Deep, Staged Transcriptomic Resources for the Novel Coleopteran Models *Atrachya menetriesi* and *Callosobruchus maculatus*

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Abstract:

Background: Despite recent efforts to sample broadly across metazoan and insect diversity, current sequence resources in the Coleoptera do not adequately describe the diversity of the clade. Here we present deep transcriptomic data generated with Illumina platform sequencing of a number of key stages in the development of two coleopteran species, the false melon beetle *Atrachya menetriesi* (Faldermann 1835) and the cowpea weevil *Callosobruchus maculatus* (Fabricius 1775)*.* These species are both agricultural pests and are of great interest to developmental biology, as well as providing additional coleopteran datapoints for comparative genomic analysis.

Results: By sampling at a range of timepoints covering ovary development and several crucial phases of embryonic development we assembled five and three timed transcriptomes for *A. menetriesi* and *C. maculatus,* respectively. We utilized this sequence data to build a transcriptomic resource combining all reads into a single, combined assembly for each species, and we analysed each of these resources in detail. The combined *A. menetriesi* assembly consists of 228,096 contigs with an N50 of 1,598 bp, while the combined *C. maculatus* assembly consists of 128,837 contigs with an N50 of 2,263 bp. For each of these assemblies, we identified 78,879 (34.6%) and 41,744 (32.4%) of contigs by BLASTx against the nr database using Blast2GO, and we found that 97% and 98.3% of the BUSCO set of metazoan orthologs were present in these assemblies. We also carried out manual analysis of 38 key developmental genes, and found that nearly all expected genes were present in our transcriptomes. Each of these analyses showed that these transcriptomes are of very high quality. Lastly, we performed read mapping using the individual timed RNA samples, allowing us to identify up- and down-regulated contigs at key stages in the development of these beetles for further analysis.

Conclusions: The data presented here represent a significant increase in the publicly available transcriptomic resources in the Coleoptera. They have also allowed us to note significant changes in expression at important embryonic stages, and will provide a firm basis for a variety of experimentation, both in developmental biology and in wider exploration of the genomic basis of growth and differentiation.

Key Words: Beetle, Coleopteran, Transcriptome, Ovary, Embryonic, Staged, *Atrachya menetriesi*, *Callosobruchus maculatus,* Chrysomelidae

Background:

The order Coleoptera is the most speciose clade of animals currently known. Despite the best efforts of generations of biologists, its species are only sparsely sampled and are yet to be comprehensively described, with approximately 90% of coleopteran diversity as yet uncategorized (e.g. [1, 2]). A similar discrepancy exists at the molecular level; while several genomic resources are available in this clade, their number and phylogenetic distribution is only just beginning to accurately sample that of the Coleoptera as a whole. A wide range of transcriptomic data is available in whole organisms (for example [3–5], among others), specific body parts (such as [6, 7]), and in several cases for staged embryos following RNA interference-mediated gene knock-down [8, 9]. The i5K project [10] will also greatly advance our knowledge of the genomic complement of Coleopterans, with 69 species of this Order listed on that database as nominated for genomic sequencing as of the 14/09/15 (url: http://arthropodgenomes.org/wiki/i5K_nominations) and at least one genome publically available

[11]. However, the majority of this information is largely still outside of the public domain, the Coleoptera are still relatively undersampled compared to the Diptera and Hymenoptera, and, in particular, timed embryonic resources (e.g. [12]) are rare in the literature.

The true phylogeny of the Coleoptera is still under investigation but, in general, four suborders, 17 superfamilies, and 168 families are recognised [13]. The structure of the coleopteran clade can be seen summarised in Fig 1A. Coleopterans have long been used for research into embryology, and in the pre-molecular era, the Chrysomelidae (summarised phylogeny shown in Fig 1B) was one of the best studied superfamilies. For example, the first functional embryonic experiments in any insect were carried out in the Colorado potato beetle, *Leptinotarsa decemlineata* (sub-family Chrysomelinae, see Fig 1B), leading to the discovery of the function of pole cells and the existence of germ plasm in insects [14, 15]. Further, the larval cuticle preparation technique, so vital for arthropod developmental biology, was first perfected in the bean beetle, *Callosobruchus maculatus* [16]. Chrysomelid beetles are also interesting from an ecological and economic viewpoint, as members of the group are usually pest species, perhaps most famously the aforementioned Colorado potato beetle, which is a major pest of potato crops in America, Asia and Europe.

The Chrysomelidae are represented in public databases by a number of ongoing transcriptomic and genomic projects. In particular, both *L. decemlineata* (Bioproject PRJNA171749) and *C. maculatus* [17] are the subject of ongoing genomic sequencing. However, while a number of transcriptomes are planned or published in this clade (e.g. [5, 6, 18–22]) none yet sample across embryonic time points in a fashion allowing insight into the genetic mechanisms behind key developmental stages and deep sampling of developmentally important genes. Here we present transcriptomic sequences for two species of chrysomelid beetle, the false melon beetle, *Atrachya menetriesi,* and the aforementioned *C. maculatus* (each of which are pictured along with their relative phylogenetic positions in Fig 1).

Compared to *C. maculatus,* the false melon beetle *A. menetriesi* (Faldermann), is a relatively unknown and understudied species. It is native to Japan, where it is an agricultural pest, and feeds on a variety of plants such as clover and lettuce. Although there has only been a small amount of research carried out on this beetle, the work that has been done has highlighted several interesting developmental traits, including the possibility of generating twin embryos after egg bisection [23], or up to four, seemingly complete, embryos following treatment with low temperatures [23, 24]. Another interesting trait exhibited by this beetle is that almost all eggs enter diapause at a certain stage, and this is only broken in the wild by winter conditions. However, a small proportion of eggs skip this diapause and continue to adulthood. The ratio of diapause to non-diapause eggs varies in different parts of Japan [25], and is a heritable trait [26]. Further, *A. menetriesi* embryos undergo a very short germ band mode of development [27], contrasting strongly with beetles such as *C. maculatus* (see below). As the last common ancestor of these two species is estimated to have existed only 80 million years ago [28], how their developmental mechanisms have diverged so greatly is a potentially fascinating area for future study. Research on these topics would greatly benefit from modern molecular studies.

C. maculatus (Fabricius) is native to West Africa [29] but is now found worldwide, and is a common pest of stored legumes. It is also known as the southern cowpea weevil, however, it is not a true weevil. As noted above, this beetle was the focus of active developmental research in the pre-molecular era, with special focus on segmentation [30, 31]. Segmentation in *C. maculatus* has also been studied more recently via immunohistochemistry for the even-skipped protein [32]. This recent work confirmed previous reports that the embryos undergo the long germ mode of development, similar to dipterans like *Drosophila melanogaster* and hymenopterans like *Apis mellifera* [33]. It is commonly believed that the long germ mode of development evolved independently in dipterans and hymenopterans, and given the phylogenetic distribution of short and intermediate germ development within the Coleoptera [34, 35], it seems likely that the long germ mode of development also evolved independently in the clade to which *C. maculatus* belongs. A comparison of the molecular basis of development in *C. maculatus* and other long germ insects, plus with more closely related species that feature short germ development, such as well studied flour beetle, *Tribolium castaneum* (super-family Tenebrionoidea, see Fig.1 A) and *A. menetriesi*, would yield crucial information on how developmental pathways have evolved to generate the long germ mode of development. *C. maculatus* has also been studied in other fields and is a useful system for undergraduate lab teaching [17].

In order to facilitate research on *A. menetriesi* and *C. maculatus*, as well as wider investigations in the Coleoptera and beyond, we present here deep, multi-stage transcriptomic resources from a range of key time points in the development of these two beetle species. Using RSEM-based methods we have compared transcript abundance across these life stages, which will allow the investigation of genes that play key roles in developmental changes in these species, particularly at the maternal/zygotic transition. We have carried out extensive searches for key genes in developmental patterning and cell signalling pathways, and from our analyses we conclude that the transcriptomes for both species are of very high coverage, with almost all expected genes being present with full open reading frames. We have already made extensive use of these resources for our own studies on the embryonic development of these two beetles, and are confident that they will be of broad utility to a range of fields in genomics and developmental biology.

Results and Discussion

RNA Extraction and Sample Selection

Ovaries and embryos were collected as described in Materials and Methods, and as seen in Fig 2. The chosen time-windows cover a variety of important stages in the development of these species, and can be expected to contain the majority of embryonically active transcripts in their expressed complement. Briefly, RNA was collected from dissected ovaries from each species and from four embryonic time windows for *A. menetriesi* and two embryonic time windows for *C. maculatus*. Samples were sequenced on the Illumina HiSeq platform (one lane per species), adaptor trimming was performed, along with preliminary assessment of read quality, and data was made available for download from an external server.

Read Quality and Assembly Metrics:

Table 1 summarises initial read information, and these reads are available from the NCBI SRA, Bioproject Accession numbers: PRJNA293391 and PRJNA293393. To confirm quality of read data, FastQC [36] was run on all read data. This showed Phred quality scores were high, with median scores always exceeding 28 through to the 101st base, and generally in the mid-30s. A slight bias was found in initial nucleotide sequence. To ensure that Illumina adaptors were removed in their entirety, Trimmomatic v.0.32 [37] was used to check for residual adaptor sequence, but none were observed. We therefore posit that this bias is due to known biases in Illumina hexamer binding [38] rather than sequencing-based artifacts.

Using Trinity [39] as an assembler, Trimmomatic-corrected read assemblies were then compared with assemblies based on uncorrected reads. Initial assays of Trimmomatic-treated read assemblies empirically found them to be less well assembled than those using un-trimmed reads, with a shorter N50 and less overall sequence recovery. Trimmed assemblies were therefore discarded in favour of untrimmed assemblies, which were used for all further analyses.

Assays of initial assemblies noted a small amount of fungal contamination in the data for both species. DeconSeq [40] was therefore run on the Trinity output for both species, with high stringency as noted in the methods. A total of 336 and 206 contigs with some homology to fungal sequence was removed from the *A. menetriesi* and *C. maculatus* total assemblies as a result, before read mapping was performed. In the *A. menetriesi* assembly, we observed particularly high contamination with the protists, notably *Dictyostelium discoideum* and *Naegleria gruberi,* and some of this almost certainly remains in the transcriptome. Metrics for final assemblies after the removal of contamination can be seen in Table 2, alongside the results of assemblies for individual time points.

