Comments on rsif-2021-0144

Wetting of the adhesive fluid controls underwater adhesion in insects
by Pranav Sudersan, Michael Kappl, Bat-El Pinchasik, Hans-Jürgen Butt, and Thomas Endlein

A previously published approach to letting female terrestrial green dock beetles walk underwater is reconsidered by the authors. Male seven-spotted ladybeetle feet (equipped with adhesive tarsal setae) were fixed and their third tarsomeres were tested on flat, smooth glass substrates in pull-off experiments. In addition, theoretical simulations are demonstrated to illustrate the significance of capillary forces in ladybeetle attachment, which is one of the multiple involved interactions occurring at the beetle foot-substrate contact site. Such knowledge-adding research could be a valuable input to explaining the fascinating insect tarsal attachment, which is not fully uncovered so far. – However, such a valuable input is not achieved by the manuscript at the present form, also because widely lacking precise, careful, biunique wording and the current state of science (substantial previous earlier and recent findings ignored). The major issue consists of the generalization drawn by a few data obtained for one sex of a single ladybird species under limited experimental, non-natural conditions with fixed legs. Such a generalization is not appropriate and should be avoided in the Title and throughout the manuscript. The description of materials and methods needs specification, including concrete definitions of devices, their origin (distinct name and location of companies), laboratory conditions, etc.

Several questions and concerns arise from this study.
Why ladybirds are investigated underwater although they do not live in aqueous environments but on plants in air? Also, schematic drawings in Figure 1 suggest that only the legs were submersed into a water film, but not the whole beetle underwater (which is different from the previous study with green dock beetles)?
How far the glue and holders affect the water condition and underwater adhesion (chemical mixing, etc.)?
Doesn´t the fixation of legs, feet, and claw interfere with the attachment mechanism and processes, and thus, tarsal adhesion? Claws are known to be essential for reliable tarsal functionality. At least some image confirmation at a higher resolution is needed to confirm the integrity of beetles' tarsomeres and adhesive pads. Also, recently published works emphasize the dynamic attachment and detachment processes in insects, which are not reflected by analyzing fixed tarsal segments. - These facts rather explain why not all setae were in contact in the present force experiments. How about the angle at which the feet and setae were aligned? And, why adhesion (pull-off force) was measured although knowing about friction and shearing predominance in insect attachment?
Why male (and) ladybirds have been used as model specimens? Why geckos are spontaneously included in theoretical simulations, however, they are no subject of the study? That's incoherent.
The fluid released by tarsal setae (hairs) is not adhesive, but adhesion-mediating. In this context, the thorough discussion of so far known chemical components and their proposed 'behavior' underwater would be more meaningful than presenting any molecular weights before and after rinsing beetle legs. First, why another beetle species is used for the estimation of molecular weights of epicuticular grease (that's incoherent)? Second, why the legs have been rinsed but not submerged, corresponding to the experimental setup? Third, why the latest best fitting approach to study insect tarsal secretion has been neglected (i.e., the analyses of left footprints, rather than washing off the legs' complex mix of epicuticular

grease than only the tarsal fluid)?
The used substrates were both glasses, which surfaces have been differently functionalized; the separation into glass and PFOTS suggests the use of two totally different substrates, which is not correct. “Normal and silanized glass” corresponds better to the given conditions. – Here, one wonders about the pretty high contact angle of Aqua millipore water (63°!) on normal (hydrophilic glass), which should be ≤ 40° - probably, transposed digits?
The differently observed contact formations are rather modes than types.

Table Headings need to be completed, reliably describing the content of the Table.

The Reference list contains several formatting mistakes and needs to be checked. Also, shouldn't be the species names written in Italics?

Towards a meaningful input and knowledge-adding research, Supplementary Material including, e.g., movies of contact formation of tarsal setae under different conditions could be valuable. Also, plenty of simulations and equations would benefit from concrete drawings at the beetle and foot level including the variables. So far this part of the manuscript appears quite abstract.

DETAILS (# page –according to ms footer–/line):

1/8, Wetting of the tarsal fluid mediates adhesion of ladybird’s hairy pads to glass submersed in water films

1/53, the tarsal fluid

1/53, adhesion on smooth glass both

2/6, “adhesion” and “underwater” are included in the Title; thus, instead, other keywords could be added; beetle > ladybird

2/14, small animals (NOT ONLY ABOREAL)

2/16, We know

2/20, “hairy” (Gorb 2001).

