

Peer Review File

Genomic data provide insights into the classification of extant termites



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Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

Hellemans et al. present a massive advance in termite taxonomy and phylogenetics. Primary data is almost exhaustive in its thoroughness while the discussion doesn't exceed the data.

My only concern is that some of the taxonomic acts relate to clades with very shallow internodes and/or weaker nodal support (as discussed in the final paragraph, lines 642 onward). Some of these, such as family elevations to resolve what was once Rhinotermitidae make sense and have been consistently recovered for many years. That there are 6 'family-like' entities within Neoisoptera other than Termitidae is obvious even if their inter relationships aren't perfectly resolved. Giving those entities formal family names makes sense. The termitid subfamilies are a harder question to resolve.

Accordingly, I'd like to see the study include some rogue-taxon analysis to test the sensitivity of resolution across these short-nodes. Phylogenomic datasets of this size cannot be reliably tested by conventional nodal support measures as they tend to converge on 100% regardless, and sensitivity metrics become more useful. There are many such approaches which could be taken but it would be useful to see 1) how stable are these relationships to rogue-taxon removal, and 2) are any of the monotypic (or otherwise low diversity) termite taxa which rendered traditional taxonomic classifications paraphyletic rogue taxa. Such analyses would reveal if any of the monotypic (or very low diversity) termitid subfamilies are rogue taxa and thus if their distinctiveness should be doubted.

Finally, Geoisoptera has the additional synapomorphy of a distinctive mitochondrial genome rare-genomic change (control region repeat structures). First noted in Cameron et al. 2012, confirmed in the great mass of subsequent mt genomes sequenced by this paper's authors.

Reviewer #2:

Remarks to the Author:

Hellemans and collaborators present a phylogeny of termites reconstructed from hundreds of ultraconserved elements based on a sample of 135 termites and three wood-feeding subsocial cockroaches as outgroups. This phylogeny addresses systematic issues at the family and subfamily levels, specifically within and around neoisopteran lineages. Accordingly, the authors revise the taxonomy of neoisopterans and propose a new classification for termite families and subfamilies.

Overall, the introduction, experimental methods, and results are adequate. However, Figure 1, which summarizes the results from 30 phylogenetic analyses, appears somewhat cluttered and overloaded with information. For instance, the inclusion of heads and morphological structures representing tentative synapomorphies for certain lineages is challenging to follow, as they are not clearly discussed in the main text. The systematic component of the work is comprehensive, with meticulous taxonomic revision and diagnoses that reflect the evolutionary history of the group, which I consider a critical aspect in taxonomic studies proposing classifications based on molecular phylogenies. The manuscript indeed presents robust results for generating a stable higher classification of termites, a significant group of insects, and is certainly suitable for publication in Nature Communication. However, considering my suggestions below, I am afraid that it requires a major revision.

My main criticism concerns the taxonomic changes proposed where the type genera of the tribes were not sampled. I also believe that the authors overstate some of the conclusions that can be drawn from their taxon sampling. I identify three major issues requiring reevaluation by the authors:

MAJOR PROBLEM 1: Excess of taxonomic changes

The authors propose new names that may not be necessary by the criterion of monophyly. For

example, based on the presented tree, the new subfamilies Crepitermitinae, Protohamitermitinae, and Cylindrotermitinae could form a single monophyletic subfamily. Yet, they are divided into separate entities, even though the authors note in their discussion that the morphological boundaries among them are not clearly defined. This is not a major flaw of the study per se, but it exemplifies an overemphasis on taxonomic authority over phylogenetic results.

Another example is the case of split of Rhinotermitidae. In Figure 1, the authors acknowledge the unresolved interfamilial relationships within Neoisoptera (previously 'Rhinotermitidae'), but split Rhinotermitidae into four distinct families anyway. This seems speculative, particularly in light of the high number of alternative topologies recovered in this part of the tree. Data have their limitations and reach where they reach, regardless of our desires to solve specific problems. I am mentioning this because many of the taxonomic changes proposed in the paper derive from parts of the tree with numerous alternative topologies, which, in my opinion, requires more cautious conclusions. Phylogenetic discordance can occur due to genuine differences in evolutionary processes, but it can also result from data or taxon sampling limitations. The authors could discuss the incongruences among the topologies and test the significance of phylogenetic discordance, especially in cases where they are proposing taxonomic changes. I strongly suggest that they assess and address the incongruences, ideally by evaluating statistically the phylogenetic discrepancies. There are a number of tools that can be used to address incongruence in phylogenomics (see Fleming et al (2023; <https://doi.org/10.1111/eva.13565>). This would allow them to make more precise taxonomic decisions, improving stability and reliability of the termite classifications by determining whether there are preferred alternative topologies/hypotheses, rather than basing decisions solely on a majority rule consensus summary tree.

MAJOR PROBLEM 2: Taxonomic changes without sampling type-genus

Following up on my previous comments on the excess of taxonomic changes, I believe that proposing new high level taxa names without sampling type-genera, as done for Protohamitermitinae, is problematic. In this particular case, the new family is not proposed based on results from their data. This subfamily was represented by a single terminal, *Orientotermes*. So my question is: how do the authors know the relationships between *Orientotermes* and the type-genus of Protohamitermitinae, Protohamitermes?

