# Geographic variation in morphological, genetic and clonal diversity of the parasitoid wasp *Pelecinus polyturator* (Hymenoptera: Pelecinidae)

**By Victor Shegelski**

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## Abstract

The wasp species *Pelecinus polyturator,* the pelecinid wasp, is a widely dispersed species that is most easily recognized by its abnormally long abdomen. The range of this organism's habitat extends from Argentina to Canada making it subject to a wide range of weather and climatic conditions. This range in environmental variation could lead to adaptations including altered morphology and unorthodox reproductive techniques such as parthenogenesis. Samples have been collected from populations in Canada, the USA and Honduras and have been compared for geographic differences in morphology and genetic relatedness. Morphological comparisons show that: 1) Specimens from Canada and the USA are larger than Honduran specimens; 2) Honduran Specimens have relatively longer antennae; 3) American specimens have relatively longer abdomens; 4) Canadian specimens have less variance in wing size and antennae length. ISSR-PCR and agarose gel electrophoresis produced DNA bands based on the $CA_{10}$ and $GT_{10}$ primers that were used; however, the resolution was too low to be analysed for genetic relatedness. With methodological adjustments these primers are likely to produce significant results that can identify relatedness within and between populations.



**Keywords:** *Pelecinus polyturator*, morphological variation, Darwin's fecundity advantage model, Bergmann's rule, selective pressure, climate change, climatic variation, population relatedness, thelytoky parthenogenesis, ISSR-PCR, Pelecinidae, Hymenoptera.


## Introduction

*Pelecinus polyturator* (Drury) is an endoparasitoid wasp species that is primarily known for its incredibly long and thin abdomen with ovipositor as well as the parasitic larval stage in its life cycle (Brues, 1928). Sexual reproduction is rare due to low male representation in the species (Johnson and Musetti, 1999). *P. polyturator* compensates for the lack of sexual partners by performing thelytoky

parthenogenesis (Brues, 1928); the female lays unfertilized eggs that produce clones of the mother. Extant species of these parasitoid wasps range from South Eastern Canada (as far West as Manitoba), through the eastern U.S.A. and the Honduran rain forests to Argentina (Johnson and Musetti, 1999). This study is designed to find relationships in the form of geographic trends in morphology, genetic diversity and clone presence in populations.

Adult *P.polyturator* primarily use *Phyllophaga spp.* grubs as the host for their larvae (Johnson and Musetti, 1999); the wasp pierces the ground with its abdomen (up to 5 cm) in order to lay its eggs on beetle grubs, generally one per grub (Aguiar, 1997). Once the eggs on the grubs hatch, the parasitic wasp larvae enter the *Phyllophagas* host. The larvae feed and develop within the host and emerge to pupate; they eventually go through complete metamorphosis to emerge as an adult and, once an adult, the wasp feeds on nectar (Shih et al, 2009).

The rarity of males is proportional to latitudinal positioning; Northern populations will have very few males (Aguiar, 1997) whereas the male presence in equatorial populations tends to be greater (Masner, 1995). Parthenogenesis is a form of reproduction in areas populated with males but the sexual reproduction also diversifies the clonal base of the community, as Vorburger (2006) found when studying parthenogenic aphids (*Myzus persicae*). One would expect lower genetic diversity in the Northern populations of *P. polyturator* as thelytoky parthenogenesis is the more common method of reproduction; however, Loxdale and Lushai (2003) found in some species (most prominently aphids) even cloned populations may still have a disparate gene pool by means of high mutation rates. With thelytokus and clonal diversity a species could easily colonize new locations and start new populations from a single individual (Loxdale and Lushai, 2003); *P. polyturator* have the largest distribution of the *Pelecinidae* (Johnson and Musetti, 1999) which may ultimately be due to their thelytoky parthenogenesis.

Because of its vast geographic distribution, *P. polyturator* is subject to varying environmental conditions such as climate, food, host sources, predators and availability of sexual reproduction. It is likely that these varying conditions and pockets of sexual reproduction could lead to varying genetic diversity when measured geographically; this is especially possible considering there are gaps in the distribution of *P.polyturator*, effectively creating large 'island' populations (Johnson and Musetti, 1999). Female *P. polyturator* body length on average can range from 20-90mm and males average 15mm (Johnson and Musetti, 1999). A factor that could affect a size trend in *P. polyturator* may be host larvae size differences in which larger larvae lead to larger wasps (Johnson and Musetti, 1999); as mentioned above, host species of *P. polyturator* are primarily of the diverse genus *Phyllophaga* which are widely distributed and can also be found in the southern areas of Canada where *P. polyturator* exist (Galloway, 2008). The larvae of *Phylophaga spp.* can range from 15-25mm in length and current known larval hosts exploited by *P. polyturator* include *Phyllophaga anxia* (LeConte), *P. inverse* (Horn), *P. drakei* (Kirby), *P. rugosa* (Melsheimer) and *Podischnus agenor* (Olivier) (Johnson and Musetti 1999).

In their research, Johnson and Musetti (1999) completed a large scale sample collection and comparison based on species variation, color and location collected. They also projected the regions of the Americas with an appropriate climate for the survival of *P. polyturator* . This area is much larger than its current dispersal and it has already been found thriving in areas even further north than what was projected (Galloway 2008) with no reported adverse effects on the ecosystem. This species is capable of adaptation that was never before predicted and the variety of grubs capable of acting as hosts for the pelecinid wasp is still not known. Clausen (1940) believed the original host of *P. polyturator* was actually a wood boring beetle similar to the mountain pine beetle (*Dendroctonus ponderosae*) currently ravaging the forests of Northern Alberta; given the climate projections by Johnson and Musetti (1999) it is possible that in the future this wasp could play a very beneficial role in

agriculture and forestry as other parasitoid wasp species do today through biological pest control (Hoffmann and Frodsham 1993).

