nature portfolio

# Peer Review File

# Identification of carnivory in the flowering plant *Saxifraga* via multidisciplinary evidence

Corresponding Author: Professor Hang Sun

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Comments
It is interesting to see the attempt to prove that a species of plant in the genus *Saxifraga* (*S. candelabrumis*) a carnivorous plant by approaching it from multiple angles, including observation, modern genetic analysis, analysis using isotopes, and scent analysis using GCMS concerning attracting pollinators, and the hypothesis that insects distinguish between predators and pollinators based on their size. Although this reviewer is not knowledgeable about genetic analysis, if the experimental results showing convergence in prey-associated genes among different families of carnivorous plants are reliable, this is a noteworthy finding for future functional analysis of carnivorous plants. This reviewer considers that it is necessary to clear 1-4 noted below.

1. Pages 23-24, Experiments for nutrient uptake: Drosophila melanogaster (fed with 15N-labeled amino acids) was counted as their population size. If it is treated quantitatively, it would be better to include the weight of the fruit fly. How about adding the average weights of fruit flies used in this study?

2. lines 51-55: The *Saxifraga* discussed by Darwin and the *Saxifraga* used in this study are different species. When discussing the genus *Saxifraga*, it is possible that an important point of discussion will be whether there is any genetic, plant ecological, or morphological similarity between *Saxifraga candelabrum* and *S. rotundifolia* and *umbrosa*. Is it possible to add insights on this point?

3. Lines 79-82, Page 18 Fig 3 (C and D): It was claimed that an association was found between prey and the number and length of inflorescence branches. However, there does not appear to be a sufficient explanation for the quantitative extent of this correlation, or whether it could lead to a causal relationship.

4. Lines 87-98 and lines 395-412: I agree that the olfactory response is important for the active attraction of insects in this plant. On the other hand, questions arose regarding the explanation of some technical aspects of the GC/MS analysis and interpretation of the data.
a) I may have overlooked this, but I was unable to find any information related to the GCMS analytical equipment. It would be better to specify the specifications of the equipment, columns, detection conditions, etc.
b) In Table S3, What do the Sample IDs DF-1 to DF-10 mean? Furthermore, it was unclear and difficult to understand how the analysis results of each sample ID and its aroma components would lead to any discussion or conclusion.
c) Has it been confirmed that the substances detected here, which are claimed to be the floral fragrance components, have any insect attractant activity (either through experiments or information from published literature)?
d) Please note the italic style in the compound names. For example, *E*, *Z*, *cis*, *trans*, *O*-decyl (O), 1*R* (R), *N*-, *n*-,
e) Was the compound name of DF-3 (Rt 9.969) “ester”? I recommend that the authors double-check the compound names and other entries in the table to ensure there are no errors.
f) Remove initial zero for the CAS numbers.

The others
・*What are the noteworthy results?*
・*Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.*

As referenced by the authors (ref-19 in the manuscript), the experimental approach and procedures, including N15 labeling and fluorescence observation, are similar with the report PNAS 118 (33) e2022724118https://doi.org/10.1073/pnas.2022724118. Therefore, the strength of this paper lies in the fact that a new carnivorous plant in the genus Saxifraga has been established at the current level of scientific methods. Regarding the novelty of the results of genome analyses, this reviewer will refrain from commenting as I do not have sufficient knowledge on the subject.

・*Does the work support the conclusions and claims, or is additional evidence needed?*
Basically, as far as this reviewer's opinion, the experiments presented in this paper demonstrate that Saxifraga candelabrum possesses characteristics of a carnivorous plant.

・*Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?*
・*Is the methodology sound? Does the work meet the expected standards in your field?*
・*Is there enough detail provided in the methods for the work to be reproduced?*
It has been argued that volatile substances derived from this Saxifraga are involved in the attraction process. However, I thought that the explanation and discussion regarding this aspect are insufficient. Therefore, as mentioned above, this reviewer believes that this section needs to be revised.

Reviewer #2

(Remarks to the Author)
This is an interesting and generally well-presented study. Using a variety of techniques, the authors provide novel evidence of carnivory in the alpine plant, Saxifraga. They further suggest that carnivory in plants may be more widespread than currently expected.

The title of the paper focuses on the identification of a new carnivorous plant lineage. This is exciting, however, a study in 2021 (Lin et al. 2021 PNAS) used some similar approaches to identify a similar mechanism of carnivory in the Triantha plant lineage. This somewhat diminishes the novelty of the current study and its significance for its research field. The current study does, however, go beyond the Lin et al. 2021 paper by including a field study and a comparison of the genomic characteristics of carnivorous plants. These aspects of the study could be further emphasised as points of difference, though it is unclear exactly how the field experiment provides evidence for carnivory in Saxifraga.

The approaches used and data presented appear to support the findings and conclusions of the study. However, some additional detail of methodology and data analysis is required for improved clarity and reproducibility. It should also be made more clear how the different components of the manuscript tie together to address the study's research questions.

More specific comments are below:

- The title and abstract both refer to Darwin's theory/hypothesis, but it may be unclear to readers what this is. It should be possible to slightly reframe the abstract/hypothesis to make clear that Darwin hypothesised that this particular plant may be carnivorous. Further explanation of Darwin's theory/hypothesis should also be included in the introduction of the manuscript.

- In the abstract, ln 22-24: This section of text needs to be reframed/reworked for clarity. D15N is not taken up, rather 15N is taken up from isotope-labelled prey, and this alters the d15N signature of the plant. Enzymatic analysis confirmed ability to digest prey, and an isotope labelling experiment with 15N-labelled prey confirmed that N was transferred from the prey to plant tissues.

- In the introduction, the authors should specifically define carnivory (vs protocarnivory/ 'murderous' plants) and specifically outline what features they aim to identify to 'prove' that Saxifraga is carnivorous.

Ln 39-41: Waterwheel plants have a snap trap (as correctly stated on ln 148). Bladderworts would be an example of a carnivorous plant using a suction trap.

Ln 41: Correct to "Despite their diverse mechanisms for…"

Ln 62: It would be more appropriate to refer to Figure 1 here, rather than specifically Figure 1A.

- At the end of the introduction, make clear what the specific research questions/hypotheses are. These could be made to nicely tie in with the subheadings that follow.

Ln 73: Use past tense throughout this section. It makes it clear that this is based on work completed in the current study.

Ln 86: Is the phrase "This insect trapping phenomenon" referring to the fact they trap insects, rather than any relationship between trap surface area and prey number? Please clarify.

It is unclear how the relationship between trap surface area and number of captured prey contributes to evidence supporting carnivory. We may see the same in non-carnivorous plants that trap insects for defensive purposes (without subsequent digestion). Make clear how this part of the work contributes to addressing the overall research questions.

Ln 87: Subheading “Active attraction and insect capture using floral odor” does not represent the content immediately below, which also includes visual attraction.

Ln 92: It is stated that more insects were attracted to the visual blocking treatment than control groups. Figure 3b shows the positive control had the same number of insects as the visual blocking treatment. Reword for clarity.

As in Triantha, it is claimed that Saxifraga balance pollination with carnivory by only capturing smaller insects that are not pollinators. There appears to be little, if any overlap of prey and flow visitors (Table S1), which supports this. However, this then raises the issue of how the experiment on floral odour vs visual attraction fits within this manuscript. If the experiment determined what attracts flower visitors, but flower visitors are not prey, then how does this relate to carnivory in Saxifraga?

Ln 95 and Table S3: Which compounds are known to be insect-attracting? Please provide references to support this.

Table S3 and ln 95: Which compounds are known to be insect-attracting? Are there refs to support this?

Ln 112: State clearly that fluorescence indicates presence of phosphatase enzyme

Ln 113: Reword to say that this indicates that the species has the capacity to directly digest trapped insects.

Ln 116-117: Rewords. Sounds like it is saying that the species doesn’t have digestive enzyme despite not capturing insects. This sentence structure doesn’t make sense, as this is exactly what you would expect.

Ln 131-132: When referring to the isotope labelling experiment, results should be expressed as enrichment in 15N, or transfer/uptake/assimilation of 15N, leading to more positive d15N values. E.g., reword to read “… highest 15N-enrichment in flowers…” (ln 131), and “lowest 15N-enrichment in basal leaves” (ln 132).

Ln 133: More prey-derived nutrients were invested in reproductive growth. Could this reflect proximity of the plant part to the captured prey, or simply the fact that this is the part of the plant that is actively growing?

Is it possible to quantify or estimate how much of the plant’s N requirement may be acquired via carnivory?

Ln 209: remove “;”

Ln 224: Amend to “are rarely captured”

Ln 224: Amend to read that “these plants therefore appear to balance carnivory with pollination”.

Ln 225: A word seems to be missing - “the alpine environment”?

Ln 226: It is unclear how this carnivory is cryptic given that sticky hairs and trapped insects are easily observable.

Ln 371-382: Restructure this section of the methodology to improve clarity. It is initially difficult to ascertain what the various herbarium vs field specimens were used for. E.g. ln 378 initially sounds like this it relates to herbarium specimens, then ultimately says that the specimens were in the field. This is confusing.

Some aspects of the methodology are unclear. E.g. How were glass boxes secured to the substrate to prevent odour escaping? Are insects only trapped by plants while flowers are present/open? Were herbarium samples flowering? Provide more information on the fruit fly labelling – how long were they fed 15N substrate for? What was the N concentration of the amino acid mix?

In the labelling experiment, were 15N labelled fruit flies added for 2 weeks, as per controls?
Using genome characteristics that were identified in this study, would it be possible to screen candidate plants for carnivory based on a ‘genetic fingerprint’ for plant carnivory?

Provide detail of statistical analyses (approach and results) that were done to support this study (e.g. How did you determine regression relationships in figures 3C and 3D? Where did you establish statistical differences in box plots?)

Figure 1 caption: Do the images truly show digested prey or just captured prey?

Figure 2 caption: Make clear what APG IV is (write APG in full).

Figure 3A: I am unclear of the value in seeing the number of specimens per year with prey, especially without knowing the total number of specimens available. Did all plants have prey? Does it matter (for this study) that there was variation among years? Any variation likely can’t be accounted for anyway, unless information on environmental conditions impacting prey availability etc. can be accounted for and collection methods and seasons were standardised.

Figure 3B and Figure 5: Indicate what the significant levels are that are indicated on the figure.

Figures 3C & D: Describe what the shaded areas represent.

Reviewer #3

(Remarks to the Author)
This manuscript tested a potentially new carnivorous plants in Saxifraga from enzymatic, isotopic and genetic analyses. The isotopic analyses, which will directly determine if a plant is carnivorous or not, suggested that Saxifraga candelabrum gained nitrogen from labelled insects. However, I have some concerns about the design of the experiment, and the results are not fully discussed, which may affect the reliability of the conclusion. The enzymatic analysis will determine if this plant is holocarnivorous (produce their own digestive enzyme) or not but lacks a positive control here.