MAJOR PROBLEM 3: Clarity with phylogenetic results

Another point that, in a way, diminishes the scientific relevance of the study is the fact that the authors are not very clear about the nodal statistical support in the trees. Some parts of the trees are not as robust as they state, e.g., those comprising the former Rhinotermitidae and the Termitidae subfamilies. In these cases, for example, it is unclear how robust the clades are because, as it is shown in Figure 1, it is not clear how low the 'UFBs <95' support is as it ranges from 0 to 94. Furthermore, as I mentioned above, the lack of discussion on the incongruences among the 30 experiments' results is a concern, given the extensive taxonomic changes proposed. Addressing these issues is vital for maintaining the study's scientific credibility.

I hope that my review and suggestions will be useful in refining and improving the manuscript. Given its potential significance to the field, with careful consideration of the raised points, I certainly recommend the manuscript for publication in Nature Communications.

Reviewer #3:

Remarks to the Author:

---Review summary----

The authors present a very impressive revision of termite phylogeny with a focus on Termitidae. Specifically, the amount of data (loci), the number of analyses, and the taxonomic attention are all exemplary. Using their results, the authors make a bold and necessary step -- they revise the current

taxonomy in light of their phylogeny using detailed morphological diagnoses. This fills a particular gap left by prior taxonomic work. Yet, more is needed in order to justify the bridge from the inferred phylogeny to the new taxonomy. Specifically, there is little discussion of the quality of their phylogenetic analyses, few formal statistical tests done, and a lack of addressing the uncertainty that short internodes bring to their results. While the results demonstrate that most of the new taxa described are probably mostly robust to errors, there are at least two where this is not the case. Regardless, the authors themselves discuss why it is advantageous to have robust phylogenetic results backing up new names, yet there is more analyses to be done towards this end.

My detailed review (below) is based mostly on the phylogenetic analyses. I can only superficially comment on the taxonomic/phenotypic/morphological content presented. However, given the author list, I would assume that these are more than sound.

- 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.

This is a large step forward considering most recent studies were done only with mitochondrial genomes or UCEs for much fewer taxa.

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

To quote from the abstract "Building the new taxonomic classification on the foundation of unambiguously supported monophyletic lineages makes it highly resilient to potential destabilization caused by future availability of novel phylogenetic markers and methods." Also, a quote from the discussion "Uncertainties in our phylogenetic reconstructions and the morphological diversity of the Neoisoptera were the principal challenges to delimit new families and subfamilies." I think at least two of the newly described subfamilies do not meet the criteria of being "highly resilient to potential destabilization". Although I specifically doubt two clades, the fact that ANY have met this criteria has not been made clear in the paper itself. I suspect that the authors have the data for this already but it hasn't been presented in the manuscript as of yet. But as I said, even if the data is presented, I still think what has been done is not enough to prove the monophyly of all subfamilies described here. Details of my perspective is below.

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

The phylogenetic analysis has a few strengths: the amount of data and the number of analyses done with different concatenation permutations. These are big advantages. The weaknesses as I see it are as follows. 1. Analyses rely completely on concatenation (more on this below). 2. Little has been done to assess the quality of the alignments analyzed and 3. Few statistical tests have been done to assess the robustness of the results.

Expanding on 2. It seems their data collection relies strongly on a previous study (Hellemens et al. 2022). This is perfectly fine and normal. But both in the previous study and in the current study I see nothing that discusses identification of poor quality loci, removal of uninformative regions, assessing that alignments meet evolutionary model assumptions etc. Shen et al. (2017) demonstrated that even a handful of rogue loci can strongly influence huge phylogenetic analyses. This is partially addressed by their multiple analyses in IQTREE but further discussion and more nuanced interpretation is necessary to fully flesh out the results. Having looked at the alignments quickly, they look very good, so I would not expect huge outliers. Yet, UCEs are highly conserved so small regions with a lot of errors might overwhelm the low proportion of informative sites (particularly on short internodes). So, it's hard to know without the analyses being done.

Shen, X. X., et al. (2017). "Contentious relationships in phylogenomic studies can be driven by a handful of genes." *Nat. Ecol. Evol.* 1(5): 126.

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

Perhaps most impressive about this study is the number of analyses done (30). Although only 4 of these analyses leverage a huge number of loci, this should not be problematic because most use a strong number of loci.

The purpose of doing so many analyses with different permutations is to ensure that your results are dependent on the data and not on the experimental choices being made. So is 30 analyses enough? It depends on the conditions that were varied and what issues those variable conditions address. For instance, did the authors examine all "internally" trimmed results for consistent differences with the "edge" trimmed results? Did they do this for DNA vs. protein analyses? Simply knowing that there are differences between these analyses is valuable because it tells us about error on nodes. But specifically, what does it tell us about the nature of the error? That is not really clear in this study. There is also the opposite problem, where certain things that are extremely meaningful were not modified at all. Specifically, all analyses rely on concatenation and IQTREE. This is important because of the key finding that there are many successive short internodes.

Fig 1. presents us with the phylogeny where the authors have illustrated support for certain nodes across the 30 analyses. Why only those nodes? As a reviewer, I would like to see the node support plots for all ancestors of families and subfamilies discussed in the paper. This would help me evaluate if their monophyly was supported across all analyses. Also, it is hard to see which nodes are being referred to in some cases because the internodes are so short.

The issue of short internodes is one of the more important findings here. As mentioned above, the point of doing 30 tests is to identify topology inferences coming from sources of error. Yet, all these 30 tests used concatenation and IQTREE inferences. This is mainly a problem because successive short internodes (and rapid radiations) are not resolvable by concatenation (Mendes & Hahn 2018). It is becoming increasingly common for empirical studies to back this up through identification of anomaly zones. Since many of the new taxa described here fall very close to, or directly on short internodes, further testing is required to establish their monophyly. While there are multiple phylogenetic statistical tests to rule out alternative hypotheses (and these should be done when needed) I think the authors must make comparisons between a coalescent method and their concatenation results, or refrain from coming to conclusions about what is occurring near short internodes. This should at least affect "Amitermitinae" and "Cylindrotermitinae". Also, based on the differential diagnoses in the main text, it is not clear how the relationships among the clades affect which characters are considered diagnostic. So, considering that the short internodes indicate uncertain relationships, the authors should ensure that their diagnoses are not dependent on the specific relationships presented here.

