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Genome Resources

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A chromosome-level reference genome for the common bed bug, *Cimex lectularius*, with identification of sex chromosomes

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Abstract

The common bed bug, *Cimex lectularius*, is a globally distributed pest insect of medical, veterinary, and economic importance. Previous reference genome assemblies for this species were generated from short-read sequencing data, resulting in a ~650 Mb composed of thousands of contigs. Here, we present a haplotype-resolved, chromosome-level reference genome, generated from an adult Harlen strain female specimen. Using PacBio long read and Omni-C proximity sequencing, we generated a 540 Mb genome with 15 chromosomes (13 autosomes and 2 sex chromosomes—X_1X_2) with an N50 > 30 Mb and BUSCO > 90%. Previous karyotyping efforts indicate an XY sex chromosome system, with $2n = 26$ and $X_1X_1X_2X_2$ females and X_1X_2Y males; however, significant fragmentation of the X chromosome has also been reported. We further use whole genome resequencing data from males and females to identify the X_1 and X_2 chromosomes based on sex biases in coverage. This highly contiguous reference genome assembly provides a much-improved resource for identifying chromosomal genome architecture, and for interpreting patterns of urban outbreaks and signatures of selection linked to insecticide resistance.

Keywords: Cimicidae, Hemiptera, indoor urban pest, Pac-Bio long read sequencing

Introduction

The common bed bug, *Cimex lectularius* (Fig. 1), is a hematophagous pest insect of medical, veterinary, and economic significance. It has a near-global distribution, primarily found infesting human dwellings in temperate regions above 30° North and South latitudes (Usinger 1966; Zorrilla-Vaca et al. 2015). In recent years, however, infestations have also been reported in tropical regions (Cambronero-Heinrichs et al. 2020; Akhoundi et al. 2022; Porras-Villamil and Olivera 2023). Derived from a lineage that is an ectoparasite of bats, the human-associated lineage diverged from its bat-host ancestor approximately 245,000 YA (Balvín et al. 2012). Despite both bat- and human-associated lineages occurring sympatrically (Booth et al. 2015) and a lack of obvious reproductive isolation mechanisms (DeVries et al. 2020; Sasínkova et al. 2023), gene flow between these host-specific lineages has been shown to be negligible (Booth et al. 2015).

Cimex lectularius was a prominent household pest prior to World War II. With the introduction of DDT in the 1940s as an indoor pest control agent, the numbers of infestations rapidly declined. Within a few years of its use, bed bugs were believed to have been eradicated from industrialized countries (Potter 2011). Although populations exhibiting resistance to DDT were reported soon after its introduction (Johnson and Hill 1948; Busvine 1958), it was not until the late 1990s that the species experienced a resurgence in cosmopolitan areas on a near-global scale (Doggett et al. 2018). Factors contributing to this resurgence included an increase in national and international travel and the frequent exchange of secondhand goods. However, the evolution of mechanisms conferring resistance to an array of insecticides is likely to have also played a significant role (Romero et al. 2007), and insecticide resistance has been reported globally (Dang et al. 2017; Booth 2024).

Several factors underscore the common bed bug as a species for which a haplotype-resolved chromosome-level reference genome would be beneficial. These include a relatively recent host-associated shift between bat- and human-associated

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Fig. 1. An adult common bed bug, *Cimex lectularius*. Photo credit—Giles San Martin.

lineages that provides an ideal experimental design for understanding the genomic factors underlying host-specific associations. Bed bugs also represent a valuable model system for studying human-associated evolution and adaptive dynamics that accompany urbanization (Johnson and Munshi-South 2017; Booth et al. 2018) that include high levels of inbreeding within populations, restricted gene flow, and a propagule-pool model of spread (Booth et al. 2012, 2015, 2018; Saenz et al. 2012; Fountain et al. 2014). A high-quality reference genome will also enhance inferences related to the evolutionary history of this important species, potentially illuminating the loci responsible for the successful shifts to human hosts and urban environments. Combined with recent evidence for rapid evolution in response to human-induced insecticidal pressures (Lewis et al. 2023), a chromosome-level genome is also particularly timely for ongoing studies of the mechanisms underlying insecticide resistance. A more contiguous genome assembly also substantially enhances inferences of gene-regulatory regions and inferences of complex repeat element structure (and polymorphism) that may be important for interpreting adaptive responses to insecticides. Although two prior draft genomes for the bed bug have been published (Benoit et al. 2016; Rosenfeld et al. 2016) and proven useful, neither are resolved to the chromosome scale or are associated with extremely long contiguous assemblies or haplotypes. Here, we present the first high-quality chromosome-level genome assembly based on Pacific Biosciences (PacBio) HiFi long read sequences scaffolded with Dovetail Omni-C data.

