Determining Cryptic Species Diversity in Feather-Footed Flies (*Trichopoda* spp.)

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Abstract

Trichopoda pennipes are parasitoid flies that attack stink bugs of economic importance. Inconsistent results in the use of *T. pennipes* as a natural biological control agent may be due to the existence of host specific pheromone strains of *T. pennipes*. Within *T. pennipes*, 2 morphotypes are present, 1 with golden wings and 1 with black wings. To discern if consistent genetic differences exist between morphotypes, the *MCS* gene of 4 *T. pennipes* specimens (2 black and 2 gold) along with 1 *Trichopoda lanipes* were chosen for DNA sequencing. Sequences were combined with a larger data set of 23 *Trichopoda* sequences, and a phylogenetic tree was reconstructed using maximum likelihood. Gold and black morphotypes were not reconstructed as individual clades, thus indicating no consistent genetic differences between phenotypes. Additionally, the *T. lanipes* clade was nested within the larger clade of *T. pennipes*, implying considerable introgression between species.

Introduction

The feather-footed fly (*Trichopoda pennipes*) is a parasitoid insect of agricultural pests such as the southern green stink bug and the brown marmorated stink bug. *Trichopoda* species have been studied as potential reducers of stink bug populations without much success, potentially due to the existence of cryptic species within this genus (Davis 1964, Coombs & Sands 2000). These possible cryptic species make it difficult to use *Trichopoda* as biological control agents due to the lack of confidence that the correct species is being utilized (Blaschke 2015). Using genetic differences to identify morphologically ambiguous *Trichopoda* species could allow for better use of these insects as biological control agents. This research used MCS gene sequences of 2 gold-winged *T. pennipes*, 2 black-winged *T. pennipes,* and 1 *T. lanipes* specimens to determine if phylogenetic analysis would produce clades that correlated with the morphological features of the specimens.

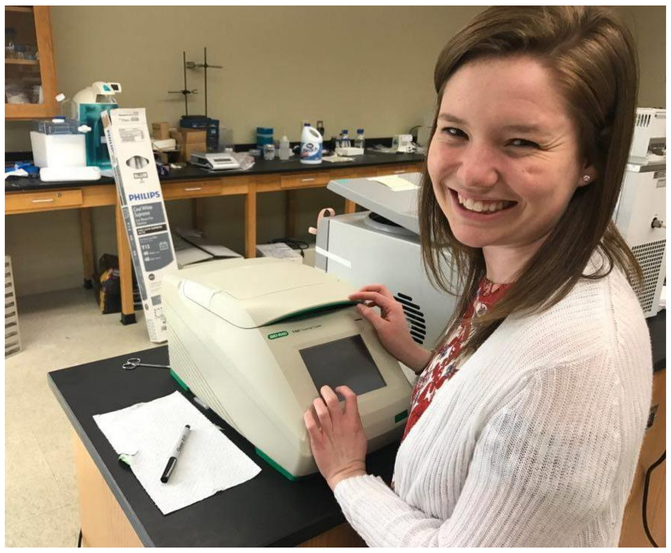

Figure 1. Placing DNA samples in PCR for amplification of MCS gene.

Methods

The specimens were collected from two areas of Great Smoky Mountains National Park, Cades Cove in Tennessee and Purchase Knob in North Carolina. The right three legs from each specimen were removed and stored in 95% ethanol until they were used for DNA extraction. The DNA was extracted using a ThermoScientific DNA extraction kit, and the *MCS* gene of 4 *T. pennipes* (2 black-winged and 2 gold-winged) and 1 *T. lanipes* specimens was isolated and amplified via PCR (Figure 1). The samples were then run on gel electrophoresis. Gel cleanup was performed, then the genes were sequenced at an outside institution. The sequences of these 5 specimens were combined with a larger data set of 23 *Trichopoda* sequences. A phylogram was reconstructed for these specimens. These results were analyzed to determine if the clades that formed correlated with the morphological features of the specimens.

Results

The 5 *Trichopoda* sequences were added to a larger data set of sequences of 9 gold *T. pennipes* (Figure 2), 7 black *T. pennipes* (Figure 3), 5 *T. lanipes* (Figure 4), 1 *T. plumipes*, and 1 *Acaulona*. A phylogram was reconstructed from these sequences (Figure 5). *T. plumipes* served as a closely related outgroup while *Acaulona* was a distantly related outgroup. All *T. pennipes* and *T. lanipes* grouped together to form a highly supported clade with a bootstrap support (bs) of 97%. This clade was sister to *T. plumipes*. All *T. lanipes* formed a clade (bs=90) nested within the black and gold *T. pennipes*. The gold *T. pennipes* specimens did not reconstruct into a distinct clade. A distinct clade of black *T. pennipes* formed (bs=94); however, it only contained 4 of the 9 black specimens.

Discussion

The two morphotypes of *T. pennipes* were not recovered as separate clades. This lack of distinction could exist because the black and gold morphotypes are in the beginning stages of a speciation event. Thus, their MCS sequences would be too similar to be separated into distinct clades at this time. Despite this similarity in DNA, a small, highly supported clade of 4 black *T. pennipes* did form. This clade could represent a population reproductively isolated from the gold phenotype.

