Peer Review history

Manuscript: A method for quantifying, visualising, and analysing gastropod shell form by Thor-Seng Liew and Menno Schilthuizen (http://dx.doi.org/10.7287/peerj.preprints.157v2)

Content   **Page**

Thank you for your submission to PeerJ. I am writing to inform you that your manuscript, "A method for quantifying, visualising, and analysing gastropod shell form" (#2013:12:1153:3:0:REVIEW), has been rejected for publication.

The comments supplied by the reviewers on this revision are pasted below. My comments are as follows:

Editor's comments

Having spoken to the referees, they have each declined to re-review the manuscript: one in writing and the other verbally. Both felt that while the rebuttal document is extensive, the authors have failed to incorporate any referee feedback into the text of their manuscript, and there was no point in repeating themselves. As I stated in my original decision, I believe that it should have been relatively simple to revise the text to include the appropriate caveats and respond appropriately to the referee comments. However, despite 3 rounds of review, and extensive rebuttal documents, there have been no substantive changes to the manuscript since the original submission. In fact, the only real changes to the text have been to add self-citation rather than to respond to referee feedback.

I was clear in my last correspondence that I did not agree with you that the presence of the reviews as supplementary materials was sufficient grounds to ignore referee comments and not incorporate any of the recommended changes into the text of your manuscript. Here referee 2 tries hard once again to provide advice and guidance for the future on how the manuscript might be revised into a valuable contribution to the field, but the authors seem determined to argue rather than consider such advise to improve their work. Despite what I believe is clear guidance from both the reviewers and the editor, as this referee points out, you have once again included a detailed rebuttal without any substantive changes to the scope or tone of the manuscript nor a reasonable scholarly treatment of the existing literature.

Given this is such an unusual case, I have consulted with two other editors for an independent opinion before returning this decision. In discussing the situation with them, each responded that they would have rejected the paper immediately upon the first resubmission if authors refused to address valid comments of the referees. One of these more senior editors raised the point that a major benefit of peer review is to curb personal arrogance that our work cannot benefit from the intellectual input of others in the field, and that just because we may disagree with a colleague does not mean that they do not have a valid point that is worthy of scholarly intellectual discussion. Such objective scholarly discussion is what advances science. In discussing this situation with them, I have to agree that the primary reason to have an editor is to have someone who serves as an objective arbitrator on the peer-review process to safeguard authors against inappropriate or unreasonable reviews and ensure that valid referee feedback is incorporated appropriately into the final manuscript.

In this case, despite 3 rounds of revision, and after extensive discussion with these other editors about this situation, I stand by my previous evaluation to support what I feel is valid referee feedback from very well qualified reviewers who are familiar with the field. Given your refusal to incorporate any of this expert feedback into your manuscript, the manuscript has quite simply not passed peer review, which is the basic criterion for acceptance of any scientific paper to a scholarly journal. Thus, I regret to inform you that I have no choice but to reject your revised manuscript, and will not send it back out for further review.

Robert Toonen
Academic Editor for PeerJ

Reviewer Comments

Reviewer 2

Basic reporting

No Comments

Experimental design

No Comments

Validity of the findings

No Comments

Comments for the author

I accepted to re-review Liew and Schilthuizen's paper, but upon examination of the text and rebuttal, I am not sure what more I am expected to do. The paper does not show the major changes that the editor requested, and by now the authors have indicated that they take my appraisals as unconstructive and incorrect. I pity this development a lot, because my sole motivation for writing my reviews was to improve their manuscript so that, when it appears, its benefit to the scientific community is maximized. These efforts, however, seem to have been a waste of time because the authors have decided to leave the paper as it is, and, hence, I doubt anything positive would amount from me performing extensive additional reviews or me bickering about comments and replies with the authors.

I regret to inform the editor and the authors that the rebuttal has not made me change my appraisal of the paper, and I feel it is not just my comments, but also those of the other reviewer, Dr. Cruz, that were not treated with the attention they deserve. Beyond this issue many of the replies in the rebuttal of the authors are beside the point (clear-cut examples are 10, 22 and 23). I will provide one last clarification, because the authors specifically requested information about

the use of landmarks on helical shells (point 12 of their rebuttal):

The authors believe 1) that no homologous points exist on gastropod shells, and 1) that this would then invalidate many of my remarks, especially considering the introduction. Both claims are wrong. The authors cite Fred Bookstein, who defined degrees of homology for landmarks, and they claim that 'Bookstein's book' does not include examples of the use of landmark-based approaches with helical shells. The authors then take the lack of an example as an argument to suggest there are no homologous points on helical shells (clearly a wrong conclusion from a set of non-exhaustive examples!). In their rebuttal they cite Johnston et al. 1991, which is a paper in which Bookstein and colleagues define landmarks, that is homologous points (!), on gastropod shells. So, I am baffled by the reply on point 12 in which first the authors acknowledge the possibility to define landmarks on helical shells, to contradict themselves 2 sentences later by saying that homologous points do not exist on such shells! The implication of the statement that no homologous points exist on helical shells is that gastropod shells altogether, even in closely related taxa, cannot be considered homologous! This makes no sense.

Johnston et al. 1991 is the first reference I provide to indicate that landmarks can be defined on gastropod shells; a second paper would be Cruz et al (2012 ZJLS). This paper has F. James Rohlf on the author list, who falls, together with Bookstein, among the most influential contributors to the field of geometric morphometrics. If the current authors would desire an example of the use of landmark morphometrics with gastropod shells from their own papers then Schilthuizen & Haase (2010 JoZ) comes to mind. From my knowledge of the literature, there are at least 50+ papers in reputable peer-reviewed journals that present landmark analyses with gastropod shells; many more exist with other mollusks. So, were all of these previous authors, including Schilthuizen himself, wrong in appraising 'homology criteria' and the use of landmarks in gastropod research?

In short I object to the bold statements of the authors which are out of sync with the existing literature, and downright baffling given that the authors themselves have applied landmark analyses to helical shells before. The above once again clarifies how the 'literature review' that is used to set up the study should be taken with more than a healthy dose of salt.

Third round of Peer Review (#2013:12:1153:2:0:REVIEW)

Editor's comments

1. After my efforts trying to guide you in the revision of your manuscript from the first submission, you asked that I pass it along to the referees as submitted, which I have done. As I predicted in my correspondence with you, both have now come back with stronger statements about the specific short comings they see with the manuscript. In particular, the second referee points out several major issues with the approach, the presentation of the data, and the scholarship of the manuscript. Thank you very much for asking the reviewers to respond to our reply in the rebuttal letter. As the editor only correspond with reviewers by phone after the first review, we could not identify which of our points in the rebuttal letter did not convince the editor and the reviewers.

2. I agree that the development of a new method likely has some value, and that it should be evaluated by the field, but the issues raised by the reviewers must be addressed by the authors before the paper can be considered for publication. As the second referee clearly states, these are serious criticisms of the scientific quality and scholarship of the work, not a question of the journal policy regarding importance. I have to agree with that sentiment and emphasize that the comments of the referees must be satisfactorily addressed for the paper to become acceptable for publication, Thank you for stating clearly your stand; we appreciate your comments. Now, we also have a clearer view on the exact issues that are of greatest concern to reviewer no. 2. However, we cannot agree with many of the points raised by the reviewers. We have already responded accordingly in our previous rebuttal letters, and we are somewhat desperate by the fact that reviewer no. 2 seems to keep GM in mind while reading our manuscript. For example, **this rebuttal letter – point 12 and point 13**.

3. Given your reluctance to revise the manuscript in response to referee feedback in the first place, and the more emphatic and specific comments returned by referees after your revision, I have to agree with the second referee that this constitutes a major revision of the manuscript. I will ask the same referees to evaluate the suitability of the revision if you decide to undertake these changes, or may ask for a new referee perspective on the manuscript at that point as well. Since we feel that the reviewers' opinions are either beside the point or the result of misapprehension (see below), we cannot bring ourselves to change our text.

4. Please be aware that we consider these revisions to be major, and your revised manuscript will probably have to be re-reviewed. We come to the conclusion that there is no way to close the gap between our viewpoint and those of the reviewers. We feel compelled to express out surprise that the Editor has changed the decision from Minor Revision to Major revision, given that the reviewer did not raise additional concerns in the manuscript besides the ones voiced in the first review round.

We would be more than happy to change or even remove the text in our manuscript if the comments are correct and constructive (for example see https://peerj.com/articles/329/reviews/). However, this is not the case in this review.

If Editor and Reviewers are willing to respond to our rebuttal, please refer to the reference number for each point. **Rebuttal Letter 1: Point 1 - Point 37; Rebuttal Letter 2: Point 1 - Point 5; Rebuttal Letter 3(this rebuttal letter): Point 1 - Point 25.** This is mentioned for the benefit of the future reader, who might read this review report.

We respect any Editor decision (either another major revision or rejection). In any case, all author-Editor exchange will be published along with this paper if this paper should be accepted in peer-

reviewed PeerJ; if this paper should be rejected, we will publish the same reports along with our paper in PrePrint https://peerj.com/preprints/157v2/, similar to, e.g., https://peerj.com/preprints/19/

We really appreciate the efforts of the Editor and reviewers towards this manuscript and we would like to see this sentiment recorded here.

5. If you are willing to undertake these changes, please submit your revised manuscript (with any rebuttal information*) to the journal within 60 days.
We apologise for the delay.

Reviewer Comments

Reviewer 1 (Ronald Allan Cruz)
Basic reporting
No Comments
Experimental design
6. "My primary concern, however, is with regard to the specimens collected. Why was only one genus, Ophistostoma, used for the study? How many specimens of each species were collected? There may be significant morphological differences even within a species. And since you have made the claim that your methodology can be used across all species, even using data from different studies, was it not important to get many different representatives of the diverse gastropod taxa?"

The above was my comment in the first review. It has been addressed with the addition of another genus, but I am still unclear on how many specimens were collected per species and whether or not it matters (I believe it does) in their particular methodology.
We have already answered this in our first rebuttal letter. **Please see Rebuttal Letter 1 – Point 4 and Point 8.**

Validity of the findings
7. "In lines 560-564, you point out that comparing radar charts between shells of different sizes is less informative, and that size differences account for certain differences in radar charts even for the same species (O. laidlawi). How then is this an advantage over geometric morphometrics, which through Procrustes transformation significantly decreases (though admittedly does not completely eliminate) size variables? I admit to being rather unfamiliar to most of your procedures, so a clarification regarding this conundrum would certainly be helpful."

The above was my comment in the first review. I do not think that this point was addressed in the revision.
We have already answered this in our first rebuttal letter. Please see **Rebuttal Letter 1 – Point 5.** Please also read the text – "**7. Visualising aperture form and trajectory changes along the shell ontogeny**"

Comments for the author
8. The manuscript and methodology has been improved, but I would like to read clarifications on those two points mentioned above.
See above.

Reviewer 2
Basic reporting
No comments
Experimental design
No comments
Validity of the findings

No comments
Comments for the author
Review of 'A method for quantifying, visualizing, and analysing gastropod shell form' by Liew and Schilthuizen.

9. I re-reviewed this paper because I think the method describes a valuable contribution towards the study of shell morphology. However, it appears that I have not been able to convince the authors of some of the flaws I pointed out in my original review. Instead, the authors have decided to largely ignore this review, certainly my objections to the rationale and introduction. I must point out that although this resubmission is a small step in the good direction in some ways, this paper could (and should!) still be improved in several other ways.
For those points for which we fundamentally disagree with the reviewer, we did not make any changes to the text.

10. Before getting into the content of the paper, however, there are a few aspects in your rebuttal that reflect practice I do not subscribe to (or at least the one that I have read, there seem to be more, which I have not found in this submission's files):
To avoid miscommunication in the future, we have compiled all the correspondence in a single pdf. We opt to publish the whole review process, see **this rebuttal letter – point 4.**

11. 1) Your point 4a does not present a scientific argumentation as to these 'facts', and I simply disagree. Many of our peers would disagree that the literature just represents facts. However, my problem is not with the literature itself, but how it is represented in your 'literature review' and what deductions you make (see below).
2) Your point 4c is not a reason to leave criticism unaddressed, certainly not at the reviewing stage (although in the author's defense there appears to be a rebuttal I have no access to). Merit to the community is evaluated at this stage, not your readiness for criticism.
3) Your point 4d as to journal policy should not be interpreted as a backdoor for attempts to inflate the impact of your paper with unjustified or unsubstantiated claims (cf. introduction; see below).
This is our response to the Editor's decision (**this rebuttal letter – point 1, Rebuttal Letter 2 – point 4b**), and we did not expect the reviewer to respond to these matters.

We read every article that we cited in the introduction carefully, and we think our review in the introduction is valid.

Our article has been evaluated both at this formal peer review stage, and by the scientific community already at https://peerj.com/preprints/157/, please see also http://figshare.com/articles/A_method_for_quantifying_visualising_and_analysing_gastropod_shell_form_/877061
https://www.facebook.com/JPaleontologicalTechniques/posts/244411532391346

12. I will re-iterate some of the important points that I made before again, but I will not re-iterate each and every aspect (i.e. please consider the original review to remain standing in a number of points not addressed here).
1) False equivalency of geometric morphometrics (GM) and theoretical modeling (TM). I am sorry but I have to insist here that these methods are designed for different purposes and that they are not equivalent (see my first review). Correcting a few sentences in the intro on the comparison of GM and TM does not change that the intro is set up in the rationale of equivalency. Though some integration between both methods is advisable, and I applaud the authors for their attempts, the choice scientists make in terms of methods in the future will necessarily reflect the kind of questions they are interested in.

IF the interest is in evolution, and hence in the variation displayed within and between populations (the raw material with which natural selection works) they will choose for a method in which data acquisition can be performed fast for a large number of specimens, hence geometric morphometrics. IF they are interested in reconstructing and visualizing a particular shell/shell shape, or in parameters of shell development then theoretical modeling presents an outcome. The method of the authors is very time-consuming and clearly not designed to go through 1000s of specimens to study variation within and between nominal species. So, I fail to see the equivalency, and I can simply not recommend maintaining sections in the introduction that make this claim and are intentionally dangling false hope in front of colleagues at this stage in the paper.

We did not say that GM and TM are equivalent. Integration of GM and TM is not possible because both are based on different philosophies. GM was developed by Bookstein (1977, 1980) based on the idea of Thompson (1917: Chapter 17). Ironically, Thompson (1917) himself did not use his comparison approach (see Chapter 17) to compare shells, instead using a logarithmic spiral approach (see Chapter 11). We do not know why he did so, but this may suggest he was aware of the limitations of his method (Chapter 17) in addressing shell shape analysis. In addition, to our knowledge, even Fred L. Bookstein placed the landmark on along the spiral geometry of the shell but not arbitrary landmarks (see Line 178 – 188 & **this rebuttal letter – (Reviewer point 15** & Johnston, Tabachnick & Bookstein, 1991, **Landmark-based morphometrics of spiral accretionary growth**, *Paleobiology* 17:19-36).

We said shells have been quantified by using both theoretical and empirical methods for quantification and comparison of the shell form. However, as we have mentioned previously (**Rebuttal Letter 1 – point 20 and point 21**), homologous points do not exist on helical shells. Could reviewer suggest an article that has proved that biologically meaningful landmarks exist on helical shells?

We have clearly mentioned in the manuscript that it takes two days to obtain the data from a single shell. Thus, we expect our colleagues to be able to estimate the amount of time needed for their own projects when using our method. Therefore, we do not think we gave false hope to the reader.

13. 2) Concerning a 'comparison' between GM and TM: I have pointed out before that such a quantitative comparison is lacking and that some of the authors' claims on methods cannot be made without comparative data. However, from this remark one should not conclude that I requested to actually make such a quantitative comparison, which would be very hard given the point made directly above. Nevertheless, many sections of the paper are still set up in a comparative mindset (see below).

Please see our reply to editor of **Rebuttal Letter 2 – point 1**.

14. 3) Lines 97-100: I disagree, scanning methods allow to obtain high resolution imagery of specimens and 3D GM are available. Line 98 reflects a subjective, comparative statement and line 99 is non sequitur; it is not because accurate morphological quantification is essential that TM is 'better'.

Regardless of whether the shape is described in two-dimensional or three-dimensional space, homologous points do not exist on a helically coiled shell. We think our statement remains correct.

15. 4) False claims of the authors lead to a misrepresentation of GM. Examples:
a) Line 178-188: there are evolutionary homologies on gastropod shells, for instance the apex (starting point of coiling), the protoconch, the protoconch-teleoconch transition, and clearly how the aperture and revolutions evolve through time. What the authors probably wish to say is that the same number of whorls in 2 different shells may reflect different intervals of growth, perhaps in different environments, and that they may not be strictly equivalent, but that is beside the point.

Points standardly used on gastropod shells follow Bookstein's categories of homology, and are recognized as usable landmarks/semi-landmarks, which has resulted in wide application. Information on curves can be included as semi-landmarks anchored to homologous landmark points. 3D objects can be sectioned by planes to define points and it is possible to define homologous sectioning methods, e.g. for snails a plane that encompasses the columellar axis and touches the aperture; a second plane can be perpendicular to the first, etc. In any case, the authors claim they are doing better (line 524-537), but are their NURBS homologous? …And how do homology and the tracing of aperture outlines (data acquisition) relate to one another in the new method which is described by the authors—does this not suffer from the same issues as articulated for GM?

Let us use the favoured example of Bookstein (1997, 1985) and Thompson (1917) – fish. Homologues landmarks can be found on fish (e.g. Bookstein 1997 – figures in Introduction), a landmark on an eye can be located, unambiguously, either on a 2D image or a 3D model. The same goes for other landmarks. In the case of the outline and surface where landmarks cannot be identified, semi-landmarks can be used, in most cases, between homologous landmarks. However, such homologous landmarks are absent from a helix. See **this rebuttal letter – point 12**. Please also see **Rebuttal Letter 1 – point 4 and point 8**.

We checked Bookstein book, there are no example of helical shells. Landmarks as well as semi-landmark have to be homologous (see *Gunz & Mitteroecker 2013 - DOI: 10.4404/hystrix-24.1-6292)*.

"*A wide taxonomy range of species evaluated does not necessarily mean a wide morphological range of shell shapes. The focus of this manuscript is to evaluate the method on shells that have regular as well as irregular shell forms. The range of shell forms that we used in our evaluation do cover a broad diversity of form. We demonstrated that the shell form can be quantified as the same set aperture ontogeny profile, regardless of the unconventionality of shell form. We aware that both reviewers are well-versed in Geometric Morphometric (GM) methodology. May we invite both reviewers to use GM on our selected shells? We would expect that this will result in the realisation that, given the shells' irregularity, GM applied on these shells will not result in meaningful comparisons.*(**Rebuttal Letter 1 – point 1**).

We estimated the Elliptical Fourier Coefficient from the outline points to quantify the aperture shape and calculate the perimeter of aperture as size. There is no homology requirement. For the geometry of the ontogeny axis, as we mentioned in the text, we followed the spiral line on the shell. Please see also https://peerj.com/articles/383/ - section "Definition of ontogeny axis". We will add this reference to the manuscript.

16. b) Moreover, the authors document various complications with tracing aperture outlines in side bar comments (which is not fully articulated in the text but should be), but continue by claiming that these issues are not caused by their methodology. Nevertheless they use similar claims to bring discredit to GM. Do the authors really want to base their claims on a double standard?
There are differences between our method and GM. GM is based on homologous landmarks, which cannot be done with helical shells, because homologous points simply **do not** exist on such shells.

Our method is based on aperture morphometric data. Sometimes, the aperture outline is difficult to be traced on a shell but it **does** exist throughout the shell ontogeny. (**Rebuttal Letter 1 – point 4**)

We do not think we apply a double standard, since these are very dissimilar claims.

17. c) The authors persist in ignoring that morphometric datasets can be modified to allow compatibility between datasets. First of all, one can always define additional points or delete some points from one dataset to make it compatible with another one. Secondly, points may have been

digitized in different orders in one dataset to the next one, but as long as each dataset was constructed consistently one can design and run programming scripts to reformat datasets to make them compatible. At the stage of compatibility the data can be all analyzed together and PCA-scores can be compared.

There are no homologous points on a helical shell. Even if arbitrary points were used, then which points were to be deleted? Can reviewer provide a published example of how this could be done on helical **shells?**

18. d) The authors used time-series analysis to compare shell forms quantitatively, and such time series analyses can be performed with several algorithms. Hence, they claim, the same data can always be analyzed in the future with better algorithms. That is ok, but just to be clear here, this is of course also true for GM.

This is probably correct, but we fail to see the implication.

19. 5) Have the authors examined reproducibility of outline tracing efforts? They claim that poor understanding of how the specimens grow will lead to poor tracing results, so perhaps the tracing results of two people may have low compatibility/comparability. If I am correct, the authors did not digitize the same specimen multiple times to estimate reproducibility. So how do they know their method is reproducible? That data obtained from different studies can be integrated does not have anything to do with reproducibility. Nevertheless in the abstract and their conclusions the authors' claim their method to have high reproducibility.

We traced the aperture outline after studying the shell aperture throughout its ontogeny (for example https://peerj.com/articles/383/. We believe that we traced the outline correctly and we do not foresee any discrepancy.

20. 6) Lines 524-537: all depends on the questions one is interested in as to whether this is 'a good investment'. This issue relates again to the point of false equivalency.

We stated this clearly in lines 533 – 535. See also **this rebuttal letter – point 12**.

21. 7) The language is at points very colloquial and imprecise. For example: the 'speed' of a computer whereas the authors clearly mean 'processing power'. This particular case is harmless, but others create confusion and hamper readability. For example the issue on 'shell size' in the discussion (e.g. line 652): size is not volume; both are not equivalent despite the authors' belief (Line 659), what the authors mean is volume as a proxy for the single-specimen biomass of soft-tissues. More colloquial language follows (line 662): The authors feel that 'a snail should grow a shell in which its entire soft body can fit…' This is overly simplistic, some snails don't have shells, some have shells that cannot accommodate the entire soft-tissue body.

We think our statement of size (**Rebuttal Letter 1 – point 4**) is correct. Could reviewer provide a reference that state that size cannot be approximated by volume?

Just to illustrate what we mean in **Rebuttal Letter 1 – point 4.**
What is the size difference between this http://borneanlandsnails.lifedesks.org/node/1095 and http://borneanlandsnails.lifedesks.org/node/1209?
The volume is the only unambiguous size metric that can be used to compare these two shells that are very different in shape.

22. 8) The conclusions are out of line with the body of the paper and the data provided. Line 675: what is 'the same aperture profiles'? See the issue on homology above. How can you guarantee exact replication between specimens can be performed—I don't think you can.

We are quite convinced that this is possible. See also **this rebuttal letter – point 15, point 16, point 19**.

Robustness in my opinion means that with the expected amounts of noise and non-biological error the methods keeps performing well (which is not tested).
Robustness in this context is that the method can be used to analyse any helical shell forms. We have mentioned this explicitly in the manuscript on lines 674-678.

Reproducibility is also not tested (see above).
See **this rebuttal letter – point 19**.

And for versatility: what is the 'data standard' required in taxonomy, functional morphology, theoretical modelling and evolutionary studies. Can the authors define this standard and what it means in this context? What about applicability with shells on which growth lines are not visible/traceable (for example a cowrie)? Can the method be applied there? I think more thought could/should go into this section.
The data required would be:
- The raw 3D shell mesh models – taxonomy - for example for visualisation of shells http://www.pensoft.net/journals/zookeys/issue/393/

- coordinates data of the vertices - theoretical modelling – point and vertices of the aperture outline, for example see Urdy et al., 2010.
- aperture ontogeny profiles - functional morphology – for example Figures 6 and 11 in https://peerj.com/articles/329/

- Dissimilarity matrix between shell forms – evolutionary studies - correlation between phylogenetic distance matrix and shell forms dissimilarity matrix of snails

These text have been included in the manuscript.

Cowrie case:
We have checked this issue with our colleague who is familiar with Cowrie and we also checked many Cowrie shells in the collection – Yes, the aperture outlines can be obtained from Cowrie shells even though these aperture outlines are not that obvious. Please see **this rebuttal letter – point 16**.

23. 9) I still think the authors do not provide a data input file (.CVS) for R. They indicate that the data analysis can be evaluated by running these scripts in R, but the source location appears to be the first author's C: drive. This obviously will not work on any other machine. In order to do so, the .CVS files outlined in the R code would be needed, or if they could be directly obtained from the .ply files, please indicate how readers could convert these .ply files to get the .CVS files needed to run your R-script.

The user can change the script to locate the file which may be saved in any location. We don't think this is more than a trivial issue.

No, the CVS is not raw data. See **Rebuttal Letter 1 – point 10. Please see the last five minutes of the video tutorial.**

24. 10) I did appreciate the tutorial video a lot. However it also shows that outlines for the 'inner' parts of the shells are actually not traced, they are estimated/reconstructed. The authors probably want to be more precise with the wording in the text. I do appreciate the time and effort that goes into creating these models, but I doubt that with the amount of tracing rigor displayed in the video you want to openly discredit other methods.

For the tracing stage, we traced the aperture outlines; it does not really matter whether it was traced from outside or inside. After we traced it from the inside, we would take into consideration the shell thickness of the overlapping whorl during the retopologising stage. This statement has been added in the text.

25. In summary, I think the method will likely be valuable for certain applications and that it is worth publishing, but I maintain that the way the paper is framed substantially decreases the value for all reasons mentioned above and in my first review. I doubt that this method will overthrow GM for mollusks or beyond, but at places in the current manuscript this intention/ambition still appears to be what the authors are after. The comparative/competitive mindset (GM and TM) is still very clearly

present in the paper, e.g. the presentation of alleged advantages and disadvantages of methods (see throughout the paper). No harm is done in simply proposing a new method with some advantages and disadvantages. But why would the authors prefer to present a not even half-baked comparative framework with GM that nota bene affects credibility? I really think the authors ought to re-frame their paper, and cut the unsupported discussion and conclusions. After all, it will be up to the scientific community (and not the authors) to decide whether this method will revolutionize malacology.
See above all points.

Second round of Peer Review (#2013:12:1153:1:1:REVIEW)

Comments from the editor (04Mar2014)

1. Having spoken to the more critical of the referees, they appreciate your detailed response to referee comments, but feel that the changes to the manuscript were superficial and insufficient. The fact that this referee felt the original text comes across as condescending is a serious criticism and one that will undoubtedly lessen the impact of your study overall. When I recommended minor revisions, I meant the manuscript needed only a conscientious rewrite of the text to address the referee comments and purge this tone from the manuscript, but the revisions to the manuscript were minimal indeed. The referee points out that the rebuttal letter is extensive, but revisions to the text of the manuscript are superficial in this regard and "do not indicate a sincere effort to respond to the referee comments in the manuscript itself."

2. I agree with that your detailed responses to referee comments helped me to better understand your manuscript, and feel that future readers of the final manuscript would benefit by having some of this information made explicit in the text also. For example, both referees commented on the use of relatively few species for this demonstration, which you have justified by saying you specifically selected shells which covered a broad range of morphological complexity to demonstrate the utility of the approach. However, you do not say that explicitly in the revised text, and I believe that future readers of your manuscript are very likely to have the same response as these two referees to the manuscript. Thus, I feel it is in your best interest to avoid that reaction from readers and provide some additional text from these response comments in the manuscript itself to convince the skeptical reader that they should apply your method. In my experience as editor, some of the most careful and likely interested readers of your manuscript will be the referees, and if they are not convinced enough to try your software on their study organisms, I do not expect it will catch on. The addition of the tutorial video is an excellent touch, but I believe that taking a hard look at your current text and working some of the information from your rebuttal into the final manuscript will result in your work being more widely accepted. Likewise, you have removed the claim of robustness, reproducibility, and versatility from the abstract, but I feel that it would be fine to provide this information in the discussion of your paper if you wanted to include it, and explain explicitly to readers why you are so enthusiastic about this approach in that context as you have done in your response to referees.

3. I know it is painful to repeatedly revise a manuscript that you have completed, but I do not see the referees changing their mind on the suitability of this manuscript without a careful revision of the text. I hope that you will take the time to look at the text of your manuscript as carefully as you have done with your response to referees in the rebuttal letter. I believe that working on the tone of the text in places where the referees reacted negatively to your manuscript, and incorporating some of the information from the rebuttal letter into the text of the manuscript directly to explain your rationale for the

approach, would be a great benefit to your future readers and the acceptance of your approach in the field.

4.

Thorseng Liew <thorsengliew@gmail.com>   4 March 2014 21:23
To: peer.review@peerj.com

Dear Dr. Robert Toonen,

Thank you very much for your advice. We would add those points which are in our rebuttal but were missing in the manuscript.

By the way, I could not find the the said referee's report.

Best,

Liew

5.

Rob Toonen <rjtoonen@gmail.com>   5 March 2014 04:24
To: thorsengliew@gmail.com, Rob Toonen <rjtoonen@gmail.com>

Dear Liew,
    In the interest of saving time to turn this around for you, I did not send it out for a formal review and just called the referee on the phone. I can have them review it if you would like to wait for that, but I assumed you would rather get it back quickly and have the time to work on the manuscript rather than have another review at this point.
    One suggestion I find can be helpful for my own lab when faced with reviews such as this is to search the manuscript for negative words and revise those sentences into a positive statement instead. I find that referees will typically react favorably to the same information presented in a positive statement whereas they react poorly to same information when stated negatively. For example, rather than saying: It is not possible to reconstruct the actual shell form from these quantitative measurements, because the accretionary growth model and spiral geometry cannot be quantified on the basis of arbitrary reference points. You could try something along the lines of: One advantage of our approach is that it is possible to reconstruct the actual shell form, because the shell's accretionary growth model and spiral geometry can be quantified... In this case you imply that the other method is incapable of doing the same thing, but don't say anything negative about it, you just espouse the advantage of your own technique. I find this a useful technique to make your paper more palatable to the people who you are trying to convince...
    Please let me know if I can provide any additional feedback.
Aloha,
    Rob

6.

