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Response paper: Morphometric article by Mejía *et al.* 2015 alluding genera *Herichthys* and *Nosferatu* displays serious inconsistencies

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In the present response paper, the article entitled "Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): How many species comprise *H. labridens* (Pellegrin, 1903)?" by Mejía *et al*. 2015 is critically reviewed. The current review pinpoints some of the more conspicuous conceptual inconsistencies and fundamental errors found in the study by Mejía *et al*. (2015), It is contended that the authors fail to provide any new insights into the complex biogeography and evolutionary history of the *Nosferatu* and *Herichthys* genus groups, and that while results of their Cox1 molecular analysis are comparable to those by De la Maza-Benignos *et al*. (2015), the conclusions of the two studies are not comparable. In addition, it is contested that, whereas the designation of genus *Nosferatu* by De la Maza-Benignos *et al*. (2015) was found on the principles of the biological and phylogenetic species concepts, the rejection of the genus by Mejía *et al*. (2015) is solely based "on the presence of (overlapping) morphometric characters" between genera. The assumption by Mejía *et al*. (2015), that because their geometric morphometrics analysis failed to provide separation of species, then *Nosferatu* genus does not correspond to a valid taxon; and their suggesting geometric morphometrics "as useful tool to discriminate species, because it allows to propose diagnostic characters" were not supported by their results. While Mejía *et al*. present some interesting thoughts on the systematics of *Nosferatu*, they unfortunately fail to provide any data that can be objectively assessed as relevant to motivate any changes in the current taxonomy.

El presente documento de respuesta analiza críticamente el artículo titulado "Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): How many species comprise *H. labridens* (Pellegrin, 1903)?" por Mejía *et al.* (2015), así como también evidencia algunas de las contradicciones conceptuales y errores fundamentales encontrados en dicho documento. Se arguye que el artículo no proporciona ningún aspecto nuevo acerca de la compleja biogeografía, ni de la historia evolutiva de los géneros *Nosferatu* y *Herichthys*, y que mientras que los resultados del análisis molecular utilizando el gen Cox1 son similares a los de la Maza-Benignos *et al.* (2015), las conclusiones de ambos estudios no son compatibles. Se contiende además que mientras que la designación del género *Nosferatu* por De la Maza-Benignos *et al.* (2015) se fundamentó en principios asociados a los conceptos biológico y filogenético de especie, el rechazo del género por Mejía *et al*., (2015) únicamente se basa "en la existencia de caracteres morfométricos (superpuestos)" entre géneros. La inferencia por parte de Mejía *et al*. de que debido a que el análisis de morfometría geométrica no logró separar a las especies, y que por lo tanto el género *Nosferatu* no corresponde a un taxón válido; así como la observación de que la morfometría geométrica corresponde a "una herramienta útil para diferenciar especies, porque permite proponer caracteres de diagnóstico" no están sustentadas por los resultados de su análisis, y mientras que Mejía *et al*., presentan algunas ideas interesantes sobre la sistemática de *Nosferatu*, lamentablemente no proporcionan ningún dato relevante que pueda ser evaluado objetivamente como para motivar cambios en la taxonomía actual.

Keywords: Cox 1, Genera, Geometric morphometrics, Monophyly requirement, Paraphyletic, Species concepts.

Introduction

In the present paper, we wish to challenge the study entitled "Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): How many species comprise *H. labridens* (Pellegrin, 1903)?" by Mejía *et al*. (2015). We review and comment on some of the more serious shortcomings of the aforementioned study. Also, we pinpoint some of the more noticeable conceptual inconsistencies and errors that characterize their manuscript.

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Material and Methods

Research article entitled "Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): How many species comprise *H. labridens* (Pellegrin, 1903)?" published by Mejía *et al.* in Neotropical Ichthyology, 13(1): 61-76, 2015 was critically reviewed. We evaluated whether the conclusions drawn from their study using DNA barcoding and geometric morphometrics are consistent with the reported results and comparable to previous molecular studies on the systematics and zoogeography of both clades reported in the scientific literature between 2007 and 2015.

