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TESE DE DOUTORADO

**IDENTIFICAÇÃO MOLECULAR E RELAÇÕES FILOGENÉTICAS DA
FAMÍLIA CALLICHTHYIDAE (ACTINOPTERYGII: SILURIFORMES)**

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TESE DE DOUTORADO

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FAMÍLIA CALLICHTHYIDAE (ACTINOPTERYGII: SILURIFORMES)

Tese apresentada ao programa de Pós-Graduação *Stricto sensu* em Ciências Biológicas (Zoologia) do Instituto de Biociências de Botucatu, Universidade Estadual Paulista “Julio Mesquita Filho”, como requisito para a obtenção do título de Doutorado.

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Palavras-chave: Biodiversidade; DNA *Barcoding*; Sistemática;
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"A NATUREZA DA GENTE NÃO CABE EM CERTEZA NENHUMA."

JOÃO GUIMARÃES ROSA

RESUMO: Callichthyidae é uma das maiores famílias de Siluriformes, endêmica da região Neotropical, com mais de 220 espécies distribuídas em duas subfamílias, Callichthyinae e Corydoradinae. Os calictídeos exibem uma ampla gama de especializações genéticas, morfológicas, fisiológicas e ecológicas, habitando os mais variados ecossistemas e adotando diferentes estratégias de vida. A grande diversidade da família proporciona material para estudos de questões fundamentais em evolução e ecologia. As relações filogenéticas de Callichthyidae vêm sendo alvo de alguns estudos sistemáticos há alguns anos, propostos com base em caracteres morfológicos e moleculares. Entretanto, diversas dúvidas ainda persistem devido ao número de caracteres utilizados na formulação das árvores em estudos morfológicos e ao baixo suporte estatístico dos ramos em estudos moleculares. O presente estudo foi desenvolvido no Laboratório de Biologia e Genética de Peixes (LBP), e teve por objetivo principal testar as relações filogenéticas de Callichthyidae, e os processos evolutivos responsáveis pela grande diversidade de espécies dessa família. Para isso, realizamos inicialmente uma delimitação molecular das espécies de ambas as subfamílias e, subsequentemente, a filogenia. Para a identificação molecular construímos um banco de dados de DNA *barcode* com mais de 1000 sequências do gene mitocondrial *citocromo c oxidase subunidade I* (COI) representando ambas as subfamílias. Nossas análises filogenéticas abrangeram mais de 60% da família, através da análise de 188 espécies e 2.536 loci através de dados de elementos ultraconservados (UCEs). Os resultados das identificações moleculares reconheceram uma diversidade antes subestimada para algumas linhagens, que deverão contribuir para ampliação do conhecimento sobre diversidade, filogenia e os processos evolutivos envolvidos na diversificação das espécies de Callichthyidae. As relações observadas com os resultados das análises filogenéticas são, de várias maneiras, diferentes das hipóteses morfológicas e moleculares previstas na literatura. Os resultados apoiam fortemente a existência de oito grupos monofiléticos dentro de Corydoradinae, que devem ser tratados como gêneros independentes. Com isso, nós propomos uma nova hipótese de relacionamento entre os gêneros, permitindo um reconhecimento de todas as linhagens monofiléticas, representando um passo importante para melhor delimitar e reconhecer a diversidade de espécies em Callichthyidae, a fim de lançar luz sobre futuras investigações taxonômicas e biogeográficas, e esclarecer a fascinante história evolutiva desses animais.

PALAVRAS-CHAVE: DNA *Barcoding*, biodiversidade, UCE, Sistemática, Taxonomia

ABSTRACT: Callichthyidae is one of the largest families of Siluriformes, endemic to the Neotropical region, with more than 220 species distributed in two subfamilies, Callichthyinae and Corydoradinae. Callitids exhibit a wide range of genetic, morphological, physiological and ecological specializations, inhabiting the most varied ecosystems and adopting different life strategies. The high diversity of the family provides material for studying fundamental issues in Evolution and Ecology. The phylogenetic relationships of Callichthyidae have been the subject of some systematic studies for some years, proposed based on morphological and molecular characters. However, several doubts still persist due to the number of characters used in the formulation of trees in morphological studies and the low statistical support of branches in molecular studies. The present study was carried out at the Laboratório de Biologia e Genética de Peixes (LBP), and its main objective was to test the phylogenetic relationships of Callichthyidae, and the evolutionary processes responsible for the great diversity of species in this family. For this, we initially performed a molecular delimitation of the species of both subfamilies and, subsequently, the phylogeny. For molecular identification we constructed a DNA barcode database with more than 1000 sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene representing both subfamilies. Our phylogenetic analyzes covered more than 60% of the family, through the analysis of 188 species and 2,536 loci through ultra-conserved element data (ECUs). The results of the molecular identifications recognized a previously underestimated diversity for some lineages, which should contribute to the expansion of knowledge about diversity, phylogeny and the evolutionary processes involved in the diversification of Callichthyidae species. The relationships observed with the results of phylogenetic analyzes are, in many ways, different from the morphological and molecular hypotheses predicted in the literature. The results strongly support the existence of eight monophyletic groups within Corydoradinae, which should be treated as independent genera. We propose a new hypothesis of relationship between genera, allowing recognition of all monophyletic lineages, representing an important step to better delimit and recognize the species diversity in Callichthyidae, in order to shed light on future taxonomic and biogeographic investigations, and to clarify the fascinating evolutionary history of these animals.

KEY WORDS: DNA *Barcoding*, Biodiversity, UCE, Systematics, Taxonomy

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1. INTRODUÇÃO

1.1 VISÃO GERAL DE CALLICHTHYIDAE

Os peixes representam a maior diversidade de táxons de vertebrados existentes do planeta, com mais de 35 mil espécies descritas (Nelson, 2006). Dentro de peixes com nadadeiras raiadas (Actinopterygii), a maioria das espécies pertence a três ordens principais: Cypriniformes (3.268 espécies; 12%), Perciformes (10.033 espécies; 36%), e Siluriformes (2.867 espécies; 10%) (Nelson, 2006). Dentro dos Siluriformes se encontra a superfamília Loricarioidea (mais de 1.500 espécies), que compreende 40% dos Siluriformes de todo o mundo (Fricke *et al.*, 2022). Essa superfamília representa um clado monofilético de peixes de água doce constituído pelas famílias Astroblepidae, Callichthyidae, Loricariidae, Nematogenyidae, Scoloplacidae e Trichomycteridae (de Pinna, 1998). Callichthyidae representa a terceira maior família dentre os Siluriformes de água doce, ficando atrás somente de Loricariidae, com 1015 espécies, e Trichomycteridae, 346 espécies.

A família Callichthyidae é representada por peixes endêmicos da região Neotropical conhecidos popularmente como “tamuatás” ou “cascudos”. Esse grupo tem sido considerado monofilético por vários autores (Nijssen & Isbrücker, 1980; de Pinna, 1998; Reis, 1998; Shimabukuro-Dias *et al.*, 2004), e é facilmente diagnosticada de outros Siluriformes por apresentar o corpo coberto por duas séries longitudinais de placas ósseas (Bonaparte, 1838), divididas pela linha lateral. Também podem ser incluídas como sinapomorfias dessa família a presença de dentes pré-maxilares pequenos ou ausentes em adultos (Günther, 1864); bexiga natatória aberta externamente, e parcialmente coberta pela expansão do pteroticosupracleitro (Eigenmann & Eigenmann, 1890); série infraorbital reduzida, com estrutura que suporta o globo ocular na sua face interna (Regan, 1911); ausência do osso lacrimal-antorbital; linha lateral reduzida, apresentando de um a seis canais tubulares; cintura peitoral apresentando processo posterior do cleitro suturado ao coracóide (Reis, 1998).

A família Callichthyidae é representada por duas subfamílias, Callichthyinae e Corydoradinae. A subfamília Callichthyinae é composta por cinco gêneros: *Callichthys* Scopoli, 1777 (quatro espécies), *Lepthoplosternum* Reis, 1997 (seis espécies), *Megalechis* Reis, 1997 (duas espécies), *Dianema* Cope, 1871 (duas espécies) e *Hoplosternum* Gill, 1858 (três espécies). A segunda, e maior subfamília, Corydoradinae

é composta atualmente por apenas três gêneros, organizados em duas tribos: Corydoradini, monotípica maior tribo, tendo como representante válido apenas o gênero *Corydoras* Lacepède 1803 (176 espécies), e Aspidoradini, composta por *Aspidoras* Ihering, 1907 (22 espécies) e *Scleromystax* Günther, 1864 (cinco espécies) (Fricke *et al.*, 2022). É inevitável que grande parte dos trabalhos existentes na literatura sejam relacionados à Corydoradinae, já que essa subfamília abrange 90% da diversidade dentro do Callichthyidae, com mais de 200 espécies válidas, e espécies novas vem sendo descritas anualmente, principalmente em *Corydoras*.

Os calictídeos tem uma ampla distribuição, podendo ser encontrados em quase todas as bacias hidrográficas da América do Sul, com exceção das drenagens do Chile. São distribuídos desde as drenagens do Norte, nos rios Orinoco e Ilha de Trinidad e Tobago e nas drenagens costeiras do escudo das Guianas, até ao Sul, nas drenagens da Argentina. Também são encontrados de Leste a Oeste da América do Sul, em todas as bacias do Brasil e nas bacias costeiras do Atlântico, bem como nos tributários da Laguna Medellín, no Uruguai. A grande maioria das espécies de Callichthyidae é originalmente descrita da bacia dos rios Amazonas e das Guianas (Cockerell, 1925; Reis, 1998).

Os calictídeos são encontrados em diferentes habitats de água-doce. Alguns podem ser encontrados no fundo dos rios, como *Callichthys*, e outros na coluna d'água, como as espécies do gênero *Dianema* e *Corydoras*. Todos os representantes dessa família apresentam respiração acessória, além da respiração habitual através das brânquias, o que os permitem respirar oxigênio atmosférico que é coletado pela boca e ingerido, realizando as trocas gasosas através do epitélio altamente vascularizado do intestino. Por esse motivo, esse órgão é considerado como órgão respiratório auxiliar desta família (Reis, 1998). Além disso, o ar atmosférico presente no intestino também é o responsável pelo controle hidrostático nesses animais (Gee & Graham, 1978). Essa peculiaridade do intestino dos calictídeos é o que os permite sobreviver em ambientes considerados hostis para a grande maioria dos peixes de água doce. Algumas espécies de calictídeos, principalmente do gênero *Hoplosternum*, podem ser encontrados em ambientes com baixa disponibilidade de ar dentro d'água, como ambientes pantanosos e salobros, graças à capacidade de realizar trocas gasosas no intestino ao captarem oxigênio atmosférico. Os calictídeos apresentam baixa importância econômica de consumo, destacando-se apenas *Hoplosternum* na pesca artesanal. Entretanto, o gênero *Corydoras* é um dos mais apreciados por aquaristas do mundo todo, e por isso, é

cultivado em cativeiro em diversos países. Embora representantes de *Corydoras* apresentem uma ampla variedade de formas, colorido e comportamento, atraindo ictiólogos ao longo da extensa história taxonômica do gênero, historicamente, problemas relacionados à sistemática de *Corydoras* remontam desde a sua descrição original, no século XIX. Assim, elucidar as relações sistemáticas dos *Corydoras* tem se mostrado uma tarefa muito desafiadora, oferecendo uma visão limitada das relações sistemáticas desse gênero rico em espécies.

Por fim, um fóssil bem preservado descoberto na Província de Juyuy, na Argentina, ajudou a esclarecer as antigas origens evolutivas dessa notável família de peixes. *Corydoras revelatus* (Cockerell, 1925) foi descrito no início do século XX da Formação Sierra del Maíz Gordo, na Argentina, colocando as origens da subfamília no final do Paleoceno, época em que já eram semelhantes morfologicamente às espécies existentes atualmente (Cockerell, 1925). Fragmentos fósseis mais recentes de calictídeos foram descobertos na Formação La Venta do Mioceno Médio de Columbia (Lundberg, 1997). Esses fragmentos consistem em um crânio e placas pré-dorsais que foram atribuídas a *Hoplosternum* sp. Restos de espinhos de nadadeiras peitorais de calictídeos claramente distinguíveis foram identificados na formação do Solimões (Reis, 1998), sugerindo que tiveram uma origem comum antiga da fauna em diferentes locais da América do Sul (Lundberg *et al.*, 1998).

1.2 HISTÓRICO DE ESTUDOS MORFOLÓGICOS EM CALLICHTHYIDAE

A grande maioria das investigações taxonômicas e sistemáticas de inter-relações de Callichthyidae foi baseada em caracteres morfológicos e análises cladísticas. O primeiro calictídeo descrito foi *Silurus callichthys*, atualmente *Callichthys callichthys*, descrito por Linnaeus (Linnaeus, 1758). A família Callichthyidae tem esse nome devido ao seu gênero-tipo *Callichthys*, de etimologia Grega, que deriva das palavras “kallis” significando beleza e “ichthys” significando peixe.

Após a descrição do primeiro calictídeo, as grandes contribuições taxonômicas envolvem, primeiramente, Hoedeman (1952), que realizou uma revisão da sistemática do grupo, onde propôs pela primeira vez a subdivisão de Callichthyidae em duas subfamílias e cinco tribos: Callichthyinae, que seria então composta pelas tribos Callichthyini, composta por *Callichthys* e *Hoplosternum*; Cascadurini composta pelo

gênero *Cascadura* Ellis, 1913; e Dianemini, composta por *Dianema* e *Cataphractops* Fowler, 1915; e a subfamília Corydoradinae, formada pelas tribos Aspidoradini, composta somente pelo gênero *Aspidoras*, e Corydoradini, composta por *Corydoras* e *Brochis* (Cope, 1872).

Em 1970, Nijssen apresentou uma revisão dos *Corydoras* do Rio Suriname, incluindo em suas análises exemplares de 17 espécies, onde descreveu sete novas espécies baseado principalmente no padrão de coloração e características morfológicas. Também apresentou uma chave de identificação de *Corydoras* do Suriname, figuras das espécies, comentários taxonômicos dos caracteres, mapa de distribuição e biogeografia. Dez anos depois, Nijssen & Isbrücker (1980) apresentaram a última revisão taxonômica realizada de Corydoradinae, abrangendo 99 espécies de *Corydoras*, mas também incluíram nas análises os gêneros *Brochis* e *Aspidoras*, definindo caracteres diagnósticos para cada gênero, e separando as espécies de *Corydoras* em cinco linhagens: “grupo *punctatus*”, “grupo *barbatus*”, “grupo *aeneus*”, “grupo *elegans*” e “grupo *acutus*”. Os autores basearam a formulação dos grupos no padrão de coloração dos indivíduos e, mesmo morfológicamente distintas, algumas espécies foram colocadas no mesmo grupo devido ao padrão de coloração semelhante. Mais de 40% das espécies atualmente reconhecidas sob o gênero *Corydoras* foram taxonomicamente caracterizadas por Nijssen (1970) e Nijssen & Isbrücker (1980) com base nos caracteres morfológicos e padrão de coloração dessas espécies.

Uma abordagem baseada na morfologia constituiu a primeira análise filogenética cobrindo todos os gêneros de Callichthyidae, apresentada por Reis (1998). O autor descreveu a anatomia esquelética dos calictídeos, estudou as inter-relações filogenéticas entre as espécies e testou a monofilia da família. Alguns dos caracteres considerados para esta análise incluem: neurocrânio, canais látero-sensoriais, suspensório e arco mandibular, série infraorbital, série opercular, arco hióide, arcos branquiais, aparelho Weberiano e esqueleto axial, nadadeiras não pareadas, nadadeira peitoral e cintura, e a barbatana pélvica e a cintura. O cladograma resultante suporta a monofilia da família Callichthyidae e a divisão das subfamílias Callichthyinae e Corydoradinae. Dentro de Callichthyinae, o primeiro grupo a divergir foi *Callichthys*, este se demonstrou sendo irmão do clado *Leptoplosternum*, *Megalechis* e *Dianema* que se mostrou mais proximamente relacionado à *Hoplosternum*. Dentro de Corydoradinae, o gênero *Aspidoras* se demonstrou como grupo irmão de um clado formado por *Corydoras* e *Brochis*. Não havia caracteres que sustentassem a monofilia de *Corydoras*,

ao passo que a monofilia de *Brochis* era sustentada por quatro características derivadas. O autor fornece, também, uma chave de identificação para todos os gêneros de Callichthyidae com base nas características morfológicas descritas. Embora a análise tenha sido robusta, faltou amostragem taxonômica dentro dos Corydoradinae; mais notavelmente dentro do gênero *Corydoras* e *Scleromystax*, gênero posteriormente revalidado. Nesse trabalho, o autor reconhece 28 sinapomorfias através de análises de caracteres osteológicos, e demonstra que os gêneros de Callichthyidae são monofiléticos, com excessão de *Corydoras*. O autor sugere, então, que uma possível solução conservadora ao não monofiletismo de *Corydoras* seria que *Brochis* fosse sinonimizado a *Corydoras*, tornando-o monofilético.

Uma filogenia morfológica da subfamília Corydoradinae foi posteriormente apresentada. Britto (2003) reanализou e redefiniu com base em 83 caracteres morfológicos, propondo uma nova hipótese de relacionamento para esta subfamília. Este estudo expandiu a lista de caracteres morfológicos e cobertura taxonômica total dentro dos gêneros *Corydoras*, *Scleromystax* e *Aspidoras*. Um dos principais resultados foi a natureza não monofilética do gênero *Corydoras* conforme definido atualmente (Britto, 2003). O autor demonstrou que havia um agrupamento de espécies de *Corydoras* mais relacionado à *Aspidoras*, e outro agrupamento de *Corydoras* mais relacionado à *Brochis*, e então propôs uma nova classificação para Corydoradinae, revalidando o gênero *Scleromystax* para posicionar as espécies de *Corydoras* relacionadas a *Aspidoras*, e sinonimizando *Brochis* a *Corydoras*. Em vez de *Brochis* e *Corydoras* formarem um grupo com *Aspidoras*, Britto propôs um clado composto por *Aspidoras* e *Scleromystax*. O autor também propôs um novo esquema de classificação abrangendo os grupos monofiléticos definidos pelo cladograma de consenso estrito resultante de suas análises (Fig. 1).

1.3 HISTÓRICO DE ESTUDOS MOLECULARES EM CALLICHTHYIDAE

Esforços em estudos moleculares em Callichthyidae na literatura se concentram em Corydoradinae, e se iniciaram com trabalhos relacionados à diversidade cariotípica e citogenética de *Corydoras*. Scheel *et al.* (1972) apresentaram contagens cromossômicas de 14 espécies de *Corydoras* revelando grande variação cariotípica resultante de duplicações do genoma dessas espécies. Os autores concluíram que havia

muita variação interespecífica entre essas espécies, comparando seus resultados com os grupos propostos por Nijssen & Isbrucker (1970).

Outros estudos relevantes relacionados à citogenética de *Corydoras* foram desenvolvidos em seguida, como Oliveira *et al.* (1988) que demonstraram variações no número de cromossomos entre populações de *C. aeneus* de quatro rios no sul do Brasil, e indicam a existência de um sistema diplóide-tetraploide nessa espécie. Posteriormente, Oliveira *et al.* (1990) examinaram três populações alopátricas de *C. nattereri* da costa sudeste do Brasil, no estado de São Paulo, que eram consideradas como pertencentes da “mesma espécie” por Nijssen & Isbrucker (1980), pois não conseguiram distinguir grupos morfológicos entre essas populações. Entretanto, os resultados de Oliveira *et al.* (1990) exibiram três contagens cromossômicas diferentes para esses indivíduos (40, 42, 44), sugerindo isolamento reprodutivo em parte devido à diferenciação cariotípica, e os rearranjos cromossômicos observados foram aparentemente mais frequentes do que as modificações morfológicas em *C. nattereri*.

Mais dados citogenéticos demonstrando números diploides e de tamanho do genoma foram apresentados em alguns estudos subsequentes relatando dados da família Callichthyidae (Oliveira *et al.*, 1993a; Oliveira *et al.*, 1993b; Shimabukuro-Dias *et al.*, 2004a). Os números diplóides variaram de 44-100, principalmente entre *Hoplosternum* e *Callichthys*, e uma nova hipótese atualizada foi apresentada sobre evolução cromossômica da família. *Dianema*, *Hoplosternum*, e *Megalechis* apresentaram cromossomos muito semelhantes, sugerindo que haja uma possível relação entre esses gêneros (Oliveira *et al.*, 1993b).

Finalmente, no século 21, a primeira filogenia molecular da família Callichthyidae foi publicada. Shimabukuro-Dias *et al.* (2004b) realizaram um importante e pioneiro estudo das relações filogenéticas da família com base em caracteres morfológicos associados à dados moleculares. Os autores utilizaram os genes mitocondriais *12 S rRNA*, *16 rRNA* e *ND4*, *tRNA ser* e *tRNA his*, e sequências completas do *ARNt his*. A análise filogenética foi realizada usando máxima parcimônia e máxima verossimilhança, e os autores compararam e combinaram seus resultados com os dados morfológicos disponíveis. O grupo interno foi formado por 28 representantes da família, incluindo os gêneros *Corydoras* (12 espécies), *Brochis* (duas espécies), *Aspidoras* (três espécies), *Dianema* (duas espécies), *Hoplosternum* (duas espécies), *Callichthys* (duas espécies), *Lepthoplosternum* (duas espécies) e *Megalechis* (duas espécies). Além disso, no mesmo ano, dados citogenéticos nas contagens de cromossomos foram mapeados,

mostrando casos de duplicação do genoma entre as linhagens *Corydoras* e *Brochis* (Shimabukuro-Dias *et al.*, 2004a)

Os autores concluíram que os Callichthyidae formam um grupo monofilético que compreende dois grupos naturais: subfamília Corydoradinae (*Aspidoras*, *Brochis* e *Corydoras*) e subfamília Callichthyinae (*Callichthys*, *Dianema*, *Hoplosternum*, *Lepthoplosternum* e *Megalechis*). Essa filogenia molecular entra em conflito com as relações morfológicas propostas no que diz respeito às relações entre grupos-irmãos. De acordo com as análises conjuntas de Shimabukuro-Dias *et al.* (2004b), *Callichthys* é grupo irmão de *Lepthoplosternum* + *Megalechis*. Além disso, *Dianema* e *Hoplosternum* demonstraram serem os gêneros mais antigos da família, e não *Callichthys*, como havia sido citado na literatura (Gosline, 1940; Hoedeman, 1952; Ribeiro, 1959; Reis, 1998). Análises citogenéticas citadas pelos autores também corroboram esses dados, devido ao fato de que o tamanho do DNA de *Dianema*, *Hoplosternum* e *Megalechis* ser menor do que nos outros callichthyídeos. Outro ponto importante demonstrado é que a topologia molecular não tem suporte morfológico para o clado *Lepthoplosternum* e *Megalechis*, bem como para o clado incluindo todos os gêneros exceto *Dianema*, resultando em uma árvore não resolvida, se somente a morfologia for usada para inferência da topologia.

fim, um terceiro grupo formado de espécies de *Corydoras*, *C. difluviatilis* e *C. sodalis*, relacionadas à *Brochis*. As análises de Reis (1998) já haviam sugerido que *Aspidoras* e *Brochis* eram grupos monofiléticos, mas que *Corydoras* estaria longe disso, devido ao gênero *Brochis* estar causando o não monofiletismo de *Corydoras*. Nas análises de Shimabukuro-Dias *et al.* (2004) o gênero *Bhochis* se mostrou representante de um grupo natural (monofilético). Contrariamente ao trabalho proposto por Britto (2003), as análises de Shimabukuro-Dias *et al.* (2004) não reconhecem o clado 9 formado pelas espécies propostas por este autor. Portanto, esse estudo concluiu que ambas as subfamílias de Callichthyidae ainda apresentam grupamentos internos mal resolvidos e ainda precisam de mais estudos relacionados às suas interrelações. A adição de dados cariotípicos adicionou uma perspectiva filogenética única, apoiando as relações basais de progenitores diplóides e a monofilia de grupos poliplóides de espécies dentro de *Corydoras* (*C. metae* e *C. araguaiensis*).

O mais recente estudo filogenético abrangendo metodologias moleculares, ecológicas e morfológicas, com membros da família Callichthyidae, foi publicado em 2011, onde Alexandrou *et al.*, visando entender as relações entre os congêneres de Corydoradinae. Os autores elaboraram uma nova hipótese filogenética, combinando dados moleculares, padrões comportamentais e morfológicos de coloração, e delimitaram nove linhagens dentro desta subfamília (figura 2). Os autores utilizaram em suas análises sequências de genes mitocondriais *12S rRNA*, *16S rRNA*, *ND4*, *Cytb*, e nucleares *Rag1* e *F-Reticulon 4*, de 425 taxons terminais, totalizando 226 espécies. Apesar de alguns nós apresentarem baixo suporte estatístico, as análises corroboraram o parafiletismo de *Corydoras* e revelaram uma nova topologia de relacionamento para a subfamília, definindo 9 linhagens dentro dessa. Em seus resultados, os autores mostraram um grande agrupamento de *Corydoras* como grupo irmão de *Scleromystax*, este clado como sendo irmão de *Aspidoras*, e este, por sua vez, irmão de um pequeno agrupamento de *Corydoras*: (((*Corydoras* + *Scleromystax*) + *Aspidoras*) + *Corydoras*). Esses resultados discordaram de todos os estudos já publicados sobre sistemática do grupo até então. Os autores observaram ainda, casos de convergência no padrão de coloração dentro das linhagens, e forneceram fortes evidências para apoiar a hipótese de mimetismo Müllleriano entre as espécies. Através disso, os autores concluíram que grande parte dos casos de padrão de coloração compartilhada entre os agrupamentos é resultado desse tipo de convergência.

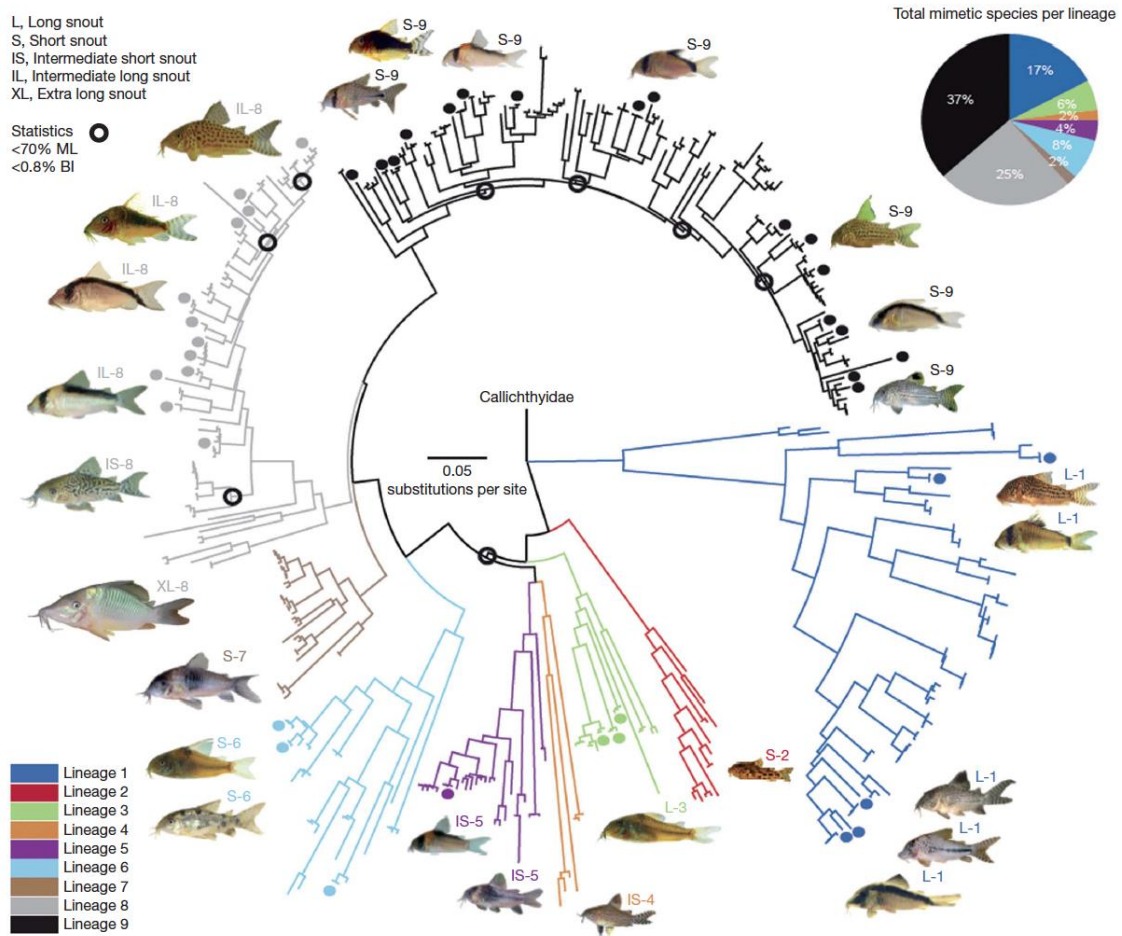


Figura 2. Hipótese de relacionamento de Corydoradinae de acordo com Alexandrou *et al.* (2011). As cores representam as nove linhagens sugeridas pelos autores.

Neste estudo, Alexandrou *et al.* (2011) utilizaram 52 espécies pertencentes a 24 grupos miméticos diferentes (cada um composto por duas ou três espécies diferentes), dos quais a distância genética é grande o suficiente para serem considerados isolados reprodutivamente. As análises também mostraram que várias espécies de linhagens diferentes ocupavam o mesmo nicho alimentar, e que as espécies miméticas simpátricas tinham padrão de coloração mais semelhante do que as alopátricas, indicando uma relação altamente significativa entre o padrão de cor e a distribuição geográfica. Além disso, os autores reportam também que os membros de diferentes linhagens sofreram duplicação genômica extensa, diferentemente do que foi reportado por Shimabukuro-Dias *et al.* (2004) na subfamília Callichthyinae, onde eles demonstram que *Dianema*, *Leptoplosternum* e *Megalechis* teriam tamanho genômico reduzido, sustentando a hipótese de que esses gêneros seriam os mais antigos da família.

1.4 IDENTIFICAÇÃO MOLECULAR DE ESPÉCIES UTILIZANDO O GENE COI

Do início do século XIX até hoje, as descrições taxonômicas de Callichthyidae, principalmente em *Corydoras*, foram baseadas, sobretudo, nos padrões de cores entre os indivíduos. Entretanto, este caráter tende a ser variável tanto intra quanto interespecificamente, dificultando sua quantificação objetiva (Nijssen, 1970), principalmente por se tratar de espécies miméticas. A maioria das descrições morfológicas depende fortemente de diferenças proporcionais, onde medidas específicas são expressas como proporções entre si. Entretanto, essas proporções são de utilidade filogenética questionável devido à alta variação entre populações geograficamente distintas e dentro de populações devido à alometria de crescimento (Nijssen, 1970). A variação alométrica em *Corydoras* foi testada por Strauss (1985) e os resultados indicaram que as mudanças na morfologia, baseados nos grupos propostos por Nijssen & Isbrucker (1980) são resultado de mudanças nas taxas de crescimento relativo entre as estruturas corporais de *Corydoras*. O autor sugere que quatro dos cinco grupos propostos por Nijssen & Isbrucker não poderiam ser discriminados adequadamente apenas com base na morfologia, devido à sobreposição na forma corporal e nas medidas merísticas propostas por esses autores. Os resultados de Strauss entram em conflito direto com os grupos de espécies propostos por Nijssen & Isbrucker que dependem fortemente de proporções e padrões de cores para delimitar as espécies.

Há uma sobreposição considerável na maioria dos grupos de espécies originalmente propostos por Nijssen & Isbrucker (1980), especialmente na forma geral do corpo e na contagem merística, como proposto por Strauss (1985). Assim, elucidar as relações sistemáticas dos *Corydoras* com base em merística e no padrão de cor tem se mostrado uma tarefa muito desafiadora, oferecendo uma visão limitada das relações sistemáticas desse gênero rico em espécies. Neste contexto, visto que a morfologia por si só muitas vezes não consegue solucionar as relações filogenéticas entre os membros de Callichthyidae, a utilização de metodologias moleculares associadas à morfologia sob uma visão taxonômica integrativa, torna-se imprescindível. A técnica de identificação molecular surgiu como uma grande ferramenta aliada às questões de taxonomia e sistemática. A análise de material genético com marcadores moleculares a fim de comparar sequências delimitando espécies permite um melhor entendimento dos taxa e, conseqüentemente, da história evolutiva dos grupos.

A metodologia DNA *barcode* (Hebert, 2003) vem sendo empregadas há mais de dez anos em diversos estudos de diferentes grupos de peixes neotropicais, revelando números muitas vezes subestimados de espécies, e se mostrando resolutivos para as questões taxonômicas de tais. Além disso, estudos recentes mostram que essa metodologia tem ajudado a identificar novas espécies e grupos de espécies crípticas (Melo *et al.*, 2011; Rossini *et al.*, 2016; Silva *et al.*, 2016; Benzaquem *et al.*, 2015; Costa-Silva *et al.*, 2015; Ochoa *et al.*, 2017; Dorini *et al.*, 2019; García-Melo *et al.*, 2019; Ochoa *et al.*, 2020a).

Callichthyidae é uma família rica em espécies que são amplamente distribuídas, como por exemplo, algumas espécies de *Corydoras*, *Hoplosternum* e *Callichthys*. Esses grupos amplamente distribuídos são os mesmos que apresentam incongruências relacionadas à sistemática. Como já foi dito por de Pinna (1998), o número de espécies de um gênero está diretamente relacionado à complexidade que ele apresenta. Dentro da família Callichthyidae existem gêneros com apenas duas espécies descritas, como *Dianema*, e, divergentemente, outros com mais de 100 espécies, como *Corydoras*. Essa discrepância no número de espécies em clados relacionados ainda não é explicada. Apesar de todos os esforços na tentativa de solucionar as relações filogenéticas dentro de Callichthyidae, problemas relevantes ainda podem ser destacados em ambas as subfamílias, mas principalmente em Corydoradinae, devido ao alto número de espécies descritas para esta subfamília. O parafiletismo de *Corydoras* é um exemplo fundamental, pois apresenta uma conformação que demonstra que as relações de Corydoradini ainda permanecem insatisfatoriamente resolvidas. *Corydoras*, sem dúvidas, é o gênero mais problemático de Callichthyidae, já que sua diversidade representa quase 80% da família.

Sendo assim, propomos neste trabalho a delimitação das espécies de Callichthyidae através do sequenciamento do gene mitocondrial citocromo c oxidase subunidade I (COI), empregando a metodologia de DNA *barcode*. Entre as análises de delimitação molecular de espécies mais utilizadas está o Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012), que foi um método criado focando em um padrão para definição de Barcode Gap, com base nas diferenças par a par. Baseado no sistema de DNA Barcoding, esse método consiste no agrupamento de sequências em grupos de espécies, de acordo com os seus sucessivos valores de gap. Nessa análise, primeiramente é realizada uma comparação par a par da distância genética entre todas as sequências de um alinhamento. Os gaps são usados para particionar os dados, e

subsequentemente, as análises recursivas são realizadas até que não haja mais particionamento, visando aguçar a delimitação dos grupos ao permitir diversos limites intra/interespecíficos. As análises se encerram apenas quando não é mais possível determinar partições naquele conjunto de dados (Puillandre *et al.*, 2012). Os resultados do ABGD enfatizam em particular a sensibilidade do método à presença de eventos de especiação recentes, através de altas taxas de especiação ou grande número de espécies.

Outra forma de se delimitar espécies com dados moleculares consiste no método, baseado em coalescência, chamado Poisson Tree Processes (PTP) (Zhang *et al.*, 2013). Esse é um método que tem como base a taxa de substituição, e cada substituição tem uma chance mínima de gerar especiação independentemente das demais (Zhang *et al.*, 2013). Além disso, a probabilidade de haver substituições em uma população é grande e, portanto, o evento segue uma distribuição de Poisson. Ou seja, a análise de PTP assume que cada substituição a mais encontrada entre taxons terminais de uma filogenia aumenta a probabilidade de que uma espécie tenha sido gerada. Assim, de acordo com os comprimentos de ramos de uma árvore filogenética, o número de substituições existentes entre taxons terminais são quantificados, e um limite entre linhagens populacionais e específicas é estabelecido (Zhang *et al.*, 2013). Portanto, a análise de PTP assume que o número de substituições encontradas entre sequências de espécies distintas será maior do que aquele entre sequências de uma mesma espécie (Zhang *et al.*, 2013; Tang *et al.*, 2014).

1.5 ELEMENTOS ULTRACONSERVADOS (UCES)

Os UCES (Ultraconserved Elements) são regiões do genoma altamente conservadas (que não sofreram variação/mutação ao longo dos anos) e compartilhadas por táxons evolutivamente distintos, como por exemplo, aves e humanos (Bejerano *et al.*, 2004). Por esse motivo, podem ser utilizados como marcadores genéticos universais devido ao fato de serem igualmente preservados em diferentes táxons, pertencentes a linhagens que sofreram divergência há milhares de anos, porém, que mantém essas regiões intactas (Dermitzakis *et al.*, 2005; Siepel *et al.*, 2005; Stephen *et al.*, 2008).

Os UCES foram descritos pela primeira vez por Bejerano *et al.* (2004), e estão associados com a regulação gênica e com o desenvolvimento (Sandelin *et al.*, 2004; Woolfe *et al.*, 2005). Apesar de serem regiões conservadas e, portanto, sem alteração, as sequências utilizadas nos estudos filogenéticos não são as regiões dos próprios UCES

em si, mas sim suas sequências flanqueadoras (que margeiam a região ultra conservada), por apresentarem alta diferenciação genética. Sondas especialmente desenhadas para esse tipo de análise reconhecem e realizam o anelamento com as sequências altamente conservadas (UCE) e, a partir delas, as sequências flanqueadoras podem ser analisadas. Por isso, os UCEs podem ser úteis na reconstrução da história evolutiva e inter-relações a nível populacional de muitos organismos (Faircloth *et al.*, 2012).

Devido ao fato de estes elementos estarem distribuídos por todo o genoma, eles podem ser tratados como loci independente em análises filogenéticas e são particularmente valiosos em métodos de elaboração de árvores, revelando o parentesco entre espécies (Knowles, 2009), e ampliando o nível de resolução, já que há um aumento no número de loci. Atualmente, está comprovado que os métodos de UCEs são mais eficientes para a reconstrução filogenética do que outras metodologias do genoma (Jarvis *et al.*, 2015). De uma maneira geral, revisões identificaram problemas importantes em análises filogenéticas, entre elas, a errônea identificação de genes ortólogos e saturação de substituições nucleotídicas, tal que, múltiplas substituições em uma dada posição de base podem obscurecer o sinal filogenético (Philippe *et al.*, 2011). Entretanto, a metodologia de UCEs têm se mostrado muito eficiente gerando filogenias robustas e bem resolvidas em vários grupos de organismos, sendo relativamente mais fácil identificar as regiões homólogas e apresentando pouca saturação (Derti *et al.*, 2006).

Os UCEs vêm sendo testados e demonstrando sua eficácia ao serem empregados em estudos com diferentes táxons ao redor do mundo (Faircloth *et al.*, 2015; Harrington *et al.*, 2016; Meiklejohn *et al.*, 2016; Starrett *et al.*, 2016; Branstetter *et al.*, 2017; Chakrabarty *et al.*, 2017; Faircloth, 2017; Alfaro *et al.*, 2018). Dentre os Siluriformes, estudos recentes têm mostrado que o uso dos UCEs em filogenias alcançam resultados extremamente satisfatórios, como por exemplos da família Heptapteridae (Silva *et al.*, 2021), Loricariidae (Roxo *et al.*, 2019), Trichomycteridae (Ochoa *et al.*, 2020b) e Pseudopimelodidae (Silva *et al.*, 2021).

Visto que os UCEs são eficientes marcadores moleculares em estudos filogenéticos, e com isso, podem ajudar a reconstruir a história evolutiva e as relações entre os indivíduos (Faircloth *et al.*, 2012), neste trabalho nós utilizamos métodos de captura e análise de sequência de UCEs para a elaboração da primeira hipótese de inter-

relacionamento dos membros da família Callichthyidae utilizando este tipo de abordagem.

1.6 JUSTIFICATIVA

A taxonomia dos calictídeos, principalmente de Corydoradinae, pode ser desafiadora, e muitas vezes frustrante, pois envolve aspectos como: morfologia muito similar, convergência adaptativa, ampla distribuição, descrições muito antigas e caracteres diagnósticos controversos das espécies nessas descrições, alta plasticidade fenotípica influenciada pelas condições ambientais (mimetismo), e chaves de identificação confusas, tornando as identificações equivocadas e imprecisas. É relevante citar também que *Corydoras* é, sem dúvida, o gênero mais problemático da família, devido ao número elevado de espécies que o compõe, e que a última revisão taxonômica do grupo foi apresentada há muitos anos (Nijssen & Isbrücker, 1980), com baixo material analisado e sem caracteres diagnósticos precisos.

Além do exposto acima, trabalhos de descrições de espécies de Callichthyidae vêm sendo publicados todos os anos, principalmente dos gêneros *Corydoras* e *Aspidoras*. Fricke *et al.* (2022) citaram que 24 espécies foram descritas nos últimos 10 anos. Lima & Britto (2020), descreveram uma espécie de *Corydoras* do rio Juruena, *C. rikbaktsa*, e Tencatt, Muriel-Cunha, Zuanon, Ferreira & Britto (2020), *Aspidoras azaghal*. Ainda mais recentemente, foram publicadas as duas espécies mais contemporâneas de *Corydoras* da bacia do rio Amazonas no Peru, *C. bethanae* Bentley, Grant & Tencatt (2021) e *C. fulleri* Tencatt, Santos, Evers & Britto (2021). Apesar de que descrições de espécies representem grandes contribuições, trabalhos de descrição de espécies aumentam ainda mais a complexidade desse gênero.

McCormack *et al.* (2013) já haviam citado que a filogenômica permite uma profundidade sem precedentes nas análises de relações entre organismos devido à grande quantidade de caracteres fornecidos ser milhares de vezes maior do que quando comparada às análises morfológicas. Portanto, diante das questões ainda relevantes referentes à sistemática de Callichthyidae, a utilização de uma metodologia molecular para definir os limites entre as espécies de Callichthyidae, com a finalidade de auxiliar a identificação morfológica de grupos e complexos de espécies, é essencial. Com isso, uma investigação inédita utilizando o que há de mais atual da metodologia de filogenia

molecular, abrangendo a máxima densidade taxonômica disponível atualmente da família com a adição de novos táxons terminais, torna-se imprescindível.

Assim, para permitir a elaboração de hipóteses mais robustas de relacionamentos, empregamos no presente estudo métodos de captura de sequências através de enriquecimento de bibliotecas de DNA da classe dos elementos ultraconservados (Faircloth *et al.*, 2012), analisando suas regiões flanqueadoras altamente polimórficas em sequenciadores de nova geração para a formulação de árvores bem suportadas que revelaram um novo posicionamento para os grupos irmãos de Callichthyidae. Portanto, neste trabalho propomos uma nova hipótese para a delimitação dessas espécies abrangendo uma grande amostragem de táxons, associada à uma filogenia robusta com a utilização de 500 a 750 mil caracteres utilizados na formulação das árvores, para auxiliar na compreensão das questões evolutivas que envolvem as grandes linhagens de Callichthyidae.

2. OBJETIVOS

O objetivo principal do presente trabalho é apresentar uma nova hipótese de monofiletismo dos grupos internos de Callichthyidae através de análises filogenéticas com elementos ultraconservadores (UCEs), com a utilização de um número de táxons terminais superior aos das filogenias atualmente propostas e grande número de caracteres para formulação das árvores, e assim, fornecer subsídios para caracterizar os grupos monofiléticos encontrados com base nos resultados alcançados. Além disso, pretendemos também analisar o maior número possível de espécies e populações da família Callichthyidae utilizando a metodologia de *DNA barcode*, para tornar disponível um método alternativo de delimitação dos grupos e para facilitar a identificação das espécies da família.

Considerando as questões ainda a serem resolvidas em Callichthyidae, os objetivos específicos do presente trabalho são:

- Testar a hipótese de monofiletismo dos gêneros de Callichthyidae, principalente *Corydoras*, através da utilização de UCEs e propor uma nova hipótese de relacionamento para esta família;

- Testar as hipóteses de relacionamento inter e intraespecífica para as espécies de Callichthyidae, estabelecendo relações entre elas e a exata inserção dos gêneros dentro da família com base em caracteres moleculares;
- Sequenciar o gene mitocondrial Citocromo c oxidase subunidade I (COI) das espécies da família Callichthyidae e revelar possíveis espécies crípticas para esta família. Para espécies com ampla distribuição, pretendemos analisar um maior número de amostras possíveis, buscando avaliar a variabilidade genética das mesmas;
- Com base na variabilidade genética observada, identificar os valores limites entre as espécies;
- Utilizar o gene COI para criar um banco genético de DNA *barcode* e depositá-las no sistema de Barcoding of Life (BOLD) para auxiliar na identificação molecular de Callichthyidae.

3. RESULTADOS

Os resultados estão organizados em três capítulos e foram elaborados em formato de artigo científico. Nós optamos por dividir as análises de delimitação de acordo com cada subfamília para facilitar a discussão dos dados. Os dois primeiros capítulos, intitulados respectivamente, “Species delimitation of the subfamily Callichthyinae (Callichthyidae: Siluriformes) with *DNA barcode* approaches” e “Molecular identification of the species of the subfamily Corydoradinae (Callichthyidae: Siluriformes) with *DNA barcode*”, reportam resultados de delimitação molecular das espécies de cada subfamília de Callichthyidae, através da utilização da metodologia de *DNA barcoding*.

Com base nesses resultados, pudemos observar que alguns gêneros de ambas as subfamílias ainda não estão suficientemente esclarecidos taxonomicamente e precisam ser revisados. Nossas análises de delimitação reportaram diferentes linhagens geneticamente distintas dentro de *Megalechis*, *Lepthoplosternum*, e *Hplosternum*. Além disso, *Corydoras* pode apresentar uma diversidade ainda maior, se consideradas as divergências genéticas encontradas pelas análises de PTP e ABGD, como por exemplo, em *C. aeneus* (linhagem 7). Espécies como *C. difluviatilis* (linhagem 8) e *C. julii* (linhagem 9) demonstraram representar mais de uma linhagem genética.

O terceiro capítulo, intitulado “A new phylogenomic hypotheses of the family Callichthyidae (Siluriformes: Loricarioidea)” propõe uma nova hipótese de relacionamento entre os membros de Callichthyidae, realizada através da utilização da metodologia de UCE. Os resultados alcançados neste capítulo sugerem que *Megalechis* não representa um grupo monofilético, e que existem oito linhagens monofiléticas em Corydoradinae, que precisam ser revisadas. Algumas linhagens apresentam nomes pré-disponíveis, porém, outras precisam ser descritas como novos gêneros. Além disso, nossos resultados sugerem que novas análises precisam ser realizadas para investigar uma possível radiação adaptativa dentro de Corydoradinae.

3.1 CAPÍTULO 1:

Species delimitation in the subfamily Callichthyinae (Callichthyidae: Siluriformes) using DNA barcode approaches¹Angelica Corrêa Dias

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Abstract

The family Callichthyidae (Siluriformes) contains two subfamilies, Callichthyinae and Corydoradinae. The Callichthyinae have a broad geographic distribution across the Neotropical region, and are represented by five genera: *Callichthys* (four species), *Dianema* (two species), *Hoplosternum* (three species), *Lepthoplosternum* (six species) and *Megalechis* (two species). The morphological definition of the species that compound this subfamily is not clear. The last systematic investigation of Callichthyinae was based on osteological characters and cladistic morphometric analyses. We present here the first molecular delimitation of Callichthyinae using the mitochondrial gene cytochrome c oxidase subunit I (COI) obtained from more than 300 samples representing all the five genera of this subfamily, based in three delimitation analysis: NJ, ABGD and PTP. The results showed with strong support that the genera *Megalechis*, *Lepthoplosternum* and *Hoplosternum* represent more than one genetic lineage. All the analysis suggested that *Megalechis* represents more than one genetic lineage. Our analysis weren't capable to recognize the biggest lineage of *Hoplosternum* from La Plata basin as different lineage. Our study improved knowledge of the diversity of Callichthyinae, and reveals the complexity and underestimated diversity of this subfamily.

Keywords: Molecular identification, biodiversity, systematics, conservation, catfish

Introduction

Loricarioidea, the largest monophyletic group among all Neotropical catfishes of the order Siluriformes (de Pinna, 1998), is composed by the families Astroblepidae, Callichthyidae, Loricariidae, Nematogenyidae, Scoloplacidae, and Trichomycteridae. The superfamily Loricarioidea includes three of the most species rich families of the entire order: Loricariidae, with 1015 species, Trichomycteridae, 346, and Callichthyidae with 223 (Frick *et al.*, 2022). The family Callichthyidae takes its name from its type genus *Callichthys*, described as *Silurus callichthys* described by Linnaeus (1758), and currently under the synonym of *Callichthys callichthys*.

Callichthyidae is a species-rich family of armored catfishes that can be distinguish from all other Siluriformes by the presence of two lateral series of bony plates on the body (Bonaparte, 1838). The broad geographic distribution and the species richness of Callichthyidae have aroused interest about the systematic of the group. Currently, the family is divided in Callochthyinae and Corydoradinae. Callichthyinae is represented by five genera, *Callichthys* Scopoli, 1777 (four species), *Dianema* Cope, 1871 (two species), *Hoplosternum* Gill, 1858 (three species), *Lepthoplosternum* (six species), and *Megalechis* (two species) (Reis, 1997).

Callichthyinae is diagnosed from Corydoradinae by a combination of features: the lateral ethmoid with a segment of the supraorbital latero-sensory canal (*vs.* dorsal face of lateral ethmoid without latero-sensory canal), the preopercle covered with skin (*vs.* preopercle exposed), dentary with teeth (*vs.* dentary without teeth), nuchal plate covered with skin (*vs.* nuchal plate exposed), snout depressed (*vs.* snout compressed), maxillary barbel long, overpassing the gill opening (*vs.* maxillary barbell short, not overpassing eye) (Reis, 1998). The callichthyinaes can be found in all Neotropical drainages, from Panamá and west of Andean chain, to the lower La Plata River, in Argentina, and reaching the Brazilian coastal drainages. Therefore, it's very common to find species of Callichthyinae in all kinds of Neotropical rivers, from large rivers of fast waters to streams with low level of available oxygen in the water (Reis, 1998).

The subfamily Callichthyinae represents the smaller in number of species of the two subfamilies that compound the family Callichthyidae, however, no less complicated. Efforts in the literature within this subfamily focus on taxonomic and systematic investigations based on morphometric analysis. Reis (1997) presented the last taxonomical revision of *Hoplosternum* based on osteological characters where he

identifies the non-monophyly of this genus. In this study, the author could observe that there were species of *Hoplosternum* more related to *Callichthys*, while others species of *Hoplosternum* were more related to *Dianema*, and therefore, the author relocated these species that was causing the non-monophyly of *Hoplosternum* to two new genera, named as *Lepthoplosternum* and *Megalechis*. In this work, the author presents an identification key for all genera of this subfamily, however, it is possible to observe overlaps and inconsistencies, and the delimitation of each genus were not clear. After that, few new species were described, but no other revision of this subfamily was proposed.

In this context, we present here the first molecular delimitation of each group of Callichthyinae based on molecular approaches, and using samples that represented all genera of this subfamily. The *DNA barcode* methodology has had its effectiveness demonstrated over the years through several papers related to fish diversity of a lot of groups of freshwater fishes (Benzaquem *et al.*, 2015; Costa-Silva *et al.*, 2015; Machado *et al.*, 2016; Rossini *et al.*, 2016; Silva *et al.*, 2016; Parente *et al.*, 2018; Melo *et al.*, 2018; Serrano *et al.*, 2018; García-Melo *et al.*, 2019; Mateussi *et al.*, 2019; Dorini *et al.*, 2020). We assume here that this methodology was also effective to demonstrate the diversity of the subfamily Callichthyinae.

Material and Methods

Taxon sampling

In this study a total of 315 specimens were used for the delimitation analyzes, representing all the genera of Callichthyinae (Supplementary Table 1). For widely distributed species, we attempted to include samples that represent their entire distribution. The specimens and tissues are deposited at LBP collection (Laboratório de Biologia e Genética de Peixes, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil).

DNA extraction

We extracted about 0.5 cm³ of a fragment of muscle tissue, gill or fin of each specimen. The tissues were preserved in 95% ethanol at -20°C and the fishes were fixed in formalin 10% and transferred posteriorly to ethanol 70% for permanent storage. The samples chosen for the analyses identified using taxonomic keys with the help of expert

taxonomists in the group. All the molecular analyses, DNA extraction, PCR amplification and DNA sequencing were performed in the LBP.

Data processing

We used the mitochondrial gene *cytochrome c oxidase subunit I* (COI) for the species delimitation analyses. Partial sequences of gene COI were amplified by polymerase chain reaction (PCR) using the primers FishF1, FishR1, FishF2, FishR2 describes by Ward *et al.* (2005), L6252-Asn and H7271-COX (Melo *et al.*, 2011), or FishF6 and FishR7 (Jennings *et al.*, 2019). The PCR solution was based on Steinke & Hanner (2010), with adjustments according to the species. We used 12.5 μ l as total volume with 7.55 μ l of double-distilled water, 1.25 μ l 5x buffer, 0.50 μ l MgCl₂ (50 mM), 0.50 μ l dNTP mix at 8 mM, 0.25 μ l of each primer at 10 μ M, 0.20 μ l PHT® Taq DNA polymerase enzyme (Phoneutria), and 2.0 μ l genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (5 min at 95°C) followed by 35 cycles of chain denaturation (45s at 95°C), primer hybridization (45s at 54°C), and nucleotide extension (60s at 68°C) and final extension (7 min at 68°C). After that, all the PCR products was visualized on 1% agarose gel and purified with ExoSap-IT (USB Corporation) following the manufacturer's instructions. For the sequencing reaction, we submitted the PCR products to the BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purified again through ethanol precipitation. After all, the products were loaded to an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems).

Delimitation analysis

The sequences that presented approximately 501 base pairs and high quality were assembled to generate consensus sequence with the software Geneious v7.1.9 (Kearse *et al.*, 2012), and then, aligned with Muscle (Edgar, 2004), under default parameters. The matrix was visualized and edited to minimize missing data and posteriorly checked for the presence of stop codons. After the editing procedure, no insertions, deletions or stop codons were verified, supporting the hypothesis that the origins of the fragments are of functional sequences and not pseudogenes of the COI gene. To evaluate the occurrence of substitution saturation we estimated the index of substitution saturation, following Xia *et al.* (2003) in software DAMBE v5.3.38.

The sequences will be deposited in the Bold System (<http://www.boldsystems.org/>) and GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). We used the best-fit model found in MEGA X-64 (Kumar *et al.*, 2018), TN93+G, to generate a neighbor-joining tree (NJ) with 1000 bootstrap pseudoreplicates (bs). The sequences were ordered into species groups following the taxonomic identification of the preliminary NJ topology in Geneious v7.1.9. The respective standard deviation values of the overall mean distance (among all specimens), intraspecific distance (among specimens of each species group) and interspecific distances (among species groups) were calculated using the best model suggested by MEGA X-64, with 1000 bootstrap pseudoreplicates (Kumar *et al.*, 2018). To formulate the NJ tree topology, we collapsed the ramus considering 2% of genetic distance.

After that, a maximum likelihood (ML) tree was constructed with RAxML v8.019 (Stamatakis, 2014) using the GTRGAMMA model (Stamatakis *et al.*, 2008), and others parameters at default. We applied the best ML tree generated by RAxML v8.019, 100,000 generations, in Poisson Tree Process (PTP) in the bPTP webserver, available at <http://species.h-its.org/ptp/> (Zhang *et al.*, 2013). Finally, we applied the Automatic Barcode Gap Discovery (ABGD) analysis (Puillandre *et al.*, 2012), using an input PHY file into the webserver (excluding the out group), <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>.

Results and Discussion

A total of 311 sequences of gene COI were analyzed including samples of all five genera of the subfamily Callichthyinae: *C. callichthys* (45), *D. longibarbis* (8), *D. urostriatum* (9), *H. littorale* (144), *H. punctatum* (4), *H. magdalenae* (38), *L. pectorale* (21), *L. aff. pectorale* (5), *M. personata* (2), *M. picta* (2), *M. thoracata* (30) and *M. aff. thoracata* (3). The species represented in the analyses corresponding to each genus was respectively: *Callichthys* (25%), *Dianema* (100%), *Hoplosternum* (100%), *Lepthoplosternum* (33%), *Megalechis* (100%). The final matrix had on average 502 base pairs (bp) with a total of 281 pb of variable sites. The frequency of adenine base was 25.9%, cytosine 25.9%, guanine 15.8% and thymine 32.3%. DAMBE indicated no saturation in either transitions or transversions in both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies. Tamura-Nei parameter model (TN-93+G) was best evolution model suggest by MEGA, which BIC = 17445,157 and AICc = 11251,634.

The overall mean distance was 0.01 ± 0.17 . The values of interspecific distances among the lineages of Callichthyinae ranged from 0.012 ± 0.004 between *C. callichthys* from Southern coastal basin and *C. callichthys* from Southeastern Brazilian coastal basin, to 0.267 ± 0.029 , between *Leptoplosternum* sp. and *H. magdalenae*; and intraspecific distances ranged from 0.001 ± 0.001 to 0.040 ± 0.008 (Table 1).