Thorseng Liew <thorsengliew@gmail.com>   7 March 2014 15:18
To: Rob Toonen <rjtoonen@gmail.com>
Cc: Peter Binfield <pete@peerj.com>

Dear Dr. Rob.

Noted with thanks.

Best,

Liew

Replies to the editor (10Mar2014)

Dear Dr. Robert Toonen,

Thank you very much for your suggestion.

Please find the changes described below in the track-change version of the manuscript.

Below are our replies to your comments.

1. Our paper proposes to provide an overview on the *status quo* of current methods in the study of shell forms, both their strength and limitation (described in the Introduction) and a new method to quantify the shell form (described in the Materials & Method, and Results & Discussions sections). We did not directly compare our method with geometric morphometrics (GM) or other existing methods. We had made a mistake in the first submission and have corrected this in the revised version and in rebuttal letter as below:

   > Previously, we claimed "*The outcome suggests that our method is* **more** *robust, reproducible, and versatile* **than the conventional traditional and geometric morphometric approaches** *for the analysis of shell form*." Now, we have changed it to "*The outcome suggests that our method is robust, reproducible, and versatile for the analysis of shell form.*" We rephrased this statement in the Abstract as well as the Conclusion.

   After we changed the only two sentences that directly compare the GM and our method, such comparison (i.e. our method is better than GM) DOES NOT occur throughout the manuscript.

   a. In the revised manuscript (as in the first submitted manuscript), we mentioned geometric morphometrics (GM) 22 times, 18 of them are in the Introduction where we reviewed the *status quo* of current methods (including GM) in the study of shell forms. In this review, we did not compare our method with GM

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and only point out the limitation of the GM in studying the shell. All the interpretation in the review was based on the references (> 50 papers). Our argument in the review was plain and based on scientific fact. We think our review is fair and not biased toward any existing methods, both strength and weakness of each method were discussed.

b. GM is mentioned once in the Methodology (Worked example), where we say that GM cannot be used on irregular shell forms. This again is the fact, not our opinion. GM is mentioned 3 times in the Discussion where we highlight the limitations of our method as compared to GM (our method is more time consuming and needs more training to read the output).

2. The reviewers' comments could be classified into two categories. One on the clarity of our method and another one on comparison between GM and our method (response to your comment 1).

a. Methodology clarity: Reviewers raised a few good points regarding the technical aspect of our method, for example, how to trace the aperture of overlapping shell whorls. We thought it might be sufficient to have our reply in the rebuttal letter because the peer review history would be published together with the paper. We apologise that we overlooked this. Now, we have clarified all these points in the manuscript (see Materials and Methods).

b. Is our method better than GM? – As we mentioned in point no. 1 above, we do not make any direct comparison between our method and GM. Nevertheless, in the rebuttal letter, we tried to help reviewers to understand the limitation of GM in shell study as compared to our method. Although this is already out of the scope of our study, we decided to spend time on this because we appreciated their comments. Although the reviewers do not respond directly to the point in the rebuttal, we are grateful that the editor (and reviewers) accept our explanations in the letter.

3. We do not make direct comparison between our method and the existing methods for the following reasons (response to your comment 1, 2):

a. The only objective way to compare GM and our method is by comparing their effectiveness in helping scientists to understand the shell form. This evaluation would only be possible when there are more studies using both methods to study the same question (such as ecological, evolutionary, or ontogenetic studies of the shells).

b. We have learnt our lesson in the first round of review on how much damage can be done by a statement such as in the textbox of point no. 1 above. Criticising one method over another is unavoidable in any direct comparison between methods. This could provoke sceptical or prejudiced readers.

c. Hence, we review the strength and weakness of each of the existing methods in the **Introduction**. We also highlight the strength and weakness of our method in the **Results & Discussion**, and **Conclusions, limitations & future directions**. By doing this, we hope that readers make their own judgements based on the plain information that we provided.

4. We do not agree to change the text in the literature review (Introduction) for the following reasons (response to your comment 2, 5):

   a. We would not change the text in the review as it reflects the fact. See also 1(a) and 3(c).
   b. The suggestion of editor in his comments of no. 5: "For example, rather than saying: It is not possible to reconstruct the actual shell form from these quantitative measurements, because the accretionary growth model and spiral geometry cannot be quantified on the basis of arbitrary reference points. You could try something along the lines of: One advantage of our approach is that it is possible to reconstruct the actual shell form, because the shell's accretionary growth model and spiral geometry can be quantified...". We say this in the Introduction which is based on the fact (literature review). We are not sure how could this suggested text fit in. Please see also 1(a), 2(b), & 3(c).
   c. Thank you for your kindness to suggest the change of text in order to attract more readers. However, we are ready for any criticism from readers and for the possibility that our method would not be widely accepted [i.e. has the same fate as the previous published models/methods (lines 105-185)]. We only hope that our paper can make others rethink the issues of shell form quantification (response to your comment 3, 5).
   d. As described in one of the PeerJ policies "*PeerJ evaluates articles based only on an objective determination of **scientific and methodological soundness**, not on **subjective determinations of 'impact,' 'novelty' or 'interest'**.*"

5. We have explained in more details about the (1) robustness, (2) and (3) versatility of our method in the manuscript. (response to your comment 2)

First round of Peer Review (#2013:12:1153:0:0:REVIEW)

Dear Dr. Robert Toonen,

We thank you and both reviewers for having dealt so diligently with our manuscript, and the time invested.

Please find our response to each of the points which were raised by you and the two reviewers. We look forward for your decision.

In addition to this rebuttal letter, please find the attached original manuscript files with tracked changes.

Sincerely,

Liew Thor-Seng and Menno Schilthuizen

Editor's comments

1. Overall, both referees are positive about the manuscript and the work, but both also have serious concerns about the generality of the model for the breadth of questions the authors feel it will address. I feel that the reviewers have done a very good job in this case, and provided constructive criticisms for the improvement of your manuscript, and I agree with their assessments. As you can see from the comments below, they both felt that the range of species, developmental times and environmental conditions under which the model was validated should be expanded, and that the enthusiasm of the authors for the broad applicability of the model is premature. I particularly agree with the statement made by each that the enthusiasm for the model needs to be balanced with carefulness - given this consistency of response, this will certainly be representative of many future readers in the field. Therefore, I would like to see that the authors take the detailed comments of the referees to heart and carefully revise the manuscript to infuse the appropriate degree of cautious optimism for the promise of the model in these "minor revisions."

- Regarding the **generality of the model, however,** we would like to stress that we did not propose any theoretical model, rather we proposed a morphometric method developed specifically for gastropod shells. Thus, the effectiveness of this method in extracting the said variables would not be influenced by any factors that have CAUSED the shell form.

- From the review reports, we are under the impression that part of this misunderstanding may have been due to the fact that reviewers did not evaluate the method by testing and using it although this can be done easily (the whole process takes literally minutes) by importing the retopologised shell models that we used in Blender, and then copying and pasting our script in Blender.

- We understand that Blender may be unfamiliar and complex to many uninitiated readers (and reviewers), so we have added a video tutorial in the supplementary materials.

- **Regarding the (taxonomic) range of the selected species.**
- A wide taxonomy range of species evaluated does not necessarily mean a wide morphological range of shell shapes. The focus of this manuscript is to evaluate the method on shells that have regular as well as irregular shell forms. The range of shell forms that we used in our evaluation do cover a broad diversity of form. We demonstrated that the shell form can be quantified as the same set aperture ontogeny profile, regardless of the unconventionality of shell form. We aware that both reviewers are well-versed in Geometric Morphometric (GM) methodology. May we invite both reviewers to use GM on our selected shells? We would expect that this will result in the realisation that, given the shells' irregularity, GM applied on these shells will not result in meaningful comparisons.

- Having said that, we agree with editor and reviewers that our sweeping claim on the biological interpretation should be toned down, because we had only preliminary data, and we have changed the manuscript accordingly. (See below – Reviewer 2 - on line 580-584). In addition, we have changed the sentence in the Abstract that claims our method is better than conventional methods. Previously, we claimed "*The outcome suggests that our method is **more** robust, reproducible, and versatile **than the conventional traditional and geometric morphometric approaches** for the analysis of shell form*." Now, we have changed it to "*The outcome suggests that our method is robust, reproducible, and versatile for the analysis of shell form."*. We rephrased this statement in the Abstract as well as the Conclusion.

- In addition, we have made the following changes to the manuscript:
    1) We added two supplemental Files which could help the reader to make an easy start in using this method.
    2) We have changed the genus name of three species from *Opisthostoma* to *Plectostoma*, based on our taxonomy paper that has been accepted by *Zookeys*. This is to avoid confusion because of inconsistent name use between different papers. The changes of name do not change any conclusion in the present manuscript.

- Finally, we would like to suggest that many of the criticisms of Reviewer 2 result from the fact that our paper argues against the use of a well-established method (GM) in favour of a novel, yet unfamiliar method. As we stress in the paper, the juxtaposition of these methods is particularly relevant for gastropod shells, because, by their nature (helically coiled tubes), they offer none of the landmarks that are the hallmark of proper GM. We do not wish to suggest that our method should replace GM for all organisms, but for the gastropod shell, we stand by our position.

Reviewer Comments

Reviewer 1 (Ronald Allan Cruz)

Basic reporting

2. The manuscript is generally written well. Sufficient background information and supporting literature are given.

   Just one comment on a statement that you made in lines 191-193: I am not sure that we can say that the data of one study on shell shape is incompatible or incomparable with those of other studies. Perhaps it has not been done before or adequately explored, but there are likely ways to circumvent this and allow for a meta-analysis of sorts? I'm just not certain that the statement should have been made with such conclusivity.

- This issue has been discussed in Klingenberg (2013) in general, and Bocxlaer & Schultheiß (2010), in particular for shells. In brief, no biologically meaningful landmarks can be identified from shells (biological meaningful landmark being the key in GM). This problem is reflected in the varying numbers of landmarks used in different studies. Hence, these landmark data from different studies cannot be analysed together.

Experimental design

3. The researchers are to be commended for making an effort to be as clear as possible in detailing the steps that they had undertaken in their procedure, particularly with the technical details.
   - Thank you. Yet, we are aware that our text descriptions of the methodology

might not be sufficient and that Blender is a quite complex software for beginners. Hence, we added a video tutorial in the Supplementary Materials.

4. My primary concern, however, is with regard to the specimens collected. Why was only one genus, Ophistostoma, used for the study? How many specimens of each species were collected? There may be significant morphological differences even within a species. And since you have made the claim that your methodology can be used across all species, even using data from different studies, was it not important to get many different representatives of the diverse gastropod taxa?

- We understand the reviewer's concerns. However, a wide taxonomic range of species does not necessarily mean a wide morphological range of shell shape. The focus of this manuscript is to evaluate the method on shells that have regular as well as irregular shell forms. The range of shell forms that we used in our evaluation do cover a very large diversity of shell form. And we demonstrated that the shell form can be quantified as the same set aperture ontogeny profile, regardless of the unconventionality of shell form.
- Our method extracts morphometric data from the aperture along the shell ontogeny. These parameters can be obtained from the aperture as long as the shell grows by unidirectional accretionary processes at the aperture. We are not aware of any shelled-gastropod species that do not grow in the way, and hence this method, by its very premises, should apply to all gastropod shells.

Validity of the findings

Just a few comments/questions:

5. 1) In lines 560-564, you point out that comparing radar charts between shells of different sizes is less informative, and that size differences account for certain differences in radar charts even for the same species (O. laidlawi). How then is this an advantage over geometric morphometrics, which through Procrustes transformation significantly decreases (though admittedly does not completely eliminate) size variables? I admit to being rather unfamiliar to most of your procedures, so a clarification regarding this conundrum would certainly be helpful.

Yes, thank you for pointing out this issue. This radar chart is simply an alternative to display

the ontogeny profiles. It does not add information.

6. 2) How do you account for the differences among the dendrogram with all four aperture ontogeny profiles (Fig. 6A) and those of the individual profiles (Fig. 6B)? What do these differences imply about the strength of your methodology?

Please see the "*Procedure 8: Quantitative comparison between shell forms*". We obtained data (i.e. aperture ontogeny profiles) directly from shell apertures. The data is a form of multivariate time series data which can be analysed in many different ways (algorithms). We applied the most recent analysis developed by Brandmaier (2012a & b) because it has an R package that can be applied and can calculate the trend similarity. That said, the same data can always be analysed by other "better" algorithms in the future.

7. 3) Given that you had only used one genus (and I am uncertain as to how many specimens per species you collected and used), can you truly conclusively say that this procedure can be used to compare all shell forms and to integrate/synthesize data from different studies?

See our reply in "**Experimental design"** above.

Comments for the author

8. I would like to say here that I am asking these questions from the viewpoint of an empirical morphologist, one of those specifically addressed by the study as coming from a particular school of thought that does not mesh with the other. In particular, I have been using geometric morphometrics on gastropods. I applaud you for your research and believe that this certainly has enough merit to be published, but I am not thoroughly convinced that your methodology significantly improves upon GM. This is primarily because of my concerns regarding your use of just one genus, but also because of your statement on size differences confounding some of your results. Perhaps there is an adequate explanation from the theoretical perspective, and if so I would be glad to be informed of it. But if the goal of this study is to encourage the communication between the empirical and theoretical perspectives--which I would like to think IS and should be the true goal--then I whole-heartedly agree and congratulate you for a job well done.

We thank the reviewer for his thoughtful and appreciative comments. We understand that it is hard to imagine how this new method can be used to address the old problem of shell quantification. We sincerely hope that the reviewer can assist by hands-on evaluation on

this new method, by using our shell example and the reviewer's own favourite shells (for example *Conus* or *Oliva*). We are quite convinced that this method would generate the same set of ontogeny profiles for *Opisthostoma*, *Conus*, and *Oliva*, and comparison of the similarity between these shells would certainly be possible.

Reviewer 2

Basic reporting

The way the paper is set up in the introduction is out of line with the data, see comments to the authors.

Experimental design

The method is original and well described.

Validity of the findings

Not all conclusions are justified given the data at hand.

Comments for the author

Review of 'A method for quantifying, visualizing, and analysing gastropod shell form' by Liew and Schilthuizen.

9. This paper presents a method for the digital reconstruction of shell shape with the aim to abstract valuable biological parameters in doing so. The methodology proposed by the authors is based on apertural shape and is interesting. Additionally, the method is explained in great detail, which should allow the community to examine and develop the method further in the future. Additionally protocols and codes will be provided in a future supplement, although these are not available for reviewing.

As mentioned in the manuscript these are available at
http://dx.doi.org/10.6084/m9.figshare.877061

10. Hence, I feel the methods described in this paper are very valuable and definitely worthwhile publishing. However, reading the manuscript in detail, many aspects remained unclear to me, and the text and figures can be much improved to better benefit the community.

Related to the method itself: part of the procedure is performed in the 3D software Blender with Python scripts, then data is saved as .CVS files for analysis in R. However, the final .CVS file is not provided according to the list of supplemental material (lines 655-676). So, am I correct then that the R input files are lacking and that the R-script cannot be run without the data? If so, please provide .CVS files in supplement too.
Indeed, we did not include the .CSV files because these are not the **raw** data. The raw data are actually the retopologised shell models. It is easy to generate raw data by simply importing the retopological shell models that we used in Blender, and then to copy and paste our script in Blender. After that, the rest of the data analysis can be performed in R (by opening the R script in R and clicking "run"). The whole process would not take more than 5 minutes.
We are aware, however, that our text descriptions of the methodology might not be sufficient, especially given the fact that Blender is quite a complex software package for the beginner. Hence, we have added a video tutorial in the Supplementary Materials.

11. The vital aspect of the entire enterprise is to reconstruct aperture shape and coiling parameters along or from the ontogenetic series that a gastropod represents. This is performed by feeding a 3D shell model into Blender. In Blender then aperture outlines are traced using growth lines, etc. These growth lines and their relative position of one to the next will then be the focus of the rest of the procedure. This makes me wonder about data quality: sure, tracing growth lines on the outer surface of the shell will be feasible, so the outer part of the aperture will not cause too much problems, however, what about inner part of the whorls (the columellar part), where in essence earlier shell layers are covered with later layers that hide any direct indication of the previous position of the aperture? Text should be provided as to how the authors dealt with this.
Indeed, as the reviewer recognises, a good understanding of the aperture outlines of the

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shell along the ontogeny is the key for this method. The 3D model is simply a guide that could facilitate this process (See Conclusion). One of the ways to deal with the situation described by the reviewer is to trace the aperture at the inner shell wall and the obscured aperture outline can then be inferred by studying the conspecific juvenile specimens. The video tutorial we have provided demonstrates how this can be done (see video tutorial 05:00 – 08:00).

13. Second, figure one seems to indicate that apertures are 2-dimensional, but often in gastropods the aperture twists in another plane making it a 3-D structure. How have the authors dealt with this? Are the apertures of Opisthostoma 2 dimensional, and if not, how do the authors believe a 2D EFA analysis affects their analysis of 3D shape change? No information is provided in the text. Third, have the authors performed robustness tests, like having two people trace the apertures of the same 3D shell model (or one person with a time lapse between the first and second tracing of the same shell?). Performing such tests is really necessary before claiming robustness and reproducibility, but I fear it has not been done (in any case the issue is not addressed in the manuscript).

We agree with the issue raised by the reviewer. In fact, we would like to refer the reviewer to lines 372-373, where we state: "For aperture form analysis, we used 3D normalised EFA algorithms (Godefroy et al., 2012) and implemented these in the custom python script."

We agree that the key to accurately trace the aperture outlines is good understanding of the way the aperture is structured. Poor tracing means poor understanding of the aperture growth on the shell. However, this is not really an issue caused by our methodology, and applies to other methods just as well. Perhaps, the reviewer's comment is prompted by the fact that this is an issue in GM. However, in our method, the orientation of the shell when the aperture is digitalised would not influence the aperture ontogeny data.

14. The 'quantitative comparison between shell forms' is confusing to me. What is exactly meant with lines 436-444? On the first line all data was combined in a three-dimensional data matrix, whatever that is (probably the authors mean with 2 different sets of variables). However on the next line they have five matrices, and I don't understand what happens after the ':' in that sentence.

See our reply to your comments on Figure 6.

15. The authors see great benefits in their method of 'quantifying' shells for a whole number

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of reasons, including taxonomy, evolutionary biology and functional morphology. However, at no place in the manuscript is this enthusiasm balanced with carefulness. Shells are formed with many constraints and tradeoffs, biological growth is often unequal through time: when times are good, growth is fast, when things turn bad, growth rates etc., and shells display much plasticity, e.g. related to environmental conditions, related to habitat, etc. Upon attempting the biological interpretation of shape data these concerns will need to be considered.

For example on line 580-584 they suggest that aperture size is periodically diminished in all of the 4 actual specimens that were documented (manipulations of the same data don't count here). They are eager to suggest that this is diagnostic for the genus, and directly conclude that this could aid taxonomists in grouping decisions. However, the changes could equally have been caused by external factors, but this is not discussed. Even if diminished aperture size would be an intrinsic feature of Opisthostoma (which would be hard to tell after the analysis of 4 specimens only), nothing suggests other closely related groups don't have the same feature (hence precluding its diagnostic value). So many more specimens would need to be analysed to reasonably confirm the hypothesis the authors make. This would also be the case for many evolutionary questions, and it poses a bit of a problem, given that collecting of these data is very time-consuming, so how realistic would it be to develop the method for a broad range of taxa, for multiple populations? Hence, I would advise the authors to be more careful in their conclusions, though I agree with the authors that it is always better to have (and report) more data than less.

In reply, we would like to address the taxonomic issue first. We apologise for the confusion because we didn't explain this properly. We have rephrased this part to:

 "In fact, these aperture size decreases during ontogeny are in accordance with the shell constriction, one of the shell characters that have been used in the taxonomy of *Opisthostoma* and *Plectostoma* (Vermeulen, 1994; Liew et al., 2014). However, the shell constriction has not been quantified previously, and we show that it could also be an important developmental homology for the two genera. This preliminary results suggest that This suggests that the constriction in aperture size profile is a diagnostic character for the genus *Opisthostoma*. In the light of this example, we believe that these aperture ontogeny profiles could aid the taxonomist in decision-making for grouping taxa based on homologous characters." We hope it is clearer now.

Second, with regard to evolutionary biology and functional morphology:

We indeed are very enthusiastic about the potential of this new method, which we feel needs not be toned down, since the retopologised shell models and dissimilarity matrix that are generated from this method comply with the data standard that is required in these two fields.

16. Many of the claims that the authors make vastly overshoot the available data. For example, the main conclusion of the abstract: 'the outcome suggests that our method is more robust, reproducible, and versatile than the conventional traditional and geometric morphometric approaches for the analysis of shell form' remains unsubstantiated. No comparison between the 'new' method and conventional traditional and geometric morphometrics in terms of robustness, reproducibility or any other aspect has been provided. We agree we may have been overenthusiastic. We have therefore changed the sentence in the Abstract as well as the Conclusions. Previously, we claimed "*The outcome suggests that our method is **more** robust, reproducible, and versatile **than the conventional traditional and geometric morphometric approaches** for the analysis of shell form*." Now, we have changed it to: "*The outcome suggests that our method is robust, reproducible, and versatile for the analysis of shell form.".* We rephrased this statement in the Abstract and the Conclusion. Thank you very much for reminding us about this issue. We agree that we should wait and let future tell whether our proposed method will be welcomed.

If you allow us, we would like to point out the following:
The philosophy, data, and results of GM and our method are all different. Thus, we are not quite sure how the data from the two methods may be compared. Nevertheless, we explain briefly here what we mean by **robustness, reproducibility, and versatility**.
Robustness – The same aperture profiles can be obtained from any form of shell. Then, these profiles from different shells and/or different studies can be analysed together. These parameters can be obtained from the aperture as long as the shell grows accretionarily at the aperture. On the other hand, studies that use GM for different shells, species, and/or studies do not use the same set of the landmarks because the "same" or "homologous" landmarks do not exist in shells – see lines 174 – 203.
Reproducibility – Aperture ontogeny profiles were obtained from the aperture outlines. This is a trait that exists in every gastropod shell. We believe that the aperture outline that is obtained by multiple experienced malacologists, on different shells, would be highly similar.

On the other hand, GM is heavily dependent on the accuracy of the landmarks that are taken from the object. As there are no biologically meaningful landmarks in shells, the "same" landmarks need to be taken from the shell that is fixed into a certain orientation.

Versatile – data generated from this method can be used for studies with different purposes. See above.

Beyond the above issues with the method I have major issues with how the paper is set up, mainly the introduction (the tension between modeling and morphometrics). I presume the writing style was chosen to add significance to the results, but it seriously detracts from the methodological aspects of the paper and devaluates the significance of the analyses in my opinion. I summarize my concerns on this below.

17. 1) The authors appear to fail and appreciate the different philosophy behind shape modeling and morphometrics and in the introduction, starting with the section 'Empirical and theoretical approach in the study of shell form' (and it should be 'approaches', not 'approach') they set up a false equivalency of both methods (see the sections starting on line 81 and 153; specific statements are for example that 'the theoretical approach faces competition from geometric morphometric methodology'). However, both approaches are philosophically very different: early shell-generating models were created for abstract purposes, namely to build models that can generate all existing morphologies within the Mollusca, and then to create a theoretical morphospace and study how living and fossil organisms occupy this morphospace. Subsequently, people have refined these models to try and reconstruct shell shape e.g. of gastropods accurately. The goal there is reproduction. Even though these shifting goals in shape modeling they have never overlapped with those goals of morphometric studies where a large number of specimens is studied to get an insight into variability within and between populations and species. These two sets of goals are very, very different things, and each set of methods is suitable for its goals, but not those of the other set of methods. However, the authors apparently feel it appropriate to ignore these differences entirely.

Yes, the brief history is correct. However, our aim in writing the Introduction in the way we did was **the goal of reproducing the shell form** Although a few cases exist where shell form is reproduced for aesthetic purposes (e.g. Fowler et al, 1992), in our opinion most of the studies reproduce the shell form in order to understand how it evolves. Many of the studies use theoretical methods to reproduce the shell form and then examine the variation between them. They asked what is the developmental constraint (Urdy et al, 2010), the geometric

constraints (e.g. Okamoto, 1988; Stone, 1996), or the functional constraints (e.g. Okajima and Chiba, 2011,12), just to name a few, that determine the shell forms. To our mind, these objectives are identical to those of "morphometric studies".

18. 2) The authors reduce the continued efforts to create standardized methods in morphometrics (which increase transparency and reproducibility) as a mere 'gift' of mathematicians/programmers to keep biologists that are unskilled in these fields on track. Moreover, they fail to note that there is a whole suite of gastropod modeling applications where a number of parameter values can be provided after which the program spits out associated shell shape (see Raup's Coiler). I would advise the authors to get rid of these misinterpretations because some readers will find that lines 154-173 have a condescending undertone and they may give up on reading the paper here.

We understand the reviewer's sentiments. We did not mean to sound condescending and would like to apologise to the reviewer and to future readers if this impression were inappropriately conveyed. Nonetheless, we have decided to retain our original wording for the following reason. We aware of Raip's Coiler, and of similar models such as Noshita (2010). However, none of these models would be able to generate the shell shapes that we have examined in the current paper.

19. 3) The authors make several highly erroneous claims on traditional and geometric morphometrics. For example lines 174-177: GM allows us to decompose shape in a standardized way to a multivariate data matrix which we can use for further statistical analyses, it is not a statistic of shape.

We admit to being puzzled by this remark. As far as we are aware, the analytical steps in GM consist of: 1) obtaining landmarks; 2) standardisation - superimposition of landmarks (e.g. by Procrustes method; 3) creating SHAPE variables (e.g. by PCA); 4) Analysis and comparison of shape variables between groups (e.g. ANOVA).

The shape variables are described as PCA component scores. So, we are unsure why the reviewer is of the opinion that GM is not a statistic of shape. Cardini and Loy (2013) also state that GM is a statistical shape analysis.

20. Lines 178-186: this section touches perhaps on difficulties of applying morphometric methods to gastropods, but it is not a reason why geometric morphometrics are 'not particularly suitable for comparison and quantification of shell form'. This is a complete

misrepresentation, morphometrics are especially well-suited for shape quantification and comparison. In fact the method used by the authors heavily relies on morphometrics. We would like to clarify again that the key point here is that GM depends on good quality landmarks. Unfortunately, given the fact that a gastropod shell is basically a helically coiled tube, such landmarks simply do not exist on a gastropod shell. See the examples that we used in this study.

21. Lines 187-193: This section really needs to be deleted. Of course data from several studies can be integrated, but the data need to be reanalyzed all together. The raw shape coordinates of two independently-collected morphometric datasets can be compared with two datasets of unaligned DNA fragments. If the datasets are collected in comparable ways (e.g. using the same landmarks or the same DNA primers) they can be combined, but that does not mean you can just analyse separate parts and then throw everything together. Like DNA fragments that need to be aligned in both datasets in the same way, all morphometric data for an analysis need to be aligned in one procedure (standardized for rotation, size and position).

We would like to clarify again that the key point here is that GM depends on good quality landmarks. Unfortunately, given the fact that a gastropod shell is basically a helically coiled tube, such landmarks simply do not exist on a gastropod shell. See the examples that we used in this study. We would like to try to explain our point with the same DNA analogy. Two fragments of DNA sequence can be aligned if there are evolutionary homologies in the sequence (for example, can be obtained by using the SAME primers). First, there are no evolutionary homologies that can be defined as landmarks on a shell, since the helical coiled tube offers no points that can be fixed across different individuals. Thus, the raw data (coordinate) from two studies can not be combined if they use different landmarks. Second, the shape variables (e.g. PCA scores) from a study cannot be compared and analysed together with other studies. The PCA scores of 1.025 in one study is NOT comparable to the value of 1.025 of any other study.

22. Line 201-203: this is wrong. In fact, analysing shell shape altogether will often blur the specific signal of interest in other components of variation, and hence produce a less clear picture about the functionality of one certain aspect compared to other aspects. This pitfall of the whole-evidence approach that the authors propose is not being reported in the paper (though it should be). In morphometrics particular signals can be easily swamped, because

31

different types of variation can interact with each other. As an example let's assume that we are studying a snail that is ornamented (with ornamentation that alters the aperture outline when it is formed) and has adult modifications of the aperture (like many have), and we want to document how strongly the adult modifications affect aperture shape. IF we compare the adult aperture with one juvenile aperture, the difference will be large, no matter which juvenile aperture has been taken, but if we compare it with 100 juvenile apertures, the adult aperture may fall in the overall range of variation of juvenile apertures, not because there are no adult modifications but because the range of variation caused by ornamentation, allometry and other factors together is so large that it swamp the signal of adult modifications. Simpler methods may recover the signal with less noise, and hence be better suited to address the particular question at hand.