Results

After a careful examination of the manuscript by Mejía *et al.* (2015), we consider that, apart from corroborating a few general aspects of both taxa that have been widely known for some time now and have already been published even in the most popular literature (for example, see Wikipedia under *Nosferatu* (fish) and *Herichthys* entries), the article fails to provide new insights into the complex evolutionary history and the biogeography of the *Nosferatu* and *Herichthys* genera. For more information on the systematics and zoogeography of both clades see Concheiro-Pérez *et al.* (2007), De la Maza-Benignos & Lozano-Vilano (2013), De la Maza-Benignos *et al.* (2015), Hulsey *et al.* (2004), López-Fernández *et al.* (2010), León-Romero *et al.* (2012), Magalhaes *et al.* (2015), McMahan *et al.* (2015) and Oldfield *et al.* (2015), to name a few.

We will begin by discussing the appropriateness of the title, "Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): How many species comprise *H. labridens* (Pellegrin, 1903)?" The title hints to a systematic revision and an update on the number of species within *Nosferatu* (De la Maza-Benignos *et al.*, 2015). However, within their paper, we were unable to identify a ground-breaking discussion which resolves the fundamental issue suggested. Instead, at best, the authors limited their contribution to a brief and often very confusing restatement of results already presented within previous studies. For example, the article concludes, "…an update of a previously published DNA barcoding study suggests the existence of three genetic clusters that included the six recognized species analyzed in this study, none of them recovered as monophyletic" and "On the other hand, geometric morphometrics arise as a useful tool to discriminate species due that [*sic*] traditional morphometrics showed a high overlap in the characters analyzed that prevents the proposal of diagnostic characters."

The first concluding statement mentioned above, that none of the species were recoveredas monophyletic, is clearly inaccurate. Based on their second concluding statement, we would have expected that their geometric morphometrics evaluation would have allowed them, at least, to disclose a discrete measure of what had passed undetected by previous authors, which would allow separating recognized nominal species without having to recur to canonical functions. Why is it then that except for *Nosferatu steindachneri*, which had already been validated as a distinct species by De la Maza-Benignos & Lozano-Vilano (2013) based on similar arguments, that the authors were incapable in their redescriptions to produce a morphometric founded diagnosis for any of the species reviewed? Instead they based their diagnosis on geographic and chromatic characteristics. In our opinion, their morphometric "findings" are contradictive to their conclusion based on geometric morphometrics.

In addition, the manuscript further acknowledges in the Introduction section that there are six "recognized" nominal species in the "group" (*i.e. N. labridens*, *N. bartoni*, *N. steindachneri*, *N. pame*, *N. pratinus*, and *N. pantostictus*), although, in the Methods section further down, they say that "we include seven taxa: *H. bartoni*, *H. labridens*, *H. steindachneri*, *H. pantostictus*, *H. pame*, *H. molango*, and *H.* cf. *labridens*, however, we cannot [*sic*] include samples from *H. pratinus*." This indicates that they constructed *a priori* determinations that their *Herichthys.* cf. *labridens* is a distinct taxon or they accounted for a species *N. pratinus*, which was excluded in the study.

As mentioned earlier, their paper provides a series of redescriptions for the nominal species *N. bartoni*, *N. labridens*, *N. steindachneri*, *N. pame*, *N. pantostictus,* and *N. molango*, and, hence, in our view, tacitly validating them (*N. pratinus* was not included in the work) and ends asserting that "…traditional morphometrics and meristic counts failed to clearly separate species…;" however, "in conclusion, the results of this study regarding geometric morphometrics allow support for the recent proposal of De la Maza-Benignos & Lozano-Vilano (2013) that *H. labridens s.l.* comprises several species…" and proposes that "further studies with other molecular markers are necessary….".

