Inner surface of *Nepenthes* slippery zone: ratchet effect of lunate cells causes anisotropic superhydrophobicity

Lixin Wang, Shuoyan Zhang, Shanshan Li, Shixing Yan and Shiyun Dong

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Revised submission: 12 February 2020
Final acceptance: 26 February 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Note: This manuscript was transferred from another Royal Society journal without peer review.

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**Review History**

RSOS-200066.R0 (Original submission)

**Review form: Reviewer 1**

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

**Recommendation?**
Accept with minor revision (please list in comments)
Comments to the Author(s)
This paper well demonstrated the anisotropic superhydrophobic of the pitcher plant’s slippery zone. The novel anisotropic phenomenon, the clear structure analyzing and the reasonable model building about the ratchet effect of the water rolling on the lunate cells are reported with the essential experiment and the logical calculations. This article fits well with the journal: Royal Society Open Science and put forward a completely new point on the Nepenthes research and the design of anisotropic superhydrophobic surfaces. The submission can be published after minor revision.

1. In the results part, only used the droplet of volume 3 \(\mu\)l to test the sliding angle. For the accuracy of the experiment, the author was advised to do more experiment with different volume.

2. It is generally believed that the pitcher plant’s surface is a kind of super-slippery surface, the author is recommended to compare the model’s difference between traditional super-slippery surface with the established model in this paper.

3. In the section 1 on the introduction of the Nepenthes surface with anisotropic wettability, the authors left out a few related works such as Adv Mater 2014, 26 (19), 313; Adv Funct Mater 2019, 29, 1904446, etc.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
This manuscript experimentally studied the anisotropic wettability of the Nepenthes slippery zone. It was found that the anisotropic superhydrophobicity was affected by structure characteristics of lunate cells, which was also verified by the derived quantitative model. This work is expected to offer inspirations for bionic design of anisotropic superhydrophobic surfaces. Yet, I have some concerns below:
1. The sliding angle towards pitcher up is around 5.22 ° while the sliding angle towards pitcher bottom is around 2.82 °. Thus, a conclusion that the Nepenthes slippery zone has a remarkable anisotropic superhydrophobicity is claimed. My argument is, such a difference of the sliding angle is large enough to support the conclusion?
2. This manuscript concentrates on the anisotropic wettability of Nepenthes slippery zone. Thus, in addition to directions of pitcher up and pitcher bottom, sliding angles of other two directions need to be measured and studied as well.

3. Chen et al. (Chen, Zhang, Zhang, Liu, Jiang, Zhang, Hang, Jiang. Continuous directional water transport on the peristome surface of Nepenthes alata. Nat. 532, 85-89.) studied the morphology of the carnivorous plant Nepenthes alata. Different from the sliding droplets observed in the present experiment, Chen et al. found that water would spread directionally on Nepenthes alata. What is the reason causing the different droplet moving behaviors?

4. From the rough morphology structure of the Nepenthes slippery zone as well as the excellent superhydrophobicity, it seems that the droplet on the slippery zone falls in the state I in the Molecular Dynamics study of Guo et al. (Guo, Tang, Kumar. Droplet morphology and mobility on lubricant-impregnated surfaces: a molecular dynamic study. Langmuir, 35, 16377-16387, 2019). More discussions of droplet morphology would deepen this work by comparing with the existing theoretical work.

Decision letter (RSOS-200066.R0)

06-Feb-2020

Dear Dr Wang

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-200066 entitled "Inner surface of Nepenthes slippery zone: Ratchet effect of lunate cells causes anisotropic superhydrophobicity" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

- Ethics statement
  If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- Data accessibility
  It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link:
http://datadryad.org/submit?journalID=RSOS&manu=RSOS-200066
• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Please ensure you have prepared your revision in accordance with the guidance at https://royalsociety.org/journals/authors/author-guidelines/ -- please note that we cannot publish your manuscript without the end statements. We have included a screenshot example of the end statements for reference. If you feel that a given heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 15-Feb-2020. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees. We strongly recommend uploading two versions of your revised manuscript:

1) Identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.
When uploading your revised files please make sure that you have:

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document";
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format);
3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account;
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript. Make sure it is clear in your data accessibility statement how the data can be accessed;
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://rs.figshare.com/). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Andrew Dunn
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Derek Abbott (Associate Editor) and R. Kerry Rowe (Subject Editor)
openscience@royalsociety.org

Reviewer comments to Author:
Reviewer: 1
Comments to the Author(s)
This paper well demonstrated the anisotropic superhydrophobic of the pitcher plant’s slippery zone. The novel anisotropic phenomenon, the clear structure analyzing and the reasonable model
building about the ratchet effect of the water rolling on the lunate cells are reported with the essential experiment and the logical calculations. This article fits well with the journal: Royal Society Open Science and put forward a completely new point on the Nepenthes research and the design of anisotropic superhydrophobic surfaces. The submission can be published after minor revision.

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2. It is generally believed that the pitcher plant’s surface is a kind of super-slippery surface, the author is recommended to compare the model’s difference between traditional super-slippery surface with the established model in this paper.

3. In the section 1 on the introduction of the Nepenthes surface with anisotropic wettability, the authors left out a few related works such as Adv Mater 2014, 26 (19), 313; Adv Funct Mater 2019, 29, 1904446, etc.

Reviewer: 2

Comments to the Author(s)

This manuscript experimentally studied the anisotropic wettability of the Nepenthes slippery zone. It was found that the anisotropic superhydrophobicity was affected by structure characteristics of lunate cells, which was also verified by the derived quantitative model. This work is expected to offer inspirations for bionic design of anisotropic superhydrophobic surfaces. Yet, I have some concerns below:

1. The sliding angle towards pitcher up is around 5.22 ° while the sliding angle towards pitcher bottom is around 2.82 °. Thus, a conclusion that the Nepenthes slippery zone has a remarkable anisotropic superhydrophobicity is claimed. My argument is, such a difference of the sliding angle is large enough to support the conclusion?

2. This manuscript concentrate on the anisotropic wettability of Nepenthes slippery zone. Thus, in addition to directions of pitcher up and pitcher bottom, sliding angles of other two directions need to be measured and studied as well.

3. Chen et al. (Chen, Zhang, Zhang, Liu, Jiang, Zhang, Hang, Jiang. Continuous directional water transport on the peristome surface of Nepethes alata. Nat. 532, 85-89.) studied the morphology of the carnivorous plant Nepenthes alata. Different from the sliding droplets observed in the present experiment, Chen et al. found that water would spread directionally on Nepenthes alata. What is the reason causing the different droplet moving behaviors?

4. From the rough morphology structure of the Nepenthes slippery zone as well as the excellent superhydrophobicity, it seems that the droplet on the slippery zone falls in the state I in the Molecular Dynamics study of Guo et al. (Guo, Tang, Kumar. Droplet morphology and mobility on lubricant-impregnated surfaces: a molecular dynamic study. Langmuir, 35, 16377-16387, 2019). More discussions of droplet morphology would deepen this work by comparing with the existing theoretical work.

Author’s Response to Decision Letter for (RSOS-200066.R0)

See Appendix A.
Review form: Reviewer 1

Is the manuscript scientifically sound in its present form? 
Yes

Are the interpretations and conclusions justified by the results? 
Yes

Is the language acceptable? 
Yes

Do you have any ethical concerns with this paper? 
No

Have you any concerns about statistical analyses in this paper? 
No

Recommendation? 
Accept as is

Comments to the Author(s) 
The authors have addressed my concerns. I'd like to recommend its acceptance in current version.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form? 
Yes

Are the interpretations and conclusions justified by the results? 
Yes

Is the language acceptable? 
Yes

Do you have any ethical concerns with this paper? 
No

Have you any concerns about statistical analyses in this paper? 
No

Recommendation? 
Accept as is

Comments to the Author(s) 
I recommend acceptance in the present form.
Decision letter (RSOS-200066.R1)

