Chromosome structural rearrangements in invasive haplodiploid ambrosia beetles revealed by the genomes of *Euwallacea fornicatus* and *Euwallacea similis* (Coleoptera, Curculionidae, Scolytinae)

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Abstract

Background. Bark and ambrosia beetles are among the most ecologically and economically damaging introduced plant pests worldwide, with life history traits including polyphagy, haplodiploidy, inbreeding polygyny and symbiosis with fungi contributing to their dispersal. Species vary in host tree ecologies, with many species attacking stressed or recently dead trees, such as the globally distributed *E. similis* (Ferrari). At the other end of the spectrum, the Polyphagous Shot Hole Borer (PSHB), *Euwallacea fornicatus* (Eichhoff), attacks over 680 host plants and is causing considerable economic damage in several countries worldwide. Despite their notoriety, publicly accessible genomic resources for *Euwallacea* Hopkins species are scarce, hampering better understanding of their invasive capabilities as well as modern control measures, surveillance and management.

Findings. Using a combination of long read and short read sequencing we assembled and annotated high quality (BUSCO > 98% complete) chromosome level genomes for these species. Comparative macro-synteny analysis showed that chromosome number increases in haplodiploid inbreeding species of *Euwallacea* due to fission events, reducing synteny with outbred diploid species. This suggests that life history traits can impact chromosome structure. Further, the genome of *E. fornicatus* had a higher relative proportion of repetitive elements, up to 17% more, than *E. similis*. We used the genome assemblies to identify the haplotype of the invasive population in Perth, Western Australia. Additionally, metagenomic analyses identified microbiota associated with both species, with fewer taxa found in association with *E. fornicatus* compared to *E. similis.*

Conclusions. Comparative analyses between closely related species with varying host tree ecologies and invasive capabilities will shed light into the molecular underpinning of species invasion. Moreover, these haplodiploid genomes will also contribute to the understanding of how life history traits contribute to their genome evolution.

Keywords: Invasive species, bark beetles, biosecurity, weevils, insect genomics, Polyphagous Shot Hole Borer

Background

Bark and ambrosia beetles (Curculionidae, Scolytinae) are some of the most ecologically damaging and economically important insect plant pests, contributing to tree mortality through infestation and the spread of pathogenic fungi (Biedermann et al., 2019; Raffa et al., 2008). Most bark beetles are phloeophagous and obtain nutrition from the phloem tissue while some lineages, termed ambrosia beetles, are xylomycetophagous and consume farmed symbiotic ambrosia fungi (Hulcr & Stelinski, 2017; Jordal, 2014). Many species are commonly transported to novel areas through human mediated

movement of wood and plant products and over one hundred species have established outside of their native range (Brockerhoff et al., 2006; Lantschner et al., 2020; Meurisse et al., 2019; Rassati et al., 2015). Life history traits including polyphagy, haplodiploidy, inbreeding polygyny and symbiosis with ambrosia fungi has been noted to contribute to the establishment of introduced species (Jordal et al., 2001; Lantschner et al., 2020; Vilardo et al., 2022), all of which characterise members of the tribe Xylebroini. Consequently, many such species have a global native or introduced distribution and are high priority pests in many regions of the world.

The genus *Euwallacea* Hopkins include several well studied polyphagous tree pests (Cognato et al., 2015; Kasson et al., 2013; O'Donnell et al., 2016). The genus is composed of over 80 species which are mostly circumtropical in their distribution (Storer et al., 2015). Species vary in their ecological relationships with host trees, with many species attacking stressed, cut and recently dead hosts. One such example is *E. similis* (Ferrari) (Figure 1b), a species native to South East Asia and possibly to Australia (Beaver et al., 2014), that has been introduced to the Americas and tropical African countries (Gomez et al., 2018) with 145 known host plants (Browne, 1961; Ruzzier et al., 2023). *Euwallacea fornicatus* (Eichhoff) (Figure 1a), also known as the Polyphagous Shot Hole Borer (PSHB) is considered one the most damaging Xyleborini species as it can infest healthy live host trees (Cook & Broughton, 2023; De Wit et al., 2022). It has been recorded to attack over 680 tree species across several plant families (Department of Primary Industries and Regional Development, Government of Western Australia, 2024; Gomez et al., 2019; Mendel et al., 2021) and is able to successfully reproduce in 168 species (Department of Primary Industries and Regional Development, Government of Western Australia, 2024 (and references therein), Mendel et al., 2021). *Euwallacea fornicatus* is widely distributed and has been introduced to California (Eskalen et al., 2012, 2013), Hawaii (Rugman-Jones et al., 2020), South Africa (Paap et al., 2018), Israel (Mendel et al., 2012), Europe (Schuler et al., 2023) and, recently, in the city of Perth, Western Australia (WA) where it is currently subject to an eradication campaign (Department of Agriculture, Fisheries and Forestry, 2024). This ambrosia beetle species is associated with several pathogenic *Fusarium* fungi, including *F. euwallaceae* Freeman *et al*. 2013 and *F. sp.* AF-18, and these fungi can induce wilt and dieback in host trees leading to tree mortality (Freeman et al., 2013). Introduced populations of *E. fornicatus* cause significant economic and ecological damage to managed and native ecosystems, and agricultural areas (Cook & Broughton, 2023; De Wit et al., 2022; Smith & Hulcr, 2015). Economic modelling has estimated the damages caused by this species to $18.45 billion over a ten-year period in South Africa (De Wit et al., 2022), while in Perth WA the cost of eradicating this species is estimated to be AUD$45 million and management of the species could reach AUD$6.8 million per annum in 30 years (Cook & Broughton, 2023).

The species *E. fornicatus* has been associated with three other species (Gomez, et al., 2018; Smith et al., 2019) representing a species complex with similar morphology, which include *E. perbrevis* (Schedl), the Tea Shot Hole Borer (TSHBa), *E. fornicatior* (Eggers), also the Tea Shot Hole Borer (TSHBb) and *E. kuroshiro* (Gomez and Hulcr), the Kuroshio Shot Hole Borer (KSHB) (Smith et al., 2019). Originally, these four species were all considered to be *E. fornicatus*, however, recent molecular phylogenetic and taxonomic works have stabilised their species status (Gomez, et al., 2018; Smith et al., 2019; Stouthamer et al., 2017). Few genomic resources exist for *E. fornicatus* and other Xyleborini, with much of the data available restricted to mitochondrial *cytochrome oxidase I* (*COI*) and a handful of genes for phylogenetic analyses (Bierman et al., 2022; Gomez et al., 2018; Stouthamer et al., 2017). Mitochondrial *COI* phylogenies have identified 21 haplotypes of *E. fornicatus* with much of the diversity found in Vietnam and Taiwan (Stouthamer et al., 2017). One haplotype, H33, has been identified in California, South Africa and Israel and is thought to have originated from Vietnam (Bierman et al., 2022; Stouthamer et al., 2017), however, other haplotypes, H35 and H38, have also been found in California and South Africa, respectively, indicating that the species could have been introduced multiple times to both countries (Bierman et al., 2022; Stouthamer et al., 2017). To date, the only sequence data available for *E. similis* is restricted to *COI* barcodes in its native South East Asian distribution (Lynn et al., 2021) Beyond this, little genomic data exists for these widespread pests and further molecular resources are vital to understand the invasion pattern of *Euwallacea* species, their invasive capabilities, and finding novel ways of controlling outbreaks and mitigating future harm.

