REGULAR PAPER





A journey through the Amazon Middle Earth reveals *Aspidoras* azaghal (Siluriformes: Callichthyidae), a new species of armoured catfish from the rio Xingu basin, Brazil

Luiz F. C. Tencatt¹ | Janice Muriel-Cunha² | Jansen Zuanon³ | Marlon F. C. Ferreira² | Marcelo R. Britto⁴

Correspondence

Luiz F. C. Tencatt, Universidade Estadual de Maringá, Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Av. Colombo, 5790, 87020-900 Maringá, Paraná, Brazil. Email: luiztencatt@hotmail.com

Funding information

The authors are supported by personal grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (LFCT: processes #141061/2014-6 and #304997/2016-1; MRB: process #309285/2018-6; JZ: process #313183/2014-7). JMC was supported by a research project for the Fundação Amazônia de Amparo a Estudos e Pesquisas (process #184/2009). MRB was supported by grants from Edital Programa Institucional de Pesquisa nos Acervos da USP and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grant #200.103/2019). This study is a partial result of the PhD project of LFCT, and an undergraduate project from the Programa de Bolsa de Iniciação Científica (PIBIC/UFPA) of MFCF

Abstract

Aspidoras azaghal n. sp. was discovered during a multitaxonomic scientific expedition to the remote Amazon Terra do Meio region in tributaries to the rio Xingu basin, Pará, Brazil. The new species can be promptly distinguished from its congeners by the following combination of features: (a) absence of the first dorsal-fin element; (b) parieto-supraoccipital fontanel located medially on bone; (c) absence of a longitudinal dark-brown or black stripe along flank midline; (d) ventral surface of trunk covered by clearly smaller, irregular and/or roundish platelets; (e) inner laminar expansion of infraorbital 1 well developed; (f) relatively wide frontal bone, with width equal to half of entire length; (g) absence of a thick, longitudinal conspicuous darkbrown stripe along dorsal portion of flank; and (h) poorly developed serrations on posterior margin of the pectoral-fin spine. Besides morphological evidence, the molecular analyses indicated significant differences between the new species and its congeners, with A. albater and A. raimundi as its closest species, showing 6.53% of genetic differentiation in both cases. The intraspecific molecular data revealed gene flow (peer fixation index, FST = 0.05249, P > 0.05, for the cytochrome oxidase I (COI) marker and FST = -0.01466, P > 0.05, for the control region) between specimens upstream and downstream from a 30-m height waterfall at the type-locality, which therefore represent a single population. Furthermore, it was possible to observe a unidirectional gene flow pattern, with genetic diversity increasing in the downstream direction.

KEYWORDS

Amazon basin, Aspidoradini, corydoradinae, Serra do Pardo, taxonomy

¹Universidade Estadual de Maringá. Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Paraná, Brazil

²Universidade Federal do Pará, Campus Bragança, Instituto de Estudos Costeiros, Braganca, Brazil

³Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, Manaus, Brazil

⁴Departamento de Vertebrados, Setor de Ictiologia, Quinta da Boa Vista, Universidade Federal do Rio de Janeiro, Museu Nacional, Rio de Janeiro, Brazil

1 | INTRODUCTION

The Aspidoras Ihering, 1907 are small-sized amored catfishes within Callichthyidae (Reis, 2003). Currently, there are 24 valid species known only from Brazil (Oliveira et al., 2017; Tencatt & Bichuette, 2017). According to Britto (2003), Aspidoras can be distinguished from the remaining Callichthyidae by having the combination of the following synapomorphies: (a) a wide posterior portion of the mesethmoid; (b) reduced frontal fontanel; (c) a circular fossa in the parieto-supraoccipital; (d) a compact opercle; and (e) a very small ossified portion of the pectoral spine, which is smaller than half of the length of the first branched ray. Despite the relatively extensive literature concerning both the taxonomy and systematics of Aspidoras published since its description (e.g., Gosline, 1940; Miranda Ribeiro, 1949; Nijssen & Isbrücker, 1976, 1980; Britto, 1998, 2000, 2003; Lima & Britto, 2001; Britto et al., 2002, 2005; Shimabukuro-Dias et al., 2004; Alexandrou et al., 2011; Wosiacki et al., 2014; Leão et al., 2015; Oliveira et al., 2017; Tencatt & Bichuette, 2017), the identity of the genus itself and also of its species is still unclear.

