

Peer Review

Overall comment – This interesting study seeks to address a real knowledge gap in sesamoid presence and distribution, with some intriguing correlations apparent. I also commend the authors for continuing this important avenue of inquiry during a difficult period (lockdown). However, I have concerns that only one interpretation is really offered in this study, with no real exploration or interrogation of the data for other, perhaps equally valid, explanations for the correlations that seem to be observed. There are also improvements that could be made in terms of structure and narrative, and the full data need to be provided or at least described.

Basic reporting

Within the article, the language used is sometimes ambiguous or would benefit from elaboration or clearer wording. I have noted instances that stand out to me in the 'Additional Comments'.

Appropriate literature and references are cited, but sometimes the citations could be discussed a bit further to given the reader more context, which would be helpful in understanding the study and authors' interpretations. I have noted instances in the 'Additional Comments' where more information and context would be useful to the reader, in my opinion.

The article is structured professionally under appropriate headings and subheadings. However, within sections (e.g. introduction, results, discussion) the narrative or logical flow of content was not always clear to me as a reader. I have noted some specifics/detail in the 'Additional Comments', however as a general recommendation I feel re-arranging the content/narrative of the Introduction and Discussion (in particular) into 'subject paragraphs' with clearer-signposted 'topic sentences' would greatly assist the reader in understanding the study's rationale, findings and interpretation.

I felt the tables and figures could be improved, or made better use of. I have noted this in more detail in the 'Additional Comments'. The raw data have not been shared, and the data presented in the some results only include those species for which evidence of a palmar sesamoid was found. In my opinion, for these species, too much descriptive anatomical data are presented – I am not sure of the value of this reporting. It would be better to present the full dataset, with anatomical findings summarized in a way that is more applicable to the questions asked by the study.

The study puts forward some interesting questions, but does not go so far as to formulate these into a hypothesis. Because of that, and the way the results are analysed, it is difficult to interpret the results presented. The authors suggest some exciting functional interpretations based on the study observations, but I feel there are other interpretations that would be equally valid and which are not considered (I have noted these in the 'Additional Comments'). In its current form, I feel we are only seeing half of a (potentially very interesting) story.

Experimental design

The study is clearly within the aims and scope of the journal, and I believe would be of interest to the journal's readership (I was very interested to read it!).

As mentioned above (in Basic Reporting), I thought re-organisation and re-writing some parts of the manuscript's introduction (with subject paragraphs and topic sentences) would assist the authors' in presenting a well-defined, relevant research question – currently, I feel the narrative and rationale of the study design is somewhat lost. It's not that the paragraphs or sentences are badly written, but their significance was not always clear to me as the reader – sometimes coming across as a series of statements and references, but not a clear point.

The research does seek to fill an intriguing knowledge gap in understanding why or why not sesamoids might be present, and inferring that for the palmar sesamoids in Anura, it might be to do with terrestrial lifestyle. The investigation and observations are consistent with the interpretations made by the authors; however, there are other interpretations which could also be consistent with these observations and which I do not feel have been rigorously explored. For example, the correlation between palmar sesamoid presence in clades and terrestrial lifestyle has been interpreted to mean that the sesamoid is functionally important to that locomotory mode/lifestyle. However, the lineages (clades) that possess the palmar sesamoid are not independent – could the fact that these closely related lineages both possess the palmar sesamoid and be terrestrial simply be due to shared ancestry, and the one not have any functional bearing on the other? This is not mentioned at all in the study. Further exploration of this possibility, and perhaps implementation of phylogenetic comparative methods, would be a way forward to more rigorously analyse the data.

In order for the study to be replicable, the full list of examined specimens from Morphosource should be detailed and reported (not just those in which there is evidence of the ossified palmar sesamoid).

Validity of the findings

Underlying data have not been fully provided.

I have concerns that the main conclusion is not well-supported by the results. The research question and main conclusion of the study seems to be that palmar sesamoid presence in Anura is related to terrestrial locomotion/lifestyle, but this is not tested or illustrated by the data (e.g. coding locomotory mode on the phylogeny to compare with sesamoid presence). A few clades or species are picked out to illustrate the apparent correlation between lifestyle and sesamoid presence, but it does not appear that the lifestyle of the other clades has been systematically described or plotted/reconstructed over the phylogeny to support that interpretation. There are also other interpretations of the study observations that don't appear to have been explored (e.g. phylogenetic non-independence, correlation vs causation between lifestyle and sesamoid presence).

