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ANDRESSA SANTOS GONÇALVES

**Chromatic polymorphism and phylogenetic independent
convergence in Bryconops Kner, 1858 (Ostariophysi:
Characiformes: Iguanodectidae)**

BELÉM – PA
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Tese apresentada ao Programa de Pós-Graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará, como parte dos requisitos exigidos para obtenção do título de mestre em Ecologia Aquática e Pesca.
Orientador: Prof. Dr. Jonathan Stuart Ready

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RESUMO

A fauna de peixes presentes em águas claras, águas pretas e águas brancas tendem a ser distintas em cada uma delas. Muitos peixes de pequeno porte na Amazônia apresentam uma variação no padrão de cor que ajuda na defesa contra predadores quando formam cardumes. Entre essas espécies temos membros do gênero *Bryconops*, Kner (1858), que necessita de revisão taxonômica, que inclui dois subgêneros claramente distinguidos por características morfológicas. Cada subgênero inclui espécies amplamente distribuídas como *B. caudomaculatus* e *B. melanurus* que apresentam variações em padrão de cor entre populações. Para determinar se o padrão de cor pode ser considerado útil para revisão taxonômica, analisamos as cores em relação à filogenia usando testes de independência de sinal filogenético e o teste de Wheatsheaf para verificar se estes caracteres apresentam convergência evolutiva. Os testes de sinal filogenético indicaram independência filogenética para a coloração das nadadeiras acima da linha mediana do corpo e o teste de Wheatsheaf apoia fortemente que dentre estas cores, a cor da nadadeira dorsal foi significativamente convergente entre espécies encontradas no mesmo tipo de água e a cor do lobo dorsal da nadadeira caudal mostrou uma tendência de convergência similar. Os resultados mostram que dependendo do tipo de água em que as espécies são encontradas eles podem apresentar uma coloração específica. A convergência de padrão de cor é esperada a refletir a seleção comum entre espécies porque deve ser resultado de seleção por predação, e assim incorporar as capacidades visuais dos predadores e a influência do ambiente visual local. Portanto a cor em si não deve ser considerada um caractere confiável para revisões taxonômicas nestes táxons.

Palavras-chave: Ambientes aquáticos. Evolução. Peixes neotropicais. Delimitação de espécies.

ABSTRACT

The fish fauna varies considerably between Amazonian clear, black and white waters with implications for evolution and conservation of this diverse biota. Many small shoaling stream fishes show shared colour patterns that are hypothesized to provide a collective defence against predators. Amongst these groups, members of the genus *Bryconops*, Kner (1858), represent a taxonomic challenge. Despite the presence of two well defined subgenera based on morphological evidence, each contains at least one species (*B. (Bryconops) caudomaculatus* and *B. (Creatochanes) melanurus*, with a very wide distribution, within which regional populations seem to present colour pattern variations. To test whether colour is plastic in those species or if phenotypic variation is related to cladogenetic events we performed tests for phylogenetic independence and determined the strength of convergence for simple colour characters in relation to water type. Colour variation characters for fins above the median line of the body were generally found to be independent from phylogeny and the Wheatsheaf test strongly supports convergence of the dorsal fin colour with the type of water from which the sample was collected and suggests a similar trend for the colour of the dorsal lobe of the caudal fin. Our results suggest that simple colour characters cannot necessarily be relied upon for use in taxonomic revision of the genus as local phenotypic variants may represent environmentally determined plasticity rather than local selection and further studies are required to determine the validity of these characters.

Key words: Aquatic light environments, Evolution, Neotropical fishes, Species delimitation

LIST OF ACRONYMS AND ABBREVIATIONS

DF - Dorsal fin;

Mel – Melanin;

Hy – Hyaline;

Y – Yellow;

O – Orange;

R – RED;

AdF – Colour of adipose fin;

DLCF – Colour of dorsal lobe of caudal fin;

VLCF – Colour of ventral lobe of caudal fin;

WT – Water types;

Transp. – Clear/transparent waters.

Few. – Waters with few dissolved tannins and a secchi disc reading of >1m;

Turbid – Turbid, sediment carrying waters.

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1. INTRODUÇÃO GERAL

1.1. DIVERSIDADE DE AMBIENTES E RELAÇÃO COM DIVERSIDADE DE PEIXES

As águas amazônicas foram inicialmente classificadas em água clara, água preta e água branca (Sioli, 1950) com base em características ópticas (Furch, 1984). Mas ao serem realizadas análises químicas nos corpos d'água de maneira individual, verificaram que haviam diferenças consideráveis em cada tipo específico (Sioli, 1968). As águas interiores são caracterizadas por variações extremamente grandes em sua composição iônica (Bayly & Williams, 1975), estão relacionados não apenas a diferentes condições hidrodinâmicas, geomorfológicas e sedimentológicas, mas também a ambientes hidroecológicos distintos (Park & Latrubesse, 2015). Devido à complexidade dos ambientes aquáticos amazônicos (Furch, 1984, Lobo et al., 2012) um estudo limnológico agrupou em águas claras com baixas concentrações de componentes opticamente ativos; águas negras ricas em carbono orgânico dissolvido; águas com grandes concentrações de sólidos suspensos inorgânicos; e águas dominadas por clorofila-a (Lobo et al., 2012).

Poderia citar diversas classificações geradas para os ambientes aquáticos amazônicos, levando em considerações diferentes características levantadas em diversos estudos, mas é importante levar em consideração o que irá ajudar a responder à pergunta da pesquisa e encontrar o que melhor se encaixa com os dados. Esses pontos levantados atentam ao fato de que ambientes aquáticos apresentam diferenças nas características química, física e biológica em cada tipo e essas características afetam diretamente as habilidades sensoriais dos organismos aquáticos (Borghezan et al. 2021).

Os cardumes de peixes de pequeno porte na Amazônia apresentam não apenas uma variação na coloração das nadadeiras como também podem estar distribuídos nos diversos tipos de água (Stevens et al., 2011; Silva, Canto & Ribeiro, 2019). Dentre eles temos o Gênero *Bryconops* Kner (1858).

1.1. CONHECIMENTO SOBRE O BRYCONOPS

Bryconops Kner (1858) é um gênero cis-andino de tetras de pequeno a médio porte amplamente distribuídos nos rios Orinoco, Amazonas, Tocantins-Araguaia, Paraná-Paraguai, São Francisco e várias bacias costeiras que drenam os escudos do Brasil e da Guiana (Lima et al., 2003; Chernoff & Machado-Allison, 2005; Wingert & Malabarba, 2011; Silva-Oliveira,

Canto & Ribeiro, 2015, 2019). Três caracteres foram propostos por Chernoff & Machado-Allison (1999) para diagnóstico do gênero: A borda ventral da maxila curvando-se acentuadamente posteriormente, formando um ângulo de 90°, estendendo-se para além da cavidade quadrada do ângulo-articular; um canal látero-sensorial infraorbital bem desenvolvido no antorbital; e, canal supraorbital do sistema cefálico látero-sensorial que se estende posteriormente nas escalas da nuca. Até recentemente, o gênero era considerado *incertae sedis* em Characidae, mas as hipóteses filogenéticas para os Characiformes sugeriam como membros de Iguanodectidae junto com *Iguanodectes* Cope, 1872 e *Piabucus* Oken, 1817 (Oliveira et al., 2011; Mirande, 2018).

