Reviewer 3

Sudersan and co-authors have submitted an interesting manuscript on the understanding of underwater adhesion seen in terrestrial insects with hairy pads. So far, it remains unclear if an air bubble is necessary for adhesion and its contribution to the net adhesion force, if any.
They present a nice experimental setup and results about the adhesion force of individual pads of a ladybug beetle in air and underwater conditions. The experiments were carried out on smooth hydrophilic and hydrophobic glass surfaces. The overall experimental details and protocol are described well in details.
In general, the manuscript reports a quite-well documented Literature and the experiments were well defined and carefully conducted. The work also includes good statistical treatments of results.
To my opinion, this paper does meet the standards of the Journal of Experimental Biology after addressing the following questions (along the manuscript) in order to improve the manuscript before being published:

- The authors detailed well the adhesion of the attachment system onto smooth hydrophilic and hydrophobic glass surfaces. Could they comment on the potential role of substrate topography?

This is an interesting aspect which we plan to study in the future. Capillary adhesion is expected to depend on the length scales of surface roughness. If the roughness amplitude is greater than the fluid thickness, the fluid will get soaked inside the crevices and prevent capillary adhesion. But for smaller roughness amplitudes, the fluid should just wet the surface while maintaining a capillary bridge between the hair and surface. In such a scenario, adhesion should be high and similar to a smooth hydrophilic surface. For the underwater case, there would be an additional air meniscus trapped between the asperities which could influence the nature of contact. Here, it is possible that the tarsal fluid capillary bridges are always “in air” at the point of contact due the trapped bubble. (p.8,ln.999-1008)

- End of §2.1.1, the authors stated that the beetles were freed after experiments by “carefully removing the epoxy glue (…)”. How was this done? Is the epoxy not touching the insect but only the Blu Tack? Please clear out this part.

The epoxy glue was only used to firmly fix the tip of the claw to the Blu Tack on the solder wire. Removing the glue was surprisingly easy using a pair of tweezers. The glue comes easily off the claw when carefully pulled on it. (p3,ln.253-256)

- Beginning of §2.1.2, the beam deflection has been calibrated using different known weights. Could those weights be mentioned and commented with regards to the insect’s weight range?

The calibration weights were in the range of 2 mg to 90 mg, well within the beetle’s body weight (34 mg) as well as the reported adhesion values (10-60 mg). (p.3,ln.262-265)

- In the experimental section on adhesion test: There is no mention of applied pre-load to measure the adhesion force values. Only the mention “a slight compression” appears in §2.1.2, resulting from the z-piezo depicted in fig. 1. Could author mention the pre-load value and how will it affect the adhesion force characteristics?

The preload force was not kept constant across the different measurements. The experimental data revealed no correlation between the applied preload and pull off force. Previous study (Kroner et.al., J. of Adh. 2011,87,447-465) on artificial fibrillary materials have shown preload dependence only on measurements done using a spherical indenter, not on a flat-on-flat contact as performed in our current study. The “slight compression” step was done only to ensure maximal contact of hairs with the surface. (p.3,ln.289-300)

- The contact area from the plot of inset down right in the fig. 1 seems

not to come back to zero at the end of the experiment. Could the authors explain this issue?
The hairs leave behind some residue of the secretion fluid after contact with glass, which unfortunately is also captured by the image processing, thus giving non-zero area values.

- Authors mention about a short pause (1 s) after contact formation to remove any viscoelastic effects. Why pause lasts for 1 s? How did the authors ensure this time was enough for materials viscous relaxation?
We did not observe any significant decay in the force due to viscoelastic effects for longer holding times (upto 10 seconds) when compared to a 1 second pause.
- In order to achieve the underwater no bubble experiments, the water was degassed in a vacuum chamber prior to tests. Although the in-situ contact pictures in fig. 3, supported by the videos, seems quite obvious, could the authors comment why this experimental protocol ensure that no air is trapped in the contact?
We believe the degassed water makes it more likely for any trapped air bubble between the hairs to get dissolved into the water. (p.3,ln.302-310)
- The contacting material is viscoelastic, as mentioned by author. Therefore, the motion speed should greatly affect the pull-off adhesion response. The manuscript mentions only 1 contacting/retraction speed. Did author also study the speed response on adhesion?
Our preliminary speed-dependent adhesion test showed higher adhesion for higher retraction speeds. The chosen speed was a compromise to be slow enough to minimize any viscous or viscoelastic effect, while large enough to maintain a relatively short experimental time-scale. Especially for underwater experiments, we wanted to avoid submerging the ladybug for long durations during the tests.
- In results (§2.2): authors show the results of adhesion force measured. Could authors also comment on the behaviour of force-distance curve during the pull-off cycle? Is this retraction cycle behaviour different for each contacting conditions?
As included in the supplementary video (S2), the force curves appear to be qualitatively similar for all three contact modes. For each condition, the retraction cycle showed a single peak corresponding to the adhesion force, beyond which the pad contact fails and snaps off from the substrate. Contact images also show a sequential detachment of hairs rather than a simultaneous contact loss.
- Moreover, since the experimental setup includes a nice in-situ visualisation, couldn't it be interesting to describe the kinetic of detachment of the contact (continuous detachment from the outer- to the inner-part of the contact, Instant snap-out of the contact,…?) as can be investigate in the videos?
The kinetics of detachment would indeed be an interesting aspect to look into. However our current setup prevents us from imaging the underwater contact satisfactorily. The poor contrast of the contact due to the presence of water (see video S2) resulted in unreliable extraction of contact areas, making such related analysis challenging. When comparing the detachment times extracted from the force curves, we don't see any significant variation between the different contact modes. A high-speed camera could perhaps be employed to study the details of the detachment kinetics, similar to a previous study by Gernay et. al. (2017, J. R Soc Interface 2017 Vol. 14 Issue 136)
- I suggest that the authors chose other colours in the plot from fig. 2 which may lead to a higher contrast in grey scale so that the plot understanding, regardless the media.
We updated the colour scheme of Figure 2 to improve readability.
- Page 10 line 4: a "." sign misses before "The substrate's wetting towards (…)"
Fixed.
- Figure 3a. Hairs are making contact in air mode as shown on right image. Here one can note two different types of grey scale values (dark and

bright spots) at real contact junctions. Could author comment on this and specify the real contacting points here?

