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## Gametophytic transcriptomics of an early embryophyte (*Blasia pusilla*) and comparative landscape of gametophyte evolution

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### Introduction:

Transcriptomics plays a central role in understanding the genetic underpinning of post-transcriptional regulation. In recent years, transcriptomics has not only acted as a model for unraveling post-transcriptional regulatory events but has also provided a basis for species-level comparative transcriptomics. In the absence of genomic data, transcriptomics not only allows for *de-novo* phylo-transcriptomics but also enables the detection of specific genes, which are specific to certain lineages or clades. We present the first reference gametophytic transcriptome of *Blasia pusilla* sequenced using Illumina Next-Seq with the aim of unravelling the gametophytic transcriptomics of an early embryophyte. We also highlight the role of transcriptomics in identifying early genes and possible phylogenetic pressures on ancestral gene evolution across embryophytes (land plants).

### Material and Methods:

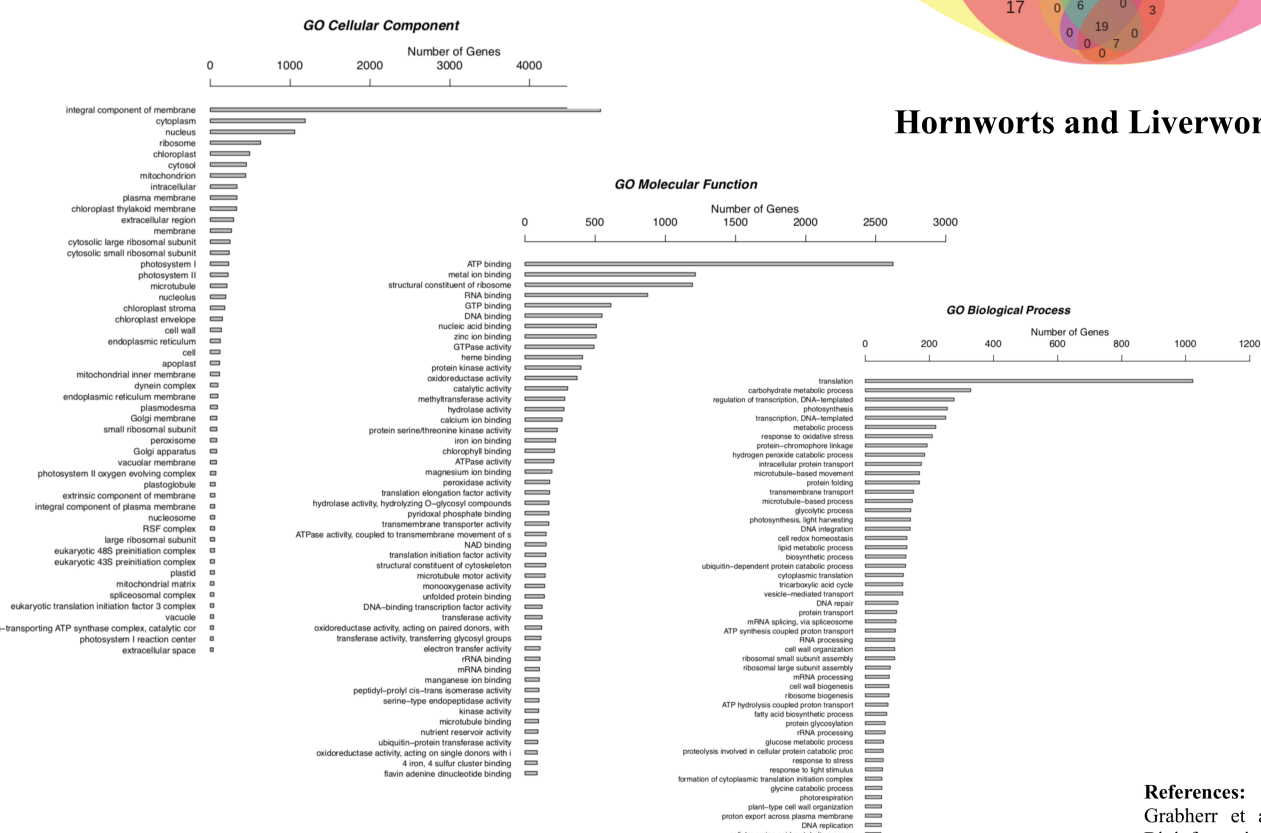
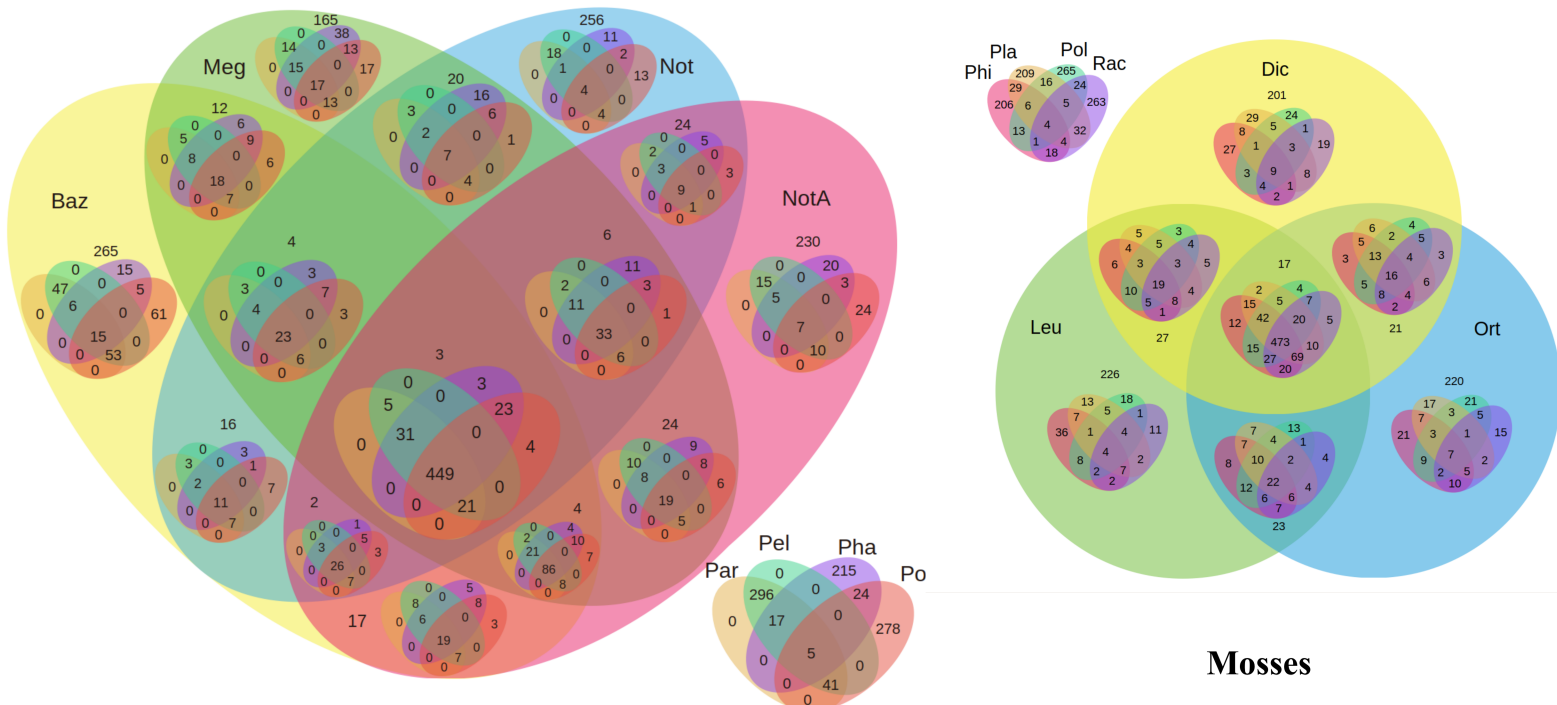
*In-vitro* grown gametophytic cultures of *B. pusilla* on MS medium were used for RNA extractions using the Biotop RNA Extraction kit (ISOLATE II RNA Plant Kit (BIO-52076)) and were subsequently used for library preparation and sequencing using Illumina Next-Seq at Institute of Biotechnology, University of Helsinki, Finland. In total, 555,696,062 M with an (87.086811%) over Q30 were further used for the transcriptome reconstruction using the Trinity assembler version 2.6.4. Gametophyte transcriptome of the representative species were downloaded from IKP (<http://www.onekp.com>) and were compared for the orthologous transcripts using ProteinOrtho (Lechner et al. 2011). Venn diagram for the shared orthologous proteins across the gametophytes were plotted using the VennPainter (Lin et al. 2016). Annotations for the assembled transcriptome were done using the eggNOG-mapper with virNOG as the backhand database (<http://eggnogdb.embl.de/#/app/emapper>). Additionally, annotations were done by performing the BLASTx searches (1E-5) against plant species UNIPROT databases available from (<https://www.uniprot.org>). Transcriptome completeness was accessed using DOGMA (Dohmen et al. 2016) and BUSCO (Waterhouse et al. 2017) using embryophytes as a lineage.