Access to high quality genomes has led to advancements in many fields, including conservation management (Formenti et al., 2022; Hogg, 2023; Theissinger et al., 2023), biodiversity genomics and monitoring (Breed et al., 2019; Cordier et al., 2021; Mc Cartney et al., 2023; The Darwin Tree of Life Project Consortium et al., 2022), and species invasion research and management (McGaughran et al., 2021, 2024; North et al., 2021). In the management of invasive species, genomic resources are facilitating accurate and time-efficient diagnostics and surveillance (Chua et al., 2023; Frey et al., 2022; Gardy & Loman, 2018; Matheson & McGaughran, 2022; McGaughran et al., 2024), understanding the origin of invasive species, invasion pathways (Atsawawaranunt et al., 2023; Liu et al., 2022; Rane et al., 2023; Zhang et al., 2023), identifying genetic (Jin et al., 2023; Parvizi et al., 2023; Yin et al., 2021) and genome structural (Stapley et al., 2015; Tepolt et al., 2022) variation related to invasion potential (Camus et al., 2024; McGaughran et al., 2024; Sillero et al., 2020). These resources are also accelerating the ways in which invasive species are controlled, such as through chemical management (Hopkinson et al., 2023; Tay et al., 2022; Thia et al., 2023), biocontrol agents (Morrow & Riegler, 2021; Nikolouli et al., 2018; Ross et al., 2022), genome-informed population control (Burgess et al., 2022; Kennedy et al., 2018; Maselko et al., 2017), or gene-editing and gene drive (Devos et al., 2022; Hammond et al., 2016, 2021).

142

143 In this study we present chromosome-scale, annotated genomes of *E. fornicatus* and *E. similis* using
144 newly developed long read sequencing technologies and performed comparative analysis of
145 macrosynteny, transposable elements, metagenomics and nuclear and mitochondrial phylogenetics.
146 These genomes will be valuable assets for tracking the invasion pathway, developing novel
147 biosecurity management plans for these invasive species and will provide essential insight into
148 comparative of invasive species genomics.
149

150 **Methods**

151

152 *Sample collection, DNA extraction and sequencing*

153

154 Specimens of *E. fornicatus* were collected by the Department of Primary Industries and Regional
155 Development (DPIRD) WA from felled *Acer negundo* trees in Perth WA in October 2022. Male and
156 females were flash frozen by storing them on dry ice and shipped to CSIRO Black Mountain Canberra
157 Australian Capital Territory and stored at -80°C. *Euwallacea similis* larvae and adult females were
158 collected from a fallen *Ficus* sp. branch in Eureka, northern New South Wales in April 2023.
159 Specimens were transported from the field at 10°C and flash frozen by storing them at -80°C.
160 Vouchers of both species have been deposited in Australian National Insect Collection (ANIC) under
161 the collection codes ANIC-25-086063 – ANIC-25-086073.
162

163 Female specimens were used for DNA extraction due to their larger size compared to haploid males.
164 First, genomic DNA (for short read sequencing) of both species was extracted using a standard
165 Qiagen DNEasy kit approach. We then used a single specimen from each species for high molecular
166 weight DNA extraction with the Qiagen MagAttract kit following a modified protocol. Samples were
167 incubated overnight at 56°C and lysates were agitated at 300 rpm. Following this, DNA was
168 suspended in two elutions of 100 µl of Buffer EB. The concentration of all extracts were assessed
169 using a DeNovix spectrophotometer and a Qubit Fluorometer with the Qubit dsDNA High Sensitivity
170 Assay kit. Fragment size distribution and genomic DNA was assessed on an Aligent Fragment
171 Analyzer system with the high sensitivity genomic DNA kit DNF-488.
172

173 For short read sequencing, sequencing libraries with 150 bp paired end reads were prepared and
174 sequenced on the Illumina NovaSeq 6000 system at Genewiz (Suzhou, China). For long read
175 sequencing, high molecular weight (HMW) DNA extracts were sequenced on the PacBio HiFi
176 circular consensus Revio system at the AGRF in Brisbane, Queensland. For two HMW extracts of
177 each species, gDNA was mechanically sheared, followed by SMRTbell Express 2.0 library

178 construction. Short DNA fragments were removed using BluePippin. Barcoded libraries were then
179 sequenced using one SMRT cell for each species on the PacBio Revio system; the libraries for *E.*
180 *fornicatus* are herein referred to as EFF26 and EFF41, and the libraries for *E. similis* are herein
181 referred to as ESF13 and ESF15.
182
183 *Genome size estimation, assembly and scaffolding*
184
185 First, genome size and heterozygosity estimates were performed with all sequence reads; kmers were
186 counted with jellyfish (Marçais & Kingsford, 2011) and genome size and heterozygosity were
187 estimated with GenomeScope v2 (Ranallo-Benavidez et al., 2020; Vurture et al., 2017). HiFi reads
188 were then filtered for HiFi adapters with HiFiAdapterFilt (Sim et al., 2022) and subsampled with
189 seqtk into randomly sampled subsets, ranging from 12x, 15x, 20x, 35x and 50x, of the estimated
190 genome size, subsets for all four libraries. Contigs were assembled with these subsets and the full
191 dataset using HiCanu v2.1.1 (Nurk et al., 2020) with the genome size estimates of 190 Mb for both
192 EFF26 and EFF42, and 170 Mb for both ESF13 and ESF15. Contigs were also assembled with
193 hifiasm v0.19.6-r595 (Cheng et al., 2021) using the -l0 flag to disable purge duplication as these
194 genomes are highly homozygous and presumably inbred. Misassembled contigs were assessed and
195 corrected with CRAQ v1.0.9a (Li et al., 2023). Haplotigs and contig overlaps were removed with
196 purge_dups v1.2.5 (Guan et al., 2020). The quality of the corrected contig assemblies were assessed
197 with QUAST v5.2.0 (Mikheenko et al., 2018) and BUSCO v5.5.0 (Manni et al., 2021) using the
198 endopterygota_odb10 dataset and the tribolium2012 dataset was specified for Augustus (Stanke et al.,
199 2008) training.
200
201 Proximity ligation information was generated with Arima HiC 2.0 kits using tissue from a single
202 individual from both species at the JCMR BRF ANU. In short, tissues were fixed in formaldehyde,
203 DNA was extracted and then digested with Arima restriction enzymes. Overhangs of digested DNA
204 were then end repaired and marked with biotinylated nucleotides. The ends were then ligated together,
205 and DNA strands were de-crosslinked and sheared for Illumina PE 150bp sequencing. The *E.*
206 *fornicatus* library was sequenced on an Illumina NovaSeq 6000 for 300 cycles and the library for *E.*
207 *similis* was sequenced on an Illumina NextSeq 2000 for 300 cycles.
208
209 The Hi-C reads were mapped to draft contig assemblies following the Arima Genomics Mapping
210 Pipeline v03. First, 5' ends of the Hi-C reads were trimmed by 5 base pairs to remove over-
211 represented 3bp barcode UMI sequences and 2 dark bases from the 5' end. Trimmed reads were then
212 mapped independently (as single-ended reads) using BWA-MEM2 (Vasimuddin et al., 2019) with the
213 -5SP flag. Only the 5' ends were retained using the "filter_five_end.pl" script provided by the Arima
214 Genomics Mapping Pipeline. As the ends of the DNA were ligated together prior to sequencing, 3'