Table 1. Pairwise comparison with TN-93 model showing genetic distances among species of Callichthyinae. Intraspecific gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	IGV	
1- <i>H. littorale</i> <i>Orinoco</i>		0,02	0,02	0,03	0,02	0,02	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,03	0,02	0,02	0,02	0,02	0,02	0,03	0,02	0,02	0,02	0,02	0,003± 0,002
2- <i>H. littorale</i> <i>Suriname</i>	0,13		0,01	0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,03	0,03	0,03	0,03	0,03	0
3- <i>H. littorale</i>	0,11	0,03		0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,03	0,02	0,02	0,03	0,03	0,001± 0,001
4- <i>H. punctatum</i>	0,23	0,24	0,24		0,01	0,01	0,02	0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,001± 0,001
5- <i>H. littorale</i> <i>Maracaibo</i>	0,21	0,22	0,23	0,04		0,01	0,02	0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,03	0,03	0,03	-
6- <i>H.</i> <i>magdalenae</i>	0,21	0,24	0,24	0,04	0,02		0,02	0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,004± 0,002
7- <i>D. urostriatum</i>	0,22	0,21	0,20	0,13	0,13	0,13		0,02	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,02	0,02	0,03	0,03	0,03	0,03	0,001± 0,004±
8- <i>D. longibarbis</i>	0,21	0,22	0,22	0,15	0,14	0,14	0,11		0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,03	0,03	0,02	0,02	0,02	0,03	0,03	0,03	0,03	0,002
9- <i>C. callichthys</i> <i>Madeira</i>	0,22	0,22	0,23	0,25	0,25	0,26	0,24	0,23		0,01	0,01	0,01	0,01	0,01	0,03	0,02	0,03	0,03	0,03	0,02	0,03	0,02	0,03	0,03	0,03	0,03	-
10- <i>C. callichthys</i> <i>Tapajos</i>	0,23	0,23	0,24	0,26	0,25	0,26	0,26	0,25	0,06		0,01	0,01	0,01	0,01	0,02	0,02	0,03	0,03	0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,03	-
11- <i>C. callichthys</i>	0,22	0,22	0,23	0,25	0,24	0,25	0,25	0,24	0,05	0,04		0,00	0,01	0,01	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,03	0,03	0,03	0,14±0 .003
12- <i>C. callichthys</i> complex <i>Costeira</i> <i>RJ, SP, Alto</i>	0,22	0,22	0,23	0,25	0,24	0,24	0,25	0,22	0,04	0,03	0,02		0,00	0,01	0,02	0,02	0,03	0,03	0,03	0,02	0,02	0,02	0,03	0,02	0,03	0,03	0,006± 0,002
13- <i>C. callichthys</i> <i>Costeira RS, PR</i>	0,21	0,21	0,22	0,24	0,23	0,24	0,25	0,22	0,04	0,03	0,02	0,01		0,01	0,02	0,02	0,03	0,03	0,03	0,02	0,02	0,02	0,03	0,03	0,03	0,03	0,004± 0,003
14- <i>C. callichthys</i> <i>Essequibo</i>	0,20	0,23	0,23	0,24	0,25	0,25	0,25	0,24	0,09	0,10	0,08	0,08	0,08		0,02	0,02	0,03	0,03	0,02	0,02	0,02	0,02	0,02	0,02	0,03	0,03	0,041± 0,008
15 - <i>Leptoplosternu</i> <i>m sp.</i>	0,21	0,24	0,24	0,25	0,25	0,27	0,22	0,22	0,22	0,21	0,22	0,21	0,21	0,22		0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,03	0,02	0,02	0,02	0,006± 0,002
16- <i>L. pectorale</i> complex	0,23	0,26	0,26	0,24	0,25	0,26	0,23	0,22	0,21	0,22	0,22	0,22	0,21	0,21	0,11		0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,002± 0,001
17- <i>M. thoracata</i> complex 4	0,19	0,23	0,22	0,23	0,23	0,25	0,23	0,20	0,23	0,24	0,23	0,22	0,22	0,23	0,17	0,18		0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,003± 0,002
18- <i>M. personata</i>	0,21	0,24	0,23	0,25	0,24	0,26	0,23	0,23	0,23	0,25	0,23	0,24	0,23	0,23	0,14	0,14	0,13		0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,010± 0,005
19- <i>M. thoracata</i> <i>Nanay River 1</i>	0,21	0,24	0,24	0,23	0,23	0,25	0,21	0,23	0,22	0,24	0,24	0,22	0,22	0,22	0,19	0,19	0,17	0,19		0,01	0,02	0,01	0,02	0,02	0,02	0,02	0,003± 0,002

To assess the genetic diversity of Callichthyinae, we used three methods to delimit the genetic lineages of our datasets: Neighbor-Joining tree (NJ), Automatic Barcode Gap Discovery (ABGD) and Poisson Tree Processes methods (PTP) (Fig. 1). The NJ was the most splitter method, indicating 23 genetic lineages for the subfamily, while ABGD partitionated the data in 14 genetic lineages, and PTP delimited 17 genetic lineages. The ABGD proposed 10 partitions: two partitions found 90 species (prior maximal distance $P=0.001$), two partitions found 33 species ($P=0.003-0.005$), one partition found 25 groups ($P=0.008$), one partition found 20 groups ($P=0.013$), one partition found 16 species ($P=0.021$) and three partitions found 14 species ($P=0.036-0.100$).

We divided the results according to each genus to better discuss the results.

Dianema

All the analyses agree that the two species of *Dianema* included in this study belonging to different lineages. The analysis of distance showed 11% of distance between them (Tab. 1). However, *H. magdalenae* and *H. punctatum* showed most similar genetically to *Dianema* than to others *Hoplosternum*, strongly supported by PTP and RaxML (Fig. 1).

Hoplosternum

The NJ analysis revealed that *H. magdalenae* and *H. punctatum* have more genetic similarity with *D. longibarbis* than to *H. littorale*. Besides that, *H. magdalena* and *H. punctatum* were recognized by ABGD and PTP as a single lineage, but NJ recognize as two different lineages (100% of bootstrap) with 4% of genetic distance between them (Tab. 1). Despite NJ showed this species presenting a considerable genetic divergence (4% of distance between *H. punctatum* and *H. magdalenae*), ABGD and PTP (0.08-0.36) recognized these two species as the same genetic lineage, and therefore, the same species. The type-locality of *H. magdalenae* is Soplaviento, Colombia, and can be found in Magdalenae and Sinus river basin, to the west of the Andean Cordilleras. *H. punctatum* is described from Marte Arnade River, in Panamá, and can be found in Pacific coastal drainages of Parana and Atrato river basin of northwestern Colombia. Furthermore, the morphology presents inconsistent diagnostic

characters that delimit the two species. *H. magdalenae* is diagnosed by present interopercle exposed on ventral surface of head as a small plate below the opercle, and is distinguished from *H. punctatum* by presenting a deeper caudal peduncle in large specimens (17.4-19.5 vs 14.3-17.2). Both species present well developed and exposed coracoid on ventral view, and *H. punctatum* differs from *H. magdalenae* by having the interopercle covered by skin, and small size of male adults of *H. punctatum*. We included in our analyses a specimen of *H. magdalenae* from Maracaibo Lake (voucher 29519, LBP 6137) that presented second infraorbital narrow, with exposed skin without plate between the eye and opercle, interopercle without skin ventrally, coracoids overlapping ventrally and four perforated plates on lateral line. A new genus was proposed by Franz (2001) to allocate the species of *H. magdalenae*, but was sinonimized to *Hoplosternum* posteriorly. Our results demonstrated that species do not represent *Hoplosternum*, but another genus, more similar genetically to *Dianema*. Our results imply in the necessity of the description of a new genus to allocate the species of *H. magdalenae* and *H. punctatum*, as they are morphologically and genetically similar (basically distinguishable by standard length, smaller in *H. punctatum*).

NJ analysis revealed three lineages of *Hoplosternum littorale*, one from Orinoco basin (11% of distance from *H. littorale* from La Plata basin), one from Suriname (13% of distance with *H. littorale* from Orinoco, Tabl 1) and other one formed by sequences from La Plata and Amazonian basin. Our analyses weren't able to differentiate genetically the *H. littorale* lineage of La Plata basin and Amazonian basin, NJ, ABGD and PTP recognized them as the same large lineage. PTP and ABGD also not differentiated the biggest lineage of *H. littorale* from Suriname, both analyses grouped these two lineages in one and didn't recognize any difference between them. On the other hand, NJ demonstrated a distance of 3% between *H. littorale* from La Plata basin.

Moreover, all the analyzes agree that *H. littorale* from Orinoco (voucher 15539, 15541, 15542, LBP 2183) are genetically distant from the others congeners (more than 10% of divergence). We analysed morphologically the specimens following the identification key proposed by Reis (1997) and we noticed that the second infraorbital bone is not well developed and neither narrow. This bone is compromise between developed and narrow, because there is a naked area between the eye and opercle. On the other hand, these specimens present six scutes of upper lateral series with lateral line perforation. Although only *H. littorale* is distributed to the east of

Andean Cordilleras, there is an available name for *Hoplosternum* from outfall of Orinoco that is currently synonymized to *H. littorale*, *H. stevardii* (Gill 1858), described from Island of Trinidad. Considering that the specimens of *H. littorale* from Orinoco analysed on this study represent a different lineage from all others *Hoplosternum*, we conclude that it's possible that this sample represents a valid species, and need to be reviewed and probably revalidated.

Callichthys

The NJ analysis suggests genetic difference between four groups of *Callichthys* (values higher than 2% between groups), but ABGD and PTP recognized all the *Callichthys* at the same lineage. The lineages of NJ recognized as genetically different between *Callichthys* was: *C. callichthys* from La Plata with São Francisco basin, *C. callichthys* from coastal rivers of Santa Catarina and Paraná States, Brazil (named as Costeira Sul basin), *C. callichthys* from Tapajos basin, a single specimen from Madeira basin, and a separated lineage of *C. callichthys* from Essequibo River, Guiana. Oliveira *et al.* (1993) had already reported cytogenetic differences between two populations of *Callichthys* from Coastal basin of Rio Grande do Sul and São Paulo State, as geographically isolated populations, and indicated the necessity of a taxonomic review of this group.

Furthermore, the analysis of distance between groups showed that *C. callichthys* from Essequibo has 10% of divergence from the others *Callichthys*. The type locality of *C. callichthys* is unknown, but this species has a broad distribution for the entire Cis-Andean drainages. There are three species of *Callichthys* that are trans-Andean, *C. oiabensis*, *C. fabricioi* and *C. serralabium*. The type-locality of *C. oiabensis* and *C. fabricioi* is Cauca River, Colombia, and *C. serralabium* is Orinoco River, in Venezuela. There are no species described for the Essequibo River. Our analysis showed *Callichthys* from Essequibo being able to represent a separated species and should be better investigated.

Megalechis

NJ analysis revealed that there are seven lineages of *Megalechis*, and they can be divided into two groups: the first one is formed by *Megalechis* more genetically similar

to *M. picta* (*M. thoracata* from Tocantins-Araguaia basin and *M. thoracata* from Nanay River, Peru), and other one composed by Amazonian and La Plata basin species and also a lineage of *M. thoracata* from Nanay River (Peru). NJ and ABGD showed 4% of genetic distance from *M. thoracata* from Nanay River (Peru) from *M. picta*, but PTP recognized both as the same lineage (0.12). The lineage of *M. thoracata* similar to *M. picta*, ABGD recognized *M. thoracata* from Tocantins-Araguaia, *M. thoracata* from Peru and *M. picta* as three genetically distinct lineages, while PTP recognized only *M. thoracata* from Tocantins-Araguaia as a different lineage (0.56) and *M. thoracata* from Peru and *M. picta* as a single lineage. In the second group, ABGD recognized all the species as genetically equals and grouped all in the same lineage, while PTP showed a different topology, where *M. thoracata* from Amazonian basin is genetically similar to *M. aff. thoracata* from the same basin.

All the analyses recognized different lineages to *Megalechis*, NJ separated *Megalechis* in seven distinct lineages, while ABGD recognized four, and PTP, five. Despite that, there are only two species described to this genus, *M. thoracata* and *M. picta*, and the type-locality is French-Guyana and Guyana, respectively. The NJ analyses divided *Megalechis* into two groups, according with their genetic similarities. In the first one, formed by *M. thoracata* from Tocantins-Araguaia, *M. thoracata* from Nanay River (Peru) and *M. picta*, NJ and ABGD recognized all as genetically distant and as distinct species, whereas PTP recognized *M. thoracata* from Nanay River (Peru) and *M. picta* as the same lineage. *M. picta* the distribution of this species do not covered the Peru drainages. According to its description (Reis, 1997), this species is distinguished of *M. thoracata* by presenting longer dorsal fin spine, anal fin with five branched rays (vs. six branched rays), and transversal dark bars on caudal fin. The distribution of this species is cis-andean (Orinoco, Amazon and Essequibo River), but one of our sample included in the analysis (voucher 34018, LBP 6976) was collected in Negro River, Amazonian River basin (São Gabriel da Cachoeira, AM, Brazil), trans-andean, therefore the distribution of this species should be increased.

Otherwise, the NJ analysis delimited four different lineages on second group of *Megalechis*, formed by *M. aff. thoracata* from Madeira River (voucher 46501, 46503, 46504) genetically similar to *M. thoracata* from La Plata system, *M. thoracata* from Amazon basin and *M. thoracata* from Nanay River (Peru). ABGD recognized all the species as genetically equals and grouped all in the same lineage, while PTP showed a different topology, where *M. thoracata* from Amazonian basin is genetically similar to

M. aff thoracata from the same basin. *M. thoracata* is described from French Guiana drainages and *M. picta* from Guiana. Our data showed is essencial that there is a taxonomical revision of this species.

Lepthoplosternum

All the analyses recognized four lineages in *Lepthoplosternum* and agrees that the *L. aff. pectorale* from Tocantins-Araguaia basin (vouchers 69426, 69427, 69428, 69429, 69430) is as a different lineage from *L. pectorale* from Paraná-Paraguai basin. The analysis of delimitation between groups showed 11% of genetic divergence between *L. aff. pectorale* from Tocantins-Araguaia basin from *L. pectorale* from Paraná-Paraguai basin (bootstrap 100% and 98%). Although, our analyses included species of *Lepthoplosternum aff altamazonicum* from Upper Amazonica basin (Colombia) that showed a distance of 18% from *L. pectorale* from Paraná-Paraguai, and 19% to *L. aff pectorale* also from Paraguay basin as different lineages from others *Lepthoplosternum* from the same basin, representing different species.

Currently, there are six described species of *Lepthoplosternum*, three from Peru: *L. altamazonicum* Reis 1997, described from Ucayali River, upper Amazon River, Peru; *L. beni* Reis 1997 was described from Madeira River basin; and *L. ucamara* Reis & Kaefer 2005, described from Ucayali River basin, Peru; *L. pectorale* (Boulenger 1895) described from Paraguai River, *L. stellatum* Reis & Kaefer 2005 from Tefé lake, Upper Amazon River, Brazil, and *L. tordilho* Reis 1997 described from Jacui River drainage, Costeira Sul basin, Brazil. No one species are described to Tocantins-Araguaia basin. Besides that, these specimens presents dorsal fin with one spine, one unbranched ray (simple) and five branched rays (*vs.* dorsal fin with one spine and seven branched rays); anal fin with one unbranched ray and four branched rays (*vs.* anal fin with one simple and five branched rays); pectoral fin spine not elongated but thickened; dorsal fin ray comparatively shorter; lower lip rounded with projections (*vs.* pointed, crenulate); body comparatively wider; 21-22 scutes on upper lateral series and 21 on lower (*vs.* 25-26 scutes on upper lateral series and 22-23 on lower in *L. pectorale* and 24 scutes on upper lateral series and 21 on lower in *L. tordilho*); skin of belly and ventral portion presenting dots (*vs.* skin of belly and ventral portion with chromatophores not forming dots). Otherwise, all the analyses agree about the *L. aff. altamazonicum* to be a differen genetic lineage, and also all the analyzis agre about there are two distincts

lineages of *L. pectorale* from Paraguai basin, the vouchers 13399, 13400 and 133401, identified as *L. aff. pectorale* from Paraguai basin are genetically distant from others *L. pectorale* from the same basin. This could be a new species, and should be better investigated.

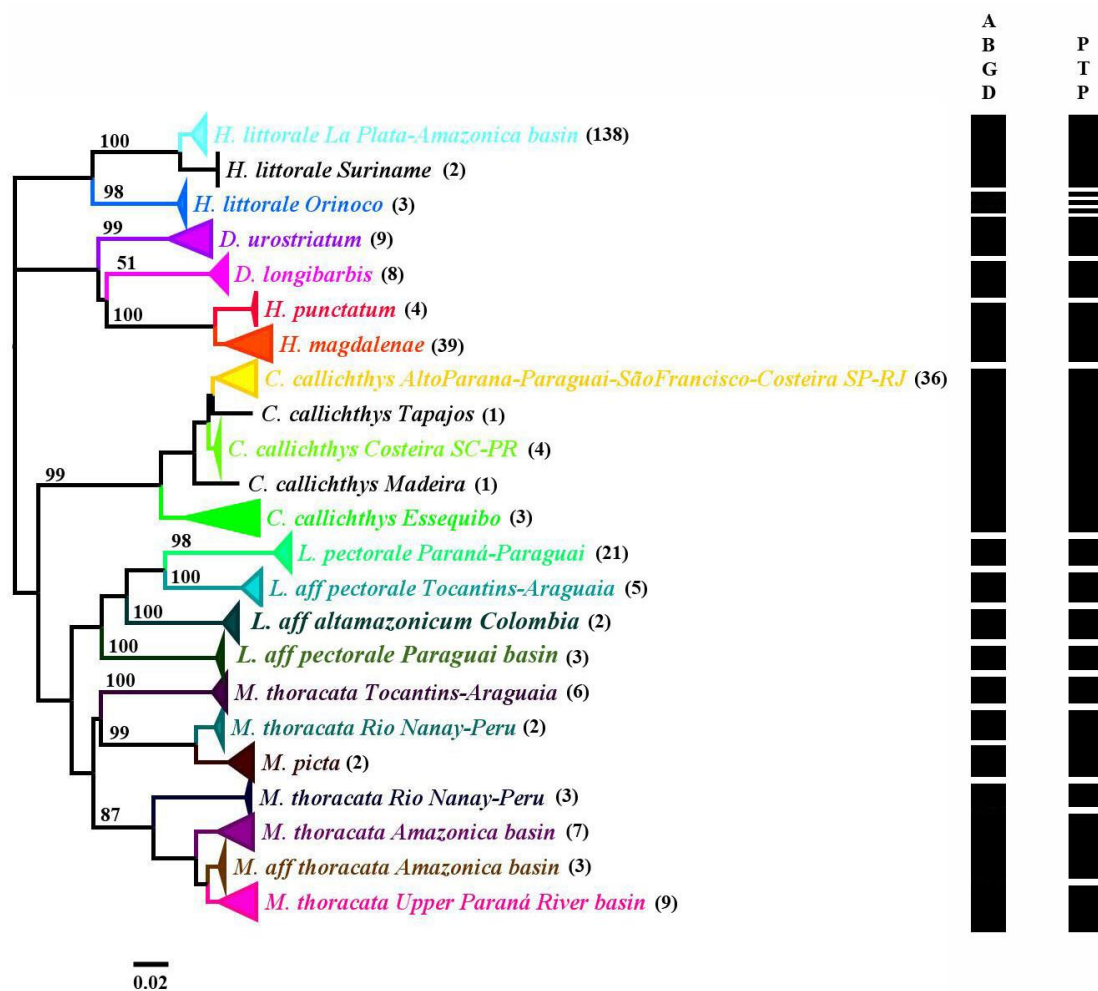


Fig 1. NJ tree of species of Callichthyinae, based on the COI gene (501 pb). Values < 50% are not shown. Vertical bars at right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Numbers of specimen are in parentheses after tip names.

Conclusion

Our study used molecular approaches to improve the knowledge of the diversity of the subfamily Callichthyinae. Herein we reveal that the complexity of the subfamily Callichthyinae, mainly *Callichthys*, *Megalechis* and *Hoplosternum*, that showed here through three analyses of delimitation the underestimated number of species and taxonomic uncertain of this subfamily. Our results reported different genetic lineages for the genera of Callichthyinae when compared with previous morphological

studies (Reis, 1997). The author refuted all the characters suggested by Gosline (1940) and presented new characters to diagnose *Hoplosternum*, and described two new genera, *Megalechis* and *Lepthoplosternum*, to allocate species of the groups *Hoplosternum*, but our results showed that are more than one genetic lineage inside *Megalechis*. In the same work, Reis (1997) suggest that *H. punctatum* as sister group of *H. magdalenae*, and *H. littorale* as sister group of that, but our results demonstrated that *H. magdalenae* and *H. punctatum* presented more genetic similarity with *Dianema* than to *Hoplosternum*, not corroborating the results of Reis (1997). Instead of, our results suggested that *H. punctatum* and *H. magdalenae* are distant genetically from others *Hoplosternum*. These species needed to be taxonomically reviewed and possibly synonymized each other, and relocated to a new genus.

The analyses also agree that *H. littorale* from Orinoco (voucher 15539, 15541, 15542, LBP 2183) represented a group genetically different from the others *H. littorale*. Beside that, our result promote evidences that *Lepthoplosternum* from Tocantins-Araguaia basin (vouchers 69426-69430) probably correspond to a new species.

The divergence between the analyses is that the ABGD takes into account the gaps between the sequences to formulate the ranked distance values, and a group the sequences based on that to create the groups of species. The bigger the gap value, the bigger the delimitation of the groups created by the analysis. The ABGD divides the dataset into as many groups as possible, respecting the divergence limit between the sequences. The ABGD results particularly emphasize the sensitivity of the method to the presence of recent speciation events, via (unrealistically) high speciation rates or large numbers of species. The PTP analysis is based on phylogenetic trees that incorporate models of population evolution, such as coalescence theory (Sites & Marshall, 2003; 2004; Pons *et al.*, 2006; Fujita *et al.*, 2012; Carstens *et al.*, 2013). This analysis is based on rate of substitutions to assess the speciation process, assuming that each additional substitution found between terminal taxa of a phylogeny increase the probability of speciation (Zhang *et al.*, 2013). It's possible that *H. magdalenae* and *H. punctatum* do not have enough gaps and low substitution rates to be considered divergent for these two analyses, despite they have divergent genetically.

The analysis showed different results for *Callichthys*. NJ suggests four distinct genetic lineages, whereas ABGD and PTP recognized all *Callichthys* sequences as the same species, although previous cytogenetic analyzes have shown that these populations are genetically distinct (Porto & Feldberg, 1988; Porto & Feldberg, 1992; Oliveira *et*

al., 1998). This result may indicate suggest that the speciation of this genus occurred recently, and the accumulation of gaps has not yet sufficient and the diversification rate maintains low to be accounted for by ABGD and PTP analysis.

Furthermore, assuming that there are at least two populations of *Hoplosternum* and *Callichthys*, we expose here the necessity of a description of a new genus to allocate *H. punctatum* and *H. magdalenae*, the complexity shown on *Megalechis* and *Lepthoplosternum*, and recognizing the high diversity of fish and values of genetic divergences are high, we suggest that a taxonomical investigation concentrating efforts on these genera is needed for this group to confirm whether the different lineages represent different species.

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Supplementary Table 1. Samples of Callichthyinae used in species delimitation analyses.

Subfamily	Species	Voucher code	Catalog number	Drainage	Country	Coordinates
Callichthyinae	<i>Callichthys callichthys</i>	46505	LBP 11150	Rio Madeira, Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"
Callichthyinae	<i>Callichthys callichthys</i>	81472	LBP 20867	Rio Tapajós, Amazonica basin	Brazil	S 14° 0'29.38" W 56°48'24.95"
Callichthyinae	<i>Callichthys callichthys</i>	28449	LBP 5964	Rio Paraguai, do Prata basin	Brazil	S 16°17'20.86" W 56°38'4.65"
Callichthyinae	<i>Callichthys callichthys</i>	FUPR274_09				
Callichthyinae	<i>Callichthys callichthys</i>	FUPR275_09				
Callichthyinae	<i>Callichthys callichthys</i>	FUPR276_09				
Callichthyinae	<i>Callichthys callichthys</i>	50971	LBP 13277	Costeira basin	Brazil	S 24°13'54.9" W 46°55'15.1"
Callichthyinae	<i>Callichthys callichthys</i>	32362	LBP 6803	Upper Rio Paraná, do Prata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyinae	<i>Callichthys callichthys</i>	32364	LBP 6803	Upper Rio Paraná, do Prata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyinae	<i>Callichthys callichthys</i>	32363	LBP 6803	Upper Rio Paraná, do Prata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyinae	<i>Callichthys callichthys</i>	32366	LBP 6803	Upper Rio Paraná, do Prata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyinae	<i>Callichthys callichthys</i>	32365	LBP 6803	Upper Rio Paraná, do Prata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyinae	<i>Callichthys callichthys</i>	GBGC6654_09				
Callichthyinae	<i>Callichthys callichthys</i>	44435	LBP 8920	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'38.2' W 52°48'54.4"
Callichthyinae	<i>Callichthys callichthys</i>	44436	LBP 8920	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'38.2' W 52°48'54.4"
Callichthyinae	<i>Callichthys callichthys</i>	12299	LBP 1555	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Callichthys callichthys</i>	10337	LBP 1008	Upper Rio Paraná, do Prata basin	Brazil	S 23°20' W 48°34'
Callichthyinae	<i>Callichthys callichthys</i>	16095	LBP 2577	Costeira basin	Brazil	S 22°14'7.0" W 41°51'44.6"
Callichthyinae	<i>Callichthys callichthys</i>	38432	LBP 8216	Costeira basin	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyinae	<i>Callichthys callichthys</i>	38212	LBP 8216	Costeira basin	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyinae	<i>Callichthys callichthys</i>	16039	LBP 2364	Costeira basin	Brazil	S 22°13'45.0" W 42°07'38.0"
Callichthyinae	<i>Callichthys callichthys</i>	16037	LBP 2364	Costeira basin	Brazil	S 22°13'45.0" W 42°07'38.0"
Callichthyinae	<i>Callichthys callichthys</i>	16038	LBP 2364	Costeira basin	Brazil	S 22°13'45.0" W 42°07'38.0"
Callichthyinae	<i>Callichthys callichthys</i>	16041	LBP 2364	Costeira basin	Brazil	S 22°13'45.0" W 42°07'38.0"
Callichthyinae	<i>Callichthys callichthys</i>	16040	LBP 2364	Costeira basin	Brazil	S 22°13'45.0" W 42°07'38.0"

Callichthyinae	<i>Callichthys callichthys</i>	7423	LBP 593	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Callichthys callichthys</i>	69177	LBP 17423	São Francisco basin	Brazil	S 19°37'56.4" W 44°02'47.4"
Callichthyinae	<i>Callichthys callichthys</i>	35537	LBP 7437	Costeira basin	Brazil	S 24°15'06.3" W 47°14'53.8"
Callichthyinae	<i>Callichthys callichthys</i>	GBGCA8649_15				
Callichthyinae	<i>Callichthys callichthys</i>	RDOCE311_15				
Callichthyinae	<i>Callichthys callichthys</i>	FPSR078_09				
Callichthyinae	<i>Callichthys callichthys</i>	FPSR079_09				
Callichthyinae	<i>Callichthys callichthys</i>	FPSR080_09				
Callichthyinae	<i>Callichthys callichthys</i>	FPSR081_09				
Callichthyinae	<i>Callichthys callichthys</i>	29865	LBP 6348	Upper Rio Paraná, do Prata basin	Brazil	S 22°47'08.4" W 45°27'20.2"
Callichthyinae	<i>Callichthys callichthys</i>	29361	LBP 6313	Costeira basin	Brazil	S 22°38'12.4" W 44°36'56.4"
Callichthyinae	<i>Callichthys callichthys</i>	31700	LBP 6543	Upper Rio Paraná, do Prata basin	Brazil	S 22°11'36.6" W 46°22'44.5"
Callichthyinae	<i>Callichthys callichthys</i>	31699	LBP 6543	Upper Rio Paraná, do Prata basin	Brazil	S 22°11'36.6" W 46°22'44.5"
Callichthyinae	<i>Callichthys callichthys</i>	BSB177_10				
Callichthyinae	<i>Callichthys callichthys</i>	61966	LBP 16315	São Francisco basin	Brazil	S 20°18'25.6" W 44°01'57.7"
Callichthyinae	<i>Callichthys callichthys</i>	41824	LBP 8984	São Francisco basin	Brazil	S 19°37'34.1" W 44°29'20.0"
Callichthyinae	<i>Callichthys callichthys</i>	52289	LBP 11454	Costeira basin	Brazil	S 26°02'23.8" W 48°38'09.4"
Callichthyinae	<i>Callichthys callichthys</i>	34251	LBP 7141	Costeira basin	Brazil	S 25°55'27.6" W 48°36'39.5"
Callichthyinae	<i>Callichthys callichthys</i>	69004	LBP 17434	Essequibo basin	Guiana	N 05°20'30.2" W 59°32'30.0"
Callichthyinae	<i>Callichthys callichthys</i>	ITAPE013_15				
Callichthyinae	<i>Callichthys callichthys</i>	RENA069_16				
Callichthyinae	<i>Dianema longibarbis</i>	87081	LBP 22527	Rio Javari, Amazonica basin	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyinae	<i>Dianema longibarbis</i>	87080	LBP 22527	Rio Javari, Amazonica basin	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyinae	<i>Dianema longibarbis</i>	7235	LBP 558	Rio Purus, Amazonica basin	Brazil	-
Callichthyinae	<i>Dianema</i> sp.	KR491520				
Callichthyinae	<i>Dianema urostriatum</i>	10703	LBP 1218	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	10704	LBP 1218	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	10707	LBP 1218	Aquario	-	-

Callichthyinae	<i>Dianema urostriatum</i>	19393	LBP 3194	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	10702	LBP 1218	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	10706	LBP 1218	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	7114	LBP 527	Upper Rio Negro, Amazonica basin	Brazil	-
Callichthyinae	<i>Dianema urostriatum</i>	10705	LBP 1218	Aquario	-	-
Callichthyinae	<i>Dianema urostriatum</i>	87079	LBP 22527	Rio Javari, Amazonica basin	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyinae	<i>Dianema urostriatum</i>	19395	LBP 3194	Aquario		
Callichthyinae	<i>Dianema urostriatum</i>	7230	LBP 557	Rio Purus, Amazonica basin	Brazil	
Callichthyinae	<i>Dianema urostriatum</i>	7232	LBP 557	Rio Purus, Amazonica basin	Brazil	
Callichthyinae	<i>Dianema urostriatum</i>	19394	LBP 3195	Aquario		
Callichthyinae	<i>Dianema</i> sp.	HEEN024_19				
Callichthyinae	<i>Hoplosternum littorale</i>	15539	LBP 2183	Orinoco basin	Venezuela	N 07°30'50.9" W 66°09'19.8"
Callichthyinae	<i>Hoplosternum littorale</i>	15542	LBP 2183	Orinoco basin	Venezuela	N 07°30'50.9" W 66°09'19.8"
Callichthyinae	<i>Hoplosternum littorale</i>	15541	LBP 2183	Orinoco basin	Venezuela	N 07°30'50.9" W 66°09'19.8"
Callichthyinae	<i>Hoplosternum littorale</i>	SU05-518	MHNG 2671.046	Rio Nickerie, Nickerie basin	Suriname	N 5°51'21.34" W 56°54'13.9811
Callichthyinae	<i>Hoplosternum littorale</i>	SU05-519	MHNG 2671.046	Rio Nickerie, Nickerie basin	Suriname	N 5°51'21.34" W 56°54'13.9811
Callichthyinae	<i>Hoplosternum littorale</i>	45763	LBP 9680	Costeira basin	Brazil	S 22°22'20.9" W 53°31'23.4"
Callichthyinae	<i>Hoplosternum littorale</i>	53513	LBP12525	Rio Itaya, Amazonica basin	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyinae	<i>Hoplosternum littorale</i>	53514	LBP 12525	Rio Itaya, Amazonica basin	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyinae	<i>Hoplosternum littorale</i>	47292	LBP 10314	São Francisco basin	Brazil	S 17°13'02.7" W 44°48'14.7"
Callichthyinae	<i>Hoplosternum littorale</i>	KM897583.1		Upper Rio Paraná, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	PDCAP287_14		Upper Rio Paraná, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	13807	LBP 2015	Upper Rio Paraná, do Prata basin	Brazil	S 22°13'34.27" W 47°34'14.11"
Callichthyinae	<i>Hoplosternum littorale</i>	47291	LBP 10314	São Francisco basin	Brazil	S 17°13'02.7" W 44°48'14.7"
Callichthyinae	<i>Hoplosternum littorale</i>	84069	LBP 21745	São Francisco basin	Brazil	S 15°46'44.0" W 43°21'27.0"
Callichthyinae	<i>Hoplosternum littorale</i>	25855	LBP 4964	Upper Rio Paraná, do Prata basin	Brazil	S 21°37'26.7" W 47°48'22.6"
Callichthyinae	<i>Hoplosternum littorale</i>	41825	LBP 8985	São Francisco basin	Brazil	S 19°37'34.1" W 44°29'20.0"
Callichthyinae	<i>Hoplosternum littorale</i>	41877	LBP 8963	São Francisco basin	Brazil	S 18°43'14.6" W 44°20'47.3"

Callichthyinae	<i>Hoplosternum littorale</i>	60949	LBP 10880	Upper Rio Paraná, do Prata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyinae	<i>Hoplosternum littorale</i>	60946	LBP 10880	Upper Rio Paraná, do Prata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyinae	<i>Hoplosternum littorale</i>	47350	LBP 10328	São Francisco basin	Brazil	S 17°13'33.7' W 44°48'27.9"
Callichthyinae	<i>Hoplosternum littorale</i>	47026	LBP 11314	São Francisco basin	Brazil	S 17°10'41.6' W 46°21'11.4"
Callichthyinae	<i>Hoplosternum littorale</i>	15165	LBP 2159	Upper Rio Paraná, do Prata basin	Brazil	S 22°50'45.71" W 48°27'34.36"
Callichthyinae	<i>Hoplosternum littorale</i>	15162	LBP 2159	Upper Rio Paraná, do Prata basin	Brazil	S 22°50'45.71" W 48°27'34.36"
Callichthyinae	<i>Hoplosternum littorale</i>	15164	LBP 2159	Upper Rio Paraná, do Prata basin	Brazil	S 22°50'45.71" W 48°27'34.36"
Callichthyinae	<i>Hoplosternum littorale</i>	19376	LBP 3186	Upper Rio Paraná, do Prata basin	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyinae	<i>Hoplosternum littorale</i>	19377	LBP 3186	Upper Rio Paraná, do Prata basin	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyinae	<i>Hoplosternum littorale</i>	84068	LBP 21745	São Francisco basin	Brazil	S 15°46'44.0" W 43°21'27.0"
Callichthyinae	<i>Hoplosternum littorale</i>	84071	LBP 21745	São Francisco basin	Brazil	S 15°46'44.0" W 43°21'27.0"
Callichthyinae	<i>Hoplosternum littorale</i>	15166	LBP 2159	Upper Rio Paraná, do Prata basin	Brazil	S 22°50'45.71" W 48°27'34.36"
Callichthyinae	<i>Hoplosternum littorale</i>	JEQUI191_13				
Callichthyinae	<i>Hoplosternum littorale</i>	FPSR268_09		Costeira basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	BSB286_10	HM405125	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	BSB229_10	HM405082	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	BSB228_10	HM405081	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	BSB225_10	HM405078	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	300198896	HM405078.1	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	300198902	HM405081.1	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	300198904	HM405082.1	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	300198990	HM405125.1	São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	13808	LBP 2015	Upper Rio Paraná, do Prata basin	Brazil	S 22°13'34.27" W 47°34'14.11"
Callichthyinae	<i>Hoplosternum littorale</i>	47293	LBP 10314	São Francisco basin	Brazil	S 17°13'02.7' W 44°48'14.7"
Callichthyinae	<i>Hoplosternum littorale</i>	47290	LBP 10314	São Francisco basin	Brazil	S 17°13'02.7' W 44°48'14.7"
Callichthyinae	<i>Hoplosternum littorale</i>	19378	LBP 3186	Upper Rio Paraná, do Prata basin	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyinae	<i>Hoplosternum littorale</i>	19380	LBP 3186	Upper Rio Paraná, do Prata basin	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyinae	<i>Hoplosternum littorale</i>	47028	LBP 11314	São Francisco basin	Brazil	S 17°10'41.6' W 46°21'11.4"

Callichthyinae	<i>Hoplosternum littorale</i>	16093	LBP 2374	Costeira basin	Brazil	S 22°00' W 41°20'
Callichthyinae	<i>Hoplosternum littorale</i>	84072	LBP 21745	Costeira basin	Brazil	S 15°46'44.0" W 43°21'27.0"
Callichthyinae	<i>Hoplosternum littorale</i>	25927	LBP 5010	Upper Rio Paraná, do Prata basin	Brazil	S 22°22'07" W 47°28'38"
Callichthyinae	<i>Hoplosternum littorale</i>	69160	LBP 17409	São Francisco basin	Brazil	S 19°37'56.4" W 44°02'47.4"
Callichthyinae	<i>Hoplosternum littorale</i>	16163				
Callichthyinae	<i>Hoplosternum littorale</i>	29268	LBP 6260	Upper Rio Paraná, do Prata basin	Brazil	S 21°17'25.6" W 46°29'42.3"
Callichthyinae	<i>Hoplosternum littorale</i>	84014	LBP 21257	Costeira basin	Brazil	S 23°16'29.7" W 46°00'00.6"
Callichthyinae	<i>Hoplosternum littorale</i>	47027	LBP 11314	São Francisco basin	Brazil	S 17°10'41.6' W 46°21'11.4"
Callichthyinae	<i>Hoplosternum littorale</i>	47348	LBP 10328	São Francisco basin	Brazil	S 17°13'33.7' W 44°48'27.9"
Callichthyinae	<i>Hoplosternum littorale</i>	47346	LBP 10328	São Francisco basin	Brazil	S 17°13'33.7' W 44°48'27.9"
Callichthyinae	<i>Hoplosternum littorale</i>	FUPR293_09				
Callichthyinae	<i>Hoplosternum littorale</i>	FUPR292_09				
Callichthyinae	<i>Hoplosternum littorale</i>	FUPR291_09				
Callichthyinae	<i>Hoplosternum littorale</i>	ANGBF24432_19				
Callichthyinae	<i>Hoplosternum littorale</i>	7424	LBP 594	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	17305	LBP 2647	Upper Rio Paraná, do Prata basin	Brazil	S 22°43'03.2" W 53°17'27.6"
Callichthyinae	<i>Hoplosternum littorale</i>	31995	LBP 6647	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'52.4' W 53°04'43.0"
Callichthyinae	<i>Hoplosternum littorale</i>	32090	LBP 6673	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'09.2' W 53°05'34.5"
Callichthyinae	<i>Hoplosternum littorale</i>	32091	LBP 6673	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'09.2' W 53°05'34.5"
Callichthyinae	<i>Hoplosternum littorale</i>	31857	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31783	LBP 6571	Upper Rio Paraná, do Prata basin	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyinae	<i>Hoplosternum littorale</i>	31856	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31858	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31860	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31861	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31946	LBP 6618	Upper Rio Paraná, do Prata basin	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyinae	<i>Hoplosternum littorale</i>	31997	LBP 6647	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'52.4' W 53°04'43.0"
Callichthyinae	<i>Hoplosternum littorale</i>	32016	LBP 6653	Upper Rio Paraná, do Prata basin	Brazil	S 22°39'45.2' W 53°06'17.0"

Callichthyinae	<i>Hoplosternum littorale</i>	32087	LBP 6673	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'09.2' W 53°05'34.5"
Callichthyinae	<i>Hoplosternum littorale</i>	32088	LBP 6673	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'09.2' W 53°05'34.5"
Callichthyinae	<i>Hoplosternum littorale</i>	32089	LBP 6673	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'09.2' W 53°05'34.5"
Callichthyinae	<i>Hoplosternum littorale</i>	7425	LBP 594	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	32206	LBP 6701	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'24.2' W 53°05'09.0"
Callichthyinae	<i>Hoplosternum littorale</i>	7284	LBP 466	Costeira basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyinae	<i>Hoplosternum littorale</i>	84015	LBP 21257	Costeira basin	Brazil	S 23°16'29.7" W 46°00'00.6"
Callichthyinae	<i>Hoplosternum littorale</i>	7128	LBP 531	Rio Negro, Amazonica basin	Brazil	S 03°04.900' W 59°59.855'
Callichthyinae	<i>Hoplosternum littorale</i>	7131	LBP 531	Rio Negro, Amazonica basin	Brazil	S 03°04.900' W 59°59.855'
Callichthyinae	<i>Hoplosternum littorale</i>	7130	LBP 531	Rio Negro, Amazonica basin	Brazil	S 03°04.900' W 59°59.855'
Callichthyinae	<i>Hoplosternum littorale</i>	53512	LBP 12525	Rio Itaya, Amazonica basin	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyinae	<i>Hoplosternum littorale</i>	11412	LBP 1358	Ilha do Marajó, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	53515	LBP 12525	Rio Itaya, Amazonica basin	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyinae	<i>Hoplosternum littorale</i>	7129	LBP 594	Rio Negro, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	32207	LBP 6701	Upper Rio Paraná, do Prata basin	Brazil	S 22°40'24.2' W 53°05'09.0"
Callichthyinae	<i>Hoplosternum littorale</i>	KU288808.1		Lower Rio Parana, do Prata basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	LARI155_12				
Callichthyinae	<i>Hoplosternum littorale</i>	JN988913.1		Upper Rio Paraná, do Prata basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	FUPR294_09				
Callichthyinae	<i>Hoplosternum littorale</i>	MUCU140_14				
Callichthyinae	<i>Hoplosternum littorale</i>	25928	LBP 5010	Upper Rio Paraná, do Prata basin	Brazil	S 22°22'07' W 47°28'38'
Callichthyinae	<i>Hoplosternum littorale</i>	EU359416.1				
Callichthyinae	<i>Hoplosternum littorale</i>	GBGC6646_09				
Callichthyinae	<i>Hoplosternum littorale</i>	LARI154_12				
Callichthyinae	<i>Hoplosternum littorale</i>	7377	LBP 569	Costeira basin	Brazil	S 30°02,820' W 51°22,347
Callichthyinae	<i>Hoplosternum littorale</i>	7383	LBP 569	Costeira basin	Brazil	S 30°02,820' W 51°22,347
Callichthyinae	<i>Hoplosternum littorale</i>	25692	LBP 4806	Costeira basin	Brazil	S 30°32'49.9" W 51°31'32.7"
Callichthyinae	<i>Hoplosternum littorale</i>	7427	LBP 594	Rio Paraguai, do Prata basin	Brazil	-

Callichthyinae	<i>Hoplosternum littorale</i>	8129	LBP 687	Rio Paraguai, do Prata basin	Brazil	S 16°25,680' W 56°25,143'
Callichthyinae	<i>Hoplosternum littorale</i>	RDOCE092_13				
Callichthyinae	<i>Hoplosternum littorale</i>	HM405080.1		São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	HM405079.1		São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	16094	LBP 2374	Costeira basin	Brazil	S 22°00' W 41°20'
Callichthyinae	<i>Hoplosternum littorale</i>	HM405079		São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	HM405080		São Francisco basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	FPSR269_09				
Callichthyinae	<i>Hoplosternum littorale</i>	RDOCE037_13				
Callichthyinae	<i>Hoplosternum littorale</i>	PRP107_16				
Callichthyinae	<i>Hoplosternum littorale</i>	ANGBF24433_19				
Callichthyinae	<i>Hoplosternum littorale</i>	7281	LBP 466	Upper Rio Paraná, do Prata basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyinae	<i>Hoplosternum littorale</i>	14219	LBP 2019	Upper Rio Paraná, do Prata basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyinae	<i>Hoplosternum littorale</i>	31989	LBP 6642	Upper Rio Paraná, do Prata basin	Brazil	S 22°39'45.2" W 53°06'17.0"
Callichthyinae	<i>Hoplosternum littorale</i>	31853	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4" W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	69317	LBP 16863	Rio Madeira, Amazonica basin	Brazil	S 09°57'11.8" W 67°44'28.2"
Callichthyinae	<i>Hoplosternum littorale</i>	11407	LBP 1358	Ilha do Marajó, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	7426	LBP 594	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	8130	LBP 687	Rio Paraguai, do Prata basin	Brazil	S 16°25,680' W 56°25,143'
Callichthyinae	<i>Hoplosternum littorale</i>	11406	LBP 1358	Ilha do Marajó, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	11411	LBP 1358	Ilha do Marajó, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	15154	LBP 2155	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	31994	LBP	Upper Rio Paraná, do Prata basin	Brazil	
Callichthyinae	<i>Hoplosternum littorale</i>	41205	LBP 12798	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°24'25.0" W 50°44'12.4"
Callichthyinae	<i>Hoplosternum littorale</i>	47347	LBP 10328	São Francisco basin	Brazil	S 17°13'33.7" W 44°48'27.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31996	LBP 6647	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'52.4" W 53°04'43.0"
Callichthyinae	<i>Hoplosternum littorale</i>	8131	LBP 687	Rio Paraguai, do Prata basin	Brazil	S 16°25,680' W 56°25,143'

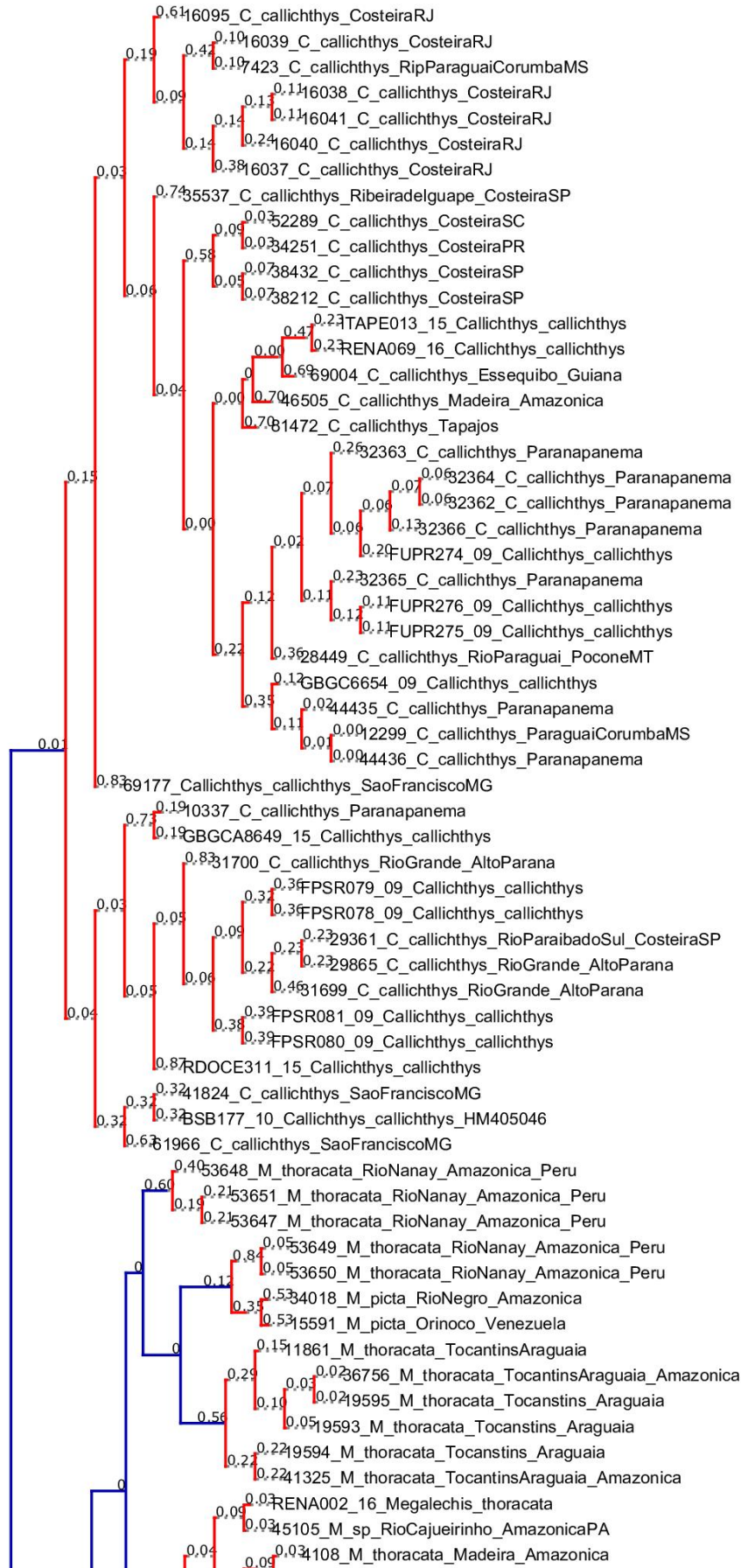
Callichthyinae	<i>Hoplosternum littorale</i>	47349	LBP 10328	São Francisco basin	Brazil	S 17°13'33.7" W 44°48'27.9"
Callichthyinae	<i>Hoplosternum littorale</i>	4134	LBP 210	Rio Madeira, Amazonica basin	Brazil	S 9°56,271' W 67°52,923'
Callichthyinae	<i>Hoplosternum littorale</i>	35532	LBP 7478	Costeira basin	Brazil	S 24°35'47.5" W 48°13'23.1"
Callichthyinae	<i>Hoplosternum littorale</i>	31852	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	31784	LBP 6571	Upper Rio Paraná, do Prata basin	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyinae	<i>Hoplosternum littorale</i>	15153	LBP 2155	Rio Paraguai, do Prata basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	11408	LBP 1358	Ilha do Marajó, Amazonica basin	Brazil	-
Callichthyinae	<i>Hoplosternum littorale</i>	21854	LBP 3705	Upper Rio Paraná, do Prata basin	Brazil	S 20°26'00.7' W 51°15'41.2"
Callichthyinae	<i>Hoplosternum littorale</i>	31854	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	7282	LBP 466	Upper Rio Paraná, do Prata basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyinae	<i>Hoplosternum littorale</i>	7283	LBP 466	Upper Rio Paraná, do Prata basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyinae	<i>Hoplosternum littorale</i>	13696	LBP 2695	Upper Rio Paraná, do Prata basin	Brazil	S 22°47.135' W 48°28.892
Callichthyinae	<i>Hoplosternum littorale</i>	4010	LBP 232	Rio Madeira, Amazonica basin	Brazil	S 10°03,038' W 67°50,874'
Callichthyinae	<i>Hoplosternum littorale</i>	31859	LBP 6585	Upper Rio Paraná, do Prata basin	Brazil	S 22°38'49.4' W 53°04'36.9"
Callichthyinae	<i>Hoplosternum littorale</i>	29519	LBP 6137	Lago Maracaibo basin	Venezuela	N 09°38'53.8' W 72°34'56.4"
Callichthyinae	<i>Hoplosternum magdalenae</i>	ADN8303				MF415655.1
Callichthyinae	<i>Hoplosternum magdalenae</i>	CIUA648_20				
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Callichthyinae	<i>Hoplosternum magdalenae</i>	ADN8296				MF415657.1
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Callichthyinae	<i>Hoplosternum magdalenae</i>	ADN8301				MF415659.1
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Callichthyinae	<i>Hoplosternum magdalenae</i>	ADN8289				MF415658.1

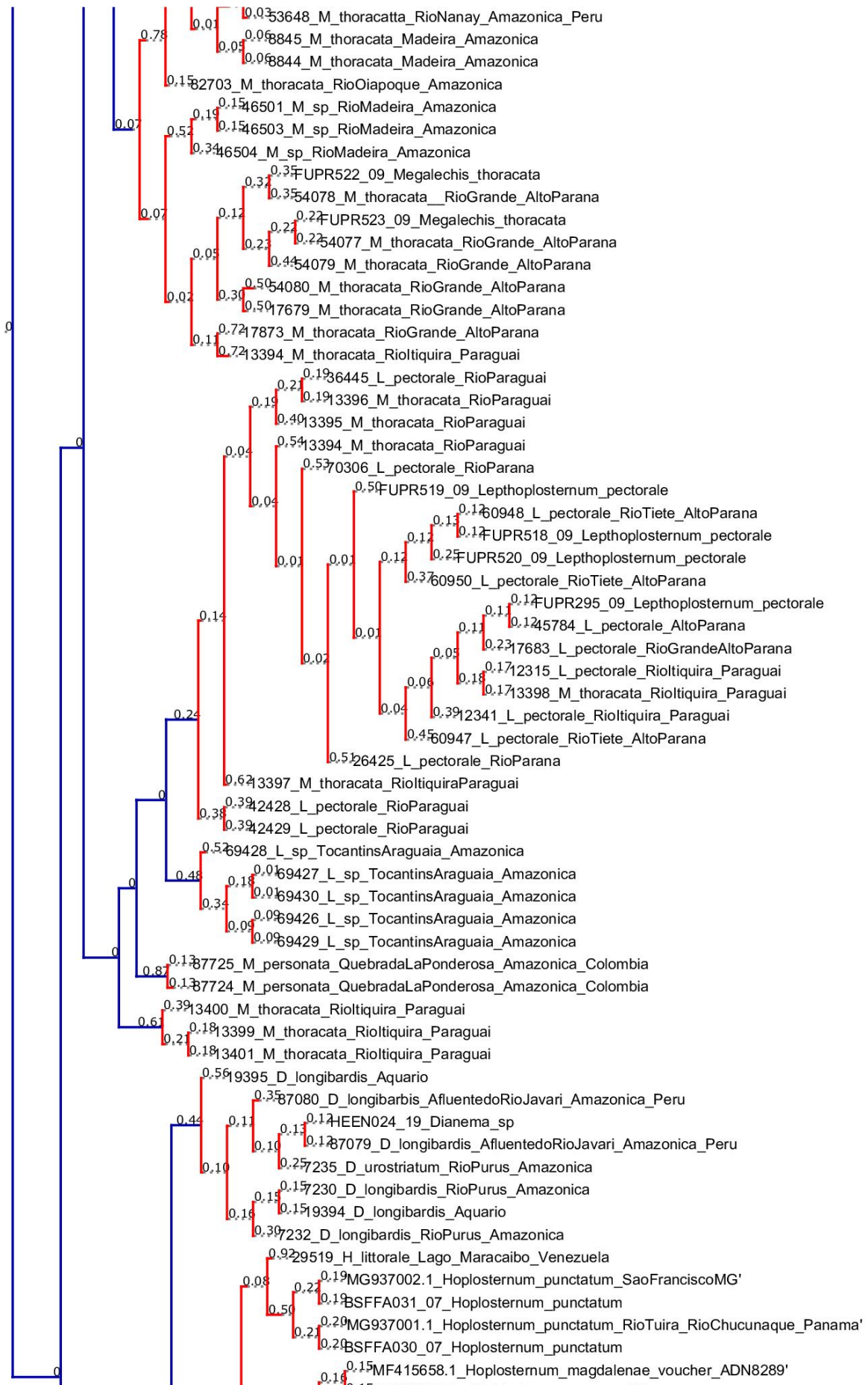
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Callichthyinae	<i>Hoplosternum magdalenae</i>	GBMIN127421_17		
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Callichthyinae	<i>Hoplosternum magdalenae</i>	CIUA682_20		
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Callichthyinae	<i>Hoplosternum magdalenae</i>	GBMIN133282_17		
Callichthyinae	<i>Hoplosternum punctatum</i>	MG937002.1	São Francisco basin	Brazil
Callichthyinae	<i>Hoplosternum punctatum</i>	BSFFA031_07		
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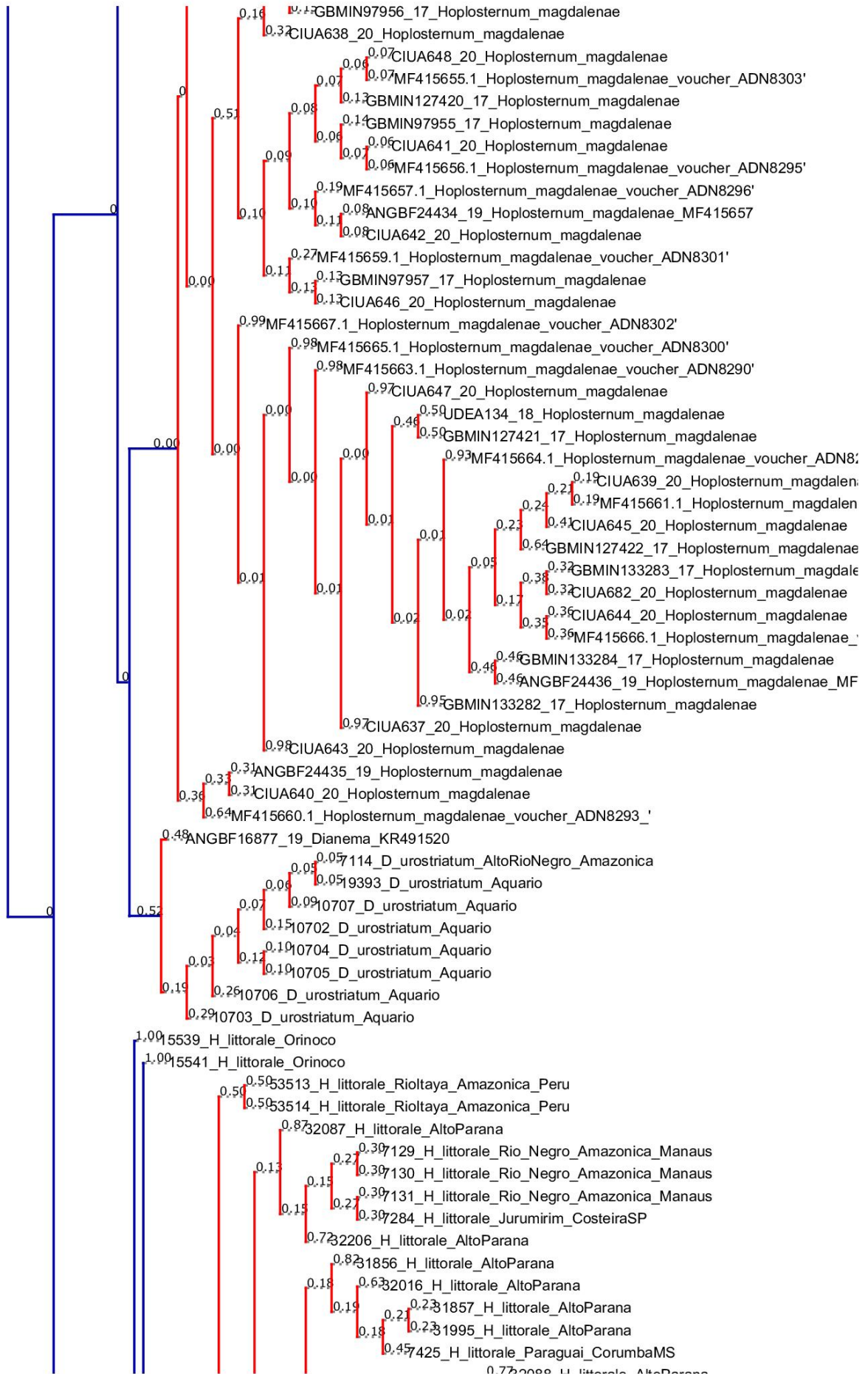
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Callichthyinae	<i>Leptoplosternum pectorale</i>	42429	LBP 8466	Rio Paraguai, do Prata basin	Brazil	S 16°03'13.6' W 57°48'31.8"
Callichthyinae	<i>Leptoplosternum pectorale</i>	42428	LBP 8466	Rio Paraguai, do Prata basin	Brazil	S 16°03'13.6' W 57°48'31.8"
Callichthyinae	<i>Leptoplosternum pectorale</i>	36445	LBP 7645	Rio Paraguai, do Prata basin	Brazil	S 15°46'03.8" W 55°30'44.5"
Callichthyinae	<i>Leptoplosternum pectorale</i>	45784	LBP 9687	Upper Rio Paraná, do Prata basin	Brazil	S 22°18'22.5' W 53°23'47.2"
Callichthyinae	<i>Leptoplosternum pectorale</i>	FUPR518_09				
Callichthyinae	<i>Leptoplosternum pectorale</i>	FUPR295_09				
Callichthyinae	<i>Leptoplosternum pectorale</i>	FUPR520_09				
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Callichthyinae	<i>Leptoplosternum pectorale</i>	60947	LBP 10880	Upper Rio Paraná, do Prata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyinae	<i>Leptoplosternum pectorale</i>	60950	LBP 10880	Upper Rio Paraná, do Prata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyinae	<i>Leptoplosternum pectorale</i>	70306	LBP 17610	Upper Rio Paraná, do Prata basin	Brazil	S 21°46'26.6" W 47°46'53.2"
Callichthyinae	<i>Leptoplosternum pectorale</i>	12315	LBP 1554	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Leptoplosternum pectorale</i>	12341	LBP 1554	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Leptoplosternum pectorale</i>	17683	LBP 2844	Upper Rio Paraná, do Prata basin	Brazil	S 20°14'10.2" W 48°40'42.0"
Callichthyinae	<i>Leptoplosternum pectorale</i>	60948	LBP 10880	Upper Rio Paraná, do Prata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyinae	<i>Leptoplosternum pectorale</i>	26425	LBP 5226	Upper Rio Paraná, do Prata basin	Brazil	S 22°47'29" W 53°20'58"
Callichthyinae	<i>Leptoplosternum sp.</i>	69428	LBP 13272	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyinae	<i>Leptoplosternum sp.</i>	69427	LBP 13272	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyinae	<i>Leptoplosternum sp.</i>	69430	LBP 13272	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyinae	<i>Leptoplosternum sp.</i>	69429	LBP 13272	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyinae	<i>Leptoplosternum sp.</i>	69426	LBP 13272	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyinae	<i>Leptoplosternum sp.</i>	61199	LBP 16957	Rio Paraguai, do Prata basin	Brazil	S 17°52'41.8" W 57°28'42.9"
Callichthyinae	<i>Megalechis personata</i>	87725	LBP 22449	Amazonica basin	Colômbia	S 04°08'24.4" W 69°56'53.4"
Callichthyinae	<i>Megalechis personata</i>	87724	LBP 22449	Amazonica basin	Colômbia	S 04°08'24.4" W 69°56'53.4"