2/40, insect tarsal hairs secrete an adhesion-mediating fluid

… IN THIS CONTEXT SUBSTANTIAL PREVIOUS FINDINGS ARE MISSED, e.g., Vötsch et al.; Betz, Federle (biphasic nature) … etc.

2/56, the tarsal fluid)

3/11, that female terrestrial green dock beetles *Gastrophysa viridula* can

3/13, tarsal hairs

3/21, oily tarsal fluid

3/25, In ladybird beetles,

3/27, of tarsal fluid by each step.

3/29, composition in green dock beetles

3/29, to be an oil-containing mixture

3/31, cholesterol in ladybirds *Hemisphaerota cyanea* and *Epilachna vingtipunctuata*,

3/35-38, THE GOAL IS NOT MATCHED! NEEDS TO BE REWORDED!

3/37, WHY *C. septempunctata*? – NEEDS TO BE EXPLAINED!

3/44-46, THE CHEMICAL PROFILE WAS NOT ANALYSED IN THIS STUDY!

4/8, on smooth normal and silanized/fluorinated glass representing flat hydrophilic and hydrophobic (= also lipophilic) surfaces, respectively.

4/12, labeled the mode of contact as

4/14, air bubble

4/18, air bubble

4/19, contact mode

4/30, Seven-spotted adult ladybug beetles
IN THIS CONTEXT INFORMATION ABOUT THE TIME SCALE OF LABORATORY KEEPING OF BEETLES (WITHOUT PREY) IS MISSED. AGE AND CONDITION (INCL. MASS) ARE REQUIRED DATA TO EVALUATE BEETLES FITNESS AND OBTAINED FORCE VALUES (ALSO IN FIXED SPECIMENS).

4/37, individual male beetles
WHY MALES??

4/48, HOW FIXED IN DETAIL, WHICH LEG SEGMENTS, WHICH SIDE, ETC?

4/52, RELATED TO CLAWS, PLEASE SEE THE COMMENT ABOVE.

5/4, adhesive hairy pads.

5/4, the ventral distal pad (4th tarsomere) to

5/21, N m^{-1}) constituted

5/25, WHICH PLASTIC MATERIAL? DIMENSIONS OF THE HOLDER?

5/41, Navitar, … CONCRETE INFORMATION NECESSARY

5/43, Heerbrugg, … CONCRETE INFORMATION NECESSARY

5/43, FLIR … CONCRETE INFORMATION NECESSARY

5/45, Basler ace U … CONCRETE INFORMATION NECESSARY

5/53, LABVIEW … CONCRETE INFORMATION NECESSARY

6/3, used simultaneously

6/7, lateral sliding motion … IN WHICH DIRECTION, AT WHICH ANGLE TO THE BODY/LEGS?

6/17, N m^{-1}

6/23, WATER LEVEL (HEIGHT)? … ONLY LEGS AND/OR FEET OR WHOLE BEETLE SUBMERSED?

6/26, THE WATER WAS DEGASSED SEPARATELY OR AFTER SUBMERSION THE BEETLE (FEET)? HOW ABOUT THE AIR TRAPPED AROUND THE BEETLE IN AIR AND REMAINED ON THE INTEGUMENT SURFACE DURING SUBMERSION?

6/31, equilibrate … HOW FAR?

6/36, male beetles

6/38, THE SAME BEETLE INDIVIDUAL WAS TESTED ON ALL SUBSTRATES UNDER EACH CONDITION? – MUST BE CLARIFIED!

7/29, GRAPH: Contact area [μm^2]

7/33, Adhesion test setup

7/48, software tool

7/56, NEGATIVE AND POSITIVE FORCE SOUNDS TRIBOLOGICALLY STRANGE – SUGGESTED TO BE REPHRASED

“final reaction step” MEANS …?

8/3, “beetle was submerged underwater” DOES NOT FIT FIG. 1

8/29, as the normal (hydrophilic) glass

8/33, … Femto … CONCRETE INFORMATION NECESSARY

8/37, For the silanized (hydrophobic, lipophobic) glass

8/49, WHY DYNAMIC CAs AND WHY THOSE OF WATER AND N-HEXADECANE WERE MEASURED?

8/51, CONCRETE INFORMATION NECESSARY FOR THE MEASUREMENT DEVICE

8/51, $\mu l\ s^{-1}$ flow rate (Table 1).

