

# Species delimitation in a highly polyphagous scale insect species complex

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## Introduction

Armored scale insects are highly specialized plant parasites in the family Diaspididae which comprises over 2600 species in 417 genera<sup>1</sup>. In the US alone they are responsible for an estimated 2-5 billion dollars annually in agricultural damage.<sup>1</sup> As of 2005, 132 species have been introduced from other countries, comprising up to 40% of the US fauna.<sup>2</sup> Many of these pest species are extremely polyphagous, capable of parasitizing many different host plant families.<sup>3</sup> Armored scales pose one of the most extreme examples of generalists, some species have been found on up to 100 different plant families. This seems to go against the evolutionary norm, as generalism is typically a strategy for predators, while specialization is more typical of herbivores. Because of this it has been suggested that extreme generalists in this case are not generalists, but rather clouds of specialist species.<sup>4</sup>

Molecular methods have revealed cryptic species in several studies of diaspidids in support of this hypothesis.<sup>11</sup> This project seeks to assess species boundaries within the *Hemiberlesia palmae/cyanophylli* insect pest complex using phylogenetic analysis of loci previously identified in Mullen et. al for scale insect systematics.

## Methods

Insects from various locations around the world were sampled, identified and preserved in ethanol. DNA extractions were performed on high and medium quality specimens using the Quiagen DNeasy Blood & Tissue Kit (Quiagen, Valencia, California) following standard methods with modifications outlined in Andersen et al.<sup>3</sup> Viable extractions were identified using PCR to amplify the large ribosomal subunit 28s gene, and samples that amplified were then tested for additional loci using primers previously developed for scale insects.<sup>5</sup> The MRE11A and MCM2 loci were selected for further testing because they have been shown to diverge at the species level in other phylogenetic analyses.<sup>7,8</sup> Samples were selected from the *Hemiberlesia palmae/cyanophylli* complex to include as many different localities and host plants as possible for each known species. Successful amplifications were purified using Exo-sap and sequenced by Psomagen.<sup>9</sup> Sequences were edited with Geneious Prime 2022.1.1 and aligned with Aliview. BPP<sup>11</sup> was then used for phylogenetic analysis to estimate the posterior probabilities of different species trees as well as the posterior probabilities of different species being present.

## Results

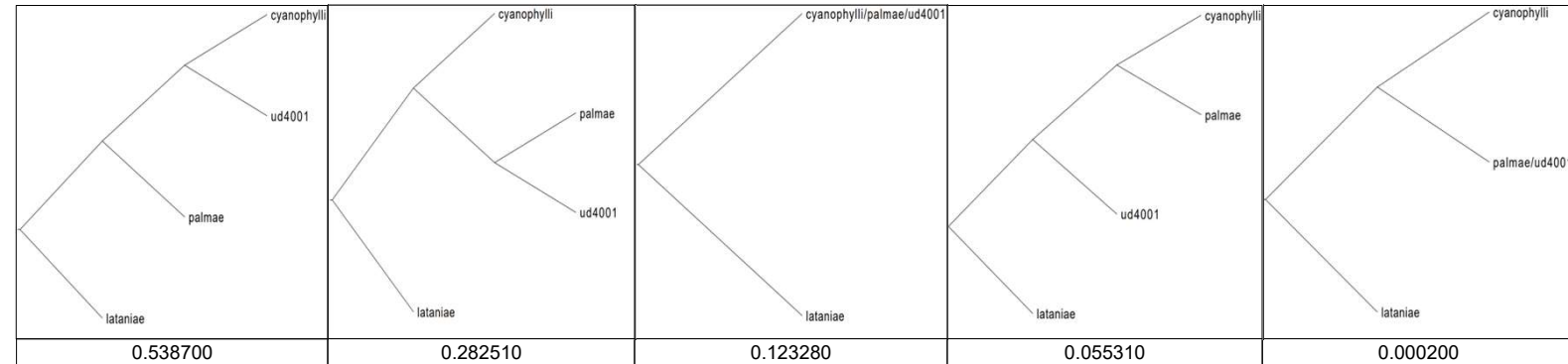


Fig. 1. Best model species trees for *Hemiberlesia cyanophylli/palmae* complex and their posterior probabilities.