Apesar do esclarecimento sobre sua posição filogenética, a monofilia e as relações interespecíficas em *Bryconops* nunca foram testadas de maneira satisfatória (Barros, Zuanon and Deus, 2016; Silva-Oliveira et al., 2020). As espécies são atribuídas tradicionalmente a dois subgêneros (Chernoff & Machado-Allison, 2005): *Bryconops*, com maxilas curtas e geralmente sem dentes superiores; e *Creatochanes* Gunther, 1864, com maxilas longas e geralmente apresentando até três dentes superiores (Chernoff & Machado-Allison, 2005). O subgênero *Bryconops* inclui as espécies *B. alburnoides* Kner, 1858, *B. caudomaculatus* Günther, 1864, *B. collettei* Chernoff & Machado-Allison, 2005, *B. disruptus* Machado-Allison & Chernoff 1997, *B. durbinae* Eigenmann, 1908, *B. gracilis* Eigenmann, 1908, *B. hexalepis* Guedes, Oliveira & Lucinda 2019, *B. magoi* Chernoff & Machado Allison, 2005, *B. piracolina* Wingert & Malabarba, 2011, *B. rheorubrum* Silva-Oliveira, Sabaj, Ota &Py-Daniel, 2019, e *B. tocantinensis* Guedes, Oliveira & Lucinda, 2016. Enquanto o subgênero *Creatochanes* inclui *B. allisoni* Silva-Oliveira, Canto & Ribeiro, 2019, *B. affinis* Günther, 1864, *B. chernoffi* Silva-Oliveira, Lima & Bogotá-Gregory, 2018, *B. colanegra* Chernoff & Machado-Allison, 1999, *B. colaroja* Chernoff & Machado Allison, 1999, *B. cyrtogaster* Norman , 1926, *B. giacopinii* Fernández-Yépez, 1950, *B. humeralis* Machado-Allison, Chernoff & Buckup, 1996, *B. imitador* Chernoff & Machado-Allison, 2002, *B. inpai* Knöppel, Junk & Géry, 1968 , *B. melanurus* Bloch, 1794, *B. sapezal* Wingert, Chuctaya & Malabarba, 2018, *B. vibex* Machado Allison, Chernoff & Buckup, 1996 e *B. marabaixo* Silva-Oliveira, Moreira, Lima & Rapp Py-Daniel 2020. No entanto, várias espécies apresentam divergências das supostas sinapomorfias que definem os subgêneros (ex. *B. disruptus*, *B. inpai*, *B. marabaixo*, *B. piracolina*, *B. tocantinensis* - onde o ponto distal da maxila não atinge a articulação com o quadrato), indicando uma demanda por uma revisão do gênero e reavaliação desses caracteres em um

quadro filogenético (Guedes, Oliveira e Lucinda, 2016, Silva-Oliveira, Canto e Ribeiro, 2019, Silva-Oliveira et al., 2020).

Além da morfologia, a identificação de espécies de *Bryconops* tem sido proposta para incorporar informações sobre a pigmentação da nadadeira caudal (Chernoff & Machado-Allison, 2005, Guedes, Oliveira e Lucinda, 2016). No entanto, considerando a ampla distribuição de alguns taxa (especialmente *B. caudomaculatus* e *B. melanurus*), o potencial de plasticidade fenotípica associada à variação ambiental (Sidlauskas, Chernoff e Machado-Allison, 2006), e a considerável gama de variação associada à intensidade e arranjo de melanóforos dentro de espécies de *Bryconops* do médio e baixo rio Xingu (Silva-Oliveira, Canto e Ribeiro, 2019), os limites entre variação geográfica intraespecífica e características em nível de espécie tornam-se intrinsecamente difíceis de distinguir.

1.2. COMO PODEMOS TESTAR PARA CONVERGÊNCIA EVOLUTIVA

Os dados moleculares têm sido aplicados a muitos estudos em peixes neotropicais (eg Torrico et al., 2009, Oliveira et al., 2011, Tagliacollo et al. 2014, Castro Paz, Batista & Porto, 2014, Dagosta & Pinna, 2017, Farias et al., 2019, Formiga, Batista & Alves-Gomes, 2021), podendo ser usadas para verificar táxons específicos com problemas de identificação mesmo em filogenias taxonomicamente incompletas, pesquisando espécies crípticas e complexos de espécies (Hebert et al., 2004), avaliar a diversidade genética presente no grupo de estudo (Amaral et al., 2013, Bellafronte et al., 2013, Pereira et al., 2013, Castro Paz et al., 2014, Escobar-Camacho, Barriga & Ron, 2015, Benzaquem et al., 2015, Silva-Oliveira, et al., 2020), testar o sinal filogenético e a convergência de características morfológicas (Comte et al. 2014; Munkmuller, 2012).

Uma maneira de determinar a extensão da autocorrelação filogenética dos nossos dados aqui estudados, como a relação do padrão de cor das nadadeiras e tipos de água, precisamos estimar o sinal filogenético. Que nada mais é do que a extensão em que os valores das características estão estatisticamente relacionados à filogenia, ou seja, até que ponto espécies intimamente relacionadas tendem a se parecer (Blomberg, Garland & Ives, 2003). Quando as características exibem um forte sinal filogenético, indica que provavelmente evoluíram por mudanças graduais ao longo do tempo (Symonds & Blomberg, 2014). Alternativamente, traços sem sinal, podem ser extremamente lábeis na escala de tempo da filogenia ou, inversamente, extremamente estáveis (Revell, Harmon & Collar, 2008). Os índices que testam o sinal

filogenético podem ser divididos em dois grupos (Munkmuller, 2012). O primeiro grupo compreende índices de autocorrelação sem um modelo evolutivo, incluindo *Cmean* de Abouheif (Abouheif, 1999) e *I* de Moran (Moran, 1950; Gittleman & Kot, 1990). Eles oferecem resultados que não podem ser usados em uma interpretação quantitativa ao comparar diferentes filogenias, pois o valor estatístico esperado sob o modelo assumido é desconhecido a priori. Mesmo assim, valores mais próximos de 1 indicam relações mais fortes entre o valor das características e a filogenia. O segundo grupo compreende índices que assumem um modelo de movimento browniano (BM) de evolução de traço, incluindo *K* de Blomberg (Blomberg, Garland & Ives, 2003) e λ de Pagel (Pagel, 1997; 1999). Para estes, valores mais próximos de zero indicam independência filogenética, enquanto valores maiores próximos ou mesmo superiores a 1 indicam que os caracteres estão distribuídos de acordo com BM. O λ de Pagel e *Cmean* de Abouheif são índices que apresenta um desempenho consideravelmente superior aos outros dois, e λ de Pagel fornece uma medida confiável do tamanho do efeito (Munkmuller, 2012; Keck et al., 2016).

Personagens identificados como evoluindo independentemente da filogenia podem então ser testados para força coevolutiva usando o índice *Wheatsheaf* (Arbuckle, Bennett & Speed, 2014), que. O índice *Wheatsheaf* pondera similaridade fenotípica próxima mais alta para espécies distantemente relacionadas, gerando distâncias fenotípicas de características entre espécies e, em seguida, penalizando-as pela distância filogenética antes de investigar a similaridade.

2. INFORMAÇÕES SOBRE O TRABALHO

A presente dissertação faz o uso de sequências mitocondriais COI-5P e análises filogenéticas para entender se o padrão de coloração das diferentes espécies do gênero *Bryconops* Kner (1858) está relacionado com o tipo de água em que são encontrados e o quão forte o sinal filogenético é para este caso. Tudo isso tentando entender se esse pode ser um dos fatores que devem ser levados em consideração quando for realizada a revisão do gênero.

3. OBJETIVO GERAL

Verificar se o padrão de cor das nadadeiras de diferentes espécies do gênero *Bryconops* Kner (1858) está relacionado com o tipo de água em que são encontrados.

3.1. OBJETIVOS ESPECÍFICOS:

- Verificar táxons específicos, espécies crípticas e complexos de espécies;
- Avaliar a diversidade genética presente no grupo;
- Testar o sinal filogenético e a convergência de características morfológicas.

