

Dear editor,

Thank you for forwarding us the reviews concerning our paper. We believe these have been of a great improvement to our manuscript. Most of the comments were based on the text and have been implemented as suggested. We have discussed the suggestion of adding additional statistical testing, however due to the limitations of our dataset have decided against this. Some references have now also been added, such as the one suggested by dr. Baeckens concerning the effect of substrate on gripping performance by Spinner et al. (2014), a reference on the optimal housing temperature of *C. calyptratus* by Andrews (2008), and a reference concerning the steplength (Fischer et al., 2008). If there are any additional comments, please feel free to forward these.

Kind regards,
Allison Luger

Reviewed by Simon Baeckens, 2020-11-03 08:31

It is with great interest and enthusiasm that I have read the manuscript by Allison Luger and colleagues, entitled "Do substrate type and gap distance impact gap-bridging strategies in arboreal chameleons". Using an experimental set-up, and focussing on both males and females of the species *Chamaeleo calyptratus*, the authors test whether gap distance, perch diameter, and perch roughness influence tail use and overall locomotor behaviour of chameleons when crossing a gap between two perches. Overall, I believe the manuscript is very well written. The authors succeed in describing the gap-bridging behaviour of the species clearly and eloquently; it was truly a pleasure to read. In addition, the authors do a great job in explaining and introducing the novelty of their study by pinpointing the current gaps in the literature and by thoroughly laying out the predictions of the planned experiments. Also, I really appreciated the "cautious" style of writing in the discussion: the authors are not too bold with strong statements and conclusion, which is an honest portrayal because the study is largely based on qualitative observations rather than quantitative results. Indeed, as mentioned by the authors (L144), the manuscript is primarily descriptive, focussing on qualitative traits in tail use. While I understand why the authors were reluctant to analyse their data qualitatively (see specific comments below), I believe there might be ways to statistically test for the predictors of tail use. Of course, I have no notion of how the actual raw data looks like, but I encourage the authors to give it a try. Alternatively, I feel the authors should provide some explanation on why they did not analyse their (valuable!) data quantitatively. Regardless, in my humble opinion, I believe this paper has ample potential and (with some relatively minor changes) will be warmly welcomed by the zoological scientific community.

Thank you very much, Simon, for your insightful comments! They are really appreciated and have given us plenty to think about. While we have decided against implementing the suggested statistical tests (as stated below), the comments concerning the text and the suggestion for an additional reference have been very useful and allowed us to improve the manuscript.

Below some more specific comments:

L19: Maybe change "...has not been studied to date." to "...has been largely neglected."

Was modified as suggested

L20: Suggestion: maybe change "type" by "roughness"? Similar for the title?

Was modified as suggested

L23: Suggestion: maybe change "material" by "surface"?

Was modified as suggested

L68: Word missing here (after "their").

Changed:

"Therefore, we studied how chameleons change the way they use their prehensile tail when confronted with different conditions including gaps of different sizes, different substrate diameters, or different substrate roughness."

L69: "We also wanted to compare ..." feels odd. Maybe change to "In addition, we examined whether this behaviour differs between sexes."

Was modified as suggested

L71-L88: It is good that the authors elaborate on their (many) predictions. In order to keep some sort of structure for the readers, I believe it would be convenient if the authors provide a numbering throughout this paragraph. For instance: "First of all, we predict ...", "Second, ...". Try also to be consistent with verb tenses (e.g. "predicted" vs. "predict").

