UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

PRISCILLA CAROLINE SILVA

SISTEMÁTICA INTEGRATIVA – DIVERSIDADE E RELAÇÕES DE *DEUTERODON* EIGENMANN 1907 (TELEOSTEI: CHARACIDAE) E GÊNEROS AFINS

PORTO ALEGRE, RS
2017

PRISCILLA CAROLINE SILVA

SISTEMÁTICA INTEGRATIVA – DIVERSIDADE E RELAÇÕES DE *DEUTERODON* EIGENMANN 1907 (TELEOSTEI: CHARACIDAE)E GÊNEROS AFINS

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Área de Concentração: Biologia Comparada

Orientador: Prof. Dr. Luiz Roberto Malabarba

Coorientador: Prof. Dr. Vinícius de Araujo Bertaco

PORTO ALEGRE, RS

2017

Sistemática integrativa – diversidade e relações de *Deuterodon* Eigenmann 1907 (Teleostei: Characidae) e gêneros afins

Priscilla Caroline Silva

Aprovada em 26 de abril de 2017.

Dr. Fernando Camargo Jerep – UEL

Dr. Fernando Rogério de Carvalho – UFMS

Dr. Jorge Abdala Dergam dos Santos – UFV

Aos meus pais Maria Helena (in memoriam) e Marcélio,
meus irmãos Marília e Marcelo e
meus avós Salvador Hugo (in memoriam) e Celípia Oliveira

"Tenho a impressão de ter sido uma criança brincando à beira-mar, divertindo-me em descobrir uma pedrinha mais lisa ou uma concha mais bonita que as outras, enquanto o imenso oceano da verdade continua misterioso diante de meus olhos."

Sir Isaac Newton

Agradecimentos

Eu nunca imaginei que acharia essa parte do trabalho a mais difícil de escrever... São muitas emoções, sentimentos, uma mistura de sensações e um medo enorme de esquecer alguém...

Em primeiro lugar devo agradecer a força maior que rege o planeta e nossa existência... Por um tempo eu duvidei... Mas você sempre esteve aí... Obrigada Deus, obrigada por tudo e por não desamparar nos momentos de dificuldade e desespero (que não foram poucos) durante essa jornada...

Minha família! Ah!! Sem vocês o que sou??? Nada! Em primeiro lugar agradeço meus pais que me deram vida, que me incentivaram, acreditaram e deram condições para que chegasse até aqui.

Agradeço minha mãe, estrela mais brilhante no meu céu, que sempre dizia nos domingos na casa de vovó Teresinha que queria uma filha doutora! Mãe como eu queria que você pudesse estar aqui conosco, pra ver essa vitória e comemorar... Meu pai... Pai, obrigada por não desistir de mim na minha dificuldade com matemática, obrigada pela educação rigorosa e pela frase que se repete até hoje na minha mente: “o estudo é tudo, sem estudo não somos nada! É a maior herança que posso deixar para vocês...” E é verdade, maior e melhor herança! Você é exemplo, tem garra, honestidade e humildade! Muito do que sou vêm de você...

Vovô Hugo, minha outra estrelinha brilhante lá no céu, e vovó Celipe, pelo amor fraternal, pelo riso terno e amparo nos momentos mais difíceis da minha vida! Quando tudo ficou escuro, vocês foram sol! Também pela ajuda financeira, que com certeza foi diferencial para que eu chegasse até aqui.

Meus irmãos, Didi e Marcelinho... Agradeço cada minuto de vida que passei e passo com vocês... São meu alicerce, minha base forte, meu porto seguro e minha certeza de sempre voltar! Isso tudo, a ausência, a privação da convivência diária, é por vocês e essa vitória é com certeza de vocês também, porque sei o quanto ela representa...

Minhas tias Jandra, Karla, Stael e Grace pelo incentivo primordial à leitura, à escrita e à cultura, tudo que sou é reflexo da infância maravilhosa que vocês nos proporcionaram (livros, idas à museus, cidades históricas, parque dos dinossauros – inesquecíveis momentos, álbuns de figurinha e almanaques, caderno de caligrafia). Meus tios Cacá e Pedro, palavra de paz e aconchego nas horas difíceis e por acreditar sempre que eu chegaria até aqui!

Vovó Teresinha pelo riso frouxo e olhar maternal que lembra tanto minha mãe... Tias queridas, especialmente minha Dinha e tia Zélia, pela lembrança viva de mamãe e por nunca se esquecerem de nós... Maria Teresa, priminha linda por cuidar tão bem da Dinha e da vovó!

Tiaguinho, meu afilhado amado! Minha primeira sensação de maternidade em algum estágio... Você sempre acreditou e me incentivou, quero ser exemplo pra você! Pepê, meu outro afilhado querido, sempre na torcida pelo meu melhor!

Meu orientador, querido professor Malabarba! Agradeço muito a sua confiança em me aceitar no seu laboratório e equipe de braços abertos mesmo sabendo que eu nunca tinha olhado um lambari numa lupa antes e que poderia dar muito errado! Hehehe Agradeço a paciência e todos os ensinamentos ao longo desses quatro anos. Eu cresci muito contigo, aprendi tanto quanto eu nunca imaginei que fosse capaz... Agradeço o incentivo para realizar coisas que até eu duvidava que poderia fazer (sanduíche, gut contends, ancient DNA, osteologia). Sua passagem pela minha vida foi marcante e certamente me mudou para melhor!

Cláudia Malabarba! Minha querida amiga e primeira aluna da bancada de molecular! Obrigada, obrigada, obrigada, por tornar os dias no lab mol menos chatos, pela parceria pra qualquer coisa! Tenho muito que agradecer todo o incentivo que recebi de ti para sair do Brasil e tentar realizar um trabalho inovador! Muito do que eu fiz, se eu segui em frente quando tudo parecia perdido, é porque você estava lá dando força, acreditando (quando eu já desacreditava)... Só tenho que dizer: que sorte a minha ter convivido com você! Tomara que nossa parceria perdure!!

Jorge Dergam! Se hoje eu trabalho com peixes, se hoje eu sou apaixonada por esses lambaris (na verdade relação de amor e ódio!) é porque você me mostrou esse mundo maravilhoso que é a ictiologia! Suas conversas apaixonadas pelo trabalho, preocupações com o futuro da bacia do Rio Doce e o entusiasmo diário com certeza me inspiraram a seguir em frente! Também sou grata por você ter dado o passo inicial nessa jornada do doutorado, quando escreveu para o Malabarba perguntando se podia me aceitar! Você sabia que era um sonho pra mim e se estou aqui hoje, devo muito a você... Também agradeço sua presteza em sempre me enviar material, sempre facilitando para que esse trabalho seguisse da melhor maneira... Obrigada!!

Maria Rita Silvério Pires, minha primeira inspiração acadêmica! Fez com que eu descobrisse o amor por anatomia comparada e ter certeza que é zoologia o caminho a seguir... Fez também com que eu tomasse gosto pela sistemática, contigo desenhei os primeiros cladogramas! Nunca vou me esquecer de quando tu me disseste: “Prisci, peixe é muito legal, tu vai lá pro Dergam e nunca mais vai deixar de trabalhar com peixe!” Você estava certa Rita! Peixe é muito legal, mas anfisbena também é! Que saudade!

Richard Vari... My dear advisor at USA... We spent few time together, but this time was intense and made all difference in my work... I need to say thank you for the amazing opportunity of work at SI, for all e-mails you sent to curators around the world that provided tissues, specimens and allowed my visits... Without you, I certainly could not have all this material and this work... I am so sorry that you had not time to see the results of this work... I am sure you are resting in peace for all you have done for everybody.

Lynne Parenti for all support at the most difficult time at US... David Johnson to support my activities when Rich was not able to do... People from Fish and Herp divisions for all funny time at coffee on Fridays, good time...

Jeff Clayton, Khris Murphy, Jeff Hunt and Lee Weigt for all support at MSC and LAB and for your patience too... Without your help part of this work could not happen.

Karsten Hartel! You were the first person that believed in my project with ancient DNA. Thank you by the incentive and all facilities that you provide at MCZ… Andrew Williston for all support during my visit at MCZ.

All curators that facilitate my work and provide material: Oliver Crimmen (BMNH), Patrice Pruvost and Philipe Keith (MNHN), Anja Palandacic (NMW), Mathew Lowe (UMZC), Mark Sabaj (ANSP), Caleb McMahan (FMNH), Marcus Anders Krag and Peter Rask Møller (ZMUC), Dave Catania (CAS), Oswaldo Oyakawa (MZUSP), Angela Zanata (UFBA), Marcelo Britto (MNRJ), Carlos Lucena and Margarete Lucena (MCP).

To all friends that I meet at USA: Janine Ziermann, Héloïse Rouzé, Matt Leray, Dan Lumbantobing, Zee Jaffar, Ai Nonaka, Natalia Agudelo, Nicoli Angeli, Bonnie Blaimer, Dan Mulcahy, Luke Tornabene, Michelle, Paul Ross, João Tonini, Lari, Lorian, Dri, André Neto-Ferreira, Carlos Oliveira! All of you guys made my days easy and happy! Miss you people! I would like to say a special thank you to David Santana and Casey Dillman that shares the same office with me and lots of good histories and unfortunately bad times... Samuel Gomides, my best friend at US, my homemade, my colleague at SI... Thank you for all good time, for all of your help, for split food, smiles and great moments... You show me another world and give me lots of reasons to believe... Thank you ever!

Às gurias da ictio: Alice Hirschmann, Ju Wingert (e Vitor lindão da tia!), Carol Hartmann e a best Karine Bonato... Gurias vocês me ensinaram muito sobre amizade nesses últimos anos, fizeram a carga parecer menos pesada, os dias mais divertidos e o mais importante, me aceitaram, me inseriram e se fizeram amigas! Que seja eterno, que seja infinito, pra mim será inesquecível! Amo vocês!

Karine, best ever and forever! Tu eu preciso agradecer em especial... Obrigada pela parceria, pelas acolhidas na sua casa, pela amizade e incentivo! Que daqui pra frente a gente siga levando só o que for bom e o que importa! Te amo!

À professora Clarice Fialho por ajudar sempre que necessário, principalmente nas burocracias quando o Mala não estava...

Ao meu coorientador Vinícius Bertaco pela rapidez em corrigir os textos e sugestões!

Ao pessoal do laboratório, pela boa convivência e pelas discussões de artigo... Em especial aos amigos da sala 119 com quem aprendo muito todos os dias: Juliano Ferrer, Dario Faustino e Júnior Chuctaya. Também em especial ao Matheus Gallas Lopes por sempre estar disposto a ajudar e pela sua curiosidade de cientista!

À Dea Thomaz, Tiago Carvalho, Udson Santos e Alice Hischmann pela ajuda na coleta do material pelas bacias costeiras...

Udson pelos ensinamentos em molecular, pelo incentivo para iniciar o doutorado e por ter amplificado muitos dos *Astyanax* do Tramandaí.

Ao Valeri por sua presteza em sempre procurar os *Astyanax* que preciso na coleção!

Ao Fabio Di Dario e seus alunos pela ajuda e animação em sair para coletar os topótipos de *Astyanax taeniatus* e pela excelente recepção em seu laboratório!

Ao CNPq pela bolsa concedida tanto no Brasil quanto no exterior, ao ensino público e de qualidade (desde a graduação)!

Ao povo gaúcho e ao Rio Grande do Sul! Não foi aqui que nasci, mas aqui me acolheram como tal! Por aqui vivi experiências inesquecíveis e grandes momentos! Jamais esquecerei! Levo comigo o chimarrão, minha bombacha e as alpargatas... Estarás sempre em mim!

Aos gaúchos do meu coração: Anderson prof, Anderson Caselani, Cris Pereira, Denise Perlim, Vini, Marco Malta, Hygor Lorençato e Rafa Menezes... Gente cada um de vocês a seu modo colaborou muito pra que este final de tese ficasse mais leve e passasse mais suave!

Wal Zart e Raíssa Moreira em especial, por tudo que significam pra mim... Já não posso viver sem vocês duas! Obrigada amigas pelo incentivo, compreensão e palavras de conforto nas horas complicadas... Vamos ter muito ainda pra comemorar!

Tommy meu bebezinho... Faz os dias mais felizes e com certeza diminuiu meu estresse com seus latidos estridentes e sua pressa para viver! Te amo muito melhor amigo, filhinho, cãopanheiro!

Aos meus amigos de Itaguara e Minas (Gustavo, Helaine, Dudu e Sandra) por compreenderem a minha ausência na maior parte do tempo e por não desistirem de mim... Em especial agradeço ao Diego, acho que você não faz ideia de como sua presença, mesmo que virtualmente, me salvou várias vezes... Que o tempo permita que nossa convivência nunca finde e que a amizade e cumplicidade cresçam com o passar dos anos!

Por último e não menos importante agradeço minha eterna morada, minha amada república Convento! Convento é força, é luta, é paz, é conforto, é segurança para o coração... Amadas irmãs conventinas, com quem aprendi muito sobre a vida, sobre viver, sobre compartilhar... Agradeço imensamente os momentos vividos e a compreensão de vocês pela minha ausência em situações especiais para a vida de cada uma... Eu prometo que vou voltar!!! Sempre irei voltar! É pra sempre!

SUMÁRIO

Resumo Geral

O objetivo principal deste trabalho foi realizar a reconstrução das relações filogenéticas das espécies do gênero *Deuterodon*, testando suas possíveis relações com espécies de outros gêneros de Characidae que possuem um arranjo similar de dentes do dentário, como em *Astyanax*, *Jupiaba* e *Myxiops*. Na análise filogenética foi utilizada uma matriz previamente publicada e com o acréscimo de 49 táxons, totalizando 233 espécies de Characidae. Vinte novos caracteres foram adicionados a esta matriz com o intuito de entender as relações dos gêneros e espécies de interesse com os demais Characidae. Um total de 219 espécimes tiveram o DNA extraído e 4 genes foram amplificados. Análises moleculares e morfológicas recuperaram um clado mais inclusivo nomeado de Probolodini, composto pelos gêneros *Deuterodon*, *Probolodus*, *Myxiops*, *Hyphessobrycon luetkenii*, espécies de *Astyanax* da região costeira do Brasil e parte das espécies de *Jupiaba*. *Deuterodon* é redefinido sustentado por 9 sinapomorfias e composto por 7 espécies. *Myxiops* é outro gênero válido sustentado por 22 autapomorfias. *Probolodus heterostomus* apresentou 10 autapomorfias na análise, e que podem eventualmente representar sinapomorfias para o gênero após a análise das demais espécies. *Astyanax* é um gênero polifilético e as espécies de *Astyanax* da região costeiras estão mais estreitamente relacionadas a espécies de outros gêneros (*Probolodus*, *Deuterodon* e *Myxiops*) do que à espécie tipo do gênero, *Astyanax mexicanus*. *Jupiaba* também é um gênero polifilético com espécies distribuídas em vários clados na árvore filogenética. *Deuterodon pedri* é mais relacionado à *Astyanax pelecus* e a duas outras espécies de caracídeos não descritos do que ao gênero *Deuterodon*. Paralelamente, como uma etapa necessária à resolução de alguns problemas taxonômicos envolvendo as espécies trabalhadas neste estudo, técnicas para recuperação de DNA antigo de espécimes coletados nos séculos passados foram aprimoradas, tornando possível a extração e amplificação de DNA de espécimes tipos. Através da aplicação destas técnicas, a identidade de *Deuterodon pedri* foi esclarecida com a extração do DNA do lectótipo, que junto com a análise morfológica possibilitou o reconhecimento da espécie em material recentemente coletado e sua redescrição. Outro resultado paralelo foi a descoberta do holótipo de *Tetragonopterus vittatus* em uma visita à coleção do Muséum national d'Histoire naturelle de Paris, considerado como desconhecido até então. O exame desse material permitiu a revalidação da espécie em combinação nova, como *Moenkhausia vittata*, sendo retirada da sinonímia de *Astyanax*

bimaulatus. O uso de técnicas tradicionais tais como estudo osteológico e taxonomia em conjunto com técnicas de biologia molecular possibilitaram o esclarecimento de relações filogenéticas neste grupo complexo e a resolução de dúvidas taxonômicas históricas.

Palavras chave: Characidae, Clado C, Dentes, Dentário, DNA antigo, Neotropical, Sistemática, Taxonomia.

Abstract

The main objective of this work was to reconstruct the phylogenetic relationships of the species of the genus *Deuterodon*, testing their possible relationships with species of other characid genera that have similar teeth arrangement, as in *Astyanax*, *Jupiaba,* and *Myxiops*. In the phylogenetic analysis, a previously published matrix was used, with the addition of 49 taxa, totaling 233 Characidae species. Twenty new characters were added to this matrix in order to better understand the relationships of the genera and species of interest with the other Characidae. A total of 219 specimens had the DNA extracted and 4 genes were amplified. Molecular and morphological analyzes recovered a larger clade named Probolodini which is composed by the genera *Deuterodon*, *Probolodus*, *Myxiops*, *Hyphessobrycon luetkenii* and by species of *Astyanax* from the coastal region of Brazil and some species of *Jupiaba*. *Deuterodon* is redefined based on nine synapomorphies and composed of seven species. *Myxiops* is another valid genus supported by 22 autapomorphies. *Probolodus heterostomus* showed 10 autapomorphies that may constitute synapomorphies for the genus if proved to occur in the remaining species. *Astyanax* is polyphyletic and most of the *Astyanax* species of the Atlantic coastal Rivers are more closely related to other genera than to *Astyanax mexicanus*, the type species of the genus. *Jupiaba* is also a polyphyletic genus with species distributed in several clades in the phylogenetic tree. *Deuterodon pedri* is more related to *Astyanax pelecus* and to two other undescribed characid species than to the genus *Deuterodon*. In parallel, as a necessary step to solve some taxonomic problems involving the species in this study, techniques for recovering ancient DNA from specimens collected in the past centuries have been improved, making possible the extraction and amplification of DNA from type specimens of taxonomically complex species of Characidae. Through the application of these techniques, the identity of *Deuterodon pedri* was clarified with the aid of the DNA of the lectotype, which together with the morphological analysis allowed the

recognition of the species in recently collected material and consequently its redescription. Another parallel result was the discovery of the holotype of *Tetragonopterus vittatus* in a visit to the collection of the Muséum national d'Histoire naturelle in Paris, considered as unknown until then. The examination of this specimen allowed the revalidation of the species in a new combination as *Moenkhausia vittata* removing from the synonym of *Astanax bimaculatus*. The use of traditional techniques such as osteological studies in conjunction with techniques of molecular biology allowed the clarification of phylogenetic relationships in these complex groups and the resolution of historical taxonomic problems as exemplified in this study.

Key words: Ancient DNA, Characidae, Clade C, Dentary, Teeth, Neotropical, Systematics, Taxonomy.

Introdução Geral

Os peixes são o maior grupo de vertebrados do mundo, com aproximadamente 33.200 espécies descritas (Froese & Pauly, 2015). É na região Neotropical que está concentrada a maior riqueza da ictiofauna de água doce do mundo, com uma estimativa entre 7.000 e 8.000 espécies (Schaefer, 1998; Albert, Reis, 2011). Dentre os Teleósteos, uma das três infraclasses de Actinopterygii, está a superordem Ostariophysi que compreende 77% de todas as espécies de peixes de água doce (Albert, Reis, 2011). Characiformes é uma das mais diversas ordens de Ostariophysi, com mais de 2.100 espécies descritas (Eschemeyer, Fong, 2017), distribuídas nas Américas do Norte, Central, do Sul e na África, com a maior diversidade de espécies concentrada na região Neotropical (Nelson, 2006).

Characidae é a maior família da ordem Characiformes, abrangendo 52% das espécies (Eschemeyer, Fong, 2017), as quais possuem uma elevada diversidade de formas, sendo constituída por gêneros de espécies diminutas e de grande porte (Nelson, 2006). Nos últimos dez anos, Characidae foi a família da ordem com o maior número de espécies descritas (Oliveira *et al*., 2011), no entanto permanece sendo a família neotropical com mais problemas taxonômicos. As relações filogenéticas entre as subfamílias e gêneros que compõem esta família permanecem incompreendidas e muitos dos gêneros não são monofiléticos (Mirande, 2010; Oliveira *et al.*, 2011).

Oitenta e oito gêneros de Characidae representados por 620 espécies das 945 reconhecidas até aquele momento foram considerados por Lima *et al*. (2003) como "*Incertae sedis*" em 2003, por não possuírem posição filogenética bem estabelecida dentro da família. Após este período alguns estudos morfológicos (Malabarba, Weitzman, 2003; Mirande, 2009, 2010) e moleculares (Calcagnotto *et al*., 2005; Javonillo *et al*., 2010; Oliveira *et al*., 2011) foram realizados e contribuíram para um maior esclarecimento das relações filogenéticas entre as espécies de Characidae. Por exemplo, na maioria destes trabalhos as mesmas hipóteses de monofiletismo e relação entre alguns gêneros da família é encontrada (e.g., Javonillo *et al*., 2010; Malabarba, Weitzman, 2003; Mirande, 2010; Oliveira *et al.*, 2011; Thomaz *et al.*, 2015a), o que fez com que a maior parte dos gêneros antes considerados "*Incertae sedis"* fossem posicionados filogeneticamente. Três clados maiores são recuperados pela maior parte destes estudos filogenéticos e são conhecidos por clados A (Stevardiinae), B e C. Dentre estes três clados o clado C é o mais rico em espécies (Eschemeyer Fong, 2017) e

também o de relações menos compreendidas, uma vez que é composto por gêneros tais como *Hyphessobrycon* Durbin, 1908, *Moenkhausia* Eigenmann 1903, *Hemigrammus* Gill 1858, *Jupiaba* Zanata 1997 e *Astyanax* Baird & Girard 1854 todos polifiléticos em estudos filogenéticos (Mirande, 2010; Oliveira *et al*., 2011).

Deuterodon Eigenmann 1907, pertence ao Clado C. Foi proposto por Eigenmann em 1907 inicialmente devido ao arranjo de dentes do dentário, decrescendo suavemente no dentário. Lucena, Lucena (2002) redefiniram o gênero novamente baseados na dentição, restringindo-o a sete espécies válidas endêmicas das bacias costeiras do Atlântico [*D. iguape* Eigenmann, *D. langei* Travassos, *D. longirostris* (Steindachner), *D. rosae* (Steindachner), *D. singularis* Lucena & Lucena, *D. stigmaturus* (Gomes), and *D. supparis* Lucena & Lucena]. As outras 3 espécies *D. parahybae* Eigenmann 1908, *D. pedri* Eigenmann 1908 e *D. potaroensis* Eigenmann 1909 foram consideradas como *incertae sedis* em Characidae por não possuírem as 3 sinapomorfias propostas por Lucena & Lucena (2002) para definir *Deuterodon*.

Apesar de Lucena & Lucena (2002) terem proposto a redefinição de *Deuterodon*, esta não foi baseada em um estudo filogenético. Nenhum trabalho feito até o momento, tanto molecular quanto morfológico, considerou todas as espécies de *Deuterodon* ou foi realizado com o intuito de compreender as relações dentro do gênero e com os gêneros relacionados a este. Coutinho-Sanches, Dergam (2015) em um ensaio sobre a citogenética de *Deuterodon pedri*, fizeram um teste filogenético com 4 espécies de *Deuterodon* (2 delas alocadas em *Incertae sedis*) e *Astyanax* das bacias costeiras do leste do Brasil utilizando dois genes. Esses autores encontraram *Deuterodon* como não monofilético e estreitamente relacionado a espécies de *Astyanax* endêmicas do leste do Brasil. Mirande (2010) encontrou uma estreita relação entre *Deuterodon iguape* e *Deuterodon langei* com duas espécies de *Jupiaba* e prediz que possivelmente o gênero *Myxiops* Zanata & Akama 2004 possa estar estreitamente relacionado a este clado, uma vez que compartilha várias características com esses outros gêneros (Mirande, 2010). Oliveira *et al.* (2011) encontraram *Deuterodon iguape* relacionado à *Probolodus heterostomus* e *Myxiops aphos*.

Myxiops é um gênero monotípico endêmico de uma drenagem no sul da Bahia. Foi descrito por Zanata & Akama (2004) principalmente pelo arranjo especial dos ossos infraorbitais. Nesse gênero os dentes do dentário decrescem gradualmente e Mirande (2010)

considerou que esse gênero esteja possivelmente relacionado à *Deuterodon* devido à esta característica.

Probolodus também é um gênero endêmico de drenagens costeiras no leste do Brasil. Composto por 3 espécies (Santos,Castro, 2014), o gênero possui um arranjo especial dos dentes relacionado ao seu hábito lepidófago (Sazima, 1977). Alguns autores (Roberts, 1970; Géry, 1977; 1980; Mirande, 2010) hipotetizaram uma relação estreita de *Probolodus* com Tetragonopterinae (composto até então por gêneros como *Deuterodon* e *Astyanax*). Sazima (1983) considera que essa estreita relação é realmente possível e que o hábito de ingerir escamas (em *Probolodus*) dentro de Tetragonopterinae pode ter evoluído devido à um comportamento agressivo em um ancestral "*Astyanax*-like" que utilizava espécies de gêneros sintópicos (*Deuterodon* e *Astyanax*) como potenciais presas. Contudo Sazima (1983) considera que é importante um teste filogenético para confirmação da estreita relação entre esses gêneros com *Probolodu*s e para testar tal afirmação.

Jupiaba Zanata 1997 é outro gênero do clado C de Characidae com algumas espécies que apresentam os dentes do dentário decrescendo gradualmente. Três das espécies que compõem esse gênero foram originalmente descritas em *Deuterodon* (*Jupiaba acanthogaster* (Eigenmann 1911), *Jupiaba pinnata* (Eigenmann 1909) e *Jupiaba minor* (Travassos 1964)). O gênero foi descrito por Zanata (1997) com o intuito de agrupar espécies de Characidae que apresentam o espinho pélvico alongado, projetando-se ou não para fora do corpo (Zanata, 1997). Dentro desse gênero existe uma ampla variação no arranjo dos dentes do dentário e padrão de coloração (Benine *et al.*, 2017). Em um estudo molecular e morfológico, Benine *et al.* (2017) sugerem *Jupiaba* como polifilético.

O gênero *Astyanax* possui a maior riqueza de espécies do "Clado C" com cerca de 147 espécies válidas (Eschemeyer *et al.*, 2016), registradas desde o sul dos Estados Unidos até o norte da Argentina (Eigenmann, 1921). A grande similaridade de formas entre as espécies deste gênero, muitas das vezes detectáveis somente em estudos osteológicos, torna difícil a definição de caracteres diagnósticos para reconhecimento de espécies (Melo, 2000). Várias mudanças taxonômicas envolvendo espécies de *Astyanax* têm ocorrido nos últimos anos; por exemplo, a sinonimização do gênero monotípico *Psalidodon* Eigenmann 1911 em *Astyanax* (Pavanelli, Oliveira, 2009), a revalidação de *Astyanax jordani* (Hubbs & Innes 1936) por muito tempo considerada sinônima de *Astyanax mexicanus* (de Filippi 1853) e o

reconhecimento de *Astyanax aeneus* (Günther 1860) anteriormente considerada sinônima de *Astyanax fasciatus* (Cuvier 1819) (Nelson, 2006). Todas estas mudanças demonstram que os limites entre as espécies de *Astyanax* não são bem determinados e que estudos taxonômicos e filogenéticos no gênero são necessários.

Dentre as 147 espécies válidas de *Astyanax*, 14 espécies (*Astyanax taeniatus* Jenyns 1842, *Astyanax jenynsii* (Steindachner 1877), *Astyanax bahiensis* (Steindachner 1877), *Astyanax giton* Eigenmann 1908, *Astyanax intermedius* Eigenmann 1908, *Astyanax ribeirae* Eigenmann 1911, *Astyanax hastatus* Meyers 1921, *Astyanax pelecus* Bertaco & Lucena 2006, *Astyanax microschemos* Bertaco & Lucena 2006, *Astyanax endy* Mirande, Aguilera & Azpelicueta 2006, *Astyanax puka* Mirande, Aguilera & Azpelicueta 2007, *Astyanax burgerai* Zanata & Camelier 2009, *Astyanax jacobinae* Zanata & Camelier 2008, e *Astyanax hamatilis* Camelier & Zanata 2014) possuem um arranjo dos dentes do dentário caracterizado pela presença de 4, 5 ou 6 dentes maiores seguidos de um intermediário em tamanho antes dos diminutos, de forma que estes ganham a impressão de diminuir gradualmente em tamanho quando comparadas às demais espécies de *Astyanax*. Algumas destas espécies de *Astyanax* são reconhecidas apenas por exemplares-tipos, não tendo sofrido revisão nos últimos séculos ou foram revisadas sem uma avaliação abrangente na área de distribuição (*e.g.* Melo, 2001). Algumas outras como *Tetragonopterus vittatus* nem ao menos possuíam o exemplar tipo reconhecido (Eschemeyer *et al.*, 2015). Todas essas espécies são endêmicas de bacias costeiras no leste do Brasil, exceto *Astyanax endy* e *Astyanax puka*, que são endêmicas de drenagens na Argentina (Mirande *et al.*, 2007).

As bacias costeiras localizadas no leste do Brasil começaram a ser formadas logo após a quebra da Gondwana, durante o Cretáceo. Estas drenagens são limitadas ao oeste pelo complexo do Espinhaço, que as isola das bacias continentais presentes no escudo Cristalino Brasileiro (Ribeiro, 2006). Estas bacias costeiras são consideradas distintas unidades biogeográficas (Vari, 1988; Weitzman *et al.*, 1988; Bizerril, 1994; Buckup, 2011) e a presença de um elevado número de espécies e gêneros endêmicos compartilhados entre sistemas de drenagens hoje isolados, dentro destas unidades biogeográficas, é explicado pela recente história paleohidrográfica (Thomaz, *et al.* 2015b).

DNA histórico ou antigo (aDNA) é aquele DNA isolado de amostras anciãs tais como subfósseis, múmias e espécimes de museus coletados nos séculos passados. Além destes, todo

tipo de DNA proveniente de amostras antigas (p. ex. espécimes de museu) que não foram especificamente fixadas para estudos moleculares deve ser considerado DNA antigo. O primeiro registro do uso de aDNA deu-se em 1984 com a finalidade de recuperação do DNA de um exemplar de *Equus quagga,* uma subespécie extinta de zebra da planície africana (Higuchi *et al.*, 1984). O espécime estava tombado há pelo menos 150 anos em um museu. O DNA extraído desse espécime ajudou não apenas na determinação do posicionamento filogenético dessa subespécie, mas permitiu o desenvolvimento de um projeto de reprodução e cruzamento com posterior reintrodução dos Quaggas em ambiente natural (http://www.quaggaproject.com/quagga-dna-results.htm).

O uso de aDNA tem sido muito útil na resolução de problemas taxonômicos, quando os espécimes tipo não tem mais as características que permitem a sua correta identificação apenas através de morfologia. Espécimes tipo de mais de um século estão frequentemente envolvidos em dúvidas nomenclaturais e ambiguidades por não possuírem na maioria das vezes os caracteres diagnósticos que permitiriam uma identificação acurada (Cappellini *et al*., 2013).

Este tipo de estudo tem se tornado cada vez mais difundido devido ao desenvolvimento de novas técnicas em biologia molecular, tais como os sequenciamentos de nova geração (Linderholm, 2016). Técnicas tradicionais como metodologia de Sanger (Sanger & Coulson, 1975), amplamente utilizada nos primórdios do aDNA, tem sido menos utilizadas, principalmente devido à natureza fragmentada deste tipo de amostra. A metodologia tradicional de sequenciamento de Sanger, no entanto, continua sendo a mais acessível para muitos grupos de pesquisa. Adicionalmente, esta metodologia fornece informações que possibilitam a comparação com um maior número de táxons, cujas sequências já estão disponíveis no GenBank ou Bold (barcode).

Considerando que muitas espécies de taxonomia problemática foram descritas nos séculos passados, o desenvolvimento de técnicas de extração e amplificação de aDNA permite estabelecer o “Genetype” para espécies descritas há séculos, possibilitando assim a resolução rápida e definitiva de várias questões taxonômicas e filogenéticas (especialmente espécies da família Characidae pertencentes ao Clado C).

De acordo com Weitzman, Malabarba (1998) o arranjo de dentes pode ser mais informativo em análises filogenéticas do que só o número de dentes em cada parte do aparato

bucal. De acordo com estes autores, considerar apenas números de dentes pode acarretar em uma organização caótica das relações filogenéticas e utilizá-los por si só para classificação pode culminar no estabelecimento de grupos polifiléticos.

A análise conjunta de caracteres morfológicos e marcadores moleculares, considerando a distribuição geográfica de espécies e gêneros que compõem o Clado C de Characidae bem como as características comuns presentes nesses gêneros pode ser a chave para o entendimento dos processos evolutivos que contribuíram para a diversificação dos mesmos.

Assim, o principal objetivo deste estudo foi fazer a reconstrução filogenética das espécies do gênero *Deuterodon*, testando suas possíveis relações com espécies de outros gêneros de Characidae que possuem um arranjo de dentes do dentário similar, como *Astyanax*, *Jupiaba* e *Myxiops*. Para isso, este estudo foi dividido em 5 capítulos:

- O primeiro capítulo trata da apresentação da filogenia obtida a partir da análise integrada de caracteres morfológicos e moleculares no estudo das relações entre espécies dos gêneros *Astyanax*, *Jupiaba*, *Deuterodon*, *Myxiops* e *Probolodus*.

- O segundo capítulo traz a redefinição do gênero *Deuterodon* e apresenta as sinapomorfias que definem o gênero. Também é apresentada uma discussão considerando a distribuição geográfica das espécies que compõe o gênero *Deuterodon sensu stricto*.

- O terceiro capítulo trata da redescoberta da identidade de *Deuterodon pedri* através da recuperação do DNA antigo do lectótipo e da redescrição da espécie com base em material recentemente coletado.

- O quarto capítulo discute a metodologia que permitiu a recuperação do DNA do lectótipo de *D. pedri*, apresentando também mais alguns exemplos de sucesso em espécies de Characidae.

- O quinto e último capítulo trata da redescoberta do holótipo de *Tetragonopterus vittatus* e da discussão de sua identidade e que somente foi possível através das visitas realizadas em museus para reconhecimento das espécies costeiras do Clado C.

Referências

Albert JS, Reis RE. Introduction to Neotropical Freshwaters. In: Albert JS, Reis RE, editors. Historical Biogeography of Neotropical Freshwater Fishes. Berkeley: University of California. 2011. p.3-19.

Benine RC, Melo BF, Oliveira C Espinho pélvico: Evidência morfológica e molecular de origens independents em Characidae (Teleostei: Characiformes). Anais XXII Encontro Brasileiro de Ictiologia. 2017.

Bizerril CRSF. Análise taxonômica e biogeográfica da ictiofauna de água doce do leste brasileiro. Acta Biological Leopoldensia.1994;16: 51-80.

Buckup PA. The Eastern Brazilian Shield. In: Albert JS, Reis RE, editors. Historical biogeography of neotropical freshwater fishes. Berkeley: University of California; 2011. p.203-210.

Calcagnotto D, Schaefer SA, DeSalle R. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Molecular Phylo Evol. 2005; 36: 135-153.

Cappellini E, Gentry A, Palkopoulou E, Ishida Y, Cram D, Roos A, *et al.*, Resolution of the type material of the Asian elephant, *Elephas maximus* Linnaeus, 1758 (Proboscidea, Elephantidae). Zool J Linn Soc. 2013; 170:222-232.

Coutinho-Sanches N, Dergam JA. Cytogenetic and Molecular Data Suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) Is a Member of an Ancient Coastal Group. Zebrafish [serial on the Internet]. 2015; 12(5):357-365. Available from: http://dx.doi.org/10.1089/zeb.2014.1068

Eigenmann CH. The American Characidae. Mem Mus Comp Zool. 1921; 43(3): 209-310.

Eschmeyer WN, Fong JD, editors: Species of fishes by family/subfamily [Internet]. Available from: [http://research.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp], 2017.

Eschmeyer WN, Fricke R, van der Laan R, editors. Catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 12 December 2015)

Eschmeyer WN, Fricke R, van der Laan R, editors. Catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2016 [updated 2016 Sep 29; cited 2016 Oct 15]. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Froese R, Pauly D. FishBase. World Wide Web electronic publication. 2015. Available from: www.fishbase.org, version (01/2016).

Géry J. Characoids of the World. Neptune: T. F. H. Publications, Inc; 1977.

Géry J. Un nouveau poisson characoide occupant la niche des mangeurs d'écailles dans le haut rio Tapajoz, Brésil: *Bryconexodon juruenae* n.g.sp. Revue Française d'Aquariologie. 1980; 7: 1-8.

Higuchi R, Bowman B, Freiberger M, Ryder O, Wilson A. DNA sequences from the quagga, an extinct member of the horse family. Nature. 1984; 213:282-284.

Javonillo R, Malabarba LR, Weitzman SH, Burns JR. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. Mol Phylo Evol. 2010; 54: 498-511.

Lima FCT, Malabarba LR, Buckup PA, Pezzi da Silva JF, Vari RP, Harold A, Benine R, Oyakawa OT, Pavanelli CS, Menezes NA, Lucena CAS, Malabarba MCSL, Lucena ZMS, Reis RE, Langeani F, Casatti L, Bertaco VA, Moreira C, Lucinda PHF. Genera *Incertae Sedis* in Characidae. In: Reis RE, Kullander SO, Ferraris CJ, editors. Check List of the Freshwater Fishes of South and Central America. Porto Alegre: Edipucrs; 2003. p. 106-169.

Linderholm A. Ancient DNA: the next generation – chapter and verse. Biol J Linn Soc. 2015; 117:150-160.

Lucena CAS, Lucena ZMS. Redefinição do gênero *Deuterodon* Eigenmann (Ostariophysi: Characiformes: Characidae). Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia. 2002; 15: 113-135.

Malabarba LR, Weitzman SH. Description of a new genus with six new species from southern Brazil, Uruguay and Argentina, with a discussion of a putative Characidae clade (Teleostei: Characiformes: Characidae). Comunicação do Museu de Ciência e Tecnologia da PUCRS, Série zoológica. 2003; 16: 67-151.

Melo, FAG. Revisão taxonômica das espécies do gênero *Astyanax* Baird & Girard, 1854, (Teleostei: Characiformes: Characidae) da região da Serra dos Órgãos. Arquivos do Museu Nacional. 2001; 59: 1-46.

Mirande JM. Weighted parsimony phylogeny of the family Characidae (Teleostei: Characiformes). Cladistics. 2009; 25: 574-613.

Mirande JM. Phylogeny of the family Characidae (Teleostei: Characiformes) from characters to taxonomy. Neotrop Ichthyol. 2010; 8: 385-568.

Mirande JM, Aguilera G, Azpelicueta MM. A new species of *Astyanax* (Characiformes: Characidae) from the endorheic basin, Tucumán, northwestern Argentina. Zootaxa. 2007; 1646: 31-39.

Nelson JS. Fishes of the world. New Jersey, Hoboken: John Wiley & Sons. 2006.

Oliveira C, Avelino GS, Abe KT, Mariguela TC, Benine RC, Ortí G, Vari RP, Castro RMC. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingrooup sampling. BMC Evol Biol. 2011; 11: 275.

Pavanelli CS, Oliveira, CAM. A redescription of *Astyanax gymnodontus* (Eigenmann, 1911), new combination, a polymorphic characid fish from the rio Iguaçu basin, Brazil. Neotro Ichthyol. 2009; 7(4): 569-578.

Ribeiro AC. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. Neotrop Ichthiol. 2006; 4: 225-246.

Roberts TR. Scale-eating American characoid fishes, with special reference to *Probolodus heterostomus*. Proc Calif Acad Sci. 1970; 20: 383-390.

Santos O, Castro RMC. Taxonomy of *Probolodus* Eigenmann, 1911 (Characiformes: Characidae) with description of two new species, and comments about the phylogenetic relationships and biogeography of the genus. Neotrop Ichthyol. 2014; 12(2): 403-418.

Sanger, F., Coulson, A. R. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology, 94: 441-448.

Sazima I. Possible case of aggressive mimicry in a neotropical scale-eating fish. Nature. 1977; 270: 510-512.

Sazima I. Scale-eating in characoids and other fishes. Environ Biol Fish. 1983; 9: 87-101.

Schaefer SA. Conflict and resolution: impact of new taxa on phylogenetic studies of the Neotropical cascudinhos (Siluroidei: Loricariidae). Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p. 375-400.

Thomaz AT, Arcila D, Ortí G, Malabarba LR. Molecular phylogeny of the subfamily Stevardiinae Gill, 1858 (Characiformes: Characidae): classification and the evolution of reproductive traits. BMC Evol Biol. 2015a; 15: 146.

Thomaz AT, Malabarba LR, Bonatto SL, Lacey L. Knowles Testing the effect of palaeodrainages versus habitat stability on genetic divergence in Riverine systems: study of a Neotropical fish of the Brazilian coastal Atlantic Forest. J Biogeogr. 2015b; 42(12): 2389-2401

Vari RP. The Curimatidae, a lowland neotropical fish family (Pisces: Characiformes): distribution, endemism and phylogenetic biogeography. In: Vanzolini PE, Heyer WR, editors. Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências; 1988. p.343-377.

Weitzman SH, Malabarba LR. Perspectives about the phylogeny and classification of the Characidae (Teleostei: Characiformes). In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p.161-170.

Weitzman SH, Menezes NA, Weitzman MJ. Phylogenetic biogeography of Glandulocaudini (Teleostei, Characiformes, Characidae) with comments on the distribution of other freshwater fishes in eastern and southeastern Brazil. In: Vanzolini PE, Heyer WR, editors. Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências; 1988. p.379-427.

Zanata AM, Akama A. *Myxiops aphos*, new characid genus and species (Characiformes: Characidae) from the rio Lençóis, Bahia, Brazil. Neotrop Ichthyol. 2004; 2: 45-54.

Zanata AM. *Jupiaba*, um novo gênero de Tetragonopterinae com osso pélvico em forma de espinho (Characidae, Characiformes). Iheringia, Série Zoologia. 1997; 83: 99-136.

Capítulo 1

Reassessment of Probolodini: an expected tribe of Characidae (Actinopterygii: Characiformes)

Artigo a ser submetido para a Revista Neotropical Ichthyology

Priscilla C. Silva, Vinícius de Araújo Bertaco and Luiz R. Malabarba

Reassessment of Probolodini: an expected tribe of Characidae (Actinopterygii: Characiformes)

Priscilla C. Silva, Vinícius de Araújo Bertaco and Luiz R. Malabarba

Departamento de Zoologia and Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91.501-970 Porto Alegre, RS, Brazil. (PCS) pricarola@gmail.com (corresponding author), (VAB) vbertaco@gmail.com, (LRM) malabarb@ufrgs.br

Abstract

Characidae is one of most diverse fish families of the Neotropical region. In the last decades, some phylogenetic studies tried to solve the relationship among the genera included in this family. In most of them, 3 clades are recovered: clades A, B and C. Clade C is the most rich-species and complex because it includes polyphyletic genera. In this clade, some species belonging to the genera *Astyanax*, *Deuterodon*, *Jupiaba*, and *Myxiops* share a peculiar feature: a special arrangement of gradually decreasing dentary teeth. To test if this feature is homolougous or evolved independently in these taxa we decide to investigate the phylogenetic relationship of these genera inside of Characidae family. Two hundred and nineteen specimens were extracted and 4 genes were amplified. Species of these genera were all included on a morphological matrix totalizing 233 specimens of Characidae and 412 characters. Both molecular and morphological analyses recovered a major clade named here as Probolodini with high statistical support. The synapomorphies that support this clade are not exclusively related to dentition. This clade is composed by *Astyanax* species from coastal drainages with gradually decreasing dentary teeth, *Hyphessobrycon luetkenii*, *Jupiaba* species with gradually decreasing dentary teeth, *Myxiops aphos*, *Probolodus heterostomus*, all *Deuterodon stricto sensu* species, *Deuterodon pedri* and two undescribed taxa. The synapomorphies that define this major clade and from all other valid genera are presented and discussed. Evolutionary patterns and biogeographic aspects are also highlighted.

Keywords: *Deuterodon*, coastal drainages, clade C, phylogeny, parsimony.

Resumo

Characidae é uma das famílias de peixes neotropicais com a maior diversidade de espécies. Nas últimas décadas, alguns trabalhos filogenéticos foram realizados com o intuito de solucionar as relações filogenéticas dentre os gêneros que compõe essa família. Na maioria destes trabalhos três clados são sempre recuperados: Clados A, B e C. Clado C é o mais especioso dos três clados e também o mais complexo, uma vez que é composto por vários gêneros polifiléticos. Neste clado algumas espécies pertencentes aos gêneros *Astyanax*, *Deuterodon*, *Jupiaba* e *Myxiops* compartilham não só, mas também uma característica peculiar: uma organização especial dos dentes do dentário que dão a impressão de que estes decrescem gradualmente. Para testar se esta característica evoluiu de maneira independente em cada gênero ou se é uma sinapomorfia que une espécimes que compartilham esse estado, as relações filogenéticas dos espécimes desses gêneros que possuem tal característica foram investigadas dentro da família Characidae. Para tanto DNA foi extraído de 219 espécimes e 4 genes foram amplificados. Espécies desses gêneros foram também incluídas em uma matriz totalizando 233 táxons e 412 caracteres morfológicos. Ambas as análises moleculares e morfológica recuperaram um grande clado, nomeado aqui de Tribo Probolodini. As sinapomorfias que sustentam essa unidade não são exclusivamente relacionadas à dentição. Este clado é composto por 11 espécies de *Astyanax* das drenagens costeiras do leste do Brasil que possuem o dentário com dentes decrescendo gradualmente, *Hyphessobrycon luetkenii*, espécies de *Jupiaba* com dentes do dentário decrescendo gradualmente, *Myxiops aphos*, *Probolodus heterostomus*, todas as espécies de *Deuterodon stricto sensu*, *Deuterodon pedri* e duas espécies não descritas. As sinapomorfias que definem esse grande clado e as sinapomorfias que definem cada gênero que o compõe são apresentadas e discutidas. Aspectos evolutivos e padrões biogeográficos são destacados.

Palavras-chave: *Deuterodon*, clado C, drenagens costeiras, filogenia, parcimônia

Running head: The Probolodini

Introduction

The Neotropical region has the richest freshwater fish fauna in the world with an estimative between 7.000 (Albert, Reis, 2011; Reis *et al.* 2016) and 8000 species (Schaefer, 1998) that represents 10% of all vertebrate species (Vari, Malabarba, 1998). Most of the Neotropical freshwater ecosystems are dominated by ostariophisan fish (Characiformes, Siluriformes and Gymnotiformes) that represents 77% of all fish species (Albert, Reis, 2011). Characiformes is one of most diverse orders with 2100 species and Characidae is the most diversified family of this order with approximately 1100 species (Eschemeyer, Fong, 2017).

Characidae is also the most problematic group inside of the Characiformes (Oliveira *et al.*, 2011) with a considerable hundred genera and species pointed as *incertae sedis* by Lima *et al.* (2003). Some recent morphological (Malabarba, Weitzman, 2003; Mirande, 2009, 2010) and molecular studies (Calcagnotto *et al*., 2005; Javonillo *et al*., 2010; Oliveira *et al*., 2011) have contributed to better understand the relationship of the genera inside of this family once congruence of monofiletism has been recovered (e.g., Javonillo *et al*., 2010; Malabarba, Weitzman, 2003; Mirande, 2010; Oliveira *et al.*, 2011; Thomaz *et al.*, 2015). These studies have placed genera considered before as *Incertae sedis* in valid subfamilies.

Three major clades are always recovered in characid phylogenies: Clade A, clade B and clade C (Javonillo *et al.*, 2010; Mirande, 2010; Oliveira *et al.*, 2011).The largest advance in the knowledge of the relationships inside of this family was the establishment of the clade A by Malabarba, Weitzman (2003). This clade encompasses characids that share 2 unbranched rays plus 8 branched rays in dorsal fin and four teeth in the internal tooth series of the premaxilla (Malabarba, Weitzman, 2003). Clade B includes Tetragonopterinae *sensu stricto* (only *Tetragonopterus*), Cheirodontinae, Aphyocharacinae, Paragoniatinae, Characinae and Aphyoditeinae.

Among these 3 major clades, the clade C is the most species-rich (Eschmeyer, Fong, 2010). This clade has very complicated relationship once it is composed by genera as *Hyphessobrycon* Durbin, 1908, *Moenkhausia* Eigenmann 1903, *Hemigrammus* Gill 1858, *Jupiaba* Zanata 1997 and *Astyanax* Baird & Girard 1854 which have been characterized as polyphyletic in phylogenetic studies (Mirande, 2010; Oliveira *et al.*, 2011). Species and genera belonging to this clade have been classified as Pristellinae (*e.g.* Eschemeyer *et al.* 2017).

Astyanax is the most diverse genus of clade C with 147 valid species (Eschmeyer *et al.*, 2016). From all know valid *Astyanax* species, a small group composed by 14 species has an interesting arrangement of dentary teeth: decreasing gradually, with 4, 5, 6 or 7 teeth followed by one tooth with intermediary size followed by small ones. All *Astyanax* species that presents this characteristic are endemic from the eastern coastal drainages in Brazil, with the exception of *Astyanax endy* Mirande *et al.*, 2006 (Mirande *et al.*, 2006) and *Astyanax puka* Mirande *et al.*, 2007, both endemic from Argentina (Mirande *et al.*, 2007).

This particular arrangement of teeth allowed Eigenmann (1907) to describe the genus *Deuterodon* Eigenmann ,1907 defined as having two series of teeth in premaxilla and dentary teeth gradually decreasingin size. Later, the genus *Deuterodon* was redefined and other synapomorphies were proposed to recognize it (Lucena, Lucena, 2002). In the redefinition, only seven species were kept as *Deuterodon* (*e.g.* Lucena, Lucena, 2002), all endemic from coastal south and southeastern drainages of Brazil (Lucena, Lucena, 1992).

Curiously some of the *Astyanax* species described by Eigenmann from coastal drainages present the same characters used by him to define *Deuterodon*, but he did not include these in that genus (*e.g*. *Astyanax ribeirae* Eigenmann 1911 and *Astyanax giton* Eigenmann 1908). Eigenmann (1908) also made comments that some species from coastal drainages are similar to *Deuterodon* as *Astyanax taeniatus* Jenyns, 1842, and suggested that this species might be closely related to *Deuterodon* species.

Jupiaba Zanata 1997 is another member of the C clade, and some of its species also have dentary teeth that gradually decrease in size. *Jupiaba* was described by Zanata (1997) to assemble species of Characidae with an elongated pelvic spine. The genus has a great morphological variation mainly in dentary teeth. Some of the *Jupiaba* species where originally described as *Deuterodon* (*e.g. Deuterodon acanthogaster* Eigenmann, 1911) or *Astyanax*, but none of them are endemic or distributed in Brazilian eastern coastal drainages.

So far, hypothesized phylogenetic relationships of *Deuterodon* with other characids have been based on the analysis with few species. Mirande (2010) found a closely relationship between two species of *Deuterodon* and two species of *Jupiaba*. A close relationship was hypothesized between *Deuterodon iguape* and *Deuterodon langei* and *Myxiops* Zanata & Akama 2004, a monotypic genus endemic from Bahia and also having dentary teeth gradually decreasing(Oliveira *et al.*, 2011). Coutinho-Sanches, Dergam (2015) demonstrate *Deuterodon*

iguape, Deuterodon supparis, Deuterodon parahybae and *Deuterodon pedri* as closely related with some *Astyanax* species endemic from eastern coastal drainages. The latter studies were based only on molecular data and none of them have a representative sampling of the species of *Deuterodon*, *Astyanax* or *Jupiaba*.

Considering the presence of a shared peculiar character among the species of these complex and polyphyletic genera, the main goal of this work is to test the relationship between these species. Morphological and molecular data were used for a better understanding of how this special tooth arrangement evolved in Characidae and wheter it is a synapomorphy within the clade C.

Material and Methods

The ingroup used to test the relationships of species from clade C with dentary gradually decreasing includes all the species of *Deuterodon sensu stricto* (Lucena, Lucena 2002, Silva *et al.* 2017: *D. iguape*, *D. langei, D. longirostris*, *D. rosae, D. singularis, D. supparis*, and *D. stigmaturus*, *D. pedri*, *D. potaroensis*), species of the genera *Myxiops, Probolodus,* and *Jupiaba* previously hypothesized as related to *Deuterodon*, and representative species of *Astyanax* and *Hyphessobrycon* from coastal Atlantic drainages. All ingroup species are included in the morphological and/or molecular analyses, but not all were available for both analyses (Supporting information Table S1 and S2).

Morphological analysis

Osteological preparations were carried out following Taylor & Van Dyke (1985). The extended matrix of Mirande *et al*. (2013) was used, excluding 53 taxa (species of *Creagrutus* and *Paleotetra*) that were not codified by several characters. Fourty nine taxa (*Astyanax bahiensis, A. brachypterygium, A. cremnobates, A. dissensus, A. douradilho, A. fasciatus, A.* aff. *fasciatus, A. giton, A. goyanensis, A. hastatus, A.* aff. *hastatus, A. henseli, A. intermedius, A. jenynsii, A. jequitinhonhae, A. lacustris, A. laticeps, A.* aff. *microschemos, A. pelecus, A. procerus, A. ribeirae, A. scabripinnis, A. taeniatus, A. xiru*, *Astyanax* sp. A, *Astyanax* sp. B, *Astyanax* sp. C, characidae sp. 1, characidae sp. 2, *Deuterodon pedri, D. potaroensis, D. rosae, D. singularis, D. stigmaturus, D. supparis, D. longirostris, Hyphessobrycon luetkenii, Jupiaba abramoides, J. acanthogaster, J. anteroides, J. asymmetrica, J.* cf. *atypindi, J.*

essequibensis, *J. ocellata*, *J. pinnata*, *J. poekotero*, *J. polylepis*, *J. potaroensis*, *Myxiops aphos*) and twenty new characters were added on the matrix previously published by Mirande *et al*. (2013), resulting in 412 characters and 233 taxa (Supporting information S3 – character matrix). The new characters were codded in all species listed above plus 29 taxa representative of the Characidae that were already available in the original matrix (*Aphyocharax anisitsi*, *Astyanax mexicanus*, *Bryconamericus agna*, *Bryconops affinis*, *Charax stenopterus*, *Cheirodon interruptus*, *Coptobrycon bilineatus*, *Cyanocharax alburnus*, *Diapoma speculiferum*, *Hasemania nana*, *Hemigrammus bleheri*, *Hollandichthys multifasciatus*, *Hyphessobrycon elachys*, *Hyphessobrycon herbertaxelrodi*, *Hyphessobrycon socolofi*, *Jupiaba mucronata*, *Jupiaba scologaster*, *Markiana nigripinnis*, *Mimagoniates rheocharis*, *Moenkhausia dichroura*, *Moenkhausia sanctaefilomenae*, *Nematocharax venustus*, *Odontostilbe paraguayensis*, *Odontostilbe pequira*, *Paracheirodon axelrodi*, *Phenagoniates macrolepis*, *Prionobrama paraguayensis*, *Pseudocorynopoma doriae*, *Xenagoniates bondi*). Although these additional characters are assigned as missing data in remaining taxa of the original matrix, it should not be considered problematic. Dillman *et al.* (2015) tested the missing data power on morphological super matrix and conclude that even with more than 60% of missing data is possible to reconstruct well supported and highly resolved hypotheses of relationship using parsimony analysis. Additionally, Prevosti & Chemisquy (2010) concluded that the inclusion of more characters could make the matrices more robust, indicating that the problem is mainly a lack of information, not just the presence of missing data *per se*.

The characters 5, 64, 73, 96, 190, 265, 342 and 347 (Mirande 2010; Mirande *et al.* 2013) were modified and are commented on Results. The codification of all characters were checked in species of *Deuterodon* and *Probolodus* available in the marix of Mirande (2010) and Mirande *et al.* (2013), and the differences found are described on Results.

The Parsimony analyses were performed following the methods described by Henning (1966) and developed by Farris (e.g. 1969, 1970, 1983). The analyses were carried out with equal weighting (Goloboff 1983) on software TNT version 1.1 (Goloboff *et al.* 2003, 2008). Heuristic searches were conducted using the new technology search options: sectorial search, ratchet, tree drifting and tree fusing as default, with the search of minimum length up to 30 times. Trees were collapsed after search. Multistate characters were considered as unordered.

Supported measures were calculated by consensus of equal weighting analysis. Implied weighting was also carried out on software TNT version 1.1 (Goloboff *et al.* 2003, 2008). Twenty one k values were considered and analysis carried out as detailed in Mirande (2009, 2010) excepted for the use of a different script for implied weighting (S4). Multistate characters were considered as unordered. Supported measures were calculated by consensus of equal weighting analysis and for k = 20 under implied weighting. For more details about k chosen, see Mirande (2009).Those measures are relative frequencies, GC values as support measures (Goloboff *et al.* 2003) and relative Bremer support (Bremer 1994; Goloboff & Farris 2001). The consensus tree, characters state changes and distribution, consistence index, retention index and Bremer support were carried out also by TNT. Consensus tree and character distribution were checked using WinClada version 1.00.08 (Nixon 2002).

Molecular phylogenetic analysis. Tissue samples of 240 specimens of the genera *Astyanax*, *Deuterodon*, *Jupiaba*, *Myxiops*, *Probolodus* and *Serrapinnus* fixed in 96% ethanol from the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS) were used in DNA extraction (Table S2). All molecular analyses were rooted with *Serrapinnus heterodon* as an outgroup. The DNA was extracted from gill filaments, muscle, or liver tissue of the samples, with "Phire Animal Tissue Direct PCR Kit" developed by Thermo Scientific® and followed manufacturer's instructions.

Two mitochondrial genes were amplified: cytochrome oxidase c subunit 1 (*COI*) with primers cocktail FishF1t1 and FishR1t1 (Ivanova *et al*. 2007) and the NADH dehydrogenase 2 (*ND2*) with primers L5216 and H6313 (Sorenson *et al*. 1999). Two nuclear genes were also amplified. The nuclear alpha-myosin 6 (*MYH6*) gene was amplified with nested-PCR using primers F459 and R1325 (1st PCR) and F507 and R1322 (2nd PCR) (Li *et al.* 2007). The SH3 and PX3 domain-containing 3 like protein (*SH3PX3*) gene was also amplified with nested-PCR using primers F461 and R1303 (1st PCR) and F532 and R1299 (2nd PCR) (Li *et al.* 2007).

The PCR reactions for all genes were carried out in a reaction volume of 20 µL [10.3 µL of H20, 2 µL of 10× reaction buffer (Platinum®Taq), 0.6 µL of MgCl2 (50 mM), 2 µL of dNTPs (2 mM), 2 µL of each primer (2 µM), 0.1 µL (5 U) of Platinum® Taq (Invitrogen), and 100 ng of template DNA].

COI was amplified using the following PCR conditions: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 52°C for 40 s, and at 72°C for 1 min, and a final extension at 72°C for 10 min. *ND2* was amplified by touchdown PCR under following PCR conditions: an initial DNA denaturation at 94°C for 4 min, followed by 9 cycles at 94°C for 30 s, at 57°C for 40 s with melting temperature decreasing one degree on each cycle, and at 72°C for 1 min and 30 seconds, 40 cycles with denaturation at 94°C for 30 s, at 47°C for 40 s and at 72°C for 1 min and 30 seconds and a final extension at 72°C for 10 min. The *MYH6* PCR conditions following: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 53°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 10 min on first PCR and an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 62°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 5 min on second PCR. The *SH3PX3* conditions following: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 55°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 10 min on first PCR and an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 65°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 5 min on second PCR. The PCR products were purified by using enzymatic method ExoSap (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50% of deionized water), and sequencing was performed on Macrogen Inc., Seoul, South Korea and Ludwig Biotec at Porto Alegre, RS, Brazil.

Sequences of each locus were independently aligned using Clustal W in MEGA 6.0 software (Tamura *et al*. 2013) and alignments were inspected by eye for any obvious misalignments that were then corrected.