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ARTIGO**Chromatic polymorphism and phylogenetic independent convergence in *Bryconops*****Kner, 1858 (Ostariophysi: Characiformes: Iguanodectidae)**Gonçalves, Andressa Santos; orcid.org/0000-0003-0935-0050

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Ready Jonathan S jonathan.ready@gmail.com orcid.org/0000-0002-9374-8661**ABSTRACT**

The fish fauna varies considerably between Amazonian clear, black and white waters with implications for evolution and conservation of this diverse biota. Many small shoaling stream fishes show shared colour patterns that are hypothesized to provide a collective defence against predators. Amongst these groups, members of the genus *Bryconops*, Kner (1858), represent a taxonomic challenge. Despite the presence of two well defined subgenera based on morphological evidence, each contains at least one species (*B. (Bryconops) caudomaculatus* and *B. (Creatochanes) melanurus*) with a very wide distribution, within which regional populations seem to present colour pattern variations. To test whether colour is plastic in those species or if phenotypic variation is related to cladogenetic events we performed tests for phylogenetic independence and determined the strength of convergence for simple colour characters in relation to water type. Colour variation characters for fins above the median line of the body were generally found to be independent from phylogeny and the Wheatsheaf test strongly supports convergence of the dorsal fin colour with the type of water from which the sample was collected and suggests a similar trend for the colour of the dorsal lobe of the caudal fin. Our results suggest that simple colour characters cannot necessarily be relied upon for use in taxonomic revision of the genus as local phenotypic variants may represent environmentally determined plasticity rather than local selection and further studies are required to determine the validity of these characters.

Key words: Aquatic light environments, Evolution, Neotropical fishes, Species delimitation

1. INTRODUCTION

Amazonian waters are classically divided into blackwaters, clearwaters and whitewaters based on their appearance and chemistry (Sioli, 1984) and the fish fauna of each water type is known to be generally distinct (Junk, Soares and Bayley, 2007). However, there is considerable variation in chemistry and the aquatic light environment both between and within these broad classifications. The role of such environmental variation in the evolution of Neotropical fishes has been revealed to be important for generating and maintaining biodiversity (Endler, 1991; Alexandrou et al. 2011; Crampton, Lovejoy & Waddell, 2011; Hurtado-Gonzales, Loew and Uy, 2014, Crampton, 2019).

Many small shoaling fishes of the Neotropics present variations on a generalized colour pattern with yellow/orange/red fins and silvery bodies with or without dark, melanin pigmented patches, suggesting that this common, generalized phenotype may have a role in defence against predation through disruptive camouflage and may also result in motion dazzle (Stevens et al., 2011). That pattern is largely shared by representatives of the Characiformes, among which the species of *Bryconops* Kner, 1858, are often found in mixed shoals with congeners, frequently with species in the *Moenkhausia lepidura* group (sensu Géry, 1977) and/or other species of *Characidae*. The genus includes Cis-andean small to medium sized tetras widely distributed in the Orinoco, Amazonas, Tocantins-Araguaia, Paraná-Paraguai, São Francisco rivers and several coastal basins draining from the Brazilian and Guiana shields (Wingert, Chuctaya and Malabarba, 2018; Silva-Oliveira, Lima and BogotáGregory, 2018; Silva-Oliveira, Canto and Ribeiro, 2019). The latest phylogenetic hypothesis for the Characiformes places *Bryconops* in the family Iguanodectidae along with *Iguanodectes* Cope, 1872 and *Piabucus* Oken, 1817 (Oliveira *et al.*, 2011; Mirande, 2018). Despite the recent clarification of its phylogenetic position, monophyly and interspecific relationships within the genus have never been tested satisfactorily (Mirande 2010, 2018; Silva-Oliveira et al. 2020), and its species have been traditionally assigned to two subgenera based on morphology: *Bryconops*, with short maxillae and usually lacking maxillary teeth; and *Creatochanes* Gunther, 1864, with long maxillae and usually presenting up to three maxillary teeth (Chernoff & Machado-Allison, 2005). The subgenus *Bryconops* includes the species *B. alburnoides* Kner, 1858, *B. caudomaculatus* Günther, 1864, *B. collettei* Chernoff and Machado-Allison, 2005, *B. disruptus* Machado-Allison & Chernoff 1997, *B. durbiniae* Eigenmann, 1908, *B. gracilis* Eigenmann, 1908, *B. hexalepis* Guedes et al., 2019, *B. magoi* Chernoff & Machado Allison 2005, *B. piracolina* Wingert and Malabarba, 2011, *B. rheorubrum* Silva-Oliveira et al., 2019, and *B. tocantinensis* Guedes et al., 2016, whereas the subgenus *Creatochanes* includes *B. allisoni* Silva-Oliveira et

al. 2019, *B. affinis* Günther, 1864, *B. chernoffi* Silva-Oliveira et al., 2018, *B. colanegra* Chernoff and Machado-Allison, 1999, *B. colaroja* Chernoff and Machado-Allison, 1999, *B. cyrtogaster* Norman, 1926, *B. giacopinii* Fernández-Yépez, 1950, *B. humeralis* Machado-Allison, Chernoff and Buckup, 1996, *B. imitator* Chernoff and Machado-Allison, 2002, *B. inpai* Knöppel, Junk and Géry, 1968, *B. melanurus* Bloch, 1794, *B. sapezal* Wingert et al., 2018, *B. vibex* Machado Allison, Chernoff and Buckup, 1996 and *B. marabaixo* Silva-Oliveira et al., 2019. However, various species present divergences from the putative synapomorphies defining the subgenera (e.g. *B. disruptus*, *B. inpai*, *B. marabaixo*, *B. piracolina*, *B. tocantinensis* - where the distal point of the maxilla does not reach the articulation with the quadrate), indicating a demand for a revision of the genus and reevaluation of those characters in a phylogenetic framework (Guedes, Oliveira and Lucinda, 2016, Silva-Oliveira, Canto and Ribeiro, 2019, Silva-Oliveira et al. 2020).

In addition to morphology, identification of *Bryconops* species has been proposed to incorporate information on the pigmentation of the caudal-fin (Chernoff & Machado-Allison, 2005, Guedes, Oliveira and Lucinda, 2016). However, considering the wide distribution of some taxa (especially *B. caudomaculatus* and *B. melanurus*), the potential for phenotypic plasticity associated with environmental variation (Sidlauskas, Chernoff and Machado-Allison, 2006), and the considerable array of variation associated with intensity and arrangement of melanophores within species of *Bryconops* from the middle and lower Xingu river (Silva-Oliveira, Canto and Ribeiro, 2019), the limits between intraspecific geographic variation and species level characteristics become intrinsically difficult to distinguish.

Molecular data have been applied to many studies on Neotropical fishes (eg Torrico et al., 2009, Oliveira et al., 2011, Tagliacollo et al., 2016, Castro Paz, Batista and Porto, 2014, Dagosta & Pinna, 2017, Farias et al., 2019, Formiga et al., 2021), and even taxonomically incomplete phylogenies can be used to verify specific taxa with identification problems, search for cryptic species and species complexes (Hebert et al., 2004), assess the genetic diversity present in the study group (Amaral et al., 2013, Bellafronte et al., 2013, Pereira et al., 2013, Castro Paz et al., 2014, Escobar-Camacho, Barriga and Ron, 2015, Benzaquem et al., 2015), and test phylogenetic signal and convergence of morphological traits (Comte et al. 2014; Munkmuller, 2012).

Indices that test phylogenetic signal can be divided into two groups (Munkmuller, 2012). The first group comprises autocorrelation indices without an evolutionary model including Abouheif's *Cmean* (Abouheif, 1999) and Moran's *I* (Moran, 1950; Gittleman & Kot, 1990). They offer results that cannot be used in a quantitative interpretation while comparing

different phylogenies, as the expected statistical value under the assumed model is unknown *a priori*. Even so, values closer to 1 indicate stronger relationships between traits value and phylogeny. The second group comprises indices that assume a Brownian Motion (BM) model of trait evolution including Blomberg's K (Blomberg, Garland & Ives, 2003) and Pagel's λ (Pagel, 1997; 1999). For these, values closer to zero indicate phylogenetic independence, while higher values approaching or even exceeding 1 indicate that the traits are distributed in accordance with BM. Characters identified as evolving independently from the phylogeny can then be tested for coevolutionary strength using the Wheatsheaf index (Arbuckle, Bennett and Speed, 2014). The Wheatsheaf index weights close phenotypic similarity higher for distantly related species by generating phenotypic distances from traits across species and then penalizing these by phylogenetic distance before investigating similarity.

