A high-quality genome assembly and annotation of the European earwig *Forficula auricularia*

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Abstract

The European earwig *Forficula auricularia* is an important model for studies of maternal care, sexual selection, sociality and host-parasite interactions. However, detailed genetic investigations of this species are hindered by a lack of genomic resources. Here we present a high-quality hybrid genome assembly for *F. auricularia*. The genome was assembled using nanopore long-reads and 10x chromium link-reads. The final assembly is 1.06Gb in length with 31.03% GC content. It consists of 919 scaffolds with an N50 of 12.55Mb. Half of the genome is present in only 20 scaffolds. Benchmarking Universal Single-Copy Orthologs scores are ~90% from three sets of single-copy orthologs (eukaryotic, insect, and arthropod). The total repeat elements in the genome are 64.62%. The MAKER2 pipeline annotated 12,876 protein-coding genes and 21,031 mRNAs. A phylogenetic analysis revealed the isolate used in our genomic analysis belongs to Subspecies B, one of the two known genetic subspecies of *F. auricularia*. The genome assembly, annotation, and associated resources will be of high value to a large and diverse group of researchers working on Dermapterans.

Introduction

Insects have been at the forefront of genetic research for various biological questions (Wilson-Sanders 2011; Mukherjee *et al.* 2015; Simons and Tibbetts 2019). However, most of the genetic studies are carried out on a small number of holometabolous insects that undergo true metamorphosis. In contrast to Holometabola, hemimetabolous insects undergo incomplete metamorphosis with a series of nymphal moults that increasingly resemble the

adult form (Truman 2019). It is widely accepted that Holometabola branched out from hemimetabolous ancestors during the Permian 300 Mya (Labandeira and Phillips 1996; Yang 2001). Yet the conserved mode of development, embryonic organisation, and the adult body plan of hemimetabolous insects offer a unique model for the study of developmental and evolutionary mechanisms. However, even with the increasing number of sequenced genomes, the majority belong to the Holometabola (Ylla *et al.* 2021). This has been a bottleneck for the exploration of the diverse biology and life history of hemimetabolous insects. To address this paucity, we report a high-quality annotated genome of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae).

The European earwig *Forficula auricularia* is widely distributed, comprising two recognised subspecies, A and B (Wirth *et al.* 1998). They are native to the western Eurasian region and were introduced to North America, Australia, and New Zealand, where they have quickly adapted and became abundant throughout the regions (Quarrell *et al.* 2018; Tourneur and Meunier 2019). Their propensity to dwell on flower and kitchen gardens can cause significant damage to crops, flowers, and commercial vegetables and make them important agricultural pests (Campos *et al.* 2011; Hill *et al.* 2019).

They have been of particular interest for many researchers not just because of their importance in the agricultural ecosystem (Binns *et al.* 2021) but also their importance as a research model for various biological and evolutionary phenomena like sexual selection, maternal care, family interactions, reproductive strategy, and social behaviour (Forslund 2000; Falk *et al.* 2014; Kramer *et al.* 2015; Van Meyel and Meunier 2020). They have been extensively studied by behavioural ecologists for the early evolution of group-living and family life (Falk *et al.* 2014). The male earwigs also show an unusual bias in their use of lateral left and right sexual organs without any conspicuous anatomical differentiation (Kamimura 2006). Like the right-handedness in humans, 90% of males of giant earwig *Labidura riparia* show a preference for the right penis for copulation, providing insights into the evolutionary origin of lateralization (Kamimura *et al.* 2021). Similarly, they are an excellent lab model to study extended phenotypes as they exhibit strange suicidal water-seeking behaviour during the late stages of infection by mermithid nematodes (Herbison *et al.* 2019). However, their use as a genetic model has been severely limited by the lack of a reference genome.

Here, we have sequenced, assembled, annotated, and analysed the genome of the European Earwig, *Forficula auricularia*, and confirmed the subspecies identity of the individuals we

used. This genome will help researchers study multiple facets of this insect's exciting biology and evolutionary characters and broaden our understanding of insect and genome evolution.

Methods and Materials

Sample collection and preparation

Earwigs (*Forficula auricularia*) were collected from the Dunedin Botanic Garden (-45° 51' 27.59" S, 170° 31' 15.56" E) and reared in a temperature-controlled room (Temperature: cycling from 15 to 12°C, day/night; Photoperiod of L:D 16:8) in the Department of Zoology, University of Otago, Dunedin. Earwigs were snap-frozen in liquid nitrogen and stored at -80°C before dissection and subsequent nucleotide extraction. Earwigs were dissected in 1x PBS buffer under a dissection microscope to check for nematode parasites, and only non-parasitized individuals were used in this study. The head, wings and muscles from the thorax region were used for DNA extraction. Juvenile instars required for RNA extraction were obtained directly from the field.

DNA extractions

DNA was extracted using either the Nanobind Tissue Big DNA kit (Circulomics, USA) or DNeasy Blood & Tissue Kit (Qiagen, Germany) by following the manufacturer's protocol. Tissues from a single individual were used for each extraction. After the extraction, RNase treatment was performed using 4µl of RNase A (10mg/ml) per 200µl of DNA elute. DNA was quantified in a Qubit 2.0 Fluorometer (Life Technologies, USA) and quality analysed using Nanodrop. Low quality DNA samples were further cleaned with 1.8x by volume AMPure XP beads (Beckman Coulter, USA), wherever applicable, following the manufacturer's instructions and eluted in 55µl of molecular grade water. High-quality DNA samples were stored at -20°C and were used within a week of extraction.

Linked read library preparation and sequencing

Linked read library was prepared at the Genetic Analysis Services (GAS), University of Otago (Dunedin, New Zealand). DNA from an adult male was extracted using the Nanobind kit and size-selected for fragments over 40kbp using Blue Pippin (Sage Science, USA). A chromium 10X linked read (10x Genomics, USA) library was prepared following the

manufacturer's instructions. The library was sequenced on the Illumina Nova-seq platform to generate 2x151bp paired-end reads (Garvan Institute, Australia).

