

Title: Differential gene expression between polymorphic zooids of the marine bryozoan *Bugulina stolonifera*

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SUPPLEMENTAL INFORMATION

Table S1: Sample preparation dates for RNA-sequencing of *Bugulina stolonifera* tissue-specific samples (three biological replicates each) and one sample of *Rhodomonas salina*.

Sample	Cultured	Fixed	Dissected	Extracted	cDNA	PCR Amp.	qPCR	Sequencing
Autozoid, Mature 1	7/1/2015	8/17/2015	4/1/2016	4/1/16	4/1/16	4/5/2016	4/25/17	5/4/17
Autozoid, Mature 2	7/1/2015	8/17/2015	2/9/2016	2/9/2016	3/11/16	4/5/2016	4/25/17	5/4/17
Autozoid, Mature 3	7/1/2015	8/17/2015	3/9/16	3/9/16	3/11/16	4/5/2016	4/25/17	5/4/17
Autozoid, Bud 1	7/1/2015	8/17/2015	4/1/16	4/1/16	3/11/16	4/5/2016	4/25/17	5/4/17
Autozoid, Bud 2	6/17/2016	8/16/2016	3/24/17	3/24/17	3/25/17	3/26/17	4/25/17	5/4/17
Autozoid, Bud 3	7/1/2015	8/17/2015	3/9/16	3/9/16	3/11/16	4/5/2016	4/25/17	5/4/17
Avicularium, Mature 1	6/17/2016	8/14/16	8/15/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Avicularium, Mature 2	6/17/2016	8/14/16	8/15/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Avicularium, Mature 3	6/17/2016	8/14/16	8/16/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Avicularium, Bud 1	6/17/2016	8/14/16	8/15/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Avicularium, Bud 2	6/17/2016	8/14/16	8/16/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Avicularium, Bud 3	6/17/2016	8/14/16	8/16/16	2/17/17	3/11/17	3/11/17	4/25/17	5/4/17
Rhizoid, Network 1	7/1/2015	8/17/2015	4/1/16	4/1/16	4/1/16	4/5/2016	4/25/17	5/4/17
Rhizoid, Network 2	6/17/2016	8/14/16	3/24/17	3/24/17	3/25/17	3/26/17	4/25/17	5/4/17
Rhizoid, Network 3	6/17/2016	8/14/16	3/24/17	3/24/17	3/25/17	3/26/17	4/25/17	5/4/17
Rhizoid, Autozoid 1	7/1/2015	8/17/2015	4/1/16	4/1/16	4/1/16	4/5/2016	5/25/16	5/27/16
Rhizoid, Autozoid 2	6/17/2016	8/14/16	3/24/17	3/24/17	3/25/17	3/26/17	4/25/17	5/4/17
Rhizoid, Autozoid 3	6/17/2016	8/14/16	3/24/17	3/24/17	3/25/17	3/26/17	4/25/17	5/4/17
<i>Rhodomonas salina</i>	6/11/2016	8/8/16	8/8/16	8/8/16	8/12/16	8/13/16	8/28/16	8/29/16

11 **Table S2: Trinity transcriptome assembly metrics for *Rhodomonas salina*.**

<i>Stats based on all transcriptome contigs:</i>			<i>Stats based on longest isoform per gene:</i>			
Genes (#)	Transcripts (#)	GC (%)	Contig N50 (bp)	Median Contig (bp)	Average Contig (bp)	Total Bases (#)
57,502	64,951	53.19	416	286	404.69	26,285,090

32 **Table S3. Annotation of differentially expressed genes up-regulated in autozoid**
33 **and avicularium tissue** (4-fold expression, FDR $p < 0.001$). Annotated transcripts of
34 selected differentially expressed genes from the 'Autozoid and Avicularium'
35 transcriptome, with annotations are listed for several functional categories including
36 WNT and Notch signaling pathways, heat response, nervous system and muscular
37 system.