The present study therefore aimed to use molecular data to test whether the phenotypic variation observed in representatives of the genus is indeed associated to plasticity as suggested by Sidlauskas, Chernoff and Machado-Allison (2006) and Silva-Oliveira, Canto and Ribeiro, (2019), or if it represents overlooked diversity. By testing if such variation is correlated to the water colour in the localities from which specimens originated, we evaluate whether colour pattern characters can be useful for taxonomic purposes considering the potential for plasticity to facilitate convergence in different environments.

2. MATERIAL AND METHODS

2.1 STUDY AREA

Bryconops samples were obtained from specimens originating from four ecoregions (*sensu* Abell et al., 2008), corresponding to independent drainages in central and eastern Amazonia (Figure 1, Table S1). These represent: coastal streams of the Atlantic region that show seasonal variability between dilute blackwater and clearwater classifications (ecoregion 323); similarly variable dilute blackwater and clearwater streams of the southern Guiana shield (ecoregion 315); more concentrated blackwater streams from the Rio Negro basin in central Amazonia, near Manaus (ecoregion 314); and streams of the Xingu river near Altamira (ecoregion 322) that are mostly clearwater but with some turbid streams.

2.2 TISSUE SAMPLING

Samples were collected using small seine nets and photographed using a Canon G12 camera as soon as possible after capture to record live colour patterns (Figures S1-S7). After euthanasia with eugenol, following Lucena et al. (2013; as approved by the Federal University of Pará Animal Ethics Committee, CEUA licence 682015), tissue samples (surface area of ~2mm²) were removed from the right side of fish in a series of distinct positions that allow subsequent identification of individuals in mixed lots, leaving the left side intact for morphological analyses. Tissues were stored in 96% ethanol, and voucher specimens fixed in 10% formalin before transferral to 70% ethanol for long term preservation and deposition at the Museu Paraense Emílio Goeldi (MPEG) fish collection.

2.3 COLOR DATA COLLECTION

Identification of the material used the morphological characters defined for the genus (Chernoff & Machado-Allison, 2005; Wingert & Malabarba, 2011; Silva-Oliveira et al., 2015; Guedes, Oliveira e Lucinda, 2016; Silva-Oliveira et al., 2019; Wingert et al., 2018). Additionally, colour pattern characters were defined including: the presence or absence of an oculus on the caudal-fin upper lobe, the overall colour of the caudal, adipose and dorsal fins (Yellow/Orange/Red), and the intensity of melanin pigmentation.

2.4 MITOCHONDRIAL SEQUENCE DATA

All new samples including representatives of *Bryconops* (N=51) as the ingroup as well as *Iguanodectes* spp. (N=7) and *Acestrorhynchus* sp. (N=1) as outgroups (Table S1) were bidirectionally sequenced for the COI-5P barcode fragment. Genomic DNA was extracted

using the Wizard Genomic DNA Purification Kit (Promega), following the manufacturers protocols. The COI-5P fragment was amplified by PCR using the primers LIICO1F 5'-GATTTTTCTCAACTAACCAAYAAAGA-3' and LIICO1R 5'-ACTTCTGGGTGTCCGAARAAYCARAA-3' in a total volume of 12.5 μ L containing 7.18 μ L ultrapure water, 1.25 μ L 10x Buffer, 0.75 μ L MgCl₂ (50 mM), 0.25 μ L dNTP mix (8 mM), 0.125 μ L of each primer (10 μ M), 0.06 μ L Taq Platinum (5 u/ μ L, Invitrogen) and 1.0 μ L of genomic DNA (10-50 ng). The PCR consisted of an initial denaturation cycle (3 min at 94°C), 40 amplification cycles (denaturation: 25 s at 94°C, annealing: 40 s at 52°C, and extension: 45 s at 72°C), and a final extension cycle (5 min at 72°C). Fragments were checked for size and band format (single vs. multiple) by electrophoresis on a 1% agarose gel before sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following standard protocols and read on the ABI 3130-Genetic Analyzer (Applied Biosystems).

2.5 mtDNA ANALISES

Chromatograms were aligned with existing COI-5P sequences from BOLD in Geneious v9 (<http://www.geneious.com>, Kearse et al., 2012) and visually inspected before production of consensus sequences for each specimen that was then also checked for stop codons. A further 19 sequences identified as *Bryconops* from BOLD and 6 sequences representing other members of the Iguanodectidae (*Piabucus melanostoma* N=2) and the outgroup *Triportheus* spp. (N=4) were added, producing a final 661bp COI-5P dataset of 84 sequences, including 70 sequences of *Bryconops*. All new sequences were submitted to BOLD under the project Ecological Ichthyofaunal Barcoding (see Table S1 for details).

2.6 ESTIMATING DIVERGENCE TIMES

The best partition parameters and evolutionary model for the dataset was determined using PartitionFinder 2 (Lanfear et al., 2017; Stamatakis, 2006). Three partitions were tested, 1) one partition for all codon positions; 2) one partition for positions 1 and 2, and another for the third codon position; and 3) a partition for each codon position. The third scheme was identified as the best evolutionary model under both AIC and BIC analyses.

Based on these model parameters, phylogenetic trees were produced to infer the evolutionary history of *Bryconops*. A Maximum likelihood (ML) tree was made in RAxML 8.2.10 (Stamatakis, 2014) using random seeds in three independent runs to avoid local topological peaks. All three trees showed the same topology, a bootstrap analysis was

performed to verify support using 1000 bootstrap pseudo-replicates. A Bayesian Inference (BI) tree was produced using MrBayes 3.2.7 (Ronquist et al., 2012). Three independent runs were performed, each with four chains and 10^6 generations with one tree sample every 1000 generations. From the total 10000 trees, we discarded the first 10% as burn-in after checking the results with Tracer 1.7 (Rambaut et al., 2018). All analyses reported the same topology.

To provide measures that are comparative with classical barcoding studies a standard Neighbor-Joining (NJ) tree was produced in MEGA X (Kumar et al., 2018), using the Kimura 2 parameter model of evolution (Kimura, 1980) and pairwise deletion of missing data among the samples. Support values were obtained based on 1000 bootstrap pseudo-replicates. Also in MEGA X (Kumar et al., 2018), distance analyses were made based on both P and Kimura 2 parameters, and using pairwise deletion of missing data among samples.

2.7 DELIMITATION SPECIES

To validate the known species of *Bryconops* and to check the existence of overlooked or cryptic species, three methodologies of species delimitation were used. 1) Automatic Barcode Gap Discovery - ABGD (Puillandre et al. 2011), a threshold methodology to delimit species was used with the COI sequences; 2) Bayesian implementation of PTP - bPTP (Zhang et al., 2013), a coalescent based methodology that uses a model-based approach upon an ML or BI gene tree; and 3) General Mixed Yule Coalescent - GYMC (Pons et al. 2006; Monaghan, Metcalfe and Torres, 2009; Fujisawa & Barraclough 2013), another model-based approach on an ultrametric gene tree.

ABDG analysis was run via the website <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html> using the default settings and Kimura (k80) model with $TS/TV = 2.0$. bPTP was run via the website <https://species.h-its.org/ptp/> using the default settings and the ML tree obtained above as input. GYMC was run via the website <https://species.h-its.org/gmyc/> using the single threshold method and a bayesian ultrametric tree, without outgroups and with only one sample per haplotype, obtained from BEAST 1.10.4 (Suchard et al., 2018).

