JOSE LUIS POVEDA CUELLAR

FILOGEOGRAFIA MOLECULAR E CITOGENÉTICA DE POPULAÇÕES DE *Lasiancistrus caucanus* EIGENMANN, 1912 NA REGIÃO TRANSANDINA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Animal, para obtenção do título de *Magister Scientiae*.

Orientador: Jorge Abdala Dergam Dos Santos

Coorientador: Francisco Antonio Villa Navarro

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Assentimento:

Jose Luis Poveda Cuellar
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Jorge Abdala Dergam dos Santos
Orientador

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RESUMO

POVEDA-CUELLAR, Jose Luis, M.Sc., Universidade Federal de Viçosa, março de 2020. **Filogeografia molecular e citogenética de populações de *Lasiancistrus caucanus* (Eigenmann, 1912) na região transandina.** Orientador: Jorge Abdala Dergam dos Santos. Coorientador: Francisco Antonio Villa Navarro.

Lasiancistrus caucanus Regan, 1904 (Loricariidae, Hipostominae, Ancistrini) é uma espécie com distribuição disjunta na região transandina. *Lasiancistrus caucanus* possui cerca de seis espécies nominais, sendo que apenas uma é reconhecida como válida (*L. caucanus*). *L. planiceps, L. mayoloi* e *L. volcanensis* são sinônimos de *L. caucanus.* Esta espécie é considerada um complexo de espécies sem variações morfológicas. O objetivo deste estudo foi desenvolver uma avaliação molecular das relações evolutivas utilizado marcadores moleculares mitocondriais na região transandina e caracterização do cariótipo baseado na citogenética clássica (Giemsa e padrões heterocromáticos) e molecular (hibridização *in situ* fluorescente (FISH) de sondas repetitivas de DNA (CA_{15} e GA_{15}) de uma população da bacia do Alto Magdalena, na Colômbia. Nossos resultados indicaram que *L. caucanus* é uma unidade monofilética na região transandina. Foram validadas três espécies que ocorrem ao longo da bacia do rio Magdalena (*L. volcanensis*), rio Cauca (*L. caucanus*) e rio Tuira/Bayano (*L. planiceps*), historicamente reconhecidas no complexo *L. caucanus*. Apresentamos o primeiro estudo citogenético para o gênero na região transandina, indicando um número cromossômico diploide 2*n* = 54 (24m + 18sm + 12st) com a ocorrência de um cromossomo metacêntrico (primeiro par) maior que os demais cromossomos homólogos.

Palavras-chave: Citogenética. Filogenia. Loricariidae. Transandina.

ABSTRACT

POVEDA-CUELLAR, Jose Luis, M.Sc., Universidade Federal de Viçosa, March, 2020. **Molecular phylogeography and cytogenetic of *Lasiancistrus caucanus* populations (Eigenmann, 1912) in the Trans-Andean region.** Adviser: Jorge Abdala Dergam dos Santos. Co-adviser: Francisco Antonio Villa Navarro.

Lasiancistrus caucanus Regan, 1904 (Loricariidae, Hipostominae, Ancistrini) is a species with disjunct distribution in the trans-Andean region. *Lasiancistrus caucanus* has about six nominal species, of which only one is recognized as valid (*L. caucanus*). *L. planiceps*, *L. mayoloi* and *L. volcanensis* are synonymous with *L. caucanus*. This species is considered a complex of species without morphological variations. The aim of this study was to develop a molecular assessment of evolutionary relationships using mitochondrial molecular markers in the trans-Andean region and characterization of the karyotype based on classical (Giemsa and heterochromatic patterns) and molecular (fluorescent in situ hybridization (FISH) hybridization of DNA probes (CA15) and GA15) cytogenetic of a population of the Upper Magdalena, Colombia. Our results indicated that *L. caucanus* is a monophyletic unit in the trans-Andean region. Three species that occur along the Magdalena river (*L. volcanensis*), Cauca river (*L. caucanus*) and Tuira/Bayano river (L. planiceps) were recognized, historically assigned in the *L. caucanus* complex. We present the first cytogenetic study for the genus in the trans-Andean region, indicating a diploid chromosome number 2n = 54 (24m + 18sm + 12st) with the occurrence of a metacentric chromosome (first pair) larger than the other homologous chromosomes.

Keywords: Cytogenetic. Loricariidae. Phylogeny. Trans-Andean.

SUMÁRIO

1. INTRODUÇÃO GERAL

Alguns problemas taxonômicos ainda são inerentes a *Lasiancistrus* devido que a maioria das espécies foram descrita usando poucos exemplares e em alguns casos exemplares e localidades são imprecisos. O gênero *Lasiancistrus* Regan, 1904 (Loricariidae: Hypostominae: Ancistrini) é composto por seis espécies com distribuição cis e transandina. As especies validas do gênero *Lasiancistrus* são: *L. heteracanthus* (espécie tipo do gênero), *L. schomburgkii* (Günther, 1864), *L. caucanus* Eigenmann, 1912, *L. guacharote* (Valenciennes, 1840)**,** *L. saetiger* Armbruster, 2005 e *L. tentaculatus* Armbruster, 2005*.* Uma destas espécies, *Lasiancistrus caucanus*, é considerada um complexo de espécies com distribuição na região transandina (ARMBRUSTER, 2005). A taxonomia desta espécie foi avaliada por Armbruster, 2005 com base em caracteres morfológicos, sugerindo que as únicas diferenças interespecíficas são dadas pelo padrão de coloração e placas abdominais. Baseados nesta informação, foram sinonimizadas as seguintes espécies: *L. planiceps* Meek & Hildebrand, 1913, *L. mayoloi* (Eigenmann, 1912) e *L. volcanensis* Dahl, 1942 dentro de *L. caucanus* Eigenmann, 1912 (ARMBRUSTER, 2005).

Atualmente, existem poucos estudos integrativos na região transandina, por exemplo, focados no uso de dados moleculares e citogenéticos, tais como estudos de código de barras de DNA dado que a maioria se concentrou na região cisandina (MARIOTTO & MOREIRA-FILHO, 2008) e a nível da família Loricariidae (RODRIGUES et al., 2011; ROXO et al., 2012, LUJAN et al., 2014) Esses estudos integrativos são importantes como ponto de partida para a taxonomia (KEKKONEN & HEBERT, 2014) e são úteis no complexo de espécies (MARQUES et al., 2013) dos peixes neotropicais (ROXO et al., 2012; GARCÍA-MELO et al., 2019). O uso de diversas análises e técnicas, moleculares e citogéneticas, melhora a detecção objetiva de unidades taxonômicas com o reconhecimento de complexo de espécies ou espécies crípticas, importante para estabelecer padrões e estimar a diversidade das especies*.* Portanto, o objetivo desta pesquisa é testar a proposta de ARMBRUSTER, 2005 baseados em dados moleculares para esclarecer a taxonomia do grupo de peixes dessa região e caracterizar citogenéticamente uma população de *L. caucanus* da Quebrada Gualanday no Alto Magdalena, Colômbia.

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CHAPTER 1.

2. CHAPTER 1. CYTOGENETIC OF *Lasiancistrus caucanus* EIGENMANN, 1912 IN THE TRANS-ANDEAN REGION

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2.1 INTRODUCTION

The Ancistrini species, one of the five tribes in the Hypostominae subfamily, are found in the diversity of regions and streams in the Neotropical region. The most rich and derived species in Ancistrini is *Ancistrus* (ARMBRUSTER, 2004), with 59 species recognized (MARIOTTO & MOREIRA-FILHO, 2008). Other Ancistrini species is *Lasiancistrus* (Loricariidae: Hypostominae: Ancistrini) with 16 species assigned to it; however, only six are valid based morphological data, *L. schomburgkii* (Günther, 1864*)*, *L. caucanus* Eigenmann, 1912, *L. guacharote*, (Valenciennes, 1840) *L. heteracanthus* (Günther, 1869*)*, *L. tentaculatus* Armbruster, 2005 and *L. saetiger* Armbruster, 2005 (ARMBRUSTER, 2005). These species are distributed from the Amazon, Essequibo, Orinoco, Magdalena, San Juan, Atrato, Tuira, and Bayano drainages and the Lake Maracaibo drainage and only differences between the species are the degree of abdominal plating and coloration (ARMBRUSTER, 2005). *Lasiancistrus caucanus* Eigenmann, 1912 is only distributed in trans-Andean region in the Magdalena, Atrato, San Juan, Baudo, Tuira, and Bayano drainages of Colombia and Panamá (AMBRUSTER, 2005).

Some cytogenetic studies (MARIOTTO & MIYAZAWA, 2006; MARIOTTO et al., 2013; BUENO et al., 2018) have been performed on Hypostominae subfamily, most of which were focused on Hypostomini and Ancistrini, specifically on the genera *Ancistrus* and *Hypostomus*. These genera highlight for their variation from the proposed basal diploid number (2n = 54) for Loricariidae (ARTONI & BERTOLLO, 2001) with diploid numbers for *Hypostomus* (2*n* = 64 to 2*n* = 84) and *Ancistrus* (2*n* = 34 to 2*n* = 54) (OLIVEIRA et al., 2009; MARIOTTO et al., 2013) and other genera, the diploid number observed is 2*n* = 52 chromosomes (ARTONI & BERTOLLO, 2001; SOUZA et al., 2004).

In general, most studies on Hypostominae analyzed the diploid numbers and nucleolar organizer regions (NORs), however, microsatellites sites and the mapping of 5S,18S rDNA is still scarce (BUENO et al., 2018). To date, cytogenetics in Ancistrini is restricted to about 10% of the total species, with analysis of the genera *Ancistrus, Lasiancistrus, Hypancistrus, Peckoltia, Hemiancistrus* and *Panaque* (ARTONI &

BERTOLLO, 2001; DE OLIVEIRA et al., 2008; MARIOTTO & MOREIRA-FILHO, 2008; BUENO et al., 2018)

The study of Mariotto & Moreira-Filho (2008) was the first study that used cytogenetic data in genus *Lasiancistrus* distributed in the cis-Andean region to compared two populations (*L. schomburgkii and L.* sp.) of species from different Brazilian watersheds. Considering the diploid number in the Ancistrini tribe and chromosomal plasticity in the *Ancistrus* genus have shown the need for further studies on this important Loricariidae taxon, which have chromosomal polymorphisms, distinct sex chromosome systems and multiple karyotype formulas (BUENO et al., 2018). This approach has been shown to be useful for the detection of cryptic species (SOUZA-SHIBATTA, et al. 2013) and provide useful information for evolutionary studies and understanding of speciation mechanism.

