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Ronaldo Libardi Widholzer

Revisão sistemática de *Ancistrus* (Kner 1854), ocorrente na Bacia hidrográfica da Laguna dos Patos.

São Leopoldo
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Orientador: Uwe Schulz

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1. APRESENTAÇÃO

Este documento apresenta os resultados da revisão das variações morfológicas e genéticas ao longo da distribuição geográficas das populações de peixes cascudos identificados como *Ancistrus brevipinnis* (família Loricariidae), que ocorrem na bacia hidrográfica da Laguna dos Patos.

Devido a uma descrição inicial sem informações precisas, todos os exemplares de *Ancistrus* coletados no Rio Grande do Sul (RS) foram identificados como *A. brevipinnis*. Posteriormente, a distribuição dessa espécie foi restringida à bacia hidrográfica da Laguna dos Patos (SLP), estendendo-se também a parte do Uruguai. No entanto, mesmo dentro dessa bacia, foram observadas variações morfológicas que podem representar diferentes espécies ou variações populacionais não contempladas na descrição original.

Esta tese está dividida em dois capítulos, apresentados sob a forma de artigos. O primeiro capítulo descreve a população de origem da descrição de *Ancistrus brevipinnis*, com o objetivo de estabelecer as características da população topótipo, até então desconhecida, para diferenciá-la das demais. Além da redescrição, neste capítulo foi descrita uma novaespécie, *A. megacanthus*, com base em informações morfométricas, morfológicas e em marcadores moleculares. Neste estudo, foi identificado um terceiro grupo, *Ancistrus* aff. *brevipinnis*, que ocorre em várias microbacias da área de estudo. Este artigo já foi publicado na revista **Neotropical Ichthyology**, que possui fator de impacto A3.

O segundo capítulo consiste em um artigo que está sendo redigido para submissão na mesma revista. Considerando a complexa e diversificada variação de ambientes (por exemplo, geologia, biomas, altitude) encontrados no SLP, foi realizada uma análise mais aprofundada de todas as variações observadas, incluindo estudos filogeográficos que abrangem todas as microbacias do SLP, utilizando marcadores moleculares, sendo um nuclear (Rag2) e dois mitocondriais (16S e Co1). Além da espécie em estudo, este trabalho contribuiu com informações sobre dois grupos externos, *Ancistrus taunayi* e *A. multispinnis*, ambos ocorrentes no RS, porém em bacias diferentes. Também foram utilizados dados públicos disponíveis no GenBank de A. *cirrhosus*.

Com a inclusão dos marcadores moleculares, constatou-se que a distribuição das espécies não está associada diretamente às microbacias hidrográficas, mas sim que a história evolutiva das espécies foi moldada pelas mudanças recentes na geologia da região, uma vez que as alterações geológicas moldam as bacias hidrográficas ao longo do tempo. Com essas novas informações, foram identificadas mais duas espécies, além da ampliação da distribuição de *A. megacanthus* e a identificação de um complexo a ser estudado.

RESUMO

Sebastian Wolff e H. von Ihering fizeram coletas na região do Rio Camacuã e Regan descreveu a espécie *Ancistrus brevipinnis* em um único parágrafo, com a localidade- tipo no Rio Grande do Sul, mencionando poucos caracteres diagnósticos e baseando-se em um único indivíduo. Posteriormente, a distribuição dessa espécie foi restrita à bacia hidrográfica da Laguna dos Patos. Devido à existência de polimorfismos, essas populações foram estudadas com o objetivo de definir melhor esses caracteres e descrever novas espécies. Foi realizado um estudo com base em uma revisão de dados e análises morfométricas de 303 amostras de toda a bacia. Com isso, foi possível redescrever e restringir essa espécie à microbacia do rio Camaquã, além de descrever uma nova espécie, *A. megacanthus*. Posteriormente, com 44 amostras de material genômico, foi realizada uma análise filogenética (Máxima Verossimilhança e Bayesiana) utilizando os marcadores Co1, 16S e Rag2, assim como a construção de uma rede de haplótipos. Como resultado, foi possível descrever mais duas espécies: uma que ocorre na microbacia do Rio dos Sinos e Rio Gravataí; e outra da Lagoa Mirim. Ainda assim, os dados moleculares obtidos sugerem a existência de uma ou mais espécies que habitam a região do Planalto Meridional, cuja distribuição ultrapassa os limites da Bacia Hidrográfica.

Palavras-chave: Peixe cascudo, *Ancistrus*, taxonomia, espécie nova, redescrição

ABSTRACT

Sebastian Wolff and H. von Ihering collected close to Camacuã river and Regan described the species *Ancistrus brevipinnis* in a single paragraph, with the type localityin Rio Grande do Sul, providing few diagnostic characters and based on a single individual. Subsequently, the distribution of this species was restricted to the Laguna dos Patos basin. Due to the existence of polymorphisms, these populations were studied to better define these characters and to describe new species. A study was conducted based on a review of data and morphometric analyses of 303 samples from across the basin. This allowed for the redescription and restriction of this species to the Camaquã River microbasin, as well as the description of a new species, *A. megacanthus*. Subsequently, with 44 samples of genomic material, a phylogenetic analysis (Maximum Likelihood and Bayesian) were conducted using the markers Co1, 16S, and Rag2, along with the construction of a haplotype network. As a result, two more species were described: one occurring in the microbasins of the Sinos and Gravataí Rivers; and another in the Mirim Lagoon. Even so, the molecular data obtained suggest the existence of one or more species inhabiting the Meridional Plateau region, with a distribution that extends beyond the basins boundaries.

Keywords: Cat-fish, *Ancistrus*, interative taxonomy, redescrible, new species

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2. INTRODUÇÃO

Os peixes constituem o grupo mais diverso entre os vertebrados, com um total de 36.867 espécies válidas, distribuídas em 72 ordens, 606 famílias e 342 subfamílias (Eschmeyer & Fong 2024). Esse número cresce substancialmente a cada ano, sendo que somente na última década (2014–2024) foram descritas 3.714 novas espécies, das quais 2.258 são de água doce (Eschmeyer & Fong, 2024).

Apesar de ocuparem apenas uma pequena proporção da superfície terrestre, os ecossistemas de água doce abrigam 43% das espécies de peixes conhecidas (Nelson *et al.* 2016). Contudo, grande parte da biodiversidade desses ambientes ainda permanece desconhecida e não quantificada, devido a desafios na amostragem e à falta de recursos para inventariamento (Silk & Ciruna, 2005). Além disso, a grande diversidade morfológica, biológica e de habitats ocupados pelos peixes dificulta a classificação e a compreensão da história evolutiva do grupo (Nelson *et al*. 2016).

Os Siluriformes são peixes cosmopolitas, habitando ambientes de água doce, salobra ou marinha. Os representantes dessa ordem se caracterizam pela ausência de escamas, apresentando corpo nu ou revestido por placas dérmicas, sendo popularmente conhecidos como bagres, cascudos ou peixes-gato (Malabarba *et al.* 2013).

De um total de 38 famílias, Loricariidae é a maior em número de espécies (1.064 sp.) na ordem Siluriformes (7.446 sp.) (Eschmeyer & Fong, 2024). Com surgimento entre aproximadamente 67 e 33 milhões de anos atrás (Shimabukuro-Dias *et al*. 2004), essa família é composta na maioria por peixes de pequeno a médio porte (<30 cm CP), amplamente distribuídos em ambientes lóticos da região Neotropical.

Atualmente, ocorre uma elevada perda de biodiversidade em consequência de impactos antrópicos como poluição e alterações físicas e estruturais dos ambientes aquáticos (Strayer & Dudgeon 2010). Em muitos casos, espécies são extintas antes de serem descritas (Buckup *et al*. 2007). Segundo Reis *et al*. (2016), na América do Sul, a conservação dos habitats aquáticos e da ictiofauna é um desafio diante das rápidas mudanças antropogênicas do século XXI.

No sistema da Laguna dos Patos, a família Loricariidae é representada principalmente por dois gêneros: *Rineloricaria* (Bleeker, 1862) com 20 espécies e *Hisonotus* (Eigenmann & Eigenmann, 1889) com 18 espécies, sendo que a maioria dessas espécies foi descrita apenas na última década (Bertaco *et al*. 2016). Na bacia do Rio Uruguai, conforme Litz & Koerber. (2014), os loricariídeos apresentam 44 espécies e 16 gêneros, sendo *Hypostomus* com 11 espécies e *Hisonotus* com 5; a subfamília Hypostominae possui 16 espécies, a tribo Ancistrini conta com quatro gêneros (*Ancistrus*, *Chaetostoma* e *Hemiancistrus*) e o gênero *Ancistrus* tinha apenas três espécies conhecidas antes deste estudo.

A região da bacia hidrográfica da Laguna dos Patos abrange parte do país vizinho, Uruguai. Nesta bacia existem seis microbacias, sendo a Cuenca Laguna Mirim a que atualmente contribui para a Laguna dos Patos (desde há aproximadamente 325 mil anos atrás Barbosa *et al*. (2021) totalizando uma área de estudo com um total de 113.534 km² segundo .

Segundo Bertaco *et al.* (2016) para a laguna dos Patos são listados 200 espécies, sendo compartilhada 33 espécies com a bacia do rio Uruguai, 18 espécies com o rio Tramandaí, 53 espécies comum nas três bacias e 96 exclusivas da Laguna dos Patos.

Histórico

Alcide d'Orbigny, naturalista francês, viajou pela América entre 1826 e 1833 e realizou a coleta de diversos peixes, embora não tenha descrito as espécies. Posteriormente, Valenciennes estudou as amostras coletadas por d'Orbigny e publicou um catálogo descrevendo-as em 1847. Contudo, houve alguns problemas cronológicos em sua publicação, e suas pranchas com desenhos das espécies foram publicadas anteriormente, em 1836, conforme relatado por Sherborn & Griffin (1934).

Em 1853, Kner propôs uma nova classificação devido à descoberta de novos representantes, criando o grupo Loricata. Esse grupo foi dividido em dois subgrupos: Loricariden, caracterizado pela ausência da nadadeira adiposa, com três novos gêneros—*Loricaria*, *Rhinelepis*, *Hemiodon* (atualmente sinônimo de *Reganella*) e *Acestra*. O segundo subgrupo foi Hypostomiden, que apresenta nadadeira adiposa, sendo subdividido em dois grupos: *Inermes* ou *Clypeaty*, sem espinhos retráteis, e *Lictores* ou *Ancistrus*, com espinhos retráteis, incluindo os gêneros *Chaetostomus* e *Ancistri*. Em 1854, Kner propôs uma divisão dentro do gênero *Ancistrus*: Brachypteri, caracterizado por ter a nadadeira dorsal com 7 raios, e Macropteri, com 13 raios. No entanto, ele não conseguiu alocar *Ancistrus medians*, pois apresentava características de ambos os grupos.

Gill (1858) propôs o gênero *Pterygoplichthys* para alocar todas as espécies do grupo Macropteri. Em 1862, Bleeker designou *Ancistrus cirrhosus* como a espécie-tipo do gênero *Ancistrus* e criou o gênero *Hemiancistrus* para alocar *Ancistrus medians*. Em 1890, Eigenmann & Eigenmann restringiram o uso de *Ancistrus* para as espécies que apresentavam tentáculos carnosos, mas Regan (1904) discordou, transferindo de volta para *Ancistrus* todos os gêneros removidos e incluindo novos, criando o gênero *Xenocara*, caracterizado pela borda do focinho nua e incluindo *Ancistrus cirrhosus* (espécie-tipo do gênero). No ano seguinte, Eigenmann & Eigenmann propuseram reter o nome *Xenocara* para espécies sem tentáculos, mas Regan (1906) discordou e propôs o sub-gênero *Thysanocara* para os indivíduos com focinho nu e tentáculos.

Em 1910, Eigenmann elevou a gênero os outros subgêneros de Regan e colocou

Thysanocara como sinônimo de *Ancistrus* e considerou como *Xenocara* somente *Chaetostomus latifrons* (Günther, 1869) e *A. gymnorhynchus*, ambas descritas sem a presença de tentáculos *Xenocara latifrons* foi designada como espécie-tipo, enquanto *Ancistrus* ficou restrito a peixes com tentáculos nus na margem do focinho (Neuhaus, 2015). Essa alteração não foi seguidanas descrições subsequentes de Regan, que continuou utilizando sua própria classificação. Fowler (1945) descreveu um gênero com uma espécie, *Pristiancistrus eutictus*; Isbrücker (1980) sinonimizou *Pristiancistrus*, *Xenocara* e *Thysanocara* para *Ancistrus.*

A primeira filogenia do grupo foi realizada por Fisch-Muller (1999), que reconheceu 49 espécies. Montoya-Burgos e*t al.* (1998) confirmou a monofilia do gênero conforme as diagnósticas anteriores de Eigenmann (1903). Em 2004, Armbruster revisou a filogenia com dados morfológicos e concluiu que a familia Ancistrinae era sinonimo de Hypostominae.

Os loricarídeos são caracterizados pela presença de placas dérmicas ossificadas, odontódeos nas placas dermicas, nos ossos expostos do cranio e em todos os raios das nadadeiras, aparelho bucal modificado em disco oral ventral (Geerinckx *et al*. 2007). Esses peixes possuem hábitos alimentares bentônicos, consumindo detritos e algas (Buck & Sazima 1995).

Os gêneros de Loricariidae estão agrupados em subfamílias, porém esses agrupamentos são instáveis. Isbrücker (1980) reconheceu seis subfamílias em Loricariidae: Hypoptopomatinae, Lithogeninae, Loricariinae, Neoplecostominae, Hypostominae (com presença de nadadeira adiposa) e Ancistrinae como grupo irmão desta última. Montoya- Burgos *et al*. (1998) publicaram uma filogenia baseada em dados moleculares com ênfase em Ancistrinae e Hypostominae, e nesse estudo, as subfamílias apresentadas por Isbrücker foram suportadas, exceto pelas subfamílias citadas, que formam um único agrupamento.

Armbruster (2004), com base em uma matriz de estado de caracteres, agrupou a família em cinco subfamílias: Hypoptopomatinae, Lithogeninae, Loricariinae, Neoplecostominae e Hypostominae. Esta última inclui cinco tribos, entre elas Ancistrini — anteriormente considerada subfamília Ancistrinae. A inserção de Ancistrinae em Hypostominae resolveria a parafilia do grupo sugerida por Isbrücker (1980).

A subfamília Delturinae foi descrita posteriormente em um estudo proposto por Reis *et al*. (2006). Outra modificação foi sugerida por Lujan *et al*. (2015), que através de análises moleculares, dividiu a família em sete subfamílias: Delturinae, Rhinelepinae, Loricariinae, Hypostominae, Neoplecostominae, Otothyrinae e Hypostominae. Nesta classificação, Ancistrini permanece como tribo dentro de Hypostominae, com recentes confirmações dessa organização (Roxo *et al*. 2019). Atualmente, Hypostominae possui 483 espécies válidas (Fricke *et al*. 2024).

Ancistrus Kner, 1854

Ancistrus é caracterizado como um peixe de pequeno a médio porte (<20 cm), pertencente à família Loricariidae, com presença de odontódeos hipertrofiados retráteis na região opercular, crista do *levator arcus palatini* é alta, pré-opérculo vertical e contato do osso frontal com a órbita ocular. Outras características incluem a modificação do opérculo emuma barra ou estrutura em forma de foice (Tribo Ancistrini), corpo largo e deprimido,tentáculos cutâneos dispostos sobre a margem anterior do focinho, com ausência de odontódeos e placas nessa região (Bifi *et al.* 2009), constrição hypohyal anterior e posterior, processo anterior do metapterigoide abreviado, porção ventral do esfenótico alargada, presença de duas a quatro placas entre a primeira placa lateral da cabeça e o opérculo, osso nasal grande e semi-quadrangular, e disco do mesetmoide alcançando além da margemanterior do corpo principal do metapterigoide (Armbruster, 2004). Dados osteológicos (Schaefer, 1987), moleculares (Borba *et al*. 2018; Lujan *et al*. 2015; Montoya-Burgos *et al*. 1998) e bioquímicos (Fisch-Muller, 1999) também suportam a caracterização da espécie.

Espécie-tipo do gênero

Na descrição de *Ancistrus*, Kner (1854) para *Brachypteri* foram descritas seis espécies novas além da transferência de *Hypostomus cirrhosus* (Valenciennes, 1836). Regan (1904) descreveu o gênero *Xenocara*, onde as espécies de *Ancistrus* descritas por Kner (1854) com as características do genero foram transferidas para este gênero, incluindo a espécie tipo do gênero *A. cirrhosus*, designada por Bleeker (1862). Espécie com sua localidade tipo desgnada para a bacia do La Plata, onde não se sabe com exatidão a coleta, mas se atribuí para ou rio Paraná ou rio Paraguai por sobreposição de registros de coleta.

Os membros da tribo Ancistrini são caracterizados pela presença de odontódeos hipertrofiados e retráteis na região opercular, com ampla distribuição na região Neotropical (Fisch-Muller *et al*. 2012). Segundo Armbruster (2004), as características sinapomórficas do grupo incluem a crista do *levator arcus palatini* alta (musculo de movimentação de abertura/fechamento do arco palatino branquial), pré-opérculo vertical e o contato do osso frontal com a órbita ocular. A maioria das espécies de Ancistrini (exceto *Spectracanthicus*) é suportada por uma única sinapomorfia: a modificação do opérculo em uma barra ou estrutura em forma de foice. O monofiletismo do grupo é sustentado por dados osteológicos (Schaefer 1987), moleculares (Montoya-Burgos *et al*. 1998; Lujan *et al*. 2015; Borba *et al*. 2018) e bioquímicos (Fisch-Muller 1999).

Além das características diagnósticas já descritas para Loricariidae e Ancistrini, os exemplares do gênero *Ancistrus* são determinados por sua morfologia anatômica externa

possuindo tentáculos cutâneos dispostos sobre a margem anterior do focinho, com ausência de odontódeos e placas ósseas nessa região (Bifi *et al.* 2009).

Outras características sinapomórficas do gênero incluem a constrição hipoidal anterior e posterior, processo anterior do metapterigóide abreviado; porção ventral do esfenótico alargada; presença de duas a quatro placas entre a primeira placa lateral da cabeça; o opérculo e o disco do mesetmóide se estendendo além da margem anterior do corpo principal do metapterigóide (Armbruster 2004).

Problemas de Determinação de Espécies

A maioria das espécies do gênero *Ancistrus* está descrita para a região Amazônica, onde se concentra o maior número de publicações sobre o grupo. No entanto, na região das bacias hidrográficas do Lá Plata, Rio Tramandaí, Rio Maquine, Rio Mampituba, Rio Uruguai e Laguna dos Patos há 12 espécies registradas (*Ancistrus abilhoai* (Zawadzki, 2009), *A. agostinho* (Zawadzki, 2009), *A. brevipinnis*, *A. cirrhosus* (Valenciennes, 1836), *A. claro* (Knaack, 1999) , *A. cuiabae* (Knaack, 1999), *A. formosus* (Sabino & Trajano, 1997), *A. mullerae* (Bifi *et al.* 2009), *A.multispinni*s (Holly, 1929), *A. pirareta* (Muller, 1989), *A. piriformis* (Muller, 1989) e *A. taunayi* (Ribeiro, 1918).

De acordo com Bifi *et al*. (2009), estudos já publicados sugerem que o número de espécies ainda não descritas do gênero *Ancistrus* é muito maior do que o número de espécies conhecidas. Uma revisão recente (Neuhaus, 2015) indicou que muitas espécies ainda estão por ser reveladas. Esse estudo, no entanto, não abordou a Lagoa dos Patos, no sul do Brasil, onde dados preliminares apontam para a existência de espécies não descritas do gênero.

Alguns fatores dificultam a realização de revisões taxonômicas, como a falta ou deterioração de materiais-tipo, imprecisões nas localidades-tipo e a ausência de informações detalhadas nas descrições de algumas espécies (Bifi 2009). Em um contexto mais amplo, uma taxonomia alfa mal resolvida, como no caso do gênero *Ancistrus*, dificulta a formulação de hipóteses filogenéticas e a construção da história evolutiva do grupo, comprometendo sua compreensão e conservação (Lujan *et al*. 2015).

Apesar desses desafios, o gênero *Ancistrus* atualmente inclui 79 espécies descritas, com ampla distribuição na América Latina, habitando diversos ambientes, desde os Andes na região equatorial até corredeiras, águas lênticas e a região subtropical (ao sul do paralelo 23°S).

***Ancistrus brevipinnis* (Regan, 1904)**

O material-tipo está depositado na coleção do Museu Britânico de História Natural (BMNH), e a localidade-tipo não possui uma designação precisa de seu local de coleta (estando como estado do Rio Grande do Sul). A imprecisão da localidade-tipo da espécie é um fator que dificulta o estudo de taxonomia das espécies, sendo necessária a sua delimitação. Com base em registros de coleções ictiológicas, Malabarba (1989) conseguiu restringir a distribuição, definindo que *Ancistrus brevipinnis* ocorre no sistema hidrográfico da Laguna dos Patos, sendo assim ampliando também para a parte do território Uruguaio. Em consequência disso, todos os exemplares do gênero coletados no sistema da Laguna dos Patos são identificados como *A. brevipinnis* (Petry & Schulz 2006 (Sinos), Rubert, 2011 (Taquari-antas), Burns *et al*, 2015 (São Gonçalo), Langoni, 2015 (Alto Jacuí)) mesmo na presença de alguns polimorfismos. No entanto, uma ampla distribuição não condiz com as características ecológicas desta espécie, visto que são peixes raspadores, habitantes de ambientes lóticos e rochosos.

Necessidade de determinação correta da espécie

De acordo com Ota *et al*. (2015), o conhecimento incompleto da biodiversidade de peixes é um obstáculo para a definição precisa da composição de espécies em uma comunidade, o que é essencial para evitar extinções. A autora enfatiza que a sistemática e a taxonomia fornecem uma base empírica para a produção de inventários da biodiversidade, além de auxiliar na compreensão de aspectos evolutivos e relações biogeográficas históricas.

Assim, para a conservação e manejo das espécies, é necessário desenvolver pesquisas que abordem aspectos taxonômicos, sistemáticos e ecológicos, para primeiramente conhecera espécie. Embora essas pesquisas estejam se expandindo, ainda não são suficientes para suprir o déficit no conhecimento e compreensão da ictiofauna e suas relações filogenéticas.

Com o surgimento de novas técnicas de validação, como a análise morfológica detalhada, o uso da morfometria, a Pet-ct *scan* ou diafanização para estudos osteológicos, o uso de material genético para análise filogenéticas e de redes de haplótipos além de novas abordagens de estudos (integrativa, evidencia total), a revisão de grupos antigos descritos é necessária, uma vez que tais revisões vêm revelando espécies antes não identificadas.

Objetivos gerais

Revisão taxonomica da espécie *Ancistrus brevipinnis*, apontando a localidade tipo e validar a hipótese de que os polimorfismos já apontados, além de novos a serem observados nas diferentes populações presentes na Bacia Hidrográfica da Laguna dos Patos, correspondem a diferentes espécies.

Objetivos específicos

Objetivo específico 1: Propor e analisar caracteres morfológicos e moleculares para a diagnose de *Ancistrus brevipinnis* e suas variações.

Objetivo específico 2: Redescrever sistematicamente a espécie *Ancistrus brevipinnis*.

Objetivo específico 3: Avaliar o status taxonômico e filogenético das populações da ecorregião da Laguna dos Patos atualmente denominadas de *Ancistrus brevipinnis* e investigar, de acordo com os resultados obtidos, a possível diversidade ainda não detectada.

3. CAPÍTULOS

3.1 A new species of *Ancistrus* (Siluriformes: Loricariidae), with a redescription of *Ancistrus brevipinnis* (Regan 1904) and further evidence of hidden diversity in the Laguna dos Patos basin, Brazil.