My main concern is from the isotopic data, which is the essential evidence to prove if a plant is carnivorous or not. I can't find the original δ15N data from the supplementary material in this manuscript, which should be there. So I can only tell from the Figure. 5, which is also not clearly labelled (for example what is the control in Fig. 5D? What does DJ mean in Fig. 5B?). The control value of δ15N from negative control (Saxifraga diversifolia and Salvia przewalskii) are between 5-7.5, which are even much higher than carnivorous plants (Drosera peltate as positive control) and the tested Saxifraga candelabrum. Usually the natural δ15N level in carnivorous plants should be higher than the non-carnivorous plants and lower than the insects in the same habitat, and that's how a plant is proved to be carnivorous or not without labelling. This high level of natural δ15N is even very rare in carnivorous plants, let along any non-carnivorous plants. The authors need to explain this unusual high level of δ15N in their negative controls. I think it's more meaningful to test the natural δ15N level of Saxifraga candelabrum, non-carnivorous plants in the same habit, and their animal preys. The isotopic analysis should be in this ecological context, that carnivorous plants gain nutrient from insects, thus should have a higher level of δ15N than non-carnivorous plants.

The consistent drop of δ15N in both lower and leaf from two weeks to 20 days (Fig. 5B) need to be explained. Were nitrogen transferred into fruits or roots later?

For Fig. 5D, are they all data from negative control? What was the control? Why there was such a dramatic increase of δ15N to 100-200 in flower after two weeks and four weeks?

From photos in Fig.1 it seems like their sticky leaves and calyx can also capture insects. The enzymatic analysis also showed that sticky leaves and calyx of Saxifraga candelabrum produce digestive enzyme (Fig. 4). But why only the stems were labelled and tested? The authors even labelled the calyx of negative control. But why not the calyx of Saxifraga candelabrum? Then we don't know if their leaves and calyx are carnivorous or not.

Other concerns:

l.36. 'including members of seven families'
Definitely more than seven, should be 14 families so far

l.40. 'suction trap in waterwheel plants'.
Waterwheel plants use snap trap, bladderworts use suction trap. Probably avoid providing examples.

l.43. 'hunting process: prey attraction, signal transduction, capture, digestion and nutrient'
I think hunting is only until capture

l.49. 'is notable for its sticky glandular trichomes'
I don't think all Saxifrage have sticky glandular trichomes. If not how many?

l.87-93. I'm wondering how this experiment set-up can distinguish the attraction of pollinator and prey. If the set-up cannot distinguish pollinator and prey, how the results can be related to the carnivory, other than pollinator attraction.

l.107-117. Probably need a positive control, a known carnivorous plants for this enzymatic test.

l.118-129. Where were Drosera peltate from? The same habitat with S. candelabrum? Different field location? Where were negative controls from? The same habitat? It makes more sense if they are from the same habitat.

l.127-129. 'To prevent contamination…' Why only the stems of S. diversifolia and S. candelabrum were excluded? How about Salvia przewalskii?

l.433-435. Why instead of upper stem from other plants, it was the calyx in Salvia przewalskii which was labelled? Need to be consistent.

In l.437 it says 'S. diversifolia…lacks glandular hairs' but in l.441 it says 'fruit flies were gently placed on the glandular surfaces'. They are contradictory.

l.450. What if the labelled insects fell on the rosette leaves, instead of the soil? How can you prevent that?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Comments
I have reviewed the revised manuscript as well as the authors' responses to the reviewers' comments. My questions have been satisfactorily addressed, and I have no further concerns at this time. I therefore recommend the manuscript for publication.

This is a minor point; however, this reviewer would appreciate it if the authors could kindly confirm whether compound names (R/S), symbols, and Greek letters should be italicized in accordance with the journal's style guidelines, and ensure that their formatting is standardized throughout the manuscript.
For example,
Line 510: n-hexane
Line 522: m/z
Supporting table S3: Please consider making S/R italic, following the other compounds.
Page 6, SC1-2:
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
Page 8, SC3-1: 4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1α,4α,7α)]-
Page 9, SC3-3:
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-

Reviewer #2

(Remarks to the Author)
Thank you to the authors for their careful consideration of my comments on the initial submission of their manuscript. I am satisfied that the authors have improved their manuscript and appropriately addressed all of my comments.

Reviewer #3

(Remarks to the Author)
The authors have carefully addressed all my concerns in the revised manuscript and the Response Letter. I have no further comments. I believe the manuscript is now suitable for publication.

REVIEWER COMMENTS AND RESPONSES

**Note on line numbers:**
For clarity, all line numbers cited in this response correspond to the revised manuscript provided in PDF format.

**Reviewer #1 (Remarks to the Author):**

Comments
It is interesting to see the attempt to prove that a species of plant in the genus *Saxifraga* (*S. candelabrum*) a carnivorous plant by approaching it from multiple angles, including observation, modern genetic analysis, analysis using isotopes, and scent analysis using GCMS concerning attracting pollinators, and the hypothesis that insects distinguish between predators and pollinators based on their size. Although this reviewer is not knowledgeable about genetic analysis, if the experimental results showing convergence in prey-associated genes among different families of carnivorous plants are reliable, this is a noteworthy finding for future functional analysis of carnivorous plants. This reviewer considers that it is necessary to clear 1-4 noted below.
**Response:** We thank the reviewer for the constructive comments and the positive overall evaluation of our study. We have revised the manuscript accordingly, and our detailed responses to the specific points are provided below.

***Comment:*** 1. Pages 23-24, Experiments for nutrient uptake: *Drosophila melanogaster* (fed with 15N-labeled amino acids) was counted as their population size. If it is treated quantitatively, it would be better to include the weight of the fruit fly. How about adding the average weights of fruit flies used in this study?
**Response:** Thank you for your constructive suggestion. To improve clarity and address the reviewer's concern, we have now measured the body mass of a subset of fruit flies used in the experiments. The average fresh weight of an adult fruit fly was 0.403 ± 0.011 mg (mean ± SD, n = 12). This information has been added to the Methods section (Now at revised manuscript (PDF) **lines 545-547**).

***Comment:*** 2. lines 51-55: The *Saxifraga* discussed by Darwin and the *Saxifraga* used in this study are different species. When discussing the genus *Saxifraga*, it is possible that an important point of discussion will be whether there is any genetic, plant ecological, or morphological similarity between *Saxifraga candelabrum* and *S. rotundifolia* and *umbrosa*. Is it possible to add insights on this point?
**Response:** Thank you for this insightful comment. We agree that *Saxifraga candelabrum* is genetically, and geographically distinct from the two species (*S. rotundifolia* and *S. umbrosa*) discussed by Charles Darwin. These species belong to different sections within *Saxifraga* (Sect. *Ciliatae*, Sect. *Cotylea*, and Sect. *Gymnopera*, respectively) and occupy distinct biogeographic regions, with the latter two occurring in temperate Europe. However, these species share the key functional trait, namely the presence of well-developed glandular hairs capable of trapping small insects, and all are herbaceous montane plants with flowering stems rising above a basal rosette. In the revised Introduction, we have added a brief clarification emphasizing that Darwin's original

hypothesis was motivated by the functional properties of glandular hairs observed in certain *Saxifraga* species. We also highlight the shared presence of well-developed, insect-trapping glandular hairs represents the key morphological and functional similarity that renders *S. candelabrum* a suitable system for testing Darwin's hypothesis using modern approaches. (Now at revised manuscript **lines 81-89**)

**lines 81-89:** "Although *S. candelabrum* is geographically and phylogenetically distinct from the two *Saxifraga* species discussed by Darwin, his original hypothesis was motivated by the functional properties of glandular hairs observed in those two *Saxifraga* species. It is also noteworthy that a number of species of *Saxifraga* have sticky glandular hairs. The shared presence of well-developed glandular hairs capable of trapping insects represents the key morphological and functional feature that motivated Darwin's experiments and renders *S. candelabrum* a suitable system for testing this long-standing hypothesis using modern approaches."

Hopefully our work will stimulate other researchers to carefully examine the species noted by Darwin--- *S. rotundifolia* and *S. umbrosa.*

***Comment:*** 3. Lines 79-82, Page 18 Fig 3 (C and D): It was claimed that an association was found between prey and the number and length of inflorescence branches. However, there does not appear to be a sufficient explanation for the quantitative extent of this correlation, or whether it could lead to a causal relationship.

**Response:** Thank you for this important comment. We have revised the manuscript to clarify both the quantitative extent of the observed association and its biological interpretation as "Field observations demonstrated that the number of insects trapped on *S. candelabrum* individuals correlated positively with both the number (Spearman's $\rho$=0.38, $p$=0.038) and length (Spearman's $\rho$=0.33, $p$=0.001) of inflorescence branches (Fig. 3)" and "This observed correlation suggested that the increased number and length of inflorescence branches may enhance insect attraction and provide a larger surface area bearing glandular hairs, potentially increasing the probability of insect capture". (Now at revised manuscript **lines 111-114 & lines 117-120**).

4. Lines 87-98 and lines 395-412: I agree that the olfactory response is important for the active attraction of insects in this plant. On the other hand, questions arose regarding the explanation of some technical aspects of the GC/MS analysis and interpretation of the data.

***Comment:*** a) I may have overlooked this, but I was unable to find any information related to the GCMS analytical equipment. It would be better to specify the specifications of the equipment, columns, detection conditions, etc.

**Response:** Thank you for pointing this out. Details of the GC/MS analytical equipment, column specifications, and detection conditions have now been added to the Methods section (Now at revised manuscript **lines 515-523**).

**lines 515-523:** "Analyses of the floral volatiles was performed with an Agilent 7890 gas chromatograph/5975 mass selective detector with a DB-5MS capillary column with a length of 30 m. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven conditions included an initial temperature of 60 °C for 1 min, the temperature increased at a rate of 10 °C/min to 130 °C, and then 6 °C/min to 250 °C for 10 min. The inlet temperature was kept constant at 250 °C, and the MS transfer line was set at 290 °C. MS acquisition parameters included scanning from m/z 50–600 in the electron impact (EI) mode for routine analysis."

***Comment:*** b) In Table S3, What do the Sample IDs DF-1 to DF-10 mean? Furthermore, it was unclear and difficult to understand how the analysis results of each sample ID and its aroma components would lead to any discussion or conclusion.