This work constitutes the first cytogenetic characterization in trans-Andean Ancistrini species. The aims of the present study were (1) characterize the karyotype of a population of *Lasiancistrus caucanus* in Upper Magdalena using standard classical cytogenetic techniques and mapping the chromosomal distribution of different domains of repetitive DNA, including microsatellites $GA_{(15)}$ and $CA_{(15)}$; (2) compare the cis-Andean and trans-Andean species using cytogenetic data to obtain novel information about chromosomal evolutionary trends and karyotypic features within this genus.

2.2 MATERIALS AND METHODS

2.2.1 Sample collection

Twelve specimens of *Lasiancistrus caucanus* (four males and eight females) were collected from the Gualanday, Upper Magdalena Basin, Colombia (04°31’ 23.6"N 074°58’ 09.2"W). Voucher specimens were deposited in the zoological collection of the Universidad del Tolima, Colombia (collection No. CZUT-IC 12802). The collected specimens were anesthetized and euthanized with clove oil overdose (300 mg/L) (LUCENA, 2013) as approved by the Universidade Federal de Viçosa Ethics Committee (permit 20405273444).

2.2.2 Cytogenetic analyses

Mitotic stimulation *in vivo* for all individuals was induced by intramuscular application of yeast (LEE & ELDER, 1980). After this, was anterior kidney removed, placed in Hank's solution with colchicine (0,0125%) and mitotic chromosomes were obtained from anterior kidney cell suspension, following Bertollo et al., 1978. Stained by conventional staining (Giemsa 10%) and classified according to their arm ratio (longer chromosome arm/shorter chromosome arm) in metacentrics (1,00–1,69), submetacentrics (1.70–2.99) and subtelocentrics (3,00–6,99) following Levan et al. (1964).

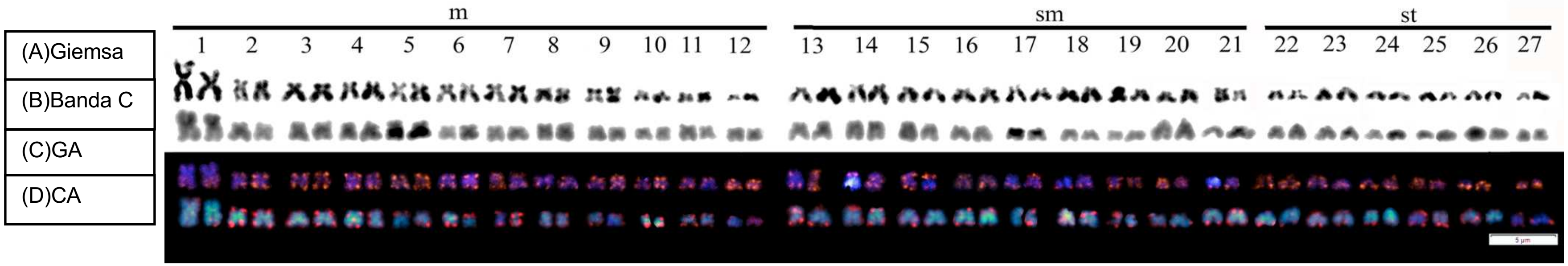
The regions of constitutive heterochromatin were evidenced using C-banding procedure (Summer, 1972). Repetitive DNA domains were characterized using microsatellite $GA_{(15)}$ and $CA_{(15)}$ probes labeled with Cy3 fluorochrome at the 5' end (Sigma). Distributions patterns of microsatellite DNA were mapped using flourecense *in situ* hybridization (FISH) fllowing Pinkel et al. (1986) with modification. The images of metaphases were obtained with a Olympus BX53 microscope with Olympus CellSens Imaging Software and measured using Image Pro Plus$^{@}$ software.

2.3 RESULTS

The analysis of mitotic metaphase cells of *L. caucanus* in the Gualanday Stream, Upper Magdalena Basin, revealed a diploid number of $2n = 54$ chromosomes with a fundamental number (FN)=92, composed of 24m+18sm+ 12st with the same chromosome composition between in females and males (Fig. 1A). In all metaphases was possible to observe the occurrence of a larger metacentric chromosome than its homologous forming the first pair (Fig. A)

The heterochromatic blocks were distributed at a metacentric pair 5 and submetacentric pair 17 revealing a conspicuous C-band at the terminal region on long and short arms, respectively (Fig. 1B). Repetitive DNA domains were characterized using microsatellite $GA_{(15)}$, $CA_{(15)}$ and were revealed preferentially localized in the terminal regions (Fig 1C-D).

Figure 1. A. Giemsa-stained karyotype; **B.** heterochromatic patterns (Banda C); **C-D** microsatellite GA$_{(15)}$, CA$_{(15)}$ of *Lasiancistrus caucanus*; M, metacentric; SM, submetacentric; ST, subtelocentric. In blue: chromosomes stained with DAPI. In red: regions hybridized with probes



Fonte: Autor

2.4 DISCUSSION

Loricariidae family, although with only 10% of species with any cytogenetic published data (KAVALCO et al., 2005), is characterized by a great karyotypic diversity with diploid number from 34 (Hypostominae) to 96 chromosomes (Delturinae) (OLIVEIRA et al., 2009). The diploid number of 2*n* = 54 chromosomes represents a plesiomorphic condition for this group of fish (ARTONI & BERTOLLO, 2001; KAVALCO et al., 2005; ALVES et al., 2006). However, diploid number by Ancistrini is 2*n* = ≤ 54, indicating probably the presence of chromosomal rearrangements in the chromosomal evolution of this taxon (OLIVEIRA et al., 2009; MARIOTTO et al., 2011). In this tribe, the most frequent diploid number is 2*n* = 52 chromosomes. With the exception of *Ancistrus* (2*n* = 34 - 54) and *Lasiancistrus* (2*n* = 54) associated to a reduction in the diploid number (MARIOTTO & MOREIRA-FILHO, 2008; OLIVEIRA et al., 2008, 2009). All this chromosomal variability found in the karyotypic formulae is promoted specially by pericentric inversions (ARTONI & BERTOLLO, 2001; SOUZA et al., 2004; ALVES et al., 2006; BUENO et al., 2012; ZIEMNICZAK et al., 2012).

Additionally, among the Ancistrini, *Ancistrus* has surprisingly high chromosomal diversity, for example, it has five of the seven mechanisms of sex chromosomes and a diverse chromosomal composition. The other genera and species of Loricariidae seem to maintain conserved chromosomal characteristics in relation to the genus *Ancistrus* (MARIOTTO & MOREIRA-FILHO, 2008). Furthermore, chromosomal rearrangements like deletions, fusions, inversions, duplications and heterocromatinization may contribute in the differentiation of the group and may be associated to morphological speciation processes (ARTONI & BERTOLLO, 2001; MILHOMEM et al., 2010; BUENO et al., 2012).

According to Ziemniczak (2011), this great karyotypic diversity may have an important role in the genetic and reproductive isolation of the Ancistrini species. *L. caucanus* presented 2*n* = 54, which was already verified in species such as *L. schomburgkii*, *Ancistrus claro*, *Pogonopoma wertheimeri*, *Rhinelepis aspera* and other (BUENO et al., 2018), corroborating the hypothesis of 2*n* = 54 on the ancestral diploid number in

Loricariidae. In all metaphases was possible to observe the occurrence of a larger metacentric chromosome than its homologous forming the first pair and similar to that observed in *L. shomburgkii* and *L.* sp of cis-Andean region (MARIOTTO & MOREIRA-FILHO, 2008). In general, there are differences in the karyotypic composition and heterochromatic patterns between the Cis and Trans Andean species.

As for the heterochromatin, there were some blocks distributed at a metacentric pair 5 and submetacentric pair 17 revealing a conspicuous C-band at the on long (pair 5) and short (pair 17) arms. A similar pattern of C-banding was observed in *L. schomburgkii* and *L.* sp of trans-Andean region where pairs 11(m) and 21(sm) also presented on almost completely heterochromatic arm (MARIOTTO & MOREIRA-FILHO, 2008), which could be a general pattern for the genus. According to Ziemniczak et al. (2012) and Gold et al. (1990) the absence of large and numerous blocks of heterochromatin may be a plesiomorphic character state in Loricariidae. This characteristic may be proven by comparing the basal genera with the derived ones (ARTONI &BERTOLLO, 2001; MARIOTTO et al., 2009; TRALDI et al., 2012). Large heterochromatic blocks are found in two or more chromosomal pairs in the genera *Scobinancistrus*, *Hypancistrus* and mainly in *Peckoltia* (SOUZA et al., 2009; CARDOSO et al., 2013; SILVA et al., 2014). This characteristic seems to be common to the Ancistrini and corroborates the suggestion proposed by Ziemniczak et al. (2012).

In Siluriformes, molecular characterization using microsatellites sites and mapping of the ribosomal genes 18S, 5S is still incipient (KAVALCO et al., 2004; CENTOFANTE et al., 2006; MENDES-NETO et al., 2011). For *Lasiancistrus caucanus*, this is the first record of molecular characterization of the microsatellites in the trans-Andean region. Microsatellites are usually located in the heterochromatic regions (telomeres/centromeres) of fish genomes, where a significant fraction of repetitive DNA is localized (CIOFFI & BERTOLLO, 2012). Indeed, this distribution pattern is found in some Siluriformes such as in the Neotropical catfishes *Rineloricaria latirostris* (Boulenger, 1900) (Loricariidae), *Imparfinis schubarti* (Gomes, 1956) (Heptapteridae) and *Steindachneridion scriptum* (Miranda-Ribeiro, 1918) (Pimelodidae) in which a remarkable accumulation of both $GA_{(15)}$ and $A_{(30)}$ microsatellites in telomeric regions occurs (VANZELA et al., 2002). A similar distribution is also present in the chromosomes of *Hoplias malabaricus* (Bloch, 1794), where 12 different microsatellite repeats, including $CA_{(15)}$ and $GA_{(15)}$, showed strong hybridization signals at subtelomeric and heterochromatic regions of several autosomes (CIOFFI et al., 2011) and the zebrafish, *Danio rerio* (Hamilton- Buchanan, 1822), showing CA(n) and GT(n) repeats clustered in the centromeric and telomeric regions (SHIMODA et al., 1999).

