms were used to establish inbred lines of *S. japonicum* (Xu & Dresden, 1990). All animal experimentation was approved by the Internal Review Board of Tongji University, School of Medicine, China.

2.2 Nucleic acid isolation and sequencing

After two rounds of inbreeding, an inbred line - designated SjF4M4, - was used for genomic DNA and total RNA extractions. Genomic DNA (10 µg for each sample) was isolated separately from male and female adult worms using a MagAttract HMW DNA Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA was quantified using a Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, USA) and a NanoDrop One Spectrophotometer (Thermo Fisher Scientific), and then examined for quality by agarose gel electrophoresis and by using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) prior to sequencing using PacBio and 10× Genomics using standardized protocols (Eid et al., 2009; Zheng et al., 2016).

Gravid adult females will harbor some eggs of the opposite sex, indicating the genomic DNA of adult female worms contains male egg DNA. However, a small amount of male eggs in female worms can be ignored as female worms are heterogametic (ZW) and males are homogametic (ZZ). Total RNA (5 µg for each stage) was isolated separately from eggs, cercariae, and female and male worms, using the Qiagen RNeasy Midi Kit (Qiagen). Paired-end (PE) RNA-seq libraries (insert sizes: 200 bp and 500 bp) were constructed using the TruSeq RNA Library Preparation Kit v2 and sequenced employing the Illumina platform.

2.3 Genome assembly

The *S. japonicum* genome size was estimated based on *k*-mer analysis ($k = 17$) of sequence data using KmerGenie (Chikhi & Medvedev, 2014). Murine host DNA contamination was removed before scaffolding using Bowtie 2 v2.3.3.1 (Langmead & Salzberg, 2012). All raw reads obtained by PacBio sequencing were aligned to each other using DALIGNER, executed using the Falcon (v0.7) assembler (Chin et al., 2016). Overlapping reads and raw sub-reads were processed to produce consensus sequences. After error-correction, overlaps were detected in the pre-assembled, error-corrected reads and used to construct a directed fragment assembly string graph. Contigs were constructed by finding the paths from the string graph. The consensus calling of the preceding assembly was performed using the consensus-calling algorithm Quiver (Chin et al., 2013). Subsequently, the paired-end, clean Illumina reads were used for further error-correction of the assembly *via* using Pilon (v1.22) (Walker et al., 2014). The 10× Genomics–scaffold extension was performed using FragScaff software (Adey et al., 2014), in which the linked reads produced from the 10× genomic library were aligned to the consensus sequence of the PacBio assembly. To obtain super-scaffolds, only consensus sequence with linked-read support was used for assembly. The genomic Illumina data were aligned to the assembled reference genome for *S. japonicum* (V3) using BWA-MEM software (v0.7.17) (Li, 2013). Core Eukaryotic Genes Mapping Approach (CEGMA) (Parra et al., 2007) and Benchmarking Universal Single Copy Orthologs (BUSCO v3) (Simao et al., 2015) were used to estimate genome completeness. Murine host genome contamination was analysed using BlobTools2 (<https://blobtoolkit.genomehubs.org/blobtools2/>).

2.4 Scaffolding of the assembly using Hi-C data to reach chromosome contiguity

Paired adult female and male worms of *S. japonicum* (n = 80) were collected, washed three times in phosphate-buffered saline (PBS) and fixed in 2% formaldehyde/PBS solution for 30 min at room temperature. Fixed worms were homogenized in liquid nitrogen, resuspended in nuclei isolation buffer and filtered using a 40 nm cell strainer. Parasite membranes were further disrupted by passing the lysate through a 30-gauge needle using a syringe. The extracted nuclei were digested with 100 U *Dpn* II (NEB, R0543) restriction enzyme by incubating overnight at 37 °C, and the digested chromatin was blunt-ended with 1 µl of 10 mM dCTP, dTTP, dGTP, 25 µl of 0.4 mM biotin-14-dATP (Invitrogen) and 100 U of Klenow fragment (NEB, M0210) for 3 h at 37 °C. Next, the ligation reaction was performed in 10 × the volume of ligation production buffer under constant shaking with 50 µl of 10 × ligation buffer and 10% Triton X-100, 2.5 µl of 20 mg/ml bovine serum albumin (BSA) and 2 µl of T4 DNA ligase (NEB, M0202). The ligation reaction was performed at 16 °C overnight. Following ligation, the nuclei were digested with proteinase K at 65 °C for 12 h. The DNA was purified and sheared to a size of 200-300 bp using a sonicator (Bioruptor™ UCD-200), and then size-selected using 0.8X - 1.1X AMPure XP beads (Beckman Coulter, A63881). The 1.1X biotin-tagged Hi-C DNA was subjected to end-repair and then bound to Dynabeads® MyOne™ Streptavidin C1 beads (Invitrogen). The C1 beads with bound DNA were resuspended, and the DNA extended with 3' A-overhangs and ligated to barcoded NextFlex adapters (Bio Scientific). To prepare sequencing libraries, the purified DNA were end-repaired, extended with 3' A-overhangs and ligated to barcoded NextFlex adapters (Bio Scientific). Libraries were amplified using KAPA HiFi HotStart ready mix (KAPA Biosystems) employing the following PCR protocol: 98 °C for 3 min, followed by 14 cycles of 98 °C for 20 sec; 65 °C for 30 s; 68 °C for 30 s, and a final extension of 68 °C for 5 min. Amplified libraries were size-selected for 300-600 bp using 2% w/v agarose gels and paired-end sequenced using an Illumina HiSeq X Ten sequencer. The cleaned Hi-C data were aligned to the primary assembly using BWA-MEM 0.7.17 software. Only read-pairs, for which both reads in the pair aligned to contigs, were considered for scaffolding. Sequences of >100 bp were selected by Lachesis (v201701) (Burton et al., 2013) to scaffold the assembly to the chromosome level.

2.5 Defining autosomal and sex chromosomes

Illumina reads representing each adult male and adult female *S. japonicum* were mapped separately to the V3 genome assembly of *S. japonicum* using BWA-MEM 0.7.17. The resultant alignments were filtered using SAMtools (Li et al., 2009) to retain only reads with a mapping quality of ≥ 33 . Then, each chromosome and scaffold was divided into 50-kb windows, with 10-kb or 5-kb windows overlapping by 1 kb to calculate the depth of male and female Illumina sequences separately in each window using SAMtools and BEDTools v2.27.1 (Quinlan & Hall, 2010). As the insert sizes of the libraries representing female and male *S. japonicum* differed from one another, we selected the longest autosome (chromosome 1) to normalise depth. We estimated depths of 33 (female) and of 44 (male) using the ratio $S_0/2L_0$, where S_0 is the sum of nucleotides that mapped, and L_0 is the length of the chromosome, excluding gaps (Ns). In each window of each chromosome or scaffold, the normalised depth value was calculated using S/dL , where S is the sum of bases that mapped in a window, L is the corresponding window-length after removing the N bases (gaps), and d is the particular absolute sequencing depth observed for a given sex (33 for females; 44 for males). Based on normalised depth, windows with male-to-female depth ratio of 1.5 to 2 were classified as being Z-linked, whereas windows with male-to-female depth ratios of < 0.5 were classified as W-linked. A chromosome/scaffold was assigned as autosomal if the majority of windows ($> 50\%$) had a male-to-female depth ratio of ~ 1.0 . A scaffold was assigned as W-chromosomal if the 'female' depth, following normalization of the majority of windows, was > 0.3 , whereas the corresponding normalised 'male' depth was less than half of the 'female' depth.