215 ends can originate from the same contiguous strands of DNA as the 5' end of the mate-pair, which can
216 manifest a ligation function and form chimeric reads after mapping. Following this, single-ended
217 mapped reads were then paired using the "two_read_bam_combiner.pl" script provided by the Arima
218 Genomics Mapping Pipeline and mapped reads with a mapping quality (MAPQ) < 10 were discarded
219 and sorted with samtools (Danecek et al., 2021). Lastly, PCR-duplicates were removed with Picard
220 Tools v3. Assemblies were then scaffolded using YahS v1.1a (Zhou et al., 2023) and assessed with
221 QUAST v5.2.0, and BUSCO v5.5.0 (Manni et al., 2021). We then compared the best scaffolded
222 assembly across the subset reads with an analogous scaffolding run using SALSA2 v2.3 (Ghurye et
223 al., 2019) and selected the best scaffolds for downstream curation using QUAST and BUSCO metrics,
224 and Hi-C mapping with the Juicebox Assembly Tools (JBAT) v1.b (Dudchenko et al., 2018; Durand
225 et al., 2016). Contaminant scaffolds, defined as contigs identified not to be of Arthropoda origin
226 through BLAST (Altschul, 1997), sequences with >15x coverage, and GC <0.3 and >0.45 were
227 removed using the BlobToolKit v4.2.1 (Challis et al., 2020). Hi-C reads were then mapped to the
228 decontaminated scaffolds using BWA-MEM2 following the Arima Genomics Mapping Pipeline v03
229 and were reviewed with JBAT v1.b (Dudchenko et al., 2018; Durand et al., 2016). Illumina short read
230 and PacBio HiFi reads were also mapped to the decontaminated scaffolds and all three mapped
231 datasets were inspected using JBrowse v2.10.3 to identify any potential misassembles (identified by
232 poor read alignments and regions of low coverage). We assessed the completeness of the final
233 scaffolded assemblies again with QUAST v5.2.0 (Mikheenko et al., 2018) and BUSCO v5.5.0 (Manni
234 et al., 2021). Lastly, the scaffolds of each library of the same species (i.e. both *E. fornicatus* and *E.*
235 *similis* assemblies) were renamed and reoriented to be syntenic with one another using BUSCOs to
236 define synteny and orientation. We identified pseudo-chromosome scaffolds by scaffolds that were >
237 500Kb in length and had an average Hi-C coverage > 30x. Pairs of syntenic pseudo-chromosomes
238 were defined by length and by BUSCO composition and these pseudo-chromosomes were named in
239 order of length by the EFF26 assembly for *E. fornicatus* and the ESF13 assembly for *E. similis*.
240 Lastly, assemblies were assessed for any remaining contamination with FCS-GX v0.5 (Astashyn et
241 al., 2024) and any contaminants identified were removed.
242
243 *Genome annotation*
244
245 Total RNA was extracted from ten adult males and females of each species, with a further eight *E.*
246 *forniacatus* females (for long read sequencing). RNA was extracted using the Zymo Research Quick-
247 RNA Miniprep Plus kit following the manufacturers tough-to-lyse sample preparation procedure.
248 RNA quantity and quality was then assessed using a DeNovix spectrophotometer and Aligent
249 Fragment Analyzer system with the HS RNA kit DNF-472. For one extract of each species and sex
250 strand-specific libraries were prepared by GENEWIZ from Azenta Life Sciences and sequenced on an
251 Illumina NovaSeq 6000 PE150. Libraries of the pooled eight *E. fornicatus* female RNA extract were

prepared for long-read sequencing following the ONT PCR-cDNA Barcoding Kit SQK-PCB111.24 by the BRF-JCMSR ANU. Libraries were sequenced on an ONT PromethIon across two FLO-PRO002 R9.4.1 flow-cells. Basecalling of the fast5 reads to fastq was performed with the Guppy basecaller (RRID:SCR_023196) v7.1.4.

Repeat annotation and masking was performed with EarlGreyTE (Baril et al., 2024). This pipeline runs RepeatModeler (Flynn et al., 2020) to identify *de novo* Transposable Element (TE) identification and automated TE curation pipelines are implemented to refine repeat element annotation. TEstrainer is used to generate maximum length *de novo* TE consensus sequences following a BLAST, Extract, Align, Trim process. This *de novo* TE library is then used to annotate the genome with RepeatMasker (Smit et al., 2013) using sensitive search settings and then full length LTRs are identified with LTR_finder (Xu & Wang, 2007). The TE annotations from the RepeatMasker run are then defragmented and combined with results from LTR_finder using RepeatCraft (Wong & Simakov, 2019). Overlapping TE annotations are then removed using the EarlGreyTE custom R script. Following repeat annotation, kimura-2 divergences between TE classes were calculated using the parseRM.pl scrip with the -l flag.

RNA-seq reads were filtered for low quality reads and adapters were removed with fastp (Chen et al., 2018) and the cleaned reads were mapped to the masked genome with hisat2 (Kim et al., 2019). Mapped reads were then sorted and indexed with samtools (Danecek et al., 2021). A protein database was constructed with sequences from the OrthoDB v11 Arthropoda dataset and annotations of the *Dendroctonus ponderosae* (Hopkins)*, D. valens* (LeConte)*, Hypothenemus hampei* (Ferrari), and *Taphrorychus bicolor* (Herbst) genome assemblies. Mapped RNA reads and the protein database were used to predict genes in repeat masked assemblies using BRAKER3 (Gabriel et al., 2023). Completeness of the annotated gene models was then assessed with BUSCO v5.5.0 (Manni et al., 2021), run in protein mode using the Endopterygota_obd10 dataset and the tribolium2012 dataset was specified for Augustus (Stanke et al., 2008) training. Lastly, genes were characterised for their putative function using eggNOG-mapper v2.1.6 (Cantalapiedra et al., 2021).

Transposable Element and chromosomal comparisons across Scolytinae

We explored repeat elements, macro-syntenic linkages and chromosomal rearrangements in an evolutionary framework across the Scolytinae. For this purpose, the, chromosome level genomes of *T. bicolor* (NCBI), *D. valens* (Liu et al., 2022), *D. ponderosae* (Keeling et al., 2022)*, Ips nitidus* (Eggers) (Z. Wang et al., 2023) and the Entiminae outgroup *Polydrsus cervinus* (L.) (Barclay et al., 2023) were downloaded from NCBI or ensemble. To compare the variation of repeat elements and macro-synteny associated with reproductive ecology within genera we included both *Dendroctonus* Erichson

assemblies to directly compare with *Euwallacea* assemblies. First, repeat annotation of downloaded genomes were performed with EarlGreyTE (Baril et al., 2024) as above. We then calculated the relative proportion of repetitive elements within the total assembly and pseudochromosomal regions to compare TEs across assemblies in R (R Core Team, 2013) with dplyr and visualised with ggplot2 (Wickham et al., 2019).