The *in situ* localization of both microsatellites $CA_{(15)}$ and $GA_{(15)}$ in the *L. caucanus* analyzed was very similar to *Rineloricaria latirostris* (VANZELA et al., 2002), being abundantly distributed on all chromosomes. Perhaps, comparative analyses among the *Lasiancistrus* species will indicate that the microsatellites have also preferential zones of accumulation at telomeric heterochromatin as is the case of naked catfishes since this particular pattern corroborates the idea that the large chromosome pair was originated through centric fusion or Robertsonian translocation during chromosomal evolution (SUPIWONG et al. 2014).

In general, *L. caucanus* conserves the karyotipic macrostructure of the Ancistrini. The maintenance of 2*n* = 54 chromosomes with a few heterochromatic blocks are evidences that this species occupies a basal position in the tribe. Our results can help to better understand the chromosomal evolution in this remarkable fish group, but the continuity of cytogenetic studies for the *Lasiancistrus* is indispensable for a better comprehension of the evolutionary trends.

A karyotypic diversity might result in great morphological diversity and color pattern in the species of Ancistrini in the Upper Magdalena. That species diversity represents an invaluable richness; therefore, it is important that there are efforts to understand the origin, evolution, behavior and ecology and the subsequent preservation of such diversity.

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CHAPTER 2

3. CHAPTER 2. PHYLOGENY AND SPECIES DELIMITATION BASED ON MOLECULAR APPROACHES ON THE GENUS *Lasiancistrus* (LORICARIIDAE: HYPOSTOMINAE: ANCISTRINI) WITH FOCUS ON *Lasiancistrus caucanus*.

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3.1 INTRODUCTION

The trans-Andean region is characterized by high levels of endemism and biodiversity (SCHAEFER, 2011), because the geomorphological history of watersheds are closely related to the diversification of freshwater fish (ALBERT & CARVALHO, 2011). Estimate biological biodiversity is especially a challenge within cryptic taxa (MELO et al., 2015; LUKHTANOV, 2019) and species complexes (BAGLEY et al., 2015; SERRANO et al., 2018) because indistinguishable morphological attributes and uninformative because speciation is not always accompanied by morphological differentiation (BICKFORD et al., 2006; ADAMS et al., 2014). In these cases, the use of additional molecular tools and methods are important to evaluate the diversity within taxonomically unresolved groups (BICKFORD et al., 2006; DE QUEIROZ, 2007; COSTA et al., 2014).

A general consensus among taxonomists is that an ‘integrative’ taxonomic (YEATES et al*.,* 2011) approach including multiple evidence (e.g., cytogenetic, behavioral, ecological, molecular, and morphological data) and multilocus phylogenetic inference are the best approaches for accurate species delimitation and understanding the phylogenetic relationships. The combined use of these allows estimating biodiversity in a more objective way (OTTONI et al., 2019). Analytical methods based on DNA barcoding allows delimit hundreds of putative species (i.e., operational taxonomic units, OTUs) (KEKKONEN & HEBERT, 2014; DUMAS et al., 2015) and are characterized for statistical robustness and population genetic inference based on coalescent theory (FUJITA et al., 2012) and distance-based methods (PUILLANDRE et al., 2012a). On the other hand, multilocus phylogenetic inference allow analyze several genes mitochondrial and nuclear simultaneously and test monophiletism and relationships between other species (SUÁREZ-VILLOTA et al., 2018; OTTONI et al., 2019).

The DNA based studies suggest that fauna of tropical region contain a large proportion of undescribed species, resulting in underestimated of species level diversity (BAGLEY et al., 2015), that have not been detected in approach based on morphology, especially freshwater fishes of the Order Siluriformes, which on characterized high levels of cryptic diversity (FERREIRA et al., 2014; CARDOSO et al., 2019; OCHOA et al., 2019). Within this region, the family Loricariidae is the most diverse family of catfishes, around 998 valid species (ESCHMEYER, 2019). These are inhabitants of lotic habitats throughout the Atlantic and Pacific slopes of South America and Central American drainages (LUJAN et al., 2014).

The loricariid systematics studies have been based on morphological and molecular data especially focused on Loricariidae family (ARMBRUSTER 2004; ROXO et al., 2019) and subfamilies (e.g., Neoplecostominae: ROXO et al., 2012; Loricariinae: COVAIN, 2011; RODRIGUES et al., 2011; Hypoptopomatinae: CHIACHIO et al., 2008; CRAMER et al., 2011; Hypostominae: LUJAN et al., 2014). In the most recent osteological phylogenetic analysis including *Lasiancistrus*, ARMBRUSTER (2004) recovered *Lasiancistrus maracaiboensis* (Schultz, 1944) *(ca. L. guacharote*) and *Lasiancistrus*. sp. as sister taxa, a group in turn sister to *Ancistrus* and *Pseudolithoxus*. Molecular phylogenetic research by Lujan et al. (2014), Collins et al. (2018) and Roxo et al. (2019) found support for the same intergeneric relationships found by Armbruster (2004). Silva et al. (2016) study of transcontinental dispersal, ecological opportunity and origins of an adaptive radiation in the Neotropical catfish genus *Hypostomus*, found monophyly of *Lasiancistrus* of cis and trans Andean region, however, this study was based only two samples of the *Lasiancistrus caucanus* Eigenmann, 1912. Until now, no study has tested the monophyly of the genus *Lasiancistrus* of cis and trans Andean regions including the most their species.

Sometimes, morphological and molecular conflict and homoplasy are much more controversial at finer taxonomic scales (e.g. genus *Pseudancistrus* comprising a single genus (ARMBRUSTER, 2008), or five genera (COVAIN & FISCH-MULLER, 2012). This dynamic is sometimes contradictory in the Hypostominae systematics, for example, Armbruster (2004, 2005) synonymized over a dozen Hypostominae genera, such is the case of genus *Lasiancistrus*. This genus (Loricariidae: Hypostominae: Ancistrini) is diagnosed by the unique presence of a ventral strut of the pterotic and the presence of whiskerlike odontodes on the snout. *Lasiancistrus* had about 16 species assigned to it; however, only six are valid based morphological data (*L. schomburgkii, L. caucanus*, *L. guacharote*, *L. heteracanthus, L. tentaculatus and L. saetiger). Lasiancistrus maracaiboensis* and *L. mystacinus* are synonyms of *L. guacharote*; *L. pictus*, *L. castelnaui*, *L. caquetae*, *L. guapore*, *L. multispinis*, and *L. scolymus* are synonyms of *L. schomburgkii* (Ambruster, 2005). These species are distributed from the Amazon, Essequibo, Orinoco, Magdalena, San Juan, Atrato, Tuyra, and Bayano drainages and the Lake Maracaibo drainage and only differences between the species are the degree of abdominal plating and coloration (AMBRUSTER, 2005).

Lasiancistrus caucanus have about three species assigned to it, *L. planiceps* Meek & Hildebrand, 1913 (Tuira, Panamá), *L. mayoloi* Eigenmann, 1912 (San Juan, Colombia) and *L. volcanensis* Dahl, 1942 (Volcán , tributary of Magdalena, Colombia) (ARMBRUSTER, 2005) with trans-Andean distribution in the Magdalena, Atrato, San Juan and Tuira drainages of Colombia and Panamá (ARMBRUSTER, 2005). Considering the issues facing the delimitation of species in *Lasiancistrus* as other genera such as *Pimelodella(*CONDE-SALDAÑA, 2016*)*, *Astroblepus* (OCHOA et al., 2019), *Chaetostoma*, *Rineloricaria* (COSTA-SILVA et al., 2015), *Bryconamericus*, *Eretmobrycon, Astyanax* and *Knodus* (GARCÍA-MELO et al., 2019) based on morphologic characteristics, the use of barcoding models (Generalized Mixed Yule Coalescent, GMYC; Poisson Tree Processes, PTP; and Automated Barcode Gap Discovery, ABGD) and complementarily multilocus molecular phylogeny would help to improve the understanding of true diversity the *L. caucanus* in the trans-Andean region and infer the biogeographic implications.

The aims of the present study were (1) test Ambruster, 2005's proposal taxonomic of genus *Lasiancistrus* performing a comprehensive molecular reappraisal of relationships and monophyly of cis and trans Andean regions by including the most representatives with emphasis on *L. caucanus*; (2) explore the molecular diversity of *Lasiancistrus caucanus* using species delimitation models based on genetic data with these established by morphology alone and to discuss species boundaries within the *L. caucanus*.

