

New Phylogenetic Tools for Scale Insects

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Introduction

Scale insects include many agricultural pests and are known for their unique genetics and abundance across many parts of the world¹. Their rapid rate of evolution, non-Mendelian inheritance, large population sizes, limited body size, and cryptic species complexes have complicated attempts to understand their phylogeny. Here we present a phylogenomic study of scale insects with a focus on armored scale insects (*Diaspididae*).

We extracted homologous single-copy genes from low-coverage genome scans and transcriptomes. Specifically, this was done using data collected as part of the 1K Insect Transcriptome Evolution project² and the software package aTRAM, automated Target Restricted Assembly Method³, which was made specifically to work with 1KITE's low-coverage data.

We expect these data and methods to be powerful tools for unraveling the many cryptic species complexes found in scale insects.



Figure 1. *Chrysomphalus aonidum* with scale covering.⁴ Figure 2. Close up of *Pseudaulacaspis cockerelli*.⁵ Figure 3. *Phoenicococcus marlatti* along a stem.⁶

Results

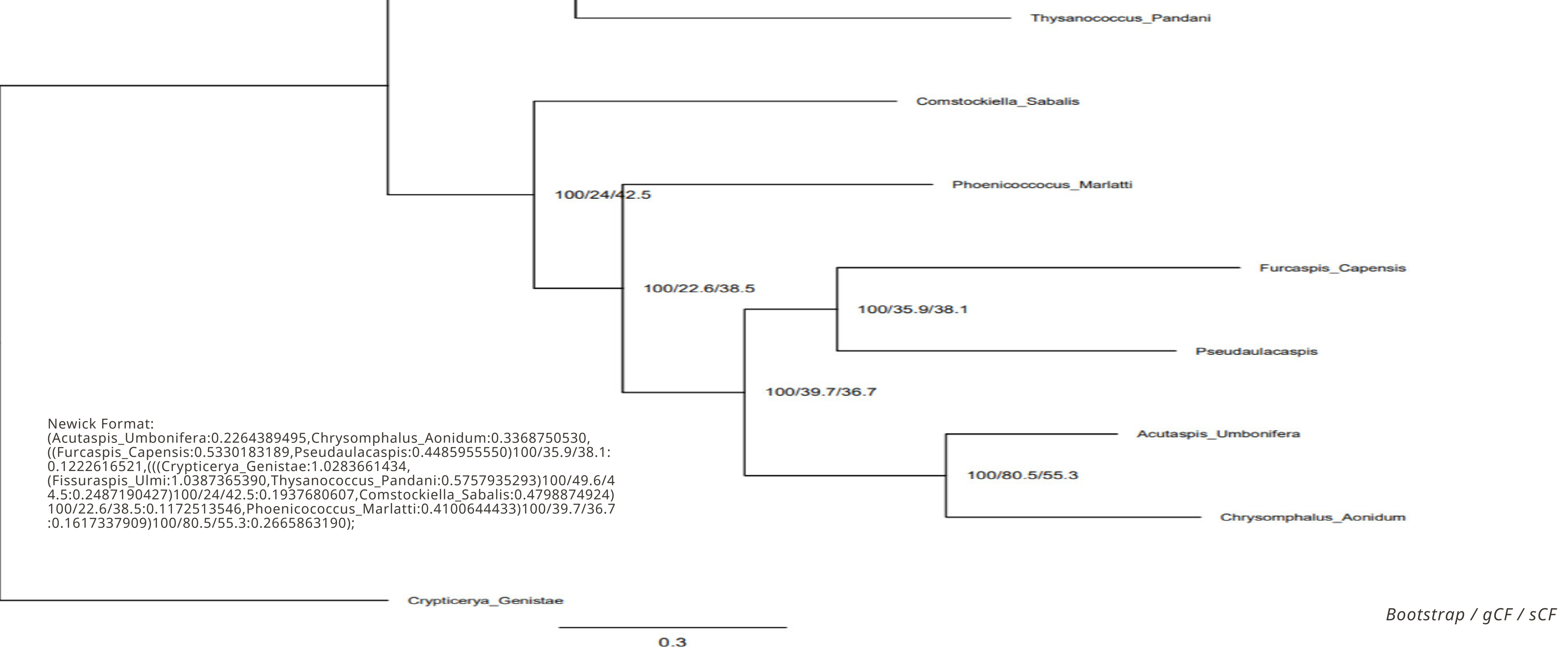


Figure 5. Phylogenetic tree of *Fissuraspis ulmi*, *Thysanococcus pandani*, *Comstockiella sabalis*, *Phoenicococcus marlatti*, *Furcaspis capensis*, *Pseudaulacaspis*, *Acutaspis umbonifera*, and *Chrysomphalus aonidum* with *Crypticerya genistae* as the root.

Methodology

- The **aTRAM preprocessor** was used to create SQLite3 and BLAST databases from prepared raw read genomes and transcriptomes of scale insect Next-Generation Sequencing data.
- aTRAM main** was used to build the assemblies from the preprocessor databases and a multi-query bait sequence from the *Phylogenomics and the evolution of hemipteroid insects*⁷ dataset. This process BLASTed the bait sequence against the aTRAM blast databases for each taxon. Then it found mate pairs for the BLAST hits in the SQLite3 database. Next, the program utilized choice de novo assemblers (Velvet and Trinity) to build contigs that would become the bait sequences for the next iteration.
- The **aTRAM stitcher** finally pieced together the assemblies using Exonerate and an iterative approach.
- PASTA**⁸, Practical Alignment using Saté and TrAnsitivity, was used to align the sequences from aTRAM with each taxon.
- AMAS**⁹, Alignment Manipulation and Summary Statistics, helped concatenate the alignments of each locus.
- IQTree**¹⁰ served to create a phylogenetic tree for the taxa from the concatenated alignments. It also determined the bootstrap support values and concordance factors.