Long read library preparation and sequencing

Five long-read sequencing libraries for Oxford minion were prepared using a ligation sequencing kit (SQK-LSK109) (Oxford Nanopore Technologies, Oxford, UK) following the manufacturer's instructions. To increase the raw nanopore read N50 the first and the second libraries were prepared using 1.75 and 0.75µg of DNA extracted via a Circulomics kit from two adult male earwigs. Both libraries were sequenced in a single minion flow cell, flushing the flow cell to remove remains of the first library before loading the second library with a Flow cell wash kit (Oxford Nanopore Technologies, Oxford, UK).

To increase the total raw output the third and the fourth libraries were prepared with DNA from two adult female earwigs, both extracted with a Qiagen kit followed by the AMPure XP beads clean-up step. Input DNA for these two libraries were 2.6 and 3.2 µg. These were each sequenced on an individual minion flow cell. The fifth library was prepared using 3.0 µg of DNA from an adult male earwig. As before DNA was extracted using a Qiagen kit followed by AMPure XP beads clean-up. However, before library preparation, the DNA was sheared five times using a 26G X 0.5" needle (Terumo, Japan). All prepared libraries were sequenced with R9 chemistry MinION flow cell (FLO-MIN106) (Oxford Nanopore Technologies, UK).

RNA extraction and sequencing

Total RNA from the different developmental stages, sex, and tissues was extracted using a Direct-zol RNA MicroPrep kit (Zymo Research) with an on filter DNAse treatment following the manufacturer's instructions. Samples included: whole body (gut removed) of juvenile instars 1-2 and juvenile instars 3-4, dissected tissues (antennae, head, thorax, abdomen, legs, and gonads) of adult males and females. RNA from each individual and tissue type was extracted separately. RNA was quantified on a Qubit 2.0 Fluorometer (Life Technologies, USA) and initially quality checked using a nanodrop. Only high-quality extracts were further processed and were stored at -80°C until use.

RNA integrity was evaluated on a Fragment Analyzer (Advanced Analytical Technologies Inc., USA) at the Otago Genomics Facility (OGF), University of Otago, Dunedin, New Zealand. As with most of the insect RNA extracts (Winnebeck *et al.* 2010) RNA quality number (RQN) values ranged from 2.5 to 10 due to the collapsing of the 28S peak; quality was thus determined via the trace rather than RQN. Four pools of samples at equimolar

concentration underwent library preparation. Pools consisted of: 8 whole body extractions for juvenile instar 1-2, 8 whole body extractions for juvenile instar 3-4, individual body tissues from five adult males, and individual body tissues from five adult females. TruSeq stranded mRNA libraries were prepared and sequenced as 2x100bp paired-end reads across two lanes of HiSeq 2500 Rapid V2 flowcell at the OGF.

Genome size estimation

Flow cytometry and k-mer based approach with short-read data were used to estimate the genome size. Flow cytometry analysis was performed on a single head of earwig with two biological replicates at Flowjoanna (Palmerston North, NZ). Briefly, the earwig's head was dissociated with a pestle in 500µl of the stock solution containing 0.1% w/v trisodium citrate dihydrate, 0.1% v/v IGEPAL, 0.052% w/v spermine tetrahydrochloride, and 0.006% sigma 7-9 (all Sigma-Aldrich, USA). Rooster red blood cells (RRBC) derived from the domestic chicken (*Gallus gallus*), stored in citrate buffer, was used as a reference sample. Test samples were filtered through 35µl filter cap and further dissociated by adding 100µl of 0.21mg/ml trypsin followed by 75µl of 2.5mg/ml trypsin inhibitor (both Sigma-Aldrich) for 10 minutes at 37°C. Nuclei were stained using 100µl of pre-stain (containing 416mg/ml propidium iodide (PI) with 500mg/ml RNAse in-stock solution). Two sample tubes, one prepared with RRBC and one prepared without, were then processed on a FACSCalibur (BD Biosciences, USA). The instrument was equipped with a 488nm laser to produce fluorescence collected using the FL-2-Area signal (585/42 BP), along with forward scatter (FSC) and side scatter (SSC) signals that enabled RRBC nuclei to be resolved from earwig nuclei. Data were analysed using Flowjo (BD Biosciences, USA) and the pg/nuclei of the sample calculated.

For k-mer based genome size estimation, we used the paired-end linked read sequences. Reads were processed with the scaff_reads script from Scaff10x (v.5.0) to remove the 10x link adapters. Quality control was carried out with Trimmomatic (v.0.39) (options: SLIDINGWINDOW:4:15 LEADING:5 TRAILING:5 MINLEN:35). We used KMC (v.3.1.1) (Kokot *et al.* 2017) with a k-mer size of 21 to produce a histogram, which was then visualised in Genomescope (v.2.0) web browser.

Bioinformatic pipeline

All the scripts used for genome assembly, *denovo* repeat library construction, and annotation are available on GitHub (https://github.com/upendrabhattarai/Earwig_Genome_Project). The

bioinformatics software and packages were run in New Zealand eScience Infrastructure (NeSI). Below is a description of the pipeline (Figure 1).

Genome assembly

Paired-end Illumina reads from the chromium library were assembled using Supernova (v.2.1.1) (Weisenfeld *et al.* 2017). Assembly quality was assessed using Quast (Gurevich *et al.* 2013) and BUSCO (Simão *et al.* 2015) metrics such as N50 values, contig/scaffold number, contiguity and ortholog completeness. Based on several trial assemblies, we down-sampled the total input to 660 million paired-end reads to produce an assembly with better completeness and contiguity. The assembled fasta sequence was obtained with "pseudohap" style of the supernova "mkoutput" function.

Nanopore reads were basecalled using guppy (v.5.0.7) and assembled using Flye (v.2.7.1) (Kolmogorov *et al.* 2019) with default parameters. The assembly statistics (as mentioned above) for the Flye assembly were better than that for the Supernova assembly. Therefore, the supernova assembly was merged into the Flye assembly using Quickmerge (Chakraborty *et al.* 2016). The resulting assembly was processed with Purgehaplotigs (Roach *et al.* 2018) to remove redundant and duplicated contigs.

