The First Recorded Host for the Assassin Fly (*Xanthomelanodes atripennis*) and a Phylogenetic Analysis of the Genus

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Abstract

Xanthomelanodes are rarely encountered endoparasitoid flies that attack assassin bugs. Only 4 species are currently recorded in the US, and only 1 of these has a known host. Here, we report the sundew assassin bug (*Zelus luridus*) as the first documented host of *Xanthomelanodes atripennis* and include the first molecular phylogeny of the genus as a whole. Forty-six specimens representing all 4 US species and encompassing diverse geographic populations were included. Available barcodes (*COI*) were downloaded from the Barcode of Life Database and added to our newly generated sequences of *COI*. These sequences were concatenated with those from the nuclear coding gene *MCS* to reconstruct the final phylogeny. In agreement with morphological hypotheses, the molecular relationships between species were reconstructed as *X. atripennis* + (*X. flavipes* + (*X. arcuatus* + *X. californicus*)).

Figure 1. Assassin bug hosts of *X. arcuatus* (a) *Pselliopus barberi*, Photo: Bonnie Ott; (b) *P. cinctus*, Photo: Judy Gallagher; (c) *Sinea spinipes*, Photo: Katja Schulz

Introduction

Xanthomelanodes are distinctively colored parasitoid flies that presumably attack assassin bugs (Figure 2). Only one species has known hosts (Shurtz and McPherson 2005, Swadener and Yonke 1975, Yonke and Medler 1970, Figure 1 and 4). Assassin bugs are unusual and dangerous hosts for parasitoids due to their nature as ambush predators equipped with paralyzing venom (Weirauch and Munro 2009). The mechanism of *Xanthomelanodes* attack and oviposition on their hosts has yet to be observed. Similar to other parasitoid flies in subfamily Phasiinae, *Xanthomelanodes* may locate their prey by detecting pheromones secreted by their hosts (Aldrich et al. 2006).

The taxonomy of *Xanthomelanodes* was last revised in 1950 by Sabrosky and includes 4 species in the United States. *Xanthomelanodes atripennis* and *X. flavipes* are only found in eastern North America, *X. californicus* is restricted to the West, and *X. arcuatus* is widely distributed throughout the whole region (O'Hara and Wood 2019). Easily identifiable characters (leg and abdomen color) distinguish *X. atripennis* and *X. flavipes* from the others, but *X. arcuatus* and *X. californicus* are difficult to differentiate from each other using morphology alone. To date, no molecular evidence has confirmed the monophyly of these species or examined the interspecific relationships within the genus. Here, we report the sundew assassin bug (*Zelus luridus*) as the first documented host of *X. atripennis* and include the first molecular phylogeny of the genus as a whole.

Methods

- DNA Extraction performed with Qiagen® DNeasy Blood and Tissue Kit
- PCR Amplification using 50 µL PCR reactions; pre-made and custom primers for the mitochondrial gene *COI* and the nuclear gene *MCS*; BIO-RAD T100™ Thermal Cycler; verification by thin gel electrophoresis
- Sequencing performed by GENEWIZ®
- Verification of sequence quality and alignment using Geneious Prime 2020.0.2
- Phylogenetic analyses performed using Maximum Likelihood (ML), RAxML v.8 on XSEDE 8.2.10, online via CIPRES portal; performed on combined data set and individual genes. *COI* sequences were obtained from BOLD Systems (26) or newly generated (20). Specimens of each species were confirmed by experts (Jim O'Hara and John Stireman III) and included to identify species clades. Trees were visualized in Geneious Prime 2020.0.2
- Calculation of intraspecific and interspecific distances and P (Randomly Distinct) values using the Species Delimitation plugin in Geneious Prime 2020.0.2