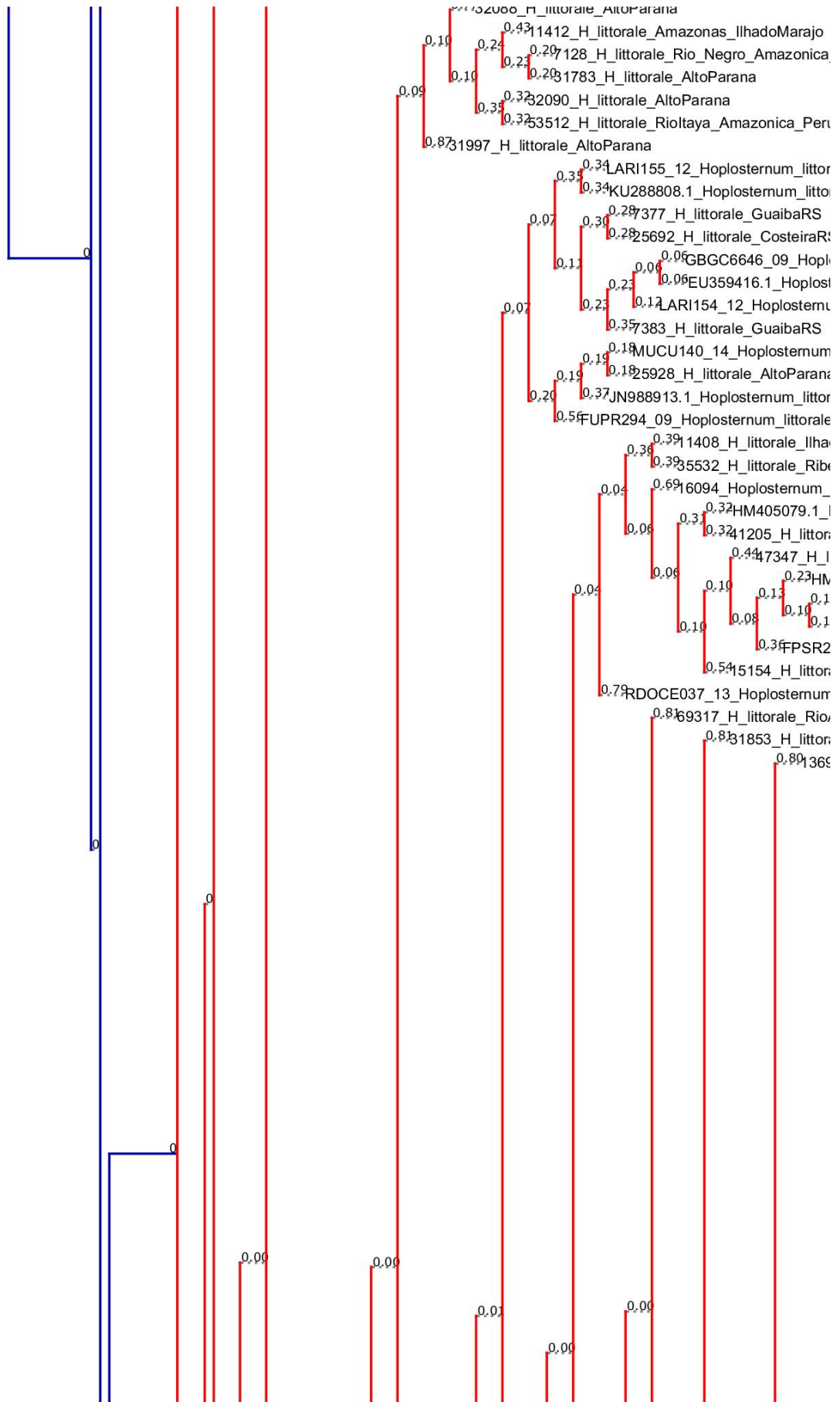
Callichthyinae	<i>Megalechis picta</i>	34018	LBP 6976	Rio Negro, Amazonica basin	Brazil	N 00°01.199' W 67°10.192'
Callichthyinae	<i>Megalechis picta</i>	15591	LBP 2201	Orinoco basin	Venezuela	N 07°30'50,9'' W 66°09'19,8''
Callichthyinae	<i>Megalechis thoracata</i>	19597	-	Rio Tocantins Araguaia, Amazonica basin	Brazil	-
Callichthyinae	<i>Megalechis thoracata</i>	13397	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13396	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13395	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13394	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13398	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13400	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13401	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	13399	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	53648	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	53647	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	53651	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	13394	LBP 1930	Rio Paraguai, do Prata basin	Brazil	S 17°28'13" W 55°14'46.7"
Callichthyinae	<i>Megalechis thoracata</i>	54080	LBP 13276	Upper Rio Paraná, do Prata basin	Brazil	S 20°24'11.6" W 48°38'08.6"
Callichthyinae	<i>Megalechis thoracata</i>	17679	LBP 2843	Upper Rio Paraná, do Prata basin	Brazil	S 20°14'10.2" W 48°40'42.0"
Callichthyinae	<i>Megalechis thoracata</i>	17873	LBP 2843	Upper Rio Paraná, do Prata basin	Brazil	S 20°14'10.2" W 48°40'42.0"
Callichthyinae	<i>Megalechis thoracata</i>	FUPR522_09				-
Callichthyinae	<i>Megalechis thoracata</i>	FUPR523_09				-
Callichthyinae	<i>Megalechis thoracata</i>	54078	LBP 13276	Upper Rio Paraná, do Prata basin	Brazil	S 20°24'11.6" W 48°38'08.6"
Callichthyinae	<i>Megalechis thoracata</i>	54079	LBP 13276	Upper Rio Paraná, do Prata basin	Brazil	S 20°24'11.6" W 48°38'08.6"
Callichthyinae	<i>Megalechis thoracata</i>	54077	LBP 13276	Upper Rio Paraná, do Prata basin	Brazil	S 20°24'11.6" W 48°38'08.6"
Callichthyinae	<i>Megalechis thoracata</i>	53648	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	4108	LBP 239	Rio Madeira, Amazonica basin	Brazil	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	RENA002_16				-
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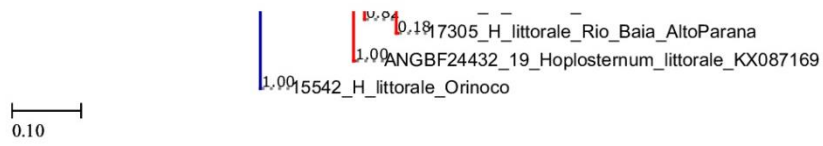
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Callichthyinae	<i>Megalechis thoracata</i>	11861	LBP 1599	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 15°48'22.6" W 52°01'53.9"
Callichthyinae	<i>Megalechis thoracata</i>	41325	LBP 9256	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°19'22.8" W 50°37'20.7"
Callichthyinae	<i>Megalechis thoracata</i>	19594	LBP 2972	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°20'05.1" W 50°42'16.2"
Callichthyinae	<i>Megalechis thoracata</i>	19593	LBP 2972	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°20'05.1" W 50°42'16.2"
Callichthyinae	<i>Megalechis thoracata</i>	36756	LBP 7797	Rio Tocantins Araguaia, Amazonica basin	Brazil	-
Callichthyinae	<i>Megalechis thoracata</i>	19595	LBP 2972	Rio Tocantins Araguaia, Amazonica basin	Brazil	S 13°20'05.1" W 50°42'16.2"
Callichthyinae	<i>Megalechis thoracata</i>	53650	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis thoracata</i>	53649	LBP 12418	Rio Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyinae	<i>Megalechis</i> sp.	46504	LBP 10811	Rio Madeira, Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"
Callichthyinae	<i>Megalechis</i> sp.	46503	LBP 10811	Rio Madeira, Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"
Callichthyinae	<i>Megalechis</i> sp.	46501	LBP 10811	Rio Madeira, Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"
Callichthyinae	<i>Megalechis</i> sp.	45105	LBP 10811	Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"



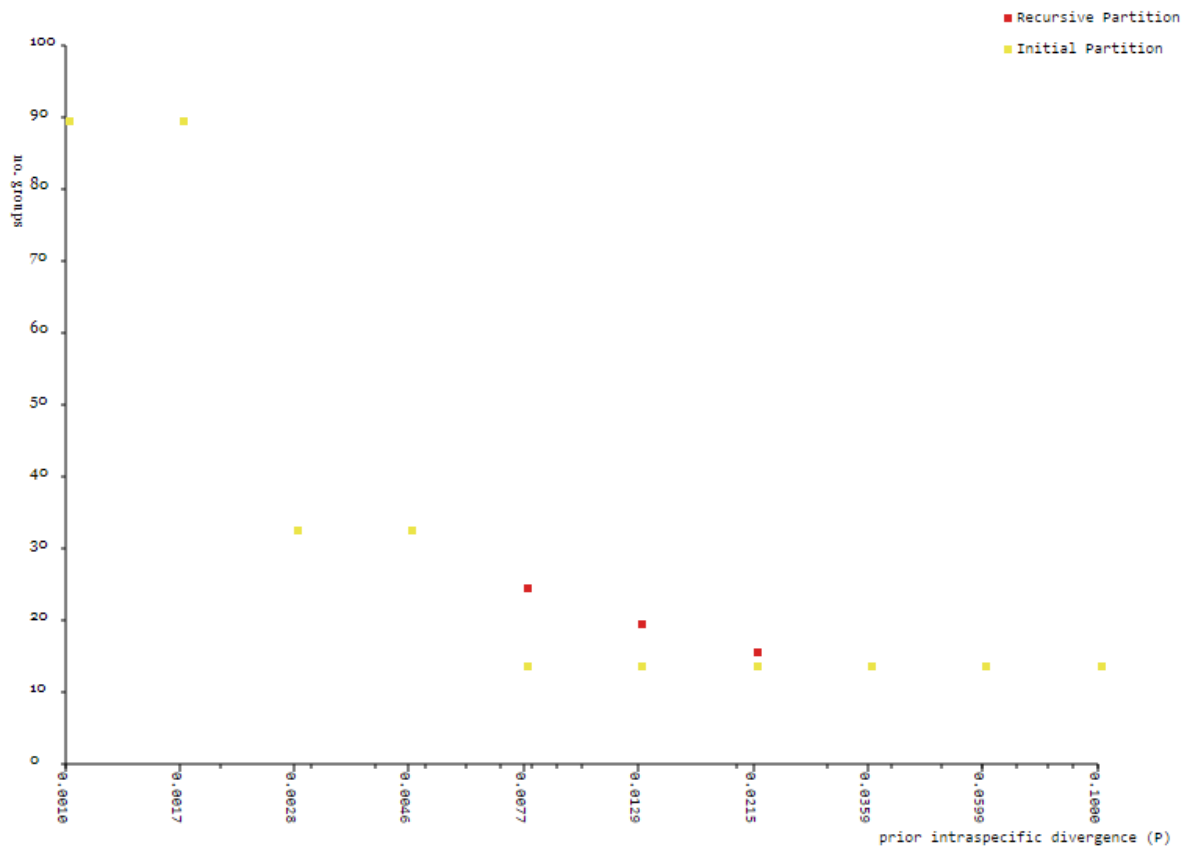




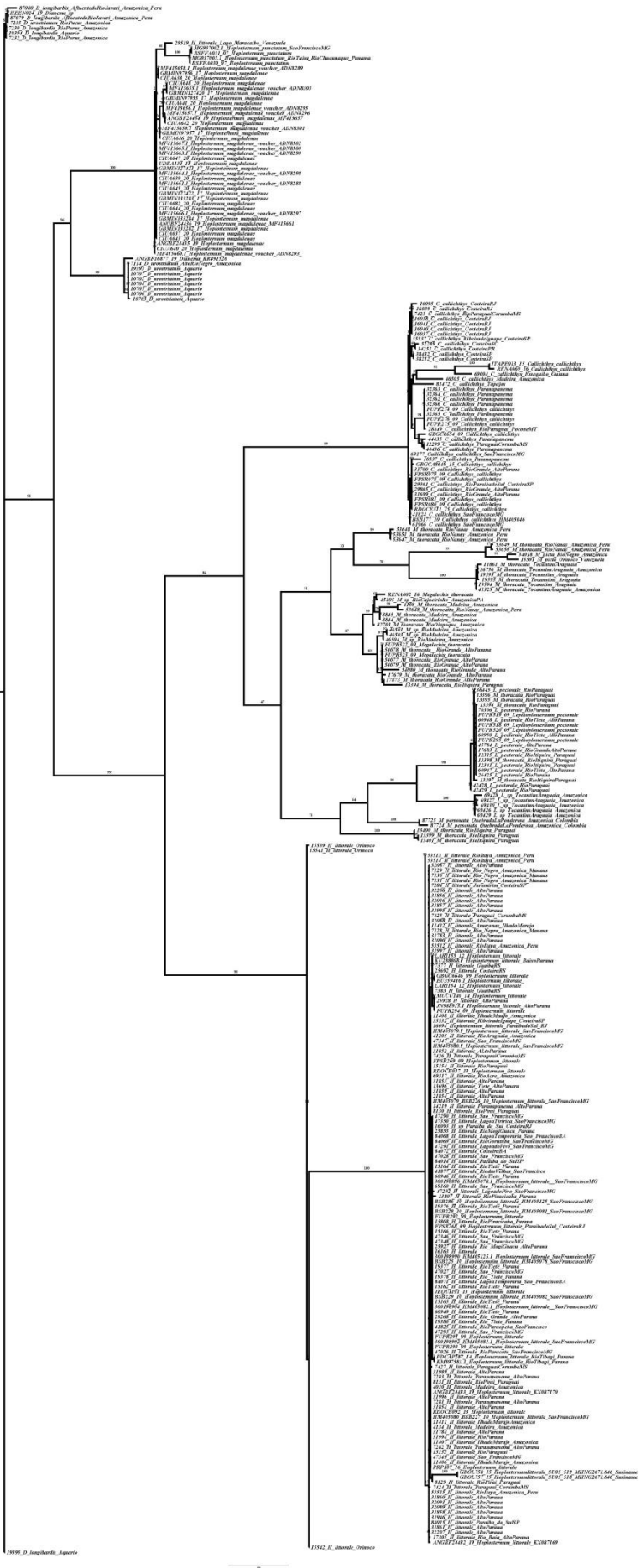




Supplementary Fig. 1. Poisson Tree Processes (PTP) delimitation tests of species of Callichthyinae using the Maximum Likelihood Phylogenetic tree (ML).



Supplementary Fig. 2. Automatic partition of the dataset (ABGD) reporting the number of groups of the subfamily Callichthyinae inside the initial and recursive partitions.



Supplementary Fig. 3. RAxML bipartitions consensus tree of Callichthyinae.

3.2 Capítulo 2

Molecular identification of the species of the subfamily Corydoradinae (Callichthyidae: Siluriformes) with DNA barcode

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Abstract

Corydoradinae (Siluriformes: Callichthyidae) is a widely distributed freshwater catfishes that represents 90% of all callichthyids, with more than 200 valid species. Although taxonomic hypotheses about relationships among Corydoradinae species are available, the molecular diversity and boundaries within some species of this subfamily remain dubious. Therefore, species of this subfamily were delimited here using the DNA barcode approach. Our goal was to assist the molecular identification of the species of Corydoradinae to reveal the genetic diversity of this large subfamily, especially *Corydoras*, which currently is composed of over 170 valid species. We sequenced the mitochondrial gene cytochrome c oxidase subunit I (COI) of more than 700 specimens of this subfamily, representing 133 operational taxonomic units, covering 69% of the subfamily. We used three delimitation analyses approaches: NJ, ABGD, and PTP, to investigate the species diversity and to provide molecular data for biodiversity studies in this subfamily. The analyzes of genetic distance ranged from 3 to 40 groups for NJ, 3 to 45 groups for PTP, and 3 to 18 groups to ABGD. Moreover, our study improves knowledge of the diversity of this subfamily, and supports several differences from hypotheses previously proposed in morphological studies. Our results also revealed that the diversity of the family is still unknown and needs to be taxonomically revised.

Keywords: Ostariophysi, catfish, species delimitation, biodiversity

Introduction

Callichthyidae is a very species-rich group of freshwater catfishes and the third largest family of the Neotropical Siluriformes. Its diversity represents 5% of the order, with 223 valid species. This number increased almost 11% in the last ten years, with 24 new species described (Frick *et al.*, 2022) and new species have been described every year (Tencatt *et al.*, 2019; Tencatt *et al.*, 2020; Bentley *et al.*, 2021; Tencatt *et al.*, 2021). Although the callictyids are easily identified from other Siluriformes by the presence of two longitudinal series of bone plates along the body (Bonaparte, 1838), they also presents a long list of misidentification in the literature, mostly in subfamily Corydoradinae. This is mainly because the members of this subfamily present almost identical colour patterns and also the coexistence of different species aggregated in large mixed shoals (Fuller and Evers, 2005; Nijssen, 1970; Sands, 1994). Besides that, the subfamily Corydoradinae represents 90% of all callichthyids, with more than 170 valid species. Currently, it's divided into three genera: *Aspidoras* Ihering, 1907, *Scleromystax* Günther, 1864, and *Corydoras* Lacepède, 1903 (Britto, 2003).

Corydoradinae represent a peculiar and complex subfamily mainly due to the members presenting similar patterns of coloration coexisting in the same niche of the streams, but also because coexisting species of Corydoradinae presents difference in the snout morphology and body size (Nijssen, 1970). Alexandrou *et al.* (2011) reported the existence of several cases of convergence in the color pattern supporting the hypothesis of Müllerian mimicry between the species of this subfamily. Moreover, they have a broad geographic distribution, inhabiting streams, rivers, and floodplains throughout all of South America (Nijsseni, 1970). Another aggravating factor is that no taxonomical revision of Corydoradinae has been presented since Nijssen & Isbrüker (1980). After that, descriptions of new species have been primarily based on color patterns. However, this character tends to be variable, thus making it difficult to quantify objectively (Nijssen, 1970). The most systematic literature on Corydoradinae in the last five years was about descriptions of new species of *Aspidoras* and *Corydoras* (Tencatt & Britto, 2016; Tencatt & Evers, 2016; Lima & Sazima, 2017; Oliveira, Zanata, Tencatt & Britto, 2017; Tencatt & Bichuette, 2017; Espíndola, Tencatt, Pupo, Villa-Verde & Britto, 2018; Tencatt & Britto, 2019; Bentley, Grant & Tencatt, 2021; Lima & Britto, 2020; Tencatt, Muriel-Cunha, Zuanon, Ferreira & Britto, 2020; Tencatt, Santos, Evers & Britto, 2021) increasing considerably the complexity of the subfamily.

In this context, since DNA barcode has been demonstrated useful in delimiting species from various groups of Neotropical fish (Benzaquem *et al.*, 2015; Costa-Silva *et al.*, 2015; Rossini *et al.*, 2016; Silva *et al.*, 2016; Machado *et al.*, 2017; Parente *et al.*, 2018; Melo *et al.*, 2018; Serrano *et al.*, 2018; García-Melo *et al.*, 2019; Mateussi *et al.*, 2019; Dorini *et al.*, 2020), our goal in the present work was to sequence the mitochondrial gene cytochrome c oxidase subunit I (COI) of more than 700 specimens of the subfamily Corydoradinae, seeking to assess their genetic variability to clarify the limit between each species of this large subfamily.

Material and Methods

Taxon sampling

The analyses included a total of 754 specimens of all genera of the subfamily Corydoradinae. Five specimens of each species were used in our analyses, whenever possible. All the sampling material belongs to the LBP collection (Laboratório de Biologia e Genética de Peixes, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil). The specimens were previously deposited in the LBP tissue collection and the vouchers of each species in the LBP collection, fixed in formalin 10% and transferred posteriorly to ethanol 70% for permanent storage. The sampling material is listed in the Supplementary Table 1, with information about voucher, catalog number, and coordinate. Some undescribed species were categorized into a group known as C-numbers and CW-numbers system (a method of recognition for aquarists). We followed Black (1987), Sands (1994), Britto (2000), Britto *et al.* (2005), Nijssen (1970), Nijssen & Isbrücker (1976), Nijssen & Isbrücker (1980), Nijssen & Isbrücker (1983), Tencatt *et al.* (2019), Weitzman (1960) and Weitzman (1963) to identify the species.

DNA extraction

The samples chosen for the analyses were always those that have been previously identified by a taxonomist of the group. All the molecular analyses, DNA extraction, PCR amplification, and DNA sequencing were performed in the LBP. We used about 10-100 mg of the fragment of muscle, gill, or fin tissue of each specimen, following the manufacturer's instruction for DNA extraction (CCDB – Canadian Center of DNA barcoding).

We amplified and sequenced fragments of the mitochondrial gene cytochrome c oxidase subunit I (COI) with the polymerase chain reaction (PCR) using the primers FishF1, FishR1, FishF2, FishR2 (Ward *et al.*, 2005), L6252-Asn, H7271-COXI (Melo *et al.*, 2011) and FishF6, FishR7 (Jennings *et al.*, 2019). A PCR total volume of 12.5 μ l was used: 7.55 μ l of double-distilled water, 1.25 μ l 5x of buffer, 0.50 μ l of MgCl₂ (50 mM), 0.50 μ l of dNTP mix at 8 mM, 0.25 μ l of each primer at 10 μ M, 0.20 μ l of PHT@Taq DNA polymerase enzyme (Phoneutria), and 2.0 μ l of genomic DNA (10–50 ng). The initial denaturation consisted in 5 min at 95°C, followed by 35 cycles of chain denaturation (45s at 95°C), primer hybridization (45s at 54°C for COI), nucleotide extension (60s at 68°C), and final extension (7 min at 68°C). At the end of this process, the PCR products were checked on 1% agarose gel, and then, purified with ExoSap-IT (USB Corporation), following the manufacturer's instructions. In the sequencing reaction, we submitted the PCR products to the BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purified again through ethanol precipitation. Finally, the sequences were loaded to an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems).

Data processing

The sequences of the mitochondrial gene cytochrome c oxidase subunit I (COI) were assembled, considering their complementary strand, in Geneious v7.1.9 (Kearse *et al.*, 2012) to create the consensus sequence for each specimen. After that, the matrix was aligned with Muscle (Edgar, 2004), under default parameters, at the same time that the consensus matrix was edited to eliminate inconsistencies such as gaps (missing data) and non-coding genes.

Delimitation analysis

We estimated the best substitution model with software MEGA X-64 (Kumar *et al.*, 2018) for each lineage. The best model was adopted for computing the standard deviation values of the overall mean distance, and before we compute within-group mean distance and between-group mean distance, was generated a previous neighbor-joining tree (NJ) as a guide for the creation of the groups, with 1000 bootstrap pseudoreplicates (bs), in MEGA X-64 (Kumar *et al.*, 2018).

To estimate a maximum likelihood (ML) tree, we used RAxML v8.019 (Stamatakis, 2014) with GTRGAMMA model (Stamatakis *et al.*, 2008), and others

parameters at default. The best ML tree was used as input in Poisson Tree Process (PTP) in the bPTP webserver (<http://species.h-its.org/ptp/>) (Zhang *et al.*, 2013), and then, was used an input FASTA file into the webserver in the Automatic Barcode Gap Discovery (ABGD) analysis (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) (Puillandre *et al.*, 2012).

Results and Discussion

We divided the analyses according to each lineage to better discuss the results. We followed the topology of UCE phylogeny (Chapter 3) to delimitate the nine genetic lineages presented below. We included 810 sequences of gene COI in our analyses specified for each lineage, with 610 bp in average, corresponding to 134 species of the three genera of the subfamily Corydoradinae: *Aspidoras* (9 species, 41% of the genus), *Corydoras* (118 species, 71% of the genus) and *Scleromystax* (3 species, 60% of the genus).

Lineage 1

We include partial sequences of the gene COI of 76 specimens of: *C. aff. areio*, *C. cf aurofrenatus*, *C. cf blochii*, *C. servinus*, *C. desana*, *C. elisae*, *C. fowleri*, *C. maculifer*, *C. narcissus*, *C. ourastigma*, *C. pastazensis*, *C. semiaquilus*, *C. serratus*, *C. simulatus*, *C. treitlii*, *C. vittatus* and we also included the undescribed species C53, C109, and *Corydoras* sp. The matrix had 615 pb with 326 pb of variable sites. The nucleotide composition of the matrix was 25% of adenine, 28% of cytosine, 17% of guanine, and 29% of thymine. The best evolution model for the matrix was TN93+G, with BIC=9661.4876 and AICc=8318.1315. The overall mean distance was 0.15 ± 0.01 . The lowest value of interspecific distances among lineage 1 was between *C. narcissus* 1 and *C. narcissus* 2 (0.0181 ± 0.0054), and the highest distance was between *Corydoras* sp. C53 from Peru and *C. ourastigma* (0.230 ± 0.0255), and intraspecific distances ranged from 0.0006 ± 0.0006 to 0.0327 ± 0.0077 (Table 1).

Table 1. Pairwise TN-93 genetic distances among species of Lineage 1. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	IGV
1. <i>C. maculifer</i>		0,018	0,019	0,019	0,017	0,018	0,018	0,019	0,018	0,018	0,018	0,020	0,020	0,018	0,022	0,023	0,025	0,020	0,023	0,025	0
2. <i>C. simulatus</i>	0,150		0,019	0,020	0,018	0,018	0,018	0,021	0,018	0,019	0,016	0,019	0,016	0,017	0,021	0,023	0,025	0,024	0,023	0,024	-
3. <i>C. desana</i>	0,158	0,161		0,014	0,017	0,018	0,017	0,019	0,017	0,017	0,018	0,022	0,016	0,020	0,024	0,024	0,023	0,023	0,024	0,024	0.0006±0.0006
4. <i>C. pastazensis</i>	0,174	0,176	0,106		0,017	0,017	0,016	0,018	0,017	0,017	0,017	0,019	0,017	0,020	0,020	0,023	0,023	0,022	0,021	0,021	-
5. <i>C. vittatus</i>	0,134	0,149	0,126	0,134		0,011	0,011	0,011	0,010	0,010	0,015	0,018	0,017	0,017	0,021	0,023	0,022	0,023	0,022	0,022	0.0017±0.0017
6. <i>C. serratus</i>	0,147	0,156	0,139	0,141	0,063		0,009	0,010	0,009	0,009	0,016	0,017	0,016	0,018	0,021	0,023	0,023	0,023	0,021	0,023	0.0088±0.0030
7. <i>C. treitlii</i>	0,142	0,148	0,133	0,131	0,063	0,049		0,008	0,006	0,006	0,016	0,018	0,016	0,018	0,022	0,022	0,023	0,023	0,021	0,022	0
8. <i>C. C109_IgarapeRio_Guama_Amazonica</i>	0,157	0,167	0,142	0,142	0,066	0,047	0,029		0,007	0,007	0,017	0,018	0,017	0,020	0,022	0,023	0,023	0,023	0,022	0,022	0
9. <i>C. narcissus2</i>	0,147	0,152	0,129	0,135	0,058	0,043	0,025	0,023		0,005	0,015	0,017	0,015	0,017	0,021	0,022	0,022	0,023	0,021	0,022	-
10. <i>C. narcissus1</i>	0,150	0,156	0,129	0,136	0,060	0,046	0,024	0,025	0,018		0,015	0,017	0,016	0,018	0,021	0,021	0,023	0,022	0,020	0,021	0.0107±0.0032
11. <i>C. elisae</i>	0,149	0,135	0,147	0,143	0,109	0,119	0,127	0,124	0,122	0,116		0,013	0,013	0,014	0,020	0,024	0,022	0,021	0,021	0,022	-
12. <i>C. ourastigma</i>	0,168	0,154	0,180	0,169	0,139	0,120	0,144	0,135	0,133	0,133	0,083		0,014	0,016	0,021	0,024	0,023	0,022	0,022	0,022	0.0158±0.0053
13. <i>C. aff_areio</i>	0,165	0,126	0,129	0,149	0,137	0,123	0,126	0,127	0,110	0,118	0,093	0,098		0,013	0,020	0,025	0,021	0,022	0,021	0,021	0.0012±0.0009
14. <i>C. cervinus</i>	0,163	0,155	0,177	0,179	0,146	0,160	0,164	0,170	0,154	0,161	0,105	0,115	0,101		0,021	0,024	0,024	0,024	0,022	0,022	0.0327±0.0077
15. <i>C. semiaquilus+C. fowleri</i>	0,205	0,199	0,204	0,185	0,181	0,183	0,192	0,191	0,180	0,178	0,173	0,180	0,169	0,194		0,022	0,020	0,019	0,019	0,019	-
16. <i>C. sp_C53_Peru</i>	0,197	0,216	0,213	0,217	0,216	0,218	0,213	0,220	0,209	0,202	0,223	0,230	0,229	0,229	0,193		0,017	0,017	0,017	0,017	0.0019±0.0007
17. <i>C. aff_C109_IgarapeAçu_Amazonica</i>	0,218	0,227	0,202	0,200	0,199	0,203	0,208	0,203	0,198	0,199	0,198	0,205	0,190	0,236	0,161	0,128		0,016	0,017	0,016	0
18. <i>C. sp_Peru</i>	0,168	0,214	0,202	0,197	0,199	0,206	0,199	0,200	0,197	0,185	0,180	0,191	0,190	0,222	0,166	0,125	0,114		0,015	0,016	-
19. <i>C. sp_Rio_Javari e_Tiquie_Negro_Amazonica</i>	0,197	0,202	0,211	0,187	0,189	0,182	0,188	0,183	0,174	0,173	0,190	0,193	0,180	0,205	0,163	0,119	0,118	0,100		0,010	0
20. <i>C. aff_C109_RioTiquie_Negro_Amazonica</i>	0,219	0,209	0,209	0,192	0,191	0,201	0,193	0,191	0,181	0,182	0,195	0,201	0,176	0,205	0,163	0,122	0,107	0,110	0,047		0.0059±0.0034
21. <i>C. aff_C109</i>	0,219	0,209	0,209	0,183	0,197	0,191	0,201	0,193	0,191	0,182	0,195	0,201	0,176	0,205	0,167	0,163	0,122	0,107	0,110	0,047	0.0019±0.0014

The NJ and PTP analysis delimited 20 groups in lineage 1, while the ABGD found 18 (Fig. 1). The ABGD analysis showed that two partitions found 26 groups (prior maximal distance $P= 0.001-0.002$), three partitions found 23 groups ($P= 0.003-0.008$), one partition found 22 groups ($P= 0.013$), one partition found 19 groups ($P= 0.021$), and one partition found 18 groups ($P= 0.036$). Despite NJ and PTP finding the same number of lineages, they disagree with the composition of the groups. The NJ results suggest that there are two genetically different groups of *C. narcissus* from the Madeira River basin (2%) (voucher 46529, 46523, 46535, 46526; LBP 10093, and voucher 66458, 46610; LBP 17116 and LBP 10094), while PTP and ABGD suggested the two groups of *C. narcissus*, *C. treitlii* and *C. C109* (voucher 43014, LBP 9122) collected from Guamá River are genetically similar and form a single lineage (0.44 of support value). By the way, *C. narcissus* was described by Nijssen & Isbrücker (1980) from Purus River, its distribution is limited to this system, and *C. treitlii* was described by Steindachner (1906) from a Coastal river of Maranhão, Parnaíba River drainage.

All the analyses agree about the *C. serratus* to be most similar genetically to the group *C. narcissus*, *C. treitlii*, and *C. C109*, but as a different lineage (more than 4% distance). Also, all the analyses agree that *C. vittatus* is a different species, genetically similar to the group *C. narcissus*, *C. treitlii*, *C. C109*, and *C. serratus* (6%). *C. pastazensis*, *C. cf. blochii* and *C. desana* were unanimous between all the analyses that represent different species, with most genetic similarity each other than to the group *C. ourastigma*, *C. elisae*, *C. cervinus*, and *C. aff areio*, but PTP analysis found two different genetic lineages between *C. ourastigma* (0.77 of support value) and *C. cervinus* (0.97).

All the analyses also agree with *C. simulates* and *C. maculifer* as different lineages, and therefore, different species. The same happened with *C. semiaquilus* and *C. fowleri*. The ABGD and NJ suggested that these two species form a single lineage, whereas PTP analysis suggested that the group is formed by two different genetic lineages. The PTP recognized *C. semiaquilus* (LBP 2828) (0.94) as a different lineage of *C. semiaquilus* + *C. fowleri* group (0.08). These species are described from Upper Solimões River and Chanco Caño, Peruvian Amazon, respectively. The pattern of coloration of this species is very similar and could result in misidentification and confusion.

The last group is formed by Amazonian species that are genetically similar to each other. All the analyses agree that *Corydoras* aff. C109 from Tiquié River, Negro

basin, Amazon system (voucher 32700, 43013, LBP 7712; and 32698, LBP 9122), and *Corydoras* sp. (voucher 87787, LBP 22532, and 32699, LBP 7712) are more genetically similar than the group composed by *Corydoras* sp. from Peru (voucher 43846, LBP 9325; and 53797, LBP 12466), *C. aff C109* from Guamá River (voucher 43011, LBP 9122) and *Corydoras* sp. From Peru (voucher 53877, 53879, LBP 12488; 53936-53939, LBP 12507).

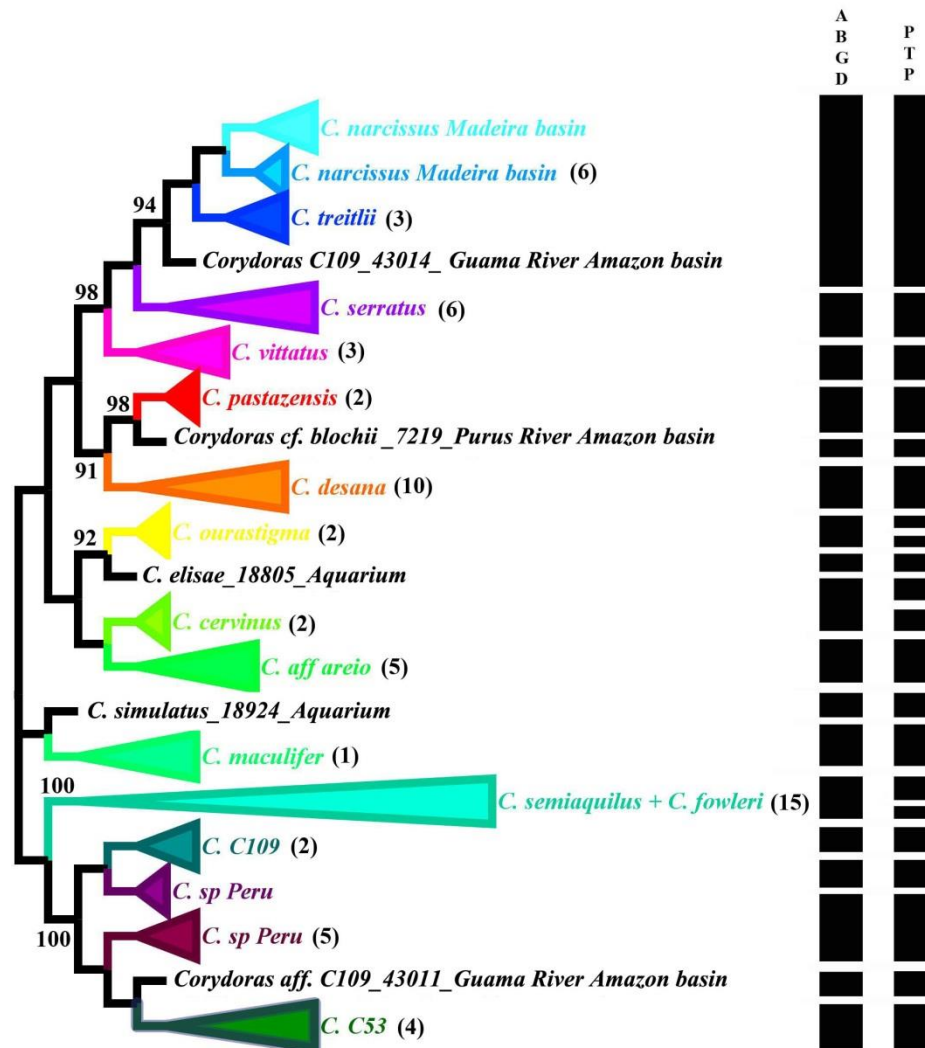


Fig. 1. NJ tree of species of Lineage 1 based on the COI gene (615 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. The numbers of the specimens used are in parentheses after tip names.

Lineage 2

This lineage correspond to the genus *Aspidoras*. We included in our analysis 44 sequences of *A. albater*, *A. depinnai*, *A. fuscoguttatus*, *A. poecilus*, *A. psammatides*, *A. raimundi*, *A. taurus*, and *A. aff poecilus*, *A. sp poecilus*, and *Aspidoras* sp. The species *A. pauciradiatus* belongs to lineage 5 of Callichthyinae and therefore was not added to in the analyses of the lineage 2.

The matrix had 617 pb with 238 pb of variable sites. The nucleotide composition of the matrix was 26% of adenine, 27% of cytosine, 17% of guanine, and 29% of thymine. The best evolution model for the matrix was T92+G. with BIC=4537.8683 and AICc=3839.8563. The overall mean distance for lineage 2 was 0.07 ± 0.001 . The lower value of interspecific distances among the lineage 2 was *A. fuscoguttatus* and *A. poecilus* from Vermelho River (0.0094 ± 0.0041), and the highest distance was between *A. poecilus* from Upper Araguaia River and *A. psammatides* (0.1842 ± 0.0226), and the intraspecific distances ranged from 0.001 ± 0.001 to 0.0026 ± 0.0018 (Table 2).

Table 2. Pairwise TN-93 genetic distances among species of Lineage 2. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	6	7	8	9	10	11	IGV
1. <i>A. depinnai</i>			0,01263	0,00843	0,01104	0,01382	0,01457	0,01387	0,00574	0,00708	0,02214	0
2. <i>A. sp. poecilus afluenta do Araguaia</i>	0,07467		0,01234	0,01314	0,00762	0,01364	0,01272	0,01163	0,01139	0,02185	0,01127	0.0012±0.0011
3. <i>A. sp. poecilus Rio Vermelho</i>	0,03593	0,07427		0,01159	0,01386	0,01400	0,01271	0,00842	0,00702	0,02253	0,00418	0
4. <i>A. raimundi</i>	0,06044	0,08400	0,06698		0,01343	0,01337	0,01256	0,01071	0,01028	0,02065	0,01074	0
5. <i>A. aff. poecilus Corrego do Sapo</i>	0,08871	0,02977	0,08765	0,08700		0,01317	0,01244	0,01253	0,01217	0,02215	0,01179	0
6. <i>A. taurus</i>	0,08662	0,08973	0,08810	0,08539	0,08736		0,00793	0,01187	0,01269	0,02081	0,01189	0.0007±0.0007
7. <i>A. poecilus</i>	0,08479	0,08167	0,07964	0,08144	0,07765	0,03361		0,01147	0,01191	0,02128	0,01156	0.0007±0.0007
8. <i>A. psammatides</i>	0,01669	0,06896	0,03925	0,05862	0,07978	0,06664	0,06633		0,00613	0,02257	0,00783	0.001±0.001
9. <i>A. albater</i>	0,02527	0,06784	0,02751	0,05717	0,07516	0,08127	0,07349	0,02302		0,02221	0,00678	0.0007±0.0006
10. <i>A. sp. poecilus Alto Araguaia</i>	0,17887	0,17747	0,18303	0,17333	0,17878	0,16960	0,17452	0,18417	0,17983		0,02176	0.0026±0.0018
11. <i>A. fuscogutattus</i>	0,03582	0,06665	0,00937	0,06226	0,07329	0,07357	0,07182	0,03648	0,02719	0,17337		0

The analysis suggests different results to *Aspidoras*. The NJ analysis recognized 11 lineages among the representatives of the Lineage 2 (values higher than 9% between groups, and bootstrap values higher than 70%), whereas ABGD and PTP found 7 lineages (Fig. 2). ABGD proposed 8 partitions: 2 partitions found 15 groups (prior maximal distance $P= 0.001-0.002$), 3 partitions found 11 groups ($P= 0.003-0.008$), 2 partitions found 7 groups ($P= 0.013-0.021$) and 1 partition found 2 groups ($P= 0.0359$). The results of ABGD and PTP (0.08 of support value) suggested there is no difference genetically between *A. albater*, *A. depinnai*, *A. psammatides*, *A. fuscoguttatus* and *Aspidoras* sp *poecilus* from the Tocantins-Araguaia basin (Fig. 2), considering them as the same clade. On the other hand, the distance between groups showed more than 10% of the distance between them.

All the analysis recognized *A. raimundi* as a lineage genetically different from the group formed by *A. albater*, *A. depinnai*, *A. psammatides*, *A. fuscoguttatus* and *A. poecilus* from Rio Vermelho, Tocantins-Araguaia basin (100% of bootstrap, more than 8% of distance genetic from the samples of the same group). *A. raimundi* showed to be genetically different from the group *A. albater*, *A. depinnai*, *A. psammatides*, *A. fuscoguttatus*, and *Aspidoras* sp *poecilus* from Rio Vermelho, Tocantins-Araguaia basin. *A. albater* was originally described from the Tocantins-Araguaia basin, and the samples used in our analyzes (voucher 63362 – 63366, LBP 15330) were collected in the same basin. The samples of *A. albater* presented dorsal and pectoral spine shorter than adjacent rays, snout in front of nostril naked, two short mental barbells, two pair of rectal barbells, the inner edge of pectoral fin spine not serrated, anterior fontanel round, the skin of anterior coracoid area without ossifications. The samples of *A. depinnai* (voucher 92698, LBP 23907) were collected from Ipojuca River, Northern Coastal basin, and presented an ossified portion of pectoral spine posterior border with serrations and presence of irregular arc-like brown blotch on caudal peduncle. The samples of *A. psammatides* (voucher 69103 – 69105, LBP 7188), from Paraguaçu River, East Costeira basin, showed an ossified portion of pectoral spine posterior border with serrations, poorly-developed pigmentation, restricted to minute scattered blotches on the dorsal region of head and body; the others diagnosis characters are osteologic and were not possible to compare. The samples of *A. fuscoguttatus* (voucher 17398, 10916, LBP 2612), from São José dos Dourados River, Upper Paraná basin, the ossified portion of pectoral spine posterior border with serrations, pectoral and dorsal spine shorter than branched rays, snout in front of nostril naked, two short mental barbells, two pair of

rectal barbels not reaching the base of pectoral fin but reaching the gill opening ventrally, the inner edge of pectoral fin spine serrated, anterior fontanel round (*vs.* elongate oval), the skin of anterior coracoid area with minute ossifications. And, the samples of *Aspidoras* sp. *poecilus* (voucher 11824 – 11823, LBP 1658) collected in Rio Vermelho, Upper Araguaia River basin, presented an ossified portion of pectoral spine posterior border with serrations, pectoral and dorsal spine shorter than branched rays, snout in front of nostril naked, two short mental barbels, two pair of rectal barbels reaching the gill opening ventrally, the inner edge of pectoral fin spine serrated, anterior fontanel round, the skin of intercoracoid area naked (*vs.* with minute ossifications).

Furthermore, our results identified four different lineages of *A. poecilus* from the Tocantins-Araguaia basin, which can be divided into two groups. All the analyses agree that one group is composed of *A. sp poecilus* (voucher 12625, 12624; LBP1456) from an affluent of Araguaia River, and *A. aff poecilus* (voucher 12304, 12308, 12338; LBP 1437) from Corrego do Sapo, Upper Tocantins-Araguaia River basin. This group showed more genetic similarity with the group formed by *A. albater*, *A. depinnai*, *A. psammatides*, *A. fuscoguttatus*, *Aspidoras* sp *poecilus*, and *A. raimundi*. The analysis showed 3% of distance between *A. sp poecilus* and *A. aff. poecilus*.

The second group of *A. poecilus* from the Tocantins-Araguaia basin was formed by *A. poecilus* (voucher 13099, 13516, 13517, 13528, 13529; LBP 1825) and *A. taurus* presenting more similarity genetically than *A. sp poecilus* from the Upper Araguaia River basin (voucher 12336, 12337, 12339; LBP 1437). The distance between *A. poecilus* and *A. taurus* was 3%. The analysis of distance between *A. sp poecilus* from Alto Araguaia River basin (voucher 12336, 12337, 12339; LBP 1437) showed 17% of distance from *A. taurus*, and *A. poecilus*. *A. poecilus* was described from the Upper Xingu basin, in Mato Grosso State, Brazil, but the range of distribution also includes the rivers of the Tocantins-Araguaia basin. The samples of *A. sp poecilus* from an affluent of Araguaia River (voucher 12625, 12624; LBP1456) used in this analysis have the anterior fontanel not round but oval (*vs.* round in *A. poecilus* and *A. albater*); eight soft rays on the dorsal fin (*vs.* six in *A. pauciradiatus*); absence of longitudinal dark brown band on dorsolateral scutes (*vs.* presence of dark band on dorsolateral scutes in *A. brunneus*); the process of supraoccipital not reaching the initial of dorsal fin, and without nuchal plate, infraorbital slender on its anterodorsal portion (*vs.* large in *A. belenos*); four pre-adipose scutes (*vs.* six in *A. lakoi*). The samples of *A. poecilus* differ from *A. taurus* by the presence of preopercle and nuchal plates exposed, not covered by

skin, and presence of lateral line on the first plate. The distance between *A. poecilus* and *A. taurus* was 3%. The analysis of distance between *A. sp poecilus* from Alto Araguaia River basin (voucher 12336, 12337, 12339; LBP 1437) showed 17% of distance from *A. taurus*, and *A. poecilus*. This species presents dorsal fin with transversal dark brown bars; infraorbital 1 with moderate expansion; conspicuous dark brown blotches on pelvic fin; the presence of dark brown stripes along of body, but not conspicuous. The ABGD and TPT analysis couldn't recognize genetic differences between the species that the morphology was. Nonetheless, our results indicate there is an underestimated knowledge of this genus and needs to be better investigated.

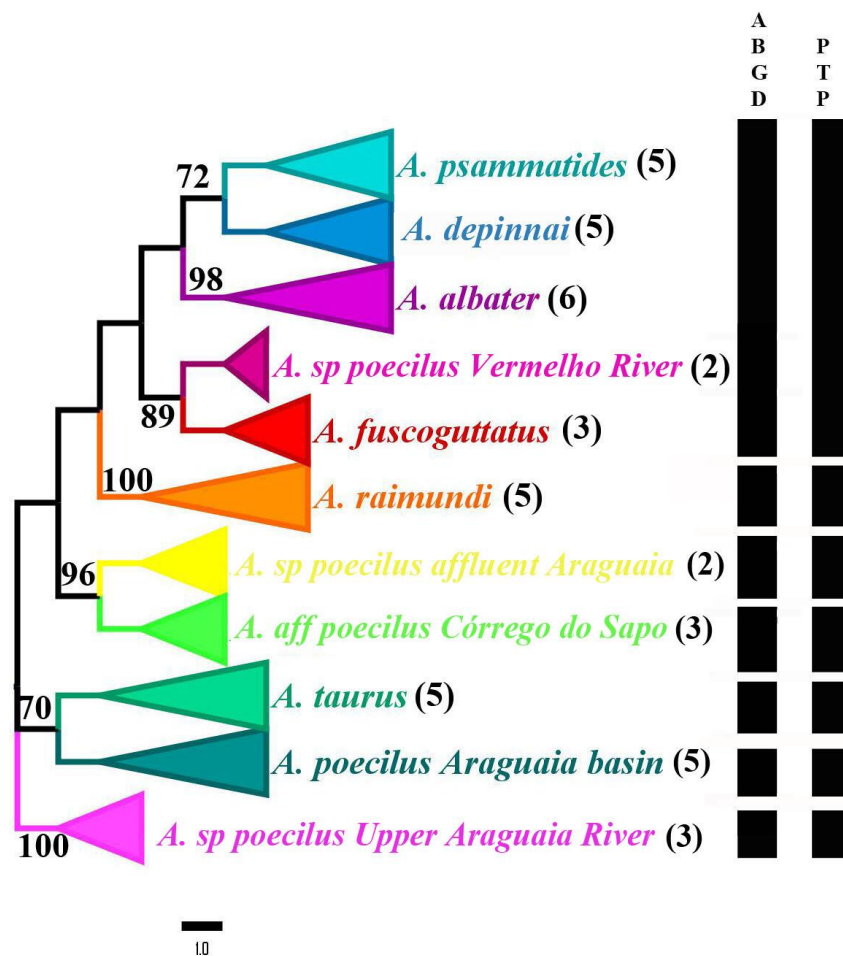


Fig. 2. NJ tree of species of Lineage 2 based on the COI gene (617 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 3

Lineage 3 corresponds to the genus *Scleromystax*. There are five described species of this genus, and we included in the present analysis *S. barbatus*, *S. macropterus* and *S. prionotus*, and two undescribed species: *Scleromystax* C112 and *Scleromystax* C113. We also included a synonymy of *S. barbatus*, the samples of *S. kronei*, and samples of *C. lacerdai*, which is closely related to this lineage (Chapter 3) and must be reallocated to this genus. The matrix was created with 64 sequences of gene COI and had 596 pb with 289 pb of variable sites. The nucleotide composition of the matrix was 26% of adenine, 27% of cytosine, 17% of guanine, and 29% of thymine. The best evolution model for the matrix was T92+G+I with BIC=5592.8434 and AICc=4455.6113. The overall mean distance for lineage 3 was 0.08±0.01. The values of interspecific distances for this lineage ranged from 0.0120±0.0039 between two populations of *S. barbatus*, to 0.1917±0.0232 between *C. lacerdai* and *Scleromystax* C113, and the intraspecific distances ranged from 0.001±0.001 to 0.072±0.013 (Table 3).

Table 3. Pairwise TN-93 genetic distances among species of Lineage 3. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	6	7	IGV
<i>S. C112</i>		0,0197	0,0196	0,0191	0,0207	0,0199	0,0196	0.072±0.013
<i>S. macropterus</i>	0,1735		0,0132	0,0208	0,0180	0,0136	0,0142	0.001±0.001
<i>S. barbatus</i> + <i>S. kronei</i>	0,1745	0,0918		0,0217	0,0162	0,0039	0,0131	0.004±0.002
<i>S. C113</i>	0,1537	0,1647	0,1816		0,0232	0,0223	0,0221	-
<i>C. lacerdai</i>	0,1822	0,1459	0,1264	0,1917		0,0160	0,0165	0.001±0.001
<i>S. barbatus</i>	0,1763	0,0944	0,0120	0,1844	0,1258		0,0129	0.003±0.001
<i>S. prionotus</i>	0,1797	0,1034	0,0853	0,1862	0,1239	0,0849		0.002±0.001

The NJ analyses recognized six distinct genetic groups between species of *Scleromystax*, whereas ABGD and PTP recognized seven lineages (Fig. 3). The ABGD proposed nine partitions: two partitions found 24 groups (prior maximal distance P= 0.001-0.002), and seven partitions found seven groups (P= 0.003-0.060). All the analysis agrees that there are at least three distinct species of *Scleromystax*: *S. barbatus*, *S. macropterus* and *S. prionotus*, but the analyses disagree about *C. lacerdai*. ABGD recognized *C. lacerdai* and *Scleromystax* C113 as the same genetic lineage, but PTP suggested 0.33 of support value for *C. lacerdai* and 1.00 for *Scleromystax* C113. Besides that, the analysis of distance showed 19% of distance between these two

species, and RAxML showed 100% of the lineage *C. lacerdai* considering a different lineage of *Scleromystax* C113 (99%). All the analyses agree about these specimens of *S. kronei* are most genetically like *S. barbatus*, forming a single genetic lineage. NJ also showed that the *S.* C112 and *S.* C113 are distinct groups, most genetically related to each other than to the others *Scleromystax*. Besides that, ABGD recognized three genetic lineages between *Scleromystax* C112 samples.

Moreover, PTP also suggests there are two genetically distinct lineages between the *Scleromystax* C112 of coastal basin from São Paulo State (1.00), and there are no available names for this basin, specifically, that may suggest that could be a new species of *Scleromystax*. The type locality and distribution of *S. barbatus* is the coastal basin of Rio de Janeiro and the Santa Catarina States. *S. macropterus* was described from the Paranaguá River, a Coastal basin from Paraná State, Brazil, and *S. prionotus* from the Espírito Santo coastal basin. Unfortunately, we couldn't add in our analysis samples of *S. reisi* and *S. salmacis*, described from Laguna dos Patos (Rio Grande do Sul) and coastal basin from Santa Catarina, respectively.

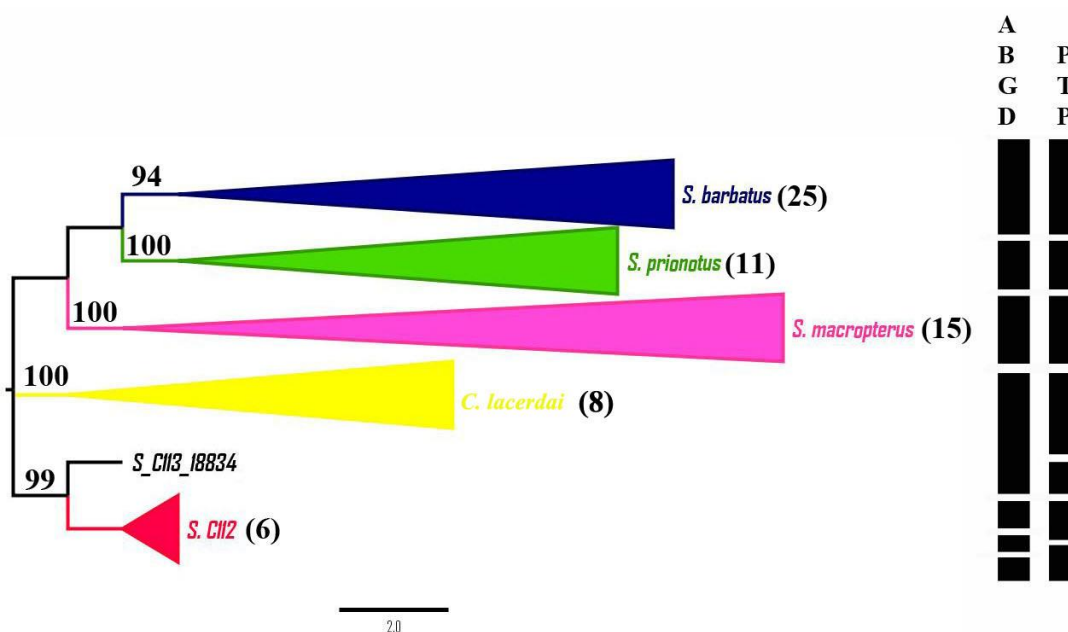


Fig. 3. NJ tree of species of Lineage 3 based on the COI gene (593 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 4

In our analyses, we included 18 sequences of COI of three species of this lineage, *C. guapore*, *C. pygmaeus* and *C. hastatus*. The matrix had 596 pb with 211 pb of variable sites. The nucleotide composition of the matrix was 24% of adenine, 27% of cytosine, 16% of guanine, and 30% of thymine. The best evolution model was TN93+G, with BIC=3309.5695 and AICc=3026.4226. The overall mean distance was 0.11 ± 0.01 . The values of interspecific distances ranged from 0.1327 ± 0.0164 between *C. pygmaeus* and *C. hastatus* to 0.1845 ± 0.0204 between *C. guapore* and *C. hastatus*, and intraspecific distances ranged from 0.0023 ± 0.0015 to 0.0057 ± 0.0019 (Table 4).

Table 4. Pairwise TN-93 genetic distances among species of Lineage 4. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	IGV
<i>C. hastatus</i>		0.016	0.020	0.0057±0.0019
<i>C. pygmaeus</i>	0.133		0.020	0.0023±0.0015
<i>C. guapore</i>	0.185	0.180		0.0033±0.0016

Our analyses demonstrated that they are genetically divergent lineages (more than 70% of bootstrap) and must be considered as three distinct species. Each species was considered as a single lineage by NJ, PTP, and ABGD analyses (Fig. 4). The ABGD proposed ten partitions: two partitions found eight groups (prior maximal distance $P= 0.001-0.002$), two partitions found seven groups ($P= 0.003-0.005$), one partition found four groups ($P= 0.008$), five partitions found three groups ($P= 0.013-0.1$). PTP showed 0.85 of support value for *C. guapore*, 0.77 for *C. pygmaeus* and 0.92 for *C. hastatus*.

