

I'm very glad to review the manuscript #106784 entitled "Revisiting the Poison Dart Frog *Ameerega ingeri* (Anura: Dendrobatidae): external morphology, tadpoles, natural history, distribution, advertisement call, phylogenetic and conservation". It's a very thorough review of the status of the species, adding several essential information and it is from high relevance to the scientific community and for the studies on Dendrobatidae. The manuscript has all the merits to be published on PeerJ, and I deeply encourage its acceptance if the authors correct some major questions.

Here I provide a review on the manuscript with comments on the text, methodology, results and discussion. I deeply suggest the authors to carefully check the grammar and wording, although the English is comprehensive, there are many confusing and repetitive parts. I suggest a revision by a native speaker.

Please include in the title that this is a redescription of the species, this is one of the main objectives of your work, and should be clear from the beginning.

The general contextualization and methodology are well written, but the description of the studied area is confuse, the authors doesn't include information about the data they used to extend the species to Ecuador and there are informations lacking about some statistical analyses.

The Diagnosis and Comparison are not right named and should be renamed do meet the criteria of the ICZN as suggested in Cifelli & Kieran-Jaworowska. Diagnosis: Differing interpretations of the ICZN. 2005.

The characterization and diagnosis are insufficient as it is, and are the parts that need most improving. Since you are working on a redescription, you should provide a more comprehensive revision on this sections, not only including more individuals but also providing more characters that could help to distinguish the species from known species, but also enough information that would help to distinguish it from species yet to be described.

There are questions on the description of the characters that should be better written, the coloration is not specific enough, and the measurements and call parameters should be presented in the format – mean  $\pm$  STDV (min-max) in all the sections. The call description is quite tricky as it presents the means of the mean values of only two specimens, this data could be better treated. The description of a single tadpole would be greatly improved using tadpoles from stages 35 to 39, without any prejudice to the data precision.

Please be careful on the assumptions about the conservation status, the discussion about the population size and stability are overestimated. It seems that there is some confusion on the definition of what is AOO and EOO on the results. Please carefully review this section. For sure the criteria A2 and A3 are not right, as they have to be

directly measured to be considered, or to consider them you need to cite the studies about this. After checking the definition of AOO and EOO, the species maybe will be categorized by the criteria B1, but the conditions should be doubled checked.

Please remember to provide the genbank accession numbers when the manuscript is accepted.

Below I provide more specific comments to the authors to consider. After the careful revision, the manuscript should be accepted.

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Line 58 – Correct to “the Amazon ecoregions are recognized”.

Line 63 – This phrase need some rewriting. The first two factors are clearly causes of the scarcity of data, but the deforestation acts removing the diversity of the area, not preventing the data collection. Please clarify this.

Line 64 – Correct to “challenges such as the complex and expensive logistics to access unexplored areas”.

Line 74 – Exclude “such as reproduction and oviposition”.

Line 80 – Correct “limited morphological variation” to “overlaps in morphology”. Se have to be careful when dealing with the “morphological” variation in Dendrobatidae, although we have this kind of problem with the coloration, most os the species present other morphological features that can distinguish them from its close relatives.

Line 89 – Digit refers to toe or finger?

Line 91 – There you describe only the throat coloration in females. How is it in males? Or the color in males is not a character for the genus?

Line 95 – Remove the italic font from “and Putumayo”.

Line 112 – I would not call it endemic to Colombia since your finding point out that the species is also present on Ecuador.

Line 114 - I cannot found anywhere where you got the new records from Ecuador, It should be stated somewhere on the Materials and methods section.

Line 124 – Change “registered” for “recorded”.

Line 124 – That is very confusing, I get which are the municipalities and the seven localities, but what are the 16 areas? Please either clarify this, or remove this information.

Line 145 – How is it 18h/person? The sum of the diurnal and nocturnal samples is 9h.

Line 146 – Now there are 15 localities? Please make it consistent throughout the text.

Line 173 – Please give the precision.

Line 181 – Please fuse Natural history and ecology, Populations and territoriality and Natural history and habitat use, they are all complementary and within the section of Natural history, it is confusing to present them separated.

Line 183 – Delete “relatively”.

Line 184 – Correct to “have not been significantly altered by anthropic activities”.

Line 194 – The sympatry with *A. hahneli* doesn't matter here. Please move this to the results or discussion.

Line 221 – Very confusing, please rewrite as follows: “For each prey category the frequency of occurrence was determined according to Stark and Schroeder (FORMULA, 1970) where the %FO is given by the percentage of appearance of a specific prey in the total content of all stomachs. The importance of each prey category was calculated with the importance index according to Biavatti et al. (2004) modified by Méndez (2020) (FORMULA) **PLEASE INCLUDE THE MEANING OF EACH PART OF THE FORMULA HERE**. Additionally the trophic niche breadth of the sampled population was determined using Levin's equation...”.