A more minor comment is that sesamoids may be ossified, mineralized, or cartilaginous. The CT modality through which most of the specimens were assigned sesamoid status in this study is often unable to discern cartilage tissues as well as bone. Therefore, interpreting sesamoids as 'absent' in certain lineages for the purposes of phylogenetic reconstruction may be misleading. The study question and discussion may need to be re-centred / re-worded to more explicitly focus on mineralized/ossified sesamoids.

PDF annotations

(Apologies, I was unable to annotate the PDF directly so have made my comments/questions here, and will attach this full review as well)

Introduction annotations:

Line 62: "are much vascularized" – this statement is within a section describing the general characteristics of sesamoids, and implies that sesamoids have relatively greater blood supply to them than other bones, but the study cited seems only to have examined a particular sesamoid of the canine forelimb, and not examined vascularization in comparison to other sesamoids or limb bones. I suggest removing or rewording this section, to avoid the implication that sesamoids are unique in their level of vascularization.

Line 65: “pivot-dispersing forces” – I have not come across a function with this terminology before; and I am not sure what it means. Could this be clarified/elaborated?

Line 66: Another major function of sesamoids appears to have been omitted, which is the altering of muscle moment arm (of the muscle whose tendon the sesamoid is embedded in). This is a function frequently attributed to sesamoids and has been demonstrated in several studies.

Line 69: “It has also been proposed...” – If in line with the journal’s guidelines, the authors may wish to change this to “We/the authors have previously proposed”, to make clearer the narrative or rationale behind this current study (which I assume to be following up on the prior study’s proposal).

Line 72: “posterior” – it’s not clear here whether posterior is meant as an anatomical direction (in which case, a veterinary/zoological term would be more appropriate e.g. dorsal) or temporally (e.g. subsequent growth).

Line 74: “constant distribution in the tetrapod taxa” – I disagree with this characterization; sesamoids are frequently noted to be variable in presence within and between individuals relative to other anatomical elements. (E.g. Vickaryous & Olson, 2007, which is cited in this current study, discuss this characteristic variability of sesamoids, as do several other surveys of sesamoid presence within taxa).

Line 76 and throughout: “hand” – I suggest using ‘manus’ rather than ‘hand’ as a more appropriate zoological term; the latter tends to be used for humans/primates only.

Line 77: “phalanx-phalanx and metacarpal-phalanx” – are these the same/equivalent in position to the distal and proximal sesamoid bones, or metacarpophalangeal sesamoids of the manus in other taxa? If so, it may be worth giving these names in parentheses which may assist readers more familiar with the other names, as well as assist future anatomical studies.

Line 81-85: Sentences “The palmar sesamoid is present...” and “The typically arboreal anuran groups...” – These sentences are particularly confusingly worded, with these two sentences seeming contradictory as currently worded. I think it is saying that most Anura have a palmar sesamoid but that it is lacking in arboreal Anura, and that in other tetrapods the palmar sesamoid and flexor plate are reduced as in arboreal anurans. If this is correct, I suggest re-wording the sentences to improve clarity; however these latter paragraphs of the Introduction also are the ones I felt were a collection of statements, and would benefit from re-planning and re-organisation into subject paragraphs with topic sentences. I feel this would greatly improve the narrative and logical rationale behind the study, which currently seems a bit lost.

Also Line 81: “The palmar sesamoid is present in most anuran groups.” – A citation is required for this statement.

Line 86: “acts by avoiding closing of the palm” – pedantic, but is this really an ‘action’ of the sesamoid or merely a consequence of its presence? Also, I do not think ‘avoiding’ is the correct term here (‘preventing’?)

Paragraph lines 88-93 – This paragraph needs a clearer point; at the moment this comes across as random information to the reader. Is this information provided to the reader in order to set up *Rhinella* as a useful group in which to study sesamoid presence/comparative anatomy? If so, a topic sentence at the head of the paragraph would be really helpful in orienting the reader and allowing them to digest this information in context.