**Response:** Thanks for this question. DF-1 to DF-10 originally referred to floral volatile samples. In the revised manuscript, Table S3 has been reorganized to clearly reflect the sampling design. The sample IDs are now defined as SC1–SC3, representing three independent flowering individuals of *Saxifraga candelabrum*, each with three replicates (–1 to –3). This has been clarified in the legend of Table S3 and in the Methods section as "Table S3 Floral volatile components detected in *Saxifraga candelabrum*. The volatile compounds listed were identified through GC-MS analysis and compared with the results from air samples and non-flowering *Saxifraga candelabrum* individuals. Only compounds with a match score (Qualitative) ≥ 80 were retained to ensure reliable compound identification. SC1–SC3 represent three individual plants of *S. candelabrum*, each with three technical replicates (-1 to -3)." (Now at revised **Supplementary materials Table S3**)

The GC–MS analysis was conducted specifically to address the first criterion required to confirm plant carnivory, namely prey attraction, as stated in the Introduction: "To investigate the potential status of *S. candelabrum* as a carnivorous plant, we tested the four key criteria required to confirm carnivory (prey attraction, capture, digestion and nutrient uptake)". The floral volatile profiles demonstrate that *S. candelabrum* emits a diverse set of aroma compounds, including compounds such as β-myrcene and eucalyptol, which have been previously reported to be associated with insect attraction (Nat. Prod. Rep., 2023, 40, 1901; Nat. Prod. Rep., 2023, 40, 819).

***Comment:*** c) Has it been confirmed that the substances detected here, which are claimed to be the floral fragrance components, have any insect attractant activity (either through experiments or information from published literature)?

**Response:** Thanks for this question. We did not directly test the insect-attractant activity of individual compounds. The floral volatiles detected by our GC–MS analyses consist predominantly of terpenoid compounds, including mono- and sesquiterpene hydrocarbons and their derivatives, together with a smaller proportion of aromatic and aliphatic hydrocarbons. Several floral volatile compounds detected by our GC–MS analyses have been reported in the literature to play roles in insect attraction or perception. For instance, β-myrcene and eucalyptol are frequently documented components of floral scents involved in insect perception in diverse plant–insect interactions (Nat. Prod. Rep., 2023, 40, 1901; Nat. Prod. Rep., 2023, 40, 819). We have added relevant references and clarified this point in the Results section. (Now at revised manuscript **lines 138-143**)

**lines 138-143:** "We further analyzed the floral volatile compounds of *S. candelabrum* using gas chromatography-mass spectrometry (GC-MS), which revealed a diverse suite of floral volatiles (Table S3). Several compounds we identified, including β-myrcene and eucalyptol, have been reported to be involved in insect perception or attraction, suggesting that the floral scent of *S. candelabrum* contains volatiles with the potential to attract insects[21,22]."

***Comment:*** d) Please note the italic style in the compound names. For example, E, Z, cis, trans, O-decyl (O), 1R (R), N-, n-,

**Response:** Thank you for pointing this out. The italic style in the compound names has now been corrected and standardized throughout the revised manuscript and supplementary materials.

(Supplementary materials **Table S3**)

***Comment:*** e) Was the compound name of DF-3 (Rt 9.969) “ester”? I recommend that the authors double-check the compound names and other entries in the table to ensure there are no errors.
**Response:** The compound name for DF-3 (retention time 9.969 min) is “Propanoic acid, 3-mercapto-, 2-ethylhexyl ester”. In the previous version of Table S3, this name was wrapped onto two lines within the same cell due to its length, which may have caused confusion. We have now reformatted Table S3 to ensure that all compound names are clearly presented. All compound names and table entries have been carefully checked.

***Comment:*** f) Remove initial zero for the CAS numbers.
**Response:** Thank you for pointing this out. The initial zeros in the CAS numbers have been removed.

The others

・What are the noteworthy results?

・Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.
As referenced by the authors (ref-19 in the manuscript), the experimental approach and procedures, including N15 labeling and fluorescence observation, are similar with the report PNAS 118 (33) e2022724118https://doi.org/10.1073/pnas.2022724118. Therefore, the strength of this paper lies in the fact that a new carnivorous plant in the genus *Saxifraga* has been established at the current level of scientific methods. Regarding the novelty of the results of genome analyses, this reviewer will refrain from commenting as I do not have sufficient knowledge on the subject.

・Does the work support the conclusions and claims, or is additional evidence needed?

Basically, as far as this reviewer’s opinion, the experiments presented in this paper demonstrate that *Saxifraga candelabrum* possesses characteristics of a carnivorous plant.

・Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

・Is the methodology sound? Does the work meet the expected standards in your field?

・Is there enough detail provided in the methods for the work to be reproduced?

It has been argued that volatile substances derived from this *Saxifraga* are involved in the attraction process. However, I thought that the explanation and discussion regarding this aspect are insufficient. Therefore, as mentioned above, this reviewer believes that this section needs to be revised.

**Response:** We appreciate the reviewer’s overall assessment and the suggestion to further strengthen

this manuscript. We have revised the Results to clarify the basis for our interpretation that floral volatile compounds may be involved in insect attraction. Specifically, several floral volatile compounds detected by our GC–MS analyses have been reported in the literature to play roles in insect attraction or perception. For instance, β-myrcene and eucalyptol are associated with insect-related functions. We have also added appropriate literature references and revised Table S3 and the Methods section to improve clarity and technical detail, as suggested.

**Reviewer #2 (Remarks to the Author):**

**Overall assessment:** This is an interesting and generally well-presented study. Using a variety of techniques, the authors provide novel evidence of carnivory in the alpine plant, *Saxifraga*. They further suggest that carnivory in plants may be more widespread than currently expected.
**Response:** We thank the reviewer for the positive overall assessment and for the constructive comments provided. Our detailed responses to the specific points raised are provided below.

**Overall assessment:** The title of the paper focuses on the identification of a new carnivorous plant lineage. This is exciting, however, a study in 2021 (Lin et al. 2021 PNAS) used some similar approaches to identify a similar mechanism of carnivory in the *Triantha* plant lineage. This somewhat diminishes the novelty of the current study and its significance for its research field. The current study does, however, go beyond the Lin et al. 2021 paper by including a field study and a comparison of the genomic characteristics of carnivorous plants. These aspects of the study could be further emphasised as points of difference, though it is unclear exactly how the field experiment provides evidence for carnivory in *Saxifraga*.
**Response:** We thank the reviewer for this thoughtful comment on novelty and the comparison with Lin et al. (2021, PNAS). We really appreciate the study of Lin et al. (2021), which provides important insights into plant carnivory. Our isotopic experiments were aligned with the methodological approach of Lin et al. (2021), which we have cited in the Methods section. While Lin et al. primarily demonstrated carnivory in *Triantha* through nutrient uptake and enzymatic evidence, our study extends this framework by explicitly testing all four criteria required to confirm plant carnivory (prey attraction, capture, digestion, and nutrient uptake) in a different lineage (*Saxifraga*). In particular, we combine field-based observations and experiments with laboratory analyses to demonstrate insect attraction and selective capture under natural conditions, and further place these traits in an evolutionary context through comparative genomic analyses revealing genome-wide convergence. We have revised the Introduction to more clearly emphasize these four criteria required to confirm plant carnivory and to clarify how the field experiments provide evidence for carnivory in *S. candelabrum*. (Now at revised manuscript **lines 50-57 & 90-101**)

**Overall assessment:** The approaches used and data presented appear to support the findings and conclusions of the study. However, some additional detail of methodology and data analysis is required for improved clarity and reproducibility. It should also be made more clear how the different components of the manuscript tie together to address the study's research questions.
**Response:** We thank the reviewer for this overall assessment and constructive suggestions. In response, we have revised the manuscript to improve methodological clarity and reproducibility by

providing additional details in the Methods section/Supplementary materials and clarifying data analysis procedures where necessary, as addressed in the specific comments below.

In the revised Introduction, we now explicitly define carnivory using the four widely accepted criteria (prey attraction, capture, digestion, and nutrient uptake) and clearly state our study objectives within this framework. Each subsequent section of the Results is organized to address these criteria in turn, making clear how the different components of the study collectively test the carnivorous status of *S. candelabrum*.

More specific comments are below:

***Comment:*** - The title and abstract both refer to Darwin's theory/hypothesis, but it may be unclear to readers what this is. It should be possible to slightly reframe the abstract/hypothesis to make clear that Darwin hypothesised that this particular plant may be carnivorous. Further explanation of Darwin's theory/hypothesis should also be included in the introduction of the manuscript.

**Response:** We thank the reviewer for this suggestion. We have revised the Abstract and Introduction to clarify the nature of Darwin's hypothesis. Specifically, we now explicitly state that Darwin hypothesized that certain *Saxifraga* species bearing glandular hairs might be capable of carnivory. We have also added a brief explanation in the Introduction to provide historical context for Darwin's hypothesis. (Now at revised manuscript **lines 29-30 & 64-69**)

**lines 29-30:** "Our findings provide the first definitive evidence of carnivory in *Saxifraga*, addressing Charles Darwin's long-standing hypothesis that some *Saxifraga* species may be capable of carnivory"

**lines 64-69:** "Inspired by his work on carnivory in *Drosera*, Darwin (1875) proposed that two *Saxifraga* species (*S. rotundifolia* and *S. umbrosa*) might similarly exhibit carnivorous behavior. Darwin regarded these two species as representing one of the most intriguing cases among plants with glandular hairs[2]. Although Darwin attempted to test this hypothesis through feeding experiments, these early attempts did not yield conclusive results[2]."

***Comment:*** - In the abstract, ln 22-24: This section of text needs to be reframed/reworked for clarity. D15N is not taken up, rather 15N is taken up from isotope-labelled prey, and this alters the d15N signature of the plant. Enzymatic analysis confirmed ability to digest prey, and an isotope labelling experiment with 15N-labelled prey confirmed that N was transferred from the prey to plant tissues.

**Response:** Thanks for this helpful clarification. We have revised the abstract to clarify that nitrogen ($^{15}N$) from isotope-labelled prey was transferred to plant tissues, rather than $\delta^{15}N$ itself being taken up. The revised text now more accurately reflects the isotope labelling experiment and its interpretation. (Now at revised manuscript **lines 23-24**)

**lines 23-24:** "and isotope labelling experiments using $^{15}N$-labelled insects demonstrated the transfer of nitrogen from prey to plant tissues"

***Comment:*** - In the introduction, the authors should specifically define carnivory (vs protocarnivory/ 'murderous' plants) and specifically outline what features they aim to identify to 'prove' that *Saxifraga* is carnivorous.