In 2010, specimens of an undetermined species of Aspidoras were collected in tributaries to the Igarapé do Pontal, within the remote Amazon Terra do Meio region, interfluvium of Xingu and Iriri rivers, during a multitaxonomic scientific expedition carried out in collaboration between the Universidade Federal do Pará (UFPA), the Museu Paraense Emílio Goeldi (MPEG), the Instituto Nacional de Pesquisas da Amazônia (INPA), the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the World Wide Fund for Nature (WWF) in the Serra do Pardo National Park, a Federal Conservation Unit.

After analysis of these specimens it was possible to conclude that an undescribed species was represented, which is formally described herein. Additionally, molecular analyses to verify intra- and interspecific genetic patterns were conducted, making it possible to provide data on genetic diversity, structure and gene flow within the species, plus genetic differences among congeneric species.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The care and use of experimental animals complied with animal welfare laws, guidelines and policies as approved by Conselho Nacional de Controle e Experimentação Animal (CONCEA, 2013) under a collection authorization granted by the Ministério do Meio Ambiente (MMA)/ ICMBio, process #24524-2. Following the recommendations of the CONCEA (2013), the sampled specimens were euthanized through immersion in a eugenol solution before obtaining tissue samples for DNA analyses and further preservation of the whole specimen.

2.2 | Taxonomic description

Morphometric and meristic data were taken following Reis (1997), with modifications of Tencatt *et al.* (2013). Measurements were

obtained using digital callipers to the nearest 0.1 mm. Morphometrics are reported as proportion of standard length (L_S) or as proportions of head length (L_H). The size of the larval specimen is exceptionally expressed in total length (L_T). The osteological analysis was performed in cleared-and-stained (c&s) specimens, prepared according to the protocol of Taylor and Van Dyke (1985). In general, osteological terminology follows Reis (1998), with the following exceptions: parietosupraoccipital instead of supraoccipital (Arratia & Gayet, 1995), compound pterotic instead of pterotic-supracleithrum (Aquino & Schaefer, 2002) and scapulocoracoid instead of coracoid (Lundberg, 1970). Additionally, the nomenclature of the bones associated with the dorsal-fin locking mechanism follows Halstead et al. (1953). The suprapreopercle sensu Huysentruyt and Adriaens (2005) will be treated here as a part of the hyomandibula according to Vera-Alcaraz (2013). Vertebral counts include only free centra, with the compound caudal centrum (preural 1 + ural 1) counted as a single element. The last two dorsal-fin rays were counted as distinct elements. Pharvngeal teeth were counted in both sides of the branchial arches. Frequencies of counts were given in the text between parentheses and after the respective characteristic, and the asterisks refer to the counts of the holotype. Nomenclature of laterosensory canals follows Schaefer and Aquino (2000), and that of preopercular pores follows Schaefer (1988). Homologies of barbels follow Britto and Lima (2003). Larval description follows Nakatani et al. (2001).

2.3 | Molecular analyses

The molecular dataset was based on sequences obtained from 38 specimens. The DNA was extracted from tissue samples obtained from pectoral and pelvic fins using the Wizard Genomic-PROMEGA commercial kit. The mitochondrial cytochrome oxidase I (COI) gene was amplified using the primers Fisher F1 and Fisher R1 (Ward et al., 2005) and the control region with primers A and G (Lee et al., 1995). The amplification reactions were performed under the following conditions: initial denaturation of DNA at 95°C followed by 35 cycles of denaturation at 95°C for 35 s, hybridization at 50°C for 40 s and extension at 72°C for 1 min and 40 s, followed by a final extension of 72°C for 3 min. The sequences were edited by CLUSTAL W (Thompson et al., 1994) implemented in BioEdit (Hall, 1999) and were submitted to the DNAsp (Librado & Rozas, 2009) to generate the input file format for the Arlequin (Excoffier & Lischer, 2010).