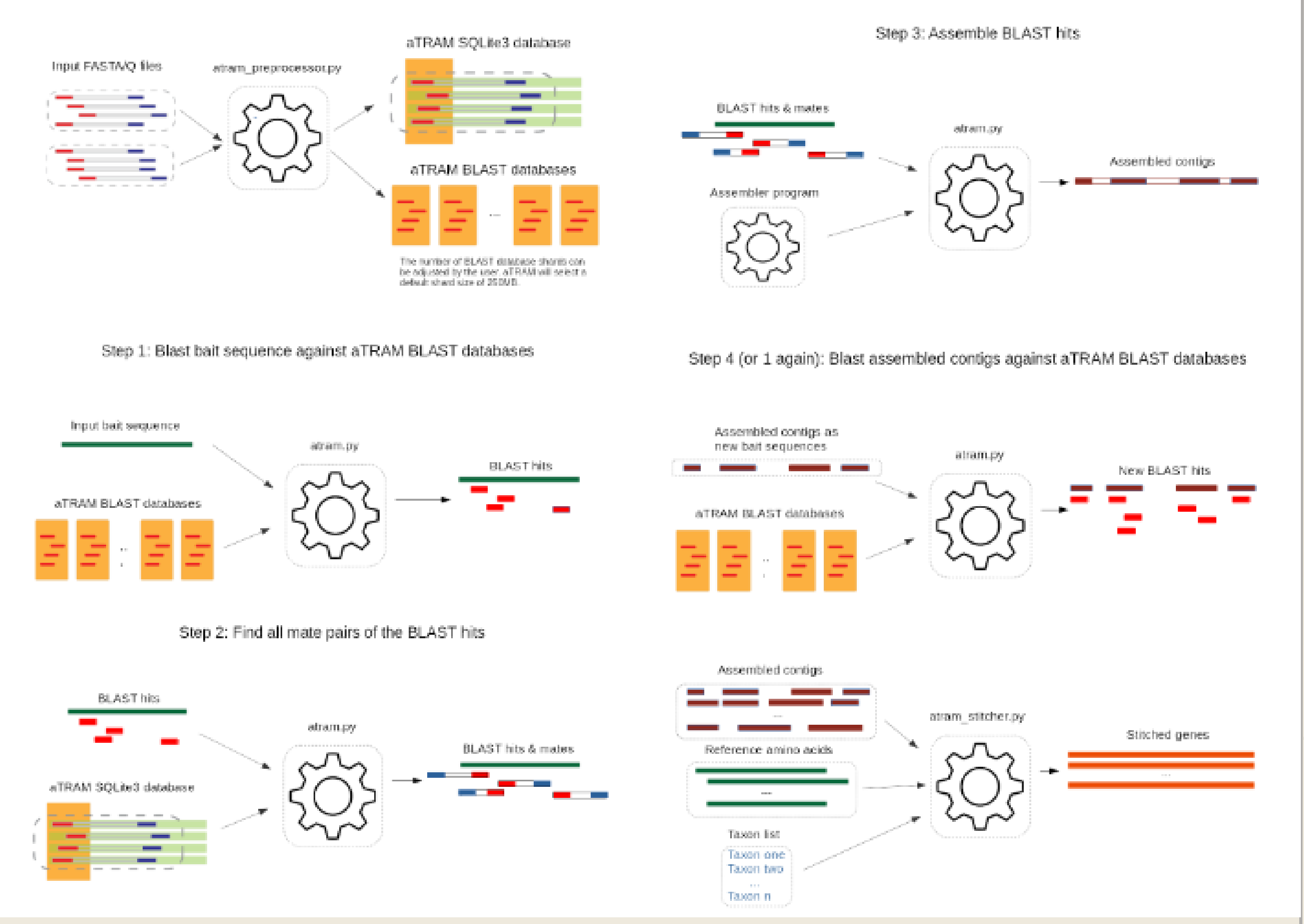


Figure 4. aTRAM steps from preprocessor to main to stitcher.³ Figure reprinted from Allen et al.³

Discussion

- An additional phylogenetic tree with the same taxa and a different query supported this tree formation as well.
- Of the 2395 loci in the *Phylogenomics and the evolution of hemipteroid insects* dataset, only the first 1650 were run through aTRAM main for this tree.
- The bootstrap support values for all of the branches are 100. However, the gene concordance factor (gCF) and the site concordance factor (sCF) differ notably from that result.
- Fissuraspis ulmi* had 93.36% ambiguity or gaps. Given this fact and its unexpected distance from other scale insects, there might be an error in the raw read for this taxon.
- Thysanococcus pandani*, *Comstockiella sabalis*, and *Phoenicococcus marlatti* are all outgroups, and their relationships to armored scales are not well resolved.
- The placement of *Pseudaulacaspis* in this tree contradicts the predicted placement in Normark et al 2019¹¹. According to that paper, *Pseudaulacaspis* should be sister to *Acutaspis umbonifera* and *Chrysomphalus aonidum*, followed by *Furcaspis capensis* being sister to *Pseudaulacaspis*.

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