Methods

Biological materials

A single virgin female specimen that originated from a Harlan strain colony maintained in the laboratory of Dr. Coby Schal (North Carolina State University) was used to generate the reference genome. The Harlan strain has been in culture because of collection from an infestation sampled at the Fort Dix US Army barracks, NJ, USA, in 1973. Specimens were starved to eliminate host blood from their digestive system, then flash frozen and sent to Dovetail Genomics (Cantata Bio, LLC.) for DNA extraction and library preparation.

Nucleic acid library preparation

DNA extraction and both PacBio and Omni-C libraries were prepared and sequenced by Dovetail Genomics, LLC (Scotts Valley, CA, now Cantana Bio). For the Dovetail Omni-C library, formaldehyde was used to fix chromatin in place in the nucleus prior to extraction, and DNAse I was used to digest the fixed chromatin. Chromatin ends were then repaired and ligated to a biotinylated bridge adapter, followed by proximity ligation of adapter containing ends. Crosslinks were subsequently reversed, and the DNA was purified. Biotin that was not internal to ligated fragments was removed from the purified DNA. NEBNext Ultra enzymes and Illumina-compatible adapters were used to generate the sequencing libraries. Prior to PCR enrichment of each library, streptavidin beads were used to isolate biotin-containing fragments.

DNA sequencing and genome assembly

The PacBio library was sequenced on the PacBio SEQUEL II system using CCS (circular Consensus Sequencing) chemistry with SMRT cells, and the Omni-C library was sequenced on an Illumina HiSeqX platform. PacBio CCS (Circular Consensus Sequencing) reads were used as an input to Hifiasm v0.15.4-r347 (Cheng et al. 2021) using default parameters. Blast results of the Hifiasm output assembly against the "nt" database were used as input for blobtools v1.1.1 (Laetsch and Blaxter 2017). Scaffolds identified as possible contamination (e.g. bacterial) were removed from the assembly. Specifically, any scaffolds that were not identified as non-Arthropoda (e.g. Protobacteria or mammalian likely resulting from undigested blood in the gut of the sequenced individual), from the "nt" database were removed. Finally, purge_dups v1.2.5 (Guan et al. 2020) was used to remove haplotigs and contig overlaps.

Assembly and scaffolding were performed using default software parameters (see Supplementary Table S1), unless otherwise noted. The input de novo assembly from a previously published genome build Clec_2.1 (NCBI GCF_000648675.2, Benoit et al. 2016) and Dovetail OmniC library reads with MQ > 50 reads were used as input data for HiRise (Putnam et al. 2016). BWA (Li and Durbin 2009) was used to align Dovetail OmniC library sequences to the draft input assembly. HiRise was used to analyze the separations of Dovetail OmniC read pairs mapped within draft scaffolds, producing a likelihood model for genomic distance between read pairs. This was then used to identify and break putative misjoins, to score prospective joins, and construct joins above a threshold.

To compare the assembly completeness, we used BUSCO v4.0.5 (Manni et al. 2021) using the eukaryota_odb10 gene set on both the current and Clec_2.1 genomes. Additionally, the previous draft Clec_2.1 genome was aligned to the current genome using minimap2 (Li 2018) with default parameters. The Clec_2.1 contigs with > 1Mbp were filtered ($n = 193$) and visualized on our chromosome-level genome using the R package circlize (Sun et al. 2020). Finally, species-specific repeats were identified using a de novo search with RepeatModeler v2.0.2 (Flynn et al. 2020). RepeatMasker v4.1.4 (Smit et al. 2013) was run sequentially on both the current assembly and on the previous bed bug assembly Clec_2.1 to annotate repeats using elements from Insecta from Repbase (Jurka et al. 2005; Bao et al. 2015) and our de novo library from RepeatModeler. Plots were visualized in R v. 4.2.3 (R Core Team 2021).