The species tree was estimated on BEAST 2.1.3 software (Bouckaert *et al*. 2014) with StarBeast template. Each DNA alignment was considered a partition and molecular models of evolution and gene trees were unlinked. The best molecular model of evolution for each DNA alignment was chosen using MrModeltest software (Nylander 2004) and this information using to set priors of site substitutions on Site Model panels. It was made to optimize the mixing and convergence of the MCMC chain. A population function constant was chosen on Mult Species Coalescent panel and a Yule Model was chosen as Species Tree prior. The tree was estimated twice and each run was performed with 800 million MCMC iterations and

80,000 trees were retained. The distribution of log likelihood scores was examined to determine stationarity for each run and achieve convergence using the program Tracer 1.5 (Rambaut & Drummond 2009) with 10% of the initial states discarded as burn-in. The program TreeAnnotator (Beast package) was used to summarize the trees with 10% of initial trees discarded as burn-in. StarBeast analyses were run on computational resources provided by Cyberinfrastructure for Phylogenetic Research (CIPRES) (Miller *et al.* 2010).

The posterior probability values of 1–0.91 and percentage values of 100–88 were considered well supported in the Bayesian and maximum parsimony analysis, respectively (Zander 2004). DNA sequences were deposited in GenBank (Access No. XXXX).

Results

Characters

The examination of all species of *Deuterodon* plus *Astyanax* coastal species allowed the description of twenty new characters, mostly related to jaw bones and teeth:

393 – Dentary shape: (0) Height nearly equal along most of its length, narrow anteriorly in the toothed portion corresponding to nearly 1/3 of its length; (1) Deepest at posteriormost portion, height diminishing progressively anteriorly in the toothed portion of the bone corresponding to half to 2/3 of its length.

The dentary in the species of *Deuterodon* is nearly triangular in lateral view (Fig. 1), whereas in most characids this bone is nearly rectangular in profile. The narrowing of the dentary is associated to the length of the distribution of teeth in this bone.

394 – Dentary, teeth, position (Fig. 2): (0) Teeth oriented dorsally not visible in ventral view; (1) Teeth oriented laterally and anteriorly, visible in ventral view.

In the species of *Deuterodon* the dentary teeth are inclined outward, forming an angle of approximately 45 degrees with the bone whereas in most of Characidae species these teeth are directed upward, forming an angle of 90 degrees relative to main dentary axis.

395 – Maxilla, length: (0) Reaching or surpassing the Meckelian cartilage, (1) Not reaching the Meckelian cartilage.

In the species of *Deuterodon*, the maxilla is short and never reaching the Meckelian cartilage. Most characids present maxilla more anteriorly positioned and vertically than in *Deuterodon* species, and this bone seems to be longer, reaching or surpassing Meckelian cartilage. The state observed in *Deuterodon* does not fit in those decribed by Mirande in his character 100, relative to the length of the maxilla (maxilla reaching posterior end of Meckelian cartilage or maxilla not reaching posterior end of Meckelian), since both assume that maxilla reaches Meckelian cartilage.

396 – Antorbital, shape: (0) Vertically elongated or tubular; (1) Triangular, lacking a vertically elongated portion or tubular region.

In most *Deuterodon* species the antorbital is triangular, lacking a vertically elongated portion or tubular region, a condition also observed in some species of *Hasemania* and *Hemigrammus*, *Bryconamericus agna*, *Bryconamericus iheringii*, *Cheirodon interruptus*, *Jupiaba mucronata*, *Jupiaba polylepis*, *Moenkhausia sanctaefilomenae*, *Moenkhausia dichroura*, *Mimagoniates rheocharis*, *Paracheirodon axelrodi*, *Paracheirodon innesi*, and *Pseudocorynopoma doriae*. In the other observed species, the anterorbital is tubular without this posterior triangular shape, except in some species of *Astyanax*, *Hyphessobrycon* and *Jupiaba* and *Deuterodon pedri* that may present have a posterior triangular extension from the anterior vertically elongated tubular format, and are coded as 0.

397 – Maxilla, ascending process: (0) Without lateral projections; (1) With a small lateroventral projection (Fig. 3).

The small lateroventral projection on ascending process of maxilla was observed only in *Deuterodon* species. This projection seems serve for the articulation of this bone with the premaxilla.

398 – Maxilla, posterior edentulous portion (modified from Lucena & Lucena 2002): (0) Longer than toothed portion; (1) Shorter than or equal to toothed portion.

The smaller size of the posterior edentulous portion of maxilla is usually related to a large number of teeth in the toothed portion (e.g. *Charax*), but it is not the case in the species

of *Deuterodon*, that have the edentulous portion smaller even not showing a significative increase in the number of teeth.

399 – Maxilla, dorsal margin: (0) Laminar, without medial laminar projection; (1) With a medial laminar projection bending medially and articulated with palatine.

The maxilla is usually a laminar bone in Characidae. The species of *Deuterodon* have a curvature in the dorsal border turning the maxilla concave medially.

400 – Maxilla, teeth, position (modified from Lucena & Lucena 2002): (0) Maxillary teeth aligned in a 45 degree angle relative to premaxillary teeth; (1) Maxillary teeth aligned continuously with pre-maxillary teeth (Fig. 4).

The state 1 of this character was originally proposed by Lucena & Lucena (2002) as a synapomorphy to define *Deuterodon*.

401 – Premaxilla, ascending process: (0) Forming 90 degree angle with toothed border; (1) Forming a 45 degree angle with the toothed border.

Usually the ascending process of pre-maxilla forms a 90 degree angle with the remaining portion of the bone in characids. In *Deuterodon*, the ascending process is inclined posteriorly in direction to interorbital area instead of directed dorsally and parallel to the nasal, as in most characids.

402 – Dentary, teeth, cusps: (0) Central cusp distinctly larger and longer than other cusps (Fig. 1); (1) All cusps nearly equal in size and shape (Fig. 2).

Tooth shape and cusp shape may vary among mouth bones and so are treated separately for tha maxilla, premaxilla and dentary in characters 402 to 407.

403 – Dentary, teeth, shape: (0) Basal portion wider than or nearly equal to apical portion; teeth juxtaposed, without space between the bases of contiguous teeth; (1) Basal portion of teeth narrower than apical portion with a gap between the bases of contiguous teeth (Figs. 1, 2).

404 - Premaxilla, teeth, cusps: (0) Central cusp distinctly larger and longer than other cusps (Fig. 1); (1) All cusps nearly equal in size and shape (Fig. 4).

405 - Premaxilla, teeth, shape: (0) Basal portion wider than or nearly equal to apical portion; teeth juxtaposed, without space between the bases of contiguous teeth; (1) Basal portion of teeth narrower than apical portion with a gap between the bases of contiguous teeth (Fig. 4).

406 - Maxilla, teeth, cusps: (0) Central cusp distinctly larger and longer than other cusps (Fig. 1); (1) All cusps nearly equal in size and shape (Fig. 3).

407 - Maxilla, teeth, shape: (0) Basal portion wider than or nearly equal to apical portion; teeth juxtaposed, without space between the bases of contiguous teeth; (1) Basal portion of teeth narrower than apical portion with a gap between the bases of contiguous teeth (Fig. 3).

408 - Maxilla, teeth, main axis: (0) Inclined towards mouth gape, not visible in lateral view; (1) Pointing anteroventrally, visible in lateral view.

409 – Fifth ceratobranchial plate, teeth: (0) Widespread in all extension of the plate; (1) Restrict to the borders of the plate.

Most examined species of Characidae have teeth in all extension of the fifth ceratobranchial plate. The distribution of these teeth restricted to the borders of the plate was observed in some *Jupiaba* (*J. abramoides*, *J. anteroides* and *J. polylepis*) and *Astyanax* species (*A. laticeps*, *A. goyanensis*, *A. henseli* and *A. jequitinhonhae*).

410 - Dentary, second tooth, insertion: (0) Tooth base inserted at a lower position in the bone; (1) All tooth bases aligned.

The second tooth of the dentary positioned in a lower position regarding the remaining teeth of the dentary is observed in the species of the *Astyanax* clade sensu Mirande, including *Astyanax mexicanus*, species of the *Astyanax fasciatus* species complex (sensu Melo, 2005), *Astyanax scabripinnis* species complex (sensu Bertaco & Lucena, 2006) and *Astyanax bimaculatus* species complex. It can also be observed in other characid fishes as for example

in the Stevardiinae (Malabarba & Weitzman 2003: 125, fig. 38F). The state 1, second tooth base aligned with the remaining ones in dentary bone is a condition observed in the species of *Deuterodon*, as well as in the majority of the species of *Astyanax* from coastal drainages, in *Hyphessobrycon luetkenii* and some species of *Jupiaba* that show gradually decreasing dentary teeth. This character has not been used previously in phylogenetic analysis.

411 - Hooks, format: (0) Small and delicate; (1) Large and robust.

There is a great variation in the shape, position and function of hooks on all rayed fins in Characidae (Malabarba and Weitzman, 2003). These variations have been partially explored in the characters proposed by Mirande (2010). One variation observed herein is in the size and robustness of hooks that may be small and delicate or large and robust. This character seems to differentiate the species of *Astyanax* from coastal drainages from large and robust ones present in Astyanax species from Astyanax clade.

412 – Dentary, teeth, number of anterior large teeth: (0) Four; (1) Five or more.

Character 142 of Mirande (2010) describes two states for the number and size of anterior dentary teeth: four or five relatively broad teeth at front of dentary or eight or more small and slender teeth at front of dentary. Although character description given by Mirande refers to the "Size and number of anterior dentary teeth", the two states just refer to the presence of absence of large teeth in the anterior portion of the dentary. The characid taxa examined with large anterior dentary teeth, however, posses more than one discrete state. The most common condition among characids corresponds to the presence of four large teeth followed by small ones. The other condition is characterized by the presence of 5, 6 or 7 large teeth anteriorly, that may be followed by one teeth intermediary of intermediate size and then by smaller teeth or that may be followed by teeth gradually decreasing in size.

Characters modified from Mirande (2010):

5 – Form of epioccipital bridge: (0) Cylindrical or vertically expanded in transverse section; (1) Depressed in its middle region, with lateral expansion only on medial portion; (2) Cylindrical with expansion on both lateral sides of the bridge, forming a loop.

Mirande (2010) described this character originally with only two states: 0) cylindrical or vertically expanded in transverse section; (1) depressed in its middle region. We have observed that in *Deuterodon pedri*, *Astyanax pelecus*, undescribed taxa 1 and 2 and *Oligosarcus jenynsii*, the epioccipital bridge is depressed in its middle region and has a lateral expansion only on medial portion. In the species of *Deuterodon* and most characid fish, this bridge has format of tube and also has a lateral expansion only on medial portion (state 0). In *Deuterodon potaroensis* and some *Jupiaba* species we could observe that the bridge is cilindrical has lateral expansion in portions, medial and radial, having a format of loop. So, we add the state two to this previously described character.

64 – Ventral extent of third infraorbital: (0) not reaching horizontal arm of preopercle, at least anteriorly; (1) reaching horizontal arm of preopercle.

This character was coded inverted, so to avoid changes in all previously coded taxon at the matrix, we only inverted the states in the text.

96 – Margins of toothed region of maxilla: (0) dorsal and ventral margins of the toothed portion of the maxilla roughly parallel; (1) anterior region of the toothed portion of the maxilla deeper than the posterior region of the toothed portion (Fig. 3).

Mirande proposed this character originally as: margins of toothed region of maxilla: (0) roughly parallel; (1) dorsally divergent. Mirande commented in the description of the character that Lucena & Lucena (2002) proposed the dorsal divergence of the margins of the maxillary lamellar portion as a synapomorphy of the genus *Deuterodon* (state 1). So, the state one was originally proposed by Lucena & Lucena. We decided to redescribed this character according with was proposed for the first time by Lucena & Lucena (2002) and considered that this new description will improve in the interpretation of this character.

190 – Anterior development of basihyal: (0) slightly surpassing anterior margin of hypohyal; (1) broadly extending beyond anterior margin of hypohyal.

This character was coded inverted, so to avoid changes in all previously coded taxon at the matrix, we only inverted the states on the text.

265 – Relative position of dorsal-fin anterior insertion: (0) posterior to vertical through pelvic-fin origin; (1) anterior to or at vertical through pelvic-fin origin.

This character was coded inverted, so to avoid changes in all previously coded taxon at the matrix, we only inverted the states on the text.

347 – Spots on each scale of the flanks: (0) absent; (1) spots forming points at the distal border of scale and located by all flank region; (2) distal border of scales above lateral line pigmented, forming a dark brown arch when chromatophores are expanded and a an unpigmented arch when chromatophores are not expanded.

Mirande proposed this character as: little spot on each scale of flanks: (0) absent; (1) present. A large variation of coloration and spots of scales can be observed in fish. To try improving this character we redescribed the states with more details and add one more state after our observations with *Deuterodon pedri* coloration pattern, the state 2.

We have identified problems on character interpretation for *Deuterodon* species in Mirande (2009, 2010) and Mirande *et al.* (2013). One of them is that in text Mirande exemplifies *Deuterodon* as having the state 1 for character 128, which is teeth with cusps aligned in straight series and without anterior concavity on inner premaxillary teeth. However, when we checked the matrix, *Deuterodon* was codified as state 0, with cusps forming anteriorly concave arch on teeth of inner premaxillary tooth row for character 128. Problems were also identified with the codification of *Probolodus heterostomus*. For example the character 118, related to form of teeth, has the state 0 for all teeth conical, caniniform, or mamiliform and state 1 for teeth multicuspidate or molariform teeth. *Probolodus* possesses mamiliform teeth (state 0), but Mirande codified as state 1. However, the multicuspidated teeth of *Probolodus* is considered no homolougous when compared with multicuspidate teeth in *Astyanax* or *Deuterodon* species. So, it is more appropriate to treat *Probolodus* teeth as mamiliform. The interpretation of characters is powerful on matrix analysis and final results (Petterson *et al.* 1993), and so we decided to reinterpret all characters previously codified by Mirande on all *Deuterodon* species and *Probolodus heterostomus*, what explain some differences between the original matrix and the new matrix presented in this work.

Molecular phylogenetic analysis. The sequence data of 219 specimens resulted in a matrix with 3091 aligned base pairs (bp). The transitions/transversions (Ti/Tv) ratio was 51 and the overall mean genetic distance (*p*-distance) was 0.14. All other information relative to each gene is summarized in Tab. 1.

StarBeast Bayesian analysis recovered a monophyletic clade composed by the genus *Deuterodon, Astyanax* species from coastal drainages with gradually decreasing dentary teeth, *Hyphessobrycon luetkenii*, *Probolodus heterostomus, Myxiops aphos* and *Jupiaba poranga.* This clade was recovered with significant posterior probability value (Fig. 5).

Deuterodon sensu stricto was recovered as monophyletic (Fig. 5, posterior probability of 0.85), including only the species assigned for the genus from southern section of the Atlantic River drainages of southern Brazil, being congruent with the restricted definition of the genus presented by Lucena, Lucena (2002) and Silva *et al.*, (2017). *Deuterodon pedri*, however, was found not closely related to *Deuterodon sensu stricto*, but recovered as sister group to two undescribed characids (Sp.1 and Sp.2) with high posterior probability (*D. pedri* clade, Fig. 1, posterior probability 0.97). *Hyphessobrycon luetkenii* appears closely related to *Astyanax ribeirae* (posterior probability 0.95) and *Probolodus heterostomus* was recovered as part of a monophyletic group (posterior probability 0.83) with *Astyanax jenynsii*, *Astyanax burgerai*, *Astyanax bahiensis* and *Astyanax* aff. *microschemos*. *Astyanax hastatus* from north Rio de Janeiro and Espírito Santo, *Astyanax intermedius* and *Astyanax giton* are a monophyletic group with posterior probability 0.82 and *Astyanax hastatus* from south Rio de Janeiro, *Astyanax taeniatus* and undescribed taxon Sp. B from Espírito Santo another monophyletic group with 0.57. The hypothesis of relationship among these clades and with *Myxiops aphos* and *Jupiaba poranga* were weakly supported and are not further commented.

All the remaining species of *Astyanax* were found to form a single clade containing *Astyanax mexicanus* (type species of the genus), *A. altiparanae, A. jacuhiensis, A. cremnobates, A. brachpterigyum, A. laticeps, A. xiru, A. bagual, A. scabripinnis, A. douradilho, A. dissensus, A. rivularis, A. fasciatus, A. eigenmaniorum, A. procerus, A. paranae, A. henseli, A. jequitinhonhae, A. lacustris,* and undescribed Sp. C and Sp. D, and would correspond to the true *Astyanax.*

Morphological analysis. The equal weighting hypothesis based on morphological data is the strict consensus among most parsimonious trees with 2884 steps (Fig. 6; CI = 0.303 and RI = 0.621). The implied weighting hypothesis (supplementary file S4) is the strict consensus of the 20th value of K (38.894; CI = 0.309 and RI = 0.645). The results and synapomorphies described herein are based on the strict consensus tree, and the reasons for not adopting the implied weigthting hypothesis are given in the discussion.

The morphological analysis under equal weighting recovered a large monophyletic clade, similar to that obtained from the analysis of molecular data. Inside this major clade, the monophyly of the genus *Deuterodon* proposed by Lucena & Lucena (2002) and Silva *et al.* 2017 was supported, including *D. rosae* not available in the molecular analysis. *Myxiops* is recovered as sister group of *Deuterodon*, differently from the hypothesis obtained from molecular data. *Deuterodon pedri* was found more closely related to *Astyanax pelecus* (not available in the molecular analysis) and to the same two undescribed characids analyzed in the molecular hypothesis. *Probolodus heterostomus*, *Hyphessobrycon luetkenii*, *Astyanax ribeirae, Astyanax* aff. *microschemos, Astyanax jenynsii, Astyanax bahiensis, Astyanax burgerai, Astyanax hamatilis, Astyanax taeniatus, Astyanax hastatus, Astyanax aff. hastatus, Astyanax giton, Astyanax intermedius,* undescribed taxon Sp. A and Sp. B, all from coastal drainages are also inserted in this clade, forming a large polytomy. Several species of *Jupiaba* with dentary teeth gradually decreasing(not available in the molecular analysis) form a monophyletic clade, and this is sister group of *Myxiops* plus *Deuterodon*. *Jupiaba poranga* together with *D. potaroensis* were found as sister group of all the taxa described above, and this is the other difference found between molecular and morphological data set analysis.

Jupiaba and *Astyanax* were found as polyphyletic genera. *Jupiaba* species appears widespread in 5 clades inside of the phylogeny. One of the clades belongs to Probolodini, whereas other 6 species are together with Probolodini clade on a polytomy and this is sister group of *Jupiaba poranga* and *D. potaroensis*. Two other species of *Jupiaba* are not close related, appearing more related to clade B and *Moenkhausia* species, most *Hyphessobrycon* species, *Hemigrammus* species and *Pristella* species. *Astyanax* species appears in three clades inside of Characidae phylogenie. 11 species are part of Probolodini. Other large monophyletic clade composed only by *Astyanax* is closely related to Probolodini plus some *Jupiaba* species and *D. potaroensis*. Remain *Astyanax* species included in the phylogeny, including *Astyanax*

mexicanus appears on a polytomy that includes *Bryconamericus scleroparius, Markiana nigripinnis, Hyphessobrycon meridionalis, Hyphesobrycon bifasciatus, Hyphessobrycon anisitsi, Psellogramus Kennedyi, Bryconamericus emperador* and the clade A.

Based on these results obtained from both molecular and morphological data, a monophyletic group is proposed amongs characids in Clade C, including *Probolodus, Deuterodon sensu stricto, Myxiops, Hyphessobrycon luetkenii,* and part of the species of the genera *Astyanax* and *Jupiaba*, especially those with dentary teeth gradually decreasingin size. This clade is named herein Probolodini, a name available from Géry, 1977. Synapomorphies supporting this clade are based on the equal parsimony analysis.

TRIBE PROBOLODINI Géry, 1977

Included taxa: *Probolodus heterostomus, Hyphessobrycon luetkenii*, *Astyanax ribeirae, Astyanax* aff. *microschemos, Astyanax jenynsii, Astyanax bahiensis, Astyanax burgerai, Astyanax hamatilis, Astyanax taeniatus, Astyanax hastatus, Astyanax giton, Astyanax intermedius Deuterodon pedri Astyanax pelecus, Myxiops aphos*, *Deuterodon iguape, Deuterodon supparis, Deuterodon stigmaturus, Deuterodon singularis, Deuterodon longirostris, Deuterodon rosae* and *Deuterodon langei*

Bremer support: 2; posterior probability: 0.63. Twenty synapomorphies support this clade:

Exclusive synapomorphy:

- Place of insertion of the second tooth of dentary teeth aligned with the insertion of other dentary teeth (410 - 0>1; 1.00; 1.00);

The state 1, second teeth aligned with remain teeth in dentary bone is a condition observed in all species from coastal drainages (including *Deuterodon* species, *Astyanax* species, *Hyphessobrycon luetkenii* and some *Jupiaba* species with gradually decreasing dentary teeth). This condition was inapplicable in *Probolodus heterostomus* because of the different arrangement of teeth in this species.

No exclusive synapomorphies:

-Supraoccipital spine extending only to anterior limit of neural complex of Weberian apparatus (53 - 0>1, 0.03, 0.71). Reversible in *Deuterodon*, *Jupiaba* species with gradually decreasing dentary teeth, *Probolodus heterostomus*, *Astyanax* aff. *microschemos* and *Astyanax jenynsii*. Parallel in *Astyanax brachypterygium*, *A. cremnobates*, Sp. C, *Astyanax goyanensis*, *A. procerus*, *A. jequitinhonhae*, *A. scabripinnis*, *Astyanax bransfordii* (Gill 1877), some *Hyphessobrycon* species, *Thayeria* Eigenmann 1908 species, some *Hemigrammus* species, *Hasemania nana* (Lütken 1875), *Bario steindachneri* (Eigenmann 1893), *Moenkhausia sanctaefilomenae* (Steindachner 1907), *Pristella maxillaris* (Ulrey 1894), *Paracheirodon axelrodi* (Schultz 1956), Aphyocharacinae (sensu Mirande 2010) species, *Grundulus cochae* Román-Valencia, Paepke & Pantoja 2003, *Gymnocharacinus bergii* Steindachner 1903, *Coptobrycon bilineatus* (Ellis 1911), Stevardiinae clade (sensu Thomaz *et al.*, 2015) and Cheirodontinae species.

-Presence of a single tube of blood vessels on lamellar portion of maxilla, parallel to dorsal margin of this bone (98 - 1>0; 0.09; 0.68). Reversible in *Deuterodon longirostris*, *Astyanax pelecus*, *Jupiaba* aff. *atypindi*, *Jupiaba poekotero*, Sp. B, *Astyanax taeniatus*, Sp. A and *Astyanax jenynsii*. Ambiguous in Sp. 1. Parallel in *Astyanax dissensus*, Sp. C, Stevardiinae clade, Cheirodontinae species, Aphyocharacinae and most of C clade (sensu Javonillo *et al.*, 2010).

- Five or more cusps on teeth on outer premaxillary row (125 - 0>1, 0.04, 0.62). Reversible in *D. pedri* and Sp. 1 and ambiguous in *M. aphos* and inapplicable in *P. heterostomus*. Parallel in *Nematocharax venustus* Weitzman, Menezes & Britski 1986, *Gymnocharacinus bergii*, some *Astyanax* species, *Jupiaba* species, *Hyphessobrycon meridionalis* Ringuelet, Miquelarena & Menni 1978, *Hyphessobrycon bifasciatus* Ellis 1911, *Knodus heteresthes* (Eigenmann 1908) and *Bryconamericus agna* Azpelicueta & Almirón 2001.

- Cusps of medial teeth on inner premaxillary row forming shallow arch or aligned in straight series from ventral view (127 - 0>1; 0.05; 0.59). Reversible in *Jupiaba essequibensis*, *J. pinnata*, *J. acanthogasther*, *J.* aff. *atypindi*, *A. burgerai* and *A. jenynsii*. Inapplicable in *P. heterostomus.* Parallel in *J. apenima*, *J. potaroensis*, *J. abramoides*, *J. anteroides*,

Astyanacinus moorii (Boulenger 1892), *Bramocharax* clade, most of Stevardiinae, Cheirodontinae and clade C species.

-Teeth of inner premaxillary tooth row with cusps aligned in straight series and without anterior concavity (128 - 0>1; 0.08; 0.78). Reversible in *Jupiaba essequibensis*, *J. pinnata*, *J. acanthogasther*, *J.* aff. *atypindi*, *A. burgerai* and *A. jenynsii*. Inapplicable in *P. heterostomus.* Parallel in *J. apenima*, *J. potaroensis*, *J. abramoides*, *J. anteroides*, *Oligosarcus menezesi* Miquelarena & Protogino 1996, *O. pintoi* Amaral Campos 1945, *Bryconamericus lethostigmus* (Gomes 1947), *Attonitus ephimeros* Vari & Ortega 2000, *Aulixidens eugeniae* Böhlke 1952, Cheirodontinae species, Aphyocharacinae species, Gymnocharacinae, *Rhoadsia altipinna* Fowler 1911, *Carlana eigenmanni* (Meek 1912), *Hemigrammus erythrozonus* Durbin 1909, *Hemigrammus bleheri* Géry & Mahnert 1986, *Paracheirodon axelrodi* and *Nematocharax venustus*.

- Absence of an abrupt decrease in size of dentary teeth (148 - 1>0; 0.05; 0.64). Reversible in *Astyanax intermedius*, *A. jenynsii*, *A. michroschemos*, Sp. 1 and Sp. 2. Even though we consider the dentary teeth decreasing abruptly, the arrangement of the teeth in these species is different from that observed in other *Astyanax* species. In other *Astyanax* and some Stevardiinae species we can observe the presence of four large teeth on dentary followed by notably smaller ones. In the species of Probolodini, we observe presence of four or five teeth followed by one with intermediate size. The small ones that follow these five or six first are small, but the difference in size is less markable when compared with that observed in fish with only four large teeth and without the fifth intermediary tooth (Fig. 7). In relation to other Probolodini, the condition of five large teeth can be considered abruptly decreasing especially when compared with *Deuterodon* species or *D. pedri* that normally have 7 teeth followed by one intermediary and other small ones. Parallel in *D. potaroensis*, *O. itau* Mirande, Aguilera & Azpelicueta 2011, *Astyanax bransfordii*, Stevardinae and Cheirodontinae.

- Anterior extension of interopercle not extending anteriorly beyond terminus of horizontal arm of preopercle (163 - 0>1; 0.05; 0.44). Reversible in *Deuterodon* species, *Myxiops aphos*, *P. heterostomus*, *Jupiaba* species, *D. pedri* clade, Sp. B, *A. burgerai*, *A. intermedius*. Parallel in *A. dissensus*, *A. jequitinhonhae*, *Creagrutus maracaiboensis* (Schultz 1944), *Microgenys minuta* Eigenmann 1913.

- Two well developed blocks of cartilage anterior to basihyal (188 - 0>1; 0.02; 0.42). Reversible in *D. singularis*, *Myxiops*, *J.* aff. *atypindi*, *J. acanthogasther*, *J. essequibensis*, *J. poekotero*, *D. pedri*, *P. heterostomus*, *A. hamatilis*. Ambiguous in *A. michroschemos* and *Deuterodon* species. Parallel in *J. poranga*, *Astyanax* species, *Oligosarcus* species, *Attonitus ephimeros*, *Knodus meridae* Eigenmann 1911, *Bryconadenos tanaothoros* (Weitzman, Menezes, Evers & Burns 2005), *Cyanocharax* sp., *Diapoma speculiferum* Cope 1894, *Hyphessobrycon bifasciatus*, *Odontostilbe microcephala* Eigenmann 1907, *Aphyocharacidium bolivianum* Géry 1973, *Microschemobrycon casiquiare* Böhlke 1953, *Hasemania nana*, *Thayeria boehlkei* Weitzman 1957, *Moenkhausia* species, *Poptella paraguayensis* (Eigenmann 1907), *Jupiaba scologaster* (Weitzman & Vari 1986), *Roeboides descalvadensis* Fowler 1932 and *Bryconops melanurus* (Bloch 1794).

- Denticles on gill rakers restricted to margins, or absent (202 - 1>0; 0.04; 0.62). Reversible in *J. poekotero*, *J. essequibensis*, *J. pinnata*, and *A. jenynsii*. Ambiguous in *A.* aff. *michroschemos* and *Myxiops*. Parallel in *D. potaroensis*, some *Astyanax* species, Stevardiinae, Cheirodontinae, Aphyocharacinae and most clade C species.

- Posterior margin of cleithrum with concavity ventral to first postcleithrum (234 - 0>1; 0.03; 0.72). Reversible in *A. burgerai*, Sp. B, *J. pinnata* and *J.* aff. *atypindi*. Parallel in *J. poranga*, *D. potaroensis*, *A. dissensus*, *A. fasciatus*, *A.* aff *fasciatus*, *A. henseli*, *A. procerus*, *A. jequitinhonhae*, *A. scabripinnis*, Stevardiinae clade, Cheirodontinae, Aphyocharacinae, some *Hyphessobrycon* species, *Hemigrammus* species, *Moenkhausia* species, *Thayeria*, *Pristella maxillaris*, *Bryconops* and Heterocharacinae.

- Five or more supraneurals (280 - 0>1; 0.02; 0.43). Reversible in *J. poekotero*, *J. pinnata*, *J. essequibensis*, *J.* aff. *atypindi*, *Myxiops* and *Probolodus*. Ambiguous in *A. giton*. Parallel in *J. potaroensis*, *J. anteroides*, *D. potaroensis*, most *Astyanax* species, *Oligosarcus* species, Stevardiinae, most clade C species, most Cheirodontinae, Aphyocharacinae, Gymnocharacinae, *Hasemania nana*, *Hemigrammus*, *Bario steindachneri*, *Hollandichthys multifasciatus* (Eigenmann & Norris 1900) and *Pseudochalceus kyburzi* Schultz 1966.

-Second humeral spot absent (342 - 1>0; 0.07; 0.25). Reversible in *A. giton*, *A. burgerai* and *A. bahiensis*. The state 0 is present in most examined species. The degree of development of the second umeral spot can influence in the determination of the presence or absence of this

feature. In most examined species the second umeral spot is diffuse and not so evident, being considered absent by some researchers. In future, this character description must be improved to describe more states related to this feature.

- Dentary deepest at most posterior portion and height diminishing progressively anteriorly, corresponding to the toothed portion of the bone (half to 2/3 of its length) (393 - 0>1; 0.11; 0.75). Reversible in *A. burgerai* and *D. pedri* clade. Parallel in *D. potaroensis*, *A. rivularis*, *A. procerus*, *Bryconamericus agna*, *Phenagoniates macrolepis* (Meek & Hildebrand 1913), *Xenagoniates bondi* Myers 1942 and *Coptobrycon bilineatus*.

-Basal portion of dentary teeth narrower than apical portion with a gap between bases of contiguous teeth (403 - 0>1; 0.16; 0.84). Reversible in *A. burgerai* and *D. pedri* clade. Parallel in *A. dissensus*, Cheirodontinae species, Aphyocharacinae, *Coptobrycon bilineatus*, *Hemigrammus bleheri* Géry & Mahnert 1986, and *Paracheirodon axelrodi*.

-Basal portion of premaxillary teeth narrower than apical portion with a gap between the bases of contiguous teeth (405- 0>1; 0.2; 0.88). Reversible in *D. pedri* and Sp. 2. Parallel in Cheirodontinae species, Aphyocharacinae, *Coptobrycon bilineatus*, *Hemigrammus bleheri*, *Paracheirodon axelrodi* and *Nematocharax venustus*.

- All cusps nearly equal in size and shape in maxillary teeth (406 - 0>1;). Reversible in *A.* aff. *michroschemos* and *A. jenynsii*. Ambiguous in *A. pelecus*. Parallel in *A. dissensus*, *Odontostilbe paraguayensis* Eigenmann & Kennedy 1903, *O. pequira* (Steindachner 1882), *C. interruptus* (Jenyns 1842) and *Paracheirodon axelrodi*.

- Basal portion of maxillary teeth narrower than apical portion with a gap between the bases of contiguous teeth (407 - 1>0; 0.2; 0.87). Ambiguous in *A. pelecus* and inapplicable in *P. heterostomus*. Parallel in *A. rivularis*, *O. paraguayensis*, *O. pequira*, *C. interruptus*, *Xenagoniates bondi*, and *P. axelrodi*.

- Maxillary teeth laterally inserted medially at the bone, visible in lateral view (408 - 0>1; 0.1; 0.64). Inapplicable in *P. heterostomus*. Parallel in *J. abramoides*, *J. potaroensis*, *D. potaroensis*, Stevardiinae clade, Cheirodontinae species, *Phenagoniates macrolepis*, *Xenagoniates bondi*, *Prionobrama paraguayensis* (Eigenmann 1914), *Aphyocharax anisitsi* Eigenmann & Kennedy 1903, *Paracheirodon axelrodi*, *Nematocharax venustus*,

Hyphessobrycon herbertaxelrodi Géry 1961, *Hyphessobrycon socolofi* Weitzman 1977, *Moenkhausia sanctaefilomenae, J. mucronata* and *Charax stenopterus* (Cope 1894).

-More than four teeth on Dentary anterior portion (412 - 0>1; 0.2; 0.77). Ambiguous in *A. michroschemos* and inapplicable in *P. heterostomus.* Parallel in *D. potaroensis*.

***Deuterodon* genus.** *Deuterodon stricto sensu* is composed by seven species (*Deuterodon iguape, Deuterodon supparis, Deuterodon stigmaturus, Deuterodon singularis, Deuterodon longirostris, Deuterodon rosae, and Deuterodon langei*) supported by 9 synapomorphies. For more details see Silva *et al.* 2017.

***Deuterodon pedri* clade.** This is a monophyletic clade composed by *Deuterodon pedri*, *Astyanax pelecus* and undescribed taxons Sp. 1 and Sp. 2. This clade was recovered also with molecular data set with support of 0.96. Twelve synapomorphies support this clade under bremmer support of 5. The following synapomorphies define this clade:

- Epioccipital bridge depressed in its middle region (5 - 0>1; 0.18; 0.5). Paralleled in *Bramocharax* clade, *A. giton, A. intermedius, J. pinnata, J. acanthogasther* and *J. poranga*.

- Posteriorly-oriented epioccipital spine absent (7 - 0>1; 0.06; 0.7). Parallel in most Characidae examined fish. The most closely related that presents the same conditions are *Deuterodon stricto sensu*, *P. heterostomus*, *A. hamatilis*, *A. burgerai*, *J. poranga*, *D. potaroensis*, *A. xiru*, *A. lacustris*, *Astyanaxcinus moori*, *Bramocharax* clade (Mirande, 2010),

-Presence of anterior paired projections of parasphenoid (40 - 0>1; 0.07; 0.57). Ambiguous in *D. pedri*. Parallel in *Deuterodon* genus, *D. potaroensis*, *J. essequibensis*, *Nematobrycon palmeri*, *Thayeria* species, some *Hyphessobrycon species*, *Hemigramus* species, *Moenkhausia* species, *Bario steindachneri*, *Poptella paraguayensis*, *Stethaprion erythrops* Cope 1870, *Paracheirodon axelrodi*, *Astyanaxcinus moorii* and *Bryconexodon juruenae* Géry 1980.

-Dilatator fossa not almost covered by sixth infraorbital that leaving a conspicuous naked area in anterior region of fossa (69 - 0>1; 0.05; 0.79). Parallel in *Deuterodon* genus, Stevardinae species, most Cheirodontinae species, *Nematobrycon palmeri* Eigenmann 1911, *Carlana eigenmanni*, *Rhoadsia altipinna*, *Hasemania nana*, *Thayeria* species, *Hemigrammus* species,

Pristella maxillaris (Ulrey 1894), some *Hyphessobrycon* species, *Moenkhausia* species, *Poptella paraguayensis*, *Gymnocorymbus ternetzi* (Boulenger 1895), *Stichonodon insignis* (Steindachner 1876), *Tetragonopterus argenteus* Cuvier 1816, some *Astyanax* species, *Nematocharax venustus*, *Psellogrammus kennedyi* (Eigenmann 1903), *Hollandichthys multifasciatus*, *Pseudochalceus kyburzi*, *Charax stenopterus*, *Phenacogaster tegatus* (Eigenmann 1911) and *Hoplocharax goethei* Géry 1966.

-Horizontal process of anguloarticular laterally covered by dentary only anteriorly (108 - 1>0; 0.03; 0.54). Parallel in most Characidae. The most related that presents the same condition are *Deuterodon* genus, *D. potaroensis*, *A. pelecus*, *J. essequibensis*, *J. pinnata*, *J.* aff. *atypindi*, *A. intermedius*, *A. michroschemos*, *M. aphos*, *P. heterostomus* and *A. hamatilis*.

-Presence of fossa for inner row of replacement premaxillary teeth (133 - 0>1; 0.12; 0.56). The presence of fossa for inner row of replacement premaxillary teeth is present on a few numbers of species and is paralleled in *D. singularis*, *A. hamatilis*, Aphyocharacinae and Aphyoditeinae (sensu Mirande, 2010).

- Anterior extension of interopercle extending anteriorly beyond tip of horizontal arm of preopercle (163 - 1>0; 0.05; 0.44). Parallel in most examined Characidae.

-Absence of bony lamella dorsal to fourth basibranchial (185 - 0>1; 0.03; 0.56). Most characid species have the bony lamella dorsal to fourth basibranchial. The absence of this is a reversion that occurs in the *Deuterodon pedri* clade and is parallel in *J. acanthogasther*, *J.* aff. *atypindi*, *J. poekotero*, *A. intermedius*, *A.* aff. *hastatus*, Sp. A, *A. goyanensis*, *Paracheirodon axelrodi*, *Nematocharax venustus*, *Hollandichthys multifasciatus*, *Jupiaba scologaster*, *Jupiaba mucronata*, *Gymnocharacinus bergii*, *Oligosarcus longirostris* Menezes & Géry 1983, *Oligosarcus pintoi*, Characinae (sensu Mirande), *Axelrodia lindeae* Géry 1973, *Prodontocharax* cf. *melanotus*, *Piabarchus analis* (Eigenmann 1914), *Knodus heteresthes*, *Mimagoniates rheocharis* Menezes & Weitzman 1990, *Carlastyanax aurocaudatus* (Eigenmann 1913) and some species of *Creagrutus* Günther 1864.

- *Adductor mandibulae* tendon inserted on vertical through middle or anterior half of Meckelian cartilage on dentary (330 - 0>1; 0.05; 0.69). Parallel in *D. iguape*, Stevardiinae

species, some *Astyanax* species, *Markiana nigripinnis* Eigenmann 1903, *Psellogrammus kennedyi*, *Phenagoniates macrolepis*, *Xenagoniates bondi* and *Gymnocharacinus bergii*.

-Spots located on the distal border of scale, forming a dark brown arch when chromatophores are expanded and a translucent arch when chromatophores are not expended, spots restrict above lateral line. (347 - 0>2; 0.28; 0.5). In *Deuterodon pedri* clade, spots appears on scales of flanks, but the spots are located on scale distal border and only on two or three series of scales immediately below to the dorsal fin. Parallel in *J. polylepis*.

-Dentary nearly equal along most of its length, narrow anteriorly in the toothed portion (nearly 1/3 of its length) (393 - 1>0; 0.11; 0.75); Parallel in most Characidae fish. Inside of Probolodini, this condition is also parallel only in *A. burgerai*.

- Basal portion of dentary teeth wider than or nearly equal to apical portion; teeth juxtaposed, without space between the bases of contiguous teeth (403 - 1>0; 0.16; 0.84). Ambiguous in *A. pelecus*. Parallel in most Characidae examined. The most related taxa that presents the same condition are *A. burgerai*, *Jupiaba* species, *D. potaroensis*, and *Astyanax* species.

***Myxiops* genus.** *Myxiops* is a valid genus also belongs to Probolodini and closely related to *Deuterodon* genus (3 synapomorhies, 2 exclusives). Twenty two autoapomorphies support *Myxiops* as a valid genus:

-Sphenotic spine not extending ventrally to articulation between sphenotic and hyomandibula (10 - 1>0; 0.05; 0.79). Parallel in *D. singularis*, *D. supparis*, *D. longirostris*, *A. bahiensis*, *D. potaroensis*, *A. cremnobates*, *A. brachpterygium*, Stevardiinae clade (sensu Thomaz *et al.* 2015), Cheirodontinae, Aphyocharacinae (sensu Mirande, 2010), Gymnocharacinae (sensu Mirande, 2010) and Characinae (sensu Mirande, 2010).

-Ventral diverging lamellae of mesethmoid absent (30 - 1>0; 0.33; 0.88). This state was only found in specimens from outgroup. Inside of examined species of Characidae this condition was only registered in *Myxiops*. Mirande, 2010 mentions that in Cheirodontinae the lamellae is extremely reduced, but present. We could not observe neither a small vestige of this lamellae in *Myxiops*.

-Bony lamellae bordering laterosensory canal of first infraorbital absent (58 - 0>1; 0.33; 0.60). Parallel in *Phenagoniates macrolepis*, *Xenagoniates bondi*, *Paragoniates alburnus* Steindachner 1876, *Prionobrama paraguayensis* and *Gymnocharacinus bergii*. The infraorbital 1 of *Myxiops aphos* can be fused or not with infraorbital 2 (Zanata, Akama, 2004). In examined specimens of *Myxiops* the infraorbital 1 was not fused and lack the bone lamellae associated. Different from other examined species, the infraorbital 1 of this species is tubular and is possible to see only a slim slice of bone that border one of the sides of infraorbital 1, but not so big to be considered a lamellae as in other examined characid species.

- Laterosensory canal of first infraorbital projects dorsally from main body of the bone (73 - 1>0; 0.09; 0.28). Parallel in *Deuterodon longirostris*, *Astyanax pelecus*, *Astyanax giton*, *Deuterodon potaroensis*, *Astyanax dissensus* and *Bryconamericus pectinatus*.

- Canal of lateral line on caudal-fin membrane absent (92 - 1>0; 0.03; 0.62). Parallel in *A.* aff. *hastatus*, *J. abramoides*, *A. goyanencis*, *Carlastyanax aurocaudatus*, *Bryconamericus indefessus*, *Bryconamericus rubropictus*, *B. thomasi*, *Diapoma* sp., *Diapoma speculiferum*, *Hyphessobrycon* species, *Serrapinus calliurus*, *Cheirodon interuuptus*, Aphyoditeinae (Mirande, 2010), Gymnocharacinae (Mirande, 2010), *Thayeria* species, *Hemigrammus* species, *Hollandichthys multifasciatus*, *Pseudochalceus kyburzi*, *Charax stenopterus*, and *Phenacogaster tegatus*.

-Ventral margin of toothed region of maxilla strongly concave (95 - 0>1; 0.20; 0.42). Parallel in *D. stigmaturus*, *Creagrutus* species, *Phenagoniates macrolepis* and *Xenagoniates bondi*. The ventral margin of toothed region of maxilla is straight or nearly straight in most examined characids. The strongly concave shape was observed only in the cited species. In *Deuterodon* we can observe a condition almost concave, or concave in some portion but not strongly, justification for the codification of almost straight and not strongly concave in this genus.

-Ascending process of premaxilla reaching just anterior end of nasal (104 - 0>1; 0.05; 0.76). Parallel in some species from Stevardiinae, Cheirodontinae, Aphyocharacinae, Gymnocharacinae, *Hyphessobrycon* species, *Hemigrammus* species, *Rhoadsia altipinna*, *Carlanna eigenmanni*, *Paracheirodon axelrodi*, *Pristella maxillaries*, *Stichonodon insignis*, *Phenacogaster tegatus* and *Charax stenopterus*.

- Alignment of ascending process of premaxilla medially shifted and separated from nasal (105 - 0>1; 0.33; 0.75). Parallel only in Aphyocharacinae.

- Medial process of dentary bordering Meckelian cartilage dorsally and medially present (115 - 0>1; 0.20; 0.20). Parallel in *J essequibensis, J.* aff. *atypindi* and *J. ocellata*. The presence of this medial process is considered a synapomorphie for Iguanodecteinae. We could observe a process in this same region, like a wall over the Meckelian cartilage, that we considered homologue to the observed condition in Iguanodectinae. Because of this the species was coded as having this process.

- Premaxillary, maxillary, and dentary teeth pedunculate and uniformly shaped (119 - 0>1; 0.25; 0.57). Parallel in *Bryconamericus lethostigmus*, *Gymnocharacinus bergii* and Cheirodontinae species. Pedunculated teeth in the upper and lower jaw is a synapomorphy proposed by Malabarba (1998) for Cheirodontinae.

- Number of one rows of teeth in premaxilla (122 - 1>0; 0.09;m0.64). Parallel in *Bryconamericus lethostigmus*, Aphyocharacinae, Cheirodontinae, *Grundulus cochae*, *Carlana eigenmanni* and *Paracheirodon axelrodi*. The presence of two rows of teeth in premaxilla is a plesiomorfic condition for Characiformes (Zanata, Vari, 2005) and is considered a synapomorphy for Characidae (Lucena, 1993). The possession of one row is a reversible condition found in few taxons of Characidae. The reversion to one row happens more than one time, once this appears in not closely related taxons as *Myxiops*, *Bryconamericus lethostigmus* and Cheirodontinae species.

-Foramen on articular condyle of quadrate present (149 - 0>1; 0.03; 0.26). Parallel in *D. stigmaturus*, *D. longirostris*, *A. taeniatus*, *A. ribeirae*, *A. intermedius*, *A.* aff. *hastatus*, *H. luetkenii*, Sp. A, *A. jenynsii*, *A. brachpterygium*, *A. xiru*, *A. douradilho*, *O. longirostris*, *Creagrutus gephyrus* Böhlke & Saul 1975, *Knodus meridae*, *Serrapinus calliurus* (Boulenger 1900), *Odontostilbe microcephala*, *Cheirodon interruptus*, *Grundulus cochae*, *Hasemania nana*, *Hyphessobrycon eques* (Steindachner 1882), *Pseudochalceus kyburzi* and Characinae.

- Contact between ectopterygoid and anterodorsal region of quadrate absent (162 - 0>1; 0.05; 0.68). Parallel in Sp. 2, Sp. B, *Probolodus heterostomus*, *A. burgerai*, *A. hamatilis*, *A. bahiensis*, *A. ribeirae*, *A. hastatus*, Stevardiinae clade, Cheirodontinae clade,

Microschemobrycon casiquiare, *Prionobrama paraguayensis*, *Aphyocharax dentatus* and *Stichonodon insignis*.

- Foramen in posterior region of metapterygoid in form of incomplete arch, bordered posteriorly by hyomandibula (168 - 1>2; 0.16; 0.74). Parallel in *D. pedri*, *D. potaroensis*, *A. cremnobates*, *A. goyanensis*, *A. laticeps*, *Eretmobrycon scleroparius* (Regan 1908), *Eretmobrycon emperador* (Eigenmann & Ogle 1907), and *Bryconops spp*.

- Denticles on gill rakers absent (201 - 0>1; 0.05; 0.48). Parallel in *P. heterostomus*, *J. apenima*, *J. abramoides*, *Creagrutus* species, *Microgenys minuta*, *Attonitus ephimeros*, *Aulixidens eugeniae*, *Knodus heterestes*, *Bryconadenos tanaothoros*, *Piabina argentea* Reinhardt 1867, *Argopleura magdalenensis* (Eigenmann 1913), *Axelrodia lindae*, *Coptobrycon bilineatus*, *Gymnocharacinus bergii*, *Grundulus cochae*, *Nematobrycon palmeri*, *Hyphessobrycon elachys* Weitzman 1985, *Hyphessobrycon herbertaxelrodi* and *Pseudochalceus kyburzi*.

- Articulation between ventral process of mesocoracoid and dorsal margin of scapula present and broad (245 - 0>1; 0.16; 0.70). Parallel in *A. rivularis*, *Creagrutus* species, *Bryconamericus pectinatus* (Vari & Siebert 1990), *Microgenys minuta* Eigenmann 1913 and *Gymnocharacinus bergii*.

- Ventral exit of laterosensory canal of supracleithrum ventral to lamella of supracleithrum and exiting on posterior margin of this bone (254 - 1>0; 0.12; 0.82). Parallel in *J. ocellata*, *A. goyanensis*, *A. laticeps*, *Markiana nigripinnis*, Aphyocharacinae and *Bryconops* species.

- Eight or more branched pelvic-fin rays (259 - 0>1; 0.16; 0.58). Parallel in *A. giton*.

- Two dorsal-fin rays articulating with first dorsal pterygiophore (266 - 1>0; 0.05; 0.80). Parallel in Stevardiinae clade, *Aphyocharacidum bolivianum*, *Axelrodia lindae*, Aphyocharacinae, *Nematobrycon palmeri*, *Paracheirodon axelrodi*, *Hyphessobrycon elachys*, *Thayeria obliqua*, *Bario steindachneri* and Characinae clade (Mirande, 2010).

- 17 or less branched anal-fin rays (287 - 1>0; 0.07; 0.72). Number of branched anal-fin rays is highly variable in Characidae family, but to have more than 17 is the most common condition. Parallel in *D. longirostris*, *A.* aff. *michroschemos*, *A. rivularis*, *A. goyanensis*, Sp. C, *A. cremnobates*, *A. brachpterygium*, some species from Stevardiinae clade,

Prodontocharax cf. *melanotus*, *Coptobrycon bilineatus*, *Gymnocharacinus bergii*, *Grundulus cochae*, *Hemigrammus bleheri*, *Hasemania nana*, and *Thayeria* species.

-Distal tip of sphenotic spine notched, limiting adductor opercula anterior and dorsally (366 - 0>1; 0.11; 0.66). Parallel in *D. longirostris*, Sp. 1, *D. potaroensis*, *J. polylepis*, some *Astyanax* species, *Oligosarcus* species and *Roeboexodon guyanensis* (Puyo 1948).

- Posterodorsal region of anguloarticular vertical (382 - 1>0; 0.14; 0.62). Parallel in *Creagrutus* species, *Carlastyanax aurocaudatus* and *Bryconamericus pectinatus*.

***Probolodus*.** *Probolodus* is a valid genus. It is closely related with *A.* aff. *microschemos*. Ten apomorphies are listed for *Probolodus heterostomus*, and may constitute synapomorphies to support *Probolodus* after the examination of all species of the genus:

-Posteriorly-oriented epioccipital spine absent (7 - 0>1; 0.06; 0.7). Parallel in most Characidae examined fish. The most closely related that presents the same conditions are *Deuterodon strict sensu*, *D. pedri* clade, *A. hamatilis*, *A. burgerai*, *J. poranga*, *D. potaroensis*, *A. xiru*, *A. lacustris*, *Astyanaxcinus moorii*, *Bramocharax* clade (Mirande, 2010).

-Epiphyseal branch of corresponding supraorbital canals oriented obliquely, opening posteriorly to epiphyseal bar (85 - 0>1; 0.14; 0.33). Parallel in *J. asymetrica*, *J. anteroides*, Sp. C, *A. goyanensis*, *A. rivularis* and *A. laticeps*.

-All teeth of premaxillary, maxillary, and dentary teeth conical, caniniform, or mamilliform (118 - 1>0; 0.12; 0.56). Parallel in *Axelrodia lindae*, *Grundulus cochae* and Characinae. Although the parallel condition of the state 1 in other taxons, we can consider that mamilliform teeth appears only in *Probolodus heterostomus* and some Characinae fish. The original character and this state should be modified with the objective to better describe the different conditions and variations of teeth.

-Mamilliform teeth outside mouth, present (120 - 0>1; 0.33; 0.60). Parallel in *Roeboides* Günther 1864 species, *Bryconexodon juruenae*, *Exodon paradoxus* Müller & Troschel 1844 and *Roeboexodon guyanensis* (Puyo 1948).

-Four or more maxillary teeth (136 - 0>1;). The number of maxillary teeth is highly variable in Characidae fish. This fact explains the elevated number of parallel condition observed at

the three. This character seems to be very homoplastic and has a high variation intra specifically.

-Teeth extending across almost entire maxillary lamella (137 - 0>1; 0.06; 0.68). Parallel in *Bramocharax* clade, *Creagrutus gephyrus*, *Creagrutus cracentis* Vari & Harold 2001, *Hemibrycon surinamensis* Géry 1962, *Prodontocharax* cf. *melanotus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*, *Paragoniates alburnus*, *Prionobrama paraguayensis*, *Grundulus cochae*, *Nematobrycon palmeri*, *Nematocharax venustus*, *Hyphessobrycon megalopterus* (Eigenmann 1915), *Hollandichthys multifasciatus*, *Pseudochalceus kyburzi* and Characinae.

-Four or fewer supraneurals (280 - 1>0; 0.02; 0.43). Number of supraneural is highly variable in Characidae. The closely taxon that presents parallel condition with *P. heterostomus* are *Jupiaba* species and *Myxiops aphos*.

- Pronounced flexion on maxilla posterior to site of attachment with premaxilla (372 - 0>1; 0.50; 0.80). Parallel on *Carlastyanax aurocaudatus* and in *Creagrutus* species.

-Three or fewer cusps of anterior dentary teeth (380 - 1>0; 0.05; 0.70). Parallel in *Oligosarcus* species, some species from Stevardiinae clade, Aphyoditeinae, Aphyocharacinae, *Grundulus cochae*, *Hasemania nana*, *Hyphessobrycon herbertaxelrodi*, *Hyphessobrycon megalopterus*, *Pristella maxillaris*, *Hollandichthys multifasciatus*, *Pseudochalceus kyburzi* and Characinae

- Cartilage-filled region anterior to scapular foramen present and wider than anterior process of scapula (387 - 1>0; 0.02; 0.46). Parallel in *D. longirostris*, *Jupiaba* species, *A. bahiensis*, *A. ribeirae*, *A. intermedius*, *H. luetkenii*, Sp. C, *A. goyanensis*, *Astyanacinus moorii*, *Oligosarcus* species, most of Stevardiinae, Cheirodontinae, Characinae and C clade species.

***Jupiaba* genus.** *Jupiaba* is a poliphyletic genus. *Jupiaba* species appears in more than four places at the tree and associated with different species inside of the Characidae. *Jupiaba* species with dentary teeth gradually decreasingform a monophyletic clade that is part of the Probolodini. This clade is closely related with *Deuterodon* genus and *Myxiops*. 6 synapomorphies define this group of *Jupiaba* with gradually decreasing dentary teeth:

-Extensive articulation of entire lateral ethmoid dorsal margin and frontal or mesethmoid (17 - 0>1; 0.14; 0.66). Parallel in *J. apenima*, *J.potaroensis*, *J. ocellata*, *A. rivularis*, *A. goyanensis* and Aphyocharacinae.

-Expansion of lamellar portion of maxilla just posterior to toothed region very pronounced (97 - 0>1; 0.10; 0.59). Parallel in *Deuterodon* genus, *A. ribeirae*, *A. hastatus*, *H. luetkenii*, *A.* aff. *hastatus*, *A. intermedius*, Cheirodontinae species and *Paracheirodon axelrodi*.

-Denticles on gill rakers distributed along entire surface of gill rakers (202 - 0>1; 0.04; 0.62). Parallel in *A. jenynsii*, *J. potaroensis*, *J. anteroides*, *J. polilepys*, *J. ocellata*, *J. poranga*, *Bramocharax* clade, *Astyanacinus moorii*, *Astyanax* species, *Hyphessobrycon megalopterus*, *Pristella maxillaris*, *Hyphessobrycon eques*, *Poptella paraguayensis*, *Gymnocorymbus ternetzi*, *Tetragonopterus argenteus*, *Hollandichthys multifasciatus*, Characinae, *Heterocharax macrolepis* Eigenmann 1912, *Lonchogenys ilisha* Myers 1927 and *Bryconops* species.

-Anterior tip of pelvic bone pointed, lacking associated cartilage and frequently projecting outside body wall (263 - 0>1; 0.16; 0.50). Parallel with other *Jupiaba* species.

-Absent or just one pair of Uroneurals (306 - 1>0; 0.03; 0.69). Parallel in most Characidae fish. Among the closely related taxon it is parallel in *A. giton*, *A.* aff. *hastatus*, Sp. A, *J. polylepis*, *J. apenima* and *J. potaroensis*.

- Dark spot covering entire depth of caudal peduncle present (348 - 0>1; 0.16; 0.50). Parallel in *A. ribeirae*, *A. hastatus*, *H. luetkenii*, *J. apenima*, *J. potaroensis* and *Moenkhausia sanctaefilomenae*.

Discussion

Both molecular and morphological analyses were congruent recovering Probolodini. The integration between different kinds of data (molecular and morphological) to generate hypothesis at species level increases the rigor in taxonomy decision (Schlick-Steiner *et al.*, 2010) and robustness. In fact the recovery of this clade twice and independently with different kinds of data, make the hypotheses of the existence of this unit strong.

Most of the species and genera that are part of this large clade are endemic from coastal drainages of East Brazil. The Atlantic coastal drainages in Brazil are considered an area of high endemism with high number of endemic genera and species of Neotropical fish (Vari, 1988; Weitzman *et al.*, 1988; Bizerril, 1994; Buckup, 2011; Carmelier, Zanata, 2014). The endemism and high diversity found inside of Probolodini can be explained by the complex history in coastal drainages that shows a series of connections and vicariant events caused by sea level fluctuations through the Pleistocene glacial periods (Weitzman *et al.*, 1988; Thomaz et al., 2015). Most of these drainages are isolated from inland continental drainages by the crystalline shield, like isolated islands.

Patterns from cladogenesis of taxons that inhabit coastal drainages where proposed by Ribeiro (2006), to illustrate levels of diversification in different periods: pattern A suggests ancient cladogenesis, exemplifying events that split subfamilies and groups of genera at family level dating from Cretaceous; pattern B illustrates events that split genera of coastal drainages from genera widespread in trans/cis andean region, dating from Tertiary; finally pattern C exemplifies recent interchanges between coastal and continental drainages, that in this case share the same species. The Probolodini seems to be an example of pattern B. The molecular hypothesis shows the endemic taxa from coastal drainages (Probolodini) form a sister group of the genus *Astyanax*, widespread from South United States to North of Argentina (pattern B). The morphological hypothesis shows *Deuterodon sensu stricto* and *Myxiops* from coastal basins are sister group of *Jupiaba* species from continental basins (pattern B). The estimated minimal age for Probolodini seems to be Tertiary, but this assumption needs to be confirmed by a molecular clock.

The main characteristic shared by the species that are included in Probolodini is the teeth arrangement. Weitzman and Malabarba (1998) considered the arrangement of teeth as

more informative in phylogenetic studies than number of teeth in each bone of the mouth. Twelve of the 20 synapomorfies that define Probolodini are related to teeth, being the most evident the particular arrangement (gradually decreasing of dentary teeth due to the presence of minimum of 4 or 5 teeth always with intermediary teeth in size before remain smaller). However, synapomorphies related with teeth and this arrangement are not exclusive from taxa within the Probolodini. Characters such as gradually decreasing dentary teeth, teeth with space in basal portion and expanded at the upper portion, high number of cusps, teeth aligned in straight series, cusps aligned in straight series, maxillary teeth inserted medially and visible in lateral position are not exclusive synapomorphies for this clade and also appears in other groups of species like Cheirodontinae and *Bryconamericus lethostigmus*. Moreover, all of these synapomorphies are absent in Probolodus, a member of the Probolodini. This is one example about the importance of the test of synapomorphies *a posteriori*. De Pinna (1991) highlights the importance of testing primary hypotheses of homology and after test this in phylogenetic approaches to identify secondary homologies, or true synapomorphies to recognize groups of taxons. In the case of Probolodini, *a posteriori* test allowed to determine that these tooth arrangements are indeed synapomorphies of the group independently adquired in other members of the Characidae.

It was not the first time that a clade formed by coastal characid species is hypothesized. Coutinho-Sanches, Dergam (2015) recovered clades with COI and RAG2 genes composed by *Deuterodon* species, *Deuterodon pedri*, *Probolodus heterostomus* and *Astyanax* from coastal drainages. All species included by them are present in this work also correspondent to Probolodini. Rossini and colleagues (2016) in a work to demonstrate the high diversity among *Astyanax* species present a phylogenetic tree based only in COI gene. These authors found 5 major clades of *Astyanax* species. They mentioned that the clade 5 is the clade with higher genetic divergence between species (8% vs. 1% in the other clades). It is interesting to highlight that their clade 5 is composed of species that in this work were found as part of Probolodini. The higher genetic distance between the species inside of this clade and between other 4 clades, are due to the fact that they are related to other genera and not *Astyanax* as currently defined.

