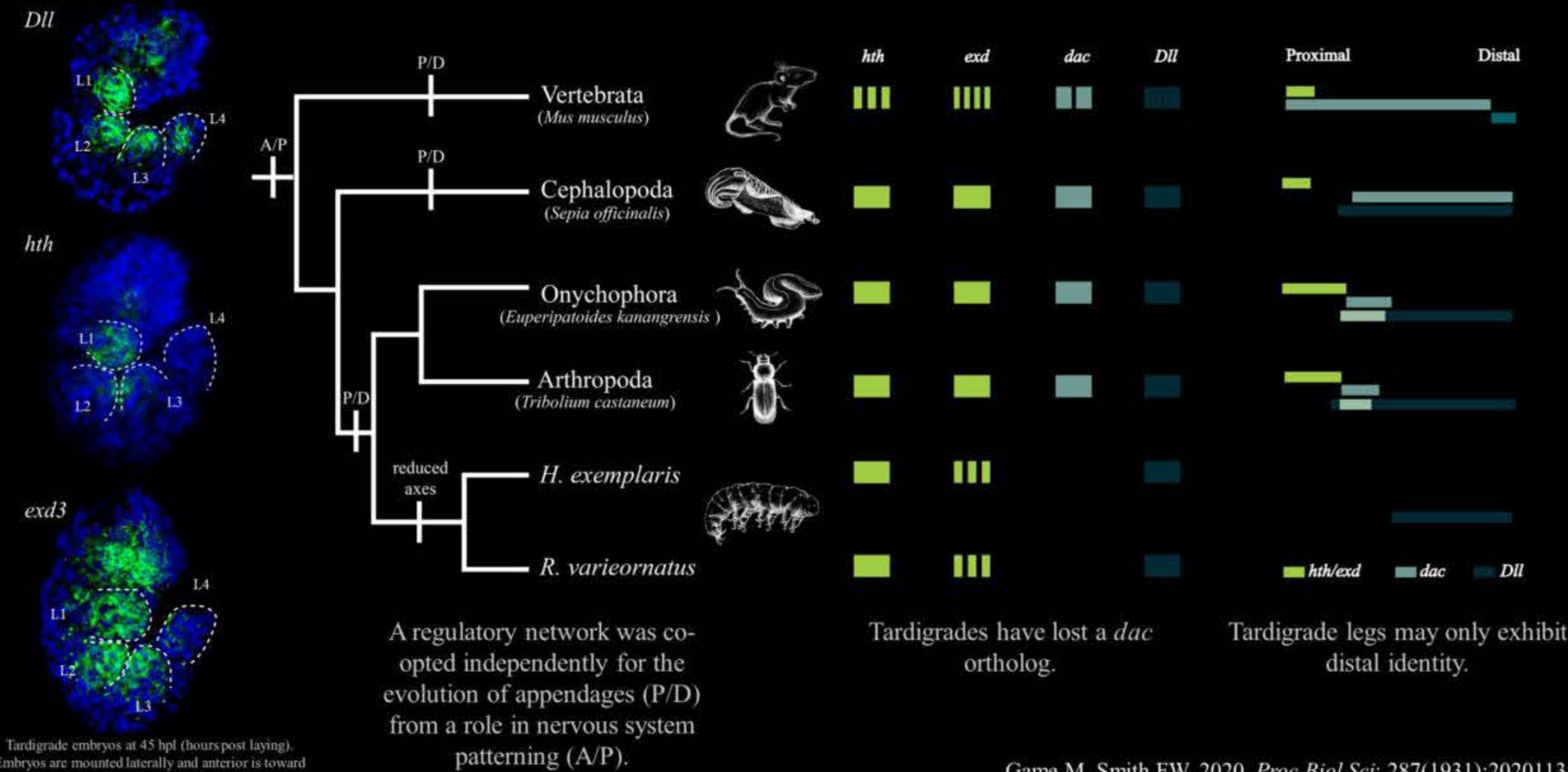