2.6 Chimeric sequence reassembly

As the Z chromosome, as initially defined, contained several W-linked regions, we designated it as a ZW-chromosome. We divided the ZW-chromosome into 5-kb windows to eliminate contamination by W fragments. Windows with a normalised female depth of ~ 1.0 and a normalised male depth of ~ 0 were termed W-linked regions, and others were termed Z-linked regions. The same method was employed for scaffolds representing W-linked fragments. Thirteen W-linked regions (Figure S2; W_region_1 to W_region_13) and 7 W-linked scaffolds (Figure S2; W_scaf_1 to W_scaf_7) were combined and re-assembled

into the W chromosome, and the Z-linked regions were re-assembled into the Z chromosome (Figure S3) using the same approach. The scaffolding used for the reassembly of each sex chromosome was performed using Scaff10X (<https://github.com/wtsi-hpag/Scaff10X>), HiC-Pro v2.10.0 (Servant et al., 2015) and SALSA v2.0 (Ghurye et al., 2019) using data from the 10× Genomics linked-read library and the Hi-C library. Then, fragments were ordered and oriented in relation to the initial *S. japonicum* V3 ZW-chromosome using RaGOO (Alonge et al., 2019). The final W-linked (ChrW_fragment) sequence assembly of 4.8 Mb does not contain the PAR region.

2.7 Gene prediction

Genes were predicted by combining *de novo*, homology-based and transcriptome-based predictions. For the *de novo* prediction, Augustus (v3.2.3) (Hoff & Stanke, 2019), GlimmerHMM (v3.0.4) (Majoros et al., 2004), SNAP (v2013.11.29) (Korf, 2004), Geneid (v1.2) (Alioto et al., 2018) and Genscan (v1.0) (Burge & Karlin, 1997) were employed. For the homology-based approach, genes of *S. mansoni* (Protasio et al., 2012; Buddenborg et al., 2021), *S. haematobium* (Stroehlein et al., 2019), *Clonorchis sinensis* (Young et al., 2021), and *Opisthorchis viverrini* (Young et al., 2014) were used as queries to search against *S. japonicum* V3 using BLAST (Johnson et al., 2008) and Genewise (Birney et al., 2004). For the transcriptome-based gene prediction TopHat mapper (v2.0.8), along with Cufflinks assembler (v2.1.1), was employed for mapping (Trapnell et al., 2012). The pseudo-ESTs were generated from RNA-Seq data by Trinity v2.7.0 (<https://github.com/trinityrnaseq/trinityrnaseq/releases>) and mapped to the assembled genome. The PASA (v2.3.3) (Haas et al., 2003) was employed to predict gene models. The gene prediction results were merged using EvidenceModeler (v1.1.1) software to generate a consensus gene set. Translated coding sequences were compared with those in known databases such as Swissprot (v20180824), a subset of Uniprot (UniProt Consortium, 2021), the non-redundant NCBI protein database (Nr; v20180716) (Sayers et al., 2021), KEGG (v20160503) (<https://www.kegg.jp/>), InterPro (v5.31-70.0) (Blum et al., 2021), GO (Harris et al., 2004) and Pfam (v31.0) (El-Gebali et al., 2019). All protein-coding genes were annotated using information from Swissprot, Nr, KEGG, InterPro, GO and Pfam.

2.8 Identifying one-to-one orthologs

The *S. mansoni* annotation (schistosoma_mansoni.PRJEA36577.WBPS13.annotations.gff3) and protein (schistosoma_mansoni.PRJEA36577.WBPS13.protein.fa) files were obtained from the WormBase ParaSite database (<https://parasite.wormbase.org/index.html>, Smansoni_v7, WBPS15, contributed by the Wellcome Sanger Institute) (Bolt et al., 2018; Buddenborg et al., 2021). Protein BLAST (Blastp) (Camacho et al., 2009) was performed using predicted proteins encoded by the *S. japonicum* Z chromosome as queries against predicted proteins encoded by the *S. mansoni* ZW-chromosome, with only the longest amino acid isoform sequence used for each *S. japonicum* and *S. mansoni* gene. In the output file, genes on the Z chromosome of *S. japonicum*, whose amino acid sequences aligned < 30% or whose alignment identity was < 50% were filtered out, retaining only the best matches. The orthologs were assigned to groups based on their genomic locations relating to *S. japonicum* specific strata (SjapS1, SjapS2, SjapS3, SjapS4), *S. mansoni* specific strata (SmanS1) and shared strata (SjapS0 and the PAR region).

2.9 Demarcation of evolutionary strata

We combined the read depth and SNP density calculated from reads from both sexes of *S. japonicum*, the sequence divergence levels between chromosomes W and Z and orthology information between *S. japonicum* and *S. mansoni*, to demarcate the evolutionary strata. Based on the depth differences between males and females, we assigned the highly divergent SDRs in the genomes of *S. japonicum* V3 and *S. mansoni*, which revealed a 2-fold depth difference between sexes. Based on the one-to-one orthologous relationship between *S. japonicum* and *S. mansoni*, their shared SDR was assigned as the 'ancient' stratum (SjapS0), and species-specific SDRs were assigned to 2 separated strata for *S. japonicum* and SmanS1 for *S. mansoni*. Although the current chrZW assembly of *S. mansoni* may contain W-linked sequences, our coverage analyses confirmed that there were few fragments showing a female-specific read coverage pattern. This suggested that we could still use the chrZW assembly to compare the syntenic

relationship between *S. mansoni* and *S. japonicum*. The W chromosome sequences were aligned to the Z chromosome sequence using nucmer v3.23 (Kurtz et al., 2004). Sequences whose alignment lengths were < 5 kb or alignment identities were < 70% were discarded. The aligned W chromosome sequences were oriented along the Z chromosome (colour-coded according to sequence divergence) in such a way that defined the boundaries or ages between strata; for instance, in our nomenclature, SjapS2 exhibited higher Z/W homology than SjapS1. Illumina reads representing adult female and male *S. japonicum* were mapped separately to the *S. japonicum* V3 genome using BWA-MEM 0.7.17. SNPs were identified from the resultant alignment files using the genomic analysis toolkit (GATK 3.8) (McKenna et al., 2010). In order to estimate Z/W divergence (dS), we extracted female-specific SNPs from genes with SNPGenie (Nelson et al., 2015). The bilateral regions of *S. japonicum* SjapS2 was assigned as the more 'recent' stratum, with the similar levels of male and female read depths, but distinct levels of female SNPs densities relative to male ones. SjapS4 exhibited lower SNPs heterozygosity in females and lower dS value compared with SjapS3, suggesting it's the most 'recent' stratum.

2.10 Prediction of repeats and Non-coding RNA

The method of Repeat sequences annotation was divided into homologous sequence alignment and de novo prediction. Based on the RepBase library (<http://www.girinst.org/replib/>), the homologous sequence alignment method used RepeatMasker and RepeatProteinMask (<http://www.repeatmasker.org/>) software to identify sequences similar to known repeat sequences. The de novo prediction method firstly established the De novo repeat sequence libraries by RepeatModeler (<http://www.repeatmasker.org/RepeatModeler.html>) software, and then RepeatMasker was used for prediction again. In addition, we also used the TRF (<http://tandem.bu.edu/trf/trf.html>) software to look for tandem repeat sequences in the genome. Based on the above method, we will provide four repeat sequences annotation files, namely Sjap.known.repeat.gff, Sjap.denovo.repeat.gff, Sjap.protein.repeat.gff and Sjap.tandem.repeat.gff.