	Zooid Type Up-regulated	Transcript ID	log Fold Change	PValue	False Discovery Rate	Uniprot ID	Protein Name	Gene Name
▼ WNT signaling pathway								
	Autozooid	138519_c0_g1	11.65	6.26E-12	6.25E-08	RN213_HUMAN	E3 ubiquitin-protein ligase	<i>RNF213</i>
	Autozooid	104535_c1_g10	9.21	4.83E-08	2.32E-05	NPHP3_XENTR	Nephrocystin-3	<i>nphp3</i>
	Autozooid	97261_c0_g3	8.99	2.63E-07	7.78E-05	RN213_MOUSE	E3 ubiquitin-protein ligase	<i>RNF213</i>
	Avicularium	144339_c0_g1	7.64	1.4E-06	0.00024827	GBLP_RAT	Receptor of activated pr otein C kinase 1	<i>Rack1</i>
▼ Notch signaling pathway								
	Autozooid	95770_c0_g2	5.99	4.6E-06	0.000578	DTX3_PONAB	Probable E3 ubiquitin-protein ligase	<i>DTX3</i>
	Avicularium	104168_c1_g1	4.35	9.21E-10	1.59E-06	NOTC1_XENTR	Neurogenic locus notch homolog pr otein 1	<i>notch1</i>
▼ Heat Response								
	Avicularium	55059_c0_g2	9.76	5.01E-09	4.63E-06	HS902_ARATH	Heat Shock Protein 90-2	<i>HSP90-2</i>
	Avicularium	190626_c0_g1	8.64	5.03E-06	0.000602	KCRM_CHICK	Creatine kinase M-type	<i>CKM</i>
	Autozooid	102041_c0_g5	7.92	4.05E-10	1.02E-06	HSP70_MAIZE	Heat Shock 70 Protein	<i>HSP70</i>
	Avicularium	102041_c0_g4	6.50	3.74E-06	0.000497	MD37C_ARATH	Heat Shock 70 kDa Protein 4	<i>HSP70-4</i>
▼ Nervous System								
	Autozooid	9660_c0_g1	11.62	8.08E-10	1.51E-06	RL10_DROME	60X ribosomal protein L10	<i>RpL10</i>
	Autozooid	104015_c0_g1	8.11	1.1E-06	0.000209	CFI1_CAEEL	AT-rich interactive domain-containing pr otein cfi-1	<i>cfi-1</i>
	Autozooid	107873_c0_g4	7.73	9.58E-09	7.51E-06	FBX38_HUMAN	F-box only protein 38	<i>FBXO39</i>
	Autozooid	106834_c0_g1	6.18	6.65E-06	0.000721	IF4G1_MOUSE	Eukaryotic translation initiation factor 4 gamma 1	<i>Elf4g1</i>
	Autozooid	109181_c0_g1	6.00	1.91E-08	1.18E-05	CPEB1_HUMAN	Cytoplasmic polyadenylation element-binding pr otein 1	<i>CPEB1</i>
	Autozooid	107498_c0_g1	5.54	6.06E-09	5.32E-06	MATN2_MOUSE	Matrilin-2	<i>Matn2</i>
	Autozooid	101264_c0_g2	4.46	3.15E-07	9.03E-05	CEL_RAT	Bile salt-activated lipase	<i>Cel</i>
	Avicularium	147413_c0_g1	9.29	4.34E-06	0.000558	APOD_BOVIN	Apolipoprotein D	<i>APOD</i>
	Avicularium	82063_c0_g1	9.26	1.45E-08	9.89E-06	APOD_RAT	Apolipoprotein D	<i>APOD</i>
	Avicularium	136775_c0_g1	8.65	4.59E-06	0.000578	IF4E_DROME	Eukaryotic translation initiation factor	<i>elf4E1</i>
	Avicularium	193557_c0_g1	8.43	3.25E-06	0.000447	TEN3_DANRE	Teneurin-3	<i>tenm3</i>
	Avicularium	165368_c0_g1	7.48	2.65E-07	7.83E-05	TENA_MOUSE	Tenascin	<i>Tnc</i>
	Avicularium	71212_c0_g1	6.90	4.57E-06	0.000577	TENA_MOUSE	Tenascin	<i>Tnc</i>
	Avicularium	190643_c0_g1	6.88	3.99E-06	0.000522	TEN1_CHICK	Teneurin-1	<i>TENM1</i>
▼ Muscular System								
	Autozooid	104015_c0_g1	8.11	1.1E-06	0.000209	DRI_DROME	Protein dead ringer	<i>retn</i>
	Autozooid	109395_c1_g3	7.36	1.33E-06	0.000242	RHOAA_DANRE	Rho-related GTP-binding protein RhoA-A	<i>rhoaa</i>
	Autozooid	88683_c0_g1	6.08	6.59E-07	0.000151	M10L1_MOUSE	RNA helicase Mov10l1	<i>Mov10l1</i>
	Avicularium	147413_c0_g1	9.29	4.34E-06	0.00055831	APOD_BOVIN	ApolipoproteinD	<i>APOD</i>
	Avicularium	82063_c0_g1	9.26	1.45E-08	9.89E-06	BLC_ECOLI	Outer membrane lipoprotein Blc	<i>blc</i>
	Avicularium	136775_c0_g1	8.65	4.59E-06	0.000578	IF4E_DROME	Eukaryotic translation initiation factor 4E1	<i>elf4E1</i>
	Avicularium	162086_c0_g1	8.32	2.03E-06	0.000321	RAC1_DROME	Res-related protein Rac1	<i>Rac1</i>
	Avicularium	85049_c0_g3	7.70	6.6E-06	0.000718	TCAF_XENLA	TRPM8 channel-associated factor homolog	<i>tcaf</i>
	Avicularium	165368_c0_g1	7.48	2.65E-07	7.83E-05	TENA_MOUSE	Tenascin	<i>Tnc</i>
	Avicularium	71212_c0_g1	6.90	4.57E-06	0.000577	TENA_MOUSE	Tenascin	<i>Tnc</i>
	Avicularium	115838_c0_g1	6.71	4.5E-06	0.000572	TITN_MOUSE	Titin	<i>Ttn</i>
	Avicularium	112663_c0_g1	4.53	3.34E-08	1.76E-05	CO4A1_CAEEL	Collagen alpha-1(IV) chain	<i>emb-9</i>
	Avicularium	98082_c1_g1	4.22	4.27E-07	0.000114	UNC87_CAEEL	Protein unc-87	<i>unc-87</i>
	Avicularium	111938_c1_g4	4.13	1.77E-07	5.86E-05	UNC87_CAEEL	Protein unc-87	<i>unc-87</i>
	Avicularium	109962_c0_g1	4.03	1.29E-09	1.92E-06	STA5B_MOUSE	Signal transducer and activator of transcription 5b	<i>Stat5b</i>