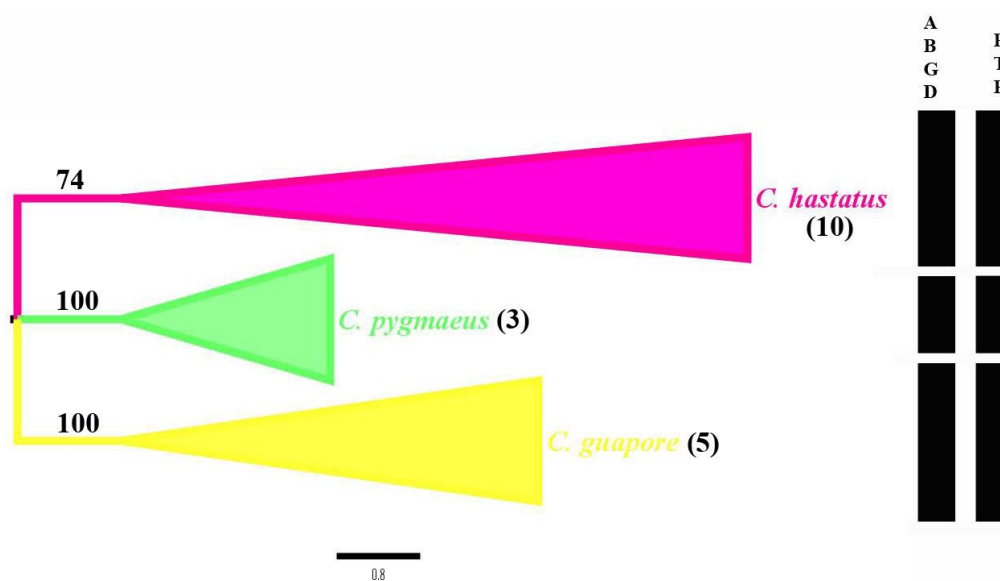


Fig. 4. NJ tree of species of Lineage 4 based on the COI gene (596 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 5

We included in the analysis of this lineage partial sequences of the gene COI from 19 specimens: *C. aff. elegans*, *C. elegans*, *C. nijsseni*, and *C. undulatus*, but also *A. pauciradiatus* from Negro River that is closely related to this lineage (Chapter 3) and must be reallocated to this genus. The matrix had 617 pb with 189 pb of variable sites. The nucleotide composition of the matrix was 27% of adenine, 26% of cytosine, 16% of guanine, and 30.5% of thymine. The best evolution model for the matrix was T92+G with BIC=3089.6119 and AICc=2878.0539. The overall mean distance was 0.05 ± 0.01 . The values of interspecific distances among the lineage 5 ranged from 0.0248 ± 0.0064 between *C. elegans* from Purus River and *C. nijsseni*, to 0.1039 ± 0.0152 between *C. undulates* and *A. pauciradiatus*, and the intraspecific distances ranged from 0.0068 ± 0.0024 to 0.0245 ± 0.0072 (Table 5).

Table 5. Pairwise TN-93 genetic distances among species of Lineage 5. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	IGV
<i>A. pauciradiatus</i>		0.0152	0.0150	0.0145	0.0132	-
<i>C. undulatus</i>	0.1039		0.0117	0.0107	0.0119	0.0245±0.0072
<i>C. elegans</i> Peru	0.0976	0.0783		0.0085	0.0090	0.0068±0.0024
<i>C. nijsseni</i>	0.0950	0.0668	0.0422		0.0064	0
<i>C. elegans</i> Purus River	0.0826	0.0768	0.0441	0.0248		0.0092±0.0035

Our analyses demonstrated agreement in almost all the groups of this lineage. The ABGD proposed six partitions: five partitions found six groups (prior maximal distance $P=0.001-0.008$), one partition found four groups ($P=0.013$). All the analyses corroborated that *A. pauciradiatus* represents a single genetic lineage. The same happened with *C. undulatus*, which showed 0.27 of support value at PTP analysis. By the way, PTP considered *C. undulatus* from Paraguay as a different lineage from *C. undulatus* from Guaíba River, Coastal basin, Rio Grande do Sul State.

The NJ analysis showed five distinct genetic lineages, and *A. pauciradiatus* was considered genetically most different from *C. undulatus*, *C. elegans*, *C. nijsseni*, and *Corydoras* aff *elegans* (Fig. 5). On the other hand, besides ABGD and PTP had recognized *C. nijsseni* and *C. aff. elegans* from Purus River (voucher 7226-7228, LBP 556) as a single lineage (0.12 of support value), the analysis of distance showed only 2% of distance between them. All the analyses also agree that *C. elegans* from Amazonian basin, Peru, as a single lineage, genetically most similar to *C. nijsseni* and *Corydoras* aff *elegans*. *C. elegans* has a history of confusing identification with *C. undulatus* and *C. nijsseni*, as they are very similar morphologically. The analysis of distance revealed 4% of distance between *C. elegans* from Peru and *C. nijsseni* and between *C. aff elegans* from the Purus, representing distinct species.

The species *C. elegans* was described from Rio Amazonas, Tefé City, and its distribution is the Upper Amazon River basin, whereas *C. nijsseni* is restricted to Negro River, but both species live in sympatry on this river. The *C. aff elegans* was collected from Purus River, the last major tributary on the right side of the Solimões River, before meeting with the Negro River. If the samples collected in the Purus represent *C. nijsseni*, that may suggest that the distribution of *C. nijsseni* must be reviewed.

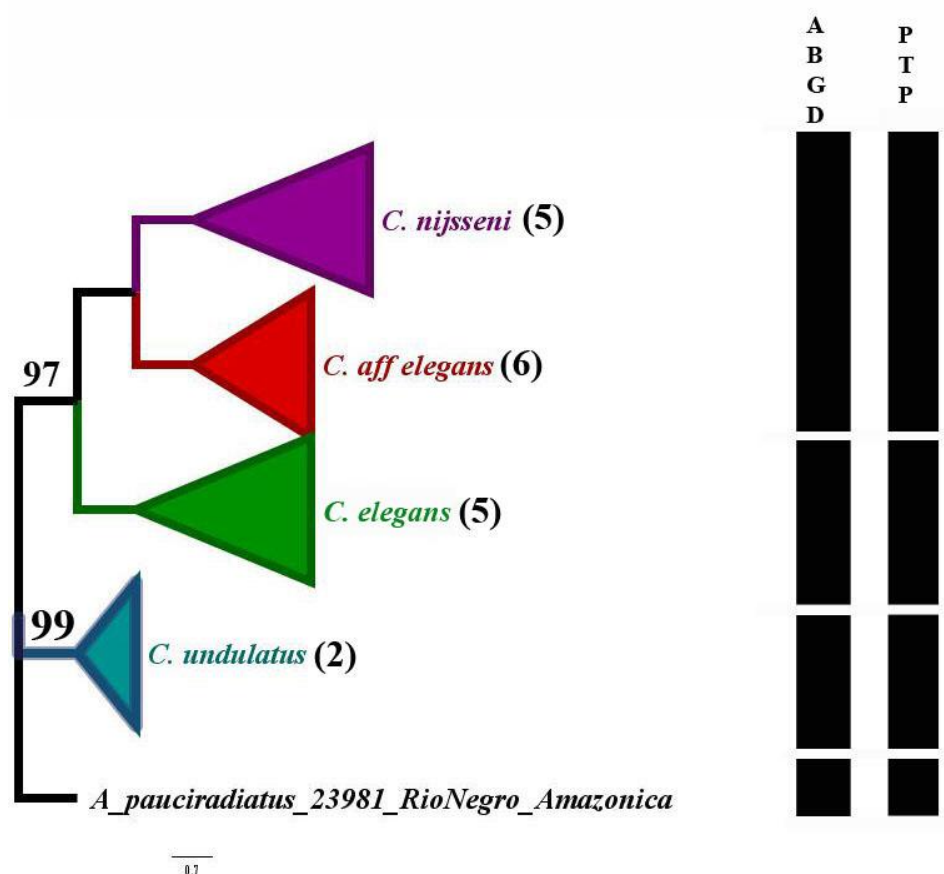


Fig. 5. NJ tree of species of Lineage 5 based on the COI gene (617 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 6

We included in our analyses 79 specimens of *C. albolineatus*, *C. ehrhardti*, *C. flaveolus*, *C. lymnades*, *C. nattereri*, *C. paleatus*, *C. potaroensis*, and the not described species *C. C68*, *C. C73*, *C. C144*, and *Corydoras* sp from Peru. The matrix had 594 pb with 256 pb of variable sites. The nucleotide composition of the matrix was 26% of adenine, 28% of cytosine, 17% of guanine, and 28% of thymine. The best evolution model found was TN93+G, with BIC=15149.9738 and AICc=11919.5960. The overall mean distance was 0.08 ± 0.01 . The values of interspecific distances ranged from 0.0053 ± 0.0031 between *C. potaroensis* and *Corydoras* C73 to 0.1583 ± 0.0192 between *C. potaroensis* and *C. flaveolus*, and intraspecific distances ranged from 0.0014 ± 0.0010 to 0.0205 ± 0.0066 (Table 6).

Table 6. Pairwise TN-93 genetic distances among species of Lineage 6. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	IGV
<i>C. lymnades</i>		0.01893	0.01823	0.01907	0.01834	0.01453	0.01995	0.01980	0.01958	0.01852	0.01873	0.01998	0.01806	0
<i>C. C68</i>	0.14259		0.01709	0.01647	0.01622	0.01950	0.01638	0.01886	0.01706	0.01937	0.01723	0.01620	0.01733	0.0014±0.0010
<i>C. ehrhardti</i>	0.12999	0.12258		0.00819	0.01004	0.01549	0.01432	0.01432	0.01500	0.01423	0.01564	0.01529	0.00394	0
<i>C. nattereri</i> Paraiba do Sul River	0.14687	0.12639	0.03926		0.00954	0.01585	0.01421	0.01459	0.01494	0.01465	0.01698	0.01420	0.00841	0.0050±0.0020
<i>C. paleatus</i>	0.13854	0.11956	0.05184	0.05408		0.01619	0.01356	0.01583	0.01406	0.01409	0.01648	0.01337	0.00978	0.0016±0.0010
<i>C. flaveolus</i>	0.09758	0.15419	0.11203	0.12288	0.12562		0.01891	0.01806	0.01920	0.01750	0.01808	0.01791	0.01562	0.0023±0.0016
<i>C. C73</i>	0.15830	0.12742	0.09577	0.09901	0.09087	0.15794		0.01507	0.00307	0.01027	0.01748	0.00986	0.01469	0
<i>C. C144</i>	0.14456	0.14102	0.08837	0.10386	0.10684	0.13919	0.09257		0.01526	0.01564	0.01789	0.01636	0.01473	0
<i>C. potaroensis</i>	0.15597	0.13094	0.10174	0.10627	0.09321	0.15835	0.00532	0.09460		0.00998	0.01723	0.00999	0.01548	0
<i>C. albolineatus</i> Paraguai basin	0.14004	0.15183	0.10190	0.10796	0.09774	0.13956	0.05698	0.10650	0.05206		0.01684	0.01196	0.01454	0.0205±0.0066
<i>C. sp</i> Peru	0.14070	0.12646	0.11041	0.13111	0.12189	0.13412	0.13265	0.13201	0.12915	0.11927		0.01666	0.01577	0.0019±0.0021
<i>C. albolineatus</i>	0.15234	0.12347	0.10458	0.10069	0.08644	0.14756	0.04936	0.10782	0.04769	0.06975	0.12378		0.01521	0
<i>C. nattereri</i> Ribeira de Iguape River	0.13035	0.12789	0.01053	0.04225	0.05084	0.11457	0.10008	0.09541	0.10738	0.10874	0.11459	0.10820		0.0037±0.0017

The ABGD and PTP analyses proposed 11 genetic lineages for this group, while NJ resulted in 12 lineages. The ABGD proposed eight partitions: two partitions found 20 groups (prior maximal distance $P= 0.001-0.002$), one partition found 17 groups ($P= 0.003$), two partitions found 12 groups ($P= 0.005-0.008$) and three partitions found 11 groups ($P= 0.013-0.036$).

The results of ABGD and PTP (0.08 of support value) recognized *C. ehrhardti* and *C. nattereri* from Ribeira de Iguape River, Coastal basin, as the same genetic lineage, but different from the second population of *C. nattereri* from Upper Paraná River (0.06 of support value, 96% of bootstrap). *C. ehrhardti* is described from Southern Coastal basin (Joinville, Santa Catarina State), and *C. nattereri* is described from Paraíba do Sul River, a Southeast coastal basin (Rio de Janeiro State). Therefore, the population of Ribeira de Iguape and the population of Upper Paraná River may not be the same species. The analysis of distance between *C. ehrhardti* and *C. nattereri* from Ribeira de Iguape River showed 1% of distance and 4% between *C. nattereri* from Ribeira de Iguape River and *C. nattereri* from Upper Paraná River. In contradiction that is presented by the genotype, the morphology and pattern of coloration of the body of *C. nattereri* from Ribeira de Iguape River is the same of the population of *C. nattereri* from Upper Paraná River, and completely different to *C. ehrhardti*.

The analyses demonstrated that *C. potaroensis* and *Corydoras* C73 represent the same lineage (0.84 of PTP value, 0.5% of distance between them), and this group is most similar genetically to a group formed by *C. albolineatus* (99% of bootstrap). *C. albolineatus* was considered by all the analyses as representing two populations genetically divergent (7% of distance), one lineage forming a group most similar to *C. potaroensis* and *Corydoras* C73, and a second group formed by samples from the Paraguai basin and Amazonian basin. The analysis of distance showed 5% of distance between *C. albolineatus* (voucher 18821, 18822; LBP 2780) from the group formed by *C. potaroensis* and *Corydoras* C73. *C. albolineatus* is described from Paraguay River, Itinez River (Guaporé River, common name in Brazil), Amazonian basin, Bolivia, and its distribution is restricted to this system. On the other hand, *C. potaroensis* is described from Essequibo, Guyana. This species could represent a complex of species and should be better investigated. *Corydoras* C144 is also genetically similar to this group, but not on the pattern of pigmentation. The analysis of distance showed at least 9% of distance between all species. Besides that, all the analysis agrees that *Corydoras* C144 could represent a new species.

Corydoras C68 from the Tocantins-Araguaia basin and *Corydoras* sp from Tapajós basin were considered distinct genetic lineages in all the analyzes (13% of distance). *C. lyrnades* and *C. flaveolus* were considered two different lineages (10% of distance), but most similar to each other than to other species from lineage 6.

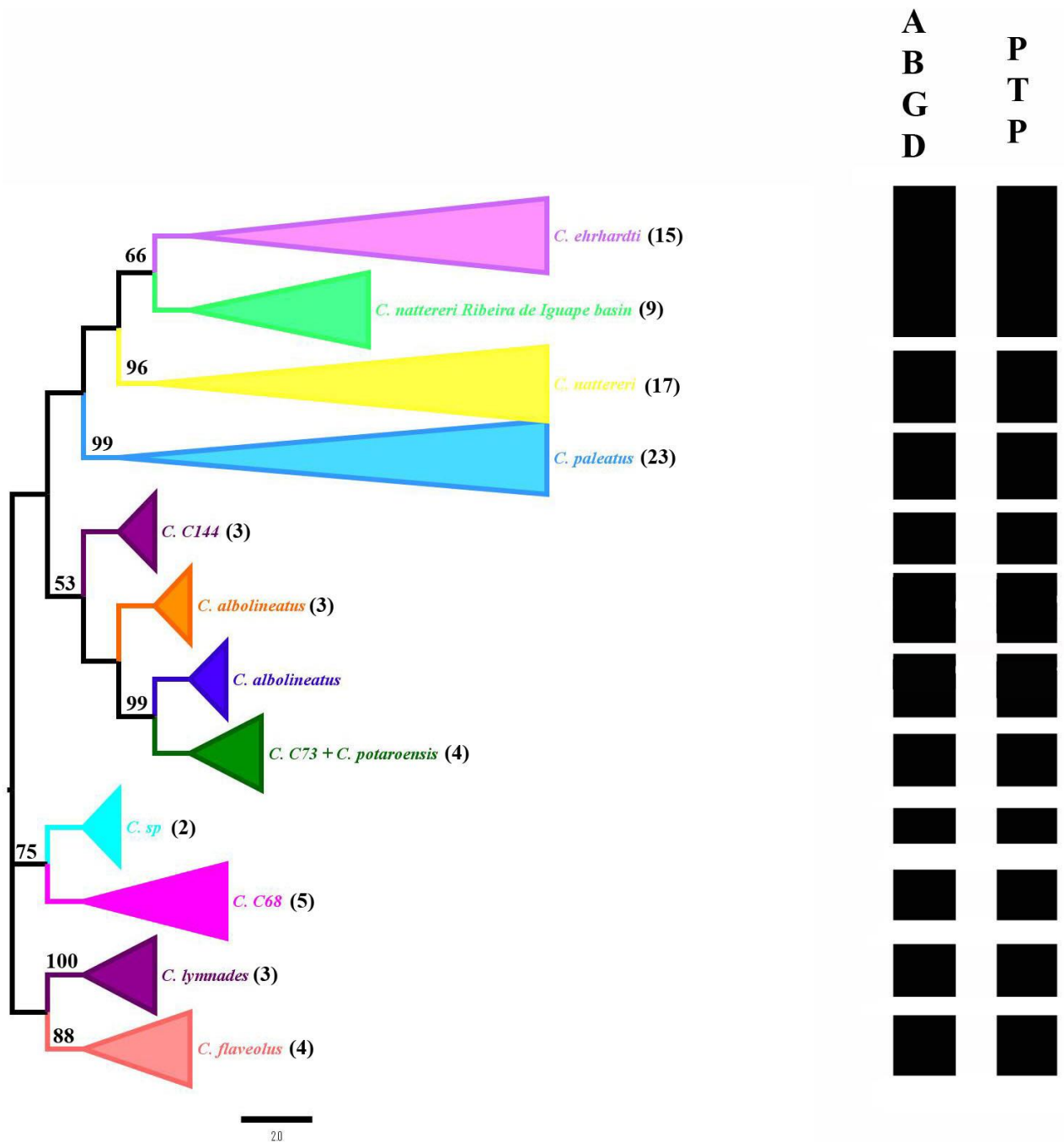


Fig. 6. NJ tree of species of Lineage 6 based on the COI gene (594 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes

represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 7

We included a high number of *C. aeneus* in the analysis of this lineage in an attempt to demonstrate the genetic complexity of this species. We also include *C. rabauti*, in total were used 88 sequences of the gene COI. The matrix presented 596 pb, and 297 pb of variable sites. The nucleotide composition was 27% of adenine, 26% of cytosine, 17% of guanine, and 30% of thymine. The best evolution model for the matrix was T92+G, with BIC=7728.3013 and AICc=6072.9094. The overall mean distance was 0.04 ± 0.00 . The lowest value of interspecific distances among this lineage ranged from 0.0154 ± 0.0040 in *C. aeneus* from Peru and *C. aeneus* “Aquario” to 0.1204 ± 0.0159 in *C. aeneus* from Acre River 2 and *C. aeneus* from *C. rabauti*, and intraspecific distances ranged from 0.0009 ± 0.0018 to 0.0117 ± 0.0030 (Table 7).

Table 7. Pairwise TN-93 genetic distances among species of Lineage 7. Intraspecific genetic variations (IGV) are highlighted in bold in the last column. Numbers below diagonal are values of interspecific distances and numbers above diagonal are respective values of standard deviation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	IGV
<i>C. aeneus</i> Amazonas River complex 1		0.0068	0.0064	0.0072	0.0110	0.0102	0.0113	0.0115	0.0117	0.0131	0.0123	0.0046	0.0153	0
<i>C. aeneus</i> Iquitos Peru	0.0306		0.0063	0.0067	0.0109	0.0102	0.0111	0.0116	0.0121	0.0124	0.0121	0.0077	0.0152	-
<i>C. aeneus</i> Aquario 2	0.0272	0.0206		0.0040	0.0101	0.0094	0.0100	0.0103	0.0119	0.0124	0.0114	0.0070	0.0153	0
<i>C. aeneus</i> Peru	0.0386	0.0292	0.0154		0.0105	0.0098	0.0106	0.0107	0.0126	0.0130	0.0121	0.0082	0.0155	0.0117±0.0030
<i>C. aeneus</i> Alto Paraná River 1	0.0737	0.0668	0.0594	0.0676		0.0047	0.0075	0.0080	0.0122	0.0134	0.0112	0.0114	0.0148	0.00091±0.0018
<i>C. aeneus</i> Alto Paraná River 2	0.0651	0.0605	0.0526	0.0619	0.0198		0.0062	0.0069	0.0116	0.0135	0.0112	0.0106	0.0149	0.0061±0.0014
<i>C. aeneus</i> Paraguai basin	0.0734	0.0664	0.0552	0.0663	0.0369	0.0267		0.0050	0.0120	0.0137	0.0112	0.0119	0.0141	0.0034±0.0013
<i>C. aeneus</i> Acre River Amazonas	0.0759	0.0723	0.0596	0.0674	0.0418	0.0327	0.0173		0.0119	0.0142	0.0115	0.0119	0.0140	0.0087±0.0040
<i>C. rabauti</i>	0.0766	0.0753	0.0715	0.0865	0.0791	0.0733	0.0760	0.0769		0.0137	0.0122	0.0114	0.0159	0.0034±0.0018
<i>C. aeneus</i> Tinidad Tobago	0.0897	0.0796	0.0777	0.0901	0.0924	0.0919	0.0895	0.0967	0.0873		0.0108	0.0148	0.0162	0
<i>C. aeneus</i> Aquario 1	0.0802	0.0742	0.0664	0.0758	0.0698	0.0694	0.0653	0.0699	0.0759	0.0588		0.0129	0.0144	0
<i>C. aff aeneus</i> Xingu River	0.0195	0.0343	0.0293	0.0436	0.0702	0.0616	0.0707	0.0730	0.0660	0.0965	0.0796		0.0161	0.0064±0.0036
<i>C. aeneus</i> Amazonas complex 2	0.1190	0.1110	0.1117	0.1180	0.1104	0.1099	0.0981	0.1020	0.1204	0.1171	0.0944	0.1143		-

The analyzes revealed a high diversity and distinct lineages related to the name *C. aeneus*, and delimited different numbers of genetic lineages of this species (Fig. 7). NJ was the most divergent analysis, showing 13 distinct lineages from this lineage, whereas PTP found eight for *C. aeneus* and ABGD found six. The ABGD proposed seven partitions for this lineage: two partitions found 44 groups (prior maximal distance $P= 0.001-0.002$), one partition found 17 groups ($P= 0.003$), three partitions found six groups ($P= 0.005-0.013$), and one partition found one group ($P= 0.021$).

C. aeneus represents different genetic lineages to all the analyses. The results revealed that the group of *C. aeneus* from Trinidad and Tobago, the type locality of this species, form a genetically distinct lineage from the other *C. aeneus* included in the analyses. NJ and ABGD suggested *C. aeneus* from Trinidad as being a single lineage (100% of bootstrap), while PTP found two different lineages in these samples (0.48 value of support). With this result, we must conclude that all other distinct lineages of *C. aeneus* from Trinidad are therefore other species.

All analyzes agree about the *C. rabauti* to be a different lineage from all *C. aeneus*, creating a polytomy with *C. aeneus* from Acre River, *C. aeneus* from Trinidad Tobago, and *C. aeneus*, though. All the analyses agree about *C. cf aeneus* 49260 from Acre River as genetically more similar to the lineage of the type locality, with 72% of bootstrap to this group, and 9% of distance between *C. aeneus* from Trinidad Tobago.

Moreover, ABGD and PTP delimited two lineages completely distant genetically to *C. aeneus* from Trinidad e Tobago: one lineage formed by samples from the La Plata system and the other formed by samples from the Amazonian basin. The analysis of distance between groups showed that *C. aeneus* from Trinidad e Tobago more than 9% of distance between *C. aeneus* from La Plata system and more than 7% of distance between *C. aeneus* from the Amazonian basin. Besides that, NJ delimited *C. aeneus* from the Paraguai basin as a different lineage to *C. aeneus* from the Upper Paraná basin (76% of bootstrap value in RAxML, Supplementary Fig 21), and this group as most similar to *C. aeneus* from the Amazonian basin (83% of bootstrap). The group formed by the Upper Paraná basin species, NJ was capable to separate the samples between Paranapanema and Tietê River basins (66% of bootstrap).

Nonetheless, *C. aeneus* is described from the Island of Trinidad, and its distribution is broad from the Colombia Rivers to the La Plata system and almost all the basin of east of Andes. There are many species under synonymy of this species: *C. microps*, described from Branco River, Paraguay basin, Brazil; *C. venezuelanus*,

described from Cabriales, Venezuela; *C. macrosteus*, described from Piracicaba River, Upper Paraná River basin, Brazil; and *C. schultzei*, described from Amazon basin. The samples of *C. aeneus* from the Paraguay basin used in this analysis belong to a different lineage than *C. aeneus* from Trinidad e Tobago (voucher 62065, LBP 6844), and could represent the species of *C. microps*. The same happened with the samples of *C. aeneus* from the Upper Paraná basin and Amazonian basin, there are available names for that morphotypes, and they could represent the species of *C. macrosteus* and *C. schultzei*, respectively.

The only difference that we found in the samples from Tiete River is the absence of contact between the nuchal plate and the first dorsal plate (*vs.* contact in *C. aeneus* from Trinidad e Tobago). By the way, the samples of voucher 18818 and 18819 were identified initially as *C. melanotaenia*, and is genetically most related to *C. aeneus* from Trinidad e Tobago than to the other group of *C. aeneus*. In our morphologic analyses, the samples presented absent of contact between the nuchal plate and the first dorsal plate, and the pattern of pigmentation of the body was suggested to be *C. melanotaenia*. The samples also presented snout shorter than *C. aeneus* from Trinidad e Tobago.

Nijsseni & Isbruchër (1980) analysed the syntypes of *C. microps* and reported that they are not conspecific. We couldn't analyze the samples because the vouchers were not found.

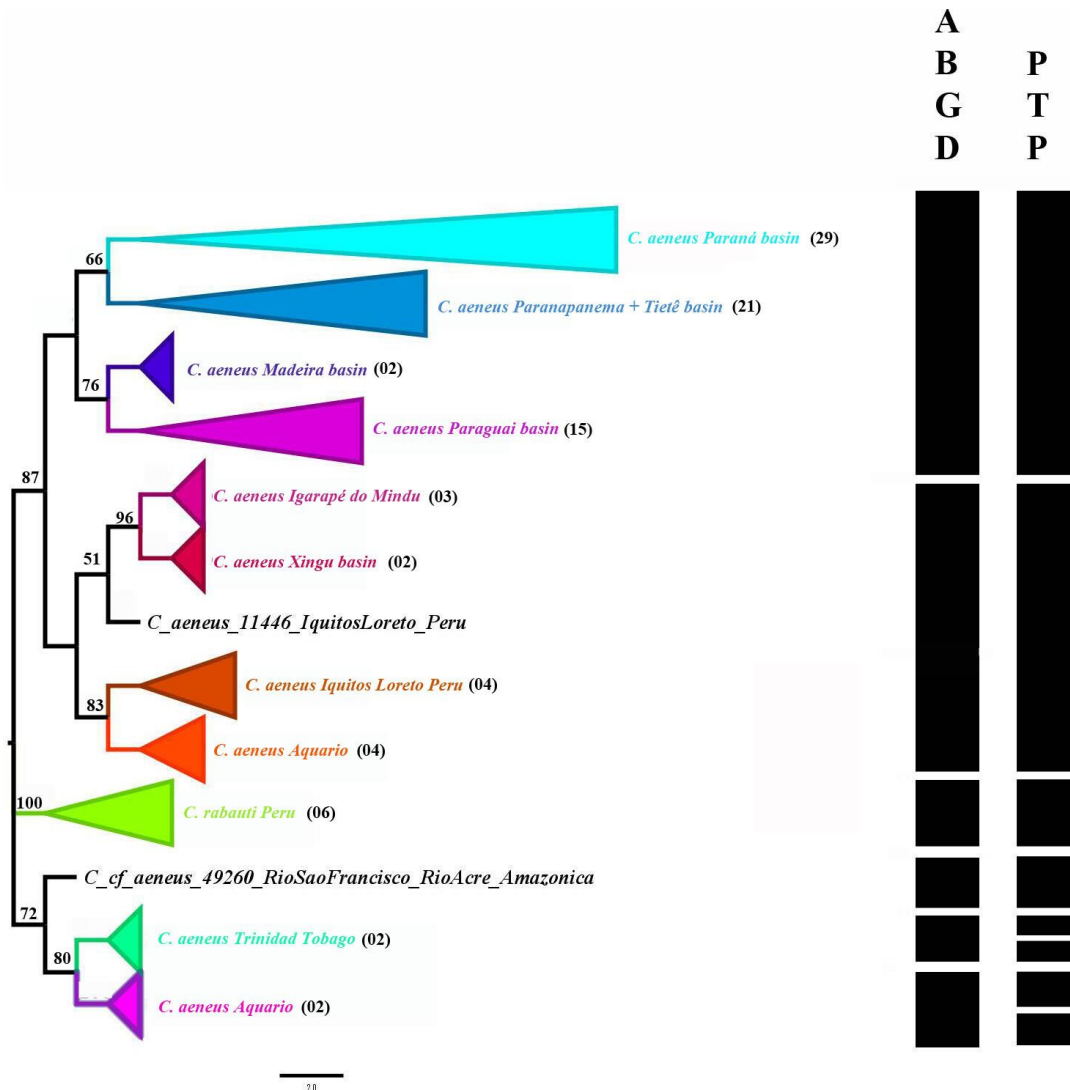


Fig. 7. NJ tree of species of Lineage 7 based on the COI gene (596 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 8

Here we included in our analyzes 158 sequences of gene COI belonging to *C. acutus*, *C. agassizii*, *C. amandajanea*, *C. ambiacus*, *C. cf ambiacus*, *C. britskii*, *C. condiscipulus*, *C. delphax*, *C. difluviatilis*, *C. heraldschultzi*, *C. imitator*, *C. latus*, *C. melanistius*, *C. aff melanistius*, *C. cf melanistius*, *C. melini*, *C. multiradiatus*, *C. orthonoptherus*, *C. pulcher*, *C. cf pulcher*, *C. punctatus*, *C. reticulatus*, *C. robinae*, *C. cf robustus*, *C. seussi*, *C. sodalis*, *C. splendens*, *C. virginiae*, and two no identified species, *C. C52*, *C. sp.* The matrix had 594 pb and 375 pb of variable sites. The nucleotide composition of the matrix was 26% of adenine, 28% of cytosine, 17% of guanine, and

29% of thymine. The best evolution model for the matrix was TN93+G, with BIC=11084.6582 and AICc=8066.1701. The overall mean distance was 0.09 ± 0.01 . The values of interspecific distances among the groups of this lineage ranged from 0.0006 ± 0.0005 in *C. melanistius* and *C. acutus* to 0.2305 ± 0.02553 in *Corydoras* C52 and *C. virginiae* from Peru (voucher 57770-57773), and intraspecific distances ranged from 0.0004 ± 0.0004 to 0.0402 ± 0.0054 (Table 8).

<i>C_condiscipulus</i>	0,20 0	0,20 9	0,10 1	0,03 1	0,02 7	0,03 6	0,03 7	0,03 7	0,04 0	0,03 4	0,04 2	0,04 2	0,04 8	0,04 8	0,06 3	0,11 1	0,11 4	0,10 8	0,03 0	0,03 4	0,04 1	0,12 8	0,14 5	0,08 9	0,13 9	0,01 3	0,01 0	0,01 2	0,01 8	0,01 0	0,01 9	-	
<i>C_amandajanae</i>	0,19 2	0,20 8	0,09 7	0,04 1	0,04 8	0,04 7	0,05 1	0,05 0	0,05 0	0,03 8	0,04 5	0,05 2	0,04 6	0,05 1	0,00 5	0,10 7	0,11 7	0,12 1	0,04 7	0,05 1	0,03 4	0,12 8	0,12 6	0,09 5	0,13 4	0,06 3	0,00 9	0,00 8	0,01 6	0,01 0	0,01 8	-	
<i>C_latus</i>	0,19 2	0,21 4	0,09 6	0,02 8	0,03 2	0,03 1	0,03 7	0,03 6	0,04 0	0,03 3	0,02 2	0,03 8	0,02 7	0,03 0	0,04 3	0,09 7	0,10 9	0,10 0	0,03 4	0,04 0	0,03 3	0,11 1	0,11 0	0,09 2	0,13 4	0,04 2	0,04 5	0,00 8	0,01 4	0,00 8	0,01 7	0,0013±0,007 6	
<i>C_melini</i>	0,20 1	0,22 5	0,10 1	0,04 0	0,03 6	0,04 7	0,04 3	0,04 1	0,03 9	0,03 4	0,03 8	0,05 2	0,05 0	0,05 6	0,04 0	0,11 6	0,10 8	0,10 8	0,04 3	0,04 3	0,02 6	0,11 4	0,12 9	0,10 1	0,14 2	0,05 5	0,03 9	0,03 9	0,01 5	0,01 0	0,01 7	0 0	
<i>C_virginiae_Peru_57749_57751</i>	0,19 3	0,20 1	0,10 3	0,09 6	0,09 7	0,10 6	0,10 4	0,10 3	0,09 9	0,09 5	0,10 5	0,10 5	0,09 9	0,11 0	0,11 4	0,10 9	0,10 0	0,04 4	0,10 4	0,10 2	0,11 2	0,04 0	0,13 3	0,10 6	0,13 2	0,11 9	0,11 7	0,09 5	0,10 2	0,01 6	0,01 2	0,0022±0,001 6	
<i>C_punctatus</i>	0,19 7	0,21 4	0,09 0	0,02 4	0,02 3	0,03 0	0,03 3	0,03 2	0,03 2	0,02 5	0,03 3	0,03 5	0,04 6	0,04 4	0,05 7	0,10 3	0,11 1	0,11 0	0,02 7	0,02 9	0,04 2	0,11 5	0,12 9	0,10 1	0,13 8	0,04 3	0,05 5	0,03 7	0,04 6	0,10 8	0,01 7	0 0	
<i>C_CS2_18815</i>	0,22 1	0,23 1	0,11 4	0,11 0	0,11 1	0,11 3	0,11 4	0,11 4	0,10 9	0,10 6	0,11 5	0,10 7	0,12 0	0,12 5	0,12 8	0,11 9	0,09 9	0,06 2	0,11 3	0,10 6	0,11 9	0,05 9	0,14 9	0,10 2	0,12 8	0,12 1	0,12 0	0,12 6	0,11 7	0,11 6	0,06 3	0,12 3	-

The analyzes delimited different groups for species of Lineage 8 (Fig. 8). NJ recognizes 31 lineages, PTP recognized 21, while ABGD proposed only nine partitions: two partitions found 29 groups (prior maximal distance $P= 0.001-0.002$), one partition found 20 groups ($P= 0.003$), three partitions found 15 groups ($P= 0.005-0.012$), one partition found 14 groups ($P=0.021$), and two partitions found three groups ($P= 0.036-0.60$).

None of the analyzes were able to recognize the genetic difference between *C. melanistius* (voucher 43088-43089, LBP 9149; 7264-7266, LBP 564; 18872-18874, LBP 2799) and *C. acutus* (43819-43823, LBP 9316), suggesting that there is no genetic difference between them, and therefore they are the same species. Nonetheless, *C. melanistius* is described from Essequibo, Guiana, by Regan (1912), while *C. acutus* was described from Ambiyacus River, in Peruvian Amazon, in 1872 by Cope. The pigmentation pattern is different in both species, suggesting they are different species. Our samples were collected in Guamá River, Upper Negro River, and Guamá River, Amazonian basin, respectively, but we couldn't analyze the samples. On the other hand, there is much confusion between *C. melanistius* and *C. brevirostris*, but these species do not belong to the same lineage. *C. melanistius* belong to lineage 8, while *C. brevirostris* belong to lineage 9, and they were not analyzed together.

ABGD and PTP recognized as a single lineage 19 species delimited by NJ: *C. acutus*, *C. agassizii*, *C. amandajanea*, *C. cf ambiacus*, *C. condiscipulus*, *C. heraldschultzi*, *C. imitator*, *C. latus*, *C. melanistius*, *C. cf melanistius*, *C. melini*, *C. cf pulcher*, *C. orthonoptherus*, *C. cf robustus*, *C. punctatus*, *C. reticulatus*, *C. robinae*, *C. seussi*, *C. sodalis*, *C. virginiae*. All the analyzes recognized at least two different lineages to *C. virginiae* from the Amazon basin, Peru (voucher 57749, 57751, voucher 14815; and 57773, 57770, LBP 14820), with 20% of distance between them. NJ even recognized the third lineage for this species, *C. virginiae* (voucher 57770, 57774, LBP 14820; 18962, 2837). ABGD and PTP suggested that *C. virginiae* voucher 57773, 57770 is most genetically similar to *Corydoras* sp C53 than to other lineages, while ABGD recognized *C. virginiae* voucher 57749, 57751, as genetically similar to *C. sp* Paraguai and *C. ambiacus*, forming a single lineage. Besides that, PTP and NJ considered these samples of *C. virginiae*, *C. sp* Paraguai and *C. ambiacus* as different species (4% of distance from the two species). *C. virginiae* was described from the Ucayali River, Peru, while *C. ambiacus* was described from Ambiyacus River, Peru, but these two species present the pattern of coloration completely different.

ABGD recognized no difference between *C. britskii* and *C. splendens*, and grouped all at the same lineage. However, the analysis of the distance between groups and PTP showed 6% of divergence between *C. britskii* and the group *C. splendens* (97% of bootstrap). *C. splendens* is described from Amazon basin (Chiquitos, Bolivia), while *C. britskii* was described from the Paraguai basin and presents consistent diagnosed characters that delimited morphologically these two species. This species was described under the genus *Brochis*, and they are currently synonymized to *Corydoras* by Britto, 2003. Despite that, our results showed that this species formed a single group most genetically related to each other than to *Corydoras*.

Furthermore, all the analyzes agree that *C. difluviatilis* represents two different genetic lineages (13% of distance between the two populations). One lineage of *C. difluviatilis* (voucher 40007-40011, LBP 8301; 100235, LBP 28258; 59918-59921, LBP 11720; 60150-60153, LBP 11669) from the Upper Paraná River and São Francisco River basin is genetically most similar to *C. acutus*, *C. agassizii*, *C. amandajanea*, *C. cf ambiacus*, *C. britskii*, *C. condiscipulus*, *C. garbei*, *C. heraldschultzi*, *C. imitator*, *C. latus*, *C. melanistius*, *C. cf melanistius*, *C. melini*, *C. multiradiatus*, *C. cf pulcher*, *C. orthonoptherus*, *C. cf robustus*, *C. punctatus*, *C. reticulatus*, *C. robinae*, *C. seussi*, *C. sodalis*, *C. virginiae* and *Corydoras* C52 (100% os bootstrap, Supplementary fig. 24). The other lineage of *C. difluviatilis* from Corumbataí River, Upper Paraná basin (voucher 100227-100234, LBP 28262) creates a polytomy between this group and *C. virginiae* from Amazon basin, Peru 2 and *Corydoras* sp C53. Besides that, PTP recognized five lineages in *C. difluviatilis* from Corumbataí River, Upper Paraná basin (0.15), and suggested that these species are most similar to *C. pulcher*.

C. difluviatilis was described from Pardo River, Upper Paraná basin, by Britto & Castro (2002), and are also distributed in Upper São Francisco River. The samples included in our analysis presented serrations on the posterior border of pectoral spine, only at the middle to the end; absence of contact between the supraoccipital and the nuchal plate; presence of expansion on first and second infraorbital; and the pattern of coloration is very similar to *C. difluviatilis* from the first lineage. The phylogeny of Dias *et al.* (in preparation) support two population of *C. difluviatilis*, one closely related to lineage 8 and another one related to lineage 9, but here we choose to analyze the two population together to demonstrate the genetic distance between them. Our analysis showed that this species represents more than one genetic lineage and needs to be reviewed.

All the analyzes agree about *Corydoras* sp. C53 represents a single lineage genetically different from all others *Corydoras* from lineage 8 (more than 9% of distance), and probably represents a new species. Besides that, PTP recognized three different lineages, while ABGD and NJ recognized *Corydoras* sp. C53 as a single lineage. These samples were collected on the Upper Amazonas River, Peru, and should be better investigated.

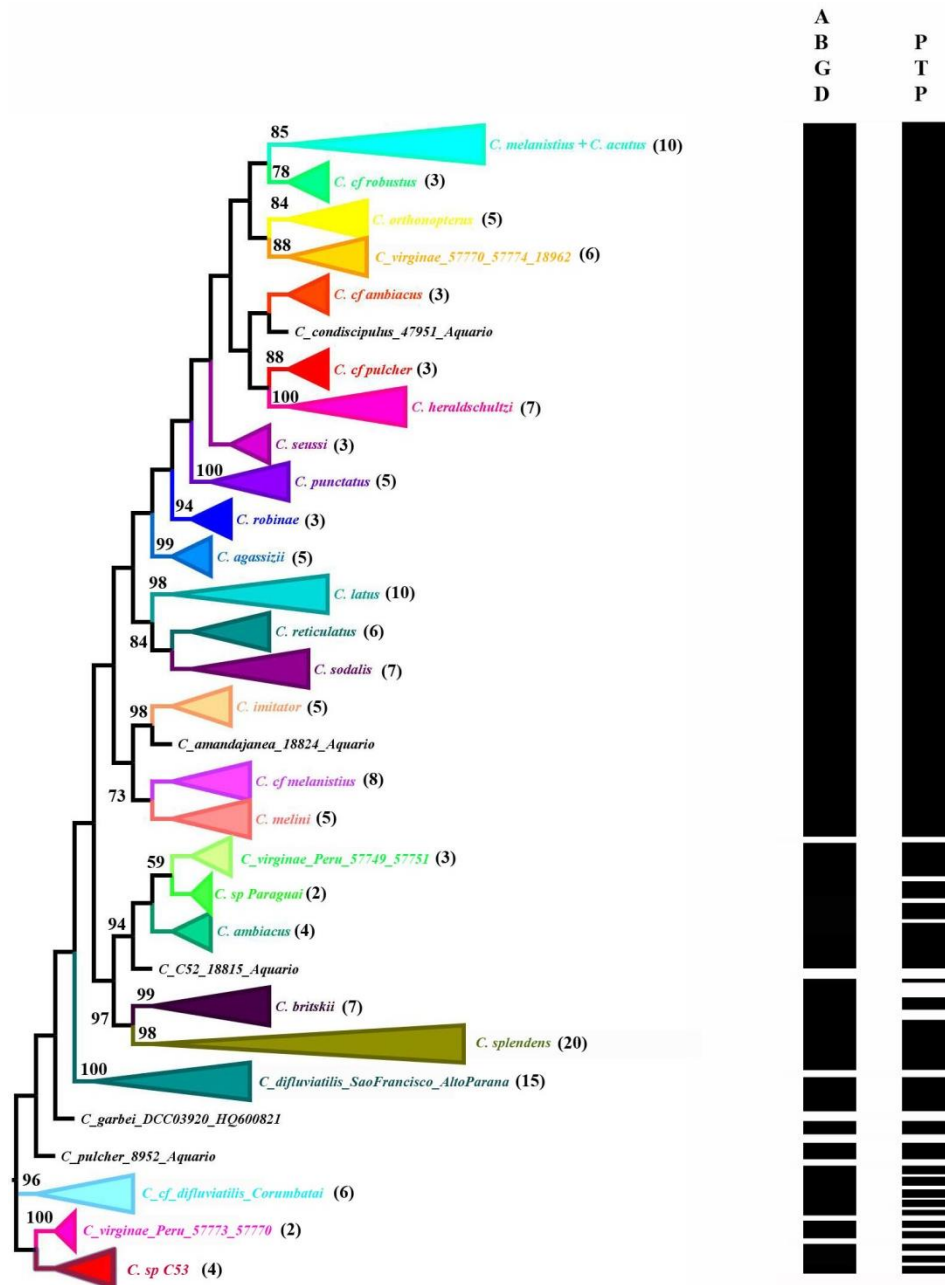


Fig. 8. NJ tree of species of Lineage 8 based on the COI gene (594 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Lineage 9

In our analyzes, we used 152 sequences of the gene COI from: *C. acrensis*, *C. adolfoi*, *C. cf adolfoi*, *C. araguaiensis*, *C. arcuatus*, *C. atropersonatus*, *C. brevirostris*, *C. cf brevirostris*, *C. burguessi*, *C. caudimaculatus*, *C. cochui*, *C. concolor*, *C. davidsandsi*, *C. cf difluviatilis*, *C. diphyes*, *C. duplicareus*, *C. grantii*, *C. aff griseus*, *C. gossei*, *C. habrosus*, *C. julii*, *C. leucomelas*, *C. metae*, *C. panda*, *C. pantanalensis*, *C. polystictus*, *C. aff polystictus*, *C. cf polystictus*, *C. schwartzi*, *C. cf schwartzi*, *C. similis*, *C. sterbai*, *C. tukano*, *C. urucu*, *C. virescens*, *C. aff virescens*, *C. weitzmani*, *C. C43*, *C. C52*, *C. C76*, *C. C91* and samples of *Corydoras* sp. The matrix had 596 pb with 368 pb of variable sites. The nucleotide composition of the matrix was 26% of adenine, 27% of cytosine, 17% of guanine, and 30% of thymine. The best evolution model was TN93+G, with BIC=14642.0702 and AICc=11683.1497. The overall mean distance was 0.10 ± 0.01 . The values of interspecific distances among this lineage ranged from 0.002 ± 0.002 between *C. grantii* and *C. adolfoi*, and 0.277 ± 0.027 , between *C. diplyes* and *C. cf. julii*, and also between *C. habrosus* and *Corydoras* sp. 53879. The intraspecific distances ranged from 0.001 ± 0.001 to 0.021 ± 0.005 (Table 9).

<i>C_sp_RioMarauia_StafsabeldoRioNegro_RioNegro</i>	0,164	0,24 7	0,16 0	0,21 1	0,20 3	0,12 6	0,04 5	0,05 3	0,05 9	0,05 8	0,06 3	0,06 9	0,05 9	0,06 7	0,06 5	0,03 3	0,01 2	0,02 6	0,06 0	0,05 8	0,05 4	0,13 1	0,13 5	0,01 2	0,01 2	0,01 0	0,01 9	0,02 4	0,01 0	0,01 3	0,02 3	0,01 1	0,01 6	0,01 1	0,02 4	0,01 1	0,02 4	0,02 5	0,01 8	0,01 8	0,01 7	0							
<i>C_atropersonatus</i>	0,169	0,23 7	0,14 4	0,22 3	0,22 1	0,11 6	0,05 0	0,05 9	0,06 7	0,06 8	0,06 8	0,05 4	0,06 8	0,06 7	0,06 4	0,05 2	0,06 9	0,06 2	0,06 7	0,04 0	0,06 3	0,12 6	0,11 9	0,06 3	0,01 1	0,01 1	0,01 8	0,02 5	0,01 1	0,01 3	0,02 6	0,01 3	0,01 6	0,01 1	0,02 5	0,01 3	0,02 5	0,02 5	0,01 6	0,01 8	0,01 7	0							
<i>C_gosseii</i>	0,151	0,25 2	0,15 3	0,22 7	0,22 3	0,12 4	0,06 3	0,06 0	0,07 9	0,07 7	0,07 4	0,07 2	0,07 7	0,07 8	0,06 6	0,06 7	0,07 5	0,03 4	0,07 7	0,07 1	0,13 5	0,14 7	0,06 6	0,05 7	0,01 1	0,01 7	0,02 7	0,01 2	0,01 3	0,02 5	0,01 4	0,01 6	0,01 1	0,02 6	0,01 2	0,02 7	0,02 7	0,01 8	0,02 0	0,01 7	0,004±0,02								
<i>C_metae</i>	0,163	0,22 9	0,14 9	0,20 4	0,20 1	0,12 7	0,05 8	0,06 3	0,06 5	0,06 2	0,07 1	0,07 9	0,07 1	0,06 6	0,07 7	0,05 4	0,04 3	0,05 1	0,06 8	0,06 4	0,13 7	0,13 6	0,04 0	0,05 9	0,06 7	0,01 8	0,02 4	0,01 2	0,01 3	0,02 0	0,01 6	0,01 2	0,01 1	0,02 2	0,01 4	0,02 4	0,02 4	0,01 8	0,01 9	0,01 6	0,014±0,04								
<i>C_sp_53879</i>	0,145	0,22 5	0,16 4	0,21 3	0,13 0	0,11 2	0,13 9	0,13 4	0,13 1	0,13 9	0,11 2	0,11 4	0,11 8	0,12 8	0,12 1	0,13 3	0,12 4	0,12 6	0,12 8	0,14 5	0,13 0	0,13 1	0,12 2	0,12 7	0,13 4	0,11 1	0,02 4	0,01 8	0,01 8	0,02 9	0,01 7	0,01 8	0,02 4	0,01 8	0,02 4	0,02 5	0,02 0	0,01 9	0,01 8	0									
<i>C_sp_53795</i>	0,214	0,21 3	0,23 5	0,14 5	0,14 1	0,20 2	0,21 7	0,21 6	0,19 6	0,19 9	0,20 2	0,19 7	0,19 9	0,18 2	0,20 9	0,20 6	0,21 9	0,22 6	0,22 7	0,19 1	0,21 7	0,20 3	0,20 8	0,20 1	0,20 7	0,21 1	0,20 0	0,02 5	0,02 3	0,02 4	0,02 5	0,02 3	0,02 4	0,02 9	0,00 4	0,00 0	0,02 9	0,02 2	0,02 3	0									
<i>C_panda_18846</i>	0,167	0,24 4	0,14 3	0,20 6	0,19 9	0,12 3	0,02 9	0,03 6	0,04 6	0,06 2	0,05 3	0,06 2	0,05 4	0,04 6	0,06 2	0,05 9	0,04 5	0,06 2	0,05 1	0,04 3	0,06 9	0,06 8	0,05 4	0,07 3	0,05 0	0,05 9	0,12 1	0,13 6	0,13 8	0,13 1	0,13 3	0,12 1	0,11 3	0,12 1	0,13 5	0,13 6	0,12 7	0,11 4	0,13 1	0,13 0	0,12 7	0,11 4	0						
<i>C_leucomelas_18942_2832</i>	0,144	0,25 6	0,15 1	0,20 3	0,19 9	0,11 3	0,05 2	0,04 8	0,06 9	0,04 5	0,06 5	0,04 6	0,02 5	0,02 6	0,04 0	0,04 3	0,04 4	0,06 2	0,05 4	0,06 1	0,06 8	0,05 9	0,06 2	0,07 4	0,06 1	0,05 8	0,13 9	0,14 5	0,05 6	0,07 5	0,07 6	0,07 4	0,12 6	0,19 3	0,05 0	0,02 4	0,01 5	0,01 1	0,01 4	0,02 6	0,00 3	0,02 2	0,01 7	0,01 7	0				
<i>C_habrosus</i>	0,233	0,25 1	0,23 7	0,25 6	0,25 7	0,23 7	0,22 8	0,22 2	0,22 9	0,22 5	0,21 2	0,22 4	0,22 2	0,22 3	0,24 1	0,23 8	0,22 4	0,21 7	0,22 8	0,23 6	0,21 2	0,23 9	0,22 1	0,23 7	0,21 6	0,21 6	0,21 7	0,23 6	0,21 3	0,23 9	0,21 4	0,27 9	0,23 6	0,23 7	0,23 6	0,23 3	0,21 9	0,02 4	0,02 9	0,02 5	0,02 5	0,02 5	0,02 5	0					
<i>C_concolor</i>	0,178	0,24 0	0,15 6	0,22 3	0,21 6	0,14 2	0,07 4	0,08 1	0,08 5	0,08 4	0,08 5	0,09 6	0,08 5	0,08 6	0,08 4	0,06 5	0,04 8	0,05 8	0,08 2	0,06 4	0,06 5	0,13 7	0,13 6	0,05 2	0,06 5	0,08 3	0,05 7	0,13 6	0,13 5	0,06 2	0,08 5	0,05 3	0,08 7	0,09 6	0,21 1	0,08 1	0,09 0	0,21 7	0,01 7	0,01 3	0,02 3	0,02 5	0,02 5	0,02 6	0,01 0	0,01 8	0		
<i>C_cf_C43</i>	0,170	0,22 4	0,15 2	0,21 4	0,20 8	0,09 6	0,10 3	0,10 3	0,10 6	0,11 1	0,10 5	0,11 3	0,10 6	0,11 7	0,10 4	0,10 3	0,10 6	0,10 7	0,10 1	0,10 7	0,10 4	0,10 3	0,12 5	0,10 9	0,11 5	0,11 2	0,11 4	0,19 7	0,10 1	0,10 5	0,26 2	0,11 2	0,01 8	0,01 3	0,02 5	0,02 3	0,02 4	0,02 9	0,01 4	0,01 9	0,01 7	0,01 5	0						
<i>C_C76_23665</i>	0,136	0,23 9	0,14 6	0,20 1	0,20 3	0,12 3	0,04 8	0,03 4	0,04 8	0,05 5	0,04 8	0,05 6	0,05 3	0,05 2	0,04 6	0,04 4	0,05 6	0,05 0	0,06 2	0,07 4	0,06 3	0,13 7	0,13 5	0,05 1	0,05 2	0,05 6	0,06 4	0,13 1	0,13 1	0,05 7	0,05 3	0,05 7	0,06 6	0,13 1	0,20 1	0,04 7	0,05 3	0,21 7	0,07 6	0,12 4	0,02 4	0,01 1	0,02 4	0,02 6	0,01 7	0,02 0	0,01 7	0	
<i>C_burgessi</i>	0,214	0,21 4	0,20 6	0,09 7	0,09 0	0,19 3	0,19 3	0,19 5	0,18 8	0,19 3	0,20 6	0,19 6	0,20 2	0,19 1	0,20 4	0,19 8	0,20 7	0,19 1	0,18 9	0,20 1	0,19 9	0,19 6	0,20 4	0,20 2	0,19 6	0,18 6	0,20 3	0,19 8	0,20 9	0,20 1	0,21 4	0,17 9	0,19 8	0,12 4	0,18 1	0,20 9	0,23 8	0,18 1	0,18 8	0,19 1	0,02 5	0,01 8	0,01 9	0,01 5	0,02 4	0,02 5	0		
<i>C_arcuatus</i>	0,155	0,25 1	0,16 0	0,20 4	0,20 3	0,11 7	0,05 5	0,05 6	0,06 9	0,05 0	0,02 8	0,03 5	0,02 0	0,03 8	0,04 4	0,04 7	0,06 1	0,05 7	0,07 8	0,06 4	0,06 4	0,13 1	0,14 5	0,06 9	0,07 9	0,07 8	0,13 4	0,20 4	0,06 4	0,02 9	0,02 1	0,23 0	0,10 1	0,10 7	0,10 3	0,05 7	0,05 3	0,21 7	0,10 0	0,10 7	0,05 3	0,21 7	0,02 4	0,02 3	0,01 7	0,01 7	0,01 7	0,020±0,06	
<i>C_sp_46620</i>	0,215	0,21 5	0,23 5	0,14 5	0,14 0	0,20 1	0,21 7	0,21 6	0,19 6	0,19 9	0,20 1	0,19 6	0,20 9	0,18 6	0,20 9	0,20 6	0,20 8	0,21 5	0,22 7	0,22 0	0,19 7	0,21 3	0,20 7	0,20 1	0,22 6	0,21 9	0,20 0	0,22 3	0,23 6	0,21 1	0,19 9	0,00 3	0,20 2	0,19 6	0,23 5	0,21 0	0,19 1	0,20 8	0,20 4	0,00 9	0,02 2	0,02 3	0,02 6	0					
<i>C_sp_32735</i>	0,226	0,21 9	0,22 8	0,14 9	0,14 4	0,20 3	0,21 1	0,21 0	0,19 3	0,20 3	0,21 5	0,20 0	0,19 5	0,20 3	0,21 4	0,20 2	0,21 9	0,23 5	0,22 0	0,20 4	0,21 5	0,21 2	0,21 0	0,22 3	0,23 4	0,20 6	0,21 4	0,22 0	0,23 6	0,20 4	0,20 8	0,04 0	0,21 8	0,19 4	0,24 1	0,22 3	0,20 2	0,21 3	0,13 0	0,20 8	0,04 4	0,02 2	0,02 3	0,02 5	0				
<i>C_diphies</i>	0,145	0,27 7	0,10 1	0,22 4	0,21 8	0,12 4	0,11 2	0,12 0	0,11 8	0,12 9	0,13 3	0,13 6	0,13 0	0,12 2	0,12 3	0,12 3	0,12 6	0,11 8	0,12 3	0,12 4	0,12 4	0,12 0	0,11 3	0,12 2	0,12 9	0,11 7	0,12 8	0,13 5	0,15 0	0,19 8	0,12 6	0,13 0	0,23 2	0,14 7	0,14 6	0,11 5	0,21 3	0,14 3	0,19 8	0,20 4	0,01 4	0,01 8	0,002±0,02						
<i>C_tukano</i>	0,116	0,24 3	0,10 6	0,23 0	0,22 3	0,13 8	0,12 7	0,13 0	0,12 9	0,14 4	0,13 7	0,14 5	0,12 9	0,12 7	0,12 5	0,13 9	0,13 7	0,12 9	0,12 6	0,12 4	0,13 8	0,13 0	0,12 6	0,12 9	0,11 4	0,12 2	0,11 3	0,12 4	0,13 9	0,12 2	0,14 4	0,13 6	0,13 5	0,20 2	0,12 4	0,13 8	0,22 4	0,14 5	0,13 4	0,14 1	0,20 9	0,14 8	0,20 2	0,21 2	0,13 1	0,12 6	0,01 8	0,003±0,02	
<i>C_pantanalensis</i>	0,167	0,21 0	0,13 5	0,21 6	0,20 6	0,07 0	0,11 8	0,10 7	0,11 3	0,13 6	0,12 6	0,13 6	0,12 3	0,12 6	0,12 1	0,12 1	0,11 8	0,12 8	0,12 8	0,11 7	0,12 0	0,11 1	0,14 7	0,14 3	0,12 4	0,11 8	0,13 8	0,11 7	0,11 0	0,14 2	0,14 3	0,12 4	0,11 8	0,11 8	0,13 7	0,22 0	0,10 2	0,11 7	0,24 8	0,12 8	0,09 2	0,11 8	0,20 3	0,13 0	0,22 1	0,21 3	0,13 5	0,12 9	0

The analyzes demonstrated different delimitations for the species of lineage 9. NJ suggests 40 groups with genetic divergence, while delimited PTP 45 groups. The least separatist analysis was ABGD, which found only four genetic lineages between all the species of lineage 9. This analysis proposed nine partitions: seven partitions found 4 groups (prior maximal distance $P= 0.001-0.022$), one partition found 3 groups ($P= 0.036$), and one partition found one group ($P= 0.060$). ABGD didn't find differences between *C. acrensis*, *C. adolfoi*, *C. cf adolfoi*, *C. araguaiensis*, *C. arcuatus*, *C. atropersonatus*, *C. brevirostris*, *C. cf brevirostris*, *C. caudimaculatus*, *C. cochui*, *C. concolor*, *C. davidsandsi*, *C. cf difluviatilis*, *C. diphyes*, *C. duplicareus*, *C. grantii*, *C. aff griseus*, *C. gossei*, *C. julii_43814_43819*, *C. leucomelas*, *C. metae*, *C. panda*, *C. pantanalensis*, *C. polystictus*, *C. aff polystictus*, *C. cf polystictus*, *C. schwartzi*, *C. cf schwartzi*, *C. similis*, *C. sterbai*, *C. tukano*, *C. urucu*, *C. virescens*, *C. aff virescens*, *C_sp_RioMarauia_StaIsabeldoRioNegro_RioNegro*, *C. C43*, *C. C52*, *C. C73*, *C. C76*, *C. C91*, *Corydoras sp_53879_Peru*, and *Corydoras cf_C43_18955*, recognizing all these species as genetically similar, and grouped all them in a single big lineage. ABGD only delimited *C. habrosus*, *C. julii_43450_43454*, and *C. burguessi* of lineage 9. Besides that, *C. burguessi* was grouped with five *Corydoras* sp. This analysis cannot found difference in the two samples of *Corydoras* sp. from Guamá and Javari Rivers, and three samples of *Corydoras* sp. from Peru (Upper Amazonas River), Negro, and Paraguai Rivers. NJ and PTP also found similarities between *Corydoras* sp 87788 and *Corydoras* sp 43014, but 9% of this species from *C. burguessi*, and more than 14% of distance from the other group of *Corydoras* sp. These two groups of *Corydoras* sp have bootstrap supported by RaxML of 98% (Supplementary Fig. 27).