This has been changed throughout the paragraph, indicated in bold:

"First, we predicted an increase in tail use on smoother substrates as this decreases the grasping ability of the hands and feet. Previous studies by Spinner et al. (2014) and Khannoon et al. (2014) showed a relationship between substrate roughness and friction. Second, we predicted that when bridging greater gaps, animals would suspend their body while gripping the perch with their hind legs and tail. When crossing greater gaps, the center of mass of the animal thus being suspended further from the points of attachment in the feet and tail. Third, we predicted that animals more often use their tail as an anchor as gap distance increases. Our fourth prediction was that when on perches with a rougher surface, chameleons should be able to cross greater gap distances compared to smoother surfaces. Finally, many chameleons, including *C. calyptratus*, are sexually dimorphic in body size, tail length and hand and feet span (Bickel and Losos, 2002). In dwarf chameleons of the species *Bradypodion* males also have larger hands and feet and longer tails resulting in a higher gripping force compared to females (Herrel et al., 2011; Da Silva et al., 2014). Consequently, we predicted differences in how chameleons use their prehensile tail based on sex-related size variation. We specifically predict that males are able to cross greater distances as their increased hand and foot length gives them a higher gripping strength, increasing friction and stability, at least on larger perches. We also predicted males to make more successful crossings without using their tail due to their expected higher grip strength."

L72: I think it would be helpful to mention that these tests were performed in an indoor experimental set-up (and that they are not e.g. observations in the wild), as on L19 in the abstract.

This has been changed in the introduction:

“We first quantified whether chameleons changed the use of their tail when substrate roughness, perch diameter, and gap distance were modified using an indoor experimental set-up.”

As well as in the Abstract:

“Using an indoor experimental set-up, where chameleons had to cross gaps of varying distances, we tested the effect of substrate diameter and roughness on tail use in *Chamaeleo calyptratus*.”

L74: I suggest changing “...substrates as this would also decrease the grasping ...” to “...substrates as this decreases grasping ...”. In addition, I suggest including a reference after the statement that smoother substrates decrease grasping ability, maybe e.g. Spinner et al. 2014 (Scientific Reports). Or are the authors truly testing the effect of substrate roughness on grasping ability?

This has been changed:

“First of all, we predicted an increase in tail use on smoother substrates as this decreases the grasping ability of the hands and feet. Previous studies such as those done by Spinner et al. (2014) and Khannoon et al. (2014) already showed a relationship between the substrate roughness and friction.”

L75: A suggestion: changing “...when bridging greater gaps animals would have to suspend ...” to “...when bridging greater gaps, animals suspend their body ...”.

Was modified as suggested

L78: A suggestion: changing “We therefore also predicted that as gap distance increases that the animals would resort to more often use their tail as an anchor.” to “We therefore predicted that animals more often use their tail as an anchor as gap distance increases.”

Was modified as suggested

L80: Change “...able to cross greater gap distances.” to “...able to cross greater gap distances than on smoother surfaces.”

Was modified as suggested

L82: Provide “,” after genus name.

Was modified as suggested

L85-86: I suggest changing “...predict that males would be able to cross ...” to “...predict that males are able to cross ...”.

Was modified as suggested

L88: Maybe change to “... due to their expected higher grip strength.”?

Was modified as suggested

L104-106: Personally, I would include table 1 as supplementary material. And also Table 2, actually.

Both tables have been added to the supplementary materials.

L108-113: Were lizards able to reach their preferred body temperature prior to the trials?

In the Methods section the following is mentioned:

“Room temperature was kept constant at 26°C, which is consistent with their natural ambient temperature.”

And

“The experiments were performed in the same room in which the animals were housed.”

Little data are available in the literature but the selected body temperature of *C. calyptratus* was suggested to be 30.4°C (Andrews, 2008). As such chameleons were tested only slightly below their selected body temperatures.

L119-120: Is the average (preferred) perch diameter in nature known for the study species? This would have helped with the choice of perch size/diameter.

While studies have been done on the preferred perch diameter of the genus *Bradypodion*, sadly none have been done yet on *Chamaeleo calyptratus*.