The purged genome underwent further scaffolding and gap-closing steps using Rails (v.1.5.1) and Cobbler (v.0.6.1) (Warren 2016), Lrscaf (v.1.1.11) (Qin *et al.* 2019) and Lrgapcloser (Xu *et al.* 2019) with the raw long-read data. The resulting assembly was scaffolded with Ragtag (v.2.1.0) (Alonge *et al.* 2021) using the Supernova assembly. The raw linked-read data were further used to scaffold the assembly with ArbitR (v.0.2) (Hiltunen *et al.* 2021), Arks (v.1.0.4) (Coombe *et al.* 2018) and Links (v.1.8.7) (Warren *et al.* 2015). mRNA-seq reads sequenced for genome annotation purposes, and total RNA-seq reads sequenced for another project (manuscript under preparation) were also used for scaffolding the assembly with the Rascaf (Song *et al.* 2016). Duplicated and redundant haplotigs were again removed using Purgehaplotigs, and discarded haplotigs were used for scaffolding the assembly using Ragtag. Blobtools2 (Laetsch and Blaxter 2017) was used to remove small (<1000bp) and low coverage contigs (<5x coverage). Contigs that were filtered out were used for re-scaffolding the assembly with Ragtag. Finally, we used Pilon (v.1.24) to polish the assembly using mRNA-seq data.

Repeat content analysis

To assist with annotation a custom repeat library was generated for the Earwig genome using different *de novo* repeat and homology-based identifiers, including LTRharvest (Ellinghaus *et al*. 2008), LTRdigest (Steinbiss *et al*. 2009), RepeatModeler (Flynn *et al*. 2020), TransposonPSI (Brian Haas 2010) and SINEBase (Vassetzky and Kramerov 2013). We concatenated the individual libraries, and sequences with more than 80% similarity were merged to remove redundancy using usearch (v.11.0.667) (Edgar 2010). It was then classified using RepeatClassifier. Sequences with unknown categories in the library were mapped against the UniProtKB/Swiss-Prot database (e-value <1e-01); if sequences were not annotated as repeat sequences they were removed from the library. The final repeat library was used in RepeatMasker (v.4.1.2) (Chen 2004) to generate a report for genome repeat content and provided to the Maker2 pipeline to mask the genome.

Genome annotation

Genome annotation was carried out with three iterations of the MAKER2 (v.2.31.9) (Holt and Yandell 2011) pipeline combining evidence-based and ab initio gene models. The first round of Maker used evidence-based models and the other two rounds were run using ab initio gene models. For the first round, we provided the MAKER2 pipeline with 180,119 mRNA transcripts *denovo* assembled via the Trinity pipeline (Grabherr *et al*. 2011) along with 26,414 mRNA and 1,529 protein sequences of dermapterans from NCBI and 779 dermapteran protein sequences from the Uniprot database.

Augustus was trained using Braker (v.2.16) (Hoff *et al.* 2019) and SNAP was trained after each round of MAKER to use for ab initio gene model prediction. For the functional annotation, we ran InterProScan (v.5.51-85.0) (Jones *et al.* 2014) for the predicted protein sequences obtained from MAKER and retrieved InterPro ID, PFAM domains, and Gene Ontology (GO) terms. Furthermore, we ran BLAST with the Uniprot database to assign gene descriptors to each transcript based on the best BLAST hit.

Phylogenetic analysis

Two sibling species of *F. Auricularia* have been described (Wirth *et al.* 1998). To assess which of these we sequenced, nucleotide sequences covering the COI and COII region from 34 isolates of *F. auricularia* were downloaded from NCBI. Those included 15 isolates reported by Wirth et al. (1998) originally used to infer sibling species A and B and other isolates from Belgian orchards submitted to NCBI. Nucleotide sequence covering COI and COII regions from the assembled genome was extracted through BLAST hits. The same

genomic region extracted from the mitochondrial genome of *Euborellia arcanum* was used as an outgroup. Nucleotide sequences were aligned using Clustal Omega (v1.2.3). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987) in the bootstrap test of 1000 replicates (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each nucleotide sequence pair (pairwise deletion option). There were a total of 799 positions in the final dataset. The optimal tree is shown (Figure 5) and the evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021).

Results and Discussion

Genome size estimates

The flow cytometer estimated the genome size of 968.22±20.747 Mb (Mean±SD) for the earwig genome. Similarly, the kmer based approach using adapter removed paired-end data from linked read sequencing estimated the male earwig to be 988 Mb. Whereas, an earlier estimation of an unknown dermapteran (earwig) species genome size was 1.4 Gb (Gregory 2005) showing a variable genome size within the order.

Genome assembly

A total of 799.6 million paired-end reads was generated for the linked-read library. Downsampled to 660 million paired-end reads, Supernova estimated the genome size of 1.22 Gb, raw coverage of 82.02%, effective coverage of 39.50% and weighted mean molecule size of 22.45 kb. The Supernova assembly was 1.15 Gb in size and had 145,055 contigs, with an N50 of 0.03 Mb and L50 of 7,500. Quast reported a complete BUSCO of 64.69% and a partial BUSCO of 9.24% from the eukaryotic database.

The nanopore sequencing yielded approximately 10.7 Gb of data, consisting of over 3 million reads. The median read length was 897 bp with an N50 length of 11,986 bp (Supplementary Table 1). The median read Phred quality was 13.34. Primary assembly from Flye produced an assembly of 1.1 Gb. There were 187,66 contigs with N50 of 0.18 Mb and L50 of 1,832. Quast reported a complete BUSCO of 82.18% and a partial BUSCO of 9.24%. The long-read assembly showed better contiguity and completeness, so we merged the linked-read assembly with the long-read assembly (Table 1).

The final hybrid assembly has a size of 1.06Gb. It has 919 scaffolds with an N50 of 12.55Mb, which shows that the assembly is highly contiguous. Half of the genome is present in just 20

scaffolds, as denoted by the L50 number (Table 1). Assembly has 846.85 “N’s” per 100kbp. The BUSCO score from the insect database (n=1,367) for the assembly was 87.1% complete, among which 4.1% were duplicated, and 3.1% fragmented BUSCO (Figure 2). Improvement in assembly statistics after each processing step is given in Supplementary Table 2.

The only other whole-genome sequence publicly available from the Dermaptera order is of the other earwig *Anisolabis maritima*. Using the insect database (n=1,367) it has 83.4% complete and 10.8% fragmented BUSCO scores. The *A. maritima* assembly has an N50 of 1.4Mb with a genome size of 649.7 Mb (Mei *et al.* 2022). So, in comparison, the *F. auricularia* genome assembly has a better gene model and contiguity from the Dermaptera order.