**Response:** We thank the reviewer for this valuable suggestion. In the revised Introduction, we have added a dedicated paragraph that explicitly defines true carnivory and protocarnivory. We also

revised the final paragraph to specifically outline the key features we tested to confirm carnivory in *Saxifraga candelabrum*: active **prey attraction** and **capture**, **enzymatic digestion**, and **nutrient uptake from prey**, in addition to convergent genomic signatures. These criteria directly correspond to the structure of our Results section. (Now at revised manuscript **lines 50-57**)

**lines 50-57:** "By definition, a carnivorous plant must be capable of absorbing nutrients derived from captured animals and possess morphological, physiological and behavioural adaptations that facilitate **prey attraction, capture, digestion and nutrient uptake**[1]. Yet some plants exhibit only a subset of carnivorous traits—most commonly prey capture without clear evidence of digestion or nutrient absorption—and are therefore described as protocarnivorous[5]. This distinction underscores the need for explicit evidence to confirm true carnivory in plants. In this context, several plant lineages bearing insect-trapping structures have remained controversial with respect to their carnivorous status."

***Comment:*** Ln 39-41: Waterwheel plants have a snap trap (as correctly stated on ln 148). Bladderworts would be an example of a carnivorous plant using a suction trap.

**Response:** Thanks for this helpful clarification. The suction trap example has been revised from waterwheel plants to bladderworts in the Introduction.

Now at revised manuscript **line 44:** "suction trap in bladderworts"

***Comment:*** Ln 41: Correct to "Despite their diverse mechanisms for…"

**Response:** Corrected as suggested **(**Now at revised manuscript **line 45)**.

***Comment:*** Ln 62: It would be more appropriate to refer to Figure 1 here, rather than specifically Figure 1A.

**Response:** Revised as suggested **(**Now at revised manuscript **line 80)**

***Comment:*** - At the end of the introduction, make clear what the specific research questions/hypotheses are. These could be made to nicely tie in with the subheadings that follow.

**Response:** We thank the reviewer for this suggestion. In the revised Introduction, we have explicitly outlined the specific research questions we aimed to address: (1) Does *S. candelabrum* actively attract and capture prey? (2) Can it directly digest prey and absorb nutrients? (3) Are there convergent genomic signatures of carnivory shared with other independent carnivorous lineages? These questions are now clearly aligned with the structure and subheadings of the Results section, thereby improving the clarity of the Introduction. **(**Now at revised manuscript **lines 90-96)**

***Comment:*** Ln 73: Use past tense throughout this section. It makes it clear that this is based on work completed in the current study.

**Response:** Thanks for this helpful suggestion. We have revised the Results section to consistently use the past tense throughout, ensuring that all descriptions clearly reflect work completed in the current study.

***Comment:*** Ln 86: Is the phrase "This insect trapping phenomenon" referring to the fact they trap insects, rather than any relationship between trap surface area and prey number? Please clarify.

**Response:** We thank the reviewer for pointing this out. In this context, "this insect-trapping

phenomenon" refers specifically to the consistent occurrence of insect trapping by glandular hairs, rather than to any quantitative relationship between inflorescence structure and prey number. We have revised the text accordingly to avoid ambiguity.

**Now at revised manuscript lines 121-126:** "In addition, examination of all available flowering herbarium specimens of *S. candelabrum* revealed that insect prey were present on glandular hairs in the vast majority of specimens (43 out of 45), spanning both the geographical range and the entire temporal span represented in the collections (Table S2)."

***Comment:*** It is unclear how the relationship between trap surface area and number of captured prey contributes to evidence supporting carnivory. We may see the same in non-carnivorous plants that trap insects for defensive purposes (without subsequent digestion). Make clear how this part of the work contributes to addressing the overall research questions.

**Response:** We agree with the reviewer that insect trapping alone is not sufficient to demonstrate carnivory. Accordingly, the relationship between trap surface area and prey number is not presented as standalone evidence of carnivory. Rather, it addresses the first criterion of carnivory—active prey attraction and capture—within the multi-criterion framework outlined in the Introduction (prey attraction, capture, digestion, and nutrient uptake). This analysis indicates that insect capture in *S. candelabrum* is consistent, rather than incidental. Evidence for digestion and nutrient assimilation is provided in subsequent sections using enzymatic assays and isotopic labeling. We have revised the text to make the specific contribution of this result clearer. **(**Now at revised manuscript **line 117-120)**

**Lines 117-120:** "This observed correlation suggested that the increased number and length of inflorescence branches may enhance insect attraction and provide a larger surface area bearing glandular hairs, potentially increasing the probability of insect capture."

***Comment:*** Ln 87: Subheading "Active attraction and insect capture using floral odor" does not represent the content immediately below, which also includes visual attraction.

**Response:** We thank the reviewer for this comment. The subheading has been revised to "Olfactory and visual cues in prey attraction" to better reflect the content. **(**Now at revised manuscript **line 129)**

***Comment:*** Ln 92: It is stated that more insects were attracted to the visual blocking treatment than control groups. Figure 3b shows the positive control had the same number of insects as the visual blocking treatment. Reword for clarity.

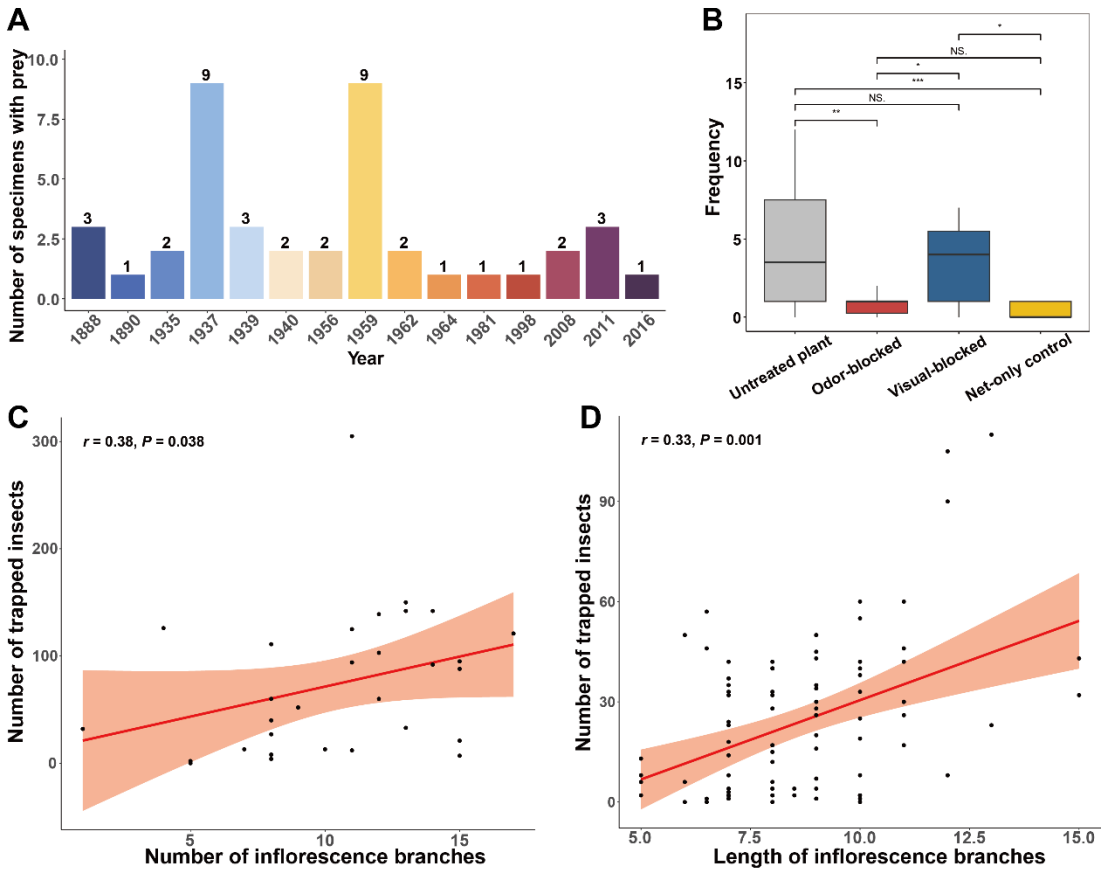
**Response:** We sincerely thank the reviewer for pointing this out. Upon careful review, we discovered that the labels for the positive and negative controls in the Methods were inadvertently reversed. We have corrected the labels in the Methods, revised the Results description accordingly, and updated the figure legend for clarity. No changes were made to the data or figures themselves. As shown in Figure 3b, insect visitation was similarly high in the untreated plants (positive control) and the visual-blocked treatment, and substantially lower in the odor-blocked treatment and the net-only control (negative control). (Now at revised manuscript **lines 131-136 & 484-492, revised Fig. 3**)

**lines 131-136:** "we enclosed the flowering individuals of *S. candelabrum* either with a glass enclosure (odor-blocked) or with a white net enclosure (visual-blocked). Significantly more flower-visiting insects were observed in the visual-blocked and the untreated groups than in both the odor-

blocked and the net-only control groups (Fig. 3B)"

**lines 484-492:** "(1) Odor-blocked: flowering individuals of *S. candelabrum* were enclosed with a transparent glass enclosure (visual attraction but no odor, with the open end of the glass enclosure sealed using transparent plastic film to minimize floral odor escape); (2) Visual-blocked: flowering individuals of *S. candelabrum* were covered with a white breathable net enclosure (permitting odor attraction); (3) Net-only control: white breathable net enclosure without *S. candelabrum* in it (testing the possibility of color attraction by the white netting); (4) Untreated: flowering individuals of *S. candelabrum* without any treatment."

**Revised Figure 3**




Note: Labels in Fig. 3B have been corrected for clarity; "Untreated" refers to flowering individuals of *S. candelabrum* without any treatment.

***Comment:*** As in *Triantha*, it is claimed that *Saxifraga* balance pollination with carnivory by only capturing smaller insects that are not pollinators. There appears to be little, if any overlap of prey and flow visitors (Table S1), which supports this. However, this then raises the issue of how the experiment on floral odour vs visual attraction fits within this manuscript. If the experiment determined what attracts flower visitors, but flower visitors are not prey, then how does this relate to carnivory in *Saxifraga*?

**Response:** We thank the reviewer for raising this important point. To avoid confusion, we have revised Table S1 by redefining insect categories as "prey" and "uncaptured visitors" and clarified these definitions in the table caption. In our study, "flower visitors" includes all insects initially attracted to the flowers, and insects categorized as "prey" represent a subset of flower visitors that

were subsequently captured by glandular hairs, whereas "uncaptured visitors" refers to flower-visiting insects that were not trapped. The floral odour versus visual attraction experiment therefore addresses the initial prey-attraction phase of carnivory, demonstrating that *S. candelabrum* actively attracts insect visitors to the trapping interface. Importantly, attraction itself does not distinguish prey from pollinators; this differentiation arises at the capture stage, where small-bodied insect visitors are trapped by the glandular hairs, while effective pollinators of *S. candelabrum* are large-bodied insects and were rarely captured. Thus, floral cues facilitate both pollination and prey availability, whereas carnivory is realized through size-selective capture and subsequent digestion. Relevant text has been revised in the Results, Methods, and Table S1 caption. **(**Supplementary materials **Table S1,** revised manuscript **lines 110 & line 455-459)**

***Comment:*** Ln 95 and Table S3: Which compounds are known to be insect-attracting? Please provide references to support this.