L114: Although I understand why, in a way, it is a shame that no statistical analyses were performed here. This is likely due to the many variables involved (especially the six different tests for gap size) making statistical analyses more challenging. However, what if the authors rearrange their input data so that (a) the PVC tests are excluded (because of the low success rate) and (b) so that the variable “gap distance” is reduced to e.g. two categories (i) small gap (i.e. lower or equal than shoulder-hip length, thus lumping gap distance 0.5 and 1) and (ii) large gap (i.e. lumping 1.25, 1.5, 1.75, and 2 gap distance). Consequently, the authors can perform a glmer model (binomial) testing whether gap size (small vs. large), perch thickness (thick vs. thin), and/or perch roughness (wood vs. sandpaper) can predict tail use (binary response variable; yes = 1; no = 0). Depending on the statistical power, the authors can do this separately per sex or combined (i.e. sex as an additional factor). Of course, the authors need to account for repeated measures (as each individual was tested multiple times). The data input file could be structured with the following columns: (column 1) individual, (column 2) tail use, (column 3) gap size, (column 4) perch thickness, (column 5) perch roughness, (and maybe (column 6) sex). Wouldn't this be worth the try?

While we really appreciate the thoughtful suggestion of the reviewer and agree that it is indeed a shame that no statistical analyses could be performed. However, this is a well considered restriction we imposed on our analysis, after having compared all the available data. The number of trials in which the conditions were sufficiently stable per setting tested, was however too low as there were quite some behavioral factors that clouded the data (though the animals were able to cross the largest distances, they were not always as willing giving us a much smaller dataset size for those set-ups). Thus, even with the suggestions given here we are convinced that the data is too fragmentary and noisy to perform reliable statistical analyses. Hence our decision to stick to descriptive observations for the results and discussion. The amount of successes between each large distance, which the reviewer suggests we should group together, still varied quite a lot (on top of the chameleons using a different strategy for the largest distances) that makes us believe that combining all distances of >1.25 shoulder-hip-length would not be suitable as a reliable category.

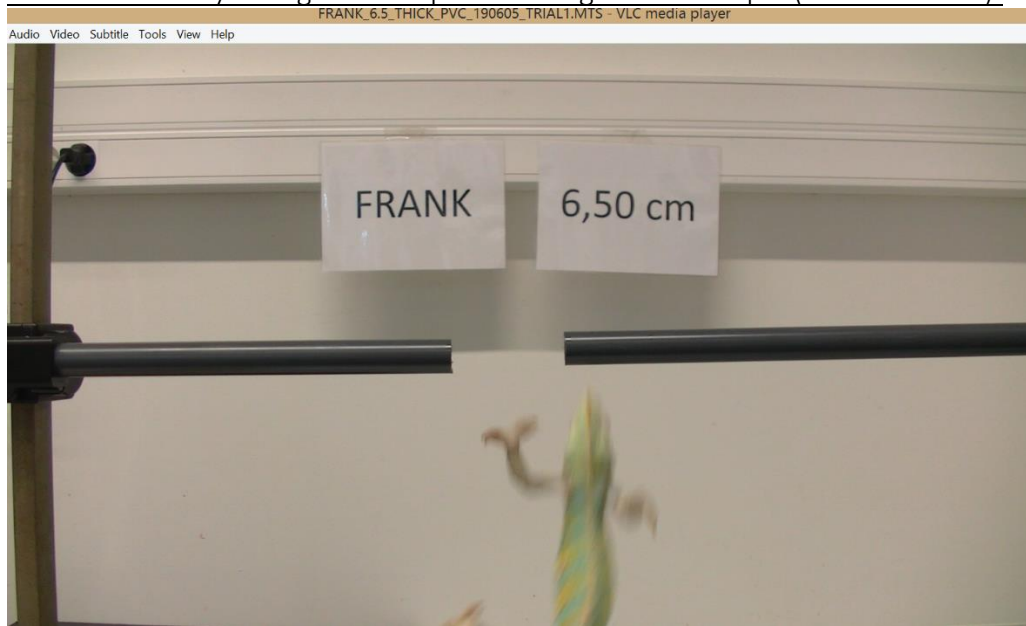
L205: Wonderful name for the observed tail strategy.
Thank you!