Genome repeat contents

Repeat analysis of the assembly showed that interspersed repeats comprised ~686 Mb (64.62%) of the *F. auricularia* genome. This includes ~248 Mb of retroelements (23.37% of the genome), ~178 Mb of DNA transposons (16.79% of the genome), ~35 Mb of rolling-circles (3.28% of the genome) and ~260 Mb of unclassified elements (Table 2). Unusually large and variable genome sizes characterise Hemimetabolans (Wu *et al.* 2017). Comparative analysis in six species of Gomphocerine grasshoppers showed a strong positive correlation between repeat content and genome size. Genome size ranged from 8.2 to 13.7 Gb in these six species with a repeat content ranging routinely from 79% to 87%, with the exception of *Stauroderus scalaris* whose genome is 96% repetitive DNA and the second-largest insect genome documented. Our estimation of genome size for *F. auricularia* doesn’t show gigantism (~968.22Mb, flow cytometer estimate). However, its repeatome (64.62%), is almost twice that of other hemimetabolous insects like *Gryllus bimaculatus* (33.69%) and *Laupala kohalensis* (35.51%) (Ylla *et al.* 2021). This fold increase in the repeatome is surprising given both *G. bimaculatus* and *L. kohalensis* have bigger genomes (~1.6 Gb) than *F. auricularia*.

Genome annotation

Combining evidence-based and *ab initio* gene models in the MAKER2 pipeline, we identified 12,876 genes and 21,031 mRNAs in the genome assembly. The mean gene length is 12,096 bp and the total gene length is 155.75 Mb, which makes 14.7% of the whole assembly. The longest gene annotated is 412,198 bp and the longest CDS is 19,035 bp (Table 3). 61.35% of total predicted mRNAs and 59.53% of predicted proteins are also functionally annotated through either one or more of InterPro, Gene ontology and Pfam databases (Supplementary

Table 3). Comparing with the Arthropoda database we got 74.4% complete BUSCO score for annotated Transcriptome and 70.9% complete BUSCO score for annotated Proteins (Figure 3). 98.3% of the gene models have AED score of 0.5 or less, assuring highly confident gene prediction (Supplementary Figure 1).

The GC content of the *F. auricularia* genome is 31.03%, markedly greater than the 19.3% GC observed in the genome of the earwig *A. maritima* (Mei *et al.* 2022). A comparison of GC content for different genomic regions of *F. auricularia* showed that exons have higher GC content (0.372±0.087) (Mean±SD) and introns have lower (0.267±0.075) when compared between intergenic regions (N = 823,037), genes (N = 12,876), exons (N = 145,003), introns (N = 123,973), and non-overlapping 10kb windows throughout the genome (N = 106,686) (Figure 4). GC content for 10kb windows was 0.308±0.032 which resembles the mean GC content of the whole genome (0.310). This finding is not unexpected as a higher GC content in exons versus introns is common across the animal and plant kingdom because of the evolutionary selection of exon regions (Amit *et al.* 2012). There was a significant different for each pairwise comparison using ANOVA followed by Tukey HSD with $P < 0.0001$.

In conclusion, the assembled and annotated *F. auricularia* genome presented here is a key resource to develop this important insect species as a genetic model. We anticipate this will enhance the genetic study on various aspects of its biology, including developmental biology, sociality, and evolutionary characteristics.

Phylogenetic analysis

The phylogenetic analysis showed two distinct subspecies groups within the *F. auricularia* (Figure 5). The two subspecies A and B inferred in our analysis is in complete agreement with the analysis by Wirth et al. (1998). One clade includes 24 individuals including 9 originally identified by Wirth and colleagues as Species A (green circle labels, Figure 5). While the remaining 11 individuals cluster into a separate clade that includes our isolate (red square label, Figure 5) and 6 individuals originally identified by Wirth and colleagues as Species B (green square labels, Figure 5. The analysis confirmed that the isolate sequenced and reported in this paper (Dunedin, NZ) belongs to the Species B of *F. auricularia* species. This is also in accordance with the report from Quarrel et al. (2018). The phylogenetic analysis carried by Quarrel and colleagues reported all Australasian (including two isolates from the New Zealand population) earwig populations as species B lineage.

Data Availability Statement

The genome assembly and annotation of *Forficula auricularia* are available through FigShare https://doi.org/10.6084/m9.figshare.19092044. The raw sequencing reads are deposited in NCBI with accession number PRJNA800435.

Code availability

The scripts used for genome assembly, repeat library preparation and masking, and genome annotation, are available at GitHub under GNU GPLv3 license. (https://github.com/upendrabhattarai/Earwig_genome_project)

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Conflict of Interest

The authors declare no competing interests.

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Figure legends

Figure 1: Schematic representation of the assembly pipeline for the *Forficula auricularia* genome. The black arrow represents the workflow and the red dotted lines represent the additional input data in the pipeline (Created with BioRender.com).

Figure 2. The BUSCO (v.5) report for the final hybrid assembly of the *Forficula auricularia* genome. BUSCO scores in percentage (x-axis) from Arthropoda, Eukaryota, and Insecta (odt10) databases (y-axis) are shown in the bar plot. The light blue portion of the bar represents Complete and single-copy orthologs, dark blue represents complete and duplicated orthologs, yellow represents fragmented BUSCO genes and red represents missing BUSCO genes.

Figure 3. Annotation completeness through BUSCO database. The plot shows the BUSCO (v.5) percentage (x-axis) for the annotated Proteins and Transcriptomes using Arthropoda_odb10 and Insecta_odb10 database as indicated in the y-axis.

Figure 4. GC percentage in different genomic regions in *F. auricularia* genome. GC content for 10kb windows was generated without regard to any genomic features. Whiskers extend to 25th and 75th percentiles. GC content in exons is higher and in introns is lower compared to the genome average.

Figure 5. The phylogenetic relationships of different isolates of *Forficula auricularia* inferred by Neighbour-Joining method and Maximum Composite Likelihood approaches using MEGA11. All ambiguous positions were removed for each nucleotide sequence pair (pairwise deletion). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Isolates labelled with the coloured squares are Species B. The red square (Dunedin NZ) is the isolate for which the genome is reported in this paper. Green squares are the species categorized as Species B by Wirth et al. (1998) and the purple squares are other isolates for which the nucleotide sequences were downloaded from NCBI. Similarly isolates labelled with coloured circles belong to Species A. Green circles represent isolates inferred as Species A by Wirth et al. (1998) and blue are other isolates for which

nucleotide sequences were downloaded from NCBI. *Euborellia arcanum* is the outgroup labelled with a bold triangle.