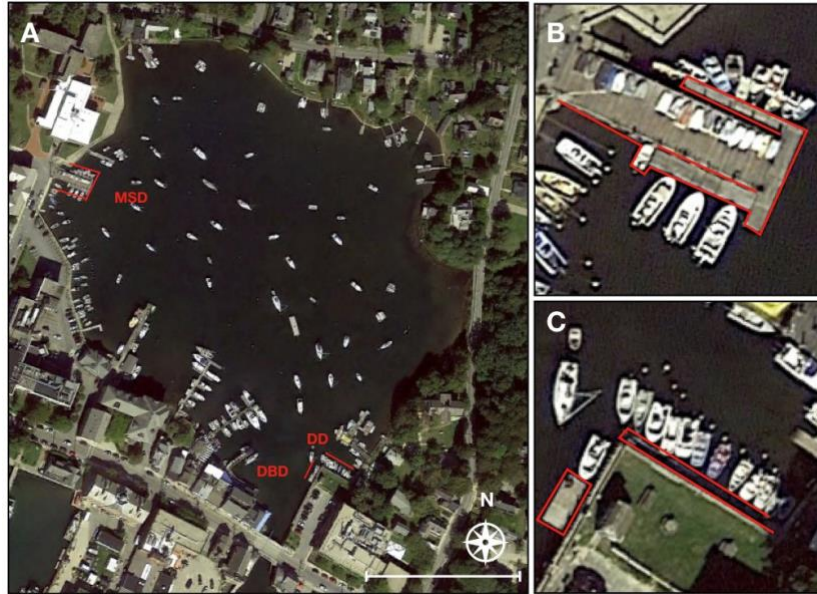
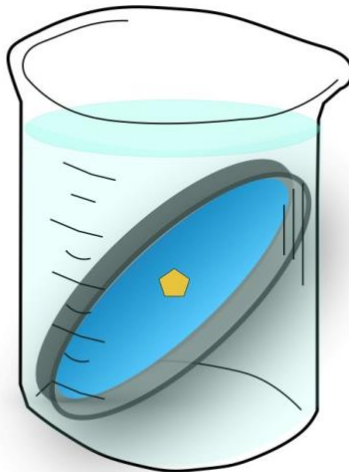


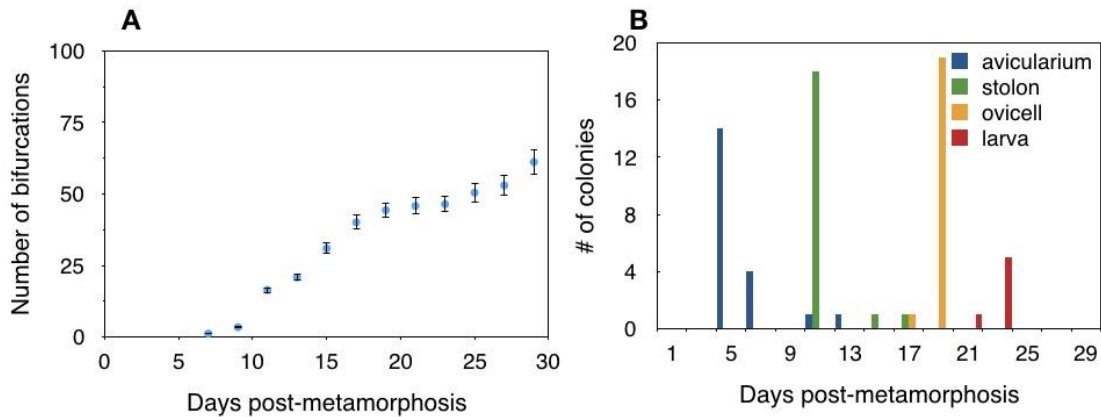
Figure S1: Bryozoan collection sites. A) Eel Pond, located at 41°31'32.40" N 70°40'13.25" W. Scale bar is 100 M. DBD- Drawbridge Dock, DD- Dinghy Dock, and MSD- MBL St. Dock. B) MBL St. Dock, zoomed in to show collection transects. C) Drawbridge Dock (to west) and Dinghy Dock (to east) collection transects. Adapted from Google Earth.



