Honors Thesis

Causes and consequences of sex ratio variation in the parasitoid wasp *Pelecinus polyturator*

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Lay Abstract: *Pelecinus polyturator* (Drury) (Hymenoptera: Pelecinidae) is a parasitoid wasp of the larval stages of June beetles of the genus *Phyllophaga* (Coleoptera: Scarabaeidae). It is morphologically variable across its range throughout continental North and South America. This species is divided into two distinct populations: a northern population (southeastern Canada, and the United States east of the Rocky Mountains) and a southern population (northern Mexico to Argentina, excluding Chile). The area of Mexico between 23° N and 28° N is apparently uninhabited by the species and distinctly separates the two populations. The northern population is almost entirely made up of females, whereas the southern population is bisexual. In this study, we extract, replicate, and sequence DNA from *P. polyturator* specimens collected in localities of both the northern and southern populations. We then examine the resulting DNA barcodes for factors related to variation in sex ratio, including relatedness of the *cox1* gene and the presence of symbiotic bacteria (*Wolbachia*). Analysis of *cox1* relatedness suggests that there is a gradient of genetic variation throughout the southern population. Specimens from the northern population were found to be nearly identical. *Wolbachia* was present in both the northern and southern populations and was nearly identical in all positive specimens. This suggests that *Wolbachia* is not the cause of sex ratio variation in *P. polyturator*.

Introduction

Pelecinus polyturator (Drury) (Hymenoptera: Pelecinidae) is a large parasitoid wasp distributed throughout the continental Americas. It is commonly found in deciduous forests, typically during late summer and early fall in colder climates or year-round in warmer climates. Females parasitize subterranean *Phyllophaga* larvae (Coleoptera: Scarabaeidae) using their highly specialized abdomens (Mason, 1984; Johnson and Musetti, 1999). The species is highly dimorphic; females have a long, slender abdomen, while males have a relatively shorter, clavate abdomen (Figure 1) (Brues, 1928). A peculiar aspect of *P. polyturator* is that a distinct difference in sex ratio exists between different populations of this species. The area of Mexico between 23° N and 28° N is apparently uninhabited by the species and distinctly separates the two populations. *P. polyturator* Nearctic populations in the southeastern provinces of Canada and in the United States east of the Rocky Mountains are virtually entirely female, whereas both sexes are common in Neotropical populations from northern Mexico to Argentina, excluding Chile (Johnson and Musetti, 1998). No prior work focuses on this phenomenon, although there has been speculation in related studies of the species (Brues, 1928; Young, 1990). In this study, we more closely investigate the factors within *P. polyturator* that relate to this sex ratio variation.

Charles T. Brues previously used *P. polyturator* as an example of geographic parthenogenesis, the appearance of parthenogenic Nearctic populations and bisexual Neotropical populations of the same species (Brues, 1928). The extreme rarity of male specimens collected from northern localities supports this concept (Young, 1990). Still, the exact manner in which this parthenogenesis takes place and the sporadic presence of male offspring requires further study.

Several different genetic variations could indicate the method through which the Nearctic *P. polyturator* population reproduces parthenogenically. Haploid females would clearly indicate a population reproducing through parthenogenesis. If females are diploid, homozygosity would