Line 99-100: “understand the functional and ecological consequences of the presence of the palmar sesamoid” – this assumes only one interpretation, which is the sesamoid’s presence carries functional and ecological consequences, and not that function or forces experienced by the tissues might be driving sesamoid formation (i.e. the opposite interpretation), no? This final sentence of the introduction is setting up only one interpretation within the study, that correlation equals causation (i.e. lifestyle and presence correlation = sesamoid must provide a function within that lifestyle).

Materials and methods

Line 103 – Where were the 13 specimens that were dissected acquired from, and was ethical approval required and obtained?

Line 103: Why were *Rhinella spp.* specifically chosen for in-depth description/dissection, and how do these data contribute to answering the study’s main question?

General comment – is the age of skeletal maturity known for these species, and is the age of the specimens known? Were both left and right sides examined? Sesamoids in other species are known for ossification later in life, and can also be variable between species/individuals. This is an important consideration when inferring sesamoid status from few representative specimens. This information would be really helpful for the reader to contextualise the study’s findings.

Line 112-114: “A movement of the flexor plate...” – Why was this done; what does eliciting this movement tell us?

Line 115: “from Ct images” – did the computer tomography images allow for discerning cartilaginous or unmineralized sesamoids? As noted in the study Introduction, sesamoids can be comprised of unmineralized connective tissue.

Line 120 and elsewhere: “ventral surface of the distal carpals” – within the manus, this would be the palmar rather than the ventral surface.

Line 124: “Maddison and Madisson” – typo; the second Maddison in this citation is spelt the same as the first.

Line 123-4: “using parsimony” – would the authors consider other methods of ancestral state reconstruction (e.g. maximum likelihood), to further explore the sensitivity of the reconstructed state?

Line 126: “Through character mapping we investigate the relationship between the palmar sesamoid and hand abilities within the anuran group.” – How exactly was the relationship investigated – was it just observation of correlation by eye at clade level, or were the species/clades coded by manual ability and statistical or other kinds of analyses performed?

Results

General comment: The formatting of the results are a little confusing in terms of subheadings, with the Anuran family name subheadings in bold/larger font than the italicized method subheadings that they fall under.

Lines 130-131: The reader is directed to the Table and figures for these results, but it could be clearer how these data relate to the study’s main aim. Some more explanatory/linking text in the Results section would be helpful. At the moment, it feels a bit like an info-dump without much guidance for the reader. In addition, the presentation and description of these data in table form is difficult to read and interpret, especially with lots of text and no boundaries in the table cells. If this

information is all critical to addressing the study question, then where there is lots of text like this, could it be condensed in the table with more detail in-text? E.g. 'the palmar aponeurosis is a sheet of connective tissue, absent in ¾ of the species examined in this study. In *R. diptchya* it was [description] [figure]', and then in the table have the character data more simply presented as 'absent / present'.

Lines 135-7: "Only those taxa with palmar sesamoid are included in this list, from which the character 1 was surveyed." – Why only the taxa with a mineralised palmar sesamoid? A full list of all species with their coding needs to be provided somewhere (even if only as a supplementary file). Additionally, I don't understand what is meant by "from which the character 1 was surveyed" – does this refer to the character 'a' in the Materials and Methods (i.e. palmar sesamoid) or the status of the sesamoid (1 = present)?

Lines 135-7: Additionally, it would be really helpful to have some description of the results here in-text rather than only directing the reader to the figures and requiring them to figure out what is being shown. The images are not sufficient on their own as a description – what do the authors want readers to take home from these images? If it is that the palmar sesamoid is variable in morphology, this could be made clearer by stating that here. It would also be helpful to have a description in-text for what the distal carpal crests and flexor plate are generally (the figure caption does not explain this sufficiently).

Lines 139-284: Data from all species and specimens should be available (e.g. a supp info table) with codings of characters 'a' and 'b', in order for this study to satisfy requirements of replicability. I'm also not convinced that this level of individual specimen anatomical description in this section is contributing to the study's question. What is the take-home message or usable data from this section? Why is this level of description useful if the characters are only going to be coded present or absent for analysis?

Lines 287-300 – This level of in-text summary with links/context to the figures is good. It would be helpful for readers unfamiliar with distal carpal crests and flexor plate anatomy to have a brief general description of these features (what is the flexor plate & distal carpal crests, perhaps general overview of Anuran manus anatomy so that readers can best appreciate the variances in anatomy described).