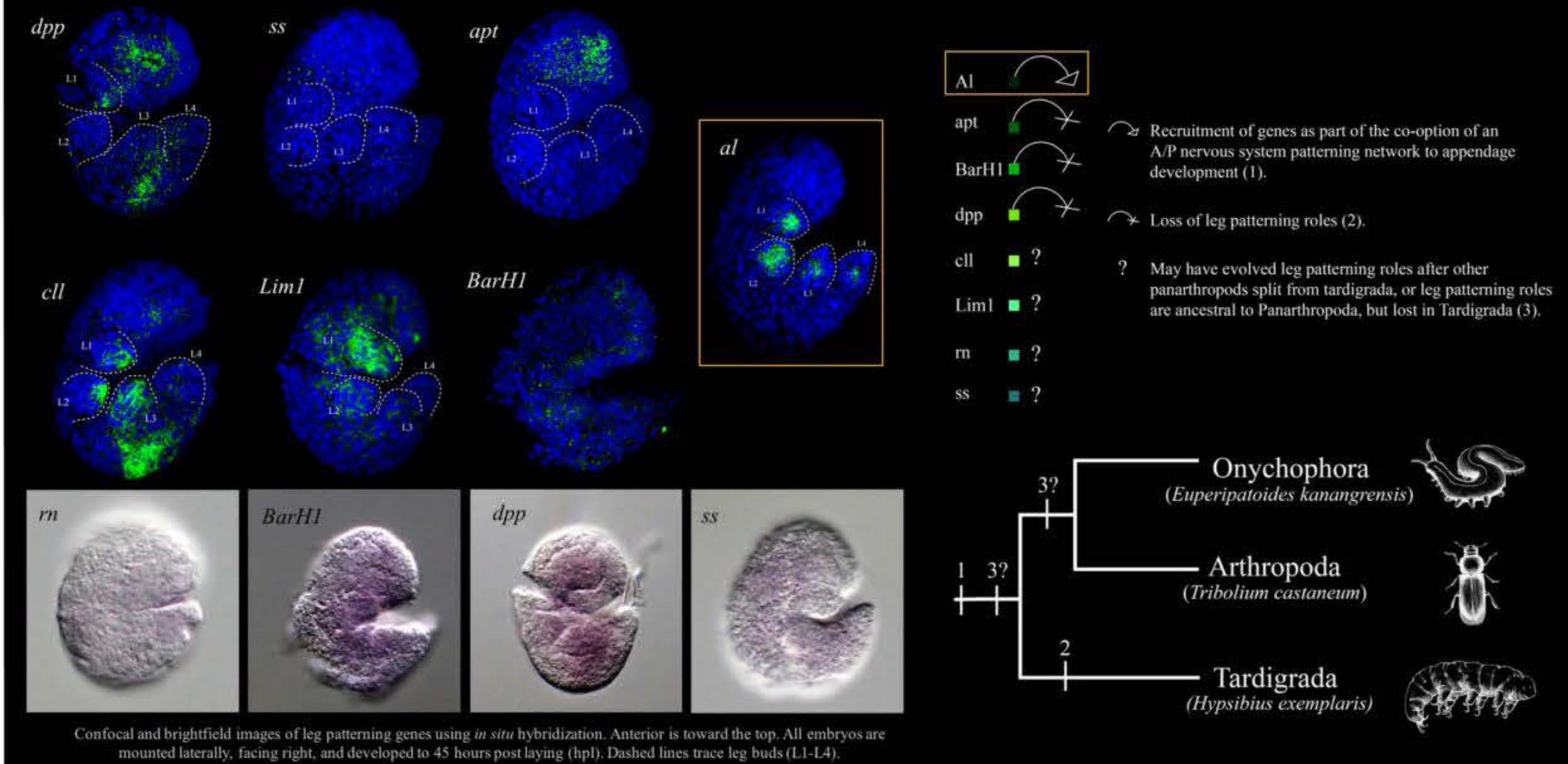


