



Detection of genes in the fern *C. richardii* using evolutionarily-conserved orthologues with known functions for moss rhizoid and angiosperm root hair development.

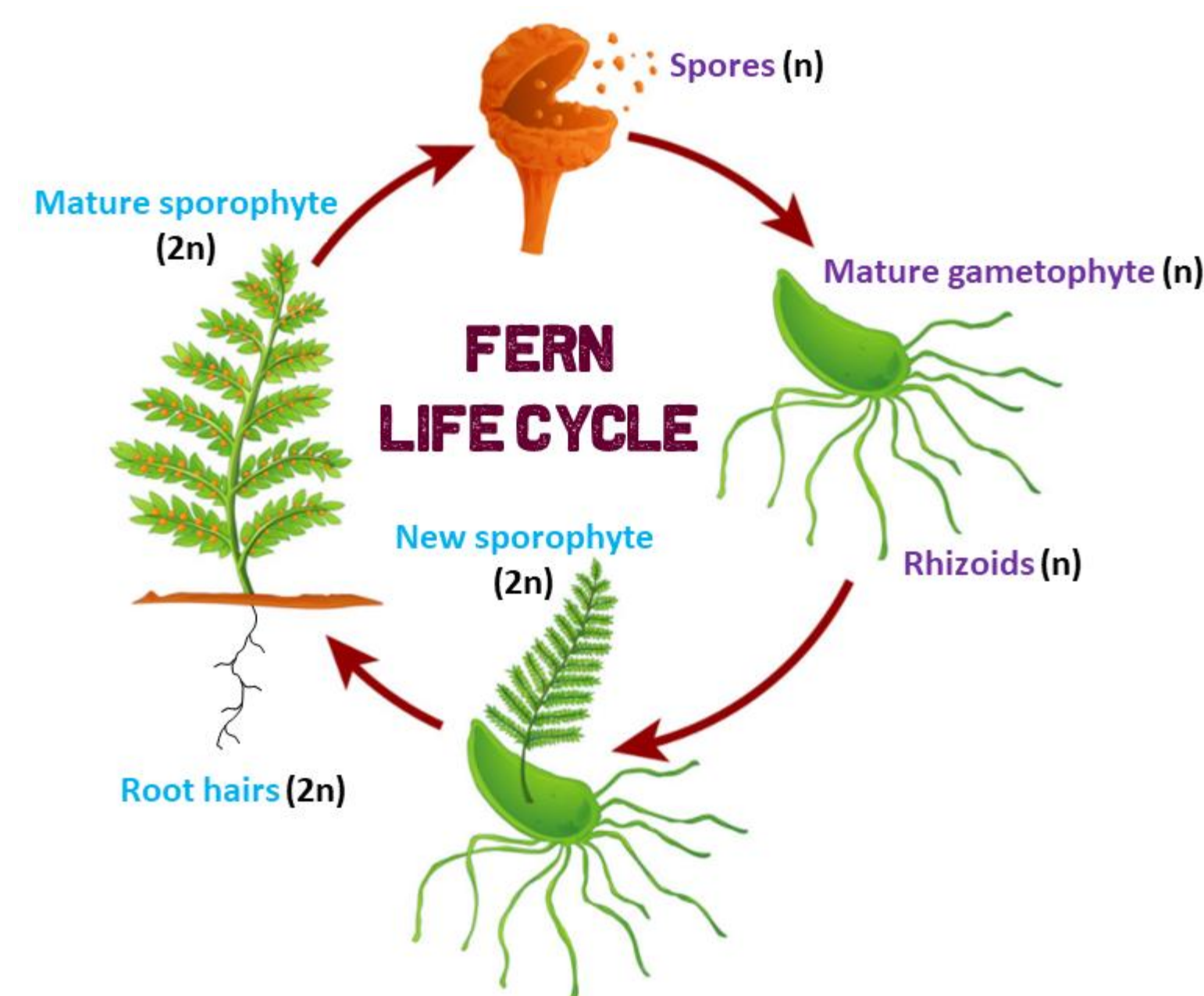
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Abstract