The sex chromosome system in *C. lectularius* is X_1X_2Y for males and $X_1X_1X_2X_2$ for females (Ueshima 1967). Based on the difference in copy number of both X_1 and X_2 chromosomes between sexes, we expect that genome resequencing data from males will have approximately ½ coverage on both X chromosomes compared with females, and also compared with autosomes in male samples. To identify the sex chromosomes in our assembly, we sequenced 4 additional adult samples, including 2 males and 2 females (see Supplementary Methods), using Illumina 250 bp paired-end reads at a coverage of ~10× each. These data were aligned to our genome using bowtie2.4.1 (Langmead and Salzberg 2012) and sequence coverage depth was estimated for 100 kb genomic windows using bedtools (Quinlan and Hall 2010). Coverage estimates were then normalized across samples by dividing 100 kb window coverage estimates by the median genomic coverage per individual. Additionally, synteny between *C. lectularius* and *C. hemipterus* (BioProject PRJNA713496) sex chromosomes was estimated using CoGe SynMap2 (https://genomevolution.org/coge/). The *C. hemipterus* sex chromosomes were previously identified via gene expression differences in males vs. females, then the differentially expressed genes were mapped back to their respective chromosomes (Law et al. 2024).

Results

The PacBio HiFi and Omni-C sequencing libraries generated 4.9 M reads with two haplotypes of 537 Mb and 540 Mb post filtering resolved. Each haplotype was sequenced to ~30× PacBio HiFi coverage based on the genome size of 650–697 Mb, estimated from previous sequencing efforts (Benoit et al. 2016; Rosenfeld et al. 2016). Contaminants identified were removed prior to genome assembly (Supplementary Fig. 1) and the overall GC content was 34.9% (range: 26.5% to 54.2%) (Fig. 2a). The assembly of this genome consists of 3,691 and 2,443 contigs spanning 835.77 and 698.24 Mb with an N50 of 27.82 Mb and 37.25 Mb, respectively (Table 1). This assembly shows enhanced contiguity compared with the previous reference genomes (Table 1, Fig. 2b). The resulting assembly was deposited in the GenBank (BioProject ID PRJNA1165749). Fifteen large scaffolds, ranging in size from 23.5 Mb to 53.5 Mb were identified (Fig. 2b), which corresponds precisely to the expected number of chromosomes: 13 autosomes + X_1X_2 sex chromosomes (Ueshima 1967). Based on our analysis of resequencing data for males and females, we identified two chromosomes that show approximately half coverage compared with other autosomes in males, and half coverage in males versus females (Fig. 3a). These predictions are precisely what we expect for X chromosomes, and we accordingly identify these