2.8 SIGNAL PHYLOGENETIC AND CONVERGENCE EVOLUTION

Colour pattern and visual environment (water type) were classified in binary states for all samples in order to perform tests of phylogenetic independence (Moran I - Gittleman & Kot, 1990; Abouheif's *Cmean* - Abouheif, 1999; Moran's *I* - Moran, 1950, Blomberg et al.'s *K* - Blomberg, Garland & Ives, 2003 and Pagel's λ - Pagel, 1999) and convergence (Wheatsheaf

index - Arbuckle, Bennett and Speed, 2014). For new samples this classification was based on live colour photographs taken immediately after collection. For samples for which existing sequence data was used, original photographs were requested from collectors, and if these were unavailable original references evaluated to use the description of colour pattern associated to the voucher specimens of those sequenced samples (or from samples collected during the same collection event). The colour pattern was recorded separately for melanic and non-melanic colour in each fin using binomial classification (0 = false, 1= true) as follows: Dorsal fin melanin (DF_Mel); Colour of dorsal fin (DF_Hy, DF_Y, DF_O, DF_R for hyaline, yellow, orange or red pigmented dorsal fin); Colour of adipose fin (AdF_Hy, AdF_Y, AdF_O, AdF_R for hyaline, yellow, orange or red pigmented adipose fin); Colour of dorsal lobe of caudal fin (DLCF_Hy, DLCF_Y, DLCF_O, DLCF_R for hyaline, yellow, orange or red pigmented caudal fin dorsal lobe); Colour of ventral lobe of caudal fin (VLCF_Hy, VLCF_Y, VLCF_O, VLCF_R for hyaline, yellow, orange or red pigmented caudal fin ventral lobe). The visual environment from which the samples were collected was classified based on field photographs and measurements and reported water characteristics, also following the binary classification (0 = false, 1= true) considering four water types: WT_transp (clear/transparent waters); WT_few (waters with few dissolved tannins and a secchi disc reading of >1m); WT_many (waters with many dissolved tannins and a secchi disc reading of <1m); and WT_turbid (turbid, sediment carrying waters). The tests for phylogenetic independence were performed using the packages *phytools* (Revell, 2012) and *adephylo* (Jombart et al, 2010), for convergence, *windex* (Arbuckle & Minter, 2015) in R (R Core Team, 2020).

3. RESULTS

All phylogenetic analyses (BI, ML, NJ) resulted in similar topologies, and we present the ML tree with support from the three methodologies (BI support above the branches, ML and NJ supports below and separated by slash, respectively - Fig 2). Although with low support from ML and NJ, all methodologies show the presence of the two subgenera as distinct monophyletic groups. Group one representing species in the subgenus *Bryconops* contains samples representing a complex of species currently identified as *B. caudomaculatus* or *B. cf. caudomaculatus* (including public sequence data), as well as *B. rheorubrum*. Group two represents the subgenus *Creatochanes* and includes samples representing the species *B. affinis*, *B. colaroja*, *B. giacopinii*, *B. melanurus* and nine specimens belonging to three unidentified species of *Bryconops* (Fig 2). One of these shows morphological similarity to *B. affinis* (*B. aff. affinis* - samples 11BA79, 11BC64 and 11BC65), whereas the other two are more distinct. Both *B. (B.) caudomaculatus* and *B. (C.) melanurus* (including public data for *B. affinis*) show substructuring of lineages based on geographical origin (sample code groups, Table S1) with deeper divergences between lineages of *B. (B.) caudomaculatus*, resulting in greater numbers of species delimited by all methods (Fig 2).

The three methodologies of species delimitation employed resulted in 10 (GYMC), 11 (ABDG) or 12 (bPTP) MOTUs. The difference in counts between the methods result from the two widespread taxa (one extra *B. (B.) caudomaculatus* lineage under bPTP, samples 10B71 and 10C01, Fig 2) and one extra *B. (C.) melanurus* lineage under GMYC) and the lack of distinction of *B. (B.) rheorubrum* and basal lineages of *B. (B.) caudomaculatus* under GMYC. Also in subgenus *Bryconops* both ABDG and bPTP shows the presence of a MOTUs composed of two unidentified specimens of *Bryconops* (*B.sp_ANGBF8811.12* and *B.sp_ANGBF8826.12*), suggesting the presence of a new species.

For the subgenus *Creatochanes*, both phylogeny and species delimitation methodologies support the monophyly of the known species *B. (C.) giacopinii* and *B. (C.) colaroja*; there is also a concordance about the monophyly and evolutive independence of three groups comprising three unidentified *Bryconops*, one formed by *B. (C.) sp. nv 1* (samples 10B90, 10C02 and 10C68), other formed by *B. (C.) sp. nv 2* (samples 11BB58, 11BB65 and 11BB41), and the last one by an unidentified species similar to *B. (C.) affinis*, herein named *B. aff. affinis* (samples 11BA79, 11BC64 and 11BC65) (Fig 2).

B. melanurus forms a monophyletic group along with online sequences for 13 unidentified *Bryconops* specimens from Maranhão state (ITAPE and RENA sample codes) and three specimens of *B. affinis* (BSB215.10, BSB216.10 and BSB217.10). While GYMC supports

that *B. melanurus* should be treated as an independent species, the support values provided by the phylogenetic analyses were low for monophyly of the group including our *B. melanurus* samples and the 13 unidentified specimens from Maranhão. Only *B. affinis* possesses strong support for both ML and NJ, but without an indicative that should be treated as a MOTU (Fig 2).

3.1. ARE ANY COLOUR CHARACTERS INDEPENDENT OF PHYLOGENETIC SIGNAL?

Colour pattern characters classified here including variation between hyaline, yellow, orange and red colour for fins above the vertical midline of the body were found to show significant independence from phylogeny (Table 2). For the autocorrelation indices Abouhief's *Cmean* and Moran's *I*, independence from phylogenetic signal was significant for the characters "Colour of dorsal fin" ($Cmean = 0.218$, $P = 0.005$; $I = 0.205$, $P = 0.001$), "Colour of adipose fin" ($Cmean = 0.724$, $P = 0.001$; $I = 0.712$, $P = 1.00E-004$) and "Colour of dorsal lobe of caudal fin" ($Cmean = 0.350$, $P = 0.001$; $I = 0.335$, $P = 1.00E-004$). Similarly, for Brownian Motion models the indices Blomberg's *K* and Pagel's λ , independence from phylogenetic signal was significant for the same characters "Colour of dorsal fin" ($K = 0.256$, $P = 0.017$; $\lambda = 0.525$, $P = 0.003$), "Colour of adipose fin" ($K = 1.287$, $P = 1.00E-004$; $\lambda = 1.081$, $P = 2.82E-027$) and "Colour of dorsal lobe of caudal fin" ($K = 0.330$, $P = 4.00E-004$; $\lambda = 0.782$, $P = 1.51E-005$).

3.2. DO COLOUR PATTERN CHARACTERS THAT ARE INDEPENDENT OF PHYLOGENETIC SIGNAL CONVERGE IN THE SAME WATER TYPES?

For the colour pattern characters that were identified as showing a high degree of independence from phylogenetic signal the Wheatsheaf index identified moderately strong strength of convergence for all three characters but with varying degrees of significance and where only "Colour of dorsal fin" was found to show significant convergence with the water type from which the samples were collected ($W = 0.898$, $Var = \pm 0.005191$, $P = 0.001$, Table 3). "Colour of dorsal lobe of caudal fin" approached significance ($W = 0.880$, $Var = \pm 0.005702$, $P = 0.074$), and with improved phylogenetic and spatial sampling coverage may be found to be convergent.

