A STUDY OF WINGLESS PHORIDS IN SINGAPORE (SCUTTLE FLIES)

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INTRODUCTION

Phorids are an ecologically diverse and successful family of flies, which can be found in many habitats. In a number of species the females have lost or reduced their wings[1]. Some wingless phorids are found on NUS campus, but nothing else is known about these tiny flies (body length ≤1.5mm). Here I first assess the species diversity, then gather life history information,before discussing the adaptive significance for such a drastic change in female morphology.

FROM ONE TO MANY

Because of their limited dispersal ability, I initially expected only one species on campus. However, collecting behind the DBS graduate cluster using dung as bait yielded flies of very different size (0.7-1.5mm). This suggested multiple species. I established 50 cultures based on single females that yielded offspring including the fully winged males. In order to determine species diversity, I sequenced Cyt B of the offspring and used clustering to estimate species diversity.

Specimens from each cluster were then imaged in order to test whether the putative species also had morphological differences.

Genetic and morphological analysis revealed five putative species; 3 in *Chonocephalus* and two in *Puliciphora* (Fig. 1).

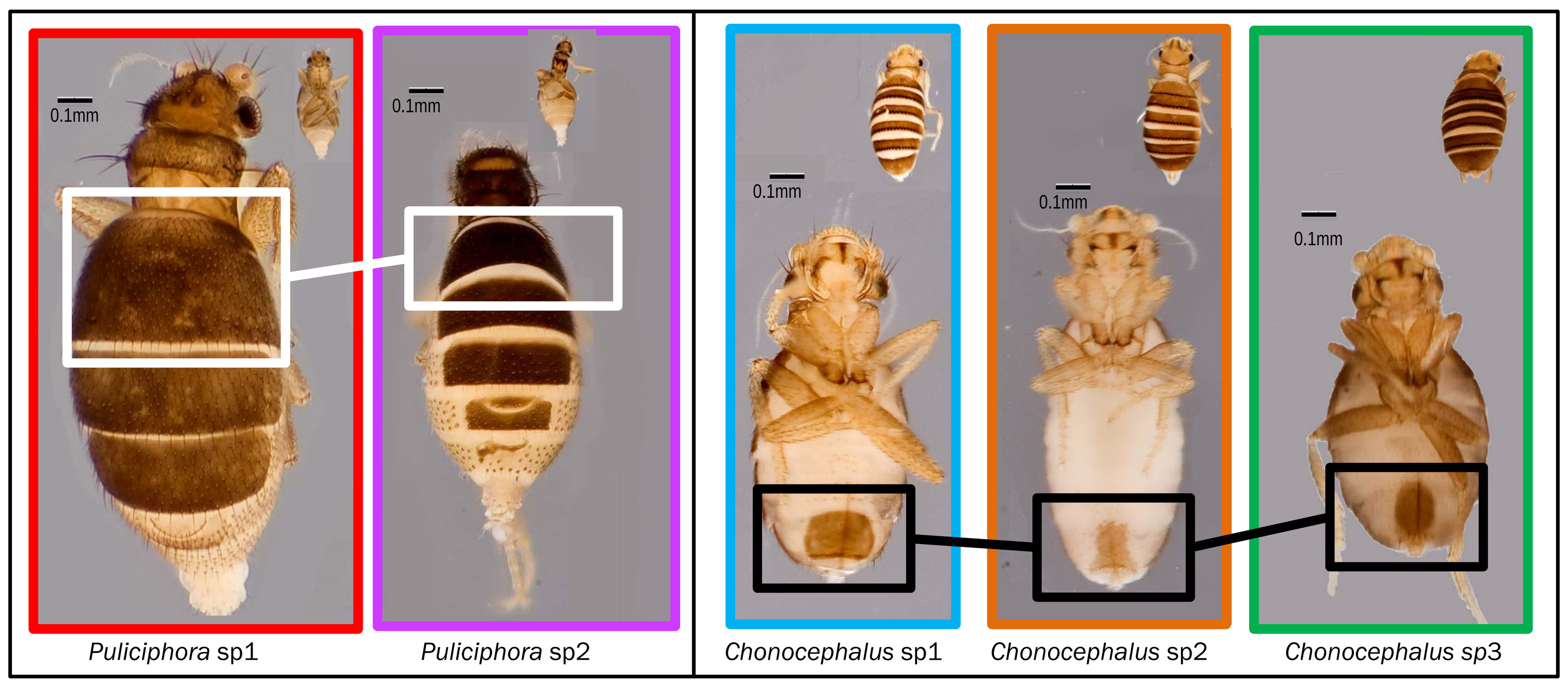

Figure 1: Dorsal and ventral images of the five putative species with main diagnostic differences highlighted

Would difference in morphology (i.e. size) result in different mating strategies?