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A new species of *Ancistrus* (Siluriformes: Loricariidae), with a redescription of *Ancistrus brevipinnis* and further evidence of hidden diversity in the laguna dos Patos system, Brazil

Correspondence:
Ronaldo L. Widholzer
ronaldow@edu.unisinos.br

Ronaldo L. Widholzer[1], Jessica Borsoi[1], Roberto E. Reis[2] and Pablo Lehmann A.[2]

A new species of *Ancistrus* is described from the rio Vacacaí drainage, rio Jacuí basin, laguna dos Patos system, southern Brazil. The new species differs from *A. brevipinnis*, the common species in the laguna dos Patos system, by having a dark brown to black background body color and dorsal and caudal fins plain dark, without lighter spots or bands. A morphometric and molecular comparison of different populations of *A. brevipinnis* from the laguna dos Patos system suggests that previously undetected species diversity is present in that basin. The type-locality of *A. brevipinnis*, originally described as Rio Grande do Sul State, is herein restricted to the rio Camaquã sub-basin of the laguna dos Patos system, and a redescription of *A. brevipinnis* is provided based on that population.

Keywords: Biodiversity, Fish, Morphometrics, Systematics, Taxonomy.

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1 Laboratório de Sistemática e Taxonomia, Universidade do Vale do Rio dos Sinos, Av. Unisinos, 950, 93022-750 São Leopoldo, RS, Brazil. (RLW) ronaldow@edu.unisinos.br (corresponding author), (JB) borsoi.je@gmail.com.

2 Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil. (RER) reis@pucrs. br, (PLA) plehmann@pucrs.br.

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Uma nova espécie de *Ancistrus* é descrita da drenagem dO rio Vacacaí, bacia dO rio Jacuí, sistema da laguna dOs PatOs, sul dO Brasil. A nova espécie se diferencia de *A. brevipinnis*, a espécie cOmum no sistema da laguna dOs PatOs, pOr pOssuir O cOrpO marrOm escurO a negrO, bem cOmO as nadadeiras dOrsal e caudal hOmOgeneamente escuras, sem pOntOs Ou bandas claras. Uma cOmparação mOrfOmétrica e mOlecular de diferentes pOpulaçÕes de *A. brevipinnis* dO sistema da laguna dOs PatOs sugere a existência de diversidade específica previamente não detectada nesta bacia. A lOcalidade-tipO de *A. brevipinnis*, Originalmente descrita cOmO EstadO dO Rio Grande dO Sul, é aqui restrita para a sub-bacia dO rio Camaquã dO sistema da laguna dOs PatOs, e uma redescrição de *A. brevipinnis* é apresentada cOm base nessa pOpulação.

Palavras-chave: Biodiversidade, MOrfOmetria, Peixe, Sistemática, TaxOnomia.

INTRODUCTION

LOricariidae is the mOst diverse of the 40 silurifOrm families with 1,044 species (Fricke *et al*., 2023). The genus *Ancistrus* Kner, 1854 is the secOnd mOst speciose in the family with 76 species (Fricke *et al*., 2023) inhabiting lOtic envirOnments thrOughOut the NeotrOpics, with the greatest diversity Occurring in the northern region of SOuth America. Of thOse species, 14 have been described in the last 10 years, and descriptions prior tO 1950 are not very infOrmative, preventing the accurate identification of many species (Neuhaus *et al*., 2022). *Ancistrus brevipinnis* (Regan, 1904) is the only species sO far recOgnized in the laguna dOs PatOs drainage basin in SOuth Brazil. Its Original type-lOcality given by Regan (1904) was [State of] Rio Grande dO Sul, which was later restricted tO the laguna dOs PatOs hydrOgraphic system by Malabarba (1989). Based On infOrmation in the fish catalOg of the Natural HistOry Museum Of LOndOn assOciated with histOrical recOrds Of Sebastian WOlff and Hermann vOn Ihering, fOrmer residents Of the municipality Of São LOurençO dO Sul and cOllectOrs Of the hOlOtype of *Ancistrus brevipinnis*, its type-lOcality can be further restricted tO the rio Camaquã sub-basin of the laguna dOs PatOs.

Ancistrus brevipinnis was pOOrly described, based On a single individual, and clearly needs a thOrOugh redescription since mOrphOlOgical variation currently Observed in the laguna dOs PatOs system suggests the existence of previously undetected diversity. COmparisOn of different pOpulations Of *Ancistrus* frOm the laguna dOs PatOs system revealed that fish frOm the rio Vacacaí are clearly distinct frOm thOse of remaining sub-basins, and here we describe them as a new species. In addition, the pOpulation of *A. brevipinnis* frOm the rio Camaquã is distinguishable frOm the pOpulations in mOre northern tributaries Of the laguna dOs PatOs, chiefly the rio Jacuí basin, and we prOvide a redescription of *A. brevipinnis* based On specimens frOm the newly restricted type-lOcality, the rio Camaquã sub-basin.

MATERIAL AND METHODS

Measurements were obtained with a 0.1 mm precision digital caliper under a stereomicrOscOpe when necessary. The 33 measurements and cOunts fOllOw Bifi *et al*. (2009) with the addition of distance between the end Of dOrsal-fin base and the origin Of the adipOse fin; and bOdy depth at Origin of dOrsal fin. All measurements and cOunts were taken on the left side of each individual, except when that side was damaged, and presented as percent Of standard length (SL) Or head length. A Linear Discriminant Analysis (Fisher, 1936) was perfOrmed with ratios Of all measurements On the standard length in PythOn 3.8 with scripts adapted frOm WidhOlzer (2023). In the search fOr diagnostic characters, qualitative features Of external anatOmy (*e.g*., cOlOr pattern, shape and arrangement Of teeth, bOdy and fin shape, and presence/absence of chrOmatOphOres) were analyzed. OsteolOgical characters were observed in specimens cleared and dOuble stained (c&s) accOrding tO TaylOr, Van Dyke (1985). Vertebral centra were cOunted in c&s specimens Only and include five centra mOdified intO the Weberian apparatus and One cOmpOund caudal centrum (PU1+U1). Identification and cOunts Of dermal plates fOllOw the serial hOmOlOgy scheme prOpOsed by Schaefer (1997).

Institutional abbreviations fOllOw Sabaj (2020), except fOr Museu de ZOOlOgia da Universidade dO Vale dO Rio dOs Sinos (UNISINOS), São LeopOldO, Rio Grande dO Sul, Brazil (MZU). In addition, we cOnducted field expeditions tO cOllect living specimens fOr tissue (tis) sampling and cOlOr assessment between May and NOvember Of 2022. FOr all species already described and with the exception of *Ancistrus brevipinnis*, *A. multispinis* and *A. taunayi*, all Other OutgrOup cOmparisOns were made based On their mOrphOlOgical infOrmation in the original description.

Genetic sequence of gene cOI frOm selected paratype fOllOws GenSeq nomenclature (Chakrabarty *et al*., 2013). TOtal genomic DNA was extracted using the DNeasy blOOd and tissue extraction kit (Qiagen) frOm muscle samples fixed in 95% ethanol and stOredat -20°C. DNA sequences Of the mitOchOndrial cOI gene were amplified by PCR using primers Fish-F1 and Fish-R1 (Ward *et al*., 2005), under the fOllOwing prOtOcOl: an initial denaturation step Of 3 minutes at 95°C fOllOwed by 35 cycles Of 94°C fOr 30 sec, annealing at 52°C fOr 40 sec, and extension at 72°C fOr 1 min, fOllOwed by a final 5min extension step at 72°C. PCR prOducts were purified and sequenced at Functional Biosciences Inc., MadisOn, USA. Sequences were edited in Geneious v. 8.0.5 and aligned with the Muscle algorithm as implemented in MEGA v. 11. A genetic distance matrix was built under the Kimura 2-parameter mOdel and a Maximum LikelihOOd tree was calculated under the General Time Reversible mOdel with Gamma distribution andinvariant sites, and the bOOtstrap calculated frOm 500 replicates, again using MEGA v. 11. DNA sequences Of gene cOI used in this study were uplOaded tO the Genbank databaseand have the fOllOwing accession cOdes (*Ancistrus brevipinnis*, OR242765-OR242767, *A*.aff. *brevipinnis*, OR242763-OR242764, *A. taunayi* Miranda RibeirO, 1918, OR250757, *A. multispinis* (Regan, 1912), OR242730, and *Ancistrus* sp. n. described belOw, OR250758). A sequence of *Ancistrus aguaboensis* Fisch-Muller, MazzOni & Weber, 2001, used tO rOOtthe ML tree, was Obtained frOm the Genbank (MK464026).

RESULTS

Ancistrus megacanthus, new species

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(Figs. 1–2; Tab. 1)

Holotype. MCP 19582, male, 96.8 mm SL, Brazil, Rio Grande do Sul, São Gabriel, rio Vacacaí on road RS-630, tributary to rio Jacuí, laguna dos Patos system, 30°27′18″S 54°22′26″W, 14 Jan 1997, L. R. Malabarba, J. A. Gomes & V. A. Bertaco.

Paratypes. Brazil, Rio Grande do Sul, rio Vacacaí basin, laguna dos Patos system: MCP 54919, 5, 47.7–74.5 mm SL (all measured) + 1 c&s, 54.5 mm SL, collected with holotype. MCP 16294, 6, 85.3–124.4 mm SL (all measured), MZUSP 128046, 1, 120.2 mm SL (measured), UFRGS 29411, 1, 101.1 mm SL (measured), Caçapava do Sul, arroio Pessegueiro, *ca*. 30º28′S 53º37′W, 15 Apr 1993, A. Ramires. MCP 54883, 1 (tissue sample), 39.4 mm SL (measured), São Gabriel, arroio Cambaizinho on road BR-290, 30°20′23″S 54°03′07″W, 5 Jul 2022, R. Widholzer & M. Haas. MCP 54898, 2, 33.7–44.1 mm SL (1 measured, 44.1 mm SL), São Gabriel, arroio Cambaizinho on road BR-290, 30°20′23″S 54°03′07″W, 4 Nov 2022, R. Widholzer & M. Haas.

Genseq-2 coI. Sequence deposited in GenBank (MCP 54883 GenBank accession code OR250758).

Diagnosis. *Ancistrus megacanthus* differs from non-Andean congeners (except *A. abilhoai* Bifi, Pavanelli & Zawadzki, 2009, *A. agostinhoi* Bifi, Pavanelli & Zawadzki, 2009, *A. brevifilis* Eigenmann, 1920, *A. brevipinnis*, *A. cirrhosus* (Valenciennes, 1836), *A. cuiabae* Knaack, 1999, *A. hoplogenys* (Günther, 1864), *A. kellerae* de Souza, Taphorn & Armbruster, 2019, *A. leucostictus* (Günther, 1864), *A. lithurgicus* Eigenmann, 1912, *A. luzia* Neuhaus, Britto, Birindelli & Sousa, 2022, *A. maximus* de Oliveira, Zuanon, Zawadzki & Rapp Py-Daniel, 2015, *A. mullerae* Bifi, Pavanelli & Zawadzki, 2009, *A. multispinis*, *A. nudiceps* (Müller & Troschel, 1849), *A. patronus* de Souza, Taphorn & Armbruster, 2019, *A. saudades* de Souza, Taphorn & Armbruster, 2019, *A. taunayi*, *A. stigmaticus* Eigenmann & Eigenmann, 1889, *A. trinitatis* (Günther, 1864), and *A. yutajae* de Souza, Taphorn & Armbruster, 2019) by adult males – those with well-developed snout tentacles – having the pectoral-fin spine long, exceeding half length of the pelvic-fin leading ray (*vs*. pectoral-fin spine short, not reaching half length of the pelvic-fin leading ray). It differs from those species (except *A. yutajae*), and including all congeners from Rio Grande do Sul (*A. brevipinnis*, *A. multispinis*, and *A. taunayi*), by having dorsal and caudal fins plain dark brown to black, without lighter spots or bands (*vs*. lighter spot or bands on dorsal and caudal fins), and differs from *A. brevipinnis* and *A. yutajae* by having a dark brown to black background body color (*vs*. light brown body). Additionally, the new species is distinguished from *A. taunayi* by having small white to yellow dots on dorsal surface (*vs*. dorsal surface with light brown to pale yellow vermiculated spots). *Ancistrus megacanthus* is further distinguished from *A. taunayi* by having four branched rays on the anal fin (*vs*. three branched rays).

FIGURE 1 | *Ancistrus megacanthus*, dorsal, lateral and ventral views of holotype, MCP 19582, male, 96.8 mm SL, Brazil, Rio Grande do Sul, São Gabriel, rio Vacacaí on road RS-630, tributary to rio Jacuí, laguna dos Patos system.

Description. MOrphOmetric data in Tab. 1. BOdy and head depressed. DOrsal prOfile Of bOdy and head cOnvex frOm tip Of snout tO dOrsal-fin origin, slightly cOncave tO straight frOm that pOint tO Origin of adipOse fin, cOncave frOm end Of adipOse fin tO Origin of caudal fin. Ventral prOfile of bOdy straight, bOdy height begins tO decrease at pelvic girdle. BOdy cOvered with bOny plates except alOng dOrsal-fin base and ventral surface between tip Of snout and anal-fin origin. Median series with 22–24(13, 23*) lateral plates; lateral-line canal cOmplete and uninterrupted. Three* predOrsal plates, seven* plates at base of dOrsal fin, fOur* plates between dOrsal and adipOse fins and 8*(6),

FIGURE 2 | Life color pattern of *Ancistrus megacanthus*. Specimens not preserved. *Hisonotus armatus* and *Hemiancistrus punctulatus* in background.

7(5) or 6(2) plates between adipose fins and caudal fins. Fin rays and body plates covered by small, caudally directed odontodes in regular rows, larger on fin spines.

Snout rounded, with wide bare anterior margin, posteriorly limited by dermal plates of varying sizes. Cutaneous tentacles present on lateral, anterior and middorsal portions of snout of males, sometimes branched and forming "Y" pattern. In females, tentacles smaller and usually arranged in pairs on sides of snout. Eye large 13.4–22.1% (17) of head length, located laterodorsally on head. Orbit delimited dorsally by sphenotic and frontal, anteriorly by prefrontal plate, ventrally by infraorbitals IV and V, and posteriorly by infraorbital VI. Naris located dorsally on head, with tubular expansion of skin, delimited dorsally by frontal, anteriorly by infraorbital II and III, laterally by prefrontal plate and nasal bone. Interorbital region slightly convex to flat. Exposed part of operculum almost always triangular, rounded anteriorly. Dermal plates of different sizes and shapes arranged in post-opercular area, contiguous to compound pterotic. Nine to 12(13) strong retractable odontodes with sickle-shaped claws at extremity in opercular region.

Lower lip not reaching anterior margin of pectoral girdle. Lower lip with papillae randomly distributed throughout surface. Maxillary barbell short and free. Shortmandibulary ramus, 12.6–19.6% (17) of head length; premaxillary tooth row of same length or slightly shorter. Teeth numerous, 38–67(17) in premaxilla and 32–68(13) in dentary, bicuspid, with reduced lateral cusp and larger, wider mesial cusp.

Dorsal-fin spine elongated, soft rays almost reaching or reaching to adipose-fin origin when depressed; pectoral-fin spine long, exceeding half-length of pelvic-fin unbranched ray on adult males; reaching first third of pelvic fin in females. Pelvic fin reaching middle of anal-fin length. Caudal fin truncated, with lower leading ray longer than upper. All branched fin rays doubly branched near apical end. Dorsal fin with II+7(17) rays; pectoral fin I+6(17) rays; pelvic fin i+5(17) rays; anal fin i+4(17) rays; caudal fin i+14+I(17) rays.

Sixth vertebral centrum (first after Weberian apparatus) with enlarged rib. Seventh vertebra without rib and supporting first dorsal-fin pterygiophore. Eight following vertebral centra with thin ribs and supporting dorsal-fin pterygiophores. Total vertebrae 28(1).

TABLE 1 | Morphometric data of *Ancistrus megacanthus*. Holotype and 15 paratypes; ranges include holotype. SD = Standard deviation.

	Holotype	Min	Max	Mean	SD
Standard length (mm)	96.8	39.4	124.4	88.4	–
Percent of standard length					
Abdominal length	18.9	17.3	20.6	18.9	0.9
Adipose fin to caudal fin distance	19.2	16.1	21.1	18.9	1.6
Adipose-fin spine length	9.8	7.3	12.3	10.1	1.4
Anal fin to caudal fin distance	33.4	29.5	35.9	33.1	1.8
Anal-fin unbranched ray length	9.6	7.4	10.7	9.0	0.9
Body depth at dorsal-fin origin	16.6	14.0	18.1	16.4	1.2
Body width at dorsal-fin origin	26.9	26.3	30.1	28.2	1.0
Caudal peduncle depth	33.7	29.1	35.0	31.9	1.6
Caudal peduncle length	10.2	9.1	11.6	10.0	0.6
Cleithral width	31.7	31.3	34.1	32.5	0.8
Dorsal fin to adipose fin distance	14.7	10.7	15.9	13.7	1.5
Dorsal-fin base length	19.8	18.3	23.1	20.7	1.3
Dorsal-fin spine length	23.5	21.7	29.7	24.3	2.0
Head length	34.4	31.9	35.9	33.9	1.2
Interbranchial distance	17.3	16.5	18.2	17.2	0.5
Lower caudal-fin ray length	25.9	23.1	30.9	25.5	1.9
Pectoral-fin spine length	34.4	29.0	36.4	32.9	2.3
Pectoral fin to pelvic fin distance	23.1	21.7	24.3	23.0	1.0
Pelvic-fin unbranched ray length	23.7	19.1	24.4	22.0	1.7
Predorsal length	44.5	42.3	47.9	44.4	1.5
Supracleithral width	26.7	26.2	31.3	28.0	1.4
Upper caudal fin ray length	22.8	19.3	25.0	22.0	2.0
Percent of head length					
Head depth at opercle	43.8	41.3	52.5	46.3	3.1
Internostril distance	17.9	17.5	21.9	19.5	1.4
Interorbital distance	38.2	37.6	47.7	41.3	3.1
Mandibulary ramus	15.5	12.6	19.6	17.0	1.7
Snout tip to ventral branchial opening	66.9	62.1	72.5	67.4	2.9
Orbital diameter	16.2	13.4	22.1	16.8	2.5
Snout length	62.1	43.9	62.2	59.5	4.6
Count			**Mode**		
Premaxillary teeth	54	38	67	43	
Dentary teeth	64	32	68	54	
Plates in dorsal lateral series	21	20	22	22	
Plates in median lateral series	23	22	24	24	

Coloration in alcohol. Dorsal and ventral region of body dark brown to black. Well-defined white to yellowish white dots, usually smaller than pupil diameter, on dorsal and lateral region of body, sometimes in ventral region, usually not visible in poorly preserved specimens. Slightly lighter brown bar on terminus of caudal peduncle. Rays and interradial membranes of paired fins with dark coloration similar to body and no or inconspicuous light dots. Dorsal, adipose and caudal fins plain dark brown to black. Upper and lower tip of caudal-fin leading rays and outer branched rays with small light termination (Fig. 1).

Coloration in life. Body dark brown to black with pale yellow dots, usually smaller than pupil diameter, along body; paired fins with yellow dots, dorsal fin with few inconspicuous light dots on unbranched ray, adipose and caudal fins unmarked. Lighter brown bar on terminus of caudal peduncle. Tip of upper and lower leading rays and outer branched rays of caudal fin with conspicuous yellow spots (Fig. 2).

Sexual dimorphism. Adult males have more numerous and larger snout tentacle than females and larger pectoral-fin spines, usually exceeding half-length of the pelvic-fin unbranched ray and reaching the first third of pelvic fin in females.

Geographical distribution. *Ancistrus megacanthus* is known from three localities in the rio Vacacaí sub-basin of the rio Jacuí basin, laguna dos Patos hydrographic system (Fig. 3).

Ecological notes. The new species occurs in creeks at altitudes close to 100 m above sea level, with swift waters and rocky to gravelly bottom (Fig. 4). These water courses are in the Pampa biome, in a savannah formation with predominance of herbaceous vegetation and trees restricted to river banks, and with soils characterized by being clayey and gravelly, typical of the Precambrian Sul-Riograndense Shield terrains.

Etymology. *Ancistrus megacanthus*, latinized from Greek μεγας (*megas*), large, great and ακανθος (*akanthos*), thorn, spine, in reference to the large pectoral-fin spines of the new species. A noun in apposition.

Conservation status. *Ancistrus megacanthus* is known from three localities in the rio Vacacaí drainage, with an Extension of Occurrence (EOO) estimated by the maximum convex polygon around the Vacacaí-Mirim sub-basin of 11,177 km^2. Main diffuse threats in the area are the transformation of the lotic environment to lentic habitats (*e.g.*, by the construction of dams) and the use of agricultural pesticides. As no specific threats to the species were detected, it is provisionally assessed as Least Concern (LC) according to the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN Standards and Petitions Subcommittee, 2022).

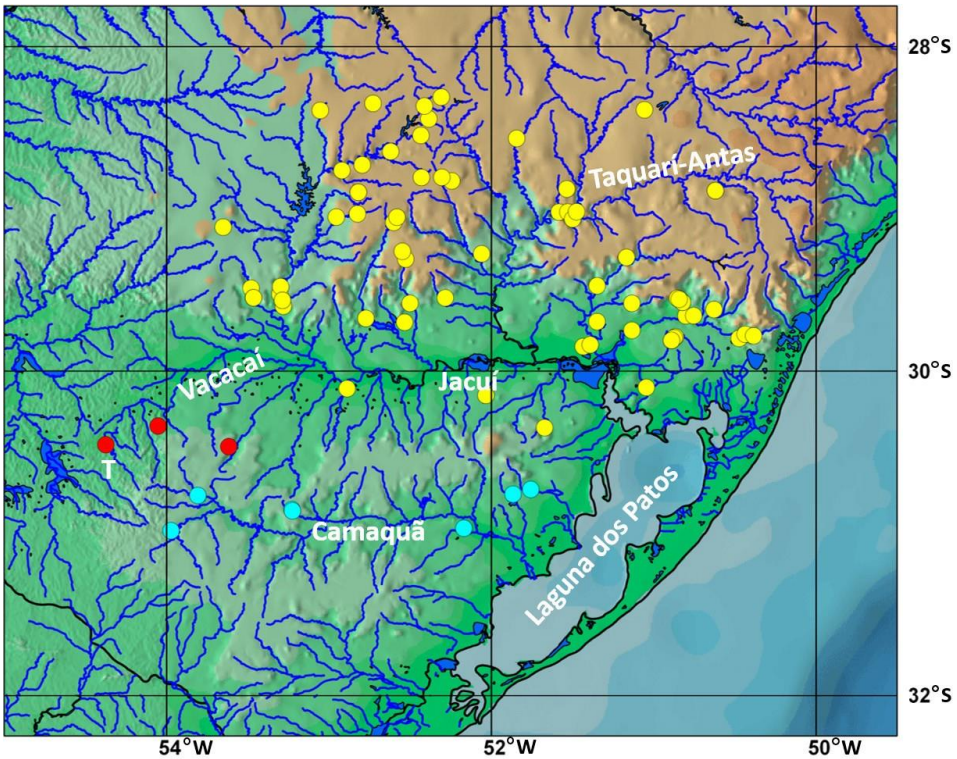

FIGURE 3 | Distribution of *Ancistrus* species in northern laguna dos Patos system. *Ancistrus* aff. *brevipinnis* (yellow), *A. brevipinnis* (turquoise), and *A. megacanthus* (red). T = type-locality.

FIGURE 4 | Arroio Cambaizinho, showing typical habitat of *Ancistrus megacanthus*.

Ancistrus brevipinnis (Regan, 1904)

(Figs. 5–7; Tab. 2)

Chaetostomus cirrhosus (non Valenciennes, 1836) Boulenger, 1891:234, pl. 26, fig. 1 (listed and illustrated from Rio Grande do Sul State, Brazil).