4. DISCUSSION

The two main clades obtained here corroborate the morphological diagnoses of the described species and subgenera (Chernoff & Machado-Allison, 1999), but clearly indicate a greater diversity in the *B. (B.) caudomaculatus* clade including at least four cryptic or overlooked species with at least partially overlapping distributions in eastern Amazonia. Much shallower, but similar divergence patterns are found in *B. (C.) melanurus* suggesting that these populations are currently exposed to isolation mechanisms that may represent the start of speciation processes. In both main clades, specimens originating from the Rio Xingu (Brazilian Shield) represent the sister groups to all other (*Bryconops*) and (*Creatochanes*), with both clades also containing lineages from all geographic regions sampled (Atlantic Coast of the Amazon, southern Guiana shield and the blackwater streams of central Amazon near Manaus). Considering the sampling limits, this follows the most common biogeographic patterns for Amazonian fishes as described by Dagosta and Pinna (2017). Moreover, the moderately large number of taxa found in each of three relatively small geographic regions (central Amazon near Manaus, Xingu river near Altamira, and the coastal rivers near Belém) suggests that dispersal capability of these taxa is high and secondary contact between species is likely to result in admixture or selective reinforcement of divergent characters. Indeed, there is an estimate of at least nine species level taxa in the genus for the lower and middle Xingu river (Silva-Oliveira, Canto and Ribeiro, 2019).

Although the described species are monophyletic, similar looking taxa or MOTUs from different localities form paraphyletic or polyphyletic groups (e.g. *Bryconops aff. affinis* vs. *B. affinis* and the various MOTUs within the *B. (B.) caudomaculatus* clade). Additionally, amongst the widespread species (or species complexes) phenotypic colour variants associated to geographic regions were found to exist in both major clades, with particularly striking variations observed within and among the *B. (C.) melanurus* and *B. (B.) caudomaculatus* clades where sampling density and geographic coverage were highest (Figures S2 and S3). The phylogenetic independence tests and Wheatsheaf index analysis showed that the phylogenetically divergent lineages show true convergence as they share similar colour patterns in the same water types, and especially across eastern Amazonia the co-collected samples confirm that this occurs syntopically (Table S1).

Convergent colour patterns associated with distinct water types may represent either a selective process (selection by predators that results in convergence of syntopic prey species that gain protection through collective disruptive camouflage and/or motion dazzle or sexual selection) or through environmental plasticity, or even as a combination of both mechanisms.

In the first case, predation on these fishes is predicted to be dominated by larger fishes or birds, both of which are visually guided predators with many species presenting colour vision involving multiple retinal cone types (Kelber, Vorobyev & Osorio, 2003). Therefore selected convergence associated with water type would be expected to be associated with the distinct light environment and transmission of the light reflected by these pigments in these water types. Sexual selection would normally be expected to result in distinct trends in colouration between sexes, but this was not observed in the analysed samples of *Bryconops*. In the case of environmental plasticity, it is important to note that pigments associated to yellows and red colouration in fishes are often derived from dietary sources of carotenoids, and that the exact hue and intensity can result from intraspecific behavioural processes such as social dominance as well as variations in diet or metabolism of carotenoids that are closely associated to environmental variation (Sefc, Brown & Clotfelter, 2014). Given the generalized hue of individuals of species at each location sampled in this study (as well as during many collection trips throughout Amazonia - pers. obs. ALN-F and JSR), the environmental effect on dietary sources or metabolism of carotenoids is the most probable cause of environmental plasticity in colour patterns in this genus. It is also possible that selection acts on existing variation resulting from phenotypic plasticity (Scoville & Pfrender, 2010). To confirm this, specific experiments that control for predation, diet and light environment are needed to fully elucidate the evolutionary processes occurring in *Bryconops*.

The generalized utility of colour pattern as a source of characters helpful to the taxonomy or identification of the species of *Bryconops* should therefore remain in question. Proposed future use of colour pattern characters in these taxa should be accompanied by rigorous analyses that refute the possibility of environmental plasticity of the proposed characters. It should be noted that the character in question is the colour itself and that characters based on the form of the colour pattern that can be clearly described (discrete spatial delimitation of the presence or absence of colour) should be more reliable. For example, *Bryconops* (C.) *aff. affinis* and *B.* (C.) sp. nv 2 from the Xingu present clearly defined ocelli in the upper caudal-fin lobe and a more diffuse, melanic pigmentation on the lower lobe, whereas *Bryconops* (C.) *melanurus* and *B.* (C.) sp. nv 1 present a generalized orange/red pigmentation across the entire caudal-fin upper lobe, with no ocellus.

Amazonian rivers are known to present a diverse range of characteristics (Sioli, 1984) and the three main water types often result in ecological clines or ecotones that are considered one of the driving forces for diversification of Amazonian fishes (Albert & Reis, 2011). Understanding the role of evolution of colour patterns of species within the context of water

types for maintaining biodiversity is particularly important considering the human impacts such as deforestation and the construction of dams that alter both the water chemistry and visual environment.

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6. FIGURES AND TABLE LEGENDS

Figure 1 - Map of *Bryconops* sampling locations with indication of the water type at each location. Blue spots = Turbid waters, Green spots = Dark (high tannin concentration) Blackwaters, Red spots = Light (low tannin concentration) Blackwaters, and Yellow spots = Clearwater.

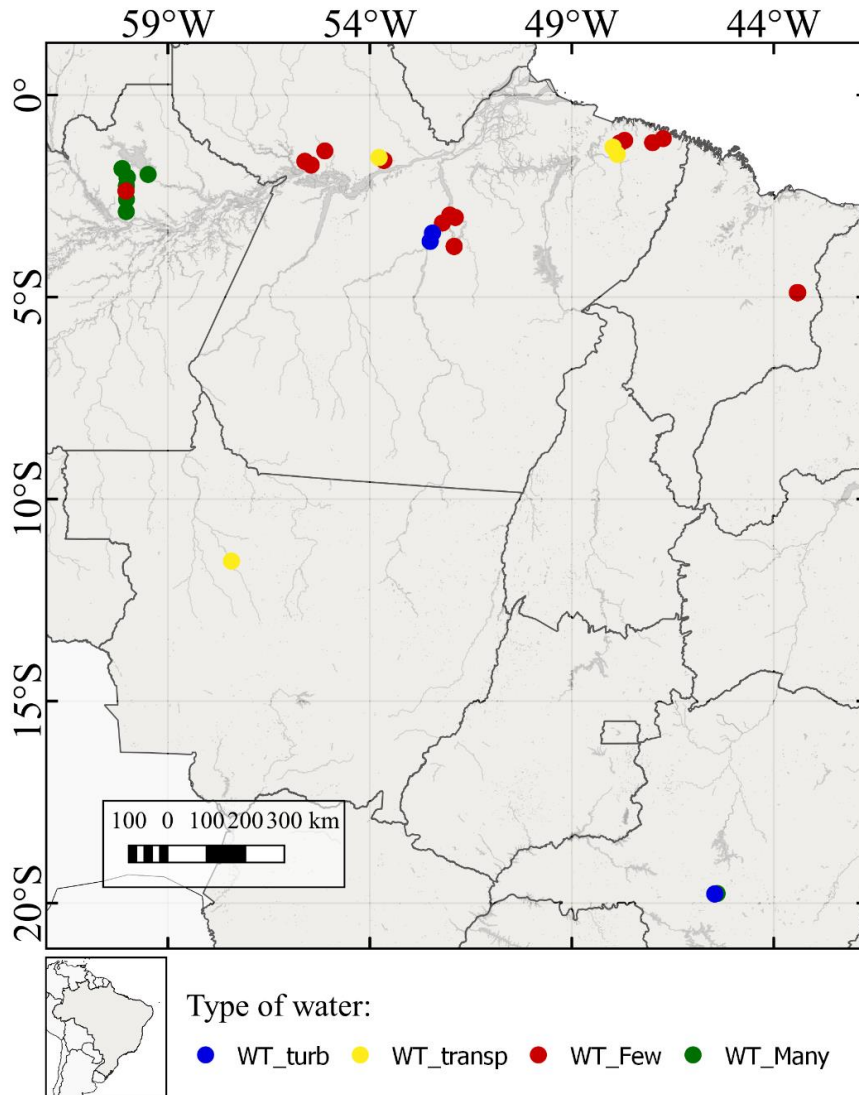


Figure 2 - Maximum likelihood phylogeny with vertical bars representing results of species delimitation analyses. Support values for nodes are based on Bayesian posterior probability (above) and bootstrap for ML and NJ analyses (below, separated by slash) respectively. The colored bars correspond to distinct MOTUs identified by ABDG, bPTP and GMYC species delimitation methodologies.* = 100% bootstrap support or BPP=1, - = no support