Population and phylogeographic analyses were performed using the software Arlequin v.3.5.1.2 (Excoffier & Lischer, 2010). The descriptive parameters were calculated for each marker studied to characterize the levels of genetic diversity, such as the number of polymorphic sites, nucleotide and haplotypic diversity (h). To assess interand intrapopulation genetic divergence levels, the *P* (uncorrected) distance was calculated using the MEGA 6 program (Tamura *et al.*, 2013) to verify the spatial and genealogical relationship of haplotypes. A haplotype network was built in the HAPLOVIEWER program (Salzburger *et al.*, 2011) by the maximum likelihood method

implemented in the Dnaml (Felsenstein, 1993). The peer fixation index (FST) (Weir & Cockerham, 1984) performed in Arlequin v.3.5.1.2 (Excoffier & Lischer, 2010) was calculated to evaluate gene flow levels within sampled localities and then infer about the pattern of population structure along sampled sites. The statistical significance of the FST was tested with 10,000 permutations. Through the Barcoding of Life Database (BOLD), the sequences of the new *Aspidoras* were compared with those of the available congeners, and a table was generated to show the genetic differences among them.

Institutional abbreviations follow Sabaj (2016).

3 | RESULTS

3.1 | Aspidoras azaghal, new species (Figure 1)

urn:lsid:zoobank.org:act:047F0D60-C105-4F48-8537-F1A496EB1443

3.2 | Holotype

MNRJ 51793, 33.4 mm $L_{\rm S}$, Brazil, Pará, Altamira, Terra do Meio Region, Parque Nacional da Serra do Pardo, unnamed Igarapé I tributary to the Igarapé do Pontal, itself a tributary to the lower rio Xingu basin, 6°00′58″S, 53°08′20″W, 9 December 2010, J.A.S. Zuanon & J. Muriel-Cunha.

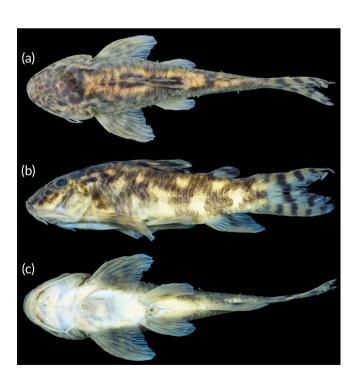


FIGURE 1 A. azaghal, holotype, MNRJ 51793, 33.4 mm SL, Brazil, Pará, Altamira, unnamed Igarapé, lower rio Xingu basin. Dorsal (a), lateral (b) and ventral (c) views. Photograph: Victor de Brito

3.3 | Paratypes

All from Brazil, Pará, Altamira, Terra do Meio Region, Parque Nacional da Serra do Pardo, Igarapé do Pontal basin, lower rio Xingu basin, J.A.S. Zuanon & J. Muriel-Cunha. ZUFMS 6373, 3, 22.2–26.6 mm $L_{\rm S}$, unnamed Igarapé II, 6°00′37″S, 53°07′16″W, 10 December 2010. INPA 59494, 4, 27.4–33.3 mm $L_{\rm S}$; MNRJ 51792, 6 ex., 7.0 mm $L_{\rm T}$ –26.3 mm $L_{\rm S}$; MPEG 38935, 4 ex., 26.1–28.0 mm $L_{\rm S}$, unnamed Igarapé I, 06°01′10″S, 53°08′16″W, 11 Dec 2010. MCP 54425, 4, 17.6–30.6 mm $L_{\rm S}$; MNRJ 51791, 3, 24.0–30.4 mm $L_{\rm S}$; MZUSP 125774, 5, 20.8–28.6 mm $L_{\rm S}$; NUP 22632, 9, 20.6–26.5 mm $L_{\rm S}$; ZUFMS 6374, 9, 9.4–30.1 mm $L_{\rm S}$, 2 c&s, 31.5–33.2 mm $L_{\rm S}$, same data as the holotype.