Following this, the composition of TEs were compared across Scolytinae genomes downloaded and the *Euwallacea* reference genomes (EFF26 & ESF13). We used BUSCO to call 2,108 single copy sequences which were aligned with MAFFT v7.490 (Katoh, 2002) and poorly aligned bases were removed with TrimAl v1.4.1r22 (Capella-Gutiérrez et al., 2009). Alignments were then concatenated with phykit v1.19.0 (Steenwyk et al., 2021) and phylogenetic analyses were conducted in IQ-tree v2.3.2 (Minh et al., 2020). Substitution models were estimated with ModelFinder Plus using a greedy strategy with gene merging (Chernomor et al., 2016; Lanfear et al., 2012) and bootstrap support was calculated with nonparametric bootstrap (Felsenstein, 1985) with 100 replicates and the phylogeny drawn in ggtree (Xu et al., 2022).

Lastly, odp (Schultz et al., 2023) was used to explore the macrosynteny and chromosomal variation across Scolytinae genomes downloaded and the *Euwallacea* reference genomes (EFF26 & ESF13). Previously used BUSCO sequences were used to describe the relationship between linkage groups and chromosomes between species. Reciprocal best hits between BUSCOs were identified with diamond blastp and the homology of scaffolds between species were inferred with pairwise p-values calculated with Fisher's exact test. Pairwise species dot plots syntenic linkage ribbon plots for all species were generated using scripts available in odp. Linkage colours in the ribbon plot follows the Bilaterian-Cnidarian-Sponge Linkage Groups (BCnS LGs) as in (Schultz et al., 2023).

Mitochondrial phylogenetics

To identify the haplotype of *E. fornicatus* present in Perth WA, previously published *COI* genes (Bierman et al., 2022; Gomez et al., 2018; Stouthamer et al., 2017) of PSHB, TSHBa&b and KSHB were downloaded from NCBI. The mitochondrial genome for all libraries were assembled using the MitoHiFi v3.2.1 pipeline (Uliano-Silva et al., 2023) which implements hifiasm (Cheng et al., 2021) to assemble reads that are mapped to a reference mitogenome (the *E. fornicatus* NC_059702 and *E. similis* ON169990 mitogenome assemblies), MitoFinder (Allio et al., 2020) to annotate mitochondrial genes and ARWEN for tRNA annotation (Laslett & Canbäck, 2008). Full length *COI* genes from these assemblies were then aligned with the sequences from NCBI using MAFFT (Katoh, 2002). Third codon positions were partitioned in the alignment and IQ-Tree v2.3.2 (Minh et al., 2020) was used to estimate the phylogeny, substitution models were identified with ModelFinderPlus (Flynn et

326 al., 2020) and bootstrap support was calculated with UFBoot (Hoang et al., 2018) with 1000
327 replicates.
328
329 *Metagenomics*
330
331 To identify the microbial communities associated with *E. fornicatus* and *E. similis*, metagenomes
332 were assembled from the total hifi sequence read dataset for each library. Metagenome-assembled
333 genomes (MAGs) were assembled using hifiasm-meta (Feng et al., 2022) with default settings.
334 BLAST was used to identify contigs and MAG identity was explored and visualised with the
335 BlobToolKit v4.2.1 (Challis et al., 2020). Host contigs and those that were unidentified were filtered
336 out of the dataset. A closed *Wolbachia* contig in was found in both *E. similis* libraries, and so the
337 strain was identified using MLST approaches implemented in the PubMLST database (Jolley et al.,
338 2018).
339
340 **Results**
341
342 *Genome assemblies and evaluation*
343
344 In total 98.23Gb of high-quality long sequence reads (PacBio HiFi) with a mean N50 of 11.6Kb were
345 obtained to assemble four genomes of two *Euwallacea* species (Table S1). The estimated genome size
346 for *E. fornicatus* was between 192.7 Mb and 208.9 Mb, while estimates of 170.7 Mb and 181.8 Mb
347 were made for *E. similis* and all are predicted to be highly homozygous (het: 0.012% – 0.112%), and
348 all short read and long read libraries consistently predicted similar results (Figure S1). The four
349 chromosome-level genomes that were selected for downstream analysis for *E. fornicaus* and *E. similis*
350 were assembled with HiCanu, as we found HiCanu yielded better assemblies than hifiasm. Genomes
351 were assembled with, post sequence read filtering and subsampling, 6.5Gb for EFF26 (29.58x, 0.35
352 subsample), 7Gb for EFF42 (24.07x, 0.35 subsample), 5.63Gb for ESF13 (30.95x, not subsampled)
353 and 8.09Gb for ESF15 (44.78x, 0.15 subsample) of PacBio HiFi reads and 3.02Gb (*E. fornicatus*) and
354 3.3Gb (*E. similis*) of Arima Hi-C paired-end reads (Table S1).
355
356 Both pipelines for scaffolding, YahS and SALSA2, identified a similar number of pseudochromosome
357 scaffolds for the *E. fornicatus* assemblies but not for *E. similis*. In the *E. similis* scaffolds, SALSA
358 failed to break incorrectly joined contigs identified through Hi-C read mapping compared to YahS
359 (Figures S2 – S5). Scaffolding, decontamination and manual curation anchored 75% and 69% of the
360 total assembly sequences into 39 pseudo-chromosomes for EFF26 (Figure S6) and EFF42 (Figure
361 S7), respectively. While the total number of assembly sequences decreased post scaffolding and
362 manual curation, N50 also decreased by 0.3Mb – 1.3Mb. This was attributed to the breakup of larger

363 contig sequences that were not supported with Hi-C alignments during manual curation of assemblies.
364 Both EFF26 and EFF42 assemblies were larger than the estimated genome sizes, at 219.83 Mb and
365 239.6 Mb, respectively. Scaffolding, decontamination and manual curation resulted in 93% and 91%
366 of the total assembly sequences successfully anchored in 37 pseudochromosomes groups for ESF13
367 (Figure S8) and ESF15 (Figure S9), respectively. The N50 of the assembly increased post scaffolding
368 by 1.44 – 1.54 Mb and the total number of assembly sequences decreased. The ESF13 assembly was
369 smaller than the estimated genome size, at 177.6 Mb, while the ESF15 genome was larger than
370 estimated at 180.07 Mb. All assemblies were found to be highly complete with BUSCO, and all
371 genomes contained over 98% complete single copy sequences (Table 1, Figure S10).
372
373 *Genome annotation*
374
375 Genome annotation with BRAKER3 predicted, on average 13,570 transcripts and an average of
376 10,845 genes for the *E. fornicatus* genomes. Annotation for the *E. similis* genomes resulted in an
377 average 16,188 transcripts and 12,141 genes predicted. Protein coding genes occupied, on average, 16
378 Mb of the *E. fornicatus* genome (6 – 7.9% of total assembly lengths) and 18 Mb of *E. similis* (10% of
379 total assembly lengths). Gene predictions were largely complete, with >90% of predicted genes
380 assigned putative homologs with functional annotation for all annotations. Moreover, most
381 annotations were largely complete with >97% complete single copy BUSCOs (Table 3). The number
382 of genes annotated for both *Euwallacea* species were slightly lower than those annotated in
383 *Dendroctonus* assemblies (Copeland et al., 2024; Liu et al., 2022) and much lower than the
384 annotations of *H. hampei* (Navarro-Escalante et al., 2021), *I. nitidus* (Wang et al., 2023)*, I.*
385 *typographus* (Powell et al., 2021) and *T. bicolor*.
386
387 *Mitochondrial and nuclear phylogenetics*
388
389 Phylogenetic analysis of mitochondrial haplotypes identified that the population of *E. fornicatus* in
390 Perth WA belong to haplotype H38, with the *COI* barcode identical to native individuals in China and
391 Vietnam, and also to the invasive population in South Africa. The H38 haplotype clustered with
392 Haplotypes 37, 39 – 42, and were found to be phylogenetically distinct from the more prevalent
393 invasive H33 haplotype (Figure S11). Phylogenomic analysis using BUSCOs of published Scolytinae
394 genomes and those of the reference *Euwallacea* yielded a topology similar to that reported by Pistone
395 *et al*. (Pistone et al., 2018) and all nodes were strongly supported (Figure 2).
396
397 *Repeat element and chromosomal variation across Scolytinae*
398

