

Chromatic polymorphism in *Trichomycterus albinotatus* (Siluriformes, Trichomycteridae), a mountain catfish from south-eastern Brazil and the role of colouration characters in trichomycterine taxonomy

Wilson J. E. M. Costa¹, José Leonardo O. Mattos¹, Pedro F. Amorim¹,
Beatriz O. Mesquita¹, Axel M. Katz¹

¹ Laboratory of Systematics and Evolution of Teleost Fishes, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

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Corresponding author: Wilson J. E. M. Costa (wcosta@acd.ufrj.br)

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Abstract

Colouration is an important tool for systematists inferring species limits and phylogenetic relationships of teleost fishes, but the use of colouration variation in trichomycterine catfish systematics has generated some controversy. We first report and describe the occurrence of four, geographically disjunct colour morphs in *Trichomycterus albinotatus*, endemic to south-eastern Brazil, as well as ontogenetic colouration change in each morph. A phylogenetic analysis using a cytb fragment (1098 bp) for 23 specimens representing all colour morphs and four outgroups did not support any correlation between colour morphs and lineages, with different colour morphs sharing identical haplotypes. This study indicated that young adult specimens found in lighter habitats had white and brown to black spots on the flank, whereas similar-sized specimens inhabiting darker habitats had white spots inconspicuous or absent and dark brown or black spots expanded. Individuals above about 65 mm SL of all populations had flank white marks less conspicuous or absent and cryptic habits during daylight, contrasting with smaller individuals with white marks and actively swimming above the substrate. Literature data indicate that ontogenetic colouration and habit changes occur in different trichomycterid lineages. Our data thus show that colouration may be problematic in taxonomical studies, although often being consistently used to diagnose species and clades. We conclude that colouration should not be discarded a priori as evidence of trichomycterine relationships and species limits, but should be used with caution in systematic studies, being necessary additional evidence, such as osteological characters or molecular data.

Key Words

Atlantic Forest, colouration ontogenetic change, mountain biodiversity, Trichomycterinae

Introduction

Colouration has been an important source of characters for systematists diagnosing species and inferring phylogenetic relationships of teleost fishes. Amongst catfishes of the Trichomycterinae, a diverse Neotropical catfish trichomycterid subfamily with over 260 species (Fricke et al. 2022), colouration has been broadly used to diagnose species since Eigenmann's (1918) monographic review of trichomycterids. Tchernavin (1944), however, did not consider colouration as valid evidence to diagnose species, us-

ing primarily morphometric data for reviewing trichomycterines from the Lake Titicaca and adjacent Andean river basins, consequently synonymising several species with different colour patterns. Following Tchernavin's view, Arratia and Menu-Marque (1981) and Arratia (1983a) recorded phenotypic colour variation in southern Andean trichomycterines related to different habitat substrate types, also synonymising nominal species with different colour patterns. However, according to Bockmann and Sazima (2004), intraspecific colour variation in trichomycterids is highly unusual and is useful to diagnose species.

Several authors have used colour patterns to distinguish trichomycterine species, with colouration playing an important role in species diagnoses of most recent taxonomical papers (e.g. Costa (1992); de Pinna (1992); Barbosa and Costa (2008); Datovo et al. (2012); Ferrer and Malabarba (2013); Rizzato and Bichuette (2014); Terán et al. (2017); Katz and Costa (2022); Costa et al. (2020a, 2021)). Colour patterns have also been used to diagnose species groups (Barbosa and Costa 2010a; Costa et al. 2020b; Vilardo et al. 2020; Costa and Katz 2021). On the other hand, colouration polymorphism and ontogenetic colour changes have been recorded for several species (Lima et al. 2008; da Silva et al. 2010; Nascimento et al. 2017; Ochoa et al. 2017; Costa et al. 2022a), as well as similar homogeneous black colouration being reported to homoplastically occur in sympatric trichomycterines with cryptic habits during daylight (Costa et al. 2020b), making species identification based on colouration problematic.

In the Atlantic Forest of south-eastern Brazil, catfishes of the genus *Trichomycterus* Valenciennes, 1832 represent the fish group with the greatest number of species adapted to life in mountain rivers, with about 60 valid species (Costa et al. 2020a; Costa and Katz 2021). Amongst these species is *Trichomycterus albinotatus* Costa, 1992, endemic to the upper section of the Rio Preto drainage, Rio Paraíba do Sul Basin (Costa 1992, 2021), which drains the Serra da Mantiqueira, a vast mountain range in south-eastern Brazil with highest peaks reaching about 2900 m. Juveniles and smaller adults of *T. albinotatus* are diurnal, being easily observed actively swimming near the river bottom during daylight (Costa 1992, 2021), but specimens above 65 mm of standard length (SL) are never seen swimming during daylight period, thus presumably considered nocturnal.