3.2 MATERIAL AND METHODS

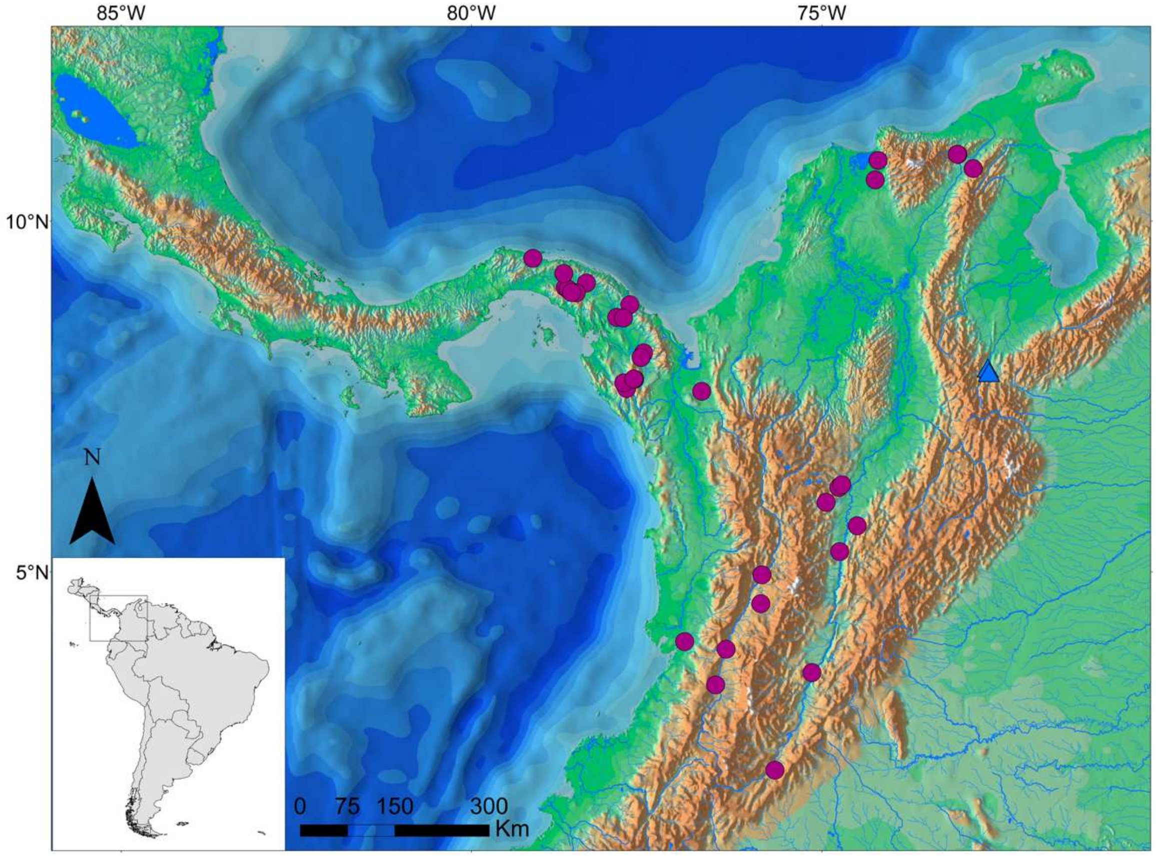
3.2.1 Study material and focal taxa

This study evaluated 96 *Lasiancistrus* tissues deposited in the fish collections of five institutions (Table 1, Fig 1) and were sequenced for barcoding and phylogeny. Sequences of type locality (La Vieja, Upper Cauca) of *Lasiancistrus caucanus* was used to delineate the specie in the resulting clusters. These species have trans and cis Andean distributions (i.e., Bayano, Tuira, San Juan, Atrato, Baudo, Magdalena-Cauca, Orinoco, Amazonas, and Maracaibo lake among others); the remaining sequences correspond to closely related genera such as *Ancistrus*.

3.2.2 DNA Extraction and Sequencing

Total genomic DNA was isolated from muscle and fin tissues of each specimen with a DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. Amplifications were performed in a total volume of 20 µl with 2 µl of 10X buffer (10 mM Tris-HCl); 0.4 µl dNTPs (20 mM of each); 1 µl each of 10 mM primer (FishF1-59, FishR1-59,Glu-5, CB2-3), 1 µl of 1 U Platinum Taq Polymerase (Invitrogen/Phoneutra), and 1 µl template DNA (aprox 10 ng); and 13.6 µl ddH2O. The PCR reactions consisted of 35–40 cycles for 3 min at 94° C, 1 min at 58–59° C, and 1 min at 72° C.

Figure 2. Distributions of *Lasiancistrus caucanus* (purple circle) and *Lasiancistrus guacharote* (blue triangle) specimens examined in this study in South America.



Fonte: Autor

All PCR products were first visually identified on a 1% agarose gel and then purified using Purification Tissue Kit (Qiagen), following the manufacturer's instructions, and the purified PCR products were sequenced using a Big Dye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems), purified again by ethanol precipitation and the sent for sequencing to the Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Table 1. *Lasiancistrus* species, voucher catalog number, country and drainage of origin for the tissue samples analyzed in this study and summary of previously published data downloaded from GenBank and Bold. CZUT-IC = Colección Zoologica, sección Ictiología de la Universidad del Tolima, Colombia; STRI = Tropical Research Institute of Smithsonian, Panamá; ANSP = The Academy of Natural Sciences of Drexel University; ROM = Royal Ontario Museum, Canada.

Taxa	Tissue #	Voucher code	Type locality	COI	CYTB	Location (River)	Drainage (River)	Pendiente	Country
Siluriformes									
Loricariidae (Ancistrini)									
Lasiancistrus caucanus	CZUT2570	CZUT103		x	x	Quebrada La Tebaida	Middle Magdalena	Atlántico	Colombia
	CZUT2640	CZUT173		x	x	Samana-Puerto Garza	Middle Magdalena	Atlántico	Colombia
	CZUT2656	CZUT189		x	x	Nare	Middle Magdalena	Atlántico	Colombia
	CZUT2026	CZUTNJ10		x	x	Melendez	Upper Cauca	Atlántico	Colombia
	CZUT2064	CZUTNJ48		x	x	Calima	San Juan	Pacífico	Colombia
	CZUT2187	CZUTNJ171		x	x	Mediacanoa	Upper Cauca	Atlántico	Colombia
	CZUT2188	CZUTNJ172		x	x	Mediacanoa	Upper Cauca	Atlántico	Colombia
	CZUT2189	CZUTNJ173		x	x	Mediacanoa	Upper Cauca	Atlántico	Colombia
	CZU2279	CZUTNJ263		x	x	Suaza	Upper Magdalena	Atlántico	Colombia
	CZUT2280	CZUTNJ264		x	x	Suaza	Upper Magdalena	Atlántico	Colombia
	CZUT1773	CZUTAG122		x	x	Rancheria	Rancheria	Atlántico	Colombia
	CZUT1758	CZUTAG107		x	x	Rancheria	Rancheria	Atlántico	Colombia
	CZUT1759	CZUTAG108		x	x	Rancheria	Rancheria	Atlántico	Colombia
	CZUT1726	CZUTAG75		x	x	Quebrada El Limon	Rancheria	Atlántico	Colombia
	CZUT1824	CZUTAG173		x		Manantial Cañaverales	Rancheria	Atlántico	Colombia
Continuation	CZUT1825	CZUTAG174		x	x	Manantial Cañaverales	Rancheria	Atlántico	Colombia

Taxa	**Tissue #**	**Voucher code**	**Type locality**	**COI**	**CYTB**	**Location**	**Drainage (River)**	**Pendiente**	**Country**
Lasiancistrus caucanus	CZUT1614	CZUTL37		x	x	Guarinó	Middle Magdalena	Atlántico	Colombia
	CZUT1615	CZUTL38		x	x	Guarinó	Middle Magdalena	Atlántico	Colombia
	CZUT3006	CZUT11214		x	x	Chirina	Lower Magdalena	Atlántico	Colombia
	CZUT1408	CZUT409			x	Anchique	Upper Magdalena	Atlántico	Colombia
	CZUT817	CZUT817		x	x	Frío	Lower Magdalena	Atlántico	Colombia
	CZUT1857	CZUT14	†	x	x	La Vieja	Upper Cauca	Atlántico	Colombia
	CZUT1858	CZUT15	†	x	x	La Vieja	Upper Cauca	Atlántico	Colombia
	CZUT265	CZUT265				León	Atrato-Leon Caribe	Atlántico	Colombia
	CZUT1826	CZUTAG175				Manantial Cañaverales	Rancheria	Atlántico	Colombia
	CZUT1664	CZUTAG13		x		Rancheria	Rancheria	Atlántico	Colombia
	CZUT1613	CZUTL36		x	x	Guarinó	Middle Magdalena	Atlántico	Colombia
	CZUT1773	CZUTAG122		x	x	Rancheria	Rancheria	Atlántico	Colombia
	STRI1647			x	x	Mandinga	Mandinga	Atlántico	Colombia
	STRI1646				x	Mandinga	Mandinga	Atlántico	Colombia
	STRI1645			x		Mandinga	Mandinga	Atlántico	Colombia
	STRI11806			x	x	Aguas Claras	Bayano	Pacífico	Colombia
	STRI3526			x	x	Chucunaque	Tuira	Pacífico	Colombia
	STRI2675			x		Q. Upper Bayano	Bayano	Pacífico	Colombia
	STRI3564			x		Chucunaque	Tuira	Pacífico	Colombia
	STRI4096			x	x	Yape	Tuira	Pacífico	Colombia
	STRI4097			x	x	Yape	Tuira	Pacífico	Colombia
	STRI4127			x	x	Capeti	Tuira	Pacífico	Colombia
	STRI4130			x	x	Capeti	Tuira	Pacífico	Colombia
Continuation	STRI908			x	x	Parti	Bayano	Pacífico	Colombia

Taxa	**Tissue #**	**Voucher code**	**Type locality**	**COI**	**CYTB**	**Location (River)**	**Drainage (River)**	**Pendiente**	**Country**
Lasiancistrus caucanus	STRI6952			x	x	Coasi	Tuira	Pacífico	Colombia
	STRI6961			x	x	Balsas	Tuira	Pacífico	Colombia
	STRI26916			x	x	Piriati	Bayano	Pacífico	Colombia
	STRI26952			x	x	Curti	Bayano	Pacífico	Colombia
	STRI6708			x	x	Membrillo_Sinai	Tuira	Pacífico	Colombia
	STRI6707			x	x	Membrillo_Sinai	Tuira	Pacífico	Colombia
	STRI11578			x	x	Cana	Tuira	Pacífico	Colombia
	STRI11688			x	x	Seteganti	Tuira	Pacífico	Colombia
	STRI11689			x		Seteganti	Tuira	Pacífico	Colombia
	STRI26917			x		Piriati	Bayano	Pacífico	Colombia
	STRI907			x	x	Parti	Bayano	Pacífico	Colombia
	STRI3646			x	x	Ipeti	Bayano	Pacífico	Colombia
	STRI11810			x		Aguas Claras	Bayano	Pacífico	Colombia
	STRI26915			x	x	Piriati	Bayano	Pacífico	Colombia
	CIUA8557			BOLD		Risaralda	Cauca	Atlántico	Colombia
	CIUA8558			BOLD		Risaralda	Cauca	Atlántico	Colombia
	CIUA8864			BOLD		San Diego Lake	Middle Magdalena	Atlántico	Colombia
Lasiancistrus guacharote	CZUT2734	CZUT30		x	x	Peralonso	Zulia	Atlántico	Colombia
	CZUT2871	CZUTLGT10		x	x	NNTabiro Creek	Zulia	Atlántico	Colombia
Continuation	CZUT2722	CZUT18		x	x	Peralonso	Zulia	Atlántico	Colombia

