HOW TAXONOMIC MISIDENTIFICATIONS MAY AFFECT PHYLOGENETIC TAXONOMY: AN ANALYSIS ON VOUCHER SPECIMENS USED IN GARCÍA-MELO ET AL. (2019)

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Abstract: I evaluated the taxonomic identifications used in García-Melo et al. (2019; GMEA hereafter) to infer the phylogenetic relationships among 372 fish species within the Stevardiinae (Characidae). The taxonomic assessment presented in this paper was based on taxa within my area of expertise, representing 13% of the species sampled in GMEA (59 of 372) and comprising species historically classified under *Bryconamericus* (21 species in GMEA), *Carlastyanax* (one species), *Eretmobrycon* (nine species), *Hemibrycon* (eight species), and *Knodus* (20 species). I detected potential taxonomic misidentifications in 17% of the taxa that were here evaluated (10 of 59 species). Finally, I provide additional details on (i) why taxa were misidentified in GMEA and (ii) how phylogenetic conclusions were potentially compromised by taxonomic errors.

Keywords: Characidae, fishes, neotropical, South America, systematics.

COMO LAS IDENTIFICACIONES TAXONOMICAS ERONEAS PODRIAN AFECTAR LAS FILOGENIAS: UN ANALISIS SOBRE LOS EJEMPLARES TESTIGO EN GARCÍA-MELO ET AL. (2019)

Resumen: Evalúo la validez de las identificaciones taxonómicas usadas en García-Melo et al. (2019; GMEA en adelante) para inferir las relaciones filogenéticas entre 372 especies de peces dentro de Stevardiinae (Characidae). La valoración taxonómica presentada en éste artículo se basa en taxones dentro de mi área de experticia, representan el 13% de las especies en GMEA (59 de 372) y comprenden especies históricamente clasificadas en *Bryconamericus* (21 especies muestreadas en GMEA), *Carlastyanax* (una especie), *Eretmobrycon* (nueve especies), *Hemibrycon* (ocho especies) y *Knodus* (20 especies). Detecto errores de identificación en 17% de los taxones evaluados en este artículo (10 de 59 especies). Finalmente, proporciono detalles adicionales sobre (i) por qué los taxones se identificaron erróneamente en GMEA y (ii) cómo potencialmente se comprometieron las conclusiones filogenéticas.

Palabras clave: Characidae, peces, neotropical, Sur América, sistemática.

A recent paper by García-Melo et al. (2019) (GMEA) evaluated the systematics within the subfamily Stevardiinae, the largest clade of neotropical characids. García-Melo et al. (2019) suggested species limits within *Bryconamericus*, *Hemibrycon*, *Knodus*, and *Eretmobrycon* based on an extensive species-and molecular-level sampling. Despite the remarkable efforts made by the authors towards establishing a novel phylogenetic hypothesis for the clade, problematic identification may have compromised the validity of several of their conclusions. Taxonomic issues were even detected by the authors of the same study: "… some morphospecies are not congruent with the exploratory delimitation (highlighted in red letters, Fig. 2) suggesting the existence of problems and errors in the identification of species of the genera studied." Here I evaluated taxonomic identification errors that may have affected the phylogenetic conclusions, and may also have important effects on the conservation status of different species, based on taxa within my area of expertise. Additionally, I propose a potential solution to this problem that can be easily incorporated in future studies.

TAXA IN CONTROVERSY

I evaluated species-level identifications in GMEA by comparing known geographical ranges and topotypical localities to collection localities in GMEA. Very often, studies defining new species include geographical information as a relevant species descriptor. Here, I use geographical overlap to assess whether sampled species in GMEA were potentially misidentified. I acknowledge that my indirect assessment based on geographical data may not be completely accurate. However, given than 84% of all Bryconamericus (n=61 especies), 92% Hemibrycon (n=51 especies), and 93% Knodus (n=28 especies) occur in allopathy (Data available in the figshare repository, see in box at the end) with species in the same genus, geographical information generally reflects taxonomic boundaries. Relevant to this discussion is also the fact that *Carlastyanax*, a monotypic genus, is restricted to the Alto Cauca River in Colombia (Román-Valencia et al., 2018). Analyzing these distributions does in fact lend taxonomic and phylogenetic studies a cutting edge, and thus the biogeographical component often takes precedence (Procheş, 2020). Here the geographical distribution is considered based on current data for each taxon. Below, I summarize information on 10 species of *Bryconamericus*, *Hemibrycon*, *Knodus*, and *Eretmobrycon* that were sampled in GMEA. I did not detect problems in the remaining 40 species sampled in the same genera.