Data on distribution and colouration of *T. albinotatus* are still restricted to its original description (Costa 1992). At that time, *T. albinotatus* was recorded for a short area of the main course of the upper Rio Preto and adjacent tributaries (Córrego Véu de Noiva and Ribeirão Santa Clara) and a colour pattern consisting of alternating white, black and brown spots on the flank was used to diagnose this species. In subsequent field studies, more specimens exhibiting the same characteristics were collected in this area, always in altitudes above about 1200 m above sea level (a.s.l.). More recently, however, specimens similar to *T. albinotatus* topotypes, but exhibiting different flank colouration were found in separate areas of the same drainage, in altitudes above about 1150 m a.s.l. Specimens from these geographically disjunct areas had other morphological characters identical to those exhibited by topotypes of *T. albinotatus*, including shape, size and relative position of eye, nares and fins, head and body proportions and number of fin rays, vertebrae, odontodes and branchiostegal rays. Interestingly, no specimen similar to *T. albinotatus* was found in slightly lower altitude areas between these sub-drainages, at about 1100 m a.s.l. or less. The primary objective of this

study is to first describe the different geographically disjunct colour morphs and ontogenetic colouration changes in *T. albinotatus*, providing data on their respective geographical distribution and habitats. Since species diversity is often high in mountain regions (Hoorn et al. 2013) and the presence of closely-related species inhabiting small neighbouring areas is not a rare phenomenon amongst mountain organisms (Dimitrov et al. 2012), the second objective of this study is to check some genetic divergence amongst geographically isolated colour morphs thorough a phylogenetic analysis using a segment of the mitochondrial gene cytochrome b (1098 bp) for 23 specimens from 11 collecting sites, representing all colour morphs.

Materials and methods

Field methods and specimen collections

Field methods and euthanasia methods were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18) and collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit number: 38553-7) and INEA (Instituto Estadual do Ambiente; permit number: 037/2018).

Field studies were made during five trips to the upper section of the Rio Preto drainage (May and July 2015; July 2017; September and December 2018). During this period, sporadic collections were also made in the middle and lower sections of the Rio Preto, where no specimen of *T. albinotatus* was found. Collections of *T. albinotatus* were made in 11 collecting sites (Table 1), in altitudes between 1155 and 1495 m a.s.l., comprising the Rio Preto main channel and all its main tributaries (Fig. 1). Specimen collections were made with small dip nets (40 × 30 cm) during daylight. Euthanasia followed AVMA Guidelines for the Euthanasia of Animals (Leary et al. 2020); specimens were euthanised using a buffered solution of tricaine methane sulphonate (MS-222) at a concentration of 250 mg/l, for a period of 10 min or more, until completely ceasing opercular movements. Just after collection, specimens were observed in small transparent plastic bottles to record general colour patterns and fin morphology and then euthanised, except for usually two or three individuals representing each collecting locality that were kept alive for about 5–10 hours, photographed in small aquaria and then euthanised as described above. Most specimens were fixed in 10% formalin for a period of 10 days and then transferred to 70% ethanol; specimens to be used in molecular analyses were fixed and preserved in 98% ethanol, indicated in the list of material examined (Appendix 1) as ‘DNA’ just after catalogue numbers. Specimens were later deposited in the ichthyological collection of the Institute of Biology, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). Geographical names

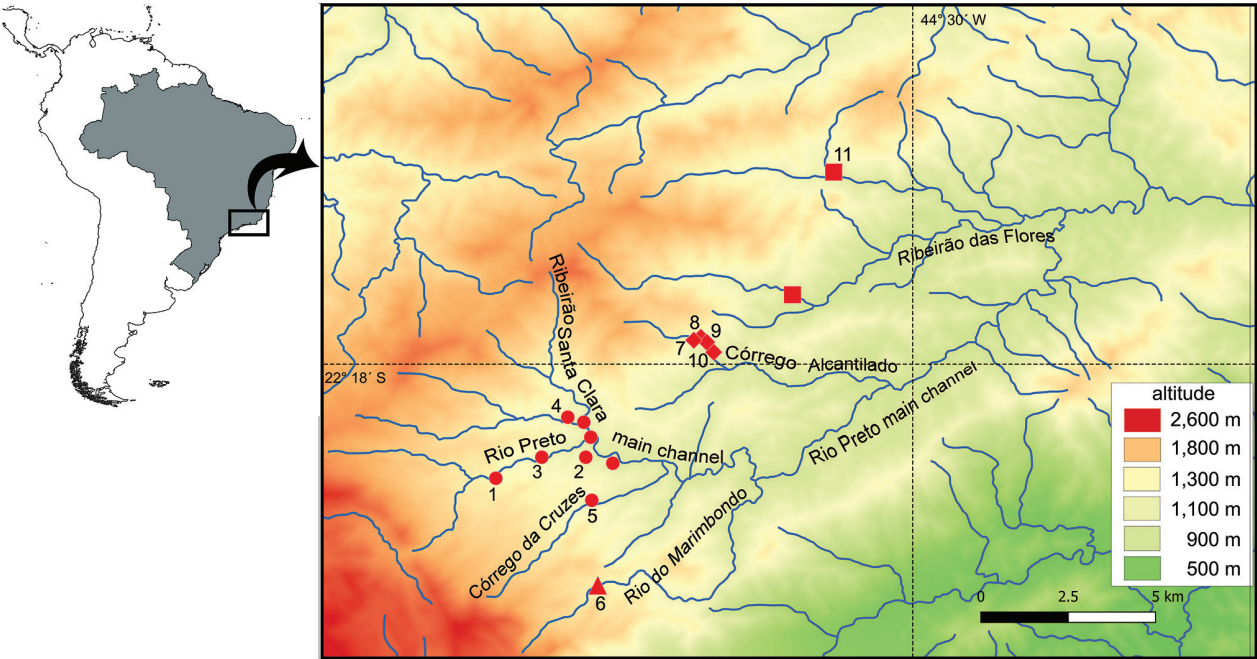


Figure 1. Geographical distribution of *Trichomycterus albinotatus* and its colour morphs (CMs): dots, CM1; triangle, CM2; diamonds, CM3; squares, CM4. Numbers are collecting sites with DNA samples.