Taxa	Tissue #	Voucher code	Type locality	COI	CYTB	Location (River)	Drainage (River)	Pendiente	Country
Lasiancistrus schomburgkii	CZU2426	CZUTNJ410		x	x	Orteguaza	Caqueta	Atlántico	Colombia
	CZUT2425	CZUTNJ409		x		Orteguaza	Caqueta	Atlántico	Colombia
	CZUT2327	CZUTNJ311		x		Orteguaza	Caqueta	Atlántico	Colombia
	CZUT2328	CZUTNJ312		x	x	Orteguaza	Caqueta	Atlántico	Colombia
	ANSP9579	ANSP9579		x	x	Ventuari	Orinoco	Atlántico	Colombia
	ANSP11071	ANSP11071		x	x	Ireng	Negro	Atlántico	Colombia
	ANSP5665	ANSP5665		x	x	Penatecaua	Xingu	Atlántico	Colombia
	ANSP11066	ANSP11066		x	x	Yuora	TakutuBranco	Atlántico	Colombia
	ANSP5664	ANSP5664		x	x	Penatecaua	Xingu	Atlántico	Colombia
	P6125	AUM45548		GenBank		Marañon	Amazon	Atlántico	Colombia
		ANSP199692				Jarauacu	Xingu	Atlántico	Brazil
Lasiancistrus tentaculatus	ROMT9014	ROMT9014		x	x	Tucupido	Tamasco	Atlántico	Venezuela
	ROMT9017	ROMT9017		x	x	Tucupido	Tamasco	Atlántico	Venezuela
	T09686	AUM53895		GenBank		Ventuari	Orinoco	Atlántico	Venezuela
Ancistrus bolivianus	T12872	ROMT90368		GenBank		Mamoré	Madeira	Atlántico	Bolivia
Ancistrus chagresi		STRI1377		GenBank		Cocle del Norte	Atlántico	Atlántico	Panamá

3.2.3 Sequencing analysis

Consensus sequences from forward and reverse strands were obtained using Geneious Pro 4.8.5 software (DRUMMOND, 2012). Sequence data were assembled, edited, aligned and concatenated following the methods of Lujan et al. (2015). Alignments were generated using Muscle (EDGAR, 2004) under default parameters. The matrix was visually checked to detect any obvious misalignments and potential cases of sequencing errors; the presence of stop codons was checked using Geneious Pro 4.8.5. Nucleotide variation, substitution patterns and genetic distances were examined using the Mega X software. The best nucleotide evolution models for the Cytochrome Oxidase Unit 1 (COI-barcoding models) and other genes were evaluated using Modeltest 3.06 (POSADA & CRANDALL, 1998) under the information theoretical Akaike information criterion (AIC). To find the best partition scheme we used PartitionFinder v 1.1.0 (LANFEAR et al., 2012) was used to determine codon-specific models of molecular evolution for each gene under the Bayesian information criterion (BIC).

3.2.4 Phylogenetic analyses

Phylogenetic analysis of the concatenated alignment was conducted using both Bayesian Inference (BI) and Maximum Likelihood (ML) methods with *Ancistrus* sp. (Loricariidae) designated as the outgroup. A Bayesian Markov chain Monte Carlo search of tree space was conducted using MrBayes (v3.2.2; RONQUIST & HUELSENBECK, 2003) on the CIPRES supercomputing cluster (MILLER et al., 2010). MrBayes was programmed to run for 50 million generations using eight chains (nchain = 8; i.e., two parallel runs with 4 cold and 4 hot chains each; temperature parameter set to default), sampling every 1500 trees with the first 25% of trees being discarded as burnin. The Bayesian search was determined to have reached stationarity when of effective sample size for all metrics exceeded 200 as determined in the Tracer software (v1.6; RAMBAUT et al., 2007). Maximum likelihood analysis was conducted using Mega X software and was generated bootstrap support values based on a 1000 generation search of tree space.

3.2.5 Species delimitation and recognition of putative species

Were applied three methods of species delimitation, including two coalescent-based methods, the generalized mixed Yule-coalescent (GMYC) method (PONS et al., 2006) and Poisson tree processes (PTP) (ZHANG et al., 2013), and one distance-based method, automatic barcode gap discovery (ABGD) (PUILLANDRE et al., 2012).

We estimated a ultrametric tree using a lognormal relaxed molecular clock and a K2P model in BEAST v.1.8.4 (DRUMMOND et al., 2007) with a gamma distribution (estimated by the program Modeltest 3.06). Bayesian inference of phylogeny with a relaxed lognormal clock and a Yule-Process speciation process rate on an arbitrary timescale was applied, and a random UPGMA tree was used as the starting tree for the Markov chain Monte Carlo searches. The length of the MCMC chain was 30.000.000 with sampling every 1.0000. The ESS values and trace files of the runs were evaluated using Tracer v1.6.0 (RAMBAUT et al., 2007), and the maximum clade credibility tree was built from the combined runs after eliminating 25% of the trees for burn-in in TreeAnnotator v1.7.2. The GMYC approach was carried out in GYMC webserver under the ‘single threshold’ model (PONS et al., 2006). In the Poisson tree processes (PTP) the Yule-coalescent transition points are modeled based on the change in substitution rates on the phylogenetic input tree. We used the ML tree as input data, and the calculations were conducted on the bPTP webserver with 500.000 MCMC generations, thinning set to 100, burn-in at 10%, and a Bayesian search.

The ABGD analysis was performed on the ABGD website with P (prior intraspecific divergence) set from 0.001 to 0.1 (which refers to the area were the barcode gap should be detected); Steps set to 10; X (minimum relative gap width, i.e., the sensitivity of the method to gap width) set to 1.5; and Nb bins (for distance distribution) set to 20. The gap could be considered the threshold of the upper limit of intraspecific distances and the lower limit of interspecific distances. We selected the Kimura (K80) model and only considered the outcome of the initial ABGD partition (PUILLANDRE et al., 2012a).

3.3 RESULTS

3.3.1 Species delimitation

After alignment and editing, the final matrix had 576 nucleotides, of which 436 positions were conserved and 140 were variable. Base composition for this fragment (COI) was 25% adenine, 28.3% cytosine, 29% thymine and 17.2% guanine. We analyzed barcode and sequences for 95 specimens with more than 550 base pairs.

We observed that the mean Kimura 2 Parameter (K2P) genetic distance within each group was 0.03%, smaller than the mean distance observed among groups (10%) (Table 2). The genetic distance observed among the groups were mostly quite high, *L. caucanus* that have three clades with genetic differentiation between lineages varies from 3% to 4% (Table 2). With genetic distance analyses, it was possible to discriminate eight lineages from the trans-Andean region within of *L. caucanus* (Table 2, Fig. 2), such as Lineage 1 (Upper Magdalena), Lineage 2 (Middle Magdalena), Lineage 3 (Rancheria), Lineage 4 (Upper Cauca), Lineage 5 (Lower Magdalena), Lineage 6 (Bayano), Lineage 7 (Tuira) and Lineage 8 (Calima) with genetic distance observed among the groups higher than 2%.

Table 2. Pairwise K2P genetic distance values among drainage-based species of *Lasiancistrus*. Bold numbers represent intraspecific genetic variation.