Non-coding RNA annotations contained tRNA, rRNA, miRNA and snRNA. According to the structure characteristics of tRNA, tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscan-SE/>) software was used to find tRNA in the genome sequence. As rRNA is highly conserved, rRNA sequences of closely related species can be selected as reference sequences to search for rRNA in the genome by the Barrnap software (<https://github.com/nickp60/barrnap>). Using Rfam family model, the Infernal (<http://eddylab.org/infernal/>) software predicted the miRNA and snRNA sequence information in the genome.

2.11 SMRT-based RNA sequencing

Total RNAs were extracted from eggs, cercariae, schistosomula, and adult female and male *S. japonicum* parasites. They were then equally mixed for library preparation. The Iso-Seq library was prepared according to the Isoform Sequencing protocol (Iso-Seq) using the Clontech SMARTer PCR cDNA Synthesis Kit and the BluePippin Size Selection System protocol as described by Pacific Biosciences (PN 100-092-800-03). Sequence data were processed using the SMRTlink 5.0 software. Circular consensus sequence (CCS) was generated from subread BAM files, parameters: min_length 200, max_drop_fraction 0.8, no_polish TRUE, min_zscore -9999, min_passes 1, min_predicted_accuracy 0.8, max_length 18000. CCS.BAM files were output, which were then classified into full length and non-full length reads using pbclassify.py script, ignore polyA false, minSeq Length 200. Nonfull length and full-length fasta files produced were then fed into the cluster step, which does isoform-level clustering (ICE), followed by final Arrow polishing, hq_quiver_min_accuracy 0.99, bin_by_primer false, bin_size_kb 1, qv_trim_5p 100, qv_trim_3p 30. Aligning consensus reads to reference using GMAP with parameters: --no-chimeras --cross-species --expand-offsets 1 -B 5 -K 50000 -f samse -n 1 against the newly assembled genome in this study.

2.12 Illumina sequencing-based transcriptome analyses

Total RNAs were extracted from eggs, cercariae, schistosomula, and adult female and male *S. japonicum* parasites. Each RNA sample was subjected to illumine-based RNA-seq. RNA-seq reads from both sexes at eight time points (14–28 dpi) used in dynamic differential transcriptome analysis between female and male parasites were obtained from GenBank (PRJNA343582) (Wang et al., 2017). Biological replicates of RNA-seq data for males and females at each stage were

combined with 16 merged libraries, and RNAseq reads were then mapped to the *S. japonicum* genome V3 using HISAT2 v2.0.4 (Kim et al., 2015). Reads were counted using HTSeq v0.11.0 (Anders et al., 2015), and transcription values - in reads per kilobase of transcript per million mapped reads (RPKM) - were calculated for each gene for each library (Mortazavi et al., 2008). All of these values are provided in the source data file (Table S19). Data on dosage compensation (DC) analysis and sex-biased genes analysis were filtered using the same threshold (RPKM > 1) for each sex and stage of *S. japonicum*. For W chromosome-linked transcription, the filtration threshold was RPKM-mean > 1 (sum of RPKMs of all libraries for the same gene was > 16). We performed edgeR to estimate gene expression changes between sexes or stages.

2.13 Gametolog analysis

First, we used BlastP to identify gene gametologs in the W chromosome of *S. japonicum* and to compare them with the predicted proteomes of *S. mansoni* and *Fasciola hepatica*. Homologs between *S. japonicum* and *S. mansoni* were identified that had a sequence alignment score of > 50 and aligned over $\geq 30\%$ of the query gene sequence, whereas homologs between *S. japonicum* and *F. hepatica* were assigned if they had an alignment score of ≥ 20 , and aligned over $\geq 10\%$ of the query gene sequence. Following filtration, we reconstructed ML (maximum likelihood) phylogenetic trees using MEGA X (Kumar et al., 2018) to establish the relationships among the groups of gametologs, with nodal support values indicated.

2.14 Gene ontology analysis

Functional annotation of protein sequences was conducted using Blast2GO software (Conesa et al., 2005) against the entire non-redundant NCBI NR database using default parameters.

2.15 Inference of gene clusters with preferential transcription in females

We used the program CROC (Pignatelli et al., 2009), selecting a window of 50 genes, ≥ 3 sequential genes representing a cluster, and a *p*-value of < 0.05. A filtration threshold of RPKM of > 1 was used for adult female genes.

3 Results

3.1 Chromosome-level genomic assembly of *S. japonicum*

To reduce heterozygosity, an inbred line (SjF4M4) of *S. japonicum* was established (Figure 1a and Figure S1), and the resultant female worms were used for genome sequencing. A genomic survey sequence (GSS) based on *k*-mer frequency data revealed an estimated genome size for *S. japonicum* of 418.8 Mb, a repetitive sequence content of 38.5%, and heterozygosity of 0.9% (Table S1); this level of heterozygosity for the SjF4M4 line is less than that of the published V2 genome (1.1%) (Luo et al., 2019).

To construct a chromosome-scale reference genome for *S. japonicum*, we employed a comprehensive *de novo* assembly strategy (Figure 1b), combining Illumina paired-end reads (~60 × coverage of the genome), PacBio single-molecule long reads (~105 × coverage), 10× Genomics (~363 × coverage) and Hi-C sequence data (~243 × coverage) (Table S2). First, we produced gapless contig sequences using PacBio reads from genomic DNA from female worms. Then, we ordered and oriented the contigs into longer scaffolds employing the linkage information obtained from the 10× and Hi-C data sets. Hi-C scaffolding resulted in 8 chromosomes (Figure S4). After gap-filling and assembly-polishing, we achieved a genome assembly (406.6 Mb) with a scaffold N50 of 49.5 Mb, and a total of 107 scaffolds. The 8 longest scaffolds, spanning 99.4% of the estimated genome size (Table 1), constitute a chromosome-scale genome assembly for *S. japonicum*, consistent with the reported karyotype of $2n = 16$ (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009). The genome size of 406.6 Mb is similar to that estimated by *k*-mer analysis (418.8 Mb), and is markedly larger than the reported V2 genome (370.5 Mb) (Luo et al., 2019). The extent of single-nucleotide polymorphism (SNP) or heterozygosity in the reference genome (0.7%) (Table S3) was also similar to that estimated in the *k*-mer analysis (0.9%) (Table S1). In addition, we performed genome contamination analysis using BlobTools2, which used BLAST and Diamond for alignment with the NCBI nt and Uniprot database at the nucleic acid and protein level, and further verified by read realignment to assembled genome. Based on sequence similarity to the host and read coverage of the genome sequence, the results indicated that this schistosoma genome assembly was not contaminated with the murine host genome (Table S4).

3.2 Identification of sex chromosome sequences in *S. japonicum*

To identify autosomal and sex-linked sequences in *S. japonicum*, we also produced Illumina reads (with 80× coverage) using DNA samples from male worms of the SjF4M4 line (Figure 1b). We assumed that equal or similar numbers of female and male reads would map to the autosomes and the pseudo-autosomal regions (PARs) of the sex chromosomes – where homologous recombination occurs (Otto et al., 2011). Furthermore, we anticipated that (i) lower numbers of female than male reads would map to sexually differentiated regions (SDRs) of the Z chromosome; (ii) a lower number of female reads would map to the SDR of the Z chromosome than to individual autosomes; and (iii) a much higher number of female reads would map to the W chromosome.