47 **Figure S2: Bryozoan culture.** Each individual (yellow), metamorphosed onto a 45
 48 mm acetate disc (blue), was seated into a 45mm diameter petri dish (grey). Dishes
 49 were oriented vertically in 150 mL beakers, such that ancestrulae were oriented
 50 downwards, and beakers were filled with 125 mL Eel Pond Seawater (EPSW). 30
 51 mm glass dishes were wedged over the petri dishes to keep them submerged and
 52 suspended in place (not depicted).

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56 **Figure S3: Bryozoan colony growth.** *B. stolonifera* colonies were cultured in the
 57 lab for one month, from metamorphosis on July 2, 2015. **A)** Colony growth
 58 (measured as number of bifurcations) is plotted over time. Error bars indicate
 59 Standard Error. **B)** Each colony was monitored every other day for the first
 60 appearance of polymorphic zooids (avicularia, stolons, ooecia) and larvae. The
 61 days post-metamorphosis of the first appearance of these polymorphic zooids is
 62 plotted over the course of one month of culturing. (n = 23 colonies)

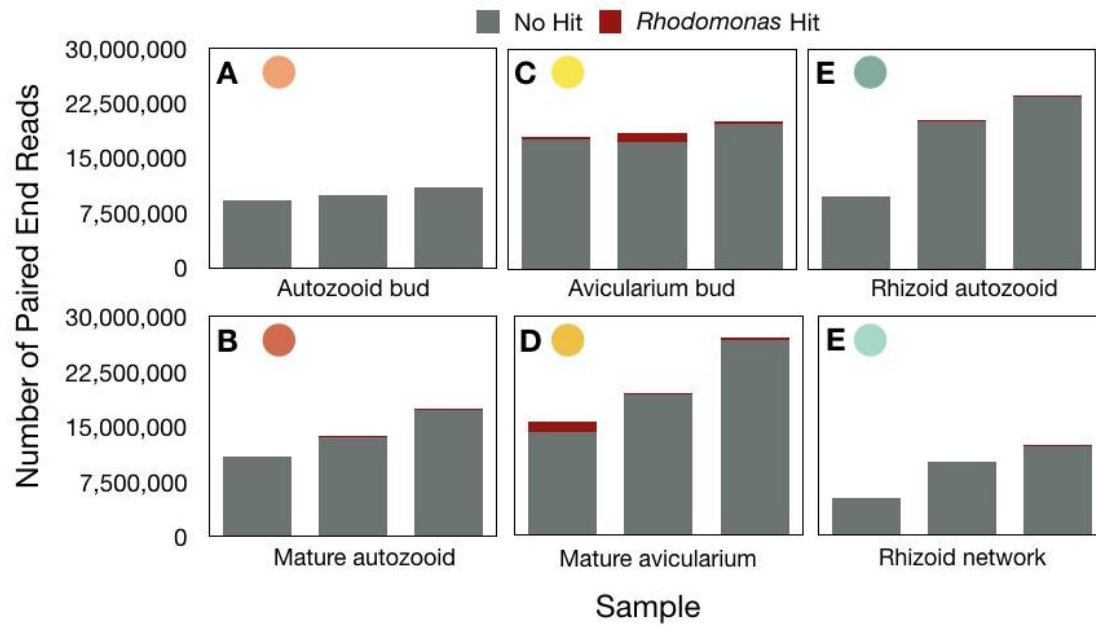


Figure S4: Removal of *R. salina* contamination All bryozoan paired reads were mapped to a transcriptome of the cryptophyte using Bowtie2. In cases where one or both pairs mapped to the *R. salina* transcriptome, those reads were removed from the dataset.