Line 287 - it is not always clear whether the en-dash (-) refers to a range of things or is part of an abbreviation. E.g. Distal carpal 3-5 in this line, and elsewhere – does this mean distal carpal bones 3, 4 & 5?

Line 290: "More developed processes are seen in, e.g. *Rhinella*..." – I think there might be some words missing (more developed processes are seen in some species, e.g. *Rhinella*...?)

Line 294: "the sesamoid rests onto the distal carpal embedded on the flexor plate" – the wording is slightly unclear here (is it the sesamoid or the distal carpal that is embedded in the flexor plate?)

Line 302: I suggest adding 'morphology of the' before "distal carpal palmar surface" in this subheading.

Line 304-5: I suggest adding 'parsimony' before optimisation (although it is not necessarily the optimisation that shows the PS is absent, but rather the study's survey of extant species). At the risk of being pedantic or devolving to semantics, can an absent feature (which is also reconstructed as absent ancestrally by this study) be considered a 'widespread character'? Perhaps consider rewording these couple of lines e.g. 'Our observations show absence of the palmar sesamoid is

widespread amongst anurans today, and our parsimony reconstructed suggest it is also ancestrally absent in many lineages.'

Line 307 and elsewhere – “reversion” – typo (reversion)

Line 317: “shows no clear relationship...” – how was this evaluated; visual impression of correlation between Figs 4A and B, or any kind of analysis performed?

Discussion

General comments: As mentioned in the ‘Basic Reporting’, I suggest re-arranging the content/narrative of the Introduction and Discussion (in particular) into ‘subject paragraphs’ with clearer-signposted ‘topic sentences’ would greatly assist the reader in understanding the study’s rationale, findings and interpretation.

In some areas, the current phrasing makes comprehension difficult. I suggest the manuscript be reviewed to improve readability (e.g. shorter, clearer sentences, checking for typos or misspellings). Some examples of sentences that I found difficult to follow or had errors include lines 323-326, 331, 333, 336, and lines 398-409. It might be helpful to have a colleague review the manuscript before resubmission, or contact a professional editing service.

Line 322 and line 326: “involves the digits tendons” and “the tendon of digit II is absent” – which digital tendons? (presumably the ones on the palmar aspect; do these have any specific name, or is there only really one tendon in each digit?)

Line 326: “always at an angle close to 60...” – is there a reference for this, or is it an observation of this current study? If the latter, does this mean the digit cannot physically adopt a different angle, even in the live animal, or is it possible that fixation has brought the digits into similar positions to one another? What is the significance of the angle, for the authors to describe it here?

Line 328-329: “tendon flexor” – other way around normally, flexor tendon

e.g. of above Line 331: “This condition allows lateral flexion of the digits to reach a grasping that makes...” – which condition? Several have been described in the preceding sentences. Additionally, what is meant by lateral flexion (isn’t flexion by definition in the palmar direction)? And I think a word is missing after ‘grasping’.

Line 339: “Sesamoid optimisation in the anuran phylogeny...” – I just wanted to query whether this is a widespread term? I would call this something like parsimony-based ancestral state reconstruction, rather than optimisation (which to me implies tree topology rather than reconstruction of character evolution).

Lines 339-350: The language and changing topic of this paragraph make it difficult to understand what point is being made about the palmar sesamoid. Some words in particular seem out of place or could have clearer alternatives to assist the authors’ message (e.g. “related” in the context of line 339, “present” in the context of line 341 & 346, “assess” in the context of line 342).

Line 346: “presenting two overlapping palmar sesamoids (Fig 4)” – Fig 4 does not show this, perhaps the wrong Fig is cited? Additionally, if this is only one specimen (or one side of one specimen), it could be that the authors are observing coalescing ossification centres of one sesamoid. It is common/known that sesamoids can mineralise from more than one centre of ossification (and I myself have seen coalescing/double sesamoids in a Lepidosauria specimen evaluated by CT, similar to the authors). Is it the same in both manus of the specimen?