Oliveira and colleagues (Oliveira *et al.*, 2011) also found close relationship between *Deuterodon*, *Myxiops* and *Probolodus* using molecular phylogenetic analysis, also

corresponding to Probolodini as defined herein. These authors make an interesting observation about all of these genera inhabiting ancient land formations in northeastern and southeastern region in Brazil (coastal drainages), “area of residence of primitive lineage in other groups of fish”. All of these previously published data recovering the same relationschip found in this work increases the robustness of the existence of this taxonomic unit that is Probolodini.

Myxiops aphos had different phylogenetic positions according with molecular and morphological data in this work. Oliveira *et al.* (2011) found *Myxiops* closely related with *Deuterodon*, a pattern also recovered here by morphological data. But with molecular data *Myxiops* was more closely related to *J. poranga*, forming together the sister group of remaining members of Probolodini. The difference found between the molecular work of Oliveira *et al.* (2011) and this work can be explained by the small taxon sampling related to Probolodini in their phylogenetic hypotheses. The addition of taxa in phylogenies increases the accuracy of the results (Heath *et al.*, 2008) leads to better understanding of the evolutionary relationship.

Althought *Myxiops* is closely related with *Deuterodon*, with 2 exclusive synapomorphies, it is a valid genus. *Myxiops* is defined by 22 autapomorphies and it is endemic from northeastern basin at Bahia (Zanata, Akama, 2004). *Deuterodon sensu stricto* is also considered valid and defined by 9 synapomorphies (one exclusive) and all species of the genus are restricted to south and southeastern of Brazil with limited north distribution to extreme south of São Paulo (see Silva *et al.*, 2017 unpublished for more detailed information).

Deuterodon pedri is not part of *Deuterodon sensu stricto* and in both analyses it was found related to two undescribed species (Sp. 1 and Sp. 2) and to *Astyanax pelecus* in the morphological analysis. Although *D. pedri* clade has high support in molecular and morphological analyses, the species that compound this clade are kept as *incertae sedis* in Characidae family.

Probolodus is a valid genus. Ten autoapomorphies support *Probolodus heterostomus.*The addition of the other two valid species in the analysis may become these apomorphies as synapomorphies for the genus, but this assumption needs to be tested. Oliveira *et al.* (2011) hypothesized that *Probolodus* is closely related to *Deuterodon* and *Myxiops*. Before Oliveira *et al.* (2011) and this work, some other studies hypothesized

Probolodus as related to Tetragonopterinae (Roberts, 1970; Géry, 1977; 1980; and sensu Mirande, 2010). From all the Probolodini species, *Probolodus* is the genus that has the most peculiar arrangement of teeth because of their lepidophagous feeding habit (Sazima, 1977). Because of this Sazima (1983) concluded that this special feeding habit of *Probolodus* within the Tetragonopterinae should be evolved because of to agressive beheaviour in some ancestral. Santos, Castro (2014) hypothesized that the specialized dentition of *Probolodus*, as well as the predatory behavior of plucking and eating scales could have evolved independently in *Bryconexodon*, *Exodon*, and *Roeboexodon*. This hypothesis is confirmed herein once *Probolodus* is more closely related to species of the genera *Deuterodon*, *Astyanax*, *Jupiaba* and *Myxiops* with completely different arrangement of teeth. The peculiar arrangement found in *Probolodus* is a morphological convergence with *Bryconexodon*, *Exodon* and *Roeboexodon* to eat scales.

Other example of morphological convergence in Characidae is the elongate pelvic spine. In 1997, Zanata (1997) hypothesized that all members of characids that presents a elongate pelvic spine (projecting for the main axis of the body or not) are part of the same unit and create the genus *Jupiaba*. Inside of this genus is possible to found specimens with variable morphological features as dentary teeth arrangement. The morphological results of this work showed *Jupiaba* as polyphyletic, with specimens appearing in more than tree clades inside of Characidae phylogeny. Benine *et al.* (2017) found the same result with morphological molecular data, but they do not mention anything about the close relationship between *Jupiaba* and *Deuterodon*. Nonetheless we found species of *Jupiaba* with dentary arrangement of four or more teeth followed by intermediary before of the small ones, as part of Probolodini. This position was not tested with molecular data, but because of the high number of synapomorphies that define Probolodini, is possible to believe that this result can be also recovered by this kind of data.

As in previous phylogenetic studies (Oliveira *et al.*, 2011; Mirande, 2010) *Astyanax* was found polyphyletic, with species appearing in at least three different clades. As with *Jupiaba* species, some species of *Astyanax* with more than 4 large anterior dentary teeth appears inside of the Probolodini. This species should actually not be considered as *Astyanax*, once they are closer to other genera (*Deuterodon*, *Myxiops* and *Probolodus*) than with *Astyanax mexicanus*. Nonotheless, they were widespread inside of the Probolodini, as part of

a big politomy. Because of these weak resolution, this species (*Astyanax giton*, *Astyanax hastatus*, *Astyanax taeniatus*, *Astyanax microschemos*, *Astyanax jenynsii*, *Astyanax intermedius*, *Astyanax pelecus*, *Astyanax hamatilis*, *Astyanax burgerai*, *Astyanax bahiensis*) should be considered as *incertae sedis* until a more decisive phylogenetic study solve it. *Astyanax* is a genus with high complexity in Characidae, and studies with multiple frameworks are needed to solve the real boundaries of the genus. In this work, a clade is high supported and includes *Astyanax mexicanus* (type species of the genus) hosted (*Astyanax mexicanus* clade). All species included in this clade have four large teeth in dentary followed by numerous teeth smaller in size (eg. *Astyanax lacustris* in Lucena, Soares, 2016; *Astyanax fasciatus* in Melo, Buckup, 2006) (Fig. 3a). The second teeth of dentary in all species of this clade are on a lower position than the remaining ones. This same clade is recovered by Rossini and colleagues (2016), and is named by them as clade 1, 2 and 3. The species that compound this clade should be considered as actually *Astyanax* species.

The tree generated under implied weighting disagreed with equal weighting and molecular data, once this shows *Deuterodon* and *Myxiops* more related with Cheirodontinae species. This analysis did not recover Probolodini. According to Congrave, Lamsdell (2016) equally weighted analyses retrieve higher frequency of polytomies but generated less erroneous topologies, due to more conservative characteristic of this analysis. Implied weighting showed a more resolved tree, without polytomies, but the results are questionable. Under this analysis the position of *Deuterodon* and *Myxiops* as closely related to Cheirodontinae is a spurious result, once these two genera are part of the clade C and Cheirodontinae belongs to clade B as supported in previous published works (Mirande, 2010; Oliveira *et al.*, 2011). Whereas implied weighting generally resolves polytomies, it also propagates errors, with a tendency towards higher rates of error when compared to equal weighting (Congrave, Lamsdell, 2016). In phylogenetic analyses, to be conservative seems to be the best choice to avoid the establishment of errors and wrong classification. In general, more robust hypotheses emerge with the use of different kinds of data sets that derive from different evolutionary constraints. A combined approach is the best choice to solve polytomies and it should be encouraged. The evolutionary process is better understood when the analyses yield testable hypotheses.

Acknowledgements

We are in debit with Jorge Dergam (MZUFV), Angela Zanata (UFBA), Hernan López Soares (ROM) and Hector Espinosa (UNAM) that provide some of the tissues and specimens to the development of this work. We also need to say thank you to Alice Hirschmann, Andréa Thomaz, Tiago Carvalho and Udson Santos for the great help and partnership during field working at Brazilan eastern coastal drainages. CNPq that provided grants and scholarship for the authors (PCS, LRM, MCM) and BRBOLD that payed the sequencing of significative part of the molecular data used here.

References

Albert JS, Reis RE. Introduction to Neotropical Freshwaters. In: Albert JS, Reis RE, editors. Historical Biogeography of Neotropical Freshwater Fishes. Berkeley: University of California. 2011. p.3-19.

Benine RC, Melo BF, Oliveira C Espinho pélvico: Evidência morfológica e molecular de origens independents em Characidae (Teleostei: Characiformes). Anais XXII Encontro Brasileiro de Ictiologia. 2017.

Bremer K. Branch support and tree stability. Cladistics. 1994; 10(3): 295-304.

Bizerril CRSF. Análise taxonômica e biogeográfica da ictiofauna de água doce do leste brasileiro. Acta Biological Leopoldensia.1994;16: 51-80.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, *et al*. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput Biol [serial on the Internet]. 2014; 10(4): e1003537. Available from: http://dx.doi.org/0.1371/journal.pcbi.1003537

Buckup PA. The Eastern Brazilian Shield. In: Albert JS, Reis RE, editors. Historical biogeography of neotropical freshwater fishes. Berkeley: University of California; 2011. p.203-210.

Calcagnotto D, Schaefer SA, DeSalle R. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Molecular Phylo Evol. 2005; 36: 135-153.

Carmelier P, Zanata AM. Biogeography of freshwater fishes from the Northeastern Mata Atlântica freshwater ecoregion: distribution, endemism, and área relationships. Neotrop Ichthyol. 2014; 12: 683-698.

Congrave CR, Lamsdell JC. Implied weighting and its utility in palaeontological datasets: a study using modelled phylogenetic matrices. Palaeontology. 2016; 59(3): 447-462.

Coutinho-Sanches N, Dergam JA. Cytogenetic and Molecular Data Suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) Is a Member of an Ancient Coastal Group. Zebrafish [serial on the Internet]. 2015; 12(5):357-365. Available from: http://dx.doi.org/10.1089/zeb.2014.1068

De Pinna, M. C. C. Concepts and tests of homology in the cladistic paradigm. Cladistics.1991; 7: 367-394.

Dillman CB, Sidlauskas BL, Vari RP. A morphological supermatrix-based phylogeny for the Neotropical fish superfamily Anostomoidea (Ostariophysi: Characiformes):phylogeny, missing data and homoplasy. Cladistics. 1:1-21.

Eigenmann CH, McAtee WL, Ward DP. On further collections of fishes from Paraguay. Annals of the Carnegie Museum. 1907; 4: 110-157.

Eigenmann CH. Preliminary descriptions of new genera and species of tetragonopterid characins. (Zoölogical Results of the Thayer Brazilian expedition.). Bull Mus Comp Zool. 1908; 52(6):91-106.

Eigenmann CH. Preliminary descriptions of new genera and species of tetragonopterid characins. (Zoölogical Results of the Thayer Brazilian expedition.). Bull Mus Comp Zool. 1908; 52: 91-106.

Eschmeyer WN, Fricke R, van der Laan R, editors. Catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2016 [updated 2016 Sep 29; cited 2016 Oct 15]. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Eschmeyer WN, Fong JD, editors: Species of fishes by family/subfamily [Internet]. Available from: [http://research.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp], 2017.

Farris JS. A successive approximations approach to character weighting. Syst Zool. 1969; 18: 374-385.

Farris JS. Methods for computerWagner trees. Syst Zool. 1970; 19: 83-92.

Farris JS. The logical basis of phylogenetic analysis. In: Platnick NI, Funk VA, editors. Advances in Cladistics II. New York: Columbia University Press; 1983. p. 7-36.

Géry J. Characoids of the World. Neptune: T. F. H. Publications, Inc; 1977.

Géry J. Un nouveau poisson characoide occupant la niche des mangeurs d'écailles dans le haut rio Tapajoz, Brésil: *Bryconexodon juruenae* n.g.sp. Revue Française d'Aquariologie. 1980; 7: 1-8.

Goloboff PA. Estimating character weights during tree search. Cladistics. 1993; 9: 83-91.

Goloboff PA, Farris JS, Nixon KC. TNT: Tree Analysis Using New Technology. [Data Analysis Software System], Versión 1.1. Available from: www.zmuc.dk/public/phylogeny.

Goloboff PA, Farris JS, Nixon KC. TNT, a free program for phylogenetic analysis. Cladistics. 2008; 24: 774-786.

Goloboff PA, Farris JS. Methods for quick consensus estimation. Cladistics. 2001; 17(1): S26-S34.

Hennig W. Phylogenetic systematics. Illinois: University of Illinois; 1966.

Heath TA, Hedtke SM, Hillis DM. Taxon sampling and the accuracy of phylogenetic analyses. J Syst Evol. 2008; 46(3): 239-257.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN. Universal primer cocktails for fish DNA barcoding. Mol Ecol Not. 2007; 7: 544-548.

Javonillo R, Malabarba LR, Weitzman SH, Burns JR. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. Mol Phylogenet Evol. 2010; 54: 498-511.

Lima FCT, Malabarba LR, Buckup PA, Pezzi da Silva JF, Vari RP, Harold A, Benine R, Oyakawa OT, Pavanelli CS, Menezes NA, Lucena CAS, Malabarba MCSL, Lucena ZMS, Reis RE, Langeani F, Casatti L, Bertaco VA, Moreira C, Lucinda PHF. Genera *Incertae Sedis* in Characidae. In: Reis RE, Kullander SO, Ferraris CJ, editors. Check List of the Freshwater Fishes of South and Central America. Porto Alegre: Edipucrs; 2003. p. 106-169.

Li C, Orti G, Zhang G, Lu G. A practical approach to phylogenomics: The phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evol Biol. 2007; 7: 44.

Lucena CAS. 1993. Estudo Filogenético da Família Characidae com uma Discussão dos Grupos Naturais Propostos (Teleostei, Ostariophysi, Characiformes). [PhD Thesis]. São Paulo, SP: Universidade de São Paulo; 1993.

Lucena CAS, Lucena ZMS. Redefinição do gênero *Deuterodon* Eigenmann (Ostariophysi: Characiformes: Characidae). Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia. 2002; 15: 113-135.

Lucena ZMS, Lucena CAS. Revisão das espécies do gênero *Deuterodon* Eigenmann, 1907 dos sistemas costeiros do sul do Brasil, com a descrição de quatro espécies novas (Ostariophysi: Characiformes: Characidae). Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia.1992; 5: 123-168.

Lucena CAS, Soares HG. Review of species of the *Astyanax bimaculatus* "caudal peduncle spot" subgroup sensu Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. Zootaxa.2016; 4072: 101-125.

Malabarba LR. Monophyly of the Cheirodontinae, Characters and Major Clades (Ostariophysi: Characidae). Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p. 193-234.

Melo FAG, Buckup PA. *Astyanax henseli*, a new name for Tetragonopterus aeneus Hensel, 1870 from southern Brazil (Teleostei: Characiformes). Neotrop Ichthyol. 2006; 4(1):45-52.

Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, USA. 2010.

Mirande JM. Weighted parsimony phylogeny of the family Characidae (Teleostei: Characiformes). Cladistics. 2009; 25: 574-613.

Mirande JM. Phylogeny of the family Characidae (Teleostei: Characiformes) from characters to taxonomy. Neotrop Ichthyol. 2010; 8: 385-568.

Mirande JM, Aguilera G, Azpelicueta MM. *Astyanax endy* (Characiformes: Characidae), a new fish species from the upper RRo Bermejo basin, northwestern Argentina. Zootaxa. 2006; 1286: 57-68.

Mirande JM, Aguilera G, Azpelicueta MM. A new species of *Astyanax* (Characiformes: Characidae) from the endorheic basin, Tucumán, northwestern Argentina. Zootaxa. 2007; 1646: 31-39.

Mirande JM, Jerep FC, Vanegas-Ríos JA. Phylogenetic relationships of the enigmatic *Carlastyanax aurocaudatus* (Eigenmann) with remarks on the phylogeny of the Stevardiinae (Teleostei: Characidae). Neotrop Ichthyol. 2013; 11: 747-766.

Nelson JS. Fishes of the World. 4th ed. New York: John Wiley & Sons Inc; 2006.

Nixon KC, Carpenter JM. On homology. Cladistics. 2012; 28: 160-169.

Nylander JAA. (2004). MrModeltest v2. Program distributed by the author. In: Uppsala Sweden, editor. Evolutionary Biology Centre. Uppsala University Available at: http://www.ebc.uu.se/systzoo/staff/nylander.html (accessed March 2010).

Oliveira C, Avelino GS, Abe KT, Mariguela TC, Benine RC, Ortí G, Vari RP, Castro RMC. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingrooup sampling. BMC Evol Biol. 2011; 11: 275.

Rambaut A, Drummond AJ. (2004). Tracer v1.3http://evolve.zoo.ox.ac.uk/ software.

Ribeiro AC. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. Neotrop Ichthiol. 2006; 4: 225-246.

Roberts TR. Scale-eating American characoid fishes, with special reference to *Probolodus heterostomus*. Proc Calif Acad Sci. 1970; 20: 383-390.

Rossini BC, Oliveira CAM, Melo FAG, Bertaco VA, Astarloa JMD, Rosso JJ, et al. Highlighting *Astyanax* Species Diversity through DNA Barcoding. Plos one [serial on the Internet]. 2016; 11(12): e0167203. Available from: http://dx.doi.org/10.1371/journal.pone.0167203

Santos O, Castro RMC. Taxonomy of *Probolodus* Eigenmann, 1911 (Characiformes: Characidae) with description of two new species, and comments about the phylogenetic relationships and biogeography of the genus. Neotrop Ichthyol. 2014; 12(2): 403-418.

Sazima I. Scale-eating in characoids and other fishes. Environ Biol Fish. 1983; 9: 87-101.

Sazima I. Possible case of aggressive mimicry in a neotropical scale-eating fish. Nature. 1977; 270: 510-512.

Schaefer SA. Conflict and resolution: impact of new taxa on phylogenetic studies of the Neotropical cascudinhos (Siluroidei: Loricariidae). Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p. 375-400.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu Rev Entomol. 2010; 55, 421-438.

Silva PC, Lucena CA, Lucena ZMS, Malabarba LR. An integrative analysis of the phylogenetic relationships of *Deuterodon* (Ostariophysi: Characidae). A ser submetido em zoological scripta. 2017

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol Phylogenet Evol. 1999; 12: 105-114.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 2013; 30: 2725-2729.

Taylor WR, Van Dyke GC. Revised procedures for staining and clearing small fishes and other vertebrates for boné and cartilage. Cybium. 1985; 9: 107-119.

Thomaz AT, Arcila D, Ortí G, Malabarba LR. Molecular phylogeny of the subfamily Stevardiinae Gill, 1858 (Characiformes: Characidae): classification and the evolution of reproductive traits. BMC Evol Biol. 2015; 15: 146.

Vari RP. The Curimatidae, a lowland neotropical fish family (Pisces: Characiformes): distribution, endemism and phylogenetic biogeography. In: Vanzolini PE, Heyer WR, editors. Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências; 1988. p.343-377.

Wiens JJ, Kuczynski CA, Smith SA, Mulcahy DG, Sites Jr. JW, Townsend TM, Reeder TW. Branch Lengths, Support, and Congruence: Testing the Phylogenomic Approach with 20 Nuclear Loci in Snakes. Syst Biol. 2008; 57(3):420-431.

Weitzman SH, Malabarba LR. Perspectives about the phylogeny and classification of the Characidae (Teleostei: Characiformes). In: Malabarba LR, Reis RE, Vari RP, Lucena

ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p.161-170.

Weitzman SH, Menezes NA, Weitzman MJ. Phylogenetic biogeography of Glandulocaudini (Teleostei, Characiformes, Characidae) with comments on the distribution of other freshwater fishes in eastern and southeastern Brazil. In: Vanzolini PE, Heyer WR, editors. Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências; 1988. p.379-427.

Zanata AM. *Jupiaba*, um novo gênero de Tetragonopterinae com osso pélvico em forma de espinho (Characidae, Characiformes). Iheringia, Série Zoologia. 1997; 83: 99-136.

Zanata AM, Akama A. *Myxiops aphos*, new characid genus and species (Characiformes: Characidae) from the rio Lençóis, Bahia, Brazil. Neotrop Ichthyol. 2004; 2: 45-54.

Zanata AM, Vari RP. The family Alestidae (Ostariophysi, Characiformes): a phylogenetic analysis of a trans-Atlantic clade. Zool J Linn Soc. 2005; 145(1): 1-144.

Zander RH. Minimal Values for Reliability of Bootstrap and Jackknife Proportions, Decay Index, and Bayesian Posterior Probability. PhyloInformatics. 2004; 2: 1-13.

Tables and Figures

Tab. 1. Information content, molecular model of evolution and characteristics of each molecular data partition

	Gene			
	COI	ND2	MYH6	SH3PX3
Number of sequences	209	111	125	40
bp after alignment	714	1049	780	723
Number of variable sites	269	792	163	185
Number of informative characters under parsimony	235	749	116	84
% informative characters under parsimony	32.9	71.4	14.8	11.6
Π_A	0.24	0.31	0.30	0.25
Π_C	0.25	0.26	0.21	0.27
Π_G	0.18	0.13	0.24	0.28
Π_T	0.32	0.29	0.25	0.20
Minimum *p*-distance among sequences	0.00	0.00	0.00	0.00
Overall mean genetic distance (*p*-distance)	0.14	0.37	0.02	0.03
maximum *p*-distance among sequences	0.25	0.72	0.11	0.23
Molecular model of evolution	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G

Fig.1. Dentary of *Deuterodon supparis* in lateral view, MCP 10632, paratype. The dentary is deepest at posteriormost portion, height dimishing progressively anteriorly in the toothed portion of the bone corresponding to half to 2/3 of its length (character 393, state 1). Teeth with all cusps nearly equal in size and shape (character 402, state 1). Basal portion of teeth narrower than apical portion with a gap between the bases of contiguous teeth (character 4033, state 1).

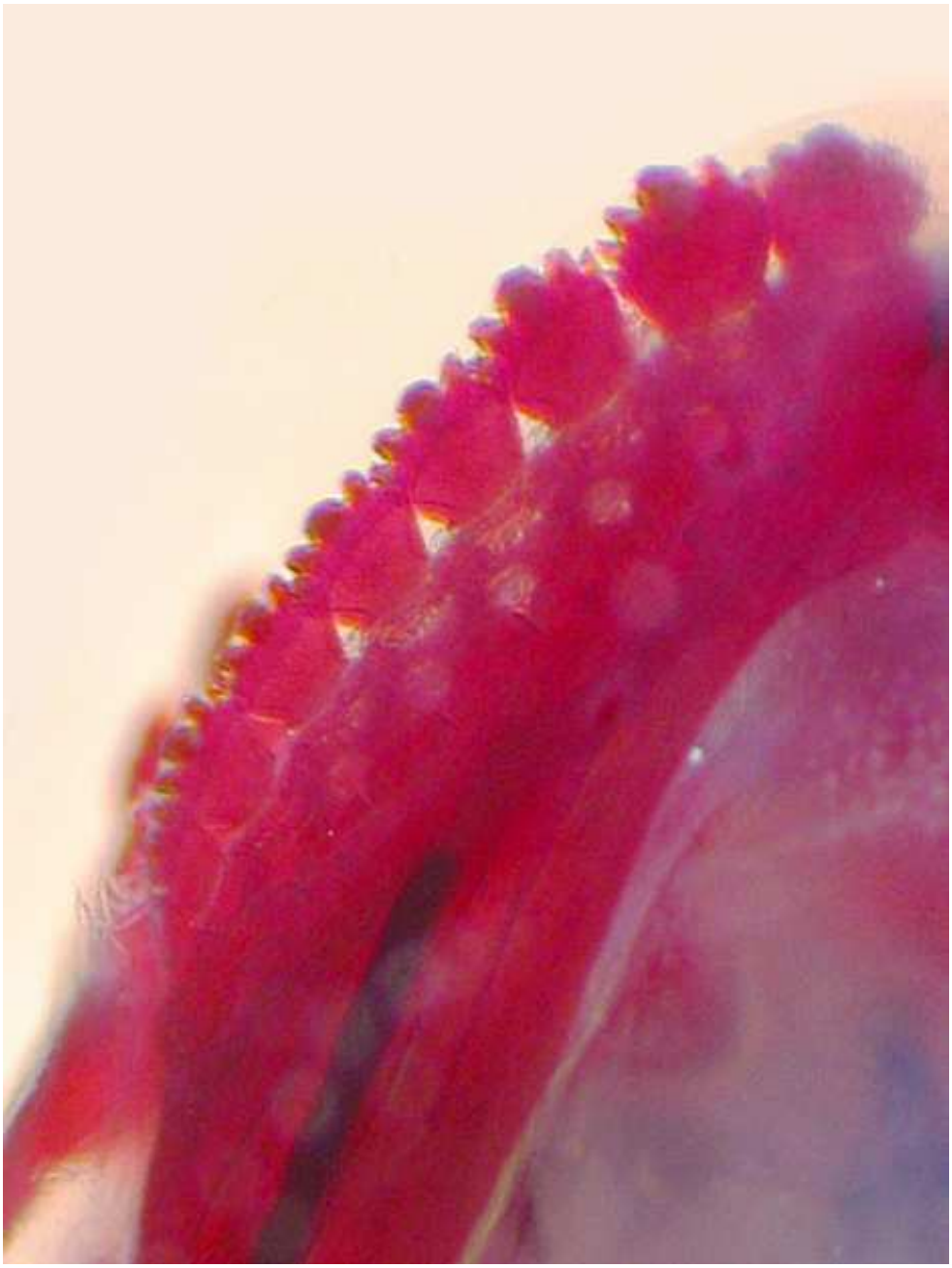


Fig.2: Dentary teeth of *Deuterodon stigmaturus* in ventral view, MCP 14678. Teeth oriented laterally and anteriorly, visible in ventral view (character 394, state 1).

Fig. 3. Maxilla of *Deuterodon supparis* in lateral view, MCP 10632, paratype. Very small lateroveltral projection (LP) (character 397, state 1). Teeth with all cusps nearly equal in size (character 406, state 1) and shape with basal portion narrower than apical portion with a gap between the bases of contiguous teeth (character 407, state 1).

Fig. 4. Lateral view of a live specimen of *Deuterodon stigmaturus* (not preserved). It is possible to see the maxillary teeth aligned continouously with pre-maxillary teeth (character 400, state 1).

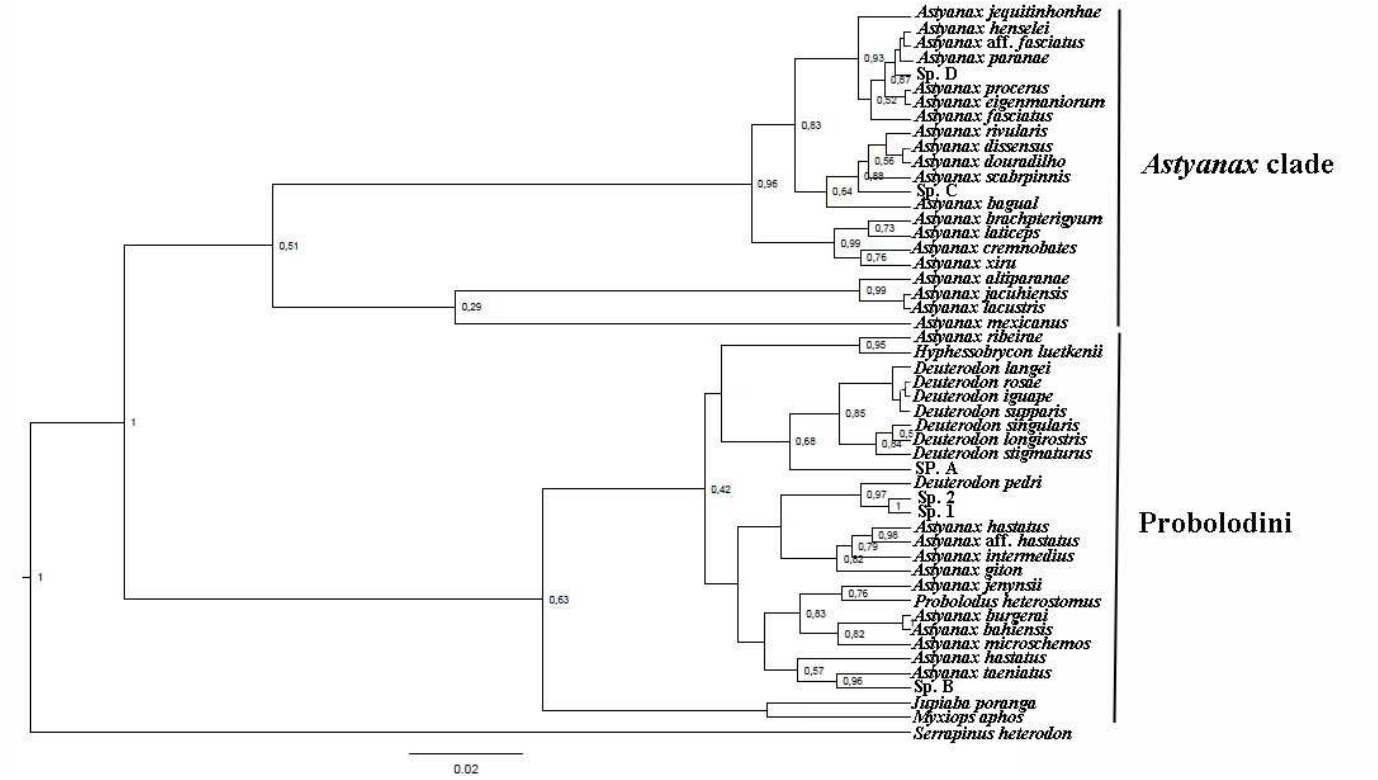


Fig.5. Species tree Bayesian based. Four genes where used, two mitochondrial (COI, ND2) and two nuclear (SH3PX3, MYH6). Numbers are posterior probability. Two major clades were found, one named here as Probolodini, is composed by *Deuterodon* species, *Astyanax species* from coastal drainages, *Hyphessobrycon luetkenii*, *Probolodus heterostomus*, *Myxiops aphos* and *Jupiaba poranga*. The other clade is composed by the remain *Astyanax* species included in the analyses and was named as *Astyanax* clade.

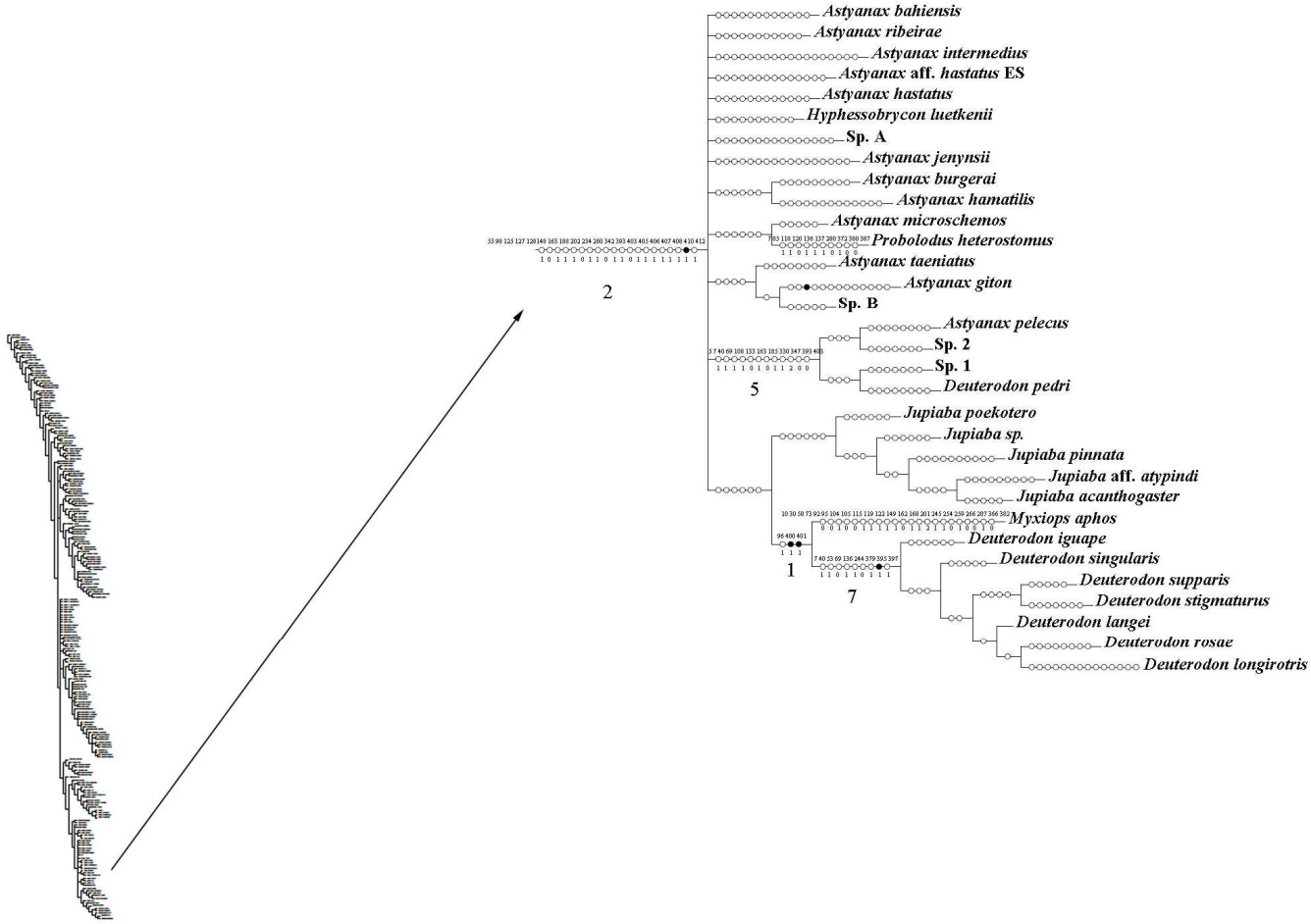


Fig.6. Consensus tree of most parsymoniose tree generated under equal weighting. The clade corresponding to Probolodini tribe is destaqued from the tree, major numbers are Bremer support.

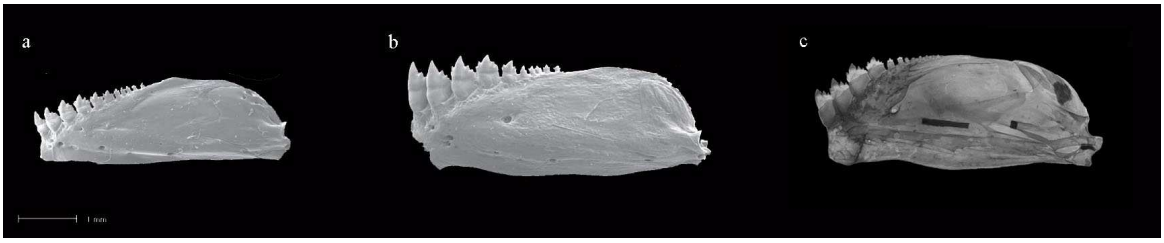


Fig.7. a) Gradually decreasing of the dentary teeth of *Astyanax pelecus* MCP 17919; b) Dentary teeth with 4 major teeth followed by one of intermediary size, given impression of 5 major teeth in *Astyanax microschemos* MCP 34366; c) Abruptaly decreasing of the dentary teeth of *Astyanax jequitinhonhae* UFRGS19070with only 4 major teeth in.

Supporting information

S1. Vouchers of speimens Clear and stained used to construct the parcimony based tree with morphological characters.

Voucher	Species	Locality
UFRGS6485	*Aphyocharax anisitsi**	Arroio do Salso, Rosário do Sul, Rio Grande do Sul, Brazil
UFRGS19044	*Astyanax cf. bahiensis*	Santa Cruz Cabrália, Bahia, Brazil
UFRGS4921	*Astyanax brachpterygium*	Rio do Marco, São José dos Ausentes, Rio Grande do Sul, Brazil
UFRGS8197	*Astyanax cremnobates*	Rio Camisa, Cambará do Sul, Rio Grande do Sul, Brazil
UFRGS17469	*Astyanax dissensus*	Cidreira, Rio Grande do Sul, Brazil
UFRGS18390	*Astyanax douradilho*	Rio do Ouro, Maquiné, Rio Grande do Sul, Brazil
UFRGS9948	*Astyanax fasciatus*	Córrego Coqueiro, Pirapora, Minas Gerais, Brazil
UFRGS 4581	*Astyanax aff. fasciatus*	Arroio Candiota., Bagé, Rio Grande do Sul, Brazil
UFRGS14814	*Astyanax giton*	Córrego Latão, rio Doce, Coimbra, Minas Gerais, Brazil
UFRGS11291	*Astyanax goyanensis*	Rio dos Couros, Alto Paraíso de Goiás, Goiás, Brazil
UFRGS18930	*Astyanax aff. hastatus*	Córrego Pratinha, Mimoso do Sul, Espírito Santo, Brazil
UFRGS18526	*Astyanax hastatus*	Rio Batatal, Peruíbe, São Paulo, Brazil
UFRGS6957	*Astyanax henseli*	Rio Carreiro, Rio Grande do Sul, Brazil
MZV4458	*Astyanax intermedius*	Rio Doce, Santa Cruz do Escalvado, Minas Gerais, Brazil
UFRGS18913	*Astyanax jenynsii*	Rio Imbé, Visconde de Imbé, Rio de Janeiro, Brazil
UFRGS19070	*Astyanax jequitinhonhae*	Lagoa Juiz de Fora, Pingo D'água, Minas gerais, Brazil
UFRGS18503	*Astyanax laticeps*	Ribeirão Passagem na saída de Iporanga, Iporanga, São Paulo, Brazil
UFRGS19054	*Astyanax lacustris*	Lagoa Tiririca, Pingo D'água, Minas gerais, Brazil
USNM310222	*Astyanax mexicanus*	Kinney County, Texas, USA
UFRGS17542	*Astyanax aff. michroschemos*	Córrego Mumbaça, Dionísio, Minas Gerais, Brazil
MCP17919	*Astyanax pelecus*	Pardo River, Candido Sales, Bahia, Brazil
UFRGS19324	*Astyanax procerus*	Rio Turvo, Espumoso, Rio Grande do Sul, Brazil
UFRGS20032	*Astyanax ribeirae*	Ribeira de Iguapé, Juquiá, São Paulo, Brazil
MNRJ36772	*Astyanax rivularis*	Santuário Caraça, Minas Gerais, Brazil
MZUFV4456	*Astyanax scabripinnis*	Rio Doce, Santa Cruz do Escalvado, Minas Gerais, Brazil
UFRGS19342	*Astyanax taeniatus*	Rio Aduelas, fazenda Sossego, Conceição de Macabu, Rio de Janeiro, Brazil
UFRGS5142	*Astyanax xiru*	Tainhas, Rio Grande do Sul, Brazil
UFRGS19407	*Bryconamericus agna**	Arroio Cuña-Piru, Província de Missiones, Argentina
UFRGS10089	*Bryconops affinis**	Balneário Pandeiros River, Balneário, Minas Gerais. Brazil
UFRGS1081	*Charax stenopterus**	Estação Ecológica do Taim, Rio Grande, Rio Grande do Sul, Brazil
UFRGS2303	*Cheirodon interruptus**	Estação Ecológica do Taim, Rio Grande, Rio Grande do Sul, Brazil
UFRGS9191	*Coptobrycon bilineatus**	Itatinga River, Bertioga, São Paulo, Brazil
UFRGS4598	*Cyanocharax alburnus**	Emboaba lake, Tramandaí, Rio Grande do Sul, Brazil
USNM437051	*Deuterodon iguape*	Iguape River, Ribeira de Iguape River basin, Road near Curitiba, São Paulo, Brazil
USNM437052	*Deuterodon supparis*	Itajaí River basin, Blumenau, Santa Catarina, Brazil
USNM297926	*Deuterodon singularis*	Tubarão River basin, Rio Fortuna, Santa Catarina, Brazil
USNM436729	*Deuterodon stigmaturus*	Grande River, Praia Grande, Santa Catarina, Brazil
UFRGS2073044	*Deuterodon pedri*	Santo Antônio River, Doce River basin, Ferros, Minas Gerais, Brazil
MCP12205	*Deuterodon longirostris*	Cedro River, Cubatão River, Sata Catarina, Brazil
ROM61441	*Deuterodon potaroensis*	Potaro River, French Guyana
UFRGS769	*Diapoma speculiferum**	Arroio dos Ratos, São Jerônimo, Rio Grande do Sul, Brazi
UFRGS9916	*Hasemania nana**	São Francisco River basin, Pirapora, Minas gerais, Brazil
UFRGS11584	*Hemigrammus bleheri**	Demeni River, Barcelos, Amazonas, Brazil
UFRGS12280	*Hyphessobrycon elachys**	Mato Grosso, Brazil
UFRGS5714	*Hyphessobrycon luetkenii*	Lagoa Negra, Viamão, Rio Grande do Sul, Brazil
UFRGS9826	*Hyphessobrycon herbertaxelrodi**	Sepotuba River, Tangará da Serra, Mato Grosso, Brazil
UFRGS11577	*Hyphessobrycon socolofi**	Turkys Aquaryium, Manaus, Amazonas, Brazil
FMNH54375	*Hollandichthys multifasciatus**	Mogy River, Raiz da Serra, São Paulo, Brazil
ROM91457	*Jupiaba abramoides*	Guyana
UFRGS13743	*Jupiaba acanthogaster*	Córrego Monjolinho Chapada dos Guimarães, Mato Grosso, Brazil
UFRGS12163	*Jupiaba apenima*	Afluente do Guaporé, Pontes e Lacerda, Mato Grosso, Brazil
ROM83417	*Jupiaba anteroides*	Peru

UFRGS13874	*Jupiaba cf. atypindi*	Rio das Mortes, Campo Verde, Mato Grosso, Brazil
ROM96089	*Jupiaba essequibensis*	Guyana
ROM96166	*Jupiaba mucronata**	Guyana
ROM98037	*Jupiaba ocellata*	Suriname
ROM91432	*Jupiaba pinnata*	Guyana
ROM88393	*Jupiaba poekotero*	Venezuela
ROM96084	*Jupiaba potaroensis*	Potaro River, French Guyana
USNM272612	*Jupiaba scologaster*	Negro River, Casiquiare River basin, Venezuela
UFRGS10682	*Markiana nigripinnis**	Poconé, Mato Grosso, Brazil
UFRGS6577	*Mimagoniates rheocharis**	Terra de areia, Rio Grande do Sul, Brazil
UFRGS2084	*Moenkhausia dichroura**	Bento Gomes River, Paraguay River basin, Poconé, Mato Grosso, Brazil
UFRGS5315	*Moenkhausia sanctaefilomenae**	Ibicuí Mirim River, Cacequi, Rio Grande do Sul, Brazil
UFRGS23403	*Myxiops aphos*	Rio Lençóis, Lençóis, Bahia, Brazil
UFRGS11046	*Nematocharax venustus**	Cachoeira River, Itapé, Bahia, Brazil
MCP12031	*Odontostilbe paraguayensis**	
UFRGS7022	*Odontostilbe pequira**	Ijuí Mirim River, Pirapó, Rio Grande do Sul, Brazil
UFRGS11580	*Paracheirodon axelrodi**	Turkys Aquaryium., Manaus, Amazonas, Brazil
ANSP150124	*Phenagoniates macrolepis**	Venezuela
MCP15580	*Prionobrama paraguayensis**	
UFRGS8968	*Pseudocorynopoma doriae**	Amaral Ferrador, Rio Grande do Sul, Brazil
UFRGS18773	*Sp. A*	Rio Ubatumirim, Ubatuba, São Paulo, Brazil
UFRGS18956	*Sp. B*	Rio Santa Maria da Vitória, Santa Maria de Jetibá, Espírito Santo, Brazil
UFRGS19746	*Sp. C*	Tripuí, Ouro Preto, Minas Gerais, Brazil
MZUFV3992	Sp.1	Doce River basin, Rio Doce, Minas Gerais, Brazil
MZUFV39335	Sp.2	Doce River basin, Rio Doce, Minas Gerais, Brazil
FMNH103538	*Xenagoniates bondi**	Rio Apure, Hato Mercedes, Barinas, Venezuela

ANSP= Academy of Natural Sciences, Philadelphia, FMNH= The Field museum of natural sciences, MCP = Museu de ciência e tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul; MNRJ= Museu Nacional do Rio de Janeiro; MZUFV = Museu de zoologia João Mojeen da Universidade Federal de Viçosa; ROM= Royal Ontario Museum ; USNM= National Museum of Natural history of Smithsonian institute; UFRGS = Universidade Federal do Rio Grande do Sul; * specimens used to examine only the new twentyteen characters add to the matrix.

S2. Table with informations and species used to construct the species tree.

Voucher	Species	Sample number	Locality	Genbank acss number
UFRGS 18508	*Deuterodon langei*	TEC4103	Paranaguá River basin	KY327419
UFRGS 18525	*Deuterodon iguape*	TEC 4138	Ribeira do Iguape River basin	KY327420
UFRGS 20032	*Deuterodon iguape*	TEC 4130	Ribeira do Iguape River basin	KY327421
UFRGS 18495	*Deuterodon suparis*	TEC 4651	Itajaí River basin	KY327422
UFRGS 18518	*Deuterodon singularis*	TEC4087	Tubarão River basin	KY327423
UFRGS 16519	*Deuterodon stigmaturus*	TEC2847	Rio Três Forquilhas	KY327424
UFRGS 16208	*Deuterodon stigmaturus*	TEC2350	Maquiné River basin	KY327425
UFRGS 18629	*Deuterodon langei*	TEC3935	Cubatão River basin	KY327426
MCP 50444	*Deuterodon longirostris*	MCP50444	Cubatão River basin	
UFRGS20644	*Deuterodon rosae*	TEC5860	Itapocu River basin	
UFRGS 17542	*Astyanax michroschemos*	CT1936 CT1940 CT1882 CT1885 CT1886 CT1890	Doce River basin	KY327428 KY327429
MCP 47661	*Deuterodon pedri*	CT2521	Doce River basin	KY327434
UFRGS17543	*Deuterodon pedri*	CT2529	Doce River basin	KY327435
MCZ17510	*Deuterodon pedri*	lectotype	Santo Antônio River, Doce River basin, Ferros, Minas Gerais, Brazil	
MZUFV3992	Sp1	CT2353 CT2765 CT2285 CT2284 CT2293 CT2345 CT2349 CT2388 CT2748 CT2749 CT2492 CT2755 CT2757 CT2758 CT2769	Doce River basin	KY327436 KY327437
MZUFV 4457	Sp2	CT2965 CT2971 CT2966 CT2968 CT2969	Doce River basin	KY327438 KY327439
MZUFV 4456	*Astyanax scabripinnis*	CT2772 CT2773 Ct2493	Doce River basin	KY327444 KY327445
UFRGS19746	Sp. D	TEC5291A TEC5291E	Tripuí River, Doce River basin	KY327447

UFRGS 18433	*Astyanax aff. fasciatus*	TEC3826	Tramandaí River basin	
UFRGS 19147		TEC4865A	Tramandaí River basin	KY327448
UFRGS 19147		TEC4865 B	Tramandaí River basin	KY327449
UFRGS 19135		TEC4853A	Tramandaí River basin	KY327450
UFRGS 19135		TEC4853B	Tramandaí River basin	KY327451
UFRGS 14913	*Astyanax fasciatus*	TEC1056	São Francisco River basin	
UFRGS 23403	*Myxiops aphos*	TEC6844A	Paraguaçu drainage	KY327452
	Myxiops aphos	TEC6844B	Paraguaçu drainage	KY327453
ROM96089	*Jupiaba essequibensis*	T15810	Essequibo River, Guyana	KY327454
ROM96166	*Jupiaba mucronata*	T16213	Guyana	KY327455
UFRGS18758	*Probolodus heterostomus*	TEC4184	Paraíbuna River, Paraíba do Sul River basin	KY327456
UFRGS22004	*Serrapinus heterodon*	TEC6956	Doce River basin	KY327457
UFRGS18431	*Hyphessobrycon luetkenii*	TEC3824A, B, C, D	Maquiné River, Tramandaí River basin	KY327458
UFRGS19226		TEC4921	Mostardas River	KY327459
UFRG16654		TEC2976	Uruguai River basin, Rosário do Sul, RS, Brazil	
UFRGS16502		TEC2830	Tramandaí River basin, Itati, RS, Brazil	
UFRGS16524		TEC2852	Tramandaí River basin, Itati, RS, Brazil	
UFRGS16543		TEC2876	Tramandaí River basin, Itati, RS, Brazil	
UFRGS17510		TEC3366	Tramandaí River basin, Cidreira, RS, Brazil	
UFRGS18603		TEC3896	Itajaí River basin, Itajaí, SC, Brazil	
UFRGS12480		TEC1288	Laguna dos Patos basin, Camaquã, RS, Brazil	
UFRGS 19342	*Astyanax taeniatus*	TEC4997	Macaé River basin	KY327460
UFRGS 19342		TEC5000	Macaé River basin	KY327461
UFRGS 18870		TEC4240	Silva Jardim, RJ, Brazil	
UFRGS 18884		TEC4253	Pirineus River, Silva Jardim, RJ, Brazil	
UFRGS 18888	*Astyanax taeniatus*	TEC4261	São João River, Silva Jardim, RJ, Brazil	
UFRGS 18516	*Astyanax ribeirae*	TEC 4112	Ribeira do Iguape River basin	KY327462
UFRGS 20032		TEC 4137	Ribeira do Iguape River basin	KY327463
UFRGS 18615		TEC3908	Piraí River, Graramirim, SC, Brazil	
UFRGS 18647		TEC3953	Guaratube River, Garuva, SC, Brazil	
UFRGS 19606		TEC4100	Matinhos, PR, Brazil	
UFRGS 18516		TEC4121	Passagem River, Iporanga, SP, Brazil	
UFRGS 18531		TEC4131	Martins River, Eldorado, SP, Brazil	
UFRGS 20032		TEC4141	Açungui River, Juquiá, SP, Brazil	
UFRGS 15350	*Astyanax altiparanae*	TEC1911	córrego do Veadão, Vitória Brasil, SP, Brazil	
UFRGS 17834	*Astyanax bagual*	TEC3513	Carreiro River, Dois Lajeados, RS, Brazil	

UFRGS 19044	*Astyanax bahiensis*	TEC4784/TEC 4786	Santa Cruz Cabrália, BA, Brazil	
UFRGS 21849	*Astyanax brachpterygium*	TEC6844	PNAS, Uruguay River basin	
UFRGS 11636	*Astyanax burgerai*	TEC1154	Santa Cru River, Belmonte, BA, Brazil	
UFRGS 18430	*Astyanax cremnobates*	TEC3823A,B,D	São Francisco de Paula, RS, Brazil	
		TEC4863E,B,C,D		
		TEC4868		
UFRGS 16521	*Astyanax dissensus*	TEC2849,	Três Forquilhas River, Itati, RS, Brazil	
		TEC3225A,B,C,D		
UFRGS 18444	*Astyanax douradilho*	TEC3837A, B,	Maquiné River, Maquiné, RS, Brazil	
UFRGS 18742		TEC3865		
UFRGS 19221	*Astyanax eigenmaniorum*	TEC4916A, B	Lagoa Bacupari, Mostradas, RS, Brazil	
MZUFV 4459	*Astyanax giton*	CT2083	Doce River basin	
		CT3461		
		CT2093		
		CT2809		
		TEC4002		
UFRGS 18952		TEC4767	Santa Maria de Jetibá, ES, Brazil	
		TEC4038		
UFRGS19058		TEC4033	Doce River basin	KY327430
MZUFV 4459		CT3464	Doce River basin	KY327431
UFRGS 18526	*Astyanax hastatus*	TEC4146	Batatal River, Peruíbe, SP, Brazil	
		TEC4155		
UFRGS 18806		TEC4212	São Pedro River, Japeri, RJ, Brazil	
		TEC4217		
UFRGS 18849		TEC4222	Paraíso River, Guapimirim, RJ, Brazil	
		TEC4229		
UFRGS 18930		TEC4279	Pratinha River, Mimoso do Sul, ES, Brazil	
UFRGS 18942		TEC4289	Nova Mantua River, Alfredo Chaves, ES, Brazil	
UFRGS 18904		TEC 4527	Macaé River basin	KY327464
UFRGS 18906		TEC 4529	Macaé River basin	KY327465
UFRGS 16525	*Astyanax henseli*	TEC2853	Três Forquilas River, Itati, RS, Brazil	
UFRGS 18427		TEC3820A, B, C, D, E	Maquiné River, Maquiné, RS, Brazil	
UFRGS 19598		TEC5189A, B	Lagoa Emboabinha, Osório, RS, Brazil	
UFRGS 19610		TEC5200	Lagoa Fortaleza, Cidreira, RS, Brazil	
UFRGS 18867	*Astyanax intermedius*	TEC4248	Silva Jardim, RJ, Brazil	
MZUFV 4458		CT2436	Doce River basin	
		CT3175		
UFRGS 18739		TEC4158	Putim River, Guararema, SP, Brazil	
MZUFV 4458		CT3205	Doce River basin	
		CT2389		

		CT2800 CT3267		
MZUFV 4458		CT3207	Doce River basin	
UFRGS 19067		TEC4051 TEC4057	Lagoa Lingüiça, Revés do Belém, MG, Brazil	
MZUFV 4458		CT2801	Doce River basin	KY327432
UFRGS18894	*Astyanax intermedius*	TEC4554	São João River basin	KY327433
UFRGS 19067		TEC4058 TEC4062 TEC4064	Lagoa Lingüiça, Revés do Belém, MG, Brazil	
UFRGS 18883		TEC4259	Pirineus River, Silva Jardim, RJ, Brazil	
UFRGS 17362		TEC3264	Lagoa dos Quadros, Capão da Canoa	
UFRGS 17509		TEC3365, D	Lagoa Fortaleza, Cidreira, RS, Brazil	
UFRGS 18429	*Astyanax jachuiensis*	TEC3822A, B	Maquiné River, Maquiné, RS, Brazil	
UFRGS 19133		TEC4852A, B	Lagoa dos Quadros, Capão da Canoa	
UFRGS 19151		TEC4869A, B	Três Forquilhas, RS, Brazil	
UFRGS18913	*Astyanax jenynsii*	TEC4271 TEC4268	Paraíba do Sul River basin, Viscondé de Imbé, SP, Brazil	KY327427
UFRGS 18917		TEC4272	Grande River, São Sebastião do Alto, SP, Brazil	
UFRGS 19052	*Astyanax jequitinhonhae*	TEC4008 TEC4010 TEC4011 TEC4014 TEC4020 TEC4027	Lagoa Tiririca, Pingo D'água, MG, Brazil	
UFRGS 19066		TEC4047	Lagoa Lingüiça, Revés do Belém, MG, Brazil	
UFRGS19070		TEC4074	Doce River basin	KY327446
UFRGS18957	*Astyanax lacustris*	TEC4772	Santa Maria da Vitória River basin	KY327440
UFRGS19055		TEC4030	Lagoa Tiririca, Doce River basin	KY327441
UFRGS 19054		TEC4009	Lagoa Tiririca, Doce River basin	
UFRGS19055		TEC4017 TEC4024 TEC4028 TEC4030	Lagoa Tiririca, Doce River basin	
UFRGS 18513		TEC4102	Matinhos, PR, Brazil	
UFRGS 18732		TEC4160	Guararema, SP, Brazil	
UFRGS 18745		TEC4180	Putim River, Guararema, SP, Brazil	
UFRGS 18910		TEC4263	Macaé, RJ, Brazil	
UFRGS 18919		TEC4273	Grande River, São Sebastião do Alto, RJ, Brazil	
UFRGS 18927		TEC4275	Pratinha River, Mimoso do Sul, ES, Brazil	
UFRGS 18957		TEC4772	Santa Maria da Vitória River, Maria de Jetibá, ES, Brazil	

UFRGS 18895		TEC4775	Farias River, Linhares, ES, Brazil	
UFRGS 19033	*Astyanax lacustris*	TEC4783	Engano River, Pedro Canário, ES, Brazil	
UFRGS 16514		TEC2842	Três Forquilhas River, Itati, RS, Brazil	
UFRGS 18251		TEC3748A, B	Maquiné River, Maquiné, RS, Brazil	
		TEC3821		
UFRGS 18428			Maquiné River, Maquiné, RS, Brazil	
UFRGS 18432	*Astyanax laticeps*	TEC3825	Maquiné River, Maquiné, RS, Brazil	
UFRGS 18503		TEC4113	Passagem River, Iporanga, SP, Brazil	
		TEC4115		
UFRGS 20031		TEC4143	Açngui River, Juquiá, SP, Brazil	
UFRGS 18503		TEC4113	Ribeira de Iguapé River basin	KY327442
UFRGS 18503		TEC4115	Ribeira de Iguapé River basin	KY327443
UFRGS 15071	*Astyanax paranae*	TEC1855	Uberlândia, MG, Brazil	
UFRGS 19143	*Astyanax procerus*	TEC4861	Lagoa dos Quadros, Capão da Canoa, RS, Brazil	
UFRGS 19213		TEC4908A, B	Lagoa Fortaleza, Cidreira, RS, Brazil	
UFRGS 11375	*Astyanax rivularis*	TEC1213	Unaí/Palmeirinha, MG, Brazil	
UFRGS 18773	Sp. A	TEC4192	Ubatumirim River, Ubatuba, SP, Brazil	
UFRGS 18795		TEC4206	Taquari River, Parati, RJ, Brazil	
		TEC4208		
UFRGS 18797		TEC4210	Mambucaia River, Angra dos Reis, RJ, Brazil	
UFRGS 18822		TEC4220	Teresópolis, RJ, Brazil	
UFRGS 18860		TEC4228	Paraíso River, Guapimirim, RJ, Brazil	
		TEC4233		
UFRGS 18502	Sp. C	TEC4116	Passagem River, Iporanga, SP, Brazil	
UFRGS23111	*Astyanax mexicanus*	TEC7407	México	
UFRGS 18931	Sp. B	TEC4282	Novo River, Vargem Alta, ES, Brazil	
UFRGS 18941		TEC4288	Nova Mantua, Alfredo Chaves, ES, Brazil	
UFRGS 18950		TEC4293	Gaviões River, Alfredo Chaves, ES, Brazil	
UFRGS 18956		TEC4299	Santa Maria da Vitória River, Maria de Jetibá, ES, Brazil	
UFRGS 18248	*Astyanax xiru*	TEC3745	Maquiné River, Maquiné, RS, Brazil	
UFRGS 18438		TEC3831	Maquiné River, Maquiné, RS, Brazil	
UFRGS 18743		TEC3866	Maquiné River, Maquiné, RS, Brazil	
UFRGS 19127		TEC4846	Carvalho River, Itati, RS, Brazil	
UFRGS 19607		TEC5197A, B	Maquiné River, Maquiné, RS, Brazil	

MCP32007	*Jupiaba poranga*	MCP32007	Kaiapá River, Nova Canaã do Norte, MT, Brazil

UFRGS = Universidade Federal do Rio Grande do Sul; MCP = Museu de ciência e tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul; MCZ= Museum of comparative zoology of Harvard University; MZUFV = Museu de zoologia João Mojeen da Universidade Federal de Viçosa.

S3. Matriz with morphological data set. Polymorphisms are denoted as z= [0 1] and y= [0 2].

Puntigrus tetrazona

???0????0?z0010?00?0zzz?1????1???00?31010100?000???0???1?1000???1?????00????0000??100??00z0?0?????0z10?11??0?0?0?000?0????????????????0
000???????????00?010???000?00000z001??011?0?000?00100??101111100?0101101???0000?00?000?00000z0001000000000?100?0zz0??10??001110?000
1000101000000?zz100000000000001001000000000????????00z1010100000??z?001z000000000000210??0?z0???1zzz00?0????????????0100?z1??0z0??????
??????????????

Acestrocephalus sardina

?00010010?01?11000000z01110000100001 0zz00000?010?0?0100000000002010001 01?01z01000100100z01100101000?10001000000z100001?000110001??0
00001111000000000?11?011100000101000010000011001001100100?01001111000101110000101101010101110001011?11000000000000000001011?000
1000001100001100001000000011111z000000101010z001z??????????2010??000010100111 1100??000000000 10??0?00????????000??0000000010010000?
0?010????????????????????