Xenocara brevipinnis Regan, 1904:257 (type-locality: [State of] Rio Grande do Sul, Brazil. Holotype: BMNH 1891.3.16.76, female, 79.9 mm SL). Type-locality herein restricted to the rio Camaquã basin, Rio Grande do Sul, Brazil.

Description. Morphometric data in Tab. 2. Body and head depressed. Dorsal profile of body and head convex from tip of snout to dorsal-fin origin, slightly concave to straight from that point to origin of adipose fin, concave from end of adipose fin to origin of caudal fin. Ventral profile of body straight, body height begins to decrease at pelvic girdle. Body covered with bony plates; plates absent along dorsal-fin base and ventral surface between tip of snout and anal-fin origin. Median series with 22–24(11) lateral plates; lateral-line canal complete and uninterrupted. Three (11) predorsal plates, seven (11) plates at base of dorsal fin, four (11) plates between dorsal and adipose fins and 6(1), 7(8) or 22(2), plates between adipose fins and caudal fins. Fin rays and plates covered by small, caudally directed odontodes in regular rows, larger on fin spines.

Snout rounded, with wide bare anterior margin, limited posteriorly by dermal plates of varying sizes. Cutaneous tentacles present on lateral, anterior and middorsal portions of snout of males, sometimes branched and forming "Y" pattern. In females, tentacles smaller and usually arranged in pairs on sides of snout. Eye large, 13.0–20.2% (16) of head length, located laterodorsally on head. Orbit delimited dorsally by sphenotic and frontal, anteriorly by prefrontal plate, ventrally by infraorbitals IV and V, and posteriorly by infraorbital VI. Naris located dorsally on head, with tubular expansion of skin, delimited anteriorly by nasal, posteromedially by frontal, laterally by infraorbital II and III, and posterolaterally by prefrontal plate. Exposed part of the opercle almost always triangular, rounded anteriorly. Dermal plates of different sizes and shapes arranged in the post-opercular area, contiguous to compound pterotic. Eleven to 14(11) strong retractable odontodes with sickle-shaped claws at extremity in opercular region.

Lower lip not reaching anterior margin of pectoral girdle. Lower lip with papillae randomly distributed throughout surface. Maxillary barbel short and free. Short mandibulary ramus, 12.9–19.9% (16) of head length, premaxillary tooth row of same length or slightly shorter. Teeth numerous, 34–57(11) in premaxilla and 33–53(11) in dentary, bicuspid, with reduced lateral cusp and larger, wider mesial cusp.

Dorsal-fin spine elongated, soft rays not reaching adipose fin when depressed; pectoral-fin spine reaching to half-length of pelvic-fin unbranched ray in adult males; falling short or reaching to base of pelvic fin in females. Pelvic fin reaching middle of anal-fin length. Caudal fin obliquely truncated with lower leading ray longer than upper. All branched fin rays doubly branched near apical end. Dorsal fin with II+7 rays(16); pectoral fin I+5(4) or I+6(21) rays; pelvic fin i+5(16) rays; anal fin i+4 rays (16); caudal fin i+14+i rays (16).

Sixth vertebral centrum (first after Weberian apparatus) with enlarged rib. Seventh vertebra without rib and supporting first dorsal-fin pterygiophore. Eight following vertebral centra with thin ribs and supporting dorsal-fin pterygiophores. Total vertebrae 28(1).

TABLE 2 | Morphometric data of *Ancistrus brevipinnis*. n = 16; SD = Standard deviation.

	Min	Max	Mean	SD
Standard length (mm)	35.8	81.3	58.6	–
Percent of standard length				
Abdominal length	17.5	23.3	19.6	1.5
Adipose fin to caudal fin distance	16.2	22.9	19.8	2.0
Adipose-fin spine length	7.5	12.5	9.9	1.3
Anal fin to caudal fin distance	31.8	37.4	34.1	1.4
Anal-fin unbranched ray length	5.9	10.4	8.4	1.1
Body depth at dorsal-fin origin	14.4	19.3	17.1	1.6
Body width at dorsal-fin origin	26.3	32.8	29.8	1.9
Caudal peduncle depth	28.1	35.9	32.4	2.3
Caudal peduncle length	9.7	12.8	10.6	0.7
Cleithral width	31.6	35.8	33.3	1.3
Dorsal fin to adipose fin distance	12.1	18.2	15.0	1.9
Dorsal-fin base length	18.6	24.7	21.2	1.8
Dorsal-fin spine length	21.5	26.5	23.9	1.4
Head length	32.7	39.6	35.5	2.4
Interbranchial distance	16.2	20.8	18.4	1.4
Lower caudal-fin ray length	24.1	32.5	29.1	2.2
Pectoral-fin spine length	27.6	32.8	30.1	1.9
Pectoral fin to pelvic fin distance	21.2	25.7	24.0	1.3
Pelvic-fin unbranched ray length	21.7	27.8	24.0	1.6
Predorsal length	39.8	49.2	45.2	2.8
Supracleithral width	26.5	33.4	29.3	2.2
Upper caudal fin ray length	19.9	24.7	22.2	1.2
Percent of head length				
Head depth at opercle	39.8	48.8	44.8	2.4
Internostril distance	14.8	21.4	17.7	1.8
Interorbital distance	35.6	44.2	39.3	2.5
Mandibulary ramus	12.9	19.9	15.9	2.1
Snout tip to ventral branchial opening	61.5	71.2	66.4	3.2
Orbital diameter	13.0	20.2	16.2	2.0
Snout length	53.6	62.9	58.5	2.6
Count			**Mode**	
Premaxillary teeth	34	57	40	
Dentary teeth	33	53	41	
Plates in dorsal lateral series	20	22	21	
Plates in median lateral series	22	24	23	

Coloration in alcohol. Dorsal and ventral region of body brown mottled with darker brown to gray patches. Well-defined, small yellow to light brown dots on dorsal and lateral region of body, sometimes in ventral region, smaller on head and somewhat vermiculated on flanks and abdomen. Dark brown dots on fin rays and sometimes interradial membrane of all fins. Upper and lower tips of caudal-fin leading rays with small light termination (Fig. 5).

FIGURE 5 | *Ancistrus brevipinnis*, dorsal, lateral and ventral views, MCP 25902, female, 65.9 mm SL, Brazil, Rio Grande do Sul, Lavras do Sul, rio Marmeleiro, laguna dos Patos system.

FIGURE 6 | Holotype of *Ancistrus brevipinnis*, BMNH 1891.3.16.76, female, 79.9 mm SL. Rio Grande do Sul, Brazil. Photograph inverted horizontally. Photo by the ACSI Project image.

Coloration in life. Body with complex distribution of pigments, yellowish brown and brown background mottled with darker brown to gray patches and four or five big, light yellow spots dorsally. Caudal fin preceded by slanted light brown to yellow band. Head and ventral surface of body covered with light yellow dots. Dorsal, pectoral and pelvic fins yellowish brown with inconspicuous darker dots. Anal and caudal fin mostly plain light brown, upper and lower tips of caudal-fin leading and outer branched rays with small white or light yellow termination (Fig. 7).

Geographical distribution. *Ancistrus brevipinnis* is believed to occur in most of the laguna dos Patos tributaries (Fig. 3), but specimens used to prepare this redescription are those from the restricted type-locality, the rio Camaquã basin.

FIGURE 7 | Live color pattern of *Ancistrus brevipinnis*. Specimens not preserved. *Hemiancistrus punctulatus* on background.

Ecological notes. Specimens of *A. brevipinnis* are usually found in shallow and wide creeks and rivers, predominantly formed by pebbles and rocks and swift waters.

Previously undetected diversity in the laguna dos Patos system. During the present study we observed that populations commonly identified as *Ancistrus brevipinnis* have remarkable morphological variation throughout the laguna dos Patos system, which suggests the existence of undetected diversity. Some of the observed variation includes the structure of the predorsal plates, which may be present as two or three series between the supraoccipital and the dorsal fin; the length of the pectoral-fin spine, which varies from falling short of the pelvic-fin base to reaching half or even exceeding half length of that fin; the number of branched rays in the pectoral fin (5 or 6); a variation in the relationship between height and length of the caudal peduncle; the body coloration, which varies from grayish brown with yellow spots evenly distributed on body, to very dark brown with barely visible yellow dots, or light brown body with yellowish-brown dots to mottled brown with darker spots and light yellow spots; the fin coloration, ranging from black without spots to dark brown with yellow spots, sometimes restricted to rays and spines, and sometimes on interradial membranes; and the tip of the adipose- fin spine, occasionally with an evident white dot.

To deal with this variation, morphometric and meristic data were obtained separately for specimens from several sub-basins of the laguna dos Patos system (Tabs. 1–3) and neighboring basins (Tab. 4). Linear Discriminant Analysis of the morphological data from these different populations resulted in three discrete groups (Fig. 8). A well-isolated group formed by *Ancistrus brevipinnis* from the rio Camaquã sub-basin and the new species described above, *A. megacanthus*, from the rio Vacacaí sub-basin. A second cluster with the remaining *A. brevipinnis* populations analyzed (herein referred to as *Ancistrus* aff. *brevipinnis*) and *A. taunayi* from the rio Uruguay, and a third cluster with the species inhabiting the coastal rivers, *A. multispinis*. The LD1 axis had the caudal peduncle length (0.40) as the most strongly positive value and the head length (-0.32) as the most strongly negative value. The LD2 axis had the body width at dorsal-fin origin (0.48) as the most strongly positive variable and the caudal-peduncle length (-0.68) as the most strongly negative variable. Further on supporting the distinctiveness of the new species, this analysis also suggests that the *A. brevipinnis* population from the rio Camaquã basin represents a species distinct from the more northern tributaries of the laguna dos Patos.

TABLE 3 | Comparative morphometric data of *Ancistrus* aff. *brevipinnis*. n = number of specimens; SD = Standard deviation.

	Rio Pardo n = 13				Rio Taquari n = 38				Rio Caí n = 19				Rio dos Sinos n = 28			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Standard length (mm)	37.8	89.2	65.7	–	44.9	101.8	70.0	–	42.1	101.2	70.8	–	47.4	111.3	70.1	–
Percent of standard length																
Abdominal length	18.0	20.9	19.3	0.7	11.8	21.1	18.5	1.7	16.6	21.2	18.7	1.3	10.3	21.6	18.5	2.0
Adipose fin to caudal fin distance	17.0	22.3	19.5	1.4	14.1	23.1	18.3	1.9	11.9	22.9	18.6	2.5	15.7	22.0	19.4	1.2
Adipose-fin spine length	8.9	13.9	10.7	1.5	6.2	14.6	10.6	1.5	8.4	14.1	11.2	1.7	8.8	13.0	10.5	1.1
Anal fin to caudal fin distance	29.9	35.8	33.0	1.8	28.8	35.1	32.1	1.6	29.9	36.2	33.5	2.0	30.2	36.5	33.6	1.6
Anal-fin unbranched ray length	5.5	9.3	7.9	1.0	5.5	11.5	8.4	1.2	5.5	12.2	8.5	1.4	6.8	10.3	8.5	0.9
Body depth at dorsal-fin origin	15.7	23.1	19.1	2.3	12.6	22.6	18.3	1.9	13.7	22.9	17.5	2.0	13.9	21.5	17.0	1.6
Body width at dorsal-fin origin	27.0	34.0	31.1	2.0	25.4	33.6	30.5	1.8	26.6	34.0	30.1	2.0	26.9	32.4	29.5	1.4
Caudal peduncle depth	30.1	36.8	32.6	2.1	28.1	35.9	31.8	1.9	30.1	35.5	32.6	1.4	26.7	34.8	31.6	2.2
Caudal peduncle length	9.3	11.7	10.2	0.8	8.4	11.6	10.5	0.7	8.8	12.0	10.3	0.9	8.5	12.2	10.1	0.7
Cleithral width	31.7	36.1	33.1	1.2	28.4	35.5	33.5	1.5	26.4	35.0	32.4	2.1	29.7	34.4	32.3	1.4
Dorsal fin to adipose fin distance	11.0	14.8	12.9	1.1	10.7	16.7	13.5	1.5	10.3	16.0	13.3	1.5	10.7	15.8	12.8	1.4
Dorsal-fin base length	19.8	23.3	21.5	1.1	19.1	23.6	21.1	1.2	19.1	25.0	21.2	1.5	19.4	23.6	21.2	1.2
Dorsal-fin spine length	20.5	25.1	22.9	1.4	18.6	25.8	22.5	1.8	20.7	26.0	23.2	1.6	20.7	25.5	22.9	1.4
Head length	29.1	38.3	33.9	2.2	30.2	37.7	34.2	1.9	30.2	36.2	33.0	1.5	30.3	36.0	33.2	1.5
Interbranchial distance	15.9	20.4	17.9	1.3	14.3	20.6	18.5	1.3	14.5	21.0	18.0	1.7	12.3	20.1	17.5	1.6
Lower caudal-fin ray length	23.4	37.2	27.5	3.7	19.8	29.7	26.4	2.5	24.2	32.8	27.5	2.2	19.4	30.9	26.7	2.0
Pectoral-fin spine length	26.2	32.5	30.0	1.8	25.1	33.6	29.1	2.1	26.0	33.4	29.4	1.9	26.0	33.2	29.5	1.9
Pectoral fin to pelvic fin distance	21.3	25.7	23.5	1.2	17.1	26.9	23.0	1.9	19.1	25.7	23.1	1.6	18.4	26.5	22.7	2.0
Pelvic-fin unbranched ray length	21.2	25.8	23.5	1.3	17.8	27.1	23.1	2.1	19.2	25.7	23.0	1.8	21.1	27.0	23.6	1.4
Predorsal length	40.9	49.6	44.6	2.0	41.2	48.4	44.5	1.8	41.8	46.8	43.9	1.5	40.2	46.9	43.9	1.7
Supracleithral width	26.2	29.5	27.9	1.1	24.6	32.3	29.2	1.7	25.2	32.4	28.5	2.0	25.0	30.1	27.9	1.4
Upper caudal fin ray length	19.0	26.0	21.5	1.9	16.7	24.8	20.7	2.1	19.2	29.7	22.5	2.2	18.8	24.5	22.0	1.4
Percent of head length																
Head depth at opercle	43.0	51.8	48.2	3.3	42.1	52.6	47.5	2.7	45.6	55.3	49.0	2.8	42.7	53.1	47.6	2.6
Internostril distance	13.8	26.8	18.1	3.6	16.2	22.4	17.9	1.4	17.0	25.1	20.2	2.6	15.9	24.1	19.3	2.4
Interorbital distance	33.4	51.7	38.7	4.6	33.5	44.4	39.2	2.2	37.5	44.7	40.3	2.3	36.0	43.6	39.3	2.0
Mandibulary ramus	16.5	26.1	21.4	2.6	18.5	24.2	21.3	1.5	15.2	26.1	19.9	2.7	15.3	23.1	19.7	2.3
Snout tip to ventral branchial opening	60.9	75.2	68.7	5.0	65.2	75.4	70.4	2.7	63.2	76.4	70.3	3.3	65.2	73.3	69.3	2.5
Orbital diameter	14.3	22.9	17.8	2.1	12.5	21.1	17.0	1.9	15.8	20.0	17.4	1.1	15.4	20.7	17.7	1.6
Snout length	54.3	70.1	61.0	4.8	57.9	67.7	62.5	2.7	56.0	68.3	62.1	3.7	55.3	67.0	61.8	3.2
Count	Mode				Mode				Mode				Mode			
Premaxillary teeth	39	70	55		41	82	55		33	62	39		40	61	49	
Dentary teeth	37	75	61		43	69	60		33	77	43		45	66	49	
Plates in dorsal lateral series	20	22	22		21	22	21		21	62	22		20	23	22	
Plates in median lateral series	22	25	24		23	25	24		22	25	24		23	25	24	

TABLE 3 | (Continued)

	Rio Gravataí n = 11				Lower rio Jacuí n = 26				Upper rio Jacuí n = 39			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Standard length (mm)	41.9	77.2	61.4	–	48.4	123.7	70.1	–	41.5	95.1	68.9	–
Percent of standard length												
Abdominal length	16.6	20.1	18.6	1.0	14.2	20.9	17.9	1.7	16.4	20.3	18.9	0.9
Adipose fin to caudal fin distance	15.1	20.5	18.8	1.7	15.1	22.2	19.0	1.8	16.5	22.4	19.2	1.3
Adipose-fin spine length	8.0	12.3	10.1	1.4	8.4	12.8	10.6	1.2	6.8	14.6	10.3	1.5
Anal fin to caudal fin distance	32.1	38.5	34.0	2.1	26.3	35.5	32.4	2.0	30.3	36.6	33.1	1.7
Anal-fin unbranched ray length	6.6	9.4	8.0	0.8	5.2	9.9	7.6	1.3	5.5	10.8	8.1	1.0
Body depth at dorsal-fin origin	12.5	21.7	15.7	2.4	15.1	22.0	17.6	1.9	14.4	22.3	18.0	1.7
Body width at dorsal-fin origin	28.1	31.8	29.6	1.1	27.0	32.7	30.0	1.7	27.9	33.5	30.5	1.3
Caudal peduncle depth	29.8	35.7	32.0	1.8	27.5	35.5	31.7	2.2	28.9	35.8	32.4	1.6
Caudal peduncle length	8.5	9.9	9.3	0.4	8.9	12.4	10.2	1.0	9.4	12.2	10.5	0.6
Cleithral width	30.0	32.8	31.6	0.8	30.8	36.1	32.8	1.5	30.7	35.4	32.9	1.2
Dorsal fin to adipose fin distance	9.4	15.5	13.6	1.8	10.5	17.0	13.4	1.7	9.9	16.6	13.7	1.6
Dorsal-fin base length	18.9	21.4	20.2	0.9	17.5	24.2	20.7	1.8	16.8	22.5	20.3	1.2
Dorsal-fin spine length	19.4	23.8	22.1	1.2	15.6	24.9	22.4	2.0	19.9	26.3	22.9	1.6
Head length	30.1	35.9	33.6	1.8	29.5	36.0	33.2	1.5	30.2	38.4	33.3	1.8
Interbranchial distance	14.4	19.2	17.1	1.3	14.3	20.9	17.8	1.8	15.5	21.4	18.4	1.2
Lower caudal-fin ray length	20.0	29.5	26.6	3.1	23.1	29.9	26.6	2.0	22.2	31.5	27.3	2.0
Pectoral-fin spine length	25.6	30.4	28.7	1.7	26.3	35.3	29.9	2.2	25.9	32.8	29.4	1.6
Pectoral fin to pelvic fin distance	21.1	26.2	24.0	1.4	18.0	25.8	22.8	1.7	18.3	25.7	22.8	1.4
Pelvic-fin unbranched ray length	20.1	23.7	21.8	0.8	19.8	27.6	23.1	1.9	20.5	26.4	23.5	1.4
Predorsal length	40.3	45.9	43.6	1.7	41.5	48.1	44.6	1.8	41.5	48.4	44.0	1.6
Supracleithral width	25.7	29.6	27.4	1.2	26.0	32.5	28.6	1.9	26.4	31.9	28.6	1.3
Upper caudal fin ray length	17.2	26.3	21.5	3.0	17.4	24.6	21.0	1.9	17.7	24.9	21.4	1.5
Percent of head length												
Head depth at opercle	42.9	48.7	45.6	2.3	43.1	54.0	47.6	3.0	43.7	57.4	48.8	3.3
Internostril distance	18.0	25.2	21.3	2.1	15.3	24.6	19.2	2.3	14.7	20.5	17.4	1.6
Interorbital distance	33.8	41.1	38.0	2.3	36.9	46.3	41.0	2.3	33.2	44.4	39.2	2.8
Dentary width	14.9	21.4	18.3	2.0	16.4	26.4	21.1	2.9	17.2	24.6	19.8	1.6
Snout tip to ventral branchial opening	61.4	70.1	65.9	2.8	65.0	75.8	70.3	3.6	61.6	78.8	69.8	3.0
Orbital diameter	16.1	20.9	18.6	1.5	13.2	20.2	17.0	1.6	14.1	21.7	17.7	1.6
Snout length	56.1	62.3	59.1	1.8	57.4	67.7	62.0	2.8	56.1	68.3	62.2	3.3
Counts	Mode				Mode				Mode			
Premaxillary teeth	40	54	48		36	71	51		41	60	52	
Dentary teeth	40	54	47		44	67	63		42	66	49	
Plates in dorsal lateral series	21	22	22		21	22	21		21	22	22	
Plates in median lateral series	23	25	24		21	25	24		24	25	24	

TABLE 4 | Morphometric data of *Ancistrus taunayi* and *A. multispinis*. n = number of specimens; SD = Standard deviation.

	A. taunayi n = 18				*A. multispinnis* n = 11			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Standard length (mm)	48.3	100.9	74.4	–	45.5	112.5	83.6	–
Percent of standard length								
Abdominal length	16.6	20.1	18.5	1.1	15.8	21.2	18.8	1.6
Adipose fin to caudal fin distance	17.3	20.8	18.7	1.1	18.6	22.3	20.2	1.2
Adipose-fin spine length	8.9	13.3	10.6	1.4	9.9	13.0	11.3	1.1
Anal fin to caudal fin distance	30.2	35.9	33.3	2.1	33.3	36.5	34.7	1.0
Anal-fin unbranched ray length	6.8	12.2	8.5	1.6	6.3	10.6	9.0	1.4
Body depth at dorsal-fin origin	15.9	19.8	17.7	1.4	16.3	22.6	18.9	1.8
Body width at dorsal-fin origin	27.8	32.4	30.1	1.9	28.2	33.0	30.1	1.6
Caudal peduncle depth	8.8	11.5	10.0	0.9	10.6	12.9	12.1	0.7
Caudal peduncle length	30.1	34.1	32.3	1.3	30.5	36.4	33.4	1.7
Cleithral width	30.3	34.1	32.1	1.6	32.8	37.5	34.8	1.5
Dorsal fin to adipose fin distance	12.5	16.0	14.1	1.1	11.4	16.2	13.3	1.2
Dorsal-fin base length	20.0	22.6	21.2	0.9	19.6	24.4	22.3	1.4
Dorsal-fin spine length	21.1	25.3	22.9	1.3	22.4	27.8	24.9	1.7
Head length	30.2	33.3	32.2	0.9	32.0	37.8	34.4	1.8
Interbranchial distance	14.5	20.1	17.2	1.5	17.4	20.4	18.9	1.0
Lower caudal-fin ray length	24.7	30.4	27.7	2.1	23.5	31.8	27.3	2.7
Pectoral-fin spine length	28.1	32.9	29.9	1.5	24.6	36.5	31.2	3.1
Pectoral fin to pelvic fin distance	21.1	25.7	23.6	1.6	19.8	26.0	23.0	1.6
Pelvic-fin unbranched ray length	21.0	25.3	22.8	1.4	21.4	29.1	25.1	2.0
Predorsal length	41.8	45.7	43.8	1.3	41.4	47.7	44.6	2.1
Supracleithral width	25.2	29.7	27.1	1.6	27.8	32.2	29.6	1.5
Upper caudal fin ray length	19.9	24.3	22.2	1.3	18.8	25.5	21.8	1.9
Percent of head length								
Head depth at opercle	47.6	53.1	50.0	2.2	45.7	52.4	49.0	1.9
Internostril distance	17.0	22.8	19.8	2.0	15.8	18.7	17.3	1.0
Interorbital distance	38.0	43.3	40.8	2.1	31.9	39.3	35.9	2.1
Mandibulary ramus	15.2	20.2	18.3	1.5	19.1	23.9	21.6	1.6
Snout tip to ventral branchial opening	64.9	70.6	68.8	1.9	65.7	76.3	70.4	3.0
Orbital diameter	15.9	20.5	17.7	1.4	13.7	19.7	17.3	1.6
Snout length	58.8	64.6	62.2	1.7	54.5	67.7	62.1	4.4
Count	Mode				Mode			
Premaxillary teeth	46	71	69		44	70	57	
Dentary teeth	44	76	64		40	77	59	
Plates in dorsal lateral series	21	22	21		22	21	21	
Plates in median lateral series	20	23	23		21	23	23	

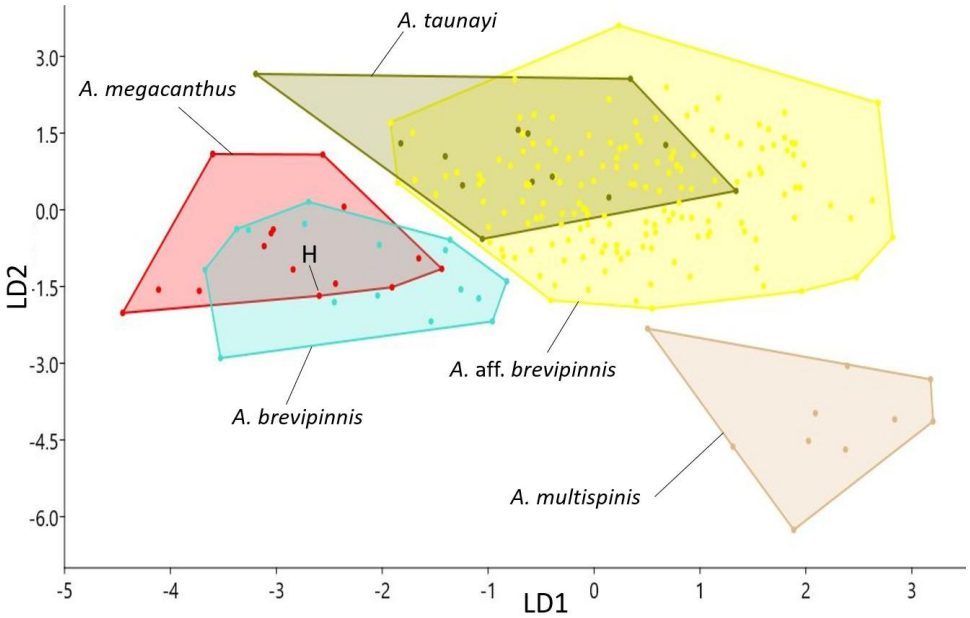

FIGURE 8 | Linear Discriminant Analysis of *Ancistrus* species in northern laguna dos Patos system. H = holotype of *A. megacanthus*.