Mendes, F. K. and M. W. Hahn (2018). "Why Concatenation Fails Near the Anomaly Zone." *Systematic Biology* 67(1): 158-169.

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

Yes but Table S2 should give more details about the alignments. Specifically the occupancy, alignment length and number of parsimony informative characters. Other information is helpful too but these are the ones I think are most important.

----Other comments----

The discussion section presents arguments against lumping taxa, but doesn't propose many arguments against splitting. We know the authors favor splitting, but they should at least let the readers know what problems this results in. What old terms now refer to more restricted taxa? What databases need to be updated? How does this affect collections etc. Showing the pros and cons of both shouldn't be damaging to the authors decision to split (since it's arbitrary in the end). It would

only better inform future scientists on how to interpret the literature.

Author list: The paper has a very long author list but only a few authors are listed in the author contributions statement. I guess the "All authors edited and accepted the final version of the manuscript" is referring to the authors who are not listed by name?

Citations lacking: throughout the paper there is sparse citation for some statements.

Pg. 6: "All ten currently recognized extant termite families (sensu Krishna et al. 2013, modified by Wang et al. 2022) are monophyletic, except for the Rhinotermitidae, which is paraphyletic with respect to Serritermitidae and Termitidae." Please cite the sources stating the families are monophyletic and paraphyletic respectively. Or mention if this is referring to the results herein.

Taxonomic diagnoses: the characters listed should either have citations to sources where these taxa were studied previously, or reference to material examined. Perhaps also the methods section should give some detail about how these diagnoses were derived. I guess these are all modified from Krishna et al 2013 but that is only mentioned in the supplement.

Biopeographic ranges of clades (in remarks subheadings) are given without citation.

Diagnostic language: Throughout, the diagnoses character states are presented without context.

Although this is commonplace it is not ideal. In the supplement I found an example of what I am talking about "Gut: esophagus distinct from the crop (as opposed to broadening into the crop as in Mastotermitidae, Teletisoptera, Serritermitidae, or Kalotermitidae);" This is type of language has the context I am referring to. It is more clear and should be used more often.

The word "systematically" is used in a few places to mean "consistently" but this is confusing because it could be misunderstood to mean "phylogenetically" as in "phylogenetic systematics".

pg. 7 "a robust classification devoid of non-monophyletic groups" double negative is confusing

Response to reviewers' comments

Thank you again for submitting your manuscript "Genomic data bring a new dawn to the classification of extant termites" to Nature Communications. We have now received reports from 3 reviewers and, after careful consideration, we have decided to invite a major revision of the manuscript.

As you will see from the reports copied below, the reviewers raise important concerns. We find that these concerns limit the strength of the study, and therefore we ask you to address them with additional work. Without substantial revisions, we will be unlikely to send the paper back to review.

If you feel that you are able to comprehensively address the reviewers' concerns, please provide a point-by-point response to these comments along with your revision. Please show all changes in the manuscript text file with track changes or colour highlighting. If you are unable to address specific reviewer requests or find any points invalid, please explain why in the point-by-point response.

RESPONSE: We thank the three reviewers for their positive evaluation of our manuscript and for providing helpful comments, all of which significantly improved our manuscript. We carried out all the requested analyses and modified our manuscript accordingly. We also provide a point-by-point response to each comment below. All modifications are highlighted in grey in the revised version of this manuscript. Briefly, we now carefully assess the topological incongruences in order to ascertain our conclusions, as suggested by all referees. To do so, we performed an additional 21 phylogenetic reconstructions within a multi-species coalescence framework (summarized in a new Figure 2). This approach allows us to overcome the limitations of the 30 concatenation analyses reported in Figure 1. We performed two different rogue taxon analyses to assess the status of monotypic subfamilies. Additionally, we performed topological tests to better assess relationships among taxa. Our new analyses confirm our amendments to the classification of Isoptera, and strengthen the conclusions of our paper.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Hellems et al. present a massive advance in termite taxonomy and phylogenetics. Primary data is almost exhaustive in its thoroughness while the discussion doesn't exceed the data.

RESPONSE: We thank Reviewer #1 for highlighting the significance of our work.

My only concern is that some of the taxonomic acts relate to clades with very shallow internodes and/or weaker nodal support (as discussed in the final paragraph, lines 642 onward). Some of these, such as family elevations to resolve what was once Rhinotermitidae make sense and have been consistently recovered for many years. That there are 6 'family-like' entities within Neosioptera other than Termitidae is obvious even if their inter relationships aren't perfectly resolved. Giving those entities formal family names makes sense. The termitid subfamilies are a harder question to resolve.

Accordingly, I'd like to see the study include some rogue-taxon analysis to test the sensitivity of resolution across these short-nodes. Phylogenomic datasets of this size cannot be reliably tested by conventional nodal support measures as they tend to converge on 100% regardless, and sensitivity metrics become more useful. There are many such approaches which could be taken but it would be useful to see 1) how stable are these relationships to rogue-taxon removal, and

2) are any of the monotypic (or otherwise low diversity) termite taxa which rendered traditional taxonomic classifications paraphyletic rogue taxa. Such analyses would reveal if any of the monotypic (or very low diversity) termitid subfamilies are rogue taxa and thus if their distinctiveness should be doubted.