We are confused by these comments. When read together with the preceding sentence, lines 201-203 say: "**Clearly, in addition to quantitatively compare shell forms, biologists want to know more about the general characteristics and physical properties of the shell form that are key elements in gaining insight into functional and ecological aspects of the shell (Evans, 2013). However, functional and ecological aspects of shell form can only be determined if the shell form can be exactly quantified**." We meant to say that **if the shell form can be exactly quantified** and NOT "**if the differences between shell forms can be exactly quantified**." We suggest the reviewer may have misread or misinterpreted this section.

23. 4) The fact that modelling and geometric morphometrics are not direct alternatives for the same purposes becomes clearer in the discussion and with this the earlier over-exaggeration of the usefulness of the approach taken is exposed. The discussion and conclusions clearly describe the new method as time-consuming and tedious compared with traditional and geometric morphometrics (for example, line 502-503 and 645-646). Given that it takes months to produce 'conventional' morphometric datasets on thousands of specimens, analyzing the same number of specimens with the here proposed approach becomes next to impossible because of time constraints and probably an endeavor of mixed success giving that specific signals may get swamped in the large amount of data generated..
(See also our reply for (1).)
We would like to emphasise here that there are no differences in the time required for data analysis between GM and our method. The only time differences are in the data **acquisition**. From our experience, two to three days are needed to collect the aperture data from the

shell. In our case, we aligned a total ca. 1500 points for each shell (ca.16 points for each of the ca. 90 apertures, see also lines 307-308). From the viewpoint of short-term cost-benefit balance, this may be seen as a waste of time, because GM requires not more than a few dozen points for each shell, which can generate the shape variables for within a study, even though these points are not comparable to other points of other shells or other studies. However, in the long run, it is a good time investment, since it will allow the understanding of shell function, growth, and evolution, as the same set of data is obtained from different shell forms and can be accumulated and analysed together. Moreover, as will all newly-developed techniques, improvements in efficiency and automation are possible and may remove these impediments in the future.

24. 5) Given all the above, and many internal repetitions, lines 13-203 would need to be strongly rewritten, or perhaps better deleted altogether. The rest of the paper would benefit from being modified to clarify issues and avoid some of the pitfalls outlined.
Given our replies to the reviewer's comments, as well as the fact that Reviewer 1 writes that **"The manuscript is generally written well. Sufficient background information and supporting literature are given"**, we have decided to stand by our position and keep the text as is (see also our replies to the Editor above).

Minor issues:
25. Line 306: please explain what these NURBS surface circles really are. It is difficult to follow why we need these?
This is explained in lines 327-332.

26. Line 510: that values have a unit does not make the biological interpretation much more straightforward. Say we measure 2 shells: one is 3.545 mm high, the other 3.497 mm (each with a standard deviation). Now on face value: what does the difference mean? Besides, any of this can be reconstructed in morphospace too, where say moving one unit along the first axis can represent a 0.09 mm change in a certain shell feature.
We would like to refer to, e.g, Okamoto 1988; Harary and Tal, 2011; Lewiner et al., 2005. We explain the meaning of the values in lines 355-362.
Please see also our reply to your comment of Line 618-625.

27. Line 512-513: The PCA score may change but should stabilize with more elements indeed. The reason is not that the differences between A and B chance by adding C, D, and E to the dataset. The reason is that the dimensions of the morphospace change somewhat and hence therefore the representation of A and B in components of the morphospace. However, if a strong biological trend exists in the data it will remain present and strong.
Yes, PCA scores is simply a statistic. See also our reply in (3).

28. Line 520-528: this is because size and shape are analysed independently
Agreed.

29. Line 540-541: this is hardly an analysis of robustness and I presume this is one of the sentences that inspired the main conclusion in the abstract. The curves in Fig 4 look similar, but still 4F does not present 2 exact series of 4D following each other. I doubt this proves anything, and it certainly does not devaluate geometric morphometrics.
4F is series of 4D. Again, GM cannot be applied since it is impossible to place the required landmarks on these two shells.

30. Line 593: I don't agree with the three groups in Fig 6A, I see a polytomy.
A dendrogram generated by a similarity (dissimilarity) matrix will not generate polytomies. The two consecutive dichotomies are actually there, but they are close together..

31. Line 618-625: size and volume are different things and should not be used as synonyms in a biological text. As linear measurements in an organisms increase with the power of one, surfaces (like muscle attachments) follow a power two, and volume a power three. Given that this is a paper on morphology, I think accuracy in such issues is key. There are a few other passages in the text where this issue plays and should be changed too.
We understand that this confusion stems from the way we define shells. We would like to refer to a text in another manuscript that we are currently preparing:
"From the developmental point of view, a snail should grow a shell in which its entire soft body can fit when the snail withdraws into the shell. From functional and developmental points of view, shell volume is a more accurate size measurement than linear dimensions, such as shell height and width (see also Gould, 1984). The conventional linear measurements are extremely effective for size comparisons between similarly-shapes

shells. However, the linear measurements have limitations when comparison is made between shells that are different in shape. For example, shell height comparison between a discoidal shell and a fusiform shell tells very little about size differences because the dimensional measurements are tied to a shell shape that results from a different coiling strategy."

32. Lines 632-638: needs to be deleted or written more carefully, as explained above.
We have to say we do not agree. See above.

33. Fig. 1. The authors indicate in the manuscript that they don't deal with ornamentation here. However, it appears they also do not deal with the earliest whorls (compare A and F). Why is this?
As noticed by the reviewer earlier in his/her report, the essential aspect of our method is to quantify the aperture which is determined by accretionary growth of the shell. The earliest whorls form the protoconch, which is an embryonic shell that may not grow accretionarily and usually has no growth lines.

34. Fig. 3. In F thickness appears to be added uniformly, but usually shell thickness is not constant throughout ontogeny.
Yes, that is true. We used the "build" function of the software, which can only "solidify" the model by uniform thickness. However, if necessary, it is possible to write a custom Python script to add the desired thickness to the shell.

35. Fig. 5. Could uniform scales be used in the radar plots? For example, the size axis jumps from 1.24 to 3.33 in units of 0.47, 0.47, 0.48, and 0.67. This issue complicates an already complex plot.
Thank you very much for pointing out this. We made a mistake here: **3.33 should be 3.13** We have now corrected the scale.

36. Fig. 6. The branches in A and B ought to be the same, but they are not. Please make A and B consistent. Also in A there is a polytomy, but it does not exist in B anymore. What is going on here?
No, the branches of A and B can differ. For A, the dendrogram was based on the similarity

between species that was calculated from all FOUR aperture ontogeny profiles. For B, the similarity was calculated from EACH of the four similarity profiles. There was no polytomy in the dendrogram. A polytomy would not be expected in a dendrogram that is generated from a similarity (dissimilarity) matrix by cluster analysis.

37. Fig. 7. It is difficult to see the occupation of points in space in the 3D plot. First of all the plot is probably close to orthogonal, but to see this clearly the page needs to be turned upside down. Also, I would label all 3-axes out of one corner. Thirdly, I would add vertical lines underneath each point to hint towards the exact position. As alternative to doing the changes, the authors may decide to delete the 3D plot, because the three panels on top provide the same insight in 2D views.

Thank you for the suggestion. We removed the 3D plot.

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Introduction

Empirical and theoretical approaches in the study of shell form

The external form diversity of organisms is the most obvious evidence for their evolution, and thus is a key element in most branches of biology. The Molluscan shell has been a popular example in morphological evolution studies because it is geometrically simple, yet diverse in form. The shell form is controlled by the shell ontogenetic process, which follows a simple accretionary growth mode where new shell material is accumulatively deposited to the existing aperture. The evolution of shell forms has been studied either by using empirical approaches that focus on the quantification of actual shell forms or by using theoretical approaches that focus on the simulation of shell ontogenetic processes and geometric forms.

Notwithstanding the active development in both empirical and theoretical approaches to the study of shell form, there has been very little integration between both schools. For the empirical approach, the quantification methods of shell form have evolved from traditional linear measurement to landmark-based geometric morphometrics and outline analyses (for an overview see Van Bocxlaer & Schultheiß, 2010). At the same time, for the theoretical approach, the simulations of shell form have evolved from simple geometry models that aimed to reproduce the form, to more comprehensive models that simulate shell ontogenetic processes (for an overview see Urdy et al., 2010). Hence, each of the two approaches has been moving forward but away from each other, where synthesis between the two schools of shell morphologists has become more challenging.

In empirical morphological studies, shell form, either in terms of heights and widths in traditional morphometrics or in terms of geometry of procrustes distances in geometric morphometrics, is quantified by a set of homologous reference points or landmarks on the shell, which can be easily obtained from the fixed dimensions of the shell. Thus, both methods could abstract the shell form in terms of size and shape of the particular shell dimensions, and the between-sample variation of shell size and shape can be assessed (in most cases only within one study). On the other hand, it is not possible to reconstruct the actual shell form from these quantitative measurements, because the shell's accretionary growth model and spiral geometry cannot be quantified on the basis of arbitrary reference points or fixed dimensions (Stone. 1997).

2

61 Nevertheless, the traditional and geometric morphometric methods have been accepted widely as

62 standard quantification methods for shell form in many different fields of research.

63

64 In contrast to empirical morphometrics in which the aim is to quantify the actual shell,

65 theoretical morphologists focus on the simulation of an accretionary growth process which

66 produces a shell form that is similar to actual shells. This field was established with the

67 theoretical shell model of D.M. Raup (Raup, 1961; Raup & Michelson, 1965). Within the first

68 two decades after these publications, only a few different versions of shell models were proposed

69 (e.g. Løvtrup & von Sydow, 1974; Bayer, 1978; McGhee, 1978; Kawaguchi, 1982; Illert, 1983).

70 The subsequent two decades, thanks to the popularity and power of desktop computing, many

71 more theoretical shell models were published (e.g., Savazzi, 1985; Okamoto, 1988; Cortie, 1989;

72 Ackerly, 1989a; Savazzi, 1990; Checa, 1991; Fowler et al., 1992; Illert & Pickover, 1992; Checa

73 & Aguado, 1992; Cortie, 1993; Savazzi, 1993; Rice, 1998; Ubukata, 2001; Galbraith,

74 Prusinkiewicz & Wyvill, 2002). Finally, we saw further improvements in the published

75 theoretical models in recent years. These recent models simulate shell forms that more accurately

76 resemble actual shells because of improved programming software, better algorithms, and 3D

77 technology (e.g. Picado, 2009, Stępień, 2009; Meinhardt, 2009; Urdy et al., 2010; Harary & Tal,

78 2011; Moulton & Goriely, 2012; Moulton, Goriely & Chirat, 2012; Faghih Shojaei et al., 2012;

79 Chacon, 2012). Here, we will not further discuss the details of the at least 29 published shell

80 models, but refer to the comprehensive overviews and descriptions of these models in Dera et al.

81 (2009) and Urdy et al. (2010).

82

83 In brief, the latest theoretical shell models are able to simulate irregularly-coiled shell forms and

84 ornamentations that resemble actual shells, whereas the earlier models could only simulate the

85 regular and general shape of shells. The major refinements that have been made during the

86 almost five decades' development of theoretical shell models are the following modifications of

87 the algorithm: 1) from a fixed reference frame to a moving reference frame system; 2) from

88 modelling based on numerical geometry parameters to growth-parameter-based modelling (e.g.

89 growth rates); 3) from three parameters to more than three parameters, which has made fine-

90 tuning of the shell simulation (e.g. aperture shape) possible. The key element of the theoretical

91 modelling of shells is the generation of shell form by simulating the aperture ontogeny in terms

3

92   of growth trajectory and form along the shell ontogeny. Hence, this has an advantage over the

93   empirical approach in the numerical representation of the shell geometry form in terms of the 3D

94   quantification and the actual shell ontogenetic processes.

95

96   Since the empirical and theoretical researchers studying shell form with two totally different

97   quantification methods, our understanding of shell evolution cannot progress solely by using

98   either empirical morphometrics or theoretical models. Ideally, theoretical models need to be

99   evaluated by empirical data of shell morphometrics, and, vice-versa, empirical morphometric

100 methods need to be improved to obtain data that better reflect the actual shell form and

101 morphogenesis which can then be used to improve the theoretical models. In this dilemma lies

102 the central problem of shell form quantification and it urgently needs to be addressed in order to

103 integrate and generalise studies of shell form evolution.

104

105 **Why empirical morphologists rarely use theoretical shell models**

106

107 Despite the fact that, since the 1980s, manyshell models have been published that are more

108 complex and versatile, the first theoretical shell model of Raup still remains the most popular.

109 There were many attempts by empirical morphologists to use the original or a modified version

110 of Raup's parameters to quantify natural shell forms (e.g. Raup, 1967; Vermeij, 1971; Davoli &

111 Rosso, 1974; Graus, 1974; Kohn & Riggs, 1975; Newkirk & Doyle, 1975; Warburton, 1979;

112 Cameron, 1981; Verduin, 1982; Ekaratne & Crisp, 1983; Saunders & Shapiro, 1986; Tissot,

113 1988; Foote & Cowie, 1988; Johnston, Tabachnick & Bookstein, 1991; Emberton, 1994; Clarke,

114 Grahame & Mill, 1999; Samadi, David & Jarne, 2000). Surprisingly, all the other shell models,

115 many of which produce more realistic forms, have received very little attention as compared to

116 Raup's model (see e.g. Savazzi, 1992; Okajima & Chiba, 2011; Okajima & Chiba, 2012, for

117 exceptions). This ironic situation might be explained by the elegance of Raup's model that is

118 intuitively and mathematically simple to be used by empirical morphologists (mostly biologists),

119 with limited mathematical and programming experience.

120

121 As discussed above, most of the theoretical models can simulate a shell that has a form

122 resembling the actual shell in a realistic 3D geometry, based on shell ontogeny processes. In

123 contrast, empirical morphometrics can only quantify and compare certain dimensions of actual

124 shells. Clearly, the theoretical approach is better than the empirical approach in its accuracy of

125 shell form quantification, because accurate morphological quantification is essential for

126 functional, ecological and evolutionary studies of shell form. Below, we identify and discuss a

127 few impediments that currently prevent empirical morphologists from adopting the theoretical

128 approach in shell form quantification.

129

130 First, the requirement of a computation resource was an impediment in the past. These

131 theoretical models may only be implemented in a computation environment. As mentioned

132 above, the advances of computation hardware in speed and 3D graphic technology have

133 promoted the development of more complex theoretical shell models. For example, the current

134 speed and storage of a desktop computer is at least four orders of magnitude greater than those

135 used by Cortie (1993) only two decades ago. Clearly, the computation hardware is no longer an

136 impediment (e.g. Savazzi, 1995) for the application and development of theoretical shell models.

137

138 Notwithstanding the hardware development, programming skills are still a prerequisite for the

139 implementation of theoretical models. Many of the early models that were published between the

140 1960s and 1990s, used third-generation programming languages such as Fortran and C++, which

141 essentially lack a graphic user interface. This situation has improved now that the simulation of

142 theoretical shell models can be done in fourth-generation programming languages such as

143 Mathematica (e.g. Meinhardt, 2009; Noshita, 2010; Okajima & Chiba, 2011; Okajima & Chiba,

144 2012) and MATLAB (e.g. Boettiger, Ermentrout & Oster, 2009; Urdy et al. 2010, Faghih

145 Shojaei et al., 2012). Most of these shell models were described with intensive mathematical

146 notation, at least from a biologist's point of view, in the publication; and some of these were

147 published together with the information on algorithm implementation. However, the actual

148 programming codes are rarely published together with the paper though they may be available

149 from the authors upon request (but see Meinhardt, 2009; Noshita, 2010; Okajima & Chiba,

150 2011). Only one theoretical modelling software package based on Raup's model has a graphic

151 user interface that is comparable to contemporary geometric morphometric software (Noshita,

152 2010). Thus, the rest of the modern theoretical models are far less approachable than the

153 morphometric software for empirical morphologists. This is because those advanced theoretical

5

154 models have not been delivered in a form that allowed empirical morphologists to have "hands-

155 on experience" with them, without extensive mathematical literacy (Savazzi, 1995; McGhee,

156 2007).

157

158 Second, theoretical shell models simulate the shell form based on the input of a set of

159 parameters, which could be non-biological or/and biologically meaningful. Non-biological

160 meaningful parameters are counter-intuitive for empirical morphologists because these

161 parameters are not extrinsic shell traits. Nevertheless, many of these non-biological parameters

162 are required for the model to fit the shell form schematically (Hutchinson, 1999). When the

163 biological parameters do represent shell traits, they are often difficult to obtain accurately and

164 directly from the actual shell because of the three-dimensional spiral geometry (Cain, 1977;

165 Ackerly, 1989a; Ackerly, 1989b; Okamoto, 1988; Schindel, 1990; Checa & Aguado, 1992,

166 Hutchinson, 1999; McGhee, 1999). Since the development of theoretical shell models, almost all

167 simulated shell models have been made by an ad hoc approach, where the parameters are chosen

168 for the model and then the simulated shells are compared with the actual shells. In almost all

169 cases, the correct parameters are chosen after a series of trial-and-error, and the parameters are

170 selected when the form of the simulated shell matches the actual shell. Okamoto (1988)

171 suggested that this ad hoc approach based on pattern matching was easier than obtaining the

172 parameters empirically from the shell.

173

174 Third, although the overall forms of the simulated shells resemble the actual shells, the simulated

175 shell is not exactly the same as the actual shell (Kohn & Riggs, 1975; Goodfriend, 1983). For

176 many models, its original parameters are not sufficient to simulate the shell form exactly

177 (Schindel, 1990; Fowler, Meinhardt & Prusinkiewicz, 1992). These simulated general shell

178 forms are adequate for theoretical morphologist interests in their exploration of general shell

179 forms. However, the subtle features on a real shell or the subtle differences among different shell

180 forms of real species that cannot be simulated by theoretical models may have significant

181 functional implications that are important for empirical morphologists.

182

183 In brief, it is clear that the implementation of current theoretical shell models is less accessible to
184 empirical shell morphologists. Yet, empirical morphologists are using traditional and geometric
185 morphometrics as a routine method for shell quantification.

186

187 **Why empirical morphologists use traditional and geometric morphometrics**

188

189 In addition to the impediments arising from the theoretical shell model itself that are limiting its
190 popularity among empirical morphologists, the theoretical approach faces competition from
191 geometric morphometric methodology. The popularisation of desktop computing that led to the
192 flourishing of theoretical shell models in the late 1980s, also promoted the development of
193 morphometric methods, such as Elliptical Fourier Analysis (EFA) and geometric morphometrics
194 (GM). Rohlf and Archie (1984) set a benchmark for the quantification of an organism's form by
195 EFA, which was improved from Kaesler and Waters (1972) and Kuhl and Giardina (1982). Rohlf
196 and Slice (1990) and Bookstein (1991) developed a complete standard protocol for GM. Soon
197 after these pioneer papers, various software with Graphic User Interface (GUI) were developed
198 for the application of EFA and GM (Cardini & Loy, 2013, see http://life.bio.sunysb.edu/morph/).
199 In contrast to the application of theoretical shell models, an understanding of mathematics and
200 programming languages is not a prerequisite for the user of these morphometric tools. Thus, EFA
201 and GM have been well received by biologists, and have been adopted in the morphometric
202 study of shell form.

203

204 These geometric morphometric software packages have standard and interactive workflows that
205 help empirical morphologists in every step of: obtaining morphometric data (e.g. placing
206 landmark coordinates), analysing data (e.g. procrustes superimposition), statistical analysis (e.g.
207 ANOVA, PCA), and visualising shape and shape changes (e.g. thin-plate spline, PCA plots).
208 This has made geometric morphometrics approachable and attractive to empirical morphologists,
209 who want to examine the similarities and differences among shell forms.

210

211 Geometric morphometrics is actually a statistic of shape that is calculated from Cartesian
212 coordinate data from a sample of objects (Cardini & Loy, 2013). However, it is not an exact

7

213 quantification of form and is not particularly suitable for comparison and quantification of shell

214 form, for the following two reasons.

215

216 First, GM analysis is based on homologous landmarks on the form, but shell has only arbitrary

217 landmarks because it has a low degree of morphological complexity (Van Bocxlaer & Schultheiß

218 2010). There are no evolutionary homologies that can be defined as landmarks on a shell, since

219 the helical coiled tube offers no points that can be fixed across different individuals. In most

220 cases, 2D landmarks are chosen at the shell apex, suture, and aperture or whorl outline that can

221 be identified from a 2D image that is taken in standard apertural view of a shell. These

222 landmarks are chosen to be analysed by GM but these points have little biological meaning.

223 Furthermore, as opposed to the form of many other organisms, 3D landmarks are even more

224 difficult to be obtained from a shell (3D model) as compared to 2D landmarks because many of

225 these landmarks, such as suture points, that are obtained from a 2D image are just artefacts of the

226 fixed 2D view of the shell.

227

228 Second, the results of separate, independent studies of shell forms cannot be integrated, even

229 though these studies use the same GM method. Statistical analysis of the Cartesian coordinate

230 data that abstractly represent the shell form is adequate in quantifying the variation of a shell

231 within a context of other shells that are included in a single study or within similar taxa where

232 similar landmarks are obtained. However, the raw coordinate data and analysed shape variation

233 from a study are incomparable and incompatible with the data from other studies (Klingenberg,

234 2013). For example, the raw data (coordinates) from two studies cannot be combined if they use

235 different landmarks and the shape variables (e.g. PCA scores) from a study cannot be compared

236 and analysed together with other studies.

237

238 Despite the fact that geometric morphometrics has been widely used by empirical morphologists,

239 it is not an ideal tool in the quantification of shell form for the reasons given above. The

240 increasing availability of the software and application in the literature might cause morphologists

241 to stray away from their initial aims of studying shell form. Hence, it is important to return to the

242 core of the question: what do biologists want to learn from the study of shell form? Clearly, in

243 addition to quantitatively compare shell forms, biologists want to know more about the general

244 characteristics and physical properties of the shell form that are key elements in gaining insight
245 into functional and ecological aspects of the shell (Evans, 2013). However, functional and
246 ecological aspects of shell form can only be determined if the shell form can be exactly
247 quantified.
248
249 **Using 3D technology to quantify shell form based on aperture ontogeny profiles**
250
251 In this paper, we propose an interactive approach to the quantification and analysis of shell forms
252 based on state of the art 3D technology and by integrating the theoretical principles of shell
253 modelling and the empirical principles of morphometric data handling. There are no theoretical
254 models that can simulate all existing shell forms. However, the theoretical background of the
255 theoretical models is biologically sound – simulating the shell form by simulating the shell
256 ontogenetic process. On the basis of this shell-ontogenesis principle, we used state-of-the-art X-
257 ray microtomography (micro-CT scan) and 3D modelling software to obtain a series of shell
258 aperture changes from the shell in an interactive workflow that is similar to empirical
259 morphometric analysis.
260
261 First, a series of shell aperture outlines were digitised directly from the reconstructed 3D shell
262 model obtained from micro-CT scanning by using open-source 3D-modelling software – Blender
263 ver. 2.63 (www.blender.org). Then, the growth trajectory and form of the shell aperture outline
264 were quantified and extracted with our custom scripts that run in Blender through its embedded
265 open-source Python interpreter (http://www.python.org/). The changes of aperture size and
266 shape, and aperture growth trajectory in terms of curvature and torsion along the shell ontogeny
267 axis length were obtained (hereafter "aperture ontogeny profiles"). The final aperture ontogeny
268 profiles are in a form of multivariate time series data, which consist of a number of instances (i.e.
269 number of quantified apertures that depends on the length of the whorled shell tube) and
270 attributes that represent the growth trajectories, aperture form, and size.
271
272 These aperture ontogeny profiles can be plotted when each shell is examined individually. On
273 the other hand, the aperture ontogeny profiles can be visually compared between different shells
274 by plotting the data as radar chart (i.e. spider and star plots). In addition, the differences between

275 shells can be assessed quantitatively by calculating the dissimilarity of aperture ontogeny profiles

276 among shells. Furthermore, the dissimilarity matrix can be used to plot the dendrogram and

277 NMDS plots, which resemble a shell morphospace. All our procedures were implemented by

278 using open source and free software.

279

280 Finally, we discuss some possible applications and implications of these shell form

281 quantification methods in theoretical morphology, functional morphology, taxonomy and shell

282 shape evolutionary studies.

283

284 **Materials and Methods**

285 **Ethics Statement**

286 Specimens were collected in Malaysia with permissions from the Economic Planning Unit,

287 Malaysia (UPE: 40/200/19/2524).

288

289 **Scanning instrumentation**

290 A micro-CT scanner (SkyScan, model 1172, Aartselaar, Belgium) and its accompanying

291 software, NRecon ver. 1.6.6.0 (Skyscan©) and CT Analyser ver. 1.12.0.0 (Skyscan©), were used

292 to generate digital shell 3D models from the actual shell specimens.

293

294 **Computation software and hardware**

295 Various commercial 3D modelling and statistical software exist for visualising, manipulating,

296 and understanding morphology, such as Amira® (Visage Imaging Inc., San Diego, CA) and

297 Autodesk Maya (San Rafael, CA) (reviewed by Abel, Laurini & Richter, 2012). However, in this

298 study, we used only two open-source 3D data modelling and processing software packages,

299 namely Blender ver. 2.63 (www.blender.org) and Meshlab ver. 1.3.2 (Cignoni, Corsini &

300 Ranzuglia, 2008, http://meshlab.sourceforge.net/). Both have been used in biology to visualise

301 and model morphology (for Meshlab: Im et al., 2012; Chaplin, Yu & Ros, 2013; Atwood &

302 Sumrall, 2012; for Blender: Pyka et al., 2010: 22); Haug, Maas & Waloszek, 2009; Cassola et

303 al., 2010; Haug et al., 2010; Andrei et al., 2012; Haug et al., 2012; Lv et al., 2013; Mayer et al.,

304 2012). However, these programs have not been used to their full extent in morphological

305 quantification and analysis of 3D data for organisms. For quantification of morphology, we used

306   the open-source Python interpreter ver. 3.2 that is embedded in Blender 2.63. In addition, we also

307   used an extension to the Python programming language – NumPy (Oliphant, 2007) which

308   consists of high-level mathematical functions.

309

310   All the morphological data were explored and analysed with the statistical open source

311   programming language R version 3.0.1 (R Core Team, 2013) in the environment of RStudio

312   (RStudio, 2012). We installed three additional packages in R, namely, "lattice": Lattice Graphics

313   (Sarkar, 2008), "pdc": Permutation Distribution Clustering (Brandmaier, 2012a; Brandmaier,

314   2012b), and "fmsb" (Nakazawa, 2010).

315

316   All the computation analyses were carried out with a regular laptop computer with the following

317   specifications: Intel®Core™i7-3612QM @ 2.1GHz, 8 GB memory (RAM), NVIDIA® GeForce

318   GT 630M with 2GB memory.

319

320   **Procedures**

321   **1. Obtaining digital 3D models from actual shells**

322   The scan conditions were as follows: voltage – 80kV or 100kV; pixel – 1336 rows × 2000

323   columns; camera binning – 2 × 2; image pixel size – 3–6 μm; rotation step – 0.4° or 0.5°; and

324   rotation – 360°. Next, the volume reconstruction on the acquired images was done in NRecon.

325   The images were aligned to the reference scan and reconstruction was done on the following

326   settings: beam hardening correction – 100%; reconstruction angular range – 360 degree;

327   minimum and maximum for CS to image conversion (dynamic range) – ca. 0.12 and ca. 20.0;

328   and result file type – BMP. Finally, 3D models were created from the reconstruction images in

329   CT Analyser with the following setting: binary image index – 1 to 255 or 70 to 255; and were

330   saved as digital polygon mesh object (*.PLY format).

331

332   **2. Pre-processing digital shell models**

333   The 3D models were then simplified by quadric edge collapse decimation implemented in

334   MeshLab (Cignoni, Corsini & Ranzuglia, 2008) to reduce computation requirements. The raw

335   polygon mesh shells in PLY format have millions of faces and a file size between 20 to 80

336   Mbytes. Thus, we reduced the number of faces for all model to 200,000 – 300,000 faces, which

11

337   range between 3 and 6 Mbytes in file size. In addition, for the sake of convenience during the

338   retopology processes, all 3D models were repositioned so that the shell protoconch columella

339   was parallel with the z-axis. This was done by using manipulator tools in MeshLab.

340

341   **3. Creating reference: Tracing aperture outlines and ontogeny axis from shell models** (Supplementary

342   Information File 1)

343   The digital shell 3D model in PLY format consists of 3D Cartesian coordinate vertices in which

344   each of the three vertices constitutes a triangular face, and all faces are connected through a

345   complex network. In order words, these vertices and faces are not biologically meaningful

346   structures, and it is not possible to extract aperture outlines data directly from a raw PLY digital

347   shell model. Monnet et al. (2009), for example, attempted to extract aperture outline

348   automatically from a digital 3D model by making a plane cross-sectioning of the shell model, but

349   its outlines do not reflect the form of the actual aperture outlines. Hence, we retopologised the

350   raw 3D mesh models according to the aperture ontogeny for later data extraction.

351

352   We used Blender, which is more flexible than the commercial software used by Monnet et al.

353   (2009). For the sake of convenience, we describe the following workflow, including the tools or

354   the function (e.g. "Import PLY") which can be called after hitting the SPACE bar while in the

355   Blender environment. However, this workflow may be modified by the user.

356

357   To begin, we imported a PLY shell model into the Blender environment ("Import PLY"). Then,

358   we resized the model 1000 × ("Resize") so that the scale of 1 Blender unit was equal to 1 mm.

