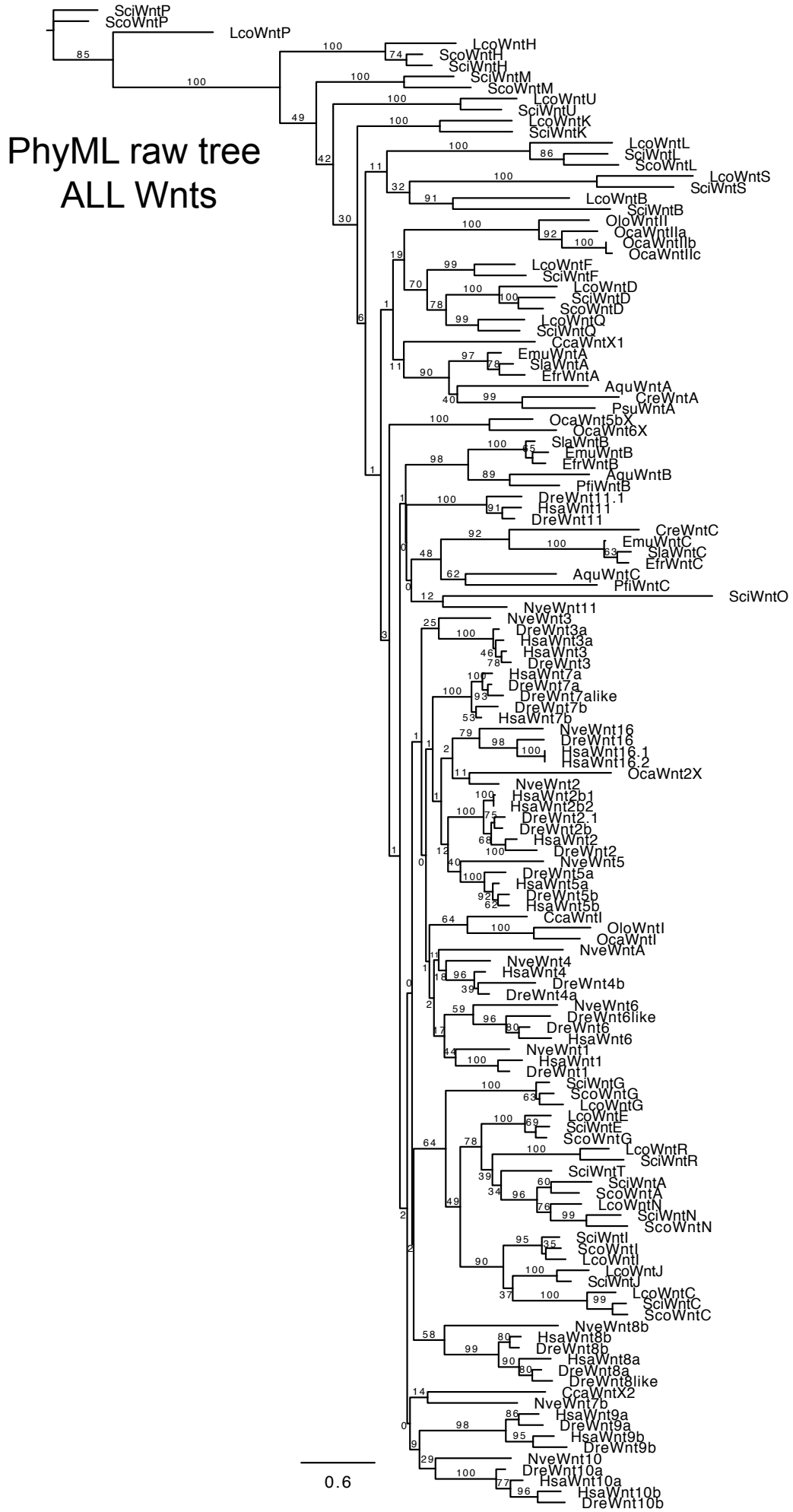Additional File 5