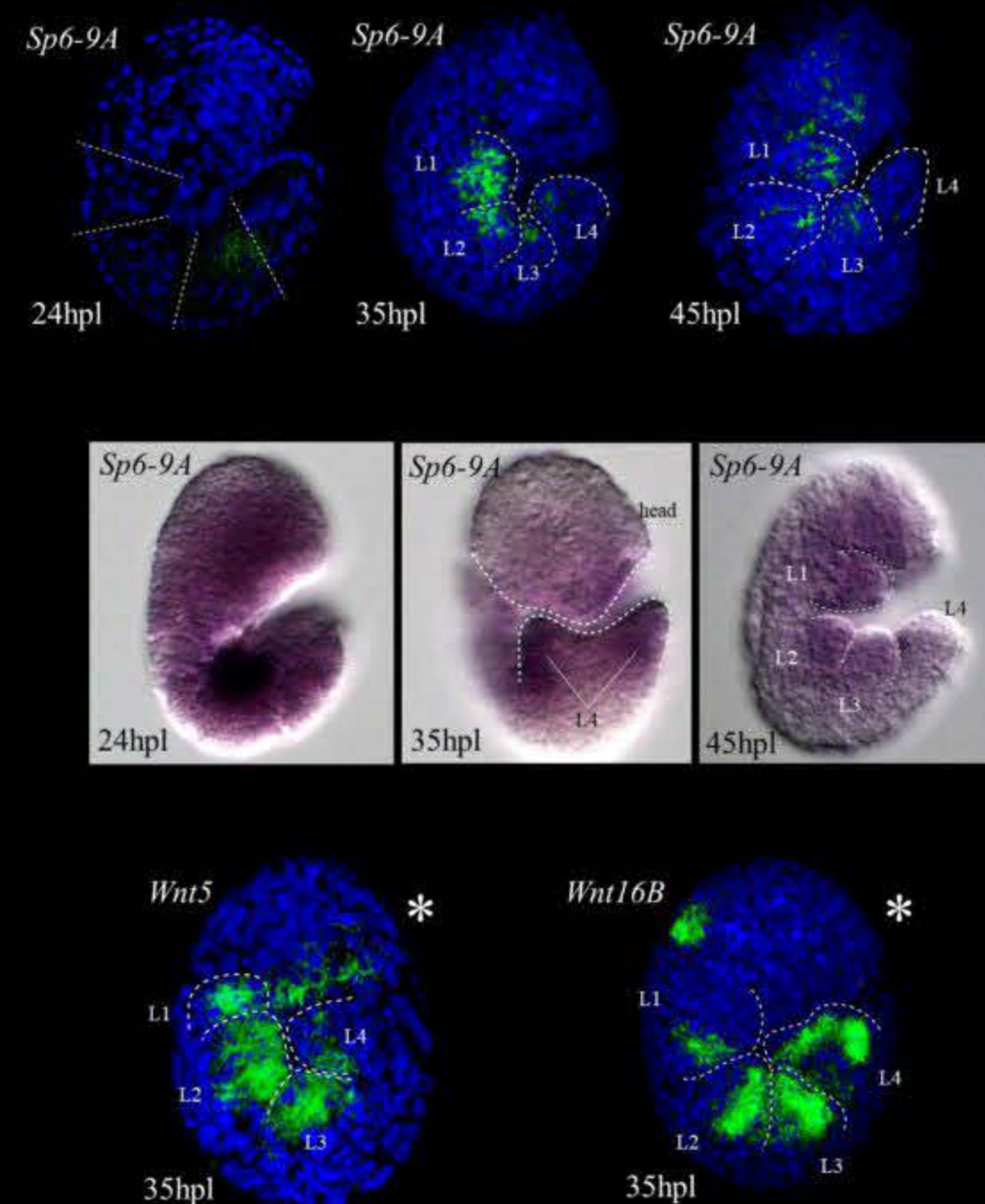
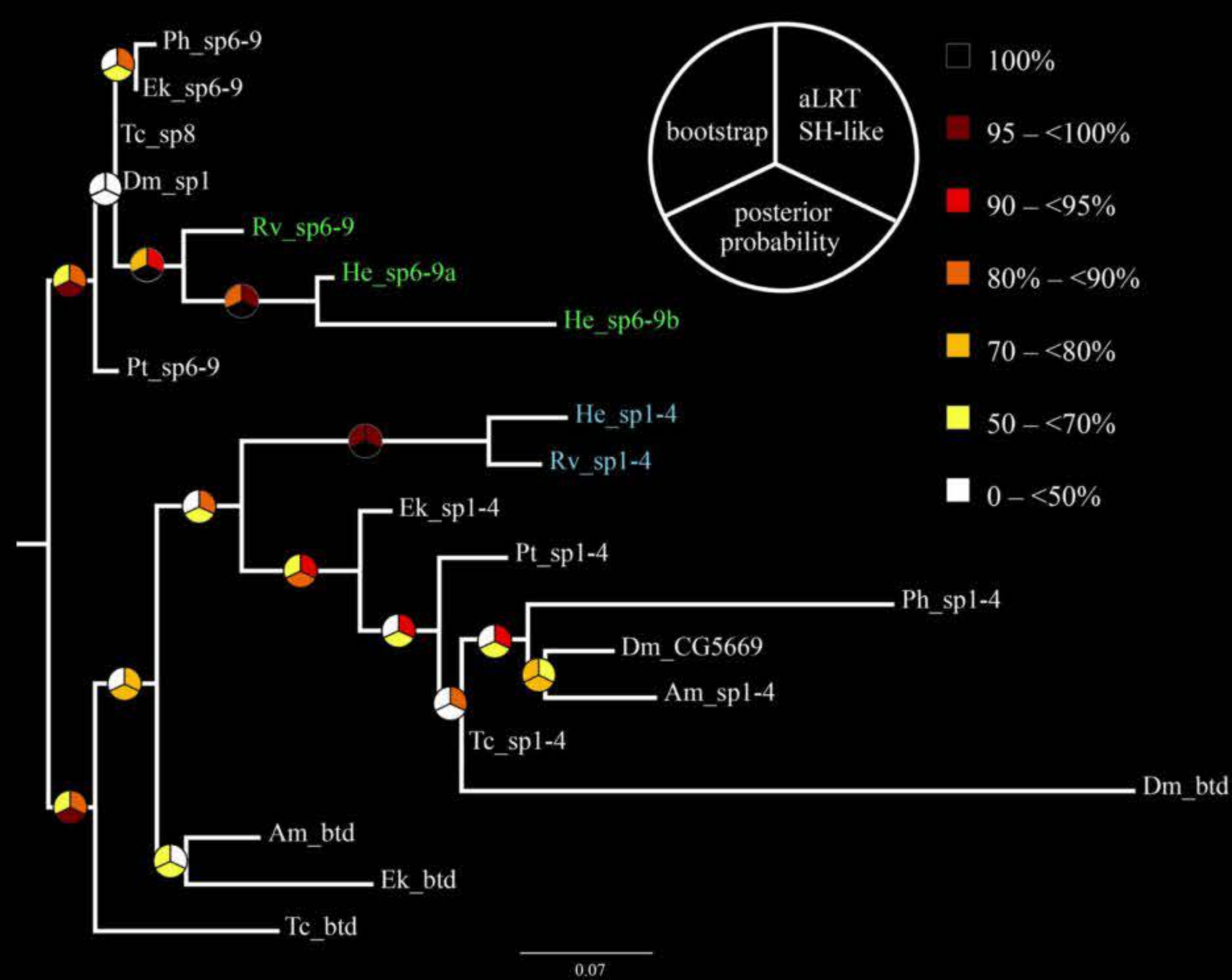
Tardigrade legs have a reduced proximodistal axis