Table 1. Assembly statistics for the genome assembly of the European earwig *Forficula auricularia.* Quast scores are to its default Eukaryota database.

	Assembly length	No. of scaffolds	N50	L50	Ns per 100 kbp	BUSCO % (Quast)	
						Complete	Partial
Supernova assembly	1,145,470,221	145,055	30,358	7,500	3,677.89	64.69	9.24
Flye assembly	1,118,374,848	18,766	180,737	1,832	0.35	82.18	9.24
Final hybrid assembly	1,062,210,345	919	12,548,649	20	846.85	87.13	2.97

Table 2. Repeat content analysis in the European earwig *Forficula auricularia* genome.

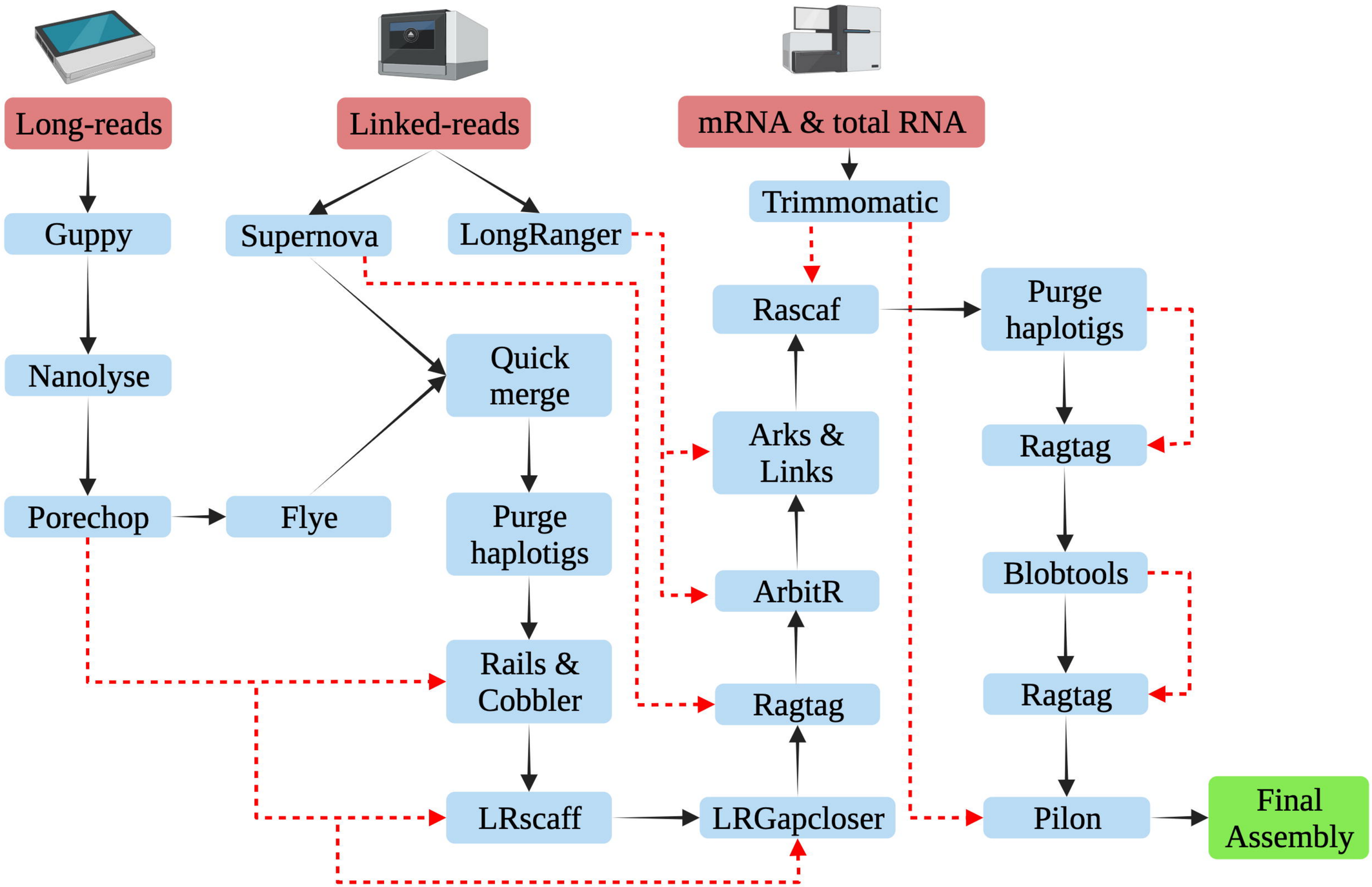
No. of sequences:				919
Total length (bp):				1062210345
GC level:				31.03%
Bases masked:				722769501 bp (68.04 %)
		Numbers	**Length (bp)**	**Percentage**
Retroelements		**1385007**	**248236495**	**23.37%**
	SINEs:	41157	5138497	0.48%
	Penelope	50409	10372837	0.98%
	LINEs:	660178	124985146	11.77%
	CRE/SLACS	0	0	0.00%
	L2/CR1/Rex	112418	20654321	1.94%
	R1/LOA/Jockey	167317	22277052	2.10%
	R2/R4/NeSL	23348	4271189	0.40%
	RTE/Bov-B	136406	28799096	2.71%
	L1/CIN4	10079	1892539	0.18%
	LTR elements:	683672	118112852	11.12%
	BEL/Pao	60561	12114300	1.14%
	Ty1/Copia	97132	14352992	1.35%
	Gypsy/DIRS1	521467	91083363	8.57%
	Retroviral	3701	443583	0.04%
DNA transposons		**1040870**	**178326460**	**16.79%**

	hobo-Activator	362395	59188939	5.57%
	Tc1-IS630-Pogo	355781	66331225	6.24%
	En-Spm	0	0	0.00%
	MuDR-IS905	0	0	0.00%
	PiggyBac	21153	2726812	0.26%
	Tourist/Harbinger	5541	1187174	0.11%
	Other (Mirage,	10240	1580945	0.15%
	P-element, Transib)			
Rolling-circles		**174964**	**34830487**	**3.28%**
Unclassified:		**1563937**	**259874747**	**24.47%**
Total interspersed repeats:			**686437702**	**64.62%**
Small RNA:		**9913**	**1406877**	**0.13%**
Satellites:		**1110**	**495561**	**0.05%**
Simple repeats:		**0**	**0**	**0.00%**
Low complexity:		**0**	**0**	**0.00%**