The final, combined timepoint, contamination-removed assemblies, comprising 228,096 and 128,837 contigs for *A. menetriesi* and *C. maculatus* respectively, contain a large number of well-assembled transcripts, with the number of contigs greater than 1 kb in length (52,217 in *A. menetriesi,* 33,250 in *C. maculatus*) and a high N50 (1,598 bp *A. menetriesi,* 2,263 bp *C. maculatus*) indicating a very well assembled dataset. This size is sufficient to span most protein coding domains, allowing easy inference of homology, and will be the full length of many transcripts. It is important to note that many of the contigs in our assembly will represent splice variants of single genes, and some genes will have multiple splice variants, which will affect these statistics. However, excellent recovery of splicing variation itself will be useful for a range of later analysis. GC content of the final assembled transcriptomes closely mirrors that of reads (32.82% in *A. menetriesi,* 38.7% in *C. maculatus*; reads 36-38%/40-43% respectively depending on library).

To gain an understanding of whether full-length transcripts were present in our data, we ran TransDecoder v 2.01 [41] to identify open reading frames (ORFs) and filtered for the results that were at least 100 amino acids long. For the *A. menetriesi* assembly, this analysis yielded 71,961 raw and 51,912 filtered contigs, while for the *C. maculatus* assembly, the analysis yielded 65,433 raw and 36,535 filtered contigs. The mean average length of the predicted polypeptides, after the filtering step, was 351 (*A. menetriesi*) and 448 (*C. maculatus*) amino acids. This is long enough for us to be confident that our assembly adequately spans coding regions, as the average eukaryotic protein length is 361 amino acids [42]. Together, these analyses suggest that we have recovered the vast majority of coding sequence in our combined assemblies, with sufficient length to adequately span ORFs, a conclusion further supported by gene annotation data as discussed further below. The numbers of ORFs presented here are considerably more than the 16,404 gene models observed in the *T. castaneum* genome (*Tribolium* Genome Sequencing Consortium, 2008), and this is likely due to both spurious ORFs in our dataset and to multiple splice variants. All assemblies are available from Figshare online (*A. menetriesi* DOI: 10.6084/m9.figshare.2056464, *C. maculatus* DOI: 10.6084/m9.figshare.2056467).

Timed RNA Expression - Differential Expression Analysis

Key developmental stages for both *A. menetriesi* and *C. maculatus* can be easily observed following fixation and nuclei staining (data not shown). Briefly, egg lay to uniform blastoderm stage takes approximately 24 hours in *A. menetriesi*, and 7 hours in *C. maculatus*. Germband formation, gastrulation and elongation occur from 24-100 hours in *A. menetriesi*, and from 7-24 hours in *C. maculatus*. The latter period was subdivided in *A. menetriesi* according to characteristic stages of short germ development pertinent to our research interests. These stages, along with assayed sample periods, are shown diagramatically in Fig 2. As well as being used for combined assemblies as described earlier, RNA extracted from mature ovaries and from embryos collected during the aforementioned time periods was also individually assembled using Trinity.

The timed transcriptome assemblies for each species often possess better assembly quality when compared to the combined assemblies by metrics such as N50 and mean contig length (Fig 2, Table 2). As a result of being made up of a subset of the total reads, the individual assemblies do not possess the breadth of the combined assemblies, with fewer contigs, especially at long contig length (e.g. 1kb <). As such, the staged transcriptomes were used solely for comparison of expression levels across time, while the combined assemblies were used for gene family analyses. We emphasise that no technical replicate was performed for these comparisons, and any conclusions drawn from them should hold this consideration in regard. With this limitation in mind, we carried out differential expression analyses and observed broad trends in expression, as can be seen in Fig 3.

First, we generated matrices from general comparison between time points in order to find the most similar samples (Fig 3 A,B). Generally, these results are congruent with steady changes in gene expression across the course of development, with most time points being most similar to those immediately preceding and following them. However, a split can be seen in *A. menetriesi* (Fig 3 A,B) between the ovary and 0-24 hour samples and all others, with the three later samples resembling each other more closely than the 0-24 hour dataset resembles the 24-48 hour sample. This could be due to the maternal:zygotic transition, which will occur at some point in this time frame. This cannot be seen for *C. maculatus*, and could be due to more admixture of RNA within the last 7-24 hour sample. Focused analysis on when the maternal:zygotic transition occurs in each species is required to resolve this question.

Next, we clustered the results of our differential expression calculations (Fig 3 C,D). The results shown are those with RSEM considering each isoform separately (rather than taking into account clustering into genes performed by Trinity). Numerous up- and down-regulated contigs can be seen at each time point, with some time points more obviously possessing or lacking a subset of genes found in the combined transcriptomes.

While replicates have not been performed and we have not analysed up and down regulated transcripts in detail, these data are available to download from Additional Files 6 and 7 attached to this document online. These data will act as a good initial guide for those interested in tracking differential expression of specific genes across development and will be useful for hypothesis building.

Basic Gene Annotation

To gain an understanding of the depth of coverage of our datasets we used the BUSCO library of well-annotated genes [43], which are known to be highly conserved in single copy across the

Metazoa, as a basis for comparison with our combined, assembled transcriptomes. Of the BUSCO set of 843 metazoan orthologs, the *A. menetriesi* assembly possesses 801 (95%) complete (of which 225, 26%, are duplicated), 16 fragmented (1.8%) and 26 missing genes (3.0%), for a total recovery of 97% of the BUSCO dataset. The *C. maculatus* assembly contains 815 (96%) complete (with 202, 23%, duplicated), 13 (1.5%) fragmented and 15 (1.7%) missing genes (98.3% recovery). This extremely high level of recovery gives us confidence that at least all housekeeping genes expected to be present in these species are found in our datasets, which strongly suggests that these transcriptomes contain the vast majority of the gene cassette of these species.

The number of duplicate genes in our BUSCO analyses likely reflects the construction of our transcriptomes from mixed RNA samples, with the allelic variation that this implies. Discerning true duplicates from allelic and splice variant data is largely contingent on the availability of well-assembled genomes. However, the recovery of these putative duplicates in our assemblies underlines that our RNA sequencing and assembly was of good depth and quality (respectively). With this information in-hand, a range of investigations will be made possible, particularly into the regulation and expression of developmentally important genes.

A further understanding of the content of our assemblies was gained from Blast2GO analysis [44, 45]. Genes were annotated using b2gpipe, on the basis of BLASTx (*E* value cutoff, 10^{-3}) comparisons made against the nr database as downloaded on the 26 January 2015. Of 228,096 (*A. menetriesi)* and 128,837 (*C. maculatus)* contigs in each assembly, 78,879 (34.6%) and 41,744 (32.4%) possessed a hit in the nr databases above the threshold. After further annotation with ANNEX and Interproscan, a total of 36,315 (15.9%) and 13,096 (10.2%) contigs were assigned to one or more GO categories. These numbers, while only a fraction of the total number of contigs within our transcriptomes, more closely reflect the expected eukaryotic protein complement in number. Blast2GO annotations are given in Additional Files 2 and 3

Fig 4A shows the distribution of species best hit by BLASTx comparison of contigs from *A. mentriesi and C. maculatus* with the nr database. For both species, *T. castaneum* is the best represented species - a reflection of both the phylogenetic position of this species and its well annotated genome. The fact that contigs in our transcriptomes match *T. castaneum* and other coleopteran and insect species more closely than those of other species suggests that gene orthology will be easy to assign in many cases, and that a higher than normal rate of molecular evolution is not observed in our species.

The distribution of GO terms within our datasets are shown in Fig 4B, alongside those of the well-annotated *D. melanogaster* and *Mus musculus* proteomes. In general, our datasets resemble one another more than they mirror that of the two sequenced genomes noted. Our transcriptomic resources empirically seem under-represented relative to *D. melanogaster* and *M. musculus* in developmentally interesting categories such as ‘Protein Binding’ (Molecular Function), ‘Multicellular Organism Development’ and ‘Cell Differentiation’ (Biological Process). Given our results for more targeted investigations, found below, we feel this is likely a result of poor annotation of these by Blast2GO, rather than true absence from the transcriptome.

At a gross level, in the intracellular ‘Cellular Component’ GO categories, our data appears more similar to that seen in the two ‘model’ species than in Molecular Function or Biological Process categories, although this has not been tested statistically. This would suggest these well-conserved structural components were more readily assigned GO categories than the developmentally interesting categories listed above. While not all GO categories are as well-annotated by this process as may be desired, the broad classification of our data into a wide range of GO categories of all levels of GO distribution demonstrates that Blast2GO annotations of our data are a useful starting point for more focussed investigations and identification of specific genes and pathways of interest.

Gene Family Recovery

To extend the semi-automated analyses presented above, we performed more targeted analysis of individual, developmentally important gene families. Both the Hox family of transcription factors and the TGF-β cassette were exceptionally well recovered in our dataset. The Hox genes, and in particular the ANTP HOXL class, which pattern the anterior-posterior body axis, are recovered almost in their entirety in both species when compared to well-catalogued databases (e.g. [46]). This can be seen in Fig 5, which shows the phylogenetic distribution of recovered ANTP HOXL class sequences from our transcriptomes alongside previously annotated members of this class.

In *A. menetriesi,* sequences for all the ANTP HOXL class genes are recovered in our transcriptome, as can be seen in Fig 5, with sequences and alignments in Additional File 1. We note, however, that the *A. menetriesi Hox 2 / maxillopedia (mxp)/ proboscipedia (pb)* sequence bears some BLAST similarity with *Hox 4 / Dfd* sequences, while the putative *Hox 4 / Deformed (Dfd)* recovered does not include the homeodomain sequence - whether it has lost this crucial domain, or if this portion of the sequence is simply not recovered in our assembly is at present unknown. The putative *A. menetriesi Hox 4 / Dfd* sequence is given in Additional File 1, although it is not shown in the phylogeny in Fig 5 due to its truncated length.

While 2 (*A. menetriesi)* and 4 (*C. maculatus*) *Hox 3 / zerknüllt (zen)* variants are seen in our species, these are identical at the coding level, and therefore seem to be splice or allelic variants, rather than the two paralogous genes seen in *T. castaneum* [47]. Similarly, no evidence of the *caudal* (*cad*) duplication seen in *T. castaneum* [48] can be found in our transcriptomic assemblies, suggesting this is perhaps specific to the flour beetle lineage. Both *zen* and *caudal* are important embryonic patterning genes, and comparison of these genes in our two species and *Tribolium* would be an excellent situation in which to study how sub- and neo-functionalisation occurs*.* Likely allelic or splice variants are also observed for other HOXL genes in both *A. menetriesi* and *C. maculatus*. It should be noted that these could represent very recent duplications, or the effect of gene conversion, although this can only be tested fully with the advent of a complete genomic resource. No *Pdx/Xlox* gene was seen, adding further circumstantial evidence for the broad scale loss of this gene across the Arthropoda.