We agree with reviewer #1 that rogue taxon analyses are needed to strengthen our paper. Therefore, we carried out new analyses addressing the problem of rogue taxa. We performed rogue taxa analyses on the phylogenetic trees reconstructed from concatenated sequence matrices, as well as on the trees reconstructed with coalescence method.

For concatenation analyses, we used RogueNaRok to identify rogue taxa based on their bootstrap tree sets. For coalescence analyses, we used TreeShrink, which identifies rogue taxa for each locus independently. Overall, two monotypic subfamilies (Crepititermitinae and Forficulitermitinae) were identified as rogues in a few analyses. While these two new subfamilies are sometimes identified as rogue taxa, most analyses did not recognize them as such. Consequently, we maintained the description of these new subfamilies and discussed their status as potential rogue taxa. Note that, in addition, as requested by reviewer #2, we also performed a series of topological tests in order to ascertain the nodes among some Termitidae subfamilies.

As noted by the referee, support values tend to converge to 100% support values. As an alternative support metric, we now provide site concordance factors (sCFs) which reflect the percentage of decisive sites supporting each split (doi: 10.1093/molbev/msaa106). We implemented this metric on majority rule consensus trees (Figures 1-2).

Finally, Geoisoptera has the additional synapomorphy of a distinctive mitochondrial genome rare-genomic change (control region repeat structures). First noted in Cameron et al. 2012, confirmed in the great mass of subsequent mt genomes sequenced by this paper's authors. **RESPONSE:** Thank you for the suggestion. We now mention this synapomorphy under the description of the clade Geoisoptera.

Reviewer #2 (Remarks to the Author):

Helleman and collaborators present a phylogeny of termites reconstructed from hundreds of ultraconserved elements based on a sample of 135 termites and three wood-feeding subsocial cockroaches as outgroups. This phylogeny addresses systematic issues at the family and subfamily levels, specifically within and around neoisopteran lineages. Accordingly, the authors revise the taxonomy of neoisopterans and propose a new classification for termite families and subfamilies.

Overall, the introduction, experimental methods, and results are adequate. However, Figure 1, which summarizes the results from 30 phylogenetic analyses, appears somewhat cluttered and overloaded with information. For instance, the inclusion of heads and morphological structures representing tentative synapomorphies for certain lineages is challenging to follow, as they are not clearly discussed in the main text. The systematic component of the work is comprehensive, with meticulous taxonomic revision and diagnoses that reflect the evolutionary history of the group, which I consider a critical aspect in taxonomic studies proposing classifications based on molecular phylogenies. The manuscript indeed presents robust results for generating a stable higher classification of termites, a significant group of insects, and is certainly suitable for publication in Nature Communication. However, considering my suggestions below, I am afraid that it requires a major revision.

RESPONSE: We agree with Reviewer #2 that the previous version of Figure 1 was hard to read because its content in information was too heavy. We now provide three separate figures instead of one, which improves the readability of the figures. Figure 1 depicts a consensus tree

summarizing the results of 30 analyses performed on concatenated sequence matrices. The new Figure 2 summarizes the results of 21 coalescence analyses. The new Figure 3 displays a cladogram depicting the most probable relationships among termite families and subfamilies, integrating the results of all analyses and topological tests. The new Figure 3 also contains morphological features and synapomorphies as discussed in the Results.

My main criticism concerns the taxonomic changes proposed where the type genera of the tribes were not sampled. I also believe that the authors overstate some of the conclusions that can be drawn from their taxon sampling. I identify three major issues requiring reevaluation by the authors:

RESPONSE: We thank Reviewer #2 for their positive assessment of our manuscript. We performed all the analyses recommended by Reviewer #2 and addressed all mentioned Major Problems. Please see below for details.

MAJOR PROBLEM 1: Excess of taxonomic changes

The authors propose new names that may not be necessary by the criterion of monophyly. For example, based on the presented tree, the new subfamilies *Crepitermitinae*, *Protohamitermitinae*, and *Cylindrotermitinae* could form a single monophyletic subfamily. Yet, they are divided into separate entities, even though the authors note in their discussion that the morphological boundaries among them are not clearly defined. This is not a major flaw of the study per se, but it exemplifies an overemphasis on taxonomic authority over phylogenetic results.

RESPONSE: Many analyses retrieved *Crepitermitinae*, *Protohamitermitinae*, and *Cylindrotermitinae* as forming a monophyletic group, which could support the erection of one subfamily instead of three. However, our phylogenetic analyses report contrasting results.

While the three new subfamilies form a monophyletic group in 18 of 30 phylogenetic analyses performed on the concatenated sequence matrices (although with low support: UFBs=51–87), the other 12 analyses did not support them forming a clade. Similarly, our new ASTRAL coalescence analyses (performed with IQ-TREE parameter “-czb” allowing for polytomies) place *Crepitermitinae* on an unresolved polytomy in most phylogenetic analyses (Figure 2; Supplementary Data). Therefore, the monophyly of *Crepitermitinae* + *Protohamitermitinae* + *Cylindrotermitinae* is unwarranted. This uncertainty led us to separate them into three subfamilies instead of one, a decision that is not only based on taxonomic authority but also on phylogenetic results, as we aimed for all described subfamilies to be unambiguously monophyletic. We report these in the Results section of the manuscript.