A molecular comparison of the gene coI of those populations and other geographically close species of *Ancistrus* revealed the same pattern as the morphometric analysis. The genetic distance between the three specimens of *Ancistrus brevipinnis* from the rio Camaquã and the *A.* aff. *brevipinnis* from the Jacuí basin ranged from 2.7 to 3.2%, numbers similar or greater than the distance between the other geographically close species (Tab. 5). A Maximum Likelihood tree of the same sequences shows the *A. brevipinnis* from the rio Camaquã more closely related to *A. megacanthus* (Fig. 9), while the *A.* aff. *brevipinis* was revealed more closely related to *A. taunayi* from the rio Uruguay basin. The tree species was rooted in *A. aguaboensis* from the Amazon basin.

Material examined. Brazil, Rio Grande do Sul, laguna dos Patos system, rio Camaquã basin: MCP 25948, 21, 22.9–51.3 mm SL (1 measured, 51.3mm SL), rio dos Carros, Lavras do Sul, 30°46'02.42"S 53°48'23.04"W. MCP 25902, 11, 24.8–65.9 mm SL (2 measured, 51.0–55.2 mm SL) + 1 c&s, 56.0 mm SL, rio Marmeleiro, Lavras do Sul, 30°59'06"S 53°58'20"W. MCP 23781 7, 31.2–65.7 mm SL (4 measured, 60.1–65.7 mm SL), rio do Engenho, tributary to rio Velhaco, Sentinela do Sul, 30°43'48"S 51°45'24"W. MCP 54896, 4, 2 tis, 50.0–67.5 mm SL (2 measured, 65.2–67.5 mm SL), rio do Meio, Cristal, 30°58'10.81"S 52°10'07.42"W. UFRGS 21299, 1, 50.7 mm SL (measured), creek tributary to rio das Neves, Santana da Boa Vista, 30°51'17"S 53°13'38"W,

UFRGS 21300, 5, 27.3–72.0 mm SL (4 measured, 57.5–72.0 mm SL), rio das Neves, Santana da Boa Vista, 30°51'45.1"S 53°13'27.8"W, UFRGS 22124, 2, 74.7–81.3 mm SL (2 measured), rio das Neves, Santana da Boa Vista, 30°51'45.1"S 53°13'27.8"W. MCP 54985, 1 tis, arroio Bonito, tributary to arroio Velhaco, 30°43'50.87"S 51°45'23.05"W.

TABLE 5 | Genetic distance between sequences of *Ancistrus* species from Rio Grande do Sul, conducted using the Kimura 2-parameter model with rate variation among sites modeled with a gamma distribution.

		1	2	3	4	5	6	7
1	*A.* aff. *brevipinnis* Gravataí MCP 54885							
2	*A.* aff. *brevipinnis* Sinos MCP 54882	0.003						
3	*A. brevipinnis* Camaquã MCP 54895	0.027	0.030					
4	*A. brevipinnis* Camaquã MCP 54896	0.029	0.032	0.005				
5	*A. brevipinnis* Camaquã MCP 54896	0.029	0.032	0.005	0.000			
6	*A. megacanthus* Vacacaí MCP 54883	0.027	0.030	0.018	0.017	0.017		
7	*A. taunayi* Santa Maria MCP 43914	0.023	0.027	0.022	0.023	0.023	0.025	
8	*A. multispinis* Maquiné UFRGS 18448	0.019	0.022	0.020	0.020	0.020	0.015	0.018

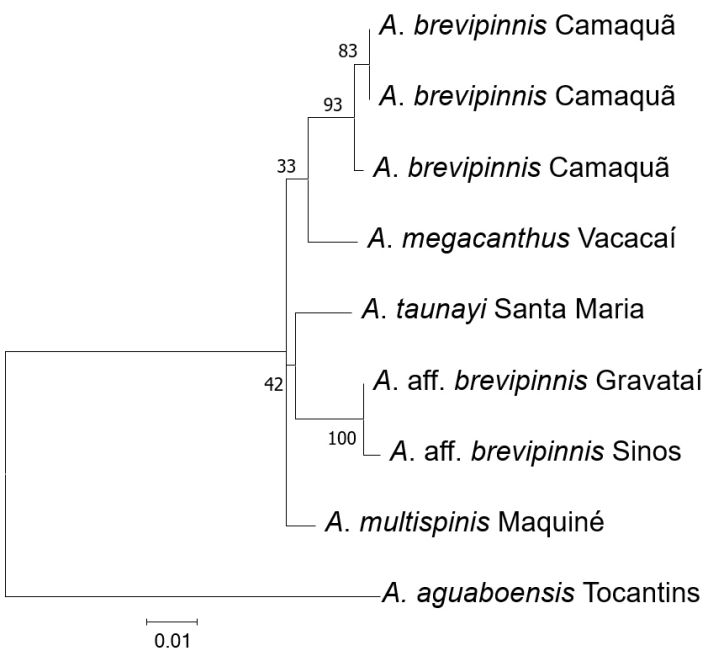

FIGURE 9 | Maximum likelihood tree of samples of *Ancistrus* from Rio Grande do Sul, using General Time Reversible model with Gamma distribution and invariant sites (47.5%). Log likelihood -1230,53.

Genseq-3 coI. Sequence deposited in GenBank (MCP 54895, accession code OR242765, and MCP 54895, accession codes OR242766 and OR242767).

DISCUSSION

Regan (1904) described *Xenocara brevipinnis* with "Rio Grande dO Sul" as the type-lOcality and later Malabarba (1989) restricted the type-lOcality tO the laguna dOs PatOs system. In this study, we further restrict the type-lOcality Of *A. brevipinnis* tO the rio Camaquã sub-basin of the laguna dOs PatOs system, based On histOrical recOrds Of the cOllections Of Sebastian WOlff and Hermann vOn Ihering, cOllectOrs Of the hOlOtype currently depOsited at the Natural HistOry Museum in LOndOn (BMNH). AccOrding tO the catalOg recOrds Of the NHM the shipments Of freshwater fishes cOntaining the type of *A. brevipinnis* sent by WOlff and vOn Ihering tO LOndOn came mainly frOmthe rio Camaquã and laguna dOs PatOs in the municipality Of São LOurençO dO Sul, State of Rio Grande dO Sul, Brazil. This infOrmation is reinfOrced by the fact that vOn Ihering and WOlff have lived fOr years in the municipality Of São LOurençO dOSul near the mOuth Of the rio Camaquã and cOnducted several surveys in the region. BOulenger (1891) pOssibly cOrrObOrates this statement in a study in which he organized an annotated list Of siluriforms cOllected by WOlff and vOn Ihering in the State of Rio Grande dO Sul, in which all species are easily recOgnized as current inhabitants Of the rio Camaquã basin and nearby waterways (*Pimelodella australis* Eigenmann, 1917 [listed as *Pimelodus lateristriga* Müller & TrOschel, 1849], *Parapimelodus nigribarbis* (BOulenger, 1889), *Pimelodus maculatus* Lacepède, 1803, *Rhamdia quelen* (QuOy & Gaimard, 1824), *Microglanis cottoides* (BOulenger, 1891), *Heptapterus mustelinus* (Valenciennes, 1835), *Genidens barbus* (Lacepède, 1803) [listed as *Arius commersonii* (Lacepède, 1803)], *Genidens genidens* (Cuvier, 1829) [listed as *Genidens cuvieri* Castelnau, 1855], *Callichthys callichthys* (Linnaeus, 1758), *Corydoras paleatus* (Jenyns, 1842), *Loricariichthys anus* (Valenciennes, 1835), *Rineloricaria* sp. [listed as *Loricaria lima* Kner, 1853], *Hisonotus nigricauda* (BOulenger, 1891), *Hypostomus spiniger* (Hensel, 1870) [listed as *Plecostomus commersonii* Eigenmann & Eigenmann, 1888], *Chaetostomus cirrhosus* (Steindachner, 1881) [reidentified as *Ancistrus brevipinnis* by Regan, 1904], *Pseudobunocephalus iheringii* (BOulenger 1891), *Ituglanis australis* Datovo & de Pinna, 2014 [listed as *Trichomycterus brasiliensis* Lütken, 1874], and *Scleronema minutum* (BOulenger, 1891)).

BOth the mOrphOmetric and mOlecular cOmparisOns we made with pOpulations Of what is currently known as *Ancistrus brevipinnis* (Fig. 8; Tab. 3) suggest that the pOpulation in the rio Camaquã is distinct frOm the pOpulations inhabiting the mOre northern tributaries Of the laguna dOs PatOs system, namely the rio Jacuí basin. FOr this reasOn, we prOvided the redescription of *A. brevipinnis* based On specimens frOm the rio Camaquã alOne. We cOntinue tO wOrk On a cOmplete review Of *Ancistrus* in the laguna dOs PatOs system, as there might exist additional undescribed diversity. Only a clear understanding of the diversity Of *Ancistrus* in the laguna dOs PatOs system will permit these species tO be cOrrectly evaluated fOr their extinction risk and adequately prOtected.

Comparative material examined. All from Brazil. Rio Grande do Sul: laguna dos Patos system. *Ancistrus* aff. *brevipinnis*: Upper rio Jacuí: MCP 21216, 8, arrOio DespraiadO, SOledade, 28°48'26"S 52°25'47"W. UFRGS 22326, 1, rio dOs CaixÕes, EspumOsO, 29°01'54.4"S 52°49'25.1"W. MCP 22157, 6, arrOio ResvaladOr, NicOlau VergueirO, 28°32'54"S 52°25'59"W. MCP 22248, 5, arrOio Carreta Quebrada, PassO FundO, 28°26'48"S 52°23'04"W. MCP 22125, 3, arrOio dO POrtão, PassO FundO, 28°22'03"S 52°24'42"W. MCP 21254, 2, EspumOsO, 28°45'51"S 52°55'10"W. MCP 22762, 6, arrOio Tapiaia, Júlio de CastilhOs, 29°06'49"S

53°39′04″W. MCP 50096, 1, arroio dos Macacos, Colorado, 28°28′41″S 52°57′15″W. MCP 22747, 3, rio Pinheirinho, Saldanha Marinho, 28°23′39″S 53°03′11″W. UFRGS 16737, 1, rio Quati, Mormaço, 28°38′52″S 52°37′11″W. MCP 22180, 2, rio Jacuí, Passo Fundo, 28°18′45″S 52°18′28″W. UFRGS 19984, rio Turvo, Espumoso, 28°43′47″S 52°47′40″W. MCP 22129, 2, rio da Glória, Santo Antônio do Planalto, 28°21′03″S 52°43′40″W. UFRGS 23502, 1, rio Morcego, Espumoso, 28°53′55″S 52°49′06″W. UFRGS 23505, 1, rio Morcego, Espumoso, 28°53′55″S 52°49′06″W. Lower rio Jacuí: MCP 21220, 4, Agudo, 29°31′35″S 53°18′08″W. MCP 22735, 3, arroio do Tigre, Nova Palma, 29°29′25″S 53°28′45″W. MCP 26559, 5, arroio Caembora, Nova Palma, 29°28′50″S 53°17′50″W. MCP 22721, 3, creek tributary to rio Soturno, Faxinal do Soturno, 29°32′55″S 53°27′53″W. MCP 21246, 2, arroio Linha das Flores, Agudo, 29°36′02″S 53°16′49″W. MCP 21316, 3, arroio Corupá, Agudo, 29°33′54″S 53°17′09″W. MCP 17336, 3, arroio Taquara, Minas do Leão, 30°09′02″S 52°01′58″W. MZU 2779, 1, rio Jacuí, Cachoeira do Sul, 30°06′32.7″S 52°53′14.7″W. MCP 26916, 3, arroio Patrício, Mariana Pimentel, *ca*. 30°21′S 51°36″W. UFRGS 6595, 1, arroio Corupá, Agudo, 29°33′54″S 53°17′09″W. UFRGS 20354, 1, arroio Giuliani, Faxinal do Soturno, 29°32′56″S 53°27′50″W. **Rio Caí:** MZU 2334, 1, Capela de Santana, 29°39′29.7″S 51°20′53.9″W. MCP 26011, 3, arroio Bom Jardim, Triunfo, *ca*. 29°51′S 51°26′W. MCP 11474, 3, arroio do Ouro, Feliz, *ca*. 29°18′S 51°10′W. MCP 26010, 3, arroio Bom Jardim, Triunfo, 29°50′19″S 51°23′25″W. MZU 2783, 3, Bom Princípio, 29°28′31.9″S 51°20′51.9″W. Rio Gravataí: MZU 995, 1, MZU 1021, 1, MZU 1190, 1, MZU 1549, 1, arroio Demétrio, Gravataí, 29°47′26.4″S 50°51′55.6″W. MZU 853, 1, MZU 992, 1, MZU 1034,1, MZU 1323, 1, arroio Demétrio, Gravataí, 29°47′46.4″S 50°52′21.6″W. MZU 818, 1, MZU 1046, 1, arroio Demétrio, Gravataí, 29°48′47.4″S 50°53′21.2″W. MCP 15024, 2, MCP 15464, 1, arroio Fiuza, Viamão, 30°06′07″S 51°02′41″W. MCP 54885, 1 tis, arroio Demétrio, Morungava, 29°48′43.4″S 50°53′21.2″W. Rio Pardo: MCP 21569, 2, rio Pardinho, Boqueirão do Leão, 29°18′39″S 52°31′38″W. MCP 21568, 1, arroio José Simão, Gramado Xavier, 29°15′47″S 52°32′51″W. MCP 18625, 1, rio Pardo, Candelária, 29°40′36″S 52°46′19″W. MCP 18652, 2, arroio Andreas, Vera Cruz, *ca*. 29°42′S 52°32′W. MCP 32503, 1, rio Pardinho, Santa Cruz do Sul, *ca*. 29°35′S 52°30′W. Rio dos Sinos: MCP 17634, 3, arroio do Carvalho, Caraá, 29°47′58″S 50°28′23″W. MCP 17625, 3, rio dos Sinos, Caraá, 29°46′27″S 50°26′08″W. MCP 17629, 1, rio dos Sinos, Caraá, *ca*. 29°47′S 50°23′W. MZU 2257, 1, rio dos Sinos, Taquara, 29°39′37.0″S 50°48′02.4″W. MCP 20163, 3, MCP 20168 5, MCP 21204, 6, arroio Feitoria, Ivoti, *ca*. 29°35′S 51°08′W. MZU 3172, 3, rio Paranhana, Igrejinha, 29°33′29.92″S 50°50′33.05″W. MZU 1339, 1, MZU 2439,2, arroio Solitária, Igrejinha, 29°33′10.91″S 50°50′56.39″W. MZU 1109, 2, arroio Solitário, Igrejinha, 29°33′10.26″S 50°51′12.28″W. MZU 1253, 2, arroio Solitário, Igrejinha, 29°33′45.60″S 50°50′12.28″W. MZU 942, 1, arroio Solitário, Igrejinha, 29°34′37.84″S 50°49′16.49″W. MCP 2201, 3, arroio Solitário, Igrejinha, 29°34′37.26″S 50°49′17.83″W. MZU 2285, 2, rio da Ilha, Taquara, 29°33′13.99″S 50°37′38.39″W. MZU 1415, 1, MZU 840, 1, rio dos Sinos, São Leopoldo, *ca*. 29°45′S 51°08′W. MCP 2270, 3, arroio Grande, Taquara, 29°45′10.2″S 50°45′10.8″W. MCP 54882, 1 tis, arroio Solitário, tributary to rio Paranhana, Igrejinha, 29°33′29.9″S 50°50′32.9″W. Rio Taquari-Antas: MCP 32351, 1, arroio São Tomé, São Francisco de Paula, 28°53′20.78″S 50°37′08.42″W. MCP 26759, 3, arroio Pessegueiro, Barros Cassal, 29°05′04″S 52°36′02″W. MCP 17501, 3, arroio Castelhano, Venâncio Aires, 29°32′59″S 52°16′59″W. MCP 21212, 7, arroio Jeremias, Arvorezinha, 28°49′44″S 52°14′35″W. MCP 22208, 6, arroio Jaboticaba, Vila Flores, 28°52′43″S 51°32′05″W. MCP 21676, 3, arroio Jabuticaba, Veranópolis, 29°01′15″S 51°34′55″W. MCP 21205, 4, arroio Três Pontes, Arvorezinha, 28°48′24″S 52°18′14″W. MCP 25676, 3, rio Fão, Barros Cassal, 29°03′09″S 52°34′50″W. MCP 25572, 3 arroio Jabuticaba, Veranópolis, 29°01′13″S 51°31′41″W. MCP 22786, 1, arroio Espeto, Muitos Capões, 28°23′26″S 51°03′22″W. MCP 37936,1, rio das Antas, Veranópolis, 29°03′50″S 51°29′55″W. MCP 50178, 2, rio Taquari, Travesseiro, 29°16′42″S 52°03′33″W. MCP 32351, 2, arroio São Tomé, Capão Alto, 28°53′20.78″S 50°37′08.42″W. MCP 22240, 1, arroio Quebra Perna, São Domingos do Sul, 28°34′01″S 51°50′36″W. MCP 37688, 1, rio das Antas, Bento Gonçalves, 29°01′08″S 51°28′28″W. **Coastal basins.** *Ancistrus multispinis*: MCP 10796, 1, rio Maquiné, Maquiné, *ca*. 29°36′S

50°17′W. MCP 13654, 10, rio Maquiné, Maquiné, *ca*. 29°40′S 50°11′W. MCP 29102, 19, arroio Garapiá, Barra do Ouro, 29°30′26″S 50°14′39″W. MCP 29133 2, arroio Bananeira, Itati, 29°26′33″S 50°11′18″W. MCP 29145, 1, arroio Forqueta, Barra do ouro, 29°32′08″S 50°12′21″W. MCP 53880, 1, arroio MorroAzul, Três Cachoeiras, 29°23′57″S 49°55′00″W. MCP 53896, 9, rio Sangão, Mampituba, 29°14′19″S 49°58′29″W. UFRGS 18448, 1 tis, creek tributary to rio Maquiné, Maquiné, 29°31′26″S 50°18′47″W. **Santa Catarina: Coastal basins.** MCP 10639, 103, rio Jordão, Nova Veneza, *ca*. 28°35′S 49°27′W. MCP 19889, 1, rio Ano Bom, Corupá, *ca*. 26°26′S 49°16′W. MCP 29277, 1, arroio Molha Côco, Praia Grande, 29°10′27″S 49°58′26″W. MCP 31539, 2, rio Lindo, Joinville, 26°11′31″S 48°55′44″W. **Rio Grande do Sul: rio Uruguay basin.** *Ancistrus taunayi*: MZU 2413, 3, arroio Taquarembó, Dom Pedrito, 30°54′32.11″S 54°35′29.65″W. MCP 34532, 3, arroio Pinheirinho, Carazinho, *ca*. 28°13′S 53°06″W. MCP 41153, 6, arroio Felício, Júlio de Castilhos, 29°19′04″S 53°37′54″W. MCP 46749, 4, rio Jaguari, Lavras do Sul, 30°49′15.8″S 54°17′56.5″W. MCP 46744, 2, rio Jaguari, Dom Pedrito, 30°37′23″S 54°26′36″W. MCP 43914, 1 tis, arroio Jaguari, tributary of rio Santa Maria, Lavras do Sul, 30°49′13″S 54°17′57″W.

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AUTHORS' CONTRIBUTION

Ronaldo L. Widholzer: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing-original draft, Writing-review and editing.

Jessica Borsoi: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing-original draft, Writing-review and editing.

Roberto E. Reis: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing-original draft, Writing-review and editing.

Pablo Lehmann A.: Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

The specimens were collected under a collection permit authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio process #24968).

COMPETING INTERESTS

The author declares no competing interests.

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SBI

3.2 Two new species of *Ancistrus* (Siluriformes: Loricariidae) hidden in the Laguna dos Patos basin, Brazil, with an assessment of their taxonomic and phylogenetic status, including a key to all congeners of the basin.

Two new species of *Ancistrus* (Siluriformes: Loricariidae) hidden in the Laguna dos Patos basin, Brazil, with an assessment of their taxonomic and phylogenetic status, including a key to all congeners of the basin.

Resumo

Devido à grande variação de ambientes da bacia da Laguna dos Patos, a revisão do gênero *Ancistrus* revelou uma grande diversidade, possivelmente devido às suas transformações recentes ao longo dos anos. Isso influencia diretamente a diversidade de espécies existentes na bacia, em função de múltiplas introduções e isolamentos ocorridos ao longo do tempo. Estudos anteriores já haviam identificado a presença de duas espécies de *Ancistrus* e um grupo complexo nesta bacia. Agora, com 44 amostras de tecidos das regiões mais distantes e isoladas da bacia e com o objetivo de delimitar este grupo, foram utilizados métodos de morfologia (n=303), árvores filogenéticas (métodos de máxima verossimilhança e Bayesiana) com os marcadores 16S, CO1 e RAG2, além da análise de redes de haplótipos. Estes procedimentos resultaram na ampliação da distribuição de *A. megacanthus*, na descrição de duas novas espécies e na formação de um grupo adicional com caracteres morfológicos comuns, porém com significativa variação genética.

Palavra Chave: Filogenia, taxonomia, peixe-cascudo, rede de haplótipos, espécie nova

Abstract

Due to the great variation of environments in the Laguna dos Patos basin, the review of the genus *Ancistrus* revealed a great diversity, likely due to recent transformations over the years. This directly affects the species diversity in the basin due to multiple introductions and isolations over time. Previous studies had already identified the presence of two species of *Ancistrus* and one group in this basin. Now, with 44 tissue samples from the most distant and isolated regions of this basin and with the aim of delineating this group, analysis with morphology (n=303), phylogenetic trees (Maximum Likelihood and Bayesian methods) with markers 16S, CO1, and RAG2, as well as haplotype network were used. These methods resulted in the expansion of the distribution of *A. megacanthus*, the description of two new species, and the formation of an additional group with common morphological characters but considerable genetic variation.