L206: Suggestion: " ... to safely cross ..."?
Was modified as suggested

L196-217: The locomotor behaviour of the chameleons is clearly and eloquently described here.
Thank you!

L247: True — the success rate on the PVC substrate was low compared to wood or sandpaper. Do you think the coloration of the perches would have had an effect on the willingness or success of the lizards to cross? The grey-ish colour of PVC tubes is likely less attractive (or less inviting) perches for animals than the more naturally brown-ish coloured wood and sandpaper wrapped around wood.

While this is a good suggestion, the animals showed no inclination to a preference for either material. Their failing to cross wasn't due to them being hesitant or unwilling to cross, but more often for actually falling off their perch during their attempts (as seen below).



L239-257: The authors studied the locomotor behaviour of one species of chameleon. Can these results be extrapolated to other species? Or do other species have more specialized skeletal or skin adaptations that allow them to cross gaps more easily/efficiently? I think it is worthwhile to elaborate a bit on this.

While we have only been studying the chameleon species, *C. calypttratus*, we can assume that other prehensile-tailed arboreal chameleon species might have similar strategies, based on their similar morphology and lifestyle. This hypothesis serves as a good starting point for future studies. Other chameleon species that have a more terrestrial lifestyle, or live in the lower shrubbery, might show

different strategies in how they utilize their tails when crossing, and could also be used for future research.

L272: Do the authors have a reference for this statement?

This has been added:

“A distance of 0.5 times shoulder-hip length appears to be slightly greater than the regular distance of a single step during a normal gait, which is in line with the step length of *C. calyptratus* found by Fischer et al. (2010), but not long enough to require a different strategy.”

L279-291: Can the authors provide an example from other animal groups in which substrate roughness is known to affect grasping ability? Also, do you think claw size and shape has an affect on grasping ability? Is there something known about this in chameleons?

The following has been added:”

“Our hypothesis that coarser substrates increase friction and thus allow chameleons to cross greater distances was supported by our data, which is consistent with the findings of Spinner et al. (2014) and Khannoon et al. (2014).”

L321: Yes — I think so, too. Is there any literature on sex difference in risk taking behaviour or exploratory behaviour in lizards, which can be used an example?

Literature was cited, but has been elaborated:

“Boldness as a personality trait has been described in other squamates, such as for the Namibian rock agama, *Agama planiceps* (Carter et al., 2012).”

Reviewed by anonymous reviewer, 2020-11-03 08:31

The authors conducted a qualitative study examining how the veiled chameleon uses the tail during various trials varying the substrate texture (for increased or reduced friction), width (wider vs narrower substrate) and also the length of the gap they must cross relative to the shoulder-hip distance of the chameleons (with all trials being able to be broken into male vs female datasets). In short, this was a rather straightforward, simple and yet fun series of trials examining how chameleons navigate in a 3dimensional environment (though this was in a lab setting, but theoretically approached the scenario chameleons must encounter time and time again when climbing through branches or bushes or even going from the ground to gripping the first of the low branches of their target plant). I for one found this a nice project that fills in some gaps on how tails are used by these derived chameleons and have only minor comments below.

- Running Title vs Title do not correlate completely, running title needs to be more broadly written about gap strategies in chameleons or something similar since it is not all due to tail use.

The running title has been changed to:

“Gap-bridging strategies in chameleons”

- Abstract: lines 26, 27 duplicated text (redundant).

Was modified as suggested

- Page 3, line 50-51 (recently coined as Chameleodactyly – though that paper is weak and is essentially self published despite being based on a conversation on Twitter by a few chameleon

biologists whom he never cited yet stole their term!) See:

- Necas. 2020. Chameleodactyly: New term to describe the unique arrangement of digits in chameleons (Reptilia: Chamaeleonidae) *Archiv für Naturgeschichte* 1(1): 4-5.