Further analysis of size differences based on environmental factors and comparisons of genetic diversity have yet to be examined for *P. polyturator*. There are obvious differences in wing size between other parasitoid wasp species based upon flight requirements such as distance and power needed to get to food sources and hosts (Price, 1973); for example, if host populations are dense the wing size may be smaller as the energy is no longer required for long, frequent flights and would be best invested elsewhere. In *Drosophila melanogaster*, Frazer *et al* (2008) found that larger wing sizes can offer a biomechanical advantage in cold weather flying. It is possible the long thin abdomen with ovipositor could be more structurally robust in areas with heavy soil that requires more strength to get through to host grubs. In other Hymenoptera, specifically the Apidae species *Bombus terrestris,* or bumblebees, antennae length has been found to be related to olfactory sensitivity and detection threshold; longer antennae allow for more complex detection, and also lower the olfactory detection threshold (Spaethe *et al* 2007). Any consistent morphological variation between regions could reveal adaptive changes in the species, and it is possible that these regional adaptations could be the early stages of speciation through the founder effect (Templeton 1980).

In order to examine genetic diversity intersimple sequence repeat-polymerase chain reaction (ISSR-PCR) was used as a form of genetic fingerprinting that targets base pair repetitions in the DNA of the *P. polyturator* specimens; this would help determine the clonal nature of geographic populations. In this case ISSR-PCR was used because there were no previously determined genetic markers for any *Pelecinus spp.* using the Scopus, Jstor and Springer Link databases, and using microsatallite markers is efficient but species specific (Selkoe and Toonen 2006). Without prior research, microsatellite determination is beyond the scope of this study.

Selection of ISSR-PCR primers was ultimately be based on testing with a slightly defined starting point. Luque et al (2002) wanted to find genetic variation between different noctuids (from the Order Lepidoptera); they found in the ISSR-PCR testing of 11 primers that $(CA)_{10}$ was the most accurate given a consistent 300ng of DNA was used in each test. Nascimento et al (2010) and Santos et al (2011) both tested 93 primers from UBC primer set #9 when testing genetic diversity and structure in bees from the Hymenoptera order. In each study $(CA)_n$ primers were among those selected for the final analysis. Uesugi and Sato (2012) found 3 $(CA)_n$ primers, $(CA)_{11}$, $(CA)_{12}$ and $(CA)_{15}$, where among the 9 most efficient in their study isolating microsatellite loci in *Encarsia smithi*, a parasitoid wasp from the family Aphelinidae (order: Hymenoptera). Lucas *et al* (2009) identified several $(GT)_n$ microsatellites in *Microstigmus nigrophthalmus*, another Hymenoptera species. CA and GT repeats seem to be a common occurrence in insects and will be a good starting point in testing primers. Upon examination of the specimens, it is expected that variation in the morphology and genetic diversity will be found relating to the geographic origin.

# Materials and Methods

## Subjects and Study Site

Samples were obtained from three ranges within the distribution of *P.* polyturator. The collection areas included: Southern Manitoba in Canada (globally Northern region); Illinois in the U.S.A. (central region); and Cusuco National Park in Honduras (tropical, equatorial region).

***Honduras***

Malaise traps were set up around Cusuco National Park at previously designated study sites. Site data that was previously collected by the organisation OPWALL and was made available for this research. General observations on soil, canopy cover and anthropogenic disturbance were made. Samples were also hand caught and stored in 75% ethanol whenever opportunity allowed.

***America***

Samples have already been collected by malaise trap at the Kibbe Life Sciences center in Illinois and were provided by Dr. Ken McCravy of the Western Illinois University. Climate, temperature, topographic, rainfall, soil and geological maps were consulted for details on the area.

***Canada***

Samples were collected by the author of this paper as well as Dr. Terry Galloway of the University of Manitoba and Michelle Duhamel, a fellow researcher in August, 2013 in Southern Manitoba. Collection site observations were made and climate, temperature, topographic, rainfall, soil and geological maps have also been used to acquire additional data.

Thirty specimens were randomly selected from each population and transferred to individual labelled containers with 75% ethanol. They were then stored at 4°C until use.

## Data Collection

***Morphological Measurements***

All measurements have been made on females as males were not numerous enough to do a proper comparison. The measurement points were as follows (Image guide available in the Appendix on page 22):

- Antennae:
    - i) Total length from base to tip
- Head:
    - ii) Width at widest point
    - iii) Height at median point of head
    - iv) Head length
    - v) Eye width at widest point

- vi) Eye width at thinnest point

- Mesosoma:
  - vii) Length from base of head to base of transition to the first metasoma joint
  - viii) Height at the tallest point of the mesosoma

- Metasoma:
  - ix) Segment diameter at the widest point of the first metasoma segment)
  - x) Length of the first metasoma segment
  - xi) Length of the second metasoma segment
  - xii) Length of the third metasoma segment
  - xiii) Length of the fourth metasoma segment
  - xiv) Length of the fifth metasoma segment
  - xv) Length of the ovipositor

- Legs:
  - xvi) Hind tarsus length
  - xvii) First leg segment from hind tarsus
  - xviii) Second leg segment from hind tarsus
  - xix) Third leg segment from hind tarsus
  - xx) Hind tibia length
  - xxi) Hind tibia width

- Wings:
  - xxii) Total length from base to tip
  - xxiii) Widest point perpendicular to length
  - xxiv) Distance from base to widest point
  - xxv) Hind wing length

Measurements for morphological data were done using two methods:

- Measurements i-iii, v-vi, xvi-xix, and xxii-xxv were made using Pro.Point 0-150mm (±0.03mm) digital calipers.
- Measurements iv,vii-xv, and xx-xxi were made using the computer software ImageJ. This was accomplished by taking digital photographs of each specimen including a scale, in this case a ruler was used, and uploading it into the program for further analyses.
- Wing size was determined through the equation: $A_w=S\bullet C$ where $A_w$ is the wing area, S is the wing span and C is the wing chord.
- Thorax size was calculated by the equation: $A_T=L_T\bullet H_T$ where $A_T$ is thorax size, $L_T$ is thorax length and $H_T$ is thorax height. Thorax measurements were taken from a profile image and therefore thorax width could not be included.
- Head size was calculated using the equation $A_H=L_H\bullet H_H\bullet W_H$ where $A_T$ is thorax size, $L_T$ is thorax length and $H_T$ is thorax height and $W_H$ is head width.

***Genetic Comparison***

ISSR-PCR procedures were done according to Nascimento et al (2010) and 4 primers ($CA_5$, $CA_{10}$, $CA_{15}$ and $GT_{10}$) were used to find the most appropriate primers. After DNA extraction and ISSR-PCR procedures were finished the samples were run through electrophoresis to determine genetic relatedness of the samples. Differences were to be characterized by the presence/absence of resulting bands. Methods used are as follows:

- DNA extraction was done using TRI Reagent® solution. Procedure for DNA extraction was followed from the Ambion (2008) TRI Reagent® laboratory manual, following procedure for use of 50mg of tissue from each specimen and 500uL of TRI Reagent® Solution. Glass homogenizers were used to homogenize specimens and were cleaned between uses.

- The initial test to determine primer validity used samples that were comprised of a mix of DNA from 3 Honduran, 3 Canadian and 3 American specimens pooled together.
- ISSR-PCR procedure will be as performed in Nascimento *et al* (2010) with slight adjustments:

  Reaction mixture (per sample)

  - 1.0µL DNA (approximately 10ng of DNA)
  - 2.5µL 10x Buffer: New England Biolabs (Cat # B9014S)
  - 0.1µL 5 U Taq: Invitrogen (Cat# 18038-042)
  - 0.5µL dNTPs (10mM): Invitrogen (Cat # 18427-013)
  - 1.0µL (12.5nmoles) of custom primer ($CA_5$, $CA_{10}$, $CA_{15}$ or $GT_{10}$): Invitrogen

  PCR Cycle

  i. 94°C – 1 minute
  ii. 40 cycles of:
    a. 92°C – 1 minute
    b. Annealing temperature (50°C in test trial, then adjusted to 37°C for actual specimens) – 2 minutes
    c. 72°C – 2 minutes
  iii. final step: 72°C – 7 minutes
  iv. kept at 4°C until agarose electrophoresis
- 1% agarose gels were used for electrophoresis and was run at 70V for 1 hour in the trial and 40 minutes for actual specimens.

## Data Analysis and Statistics

### *Morphological Data*

- Mean and standard deviation were calculated for each site.

- Single factor ANOVA testing was done to determine the existence of differences between populations.
- Tukey-Kramer tests were used for pairwise testing.
- Regression analyses were used to determine the presence of relationships between thorax size (used as the standard for specimen size) and various appendages.
- Two sample F-tests were used to determine pairwise differences in variance between populations.

***Genetic Data***

- Presence and absence of bands were to be recorded for each individual within a site (Table 1) and relatedness was to be calculated on how many bands/absence of bands are shared between the individuals (Table 2); this would yield the relatedness in a percentage for each individual.

**Table 1 – Example of individuals A, B and C and the presence/absence of bands 1, 2 and 3**

| Bands | A | B | C |
|---|---|---|---|
| 1 | 0 | 1 | 0 |
| 2 | 1 | 1 | 1 |
| 3 | 1 | 1 | 0 |

**Table 2 – Example of percentage of bands shared between individuals A, B and C.**

| Individuals | A | B | C |
|---|---|---|---|
| A | 0 | | |
| B | 0.67 | 0 | |
| C | 0.67 | 0.33 | 0 |

- This relatedness would then be averaged and compared to the other sites by a $X^2$ test to determine whether or not statistical difference exists between the sites.
- The frequency of band appearance were also to be compared using a $X^2$ test. This would show if some bands are more prominent in different populations.
- The number of clone groups and individuals within each clone group were also to be compared among the three populations using $X^2$ tests.

- Individuals with >95% relatedness were also to be noted as possible clones with genetic mutation and compared between populations using a $X^2$ test.

## Results

There was no bias found between the trapping methods used in Honduras ($X^2$: df=1; p=0.97). Overall length is greater in the Canadian and USA populations while the Honduran population is the shortest (Figure 1; A NOVA: F(2, 87)= 31.04; p<0 .05; Tukey: A=C>H; α=0.05). This trend was seen in many other anatomical features: thorax length and head size are also greater in the Canadian and USA populations (ANOVA: F(2, 87)=10.63; p<0.05; Tukey: A=C>H; α=0.05; and ANOVA: F(2, 87)=44.24; p<0.05; Tukey: A=C>H, respectively).

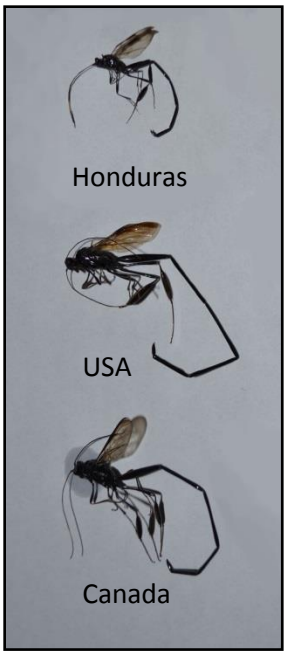


Figure 1 – *P. polyturator* collected from Honduras, USA and Canada.