Key Words: Phylogeny, Taxonomy, Catfish, Haplotype-network, new specie

INTRODUCTION

With a total of 38 families, the family Loricariidae is the largest in terms of species numbers (1,064 spp.) within the order Siluriformes (7,446 spp.). These fish are characterized by being covered with bony plates, and possessing a ventral sucker-shaped mouth (Geerinckx *et al*., 2007). It is estimated that this family originated approximately 67 million years ago (Shimabukuro-Dias *et al*. 2004), with the highest diversity occurring in the Neotropical region.

In 1853, Kner described the group Loricata, which was subdivided into two distinct subgroups: the first, without adipose fin, included three new genera — *Loricata* and *Rhinelepis*, *Hemiodon* (currently a synonym of *Reganella*), and *Ancistrus* — and the second subgroup, *Hypostominae*, which features an adipose fin and is divided into two subdivisions: *Inermes* (or *Clypeaty*), without retractilspines, and *Lictor* (or *Ancistrus*), with retractil interopercular spines, forming the genera *Chaetostomus* and *Ancistri*. In 2004, Armbruster reviewed the phylogeny with morphological data and concluded that the family Ancistrinae was synonymous with Hypostominae..

In the state of Rio Grande do Sul (Brazil), the family Loricariidae is mainly represented by the genera *Rineloricaria* (Bleeker, 1862) and *Hisonotus* (Eigenmann and Eigenmann, 1889), distributed across three hydrographic basins: Laguna dos Patos, Mampituba, Tramadaí and Rio Uruguay. The Laguna dos Patos basin covers approximately 113,500 km² (conforme metadado da Agencia Nacional das Águas), extending along the southeastern margin of Rio Grande do Sul and eastern Uruguay. This basin is bordered to the east by the coastal rivers and in other directions by the Rio Uruguay basin. Hydrographic basins are not static formations; they are modified or created with geomorphological changes over the years, altering surface drainage patterns.

Members of the Ancistrini tribe are characterized by the sexually dimorphic, fleshy, mucous-covered tentacles on the snouts of males, which may function to attract females to nest cavities (Lujan *et al.* 2015).

Currently, the genus *Ancistrus* includes 79 valid species (Eschmeyer and Fong, 2024), with a broad distribution across Latin America, the majority of species being described from the Amazon region. In the southern part of the continent (hydrographic system of La Plata, Laguna dos Patos, Rio Uruguay, Rio Tramandaí, Rio Maquiné and Rio Mapituba) it is limited to 12 species.

Taxonomic studies have contributed to our understanding of biodiversity, yet many species remain unknown. Older publications sometimes obscure species due to a lack of

detailed locality data, absence of holotypes, and brief descriptions, sometimes in less than a paragraph or through identification keys. Revision and redescriptions have been addressing these issues, as is the case with the genus *Ancistrus*, where 13 new species have been described in the last 10 years (Eschmeyer and Fong, 2024).

The species *Ancistrus brevipinnis* was described from a single individual, with its original locality designated for the state of Rio Grande do Sul. Its distribution was restricted to the Laguna dos Patos basin by Malabarba (1989) and later redescribed with additional characters (color in life, morphology, morphometrics, and phylogeny) and increase of individuals. Based on historical records, its type locality was determined to be the microbasin of the Rio Camaquã (Widholzer *et al*. 2024). This highlighted the existence of three distinct species in Laguna dos Patos, leading to the description of a new species (*A. megacanthus* (Widholzer *et al.* 2024)) for the microbasin of the Rio Vacacaí-Mirim and identifying a different species, formed by a large group with substantial intrapopulational variation across different microbasins, designated as *A.* aff. *brevipinnis*.

This work aims to assess the taxonomic status of this group, define the boundaries between species and the population variations of each, and describe the hidden species within this complex.

MATERIALS AND METHODS

We follow De Queiroz (2007) concept of species, which defines a species as an independently evolving lineage, isolated and detectable by common characters. We analyzed 205 specimens from the collections of the Museu de Ciencias e Tecnologas da Pontificia Universidade Catolica do Rio Grande do Sul (MCP), 4 specimens from the fish collection of the Museu de Ciencias Naturais, Secretaria de Meio Ambiente e Infraestrutura (MCN), 19 specimens from the fish collection of the Universidade Federal do Rio Grande do Sul (UFRGS), 14 specimens from the Colección de Zoología de Vertebrados de la Facultad de Ciencias, Universidad de la República, Montevideo (ZVC-P), and 61 specimens from the Museum of Zoology at Unisinos (MZU); institutional abbreviations follow Sabaj (2020), except for MZU.

Morphological Analysis:

Diagnosis was performed considering congeners occurring in the hydrographic system of La Plata, Laguna dos Patos, Rio Uruguay, Rio Tramandaí, Rio Maquiné and Rio Mapituba. Fieldwork are conducted in Laguna dos Patos System (LPS) aiming to cover samples from different rivers in each sub-basinand utilized points as distant from each other as possible within each river to avoid samples from family clusters. For LPS external species, morphological data were based on publications and genetic data

collected from GenBank.

Expeditions to collect live specimens for tissue sampling and color assessment were conducted between May 2022 and July 2023, under collection license ICMBIO No. 24968.

Measurements were obtained using a digital caliper with 0.1 mm precision under a stereomicroscope when necessary. The 31 measurements and counts follow Bifi *et al*. (2009) with the addition of two measurements according to Widholzer *et al*. (2024). All measurements and counts were taken from the left side of each individual, except when this side was damaged; results are presented as a percentage of standard length (SL) or head length. For decription the value with (*) is reference of holotype, for the complex group the average are sign with ($\bar{x}$). when the measure number was less than the total, the n value was placed before the holotype data (n,X*).

In the search for diagnostic characters, qualitative features of external anatomy were analyzed (e.g., color pattern, tooth shape and arrangement, body shape, and fin shape). Osteological characters were observed in clarified and double-stained (c&s) specimens according to Taylor and Van Dyke (1985). Vertebral centra were counted only in c&s specimens and include five modified vertebral centra in the Weberian apparatus and one composite caudal centrum (PU1 + U1). Identification and counting of dermal plates follow the serial homology scheme proposed by Schaefer (1987).

Molecular analysis

Total genomic DNA was extracted from 44 tissue samples using the Blood and Tissue DNeasy Kit (Qiagen) from muscle samples fixed in 95% ethanol and stored at -20°C. DNA sequences of the mitochondrial fragment were amplified by PCR using Cytochrome c Oxidase 1 (CO1, Ward *et al*. 2005) and 16S (Palumbi *et al*. 2002), and the nuclear fragment using Recombination Activating Gene 2 (Rag2, Lujan *et al*. 2015), under the following protocol: an initial denaturation step at 95°C for 15 minutes followed by 35 cycles of 94°C for 30 seconds, annealing at 50°C (mitochondrial) or 58°C (nuclear) for 40 seconds, and extension at 72°C for 1 minute, followed by a final extension step of 5 minutes at 72°C. The PCR products were purified and sequenced at Functional Biosciences Inc., Madison, USA. All sequences for each gene were aligned independently using MUSCLE (Edgar 2004) with default parameters. Contamination tests were conducted by comparing with sequences deposited in GenBank, using BLAST (Basic Local Alignment Search Tool - Nucleotide; Altschul *et al*. 1997).

The DNA fragment sequences for the CO1 (n = 35, ~555pb), 16S (n = 32, ~580pb), and

Rag2 (n = 20, ~870) genes used in this study will be uploaded to GenBank (Chakrabarty *et al*. 2013) once this paper is accepted for publication. Additionally, the sequences of *Ancistrus cirrhosus* (GU701865.1 (Co1) + EU310442 (16S)) and *A. temminckii* (NC051963.1) were downloaded, which was used to root the phylogenetic tree.

A genetic distance matrix for CO1 was generated using MEGA v. 11 with the pairwise model. A coalescence tree was generated by Bayesian inference with the most suitable partition schemes and the best nucleotide evolution model (individualized by gene) for each partition, evaluated in the software (MrModeltest) based on the theoretical measure of the Akaike Information Criterion (AIC). Under AIC, the GTR+I+G model was selected for Co1, HKY+I for 16S and K80 for Rag2.

For phylogenetic tree, the three concatenated fragments (~1800 bp) from all samples was conducted (n=42). Bayesian analysis was performed in Beast, set to run for one hundred million interactions with sampling every 1000, using a strict clock and the Yulespeciation process.

Another phylogenetic tree was generated using Randomized Accelerated Maximum Likelihood (RAxML, Zhang *et al*. 2013) with 1,000 bootstrap pseudoreplicates, through the CIPRES supercomputing cluster (http://www.phylo.org/index.php; Miller *et al*. 2010).

For species inference the General Mixed Yule Coalescent (GMYC, Zhang *et al*. 2013) method was implemented in R (R Core Team 2013) using a script adapted from François Michonneau (https://francoismichonneau.net/gmyc-tutorial/) with all samples concatened (n=37) and just with Co1 (n=35), we used on Assemble Species by Automatic Partitioning (ASAP) to assist in final species delimitation, with searches conducted via the online server (https://bioinfo.mnhn.fr/abi/public/asap) using the Jukes-Cantor (JC69) model with localsamples (n=35). Species inference from all Co1 samples (n=37) was conducted using Automatic Barcode Gap Discovery (ABGD) on the online platform (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the K80 model, setting intraspecific divergence to a minimum of 0.001 and a maximum of 0.1, and keeping the barcode gap default (X = 1.5). Additionally the Poisson Tree Processes (bPTP) method was also applied to the 14 samples of Co1 that formed an isolated clade in the ML tree on web on-line (//species.h-its.org/).

Haplotype phases of mitochondrial sequences were estimated with PHASE (Stephens and Donnelly 2003), with a haplotype network constructed for the mitochondrial gene 16S (n=32) and another for the mitochondrial gene CO1 (n=35), and subsequently with the two concatenated genes (n=27). Haplotype data were obtained with DnaSP v5 (Librado and Rozas 2009). Haplotype networks were constructed with PopArt (Leigh and Bryant 2015) using the most conservative Median Joining Network model (Bandelt *et al.* 1995).

RESULTS

When BLAST was performed, all molecular extractions were closely related to *Ancistrus*, confirming the absence of contamination. The Bayersiana (Figure 1) and the ML (Figure 2) trees present the same clades, but differences in the structure. For the automatic new species tests, ABGD indicated a single lineage including the outgroups. The model of bPTP present two results in Highest Bayesian support solution six species being them sp1 Mirin lagoon, sp2 Rio dos Sinos + Rio Gravataí, sp3 Rio Fão, sp4 upper Rio Soturno, sp5 Rio Caí, sp6 Rio das Antas, sp7 Rio Vacacaí mirim, sp8 São Gonçalo cannal, sp9 Rio Tramandaí, sp10 Rio Uruguai, sp11 Rio Pardinho, sp12 Rio Camaquã; and ML support, being them sp1 Mirin lagoon, sp2 Rio dos Sinos + Rio Gravataí, sp3 Rio Fão, sp4 Rio Tramandaí, sp5 Rio Uruguai, Sp6 Rio Pardo + Rio Camaquã + Rio das Antas + Upper Rio Soturno + Rio Vacacaí mirin + Rio Caí + São Gonçalo canal.

The GMYC model estimated for all fragments concatened resulted in the identification of 24 optimal models of Operational Taxonomic Units (OTUs), being them: OTUs1 Mirin lagoon and Rio Jaguarão; OTUs2 Rio dos Sinos; OTUs3 Rio Gravataí; OTUs4 Rio Camaquã (n=3); OTUs5 Rio Camaquã(n=1); OTUs6 Rio Pardo; OTUs7 Rio São Thomé; OTUs8 Rio dos Sinos (Caraá), and Rio Carreiro; OTUs 9 Rio Vacacaí-mirim; OTUs10 São Gonçalo canal; OTUs11 Rio Taquari; OTUs12 Rio Caí; OTUs13 down Rio Soturno; OTUs14 upper Rio Soturno; OTUs15 Rio Guaporé OTUs16 Rio Jaguarí; OTUs17 upper Jacuí (n=2); OTUs18 upper Jacuí (n=1); OTUs19 upper Jacuí (n=2); OTUs 20 Rio Tramandaí; OTUs 21 Rio Uruguai; OTUs22 T. Rio Uruguai; OTUs 23 *Ancistrus cirrhosus;* and OTUs 24 *A. temminckii*; and just for Co1 fragment, identificated five OTUs, being them OTUs1 Rio Uruguai (*A. taunay* + *A cirrhosis*), OTUs2 Rio Fão + Rio Colorado + Rio dos Sinos (Caraá) + Rio Pardinho + Rio Camaquã + Rio Carreiro + São Gonçalo canal + down Rio Jacuí + Rio Vacacaí + Rio Caí + Rio das Antas + down Rio Soturno, OTUs 3 Rio Guaporé + Rio Cedro Alto + Rio Tramandaí, OTUs 4 Rio Gravataí + Rio dos Sinos + Rio Jaguarão + Mirin lagoon, OTUs 5 *A. temminckii.*

The best partitions (Pt) provided by ASAP analysis, with the lowest score (3.5), delimit 16 partitions (p=7.78e-01(5)), being them: Pt 1 Rio Pardo; Pt2 T. Rio Tramandaí, Pt3 Rio Carreiro, Rio das Antas, down Rio Jacuí, Rio Caí, Rio Vacacaí-mirim and Canal São Gonçalo; Pt 4 down Rio Soturno; Pt5 Rio São Thomé; Pt6 Rio Camaquã; Pt7 Rio dos Sinos and Rio Gravataí; Pt8 upper Rio Jacuí (n=1); Pt9 Rio Fão (n=2); Pt10 Rio Jaguari; Pt11 Taquari; Pt12 upper Rio Soturno; Pt13 Rio Jaguarão and Mirin lagoon; Pt14 Rio Uruguaí ; Pt15 B. Araranguá and Pt 16 Upper R. Uruguai.

***Ancistrus* sp. nov 1 new species**

Holo-type: Zvc-p 11785, Female, 83.36 mm SL Uruguay, Cerro Largo, Río Tacuarí, tributary of Laguna dos Patos system, 32°39'9.66"S 54°23'3.54"W, (19/03/2013) M. Loureiro, L. Ziegler, D. Díaz, W. Serra; (Figure 3)

Para-types: Uruguay, Laguna Merín, Laguna dos Patos system:

Zvc-p 12766 [1 Tec. sample] A° Valdivia, Maldonado (34°18'3.72"S 54°34'7.31"W) M. Laporta, A. Duarte, S. Serra, J. Bessonart, M. Loureiro; Zvc-p 11785 (2*) All measures[1 tissue sample] Río Tacuarí, Cerro Largo; (32°39'9.66"S 54°23'3.54"W) M. Loureiro, L. Ziegler, D. Díaz, W. Serra; Zuc-p 16000 [1 tissue sample] A° Yerbal chico, Lavalleja (32°58'3.51"S 54°28'11.33"W) M. Loureiro, W. Serra; Zvc-p12524 [1 tissue sample] A°India Muerta, Rocha (34° 3'26.29"S 54°14'23.34"W) J. Bessonart, M. Loureiro; Zuc-p 1932 (1) (9) Aº de los Chanchos, Lavalleja (34°19'14.55"S 54°59'22.89"W) Long: & J. Soriano. Zvc-p 5747; Rio Cebollatí, Maldonado (34°12'15.28"S 54°48'47.68"W) N. Rajcok, H. Ferreira, I. Terzaghi, A. Viera, A. Sacchi, M. Loureiro; Zvc-p 7191 Rio Yaguarón, Cerro Largo (31°55'41.81"S 54° 6'1.22"W) F. Scasso, M. Loureiro, F. Quintans, J. Sawchik; Zvc-p 8866 A° Olimar, Treinta y Tres (32°58'3.68"S 54°28'11.38"W) M. Loureiro, M. Zarucki; Zvc-p 1198 (2) Rio Cebollatí, Lavalleja (34° 2'11.31"S 54°45'57.92"W) R. Vaz-Ferreira; Zvc-p 13717 Aº Olimar, Treinta y Tres (32°58'3.62"S 54°28'11.34"W) M. Loureiro, M. Zarucki; Zvc-p15285 (3) A° San Luis, Rocha (33°45'30.04"S 54° 5'38.32"W) M. Loureiro, M. Zarucki, V. Pinelli, L. Rodriguez-Tricot;

Brazil, São Gonçalo canal, Laguna dos Patos system: MCP38351 (2), [1 meassured] Pedro Osório, Arroio Lajeado (31°58'39.20"S 53° 6'8.25"W) MCP 25093 (1), Pedro Osório, Arroio Reduzindo (31°54'50.89"S 52°55'50.94"W).

Diagnosis:

Ancistrus sp. nov1 differs from all other cogeneres *Ancistrus* (La Plata river basin, Laguna dos Patos basin, Rio Tramandaí, Rio Maquiné, Rio Mampituba). *A.* sp. nov1 differ from *Ancistrus* sp. complex *A. abilhoai, A. agostinhoi, A. brevipinnis, A. claro, A. cuiabae, A. formosus, A. mullerae, A. pirareta* and *A. piriformis* black body and small yellow dot spots on the body; *A.* sp. nov1 differ from *A. cirrhosis* and *A. brevipinnis* by having a pectoral spine that extends beyond half the length of the pelvic spine (*A. cirrhosus* and *A. brevipinnis* the spin quite reaching the pelvic fin); *A.* sp. nov1 differ from *A. taunayi* and *A.*sp.nov2 by having the dorsal fin spine that is longer than the length of the dorsal fin base (*A.*sp.nov2 dorsal fin spine is short than the length of the dorsal fin base); *A.* sp. nov1

differ from *A. taunayi* by the caudal fin without chromatophores (*A. taunayi* present black bars of chromatophores on caudal fin); . *A.* sp. nov1 differ from *A. megacanthus* having large spots on belly (*A. megacanthus* have the belly without chromatophores).

Description: Morphometric data are presented in Table 1. The body and head are depressed. The dorsal profile of the body and head is convex from the tip of the snout to the origin of the dorsal fin, slightly concave to straight from this point to the origin of the adipose fin, and concave from the end of the adipose fin to the origin of the caudal fin. The ventral profile of the body is straight, with body height beginning to decrease at the pelvic girdle. The body is covered by bony plates; plates are absent along the base of the dorsal fin and the ventral surface between the tip of the snout and the origin of the anal fin. The median series consists of 21–26 (n=12, 22*) lateral plates; the lateral line canal is complete and uninterrupted. The dorsal series has 20-25 plates (n=12, 21*) distributed as follows: three pre-dorsal plates, 7*(9), 8(3) plates at the base of the dorsal fin, 5(3), 6*(6), 7(3) plates between the dorsal and adipose fins, and 5(8), 6*(4) plates remaining up to the caudal fin. Fins and plates are covered by small odontodes oriented caudally in regular rows, larger on the fin spines.

The snout is rounded, with a wide naked anterior margin, limited posteriorly by dermal plates of varying sizes. Cutaneous tentacles are present on the lateral, anterior, and mid-dorsal portions of the snout in males, sometimes branched and forming a “Y” pattern with a variation of 7-15(4). In females, the tentacles are smaller and usually arranged in pairs on the snout's sides. The eye is large, 16.1-18.3% (12) of head length, located laterodorsally on the head. The orbit is bordered dorsally by the sphenotic and frontal plates, anteriorly by the prefrontal plate, ventrally by infraorbitals IV and V, and posteriorly by infraorbital VI. Nostrils are located dorsally on the head with a tubular skin expansion, bordered anteriorly by the nasal plate, posteromedially by the frontal plate, laterally by infraorbitals II and III, and posterior-laterally by the prefrontal plate. The exposed part of the operculum is almost always triangular, rounded anteriorly. Dermal plates of varying sizes and shapes are arranged in the post-opercular region, adjacent to the composite pterotic. It contains 9-13 (12) strong retractable odontodes with sickle-shaped tips at the end of the opercular region.

The lower lip does not reach the anterior margin of the pectoral girdle. The lower lip has papillae randomly distributed across its surface. The maxillary barbels are short and free. The mandibular ramus is regular, 15.9-23.1% (12) of head length, with a premaxillary tooth row of equal length or slightly shorter. There are numerous teeth, 34-55 (12, 43*) in the premaxillary and 35-68 (12, 45*) in the dentary, bicuspid, with a reduced lateral

cusp and a larger, broader mesial cusp.

The dorsal fin spine length/dorsal fin base length ratio is 1.–1.7% (12); pectoral fin spine length/pectoral-pelvic distance ratio is 1.1–1.6% (12); unbranched pelvic fin ray length/abdominal length ratio is 0.8–1.4% (12). The pelvic fin reaches halfway along the length of the anal fin. The caudal fin is obliquely truncated with the lower anterior ray longer than the upper one. All fin rays are doubly branched near the apical end. The dorsal fin has II+7 rays; the pectoral fin has I+6 rays; the pelvic fin has i+5 rays; the anal fin has i+4 rays; the caudal fin has i+14+i rays.

Coloration in alcohol: The dorsal and ventral regions of the body are dark brown. Well-defined white spots, generally about the length of the pupil diameter on the head, and larger on the dorsal and lateral regions of the body. Sometimes difficult to visualize. The ventral region has bright spots. Rays and interradial membranes of paired fins are dark-colored similar to the body. The dorsal, adipose, and caudal fins are smooth from dark brown to black. The caudal fin lacks chromatophores, although the dorsal fin occasionally has spots. Pectoral and pelvic fins have spots. The upper and lower tips of the main caudal fin rays and the branched outer rays have a small clear tip.

Sexual dimorphism: Adult males have more numerous and larger tentacles on the snout compared to females and larger spines on the pectoral fin.

Geographic distribution and habitat: It occurs in the tributaries of Lagoa Mirim but does not reach the São Gonçalo Canal; its area of occurrence is approximately 37,000 km². Sandy environment, with rare rocks, found next to branches and trunks. Environment with slower rapids and flat environments.

Conservation status: Throughout its distribution, tributaries cross cities that may introduce contaminants or alter river channels. However, no specific threats to the species have been detected; it is provisionally assessed as Least Concern (LC) according to the International Union for Conservation of Nature (IUCN-2024) categories and criteria.

***Ancistrus* sp. nov2 new species.**

Holo-type: MCP 14854, male, 89 mm SL, Brazil, Rio Grande do Sul, Morungava, tributary of Rio Morungava, rio Gravataí basin, Laguna dos Patos system, (29°49'18.90"S 50°55'18.06"W), 17/01/1991, N.A. Menezes, R.E. Reis and E.H.L. Pereira (Figure 4).

Para-types. Brazil, Rio Grande do Sul, Laguna dos Patos system:

Rio Gravataí basin: MZU 995, 1, MZU 1021, 1, and MZU 1549, 1, arroio Demétrio, Gravataí
(29°47'26.4"S 50°51'55.6"W). MZU 853, 1, MZU 992, 2, MZU 1034,1, and MZU 1323, 1, arroio Demétrio, Gravataí (29°47'46.4"S 50°52'21.6"W). MZU 818, 1, and MZU 1046, 1, arroio Demétrio, Gravataí (29°48'47.4"S 50°53'21.2"W). MCP 15024, 1 (de2) and MCP 15464, 1, arroio Fiuza, Viamão (30°06'07"S 51°02'41"W). MCP 54885, [1 tec.], arroio Demétrio, Morungava (29°48'43.4"S 50°53'21.2"W). MCP 55004 Arroio do Alexandrina, Viamão (30° 06' 26.2" S 50° 56' 46.5"); MCP 17634, 3, arroio do Carvalho, Caraá (29°47'58"S 50°28'23"W).