Acestrorhynchus pantaneiro

?0101001z?z1111000000z0?01000010?011001100000000110101110000101201000100101z001000001000001001110 00?10001000000001000000000 1???????
???001111000000000011?011100010101010 0200?001001000011100101100 00002?1101010000101100??1001110000110010010000100010000000011000
01000001100001100101 00z1101011100000000z00010z00110????????0000??0000001000111100001000000001 0??0?0000?11000000??000000001001000
010?010????????????????????

Agoniates anchovia

?01010001100011000000001110000100001001100000000100001100100101?000000000011001000 00?00100100??1000?10001?000000100000010001 0001?
?10?00111100000??00?001?011101000000000201?001001000010 10?1011000100000101010000100100000011111001100?1001100010001000000 1111?0
000001001100001100101 00111z011111000000?00101000011??????????001100000000 1001?111000000000000010??0?00????????000??000000001001000
010?010????????????????????

Alestes macrophthalmus

?0?0100?11100????010010?1100000?00?121?1?000?000???01000?100??????0?0?0?01??1000?11?0??0?110??0???210000101010?110000010001 0?020000
0000000??000010000000000001000?10?0?020???01?0000001??100?1100??000000???1000010010?0000?1?1?001?00??00?000010001000000z0??0000110
000?00000111000000110?000110000?01111000000 1010?????????00?1010000001110?1?0000?000000000?0??0?0?????????000????????????????????01?
????????????????????

Apareiodon affinis

??1010001101010?1010111?10???10??001110101000000?0?0110001000??0000100010100000000 11000?000000?10100010011?000010?100001?00????????
??1?1100110????????00001000000000011000200110100000000001100?1101110010?0101?0??01000000000?0?1000010010001100010000000000100000001
00?0000100011100011 0z101000000000100110101000010?????????0001010100000????10110000000000110??0?00???111100?0??00000000???10000?0
?01?????????????????????

Aphyocharacidium bolivianum

?00110010?z001100010010?110100110001000 00001?0110000110000000010000 1011?0100000000010000 11000?100001100110010001000000100000??11
1110011110100010000000010100000000000000101110100011001010001010011110000001100001001010001010100010 1z?1001000000000000000101010
0010000001100001100001000000000z1100z00000101010z001011100010 10001???0000001000?1z??010000000000?01?0?00????????000??000000001001000
0???010????????????????????

Aphyocharax anisitsi

?00110010?0011101111010?110000??100001000010011100101100000000 10001??01?0100011000z10000110110100001001111010001000000100000??1??
1000111z0000001?000000101000000000000000 101010100011001010 00?010001110000001100001001000000101010001011010010000000001000z00110000
000000011000011000010001000011z000000001010100001011 10001?100011200000001000011100000000 1000000100?00?0?11000000??00000000100100
11?00000z00000000??????1????

Aphyocharax dentatus

?00110010?0011101110010?110000??100001000010011100101100000000 10001??01?01000110000100001101101000010011110100010000001 00000??1?
?1z0011110000001?000000101000000000 100000101010100011z010100000100011100000011000010010000010101000101101001000000000100000011000
0010000001100001100001000100001110000000010101000010111000000000011200000000 10000111z00000001000000100?00???11000000??0000000010010
011?00010????????????????????

Aphyocharax nattereri

?00110010?0011101110000z110000???000010000100110?0?0110000000010001???1?01000?11?0?1000011010?1000?100111100000100000010000 0??1??
10001111000000 1?00000010100000000000000101?0010001100101000001000111000000110000100100000101010001011010010000000001000000110?00
0z00000110000010000100010000111000000000101010000101110000000000112000000010000111?00000000000000000?0000??????000??00000000100100?
1?10000????????????????????

Aphyodite grammica

?00110010?1001100010000011010010000100000000011z00011000000000010101??11?0100000000001000011010?1000011001100000010000001000 00??1?
?1100?1z00000011?00000010100000000000000101110100011001010 00?010011100000001100001001000001010100010110100100000000010000010101 0
0010000011z00011000010001000011100000000101010000 10?????????001120000001 1001?1110010000000000?0??0?00????????000??000000001001 00?
0???000????????????????????

Argopleura magdalenensis

?00010010?0001101010000011000011000100000000101100001000000000010000z0z1?0100000000001000011 00??10000100010000z1z1000000100010001z0
000001z00100001?00000000100000000100000101 00z1000110010zz00?0100111000001011000010010 00001z1010001z10010011000000 00z00000z0101000
z000z011000001000000001000011110000000101010z0010110000000000112000000010000111000000000000000011110z111?????000?10000000011010000
?0?010????????????????????

Astyanax abramis

?00110010?1001100010000011000010000100000000000 10?0?0z00000000000 1000001?0110000000001000011001 01???11000100001 1?1000000100010001 0
0100000000??0001?000z0000100000000000000010000010001100101000?010001z000000011000010010000010101000101001000000000000000000101010
001000001110001100001000001001111000000010101000010111000010000012000000001100011100001000000100 10000?01???11000000?10000000011010
000000010????????????????????

Astyanax asuncionensis

?00110010?100110001000001100001000010000000000010?0?01000000000000 1001001?0110000000001000011001 01???11000100001111000000100010001 0
0100000000??0001?000000001000000000000000 10z?0z10001100101000?0100011000000011000010010000010101000101001000000000000000000101010
0010000011100011000010 00z0100111100000000101010000101110000000000012z00000011000111000010000001001 0000?01???11000000?100000000z1010
000000010????????????????????

Astyanax cf. *abramis*

?00110010?100110001000001100001000010000000000010?0?0z0000000000001000001?0110000000001000011001 01???11000100001111000000100010001 0
0100000000??0001?0000000010000000000000001 0z00z1000110010100000 10001z00000001100001001000001010100010100100000000000000000010101 0
0010000011100011000010 00z0100111100000000101010000 10111zzz000000012z0000001100011100001000000100 10000?01????????000?100000000z101000
0z00010????????????????????

Astyanax cf. *asuncionensis*

?00110010?100110001000001100001000010000000000010?0?0100000000000 01001001?011000000000100001100101???11000100001111000000100010001 0
0100000000??0001?000z000010000000000000001010001000110 0101z00001000110000000110000100 1z00001010100010100100000000000000000010101 0
0010000011100011000010 00z0100111z00000001010100001011111101 0000012z0000001100011100001000000 10000000?01????????000?100000000z10100
00000010????????????????????

Astyanax cf. *eigenmanniorum*1

?00110010?1001100010000111000010000100000000000110000100000000000001001001?0100000000001000011001 01??0110001000001010000000100010011 1
01000010001z0001?00z000001000000000000000101000100011001 01z0010100011000000001100001001000001010100010100100000000000000000010101 0
001000001110001100001000101001110000000001010100001011100000000001120000000100001110000000000000?0000?00????????000?10000000011010
000000010????????????????????

Astyanax cf. *eigenmanniorum*2

?00110010?1001100010000011000010000 10000z000001100001000000000000010 01001?0100000000001000011001 01??011000100000 1z1000000100010011 1
01000010001z0001?00000000100000000000000010100010001100101000z0100011000000011000010010000010101000101001000000000000000000101010
001000001110001100001000101001110000000001010100001011100000000001120000000100001110000000000000?0000?00????????000?10000000011010
000000010????????????????????

Astyanax cf. *rutilus*

?00110010?1001100010000111000010000100000000001100001000000000001001001?0100000000010000110010l??011000100000zz10000001000100z11
0100001000110001?0010001010000000000000001010001000110010100010100010000000011000010010000010lz100010100100000000000000000000101010
001000000111000110000100010100111100000001010100001011100000000001120000000100001110000000000000000000?00????????000?10000000011010
000000010????????????????????

Astyanax chico

?00110010?1001100010000011000010000100000000001100001000000000001001001?01z000000000100001100101??011000100000111000000010001001l1
01000010001z0001?000000001000000000000000101?0010001100101000101000110000000111000010010000010101000101001000000000000000000101010
00100000011100011000010001010011100000000010101000010111111000000112000000001100011100000000000000010000?00????????000?100000000z1010
000000010????????????????????

Astyanax correntinus

?00110010?100110001000011100001000010000000000001z0000z000000000001001001?0100000000010000110012l??0110001000001110000000100010011O
0100001000110001?0000000010000000000000001010001000110010100010100011000000111000010010000010101000101001000000000000000000010101
0001000000111000110000100000100111100000001010100001011100????0011200000000100001110000z0000000000000?01????????000?10000000011010
000000010????????????????????

Astyanax endy

?00110010?1001100010000011000010000100000000001100001000000000001001001?01000000000100001100101000110001000001z10000001000100111
0100001z001z0001?00000000100000000000000010100010001100101000z010001100000001100001001000001010100010100100000000000000000000101010
001000000111000110000100010100111000000000101010000101110000000000112000000001100011100000000000000000000?00????????000?10000000011010
000000010????????????????????

Astyanax latens

?00110010?1001100010000011000010000100000z000001z0000100000000000001001011?0100000000010000110010100011000100000zz10000001000100011
01000011z0100001?00010010100000000000000010100010001100101000z01000100000000110000100100000101010001010lz01000000000000000000101010
00100000011100011000010001010011110000000101010000101110000000000112000000001000?1110000000000000000000?00????????000?10000000011010
000100010????????????????????

Astyanax lineatus

?00110010?z00110001000001100001000010000000000010?0?01000000000001000001?0110000000010000z100101??011000100001111000000010001000l0
01000010001z0001?000z0000100000000000000101?1010001100101000?01000110000000110000100100000101010001010010000000000000000000101010
0010000001110001100001000z0100111z000000010101000010111111000000012000000001100011100000000000000000000?0100?11000000?100000000z1010
000000010????????????????????

Astyanax mexicanus

?00110010?1001100010 0z00110000100001000000000?0110000100000000000001000001?0100000000010000110010l??0110001000001z10000001000100011
0100001000110001?000000101000000000000000101000100011001010000010001100000001100001001000001010100010100100000000000000000000101z10
00100000011100011000010 00z010011100000000101010000101110000000000012000000001000011100000000000000?0000?00???1z000000?10000000011010
0000000100000000000000000?010

Astyanax paris

?00110010?1001100010000011000010000100000000001100001000000000001000001?01000000000100001100101000110001000000110000000100010001l
01000011z0100001?00z1000010000000000000001010001000110010100000100011000000011000010010000010101000101001000000000000000000101010
001z000011z000110000100010100111000000000101010000100????????0011200000000100001110000000000000000?0?00????????000?1000000001z01000
0000010????????????????????

Astyanax pelegrini

?00110010?1001100010000011000010000100000000001100001000000000001001001?0100000000010000110011l??0110001000011110000000100010001O
0100001000110001?000000001000000000000000101010100011001010001010001000000000110000100100000101010001010010000000000000000000010101
0001000000111000110000100000100111110000001010100001011100000000000120000000110001110000000000000000000?01????????000?100000000z101
0000000010????????????????????

Astyanax puka

?00110010?1001100010000011000010000100000z00000110000100000000000001001001?0100000000010000110010l??011000100000lz10000001000100111
0100001000110001?000000001000000000000000101000100011001010001010001100000011100001001000001010100010100100000000000000000000010101

0001000001110001100001000z0100111z000000010101000010111000000000112000000011000111000000000000000000?00????????000?1000000001101 0
000100010????????????????????

Astyanax troya

?00110010?100z100010000011000010000100000z0000011000010000000000001000001?0110000000010000110010 1??0110001000001110000001000100111
010000100011000 1?000000000100000000z000001010001z001100101000z0100011z000001110000100100000101010001010010000000000000000000101z100
010000011100011000 01000z010011100000000101010000101111110 00?00112000000011000111000 0z00000000z0000?00????????000?1000000001101000
0100010????????????????????

Attonitus ephimeros

?00110010?00111010100z0?1100001100010000000001010?0?0110000000010000001 1?0100010000011??0110010100001100110000011000000 1000100011
1000001110100001?0000000010001000010000010101010001100100000101011111000010110000100100000101010001010010011000000000000000000101
0001000001100000100000000100001100000000010101000010111000110000112000000010000111100000000000?0100000110????000?100000000z001
000010?010????????????????????

Aulixidens eugeniae

?0011001??z001001010010?1100001000010000000001011000011000000000100000011?010000000001 1??01100100???011001100001110000000100000??111
000000000??0001?001000101001 0000?100000101?101000110010 1000?011011110000101100001 1?10000010101000101101001000000000z000000000100
0100000110000z1000000000100001110000000001010100001011100011100011200000001110??1???0?000000000000??0?00????????000??10z000000101000z
?0?010????????????????????

Axelrodia lindeae

?00110010?z001100010000111010010000100000001?0110000110000000 0?010010?1?0100000000010000110 10?1000011001100000010000000000 00??1??
110011110000011?010000101000000000z0000[12]0111010?011001001000010001110000101000001001010001010100010 11?1001000000000000000010101
000000000110000110000100000000111z010000010101 1z101011100011000 01???000000100??1z??010000000000?01?0?00????????000??000000001001 00
010??010????????????????????

Bario steindachneri

?00110010?1001100010010?110000100001001010000011z000110000000000100z0?1?0100000000 1100?01100??100001000100000101000000 1000100011
0100001z001z0001?000100001000000000000000101?0010001100101000?010001100000000110000100100000101010001010010000000000000000000101010
00100000110000110000100010000111000000000101010000111111110 1120z111100001110000 11??0?00000000z0?0??0?0?????????000?1000000001101 00
0z0z?000????????????????????

Bramocharax bransfordii

?0?111010?10011000100000110000100001000000000000111000110000000000001000001?0100000000 0100?01100??1000?10001000001z1000000 1000100011
0z000011111100 01?0000000011000000000000010100010001100101000?010001100000001110000100100000101010001010010000000000000000000101110
0010000011100011000010000010011110000000010101000010 1????????001120000000100?011??000000000000?0??0?00????????000?1000000001101 00
00?0?010????????????????????

Brycinus carolinae

???01??111?001???0?0011?1100000?000121?1?100?000?0?0110001000?000000000101001000?11????0?1100?0???21?00010101011100000100010?02000
00000000??0000100000000000001000?100000201?101?0?000?1??100?11???1000000???1000010010??000?111?000000?00000000 1000100000010???0001
10000?00000111000000011000001000000001111?000001010?????????00010100000011 1??11??00?000000000 10??0?0????10000000??0???????01100100?1
?010????????????????????

Brycon falcatus

?010100z0?100z10001000011100001 0?00100?100000001?0?011000000???01000000111101000010000?00100111000?10000?000?011000000100011?0110
10100111110000010 00z00001?000000?000000200?001?0000001??100?0100010000100111000010010000000111000110000000000010001000000??11000
0100000111000110000100111000111000000000101010000111?????????0001200000101000?1?????0000000000?0??0?0????11000001??000000001101000
000?010????????????????????

Brycon meeki

?0?0100z0?100010??10000111000010?00100?100000001???010?000000?0010000000100101000??0000?0?100??1000?100000000?011000000100011?01?0
10?0011111000001000??0?01000?000?00000020??001000?000111100?0100??0000?001?10?0010010000000111?00z100??0000000?0001000000001?0000
10000011000011000010011 1?001111000000011101000011??????????00????0000?01??0?????000000000000?0??0?0?????????00???00???0??1101000 0?0
?010????????????????????

Brycon orbignyanus

?01010000?10011000100001110000100001 00?100000000?0?0z000000001001000000010z101000010000?00100111000?1000000001011000000100011?111
0111001111110000100000000010z00000100000020000010000100111100?0101110000100111000010010000000111000110000000000001000000000000001100
0010000001110001100001001110001 11z00000001010100001 1??????????0001200000?0100001100000100000000?0??0?00???11000001??00000000110100
0000?010????????????????????

Brycon pesu

?0?0100z0?1001100010011?11000010?0010011010000011000110000000100000000010z1010000?0000000100111000?1000000001011000000100011?011
0101001111100000 1000z00001z000000z000000200000 10000z00111100?0100011000100 1110z0010010000000111000100000000000010001000000000 10000
010000011z00011000010011100011100000000101010000 11?????????000120010010100?01?1?000000000000 10??0?0000?110000011?000000001101000
000?010????????????????????

Bryconaethiops macrops

??101000111001??001001z?1100000?000121?10000?000?0?010000100??00000?0001?1001000?1100??0?1000?0???2100001010101110000 0100011?02000
00000000??000010000000000001000?100000201?10100?000?1??100?11?111000000??110??010010??0001111?001?00?000000001000100000 0z01000001
110?000000011100000001 00?000110000000111100000 01010?????????000101000000 1110?11??00?000000000?0??0?0?????????00???0???????01?00100?1?
010????????????????????

Bryconamericus agna

?00110010?0001101010000001100001100010000000001011000011000000000100000011?0100000000011??011001010z0010001100011110000001000100111
0000001100110001?000000001000000000100000101010100011001010000010111110000001100001001000000101010001010010010000000000z00000001010
0010000001100000100000000100001110000000001010100001011100000000001120000000110001110000000000000000100000????????000?10000000001010
000?0?0101001000000000z01????

Bryconamericus alpha

?00110010?z0011010100z0?110000110001 0000z000101100001100000000100000011?01000z0000011??011001010000100010000111100000001000100z110
000001100110001?0000000010000000001000001010101000110010100000101111100000011000010010000010101000101101001000000000000000000 1010
0010000001100000100000000z0000111z00000001010100001011 1000z00000112000000z11000111000000000000000?0??0000????????000?100000000110100
0000?010????????????????????

Bryconamericus cf. *iheringii*

?00110010?000110101000000110000110001000000000101100001100000000010000001 1?01000z0z00011??011001010000100011000011100000001000100011
00000011z0100001?000000001000000000z0000010101010001100101000001111110000000110000100100000101010001010010010000000000000000001010
001000000110000010000000001000011z000000001010100001011111011000011200000000110001110000000000000000100 0000??11100000?100000000z1010
00000?010????????????????????

Bryconamericus cf. *rubropictus*

?00110010?0001101010000111000011000z0000000010110000110000000001000000 11?01000z0000011??011001010000100011000011100000001000100011
0z00001110100001?000000001000000000z0000010101010001100101000001111111000000110000100100000101010001 01z0100100000000000000000001010
0010000001100000100000000100001100000000001010100001011100011000011200000000110001110000000000000100000 00????????000?10000000001010
00100?010????????????????????

Bryconamericus mennii

?00110010?000110101000000110000110001000000000101100001100000000010000001 1?01000z0000011??01100101000010001 0000z111000000 01000100011
00000011z0100001?000000001000000000100000101010100011001010000010001110000001100001001000000101010001010010010000000000000000001010
0010000001100000100000000z000011100000000010101000 0100?????????00112000000011000111000000000000000??0000?0??????000?100000000z10100
0z00?010????????????????????

Bryconamericus rubropictus

?00110010?0001101010000z11000011000100000000010110000110000000001010000 11?01000101?0011??011000?1000010001100001110000000 1000100011
00000011z0100001?000000001000000000z00000101010100011001010000011 1111z0000001100001001000000101010001011010010000000000000000001010
0010000001100000100000000100001100000000001010100001011111011000011200000000110001110000000000000001000000 0????????000?10000000001010
00z00?010????????????????????

Bryconamericus scleroparius

?00110010?100110101001O?11000010000100000000010?0?0100000000000000001?0110000000010000110010100011000100000111000000100010z011
0000001100110001?000000001000000000000002010001000110010100000100011z000000110000100100000010101000101001000000000000000000101110
0010000011100011000010001010011110000000101010000011??????????00112000000010000111000000000000?0?00?00????????000?100000000110100
0000?010?????????????????????

Piabina thomasi

?00110010?000110101000z11000011000100000000101100001100000000100000011?0100010000011??011000?10000100011000011100000010001000110
00000011z0100001?000000001000000000z000000101010001100101000001111111000000110000100100000010101000101001001000000000000000001010
0010000011000001000000001000011z000000000101010z00101110101100001120000000110001110000000000000z0100000?????????000?100000000z10100
0000?010?????????????????????

Bryconexodon juruenae

?001100z??0z00100010000111000010000100101000001110001010000100101000001?011010000001000011001010007?10001000000z10000001010???????
???00111100000000??000010000000000000001010001000110010010000100111110000011100001001000001z1010001010?100000000000z00000101?100
0100000011100011000010001000011100000000101010000111?????????00112000000010??1???0??000000000??0?00????????000??000000007?010000
001010?????????????????????

Bryconops affinis

?01010010?100110001000011100001000010011000000011000110000001100100100001110100001010000011007?10002100010000000100000010001007?01z
01000011001z0001?000100001000000000000002011001000010011110010100011100000111000010010000011101000110000001000010001000000001000
0010000010000011000010011000011110000000101010100117?????????00112000000010007?01???0?000000000?00?0?00000100000007100000000110100
00z0?010?????????????????????

Bryconops melanurus

?01010010?100110001000011100001000010011000000011000110000001120100z000011101000010100000110?10002100010000000100000010001001110
01000011001z0001?000100001100000000000002011001000010011110010100011100000111000010010000011101000110000001000010001000000001000
0010000010000011000010011000011110000000101010100117?????????00112000000010007?01???0?000000000?00?0?00000100000007100000000110100
00z0?010?????????????????????

Carlana eigenmanni

?00110010?z0011000100z00110110100001000000000011100010000000000000001??11?0100000000100001101O?100z?1z10110000001000000100000??111
1000011zz111101?0000z0001zz000000000000101100100011001010000010111110000001100001001000001O1z1000101z??000000000000000000010111000
10000011z0101100001000101001111000000010101001000001z1110000000001???000000100??11??0?0000000000?00?0?00????????000??00000000z10100?0?
0?010?????????????????????

Carnegiella strigata

??01????0?00010?1100011?1100000??0010001010000007O?011001????1?00?1????1?0?????0000??00?0???10?100001000111000001000000100000??11011
000100000000001?000101?01000100000z000020101010?0000010100001000111100?000100100100100000000?100010100000??001100?00?0111????010000
00101100000000110000011001011z00000201010z000000110000???000110101000010001111000000000000100?0?10???11000000?100000000100000?1??
?000?????????????????????

Chalceus macrolepidotus

?01110010?100110001001O?110100100001Oz1100000000?0?011000000000100000001111100000100000001100?1000?100001000101100000010001?011
0100001111100000100000000100010000000000201?001000000011110O?11000110000000110000100100000011110000000000000000010001000000101000
0011000010000011101011011100000000000000111100000001??????????000101010000100011100000000000000?0??0?00???111z0000??000000001101000
00?1?010?????????????????????

Characidium borellii

??1010011OO??1O?0010011??0???10??001001100000000110001100010000??0100?0?0001?00?0000000??100000?1???0110011000001O?00000100000??????
??0?0000??0001?000010001000001000010002010101000000001110000110111111007010100000100110000007100000001000110000?001001000010100000
1100000010001000001000100000000000002110101000000101000000?00112000000000????111110000000000011000?00???11000000??000000001001007
010?010?????????????????????

Characidium rachovii

??1010011OO??1O?00?0011??0???10??001001100000000110001100010000?01?0???0001????0000?00??11??10?1???011001?00000z0?00000100000???????
?0?0000??0001?000010101000001000?10002010101000000001110000110111111007010100000100100000007z?1000001010001000?001001000010100000 1

1000000100010000011101000000000000002110101000000?0?????????0011200000000??0?1111110000000000011000?z0???11000000??00000000000100?1?
0?000????????????????????

Charax stenopterus

?z0110010?01011000000001110000100001000000000011z?00000000000002010z1011?0100000010010000011000?1000?1000110000001000001000?1???????
???001111000011?000011?011100000?0100001011001 0?010001001000010001110001011100001011010101011100010110111000000000000000001011100
0100000110000110000100000101111110001001010100001011100000?0010??0?10001001111100100000000000000?0?00???????000??000000001001000
100?010000001000??????1????

Cheirodon interruptus

?00110010?0001101010010?1101001?00010000000000110000110000000010000101 1?0100000000?1000011010?1??101100110010001000000110000??11
1100001000110001?000010101000000000z010001010101 0?01110101000z01z111100000001100001001000000101010001011010000000000000000000101010
00000000111000110000100010000 11z000000001010111101011100011000011200000001000?111001000000000010100?00000????000??0000000001010
001z0?0100z01000001111111????

Coptobrycon bilineatus

?00110010?000110101000001100001001?10000z0000010?0?011000????????00???1?0??????1?00???00?1010?1???010001??0000z1000000100010?011100
0000000??0001?0000001010000000z00000101010 10?011001000000010z111z00001011000010010000010101000101z?100000000010000000001010?00000
0000111000010000100010000110000100001010100001z0?????????001???0000001000?11??0??00010000001??0?10????????000?100000000010100?10z?
0z01???0?0??1111???????

Cyanocharax sp.

?00110010?0001101010000011000011000100000000101z000011000000000010000z0z1?0100000000011??011010?1000010001000001z10000001000100 0110
z000011z0z00001?0000000010000000010000010101z10001100101000101 0z1110000000011000010010000010101000101001 00??00000000z00100zz101000
000000110000010000000 0z0011111z000000010101000010111000110000112000000010000111000000000000010000000????????000?1000000001101000z
10?010????????????????????

Cynopotamus argenteus

?100100z0?011110000000011100001000010100000000 10?0?110100000000201000101?010010001001001011001 11000?100010000000010000100001???????
???001111000001?000011?0111000001010000100000110010011011000010011 1z01?1011100001011010101011100011 1101z100000000000000001011100
01000001100001100001000 00z0111111000100101010100 10??????????2010??00001010011111000000000000000?0??0?00???11100000??000000001001000
000?010????????????????????

Cyphocharax spilotus

?01110011100010?0010000110???10??001010100000000?0?0110001000?000z01000001z000000?00000000000?1???0010011000001 0?00000????????????
????0000?????1?????0010000000000000100000111010000100001100?111111??00?0101111110001 00000000?1000001000001000010010000000101000001
1000000100011100011011010000000000000111101000001 0?????????0000??0000000??1?100?00000000000 2?0??0?0????111100?0??0?000000??0100?100
?000????????????????????

Distichodus maculatus

?010100110z0011?0010010?10???10??00131010000?000???0110001000?00000100000100?????000000000000?1???0000?01?000?01?00000??00100?????
???00000??00100000000001000000 10?0000000001101100000011110011101110000001011000010010000000z?00000100?10010000100100000000????0?001
110?000100010101011111110010000000001100110000000?????????1000??0000010??1?001100000000000010??0?0?????????0?0??0?000?001???00?000?
010????????????????????

Engraulisoma taeniatum

?01010010?00010?z0?00z001100000?000101010000?000?0?0110010010?00001??00000000000010z??00?1100?1???0100010100000100000 0100010?01100
00000000??0001?000100101002000001000002010101000000010110000101111100?0??10010011?000000?10?1000z011010010000000000000011?????1000
00110110000010000000010010011000000000001000000000??????????00110101000011 00?11100000000000000010??0?00????????000?100000100110100?11
??010????????????????????

Exodon paradoxus

?00110010?z001100010010?1100001000010000000000011100010000000100201000001?0110100000001000011001010 00?1000100000000100000000010???????
???00111100000000000?01?0111000001000000101000100011001 00z000010011110000001110000100100000101010001010010000000000000000000001010100
0100000101000110000100 0z010011100000000010101000011 0?????????0001200000000100001111000000000000?0?0??0?00???11100000??000000001001000
000?000????????????????????

Galeocharax humeralis

?z0010010?0101100000010?1100001000110110000000010?0?0101001000020100010 1?0100100010010z10110011100 0?1000100000001000010000 1???????
???00111100000000000011?0111000001010000100000 1z0010011001000010011 1z01?10111000010110101 0z01110001z11011z0000000000000000010111000
1000001100001100001000000101111110000001010101001111100000002010??0000101001111100?000000000010??0?00???11100000??000000001001000
000?010?????????????????????

Grundulus cochae

?0011011??000110101000001100001100010000000000010???01100000000??100???1?01?0??01?00????0????10?1000?10001100000z1000000000000??1??1
100?11110000z1?000010101000000?000000101?10100011001010 00?0101111z0000101100001001000001010 1?00z0100100000000010000000z01010000
000001110001100001000110001100000000001010100001 00?????????0011200?1000100??1???0?000000000001??0?1?????????000??000000001001 00?000
?0z0?????????????????????

Gymnocharacinus bergii

?0011001 0?0011?010z000001100001100010000000000010?0?01100001000??1?0???1?010001000?0?00001100??1000010001100000110000001100100111 1
000001100110001?000000101000000000000000010100010001100100100001011110000010110000100100000100101000001001000000000100010000011010
000000000111000110000100011010110000000000101010z00100?????????001???0?10001100?111000000000000?0000?10????????000?1000000000101 00?
00??010?????????????????????

Gymnocorymbus ternetzi

?00110010?1001100010000011100001000010010z000001110010000000000001001011?0100000000010000110010 1??0010001000000010000001000100011
0100001000100001?0011001010000000000000000101?0010001100101000?0100011000000111000010010000010101000010100100000000000000000010101 0
000000000111000110000100000100111111000110z01010000101?????????0011200?10111000011100000100000000z0??0?0????1zz00000?100000000z101000
00z?000?????????????????????

Hasemania nana

?001z0010?1001100010000011000010000100000000001110001100000000000001??11?0100000000010000110 10?1??0010001000000010000001000100011
010000z000??0001?0000101010000000000000000010110010?0110010100010100011z0000001100001001000001010100010110100000000000000000001000 10
000000000111000110000100?1000011000010000101010000100?????????0011200000001000?1110000000000000?0??0?z0???1z000000?100000000100100
1100?00000010?0000000??0????

Hemibrycon dariensis

?001100z0?z0011010100000110000110001000000000011000011000000001000000 11?0100000000011??0110010100001000100000111000000100010zz110
000001110110001?000100001000000000000000010100110001100101000001000111000000110000100100000101010001010010010000000000000000z010100
0100000110000010000000010000111100000001010101000010 1?????????0011200000001000011100000000000000?0??000000??????000?1000000001101000
000?010?????????????????????

Hemigrammus bleheri

?00110010?z001100010010?1100001000010010100000111000110000000000010010 11?0100000000010000110 10?1??0010001100000110000001000100011
110000z000100001?0000001010000000000000000010110010001100101000001000 11z0000001100001001000001010100010100100100000000000000001010100
001z0000111000110000100000000110000000001010100001011?????????0011200000011000011100000000000000?0??0?00???11000000?100000000010100
0000?00000010?0?01111??0????

Hemigrammus erythrozonus

?00110010?1001100010000111000010000100001000001110001100000000000101??11?01000?00000100?011010?1000010001100000110000001000100011
11000011z0110001?0000001010000000000000000010110010001100101000001 00?1110000001100001001000001010100010110100100000000000000001000100
00z0000011100011000010000000011z0000000010101000010111010z01000?12000000z1000?1?1000000000000000??0?000001zz00000?100000000110100
?11z?000?????????????????????

Hemigrammus ulreyi

?00110010?1001100010000111000010?0001001010000011100010000000000001001011?0100000000010000110110100001000100000001000000100010001 1
01000011z0100001?0001001010000000000000000010110010001100101000001 00011z0000001100001001000001010100010110100100000000000000001010100
0010000011100011000010000 0z0011100000000101010000101110000010001120000007100001110000?0z000000?0??0?00?????????000?100000000110100
010z?000?????????????????????

Hemigrammus unilineatus

?00110010?1001100010000z11000010000100?010000011z00010000000000001001011?01000000000100001101 0?10000100010000000100000010001000 11
01000011101z0001?000z0010100000000z00000101?001?001100101z00?0100011z00000011000010010000010101000101 10100z00000000000000001010100
01000001110001100001000100001111 00000?0z010100001011100000000001120000001100001 1?????001000000000?0?00???11100000?100000000110100?
0?z?0z0????????????????????

Hemiodus cf. *thayeria*

?01010011100011?00100001110001 0??001001100000001100011000100000000100000001111000011000000 1000?100z0010011000001 0?00000100000?????
???1?1110110??00????00101000000000000000200010100001000111000110111000000101100001001000000111100001000000010000100100000001010000
01100000000001110001001110000000000000111011000001 0?????????0001000000000????10110000000200001 0??0?00???111100?0??00000000??01000
000?010????????????????????

Heterocharax macrolepis

?00110011010011000100001110000 10?0010010z00001111000100000001000000001001??000100001 0?01101100111000?1000110010001000000000 0???????
???00111100000000000?00001110000000010000[12]00?00000z0z001111000110001100000001110000100100000111011001010?1001000010001000000000101
000100?001100001100001001101001111z00000010z01000010?????????001???0000001000?111000?000100000100?0?00????????000??00000000?00100
?0z0?000????????????????????

Hollandichthys multifasciatus

?00110010?zz0110001000001100001000010z0000000010?z?0100000000120101??11?0100000000010000110 10?1000?10001000000z1000000100010001 10
1000011111000 01?0000z1?01110000000000000101100 10?01100100100z0100011z000z0111000010010000010 1z1000101011000000000000000000010111010
00000011z000110000100010100111100000000101010000101110001000000112000000001 00?011??0?0000000000000?0?0011111000000?1000000001001000
100?01000??????00000???????

Hoplias cf. *malabaricus*

??101?010????10?0010011?01000000?01101110100?000???01101???0010000000?1???000?00010000000000 0?1000?100010000000100000000 00????????
??0011110000000000001?011?000110010000?0000010?00001111100?11?11100?0110111000010 1110??0000?10000z000?0000000?11?0110000?01100001
0001000000001000001110100000000000002100000000000?????????0001010100001000?1101?000000000001 0??0?1000?00000000??0000?????001000??
0?010????????????????????

Hoplocharax goethei

?00110011010011000100z0111000010?001001zz00001111000100000000000001??11?0000??00010?00?01101101000?1000110010001000000000 0????????
??0011110000000000?000011000000001 0000[12]0??000000zz001001000110 1111z0000001100001001000001z1011001011??0z1000010000000000001 0100
00000001100000100000001100001 11z00000001010100101 0??????????001???0000001001?111000?000100000100?0?00????????000??00000000?00100?0
1??000????????????????????

Hyphessobrycon anisitsi

?00110010?10011000100000110000100001 0000z000001100001000000000001001001?01000000000100001101 0?1000110001000001z1000000100010001 1
0100001z001z0001?0000000010000000000000001010001000110010100000100011z000000110000100100000101010001010010000000000000000000101010
00100000111000110000100010100111000000000101010000101110000000000112000000001000011100000000000000000?00???11000000?10000000001010
000?0?000????????????????????

Hyphessobrycon bifasciatus

?00110010?100110001000001100001000010000000000 1z000010000000000001001011?01000000000100001101 0?1??01100011000000100000010001001 11
0100001000110001?0000001010000000000000001010001000110010100010100011z0000001100001001000001010100010110100000000000000000000101z10
00100000111000110000100000100111100000000101010000101110000000000112000000001000011100010100000000000?00???11000000?10000000001010
00000?010????????????????????

Hyphessobrycon elachys

?00110z10?10011000100000110000 1001?10000z0000011z0001100000000000100???1?01?0??01?0?1000011010?1???010001??00000100000010001000 1101
00000000??0001?000000101000000000000000101100100011001010000010111100000101100001001000001010100010110100100000000000000010001000
0000001100101100001000z000011z000000001010100001 00????????00112000000001000?10100?2000000000?0??0?00????????000?100000000z10100??0
1?0000000000000010???????

Hyphessobrycon eques

?00110010?1001100010000111000010000100001000001110001000000000000100z1011?0100000000110000110 10?1000010001100000010000000100010001 1
01000011101z0001?00001010100000000000000101100100011001010000010001100000001110000100100000101010001011010000000000000000000101z10

0010000011100011000010000000011110000010z0101000010110000???2001120000000100001110011001000000000?00?00???1zz00000?100000000110100
0100?010????????????????????

Hyphessobrycon herbertaxelrodi

?00110010?1001100010000011000010000100001000001110001100000000000010010?1?010000000001000011010?1000010001000000010000001000100011
0100001110100001?00010010100000000z0000010100010001100101z00001000111000010110000100100000010101000101101001000000000100000100010
0010000011z0001100001z0000z001110000000001010100001011100000100011200000001000?1?10000000000000000?0?00???11100000?100000000100100
011??0z000000000000000001????

Hyphessobrycon meridionalis

?00110010?100110000000000110000100001000000000001100001000000000000100???1?01?0??01?001000011010?1000110001100000010000001000100111
0100001100110001?000000101000000000000000101000100011001010000010001100000001100001001000000101z10001011010000000000000000000101010
00000000111000110000100010z001111000000010101000010110111???2001120000000100001z1000000000000000000?00????????000?100000000z10100
0100?010????????????????????

Hyphessobrycon megalopterus

?00110110?1001100010000111000010000000000000000111000100000?00011001???1?01?????1?011000011010?100??10001100000010000001000z0?????
110001111100011?0000z0101000000000000000010110010?01100101000001000110000z01110000100100000101z100010110100000000000000000000101z100
0100000111010110000100000100z1100000010100110000100?????????00102?00000010000111001?00?00000000000?00???1zz00000??000000000100100?1
1z?010????????????????????

Hyphessobrycon pulchripinnis

?00110010?1001100010010?1100001000010000z0000011z0001100000000000001??01?01z000000001000011010?10000100011000000100000010001000110
100001100110001?0000001010000000000000001011101000110010100000100011100000011000010010000010101000101z01001000000000000000001010100
0100000111000110000100000000111z000000010101000010??????????00112000000001000?1?1000100100000000??0?00???1z000000?1000000001101000
01??000????????????????????

Hyphessobrycon socolofi

?00110010?100110001000001100001000010000z0000011100z1z00000000010001011?010000000001000011010?1000010001100000010000001000100011
0100001110100001?0000001010000000000000010110010001100101000z010001000000001100001001000000101010001011010000000000000000000101010
0010000011100011000010000000011110000010101010000101111110102001120000000100001110011001000000?0??0?00????????000?10000000011010
000???0000000000000000001????

Iguanodectes geisleri

?01010010?1001100010010?11000010000100110000??11100011?000000?000001010011000000001000000?100??1???2000?11000101100110010001000110
100000000??0001?0000?0110000?100?000001201?10100001001?1100?010111110000?0?00z0010010000011101000z1z?0?00110001000z000?z0001000001
000001100001100001000110?00110000010010101000011?????????0000??0000001121?11100??000000001?0??0?00????????000?10????0??0101000000
?010????????????????????

Inpaichthys kerri

?00110010?100110?010010?11000011?00000000000001101001101?0000010001???1??100?0000001000011010?1000010001100000z1000000100000??1??
1z0011110100001?0000001010000000000010001011101000110010100000100011100000011000010010000010101000101101000000000000000000001010100
000000011z0001100001z001000011100000000101010000100?????????00112000000001000?1010000000000000?0??0?00???11100000??000000000100100?
110?010????????????????????

Jupiaba mucronata

?00110010?10011000?000011100001000010010z00000111000100000000000001001011?01000000000100001100101000010001000000z10000001000100111
01000011z0110001?0000000010000000z000000101000100011001011000010??11z000000110?0010010000010101?001010010000000000000000000101010
001000?1111000110000100000z00111z000000010101000011??????????00112000000000100??1???0?0100000000?0??0?00????????000?1000000001101000
000?0100001000000000000001????

Jupiaba scologaster

?00110010?100110001000011100001000010010000000111000100000000000001001011?010000000001000011001010000100010000000010000001000100111
0100001110110001?000z000010000000zz000001010001000110010010010100011100000z1100001001000000101010001010010000000000000000000101010
001000?111100011000010000010011100000000101010000100?????????00112000000001000011100000000000000?0??0?00????????000?100000000110100
0z00?0100000000000?????????

Knodus breviceps

?00110010?z001101010010?1100001000010000000010110000110000000010000z011?0100000000011??01100??1000010001000011110000000100010z0110
000001100110001?00000000100000000100000101?1010001100101000?010111110000001100001001z0000101010001010010010000000000000000000100
0100000110000010000000001000011z0000000010101000010111000z000001120000001110001???0?0000000000?0??00000??11100000?1000000001101000
1?0?010????????????????????

Knodus heterestes

?00110010?00011010100z0111000011000100000000101100001100000000100000011?01000z0000011??01100??10000100011000z111000000010001001110
000001100110001?000000101000000000100000101?1110001100100100?0100111100001011000010010000010101000101101001000000000000000000100
0100000110000010000000001000011z00000000101010000101110?0z0000????????001110?01???0?000???000?????0000????????000?10000000011010001
00?010????????????????????

Knodus meridae

?00110010?0001101010010?11000011000100000000101100001100000000100000011?01000z0000011??011001010000100010000111100000001000100z11
0000001100110001?00001000100000000100000101z101000110010100010101111100000011000010010000010101000101z01001100000000?00000000010
0010000011000001000000000000011000000000101010000101110001100001120000001110?01???0?000???000???0?0?0001??????000?100000000z10100
01?0?010????????????????????

Leporinus striatus

?01010010?00010?0010100111000100001001?100000000???011?001000??01001000001101000?110000000000?1???0010011000???0?00000?000????????
??0?0000??0001?00?0000100000000?0?z0?0000?1010000100001100?1101111000101011011000000000?0000?10000000000010000?0010100000101000001
z0000001000111000110010100000000000001111010000010?????????000101000000???1?1001000000000002?1??0?00???11110000??0????0000??00000?0
?010????????????????????

Lonchogenys ilisha

?00110011010011000100001110000z??001001000000111100011000000000000001000000001000010?00101100111000?10001100000010000000000???????
???0011110000000000?100011000000001000010?100000001001111000110001100000011100001001000001z1011001011?00010000100000000000000010100
01000001100001100001001101001111z00000010101000010??????????001???0?10001001?1z1000?000100000100?0?00????????000??00000000000010000
00?000????????????????????

Markiana nigripinnis

?001100z0?100z1100100000110000100001010000000010?0?0z0100000000100000001?0110100001010z00z100101???21000100001111000000010001000110
000000000??0001?000000001000000000000000101010111011001010000010001100000001100001001000001010100010100100000000000000000001011000
010000011z0001100001000101001111100100010101000011110000???00110??000011110001100000000000000000?0?01???11100000?1000000000101000
010?0100001000000000???????

Metynnis maculatus

?01110010?1001010010000z110000100001010100000000?0?1000000000100101???000100000000000000001000?1???21000100001011000000100010000??
001000000??0000110000001010000000000000?10?1010000?001001001010111100010001000001001000000001110011011000000000100000000001010000
000000010100010000111111010011111?0002010001000001??????????0000??0?11101110?1?010?000000100010??0?0000?11111000?10?000?00??110000
?0?010????????????????????

Micralestes stormsi

?01110011110011?0010010?1100000?000101110000?000?0?01100010000000100001?01000100011z000001100?0???2100011010111100000010001001110
000000000??00001000000101000100001000002010101000010010010011100111z000000110000100100000011110001010000010000100010000000010000
0100000000000111010000010100010000000111100000001010????????0001010000001100?1100000000000000?0??0?00????????000??z?000?0001110000
11?010????????????????????

Microschemobrycon casiquiare

?00110010?0001100010010?11010010000100000000z0011z000110000000001000000011?0100000000011??011010?100001100110000001000000100000??1??
110011110100011?0000001010000000001000001011101z0011001010001010011110000001100001001000001110100010110100100000000000000001010100
010000011100011000010000000011z0000000010101000010??????????00112100000z100001110010000000000?0??0?00???00000000??0?0000001001000
00??0z0????????????????????

Mimagoniates rheocharis

?00110010?z00110?0?000001100001??0000000z0000z1100001100000001100z?011?010?00?000?1???0?101??1000010001000001010000000100010001101
000011101000001?000000001000000000z0000010101z10?01100101100?0100011z00000011000010010000010101000101010?100??000000000000100001010z00
0000011000010000010001100111110000000101010000101010011110?00112000000?1000?11??0?0000000000000010000111????000?1000000000100100?1?
0?0100001000000000??1????

Moenkhausia cf. *intermedia*

?00110010?10011000100001110000100001001z00000111000100000000000001001011?0100000000010000110010100001000100000000100000001000100011
0100001z00100001?00010010100000000000000010110010001100101000101001110000000011000010010000010101010101001001100000000000000000100010
0010000011z000110000100000z001110000000001010100001z0?????????00112000000110000111000000000300000??0?00???11000000?100000000110100
000z?000????????????????????

Moenkhausia dichroura

?00110010?100110001000011100001000010010100000111000100000000000001001011?0100000000010000110010100001000100000000100000001000100010
010000zz00100001?000100101000000000000000101000100011001010001010011000000000110000100100000101z1010101000001000000000000000000101010
00100000011100011000010000z0100111z0000000101010z00110?????????00112000000110000111000000000300000??0?00???11000000?100000000110100
00?0?0100001000000000???????

Moenkhausia sanctaefilomenae

?00110010?10011000100000110000100001000z0000011z0001100000000000000z011?0100000000010000110110100001000100000000z1000000100010001 1
0100001z00110001?000100101000000000000000101?00100011001010000?010001110000000110000100100000101010001010010000000000000000000101010
00100000011100011000010000z00001110000000001010100001z110000????0011110000011000?1110000000000001000??0?000001z000000?100000000z10100
00?1?0000??1000000000001????

Moenkhausia xinguensis

?001100z0?1001100010000111000010000100101000001110001000000000000010010 11?01z0000000z10000110010100001000100000000100000001000100011
0100001z00110001?000100001000000000000000101000100011001010001010001100000 0z1100001001000001z1010001010010000000000000000000101010
00100000101000110000100000100111000000000101010000111100 00????00112100000110000111000000000000000000?0?00????????000?100000000110100
000z?000????????????????????

Nematobrycon palmeri

?00110010?z00110001 0010?1100001100010000 10000z111000100000000000000001??11?0100000000?100001100??1000?10001z000011100000001000100 0110
0000?1111100001?0000001010000000000000000101?101000110010??00?0100111z000010110000100100000101010001010010000000000000z000001010100
010000011000011000010001000011110000000010101000010 0?????????001???0000?0100??1???0??00000000?????0?1000011000000?1000000000110100?1
00?010????????????????????

Nematocharax venustus

?00110010?10011000100001110000100001000000000001110001000000000000001000011?0100000000010000 11010?1000?10001000000z10000001000100111
110000111111?001?000000101zz00000000000001011001000110010110000100011100000001100001001000001010100010100100000000000000000000101010
0010000011101011000010000000 0111z0000000101010000101100z0?? ?0001z20000001100??11??000000000000?0000?00000????000?100000000110100
0z000010zz000100000z1001????

Odontostilbe microcephala

?00110010?0001101010010?11010010000100000000000110000110000000000100001001?0100100000001000011001010010110011001000100000001100 00??11
11000011001100 01?00001010100000000010100010101010001110101000101011110000000011000010010000010101000101101001000000000000000010101
0011000000111001110000100010000111000000000101010000101110000100001120000000100001110010000000000000100?00???11100000??00000000z101
000000?010????????????????????

Odontostilbe paraguayensis

?00110010?0001101010010?1101001000010000z0000011000010000000000100001001?0100000000110000110010100101100110010001000000 0110000??11
1100001100110001?0000z01010000000001010001010101000111010100000100011100000001100001001000001010100010110100100000000010000010 1010
0110000001110011100001000001001110000000001010100001011100001000011200000001000010100100000000000z0100?0000011100000??00000000z1010
000?z?0000100000001111111????

Odontostilbe pequira

?00110010?0001101010010?1101001?00010000000000110000110000000001000010 01?0100000000010000110010100101000110010001000000110000??11
1100001100110001?0000001010000000 0z0z000101?10100011001010000010001100000000110000100100000101010001011010010000000000000000101010

0110000011100111000010001000011100000000101010000101110000100001120000000100001110010000000000000100?0000011100000??00000000z1010
00010?0100z00000001111111????

Paracheirodon axelrodi

?00110110?1001100010000111010010000100001000001110001100000000000100???1?01000?0000010000?1010?1??1010001100000010000001000000??11
1100001000110001?0000001010000000000000010111010?01100101100001z00110000000110000100100000101010001010010010000000000000000100010
0010000011000011000010000z000001z000000001010100001O1?????????00????000000100??1?100?2000000000?0??0?00?0?1zz00000??00000000010100?
00z?00001010000002111111????

Paragoniates alburnus

?00110010?0001101110010?110000??1000010000?0??10?0?01000??1?001?00000?1?0100011000?100001101101000?100?11?0000010000001000z0??1??1
000?1111100001?000000101000??00?0000?0101??0100?11001010?0?0100??1000000011000010010000010101000101???0010001000001000zz????00001
00?00110000110000100011001111110000101010100001OO????????001???0000101000???100??000000000?0??0?00000?????000??0000?0??10010000?0
?010????????????????????

Parecbasis cyclolepis

?00110010?z001100010010?110100100001000000000011100010000000000100000001?0100000000110O?01100??1???0110011001000100000010000O??1??
110010000??0011?00000010100000000000000101?001000110010100O?0101110000000011000010010000011101010101001001000000000000000001010100
010000010z00011000010000010011100000000z0101000010?????????00112000000011000?11100?0000000000?0??0?0?????????000??0?000000000100OO
00?010????????????????????

Parodon nasus

??1010001101010?1010111?10???10??0011101010000000?0?0110001000??000000001010000000011000?000000?101000100110000010?100001?00??????
???1?1100110????????00001000000000010000200110 1?000000001100011011100 10?010110100100100000000?1000000010001100010000000000010000000
1000000010001110001 10z1z100000000010011010100000 10?????????000101010 0000????1011000000000000110??0?0????111100?0??0????0??0?010000z0
?010????????????????????

Phenacogaster tegatus

?00110010?000110001000011100001000010000000001110000100000000020100101 1?0100000000001000011010?1000?10001100000010000001000100011
0110001111000001?0000001010000000 0z100001011001000110010100000100011100010111000010010100010111000101101000000000000000000101010
0010000011000011000010001010111111000000101010000101101 00???00011200000001000011100100000000000?01?0?00????????000?100000000100100
0000?010????????????????????

Phenagoniates macrolepis

?00110010?00111011100z0011000010?00001000010?z10???010000010001000000 11?0100001000010 0?01101101110?100?101000001001000100000??111
1000?1111100001?000000111000000000000000101?1010001100101000?010001110000001100001001000001010100 0111??100100000000000100000??10000
0000?00110000010000000011001111110000101010100001 0??????????0000??0000?01100?11?000?00000000010??0?10????????000??0000000010010001
?0?010100001100000000 1????

Piabucus melanostomus

?11010010?10011000100z00110000100001001100 00?1011000100000000?00010101000100000000101000011001010112000?1100010110001001000 00??11
010000110011000 1?0000001100001100000000120101010 00z10010010010101111000000001000001001000001010100001010001100011000100 0011??1z00
00100010110000010010000011z111111100010010101000011110000???00000??0000001121?10100000000000100010?0?????????000??0000?000110100
0010?010????????????????????

Piaractus mesopotamicus

?01110010?10010100100z01110000100001010100000000?0?0z0000000010010000000011010000100000000100101???21000000001011100000100010000??
001000000??00001000000000100000000010000210010100 00?00100100001011100001001100100100110000001110011010001000000010000000000 1011000
00000001100001010001111 1z1001110000000201000100000 1??????????0000??0?11101000?1101000000001000?0??0?00???11110000??00000?000?110000
?0?010????????????????????

Poptella paraguayensis

?00110010?10011000100001110000100001001010000011100100000000000001001011?01000000000100001100101000010001000000010000001000100011
01000011z01z0001?00110000100000000000000101000100011001 01z00101000110000001110000100100000101010001010010000000000000000000101010
000000001011001100001000000100111100000101010100001111101000020011200000z11000011100000z000000010000?0?00?11000000?10000000001010
00000?000????????????????????

Prionobrama paraguayensis

?00110010?0011101110010?110000??100001000010011100101100001000010001???1?0100011000010000110110100?10001110000010000000100000??1??
1100111110000001?00000010100000000100000101010100011001010000010001100001001100001001000001010100010 11?1001000000000101000?010000
00000001100000100000000100011111000000000101000010111000100000112000000001000?101000000000000010100?0000011100000??00000000100100
?1?0?010000000000??????1????

Pristella maxillaris

?00110010?1001100010000z11000010000000000000000z111000110000000001000101 1?01000??000110000?1010?100001z001100000001000000100010?0110
1000011zz000001?0010001010100000000000001011001000110010100000 1z0z1100001011100001001000001010100010100100100000000000000001010100
01000001110001100001000000001110000000 0z010100001011100000100011200000011000010100000001000000000?0?00???1zz00000??000000001001000
1?1?010????????????????????

Prochilodus lineatus

?01010010?1001??0010000110???10??001010100000000?0?0100001000??010010001011010000110000000000?1????0?00110000000?00000????????????
????0000?????1?????0001000000000?100?01011010000100001100011011100001010111111000100000000?10000100000000000100100000000101100001
10?00001000111000110z1010000000000001111010000010?????????0001010100000??0?100100000000000210??0?0000?111100?0??00???0?????10000?0
?010????????????????????

Prodontocharax cf. *melanotus*

?00110010?000110z010010?1101001100010000z000?01100001100000000010000101 1?0100000000z100001100101010?11001100100z1000000100000??111
1000011111z0101?00000010100000000101000101110100011001011000011111z00000001100001001000001010100010110100100000000000000001010100
11000001110001100001000101001100000000010101000010111000z000001120000000 1000?101001000000000000100?00????????000??00000000100100?
00??010????????????????????

Psellogrammus kennedyi

?00110010?10011000100000110000100001000 0z000001100000000000000000100101 1?0100000000010000110z101???110001000011110000000100010001 0
0100000000??0001?000000101000000000000001010101000110010100 0z01000110000000110000100100000101010001 01z010000000000000000000101010
00100000111000110000100000100111110000101010100001011000 0???0200120000000110001110 0?000000000000000?01???11000000?10000000001010
00000?010????????????????????

Pseudochalceus kyburzi

?00110010?10011000100z00110000100001000000000001110 00z0000000000000 1??11?0?0000000001000011010?1000?10001000000z100000010001000110
1000011111000 01?000z11?01110000000000000101000100011001010000010001100000101100001011000001010100010101100000000000000000000011100
010000011101011000010001 0z001111000000010101000010 0?????????0011200000??100?01???0?000000000?00??0?00000?????000?10000000010010000
00?010????????????????????

Pyrrhulina australis

??100??10?00010?0010011?0100010??011010001000000?0?01100010000100101 0?1?0100010001?000?0?0010?100001000110000011000000000???????
???001110000010000000001010?000000000100??0101010?00z00100100?0101111100001011000010000000?0000?100000000?0000000100000?0001??00?000
10000001000011000010001100000000000021001000000000?????????0001010100001001?1001?0000000000010??0?10???000000?0??0000?000100100??
?1?010????????????????????

Rhaphiodon vulpinus

?110100110000100000000001010000100010011100000000110101 1?0100001?01000100101001000100000?00000111000?10001000000110000000001??????
????001111000001?000011?01110000110101002 01?001101000111010 0?110111002?11011100001011110010 00?1?00010001000001110 00???0011??11100
000001011000011111010011101111111000000 1000?0000110?????????0000??0?z0101000111110000000000000 10??0?00???111100?0??000000001001000
0?0?010????????????????????

Rhoadsia altipinna

?00010010?00011000100z01110110100001000000000 1111000z00000000000001??11?01000000000010000z1010?101z01z101100000001000000 10001000111
1000011zz111101?0000z0001zz0000000000000101100100011001010 00?010011100000??11000010010000010101000101001000000000000000000000111000
10000011101011000010001010011110000000101010000100????????? 001???0000001000?111?0?0000000000?0??0?0?????????000??00000000z10100000
0?010????????????????????

Roeboexodon guyanensis

?00110010?0001110010000111100?10001100000000001110001z000001?0?00000001?0100z00?000100001100??1000?10001??0000z10000?01010????????
?0001111000001?0000001011100000?000000101?0010001100100100?01000111000000110000100100000111010001010010010000000000000000010101000
10000011000011000010001000011100000000010101000010?????????0011200000001??0?1110010000000000?0??0?0?????????1?0??00000000?00100000
0?0z0????????????????????

Roeboides descalvadensis

?10110000?110111000000011100001000010000000000111000000100000000020101??01?01101000100100z01100111000?100010000000010000010010???????
???001111000001?000011?01100000010100001010001000100010010010100011z00010111000010110101010111000101101z10000000000z000001011100
010000011z0001100001000000101111110001101010100001z1?????????0011200?10001001111100100000000000000?0?00???11100000??00000000100100?
010?010????????????????????

Roeboides microlepis

?10110000?110110000000001110000100001010000000110?0?00010000000020101??01?01101000100100001100111000?100010000000010000010010???????
???001111000001?000011?011100000101000010000011001000100100001000110000101110000101101010101110001011011100000000000000000001011100
010000011100011000010000010111111000110101010z0011110000???000012000001010011111001010000000000000?0?00???11100000??00000000100100
0000?010????????????????????

Salminus brasiliensis

??1010000?01000000z001z?1100001000010zz10000?000???011000000012010000000101101000?10000?00100111000?10001000000001000000100010001??
110001111z000000000011?011100000?000000100?0010000101111100?010001000011010100001001000000011100001000000000001000000000001011000
0100000111000110000100111000111z0000000101010100111?1???????00012000000001000011000000000000000??0?00???11000000??000000??1001000
000?010????????????????????

Serrapinnus calliurus

?00110010?0001101010010?1101001000010000z000001100001100000000100001001?010000000011000011010?100101100110010001000000110000??11
1100001100110001?000010101000000000101000101010100011101010000011001110000000110000100100000101010001010010010000000000000000010101
00010000001110001100001000000100111001000001010111101011100011010011200000000100001010010000000000z0100?0000011100000??000000000101
00001z?000????????????????????

Serrasalmus maculatus

??011?010?110101?010010?11000010000101010000?100?0?0000000000100101??000010000000100000011000?1???210001000010110000001000???????
???000000??0001?10000000100000100000000021001010000?001001000010111100010011100001001000000011100110100010000000100000000001011000
000000010000010100111111010011110000020100110000010?????????0000??0?11101110?110100?00000100010??0?00???11111000??000000000?10000
0?0?010????????????????????

Stethaprion erythrops

?00110010?1001100010000111000010000100101000?011100100000000000001001001?010000000001000011001010000100010000000100000001000100011
0100001z00100001?00110000100000000000000101?0010001100101000?0100??10000000?10000100?0000010101000101???0000000000000000000101010
000000101011001100001100001001111100001010101000011110??????20011201000111000?11??0??000000000?00?0?0?????????000?100000000110100
0000?010????????????????????

Stichonodon insignis

?0?110010?100110?010000?110?0010000100?00000?011100100?0000000000001011?010000000?1100001100101???0100011000000100000010001000110
100010000??0001?001000101000?000?100000101?0010001000101000?01000100000?0011000010010000010101010101???0000000000000000000101?10?
0000?001010001100001100001001111000001010101000011??????????0011210?1011100??1???0??000000000?0??0?00????????000?10000000011010000
10?010????????????????????

Thayeria boehlkei

?00110010?1001100010000z11000010000100101000001110001100000000001001011?01000001?001000011010?1??00100010000001100000?1000100011
0100001000100001?000100101000000000z000001011001000110010100010100011z0000001100001001000001010100010100000100000000000000000100010
001000000111000110000100000000011000000000101010000100?????????0011200000011000?1110000000020000?0??0?00???11000000?100000000110100
0101?000????????????????????

Thayeria obliqua

?00110010?z001100010010?11000010000100101000001110001100000000001001011?010000000001000011010?1??0010001000000z10000001000100011
010000z000100001?00010010100000000000000010110010001100101000?01100110000000110000100100000111010001010000010000000001000000100010

0010000011000011000010000000011000000000101010000100?????????0011200000011000?11100?0000020000?0??0?00???1z0000?0?100000000110100
0001?000????????????????????

Thoracocharax stellatus

??01????0?00010?1100011?1100000??001000001000000?0?01100100001?01?1???1?000???0000?000?0????0?100001000111000001000000100010001101
100011z0000001?000001?01010100000000000201010100000000001000?0000111000?000100100100110??0000?100010100000??001?00?00?0111????01000
0001011000010011011101100101111000020001010000000110000???0001101010010100011100000000000000110?00?00???11zz0000?100000000100000?1?
??000????????????????????