3.4 | Diagnosis

Aspidoras azaghal can be distinguished from all of its congeners by the absence of the median foramen at the dorsal-fin spine base (Figure 2a) (vs. presence, Figure 2b). Additionally, the new species can be distinguished from its congeners, with exception of A. velites Britto et al., 2002, by the absence of the first dorsal-fin element, the spinelet (Figure 3) (vs. presence, see Tencatt & Bichuette, 2017: 7, fig. 4a); from A. velites it differs by having the parieto-supraoccipital fontanel located medially on bone (Figure 4a) (vs. close to origin of posterior process, see Tencatt & Bichuette, 2017: 7, fig. 4b), absence of a longitudinal dark brown or black stripe along flank midline (Figure 1) (vs.

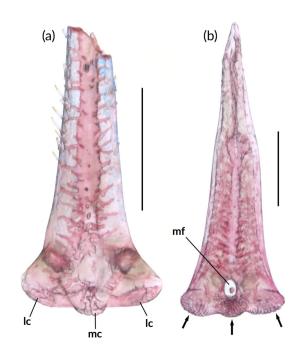


FIGURE 2 Dorsal-fin spine in CS specimens of (a) *A. azaghal*, paratype, ZUFMS 6374, 31.5 mm SL, and (b) *Aspidoras lakoi*, MNRJ 5293, 30.0 mm SL. lc, lateral condyle; mc, median condyle; mf, median foramen. Arrows indicate the conspicuous grooves on condyles. Scale bar = 1 mm

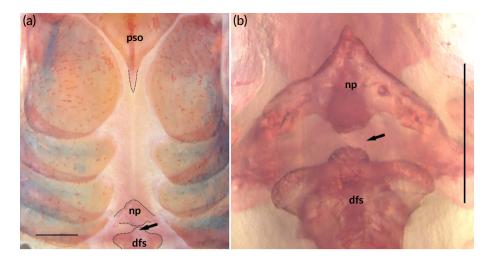


FIGURE 3 (a) Predorsal region of trunk of a CS paratype of A. azaghal, ZUFMS 6374, 33.2 mm SL, showing the absence of contact between the posterior process of the parieto-supraoccipital and the nuchal plate. (b) Detail of the area between dorsal-fin spine base and nuchal plate, showing the absence of the dorsal-fin spinelet. Dotted lines represent the bony surfaces entirely covered by thick layer of skin. Arrows indicate the place where the dorsal-fin spinelet should be. dfs, dorsal-fin spine; np, nuchal plate; pso, parieto-supraoccipital. Scale bars = 1 mm

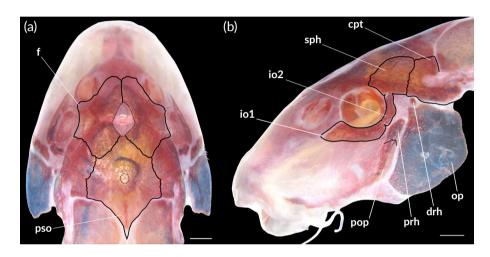


FIGURE 4 Head of a CS paratype of A. azaghal, ZUFMS 6374, 31.5 mm SL, in (a) dorsal and (b) lateral views. Solid lines represent the suture between bones. Dotted lines in (a) delimit frontal and parieto-supraoccipital fontanels between frontal bones and located medially on parieto-supraoccipital bone, respectively. cpt, compound pterotic; dhr, dorsal ridge of hyomandibula; f, frontal; io1, infraorbital 1; io2, infraorbital 2; op, opercle; prh, posterodorsal ridge of hyomandibula; pop, preopercle; pso, parieto-supraoccipital; sph, sphenotic. Scale bars = 1 mm