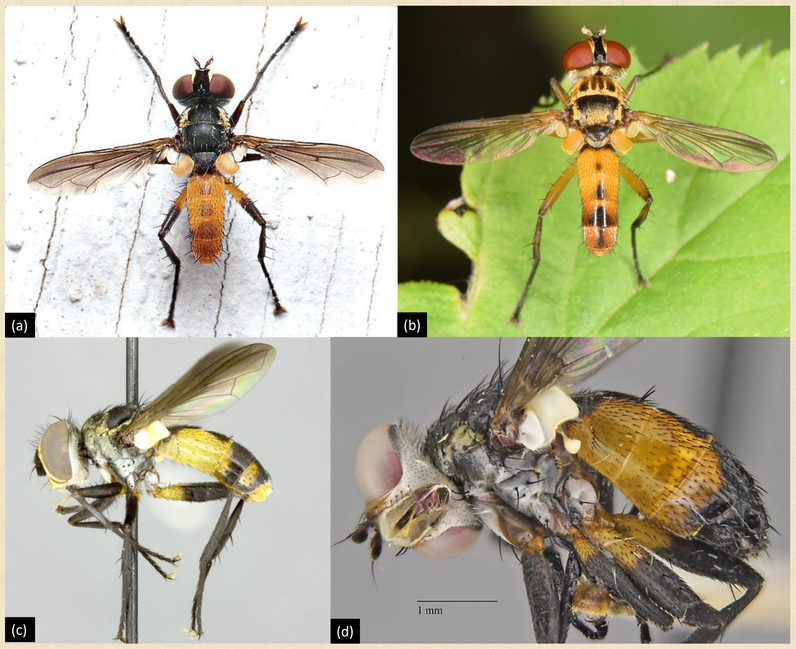

Figure 2. Nearctic *Xanthomelanodes* (a) *X. atripennis*, Photo: Ken Childs; (b) *X. flavipes*, Photo: Christina Butler; (c) *X. arcuatus*, Photo: Jim O'Hara and Shannon Henderson; (d) *X. californicus*, Photo: Gary Alpert.

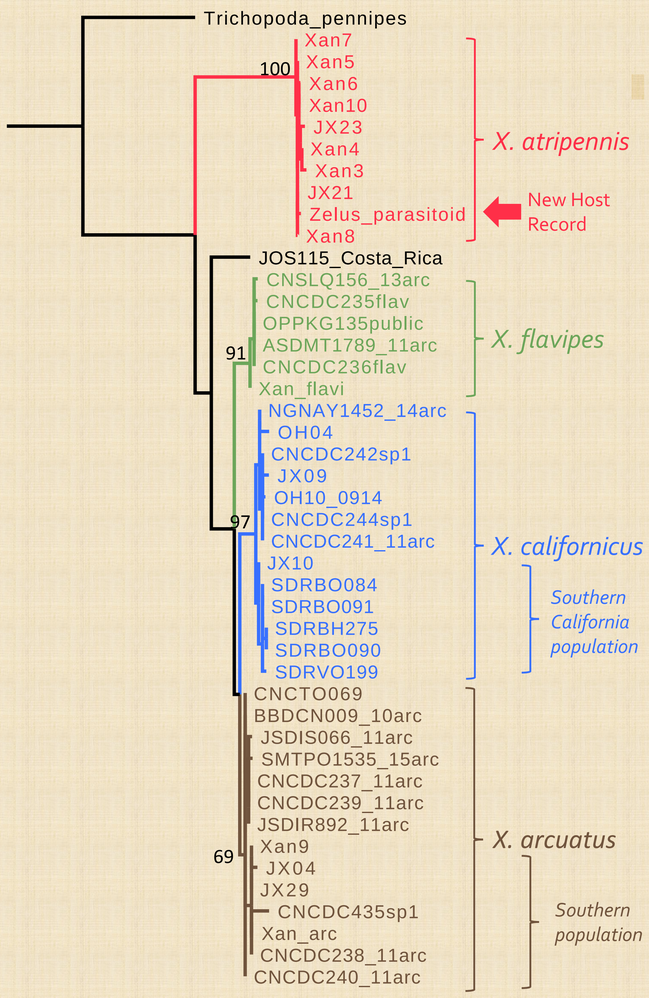

Figure 3. ML Phylogeny with combined sequence data from *COI* and *MCS* showing 4 distinct clades of North American *Xanthomelanodes* and identifying *Zelus luridus* as a new host for the *X. atripennis*.