1. *Bryconamericus andresoi* Román-Valencia, 2003a

Examined material (GMEA): MPUJ 11047; Colombia, Cauca, Patia, Timbío, 800 m arriba del casco urbano.

Comments: *Bryconamericus andresoi* is identified in the upper Patia River in small creeks of the Junaba and Molinoyaco rivers, Colombia (Román-Valencia, 2003a). GMEA did not examine samples of *B. andresoi*. Although taxonomic identity of the material examined in GMEA is still ambiguous, their samples clearly do not correspond to *B. andresoi*. Furthermore, previous research by Román-Valencia (2003a) has suggested that *B. andresoi* in GMEA should be *B. guaytarae*. What GMEA classifies as *H. guaytarae* might correspond to an *Eretmobrycon* or *Bryconamericus.*

2. *Bryconamericus caldasi* Román-Valencia, Ruiz-C, Taphorn & García-Alzate, 2014

Examined material (GMEA): MPUJ 11134; Colombia, Magdalena-Cauca, La Libertad Creek, vereda La Libertad en el puente, corregimiento de Arauca, Municipio de Palestina, Caldas.

Comments: *Bryconamericus caldasi* is only known to live on the middle Cauca River Basin in La Libertad Creek, San José county, Caldas, Colombia (Román-Valencia et al., 2014a; Román-Valencia et al., 2017a). Collection site in GMEA does not correspond to *B. caldasi*. In an earlier work, Román-Valencia et al. (2014a) concluded that the *Bryconamericus* from La Libertad Creek, La Libertad village in the Arauca bridge (municipality of Palestine), correspond to *B. caucanus*. Here I suggest that GMEA examined samples of *B. caucanus* instead of *B. caldasi*. Román-Valencia et al. (2014a; 2017a) meaning that samples in GMEA for *B. caldasi* and *H. boquiae* are both actually *B. caucanus*.

3. *Bryconamericus galvisi* Román-Valencia, 2000

Examined material (GMEA): MPUJ 11046; Colombia, Amazonas, Putumayo, Colón, Vereda Las Palmas, Zanjón, afluente del Putumayo River.

Comments: *Bryconamericus galvisi* is only identified to be from the Sibundoy Valley, Putumayo in Colombia (Román-Valencia, 2000; Román-Valencia & Muñoz, 2001; Román-Valencia et al., 2017a). GMEA did not examine samples of *B. galvisi*. Román-Valencia (2000) and Román-Valencia & Muñoz (2001) suggested that *B. galvisi* in GMEA corresponds to another species of *Bryconamericus* or *Knodus*.

4. *Bryconamericus huilae* Román-Valencia, 2003a

Examined material (GMEA): MPUJ 11033; Colombia, Magdalena River, Garzón Creek, casco urbano de Garzón, Huila.

Comments: *B. huilae* was described from the Alto Magdalena River, Suaza, Guachico and Timaná River system (Román-Valencia, 2003a; Román-Valencia et al., 2017a). Sampling for *B. huilae* in GMEA does not correspond with *B. huilae*. However, sampling sites for *B. huilae* in GMEA overlap with the distribution of *B. tolimae*. Previous research by Román-Valencia (2003a; 2004) and Román-Valencia et al. (2017a) suggested that *B. huilae*, in GMEA, must correspond with *B. tolimae*.

5. *Bryconamericus macarenae* Román-Valencia, García-Alzate, Ruiz-C. and Taphorn, 2010

Examined material (GMEA): MPUJ 76479, 76478, 76481 and 76482; Colombia, Orinoco, Guaviare.