Table 3. Genome annotation summary for the European earwig *Forficula auricularia*

Total sequence length	1,062,210,345
Number of genes	12,876
Number of mRNAs	21,031
Number of exons	145,003
Number of introns	123,973
Number of CDS	21,030
Total gene length	155,753,058
Total mRNA length	271,884,000
Total exon length	32,584,454
Total intron length	239,538,939
Total CDS length	23,936,568

Shortest gene	3
Shortest mRNA	3
Shortest exon	1
Shortest intron	4
Shortest CDS	3
Longest gene	412,198
Longest mRNA	412,198
Longest exon	10,240
Longest intron	319,382
Longest CDS	19,035
mean gene length	12,096
mean mRNA length	12,928
mean exon length	225
mean intron length	1,932
mean CDS length	1,138
% of genome covered by genes	14.7
% of genome covered by CDS	2.3
mean mRNAs per gene	2
mean exons per mRNA	7
mean introns per mRNA	6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Linked-reads
mRNA & total RNA
Guppy
Supernova
LongRanger
Trimmomatic
Rascaf
Purge haplotigs
Nanolyse
Quick merge
Arks & Links
Ragtag
Porechop
Flye
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ArbitR
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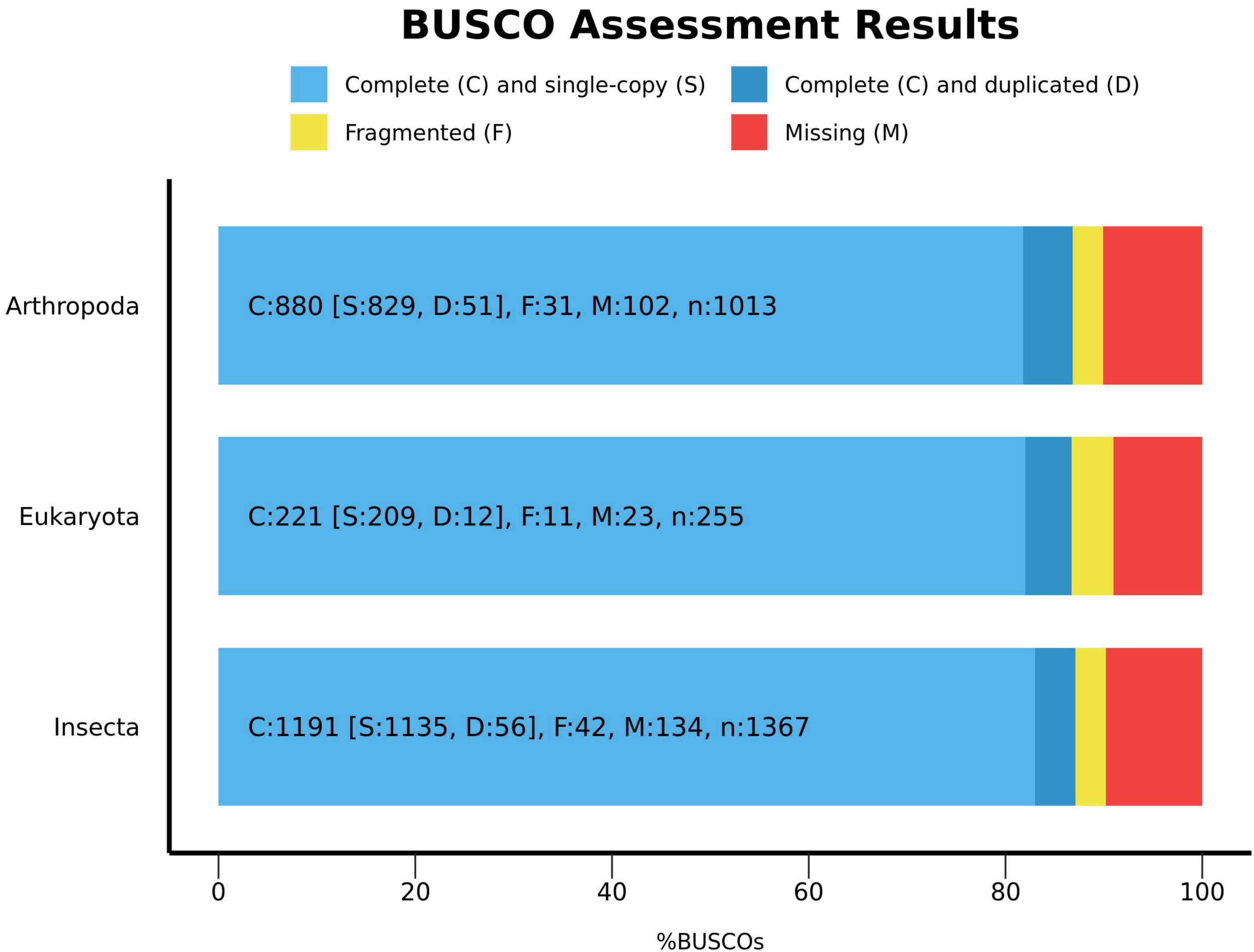
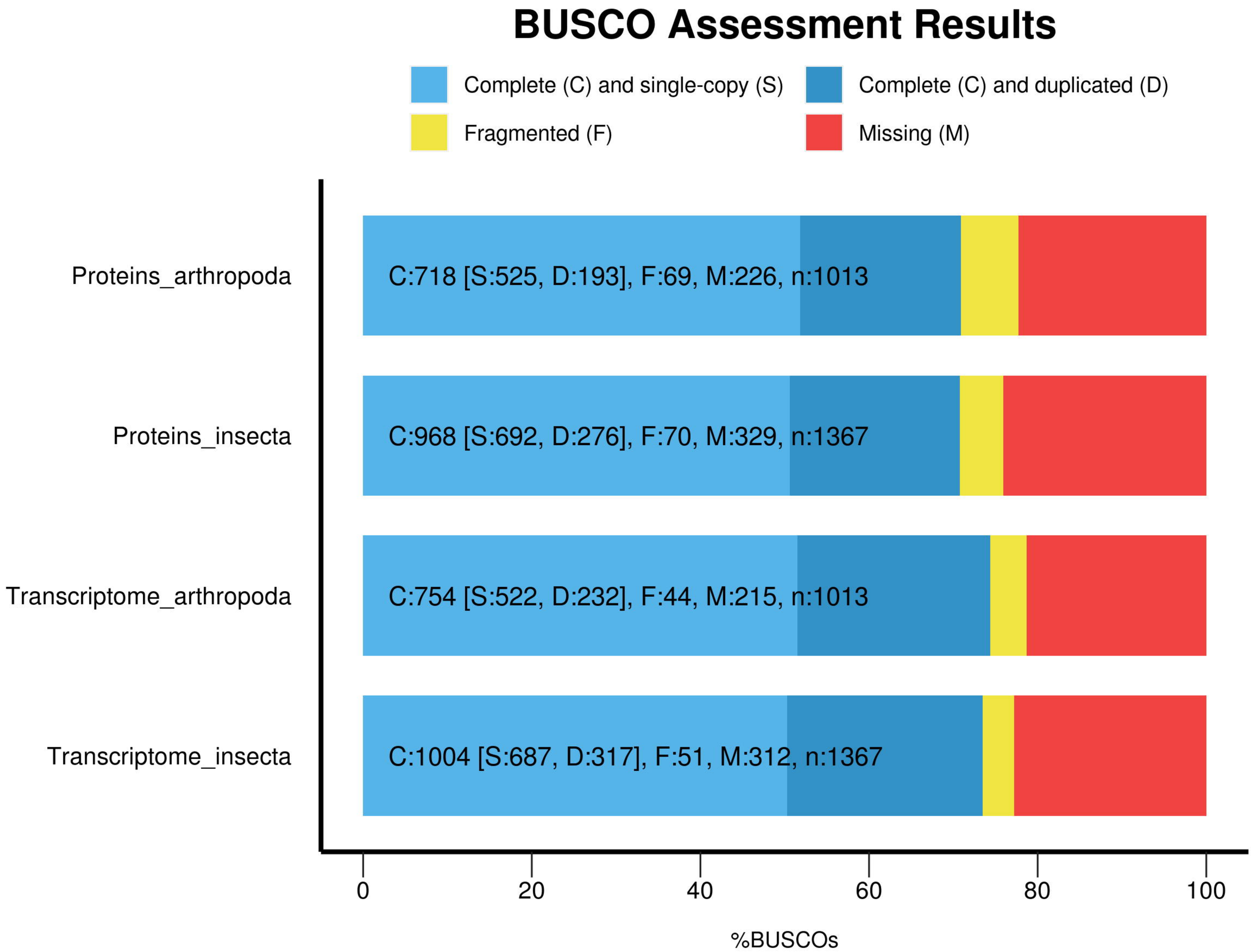
Ragtag
Ragtag
LRscaff
LRGapcloser
Pilon
Final Assembly