Line 233 – I think that two males are too little to define the call with confidence, but is enough to start, please ensure that you are aware of this on your conclusions.

Line 251 – Please do not inform the name of the files that you analyzed, it doesn't add any relevant information to the text and is confusing, just provide the vouchers on the FNVJ.

Line 256 – This is a problem, I know that sometimes in taxonomy is common to use the males as the unit, but in your case, since you have only two males, it is strange to calculate any mean or STDV. As well as the coefficient of variation between two males doesn't sound good statistically. I think that the coefficient of variation should be deleted, as you don't sample enough to consider it, and the mean and STDV should be informed based on the total of calls and not on the males, so that you consider the real variation between calls, and reduce the effect of having only two males.

Line 271 – It doesn't make sense, as the EOO is literally the Extent of Occurrence while the AOO is the Area of Occupancy, so how the EOO would define the area occupied? Please clarify.

Line 272 – Correct to "it allows to determine".

Line 275 – Correct to "such as land cover type and elevation range".

Line 287 – Please reorder the section as follows: Paragraph 1. Extraction and PCR; P. 2. Genbank, alignment and deposition; P. 3. Delimitation methods and p-distance; P. 4. Model selection and lastly phylogenetic reconstruction.

Line 290 – Correct to "of each primer".

Line 291 – Correct to "forward and reverse".

Line 291 – Correct to "and Milli-q water to complete 15ul of final reaction volume.

Line 329 – I think that Niceforo's Poison Frog mean should be put in a distinct section called Vernacular Name.

Line 332 – There is an ongoing change on the treatment of the sections on taxonomic works to prevent confusion on the text of species descriptions (see Cifelli & Kieran-Jaworowska. Diagnosis: Differing interpretations of the ICZN. 2005), so that what you are calling Revised Diagnosis is in fact Revised Characterization and the Comparison with other species is the Diagnosis. This is because the ICZN clearly states that the diagnosis should compare the species to its closest relatives, and thus the current organization could be confusing.

Line 332 – The Characterization and diagnosis are not sufficient as they are in the MS. Please include more information, e.g. others relevant measurements, the main measures of the tadpole and the main temporal and spectral parameters of the call.

Line 336 – It's strange the male with 14.2 mm it seems to be an outlier, possible a juvenile, if this is the case I would remove this specimen, and any other juvenile from the data used in the ranges and mean. It would help on the comparison.

Line 339 – The numbers of the characters are wrong, please correct them.

Line 343 – The Diagnosis is insufficient, the only measurement that you include is the SVL and it presents a great overlap, that do not differentiate *A. ingeri* from *A. hahneli* and *A. bilinguis*. The color is a complementary character that should be informed in this section, but please don't rely this much on it, is that what lead the Dendrobatidae taxonomy to its current problems. Use more measurements on the comparison, as well as the data of the call that should help a lot to distinguish the species. Also, please don't compare to only the other Colombian species, compare also to its closest relatives on the phylogeny at least to *A. silverstonei* and *A. bassleri*. Also, I think that there are more

species that occur in Colombia, as *A. trivittata*, right? The improvement of this section would greatly improve your manuscript, as you aim to redescribe the species. On a redescription, you should work not only on more individuals but also on more data from the species.

Line 346 – What is dark dorsum? Black? Brown? Blue? Gray? Please be more specific on your color descriptions.

Line 347 – Please exclude the second time you write also.

Line 361 – Again, what is dark? I suggest you to use the Color Catalogue for Field Biologists from Kohler 2012, to choose the correct category for each color, and make it comparable.

Line 369 – I can only see the darker gular region on fig 4B, not in 7B. Did you observe if this occurs only in active/calling males? It is a common report from my colleagues as well as a observation of my fieldwork's that the active males of most of the *Ameerega* species has a darker coloration in the gular region, so, if this is the case, please state it on the text, although it is common to observe it is very badly documented.

Line 371 – You already cited that the adults have bright orange spots before on this paragraph, please exclude to avoid redundancy.

Line 371 – I see a black canthal region with a labial region bright blue, please check it.

Line 375 – Please cite the ranges for all the measurements and rates that you include in this section, give only the max or only the mean could lead to wrong interpretations.

Line 379 – It may sound obvious but cite that the vocal slits are present on the males.

Line 379 – You only cite the texture of the dorsal skin on the head, please include the dorsum texture.

Line 382 – How the dorsal and ventral view can have different formats, for me is only rounded.

Line 389 – Correct “addressed” to “appressed”.

Line 404 – I can only see the tarsus on the fig. 9B please correct.

Line 407 – Same as above, what is dark? Please be more specific on the color descriptions.

Line 408 – correct “while” to “and”.

Line 411 – I don't see white spots, only pale cream or gray, please correct.

Line 413 – I don't like the idea of describing the tadpoles with only one specimen, specially when you have collected a great series. I suggest you to use the stages from

35-39 to describe, specially as they seem to be very similar. Please correct and include the ranges.