All *T. lanipes* specimens formed a clade; however, that clade was not distinctly separated from the *T. pennipes* specimens. It is possible that the *T. lanipes* clade was nested within the *T. pennipes* specimens because *T. lanipes* is in the beginning stages of a speciation event but is not yet currently reproductively isolated. Therefore, it would be backcrossing with *T. pennipes*. This introgression could result in offspring that morphologically resemble *T. lanipes* but are genetically similar to *T. pennipes*. Although MCS has been determined to be the best barcoding gene for tachinid flies, it is possible that the genetic differences between *T. lanipes* and *T. pennipes* are so minute that they are undetectable by the MCS gene (Winkler et. al. 2015).

Two different conclusions can be drawn from this tree. The first is that the black and gold morphotypes of *T. pennipes* should remain classified as the same species because they did not reconstruct into distinct clades that correlated with their morphologies. The second conclusion is that *T. pennipes* and *T. lanipes* should be classified as the same species because the *T. lanipes* clade was not separated from the large *T. pennipes* clade. However, due to small sample sizes, these conclusions are extremely preliminary. Additional sequences would be needed before any taxonomic changes could be made.

Literature Cited

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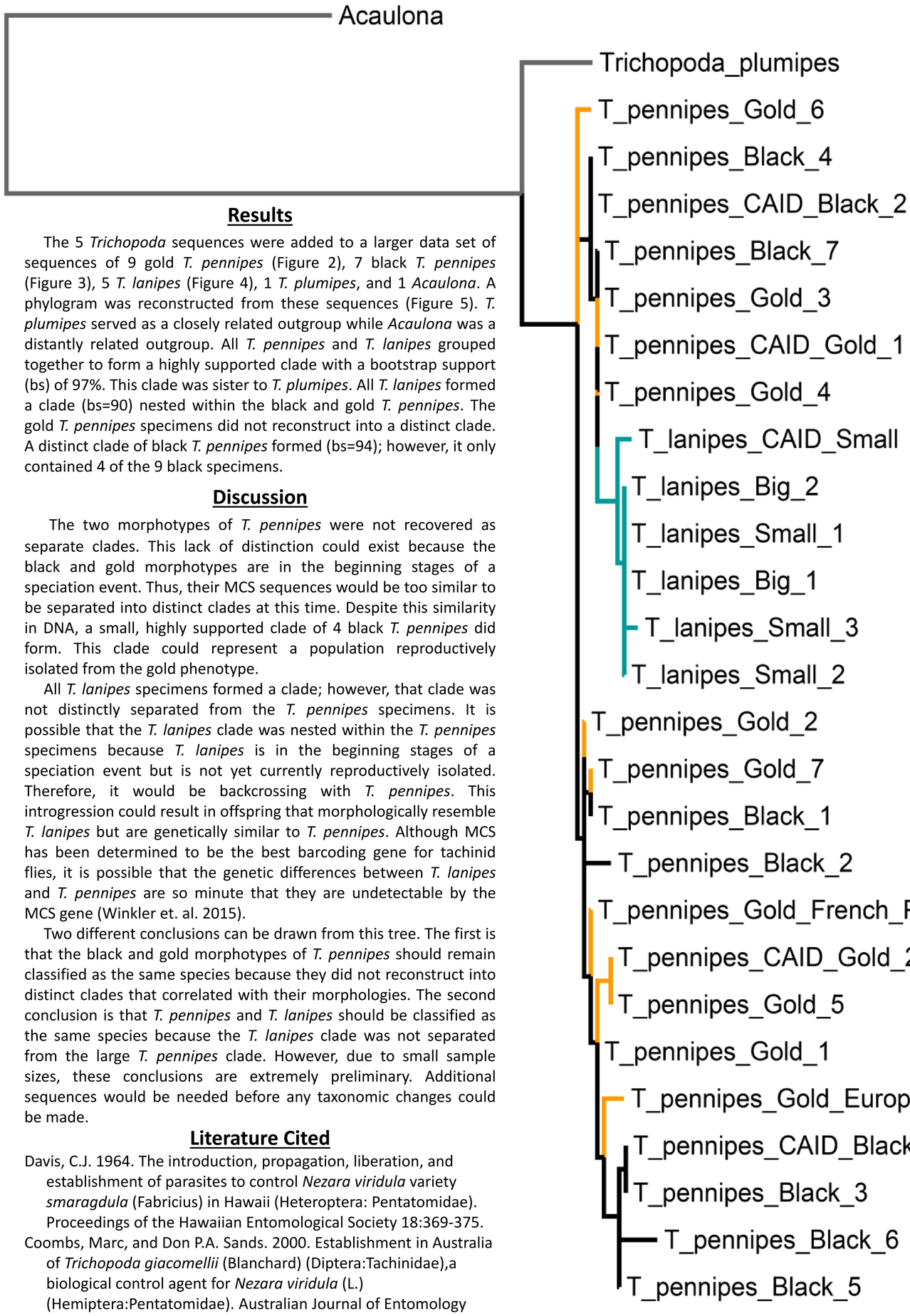

Figure 5. A phylogram reconstructed using the previously sequenced *MCS* gene of *T. pennipes* and *T. lanipes* specimens. *Acaulona* and *T. plumipes* were sequenced to be compared as outgroups.

Figure 2. Gold-winged morphotype of *T. pennipes.* Photo: Rui Andrade

Figure 4. *Trichopoda lanipes.* Photo: Richard Orr

Figure 3. Black-winged morphotype of *T. pennipes.* Photo: Jim McCulloch

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