Another example is the case of split of *Rhinotermitidae*. In Figure 1, the authors acknowledge the unresolved interfamilial relationships within *Neoisoptera* (previously '*Rhinotermitidae*'), but split *Rhinotermitidae* into four distinct families anyway. This seems speculative, particularly in light of the high number of alternative topologies recovered in this part of the tree. Data have their limitations and reach where they reach, regardless of our desires to solve specific problems. I am mentioning this because many of the taxonomic changes proposed in the paper derive from parts of the tree with numerous alternative topologies, which, in my opinion, requires more cautious conclusions. Phylogenetic discordance can occur due to genuine differences in evolutionary processes, but it can also result from data or taxon sampling limitations. The authors could discuss the incongruences among the topologies and test the significance of phylogenetic discordance, especially in cases where they are proposing taxonomic changes. I strongly suggest that they assess and address the incongruences, ideally by evaluating statistically the phylogenetic discrepancies. There are a number of tools that can be used to address incongruence in phylogenomics (see Fleming et al

(2023; <https://doi.org/10.1111/eva.13565>). This would allow them to make more precise taxonomic decisions, improving stability and reliability of the termite classifications by determining whether there are preferred alternative topologies/hypotheses, rather than basing decisions solely on a majority rule consensus summary tree. RESPONSE: Indeed, the interfamilial relationships within the Neoisoptera are unresolved, which may be due to genuine differences in evolutionary processes. However, the new families we propose are always retrieved as monophyletic groups, satisfying the criterion of monophyly. Only the relationships between these new monophyletic families are unresolved. That said, we agree with Reviewer #2 that our modifications to the termite classification, solely based on the monophyly and the majority rule consensus, lacked robustness. We addressed this problem. We performed a series of topological tests (Shimodaira's Approximately Unbiased AU tests) to assess the topological incongruences among early-diverging Neoisoptera and Termitidae. In addition, we provide topology and support values for the 21 new coalescence analyses reported in this revised manuscript, which provide a better contrast of the reported incongruences. We specifically address this problem in the Results section of the manuscript.

MAJOR PROBLEM 2: Taxonomic changes without sampling type-genus

Following up on my previous comments on the excess of taxonomic changes, I believe that proposing new high level taxa names without sampling type-genera, as done for Protohamitermitinae, is problematic. In this particular case, the new family is not proposed based on results from their data. This subfamily was represented by a single terminal, *Orientotermes*. So my question is: how do the authors know the relationships between *Orientotermes* and the type-genus of Protohamitermitinae, *Protohamitermes*? RESPONSE: We agree with Reviewer #2 that the type genus should ideally be sampled. However, although the type-genus *Protohamitermes* was not included in our phylogenetic analyses, it is known to be closely related to *Orientotermes* from previous phylogenetic analyses (Inward *et al.*, 2007). One key feature of both genera is the absence of the soldier caste, a rare feature only found in the Apicotermitinae and perhaps in the Amitermitinae *Invasitermes*. This is now explicitly stated in the Protohamitermitinae's Systematics section.

MAJOR PROBLEM 3: Clarity with phylogenetic results

Another point that, in a way, diminishes the scientific relevance of the study is the fact that the authors are not very clear about the nodal statistical support in the trees. Some parts of the trees are not as robust as they state, e.g., those comprising the former Rhinotermitidae and the Termitidae subfamilies. In these cases, for example, it is unclear how robust the clades are because, as it is shown in Figure 1, it is not clear how low the 'UFBs <95' support is as it ranges from 0 to 94. Furthermore, as I mentioned above, the lack of discussion on the incongruences among the 30 experiments' results is a concern, given the extensive taxonomic changes proposed. Addressing these issues is vital for maintaining the study's scientific credibility. RESPONSE: We agree our study could have clarified better how robust each clade was, as variation in UFB from 0 to 94 are too large to properly assess node support. To address this issue, we now provide each individual tree as Supplementary Data (in Dryad), which allows readers to assess the support of each node for each tree. In addition, we now provide the range of node support across all our analyses in the Results section for each newly defined family and subfamily. Finally, the incongruences are now assessed in the Results section.

I hope that my review and suggestions will be useful in refining and improving the manuscript. Given its potential significance to the field, with careful consideration of the raised points, I certainly recommend the manuscript for publication in Nature Communications. RESPONSE: We thank again reviewer #2 for their thorough review and helpful comments. We

believe we have addressed all concerns and that the quality of our manuscript significantly improved.

Reviewer #3 (Remarks to the Author):

---Review summary---

The authors present a very impressive revision of termite phylogeny with a focus on Termitidae. Specifically, the amount of data (loci), the number of analyses, and the taxonomic attention are all exemplary. Using their results, the authors make a bold and necessary step -- they revise the current taxonomy in light of their phylogeny using detailed morphological diagnoses. This fills a particular gap left by prior taxonomic work. Yet, more is needed in order to justify the bridge from the inferred phylogeny to the new taxonomy. Specifically, there is little discussion of the quality of their phylogenetic analyses, few formal statistical tests done, and a lack of addressing the uncertainty that short internodes bring to their results. While the results demonstrate that most of the new taxa described are probably mostly robust to errors, there are at least two where this is not the case. Regardless, the authors themselves discuss why it is advantageous to have robust phylogenetic results backing up new names, yet there is more analyses to be done towards this end.

RESPONSE: We thank reviewer #3 for the insightful review. We have addressed all comments in this revised version of our manuscript. We performed a series of new analyses (coalescence analyses and topological tests) investigating the topological uncertainties (especially those of short internodes). We specifically examined the two subfamilies for which reviewer #3 raised doubts (Amitermitinae and Cylindrotermitinae; see our response below). We believe that we adequately addressed reviewer #3's comments in this revised version of our manuscript.