Our *C. maculatus* transcriptome also contains the full complement of ANTP HOXL genes, although, similar to the case of *Hox 4 / Dfd* in *A. menetriesi*, the 4 recovered *abdominal-A (abd-A)* homologs lack the whole homeodomain sequence, with several residues missing. These truncations excluded them from the phylogeny shown in Fig 5, but the sequences for these putative homologs are given in Additional File 1.

Even more so than in *A. menetriesi,* a remarkable diversity of potential splice/allelic variants are noted in *C. maculatus,* particularly for the *Hox 6/8* superfamily and *Hox 9-13* / *Abdominal-B* (*Abd-B*) gene family. Of the *Hox 6/8* gene superfamily, normally represented by four genes in the beetle (*prothoraxless* (*ptl)*, *fushi tarazu* (*ftz)*, *Ultrathorax* (*Utx)* and *abd-A*), 12 *ptl*, 1 *ftz*, 4 *Utx* and 4 *abd-A* representatives were found in our analysis. Furthermore, up to 26 different potential allelic or splice variants of abdB are recorded. As our transcriptome is made of mixed embryonic samples, it is perhaps not surprising that a diversity of splice/allelic variants are observed, but the excellent recovery of this data confirms the deep coverage provided by our sequencing and assembly.

The TGF-β cassettes of the insects have been very well described previously (e.g. [49, 50]). Our datasets recover almost the full expected complement of the Coleoptera. A slight exception to this is *Activin (Act)*, a partial sequence of which is recovered for both species: a portion of the propeptide which does not span the mature signal peptide sequence. Whether this is a consequence of loss of the mature domain in these species or low levels of expression at the sampled timepoints remains to be established. A *BMPx* ortholog of clear homology to genes of that family can be found in *C. maculatus,* but has been excluded from the tree seen in Fig 6 as it is incomplete in length. Its sequence can be found in Additional File 1, and we have no doubt as to its identity due to high levels of sequence conservation between it and the *T. castaneum* and *A. menetriesi* orthologs of this gene.

The *glass bottom boat (gbb)* duplication observed in *T. castaneum* cannot be found in our data, but we can recover a range of splice or allelic variants for other genes, especially in *A. menetriesi*. These do not differ in the protein coding regions, which leads us to suspect that these are not from gene duplications (unless the duplication(s) occurred very recently). The phylogeny shown in Fig 6 confirms the homology of all genes, and splice/allelic variant numbers observed are given there in brackets, with all sequences available in Additional File 1.

We also note the discovery of an additional TGF-β ligand in *C. maculatus*. This gene has been previously automatically annotated as *derriere* in *T. castaneum*, (XP_008191586.1) and if it is truly of this family, which is also known as *GDF1/3/Univin/Vg1*, this would be a surprise, as its presence outside the Deuterostomia is controversial [51]. If proof could be found for this being a *bona fide GDF1/3/Univin/Vg1*, the presence of this gene in more than one coleopteran could suggest that this might in fact be ancestrally present in all bilaterian species, but further investigation is warranted before strong conclusions can be drawn in this regard. We could gain no phylogenetic support for placing either of these beetle sequences in the GDF1/3 clade, and it may well be that these sequences instead represent a coleopteran novelty.

Our recovery of not only the full expected complements of these vital developmental genes, but also a remarkable diversity of alternative variants, demonstrates the depth of our assemblies as a resource. Whether used as the basis for simple cloning or more sophisticated analysis of patterns of gene variation and diversification, these transcriptomes will be of wide utility to the field of coleopteran and insect developmental biology.

Pathway Recovery

As well as examining specific gene families, we investigated a number of broader pathways commonly studied in insects [52]. This allows us to both note how well-recovered such pathways are in our species as a measure of transcriptome utility, as well as note interesting differences between these pathways in our species when compared to others. We did this using both automated (KEGG KAAS mapping) and manual (BLAST based) methods. Some representative results of KEGG KAAS mapping can be seen in Fig 7, and all KEGG annotations can be downloaded from Additional Files 4 and 5.

KEGG KAAS mapping uses BLAST results to annotate known pathways, and gives a rapid overview of the recovery of these. Here we have shown the well-known Wnt, Notch and Hedgehog pathways to indicate the depth of our transcriptomes, and show how they may be useful for future research. However, these maps often use terminology based on vertebrate nomenclature, and contain genes known to be absent from particular clades. We have therefore indicated in Fig 7 (using unshaded boxes as shown in the Key) genes that may be absent ancestrally in the Coleoptera, based on their absence from the *T. castaneum* pathway. Of genes expected to be present in the Coleoptera we find almost total recovery in our transcriptomes. In the three pathways examined, only three genes noted to be present in *T. castaneum* were noted as absent from both of our transcriptome datasets, all in the Wnt cascades (Fig 7A). The expected Hedgehog cassette was recovered *in toto* (Fig 7B) and in the Notch signalling cascade (Fig 7C), only APH-1 was noted to be absent, and only from the *C. maculatus* transcriptome. We must note that these may not be true absences - KEGG KAAS mapping is based on automatic BLAST assignation, and if these sequences are divergent in our transcriptomes they may have been missed by this analysis.

We also examined pathways manually, using reciprocal BLAST hits and closer manual investigation to confirm identity of individual genes, the results of which can be seen in Table 3. The anterior-posterior patterning genes *cad* (mentioned earlier) and *hunchback (hb)* are present in both species. Of the germline establishment and localization genes examined, *nanos* was surprisingly absent in both species, while *bruno (bru;* also known as *arrest)*, *exuperantia (exu)*, *tudor* (*tud;* 2 copies in *A. menetriesi)*, *oskar (osk)*, *vasa (vas)* and *valois (vls)* were present. Interestingly, *pumilio (pum)* is present in *C. maculatus* in single copy (although it is divided across two contigs), while *A. menetriesi* possesses a total of seven copies. The different *A.*

menetriesi pum copies varied both at the nucleotide level and in their amino acid sequences, strongly suggesting that they are in fact paralogs. In depth analysis of these genes is required to uncover why they have undergone several rounds of duplication. Orthologs of the *Drosophila* gene *swallow (swa)* could not be found in either of our transcriptome resources, nor is it present in several other insects (data not shown) and we suggest it may therefore be a schizophoran novelty.

Canonical gap genes *Krüppel (Kr)*, *knirps (kni)*, *giant (gt)*, *huckebein (hkb)*, *tailless* (*tll;* 2 paralogs in *C. maculatus*), *buttonhead (btd)*, *empty spiracles (ems)* and both *orthodenticle* orthologs (*Otd* and *Otd2*) were recovered in both species examined here. The *C. maculatus* specific duplication of *tll* would be another excellent opportunity to study gene duplication and evolution. The pair rule genes e*ven skipped (eve), hairy (h), fushi tarazu (ftz), odd paired (opa), odd skipped (odd), paired (prd), runt (run)* and *sloppy paired 1 (slp1)* were present in single copy. The segment polarity gene cassette was also present in both species, with the notable absence of *gooseberry-neuro (gsb-n)* from our datasets. The genes *armadillo (arm), cubitis interruptus (ci), Engrailed (En), fused (fu), gooseberry (gsb), hedgehog (hh), pangolin (pan), patched (ptc)* and *wingless (wg)* were all present, and their sequences can be found in Additional File 1.

All of these pathways are commonly studied in insects, and the annotations provided here, along with preliminary timed expression data, will provide a basis for a wide range of targeted investigations into the embryonic development of these two species, and how these pathways have changed over the course of evolution. Furthermore, the excellent recovery of these pathways by both automated (KEGG-KAAS) and manual annotation gives us high confidence in the completeness of our transcriptomic resources. This confirms the results of our BUSCO analysis, and our datasets are therefore likely to contain the vast majority of transcribed genes in these two species, with only lowly expressed and temporally restricted genes absent from these transcriptome resources.

Conclusions:

Our production of deep transcriptomic sequence data for *A. menetriesi* and *C. maculatus* will assist in the inference of character gain and loss across the Coleoptera, aid in future phylogenetic efforts, and allow a range of investigations into the embryonic development of these species at the molecular level. The status of these organisms as common agricultural pests also suggests that such resources may allow targeted control mechanisms to be developed for these species. This data will be another key building block in our understanding of the transcriptomic basis to embryological development, and provide a window into the basic biology of the most successful clade of animals.

Methods:

Animal Husbandry

A. menetriesi eggs were kindly provided by Dr Yoshikazu Ando, and were reared at 25°C on wet sand or soil and fed fresh lettuce. *C. maculatus* beetles were kindly provided by Dr Joel Savard, and were reared at 30°C on dry black-eyed peas.

RNA Extraction and Sequencing

RNA was extracted from ovary and timed embryonic samples using a TRIzol RNA extraction kit according to the manufacturer’s protocols. RNA quantity and quality was checked using a Thermo Scientific Nanodrop 2000C Spectrophotometer and 1 µg was sent for sequencing by the Cologne Centre for Genomics. Adaptor trimming and initial quality control was performed by the commercial provider according to their internal standards. This cleaned data was then made available to us for download from an external server. Paired end read quality after sequencing was assessed using the FastQC program [36].

Transcriptome Assembly and Comparative Expression Analyses

Assemblies used in our final analysis were made using Trinity version 2013_08_14 [39], with the default settings. Full assemblies were made using reads from all time points, and individual assemblies were then constructed using each sampled time point individually. All assemblies are available from Figshare online (*A. menetriesi* DOI: 10.6084/m9.figshare.2056464, *C. maculatus* DOI: 10.6084/m9.figshare.2056467). DeconSeq standalone version 0.4.3 [40] was run on full assemblies with settings -i 95 -c 95, using the bact, fungi, hsref, and prot databases. Comparative expression analyses were performed using RSEM [53] as packaged in the Trinity module, and results shown here are the ‘as-isoform’ data, although ‘as-gene’ data is provided in Additional Files. BUSCO v1.1b1 [43] was used to assess gene complement completeness.

Functional Annotation

Our combined assemblies were automatically assigned homologs and annotated according to gene ontology (GO) terms using Blast2GO [44, 45]. Initially, BLASTx was run using BLAST 2.2.29+ against the NCBI nr database as downloaded to a local server on the 17/01/2015, with settings -evalue 0.001 -max_target_seqs 5 -outfmt 5. GO term distribution within the *D. melanogaster* and *H. sapiens* genomes were downloaded from B2GO-FAR [54] and calculated using the Combined Graph function of Blast2GO. KEGG KAAS mapping was automatically performed using the KEGG KAAS tool (http:// www.genome.jp/tools/kaas/), single-directional best hit with default BLAST settings, and with the eukaryote dataset as a basis for annotation.