Many known distal-leg patterning genes may not play general roles in tardigrade legs



Upstream components of leg patterning appear to be conserved



Confocal and brightfield images using *in situ* hybridization. Anterior is toward the top. Dashed lines trace segment boundaries or leg buds (L1-L4).

* *Wnt in situ* hybridization performed by Raul Chavarria

Conclusions

| Gene | Gene expression |
|----------------|-----------------|
| <i>Dll</i> | ● |
| <i>hth</i> | ● |
| <i>exd</i> | ● |
| <i>dac</i> | ○ |
| <i>al</i> | ● |
| <i>apt</i> | ○ |
| <i>BarH1</i> | ○ |
| <i>cll</i> | ○ |
| <i>dpp</i> | ● |
| <i>Lim1</i> | ○ |
| <i>nub</i> | ○ |
| <i>rn</i> | ○ |
| <i>ss</i> | ○ |
| <i>Sp1-4</i> | ○ |
| <i>btd/Sp5</i> | ○ |
| <i>Sp6-9</i> | ● |
| <i>Wnt4</i> | ○ |
| <i>Wnt5</i> | ● |
| <i>Wnt16B</i> | ● |

● Presence/conserved expression
○ Absence/expression not conserved
? Currently unknown

While many highly conserved leg patterning genes are present in the genomes of both tardigrades and their outgroups, expression patterns of many of these genes are not conserved in tardigrade legs. This supports our hypothesis that the tardigrade body plan evolved through secondary simplification.

Future directions

- Investigate leg patterning gene expression in other developmental stages
- Use RNAi to determine the function of leg patterning genes in tardigrades
- Investigate whether interactions between leg patterning genes are conserved in Tardigrada

Tree shows relationships of *Sp* genes in Panarthropoda. Tardigrade genes are in blue and green. Bootstrap support out of 500 replicates.