Results

- Amplification – 10 *MCS* sequences (Max=960bp, Min=662, Avg=923.6); 12 *COI* sequences (Max=654bp, Min=423, Avg=617.5); coverage=0.61.
- Phylogenetics – *COI*, *MCS*, and combined trees were congruent; *X. atripennis* formed a distinct clade from other species (BS=100, P ID(Strict)=0.96); *X. flavipes* formed a distinct clade within a group containing *X. flavipes, X. californicus,* and *X. arcuatus* (BS=91, P ID(Strict)=0.87); *X. californicus* and *X. arcuatus* formed clades (BS=97, 69, and P ID(Strict)=0.87, 0.89, respectively) (Figure 3).
- Species Delimitation - intraspecific distances were low (*X. atripennis*: 0.002; *X. flavipes*: 0.002; *X. californicus*: 0.005; *X. arcuatus*: 0.004); P (Randomly Distinct) values were 1.00 for *X. atripennis* and *X. californicus*, 0.56 for *X. flavipes*, and 0.57 for *X. arcuatus.*

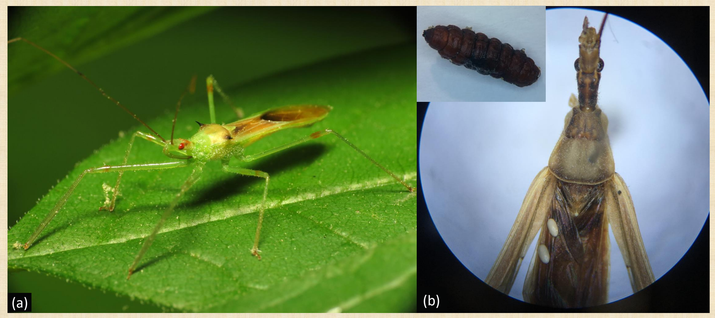

Figure 4. (a) Adult *Zelus luridus*, a newly recorded host of *X. atripennis*, Photo: Katja Schulz; (b) Adult *Zelus luridus* with *X. atripennis* larva. Inset: Pupa of *X. atripennis* parasitoid emerged from *Z. luridus* host

Discussion

During a study on sundew assassin bugs, Smith and Blaschke (2018) noted the emergence of a dipteran parasitoid from one of their *Z. luridus* specimens. The fly failed to mature to adulthood, making identification to species unlikely. DNA from that specimen was sequenced and included in our phylogeny where it nests firmly within the *X. atripennis* clade. This is the first confirmed host for *X. atripennis* and provides further evidence that *Xanthomelanodes* are highly specialized assassin bug parasitoids (Figure 3).

Our results represent the first phylogenetic analysis of *Xanthomelanodes* in the US and confirm the existence of at least four distinct species. Sabrosky (1950) was the last to provide an expanded discussion of the interspecific relationships within the genus and our results support his morphological findings, specifically that *X. atripennis* is quite distinct from other US species, that *X. flavipes* is most similar to *X. arcuatus*, and that *X. arcuatus* shares a close affinity to *X. californicus.* The monophyly of each species was robustly supported by individual gene analyses and the combined dataset.

Within *X. californicus* and *X. arcuatus,* two subpopulations were recovered corresponding to distinct geographical regions. *Xanthomelanodes californicus* contained a genetic cluster of specimens from southern California that may represent a novel species or an existing Neotropical species whose range has expanded into the US. Within *X. arcuatus*, a delineation between specimens collected in Canada (northern) and those collected in the US (southern) was uncovered and may indicate an ongoing speciation event. Morphological studies and an expanded phylogeny including additional *X. arcuatus* and *X. californicus* specimens are needed to determine whether there are significant enough differences between populations to warrant a separate species designation.

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