359   After that, we examined the traces of aperture outlines (i.e. growth lines, ribs, spines) (Figure

360   1A) and ontogeny axis (i.e. spiral striation, ridges, colour lines) (Figure 1B) of the actual shells.

361   However, it is not possible to trace apertures from the shell protoconch because the protoconch is

362   an embryonic shell that may not grow accretionarily and usually has no growth lines. In many

363   cases, the aperture of the overlapping whorls cannot be traced from the outer shell wall. One of

364   the ways to deal with this situation is to trace the aperture at the inner shell wall and the obscured

365   aperture outline can then be inferred by studying conspecific juvenile specimens (see video

366   tutorial 05:00–08:00 of Supplementary Information File 1). It does not really matter whether the

367   aperture outline was traced from outside or inside. After it was traced from the inside, the

368 subsequent retopologising stage would need take into consideration the shell thickness of the

369 overlapping whorl.

370

371 After these aperture traits were identified, we selected the 3D model (by clicking "right mouse

372 button"), and traced all these traits on the surface of the raw 3D mesh model in Blender by using

373 the "Grease Pen Draw" tool. After that, the grease pen traced aperture traits were converted to

374 Bezier curves with "Convert Grease Pencil" (Figure 1C).We would like to emphasise that this is

375 the most critical step that determines the efficiency of this shell quantification method. Thus, the

376 key lies in the good understanding of the way the aperture is structured, which is essential to

377 trace the aperture outlines accurately. However, the orientation of the shell when the aperture is

378 digitalised would not influence the aperture ontogeny data.

379

380 **4. Retopologising aperture outlines from the reference and generating retopologised shell models**

381 (Supplementary Information File 1 and File 4)

382 For each shell, we created a set of new Non Uniform Rational B-Splines (NURBS) surface

383 circles ("Add Surface Circle") and modified these ("Toggle Editmode") according to the aperture

384 outlines. We created a 16 points NURBS surface circle and aligned the circle to the aperture

385 outline by translation ("Translate"), rotation ("Rotate"), and resizing ("Resize") (Figure 1D).

386 After the NURBS surface circle was generally aligned, each of the 16 points of the NURBS

387 surface circle were selected and adjusted by translation ("G") one by one, so that the outline of

388 the NURBS surface circle was exactly the same as the aperture outline. At the same time, the

389 second point of the NURBS surface circle was aligned to the ontogeny axis (Figures 1B and 1C).

390

391 After the first aperture outline was retopologised as a NURBS surface circle, the NURBS surface

392 circle was duplicated ("Duplicate Objects") and aligned to the next aperture outline as the

393 previous one. This step was repeated until all the aperture outlines were retopologised into

394 NURBS surface circles (Figures 1D and 1E). Then the shell surface was created in the form of a

395 NURBS surface based on the digitised aperture NURBS surface circle ("(De)select All" and

396 "Make Segment" in "Toggle Editmode") (Figures 1F and 1G). Lastly, we made the surface meet

397 the end points in U direction and increased the surface subdivision per segment (resolution U =

398   8) through the properties menu of the object (Properties (Editor types)>Object Data>Active

399   Spline).

400

401   After that, we converted the NURBS surface 3D model into a 3D Mesh model that consists of

402   vertices, edges, and faces ("Convert to" - "Mesh from Curve/Meta/Surf/Text"). The final

403   retopologised 3D shell Mesh consists of X number of apertures outlines and each aperture

404   outline has Y number of vertices and then a total of X*Y vertices. Each of the vertices is

405   connected to four other nearest vertices with edges to form a wireframe shell and face (Figure

406   1H).

407

408   It is important to note that the NURBS surface circle is defined by a mathematic formula which

409   does not imply any biology perspective of the shell. We choose NURBS surface circle because

410   the 3D aperture outline form can be digitalised by a small number of control points and shell

411   surface can be recreated by NURBS surface based on the digitised aperture NURBS surface

412   circle. The final 3D polygon mesh model is more simplified than the raw PLY 3D model and

413   each of its vertex data resemble the actual accretionary process of the shell (Figures 1A and 1H).

414

415   **5. Quantifying aperture growth trajectory**

416   The aperture ontogeny profiles were quantified as described in Liew et al. (2014a) with slight

417   modifications where both aperture growth trajectory and aperture form were quantified directly

418   from the retopologised 3D shell model. This aperture growth trajectory was quantified as a

419   spatial curve, which is the ontogeny axis as represented by a series of first points of the aperture

420   outlines. We estimated two differential geometry parameters, namely, curvature (κ) torsion (τ),

421   and ontogeny axis length for all apertures (Okamoto, 1988; Harary & Tal, 2011). The local

422   curvature and torsion, and accumulative ontogeny axis length were estimated from the aperture

423   points along the growth trajectory by using weighted least-squares fitting and local arc length

424   approximation (Lewiner et al., 2005). All the calculations were done with a custom-written

425   Python script which can be implemented in Python interpreter in the Blender ver. 2.63

426   environment. The whole workflow was: (1) selecting the retopologised 3D shell Mesh (by

427   clicking "right mouse button"), (2) input parameters for number of sample points "q = ##" in the

428   python script, and (3) paste the script into the Python interpreter (Supplementary Information

14

429   File 2). The final outputs with torsion, curvature and ontogeny axis reference for each aperture

430   were saved as CSV files.

431

432   We found a convergence issue in curvature and torsion estimators. The accuracy of the curvature

433   and torsion estimates depends on the number and density of the vertices in the ontogeny axis (i.e.

434   number of aperture outlines), and the number of sample points. Nevertheless, different numbers

435   of sample points can be adjusted until good (i.e. converged) curvature and torsion estimates are

436   obtained. We used 10% of the total points as number of sample points of the ontogeny axis,

437   which gave reasonably good estimates for curvature and torsion.

438

439   Notwithstanding the algorithm issue, the curvature and torsion estimators are informative in

440   describing the shell spiral geometry growth trajectory. Curvature is always larger or equal to zero

441   ($\kappa \geq 0$). When $\kappa = 0$, the spatial curve is a straight line; the larger the curvature, the smaller the

442   radius of curvature ($1/\kappa$), and thus the more tightly coiled the spatial curve. On the other hand,

443   the torsion estimator can be zero or take either negative or positive values ($-\infty \leq \tau \leq \infty$). When τ

444     $= 0$, the spatial curve lies completely in one plane (e.g. a flat planispiral shell), negative torsion

445   values correspond to left-handed coiling and to right-handed coiling for positive torsion values;

446   the larger the torsion, the smaller the radius of torsion ($1/\tau$), and thus the taller the spiral.

447

448   **6. Quantifying aperture form**

449

450   We quantified the aperture outline sizes as perimeter and form as normalised Elliptic Fourier

451   coefficients (normalised EFA) by using a custom-written Python script which can be

452   implemented Python interpreter embedded in the Blender environment. The workflow was (1)

453   selecting the retopologised 3D shell mesh (by clicking "right mouse button"), (2) input

454   parameters for "number_of_points_for_each_aperture = ##" in the python script, and (3) paste

455   the script into the Python interpreter of Blender (Supplementary Information File 2). The final

456   outputs were saved as CSV files.

457

458   Aperture outline perimeter was estimated from the sum of lengths (mm) for all the edges that are

459   connecting the vertices (hereafter "aperture size"). For aperture form analysis, we used 3D

460   normalised EFA algorithms (Godefroy et al., 2012) and implemented these in the custom python

15

461   script. Although many algorithms exist for describing and quantifying the form of a closed
462   outline (Claude, 2008), we used EFA because it is robust to unequally spaced points, can be
463   normalised for size and orientation, and can capture complex outline form with a small number
464   of harmonics (Rohlf & Archie, 1984; Godefroy et al., 2012). In this study, we used five
465   harmonics, each with six coefficients which were sufficient to capture the diverse aperture
466   shapes of our shells. For quantification of apertures shape that are invariant to size and rotation,
467   we normalised EFA of aperture outlines for orientation and size. If needed for comparison with
468   other studies, the normalised EFA can be repeated for the same dataset with higher or lower
469   numbers of harmonics.
470
471   After normalisation, we ran principal components analysis (PCA) to summarise the 30
472   normalised Fourier coefficients as principal components scores (hereafter "aperture shape
473   scores"). After that, we selected the major principal components (explaining > 90 % of the
474   variance) for further analysis. The aperture shape scores of each selected principal component
475   were plotted and analysed against the ontogeny axis.
476
477   **7. Visualising aperture form and trajectory changes along the shell ontogeny**
478   For exploration of data, we used two graphical techniques for representing aperture ontogeny
479   profile changes along the shell ontogeny. For each shell, we made a vertical four-panels scatter
480   plot in which each of the four variables (namely, curvature, torsion, aperture size, and the first
481   principal component aperture shape score) were plotted against the ontogeny axis. When
482   necessary, the second and third principal component aperture shape scores were also included. In
483   addition, the axis of each variable was rescaled so that it was the same for the same variable of
484   all shells. After standardisation of the axis, the aperture ontogeny profiles of several shells could
485   be quantitatively compared side by side.
486
487   However, comparison of between plots would become less effective with a larger number of
488   shells. Alternatively, therefore, all aperture ontogeny profile variables of each shell can also be
489   represented in a radar chart, instead of scatter plots. This chart is effective in showing the
490   variable outliers within a chart and the overall similarity between charts. Before plotting the data
491   in a radar chart, the datasets of all shells need to be restructured because the dataset of different

16

492 shells could differ in the number of data points (i.e. quantified aperture), which depends on the

493 ontogeny axis length of each shell.

494

495 We did this by dividing the ontogeny axis of each shell into 20 equal length intervals, and then

496 by sampling the variable values at the end of every interval. In the restructured dataset, the trend

497 of the aperture ontogeny profile of each variable is retained and all radar charts have the same

498 number of data points. Thus, the changes of aperture variables between each subsequent 1/20 of

499 the ontogeny axis can be examined within a shell and be compared among different shells in a

500 synchronistic manner. We suggest to use 20 points to summarise hundreds variable points of the

501 aperture ontogeny profile variables along ontogeny axis because the radar would be

502 overwhelming with too many points and hard to interpret. Similar to the scatter plot, we

503 standardised the axis scales of each variable of all radar charts.

504

505 In addition, we added a new variable which represents the ontogeny axis interval length in order

506 to compensate for the loss of shell size information during the standardisation of ontogeny axis

507 length. Finally, we plotted the variables, namely, curvature, torsion, aperture size, and ontogeny

508 axis length, and aperture shape scores in a radar chart for each shell by using the "fmsb" library

509 (Nakazawa, 2010) with R version 3.0.1 (R Core Team, 2013) (Supplementary Information File

510 5).

511

512 **8. Quantitative comparison between shell forms**

513 In addition to the qualitative comparison between shells forms as described above, the

514 dissimilarity between different shells can be analysed quantitatively. We used Permutation

515 Distribution Clustering (PDC) which finds similarities in a time series dataset (Brandmaier,

516 2012a; Brandmaier, 2012b). PDC can be used for the analysis of the changes in a variable along

517 shell ontogeny between different shells (i.e. two-dimensional dataset: number of shells × number

518 of apertures) and multiple variable changes between shells (i.e. three-dimensional dataset:

519 number of shells × number of variables × number of apertures). We applied the most recent

520 analysis developed by Brandmaier (2012a & b) because it has an R package that can be applied

521 and can calculate the trend similarity. That said, the same data can always be analysed by other

522 "better" algorithms in the future.

523

524   Although PDC is robust to the length differences between datasets, our preliminary analysis

525   showed that the PDC output would be biased when there was a great (around two-fold) length

526   difference in the total ontogeny axis length. Hence, we standardised the data as in procedure 7,

527   but dividing the ontogeny axis of each shell into 50, instead of 20, equal length intervals. This

528   standardisation procedure allows comparison of trends in variable changes along the shell

529   ontogeny without the influences of size. In other words, the dissimilarity is zero between two

530   shells that have exactly the same shape, but differ only in size. In addition to the shape

531   comparison, we obtained the shell size in terms of volume by using "Volume" function in

532   Blender after the 3D shell model was closed at both ends by creating faces "Make edge/Face")

533   on selected apertures at both end ("Loop Select") in EDIT mode.

534

535   The aperture ontogeny profiles of all shells were combined into a three-dimensional data matrix

536   consisting of n shells × four variables × 50 aperture data points. We ran four PDCs, each for the

537   five data matrices with: 1) all four variables, 2) torsion, 3) curvature, 4) aperture size, and 5)

538   aperture shape scores. The parameter settings for the PDC analysis were as follows: embedding

539   dimension = 5; time-delay of the embedding = 1; divergence measure between discrete

540   distributions = symmetric alpha divergence; and hierarchical clustering linkage method = single.

541   The dissimilarity distances between shells were used to produce the dendrogram. PDC analysis

542   was performed with the "pdc" library (Brandmaier, 2012b) in R version 3.0.1 (R Core Team,

543   2013) (Supplementary Information File 5).

544

545   In addition to the dendrogram representation of the output from PDC, we plotted the

546   dissimilarity as a non-metric multidimensional scaling (NMDS) plot which resembles a

547   morphospace. NMDS was performed by using "MASS" library (Venables & Ripley, 2002) in R

548   version 3.0.1 (R Core Team, 2013) (Supplementary Information File 5).

549

550   **Worked example: Comparative analysis of *Opisthostoma* and *Plectostoma* species shell form**

551   **and simulated shell form**

552   We evaluated the above-described shell form quantification method by using the shells of

553   *Opisthostoma* and *Plectostoma*, which exhibit a great variability in shell form. Some of the

554 species follow a regular coiling regime whereas others deviate from regular coiling in various

555 degrees. It remains a challenging task to quantify and compare these shell forms among species,

556 either by using traditional or geometric morphometrics, because a standard aperture view for the

557 irregular and open coiled shells cannot be determined.

558

559 We selected four species, namely, *Plectostoma laidlawi* Skyes 1902 (Figure 2A), *Plectostoma*

560 *crassipupa* van Benthem Jutting, 1952 (Figure 2B), *Plectostoma christae* Maassen 2001 (Figure

561 2C), and *Opisthostoma vermiculum* Clements and Vermeulen, 2008 (in Clements et al., 2008)

562 (Figure 2D), for which the shell forms are, respectively: regularly coiled, slight distortion of the

563 last whorl, strong distortion of the last whorl, and complete distortion of most of the whorls.

564 Despite the narrow taxonomic range of the selected species, the range of shell forms of these

565 four species do cover a very large diversity of shell form. We retopologised these four shells by

566 following the procedures 1 to 4 (Supplementary Information Files 6).

567

568 In addition to the four retopologised 3D shell models, we manually created another four shell

569 models by transforming three out of the four retopologised NURBS surface 3D shell models by

570 using the "Transform" function in Blender. These models are: 1) *Plectostoma laidlawi* that was

571 resized to half the original size and given slight modification of the aperture size (Figure 2E); 2)

572 *Plectostoma christae* that was reshaped into an elongated form by reducing the model size (linear

573 dimension) to one-half along the x and y axes, and by doubling the size along the z axis (Figure

574 2F); 3) *Plectostoma christae* that was reshaped into a depressed form by multiplying by 1.5 the

575 model size along the x and y axes, and by reducing to one-half along the z axis (Figure 2G); and

576 4) *Opisthostoma vermiculum* that consists of one *Opisthostoma vermiculum* original 3D model of

577 which we connected the aperture to another, enlarged, *Opisthostoma vermiculum* (Figure 2H).

578 Finally, we analysed all these eight shell models by following the procedures 5 to 8.

579

580 **Results and Discussion**

581 **Retopologied 3D shell models**

582 All the final retopologised 3D shell models can be found in Supplementary Information (Files 7

583 to 14) in PLY ASCII mesh format, with the raw data as a list of vertices, followed by a list of

584 polygons, which can be accessed directly without the need of any 3D software. Each vertex is

19

585 represented by x, y, z coordinates. Each polygon face consists of four vertices. This simplified
586 yet biologically informative 3D mesh shell model allows the quantification of aperture form and
587 growth trajectory. Moreover, the 3D shell models and their raw vertices data could potentially be
588 used in studies of functional morphology and theoretical modelling of shell form, respectively.
589
590 Malacologists have been focusing on empirical shell morphological data, from which the
591 functional, ecological and evolutionary aspects were then extracted. The physical properties were
592 then determined by its form (e.g. Okajima & Chiba 2011; Okajima & Chiba, 2012). By using the
593 3D models, the shell properties and function can be analysed *in silico*. For example, the thickness
594 of the shell can be added to the 3D shell model (Figure 3E and Figure 3F) in order to obtain the
595 shell material's volume, the shell's inner volume, its inner and outer surface area, and centre of
596 gravity. We used the "build" function of the software, which can only "solidify" the model by
597 uniform thickness. However, if necessary, it is possible to write a custom Python script to add
598 the desired thickness to the shell. Quantification of shell properties may then be done by using
599 the geometry approach in Meshlab or Blender, as compared to the pre-3D era where
600 mathematical descriptions of the shell form were required (e.g. Moseley, 1838; Raup & Graus,
601 1972; Stone, 1997). Furthermore, it is possible to convert the 3D models to a 3D finite element
602 (FE) model, of which the physical properties (e.g. strength) can be tested (e.g. Faghih Shojaei et
603 al., 2012).
604
605 In addition to the potential use of 3D shell models in functional morphology, the coordinate data
606 of the vertices of 3D models could be used directly by theoretical morphologists (see Figure
607 1 in Urdy et al., 2010). For example, these data can be extracted in different formats that fit the
608 data requirement of different types of theoretical shell models, namely, generating curve models
609 using a fixed reference frame or moving reference frame (Figure 3C), helicospiral or multivector
610 helicospiral models using a fixed reference frame (Figure 3A, Figure 3B and Figure 3D) or
611 growth vector models using a moving reference frame (Figure 3A and Figure 3B).
612
613 The retopologising of the aperture ontogeny from a raw 3D shell model (procedures 1 to 4) is a
614 time-consuming and tedious process compared with traditional and geometric morphometrics.
615 There are no differences in the time required for data analysis between GM and our method. The

only time differences are in the data acquisition. In our experience, two to three days are needed
to collect the aperture data from the shell. For example, the four shell models were created by
retopologising between 73 and 96 separate apertures (ca. 1500 points for 90 apertures). From the
viewpoint of short-term cost-benefit balance, this may be seen as a waste of time, because GM
requires not more than a few dozen points for each shell, which can generate the shape variables
for a study, even though these points are not comparable to other points of other shells or other
studies. However, in the long run, it is a good time investment, since it will allow the
understanding of shell function, growth, and evolution, as the same set of data is obtained from
different shell forms and can be accumulated and analysed together. Moreover, as with all
newly-developed techniques, improvements in efficiency and automation are possible and may
remove these impediments in the future.

Comparing shell form from the view of shell ontogeny

Figure 4 gives an overview of the aperture ontogeny profile and shell volume for each species.
The curvature, torsion perimeter, and ontogeny axis are represented by true numerical values
with the unit of mm^{-1} and mm, and thus can be interpreted directly. In contrast, the aperture
shape scores are just statistics of Fourier coefficients and are not the absolute quantification of
aperture shape. The PCA score of an aperture shape depends on the shape of other aperture
outlines and thus it might change whenever other aperture outlines are added into the analysis.
Nevertheless, the aperture scores will stabilise as data of more shells become available and when
most of the extreme aperture forms are included. In this study, the first principal component
explained 92% of the total variance; the second and third principal component explained only
3% or 1% of the total variance. We showed that the shell form can be represented by the
ontogeny changes of the aperture growth trajectory in terms of curvature and torsion, and
aperture form, in terms of perimeter and shape.

Our first example evaluates this method in illustrating the differences between two shells that
have the same shape but differ in shell size – the half-size *Plectostoma laidlawi* (Figure 4E) shell
and the original *Plectostoma laidlawi* shell (Figure 4C). As revealed by their aperture ontogeny
profiles, the size difference between the two shells has had an effect on the curvature, torsion,
ontogeny axis length and aperture size. For the resized *Plectostoma laidlawi* shell, the values of

647   curvature and torsion are twice as large as for the original, whereas the ontogeny axis length and

648   aperture size are only half those of the original shell. However, there is no discrepancy in the

649   aperture shape statistics. Despite this scalar effect, the overall trends in the changes of these

650   variables along the ontogeny axis are comparable between these two shells (Figure 6B).

651

652   Another example shows the ontogeny profiles of three shells, namely, the elongated (Figure 4G),

653   depressed (Figure 4H), and original (Figure 4A) versions of the *Plectostoma christae* shell.

654   Comparison of aperture profiles among these show the most obvious discrepancies in greater

655   torsion values for the elongated shell, which change in a more dramatic trend along the shell

656   ontogeny. In addition, each of the three shells has its unique aperture shape scores, though there

657   are no big discrepancies in the aperture size. The differences in ontogeny axis length, curvature

658   and torsion are related to the differences of the aperture shape statistics among the three shells.

659   However, our small dataset with only three shells is not sufficient for thorough disentangling of

660   the interplay between aperture size, shape, and growth trajectory in relation to the shell form.

661

662   Our last example is the comparison between the original (Figure 4D) and the composite (Figure

663   4F) *Opisthostoma vermiculum* shell . It is clear that our method has high sensitivity and

664   robustness in the analysis of such bizarre shell forms. As shown in Figure 4F, the start of the

665   aperture ontogeny profile of the composite shell was the same as for the original shell (Figure

666   4D). In addition, the later parts of the ontogeny profile trends are still comparable to the first

667   part, but different in value because of the scalar effect.

668

669   As an alternative visualisation, Figure 5 shows the radar charts that summarise the same aperture

670   ontogeny profiles of each species. The polygon edges in each chart show how dramatically the

671   aperture form (size and shape), and growth trajectory (curvature and torsion) are changing at

672   each of the subsequent 5% intervals of the shell ontogeny. The aperture size (mm) and the

673   ontogeny segment length (mm) variables indicate the shell size (i.e. volume). To illustrate this,

674   aperture size and ontogeny axis length can be seen as the circle size and height of a cylinder.

675   This chart is useful for the visual comparison between shells that are similar in size, for example,

676   *Plectostoma christae* (2.43 mm^3), *Plectostoma laidlawi* (2.39 mm^3), and the depressed

677   *Plectostoma christae* (2.73 mm^3). The radar chart shows that (1) the depressed *Plectostoma*

678   *christae* is the largest and has a very different aperture shape as compared to the other two shells;

679   (2) most of the shell whorls' form of *Plectostoma christae* is very similar to *Plectostoma*

680   *laidlawi* (i.e. most of the polygons in the chart were similar), but the *Plectostoma laidlawi* shell

681   differs from *Plectostoma christae* shell by having distorted whorls at the last part of the shell

682   ontogeny (magenta lines at torsion) and a more open umbilicus at the beginning of the shell

683   ontogeny (red lines at curvature and aperture size).

684

685   However, comparison of radar charts between shells that differ greatly in size would be less

686   informative. For example, the radar charts between the resized *Plectostoma laidlawi* shell and

687   the original *Plectostoma laidlawi* shell are very different, though the resized one has the same

688   shell shape as the original. The difference in radar charts between the two shells was therefore

689   mainly caused by the size difference.

690

691   As we have shown in both graphical techniques (Figures 4 and 5), the shell forms can be

692   explored and compared qualitatively on the basis of aperture ontogeny profiles. Users might need

693   some training in the interpretation of the plots because they are different from both linear

694   dimension measurement plots and geometric morphometric shape coordinate plots. Our

695   evaluation suggested that both data visualisation methods are sensitive and robust in capturing

696   the aperture ontogeny profile for any shell form and thus make the qualitative comparison across

697   gastropod taxa and studies possible.

698

699   This method could be applied in malacological taxonomy because its core business is the

700   description of shell form. Despite hundreds of years of taxonomic history of shells, there has

701   been little change in the way shell form is being described. For example, shell from is usually

702   described in terms of linear dimensions: shell width and height; number of whorls; shell shape –

703   flat, depressed, globose, conical, or elongated; whorls shape – from flat to convex. Here, we

704   suggest that the aperture ontogeny profiles would be a great supplement to the classical approach

705   to shell description. For example: (1) the size of the shell (its volume) depends on the ontogeny

706   axis length and aperture size; (2) the shell shape depends on the growth trajectory in terms of

707   curvature and torsion; (3) the shape of the whorls depends on the shape of the aperture (Figure

708   4). In our case of the four shells (Figures 2A – 2D), it is clear that aperture size of each shell is

23

709 constricted at roughly the same part of the respective shell ontogeny, namely between 70% and

710 85%, regardless of the dissimilar shell sizes and shapes (Figures 4A – 4D, and aperture size

711 profiles in Figure 5B). In fact, these aperture size decreases during ontogeny are in accordance

712 with the shell constriction, one of the shell characters that have been used in the taxonomy of

713 *Opisthostoma* and *Plectostoma* (Vermeulen, 1994; Liew et al., 2014b). However, the shell

714 constriction has not been quantified previously, and we show that it could also be an important

715 developmental homology for the two genera. This preliminary results suggest that these aperture

716 ontogeny profiles could aid the taxonomist in decision-making for grouping taxa based on

717 homologous characters.

718

719 **Quantitative comparison between different shell forms**

720 Figure 6 shows dendrograms resulting from a permutation distribution clustering analysis of the

721 eight shells in terms of their aperture ontogeny profiles. Figure 6A shows the hierarchical

722 clustering of the eight shells based on all four aperture ontogeny profiles. From this dendrogram,

723 the composite *Opisthostoma vermiculum* is completely separate from the other shells. The

724 remaining seven shells are clustered into two groups. One consists of the more regularly coiled

725 shells, namely, *Plectostoma christae* and its two transformed shells, and *Plectostoma crassipupa*;

726 the other group consists of the shells that deviate from regular coiling, namely *Plectostoma*

727 *laidlawi* and its transformed shell, and *Opisthostoma vermiculum*. Nevertheless, there were high

728 dissimilarities between shells within each group as revealed by the long branch lengths in Figure

729 6A, except for the two *Plectostoma laidlawi* shells (Table 1). The aperture ontogeny profiles for

730 the *Plectostoma laidlawi* shell and its reduced version are almost the same. The high

731 dissimilarity among the other six shells can be explained when each of the variables in the

732 aperture ontogeny profile is analysed separately as shown in Figure 6B.

733

734 Figure 6B shows the dendrograms of aperture ontogeny profiles for each of the four variables.

735 All four dendrograms have a different topology than the one in Figure 6A. Among the variables,

736 the aperture ontogeny profile of the curvature has the smallest discrepancies among shells. The

737 two *Plectostoma laidlawi* shells are the only pair that clusters together in all the dendrograms of

738 Figures 6A and 6B because they are identical in every aspect of aperture ontogeny profile except

739 torsion. Hence, the independent analysis of aperture ontogeny profile variables corresponds well

740 to the overall analysis of aperture ontogeny profiles.

741

742 Figure 7 shows a three-dimensional NMDS plot of the distance matrix (Table 1) that was

743 generated from PDC analysis on all four aperture ontogeny profiles. The very low stress level

744 (0.000) indicates that this 3D plot is sufficient to represent the distance matrix of the aperture

745 ontogeny profiles. This NMDS plot can therefore be regarded as a morphospace of the shell

746 shape, as derived from aperture ontogeny profiles. However, neither the dendrogram nor the

747 NMDS plot contains information about the shell size because the analysis of PDC is based on the

748 standardised ontogeny profiles and their trends. Thus, both plots are useful for the comparative

749 analysis of shell shape, but not shell size. Nevertheless, the size comparison between shells is

750 rather straightforward.

751

752 The conventional quantification of shell size is based on the linear measurement of two or three

753 dimensions of a shell, for example, shell height and shell width. These measurements are

754 extremely effective for size comparisons between similarly-shapes shells. However, the linear

755 measurements have limitations when comparison is made between shells that are different in

756 shape. For example, shell height comparison between a discoidal shell and a fusiform shell tells

757 very little about size differences because the dimensional measurements are tied to a shell shape

758 that results from a different coiling strategy.

759

760 Thus, shell size may be more appropriately given as shell volume, which can be estimated easily

761 from retopologised 3D shell models (Figure 4). This quantification of shell size in terms of

762 volume is more meaningful from the functional and developmental point of view because a snail

763 should grow a shell in which its entire soft body can fit when the snail withdraws into the shell.

764 In addition to the exact volume, a shorthand to qualitatively comparing size between two shells is

765 by examining the ontogeny axis length and aperture size in the radar chart (Figure 5). We can

766 then compare the form between shells when the dendrograms or NMDS plot are interpreted

767 together with shell size (volume) data. For example, the *Plectostoma laidlawi* shell has the same

768 shape as, but is eight times larger than, the resized *Plectostoma laidlawi*.

769

770 In addition to the construction of morphospace, the dissimilarity matrix can be used in

771 phylogenetic signal tests (Hardy & Pavoine, 2012). Furthermore, it can also be analysed together

772 with other distance matrices, such as for geographical or ecological distance, to improve our

773 understanding of the evolutionary biology of shell forms.

774

775 **Conclusions, limitations and future directions**

776 We demonstrated an alternative workflow for data acquisition, exploration and quantitative

777 analysis of shell form. This method has several advantages: (1) robustness – this method can be

778 used to compare any shell form: The same aperture profiles can be obtained from any form of

779 shell. Then, these profiles from different shells and/or different studies can be analysed together.