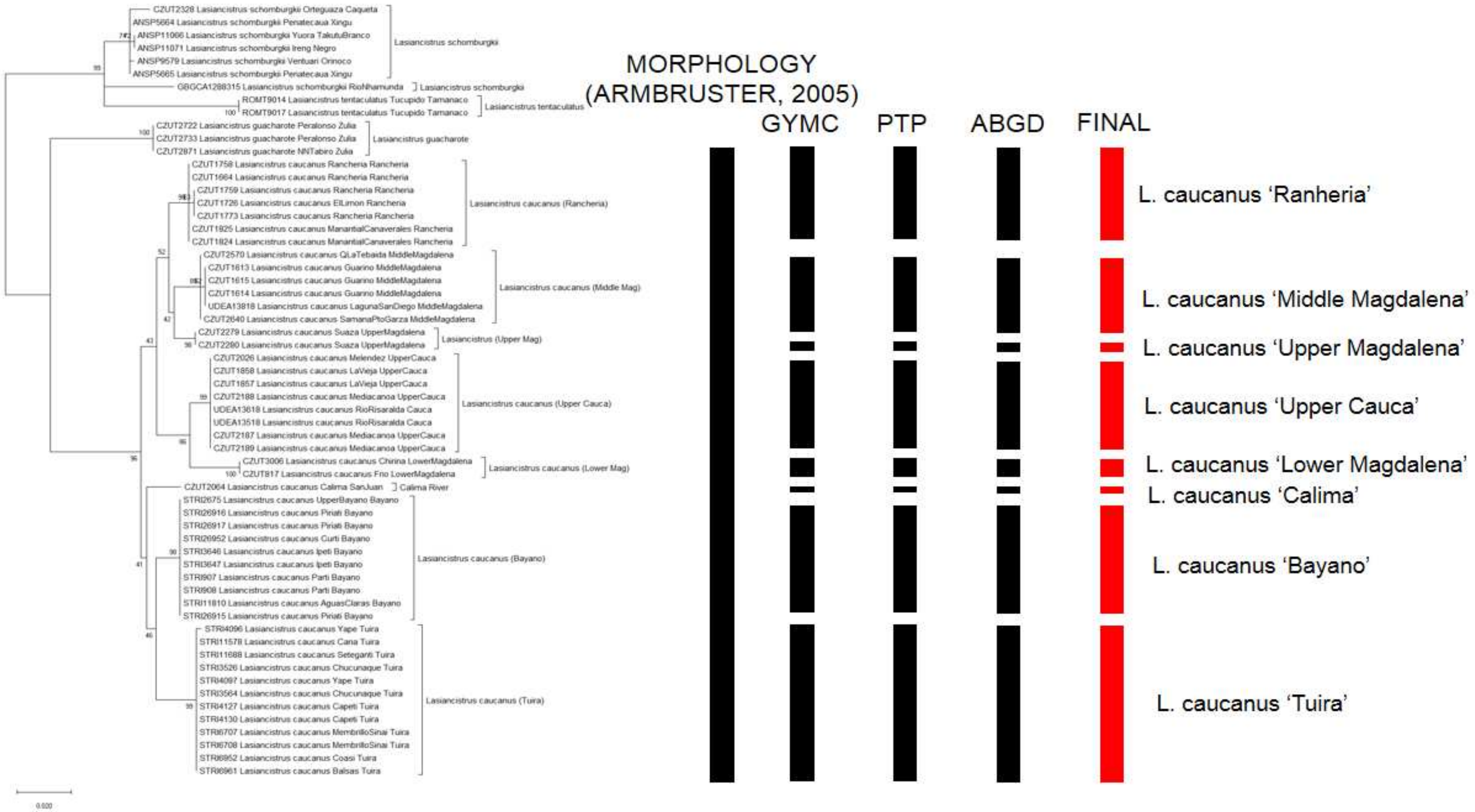
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1. *Lasiancistrus schomburgkii*	**0.016 ± 0.005**										
2. *Lasiancistrus caucanus* (Middle Magdalena)	0.103 ± 0.015	**0.01 ± 0.004**									
3. *Lasiancistrus caucanus* (Rancheria)	0.090 ± 0.013	0.022 ± 0.007	**0.001 ± 0.001**								
4. *Lasiancistrus caucanus* (Upper Cauca)	0.091 ± 0.014	0.038 ± 0.009	0.030 ± 0.008	**0.00 ± 0.00**							
5. *Lasiancistrus caucanus* (Calima)	0.091 ± 0.014	0.038 ± 0.009	0.034 ± 0.008	0.029 ± 0.008	**0.00 ± 0.00**						
6. *Lasiancistrus caucanus* (Upper Magdalena)	0.100 ± 0.015	0.016 ± 0.006	0.020 ± 0.007	0.036 ± 0.009	0.035 ± 0.008	**0.01 ± 0.004**					
7. *Lasiancistrus guacharote* (Maracaibo)	0.078 ± 0.013	0.081 ± 0.015	0.077 ± 0.014	0.077 ± 0.014	0.071 ± 0.012	0.081 ± 0.014	**0.00 ± 0.00**				
8. *Lasiancistrus caucanus* (Lower Magdalena)	0.109 ± 0.016	0.037 ± 0.009	0.042 ± 0.010	0.027 ± 0.008	0.044 ± 0.010	0.039 ± 0.010	0.097 ± 0.016	**0.00 ± 0.00**			
9. *Lasiancistrus tentacualtus*	0.056 ± 0.010	0.106 ± 0.017	0.093 ± 0.015	0.098 ± 0.015	0.092 ± 0.014	0.100 ± 0016	0.090 ± 0.014	0.100 ± 0.016	**0.027 ± 0.135**		
10. *Lasiancistrus caucanus* (Tuira)	0.100 ± 0.015	0.026 ± 0.007	0.034 ± 0.009	0.041 ± 0.010	0.030 ± 0.008	0.030 ± 0.008	0.077 ± 0.014	0.045 ± 0.010	0.108 ± 0.016	**0.00 ± 0.00**	
11. *Lasiancistrus caucanus* (Bayano)	0.092 ± 0.015	0.035 ± 0.009	0.037 ± 0.009	0.040 ± 0.010	0.023 ± 0.007	0.037 ± 0.009	0.071 ± 0.013	0.052 ± 0.012	0.099 ± 0.016	0.099 ± 0.007	**0.00 ± 0.00**

3.3.2 Congruence among methods

The numbers of putative species delimited for *L. caucanus* was highly congruent for all three methods. For the given dataset, GMYC, PTP and ABGD yield almost identical results. Nevertheless, the congruence of groupings is very high among the three methods (100%).

Species delimitation analyses using the GMYC model estimated under a Yule prior of branching rates shows a threshold time of 0.005, which indicates the time before which all nodes reflect diversification events and after which all nodes in the tree reflect coalescent events. The maximum likelihood for the null model was 314.265, and the maximum likelihood for the GMYC model was 312.901. A single-threshold model from GMYC suggests the presence of eight species with a confidence interval of 6–10 species. As such, the number of species of *L. caucanus* under this model was the same to than the one obtained using the K2P pairwise distance (Table 2) and ML analysis (Fig. 2; APÊNDICE B). The threshold shows putative species splits within *L. caucanus*: Upper Cauca (*Sensu Stricto*), Lower Magdalena, Upper Magdalena, Middle Magdalena, Rancheria, Calima, Bayano and Tuira. For the PTP and ABGD procedures, 8 putative species clusters were inferred for the analyses based on the best-fit ML tree and “barcoding gap” within *L. caucanus*, respectively (Fig. 2).

Figure 3. Maximum Likelihood (ML) phylogenetic tree of *Lasiancistrus* obtained with COI data. The values in the node branches represent bootstraps. The first four black vertical columns represent, respectively, the status of the identification of species delimitation using the Morphologhy (Armbruster, 2005), GMYC, PTP and ABGD, while the fifth column represents the final species delimitation.



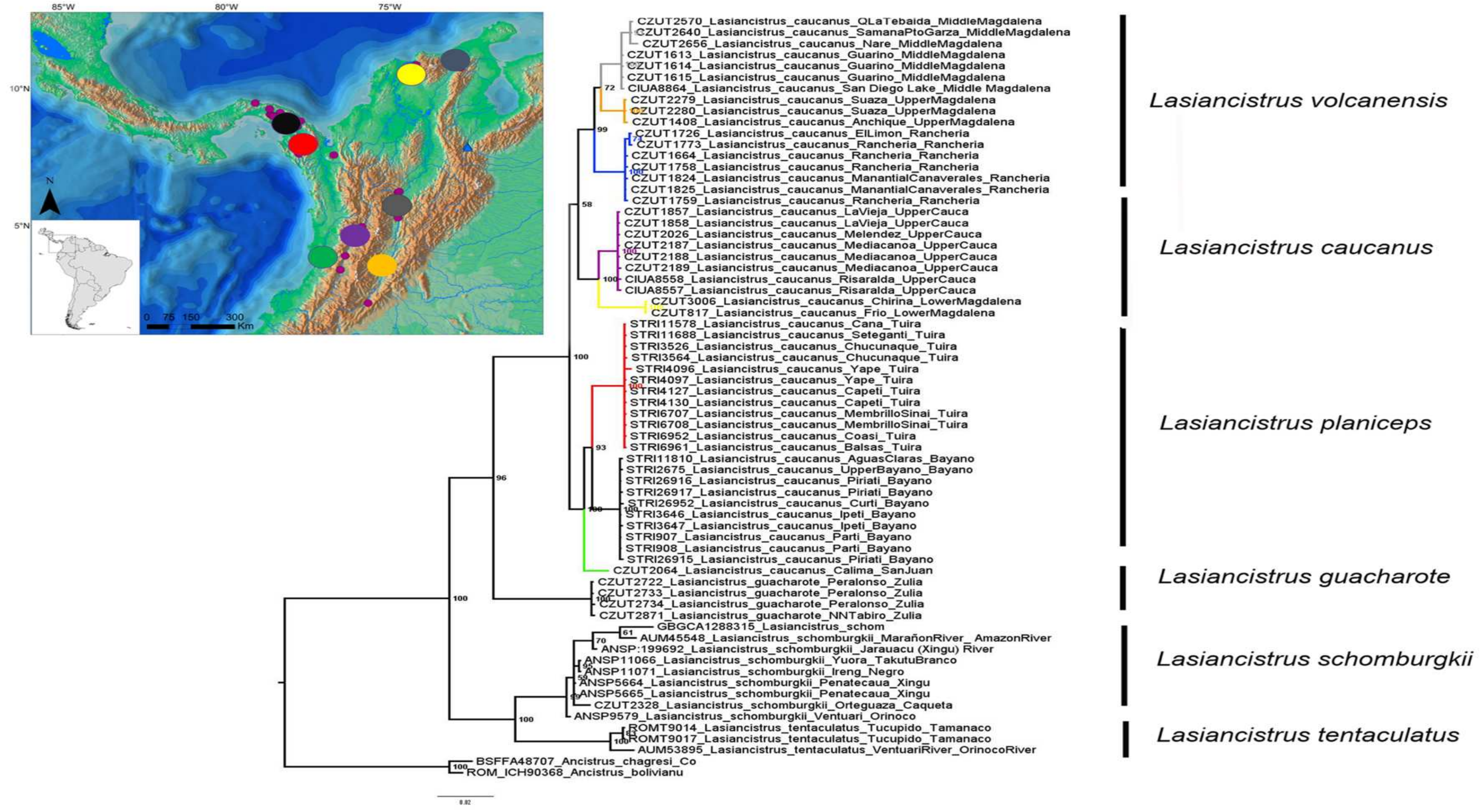
Fonte: Autor

3.3.3 Phylogenetic analyses

Relationships among sequences were represented by ML and BI trees (Fig. 2-3). The monophyly of the *Lasiancistrus* trans-Andean (*L. caucanus* and *L. guacharote*) and cis-Andean (*L. tentaculatus* and *L. schomburgkii*) species were supported with maximum node support value (Fig. 3). The sister clade of the *L. caucanus* was the clade comprising *L. guacharote* Maracaibo Basin supported by 97% of posterior probability (Fig. 3; APÊNDICE A). *L. caucanus* is specie monophyletic. Nevertheless, several strongly supported and monophyletic lineages emerge, most of them with long branches and concordant with patterns of drainage basin isolation generated from geological events.

Species delimitation methods supported the same eight lineages within the *L. caucanus* group. However, using hypothetical relationship of *L. caucanus* in the trans-Andean region based on multilocus bayesian inference (Fig. 3) we propose the definition of three main clades: first, *Lasiancistrus volcanensis* occurring along the La Tebaida, Samaná, Náre and Guarinó rivers (Middle Magdalena), Suaza river (Upper Magdalena) and El Limon Creek, Manantial Cañaverales and Rancheria rivers (Rancheria). Second, *Lasiancistrus caucanus 'Sensu Stricto'* occurring along the Frio and Chirina rivers (Lower Magdalena) and La Vieja (type locality), Mediacanoa and Melendez rivers (Upper Cauca). Third, *Lasiancistrus planiceps* distributed along the Upper Bayano, Parti, Ipeti, Curti, Piriati, Aguas Claras river (Bayano) and Chucunaque, Yape, Capeti, Coasi, Balsas, Membrillo Sinai, Cana, Seteganti rivers (Tuira). The Calima River is probably another specie, but we need to increase the representativeness of the Colombian Pacific to define its validity. Apparently, within the three clades there are other new species that are being examined with morphological data. The present study recognizes eight lineages (species) within the *L. caucanus* species group, depending on the species delimitation method, grouped into three distinct and highly supported clades of trans-Andean region: *L. volcanensis*, *L. caucanus* and *L. planiceps*.