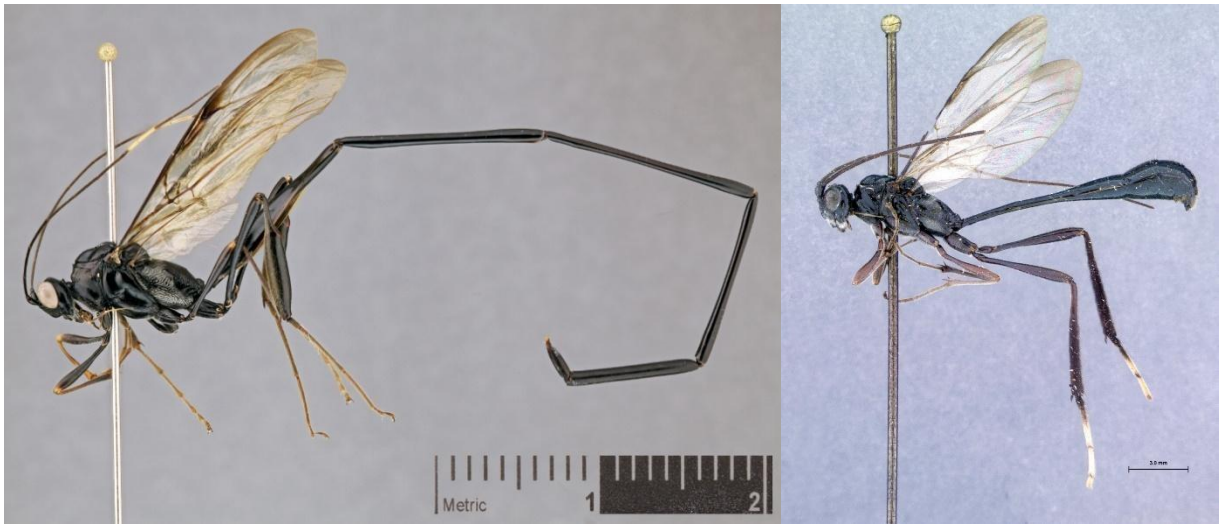

Figure 1 – Female P. polyturator *(OSUC 162167) on left, male* P. polyturator *(OSUC 1076) on right. Additional images can be found on the Hymenoptera Online database (hol.osu.edu).*

indicate a thelytokous population in which chromosomes replicate following the parthenogenic production of a haploid egg, resulting in a diploid individual (Ma et al., 2015). Previous work also speculates that *P. polyturator* females could be polyploid (Brues, 1928). Alternatively, heterozygous diploid females would indicate that females are, indeed, mating with males to produce offspring. The presence of spermatozoa in the female's spermatheca would similarly indicate a sexually reproducing population.

There is also the possibility that *Wolbachia* infection in different populations of P. polyturator contributes to sex ratio variation. *Wolbachia* is a genus of intracellular symbiotic bacteria that is extremely common in insects and other arthropods (Werren, 1997). It is capable of altering the host reproductive system through many different means, including killing male embryos, causing cytoplasmic incompatibility (CI), instigating parthenogenesis, and feminizing individuals that are genetically male (Stouthamer, 1999; Werren, 1997). Although there is no prior work on the relationship between *Wolbachia* and *Pelecinus* specifically, the symbiont's presence in other parasitoid wasps such as Nasonia (Hymenoptera: Pteromalidae) is well documented (Dittmer, 2016).

Neotropical specimens of *Pelecinus polyturator* show much greater variation in color, size, and morphological proportions, but virtually all newly proposed species and subspecies are currently thought to be nothing more than phenotypic variants (Johnson and Musetti, 1999). Because of this, it is more useful to look at underlying genetic variation through barcoding the mitochondrial DNA (Hebert, 2003). Interestingly, the introduction of CI-inducing strains of *Wolbachia* into a population can cause a selective sweep of mitochondrial DNA variants (Johnstone and Hurst, 1996). This suggests that Nearctic populations may exhibit less mtDNA variation than their Neotropical counterparts. Because of this, determining speciation between P. polyturator populations solely by examining mtDNA variation should be considered with caution.

The main objective of this study is to examine differences between the *Wolbachia* strains and the *cox1* gene within the Nearctic and Neotropical *P. polyturator* populations in order to obtain a better understanding of the source of the variation in sex ratio that exists between different populations of the species. The following were our hypotheses about the findings of this study:

- *Wolbachia* will be present in both the Nearctic and Neotropical populations.
- The strains of *Wolbachia* present in Nearctic specimens will differ from those in Neotropical specimens.
- There will be greater *cox1* gene variation within Neotropical specimens than within Nearctic specimens.
- The *cox1* gene sequence will be relatively consistent between the Nearctic and Neoptropical populations, suggesting that both populations belong to the same species.

Materials and Methods

We first obtained twenty-five specimens of *Pelecinus polyturator* (Table 1). Specimens were kept in 95% ethanol in a freezer at -20° C. We extracted DNA from the specimens using nondestructive DNA extraction protocols for Hymenoptera (Taekul et al., 2014). Whole specimens were placed individually in 1.5 mL tubes with a mixture of 180 µL buffer ATL and 40 µL proteinase K and incubated for 8 hours at 56° C. An additional 20 µL proteinase K was added to each tube and the specimens were incubated for an additional 16 hours. Specimens were then moved to a freezer for at least 2 hours at -20°C.

The specimens were removed from the supernatant and placed in distilled water for 30 minutes before being transferred back to 95% ethanol. Specimens were prepared for standard mounting and are currently deposited as DNA vouchers at the C.A. Triplehorn Insect Collection.