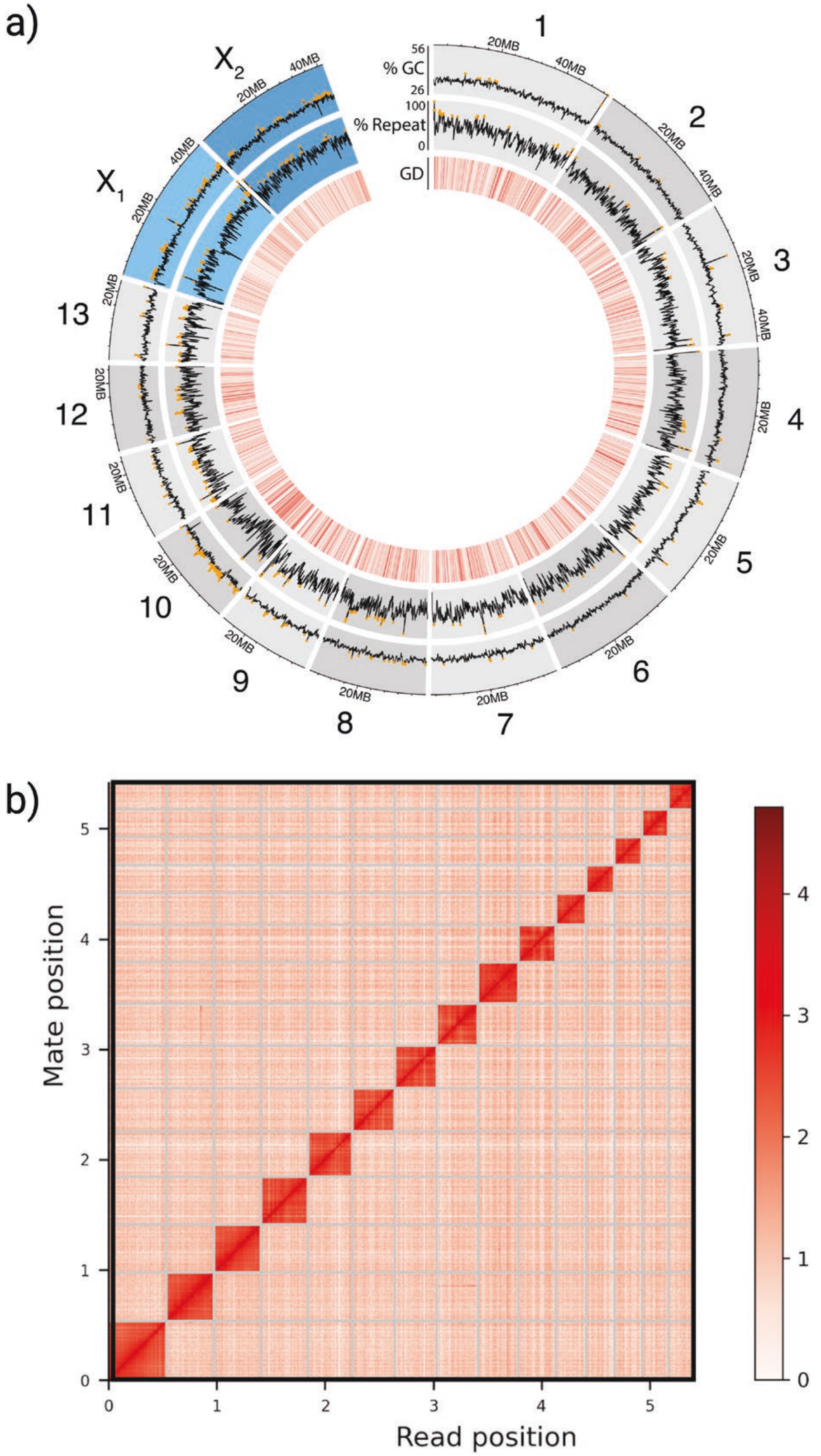

Fig. 2. a) Circos plot of 15 chromosomes (13 autosomes and 2 sex chromosomes—X_1X_2). Outer and middle tracks represent GC and repeat content for 100 kb windows, respectively, with orange dots highlighting windows in the top 5% highest values. The inner track shows gene density for 100 kb windows, with low density shown in white and high density shown in red. b) Omni-C Contact maps that translate the proximity of genomic regions in 3D space to contiguous linear organization. Each cell represents sequence data supporting the joining between two regions. The darker red clustering represents the 15 chromosomes.

Table 1. Assembly quality comparisons among this and previous draft *Cimex lectularius* genomes.

	Total sequence	Total Contigs	Contig N50	Total scaffolds	Scaffold N50
This assembly haplotype 1	537.87 Mb	3,691	37.25 Mb	15	671 kb
This assembly haplotype 2	540.74 Mb	2,443	27.82 Mb	15	967 kb
Benoit et al. (2016)	650.47 Mb	1,402	7.17 Mb	45,073	23.5 kb
Rosenfeld et al. (2016)	697.87 Mb	13,151	947 kb	77,082	12.6 kb