are according to the regional popular use in Portuguese (e.g. Córrego, Ribeirão, Rio), making easier recognition of localities in the field and avoiding common equivocal generalisations when tentatively translating them to English. A complete list of specimens analysed appears in the Appendix 1.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscular tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's protocol. The analyses included a fragment of the mitochondrial encoded gene cytochrome b (CYTB), which have been successfully used to delimit trichomycterine species (Costa and Katz 2021). Amplification was made through the polymerase chain reaction (PCR) method, using the primers Cytb Siluri F and Cytb Siluri R (Villa-Verde et al. 2012). Double-stranded PCR amplifications were performed in 60 µl reactions with reagents at the following concentrations: 5× GreenGo-Taq Reaction Buffer (Promega), 1 mM MgCl₂, 1 mM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1 U of standard Taq polymerase or Promega GoTaq Hot Start polymerase. The thermocycling profile was as follows: initial denaturation for 2 min at 94–95 °C; 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 48.0–60.0 °C and extension for 1–2 min at 72 °C; and terminal extension for 4 min at 72 °C. Negative controls were used to check on contaminations. The PCR products were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sanger Sequencing reactions were made by Mac-

Table 1. Collecting sites, coordinates, altitude and UFRJ catalogue numbers of specimens of *Trichomycterus albinotatus* used in the molecular analysis. All localities are situated in the upper Rio Preto drainage, Rio Paraíba do Sul Basin, south-eastern Brazil.

Collecting sites	Coordinates and altitude	Voucher number
Rio Preto main channel		
CS1 Cachoeira do Escorrega	22°19'54"S, 44°36'57"W, 1495 m	11659
CS2 Rio Preto, Maromba 1	22°19'33"S, 44°35'27"W, 1305 m	9873, 11976
CS3 Rio Preto, Maromba 2	22°19'33"S, 44°36'11"W, 1250 m	11661-2, 10476
Ribeirão Santa Clara subdrainage		
CS4 Cachoeira Santa Clara	22°18'53"S, 44°35'45"W, 1215 m	11665
Córrego da Cruzes subdrainage		
CS5 Córrego das Cruzes	22°20'16"S, 44°35'21"W, 1195 m	12035
Rio do Marimondo subdrainage		
CS6 Cachoeira do Marimondo	22°21'41"S, 44°35'15"W, 1435 m	11932, 11983
Córrego Alcantilado subdrainage		
CS7 Córrego Alcantilado 1	22°17'36"S, 44°33'39"W, 1320 m	11658, 11663
CS8 Córrego Alcantilado 2	22°17'33"S, 44°33'32"W, 1295 m	11664
CS9 Cachoeira da Muralha	22°17'38"S, 44°33'26"W, 1170 m	11964
CS10 Córrego Alcantilado 3	22°17'48"S, 44°33'19"W, 1155 m	11963
Ribeirão das Flores subdrainage		
CS11 Cachoeira da Prata	22°14'48"S, 44°31'19"W, 1220 m	11931

rogen Inc. (South Korea). Sequencing chromatograms and sequences were assessed using MEGA 7 (Kumar et al. 2016). The generated DNA sequences were translated into amino acids residues to verify the absence of premature stop codons or indels using the programme MEGA 7. A list of GenBank access numbers is available in Online Resource 2.

Phylogenetic analysis

A phylogenetic analysis was performed using a CYTB fragment, 1098 bp, for 23 specimens of *T. albinotatus* from 11 collecting sites (CS1–11), representing all colour morphs (CM1–4). Outgroups included single sequences of four congeners, including *Trichomycterus vitalbrazili* Vilardo, Katz & Costa, 2020, the sister group of *T. albinotatus*, the only other member of the subgenus *Humboldtglanis* Costa, 2021; *Trichomycterus brasiliensis* Lütken, 1874 and *Trichomycterus mirissumba* Costa, 1992, two species of the subgenus *Cryptocambeva* Costa, 2021, the sister group of *Humboldtglanis*; and *Trichomycterus auroguttatus* Costa, 1992, a member of the subgenus *Psammocambeva* Costa, 2021, the sister group of the clade comprising *Cryptocambeva* plus *Humboldtglanis*. The data were partitioned according to codon positions; the best-fitting models of molecular evolution for each partition was found using the Bayesian Information Criterion (BIC) of ModelFinder (Kalyaanamoorthy et al. 2017), implemented in IQ-TREE 1.6.11 (Nguyen et al. 2015), which found the K2P+FQ+I+G4 model for the 1st codon position, the TN+F+I model for 2nd codon position and the GTR+FI+G4 model for the 3rd codon position. The dataset was analysed using Maximum Likelihood (ML) methods implemented in IQ-TREE, with two methods for assessing the reliability of internal branches: the Shimodaira-Hasegawa-like procedure support (SH-aLRT; Guindon et al. (2010)) and the ultrafast bootstrap support (UFBoot; Minh et al. (2013); Hoang et al. (2017)), using 1000 replicates and default parameters. Genetic distances were calculated to illustrate genetic diversity amongst taxa using Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA 7.