Figure 4. Hypothetical relationship of *L. caucanus* in the trans-Andean region, based on multilocus bayesian inference; node numbers indicate posterior probability values



Fonte: Autor

3.4 DISCUSSION

3.4.1 Species delimitation and species diversity

The genus *Lasiancistrus* in the trans and cis Andean region has been limited to purely morphological approaches (ARMBRUSTER, 2005). These molecular results, -- species delimitation models and multilocus phylogeny --, have defined eight lineages (species) within the *L. caucanus* species group, using on the species delimitation method, grouped into three distinct and highly supported clades of trans-Andean region: *L. volcanensis*, *L. caucanus* and *L. planiceps* that evolve separately (DE QUEIROZ, 2007). The taxonomic status and geographical distribution of *Lasiancistrus* species in the trans-Andean region is reformulated and the following hypothesis is suggest: *L. volcanensis* Dahl, 1942, *L. caucanus* Eigenmann, 1912 and *L. planiceps* Meek & Hildebrand, 1913 are recognized as species valid. It is important to emphasize that every species is a hypothesis, which potentially is refuted or corroborated by subsequent studies (DE QUEIROZ, 2007)

Three linages were recognized species did yet reach the reciprocal monophyly, demonstrating that multilocus phylogeny and DNA barcoding can help to identify new taxa in complex groups, as previously observed in Characins (Stevardiinae) (GARCÍA-MELO et al., 2019), *Rineloricaria* (COSTA-SILVA et al., 2015) *Tetragonopterus* (MELO et al., 2011), *Neoplecostomus* (ROXO et al., 2012), and other genera. As time passes in the speciation process, the boundaries between new species become increasingly evident (DE QUEIROZ, 2007). However, at the beginning of this process (named grey zone), the boundaries among species were difficult identified, making the species boundary very subjective and dependent on the concept of species (DE QUEIROZ, 2007).The results obtained with other species such as *Rineloricaria* showed that the species limits do not appear in a fixed order; however with *L. caucanus* the genetic limit preceded the morphological, -- based strictly on morphological *Lasiancistrus*'s study done by Armbruster (2005)--, nonetheless, a morphometric, meristic and osteological review is being performed.

Currently, DNA barcoding techniques are used as an additional methodology to help with species delimitation in Neotropical fishes and to support new species descriptions (e.g., MELO et al., 2011; ROXO et al., 2012; GARCÍA-MELO et al., 2019). Moreover, the barcoding techniques could be an important first step to traditional taxonomy work (KEKKONEN & HEBERT, 2014) and are frequently helpful to work on species complexes (MARQUES et al., 2013).

The genetic divergence between the lineages that are morphologically similar (ARMBRUSTER, 2005) characterizes of cryptic species (BELLAFRONTE et al. 2013; MARQUES et al., 2013), and this is the case found in *L. caucanus* that have three clades with genetic differentiation between lineages varies from 3% to 4%. The variation found among lineages was lower than the variation found between distinct species of *Lasiancistrus* (Table 2). This variation could be due to mutation accumulation over time in geographical isolation (KEKKONEN & HEBERT, 2014). For example, the Upper Magdalena, Upper Cauca and Tuira/Bayano basins, respectively, are separated by physical barriers as the mountain ranges of the Andes

DNA barcoding using an integrative approach of species delimitation methods and multilocus phylogeny was very efficient for delimiting of *L. caucanus* group (trans-Andean region) mainly in groups with low morphological variation (i.e., cryptic species). The recognition of different genetic structures in groups with very similar morphology has exposed a common pattern across the life tree, and it is observed regularly in species-rich genera, as observed in sharks (QUATRO et al., 2006) and neotropical fishes (ROXO et al., 2012; GARCÍA-MELO et al., 2019). The genetic diversity of the lineage of *L. caucanus* showed that were widely distributed and fragmented into several local lineages. Thus, a great effort is needed to preserve the diversity of the genus and maintain local lineages such as conservation politics and the delineation of preservation areas.

3.4.2 Monophyly, phylogeny and biogeographic history of the *Lasiancistrus caucanus* species group

DNA-based phylogenies have provided more objective assessments of the evolutionary history of closely related Neotropical freshwater fish lineages and their geographic distribution (CONDE-SALDAÑA, 2016, LUJAN, et al., 2015). Our phylogenetic analysis of the mtDNA such as cytochrome b and cytochrome oxidase genes support the separation of *Lasiancistrus* into two clades. The mtDNA based phylogeny supports a cis-, trans-Andean split in the genus, and indicates that separation of *L. caucanus*, *L. guacharote* (trans-Andean) of *L. tentacualtus* and *L. schomburgkii* (cis-Andean).

The mean level of mtDNA sequence divergence separating cis- and trans-Andean *Lasiancistrus* is >20%, and application of mtDNA molecular clocks determined by Silva et al., 2016 indicate that the age of this split was approximately 13 million of years (Ma). These mtDNA-based dates are concordant with based ages for the emergence of the Eastern Cordillera that isolated the Magdalena System (including the Magdalena, Cauca, Sinú, San Jorge) and the Ranchería, which together form the Magdalena Domain (RODRIGUEZ-OLARTE et al., 2011), from the Maracaibo Basin. Later, the Eastern Cordillera diverged, forming the Serranía del Perijá and the Mérida Andes that finalized the separation of the Maracaibo basin from the proto-Orinoco-Amazon (SCHAEFER, 2011). The closure of the Eastern Cordillera caused the final separation of the continental systems into the cis- and trans-Andean regions (ALBERT & REIS, 2011). Some phylogenetic hypotheses have suggested that genera such as *Rhamdia* (PERDICES, 2002), *Gymnotus* (LOVEJOY et al., 2010) and *Hypostomus* (MONTOYA-BURGOS, 2003) were affected by the Miocene tectonic, especially Eastern Cordillera and other events that defined the landscape of northern South America (LUNDBERG et al., 1998). Patterns of species diversity in Neotropical fishes are heterogeneous due to landscape reconfigurations that have resulted from the complex geologic history of the region (LUNDBERG et al., 1998; RODRIGUEZ-OLARTE et al., 2011; SCHAEFER, 2011; ANTONELLI et al., 2018a; ANTONELLI. et al., 2018b).

The hybrid origin of the Maracaibo ichthyofauna has two explanations. One explanation involves a partial extinction of the original (cis- Andean) Maracaibo ichthyofauna in the Late Miocene (LUNDBERG et al., 1998; LUNDBERG & AGUILERA, 2003) product of local marine incursion that inundated modern Maracaibo Basin (ALBERT et al., 2006). The other explanation, includes secondary replacement of freshwater fish taxa from the adjacent (trans-Andean) Magdalena Basin, because colonization of newly exposed freshwater habitats in the Maracaibo by Magdalena taxa could have occurred by coastal stream capture along their common Caribbean shorelines, especially during periods of marine regression (ALBERT et al., 2006). According to this model, Maracaibo taxa with Magdalena affinities (e.g. clades within *Creagrutus*, *Ctenolucius*, *Cyphocharax, Gilbertolus*, *Apteronotus*, *Hoplosternum*, and *Rhamdia*) date perhaps to the Late Miocene or Pliocene, whereas other taxa (e.g. clades within *Roeboides*, *Apteronotus*, and *Brachyhypopomus*) with cis-Andean affinities date (ALBERT et al., 2006). The presence of *L. guacharote* in the Maracaibo basin, sister species of *L. caucanus* in the trans-Andean region, is probably the product of its isolation and dispersion following processes of drainage anastomosis during periods of sea level rise (RODRÍGUEZ-OLARTE et al., 2011), colonization of newly exposed freshwater habitats in the Maracaibo by Magdalena taxa (ALBERT et al., 2006; ALBERT & REIS, 2011) or the Serranía del Perijá that finalized the separation of the Maracaibo basin from the proto-Orinoco-Amazon (SCHAEFER, 2011). On the other hand, vicariant processes along the western slope of the Eastern cordillera may have been the limiting factors *L. caucanus* populations in the Magdalena Domain, Colombian Biogeography Chocó, Tuira and Bayano basins.

The Magdalena drainage forms an extensive inter-mountain valley between the Eastern and Central Cordilleras. The most important tributary, the Cauca runs parallel to the Magdalena’s main channel between the Western and Central Cordilleras. The Cauca from its origin down to about 2,000 m is torrential, but between 1,500 and 900 m the valley widens, slopes lessen, and the meanders through an ample floodplain (RINCON-SANDOVAL et al., 2019). About 500 km farther downstream it flows through a deep, narrow canyon and passes through a series of rapids that are an insurmountable geographic barrier for many species of fishes (RINCON-SANDOVAL et al., 2019) such as *Geophagus steindachneri* (Eingenmann, 1922), *Hoplias malabaricus* (Bloch 1794) and *Pimelodella floridablancaensis* (Ardila Rodriguez, 2017*)*. This barrier could have been a important impediment to the *L. caucanus*, generating the isolation of the populations of the Upper Cauca and the consequent allopatric speciation between these populations (*L. caucanus* 'Upper Cauca') and the populations of the Magdalena (*L. volcanensis*).

Also, during the Miocene–Pliocene, plate reorganizations ultimately resulted in the closing of the Panamá Isthmus (SCHAEFER, 2011; O'DEA et al., 2016). Before the uplift of the Serranía del Darién and continuous climatic changes between dry and wet periods during the Pleistocene (MALDONADO-OCAMPO et al., 2012), the Atrato flowed into the Tuira Gulf on the Pacific slope of Panamá (MATAMOROS et al., 2015). This barrier could have generated the isolation of the populations of the Colombian Biogeography Chocó and the allopatric speciation between these populations (*L. caucanus* 'Calima') and the populations of *L. planiceps* 'Bayano/Tuira. Several phylogeographic analysis distinguished multiple *Rhamdia* mtDNA lineages, and geographic congruence across evolutionarily independent clades indicated that vicariance has played a strong role in the Mesoamerican fast diversification of this genus (BERMINGHAM & MARTIN, 1998; PERDICES et al., 2002). The genus *Pimelodella* presents a complex mosaic distribution in this region, probably caused by multiple processes such as local extinction and dispersion (MARTIN & BERMINGHAM, 2000). Additionally, variations in sea level could flood the nascent isthmus, causing local extinctions and allopatric separation in eastern and central Panama (SMITH & BERMINGHAM, 2005).