The USA population relatively has the longest metasoma, the Honduran population has the shortest and the Canadian population is between the two (Figure 2; ANOVA: F(2, 87)=86.95; p<0.05; Tukey: A>C>H).

The Honduran population has relatively longer antennae than the USA population (Figure 3; ANOVA: F(2, 87)=3.23; p<0.05;Tukey: H>A).

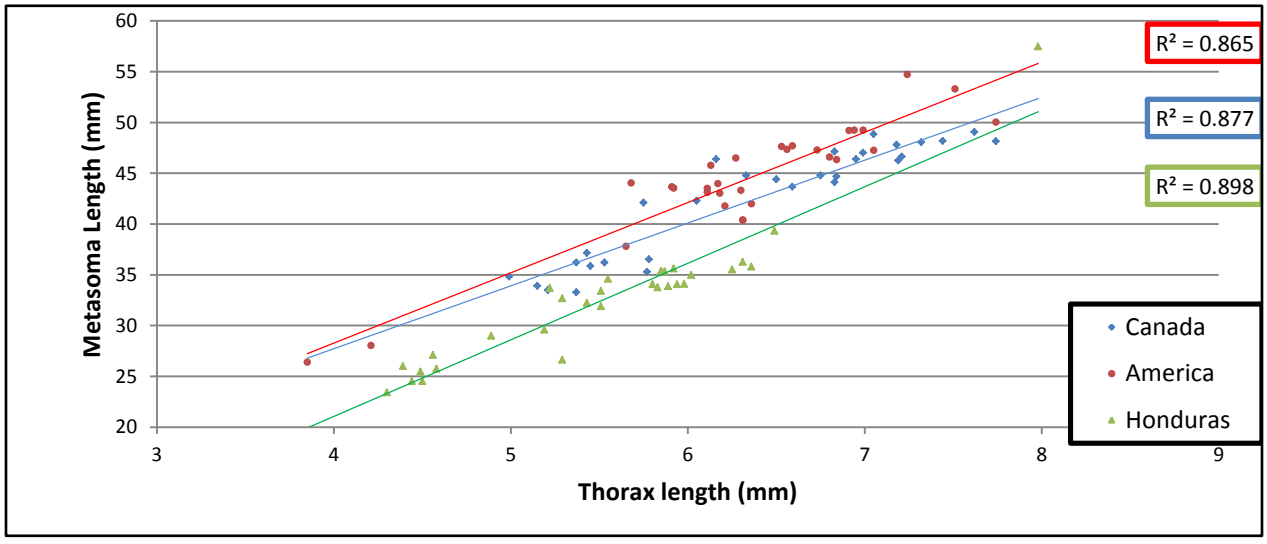


Figure 2 – Metasoma length vs thorax length; these are relatively proportional in all populations (Regression: p<0.05).

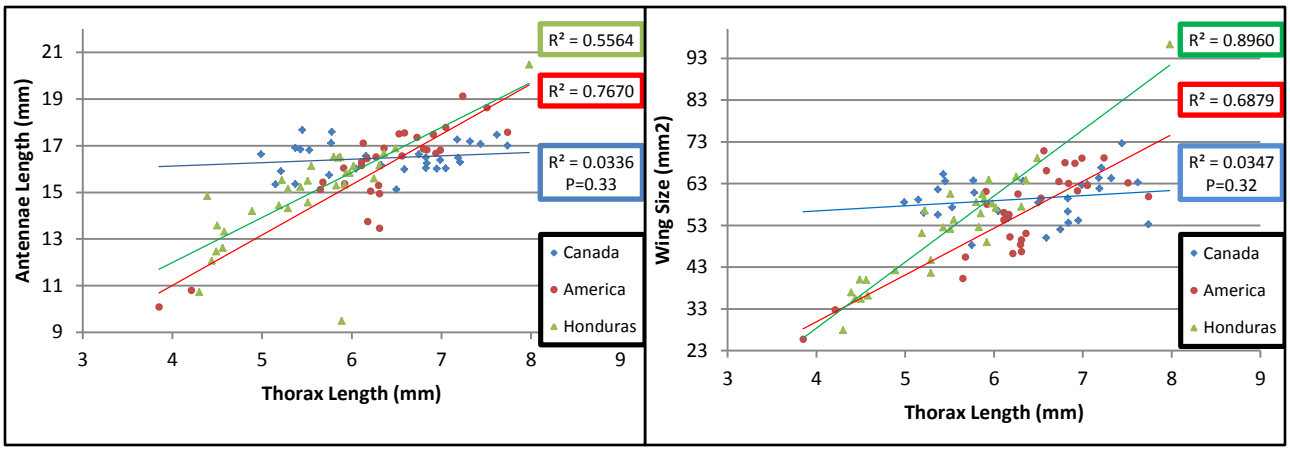


Figure 3 – Both wing size vs thorax length (left) and antennae length vs thorax length (right) show the lack of regression in the Canadian population. If a p-value is not shown under the $R^2$ value, then $p<0.05$.