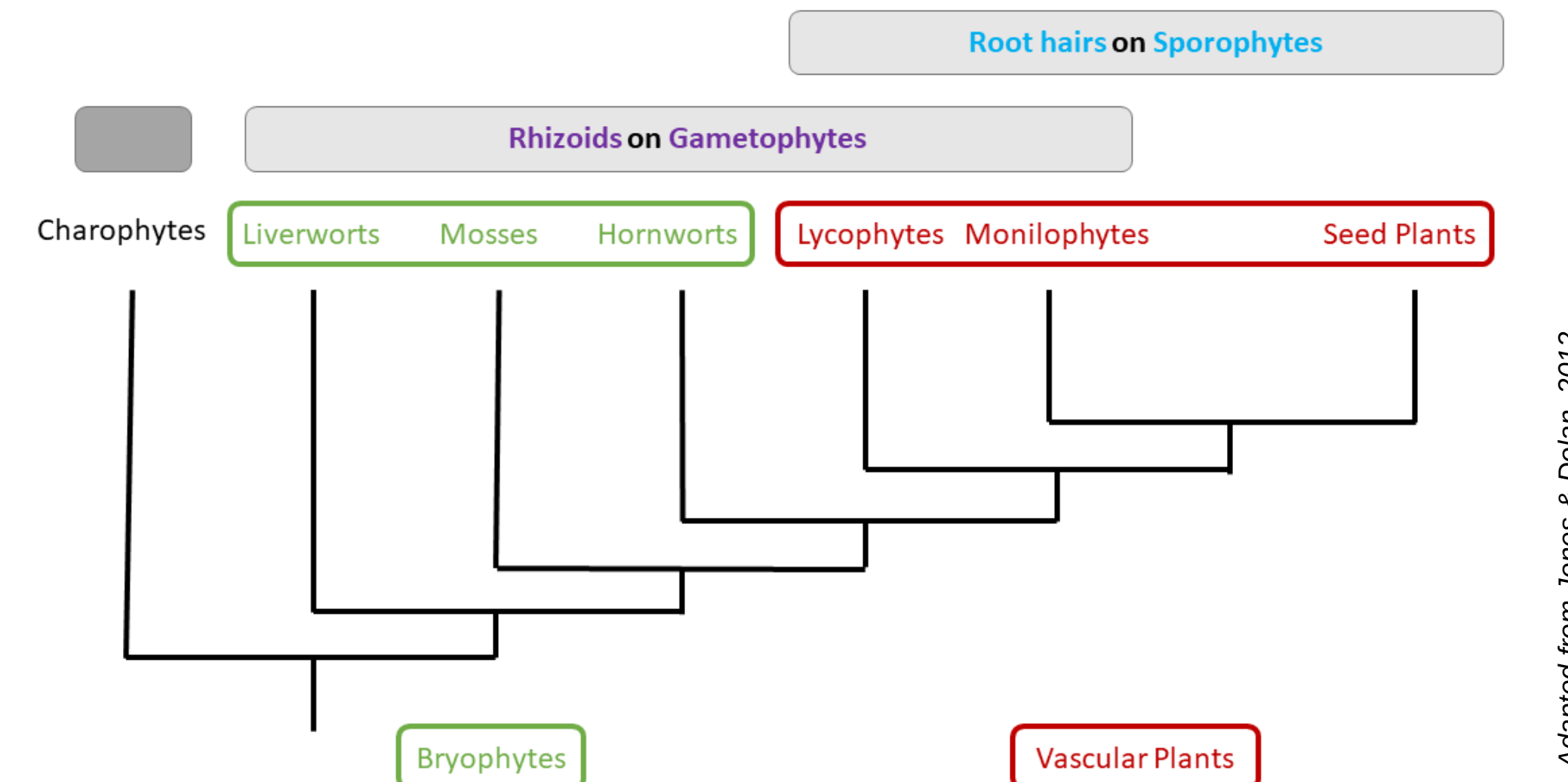
The evolution of terrestrial plants is an approximately 500-million-year story. Although all land plants execute an alternation of generations between a haploid gametophyte and a diploid sporophyte stage, the amount of time spent and dominance of each generation varies across clades. For instance, the bryophytes typified by *Physcomitrella patens*, are gametophyte-dominant and produce gametophyte structures known as rhizoids for anchoring to substrates as well as for nutrient absorption. Moss sporophytes are dependent on the moss gametophyte, and are diminutive in size, entirely lacking roots. In contrast, angiosperms like *Arabidopsis thaliana* are characterized by sporophyte-dominant stages with an exceptional spatiotemporal reduction in gametophyte structures. Such is the reduction that flowering plants rely on sporophyte root systems for anchorage and nutrient absorption, forgoing any production of rhizoids. In the middle of this plant evolutionary saga lies *Ceratopteris richardii*, a fern capable of not only producing gametophyte rhizoids but also sporophyte root systems. Great strides have been made in the elucidation of the genes required in the specification and development of sporophyte root hairs in *Arabidopsis* as well as for the development of *Physcomitrella* gametophyte rhizoids, but little is known of the specific genes utilized in ferns for similar processes. Recent advances in genomics and transcriptomics for *Ceratopteris richardii* permits orthologous gene-function searches. Here we showcase novel fern genes that are expressed in gametophyte and sporophyte stages that share predicted-peptide conservation with known moss and cress genes that are key molecular players in rhizoid and root hair development, respectively.