All the analyses recognized *C. habrosus* as a single lineage, but this species create a polytomy with the group formed by *C. julii* 43450-43454, *C. burguessi*, *Corydoras* sp. from Guamá and Javari Rivers, and *Corydoras* sp. from Peru (Upper Amazonas River), Negro, and Paraguai Rivers. The ABGD recognized *C. cf julii* (voucher 43450-43454, LBP 9327) from Guamá River, Amazonian basin, as a single lineage, but PTP found two different lineages composing *C. cf julii*, one formed by the samples 43454 and 43453, with 0.49 of support value, and other one formed by 43450, 43451 and 43452, 0.35. On the other hand, NJ recognized two lineages of *C. julii* from Guamá River, one lineage formed by *C.cf julii_43814_43819* that showed 25% of distance from *C. cf julii* (voucher 43450-43454). ABGD and PTP grouped *C.cf julii_43814_43819* with *C. arcuatus*, *C. schwartzi*, and *C. urucu*, recognizing all as the

same species (0.03). *C. julii* is described from Parnaíba River, a Coastal basin from Maranhão State, Brazil, and its distribution is lower Amazon Rivers and coastal rivers of northeastern Brazil and could represent more than one morphotype. The samples used in the analysis of delimitation of lineage 9 were collected all on the same river, Guamá River, Amazonas basin, and even though represents distinct. *C. leucomelas*, *C. araguaiensis*, and *C. acrensis* were delimited by NJ and PTP as distinct lineages, most genetically similar to *C. julii*, *C. arcuatus*, *C. schwartzi*, and *C. urucu* than to other species of the lineage 9.

NJ and PTP recognized *C. grantii* (voucher 32587-32591, LBP 7709) and *C. adolfoi* (voucher 7103, 7102, LBP 525) as the same lineage (0.12 of support value on PTP, and 99% of bootstrap on RAxML). The analysis of distance showed low support between this species (0.02%), suggesting that they are the same species. *C. grantii* is a recent species described from the Upper Negro River, São Gabriel da Cachoeira, Amazon basin and the samples of *C. grantii* used in this analysis belong to the type-series of this species description. On the other hand, *C. adolfoi* is also described from Upper Negro River (São Gabriel da Cachoeira) and distributed to all Negro River. The samples of *C. adolfoi* included in this analysis presented small spines on pectoral fin, as mentioned by Sands (1994) (*vs.* pectoral fin without small spines in *C. duplicareus*); longitudinal band on the base of dorsal fin with conspicuous border, not extending to the middle of the body (*vs.* band on the longitudinal base of dorsal fin with inconspicuous border and extending to the middle of the body in *C. duplicareus*). The pattern of the color of *C. adolfoi*, *C. duplicareus*, and *C. davidsandsi* is similar but different to *C. grantii*. Besides that, *C. adolfoi* showed to be genetically more similar to *C. grantii* than to *C. duplicareus*, instead. *C. duplicareus*, on the other hand, is most similar to *C. davidsandsi*, and the PTP analysis splits *C. duplicareus*, recognizing two distinct lineages for this species (0.50 and 0.76).

C. caudimaculatus and *Corydoras* C43 were demonstrated to be the same species by all the analyses (98% of bootstrap value). On the other hand, *Corydoras* C76 and *Corydoras* C91 were considered distinct lineages. The pattern of the color of *Corydoras* C91 is similar to *C. julii*, but the analysis of distance indicated that they represent 24% of distance, and *Corydoras* C91 is genetically most similar to *C. atropersonatus* (0.76 of support value on PTP).

C. polystictus demonstrated to be more than one lineage by PTP and NJ analysis. One lineage is most similar to *C. panda* and *C. similis*, while there is another

lineage (voucher 8111, 8057; LBP 689) similar to *C. pantanalensis* and another one (voucher 13659, LBP 1958; 46600-46604; LBP 8322) similar to *C. aff griseus*.. Morphologically, *C. polystictus* present a similar pattern of color to *C. similis*. However, *C. polystictus* is described from the Paraguai basin and has *C. virescens* in synonymous with this species. Although *C. virescens* is currently synonymized with *C. polystictus*, we included in our analysis samples of *C. aff virescens* (voucher 46616 and 46618, LBP 10096) that demonstrated to be similar to *C. diphyes*, and distant to *C. polystictus*. The analysis of distance indicates that *C. virescens* is 14% between *C. polystictus*, and from *C. cf polystictus*, and 12% from *C. aff polystictus*. This result indicates that *C. virescens* could be a valid species and need to be reviewed.

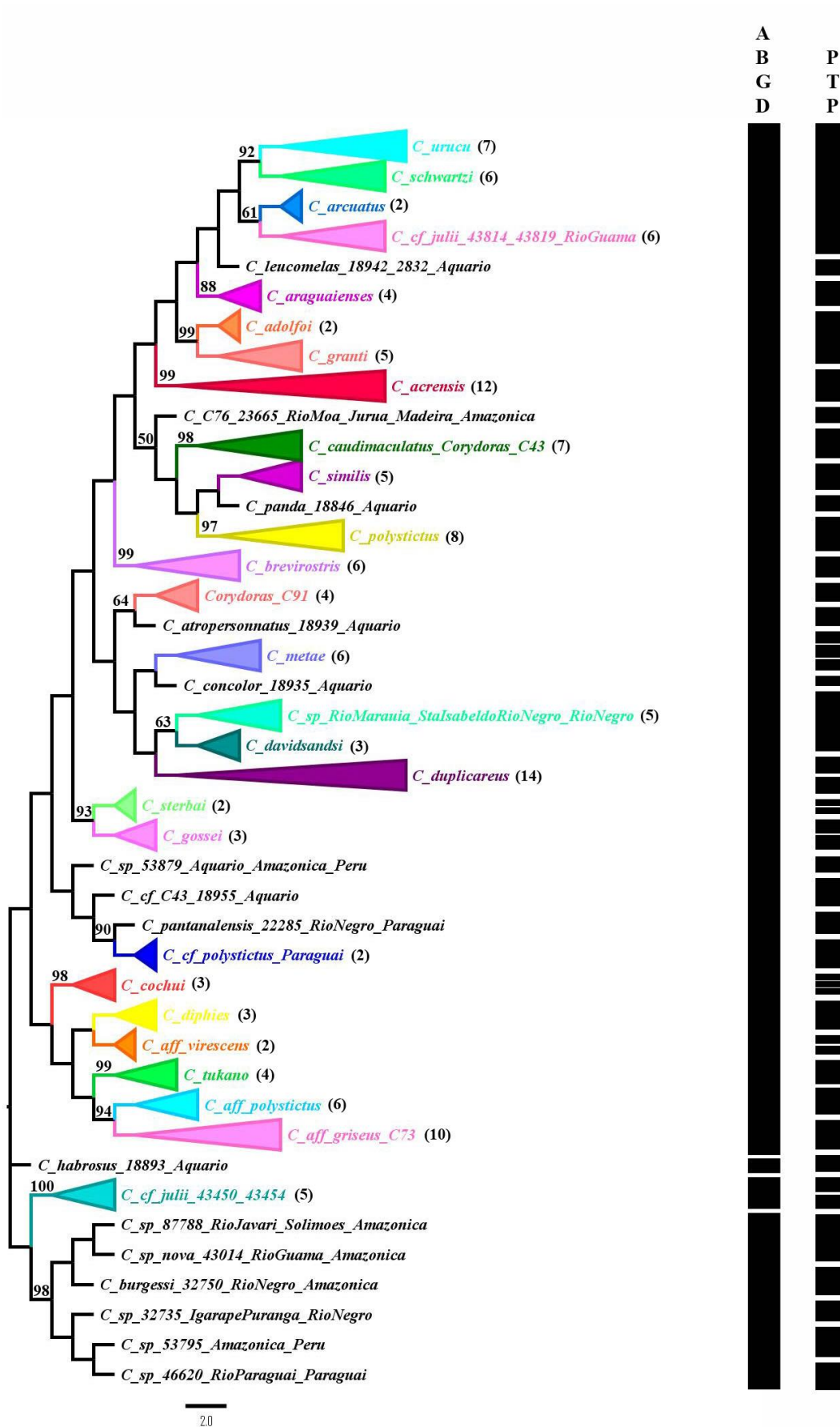


Fig. 9. NJ tree of species of Lineage 9, based on the COI gene (596 pb). Vertical bars at the right represent the number of species obtained by the ABGD and PTP analyses. Numbers near nodes represent

bootstrap support. Values < 50% are not shown. Numbers of the specimen are in parentheses after tip names.

Conclusion

We presented here the first analysis of molecular delimitation of species of the subfamily Corydoradinae, with more than 800 sequences, and demonstrated the taxonomic uncertain of this subfamily. The results showed several potentially valid taxonomic units and also the use of the same valid name for several genetically distinct groups. Our data also demonstrated that some valid names need to be relocated to another genus, or synonymized.

Many species, especially in the lineage 9, presented similarity in their mitochondrial genome and pattern of the color of the body, but morphological divergence in body shape and snout. The ABGD and PTP methods recognized as a single lineage several species mainly on lineages 7, 8, and 9. These two methods only agree on the number of lineages in lineage 2, 3, 4, 5, 6 and 7, and disagree about lineage 1, 8, and 9. The more split method was PTP, which recognized the highest number of groups between the lineages. The ABGD was the most conservative method when compared with PTP and NJ. Especially in lineage 9, the ABGD recognized only four groups and gathered as a single lineage many morphologically distinct species. This happened because the nature of each method is different in the way they work. The ABGD uses the accumulation of gaps between the sequences to formulate the ranking of distance values and to create the groups of species. The bigger the gap value, the bigger the delimitation of the groups created by the analysis. This method depends on the accumulation of gaps to recognize the difference between the sequences. If a group of species suffers rapid speciation, the gap accumulation has not yet been fixed in the genotype of the population, and therefore, it will not be recognized by the ABGD method.

On the other hand, the PTP analysis creates groups based on phylogenetic trees that incorporate models of population evolution, such as coalescence theory. The PTP uses the rate of substitution of bases to delimit each group. The greater the rate of substitution in the matrix, the greater is the number of groups this method will delimit. Each substitution found between the terminal taxa of a phylogenetic tree increases the probability of the method recognizing speciation, and the number of lineages created by

it. Another aggravating factor is that species in lineages 7, 8, and 9 have more chromosomes than species in other lineages, which can accelerate diversification rates and contribute to reproductive isolation (Oliveira *et al.*, 1992; Oliveira *et al.*, 1993), adding further support to the recognition of so many genetically distinct groups by PTP analysis.

Nevertheless, from a morphological point of view, Corydoradinae is composed of species that present a varied pattern of colors, as well as a high variation in snout morphology, a result that contrasts with molecular analysis, which demonstrates genetic similarity of some lineages. Traditionally, species of Corydoradinae have been described based on color patterns, as morphological differences between species are subtle (Nijssen & Isbrücker, 1980; Britto, 2003). Therefore, the species of Corydoradinae have a long history of misidentification mainly because the last taxonomic revision was carried out many years ago (Nijssen & Isbrücker, 1980). In that time, there was still little material collected in collections, which made it difficult for the authors to analyze all the population variations. Furthermore, the authors didn't present diagnostic characters for each species. After that, there were only descriptions of new species in the literature, creating a lumping of species with similar color, but in actuality they are genetically and morphologically similar, and also creating a splitting of geographic variants of single species, increasing substantially the complexity of this subfamily.

Our results provide knowledge to guide future studies of systematic and phylogenetic of Corydoradinae, strongly supporting the necessity a taxonomic review of the subfamily to reinforce the boundaries that define each species and each genus, and to identify the taxonomic status and possibly relocate the species of some genus. Our results also revealed some potentially new species delimited by the molecular analysis that need to be described. In addition, we demonstrated the necessity of further studies related to phylogenetic inference with time-calibrated genome-scale analysis associated with a test of the hypothesis of biogeography and ancestral diversification of Corydoradinae to better understand the correlation of the mimicry and snout size with the pattern of dispersion of this species.

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Supplementary Table 1. List of the all specimens used in species delimitation analyses.

Family	Subfamily	Lineage	Species	Voucher code	Catalog number	Drainage	Country	Coordinates
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff areio</i>	46623	LBP 9316	Ribeirao dos Veados Paraguai	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff areio</i>	46624	LBP 9316	Ribeirao dos Veados Paraguai	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff areio</i>	46625	LBP 9316	Ribeirao dos Veados Paraguai	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff areio</i>	43844	LBP 9325	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff areio</i>	46625	LBP 10098	Ribeirao dos Veados Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cf aurofrenatus</i>	13566	LBP 1960	Ribeirao dos Veados Rio Taquari Paraguai	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cf blochiii</i>	7219	LBP 554	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cf blochiii</i>	7216	LBP 554	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cf blochiii</i>	7218	LBP 554	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cervinus</i>	46621	LBP 9316	Ribeirao dos Veados Paraguai	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. cervinus</i>	46622	LBP 9316	Ribeirao dos Veados Paraguai	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32694	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32695	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32724	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32714	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32696	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32711	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32699	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32697	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32698	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. desana</i>	32700	LBP 7712	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. ellisae</i>	18805	LBP 2774	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53973	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53974	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53975	LBP 12517	Rio Amazonas	Peru	-

Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53976	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53977	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53798	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53799	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53800	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. fowleri</i>	53801	LBP 12517	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. maculifer</i>	32890	LBP 7213	Corrego Taquaral, Tocantins-Araguaia, Amazonas	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	66458	LBP 17116	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	46610	LBP 10094	Rio Purus Madeira Amazonica	Brazil	S 07°56.110' W 63°27.353'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	46529	LBP 10144	Rio Purus Madeira Amazonica	Brazil	S 09°66.7195" W 65°43.7438"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	46530	LBP 10144	Rio Purus Madeira Amazonica	Brazil	S 09°66.7195" W 65°43.7438"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	46531	LBP 10144	Rio Purus Madeira Amazonica	Brazil	S 09°66.7195" W 65°43.7438"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. narcissus</i>	46532	LBP 10144	Rio Purus Madeira Amazonica	Brazil	S 09°66.7195" W 65°43.7438"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. ourastigma</i>	49493	LBP 10659	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. ourastigma</i>	49494	LBP 10659	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. pastazensis</i>	18956	LBP 2835	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. pastazensis</i>	18957	LBP 2836	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilus</i>			Aquario	-	
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilis</i>	53798	LBP 12467	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilus</i>	53799	LBP 12467	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilus</i>	53800	LBP 12467	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilus</i>	53801	LBP 12467	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. semiaquilus</i>	53802	LBP 12467	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32563	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32564	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32565	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32577	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'

Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32578	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. serratus</i>	32579	LBP 6869	Igarape Yamirim Rio Negro	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>C. simulatus</i>	18924	LBP 2820	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. treitlii</i>	18918	LBP 2818	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. treitlii</i>	18919	LBP 2818	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. treitlii</i>	18920	LBP 2818	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. vittatus</i>	18810	LBP 2776	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. vittatus</i>	18811	LBP 2776	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. vittatus</i>	18812	LBP 2776	Aquario Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. sp</i>	53877	LBP 12488	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. sp</i>	53879	LBP 12488	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. sp</i>	87787	LBP 22532	Rio Javari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. sp</i>	53796	LBP 12466	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. sp</i>	53797	LBP 12466	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C53</i>	53936	LBP 12507	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C53</i>	53937	LBP 12507	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C53</i>	53938	LBP 12507	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C53</i>	53939	LBP 12507	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C109</i>	43014	LBP 9122	Igarape Açu Rio Guama Amazonica	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. C109</i>	43846	LBP 9325	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff C109</i>	43011	LBP 9122	Igarape Açu Rio Guama Amazonas	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 1	<i>C. aff C109</i>	43013	LBP 9122	Igarape Açu Rio Guama Amazonas	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63362	LBP 15330	Rio CachoeiradeTaquarucu Rio Tocantins	Brazil	S 10°18'56.6" W 48°13'00.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63363	LBP 15330	Rio CachoeiradeTaquarucu Rio Tocantins	Brazil	S 10°18'56.6" W 48°13'00.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63364	LBP 15330	Rio CachoeiradeTaquarucu Rio Tocantins	Brazil	S 10°18'56.6" W 48°13'00.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63365	LBP 15330	Rio CachoeiradeTaquarucu Rio Tocantins	Brazil	S 10°18'56.6" W 48°13'00.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63366	LBP 15330	Rio CachoeiradeTaquarucu Rio Tocantins	Brazil	S 10°18'56.6" W 48°13'00.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. albater</i>	63260	LBP 15292	Rio SaoBernardo TocantinsAraguaia	Brazil	S 13°44'15.9" W 46°21'48.8"

Callichthyidae	Corydoradinae	Lineage 2	<i>A. depinnai</i>	92696	LBP 23907	Rio Ipojuca CosteiraPE	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. depinnai</i>	92697	LBP 23907	Rio Ipojuca CosteiraPE	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. depinnai</i>	92698	LBP 23907	Rio Ipojuca CosteiraPE	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. depinnai</i>	92699	LBP 23907	Rio Ipojuca CosteiraPE	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. depinnai</i>	92700	LBP 23907	Rio Ipojuca CosteiraPE	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. fuscoguttatus</i>	17398	LBP 2612	Rio SaoJosedosDourados Parana	Brazil	S 20°37'14.3" W 49°53'40.6"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. fuscoguttatus</i>	17399	LBP 2612	Rio SaoJosedosDourados Parana	Brazil	S 20°37'14.3" W 49°53'40.6"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. fuscoguttatus</i>	10916	LBP 1295	Rio Araponga, Tietê, Paraná	Brazil	-
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	12338	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	12624	LBP 1456	AfluentedoRio Araguaia	Brazil	S 17°19'03.9" W 53°15'51.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	12625	LBP 1456	AfluentedoRio Araguaia	Brazil	S 17°19'03.9" W 53°15'51.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	13516	LBP 1825	Araguaia	Brazil	S 15°51'36.4" W 52°12'06.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	13517	LBP 1825	Araguaia	Brazil	S 15°51'36.4" W 52°12'06.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	13528	LBP 1825	Araguaia	Brazil	S 15°51'36.4" W 52°12'06.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	13529	LBP 1825	Araguaia	Brazil	S 15°51'36.4" W 52°12'06.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. poecilus</i>	13099	LBP 1825	Araguaia	Brazil	S 15°51'36.4" W 52°12'06.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. aff poecilus</i>	12304	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. aff poecilus</i>	12308	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. sp poecilus</i>	12336	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. sp poecilus</i>	12337	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. sp poecilus</i>	12339	LBP 1437	CorregodoSapo Rio Araguaia	Brazil	S 17°33'42.4" W 53°18'29.7"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. sp poecilus</i>	11823	LBP 1658	Rio Vermelho Araguaia	Brazil	S 15°55'01.5" W 50°07'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. sp poecilus</i>	11824	LBP 1658	Rio Vermelho Araguaia	Brazil	S 15°55'01.5" W 50°07'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. psammatides</i>	69101	LBP 7188	Rio Lapao Rio Paraguacu Costeira	Brazil	S 12°32'33.6" W 41°22'51.5"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. psammatides</i>	69102	LBP 7188	Rio Lapao Rio Paraguacu Costeira	Brazil	S 12°32'33.6" W 41°22'51.5"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. psammatides</i>	69103	LBP 7188	Rio Lapao Rio Paraguacu Costeira	Brazil	S 12°32'33.6" W 41°22'51.5"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. psammatides</i>	69104	LBP 7188	Rio Lapao Rio Paraguacu Costeira	Brazil	S 12°32'33.6" W 41°22'51.5"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. psammatides</i>	69105	LBP 7188	Rio Lapao Rio Paraguacu Costeira	Brazil	S 12°32'33.6" W 41°22'51.5"

Callichthyidae	Corydoradinae	Lineage 2	<i>A. raimundi</i>	69106	LBP 5568	Rio Tapuiu Rio Parnaiba	Brazil	S 09°06'35' W 45°55'20'
Callichthyidae	Corydoradinae	Lineage 2	<i>A. raimundi</i>	69107	LBP 5568	Rio Tapuiu Rio Parnaiba	Brazil	S 09°06'35' W 45°55'20'
Callichthyidae	Corydoradinae	Lineage 2	<i>A. raimundi</i>	69108	LBP 5568	Rio Tapuiu Rio Parnaiba	Brazil	S 09°06'35' W 45°55'20'
Callichthyidae	Corydoradinae	Lineage 2	<i>A. raimundi</i>	69109	LBP 5568	Rio Tapuiu Rio Parnaiba	Brazil	S 09°06'35' W 45°55'20'
Callichthyidae	Corydoradinae	Lineage 2	<i>A. raimundi</i>	69110	LBP 5568	Rio Tapuiu Rio Parnaiba	Brazil	S 09°06'35' W 45°55'20'
Callichthyidae	Corydoradinae	Lineage 2	<i>A. taurus</i>	12306	LBP 1427	Rio Itiquira Paraguai	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. taurus</i>	12333	LBP 1427	Rio Itiquira Paraguai	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. taurus</i>	12334	LBP 1427	Rio Itiquira Paraguai	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. taurus</i>	12335	LBP 1427	Rio Itiquira Paraguai	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>A. taurus</i>	12317	LBP 1427	Rio Itiquira Paraguai	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13695	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13678	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13679	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13705	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13694	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13677	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13706	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>C. lacerdai</i>	13705	LBP 1966	Ribeirada Terra Firme Costeira BA	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	46493	LBP	Costeira	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	21369	LBP 2126	Costeira Mongagua	Brazil	S 24°05'18.3" W 46°43'53.6"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	21370	LBP 2126	Costeira Mongagua	Brazil	S 24°05'18.3" W 46°43'53.6"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	21372	LBP 2126	Costeira Mongagua	Brazil	S 24°05'18.3" W 46°43'53.6"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	21373	LBP 2126	Costeira Mongagua	Brazil	S 24°05'18.3" W 46°43'53.6"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	11088	LBP 1229	Costeira Bertioga	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	4523	LBP 407	Vale do Ribeira Costeira	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	4588	LBP 407	Vale do Ribeira Costeira	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5160	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5161	LBP 421	Costeira JuquiaSP	Brazil	-

Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5164	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5166	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5167	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5168	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5169	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	5171	LBP 421	Costeira JuquiaSP	Brazil	-
Callichthyidae	Corydoradinae	Lineage 3	<i>S. barbatus</i>	11163	LBP 1250	Ribeirao Grande Ribeira de Iguape Costeira	Brazil	S 24°16,077' W 47°24,455'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	35441	LBP 7372	Rio Betari RibeiradeIguape Costeira	Brazil	S 24°33'42.1" W 48°40'05.7"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	35442	LBP 7372	Rio Betari RibeiradeIguape Costeira	Brazil	S 24°33'42.1" W 48°40'05.7"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	32796	LBP 7716	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	32797	LBP 7716	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	35720	LBP 7427	Rio Pindauba RibeiradeIguape Costeira	Brazil	S 24°45'25.0" W 48°04'07.2"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. kronei</i>	35721	LBP 7427	Rio Pindauba RibeiradeIguape Costeira	Brazil	S 24°45'25.0" W 48°04'07.2"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	36036	LBP 7550	Rio Mumuna Rio RibeiradeIguape Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	36037	LBP 7550	Rio Mumuna Rio RibeiradeIguape Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	36038	LBP 7550	Rio Mumuna Rio RibeiradeIguape Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	36039	LBP 7550	Rio Mumuna Rio RibeiradeIguape Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	36040	LBP 7550	Rio Mumuna Rio RibeiradeIguape Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	38214	LBP 8217	Rio semnome Costeira SP	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	38215	LBP 8217	Rio semnome Costeira SP	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	38216	LBP 8217	Rio semnome Costeira SP	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	32803	LBP 7718	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10.890' W 46°50.563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	32804	LBP 7718	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10.890' W 46°50.563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	32805	LBP 7718	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10.890' W 46°50.563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	32807	LBP 7718	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10.890' W 46°50.563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	32808	LBP 7718	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10.890' W 46°50.563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	38433	LBP 8217	Riacho semnome Costeira ItanhaemSP	Brazil	S 24°13'55.0" W 46°45'14.9"

Callichthyidae	Corydoradinae	Lineage 3	<i>S. macropterus</i>	38434	LBP 8217	Riacho semnome Costeira ItanhaemSP	Brazil	S 24°13'55.0" W 46°45'14.9"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32786	LBP 7715	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32787	LBP 7715	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32788	LBP 7715	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32789	LBP 7715	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	11106	LBP 1267	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	11108	LBP 1267	Rio Fau Ribeira de Iguape Costeira	Brazil	S 24°12.441' W 47°28.616'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	35381	LBP 17381	Igarape YaMirim Rio Negro Amazonas	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	35383	LBP 17381	Igarape YaMirim Rio Negro Amazonas	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	35384	LBP 17381	Igarape YaMirim Rio Negro Amazonas	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32800	LBP 7717	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10,890' W 46°50,563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. prionotos</i>	32801	LBP 7717	Rio Preto Rio Itanhaem Costeira	Brazil	S 24°10,890' W 46°50,563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. C112</i>	11096	LBP 1224	CosteiraSP	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. sp C112</i>	11067	LBP 1224	Costeira BertiogaSP	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. sp C112</i>	11070	LBP 1224	Costeira BertiogaSP	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. sp C112</i>	11071	LBP 1224	Costeira BertiogaSP	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. sp C112</i>	11072	LBP 1224	Costeira BertiogaSP	Brazil	S 23°57,769' W 46°10,625'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. sp C112</i>	11123	LBP 1237	CosteiraSP	Brazil	S 24°10,890' W 46°50,563'
Callichthyidae	Corydoradinae	Lineage 3	<i>S. C113</i>	18834	LBP 2785	Aquario	Brazil	S 24°10,890' W 46°50,563'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. guapore</i>	46592	LBP 10089	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. guapore</i>	46593	LBP 10089	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. guapore</i>	46594	LBP 10089	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. guapore</i>	46595	LBP 10089	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. guapore</i>	46596	LBP 10089	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	22205	LBP 3772	Rio Negro Paraguai	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	22206	LBP 3772	Rio Negro Paraguai	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	22207	LBP 3772	Rio Negro Paraguai	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	22208	LBP 3772	Rio Negro Paraguai	Brazil	S 19°34'33.7" W 56°14'49.5"

Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	22209	LBP 3772	Rio Negro Paraguai	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	46636	LBP 10103	AfluentedoMadeira	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	46637	LBP 10103	AfluentedoMadeira	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	46638	LBP 10103	AfluentedoMadeira	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	46639	LBP 10103	AfluentedoMadeira	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. hastatus</i>	46640	LBP 10103	AfluentedoMadeira	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 4	<i>C. pygmaeus</i>	18848	LBP 2791	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 4	<i>C. pygmaeus</i>	18849	LBP 2791	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 4	<i>C. pygmaeus</i>	18850	LBP 2791	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 5	<i>A. pauciradiatus</i>	23981	LBP 4308	Rio Negro Amazonica	Brazil	S 00°51'57" W 62°46'24.7"
Callichthyidae	Corydoradinae	Lineage 5	<i>A. pauciradiatus</i>	23982	LBP 4308	IgarapeZamula Rio Negro Amazonica	Brazil	S 00°51'57" W 62°46'24.7"
Callichthyidae	Corydoradinae	Lineage 5	<i>A. cf pauciradiatus</i>	7188	LBP 548	BarcelosAM	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>A. cf pauciradiatus</i>	7189	LBP 548	BarcelosAM	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>A. cf pauciradiatus</i>	7190	LBP 548	BarcelosAM	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. elegans</i>	57698	LBP 14804	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. elegans</i>	57699	LBP 14804	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. elegans</i>	57700	LBP 14804	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. elegans</i>	57701	LBP 14804	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. elegans</i>	57702	LBP 14804	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	46608	LBP 10092	RibeiraodosVeados Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	46609	LBP 10092	RibeiraodosVeados Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	7226	LBP 5568	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	7227	LBP 5568	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	7228	LBP 5568	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. aff elegans</i>	46607	LBP 10092	RibeiraodosVeados Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. nijsseni</i>	32532	LBP 6861	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. nijsseni</i>	32533	LBP 6861	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. nijsseni</i>	32622	LBP 6861	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'

Callichthyidae	Corydoradinae	Lineage 5	<i>C. nijsseni</i>	32645	LBP 6861	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 5	<i>C. cf nijsseni</i>	5186	LBP 419	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 5	<i>C. undulatus</i>	7386	LBP 566	Rio Guaiba CosteiraRS	Brazil	S 30°02,820' W 51°22,347
Callichthyidae	Corydoradinae	Lineage 6	<i>C. albolineatus</i>	26290	LBP 5153	Rio Bugres Paraguai	Brazil	S 15°04'37" W 57°10'51"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. albolineatus</i>	18821	LBP 2780	Aquario	Brazil	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. albolineatus</i>	18822	LBP 2780	Aquario	Brazil	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	21668	LBP 3635	RibeiraoCavalo CosteiraSC	Brazil	S 26°28'17.2" W 49°10'55.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	21670	LBP 3635	RibeiraoCavalo CosteiraSC	Brazil	S 26°28'17.2" W 49°10'55.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	21671	LBP 3635	RibeiraoCavalo CosteiraSC	Brazil	S 26°28'17.2" W 49°10'55.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	21672	LBP 3635	RibeiraoCavalo CosteiraSC	Brazil	S 26°28'17.2" W 49°10'55.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	14437	LBP 2086	Rio Guabiroba Parana	Brazil	S 25°15'07.5" W 50°01'01.8"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32753	LBP 7713	CorregoLambari Paranapanema Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32755	LBP 7713	CorregoLambari Paranapanema Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32756	LBP 7713	CorregoLambari Paranapanema Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	21629	LBP 3620	Rio Itapucu CosteiraSC	Brazil	S 25°26'49.3" W 49°09'37.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	36124	LBP 17363	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	36126	LBP 17363	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	36128	LBP 17363	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32757	LBP 7713	CorregoLambari Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32756	LBP 7713	CorregoLambari Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. ehrhardti</i>	32759	LBP 7713	CorregoLambari Parana	Brazil	S 23°56'17.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. flaveolus</i>	17225	LBP 2613	Rio Capivara Tiete Parana	Brazil	S 22°53.963' W 48°23.204'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. flaveolus</i>	17226	LBP 2613	Rio Capivara Tiete Parana	Brazil	S 22°53.963' W 48°23.204'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. flaveolus</i>	21755	LBP 3670	Rio Capivara Tiete Parana	Brazil	S 22°45'48.2" W 48°15'41.8"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. flaveolus</i>	21756	LBP 3670	Rio Capivara Tiete Parana	Brazil	S 22°45'48.2" W 48°15'41.8"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. lymnades</i>	99929	LBP 28312	Rio Escuro SaoFrancisco	Brazil	S 17°44'53.1" W 47°05'40.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. lymnades</i>	99930	LBP 28312	Rio Escuro SaoFrancisco	Brazil	S 17°44'53.1" W 47°05'40.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. lymnades</i>	99931	LBP 28312	Rio Escuro SaoFrancisco	Brazil	S 17°44'53.1" W 47°05'40.1"

Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	29094	LBP 6466	ParaibadoSul Costeira	Brazil	S 23°22'08.5" W 46°01'28.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32331	LBP 6794	Rio Piraitinguinha Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32332	LBP 6794	Rio Piraitinguinha Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32333	LBP 6794	Rio Piraitinguinha Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32334	LBP 6794	Rio Piraitinguinha Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	40235	LBP 8886	Rio Tiete Parana	Brazil	S 23°34'35.0" W 45°58'28.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	40236	LBP 8886	Rio Tiete Parana	Brazil	S 23°34'35.0" W 45°58'28.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	40237	LBP 8886	Rio Tiete Parana	Brazil	S 23°34'35.0" W 45°58'28.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	40238	LBP 8886	Rio Tiete Parana	Brazil	S 23°34'35.0" W 45°58'28.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	40239	LBP 8886	Rio Tiete Parana	Brazil	S 23°34'35.0" W 45°58'28.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32330	LBP 6794	Rio Piraitinguinha Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	28177	LBP 5777	Rio Paraiuna ParaibadoSul Costeira	Brazil	S 22°01'53.8" W 43°08'58.7"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	28178	LBP 5777	Rio Paraiuna ParaibadoSul Costeira	Brazil	S 22°01'53.8" W 43°08'58.7"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	28179	LBP 5777	Rio Paraiuna ParaibadoSul Costeira	Brazil	S 22°01'53.8" W 43°08'58.7"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	28180	LBP 5777	Rio Paraiuna ParaibadoSul Costeira	Brazil	S 22°01'53.8" W 43°08'58.7"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	28181	LBP 5777	Rio Paraiuna ParaibadoSul Costeira	Brazil	S 22°01'53.8" W 43°08'58.7"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	11109	LBP 1266	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	11110	LBP 1266	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	35382	LBP 7392	Rio Jaguaru RibeiradeIguape Costeira	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	35385	LBP 7392	Rio Jaguaru RibeiradeIguape Costeira	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32776	LBP 7714	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32777	LBP 7714	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32778	LBP 7714	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32779	LBP 7714	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	32780	LBP 7714	Rio Fau RibeiradeIguape Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>C. nattereri</i>	17106	LBP 2579	CosteiraRJ	Brazil	S 22°14'7.0" W 41°51'44.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36114	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9" W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36119	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9" W 50°43'44.5"

Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36121	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	29047	LBP 6036	Rio Maquine CosteiraRS	Brazil	S 29°42'04.5'' W 50°11'12.0''
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	29050	LBP 6036	Rio Maquine CosteiraRS	Brazil	S 29°42'04.5'' W 50°11'12.0''
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	29051	LBP 6036	Rio Maquine CosteiraRS	Brazil	S 29°42'04.5'' W 50°11'12.0''
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60762	LBP 14511	CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60763	LBP 14511	CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	55246	LBP 13210	Rio Piraquara Rio Iguacu	Brazil	S 25°28'49.0" W 49°06'10.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	55247	LBP 13210	Rio Piraquara Rio Iguacu	Brazil	S 25°28'49.0" W 49°06'10.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	55248	LBP 13210	Rio Piraquara Rio Iguacu	Brazil	S 25°28'49.0" W 49°06'10.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	55249	LBP 13210	Rio Piraquara Rio Iguacu	Brazil	S 25°28'49.0" W 49°06'10.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	55250	LBP 13210	Rio Piraquara Rio Iguacu	Brazil	S 25°28'49.0" W 49°06'10.2"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60762	LBP 14511	Riachosemnome CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60763	LBP 14511	Riachosemnome CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60765	LBP 14511	Riachosemnome CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36116	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36117	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	36122	LBP 17362	CorregoLambari Parana	Brazil	S 23°56'16.9' W 50°43'44.5"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60761	LBP 14511	Riachosemnome CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	60764	LBP 14511	Riachosemnome CosteiraRS	Brazil	S 33°41'22.6" W 53°26'22.3"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	29048	LBP 6036	Rio Maquine CosteiraRS	Brazil	S 29°42'04.5'' W 50°11'12.0''
Callichthyidae	Corydoradinae	Lineage 6	<i>C. paleatus</i>	29049	LBP 6036	Rio Maquine CosteiraRS	Brazil	S 29°42'04.5'' W 50°11'12.0''
Callichthyidae	Corydoradinae	Lineage 6	<i>C. potaroensis</i>	18807	LBP 2775	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C68</i>	32901	LBP 7214	CorregoTaquaral TocantinsAraguaia Amazonica	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C68</i>	32902	LBP 7214	CorregoTaquaral TocantinsAraguaia Amazonica	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C68</i>	32903	LBP 7214	CorregoTaquaral TocantinsAraguaia Amazonica	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C68</i>	32904	LBP 7214	CorregoTaquaral TocantinsAraguaia Amazonica	Brazil	S 15°40.678' W 52°17.863"

Callichthyidae	Corydoradinae	Lineage 6	<i>C. C68</i>	32926	LBP 7214	Corrego Taquaral Tocantins Araguaia Amazonica	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C73</i>	18858	LBP 2794	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C73</i>	18859	LBP 2794	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C73</i>	18857	LBP 2794	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C144</i>	57194	LBP 13806	Rio Tapajos Amazonica	Brazil	S 04°33'09.7" W 56°17'59.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C144</i>	57195	LBP 13806	Rio Tapajos Amazonica	Brazil	S 04°33'09.7" W 56°17'59.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. C144</i>	57196	LBP 13806	Rio Tapajos Amazonica	Brazil	S 04°33'09.7" W 56°17'59.6"
Callichthyidae	Corydoradinae	Lineage 6	<i>C. sp</i>	53881	LBP 12488	Aquario Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 6	<i>C. sp</i>	53880	LBP 12488	Aquario Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	22470	LBP 3896	Rio Novo Paranapanema Parana	Brazil	S 23°01'27.4" W 48°49'41.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	7138	LBP 535	Igarapedo Mindu Amazonica	Brazil	S 03°04.900' W 59°59.855'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	7139	LBP 535	Igarapedo Mindu Amazonica	Brazil	S 03°04.900' W 59°59.855'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	11446	LBP 1349	Iquitos Loreto	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	11447	LBP 1349	Iquitos Loreto	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	11448	LBP 1349	Iquitos Loreto	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	11449	LBP 1349	Iquitos Loreto	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	11451	LBP 1349	Iquitos Loreto	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45783	LBP 9686	Corregodo Baile Parana	Brazil	S 22°18'22.5' W 53°23'47.2"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18827	LBP 2782	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18828	LBP 2782	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18829	LBP 2782	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18875	LBP 2800	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17236	LBP 2595	Rio Tiete Parana	Brazil	S 21°00'46.6" W 49°41'25.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	22466	LBP 3896	Rio Novo Paranapanema Parana	Brazil	S 23°01'27.4" W 48°49'41.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	22467	LBP 3896	Rio Novo Paranapanema Parana	Brazil	S 23°01'27.4" W 48°49'41.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	22468	LBP 3896	Rio Novo Paranapanema Parana	Brazil	S 23°01'27.4" W 48°49'41.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	20396	LBP 3401	Rio Capivara Tiete Parana	Brazil	S 22°52'20.9" W 48°22'27.3"

Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	20397	LBP 3401	Rio Capivara Tiete Parana	Brazil	S 22°52'20.9" W 48°22'27.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	20398	LBP 3401	Rio Capivara Tiete Parana	Brazil	S 22°52'20.9" W 48°22'27.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17234	LBP 2595	Rio Tiete Parana	Brazil	S 21°00'46.6" W 49°41'25.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	22469	LBP 3896	Rio Novo Paranapanema Parana	Brazil	S 23°01'27.4" W 48°49'41.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17235	LBP 2595	Rio Tiete Parana	Brazil	S 21°00'46.6" W 49°41'25.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	19371	LBP 3183	Rio Conchas Tiete Parana	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	19373	LBP 3183	Rio Conchas Tiete Parana	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	19375	LBP 3183	Rio Conchas Tiete Parana	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	24837	LBP 4691	RibeiraoCubatao Tiete Parana	Brazil	S 21°11'35' W 49°07'22'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	24838	LBP 4691	RibeiraoCubatao Tiete Parana	Brazil	S 21°11'35' W 49°07'22'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	24839	LBP 4691	RibeiraoCubatao Tiete Parana	Brazil	S 21°11'35' W 49°07'22'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	24840	LBP 4691	RibeiraoCubatao Tiete Parana	Brazil	S 21°11'35' W 49°07'22'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17232	LBP 2595	Rio Tiete Parana	Brazil	S 21°00'46.6" W 49°41'25.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	19590	LBP 2970	Rio Pardo Paranapanema Parana	Brazil	S 22°59'25.3" W 48°25'37.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	32002	LBP 6617	CorregodasTresLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	31944	LBP 6617	CorregodasTresLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	31945	LBP 6617	CorregodasTresLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	47753	LBP 10210	Rio Parana	Brazil	S 20°42'21.0' W 51°45'23.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	47754	LBP 10210	Rio Parana	Brazil	S 20°42'21.0' W 51°45'23.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	47755	LBP 10210	Rio Parana	Brazil	S 20°42'21.0' W 51°45'23.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	49855	LBP 10775	RibeiraodosVeados Rio Taquari Parana	Brazil	S 18°25'24.4" W 54°50'05.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	44402	LBP 8930	Rio Paranaparema Parana	Brazil	S 22°38'05.0' W 52°49'19.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45619	LBP 9633	CorregoOuroVerde Parana	Brazil	S 22°07'59.7' W 53°46'08.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45620	LBP 9633	CorregoOuroVerde Parana	Brazil	S 22°07'59.7' W 53°46'08.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45621	LBP 9633	CorregoOuroVerde Parana	Brazil	S 22°07'59.7' W 53°46'08.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45622	LBP 9633	CorregoOuroVerde Parana	Brazil	S 22°07'59.7' W 53°46'08.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	45623	LBP 9633	CorregoOuroVerde Parana	Brazil	S 22°07'59.7' W 53°46'08.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	10970	LBP 1327	Rio Keller Rio Ivai Parana	Brazil	S 23°38'30.1" W 51°51'32.3"

Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	31943	LBP 6617	CorregodasTresLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17375	LBP 2602	SaoJosedosDourados Parana	Brazil	S 20°44'43.7" W 49°46'45.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17376	LBP 2602	SaoJosedosDourados Parana	Brazil	S 20°44'43.7" W 49°46'45.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17377	LBP 2602	SaoJosedosDourados Parana	Brazil	S 20°44'43.7" W 49°46'45.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17378	LBP 2602	SaoJosedosDourados Parana	Brazil	S 20°44'43.7" W 49°46'45.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	17379	LBP 2602	SaoJosedosDourados Parana	Brazil	S 20°44'43.7" W 49°46'45.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	10971	LBP 1327	Rio Keller Rio Ivai Parana	Brazil	S 23°38'30.1" W 51°51'32.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	10974	LBP 1327	Rio Keller Rio Ivai Parana	Brazil	S 23°38'30.1" W 51°51'32.3"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	32001	LBP 6617	CorregodasTrêsLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	32002	LBP 6617	CorregodasTrêsLagoas Parana	Brazil	S 22°37'57.3' W 53°03'09.4"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	44403	LBP 8930	Rio Paranaparema Parana	Brazil	S 22°38'05.0' W 52°49'19.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	44404	LBP 8930	Rio Paranaparema Parana	Brazil	S 22°38'05.0' W 52°49'19.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	44405	LBP 8930	Rio Paranaparema Parana	Brazil	S 22°38'05.0' W 52°49'19.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	44406	LBP 8930	Rio Paranaparema Parana	Brazil	S 22°38'05.0' W 52°49'19.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	19372	LBP 3183	Rio Conchas Tiete Parana	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41546	LBP 8417	Rio semnome Paraguai	Brazil	S 14°27'26.3' W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41547	LBP 8417	Rio semnome Paraguai	Brazil	S 14°27'26.3' W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41548	LBP 8417	Rio semnome Paraguai	Brazil	S 14°27'26.3' W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41549	LBP 8417	Rio semnome Paraguai	Brazil	S 14°27'26.3' W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41550	LBP 8417	Rio semnome Paraguai	Brazil	S 14°27'26.3' W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	19374	LBP 3183	Rio Conchas Tiete Parana	Brazil	S 23°00'46.0" W 47°59'51.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	41653	LBP 8437	Rio Sepotuba Paraguai	Brazil	S 14°30'04.4' W 57°34'38.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	19512	LBP 2940	Rio Tiete Parana	Brazil	S 23°30'40.3" W 45°51'32.6"
Callichthyidae	Corydoradinae	Lineage 7	<i>C.aeneus</i>	49258	LBP 10562	Rio Acre, Amazonica	Brazil	S 09°55'52.9" W 67°53'07.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18818	LBP 2779	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	18819	LBP 2779	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	62055	LBP 6844	BaixoTacarigua Atlantico TrinidadTobago	Brazil	N 10°38.495' W 61°22.472'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aeneus</i>	Rack8H2	LBP 6844	BaixoTacarigua Atlantico TrinidadTobago	Brazil	N 10°38.495' W 61°22.472'

Callichthyidae	Corydoradinae	Lineage 7	<i>C. aff aeneus</i>	66639	LBP 17314	Rio Xingu Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	27403	LBP 5640	Rio Coxipo Rio Cuiaba Paraguai	Brazil	S 15°36'20.1" W 56°03'06.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	27405	LBP 5640	Rio Coxipo Rio Cuiaba Paraguai	Brazil	S 15°36'20.1" W 56°03'06.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	27406	LBP 5640	Rio Coxipo Rio Cuiaba Paraguai	Brazil	S 15°36'20.1" W 56°03'06.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	27407	LBP 5640	Rio Coxipo Rio Cuiaba Paraguai	Brazil	S 15°36'20.1" W 56°03'06.9"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	8122	LBP 17379	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	8110	LBP 17379	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	8059	LBP 17379	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	49257	LBP 10562	Rio Acre, Amazonica	Brazil	S 09°55'52.9" W 67°53'07.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. cf aeneus</i>	49260	LBP 10562	Rio Acre, Amazonica	Brazil	S 09°55'52.9" W 67°53'07.8"
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aff aeneus</i>	66638	LBP 17314	Rio Xingu Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. aff aeneus</i>	66640	LBP 17314	Rio Xingu Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	18942	LBP 2827	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	57752	LBP 14816	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	57753	LBP 14816	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	57754	LBP 14816	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	57755	LBP 14816	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. rabauti</i>	57756	LBP 14816	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>C. zygatus</i>	5277	LBP 422	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. acutus</i>	43819	LBP 9316	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. acutus</i>	43820	LBP 9316	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. acutus</i>	43821	LBP 9316	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. acutus</i>	43822	LBP 9316	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. acutus</i>	43823	LBP 9316	Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. agassizii</i>	57762	LBP 14818	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. agassizii</i>	57763	LBP 14818	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. agassizii</i>	57764	LBP 14818	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. agassizii</i>	57765	LBP 14818	Rio Amazonas	Peru	-

Callichthyidae	Corydoradinae	Lineage 8	<i>C. agassizii</i>	57766	LBP 14818	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. amandajanea</i>	18824	LBP 2781	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. ambiacus</i>	57767	LBP 14819	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. ambiacus</i>	57768	LBP 14819	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. ambiacus</i>	57769	LBP 14819	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. ambiacus</i>	18952	LBP 2833	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf ambiacus</i>	7197	LBP 13331	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf ambiacus</i>	7198	LBP 13331	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf ambiacus</i>	7199	LBP 13331	Rio Purus Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. britskii</i>	8113	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. britskii</i>	8116	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. britskii</i>	8065	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. britskii</i>	8112	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. britskii</i>	8117	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. aff britskii</i>	8108	LBP 688	AfluentePirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. aff britskii</i>	3601	LBP 15	Rio Miranda Paraguai	Brazil	S 19°34,630' W 57°01,123'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. condiscipulus</i>	47950	LBP 9311	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. condiscipulus</i>	47951	LBP 9311	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. delphax</i>	18832	LBP 2784	Aquario	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	59918	LBP 11720	Rio Parnaiba Parana	Brazil	S 19°09'05.0" W 46°15'47.0"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	59919	LBP 11720	Rio Parnaiba Parana	Brazil	S 19°09'05.0" W 46°15'47.0"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	59920	LBP 11720	Rio Parnaiba Parana	Brazil	S 19°09'05.0" W 46°15'47.0"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	59921	LBP 11720	Rio Parnaiba Parana	Brazil	S 19°09'05.0" W 46°15'47.0"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	59922	LBP 11720	Rio Parnaiba Parana	Brazil	S 19°09'05.0" W 46°15'47.0"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	60150	LBP 11669	Rio GuardaMor SaoFrancisco	Brazil	S 17°46'16.8" W 47°06'15.8"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	60151	LBP 11669	Rio GuardaMor SaoFrancisco	Brazil	S 17°46'16.8" W 47°06'15.8"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	60152	LBP 11669	Rio GuardaMor SaoFrancisco	Brazil	S 17°46'16.8" W 47°06'15.8"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	60153	LBP 11669	Rio GuardaMor SaoFrancisco	Brazil	S 17°46'16.8" W 47°06'15.8"

Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	40007	LBP 8301	Rio Araras Parana	Brazil	S 22°22'42.4" W 47°25'37.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	40008	LBP 8301	Rio Araras Parana	Brazil	S 22°22'42.4" W 47°25'37.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	40009	LBP 8301	Rio Araras Parana	Brazil	S 22°22'42.4" W 47°25'37.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	40010	LBP 8301	Rio Araras Parana	Brazil	S 22°22'42.4" W 47°25'37.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	40011	LBP 8301	Rio Araras Parana	Brazil	S 22°22'42.4" W 47°25'37.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. difluviatilis</i>	100235	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. garbei</i>	DCC03920	LBP	HQ600821	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	18890	LBP 2807	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	18891	LBP 2807	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	18892	LBP 2807	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	46597	LBP 10090	AfluyenteRio Guapore Madeira Amazonica	Brazil	S 14°96.904' W 59°98.319'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	46598	LBP 10090	AfluyenteRio Guapore Madeira Amazonica	Brazil	S 14°96.904' W 59°98.319'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	46599	LBP 10090	AfluyenteRio Guapore Madeira Amazonica	Brazil	S 14°96.904' W 59°98.319'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. heraldschultzi</i>	46631	LBP 10100	Rio Guapore Madeira Amazonica	Brazil	S 15°01.0642" W 59°95.762'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. imitator</i>	32502	LBP 6862	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. imitator</i>	32503	LBP 6862	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. imitator</i>	32504	LBP 6862	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. imitator</i>	32505	LBP 6862	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. imitator</i>	32506	LBP 6862	IgarapePuranga Rio Negro Amazonica	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8120	LBP 690	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8125	LBP 691	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8126	LBP 691	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8127	LBP 691	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8064	LBP 691	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	46566	LBP 9890	Rio Abobral Paraguai	Brazil	S 19°26'02.1" W 57°03'08.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	46567	LBP 9890	Rio Abobral Paraguai	Brazil	S 19°26'02.1" W 57°03'08.9"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	46569	LBP 9890	Rio Abobral Paraguai	Brazil	S 19°26'02.1" W 57°03'08.9"

Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	28267	LBP 5809	BaiadasPiranhas Paraguai	Brazil	S 15°44'3.60" W 55°52'48.7"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. latus</i>	8061	LBP 690	AfluentedoPirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melanistius</i>	43088	LBP 9149	Rio Guama Amazonica	Brazil	S 01°32'43.6" W 47°07'37.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melanistius</i>	43089	LBP 9149	Rio Guama Amazonica	Brazil	S 01°32'43.6" W 47°07'37.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melanistius</i>	7264	LBP 5640	AltoRio Negro Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melanistius</i>	7265	LBP 5640	AltoRio Negro Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melanistius</i>	7266	LBP 5640	AltoRio Negro Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. aff melanistius</i>	18872	LBP 2799	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. aff melanistius</i>	18873	LBP 2799	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. aff melanistius</i>	18874	LBP 2799	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf melanistius</i>	46733	LBP 9962	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf melanistius</i>	46734	LBP 9962	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf melanistius</i>	46735	LBP 9962	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf melanistius</i>	46736	LBP 9962	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf melanistius</i>	46737	LBP 9962	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melini</i>	32652	LBP 7711	Rio Tiquie Rio Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melini</i>	32653	LBP 7711	Rio Tiquie Rio Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melini</i>	32654	LBP 7711	Rio Tiquie Rio Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melini</i>	32655	LBP 7711	Rio Tiquie Rio Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. melini</i>	32662	LBP 7711	Rio Tiquie Rio Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53768	LBP 12460	Amazonica	Peru	S 03°18'46.1" W 71°50'58.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53770	LBP 12460	Amazonica	Peru	S 03°18'46.1" W 71°50'58.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53533	LBP 12531	Rio Itaya Amazonica	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53534	LBP 12531	Rio Itaya Amazonica	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53535	LBP 12531	Rio Itaya Amazonica	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. multiradiatus</i>	53536	LBP 12531	Rio Itaya Amazonica	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. orphnopterus</i>	53945	LBP 12509	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. orphnopterus</i>	53946	LBP 12509	Rio Amazonas	Peru	-

Callichthyidae	Corydoradinae	Lineage 8	<i>C. orphnopterus</i>	53947	LBP 12509	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. orphnopterus</i>	53948	LBP 12509	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. orphnopterus</i>	53949	LBP 12509	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. pulcher</i>	8957	LBP 909	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. pulcher</i>	8952	LBP 909	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf pulcher</i>	7244	LBP 560	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf pulcher</i>	7245	LBP 560	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf pulcher</i>	7246	LBP 560	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C punctatus</i>	53837	LBP 12476	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C punctatus</i>	53838	LBP 12476	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C punctatus</i>	53839	LBP 12476	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C punctatus</i>	53840	LBP 12476	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C punctatus</i>	53841	LBP 12476	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. reticulatus</i>	57744	LBP 14814	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. reticulatus</i>	57746	LBP 14814	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. reticulatus</i>	57747	LBP 14814	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. reticulatus</i>	57748	LBP 14814	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. reticulatus</i>	57745	LBP 14814	Aquario Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. robinae</i>	18880	LBP 2803	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. robineae</i>	8951	LBP 908	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. robineae</i>	8959	LBP 908	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf robustus</i>	18930	LBP 2822	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf robustus</i>	18931	LBP 2822	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. cf robustus</i>	18932	LBP 2822	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. seussi</i>	7173	LBP 545	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. seussi</i>	7174	LBP 545	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. seussi</i>	7175	LBP 545	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	7211	LBP 553	Rio Purus Amazonica	-	-

Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	7213	LBP 553	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	7214	LBP 553	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	7125	LBP 530	AltoRio Negro	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	18851	LBP	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	18852	LBP	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. sodalis</i>	18853	LBP	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53936	LBP 12507	Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53937	LBP 12507	Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53938	LBP 12507	Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53939	LBP 12507	Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	7222	LBP 555	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53532	LBP 12507	Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	41220	LBP 12794	Rio Araguaia Amazonica	Brazil	S 13°18'37.3" W 50°36'47.6"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	41220	LBP 12794	Rio Araguaia Amazonica	Brazil	S 13°18'37.3" W 50°36'47.6"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	41220	LBP 12794	Rio Araguaia Amazonica	Brazil	S 13°18'37.3" W 50°36'47.6"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	41220	LBP 12794	Rio Araguaia Amazonica	Brazil	S 13°18'37.3" W 50°36'47.6"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	41220	LBP 12794	Rio Araguaia Amazonica	Brazil	S 13°18'37.3" W 50°36'47.6"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	36755	LBP 7796	Rio Araguaia Amazonica	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53769	LBP 12498	Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	87578	LBP 22528	AfluentedoJavari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	87579	LBP 22528	AfluentedoJavari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	87580	LBP 22528	AfluentedoJavari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	5585	LBP 424	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	87582	LBP 22528	AfluentedoJavari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	5586	LBP 424	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	14216	LBP 2017	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	87581	LBP 22528	Rio Javari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. splendens</i>	53533	LBP 12507	Amazonas	-	-

Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57770	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57771	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57772	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57774	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57773	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	57770	LBP 14820	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. virginiae</i>	18962	LBP 2837	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. C52 aff sterbai</i>	18815	LBP 2778	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp	87786	LBP 22532	Rio Javari Amazonica	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp	8115	LBP 688	Afluente Pirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp	41751	LBP 8503	Rio Salobra Paraguai	Brazil	S 15°19'53.5' W 57°11'31.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	22987	LBP 4075	Rio Japim Rio Jurua Amazonica	Brazil	S 07°34'28.8' W 72°55'24.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	22988	LBP 4075	Rio Japim Rio Jurua Amazonica	Brazil	S 07°34'28.8' W 72°55'24.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	22989	LBP 4075	Rio Japim Rio Jurua Amazonica	Brazil	S 07°34'28.8' W 72°55'24.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	22990	LBP 4075	Rio Japim Rio Jurua Amazonica	Brazil	S 07°34'28.8' W 72°55'24.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	22991	LBP 4075	Rio Japim Rio Jurua Amazonica	Brazil	S 07°34'28.8' W 72°55'24.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	18947	LBP 2831	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	18948	LBP 2831	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	23687	LBP 4149	Rio Moa Rio Jurua Amazonica	Brazil	S 07°26'35.5' W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	23688	LBP 4149	Rio Moa Rio Jurua Amazonica	Brazil	S 07°26'35.5' W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	23689	LBP 4149	Rio Moa Rio Jurua Amazonica	Brazil	S 07°26'35.5' W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	23690	LBP 4149	Rio Moa Rio Jurua Amazonica	Brazil	S 07°26'35.5' W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. acrensis</i>	23691	LBP 4149	Rio Moa Rio Jurua Amazonica	Brazil	S 07°26'35.5' W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32735	LBP 6863	Igarape Puranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32527	LBP 6863	Igarape Puranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32528	LBP 6863	Igarape Puranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32529	LBP 6863	Igarape Puranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32530	LBP 6863	Igarape Puranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'

Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32543	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32544	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32545	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32546	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32547	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32621	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32650	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32646	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. adolfoi</i>	32649	LBP 6863	IgarapePuranga Rio Negro	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf adolfoi</i>	70137	LBP 17850	Rio Marauia Rio Negro Amazonica	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf adolfoi</i>	70138	LBP 17850	Rio Marauia Rio Negro Amazonica	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf adolfoi</i>	70139	LBP 17850	Rio Marauia Rio Negro Amazonica	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf adolfoi</i>	70140	LBP 17850	Rio Marauia Rio Negro Amazonica	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf adolfoi</i>	70141	LBP 17850	Rio Marauia Rio Negro Amazonica	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. araguaiensis</i>	13287	LBP 1861	Rio Insula Rio Araguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. araguaiensis</i>	13288	LBP 1861	Rio Insula Rio Araguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. araguaiensis</i>	13289	LBP 1861	Rio Insula Rio Araguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. araguaiensis</i>	13291	LBP 1861	Rio Insula Rio Araguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. arcuatus</i>	18843	LBP 2789	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. arcuatus</i>	11443	LBP 1348	Iquitos	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. arcuatus</i>	18917	LBP 2817	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. arcuatus</i>	50789	LBP 11093	Rio Madeira Amazonica	Brazil	S 07°34'25.9" W 63°06'40.8"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. atropersonatus</i>	18939	LBP 2826	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	18866	LBP 2797	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	19704	LBP 3207	Rio Orinoco	Venezuela	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	19705	LBP 3207	Rio Orinoco	Venezuela	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	19706	LBP 3207	Rio Orinoco	Venezuela	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	19707	LBP 3207	Rio Orinoco	Venezuela	-

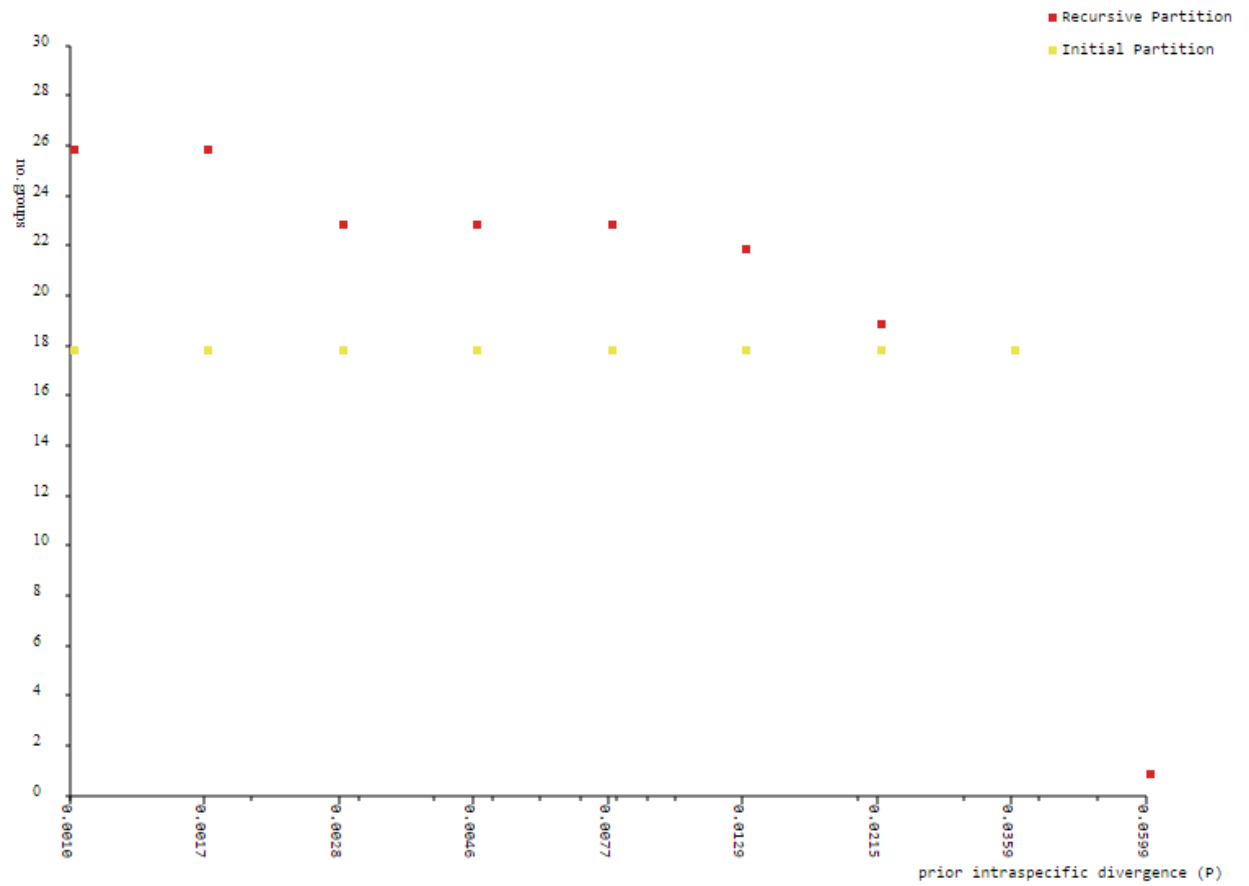
Callichthyidae	Corydoradinae	Lineage 9	<i>C. brevirostris</i>	19708	LBP 3207	Rio Orinoco	Venezuela	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf brevirostris</i>	46732	LBP 9961	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf brevirostris</i>	46731	LBP 9961	Rio Pelehojo Orinoco	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. burgessi</i>	32741	LBP 6867	Rio Negro Amazonas	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. burgessi</i>	32742	LBP 6867	Rio Negro Amazonas	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. burgessi</i>	32743	LBP 6867	Rio Negro Amazonas	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. burgessi</i>	32750	LBP 6867	Rio Negro Amazonas	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. burgessi</i>	32747	LBP 6867	Rio Negro Amazonas	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. caudimaculatus</i>	7253	LBP 562	Rio Paraguai Paraguai	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. caudimaculatus</i>	7254	LBP 562	Rio Paraguai Paraguai	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. caudimaculatus</i>	7256	LBP 562	Rio Paraguai Paraguai	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. caudimaculatus</i>	18869	LBP 2798	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. caudimaculatus</i>	18870	LBP 2798	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cochui</i>	13272	LBP 1853	Rio Insula Rio Araguaia TocantinsAraguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cochui</i>	13273	LBP 1853	Rio Insula Rio Araguaia TocantinsAraguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cochui</i>	13274	LBP 1853	Rio Insula Rio Araguaia TocantinsAraguaia	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. concolor</i>	18935	LBP 2824	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. davidsandsi</i>	7201	LBP 551	AltoRio Negro Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. davidsandsi</i>	7202	LBP 551	AltoRio Negro Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. davidsandsi</i>	7203	LBP 551	AltoRio Negro Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. davidsandsi</i>	7204	LBP 551	AltoRio Negro Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. davidsandsi</i>	7205	LBP 551	AltoRio Negro Amazonas	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100227	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100228	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100229	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100230	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100231	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf. difluviatilis</i>	100234	LBP 28258	RibeiraodeFurnas Rio Parana	Brazil	S 22°23'00.9" W 47°25'49.2"

Callichthyidae	Corydoradinae	Lineage 9	<i>C. diphyes</i>	18904	LBP 2813	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. diphyes</i>	18905	LBP 2813	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. diphyes</i>	18906	LBP 2813	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. duplicareus</i>	7102	LBP 525	AltoRio Negro Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. duplicareus</i>	7103	LBP 525	AltoRio Negro Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. grantii</i>	32587	LBP 7709	IgarapeYamirim Rio Negro Amazonica	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. grantii</i>	32588	LBP 7709	IgarapeYamirim Rio Negro Amazonica	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. grantii</i>	32589	LBP 7709	IgarapeYamirim Rio Negro Amazonica	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. grantii</i>	32590	LBP 7709	IgarapeYamirim Rio Negro Amazonica	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. grantii</i>	32591	LBP 7709	IgarapeYamirim Rio Negro Amazonica	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46628	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46629	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46630	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46631	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46632	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46633	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46634	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46635	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff griseus</i>	46627	LBP 10099	Rio JaciParana Madeira Amazonica	Brazil	S 09°25.644' W 64°38.762'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. gossei</i>	7169	LBP 544	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. gossei</i>	7171	LBP 544	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. gossei</i>	18898	LBP 2811	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. habrosus</i>	18893	LBP 2808	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43450	LBP 8613	Rio Guama, Amazonas	Brazil	S 14°08'39.8' W 56°05'48.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43451	LBP 8613	Rio Guama, Amazonas	Brazil	S 14°08'39.8' W 56°05'48.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43452	LBP 8613	Rio Guama, Amazonas	Brazil	S 14°08'39.8' W 56°05'48.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43453	LBP 8613	Rio Guama, Amazonas	Brazil	S 14°08'39.8' W 56°05'48.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43454	LBP 8613	Rio Guama, Amazonas	Brazil	S 14°08'39.8' W 56°05'48.6"

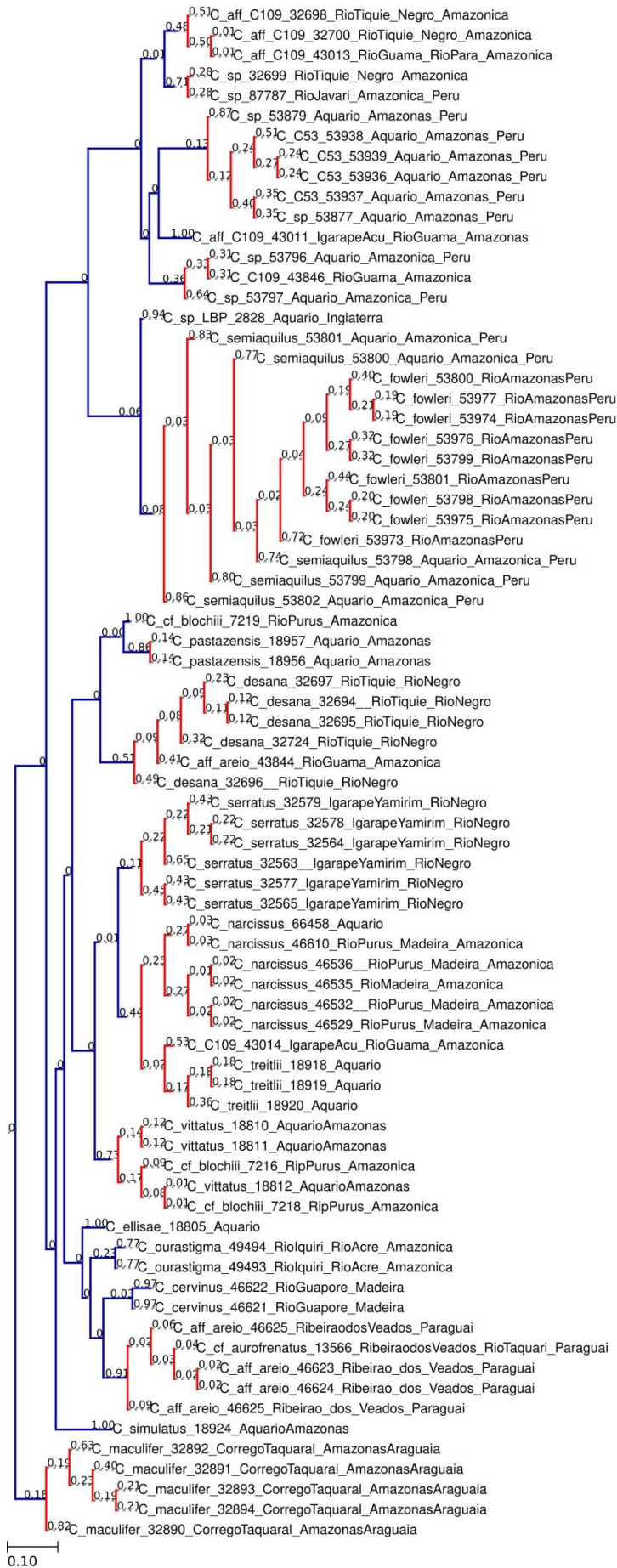
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43814	LBP 9315	IgarapeJoaoAlves Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43815	LBP 9315	IgarapeJoaoAlves Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43816	LBP 9315	IgarapeJoaoAlves Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43817	LBP 9315	IgarapeJoaoAlves Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43818	LBP 9315	IgarapeJoaoAlves Rio Guama Amazonica	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. julii</i>	43019	LBP 9124	IgarapeAcu Rio Guama Amazonica	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. leucomelas</i>	18942	LBP 2827	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5608	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5601	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5602	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5606	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5610	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. metade</i>	5599	LBP 428	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. panda</i>	18846	LBP 2790	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>C. pantanalensis</i>	22283	LBP 3773	Rio Negro Paraguai AquidauanaMS	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. pantanalensis</i>	22285	LBP 3773	Rio Negro Paraguai AquidauanaMS	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>C. pantanalensis</i>	22284	LBP 3773	Rio Negro Paraguai AquidauanaMS	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. polystictus</i>	13659	LBP 1958	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. polystictus</i>	13645	LBP 1958	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. polystictus</i>	13564	LBP 1958	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. polystictus</i>	13600	LBP 1958	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	46600	LBP 10091	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	46601	LBP 10091	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	46602	LBP 10091	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	46603	LBP 10091	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	46604	LBP 10091	RibeiraodosVeados Rio Taquari Paraguai	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	8123	LBP 689	AfluentedoRio Pirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff polystictus</i>	8056	LBP 689	AfluentedoRio Pirai Paraguai	Brazil	S 16°25,680' W 56°25,143'

Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf polystictus</i>	8111	LBP 689	AfluentedoRio Pirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf polystictus</i>	8057	LBP 689	AfluentedoRio Pirai Paraguai	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	7119	LBP 1783	AltoRio Negro	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	7120	LBP 1783	AltoRio Negro	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	7121	LBP 1783	AltoRio Negro	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	18840	LBP 2788	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	58742	LBP	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. schwartzi</i>	58744	LBP	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf schwartzi</i>	7251	LBP 561	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf schwartzi</i>	7250	LBP 561	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf schwartzi</i>	7248	LBP 561	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf schwartzi</i>	7249	LBP 561	Rio Purus Amazonica	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. similis</i>	49457	LBP 10648	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. similis</i>	49458	LBP 10648	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. similis</i>	49459	LBP 10648	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. similis</i>	49460	LBP 10648	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. similis</i>	49461	LBP 10648	Rio Iquiri Rio Acre Amazonica	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sterbai</i>	7164	LBP 543	Paraguai CuiabaMT	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sterbai</i>	7165	LBP 543	Paraguai CuiabaMT	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sterbai</i>	18814	LBP 2777	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32690	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32687	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32688	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32689	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32683	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	18885	LBP 2804	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	7193	LBP 549	AltoRio Negro	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32681	LBP 6872	Rio Tiquie Negro Amazonica	Brazil	N 00°15' W 69°50'

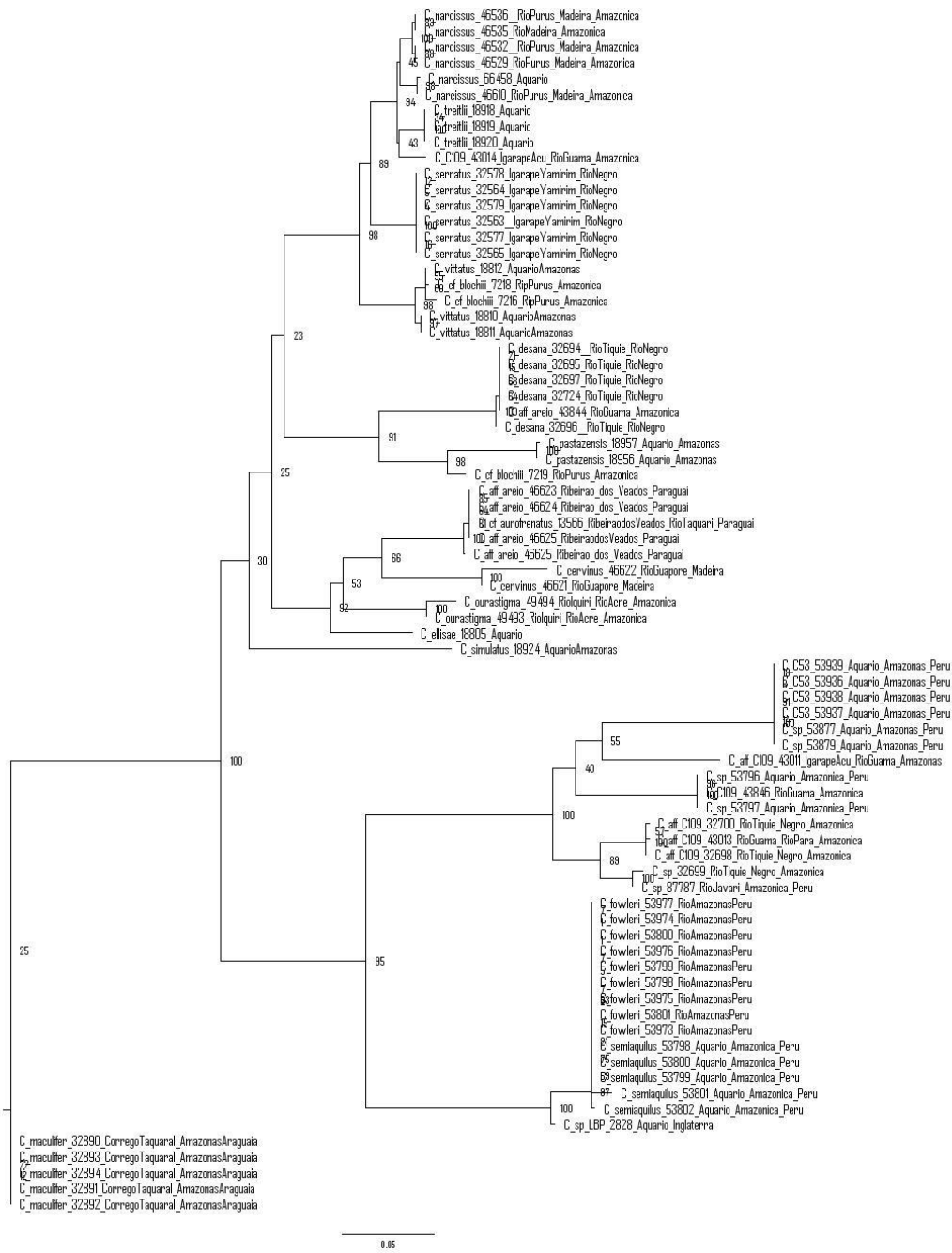
Callichthyidae	Corydoradinae	Lineage 9	<i>C. tukano</i>	32682	LBP 6872	Rio Tiquie Negra Amazonica	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. urucu</i>	46612	LBP 10095	Rio Madeira Amazonica	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. urucu</i>	46613	LBP 10095	Rio Madeira Amazonica	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. urucu</i>	46614	LBP 10095	Rio Madeira Amazonica	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. urucu</i>	46615	LBP 10095	Rio Madeira Amazonica	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. virescens</i>	46619	LBP 10096	Rio Paraguai	Brazil	S 16°06.352' W 57°70.750'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff virescens</i>	46620	LBP 10096	Rio Paraguai	Brazil	S 16°06.352' W 57°70.750'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff virescens</i>	46616	LBP 10096	Rio Paraguai	Brazil	S 16°06.352' W 57°70.750'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff virescens</i>	46617	LBP 10096	Rio Paraguai	Brazil	S 16°06.352' W 57°70.750'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. aff virescens</i>	46618	LBP 10096	Rio Paraguai	Brazil	S 16°06.352' W 57°70.750'
Callichthyidae	Corydoradinae	Lineage 9	<i>C. weitzmani</i>	57749	LBP 14815	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. weitzmani</i>	57750	LBP 14815	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. weitzmani</i>	57751	LBP 14815	Rio Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C43</i>	18953	LBP 2834	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C43</i>	18954	LBP 2834	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. cf C43</i>	18955	LBP 2834	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C52</i>	18815	LBP 2778	Aquario	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C76</i>	23665	LBP 4140	Rio Moa Jurua Madeira Amazonica	Brazil	S 07°26'35.5" W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C91</i>	53742	LBP 12495	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C91</i>	53743	LBP 12495	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C91</i>	53744	LBP 12495	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. C91</i>	53760	LBP 12495	Amazonas	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sp</i>	87788	LBP 22532	Rio Javari Solimoes Amazonica	Brazil	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sp</i>	43014	LBP 9122	Rio Guama Amazonica	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sp</i>	53795	LBP 12466	Amazonica	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sp</i>	87784	LBP 22532	Rio Javari Solimoes Amazonica	Brazil	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>C. sp</i>	53879	LBP 12488	Aquario Amazonica	Peru	-



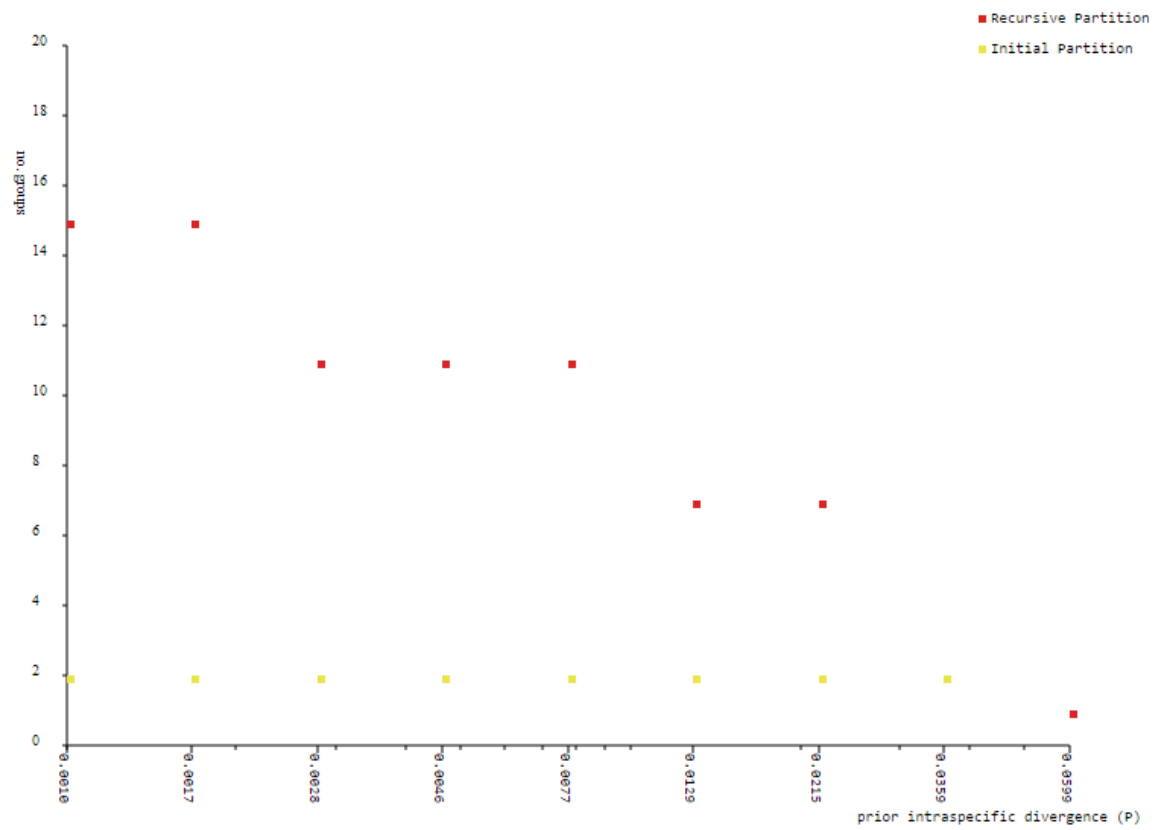
Supplementary Fig. 1. Automatic partition of the dataset reporting the number of groups of lineage 1 inside the initial and recursive partitions.



Supplementary Fig. 2. Poisson Tree Processes (PTP) delimitation tests of species of lineage 1 using the maximum likelihood phylogenetic tree.



Supplementary Fig. 3. RAxML bipartitions consensus tree of lineage 1.



Supplementary Fig. 4. Automatic partition of the dataset reporting the number of groups of lineage 2 inside the initial and recursive partitions.

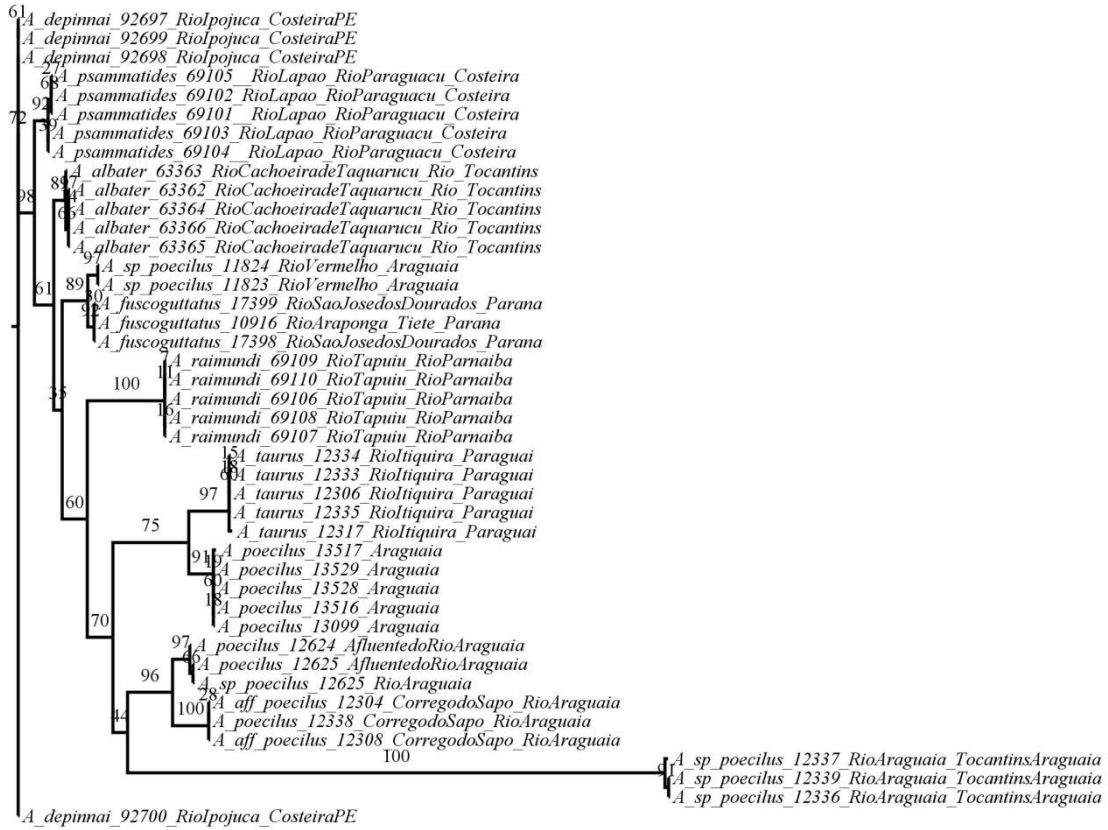
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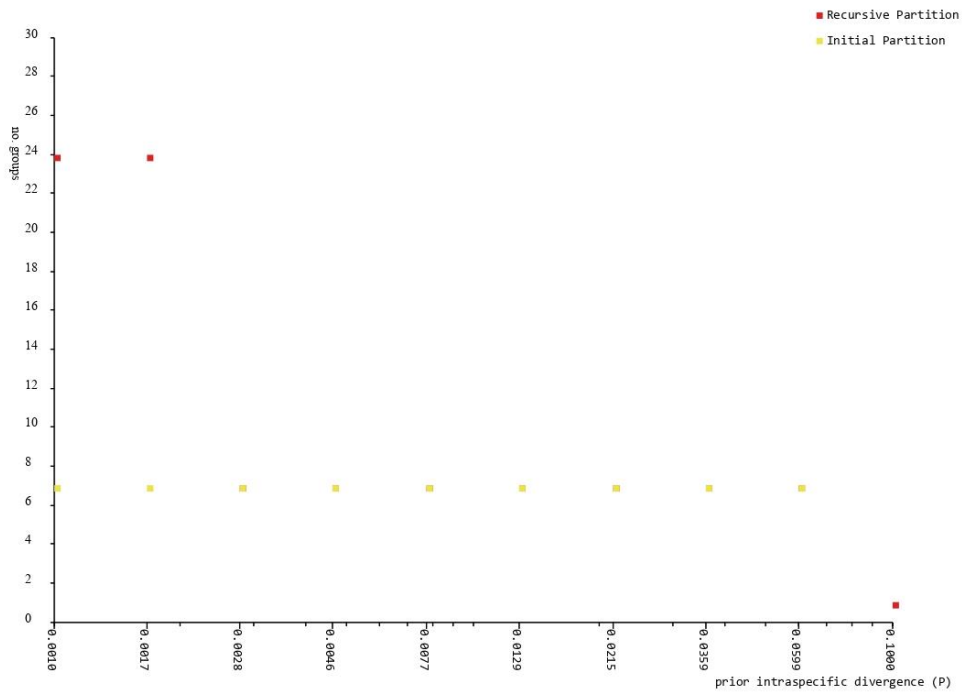
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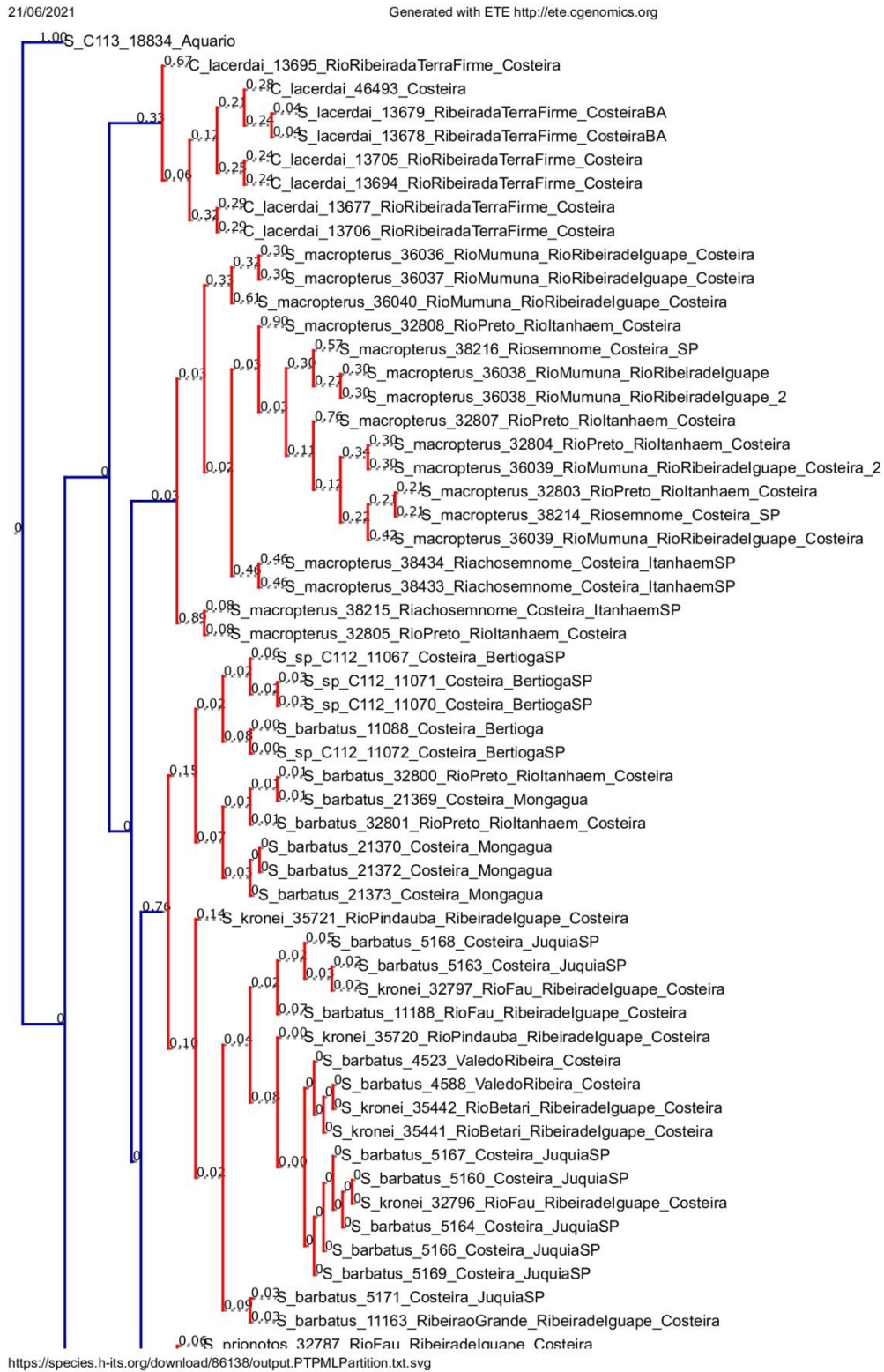
Supplementary Fig. 5. Poisson Tree Processes (PTP) delimitation tests of species of lineage 2 using the maximum likelihood phylogenetic tree.

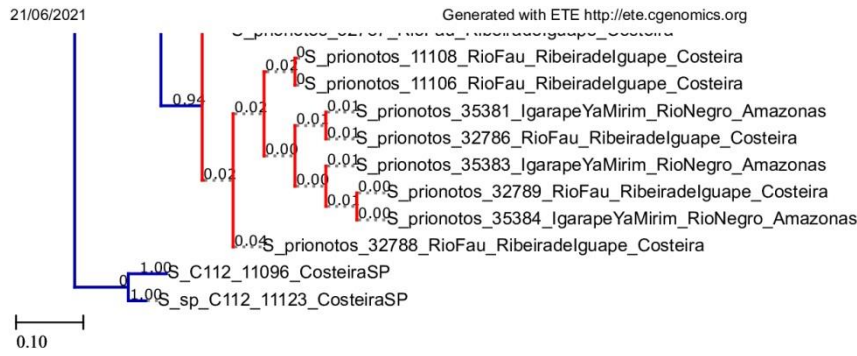


Supplementary Fig. 6. RAxML bipartitions consensus tree of lineage 2.

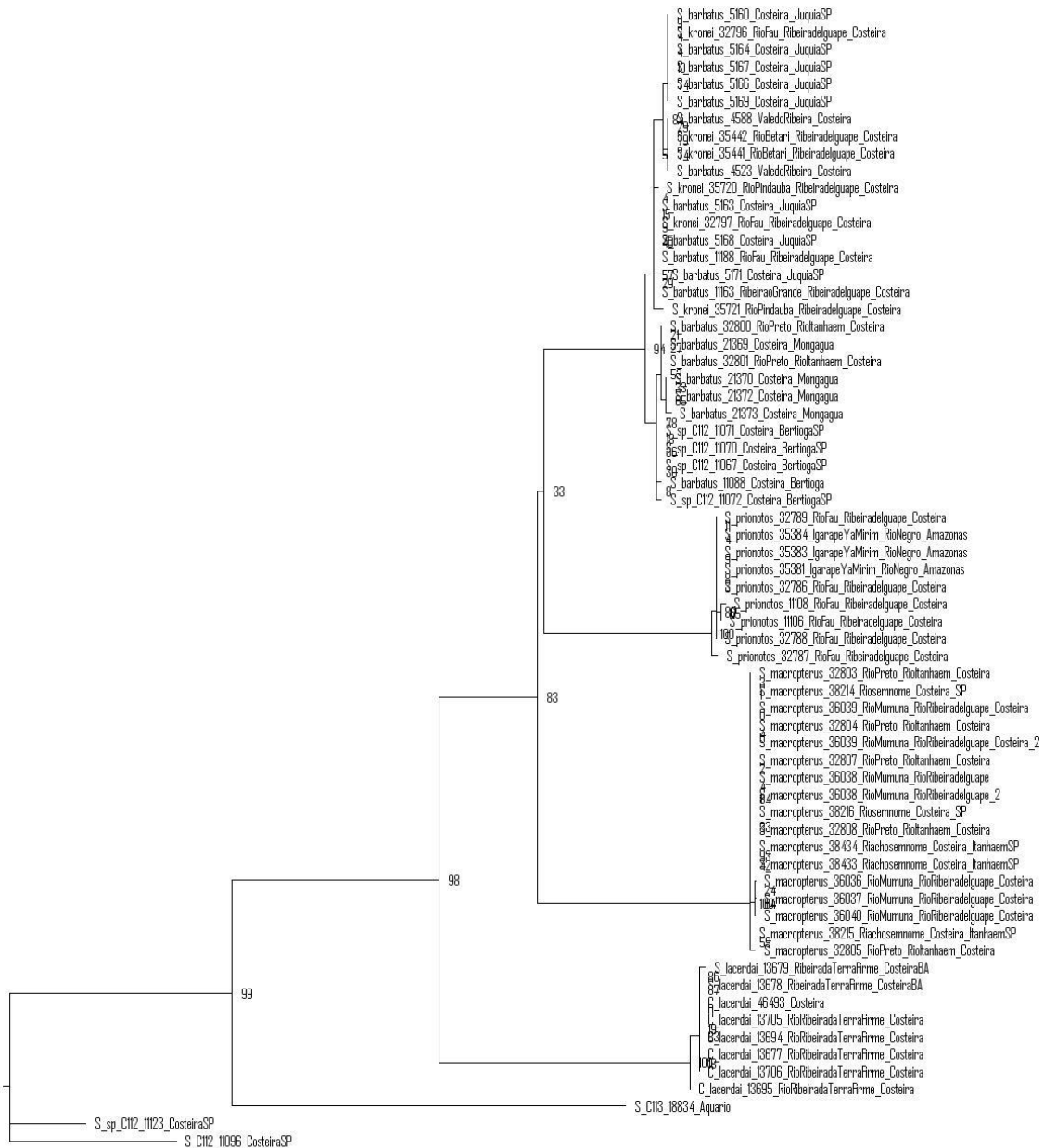


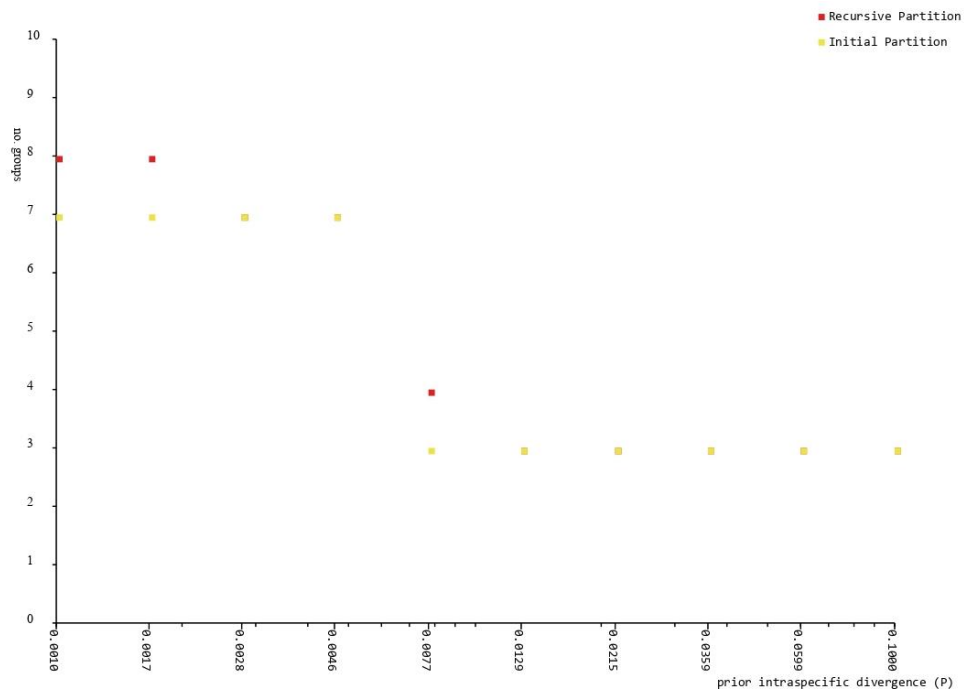
Supplementary Fig. 7. Automatic partition of the dataset reporting the number of groups of lineage 3 inside the initial and recursive partitions.

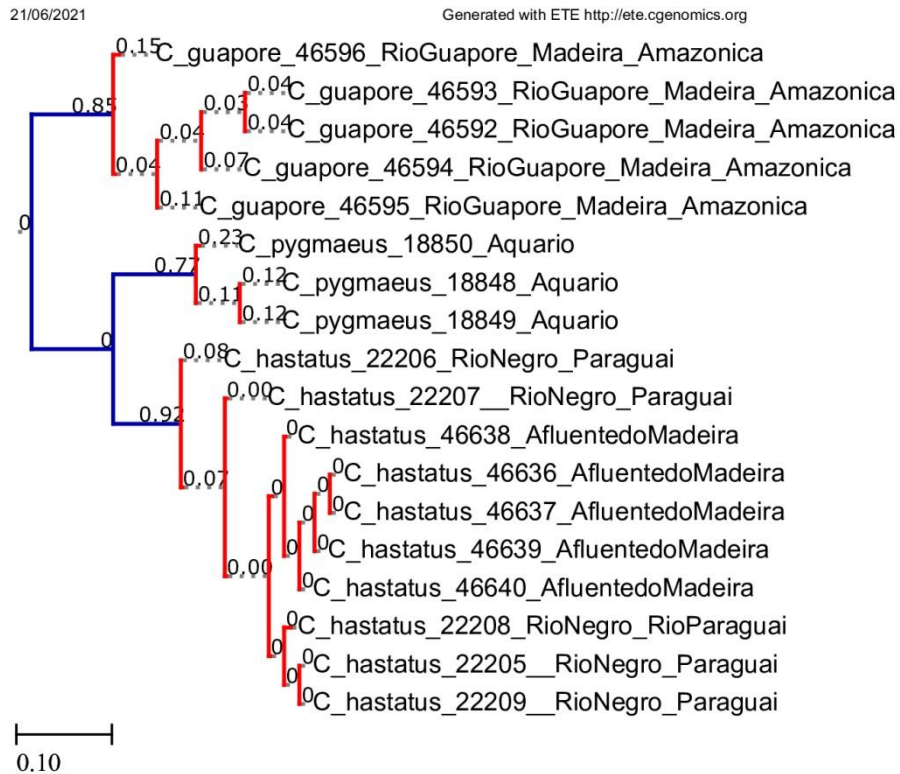




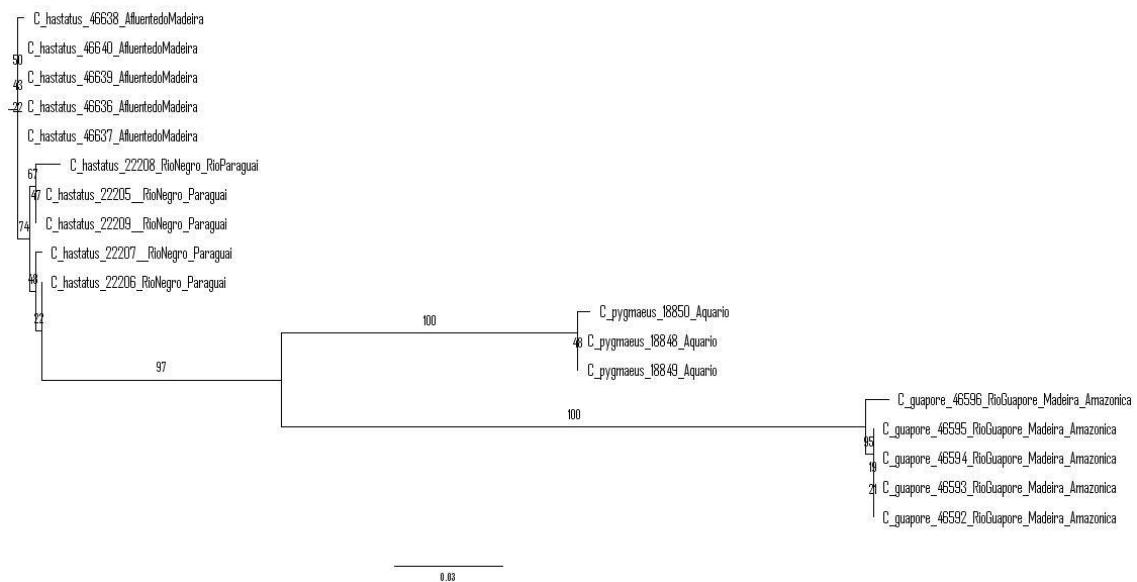
Supplementary Fig. 8. Poisson Tree Processes (PTP) delimitation tests of species of lineage 3 using the maximum likelihood phylogenetic tree.



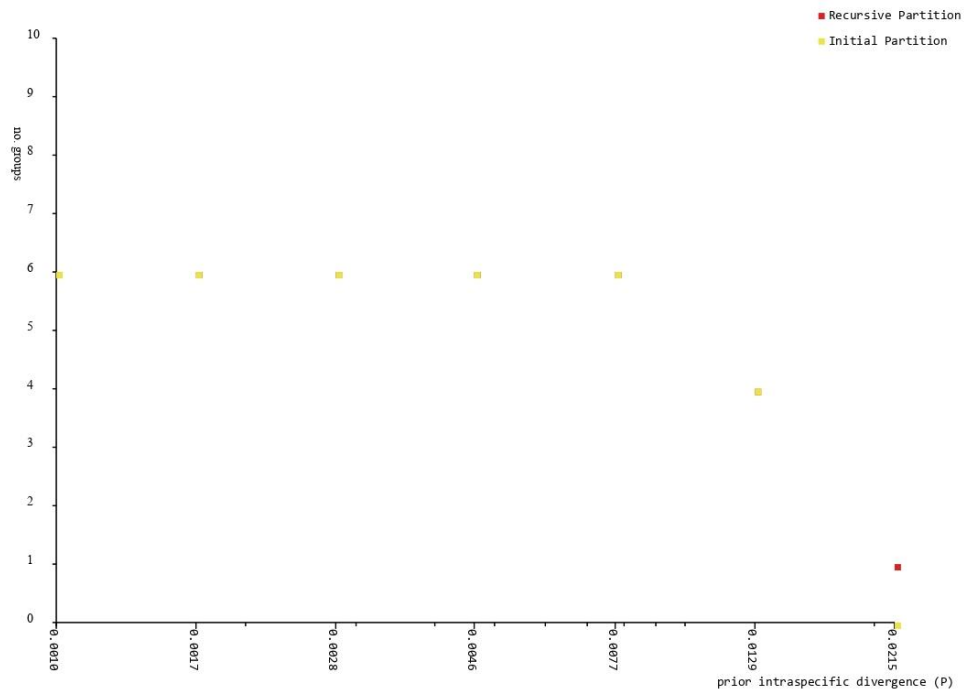
Supplementary Fig. 9. RAxML bipartitions consensus tree of lineage 3.**Supplementary Fig. 10.** Automatic partition of the dataset reporting the number of groups of lineage 4 inside the initial and recursive partitions.



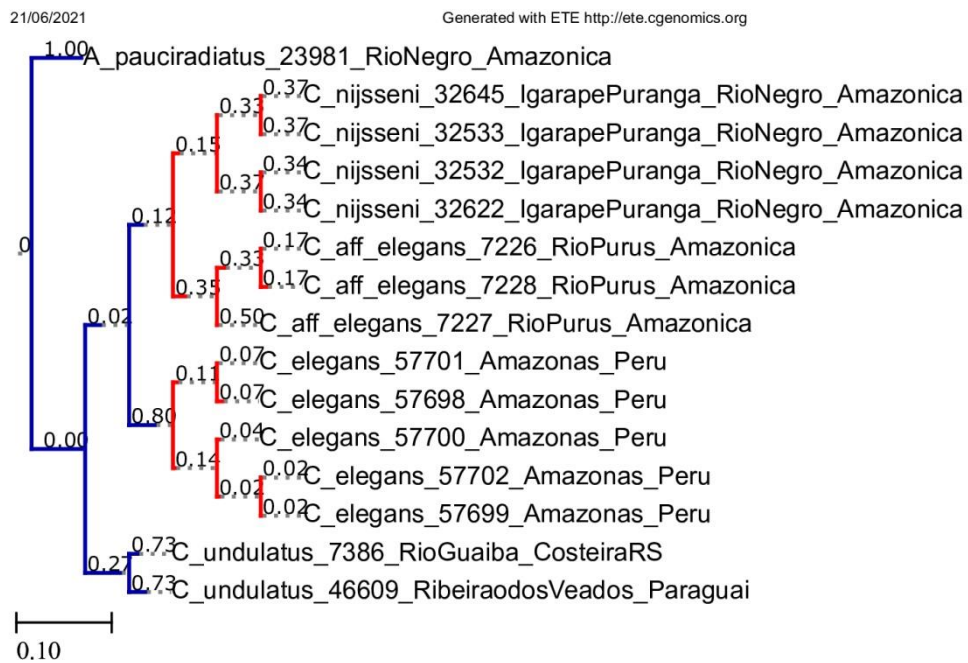
Supplementary Fig. 11. Poisson Tree Processes (PTP) delimitation tests of species of lineage 4 using the maximum likelihood phylogenetic tree.



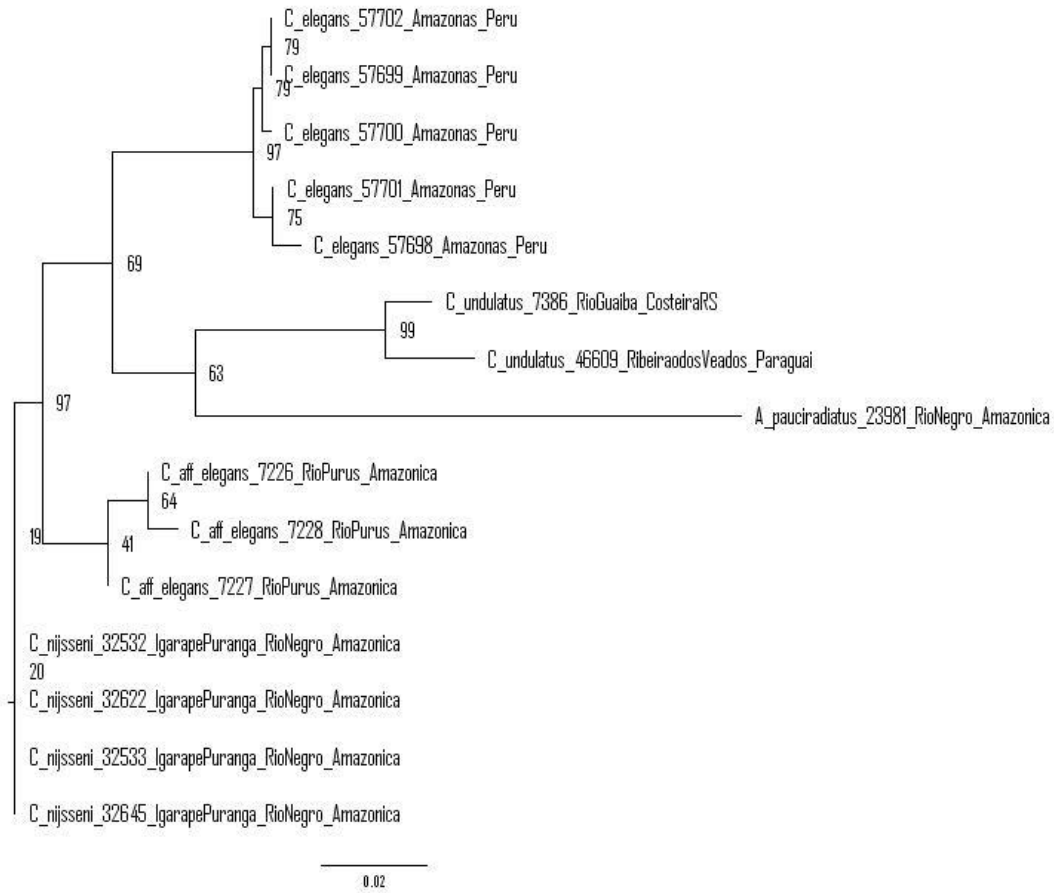
Supplementary Fig. 12. RAxML bipartitions consensus tree of lineage 4.



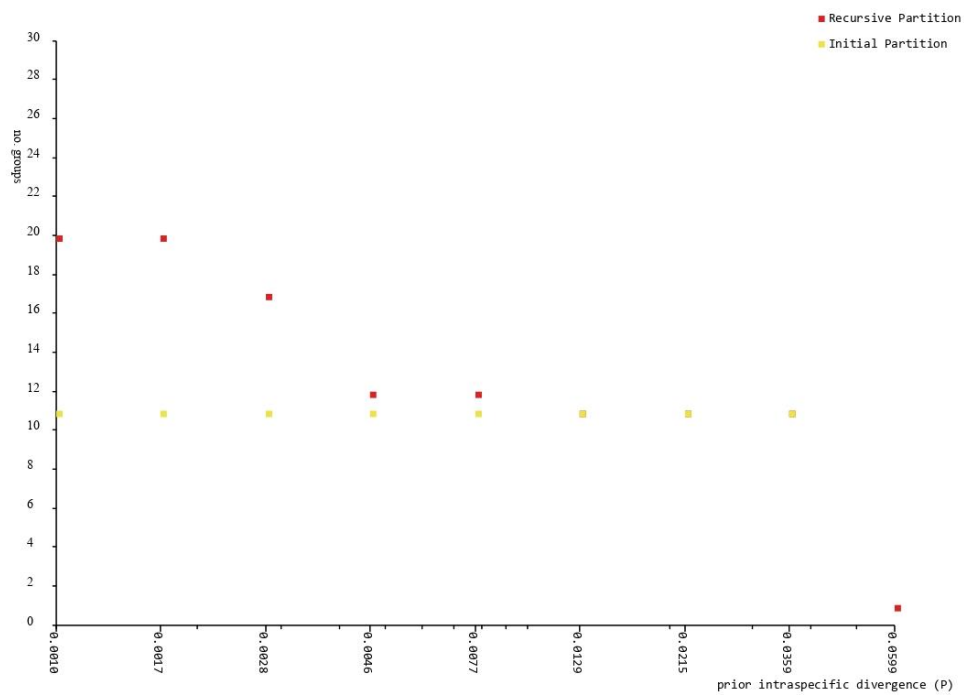
Supplementary Fig. 13. Automatic partition of the dataset reporting the number of groups of lineage 5 inside the initial and recursive partitions.



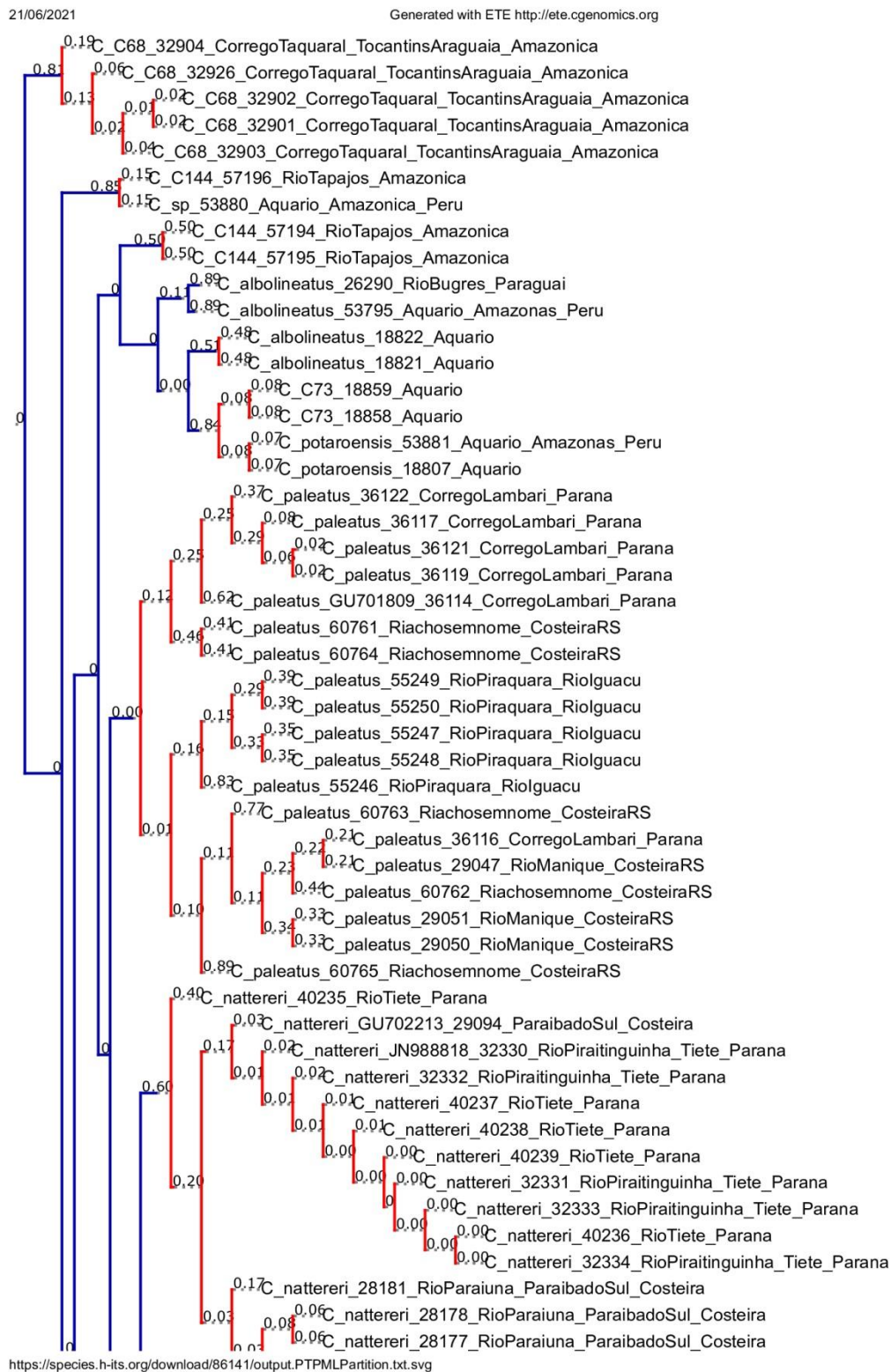
Supplementary Fig. 14. Poisson Tree Processes (PTP) delimitation tests of species of lineage 5 using the maximum likelihood phylogenetic tree.