26-Feb-2020

Dear Dr Wang,

It is a pleasure to accept your manuscript entitled "Inner surface of Nepenthes slippery zone: Ratchet effect of lunate cells causes anisotropic superhydrophobicity" in its current form for publication in Royal Society Open Science. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Please ensure that you send to the editorial office an editable version of your accepted manuscript, and individual files for each figure and table included in your manuscript. You can send these in a zip folder if more convenient. Failure to provide these files may delay the processing of your proof. You may disregard this request if you have already provided these files to the editorial office.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org) and the production office (openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

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Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
Anita Kristiansen
Editorial Coordinator

Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Derek Abbott (Associate Editor) and R. Kerry Rowe (Subject Editor)
openscience@royalsociety.org

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
The authors have addressed my concerns. I'd like to recommend its acceptance in current version.

Reviewer: 2

Comments to the Author(s)
I recommend acceptance in the present form.
Appendix A

Dear editors,

Thanks for your effort to this manuscript, also appreciate the reviewers for their valuable and constructive comments. In the following, we try our best to reply the comments in detail.

Reviewer 1-Comment 1: In the results part, only used the droplet of volume 3μl to test the sliding angle. For the accuracy of the experiment, the author was advised to do more experiment with different volume.

Reply: Thanks for the suggestion. In fact, in measuring all the sliding angles, considering the evaporation and the syringe accuracy, the volume of ultrapure-water droplet is not strictly limited to 3μl, sometimes more than 3μl, sometimes less than 3μl. In order to express this point accurately, in the revised manuscript, we put a word ‘approximate’ before 3μl and have highlighted this changed point with red colour.

Reviewer 1-Comment 2: It is generally believed that the pitcher plant’s surface is a kind of super-slippery surface, the author is recommended to compare the model’s difference between traditional super-slippery surface with the established model in this paper.

Reply: Exactly, the Nepenthes pitcher consists of lid, peristome, slippery zone and digestive zone. The well-known ‘super-slippery surface’ is the inner surface of slippery zone. It shows super-slippage properties to insects (Roy. Soc. Open Sci. 2018, 5, 180766.). The super-slippery surface causes the water-droplet to present a sliding angle of about 3° when sliding toward pitcher bottom, and about 6° when sliding toward pitcher up. In the manuscript, our established model gives an explanation to the difference in the two types of sliding angles. Compared with the previous theories, our model is the first to focus on the difference in sliding angles.

According to the reviewer’s suggestion, in the revised manuscript, we analyze the model’s difference between the traditional super-slippery surface and our established model, as follows:

Previous studies have shown some extremely important theoretical models to characterize the super-slippage properties of the Nepenthes slippery zone, in the aspects of superhydrophobicity [17,24,33], insect attachment ability [15,24,25], and static contact angle [22]. Here, our proposed model firstly explains how the structure characteristics of lunate cells, including the slope/precipice angle and the slope/precipice height, affect the anisotropic superhydrophobic wettability of Nepenthes slippery zone.

The changed point has been highlighted with blue colour in the revised manuscript.

Reviewer 1-Comment 3: In the section 1 on the introduction of the Nepenthes surface with anisotropic
wettability, the authors left out a few related works such as Adv Mater 2014, 26 (19), 313; Adv Funct Mater 2019, 29, 1904446, etc.

**Reply:** Thanks for the valuable suggestion. We have read the articles suggested by the Reviewer, and have added in the revised manuscript, as Refs [20] and [21]. And, the latter references are renumbered.

20. Li P, Cao MY, Bai HY, Zhao TH, Ning YZ, Wang XS, Liu KS, Jiang L. 2019 Unidirectional liquid manipulation via an integrated mesh with orthogonal anisotropic slippery tracks. *Adv. Funct. Mater.* 29, 1904446. (doi: 10.1002/adfm.201904446)

21. Zhang PC, Liu HL, Meng JX, Yang G, Liu XL, Wang ST, Jiang L. 2014 Grooved organogel surfaces towards anisotropic sliding of water droplets. *Adv. Mater.* 26, 3131-3135. (doi: 10.1002/adma.201305914)

All the changed points have been highlighted with green color in the revised manuscript.