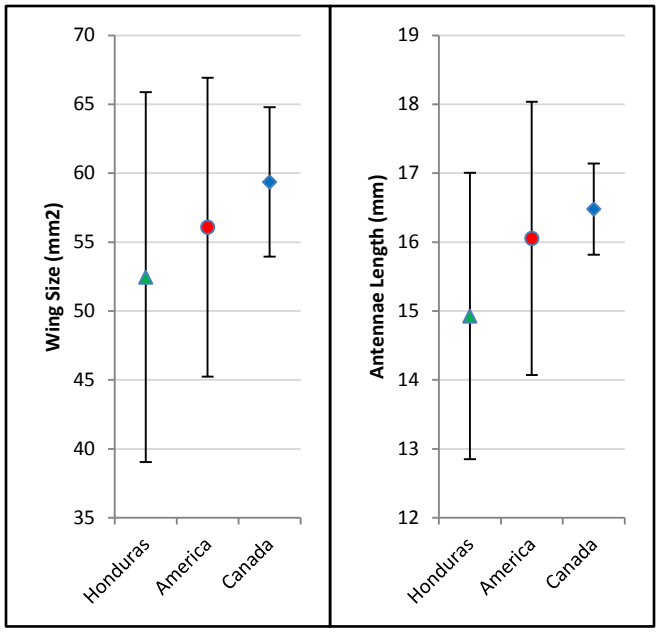


Figure 4 – Wing size (left) and antennae length (right) both show a significantly lower variance in the Canadian population. Error bars show standard deviation.

There is no change in wing aspect ratio between the populations (ANOVA: $F(2, 87)=0.09$; $p=0.91$); however, The Canadian population displayed significantly less wing size variance than the other populations (Figure 4; F-test: $p<0.05$). It also showed no relationship between wing size and thorax length (Figure 3; $R^2=0.035$; $p=0.32$), while the USA and Honduran populations showed a relationship (Figure 3; $R^2=0.896$; $p<0.05$ and $R^2= 0.688$; $p<0.05$ respectively).

The Canadian population displayed significantly less antennae length variance than the other populations (Figure 4; F-test: $p<0.05$). It also showed no relationship between antennae length and thorax length (Figure 3; $R^2=0.034$; $p=0.33$), while the USA and Honduran populations showed a relationship (Figure 3; $R^2=0.767$; $p<0.05$ and $R^2= 0.556$; $p<0.05$ respectively).

There was no differences in variance considering thorax size(F-test: USA/Honduras: p=0.499; USA/Canada: p=0.433; Canada/Honduras: p0.433) and overall length (F-test: USA/Honduras: p=0.396; USA/Canada: p=0.368; Canada/Honduras: p=0.274).

The initial test to determine which primer would produce the clearest bands did not produce any visible DNA (Figure 5). This gel was run for a full hour and the annealing temperature of the ISSR-PCR reaction was set to 50°C. The subsequent gels (Figures 6, 7 and 8) were only run for 40 minutes and the ISSR-PCR annealing temperature was adjusted to 37°C. In these gels bands of DNA were visible but resolution was poor and the bands were not comparable for the purposes of this study.

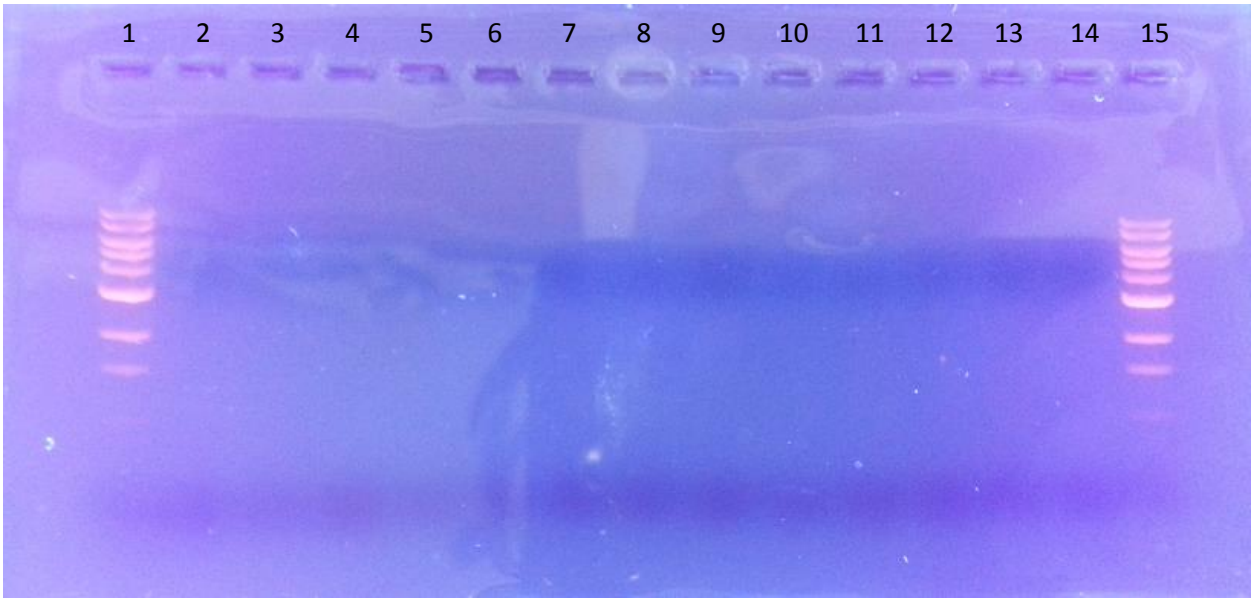


**Figure 5 – The primer trial electrophoresis results: Lanes 1 and 15 are DNA ladder; lanes 2-4 are samples tested with $CA_5$ primer; lanes 5-7 are samples tested with $CA_{10}$ primer; lanes 8-10 are samples tested with $CA_{15}$ primer; and lanes 11-14 are samples tested with $GT_{10}$ primer.**