Triportheus nematurus

?01110000?1001100010010?110000100001001000000000100001100100010000000100010000100001000100010011100021000000001011000000100011?010
0101001100100000100000010100000000z000010201010110000000111000010000100001000110000100100100011111001000010000111100000?0011??1100
000001101100001100101001111z0011110000000010101000011 0????????001101000000100001110000000000000010??0?00???11100000??00000000010100
0010?010????????????????????

Triportheus pantanensis

?01110000?1001100010010?110000100001001000000000100001100100010000000100010000100001000100010011100021000000001011000000100011?010
0101001100100000100000010100000000z000010201010110000000111000010000100001000110000100100100011111001010010000111100000?0011??1100
000001101100001100101001111z0011110000000010101000011 0????????001101000000100001110000000000000010??0?00????????000??00000000010100
0010?010????????????????????

Xenagoniates bondi

?00110010?0011101110010?110000??10000100001001 10?1?010000?100010?00?0?1?0?00011000?1???01100??1110??00?1??0011?1001000100000??1111
00001111100001?0000001110000?100000000101?1010001100100?10001000111000000110000100100000101010001111010011000000000000z1????0000
000?0011000001000000000110111111100001010101000010 0????????0011200000000111??11??0?0000000000?0??0?00000?????0?0??0000?000z00100?00
0?010100001100111 1011????

Astyanacinus moorii

?00110010?10011010100000110000100001000010 00?0110000z000000000y01000001?0110000000001000011001010001100010 0000z11000000100010 0011
01000011z0110001?000100001z0000000000000101000100011001010000010001100000001110000100100000101010001010010000000000000000000101110
0?1000001110??11000010000000011110000000010101000010?????????00012000000001000011100001000000000??0?01????????000?100000000110100
0000?010????????????????????

Bryconamericus emperador

?00110010?1000101010000011000010000100000000z010?0?01000000000z00000001?01000000000100001100101000110001000001110000000100010z011
0000001100 10?001?00010000100000000000000201000100011001010000010001100000000110000100100000101010001010?1000000000000100000010101 0
0010000011100011000010001000011110000000101010000 11?????????0011200000000100001110000000000000010??0?00????????000?000000000110100
0000?010????????????????????

Oligosarcus bolivianus

?00111010?z0?0100010000z1100001000010000000000001100001000000000201000101?010000001001000011001010 00?1000100000011000000 1001???????
??0001111100001?000101?011100010100000001000001110110010100010100011000010111000010010000010101000101001000000000000000000001011100
0100000111000110000100010000111000000000101010000111110001100001120000000100001110000000000000010000?00????????100??00000000100100
0010?010????????????????????

Oligosarcus jenynsii

?00111010?10?01000z00z0z11000010000100000000000110000100000000020 1000101?0100100010010000110010100 0?100010000001100000010 01???????
??0001111100001?000101?011100010100000001010001110110010100010100011000010111000010010000010101000101001000000000000000000001011100
010000011z0001100001000100001 11z000000010101000011111000z100001120000000100001110000000000000010000?00???11000100??000000001001000
000?010????????????????????

Oligosarcus itau

?00111010?10001000100000110000100001000000000001100001000000000201000001?010000000001000001001 01000?1000100000011000000100010 0011
01000011111100001?000000001100001010000000100000 1z10110010100000100011000000111000010010000010 1z100010100100000000000000000000101110
0010000011100011000010001010011100000000101010000111110000000000112000000001000011100000000000000?0000?00????????000?10000000011010
00000?010????????????????????

Oligosarcus longirostris

?00111010?10?0100000010?1100001000010010000000110000100000000002010001z1?01000000100100001100101000?10001000000110000000z001???????
???001111100001?000111?011000010100000010z000111011001011000010001100001010100001011000001010100010100100000000000000000000011100
010000011z0??110000100010000111z000000010101000011?????????0011200000001000011100000000000000000?00????????100??0000000010010000
00?010????????????????????

Oligosarcus menezesi

?00z11010?10?0100000010?110000100001000000000001100001000000000020101??z1?01000000100100001100101000?10001000000110000001001100011
100?001111100001?000101?011100010100000010z00011101100101000?0100011000000111000010110000010101000101001000000000000000000101110
001000z011z0??110000100010000111z000000010101000011?????????001z200000001000011100000000000000000?00????????100??000000001001000
000?010????????????????????

Oligosarcus pintoi

?00111010?10?110001000011100001000010000000000011000010000000000201000z01?01000000100100001100101000?10001000000z10000001001???011
1000001111100001?000z01?011000010000000010z000111011001011001010001100000011100001001000001010100010100100000000000000000000101110
0?1000001110??11000010001000011110000000010101000010?????????001120000000100001110000000000000010??0?00???11000100??00000000110100
0000?010????????????????????

Creagrutus anary

?00110010?100110101000001110001100010000000001011000011000000000100000011?01000100000100001100101010010001000011110000000100011?010
00000011z0100001?000000011000000001000001010101z001100100z00001000110000010100000100100000101z1000101001001100000000010000000010
00100000010000001000000000101000z00000000010101010010110000???000112000000011100100000000000000?0??0000????????0011011101111001011
00100010????????????????????

Creagrutus atrisignum

?0011000z1000z1010100z011110001z0001000000000101z000011000000001100000011?01000z00000100001100101010011001000011110000000100011?0100
0000011z0100001?00000001100000000100000100000100011001000000001000111000000000000010010000010101000101001001100000000z100000011100
010000010000001000000001000001000000000101010000101110000000001120000000111?01????00100000000?0??0000????????00110111011110010110
0100010????????????????????

Creagrutus meridionalis

?001100011000z0010100001111000110001000000000101100001000000001101000011?010000000000100001100101010010001000011110000000100011?010
0000001100100001?000000011000000001000010100010001100100000000100011100001010010010010000010101000101001001100000000z10000001010
001000000100000010000000000100z1000000000101010z00101110001000001120000000111?01????00000000000?0??0000????????0011011101111001011
00100010????????????????????

Creagrutus taphorni

?00110010?0001101010000011100010000100000000?0110000110000000001010000011?01000100000100001100??1010010001000011110000000100011?011
00000011z0100001?00000001100000000?100000100?101?00110010?100?01?0?11100001010010010010000010101000101???0011000000001100000010100
0100000110000010000000010z000z0000000001010101001011100011000011200000001110?111000?000000000?0??000?????????00110111011110010110
0100010????????????????????

Creagrutus cracentis

?00110010?0000101010000111000011000000000000001010?0?011000000000100000011?0100010000110000110010100001?001000?1????00??01000101011
0000001111100001?000000011000000001000001010001000110010000000010011100000101001001001000001010100010100100110000000000100000??010
00100000010000001000000000100000000000000101010000000111000000000011200000000???0?1?0??00000000000?0?00000?????????0?101101111111111
00101010????????????????????

Creagrutus gephyrus

?00110010?0000101010010?11000010000000000000001010?0?0110000000000000000011?01000110000100001100101000010001000010110000000100011?011
0000001111100001?0000101110000000010000010100010001100110100001000110000000010010010010000010101000101001001100000000011000000?010
00100000010000001000000000000000100000000010101010000111000110000112000000001110?111?000000000000?0?00000?????????0?011101111111101
00100010????????????????????

Creagrutus maracaiboensis

?00110010?0001101010010?1110001000000000000001010?0?0110000???1101101011?0?100100000100001100101110010001000011110000000100011?011
0000001110100001?000000011000000011100001010001000110010100000100111000001011010010Oz000001010100010110100100000000011010000?010
0010000011000001000000000000001000000000101010000001110000000000112000000???0?110?00000000000010?00000??????????10011100111001011
01110000????????????????????

Creagrutus muelleri

?00110010?0001101010010?1110001000000000000001010?0?011000000?1000000011?0110010000010?001100101100010001000010110000000100011?011
0000001100100001?00000011100100000100000100001100011001110000010001110000101001001001000001010100010100100100000000001000000?010
0010000001000000100000000000000z0000000001010101000000????????0011200000001110?110?000000000000?0?00000??????????100111001110010110
0100010????????????????????

Creagrutus ouranonastes

?00110010?0001101010010?11100010000000000000001?10?0?011000000010000000011?01000100000100001100101110010001000010110000000100011?011
00000011z0100001?00000011100100000100000100000100011001010000010001100000101001001001000001010100001z100100100000000011000000?010
0010000001100000100000000010000010000000000101010100000????????0011200000001110?110?000000000000?0?00000??????????10011100111001011
00100010????????????????????

Creagrutus peruanus

?00110010?0001101010000011100010000000000000001010?0?01100000001001100011?0100011000010000110010110001000100001101000000100011?011
00000011z0100001?00000011100000000100000100001100011001010000010001110000101001001001000001010100010100100100000000001010000?010
00100000010000001000000000100000z000000000101010100000????????0011200000001100?110?000000000000?0?00000??????????100111001110010110
0100010????????????????????

Carlastyanax aurocaudatus

?00110010?0001101010010?11100010000100000000001010?00010000????010101??11?0?000111?001000011000?1010010001000010110000000100011?0100
0000001110100001?000000011000000001000001010z010?01100100100001000111000000001010010010000011101000101z010000000000000z0z00z0111000
0000z01100000100000000z00001110000000001z1010000z00????????001120000000110001100000000000000000000000???????0110001100101110010?10
0?010????????????????????

Microgenys minuta

?00110010?00011?101000001100001100010000000001010?0?01100000000100000011?01000110000100000100101010011001100010110000000100010?0??
?00?001100100001?000000001000000001100001010101000110010100000100111100001?110000100100000010101000101001001000000000110000001010
001000001100??01000000000100000000000z00010001000010?????????0011200000001110011000000000000000000??0000????????000?0000000000001000
0?z0010????????????????????

Piabina argentea

?00110010?010z10101000001110001000010000000001011000011000000000100000011?01000z000001000011001010z0010001000011110000000100011?010
0000001100100001?0000000010000000010000010100010001100111000001000110000010110000100100000010101000101101001100000000000000000101
00010000011000001000000001000011z00000000z01010000101110001?000011200000001110?1z1100000000000000010000001?1110000100000001000001
0010000010????????????????????

Acrobrycon tarijae

?00z10010?0001101010000z11000011000100000000z0z10000110000000000000000011?0100000000011??01100101000010001000001z100000010001000110
0000001110100001?000000001000000000z0000010100110001100101000000100011000000011000010010000001010100010z0010010000000000000000010100
0100000110000010000000011000111z00000000101010000101110011100001120000000100001110000000000000000111100111????000?100000000110100
0010?010????????????????????

Bryconadenos tanaothoros

?00110010?0001101010010?110000110001000000001011000011000000000100000011?01000z0000011??01100101000010001z00011110000001000100011
0000001100110001?0000000010z00000010000010101010001100100z0010100111z00001?110000100100000010101000101z010011000000000z00000010100
0100000110000010000000001000011100z0000010101000010110000???00011200000000110?01????00000000000?010000011??????000?1000000000101000
z000010????????????????????

Piabarchus analis

?00110010?000110101000001100z01100010000000001011000011000000000100000011?0100010000011??01100101000010001000011110000001000100011
0000001100100001?000000001000000001000001010101000110010010000100111100000001100001001000001z10100010100100100000000000000000001010

000000001100000100000000z00111111000000101010z001z101000000?00112000000110?011???00000000000?0000000????????000?100000000z101000
1000010????????????????????

Diapoma alburnus

?00110010?z00110101000001100001100010000000010110000110000000010000001?0100000000011??011001010000100010000001z10000001000100011
0z000011z0100001?0000001010000000010000010101010001100101000001 0z11100000001100001001z000011101000101001001000000000000000001010
000000001100000100000000100001110000000010101000010111000000000112000000010000111000000000000?010000001??????000?10000000011010
00000?01001000000000000001????

Diapoma speculiferum

?00110010?00011010100000110000110001000000000101100001100000000010000001?0100000000011??011010?10000100010000011100000010001000 11
0000001110100001?0000001010000000010000010101010001100101000101z0011000000011000010010000001z10100010100100??000000000000100000010
000000001100000z100000001z01111110000000101010000101110000??0000112000000?10000111000000000000000?0111100111?????000?100000000110100
0100?010000000000000000????

Hemibrycon surinamensis

?00110010?0001101010000011000011000100000000000011000011000000000100000 0?1?01???00000011??01100??1000?10001000001110000000100010z0110
z00001111100001?0001000010000000z00000101001100011001010000?0100011z000000110000100100000101010001010?1001000000000000000z0??1000
1000001100000100000000100001111000000010101000001 01????0????00112000000010000111000000000000?01?00000???????0?0?1000000001 10100?0
00?01????????????????????

Odontostoechus lethostigmus

?00110010?z0011010100z0?11000010000100000000101100001100000000010000001 1?0100010000011??0110010101001000110000111000000110000??11
1100001110110001?000000001000000001000001010101000110010100 0?0101111z00000011000010010000001z1010001010?100100000000000000000001010
0010000011000001000000001000011z000000001010100001z1110001zz00011200000001100011??00000000000000010000001??????000??000000000 10100
0000010????????????????????

Pseudocorynopoma doriae

?00110010?00011010100z00110000100001000000000z000?0?01100000000100000 0z1?01000z0000011??0110010100001 0001z00001z10000000100010001 10
1z00011z01z0001?0000001010000000000000000[12]010z01000110010100000110z110000000110z001001z000010101000101z0100??001000000000110001 0?0
0000000011001011000000000110011111z000010101010000101 1000z???00011200000001000011100000000000000000111001 11?????000?100000000110100
0110?010000100000000000001????

Tetragonopterus argenteus

?00110010?10011000100001110000100001001000000011z0000000000000001001011?0100100000010000110011100011000100000000100000010001 00011
01000011z0100001?00110000100000000000000101100100011001010000010001100000011100001001000001010100010100000000000000000000000001010
001000001110001100001000001001111z000010101010z0011111000000000111210000111000011100000100000000100?0?0000?11z00000?100000000z10100
000z?0z0????????????????????

Bryconamericus pectinatus

?00110010?0000101010010011000010000000000000001010?0?011000000000000000011?000?010000011??011001010000100010000100100000010001 00011
0000001110100001?0000000010010000010000010000100001100100000001001110000000110000100100000111010001010010010000000001100001 0001
000000000110000010000000010000110001000000101010z00000?????????00112000000001110?1?1?000000000000?0?000001?????????0??000000000100010
010z0010????????????????????

Bryconamericus indefessus

?00110010?00011010100000110000110001000000001010?0?01100000000?010z0011?01000z0z00011??0?1000?10z00100011000z01100000001000100 0110
100001110100001?000000z010z000000z00000[12]0101010001100101000001011111000000110000100 1z000010101000z01z0100z000000000000000001z10
0010000011000001000000001000 01z0000000001 01z10000101z1000z1z?001120000000100001110000000000000z0000000????????000?100000000 100100?
z000010????????????????????

Bryconamericus exodon

?00110010?0001101010000z11000011000100000000101100001100000000010000001 1?0100000000011??011001010000100010000111100000001000101011
0000001100100001?0000000010000000010000010101z10001100101z00001z0z11z0000001100001001000001010100010110100110000000000000000000 100
0100000110000010000000010000111000000000101010000 1z1110001000001120000000110001110000000 0[03]0000001000000 1?11100000?0000000001101
000z00?010????????????????????

Sp.1

?00111010?10011000100001110000110001000010000011100011000000000001000011?010?00000001000011001010000z10?0100000111000000010001000011
110001110011?001?00110010z0000000000000010100010001100111100101000110000000110000100100000111010001010z1001000000001100000100110
0010000011100011z00010?01?100111100000001010100001111000000000001120000000111001110000?000000200000000?0?011000100?10000000001010
000100010000100000000111110101

Deuterodon pedri

?00111010?1001100000010z1100001110010?0z000001110001110000000000100011?0110000000001000011001010000100?0100000111000000010001000011
110001110011?001?000000101000000000z000002010001000110011110000100011000000011000010010000010101000101001001000000001100000100110
001000000111000110000100010100111000000000101010000111100000000000112000000011000111000?100000200000000?0?011000000?10000000011010
000100010000100000000101110101

Astyanax pelecus

?00111010?10011000100001110000100001000?01010001110001100000000000100011?0010000000001000011001010000210?0100000111000000010001001111
110001110011?001?000000101010000000000000101000100011001?1100101000111000000011000010010000011101000101001001000?00000100000100110
0010000011100011000010001010011100000000101010000110?????????00112000000000110?01???00?100000200000000?0?0?????000?100000000110100
00100010???????????????101-1

Sp. 2

?00111010?10011000100z01110000100001000010000011100011000000000000000011?010?00000001000011001010000010?0100000111000000010001001111
1100011100110001?001100001010000000100000101100100011001100??0101000?100000001?0000100100000101010001010010010000000011000001011110
00100000111000110000100010100111000010001010100001110?????????00112z000000110??1????0010000020000000000?0??11000000?100???000010100
00?000100000000000001011101-1

Deuterodon iguape

?00110010?10011000100z0111000010000?00?0100000111?00100000000000001000011?010?000000010000?100101010010?0100000011000000010001001111
110100111011000001?000000001000000000?000000101?001000110010100000?010001100000001100001001000000101010001010010001?00000000100000000101010
001000000111000110000100010100111000?0000101010000111?????????00112z0000001101?1110000100000000010000000?0???????000?100???0001101000
0?000101111101112111111 0101

?00110000?1001100010000011000010000100?0100000111?001000000?0??000000?1?0110??0010?1000011001010110?0?0100001?1100000010001001111
100001110110001?0000000010100000?000000101?0010001100101000?01000110000000110000100100000111010001010010010000000011000001010100
010000001?000110000100010100011000?0?0010101000011??????????00112z0000001?0??1????0010000000000000000?0???????000?100???00011010000
?00010111111111111111110101

Deuterodon longirostris

?00110010?0001100010010011000010000100001000001110001100000010001000011?000?00000001?0001100101011210?01000011110000001000100111
1100001110110001?0000100010000000?00000010110010001100101000?0100011000000011000010010000010101000101001001000000000100000100010
0010000000110001100000000101001100001000010101000001??????????001011000000???????1????1000?0000?000000?0???????100?100???0??11010000
0000101111111111211111110101

Deuterodon potaroensis

?00112010?010110001001011100001100z100001000001110001z00000000201000001?001100000001?00000?0101000010?01000001010000001000100010
0100011110110001?000000101000000000000002010001000110010100000100011000000011000010110000000010110010100100110000000010000010111
00010000001110001100000000101000110000000010101000001??????????0010110000001??????1????1101?01000000000?0???????100?100???0??110100
000001101000001100000000010001

Jupiaba asymetrica

?00112000-10011011000000111000011000100?10001101110001000000000200000001-010-
0000001101000100101000210?0100000?110000001000100011110000110011?00100001011010000000z00000010100010001100111000000100011000001-
110000100100000001101000101001000000000001100000001110001000-
11110001100001000001000111000000010101000010??????????00112100000010????????00?000?0010100000000000?????000-
1000?00000101000000000100001000000000000000?0

Jupiaba essequibensis

?00110000-1z011001?0000111000010000100?01000001110001000000000000000001-010-
0000000100001100101001010?01000000110001001000100110010000110011?0010000000101000000000000000101000100011001010000010001000000011
10000100100000011101000101001001100000000100000100010001000-
1111000110000100000100111000000000101010000101100000000000112000000010????????001000?00100000000?000?????000-
1000?000001010000000001011010010011111110101

Jupiaba potaroensis

?00110000-1001100100000111000010000100000000101110001000000000000001001-010-
0000001100001100101000110?010000011100000001000101011110000110011?001000010010100000000000000101001100011001010000010001100000011
10000100100000001010001010010000000000001100000101010001000-
0111000110000100010100111100000000101010000101100000000000112100000010????????001100?011010000000000?????000-
1000?00000101000010001000001000000000000100?0

Jupiaba aff. *atypindi*

?10112000-1000100110010011000010000100?00000101110001000000000000000001-010-
0000000100001100101001110?01000000110001001000100110010000110011?0010000000101000000000000001010001000110010110-
00100011100000001100001001000001010110010100100000000000100000101110001000-
111100011000010000010011100000000010101000010??????????00112000000010????????00?000?0010100000000000?????000-
1000?00000101000010001011010000001111111101?1

Jupiaba abramoides

?00110000-100z10??00010111000010000100?00000101111000100000000000001000001-010-00000011000011000-
1000110?01000011110000001000101011110000110010?00100001001010000000?00000010000010001100111000000100011000001-
1101001001000000011010001010010000000000001100000101110001000-
111100011000010000010011110000000010101000011??????????00112100000010????????00111030100000000?000?????000-
1000?00000101000000000100001100000000000110?0

Jupiaba poekotero

?00110000-100110z110010111000010000100100000001110001100000000001001001-010-
0000001100001100101001110?01000010110000001000100111110000110011?0010000000001000000000000001010001000110011010-
0010001100000001110000100100000101010001010010011000000000100000101010001000-

111100011000010000010011100000000010101000010110011000000112z00000010????????001000?00100000000?000?????000-
1000?000001010000000001010010010011111110101

Jupiaba pinnata

?00111000-1001100110010111000010000100?0000001111000100000000000001001001-010-
0000000100001100111001010?01000000110000001000100110010000110011?0010000000101000000000000000101001100011000-000-
1000001110000001110000100100000011011001010010000000000000100000101110001000-
111100011000010000010011100000000010101000011??????????0011210000001?????????001000?00100000000?000?????000-
1000?00000101000000000101000001000111111101?1

Jupiaba anteroides

?00110000-100110??10010111000010000100?000010101000010000000000001000001-010-
0100001101001100101000110?01000010110000001000100011110000110011?00100001001010100000?000000101000100011001010100010001100000011
100001001000000001011001010010000000000001100000101110001000-
011100011000010001010011110000000010101000011??????????00112100000010????????001000?000000000000000?????000-
1000?000001010000000001000010000000000000010?0

Jupiaba acanthogasther

?00111000-1000100110000111000010000100000000110111000100000000000001001001-010-
0000000100001100101001010?01000010110000001000100110010000110011?001000000000010000000z0000001010001000110010110-
0010001110000000110000100100000101010001010010010000000000100000101110001000-
111100011000010001010011100000000010101000010??????????00112100000010????????00?000?00100000000?000?????000-
1000?00000101000000000101101001000111111101?1

Jupiaba polylepis

?00110000-100?10?010000111000010000100?0?0001011100010000000000001000001-010-
0000000100001100101000110?01000010010000001000101010010000110011?00100001001010000000?0000001010001000110010110-
?0100011000000111000010010000000010100010100100000000000011000001011100000000-
1111000110000100000000011100000000010101000010??????????00112100000010????????001100?02000000000?000?????100-
1000?000001010000000001000010000000000000010?0

Jupiaba ocellata

?00110000-100?1001100z011100001000010000000000011100010000000000001000001-010-
0000000100001100111000110?01000000110001001000101110010000110011?001000010010100000000z000000101000100011001010000010001100000011
100001001000000101010001010010000000000000100000101100001000-
011100011000010000010011100000000010101000011??????????00112100000010????????001100?00001000000?000?????000-
1000?000001010000100010000100000000011000?0

Astyanax giton

?00111000-1z0z100010010011000010000100000000210111000110000000000001001001-
001000000000100001100101000010?0100001?1100000001000100111110000110011?001000000000010000000001000010110010001100101000101000110000
0001100001001000000001010001011010010000000001100000101110001100000110001100001000z01001110000000001010100001011001100000001120000000
010????????0011000000000000000100011000000-1000?00000101000010000010010010001011110101

Astyanax bahiensis

?00110000-000?100010010111000010000100?000000011100011000000000001001001-010-
0000000100001100101000010?010000111100000001000100111110000110011?001000000010100000000?110000101000100011001010001010001100000001
100001001000000101010001010010010000000000100000101010001000000011000110000100010000111000000000101010000111100000000000112000000010
????????00110000000000000?000?????000-1000?00000101000000000010010000001011110101

Astyanax ribeirae

?00110000-110?1000100z0011000010000100?000000011100011000000000z001001-010-
000000010000110z101001010?010000111100000001000100111110000110011?0010000010101000000000110000101000100011101010001010001100000001
1000010010000000010100010100100100000000010000010111000000000111000110000100010000111000000001z101000011111111000000112z00000010?
???????0010000000100000000?00011000000-1000?00000101000000000010010000001111110101

Astyanax intermedius

?00111001011011000100001110000100001000000000011000011000000000000001001-010-
000000010000110010100101 0?01000001110000001000100111110 0?0110011?001000011000100000000000000101000100011001 0010-
101000110000000110000100100000111010001010010010000000??100000101110001000001110001100001000101001110000000010101000011111111000
000112z000000?????????00100000000000000?000?????000-1000?00000101000000001010011000001111110101

Astyanax aff. *hastatus*

?0011000??100110001000011100001100010 0?00000001100001100000000001000001-010-000000010000110z0-
1001010?01000011110000000100010011111000011001 1?001000000100010000000?010000101000100001001 0110-
101100110000000110000100100000000101000101101001000000000100000101110001000000110001100001000100001110000000010101000010?????????
?00112z00000010????????0010000000000000000?000?????000-1000?00000101000010000010011000001011110101

Astyanax hastatus

?0011000111000100010000011000010000100000000001110001100000000001001001-
0110000000110000110z101001010?010000101100000001000100111110000110011?0010000000z0100000000110000101000100011001010001010001100000
000110000100100000101010001010010010000000001000001010100010000011100011000010001000011100000000101010000111111110000000112z00000
010????????00100000001000000000?000?????000-1000?000001010000100000110100000011111110101

Hyphessobrycon luetkenii

?00110000-1001100010000z11000010000100000000001100001100000000001001001-010-
000000010000110z101011010?01000010110000000100010011111000011001 1?00100000101010000000000100001010001000110010100010100011000000001
100001001000000011010001010010010000000001100000101110001000001110001100001000100001110000000001010100001111111000000112z00000010?
???????0010000000100000000 1000?????000-1000?0000010100000000101101000000111111 0101

Sp. B

?00110000-100?1000100101110000100001 00?000000011100011000000000001000001-
0110000000010000110010100011 0?01000010110000000100010011111000011001 1?0010000000010100000000?100000101000100001001110001010001100000
0001100001001000000001011001010010000000000001100000101110001000000110001100001000101001110000000001010100001111111000000112z00000
010????????0010000000000000000?000?????000-1000?0000010100001000101001001000101111 0101

Astyanax taeniatus

?00110000-1101100010000?11000010000100000000001110001100000000200000001-
0110000000110000?100101000110?010000101100000001000100111110000110011?0010000010001000000000z100001010001000110011100010100011 0000
00011000010010000000010100010100100100000000011000001010100010000000110001100001000101001110000000001010100001 10---------
00112z00000010????????001000000000000000001000?????000-1000?0000010100001000101001101000101111 01-1

Sp. A

?0011000?-10001??0?0000011000010000100?00000001110001100000000210000001-
011000000000100001100101 01?110?0100001111000000010001001111100001100 11?0010000010001000000000100001010001000010011110-
1010001100000000110000100100000?0??10001010010010000000001000001011100010000011100011000010001010011100000000101010000 10?????????
?00112z00000010????????0010000000000000000?000?????000-1000?00000101000010001010011000001011110 1-1

Astyanax jenynsii

?0?11000?-10001000?00100110000100001 00?00000001110001000?0000000000000 1-
0?10?000001100001100??100011???1000010?1000000100010011001000011001 1?0010000110101000000000010000101?0010000100111000?01000110000
0011100001001 00000????10001010010010000000001000001 0??10001000000111000110000100010100111000000000101010000111?????????00112z000000
?????????00100000000000000000?000?????000-1000?00000101000010000 01?0100000010101101-1

Astyanax microschemos

?00010000-?00110?000000011000010000100?0000000111?0010?0???????????????1-
?????00000?1000011001010000 1???1??000011000000100010011111000011001 1?001000?100?????0?000????000101???100???????????????????????????
??????????????0110110010100100100000000000000?0?1???1000100000111000110000100010000110000000001010100001 1?1?000000000112z000000?????
?????00100000000000000000?000?????000-1000?0000010100??1000101?????0000101011????

Myxiops aphos

?00110000-00011000100101110000000000100?000000011100011000 01000z0100--01-000-0000000100001 1000-1110010?01110000110001001 10000--
11110000110011?00100000100010000000001000002010001000110010100000100011000001-

1100001001000000101010001010010010000000001100001011000011000011000011000010000000011000000000101010000111?????????0??????0000010?
???????00100000000000000000?000?????100--?00?00000100000010001011010001111111110101

Astyanax rivularis

?00110000-z0011011100100110000100001 00?1000000110000100000000000001000001-010-
000000010100110z101000110?0100001111000000100010001001000011 0010?00100001001010000000000000010100010001100101000101000110000000 1
1000010010000001010100010100100000000000001100001010100010000011100011000010001000011000000000101010000 10?1110000000001120000000 10?
???????00110000000000000000?000?????100-1000?000001010000100010100000000000000100011

Astyanax laticeps

?001100011110010001000 0z11000010000100?000000010---01000000010001000001-010-
0000000101001100101000z10?010000101100000010001000100100001 00010?0010000100001000000000000002010001000110011100010100011000000 01
10000100100000011101000101001000000000000110000000110000100000111000110000100010000111000000001010100001 01????????00112100000010?
???????0010000000001000000 1000?????100-1000?000001010000100010000000000000000010?0

Astyanax brachypterygium

?00110000-00001010100100110000100001000000000010---0110000000000000000 1-
011001101001000001 0z101000110?010001101100000001000 10?01001000011 0010?00100001101010000000000000010100010001100101000101000110000
000010000100100000 1?101000101001000000000000000000010111000100000111000110000100010000110000000001010100000011100000000011200000
0010????????0010000000000000000001000?????100-1000?0000010100001000100000000000000000000 11

Astyanax cremnobates

?00110001100001010000100110000100001000000000101100001100000000000 00z001-
01100110100100001 10z101000110?0100011011000000 1000100110010000110010?001000010010100000000000000201000100011001110000010001 10000
00011000010010000011101000101101000000000001100000101110001000001110001100001000101001100000000 0z0101000010111000000000112000000
010????????0010000000000000000001000?????100-1000?00000101000010001000000000000000000000 11

Sp. D

?00112000-z00111101001001100001100013001000000110000110000000000 100z001-010-
000000010100110z101000010?0100011111000000100010001001000011 0011?0010000101101000000000001000101000100001001110001001001100000011
1000010010000001010100010100100000000000010000010111000100000111000110000100010100110000000000z0101000010110100000000112z00000010?
???????00100000000000000000?000??????00-1000?0000010100000000100001000000000000000 0

Astyanax goyanensis

?00112000-1000101110010011000010000100000000001 0---0110000000000100z011-010-000000010100010z0-
1000110?0100010011000000100010001001000011 0011?00100001001010000000000000002010001000110010-10-
10100011000000011000010010000011101000101001000000000000010000010110000100000111000110000100010100110000000001010100001 0?1000000
0000112000000010????????00100000000000000000?000?????100-1000?00000101001000001000010000000000001010

Astyanax xiru

?0011201?-1100100z?00101110000100001000000000001100001000000000000100z001-
0110000000010z001100101000210?010001011100000001000100110010000110010?001000011010101000 00z0000001010001000010011100000010001 10000
0011100001001000 00??1?1?00101001000000000000110000010111000100000111000110000100010100111000000000101010000 10?????????0011200000 00
10????????00110000000000000000?000?????100-1000?00000101000010001000000100000000000?0-1

Astyanax douradilho

?0011000?-110010001000011100001000010000000??0110000100000000000001000001-
01100000000100000100101000210?01000101110000000100010011001000010 0010?001000011010100000000z0000001010001000110010100010000011 0000
001110000100100000111010001010010000000000001100000101110001000000110001100001000101001110000000001010100001 0?????????00112z00000
010????????00110000000000000000?000?????100-1000?000001010000100010000110000000000000?1

Astyanax procerus

?0011000?-z1011000100101110000100001 00?00000001100001100000000000000001-010-
000000010000110010100021 0?01000001110000000100010001001000010 0010?001000010000100 00000?000000101000100011001010001010001100000011
10000100100000010101100101001001000000000110000010111000100000011000110000100010z0011100000000010101000011 1????????00112100000010??
??????00110000000001000000?000?????100-1000?000001010000100010100000000000000000?0

Astyanax dissensus

?0011000?-10011000100z01110000110001000000000001100001000000000000001001001-000-
0000001100001100101000010?01000011110000001000100110010000100011?00100001001010000000?010000101000100011101010001010001100000001
100001001000000111010001010010010000000011000001011100010000000110001100001000100001110000000001010100001111000000000000112100000010
????????00110000000001000000?000?????100-1000?0000010100001000100000000000001101000010

Astyanax fasciatus sao francisco

?0011000?-z00110zz000001110000100001000?0000000110000100000000000001001011-010-
0000000100001100101000110?01000011110000001000100110010000100010?00100011001010000000?000000101000100011001010001010001000000011
1000010010000001110100010100100100000000110000010111000100000001100011000010001010011100000000101010000111110000000000112z00000010?
???????00100000000000000001000?????100-1000?000001010000100010000100000000000000010

Astyanax aff. *fasciatus* rio grande do sul

?0011000?-1001100010000111000010000100?000000011000010000??????????????1-???-
???0000100001100??1000110??1000001110000001000100010010000100010?00100011001?10000000z00000010100010001100101000?010001000000001
100001001000000111010001010??0010000000000100000010??10001000000011000110000100010100?1100000000101010000101110000000000112z000000???
???????00100000000?000000?000?????100-1000?000001010000100010000?0000000000000010

Astyanax henseli

?0011000?-10011000100101110000100001000000000000110000100000?????00??????1-??1-
?000000100001100??1000110?01??01010100000001000101110010000110010?00100011001010000000z0??000101000100011001010001010001000000001
10000100100000011101000101001001000000000010000010111000100000001100011000010000000011100000000010101?????0111000000000112z00000010?
???????00100000000000000000?000?????100-1000?000001010000100010000?000000000001010

Astyanax jequitinhonhae

?0011000?-1001100010000111000010000100000000101100001110000000001001001-
01100000100100001100101000210?01000001110000011000101010010000100011?00100001001010000000001000010100010001100101000101000100000
00111000010010000010101000101001001000000000110000010111000100000001100011000010001000011100000000101010000101110000000000112z00000
010????????00100000000z000000?000?????000-1000?00000101000010001000000000000000000001010

Astyanax scabripinnis

?0011000?-10011000100001110000100001000000000000110000110000000000001000001-
01100000000100001100101000z10?01000111110000001000101110010000100011?001000010010100000000000000010100010001100101000101000110000
0011100001001000000101010001010010010000000011000001011100010000000110001110001000101001110000000001010100001011111111100112z00000
010????????00100000000000000000?000?????100-1000?000001010000z00010000001000000000000010

Astyanax lacustris

?0??1001?-?00??010?0000011000010000100?00010001????01000000000201000001-01100000001100001100??100-
210?0100001111000000100010101001000000000--
?001000010010100000000000000010100010001100101000101000110000001110000100100000????1?001010010000000000000100000001010001000000110
00110000100000100111000000000101010000110---------00112z00000010????????00100000000000000000?000?????100-1000?000001010000z000100000000-
0000000000-0

Jupiaba poranga

?00111010-10011000000000011000010000100100000?00111000100000000000001000001-
011100000001000000100101000110?010000011100000001000100010010000110011?00100001001010000000z00001010100010001100101000101000110000
001110000101100000001111000101101001000000000100000101110001000-
11110001100001000000100111000000000101010000111111110000000112000000010????????0011000000000000000?000?????000-
1000?00000101100010000000000000000000000001000

Astyanax burgerai

?00010010-100110001000011100001?00010000000000011100011000000000001000001-010-
0000000100001100101000010?010000101100000001000100110010000110011?00100000000010000000001000001010001000110010100010100011000000001
10000100100000010101100101001000000000000010000010101000100000111000110000100010000111000000000101010000111100110000000112100000010
????????00110000000000000000?000?????000-1000?000001010000?00000000100000000111110101

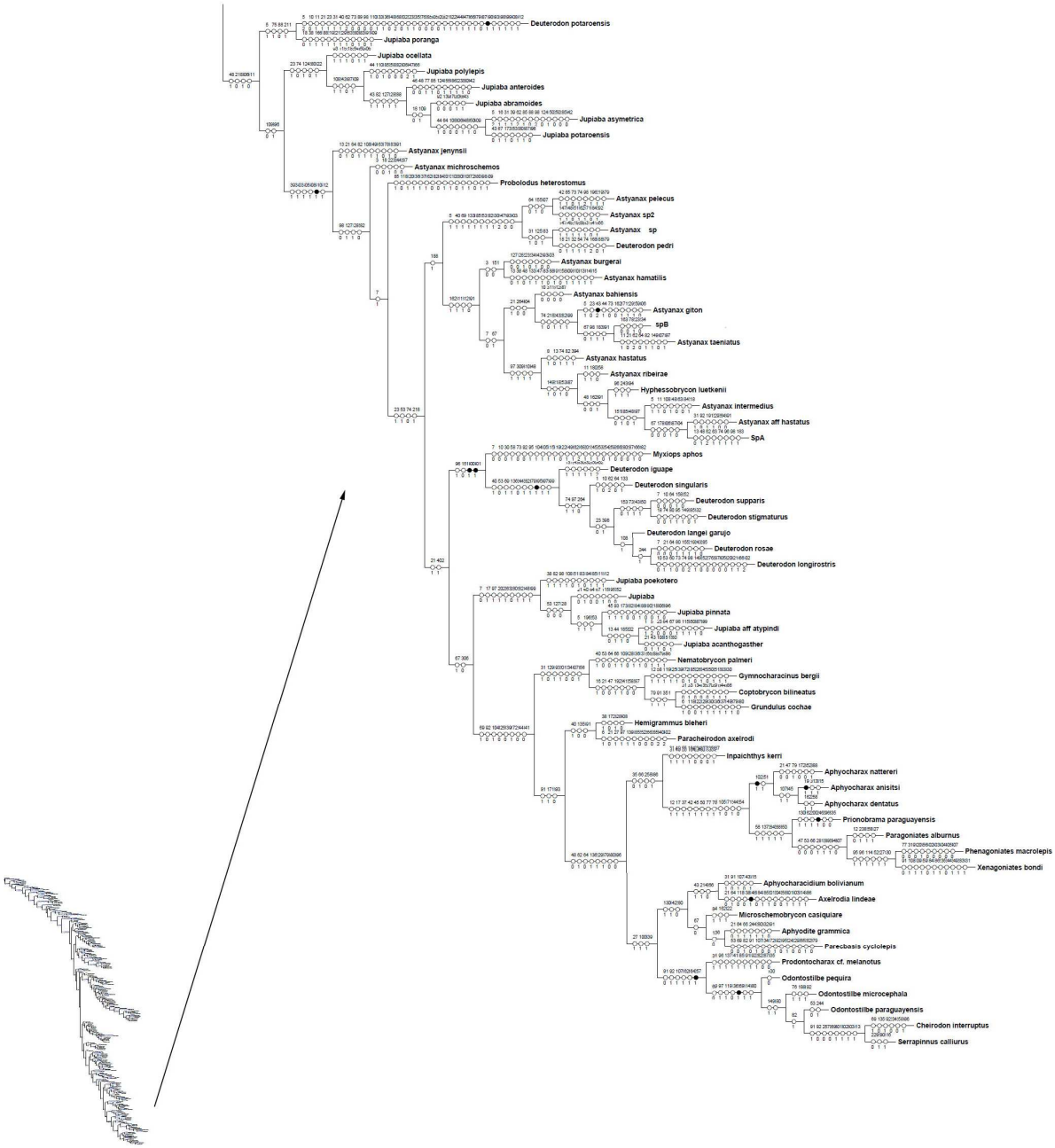
Probolodus heterostomus

?00110010-10011000100000110000100001000000000011100010000000000001000001-01100000000101001100101000010?0100000011000000001----------11111-?---00---
00101000000001000001010001000110000000000100011000001011000011010000001101000101001001000000000100000101010001000001110001100001000000001110000000010101000011111100000000112z00000010????????00100000000000000000?000?????000--010?000000010000000001-0101000-------1-0-

Astyanax hamatilis

?00010010-10001000100001110000100001001000000011000011000000000001000001-010-
00000001000011001010000010?0100000111000000100010011111000111001?00100010000010000000011000010100010001100111000001100110000000110000100100000101010001010010010000000001000001010100000000011100011000010001000011100000000101010000111111111110001l2z00000010?
???????00100000000000000000?000?????000-1000?000001010000100000100100000011111101 01

S4. Consensus tree of the implied weighting hypotheses.



S5. Comparative material. C&S = cleared and stained according to Taylor & Van Dyke (1985). * = specimens not measured because of damage or clear and stained and not previously measured or tissue sample (specimen fixed at ethanol absolute).

Specimens were examined from the following institutions: Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, MCP ; MCZ, Museum of Comparative Zoology of Harvard University, Cambridge; MNRJ, Museu Nacional do Rio de Janeiro, Rio de Janeiro; MZUFV, Museu de Zoologia João Mojeen, Universidade Federal de Viçosa, Viçosa; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; UFRGS, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre; USNM, National Museum of Natural History of Smithsonian Institution, Washington D.C.

Comparative material
***Astyanax fasciatus* (MOL):**
UFRGS 19070, TEC4074, Doce River basin;
UFRGS 19147, TEC4865A, Tramandaí River basin;
UFRGS 19147, TEC4865B, Tramandaí River basin ;
UFRGS 19135, TEC4853A Tramandaí River basin;
UFRGS 19135, TEC4853B, Tramandaí River basin.
Astyanax giton (MOL):
MCZ 20936, lectotype, 63,42 mm SL, rio Paraíba do Sul, Rio de Janeiro, Brazil;
CAS 42482, **paralectotype**, 55,81 mm SL, rio Paraíba do Sul, Rio de Janeiro, Brazil;
UFRGS 14814, 2 C&S, 47.9-49.9 mm SL, córrego Latão, tributary of rio Doce, Coimbra, Minas Gerais.
***Astyanax hastatus*:** USNM 92952, **holotype**, 37.16 mm SL, Rio de Janeiro, Brazil;
USNM 94312, 29 **paratypes** of 29, 21.15-42.18 mm SL, Rio de Janeiro, Brazil;
UFRGS 10257, 2 C&S, 49.3-49.9 mm SL, Macacu, Rio de Janeiro.
***Astyanax intermedius* (MOL):** MCZ 20684, **lectotype**, 45,86 mm SL, rio Parahyba, Rio de Janeiro, Brazil;
MCZ 20684, 3 **paralectotypes** of 5, 34.12-37.33 mm SL, rio Parahyba, Rio de Janeiro, Brazil;
UFRGS 10821, 2 C&S, 59.3-62.5 mm SL, Santa Virginia, São Paulo.
***Astyanax jenynsii* (MOL):**
***Astyanax lacustris* (MOL):**
UFRGS 18957, TEC4772, Santa Maria da Vitória River basin ;
UFRGS 19055, TEC4030, Tiririca lake, Doce River basin.
Astyanax laticeps:
UFRGS 18503, TEC4113, Ribeira de Iguape River basin ;
UFRGS 18503, TEC4115, Ribeira de Iguape River basin .
***Astyanax mexicanus*:** USNM 310222, 2 of 22 C&S, Kinney County, Texas, USA.

***Astyanax microschemos* (MOL):**
UFRGS 15358, 3 of 4 specimens, 81.6-89.1 mm SL, rio Mumbaça, Dionísio, Minas Gerais, Brazil;
UFRGS 17542, 2 of 4 specimens, 92.2-93.6 mm SL, Baixa Verde, Dionísio, Minas Gerais, Brazil.
***Astyanax novae*:**
FMNH 54641 8 **syntypes** from 11, 28.77-33.70 mm SL, rio Sapon, Prazer Bahia, Brazil;
FMNH 14928 1 **syntypes**, 31.88 mm SL, above Cachoeira Velha, rio Nova, Góias, Brazil;
FMNH 54642 7 **syntypes**, 27.90-67.36 mm SL, above Cachoeira Velha, Rio Nova, Góias, Brazil.
***Astyanax parahybae*:**
MCZ 20685 **lectotype**, 100.84 mm SL, rio Paraíba do Sul, Rio de Janeiro, Brazil;
USNM 120245 3 **paralectotypes** of 3, 87.07-103.09 mm SL, Rio Paraíba do Sul, Rio de Janeiro, Brazil.
***Astyanax pelecus*:**
MCP 37570 **holotype**, 56.4 mm SL, rio Pardo, Cândido Sales, Bahia, Brazil;
MCP 17919, 8 **paratypes** of 8, 1 c&s, 26.8-60.0 mm SL, rio Pardo, Cândido Sales, Bahia, Brazil.
***Astyanax ribeirae*:** FMNH 54725 **holotype**, 50.66 mm SL, Xiririca, Brazil;
FMNH 149631 **paratype** of 1, 39.51 mm SL, Morretes, Paraná, Brazil;
FMNH 14959 1 **paratype** of 1, 42.43 mm SL, Morretes, Paraná, Brazil;
FMNH 14962 1 **paratype** of 1, 45.96 mm SL, Morretes, Paraná, Brazil;
FMNH 14961 1 **paratype** of 1, 46.98 mm SL, Morretes, Paraná, Brazil;
FMNH 14960 1 **paratype** of 1, 41.93 mm SL, Morretes, Paraná, Brazil;
FMNH 54726 40 **paratypes** of 47, 18.98-55.81 mm SL, Morretes, Paraná, Brazil.
***Astyanax scabripinnis* (MOL):MZUFV4456**, Santa Cruz do Escalvado, Minas Gerais, Brazil.
***Astyanax taeniatus*:** UCMZ 6975, 2 **syntypes**, 41.10-41,25 mm SL, Sosego, Conceição de Macabu, Rio De Janeiro, Brazil.
Astyanax scabripinnis :
MZUFV 4456, CT2772, Doce River basin;
MZUFV 4456, CT2773, Doce River basin;
Sp.D
UFRGS 19746, TEC5291 E, Tripuí River, Doce River basin .
***Deuterodon acanthogaster*:**
FMNH 54750 5 **paratypes** of 5, 27.32-41.91 mm SL, Ria Jauru, Mato Grosso, Brazil;
FMNH54749 9 **paratypes** of 9, 33.16-40.30 mm SL, Corumbá, Brazil.
***Deuterodon cf. longirostris*:**
UFRGS 18629 1 specimen of 1, tissue*, rio Prata, rio Cubatão basin, Santa Catarina, Brazil.
***Deuterodon iguape*:**
USNM 437051 1 specimen of 1, C&S*, rio Iguape, rio Ribeira de Iguape basin, Road near Curitiba, São Paulo, Brazil;
USNM 354704 4 of 4 specimens, rio Iguape, rio Ribeira de Iguape basin, Road near Curitiba, São Paulo, Brazil;
MCP 12175 10 of 1º, 2 C&S, 44.4-95.4 mm SL, São Paulo, Brazil;

UFRGS 18525 1 specimen of 32, tissue*, Iporanga, rio Ribeira de Iguape basin, São Paulo, Brazil.
UFRGS 20032, TEC 4130, Ribeira do Iguape River basin.
UFRGS 18525, TEC 4138, Ribeira do Iguape River basin.

***Deuterodon langei*:**
UFRGS 18508, 1 specimen of 24, TEC4103, rio Paranaguá basin, Paraná, Brazil;
USNM 436728 3 specimens of 3, C&S*, rio Lindo, rio Cubatão basin, Joinvile, Santa Catarina, Brazil;
USNM 437050, 3 specimens of 3, C&S*, rio Marumbi, Morretes, Paraná, Brazil.

***Deuterodon longirostris*:**
MCP 12205 rio Cedro, rio Cubatão, Santa Catarina, Brazil;
UFRGS 18629, TEC3935, Rio da Prata, Cubatão River basin.

***Deuterodon pedri*:**
UFRGS 2073044 rio Santo Antônio, rio Doce basin, Ferros, Minas Gerais, Brazil;
MCP 47661, CT2521, Rio Santo Antônio, rio Doce basin, Ferros, Minas Gerais, Brazil;
UFRGS 17543, CT2529, Rio Santo Antônio, rio Doce basin, Ferros, Minas Gerais, Brazil.

***Deuterodon pinnatus*:**
FMNH 53525 **holotype**, 49.43 mm SL, Lower Potaro River, Amatuk, Guyana;
FMNH 53527 5 **paratypes** of 5, 19.07-32.00 mm SL.

***Deuterodon potaroensis*:**
FMNH 52967 **holotype**, 32.33 mm SL, Potaro River, Amatuk, Guyana;
FMNH 52968 1 **paratype** of 1, 37.60 mm SL, Potaro River, Amatuk, Guyana;
MCZ 29954,1 **paratype** of 1, 23.70 mm SL, Potaro River, Amatuk, Guyana;
ROM 61441 10 of 384, 3 C&S, 40.62-59.39 mm SL, Potaro River, Guyana.

***Deuterodon rosae*:**
USNM 64901 rio Humboldt, Joinville, Santa Catarina, Brazil

***Deuterodon rosae*:**
MCP 12209 15 specimens of 15, 1 C&S, 76.5-101.4 mm SL, rio Itapocú, Santa Catarina, Brazil;
USNM 649011 specimen of 1, C&S*, rio Humboldt, Joinville, Santa Catarina, Brazil.

***Deuterodon singularis*:**
USNM 297926 rio Tubarão basin, Rio Fortuna, Santa Catarina, Brazil.
UFRGS 18518, TEC4087, Tubarão River basin.
Deuterodon singularis:
MCP 14753 **holotype**, 88.1 mm SL, rio Sanga de Areia, Santa Catarina, Brazil;
MCP 11084 85 **paratypes** of 85, 3 C&S, 33.4-78.8 mm SL, rio Capivari, Gravatal, Santa Catarina, Brazil;
USNM 297926 3 specimens of 47, C&S*, rio Tubarão basin, Rio Fortuna, Santa Catarina, Brazil;
UFRGS 18518 1 of 12 specimens, tissue*, rio Tubarão basin, Rio Fortuna, Santa Catarina, Brazil.

***Deuterodon stigmaturus*:**
MCP 12207 13 specimens of 13, 2 C&S, 10.8-107 mm SL, rio Três Forquilhas, Chapéu, Torres, Rio Grande do Sul, Brazil;
UFRGS 16208 1 specimen of 6, tissue*, rio Maquiné basin, Rio Grande do Sul, Brazil.
UFRGS 16208, TEC2350,rio Maquiné basin

UFRGS 16519, 1 specimen of 1, tissue*, Rio Três Forquilhas, Rio Grande do Sul, Brazil, UFRGS 16519, TEC2847,rio Três Forquilhas
USNM 297956 11 specimens of 11, not measured, Praia Grande, Santa Catarina, Brazil;
USNM 436729, 1 specimen of 1, C&S*, rio Grande, Praia Grande, Santa Catarina, Brazil; rio Grande,

***Deuterodon supparis*:**

MCP 14752 **holotype**, 86.75 mm SL, rio Itajaí basin, Blumenau, Santa Catarina, Brazil;
MCP 10632, 43 **paratypes** of 43, 2 C&S, 51.8-102.4 mm SL, rio Itajaí basin, Blumenau, Santa Catarina, Brazil;
USNM 437052 1 specimen of 1, C&S*, rio Itajaí basin, Blumenau, Santa Catarina, Brazil;
USNM 279630 28 specimens of 28, not measured, rio Itajaí basin, Blumenau, Santa Catarina, Brazil;
UFRGS 18495, 1 specimen of 14, tissue*, rio Itajaí basin, Blumenau, Santa Catarina, Brazil.
UFRGS 18495, TEC 4651, Itajaí River basin.

***Hyphessobrycon luetkenii*:**

BMNH 1886.3.15.35, **lectotype**, 1, 55.8 mm SL, San Lorenzo, Rio Grande do Sul, Brazil;
BMNH 1886.3.15.36-38, 3 **paralectotypes** of 3, 55.9-62.5 mm SL, San Lorenzo, Rio Grande do Sul, Brazil;
BMNH 1885.2.3.78-79, 2 **paralectotypes** of 2, 34.3-35.6 mm SL, San Lorenzo, Rio Grande do Sul, Brazil;
UFRGS 5270, 5 C&S, 34.7-54.2 mm SL, Viamão, Rio Grande do Sul, Brazil;
UFRGS 5294, 2 c&s, 33.1-35.3 mm SL, rio Salso, Rosário do Sul, Rio Grande do Sul; Brazil.

Jupiaba scologaster

USNM 272612 rio Negro, rio Casiquiare basin, Venezuela

***Probolodus heterostomus*:**

FMNH 54330 2 **paratypes** of 2, one measured 60.29 mm SL, Iporanga, São Paulo, Brazil.

Serrapinnus heterodon: UFRGS 22004, ,TEC6956,Lagoa marginal, rio Doce basin, Santa Cruz de Escalvado, Minas Gerais, Brazil.

Sp. 1: MZUFV 3992, CT2353, CT2765, 5 C&S from 100, rio Doce basin, Rio Doce, Minas Gerais, Brazil.

Sp. 2: MZUFV 4457, CT2965, CT2969 (MOL); 1 C&S of 10, rio Doce basin, Rio Doce, Minas Gerais, Brazil

Capítulo 2

An integrative analysis of the phylogenetic relationships of *Deuterodon* (Ostariophysi: Characidae)

Artigo a ser submetido para a Revista Zoologica scripta

Priscilla C. Silva, Carlos A. Lucena, Zilda M. S. Lucena and Luiz R. Malabarba

Priscilla Caroline Silva
Programa de Pós-Graduação em Biologia Animal, Laboratório de Ictiologia, Avenida Bento Gonçalves, 9500, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil.
Phone: +55 51 33087727
e-mail: pricarola@gmail.com
Fax number: +55 51 3308 7696

An integrative analysis of the phylogenetic relationships of *Deuterodon* (Ostariophysi: Characidae)

PRISCILLA C. SILVA, CARLOS A. LUCENA, ZILDA M. S. LUCENA & LUIZ R. MALABARBA

Running title: Phylogeny of *Deuterodon*
P. C. Silva *et al.*

Silva P.C., Lucena C.A., Lucena M.S.Z., Malabarba, L.R. (2017). An integrative analysis of the phylogenetic relationships of *Deuterodon* (Ostariophysi, Characidae). *Zoologica Scripta*, 00, 000-000. *Deuterodon* was described in 1907 with *Deuterodon iguape* as the type species by monotypy. The genus was initially diagnosed by the presence of two rows of premaxillary teeth, teeth multicuspidate expanded on the distal portion and dentary teeth gradually decreasingin size posteriorly, being the last character long used to define the genus. Later, in 2002, the genus was diagnosed based in three characters: (1) the anterior region of the toothed portion of the maxilla deeper than the posterior region of the toothed portion; (2) the ventral margin of toothed portion of maxilla arched toward the ventral margin of the premaxilla, showing an alignment between maxillary and premaxillary teeth; and (3) posterior region of the maxilla without teeth smaller than anterior toothed region. In this new definition, *Deuterodon sensu stricto* included seven valid species. In order to test the monophyly of *Deuterodon* and the relationships among its species, the relationships are investigated based on an integrative approach using morphological characters (supermatrix with 412 characters) and a molecular data set (4 genes). Both kinds of data were congruent and showed *Deuterodon* as monophyletic. New characters are described and new synapomorphies proposed to define *Deuterodon*. The biogeographic history related to the evolution of the diferent lineages of the genus are further discussed.

Priscilla C. Silva, Laboratório de Ictiologia, Programa de Pós-graduação em Biologia Animal, Avenida Bento Gonçalves, 9500, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil E-mail:pricarola@gmail.com
Carlos A. S. Lucena, PUCRS, Museu de Ciências e Tecnologia. Av. Ipiranga, 6681, P.O. Box 1491, 90619-900 Porto Alegre, RS, Brazil E-mail: lucena@pucrs.br
Zilda M. S. Lucena, PUCRS, Museu de Ciencias e Tecnologia. Av. Ipiranga, 6681, P.O. Box 1491, 90619-900 Porto Alegre, RS, Brazil E-mail: margaret@pucrs.br
Luiz R. Malabarba Laboratório de Ictiologia, Programa de Pós-graduação em Biologia Animal, Avenida Bento Gonçalves, 9500, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil E-mail: malabarb@ufrgs.br

INTRODUCTION

The Neotropical fish *Deuterodon* Eigenmann genus was described in Eigenmann *et al*. (1907), with *Deuterodon iguape* Eigenmann, 1907 as the type species by monotypy. The genus was diagnosed initially by the presence of two rows of premaxillary teeth, teeth multicuspidate expanded on the distal portion and dentary teeth gradually decreasingin size posteriorly, being the last character long used to define the genus. After Eigenmann, 17 species from several regions in South America were assigned to *Deuterodon*, but five of them were subsequently moved to the genera *Jupiaba* Zanata, 1997 [*Jupiaba acanthogaster* (Eigenmann, 1911), *Jupiaba minor* (Travassos, 1964) and *Jupiaba pinnata* (Eigenmann, 1909)], *Gephyrocharax* Eigenmann, 1912 by Eigenmann (1914) [*Gephyrocharax atracaudatus* (Meek & Hildebrand, 1912)], and *Odontostilbe* by Malabarba (2003) [*Odontostilbe euspilura* (Fowler, 1945)] based on other characters grouping these species to those genera.

Lucena & Lucena (2002) were the first to redefine the genus based on synapomorphies, listing three characters to diagnose *Deuterodon*: (1) the anterior region of the toothed portion of the maxilla deeper than the posterior region of the toothed portion; (2) the ventral margin of toothed portion of maxilla arching toward the ventral margin of the premaxilla, determining an alignment between maxillary and premaxillary teeth; and (3) posterior region of the maxilla without teeth smaller than anterior toothed region. In this new definition, *Deuterodon* included seven valid species, with two junior synonyms. All the species of *Deuterodon* in this restricted sense are endemic from Atlantic coastal drainages along the Atlantic Forest Biome of south and south-eastern Brazil [*D. iguape*, *D. langei* Travassos 1957 (including *D. amniculus* Lucena & Lucena 2992 and *D. garujo* Lucena & Lucena 2992 as junior synonyms), *D. longirostris* (Steindachner 1907), *D. rosae* (Steindachner 1908), *D. singularis* Lucena & Lucena 1992, *D. stigmaturus* (Gomes 1947), and *D. supparis* Lucena & Lucena 1992].

The three remaining species, *D. parahybae* Eigenmann 1908, *D. pedri* Eigenmann 1908 and *D. potaroensis* Eigenmann 1909 were assigned by Lucena & Lucena (2002) as *incertae sedis* within Characidae due to the lack of the three synapomorphies proposed to define the genus. The identity of *Deuterodon pedri* remained uncertain until recently with the rediscovery of the species based on the extraction of DNA from old types and examination of

recently collected specimens, being now considered a species restricted to the Rio Doce drainage (Silva *et al.* 2017). The identity of *D. parahybae*, described from the Rio Itapemirim, Espírito Santo, Brazil, remains uncertain, and the third species, *D. potaroensis*, is known from Guyana.

Two of the three synapomorphies proposed by Lucena & Lucena (2002) to diagnose *Deuterodon* have never been tested in a congruence phylogenetic analysis. Only the first synapomorphy has been tested in a parsimony analysis in the family Characidae (Mirande 2009, 2010), but including only two species of the genus. Mirande (2010) found *Deuterodon iguape* and *D. langei* related to *Jupiaba* Zanata and further hypothesized as possibly related to *Myxiops* Zanata & Akama, but the only species of the last genus was not included in his analysis.

In a molecular phylogeny of the family Characidae including the type species of the genus, Oliveira *et al.* (2011) found *Deuterodon iguape* forming a clade along with *Probolodus heterostomus* Eigenmann and *Myxiops aphos* Zanata & Akama. More recently and based only on molecular evidence (with two genes) and with four *Deuterodon* species analysed, two of them previously considered by Lucena & Lucena (2002) as *incertae sedis*, Coutinho-Sanches & Dergam (2015) concluded that *Deuterodon* is not monophyletic.

The monophyly and the relationships among the seven species of the genus *Deuterodon sensu* Lucena & Lucena (2002) are investigated herein based on an integrative approach, including *D. pedri*, *D. potaroensis* and other characid species and genera. Morphological characters previously used to define the genus and new characters are tested under a comprehensive parsimony analysis, including 412 morphological characters and 233 characid taxa. These species were further subject to Bayesian analyses with mitochondrial and nuclear DNA sequences in order to reciprocally test the hypotheses of relationships obtained with the use of different methods.