Comments: *B. macarenae* has only been identified in the Güejar River basin in the Meta department, Macarena Mountain range, Orinoco system in Colombia (Román-Valencia et al., 2010; Román-Valencia et al., 2017a). Samples examined by GMEA do not correspond to *B. macarenae*. However, samples in GMEA were collected of *B. alpha* (Román-Valencia, 2003b; Román-Valencia et al., 2008a; Fricke et al., 2020). I also note that previous research by Román-Valencia (2003b), Román-Valencia et al. (2008a), Fricke et al., (2020), and Román-Valencia et al. (2010; 2017a) has shown that *B. macarenae* in GMEA may correspond to another species of *Bryconamericus* or *Knodus*.

6. *Bryconamericus orinocoense* Román-Valencia, 2003c

Examined material (GMEA): LBP 16928; Brasil, Tocantins, Araguaia.

Comments: *B. orinocoense* is identified in Amazonas State, Venezuela, from the Rio Mavaca in the south to the Rio Cataniapo in the north (Román-Valencia, 2003c). The material examined in GMEA correspond to *B. breviceps* (Román-Valencia, 2005; Fricke et al., 2020). The samples probably was collected from localities outside the distribution of *B. orinocoense*.

7. *Hemibrycon boquiae* Eigenmann, 1913

Examined material (GMEA): MPUJ 11050; Colombia, Alto Cauca, Quindío River.

Comments: *H. boquiae* was described from Boquia Creek, tributary of the Quindío River in Boquia, Salento, Quindío, Colombia (Eigenmann, 1913; Román-Valencia, 2001; Román-Valencia et al., 2008b; 2009a). Román-Valencia et al.(2009a;b; 2018) also identified in other drainages of Quindío River (e.g. Hojas anchas, La Sonadora, La Vibora, Doña Juana, Villa Paola, Canceles, La Florida, La Venada, El Macho and El Broche creeks; 1500-1800 meters above sea level). However, given that the collection locality in GMEA for H. aff. boquiae is extremely inaccurate, samples are probably from a more widespread species in the area (*B. caucanus*).

8. *Hemibrycon mikrostiktos* Bertaco and Malabarba, 2010

Examined material: CZUT 12038; Colombia, Amazonas, Caquetá, on the road from Florencia to Neiva, Quebrada La Sardina; CZUT 12048, Colombia, Amazonas, Caquetá, Belén de los Andaquíes, La Arenosa Creek; CZUT 12052, Caquetá, Morelia, quebrada Aguascalientes.

Comments: *H. mikrostiktos* is identified from the upper Ucayali River drainage, Ucayali, Peru (Fricke et al., 2020; Bertaco & Malabarba, 2010). Collection locality reported in GMEA for these samples of this species do not correspond to *H. mikrostiktos*. More importantly, *H. mikrostiktos* is not known from Colombia (Do Nascimiento et al., 2017). Therefore, *H. mikrostiktos* in GMEA must correspond to another species of *Hemibrycon*.

9. *Hemibrycon raqueliae* Román-Valencia and Arcila-Mesa, 2010

Examined material (GMEA): CZUT 11920; Colombia, Magdalena River, Bocaya Creek; CZUT 15751, Colombia, Magdalena River, Samaná, vereda Modín, Tasajos River.

Comments: *H. raqueliae* is identified in the La Miel River, middle basin of the Magdalena River (Román-Valencia & Arcila-Mesa, 2010; Román-Valencia et al., 2017a). Therefore, *Hemibrycon* aff. *raqueliae* in GMEA must correspond to another species of *Hemibrycon*. GMEA do not examine topotypic material, and samples were collected from localities outside of the geographic range for this species.

10. *Knodus meridae* Eigenmann, 1911

Examined material (GMEA): LBP 15818; Venezuela, Orinoco

Comments: *K. meridae* is identified in the Lake Maracaibo basin, Venezuela and Colombia (Román-Valencia, 2005; Fricke et al., 2020). Furthermore, Román-Valencia (2005) and Fricke et al., (2020) suggest that *K. meridae* in GMEA must correspond to another species of *Bryconamericus* or *Knodus*. GMEA did not examine topotypic material and sampling sites for their samples do not overlap with the geographical range for this species. Because distribution data recorded in GMEA are more congruent with species from the Orinoco basin.