With these considerations in mind, we identified seven autosomal sequences, and one sex chromosome sequence, although many regions showed a chimeric assembly of Z and W chromosomes, i.e., a female-specific read coverage pattern within the adjacent regions showing a similar coverage level between sexes (Figure S3). Chimeric assemblies of Z- and W-linked sequences were also reported for the previously published genomes of *S. japonicum* (Picard et al., 2018), suggesting that some sex-linked regions are not clearly demarcated from each other, thereby confounding the genome assembly. To circumvent this issue, we extracted the W-linked sequence fragments from the genome assembly, according to their female-specific read mapping patterns, and performed separate scaffold assemblies for the Z- or W-linked sequences, again using the 10× and Hi-C linkage data. The final assembly contains one Z-chromosome of 87 Mb, and a W-linked (ChrW) scaffold of 4.8 Mb (Figure 2). Overall, the autosomes, the Z chromosome and the W chromosome constitute 76.0%, 21.4% and 1.2% of the *S. japonicum* genome assembly (version 3, V3), respectively; only 1.4% of the assembled sequences (~5.9 Mb in length) could not be assigned to chromosomes due to their repetitive nature.

3.3 Genome assembly quality and gene annotation

The present *S. japonicum* genome (V3) is significantly more intact than both previously published versions (V1 and V2) (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009; Luo et al., 2019), as it shows a higher percentage (97.5% for V3 versus 90.3% for V2) of concordantly mapped Illumina reads (Table S5); 98.6% of V3 had a sequence depth of > 10 (Table S5), indicating high accuracy at the nucleotide level. There was a high

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degree of synteny between versions V2 and V3 (Figure 3a), and between V3 and the *S. mansoni* genome V7 (Figure 3b). The W chromosome sequence matched the combined ChrZ/W sequence for *S. mansoni*. As *S. mansoni* chromosomes were not used to order or orient the scaffolds for the *S. japonicum* genome, these findings provide independent evidence that the contig assemblies and scaffolding are accurate.

Approximately 46.0% of the V3 genome of *S. japonicum* represent repetitive sequences, comparable to 46.6% reported previously for the V2 assembly (Table S6). Due to the suppression of recombination (Charlesworth & Charlesworth, 2000), the W chromosome of *S. japonicum* has accumulated more simple repeats and LTR retrotransposons than the Z and autosomal chromosomes (Figure S5). Using *de novo*-, homology- and transcriptome (SMRT- or Illumina-based RNA-sequencing)-based approaches, we predicted 10,158 protein-coding genes in the *S. japonicum* V3 assembly, which is similar to the number (10,089) estimated for V2 (Table 1 and Table S7). The CEGMA analysis identified 214 of the 248 core eukaryotic genes (86.3%) (Table 1 and Table S8). An analysis of BUSCO showed that 62.2% of complete genes (*i.e.* 58.4% single-copy genes, and 3.8% duplicated genes) of *S. japonicum* are represented in its transcriptome, compared with 59.8% for *S. mansoni* and 55.1% for *S. haematobium* (Table 1 and Table S9). The average gene and coding DNA sequence (CDS) lengths were 16,941 bp and 1,573 bp, respectively (Table S10). Numerous non-coding RNAs were identified, including 3892 microRNAs (miRNAs), 176 transfer RNAs (tRNAs), 29 small nuclear RNAs (snRNAs) and 40 ribosomal RNAs (rRNAs) (Table S11).

3.4 'Evolutionary strata' on the Z chromosome

A previous study inferred the presence of PARs and SDRs in *S. japonicum* using the Z chromosome of *S. mansoni* (Figure S6) as a reference (Picard et al., 2018). Due to the Z-linked rearrangements between these two species, and a lack of W chromosome sequences, our understanding of the evolutionary history of schistosome sex chromosomes remains incomplete. By combining the information on comparative alignment of chrZ between *S. mansoni* and *S. japonicum* (Figure 4a), assembled W-linked sequences and Z/W sequence similarities (Figure 4b), read-coverage and SNPs density for each sex of *S. japonicum* (Figure 4c-d), we reconstructed the successive steps of recombination suppression (evolutionary strata) (Lahn & Page, 1999; Vicoso et al., 2013; Zhou

et al., 2014; Li et al., 2021) for the sex chromosomes of both *S. japonicum* and *S. mansoni*. Evolutionary strata are a sequence pattern between sex chromosomes that reflects stepwise suppression of recombination caused by presumably successive chromosomal inversions. In vertebrates (birds and mammals) this process is recognised as resulting from a stepwise linkage of sexually antagonistic genes to sex-determining genes (Handley et al., 2004; Zhou et al., 2014; Zhou et al., 2021). Along the Z-linked SDR that has completely suppressed recombination, we expected a 2-fold higher read coverage in male than in female if the W-linked sequences have become fully degenerated, or a female-biased SNP pattern in the Z-linked SDR if the homologous W-linked region has only lost recombination very recently and just started to diverge in sequence. Older evolutionary strata, if any, were expected to contain less assembled W-linked sequences, and lower Z/W pairwise sequence similarity due to sequence deletion or more serious accumulation of repeat sequences that hamper the genome assembly, than younger strata.

For the Z-linked SDRs of *S. japonicum*, to which two-fold more male than female reads mapped (Figure 4a), 20.5% were shared with the partial SDR of *S. mansoni* (Figure S6), whereas the remainder were homologous to the PARs of *S. mansoni* (Figure 4a, c). We defined this shared SDR between two schistosome species as the S0 region (designated 'evolutionary stratum 0' = S_{jap}S0). As expected, we were able to assemble very few W-linked fragments at S0 compared (Table S12) to other evolutionary strata (Figure 4b), except for some that were possibly unannotated repeat sequences and showed high levels of Z/W sequence identity. This result indicated that only the S_{jap}S0 stratum suppressed recombination on the proto-sex chromosomes in the ancestor of *S. japonicum* and *S. mansoni*, followed by the independent formation of the evolutionary stratum after these species diverged from one another (Picard et al., 2018). One would expect the master female-determining gene(s), if present, to be located on W-linked sequences, or that a dosage-dependent Z-linked gene occurs in this S_{jap}S0 region (Table S13). However, we were unable to identify any genes on the very few assembled W-linked sequences at S0. A recent study used *de novo* assembled transcriptome sequences and identified a splicing factor *U2AF2* at W-linked S0 as a candidate female-determining gene (Elkrewi et al., 2021).

We inferred two younger evolutionary strata S_{jap}S1 and S_{jap}S2 of *S.*

japonicum that were mapped to the PAR of *S. mansoni*, and also exhibited a 2-fold male-biased read coverage. SjavS1 had no homologous W-linked fragments assembled and there were 945 kb of W-linked fragments assembled for SjavS2. A Z-linked inversion probably reversed the order of SjavS2 and SjavS3, the latter of which showed a similar level of read coverage between sexes, but a higher SNP density in female (*i.e.*, the Z/W sequence divergence level, Figure 4d) than in male. The youngest stratum SjavS4 also had a similar level of read coverage between sexes, but a lower degree of female-biased SNP pattern compared to SjavS3. We hypothesized that SjavS3 and SjavS4 represented evolutionary strata that have recently lost homologous recombination. Therefore, the W chromosome regions are expected to show very limited sequence divergence, which would explain the similar coverage level between sexes, and chimeric assembly earlier observed in the *S. japonicum* V3 chimeric assembly (Figure S3). We found very few Z- and W-linked gametolog pairs for the older strata SjavS0-2; however, we did confirm that the divergence level at the silent sites were indeed higher in SjavS3 than in SjavS4, calculated by female-specific SNPs within the Z-linked genes of these two regions (Figure S7).