**Response:** We thank the reviewer for this comment. Several compounds detected in our GC–MS analysis (Table S3) have been reported in previous studies to function as insect-attracting or insect-associated floral scent components. For instance, β-myrcene and eucalyptol are frequently documented components of floral scents involved in insect perception in diverse plant–insect interactions (Nat. Prod. Rep., 2023, 40, 1901; Nat. Prod. Rep., 2023, 40, 819). We have now cited these references to support our argument and clarified in the Results section that the presence of such compounds suggests that the floral scent of *Saxifraga candelabrum* contains volatiles with the potential to attract insects. **(**Supplementary materials **Table S3,** revised manuscript **lines 140-143)**

**lines 140-143:** "Several identified compounds we identified, including β-myrcene and eucalyptol, have been reported to be involved in insect perception or attraction, suggesting that the floral scent of *S. candelabrum* contains volatiles with the potential to attract insects[21,22]."

***Comment:*** Table S3 and ln 95: Which compounds are known to be insect-attracting? Are there refs to support this?

**Response:** We have revised the Results section to explicitly indicate that several detected floral volatile compounds are known to be associated with insect attraction or perception, such as β-myrcene and eucalyptol (Nat. Prod. Rep., 2023, 40, 1901; Nat. Prod. Rep., 2023, 40, 819). Relevant references have now been added to the corresponding Results text **(**Supplementary materials **Table S3,** revised manuscript **lines 140-143)**

***Comment:*** Ln 112: State clearly that fluorescence indicates presence of phosphatase enzyme

**Response:** We have revised the text at Ln 112 to explicitly state that the observed fluorescence indicates phosphatase activity. **(**Now at revised manuscript **line 159-161)**

**line 159-161:** "Following treatment with the ELF97 phosphatase substrate, yellow-green fluorescence indicative of phosphatase activity was detected in the glandular hairs of the carnivorous plant *Pinguicula primuliflora* and *S. candelabrum*."

***Comment:*** Ln 113: Reword to say that this indicates that the species has the capacity to directly digest trapped insects.

**Response:** Thank you for this helpful suggestion. We have reworded the sentence as "This enzymatic activity indicated that *S. candelabrum* has the capacity to directly digest trapped insects,

rather than relying on alternative decomposition processes." **(**Now at revised manuscript **line 162-163)**

***Comment:*** Ln 116-117: Rewords. Sounds like it is saying that the species doesn't have digestive enzyme despite not capturing insects. This sentence structure doesn't make sense, as this is exactly what you would expect.

**Response:** We thank the reviewer for pointing out this issue. We have reworded the sentence to clarify that the lack of detectable phosphatase activity in *S. filicaulis* is consistent with its glandular hairs rarely capturing insects. **(**Now at revised manuscript **lines 164-167)**

**lines 164-167:** "In contrast, *S. filicaulis*, a closely related and often sympatric species in Shangri-La, showed no detectable fluorescence under identical conditions, consistent with the observation that its glandular hairs rarely captured insects (Fig. 4)."

***Comment:*** Ln 131-132: When referring to the isotope labelling experiment, results should be expressed as enrichment in 15N, or transfer/uptake/assimilation of 15N, leading to more positive d15N values. E.g., reword to read "… highest 15N-enrichment in flowers…" (ln 131), and "lowest 15N-enrichment in basal leaves" (ln 132).

**Response:** We thank the reviewer for this helpful suggestion. We have reworded the relevant sentences to express the results as differential $^{15}N$ enrichment among plant organs.

Now at revised manuscript **lines 185-187:** "We further compared the $\delta^{15}N$ enrichment in different organs of *S. candelabrum* and found the highest $\delta^{15}N$ enrichment in flowers, followed by cauline leaves, with the lowest $\delta^{15}N$ enrichment in basal leaves"

***Comment:*** Ln 133: More prey-derived nutrients were invested in reproductive growth. Could this reflect proximity of the plant part to the captured prey, or simply the fact that this is the part of the plant that is actively growing?

**Response:** We thank the reviewer for this insightful comment. We agree that the higher $\delta^{15}N$ enrichment observed in reproductive organs could potentially reflect factors such as spatial proximity to captured prey or organ-specific growth activity. To avoid over-interpretation, we have revised the Results to present this pattern more conservatively and to relate it to the monocarpic life-history strategy of *S. candelabrum*. We now clarify that, while proximity and growth activity may contribute to the observed pattern, the significantly stronger enrichment in flowers relative to cauline leaves (which are similarly proximate to prey capture sites and actively growing) is consistent with preferential allocation of prey-derived nitrogen to reproductive tissues.

Now at revised manuscript **lines 187-193** "The higher content of $^{15}N$ accumulated in reproductive organs than vegetative organs could not be fully explained by their proximity to glandular trichomes that absorb $^{15}N$, given that cauline leaves, which were similarly close to the nitrogen sources, exhibited significantly lower $^{15}N$ levels. This contrast indicated that plants actively allocate more nitrogen to their reproductive organs, a strategy that optimizes resource use to maximize reproductive output of this monocarpic plant."

***Comment:*** Is it possible to quantify or estimate how much of the plant's N requirement may be acquired via carnivory?

**Response:** We thank the reviewer for this insightful question. In principle, the contribution of prey-

derived nitrogen to plant nutrition can be quantified or estimated in some carnivorous plants using natural-abundance $\delta^{15}N$ mixing models (e.g. Schulze et al. 2001). However, such approaches rely on several assumptions that are not met in our study system. In particular, the $\delta^{15}N$ values of potential prey insects span a wide range that can exceed the variation observed in both soils and non-carnivorous reference plants, a limitation explicitly noted by Schulze et al. (2001) that renders simple two-end-member mixing models inapplicable. In addition, *Saxifraga candelabrum* grows in spatially isolated rock crevices with a distinct soil nitrogen baseline, and our data show that both plant and soil natural-abundance $\delta^{15}N$ values differ from those of nearby non-carnivorous reference plants (Supplementary materials Table S4). For example, soils surrounding the non-carnivorous plant *S. diversifolia* exhibited higher natural $\delta^{15}N$ values (mean ± SD: 6.78 ± 0.19‰) than those associated with *S. candelabrum* (3.07 ± 1.54‰). Under these conditions, the numerical estimate of prey-derived nitrogen contribution would be unreliable.

***Comment:*** Ln 209: remove ";"
**Response:** Thanks for this correction. The sentence has been revised accordingly. We have checked throughout the manuscript to avoid such mistakes. **(**Now at revised manuscript **line 271)**

***Comment:*** Ln 224: Amend to "are rarely captured"
**Response:** Thanks for this suggestion. We have revised the statement. **(**Now at revised manuscript **line 287)**

***Comment:*** Ln 224: Amend to read that "these plants therefore appear to balance carnivory with pollination".
**Response:** Thanks for this suggestion. We have revised this statement. **(**Now at revised manuscript **line 287-288)**

***Comment:*** Ln 225: A word seems to be missing - "the alpine environment"?
**Response:** Thanks for pointing this out. We have corrected this sentence. **(**Now at revised manuscript **line 289)**

***Comment:*** Ln 226: It is unclear how this carnivory is cryptic given that sticky hairs and trapped insects are easily observable.
**Response:** We thank the reviewer for highlighting this potential confusion. While glandular hairs and trapped insects in *S. candelabrum* are morphologically conspicuous, carnivory in this species has historically been overlooked because such structures in many angiosperms are typically interpreted as defensive rather than carnivorous. Therefore, the term "cryptic" refers to this previous oversight, not to the visual conspicuity of the hairs or captured insects. To eliminate any ambiguity, we have revised the sentence to: "The long-overlooked carnivory in *S. candelabrum*—despite the conspicuous glandular hairs and trapped insects—suggests the possibility of broader occurrence of carnivory in flowering plants.". **(**Now at revised manuscript **lines 289-292)**

***Comment:*** Ln 371-382: Restructure this section of the methodology to improve clarity. It is initially difficult to ascertain what the various herbarium vs field specimens were used for. E.g. ln 378 initially sounds like this it relates to herbarium specimens, then ultimately says that the specimens

were in the field. This is confusing.

**Response:** Thanks for this helpful suggestion. We have restructured the paragraph by reorganizing the sentences and adding transitional phrases to clearly distinguish between the use of field-collected insects and living plants from the examination of herbarium specimens. This eliminates confusion about the distinct purposes of field and herbarium samples. **(**Now at revised manuscript **lines 462-477)**

**lines 462-477:** "To quantify prey capture in living plants and test the relationship between the number of trapped insects and trapping surface, we recorded the number and length of inflorescence branches, as well as the number of trapped insects on each branch, for 32 full-blooming individuals of *S. candelabrum* from Wufeng Mountain of Yunnan province in China. The relationships between the number of trapped insects and inflorescence branch number and length were assessed using Spearman's rank correlation. Linear models were used solely to visualize trends, with shaded areas indicating 95% confidence intervals.

Additionally, to assess the consistency of insect trapping across the species' geographical and temporal distribution, all available flowering specimens of *S. candelabrum* from the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN) were examined, and the number of trapped insects (prey) on glandular hairs was recorded (45 specimens spanning 1888-2016; Supplementary materials Table S2)."

***Comment:*** Some aspects of the methodology are unclear. E.g. How were glass boxes secured to the substrate to prevent odour escaping? Are insects only trapped by plants while flowers are present/open? Were herbarium samples flowering? Provide more information on the fruit fly labelling – how long were they fed 15N substrate for? What was the N concentration of the amino acid mix?

**Response:** We thank the reviewer for pointing out these unclear aspects of the methodology.

**1): How were glass boxes secured to the substrate to prevent odour escaping?**

We have revised the Methods section to provide additional details and improve clarity. Specifically, we now describe how the open end of the glass enclosure was sealed to the substrate using transparent plastic film to minimize the escape of floral odors while maintaining light penetration. **(**Now at revised manuscript **lines 486-487):** "with the open end of the glass enclosure sealed using transparent plastic film to minimize floral odor escape"

**2): Are insects only trapped by plants while flowers are present/open? Were herbarium samples flowering?**

We now clarify that insect trapping in *Saxifraga candelabrum* occurs predominantly during the flowering stage, with only occasional captures outside the flowering period, and that all examined herbarium specimens were flowering individuals **(**Now at revised manuscript **lines 465 & 473)**.