**Reviewer 2-Comment 1:** 1. The sliding angle towards pitcher up is around 5.22° while the sliding angle towards pitcher bottom is around 2.82°. Thus, a conclusion that the *Nepenthes* slippery zone has a remarkable anisotropic superhydrophobicity is claimed. My argument is, such a difference of the sliding angle is large enough to support the conclusion?

**Reply:** Thanks for pointing the inappropriate aspect. Indeed, the word ‘remarkable’ is not suitable for describing the difference between 5.22° and 2.82°. We have changed the word ‘remarkable’ with ‘observable’ or ‘obvious’ in the revised manuscript, thank you. And, the changed points are highlighted with dark-red color.

**Reviewer 2-Comment 2:** This manuscript concentrates on the anisotropic wettability of *Nepenthes* slippery zone. Thus, in addition to directions of pitcher up and pitcher bottom, sliding angles of other two directions need to be measured and studied as well.

**Reply:** As shown in Figure 2b and Figure 2a, based on the structure characteristics of lunate cells in the direction towards pitcher up (forming ‘precipice’) and bottom (forming ‘slope’), sliding angles of the two types can almost adequately characterize the anisotropy of *Nepenthes* slippery zone. Further, in previous studies, many authors have used the two directions to characterize the anisotropy of *Nepenthes* slippery zone in insect attachment behaviors (*Beilstein J. Nanotechnol.* 2, 302–310. *J. Bionic Eng.* 12, 79–87. *J. Bionic Eng.* 13, 373–387. *Roy. Soc. Open Sci.* 5, 180766.).
Reviewer 3-Comment 1: Chen et al. (Chen, Zhang, Zhang, Liu, Jiang, Zhang, Hang, Jiang. Continuous directional water transport on the peristome surface of Nepenthes alata. Nature 532, 85-89.) studied the morphology of the carnivorous plant Nepenthes alata. Different from the sliding droplets observed in the present experiment, Chen et al. found that water would spread directionally on Nepenthes alata. What is the reason causing the different droplet moving behaviors?

Reply: The *Nepenthes* pitcher consists of four parts: lid, peristome, slippery zone and digestive zone. In Chen’s article (*Nature*, 2016, 532, 85-89), the authors focused on the peristome, they studied the droplet moving behaviors on the peristome (Figure R1). In our manuscript, we investigated the sliding behaviors of water droplet on the slippery zone (Figure R1).

![Figure R1: Peristome and slippery zone of the *Nepenthes* pitchers](image)

Reviewer 2-Comment 4: From the rough morphology structure of the Nepenthes slippery zone as well as the excellent superhydrophobicity, it seems that the droplet on the slippery zone the state I in the Molecular Dynamics study of Guo et al. (Guo, Tang, Kumar. Droplet morphology and mobility on lubricant-impregnated surfaces: a molecular dynamic study. *Langmuir*, 35, 16377-16387, 2019). More discussions of droplet morphology would deepen this work by comparing with the existing theoretical work.

Reply: Thanks for the valuable suggestion, and we have read this article. According to this article, we discussed the ultra-water droplet morphology on the slippery zone, as the follows:
According to a molecular dynamic investigation of droplet morphology on lubricant-impregnated surface [35], when an ultrapure-water droplet is sliding on the *Nepenthes* slippery zone towards pitcher up/bottom, the ultrapure-water droplet neither completely infiltrates into nor floats on the micro-nano scaled structures of slippery zone. Instead, the ultrapure-water droplet partly infiltrates into the isotropic wax coverings and anisotropic lunate cells.

And, this changed point has been highlighted with orange color in the revised manuscript.

The above are our reply to the comments. However, we are not sure that the reviewers can completely accept our reply. If not, we will revise this manuscript again, thank you.

Best regards,

Lixin Wang