Results

Colour morphs

Four colour morphs were found, as below described (see Fig. 1 for geographical distribution of colour morphs).

Colour morph 1 (CM1). Description: In specimens between about 30 and 60 mm SL (Fig. 2B–E), flank pale brownish-yellow, darker above longitudinal body mid-line; two horizontal rows of rounded to horizontally elongated brown to black spots, alternated with similar-shaped white spots, sometimes with faint golden iridescence. Horizontal row of pale brown spots irregularly shaped on ventral part of flank, sometimes inconspicuous. In larger adult specimens above about 65 mm SL (Fig. 2A), dark spots on flank enlarged and coalesced to form vermiculate pattern; light marks paler.

Distribution and habitat: Areas above 1150 m a.s.l. of the upper course of the Rio Preto, the Ribeirão Santa Clara and Córrego das Cruzes drainage (CS1–6; Fig. 1). Rivers and streams about 2–15 m wide, deepest areas about 5 m deep, running in broad valleys, making the riverine environment highly exposed to sunlight. Water clear, hyaline. Common presence of large rocks favours

formation of small pools where most specimens were concentrated (Fig. 3A, B). Bottom with light grey rocks and multicolour gravel, predominating white, light grey and light brownishyellow colours.

Colour morph 2 (CM2). Description: Similar to CM1, except for white marks irregularly shaped and dark marks highly coalesced even in smaller specimens (Fig. 2F). In a single specimen found above 65 mm SL, white spots absent (Fig. 2G).

Distribution and habitat: Upper Rio do Marimbondo, altitude about 1435 m a.s.l. (CS6; Fig. 1). River about 5–10 m wide, deepest areas about 3 m deep, running in broad valley, riverine environment highly exposed to sunlight. Water clear, greenish hyaline. Common presence of large rocks favours formation of small pools below waterfalls where most specimens were concentrated (Fig. 3C). Bottom predominantly with light grey rocks of different sizes.

Colour morph 3 (CM3). Description: Similar to CM1, but white spots on body mid-line highly coalesced, usually with intense golden iridescence (Fig. 2H).

Distribution and habitat: Rio das Flores subdrainage at about 1220 m a.s.l. (Fig. 1; CS11). Rivers about 5–10 m wide, deepest areas about 2 m deep, running in broad valley, riverine environment highly exposed to sunlight. Water clear, yellowish hyaline. Rocks small, rare small waterfalls where most specimens are concentrated (Fig. 3E). Bottom predominantly with small light grey rocks, light grey to light yellow gravel and sand.

Colour morph 4 (CM4). Description: Flank and head pale yellow, with dark brown to black spots (Fig. 2I–J); flank dark spots highly coalesced in larger specimens (Fig. 2J), forming interconnected vermiculate pattern, flank white spots almost inconspicuous in smaller specimens (Fig. 2I), always absent in specimens above about 65 mm SL (Fig. 2J).

Distribution and habitat: Upper section of the Córrego do Alcantilado (CS7–10; Fig. 1), at altitudes between 1155 and 1320 m a.s.l. Stream about 2–5 m wide, deepest areas barely reaching about 2 m deep, running in narrow and steep valley, with numerous small waterfalls (Fig. 3D); riverine environment poorly exposed to sunlight. Water clear, yellowish hyaline. Bottom predominantly composed of dark grey rocks.

Phylogeny

The phylogenetic analysis highly supported *T. albinotatus* as a unique lineage, with maximum values for both SH-aLRT and UFBoot (Fig. 4). The tree topology did not support any correlation between colour morphs and lineages, with colour morphs not forming unique lineages. Genetic distances amongst haplotypes were low (0.1–0.4%). The eleven haplotypes found amongst the 23 sequenced individuals were scattered in different localities. H1 was the most common haplotype, present in seven analysed specimens collected in seven separate localities and exhibited all the four colour morphs.