presence, see Britto et al., 2002: 728, fig. 2) and ventral surface of trunk covered by relatively small irregular and/or roundish platelets (similar to Tencatt & Bichuette, 2017: 8, fig. 6a) (vs. relatively large, irregular and/or elongated platelets, see Tencatt & Bichuette, 2017: 8, fig. 6b). Furthermore, it can be distinguished from A. belenos Britto, 1998, A. gabrieli Wosiacki et al., 2014, A. lakoi Miranda Ribeiro, 1949, A. maculosus Nijssen & Isbrücker, 1976, A. menezesi Nijssen & Isbrücker, 1976, A. mephisto Tencatt & Bichuette, 2017, A. psammatides Britto et al., 2005, A. raimundi (Steindachner, 1907), A. spilotus Nijssen & Isbrücker, 1976 and A. velites Britto et al., 2002 by having inner laminar expansion of infraorbital 1 well developed (vs. moderately developed in A. belenos, A. maculosus, A. menezesi, A. mephisto, A. raimundi and A. spilotus; extremely well developed in A. gabrieli and A lakoi; poorly developed in A. psammatides and A. velites). The new species can also be distinguished from A. depinnai, A. marianae Leão, Britto & Wosiacki, 2015, A. microgalaeus Britto, 1998, A. poecilus Nijssen & Isbrücker, 1976, A. psammatides, A. raimundi and A. velites by having relatively wide frontal bone, with width equal to half of entire length (vs. narrow, with width slightly smaller than half of entire length, in A. depinnai, A. marianae, A. menezesi, A. microgalaeus, A. poecilus, A. raimundi and A. velites; strongly narrow, with width conspicuously smaller than half of entire length, in A. psammatides). A. azaghal can be distinguished from

A. brunneus Nijssen & Isbrücker, 1976 by lacking a thick, longitudinal conspicuous dark-brown stripe along dorsal portion of flank (vs. presenting); from A. pauciradiatus (Weitzman & Nijssen, 1970) and A. virgulatus Nijssen & Isbrücker, 1980 by having poorly developed serrations on posterior margin of the pectoral-fin spine (vs. strongly developed).

3.5 | Description

Morphometric data are presented in Table 1. Head compressed with convex dorsal profile; somewhat trapezoid in dorsal view. Snout moderately developed and pointed; rounded in some specimens. Head profile convex from tip of snout to anterior nares; region of frontals slightly concave in some specimens; ascending slightly convex from this point to dorsal-fin origin. Profile slightly convex along dorsal-fin base. Post-dorsal-fin body profile slightly concave or nearly straight to adipose-fin spine; slightly concave or nearly straight from this point to caudal-fin base. Ventral profile of body slightly convex from isthmus to pelvic-fin origin; region of gill opening slightly concave in some specimens; nearly straight from this point to anal-fin origin; slightly concave until caudal-fin base. Body roughly elliptical in cross-section at pectoral girdle, gradually becoming more compressed towards caudal fin.

TABLE 1 Morphometric data of the holotype and 25 paratypes of A. azaghal

	Holotype	Low-high	Mean ± S.D.
Standard length (mm)	33.4	20.8-33.4	27.5 ± 3.1
Percentage of standard length			
Depth of body	28.4	22.6-31.0	27.8 ± 2.0
Predorsal distance	49.4	42.4-49.8	46.7 ± 2.1
Prepelvic distance	52.1	45.4-53.3	50.2 ± 1.9
Preanal distance	81.4	68.3-81.6	76.0 ± 3.7
Preadipose distance	85.9	78.2-89.7	84.9 ± 3.1
Length of dorsal spine	11.7	6.0-16.6	11.8 ± 2.3
Length of pectoral spine	11.1	10.4-17.8	12.4 ± 1.8
Length of adipose-fin spine	10.2	9.0-12.5	10.3 ± 0.8
Depth of caudal peduncle	14.7	14.7-17.2	15.9 ± 0.7
Length of dorsal-fin base	15.0	14.1-19.7	16.4 ± 1.6
Dorsal to adipose distance	23.1	19.9-27.4	24.3 ± 2.0
Maximum cleithral width	24.9	22.8-26.8	25.1 ± 1.0
Head length	37.4	32.5-39.2	36.1 ± 1.5
Length of maxillary barbel	19.5	13.7-20.6	17.7 ± 2.0
Percentage of head length			
Head depth	68.0	59.7-75.8	69.2 ± 3.3
Least interorbital distance	32.8	30.4-42.4	36.7 ± 2.3
Horizontal orbit diameter	17.6	11.1-21.8	16.9 ± 3.3
Snout length	48.0	43.8-53.0	47.7 ± 1.7
Least internarial distance	19.2	18.8-31.8	23.2 ± 3.6