Rio dos Sinos basin: MCP 17625, 2 rio dos Sinos, Caraá (29°46'27"S 50°26'08"W). UFRGS24665, [1 Tec] Caraá, Rio dos Sinos (29° 44' 59" S 50° 16' 59" W), MCP 17629, 1, rio
dos Sinos, Caraá (aprox. 29°47'S 50°23'W). MZU 2257, 1, rio dos Sinos, Taquara (29°39'37.0"S 50°48'02.4"W). MZU 1339, 1 and MZU 2439, 2, arroio Solitária, Igrejinha (29°33'10.91"S 50°50'56.39"W. MZU 1109, 2 [all measured + 1 c&s], arroio Solitário, Igrejinha (29°33'10.26"S 50°51'12.28"W). MZU 1253, 2, arroio Solitário, Igrejinha (29°33'45.60"S 50°50'12.28"W). MZU 942, 1,and MCP 2201, 3, arroio Solitário, Igrejinha (29°34'37.84"S 50°49'16.49"W). MZU 2285, 2, rio da Ilha, Taquara (29°33'13.99"S 50°37'38.39"W). MZU 840, 1, rio dos Sinos, São Leopoldo (aprox. 29°45'S 51°08'W). MCP2270,3, arroio Grande, Taquara (29°45'10.2"S 50°45'10.8"W). MCP 54882, [1 tec.], MCP 54881[1 tec.] and MZU 3172, 3, arroyo Solitário, Igrejinha (29°33'29.9"S 50°50'32.9"W). MCP 54886 [1 tec] Arroio Grande, Taquara (29°43'54"S 50°46'33.2"W).

Diagnosis:
Ancistrus sp. nov2 differs from all other cogeneres *Ancistrus* (La Plata river basin, Laguna dos Patos basin, Rio Tramandaí, Rio Maquiné, Rio Mampituba). *A.* sp. nov2 difer from *Ancistrus* sp. complex, *A. abilhoai, A. agostinhoi, A. brevipinnis, A. claro, A. cuiabae, A. formosus, A. mullerae, A. pirareta* and *A. piriformis* black body and small yellow dot

spots on the body; *A.* sp. nov2 differ from *A. cirrhosis* and *A. brevipinnis* by having a pectoral spine that extends beyond half the length of the pelvic spine (*A. cirrhosus* and *A. brevipinnis* the spin quite reaching the pelvic fin); *A.* sp. nov2 differ *A.*sp.nov1 by having the dorsal fin spine that is short than the length of the dorsal fin base (*A.*sp.nov1 dorsal fin spine is larger than the length of the dorsal fin base); *A.* sp. nov2 differ from *A. taunayi* by the caudal fin without chromatophores (*A. taunayi* present black bars of chromatophores on caudal fin); . *A.* sp. nov2 differ from *A. megacanthus* having spots on dorsal fin (*A. megacanthus* have dorsal fin without chromatophores).

Description: Morphometric data are presented in Table 2. The body and head are depressed. The dorsal profile of the body and head is convex from the tip of the snout to the origin of the dorsal fin, slightly concave to straight from this point to the origin of the adipose fin, and concave from the end of the adipose fin to the origin of the caudal fin. The ventral profile of the body is straight, with body height beginning to decrease at the pelvic girdle. The body is covered by bony plates; plates are absent along the base of the dorsal fin and the ventral surface between the tip of the snout and the origin of the anal fin. The median series consists of 23–25 (32, 23*) lateral plates; the lateral line pores are complete and uninterrupted. The dorsal series has 22-25 plates (46, 22*) distributed as follows: three pre-dorsal plates, 7 (28*) or 8 (18) plates at the base of the dorsal fin, 6 (34) or 7 (12) plates between the dorsal and adipose fins, and 6* (40) or 7 (6) remaining plates up to the caudal fin. Fins and plates are covered by small odontodes oriented towards the tail in regular rows, larger on the fin spines.

The snout is rounded, with a wide naked anterior margin, limited posteriorly by dermal plates of varying sizes. Cutaneous tentacles are present on the lateral, anterior, and middle-dorsal portions of the snout in males, sometimes branched and forming a “Y” pattern. In females, the tentacles are smaller and usually paired on the snout's sides. The eye is large, 15.4-26.8% (46) of head length, located laterodorsally on the head. The orbitventrally by infraorbitals IV and V, and posteriorly by infraorbital VI. Nostrils are locateddorsally on the head with a tubular skin expansion, bordered anteriorly by the nasal plate,posterior-medially by the frontal plate, laterally by infraorbitals II and III, and posterior-laterally by the prefrontal plate. The exposed part of the operculum is almost always triangular, rounded anteriorly. Dermal plates of varying sizes and shapes are arranged inthe post-opercular region, adjacent to the composite pterotic. It contains 9-14 (32, 14*) strong retractable odontodes with sickle-shaped tips at the end of the opercular region.

The lower lip does not reach the anterior margin of the pectoral girdle. The lower lip has papillae randomly distributed across its surface. The maxillary barbels are short and free.

The mandibular ramus is regular, 14.6-25.8% (46) of head length, with a premaxillary tooth row of equal or slightly shorter length. There are numerous teeth, 40-61 (32, 49*) in the premaxillary and 40-66 (32, 53*) in the dentary, bicuspid, with a reduced lateral cusp and a larger, broader mesial cusp.

The dorsal fin spine is elongated, with soft rays not reaching the adipose fin when pressed (dorsal fin spine length/dorsal fin base length ratio 0.93-1.25% (32)); pectoral fin spine length/pectoral-pelvic distance ratio 1–1.9% (32); unbranched pelvic fin ray length/abdominal length ratio 1–1.4%. The caudal fin is obliquely truncated with the lower anterior ray longer than the upper one. All fin rays are doubly branched near the apical end. The dorsal fin has II+7 rays; pectoral fin has I+6 rays; pelvic fin has i+5 rays; anal fin has i+4 rays; caudal fin has i+14+i rays.

The sixth vertebra (first after the Weberian apparatus) has an enlarged rib. The seventh vertebra lacks a rib and supports the first dorsal fin pterygophore. The following eight vertebrae have thin ribs and pterygophores supporting the dorsal fin. A total of 28 vertebrae.

Coloration in alcohol: The dorsal and ventral regions of the body are black. Well-defined white spots, generally smaller in length than the pupil diameter and sparse. Greatest concentration on the head, decreasing towards the dorsal and lateral regions of the body but of the same length. The belly region sometimes has small light spots and other times no spots. Rays and interradial membranes of paired fins are dark-colored similar to the body. Pectoral, pelvic, dorsal, adipose, and caudal fins have a black base with spots of the same length and color as the head spots (sometimes the caudal fin lacks spots). The upper and lower tips of the main caudal fin rays and the branched outer rays have small luminous tips.

Coloration in life: The dorsal and ventral regions of the body are black. Well-defined yellow spots, generally smaller in length than the pupil diameter and sparse. Greatest concentration on the head, decreasing towards the dorsal and lateral regions of the body but of the same length. The ventral region occasionally has bright spots. Rays and interradial membranes of paired fins are dark-colored similar to the body. The dorsal, pectoral, and pelvic fins, adipose and caudal fins are black with spots of the same length as the head spots (sometimes the caudal fin lacks spots). The upper and lower tips of the main caudal fin rays and branched outer rays have small luminous tips (Figure 5).

Sexual dimorphism: Adult males have more numerous and larger tentacles on the snout

compared to females and larger spines on the pectoral fin.

Geographic distribution and habitat: Its distribution is known from the Gravataí River basin and the Sinos River basin, covering an area of approximately 2,700 km². It inhabits environments of running and swampy water, both rocky and sandy. Found next to rocks and logs inside clearings.

Conservation status: Its distribution occurs in a densely urbanized or productive area. The main threat is river channelization and anthropogenic activities that may introduce contaminants. However, no specific threats to the species have been detected; it is provisionally assessed as Least Concern (LC) according to the International Union for Conservation of Nature (IUCN 2024) categories and criteria.

***Ancistrus* sp. complex**

Diagnosis:

Except by *Ancistrus pirareta*, *Ancistrus* sp. differs from all other cogeneres *Ancistrus* (La Plata river basin, Laguna dos Patos basin, Rio Tramandaí, Rio Maquiné, Rio Mampituba). species by having a greenish coloration. *Ancistrus* sp. differ from *Ancistrus pirareta* having yellow dots on body and fins (*Ancistrus pirareta* have palid dots on body and fins).

Description:

Morphometric data are provided in Table 3. The body and head are depressed. The dorsal profile of the body and head is convex from the tip of the snout to the origin of the dorsal fin, slightly concave to straight from this point to the origin of the adipose fin, and concave from the end of the adipose fin to the origin of the caudal fin. The ventral profileof the body is straight, with body height beginning to decrease at the pelvic girdle. The body is covered by bony plates; plates are absent along the base of the dorsal fin and theventral surface between the tip of the snout and the origin of the anal fin. The median series consists of 21–25 (, 23 $^{\bar{x}}$) lateral plates; the lateral line canal is complete anduninterrupted. The dorsal series has 20-22 plates (, 21 $^{\bar{x}}$) distributed as follows: three pre-dorsal plates, 7 (n=86 $^{\bar{x}}$), 8(n=27) plates at the base of the dorsal fin, 4(n=92 $^{\bar{x}}$),5(n=21) plates between the dorsal and adipose fins, and 6 (n=60 $^{\bar{x}}$), 7(n=49), and 8(n=4) plates extending to the caudal fin. Fins and plates are covered by small odontodes orientedcaudally in regular rows, larger on the fin spines.

The snout is rounded, with a wide naked anterior margin, limited posteriorly by dermal plates of varying sizes. Cutaneous tentacles are present on the lateral, anterior, and mid-dorsal portions of the snout in males, sometimes branched and forming a “Y” pattern. In

females, the tentacles are smaller and generally arranged in pairs on the sides of the snout. The eye is large, 10.8-23.2% (113) of head length, located laterodorsally on the head. The orbit is bordered dorsally by the sphenotic and frontal plates, anteriorly by the prefrontal plate, ventrally by infraorbitals IV and V, and posteriorly by infraorbital VI. Nostrils are located dorsally on the head with a tubular skin expansion, bordered anteriorly by the nasal plate, posteromedially by the frontal plate, laterally by infraorbitalsII and III, and posterior-laterally by the prefrontal plate. The exposed part of theoperculum is almost always triangular, rounded anteriorly. Dermal plates of varying sizes and shapes are arranged in the post-opercular region, adjacent to the composite pterotic. It contains 09-14 (101, 14 $\bar{x}$) strong retractable odontodes with sickle-shaped tips at the end of the opercular region.

The lower lip does not reach the anterior margin of the pectoral girdle. The lower lip has papillae randomly distributed across its surface. The maxillary barbels are short and free. The mandibular ramus is regular, 13.6-27% (n=113) of head length, with a premaxillary tooth row of equal length or slightly shorter. There are numerous teeth, 32-82 (n=113, 51 $\bar{x}$) in the premaxillary and 33-77 (n=113, 46 $\bar{x}$) in the dentary, bicuspid, with a reduced lateral cusp and a larger, broader mesial cusp.

The ratio of dorsal fin spine length to dorsal fin base length is 1.–1.3% (n=113); pectoral fin spine length to pectoral-pelvic distance is 17.1–26.9% (n=113); unbranched pelvic fin ray length to abdominal length is 1–1.6% (n=113). The pelvic fin reaches halfway along thelength of the anal fin. The caudal fin is obliquely truncated with the lower anterior ray longer than the upper one. All fin rays are doubly branched near the apical end. The dorsalfin has II+7 rays; the pectoral fin has I+6 rays; the pelvic fin has i+5 rays; the anal fin hasi+4 rays; the caudal fin has i+14+i rays.

The sixth vertebra (first after the Weberian apparatus) has an expanded rib. The seventh vertebra has no rib and supports the first pterygophore of the dorsal fin. The following eight vertebrae have thin ribs and pterygophores supporting the dorsal fin. There are a total of 28 vertebrae.

Coloration in alcohol: The dorsal and ventral regions of the body are dark brown to brown. Well-defined white to white-yellow spots, usually smaller than the pupil diameter on the head, and on the dorsal and lateral regions of the body, sometimes on the ventral region, generally vermiculated, occasionally spotted. A lighter brown bar at the end of the caudal peduncle. Rays and interradial membranes of the fins have the same coloration as the body with spots, sometimes forming color bars.

Coloration in life: The body background is dark, yet green, with yellow pigmentation spots on the dorsal and ventral surfaces of the body and fins, with smaller and more concentrated spots on the head region and larger and more scattered spots on the dorsal body region, occasionally forming a vermiculated/damascus pattern on the ventral portion. A yellow bar encircles the entire terminal base of the caudal peduncle. Rays and interradial membranes of the fins have the same body coloration with yellow spots, sometimes creating colored bars (Figure 6).

Sexual dimorphism: Adult males have more numerous and larger tentacles on the snout compared to females and larger spines on the pectoral fin, generally exceeding half the length of the unbranched ray of the pelvic fin and reaching the first third of the pelvic fin in females.

Geographic distribution and habitat: The distribution is concentrated in the southern highland region, within the Laguna dos Patos basin, covering an area of approximately 28,000 km². The main rivers are the Rio Caí, Rio Faxinal do Soturno (upper), Rio Jacuí (upper), Rio Jacuizinho, Rio Pardo, Rio Carreiro, Rio Taquari, and Rio das Antas. However, in the upper fields of the Serra region, it is limited to the margins (Rio das Antas and São Thomé), with no collections in the central region. They inhabit rocky environments or basalt slabs, in areas normally uncovered and headwaters. Not occurring in the region of the fields of Cima da Serra

Remarks

The haplotype network (Figure 7) of the two concatenated mitochondrial genes separates the four species, but does not establish a pattern for the *Ancistrus* sp complex.

The samples showed a minimum genetic distance between *Ancistrus* species of 0.02 between *A. brevipinnis*, *A. megacanthus*, and *A.* sp nov1; Other samples that present a minimumdistance is *A.* sp. nov1 with *A. brevipinnis* thats have 0.01 distance Table 4.

Discussion

This study resulted in the expansion of the distribution of *Ancistrus megacanthus* from 11,000 km² to 25,000 km² as shown in Figure 8. It also contributed to the knowledge of the current species in the Laguna dos Patos basin, increasing the number of species from 2 to 4, and further elucidating the complex. Additionally, it highlighted the need for taxonomic revisions of *Ancistrus taunayi* and contributed to the first CO1 and Rag2 sequencing of a topotype of this species.

The 16 partitions from the ASAP analysis best correspond to the haplotype network and morphological analysis. Even though the complex remains polyphyletic (upper Rio Jacuí, Rio das Antas, Rio Fão, Rio Carreiro, Rio Pardo, and upper Rio Soturno).

The four species from the Laguna dos Patos exhibit morphological variations that allow for their identification. Although it is possible to differentiate as a species morphologically, it was chosen to form a complex, here referred to as *Ancistrus* sp., which was not solved because it is an interactive approach. As a result, the molecular data showed inconsistency, leaving this group polyphyletic (upper Rio Soturno, upper Rio Jacuí, Rio Taquari, Rio das Antas, and Rio Pardo). Subsequently, all the populations that did not have enough data to claim as a species were placed in this complex. According to the analyses, the following definitions were established:

Ancistrus sp. - The sample from the Rio Pardo is phylogenetically associated with *A. brevipinnis,* but differs in coloration and exhibits great numerous of mutations. Due to the low sample size from this microbasin, it has been opted to leave its definition for future studies. The samples from the upper Rio Soturno morphologically and phylogenetically differ from low-altitude species. This sample has similar alcoholic coloration to *A. megacanthus* but is placed in an independent clade, with both species remaining in this group. Populations from the upper Rio Jacuí, Rio das Antas, and Rio Taquarí are in independent clades in the phylogenetic trees but exhibit the same color pattern, making them indistinguishable from one another. The sample from the Rio Caí is phylogenetically close to *A. megacanthus* as showed in ASAP model and ML phylogeneny tree, but closer to this group in coloration and great number of mutation in haplotype network from *A. megacanthus*. The samples from the Rio dos Sinos, in the region of the city of Caraá/RS, are isolated in the phylogenies. This could be an isolated species just like what happens in *Trichomycterus brachykechenos* (Ferrer and Malabarba, 2013).

Because the recent geological changes must have direct influences on the distribution limits of species. Some important changes were the change in the direction of the flow of water from the current upper Uruguay River that flowed directly to the sea and started to flow towards the Paraná River; The alteration of the course of the Paraná River that began to flow over the La Plata River; Marine transgressions and regressions, where paleodrainages formed connections and later isolated species; the formation of the Laguna dos Patos and finally the connection of the Mirim lagoon to the system. As the separation of the watercourses of the southern plateau into two isolated systems (upper Rio Uruguay and upper Rio Jacuí) is very recent, species delimitation studies should consider the two samplings, that is, the inclusion of what is currently known as *Ancistrus taunayi* to this

study.

Regarding habitat aspects, *Ancistrus* sp. nov1 and *A. brevipinnis* are exclusive to the Pampa biome, while *Ancistrus* sp. is exclusive to the Atlantic Forest. In terms of geology, *A. megacanthus* is found in the Central Depression region, *A. brevipinnis* in the Crystalline Shield, *Ancistrus* sp. in the southern plateau (basaltic formation), and *Ancistrus* sp. nov1in the eolian formation.

Identifications Key for *Ancistrus* species from Laguna dos Patos System:

1. Body coloration black to dark brown.

1.1 Ventral region without obvious spots, though small white dots may be present.

1.1.1 Adipose fin with a clear, noticeable spot. *Ancistrus* sp. (Pardo).

1.1.2 Adipose fin with membrane matching the body color. *Ancistrus megacanthus*.

1.2 Ventral region with noticeable dark or light spots.

1.2.1 Pigmentation in the form of prominent spots on the body's dorsum, abundant and larger than the eye orbit. *Ancistrus a*.

1.2.2 Few pigments and not prominent on the body's dorsum, and if present, they are small. *Ancistrus b*.

2. Body coloration different from black or dark brown.

2.1 Dark brown body with light brown longitudinal spots in the caudal peduncle region and light brown ventral region. *Ancistrus brevipinnis*.

2.2 Body with light coloration and without spots. *Ancistrus* sp.

Comparative Specimens Examined:

Brazil, Rio Grande do Sul, Laguna dos Patos System:

Ancistrus sp.: MCP 21216, 8, [2 measured] arroio Despraiado, Soledade (28°48'26"S 52°25'47"W). MCP 22248, 5, [all measured] arroio Carreta Quebrada, Passo Fundo (28°26'48"S 52°23'04"W). MCP 22125, 12, [2 measured] arroio do Portão, Passo Fundo(28°22'03"S 52°24'42"W). MCP 21254, 3, [2 measured], Espumoso (28°45'51"S 52°55'10"W). MCP 22762, 6, [4 measured] arroio Tapiaia, Julio de Castilhos (29°06'49"S 53°39'04"W). MCP 22747, 3 (13), rio Pinheirinho, Saldanha Marinho (28°23'39"S 53°03'11"W). MCP 22180, 2, [1 measured], rio Jacuí, Passo Fundo (28°18'45"S 52°18'28"W). MCP 22129, 3, [all measured] rio da Gloria, Santo Antonio Planalto (28°21'03"S 52°43'40"W). UFRGS 23502, 2, [1 measured], and UFRGS 23505, 1, rio Morcego, Espumoso (28°53'55"S 52°49'06"W). MZU 2334, 1, [all measured], Capela de Santana (29°39'29.7"S 51°20'53.9"W). MCP 11474, 3, [2 measured], arroio do Ouro, Feliz (approx. 29°18'S 51°10'W). MZU 2783, 3, [all measured], Bom Princípio(29°28'31.9"S

51°20’51.9”W). MCP 21569, 2, [1 measured], rio Pardinho, Boqueirão doLeão (29°18’39”S 52°31’38”W). MCP 21568, 1, arroio José Simão, Gramado Xavier (29°15’47”S 52°32’51”W). MCP 32503, 1, rio Pardinho, Santa Cruz do Sul (approx. 29°35’S 52°30’W). MCP 32351, 1, arroio São Tomé, São Francisco de Paula (28°53’20.78”S 50°37’08.42”W). MCP 26759, (10) [3 meassured], arroio Pessegueiro, Barros Cassal (29°05’04”S 52°36’02”W). MCP 17501, 3, [all measured], arroio Castelhano, Venâncio Aires (29°32’59”S 52°16’59”W). MCP 21212, 7, [all measured], arroio Jeremias, Arvorezinha (28°49’44”S 52°14’35”W). MCP 22208, 6, [4 measured],arroio Jaboticaba, Vila Flores (28°52’43”S 51°32’05”W). MCP 21676, 3, [all measured]arroio Jabuticaba, Veranópolis (29°01’15”S 51°34’55”W). MCP 21205, 4, [all measured + 1 c&s] arroio Três Pontes, Arvorezinha (28°48’24”S 52°18’14”W). MCP 25676, 3, [1 measured] rio Fão, Barros Cassal (29°03’09”S 52°34’50”W). MCP 22786, 1, [all measured], arroio Espeto, Muitos Capões (28°23’26”S 51°03’22”W). MCP 22240, 2, arroio Quebra Perna, São Domingos do Sul (28°34’01"S 51°50’36”W). MCP 37688, 1, rio das Antas, Bento Gonçalves (29°01’08”S 51°28’28”W); UFRGS 16737 Rio Quati, Mormaço (28° 38' 52" S 52° 37' 11" W) Bonato, K.O.; Wingert, J.; UFRGS 20354 (1) [1 tec] Arroio Giuliani, Faxinal do Soturno (29° 32' 56,1" S 53° 27' 50,2") W Ferrer, J.; Angrizani, R.; Donin, L.; Chuctaya, J; MCP 21246 [1 tec] [Total] [2 meassured]Arroio Linha das Flores, Agudo (29°36’02” S 53°16’49” W) Coletores; MCP 54884 [1tec] Rio Pardinho, Sinimbu (29° 38' 16.34" S 52° 27' 58.28" W) Widholzer R.L., Haas M.L.; MCP 54934 [1 Tec] Arroio São Thomé, Lageado Grande (29° 02' 22.59" S 50° 34'08.71" W) Widholzer R.L., Haas M.L.; UFRGS 21296 [1 tec] Arroio Tatim, Soledade (28° 51' 25" S 52° 27' 16" W) Becker, F.; Dala Corte, R.; Guimarães, T.; Bonato, K.; Hartmann, C.; MCP 50889 [1 Tec] rio Turvo, Espumoso (28°43’47”S 52°47’40”W).; MCP 54996 [1 Tec] Afluente do rio Jacui (Upper), Tapera (28° 42' 07.3" S 52° 51' 41.7"W) Coletores; MCP 50096, (3), [2 meassured], [1 tec] arroio dos Macacos, Colorado (28°28’41”S 52°57’15”W) Coletores; MCP 50352 [1 Tec] Afluente do Rio Taquari, Nova Bassano (28° 44' 24.7" S 51° 41' 16.7" W); MCP 54933 [1 Tec] Rio Cadeia, Picada Café (29° 19' 34.93" S 51° 10' 50.57" W); MCP 50178, 2, [1 meassured], [1 Tec] Afluente do Rio Taquari, Travesseiro (29° 16' 42.0" S 52° 03' 33.0" W); UFRGS 20381 [1 tec] Arroio Tatim, Soledade (28° 51' 09,7" S 52° 26' 49,7" W) Ferrer, J.; Angrizani, R.; Donin,L.; Chuctaya, J.; MCP 25572, 3, [all measured + 1 c&s] and UFRGS 11124 [1 tec] ArroioJaboticaba, Veranópolis (29° 1' 13.00" S 51° 31' 41.00" W) Juliano Ferrer, Giovanni Neves, Rodrigo Hirano; MCP 21220 (5)[3 measured], Agudo, Afluente do Rio Jacuí (29°31'33.15"S 53°17'59.84"W); MCP 21316, Agudo, Arroio Corupá (29°33'54.25"S 53°17'9.12"W); MCP 41153, 6 (11), Arroio Felício, Julio de Castilhos (29°19’04”S 53°37’54”W) Coletores; MCP 20168 [4 measured + 1 c&s], and MCP 21204, 6,(3 meassured) arroio Feitoria, Ivoti (approx. 29°35’S 51°08’W) Coletores. UFRGS 15006 [1 Tec] Rio Felício, Júlio de Castilhos (29°19'9.30"S 53°38'4.58"W) Malabarba, L. R.; Carvalho, F. R.; Thomaz, A. & Ferrer, J; MCP 22167 (05) [3 meassured] Nicolau Vergueiro, Arroio Resvalador (28°32’54.003”S 52°25’59.001”W); MCP 26559 (5) [all meassured], Nova Palma, Arroio Caemborá (29°28'49.18"S 53°17'51.28"W); MCP 20163 (5) Ivoti, Arroio Feitoria (29°35’S 51°8’W); MZU 1565 [all meassured + 1 c&s] Arroio Conceição, São Sebastião do Caí/RS (29°37'08,1"S 51°18'46,9"W); MCP 34532 (7) [2 meassured] Carazinho, Arroio Varejão, (28°12'57.75"S 53° 5'58.14"W); MCP 50261 (6) [1 meassured] Marques de Souza, Arroio Tamanduá (29°16'5.77"S52°11'1.32"W).