Gene Identification

Gene sequences were manually identified and their homology confirmed by independently using tBLASTn [55] searches using gene sequences of known homology downloaded from the NCBI nr database as queries against standalone databases created on a local server using BLAST

2.2.29+ or the CLC Main workbench. Genes putatively identified using this method were reciprocally BLASTed against the online NCBI nr database using BLASTx to confirm their identity. Where identity was uncertain, phylogenetic analysis was used to confirm identity.

Phylogenetic Tree Construction

Sequences were aligned using MAFFT 7 [56] unless otherwise stated under the L-INS-i strategy. Alignments were then saved and exported to MEGA 6, where regions of poor alignment were manually excluded and maximum likelihood phylogenetic trees were constructed using the LG model, 1000 bootstrap replicates as indicated, 4 gamma categories and invariant sites, and all other default prior settings [57].

List of Abbreviations Used:

BLAST: Basic Local Alignment Search Tool, BUSCO: Benchmarking Universal Single-Copy Orthologs, GDF: Growth Differentiation Factor, KEGG-KAAS: Kyoto Encyclopaedia of Genes and Genomes - Automatic Annotation Server, MAFFT: Multiple Alignment using Fast Fourier Transform, NCBI: National Centre for Biotechnology Information,ORF: Open Reading Frame, RNA: Ribonucleic Acid, RSEM: RNA-Seq by Expectation-Maximization, SRA: Short Read Archive, TGF: Transforming Growth Factor

Competing Interests:

The authors declare that they have no competing interests.

Authors' contributions:

MAB, KC and JAL maintained animals, extracted RNA and arranged sequencing. MAB and NJK assembled transcriptomes, analysed raw data and wrote the manuscript. MAB, NJK, KC, JAL and SR designed experiments and contributed to gene family analyses presented in this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are available in the NCBI SRA repository [Bioproject Accession numbers: PRJNA293391 and PRJNA293393] and in the Figshare repository [DOIs: 10.6084/m9.figshare.2056464 and 10.6084/m9.figshare.2056467]. NOTE: DATA EMBARGOED UNTIL PUBLICATION

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Figure legends

Figure 1: A) Cladogram of Coleoptera simplified from that determined by Hunt *et al.* (2007) [13]. Red asterisk indicates the superfamily in which the Chrysomelidae are located. B) Cladogram of Chrysomelidae simplified from that determined by Gomez-Zurita *et al*. (2007) [28]. Black and grey asterisks indicate the sub-families in which *A. menetriesi* and *C. maculatus* are located (respectively). C) *A. menetriesi* and D) *C. maculatus* adults.

Figure 2: Summary of RNA sources, life stages sampled and sequencing results, with *A.menetriesi* data presented at left and *C. maculatus* data at right.

Figure 3: Overview of results of differential expression analysis performed by RSEM within the Trinity framework. A) and B) show the sample correlation matrix for *A. menetriesi* and *C. maculatus* respectively. C) and D) show relative expression of each differentially expressed contig, considered as isoforms, across time.

Figure 4: Blast2GO results A) Distribution of BLASTx best hits by species, showing metazoans only, for *A. menetriesi* in orange, *C. maculatus* in blue. B) Distribution of GO terms expressed as a percentage of annotated contigs which were assigned a term within each of the three (Molecular Function, Cellular Component, Biological Process) categories of GO ID.

Figure 5: Phylogenetic inter-relationships of ANTP HOXL class genes*,* as reconstructed by MEGA 6 using the LG+Freqs model with 4 gamma categories and invariant sites, based on a 59 amino acid alignment spanning the homeodomain. Numbers at base of nodes represent bootstrap percentages of 1000 replicates. Scale bar at base of phylogeny gives substitutions per site at given unit distance. Red underline indicates *A. menetriesi* and *C. maculatus* sequences, coloured boxes are used to delineate known gene families (and in the case of Hox 6/7/8, a superfamily).

Figure 6: Maximum Likelihood Phylogeny of TGF-β ligands, as determined using MEGA under the LG+Freqs model with 4 gamma categories and invariant sites, on the basis of a 72 amino acid alignment of mature peptide sequences. The given scale depicts the number of substitutions per site per unit length. Bootstrap percentages (of 1000 replicates) are given at base of nodes. *A. menetriesi* and *C. maculatus* sequences are underlined in red. Coloured boxes represent known gene families with representatives in our transcriptomic resources, while all gene families, including those not found in our datasets, are indicated at right.

Figure 7: KEGG style pathway maps showing recovery in our transcriptome resources of A) the Wnt signalling pathway in canonical and non-canonical contexts, B) the Hedgehog signalling pathway and C) the Notch signalling pathway. Coloration of genes indicates presence, absence or ancestral absence from the Coleoptera as detailed in the key, which also gives other information as noted.

Tables

Table 1: Basic read data metrics

	A. menetriesi					*C. maculatus*		
Library: (hpf= hours post fertilization)	*Ovary*	*0-24 hpf*	*24-48 hpf*	*48-72 hpf*	*72-100 hpf*	*Ovary*	*0-7 hpf*	*7-24 hpf*
Number of Paired-end Reads	44,695,199	56,139,626	39,911,618	46,217,614	45,213,862	67,026,913	61,060,357	55,273,927
Read Length (bp)	101	101	101	101	101	101	101	101
Amount of Data (Gigabytes)	7.3	9.5	6.8	7.8	7.7	10.8	9.8	8.8
GC content (%)	38	36	37	37	37	43	41	40

Table 2: Assembly metrics for individual time point and combined transcriptomic resources

	A. menetriesi						*C. maculatus*			
	Ovary	0-24 hpf	24-48 hpf	48-72 hpf	72-100 hpf	Combined	Ovary	0-7 hpf	7-24 hpf	Combined
Number of contigs	100,084	106,548	120,497	140,868	126,400	228,096	98,082	57,879	80,665	128,837
Max contig length (bp)	20,759	17,765	18,398	17,591	17,180	20,757	30,742	22,941	22,956	29,933
Mean contig length (bp)	983.95	1027.36	924.27	879.03	914.2	844.66	1074.86	1216.55	1106.56	991.04
Median contig length (bp)	471	490	453	426	448	401	416	526	474	391
N50 contig length (bp)	1,894	1,993	1,721	1,643	1,686	1,598	2,450	2,570	2,308	2,263
# contigs in N50	14,196	15,200	17,511	19,814	18,105	31,196	11,695	7,874	10,978	15,155
# contigs > 1kb	28,255	31,944	32,307	34,520	33,002	52,217	27,763	19,919	25,529	33,250
# bases, total	98,477,173	109,463,362	111,371,655	123,827,226	115,555,146	192,664,213	105,424,220	70,412,726	89,260,499	127,682,507
# bases in contigs > 1kb	68,737,074	78,526,348	75,208,106	80,796,007	77,199,414	122,986,547	78,519,052	55,032,546	67,296,566	91,466,099

GC Content % (2dp)	33.54	33.14	33.14	33	33.17	32.82	39.26	39.21	38.59	38.7

Table 3: Genes identified by manual annotation

Pathway/Gene	*A. menetriesi*	*C.maculatus*	*Pathway/Gene*	*A. menetriesi*	*C.maculatus*
Maternal Effect:			Pair rule:		
caudal	present	present	*even skipped*	present	present
hunchback	present	present	*fushi tarazu*	present (see Hox)	present (see Hox)
			hairy	present	present
Germline:			*odd paired*	present	present
bruno/arrest	present	present	*odd skipped*	present	present
exuperantia	present	present	*paired*	present	present
nanos	absent	absent	*runt*	present	present
oskar	present	present	*sloppy paired 1*	present	present
pumilio	present - 7 copies	present			
swallow	absent	absent	Segment Polarity:		
tudor	present - 2 copies	present	*armadillo*	present	present
valois	present	present	*cubitis interruptus*	present	present
vasa	present	present	*Engrailed*	present	present

			fused	present	present
Gap genes:			*gooseberry*	present	present
buttonhead	present	present	*gooseberry-neuro*	absent	absent
empty spiracles	present	present	*hedgehog*	present	present (but on 3 contigs)
giant	present	present	*pangolin*	present	present
huckebein	present	present	*patched*	present	present
knirps	present	present	*wingless*	present	present
Krüppel	present	present			
orthodenticle 1	present	present			
orthodenticle 2	present	present			
tailless	present	present - 2 copies			

Additional Files:

Additional File 1: Sequences of all genes referred to in text, and alignments used in phylogenetic analyses (.xls)

Additional File 2: Annotations of transcriptome (Blast2GO .annot file) *A. menetriesi*