In short, 17% of the species examined in this study have problematic taxonomic identifications (10 of 59 species) (Fig.1 in this study). As a consequence of uncoupling between the identity for these 10 species and the collection localities in GMEA, conclusions reached by the authors on the phylogenetic relationships within the Stevardiinae are potentially problematic. Below, I discuss how taxonomic misidentifications may have influenced the inferred phylogenetic patterns within clades 2, 4, and outside of the four major clades proposed by GMEA (Fig.1 and 2).

First, problematic identifications that were previously corrected elsewhere were not accounted for in GMEA (Román-Valencia, 2002; Román-Valencia, 2003a; b; c; 2004; Román-Valencia & Vanegas-Ríos, 2009; Román-Valencia et al., 2008; 2009a;b; 2014a;

2017a;b; Román-Valencia & Arcila-Meza, 2010). A species-by-species outline of the taxonomic omission made in GMEA is presented in this manuscript under results. In short, the fact that GMEA did not account for taxonomic errors corrected elsewhere raises doubts on the accuracy of the conclusions on the systematics of the group.

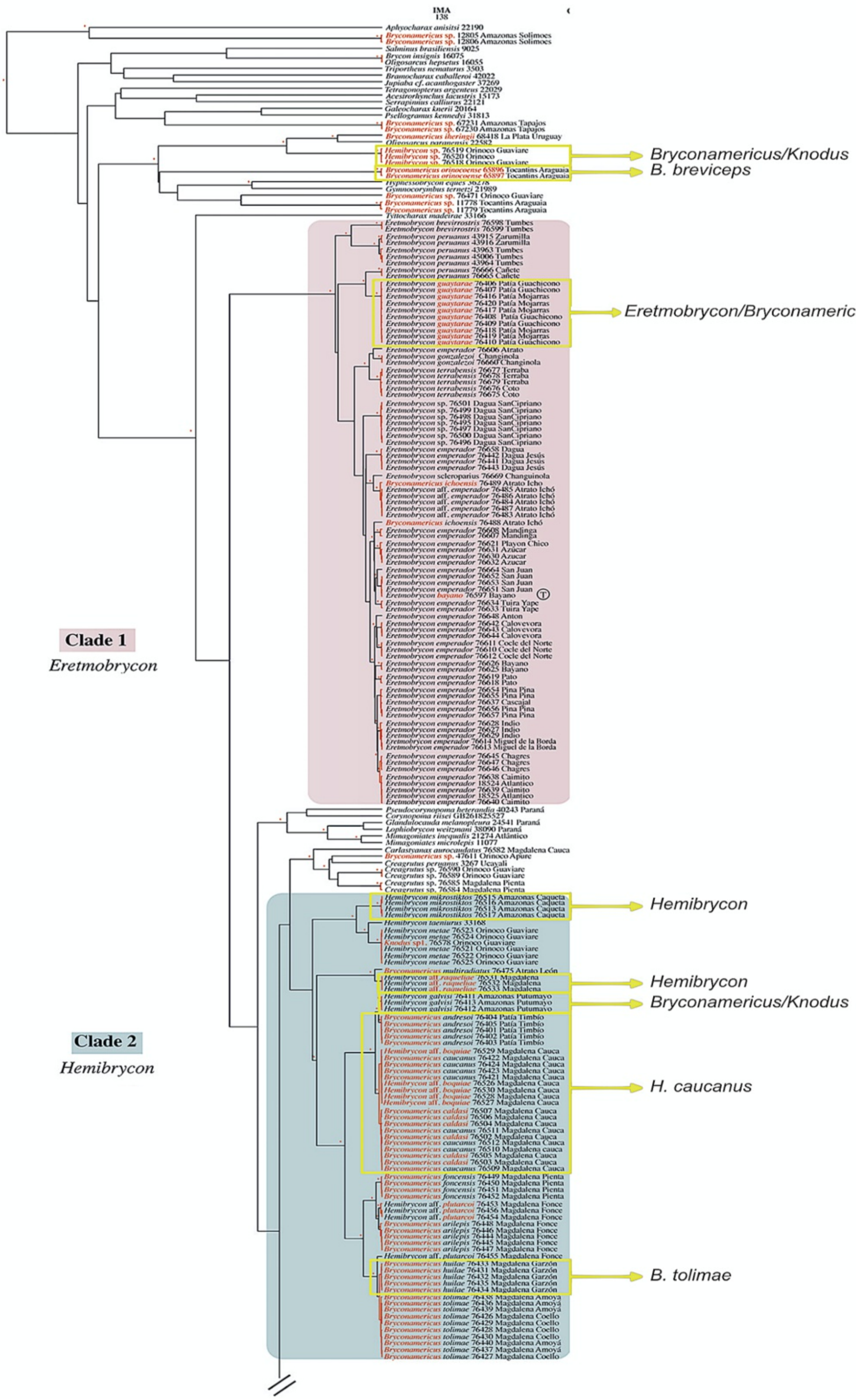

Fig. 1. Bayesian phylogenetic tree of *Eretmobrycon*, *Bryconamericus*, and *Hemibrycon* obtained with COI data. Modified of Garcia-Melo et al. (2019).