To search for further evidence of an independent evolution of strata between *S. japonicum* and *S. mansoni*, we reconstructed phylogenetic trees (maximum likelihood) for all seven genes with Z-linked orthologous gene sequences in *S. mansoni* and both the Z/W gametologs available for *S. japonicum*. If the strata formed after the divergence of the two species, one would expect the gametologs to group according to species; otherwise, the Z- or W-linked gametologs of different species would be expected to group together. Five of seven genes from SjavS4 and the 2 other genes from SjavS2 supported the independent evolution of strata (Figure 4f, Figure S8 and Table S13).

For Z-linked genes of strata SjavS0/1/2/3, we revealed overall male-biased transcription profiles (~ 2-fold difference between sexes) (Figure 4e, g), which may be explained by a lack of global dosage compensation (Vicoso & Bachtrog, 2011) and/or an almost complete loss of W-linked genes (Charlesworth & Charlesworth, 2000). For stratum SjavS4, we inferred a largely unbiased transcription profile (with slightly more male-biased genes compared with PARs and autosomes) (Figure S9, S10c, S11). Based on these findings, we concluded that the sex chromosomes of *S. japonicum* shared one recombination suppression

event (SjapS0) with those of *S. mansoni*, followed by three other recombination suppression events occurring specifically in *S. japonicum*.

3.5 W-linked transcription profiles

We annotated 35 W-linked genes on the 4.8 Mb of W-linked sequences (Figure 5a and Table S14), 20 of which were confirmed by qPCR using (separately) female and male genomic DNA and cDNA (Figure S12 and Table S15). The dynamic expression pattern of these genes was analyzed separately using the transcriptome data for each male and female worm of *S. japonicum* at eight time points (14–28 days post infection; dpi) throughout the sexual developmental process (Wang et al., 2017). As expected, these W-linked genes were specifically or highly transcribed in female worms. The transcription patterns of W-linked genes in both the egg and cercarial stages were similar to those in the schistosomula and adult stages. Although we were unable to differentiate the transcription of these genes in female eggs and cercariae, our results indicated that they might be stably transcribed in females of all stages. Gene ontology (GO) analysis assigned the W-linked genes to metabolic and cellular processes, binding and cellular anatomy (Figure S13). Of the 35 W-linked genes, 11 were linked to metabolic enzymes (such as glycogen phosphorylase, beta-1, 3-galactosyltransferase and Ras-related protein Rab-14), three transcription factors or co-activators, two receptors (a rhodopsin orphan GPCR and a growth hormone secretagogue receptor) and one chromatin regulatory protein (Sir2).

3.6 Female-biased gene clusters on autosomes and the Z chromosome

We did not identify any genes associated with egg-production encoded in the W-chromosome, except for a gene belonging to the vitellogenin-N superfamily. We found 257 genes on seven autosomes or the Z chromosome which were transcribed at a higher level (≥ 5 -fold) in adult females than in adult males (Table S16); notably, 42.0% (108 genes) of them formed 17 gene clusters (Figure 5b and Table S16); four genes on chromosome 2 (cluster 6) and six on chromosome 3 (cluster 9) were located in arrays (Figure S14). These genes are short and contain only one or two exons. All four adjacent genes of cluster 6 encode eggshell proteins; the six adjacent genes in cluster 9 encode a chorion protein, a trematode eggshell synthesis protein, two DNA damage-responsive proteins, an asparagine-rich antigen and a gene without annotation.

Unlike W-linked genes that were almost constitutively transcribed in females in all developmental stages studied, the 257 non-W-chromosomal genes with female-biased transcription were preferentially and highly transcribed in schistosomula (from 16 dpi) and in adult worms. It is noteworthy that the transcription of 156 (60.7%) of these 257 genes was markedly down-regulated on 20 dpi (Figure 5c), when ~ 50% of worms were paired. This transcriptional down-regulation was unique, and was not observed for genes on chromosomes 1, or the Z and W chromosomes (Figure 5a and Figure S15).

We performed edgeR to estimate the significant gene expression changes. For 108 female-biased gene clusters on autosomes and the Z chromosome, 104 of them showed a significantly higher expression in adult females compared with adult males ($p < 0.05$); the other 4 genes were filtered out caused by low reads count (Table S17, sheet 1). For the 156 female down-regulated genes at dpi20 stage, 146 of these genes showed a significantly lower expression at dpi20 stage compared with dpi18 and dpi22 stages ($p < 0.05$); the other 10 genes were filtered out caused by low reads count (Table S17, sheet 2).

4 Discussion

Here, by combining PacBio, 10 × Genomics, Hi-C and Illumina sequence data from a clonal line of *S. japonicum*, we produced an improved high-quality chromosome-level genome for this species. We created a new pipeline and separately assembled the Z- and W-chromosomes. Then, we reconstructed the evolutionary history of the sex chromosomes in *Schistosoma* spp., revealing a partially overlapping pattern of evolutionary strata and found considerably more regions with suppressed recombination between sex chromosomes than inferred previously (Figure S16) (Picard et al., 2018). Published genomes for *S. mansoni* and *S. japonicum* are chimeric, with some mixed sequences representing both the Z and W chromosomes. The order of sequences within the two chromosomes reported here for both species is significantly improved (Picard et al., 2018), allowing us to reveal unique observations regarding the evolutionary strata of schistosome sex chromosomes.

We inferred that, besides one evolutionary stratum, or recombination suppression event, shared by *S. mansoni* and *S. japonicum*, the latter species has formed four independent evolutionary strata (Figure 4). Two of four strata (SjapS3 and SjapS4) likely formed recently, given the similarity in read coverage

between the sexes, but significantly more heterozygosity in females than in males. However, as the *W* chromosome sequences are not completely assembled, the mechanism(s) of formation of the evolutionary strata cannot be established. A specific reduction in read-coverage in females, but not in males, at the boundary between S_{jap}S3 and S_{jap}S2 and between S_{jap}S4 and S_{jap}S2, suggests that, similar to the sex chromosomes of humans and birds (Lahn & Page, 1999; Zhou et al., 2014), a chromosomal inversion on the *W* chromosome might account for the formation of S_{jap}S2 (Figure S16a).

The *W*-chromosomal genes of *S. japonicum* were observed to be transcribed in several, and perhaps all, female developmental stages. Although our study did not include an analysis of the developmental stages in the snail intermediate host, we observed a stable transcription of these genes in eggs, cercariae, female schistosomula and female adults, providing some evidence for sex determination by the *W* chromosome. The continuous transcription/expression of these *W* chromosome-specific genes could determine the sex of the parasite after the egg is fertilized.

Recently, Elkrewi et al. applied a *k*-mer-based pipeline to assemble female-specific transcripts in *S. mansoni* and *S. japonicum* (Elkrewi et al., 2021). We searched the reported *W*-linked transcripts of the two Schistosome species annotated by Elkrewi et al. against the *S. japonicum* genome V3 by BLAST (Table S18, sheet 1). 81 of the 94 or 86.1% of the *W*-linked *S. japonicum* transcripts found by Elkrewi et al. can be found in our *S. japonicum* genome V3 (Table S18, Sheet 1). Besides the 35 *W*-linked genes we annotated and analyzed on the chromosomal assembly, another 80 *W*-linked genes can be found on the contig sequences that cannot be incorporated into the chromosomal sequences, due to the lack of 10x or Hi-C linkage evidence. For the 90 *S. mansoni* *W*-linked transcripts identified by Elkrewi et al., we found 12 of them have homologous sequences in *S. japonicum* genome V3 located on the chrZ or autosomes (Table S18, Sheet 2). For *U2AF2*, we only found its gametolog on *S. japonicum* genome V3 chrZ.