SWEPT OFF HER FEET

Microscope video analysis revealed that:

a) *In copula, all Chonocephalus males lift females off the substrate using their genitalia, while Puliciphora females remain on the substrate (Fig 2).*

b) *Chonocephalus* couples fly *in copula*, while this was not observed for *Puliciphora*, although it is described for *Puliciphora borinquenensis*[2].

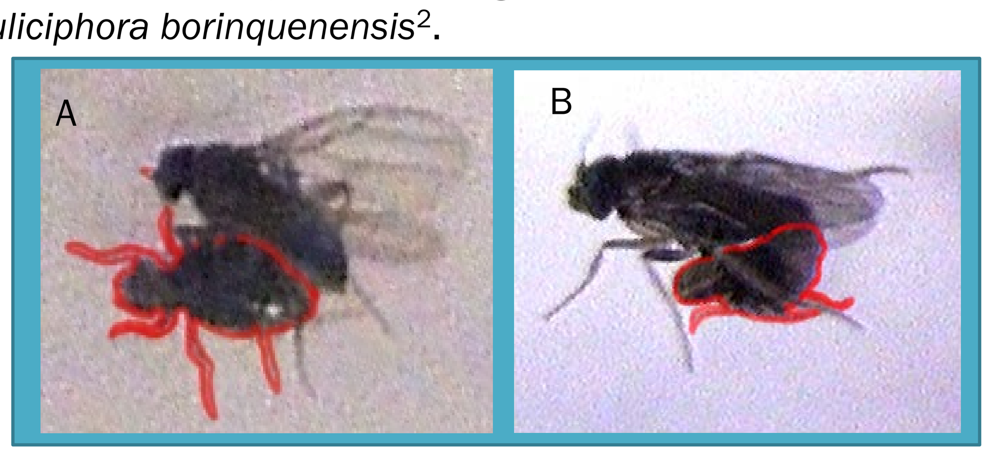

Figure 2: Image of (A) *Puliciphora* and (B) *Chonocephalus* ('pick-up' behaviour) *in copula*.

I performed field experiments (Fig. 3) to determine which species can transport females over a water barrier:

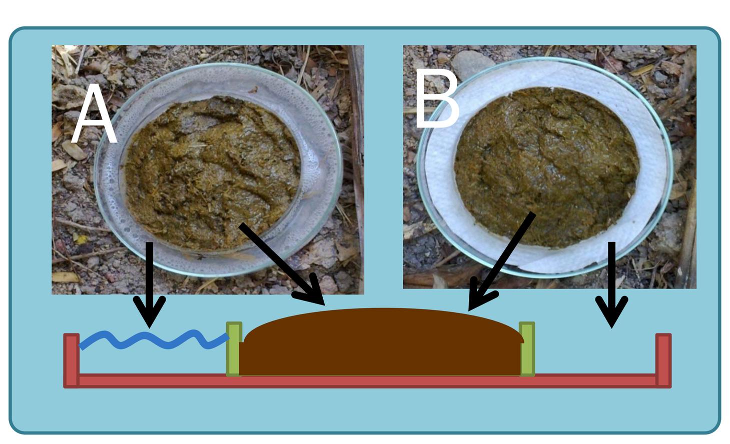

Figure 3:
(A) Experimental set-up of dung surrounded by detergent water;
(B) control

Results:
Both genera can transport females to oviposition sites (Fig 4).
However, *Puliciphora* sp1 were not observed in the experiments.

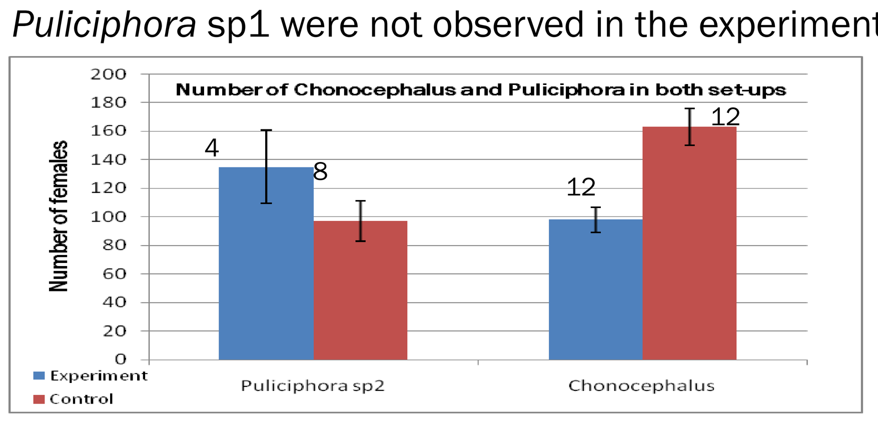

Figure 4: Number of females from both genera in experimental and control set-ups (> 4 hour trapping) for 15 experiments at 2 sites on campus; number of trials with specimens above the bars

Size matters: only species with small females are observed to exhibit 'transport' behavior. This trait is ***not*** genus-specific.