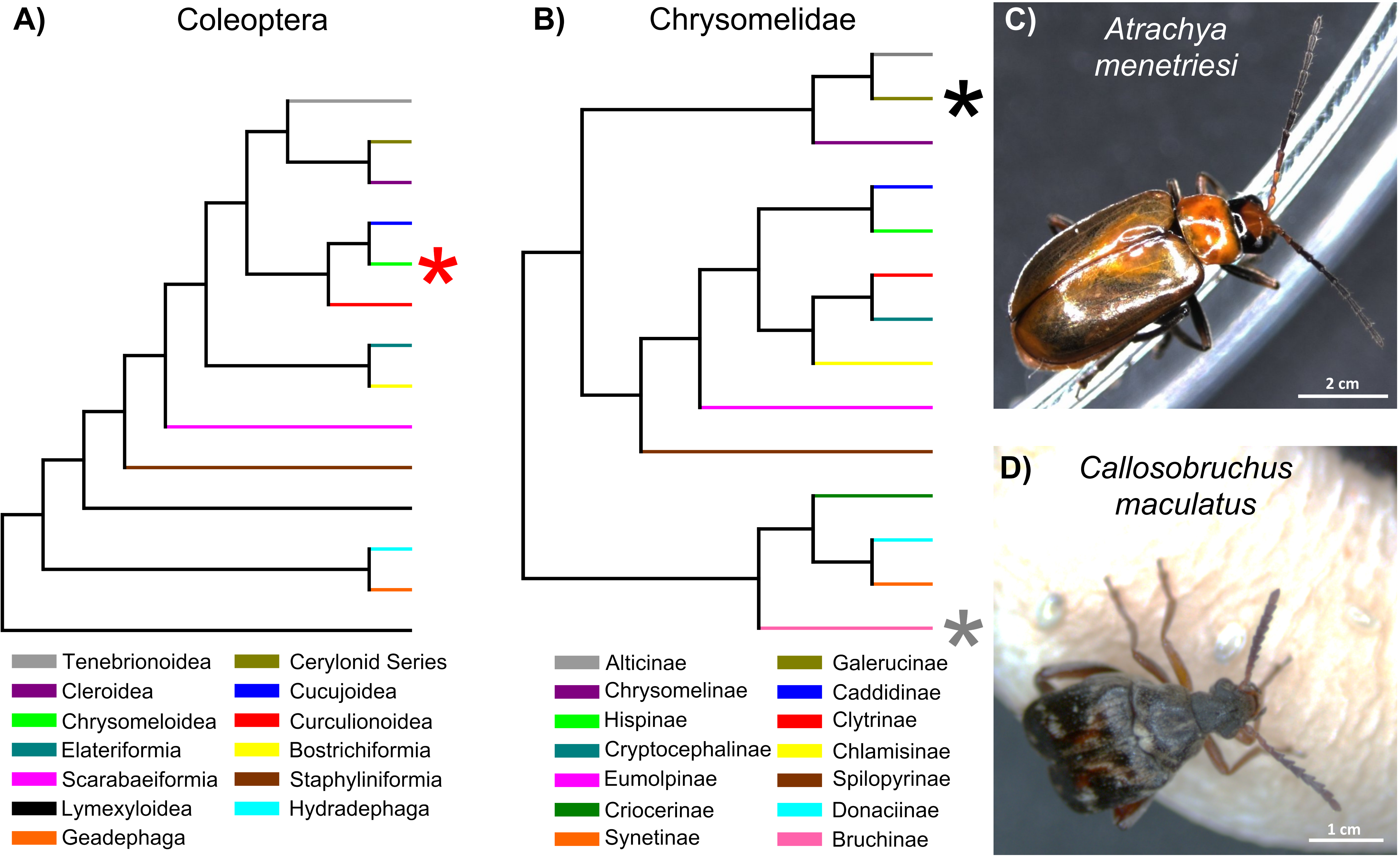
Additional File 3: Annotations of transcriptome (Blast2GO .annot file) *C. maculatus*

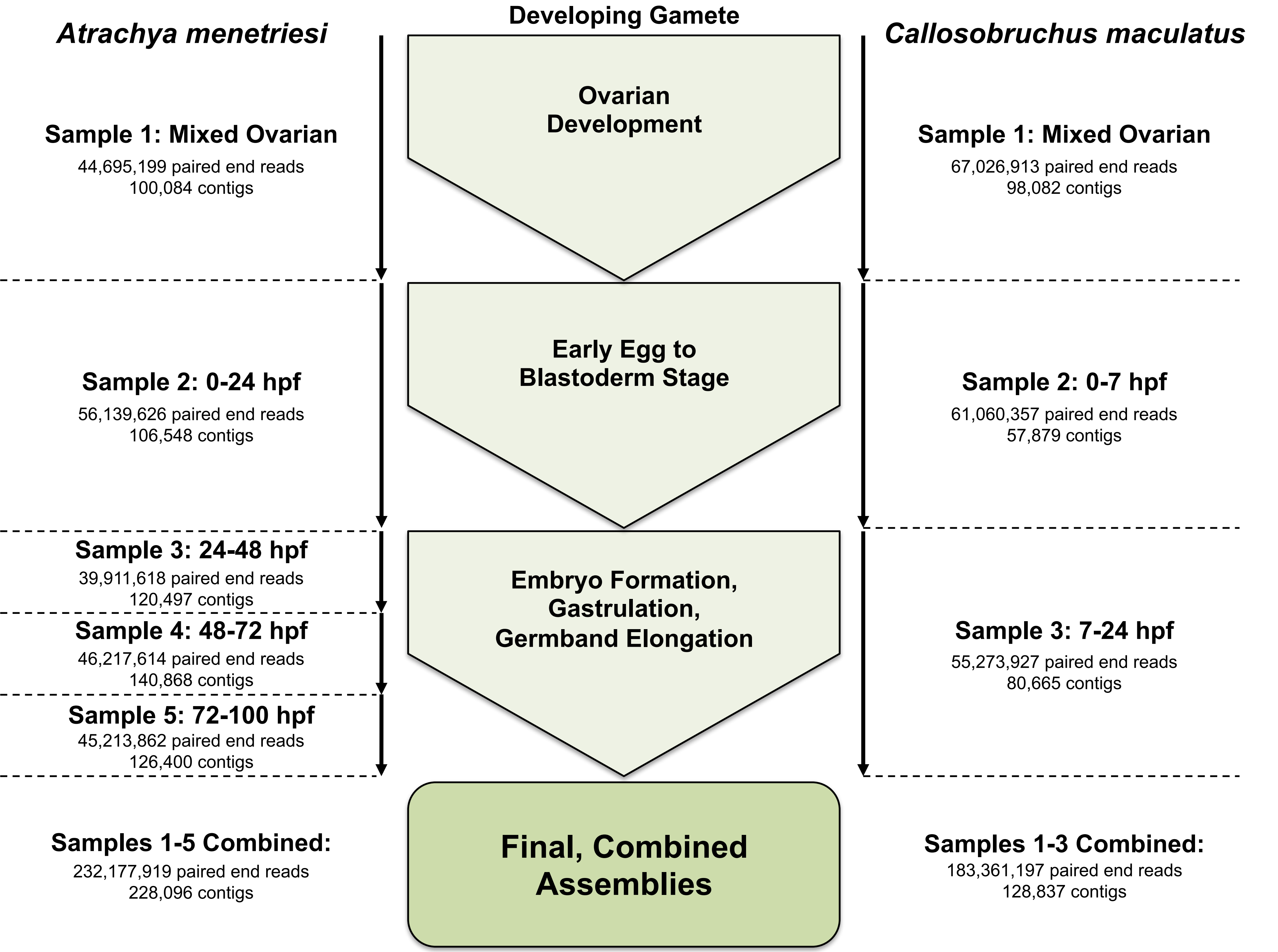
Additional File 4: KEGG data, *A. menetriesi*

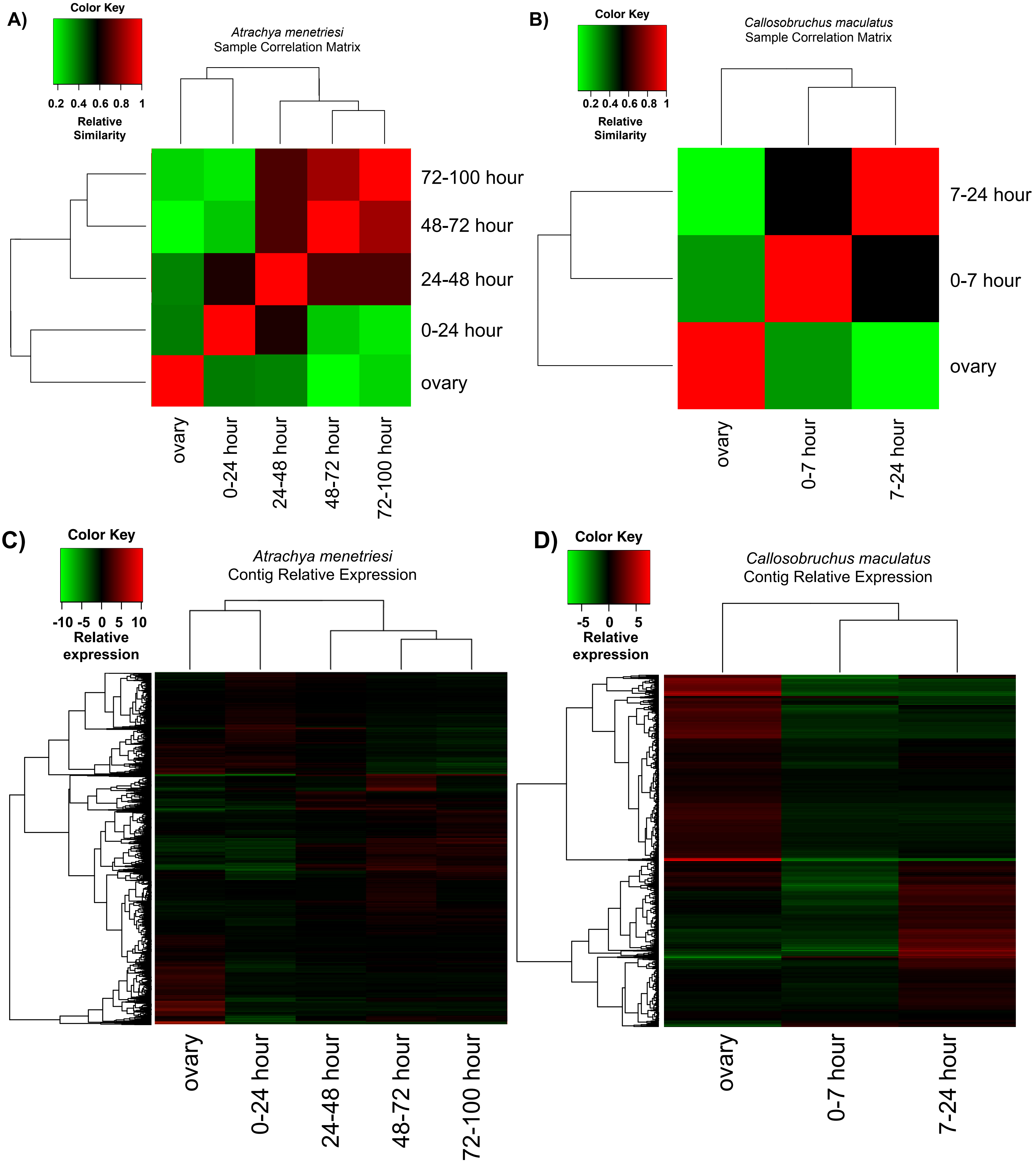
Additional File 5: KEGG data, *C. maculatus*

