

***Coenonympha glycerion* genome annotation report**

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Methods

Genome annotation

Repeats present in the genome assembly were annotated with RepeatMasker v4-1-2 (<http://www.repeatmasker.org>) using the custom repeat library available for *insecta*. Moreover, a new repeat library specific for our assembly was made with RepeatModeler v1.0.11. After excluding those repeats that were part of repetitive protein families (performing a BLAST¹ search against Uniprot) from the resulting library, RepeatMasker was run again with this new library in order to annotate the specific repeats.

The gene annotation of the *species* genome assembly was obtained by combining transcript alignments, protein alignments and *ab initio* gene predictions. A flowchart of the annotation process is shown in **Figure ANN1**.

RNA from two different tissues (20220719_Coenchympha_Wing_CBP21 and 20220719_Coenchympha_Leg_CBP21) was obtained and sequenced with ONT direct cDNAseq. The long reads were aligned to the genome using MINIMAP2² v2.14 with the splice option. Transcript models were subsequently generated using Stringtie. Transcript models were subsequently generated using Stringtie³ v2.2.1 and merged using TACO⁴ v0.7.3. High-quality junctions to be used during the annotation process were obtained by running ESPRESSO⁵ v1.3.0 after mapping with MINIMAP2. Finally, PASA assemblies were produced with PASA⁶ v2.5.2. The *TransDecoder* program, which is part of the PASA package, was run on the PASA assemblies to detect coding regions in the transcripts. Secondly, the proteomes of the Kamehameha, Danaus and “Squinting bush” butterflies and the “swissprot invertebrates” proteins were downloaded from Uniprot in February 2023. Furthermore, we also downloaded the RefSeq proteomes of all butterflies of the superfamily Papilionoidea from the NCBI available in February 2023. These five protein data sets were combined and aligned to the genome using Miniprot⁷ 0.6. *Ab initio* gene predictions were performed on the repeat-masked assembly with three different programs: GeneID⁸ v1.4, Augustus⁹ v3.5.0 and Genemark-ET¹⁰ v4.71 with and without incorporating evidence from the RNAseq data. The gene predictors were run with trained parameters for the honey bee, except Genemark, which runs in a self-trained mode. Finally, all the data were combined into consensus CDS models using EvidenceModeler-1.1.1 (EVM)⁶. Additionally, UTRs and alternative splicing forms were annotated via two rounds of PASA annotation updates. Functional annotation was performed on the annotated proteins with Pannzer’s¹¹ online server.

Results

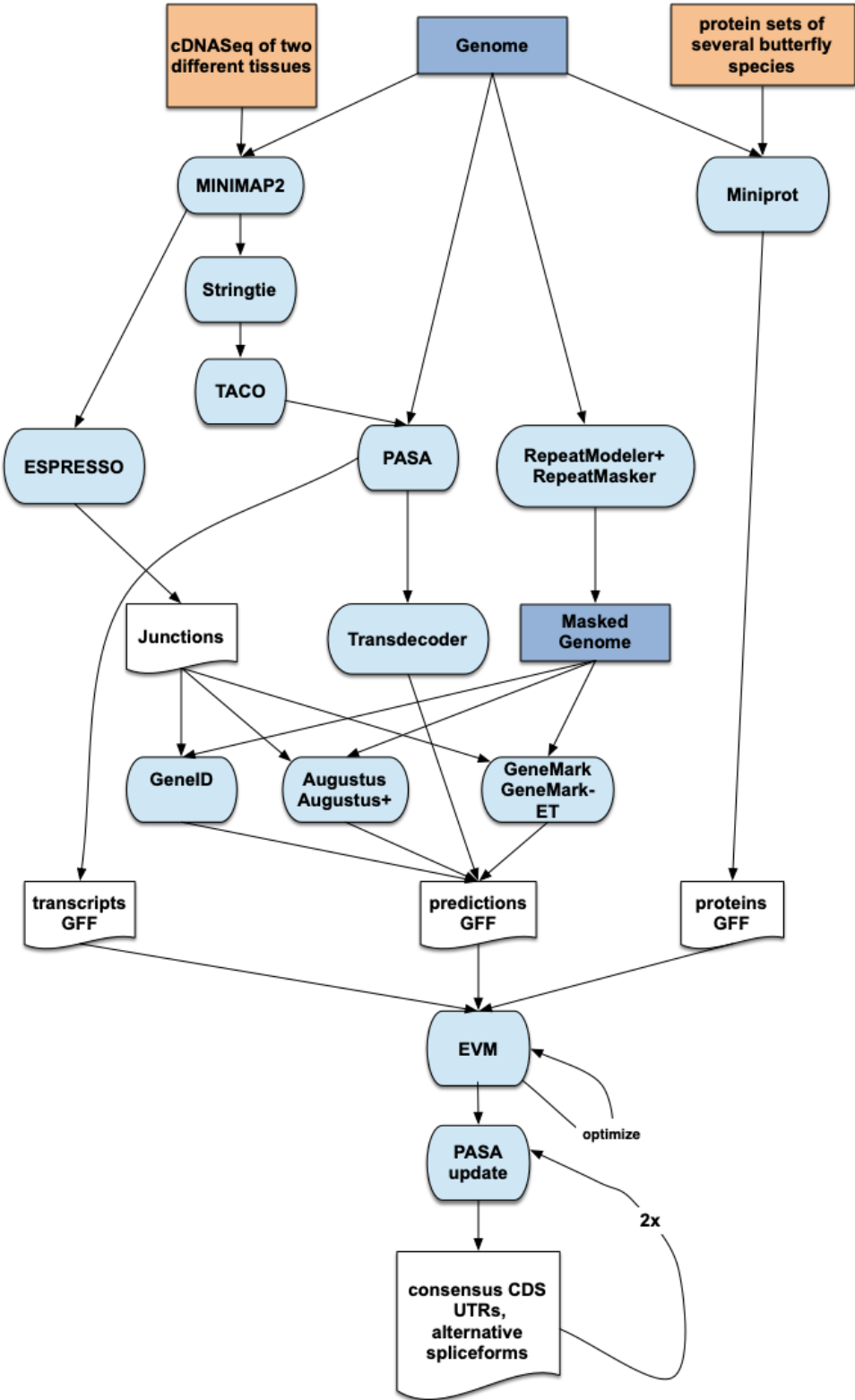
Genome annotation

In total, we annotated 20,312 protein-coding genes that produce 22,015 transcripts (1.08 transcripts per gene) and encode for 21,766 unique protein products. We were able to assign functional labels to 59.2% of the annotated proteins. The annotated transcripts contain 6.08 exons on average, with 78% of them being multi-exonic (**Table ANN1**).

Table ANN1: Genome annotation statistics

	COGLY1B annotation
Number of protein-coding genes	20,312
Median gene length (bp)	3,910
Number of transcripts	22,015
Number of exons	120,434
Number of coding exons	116,925
Median UTR length (bp)	620
Median intron length (bp)	626
Exons/transcript	6.07703838292074
Transcripts/gene	1.08384206380465
Multi-exonic transcripts	0.784465137406314
Gene density (gene/Mb)	44.3763442048429

Figure ANN1: workflow of the genome annotation process



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