We showed that some genes encoding transcription factors, RNA-binding proteins, GPCRs and chromatin regulatory proteins are located on the *W* chromosome, features which merit further study to determine their precise functions in the regulation of gene transcription during female reproductive

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development. Of these, ChrW.662 encodes a chromatin regulatory protein, Sir2. Sir2 is a member of the class of histone deacetylases (HDACs), which coordinate the DNA transcription by deacetylation of histones (Strahl & Allis, 2000). Sir2s are widely present in all phyla, and they play different roles depending on the organism (Religa & Waters, 2012; Gomes et al., 2021). In *S. mansoni*, *SmSir2* was firstly reported by Lancelot et al. (Lancelot et al., 2013). *SmSir2* was considered as a novel drug target. Inhibitors of *SmSir2* showed potency against both larval schistosomes (viability) and adult worms (pairing, egg laying) in culture (Monaldi et al., 2019). A role for Sir2 in schistosome sex determination and differentiation has not been reported. However, *Drosophila* Sir2 was found to be an essential gene whose loss of function resulted in both segmentation defects and skewed sex ratios, associated with reduced activities of the Hairy and Deadpan bHLH repressors (Rosenberg & Parkhurst, 2002).

Some autosomal genes that are highly transcribed in females are clustered and stage-specific. We identified four adjacent single-exon genes encoding eggshell proteins (ESPs) on autosomal chromosome 2, i.e. Chr2.57 (encoding ESP2a), Chr2.58 (encoding ESP1a) Chr2.59 (encoding ESP1b) and Chr2.60 (encoding ESP2b). A previous study (Henkle et al., 1990) reported that a female-specific component in nuclear extracts of *S. japonicum* bound to conserved DNA sequences (i.e. TCACGT, TCAGCT and GTAGAAT) upstream of the transcription start sites of the ESP genes, indicating the clustering of genes preferentially transcribed in females, including ESPs, might facilitate transcriptional regulation.

Direct male–female interaction in schistosomes usually occurs after 14 dpi (Wang et al., 2017), such that the high level of transcription in female worms at 16 and 18 dpi (and low transcription in eggs and cercariae) indicates a dependence on worm pairing. The marked down-regulation of most of these genes on 20 dpi we observed was unexpected. We hypothesize that mRNAs transiently accumulate in young females in large quantities and are required for egg production and/or for the eggs themselves. Notably, from 22 dpi onward, as egg production stabilizes, these genes are upregulated and regain high levels of transcription.

5 Conclusion

The enhanced genome assembly (V3) for *S. japonicum* and the genetic analyses of the Z and W chromosomes presented here provide a solid platform for future

functional investigations of *S. japonicum* to unravel the processes of sex determination, differentiation, and pairing, which could enable the discovery and development of new interventions for the control of schistosomiasis.

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DATA AVAILABILITY STATEMENT

All the data have been deposited in the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences / China National Center for Bioinformation, under BioProject ID PRJCA010213 that is publicly accessible at <https://ngdc.cnbc.ac.cn/gwh>.

COMPETING INTERESTS

No competing interests.

AUTHOR CONTRIBUTIONS

X.X. cloned parasites and prepared genomic DNAs or RNAs for sequencing. Y.W., Q.G., G.G., G.J., and Z.Z. performed the high-throughput sequencing, genome assembly, gene annotation and other bioinformatic analysis. Y.D., Y.L., G.W., C.W., and X.H. constructed Hi-C-seq and RNA-seq libraries. X.X., Y.W., D.P.M., J.C., Q.Z., and Q.Z. wrote the manuscript with crucial contributions from R.B.G., N.D.Y., W.Z., S.W., A.P., and other co-authors. Q.Z., Q.Z., J.C., and D.P.M. managed the project. All

authors read and approved the final manuscript.

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Figure Legends

Figure 1. Establishment of an inbred line of *Schistosoma japonicum* and the genome assembly pipeline used. (a) Establishment of a clonal line of *S. japonicum*. Individual *Oncomelania hupensis* snails were infected with a single miracidium of *S. japonicum*. Then, the sex of the clonal cercariae shed from each individual snail was determined by PCR (**Figure S1**). Ten female and ten male cercariae were used to infect individual mice. Eggs were collected to establish a clonal parasite (SjF4M4) after 2 rounds of inbreeding; the resultant parasite line was used for DNA and RNA sequencing. **(b)** A schematic overview of the *S. japonicum* assembly pipeline. Two major aspects are shown; the initial assembly procedure (yellow box) and then sex chromosome validation and reassembly (green box). Female PacBio long reads were used to generate contigs; then, with 10× and Hi-C data linkage information, we generated a chromosome-length genome assembly (yellow box). Using male and female Illumina reads mapping to the assembly, these scaffolds were assigned to autosomal or sexual types based on their normalized depth value. Chimeric sequences were extracted from the Z chromosome, and split into Z and W regions, which were then combined with other Z and W sequences and reassembled into the W chromosome and the new Z chromosome, respectively (green box).

Figure 2. Coverage plots of *Schistosoma japonicum* V3 autosomal chromosomes and sex chromosomes Z and W. Sequence read-coverage plots of *S. japonicum* chromosomes. Male (blue dot) and female (red dot) normalized depth values (y-axis) along *S. japonicum* chromosomes (x-axis) are shown across 50 kb windows.

Figure 3. Circos plot of synteny between *Schistosoma japonicum* genome versions (V3 and V2) and between *S. japonicum* genome V3 and *S. mansoni* genome version V7. (a) A total of 98.1% of contigs longer than 1M bp in *S. japonicum* genome V2 (BioProject PRJNA520774) were assembled in *S. japonicum* genome V3. **(b)** All *S. japonicum* V3 chromosomes matched the corresponding *S. mansoni* chromosomes V7 (BioProject PRJEA36577).

Figure 4. Evolutionary strata on the Z chromosome. (a) Genes orthologous between the *Schistosoma japonicum* (Sjap) Z chromosome and the *S. mansoni*

(Sman) (fused) ZW chromosome were plotted. Each line represents one pair of orthologs, and different line colours represent orthologs with varied genomic locations. The inferred region of each evolutionary stratum of *S. japonicum* and *S. mansoni* (**Figure S6**), and also PAR of the Z chromosome are shown. Since the W chromosomes are expected to share the PAR with the Z chromosome, the PAR of the W are not shown. **(b)** *S. japonicum* (Sjap) sequence similarity between the W and Z chromosomes were plotted; colour from green to red represents low (70%) to high (100%) similarity. **(c-e)** *S. japonicum* read-coverage on the Z chromosome, SNPs and gene transcription plots. Each dot represents a 50-kb window. Coordinates on the Z chromosome of *S. japonicum* are used in these Figures. **(f)** The ML (maximum likelihood) phylogenetic relationship of *S. japonicum* gametologs; *Fasciola hepatica* (Fhеп) was used as an outgroup. **(g)** Sex-biased transcription of genes shown as proportions. The asterisks illustrate significant numbers of differences between the sex-biased genes (F/M > 2 or M/F > 2) on the sex and autosomal chromosomes: *** $p < 0.001$, estimated using the Chi-Squared test.