The different grey scales correspond to the different kinds of hairs making contact with the surface, where, the darker region in the center are due to the "discoidal" hairs, while the lighter region at the sides are due to the "pointed" hairs.

- Young-Duprè equation mentioned in §3.1 should be written Young-Dupré.

Fixed

- Please consider replacing "w.r.t." acronyms by "with respect to". (twice in page 15)

Fixed.

- "The contact area fraction of the hairs relative to the pad, α = NDh2/Dp2, hair aspect ratio, L/Dh, and fluid size parameter, φf , were fixed to values typical for a ladybug's hairy pad." Could the authors provide a corresponding reference from literature?

The model parameters have been updated (see Table 1) and the corresponding references are cited (p.5,ln.546-554)

- I suggest the authors mention in the text that the §3.2 corresponds to modeling results obtained in air.

The results in the section "Capillary force of a single liquid bridge" is meant to expand also for other cases and thus applicable to any medium. In fact, we used these same simulation results (Figure 4) to estimate adhesion of the beetle for all contact modes, including underwater. (Figure-5).

- In Figure 5: the force-distance curve for different contacting conditions are shown. Could the authors comment on the deviation from zero, above or below zero line for large distance? In an ideal situation it should reach back to zero force.

The force curves go back to zero at a higher separation distance. The plots have been updated to show that (Figure 5). For the case of underwater contact with bubble, the bubble loses contact at a much higher separation distance. Hence, the forces for the red curve don't go exactly to zero for the plotted distance.

- In the theoretical analysis of contact: no parameter to consider the mechanical properties of hairs are implemented? Wouldn't it be useful to consider it in your model?

The mechanical properties will indeed influence adhesion, especially when considering a "spatula" or "pointed" shaped hairs, where, contact area would also depend on the modulus. A more rigorous model would probably involve other aspects like by the work of Gilet et. al. (Soft Matter, 2019, 15, 3999-4007), considering an elasto-capillary model, which is beyond the scope of our present work. However, when comparing the adhesion on smooth surfaces in air and underwater conditions, we expect that the mechanical contributions should remain similar for each contact mode. Since the goal of the model was to understand how underwater adhesion would be different from that in air, neglecting any elastic effects is an assumption that we made to simplify our analysis. (p.8,ln.887-892)

- During the underwater (no bubble situation): I believe there might be another layer in between hair, and substrate, apart from the adhesive fluid. How do you make sure that the intermediate water layer is completely squeezed out from the contact interface (between adhesive fluid and substrate)?

We believe that the adhesive fluid secreted by the insects can dewet the surface to a great part (depending on the exact nature of the liquid and substrate energy). However, we cannot exclude that a very thin layer of water remains in the contact space. It would be interesting to investigate this aspect in a further study. (p.9,ln.916-926)

- Since the work deals with adhesion depending on (notably) wettability situation, a reference to the Cassie-Baxter/Wenzel wetting modes could to my opinion strengthen the context of discussion. For instance, discussing the imbibition situation vs the literature criteria ([J. Bico et al. Europhys. Lett. 2001, 55, 214−220] for spontaneous wetting of structured surfaces for instance) shall make sense when considering the bubble's role.

We thank the referee for the valuable suggestion. In our case, we always get only partial impalement of water during immersion of the toe pad into the liquid, there is always a bubble forming ("Cassie state"). This bubble itself is then very stable against further impalement due the combination of strong contact line pinning within the hairy structure and the low hydrostatic pressure that cannot overcome the Laplace pressure of the bubble. Therefore, full impalement occurs only in the case where we degassed the water and the bubble then dissolved into water by gas diffusion ("Wenzel state"). The actual mechanics of this impalement process is however beyond the scope of the present study. We are only interested in the underwater adhesion of the hairy pad while under the "Cassie" and "Wenzel" states.

- In this respect the value of L (height of cylindrical rods) as been largely ignored in the modelling / discussion. Actually, rods' aspect ratio has been extensively investigated in literature (for both adhesion and wettability). Could this point be addressed, maybe in further work, or at least commented or pointed out in this work? Addressing such questions might leads to some scale effect (like in [V. Hisler et al., Langmuir 2014, 30, 9378−9383]) which might emphasize the application of the results of this work in broader scale range.

The increase in pull-off strength for higher aspect ratio pillar arrays (Greiner et. al., Langmuir 2007, 23, 3495-3502) is understood to be a result of elastic dissipation during contact loss. The model presented in our study does not consider any elastic effect of the hairs, as mentioned in a previous comment. Based on the model, the aspect ratio can only influence the bubble's contribution to adhesion by changing the bubble capillary bridge height. But, this effect is not significant when compared to the adhesion contribution of the tarsal fluid secretions (see also model sensitivity analysis, Table S4).