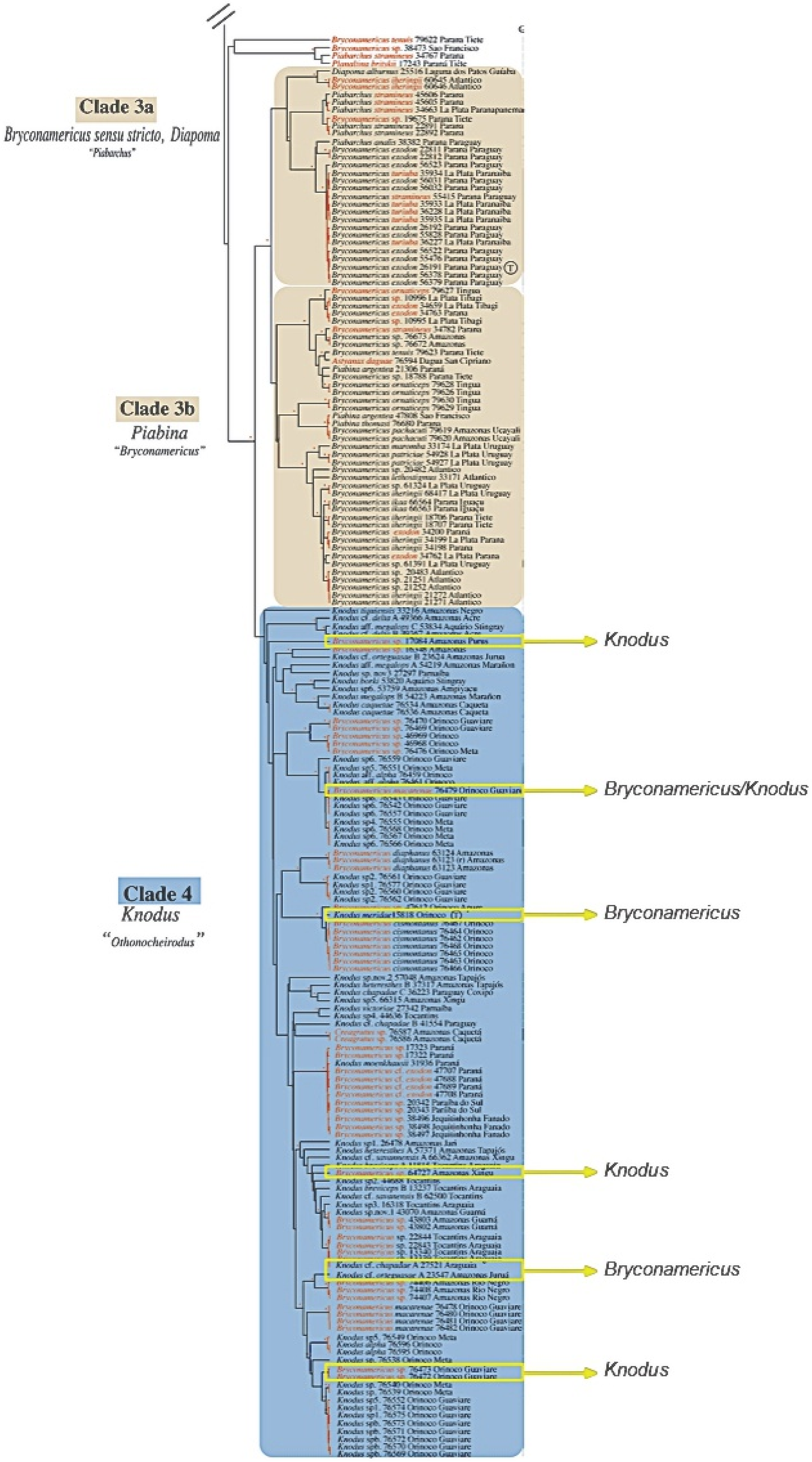

Fig. 2. Bayesian phylogenetic tree of *Bryconamericus*, and *Knodus* obtained with COI data. Modified of Garcia-Melo et al. (2019).

Secondly, GMEA synonymized one *Bryconamericus* species (*B. turiuba* to *B. exodon*) and transferred 12 *Bryconamericus* species to other genera (*Eretmobrycon*, *Hemibrycon* and *Knodus*; Table 2 therein): *B. andresoi* (to *H. andresoi*), *B. arilepis* (to *H. arilepis*), *B. cismontanus* (to *K. cismontanus*), *B. diaphanus* (to *K. diaphanus*), *B. foncensis* (to *H. foncensis*), *B. ichoensis* (to *E. ichoensis*), *B. macarenae* (to *K. macarenae*), *B. multiradiatus* (to *H. multiradiatus*), *B. tolimae* (to *H. tolimae*), *B. dahli* (to *E. guaytarae*), *B. caldasi* (to *H. caucanus*), and *B. huilae* (to *H. tolimae*). Among these taxonomic changes, 42% may be invalid due to taxonomic misidentifications (i.e. *B. andresoi*, *B. macarenae*, *B. tolimae*, *B. huilae*, and *B. caldasi*). Taxonomic changes in GMEA (table 2, fig. 2 therein) are poorly supported according to the rules of the International Commission on Zoological Nomenclature (1999: chapter 6 article 23, recommendation 24A). Thus, I suggest that taxonomic changes proposed in GMEA for these five species are invalid.

Third, unsupported taxonomic changes in GMEA may have compromised their phylogenetic conclusions. Below I summarize the implications that incorrect taxonomic identifications may have placed on the composition of clades proposed by GMEA (Fig. 1 and 2):

Clade 1: No taxonomic misidentifications were observed.

Clade 2: First, samples assigned to *H. nikrostiktus* in GMEA were probably misidentified. Consequently, the closest relative to *H. metae* and *H. taeniurus* is not *H. nikrostiktus* but probably another Hemibrycon species. Second, the closest relative of *B. multiradiatus* and *B. andresoi* is potentially a *Hemibrycon* or *Bryconamericus* species but probably not *H.* aff. *raqueliae* and not *H. galvisi* respectively as stated in GMEA. Third, *Bryconamericus caucanus* is actually monophyletic if the correct taxonomy is used for *H.* aff. *boquiae* and *B. caldasi* (i.e. both species are *B. caucanus*). Fourth, under the corrected taxonomy, *B. tolimae* comprise samples that GMEA assigned to *B. huilae*. Therefore, these two species are not sister lineages. Fifth, *B. tolimae* is monophyletic if the correct taxonomy is used.