Figure 5. Sex-linked gene expression and female-preferentially transcribed gene clusters. **(a)** Transcription of W-chromosomal genes in different developmental stages. **(b)** Female-preferentially transcribed gene (FTG) cluster distribution (C1-C17); each solid circle represents one gene, and F/M expression differences are shown by colour variation. **(c)** Female-preferentially transcribed gene (FTG) cluster expression across various developmental stages. dpi, days post infection.

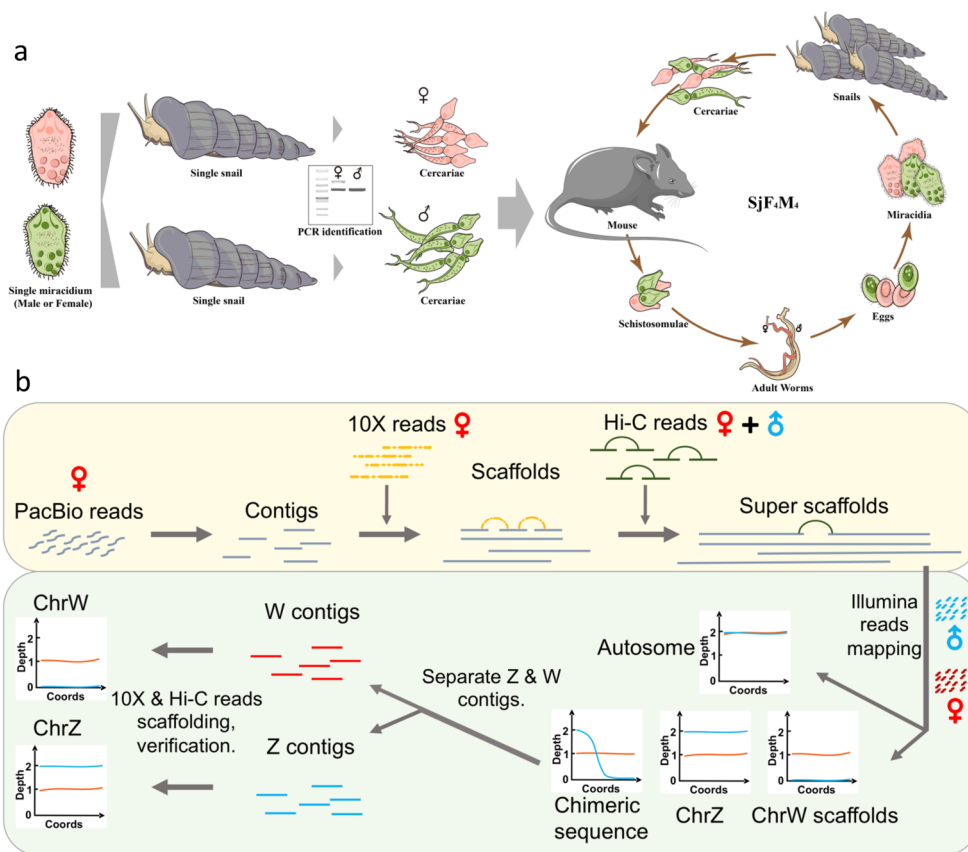
Table 1. Features of the chromosome-level assembled genome of *S. japonicum* (version V3) compared with the previously published genome (V2, BioProject PRJNA520774) for the same species, *S. mansoni* (V9) and *S. haematobium* (V2, BioProject PRJNA78265)

	<i>S. japonicum</i> V3	<i>S. japonicum</i> V2	<i>S. mansoni</i> V9 ^a	<i>S. haematobium</i> V2
Genome size (Mb)	406.6	369.9	391.4	371.4
Number of scaffolds	107	1,789	9	666
Number of contigs	628	2,108	368	12,937
Longest scaffold (Mb)	90.6	6.3	89.1	14.3
Average scaffold length (bp)	3,799,739	210,145	43,488,369	557,649
Number of scaffolds: >10 kb	98	1,052	9	/
Scaffold N50 (bp)	49,539,924	1,093,989	45,716,228	4,779,868
Contig N50 (bp)	2,378,822	871,911	4,473,015	21,744
GC content (%)	34.0	33.8	35.5	34.5
Repeat content (%)	46.3	46.9	54.5	47.2
CEGMA Assembly (n=248)	86.3	/	/	/
Putative coding genes (N)	10,158 ^b	10,089	9,794	9,314
BUSCO Annotation (n=1658)	68.2	/	53.3	63.9
Chromosomes	7 + Z+W	NA	7 + Z+W	7 + Z/W

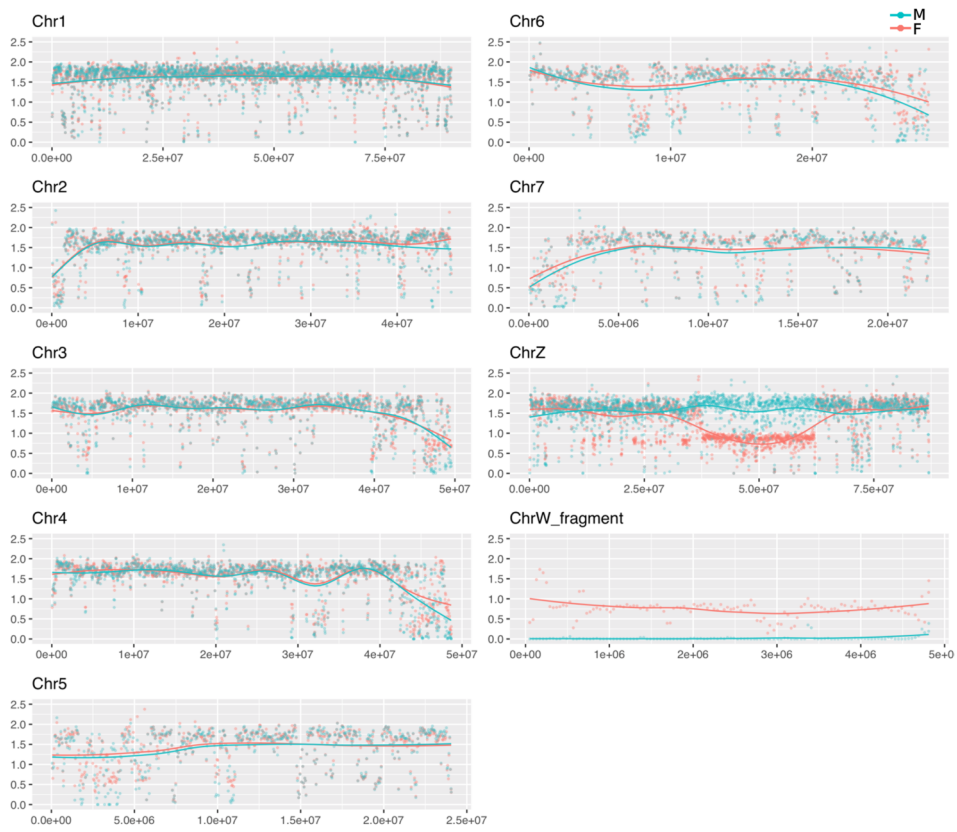
^a *S. mansoni* V9 genome data were download from NCBI

(https://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/237/925/GCA_000237925.5_SM_V9/)