9/3, Dynamic contact angles (xx µl) of Aqua millipore water and n-hexadecane on different test substrates. Mean ± SD, n = … etc

9/5-8, Glass treatment

> oxygen plasma
> (normal glass)
>
> PFOTS
> (silanized glass)

WHY NO DISTINCT VALUES PROVIDED FOR n-hexadecane?

9/17, WHY ARACHNIDS ARE REFERRED TO, BUT NOT ADEQUATE STUDIES ON (LADYBIRD) BEETLES, HOWEVER, THEY ARE AVAILABLE – AT LEAST INFORMATION ABOUT THE FLUID’S CHEMISTRY, WHICH COULD BE EVALUATED RELATED TO SOLUBILITY?.

9/23, Instruments … CONCRETE INFORMATION NECESSARY

9/25, WHY rinsing, NOT SUBMERSION?

9/25, WHY *Harmonia axyrides*, NOT *C. SEPTEMPUNCTATA*?

9/27, xxx FULL NAME (THF)

9/17-35, LEG OR FOOT OR BOTH? WHY NO SEPARATE FOOTPRINTS ENSURING THE ANALYSIS OF THE TARSAL FLUID ONLY, i.e., NOT MIXED WITH EPICUTICULAR GREASE?

9/41, RESULTS, NOT MM!
Adhesion of tarsal pads

9/44 against normal and silanized glass were similar,

9/46, detected between ?

9/48, on a silanized glass surface was

9/50, adhesion on silanized glass was

9/54, on normal glass,

10/3, on normal glass

10/6-8, normal glass (blue)
silanized glass (red)

10/38, showing data (median, quartiles, outliers??) of ladybug's third tarsal adhesive pad adhesion on normal and silanized glass substrates

10/42, theoretical predictions relying on xxx?

10/45, hair length

10/46, the tarsal fluid

10/47, mN m^{-1} mN m^{-1}

10/48, mN m^{-1}

10/52, contact modes

11/3, either normal or silanized glass substrate.

11/5, a "bad" contact
ACTUALLY, THAT NOT ALL SETAL ADHESIVE TERMINALS ARE IN CONTACT WITH THE SUBSTRATE IN THIS EXPERIMENT DOES NOT MEAN A "BAD CONTACT" PER SÉ. IN CONTRAST, UNDER NATURAL CONDITIONS BEETLES SELDOM OR NEVER HAVE ALL ADHESIVE TERMINALS SIMULTANEOUSLY IN CONTACT; … AS DESCRIBED IN PREVIOUS PUBLICATIONS …

11/5-13, = DISCUSSION; + NON-NATURAL CONDITIONS, FIXED FEET, ETC.

11/17, RESULTS, NOT MM!
The molecular weight of the leg's epicuticular grease

11/20, leg after rinsed with water (

11/21, molecular weight of the epicuticular grease extracted from the beetle's leg

11/23, after rinsing with water,

11/24-25, except for 406.8 g mol^{-1} and 331.6 g mol^{-1}, the molecular weight remained unchanged (Table 2).

11/27, This let assume that the tarsal fluid was

11/29, HOW THE PROBABLE COMPOUNDS CORRESPONDING TO MOLECULAR WEIGHT WERE DETECTED/DEFINED? - THOSE COMPOUNDS NEED FURTHER DESCRIPTION AND CONSIDERATIONS IN THE DISCUSSION.

11/33, let us conjecture that,

11/34, the tarsal fluid was not

11/38, of tarsal fluid/secretion (either fluid or secretion)

axyridis extracted without and after rinsing … MORE DETAILS MUST BE PROVIDED IN THE HEADING!

11/41, g mol^{-1} g mol^{-1}

11/41-51, WHAT DO US TELL THE 8 LINES OF THE TABLE? … QUITE CONFUSING

12/15, tarsal fluid

12/10-38, SUBSTRATES AND CONTACT MODE SHOULD BE INDICATED

12/40, The tarsal hairs
WHICH substrate? … AND, HOW ABOUT CONTACT FORMATION?

12/41, the tarsal fluid

12/42, tarsal fluid

12/44, tarsal fluid

12/43-45, FURTHER EXPLANATION NEEDED.