Furthermore, their article contains some misreports. For example, in the work by De la Maza-Benignos & Lozano-Vilano (2013) and De la Maza-Benignos *et al.* (2015), the authors clearly indicate that *N. molango* is restricted to Laguna Azteca and suggest that this species is the only native cichlid found in this place. In addition, De la Maza-Benignos *et al.* (2015) suggest that "the species could correspond to a phenomenon of secondary contact between both genera, as *N. molango* exhibited mitochondrial DNA affinity to *Herichthys*."

In contrast, the paper by Mejía *et al.* (2015) amends the distribution range of *N. molango* from the original description and arbitrarily assign the species to "…widely distributed in rivers windward of the Sierra Madre Oriental in the states of Hidalgo and San Luis Potosí," without any explanation. Hence, the interpretation by Mejía *et al.* (2015) of *N. molango* is fundamentally in disagreement with the nominal species *sensu stricto*, and their analysis based on a composite of *N. molango* and *N. pantostictus* (see

Material Examined on page 74 of their paper). Moreover, it can be inferred that *N. molango* was not included in their genetic analysis, "because this species was formalin fixed and preserved." Hence, the redescription and their morphometric and molecular conclusions regarding *N. molango* are specious and misleading.

In the introductory section of the paper, Mejía *et al.* (2015) indicate that there are "12 currently recognized species (in *Herichthys* + *Nosferatu*)," followed by the idea that "recent phylogenetic studies have confirmed the monophyly of the genus but not of the species included in it…." It can in addition be inferred that the authors believe that there remain taxonomically uncertain populations within the group found in the mainstream of the Pánuco and Tamesí rivers at the sites marked with a † on the map in Figure 1, a very improbable situation in our opinion. Thus, we will contend that it is reasonable to assume that the authors could be dealing with a mislabeling and not a taxonomic problem.

Because Mejía *et al.* (2015) were unable to identify "unique autapomorphies that allow" to distinguish between *N. labridens* and *N. pantostictus* "from the rest of the species of the group," and because they basically disregarded the work by De la Maza-Benignos & Lozano-Vilano (2013), they appear to have chosen to treat the *H.* cf. *labridens* tag as a taxonomic unit. Although, based on the localities provided, and the position of such specimens in the phylogenetic tree, it can be inferred that the species labeled as *Herichthys* sp. *labridens* corresponds to mislabeled specimens of *N. pantostictus sensu* De la Maza-Benignos & Lozano-Vilano (2013). Consequently, the apparent polyphyly discrepancy discussed in Mejía *et al.* (2015) essentially becomes an easily resolved label/tag problem.

The inefficacy of a particular method or genetic marker to discriminate between species, particularly within *Herichthys,* for example in *H. carpintis vs. H. tamasopoensis* (see De la Maza-Benignos *et al*., 2015), does not signify that differences do not exist. Hence, unlike higher taxonomic ranks, for which we believe phylogenetic analysis should clearly demonstrate both monophyly and validity as a separate lineage in phylogenetic trees, species and subspecies can appear paraphyletic (Crisp & Chandler, 1996), for example, *N. bartoni vs. N. labridens*, depending on the levels of resolution of the genetic markers used. In fact, species-level paraphyly has been reported to occur in 19% to 23% of animal species on the basis of a meta-analysis of published mitochondrial gene trees [see: Funk & Omland (2003), Hörandl (2006), and Ross (2014)].

In the introduction section of their paper, Mejía *et al.* (2015) "rejected the recent proposal of De la Maza-Benignos *et al.* (2015), who suggested that the species included in the *H. labridens* species group must be segregated into a new genus named *Nosferatu* because the morphological characters that support both genera are also present in species of the other genus." No evidence for the above claim is provided, for nowhere in the manuscript is the separation between *Nosferatu* and *Herichthys* even analyzed or discussed. Instead, the authors cite an unpublished paper "in preparation" by Pérez-Miranda. The fact is that both genera can be easily distinguished from each other (see De la Maza-Benignos *et al*., 2015) even by the inexperienced eye. Mejía *et al.* (2015) are of course entitled to "reject" the existence of a genus; however, in academia the *onus probandi* should fall upon those who make claims.