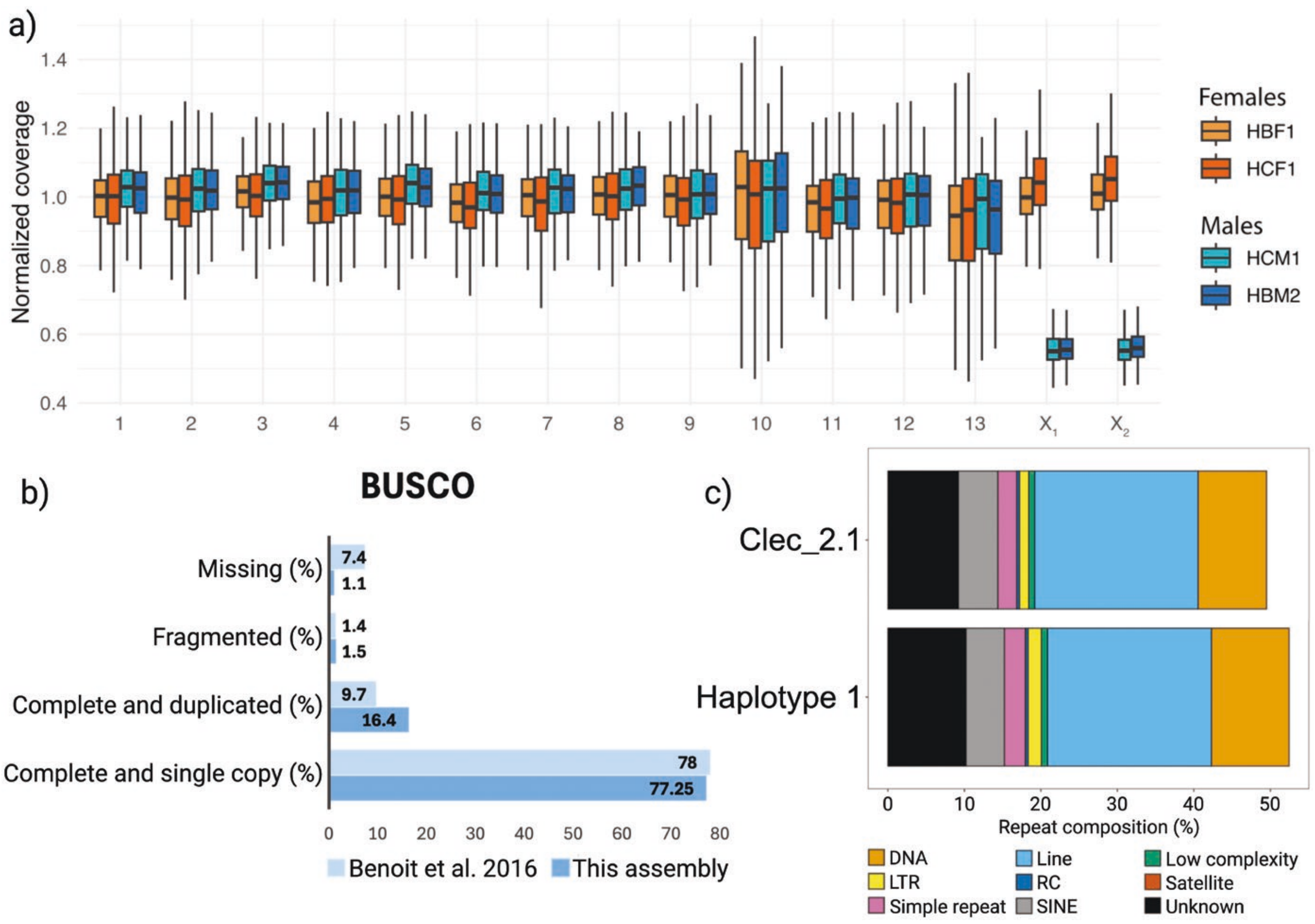

Fig. 3. a) Distribution of normalized sequencing coverage for 100kb windows across chromosomes for four re-sequenced individuals (outlier values not shown). b) BUSCO percentages for this assembly (darker bar) are comparable or higher than the previous draft genome (lighter bar). c) Repeat content across the previous draft genome and this assembly. Repeat proportions are similar between the two assemblies, however the new build produced more unknown repeats. Both assemblies have the greatest proportion of repeats belonging to LINEs.

two chromosomes as X_1 and X_2. Synteny analysis revealed a match between the X_1 and X_2 sex chromosomes identified here and sex chromosomes 2 and 3 of the *C. hemipterus* genome (Supplementary Fig. 2) (Law et al. 2024).