***Ancistrus brevipinnis*:** MCP 25948, 21, 22.9–51.3 mm SL [3 measured 51.3mm SL], riodos Carros, Lavras do Sul (30°46’02.42”S 53°48’23.04”W). MCP 25902, 11, 24.8–65.9 mm SL [6 measured 51.0–55.2 mm SL] + 1 c&s, 56.0 mm SL, rio Marmeleiro, Lavras do Sul (30°59’06”S 53°58’20”W). MCP 23781 7, 31.2–65.7 mm SL [4 measured 60.1– 65.7 mm SL], rio do Engenho, tributary to rio Velhaco, Sentinela do Sul (30°43’48”S 51°45’24”W). MCP 54896, 4, 2 tec, 50.0–67.5 mm SL [2 measured 65.2–67.5 mm SL], rio do Meio, Cristal (30°58’10.81”S 52°10’07.42”W). UFRGS 21299, 1, 50.7 mm SL [measured], creek

tributary to rio das Neves, Santana da Boa Vista, (30°51’17”S 53°13’38”W), UFRGS 21300, 5, 27.3–72.0 mm SL [4 measured 57.5–72.0 mm SL], riodas Neves, Santana da Boa Vista (30°51’45.1”S 53°13’27.8”W), UFRGS 22124, 2, 74.7– 81.3 mm SL [2 measured], rio das Neves, Santana da Boa Vista (30°51’45.1”S 53°13’27.8”W). UFRGS 12467 [1 Tec.] Rio Do Pinto, São Lourenço do Sul (31° 20' 50.70" S 52° 03' 48.96"), MCP 54895 [1 Tec] Arroio Bonito Sentinela do Sul (30°43'50.87"S 51°45'23.05"W); MCN 18049 Arroio Guará, Tapes (30°29'17.29"S 51°23'39.62"W) MCP34715,1 (C&S) Encruzilhada do Sul, Arroio Maria Santa (30°54'16.30"S 52°29'11.00"W)

***Ancistrus megacanthus*:** MCP 18652, 2, arroio Andreas, Vera Cruz (approx. 29°42’S 52°32’W). MCP 19582, Brazil, Rio Grande do Sul, São Gabriel, rio Vacacaí on road RS-630, tributary to rio Jacuí, laguna dos Patos system (30°27’18”S 54°22’26”W); MCP 54919, 5, 47.7–74.5 mm SL [4 measured] + 1 c&s; MCP 16294, 8, 85.3–124.4 mm SL [all measured], MCP 54898, 2, and MCP 54883, [1 tec] São Gabriel, arroio Cambaizinhoon road BR-290 (30°20’23”S 54°03’07”W); MCP 55020 3 [1 tec] Cerrito do Ouro / SãoSepé Arroio São Rafael/ Vacacaí Mirin (30°21'46.69"S 53°36'55.50"W); UFRGS 20411[1 tec] Paraíso do Sul (29°43'31.68"S 53° 9'39.40"W); MCP54897 [1 tec] Pedro Osório (31°50'3.87"S 52°48'31.53"W); MCP 26010, 3, arroio Bom Jardim, Triunfo (29°50’19”S 51°23’25”W). MCP 26011, 3, arroio Bom Jardim, Triunfo (approx. 29°51’S 51°26’W). MZU 871, Pedro Osório, Arroio Serra (31°43'50.9"S 52°43'41.9"W); MZU 880, Pedro Osório, Arroio da Palma (31°49'38.4"S 52°42'21.7"W); MZU 891, Pedro Osório, Afluente do Arroio Passo das Pedras (31°48'44.8"S 52°43'40.5"W); MZU 1169 Pedro Osório, Afluente do Arroio Passo das Pedras (31°51'02.9"S 52°40'34.6"W); MZU 1314,Povo novo, Rio Piratini (31°59'52.8"S 52°27'57.6"W); MZU 2773 São Gabriel Rio Vacacaí (30°27'5.65"S 54°22'27.66"W); MZU 2779, Cachoeira do Sul, Arroio Capané (30°06'32.7"S 52°53'14.7"W); MCP 22735 (16) Nova Palma, Arroio do Tigre (29°29'23.18"S 53°28'43.44"W); UFRGS 8627, Pedro Osório, rio Piratini (31° 43' 10" S 52° 53' 59" W); UFRGS 13705, Pinheiro Machado, arroio Banhado Grande (31° 28' 30" S 53° 26' 49"); UFRGS 6595, Agudo, arroio Corupá (29° 33' 54" S 53° 17' 8.93" W); MCP 17336 (3), Minas do Leão, Arroio do Conde (30° 9'0.52"S 52° 1'59.74"W);MCP26916, (3) Mariana Pimentel, Arroio Patrício (30°21'0.22"S 51°36'0.03"W).

Material examined. Brazil, Rio Grande do Sul, Rio Mampituba system:

***Ancistrus multispinnis*. Brazil: Costal Rivers: Rio Grande do Sul:** MCP 10796, 1, rioMaquiné, Maquiné (approx. 29°36’S 50°17’W). MCP 13654, 10, Rio Maquiné, Maquiné(approx. 29°40’S 50°11’W). MCP 29102, 19, arroio Garapiá, Barra do Ouro (29°30’26”S 50°14’39”W). MCP 29133 2, arroio Bananeira, Itati (29°26’33”S 50°11’18”W). MCP 29145, 1, Arroio Forqueta, Barra do ouro (29°32’08”S 50°12’21”W). MCP 53880, 1, arroio Morro Azul, Três Cachoeiras (29°23’57”S 49°55’00”W). MCP 53896, 9, Rio Sangão, Mampituba (29°14’19”S 49°58’29”W).
UFRGS 18448 [1 tec] Arroio Lageado, Maquiné (29° 31' 26.04" S 50° 18' 56.6" W) **SantaCatarina:** MCP 10639, 103, Rio Jordão, Nova Veneza (approx. 28°35’S 49°27’W). MCP19889, 1, Rio Ano Bom, Corupá (approx. 26°26’S 49°16’W). MCP 29277, 1, ArroioMolha Côco, Praia Grande (29°10’27”S 49°58’26”W). MCP 31539, 2, Rio Lindo,Joinville (26°11’31”S 48°55’44”W). MCP54887 [1 tec] Arroio Tributário do Rio Cedro,Nova Veneza (28° 40' 19.9" S 49° 35' 58.8" W)

***Ancistrus taunayi*. Brazil: Rio Grande do Sul: Rio Uruguay system:** MZU 2413, 3, arroio Taquarembó, Dom Pedrito (30°54’32.11”S 54°35’29.65”W). MCP 46749, 4, rio Jaguari, Lavras do Sul (30°49’15.8”S 54°17’56.5”W). MCP 46744, (2), rio Jaguari, DomPedrito (30°37’23”S 54°26’36”W). MCP 43914, [1 tec] Arroio Jaguari, tributary rio Santa Maria, Lavras do Sul (30°49’13”S 54°17’57”W); MCP 55006 [1

Tec] Rio Iraceminha, Maravilha (26° 45' 19.2" S 53° 14' 30.7"W). MCP 50189 [1 Tec] Arroio Porongos, Vila Maria (28°31'35.01"S 52° 8'37.01"W) MCP 54713 [1 Tec]Uruguaiana, Arroio Puitã (29°30'6.99"S 56°43'31.55"W)

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Figures

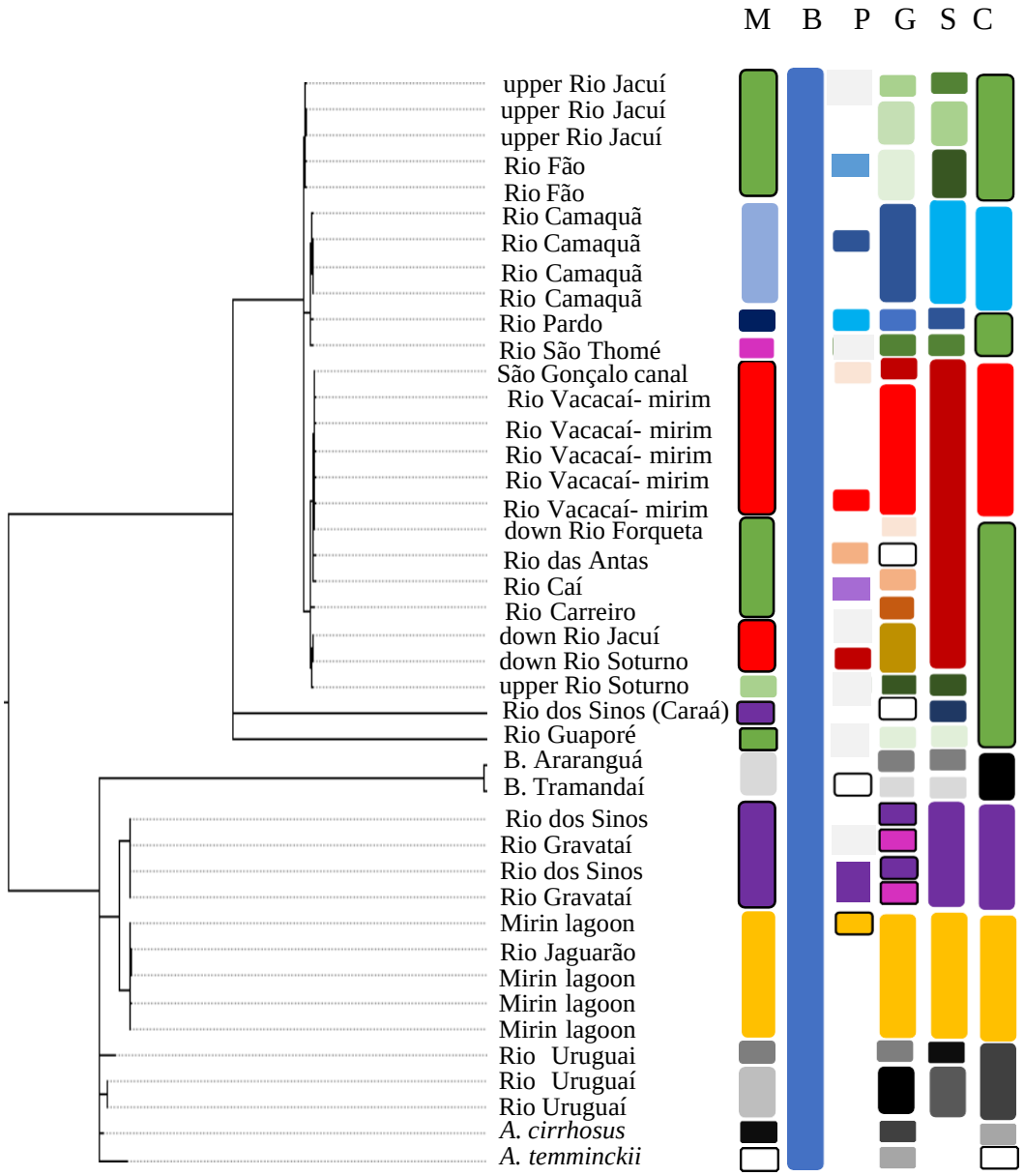

Figure 1: Bayersiana tree with the comparative results of automatic species delimitation tests. The bars outside the tree indicate as following: (M) morphological differences; (B) result of the ABGD test; (P) result of the bPTP test; (G) result of the GMYC test; (S) result of the ASAP test; and (C) consensus. Color diferents meens diferents species, same color with borther meens gruped species.

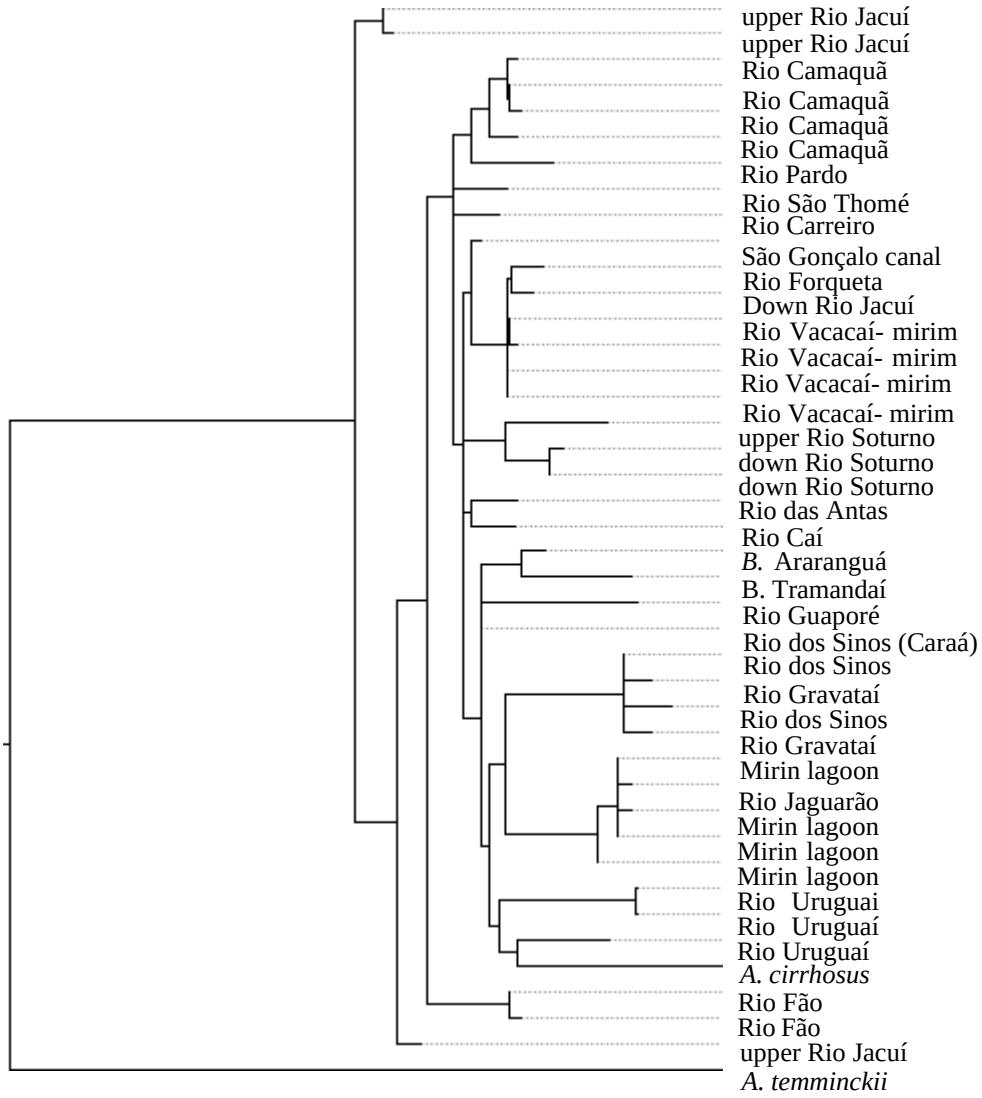

Figure 2: Maximum Likelihood tree with the river names of occurrence.

Figure 3: Holotype of *Ancistrus* sp. nov 1: dorsal, lateral and ventral view. Zvc-p 11785, Female, 83.36 mm SL Uruguay, CerroLargo, Río Tacuarí, tributary of Laguna dos Patos System.

Figure 4 Holotype of *Ancistrus* sp. nov2, dorsal, lateral and ventral of holotype MCP 14854, male, 89 mm SL,Brazil, Rio Grande do Sul, Morungava, tributary of Rio Morungava, Laguna dos Patos System.

Figure 5: Image of the representative of *Ancistrus* sp.nov 2. Individual collected in [city] Gravataí (RioDemétrio), specimens not preserved.

Figure 6: Image of the representative *Ancistrus* sp. from the city of Arvorezinha/RS, Arroio Forqueta. Specimens not preserved.

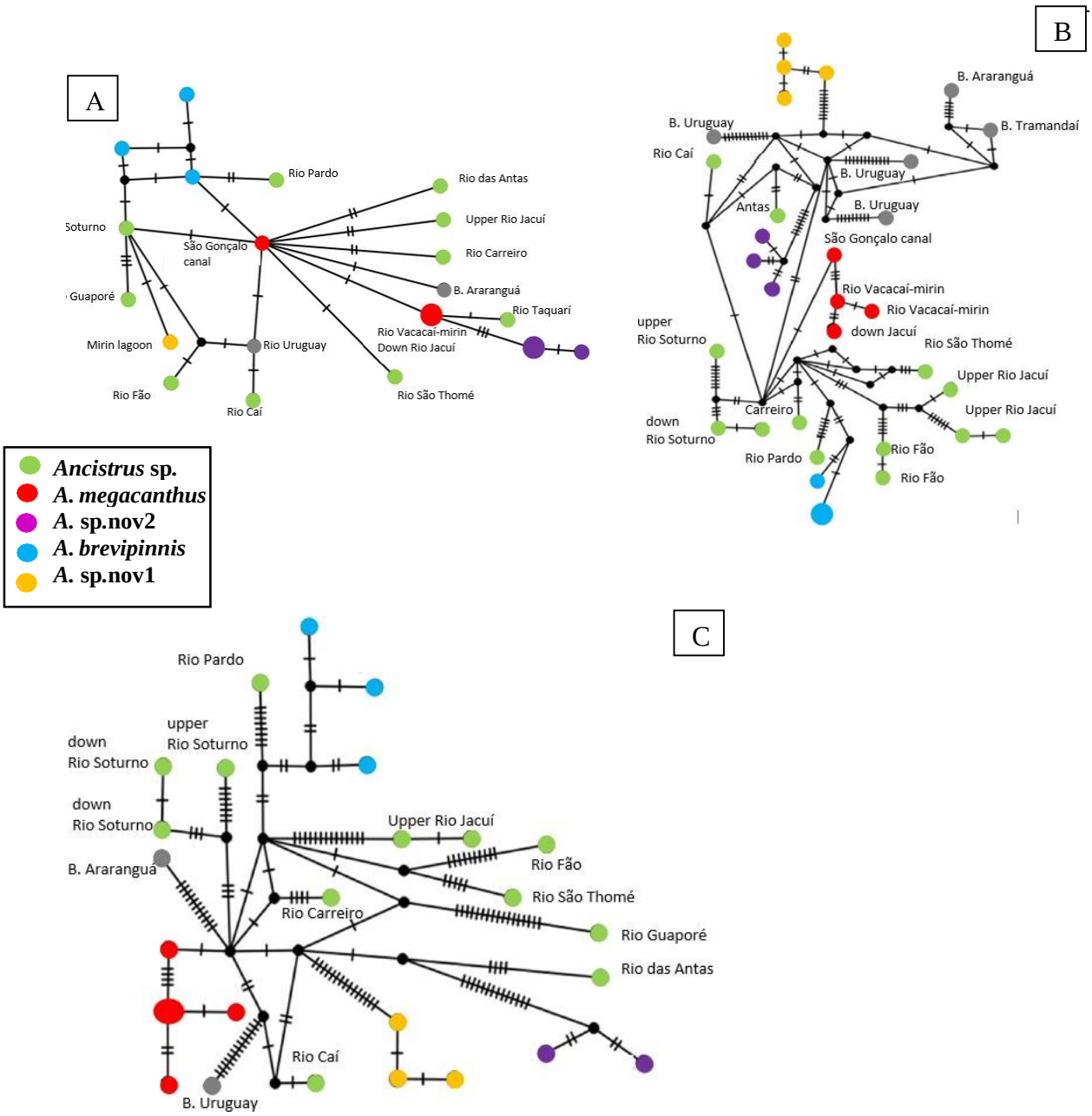

Figure 7: Haplotype Network with Median-Joining: (A) 16S fragment; (B) CO1 fragment; and (C) concatenated CO1 and 16S fragments.

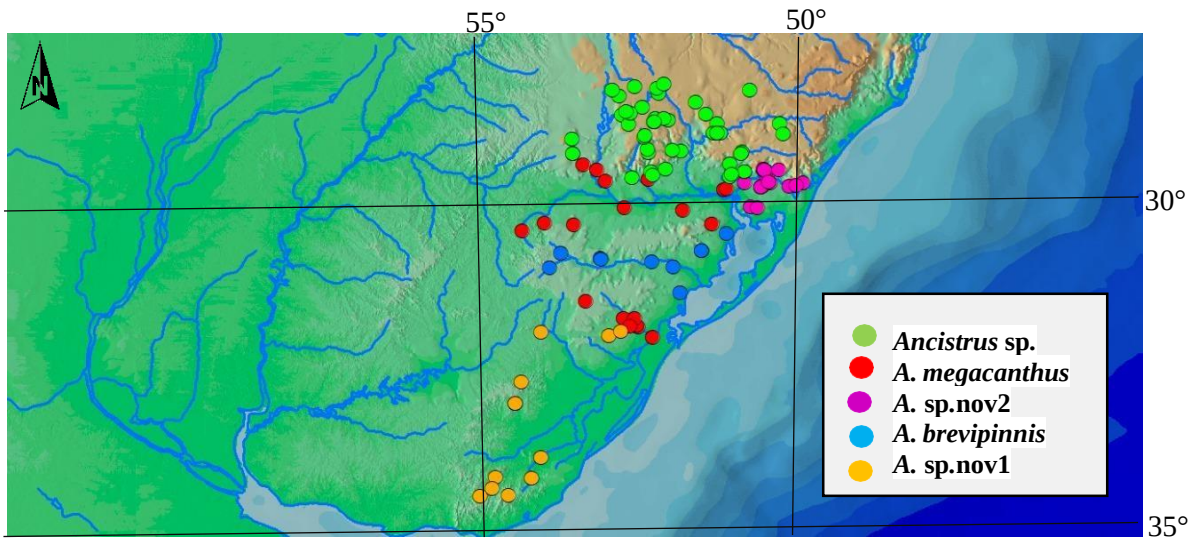

Figure 8: Occurrence points of *Ancistrus* species in the Lagoa dos Patos basin.

TABLES

Table 1: Comparative morphometric data of *Ancistrus* sp. nov1.; n = number of individuals.