**3): how long were they fed $^{15}$N substrate for? What was the N concentration of the amino acid mix?**

We have expanded the description of the fruit fly labelling protocol by specifying the duration of rearing on the $^{15}$N-labeled diet (two months) and by providing the certified isotopic enrichment (98 atom % $^{15}$N) of the algal amino acid mixture used.

**Now at revised manuscript lines 540-544:** "fruit flies (*Drosophila melanogaster*) were fed with $^{15}$N-labeled amino acids for approximately two months, allowing multiple generations to develop and ensuring stable isotopic enrichment. The labeled diet was prepared by adding 100 mg algal

amino acid mixture (Sigma-Aldrich, 98 atom%$^{15}$N) to 100 g standard *Drosophila* medium."

***Comment:*** In the labelling experiment, were 15N labelled fruit flies added for 2 weeks, as per controls?

**Response:** We thank the reviewer for this clarification request. The $^{15}$N-labeled fruit flies were applied for the same two-week duration as the unlabeled controls for all species. To avoid ambiguity, we have now explicitly stated the treatment duration in the Methods section. In addition, for *Saxifraga candelabrum*, we further sampled tissues at 20 and 28 days after treatment to examine temporal changes in prey-derived nitrogen uptake. **(**Now at revised manuscript **line 564-569)**

**line 564-569:** "Five to ten individuals of each species were treated with ten unlabeled fruit flies for two weeks as controls. Five to ten individuals of each species were treated with ten 15N-labeled fruit flies for each treatment, and tissues were harvested two weeks after treatment for isotopic analysis. In addition, for *S. candelabrum*, additional samples were collected 20 and 28 days after treatment to examine temporal changes in prey-derived nitrogen uptake."

***Comment:*** Using genome characteristics that were identified in this study, would it be possible to screen candidate plants for carnivory based on a 'genetic fingerprint' for plant carnivory?

**Response:** Thanks for this question. While our genomic analyses identified convergent signals (e.g., gene family expansions, rate acceleration, and amino acid substitutions) associated with carnivory, these genes are often present in non-carnivorous plants and may have been co-opted from ancestral functions such as herbivore defense or stress responses (e.g., Birkeland, S. et al. Mol. Biol. Evol. 2020). Therefore, we do not consider these genomic features sufficient to serve as a reliable "genetic fingerprint" for screening candidate carnivorous plants, as functional validation (e.g., of digestion and nutrient uptake) remains essential (Hedrich and Fukushima, Annu. Rev. Plant Biol. 2021). Rather, the candidate genes identified here provide hypotheses for future functional studies aimed at elucidating the molecular basis of carnivory. Using such genomic signatures to screen for carnivory may represents an intriguing avenue for future research.

***Comment:*** Provide detail of statistical analyses (approach and results) that were done to support this study (e.g. How did you determine regression relationships in figures 3C and 3D? Where did you establish statistical differences in box plots?)

**Response:** We thank the reviewer for this comment. We have now clarified the statistical approaches and results by adding relevant details directly to the corresponding Methods sections, and significance levels are now explicitly defined in the figure captions.

**Fig. 3 caption:** "Significance levels: NS, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$"

**Revised manuscript lines 467-470:** "The relationships between the number of trapped insects and inflorescence branch number and length were assessed using Spearman's rank correlation. Linear models were used to visualize trends, with shaded areas indicating 95% confidence intervals."

**Revised manuscript lines 111-114:** "Field observations demonstrated that the number of insects trapped on *S. candelabrum* individuals correlated positively with both the number (Spearman's $\rho = 0.38$, $P = 0.038$) and length (Spearman's $\rho = 0.33$, $P = 0.001$) of inflorescence branches (Fig. 3)."

***Comment:*** Figure 1 caption: Do the images truly show digested prey or just captured prey?

**Response:** We thank the reviewer for pointing this out. The images in Fig. 1 show insect prey

captured by the sticky glandular hairs rather than direct evidence of digestion. We have therefore revised the figure caption to avoid overinterpretation.
**Figure 1 caption:** "These photos (especially C, D, and E) show the sticky reddish glandular hairs and captured insect prey."

***Comment:*** Figure 2 caption: Make clear what APG IV is (write APG in full).
**Response:** We thank the reviewer for this suggestion. We have revised the Figure 2 caption to write out APG IV in full as Angiosperm Phylogeny Group IV.
**Figure 2 caption:** "Phylogenetic tree based on the Angiosperm Phylogeny Group IV (APG IV, 2016) illustrating multiple origins and trap types of carnivorous plants in angiosperms"

***Comment:*** Figure 3A: I am unclear of the value in seeing the number of specimens per year with prey, especially without knowing the total number of specimens available. Did all plants have prey? Does it matter (for this study) that there was variation among years? Any variation likely can't be accounted for anyway, unless information on environmental conditions impacting prey availability etc. can be accounted for and collection methods and seasons were standardised.
**Response:** We thank the reviewer for this thoughtful comment. We agree that year-to-year variation in the number of herbarium specimens with trapped insects cannot be interpreted quantitatively, as sampling effort, collection season, and environmental conditions were not standardized. The purpose of examining herbarium specimens and presenting Fig. 3A was therefore not to assess interannual variation in prey capture intensity, but to document the long-term persistence of insect trapping in *Saxifraga candelabrum*. Importantly, all available flowering specimens at KUN were examined (45 specimens spanning 1888–2016), and insect prey were present on glandular hairs in the vast majority of specimens (43/45), across both time and space (Supplementary materials Table S2). We have revised the Results text to clarify that herbarium data are used as qualitative evidence that prey capture is a recurrent and widespread feature of the species, rather than to infer temporal trends.
**Now at revised manuscript lines 121-128:** "In addition, examination of all available flowering herbarium specimens of *S. candelabrum* revealed that insect prey were present on glandular hairs in the vast majority of specimens (43 out of 45), spanning both the geographical range and the entire temporal span represented in the collections (Table S2). This finding indicates that insect trapping by glandular hairs is a recurrent and widespread feature of the species, rather than a sporadic or recent phenomenon."

***Comment:*** Figure 3B and Figure 5: Indicate what the significant levels are that are indicated on the figure.
**Response:** We thank the reviewer for this comment. We have revised the figure captions for Figures 3B and 5 to explicitly define the significance levels corresponding to the symbols (NS, *, **, ***).
**Figure 3B caption:** "Significance levels: NS, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$"
**Figure 5 caption:** "Significance levels: NS, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$."

***Comment:*** Figures 3C & D: Describe what the shaded areas represent.
**Response:** We thank the reviewer for this comment. We have revised the figure legend of Fig. 3C and 3D to explicitly state that the shaded areas represent the 95% confidence intervals.

**Figures 3 caption:** "…with shaded areas indicating the 95% confidence intervals."

**Reviewer #3 (Remarks to the Author):**

This manuscript tested a potentially new carnivorous plants in *Saxifraga* from enzymatic, isotopic and genetic analyses. The isotopic analyses, which will directly determine if a plant is carnivorous or not, suggested that *Saxifraga candelabrum* gained nitrogen from labelled insects. However, I have some concerns about the design of the experiment, and the results are not fully discussed, which may affect the reliability of the conclusion. The enzymatic analysis will determine if this plant is holocarnivorous (produce their own digestive enzyme) or not but lacks a positive control here.

**Response:** We thank the reviewer for this overall assessment of our study and for highlighting key concerns regarding experimental design, interpretation of the isotopic evidence, and the enzymatic analysis. We take these points seriously. All of these issues have been carefully addressed in our responses to the specific comments below, and corresponding clarifications and revisions have been made in the revised manuscript and supplementary materials to improve the transparency and robustness of our conclusions. In particular, we have expanded the discussion of the isotopic experiment and included a known carnivorous plant (*Pinguicula primuliflora*) as a positive control for the enzymatic analysis to strengthen the reliability of our conclusions.

***Comment:*** My main concern is from the isotopic data, which is the essential evidence to prove if a plant is carnivorous or not. I can't find the original δ15N data from the supplementary material in this manuscript, which should be there. So I can only tell from the Figure. 5, which is also not clearly labelled (for example what is the control in Fig. 5D? What does DJ mean in Fig. 5B?).

**Response:** We thank the reviewer for highlighting these important points. We have now added the raw $\delta^{15}N$ data as a new supplementary table (Supplementary materials Table S4). In addition, we have revised and clarified the legends for Fig. 5 to explicitly define all treatments and controls. Specifically, in all panels, control samples represent individuals treated with unlabeled fruit flies for two weeks, whereas labeled treatments correspond to exposure to $^{15}N$-labeled *Drosophila* for the durations indicated. We have made this definition explicit in the Fig. 5 caption to avoid confusion. In addition, the abbreviation "DJ" has been replaced with "SC" (*Saxifraga candelabrum*), and all abbreviations are now clearly defined in the figure caption to avoid ambiguity. **(Supplementary materials Table S4, Fig. 5, revised manuscript lines 564-569)**

**Table S4** Raw data of $\delta^{15}N$ values for all plant tissues measured in the isotopic experiments. Data include different species, organs, localities, treatment durations, and treatments with either $^{15}N$-labeled or unlabeled *Drosophila* (as control).

**Fig. 5 caption: C:** $\delta^{15}N$ enrichment in individual organs (flower, cauline leaf, basal leaf) under control, two-week labeled, and four-week labeled treatments. **D:** Comparison of $\delta^{15}N$ enrichment across organs within each treatment group. In all experiments, control individuals were treated with unlabeled fruit flies for two weeks, whereas labeled treatments involved exposure to $^{15}N$-labeled *Drosophila* for the durations indicated. Significance levels: NS, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

**lines 564-569:** Five to ten individuals of each species were treated with ten unlabeled fruit flies for

two weeks as controls. Five to ten individuals of each species were treated with ten $^{15}$N-labeled fruit flies for each treatment, and tissues were harvested two weeks after treatment for isotopic analysis. In addition, for *S. candelabrum*, additional samples were collected 20 and 28 days after treatment to examine temporal changes in prey-derived nitrogen uptake.

***Comment:*** The control value of $\delta^{15}$N from negative control (*Saxifraga diversifolia* and *Salvia przewalskii*) are between 5-7.5, which are even much higher than carnivorous plants (*Drosera peltata* as positive control) and the tested *Saxifraga candelabrum*. Usually the natural $\delta^{15}$N level in carnivorous plants should be higher than the non-carnivorous plants and lower than the insects in the same habitat, and that's how a plant is proved to be carnivorous or not without labelling. This high level of natural $\delta^{15}$N is even very rare in carnivorous plants, let along any non-carnivorous plants. The authors need to explain this unusual high level of $\delta^{15}$N in their negative controls. I think it's more meaningful to test the natural $\delta^{15}$N level of *Saxifraga candelabrum*, non-carnivorous plants in the same habit, and their animal preys. The isotopic analysis should be in this ecological context, that carnivorous plants gain nutrient from insects, thus should have a higher level of $\delta^{15}$N than non-carnivorous plants.