Compared with the previously available *C. lectularius* genome (Clec_2.1), our new genome assembly has a higher percentage (93.65% vs. 87.7%) of BUSCO's ortholog genes, suggesting it is more complete than prior reference genomes for this species (Fig. 3b). This increase in completeness is due to fewer missing BUSCO genes and an increase in complete and duplicated genes compared with the previous draft genome. Our de novo identification of repeat elements using RepeatModeler identified a total of 1,170 known and 1,202 unknown repeat element consensus sequences. Our analysis of repeat element content (integrating this consensus library with Repbase) suggests that LINE retroelements represent the most abundant class of repeat elements (by total bases; Fig. 3c), consistent with inferences from the previous draft genome.

Discussion

We present the first haplotype-resolved chromosome-scale genome assembly for the global human pest *C. lectularius*. The total length of our Dovetail HiRise assembly was 835.77 Mb, consistent with the 864.5 Mb total estimated through comparison with other insects by propidium iodide analyses (Benoit et al. 2016). The total size of scaffolds assigned to chromosome-sized contigs (<1Mb), postfiltering and scaffolding, was 540 Mb and represents a coverage depth of 30×. This is somewhat smaller than previous genome draft assemblies of 650 Mb (Benoit et al. 2016) and 697 Mb (Rosenfeld et al. 2016) but shows an increase in the overall completeness and N50 (Table 1). The unplaced and shorter-length scaffolds appear to account for the difference in expected genome size. Our assembly constructed two distinct haplotypes of similar size. The proportion of single-copy orthologs (eukaryota_odb10 BUSCO lineage) improved in this assembly, compared with previous references, and is similar to that of the core eukaryotic genes (CEGs) in the Rosenfeld et al. (2016) study. Thus, our assembly did not lose significantly conserved gene orthologs during the process of genome scaffolding, suggesting our chromosome-level scaffolds are remarkably complete in terms of genes.

Repetitive elements often comprise major components of eukaryotic genomes, and the proportions of these elements within the genome can vary widely across organisms (Chalopin et al. 2015). Repetitive elements may influence genome architecture and evolution, contribute to rewiring gene-regulatory networks (Feschotte 2023), and may even be involved in insecticide resistance (e.g. Gahan et al. 2001; Rostant et al. 2012). Here, we found that the most abundant class of repetitive elements (by bases occupied) were LINE Non-LTR retroelements, which is consistent with inferences from prior genome assemblies of this species (Petersen et al. 2019). There is considerable variation in repetitive element content across Hemiptera (Petersen et al. 2019), and it is unclear if the relatively large abundance and diversity of LINEs is specific to *Cimex*. The recent chromosome-level genome of the tropical bed bug, *Cimex hemipterus*, identified genomic repeat elements and found similar levels of LINEs in that species (Law et al. 2024). The lineages to which these two species belong split from their most recent common ancestor ~50 MYA (Roth et al. 2019). As such, these findings suggest that this high LINE density may be a characteristic common to *Cimex*. However, additional genome sequences of other Cimicid species will be needed to resolve this question.

This study identified two chromosomes with characteristics consistent with being sex chromosomes (X_1X_2) (i.e. sequencing coverage in male specimens approximately half of that of females). This was confirmed through synteny analysis that revealed a syntenic match to chromosomes 2 and 3 of the *C. hemipterus* genome (Supplementary Fig. 2) (Law et al. 2024). Although the genome of *C. lectularius* consists of 13 pairs of autosomes, the sex chromosomes have proven complex, with X fragmentation resulting in over 20 distinct fragments (Sadílek et al. 2013). Intraspecific variation in the number of sex chromosomes has been reported in other members of the Cimicidae, to which *C. lectularius* phylogenetically belongs (Ueshima 1968).

This genome provides a new valuable resource for enhancing investigations of a broad range of questions of this medically and economically significant resurging pest insect. With this, the common bed bug now represents a promising emerging model for which to study patterns of human-associated evolution and adaptation that accompanies urbanization.

Supplementary material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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Data availability

Fasta files for the chromosome-level assembly and the SRA fasta files are deposited in GenBank under BioProject PRJNA1160271.

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