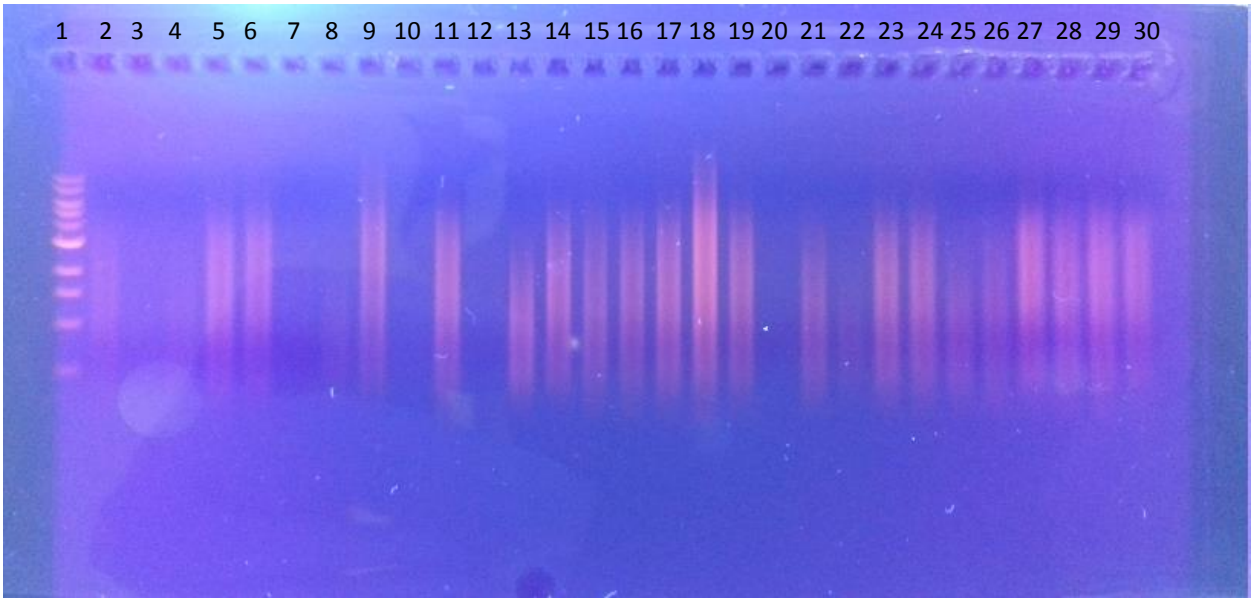


**Figure 6 - The first gel electrophoresis results: lane 1 is the DNA ladder; lanes 2-14 are Honduran samples treated with $GT_{10}$ primer; lanes 15-26 are Canadian samples treated with $GT_{10}$ primer; and lanes 27-30 are American samples treated with $GT_{10}$ primer.**

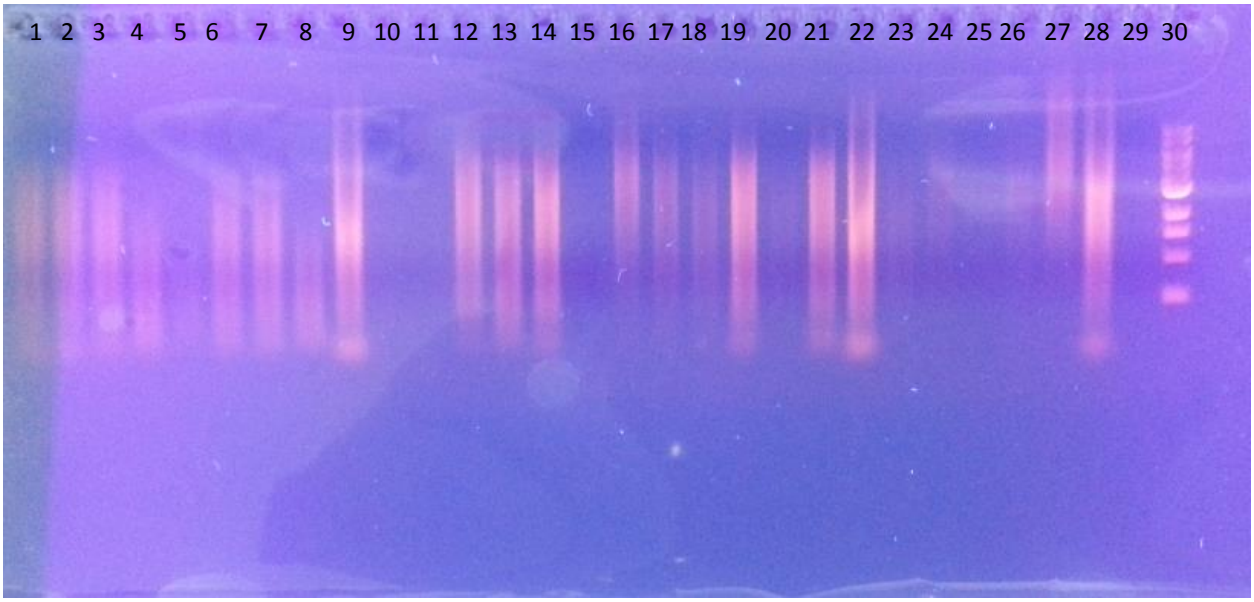


**Figure 7 - The second gel electrophoresis results: lanes 1-8 are American samples treated with $GT_{10}$ primer; lanes 9-18 are Honduran samples treated with $CA_{10}$ primer; lanes19-28 are Canadian samples treated with $CA_{10}$ primer; lane 29 is an American sample treated with $CA_{10}$ primer; and lane 30 is the DNA ladder.**