BUSCO Assessment Results
Complete (C) and single-copy (S)
Complete (C) and duplicated (D)
Fragmented (F)
Missing (M)
Arthropoda
C:880 [S:829, D:51], F:31, M:102, n:1013
Eukaryota
C:221 [S:209, D:12], F:11, M:23, n:255
Insecta
C:1191 [S:1135, D:56], F:42, M:134, n:1367
0
20
40
60
80
100
%BUSCOs


BUSCO Assessment Results
Complete (C) and single-copy (S)
Complete (C) and duplicated (D)
Fragmented (F)
Missing (M)
Proteins_arthropoda
C:718 [S:525, D:193], F:69, M:226, n:1013
Proteins_insecta
C:968 [S:692, D:276], F:70, M:329, n:1367
Transcriptome_arthropoda
C:754 [S:522, D:232], F:44, M:215, n:1013
Transcriptome_insecta
C:1004 [S:687, D:317], F:51, M:312, n:1367
0
20
40
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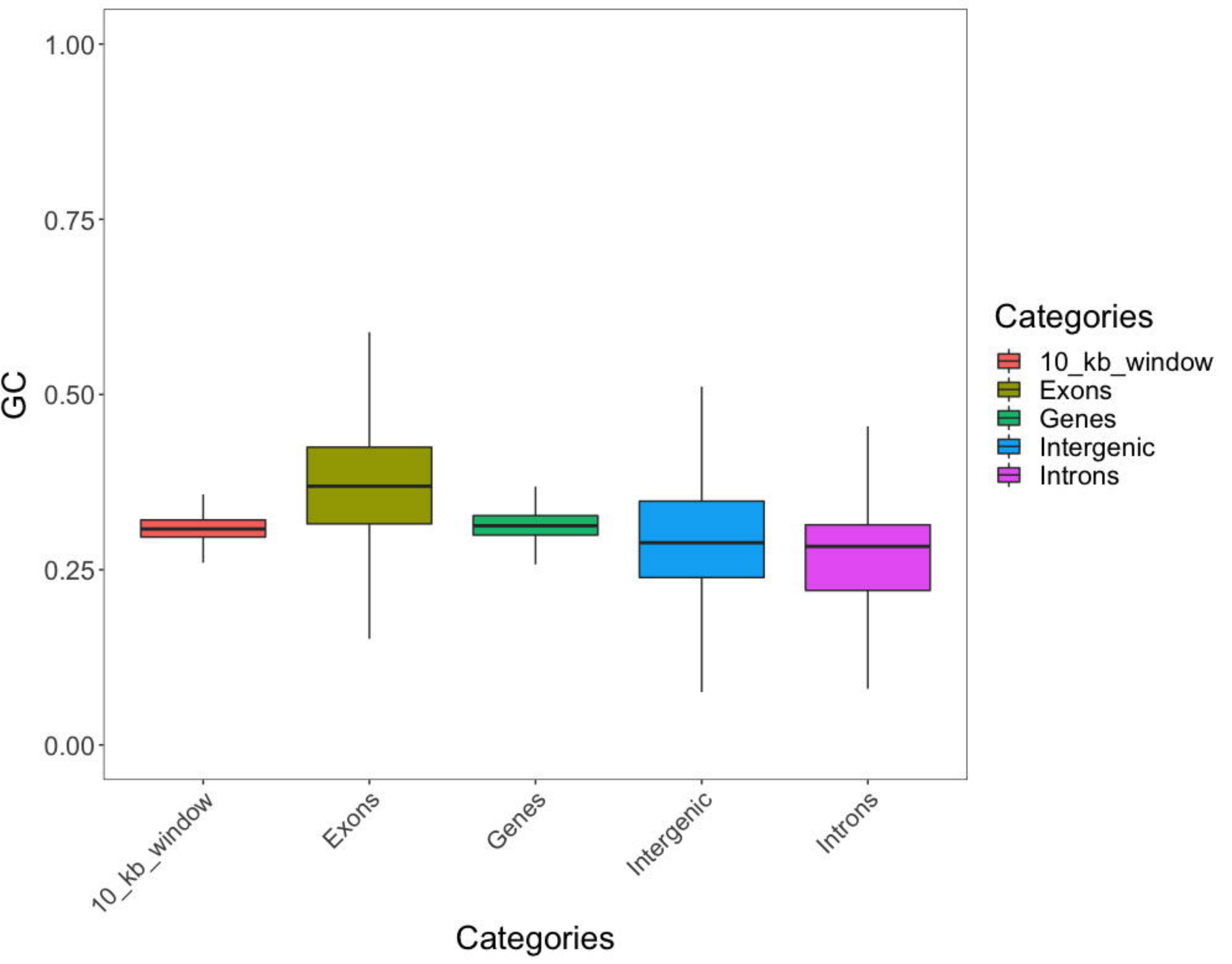
80
100