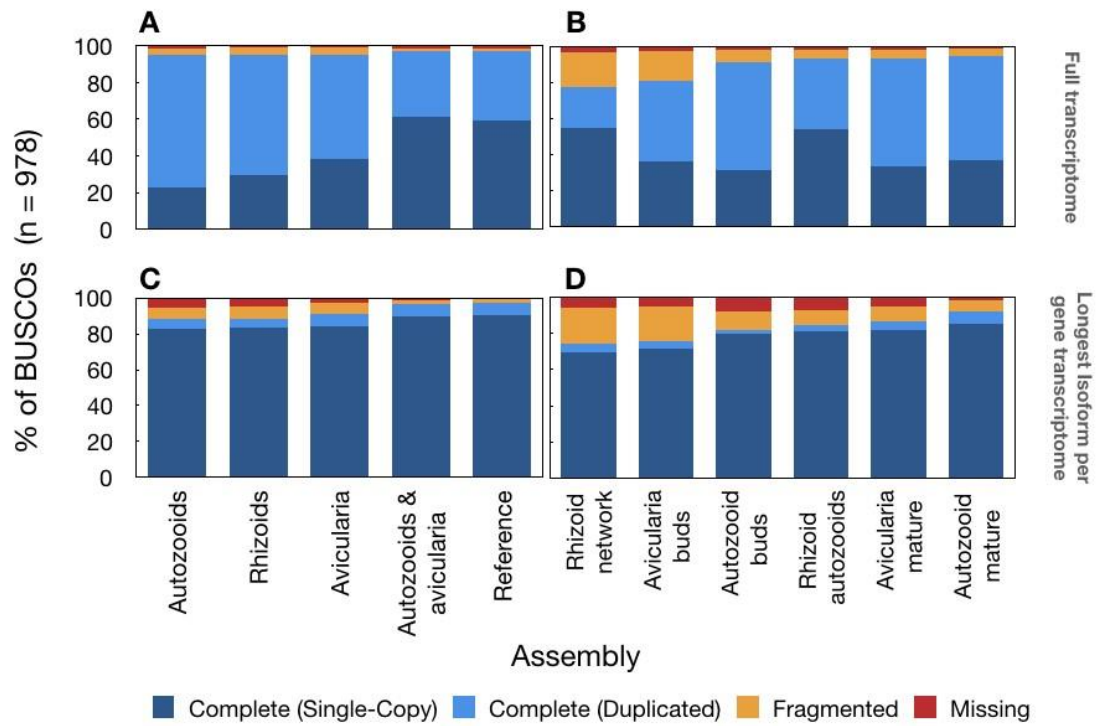


Figure S5: BUSCO analysis of assembly completeness for A, C) tissue-specific and B,D) sample-specific assemblies of *B. stolonifera*, comparing BUSCO results for the complete transcriptome dataset (all isoforms included) A, B) with BUSCO results for a dataset composed of the longest isoform per gene C, D). Transcriptomes were searched using the *metazoa odb9* database which contains a total of 978 curated single-copy gene orthologs.

