Comparative genomics of secreted proteins associated with the virulence of *Seiridium cardinale*

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Seiridium spp. isolates

Seiridium cardinale   Seiridium unicorne   Seiridium cupressi

Figure 1: *Seiridium* (Xylariales, *Sporocadaceae*) is an important plant pathogenic ascomycete genus, and is known to be the causal agent of Cypress Canker Disease (CCD). In this study, genomes were assembled and annotated. We present a comparison of predicted proteins among three phylogenetically related species. The growth of the three species on Potato Dextrose Agar (PDA) allows us to observe morphological differences among them.

Prediction of protein coding genes

Protein coding genes	*S. unicorne* CBS 538.82	*S. cardinale* BM-138-000 494	*S. cardinale* BM-138-000 479	*S. cupressi* BM-138–000 515	*S. cupressi* BM-138–000 234
Predicted genes	14,108	14,281	14,050	13,376	13,446
Secreted proteins	1,504	1,536	1,520	1,502	1,504
Effector proteins	3,390	3,398	3,323	3,359	3,387
Cytoplasmic effectors	2,893	2,883	2,821	2,917	2,938
Apoplastic effectors	497	515	502	442	449

Table 1: Evaluation of predicted proteins of three *Seiridium* species based on our recent genome assemblies. The table compares the number of predicted proteins and the number of secreted proteins. The predicted proteins were further classified by identifying the number of cytoplasmic and apoplastic effectors using Effectorp. **Effectors** are secreted proteins that modulate the immune response of hosts. Effectors in fungi have been shown to be extremely species-specific.

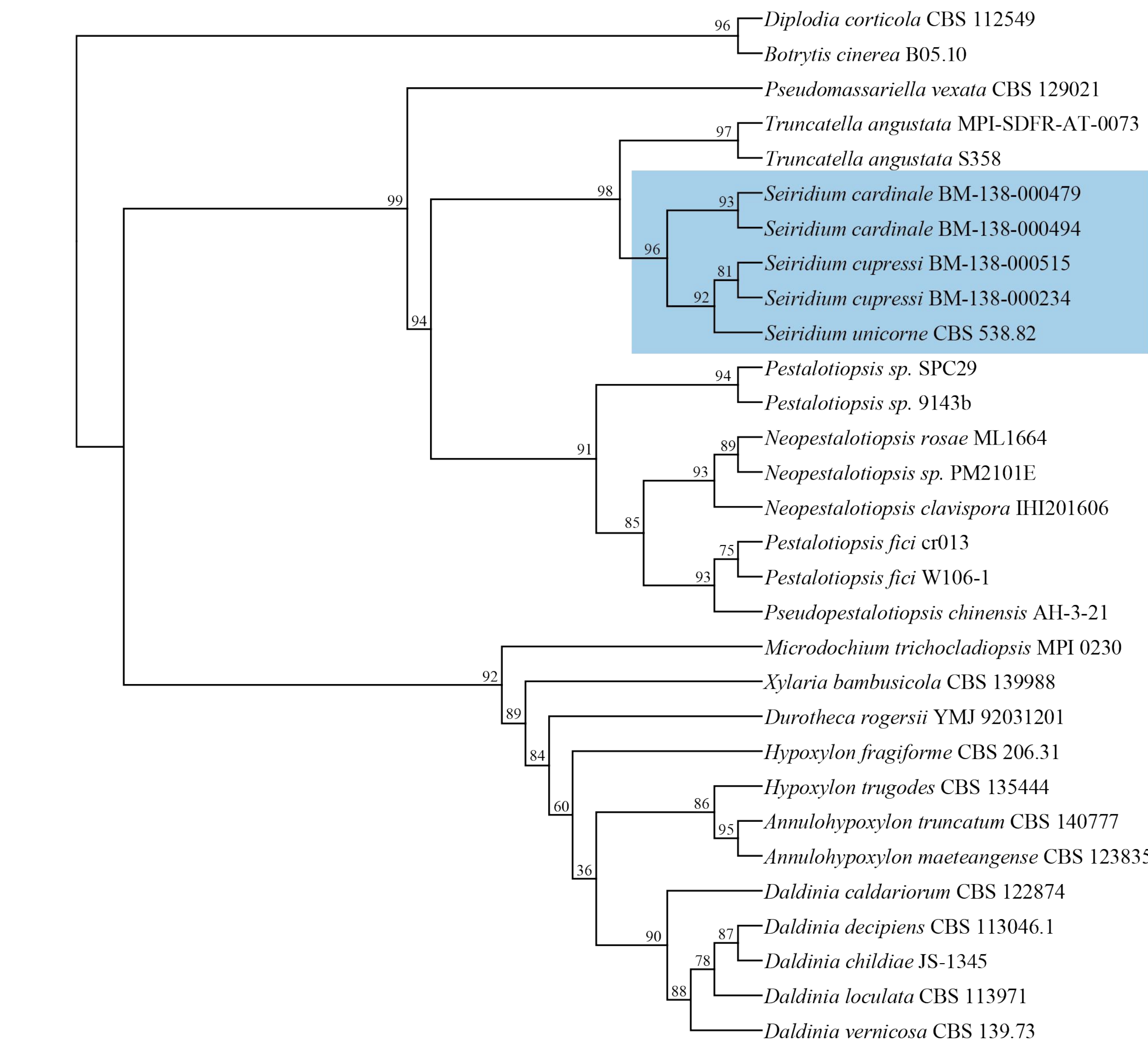## Phylogenetic orthology inferred from comparative genomics

Figure 2: Phylogenetic tree constructed by comparing the predicted proteins of fungi closely related to those belonging to the Xylariales order and Sporocadaceae. *Botrytis cinerea* and *Diplodia corticola* have been selected as outgroups.