399 Roughly half (47 – 50%) of the *E. fornicatus* assemblies and over one third (33 - 34%) of the *E.*
400 *similis* assemblies were masked by the EarlGreyTE pipeline (Table 3, Figure 2, Figure S12). DNA
401 transposons and long terminal repeats (LTR) were the most abundant repeat elements identified, with
402 unclassified elements only accounting for a small proportion of all annotations (Table 3). Few rolling
403 circle elements were identified in all assemblies and SINEs were only found in the *E. similis*
404 assemblies (Table S1). Kimura 2 divergence landscapes of repetitive elements indicate a recent burst
405 of DNA transposons and LTR elements, while LINE and rolling circle elements are experiencing
406 ongoing proliferation in the *Euwallacea* assemblies (Figures S13 – 16). The relative proportion of
407 annotated TEs varied throughout Scolytinae assemblies, with over 60% of the *T. bicolor* assembly
408 occupied by TEs, and the assembly of *D. ponderosae* possessed the lowest relative proportion of
409 annotated TEs (25%). The relative proportions of the types of TEs also varied significantly throughout
410 Scolytinae as well, and TE annotations were largely composed of unclassified elements, DNA
411 transposons and LINEs. All published Scolytinae assemblies possessed greater amounts of Satellite
412 sequences than the *Euwallacea* assemblies (Figure 2). Lastly, the Entiminae outgroup *P. cervinus* had
413 a larger proportion of the genome of annotated TEs compared to Scolytinae.
414
415 The assemblies of *E. fornicatus* and *E. similis* were more syntenic with one another than other
416 Scolytinae. Largely, synteny was retained throughout the Scolytinae assemblies with several
417 translocations of genomic material. However, extensive translocations, rearrangements, and fissions
418 of syntenic blocks were identified between the assemblies of *Euwallacea* and other Scolytinae.
419 Further, the X chromosome was identified to be synentic throughout all assemblies, however, blocks
420 corresponding to these scaffolds were broken apart in the *Euwallacea* assemblies (Figure 3).
421
422 *Metagenomics*
423
424 The *E. similis* libraries produced more MAG contigs (730 and 1,200) than the *E. fornicatus* libraries
425 (103 and 109). Across all libraries, many more Bacteria were identified than other microbial
426 Kingdoms, with Actinomycetota and Pseudomonadota highly prevalent (Figure S17). Several
427 complete circularised Bacteria assemblies were also identified across all libraries (Table S2),
428 including *Wolbachia* for both *E. similis* libraries. MLST profiling identified the *Wolbachia* strain as
429 closely related to *w*Ei (Kawasaki et al., 2016), and unique SNPs were found in the *hcpA* and *gatB* loci.
430 Consequently, this represents a novel strain, herein referred to as *w*Es. Several fungal taxa were
431 identified for both species (Figure S17), and mitogenomic contigs of *Fusarium* belonging to the
432 *Euwallacea Fusarium* Clade (EFC) were identified by BLAST for all libraries except for ESF13. The
433 only fungi found in *E. fornicatus* MAGs were *Fusarium*, while a richer diversity of fungi were found
434 in association with *E. similis*, with 13 genera classified including *Leptographium, Ophiostoma,*

Ceratocystis and *Sporothrix* (Table S1). Several viral contigs were also identified in both species, all belonging to Caudoviricetes.

Discussion

Genome synteny across Scolytinae

We sequenced, assembled and annotated the genomes of *Euwallacea fornicatus* and *E. similis*. These genomes are highly contiguous, complete and accurate as we sequenced the libraries of two female specimens per species and employed multiple assembly and scaffolding pipelines to validate their quality. The *E. fornicatus* assemblies presented herein were accepted and published in the NCBI GenBank on the same day as two other contig-level assemblies of the same species from South Africa, GCA_040114445 & GCA_040114495, from a female and male, respectively. The assembly sizes of these contig-level genome assemblies are similar to our chromosome-scaffold assemblies, further validating the quality of our assemblies. These genomes represent the first chromosome scaffold genomes for these pests and also the first genomes of diploid females from a haplodiploid inbreeding beetle lineage. They are among the smallest Scolytinae genomes published and contain the highest number of pseudo-chromosomal scaffolds. Additionally, 17 – 81% fewer protein coding genes were predicted for these *Euwallacea* assemblies compared to previously published Scolytinae genomes (Copeland et al., 2024; Liu et al., 2022; Navarro-Escalante et al., 2021; Powell et al., 2021; Wang et al., 2023).

The number of pseudo-chromosomal scaffolds identified for both *Euwallacea* species (n = 37 – 39) were higher than the typical number of chromosomes in Scolytinae (n = 7 – 16) (Virkki & Smith, 1978). Chromosome number variation has previously been identified across and within genera of scolytines. For example, the karyotype of all 19 *Dendroctonus* species have been characterised, with chromosome numbers ranging from 2n = 12 – 30, and chromosomal fusion resulting in chromosome number reduction (Keeling et al., 2022; Lanier, 1981; Six & Bracewell, 2015). Similarly, chromosome number variation has also been identified across 32 species of *Ips*, with numbers ranging from 2n = 16 – 32 (Virkki & Smith, 1978). In Curculionidae, a larger variation in chromosome numbers have been characterised in those that are parthenogenetic due to polyploidy (Blackmon & Demuth, 2015; Virkki & Smith, 1978). For example, parthenogenetic pseudogamy resulting in triploid females have been characterised in *Ips acuminatus* Gyllenhal and *I. tridens* Mannerheim (Lanier & Kirkendall, 1986). Similar observations have been made for several species of obligate inbreeding haplodiploid Xyleborini (Takenouchi & Takagi, 1967; Virkki & Smith, 1978). Species of the genus *Xylosandrus* Reitter have fewer chromosomes than other Scolytinae, with *X. germanus* (Blandford) 2n = 8 and *X. compactus* (Eichhoff) 2n = 10 (Takenouchi & Takagi, 1967). In contrast,