**Response:** We thank the reviewer for this thoughtful and detailed comment. We agree that the relatively high natural $\delta^{15}$N values observed in the negative controls are unusual when viewed under the classical natural-abundance framework used for many carnivorous plants. However, the relatively high $\delta^{15}$N values observed in the negative controls (*Saxifraga diversifolia* and *Salvia przewalskii*) likely reflect variation in background soil $\delta^{15}$N. As indicated in Table S4 (Raw data of $\delta^{15}$N values), we have conducted the measurements of natural $\delta^{15}$N in soils associated with *S. diversifolia* and *S. candelabrum* (Supplementary materials Table S4). Notably, soils surrounding *S. diversifolia* exhibited higher $\delta^{15}$N values (mean ± SD: 6.78 ± 0.19‰) than those associated with *S. candelabrum* (3.07 ± 1.54‰). This pattern is consistent with previous studies demonstrating that plant $\delta^{15}$N values are strongly influenced by local soil nitrogen isotopic composition and microhabitat conditions (e.g. Kahmen et al., 2008). Although all plant species examined in Fig. 5A were collected from the same locality (Wufeng Mountain, Shangri-la, China), *S. candelabrum* grows exclusively in rock crevices with limited soil accumulation, whereas the negative controls (*Saxifraga diversifolia* and *Salvia przewalskii*) occur in soil-rich microsites. Such fine-scale habitat heterogeneity likely contributes to the observed baseline $\delta^{15}$N differences among species.

We agree that comparisons of natural $\delta^{15}$N values among co-occurring plant species can provide ecological context, and all species included in our interspecific comparisons were sampled from the same locality (Wufeng Mountain). However, we also note that natural $\delta^{15}$N values alone do not provide a definitive criterion for diagnosing carnivory, because both plant baseline values and prey $\delta^{15}$N can vary widely. For example, $\delta^{15}$N values of prey insects may span a broad range (e.g. −4.5‰ to +7.2‰; Schulze et al., 2001), potentially overlapping with values observed in both carnivorous and non-carnivorous plants. For this reason, we relied primarily on $^{15}$N-labeled feeding experiments to directly trace nitrogen transfer from insects to plant tissues, which provides a more robust test of nutrient uptake from prey than comparisons based solely on natural $\delta^{15}$N values. To avoid ambiguity regarding this point, we have revised the Methods and Results sections. **(Supplementary materials Table S4, revised manuscript lines 172-177 & 558-560)**

**lines 172-177:** "All samples used for interspecific comparisons were collected from the same locality (Wufeng Mountain, Shangri-La), although minor microhabitat differences were present

within the site, as reflected by variation in natural $\delta^{15}N$ values of local soils (Table S4), with negative control species exhibiting higher baseline $\delta^{15}N$ values than *S. candelabrum* and *D. peltata* (Fig. 5A)."
**lines 558-560:** "For interspecific comparisons, *S. candelabrum*, *S. diversifolia*, *S. przewalskii*, and *D. peltata* were all sampled from the same locality (Wufeng Mountain, Shangri-La, Yunnan, China)."

***Comment:*** The consistent drop of δ15N in both lower and leaf from two weeks to 20 days (Fig. 5B) need to be explained. Were nitrogen transferred into fruits or roots later?
**Response:** We thank the reviewer for raising this point. The decline in $\delta^{15}N$ observed from two weeks to 20 days likely reflects isotopic dilution following a pulse input of $^{15}N$-labeled prey, as labeled insects were supplied only at the beginning of the experiment while plants continued to assimilate unlabeled nitrogen thereafter. As the experiment progressed, continued uptake of unlabeled nitrogen from the soil diluted the $^{15}N$ signal in plant tissues, resulting in lower $\delta^{15}N$ values at later time points. We agree that reallocation of nitrogen to other sinks, such as developing reproductive organs or roots, could in principle also contribute to declining $\delta^{15}N$ values. However, due to current limitations, we cannot directly quantify nitrogen transfer to these organs. Given the single pulse of labeled nitrogen and the continued availability of unlabeled nitrogen, isotopic dilution is expected to be the dominant process shaping the temporal $\delta^{15}N$ pattern observed here. We have clarified this interpretation to the Results sections. **(Now at revised manuscript line196-200)**
**line196-200:** "The decline in $\delta^{15}N$ enrichment from two weeks to 20 days likely reflects the complete absorption of the limited labeled prey (10 fruit flies) within the first two weeks (Fig. 5), after which unlabeled soil nitrogen continued to be incorporated, diluting the isotopic signal."

***Comment:*** For Fig. 5D, are they all data from negative control? What was the control? Why there was such a dramatic increase of δ15N to 100-200 in flower after two weeks and four weeks?
**Response:** We thank the reviewer for this question.
**For the first question:** are they all data from negative control? What was the control?
We apologize for the potential confusion caused by the presentation of Fig. 5D. The data shown in Fig. 5D are not all from negative controls. Instead, Fig. 5D represents a reorganization of the same dataset shown in Fig. 5C, with data grouped by treatment rather than by organ. Specifically, "Control" refers to plants fed with unlabeled fruit flies for two weeks, whereas "Two weeks" and "Four weeks" refer to plants exposed to $^{15}N$-labeled *Drosophila* for two and four weeks, respectively. **(Fig. 5, revised manuscript lines 564-569)**
**For the second question:** Why there was such a dramatic increase of δ15N to 100-200 in flower after two weeks and four weeks?
The pronounced $\delta^{15}N$ enrichment (up to ~100–200‰) observed in flowers after two and four weeks therefore reflects assimilation of highly enriched insect-derived nitrogen under labeled treatments, rather than temporal changes in control plants, as shown in Fig. 5C (temporal changes per organ) and Fig. 5D (organ comparison per treatment). We have revised the figure legend to explicitly clarify the treatment definitions in **Fig. 5C&D** to avoid further ambiguity.
**Fig. 5 caption: "**C: $\delta^{15}N$ enrichment in individual organs (flower, cauline leaf, basal leaf) under control, two-week labeled, and four-week labeled treatments. D: Comparison of δ15N enrichment across organs within each treatment group. In all experiments, control individuals were treated with unlabeled fruit flies for two weeks, whereas labeled treatments involved exposure to $^{15}N$-labeled

*Drosophila* for the durations indicated."
**lines 564-569:** "Five to ten individuals of each species were treated with ten unlabeled fruit flies for two weeks as controls. Five to ten individuals of each species were treated with ten 15N-labeled fruit flies for each treatment, and tissues were harvested two weeks after treatment for isotopic analysis. In addition, for *S. candelabrum*, additional samples were collected 20 and 28 days after treatment to examine temporal changes in prey-derived nitrogen uptake."

***Comment:*** From photos in Fig.1 it seems like their sticky leaves and calyx can also capture insects. The enzymatic analysis also showed that sticky leaves and calyx of *Saxifraga candelabrum* produce digestive enzyme (Fig. 4). But why only the stems were labelled and tested? The authors even labelled the calyx of negative control. But why not the calyx of *Saxifraga candelabrum*? Then we don't know if their leaves and calyx are carnivorous or not.
**Response:** We thank the reviewer for this question. We agree that both leaves and calyces of *Saxifraga candelabrum* are capable of capturing insects, as shown by field observations (Fig. 1) and supported by the detection of digestive enzyme activity in these organs (Fig. 4).
**For the first question:** why only the stems were labelled and tested?
In the isotope-labeling experiments, insects were applied exclusively to the upper stems of *S. candelabrum* for methodological reasons rather than because leaves or calyces were assumed to be non-carnivorous. The cylindrical stems allowed secure fixation of labeled insects using parafilm without physical damage, whereas the small surface area of calyces and the fragility of fleshy leaves in this species made comparable labeling difficult without risking tissue damage, leaf abscission, or interference with normal physiological function. Nutrient uptake from these structures was not tested in the current study and remains an avenue for future research.
**For the second question:** The authors even labelled the calyx of negative control. But why not the calyx of *Saxifraga candelabrum*?
The calyx of the negative control species (*Salvia przewalskii*) was labeled because this species bears sticky glandular hairs exclusively on the calyx, as stated in the Methods (**lines 556–558**). In addition, the calyx of *S. przewalskii* is morphologically larger and structurally more robust than that of *S. candelabrum*, allowing secure attachment of labeled insects without tissue damage. Consequently, the calyx was the only organ in *S. przewalskii* where labelled insects could be experimentally attached.
**Revised manuscript lines 556-558:** "*Salvia przewalskii* was chosen as a control because it often co-occurs with *S. candelabrum* in Shangri-La, and has sticky glandular hairs restricted to on its calyx but no evidence of carnivory."

Other concerns:

***Comment:*** l.36. 'including members of seven families'
Definitely more than seven, should be 14 families so far
**Response:** We thank the reviewer for pointing this out. We have revised the text accordingly.
**Now at revised manuscript line 40:** "Carnivory has evolved multiple times in angiosperms, including members of seven 14 families"

***Comment:*** l.40. 'suction trap in waterwheel plants'.

Waterwheel plants use snap trap, bladderworts use suction trap. Probably avoid providing examples.
**Response:** We thank the reviewer for pointing this out. The suction trap example has been revised from waterwheel plants to bladderworts in the Introduction.
**Now at revised manuscript line 44:** "suction trap in bladderworts"

***Comment:*** l.43. 'hunting process: prey attraction, signal transduction, capture, digestion and nutrient'
I think hunting is only until capture
**Response:** We agree with the reviewer that the term "hunting process" may be misleading if extended beyond prey capture. We have therefore revised this sentence to avoid the term "hunting" and now describe a common functional sequence during prey utilization.
**Now at revised manuscript lines 45-47:** "Despite their diverse mechanisms for trapping prey, most carnivorous plants share a common functional sequence during prey utilization, encompassing prey attraction, capture, digestion and subsequent nutrient absorption[12]."