MATERIAL AND METHODS

The ingroup used to test the monophyly and relationships of *Deuterodon* includes all species of *Deuterodon sensu* Lucena & Lucena (2002: *D. iguape*, *D. langei*, *D. longirostris*, *D. rosae*, *D. singularis*, *D. stigmaturus*, and *D. supparis*), species referred to the genus but presently assigned as *incertae sedis* in Characidae (*D. pedri* and *D. potaroensis*), species of

the genera *Myxiops*, *Probolodus*, and *Jupiaba* previously hypothesized as related to *Deuterodon*, and representative species of *Astyanax* and *Hyphessobrycon*, mainly from coastal Atlantic drainages. Two undescribed taxa (here mentioned as characid sp. 1 and characid sp. 2) were also included to the matrix due to the presence of some morphological similarities with *D. pedri*. All ingroup species are included in the morphological and/or molecular analyses, but not all were available for both analyses (Supporting information Table S1 and S2).

Morphological analysis

Osteological preparations were carried out following Taylor & van Dyke (1985). The extended matrix of Mirande *et al*. (2013) was used, excluding 53 taxa (species of *Creagrutus* and *Paleotetra*) that were not codified by several characters. Fourty nine taxa (*Astyanax bahiensis*, *A. brachypterygium*, *A. cremnobates*, *A. dissensus*, *A. douradilho*, *A. fasciatus*, *A.* aff. *fasciatus*, *A. giton*, *A. goyanensis*, *A. hastatus*, *A.* aff. *hastatus*, *A. henseli*, *A. intermedius*, *A. jenynsii*, *A. jequitinhonhae*, *A. lacustris*, *A. laticeps*, *A.* aff. *microschemos*, *A. pelecus*, *A. procerus*, *A. ribeirae*, *A. scabripinnis*, *A. taeniatus*, *A. xiru*, *Astyanax* sp. A, *Astyanax* sp. B, *Astyanax* sp. C, characid sp. 1, characid sp. 2, *Deuterodon pedri*, *D. potaroensis*, *D. rosae*, *D. singularis*, *D. stigmaturus*, *D. supparis*, *D. longirostris*, *Hyphessobrycon luetkenii*, *Jupiaba abramoides*, *J. acanthogaster*, *J. anteroides*, *J. asymmetrica*, *J.* cf. *atypindi*, *J. essequibensis*, *J. ocellata*, *J. pinnata*, *J. poekotero*, *J. polylepis*, *J. potaroensis*, *Myxiops aphos*) and twenty new characters were added on the matrix previously published by Mirande *et al*. (2013), resulting in 412 characters and 233 taxa (Supporting information S3 – character matrix in Silva *et al.* 2017a). For more detail of methodology see Silva *et al.* 2017 (unpublished).

Molecular phylogenetic analysis

Tissue samples from 48 specimens of the genera *Astyanax*, *Deuterodon*, *Jupiaba*, *Myxiops*, *Probolodus* and *Serrapinnus* fixed in 96% ethanol from the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS) were used in DNA extraction (Table S1). All molecular analyses were rooted with *Serrapinnus heterodon*. The DNA was extracted from gill filaments, muscle, or liver tissue of the samples,

with “Phire Animal Tissue Direct PCR Kit” developed by Thermo Scientific® under commercial recommendations.

Two mitochondrial genes were amplified: cytochrome oxidase c subunit 1 (*COI*) with primers cocktail FishF1t1 and FishR1t1 (Ivanova *et al.* 2007) and the NADH dehydrogenase 2 (*ND2*) with primers L5216 and H6313 (Sorenson *et al.* 1999). Two nuclear genes were also amplified. The nuclear alpha-myosin 6 (*MYH6*) gene was amplified with nested-PCR using primers F459 and R1325 (1st PCR) and F507 and R1322 (2nd PCR) (Li *et al.* 2007). The SH3 and PX3 domain-containing 3 like protein (*SH3PX3*) gene was also amplified with nested-PCR using primers F461 and R1303 (1st PCR) and F532 and R1299 (2nd PCR) (Li *et al.* 2007).

The PCR reactions for all genes were carried out in a reaction volume of 20 µL [10.3 µL of H20, 2 µL of 10× reaction buffer (Platinum®Taq), 0.6 µL of MgCl2 (50 mM), 2 µL of dNTPs (2 mM), 2 µL of each primer (2 µM), 0.1 µL (5 U) of Platinum® Taq (Invitrogen), and 100 ng of template DNA].

COI was amplified using the following PCR conditions: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 52°C for 40 s, and at 72°C for 1 min, and a final extension at 72°C for 10 min. *ND2* was amplified by touchdown PCR under following PCR conditions: an initial DNA denaturation at 94°C for 4 min, followed by 9 cycles at 94°C for 30 s, at 57°C for 40 s with melting temperature decreasing one degree on each cycle, and at 72°C for 1 min and 30 seconds, 40 cycles with denaturation at 94°C for 30 s, at 47°C for 40 s and at 72°C for 1 min and 30 seconds and a final extension at 72°C for 10 min. The *MYH6* PCR was performed in the following conditions: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 53°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 10 min on first PCR and an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 62°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 5 min on second PCR. The *SH3PX3* conditions following: an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 55°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 10 min on first PCR and an initial DNA denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, at 65°C for 45 s, and at 72°C for 1 min and 30 s, and a final extension at 72°C for 5 min on second PCR. The PCR products were purified by using

enzymatic method ExoSap (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50% of deionized water), and sequencing was performed on Macrogen Inc., Seoul, South Korea and Ludwig Biotec at Porto Alegre, RS, Brazil.

Sequences of each locus were independently aligned using Clustal W in MEGA 6.0 software (Tamura *et al.* 2013) and alignments were inspected by eye for any obvious misalignments that were then corrected.

The species tree was estimated on BEAST 2.1.3 software (Bouckaert *et al.* 2014) with StarBeast template. Each DNA alignment was considered a partition and molecular models of evolution and gene trees were unlinked. The best molecular model of evolution for each DNA alignment was selected with MrModeltest software (Nylander 2004) and this information using to set priors of site substitutions on Site Model panels. It was made to optimize the mixing and convergence of the MCMC chain. A population function constant was chosen on Mult Species Coalescent panel and a Yule Model was chosen as Species Tree prior. The tree was estimated twice and each run was performed with 200 million MCMC iterations and 20,000 trees were retained. The distribution of log likelihood scores was examined to determine stationarity for each run and achieve convergence using the program Tracer 1.5 (Rambaut & Drummond 2009) with 10% of the initial states discarded as burn-in. The program TreeAnnotator (Beast package) was used to summarize the trees with 10% of initial trees discarded as burn-in. StarBeast analyses were run on computational resources provided by Cyberinfrastructure for Phylogenetic Research (CIPRES) (Miller *et al.* 2010).

The posterior probability values of 1–0.91 and percentage values of 100–88 were considered well supported in the Bayesian and maximum parsimony analysis, respectively (Zander 2004). DNA sequences were deposited in GenBank (Access No. XXXX).

RESULTS

Molecular analysis.

The combined sequence data set of 48 specimens resulted in a matrix with 3.103 aligned base pairs (bp). The transitions/transversions (Ti/Tv) ratio was 111 and overall mean genetic distance (*p*-distance) was 0.13. All other information relative to each gene is summarized in Table 1.

StarBeast Bayesian analysis recovered the genus *Deuterodon* including only the species from southern section of the Atlantic River drainages of southern Brazil as monophyletic (*Deuterodon sensu stricto*, Fig. 1), being congruent with the restricted definition of the genus as presented by Lucena & Lucena (2002). *Deuterodon pedri* was found not closely related to this *Deuterodon sensu stricto*, but recovered as sister group to two undescribed species (characid sp. 1 and characid sp. 2) with high posterior probability and bootstrap values (*D. pedri* clade, Fig. 1).

Myxiops and *Probolodus*, previously hypothesized as related to *Deuterodon*, were recovered as forming a clade with high posterior probability containing *Deuterodon* and some species of *Astyanax* from Atlantic coastal River drainages in Brazil, demonstrating a common phylogenetic history, but not as sister groups to *Deuterodon sensu stricto*. The species of *Jupiaba*, however, were not found closely related to the genus.

The analysis further demonstrates the polyphyletic nature of *Astyanax* whose species appears in three different clades. The clade containing *Astyanax mexicanus* (type species of the genus), *A. laticeps*, *A. scabripinnis*, *A. fasciatus* and *A. lacustris* would correspond to the true *Astyanax*. The other examined species were found closely related to *Probolodus* or as sister group to *Deuterodon.*

Morphological analysis.

The equal weighting hypothesis based on morphological data is the strict consensus among most parsimonious trees with 2884 steps (Fig. 2; CI = 0.303 and RI = 0.621). The implied weighting hypothesis is the strict consensus between 2 trees generated under the 20th value of K (38.894, more stable value) (S4; CI = 0.309 and RI = 0.645). For more details about k chosen, see Mirande (2009). We opted to work with the equal weighting generated tree to be as conservative as possible. The tree of implied weighting is presented as supplementary file S4 for comparison. Both analyses further supported the monophyly of the genus *Deuterodon sensu* Lucena & Lucena (2002), including *D. rosae* (not available in the molecular analysis). *Deuterodon pedri* and *D. potaroensis* were found not belonging to *Deuterodon*, but more closely related to *Astyanax pelecus* (not available in the molecular analysis) and two undescribed characids, and to *Jupiaba poranga*, respectively.

Based on these results, a complemented diagnosis is presented for *Deuterodon*, including *D. iguape*, *D. langei*, *D. longirostris*, *D. rosae*, *D. singularis*, *D. stigmaturus* and *D. supparis*.

Deuterodon Eigenmann 1907

Deuterodon Eigenmann in Eigenmann, McAtee & Ward 1907: 140 (Type species: *Deuterodon iguape* by monotypy).

Joinvillea Steindachner, 1908: 29 (Type species: *Joinvillea rosae* by monotypy).

Distoechus Gomes, 1947:12 (Type species: *Distoechus stigmaturus* by original designation).

Diagnosis. The following synapomorphies indicate the monophyly and diagnose the genus. Numbers on final of each synapomorphy description is the corresponding numeration of characters proposed by Mirande (2010), Mirande *et al.* (2011) and Mirande *et al.* (2013), followed by the state change in this node, the consistence index and the retention index of the character.

Exclusive synapomorphy. - Maxilla not reaching the Meckelian cartilage (395 – 0>1; 1.00; 1.00). This synapomorphy is exclusive of the genus *Deuterodon.* Originally Mirande (2010) described a character that has two states: maxilla reaching posterior end of Meckelian cartilage and maxilla not reaching posterior end of Meckelian cartilage. In *Deuterodon*, the maxilla is short and does not reach the Meckelian cartilage in neither portion. All of the remain examined characids have the maxilla reaching the Meckelian cartilage on the posterior region.

Non-exclusive synapomorphies. The following synapomorphies although diagnosing *Deuterodon*, can be observed in distantly related taxa, being by parsimony considered non-homologous with those taxa.

- Maxillary ascending process with a small lateroventral projection (397 – 0>1; 0.11; 0.42). Ambiguous in *Deuterodon stigmaturus* and parallel in *Astyanax intermedius*, *Astyanax* aff. *hastatus*, Sp. A, *Astyanax taeniatus*, *Jupiaba abramoides*, *Jupiaba potaroensis*, *Astyanax scabripinnis*, *Astyanax douradilho* and *Astyanax xiru.*

-Posteriorly oriented epioccipital spine absent (7 – 0>1; 0.06; 0.70). Reversed in *Deuterodon supparis* and *D. rosae*. Most Characidae species lack the posteriorly oriented epioccipital spine, but other characid species belonging to the clade that includes *Deuterodon* have this projection.

-Presence of anterior paired projections of parasphenoid (40 – 0>1; 0.07; 0.57). Parallel in *D. potaroensis*, *A. pelecus*, *J. essequibensis*, *Nematobrycon palmeri*, *Thayeria* species, some *Hyphessobrycon species*, *Hemigrammus* species, *Moenkhausia* species, *Bario steindachneri*, *Poptella paraguayensis*, *Stethaprion erythrops*, *Paracheirodon axelrodi*, *Astyanacinus moori* and *Bryconexodon juruena*.

-Supraoccipital spine extending posteriorly to, at least, middle length of neural complex of Weberian apparatus (53 - 1>0; 0.03; 0.71). Reversed in *Deuterodon longirostris*. Most examined Characidae present the same condition found in the genus *Deuterodon*. The most closely related are *Jupiaba*, *Astyanax microschemos*, *Astyanax jenynsii* and *Probolodus heterostomus*.

-Dilatator fossa not covered by sixth infraorbital, leaving a conspicuous naked area in anterior region of fossa (69 - 0>1; 0.05; 0.79). Parallel in *D. pedri*, *A. pelecus*, Stevardiinae species, most Cheirodontinae species, *Nematobrycon palmeri*, *Carlana eigenmanni*, *Rhoadsia altipinna*, *Hasemania nana*, *Thayeria* species, *Hemigrammus* species, *Pristella maxillaries*, some *Hyphessobrycon* species, *Moenkhausia* species, *Poptella paraguayensis*, *Gymnocorymbus ternetzi*, *Stichonodon insignis*, *Tetragonopterus argenteus*, some *Astyanax* species, *Nematocharax venustus*, *Psellogrammus kennedyi*, *Hollandichthys multifasciatus*, *Pseudochalceus kyburzi*, *Charax stenopterus*, *Phenacogaster tegatus*, and *Hoplocharax goethei*.

- Four or more teeth on maxilla (136 - 0>1; 0.04; 0.71). Parallel on *Probolodus heterostomus*, *Jupiaba scologaster*, *Nematobrycon palmeri*, *Axelrodia lindae*, *Aphyocharacidium bolivianum*, *Prodontocharax* cf. *melanotus*, *Inpaichthys kerri* and aphyocharacine species.

-Presence of a process of scapula forming anterior border of scapular foramen (244 - 1>0; 0.05; 0.74). Reversed in *D. longirostris* and *D. rosae*. On the clade where *Deuterodon* is inserted, only *Deuterodon* species have this condition. In most characid species of other clades, the condition observed is the same found in *Deuterodon*.

-Ten or more teeth on anterior row of dentary (379 - 0>1; 0.03; 0.64).

DISCUSSION

Morphological and molecular phylogenies generated at this work were congruent in demonstrating the monophyly of *Deuterodon sensu stricto* according to Lucena & Lucena (2002), excluding *Deuterodon pedri* and *D. potaroensis*. The integration between different kind of data (molecular and morphological) to generate hypothesis at species level increases the rigor in the taxonomy decision (Schlick-Steiner *et al.* 2010). The congruence in independently analysed molecular and morphological datasets makes the hypothesis generated by our data set very strong and rigorously tested.

Coutinho-Sanches & Dergam (2015) have also concluded that *Deuterodon sensu lato* is not a monophyletic group. Their first hypothesis is based exclusively on cytochrome oxidase subunit I sequences, that should be not used alone to reconstruct phylogenies (Will & Rubinoff, 2003), showing quite different hypotheses of relationships than those described here (e.g. *Deuterodon pedri* as sister group to *D. singularis*). Interestingly, their second hypothesis using only RAG-2 is congruent with our hypothesis in placing *Deuterodon iguape*, *D. pedri*, *Astyanax giton* and *Probolodus heterostomus* in a single clade. The corresponding clade in our study has 15 terminals including the four listed above, and the differences between the two trees topologies may be related to the small taxon sampling of Coutinho-Sanches & Dergam (2015), since the increased taxon sampling has a clear and strongly positive effect on the accuracy of phylogenetic analyses (Zwickl & Hillis 2002; Hillis *et al.* 2003).

The relationships of *D. pedri* with two undescribed characid taxa (Sp. 1 and Sp. 2) and to *Astyanax pelecus* was also congruent in both morphological and molecular phylogenies,

further supporting *D. pedri* as belonging to a clade separate from *Deuterodon sensu stricto.* Molecular data on *Deuterodon potaroensis* were not available, but morphological data also placed this species apart from *Deuterodon sensu stricto.* This species was closely related to *Jupiaba poranga*. Even though it appears as sister group of *Jupiaba poranga*, the number of autapomorphies observed in *D. potaroensis* is elevated (31 unambiguous autapomorphies). Unfortunately, we could not identify recent material as *Deuterodon parahybae* to include in our analyses, but we have examined the type specimens, allowing to confirm that this species does not have the synapomorphies of the genus *Deuterodon*. In order to be more conservative, and in the lack of a more inclusive morphological analyses including more genera of the clade C (Javonillo, 2009) of Characidae, we decide to keep *D. pedri*, *D. potaroensis* and *D. parahybae* as *incertae sedis* in Characidae as previously proposed by Lucena and Lucena (Lucena & Lucena, 2002).

The species of *Deuterodon sensu stricto* occur only in River drainages of the Atlantic forest and southern to the magmatic lineament of Cabo Frio (Riccomini *et al.* 2005) in Brazil: Ribeira de Iguape (*D. iguape*), South-eastern Atlantic Forest (*D. singularis, D. rosae*, *D. longirostris, D. langei* and *D. supparis*) and Tramandaí-Mampituba (*D. stigmaturus*) (Fig. 3). The magmatic lineament of Cabo Frio seems to be an important barrier that restricts the distribution of other genera to southern drainages (e.g. *Diapoma* Cope 1894, *Pseudocorynopoma* Perugia 1891, *Chasmocranus* Eigenmann 1912, *Rhamdioglanis* Ihering 1907, *Epactionotus* Reis & Schaefer 1998, *Lampiella* Isbrücker 2001, and *Pseudotothyris* Britski & Garavello 1984). The magmatic lineament of Cabo Frio also affects the distribution patterns of wide distributed species in phylogeographic studies (e.g. *Hoplias*, in Pereira *et al.* 2012).

Despite of the low support for the hypothesis of relationships among the species included in the *Deuterodon sensu stricto*, *D. stigmaturus* and *D. singularis* form a separate clade representing the southernmost distribution of the genus, and a sister group relationship to the other species that occur to the north of the distribution (Fig. 3). These two species are endemic from Maquiné, Três Forquilhas and Mampituba rivers and Tubarão River basin respectively. These drainages are located in the same palaeodrainage region in Atlantic forest in Brazil (e.g. Thomaz *et al.* 2015). The other *Deuterodon* species (north) are distributed in other three palaeodrainages. Palaeodrainages have an important role in interpreting general

patterns of diversity in Riverine organisms (Thomaz *et al.* 2015). Additionally, Thomaz and colleagues (Thomaz, *et al.* 2015) highlighted the possibility that palaeodrainage connections could influence the structure patterns of present populations. A more inclusive study of *Deuterodon* species may corroborate this hypotheses, once the distribution patterns and species relationships found by us suggest some influence in the species range along coastal river drainages of southern Brazil.

The tree synapomorphies proposed by Lucena and Lucena (2002) were not recovered as synapomorphies for *Deuterodon*. Their redefinition of the genus was based on primary homology hypotheses. According to de Pinna (1991), the test of synapomorphies is split in two steps: first when similarities are observed and supposed to be synapomorphies (primary homology, hypotheses) and second when the primary homologies are tested in more inclusive phylogenies and are found as actual synapomorphies to support clades (secondary homologies). The inclusion of the synapomorphies proposed by Lucena and Lucena on more extensive and exhaustive test is a clear example of the importance of testing primary homologies. The phylogenetic test allows us to better understand trait evolution flowing by the history of organisms, and point us which characters should be considered as synapomorphies to define clades and to recognize genera, families, sub families, orders. Despite these, the seminal work of observation of similarities (primary homology search) is primordial for further analyses. Although the tree characteristics proposed by Lucena and Lucena were not recovered as synapomorphies to define *Deuterodon*, the monophyly of the genus, previously proposed by these authors was recovered with the support of other synapomorphies. Actually, the character observed by Lucena & Lucena proved to be synapomorphies at higher levels, helping to solve relationships among inclusive clades.

From the twenty characters created to improve the understanding of *Deuterodon* and related genera relationships, only 3 of them are synapomorphies for *Deuterodon* genus. Some of the new characters proposed based on the dentition proved to be homoplastic and occur among different characid lineages. The teeth with cusps nearly equal in size, basal tooth portion narrower than apical portion, dentary teeth inserted laterally and visible in ventral view, maxillary teeth located ventrally to the bone were observed in other, distantly related Characidae taxa such as *Deuterodon* spp., *Bryconamericus iheringii*, *Jupiaba polylepis*, and Cheirodontinae. It suggests that characters related with mouth, especially teeth, can be highly

plastic features associated with feeding habits and environmental conditions in which species are inserted. According with ecological studies the cited species have similar feeding preferences. Cheirodontines have a tendency to herbivory with zooplanctivory habit (Dias, 2007). *Bryconamericus iheringii* has preference to eat algae and microcrustaceans (Escalante, 1983) and could be considered planctophagous (Borges *et al.* 2006). *Deuterodon stigmaturus* is algae feeder and should be considered herbivorous (Dala Corte, 2012). This similarity in feeding habits suggest a similar niche occupation that may contribute to the independent development of the similar teeth morphology in these distantly related characids.

ACKNOWLEDGEMENTS

We are grateful to Karsten Hartel that allowed us the possibility of extract tissues from MCZ type specimens, sent material to analyse at Smithsonian Institution and helped the first author (P.C.S) during a visit to MCZ. We would like to say thank you to Richard Vari (*in memoriam*) that "open the gates" of NMHN and allowed the development of an important part of this study at Smithsonian. We are also grateful to Jorge Dergam that collected fresh material and made it gently available for us. Lynne Parenti and Dave Johnson by all help at Smithsonian. Jeff Clayton and Chris Murphy by support at NMHN and MSC respectively; Lee Weigh and Jeff Hunt by support at LAB. Cláudia Malabarba that generated some of sequences. BRBOLD to the financial support to sequencing and CNPq XXXXXXX, CAPES and FAURGS also by financial support and scholarships.

REFERENCES

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J. V., Heibel, T. J., Wikramanayake, E., Olson, D., López, H. L., Reis, R. E., Lundberg, J. G., Pérez, M. H. S., & Petry, P. (2008). Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, 58, 403–414.

Barros, L. C., Santos, U., Zanuncio, J. C. & Dergam, J. A. (2012). *Plagioscion squamosissimus* (Sciaenidae) and *Parachromis managuensis* (Cichlidae): A Threat to Native Fishes of the Doce River in Minas Gerais, Brazil. *PLoS ONE*, 7, e39138. doi:10.1371/journal.pone.0039138

Bertaco, V. A. & Lucena, C. A. S. (2006). Two new species of *Astyanax* (Ostariophysi: Characiformes: Characidae) from eastern Brazil, with a synopsis of the *Astyanax scabripinnis* species complex. *Neotropical Ichthyology, 4*, 53–60.

Borges, R. Z., Assakawa, L. F., Cunha. A. B., Bialetzki, A. & Nakatani, K. (2006). Morfologia do trato digestório e dieta de larvas de *Bryconamericus* aff. *iheringii* (Boulenger, 1887) (Osteichthyes, Characidae). *Acta Scientiarum. Biological Sciences*, 28, 51–57.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu C-H, *et al*. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computation Biology 10*, e1003537.doi: 10.1371/journal.pcbi.1003537

Bührnheim, C. M. & Malabarba, L. R. (2006). Redescription of the type species of *Odontostilbe* Cope, 1870 (Teleostei: Characidae: Cheirodontinae), and description of three new species from the Amazon basin. *Neotropical Ichthyology*, 4(2), 167–196.

Camelier, P. & Zanata, A. M. (2014). Biogeography of freshwater fishes from the Northeastern Mata Atlântica freshwater ecoregion: distribution, endemism, and area relationships. *Neotropical Ichthyology*, 12, 683–698.

Coutinho-Sanches, N. & Dergam, J. A. (2015). Cytogenetic and Molecular Data Suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) Is a Member of an Ancient Coastal Group. *Zebrafish*, 00, doi: 10.1089/zeb.2014.1068

Dala Corte, R. B. (2012). História Natural de *Deuterodon stigmaturus* (Gomes, 1947) (Teleostei: Characidae) em um riacho costeiro da floresta atlântica, sul do Brasil. Dissertação (Mestrado em Biologia Animal) – Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2007. 100p.

De Pinna, M. C. C. (1991). Concepts and tests of homology in the cladistic paradigm. *Cladistics*, 7, 367–394.

Dias, T. S. (2007). Estudo da dieta de oito espécies da Subfamília Cheirodontinae (Characiformes: Characidae) em diferentes sistemas lacustres nos Estados do Rio Grande

do Norte e Rio Grande do Sul. Dissertação (Mestrado em Biologia Animal) – Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2007. 89 f.

Dillman, C. B., Sidlauskas, B. L. & Vari, R. P. (2015). A morphological supermatrix-based phylogeny for the Neotropical fish superfamily Anostomoidea (Ostariophysi: Characiformes): phylogeny, missing data and homoplasy. *Cladistics 2015,* 1–21.

Eigenmann, C. H. (1908). Preliminary descriptions of new genera and species of tetragonopterid characins. (Zoological Results of the Thayer Brazilian expedition.). *Bulletin of the Museum of Comparative Zoology, 52,* 91–106.

Eigenmann, C. H. (1909). Reports on the expedition to British Guiana of the Indiana University and the Carnegie Museum, 1908. Report no. 1. Some new genera and species of fishes from British Guiana. *Annals of the Carnegie Museum,* 6, 4–54.

Eigenmann, C. H. (1914). Some results from studies of South American fishes. II. The Glandulocaudinae (a new subfamily of characid fishes with innate potentialities for sexual dimorphism). *Indiana University Studies,* 20, 31–42.

Eigenmann, C. H., McAtee, W. L. & Ward, D. P. (1907). On further collections of fishes from Paraguay. *Annals of the Carnegie Museum, 4,* 110-157, Pls. 31–45.

Escalante, A. H. (1983). Contribución al conocimiento de las relaciones tróficas de peces de agua dulce del Área Platense. II. Otros Tetragonopteridae. *Limnobios,* 2, 379–402.

Eschmeyer, W. N. (ed). Catalog of fishes: Genera, Species, References. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 27 April 2015.

Farris, J. S. (1969). A successive approximations approach to character weighting. *Systematic Zoology,* 18, 374–385.

Farris, J. S. (1970). Methods for computer Wagner trees. *Systematic Zoology,* 19, 83–92.

Farris, J. S. (1983). The logical basis of phylogenetic analysis. In: N.I. Platnick, V.A. Funk (Eds) *Advances in Cladistics II* (pp. 7–36). New York: Columbia University Press.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* 39, 783–791.

Fink, W. L. & Weitzman, S. H. (1974). The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). *Smithsonian Contributions to Zoology*, 172, 1–46.

Goloboff, P. A. (1993). Estimating character weights during tree search. *Cladistics*, 9, 83–91.

Goloboff, P. A., Farris, J. S. & Nixon, K. C. (2003a). TNT: Tree Analysis Using New Technology. Version 1.1, February 2007. Software and documentation available from the authors and at www.zmuc.dk/public/phylogeny.

Goloboff, P. A., Farris, J. S. & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics*, 24, 774–786.

Hennig, W. (1966). Phylogenetic systematics. Ed. University of Illinois, Illinois, 263 p.

Higgins, D., Thompson, J., Gibson, T., Thompson, J. D., Higgins, D. G. & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A. & Wilson, A. C. (1984). DNA sequences from the quagga, an extinct member of the horse family. *Nature*, 213, 282–284.

Hillis, D. M., Pollock, D. D., McGuire, J. A. & Zwickl, D. J. (2003). Is Sparse Taxon Sampling a Problem for Phylogenetic Inference? *Systematic Biology*, 52, 124–126.

Huelsenbeck, J. P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogeny. *Methods of Biochemical Analysis*, 17, 754–755.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H. & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7, 544–548.

Javonillo, R., Malabarba, L. R., Weitzman, S. H. & Burns, J. R. (2010). Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Molecular Phylogenetic and Evolution*,54, 498–511.

Lambertz, M. & Dergam, J. A. (2015). Mining disaster: huge species impact. *Nature*, 528, 39.

Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012). PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution*, 29, 1695–1701.

Li, C., Orti, G., Zhang, G. & Lu, G. (2007). A practical approach to phylogenomics: The phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evolutionary Biology*, 7, 44.

Linderholm, A. (2015). Ancient DNA: the next generation – chapter and verse. Biological journal of the Linnean society, 117, 150–160.

Lucena, C. A. S. & Lucena, Z. M. S. (2002). Redefinição do gênero *Deuterodon* Eigenmann (Ostariophysi: Characiformes: Characidae). *Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia,* 15, 113–135.

Lucena, C. A. S. & Lucena, Z. M. S. (2013). Designation of a lectotype for *Deuterodon pedri* Eigenmann, 1908 (Characiformes: Characidae). *Zootaxa,* 3635, 598.

Lucena, C. A. S. & Soares, H. G. (2016) Review of species of the *Astyanax bimaculatus* "caudal peduncle spot" subgroup sensu Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. *Zootaxa,* 4072, 101–125.

Malabarba, L. R. (2003). Subfamily Cheirodontinae (Characins, tetras). In: Reis, R. E., Kullander, S. O., Ferraris, Jr., C. J. (Eds) *Check list of the freshwater fishes of South and Central America.* (pp. 215-221). Porto Alegre: Edipucrs.

Malabarba, L. R. & Weitzman, S. H. (2000). A new genus and species of inseminating fish (Teleostei: Characidae: Cheirodontinae: Compsurini) from South America with uniquely derived caudal-fin dermal papillae. *Proceedings of the Biological Society of Washington*, 113(1), 269–283.

Malabarba, L. R. & Weitzman, S. H. (2003). Description of a new genus with six new species from Southern Brazil, Uruguay and Argentina, with a discussion of a putative characid clade (Teleostei: Characiformes: Characidae). *Comunicações do Museu de Ciências e Tecnologia, PUCRS, Série Zoologia,* 16, 67–151.

Melo, F. A. G. (2005). Revisão taxonômica do complexo de espécies *Astyanax fasciatus* (Cuvier, 1819) (Teleostei: Characiformes: Characidae). Unpublished Ph.D. Dissertation. Museu Nacional, UFRJ.

Menezes, N. A., Weitzman, S. H., Oyakawa, O. T., Lima, F. C. T., Castro, R. M & Weitzman, M. J. (2007). Peixes de água doce da Mata Atlântica. São Paulo: Museu de Zoologia, Universidade de São Paulo Press.

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for inference of large phylogenetic trees.* Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, USA.

Mirande, J. M. (2009). Weighted parsimony phylogeny of the family Characidae (Teleostei: Characiformes). *Cladistics*, 25, 574–613.

Mirande, J. M. (2010). Phylogeny of the family Characidae (Teleostei: Characiformes) from characters to taxonomy. *Neotropical Ichthyology*, 8, 385–568.

Mirande, J. M., Aguilera G. & Azpelicueta, M. de las M. (2011). A threatened new species of *Oligosarcus* and its phylogenetic relationships, with comments of *Astyanacinus* (Teleostei: Characidae). *Zootaxa*, 2994, 1–20.

Mirande, J. M., Jerep, F. C. & Vanegas-Ríos, J. A. (2013). Phylogenetic relationships of the enigmatic *Carlastyanax aurocaudatus* (Eigenmann) with remarks on the phylogeny of the Stevardiinae (Teleostei: Characidae). *Neotropical Ichthyology*, 11, 747–766.

Nilsson, R. H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K. H. & Kõljalg, U. (2006).Taxonomic Reliability of DNA Sequences in Public Sequence Databases: A Fungal Perspective. *PLoS ONE* 1, e59. doi:10.1371/journal.pone.0000059

Nixon, K. C. & J. M. Carpenter. (2012). On homology. *Cladistics*, 28, 160–169.

Nylander, J. A. A. (2004). MrModeltest v2. Program distributed by the author. In: Uppsala Sweden, editor. Evolutionary Biology Centre. Uppsala University Available at: http://www.ebc.uu.se/systzoo/staff/nylander.html (accessed March 2010).

Oliveira, C., Avelino, G.S., Abe, K.T., Mariguela, T.C., Benine, R.C., Ortí, G., Vari, R.P. & Castro, R.M.C. (2011). Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. *BMC Evolutionary Biology*, 11, 275, 1–25.

Ornelas-Garcia, C. P., Domínguez-Domínguez, O. & Doadrio, I. (2008). Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. *BMC Evolutionary Biology*, 8, 340–357.

Patterson, C., Williams, D. M. & Humphries, C. J. (1993). Congruence between molecular and morphological phylogenies. Annual Review of Ecology, Evolution, and Systematics, 24, 153–88.

Pereira, T. L., Santos, U., Schaefer, C. E., Souza, G. O., Paiva, S. R., Malabarba, L. R., Schmidt, E. E. & Dergam, J. A. (2012). Dispersal and vicariance of Hoplias malabaricus (Bloch, 1794) (Teleostei, Erythrinidae) populations of the Brazilian continental margin. *Journal of Biogeography*, 40, 1–10.

Prevosti, F. J. & Chemisquy, M. A. (2010). The impact of missing data on real morphological phylogenies: influence of the number and distribution of missing entries. Cladistics, 26, 326–339.

Rambaut, A. & Drummond, A.J. (2004). Tracer v1.3http://evolve.zoo.ox.ac.uk/ software.

Riccomini, C., Velázquez, V. F., Gomes, C. B. (2005). Tectonic controls of the Mesozoic and Cenozoic alkaline magmatism in central-southeastern Brazilian Platform. In: Comin-Chiaramonti, P. & Gomes, C. B. (Eds) Mesozoic to Cenozoic alkaline magmatism in the Brazilian Platform (pp. 31–56).São Paulo: Edusp-Fapesp Press.

Ronquist, F., Teslenko, M., Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. John, P. & Huelsenbeck, J. P. (2012). MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology*, 61, 539–542.

Rothwell, G. W. & Nixon, K. C. (2006). How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? *International Journal of Plant Sciences*, 167, 737–749.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology*, 55, 421–438.

Silva, P.C., Bertaco, V.A., Malabarba L.R. (2017). Are the dentary teeth gradually decreasing and all arrangements that its provide a synapomorphy for a major group inside of Characidae family?. Article to be submitted to journal Neotropical Ichthyology.

Sorenson, M. D., Ast, J. C., Dimcheff, D.E., Yuri, T. & Mindell. D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution*,12, 105–114.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution,* 30, 2725–2729.

Taylor, W. R. & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage. *Cybium*, 9, 107–119.

Thomaz, A. T., Arcila, D., Ortí, G. & Malabarba, L. R. (2015) Molecular phylogeny of the subfamily Stevardiinae Gill, 1858 (Characiformes: Characidae): classification and the evolution of reproductive traits. *BMC Evolutionary Biology*, 15, 146.

Will, K. W., Rubinoff, D. (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*, 20, 47–55

Zanata, A. M. (1997). *Jupiaba*, um novo gênero de Tetragonopterinae com osso pélvico em forma de espinho (Characidae, Characiformes). Iheringia, Série Zoologia (Porto Alegre) No. 83: 99-136.

Zander, R. H. (2004). Minimal Values for Reliability of Bootstrap and Jackknife Proportions, Decay Index, and Bayesian Posterior Probability. *Phyloinformatics*, 2, 1–13.

Zwickl, D.J., & Hillis, D.M. (2002). Increased taxon sampling greatly reduces phylogenetic error. Systematic Biology; 51, 588–598.

Tables and Figures

Table 1 Information content, molecular model of evolution and characteristics of each molecular data partition

	Gene			
	COI	ND2	MYH6	SH3PX3
Number of sequences	40	30	36	25
bp after alignment	699	903	779	724
Number of variable sites	233	618	110	175
Number of informative characters under parsimony	205	574	53	73
% informative characters under parsimony	29.3	63.5	6.8	10
Π_A	0.24	0.32	0.30	0.25
Π_C	0.25	0.26	0.21	0.27
Π_G	0.18	0.13	0.24	0.28
Π_T	0.32	0.29	0.25	0.20
Minimum *p*-distance among sequences	0.00	0.00	0.00	0.00
Overall mean genetic distance (*p*-distance)	0.13	0.27	0.02	0.04
maximum *p*-distance among sequences	0.22	0.45	0.07	0.18
Molecular model of evolution	GTR+I+G	GTR+G	GTR+I+G	GTR+G

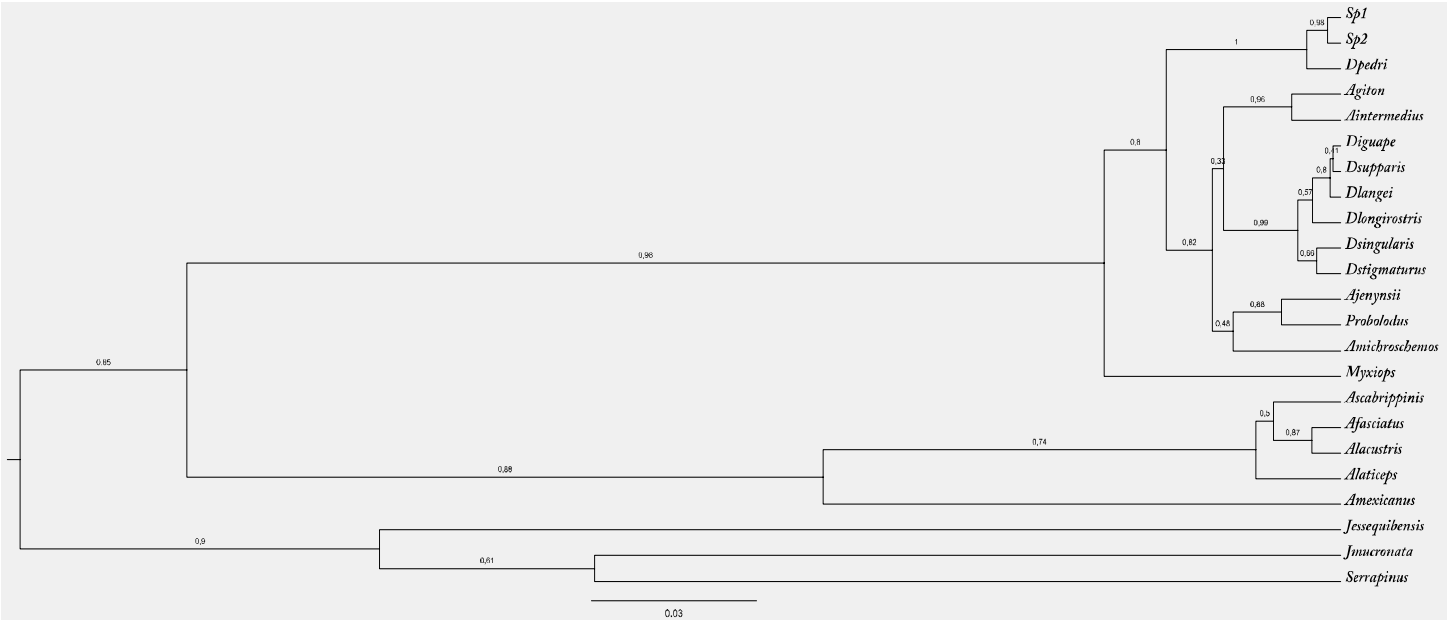


Figure 1: Species tree Bayesian based generated with 4 genes: COI, ND2, MYH6 and SH3PX3. The numbers above the branches are the posterior probability.

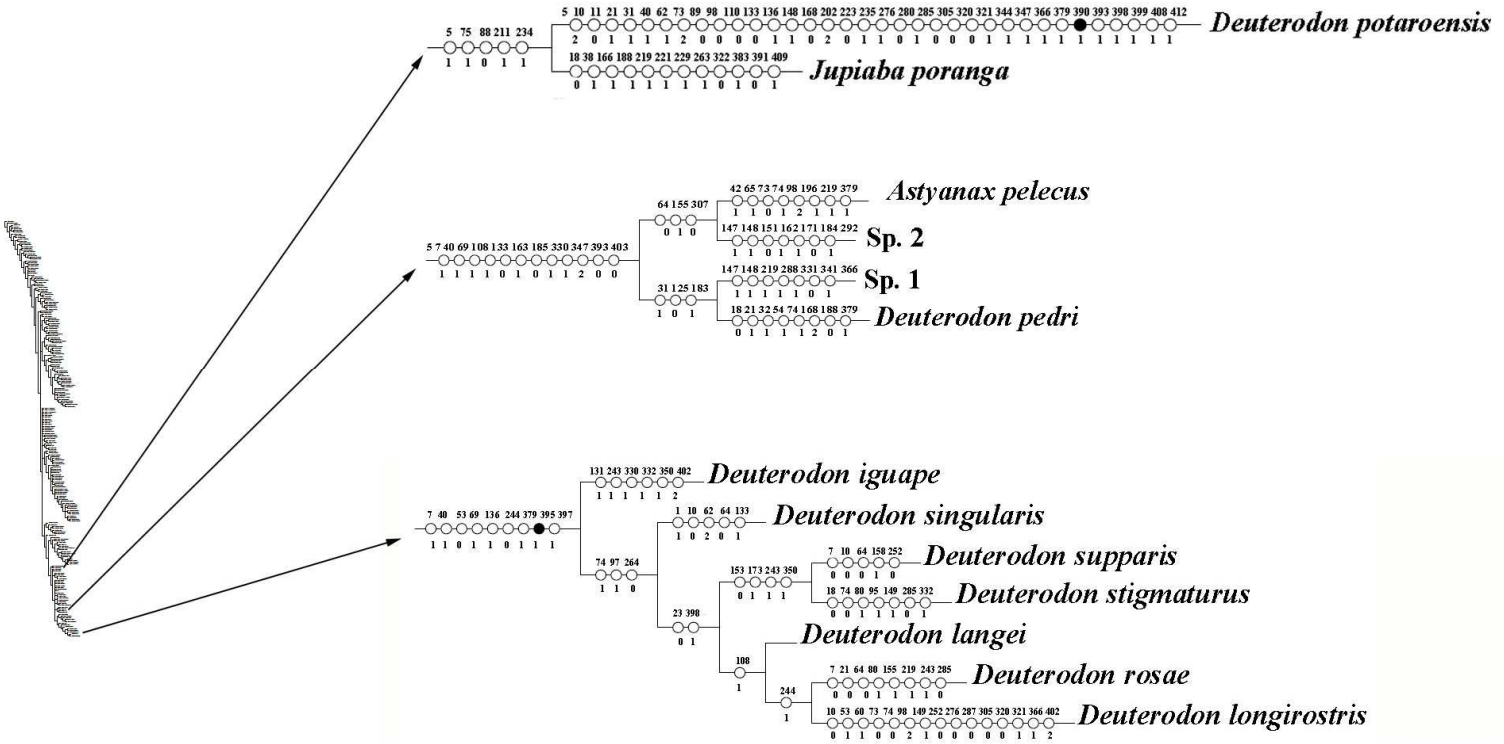


Figure 2: Consensus of most parcimonious trees under equal weighting. The analysis recovered *Deuterodon sensu lato* as poliphyletic.

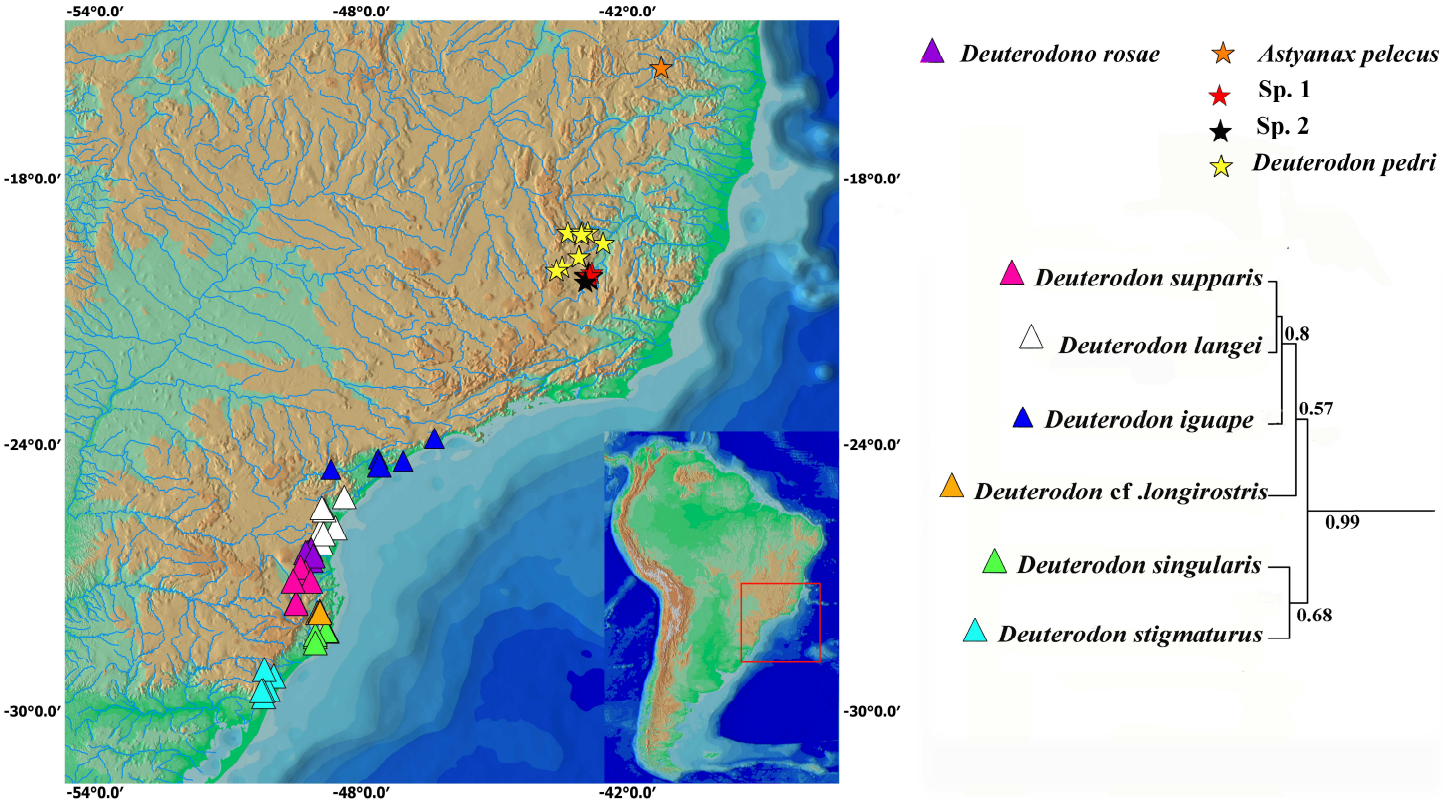


Figure 3: Distribution of the species of *Deuterodon sensu stricto*. The phylogeografic patterns is recovered by the species tree bayesian based: species of *Deuterodon* with distribution restrict to river basins at southest of distribution and belonging to the same paleodrainage (*D. singularis* and *D. stigmaturus*) are forming a monofiletic clade that is sister group of the remain species that are distributed to river basins that belong to the same paleodrainage located at North. Triangles are representing the occurrence area of each species considered in the study.

Appendix material

S1. Specimens used to molecular analyses

Voucher	Species	Sample number	Locality	Genbank acss number
UFRGS 18508	*Deuterodon langei*	TEC4103	Paranaguá River basin	KY327419
UFRGS 18525	*Deuterodon iguape*	TEC 4138	Ribeira do Iguape River basin	KY327420
UFRGS 20032	*Deuterodon iguape*	TEC 4130	Ribeira do Iguape River basin	KY327421
UFRGS 18495	*Deuterodon suparis*	TEC 4651	Itajaí River basin	KY327422
UFRGS 18518	*Deuterodon singularis*	TEC4087	Tubarão River basin	KY327423
UFRGS 16519	*Deuterodon stigmaturus*	TEC2847	Rio Três Forquilhas	KY327424
UFRGS 16208	*Deuterodon stigmaturus*	TEC2350	Maquiné River basin	KY327425
UFRGS 18629	*Deuterodon langei*	TEC3935	Cubatão River basin	KY327426
UFRGS18913	*Astyanax jenynsii*	TEC4271	Paraíba do Sul River basin	KY327427
UFRGS 17542	*Astyanax michroschemos*	CT1936	Doce River basin	KY327428
UFRGS 17542	*Astyanax michroschemos*	CT1940	Doce River basin	KY327429
UFRGS19058	*Astyanax giton*	TEC4033	Doce River basin	KY327430
MZUFV 4459	*Astyanax giton*	CT3464	Doce River basin	KY327431
MZUFV 4458	*Astyanax intermedius*	CT2801	Doce River basin	KY327432
UFRGS18894	*Astyanax intermedius*	TEC4554	São João River basin	KY327433
MCP 47661	*Deuterodon pedri*	CT2521	Doce River basin	KY327434
UFRGS17543	*Deuterodon pedri*	CT2529	Doce River basin	KY327435
MZUFV3992	Sp1	CT2353	Doce River basin	KY327436
MZUFV3992	Sp1	CT2765	Doce River basin	KY327437
MZUFV 4457	Sp2	CT2965	Doce River basin	KY327438
MZUFV 4457	Sp2	CT2971	Doce River basin	KY327439
UFRGS18957	*Astyanax lacustris*	TEC4772	Santa Maria da Vitória River basin	KY327440
UFRGS19055	*Astyanax lacustris*	TEC4030	Tiririca lake, Doce River basin	KY327441
UFRGS 18503	*Astyanax laticeps*	TEC4113	Ribeira de Iguapé River basin	KY327442
UFRGS 18503	*Astyanax laticeps*	TEC4115	Ribeira de Iguapé River basin	KY327443
MZUFV 4456	*Astyanax scabripinnis*	CT2772	Doce River basin	KY327444
MZUFV 4456	*Astyanax scabripinnis*	CT2773	Doce River basin	KY327445
UFRGS19070	*Astyanax* aff. *fasciatus*	TEC4074	Doce River basin	KY327446
UFRGS19746	*Astyanax* N sp	TEC5291	Tripuí River, Doce River basin	KY327447
UFRGS 19147	*Astyanax fasciatus*	TEC4865A	Tramandaí River basin	KY327448
UFRGS 19147	*Astyanax fasciatus*	TEC4865 B	Tramandaí River basin	KY327449
UFRGS 19135	*Astyanax fasciatus*	TEC4853A	Tramandaí River basin	KY327450
UFRGS 19135	*Astyanax fasciatus*	TEC4853B	Tramandaí River basin	KY327451
UFBA 07798	*Myxiops aphos*	A	Paraguaçu drainage	KY327452
UFBA 07798	*Myxiops aphos*	B	Paraguaçu drainage	KY327453
ROM96089	*Jupiaba essequibensis*	T15810	Essequibo River, Guyana	KY327454
ROM96166	*Jupiaba mucronata*	T16213	Guyana	KY327455
UFRGS18758	*Probolodus heterostomus*	TEC4184	Paraíbuna River, Paraíba do Sul River basin	KY327456
UFRGS22004	*Serrapinus heterodon*	TEC6956	Doce River basin	KY327457

Voucher	Species	Sample number	Locality	Genbank acss number
UFRGS18431	*Hyphessobrycon luetkenii*	TEC3824	Maquiné River, Tramandaí River basin	KY327458
UFRGS19226	*Hyphessobrycon luetkenii*	TEC4921	Mostardas River	KY327459
UFRGS 19342	*Astyanax taeniatus*	TEC4997	Macaé River basin	KY327460
UFRGS 19342	*Astyanax taeniatus*	TEC5000	Macaé River basin	KY327461
UFRGS 18516	*Astyanax ribeirae*	TEC 4112	Ribeira do Iguape River basin	KY327462
UFRGS 20032	*Astyanax ribeirae*	TEC 4137	Ribeira do Iguape River basin	KY327463
UFRGS 18904	*Astyanax hastatus*	TEC 4527	Macaé River basin	KY327464
UFRGS 18906	*Astyanax hastatus*	TEC 4529	Macaé River basin	KY327465
MCZ17510	*Deuterodon pedri*	lectotype	Santo Antônio River, Doce River basin, Ferros, Minas Gerais, Brazil	

Capítulo 3

Using ancient DNA to unravel taxonomic puzzles: the identity of *Deuterodon pedri* (Ostariophysi: Characidae)

Artigo pubicado na Revista Neotropical Ichtiology

Print version **ISSN 1679-6225**
On-line version **ISSN 1982-0224**

Volume: 15, número:1, 2017

Doi: http://dx.doi.org/10.1590/1982-0224-20160141

Priscilla C. Silva, Maria C. Malabarba and Luiz R. Malabarba

Neotropical Ichthyology, 15(1): e160141, 2017
DOI: 10.1590/1982-0224-20160141

Journal homepage: www.scielo.br/ni
Published online: 03 April 2017 (ISSN 1982-0224)
Printed: 31 March 2017 (ISSN 1679-6225)

Using ancient DNA to unravel taxonomic puzzles: the identity of *Deuterodon pedri* (Ostariophysi: Characidae)

Priscilla C. Silva, Maria C. Malabarba and Luiz R. Malabarba

Accurate identification is essential for any study exploring biodiversity. Unfortunately, museum type specimens preserved for more than a hundred years are often not informative enough for precise identification of the species represented by the name-bearing type. The use of ancient DNA can help solve taxonomic problems when name-bearing types no longer have diagnostic morphological features that allow for an accurate identification of the species involved. That is the case for *Deuterodon pedri*, an endemic species from a small drainage in the rio Doce basin in Minas Gerais, Brazil, for which the type material is in poor condition. Specimens of *D. pedri* were collected in 1865 by the Thayer Expedition to Brazil and fixed in spirits, enabling them to yield viable DNA. As the morphology alone of the type material does not allow for an accurate identification, we used both morphological and ancient DNA (aDNA) methods to decisively establish the identity of *D. pedri*. This identification allowed us to recognize the species among recently collected specimens and then, based on them, redescribe the species. A genetype for the lectotype of *D. pedri* is presented.

Keywords: Lectogenetype, Mini-Barcode, Primers, Rio Doce, Thayer Expedition.

Uma identificação acurada é fundamental para qualquer estudo que explora a biodiversidade. Infelizmente, espécimes de museu descritos há mais de cem anos, algumas vezes não são informativos o suficiente para uma identificação precisa da espécie representada pelo tipo. O uso de DNA antigo pode ajudar a resolver problemas taxonômicos, quando espécimes tipos não apresentam mais as características morfológicas diagnósticas que permitem a identificação precisa das espécies. Esse é o caso de *Deuterodon pedri*, uma espécie endêmica de uma pequena drenagem na bacia do rio Doce, em Minas Gerais, Brasil cujo material tipo encontra-se em condições precárias. Espécimes de *D. pedri* foram coletados em 1865 pela Expedição Thayer ao Brasil e fixados em "cachaça", o que permite apresentar DNA viável. Como apenas o exame morfológico do material tipo não permitiria a identificação precisa, nós usamos ambos os dados de análises morfológicas e DNA antigo (aDNA) para estabelecer decisivamente a identidade de *D. pedri*. Esta identificação permitiu reconhecer a espécie entre exemplares coletados recentemente e, com base neles, redescrever a espécie. É apresentado um genetipo para o lectótipo de *D. pedri*.

Palavras-chave: Expedição Thayer, Lectogenetipo, Mini-Barcode, Primers, Rio Doce.

Introduction

Taxonomy is fundamental to the biological sciences. More than merely labeling biodiversity, taxonomy is essential for any study exploring biodiversity. Thus, the accurate identification of life forms is crucial not only to understanding biodiversity but also for any taxonomy-based study, whether phylogenetic, evolutionary, inventorial, ecological or conservation-focused (Buerki, Baker, 2016; Vecchione *et al.*, 2000). As the traditional repository for biological specimens, and recent years also for tissue samples, museum collections are a valuable resource for mapping and naming biodiversity. However, independent of the collecting and storage methods, museum materials tend to degrade over time. Century-old name-bearing types are often involved in nomenclatural doubts and ambiguities because they no longer exhibit the diagnostic features that allow an accurate identification (Cappellini *et al.*, 2013). The use of ancient DNA (aDNA) can help to solve these taxonomical problems.

Ancient DNA techniques were first used in 1984 to recover DNA from a 150-year-old museum specimen of an extinct subspecies of the Plains Zebra *Equus quagga* (Higuchi *et al.*, 1984). In that study, aDNA was used to solve identification problems and was sufficient for determining the phylogenetic relationships of the species, which allowed the development of a project for breeding and re-introducing the Quaggas (http://www.quaggaproject.com/quagga-dna-results.htm).

Departamento de Zoologia and Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil. (PCS) pricarola@gmail.com (corresponding author), (MCM) claudia.malabarba@ufrgs.br, (LRM) malabarb@ufrgs.br

Deuterodon pedri Eigenmann, 1908 was collected in 1865 by Ward during the Thayer Expedition to Brazil (see www.mcz.harvard.edu/Departments/Ichthyology/expeditions_thayer_hassler.html; Higuchi, 1996). As usual then, the specimens were fixed in an available spirit ("cachaça" in this case), which could yield viable DNA sequences (De Bruyn *et al*., 2011). The adoption of formaldehyde fixation made DNA difficult to amplify from more recent collections because formaldehyde fixation degrades DNA and cross-links DNA, DNA to protein, and protein to protein (Schander, Halanych, 2003). Eigenmann (1908) briefly described the species based on seven specimens, which were in very poor conditions (Fig. 1). Later, Eigenmann (1927:348) provided a more complete description of the species, indicating the same seven damaged specimens as cotypes (=syntypes) and again emphasized their poor conditions. In a more recent redefinition of the genus *Deuterodon* Eigenmann, 1907, Lucena, Lucena (2002) placed the species *D. pedri* as *incertae sedis* in Characidae. After inspection of two of the syntypes, Lucena, Lucena (2013) designated a lectotype (MCZ 21081) and consequently four paralectotypes (MCZ 170510) for *D. pedri*, observing that only five specimens (not seven) were registered as syntypes at the collection database of the Museum of Comparative Zoology. Lucena, Lucena (2013) also commented that the only paralectotype they examined clearly belonged to a species different from the lectotype.

In 2015, as part of her doctoral dissertation, one of the authors (PCS) had the opportunity to examine the types in an attempt to identify *D. pedri* and recognize it among recently collected material from the type locality. However, more than a hundred years after the description, the original specimens had deteriorated so severely that they no longer provide morphological information sufficient for a definitive identification of the species based on morphological traits alone. Thus, we sought to resolve the identity of *D. pedri* by combining the scant morphological information available with DNA data from the type specimens.

Ancient specimens usually contain highly degraded nucleic acid molecules (Linderholm, 2016), which directly complicate the amplification process. In addition, probably because of their poor conditions, in 1978 the syntypes of *D. pedri* (Fig. 1) were re-fixed in 10% formalin for 28 days, which further degraded the DNA and made the amplification and sequencing even more difficult. We applied a DNA protocol that proved successful for accessing the ancient information stored in the genomes of these museum materials. The sequences obtained from selected *Cytochrome oxidase c subunit 1* (COI) regions of the type specimens were compared with those of fresh specimens from the type locality and nearby localities to find a match and definitively identify the species. The aDNA sequences from the lectotype matched those from some of the fresh specimens, enabling the correct recognition and redescription of *D. pedri*. The *D. pedri* lectogenetype here presented corresponds to the DNA sequence obtained from the lectotype. The term was proposed by Chakrabarty (2010), to indicate DNA sequences generated from type specimens.

Fig. 1. *Deuterodon pedri*, MCZ 21081, lectotype, 78.56 mm SL and original labels.