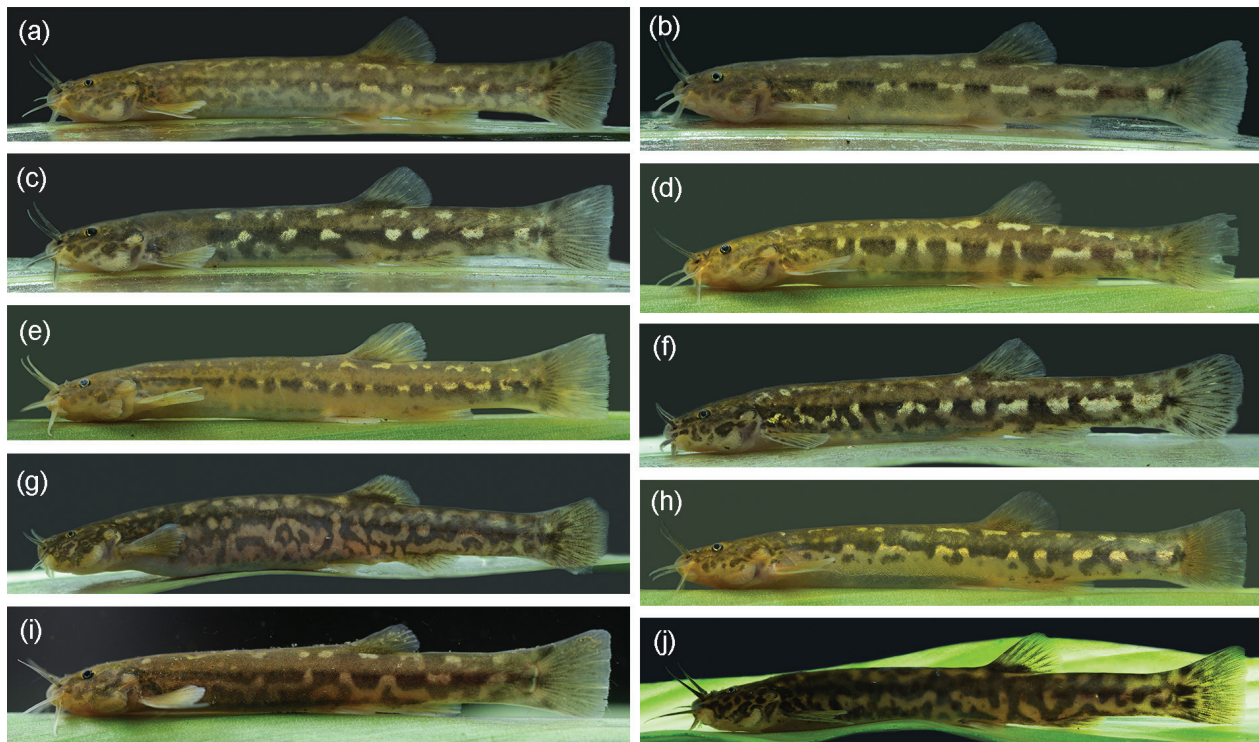


Figure 2. Colour morphs (CMs) of *Trichomycterus albinotatus*, with respective collecting sites (CSs) and collection catalogue numbers: CM1 (a–e); CM2 (f–g); CM3 (h); CM4 (i–j). a. CS3, UFRJ 11062, 66.8 mm SL; b. CS3, UFRJ 11062, 40.5 mm SL; c. CS3, UFRJ 11976, 44.3; d. CS5, UFRJ 12035, 44.9 mm SL; e. CS5, UFRJ 12035, 46.2 mm SL; f. CS6, UFRJ 12064, 45.2 mm SL; g. CS6, UFRJ 12064, 67.0 mm SL; h. CS11, UFRJ 12052, 49.2 mm SL; i. CS9, UFRJ 11670, 44.8 mm SL; j. CS10, UFRJ 12063, 71.5 mm SL.

Discussion

Distribution of colour morphs

High levels of intraspecific colouration polymorphism have been recorded in taxonomical papers on some trichomycterines, including both discrete and indiscrete colouration variation in syntopic specimens (e.g. Arratia et al. (1978); Sarmiento-Soares et al. (2005); Lima et al. (2008)). On the other hand, according to Arratia and Menu-Marque (1981) and Arratia (1983a), some Andean trichomycterine species exhibit distinct colour morphs in disjunct habitats with different river substrates. In the present study, we found specimens of *T. albinotatus* exhibiting different colour morphs in disjunct areas of the upper Rio Preto drainage, with slightly different ecological conditions (Fig. 2; Table 2). In the most extreme case (compare Fig. 2C with Fig. 2I for specimens about 45 mm SL and Fig. 2A with Fig. 2J for specimens about 65–70 mm SL), specimens found in habitats with light substrate, situated in wide valleys highly exposed to sunlight, had a colour pattern consisting of alternating white and brown to black spots (CM1, Fig. 2A–C). Contrastingly, specimens found in a stream with dark grey rocks on the bottom, situated in a narrow steep valley weakly exposed to sunlight were darker, with dark spots elongated and coalesced and white spots inconspicuous or absent (CM4, Fig. 2I–J). It is remarkable that, during daylight field studies, CM1 specimens when swimming close to multicolour gravel substrate were often almost invisible,

showing that CM1 may favour camouflage in this kind of habitat. These data suggest that the existence of different colour morphs may be related to specific kinds of habitat, a hypothesis to be tested only after detailed inventory on physical and chemical factors of the riverine habitats being available.

Ontogenetic colouration and habitat change

In all colour morphs of *T. albinotatus*, white marks on the flank were less conspicuous or had completely disappeared, whereas dark marks have expanded their extent in individuals above 65 mm SL (Fig. 2A, G, J). This ontogenetic colour change may be related to field evidence indicating that specimens about 60 mm or less are typically diurnal, whereas larger ones are apparently nocturnal. The presence of rows of alternating dark and light spots on the body side is a common colour pattern occurring in diurnal trichomycterids living in sandy or gravel substrate, which contribute to making them little visible for predators (Zuanon and Sazima 2004). For example, in *T. auroguttatus*, a typical diurnal psammophilic species sympatric to *T. albinotatus* (Costa 2021), there are alternating dark brown and golden spots on the flank (Costa 1992). On the other hand, different species having cryptic habits during daylight have dark colouration and no bright mark (Costa et al. 2020b). A similar ontogenetic change was recorded for *T. vitalbrazili*, the sister group of *T. albinotatus* (Vilardo et al. 2020).

Table 2. List of specimens used in the molecular analysis, with respective voucher number in the ichthyological collection of the Biology Institute, Federal University of Rio de Janeiro (UFRJ) and GenBank accession numbers. In specimens of *T. albinotatus*, CS means collecting site (see Table 1); CM, colour morph; H, haplotype.