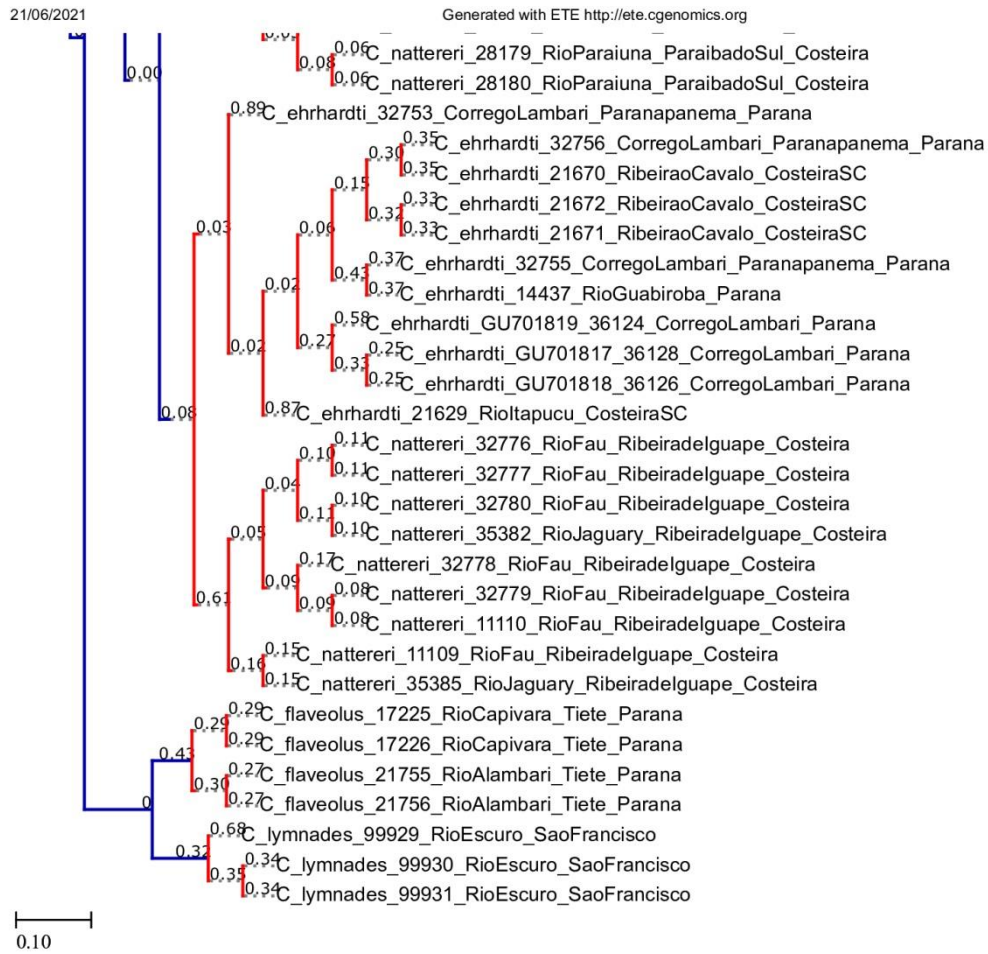


Supplementary Fig. 15. RAxML bipartitions consensus tree of lineage 5.



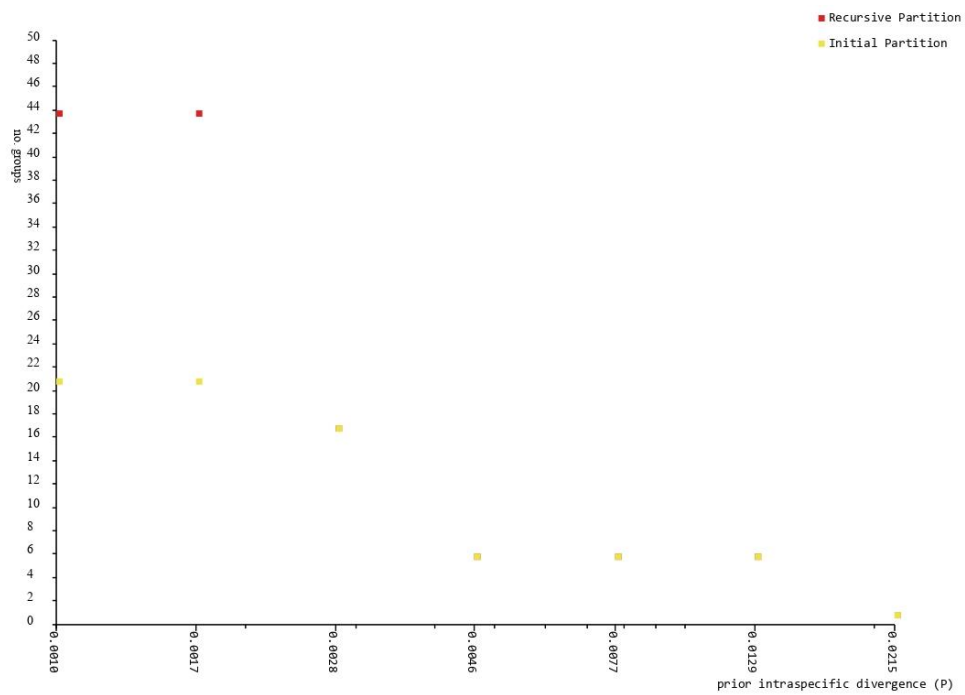
Supplementary Fig. 16. Automatic partition of the dataset reporting the number of groups of lineage 6 inside the initial and recursive partitions.





Supplementary Fig. 17. Poisson Tree Processes (PTP) delimitation tests of species of lineage 6 using the maximum likelihood phylogenetic tree.



Supplementary Fig. 18. RAxML bipartitions consensus tree of lineage 6.**Supplementary Fig. 19.** Automatic partition of the dataset reporting the number of groups of lineage 7 inside the initial and recursive partitions.

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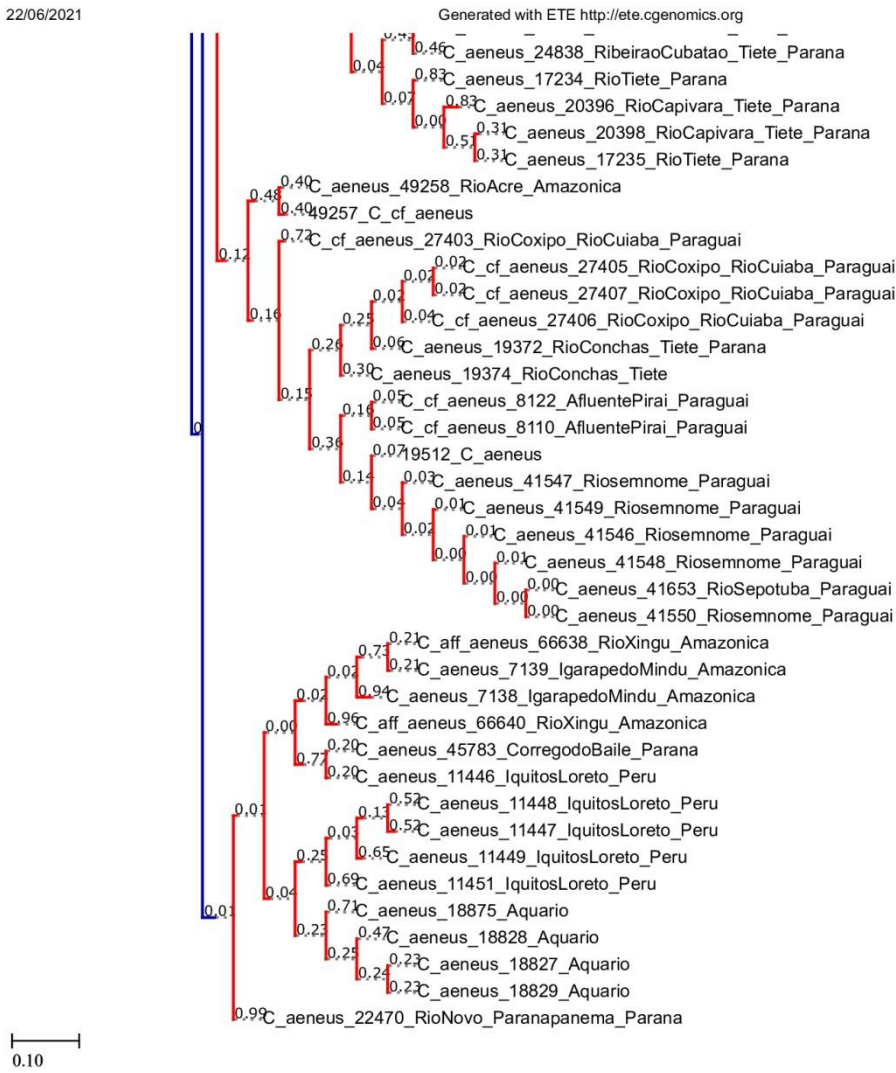
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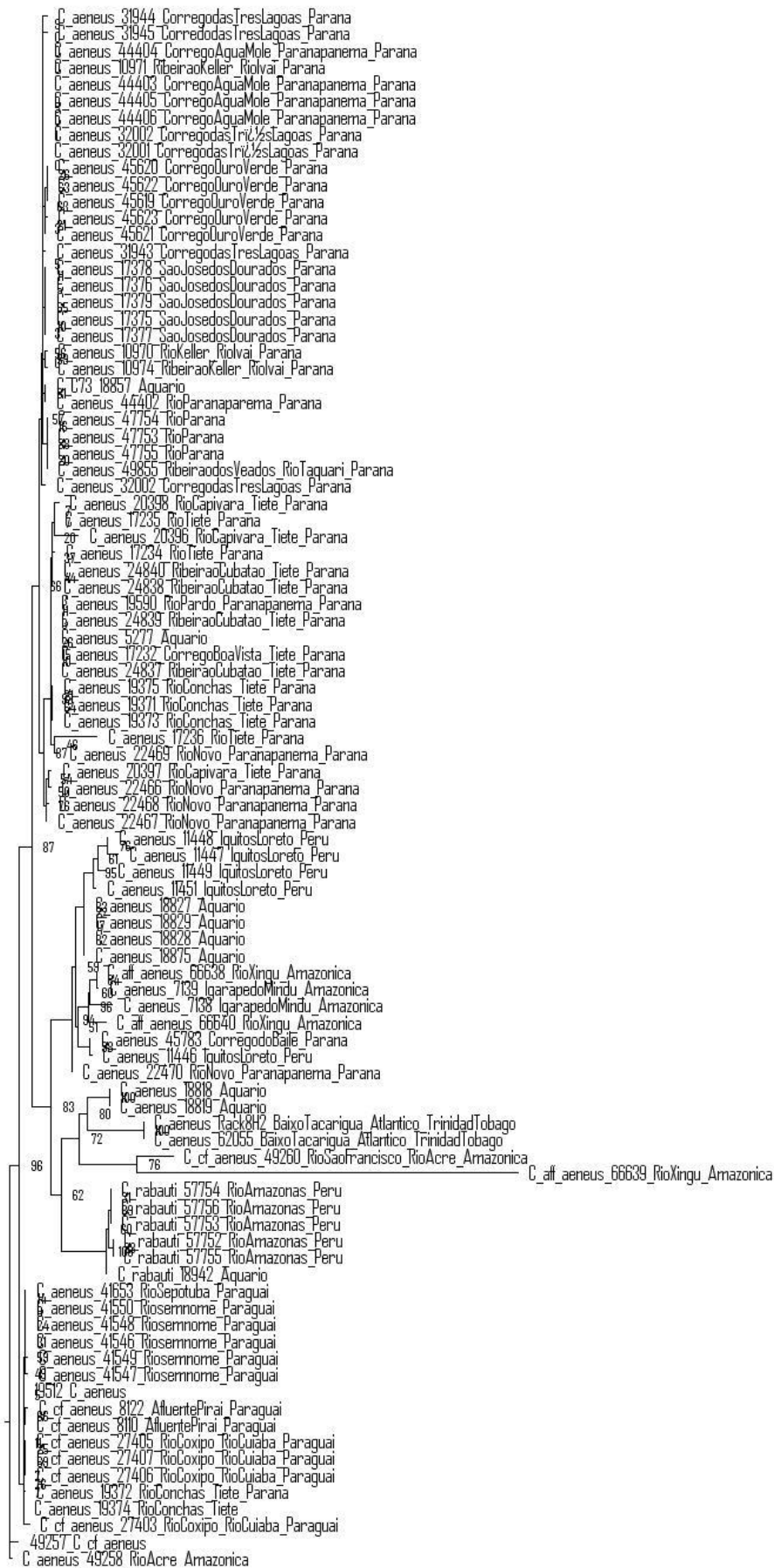
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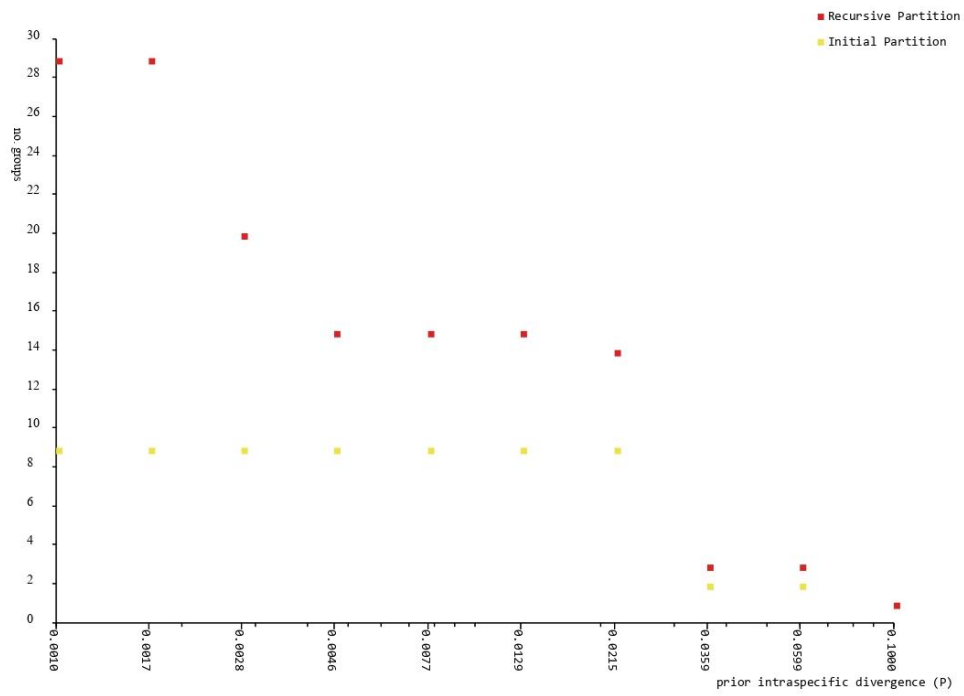
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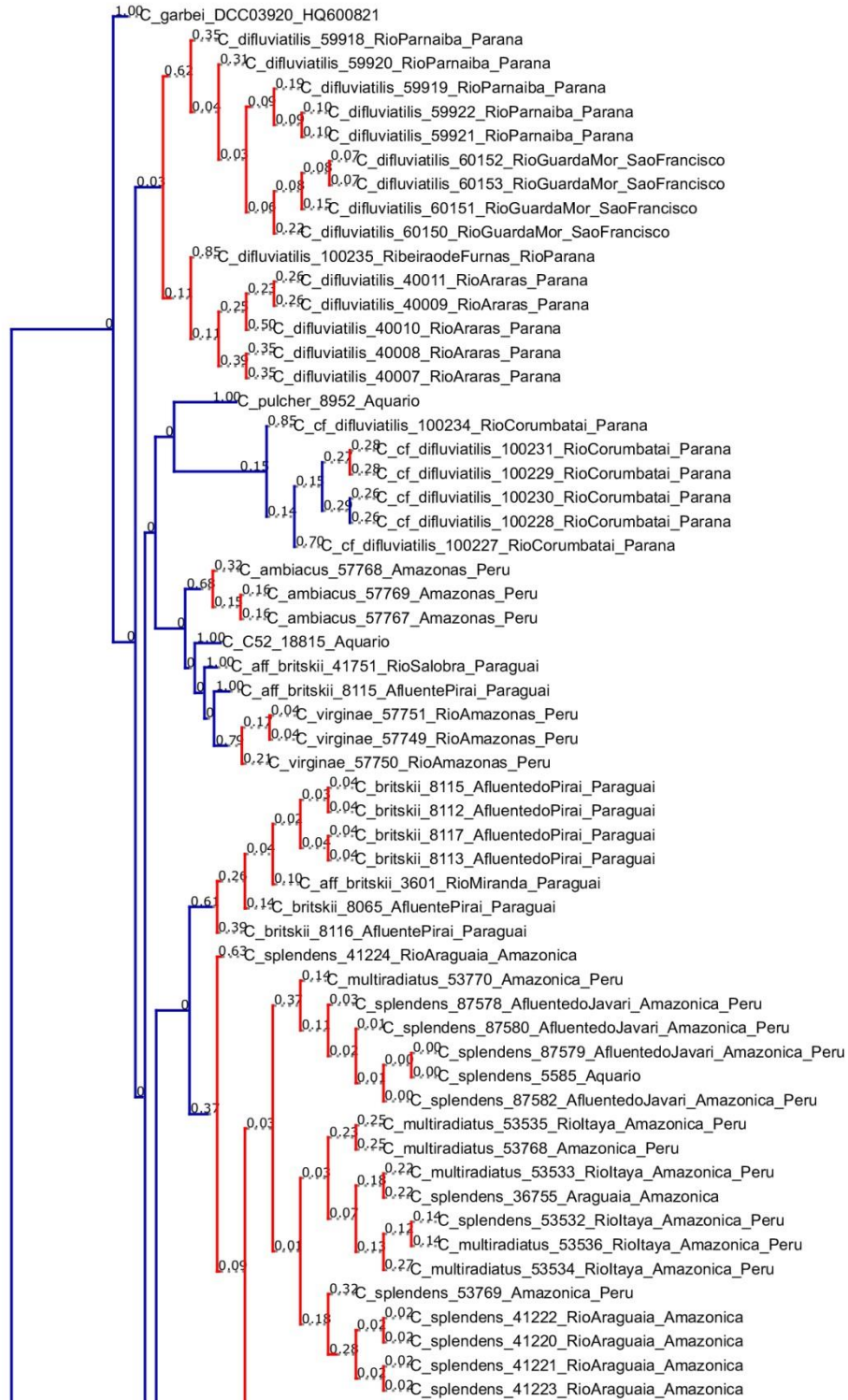
Supplementary Fig. 20. Poisson Tree Processes (PTP) delimitation tests of species of lineage 7 using the maximum likelihood phylogenetic tree.



Supplementary Fig.21. RAxML bipartitions consensus tree of lineage 7.

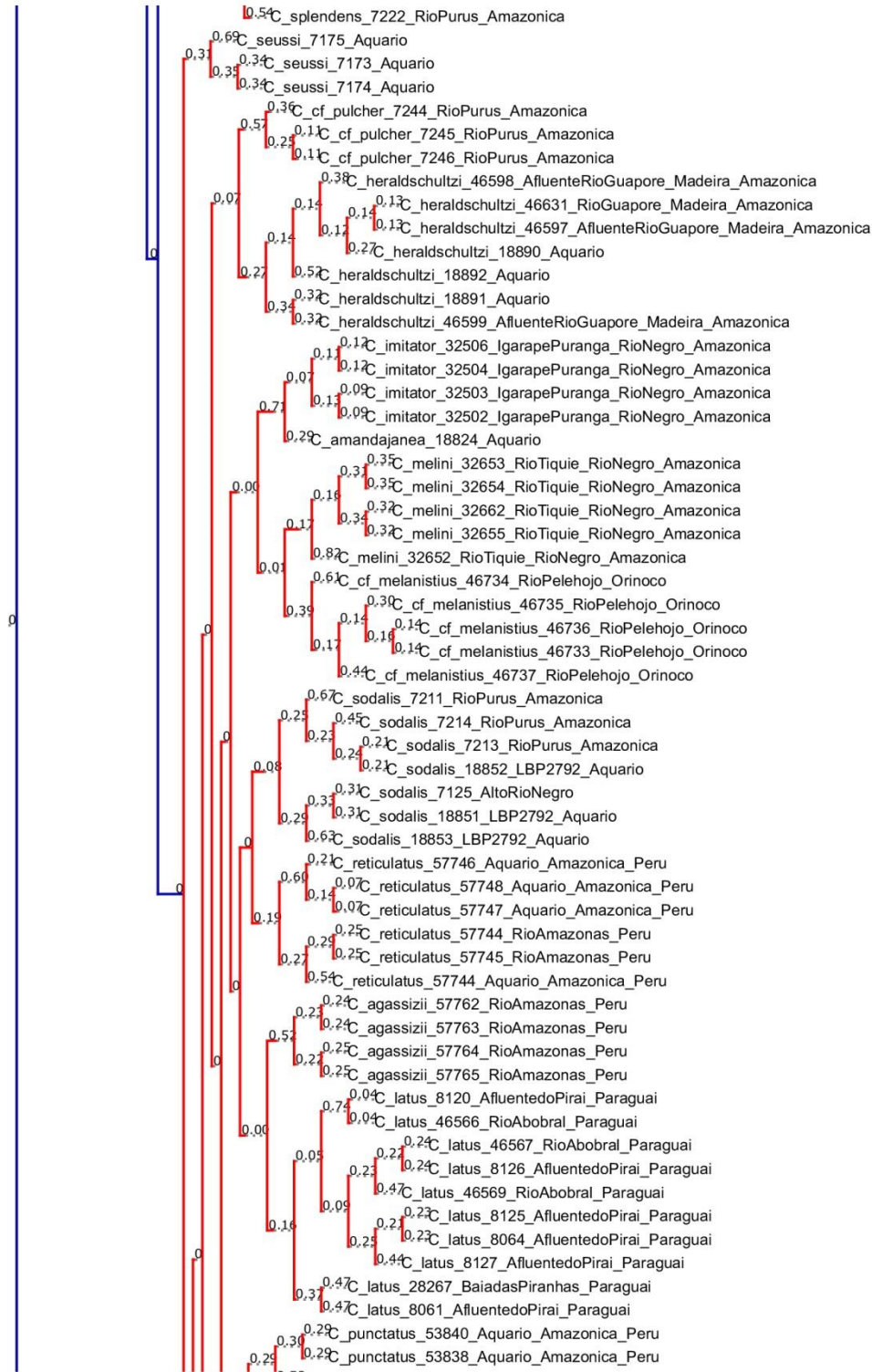


Supplementary Fig. 22. Automatic partition of the dataset reporting the number of groups of lineage 8 inside the initial and recursive partitions.



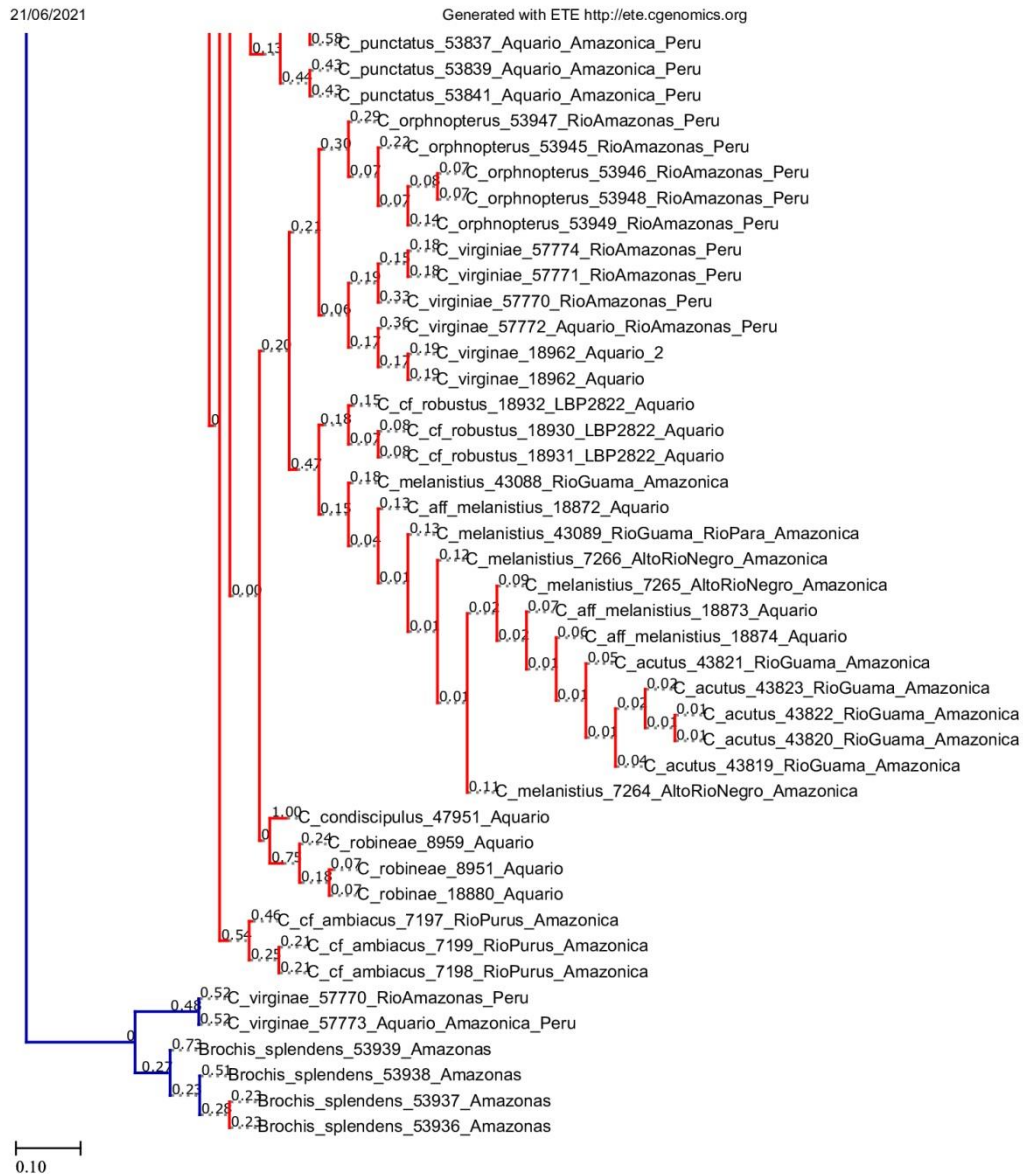
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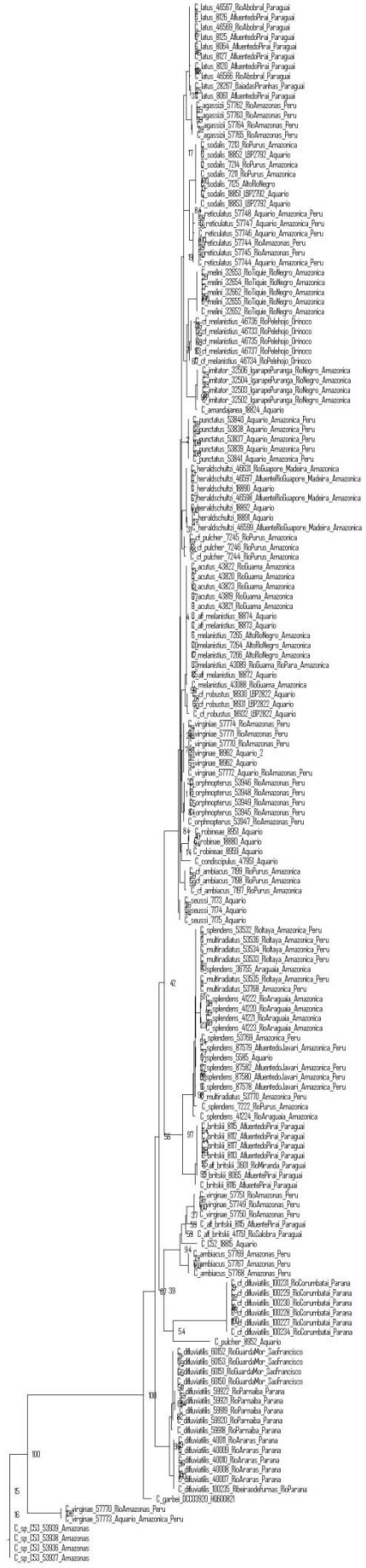


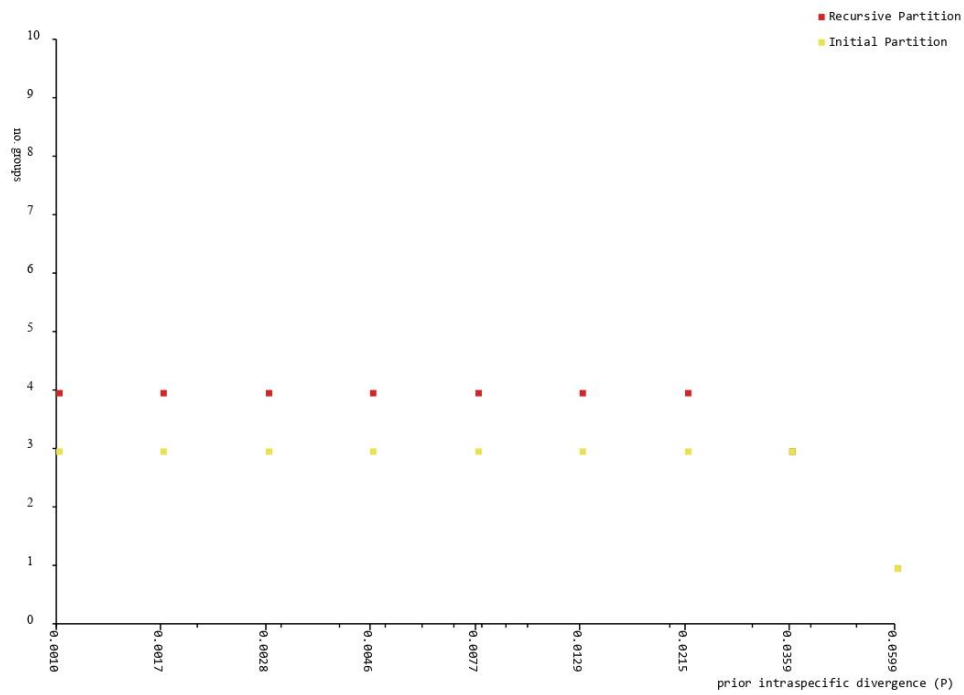
<https://species.h-its.org/download/86147/output.PTPMLPartition.txt.svg>

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Supplementary Fig. 23. Poisson Tree Processes (PTP) delimitation tests of species of lineage 8 using the maximum likelihood phylogenetic tree.



Supplementary Fig. 24. RAxML bipartitions consensus tree of lineage 8.**Supplementary Fig. 25.** Automatic partition of the dataset reporting the number of groups of lineage 9 inside the initial and recursive partitions.

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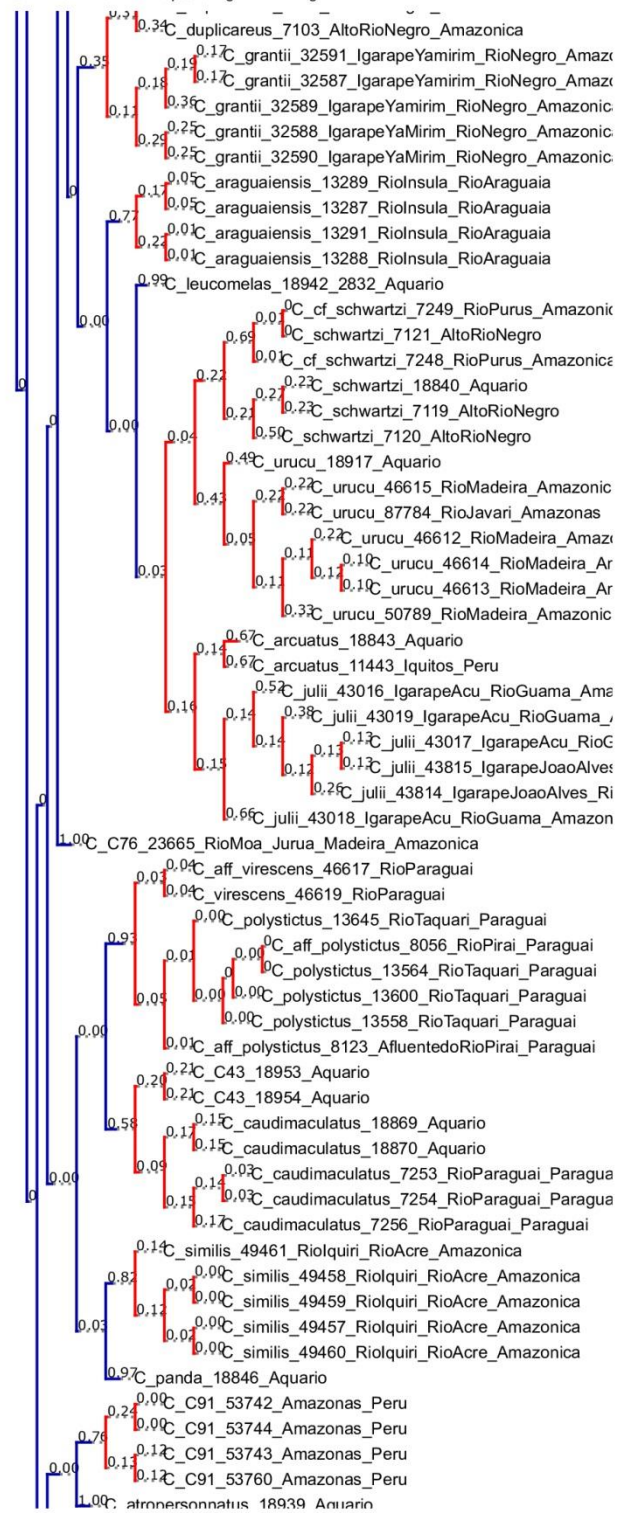


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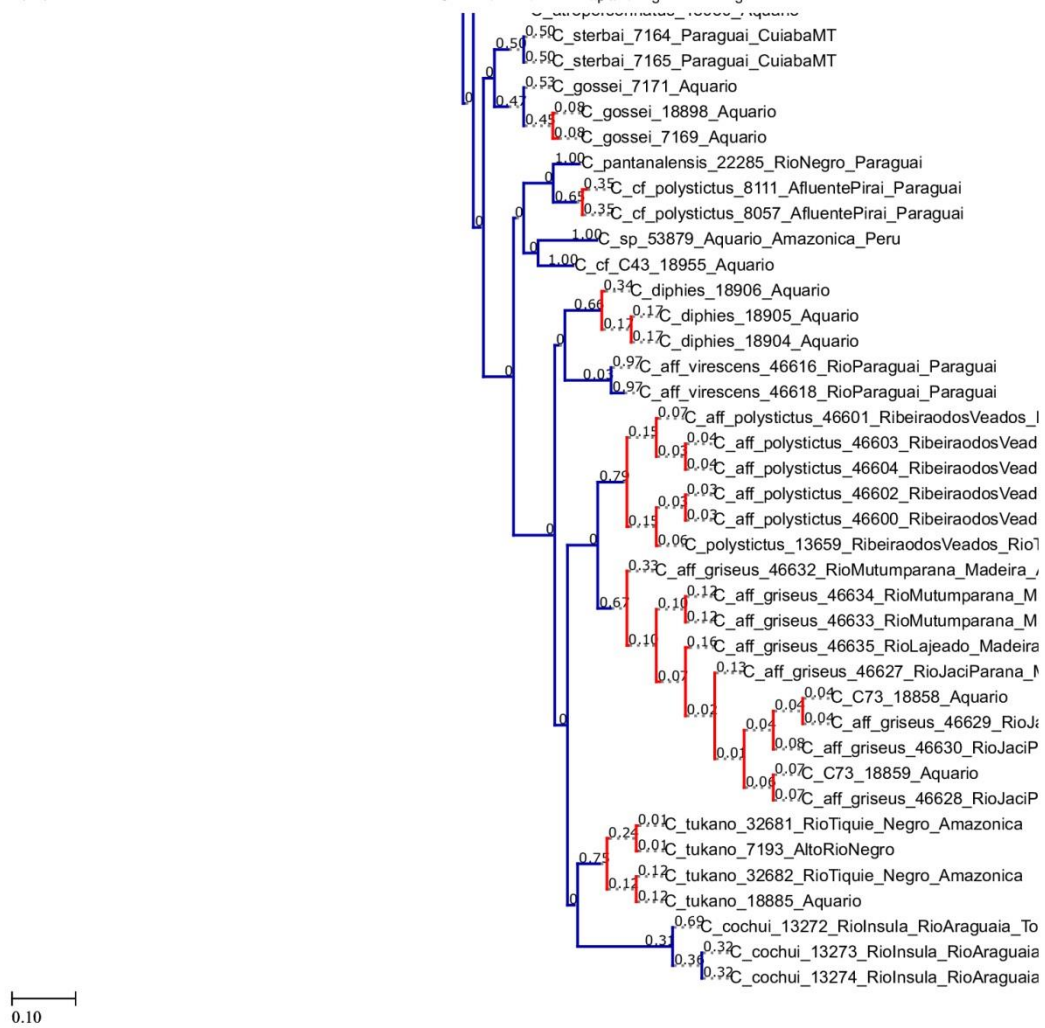
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Supplementary Fig. 26. Poisson Tree Processes (PTP) delimitation tests of species of lineage 9 using the maximum likelihood phylogenetic tree.

Supplementary Fig. 27. RAxML bipartitions consensus tree of lineage 9.

3.3 Capítulo 3

A new phylogenetic perspective of the family Callichthyidae (Siluriformes: Loricarioidea)

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Abstract

Neotropical freshwater fish comprise the most species-rich assemblage of vertebrates on the planet. Callichthyidae catfishes are widely distributed throughout the neotropics, and represent the third largest family of Siluriformes, with more than 220 species. They represent particular interest evolutionarily due to the mechanisms that have enabled their high diversification, making them ideal candidates for an investigation of the phylogenetic relationships among species. Despite the monophyly of both subfamilies having been tested sometimes over the years, the relationship of its members is not completely clear, mainly between Corydoradinae. Herein, we established the first comprehensive molecular phylogenetic framework for Callichthyidae, supporting eight lineages in Corydoradinae, and demonstrating the non-monophyly of some genera in both subfamilies. We presented a new phylogenetic hypothesis to the family using sequence data from ultraconserved elements (UCEs) of the genome from 188 taxa of Callichthyidae, representing 140 operational taxonomic units (61% of the family). Our results were demonstrated by maximum likelihood (ML), Bayesian (BI), and Astral-II analysis, which strongly supported the branches that corroborated the monophyly of almost all genera. Although, the relationships observed within these subfamilies are in several ways different from those hypotheses previously proposed by morphological and molecular data. Our results promoted knowledge about the unknown diversity of the family revealing a relationship never proposed before through UCE methodologies and

represent new information to be considered, allowing a new view on the complexity of Callichthyidae and the processes involved in the evolutionary history of the family.

Keywords: Phylogenomic, biodiversity, UCE, catfish, *Corydoras*

Introduction

The superfamily Loricarioidea (Siluriformes) is composed of the families Astroblepidae, Callichthyidae, Loricariidae, Nematogenyidae, Scoloplacidae, and Trichomycteridae. The family Callichthyidae represents the third largest family of the Siluriformes, with 223 valid species, and many species have been described every year. The callitids have a wide distribution and can be found in almost all hydrographic basins in South America, from the northern drainages, in the Orinoco and Trinidad and Tobago rivers, as well as in the coastal drainages of the Guyana shield, to the south, in the drainages of Argentina, but the vast majority of Callichthyidae species are originally described from the Amazon and Guyana river basins. Currently, the family is divided into two subfamilies, Callichthyinae composed by *Callichthys* Scopoli, 1777, *Dianema* Cope, 1871, *Hoplosternum* Gill, 1858, *Lepthoplosternum* Reis, 1997, and *Megalechis* Reis, 1997; and Corydoradinae composed by *Aspidoras* Ihering, 1907, *Corydoras* Lacepède 1803, and *Scleromystax* Günther, 1864.

In 1998, Reis proposed the first hypothesis of relationship of Callichthyidae focusing on morphological characters and skeletal anatomy and corroborating the monophyly of the family. The resulting cladogram of Reis supports the monophyly of the entire genus within Callichthyinae, but Corydoradinae demonstrated to be non-monophyletic. The topology of the tree showed the genus *Aspidoras* as sister-group of a clade formed by *Corydoras* and *Brochis*. The author couldn't find characters to support the monophyly of *Corydoras*, whereas the monophyly of *Brochis* was supported by four derived features, even though, the author suggests *Brochis* as synonymous of *Corydoras*. Thereafter, Britto (2003) presented a new hypothesis of the relationship of the subfamily Corydoradinae, which was reanalyzed and re-defined based on 83 morphological characters, and corroborated the division of the subfamily into two tribes, Aspidoradini and Corydoradini. Aspidoradini was considered a monophyletic clade, formed by *Aspidoras* and *Scleromystax*, a revalidated genus; while the tribe Corydoradini, proposed as a monogenic tribe, was composed by only *Corydoras*, and synonymizing *Brochis* to this genus. One of the main results of Britto was the non-monophyletic nature of the genus *Corydoras* as currently defined. The author also proposed a new classification scheme encompassing the monophyletic groups defined by the consensus cladogram.

The first molecular investigation of the family, by Shimabukuro-Dias *et al.*, (2004), combined and compared results of multilocus analysis, morphological and molecular data to corroborate the monophyly of Callichthyidae and the existence of two natural groups: Callichthyinae and Corydoradinae. Posteriorly, in a large study of Corydoradinae, Alexandrou *et al.* (2011) suggested the presence of nine lineages in this subfamily, not corroborating with the previously proposed groups of Nijssen & Isbrücker (1980) using morphological data. Alexandrou *et al.* (2011) also provided evidence of Müllerian mimicry between several species of Corydoradinae and showed that mimicry can increase the diversification rates among allopatric communities and lead to convergence of coloration among sympatric taxa.

Efforts in the literature over the years have focused on clarifying the phylogenetic relationships of Corydoradinae due because is the largest and most problematic among the two subfamilies. Even though, Corydoradinae's relationships remain uncertain. Corydoradinae is the most complex subfamily of Callichthyidae, due to several factors, such as a large number of valid species, and the lack of a recent taxonomic revision including a higher number of samples with precisely defines the boundaries of each species. *Corydoras* comprise a significant proportion of these problems, due to the huge diversity of this genus that includes the freshwater ichthyofaunal of almost all South America. They represent particular interest evolutionarily due to the genetic and ecological mechanisms that have enabled their diversification.

The molecular techniques offer new sets of characters to elaborate phylogenetic hypotheses and have been useful to understand the inter-relationship between many groups, including fishes. Moreover, the UCE methodology has been widely used worldwide and has been demonstrated effectiveness in helping to understand complex phylogenies of freshwater fishes (Faircloth *et al.*, 2013; Harrington *et al.*, 2016; Chakrabarty *et al.*, 2017; Alfaro *et al.*, 2018; Roxo *et al.*, 2019; Ochoa *et al.*, 2020; Silva *et al.*, 2021). In face of the questions regarding the phylogenetic relationships of Callichthyidae, we proposed here an investigation using the most current molecular phylogenetic methodology and covering the greatest taxonomic density available for the family to test the monophyly of Corydoradinae, and also to test if Callichthyinae remains as monophyletic on this perspective. In this context, we present here the first phylogenetic hypothesis of the relationship of Callichthyidae using UCEs data approach.

Material and Methods

Taxon sampling

The samples were previously collected and deposited on LBP collection (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu, Brazil). Additional samples were obtained from the Academy of Natural Science of Philadelphia – ANSP, or collected. We prioritized using the tissues that were previously identified by expert taxonomists of the group. Even though, we morphologically identified the species by consulting the taxonomic literature and identification keys (Reis, 1997, 1998; Britto, 2004; Tencatt *et al.*, 2013).

DNA extraction

Whole genomic DNA was extracted from muscle tissue, fin, or gills preserved with ethanol using the DNeasy Tissue kit (Qiagen Inc.), cleaned through RNase treatment, and then passed through cleaning columns. After all the cleaning process, 2µl of the genomic DNA was quantified with fluorometry, following the manufacturer's instructions. The extraction with a concentration bigger than 10 ng/µl was sent to Arbor Biosciences (AB; arborbiosci.com; Ann Arbor, MI, USA) to be sequenced and to prepare the libraries.

Data sequence and processing

The sequencing was performed across two Illumina HiSeq paired-end 100 bp lanes using v4 chemistry in Arbor Biosciences, and then sent to our laboratory for the subsequent analyses. First of all, the sequences were downloaded from the server system and contaminations from the adapters were removed, as well as the DNA segments with low-quality sequences, and the sequences containing ambiguous bases, following Dr. Brant Faircloth and collaborators' protocol (<https://github.com/faircloth-lab/illumiprocessor>). The assembly of the reads was carried out by the program Velvet (Zerbino & Birney 2008) and VelvetOptimiser (<https://github.com/Victorian-Bioinformatics-Consortium/VelvetOptimiser>).

Subsequently, the alignment of species-specific to our probe-UCE set that was used in the enrichment was performed using the PHYLUCE package (Faircloth, 2016; <https://github.com/faircloth-lab/phyluce>), integrating Python program

(`match_contigs_to_probes.py`) and LASTZ (Harris, 2007). During the check, this program creates a database to store the UCE locus for each taxon. After generating the UCE/tax matrices, other components of the PHYLUCE program (`get_match_counts.py`) were used to check the databases and generate .fasta files for the UCE loci identified for all the taxa. Afterward, a customized program for Python (`seqcap_align_2.py`) was used to align the contigs with the MUSCLE program alignment algorithm (Edgar, 2004) and to perform edge trimming and internal trimming, cutting the contigs that represented the UCEs, in parallel, among all the selected taxa before the phylogenetic analysis (Faircloth *et al.*, 2012).

Phylogenetic analysis

We performed phylogenetic analyses with different amounts of missing data-keeping 75% and 90% of UCEs present in the complete alignment matrix. The hypothesis of phylogenetic reconstruction was performed using maximum likelihood (ML) in RAxML v8.019 (Stamatakis, 2014) and Bayesian inference (BI) in ExaBayes v1.4 (Aberer *et al.*, 2014) approaches on the 2×10 CPU, 256 GB Zungaro server at IBB-UNESP. The RAxML best tree search was performed under the parameter $-N = 5$ which specifies the number of alternative runs on distinct parsimony starting trees, using the GTRGAMMA model (Stamatakis *et al.*, 2008). The concatenated alignment was also used to perform bootstrap replicates using the autoMRE function for the extended majority-rule consensus tree criterion (available in RAxML v8; Stamatakis, 2014) to assess support for individual nodes, with intent to allow the bootstrap convergence test to be conducted, which determines whether replicates are sufficient to obtain stable support values (Pattengale *et al.*, 2010). The ML analysis was performed on 75% and 90% complete matrices without partitions.

The BI analysis of 75% and 90% complete matrices were performed with ExaBayes v1.4 (Aberer *et al.*, 2014) with two independent runs, with two chains (one cold and one heated) each one, with 3.000.000 generations using the GTR+G model. The tree space was sampled every 100 generations to yield a total of 10,001 trees. Parameter estimates and ESS values were visualized in Tracer v1.6 (Rambaut *et al.*, 2014) and the last 6001 trees were sampled after checking results for convergence. The average standard deviation of split frequencies was <1%, effective sample sizes (ESS) were >200, and the potential scale reduction factor for estimated parameters was approximately 1.0. We used the consensus algorithm of ExaBayes (burn-in: 20%;

thinning: 1000) to generate the most credible set of trees from the posterior distribution of possible topologies.

Coalescence analysis

We inferred a species tree from individual gene trees using the PHYLUCe to resample loci available for 75% and 90% of taxa and used RAxML to analyze each of these alignments and generate a set of best trees for each matrix. These best trees were then analyzed using ASTRAL-II (Mirarab & Warnow, 2015) to infer majority-rule species trees have a minimum clade frequency of 0.7.

Results and Discussion

We included in our analysis a total of 188 taxa (Supplementary Table 1), 75,406,628 characters of the entire matrix, which of 60,294,177 were nucleotides and 15,112,451 (20%) were missing data. The matrix of 75% presented 770 individual UCEs alignments, and the matrix of 90% presented 174 alignments. The ingroup was formed by 174 taxa, or 140 operational taxonomic units of Callichthyidae (61% of the family), representing all the genera of the family. Our analysis was constructed from 129 species of Corydoradinae (68% of the subfamily) and 11 species of Callichthyinae (65% of the subfamily). The outgroup was represented by 14 species from five families: Astroblepidae, Loricariidae, Nematogenyidae, Scoloplacidae, and Trichomycteridae. Sequences of *Nematogenys inermis* (Guichenot 1848) were used to root the tree. Supplementary Table 1 summarizes data from all the samples that represent the ingroup and outgroup. Therefore, we based our discussion on results of the topology of ML consensus analysis, BI, and Astral-II (Figs. 1-6) of the edge-trimmed, 75% and 90% complete, unpartitioned matrix. The analysis yielded nodes with strong support (ML > 100%, BI > 1%, Astral > 1%).

The results of ML and BI analyses were very similar to each other, although the results of Astral-II were topologically different from ML and BI. The differences in the topology of the analyses are discussed below. Our results supported the monophyly of the subfamily Callichthyinae, but not Corydoradinae, corroborating the phylogenetic hypothesis proposed by the morphological studies (Reis, 1998, with the family Callichthyidae; Britto, 2003, with the subfamily Corydoradinae), and molecular studies (Shimabukuro-Dias *et al.*, 2004, with the family Callichthyidae; Alexandrou *et al.*, 2011

with Corydoradinae), although, the relationships regarding the assemblages of several groups were different from those studies. We divided the results according to the subfamilies to better discuss the results.

The subfamily Callichthyinae

All the topologies of all the matrices demonstrated *Dianema* was the first genus to diverge inside Callichthyidae (ML > 100%; BI = 1; Astral-II = 1). *Dianema* represented the sister group of all others callichthyines analyzed, not corroborating the morphological hypothesis of Reis (1998), but agreeing with the molecular hypothesis of Shimabukuro-Dias *et al.* (2004). Our results of BI and ML suggested *Dianema* + (*Hoplosternum* + (*Callichthys* + (*Megalechis* + (*Megalechis* + (*Lepthoplosternum* + *Megalechis*))))). However, Astral-II analysis showed a different topology, with *Dianema* + *Hoplosternum* as sister group of all other callichthyines (Fig. 5-6). Reis (1998) suggested *Dianema* as sister group only of *Hoplosternum* and, according to his hypothesis, the first genus to diverge was *Callichthys*. Our analysis didn't recover this position of *Callichthys*, this genus showed as sister group of (*Megalechis* + (*Lepthoplosternum* + *Megalechis*)).

Our results corroborated the molecular analyzes of Shimabukuro-Dias *et al.*, (2004) in almost all the positions of the clades of Callichthyinae, but disagree with two genera. The topologies of ML, BI, and Astral-II analyzes supported the monophyly of *Dianema*, *Hoplosternum*, and *Callichthys*, but didn't recover the monophyly of the genus *Megalechis*. Reis (1997) presented a revision of *Hoplosternum* where he demonstrated the non-monophyly of this genus, and, in an attempt to make *Hoplosternum* monophyletic, in the next year, Reis (1998) proposed the new genus *Megalechis* and *Lepthoplosternum* relocating some species from *Hoplosternum* and *Callichthys* to *Megalechis*, in an attempt to solve the non-monophyly of *Callichthys*. However, our results showed with strong support that *Megalechis* not forming a monophyletic clade. There are three clades of *Megalechis*, one closely related to *Lepthoplosternum*, one clade of *Megalechis thoracata* as sister of (*Lepthoplosternum* + *M. picta*), and a third clade of *M. thoracata*, as sister group of (*M. thoracata* + (*Lepthoplosternum* + *M. picta*)). *M. picta* (Müller & Troschel 1849) was described from the Guyana system and its distribution broad the almost all East of Andes, Essequibo, and Orinoco systems, and *M. thoracata* was described from Mana River, French

Guyana by Valenciennes (1840), but its distribution broad almost all South America, including Amazon, Orinoco and Paraguay River basins, coastal Rivers from Guyana and northern Brazil. Our samples of *M. picta* were collected on Orinoco River, Venezuela (voucher 15591), Upper Negro River, São Gabriel da Cachoeira, Brazil (voucher 34018), and Purus River, Amazonian basin (voucher 7209). These species need to be reviewed and probably relocated to a new genus, for *Megalechis* to achieve the monophyly.

On the other hand, Reis (1998) proposed *Megalechis* as sister group of *Dianema* + *Hoplosternum*, and *Lepthoplosternum* sister group of that. But our analysis didn't recover these positions and corroborated Shimabukuro-Dias *et al.*, (2004), who proposed *Megalechis* and *Lepthoplosternum* as sister groups. However, ML, BI, and Astral-II analyzes showed that there is a clade of *Megalechis*, identified as *M. thoracata* (voucher 19593, LBP 2972), as sister group of (*M. thoracata* + (*Lepthoplosternum* + *Megalechis picta*)). *M. thoracata* was originally described as *Callichthys thoracatus* from Mana River, French Guiana, synonymized to *Hoplosternum thoracatum*, and then, transferred to a new genus *Megalechis* by Reis (1997). This species has a long synonymous list, the author considered as junior synonymous of *M. thoracata*: *Callichthys exaratus* Müller & Troschel 1849, type locality British-Guiana; *C. longifilis* Valenciennes in Cuvier & Valenciennes described from Cayenne, French Guiana; *C. pictus* type locality British-Guiana, but later reallocated to *Megalechis*; *C. sulcatus* Kneer 1855, type locality Rio Branco and Marabitanas; *H. orinocoi* type locality La Pedrita, Venezuela; and *C. personatus*, type locality unknown, later relocated to synonymy of *M. personata*, a junior synonymous of *M. thoracata*. Our sample was collected from the Araguaia River. The distribution of *M. thoracata* broad this basin, but the position recovered to this sample demonstrated that this genus needs to be better investigated.

There is a sample of *Megalechis* related to *Lepthoplosternum*, identified as *M. personata* (Ranzani 1841) (LBP 22449). However, *M. personata* is currently synonymous with *M. thoracata*. This species was originally described as *Callichthys personatus* (Ranzani 1841), whose type locality is unknown. Our sample was collected on Amazon River, Leticia, Colombia, but instead of being demonstrated to be most related to the others *Megalechis*, it showed close related to *Lepthoplosternum*, although there are no species of *Lepthoplosternum* described to Colombia Rivers systems. The species of *Lepthoplosternum* are described as *L. altamazonicum* Reis 1997, Ucayali

River, Peru, distribution Upper Amazon River, *L. beni* Reis 1997, Beni River, Madre de Dios, Peru, distribution Madeira River basin, *L. pectorale* Boulenger 1895, Paraguai River, distribution Paraguai River basin, Brazil, *L. stelatum* Reis & Kaefer 2005, Tefé River, Upper Amazonian basin, distribution Tefé River, Upper Amazonian basin, Brazil, *L. tordilho* Reis 1997, Coast River from the Rio Grande do Sul State, distribution Jacui River basin, Brazil, and *L. ucamara* Reis & Kaefer 2005, Pacaya River, Loreto, Peru, distribution Ucally River, Peru, and Japurá River mouth, Brazil and Peru. The samples are too small and impossible to identify correctly. Our results suggest this sample identified *M. personata* could represent a new species of *Lepthoplosternum* from Colombia.

Figure 1. Maximum likelihood tree (ML) of 75% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

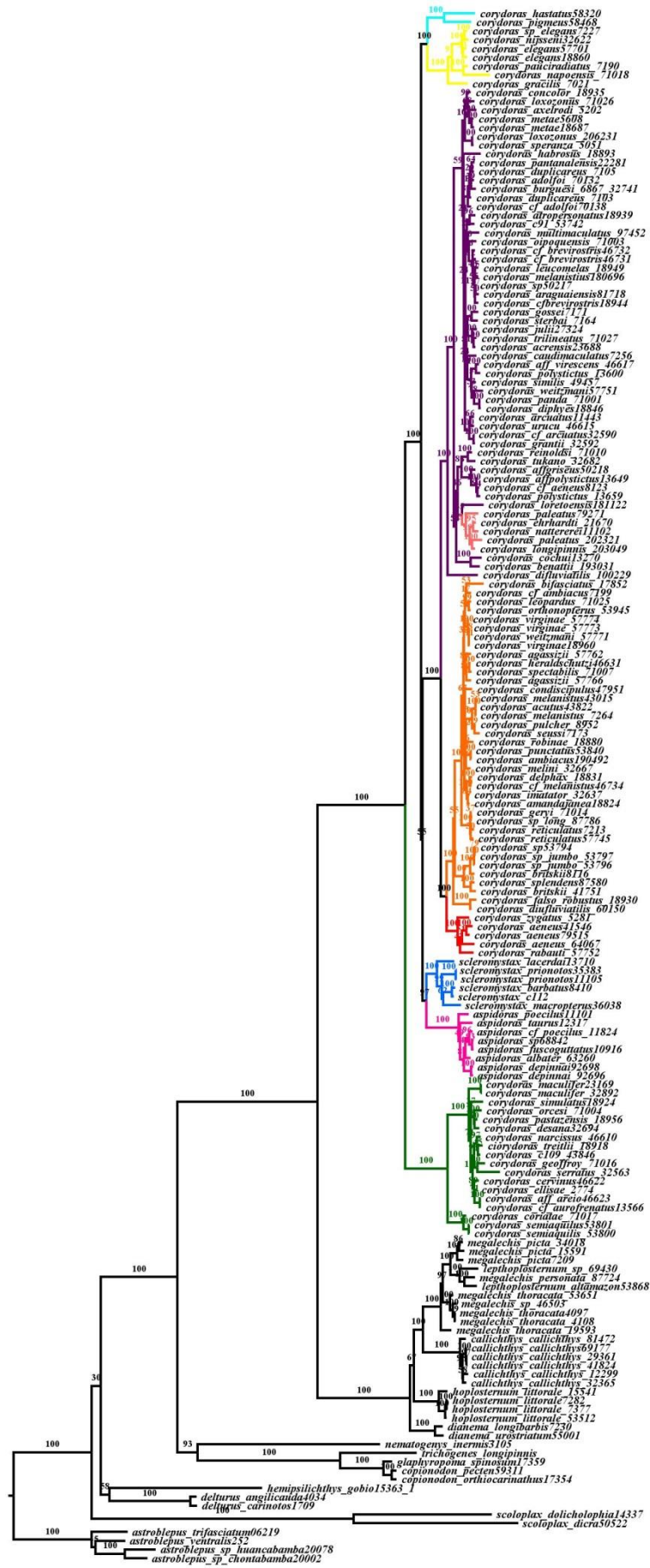


Figure 2. Maximum likelihood tree (ML) of 90% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

Figure 3. Bayesian tree (BI) of 75% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

Figure 4. Bayesian tree (BI) of 90% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

The Subfamily Corydoradinae

Our analyses supported topologies for the subfamily Corydoradinae that demonstrated to be similar to the recent multilocus phylogeny by Alexandrou *et al.* (2011), but we didn't recover the same sister groups. Our results also didn't corroborate the clades proposed by Shimabukuro-Dias *et al.* (2004), and the relationships of Corydoradinae were also different from the morphological hypothesis suggested by Nijssen & Isbrücker (1980), Reis (1998), and Britto (2003).