Material and Methods

Tissue collection, DNA extraction and sequence generation for museum specimens. A 2 mg fragment of epaxial muscle tissue was removed from the right side of the body by incision from the lectotype (MCZ 21081) and one of the paralectotypes (MCZ 170510, 58.6 mm SL) of *D. pedri*. The tissue samples were processed in the molecular biology facilities of the Smithsonian National Museum of Natural History. DNA was extracted using the QIAamp DNA micro kit (Qiagen) following the manufacturer's protocol. A negative control containing no sample was prepared and analyzed following the same procedure used for the ancient samples. The extractions were conducted in a dedicated laboratory area free from DNA and PCR products (amplicons); meanwhile PCRs were carried out in an isolated section of a different laboratory to avoid contamination from other DNA extracts (Gilbert *et al*., 2005). Given the highly fragmented nature of the aDNA (Linderholm, 2016), the primers were designed in this study to flank small fragments of 100 - 150 bp of the COI gene (see Tab. 1). We designed 5 sets of primers to cover the entire COI gene, but only the two first sets were successfully amplified. PCRs were performed in a volume of

10 µL of a Promega Hotstart Master Mix under commercial recommendations. The PCR products were purified by the Exosap enzymatic method (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50% deionized water), and sequencing was performed at the Laboratory of Analytical Biology at National Museum of Natural History, Smithsonian, Washington DC. Each fragment of sequence was independently aligned using Clustal W in MEGA 6.0 software (Tamura *et al*., 2013). The p-distance between the ancient sequence and modern ones was estimated using the default conditions (Kimura 2-parameter model; d: Transitions + Transversions; uniform rates; Pairwise deletion; three codon positions selected) of the MEGA 6.0 software (Tamura *et al*., 2013). To illustrate the relationship among the sequences, a Neighbor Joining tree (using the same default conditions for calculating of p-distance) was constructed in MEGA. Additionally, polymorphic sites were identified using DnaSP software (Librado, Rosas, 2009) and a haplotype network was drawn using Network 5.0 software (Fluxus technology Ltd.).

Tab. 1. COI DNA primers designed for this study.

	Primer Sequence Left	Primer Sequence Right
COI - 1	5' GTATTYGTTGCCTGAGCYGG 3'	5' TATRACRAARGCATGTGCGG 3'
COI - 2	5' WTCCCTTTTAGGTGAYGACC 3'	5' KGGRGGAAGAAGYCARAAGC 3'

Tissue collection, DNA extraction and sequencing for modern specimens. For the comparison with museum samples, we sequenced 699 base pairs of the COI gene for 47 individuals from 30 species (**S1**- Available only as online supplementary files accessed with the online version of the article at http://www.scielo.br/ni). Tissues previously fixed in 96% ethanol from the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Museu de Zoologia João Moojen da Universidade Federal de Viçosa (MZUFV), Royal Ontario Museum (ROM), and Universidade Federal da Bahia (UFBA). DNA was extracted from the gill filaments, muscles, or liver tissue of the samples using the "Phire Animal Tissue Direct PCR Kit" developed by Thermo Scientific® under commercial recommendations. COI was amplified with the primer cocktails FishF1t1 and FishR1t1 (Ivanova *et al*., 2007).

The PCR reactions were conducted in a reaction volume of 20 µL [10.3 µL of H2O, 2 µL of 10× reaction buffer (Platinum®Taq), 0.6 µL of MgCl2 (50 mM), 2 µL of dNTPs (2 mM), 2 µL of each primer (2 µM), 0.1 µL (5 U) of Platinum® Taq (Invitrogen), and 100 ng of template DNA]. The PCR conditions were as follows: an initial DNA denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 30 s, 52°C for 40 s, and 72°C for 1 min and a final extension at 72°C for 10 min.

The PCR products were purified using the Exosap enzymatic method (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50% deionized water), and the sequencing was performed by Macrogen Inc, Seoul, South Korea, and by Ludwig Biotec at Porto Alegre, Brazil. The sequences were aligned using Clustal W in MEGA 6.0 software (Tamura *et al*., 2013), and the alignments were visually inspected for any obvious base miscall (base incorporated at the sequence different from the color pic showed at chromatograms). All work involving modern DNA was performed at the molecular biology laboratory of Departamento de Zoologia (UFRGS, Porto Alegre, RS, Brazil), with separately ordered primers (Ishida *et al*., 2011).

Morphological techniques. Measurements and counts followed Fink, Weitzman (1974), with the exception of the number of scale rows below the lateral line, which were counted from the scale row ventral to the lateral line to the scale row nearest to the origin of the first pelvic-fin ray.

The measurements were taken point to point with an electronic caliper on the left side of specimens. Measurements are expressed as the percentage of standard length (SL) except for subunits of the head, which are recorded as percents of head length (HL). The counts of vertebrae, supraneurals, and procurrent caudal-fin rays were taken from cleared and stained specimens (c&s). The vertebral counts included the four vertebrae of the Weberian apparatus, and the terminal centrum counted as a single element.

Institutional abbreviations. MCN, Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre; MCP, Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MCZ, Museum of Comparative Zoology of Harvard University, Cambridge; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; UFRGS, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre.

Results

Molecular analyses of type sequences. In the lectotype (MCZ 21081), only two sets of the five designed primers directly in-line succeeded in recovering the first third of the COI sequence with 136 (COI-1: starting at bp1) and 179 (COI-2: starting at bp115) base pairs each. Since the COI-1 sequence showed a p-distance 0.1 or 10% from recently collected material from the Santo Antônio River basin, which is the type locality of *D. pedri*, and a distance greater than 0.1 from all other characid species included in the alignment, it was excluded from further analysis (see

additional comments on the Discussion). Concerning the paralectotype (MCZ 170510, 58.6 mm SL), although the DNA extraction was successful, the amplification (PCR) failed. The COI-2 lectotype sequence was compared separately with the characid sequences of 48 species of *Astyanax* Baird & Girard, *Deuterodon*, *Hyphessobrycon* Durbin, *Jupiaba* Zanata, *Myxiops* Zanata & Akama, and *Probolodus* Eigenmann from the coastal and the rio Doce drainages. All data referring to this comparative material, including the GenBank accession numbers, (are listed in **S1** - Available only as online supplementary files accessed with the online version of the article at http://www.scielo.br/ni). The lectotype COI-2 sequence (accession number KY345055) showed the lowest p-distance (p=0.01=1%; **S2** - Available only as online supplementary files accessed with the online version of the article at http://www.scielo.br/ni) to a characid fish population recently collected from the rio Santo Antônio in the rio Doce basin (MCP 47661 and UFRGS 17543), indicating that these specimens actually correspond to *D. pedri* (Fig. 2).

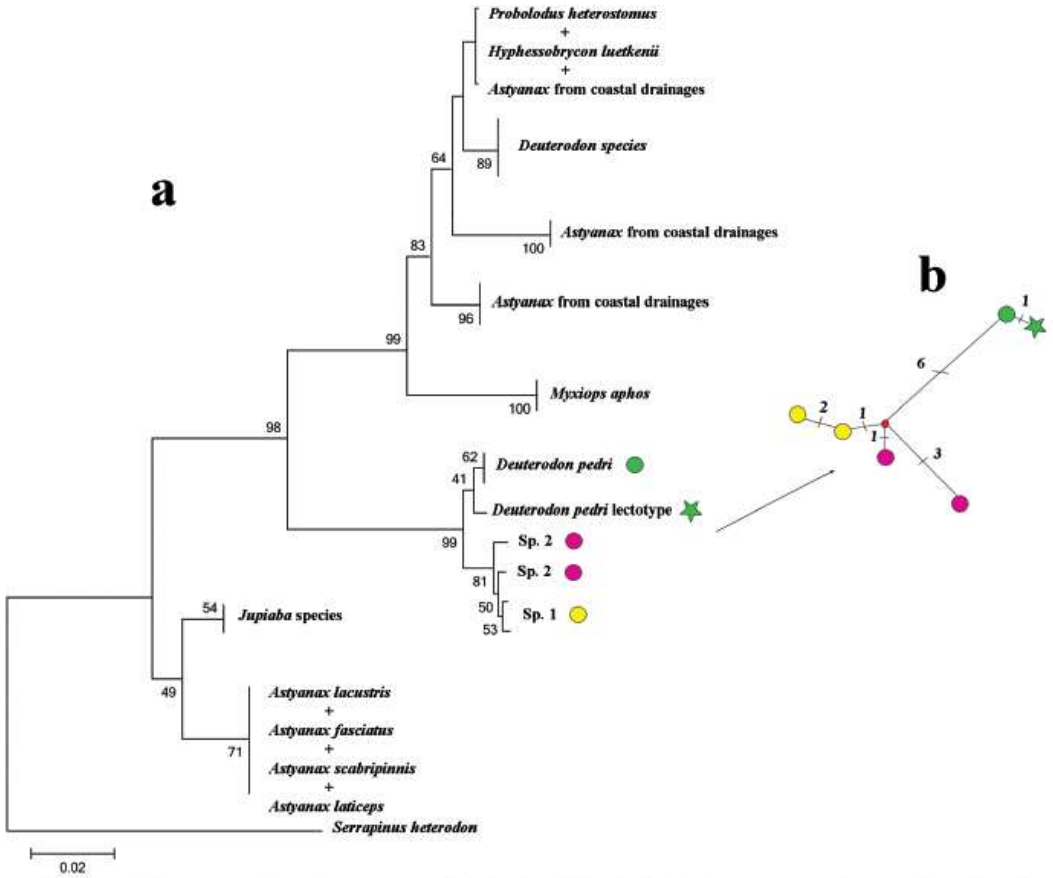


Fig. 2. Neighbor Joining tree and Haplotype network showing high similarity between specimens collected at rio Santo Antônio basin and sequence of the lectotype of *Deuterodon pedri*. **a.** Neighbor joining tree with bootstrap values. **b.** Haplotype network of *D. pedri* clade. Numbers in each branch of the net refer to number of mutational steps between haplotypes.

Morphological analyses of type specimens and of specimens identified by Eigenmann as *Deuterodon pedri*. The examination of the type series of *D. pedri* detected two different species among the syntypes. The lectotype (MCZ 21081, 78.6 mm SL) and two of the paralectotypes (MCZ 170510, 72.1 and 76.3 mm SL) have dentary teeth decreasing gradually in size posteriorly, bearing seven cusps each (Figs. 3a,c). This same tooth arrangement is observed in the characid samples from the rio Santo Antônio and rio Piracicaba populations, both of which are western tributaries of the rio Doce. That similarity is coincident with the analysis of the aDNA amplified from the lectotype, and also matches the DNA from the Santo Antônio river population. As with the lectotype, these two paralectotypes and the fresh specimens from these populations do not differ in other counts and measurements. Thus they are considered conspecific and the newly-collected samples from the rio Doce basin are used below in the redescription of *D. pedri*.

Two of the paralectotypes (MCZ 170510, 58.6 and 71.4 mm SL), however, have dentary teeth decreasing abruptly in size after the fifth (Figs. 3b,d), with five cusps on the three anterior dentary teeth instead of seven. These do not belong to *D. pedri*. Previously, Lucena, Lucena (2013) noted that at least one paralectotype was distinct from the lectotype, but it was left as an unidentified species. Comparison with recent material from Santana de Ferros (Ferros, state of Minas Gerais) and with the type series of *Astyanax intermedius* Eigenmann, 1908, allowed the identification of these two paralectotypes of *D. pedri*, as *A. intermedius*.

Furthermore in the original description of *Deuterodon pedri*, Eigenmann listed five lots (MCZ 20956-20960; Fig. 4) collected by Dom Pedro II, the Brazilian Emperor, at Santa Cruz, Rio de Janeiro State. Although Eigenmann (1908, 1927) did not include this additional material as part of the type series, he mentioned the possibility that they belong to *D. pedri*. Due to the poor conditions of the types, Eigenmann (1908:99, 1927:348) claimed that an "absolute morphologically identification is impossible." Examination of two of these five lots (MCZ 20956 and 20958) allowed us to reject their assignment to *D. pedri*. These specimens present bony hooks distributed on the anal-, dorsal-, pectoral-, pelvic- and caudal-fin rays, instead of bony hooks only on the anal-fin rays, as observed in *D. pedri*. In addition, these specimens have fewer lateral line scales (35-37 *vs*. 39-41 in *D. pedri*) and a humeral spot with the dorsal portion expanded like a large dot and the ventral portion narrow and curved anteriorly (like a comma) rather than bar-shaped as in *D. pedri*. In view of that, we propose that these lots collected by Dom Pedro II be assigned to *Astyanax hastatus* Myers, 1928, which presents these same features.

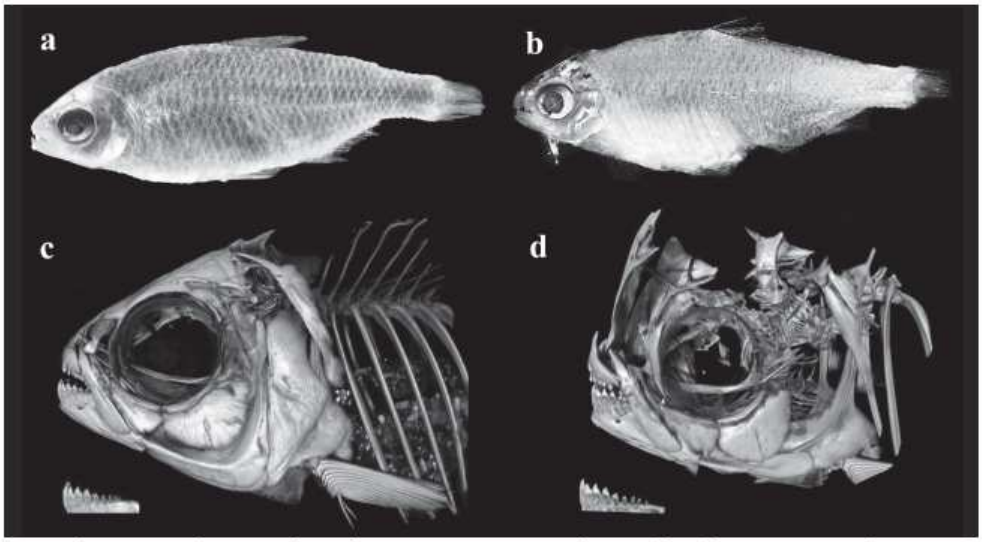


Fig. 3 Two paralectotypes of *Deuterodon pedri*, MCZ 17510. **a-c.** Photograph and X-ray computed tomography of the paralectotype with 72.1 mm SL, showing the dentary teeth decreasing gradually in size, corresponding to *D. pedri*. **b-d.** Photograph and X-ray computed tomography of the paralectotype with 58.6 mm SL, showing the dentary teeth decreasing abruptly, corresponding to *Astyanax intermedius*.

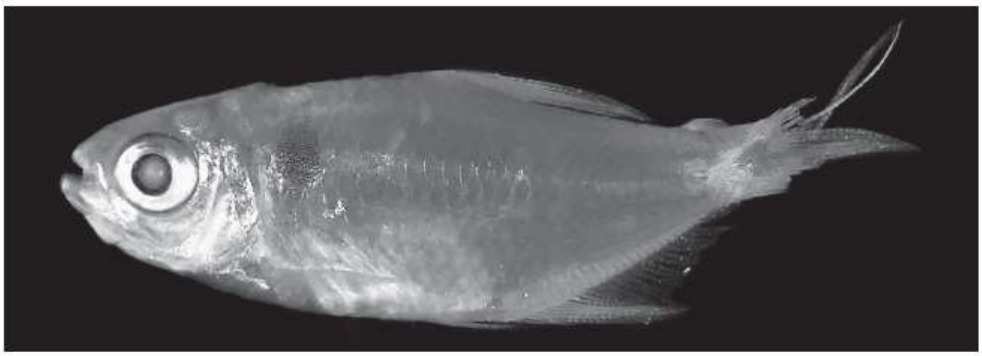


Fig. 4. Specimen of *Astyanax hastatus* from Santa Cruz, Rio de Janeiro (MCZ 20958, 33.0 mm SL), tentatively identified as *Deuterodon pedri* by Eigenmann (1908).

***Deuterodon pedri* Eigenmann, 1908**

Figs. 1, 3a-b, 5-7, Tab. 2

Deuterodon pedri Eigenmann, 1908:98 (brief description). -Eigenmann, 1927:348 (description; type locality: Santa Anna de Ferros, Minas Gerais, Brazil). -Lucena, Lucena, 2002: 119 (placed as *incertae sedis* in Characidae). -Lucena, Lucena, 2013: 598 (MCZ 21081 designated as lectotype). -Coutinho-Sanches, Dergam, 2015: 9 (Cytogenetic data).

Diagnosis. *Deuterodon pedri* can be distinguished from all congeners by the following combination of characters: a characteristic pigmentation on the two or three longitudinal and dorsolateral series of scales below the dorsal fin, each scale showing an arched and well-delineated strip at the distal margin of the free border; this strip is either dark brown when chromatophores are expanded (Fig. 5a) or translucent when chromatophores are contracted (Fig. 5b) – in either case it is clearly distinguishable from the pigmentation of the whole scale; dentary teeth decreasing gradually in size; number of anal-fin rays 18-21 in females (n=26) and 22-24 in males (n=3); longitudinal lateral silver band starting 5 or 6 scales posterior to upper margin of the opercle; humeral spot bar-shaped, vertically elongated above and below the lateral line; bony hooks only on anal-fin rays of mature males.

Fig. 5. Detailed images of the dorsolateral series of scales in *Deuterodon pedri*, showing their pigmentation pattern, each scale showing an arched and well delineated strip at the distal margin of the free border that is either dark brown when chromatophores are expanded (**a.** MNRJ 38463, 51.18 mm SL) or translucent when chromatophores are contracted (**b.** MCN 19698, 85.70 mm SL).

Deuterodon pedri can be further differentiated from morphologically similar species of the genus *Astyanax* that occur in Atlantic coastal river basins by the higher number of perforated scales in the lateral line (39-41 *vs.* 35-38 in most of species), except *A.* aff. *fasciatus* (Cuvier, 1819), *A. parahybae* Eigenmann, 1908 and *A. taeniatus* (Jenyns, 1842). From *A.* aff. *fasciatus*, *A. parahybae* and *A. scabripinnis* (Jenyns, 1842) it can be distinguished by the dentary teeth decreasing gradually in size posteriorly (*vs.* dentary teeth decreasing abruptly after the fourth tooth). From *A. taeniatus*, *D. pedri* can be distinguished by the absence of a gap between the symphyseal teeth of dentary and by a rectangular and vertically elongate humeral spot (*vs.* the presence of a gap between the symphyseal dentary teeth and humeral spot shaped like a comma).

Description. Morphometric data are summarized in Tab. 2. Body compressed and elongated; deepest at dorsal-fin origin. Snout profile slightly rounded from margin of upper lip to vertical through anterior nostrils. Dorsal profile of head straight between vertical through posterior nostril and tip of supraoccipital spine. Body profile convex from tip of supraoccipital spine to dorsal-fin base; ventrally slanted from this point to caudal peduncle. Ventral profile of body convex from margin of lower lip to pelvic-fin origin, and straight from that point to anal-fin origin. Body profile along anal-fin base dorsally slanted. Caudal peduncle elongated and nearly straight to slightly concave along both dorsal and ventral margins.

Head small. Mouth terminal or slightly sub-terminal. Maxilla extending posteriorly to vertical through anterior margin of orbit, slightly oblique. Anterodorsal border of maxilla slightly concave, posterodorsal border slightly convex, and ventral border convex.

Premaxilla with two tooth rows; outer row with three (6) or four (5) teeth bearing four, five or six cusps with central cusp longer. Five teeth (11) on inner row, gradually decreasing in size from first to fifth teeth. Symphyseal premaxillary teeth of inner series distinctively narrower than other teeth and asymmetrical, with two or three short cusps shorter on medial side near symphysis, followed by a high cusp and another three or four short cusps on lateral side of tooth. Teeth with five to nine cusps, with central cusp longer and as broad as other cusps. Maxilla with three (10) or four (1) teeth with five to seven cusps (usually 5 or 6), central cusp longest. Seven or six anteriormost dentary teeth larger than other teeth, with five to eight cusps, followed by three or four teeth gradually decreasing with three to five cusps. Central cusp in all teeth as long and broad as other cusps. Symphyseal teeth of dentary narrower than others with seven or eight cusps.

Dorsal-fin rays ii,9(27). Distal margin of dorsal fin straight or slightly convex. Dorsal-fin origin approximately at middle of SL. Anal-fin rays ii-iv, 18(5), 19(15), 20(4), 21(2) in females and 22(1), 23(1), 24(1) in males. Anal-fin distal border concave, with rays decreasing in size, with anterior-most rays much longer than others. Anal-fin origin located approximately on vertical through base

of posterior third portion of dorsal-fin. Pectoral-fin rays i, 11(4), 12(15), 13(9). Pectoral-fin tip falls one or two scales short of vertical through pelvic-fin insertion or reaching pelvic-fin origin in some specimens. Pelvic-fin rays i(9), ii(18), 7(27). Dorsal-fin origin located at vertical line through first third part of pelvic-fin. Tip of adpressed pelvic fin falls one or two scales short of anal-fin origin. Caudal-fin forked with 18(1), 19(22), 20(2) principal rays. Dorsal procurrent rays 11(1) or 12(1). Ventral procurrent rays 10(2).

Lateral line slightly curved anteriorly, completely pored, with 38(2), 39(10), 40(13) or 41(4) (mean= 39.7, n = 29) perforated scales. Horizontal scale rows between dorsal-fin origin and lateral line 5(26) or 6(1). Horizontal scale rows between lateral line and pelvic-fin origin 4(27). Pre-dorsal scales 9(1), 10(5), 11(15), 12(6), arranged in regular or irregular series. Thirteen (12) or fourteen (13) scale rows around caudal peduncle. Scale sheath along anal-fin base formed by six to ten scales in a single series and covering base of anteriormost rays.

Precaudal vertebrae 16(2); caudal vertebrae 21(2); total vertebrae 37(2). Supraneurals 5(2). First gill-raker upper limb of 6(9), 7(4), or 8(2) + lower branch 11(8), 12(6) or 13(1). Anal pterygiophores 20(2). Dorsal pterygiophores 10(2).

Coloration in alcohol. Dorsal and dorsolateral portions of head light brown. Infraorbitals, preopercle and opercular bones silver, without chromatophores or rarely a few. Lips yellow to light brown, snout with concentration of few chromatophores. Dorsal and dorsolateral portion of body dark brown. Scales above lateral band showing an arched and well-delineated strip bordering the posterior margin. This strip is either dark brown when chromatophores are expanded (Fig. 5a) or translucent when chromatophores are contracted (Fig. 5b); in any case it is clearly distinguishable from the pigmentation of the whole scale. A conspicuous dark or silver midlateral band extending from two scales after humeral spot to the middle caudal-fin rays crossing a slightly rectangular caudal spot. Humeral spot vertical and bar-shaped, extended for two or three scales above and one or two scales below lateral line. Pectoral-, pelvic-, and anal-fins hyaline. Dorsal fin usually hyaline; in some cases with disperse chromatophores on distal portion of rays. Caudal-fin border slightly black (Fig. 6).

Sexual dimorphism. One pair of bony hooks per segment are present on each lepidotrichium along the anal-fin rays of mature males. They are delicate and narrow, distributed from the 4th unbranched ray to the 12th to 19th branched rays. The number of segments bearing bony hooks decreases gradually from the anterior to the posterior rays on all observed males. Males have a higher number of rays (22-24) than females (18-21). Gill glands were not observed on first gill arch in either males or females.

Geographical distribution. Until recently, *Deuterodon pedri* was considered endemic to the rio Santo Antônio basin (its type locality). The latest collecting trips have found the species in the rio Guanhães, which is a tributary of the rio Santo Antônio, and at the confluence of the rio Brumadinho and the rio Caraça, which are tributaries of the rio Piracicaba. All of these are sub-drainages of the rio Doce (Fig. 7).

Tab. 2. Morphometric data for *Deuterodon pedri*, Lectotype (Lec), Paralectotypes (Par) and non-type specimens. SD = standard deviation.

Character	Lec	Par (n=2)				Males (n= 2)				Females (n= 23)			
		Low	High	Mean	SD	Low	High	Mean	SD	Low	High	Mean	SD
Standard length (mm)	78.5	72.06	76.27	74.17	-	86.9	87.18	87.04	-	43.5	89.23	72.40	-
Percents of standard length													
Head length	24.3	23.7	24.8	24.2	0.78	21.8	23.4	22.6	1.11	22.3	27.4	24.4	1.50
Predorsal distance	48.0	49.5	50.7	50.1	0.87	48.1	50.00	49.00	1.34	46.6	52.7	50.3	1.40
Prepelvic distance	45.7	42.9	47.9	45.4	3.50	43.4	44.4	43.9	0.73	43.4	49.9	46.9	1.32
Prepectoral distance	24.5	23.00	23.6	23.3	0.48	23.3	25.3	24.3	1.44	21.6	25.9	23.6	1.27
Preanal distance	65.1	64.4	65.3	64.8	0.67	62.7	63.5	63.1	0.53	62.9	71.5	66.6	1.94
Depth at dorsal-fin origin	32.3	30.6	30.7	30.7	0.12	31.1	31.9	31.5	0.61	27.6	37.6	32.0	2.32
Caudal peduncle depth	10.4	10.7	11.0	10.9	0.20	10.0	10.2	10.1	0.14	9.6	12.0	10.9	0.64
Caudal peduncle length	15.9	11.8	14.5	13.2	1.95	12.6	14.4	13.5	1.29	13.1	16.4	14.8	0.96
Anal-fin base	23.9	-	24.3	-	-	28.9	30.5	29.7	1.12	20.3	26.2	24.3	1.16
Dorsal fin length	-	21.8	23.8	22.8	1.43	24.2	24.6	24.4	0.31	19.0	26.3	23.8	1.59
Pelvic fin length	14.3	15.5	15.6	15.5	0.05	16.4	16.9	16.6	0.33	14.5	16.7	15.5	0.66
Pectoral fin length	15.7	17.5	20.61	19.0	2.17	21.0	21.9	21.5	0.60	15.8	23.3	21.0	1.45
Percents of head length													
Snout length	24.0	22.9	23.8	23.4	0.62	24.1	24.9	24.5	0.58	20.7	27.8	23.6	1.75
Upper jaw length	39.3	38.9	39.0	38.9	0.62	34.4	41.1	37.8	4.75	30.4	38.3	34.7	1.76
Orbital diameter	32.3	33.9	34.9	34.4	0.67	37.0	41.3	39.2	3.02	38.6	44.0	40.9	1.45
Interorbital width	32.3	33.8	33.9	33.9	0.09	33.6	37.0	35.3	2.44	27.6	35.8	32.2	2.34

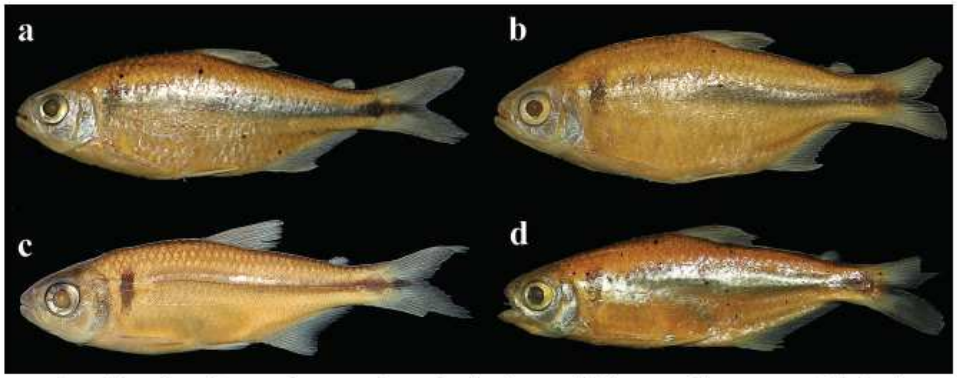


Fig. 6. Recently collected specimens of *Deuterodon pedri* showing variability according to sex and body size: a, female, MCN 19697, 79.82 mm SL; b, female, MCN 19698, 85.70 mm SL; c, juvenile, MNRJ 38463, 51.18 mm SL; d, male, UFRGS 17543, 86.47 mm SL.

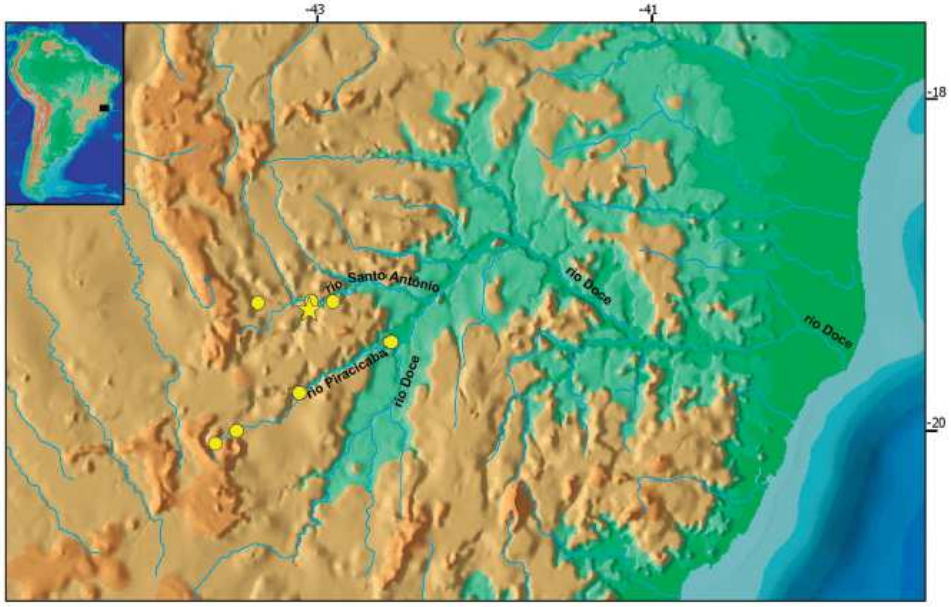


Fig. 7. Rio Doce drainage with the distribution of *Deuterodon pedri*, southeastern Brazil. The star indicates the type locality.

Ecological notes. *Deuterodon pedri* is found in localities with rapid-to-median speed dark waters with substrates of rocks and sand. At the type locality, this species is syntopic with other endemic species from the rio Santo Antônio basin (*e.g.*, *Henochilus wheatlandii* Garman).

Conservation status. According to IUCN criteria, we recommend that *D. pedri* be classified as an Endangered species (EN). The species is known from an Extent of Occurrence (EOO) of approximately 4,900 km^2 (B1). There is a significant continued decline in habitat quality [criterion B1b(iii)] from continuous extensive iron ore quarrying and from the rupture of two dams in 2015 that released a large amount of iron ore waste, which contaminated most of the rio Doce, including the area between the rio Santo Antônio and rio Piracicaba, where the species is found. This accident has been reported to have eliminated all endemic flora and fauna in the affected waterways (Lambertz, Dergam, 2015). Additionally, the habitat of the species has been severely fragmented by hydroelectric power dams that modify river hydrodynamics, by quarrying activities that renders river stretches uninhabitable and due to the presence of 35 exotic fish species in that drainage (Barros *et al.*, 2012).

Discussion

The use of aDNA recovered from the lectotype associated with the examination of new and representative samples of characid fish species from the type locality and nearby proved to be a powerful framework to solve the taxonomical puzzle of *Deuterodon pedri*. According to Marinho *et al*. (2015), the most challenging genera of the Characidae (*Astyanax*, *Bryconamericus* Eigenmann, *Hemigrammus* Gill, *Hyphessobrycon* and *Moenkhausia* Eigenmann) present serious taxonomic problems mainly due to the existence of poorly known widespread species complexes, old and short descriptions, and poorly preserved type specimens, most of which have unknown type localities and uncertain geographical distributions. The case study of *D. pedri* typifies the problems exposed by Marinho *et al*. (2015). The species was described based on poorly preserved material (Eigenmann, 1908); the type series included different species (Lucena, Lucena, 2013 and our results), and its assumed distribution was overly broad because of the incorrect assignation of specimens of a third species (our results). Not surprisingly, the species has not been cited in the literature for a long time, and it has remained an enigma for ichthyologists. Recently, the description of a new species of Diptera (Marshall, Evenhuis, 2015) that designated a photo as the holotype has triggered the discussion of whether preserved specimens are needed for species descriptions (Amorim *et al*., 2016; Pape, 2016; Krell, 2016; Ceríaco *et al*., 2016). *Deuterodon pedri* provides a sound argument for the importance and utility of preserved types in this discussion.

The result obtained from the first fragment (COI-1), with a p-distance equal or larger than 0.1 when compared with all characid species included at the alignment, may be explained by nucleotide misincorporation in aDNA (Sawyer *et al*., 2012). Studies with this kind of sample have revealed an increased occurrence of depurination of the DNA followed by hydrolysis of the phosphate-sugar backbone (Briggs *et al*., 2007). An alternative explanation for COI-1 result is that the primers amplified a numt instead COI-1. The amplification of numts seems to increase with the usage of primers to amplify small fragments and is common in studies with aDNA (Tex *et al*., 2010).

The second fragment (COI-2), although short (179 base pairs), is located in a variable region of the gene and was sufficient to determine the identity of the lectotype of *D. pedri*. Long DNA barcode sequences are no longer considered essential, since even a few base pairs may be sufficiently informative in solving taxonomical questions (see Hajibabaei *et al*., 2006). The use of such a mini-barcode has been proposed as an alternative when it is not possible to obtain the entire fragment because of the degraded nature of the samples (Boyer *et al*., 2012). Our data further demonstrates that mini-barcodes can be as effective as the entire COI gene in determining species identities.

Deuterodon pedri is a valid species but should be maintained as *incertae sedis* in Characidae as previously proposed by Lucena, Lucena (2002) since it does not share the synapomorphies that define the genus: (1) the anterior region of the toothed portion of the maxilla deeper than the posterior region of the toothed portion; (2) the ventral margin of toothed portion of maxilla arching toward the ventral margin of the premaxilla, determining an alignment between maxillary and premaxillary teeth; and (3) posterior region of the maxilla without teeth smaller than anterior toothed region. An ongoing and more comprehensive study is being developed to reconstruct *D. pedri's* relationships and determine its most appropriate generic assignment.

Increasing advances are making molecular techniques more accessible, facilitating the use of aDNA (Linderholm, 2016) as a complement to solve taxonomical problems. In cases such as that exemplified herein with *D. pedri* (see discussion in Marinho *et al*., 2015), the aDNA of museum types associated with DNA and morphological studies of new samples will be the key to help solving problems related to the species identity and relationships. Notwithstanding the degraded nature of aDNA prevents the amplification of full COI fragments, we herein demonstrate that the usage of mini-barcode is a powerful tool in the resolution of taxonomical problems. So, we strongly recommend the use of small fragments of aDNA for taxonomic resolution in cases with high complexity.

Material examined. *Deuterodon pedri*: Lectotype: MCZ 21081, 78.6 mm SL, Brazil, Minas Gerais State, Santa Anna de Ferros, rio Doce basin at rio Santo Antônio, approx. 19°17'S 43°02'W, T. Ward, 1865. Lectogenetype: GenBank accession number KY345055. Paralectotypes: MCZ 170510, 2 of 4, 58.64-76.27 mm SL, collected with the lectotype.

Non type-specimens. All from Brazil, Minas Gerais State: MCP 47661, 7, 76.71-86.90 mm SL, Ferros, rio Santo Antônio basin, 19°13'55"S 43°01'17"W. UFRGS 17543, 6, 88.07-74.97 mm SL, and UFRGS 17544, 1 c&s, 89.23 mm SL, same locality as MCP 47661. MCN 19700, 1, 69.99 mm SL, Ferros, rio Esmeralda, 19°14'01"S 42°53'30"W. MCN 19697, 4, 76.4-79.4 mm SL (counts only), Ferros, rio Santo Antônio, 19°13'55"S 43°01'17"W. MCN 19698, 2, 85.82-73.61 mm SL, Ferros, rio Santo Antônio, 19°13'55"S 43°01'17"W. MNRJ 38463, 10 of 90, 30.49-54.51 mm SL (4, 43.52-54.51 mm SL), Catas Altas, confluence of the rio Brumadinho and rio Caraça, 20°00'34"S 43°28'15"W. MNRJ 38444, 6, 29.73-61.55 mm SL (3, 44.75-61.55 mm SL), Catas Altas, rio Conceição, 20°05'11"S 43°35'54"W. MZUSP 75389, 15, 56.51-72.08 mm SL, Morro do Pilar, rio Preto, on the road between Morro do Pilar and Santo Antônio, 19°14'29"S 43°20'29"W. MZUSP 73133, 5, 52.30-37.53 mm SL (counts only), Dores de Guanhães, rio Guanhães, road km 351, 19°03'22"S 42°55'53"W. MZUSP 110653, 1, 66.59 mm SL (counts only), João Monlevade, rio Santa Bárbara, 19°46'50"S 43°05'W. MZUSP 110671, 4, 32.69-64.65 mm SL (counts only), João Monlevade, rio Piracicaba, 19°46'55"S

43º05'38"W. MZUSP 75340, 30 of 134 specimens 43.70-60.79 mm SL (counts only), Dores de Guanhães, rio Guanhães, road to quarry, Maria das Dores farm, approximately 8 km N of Dores de Guanhães, 19°00'33.23"S 42°56'20"W. MZUSP 104709, 6, 53.20-62.67 mm SL (counts only), Conceição do Mato Dentro, córrego São João, 19º02'29"S 49º20'34"W.

Acknowledgments

We are indebted to Karsten Hartel (MCZ) for the permission to study and to sample tissues from type specimens, and to Andrew Williston (MCZ) for providing CTscans; both of them supported the visit by PCS to MCZ. The present study was largely accomplished during a 10-month visit of the first author to the NMNH (Smithsonian Institution, Washington DC) in 2015-2016, financially supported by a CNPq scholarship (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). In this sense, we are indebted to the NMNH staff, in particular that of the Fish Division, Lynne Parenti and David Johnson, for guidance. The authors would like to thank Richard Vari (*in memoriam*) who encouraged, facilitated and supported the execution of this study at the Smithsonian. The molecular work was undertaken at LAB (SI), and we thank Lee Weight and Jeff Hunt for their support and Jeff Clayton and Chris Murphy for help with fish collection at NMNH and MSC, respectively. We are also grateful to Jorge Dergam (UFV) for collecting an important amount of fresh material and kindly making it available for this study; to Oswaldo Oyakawa for taking photos and tissue samples from the lectotype; and to Angela Zanata for providing tissues from *Myxiops*. We thank the editor Brian Sidlauskas and three anonymous reviewers for comments that greatly improved this manuscript. Financial support was provided by BRBOLD Project for the sequencing. CNPq, CAPES and FAURGS provided grants and scholarships to the authors (PCS, MCM, LRM).

References

Amorim DS, Santos CMD, Krell FT, Dubois A, Nihei SS, Oliveira OMP *et al*. Timeless standards for species delimitation. Zootaxa. 2016; 4137(1):121-28.

Barros LC, Santos U, Zanuncio JC, Dergam JA. *Plagioscion squamosissimus* (Sciaenidae) and *Parachromis managuensis* (Cichlidae): a threat to native fishes of the Doce River in Minas Gerais, Brazil. PlosOne [serial on the Internet]. 2012; 7(6):e39138. Available from: http://dx.doi:10.1371/journal.pone.0039138

Boyer S, Brown SDJ, Collins RA, Cruickshank RH, Lefort MC, Malumbres-Olarte J, Wratten SD. Sliding window analyses for optimal selection of mini- barcodes, and application to 454 pyrosequencing for specimen identification from degraded DNA. PlosOne [serial on the Internet]. 2012; 7(5):e38215. Available from: http://dx.doi:10.1371/journal.pone.0038215

Briggs A, Stenzel U, Johnson PL, Green R, Kelso J, Prüfer K *et al*. Patterns of damage in genomic DNA sequences from a Neanderthal. Proc Natl Acad Sci. 2007; 104(37):14616-21.

De Bruyn M, Parenti LR, Carvalho GR. Successful extraction of DNA from archived alcohol-fixed white-eye fish specimens using an ancient DNA protocol. J Fish Biol. 2011; 78(7):2074-79.

Buerki S, Baker WJ. Collections based research in the genomic era. Biol J Linn Soc. 2016;117(1):5-10.

Cappellini E, Gentry A, Palkopoulou E, Ishida Y, Cram D, Roos AM, *et al*. Resolution of the type material of the Asian elephant, *Elephas maximus* Linnaeus, 1758 (Proboscidea, Elephantidae). Zool J Linn Soc. 2013; 170(1):222-32.

Ceríaco LMP, Gutiérrez EE, Dubois A, Abdala CS, Alqarni AS, Adler K, *et al*. Photography-based taxonomy is inadequate, unnecessary, and potentially harmful for biological sciences. Zootaxa. 2016; 4196(3):435-45.

Chakrabarty P. Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. Zootaxa. 2010; 2632(1):67-68.

Coutinho-Sanches N, Dergam J. Cytogenetic and molecular data suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) is a member of an ancient coastal group. Zebrafish. 2015; 12(15):357-65.

Eigenmann CH. Preliminary descriptions of new genera and species of tetragonopterid characins. (Zoölogical Results of the Thayer Brazilian expedition.). Bull Mus Comp Zool. 1908; 52(6):91-106.

Eigenmann CH. The American Characidae. Cambridge: Printed for the Museum; 1927. (Memoirs of the Museum of Comparative Zoology at Harvard College; vol 43, part 4).

Fink W, Weitzman S. The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). Washington (DC): Smithsonian Institution Press; 1974. (Smithsonian contributions to Zoology; No. 172).

Gilbert MT, Bandelt HJ, Hofreiter M, Barnes I. Assessing ancient DNA studies. Trends Ecol Evol. 2005; 20(10):541-44.

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN. A minimalist barcode can identify a specimen whose DNA is degraded. Mol Ecol Notes. 2006; 6(4):959-64.

Higuchi H. An updated list of ichthyological collecting stations of the Thayer Expedition to Brazil (1865–1866). 1996. Available from: www.mcz.harvard.edu/Departments/Ichthyology/docs/Higuchi_1996_Thayer_Formated_prelim.pdf (30/Sep/2016).

Higuchi R, Bowman B, Freiberger M, Ryder O, Wilson A. DNA sequences from the quagga, an extinct member of the horse family. Nature. 1984; 312(5991):282-84.

Ishida Y, Demeke Y, van Coeverden de Groot PJ, Georgiadis NJ, Leggett KE, Fox VE, Roca AL. Distinguishing forest and savanna African elephants using short nuclear DNA sequences. J Hered . 2011; 102(5):610-16.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN. Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes. 2007; 7(4):544-48.

Krell FT. Taxonomy: preserve specimens for reproducibility. Nature. 2016; 539(7628):168.

Lambertz M, Dergam JA. Mining disaster: huge species impact.

Nature. 2015; 528(7580):39.

Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25(11):1451-52.

Linderholm A. Ancient DNA: the next generation - chapter and verse. Biol J Linn Soc. 2016; 117(1):150-60.

Lucena CAS, Lucena ZMS. Redefinição do gênero *Deuterodon* Eigenmann (Ostariophysi: Characiformes: Characidae). Comum Mus Ciênc Tecnol, Sér Zool. 2002; 15(1):113-35.

Lucena C, Lucena Z. Designation of a lectotype for *Deuterodon pedri* Eigenmann, 1908 (Characiformes: Characidae). Zootaxa. 2013; 3635(5):598.

Marshall SA, Evenhuis NL. New species without dead bodies: a case for photo-based descriptions, illustrated by a striking new species of *Marleyimyia* Hesse (Diptera, Bombyliidae) from South Africa. Zookeys [serial on the Internet]. 2015; 525:117-27. Available from: http://dx.doi.org/10.3897/zookeys.525.6143

Marinho MMF, Camelier P, Birindelli JLO. Redescription of *Astyanax guianensis* Eigenmann 1909 (Characiformes: Characidae), a poorly known and widespread fish from the Amazon, Orinoco and Guiana Shield drainages. Zootaxa. 2015; 3931(4):568-78.

Pape T. Taxonomy: species can be named from photos. Nature. 2016; 537(7620):307. Available from: http://dx.doi.org/10.1038/537307b

Sawyer S, Krause J, Guschanski K, Savolainen V, Pääbo S. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. PlosOne [serial on the Internet]. 2012; 7(3):e34131.Available from: http://dx.doi.org/10.1371/journal.pone.0034131

Schander C, Halanych KM. PCR and formalinized animal tissue - a short review and protocols. Org Divers Evol. 2003; 3(3):195-205.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 2013; 30(12):2725-29.

Tex R-Jd, Maldonado JE, Thorington R, Leonard JA. Nuclear copies of mitochondrial genes: another problem for ancient DNA. Genetica [serial on the Internet]. 2010; 138(9-10):979-84 Available from: http://dx.doi.org/10.1007/s10709-010-9481-9

Vecchione M, Mickevich MF, Fauchald K, Collette BB, Williams AB, Munroe TA, Young RE. Importance of assessing taxonomic adequacy in determining fishing effects on marine biodiversity. ICES J Mar Sci. 2000; 57(3):677-81.

Submitted October 6, 2016
Accepted March 8, 2017 by Brian Sidlauskas

Capítulo 4

Solving taxonomic puzzles using ancient DNA: How to do it better?

Artigo a ser submetido na Revista Neotropical Ichtiology

Priscilla C. Silva, Maria C. Malabarba and Luiz R. Malabarba

This chapter is especially dedicated to Dr. Richard Vari (in memorian) who believed and gave all support for it to happen, but sadly could not see the results.

Solving taxonomic puzzles using ancient DNA: How to do it better?

Priscilla C. Silva, Maria Claudia Malabarba and Luiz R. Malabarba

Departamento de Zoologia and Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91.501-970 Porto Alegre, RS, Brazil. (PCS) pricarola@gmail.com (corresponding author), (MCM) claudia.malabarba@ufrgs.br, (LRM) malabarb@ufrgs.br

Abstract

Ancient (aDNA), also known as historical DNA, is DNA isolated from ancient samples as subfossils, mummies, or museum specimens. The use of ancient DNA in archived specimens helps to resolve queries such as the evolutionary relationships between species, the rescue of extinct populations, and the historical taxonomic problems. This new technique reinvents the biological collections, giving new purposes to the museum specimens. Despite the increasing use of the new generation sequencing, the traditional methodologies like Sanger are still an accessible option for aDNA. This contribution reports the experience of extracting and amplifying DNA of 53 type specimens of the Characidae, stored in museums around the word. Two kits and two spaces, regular and isolated, were tested in the extraction and PCRs processes. The samples yielded a mean of 120 ng/ul of DNA in the extractions and no correlation between amount of DNA and time from tissue fixation was observed. So far, 14 samples were amplified, and nine of them generated viable sequences. Based on this experience, guidelines and protocols to perform and succeed in aDNA studies are presented. Our findings provide good support for the use of short and highly variable regions in the identification of ancient samples. We conclude that in aDNA studies an isolated place is not an option but is mandatory.

Resumo

DNA antigo (aDNA), também chamado DNA histórico, é aquele DNA extraído de amostras antigas como subfósseis, múmias ou espécimes tombados em museus e não fixados para essa finalidade. O uso de aDNA em espécimes tombados em museus ajuda a resolver vários tipos de questão tais como relações evolutivas entre espécies, recuperação de populações extintas e resolução de problemas taxonômicos históricos. Essa nova técnica reinventa as coleções biológicas, dando novo uso aos espécimes de museu. Apesar do crescente uso de sequenciamento de Nova geração para acessar este tipo de dado, a metodologia de Sanger ainda é a mais acessível para a maioria dos pesquisadores. Este estudo reporta a experiência de extração e amplificação de DNA de 53 espécimes tipos pertencentes à Characidae, de museus de várias localidades no mundo. Dois kits e dois espaços físicos diferentes, um de uso regular e comum e outro isolado e controlado, foram testados para extração e processo de PCR. Uma média de 120 ng/ul de DNA foi obtida e nenhuma correlação entre quantidade de DNA e tempo de fixação foi observada. Até o momento 14 amostras foram amplificadas e nove geraram sequências viáveis. Baseados nesta experiência, guias e protocolos para realizar trabalhos de forma efetiva em estudos com aDNA são apresentados. Nossos resultados são um forte suporte de que o uso de regiões com poucos pares de bases, mas hipervariáveis são suficientes na identificação de amostras antigas. Concluímos que em estudos de aDNA o uso de local isolado para trabalhar não é opcional, mas obrigatório.

Keywords

Characidae; Genetype; Sanger methodology; Neotropical fish;

Palavras chave:

Characidae; Genetipo; Metodologia de Sanger; Peixes neotropicais,

Running head

Ancient DNA doing it in the best way

Introduction

Ancient DNA also known as historical or antique DNA, is that DNA isolated from old samples as subfossil bones, mummies, or museum specimens, which were not properly preserved for DNA extraction. As traditional repositories for biological specimens and tissue samples, museum collections are valuable resources for mapping and naming biodiversity. The possibility of extracting DNA from archived specimens has reinvented the museum collections, turning them into powerful genetic storehouses for molecular studies (Gee, 1988; Graves, Braun, 1992), sometimes including samples of populations no longer available in nature.

The first record of the aDNA usage was in 1984 to recover DNA from a 150-year-old museum specimen of an extinct subspecies of the plain zebra: *Equus quagga* (Higuchi *et al.*, 1984). This experience proved to be sufficient to determine the phylogenetic relationships of the species in question, which allowed the development of a project for the breeding and re-introduction of the Quaggas (http://www.quaggaproject.com/quagga-dna-results.htm).

This publication triggered an explosion of works claiming the recovery of aDNA from amber preserved species (Cano *et al.*, 1993), dinosaurs (Woodward *et al.*, 1994), and Neanderthal (Caramelli *et al.*, 2003), among other famous examples. The effervescence in the aDNA field coincided with the enhancement of PCR-based techniques and pyro-sequencing in the 1980´s (Linderholm, 2016). At the end of the XX and beginnings of the XXI centuries, the boom of aDNA works start to decrease substantially due to the emerging of criticism pointing out the unrepeatability and contaminations of the data in some previously published articles questioning the reliability of the results (Cooper, Poinar, 2000; Gilbert *et al.*, 2005). Subsequently, some measures in the proceedings involving aDNA were proposed in order to produce accurate and reliable results (Cooper, Poinar, 2000).

In the taxonomy, the use of aDNA may help to solve those problems wherein the type specimens no longer preserve informative features for a correct identification. Very old name-bearing types are often involved in nomenclatural doubts and ambiguities because they do not exhibit the diagnostic features anymore (Cappellini *et al.*, 2013). As very recently demonstrated by Silva *et al.* (2017), aDNA can be a powerful tool for solving

such taxonomical problems when associated to, even if meager, morphological information.

Due to the high fragmentation of the aDNA, Sanger is not the most appropriate methodology to sequence the molecule. However, as we demonstrate in this paper it can be used under restrict guidelines and for specific results. Also, this technique allows to establish a genetype (Chakrabarty, 2010), which can be very useful for further studies (i.e., phylogeny, ecology) involving the species.

In this paper, we present our experience extracting and amplifying DNA from old museum type specimens of Characidae fish family using Sanger methodology. Based on our experience, we present a detailed protocol including guidelines and facilities, intend to make the use of aDNA easier, more successful and reliable.

Material and Methods

Taxon sampling. A guideline with detailed information about all the process (since tissue extraction until DNA work details) was prepared (see S1) and sent to the museum curators where the specimens were deposited. We were authorized to sampling tissues and extract DNA from 53 Characidae type specimens belonging to different collections: ANSP, CAS, Field museum, MCZ, MNHN, NWM, BMNH, ZMUC.

Tissue extraction was done with maximum careful to avoid damage of the specimens and contamination of the samples. The bench utilized for the process was previously cleaned with household bleach solution (sodium hypochlorite 10%) and covered with absorbent paper (dog pad type) to avoid wet surface during the extraction process. Every surgical material was previously washed with soap and water and then immersed in a bleach solution bath overnight. After bath, all material was dried and then exposed to UV for 30 minutes to be individually stored in clean plastic bags. The material used in one specimen was cleaned in the process described before to be used again in other specimen. Nitrile gloves, lab coach, mask and hair stuck, were used throughout the process, and replaced for each specimen.

The ideal region to be sampled was choose according to the conditions of the specimen: a) in case of good conditions, with scales all over the body, part of the gill filaments on right side of the body was removed; b) in case of not so good conditions,

lacking scales at the body, amounts of muscle were removed by small incision below the dorsal fin on right side of the body or under the pelvic fins.

Each extracted tissue was immediately inserted in a 2.5 ml microtube with alcohol absolute and put under cold storage. The extracted tissues were sufficient for three DNA extractions, foreseeing the possibility of repeating the process (Gilbert *et al.*, 2005).

DNA extraction. We tested two kits for DNA extraction: Microamp Qiagen and First DNA from Gen-Ial. Both kits were used under their commercial recommendations. After the extraction, the DNA was quantified using Epoch Microplate spectrophotometer (Biotek) and checked for fragmentation in agarose gel with concentration of 0.8%.

Pre PCR room. The first two extractions were done in the dedicated Pre-PCR room of the Laboratory of Analytical Biology (LAB), at National Museum of Natural History (NMNH) of the Smithsonian Institution (SI, Washington DC). With the objective to avoid the contamination of the extracted DNA, this room is designated only for DNA extraction and materials from PCR room are strictly forbidden.

In this room, the extractions were performed in a chapel equipped with filter and UV light. As the chapel was not exclusive for us, before the use it was cleaned with bleach solution, and irradiated with UV lights for 30 minutes. All tubes and box of reagents were previously cleaned with bleach 10% solution before enter inside of the chapel. The centrifuge and incubator block were not inside of the chapel.

Isolated room. The following 6 extractions were processed in a different room located at Museum Support Center (MSC), which is located about 9 km from National Museum of Natural Sciences and out of Washington DC area. This isolated MSC room had ever been used for any DNA procedure (neither for PCR, extraction or sequencing). Even so, before usage, the room was entirely, from top to bottom, cleaned and disinfected with bleach solution. After that, it was equipped with two chapels, both with filter and UV lights, an exclusive refrigerator for storing reagents, a centrifuge, and a dry bath incubator. One of the chapels was just for the extraction proceedings, and the other one to store incubator and

centrifuge. The entrance of this room was restricted to researcher (PCS) and LAB manager.

DNA amplification.

Primers design. Because of the fragmented nature of the ancient DNA, we designed 5 sets of primers (COI-1, COI-2, COI-3, COI-4 and COI-5; Tab. 1) to amplify small sections, 150-200 bp of the COI gene, which combined would recover the entire gene. We previously prepared an alignment of COI sequences with 217 modern samples (S3) with mean of 600 pair bases trying to sample the maximum of variability of the specimens at the occurrence area. We used the tool Oligo Explorer 1.4 (Javed *et al.*, 2004) to design the intern primers trying to establish the sets of primers to amplify the maximum of 200 bp. The sets of primers were checked at Oligo Analyzer 1.0.2 (Kuulasmaa, 2002), to confer the quality of primers: absence of hairpins, melting temperature amplitude between the forward and reverse, and absence of self annealing.

PCR conditions. Two brands of reagents were tested for PCR reactions: Phire Hot Start Taq polymerase (ThermoFisher Scientific) and Hot Start Master mix (Promega). PCR with Phire Hot Start Taq was carried out in a volume of 20 µl containing 11.6 µL of H20, 4 µl of 10× reaction buffer, 1 µl of dNTPs (2 mM), 1 µlof each primer (10 µM), 0.4 µl (5 U) of Taq and 1µl of template DNA.

In the PCR using Promega Hot Start Master mix was produced a total volume of 10 µL, which included 3.45 µL of H20, 5 µL of Master mix (Promega), 0.15 µL of each primer (10 µM), and 1.25 ul of template DNA. The PCR thermal profile used was the same for both mixes: 94°C for 3 min for initial denaturation, followed by 5 cycles at 94°C for 30 s, high melting temperature (see Tab. 1) for temperature of each set of primers) for 40 s, and at 72°C for 1 min, followed by 55 cycles at 94°C for 30 s, low melting temperature (see Tab. 1 for temperature of each set of primers) for 40 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

The PCR reaction was loaded to a 1% agarose gel in TBE with EtBr together with KAPA universal ladder (Kapa Biosystem). The PCR products were purified by the Exosap enzymatic method (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50%

deionized water). Sequences were obtained using the Big-Dye reaction on an ABIPrism 3770 automated sequencer from the LAB (NMNH-SI).

Regular PCR room. The first 10 extracted samples (Tab. 2) were amplified at PCR room of Smithsonian National Museum of Natural History. This is a common use space with 77 PCRs stations, without chapels. Initially, we used one of these stations (Fig. 1) with not exclusive pipes. The mixes were prepared without chapel and reagents were storage on a common use freezer.

Isolated chapel. The remaining 8 samples were amplified at the same PCR room, but on a specially prepared chapel equipped with UV lights and filter space (Fig. 2). The mix was done inside this chapel, isolated from the rest of the laboratory. The reagents were storaged at the common use freezer, but inside of box protected by plastic bags. Before manipulation and preparing the mixes, the chapel was cleaned with 10% bleach solution and the containing reagent and DNA boxes, and the pipes were irradiated for 5 min by UV light. After use, all reagents tubes, boxes, pipes and the chapel were again cleaned with 10% bleach solution and then put inside of plastic bags before leave the chapel. Both boxes (DNA and reagents) were never open out of the chapel. The pipes were from our exclusive use and always were kept inside the chapel.

Molecular Data Analyses. The sequences obtained for each set of primers were separately aligned using only a full COI sequence (positive control) of 600 pair base in the Mega 6 software with algorithm Clustal W (Tamura *et al.*, 2013). After that, these sequence fragments were combined to form a more complete and independent alignment.

These generated sequences were added to a previously prepared file containing the COI gene alignment for 217 characid specimens. Sequences were compared by p-distance in Mega 6 (Tamura *et al.*, 2013) using the default conditions (Kimura 2-parameter model; d: Transitions + Transversions; uniform rates; Pairwise deletion; three codon positions selected). To illustrate the relationships among specimens, polymorphic sites were identified using DnaSP software (Librado, Rosas, 2009) and a haplotype network was drawn using Network 5.0 software (Fluxus technology Ltd.).

All sequence identities, including those of the positive controls, were checked with the Blast tool at Genbank.

Results

Taxon sampling.

The conditions of the specimens allowed to work (53 samples) is, in general, bad preserved, poor. Usually, the body presents an yellowish shade, with missing scales and no coloration preserved. In most of them, the muscle tissue is decayed and frayed when it is sampled. We managed to sample muscle and branchial tissues from almost all types and always at the right side of the fish body. Incisions to cut the muscle were done below the dorsal-fin (Fig. 3); for the branchial tissue the first arch was entirely removed.

DNA extraction. The 53 extractions showed a mean of 120 ng/ul of DNA (Tab. 3) with no correlation between collected year and amount of DNA observed (Fig. 4). Both kits worked successfully for DNA extracting processes, but their PCR and post PCR procedures run differently. Samples extracted with First-DNA all kit (*Astyanax rutilus jequitinhonhae* syntypes NMW57759, and *Tetragonopterus lacustris* syntype NMW57540) showed presence of DNA in spectrophotometer quantification and also in the agarose gel (Fig. 5), but the sequencing failed. However, when these same samples were extracted with Qiamp micro kit, they showed presence of DNA in spectrophotometer and agarose gel, and sequencing work effectively generating good quality sequences for *Astyanax rutilus jequitinhonhae* syntypes NMW57759and NMW57760-2.

Primers. The COI-1 primer was used 217 times to amplify DNA (including ancient samples and positive control in amplified reactions), of which 47% (102) was checked presence of bands in agarose and sequenced. Sequencing worked for 21% (22 samples). The COI-2 set was tested in 56 samples and bands were confirmed in 37.5% (21) of them. The sequencing succeed for 90.47% (19) of these samples. The COI-3 set amplified 29 samples and bands were observable in 51.72% (15) of them, but except for two samples

(Tab. 2) the sequencing failed. The COI-4 set always showed double bands in the agarose gel, in despite of our efforts to increase the product specificity. So, no sample was sequenced for COI-4 set. The COI-5 set was used in 29 samples, forming bands in 34.48% (10); and successfully sequenced for only 20% (2) of the samples.

Regarding to variability, COI-1 and COI-5 showed to be more conservative than COI-2 and COI-3 fragments (Fig. 6). For example, COI-2 fragment presents 6 mutational steps from the modern population of *Deuterodon pedri* to other species (Fig. 6a). In the COI-1 and COI-5 fragments, there is only 1 mutational step between *Astyanax rutilus jequitinhonhae* and the remaining samples; whereas in the COI-2 fragment there are 9 steps (Fig. 6b) between them. Also, COI-3 fragment of *Tetragonopterus eigenmaniorum*, 19 mutational steps are counted between this species and remaining samples (Fig. 6c). In short, COI-2 and COI-3 are more variables, and therefore more informative for barcode identifications.

