

Reviewer Comments for PeerJ on Submitted MS “Have female twisted-wing parasites (Insecta: Strepsiptera) evolved tolerance traits as response to traumatic penetration?” (#71588)

General:

This MS represents a creative, multifaceted contribution on the reproductive biology of a poorly known but fascinating order of insects. An attractive feature of the submission is its melding of high-tech instrumental resolution, appropriate analytic methods, and standard entomological procedures to tease out conclusions about both female and male sexual behaviors and evolution of the Strepsiptera.

The title of the MS understates its contents, i.e., the demonstration of an extra layer of cuticle where the male intromittent organ penetrates the female cephalothorax represents only one portion of the Results. The Discussion is relatively conservative, e.g., avoiding controversial topics that arise in the Introduction and Results, such as lock and key (mechanism) and coevolution. The observation of this conservative approach is not a criticism, but the MS might attract a broader readership through discussion of the (potentially) controversial terms of sexual selection.

In general the MS is well written and organized, although some tweaking of English expressions, and adding an extra phrase or two where needed, could improve readability. These suggested changes, as well as a few modifications of the Statistical analysis section, are included as suggestions under Specific Comments below.

Specific:

I.94-95: The meaning of “The female represents a functional unit of the exuviae.....” is unclear. I suggest that you rephrase to indicate that the exuviae of second and third larval instars are incorporated into the female exoskeleton.

Fig.1: A measurement scale is needed for each panel (A-D).

Fig.2: To the legend, add ‘female’ cephalothorax.

I.114: “intraspecific interbreeding” seems (to me) to be a tautology; please rephrase

I.160: “to prevent males from hatching”....indicate males of which species, e.g., *A. vega* or *S.ovinae*.

I.172: an ‘aerarium’ was a public treasury in ancient Rome; perhaps use “cage”, instead?

I.212: were females alive during the ‘micro-indentation experiments’?

I.217: the μm notation is repeated twice in succession.

I.218: Include variance terms and ‘n’ with the 4.0 μm and 0.7 μm measurements.

I.233-247: consider broadening the Statistical analysis section to include the PCA. (If necessary, this section could be transposed to later in the Materials and Methods.)

I.243: I suspect that the ‘pairwise Wilcoxon test’ should be the Wilcoxon pairwise comparison.

l.256-258 & l.262-264: If the methods for attracting male Stylopodia in the field and for cooling live males to keep them vigorous for experiments have been published previously, please provide citations.

l.311: change 'try' to "tried"

l.357: 'decimated' means annihilated or obliterated; if you meant reduced to a decimal, try "decimalized"

l.382: change 'San Jose, USA' to San Jose CA, USA because other states in the USA have cities named San Jose.

Table 1: in a footnote, explain 'mN' and 'GPa'; numbers of observations should be recorded for each line.

Table 2 & l.398-406, l.404-405, l.411-412, l.415-416: It is not clear why both K-W and Wilcoxon paired comparisons results are given for some comparisons. The Kruskal-Wallis (note spelling) test is appropriate only if numbers of groups are three or more.

Fig.6: Legend should explain box plot details, such as the red and yellow dots above or below three plots.

l.428-429: Is this section meant to apply to both *S. ovinae* and *X. vesparum*? (perhaps use species names in the sentence.)

Videos: these are valuable supplemental evidence of interspecific and conspecific male mating attempts with *S. ovinae* females.

Fig. 7: Legend should explain 'cephalothoraces of female *S. ovinae*.'

l.442-447: is coevolution appropriately invoked here? (See, e.g., Tong & Huang 2019). Do the authors see this as Sexually Antagonistic Coevolution (SAC) as described by Tataric et al. (2014)?

l.451,465: the appropriate English is coefficient of "variation" not, 'variance'

l.469: change 'straightening' to "straightened"

l.488: remove 'then'

l.498: what is meant by a 'noteworthy' resistance barrier?

l.511: omit "one"

l.512: In English, the capitol of Libya is "Tripoli"

l.515: insert "a" after 'during' and before 'few'

l. 517: is polygamy documented in Strepsiptera?

l.564-565: Might the evolution of 'tolerance traits' be an example of coevolution?

l. 594-607: 7/8 of the data sets were not available for review, because these will not be released until 'after acceptance'.

New Citation:

Tong, Z-Y & SQ Huang. 2019. The development, misuse, and evidence of the concept "coevolution". *Scientia Sinica* 49: 421-435.