Alternation of Generations



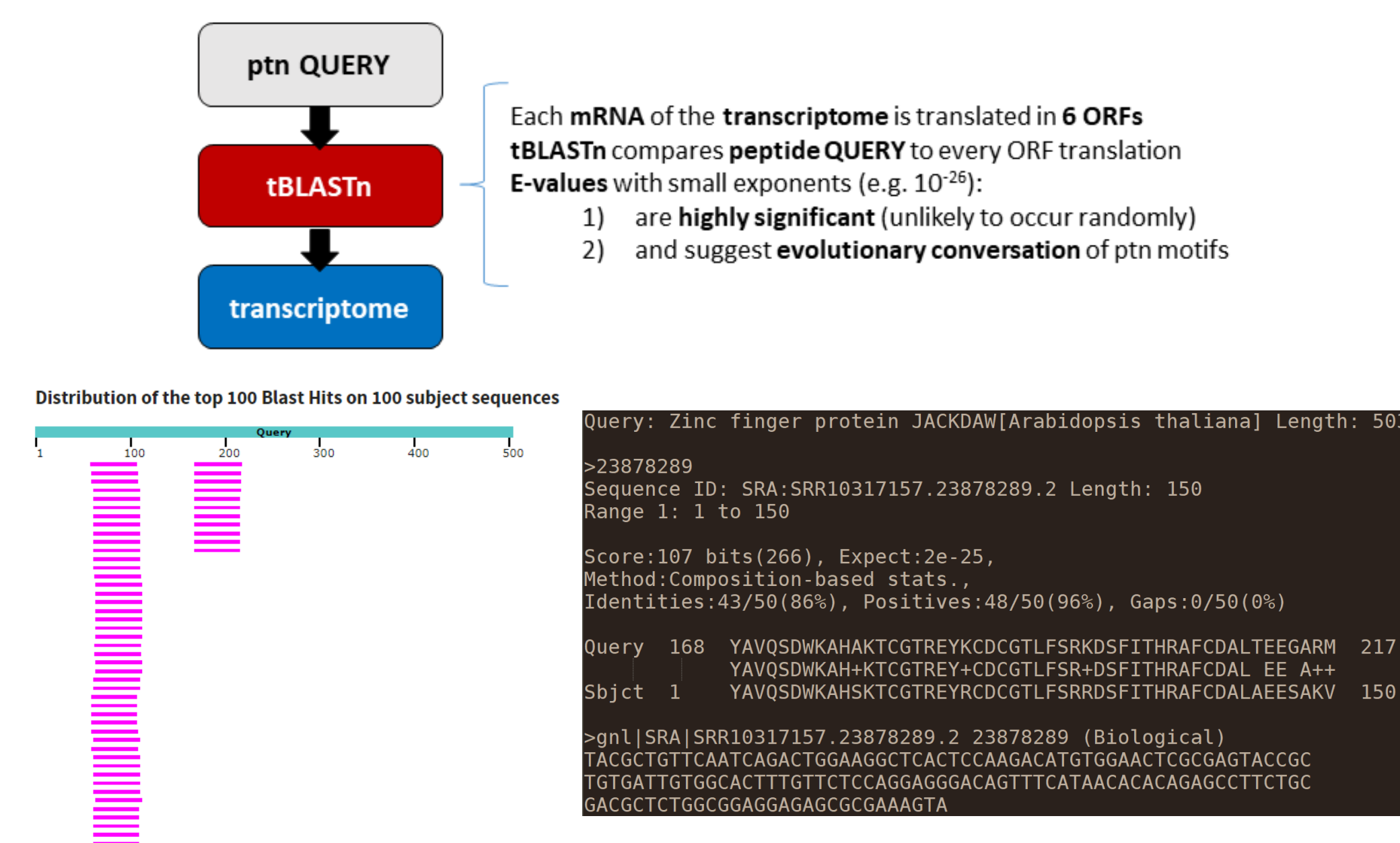
All plant lifecycles execute alternation of generations. Briefly, a haploid spore germinates into a multicellular gametophyte. Flagellated sperm fertilize eggs in archegonial chambers found on gametophytes to form a diploid zygote. Mitoses yield a mature, diploid sporophyte capable of producing haploid spores via meiosis. Ferns are uniquely capable of producing both haploid rhizoids during gametophyte stages and diploid root hairs as sporophytes.