Line 413 – Since you already give all the abbreviations to the measurements, you may cite only the abbreviations here.

Line 413 – Please include a photo of the dorsum of the tadpole on fig. 10.

Line 427 – The Tail Length is wrong, please correct. Please also presents the ratio between the TAL and BL.

Line 442 – Finally dark brown, that sounds good hahahaha.

Line 445 – You cite the tail musculature two times, please rewrite this entire section to make it clearer. Start with the body, the venter, then the tail and finally the legs.

Line 447 – Include that the lower fin is less marked than the upper.

Line 451 – Please write as follows “The body is almost uniformly heavily dark brown pigmented”.

Line 455 – This section is very nice, I like that you included it.

Line 459 – Was either in all stages? If it doesn't vary trough the stages I would include at least the number of individuals of each one.

Line 468 – Please make clear the origin of these specimens on the methods.

Line 468 – Rewrite as follows “Given that, the specimens collected by INABIO (2024) extends the occurrence of the species to Ecuador (**include the coordinates here**) in a mean distance of 195 km of the type locality (Fig. 1B and 1C).” Please move this sentence to the end of the paragraph, it is very confusing as it is.

Line 468 – Please thicken the country borders on the map, so that it is easier to distinguish between the countries and department borders, the maps is a little bit confusing to someone that doesn't know this region very well.

Line 484 – The second coma in the km should be a dot.

Line 487 – Oh that's not good, you already write this above, and with other distance, please correct it and delete one of the sentences.

Line 491 – I don't understand that, you have captured 94 or 126? Please state first the total number, and then cite the number of individuals captured in each locality.

Line 493 – Please exclude “taking”.

Line 494 – With only “??” Please cite how many in absolute numbers.

Line 495 – Correct “shows” to “indicates”.

Line 497 – You don't describe the tukey test on the methodology, please include it. Also, all the other statistic results are from tukey's test?

Line 499 – I think that it is confusing to cite the effect of the environment on the proportion of male and females and the differences in body size together.

Line 500 – Correct "like" to "and".

Line 503 – Correct to "non-open".

Line 504 – I would put this paragraph together with the first sentence of the last paragraph, linking sex ratio results.

Line 506 – Oh no! Be careful, you have to be clear, this is common to Amazonian *Ameerega*, this happens because they usually sleep at green leaves and call on the leaf litter or in fallen branches. I think is better to explain this behavior together with your results.

Line 511 – Inactive during the daytime? That's nice.

Line 518 – Please exclude "within and among localities" it is not necessary here.

Line 524 – Correct to "higher". Also, specify how greater is the sampling in this locality, in hours or percentage.

Line 535 – Ok, but what a nice width of 0.087 means?

Line 537 – Please provide the means together with the ranges.

Line 543 – How many notes from the first part are shorter?

Line 556 – Which other traits?

Line 567 – Correct to "... similarity in other temporal traits (Jungfer, 1989)".

Line 588 – Oh that's great, I deeply appreciate that you recognize the problems of your data. But you can discuss your results even though they are incongruent with Guillory's results. Even though it is a great work, it still have some problems.

Line 601 – Hm, how they are consistent if one delimited 12 and the other delimited 37? Also these information doesn't seem to be congruent with the table of results.

Line 609 – Small population of mature individuals and limited information on stable populations outside the type locality? Well you didn't do population genetics to confirm, and the closest that you reach to this information is with the number of recaptured individuals, and 0.83% could indicate, as you stated before, that the population is very stable. I would be more cautious on this result.

About the figures and tables:

Fig. 1. Please thicken the country borders in part A and B.

Fig. 3. The pictures doesn't represent the two environment adequately.

Fig. 4. It is not an obligation, but it would be nice if you include pictures of more individuals on this figure.

Fig. 6. Correct the legend to state that those are the species of *Ameerega* that occur in sympatry on the Colombian forest.

Fig 7. Please make clear that the insets 1, 2 and 3 are from juveniles, not from the same specimen. Please include a scale bar.

Fig. 8. Please include a scale bar.

Fig. 9. Please include a scale bar.

Fig. 10. Please include a picture of the dorsum, and a scale bar

Fig. 11. 96, it wasn't 94?.

Fig. 12. It would be nice to see the pluviosity together in this graph, even if it is taken from the region, and not from the specific locality.

Fig. 14. I love when the habitat is shown, congrats on doing that!

Fig. 17. You don't need to provide the file name.

Fig. 18. *Ameerega silverstonei* group? There is some paper defining this group? If not I would prefer to cite as "relationships between *Ameerega ingeri* and its closest relatives". Please work better on the preparation of the image, the area erased in the back of *A. ingeri* is visible, it looks like you take this three from other work already published. Also, indicate that the arrow indicate the three to the other *Ameerega* species.

Table S4. Correct Old to Previous.