Pre PCR room extraction and Regular PCR room usage. From 53 samples, 10 samples (19%) were extracted at the beginning of the study at Pre-PCR room. The amplification process of these samples was done at Regular PCR room. The amplification worked, but the sequencing showed contamination with positive control in the 10 samples. As the extraction and PCR positive controls were the same, we re-extracted the 10 samples again. Of the 20 sequences generated (10 COI-1 + 10 COI-2), 50% (10) presented chromatograms with highly noisy and incoherent peaks; the other 50% of sequences (10) were identical to the positive control (suggestive of contamination at some level) and chromatograms with intense back ground noise (suggestive of a poor signal) making the sequence unreadable. Negative control of PCRs sometimes showed bands and sometimes not. In order to test for contamination in the polymerase chain reactions, they were redone using new reagents and generating 24 PCR products, which were sent to sequencing (forward and reverse complements). In 29% of the samples, the sequencing barely (highly noisy chromatograms) or did not worked at all (sequencing reaction did not start). 46 % sequenced identical to the positive control. The remaining 25% samples, generated sequences different from positive control, but with highly matching with marine fish (*Citharus linguatula*), bird (*Aquila chrysaetos canadensis*), bacteria (*Pandoraea*

thiooxydans), freshwater fish not included in our samples (*Hyphessobrycon itaparicensis*) and a specific frog parasite (*Protopolystoma xenopodis*). Shortly, contamination occurred in two stages: during extractions evidenced by persistence of positive control DNA in the products; and during the polymerase chain reactions evidenced by the amplicons (presence of exogene DNA) in the products.

Isolated room for DNA extraction and isolated chapel for DNA amplification.

The detection of contamination in the DNA extraction and amplification required the adoption of new procedures to confer on utmost care and isolation possible to the process. Since then, the extractions and amplifications were made under controlled conditions in isolated room and chapel, respectively. All the 53 samples (which include those previously processed without controlled conditions) were processed under these new conditions. To detect any contamination, negative controls were used in all extractions, but no positive control was used, since this isolated room was used exclusively to manipulate ancient samples. Of the 53 extractions, PCR amplification worked only for 8 samples (15%). The PCR positive control, *Probolodus heterostomus*, was extracted separately in a regular Pre-PCR room, given that it is a modern sample. No positive control nor any modern material can go in the isolated chapel, its utilization is restrict to ancient material and reagents. Because of that, the positive control DNA was always added outside of the isolated chapel in other bench located in different place. Of the 15 sequences generated under these isolated conditions, two of them showed noisy chromatograms preventing the reading. Both sequences belong to a sample whose DNA was extracted using Gen Ial kit (without silica columns). Of the 11 sequences, two of them presented very long branches on the tree and were translatable to protein (stop codons and without similarity at Genbank). The other 9 sequences were in working conditions showing good quality chromatograms; differing from positive control sequences; and clean negative controls at all levels; consistent results from the Genbank comparisions, with similarity with members of Characidae (for example: *Tetragonopterus rutilus jequitinhonhae* with 97% of similarity with *Astyanax jequitinhonhae,* and *Deuterodon pedri*, see Silva *et al.* 2017).

Discussion

Our experience using historical specimens above reported demonstrates that even very small samples may generate viable DNA sequences if handled with care under controlled conditions. The specimens here studied were collected more than a century ago by naturalists or scientific expeditions in South America, more specifically in Brazil. The Thayer Expedition (1865-1866;), Charles Darwin in the Beagle´s voyage (1832), and Castelnau, as consul of the France in Brazil (Higuchi, 1996; Kury, 2001; Keynes, 2004; Simões, 2010, Silva, Malabarba, 2016) collected and sent to European museums a significant amount of material, which later were used to describe new species. Usually, these earlier naturalists fixed the collected specimens putting them in jars with spirits as rum, brandy, Brazilian cachaça, or whisky (see Malabarba, Reis, 1987, Fortey, 2008). As spirits are essentially alcohol, this fixation certainly collaborated to make it possible obtain viable DNA from such an old material (De Bruyn *et al.*, 2011). Although the DNA extracting from formalin-fixed material is increasing (see Ruane, Austin, 2017), it is not an easy practical. Research with ancient DNA is facilitated if the material is frozen or fixed in alcohol instead of formalin (De Bruyn *et al.*, 2011; Smith *et al.*, 2003).

Successful DNA extracting from ancient samples requires some cares. Ancient samples that were not properly fixed for molecular studies usually yield smaller amounts of a highly fragmented DNA, if compared to modern and adequately fixed materials (Cooper, Poinar, 2000). As a rule, the traditional protocols used for modern samples, like CTAB and fenol chloroform, do not provide good results when extracting DNA from ancient samples (Yang *et al.*, 1998). Indeed, the access to ancient DNA is facilitated with the use of extracting kits for forensic studies which are designed to optimize the quality and quantity of DNA extracted (specially from small samples).

Although both extraction kits here tested quantified positively for DNA in the spectrophotometer, only the Qiagen kit, which uses silica columns, produced viable sequences. The sequences generated from those samples extracted with the Gen-Ial kit (without silica columns) showed an intense noise and weak signal preventing the reading. We conclude from this that, the use of silica columns during extraction results in a cleaner material and free of impurities DNA (PCR and sequencing inhibitors, tissue remains, and

extremely small DNA fragments), improving the amplification and the sequencing processes.

No correlation was detected between the amount of DNA extracted and year of collection (age of the sample). Instead, the amount and quality of the extracted DNA may be more related with the conservation history and conditions which the specimens were exposed to (alcoholic degree at fixation, amount of specimens fixed together, evaporation, dehydration, among others). As a viable sequence appears to be dependent of the fragmentation degree of the DNA, a good quantity of DNA in the sample it is not a guarantee that the amplification and sequencing processes will succeed.

During extraction at Pre-PCR room and amplification in regular PCR room, two events of contamination were detected: with the positive control during extraction and with amplicons during the PCRs preparations. This conclusion was possible because different species generated sequences identical to that of the extraction positive control (even when PCR reagents were replaced). Also, a same sample sequenced initially equal to positive extraction control, and subsequently its sequences were identical to bacteria, birds and marine fish. We believe that amplicons at the laboratory could influence and contaminate our amplified product. Amplicons are accumulations of PCR products in the laboratory environmental by repeated amplification of a same target sequence and it can stay at the equipments, or even in the air, as a contaminant source (Persing, 1991). In regular samples, in which the DNA is in good concentration and quality, the low concentration of the amplicons is not enough to jeopardize the results. However, because the ancient DNA is usually in very low concentrations and highly fragmented, the presence of amplicons will be decisive. In this case, the physical destruction of the molecule, will increases the risk of preferentially amplify a contaminant sequence (Gilbert *et al.*, 2005) and disabling the results. The contamination is the main problem when treating with ancient DNA. That is why isolated spaces and special procedures are mandatory (Cooper, Poinar, 2000). Nine basic procedures, proposed by Cooper, Poinar (2000), should be followed to provide reliable results and, therefore, allowing your use to take scientific decisions such as taxonomic status and nomenclatural acts.

Among the species here studied, the sequences from *Deuterodon pedri*, *Tetragonopterus rutilus jequitinhonhae* and *Tetragonopterus eigenmaniorum* will be

valuable for an accurate identification and, possibly, the redescription of these species. Actually, the *D. pedri* lectotype sequence (COI-2) was very recently used to solve the identification problem and to redescribe the species (Silva *et al.*, 2017). On the other hand, the discarded COI-1 sequences from *D. pedri* lectotype and paralectotype (Silva *et al.*, 2017) are possibly product of the numts. Numts are sequences of mitochondrial DNA that migrate to nuclear genome where they start to evolve without a repair mechanism (Hazkani-Covo *et al.*, 2010). Tex *et al.* (2010) reported that the presence of numts in ancient DNA has a higher rate than predicted before. These authors believe that the use of short length universal primers may improve these results. For this study, we designed a kind of short universal primers, because it was derived from many different species. Numts sequences can be detected by presence of stop codons in the translation to protein, double peaks on electropherograms, differences in length of the branch on trees and misplaces in the tree (Cristiano *et al.*, 2012). All these evidences were observed in the COI-1 sequences of *D. pedri* lectotype and paralectotype, strongly suggesting they are numts, and thus considered as not valid sequences.

Examples of aDNA studies with fish organisms are scarce, and mostly involving North American or European fishes (see Nikulina, Schmölcke 2016; Ludwig *et al.*, 2016; Metcalf *et al.* 2012; Ketmaier *et al.* 2004). For Neotropical fishes, the literature is even meager (Garrigos *et al.* 2013; Silva *et al.*, 2017). Considering that, the guidelines here presented aim to stimulate and encourage the development of ancient DNA studies with Neotropical fishes. This study pointed ways of how to work on aDNA, showing some problems that can occur in case of disregarding care and rules basic. The development of aDNA study is especially important in Neotropical region once this is the most diverse ichthyological region of the world, housing a great number of taxonomically complicated species (like Characidae fish family) and some of them known only by museum types. Ancient DNA studies are fascinating, since that the data generated will help to recover almost extinct or even extinct organisms (Higuchi, 1984; Shapiro, 2016); to understand the relationship of extinct organisms with alive taxa (Mitchel *et al.*, 2016); to better understand the evolutionary process through the incorporation of extinct population data (D'Elia *et al.*, 2016; Eda *et al.*, 2016; Nikulina, Schmölcke, 2016); and the possibility of solving taxonomical puzzles (Silva *et al.*, 2017). The increasing advances in molecular biology

have facilitated the usage and procedures in ancient DNA studies (Linderholm, 2016). Likewise, we hope that the above reported experience encourages other groups to start these kind of research, to better understand, map and help our biodiversity resources.

Acknowledgements

We are indebted to Karsten Hartel (MCZ), Oliver Crimmen (BMNH), Patrice Pruvost and Philipe Keith (MNHN), Anja Palandacic (NHMW), Mathew Lowe (UMZC), Mark Sabaj (ANSP), Caleb McMahan (FMNH), Marcus Anders Krag and Peter Rask Møller (ZMUC) and Dave Catania (CAS) for the permission to study and to sample tissues from type specimensand the 6 first also supported the visit by PCS to MCZ, BMNH, MNHN, NHMW and UMZC. The present study was largely accomplished during a 10-month visit of the first author to the NMNH (Smithsonian Institution, Washington DC) in 2015-2016, financially supported by a CNPq scholarship (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). In this sense, we are indebted to the NMNH staff, in particular that of the Fish Division, Lynne Parenti and David Johnson, for guidance. The authors would like to thank Richard Vari (*in memoriam*) who encouraged, facilitated and supported the execution of this study at the Smithsonian. The molecular work was undertaken at LAB (SI), and we thank Lee Weight and Jeff Hunt for their support and Jeff Clayton and Chris Murphy for help with fish collection at NMNH and MSC, respectively. We are also grateful to Jorge Dergam (UFV) for collecting an important amount of fresh material and kindly making it available for this study and to Angela Zanata for providing tissues from *Myxiops*. Financial support was provided by BRBOLD Project for the sequencing. CNPq, CAPES and FAURGS provided grants and scholarships to the authors (PCS, MCM, LRM).

References

Cano RJ, Poinar HN, Pieniazek NJ, Acra A, Poinar GO. Amplification and sequencing of DNA from a 120–135-million-year-old weevil. Nature. 1993; 363: 563-568.

Cappellini E, Gentry A, Palkopoulou E, Ishida Y, Cram D, Roos A, *et al.*, Resolution of the type material of the Asian elephant, *Elephas maximus* Linnaeus, 1758 (Proboscidea, Elephantidae). Zool J Linn Soc. 2013; 170:222-232.

Caramelli D, Lalueza-Fox C, Vernesi C, Lari M, Casoli A, Mallegni F, Chiarelli B, Dupanloup I, Bertranpetit J, Barbujani G, and Bertorelle G. Evidence for a genetic discontinuity between Neandertals and 24,000-year-old anatomically modern Europeans. PNAS. 2003; 100 (11): 6593-6597.

Chakrabarty P. Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. Zootaxa. 2010; 2632:67-68.

Cooper A, Poinar HN. Ancient DNA: do it right or not at all. Science. 2000; 289: 1139-1139.

Cristiano MP, Fernandes-Salomão TM, Yotoko KSC. Nuclear mitochondrial DNA: an Achilles' heel of molecular systematics, phylogenetics, and phylogeographic studies of stingless bees. Apidologie. 2012; 43 (5): 527–538. DOI: 10.1007/s13592-012-0122-4

De Bruyn M, Parenti LR, Carvalho GR. Successful extraction of DNA from archived alcohol fixed White eye fish specimens using an ancient DNA protocol. J Fish Biol. 2011; 78:2074-2079.

D'Elia J, Haig SM, Mullins TD, Miller MP. Ancient DNA reveals substantial genetic diversity in the California Condor (*Gymnogyps californianus*) prior to a population bottleneck. The Condor. 2016; 118 (4):703-714. DOI: 10.1650/CONDOR-16-35.1

Eda Masaki, Koike H, Higuchi H. Understanding prehistoric maritime adaptations in northern Japan: Indirect evidence from ancient DNA and histological observations of albatross (Aves: Diomedeidae) bones. Quaternary International. 2015; 1e6 http://dx.doi.org/ 10.1016/j.quaint.2015.06.067

Fortey R.. Dry Store Room No. 1: The Secret Life of the Natural History Museum. (2008). London: Harper Perennial.

Garrigos YE, Hugueny B, Koerner K, Ibañez C, Bonillo C, Pruvost P, Causse R, Cruaud C, Gaubert P. Non-invasive ancient DNA protocol for fluid-preserved specimens and phylogenetic systematics of the genus Orestias (Teleostei: Cyprinodontidae). Zootaxa. 2013; 3640 (3): 373-394.

Gee H. Natural History Museum to build DNA database in London. Nature. 1988; 336: 707-707.

Graves GR, Braun MJ. Museums: Storehouses of DNA? Science. 1992; 255: 1335-1336.

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I. Assessing ancient DNA studies. Trends Ecol Evol. 2005; 20: 541-544.

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I. Assessing ancient DNA studies. Trends in Ecology & Evolution. 2005; 20: 541-544.

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. Recharacterization of ancient DNA miscoding lesions: Insights in the era of sequencing-by-synthesis. Nucleic Acids Res. 2007; 35: 1-10 https://doi.org/10.1093/nar/gkl483

Hazkani-Covo Einat, Zeller RM, Martin W. Molecular Poltergeists: Mitochondrial DNA Copies (numts) in Sequenced Nuclear Genomes. PLos Genet [serial on the internet]. 2010; 6(2): e1000834. Available from: http://dx.doi.org/10.1371/journal.pgen.1000834

Higuchi R, Bowman B, Freiberger M, Ryder O, Wilson A. DNA sequences from the quagga, an extinct member of the horse family. Nature. 1984; 213:282-284.

Higuchi H. An updated list of ichthyological collecting stations of the Thayer Expedition to Brazil (1865–1866). 1996. Available from: www.mcz.harvard.edu/Departments/Ichthyology/docs/Higuchi_1996_Thayer_Formated_prelim.pdf (30/Sep/2016).

Javed A, Kuulasmaa T, Muthusamy A. Oligo Explorer 1.2. *Genelink, Hawthorne, New York* (2004).

Ketmaier V, Bianco PG, Cobolli M, Krivokapic MJ,Caniglia R, De Matthaeis E. Molecular phylogeny of two lineages of Leuciscinae cyprinids (Telestes and Scardinius) from the peri-Mediterranean area based on cytochrome b data. Mol Phylo Evol. 2004. 32:1061-1071.

Kuulasmaa T. Oligo Analyzer 1.0.2. 2002. Distributed by the author. Kuopio

Keynes R. Aventuras e descobertas de Darwin a bordo do Beagle: 1832-1836. Rio de Janeiro: Jorge Zahar, 2004.

Kury L. “Viajantes e naturalistas do século XIX”. In: PEREIRA, P. R. (org.). Brasiliana da Biblioteca Nacional. Rio de Janeiro: Nova fronteira / MEC / Fundação Biblioteca Nacional. 2001.

Linderholm A. Ancient DNA: the next generation – chapter and verse. Biol J Linn Soc. 2015; 117:150-160.

Ludwig A, Makowiecki D, Benecke N. Further evidence of trans-Atlantic colonization of Western Europe by American Atlantic sturgeons. Archaeofauna. 2009; 18: 185-192.

Malabarba LR, Reis RE. Manual de técnicas para a preparação de coleções zoológicas. Soc bras zool. 1987; 36(1):1-14.

Metcalf JL, Stowell SL, Kennedy CM, Rogers KB, Mcdonald D, Epp J, Keepers K, Cooper A, Austin JJ, Martin AP. Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. Molecular Ecology. 2012; 21(21): 5194-5207.

Mitchell KJ, Scanferla A, Soibelzon E, Bonini R, Ochoa J, Cooper A. Ancient DNA from the extinct South American giant glyptodont Doedicurus sp. (Xenarthra: Glyptodontidae) reveals that glyptodonts evolved from Eocene armadillos. Molecular ecology. 2016; 25 (14): 3499-3508. doi: 10.1111/mec.13695

Nikulina EA, Schmölcke U. Reconstruction of the historical distribution of sturgeons (Acipenseridae) in the eastern North Atlantic based on ancient DNA and bone morphology of archaeological remains: implications for conservation and restoration programmes. Diversity and Distributions. 2016; 22(10): 1036–1044.

Nagy ZT. A hands-on overview of tissue preservation methods for molecular genetic analyses. Organisms Diversity & Evolution. 2010; 10 (1): 91-105.

Pääbo S. Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. Proc. Natl Acad. Sci. USA.1989; 86: 1939-1943.

Persing DH. Polymerase Chain Reaction: Trenches to Benches. journal of clinical microbiology. 1991; 29(7): 1281-1285.

Silva & Malabarba, 2016

Smith CI, Chamberlain AT, Riley MS, Stringer C, Collins MJ. The thermal history of human fossils and the likelihood of successful DNA amplification. Journal of Human Evolution. 2003; 45: 203-217.

Shapiro B. Pathways to de-extinction: how close can we get to resurrection of an extinct species? Functional Ecology. 2016; doi: 10.1111/1365-2435.12705

Woodward S, Weyand N, Bunnell M. DNA sequence from Cretaceous period bone fragments. Science. 1994; 266: 1229-1232.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013; 30: 2725-2729.

Tex R-Jd, Maldonado JE, Thorington R, Leonard JA. Nuclear copies of mitochondrial genes: another problem for ancient DNA. Genetica. 2010; 138:979-984 10.1007/s10709-010-9481-9

Silva PC, Malabarba CM, Malabarba LR. Using ancient DNA to unravel taxonomic puzzles: the identity of Deuterodon pedri (Ostariophysi: Characidae). Neotropical Ichthyology. 2017; 15(1): e160141- e160141[11]

Yang DY, Eng B, Waye JS, Dudar, JC, Saunders SR. Improved DNA extraction from ancient bones using silica-based spin columns. American Journal of Physical Anthropology. 1998; 105: 539-543.

Tables and figures

Tab. 1. COI DNA primers designed for this study and their melting temperatures used in PCR.

	Primer Sequence Left	Primer Sequence Right	High Melting temperature	Low Melting temperature
COI - 1	5'GTATTYGTTGCCTGAGCYGG3'	5'TATRACRAARGCATGTGCGG3'	58°C	56°C
COI - 2	5'WTCCCTTTTAGGTGAYGACC3'	5'KGGRGGAAGAAGYCARAAGC3'	56°C	54°C
COI - 3	5'GTRATAATYGGRGGRTTTGG3'	5'CCTARAATTGAAGADACACC3'	53°C	49°C
COI - 4	5'GTTTACCCYCCTYTWGCYGG3'	5'ATYCCTGCTGCYAGAACBGG3'	60°C	56°C
COI - 5	5'HCCAGCYATTTCRCARTACC3'	5'ARRTGTTGATAAAGRATGGG3'	58°C	54°C

Tab. 2. Results of DNA extraction and amplification for ancient samples at Pre-PCR room and isolated room.

Species	Taxonomic status / Museum catalog number	Extraction		PCR		Primers set working information Blast/pair basis/identity with controls				
		Pre PCR room	Isolated room	Regular PCR room	Isolated chapel	C1	C2	C3	C4	C5
Astyanax giton	Lectotype / MCZ20936	x		x		Chromats with noise, showing clearly more than one squence; 100% identity with *Citharus linguatula*	Identical to positive extraction control	Chromats with noise, showing clearly more than one squence;	-	Chromats with noise, showing clearly more than one squence;
Deuterodon pedri	Lectotype / MCZ21081	x	x	x	x	**Regular PCR room:** identical to the positive extraction control **Isolated chapel:** 136 pair bases, different from positive control, and similar to the modern sample supposed to be *Deuterodon pedri*, 100% Blast identity with *Astyanax* sp.	**Regular PCR room:** identical to the positive extraction control **Isolated chapel:** pair bases, and two pair bases different from to the modern sample supposed to be *Deuterodon pedri*, 100% Blast identity with D. singularis	-	-	-
Deuterodon pedri	Paralectotype / MCZ170510	x	x	x	x	**Regular PCR room:** identical to the positive extraction control **Isolated chapel:** sequence with no similarities at Genbank using blast tool	**Regular PCR room:** - **Isolated chapel:**-	-	-	-
Astyanax brevirhinus	Holotype / MCZ20905	x		x		90% of identity with *Pandoraea thiooxydans*	Identical to positive extraction control	-	-	-

Species	Taxonomic status / Museum catalog number	Extraction		PCR		Primers set working information Blast/pair basis/identity with controls				
		Pre PCR room	Isolated room	Regular PCR room	Isolated chapel	C1	C2	C3	C4	C5
Astyanax janeiroensis	Holotype / MCZ21057	x		x		Foward 100% Blast identity with *Hyphessobrycon itaparicensis*; Reverse 100% of identity with *Protopolystoma xenopodis*	Identical to positive extraction control	-	-	-
Deuterodon parahybae	Syntype / MCZ 20933A	x		x		-	Identical to positive extraction control	-	-	-
Deuterodon parahybae	Syntype / MCZ 20933B	x		x		-	Identical to positive extraction control	-	-	-
Astyanax scabripinnis intermedius	Lectotype / MCZ20684	x		x		-	Identical to positive extraction control	-	-	-
Astyanax scabripinnis intermedius	Paralectotype / MCZ20635	x		x		90% of Blast identity with *Pandoraea thiooxydans*	100% of Blast identity with *Aquila chrysaetos canadensis*	-	-	-
Astyanax fasciatus parahybae	Paralectotype / MCZ20891	x		x	x	**Regular PCR room:** - **Isolated chapel:** Identical to positive extraction control	**Regular PCR room:** - **Isolated chapel:** Identical to positive extraction control	-	-	-
Astyanax rutilus jequitinhonhae	Syntype / NWM57759		x		x	129 pair bases, 98% of identity with *Astyanax bockemani*	-	-	-	-
Astyanax rutilus jequitinhonhae	Syntype / NWM57760:1		x		x	134 pair bases, 98% of identity with *Astyanax bockemani*	184 pair basis 97% of identity with *Astyanax fasciatus jequitinhonhae*	-	-	-
Astyanax rutilus	Syntype /		x		x	134 pair bases, 98%	184 pair basis 97% of	-	-	180 pair basis 99% of

Species	Taxonomic status / Museum catalog number	Extraction		PCR		Primers set working information Blast/pair basis/identity with controls				
		Pre PCR room	Isolated room	Regular PCR room	Isolated chapel	C1	C2	C3	C4	C5
jequitinhonhae	NWM57760:2					of identity with *Astyanax bockemani*	identity with *Astyanax fasciatus jequitinhonhae*			identity with *Astyanax fasciatus*
Tetragonopterus lacustris	Syntype / NWM57540		x		x	chromats with noise	chromats with noise	-	-	-
Tetragonopterus eigenmaniorum	Holotype / ANSP		x		x	94 pair bases, 96% identity with *Oligosarcus paranensis*	-	267 pair bases, 87% identity with *Astyanax bockemani*	-	-

Tab. 3- Type specimens allowed by museums to be extracted and amplified in this stuy. Means of DNA extractions are presented: 1st clean is relative to the amount of the first step clean of the silica column; 2nd clean is relative to the amount of the second step clean of the silica column; 3rd clean is relative to the amount of the third step clean of the silica column.

		Museum		Extraction ng/ul		
Specimen	Taxonomical status	Catalog number	Description year	1st clean	2nd clean	3rd clean
Astyanax giton	Lectotype	MCZ 20936	1908, Eigenmann	71.745	12.587	3.121
Deuterodon pedri	Lectotype	MCZ 21081	1908, Eigenmann	81.921	78.681	65.67
Deuterodon pedri	Paralectotype	MCZ 170510	1908, Eigenmann	117.807	115.423	96.813
Astyanax brevirhinus	Holotype	MCZ 20905	1908, Eigenmann	11.94	5.939	27.379
Astyanax janeiroensis	Holotype	MCZ 21057	1908, Eigenmann	25.576	69.793	58.974
Deuterodon parahybae	Syntype	MCZ 20933 A	1908, Eigenmann	57.329	18.806	- 5.711
Deuterodon parahybae	Syntype	MCZ 20933 B	1908, Eigenmann	123.838	51.864	14.596
Astyanax scabripinnis intermedius	Lectotype	MCZ 20684	1908, Eigenmann	74.548	6.526	1.745
Astyanax scabripinnis intermedius	Paralectotype	MCZ 20635	1908, Eigenmann	16.286	2.444	41.391
Astyanax scabripinnis intermedius	Paralectotype	MCZ 20919 A	1908, Eigenmann	33.81	8.633	3.358
Astyanax scabripinnis intermedius	Paralectotype	MCZ 20919 B	1908, Eigenmann	67.312	12.331	22.141
Tetragonopterus rutilus jequitinhonhae	Syntype	NMW 57759	1877, Steidachner	76.259	8.93	45.262
Tetragonopterus rutilus jequitinhonhae	Syntype	NMW 57760:1	1877, Steidachner	87.558	54.762	67.842
Tetragonopterus rutilus jequitinhonhae	Syntype	NMW 57760:2	1877, Steidachner	176.907	22.972	3.495
Tetragonopterus jenynsii	Syntype	NMW 57534:1	1877, Steidachner	62.712	2.118	0.174
Tetragonopterus jenynsii	Syntype	NMW 57534:3	1877, Steidachner	96.079	36.357	4.551
Tetragonopterus jenynsii	Syntype	NMW 57535:1	1877, Steidachner	278.224	21.559	- 0.367
Tetragonopterus bahiensis	Syntype	NMW 57251:1	1877, Steidachner	74.212	67.311	16.702
Tetragonopterus bahiensis	Syntype	NMW 57252	1877, Steidachner	194.803	8.003	17.796
Tetragonopterus rivularis	Syntype	USNM 44960 S	1875, Lutken	61.404	15.155	12.837
Tetragonopterus rivularis	Syntype	USNM 44960 B	1875, Lutken	116.904	110.624	41.843
Tetragonopterus rivularis	Syntype	NMW 57707:1	1875, Lutken	165.913	39.635	30.909
Tetragonopterus rivularis	Syntype	NMW 57708:1	1875, Lutken	143.09	69.958	5.667
Tetragonopterus rivularis	Syntype	ZMUC 2074411 P.241372	1875, Lutken	54.506	180.107	77.898
Tetragonopterus rivularis	Syntype	ZMUC 2074411 P.241376	1875, Lutken	167.591	62.14	24.682
Hemigrammus santae	Syntype	USNM 55652 B	1907, Eigenmann	127.879	24.061	5.856
Hemigrammus santae	Syntype	USNM 55652 S	1907, Eigenmann	138.739	23.451	13.867
Salmo bimaculatus	Syntype	BMNH 1853.11.12.34	1758,Linneus	158.593	112.336	9.06
Astyanax bimaculatus novae	Cotype	FMNH 54641 A	1911, Eigenmann	146.536	15.161	-1.066
Astyanax bimaculatus novae	Cotype	FMNH 54641 F	1911, Eigenmann	142.272	32.193	8.574
Tetragonopterus jacuhiensis	Lectotype	ANSP 21912	1894, Cope			
Tetragonopterus lacustris	Syntype	NMW 57540	1875, Lutken	75.816	24.628	9.294
Tetragonopterus lacustris	Syntype	ZMUC 382 P. 241322	1875, Lutken	111.16	18.592	2.247
Astyanax fasciatus parahybae	Paralectotype	USNM 120245 1	1908, Eigenmann	112.428	30.261	22.619
Astyanax fasciatus parahybae	Paralectotype	USNM 120245 2	1908, Eigenmann	128.443	13.263	18.272
Astyanax fasciatus parahybae	Lectotype	MCZ 20685	1908, Eigenmann	90.195	37.186	4.286
Astyanax fasciatus parahybae	Paralectotype	MCZ 20891	1908, Eigenmann	109.832	6.524	17.649
Astyanax fasciatus parahybae	Paralectotype	MCZ 20890	1908, Eigenmann	40.185	46.916	29.222
Tetragonopterus curvieri	Syntype	ZMUC P. 241294	1875, Lutken	171.82	47.068	23.602
Tetragonopterus mexicanus	Syntype	ZMUC P. 241247	1853, De Fillipi	131.64	45.658	57.279
Cheirodon ribeiroi	Holotype	CAS 59778	1907, Eigenmann	158.475	41.533	31.3
Cheirodon ribeiroi	Paratype	CAS 59779	1907, Eigenmann	145.97	18.792	21.389
Tetragonopterus luetkenii	Paralectotype	BMNH 1886.3.15.35	1887, Boulenger	205.847	50.485	3.668
Hyphessobrycon luetkenii	Lectotype	BMNH 1886.3.15.80	1887, Boulenger	160.083	77.333	21.334
Oligobrycon microstomus	Paratype	FMNH 57914	1915, Eigenmann	139.402	37.051	13.206
Probolodus heterostomus	Paratype	FMNH 54329	1911, Eigenmann	186.116	54.718	28.085
Tetragonopterus taeniatus	Syntype	UCMZ F.6975.2	1842, Jenyns	140.913	26.306	29.52
Tetragonopterus fasciatus longirosris	Syntype	NMW 57508	1907, Steindachner	108.861	36.822	16.723
Tetragonopterus laticeps	Holotype	ANSP 21852	1894, Cope	174.089	24.112	9.966
Deuterodon potaroensis	Paralectotype	FMNH 52968	1909, Eigenmann	132.408	22.806	19.556
Tetragonopterus scabripinnis	Holotype	BMNH 1917.7.14.15	1842, Jenyns	116.081	32.531	17.286
Astyanax scabripinnis paranae	Holotype	CAS 22555	1914, Eigenmann	102.553	22.361	9.007
Astyanax ribeirae	Paratype	FMNH 54726	1911, Eigenmann	93.558	33.554	10.197

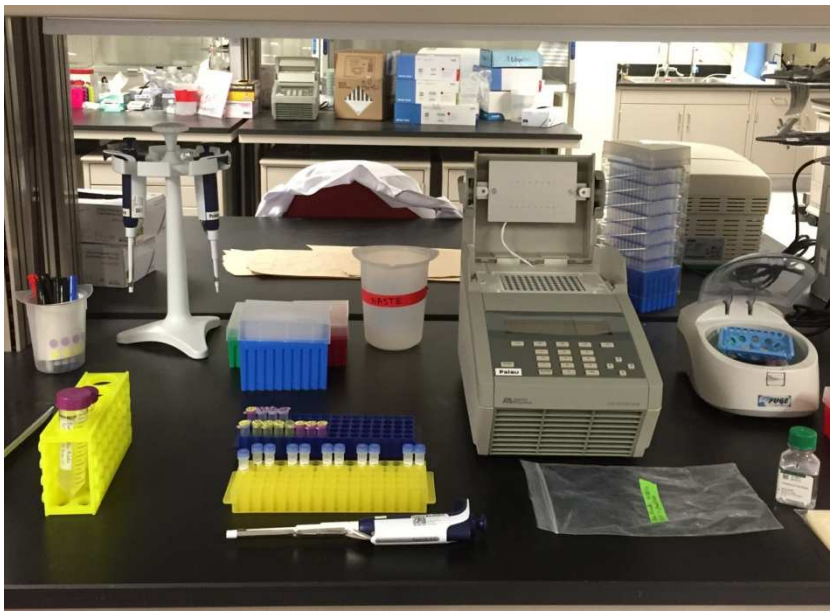


Fig. 1- Bench used at Regular PCR-room, similar to other 77.

Fig. 2- Bench with chapel used at isolated room.

Fig. 3- Right side of the lectotype of *Deuterodon pedri*: a) before the incision and b) after the incision, exemplifying the low level of damage of the specimen.

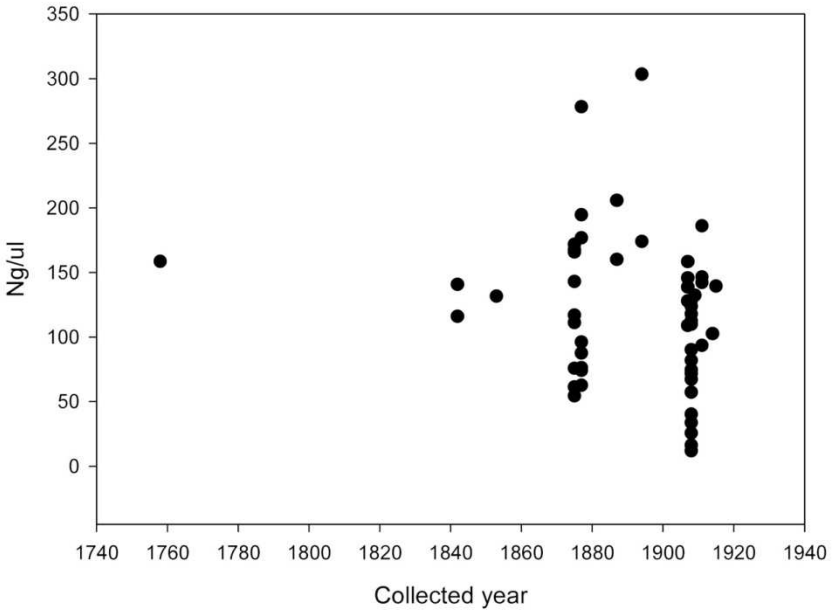


Fig. 4- Correlation between year of collected samples and mount of DNA extracted. The graphic shows that there is no correlation between these two variables.

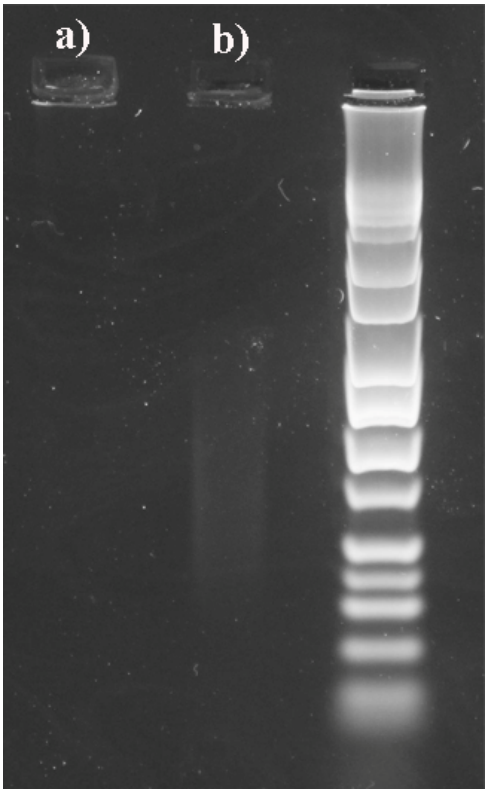


Fig. 5- Agarose gel of extracted DNA of *Tetragonopterus rutilus jequitinhonhae* NWM57760:2: a) extracted with Qiamp micro kit b) extracted with DNA first all Kit.

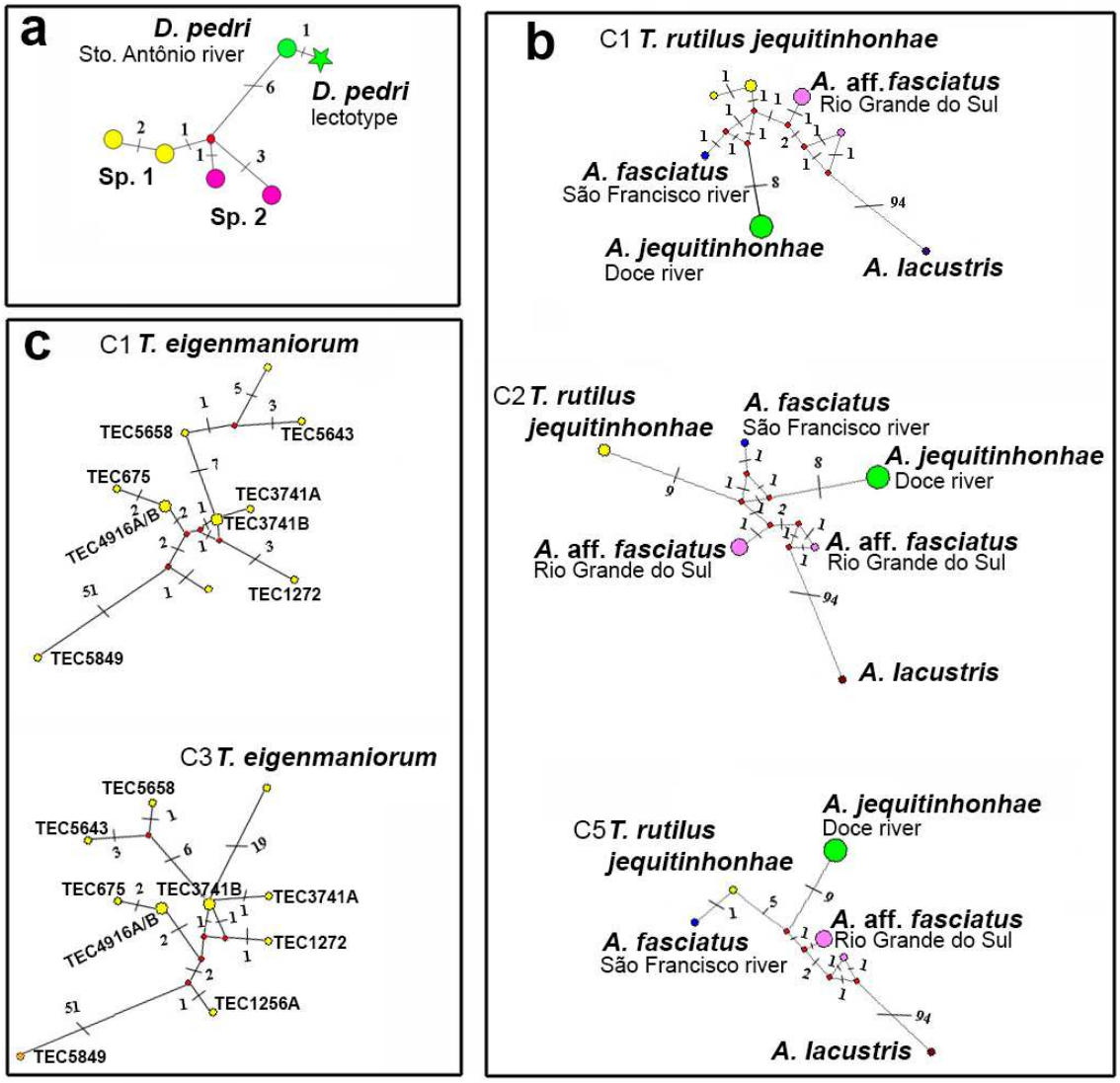


Fig. 6- Haplotype networks constructed for valid sequences obtained in this study for ancient samples: a) Haplotype network drawn with C2 of *D. pedri* lectotype and sequences with low p-distance on the matrix. b) Haplotype networks for *T. rutilus jequitinhonhae*. C1 network shows more similarity with *Astyanax fasciatus* from São Francisco and *Astyanax* aff. *fasciatus* from Rio Grande do Sul. C2 network shows a high variable and high number of mutational steps (9) between species with the low p-distance on the matriz, indicating absence of the samples that matchs with the sequence. C5 network shows more similarity with *Astyanax fasciatus* from São Francisco.c) C1 and C3 network drawn for *T. eigenmaniorum* sample showing high number of mutational steps (5 and 19) between the holotype and samples with the lower value of p-distance on the matriz. The patterns found in b) and c) is strongly indicating the absence of the sample that match with the sequences of syntypes (b) and holotype (c).

Supporting information

S1. Model of request to sample ancient specimens for aDNA research

Request for invasive sampled procedures: Ancient DNA from type specimens from (collection name Ex: ZMUC)

Project title: Extraction of ancient DNA to provide taxonomic solutions in identifying *Astyanax* species, a complex group of Neotropical fish

Proponent: Priscilla Caroline Silva, PhD candidate

Advisors: Dr. Richard Vari, Smithsonian Institution and Dr. Luiz Roberto Malabarba, Universidade Federal do Rio Grande do Sul

Briefly introduction about the project:

Astyanax is one of the most speciose fish genera of the Neotropical Region with approximately 158 valid species. The lack of synapomorphies defining the genus results in a taxonomically complex group of species. The great part of *Astyanax* species was described in the 19th and first half of 20th Centuries, with poor details. Most type specimens are in poor preservation conditions. The lack or partial information from type specimens increases the possibility of errors in species recognition due to morphological misinterpretations. Genetype is a recent proposal to link taxonomy and molecular systematic. It consists of DNA sequences from type specimens. The Genetype establishment increases the resolution in the recognition of difficult species. Ancient DNA is the sequencing of DNA from ancient organisms like fossils and specimens collected in past centuries. The establishment of Genetypes from Ancient DNA has solved historical taxonomical problems. The amplification of citochrome oxidase subunit 1 from Ancient DNA of *Astyanax* type specimens and types of other related genera will allow barcoding comparisons with recent collected specimens. This certainly will help to solve many taxonomical problems.

Guidelines to procedures:

The tissues will be extracted from type specimens deposited in collections around the world. The extraction will be made with total care to damage less as possible the specimens. We will evaluate the best way in each case, which could be done by:

1) removing part of the gill filaments on right side of the body (when specimens are in excellent conditions, with scales in all body)

2) removing small amounts of muscle by small incision below the dorsal fin on right side of the body or under pelvic fins (when specimens are in not so good conditions of preservation, lacking scales in the body).

These regions has been chosen because are not taxonomic informative for these specimens.

All procedure will be photographed before and after incision/filaments extraction with a small report that will send to the curator of the collection.

Molecular techniques will be developed on Smithsonian Institution, under supervision of a technical team with at least ten professionals. Kits for forensic DNA will be utilized for DNA

extraction. The citochrome oxidase subunit 1 COI fragment will be recover by PCR made with primers, specially designed, that can amplified small fragments of DNA because of the fragmentation condition of the Ancient DNA.

All results will be available for collections of origin of each specimen. The sequences generated will be deposited on public banks, like Gen Bank and BOLD.

About the proponent:

Priscilla is a Brazilian PhD. Candidate of Universidade Federal do Rio Grande do Sul in Brazil under supervision of professor Luiz Malabarba. She is developing part of her research on National Museum of Natural History of Smithsonian Institution under supervision of professor Richard Vari. On Smithsonian she is working with Ancient DNA of fish species up to approximately 300 years old. She has experience on molecular phylogenetic studies and difficult DNA. She is specializing on taxonomic and phylogenetic studies of some Neotropical fish species from Characidae family, popularly knowing as Tetras.

She gets excellent results (DNA extraction, PCR and sequencing) with the holotype of *Tetragonopterus eigenmanniorum* collected before 1894 and deposited on Academy of Natural Sciences, Philadelphia, Pennsylvania, U.S.A. The experience with this specimen allow us confiability and a previously tested protocol what increases the go of success.

(Briefly comments about the interest specimens of that museum Ex: The specimens of *Tetragonopterus cuvieri*, ZMB 9198; *Tetragonopterus lacustris*, ZMB 9200 and *Tetragonopterus rivularis*, ZMB 9199 from the Museum für Naturkunde Berlin, are type specimens of species know only by the original descriptions. Although the knowledge of the type localities and elevated number of types on other museums, anyone in two centuries could advance in determine correctly these species on recent collected material. These species are nowadays part of *Astyanax* a complex and undefined genus from Neotropical region. The Ancient DNA from these specimens can be an additional tool on the decision and better re description of the species that these represent. Additionally the establishment of Ancient DNA to try solves the complex species can be the start of resolution by major problems in phylogenetics studies. The most basal and truly statement for any scientific work in biology is the correct identification of the considered taxons, only if it occurs is possible to answer major questions in correct way.)

Washington DC, june, 11th, 2015

Priscilla Caroline Silva

Proponent

Luiz Roberto Malabarba

Brazilian Advisor

Capítulo 5

Rediscovery of the holotype of *Tetragonopterus vittatus* Castelnau 1855, a senior synonym of *Moenkhausia doceana* (Steindachner 1877) (Characiformes: Characidae)

Artigo publicado na Revista Zootaxa

ISSN 1175-5326 (print edition)

ISSN 1175-5334 (online edition)

Volume: 4132, Número: 2, 2016

Doi: http://doi.org/10.11646/zootaxa.4132.2.6

Priscilla C. Silva and Luiz R. Malabarba

Zootaxa 4132 (2): 269–271
http://www.mapress.com/j/zt/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4132.2.6
http://zoobank.org/urn:lsid:zoobank.org:pub:C95C0D7B-1DCF-4EBE-9C0E-E4ADB2B37093

Rediscovery of the holotype of *Tetragonopterus vittatus* Castelnau 1855, a senior synonym of *Moenkhausia doceana* (Steindachner 1887) (Characiformes: Characidae)

PRISCILLA CAROLINE SILVA[1] & LUIZ ROBERTO MALABARBA[2]
Universidade Federal do Rio Grande do Sul, Departamento de Zoologia, Programa de pós graduação em Biologia Animal, Laboratório de Ictiologia. Avenida Bento Gonçalves, 9500, prédio 43435, sala 104, Porto Alegre, Rio Grande do Sul, Brazil.
E-mail: [1]pricarola@gmail.com [2]malabarb@ufrgs.br

The description of *Tetragonopterus vittatus* presented by Francis L. Castelnau (1855) was very concise and apparently based on a single specimen (not explicitly stated in the text, but deducible according to the single counts for the meristic data presented in the description). The type locality is recorded simply as "Bahia", with no specification of the drainage or nearby city. The existence of type specimens has been considered unknown (Lima *et al.*, 2003; Eschmeyer & Fricke, 2015; Lucena & Soares, 2016), and have not been mentioned in published catalogues for type specimens of MNHN (*e.g.* Bertin, 1948).

In a revision of the genus *Astyanax*, Eigenmann (1921) examined several specimens belonging to the *Astyanax bimaculatus* group from Bahia, and considered that "the only variety of this genus from Bahia that can possibly have been used by Castelnau for his figure of *A. vittatus* is the one that was later designated as *A. bahiensis* by Steindachner [1877]." Eigenmann (1921) considered the species described by Castelnau (1855) as a valid subspecies, *Astyanax bimaculatus vittatus*, with *Astyanax bahiensis* as a junior synonym. Eigenmann (1921) further mentioned that *A. bimaculatus vittatus* is likely closely related to *Astyanax bimaculatus lacustris*. Subsequent authors dealing with the systematics of species belonging to the *Astyanax bimaculatus* complex (Garutti & Britski, 1997; Garutti, 1998; Garutti, 1999) followed Eigenmann's decision and presented, along with the descriptions of new characid species from the *Astyanax bimaculatus* complex, diagnoses to distinguish *Astyanax bimaculatus vittatus* from the other members of the complex. Lima *et al.* (2003) considered *Tetragonopterus vittatus* as a provisional synonym of *Astyanax bimaculatus*. More recently, Lucena & Soares (2016) considered *T. vittatus* as a *species inquirenda* and *A. bahiensis* as a valid species.

FIGURE 1. MNHN 0000-3088, holotype of *Tetragonopterus vittatus*.

During a visit to the Muséum National D'Histoire Naturelle (MNHN), Paris, France, one of us (PC) found a lot (MNHN 0000-3088) containing a single characid specimen from Bahia collected by Castelnau (Fig. 1). The modern label stuck outside the jar bears the identification of *Astyanax bimaculatus*. In the original catalog the lot MNHH 0000 – 3088 is assigned the designation of *Tetragonopterus vittatus*, identified and collected by Castelnau at "Bahia". This lot is

Accepted by F. Lima: 10 May 2016; published: 29 Jun. 2016

further identified as "type of Castelnau" in the catalog, with an apparently latter addition that says "= *T. maculatus*". Castelnau (1855) provided a brief description and a full depiction (plate XXXIII, Fig. 2) of a single specimen, presumably the holotype, measuring 10 cm in total length and 3 cm depth, and these measurements approximately fit the measurements of the discovered specimen. The specimen measures 68 mm in standard length, but it lacks part of the caudal fin and has the appearance of having dried up at least once, making it impossible to retrieve the actual total length (presumably 10 cm). Other data taken from specimen MNHN 0000–3088 that fit in the description and illustration of *Tetragonopterus vittatus* are (information given by Castelnau follows in parentheses): the measured depth of 27.5 mm (3 cm); the presence of 3 unbranched rays with anteriormost only visible under stereomicroscope examination and 33 branched rays in the anal fin (Castelnau described one little spine and 34 rays). The latter number seems to include the longest unbranched ray plus 33 branched rays. The little spine likely refers to the second unbranched ray (the first unbranched ray seems to not have been observed by Castelnau), dorsal-fin elongated with 2 unbranched and 9 branched rays (1 spine and 9 rays corresponding to the longest unbranched ray plus 9 branched rays; the first, shorter unbranched ray of the dorsal-fin was apparently not counted or observed by Castelnau), one unbranched and 7 branched rays at pelvic-fins (8 rays, including the longest unbranched and all branched rays), pectoral-fins with 1 unbranched and 13 branched rays (14 rays, including the longest unbranched and all branched rays). The body shape and form of the humeral spot of the presumed holotype also fits the description and illustration of Castelnau (compare Figs. 1 and 2).

FIGURE 2. The original drawing of the holotype of *Tetragonopterus vittatus* presented in Castelnau (1855).

The discovery of this type specimen makes it possible to reevaluate the status of *Tetragonopterus vittatus*. In fact, the examination of the holotype easily allows its identification as *Moenkhausia doceana* (Steindachner 1877), a species recently redescribed from coastal river systems from northern Espírito Santo state to southern Bahia State, Brazil (Carvalho *et al.*, 2014).

Characters that allows to recognize *Tetragonopterus vittatus* and *Moenkhausia doceana* as the same species are the high number of teeth in maxillary bone (7 in the holotype and 4–7, mode 5 in *M. doceana*); the number of branched anal-fin rays (33 in the holotype and 29–34, mode 32 in *M. doceana*); and the number of scales above lateral line (7 in the holotype and 7–8, mode 7 in *M. doceana*). The holotype of *Tetragonopterus vittatus* also fits with *M. doceana* in scales counts (35 perforated scales in lateral line), gill rakers (8 on epibranchial, 1 between epibranchial and ceratobranchial and 11 on ceratobranchial/hypobranchial), and the vertically-elongated oval shape of the humeral spot.

Similar to *Moenkhausia doceana*, the holotype of *T. vittatus* has the outer row of teeth in premaxilla with 5 tricuspid teeth; inner row with 5 teeth with 4 to 6 cusps (5 cusps in *M. doceana* according to Carvalho *et al.*, 2014); dentary with 5 large pentacuspid teeth, followed by 12 conical or tricuspid teeth, abruptly smaller than the 5 anteriormost teeth; 7 teeth with 3–5 cusps in maxillary bone (3–4 cusps in *M. doceana* according to Carvalho *et al.*, 2014). These small differences between the holotype of *T. vittatus* and *M. doceana* are not considered to be significative enough to justify the recognition of two species. Consequently, since *Tetragonopterus vittatus* has priority over *Moenkhausia doceana*, the valid name for the species is *Moenkhausia vittata*, new combination, with *Moenkhausia doceana* as a junior synonym. The ending of the name of the species has been changed to agree in gender with the name of the genus (Code art. 31.2).

Castelnau (1855) described *M. vittata* and several other fish species (including *Crenicichla lacustris*) from specimens collected during his stay as consul of France at Salvador, Bahia. The type locality assigned for *M. vittata* in the original description is "Bahia", but Lucena & Soares (2016) considered it as "=Salvador" [sic] according to Kullander & Lucena (2006). The last authors considered the type locality of *Crenicichla lacustris* (Castelnau, 1855),

originally assigned as "Dique, ou étang près de Bahia" as equal to Salvador, Bahia, and probably an error due to the absence of this species at Salvador or nearby this location. Kullander & Lucena (2006) considered the correct type locality as "somewhere near Rio de Janeiro", but this conclusion does not seems to extend to *M. vittata*. This species does not occur in Rio de Janeiro state nor Salvador and its surroundings [the many localities listed near Salvador by Eigenmann (1921) for *Astyanax bimaculatus vittatus* actually refer to *Astyanax bahiensis* or *Astyanax lacustris*]. It ranges from rio Riacho, a small coastal basin south of rio Doce in Espírito Santo State, to rio João de Tiba, on southern coastal Bahia State, Brazil (Carvalho *et al*. 2014). We have no reason to consider a type locality distinct from Bahia as stated in the original description for *M. vittata*, and we consider that the type specimen has been probably collected in the southern portion of this State, and not in the surroundings of either Salvador or Rio de Janeiro, where the species is absent.

Acknowledgements

We are grateful to Patrice Pruvost and Philipe Keith for allowing access to the MNHN collection, and to Claude Ferrara and Michel Jégu for the support at MNHN during the visit of the first author, and finally to Casey Dilman for the English review of the manuscript.

Literature cited

Bertin, L. (1948) Catalogue des types de poissons du Museum National d'Histoire Naturelle. Paris, 1939–1950 (3e partie: Ostariophysaires (Characiniformes, Gymnotiformes). *Bulletin du Museum national d'histoire naturelle*, 1, 1–49.

Carvalho, F.R., Sarmento-Soares, L.M. & Martins-Pinheiro, R.F. (2014) Redescription of *Moenkhausia doceana* (Steindachner, 1877) (Ostariophysi: Characiformes): a characid from the Northeastern Mata Atlântica ecoregion, Brazil. *Neotropical Ichthyology*, 2, 377–388.

Castelnau, F.L. (1855) Poissons. *In: Animaux nouveaux or rares recueillis pendant l'expédition dans les parties centrales de l'Amérique du Sud, de Rio de Janeiro a Lima, et de Lima au Para; exécutée par ordre du gouvernement Français pendant les années 1843 a 1847. Zoologie*. P. Bertrand, Paris, 7 (2), xii + 112 pp.

Eigenmann, C.H. (1921) The American Characidae. Part 3. *Memoirs of the Museum of Comparative Zoology*, 43, 209–310.

Eschmeyer, W.N. & Fricke, R. (2015) *Catalog of fishes: genera, species, references. Eletronic version*. California Academy Sciences. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 12 December 2015)

Garutti, V. & Britski, H.A. (1997) Descrição de uma espécie nova de *Astyanax* (Teleostei, Characidae), com mancha umeral horizontalmente ovalada, da bacia do rio Guaporé, Amazônia. *Papéis Avulsos de Zoologia*, 40, 217–229.

Garutti, V. (1998) Descrição de uma espécie nova de *Astyanax* (Teleostei, Characidae) da Bacia do Tocantins, Brasil. *Iheringia, Série Zoologia*, 85, 115–122.

Garutti, V. (1999) Descrição de *Astyanax argyrimarginatus* sp. n. (Characiformes, Characidae) procedente da bacia do Rio Araguaia, Brasil. *Revista Brasileira de Biologia*, 59, 585–591.

Kullander, S.O. & Lucena, C.A.S. (2006) A review of the species of *Crenicichla* (Teleostei: Cichlidae) from the Atlantic coastal rivers of southeastern Brazil from Bahia to Rio Grande do Sul States, with descriptions of three new species. *Neotropical Ichthyology*, 4, 127–146.

Lima, F.C.T., Malabarba, L.R., Buckup, P.A., Pezzi da Silva, J.F., Vari, R.P., Harold, A., Benine, R., Oyakawa, O.T., Pavanelli, C.S., Menezes, N.A., Lucena, C.A.S., Malabarba, M.C.S.L., Lucena, Z.M.S., Reis, R.E., Langeani, F., Casatti, L., Bertaco, V.A., Moreira, C. & Lucinda, P.H.F. (2003) Genera *Incertae Sedis* in Characidae (2003). *In:* Reis, R. E., Kullander, S.O. & Ferraris Jr., C.J. Rio de Janeiro (Eds.), *Check list of the freshwater fishes of South and Central America*. EDIPUCRS, Porto Alegre, pp. 106–169.

Lucena, C.A.S. & Soares, H.G. (2016) Review of species of the *Astyanax bimaculatus* "caudal peduncle spot" subgroup sensu Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. *Zootaxa*, 4072 (1), 101–125. http://dx.doi.org/10.11646/zootaxa.4072.1.5

Conclusões Gerais

- Análises moleculares e morfológicas recuperaram um grupo monofilético no clado C, nomeado Probolodini Géry, composto pelos gêneros *Deuterodon*, *Probolodus*, *Myxiops*, por *Hyphessobrycon luetkenii*, espécies de *Astyanax* da região costeira do Brasil e espécies de *Jupiaba* com arranjo de dentes do dentário similar ao encontrado nos demais gêneros. A recuperação desse mesmo clado por conjuntos de dados diferentes torna a hipótese de existência do mesmo mais robusta (sistemática integrativa).
- Foi demonstrado através de caracteres morfológicos e moleculares que o gênero *Deuterodon* é polifilético na sua composição atual. Propõe-se o reconhecimento de um gênero *Deuterodon sensu stricto* sustentado por 9 sinapomorfias e composto por 7 espécies [*D. iguape* Eigenmann, *D. langei* Travassos, *D. longirostris* (Steindachner), *D. rosae* (Steindachner), *D. singularis* Lucena & Lucena, *D. stigmaturus* (Gomes), and *D. supparis* Lucena & Lucena].
- *Myxiops* é um gênero válido sustentado por 22 autapomorfias.
- A espécie-tipo do gênero *Probolodus* apresenta 10 autapomorfias, sendo necessária a sua análise nas demais espécies do gênero para avaliar quais correspondem a sinapomorfias do gênero.
- *Astyanax* é um gênero polifilético e a maioria das espécies de *Astyanax* da região costeira estão mais estreitamente relacionadas a outros gêneros do que à *Astyanax mexicanus*, espécie-tipo do gênero, devendo ser consideradas como *Incerta sedis*. *Astyanax stricto sensu* deve ser considerado composto por apenas as espécies que compõe *Astyanax* clade encontrado neste estudo e que corresponde ao clado 1 de Rossini *et al.*, 2016.
- *Jupiaba* é um gênero polifilético com espécies distribuídas por toda a árvore filogenética. O espinho pélvico característico utilizado para justificar a proposta desse gênero por Zanata (1997) evoluiu independentemente, sendo, portanto, mais um exemplo de convergência adaptativa de um caráter morfológico em Characidae.

- *Deuterodon pedri* é mais relacionado à *Astyanax pelecus* e duas outras espécies de caracídeos não descritos do que às espécies do gênero *Deuterodon sensu stricto*.
- É possível recuperar DNA antigo de espécimes coletados nos séculos passados através de metodologia de Sanger. O uso dessas técnicas permitiu o reconhecimento de *Deuterodon pedri* que teve a identidade esclarecida com o auxílio do DNA extraído do lectótipo juntamente com análise taxonômica tradicional.
- A redescoberta do holótipo de *T. vittatus*, considerado como desconhecido, permitiu a revalidação da espécie em uma nova combinação, como *Moenkhausia vittata*.
- O uso de técnicas tradicionais tais como estudo osteológico em conjunto com técnicas de biologia molecular permitiram a formulação de hipóteses filogenéticas mais robustas de relações entre táxons problemáticos em Characidae, corroboradas por dois métodos distintos de análise.