Peer J Review of

***Didymium arenosum,* a myxomycete new to science from the confluence of desert in northwestern China.**

The authors have modified the title and abstract in line with my previous comments. This is now acceptable. This is much, much better!

The Introduction has been revised so that previous classification details were removed. The authors may choose to use their literature source of classification but I still recommend that they consider Amoebosa as the most recent choice. However, this change is optional.

The authors have included references of other publications in arid regions of the world where I previously suggested. This coverage is much better now and summarizes and includes these regions now especially in South America.

I strongly urge the authors consider rearranging the text by moving lines 71 to 78 on the life cycle, which seems out of place, and insert this at line 111. Why? This keeps all of the geographical narrative on arid distribution together and the life cycle at the end of the Introduction. The logical flow is much better if this change is made. Again, this is optional. I like the narrative content in this section now.

Please note lines 60 to 62. This text is okay and worth mentioning but there is a publication worth reading and citing here since there is a table that highlights additional related metabolite data. Please see the following reference: Keller, H. W**.** and S.E. Everhart. 2010. Importance of Myxomycetes in biological research and teaching. FUNGI 3 (1): 29–43. This reference relates directly to the author's statement here.

Lines 103 to 110. I really like this section now. In line 104 I suggest the word typical be deleted.

Material and Methods

This topical section still needs revision. Line 117 ..Basidiospores were observed must be changed to Myxomycete spores were observed….Line 118 sentence needs rewording. These spores were not basidiospores. Revise sentence to read One hundred myxomycete spores were measured using the….Did you do this? Lines 118 starting with The dimensions are expressed as…and extending to Line 121 ending with Roniker must be deleted. Why. Myxomycete spores are mostly spherical not oblong with lengths and widths like basidiospores. What you have inserted here simply confuses and is incorrect. This is a major error. All you need to say is that you measured 100 spores and the spore dimensions are based on this. Did you do this?

Line 126, Change sentence to Water agar cultures were prepared…and summarized as follows: Did you use tap water. If you did then you cannot change this but you should never, never use tap water which is clorinated. Always use sterile glass distilled water. Tap water has chlorine and perhaps other foreign elements and this is not acceptable laboratory protocol. If this is what you did leave it but this is not acceptable laboratory protocol and should not be repeated. Check the publications by Keller especially his thesis to see how agar cultures were prepared. You said boiled but do you have an autoclave? It is standard protocol to autoclave flasks with agar before you pour the plates. Everything should have been autoclaved: Petri dishes, ground oats, so that once the agar was cooled and gelled no contaminating microorganisms were present. You will have to state what you did, good or bad, and not make up something just to meet the requirements of publishing this paper. After sterilization…I assume you used an autoclave? See Line 134, Were the pipettes sterile? The plasmodia were continuously monitored during development. Sterile oatmeal powder was introduced using sterile tweezers. This topical section is confusing and needs careful revision before acceptable. Why is controlled

laboratory protocol so important? Controlled laboratory conditions ensures repeatability and eliminates variables that cannot be duplicated.

Line 143-144, Wherever Petri dish appears Petri must be capitalized because this is a proper name of someone who invented this dish.

Line 175 …which means sandy place…

Line 182 revise wording Sporocarps delete The…are either.. delete …of this species (not needed),

Line 190-191, Lav …Wav..Q… Qav not needed, please delete. All that is needed is the measurements given in length and width.

Response Point 2, Concerns about literature citations in reference to morphology were addressed here. In addition, the stellate crystals and photographs demonstrate the crystalline nature. This improves the structural evidence for this taxonomic character.

Point 3, Response 3, Illustrations, no captions were given nor figure numbers? However, the two SEMs of the stellate crystals now clearly show the morphology of these structures. The four LMs of the crystals are out of focus and do not sharply focus the pointed crystals and no captions are given to describe the black pointers which designate the crystals. The sharp points evident in the SEMs cannot be clearly seen in the LMs? Four photographs are not needed just use the two lower ones. Also, the revised plates incorporated with these new photographs should be shown.