My detailed review (below) is based mostly on the phylogenetic analyses. I can only superficially comment on the taxonomic/phenotypic/morphological content presented. However, given the author list, I would assume that these are more than sound.

RESPONSE: We have responded point-by-point to the comments and suggestions of reviewer #3.

- 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.

This is a large step forward considering most recent studies were done only with mitochondrial genomes or UCEs for much fewer taxa.

RESPONSE: Thanks for your positive assessment.

- Does the work support the conclusions and claims, or is additional evidence needed? To quote from the abstract "Building the new taxonomic classification on the foundation of unambiguously supported monophyletic lineages makes it highly resilient to potential destabilization caused by future availability of novel phylogenetic markers and methods." Also, a quote from the discussion "Uncertainties in our phylogenetic reconstructions and the morphological diversity of the Neoisoptera were the principal challenges to delimit new families and subfamilies." I think at least two of the newly described subfamilies do not meet the criteria of being "highly resilient to potential destabilization". Although I specifically doubt two clades, the fact that ANY have met this criteria has not been made clear in the paper itself. I suspect that the authors have the data for this already but it hasn't been presented in the manuscript as of yet. But as I said, even if the data is presented, I still think what has been done is not enough to prove the monophyly of all subfamilies described here. Details of my perspective is below.

RESPONSE: We agree that additional analyses testing the monophyly of all families and subfamilies newly defined here were needed. In the revised version of this manuscript, we performed an additional 21 coalescence analyses with ASTRAL, two implementations of rogue taxa (in RogueNaRok and TreeShrink), and Shimodaira's Approximately Unbiased (AU) tests to ascertain key relationships. We also now report the node support values (UFBs and LPPs) for each clade in the Results section; as well as site concordance factors (sCFs), which reflect the percentage of decisive sites supporting each node (doi: 10.1093/molbev/msaa106). We specifically discussed how we addressed the concerns of reviewer #3 about Amitermitinae and Cylindrotermitinae below.

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

The phylogenetic analysis has a few strengths: the amount of data and the number of analyses done with different concatenation permutations. These are big advantages. The weaknesses as I see it are as follows. 1. Analyses rely completely on concatenation (more on this below). 2. Little has been done to assess the quality of the alignments analyzed and 3. Few statistical tests have been done to assess the robustness of the results.

RESPONSE: We addressed all three weaknesses mentioned by Reviewer #3. (1) We performed coalescence analyses with ASTRAL. (2) We assessed the quality of internally- and edge-trimmed alignments using three compositional heterogeneity tests. (3) Finally, we tested the robustness of our results with two analyses of rogue taxa and Shimodaira's Approximately Unbiased AU tests.

Expanding on 2. It seems their data collection relies strongly on a previous study (Hellemens et al. 2022). This is perfectly fine and normal. But both in the previous study and in the current study I see nothing that discusses identification of poor quality loci, removal of uninformative regions, assessing that alignments meet evolutionary model assumptions etc. Shen et al. (2017) demonstrated that even a handful of rogue loci can strongly influence huge phylogenetic analyses. This is partially addressed by their multiple analyses in IQTREE but further discussion and more nuanced interpretation is necessary to fully flesh out the results. Having looked at the alignments quickly, they look very good, so I would not expect huge outliers. Yet, UCEs are highly conserved so small regions with a lot of errors might overwhelm the low proportion of informative sites (particularly on short internodes). So, it's hard to know without the analyses being done.

Shen, X. X., et al. (2017). "Contentious relationships in phylogenomic studies can be driven by a handful of genes." *Nat. Ecol. Evol.* 1(5): 126.

RESPONSE: As mentioned by Reviewer #3, we did not originally attempt to identify potential poor-quality or rogue loci. We addressed this issue in this revised version. We performed Chi-square tests of nucleotide homogeneity (in BaCoCa) and normalized relative composition frequency variability (nRCFV from RCFV_Reader). We performed these tests on all loci retained in our phylogenetic analyses, both after internal- and edge-trimming. The results for individual loci are reported in two new Supplementary Tables (Tables S2-S3) and the main text. Additionally, we performed 21 new multi-species coalescence analyses (ASTRAL-III). As each individual gene tree has equal weight in coalescence analyses, they are less impacted by rogue loci. Finally, we provide an alternative support metric, the site concordance factors (sCFs), which reflects the percentage of decisive sites supporting each node (doi: 10.1093/molbev/msaa106) (see Figures 1-2). We believe these new analyses address the concerns about rogue and poor-quality loci.

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

Perhaps most impressive about this study is the number of analyses done (30). Although only 4 of these analyses leverage a huge number of loci, this should not be problematic because most use a strong number of loci.

The purpose of doing so many analyses with different permutations is to ensure that your results are dependent on the data and not on the experimental choices being made. So is 30 analyses enough? It depends on the conditions that were varied and what issues those variable conditions address. For instance, did the authors examine all "internally" trimmed results for consistent differences with the "edge" trimmed results? Did they do this for DNA vs. protein analyses? Simply knowing that there are differences between these analyses is valuable because it tells us about error on nodes. But specifically, what does it tell us about the nature of the error? That is not really clear in this study. There is also the opposite problem, where certain things that are extremely meaningful were not modified at all. Specifically, all analyses rely on concatenation and IQTREE. This is important because of the key finding that there are many successive short internodes.