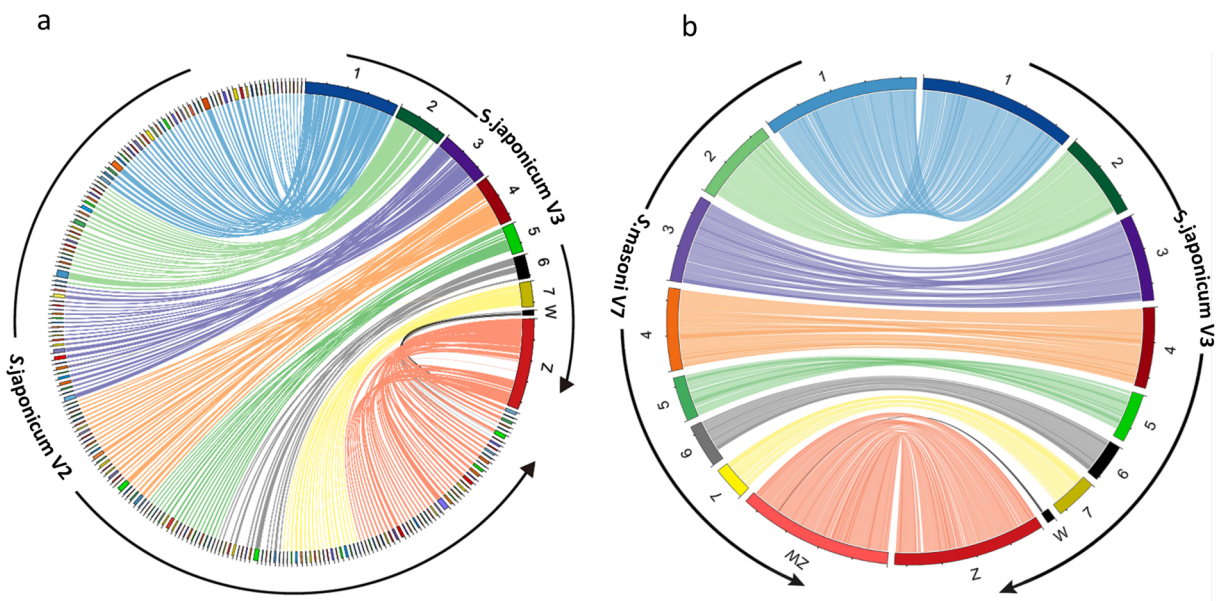
^b 9,760 full-length CDS



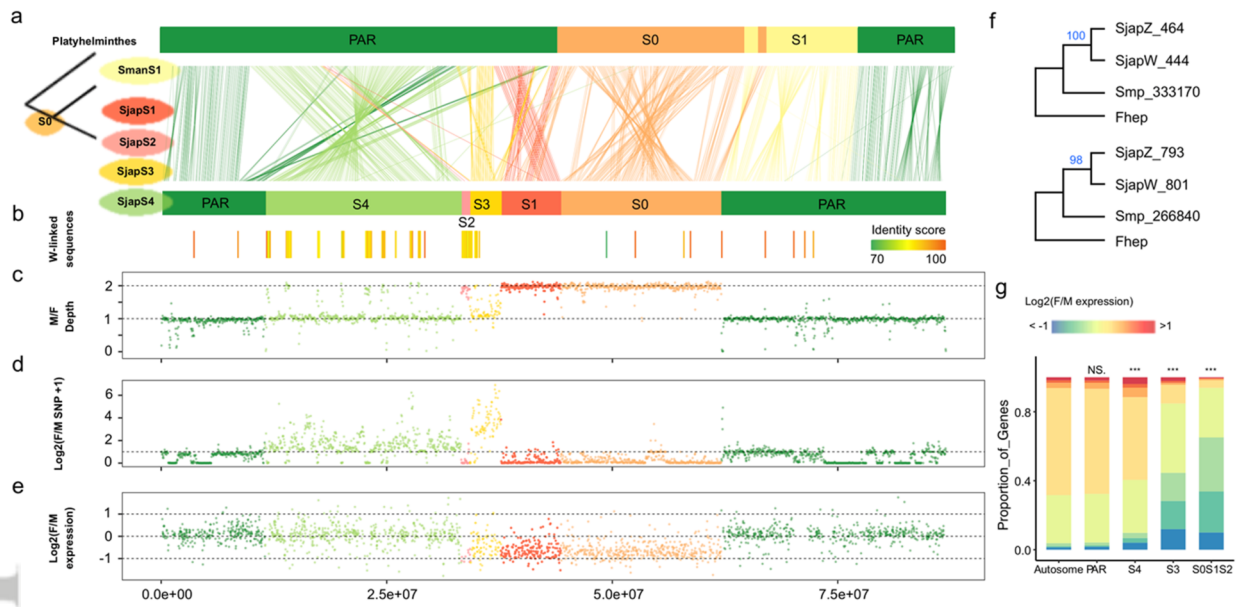
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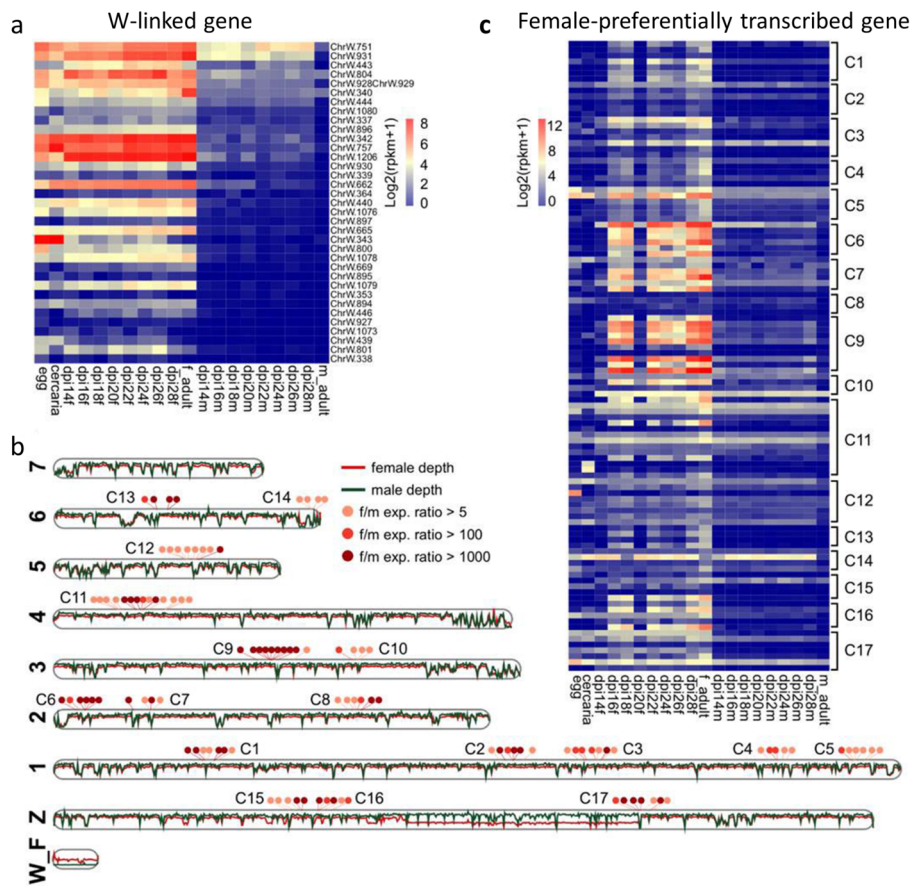
MEN_13689_Figure 2.tif



MEN_13689_Figure 3.tif



MEN_13689_Figure 4.tif



MEN_13689_Figure 5.tif