472 the chromosomes of *Anisandrus dispar* (F.) are small in size and numerous, n = 40 (Virkki & Smith,
473 1978), similar to the *Euwallacea* species genomes.
474
475 We identified extensive chromosomal rearrangements between haplodiploid inbreeding and diploid
476 outbreeding Scolytinae. The synteny of autosomal chromosomes and the ancestrally conserved X
477 chromosome between diploid outbreeding species are largely conserved across genera. Most
478 chromosomes shared 1:1 conserved synteny and several blocks are either translocated within or across
479 scaffolds. Further, despite the difference in chromosomal numbers between *D. ponderosae* (2n = 12)
480 and *D. valens* (2n = 14) (Six & Bracewell, 2015), synteny across assemblies are largely conserved,
481 identified herein and previously (Copeland et al., 2024; Liu et al., 2022). Between the two
482 haplodiploid inbreeding *Euwallacea* species, the assemblies are less syntenic than the diploid
483 outbreeding species. Few 1:1 conserved syntenic chromosomes exist due to widespread translocations
484 and inversions of orthologous genes. Furthermore, extensive chromosomal rearrangement
485 characterised the variation in genomic architecture between *Euwallacea* and diploid outbreeding
486 species, with widespread translocation events shuffling syntenic blocks and fission events breaking up
487 chromosomes (including the ancestrally conserved X chromosome). Comparative analyses of
488 Coleopteran karyotypes have identified that small effective population sizes and genetic drift greatly
489 impact chromosome number variation across species (Blackmon et al., 2024). As *Euwallacea,*
490 obligately engage in inbreeding and haplodiploidy, species are highly homozygous, as identified
491 herein and in Storer et al., 2017; Urvois et al., 2023. As such, the reproductive ecology of Xylebroini
492 species may contribute to the increased chromosome number identified in *Euwallacea* and extensive
493 rearrangements across assemblies.
494
495 Comparative analyses indicate that the *E. fornicatus* genome has one of the highest proportions of
496 repeat and transposable elements relative to total assembly among the Scolytinae assessed, with
497 roughly 50% of the assembly composed by TEs. While the relative proportion of these elements in the
498 genome of *E. similis* only occupy about 30% of the total assembly, DNA transposons are among the
499 most prevalent transposable elements in both *Euwallacea* species. DNA transposons have been
500 identified to play a significant role in Eukaryotic genome and chromosomal evolution (Feschotte &
501 Pritham, 2007). Moreover, it has been identified in plants (Ren et al., 2018) that genomic
502 rearrangement and recombination can result in net sequence loss at newly formed telomeric regions in
503 the chromosome. Given that we have identified recent bursts in the proliferation of DNA transposons
504 in the genomes of *Euwallacea*, these repetitive elements could be involved in the widespread
505 structural variation, high number of chromosomes and smaller genome size and content in this genus.
506 Further karyotyping and chromosome level assemblies of haplodiploid inbreeding Scolytinae are
507 needed to explore these genomic rearrangements further.
508

Co-evolution with symbiotic ambrosia fungi could also contribute to chromosomal rearrangements in Xyleborini, as observed in *Euwallacea* assemblies. Genomic erosion is particularly prevalent in the genomes of symbionts that provide nutrition to their hosts, and genes of biosynthesis pathways producing amino acids obtained from the host are commonly lost (Beasley-Hall et al., 2023; McCutcheon & Moran, 2012; Sloan & Moran, 2012). This has been identified to occur in host animals as well; in the deep-sea bone-eating worm, *Osedax frankpressi* Rouse, Goffredi & Vrjenhoek (Annelida: Polychaeta), endosymbionts provide key nutritional biosynethetic pathways which has subsequently resulted in host genome reduction and gene loss (Moggioli et al., 2023). Similarly, in socially parasitic ant species, gene loss of olfactory receptor repertoires corresponding with reduced social behavioural has evolved repeatedly (Schrader et al., 2021). Host genome rearrangements have also been reported in ants, with high levels of structural rearrangements, loss of synteny across assembled genomes, gene family contraction, expansion and a gain of novel genes identified in the *Atta* fungal farming ant lineage (Nygaard et al., 2011, 2016). Similar processes could have acted on the genomes of fungal farming Scolytinae in the coevolution with their symbionts, leading to the observed small genome size, genomic rearrangements and loss of synenty with other Scolytinae in *Euwallacea* genomes. It should be noted that the evolution of obligate inbreeding and haplodiploidy, in Dryocoetini, seems to preceded the development of the ambrosia fungal symbiosis in Xyleborini (Jordal & Cognato, 2012), and as such, further chromosome scale assemblies of non-fungus farming inbreeding haplodiploid species (Dryocoetini) are needed to assess the impact of the ambrosia symbiosis on chromosome structure.

Genomic resources for biosecurity

The genomes assembled for *Euwallacea* will be essential resources for the management of these pests. A vital component in managing an invasive species is understanding where the species originates and how it arrived. Through mitochondrial haplotyping, we have identified that the population of *E. fornicatus* introduced to Perth, Western Australia corresponds to the H38 haplotype, which has also been introduced to California and South Africa. While further markers are needed for population genomic analyses in order to track the invasion pathway from the native to the introduced range (Rane et al., 2023), the assembled genomes will be vital in understanding population differentiation at the gene and genomic level. These genomes will also be a vital resource in the diagnostics and detection of these pests. While the mitochondrial genome for both species have already been published (Guo et al., 2023; Wang et al., 2020), the ability to identify nuclear markers or sequences will be important for the diagnostics and detection (such as through eDNA techniques) of the pests. Diagnostic protocols exist for the detection of *E. fornicatus* through identification of the symbiont *Fusarium euwallaceae* (De Jager & Roets, 2022), however, the protocol operates on the detection of fungus in host plant woody tissues, which indicates that the species is already present and likely reproducing in a given

546 locality. Using eDNA techniques and these reference genomes, these pests could be detected early,
547 increasing the chances of eradication or containment, and reducing the time and resources necessary
548 to achieve this.
549
550 Beyond tracking, detection and diagnostics, these genomes will also provide needed information for
551 genome guided management of these pest. The endosymbiont gram-negative bacteria *Wolbachia* has
552 been successfully deployed as a control agent for several insect pests (Morrow & Riegler, 2021;
553 Nikolouli et al., 2018; Ross et al., 2022) and may prove useful in the control of *Euwallacea* pests.
554 While strains have not yet been identified to be associated with *E. fornicatus*, further sequencing and
555 MLST identification could identify naturally present strains, or strains from closely related species
556 (such as *w*Es identified in this study) that could be artificially transferred (Grenier et al., 1998; Xi et
557 al., 2005). However, as noted in previous studies, the impacts of *Wolbachia* on haplodiploid
558 inbreeding Scolytinae are not yet fully understood (Bickerstaff et al., 2023; Kawasaki et al., 2016).
559 Gene drive through CRISPR gene editing may be an avenue to control populations of invasive *E.*
560 *fornicatus* and *E similis.* As demonstrated in invasive wasps (Lester et al., 2020; Meiborg et al.,
561 2023), the ability to control population growth with this technique is limited due to haplodiploidy,
562 with only a single gene targeting female fertility identified as useful and infertile individuals were not
563 as competitive as fertile counterparts. Outbreeding is rare in the inbreeding Scolytinae lineages and
564 comes at a fitness cost (Peer & Taborsky, 2005), and as such the penetration of gene drive through
565 populations may be limited. Further insights are needed to first identify candidate genes in these
566 genomes and then test the suitability of this management approach.
567
568 These genomes will also be of value in understanding mechanisms driving species invasion and pest
569 impact from a genomic perspective. Preliminary comparative analyses of transposable element
570 variation indicate that the relative proportion of genome content is similar to other beetle genomes
571 (Gilbert et al., 2021; Petersen et al., 2019), however, a higher number of TEs are present in the *E.*
572 *fornicatus* genome than in the *E. similis* genome. Transposable elements have been identified to be
573 adaptive in species (Gilbert et al., 2021) and promote genetic diversity and rapid adaption in invasive
574 species. The higher accumulation of TEs present in *E. fornicatus* may explain why the species can
575 reproduce in a wider breadth of host trees and successfully establish galleries in healthy live hosts,
576 unlike *E. similis*. Further comparative genomic analysis, such as gene family evolution (McKenna et
577 al., 2016), structural variation (North et al., 2021; Stapley et al., 2015), and pangenomics (Gerdol et
578 al., 2020) of both beetles and their symbionts may yield greater insights into why these species are
579 such successful invaders compared to other haplodiploid inbreeding ambrosia beetles, understanding
580 variation in host tree ecology and why *E. fornicatus* is more destructive than other members
581 *Euwallacea*.
582