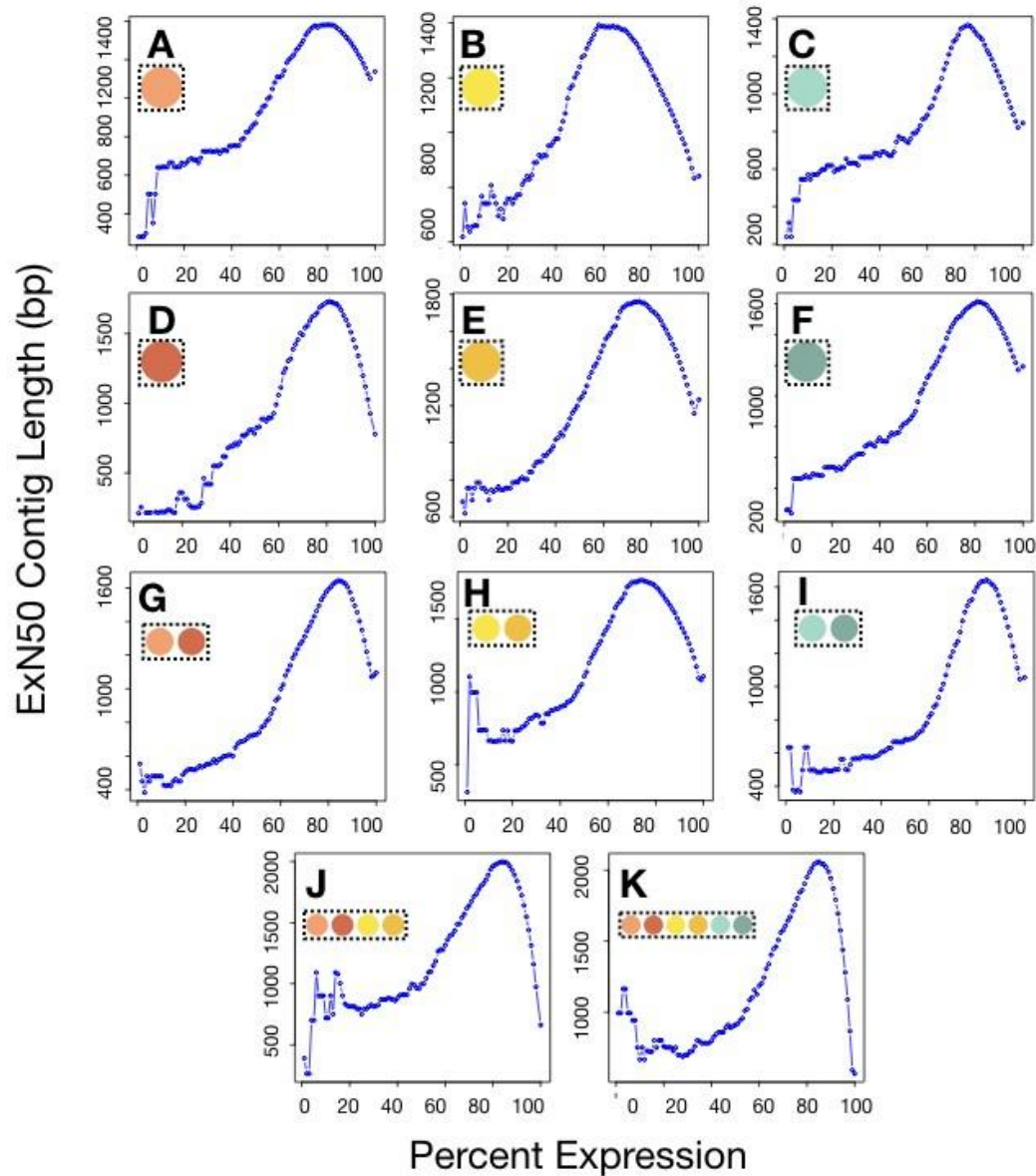


Figure S6: ExN50 Plots. Contig N50 (the size of contigs where half of the assembled bases are found) is plotted by percent (normalized) gene expression for each transcriptome assembly. **A)** Autozoid bud assembly, **B)** Avicularium bud assembly, **C)** Rhizoid network assembly, **D)** Mature autozoid assembly, **E)** Mature avicularium assembly, **F)** Rhizoid autozoid assembly, **G)** Autozoid assembly, **H)** Avicularium assembly, **I)** Rhizoid assembly, **J)** Autozooids and avicularia assembly, and **K)** Reference assembly.