Has winglessness and sexual dimorphism evolved twice in the two genera?

MULTIPLE ORIGIN OF WINGLESSNESS & SEXUAL DIMORPHISM

I sequenced five mitochondrial and nuclear gene fragments (Cyt B, COI, 12S, 16S and 28S) and reconstructed the phorid phylogeny using 27 specimens from my experiments and 24 phorid species with data in GenBank (Fig 5).

Results:

(1) 'Winglessness' and dropping behaviour appears to have evolved twice
(2) The 'pickup' mating behavior is *Chonocephalus* specific.
(3) Sexual size dimorphism has evolved in *Chonocephalus* while female *Puliciphora* are only slightly smaller than males

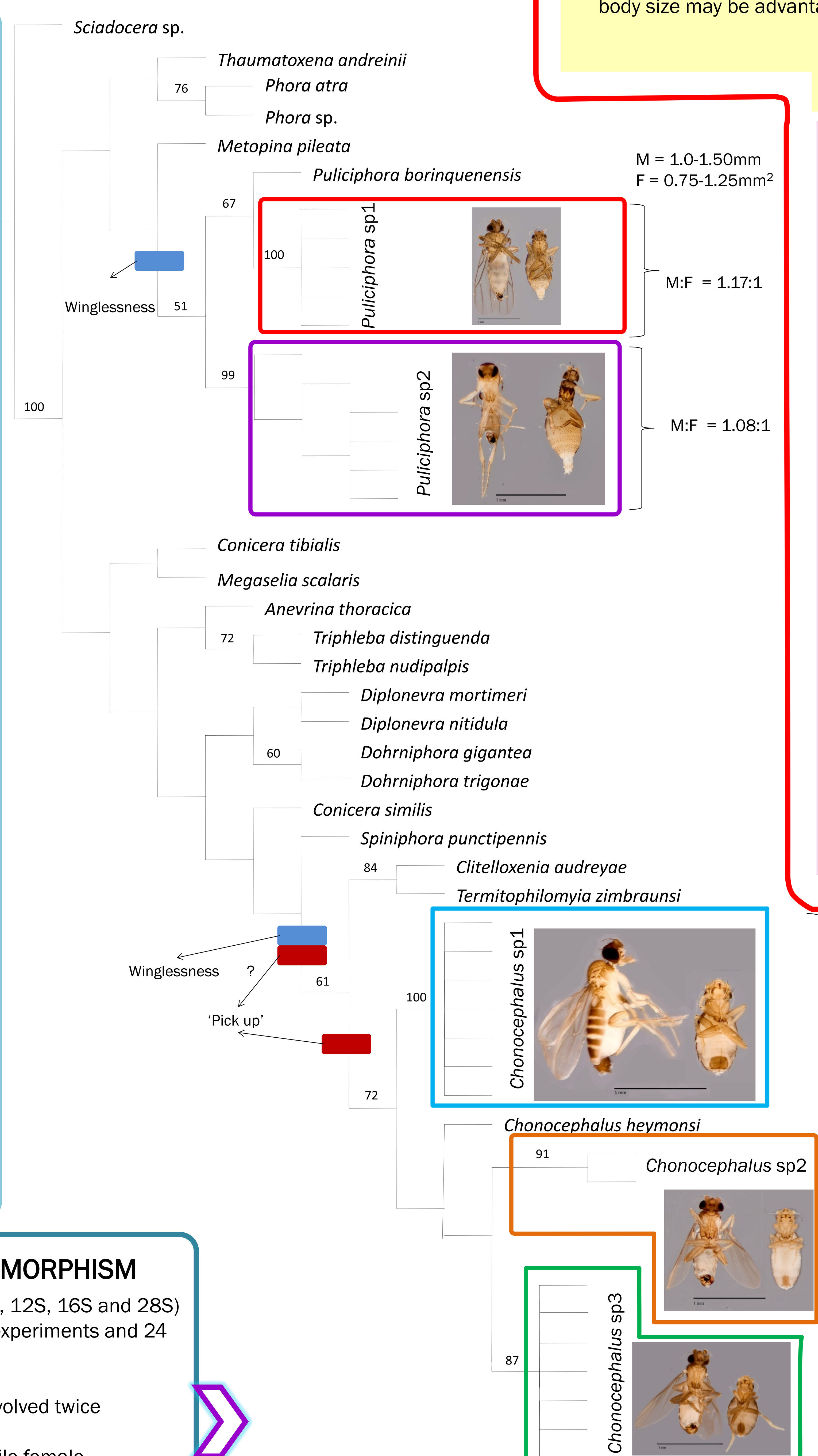

Figure 5: Strict consensus tree based on Cyt B, COI, 12S, 16S and 28S gene sequences with *Sciadocera* sp. used as outgroup. Support for the tree node and male to female size ratio are listed.

FITNESS BENEFITS AND COSTS

What is the adaptive significance for such drastic morphological reductions (size reduction in females & wing loss)? Here I discuss some costs and benefits:

BENEFITS

a) Faster Development

Being smaller and simpler in morphology should allow females to develop faster. This would be especially useful when resources are ephemeral.

Ten females per species were allowed to oviposit for 4 hours on a substrate dish (5 replicates). A total of 455 offspring puparia were placed individually in PCR tubes and checked for eclosion time.