%BUSCOs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Categories
Categories
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Exons
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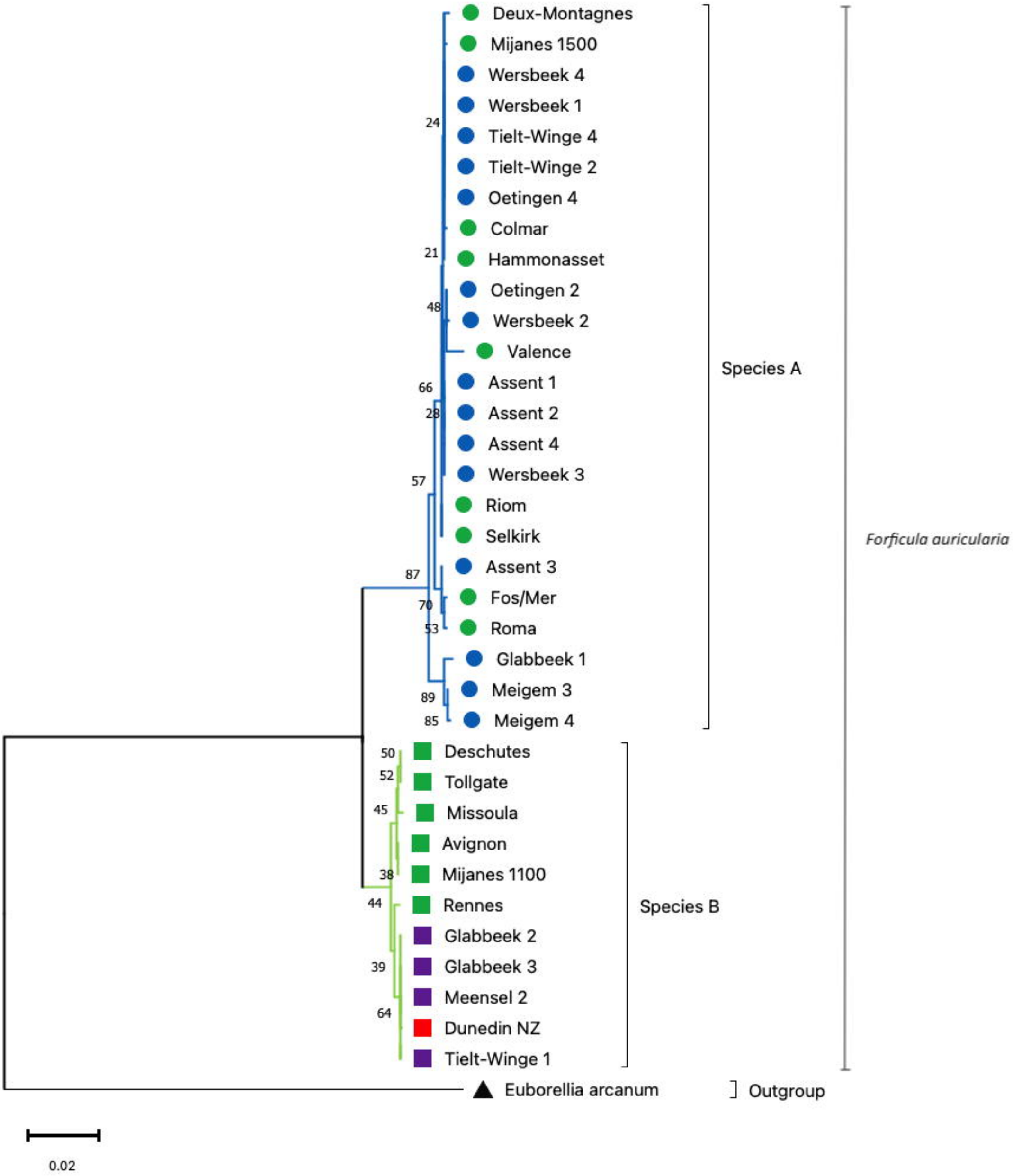
Intergenic
Introns


Deux-Montagnes
Mijanes 1500
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Wersbeek 1
Tielt-Winge 4
Tielt-Winge 2
Oetingen 4
Colmar
Hammonasset
Oetingen 2
Wersbeek 2
Valence
Assent 1
Assent 2
Assent 4
Wersbeek 3
Riom
Selkirk
Assent 3
Fos/Mer
Roma
Glabbeek 1
Meigem 3
Meigem 4
Deschutes
Tollgate
Missoula
Avignon
Mijanes 1100
Rennes
Glabbeek 2
Glabbeek 3
Meensel 2
Dunedin NZ
Tielt-Winge 1
Euborellia arcanum
24
21
48
66
28
57
87
70
53
89
85
50
52
45
38
44
39
64
Species A
Species B
Forficula auricularia
Outgroup
0.02