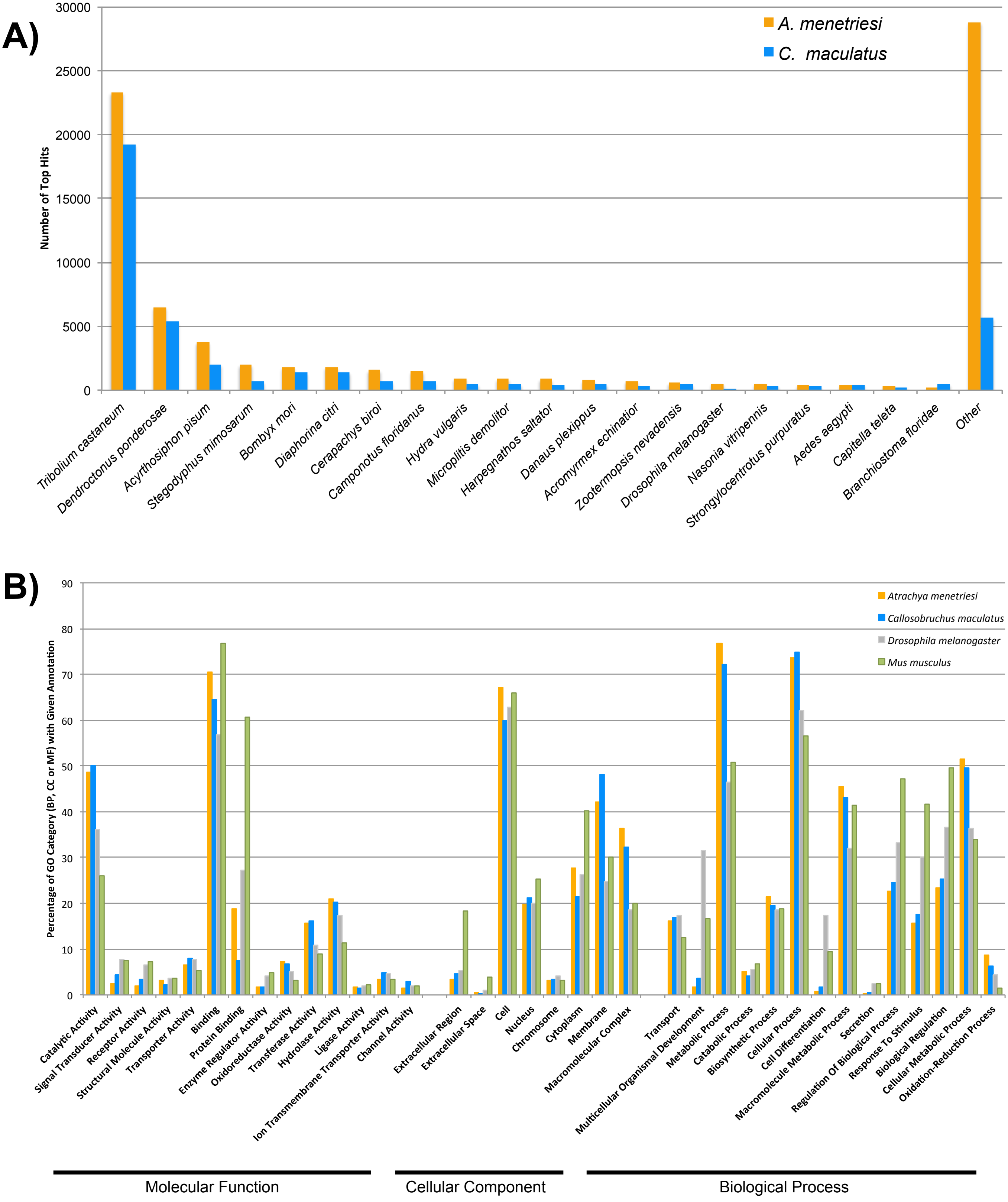
Additional File 6: Comparative expression data, *A. menetriesi*

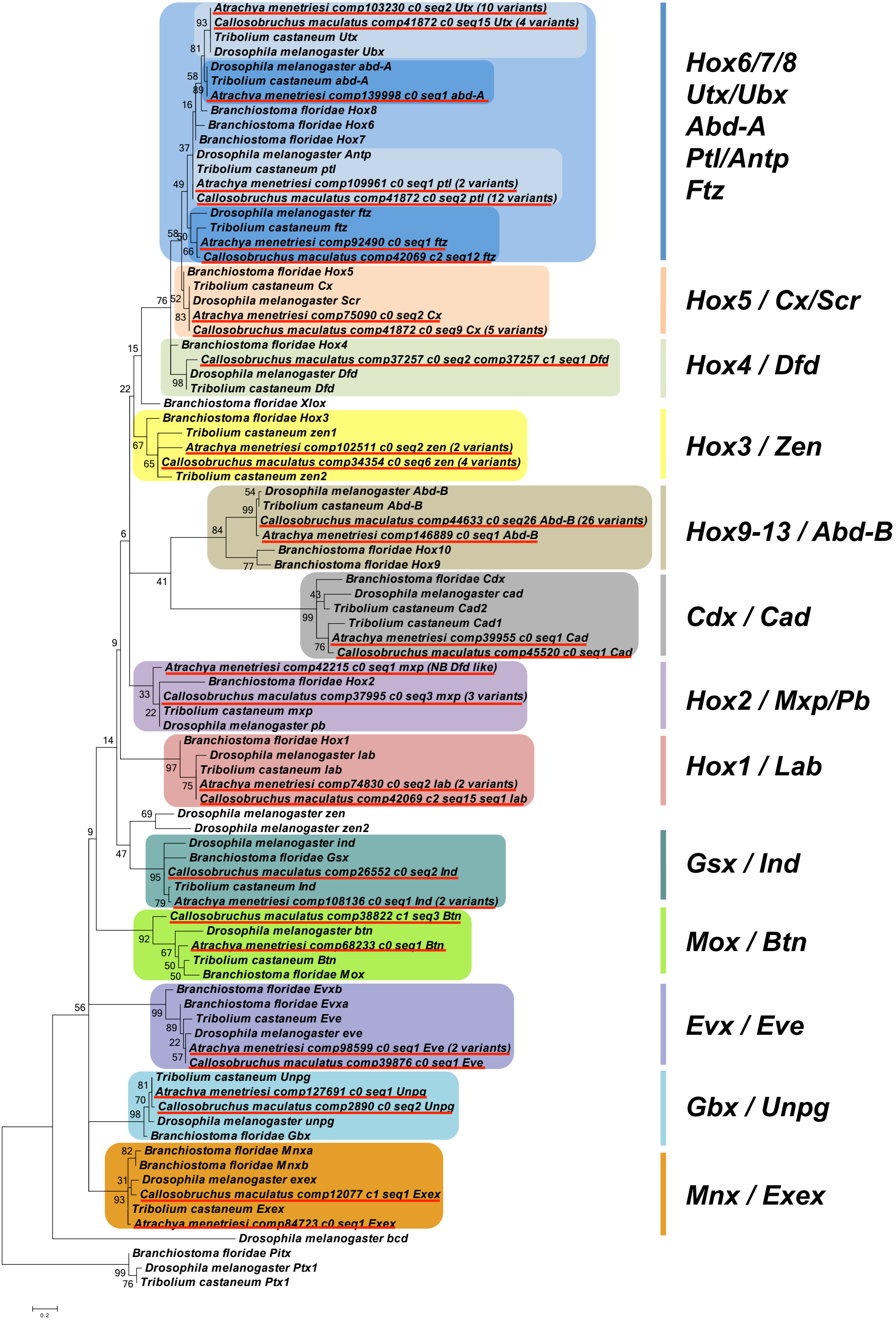
Additional File 7: Comparative expression data, *C. maculatus*

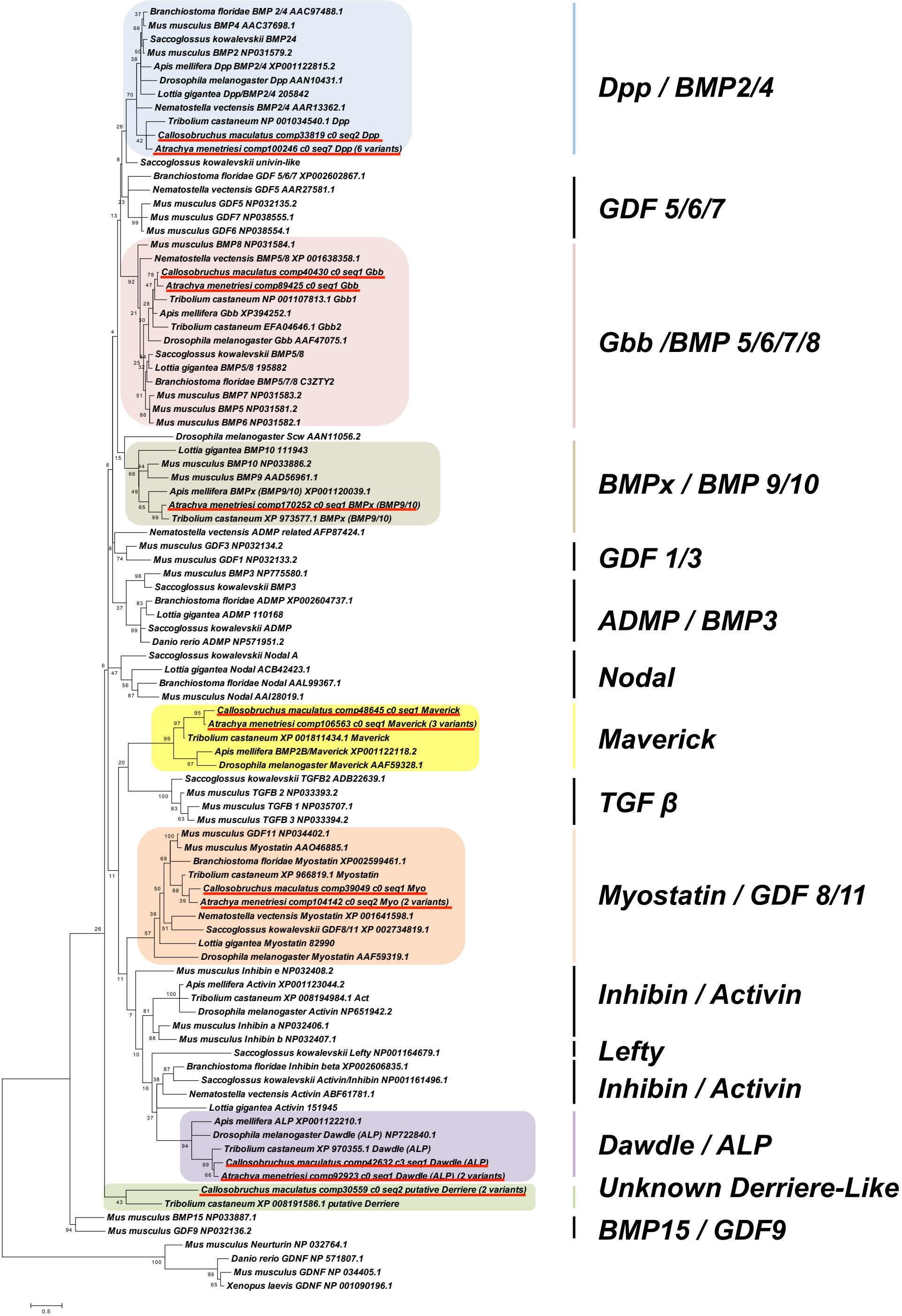

A)
Coleoptera
*
Tenebrionoidea
Cleroidea
Chrysomeloidea
Elateriformia
Scarabaeiformia
Lymexyloidea
Geadephaga
Cerylonid Series
Cucujoidea
Curculionoidea
Bostrichiformia
Staphyliniformia
Hydradephaga
B)
Chrysomelidae
*
*
Alticinae
Chrysomelinae
Hispinae
Cryptocephalinae
Eumolpinae
Criocerinae
Synetinae
Galerucinae
Caddidinae
Clytrinae
Chlamisinae
Spilopyrinae
Donaciinae
Bruchinae
C)
Atrachya menetriesi
2 cm
D)
Callosobruchus maculatus
1 cm