RESPONSE: We agree that the issues mentioned by Reviewer #3 must be addressed. We performed 21 coalescence analyses, which specifically address the key issues related to successive short internodes and supplement our original IQTREE analyses relying on concatenation. Our analyses again revealed topological variations for short internodes across analyses (Figure 2). Therefore, these new analyses provide evidence of rapid diversification leading to short internodes and indicative of the presence of many gene trees differing from the species tree. In the revised version of this manuscript, we now discuss in more details the topological alternatives. For instance, we found four different topologies for Termitogetonidae across all analyses and trimming methods. We now provide in the manuscript this level of detail when relevant to assess topological incongruences. In addition, we performed a series of topological tests using Shimodaira's AU test (Table S6) (see results and discussion).

Fig 1. presents us with the phylogeny where the authors have illustrated support for certain nodes across the 30 analyses. Why only those nodes? As a reviewer, I would like to see the node support plots for all ancestors of families and subfamilies discussed in the paper. This would help me evaluate if their monophyly was supported across all analyses. Also, it is hard to see which nodes are being referred to in some cases because the internodes are so short.

RESPONSE: We agree that our original Figure 1 was difficult to read because it contained too much information. This issue was also pointed out by Reviewer #2. We now present three separate figures instead of one. The first and second Figures synthesize node supports for our IQTREE analyses performed on concatenated alignments and the multi-species coalescence analyses performed with ASTRAL, respectively. The third Figure displays a summary cladogram together with drawings highlighting the main morphological characteristics of each new family and subfamily. We agree with Reviewer #3 that our study lacked a clear overview of how robust each family and subfamily is, because we did not present nodal support for each node. To resolve this issue, we now provide each individual reconstruction as Supplementary Data (in the Dryad package). In addition, we indicate in Figures 1 and 2 all nodal support values underlying families and subfamilies and discuss the support for these families in the Results section.

The issue of short internodes is one of the more important findings here. As mentioned above, the point of doing 30 tests is to identify topology inferences coming from sources of error. Yet,

all these 30 tests used concatenation and IQTREE inferences. This is mainly a problem because successive short internodes (and rapid radiations) are not resolvable by concatenation (Mendes & Hahn 2018). It is becoming increasingly common for empirical studies to back this up through identification of anomaly zones. Since many of the new taxa described here fall very close to, or directly on short internodes, further testing is required to establish their monophyly. While there are multiple phylogenetic statistical tests to rule out alternative hypotheses (and these should be done when needed) I think the authors must make comparisons between a coalescent method and their concatenation results, or refrain from coming to conclusions about what is occurring near short internodes. This should at least affect "Amitermitinae" and "Cylindrotermitinae". Also, based on the differential diagnoses in the main text, it is not clear how the relationships among the clades affect which characters are considered diagnostic. So, considering that the short internodes indicate uncertain relationships, the authors should ensure that their diagnoses are not dependent on the specific relationships presented here.

Mendes, F. K. and M. W. Hahn (2018). "Why Concatenation Fails Near the Anomaly Zone." *Systematic Biology* 67(1): 158-169.

RESPONSE: We agree with Reviewer #3. Our finding of short internodes at some branching, such as that of Amitermitinae and Cylindrotermitinae, is not new. Short internodes, leading to topological uncertainties and suggesting rapid diversification, were already reported for early divergences within Rhinotermitidae *sensu lato* and branchings around the Termitinae (Bucek et al. 2019; doi: 10.1016/j.cub.2019.08.076). To address the problem of short internodes, we performed coalescence analyses and topological tests (more details are provided above).

Concerning the Amitermitinae and Cylindrotermitinae, our new coalescence analyses confirm that they are both monophyletic groups. Other subfamilies defined or redefined here are also unambiguously monophyletic. However, the relationships of Amitermitinae and Cylindrotermitinae with other subfamilies of Termitidae remain partly unresolved (see also topological tests in Table S6). As Reviewer #3 noted, the morphological boundaries of some subfamilies are difficult to define. Despite the difficulty of identifying diagnostic characters for each subfamily of Termitidae, we were able to define a combination of characters diagnostic of each subfamily. For instance, while Cylindrotermitinae and Protohamitermitinae share a characteristic ventral loop in their digestive tracts (see Figure 3), Cylindrotermitinae possess a soldier caste while Protohamitermitinae lost the soldier caste, a feature otherwise only known from most genera of Apicotermitinae and possibly from *Invasitermes*, one genus of Amitermitinae. Therefore, Cylindrotermitinae and Protohamitermitinae can be distinguished from other subfamilies by a combination of diagnostic characters. The same was true for other subfamilies of Termitidae, indicating that both molecular and morphological data support them as separate entities. Additionally, we now provide differential diagnoses under each subfamily description.

- Is there enough detail provided in the methods for the work to be reproduced? Yes but Table S2 should give more details about the alignments. Specifically the occupancy, alignment length and number of parsimony informative characters. Other information is helpful too but these are the ones I think are most important.

RESPONSE: We agree and now provide a new Supplementary Table S4 providing more details (*i.e.*, taxon occupancy, length, number of sites, and number of parsimony informative sites) for each supermatrix. In addition, as mentioned above, we provide new Supplementary Tables (S2-S3) evaluating the compositional heterogeneity of loci after internal or edge-trimming.

---Other comments---

The discussion section presents arguments against lumping taxa, but doesn't propose many arguments against splitting. We know the authors favor splitting, but they should at least let the

readers know what problems this results in. What old terms now refer to more restricted taxa? What databases need to be updated? How does this affect collections etc. Showing the pros and cons of both shouldn't be damaging to the authors decision to split (since it's arbitrary in the end). It would only better inform future scientists on how to interpret the literature.