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Affinity of *Seiridium cardinale* predicted proteins with fungi in the Xylariales and *Sporocadaceae*
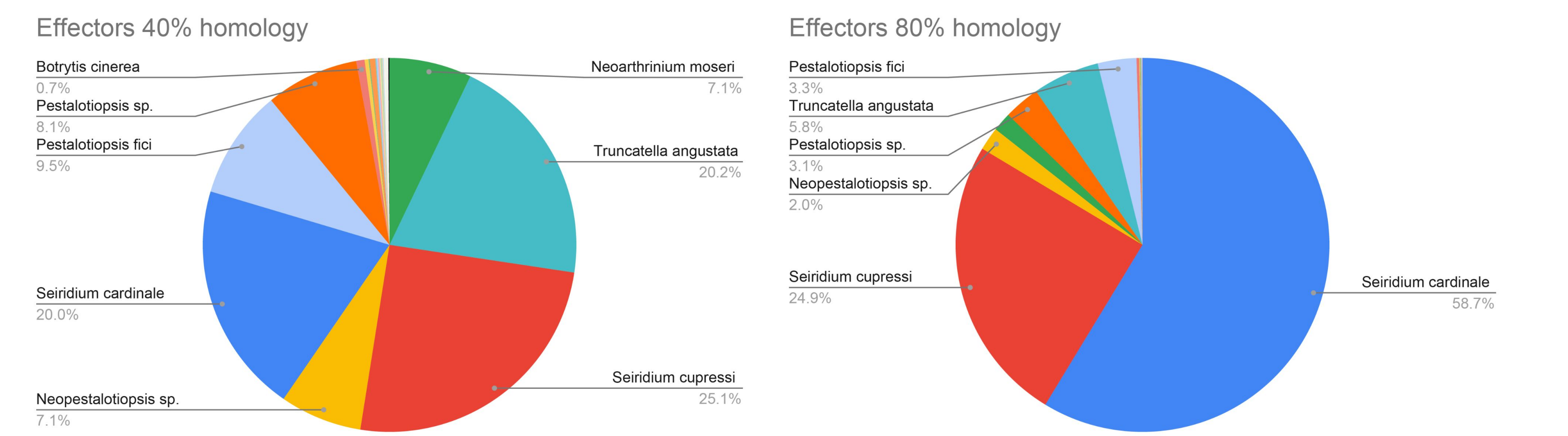

Figure 3: Our analysis employing a database of all known fungal effectors shows that, at least in the *Sporocadaceae,* effectors are conserved beyond the species boundaries. When comparing effectors at the significant, but low, 40% homology threshold, the percentage of shared effectors is positively correlated to the phylogenetic signal, with closely related taxa sharing a higher percentage of effectors. When increasing the threshold of homology to 80%, we surprisingly show that highly homologous effectors are still shared among related taxa, and, in particular, we show that 25% of effectors are the same or almost in *S. cardinale* and *S. cupressi*. We note that the percentage of effectors shared between *S. cardinale* and *S. cupressi* is the same, independent of the homology threshold, suggesting that effectors shared between the two species are all highly homologous (homology >80%) or are almost not homologous (homology < 40%).

Conclusions

- The phylogenetic position of the genus *Seiridium* within the Xylariales order is still uncertain. Our phylogenetic tree based on orthologous proteins shows *Seiridium* to be monophyletic and a sister genus to *Truncatella.* The closest neighbors are the genera *Pestalotiopsis* and *Pseudomassariella.*

- Although it is reported that a relatively low number of effectors are shared among species, we find a clear phylogenetic signal across multiple related taxa in the *Sporocadaceae*, with a number of shared homologous effectors (40% homology) that is relatively high (range 0.7- 25.1 %) and growing with increasing relatedness among taxa. We note that effectors are shared well beyond taxa that would be regarded as related (e.g *Seiridium* and *Pestalotiopsis)*, but that all taxa that share effectors are known to have a pathogenic lifestyle.

- Using the high homology threshold comparison, we do find that approximately 60% of effectors are specific to *S. cardinale,* but we also find that 25% of effectors are shared with *S. cupressi*. This suggests that very closely related taxa maintain a significant number of identical, or nearly identical, effectors and that these effectors may be progressively lost or may themselves evolve in a non-linear fashion only as taxa further diverge evolutionarily.