Clade 3: No taxonomic misidentifications were observed. However, Menezes et al. (2020), analyze that the close relationship of *B pachacuti* with *B. exodon* in GMEA may be an analytical artifact.

Clade 4: First, *Knodus meridae* is actually a *Bryconamericus* or another *Knodus* species. Second, *B. macarenae* is actually another *Byconamericus* species. Third, Menezes & Netto-Ferreira (2019) propose that *Knodus* sp. nov2, *K. heteresthes*, *K. chapadae*, and *Knodus* sp.5, in GMEA, are likely to be within the *Rhinopetitia*.

Species outside of Clades 1-4: Taxa outside of the four clades in GMEA include species in *Bryconamericus*, *Hemibrycon*, and *Knodus* (*B. orinocoense* is *B. breviceps* or potentially *Knodus*). Specifically, GMEA identifies several samples collected from lowland localities (<1000 m. asl) in the Orinoco basin as *Hemibrycon* sp. (fig. 2, S1 table 1 therein). However, these samples must correspond to *Knodus* and not *Hemibrycon* given that most *Hemibrycon* are distributed in highland creeks (>1500 m; the only exception being *H. dariensis*, Román-Valencia & Arcila-Meza, 2009; Román-Valencia et al., 2009a; b; 2014a; b).

Taxonomic errors as the ones summarized in this paper also imply significant changes to the conclusions in previous studies from the same authors (e.g. García-Melo et al., 2018, García-Melo, 2017). For instance, samples in García-Melo et al. (2017; 2018) assigned to *H. cardalensis* and *H. jabonero* do not correspond to the same species (see Román-Valencia et al., 2013; see Román-Valencia et al., 2018). Nevertheless, none of these species were sampled in GMEA.

I also note that taxonomic misidentifications not only affect phylogenetic conclusions in GMEA but also may have important effects on the conservation status of different species. For instance, treating *B. caldasi* under a single widespread species such as *B. caucanus* would likely change the conservation status of *B. caldasi* to a less threatened category. Taxonomic changes like this will have major implications in future conservation actions that take place within the geographical ranges of different taxa (Lamprea, 2019).

In sum, I show that the inclusion of type material is critical for supporting the validity of potential taxonomic changes that are based on phylogenetic analysis. Nevertheless, I did not discuss whether the inferred phylogenetic relationships based on COI (Cytochrome c Oxidase I) are correct or not. This aspect should be addressed in future studies. The taxonomic sampling in Garcia-Melo et al. (2019) is inadequate for the proposed nomenclature changes (see also Reis et al. 2019). Several of the species potentially misidentified in Garcia-Melo et al. (2019), *B. andresoi*, *B. caldasi*, *B. galvisi*, *B. huilae*, *B. macarenae*, *B. orinocoense*, *H. cardalensis* and *H. raqueliae*, have type series deposited at the *Laboratorio de Ictiología de la Universidad del Quindío*, Armenia, Colombia (IUQ) (Román-Valencia et al., 2017a). These samples, along with others that may be of interest to researchers in the field, are available for future studies. Phylogenetic studies in the field should rely on the inclusion of type material from as many species as possible to avoid issues like those summarized in this document. Similarly, collaboration and cooperation among researchers may also help to alleviate the different problems like the ones highlighted in this article.

ACKNOWLEDGEMENTS

Cristian Román-Palacios and Heidi E. Steiner (University of Arizona, Tucson, USA) read versions of this paper and raised valuable suggestions and comments. Three anonymous reviewers read the final version of this work and made excellent suggestions and criticisms. C.R-V received support from the Universidad del Quindío, vice-rectory for research (grants 824 and 993).

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Editor Cientifico / Scientific Editor: David Veliz, UChile, Chile
Recebido / Recibido / Received: 09.08.2020
Revisado / Revised: 17.12.2020
Aceito / Aceptado /Accepted: 18.12.2020
Publicado / Published: 04.01.2021

Dados disponíveis / Datos disponibles / Available data: https://figshare.com/articles/dataset/Table_1_Geographical_distribution_of_Bryconamericus_Hemibrycon_and_Knodus_species_xlsx/13503390