780 These parameters can be obtained from the aperture as long as the shell grows accretionarily at

781 the aperture; (2) scalability and reproducibility – the data obtained from different studies and

782 different gastropod taxa can be integrated: Aperture ontogeny profiles were obtained from the

783 aperture outlines. This is a trait that exists in every gastropod shell. We believe that the aperture

784 outline that is obtained by multiple experienced malacologists, on different shells, would be

785 highly similar; (3) versatility – outputs from this method are comply with data standard that is

786 required in taxonomy (e.g., functional morphology, theoretical modelling, and evolutionary

787 studies: the raw 3D shell mesh models can be used for visualisation of shells in taxonomic

788 research (e.g. Liew et al., 2014b), coordinates data of the vertices can be used for theoretical

789 modelling (e.g. Urdy et al., 2010), aperture ontogeny profiles can be used for shell functional

790 studies (e.g. Liew & Schilthuizen, 2014), and dissimilarity matrix between shell forms can

791 analysed with phylogenetic distance matrix. the raw 3D shell mesh models, coordinates data of

792 the vertices, aperture ontogeny profiles, and dissimilarity matrix between shell forms comply

793 with the data standard that is required in taxonomy, functional morphology, theoretical

794 modelling, and evolutionary studies.

795

796 Yet, our method has its limitations. Firstly, our retopology procedures rely on a 3D shell model

797 that requires CT-scan technology. In fact, although a CT-scan 3D shell model can certainly

798 facilitate the retopology process of a shell, it is not indispensable. The key of the retopology

799 processes is to digitise the aperture along the shell ontogeny, and thus a shell can be

800 retopologised fully in Blender with a good understanding of the aperture ontogeny profiles by

801 studying the real specimens even without a reference shell model. Secondly, the retopology

802 procedure which is essential for our data acquisition is more time-consuming than traditional and

803 geometric morphometric where data can be obtained from an image taken from a shell. Thirdly,

804 our method is effective in the analysis of overall shell form, but not of the shell ornamentation.

805

806 In the future, our method can be improved to accommodate the shell ornamentation analysis.

807 Parts of our method (i.e. procedures 1 – 6) can be used to obtain shell ornamentation data, such

808 as radial ribs (*i.e.,* commarginal ribs), but these data cannot be analysed with our qualitative and

809 quantitative approaches that focus on longitudinal growth (i.e. procedures 7 – 8). Finally, we

810 hope this shell form quantification method will simulate more collaboration within malacologists

811 that work in different research fields, and between empirical and theoretical morphologists.

812

813 **Supplementary Information (http://dx.doi.org/10.6084/m9.figshare.877061)**

814 File 1 – Video tutorial for procedure 3 and 4.

815 File 2– A python script for procedures 5 and 6 – Aperture form and growth trajectory analysis on

816 retopologised 3D shell mesh in Blender.

817 File 3– A python script to convert normalised elliptical Fourier coefficients to polygon mesh in

818 Blender.

819 File 4 – Python script for retopologising procedure.

820 File 5 – An R script for data analysis as described in procedures 7 and 8.

821 File 6 – A Blender file consisting of raw data of 8 shells of procedures 1 – 4.

822 File 7 – PLY ASCII mesh 3D model of *Plectostoma laidlawi* Sykes 1902.

823 File 8 – PLY ASCII mesh 3D model of *Plectostoma crassipupa* van Benthem Jutting, 1952.

824 File 9 – PLY ASCII mesh 3D model of *Plectostoma christae* Maassen 2001.

825 File 10 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* Clements and Vermeulen,

826 2008.

827 File 11 – PLY ASCII mesh 3D model of *Plectostoma laidlawi* that was reduced in size by one-

828 half and with slight modification of the last aperture size.

829 File 12 – PLY ASCII mesh 3D model of *Plectostoma christae* that was reshaped into an

830 elongated form by reducing the model size (linear dimension) by one-half along the x and y axes,

831 and by doubling the size along the z axis.

832 File 13 – PLY ASCII mesh 3D model of *Plectostoma christae* that was reshaped into a depressed

833 form by doubling the model size along the x and y axes, and by reducing the size by one-half

834 along the z axis.

835 File 14 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* that consists of one

836 *Opisthostoma vermiculum* original 3D model of which the aperture was connected to a second

837 enlarged *Opisthostoma vermiculum*.

844 **Author Contributions**

845 Conceived and designed the experiments: LTS. Performed the experiments: LTS. Analyzed the

846 data: LTS. Contributed reagents/materials/analysis tools: LTS MS. Wrote the paper: LTS MS.

847

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Introduction

Empirical and theoretical approaches in the study of shell form

The external form diversity of organisms is the most obvious evidence for their evolution, and thus is a key element in most branches of biology. The Molluscan shell has been a popular example in morphological evolution studies because it is geometrically simple, yet diverse in form. The shell form is controlled by the shell ontogenetic process, which follows a simple accretionary growth mode where new shell material is accumulatively deposited to the existing aperture. The evolution of shell forms has been studied either by using empirical approaches that focus on the quantification of actual shell forms or by using theoretical approaches that focus on the simulation of shell ontogenetic processes and geometric forms.

Notwithstanding the active development in both empirical and theoretical approaches to the study of shell form, there has been very little integration between both schools. For the empirical approach, the quantification methods of shell form have evolved from traditional linear measurement to landmark-based geometric morphometrics and outline analyses (for an overview see Van Bocxlaer & Schultheiß, 2010). At the same time, for the theoretical approach, the simulations of shell form have evolved from simple geometry models that aimed to reproduce the form, to more comprehensive models that simulate shell ontogenetic processes (for an overview see Urdy et al., 2010). Hence, each of the two approaches has been moving forward but away from each other, where synthesis between the two schools of shell morphologists has become more challenging.

In empirical morphological studies, shell form, either in terms of heights and widths in traditional morphometrics or in terms of geometry of procrustes distances in geometric morphometrics, is quantified by a set of homologous reference points or landmarks on the shell, which can be easily obtained from the fixed dimensions of the shell. Thus, both methods could abstract the shell form in terms of size and shape of the particular shell dimensions, and the between-sample variation of shell size and shape can be assessed (in most cases only within one study). On the other hand, it is not possible to reconstruct the actual shell form from these quantitative measurements, because the shell's accretionary growth model and spiral geometry cannot be quantified on the basis of arbitrary reference points or fixed dimensions (Stone. 1997).

2

61   Nevertheless, the traditional and geometric morphometric methods have been accepted widely as

62   standard quantification methods for shell form in many different fields of research.

63

64   In contrast to empirical morphometrics in which the aim is to quantify the actual shell,

65   theoretical morphologists focus on the simulation of an accretionary growth process which

66   produces a shell form that is similar to actual shells. This field was established with the

67   theoretical shell model of D.M. Raup (Raup, 1961; Raup & Michelson, 1965). Within the first

68   two decades after these publications, only a few different versions of shell models were proposed

69   (e.g. Løvtrup & von Sydow, 1974; Bayer, 1978; McGhee, 1978; Kawaguchi, 1982; Illert, 1983).

70   The subsequent two decades, thanks to the popularity and power of desktop computing, many

71   more theoretical shell models were published (e.g., Savazzi, 1985; Okamoto, 1988; Cortie, 1989;

72   Ackerly, 1989a; Savazzi, 1990; Checa, 1991; Fowler et al., 1992; Illert & Pickover, 1992; Checa

73   & Aguado, 1992; Cortie, 1993; Savazzi, 1993; Rice, 1998; Ubukata, 2001; Galbraith,

74   Prusinkiewicz & Wyvill, 2002). Finally, we saw further improvements in the published

75   theoretical models in recent years. These recent models simulate shell forms that more accurately

76   resemble actual shells because of improved programming software, better algorithms, and 3D

77   technology (e.g. Picado, 2009, Stępień, 2009; Meinhardt, 2009; Urdy et al., 2010; Harary & Tal,

78   2011; Moulton & Goriely, 2012; Moulton, Goriely & Chirat, 2012; Faghih Shojaei et al., 2012;

79   Chacon, 2012). Here, we will not further discuss the details of the at least 29 published shell

80   models, but refer to the comprehensive overviews and descriptions of these models in Dera et al.

81   (2009) and Urdy et al. (2010).

82

83   In brief, the latest theoretical shell models are able to simulate irregularly-coiled shell forms and

84   ornamentations that resemble actual shells, whereas the earlier models could only simulate the

85   regular and general shape of shells. The major refinements that have been made during the

86   almost five decades' development of theoretical shell models are the following modifications of

87   the algorithm: 1) from a fixed reference frame to a moving reference frame system; 2) from

88   modelling based on numerical geometry parameters to growth-parameter-based modelling (e.g.

89   growth rates); 3) from three parameters to more than three parameters, which has made fine-

90   tuning of the shell simulation (e.g. aperture shape) possible. The key element of the theoretical

91   modelling of shells is the generation of shell form by simulating the aperture ontogeny in terms

3

92   of growth trajectory and form along the shell ontogeny. Hence, this has an advantage over the

93   empirical approach in the numerical representation of the shell geometry form in terms of the 3D

94   quantification and the actual shell ontogenetic processes.

95

96   Since the empirical and theoretical researchers studying shell form with two totally different

97   quantification methods, our understanding of shell evolution cannot progress solely by using

98   either empirical morphometrics or theoretical models. Ideally, theoretical models need to be

99   evaluated by empirical data of shell morphometrics, and, vice-versa, empirical morphometric

100 methods need to be improved to obtain data that better reflect the actual shell form and

101 morphogenesis which can then be used to improve the theoretical models. In this dilemma lies

102 the central problem of shell form quantification and it urgently needs to be addressed in order to

103 integrate and generalise studies of shell form evolution.

104

105 **Why empirical morphologists rarely use theoretical shell models**

106

107 Despite the fact that, since the 1980s, manyshell models have been published that are more

108 complex and versatile, the first theoretical shell model of Raup still remains the most popular.

109 There were many attempts by empirical morphologists to use the original or a modified version

110 of Raup's parameters to quantify natural shell forms (e.g. Raup, 1967; Vermeij, 1971; Davoli &

111 Rosso, 1974; Graus, 1974; Kohn & Riggs, 1975; Newkirk & Doyle, 1975; Warburton, 1979;

112 Cameron, 1981; Verduin, 1982; Ekaratne & Crisp, 1983; Saunders & Shapiro, 1986; Tissot,

113 1988; Foote & Cowie, 1988; Johnston, Tabachnick & Bookstein, 1991; Emberton, 1994; Clarke,

114 Grahame & Mill, 1999; Samadi, David & Jarne, 2000). Surprisingly, all the other shell models,

115 many of which produce more realistic forms, have received very little attention as compared to

116 Raup's model (see e.g. Savazzi, 1992; Okajima & Chiba, 2011; Okajima & Chiba, 2012, for

117 exceptions). This ironic situation might be explained by the elegance of Raup's model that is

118 intuitively and mathematically simple to be used by empirical morphologists (mostly biologists),

119 with limited mathematical and programming experience.

120

121 As discussed above, most of the theoretical models can simulate a shell that has a form

122 resembling the actual shell in a realistic 3D geometry, based on shell ontogeny processes. In

4

123 contrast, empirical morphometrics can only quantify and compare certain dimensions of actual

124 shells. Clearly, the theoretical approach is better than the empirical approach in its accuracy of

125 shell form quantification, because accurate morphological quantification is essential for

126 functional, ecological and evolutionary studies of shell form. Below, we identify and discuss a

127 few impediments that currently prevent empirical morphologists from adopting the theoretical

128 approach in shell form quantification.

129

130 First, the requirement of a computation resource was an impediment in the past. These

131 theoretical models may only be implemented in a computation environment. As mentioned

132 above, the advances of computation hardware in speed and 3D graphic technology have

133 promoted the development of more complex theoretical shell models. For example, the current

134 speed and storage of a desktop computer is at least four orders of magnitude greater than those

135 used by Cortie (1993) only two decades ago. Clearly, the computation hardware is no longer an

136 impediment (e.g. Savazzi, 1995) for the application and development of theoretical shell models.

137

138 Notwithstanding the hardware development, programming skills are still a prerequisite for the

139 implementation of theoretical models. Many of the early models that were published between the

140 1960s and 1990s, used third-generation programming languages such as Fortran and C++, which

141 essentially lack a graphic user interface. This situation has improved now that the simulation of

142 theoretical shell models can be done in fourth-generation programming languages such as

143 Mathematica (e.g. Meinhardt, 2009; Noshita, 2010; Okajima & Chiba, 2011; Okajima & Chiba,

144 2012) and MATLAB (e.g. Boettiger, Ermentrout & Oster, 2009; Urdy et al. 2010, Faghih

145 Shojaei et al., 2012). Most of these shell models were described with intensive mathematical

146 notation, at least from a biologist's point of view, in the publication; and some of these were

147 published together with the information on algorithm implementation. However, the actual

148 programming codes are rarely published together with the paper though they may be available

149 from the authors upon request (but see Meinhardt, 2009; Noshita, 2010; Okajima & Chiba,

150 2011). Only one theoretical modelling software package based on Raup's model has a graphic

151 user interface that is comparable to contemporary geometric morphometric software (Noshita,

152 2010). Thus, the rest of the modern theoretical models are far less approachable than the

153 morphometric software for empirical morphologists. This is because those advanced theoretical

154   models have not been delivered in a form that allowed empirical morphologists to have "hands-
155   on experience" with them, without extensive mathematical literacy (Savazzi, 1995; McGhee,
156   2007).
157

158   Second, theoretical shell models simulate the shell form based on the input of a set of
159   parameters, which could be non-biological or/and biologically meaningful. Non-biological
160   meaningful parameters are counter-intuitive for empirical morphologists because these
161   parameters are not extrinsic shell traits. Nevertheless, many of these non-biological parameters
162   are required for the model to fit the shell form schematically (Hutchinson, 1999). When the
163   biological parameters do represent shell traits, they are often difficult to obtain accurately and
164   directly from the actual shell because of the three-dimensional spiral geometry (Cain, 1977;
165   Ackerly, 1989a; Ackerly, 1989b; Okamoto, 1988; Schindel, 1990; Checa & Aguado, 1992,
166   Hutchinson, 1999; McGhee, 1999). Since the development of theoretical shell models, almost all
167   simulated shell models have been made by an ad hoc approach, where the parameters are chosen
168   for the model and then the simulated shells are compared with the actual shells. In almost all
169   cases, the correct parameters are chosen after a series of trial-and-error, and the parameters are
170   selected when the form of the simulated shell matches the actual shell. Okamoto (1988)
171   suggested that this ad hoc approach based on pattern matching was easier than obtaining the
172   parameters empirically from the shell.
173

174   Third, although the overall forms of the simulated shells resemble the actual shells, the simulated
175   shell is not exactly the same as the actual shell (Kohn & Riggs, 1975; Goodfriend, 1983). For
176   many models, its original parameters are not sufficient to simulate the shell form exactly
177   (Schindel, 1990; Fowler, Meinhardt & Prusinkiewicz, 1992). These simulated general shell
178   forms are adequate for theoretical morphologist interests in their exploration of general shell
179   forms. However, the subtle features on a real shell or the subtle differences among different shell
180   forms of real species that cannot be simulated by theoretical models may have significant
181   functional implications that are important for empirical morphologists.
182

183   In brief, it is clear that the implementation of current theoretical shell models is less accessible to

184   empirical shell morphologists. Yet, empirical morphologists are using traditional and geometric

185   morphometrics as a routine method for shell quantification.

186

187   **Why empirical morphologists use traditional and geometric morphometrics**

188

189   In addition to the impediments arising from the theoretical shell model itself that are limiting its

190   popularity among empirical morphologists, the theoretical approach faces competition from

191   geometric morphometric methodology. The popularisation of desktop computing that led to the

192   flourishing of theoretical shell models in the late 1980s, also promoted the development of

193   morphometric methods, such as Elliptical Fourier Analysis (EFA) and geometric morphometrics

194   (GM). Rohlf and Archie (1984) set a benchmark for the quantification of an organism's form by

195   EFA, which was improved from Kaesler and Waters (1972) and Kuhl and Giardina (1982). Rohlf

196   and Slice (1990) and Bookstein (1991) developed a complete standard protocol for GM. Soon

197   after these pioneer papers, various software with Graphic User Interface (GUI) were developed

198   for the application of EFA and GM (Cardini & Loy, 2013, see http://life.bio.sunysb.edu/morph/).

199   In contrast to the application of theoretical shell models, an understanding of mathematics and

200   programming languages is not a prerequisite for the user of these morphometric tools. Thus, EFA

201   and GM have been well received by biologists, and have been adopted in the morphometric

202   study of shell form.

203

204   These geometric morphometric software packages have standard and interactive workflows that

205   help empirical morphologists in every step of: obtaining morphometric data (e.g. placing

206   landmark coordinates), analysing data (e.g. procrustes superimposition), statistical analysis (e.g.

207   ANOVA, PCA), and visualising shape and shape changes (e.g. thin-plate spline, PCA plots).

208   This has made geometric morphometrics approachable and attractive to empirical morphologists,

209   who want to examine the similarities and differences among shell forms.

210

211   Geometric morphometrics is actually a statistic of shape that is calculated from Cartesian

212   coordinate data from a sample of objects (Cardini & Loy, 2013). However, it is not an exact

213 quantification of form and is not particularly suitable for comparison and quantification of shell
214 form, for the following two reasons.

215

216 First, GM analysis is based on homologous landmarks on the form, but shell has only arbitrary
217 landmarks because it has a low degree of morphological complexity (Van Bocxlaer & Schultheiß
218 2010). There are no evolutionary homologies that can be defined as landmarks on a shell, since
219 the helical coiled tube offers no points that can be fixed across different individuals. In most
220 cases, 2D landmarks are chosen at the shell apex, suture, and aperture or whorl outline that can
221 be identified from a 2D image that is taken in standard apertural view of a shell. These
222 landmarks are chosen to be analysed by GM but these points have little biological meaning.
223 Furthermore, as opposed to the form of many other organisms, 3D landmarks are even more
224 difficult to be obtained from a shell (3D model) as compared to 2D landmarks because many of
225 these landmarks, such as suture points, that are obtained from a 2D image are just artefacts of the
226 fixed 2D view of the shell.

227

228 Second, the results of separate, independent studies of shell forms cannot be integrated, even
229 though these studies use the same GM method. Statistical analysis of the Cartesian coordinate
230 data that abstractly represent the shell form is adequate in quantifying the variation of a shell
231 within a context of other shells that are included in a single study or within similar taxa where
232 similar landmarks are obtained. However, the raw coordinate data and analysed shape variation
233 from a study are incomparable and incompatible with the data from other studies (Klingenberg,
234 2013). For examples, the raw data (coordinates) from two studies cannot be combined if they use
235 different landmarks and the shape variables (e.g. PCA scores) from a study cannot be compared
236 and analysed together with other studies.

237

238 Despite the fact that geometric morphometrics has been widely used by empirical morphologists,
239 it is not an ideal tool in the quantification of shell form for the reasons given above. The
240 increasing availability of the software and application in the literature might cause morphologists
241 to stray away from their initial aims of studying shell form. Hence, it is important to return to the
242 core of the question: what do biologists want to learn from the study of shell form? Clearly, in
243 addition to quantitatively compare shell forms, biologists want to know more about the general

Comment [points1]: First, there are no evolutionary homologies that can be defined as landmarks on a shell, since the helical coiled tube offers no points that can be fixed across different individuals. Thus, the raw data (coordinate) from two studies cannot be combined if they use different landmarks. Second, the shape variables (e.g. PCA scores) from a study cannot be compared and analysed together with other studies. The PCA scores of 1.025 in one study is NOT comparable to the value of 1.025 of any other study.

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Comment [points2]: In brief, no biologically meaningful landmarks can be identified from shells (biological meaningful landmark being the key in GM). This problem is reflected in the varying numbers of landmarks used in different studies. Hence, these landmark data from different studies cannot be analysed together.

244 characteristics and physical properties of the shell form that are key elements in gaining insight

245 into functional and ecological aspects of the shell (Evans, 2013). However, functional and

246 ecological aspects of shell form can only be determined if the shell form can be exactly

247 quantified.

248

249 **Using 3D technology to quantify shell form based on aperture ontogeny profiles**

250

251 In this paper, we propose an interactive approach to the quantification and analysis of shell forms

252 based on state of the art 3D technology and by integrating the theoretical principles of shell

253 modelling and the empirical principles of morphometric data handling. There are no theoretical

254 models that can simulate all existing shell forms. However, the theoretical background of the

255 theoretical models is biologically sound – simulating the shell form by simulating the shell

256 ontogenetic process. On the basis of this shell-ontogenesis principle, we used state-of-the-art X-

257 ray microtomography (micro-CT scan) and 3D modelling software to obtain a series of shell

258 aperture changes from the shell in an interactive workflow that is similar to empirical

259 morphometric analysis.

260

261 First, a series of shell aperture outlines were digitised directly from the reconstructed 3D shell

262 model obtained from micro-CT scanning by using open-source 3D-modelling software – Blender

263 ver. 2.63 (www.blender.org). Then, the growth trajectory and form of the shell aperture outline

264 were quantified and extracted with our custom scripts that run in Blender through its embedded

265 open-source Python interpreter (http://www.python.org/). The changes of aperture size and

266 shape, and aperture growth trajectory in terms of curvature and torsion along the shell ontogeny

267 axis length were obtained (hereafter "aperture ontogeny profiles"). The final aperture ontogeny

268 profiles are in a form of multivariate time series data, which consist of a number of instances (i.e.

269 number of quantified apertures that depends on the length of the whorled shell tube) and

270 attributes that represent the growth trajectories, aperture form, and size.

271

272 These aperture ontogeny profiles can be plotted when each shell is examined individually. On

273 the other hand, the aperture ontogeny profiles can be visually compared between different shells

274 by plotting the data as radar chart (i.e. spider and star plots). In addition, the differences between

275 shells can be assessed quantitatively by calculating the dissimilarity of aperture ontogeny profiles

276 among shells. Furthermore, the dissimilarity matrix can be used to plot the dendrogram and

277 NMDS plots, which resemble a shell morphospace. All our procedures were implemented by

278 using open source and free software.

279

280 Finally, we discuss some possible applications and implications of these shell form

281 quantification methods in theoretical morphology, functional morphology, taxonomy and shell

282 shape evolutionary studies.

283

284 **Materials and Methods**

285 **Ethics Statement**

286 Specimens were collected in Malaysia with permissions from the Economic Planning Unit,

287 Malaysia (UPE: 40/200/19/2524).

288

289 **Scanning instrumentation**

290 A micro-CT scanner (SkyScan, model 1172, Aartselaar, Belgium) and its accompanying

291 software, NRecon ver. 1.6.6.0 (Skyscan©) and CT Analyser ver. 1.12.0.0 (Skyscan©), were used

292 to generate digital shell 3D models from the actual shell specimens.

293

294 **Computation software and hardware**

295 Various commercial 3D modelling and statistical software exist for visualising, manipulating,

296 and understanding morphology, such as Amira® (Visage Imaging Inc., San Diego, CA) and

297 Autodesk Maya (San Rafael, CA) (reviewed by Abel, Laurini & Richter, 2012). However, in this

298 study, we used only two open-source 3D data modelling and processing software packages,

299 namely Blender ver. 2.63 (www.blender.org) and Meshlab ver. 1.3.2 (Cignoni, Corsini &

300 Ranzuglia, 2008, http://meshlab.sourceforge.net/). Both have been used in biology to visualise

301 and model morphology (for Meshlab: Im et al., 2012; Chaplin, Yu & Ros, 2013; Atwood &

302 Sumrall, 2012; for Blender: Pyka et al., 2010: 22); Haug, Maas & Waloszek, 2009; Cassola et

303 al., 2010; Haug et al., 2010; Andrei et al., 2012; Haug et al., 2012; Lv et al., 2013; Mayer et al.,

304 2012). However, these programs have not been used to their full extent in morphological

305 quantification and analysis of 3D data for organisms. For quantification of morphology, we used

306   the open-source Python interpreter ver. 3.2 that is embedded in Blender 2.63. In addition, we also

307   used an extension to the Python programming language – NumPy (Oliphant, 2007) which

308   consists of high-level mathematical functions.

309

310   All the morphological data were explored and analysed with the statistical open source

311   programming language R version 3.0.1 (R Core Team, 2013) in the environment of RStudio

312   (RStudio, 2012). We installed three additional packages in R, namely, "lattice": Lattice Graphics

313   (Sarkar, 2008), "pdc": Permutation Distribution Clustering (Brandmaier, 2012a; Brandmaier,

314   2012b), and "fmsb" (Nakazawa, 2010).

315

316   All the computation analyses were carried out with a regular laptop computer with the following

317   specifications: Intel®Core™i7-3612QM @ 2.1GHz, 8 GB memory (RAM), NVIDIA® GeForce

318   GT 630M with 2GB memory.

319

320   **Procedures**

321   **1. Obtaining digital 3D models from actual shells**

322   The scan conditions were as follows: voltage – 80kV or 100kV; pixel – 1336 rows × 2000

323   columns; camera binning – 2 × 2; image pixel size – 3–6 μm; rotation step – 0.4° or 0.5°; and

324   rotation – 360°. Next, the volume reconstruction on the acquired images was done in NRecon.

325   The images were aligned to the reference scan and reconstruction was done on the following

326   settings: beam hardening correction – 100%; reconstruction angular range – 360 degree;

327   minimum and maximum for CS to image conversion (dynamic range) – ca. 0.12 and ca. 20.0;

328   and result file type – BMP. Finally, 3D models were created from the reconstruction images in

329   CT Analyser with the following setting: binary image index – 1 to 255 or 70 to 255; and were

330   saved as digital polygon mesh object (*.PLY format).

331

332   **2. Pre-processing digital shell models**

333   The 3D models were then simplified by quadric edge collapse decimation implemented in

334   MeshLab (Cignoni, Corsini & Ranzuglia, 2008) to reduce computation requirements. The raw

335   polygon mesh shells in PLY format have millions of faces and a file size between 20 to 80

336   Mbytes. Thus, we reduced the number of faces for all model to 200,000 – 300,000 faces, which

337   range between 3 and 6 Mbytes in file size. In addition, for the sake of convenience during the

338   retopology processes, all 3D models were repositioned so that the shell protoconch columella

339   was parallel with the z-axis. This was done by using manipulator tools in MeshLab.

340

341   **3. Creating reference: Tracing aperture outlines and ontogeny axis from shell models** (Supplementary

342   Information File 1)

343   The digital shell 3D model in PLY format consists of 3D Cartesian coordinate vertices in which

344   each of the three vertices constitutes a triangular face, and all faces are connected through a

345   complex network. In order words, these vertices and faces are not biologically meaningful

346   structures, and it is not possible to extract aperture outlines data directly from a raw PLY digital

347   shell model. Monnet et al. (2009), for example, attempted to extract aperture outline

348   automatically from a digital 3D model by making a plane cross-sectioning of the shell model, but

349   its outlines do not reflect the form of the actual aperture outlines. Hence, we retopologised the

350   raw 3D mesh models according to the aperture ontogeny for later data extraction.

351

352   We used Blender, which is more flexible than the commercial software used by Monnet et al.

353   (2009). For the sake of convenience, we describe the following workflow, including the tools or

354   the function (e.g. "Import PLY") which can be called after hitting the SPACE bar while in the

355   Blender environment. However, this workflow may be modified by the user.

356

357   To begin, we imported a PLY shell model into the Blender environment ("Import PLY"). Then,

358   we resized the model 1000 × ("Resize") so that the scale of 1 Blender unit was equal to 1 mm.

359   After that, we examined the traces of aperture outlines (i.e. growth lines, ribs, spines) (Figure

360   1A) and ontogeny axis (i.e. spiral striation, ridges, colour lines) (Figure 1B) of the actual shells.

361   However, it is not possible to trace apertures from the shell protoconch because the protoconch

362   is an embryonic shell that may not grow accretionarily and usually has no growth lines. In many

363   cases, the aperture of the overlapping whorls that could cannot be traced from the outer shell

364   wall. One of the ways to deal with this situation is to trace the aperture at the inner shell wall and

365   the obscured aperture outline can then be inferred by studying the conspecific juvenile specimens

366   (see video tutorial 05:00–08:00 of Supplementary Information File 1).