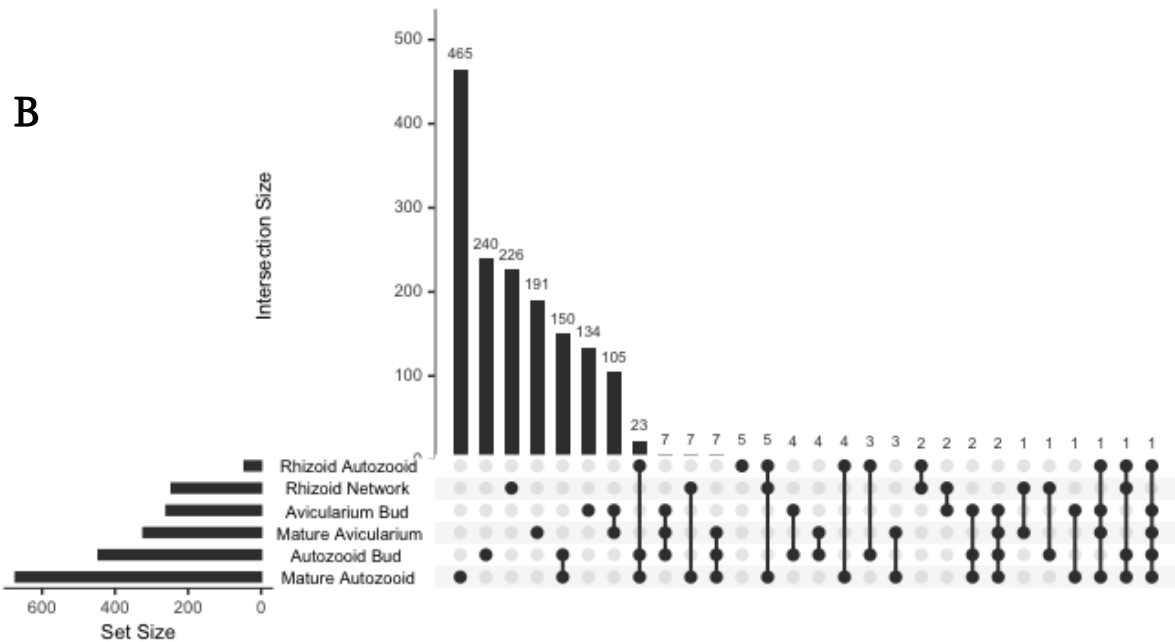
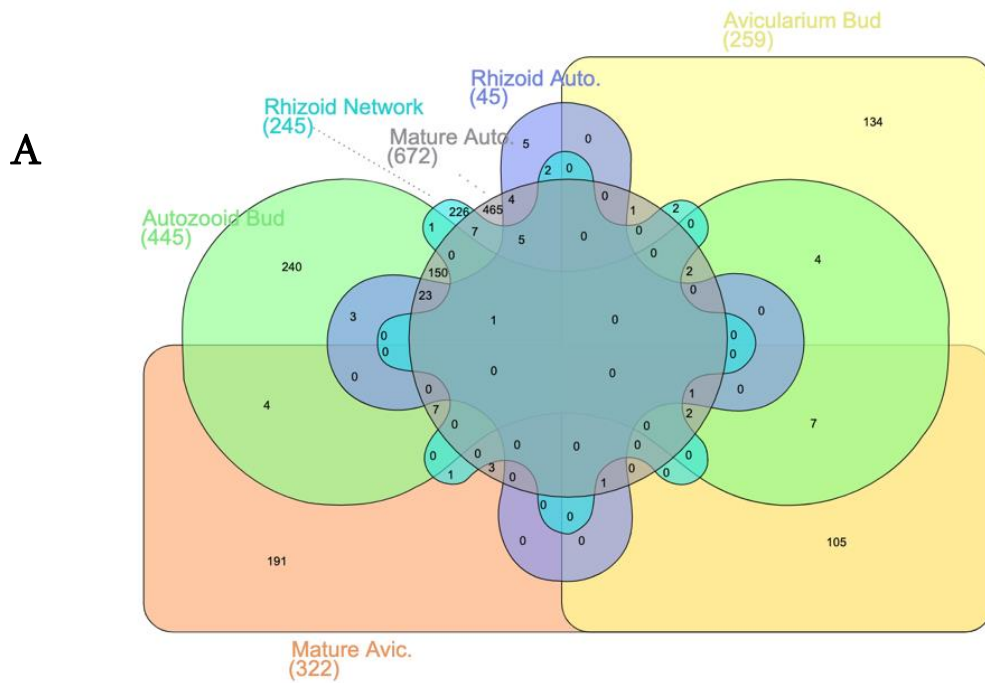
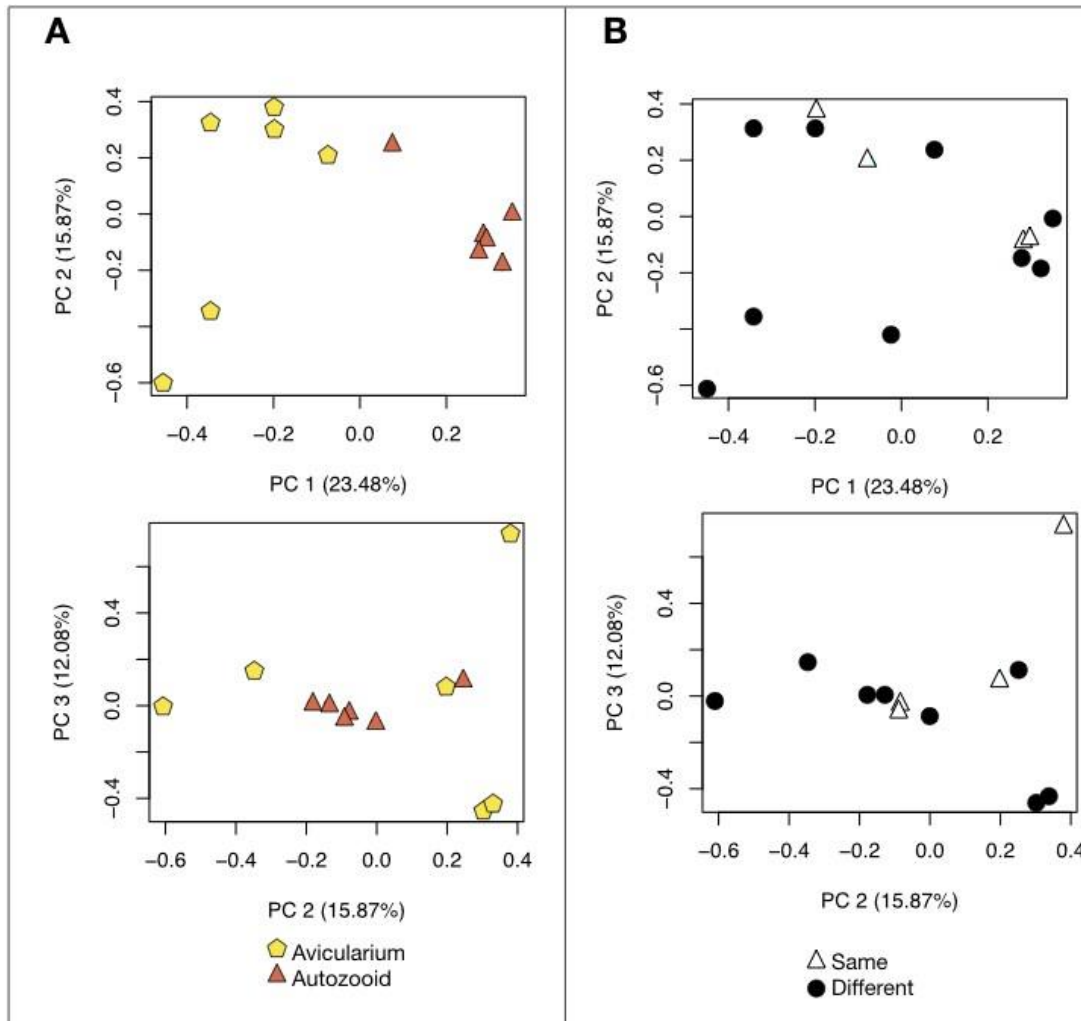


Figure S7: Shared and significantly up-regulated gene transcripts of each zooid type. Average expression (at 4-fold or greater level, FDR $p < 0.001$) was calculated across sample replicates from pairwise comparisons in the reference transcriptome and summarized as sample-specific up-regulated gene expression