Voucher number	Species	Accession numbers
9873.1	<i>Trichomycterus albinotatus</i>	CS2 CM1 H2 MT459172
10476.1	<i>Trichomycterus albinotatus</i>	CS3 CM1 H1 MT459173
11662.1	<i>Trichomycterus albinotatus</i>	CS3 CM1 H3 MT459174
11663.1	<i>Trichomycterus albinotatus</i>	CS7 CM4 H1 MT459175
11664.1	<i>Trichomycterus albinotatus</i>	CS8 CM4 H2 MT459176
11658.2	<i>Trichomycterus albinotatus</i>	CS7 CM4 H2 MT459177
11659.2	<i>Trichomycterus albinotatus</i>	CS1 CM1 H4 MT459178
11665.1	<i>Trichomycterus albinotatus</i>	CS5 CM1 H2 MT459179
11665.2	<i>Trichomycterus albinotatus</i>	CS5 CM1 H5 MT459180
11931.1	<i>Trichomycterus albinotatus</i>	CS11 CM3 H6 MT459181
11931.2	<i>Trichomycterus albinotatus</i>	CS11 CM3 H1 MT459182
11932.2	<i>Trichomycterus albinotatus</i>	CS6 CM2 H2 MT459183
11932.3	<i>Trichomycterus albinotatus</i>	CS6 CM2 H2 MT459184
11963.1	<i>Trichomycterus albinotatus</i>	CS10 CM4 H8 MT459185
11963.2	<i>Trichomycterus albinotatus</i>	CS10 CM4 H7 MT459186
11963.3	<i>Trichomycterus albinotatus</i>	CS10 CM4 H1 MT459187
11963.4	<i>Trichomycterus albinotatus</i>	CS10 CM4 H8 MT459188
11964.1	<i>Trichomycterus albinotatus</i>	CS9 CM4 H9 MT459189
11964.2	<i>Trichomycterus albinotatus</i>	CS9 CM4 H1 MT459190
11976.1	<i>Trichomycterus albinotatus</i>	CS2 CM1 H10 MT459191
11976.2	<i>Trichomycterus albinotatus</i>	CS2 CM1 H11 MT459192
11983.1	<i>Trichomycterus albinotatus</i>	CS6 CM2 H1 MT459193
12035.1	<i>Trichomycterus albinotatus</i>	CS5 CM1 H1 MT459194
11968.2	<i>Trichomycterus auroguttatus</i>	MT470410
10642	<i>Trichomycterus brasiliensis</i>	MT470418
11672.2	<i>Trichomycterus mirissumba</i>	MT470411

Little is still known about natural history of trichomycterines; therefore, it is not possible to infer securely if the synchronic change of colouration and period of activity in *T. albinotatus* and *T. vitalbrazili* corresponds to a unique evolutionary event or if it is a widespread condition amongst trichomycterines. Miranda-Ribeiro (1905) first reported conspicuous differences in the colouration of juvenile and adult specimens of trichomycterines. Since then, several papers have provided evidence of ontogenetic colouration change in *Trichomycterus* and the closely-related genus *Cambeva* (Costa 1992; Barbosa and Costa 2008, 2010b; da Silva et al. 2010; Ferrer and Malabarba 2013; Reis et al. 2020; Costa et al. 2021, 2022b). Amongst these studies, only da Silva et al. (2010) have demonstrated that small and large individuals of *Cambeva iheringi* (Eigenmann, 1917) besides having a distinct colour pattern, are also found in different habitats. According to these authors, spotted juveniles of *C. iheringi* live partially or totally buried in light sandy substrate, whereas dark-coloured larger adults are found in areas with dark stones. Arratia (1983b) reported juveniles and small adults of three Chilean trichomycterines living in shallower and more exposed habitats than larger adults, but no mention was made of possible ontogenetic change in colouration. Furthermore, recent studies in species of the trichomycterid subfamilies

Copionodontinae and Trichogeninae indicate that ontogenetic changes in the activity period is followed by colouration change (Sazima 2004; Zanata and Primitivo 2013). Sazima (2004) noted that juveniles and small adults of *Trichogenes longipinnis* Britski & Ortega, 1983 are more active at the daytime, whereas large adults with darker colours are mainly seen at night. Similarly, Zanata and Primitivo (2013) reported diurnal nektonic habits in small juveniles of *Copionodon pecten* de Pinna, 1992, as well as nocturnal benthonic habits in sub-adults and adults, which may exhibit a darker coloured phenotype. Therefore, since copionodontines and trichogenines together form a clade distantly related to trichomycterines (Katz et al. 2018), it is possible that synchronic change of colouration and habits during ontogeny have occurred independently in the clade comprising copionodontines and trichogenines and in the clade comprising *T. albinotatus* and *T. vitalbrazili*.

Role of colouration in trichomycterine taxonomy

This study firstly reported different colour morphs of *T. albinotatus* inhabiting geographically disjunct areas, which could induce taxonomists to recognise them as different species. Although the occurrence of geographically disjunct colour morphs is possibly uncommon amongst trichomycterids, data on colouration polymorphism provided by Arratia & Menu-Marque (1981) and Arratia (1983a) suggest that colouration may vary according to local environmental conditions in different trichomycterid lineages, attesting against the use of colouration characters in trichomycterid systematics.