Females do develop faster than males and this correlates with the size ratio between the sexes (Fig 6). An increase in size difference increases the difference in development time.

However, the reduction in female development time appears small relative to the big difference in body size (e.g., *Chonocephalus*).

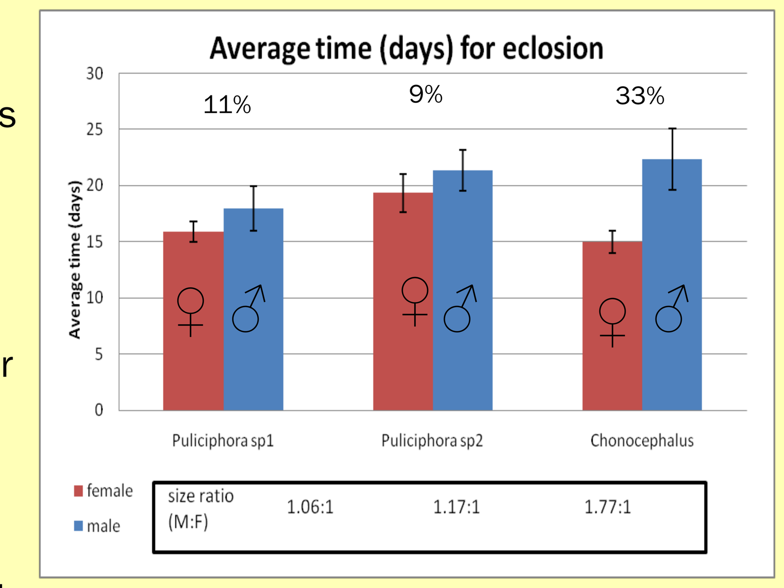

Figure 6: Comparison and percentage differences between male and female development time in *Puliciphora* and *Chonocephalus* with association to male:female size ratio.

b) Transport to distant resources.

Small females are more easily carried over longer distances by males. This increases their chances of getting to more distant oviposition sites (Fig 7).

The literature also describes that *Puliciphora borinquenensis* males drop large females in transport when oviposition sites are hard to find[2]. Being small reduces the risk of being dropped into an unsuitable habitat.

Figure 7: *Chonocephalus in copula*

c) Burrowing Capability

My observations show that females often burrow into substrate. The loss of wings and the reduction of body size may be advantageous. Burrowing allows females to:

(1) Exploit resources beneath the substrate surface
(2) Evade surface predators such as ants
(3) Lay eggs in the substrate, keeping the eggs in moist habitats

COSTS

a) Lower fecundity?

Size limits fecundity, and small clutch sizes were observed in all species.

Clutch sizes were 2-4, 2-5 and 7-10 for *Chonocephalus*, *Puliciphora* sp2 and *Puliciphora* sp1 respectively (Fig 8).