Plant Phylogeny



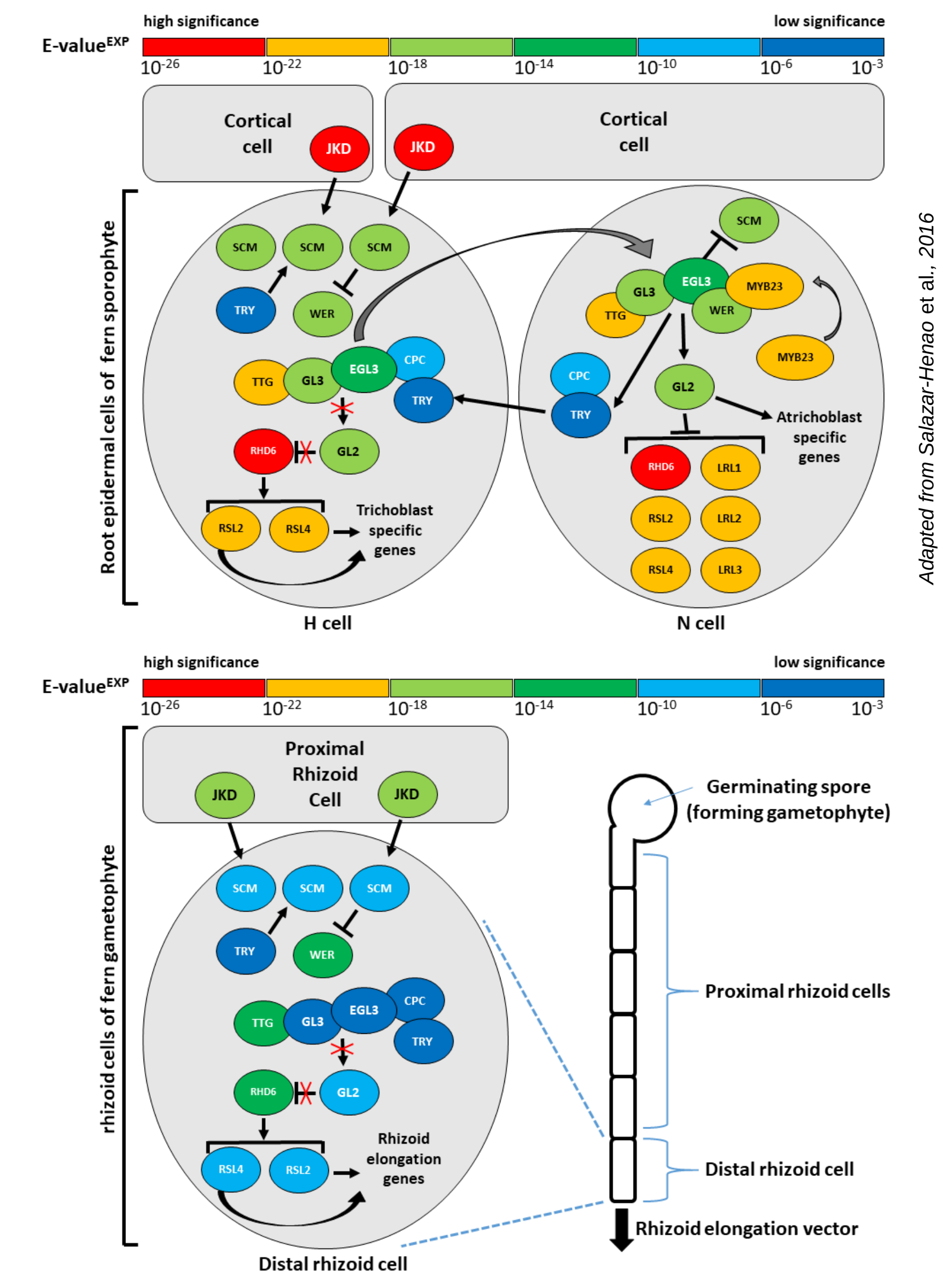
Land plants are embryophytes that descended with modification from an ancestral charophyte. Bryophytes are non-vascular plants with a dominant gametophyte life-cycle stage. All Bryophytes rely on haploid rhizoids for nutrient assimilation. Vascular plants in contrast exhibit a sporophyte-dominated life-cycle, and all vascular plants contain lignified tissue that permits xylem and phloem to conduct water and food, respectively. Some vascular plants are seedless (e.g. ferns of Monilophyta) while others evolved seeds (e.g. gymnosperms and angiosperms). The flowering plants (angiosperms) lack rhizoids and assimilate nutrients via diploid root hairs. Ferns are a transition organism: gametophyte-stage ferns are bryophyte-like and have rhizoids; sporophyte-stage ferns are angiosperm-like with root hairs.