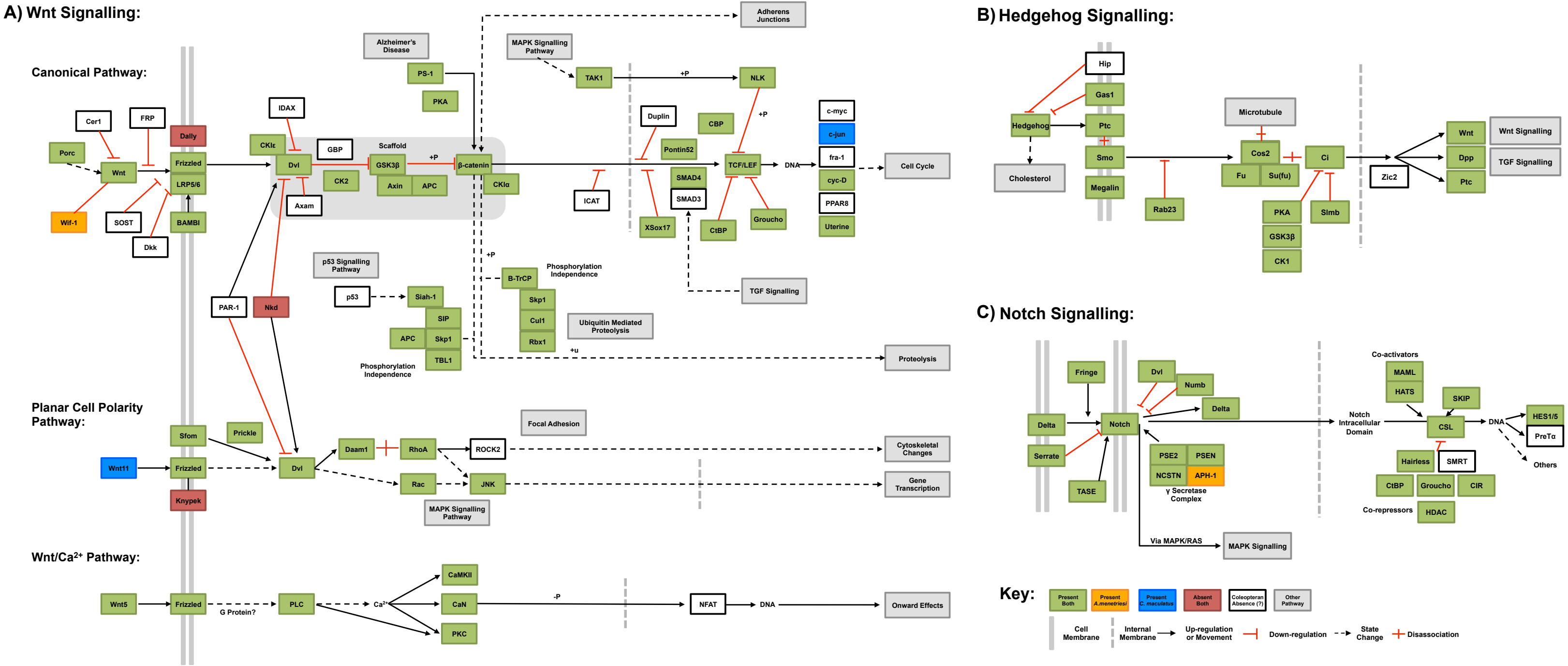

Atrachya menetriesi
Developing Gamete
Callosobruchus maculatus
Ovarian Development
Sample 1: Mixed Ovarian
44,695,199 paired end reads
100,084 contigs
Sample 1: Mixed Ovarian
67,026,913 paired end reads
98,082 contigs
Early Egg to Blastoderm Stage
Sample 2: 0-24 hpf
56,139,626 paired end reads
106,548 contigs
Sample 2: 0-7 hpf
61,060,357 paired end reads
57,879 contigs
Embryo Formation, Gastrulation, Germband Elongation
Sample 3: 24-48 hpf
39,911,618 paired end reads
120,497 contigs
Sample 4: 48-72 hpf
46,217,614 paired end reads
140,868 contigs
Sample 5: 72-100 hpf
45,213,862 paired end reads
126,400 contigs
Sample 3: 7-24 hpf
55,273,927 paired end reads
80,665 contigs
Final, Combined Assemblies
Samples 1-5 Combined:
232,177,919 paired end reads
228,096 contigs
Samples 1-3 Combined:
183,361,197 paired end reads
128,837 contigs


A)
Color Key
0.2 0.4 0.6 0.8 1
Relative Similarity
Atrachya menetriesi
Sample Correlation Matrix
72-100 hour
48-72 hour
24-48 hour
0-24 hour
ovary
ovary
0-24 hour
24-48 hour
48-72 hour
72-100 hour
B)
Color Key
0.2 0.4 0.6 0.8 1
Relative Similarity
Callosobruchus maculatus
Sample Correlation Matrix
7-24 hour
0-7 hour
ovary
ovary
0-7 hour
7-24 hour
C)
Color Key
-10 -5 0 5 10
Relative expression
Atrachya menetriesi
Contig Relative Expression
ovary
0-24 hour
24-48 hour
48-72 hour
72-100 hour
D)
Color Key
-5 0 5
Relative expression
Callosobruchus maculatus
Contig Relative Expression
ovary
0-7 hour
7-24 hour


A)
30000
25000
20000
15000
10000
5000
0
Number of Top Hits
A. menetriesi
C. maculatus
Tribolium castaneum
Dendroctonus ponderosae
Acyrthosiphon pisum
Stegodyphus mimosarum
Bombyx mori
Diaphorina citri
Cerapachys biroi
Camponotus floridanus
Hydra vulgaris
Microplitis demolitor
Harpegnathos saltator
Danaus plexippus
Acromyrmex echinatior
Zootermopsis nevadensis
Drosophila melanogaster
Nasonia vitripennis
Strongylocentrotus purpuratus
Aedes aegypti
Capitella teleta
Branchiostoma floridae
Other
B)
90
80
70
60
50
40
30
20
10
0
Percentage of GO Category (BP, CC or MF) with Given Annotation
Atrachya menetriesi
Callosobruchus maculatus
Drosophila melanogaster
Mus musculus
Catalytic Activity
Signal Transducer Activity
Receptor Activity
Structural Molecule Activity
Transporter Activity
Binding
Protein Binding
Enzyme Regulator Activity
Oxidoreductase Activity
Transferase Activity
Hydrolase Activity
Ligase Activity
Ion Transmembrane Transporter Activity
Channel Activity
Extracellular Region
Extracellular Space
Cell
Nucleus
Chromosome
Cytoplasm
Membrane
Macromolecular Complex
Transport
Multicellular Organismal Development
Metabolic Process
Catabolic Process
Biosynthetic Process
Cellular Process
Cell Differentiation
Macromolecule Metabolic Process
Secretion
Regulation Of Biological Process
Response To Stimulus
Biological Regulation
Cellular Metabolic Process
Oxidation-Reduction Process
Molecular Function
Cellular Component
Biological Process


Atrachya menetriesi comp103230 c0 seq2 Utx (10 variants)
93
Callosobruchus maculatus comp41872 c0 seq15 Utx (4 variants)
Tribolium castaneum Utx
81
Drosophila melanogaster Ubx
Drosophila melanogaster abd-A
58
Tribolium castaneum abd-A
89
Atrachya menetriesi comp139998 c0 seq1 abd-A
16
Branchiostoma floridae Hox8
Branchiostoma floridae Hox6
Branchiostoma floridae Hox7
37
Drosophila melanogaster Antp
Tribolium castaneum ptl
49
Atrachya menetriesi comp109961 c0 seq1 ptl (2 variants)
Callosobruchus maculatus comp41872 c0 seq2 ptl (12 variants)
Drosophila melanogaster ftz
Tribolium castaneum ftz
58
50
Atrachya menetriesi comp92490 c0 seq1 ftz
66
Callosobruchus maculatus comp42069 c2 seq12 ftz
Branchiostoma floridae Hox5
Tribolium castaneum Cx
76
52
Drosophila melanogaster Scr
83
Atrachya menetriesi comp75090 c0 seq2 Cx
Callosobruchus maculatus comp41872 c0 seq9 Cx (5 variants)
15
Branchiostoma floridae Hox4
Callosobruchus maculatus comp37257 c0 seq2 comp37257 c1 seq1 Dfd
Drosophila melanogaster Dfd
98
Tribolium castaneum Dfd
22
Branchiostoma floridae Xlox
Branchiostoma floridae Hox3
Tribolium castaneum zen1
67
Atrachya menetriesi comp102511 c0 seq2 zen (2 variants)
65
Callosobruchus maculatus comp34354 c0 seq6 zen (4 variants)
Tribolium castaneum zen2
54
Drosophila melanogaster Abd-B
99
Tribolium castaneum Abd-B
Callosobruchus maculatus comp44633 c0 seq26 Abd-B (26 variants)
84
Atrachya menetriesi comp146889 c0 seq1 Abd-B
6
Branchiostoma floridae Hox10
77
Branchiostoma floridae Hox9
Branchiostoma floridae Cdx
41
43
Drosophila melanogaster cad
Tribolium castaneum Cad2
99
Tribolium castaneum Cad1
Atrachya menetriesi comp39955 c0 seq1 Cad
76
Callosobruchus maculatus comp45520 c0 seq1 Cad
9
Atrachya menetriesi comp42215 c0 seq1 mxp (NB Dfd like)
Branchiostoma floridae Hox2
33
Callosobruchus maculatus comp37995 c0 seq3 mxp (3 variants)
22
Tribolium castaneum mxp
Drosophila melanogaster pb
14
Branchiostoma floridae Hox1
Drosophila melanogaster lab
97
Tribolium castaneum lab
75
Atrachya menetriesi comp74830 c0 seq2 lab (2 variants)
Callosobruchus maculatus comp42069 c2 seq15 seq1 lab
69
Drosophila melanogaster zen
9
Drosophila melanogaster zen2
47
Drosophila melanogaster ind
Branchiostoma floridae Gsx
95
Callosobruchus maculatus comp26552 c0 seq2 Ind
Tribolium castaneum Ind
79
Atrachya menetriesi comp108136 c0 seq1 Ind (2 variants)
Callosobruchus maculatus comp38822 c1 seq3 Btn
92
Drosophila melanogaster btn
67
Atrachya menetriesi comp68233 c0 seq1 Btn
50
Tribolium castaneum Btn
50
Branchiostoma floridae Mox
56
Branchiostoma floridae Evxb
99
Branchiostoma floridae Evxa
89
Tribolium castaneum Eve
Drosophila melanogaster eve
22
Atrachya menetriesi comp98599 c0 seq1 Eve (2 variants)
57
Callosobruchus maculatus comp39876 c0 seq1 Eve
Tribolium castaneum Unpg
81
Atrachya menetriesi comp127691 c0 seq1 Unpg
70
Callosobruchus maculatus comp2890 c0 seq2 Unpg
98
Drosophila melanogaster unpg
Branchiostoma floridae Gbx
82
Branchiostoma floridae Mnxa
Branchiostoma floridae Mnxb
31
Drosophila melanogaster exex
93
Callosobruchus maculatus comp12077 c1 seq1 Exex
Tribolium castaneum Exex
Atrachya menetriesi comp84723 c0 seq1 Exex
Drosophila melanogaster bcd
Branchiostoma floridae Pitx
99
Drosophila melanogaster Ptx1
76
Tribolium castaneum Ptx1
0.2
Hox6/7/8
Utx/Ubx
Abd-A
Ptl/Antp
Ftz
Hox5 / Cx/Scr
Hox4 / Dfd
Hox3 / Zen
Hox9-13 / Abd-B
Cdx / Cad
Hox2 / Mxp/Pb
Hox1 / Lab
Gsx / Ind
Mox / Btn
Evx / Eve
Gbx / Unpg
Mnx / Exex