***Comment:*** l.49. 'is notable for its sticky glandular trichomes'
I don't think all Saxifrage have sticky glandular trichomes. If not how many?
**Response:** We thank the reviewer for pointing this out. We have revised the sentence to clarify that this trait is present in some species of *Saxifraga*, rather than implying this as a genus-wide trait. This revision is consistent with previous observations that insect-trapping glands occur in some *Saxifraga* species. **(Now at revised manuscript lines 61-64)**
**lines 61-64:** "*Saxifraga* L. is a predominantly north temperate genus, including species notable for sticky glandular trichomes and many members that thrive in nutrient-poor habitats such as exposed rock outcrops and crevices"

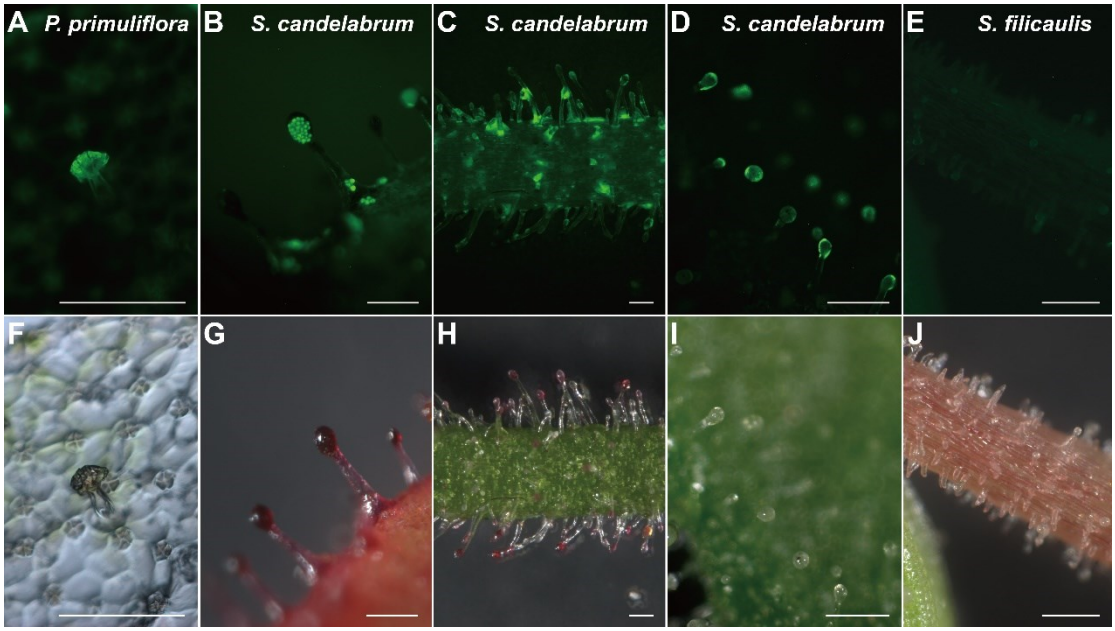
***Comment:*** l.87-93. I'm wondering how this experiment set-up can distinguish the attraction of pollinator and prey. If the set-up cannot distinguish pollinator and prey, how the results can be related to the carnivory, other than pollinator attraction.
**Response:** We thank the reviewer for this important question. We agree that the attraction experiment itself does not distinguish pollinators from prey. This limitation is inherent to the attraction stage, because both pollinators and potential prey are initially attracted as flower visitors. To avoid confusion, we have revised Table S1 by redefining insect categories as "prey" and "uncaptured visitors" and clarified these definitions in the table caption. In our framework, insects categorized as "prey" represent a subset of flower visitors that are subsequently captured by glandular hairs. Thus, the distinction between pollinators and prey arises at the capture stage, not during initial attraction.
Accordingly, the floral odour versus visual attraction experiment addresses the first criterion of carnivory—active prey attraction—within a multi-criterion framework that also includes capture, digestion, and nutrient uptake. Attraction per se is therefore not presented as standalone evidence of carnivory, but as a necessary prerequisite that increases prey availability at the trapping interface. We have clarified this logic in the Methods and Results. We have clarified this logic in the Methods and Results. **(Supplementary materials Table S1, revised manuscript lines 447-451)**
**Table S1 caption:** Insects categorized as "prey" are flower visitors captured by glandular hairs, whereas "uncaptured visitors" are flower visitors not captured.

**line 455-459:** "In *Saxifraga candelabrum*, insect capture occurs predominantly during the flowering (reproductive) stage, whereas only occasional insect trapping is observed during the vegetative (rosette) stage. Accordingly, insect visitors representing both uncaptured floral flower visitors and prey organisms trapped by glandular hairs of *S. candelabrum* were collected from flowering individuals in the field."

***Comment:*** l.107-117. Probably need a positive control, a known carnivorous plant for this enzymatic test.

**Response:** We thank the reviewer for this constructive suggestion. We have now included a positive control using the well-established carnivorous plant *Pinguicula primuliflora*. Clear yellow-green fluorescence was detected in the glandular hairs of *P. primuliflora*, consistent with phosphatase activity reported in carnivorous plants. These results are shown in the revised Fig. 4. The corresponding Methods and Results sections have been updated accordingly. **(Fig. 4, revised manuscript lines 160-161 & 527-530)**

**Revised Fig. 4:**




Note: Panels A and F in Fig. 4 show the newly added positive control (*Pinguicula primuliflora*).

***Comment:*** l.118-129. Where were *Drosera peltata* from? The same habitat with *S. candelabrum*? Different field location? Where were negative controls from? The same habitat? It makes more sense if they are from the same habitat.

**Response:** Thanks for this question. All species included in our interspecific comparisons—*Saxifraga candelabrum*, *Drosera peltata*, *Saxifraga diversifolia*, and *Salvia przewalskii*—were sampled from the same locality (Wufeng Mountain, Shangri-la, China). We have now clarified this explicitly in the Methods and Results sections. **(Now at revised manuscript lines 172-174 & 550-552)**

**lines 172-174:** "All samples used for interspecific comparisons were collected from the same locality (Wufeng Mountain, Shangri-La)"

**lines 558-560:** "For interspecific comparisons, *S. candelabrum*, *S. diversifolia*, *S. przewalskii*, and

*D. peltata* were all sampled from the same locality (Wufeng Mountain, Shangri-La)."

***Comment:*** l.127-129. 'To prevent contamination…' Why only the stems of *S. diversifolia* and *S. candelabrum* were excluded? How about *Salvia przewalskii*?

**Response:** We thank the reviewer for pointing this out. We have revised the Methods to clarify that, in all species, tissues directly exposed to labeled fruit flies were excluded from $\delta^{15}N$ measurements. In *Salvia przewalskii*, only the calyx bears sticky glandular hairs capable of retaining insects; therefore, the calyx was used as the exposure site for this species and excluded accordingly. Relevant text has been revised **(**Now at revised manuscript **lines 575-579).**

**lines 575-579:** "To avoid potential overestimation caused by residual labeled material remaining on glandular surfaces, the upper stems of *Saxifraga*, calyces of *Salvia*, and leaves of *D. peltata* directly exposed to labeled fruit flies were excluded from $\delta^{15}N$ measurements."

***Comment:*** l.433-435. Why instead of upper stem from other plants, it was the calyx in *Salvia przewalskii* which was labelled? Need to be consistent.

**Response:** Thanks for this question. In *Salvia przewalskii*, only the calyx bears sticky glandular hairs capable of retaining insects; stems lack adhesive structures. Accordingly, the calyx was used as the exposure site. We have clarified this point in the Methods. **(Now at revised manuscript lines 556-558)**

**lines 556-558:** "*Salvia przewalskii* was chosen as a control because it often co-occurs with *S. candelabrum* in Shangri-La, and has sticky glandular hairs restricted to on its calyx but no evidence of carnivory."

***Comment:*** In l.437 it says '*S. diversifoli*a…lacks glandular hairs' but in l.441 it says 'fruit flies were gently placed on the glandular surfaces'. They are contradictory.

**Response:** We thank the reviewer for pointing this out. *Saxifraga diversifolia* does possess glandular trichomes on the stem, however, based on our observations, but these glands show no evidence of insect capture. We have revised the text to avoid confusion. **(Now at revised manuscript lines 554-556)**

**lines 554-556:** "*S. diversifolia* was selected as a negative control as it is a close relative of *S. candelabrum* and, based on our observations, shows no evidence of insect capture, despite possessing glandular hairs."

***Comment:*** l.450. What if the labelled insects fell on the rosette leaves, instead of the soil? How can you prevent that?

**Response:** We thank the reviewer for this question. If labeled insects fell onto the rosette leaves, such events would have been readily detectable during routine observations, as the dark-colored fruit flies are easily visible against the green leaf surface and are immobilized by glandular hairs. Any such individuals would have been excluded from analysis, although no such events were observed during the experiment. In contrast, fruit flies falling into soil would be more difficult to detect visually and could contribute labeled nitrogen via root uptake after decomposition, which is why we specifically measured soil $\delta^{15}N$ to evaluate this pathway.

REVIEWER COMMENTS AND RESPONSES

**Reviewer #1 (Remarks to the Author):**

**Comments**

I have reviewed the revised manuscript as well as the authors' responses to the reviewers' comments. My questions have been satisfactorily addressed, and I have no further concerns at this time. I therefore recommend the manuscript for publication.

**Response:** We thank the reviewer for the positive evaluation of our manuscript and for recommending it for publication. We sincerely appreciate the time and effort devoted to improving our work.

***Comment:*** This is a minor point; however, this reviewer would appreciate it if the authors could kindly confirm whether compound names (R/S), symbols, and Greek letters should be italicized in accordance with the journal's style guidelines, and ensure that their formatting is standardized throughout the manuscript.

For example,

Line 510: n-hexane

Line 522: m/z

Supporting table S3: Please consider making S/R italic, following the other compounds.

Page 6, SC1-2:

(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

Page 8, SC3-1: 4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1α,4α,7α)]-

Page 9, SC3-3:

Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-

Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-

**Response:** We thank the reviewer for this suggestion. The formatting of stereochemical descriptors (R/S) and Greek letters has been standardized throughout the manuscript and Supplementary Information. The instances noted at Lines 510 (n-hexane) and 522 (m/z) have been corrected and now appear at Lines 328 and 340, respectively, in the revised manuscript (clean PDF version). The formatting in Supplementary Table S3 has also been standardized (see Supplementary Data 1).

**Reviewer #2 (Remarks to the Author):**

***Comment:*** Thank you to the authors for their careful consideration of my comments on the initial submission of their manuscript. I am satisfied that the authors have improved their manuscript and appropriately addressed all of my comments.

**Response:** We thank the reviewer for the careful evaluation of our manuscript and sincerely appreciate the time and effort devoted to improving our work. We are pleased that our revisions have satisfactorily addressed all comments.

**Reviewer #3 (Remarks to the Author):**

***Comment:*** The authors have carefully addressed all my concerns in the revised manuscript and the Response Letter. I have no further comments. I believe the manuscript is now suitable for publication.

**Response:** We thank the reviewer for the thoughtful assessment of our manuscript and sincerely appreciate the time and effort invested in the review. We are pleased that our revisions have addressed all concerns.