- Also, see the work of Diaz and Trainor 2015 for extensive work on the autopodium.

As most of our cited literature refers to the arrangement of the digits of chameleons as zygodactylous, to avoid confusing we prefer to continue using this terminology.

- Page 4, line 68: should read "...their tail when confronted...".

Was modified as suggested

- Page 5, line 95: which vegetables were the veiled chameleons fed?

This has been added:

"The animals were kept in wire cages of 90 x 45 x 46 cm and fed *ad libitum* on a diet of vegetables (lettuce) and crickets dusted with mineral and vitamin supplements."

- While the only trials presented were those where successful crossings were made, I find it would still be of interest to present how many total attempts were conducted since that number may affect animal behavior and is also of interest (though you can still present the successful crossings as you did in figures).

While we do believe that this would indeed be very useful information and ideal for future experiments, the number of suitable trials obtained for each set-up also depended on the willingness of the chameleons to cross. As the animals were more hesitant to cross larger distances (though they had proven to be capable to do so), and manipulating the animals to cross would influence their natural behavior, we ended up with inconsistencies in the number of trials for each set-up. This is the reason why we focused only on the successful crossings in this paper.

- I liked the use of shoulder-hip length as a measure.

Thank you!

- Page 12, line 206: I feel that the page would flow better if you split into two paragraphs, with the second paragraph beginning with "With gap distances above 1.25 times shoulder-hip length...."

This section has now been split into two paragraphs, as per suggestion.

Reviewed by anonymous reviewer, 2020-11-03 08:31

Dear Editor,

First, I want to apologize for the delay in my reply.

I have read the manuscript by Luger et al. entitled "Do substrate type and gap distance impact gap-bridging strategies in arboreal chameleons?". This is a straightforward and well written, but entirely descriptive manuscript on gap-crossing behavior. Hypotheses are clearly formulated (yet

not really 'stunning') and the performed video-analyses seem to support the hypotheses. Apparently, no real testing is (or could be) carried out (probably because of a too fragmentary data set?). The discussion is adequate for the descriptive data available. As such, little or no comments can be formulated. This is a useful, yet not overwhelming contribution.

Line 68: 'tail' missing

Was modified as suggested

Line 148: Is the number of windings of the coiling tail not relevant?

Though we agree that this indeed would be interesting data to collect for any future experiment, to get an exact number of windings of the coiling tail (or a measurement of how much length of the tail is used for coiling), we would also need to have video recordings from the other side of the set-up. Especially with the thicker perches it is difficult to make an estimate, as part of the tail disappears behind the perch on the video.

Line 166-167 (and 172-173): Syntax is a bit strange [suggestion: "Percentages giving of the number of times tails were used or not used on the three different materials (PVC, sandpaper and wood) and for the two different perch diameters (narrow and broad)."].

This has been changed for both figures:

"Percentages given are the number of times a tail was used or not for each of the three different materials (PVC, sandpaper and wood) and the two different perch diameters (narrow and broad). Distances without any data indicate that no successful crossings were made for the trials with that perch diameter and material."

Line 169 (and 175): I suggest to add the absolute numbers, too (or at least to refer to table 2 for these numbers). A graphical representation of '100%' for a single count is otherwise somewhat misleading.

For both figures the following has been added:

"Frequency of tail use plotted against perch thickness and material in male *Chamaeleo calytratus*, the absolute number of successful trials can be found in Suppl. Table 2."

Line 189 (table 2): Is it not relevant to refer to the individuals? Are some of the individuals more 'participative' than the others?

We decided to group sexes together as to avoid spreading out our data even more. As multiple individuals for each sex were used, we believe this should diminish most individual variation.

Line 307: How do we know this is 'significant'?

This has been changed to the following:

"As the distances were adjusted to the average length per sex, it seems that there is a difference in performance difference irrespective of size."