367

368 After these aperture traits were identified, we selected the 3D model (by clicking "right mouse
369 button"), and traced all these traits on the surface of the raw 3D mesh model in Blender by using
370 the "Grease Pen Draw" tool. After that, the grease pen traced aperture traits were converted to
371 Bezier curves with "Convert Grease Pencil" (Figure 1C).
372 We would like to emphasise that this is the most critical step that determines the efficiency of
373 this shell quantification method. Thus, the key lies ~~o~~in the good understanding of the way the
374 aperture is structured, which is essential to trace the aperture outlines accurately. However, the
375 orientation of the shell when the aperture is digitalised would not influence the aperture ontogeny
376 data.
377
378 **4. Retopologising aperture outlines from the reference and generating retopologised shell models**
379 (Supplementary Information File 1 and File 4)
380 For each shell, we created a set of new Non Uniform Rational B-Splines (NURBS) surface
381 circles ("Add Surface Circle") and modified these ("Toggle Editmode") according to the aperture
382 outlines. We created a 16 points NURBS surface circle and aligned the circle to the aperture
383 outline by translation ("Translate"), rotation ("Rotate"), and resizing ("Resize") (Figure 1D).
384 After the NURBS surface circle was generally aligned, each of the 16 points of the NURBS
385 surface circle were selected and adjusted by translation ("G") one by one, so that the outline of
386 the NURBS surface circle was exactly the same as the aperture outline. At the same time, the
387 second point of the NURBS surface circle was aligned to the ontogeny axis (Figures 1B and 1C).
388
389 After the first aperture outline was retopologised as a NURBS surface circle, the NURBS surface
390 circle was duplicated ("Duplicate Objects") and aligned to the next aperture outline as the
391 previous one. This step was repeated until all the aperture outlines were retopologised into
392 NURBS surface circles (Figures 1D and 1E). Then the shell surface was created in the form of a
393 NURBS surface based on the digitised aperture NURBS surface circle ("(De)select All" and
394 "Make Segment" in "Toggle Editmode") (Figures 1F and 1G). Lastly, we made the surface meet
395 the end points in U direction and increased the surface subdivision per segment (resolution U =
396 8) through the properties menu of the object (Properties (Editor types)>Object Data>Active
397 Spline).
398

399 After that, we converted the NURBS surface 3D model into a 3D Mesh model that consists of
400 vertices, edges, and faces ("Convert to" - "Mesh from Curve/Meta/Surf/Text"). The final
401 retopologised 3D shell Mesh consists of X number of apertures outlines and each aperture
402 outline has Y number of vertices and then a total of X*Y vertices. Each of the vertices is
403 connected to four other nearest vertices with edges to form a wireframe shell and face (Figure
404 1H).

405

406 It is important to note that the NURBS surface circle is defined by a mathematic formula which
407 does not imply any biology perspective of the shell. We choose NURBS surface circle because
408 the 3D aperture outline form can be digitalised by a small number of control points and shell
409 surface can be recreated by NURBS surface based on the digitised aperture NURBS surface
410 circle. The final 3D polygon mesh model is more simplified than the raw PLY 3D model and
411 each of its vertex data resemble the actual accretionary process of the shell (Figures 1A and 1H).

412

413 **5. Quantifying aperture growth trajectory**
414 The aperture ontogeny profiles were quantified as described in Liew et al. (2014) with slight
415 modifications where both aperture growth trajectory and aperture form were quantified directly
416 from the retopologised 3D shell model. This aperture growth trajectory was quantified as a
417 spatial curve, which is the ontogeny axis as represented by a series of first points of the aperture
418 outlines. We estimated two differential geometry parameters, namely, curvature (κ) torsion (τ),
419 and ontogeny axis length for all apertures (Okamoto, 1988; Harary & Tal, 2011). The local
420 curvature and torsion, and accumulative ontogeny axis length were estimated from the aperture
421 points along the growth trajectory by using weighted least-squares fitting and local arc length
422 approximation (Lewiner et al., 2005). All the calculations were done with a custom-written
423 Python script which can be implemented in Python interpreter in the Blender ver. 2.63
424 environment. The whole workflow was: (1) selecting the retopologised 3D shell Mesh (by
425 clicking "right mouse button"), (2) input parameters for number of sample points "q = ##" in the
426 python script, and (3) paste the script into the Python interpreter (Supplementary Information
427 File 2). The final outputs with torsion, curvature and ontogeny axis reference for each aperture
428 were saved as CSV files.

429

430   We found a convergence issue in curvature and torsion estimators. The accuracy of the curvature

431   and torsion estimates depends on the number and density of the vertices in the ontogeny axis (i.e.

432   number of aperture outlines), and the number of sample points. Nevertheless, different numbers

433   of sample points can be adjusted until good (i.e. converged) curvature and torsion estimates are

434   obtained. We used 10% of the total points as number of sample points of the ontogeny axis,

435   which gave reasonably good estimates for curvature and torsion.

436

437   Notwithstanding the algorithm issue, the curvature and torsion estimators are informative in

438   describing the shell spiral geometry growth trajectory. Curvature is always larger or equal to zero

439   ($\kappa \geq 0$). When $\kappa = 0$, the spatial curve is a straight line; the larger the curvature, the smaller the

440   radius of curvature ($1/\kappa$), and thus the more tightly coiled the spatial curve. On the other hand,

441   the torsion estimator can be zero or take either negative or positive values ($-\infty \leq \tau \leq \infty$). When τ

442    $= 0$, the spatial curve lies completely in one plane (e.g. a flat planispiral shell), negative torsion

443   values correspond to left-handed coiling and to right-handed coiling for positive torsion values;

444   the larger the torsion, the smaller the radius of torsion ($1/\tau$), and thus the taller the spiral.

445

446   **6. Quantifying aperture form**

447

448   We quantified the aperture outline sizes as perimeter and form as normalised Elliptic Fourier

449   coefficients (normalised EFA) by using a custom-written Python script which can be

450   implemented Python interpreter embedded in the Blender environment. The workflow was (1)

451   selecting the retopologised 3D shell mesh (by clicking "right mouse button"), (2) input

452   parameters for "number_of_points_for_each_aperture = ##" in the python script, and (3) paste

453   the script into the Python interpreter of Blender (Supplementary Information File 2). The final

454   outputs were saved as CSV files.

455

456   Aperture outline perimeter was estimated from the sum of lengths (mm) for all the edges that are

457   connecting the vertices (hereafter "aperture size"). For aperture form analysis, we used 3D

458   normalised EFA algorithms (Godefroy et al., 2012) and implemented these in the custom python

459   script. Although many algorithms exist for describing and quantifying the form of a closed

460   outline (Claude, 2008), we used EFA because it is robust to unequally spaced points, can be

461   normalised for size and orientation, and can capture complex outline form with a small number

15

462 of harmonics (Rohlf & Archie, 1984; Godefroy et al., 2012). In this study, we used five

463 harmonics, each with six coefficients which were sufficient to capture the diverse aperture

464 shapes of our shells. For quantification of apertures shape that are invariant to size and rotation,

465 we normalised EFA of aperture outlines for orientation and size. If needed for comparison with

466 other studies, the normalised EFA can be repeated for the same dataset with higher or lower

467 numbers of harmonics.

468

469 After normalisation, we ran principal components analysis (PCA) to summarise the 30

470 normalised Fourier coefficients as principal components scores (hereafter "aperture shape

471 scores"). After that, we selected the major principal components (explaining > 90 % of the

472 variance) for further analysis. The aperture shape scores of each selected principal component

473 were plotted and analysed against the ontogeny axis.

474

475 **7. Visualising aperture form and trajectory changes along the shell ontogeny**

476 For exploration of data, we used two graphical techniques for representing aperture ontogeny

477 profile changes along the shell ontogeny. For each shell, we made a vertical four-panels scatter

478 plot in which each of the four variables (namely, curvature, torsion, aperture size, and the first

479 principal component aperture shape score) were plotted against the ontogeny axis. When

480 necessary, the second and third principal component aperture shape scores were also included. In

481 addition, the axis of each variable was rescaled so that it was the same for the same variable of

482 all shells. After standardisation of the axis, the aperture ontogeny profiles of several shells could

483 be quantitatively compared side by side.

484

485 However, comparison of between plots would become less effective with a larger number of

486 shells. Alternatively, therefore, all aperture ontogeny profile variables of each shell can also be

487 represented in a radar chart, instead of scatter plots. This chart is effective in showing the

488 variable outliers within a chart and the overall similarity between charts. Before plotting the data

489 in a radar chart, the datasets of all shells need to be restructured because the dataset of different

490 shells could differ in the number of data points (i.e. quantified aperture), which depends on the

491 ontogeny axis length of each shell.

492

493 We did this by dividing the ontogeny axis of each shell into 20 equal length intervals, and then

494 by sampling the variable values at the end of every interval. In the restructured dataset, the trend

495 of the aperture ontogeny profile of each variable is retained and all radar charts have the same

496 number of data points. Thus, the changes of aperture variables between each subsequent 1/20 of

497 the ontogeny axis can be examined within a shell and be compared among different shells in a

498 synchronistic manner. We suggest to use 20 points to summarise hundreds variable points of the

499 aperture ontogeny profile variables along ontogeny axis because the radar would be

500 overwhelming with too many points and hard to interpret. Similar to the scatter plot, we

501 standardised the axis scales of each variable of all radar charts.

502

503 In addition, we added a new variable which represents the ontogeny axis interval length in order

504 to compensate for the loss of shell size information during the standardisation of ontogeny axis

505 length. Finally, we plotted the variables, namely, curvature, torsion, aperture size, and ontogeny

506 axis length, and aperture shape scores in a radar chart for each shell by using the "fmsb" library

507 (Nakazawa, 2010) with R version 3.0.1 (R Core Team, 2013) (Supplementary Information File

508 5).

509

510 **8. Quantitative comparison between shell forms**

511 In addition to the qualitative comparison between shells forms as described above, the

512 dissimilarity between different shells can be analysed quantitatively. We used Permutation

513 Distribution Clustering (PDC) which finds similarities in a time series dataset (Brandmaier,

514 2012a; Brandmaier, 2012b). PDC can be used for the analysis of the changes in a variable along

515 shell ontogeny between different shells (i.e. two-dimensional dataset: number of shells × number

516 of apertures) and multiple variable changes between shells (i.e. three-dimensional dataset:

517 number of shells × number of variables × number of apertures). We applied the most recent

518 analysis developed by Brandmaier (2012a & b) because it has an R package that can be applied

519 and can calculate the trend similarity. That said, the same data can always be analysed by other

520 "better" algorithms in the future.

521

522 Although PDC is robust to the length differences between datasets, our preliminary analysis

523 showed that the PDC output would be biased when there was a great (around two-fold) length

524   difference in the total ontogeny axis length. Hence, we standardised the data as in procedure 7,

525   but dividing the ontogeny axis of each shell into 50, instead of 20, equal length intervals. This

526   standardisation procedure allows comparison of trends in variable changes along the shell

527   ontogeny without the influences of size. In other words, the dissimilarity is zero between two

528   shells that have exactly the same shape, but differ only in size. In addition to the shape

529   comparison, we obtained the shell size in terms of volume by using "Volume" function in

530   Blender after the 3D shell model was closed at both ends by creating faces "Make edge/Face")

531   on selected apertures at both end ("Loop Select") in EDIT mode.

532

533   The aperture ontogeny profiles of all shells were combined into a three-dimensional data matrix

534   consisting of n shells $\times$ four variables $\times$ 50 aperture data points. We ran four PDCs, each for the

535   five data matrices with: 1) all four variables, 2) torsion, 3) curvature, 4) aperture size, and 5)

536   aperture shape scores. The parameter settings for the PDC analysis were as follows: embedding

537   dimension = 5; time-delay of the embedding = 1; divergence measure between discrete

538   distributions = symmetric alpha divergence; and hierarchical clustering linkage method = single.

539   The dissimilarity distances between shells were used to produce the dendrogram. PDC analysis

540   was performed with the "pdc" library (Brandmaier, 2012b) in R version 3.0.1 (R Core Team,

541   2013) (Supplementary Information File 5).

542

543   In addition to the dendrogram representation of the output from PDC, we plotted the

544   dissimilarity as a non-metric multidimensional scaling (NMDS) plot which resembles a

545   morphospace. NMDS was performed by using "MASS" library (Venables & Ripley, 2002) in R

546   version 3.0.1 (R Core Team, 2013) (Supplementary Information File 5).

547

548   **Worked example: Comparative analysis of *Opisthostoma* and *Plectostoma* species shell form**

549   **and simulated shell form**

550   We evaluated the above-described shell form quantification method by using the shells of

551   *Opisthostoma* and *Plectostoma*, which exhibit a great variability in shell form. Some of the

552   species follow a regular coiling regime whereas others deviate from regular coiling in various

553   degrees. It remains a challenging task to quantify and compare these shell forms among species,

18

554 either by using traditional or geometric morphometrics, because a standard aperture view for the
555 irregular and open coiled shells cannot be determined.

556

557 We selected four species, namely, *Plectostoma laidlawi* Skyes 1902 (Figure 2A), *Plectostoma*
558 *crassipupa* van Benthem Jutting, 1952 (Figure 2B), *Plectostoma christae* Maassen 2001 (Figure
559 2C), and *Opisthostoma vermiculum* Clements and Vermeulen, 2008 (in Clements et al., 2008)
560 (Figure 2D), for which the shell forms are, respectively: regularly coiled, slight distortion of the
561 last whorl, strong distortion of the last whorl, and complete distortion of most of the whorls.
562 Despite the narrow taxonomyic range of the selected species, the range of shell forms of these
563 four species do cover a very large diversity of shell form. We retopologised these four shells by
564 following the procedures 1 to 4 (Supplementary Information Files 6).

565

566 In addition to the four retopologised 3D shell models, we manually created another four shell
567 models by transforming three out of the four retopologised NURBS surface 3D shell models by
568 using the "Transform" function in Blender. These models are: 1) *Plectostoma laidlawi* that was
569 resized to half the original size and given slight modification of the aperture size (Figure 2E); 2)
570 *Plectostoma christae* that was reshaped into an elongated form by reducing the model size (linear
571 dimension) to one-half along the x and y axes, and by doubling the size along the z axis (Figure
572 2F); 3) *Plectostoma christae* that was reshaped into a depressed form by multiplying by 1.5 the
573 model size along the x and y axes, and by reducing to one-half along the z axis (Figure 2G); and
574 4) *Opisthostoma vermiculum* that consists of one *Opisthostoma vermiculum* original 3D model of
575 which we connected the aperture to another, enlarged, *Opisthostoma vermiculum* (Figure 2H).
576 Finally, we analysed all these eight shell models by following the procedures 5 to 8.

577

578 **Results and Discussion**
579 **Retopologied 3D shell models**
580 All the final retopologised 3D shell models can be found in Supplementary Information (Files 7
581 to 14) in PLY ASCII mesh format, with the raw data as a list of vertices, followed by a list of
582 polygons, which can be accessed directly without the need of any 3D software. Each vertex is
583 represented by x, y, z coordinates. Each polygon face consists of four vertices. This simplified
584 yet biologically informative 3D mesh shell model allows the quantification of aperture form and

Comment [points7]: A wide taxonomy range of species evaluated does not necessarily mean a wide morphological range of shell shapes. The focus of this manuscript is to evaluate the method on shells that have regular as well as irregular shell forms. The range of shell forms that we used in our evaluation do cover a broad diversity of form.

The range of shell forms that we used in our evaluation do cover a very large diversity of shell form. And we demonstrated that the shell form can be quantified as the same set aperture ontogeny profile, regardless of the unconventionality of shell form.

585 growth trajectory. Moreover, the 3D shell models and their raw vertices data could potentially be

586 used in studies of functional morphology and theoretical modelling of shell form, respectively.

587

588 Malacologists have been focusing on empirical shell morphological data, from which the

589 functional, ecological and evolutionary aspects were then extracted. The physical properties were

590 then determined by its form (e.g. Okajima & Chiba 2011; Okajima & Chiba, 2012). By using the

591 3D models, the shell properties and function can be analysed *in silico*. For example, the thickness

592 of the shell can be added to the 3D shell model (Figure 3E and Figure 3F) in order to obtain the

593 shell material's volume, the shell's inner volume, its inner and outer surface area, and centre of

594 gravity. We used the "build" function of the software, which can only "solidify" the model by

595 uniform thickness. However, if necessary, it is possible to write a custom Python script to add

596 the desired thickness to the shell. Quantification of shell properties may then be done by using

597 the geometry approach in Meshlab or Blender, as compared to the pre-3D era where

598 mathematical descriptions of the shell form were required (e.g. Moseley, 1838; Raup & Graus,

599 1972; Stone, 1997). Furthermore, it is possible to convert the 3D models to a 3D finite element

600 (FE) model, of which the physical properties (e.g. strength) can be tested (e.g. Faghih Shojaei et

601 al., 2012).

602

603 In addition to the potential use of 3D shell models in functional morphology, the coordinate data

604 of the vertices of 3D shell models could be used directly by theoretical morphologists (see Figure

605 1 in Urdy et al., 2010). For example, these data can be extracted in different formats that fit the

606 data requirement of different types of theoretical shell models, namely, generating curve models

607 using a fixed reference frame or moving reference frame (Figure 3C), helicospiral or multivector

608 helicospiral models using a fixed reference frame (Figure 3A, Figure 3B and Figure 3D) or

609 growth vector models using a moving reference frame (Figure 3A and Figure 3B).

610

611 The retopologising of the aperture ontogeny from a raw 3D shell model (procedures 1 to 4) is a

612 time-consuming and tedious process compared with traditional and geometric morphometrics.

613 There are no differences in the time required for data analysis between GM and our method. The

614 only time differences are in the data acquisition. ~~From~~In our experience, two to three days are

615 needed to collect the aperture data from the shell. For example, the four shell models were

Comment [points8]: Yes, that is true. We used the "build" function of the software, which can only "solidify" the model by uniform thickness. However, if necessary, it is possible to write a custom Python script to add the desired thickness to the shell.

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Comment [points9]: We would like to emphasise here that there are no differences in the time required for data analysis between GM and our method. The only time differences are in the data **acquisition**. From our experience, two to three days are needed to collect the aperture data from the shell. In our case, we aligned a total ca. 1500 points for each shell (ca.16 points for each of the ca. 90 apertures, see also lines 307-308). From the viewpoint of short-term cost-benefit balance, this may be seen as a waste of time, because GM requires not more than a few dozen points for each shell, which can generate the shape variables for within a study, even though these points are not comparable to other points of other shells or other studies.
However, in the long run, it is a good time investment, since it will allow the understanding of shell function, growth, and evolution, as the same set of data is obtained from different shell forms and can be accumulated and analysed together. Moreover, as will all newly-developed techniques, improvements in efficiency and automation are possible and may remove these impediments in the future.

616 created by retopologising between 73 and 96 separate apertures. (ca. 1500 points for 90

617 apertures). Nevertheless, the final product of a clean 3D shell mesh is versatile for many

618 different kinds of analysis and thus has great potential for improving our understanding of shell

619 form.From the viewpoint of short-term cost-benefit balance, this may be seen as a waste of time,

620 because GM requires not more than a few dozen points for each shell, which can generate the

621 shape variables for within a study, even though these points are not comparable to other points of

622 other shells or other studies. However, in the long run, it is a good time investment, since it will

623 allow the understanding of shell function, growth, and evolution, as the same set of data is

624 obtained from different shell forms and can be accumulated and analysed together. Moreover, as

625 willth all newly-developed techniques, improvements in efficiency and automation are possible

626 and may remove these impediments in the future.

627

628 **Comparing shell form from the view of shell ontogeny**

629 Figure 4 gives an overview of the aperture ontogeny profile and shell volume for each species.

630 The curvature, torsion perimeter, and ontogeny axis are represented by true numerical values

631 with the unit of mm^{-1} and mm, and thus can be interpreted directly. In contrast, the aperture

632 shape scores are just statistics of Fourier coefficients and are not the absolute quantification of

633 aperture shape. The PCA score of an aperture shape depends on the shape of other aperture

634 outlines and thus it might change whenever other aperture outlines are added into the analysis.

635 Nevertheless, the aperture scores will stabilise as data of more shells become available and when

636 most of the extreme aperture forms are included. In this study, the first principal component

637 explained 92% of the total variance; the second and third principal component explained only

638 3% or 1% of the total variance. We showed that the shell form can be represented by the

639 ontogeny changes of the aperture growth trajectory in terms of curvature and torsion, and

640 aperture form, in terms of perimeter and shape.

641

642 Our first example evaluates this method in illustrating the differences between two shells that

643 have the same shape but differ in shell size – the half-size *Plectostoma laidlawi* (Figure 4E) shell

644 and the original *Plectostoma laidlawi* shell (Figure 4C). As revealed by their aperture ontogeny

645 profiles, the size difference between the two shells has had an effect on the curvature, torsion,

646 ontogeny axis length and aperture size. For the resized *Plectostoma laidlawi* shell, the values of

21

647   curvature and torsion are twice as large as for the original, whereas the ontogeny axis length and

648   aperture size are only half those of the original shell. However, there is no discrepancy in the

649   aperture shape statistics. Despite this scalar effect, the overall trends in the changes of these

650   variables along the ontogeny axis are comparable between these two shells (Figure 6B).

651

652   Another example shows the ontogeny profiles of three shells, namely, the elongated (Figure 4G),

653   depressed (Figure 4H), and original (Figure 4A) versions of the *Plectostoma christae* shell.

654   Comparison of aperture profiles among these show the most obvious discrepancies in greater

655   torsion values for the elongated shell, which change in a more dramatic trend along the shell

656   ontogeny. In addition, each of the three shells has its unique aperture shape scores, though there

657   are no big discrepancies in the aperture size. The differences in ontogeny axis length, curvature

658   and torsion are related to the differences of the aperture shape statistics among the three shells.

659   However, our small dataset with only three shells is not sufficient for thorough disentangling of

660   the interplay between aperture size, shape, and growth trajectory in relation to the shell form.

661

662   Our last example is the comparison between the original (Figure 4D) and the composite (Figure

663   4F) *Opisthostoma vermiculum* shell . It is clear that our method has high sensitivity and

664   robustness in the analysis of such bizarre shell forms. As shown in Figure 4F, the start of the

665   aperture ontogeny profile of the composite shell was the same as for the original shell (Figure

666   4D). In addition, the later parts of the ontogeny profile trends are still comparable to the first

667   part, but different in value because of the scalar effect.

668

669   As an alternative visualisation, Figure 5 shows the radar charts that summarise the same aperture

670   ontogeny profiles of each species. The polygon edges in each chart show how dramatically the

671   aperture form (size and shape), and growth trajectory (curvature and torsion) are changing at

672   each of the subsequent 5% intervals of the shell ontogeny. The aperture size (mm) and the

673   ontogeny segment length (mm) variables indicate the shell size (i.e. volume). To illustrate this,

674   aperture size and ontogeny axis length can be seen as the circle size and height of a cylinder.

675   This chart is useful for the visual comparison between shells that are similar in size, for example,

676   *Plectostoma christae* (2.43 mm^3), *Plectostoma laidlawi* (2.39 mm^3), and the depressed

677   *Plectostoma christae* (2.73 mm^3). The radar chart shows that (1) the depressed *Plectostoma*

678   *christae* is the largest and has a very different aperture shape as compared to the other two shells;

679   (2) most of the shell whorls' form of *Plectostoma christae* is very similar to *Plectostoma*

680   *laidlawi* (i.e. most of the polygons in the chart were similar), but the *Plectostoma laidlawi* shell

681   differs from *Plectostoma christae* shell by having distorted whorls at the last part of the shell

682   ontogeny (magenta lines at torsion) and a more open umbilicus at the beginning of the shell

683   ontogeny (red lines at curvature and aperture size).

684

685   However, comparison of radar charts between shells that differ greatly in size would be less

686   informative. For example, the radar charts between the resized *Plectostoma laidlawi* shell and

687   the original *Plectostoma laidlawi* shell are very different, though the resized one has the same

688   shell shape as the original. The difference in radar charts between the two shells was therefore

689   mainly caused by the size difference.

690

691   As we have shown in both graphical techniques (Figures 4 and 5), the shell forms can be

692   explored and compared qualitatively on the basis of aperture ontogeny profiles. Users might need

693   some training in the interpretation of the plots because they are different from both linear

694   dimension measurement plots and geometric morphometric shape coordinate plots. Our

695   evaluation suggested that both data visualisation methods are sensitive and robust in capturing

696   the aperture ontogeny profile for any shell form and thus make the qualitative comparison across

697   gastropod taxa and studies possible.

698

699   This method could be applied in malacological taxonomy because its core business is the

700   description of shell form. Despite hundreds of years of taxonomic history of shells, there has

701   been little change in the way shell form is being described. For example, shell from is usually

702   described in terms of linear dimensions: shell width and height; number of whorls; shell shape –

703   flat, depressed, globose, conical, or elongated; whorls shape – from flat to convex. Here, we

704   suggest that the aperture ontogeny profiles would be a great supplement to the classical approach

705   to shell description. For example: (1) the size of the shell (its volume) depends on the ontogeny

706   axis length and aperture size; (2) the shell shape depends on the growth trajectory in terms of

707   curvature and torsion; (3) the shape of the whorls depends on the shape of the aperture (Figure

708   4). In our case of the four shells (Figures 2A – 2D), it is clear that aperture size of each shell is

23

709   constricted at roughly the same part of the respective shell ontogeny, namely between 70% and

710   85%, regardless of the dissimilar shell sizes and shapes (Figures 4A – 4D, and aperture size

711   profiles in Figure 5B). In fact, these aperture size decreases during ontogeny are in accordance

712   with the shell constriction, one of the shell characters that have been used in the taxonomy of

713   *Opisthostoma* and *Plectostoma* (Vermeulen, 1994; Liew et al., 2014). However, the shell

714   constriction has not been quantified previously, and we show that it could also be an important

715   developmental homology for the two genera. This preliminary results suggest that these aperture

716   ontogeny profiles could aid the taxonomist in decision-making for grouping taxa based on

717   homologous characters.

718

719   **Quantitative comparison between different shell forms**

720   Figure 6 shows dendrograms resulting from a permutation distribution clustering analysis of the

721   eight shells in terms of their aperture ontogeny profiles. Figure 6A shows the hierarchical

722   clustering of the eight shells based on all four aperture ontogeny profiles. From this dendrogram,

723   the composite *Opisthostoma vermiculum* is completely separate from the other shells. The

724   remaining seven shells are clustered into two groups. One consists of the more regularly coiled

725   shells, namely, *Plectostoma christae* and its two transformed shells, and *Plectostoma crassipupa*;

726   the other group consists of the shells that deviate from regular coiling, namely *Plectostoma*

727   *laidlawi* and its transformed shell, and *Opisthostoma vermiculum*. Nevertheless, there were high

728   dissimilarities between shells within each group as revealed by the long branch lengths in Figure

729   6A, except for the two *Plectostoma laidlawi* shells (Table 1). The aperture ontogeny profiles for

730   the *Plectostoma laidlawi* shell and its reduced version are almost the same. The high

731   dissimilarity among the other six shells can be explained when each of the variables in the

732   aperture ontogeny profile is analysed separately as shown in Figure 6B.

733

734   Figure 6B shows the dendrograms of aperture ontogeny profiles for each of the four variables.

735   All four dendrograms have a different topology than the one in Figure 6A. Among the variables,

736   the aperture ontogeny profile of the curvature has the smallest discrepancies among shells. The

737   two *Plectostoma laidlawi* shells are the only pair that clusters together in all the dendrograms of

738   Figures 6A and 6B because they are identical in every aspect of aperture ontogeny profile except

739   torsion. Hence, the independent analysis of aperture ontogeny profile variables corresponds well

740   to the overall analysis of aperture ontogeny profiles.

741

742   Figure 7 shows a three-dimensional NMDS plot of the distance matrix (Table 1) that was

743   generated from PDC analysis on all four aperture ontogeny profiles. The very low stress level

744   (0.000) indicates that this 3D plot is sufficient to represent the distance matrix of the aperture

745   ontogeny profiles. This NMDS plot can therefore be regarded as a morphospace of the shell

746   shape, as derived from aperture ontogeny profiles. However, neither the dendrogram nor the

747   NMDS plot contains information about the shell size because the analysis of PDC is based on the

748   standardised ontogeny profiles and their trends. Thus, both plots are useful for the comparative

749   analysis of shell shape, but not shell size. Nevertheless, the size comparison between shells is

750   rather straightforward.

751

752   The conventional quantification of shell size is based on the linear measurement of two or three

753   dimensions of a shell, for example, shell height and shell width. Thisese measurements are

754   extremely effective for size comparisons between similarly-shapes shells. However, the linear

755   measurements have limitations when comparison is made between shells that are different in

756   shape. For example, shell height comparison between a discoidal shell and a fusiform shell tells

757   very little about size differences because the dimensional measurements are tied to a shell shape

758   that results from a different coiling strategy.

759

760   HoweverThus, shell size may be more appropriately given as shell volume, which can be

761   estimated easily from retopologised 3D shell models (Figure 4). This quantification of shell size

762   in terms of volume is more meaningful from the functional and developmental point of view

763   because a snail should grow a shell in which its entire soft body can fit when the snail withdraws

764   into the shell. In addition to the exact volume, Aa shorthand to qualitatively comparing size

765   between two shells is by examining the ontogeny axis length and aperture size in the radar chart

766   (Figure 5). We can then compare the form between shells when the dendrograms or NMDS plot

767   are interpreted together with shell size (volume) data. For example, the *Plectostoma laidlawi*

768   shell has the same shape as, but is eight times larger than, the resized *Plectostoma laidlawi*.

769

770 In addition to the construction of morphospace, the dissimilarity matrix can be used in

771 phylogenetic signal tests (Hardy & Pavoine, 2012). Furthermore, it can also be analysed together

772 with other distance matrices, such as for geographical or ecological distance, to improve our

773 understanding of the evolutionary biology of shell forms.

774

775 **Conclusions, limitations and future directions**

776 We demonstrated an alternative workflow for data acquisition, exploration and quantitative

777 analysis of shell form. This method has several advantages: (1) robustness – this method can be

778 used to compare any shell form: The same aperture profiles can be obtained from any form of

779 shell. Then, these profiles from different shells and/or different studies can be analysed together.