### *Blasia pusilla* gametophyte transcriptome summary statistics

<i>Blasia pusilla</i> assembled transcripts	130871
<i>Blasia pusilla</i> Supertranscripts	80880
Percent GC	46.55
* <i>Blasia pusilla</i> Transcriptome statistics based on ALL transcript contigs	
Contig N50:	3191bp
Median contig length	653bp
Average contig	1533.29bp
* <i>Blasia pusilla</i> Transcriptome statistics based on longest isoform	
Contig N50	2334bp
Median contig length	395bp
Average contig	921.32bp
Number of CDS predicted using GeneMark-ST (Transcriptome)	60812
Number of CDS predicted using GeneMark-ST (Supertranscripts)	24336
Number of transcripts associated virNOG:	39499
Number of supertranscripts associated virNOG:	11696
Number of transcripts mapped to KEGG:	22996
Number of supertranscripts mapped to KEGG:	7199
Domain assessment of transcriptome completeness: 59.28% completeness representing 77.25% (CDAsize 1), 55.39% (CDAsize2)	
BUSCO assessment of transcriptome completeness: 44% completeness representing 635 (CB), 610 (CSB)	

Venn diagram showing the distribution of the shared *Blasia pusilla* transcripts across the mosses, hornworts and liverworts 1KP gametophyte transcriptome. \* The first three letter defines the species in the table with (A) added to same genus name.

Species	Family	Class
<i>Philonotis fontana</i>	Bartramiaceae	Mosses
<i>Plagiomnium insignie</i>	Mielichhoferiaceae	Mosses
<i>Racomitrium elongatum</i>	Grimmiaceae	Mosses
<i>Racomitrium varium (A)</i>	Grimmiaceae	Mosses
<i>Dicranum scoparium</i>	Dicranaceae	Mosses
<i>Leucobryum albidum</i>	Leucobryaceae	Mosses
<i>Stereodon subimponens</i>	Pylaisiaceae	Mosses
<i>Orthotrichum lyellii</i>	Orthotrichaceae	Mosses
<i>Polytrichum commune</i>	Polytrichaceae	Mosses
<i>Syntrichia princeps</i>	Pottiaceae	Mosses
<i>Sphagnum lescurii</i>	Sphagnaceae	Mosses
<i>Takakia lepidiozoides</i>	Takakiaceae	Mosses
<i>Timmia austriaca</i>	Timmiaceae	Mosses
<i>Paraphymatoceros hallii</i>	Notothyladaceae	Hornworts
<i>Phaeoceros carolinianus</i>	Notothyladaceae	Hornworts
<i>Nothoceros aenigmaticus</i>	Dendrocerotaceae	Hornworts
<i>Nothoceros vincentianus (A)</i>	Dendrocerotaceae	Hornworts
<i>Megaceros flagellaris</i>	Dendrocerotaceae	Hornworts
<i>Bazzania trilobata</i>	Lepidoziaceae	Liverworts
<i>Pellia neesiana</i>	Pelliaceae	Liverworts
<i>Porella navicularis</i>	Porellaceae	Liverworts



### Gene ontology classification at cellular, biological and molecular functions.

### References:

Grabherr et al. Nat Biotechnol. 2011; 29:644-652; Lin et al. PLoS One. 2016;11(4): e0154315.; Lechner et al. BMC Bioinformatics. 2011; 12:124. Huerta-Cepas et al. Nucleic Acids Res. 2019;47(D1): D309–D314. Dohmen et al. Bioinformatics, 2016; 32:2577-81; Waterhouse et al. Mol Biol Evol. 2017; 35:543–548.

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