According to the present study, is clear that the *L. caucanus* species group represents a valid and monophyletic group, recognizing eight lineages (species) depending on the species delimitation method, grouped into three distinct and highly supported clades of trans-Andean region: *L. volcanensis*, *L. caucanus* and *L. planiceps*. Our results have contributed to the clarification of the taxonomy and distribution of the genus *Lasiancistrus* in the trans-Andean region. However, we need to focus our efforts on finding diagnostic characters for the description of the new possible species not recognized for the Rancheria, Upper, Lower Magdalena and Bayano basins, as well as the revalidate *L. volcanensis*, *L. planiceps* and *L. mayaloi* (in preparation). Additionally, increase the representativeness of Colombian Biogeography Chocó sample and genes and thus clarify the phylogenetic relations with the Panamá basins.

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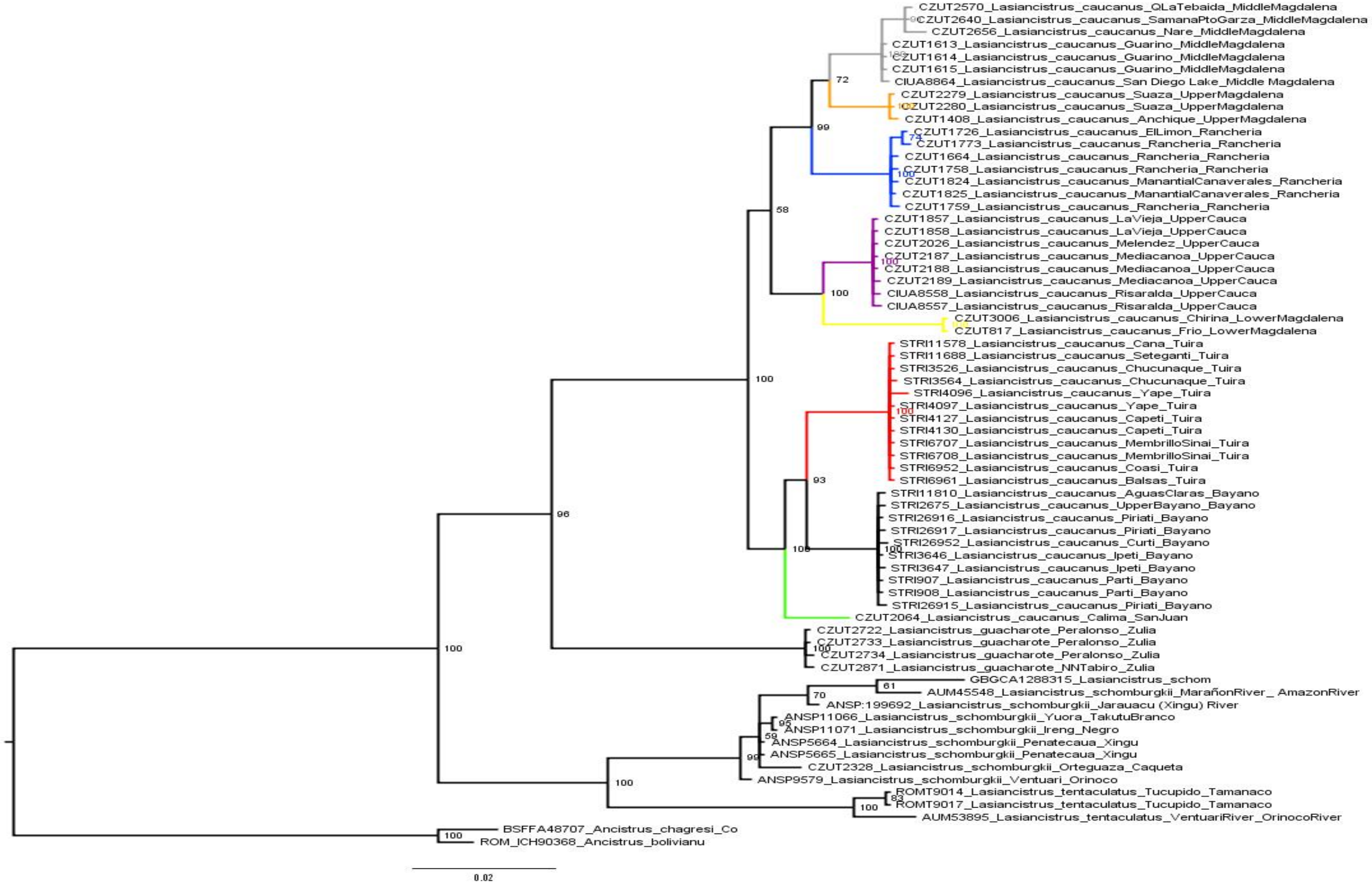
4. CONCLUSÕES GERAIS

O gênero *Lasiancistrus* é um grupo monofilético na região cis e transandina. Foram consideradas espécies validas *L. volcanensis*, *L. mayoloi* and *L. planiceps* antiguamente reconhecidas como especies sinônimas de *L. caucanus*. Provavelmente, existem outras espécies nas bacias Rancheria, Alto Magdalena e Bajo Magdalena que estão sendo avaliadas morfologicamente.

Esta pesquisa representa a primeira caracterização citogenética do gênero *Lasiancistrus* na região transandina. Em geral, *Lasiancistrus caucanus* conserva uma macroestrutura cariotípica similar da tribu Ancistrini, com 2n= 54 e poucos blocos de heterocromatina, ocupando uma posição basal na tribo, além disso, apresentou um padrão comum com as espécies cisandinas.

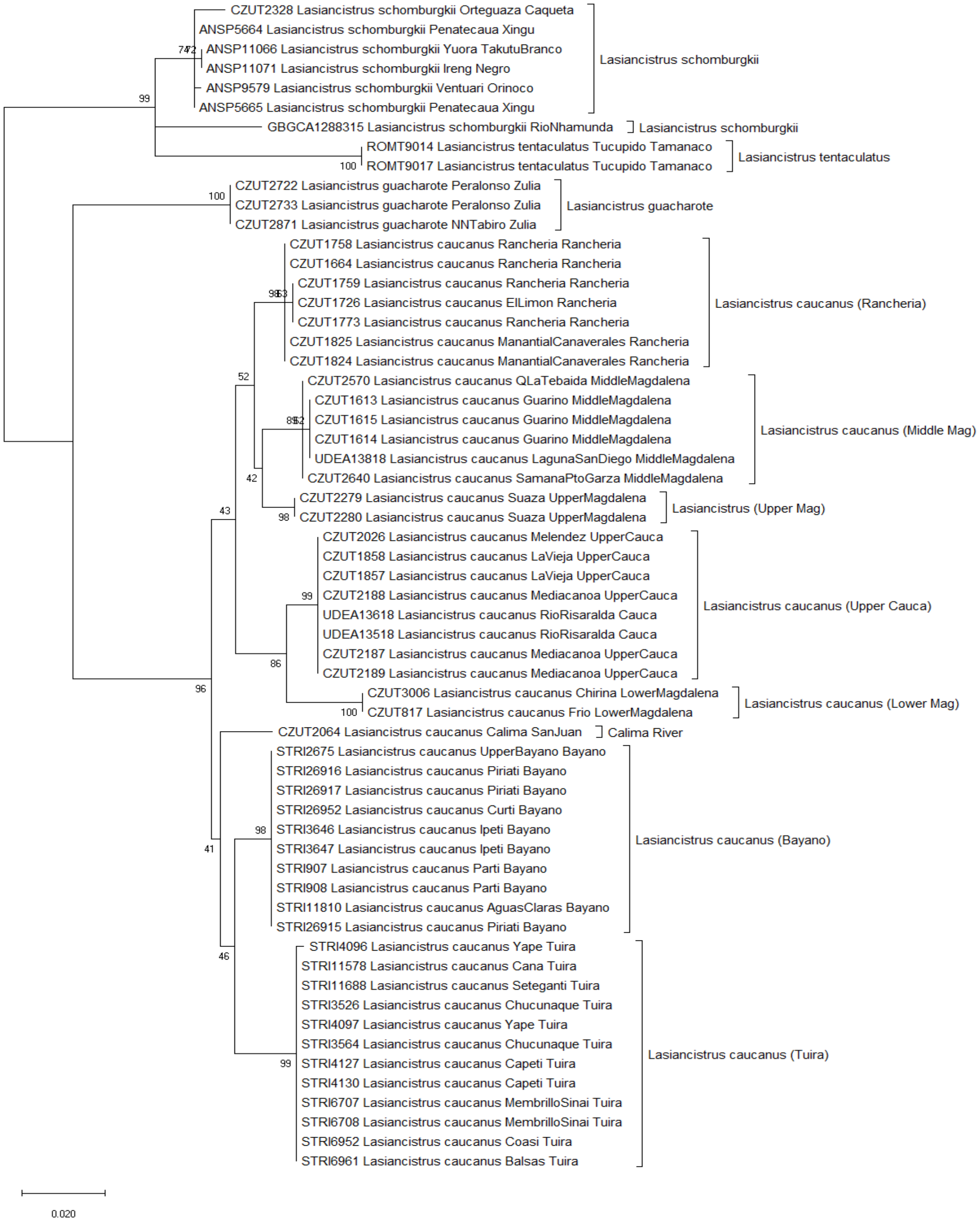
APÊNDICES

APÊNDICE A. Hypothetical relationship of *L. caucanus* in the trans-Andean region, based on multilocus bayesian inference; node numbers indicate probability values.



Fonte: Autor

APÊNDICE B. Maximum Likelihood (ML) phylogenetic tree of *Lasiancistrus* obtained with COI data. The values in the node branches represent bootstraps.



Fonte: Autor