Bioinformatics Methodology: tBLASTn



The tBLASTn method aligns peptide sequences. As such, the algorithm tolerates synonymous mutations (i.e. of redundancy in the genetic code). Over evolutionary time, as nucleic acids change, conserved protein domains and motifs of essential processes remain constant at the amino acid level.

Putative fern orthologous genes



tBLASTn results are shown using known genes (as ptn queries) for root hair development in the angiosperm, *Arabidopsis thaliana* against existing sporophyte and gametophyte transcriptome databases (as 6-ORF predicated peptides). Our search yields ~25 fern orthologues (not all are shown above) with e-values < 10⁻¹⁰ significance.

References

- ¹Jones and Dolan, 2012. The evolution of root hairs and rhizoids. *Annals of Botany*. 110: 205-212.
- ²Salazar-Henao *et al.*, 2016. The regulation and plasticity of root hair patterning and morphogenesis. *The Company of Biologists | Development*. 143: 1848-1858.
- ³Menand *et al.*, 2007. An ancient mechanism controls the development of cells with a rooting function in land plants. *Science*. 316 (5830): 1477-1480.
- ⁴Plackett *et al.*, 2018. *LEAFY* maintains apical stem cell activity during shoot development in the fern *Ceratopteris richardii*. *eLife*. 7: e39625: 1-34.
- ⁵Shibata & Sugimoto, 2019. A gene regulatory network for root hair development. *Journal of Plant Research*. 132: 301-309.
- ⁶Marzec *et al.*, 2015. Root hair development in the grasses: what we already know and what we still need to know. *Plant Physiology*. 168: 407-414.
- ⁷Rodriguez *et al.*, 2019. Systems biology of plant-microbiome interactions. *Molecular Plant*. 12: 804-821.
- ⁸Jang *et al.*, 2011. RSL genes are sufficient for rhizoid system development in early diverging land plants. *Development*. 138: 2273-2281.
- ⁹Proust *et al.*, 2016. RSL class I genes controlled the development of epidermal structures in the common ancestor of land plants. *Current Biology*. 26(1): 93-99.
- ¹⁰Tam *et al.*, 2015. Conserved regulatory mechanism controls the development of cells with rooting functions in land plants. *PNAS*. 10.1073: e3959-e3968.