Specimen Barcode	Specimen Sex	Locality	Genes Sequenced
OSUC 553231	♀	Ohio, USA	*ftsZ*
OSUC 571247	♀	West Virginia, USA	*cox1, ftsZ*
OSUC 571248	♀	West Virginia USA	*cox1, ftsZ*
OSUC 571249	♀	Wisconsin, USA	*ftsZ*
OSUC 571303	♀	West Virginia, USA	*ftsZ*
OSUC 571304	♂	Oaxaca, Mexico	*none*
OSUC 571305	♀	Ontario, Canada	*ftsZ*
OSUC 571306	♀	Ohio, USA	*cox1, ftsZ*
OSUC 571307	♀	Salesópolis, Brazil	*cox1*
OSUC 571308	♀	Ohio, USA	*ftsZ*
OSUC 571309	♀	Oaxaca, Mexico	*ftsz*
OSUC 571310	♂	Oaxaca, Mexico	*ftsZ*
OSUC 571311	♀	Oaxaca, Mexico	*cox1*
OSUC 571312	♂	Oaxaca, Mexico	*ftsZ*
OSUC 571313	♀	Oaxaca, Mexico	*ftsZ*
OSUC 571314	♂	Oaxaca, Mexico	*ftsZ*
OSUC 571315	♀	Oaxaca, Mexico	*cox1, ftsz*
OSUC 571316	♀	Oaxaca, Mexico	*cox1, ftsz*
OSUC 571317	♂	Oaxaca, Mexico	*cox1, ftsz*
OSUC 571318	♂	Oaxaca, Mexico	*ftsZ*
OSUC 571319	♀	Oaxaca, Mexico	*ftsZ*
OSUC 571320	♂	Oaxaca, Mexico	*ftsZ*
OSUC 655026	♀	Ohio, USA	*ftsZ*
OSUC 688148	♀	Ontario, Canada	*ftsZ*
OSUC 697905	♀	Matagalpa, Nicaragua	*cox1, ftsZ*

Table 1 – Specimens are designated by their barcode numbers in the HOL database (hol.osu.edu) Also shown is specimen sex, collection locality, and the target genes that were successfully sequenced for each specimen.

200 µL buffer AL was added to each tube of supernatant and mixed by vortexing, then incubated at 70° C for 10 minutes. 200 µL cold (-20° C) 100% ethanol was added to each tube of supernatant and mixed by vortexing. Each supernatant was then transferred into DNeasy columns that were then placed into 2 mL collection tubes and centrifuged for 1 minute at 8000 rpm. Flow-through and collection tubes were discarded, and the DNeasy columns were placed in new collection tubes. 500 µL buffer AW1 was added to each column and centrifuged for 1 minute at 8000 rpm. Flow-through and collection tubes were discarded, and the DNeasy columns were placed in new collection tubes. 500 µL buffer AW2 was added to each column and centrifuged for 3 minutes at 14000 rpm. Flow through and collection tubes were discarded, and the DNeasy columns were placed in 1.5 mL tubes. 50 µL warm (55° C) buffer AE was added and incubated at room temperature (25° C) for 1 minute, then centrifuged for 1 minute at 8000 rpm. Another 50 µL warm buffer AE was added and incubated at room temperature for 1 minute, then centrifuged for 1 minute at 8000 rpm. Extract was then stored at -20°C.

We targeted the mitochondrial cytochrome oxidase I (*cox1*) gene from *Pelecinus polyturator* and the *ftsZ* gene from any associated *Wolbachia*. The *cox1* gene was amplified with the C1-N-2328/C1-J-1718 primer pair (Simon et al., 1994). The *ftsZ* gene was amplified with the ftsZ-R1/ftsZ-F1 primer pair (Baldo et al., 2006). Initial denaturation was conducted for 4 minutes at 94° C. The thermocycling protocol consisted of 45 seconds at 95° C for denaturation, 45 seconds at 54° C for annealing, and 45 seconds at 72° C for extension. This thermocycling protocol was run for 35 cycles. The final extension was initiated for 5 minutes at 72°C. Products were sequenced in both directions by Beckman Coulter Genomics (Danvers, MA) and assembled using Sequencher v4.0 (Gene Codes Corporation, Ann Arbor, MI).

Sequences were aligned by codons using MUSCLE implemented in MEGA6 (Tamura et al., 2013). Distance matrices were produced by MEGA6 after alignment. Bayesian analysis was performed using MrBayes 3.2.6 (Ronquist et al., 2012) using *Trichopria basalis* as an outgroup.

Results

The *cox1* gene was successfully replicated and sequenced from nine of the twenty-five specimens, and the *ftsZ* gene was successfully replicated and sequenced from twenty-two of the twenty-five specimens (Table 1).