RESPONSE: We agree and wrote an additional paragraph discussing the consequences of splitting Rhinotermitidae and Termitinae.

Author list: The paper has a very long author list but only a few authors are listed in the author contributions statement. I guess the "All authors edited and accepted the final version of the manuscript" is referring to the authors who are not listed by name?

RESPONSE: Yes, this is correct. To clarify this point, we now provide a list with the abbreviation of all author names instead of only referring to "all authors."

Citations lacking: throughout the paper there is sparse citation for some statements. Pg. 6: "All ten currently recognized extant termite families (sensu Krishna et al. 2013, modified by Wang et al. 2022) are monophyletic, except for the Rhinotermitidae, which is paraphyletic with respect to Serritermitidae and Termitidae." Please cite the sources stating the families are monophyletic and paraphyletic respectively. Or mention if this is referring to the results herein. Taxonomic diagnoses: the characters listed should either have citations to sources where these taxa were studied previously, or reference to material examined. Perhaps also the methods section should give some detail about how these diagnoses were derived. I guess these are all modified from Krishna et al 2013 but that is only mentioned in the supplement. Biogeographic ranges of clades (in remarks subheadings) are given without citation.

RESPONSE: We agree that some references were missing. The statement "except for the Rhinotermitidae, which is paraphyletic with respect to Serritermitidae and Termitidae" is supported by our results and previous studies. We added the corresponding citations, which support the splitting of Rhinotermitidae into four distinct families (see Results). As reviewer #3 noted, many diagnoses were modified from Krishna *et al.* (2013), so were the biogeographic ranges. We now indicate this more clearly in the main text.

Diagnostic language: Throughout, the diagnoses character states are presented without context. Although this is commonplace it is not ideal. In the supplement I found an example of what I am talking about "Gut: esophagus distinct from the crop (as opposed to broadening into the crop as in Mastotermitidae, Teletisoptera, Serritermitidae, or Kalotermitidae);" This is type of language has the context I am referring to. It is more clear and should be used more often.

RESPONSE: We revised our diagnoses and included context when needed under the description of each subfamily, as suggested by Reviewer #3. Furthermore, some key features are illustrated in the new Figure 3.

The word "systematically" is used in a few places to mean "consistently" but this is confusing because it could be misunderstood to mean "phylogenetically" as in "phylogenetic systematics".

RESPONSE: We agree that this may be confusing and consistently replaced "systematically" with "consistently" throughout the manuscript.

pg. 7 "a robust classification devoid of non-monophyletic groups" double negative is confusing

RESPONSE: We rephrased this statement as "a robust classification only composed of monophyletic groups".

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

Revised manuscript addresses the concerns raised in my former review very thoroughly. The other reviewers made more extensive suggestions on the manuscript which similarly appear to have been handled satisfactorily - however those reviewers can be the better judge of that.

Congratulations on a very significant work.

Reviewer #2:

Remarks to the Author:

Dear Editor and authors,

The authors have thoughtfully considered and incorporated all the suggestions I made in my initial review. I also wish to commend the authors for the changes made in response to the suggestions provided by the other reviewers, which have further improved various aspects of the manuscript and its overall quality.

Following my suggestions, they refined the manuscript's illustrations by dividing their initial Figure 1 into three figures, which certainly enhanced the readability of their results. They also performed a series of statistical tests to assess the topological incongruences among early-diverging Neoisoptera and Termitidae and added more details in the Results and Discussion sections, providing greater clarity and support for the taxonomic changes proposed in the paper, including that involving Prothamitermitinae, which I specifically commented in my initial review.

With these changes, I stand by my previous assessment: this manuscript represents a valuable contribution to the field. I have no additional suggestions and believe the paper is ready for publication. I congratulate the authors on their impressive work and thank you for the opportunity to review this manuscript. I am looking forward to seeing it published in Scientific Reports.

Best regards

Reviewer #3:

Remarks to the Author:

I am impressed by how much work the authors have done to address each of the reviewers comments. More could always be done but the authors have adequately addressed my comments. If the monophyly of each of the relevant clades is consistent in rogue taxon analyses and coalescent inference, then I think due diligence has been achieved. My congratulations once again.

Response to reviewers' comments

Thank you for submitting your manuscript "Genomic data bring a new dawn to the classification of extant termites" to Nature Communications. I am delighted to say that we are happy, in principle, to publish it under the open access CC BY license (Creative Commons

Attribution 4.0 International License).

First, we ask you to revise your paper to address our editorial requests (in the attached Author Checklist) and any remaining comments from reviewers (included at the end of this email, if applicable).

[RESPONSE: We thank the three reviewers for their positive evaluation of our revisions. We attached to this resubmission the completed Author Checklist.](#)

REVIEWERS' COMMENTS

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Congratulations on a very significant work.

[RESPONSE: We thank Reviewer #1 for their positive assessment of our revisions.](#)

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With these changes, I stand by my previous assessment: this manuscript represents a valuable contribution to the field. I have no additional suggestions and believe the paper is ready for publication. I congratulate the authors on their impressive work and thank you for the opportunity to review this manuscript. I am looking forward to seeing it published in Scientific Reports.

Best regards

[RESPONSE: We thank Reviewer #2 for their positive assessment of our revisions.](#)

Reviewer #3 (Remarks to the Author):

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comments. More could always be done but the authors have adequately addressed my comments. If the monophyly of each of the relevant clades is consistent in rogue taxon analyses and coalescent inference, then I think due diligence has been achieved. My congratulations once again.

RESPONSE: We thank Reviewer #3 for their positive assessment of our revisions..