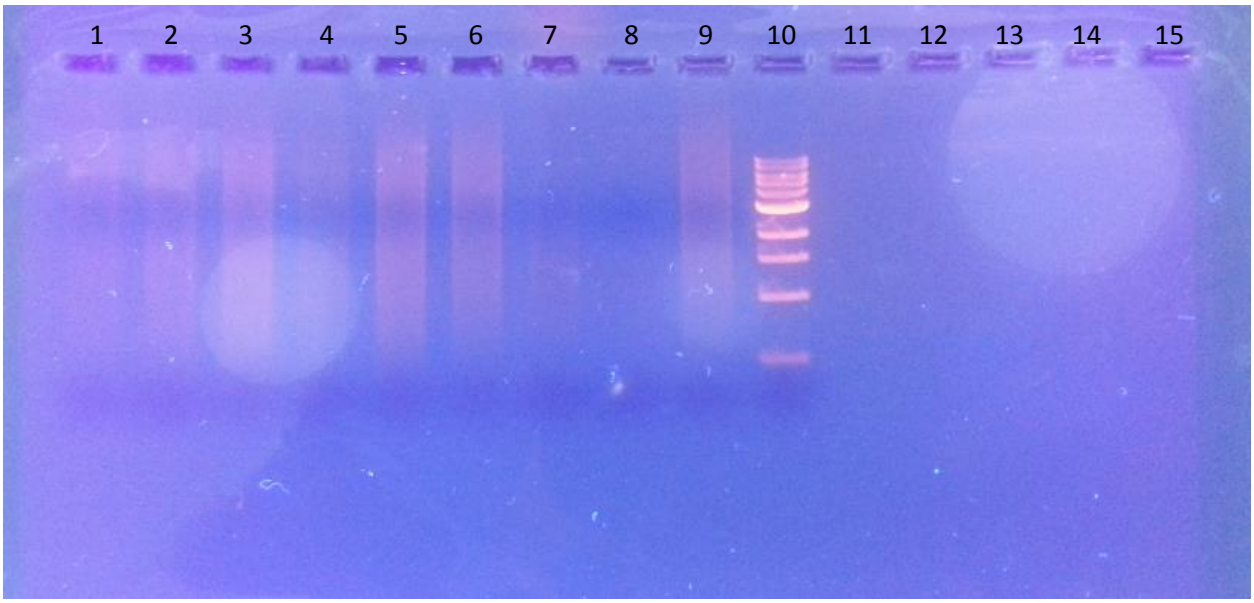


**Figure 8 - The second gel electrophoresis results: lanes 1-9 are American specimens treated with $CA_{10}$ primer; lane 10 is the DNA ladder; and lanes 11-15 are empty.**

## Discussion

The overall trend seen in the populations shows that the more Northern populations (Canada and USA) display an increase in overall size, which could provide several benefits to the species. Two such benefits are illustrated in Darwin's fecundity advantage model and in Bergmann's rule. Firstly, Darwin believed that increased female size could be related to increased fecundity; in several entomological studies larger females were found to have higher fecundity (Afaq and Afaq 2013) largely due to increased egg producing ability (Agarwala *et al* 2001: Berger *et al* 2008). Secondly, Bergmann's rule describes how increased size decreases relative surface area, making the larger organism more capable of environmental resistance due to reduced heat loss. Adult size in holometabolous insects is entirely determined by growth during the premetamorphic larval stages and pupation. Although the underlying mechanisms are not fully understood, it is found that often insects in colder climates have a larger developmental phase which appears to lead to larger body sizes (Atkinson and Sibly 1997), hence it is phenotypic plasticity in the organism; the large body size of the Canadian and USA populations of *P. polyturator* seem to follow this trend.

Assuming there is no previously undescribed function of the metasoma in female *P. polyturator,* the longer metasoma of the USA populations likely adds a mechanical advantage to the parasite's ability to access hosts in the region. It would be assumed that this must be an energetically valuable adaptation as the length of the tail appears to be disadvantageous in flight, resulting in awkward and slow aerial movement.

It is possible that the relatively larger antennae length in the Honduran population can offer an olfactory advantage in not only detection, but sensitivity to discerning between the plethora of ambient organic chemicals. This phenomenon has been previously described by Spaethe *et al* 2007) in the study of *Bombus terrestris*. Honduras is a biologically complex environment (Townsend *et al* 2008) with an untold number of species and biological complexity; such discretionary capabilities would prove highly beneficial.

While the Canadian population did not display a relatively larger antennae length, there was a lack of antennae length and wing size variation in this population; low variance of a trait may be indicative of selective pressure (Lenormand 2002). It would be expected that if the founder effect was a factor that this low variation would be shared by more traits, but no variation difference between the populations were observed in overall length or thorax size, indicating that only these anatomical features (perhaps including others not observed in this study) were acted upon. Alternatively, it should also be considered that this Canadian population has only recently immigrated (Galloway 2008); it may be possible that the founder effect has taken place leading to low morphology variation but high mutation rates have renewed variation in less crucial anatomical features for this specific environment, such as the thorax size.

The lack of variance seemed to show a higher minimum wing size and antennae length; it is possible that for these traits a minimum value is required to function efficiently in Canada's sparse environment. Spaethe *et al* (2007) described how antennae length is proportional to olfactory

sensitivity and inversely proportional to detection threshold; the relative abundance and density of *P. polyturator* have not been examined in these study sites, but it is possible that a minimum antennae length, and therefore olfactory detection threshold, may be required to detect hosts and/or pheromones considering Canada's sparse environment. This sparse environment could also require more flight to access resources and potential mates as males are considered to be relatively rare in the more polar regions (Brues 1928). Larger wing size could offer a biomechanical advantage for not only distance flight, but also cold weather performance (Frazer *et al* 2008).

The exact benefits of the morphological differences between the populations can only be speculated on at this point; they may be directly related to lower host availability and decreased seasonal time in which to reproduce. Increase in size can enhance fecundity, higher minimum wing size and antennae length could enhance the ability to find potential mates and hosts efficiently. This plasticity is likely a determining factor in the large dispersal of *P. polyturator*.