Similarly, the use of colouration alone to distinguish sympatric species may induce misidentifications. For example, sympatric juveniles of *T. itatiayae* Miranda Ribeiro, 1906 and *T. nigroauratus* Barbosa & Costa, 2008, two species belonging to different subgenera (Costa 2021), have similar colouration, making it difficult to distinguish them in the field (Barbosa and Costa 2008), as well as the non-closely related, sympatric *T. immaculatus* (Eigenmann & Eigenmann, 1898) and *T. quintus* Costa, 2020 that share a similar overall black colouration, are only distinguishable under careful examination (Costa et al. 2020b).

These data do not diminish the value of colour pattern characters to diagnose species and trichomycterine clades. For example, even with the occurrence of chromatic polymorphism herein reported for *T. albinotatus*, the presence of white marks on the flank of juveniles uniquely shared by *T. albinotatus* and *T. vitalbrazili* amongst trichomycterines, clearly corroborated monophyly of this clade formally named as subgenus *Humboldtglanis* Costa, 2021, which is highly supported in a phylogenetic study integrating morphology and DNA sequences (Costa 2021). Similarly, apomorphic colour patterns are shared by trichomycterine clades corroborated by both osteological and molecular data (Costa 2021;



Figure 3. Habitats of *Trichomycterus albinotatus*: **a.** Upper Rio Preto, the type locality of *T. albinotatus*; **b.** Upper Córrego das Cruzes; **c.** Cachoeira do Marimbondo, upper Rio do Marimbondo subdrainage; **d.** Cachoeira da Muralha, upper Córrego do Alcantilado subdrainage; **e.** Cachoeira das Antas, upper Ribeirão das Flores subdrainage.

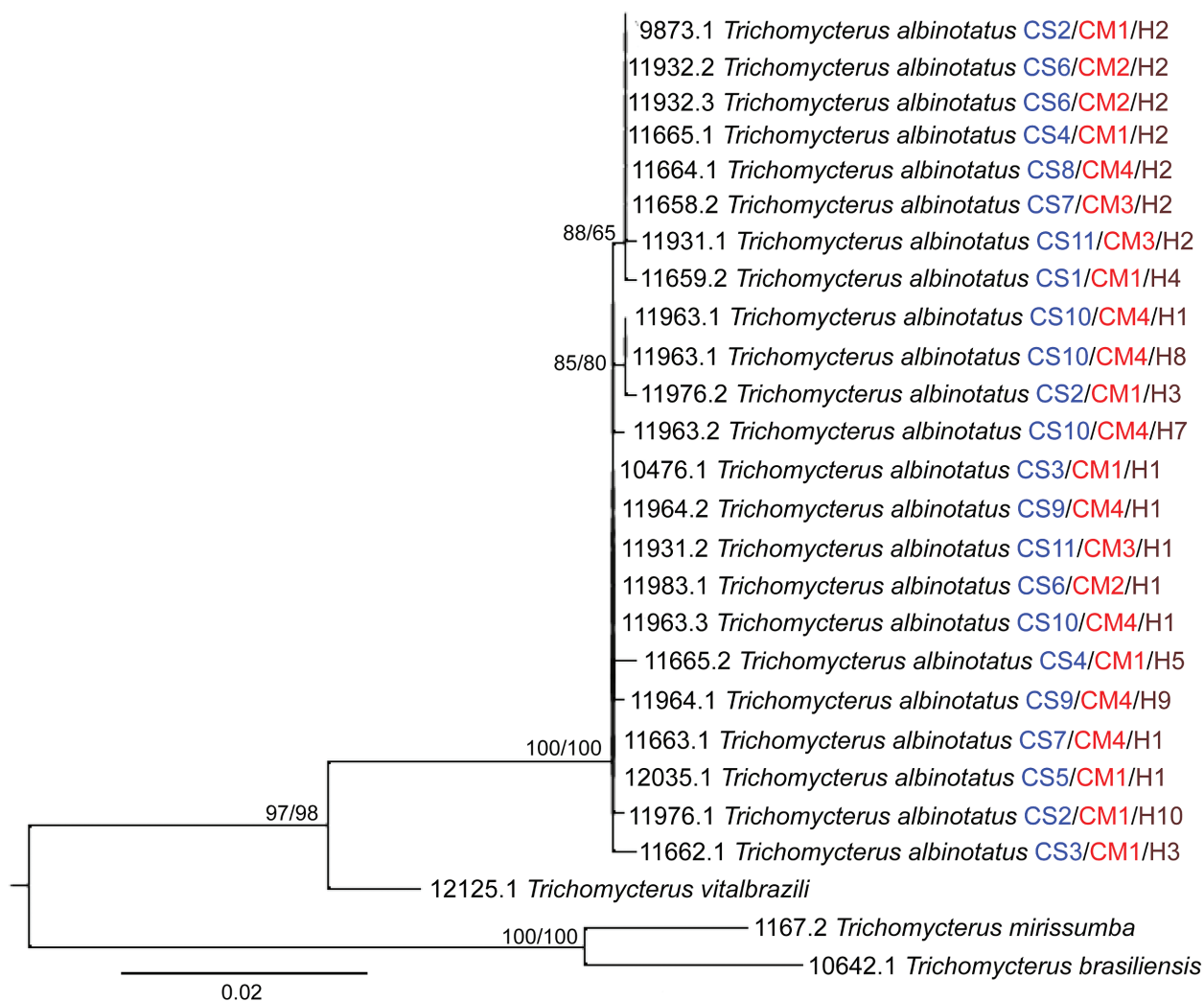


Figure 4. Phylogenetic relationships amongst 23 specimens of *Trichomycterus albinotatus* and three outgroups, inferred by Maximum Likelihood analysis performed in IQ-TREE using a cyt b fragment 1098 bp. Numbers close to nodes are support values: SH-aLRT support (%) and ultrafast bootstrap support (%). CS1–11, collecting sites 1–12; CM1–4, colour morphs 1–4; H1–11, haplotypes 1–11. The most external outgroup is not represented in the figure.