95 for each zooid type. The set of 1,597 differentially differently expressed genes
96 are depicted as a **A**) six-way Venn diagram (Heberle *et al.*, 2015), with shared
97 genes between tissue types depicted as areas of overlap and the count of unique
98 genes for each tissue type depicted as areas without overlap, and as an **B**) UpSet
99 plot (Conway *et al.*, 2017) where unique differentially expressed gene counts are
100 depicted for each unique tissue type as well as for subsets of multiple tissue types.
101 Sample abbreviations are as follows: “Mature Auto.” = Mature autozooid, “Mature
102 avic.” = Mature avicularium, “Rhizoid auto.” = Rhizoid autozooid.
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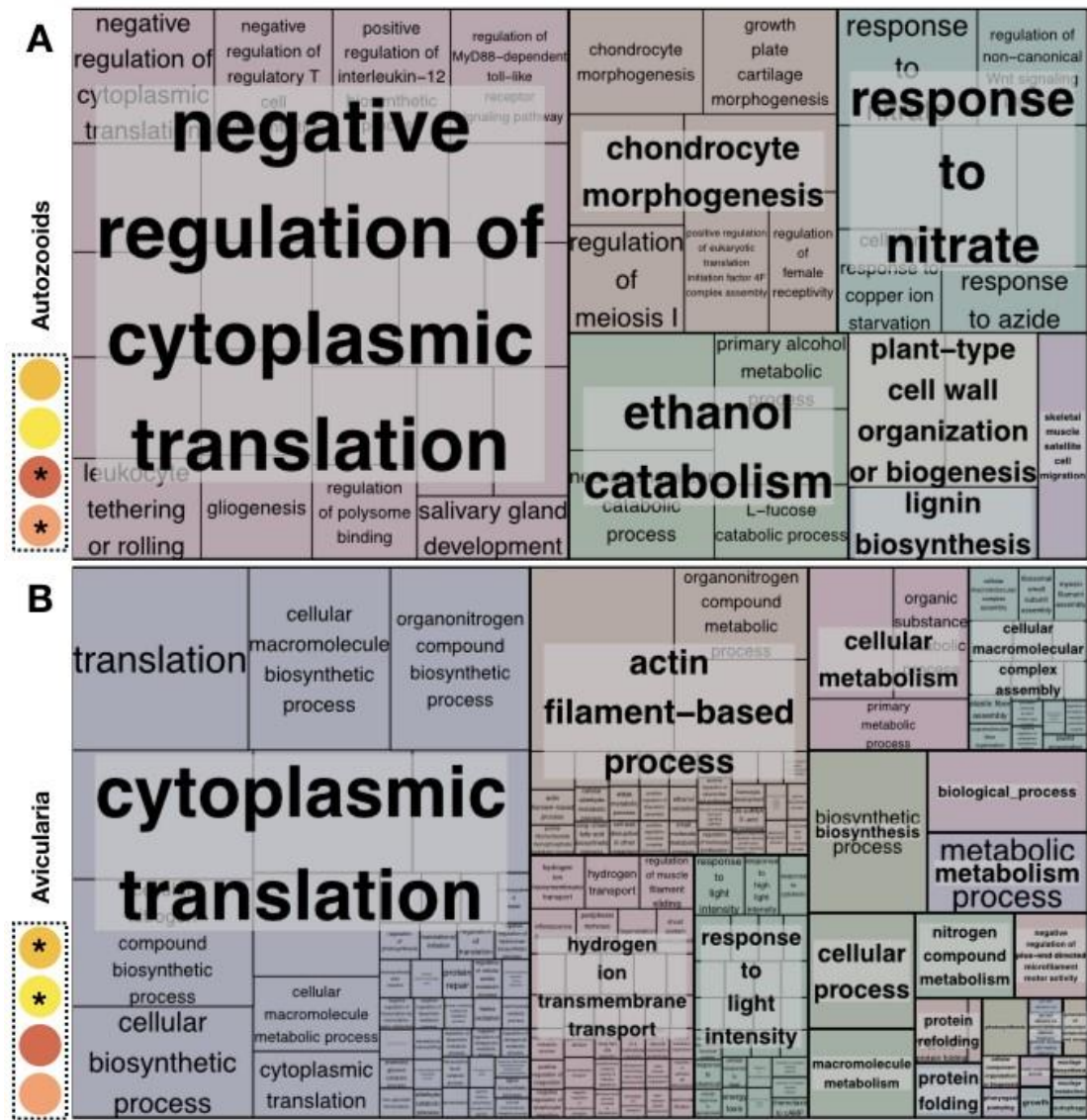


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107 **Figure S8: Autozoid and avicularium transcriptome sample relationships.**
 108 Principal components analysis (plotted as PC1 vs. PC2 and PC2 vs. PC3) of the
 109 log₂ gene count matrix demonstrates the relative similarity of transcripts in each
 110 sample in the autozoid and avicularium transcriptome assembly. **A)** Samples are
 111 labeled by tissue type and developmental stage ('auto' = mature autozoid and
 112 autozoid bud samples, 'avic' = avicularium bud and mature avicularium
 113 samples). **B)** Samples are labeled by genetic individual, where samples labeled
 114 'diff.' originate from genetically distinct colonies and samples labeled 'same'

represent six tissue samples of different zooid type and stage, that originated from the same genetic individual.

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121 **Figure S9: Autozoooid and avicularium transcriptome gene ontology, biological**
122 **processes. A) enriched GO terms found in genes that are up-regulated in**