Eye rounded, located dorso-laterally on head; orbit delimited dorsally by lateral ethmoid, frontal and sphenotic, ventrally by infraorbitals. Anterior and posterior nares close to each other, only separated by flap of skin. Anterior naris tubular. Posterior naris close to anterodorsal margin of orbit, separated from it by distance similar to naris diameter. Mouth small, subterminal, width slightly larger than bony orbit diameter. Maxillary barbel generally large, reaching to or slightly surpassing anteroventral limit of gill opening. Outer mental barbel ranging from slightly smaller to slightly larger than maxillary barbel. Inner mental barbel fleshy, with base close to its counterpart. Lower lip moderately developed, forming small semicircular or triangular fleshy flap. Small rounded papillae covering entire surface of all barbels, upper and lower lips, snout and isthmus.

Mesethmoid short; anterior tip well developed, slightly larger than 50% of bone length (see Britto, 2003: 123, character 1, state 0; fig. 1A); posterior portion wide, entirely covered by thick layer of skin (Figure 4a). Nasal slender, curved laterally, inner and outer margins with poorly developed laminar expansions; mesial border contacting only frontal.

Frontal elongated, relatively wide, with width equal to half of entire length; anterior projection short, with size smaller than nasal length; anterior margin generally covered by thick layer of skin (Figure. 4a). Frontal fontanel relatively small, ellipsoid or somewhat rhomboid; posterior tip extension not entering anterior margin of parieto-supraoccipital (Figure 4a). Sphenotic somewhat trapezoid, contacting

parieto-supraoccipital dorsally, compound pterotic posteriorly, second infraorbital ventrally and frontal anteriorly (Figure 4b). Compound pterotic roughly pipe-shaped, with posteriormost portion contacting first lateral-line ossicle, and ventral margin contacting opercle and cleithrum; posterior expansion almost entirely covering lateral opening of swimbladder capsule, leaving slender area on its dorsal margin covered only by thick layer of skin (Figure 4b). Parieto-supraoccipital wide, posterior process poorly developed, not contacting nuchal plate (Figure 4a). Parieto-supraoccipital medial keel expanded ventrally; laminar, with posterior portion at same level as posterior process tip. Parieto-supraoccipital fontanel small, roundish; located medially on parieto-supraoccipital (Figure 4a).

Two laminar infraorbitals with minute odontodes; infraorbital 1 large, ventral laminar expansion generally ranging from poorly developed to moderately developed; well developed in some specimens; anterior portion with moderately developed expansion, reaching middle of nasal capsule; inner laminar expansion well developed, with anterior portion clearly larger than posterior portion; small portions of external surface covered by thick layer of skin (Figure 4b); infraorbital 2 small, slender; with posterior laminar expansion poorly developed; conspicuously reduced in some specimens; inner laminar expansion moderately developed; posteroventral margin close but not directly contacting posterodorsal ridge of hyomandibula; contacting in some specimens; dorsal tip contacting only sphenotic; small portions of external surface covered by thick layer of skin (Figure 4b).