Line 351 and elsewhere: “The scattered presence of the palmar sesamoid among anuran clades suggests that its genesis can be explained not only by phylogenetic inheritance but also by epigenetic stimulus probably related to anuran life's mode” – is the word genesis here meant in terms of the individual animal (ontogeny/development) or the evolutionary origin of the sesamoid within the lineage? This differentiation is important to be clear about. The first meaning is consistent with what we know about sesamoids generally, but I think the authors intend the second meaning, which is not the only valid interpretation.

Lines 351-358 and elsewhere – The main finding of the study being presented in the Discussion seems to be that palmar sesamoid presence in Anura is related to terrestrial locomotion/lifestyle, but this is not tested or illustrated by the data (e.g. coding locomotory mode on the phylogeny to compare with sesamoid presence). A few clades or species are picked out to illustrate the apparent correlation between lifestyle and sesamoid presence, but it does not appear that the lifestyle of the other clades has been systematically described or plotted/reconstructed over the phylogeny to support that interpretation.

Line 358 – How do the authors reconcile the inferred terrestrial ancestral mode of life of frogs with the ancestral absence of the palmar sesamoid?

Lines 360-362: These two last lines of the paragraph seem to directly contradict each other. I’m also not too sure how they link up with the preceding sentences/topics in the paragraph.

Line 367 and elsewhere – What is meant by cell “distortion”?

Line 367-8: “fast development and ossification” – I don’t agree with this interpretation; evidence suggests the opposite – that sesamoids actually develop and ossify slower/later than other skeletal elements.

Line 398-406 – as with other parts of the Discussion (e.g. lines 339-350), the language and changing topic of this paragraph make it difficult to follow. Some words in particular seem out of place or could have clearer alternatives to assist the authors’ message (e.g. “betweeness” in the context of line 399, “canonical” in the context of line 400, “outstanding” in the context of line 402).

Lines 407-409 – I don’t follow the reasoning here; could it be elaborated or made clearer? Why would a branch of a nerve passing through the brachial plexus show that the forelimb has special sensory function (any more so than other sensory nerves of the forelimb), and why is this relevant to a sesamoid that this nerve does not innervate?

Conclusion

Line 412: “presence is strongly related to specific clades” – to me, using the words ‘strongly related’ are not particularly appropriate – it suggests the palmar sesamoid is universal in the clades it occurs in, which does not appear to be the case from Fig 4. There is one large clade where most (but not all) species possess it, and other instances where the sesamoid is found in several species of a clade. It also implies that some statistical analysis of sesamoid relationship (to another factor) has been done.

Line 419-412 – This final sentence is the take-home message of the study. However, I am concerned that this conclusion cannot be confidently made on the basis of the evidence and analysis of the study currently. There are other interpretations that are not explored (described in previous comments), and/or further analyses that would be needed to demonstrate i) that there is a correlation between sesamoid presence and life mode and ii) the nature of that correlation (phylogenetic independence).

Figures

The scale bars are not shown in a consistent position on figures, and very close to edges in some images. Could these be placed in an inset somewhere consistent in each figure?

The images are also not aligned in some Figs (e.g. 1 E&F, Fig 2).

Figure 2 caption (and elsewhere in-text) – it is not always clear whether the en-dash (-) refers to a range of things or is part of an abbreviation. E.g. D-C 3-5 – does this mean distal carpal bones 3, 4 & 5? Is a dash also necessary between D-C in this abbreviation? It just makes things a bit tough to read. There are some typos in the caption (e.g. paralell)

Figure 2 image – it is hard to appreciate borders/distinguish the bones due to the lighting of the models (especially when the images are printed). Could the lighting of the models be altered? Another solution could be to have line drawings/tracing of the anatomical elements as an inset.

Fig 3 Caption – a brief explanation of the figure would assist the reader who is looking at the figures in isolation (i.e. why do the authors consider this important to show the reader – this could be stated e.g. This figures shows the different possible configurations of the flexor plate and sesamoid observed in Anura/Rhinella). Possible typo in caption (diaphanaized).

Fig 3 A – image is very dark, plus arrows and scale bars are barely visible.

Figure 4 caption typo (ûat)

Figure 4 image – the horizontal orientation makes the trees smaller and hard to read. The size means you cannot actually read much of the text. I suggest orienting the trees vertically and of sufficient size and resolution to discern the text. It would support the conclusion of the study to map lifestyle (e.g. terrestriality) to the clades/species, to demonstrate the correlation proposed by the authors.