780 These parameters can be obtained from the aperture as long as the shell grows accretionarily at

781 the aperture; (2) scalability and reproducibility – the data obtained from different studies and

782 different gastropod taxa can be integrated: Aperture ontogeny profiles were obtained from the

783 aperture outlines. This is a trait that exists in every gastropod shell. We believe that the aperture

784 outline that is obtained by multiple experienced malacologists, on different shells, would be

785 highly similar; (3) versatility – the raw 3D shell mesh models, coordinates data of the vertices,

786 aperture ontogeny profiles, and dissimilarity matrix between shell forms are comply with the

787 data standard that is required can be used for in taxonomy, functional morphology, theoretical

788 modelling, and evolutionary studies.

789

790 Yet, our method has its limitations. Firstly, our retopology procedures rely on a 3D shell model

791 that requires CT-scan technology. In fact, although a CT-scan 3D shell model can certainly

792 facilitate the retopology process of a shell, it is not indispensable. The key of the retopology

793 processes is to digitise the aperture along the shell ontogeny, and thus a shell can be

794 retopologised fully in Blender with a good understanding of the aperture ontogeny profiles by

795 studying the real specimens even without a reference shell model. Secondly, the retopology

796 procedure which is essential for our data acquisition is more time-consuming than traditional and

797 geometric morphometric where data can be obtained from an image taken from a shell. Thirdly,

798 our method is effective in the analysis of overall shell form, but not of the shell ornamentation.

799

26

Comment [points11]: Robustness – The same aperture profiles can be obtained from any form of shell. Then, these profiles from different shells and/or different studies can be analysed together. These parameters can be obtained from the aperture as long as the shell grows accretionarily at the aperture. On the other hand, studies that use GM for different shells, species, and/or studies do not use the same set of the landmarks because the "same" or "homologous" landmarks do not exist in shells – see lines 174 – 203. **Reproducibility** – Aperture ontogeny profiles were obtained from the aperture outlines. This is a trait that exists in every gastropod shell. We believe that the aperture outline that is obtained by multiple experienced malacologists, on different shells, would be highly similar. On the other hand, GM is heavily dependent on the accuracy of the landmarks that are taken from the object. As there are no biologically meaningful landmarks in shells, the "same" landmarks need to be taken from the shell that is fixed into a certain orientation.
Versatile – data generated from this method can be used for studies with different purposes. See above.

Comment [points12]: Our method extracts morphometric data from the aperture along the shell ontogeny. These parameters can be obtained from the aperture as long as the shell grows by unidirectional accretionary processes at the aperture. We are not aware of any shelled-gastropod species that do not grow in the way, and hence this method, by its very premises, should apply to all gastropod shells.

Comment [points13]: Second, with regard to evolutionary biology and functional morphology:
We indeed are very enthusiastic about the potential of this new method, which we feel needs not be toned down, since the retopologised shell models and dissimilarity matrix that are generated from this method comply with the data standard that is required in these two fields.

800   In the future, our method can be improved to accommodate the shell ornamentation analysis.

801   Parts of our method (i.e. procedures 1 – 6) can be used to obtain shell ornamentation data, such

802   as radial ribs (*i.e.,* commarginal ribs), but these data cannot be analysed with our qualitative and

803   quantitative approaches that focus on longitudinal growth (i.e. procedures 7 – 8). Finally, we

804   hope this shell form quantification method will simulate more collaboration within malacologists

805   that work in different research fields, and between empirical and theoretical morphologists.

806

807   **Supplementary Information (http://dx.doi.org/10.6084/m9.figshare.877061)**

808   File 1 – Video tutorial for procedure 3 and 4.

809   File 2– A python script for procedures 5 and 6 – Aperture form and growth trajectory analysis on

810   retopologised 3D shell mesh in Blender.

811   File 3– A python script to convert normalised elliptical Fourier coefficients to polygon mesh in

812   Blender.

813   File 4 – Python script for retopologising procedure.

814   File 5 – An R script for data analysis as described in procedures 7 and 8.

815   File 6 – A Blender file consisting of raw data of 8 shells of procedures 1 – 4.

816   File 7 – PLY ASCII mesh 3D model of *Plectostoma laidlawi* Sykes 1902.

817   File 8 – PLY ASCII mesh 3D model of *Plectostoma crassipupa* van Benthem Jutting, 1952.

818   File 9 – PLY ASCII mesh 3D model of *Plectostoma christae* Maassen 2001.

819   File 10 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* Clements and Vermeulen,

820   2008.

821   File 11 – PLY ASCII mesh 3D model of *Plectostoma laidlawi* that was reduced in size by one-

822   half and with slight modification of the last aperture size.

823   File 12 – PLY ASCII mesh 3D model of *Plectostoma christae* that was reshaped into an

824   elongated form by reducing the model size (linear dimension) by one-half along the x and y axes,

825   and by doubling the size along the z axis.

826   File 13 – PLY ASCII mesh 3D model of *Plectostoma christae* that was reshaped into a depressed

827   form by doubling the model size along the x and y axes, and by reducing the size by one-half

828   along the z axis.

829 File 14 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* that consists of one

830 *Opisthostoma vermiculum* original 3D model of which the aperture was connected to a second

831 enlarged *Opisthostoma vermiculum*.

838 **Author Contributions**

839 Conceived and designed the experiments: LTS. Performed the experiments: LTS. Analyzed the

840 data: LTS. Contributed reagents/materials/analysis tools: LTS MS. Wrote the paper: LTS MS.

841

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Introduction

Empirical and theoretical approaches in the study of shell form

The external form diversity of organisms is the most obvious evidence for their evolution, and thus is a key element in most branches of biology. The Molluscan shell has been a popular example in morphological evolution studies because it is geometrically simple, yet diverse in form. The shell form is controlled by the shell ontogenetic process, which follows a simple accretionary growth mode where new shell material is accumulatively deposited to the existing aperture. The evolution of shell forms has been studied either by using empirical approaches that focus on the quantification of actual shell forms or by using theoretical approaches that focus on the simulation of shell ontogenetic processes and geometric forms.

Notwithstanding the active development in both empirical and theoretical approaches to the study of shell form, there has been very little integration between both schools. For the empirical approach, the quantification methods of shell form have evolved from traditional linear measurement to landmark-based geometric morphometrics and outline analyses (for an overview see Van Bocxlaer & Schultheiß, 2010). At the same time, for the theoretical approach, the simulations of shell form have evolved from simple geometry models that aimed to reproduce the form, to more comprehensive models that simulate shell ontogenetic processes (for an overview see Urdy et al., 2010). Hence, each of the two approaches has been moving forward but away from each other, where synthesis between the two schools of shell morphologists has become more challenging.

In empirical morphological studies, shell form, either in terms of heights and widths in traditional morphometrics or in terms of geometry of procrustes distances in geometric morphometrics, is quantified by a set of homologous reference points or landmarks on the shell, which can be easily obtained from the fixed dimensions of the shell. Thus, both methods could abstract the shell form in terms of size and shape of the particular shell dimensions, and the between-sample variation of shell size and shape can be assessed (in most cases only within one study). On the other hand, it is not possible to reconstruct the actual shell form from these quantitative measurements, because the shell's accretionary growth model and spiral geometry cannot be quantified on the basis of arbitrary reference points or fixed dimensions (Stone. 1997).

2

61 Nevertheless, the traditional and geometric morphometric methods have been accepted widely as

62 standard quantification methods for shell form in many different fields of research.

63

64 In contrast to empirical morphometrics in which the aim is to quantify the actual shell,

65 theoretical morphologists focus on the simulation of an accretionary growth process which

66 produces a shell form that is similar to actual shells. This field was established with the

67 theoretical shell model of D.M. Raup (Raup, 1961; Raup & Michelson, 1965). Within the first

68 two decades after these publications, only a few different versions of shell models were proposed

69 (e.g. Løvtrup & von Sydow, 1974; Bayer, 1978; McGhee, 1978; Kawaguchi, 1982; Illert, 1983).

70 The subsequent two decades, thanks to the popularity and power of desktop computing, many

71 more theoretical shell models were published (e.g., Savazzi, 1985; Okamoto, 1988; Cortie, 1989;

72 Ackerly, 1989a; Savazzi, 1990; Checa, 1991; Fowler et al., 1992; Illert & Pickover, 1992; Checa

73 & Aguado, 1992; Cortie, 1993; Savazzi, 1993; Rice, 1998; Ubukata, 2001; Galbraith,

74 Prusinkiewicz & Wyvill, 2002). Finally, we saw further improvements in the published

75 theoretical models in recent years. These recent models simulate shell forms that more accurately

76 resemble actual shells because of improved programming software, better algorithms, and 3D

77 technology (e.g. Picado, 2009, Stępień, 2009; Meinhardt, 2009; Urdy et al., 2010; Harary & Tal,

78 2011; Moulton & Goriely, 2012; Moulton, Goriely & Chirat, 2012; Faghih Shojaei et al., 2012;

79 Chacon, 2012). Here, we will not further discuss the details of the at least 29 published shell

80 models, but refer to the comprehensive overviews and descriptions of these models in Dera et al.

81 (2009) and Urdy et al. (2010).

82

83 In brief, the latest theoretical shell models are able to simulate irregularly-coiled shell forms and

84 ornamentations that resemble actual shells, whereas the earlier models could only simulate the

85 regular and general shape of shells. The major refinements that have been made during the

86 almost five decades' development of theoretical shell models are the following modifications of

87 the algorithm: 1) from a fixed reference frame to a moving reference frame system; 2) from

88 modelling based on numerical geometry parameters to growth-parameter-based modelling (e.g.

89 growth rates); 3) from three parameters to more than three parameters, which has made fine-

90 tuning of the shell simulation (e.g. aperture shape) possible. The key element of the theoretical

91 modelling of shells is the generation of shell form by simulating the aperture ontogeny in terms

92   of growth trajectory and form along the shell ontogeny. Hence, this has an advantage over the

93   empirical approach in the numerical representation of the shell geometry form in terms of the 3D

94   quantification and the actual shell ontogenetic processes.

95

96   Since the empirical and theoretical researchers studying shell form with two totally different

97   quantification methods, our understanding of shell evolution cannot progress solely by using

98   either empirical morphometrics or theoretical models. Ideally, theoretical models need to be

99   evaluated by empirical data of shell morphometrics, and, vice-versa, empirical morphometric

100   methods need to be improved to obtain data that better reflect the actual shell form and

101   morphogenesis which can then be used to improve the theoretical models. In this dilemma lies

102   the central problem of shell form quantification and it urgently needs to be addressed in order to

103   integrate and generalise studies of shell form evolution.

104

105   **Why empirical morphologists rarely use theoretical shell models**

106

107   Despite the fact that, since the 1980s, manyshell models have been published that are more

108   complex and versatile, the first theoretical shell model of Raup still remains the most popular.

109   There were many attempts by empirical morphologists to use the original or a modified version

110   of Raup's parameters to quantify natural shell forms (e.g. Raup, 1967; Vermeij, 1971; Davoli &

111   Rosso, 1974; Graus, 1974; Kohn & Riggs, 1975; Newkirk & Doyle, 1975; Warburton, 1979;

112   Cameron, 1981; Verduin, 1982; Ekaratne & Crisp, 1983; Saunders & Shapiro, 1986; Tissot,

113   1988; Foote & Cowie, 1988; Johnston, Tabachnick & Bookstein, 1991; Emberton, 1994; Clarke,

114   Grahame & Mill, 1999; Samadi, David & Jarne, 2000). Surprisingly, all the other shell models,

115   many of which produce more realistic forms, have received very little attention as compared to

116   Raup's model (see e.g. Savazzi, 1992; Okajima & Chiba, 2011; Okajima & Chiba, 2012, for

117   exceptions). This ironic situation might be explained by the elegance of Raup's model that is

118   intuitively and mathematically simple to be used by empirical morphologists (mostly biologists),

119   with limited mathematical and programming experience.

120

121   As discussed above, most of the theoretical models can simulate a shell that has a form

122   resembling the actual shell in a realistic 3D geometry, based on shell ontogeny processes. In

4

123　contrast, empirical morphometrics can only quantify and compare certain dimensions of actual

124　shells. Clearly, the theoretical approach is better than the empirical approach in its accuracy of

125　shell form quantification, because accurate morphological quantification is essential for

126　functional, ecological and evolutionary studies of shell form. Below, we identify and discuss a

127　few impediments that currently prevent empirical morphologists from adopting the theoretical

128　approach in shell form quantification.

129

130　First, the requirement of a computation resource was an impediment in the past. These

131　theoretical models may only be implemented in a computation environment. As mentioned

132　above, the advances of computation hardware in speed and 3D graphic technology have

133　promoted the development of more complex theoretical shell models. For example, the current

134　speed and storage of a desktop computer is at least four orders of magnitude greater than those

135　used by Cortie (1993) only two decades ago. Clearly, the computation hardware is no longer an

136　impediment (e.g. Savazzi, 1995) for the application and development of theoretical shell models.

137

138　Notwithstanding the hardware development, programming skills are still a prerequisite for the

139　implementation of theoretical models. Many of the early models that were published between the

140　1960s and 1990s, used third-generation programming languages such as Fortran and C++, which

141　essentially lack a graphic user interface. This situation has improved now that the simulation of

142　theoretical shell models can be done in fourth-generation programming languages such as

143　Mathematica (e.g. Meinhardt, 2009; Noshita, 2010; Okajima & Chiba, 2011; Okajima & Chiba,

144　2012) and MATLAB (e.g. Boettiger, Ermentrout & Oster, 2009; Urdy et al. 2010, Faghih

145　Shojaei et al., 2012). Most of these shell models were described with intensive mathematical

146　notation, at least from a biologist's point of view, in the publication; and some of these were

147　published together with the information on algorithm implementation. However, the actual

148　programming codes are rarely published together with the paper though they may be available

149　from the authors upon request (but see Meinhardt, 2009; Noshita, 2010; Okajima & Chiba,

150　2011). Only one theoretical modelling software package based on Raup's model has a graphic

151　user interface that is comparable to contemporary geometric morphometric software (Noshita,

152　2010). Thus, the rest of the modern theoretical models are far less approachable than the

153　morphometric software for empirical morphologists. This is because those advanced theoretical

154 models have not been delivered in a form that allowed empirical morphologists to have "hands-

155 on experience" with them, without extensive mathematical literacy (Savazzi, 1995; McGhee,

156 2007).

157

158 Second, theoretical shell models simulate the shell form based on the input of a set of

159 parameters, which could be non-biological or/and biologically meaningful. Non-biological

160 meaningful parameters are counter-intuitive for empirical morphologists because these

161 parameters are not extrinsic shell traits. Nevertheless, many of these non-biological parameters

162 are required for the model to fit the shell form schematically (Hutchinson, 1999). When the

163 biological parameters do represent shell traits, they are often difficult to obtain accurately and

164 directly from the actual shell because of the three-dimensional spiral geometry (Cain, 1977;

165 Ackerly, 1989a; Ackerly, 1989b; Okamoto, 1988; Schindel, 1990; Checa & Aguado, 1992,

166 Hutchinson, 1999; McGhee, 1999). Since the development of theoretical shell models, almost all

167 simulated shell models have been made by an ad hoc approach, where the parameters are chosen

168 for the model and then the simulated shells are compared with the actual shells. In almost all

169 cases, the correct parameters are chosen after a series of trial-and-error, and the parameters are

170 selected when the form of the simulated shell matches the actual shell. Okamoto (1988)

171 suggested that this ad hoc approach based on pattern matching was easier than obtaining the

172 parameters empirically from the shell.

173

174 Third, although the overall forms of the simulated shells resemble the actual shells, the simulated

175 shell is not exactly the same as the actual shell (Kohn & Riggs, 1975; Goodfriend, 1983). For

176 many models, its original parameters are not sufficient to simulate the shell form exactly

177 (Schindel, 1990; Fowler, Meinhardt & Prusinkiewicz, 1992). These simulated general shell

178 forms are adequate for theoretical morphologist interests in their exploration of general shell

179 forms. However, the subtle features on a real shell or the subtle differences among different shell

180 forms of real species that cannot be simulated by theoretical models may have significant

181 functional implications that are important for empirical morphologists.

182

183 In brief, it is clear that the implementation of current theoretical shell models is less accessible to
184 empirical shell morphologists. Yet, empirical morphologists are using traditional and geometric
185 morphometrics as a routine method for shell quantification.

186

187 **Why empirical morphologists use traditional and geometric morphometrics**

188

189 In addition to the impediments arising from the theoretical shell model itself that are limiting its
190 popularity among empirical morphologists, the theoretical approach faces competition from
191 geometric morphometric methodology. The popularisation of desktop computing that led to the
192 flourishing of theoretical shell models in the late 1980s, also promoted the development of
193 morphometric methods, such as Elliptical Fourier Analysis (EFA) and geometric morphometrics
194 (GM). Rohlf and Archie (1984) set a benchmark for the quantification of an organism's form by
195 EFA, which was improved from Kaesler and Waters (1972) and Kuhl and Giardina (1982). Rohlf
196 and Slice (1990) and Bookstein (1991) developed a complete standard protocol for GM. Soon
197 after these pioneer papers, various software with Graphic User Interface (GUI) were developed
198 for the application of EFA and GM (Cardini & Loy, 2013, see http://life.bio.sunysb.edu/morph/).
199 In contrast to the application of theoretical shell models, an understanding of mathematics and
200 programming languages is not a prerequisite for the user of these morphometric tools. Thus, EFA
201 and GM have been well received by biologists, and have been adopted in the morphometric
202 study of shell form.

203

204 These geometric morphometric software packages have standard and interactive workflows that
205 help empirical morphologists in every step of: obtaining morphometric data (e.g. placing
206 landmark coordinates), analysing data (e.g. procrustes superimposition), statistical analysis (e.g.
207 ANOVA, PCA), and visualising shape and shape changes (e.g. thin-plate spline, PCA plots).
208 This has made geometric morphometrics approachable and attractive to empirical morphologists,
209 who want to examine the similarities and differences among shell forms.

210

211 Geometric morphometrics is actually a statistic of shape that is calculated from Cartesian
212 coordinate data from a sample of objects (Cardini & Loy, 2013). However, it is not an exact

7

213   quantification of form and is not particularly suitable for comparison and quantification of shell

214   form, for the following two reasons.

215

216   First, GM analysis is based on homologous landmarks on the form, but shell has only arbitrary

217   landmarks because it has a low degree of morphological complexity (Van Bocxlaer & Schultheiß

218   2010). In most cases, 2D landmarks are chosen at the shell apex, suture, and aperture or whorl

219   outline that can be identified from a 2D image that is taken in standard apertural view of a shell.

220   These landmarks are chosen to be analysed by GM but these points have little biological

221   meaning. Furthermore, as opposed to the form of many other organisms, 3D landmarks are even

222   more difficult to be obtained from a shell (3D model) as compared to 2D landmarks because

223   many of these landmarks, such as suture points, that are obtained from a 2D image are just

224   artefacts of the fixed 2D view of the shell.

225

226   Second, the results of separate, independent studies of shell forms cannot be integrated, even

227   though these studies use the same GM method. Statistical analysis of the Cartesian coordinate

228   data that abstractly represent the shell form is adequate in quantifying the variation of a shell

229   within a context of other shells that are included in a single study or within similar taxa where

230   similar landmarks are obtained. However, the raw coordinate data and analysed shape variation

231   from a study are incomparable and incompatible with the data from other studies (Klingenberg,

232   2013).

233

234   Despite the fact that geometric morphometrics has been widely used by empirical morphologists,

235   it is not an ideal tool in the quantification of shell form for the reasons given above. The

236   increasing availability of the software and application in the literature might cause morphologists

237   to stray away from their initial aims of studying shell form. Hence, it is important to return to the

238   core of the question: what do biologists want to learn from the study of shell form? Clearly, in

239   addition to quantitatively compare shell forms, biologists want to know more about the general

240   characteristics and physical properties of the shell form that are key elements in gaining insight

241   into functional and ecological aspects of the shell (Evans, 2013). However, functional and

242   ecological aspects of shell form can only be determined if the shell form can be exactly

243   quantified.

244

Using 3D technology to quantify shell form based on aperture ontogeny profiles

246

247 In this paper, we propose an interactive approach to the quantification and analysis of shell forms

248 based on state of the art 3D technology and by integrating the theoretical principles of shell

249 modelling and the empirical principles of morphometric data handling. There are no theoretical

250 models that can simulate all existing shell forms. However, the theoretical background of the

251 theoretical models is biologically sound – simulating the shell form by simulating the shell

252 ontogenetic process. On the basis of this shell-ontogenesis principle, we used state-of-the-art X-

253 ray microtomography (micro-CT scan) and 3D modelling software to obtain a series of shell

254 aperture changes from the shell in an interactive workflow that is similar to empirical

255 morphometric analysis.

256

257 First, a series of shell aperture outlines were digitised directly from the reconstructed 3D shell

258 model obtained from micro-CT scanning by using open-source 3D-modelling software – Blender

259 ver. 2.63 (www.blender.org). Then, the growth trajectory and form of the shell aperture outline

260 were quantified and extracted with our custom scripts that run in Blender through its embedded

261 open-source Python interpreter (http://www.python.org/). The changes of aperture size and

262 shape, and aperture growth trajectory in terms of curvature and torsion along the shell ontogeny

263 axis length were obtained (hereafter "aperture ontogeny profiles"). The final aperture ontogeny

264 profiles are in a form of multivariate time series data, which consist of a number of instances (i.e.

265 number of quantified apertures that depends on the length of the whorled shell tube) and

266 attributes that represent the growth trajectories, aperture form, and size.

267

268 These aperture ontogeny profiles can be plotted when each shell is examined individually. On

269 the other hand, the aperture ontogeny profiles can be visually compared between different shells

270 by plotting the data as radar chart (i.e. spider and star plots). In addition, the differences between

271 shells can be assessed quantitatively by calculating the dissimilarity of aperture ontogeny profiles

272 among shells. Furthermore, the dissimilarity matrix can be used to plot the dendrogram and

273 NMDS plots, which resemble a shell morphospace. All our procedures were implemented by

274 using open source and free software.

9

275

276 Finally, we discuss some possible applications and implications of these shell form

277 quantification methods in theoretical morphology, functional morphology, taxonomy and shell

278 shape evolutionary studies.

279

280 **Materials and Methods**

281 **Ethics Statement**

282 Specimens were collected in Malaysia with permissions from the Economic Planning Unit,

283 Malaysia (UPE: 40/200/19/2524).

284

285 **Scanning instrumentation**

286 A micro-CT scanner (SkyScan, model 1172, Aartselaar, Belgium) and its accompanying

287 software, NRecon ver. 1.6.6.0 (Skyscan©) and CT Analyser ver. 1.12.0.0 (Skyscan©), were used

288 to generate digital shell 3D models from the actual shell specimens.

289

290 **Computation software and hardware**

291 Various commercial 3D modelling and statistical software exist for visualising, manipulating,

292 and understanding morphology, such as Amira® (Visage Imaging Inc., San Diego, CA) and

293 Autodesk Maya (San Rafael, CA) (reviewed by Abel, Laurini & Richter, 2012). However, in this

294 study, we used only two open-source 3D data modelling and processing software packages,

295 namely Blender ver. 2.63 (www.blender.org) and Meshlab ver. 1.3.2 (Cignoni, Corsini &

296 Ranzuglia, 2008, http://meshlab.sourceforge.net/). Both have been used in biology to visualise

297 and model morphology (for Meshlab: Im et al., 2012; Chaplin, Yu & Ros, 2013; Atwood &

298 Sumrall, 2012; for Blender: Pyka et al., 2010: 22); Haug, Maas & Waloszek, 2009; Cassola et

299 al., 2010; Haug et al., 2010; Andrei et al., 2012; Haug et al., 2012; Lv et al., 2013; Mayer et al.,

300 2012). However, these programs have not been used to their full extent in morphological

301 quantification and analysis of 3D data for organisms. For quantification of morphology, we used

302 the open-source Python interpreter ver. 3.2 that is embedded in Blender 2.63. In addition, we also

303 used an extension to the Python programming language – NumPy (Oliphant, 2007) which

304 consists of high-level mathematical functions.

305

306 All the morphological data were explored and analysed with the statistical open source

307 programming language R version 3.0.1 (R Core Team, 2013) in the environment of RStudio

308 (RStudio, 2012). We installed three additional packages in R, namely, "lattice": Lattice Graphics

309 (Sarkar, 2008), "pdc": Permutation Distribution Clustering (Brandmaier, 2012a; Brandmaier,

310 2012b), and "fmsb" (Nakazawa, 2010).

311

312 All the computation analyses were carried out with a regular laptop computer with the following

313 specifications: Intel®Core™i7-3612QM @ 2.1GHz, 8 GB memory (RAM), NVIDIA® GeForce

314 GT 630M with 2GB memory.

315

316 **Procedures**

317 **1. Obtaining digital 3D models from actual shells**

318 The scan conditions were as follows: voltage – 80kV or 100kV; pixel – 1336 rows × 2000

319 columns; camera binning – 2 × 2; image pixel size – 3–6 μm; rotation step – 0.4° or 0.5°; and

320 rotation – 360°. Next, the volume reconstruction on the acquired images was done in NRecon.

321 The images were aligned to the reference scan and reconstruction was done on the following

322 settings: beam hardening correction – 100%; reconstruction angular range – 360 degree;

323 minimum and maximum for CS to image conversion (dynamic range) – ca. 0.12 and ca. 20.0;

324 and result file type – BMP. Finally, 3D models were created from the reconstruction images in

325 CT Analyser with the following setting: binary image index – 1 to 255 or 70 to 255; and were

326 saved as digital polygon mesh object (*.PLY format).

327

328 **2. Pre-processing digital shell models**

329 The 3D models were then simplified by quadric edge collapse decimation implemented in

330 MeshLab (Cignoni, Corsini & Ranzuglia, 2008) to reduce computation requirements. The raw

331 polygon mesh shells in PLY format have millions of faces and a file size between 20 to 80

332 Mbytes. Thus, we reduced the number of faces for all model to 200,000 – 300,000 faces, which

333 range between 3 and 6 Mbytes in file size. In addition, for the sake of convenience during the

334 retopology processes, all 3D models were repositioned so that the shell protoconch columella

335 was parallel with the z-axis. This was done by using manipulator tools in MeshLab.

336

3. Creating reference: Tracing aperture outlines and ontogeny axis from shell models (Supplementary Information File 1)

The digital shell 3D model in PLY format consists of 3D Cartesian coordinate vertices in which each of the three vertices constitutes a triangular face, and all faces are connected through a complex network. In order words, these vertices and faces are not biologically meaningful structures, and it is not possible to extract aperture outlines data directly from a raw PLY digital shell model. Monnet et al. (2009), for example, attempted to extract aperture outline automatically from a digital 3D model by making a plane cross-sectioning of the shell model, but its outlines do not reflect the form of the actual aperture outlines. Hence, we retopologised the raw 3D mesh models according to the aperture ontogeny for later data extraction.

We used Blender, which is more flexible than the commercial software used by Monnet et al. (2009). For the sake of convenience, we describe the following workflow, including the tools or the function (e.g. "Import PLY") which can be called after hitting the SPACE bar while in the Blender environment. However, this workflow may be modified by the user.

To begin, we imported a PLY shell model into the Blender environment ("Import PLY"). Then, we resized the model 1000 × ("Resize") so that the scale of 1 Blender unit was equal to 1 mm. After that, we examined the traces of aperture outlines (i.e. growth lines, ribs, spines) (Figure 1A) and ontogeny axis (i.e. spiral striation, ridges, colour lines) (Figure 1B) of the actual shells. After these aperture traits were identified, we selected the 3D model (by clicking "right mouse button"), and traced all these traits on the surface of the raw 3D mesh model in Blender by using the "Grease Pen Draw" tool. After that, the grease pen traced aperture traits were converted to Bezier curves with "Convert Grease Pencil" (Figure 1C).

4. Retopologising aperture outlines from the reference and generating retopologised shell models (Supplementary Information File 1 and File 4)

For each shell, we created a set of new Non Uniform Rational B-Splines (NURBS) surface circles ("Add Surface Circle") and modified these ("Toggle Editmode") according to the aperture outlines. We created a 16 points NURBS surface circle and aligned the circle to the aperture outline by translation ("Translate"), rotation ("Rotate"), and resizing ("Resize") (Figure 1D).

368   After the NURBS surface circle was generally aligned, each of the 16 points of the NURBS

369   surface circle were selected and adjusted by translation ("G") one by one, so that the outline of

370   the NURBS surface circle was exactly the same as the aperture outline. At the same time, the

371   second point of the NURBS surface circle was aligned to the ontogeny axis (Figures 1B and 1C).

372

373   After the first aperture outline was retopologised as a NURBS surface circle, the NURBS surface

374   circle was duplicated ("Duplicate Objects") and aligned to the next aperture outline as the

375   previous one. This step was repeated until all the aperture outlines were retopologised into

376   NURBS surface circles (Figures 1D and 1E). Then the shell surface was created in the form of a

377   NURBS surface based on the digitised aperture NURBS surface circle ("(De)select All" and

378   "Make Segment" in "Toggle Editmode") (Figures 1F and 1G). Lastly, we made the surface meet

379   the end points in U direction and increased the surface subdivision per segment (resolution U =

380   8) through the properties menu of the object (Properties (Editor types)>Object Data>Active

381   Spline).

382

383   After that, we converted the NURBS surface 3D model into a 3D Mesh model that consists of

384   vertices, edges, and faces ("Convert to" - "Mesh from Curve/Meta/Surf/Text"). The final

385   retopologised 3D shell Mesh consists of X number of apertures outlines and each aperture

386   outline has Y number of vertices and then a total of X*Y vertices. Each of the vertices is

387   connected to four other nearest vertices with edges to form a wireframe shell and face (Figure

388   1H).