The results of the ISSR-PCR were inconclusive. Bands of DNA were present in nearly all specimens, however, the resolution was very poor and individual bands were not prominent. There were several factors that may have contributed to this: 1) the method of DNA isolation involved homogenizing whole specimens which included the hard exoskeleton. In future studies it would be beneficial to extract materials, such as thorax muscle, for a more consistent DNA extraction yield; 2) The most efficient annealing temperatures of the custom primers were unknown. The temperatures used in the ISSR-PCR were more or less an educated guess; further testing of these primers would determine the most effective annealing temperature. Also, the validity of these primers is unknown in this species and genetic mapping could help determine what primer would be most effective for this process. Microsatellite mapping would be the most ideal method (Selkoe and Toonen 2006) and should be considered in future studies if this organism's genome is mapped; 3) Gel thickness should be reduced for increased resolution. The gels used in this study were approximately 7-8mm thick but this could be

reduced; 4) The primers used in this study may not be specific enough to yield prominent bands if they are causing too many fractions to be replicated in the ISSR-PCR. Further studies should include some more specific and longer ISSR-PCR primers.

The ISSR-PCR and gel electrophoresis methods used in this study were exploratory and considered to be preliminary to further research. These results show that primers of this type may be used, with some methodical modification, to produce DNA bands within the Genome of *Pelecinus polyturator*.

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*1. Concordia University College of Alberta*

*2. Western Illinois University*

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*4. Operation Wallacea*

*5. University of Manitoba*

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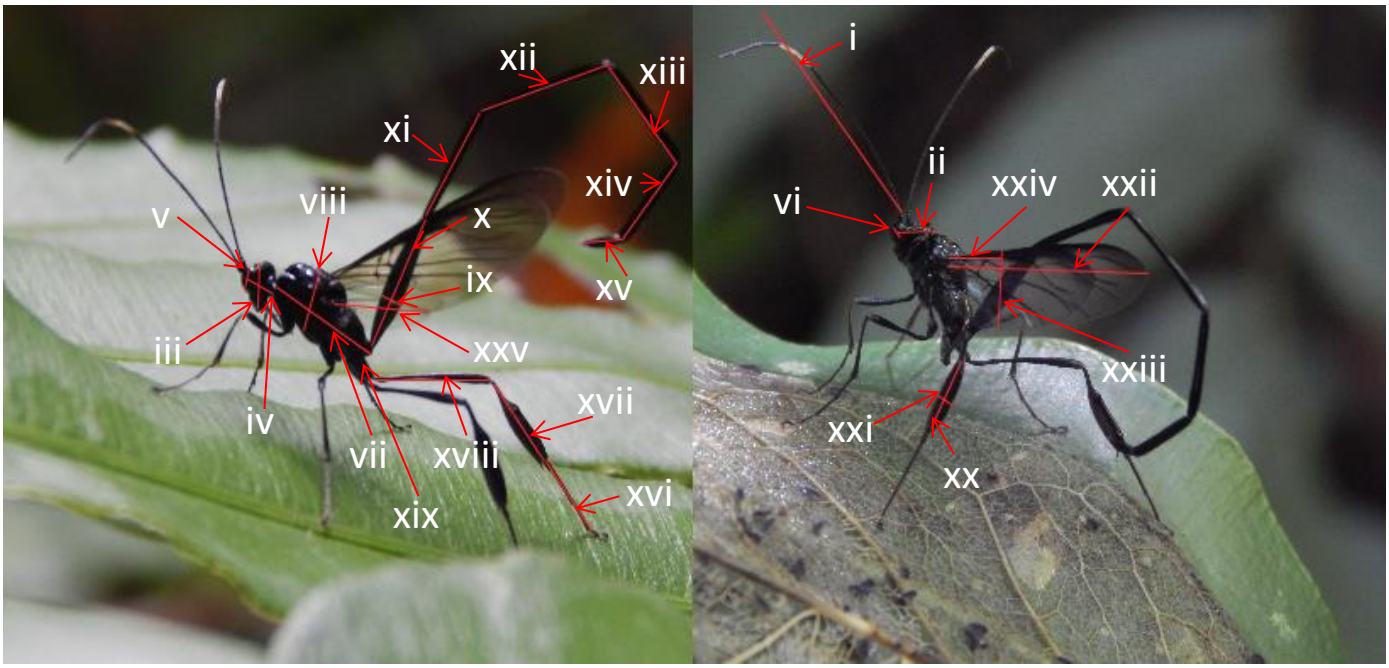
**Appendix:** Measurement Points




Antennae:

i) Total length from base to tip

Head:

ii) Width at widest point

iii) Height at median point of head

iv) Head length

v) Eye width at widest point

vi) Eye width at thinnest point

Mesosoma:

vii) Length from base of head to base of transition to the first metasoma joint

viii) Height at the tallest point of the mesosoma

Metasoma:

ix) Segment diameter at the widest point of the first metasoma segment)

x) Length of the first metasoma segment

xi) Length of the second metasoma segment

xii) Length of the third metasoma segment

xiii) Length of the fourth metasoma segment

xiv) Length of the fifth metasoma segment

xv) Length of the ovipositor

Legs:

xvi) Hind tarsus length

xvii) First leg segment from hind tarsus

xviii) Second leg segment from hind tarsus

xix) Third leg segment from hind tarsus

xx) Hind tibia length

xxi) Hind tibia width

Wings:

xxii) Total length from base to tip

xxiii) Widest point perpendicular to length

xxiv) Distance from base to widest point

xxv) Hind wing length