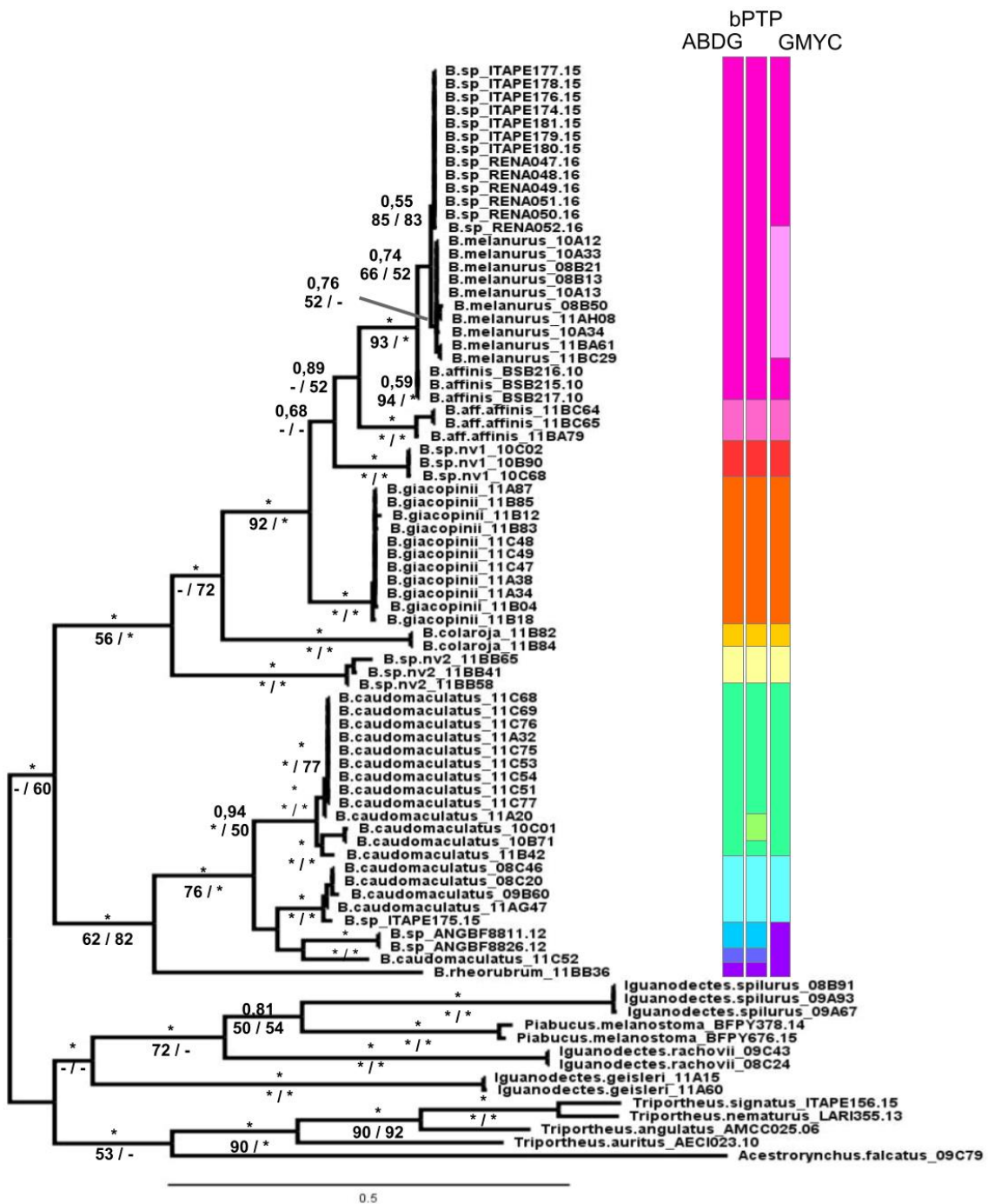


Figure 3 - Second Phylogeny with images of fish and colour pattern of water types

Table 1 - Summary data for sample numbers by species and the water types and number of localities from which they were sampled

Species	N	Water types	Number of localities sampled
<i>B. (B.) caudomaculatus</i>	21	Clearwater Dark Blackwater Light Blackwater	11
<i>B. (B.) rheorubrum</i>	1	Turbid	1
<i>B. (C.) affinis</i>	3	Dark Blackwater Turbid	2
<i>B. (C.) aff. affinis</i>	3	Light Blackwater Turbid	2
<i>B. (C.) colaroja</i>	2	Dark Blackwater	1
<i>B. (C.) giacopini</i>	11	Dark Blackwater	7
<i>B. (C.) melanurus</i>	23	Clearwater Light Blackwater	10
<i>B. (C.) sp nov 1</i>	3	Light Blackwater	2
<i>B. (C.) sp nov 2</i>	3	Light Blackwater	1

Table 2 - Characters for which tests showed statistical phylogenetic independence using Autocorrelation (Abouheif *C mean* and Moran *I*), and Brownian Motion models (Blomberg *K* and Pagel's λ)

Variable	Abouheif <i>C mean</i>	P	Moran <i>I</i>	P	Blomberg <i>K</i>	P	Pagel's λ	P
Colour of dorsal fin	0.218	0.005	0.205	0.001	0.256	0.017	0.525	0.003
Colour of adipose fin	0.724	0.001	0.712	1.00 E-04	1.287	1.00 E-04	1.081	2.82 E-27
Colour of dorsal lobe of caudal fin	0.350	0.001	0.335	1.00 E-04	0.330	4.00 E-04	0.782	1.51 E-05

Table 3 - Coevolutionary strength for the association of colour characters with water type as determined by the Wheatsheaf test. *Significant p-value.

Colour character	Wheatsheaf	Lower bound	Upper bound	P
Colour of dorsal fin	0.898	0.895	0.906	0.001*
Dorsal fin melanic patch	0.830	0.827	0.841	0.854
Colour of adipose fin	0.861	0.858	0.870	0.38
Colour of dorsal lobe of caudal fin	0.880	0.877	0.888	0.074
Colour of ventral lobe of caudal fin	0.836	0.834	0.847	0.894

Figure S1 - Variation in colour of the *B. (Creatochanes)* clade across sample locations. a) *Bryconops (C.) giacopinii* (Manaus - dark Blackwaters); b) *Bryconops (C.) melanurus* (Manaus - light Blackwaters); c) *Bryconops (C.) melanurus* (Coastal - light Blackwaters); d) *Bryconops (C.) aff. affinis* (Xingu - Clearwaters);