389

390   It is important to note that the NURBS surface circle is defined by a mathematic formula which

391   does not imply any biology perspective of the shell. We choose NURBS surface circle because

392   the 3D aperture outline form can be digitalised by a small number of control points and shell

393   surface can be recreated by NURBS surface based on the digitised aperture NURBS surface

394   circle. The final 3D polygon mesh model is more simplified than the raw PLY 3D model and

395   each of its vertex data resemble the actual accretionary process of the shell (Figures 1A and 1H).

396

397   **5. Quantifying aperture growth trajectory**

398 The aperture ontogeny profiles were quantified as described in Liew et al. (in press, b20104)

399 with slight modifications where both aperture growth trajectory and aperture form were

400 quantified directly from the retopologised 3D shell model. This aperture growth trajectory was

401 quantified as a spatial curve, which is the ontogeny axis as represented by a series of first points

402 of the aperture outlines. We estimated two differential geometry parameters, namely, curvature

403 (κ) torsion (τ), and ontogeny axis length for all apertures (Okamoto, 1988; Harary & Tal, 2011).

404 The local curvature and torsion, and accumulative ontogeny axis length were estimated from the

405 aperture points along the growth trajectory by using weighted least-squares fitting and local arc

406 length approximation (Lewiner et al., 2005). All the calculations were done with a custom-

407 written Python script which can be implemented in Python interpreter in the Blender ver. 2.63

408 environment. The whole workflow was: (1) selecting the retopologised 3D shell Mesh (by

409 clicking "right mouse button"), (2) input parameters for number of sample points "q = ##" in the

410 python script, and (3) paste the script into the Python interpreter (Supplementary Information

411 File 21). The final outputs with torsion, curvature and ontogeny axis reference for each aperture

412 were saved as CSV files.

413

414 We found a convergence issue in curvature and torsion estimators. The accuracy of the curvature

415 and torsion estimates depends on the number and density of the vertices in the ontogeny axis (i.e.

416 number of aperture outlines), and the number of sample points. Nevertheless, different numbers

417 of sample points can be adjusted until good (i.e. converged) curvature and torsion estimates are

418 obtained. We used 10% of the total points as number of sample points of the ontogeny axis,

419 which gave reasonably good estimates for curvature and torsion.

420

421 Notwithstanding the algorithm issue, the curvature and torsion estimators are informative in

422 describing the shell spiral geometry growth trajectory. Curvature is always larger or equal to zero

423 ($\kappa \geq 0$). When $\kappa = 0$, the spatial curve is a straight line; the larger the curvature, the smaller the

424 radius of curvature ($1/\kappa$), and thus the more tightly coiled the spatial curve. On the other hand,

425 the torsion estimator can be zero or take either negative or positive values ($-\infty \leq \tau \leq \infty$). When τ

426 = 0, the spatial curve lies completely in one plane (e.g. a flat planispiral shell), negative torsion

427 values correspond to left-handed coiling and to right-handed coiling for positive torsion values;

428 the larger the torsion, the smaller the radius of torsion ($1/\tau$), and thus the taller the spiral.

429

430 **6. Quantifying aperture form**

431

432 We quantified the aperture outline sizes as perimeter and form as normalised Elliptic Fourier

433 coefficients (normalised EFA) by using a custom-written Python script which can be

434 implemented Python interpreter embedded in the Blender environment. The workflow was (1)

435 selecting the retopologised 3D shell mesh (by clicking "right mouse button"), (2) input

436 parameters for "number_of_points_for_each_aperture = ##" in the python script, and (3) paste

437 the script into the Python interpreter of Blender (Supplementary Information File 21). The final

438 outputs were saved as CSV files.

439

440 Aperture outline perimeter was estimated from the sum of lengths (mm) for all the edges that are

441 connecting the vertices (hereafter "aperture size"). For aperture form analysis, we used 3D

442 normalised EFA algorithms (Godefroy et al., 2012) and implemented these in the custom python

443 script. Although many algorithms exist for describing and quantifying the form of a closed

444 outline (Claude, 2008), we used EFA because it is robust to unequally spaced points, can be

445 normalised for size and orientation, and can capture complex outline form with a small number

446 of harmonics (Rohlf & Archie, 1984; Godefroy et al., 2012). In this study, we used five

447 harmonics, each with six coefficients which were sufficient to capture the diverse aperture

448 shapes of our shells. For quantification of apertures shape that are invariant to size and rotation,

449 we normalised EFA of aperture outlines for orientation and size. If needed for comparison with

450 other studies, the normalised EFA can be repeated for the same dataset with higher or lower

451 numbers of harmonics.

452

453 After normalisation, we ran principal components analysis (PCA) to summarise the 30

454 normalised Fourier coefficients as principal components scores (hereafter "aperture shape

455 scores"). After that, we selected the major principal components (explaining > 90 % of the

456 variance) for further analysis. The aperture shape scores of each selected principal component

457 were plotted and analysed against the ontogeny axis.

458

459 **7. Visualising aperture form and trajectory changes along the shell ontogeny**

460   For exploration of data, we used two graphical techniques for representing aperture ontogeny

461   profile changes along the shell ontogeny. For each shell, we made a vertical four-panels scatter

462   plot in which each of the four variables (namely, curvature, torsion, aperture size, and the first

463   principal component aperture shape score) were plotted against the ontogeny axis. When

464   necessary, the second and third principal component aperture shape scores were also included. In

465   addition, the axis of each variable was rescaled so that it was the same for the same variable of

466   all shells. After standardisation of the axis, the aperture ontogeny profiles of several shells could

467   be quantitatively compared side by side.

468

469   However, comparison of between plots would become less effective with a larger number of

470   shells. Alternatively, therefore, all aperture ontogeny profile variables of each shell can also be

471   represented in a radar chart, instead of scatter plots. This chart is effective in showing the

472   variable outliers within a chart and the overall similarity between charts. Before plotting the data

473   in a radar chart, the datasets of all shells need to be restructured because the dataset of different

474   shells could differ in the number of data points (i.e. quantified aperture), which depends on the

475   ontogeny axis length of each shell.

476

477   We did this by dividing the ontogeny axis of each shell into 20 equal length intervals, and then

478   by sampling the variable values at the end of every interval. In the restructured dataset, the trend

479   of the aperture ontogeny profile of each variable is retained and all radar charts have the same

480   number of data points. Thus, the changes of aperture variables between each subsequent 1/20 of

481   the ontogeny axis can be examined within a shell and be compared among different shells in a

482   synchronistic manner. We suggest to use 20 points to summarise hundreds variable points of the

483   aperture ontogeny profile variables along ontogeny axis because the radar would be

484   overwhelming with too many points and hard to interpret. Similar to the scatter plot, we

485   standardised the axis scales of each variable of all radar charts.

486

487   In addition, we added a new variable which represents the ontogeny axis interval length in order

488   to compensate for the loss of shell size information during the standardisation of ontogeny axis

489   length. Finally, we plotted the variables, namely, curvature, torsion, aperture size, and ontogeny

490   axis length, and aperture shape scores in a radar chart for each shell by using the "fmsb" library

491 (Nakazawa, 2010) with R version 3.0.1 (R Core Team, 2013) (Supplementary Information File
492 5̲3).

493

494 **8. Quantitative comparison between shell forms**
495 In addition to the qualitative comparison between shells forms as described above, the
496 dissimilarity between different shells can be analysed quantitatively. We used Permutation
497 Distribution Clustering (PDC) which finds similarities in a time series dataset (Brandmaier,
498 2012a; Brandmaier, 2012b). PDC can be used for the analysis of the changes in a variable along
499 shell ontogeny between different shells (i.e. two-dimensional dataset: number of shells × number
500 of apertures) and multiple variable changes between shells (i.e. three-dimensional dataset:
501 number of shells × number of variables × number of apertures).

502

503 Although PDC is robust to the length differences between datasets, our preliminary analysis
504 showed that the PDC output would be biased when there was a great (around two-fold) length
505 difference in the total ontogeny axis length. Hence, we standardised the data as in procedure 7,
506 but dividing the ontogeny axis of each shell into 50, instead of 20, equal length intervals. This
507 standardisation procedure allows comparison of trends in variable changes along the shell
508 ontogeny without the influences of size. In other words, the dissimilarity is zero between two
509 shells that have exactly the same shape, but differ only in size. In addition to the shape
510 comparison, we obtained the shell size in terms of volume by using "Volume" function in
511 Blender after the 3D shell was closed at both ends by creating faces "Make edge/Face")
512 on selected apertures at both end ("Loop Select") in EDIT mode.

513

514 The aperture ontogeny profiles of all shells were combined into a three-dimensional data matrix
515 consisting of n shells × four variables × 50 aperture data points. We ran four PDCs, each for the
516 five data matrices with: 1) all four variables, 2) torsion, 3) curvature, 4) aperture size, and 5)
517 aperture shape scores. The parameter settings for the PDC analysis were as follows: embedding
518 dimension = 5; time-delay of the embedding = 1; divergence measure between discrete
519 distributions = symmetric alpha divergence; and hierarchical clustering linkage method = single.
520 The dissimilarity distances between shells were used to produce the dendrogram. PDC analysis

17

521 was performed with the "pdc" library (Brandmaier, 2012b) in R version 3.0.1 (R Core Team,
522 2013) (Supplementary Information File 53).
523
524 In addition to the dendrogram representation of the output from PDC, we plotted the
525 dissimilarity as a non-metric multidimensional scaling (NMDS) plot which resembles a
526 morphospace. NMDS was performed by using "MASS" library (Venables & Ripley, 2002) in R
527 version 3.0.1 (R Core Team, 2013) (Supplementary Information File 53).
528
529 **Worked example: Comparative analysis of *Opisthostoma* and *Plectostoma* species shell form**
530 **and simulated shell form**
531 We evaluated the above-described shell form quantification method by using the shells of
532 *Opisthostoma* and *Plectostoma*, which exhibit a great variability in shell form. Some of the
533 species follow a regular coiling regime whereas others deviate from regular coiling in various
534 degrees. It remains a challenging task to quantify and compare these shell forms among species,
535 either by using traditional or geometric morphometrics, because a standard aperture view for the
536 irregular and open coiled shells cannot be determined.
537
538 We selected four ~~Opisthostoma~~ species, namely, ~~Opisthostoma~~*Plectostoma laidlawi* Skyes 1902
539 (Figure 2A), ~~Opisthostoma~~*Plectostoma crassipupa* van Benthem Jutting, 1952 (Figure 2B),
540 ~~Opisthostoma~~*Plectostoma christae* Maassen 2001 (Figure 2C), and *Opisthostoma vermiculum*
541 Clements and Vermeulen, 2008 (in Clements et al., 2008) (Figure 2D), for which the shell forms
542 are, respectively: regularly coiled, slight distortion of the last whorl, strong distortion of the last
543 whorl, and complete distortion of most of the whorls. We retopologised these four shells by
544 following the procedures 1 to 4 (Supplementary Information Files 612).
545
546 In addition to the four retopologised 3D shell models, we manually created another four shell
547 models by transforming three out of the four retopologised NURBS surface 3D shell models by
548 using the "Transform" function in Blender. These models are: 1) ~~Opisthostoma~~*Plectostoma*
549 *laidlawi* that was resized to half the original size and given slight modification of the aperture
550 size (Figure 2E); 2) ~~Opisthostoma~~*Plectostoma christae* that was reshaped into an elongated form
551 by reducing the model size (linear dimension) to one-half along the x and y axes, and by

18

552 doubling the size along the z axis (Figure 2F); 3) ~~Opisthostoma~~<u>Plectostoma</u> *christae* that was

553 reshaped into a depressed form by multiplying by 1.5 the model size along the x and y axes, and

554 by reducing to one-half along the z axis (Figure 2G); and 4) *Opisthostoma vermiculum* that

555 consists of one *Opisthostoma vermiculum* original 3D model of which we connected the aperture

556 to another, enlarged, *Opisthostoma vermiculum* (Figure 2H). Finally, we analysed all these eight

557 shell models by following the procedures 5 to 8.

558

559 **Results and Discussion**

560 **Retopologied 3D shell models**

561 All the final retopologised 3D shell models can be found in Supplementary Information (Files <u>7</u>~~4~~

562 to ~~11~~<u>14</u>) in PLY ASCII mesh format, with the raw data as a list of vertices, followed by a list of

563 polygons, which can be accessed directly without the need of any 3D software. Each vertex is

564 represented by x, y, z coordinates. Each polygon face consists of four vertices. This simplified

565 yet biologically informative 3D mesh shell model allows the quantification of aperture form and

566 growth trajectory. Moreover, the 3D shell models and their raw vertices data could potentially be

567 used in studies of functional morphology and theoretical modelling of shell form, respectively.

568

569 Malacologists have been focusing on empirical shell morphological data, from which the

570 functional, ecological and evolutionary aspects were then extracted. The physical properties were

571 then determined by its form (e.g. Okajima & Chiba 2011; Okajima & Chiba, 2012). By using the

572 3D models, the shell properties and function can be analysed *in silico*. For example, the thickness

573 of the shell can be added to the 3D shell model (Figure 3E and Figure 3F) in order to obtain the

574 shell material's volume, the shell's inner volume, its inner and outer surface area, and centre of

575 gravity. Quantification of shell properties may then be done by using the geometry approach in

576 Meshlab or Blender, as compared to the pre-3D era where mathematical descriptions of the shell

577 form were required (e.g. Moseley, 1838; Raup & Graus, 1972; Stone, 1997). Furthermore, it is

578 possible to convert the 3D models to a 3D finite element (FE) model, of which the physical

579 properties (e.g. strength) can be tested (e.g. Faghih Shojaei et al., 2012).

580

581 In addition to the potential use of 3D shell models in functional morphology, the coordinate data

582 of the vertices of 3D shell models could be used directly by theoretical morphologists (see Figure

583 1 in Urdy et al., 2010). For example, these data can be extracted in different formats that fit the

584 data requirement of different types of theoretical shell models, namely, generating curve models

585 using a fixed reference frame or moving reference frame (Figure 3C), helicospiral or multivector

586 helicospiral models using a fixed reference frame (Figure 3A, Figure 3B and Figure 3D) or

587 growth vector models using a moving reference frame (Figure 3A and Figure 3B).

588

589 The retopologising of the aperture ontogeny from a raw 3D shell model (procedures 1 to 4) is a

590 time-consuming and tedious process compared with traditional and geometric morphometrics.

591 For example, the four shell models were created by retopologising between 73 and 96 separate

592 apertures. Nevertheless, the final product of a clean 3D shell mesh is versatile for many different

593 kinds of analysis and thus has great potential for improving our understanding of shell form.

594

595 **Comparing shell form from the view of shell ontogeny**

596 Figure 4 gives an overview of the aperture ontogeny profile and shell volume for each species.

597 The curvature, torsion perimeter, and ontogeny axis are represented by true numerical values

598 with the unit of mm^{-1} and mm, and thus can be interpreted directly. In contrast, the aperture

599 shape scores are just statistics of Fourier coefficients and are not the absolute quantification of

600 aperture shape. The PCA score of an aperture shape depends on the shape of other aperture

601 outlines and thus it might change whenever other aperture outlines are added into the analysis.

602 Nevertheless, the aperture scores will stabilise as data of more shells become available and when

603 most of the extreme aperture forms are included. In this study, the first principal component

604 explained 92% of the total variance; the second and third principal component explained only

605 3% or 1% of the total variance. We showed that the shell form can be represented by the

606 ontogeny changes of the aperture growth trajectory in terms of curvature and torsion, and

607 aperture form, in terms of perimeter and shape.

608

609 Our first example evaluates this method in illustrating the differences between two shells that

610 have the same shape but differ in shell size – the half-size ~~Opisthostoma~~_Plectostoma_ _laidlawi_

611 (Figure 4E) shell and the original ~~Opisthostoma~~_Plectostoma_ _laidlawi_ shell (Figure 4C). As

612 revealed by their aperture ontogeny profiles, the size difference between the two shells has had

613 an effect on the curvature, torsion, ontogeny axis length and aperture size. For the resized

20

614   *Opisthostoma*Plectostoma* laidlawi shell, the values of curvature and torsion are twice as large as

615   for the original, whereas the ontogeny axis length and aperture size are only half those of the

616   original shell. However, there is no discrepancy in the aperture shape statistics. Despite this

617   scalar effect, the overall trends in the changes of these variables along the ontogeny axis are

618   comparable between these two shells (Figure 6B).

619

620   Another example shows the ontogeny profiles of three shells, namely, the elongated (Figure 4G),

621   depressed (Figure 4H), and original (Figure 4A) versions of the *Opisthostoma*Plectostoma*

622   *christae* shell. Comparison of aperture profiles among these show the most obvious

623   discrepancies in greater torsion values for the elongated shell, which change in a more dramatic

624   trend along the shell ontogeny. In addition, each of the three shells has its unique aperture shape

625   scores, though there are no big discrepancies in the aperture size. The differences in ontogeny

626   axis length, curvature and torsion are related to the differences of the aperture shape statistics

627   among the three shells. However, our small dataset with only three shells is not sufficient for

628   thorough disentangling of the interplay between aperture size, shape, and growth trajectory in

629   relation to the shell form.

630

631   Our last example is the comparison between the original (Figure 4D) and the composite (Figure

632   4F) *Opisthostoma vermiculum* shell . It is clear that our method has high sensitivity and

633   robustness in the analysis of such bizarre shell forms. As shown in Figure 4F, the start of the

634   aperture ontogeny profile of the composite shell was the same as for the original shell (Figure

635   4D). In addition, the later parts of the ontogeny profile trends are still comparable to the first

636   part, but different in value because of the scalar effect.

637

638   As an alternative visualisation, Figure 5 shows the radar charts that summarise the same aperture

639   ontogeny profiles of each species. The polygon edges in each chart show how dramatically the

640   aperture form (size and shape), and growth trajectory (curvature and torsion) are changing at

641   each of the subsequent 5% intervals of the shell ontogeny. The aperture size (mm) and the

642   ontogeny segment length (mm) variables indicate the shell size (i.e. volume). To illustrate this,

643   aperture size and ontogeny axis length can be seen as the circle size and height of a cylinder.

644   This chart is useful for the visual comparison between shells that are similar in size, for example,

21

645 ~~*Opisthostoma*~~*Plectostoma christae* (2.43 mm^3), ~~*Opisthostoma*~~*Plectostoma laidlawi* (2.39 mm^3),

646 and the depressed ~~*Opisthostoma*~~*Plectostoma christae* (2.73 mm^3). The radar chart shows that (1)

647 the depressed ~~*Opisthostoma*~~*Plectostoma christae* is the largest and has a very different aperture

648 shape as compared to the other two shells; (2) most of the shell whorls' form of

649 ~~*Opisthostoma*~~*Plectostoma christae* is very similar to ~~*Opisthostoma*~~*Plectostoma laidlawi* (i.e.

650 most of the polygons in the chart were similar), but the ~~*Opisthostoma*~~*Plectostoma laidlawi* shell

651 differs from ~~*Opisthostoma*~~*Plectostoma christae* shell by having distorted whorls at the last part

652 of the shell ontogeny (magenta lines at torsion) and a more open umbilicus at the beginning of

653 the shell ontogeny (red lines at curvature and aperture size).

654

655 However, comparison of radar charts between shells that differ greatly in size would be less

656 informative. For example, the radar charts between the resized ~~*Opisthostoma*~~*Plectostoma*

657 *laidlawi* shell and the original ~~*Opisthostoma*~~*Plectostoma laidlawi* shell are very different, though

658 the resized one has the same shell shape as the original. The difference in radar charts between

659 the two shells was therefore mainly caused by the size difference.

660

661 As we have shown in both graphical techniques (Figures 4 and 5), the shell forms can be

662 explored and compared qualitatively on the basis of aperture ontogeny profiles. Users might need

663 some training in the interpretation of the plots because they are different from both linear

664 dimension measurement plots and geometric morphometric shape coordinate plots. Our

665 evaluation suggested that both data visualisation methods are sensitive and robust in capturing

666 the aperture ontogeny profile for any shell form and thus make the qualitative comparison across

667 gastropod taxa and studies possible.

668

669 This method could be applied in malacological taxonomy because its core business is the

670 description of shell form. Despite hundreds of years of taxonomic history of shells, there has

671 been little change in the way shell form is being described. For example, shell from is usually

672 described in terms of linear dimensions: shell width and height; number of whorls; shell shape –

673 flat, depressed, globose, conical, or elongated; whorls shape – from flat to convex. Here, we

674 suggest that the aperture ontogeny profiles would be a great supplement to the classical approach

675 to shell description. For example: (1) the size of the shell (its volume) depends on the ontogeny

676   axis length and aperture size; (2) the shell shape depends on the growth trajectory in terms of

677   curvature and torsion; (3) the shape of the whorls depends on the shape of the aperture (Figure

678   4). In our case of the four ~~Opisthostoma~~ shells (Figures 2A – 2D), it is clear that aperture size of

679   each shell is constricted at roughly the same part of the respective shell ontogeny, namely

680   between 70% and 85%, regardless of the dissimilar shell sizes and shapes (Figures 4A – 4D, and

681   aperture size profiles in Figure 5B). In fact, these aperture size decreases during ontogeny are in

682   accordance with the shell constriction, one of the shell characters that have been used in the

683   taxonomy of *Opisthostoma* and *Plectostoma* (Vermeulen, 1994; Liew et al., 2014). However, the

684   shell constriction has not been quantified previously, and we show that it could also be an

685   important developmental homology for the two genera. This preliminary results suggest that ~~This~~

686   ~~suggests that the constriction in aperture size profile is a diagnostic character for the genus~~

687   ~~Opisthostoma. In the light of this example, we believe that~~ these aperture ontogeny profiles could

688   aid the taxonomist in decision-making for grouping taxa based on homologous characters.

689

690   **Quantitative comparison between different shell forms**

691   Figure 6 shows dendrograms resulting from a permutation distribution clustering analysis of the

692   eight shells in terms of their aperture ontogeny profiles. Figure 6A shows the hierarchical

693   clustering of the eight shells based on all four aperture ontogeny profiles. From this dendrogram,

694   the composite *Opisthostoma vermiculum* is completely separate from the other shells. The

695   remaining seven shells are clustered into two groups. One consists of the more regularly coiled

696   shells, namely, ~~Opisthostoma~~*Plectostoma christae* and its two transformed shells, and

697   ~~Opisthostoma~~*Plectostoma crassipupa*; the other group consists of the shells that deviate from

698   regular coiling, namely ~~Opisthostoma~~*Plectostoma laidlawi* and its transformed shell, and

699   *Opisthostoma vermiculum*. Nevertheless, there were high dissimilarities between shells within

700   each group as revealed by the long branch lengths in Figure 6A, except for the two

701   ~~Opisthostoma~~*Plectostoma laidlawi* shells (Table 1). The aperture ontogeny profiles for the

702   ~~Opisthostoma~~*Plectostoma laidlawi* shell and its reduced version are almost the same. The high

703   dissimilarity among the other six shells can be explained when each of the variables in the

704   aperture ontogeny profile is analysed separately as shown in Figure 6B.

705

706   Figure 6B shows the dendrograms of aperture ontogeny profiles for each of the four variables.

707   All four dendrograms have a different topology than the one in Figure 6A. Among the variables,

708   the aperture ontogeny profile of the curvature has the smallest discrepancies among shells. The

709   two ~~Opisthostoma~~*Plectostoma* *laidlawi* shells are the only pair that clusters together in all the

710   dendrograms of Figures 6A and 6B because they are identical in every aspect of aperture

711   ontogeny profile except torsion. Hence, the independent analysis of aperture ontogeny profile

712   variables corresponds well to the overall analysis of aperture ontogeny profiles.

713

714   Figure 7 shows a three-dimensional NMDS plot of the distance matrix (Table 1) that was

715   generated from PDC analysis on all four aperture ontogeny profiles. The very low stress level

716   (0.000) indicates that this 3D plot is sufficient to represent the distance matrix of the aperture

717   ontogeny profiles. This NMDS plot can therefore be regarded as a morphospace of the shell

718   shape, as derived from aperture ontogeny profiles. However, neither the dendrogram nor the

719   NMDS plot contains information about the shell size because the analysis of PDC is based on the

720   standardised ontogeny profiles and their trends. Thus, both plots are useful for the comparative

721   analysis of shell shape, but not shell size. Nevertheless, the size comparison between shells is

722   rather straightforward.

723

724   The conventional quantification of shell size is based on the linear measurement of two or three

725   dimensions of a shell, for example, shell height and shell width. However, shell size may be

726   more appropriately given as shell volume, which can be estimated easily from retopologised 3D

727   shell models (Figure 4). A shorthand to qualitatively comparing size between two shells is by

728   examining the ontogeny axis length and aperture size in the radar chart (Figure 5). We can then

729   compare the form between shells when the dendrograms or NMDS plot are interpreted together

730   with shell size (volume) data. For example, the ~~Opisthostoma~~*Plectostoma* *laidlawi* shell has the

731   same shape as, but is eight times larger than, the resized ~~Opisthostoma~~*Plectostoma* *laidlawi*.

732

733   In addition to the construction of morphospace, the dissimilarity matrix can be used in

734   phylogenetic signal tests (Hardy & Pavoine, 2012). Furthermore, it can also be analysed together

735   with other distance matrices, such as for geographical or ecological distance, to improve our

736   understanding of the evolutionary biology of shell forms.

737

Conclusions, limitations and future directions

We demonstrated an alternative workflow for data acquisition, exploration and quantitative

analysis of shell form. This method has several advantages ~~over traditional and geometric~~

~~morphometries in the analysis of shell forms, namely in terms of~~: (1) robustness – this method

can be used to compare any shell form; (2) scalability and reproducibility – the data obtained

from different studies and different gastropod taxa can be integrated; (3) versatility – the raw 3D

shell mesh models, coordinates data of the vertices, aperture ontogeny profiles, and dissimilarity

matrix between shell forms can be used for taxonomy, functional morphology, theoretical

modelling, and evolutionary studies.

Yet, our method has its limitations. Firstly, our retopology procedures rely on a 3D shell model

that requires CT-scan technology. In fact, although a CT-scan 3D shell model can certainly

facilitate the retopology process of a shell, it is not indispensable. The key of the retopology

processes is to digitise the aperture along the shell ontogeny, and thus a shell can be

retopologised fully in Blender with a good understanding of the aperture ontogeny profiles by

studying the real specimens even without a reference shell model. Secondly, the retopology

procedure which is essential for our data acquisition is more time-consuming than traditional and

geometric morphometric where data can be obtained from an image taken from a shell. Thirdly,

our method is effective in the analysis of overall shell form, but not of the shell ornamentation.

In the future, our method can be improved to accommodate the shell ornamentation analysis.

Parts of our method (i.e. procedures 1 – 6) can be used to obtain shell ornamentation data, such

as radial ribs (*i.e.*, commarginal ribs), but these data cannot be analysed with our qualitative and

quantitative approaches that focus on longitudinal growth (i.e. procedures 7 – 8). Finally, we

hope this shell form quantification method will simulate more collaboration within malacologists

that work in different research fields, and between empirical and theoretical morphologists.

Supplementary Information (http://dx.doi.org/10.6084/m9.figshare.877061)

File 1 – Video tutorial for procedure 3 and 4.

767   File S~~1~~2– A python script for procedures 5 and 6 – Aperture form and growth trajectory analysis

768   on retopologised 3D shell mesh in Blender.

769   File S~~3~~2– A python script to convert normalised elliptical Fourier coefficients to polygon mesh

770   in Blender.

771   File 4 – Python script for retopologising ~~repotopologing~~ procedure.

772   File S~~5~~3 – An R script for data analysis as described in procedures 7 and 8.

773   File S~~6~~12 – A Blender file consisting of raw data of 8 shells of procedures 1 – 4.

774   File S~~7~~4 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *laidlawi* Sykes 1902.

775   File S~~8~~5 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *crassipupa* van Benthem

776   Jutting, 1952.

777   File S~~9~~6 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *christae* Maassen 2001.

778   File S~~10~~7 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* Clements and Vermeulen,

779   2008.

780   File S~~11~~8 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *laidlawi* that was reduced

781   in size by one-half and with slight modification of the last aperture size.

782   File S~~12~~9 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *christae* that was reshaped

783   into an elongated form by reducing the model size (linear dimension) by one-half along the x and

784   y axes, and by doubling the size along the z axis.

785   File S~~1~~30 – PLY ASCII mesh 3D model of *~~Opisthostoma~~Plectostoma* *christae* that was reshaped

786   into a depressed form by doubling the model size along the x and y axes, and by reducing the

787   size by one-half along the z axis.

788   File S~~14~~1 – PLY ASCII mesh 3D model of *Opisthostoma vermiculum* that consists of one

789   *Opisthostoma vermiculum* original 3D model of which the aperture was connected to a second

790   enlarged *Opisthostoma vermiculum*.

791   ~~File S12 – A Blender file consisting of raw data of 8 shells of procedures 1 – 4.~~

792

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799

Author Contributions

801 Conceived and designed the experiments: LTS. Performed the experiments: LTS. Analyzed the

802 data: LTS. Contributed reagents/materials/analysis tools: LTS MS. Wrote the paper: LTS MS.

803

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