

# Have female twisted-wing parasites (Insecta: 1 Strepsiptera) evolved tolerance traits as response to traumatic penetration? (#71588)

**BASIC REPORTING** Clear, professional English language used throughout. Relevant literature is lacking. The structure conforms to [PeerJ standards](#). High quality figures, well labelled. Raw data supplied.

**EXPERIMENTAL DESIGN** Original primary research within [Scope of the journal](#). Research question in *Stylops* well defined, not equally in *Xenos*. High technical & ethical standard. Methods detailed.

**VALIDITY OF THE FINDINGS** High impact and novelty. Literature is clearly stated for *Stylops* but incomplete for *Xenos*. Data are robust and statistically controlled. Conclusions support results.

## General comments, criticisms and suggestions

### INTRODUCTION

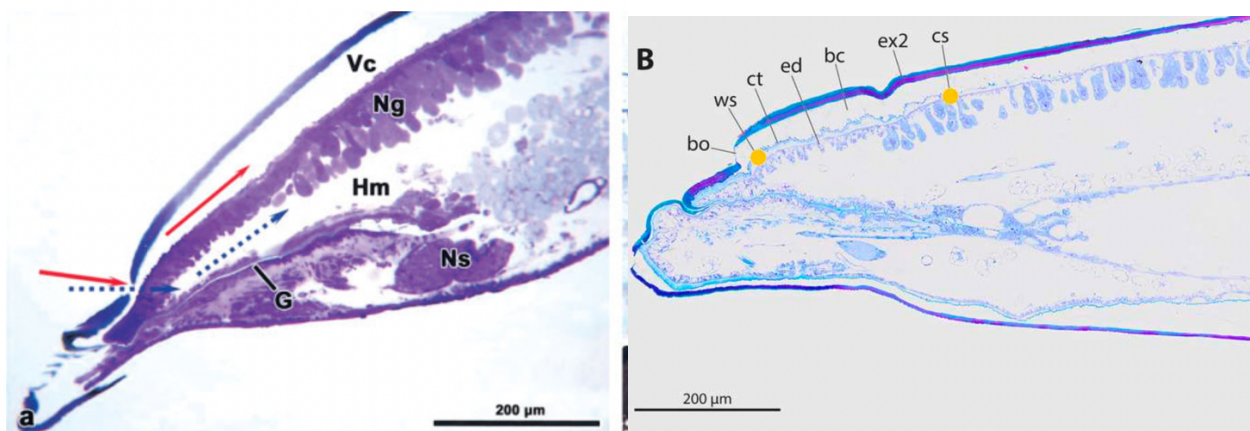
Abstract is perfect, while Introduction could be shortened to be more effective. For example, omit any information on Mengenillidae (lines 50-52, 77-78, 82-83, 111-113).

Given the topic – traumatic insemination - I suggest avoiding the term “copulate” (76), “copulation” (90), “copulate” (311), *sensu* Eberhard (1995, p.69): “copulation (genitalic coupling of male and female) is not equivalent to insemination.”

89-90: “the secondary copulation opening”: unclear. Do you mean that this opening “is used in most species for insemination” (93-94)? My suggestion is to quote and discuss a study on *Xenos vesparum* in which this alternative sperm routes have been described, besides traumatic insemination. This study describes - with scanning and transmission electron microscopy - two routes by which sperm cells can reach the hemocoel, hypodermic insemination and/or extragenital insemination via ventral canal (compare Fig.5 with Fig.2B in this ms).

Beani, L., Giusti, F., Mercati, D., Lupetti, P., Paccagnini, E., Turillazzi, S., & Dallai, R. (2005). Mating of *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited. *Journal of Morphology*, 265(3), 291-303.

### *XENOS VESPARUM* REP.



## MATERIAL AND METHODS

161-163: preference of *Xenos vesparum* for *Campsis radicans* has been described in detail in a recent study. Add this reference, please, and briefly comment the altered feeding of parasitized wasps. I wonder if *Andrena vaga* bees change their behavior if parasitized as well as *X. vesparum*, for example if they aggregate and thus facilitate parasite's mating.

Beani, L., Cappa, F., Manfredini, F., & Zaccaroni, M. (2018). Preference of *Polistes dominula* wasps for trumpet creepers when infected by *Xenos vesparum*: A novel example of co-evolved traits between host and parasite. *PLoS one*, 13(10), e0205201.

I appreciated the integrated methods used in this study, both observational and experimental: confocal laser scanning microscope, micro-indentation experiments, scanning electron microscopy, attraction experiments, specimen identification, mating experiments, X-ray computed tomography, 3D-reconstruction. I wonder if reading this beautiful research could be facilitated by transferring the very technical paragraphs "geometric morphometrics, landmarks and image processing" in Supplementary Methods, since "the males' intromittent organs of *S. ovinae* and *X. vesparum* vary to a similar extent." (549-552).

## RESULTS and DISCUSSION

The main results of this study are the thicker resilin-rich integument and the higher forces required to penetrate in areas where traumatic insemination takes place rather than in control areas, either in *Stylops* or in *Xenos*, although the latter is less sclerotized. Co-evolution of the penises with the females' paragenital organs in *Stylops* is a further intriguing result, as well as the alternative hypothesis that Strepsiptera have evolved tolerance rather than resistance against traumatic insemination: lower risk of integument rupture, sealing of the copulation wounds and reduced haemolymph loss (504-505).

As regards Attraction and Mating experiments in *Stylops*, you could quote similar field observations carried out in *X. vesparum*: five volant *X. vesparum* males, one mating on a leaf 20 cm from an aggregation and seven males close to a caged receptive female.

Hughes, D. P., Kathirithamby, J., Turillazzi, S., & Beani, L. (2004). Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation? *Behavioral Ecology*, p.1042.

Again, in lab we described the direct approach by a male towards a receptive female, host interference by wings and legs movements, quick mating, and subsequent death of the male.

Beani et al. (2005). Mating of *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited (p.297)

Moreover, wasps parasitized by one or two *X. vesparum* females, individually enclosed into vials covered with a mesh, attracted male parasites inside the vials.

Beani et al. (2018). Preference of *Polistes dominula* wasps for trumpet creepers when infected by *Xenos vesparum*.

Another original information is the sympatric occurrence of congeneric species, which increased interspecific competition (506-517) in *Stylops* but not in *X. vesparum*, released "over a period of several weeks" (518-519). More exactly, males emerge from mid-July until mid-August, the peak of the mating period, as indicated by the maximum number of empty puparia.

Hughes et al. (2004). Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation? *Behavioral Ecology*. Fig.2, p.1039.

According with Kathirithamby (2015), females of Strepsiptera produce species-specific pheromones to attract conspecific and to exclude heterospecific males (526-527). Although further prezygotic barriers may reduce heterospecific mating (527-545), I think that you could describe the Nasonow's gland, which opens behind the brood canal up to the neck region. Dallai, R., Lupetti, P., Giusti, F., Mercati, D., Paccagnini, E., Turillazzi, S., Beani, L., Kathirithamby, J. (2004). Fine structure of the Nasonow's gland in the neotenic endoparasitic of female *Xenos vesparum* (Rossi) (Strepsiptera, Insecta). *Tissue and Cell*, p.214.