Wolbachia was present in both the northern and southern populations. The *ftsZ* distance matrix showed that the *Wolbachia* strains found throughout both *Pelecinus* populations are nearly identical (≥99.898% relatedness), suggesting that there is a single *Wolbachia* strain associated with *Pelecinus* throughout its range (Figure 2).

A Bayesian tree was generated to show the possible phylogenetic relationship between the populations of *P. polyturator* (Figure 3). The Bayesian analysis suggests that there exist distinct populations within the United States, Mexico, Brazil, and Nicaragua, and that the northern population is more closely related to the Mexican population than to the rest of the southern population.

The *cox1* distance matrix showed that all Mexican *P. polyturator* specimens are nearly identical (≥99.684% relatedness) and all United States specimens are nearly identical (≥99.681% relatedness) (Figure 4). The Brazilian and Nicaraguan specimens showed even greater sequence variation. They were 91.891% related to each other but were only ≥87.154% related to the other populations.

OSUC #	655026	571247	571248	571249	688148	571317	571316	571315	571314	571313	571312	571310	571309	571308	571306	571305	571303	697905	655026
655026																			
571247	99.899																		
571248	99.899	99.9																	
571249	99.899	99.9	99.901																
688148	99.899	99.9	99.901	99.901															
571317	99.898	99.898	99.898	99.898	99.898														
571316	99.898	99.898	99.898	99.898	99.898	99.898													
571315	99.898	99.898	99.898	99.898	99.898	99.898	99.898												
571314	99.898	99.898	99.898	99.898	99.898	99.898	99.898	99.898											
571313	99.898	99.898	99.898	99.898	99.898	99.898	99.898	99.898	99.898										
571312	99.899	99.899	99.899	99.899	99.899	99.898	99.898	99.898	99.898	99.898									
571310	99.899	99.9	99.9	99.9	99.9	99.898	99.898	99.898	99.898	99.898	99.899								
571309	99.898	99.899	99.899	99.899	99.899	99.898	99.898	99.898	99.898	99.898	99.898	99.899							
571308	99.898	99.899	99.899	99.899	99.899	99.898	99.898	99.898	99.898	99.898	99.898	99.899	99.899						
571306	99.899	99.9	99.9	99.9	99.9	99.898	99.898	99.898	99.898	99.898	99.899	99.9	99.899	99.899					
571305	99.898	99.899	99.899	99.899	99.899	99.898	99.898	99.898	99.898	99.898	99.898	99.899	99.899	99.899	99.899				
571303	99.899	99.9	99.9	99.9	99.9	99.898	99.898	99.898	99.898	99.898	99.899	99.9	99.899	99.899	99.9	99.899			
697905	99.899	99.899	99.899	99.899	99.899	99.898	99.898	99.898	99.898	99.898	99.899	99.899	99.898	99.898	99.899	99.898	99.899		
655026	99.899	99.9	99.9	99.9	99.9	99.898	99.898	99.898	99.898	99.898	99.899	99.9	99.899	99.899	99.9	99.899	99.9	99.899	

Figure 2 – Distance matrix showing the relatedness between the ftsZ *genes sequenced from associated Wolbachia.*