The authors further state in the results section that "the phylogenetic analysis of the mitochondrial COI confirms the previously published results using COI and other molecular markers that the genus *Herichthys* comprises a well-supported monophyletic group (BPP = 1.0), and that this genus includes two well-supported clades: the group of species related to *H. cyanoguttatus* (BPP = 1.0) (=*Herichthys*) and the group of species related to *H. bartoni* analyzed in the current study (BPP = 1.0) (=*Nosferatu*)."

Whereas the scope of this note is not to provide a full discussion of the trite species problem, a quick review of the biological and the phylogenetic species concepts is relevant to our point. For a more comprehensive discussion regarding the species problem, see Mayr (1970, 1996), Wiley (1978), Cracraft (1983, 1997), Crisp & Chandler (1996), Kullander (1999), Mishler & Theriot (2000), Noor (2002), and Wiens (2004), among others.

Discussion

The classical biological species concept defines species as groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr, 1970), whereas the phylogenetic species concept considers a species as the smallest recognizable organismal unit in a phylogenetic genealogy, and because there should be no structural difference between taxa of different categories, we will argue that both concepts also apply to the genus rank. For a more comprehensive discussion on the "monophyly requirement" for species rank see Crisp & Chandler (1996), and Kullander (1999).

In that order of ideas, genera should be composed of phylogenetic units of the same kind as other genera; and both *Nosferatu* (BPP = 1.0 according to Mejía *et al*., 2015) and *Herichthys* (BPP = 1.0 according to Mejía *et al*., 2015) genus groups *sensu* De la Maza-Benignos *et al.* (2015) fully meet the underlying criteria of the two concepts. Furthermore, separate molecular analysis by Concheiro-Pérez *et al.* (2007), De la Maza-Benignos *et al.* (2015), Hulsey *et al.* (2004), López-Fernández *et al.* (2010), León-Romero *et al.* (2012), Mejía *et al.* (2015), Magalhaes *et al.* (2015), McMahan *et al.* (2015) and Oldfield *et al.* (2015) support the monophyly of the *Nosferatu* + *Herichthys* clade as well as separation between both genera. Moreover, the separate analysis of the mitochondrial gene Cox1 by León-Romero *et al.* (2012), De la Maza-Benignos *et al.* (2015), and Mejía *et al.* (2015) reveal the existence of three clades in *Nosferatu*: 1) the bartoni clade (= phylogenetic group I *sensu* Mejía *et al*., 2015); 2) the steindachneri clade (= phylogenetic group II *sensu* Mejía *et al.*, 2015); and 3) the pantostictus clade (in the study by León-Romero *et al.* (2012) and Mejía *et al.* (2015) some of the lineages of *N. pantostictus* are misidentified as *H.* cf. *labridens*, and consequently, they reported polyphyly).

In conclusion, while morphometric and molecular analysis results by Mejía *et al.* (2015) and De la Maza-Benignos *et al.* (2015) are comparable, the conclusions drawn by the two papers are contradictory. The designation of genus *Nosferatu* by De la Maza-Benignos *et al.* (2015) was found on the principles of the biological and phylogenetic species concepts, whereas the rejection of the genus by Mejía *et al.* (2015) is based solely on the lay understanding of what a species is and the presence of (overlapping) morphological characters in both genera, according to an unpublished paper still in preparation. Because geometric morphometrics analysis failed to provide separation of the species, the conclusion from their study that geometric morphometrics is a useful tool to discriminate species as it allows the proposition of diagnostic characteristics is not consistent with their results. Moreover, both papers agree that further molecular analyses using different markers are required. Mejía *et al.* (2015) present some interesting thoughts on the systematics of *Nosferatu*; however, unfortunately, they do not provide any data that can be objectively assessed as relevant for motivating any changes in the current taxonomy.

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