Costa and Katz 2021), thus demonstrating that colour patterns may be effectively used for diagnosing trichomycterine species and groups. These data in fact show that colouration should not be discarded a priori as evidence of species limits or phylogenetic relationships in trichomycterines, as well as colouration alone should not be used to infer species limits, being necessary to reinforce species delimitation hypotheses using additional morphological evidence (e.g. osteological characters) or molecular data.

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Appendix 1

List of specimens, with respective catalogue numbers of the ichthyological collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ). DNA, means specimens fixed and preserved in 98% ethanol; other specimens were fixed in 10% formalin and then preserved in 70% ethanol. All specimens collected in the Rio Preto drainage, Rio Paraíba do Sul basin, south-eastern Brazil.

Rio de Janeiro State: Itatiaia Municipality: – UFRJ 10476, 2 (DNA); Rio Preto about 650 m above the village of Maromba, 22°19'33"S, 44°36'11"W, about 1240 m asl (type locality); W. J. E. M. Costa et al., 3 May 2015. – UFRJ 11062, 4; UFRJ 11976, 5 (DNA); same locality as anterior; W. J. E. M. Costa et al., 27 Sep. 2018. – UFRJ 11661, 1 (DNA), UFRJ 11662, 1 (DNA), UFRJ 11668, 6; same locality as anterior; W. J. E. M. Costa et al., 25 Jul. 2017. – UFRJ 9873, 2 (DNA); Rio Preto, about 930 m below Cachoeira do Escorrega, 22°19'33"S, 44°35'27"W, about 1305 m asl; same locality as UFRJ 8579; W. J. E. M. Costa & C. P. Bove, 2 Feb. 2014. – UFRJ 10485, 3; UFRJ 11667, 5; UFRJ 11659, 3 (DNA); Rio Preto about 100 m above Cachoeira do Escorrega, 22°19'54"S, 44°36'57"W, about 1495 m asl; W. J. E. M. Costa et al., 25 Jul. 2017. **Resende Municipality:** – UFRJ 12035, 12 (DNA); UFRJ 12060, 7; Córrego das Cruzes close to the end of Vale das Cruzes road, 22°20'16"S, 44°35'21"W, about 1200 m asl; W. J. E. M. Costa et al., 2 Nov. 2018. – UFRJ 11932, 3 (DNA); UFRJ 12064, 7; UFRJ 11983, 1 (DNA); Poço do Marimbondo, Rio Marimbondo, 20°21'41"S,

44°35'15"W, about 1435 m asl; W. J. E. M. Costa et al., 27 Sep. 2018. **Minas Gerais State: Bocaina de Minas Municipality:** UFRJ 11665, 5 (DNA); Cachoeira Santa Clara, 22°18'53"S, 44°35'45"W, about 1215 m asl; W. J. E. M. Costa et al., 24 Jul. 2017. – UFRJ 11666, 15, Ribeirão Santa Clara, 22°18'58"S, 44°35'29"W, about 1160 m; W. J. E. M. Costa et al., 25 Jul. 2017. – UFRJ 11931, 3 (DNA); UFRJ 12061, 7; Cachoeira da Prata, Rio da Prata, 22°14'48"S, 44°31'19"W, about 1220 m asl; W. J. E. M. Costa et al., 26 Sep. 2018. – UFRJ 12059, 3; Cachoeira das Antas, 22°16'52"S, 44°32'02"W, about 1090 m asl; P. F. Amorim & B. Mesquita, 1 Sep. 2018. – UFRJ 12063, 4; Córrego do Alcantilado, 22°17'47"S, 44°33'19"W, about 1155 m asl; W. J. E. M. Costa et al., 25 Sep. 2018. – UFRJ 11664, 1 (DNA); UFRJ 11670, 3; Córrego do Alcantilado about 300 m from Cachoeira do Alcantilado, 22°17'33"S, 44°33'32"W, about 1295 m asl; W. J. E. M. Costa et al., 26 Jul. 2017. – UFRJ 11663, 1 (DNA); Córrego do Alcantilado, close to cave about 150 m from Cachoeira do Alcantilado, 22°17'36"S, 44°33'39"W, about 1320 m asl; W. J. E. M. Costa et al., 26 Jul. 2017. – UFRJ 11658, 4 (DNA); idem; W. J. E. M. Costa et al., 25 Sep. 2018. – UFRJ 11964, 3 (DNA); Cachoeira da Muralha, Córrego do Alcantilado, 22°17'38"S, 44°33'26"W, about 1170 m asl; W. J. E. M. Costa et al., 25 Sep. 2018. – UFRJ 11963, 4 (DNA); Córrego do Alcantilado, 22°17'48"S, 44°33'19"W, about 1155 m asl; W. J. E. M. Costa et al., 25 Sep. 2018.