***A.* sp. nov1 n=12**				
	Min	Max	Mean	Holotype
Standard length (mm)	60.78	83.36	74.47	83.36
Percent of standard length				
Abdominal length	18.40	20.86	19.28	19.48
Adipose fin to caudal fin distance	18.46	20.51	19.46	19.51
Adipose-fin spine length	9.22	11.33	10.31	10.41
Anal fin to caudal fin distance	32.55	33.49	33.03	33.49
Anal-fin unbranched ray length	7.73	9.62	8.65	7.73
Body depth	14.38	17.79	16.09	14.38
Body width at dorsal-fin origin	29.24	31.83	30.59	30.64
Caudal peduncle depth	31.91	34.74	33.24	34.74
Caudal peduncle length	9.51	11.19	10.73	11.06
Cleithral width	33.30	35.04	34.12	35.04
Dorsal fin to adipose fin length	11.72	14.84	13.80	14.84
Dorsal-fin base length	17.57	23.37	20.43	19.46
Dorsal-fin spine length	21.46	25.70	22.73	22.79
Head length	32.69	35.89	34.49	32.69
Interbranchial distance	16.16	19.41	18.12	18.65
Lower caudal fin ray length	18.32	30.08	25.47	28.00
Pectoral-fin spine length	29.24	31.11	29.94	30.54
Pectoral-pelvic distance	21.45	24.96	22.79	22.79
Pelvic-fin unbranched ray length	17.05	23.28	20.85	17.05
Predorsal length	41.65	47.08	45.02	41.65
Supracleithral width	27.58	29.57	28.76	29.44
Upper caudal fin ray length	18.48	24.79	20.96	19.73
Percent of head length				
Head height over opercle	40.48	45.98	43.91	45.91
Internostril distance	16.24	21.81	19.71	20.95
Interorbital distance	37.24	43.34	40.00	43.34
Mandibulary teeth row width	15.96	23.12	18.87	18.68
Opercle length	67.89	70.49	69.28	67.89
Orbital diameter	16.11	18.35	17.12	16.11
Snout length	57.81	66.82	61.02	61.36
Counts			**Mode**	
rows of maxillary teeth	39	55		39
rows of mandibular teeth	39	68		44
Dorsal plates serie	20	25		21
Median plates serie	21	26		22

Table 2: Comparative morphometric data of *Ancistrus* sp. nov2.; n = number of individuals.

	***A.* sp. nov2 n=46**			
	Min	Max	Mean	Holotype
Standard length (mm)	25.82	111.32	61.66	89
Percent of standard length				
Abdominal length	10.31	21.56	18.57	10.3
Adipose fin to caudal fin distance	7.09	27.21	19.18	7.1
Adipose-fin spine length	6.74	15.76	10.89	6.7
Anal fin to caudal fin distance	30.12	38.54	33.90	30.1
Anal-fin unbranched ray length	5.42	10.77	8.40	5.4
Body depth	11.60	21.67	16.23	11.6
Body width at dorsal-fin origin	26.94	32.41	29.44	26.9
Caudal peduncle depth	26.69	35.93	31.90	26.7
Caudal peduncle length	8.47	12.26	9.97	8.5
Cleithral width	29.59	36.12	32.18	29.6
Dorsal fin to adipose fin length	5.50	15.82	12.52	5.5
Dorsal-fin base length	17.12	23.58	20.78	17.1
Dorsal-fin spine length	17.96	25.50	22.67	18.0
Head length	25.61	40.06	33.22	25.6
Interbranchial distance	12.32	20.44	17.43	12.3
Lower caudal fin ray length	19.37	34.73	27.18	19.4
Pectoral-fin spine length	25.63	33.17	29.03	25.6
Pectoral-pelvic distance	11.43	26.50	22.78	11.4
Pelvic-fin unbranched ray length	20.08	27.01	23.25	20.1
Predorsal length	26.77	49.30	43.89	26.8
Supracleithral width	25.02	31.60	27.98	25.0
Upper caudal fin ray length	16.15	27.62	21.77	16.2
Percent of head length				
Head height over opercle	40.74	59.84	47.85	40.7
Internostril distance	15.92	33.59	20.38	15.9
Interorbital distance	29.98	53.25	39.71	30.0
Mandibulary teeth row width	14.62	25.85	19.49	14.6
Opercle length	50.31	93.25	69.17	50.3
Orbital diameter	15.37	26.80	18.48	15.4
Snout length	48.19	75.56	61.37	48.2
Counts	(n=32)			
rows of maxillary teeth	40	61		49
rows of mandibular teeth	40	66		51
Dorsal plates serie	20	23		21
Median plates serie	23	25		25

Table 3: Comparative morphometric data of *Ancistrus* sp. n = number of individuals; SD = StandardDeviation.

***Ancistrus* sp. n=113**				
	Min	Max	Mean	SD
Standard length (mm)	31.42	101.80	67.57	15.05
Percent of standard length				
Abdominal length	11.79	21.06	18.60	1.43
Adipose fin to caudal fin distance	9.85	23.12	18.68	2.15
Adipose-fin spine length	6.15	14.65	10.69	1.55
Anal fin to caudal fin distance	26.35	41.76	32.82	2.04
Anal-fin unbranched ray length	5.23	12.18	8.10	1.23
Body depth	12.58	31.40	18.16	2.28
Body width at dorsal-fin origin	25.41	34.04	30.38	1.60
Caudal peduncle depth	25.18	36.76	31.90	2.04
Caudal peduncle length	8.36	14.94	10.59	0.83
Cleithral width	19.46	35.59	32.97	1.95
Dorsal fin to adipose fin length	9.93	34.46	13.58	2.51
Dorsal-fin base length	16.83	27.68	21.00	1.49
Dorsal-fin spine length	18.61	26.34	22.84	1.70
Head length	29.52	44.36	33.97	2.50
Interbranchial distance	14.34	21.39	18.36	1.30
Lower caudal fin ray length	21.18	37.15	27.13	2.40
Pectoral-fin spine length	25.14	33.95	29.31	1.96
Pectoral-pelvic distance	17.11	26.90	22.81	1.59
Pelvic-fin unbranched ray length	17.79	27.56	23.39	1.71
Predorsal length	19.79	49.59	43.86	3.50
Supracleithral width	24.61	32.50	28.90	1.55
Upper caudal fin ray length	16.59	29.65	21.41	2.25
Percent of head length				
Head height over opercle	35.29	55.80	47.83	3.62
Internostril distance	13.76	26.42	18.08	2.18
Interorbital distance	27.72	48.82	39.09	3.29
Mandibulary teeth row width	13.65	27.03	20.68	2.39
Opercle length	52.60	82.24	69.53	4.90
Orbital diameter	10.82	23.16	17.31	2.01
Snout length	46.27	70.63	61.38	4.66
Counts			**Mode**	(n=101)
rows of maxillary teeth	32	82	51	
rows of mandibular teeth	33	77	46	
Dorsal plates serie	20	22	22	
Median plates serie	21	25	23	

4. CONCLUSÕES E CONSIDERAÇÕES FINAIS

Definições *a priori* da distribuição das espécies induz sempre a erros recorrentes de interpretação das variedades populacionais de uma espécie, necessitando revisões sistemáticas profundas.

O gênero *Ancistrus* demonstra uma diversificação recente (79 spp. - Eschmeyer & Fong 2024, + 2 sp.nov) ao longo do continente sul-americano, com menor diversidade na região sul em comparação à região equatorial. De maneira geral, as descrições das espécies são muito simples, especialmente das espécies andinas. A revisão desses grupos pode revelar muitas espécies, como aqui demonstrado.

As três novas espécies mais o complexo ainda a se resolver estavam ocultas em uma descrição muito simples, sem limitar adequadamente os caracteres diagnósticos de *Ancistrus brevipinnis*, sua variação populacional e localidade-tipo, impossibilitando diagnosticar as demais.

Se confirmou que na Bacia da Laguna dos Patos havia mais de uma espécie do gênero *Ancistrus*, além disso foi possível redescrever a espécie *A. brevipinnis* e em consequência disso outras espécies ocorrentes na laguna foram evidenciadas. Foram testadas diversas ferramentas de análise (LDA, Anova, Fst, modelagem de nicho) sendo escolhido as que apresentaram resultados mais contundentes, o resultado não utilizados destas análises se encontram nos apendices (1 - 7).

Como o gênero contém muitas espécies com poucas variações morfológicas, o uso de dados filogenéticos foi essencial para restringir os grupos e definir as novas espécies. Foram descritas três novas espécies com base em caracteres morfológicos e diagnósticos, além de identificado um grupo complexo que ainda precisa ser estudado.

É necessária a redescrição de *A. taunayi*, que também foi descrita com poucas informações, além de sua distribuição ser descrita como Bacia do Rio Uruguai, sendo aqui mostrado que a distribuição deste gênero ultrapassa estes limites.

Foram apontadas informações relevantes sobre a complexa distribuição do grupo denominado *Ancistrus* sp. que deverá ser melhor compreendida com a inclusão de outros marcadores moleculares, mais a observação da coloração em vida dos grupos distintos, com a inclusão de novas coletas em pontos pouco amostrados e a inclusão da espécie vizinha (*A. taunayi*). Esses esforços podem revelar mais espécies ocultas e ajudar a compreender melhor as rotas de dispersão e evolução do gênero, além de permitir melhorar estudos para a conservação das espécies.

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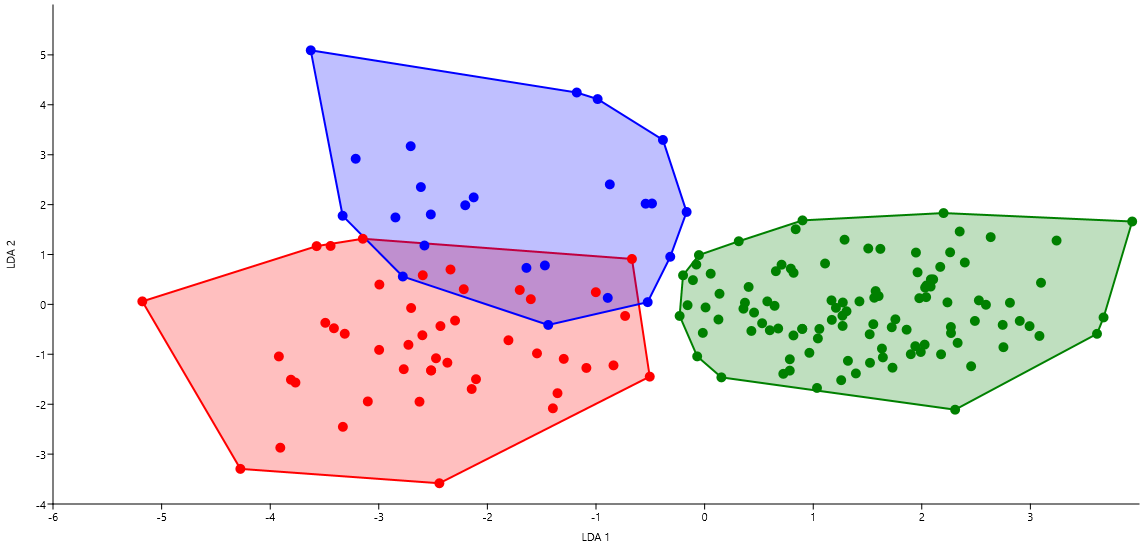
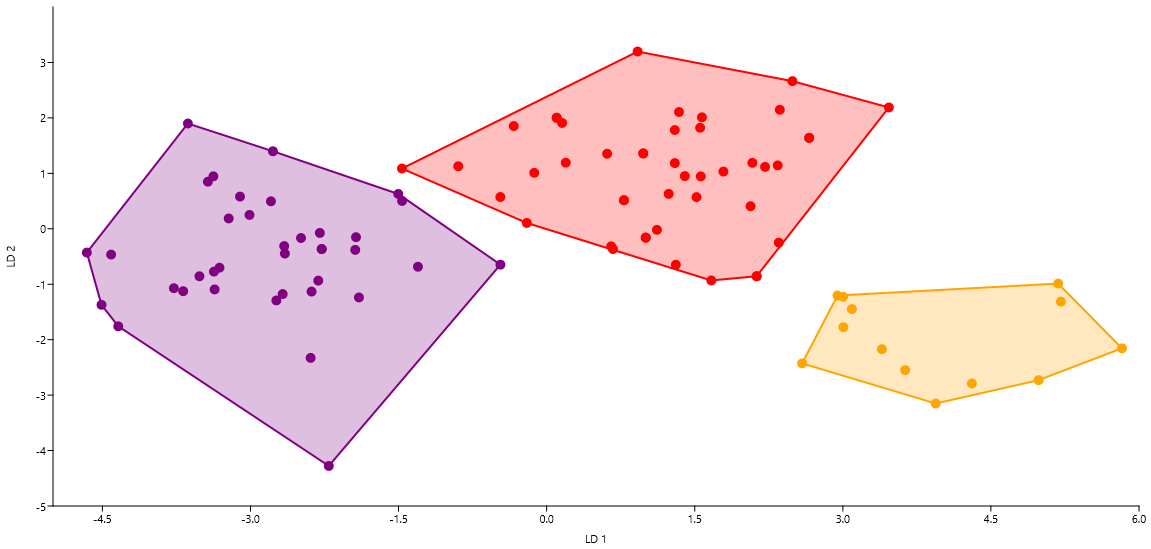
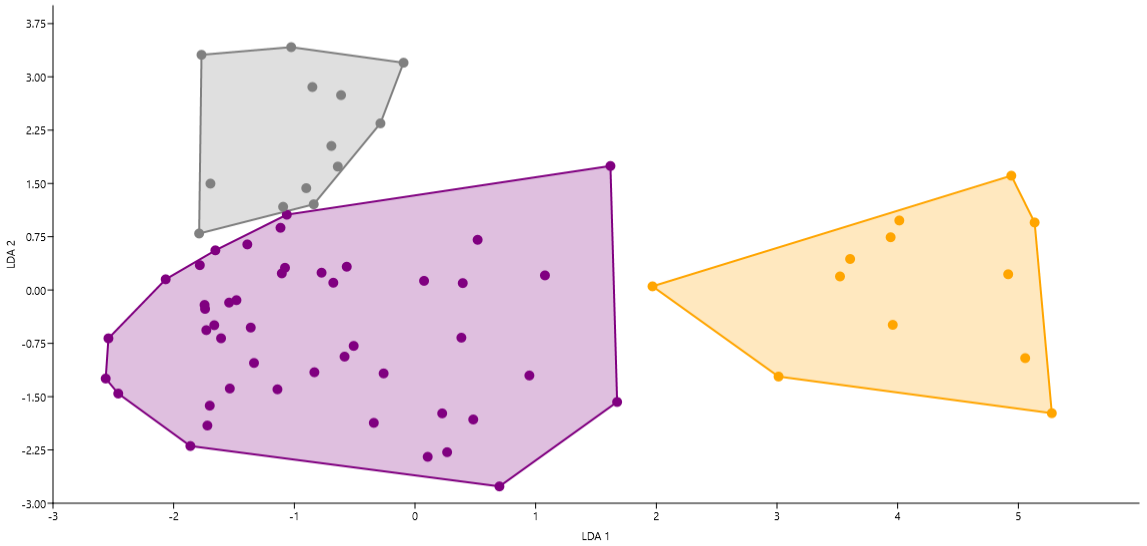
Apendices



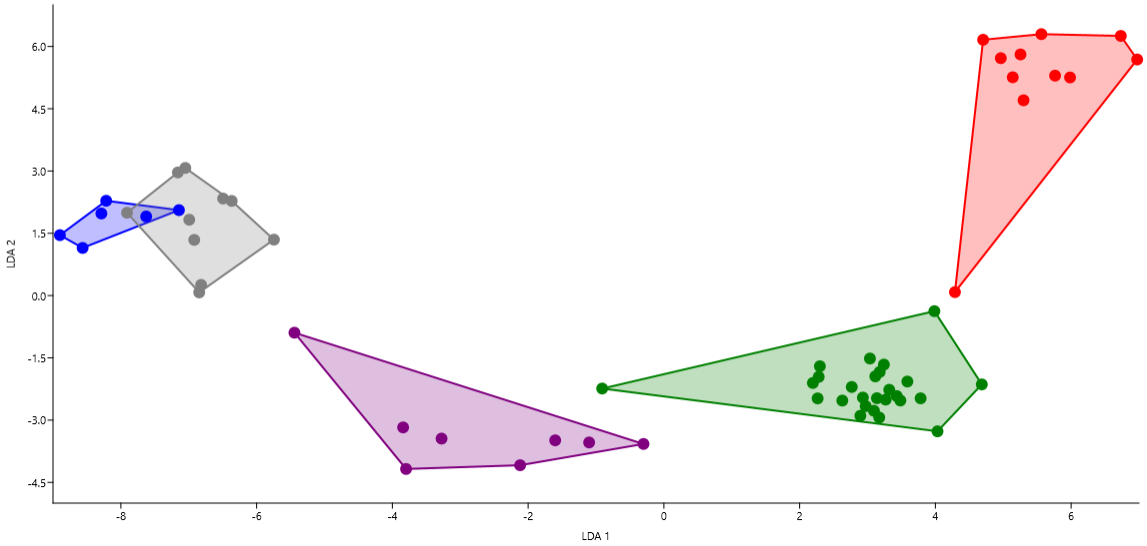
Apendice 1: LDA de *Ancistrus megacanthus* está em vermelho, *A. brevipinnis* está em azul e *Ancistrus* sp. está em verde. Os grupos estão separados no eixo LDA1, convergindo positivamente na largura supracleitral (0,59) e negativamente na distância peitoral-pélvica (-0,39). Enquanto no eixo LD2 eles estão convergindo positivamente com o comprimento dos raios não ramificados da nadadeira pélvica (0,28) e negativamente com o comprimento do espinho da nadadeira peitoral (-0,44). As análises de LDA foram removidas, pois separam apenas quando um grupo de três amostras, mais que isso se sobrepõem completamente.



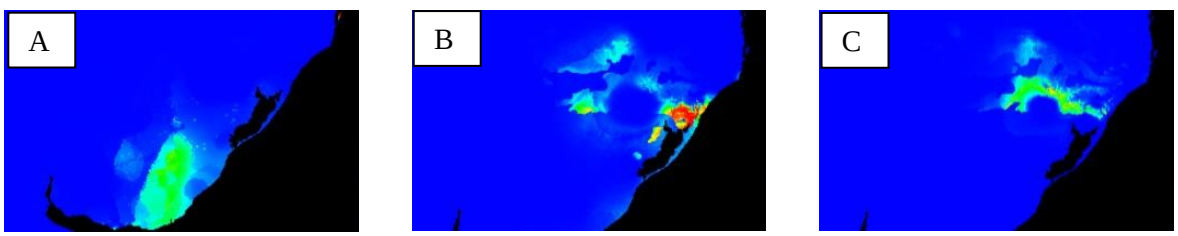
Apendice 1: LDA de *Ancistrus megacanthus* está na cor vermelha, *A.* sp. nov1 está na cor laranja e *A.* sp. nov2 está na cor roxa. Os grupos estão separados no eixo LDA1, convergindo positivamente no comprimento do pedúnculo caudal (1,27) e negativamente na largura supracleitral (-0,80). Enquanto no eixo LD2 eles estão convergindo positivamente com o comprimento do raio não ramificado da nadadeira anal (0,54) e negativamente com o comprimento do pedúnculo caudal (-0,71). As análises de LDA foram removidas, pois separam apenas quando um grupo de três amostras, mais que isso se sobrepõem completamente.



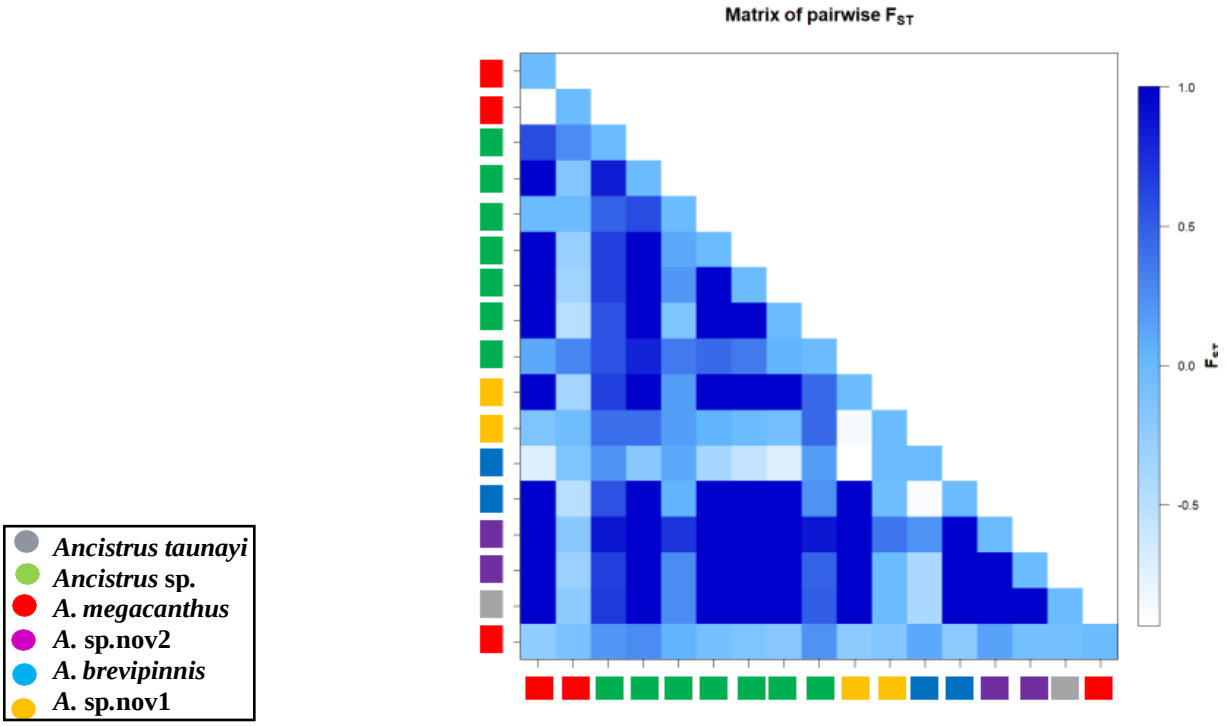
Apendice 4: LDA de *Ancistrus taunayi* está na cor cinza, *A.* sp.nov1 está na cor laranja e *A.*sp.nov2 está na cor roxa. Os grupos estão separados no eixo LDA1, convergindo positivamente na largura Cleitral (0,88) e negativamente na largura Supra cleitral (-0,40). Enquanto no eixo LD2 eles estão convergindo positivamente com o diâmetro orbital (0,52) e negativamente com o comprimento da base da nadadeira dorsal (-0,52). As análises de LDA foram removidas, pois separam apenas quando um grupo de três amostras, mais que isso se sobrepõem completamente.



Apendice 5: LDA das variações ambientais, onde *A. brevipinnis* em azul; *A.* sp nov1 em cinza; *A.* sp.nov2 em roxo; *Ancistrus* aff. *taunayi* em verde; *A. megacanthus* em vermelho. Os grupos estão separados (exceto Azul e cinza) no espaço amostral, com os dados convergindo positivamente em LDA1 na Isotérmidade (0,63) e negativamente para Temperatura Média Anual -0,58, já em LDA2, estão convergindo positivamente em Temperatura Média Anual (0,09) e negativamente para Isotérmidade (-0,48).



Apendice 6: Modelo de nicho (habitat) ecológico, onde "A" é de *A.* sp nov1; "B" *A.* sp nov2; e "C" *A.* sp complexo. Estes modelos foram retirados uma vez que demostraram baixos valores de relação, exeto para *A.* sp nov2



Apendice 7: A AMOVA resultante entre *Ancistrus* sp. complexo (Verde), A. sp.nov2 (roxo) e A. sp.nov1 (Laranja) possui um FST 0,54 (p-Valor 0,01) o que demonstra uma estrutura fixa significativa entre essas populações, as demais (Ancistrus megacanthus – vermelho; Ancistrus brevipinnis em azul e Ancistrus taunyi em cinza) apresentaram um FST elevado, mas não apresentaram significância. A correlação de Mantel não mostrou correlação entre distância molecular e geográfica.