Line 200 Delete The …Spore germination…Revise sentence, Spore germination was by the split method creating a v-shape opening in sterile water…Line 203, ..change posterior end to anterior end…while shorter projections were often attached to the side…these are not flagella line 204,…the spore released their internal contents replace the word material, line 207…spore suspension of the zoospores (myxomycetes do not have zoospores change to swarm cells. Line 211 oat feeding after 3-6 days. Delete cultivated for. Line 213, reword …and distributed on the water agar surface as a network. Line 216…reword …began to form sporocarps…delete to gather

Phylogenetic analyses is excellent. No changes

Line 234, …reword…and the life cycle was completed on water agar culture. This part was rewritten but the English grammar needs revision.

Line 236, **The DISCUSSION** wording is confusing in places and needs rewording. This is because this section of the narrative was rewritten. For example, Line 234, English needs correction. I suggest the following: …add.. completed its life cycle on water agar culture. Line 236, …pale yellow, yellow brown, or light orange-brown. Delete other words. Much of this section is awkwardly worded. Line 238, ..the hyphae of…myxomycetes DO NOT HAVE HYPHAE BUT FUNGI DO. I think you mean plasmodial veins but I am not sure. Much of this section is new and the English needs careful revision.

Line 240 to 244, This sentence needs rewording. Line 241… similar to this species. Use period and start a new sentence, There are many ridges…..connected by a incomplete network. In contrast, *Didymium arenosum* has irregular spines and warts which can be combined into short lines. Delete other words. Line 146, inconspicuous columella, separate into two words, Lines 248 and 249, …has close affinity with *D. panniform*e…consistent with the morphological study of ….

The rewritten portions of the narrative need someone fluent in English to read and edit the content because the English is not acceptable now.

Response 4 is noted and accepted. However, the English needs careful editing that I have tried to do. The references are noted and accepted as a survey of the relevant literature.

Point 5 and Response 5. Content of the species description is much improved. See my comments on this topical section. The individual measurements given are not necessary but perhaps that could go in an appendix. I defer to the editors of Peer J for this or simply delete?

Point 7, Response 7, I have commented on the images previously but the new photographs need to be organized into plates and incorporated into the text. This requires a new submission as soon as possible.

Point 8, Response 8, Hoyer's Mounting Medium was used for mounting myxomycete spores. This should go in Materials and Methods.

Point 9, Response 9. Full page of eight photogmicrographs of what look like swarm cells. There were no captions. The image in the lower right hand corner is a swarm cell. You do not need all of these photographs.

Points 10, 11, and 12, Yes, the revised manuscript descriptive content has improved the case for a new species. However, the English grammar of this new narrative is substandard and needs to be carefully edited. This require corrections and reorganization of a new manuscript with figures and captions. The 12 images of the dark fruiting bodies on water agar show the variation if fruiting body morphology. However, you do not need all of these. I am not sure how this plate will fit in the manuscript without captions. You do not need all of these images. I assume they will be in color? I suggest you use the following images only: upper right hand corner, second level right hand third image, third level left hand side only and the bottom three images perhaps do not use because they are not clear enough?

Point 13, Response 13, This plates of photographic images would look nice in color if that is possible. There were no captions that are needed to document the different site habitats. The collection site should have as much as possible the rainfall, fluctuating seasonal temperatures, description and species identification of the plants, literature citations from publications on this desert region. Coordinates were provided.

I like the CONCLUSTIONS which correlate the life cycle stages with arid environments. This topical section is the best part of the entire paper. CONGRATULATIONS!

The narrative in this revised version is greatly improved but still lacks acceptable English grammar. The authors addressed many of my concerns in their Points and Responses. Peer J editors should request another revised re-submission with my suggestions and English corrections included along with the Figures. I encourage the authors to find a reader fluent in English to approve the sentence syntax and grammar before submitting the paper back to Peer J.

I am sorry this has taken me so long to prepare this review but medical complications slowed my time spent proofing the text. The paper in its present form is not acceptable. However, the inclusion of the molecular data and phylogram enhance the value of this paper and I believe the improved content should be published if the narrative can be revised.

Harold W. Keller, Professor Emeritus, Research Scientist, Botanical Research Institute of Texas. You have my permission to release my name to the authors. October 10, 2023.