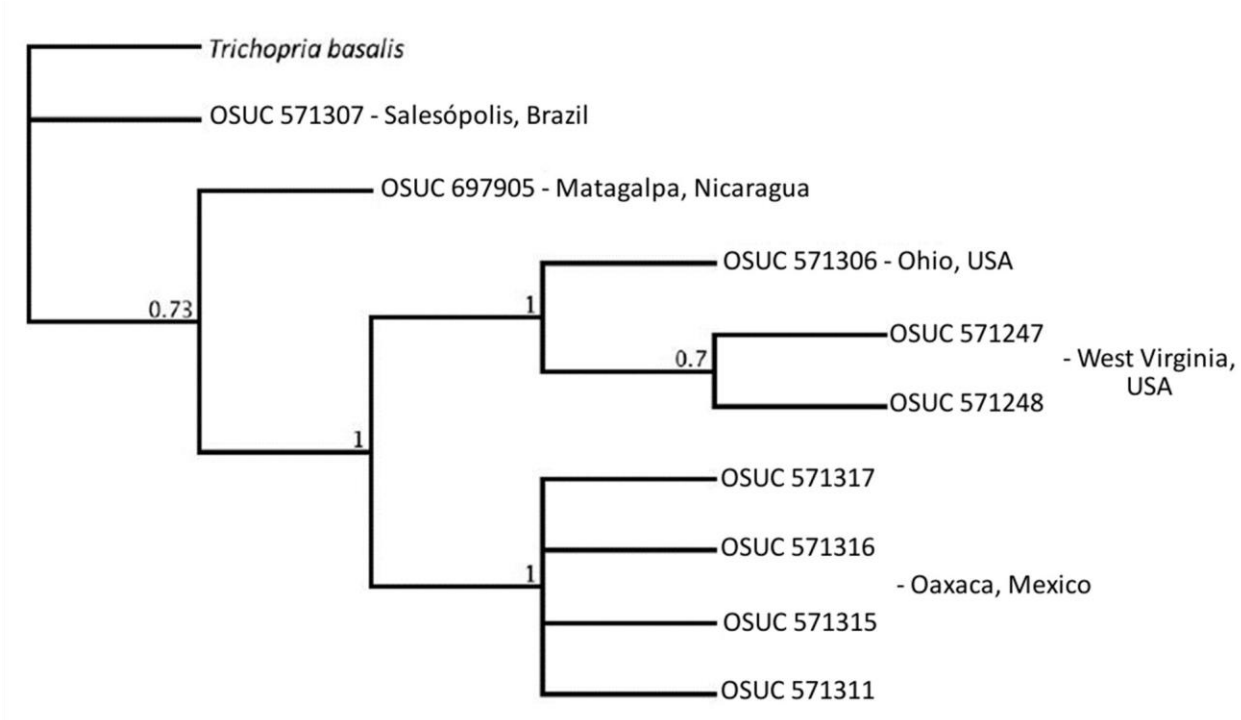

Figure 3 – Bayesian tree of the specimens based on cox1 *analysis, with* Trichopria basalis *as an outgroup. The numbers at the nodes indicate posterior probabilities.*

OSUC #	571317	571316	571311	571315	571307	697905	571248	571247	571306
571317									
571316	100.000								
571311	100.000	100.000							
571315	99.684	99.684	99.684						
571307	88.942	88.942	88.942	88.626					
697905	89.258	89.258	89.258	88.942	91.891				
571248	92.173	92.173	92.173	92.173	87.380	87.380			
571247	92.195	92.195	92.195	92.195	87.154	87.480	99.836		
571306	92.575	92.575	92.575	92.259	87.362	87.520	99.681	99.837	

Figure 4 – Distance matrix showing the relatedness between the cox1 *genes.*

Discussion

One possible explanation for the variation in *cox1* relatedness between northern and southern populations is that this species actually represents a species complex consisting of multiple species. While the northern population likely represents a single species, as is demonstrated by the nearly identical *cox1* relatedness, the southern population may represent one or more closely related species.

Alternatively, the pattern of *cox1* relatedness throughout the southern population also suggests that this population represents a single species that displays a gradient of genetic variation across its range and that geographic location correlates with *cox1* genotype. A paired t-test, where $p<.05$ indicates statistical significance, was performed to determine the correlation between *cox1* similarity and geographic distance between specimens. The resulting p-value of 0.00005 indicates that there is a statistically significant correlation between specimen distance and *cox1* similarity. This explanation would contribute to our understanding of why there is such great variation in color, size, and morphological proportions throughout the Neotropical population.

Cox1 similarity suggests that the Nearctic population is monophyletic, but the Neotropical population is not. This implies that there is a single origin of parthenogenesis in the Nearctic population. The *Wolbachia* strain present in Nearctic specimens did not greatly differ from that present in Neotropical specimens. This suggests that this particular strain of *Wolbachia* is not the cause of the sex ratio variation between *P. polyturator* populations. It is still unknown whether this strain of *Wolbachia* causes cytoplasmic incompatibility or another effect that could impact sex ratio. It is also important to note that not all specimens tested positive for the presence of *Wolbachia*. Whether this is due to actual absence or error in *ftsZ* extraction and replication is unknown.

Future research could aim to examine possible mechanisms of thelytokous reproduction in the northern population, including looking for spermatozoa in the spermatheca of females and determining the ploidy of both male and female specimens.

Material available for study limited the density of geographic sampling across the range of *Pelecinus*. Morphological analysis suggested significantly greater variability in southern populations. More intensive sampling for DNA variability may corroborate this at the genetic level. Future research could also elaborate on the genetic variation that exists throughout the southern population by sequencing additional genes and expanding the geographic coverage of studied specimens. Finally, analysis of *P. polyturator* morphology from different localities could be paired with further phylogenetic analysis to establish potential new morphospecies.

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