**Supplementary file 3. Folder structure of the TrAnnoScope pipeline**

This supplementary file provides an overview of the directory structure generated by the TrAnnoScope pipeline, detailing the outputs from each stage of the workflow.

├── illumina/

(B) Quality Control

│ ├── qc/

│   │   ├── rawFastQC/

│   │ └── rawMultiQC/

│ ├── filtered\_fastqscreen/

│   ├── trimmed\_fastp/

│   │   ├── brain2\_1.trimmed.fq.gz

│   │   ├── brain2\_2.trimmed.fq.gz

│   │   ├── brain2.html

│   │   ├── brain2.json

│   │   ├── brain5\_1.trimmed.fq.gz

(E) Illumina Preprocessing

│   │   ├── brain5\_2.trimmed.fq.gz

│   │   ├── brain5.html

│   │   ├── brain5.json

│   │   ├── ovary2\_1.trimmed.fq.gz

│   │   ├── ovary2\_2.trimmed.fq.gz

│   │   ├── ovary2.html

│   │   ├── ovary2.json

│   │   ├── testis5\_1.trimmed.fq.gz

│   │   ├── testis5\_2.trimmed.fq.gz

│   │   ├── testis5.html

│   │   └── testis5.json

│   ├── trimmedFastQC/

│   └── trimmedMultiQC/

├── pacbio/

(D) PacBio Preprocessing

│   ├── ccs/

│   ├── fl/

│   ├── flnc/

│   ├── clustered/

│   ├── remove\_contaminants/

│   │ ├── blastn/

│   │ ├── blobtools/

(F) Post-processing

│   │ ├── busco\_clustered/

│   │ ├── clean\_reads/

│   │ └── coverage/

│   ├── error\_correction/

│   ├── classification/

│   │   ├── cdhit/

│   │   └── evigene/

├── quality\_assessment/

│   ├── busco/

(D) Quality Assessment

│   │   └── vertebrata\_odb10\_transcriptome/

│   ├── fl\_representation/

│   │   ├── against\_zebrafinch/

│   │   ├── transcriptome\_uniprot\_blastp.outfmt6

│   │   ├── transcriptome\_uniprot\_blastp.outfmt6.coverage.hist

│   │   ├── transcriptome\_uniprot\_blastx.outfmt6

│   │   └── transcriptome\_uniprot\_blastx.outfmt6.coverage.hist

│   └── stats/

│   ├── protein\_completeness.csv

│   ├── protein\_completeness\_plot\_detailed.png

│   ├── protein\_completeness\_plot.png

│   ├── transcriptome\_nt\_NanoPlot-report.html

│   ├── transcriptome\_nt\_NanoStats.txt

│   ├── transcriptome\_nt\_Non\_weightedHistogramReadlength.{html/png}

│   ├── transcriptome\_nt\_Non\_weightedLogTransformed\_HistogramReadlength.{html/png}

│   ├── transcriptome\_nt\_WeightedHistogramReadlength.html

│   ├── transcriptome\_nt\_WeightedHistogramReadlength.png

│   ├── transcriptome\_nt\_WeightedLogTransformed\_HistogramReadlength.{html/png}

│   ├── transcriptome\_nt\_Yield\_By\_Length.{html/png}

│   ├── transcriptome\_prot\_dist\_plot.png

│   └── transcriptome\_prot\_summary.txt

└── transcriptome/

│ ├── gene\_trans\_map.txt

(G) Annotation

│ ├── split/

│ ├── transcriptome\_nt.fasta

│ ├── transcriptome\_prot.fasta

│ └── transcriptome\_prot\_modified.fasta

├── annotation/

│   ├── eggnog\_mapper/

│   ├── infernal/

│   ├── nr\_blastp/

│   ├── nr\_blastx/

│   ├── nt\_blastn/

│   ├── pfam/

│   ├── plots/

│   │   ├── zebrafinch\_full\_ALL\_GOSlims.pdf

│   │   ├── zebrafinch\_full\_Biological\_Process\_GOSlims.pdf

│   │   ├── zebrafinch\_full\_Cellular\_Component\_GOSlims.pdf

│   │   ├── zebrafinch\_full\_COG\_dist.pdf

│   │   ├── zebrafinch\_full\_KEGG\_dist.pdf

│   │   ├── zebrafinch\_full\_KEGG\_slim\_dist.pdf

│   │   ├── zebrafinch\_full\_Molecular\_Function\_GOSlims.pdf

│   │ ├── zebrafinch\_full\_top10\_species\_dist\_NR\_blastx.pdf

│   ├── signalp6/

│   ├── swissprot\_blastp/

│   ├── swissprot\_blastx/

│   ├── tmhmm2/

│   ├── zebrafinch\_full\_trinotate\_report\_trans.goslims

│   └── zebrafinch\_full\_trinotate\_report.xls

│

**Analysis Details and Runtime**

The transcriptome analysis was executed on a SLURM-based high-performance computing cluster. Each step of the Snakemake pipeline was submitted as an independent SLURM job, running in parallel to optimize performance and reduce runtime.

**Total runtime**: The entire pipeline took approximately 8 hours to complete, with various jobs running in parallel.

**SLURM job submission**:

Each Snakemake rule was executed as a separate SLURM job.

**Number of jobs**: 517

**Resources per job**: Resources were defined in the config.yaml file.

**SLURM job parameters**:

**CPUs per job**: defined in the config.yaml file.

**Memory per job**: defined in the config.yaml file.