583 **Conclusions**

584

585 Pest species of *Euwallacea* are globally invasive and can cause significant ecological and economic
586 impacts in the ranges where they are established. Our study presents the first chromosome-level
587 genome assemblies of these pests and also for haplodiploid inbreeding species. These assemblies will
588 not only offer a deeper understanding of the genome structural variation that due to species ecology
589 but also serve as essential resources for biosecurity management and control strategies. The
590 comparative analyses of repeat and transposable elements, as well as the macro-synteny of
591 chromosome assemblies, shed light on the potential impacts of haplodiploidy and inbreeding on
592 genome evolution in these beetles. Our findings underscore the importance of high-quality genome
593 assemblies in advancing our knowledge of invasive species and contributing to their effective
594 management and control.

595

596 **Data Availability**

597

598 All genome and raw sequencing reads produced in this have been deposited with the NCBI, under the
599 BioProject: PRJNA1086994. Specific accession numbers for raw sequence reads and genome
600 assemblies are available in Table S1 and Table 1, respectively.

601

602 **Additional Files**

603

604 **Supplementary File 1.** The supplementary figures produced through assembly QC and analyses that
605 complement the main text.
606 **Supplementary Table S1.** Sequence read output of all libraries sequenced.
607 **Supplementary Table S2.** Classes of Repeat and Transposable Elements identified by EarlGreyTE.

608

609 **Abbreviations**

610

611 **ANIC:** Australian National Insect Collection
612 **ANU**: Australian National University
613 **BRF-JCSMR**: Biomolecular Resource Facility – John Curtin School of Medical Research
614 **BLAST:** Basic Local Alignment Search Tool
615 **BUSCO**: Benchmarking Universal Single Copy Orthologs
616 ***COI*:** Cytochrome Oxidase I
617 **CSIRO**: Commonwealth Scientific Industrial Research Organisation
618 **DPIRD WA:** Department of Primary Industries and Regional Development, Western Australia
619 **EFC:** *Euwallacea Fusarium* Clade

620 **EFF**: *Euwallacea fornicatus* Female
621 **ESF**: *Euwallacea similis* Female
622 **Hi-C:** High-throughput chromosome conformation capture
623 **HiFi**: High Fidelity
624 **HMW**: High Molecular Weight
625 **MAG:** Metagenome-assembled genomes
626 **MLST:** Multi-locus Sequence Typing
627 **NCBI:** National Centre for Biotechnology Information
628 **ONT**: Oxford Nanopore
629 **PacBio**: Pacific Biosciences
630 **PSHB**: Polyphagous Shot-Hole Borer
631 **REs:** Repeat Elements
632 **TEs:** Transposable Elements
633
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635
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637
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642
643 **Author's Contributions**
644
645 JB, HE, TW, RR, VC, AS and TW conceptualised the research. DPIRD and KI provided PSHB
646 specimens. TW, RR, LC and GP provided advice for HWM DNA extraction and genome assembly
647 pipelines. JB collected the data. JB and HE analysed and interpreted the data. JB wrote the manuscript
648 with input from all authors. All authors agree to the submitted manuscript.
649
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Figures

Figure 1. The ambrosia beetles A) *Euwallacea fornicatus* (PSHB) and B) *Euwallacea similis* whose genomes were sequenced herein. Photo taken by Hermes Escalona.

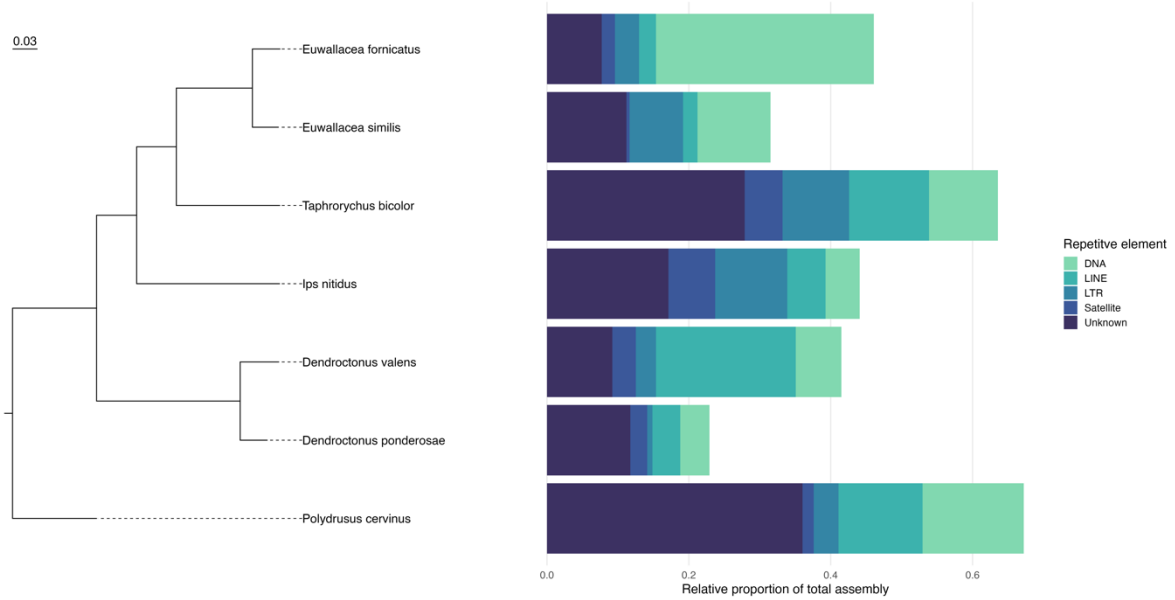

Figure 2. Maximum-likelihood phylogenetic tree of the concatenated 2,108 BUSCO single copy sequences of available pseudo-chromosome level assemblies for Scolytinae based using 2,108 single copy complete BUSCO sequences. All nodes were strongly supported with 100 bootstrap support. Scale bar indicated 0.03 substitutions per nucleotide position. To the right of the tips are the relative proportion of repetitive element composition as annotated in the species' genome.