Branchiostoma floridae BMP 2/4 AAC97488.1
Mus musculus BMP4 AAC37698.1
Saccoglossus kowalevskii BMP24
Mus musculus BMP2 NP031579.2
Apis mellifera Dpp BMP2/4 XP001122815.2
Drosophila melanogaster Dpp AAN10431.1
Lottia gigantea Dpp/BMP2/4 205842
Nematostella vectensis BMP2/4 AAR13362.1
Tribolium castaneum NP 001034540.1 Dpp
Callosobruchus maculatus comp33819 c0 seq2 Dpp
Atrachya menetriesi comp100246 c0 seq7 Dpp (6 variants)
Saccoglossus kowalevskii univin-like
Branchiostoma floridae GDF 5/6/7 XP002602867.1
Nematostella vectensis GDF5 AAR27581.1
Mus musculus GDF5 NP032135.2
Mus musculus GDF7 NP038555.1
Mus musculus GDF6 NP038554.1
Mus musculus BMP8 NP031584.1
Nematostella vectensis BMP5/8 XP 001638358.1
Callosobruchus maculatus comp40430 c0 seq1 Gbb
Atrachya menetriesi comp89425 c0 seq1 Gbb
Tribolium castaneum NP 001107813.1 Gbb1
Apis mellifera Gbb XP394252.1
Tribolium castaneum EFA04646.1 Gbb2
Drosophila melanogaster Gbb AAF47075.1
Saccoglossus kowalevskii BMP5/8
Lottia gigantea BMP5/8 195882
Branchiostoma floridae BMP5/7/8 C3ZTY2
Mus musculus BMP7 NP031583.2
Mus musculus BMP5 NP031581.2
Mus musculus BMP6 NP031582.1
Drosophila melanogaster Scw AAN11056.2
Lottia gigantea BMP10 111943
Mus musculus BMP10 NP033886.2
Mus musculus BMP9 AAD56961.1
Apis mellifera BMPx (BMP9/10) XP001120039.1
Atrachya menetriesi comp170252 c0 seq1 BMPx (BMP9/10)
Tribolium castaneum XP 973577.1 BMPx (BMP9/10)
Nematostella vectensis ADMP related AFP87424.1
Mus musculus GDF3 NP032134.2
Mus musculus GDF1 NP032133.2
Mus musculus BMP3 NP775580.1
Saccoglossus kowalevskii BMP3
Branchiostoma floridae ADMP XP002604737.1
Lottia gigantea ADMP 110168
Saccoglossus kowalevskii ADMP
Danio rerio ADMP NP571951.2
Saccoglossus kowalevskii Nodal A
Lottia gigantea Nodal ACB42423.1
Branchiostoma floridae Nodal AAL99367.1
Mus musculus Nodal AAI28019.1
Callosobruchus maculatus comp48645 c0 seq1 Maverick
Atrachya menetriesi comp106563 c0 seq1 Maverick (3 variants)
Tribolium castaneum XP 001811434.1 Maverick
Apis mellifera BMP2B/Maverick XP001122118.2
Drosophila melanogaster Maverick AAF59328.1
Saccoglossus kowalevskii TGFB2 ADB22639.1
Mus musculus TGFB 2 NP033393.2
Mus musculus TGFB 1 NP035707.1
Mus musculus TGFB 3 NP033394.2
Mus musculus GDF11 NP034402.1
Mus musculus Myostatin AAO46885.1
Branchiostoma floridae Myostatin XP002599461.1
Tribolium castaneum XP 966819.1 Myostatin
Callosobruchus maculatus comp39049 c0 seq1 Myo
Atrachya menetriesi comp104142 c0 seq2 Myo (2 variants)
Nematostella vectensis Myostatin XP 001641598.1
Saccoglossus kowalevskii GDF8/11 XP 002734819.1
Lottia gigantea Myostatin 82990
Drosophila melanogaster Myostatin AAF59319.1
Mus musculus Inhibin e NP032408.2
Apis mellifera Activin XP001123044.2
Tribolium castaneum XP 008194984.1 Act
Drosophila melanogaster Activin NP651942.2
Mus musculus Inhibin a NP032406.1
Mus musculus Inhibin b NP032407.1
Saccoglossus kowalevskii Lefty NP001164679.1
Branchiostoma floridae Inhibin beta XP002606835.1
Saccoglossus kowalevskii Activin/Inhibin NP001161496.1
Nematostella vectensis Activin ABF61781.1
Lottia gigantea Activin 151945
Apis mellifera ALP XP001122210.1
Drosophila melanogaster Dawdle (ALP) NP722840.1
Tribolium castaneum XP 970355.1 Dawdle (ALP)
Callosobruchus maculatus comp42632 c3 seq1 Dawdle (ALP)
Atrachya menetriesi comp92923 c0 seq1 Dawdle (ALP) (2 variants)
Callosobruchus maculatus comp30559 c0 seq2 putative Derriere (2 variants)
Tribolium castaneum XP 008191586.1 putative Derriere
Mus musculus BMP15 NP033887.1
Mus musculus GDF9 NP032136.2
Mus musculus Neurturin NP 032764.1
Danio rerio GDNF NP 571807.1
Mus musculus GDNF NP 034405.1
Xenopus laevis GDNF NP 001090196.1
0.5
Dpp / BMP2/4
GDF 5/6/7
Gbb /BMP 5/6/7/8
BMPx / BMP 9/10
GDF 1/3
ADMP / BMP3
Nodal
Maverick
TGF β
Myostatin / GDF 8/11
Inhibin / Activin
Lefty
Inhibin / Activin
Dawdle / ALP
Unknown Derriere-Like
BMP15 / GDF9


A) Wnt Signalling:
Canonical Pathway:
Cer1
FRP
Dally
Porc
Wnt
Frizzled
LRP5/6
Wif-1
SOST
Dkk
BAMBI
IDAX
CKIε
GBP
Scaffold
Dvl
+P
GSK3β
β-catenin
CK2
Axin
APC
CKIα
Axam
Alzheimer's Disease
PS-1
PKA
MAPK Signalling Pathway
TAK1
+P
NLK
Adherens Junctions
Duplin
CBP
Pontin52
+P
TCF/LEF
DNA
SMAD4
SMAD3
ICAT
XSox17
CtBP
Groucho
c-myc
c-jun
fra-1
cyc-D
PPARδ
Uterine
Cell Cycle
TGF Signalling
+P
B-TrCP
Phosphorylation Independence
Skp1
Cul1
Rbx1
Ubiquitin Mediated Proteolysis
+u
Proteolysis
p53 Signalling Pathway
p53
Siah-1
SIP
APC
Skp1
TBL1
Phosphorylation Independence
PAR-1
Nkd
Planar Cell Polarity Pathway:
Sfom
Prickle
Wnt11
Frizzled
Knypek
Dvl
Daam1
RhoA
ROCK2
Focal Adhesion
Cytoskeletal Changes
Rac
JNK
Gene Transcription
MAPK Signalling Pathway
Wnt/Ca2+ Pathway:
Wnt5
Frizzled
G Protein?
PLC
Ca2+
CaMKII
CaN
PKC
-P
NFAT
DNA
Onward Effects
B) Hedgehog Signalling:
Hip
Gas1
Hedgehog
Ptc
Smo
Megalin
Cholesterol
Rab23
Microtubule
Cos2
Fu
Su(fu)
Ci
PKA
GSK3β
CK1
Slmb
Zic2
Wnt
Dpp
Ptc
Wnt Signalling
TGF Signalling
C) Notch Signalling:
Fringe
Dvl
Numb
Delta
Delta
Notch
Serrate
TASE
PSE2
PSEN
NCSTN
APH-1
γ Secretase Complex
Notch Intracellular Domain
Co-activators
MAML
HATS
SKIP
CSL
DNA
HES1/5
PreTα
Others
Hairless
SMRT
CtBP
Groucho
CIR
Co-repressors
HDAC
Via MAPK/RAS
MAPK Signalling
Key:
Present Both
Present A.menetriesi
Present C. maculatus
Absent Both
Coleopteran Absence (?)
Other Pathway
Cell Membrane
Internal Membrane
Up-regulation or Movement
Down-regulation
State Change
Disassociation