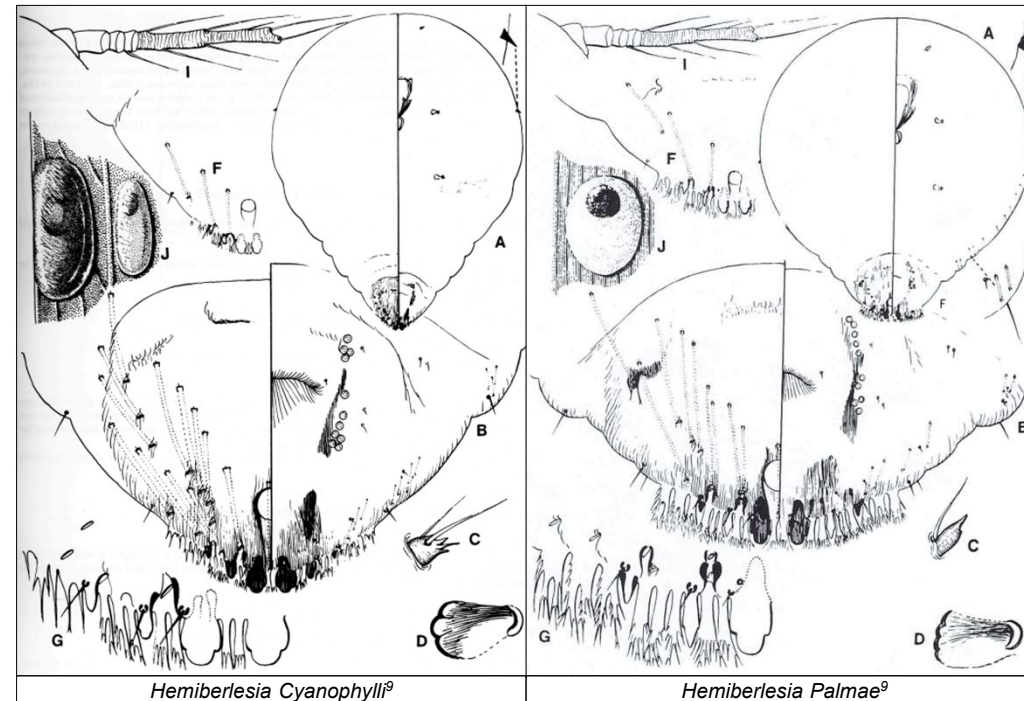


Fig. 2. Illustrations of *Hemiberlesia cyanophylli* and *Hemiberlesia palmae* with identifying characters. ud4001 is believed to be an undescribed species with most characters being an intermediate between the two. Before it was hypothesized to be a cryptic species ud4001 samples were previously identified as *cyanophylli* or *nr. Palmae*. A = top(left) and bottom(right) of uncovered female scale B = top(left) and bottom(right) of rear portion of female scale C = antennae D = anterior spherical F = 2<sup>nd</sup> instar pygidial margin G = pygidial margins I = antennae of 1<sup>st</sup> instar J = female(left) and male(right) with scale coverings, only female for palmae<sup>9</sup>

Posterior Probabilities of Species Delimitations			Posterior Probabilities for each Delimited Species	
Species Delimitation	# of Species	Posterior Probability	Species	Posterior Probability
c   p u	4	0.87652	l	1
cpu	2	0.12328	c	0.87672
c   pu	3	0.00020	u	0.87652
			p	0.87652
			cpu	0.12328
			pu	0.00020

Table 1. Posterior probabilities of species delimitations and each delimited species.

c = cyanophylli, l = lataniae, cpu = cyanophylli/palmae/ud4001  
p = palmae, u = ud4001 pu = palmae/ud4001

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