The analysis of UCE phylogeny strongly supported eight monophyletic lineages in this subfamily (ML > 100; BI = 1; Astral-II = 1), instead of nine, as proposed by Alexandrou *et al.* (2011), thereby potentially requiring a new generic name to host the species that do not have pre-available genus. Our results also didn't recover the position of *Aspidoras* as being sister group of *Corydoras* + *Brochis*, as suggested by Reis (1998). Instead, all analyzes corroborate that the clade that diverged firstly was lineage 1 (green on Fig. 1-6), and *Aspidoras* demonstrated to be close related to *Scleromystax* in all the topologies. Therefore, lineage 1 demonstrated to be the sister group of all others lineages of this subfamily. Lineage 1 represents the “true *Corydoras*”, due to carries the type species of the genus, *C. geoffroy* Lacépède (1803). The species of this clade are known for presenting long-snouted (‘saddle-nosed’), such as *C. desana* Lima & Sazima 2017, *C. narcissus* Nijssen & Isbrücker 1980 and *C. aurofrenatus* Eigenmann & Kennedy 1903. All ‘saddle nosed’ species occur within this lineage, and genetic differences among species are in general very large, which means many species diverged a long time ago.

Our results showed with strong support that the first species to diverge within this lineage were *C. coriatae* Burgess 1997 and *C. semiaquilus* Weitzman 1964, corroborating Alexandrou *et al.* (2011). *C. coriatae* and *C. semiaquilus* are the sister group of all other species of lineage 1. Our results also recovered *C. ellisae*, *C. geoffroy*, and *C. serratus* Sands 1995 as a monophyly group, sister of *C. areio*, *C. aurofrenatus*,

C. simulatus, *C. narcissus*, *C. treitlii*, *C. cervinus*, *C. maculifer*, and *Corydoras* C109. The results also corroborated Alexandrou *et al.* (2011) in belonging to the lineage 1 the species *C. coriatae*, *C. semiaquilus*, *C. treitlii*, *C. narcissus*, *C. serratus*, *C. simulatus*, *C. aurofrenatus*, *C. ellisae*, *C. pastazensis*, *C. areio*, *C. cervinus*, *C. geoffroy*, *C. maculifer*, *C. orcesi*, and *Corydoras* C109, and Britto (2003) proposed synapomorphies to his Clade III, and included in the analysis: *C. elisae*, *C. acutus*, *C. stenocephalus*, *C. septentrionalis*, *C. aurofrenatus*, and *C. vittatus*, but the results of ML, BI and Astral-II didn't recover the position of *C. acutus*. In our UCE phylogeny, *C. acutus* demonstrated with strong support to be related to the species of the lineage 8, not corroborating with Britto (2003) and Alexandrou *et al.* (2011). Nijsseni & Isbrücker (1980) grouped some species of lineage 1 within the “*acutus* group” *sensu* Nijssen (1970): *C. aurofrenatus*, *C. treitlii*, *C. spirulus*, *C. septentrionalis*, *C. elisae*, *C. fowleri*, *C. cervinus*, *C. pastazensis*, *C. orcesi*, *C. semiaquilus*, *C. oxyrhynchus*, *C. octocirrus*, *C. saramaccensis*, *C. simulatus*, *C. maculifer*, *C. blochi*, *C. vittatus*, *C. amapaensis*, *C. ourastigma*, and *C. narcissus*. Our analysis corroborated with *C. aurofrenatus*, *C. treitlii*, *C. ellisae*, *C. cervinus*, *C. pastazensis*, *C. orcesi*, *C. semiaquilus*, *C. simulatus*, *C. maculifer* and *C. narcissus* as belonging to this lineage.

C. orcesi are currently synonymous with *C. pastazensis*, our analysis demonstrated that these two species are paraphyletic. *C. orcesi* and *C. pastazensis* are related to each other, but they also are related with *C. desana* Lima & Sazima 2017. The same was presented by the multilocus phylogenetic hypothesis of Alexandrou *et al.* (2011). Besides that, *C. ellisae* are currently synonymous with *C. aurofrenatus*, but our analysis showed they are closely related to *C. cervinus* and *C. areio*, forming a paraphyletic group. On the other hand, this result didn't corroborate Alexandrou *et al.* (2011), where *C. elisae* is close related to *C. aurofrenatus*, but they didn't include *C. areio* in their analysis.

The second lineage is formed by all known species of the genus *Aspidoras*, except *A. pauciradiatus* (Weitzman & Nijssen. 1970), which is closely related to Lineage 5 (discussed below). The type species designated for this genus is *A. rochai* Ihering. 1907. All the analysis demonstrated that *A. poecilus* was the first species to diverge within this clade, not corroborating Alexandrou *et al.* (2011). Shimabukuro-Dias *et al.* (2004) that proposed *A. poecilus* is close related to *C. macropterus*, but our analysis didn't recover this position. Instead, *C. macropterus* demonstrated to be close related to lineage 3. This lineage is composed of species of the genus *Scleromystax*, but

also *Corydoras lacerdai* Hieronimus 1995, that should be relocated to this genus. The species described for lineage 3 are *S. barbatus*, *S. macropterus*, *S. prionotus*, *S. reisi*, and *S. salmacis*. Nijssen & Isbrücker (1980) had already reported this lineage as the “*barbatus* group”, but included *C. barbatus*, *C. paleatus*, *C. nattereri*, *C. ehrhardti*, *C. flaveolus*, *C. garbei*, *C. micracanthus*, *C. macropterus*, *C. cochui*, *C. steindachneri*, and *C. prionotos*.

Lineages 2 and 3 corroborated Britto (2003) and Alexandrou *et al.* (2011) as monophyletic clades, representing the genera *Aspidoras* and *Scleromystax*, respectively. However, the analyzes were partially congruent regarding the positioning of these two lineages. Alexandrou *et al.* (2011) proposed *Aspidoras* as being the sister group of *Scleromystax* + the lineages 4-9, but the ML, BI and Astral-II trees of 75% of complete matrix showed *Aspidoras* forming a monophyletic clade between *Aspidoras* and *Scleromystax*, closely related to each other, and this clade as sister group of the lineages 4-9 (Fig. 1, 3 and 5). On the other hand, the ML, BI and Astral-II trees of 90% of the complete matrix proposed a different topology for the genera *Aspidoras* and *Scleromystax*, where were proposed that the lineages 4 and 5 as the sister group of the lineages *Aspidoras* and *Scleromystax*, and 6, 7, 8, 9 (Fig. 2, 4 and 6). Instead, neither analysis recovered the position of *Aspidoras* and *Scleromystax* suggested by Alexandrou *et al.* (2011). Besides that, our analysis partially corroborates the composition of the clades proposed by Britto (2003) on his clade 1 (*Scleromystax* clade). In our analysis, all the analysis agrees that *C. lacerdai* belongs to lineage 3, and demonstrated to be the first species to diverge within *Scleromystax*. However, our results corroborated Alexandrou *et al.* (2011) and recovered the position of *C. lacerdai* in the lineage 3.

The next lineage, lineage 4, comprises all species called “dwarf *Corydoras*”. Despite that, not all the species within this group are dwarfs, but they are closely related and share similar color patterns throughout larval development. All the species of this group present similar in color pattern, snout shape and body size. Therefore, it would be necessary to resurrect the genus name of *Microcorydoras* (Myers, 1953), with the designated type species: *C. hastatus* Eigenmann & Eigenmann 1888. The valid names of the species in this lineage include (*sensu* Alexandrou *et al.*, 2011): *C. hastatus*, *C. pygmaeus*, *C. mamore*, *C. guapore*, and *C. paucerna*, and we included in our analysis sequences of *C. hastatus* and *C. pygmaeus*. Britto (2003) described synapomorphies to the clade IV (lineage 4), based on *C. hastatus* and *C. pygmaeus* Knaack 1966, and

proposed its monophyly. Nijssen and Isbrucker (1980) had already suggested that this species belongs to “*elegans* group”. All of our analyses strongly support the monophyly of this lineage and demonstrated this clade is closely related to lineage 5 (that includes *C. elegans*) in all topologies.

Lineage 5 formed a monophyletic group that demonstrated to be closely related to clade 4 in all of our analyses. The species of clade 5 are known as “*elegans* group”. *Corydoras elegans* Steindachner, 1876 was moved to the new genus *Gastrodermus* by Cope (1878) but this genus was synonymized as *Corydoras* by Nijssen and Isbrucker (1980). The type species designated was *C. elegans* Steindachner 1876 and this is the reason this group is known as “*elegans* group” *sensu* Nijssen (1970). The author included in this group the species: *C. elegans*, *C. hastatus*, *C. undulatus*, *C. latus*, *C. guapore*, *C. pygmaeus*, *C. nanus*, and *C. gracilis*. We do not corroborate the composition of the species suggested by Nijssen and Isbrucker (1980) to this clade. Our results demonstrated *C. gracilis* as the first species that diverge in lineage 5, corroborating the results of molecular analysis of Alexandrou *et al.* (2011). Moreover, all the analyses demonstrated *A. pauciradiatus* belongs to this lineage rather than to *Aspidoras* (Lineage 2). This species was described in *Corydoras*, and then synonymized to *Aspidoras* by Weitzman & Nijssen (1970), but Weitzman & Balph (1979) already reported the inconsistency of the relationship of *A. pauciradiatus* and *Corydoras*, and commented about the inconsistent characters used to separate these two genera. The same authors added samples of *A. pauciradiatus* from Negro River in the analysis for the species and reported that the diagnosed character of *Aspidoras*, the two fontanels (cranial and supraoccipital), was not a consistent enough character to diagnose the genus. Besides the holotype are described from the Araguaia River, it presented small and circular frontal fontanel, whereas the paratypes of the same River presented elongate frontal fontanel, similar to the samples of the Rio Negro River and some species of *Corydoras*. Our results reinforce that *A. pauciradiatus* from Rio Negro does not belong to *Aspidoras*, but *Corydoras*.

The lineage 6, *sensu* Alexandrou *et al.* (2011), forming a monophyletic sister group of the lineage 7-9. However, our results do not corroborate this position. Our analysis demonstrated with strong support that the clade formed by *C. ehrhardti*, *C. paleatus*, *C. longipinis*, and *C. nattereri* (lineage 6) is closely related to lineage 9. Besides that, the matrices of ML, BI, and Astral-II 75% and 90% didn't agree in the position of the lineage 6. ML, BI and Astral-II trees of 75% of complete matrix showed

the species of lineage 6 forming a monophyletic clade with *C. polystictus*, *C. griseus*, *C. tukano*, and *C. reinoldsi* with *C. benatti* and *C. cochui* sister of this first clade (Figs. 1, 3 and 5). On the other hand, ML, BI and Astral-II trees of 90% of complete matrix demonstrated the lineage 6 forming a monophyletic clade with *C. polystictus*, *C. griseus*, *C. tukano*, *C. reinoldsi*, *C. benatti*, and *C. cochui*, close related to *C. loretoensis*, with this species as sister of this clade (Figs. 2, 4 and 6). All the topologies agree with the non-monophyly of *C. paleatus*. The species under this clade were always classified as *Corydoras*, and because of that, there is no type species available to designate and must be morphologically reviewed. Thereby, it would be necessary to describe a new genus with a new type species to relocate this species.

The lineage 7 comprises mainly the “*aeneus* group”. *C. aeneus* represents a complex of species, mainly due to extensive geographic distribution throughout the neotropical region and pattern of coloration. A morphological revision would imply resurrecting the genus *Osteogaster* (Cope, 1894), and the type species *C. eques* Steindachner 1876. Nijssen & Isbrücker (1980) grouped these species in what they called “*aeneus* group”, with the species: *C. eques*, *C. melanotaenia*, *C. metae*, *C. potaroensis*, *C. melini*, *C. arcuatus*, *C. bondi*, *C. copenamensis*, *C. griseus*, *C. rabauti*, *C. zygatus*, *C. osteocarus*, *C. reynoldsi*, *C. habrosus*, *C. axelrodi*, *C. boesemani*, *C. sanchesii*, *C. baderi*, *C. guianensis*, *C. heteromorphus*, *C. panda*, *C. weitmani*, *C. gossei*, *C. oiapoquensis*, and *C. condiscipulus*, but our analysis didn't recover the same species.

Oliveira *et al.* (1988) demonstrated that the chromosome number variation within the *C. aeneus* populations ranged from 58-64, and the existence of a diploid-tetraploid system within the species group. Oliveira *et al.* (1990) reported that differences in chromosome counts suggest reproductive isolation partly due to karyotypic differentiation. In this paper, the authors explain the cytogenetic difference may suggest that the occurrence of tandem translocation events was responsible for the observed variability between the morphotypes. Their analysis shows that chromosomal rearrangements were more frequent than morphological modifications, and Nijssen & Isbrücker couldn't distinguish the morphological difference between groups of species (Nijssen & Isbrücker, 1980a; Nijssen & Isbrücker, 1980b). Oliveira *et al.* (1992) also demonstrated that there are at least five groups of species sharing similar chromosome morphology, diploid numbers, and DNA content. A diagram suggesting hypothetical cytogenetic interrelationships in the genus *Corydoras* was presented which also conflicted with the morphological groups previously proposed.

Our analysis demonstrated the no monophyly of *C. aeneus* that showed to be paraphyletic and close related to *C. rabauti*. Originally described by Gill (1858) for the island of Trinidad, *C. aeneus* encompasses a wide range of morphologically similar populations distributed in the various cis-Andean basin, occurring from Colombia and the island of Trinidad to the La Plata river basin, Argentina, east of the Andes (Reis, 2003), with overlaps between areas of occurrence of other species with similar patterns of coloration of body. The capacity of producing toxins of Corydoradinae has been reported by aquarists through anecdotal evidence, scientifically unconfirmed until the investigations of Greven *et al.* (2006). The authors reported that *C. aeneus* and *C. sterbai* can produce toxins through the axillary gland at the pectoral spine base, and the productions of toxic secretions (containing a variety of bactericidal substances) are stimulated by stress. Alexandrou *et al.* (2011) have already introduced the hypothesis of mimetic rings among Corydoradinae species, and the non-monophyly of *C. aeneus* may be related to the toxin production capacity of this species. In this paper, the authors also reported the non-monophyly of *C. aeneus*. More recently, Wright (2009) has shown that many more Corydoradinae species are toxic than previously hypothesized. Although all *Corydoras* may possess the ability to produce toxins, some species are likely to be more toxic than others, and this could be influenced by the similar pattern of color between other species. Furthermore, the taxonomic problem in this species implies not only a lack of knowledge of the interspecific differences of the "*aeneus* group" and the variations within the population, but also an understanding of their geographic distribution.

In 2003, the results of Britto suggested the monophyly of the lineage 7 (clade IX), including in the analysis samples of *C. aeneus*, *C. zygatus*, *C. rabauti*, *C. eques*, and the species related to *Brochis*, and also presented synapomorphies that support the clade. Our analysis confirms that this lineage is a monophyletic clade, and closely related to lineage 8. *C. zygatus* demonstrated to be the first species to diverge in this lineage. *C. zygatus* and *C. rabauti* showed to be more closely related to each other than to other species of this lineage. Britto (2003) had already reported the non-monophyly of *Brochis* related to this lineage, and our analysis demonstrated that *Brochis* do not belong to this lineage, but lineage 8 (corroborating Alexandrou *et al.* 2011).

This lineage includes the synonymized genus *Brochis* (Cope, 1871). Species whitening this group have deep bodies and the longest length between all Corydoradinae. They also have the "intermediate long-snout" of this subfamily. A taxonomical revision

of this subfamily would involve the revalidation of *Brochis*, with the designated type species *B. splendens*. Lineage 8 is a species-rich group divided into more monophyletic sub-clades. Alexandrou *et al.* (2011) proposed four subclades: the Sub-clade 1 would be formed by species of the genus *Brochis*: *B. britskii*, *B. multiradiatus*, and *B. splendens*. Furthermore, another genus would have to be named for the next subclades within this species-rich lineage. Sub-clade 2, *sensu* Alexandrou *et al.* (2011) would be formed by *C. garbei* and *C. difluviatilis*. Our analysis corroborated only *C. difluviatilis* that demonstrated to be the first species to diverge into this lineage. The Sub-clade 3 would be composed of *C. latus*, *C. sodalis*, *C. reticulatus*, *C. geryi*, and *C. pantanalensis*. We only corroborated *C. reticulatus* and *C. geryi* as belonging to the same clade. *C. pantanalensis* was demonstrated to be closely related to *C. adolfoi* and *C. duplicareus* in lineage 9. The Sub-clade 4 would be composed by *C. crypticus*, *C. imitator*, *C. virginiae*, *C. amandajanea*, *C. condisciplus*, *C. ornatus*, *C. orphnopterus*, *C. pulcher*, *C. agassizii*, *C. ambiacus*, *C. crimmeni*, *C. delphax*, *C. ephippifer*, *C. incolicana*, *C. robustus*, *C. leopardus*, *C. gomezi*, *C. haraldschultzi*, *C. isbrueckeri*, *C. noelkempffi*, *C. pinheiroi*, *C. robinae*, *C. seussi*, *C. spectabilis*, *C. approuaguensis*, *C. filamentosus*, *C. sychri*, *C. melanistius*, *C. lamberti*, *C. spilurus*, and *C. bifasciatus*. Our analysis didn't recover the position of almost all the species of this sub-clade. Our results suggested more than four monophyletic groups. Only *C. imitator*, *C. amandajanea*, *C. condisciplus*, *C. delphax*, *C. melanistius*, *C. robinae* and *C. melini* demonstrated to be clode related to each other, and would be considered Sub-clade 4; *C. virginiae*, *C. orphnopterus*, *C. leopardus*, *C. bifasciatus* and *C. weitzmani* formed a monophyletic group (Sub-clade 5); *C. pulcher*, *C. seussi*, *C. acutus*, *C. melanistus*, *C. ambiacus*, and *C. punctatus* also formed a monophyletic group and would be considered the Sub-clade 6; *C. agassizii*, *C. haraldschultzi*, and *C. spectabilis* would be considered the Sub-clade 7.

Although Britto (2003) found synapomorphies that supported the monophyly of *Brochis*, the author opted for a conservative approach to synonymizing this genus to *Corydoras*, in an attempt to make *Corydoras* monophyletic, instead of revalidating the four genera of sister species of *Brochis*. Different from our analysis proposed, his results suggested *Brochis* were close related to *C. aeneus*, *C. zygatus*, and *C. rabauti*, forming a tritomy with this species. The ML, BI, and Astral-II strongly supported the monophyly of *Brochis*, and as being the second clade to diverge in lineage 8, behind *C. cf. robustus* and *C. difluviatilis*. On the other hand, in our analysis, *C. britskii* recovered

as non-monophyly. The samples of *C. britskii* were collected in the Paraguay River basin, besides that, the same voucher 41751 (LBP 8503) was demonstrated to be closely related to *C. splendens*, while *C. britskii* voucher 8116 (LBP 688) is closely related to *Corydoras* sp.

Besides that, Britto also reported *C. reticulatus* as forming a polytomy with a large assemblage with basal taxa. Our results didn't recover this position and reported *C. reticulatus* forming a monophyletic group closely related to *C. geryi* and *Corydoras* sp. collected from the Upper Amazon River, in Peru. Besides that, the short branch length of this lineage and the high morphological diversity against genetical similarity (Chapter 2) suggested that morphological and ecological diversities may be the result of colonization followed by rapid adaptive radiation of the lineage 8 and 9, not corroborating Britto (2003) as this lineage are formed by basal taxa.

In the phylogenetic hypothesis presented by Britto (2003), the author proposed *C. difluviatilis* as sister of all other species of the tribe Corydoradinae and reported several plesiomorphic characteristics. Our analysis didn't recover this position of *C. difluviatilis*. In strong contrast to Britto's results, our data showed that *C. difluviatilis* is not monophyletic. The species of *C. difluviatilis* from Upper Paraná River, Minas Gerais State (voucher 60150), demonstrated to be sister group of the lineage 8 (Fig. 1-6, Supplementary Tab. 1). ML, BI and Astral-II recovered *C. difluviatilis* and *Corydoras* cf. *robustus* identified temporarily as "false *robustus*" (voucher 18930, LBP 2822) as the first clade to diverge into this lineage. On the other hand, the specimens from Corumbataí River, São Paulo State (also Upper Paraná River basin) demonstrated to be sister group of lineage 9 (the "*adolfoi* group"). Multiple dispersal events within lineage 8 and 9 seem to have occurred and need to be better investigated.

Lineage 9 is by far the richest species group of the Corydoradinae, widely distributed across all basins except for the East coast of Brazil and Sao Francisco. This lineage is comprised of all of the classic "short-snouted" species of Corydoradinae, known as "*adolfoi* group" *sensu* Nijssen & Isbrücker (1980). A revision would involve the resurrection of the name *Hoplosoma* (Agassiz, 1846), with the designated type species: *C. punctatus*, currently a synonym of *C. paleatus* (Jenyns 1842). This is a complicated clade mainly because this lineage is a very species-rich lineage and the short length of branch shown on the topology of the trees suggests many of these species are relatively recently evolved. There is a large diversity of color patterns within this lineage and some color patterns have evolved multiple times such as the '*arcuatus*'

pattern, which appears to have evolved at least 4 times within this lineage (Alexandrou *et al.*, 2011).

Our analysis didn't recover the position of *C. atropersonatus* close related to *C. loretoensis* as mentioned by Britto (2003) (clade V). ML, BI, and Astral-II agree that two species belong to the lineage 9. However, in our analysis, they are not related to each other, *C. loretoensis* is most related to *C. axelrodi*, *C. habrosus* and *C. metae*. Britto (2003) grouped *C. axelrodi* in clade VI and *C. habrosus* in clade VII, with *C. cochui*, *C. julii*, and *C. bicolor*. The clade VIII suggested by the author was composed by *Corydoras xinguensis*, *C. panda*, *C. trilineatus*, *C. arcuatus*, *C. baderi*, *C. flaveolus*, *C. gracilis*, *C. undulatus*, and *C. garbei* as a monophyletic clade, and *C. panda*, *C. trilineatus*, *C. arcuatus*, *C. baderi*, *C. flaveolus*, *C. gracilis*, *C. undulatus*, and *C. garbei* forming a second monophyletic clade, but the author reported that this group formed a trytomy with *C. panda*. Our results do not recover the position of this species. Our analysis suggested three monophyletic sub-clades to this lineage: the first one is composed of *C. difluviatilis* that demonstrated to be sister of all species, the first species to diverge into the lineage 9; a second formed by the species of lineage 6 as sister of a clade formed by *C. reinoldsi*, *C. tukano*, *C. cf. griseus*, *C. aff. polystictus*, *C. cochui* and *C. benatii*; and the third monophyletic sub-clade formed by *C. loretoensis* as sister of *C. habrosus*, *C. axelrodi*, *C. metae*, *C. concolor*, *C. loxozonus*, *C. speranza*, *C. urucu*, *C. arcuatus*, *C. grantii*, *C. julii*, *C. trilineatus*, *C. acensis*, *C. caudimaculatus*, *C. weitzmani*, *C. similis*, *C. aff. virescens*, *C. polystictus*, *C. panda*, *C. diphyses*, *C. sterbai*, *C. gossei*, *C. oiapoquensis*, *C. multimaculatus*, *Corydoras* C91, *Corydoras* sp., *C. atropersonatus*, *C. cf. brevirostris*, *C. araguaiensis*, *C. leucomelas*, *C. melanistius*, *C. burguesi*, *C. duplicareus*, *C. pantanalensis* and *C. adolfoi* are all species closely related to each other. Nijssen & Isbrücker (1983) described *C. imitator* as being close related morphologically to *C. adolfoi*, but our phylogenetic hypothesis demonstrated that these species don't belong to the same lineage. *C. imitator* belongs to lineage 8, and is closely related to *C. agassizi*, *C. amandajanae*, *C. spectabilis*, while *C. adolfoi* belong to lineage 9. However, *C. arcuatus*, *C. adolfoi*, *C. brevirostris*, *C. metae*, *C. loxozonus*, and *C. duplicareus* demonstrated to be non-monophyletic in all the topologies of our analysis.

Figure 5. Topology of Astral of 75% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

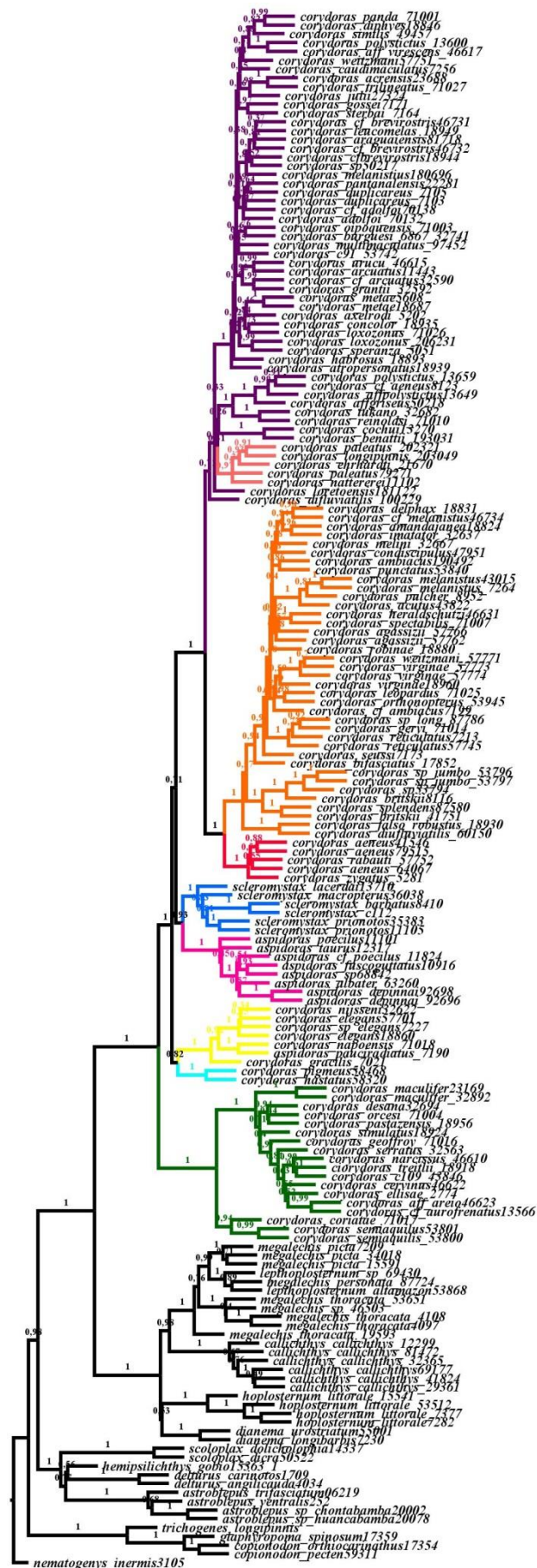


Figure 6. Topology of Astral of 90% complete matrix based on 188 taxa and 2.536 UCE loci showing interspecific relationships of the family Callichthyidae. The subfamily Corydoradinae is represented subdivided into nine lineages: lineage 1, green; lineage 2, pink; lineage 3, blue; lineage 4, cyan; lineage 5, yellow; lineage 6, salmon; lineage 7, red; lineage 8, orange; and lineage 9, purple. All nodes are supported with 100% bootstrap values.

Conclusion

We propose here a new phylogenetic hypothesis for the family Callichthyidae elaborated through ultra-conserved element sequence data (UCEs) and tested its monophyly through Bayesian Inference, Maximum Likelihood, and Astral-II coalescence analysis to help clarify the relationship within their subfamilies. All the analyses showed strong support branches that corroborated the monophyly of almost all genera. The results represent new information to be considered when compared with pre-available morphological data and allow a new view on the complexity of species of Callichthyidae and on the processes involved in the evolutionary history of the group.

Our results corroborate with Reis (1998) about the monophyly of both subfamilies of Callichthyidae but do not corroborated in the relationship of their sister groups. Our analysis is according to the molecular hypothesis of Shimabukuro-Dias *et al.* (2004) about *Dianema* being the first genus to diverge within the subfamily Callichthyinae. We revealed the non-monophyletic of *Megalechis*, which proved to be the most problematic within this subfamily, and the necessity of a new taxonomical review within this genus to uncover its monophyletic subgroups, and possibly split this genus into more than one monophyletic clade. Reis (1998) described *Megalechis* in an attempt to resolve the non-monophyly of *Hoplosternum*, however, a taxonomic revision is needed to describe another genus to relocate the species that are causing the non-monophyly of *Megalechis*.

Our results showed topological differences among trees were highly unrelated to previous phylogenetic hypotheses of Corydoradinae. The main difference with the morphological hypothesis is in the topology of lineage 1 that showed to be a sister clade to all other lineages of this subfamily. Lineage 1 host the type species of *Corydoras*, *C. geoffroy*, so we must conclude that all other species that do not belong to lineage 1 do not represent species of *Corydoras*, but to other genera. In addition, our topologies did not recover the same lineages proposed by the most recent molecular phylogeny to this

subfamily (Alexandrou *et al.*, 2011). Our results retrieved eight natural groups within Corydoradinae, instead of nine, which must be taxonomically revised. Some of these lineages represented greater complexity in their interrelationships, such as lineage 7, 8, and 9.

The analyzes managed to evidence at least four candidates for new genus, considering the monophyletic subclades of the lineage 8, and the revalidation of five synonymized genera: *Microcorydoras* (lineage 4), *Gastrodermus* (lineage 5), *Osteogaster* (lineage 7), *Brochis* (sub-clade 1, lineage 8) and *Hoplossoma* (lineage 9). The lineage 6, *sensu* Alexandrou *et al.* (2011), must be considered *Hoplossoma* (lineage 9), as demonstrated by our analyses. The revalidation and description of new genus must involve a morphological revision of the subfamily.

When Reis (1998) presented his phylogenetic hypothesis of the family Callichthyidae and corroborated the monophyly of both subfamilies, the author proposed the genus *Aspidoras* as sister-group of a clade formed by *Corydoras* and *Brochis*. The author couldn't find characters that supported the monophyly of *Corydoras*, whereas the monophyly of *Brochis* was supported by four derived features. Besides that, the author suggested *Brochis* was synonymized to *Corydoras* in an attempt to become this genus monophyletic. However, Britto (2003) presented a new hypothesis of the relationship of Corydoradinae, and instead of *Brochis* and *Corydoras* forming a group with *Aspidoras* as its sister assemblage, the author proposed a clade consisting of *Aspidoras* and *Scleromystax* (Britto, 2003) and synonymized *Brochis* to *Corydoras*. The author observed a tritomy between *C. aeneus*, *C. zygatus* and *C. rabauti* mainly caused by *Brochis*. Species of Corydoradinae, usually belonging to different lineages, live in sympatry, competing for the same habitats, and these species assemblages tend to display identical color patterns, mimicking each other (Alexandrou *et al.*, 2011). This species presents variations in snout morphology due to competition for food and resources in these environments, thus enabling niche differentiation between species. The morphology of the snout, particularly in lineage 8, can change grossly amount the sub-clades. This character seems to have evolved multiple times in different lineages, independently. Ecological factors such as rivers and streams of the Amazon basin have a lot of influence on it because provide a huge amount of habitats, climates, and niches. Short snouts are commonly found in lineages 2, 4, 5, 6, 7, 8, and 9, while long snouts are presented in "basal" lineages like 1 (the first lineage to diverge), 3, and 8 (sub-clade 1). However, the members of lineage 8 presented a different kind of snout morphology:

since long-snouted sub-clades (*Brochis*), short-snouted sub-clades (as *C. difluviatilis*, *C. reticulatus* and *C. geryi*), and the rest of the species (comprising the majority of diversity) presenting intermediate long snouts. This variation on the morphology of the snout in the sub-clades of lineage 8 may have confused the cladistics analysis of Britto, which led him to synonymize *Brochis* with *Corydoras*. Perhaps due to these changes in snout morphology, it has been particularly challenging to identify osteological synapomorphies that could be used to delineate different genetic lineages of Corydoradinae in the morphologic approach.

The topologies of lineages 8 and 9 demonstrated very short branch lengths, suggesting high rates of diversification in a short period of time. Besides that, lineage 8 and 9 showed high phenotypic disparity against low genotypic disparity, indicating that there was adaptative radiation of these lineages. Cytogenetic data, such as Oliveira *et al.* (1992; 1993) and Shimabukuro-Dias *et al.* (2004), reported variation in genome size and chromosome number of lineages 8 and 9 of *Corydoras* that accelerate diversification rates. Oliveira *et al.* (1992) demonstrated cases of genome duplication among *Corydoras* and *Brochis* and the influence of polyploidy in the diversification of this species. The genome duplication and polyploidy in ancient fish lineages is regarded to be one of the key genetic mechanisms responsible for the rapid evolution of vertebrates (Van de Peer *et al.*, 2009). Besides that, genomic duplication increases resistance to ecological limits imposed by adding extra genetic material, promoting adaptation, which may also explain why polyploids tend to be successful and have some kind of advantage in the environment, as suggested by Fawcett *et al.* (2009) in a study with plants. In addition to genomic duplication acting directly on the rapid speciation of Corydoradinae lineages, the occurrence of mimetic species, as demonstrated by Alexandrou *et al.* (2011) can increase the diversification rates among allopatric communities. Thereby, if a heritable trait is related to a mimetic gene that gives the high aptitude to the individual and confers a greater chance of survival in an environment where selection is led by predation, consequently the frequency of this gene increase over generations, and this character quickly settles in the population in a short period of time, promoting rapid phenotype differentiation but not genotype differentiation, promoted by stabilizing selection.

Mallet & Joron (1999) hypothesized that mimicry strongly contributes to the speciation and maintenance of species. If mimicry contributes to speciation, and genome duplication guarantees advantage to the individuals that provide these

characteristics promoting rapid differentiation, possibly these events were responsible for the high diversity of species observed in lineages 8 and 9. Colour pattern seems to have a strong adaptive value in Corydoradinae, and the genes associated with color patterns also are duplicated with the genome, increasing the aptitude. All these factors seem to have been responsible for the high diversification of lineages 8 and 9, increasing the chances of survival and reproduction, favoring mimetic and polyploid individuals. Although adaptive radiation and stabilizing selection acting on mimetic species are a possible explanation for cases of high diversification in a short space of time, as shown in lineages 8 and 9, adaptive radiation events are attributed to the colonization of a new territory where there are no competitors, to the extinction of competitors or the breaking of adaptive barriers, and therefore, our explanation remains speculative and needs further study to test whether adaptive radiation was the responsible factor, and thus confirm or reject this hypothesis.

In the light of the results of the UCE phylogeny proposed here, we reinforce the urgency to revalidate five synonymized genera of Corydoradinae, and the necessity of description of at least one new genus in Callichthyinae and four in Corydoradinae to host the species that are causing the non-monophyly of *Megalechis* and *Corydoras*, respectively. Despite the extensive sampling carried out in this work, we recognized that some species need to be better sampled for a more complete phylogenetic approach to investigate the non-monophyly of this species. Nevertheless, our results indicate that the non-monophyly of some species in the molecular approach may evidence the existence of cryptic species, and reveals that a morphological review will be extremely important for a better understanding of the evolution of the group, as well as the reassessment of the current taxonomic arrangement together with the new clades presented, in order to increase the knowledge of the group. Besides that, a multidisciplinary approach considering the interface of ecology and evolution biogeography to assess likely patterns of geographic diversification, variety of phylogenetic comparative methods, including relaxed molecular clock analyses, ancestral reconstructions, diversification rate tests, and dispersal-extinction with a time calibrated tree is necessary in order to investigate the mechanisms of speciation, coexistence and adaptative radiation in this large family Callichthyidae.

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Supplementary Table 1. Taxonomic sampling, voucher, catalog number, and drainage of the samples.

Family	Subfamily	Lineage	Species	Voucher	Catalog number	Drainage	Country	Coordinates
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras</i> aff. <i>areio</i>	46623	LBP 10098	Rio Paraguai, La Plata basin	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras</i> cf. <i>aurofrenatus</i>	13566	LBP 1960	Rio Paraguai, La Plata basin	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras cervinus</i>	46622	LBP 10097	Rio Madeira, Amazonica basin	Brazil	S 14°96.904' W 59°98.319'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras coriatae</i>	71017	LBP 18433	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras desana</i>	32694	LBP 7712	Rio Negro, Amazonica basin	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras ellisae</i>	16804	LBP 2774	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras geoffroy</i>	71016	LBP 18432	Guiana basin	Guiana	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras maculifer</i>	32892	LBP 7213	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras maculifer</i>	23169	LBP 6874	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 15°40.678' W 52°17.863"
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras narcissus</i>	46610	LBP 10094	Rio Purus, Amazonica basin	Brazil	S 07°56.110' W 63°27.353'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras orcesi</i>	71004	LBP 18420	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras pastazensis</i>	18956	LBP 2835	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras semiaquilus</i>	53800	LBP 12467	Lago Nanay, Amazonica basin	Peru	S 3°42'1.32" W 73°16'36.90"
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras semiaquilus</i>	53801	LBP 12467	Lago Nanay, Amazonica basin	Peru	S 3°42'1.32" W 73°16'36.90"
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras serratus</i>	32563	LBP 6869	Rio Negro, Amazonica basin	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras simulatus</i>	18924	LBP 2820	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras treitlii</i>	18918	LBP 2818	-	-	-
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras urucu</i>	46614	LBP 10095	Rio Madeira, Amazonica basin	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 1	<i>Corydoras</i> C109	43846	LBP 9325	Rio Guamá, Amazonica basin	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras albater</i>	63260	LBP 15292	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 13°44'15.9" W 46°21'48.8"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras</i> cf. <i>poecilus</i>	11824	LBP 1658	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 15°55'01.5" W 50°07'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras depinnai</i>	92696	LBP 23907	Rio Ipojuca, Costeira basin	Brazil	S 08°22'59.5" W 35°26'41.0"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras depinnai</i>	92698	LBP 23907	Rio Ipojuca, Costeira basin	Brazil	S 08°22'59.5" W 35°26'41.0"

Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras fuscoguttatus</i>	10916	LBP 1295	Upper Rio Paraná, La Plata basin Rio Tocantins-Araguaia,	Brazil	S 21°23'2.99" W 50°2'32.99"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras poecilus</i>	11101	LBP 1272	Amazonica basin	Brazil	S 15°53'56.36" W 52°11'20.57"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras raimundi</i>	69110	LBP 5568	Rio Parnaíba, Costeira basin	Brazil	S 09°06'35" W 45°55'20"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras taurus</i>	12317	LBP 1427	Rio Paraguai, La Plata basin Rio Tocantins-Araguaia,	Brazil	S 17°02'36.3" W 53°28'43.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras velites</i>	18554	LBP 1443	Amazonica basin	Brazil	S 17°19'19.4" W 53°14'25.9"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras</i> sp.	64571	LBP 15714	Rio Xingu, Amazônica basin	Brazil	S 12°57'05.8" W 51°52'28.2"
Callichthyidae	Corydoradinae	Lineage 2	<i>Aspidoras</i> sp.	68842	LBP 17226	Upper Rio Paraná, La Plata basin	Brazil	S 17°17'36.0" W 48°31'51.3"
Callichthyidae	Corydoradinae	Lineage 2	<i>Corydoras</i> sp.	25H6	LBP 18650	Rio Guaviare, Orinoco basin	Colombia	-
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax barbatus</i>	8410	LBP 743	Rio São João, Costeira basin Rio Ribeira de Iguape, Costeira	Brazil	S 25°58,649' W 48°52,993'
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax kronei</i>	6317	LBP 2696	basin Rio Ribeira da Terra Firme,	Brazil	S 24°33.738' W 48°40.166'
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax lacerdai</i>	13710	LBP 1966	Costeira basin	Brazil	S 15°32'17.9" W 39°00'28.5"
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax macropterus</i>	36038	LBP 7550	Rio Ribeira de Iguape, Costeira	Brazil	S 24°42'57.8" W 47°41'28.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax prionotos</i>	11105	LBP 1267	basin Rio Ribeira de Iguape, Costeira	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax prionotos</i>	35383	LBP 17381	basin Rio Ribeira de Iguape, Costeira	Brazil	S 24°35'41.1" W 48°12'53.3"
Callichthyidae	Corydoradinae	Lineage 3	<i>Scleromystax</i> C112	11125	LBP 1237	basin	Brazil	S 24°10,890' W 46°50,563'
Callichthyidae	Corydoradinae	Lineage 4	<i>Corydoras hastatus</i>	58320	LBP 14008	Rio Paraguai, La Plata basin	Brazil	S 17°49'26.7" W 57°31'03.0"
Callichthyidae	Corydoradinae	Lineage 4	<i>Corydoras pygmeus</i>	58468	LBP 14076	Rio Tapajós, Amazonica basin	Brazil	S 04°55'58.8" W 56°51'51.6"
Callichthyidae	Corydoradinae	Lineage 5	<i>Aspidoras pauciradiatus</i>	7190	LBP 548	-	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras elegans</i>	18860	LBP 2795	-	-	-
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras elegans</i>	57701	LBP 14804	Rio Amazonas, Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras</i> cf. <i>elegans</i>	46608	LBP 10092	Rio Paraguai, La Plata basin	Brazil	S 18°42.338' W 54°83.472'
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras</i> cf. <i>elegans</i>	7227	LBP 556	Rio Purus, Amazonica basin	Brazil	-
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras gracilis</i>	71021	LBP 18437	-	-	-

Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras napoensis</i>	71018	LBP 18434	-	-	-
Callichthyidae	Corydoradinae	Lineage 5	<i>Corydoras nijsseni</i>	32622	LBP 6861	Rio Negro, Amazonica basin	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 6	<i>Corydoras ehrhardti</i>	21670	LBP 3635	Rio Itapocu, Costeira basin	Brazil	S 26°28'17.2" W 49°10'55.1"
Callichthyidae	Corydoradinae	Lineage 6	<i>Corydoras longipinnis</i>	203049	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 6	<i>Corydoras nattererei</i>	11102	LBP 1266	Rio Ribeira de Iguape, Costeira basin	Brazil	S 24°12,441' W 47°28,616'
Callichthyidae	Corydoradinae	Lineage 6	<i>Corydoras paleatus</i>	202321	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 7	<i>Corydoras aeneus</i>	64067	LBP 16579	Rio Bacajá, Amazonica basin	Brazil	S 4°31'51.41" W 51°23'38.97"
Callichthyidae	Corydoradinae	Lineage 7	<i>Corydoras aeneus</i>	41546	LBP 8417	Rio Paraguai, La Plata basin	Brazil	S 14°27'26.3" W 57°34'34.0"
Callichthyidae	Corydoradinae	Lineage 7	<i>Corydoras aeneus</i>	79515	LBP 18917	Rio Paraná, La Plata basin	Brazil	S 23°11'09.7" W 47°14'19.1"
Callichthyidae	Corydoradinae	Lineage 7	<i>Corydoras rabauti</i>	57752	LBP 14816	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 7	<i>Corydoras zygatus</i>	5281	LBP 422	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras acutus</i>	43822	LBP 9316	Rio Guamá, Amazonica basin	Brazil	S 01°34'00.5" W 47°09'51.4"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras agassizii</i>	57762	LBP 14818	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras agassizii</i>	57766	LBP 14818	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras amandajanea</i>	18824	LBP 2781	Amazonica basin	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras ambiacus</i>	190492	LBP 2787	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras bifasciatus</i>	72052	LBP 17852	Rio Tapajós, Amazonica basin	Brazil	S 4°42'3.71" W 56°37'20.15"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras britskii</i>	41751	LBP 8503	Rio Paraguai, La Plata basin	Brazil	S 15°19'53.5' W 57°11'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras britskii</i>	8116	688	Rio Paraguai, La Plata basin	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras cf. ambiacus</i>	7199	LBP 13331	Rio Purus, Amazonica basin	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras cf. melanistus</i>	46734	LBP 9962	Rio Pelohojo, Orinoco basin	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras condiscipulus</i>	47951	LBP 9311	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras delphax</i>	18831	LBP 2784	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras difluviatilis</i>	60150	LBP 11669	Upper Rio Paraná, La Plata basin	Brazil	S 17°46'16.8" W 47°06'15.8"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras geryi</i>	71014	LBP 18430	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras heraldschutzi</i>	46631	LBP 10100	Rio Madeira, Amazonica basin	Brazil	S 15°01.0642" W 59°95.762'

Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras imitator</i>	32637	LBP 7710	Rio Negro, Amazonica basin	Brazil	N 00°00.321' W 66°55.357'
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras leopardus</i>	71025	LBP 18441	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras melanistus</i>	7264	LBP 564	Alto Rio Negro, Amazonica basin	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras melanistus</i>	43015	LBP 9123	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 01°34'28.3" W 47°02'03.5"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras melini</i>	32667	LBP 7711	Rio Negro, Amazonica basin	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras orthonopterus</i>	53945	LBP 12509	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras pulcher</i>	8952	LBP 909	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras punctatus</i>	53840	LBP 12476	Lago Nanay, Amazonica basin	Peru	S 3°42'1.32" W 73°16'36.90"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras reticulatus</i>	57745	LBP 14814	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras reticulatus</i>	7213	LBP 553	Rio Purus, Amazonica basin	Brazil	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras robiniae</i>	18880	LBP 2803	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras cf. robustus</i>	18934	LBP	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras seussi</i>	7173	LBP 545	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras spectabilis</i>	71007	LBP 18423	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras splendens</i>	87580	LBP 22528	Amazonica basin	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras virginiae</i>	57773	LBP 14820	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras virginiae</i>	57774	LBP 14820	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras virginiae</i>	18960	LBP 2837	-	-	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras virginiae</i>	57771	LBP 14820	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp.	87786	LBP 22532	Rio Javari, Amazonica basin	Peru	S 04°19'45.9" W 70°00'30.2"
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp.	53796	LBP 12466	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp.	53797	LBP 12466	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 8	<i>Corydoras</i> sp.	53794	LBP 12466	-	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydora adolfoi</i>	32619	LBP 6863	Rio Negro, Amazonica basin	Brazil	N 00°04.665' W 66°49.546'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras acrensis</i>	23688	LBP 4149	Rio Juruá, Amazonica basin	Brazil	S 07°26'35.5" W 73°03'33.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras adolfoi</i>	70132	LBP 17851	Rio Negro, Amazonica basin	Brazil	S 00°41'53.2" W 66°14'55.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. adolfoi</i>	70138	LBP 17850	Rio Negro, Amazonica basin	Brazil	S 00°41'53.2" W 66°14'55.6"

Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. aeneus</i>	8123	LBP 689	Rio Paraguai, La Plata basin	Brazil	S 16°25,680' W 56°25,143'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cochui</i>	13270	LBP 1853	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 15°32'54.2" W 52°12'17.7"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras aff. griseus</i> <i>Corydoras aff.</i>	50218	LBP 10897	Rio Madeira, Amazonica basin	Brazil	S 09°15'23.3" W 64°23'13.6"
Callichthyidae	Corydoradinae	Lineage 9	<i>Polystictus</i>	13649	LBP 1957	Rio Paraguai, La Plata basin Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras araguaiensis</i>	81718	LBP 20799	Amazonica basin	Brazil	S 15°42'43.4" W 52°15'32.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras arcuatus</i>	11443	LBP 1348	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras atropersonatus</i>	18939 IAvH-P	LBP 2826	Amazonica basin	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras axelrodi</i>	t15202	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras burguesi</i>	32741	LBP 6867	Rio Negro, Amazonica basin	Brazil	S 00°08.156' W 67°05.057'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras caudimaculatus</i>	7256	LBP 562	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. arcuatus</i>	32590	LBP 7709	Rio Negro, Amazonica basin	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras benattii</i>	193031	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. brevirostris</i>	46731	LBP 9961	Rio Pelohojo, Orinoco basin	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. brevirostris</i>	46732	LBP 9961	Rio Pelohojo, Orinoco basin	Venezuela	N 7°32'22.6" W 66°08'31.1"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. brevirostris</i>	18944	LBP 2829	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. melanistius</i>	4577	LBP 404	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras concolor</i>	18935	LBP 2824	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras diphyes</i>	18846	LBP 2790	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras cf. difluviatilis</i>	100229	LBP 28262	Upper Rio Paraná, La Plata basin Upper Rio Negro, Amazonica basin	Brazil	S 22°20'51.8" W 47°34'14.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras duplicareus</i>	7103	LBP 525	Upper Rio Negro, Amazonica basin	Brazil	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras duplicareus</i>	7105	LBP 525	Upper Rio Negro, Amazonica basin	Brazil	-

Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras gossei</i>	7171	LBP 544	Amazonica basin	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras grantii</i>	32592	LBP 7709	Rio Negro, Amazonica basin	Brazil	N 00°16.259' W 66°38.365'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras habrosus</i>	18893	LBP 2808	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras julii</i>	27324	LBP 5598	Rio Parnaíba, Costeira basin	Brazil	S 07°46'16" W 46°01'45"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras leucomelas</i>	95926	LBP 26118	Amazonica basin	Brazil	S 04°26'37.0" W 70°14'11.0"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras loretoensis</i>	71023	LBP 18439	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras loxozonus</i>	206231	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras loxozonus</i>	71026	LBP 18442	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras metae</i>	27D6	LBP 18687	Rio Meta, Orinoco basin	Colombia	N 3°52'58.86" W 73°46'51.60"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras metae</i>	5608	LBP 428	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras metae</i>	rack27F4	LBP 18692	Rio Meta, Orinoco basin	Colombia	N 4°11'49.80" W 73°38'4.09"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras multimaculatus</i>	97452	LBP 28098	São Francisco basin	Brazil	S 15°07'30.7" W 44°47'26.2"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras oiapoquensis</i>	71003	LBP 18419	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras paleatus</i>	79271	LBP 19920	Rio Paraná, La Plata basin	Argentina	S 31°33'45.95" W 60°28'51.20"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras panda</i>	71001	LBP 18417	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras pantanalensis</i>	22281	LBP 3773	Rio Paraguai, La Plata basin	Brazil	S 19°34'33.7" W 56°14'49.5"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras polystictus</i>	13600	LBP 1958	Rio Paraguai, La Plata basin	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras polystictus</i>	13659	LBP 1958	Rio Paraguai, La Plata basin	Brazil	S 18°25'21.8" W 54°50'06.3"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras reinoldsi</i>	71010	LBP 18426	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras similis</i>	49457	LBP 10648	Rio Acre, Amazonica basin	Brazil	S 10°04'44.3" W 67°32'33.9"
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras speranza</i>	t15051	ANSP	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras sterbai</i>	7164	LBP 543	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras trilineatus</i>	71027	LBP 18443	-	-	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras tukano</i>	32682	LBP 6872	Rio Negro, Amazonica basin	Brazil	N 00°15' W 69°50'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras urucu</i>	46615	LBP 10095	Rio Madeira, Amazonica basin	Brazil	S 07°57.385' W 63°11.133'
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras aff. virescens</i>	46617	LBP 10096	Rio Paraguai, La Plata basin	Brazil	S 16°06.352' W 57°70.750'

Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras weitzmani</i>	57771	LBP 14820	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras</i> C91	53742	LBP 12495	Amazonica basin	Peru	-
Callichthyidae	Corydoradinae	Lineage 9	<i>Corydoras</i> sp.	50217	LBP	-	-	-
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	12299	LBP 1555	-	-	-
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	29361	-	-	-	-
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	32365	LBP 6803	Rio Paraná, La Plata basin	Brazil	S 22°26'12.2" W 49°12'40.5"
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	41824	LBP 8984	Rio São Francisco basin	Brazil	S 19°37'34.1" W 44°29'20.0"
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	81472	LBP 20867	Rio Paraguai, La Plata basin	Brazil	S 14° 0'29.38" W 56°48'24.95"
Callichthyidae	Callichthyinae	-	<i>Callichthys callichthys</i>	69177	LBP 17423	Rio São Francisco basin	Brazil	S 19°37'56.4" W 44°02'47.4"
Callichthyidae	Callichthyinae	-	<i>Dianema longibarbis</i>	7230	LBP 557	Rio Purus, Amazonica basin	Brazil	-
Callichthyidae	Callichthyinae	-	<i>Dianema urostriatum</i>	55001	LBP 9917	-	-	-
Callichthyidae	Callichthyinae	-	<i>Hoplosternum littorale</i>	15541	LBP 2183	Laguna de Castelleros, Rio Orinoco basin	Venezuela	N 07°30'50,9'' W 66°09'19,8''
Callichthyidae	Callichthyinae	-	<i>Hoplosternum littorale</i>	29519	LBP 6137	Rio Santa Rosa, Lago Maracaibo	Venezuela	N 09°38'53.8' W 72°34'56.4"
Callichthyidae	Callichthyinae	-	<i>Hoplosternum littorale</i>	53512	LBP 12525	Rio Itaya, Upper Amazonica basin	Peru	S 03°43'39.4" W 73°13'58.5"
Callichthyidae	Callichthyinae	-	<i>Hoplosternum littorale</i>	7377	LBP 569	Rio Guaiba, Costeira basin	Brazil	S 30°02,820' W 51°22,347'
Callichthyidae	Callichthyinae	-	<i>Hoplosternum littorale</i>	7282	LBP 466	Rio Paraná, La Plata basin	Brazil	S 19°34,630' W 57°01,123'
Callichthyidae	Callichthyinae	-	<i>Lepthoplosternum altamazon</i>	11244	LBP 1338	-	Peru	-
Callichthyidae	Callichthyinae	-	<i>Lepthoplosternum pectorale</i>	60945	LBP 10880	Rio Paraná, La Plata basin	Brazil	S 21°52'07.0" W 48°16'32.0"
Callichthyidae	Callichthyinae	-	<i>Lepthoplosternum</i> sp.	69430	LBP 13272	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 13°19' W 50°37'
Callichthyidae	Callichthyinae	-	<i>Megalechis personata</i>	87724	LBP 3809	Rio Itatinga, Costeira basin	Brazil	S 23°45'01.2' W 46°09'52.9"
Callichthyidae	Callichthyinae	-	<i>Megalechis picta</i>	15591	LBP 2201	Laguna de Castelleros, Rio Orinoco basin	Venezuela	N 07°30'50,9'' W 66°09'19,8''
Callichthyidae	Callichthyinae	-	<i>Megalechis picta</i>	34018	LBP 6976	Rio Negro, Amazonica basin	Brazil	N 00°01.199' W 67°10.192'
Callichthyidae	Callichthyinae	-	<i>Megalechis picta</i>	7209	LBP 552	Rio Purus, Amazonica basin	Brazil	-
Callichthyidae	Callichthyinae	-	<i>Megalechis thoracata</i>	19593	LBP 2972	Rio Tocantins-Araguaia, Amazonica basin	Brazil	S 13°20'05.1" W 50°42'16.2"
Callichthyidae	Callichthyinae	-	<i>Megalechis thoracata</i>	4108	LBP 239	Costeira basin	Brazil	S 10°02' W 67°52'

Callichthyidae	Callichthyinae	-	<i>Megalechis thoracata</i>	53651	LBP 12418	Lago Nanay, Amazonica basin	Peru	S 03°50'25.3" W 73°22'51.6"
Callichthyidae	Callichthyinae	-	<i>Megalechis thoracata</i>	4097	LBP 239	Costeira basin	Brazil	S 10°02' W 67°52'
Callichthyidae	Callichthyinae	-	<i>Megalechis</i> sp.	46503	LBP 10811	Rio Madeira, Amazonica basin	Brazil	S 14°58'08.5" W 59°58'59.5"
Astroblepidae	-	-	<i>Astroblepus</i> sp.					
			<i>Chontabamba</i>	20002	LBP 3254	Rio Ucayali, Amazonica basin	Peru	S 10°36'06,6'' W 075°29'10,8''
			<i>Astroblepus</i> sp.					
Astroblepidae	-	-	<i>huancabamba</i>	20078	LBP 3275	Rio Ucayali, Amazonica basin	Peru	S 10°02'57,6'' W 075°32'28,8''
Astroblepidae	-	-	<i>Astroblepus trifasciatum</i>	90786	LBP 29296	-	Colombia	-
Astroblepidae	-	-	<i>Astroblepus ventralis</i>	252	-	-	-	-
Loricariidae	Delturinae	-	<i>Delturus angilicauda</i>	4034	-	-	-	-
Loricariidae	Delturinae	-	<i>Delturus carinotos</i>	1709	-	-	-	-
Loricariidae	Delturinae	-	<i>Hemipsilichthys gobio</i>	15363_1	-	-	-	-
Nematogenyidae	-	-	<i>Nematogenys inermis</i>	19763	LBP 3105	Río Andalién, Costeira do Pacífico basin	Chile	S 36°50.304' W 72°55.642'
Scoloplacidae	-	-	<i>Scoloplax dicra</i>	50522	LBP 11001	Rio Madeira, Amazonica basin	Brazil	S 10°26'23.5" W 65°20'34.1"
Scoloplacidae	-	-	<i>Scoloplax dolicholophia</i>	14337	LBP 2014	Rio Negro, Amazonica basin	Brazil	-
Trichomycteridae	Copionodontinae	-	<i>Copionodon pecten</i>	59311	LBP 17361	Rio Paraguaçu, Costeira basin	Brazil	S 13°17'20" W 41°16'04"
			<i>Copionodon</i>					
Trichomycteridae	Copionodontinae	-	<i>orthiocarinathus</i>	57982	LBP 17354	Rio Paraguaçu, Costeira basin	Brazil	S 12°57'04" W 41°16'38"
			<i>Glaphyropoma</i>					
Trichomycteridae	Copionodontinae	-	<i>spinosum</i>	61425	LBP 17359	Rio Paraguaçu, Costeira basin	Brazil	S 12°52'40" W 41°18'43"
Trichomycteridae	Trichogeninae	-	<i>Trichogenes longipinnis</i>	22412	LBP 3862	Costeira basin	Brazil	S 23°21'06.8' W 44°45'48.9"

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