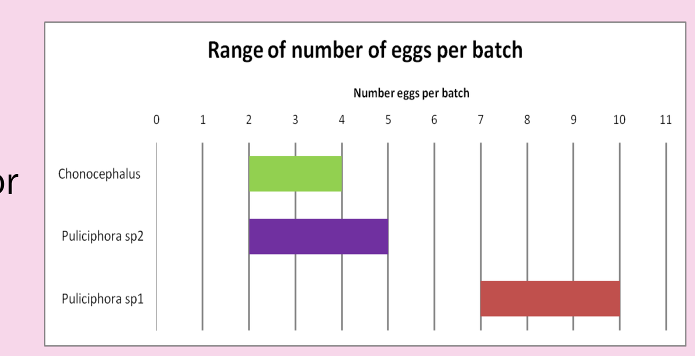

Figure 8: Range of number of eggs per batch from single female set-ups and dissection.

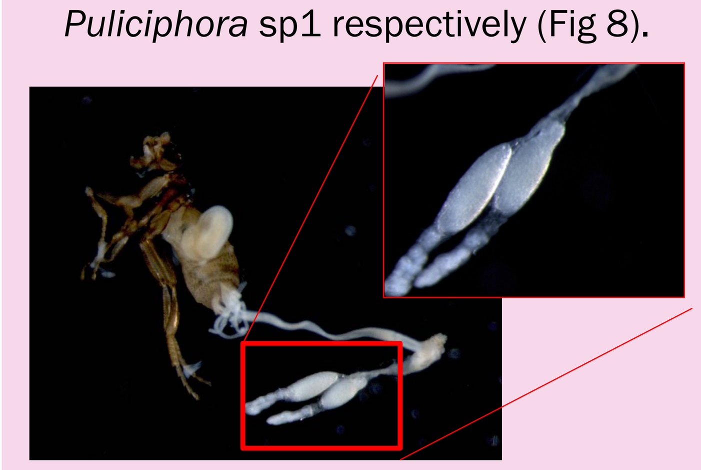

Figure 9: Image of dissected *Puliciphora* sp2 female with two fully developed eggs

Presence of eggs was checked daily for 70 single female set-ups.

In addition and due to high mortality (~50% within 4 days), I also dissected 10 females per species in order to determine clutch size (Fig 9).

To test if small clutch sized is compensated with longer lifespan, I carried out experiments on longevity.

Larvae from parental cultures were allowed to pupate in a container. Daily checks for eclosion were carried out and flies were removed to another cage. These were monitored every two days and fresh dung was given every five days.

Longevity experiments showed that there is no significant correlation between longevity and batch size (Fig. 10).

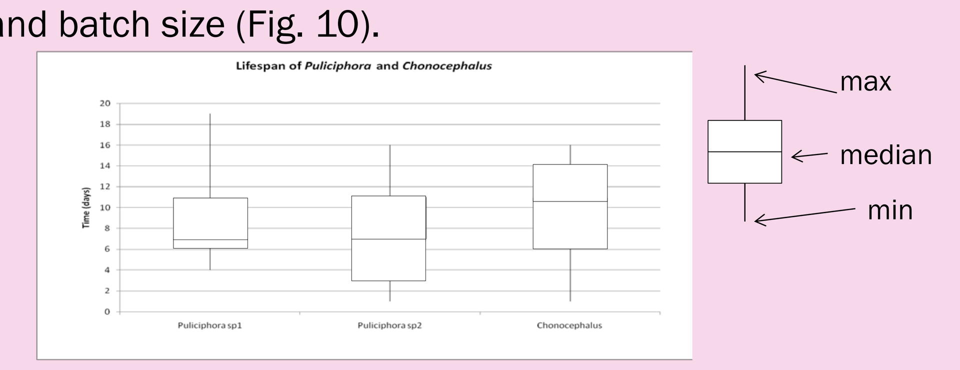

Figure 10: Box plot of female lifespan for *Puliciphora* and *Chonocephalus*.

b) Reliance on males

Females are largely dependent on males for transport to distant resources.

The small reduction in development time is unimpressive compared to the large loss of fecundity. Thus, more research is needed on the advantages of the reduction in female body size.

CONCLUSION

(1) Multiple species were discovered in two sampling sites
(2) I demonstrate two origins for wing loss in females
(3) Wing loss and size dimorphism is correlated with unique mating behavior
(4) Reduced size and complexity may be an adaptation for developing faster in more ephemeral resource patches. However, it appears unlikely that all benefits are known unless the transport to substrate constitutes a huge advantage.

REFERENCES

1. Disney, R.H.L. (1994). Scuttle Flies: The Phoridae. London, Chapman and Hall, 467pp.
2. Miller, P.L. (1984). Alternate reproductive routines in a small fly, *Puliciphora borinquenensis* (Diptera: Phoridae). Ecological Entomology **9**, 293-302.