12/54, a tarsal fluid

12/56, …, mediating contact with

13/17, DELETE THE SPACE AFTER /

13/19, DELETE THE SPACE AFTER /

14/9, the tarsal fluid

14/26, the tarsal fluid

14/28, the tarsal fluid

14/30, WHICH lengths?

14/38, the tarsal fluid to be an oil-containing substance

14/40, were assumed to correspond

15/4, FURTHER EXPLANATION NEEDED.

15/8, Contact area

15/9, Adhesive hair

15/12, Tarsal fluid-

15/13, Tarsal fluid

15/23, Tentative forces due

15/37, the tarsal fluid

15/39…, “positive” and “negative” curvature – PROBABLY, CONVEX AND CONCAVE IS MEANT IN THIS CONTEXT – NEEDS REVISION THROUGHOUT THE MS

16/22, Simulation of normalized capillary …

16/26, the tarsal liquid with

16/33, of WHAT being repulsive?

16/…, FIGURE 4b SHOULD BE REFERRED TO IN THIS PARAGRAPH.

17/16, Hydrophilic glass   Hydrophobic glass

17/47, the tarsal fluid wets

18/21, normal glass

18/23, mN m^{-1} mN m^{-1}

18/26, silanized glass

18/28, mN m^{-1} mN m^{-1}

18/30, with normal and silanized glass in air ..

19/7, air bubble

19/40, trapped air bubble

19/56, tarsal hairs

21/7, Hydrophilic glass   Hydrophobic glass

21/23, Hair tip diameter   Hair tip diameter

21/30, DELETE: system

21/31, hair tip diameter

21/35, The air bubble

21/37, the length of all tarsal hairs

21/41, Tarsal hairs on ladybug’s adhesive pad terminate in various shapes, such as …

21/53-56, = DISCUSSION

22/39, tarsal hairs

22/41, an air bubble

22/41, in female dock beetles totally submersed in water walking on different substrates (others than in the present study).

23/7, air bubble

23/11, oily tarsal secretion

23/17, hydrophobic silanized glass surface

23/21, mN m^{-1} mN m^{-1}

23/5-35, ATTACHMENT DYNAMICS/KINETICS NEED TO BE CONSIDERED CAREFULLY.

23/25-26, CORRESPONDING MEASUREMENTS HAVE BEEN RECENTLY CARRIED OUT BY COLLEAGUES AROUND TRISTAN GILET AND OTHERS ..

23/29, HERE, OTHER EFFECTS THAN SURFACE IRREGULARITIES ON FLAT GLASS MATTER MORE (SEE COMMENTS ABOVE ON FIXED FEET, DYNAMICS, NON-NATURAL CONDITIONS, ETC.)

23/35, a contact of all adhesive hairs' terminals

23/37, NOT ONLY IN "our experiments", BUT IN INSECT ATTACHMENT PER SÈ

23/43, the detachment under natural conditions,

23/47, the male beetle's

23/55, govern ladybird's adhesion

24/5, enable ladybird attachment

24/11, This effect can be explained by

24/13, HOW FAR FDMS RESULTS PROVIDE VALIDATION?

24/13, provide

24/15, the tarsal fluid

24/19, To some extent, the findings could be transferred to

24/33, mN m^{-1}

24/37, mN m^{-1}

24/38, mN m^{-1}

24/47-25/43, THE MIXING OF ADHESION AND FRICTION AND DETAILS ON GECKOS ARE CONFUSING TO THE READERS, AND DO NOT FIT THIS STUDY.

25/49, controlling ladybird beetle traction force.

26/5, of tarsal adhesion-mediating fluid can

26/7, performance in bio-inspired fibrillar adhesive systems.

26/15, relay on both tarsal setae and oily tarsal fluid to adhere to

26/31, underwater adhesion force measurements

26/32-35, THE STATEMENT ON GECKOS DOES NOT FIT. … AND, HOW ABOUT THE PREVIOUSLY REPORTED, ALWAYS PRESENT SURFACE HUMIDITY AND ITS INFLUENCE ON ADHESION?

26/37, of the ladybird beetle adhesion.

26/15-37, A MEANINGFUL CONTEXTUALIZATION WITH BIOLOGICAL SIGNIFICANCE IS MISSED.

26/44, THE AFFILIATION FOR SUPPORTERS SHOULD BE PROVIDED.

SUPPLEMENTAL FILE: THE SAME DETAILS TO CONSIDER AS MENTIONED ABOVE!