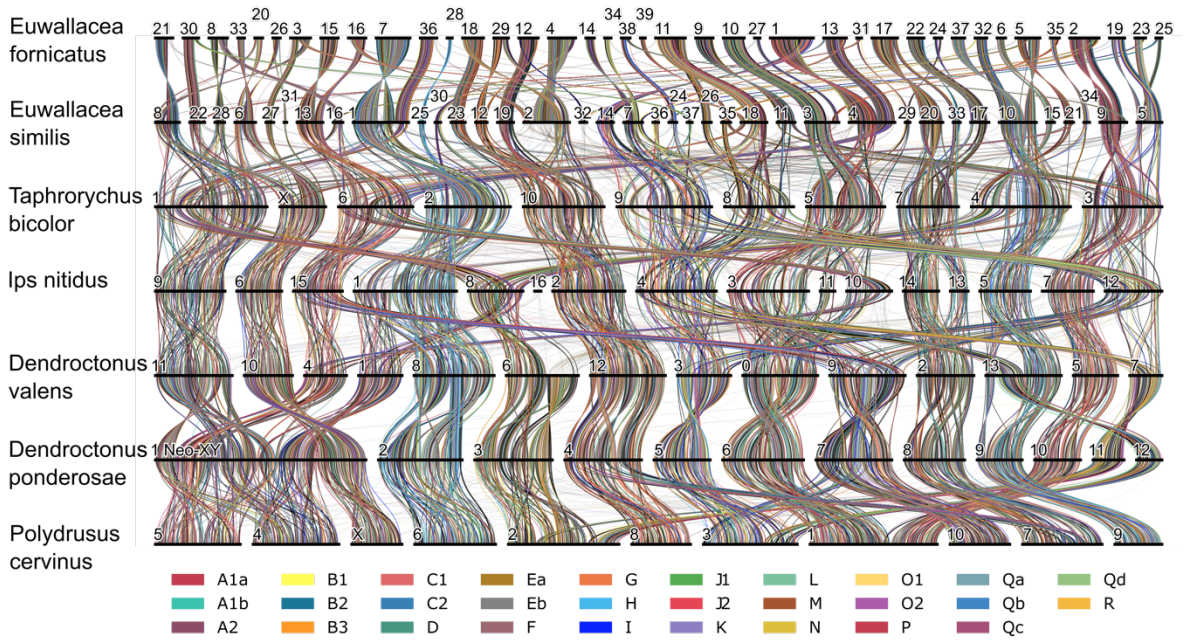

Figure 3. Ribbon plot showing the synteny and structural and chromosomal rearrangement in Scolytinae chromosomal assemblies. Syntenic blocks of single copy complete BUSCO sequences link pseudo-chromosome scaffolds between assemblies. Black bars represent individual pseudo-chromosome scaffolds and are labelled according to the name provided in the assembly. Scaffolds are ordered following the *Dendroctonus ponderosae* assemblies and the lengths of the bars representing scaffolds indicate total number of BUSCOs and not scaffold length. Coloured lines connect orthologous genes across scaffolds and the colour code follows the Bilaterian-Cnidarian-Sponge Linkage Groups (BCnS LGs) as in (Schultz et al., 2023).

1312 **Table 1.** Contig and scaffold summary statistics for the assemblies produced. Subsampled library refers to the level of subsampling and sequence statistics
1313 therein post subsampling with seqtk. Number of assembly seqs refers to the number of contigs (contig assembly) or scaffolds (scaffold assembly) present the
1314 assemblies. Mean HiFi coverage was calculated by dividing the number of base pairs in the filtered subsampled HiFi libraries by the total assembly length.
1315 Mean Hi-C coverage was calculated by dividing the number of base pairs in the filtered Hi-C libraries by the total assembly length.
1316

Species	*Euwallacea fornicatus*				*Euwallacea similis*			
Library	EFF26		EFF42		ESF13		ESF15	
Assembly	Contig	Scaffold	Contig	Scaffold	Contig	Scaffold	Contig	Scaffold
NCBI Accession								
Estimated Genome Size (Mb)	192.7	192.7	197.7	197.7	181.83	181.83	170.77	170.77
Library subsampling strategy	0.35	0.35	0.35	0.35	1	1	0.15	0.15
Subsampled seqs (K)	609.62	609.62	578.21	578.21	0.54	0.54	659.71	659.71
Subsampled bases (Gb)	6.5	6.5	7	7	5.63	5.63	8.09	8.09
Total assembly Length (Mb)	268.98	219.83	290.46	239.6	210.3	177.6	210.22	180.07
Longest assembly seq (Mb)	9.93	8.67	10.78	8.89	7.16	10.09	7.19	10.04
Number of assembly seqs	530	185	422	179	654	128	480	140
Number of pseudo-chromosomes		39		39		37		37
% assembly anchored in pseudo-chromosomes		75%		69%		93%		91%
N50 (Mb)	4.1	3.8	4.9	3.6	3.6	5.14	3.7	5.14
L50	22	20	21	23	22	13	21	13

N's per 100 kpb	0	0.36	0	0.42	0	2.49	0	1.9
Mean HiFi coverage (x)	24.18	29.58	24.07	31.80	26.79	30.95	38.50	44.78
Mean Hi-C coverage (x)	11.90	14.56	11.02	13.36	15.69	18.12	15.70	18.26
BUSCO: genome (BUSCO v5.5.0; endopterygota_odb10, n = 2124)								
Complete single copy	2080 (97.9%)	2080 (97.9%)	2082 (98%)	2079 (97.9%)	2062 (97.1%)	2084 (98.1%)	2096 (97.4%)	2084 (98.1%)
Complete duplicated	7 (0.3%)	8 (0.4%)	8 (0.4%)	8 (0.4%)	28 (1.3%)	9 (0.4%)	19 (0.9%)	9 (0.4%)
Fragmented	11 (0.5%	15 (0.7%)	12 (0.6%)	15 (0.7%)	10 (0.5%)	7 (0.3%)	9 (0.4%)	8 (0.4%)
Missing	26 (1.3%)	21 (1%)	22 (1%)	22 (1%)	24 (1.1%)	24 (1.2%)	27 (1.3%)	23 (1.1%)

1317

1318 **Table 2.** Protein and repeat annotation statistics for the assemblies produced. Proteins were identified and annotated by BRAKER3 and completeness
1319 assessed with BUSCO. Repeats were identified by EarlGreyTE.
1320

Species	*Euwallacea fornicatus*		*Euwallacea similis*	
Library	EFF26	EFF42	ESF13	ESF15
Predicted transcripts	16,390	10,749	16,170	16,206
Predicted genes	11,589	10,101	12,128	12,153
Total coding region (bp) (%)	17,388,659 (7.9%)	14,946,768 (6%)	18,078,735 (10%)	18,139,032 (10%)
Functionally annotated transcripts	14,771	9,584	14,600	14,600
Functionally annotated genes	10,767	9,375	11,327	11,285
BUSCO: protein (BUSCO v5.5.0; endopterygota_odb10, n = 2124)				
Complete single copy	2089 (97.7%)	1889 (88%)	2075 (97.7)	2075 (97.7)
Complete duplicated	14 (0.7%)	19 (0.9%)	18 (0.8%)	17 (0.8%)
Fragmented	10 (0.5%)	16 (0.8%)	4 (0.2%)	4 (0.2%)
Missing	25 (1.1%)	219 (10.3%)	27 (1.3%)	28 (1.3%)
Repeats				
DNA (bp) (%)	68,089,859 (30.9)	31,487,152 (13.1)	18,861,021 (10.6)	18,580,216 (10.3)
LINE (bp) (%)	5,329,747 (2.4)	7,068,963 (3.0)	3,325,503 (1.9)	3,673,824 (2.0)
LTR (bp) (%)	7,594,481 (3.5)	9,293,361 (3.9)	13,488,481 (7.6)	16,621,346 (9.1)
Unclassified (bp) (%)	18,446,822 (8.4)	19,202,615 (8.0)	21,972,489 (12.4)	21,763,152 (12.0)
Total masked (bp) (%)	103,661,639 (47.1)	122,116,844 (50.9)	58,944,136 (33.2)	61778298 (34.2)

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