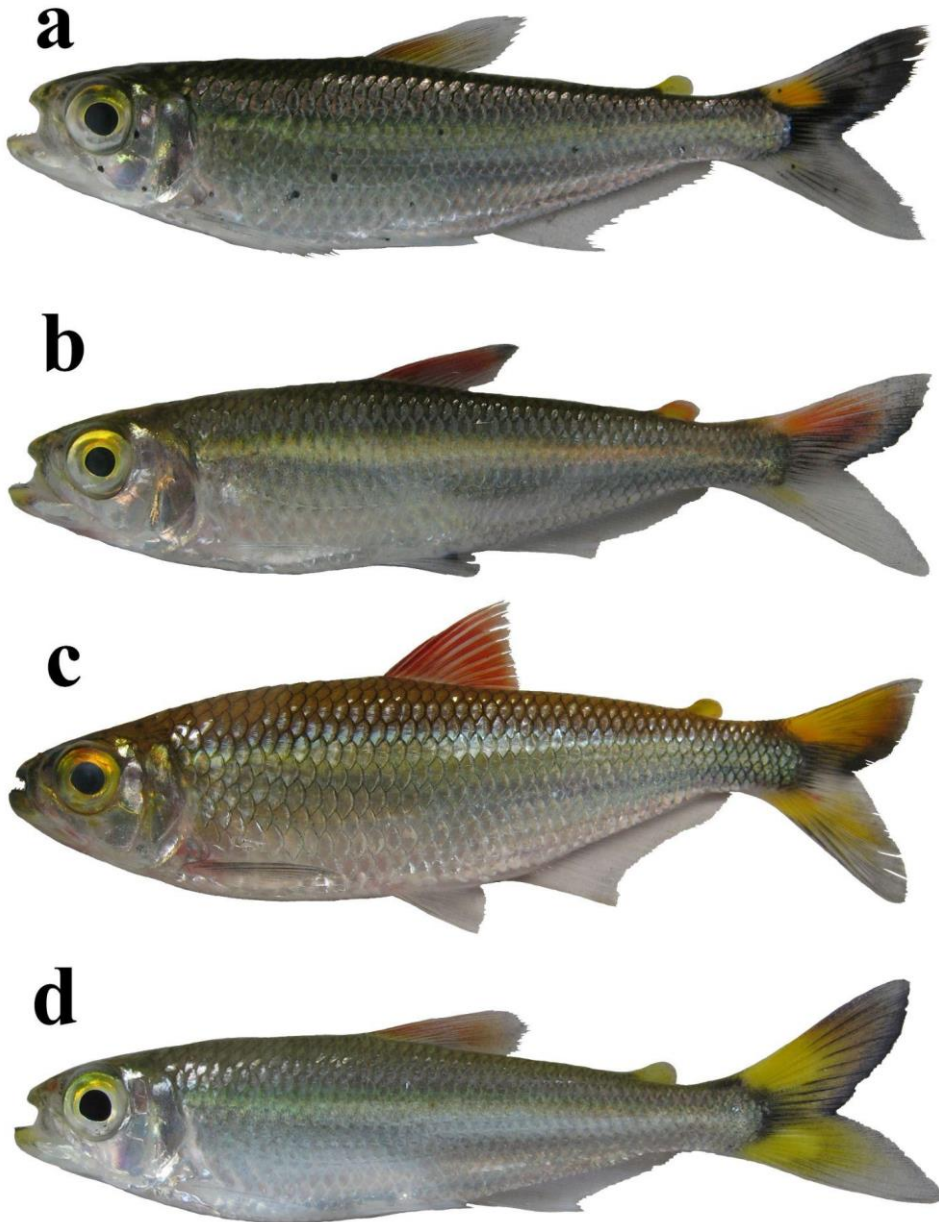


Figure S2 - Variation in colour of the *B.* (*Bryconops*) clade across sample locations a) *Bryconops* (*B.*) *caudomaculatus* (Manaus - dark Blackwaters); b) *Bryconops* (*B.*) *caudomaculatus* (S Guiana shield - lighter Blackwaters); c) *Bryconops* (*B.*) *caudomaculatus* (Coastal - lighter Blackwaters); d) *Bryconops* (*B.*) *rheorubrum* (Xingu - Clearwaters and turbid waters).

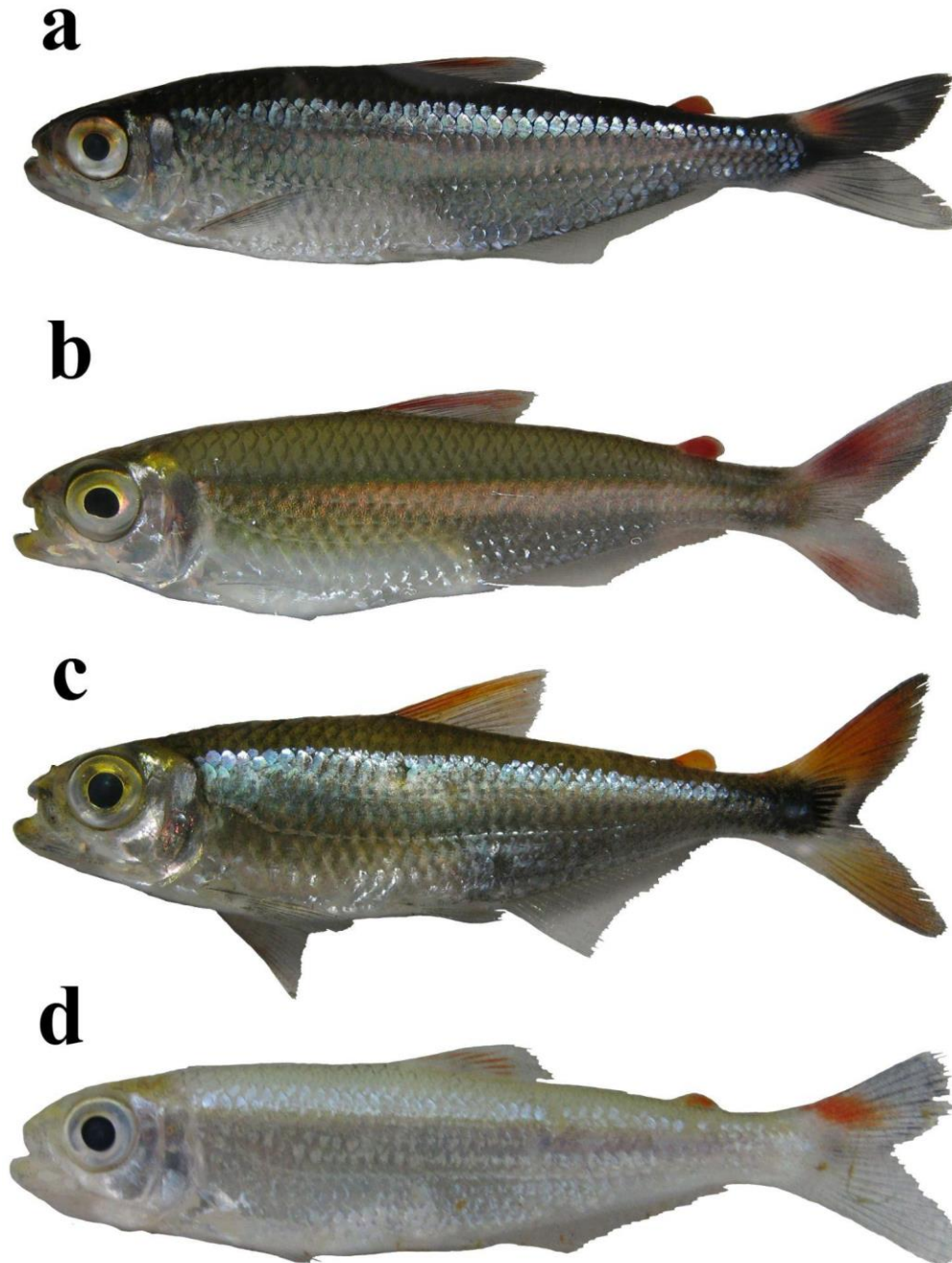


Table S1- [Excel file](#) containing sample data and character states. Samples with sequences from BOLD included. Photographs of samples identified from BOLD samples obtained from Daniel Cardoso Carvalho and Luis Malabarba.