Posterodorsal ridge of hyomandibula close to its articulation with opercle oblong; exposed, relatively slender; dorsal ridge of hyomandibula between compound pterotic and opercle exposed (Figure 4b); covered by thick layer of skin in some specimens; exposed areas generally bearing small odontodes. Interopercle generally entirely covered by thick layer of skin (Figure 4b); somewhat triangular, anterior projection moderately developed. Preopercle relatively slender, elongated (Figure 4b), minute odontodes sparse on external surface. Opercle compact in shape, width larger than half of its length; free margin convex; posterodorsal region with smoothly concave area in some specimens; without serrations and covered by small odontodes; some portions of bony distal margin irregular in some specimens (Figure 4b).

Four branchiostegal rays decreasing in size posteriorly. Hypobranchial 2 somewhat triangular, tip ossified and directed towards anterior portion, posterior margin cartilaginous; ossified portion well developed, about twice size of cartilaginous portion. Five with ceratobranchials expansions increasing posteriorly: ceratobranchial 1 with strongly reduced process on anterior margin of mesial portion; absent in some specimens; ceratobranchial 3 with continuous posterolateral margin; ceratobranchial 5 toothed on posterodorsal surface, 28 to 30 (2) teeth aligned in one row. Four epibranchials with similar size; epibranchial 2 slightly larger than others, with small pointed process on laminar expansion of posterior margin: epibranchial 3 with triangular uncinate process on laminar expansion of posterior margin. Two wide pharyngobranchials (3 and 4), pharyngobranchial 3 with triangular laminar expansion on posterior margin; triangular laminar expansion with notches in some specimens. Upper tooth plate oval; 30 to 32 (2) teeth aligned in two rows on posteroventral surface.

Lateral-line canal entering neurocranium through compound pterotic, branching twice before entering sphenotic: pterotic branch, with single pore; preoperculomandibular branch conspicuously reduced, with a single pore opening close to postotic main canal; postotic main canal becoming widened just posterior to pterotic branch. Sensory canal continuing through compound pterotic, entering sphenotic as temporal canal, which splits into two branches: one branch giving rise to infraorbital canal, the other branch entering frontal through supraorbital canal, both with single pore. Supraorbital canal branched, running through nasal bone. Epiphyseal branch of supraorbital canal relatively long; pore opening close to frontal fontanel. Nasal canal with three openings, first on posterior edge, second on posterolateral portion and third on anterior edge; second pore variably fused with first pore, or nasal canal with two openings, with single pore at each edge. Infraorbital canal running through entire second infraorbital, extending to infraorbital 1 and opening into two or three pores. Preoperculomandibular branch giving rise to preoperculo-mandibular canal, which runs through entire preopercle with three openings, leading to pores 3, 4 and 5, respectively; pore 3 variably opening at posterodorsal ridge of hyomandibula.

Dorsal fin somewhat triangular, located just posterior to third dorsolateral body plate. Dorsal-fin rays I,8* (14), I,9 (1), posterior margin of dorsal-fin spine smooth. Nuchal plate poorly developed in length;

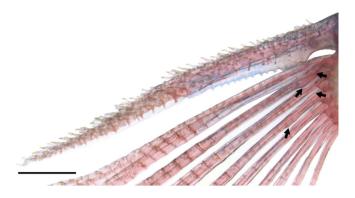


FIGURE 5 Left pectoral-fin spine and soft rays of a CS paratype of A. *azaghal*, ZUFMS 6374, 31.5 mm SL, showing the serration pattern on posterior margin of the spine. Arrows indicate the small laminar expansions on base of first branched rays. Scale bar = 1.0 mm

entirely covered by thick layer of skin; spinelet absent (Figure 3); spine poorly developed, adpressed distal tip reaching to middle portion of dorsal-fin base; or moderately developed, adpressed distal tip slightly surpassing middle portion of dorsal-fin base; anterior margin with small odontodes. Pectoral fin roughly triangular, its origin just posterior to gill opening. Pectoral-fin rays I,8 (1), I,9* (14); posterior margin of pectoral spine with 17 (2) poorly developed serrations along almost its entire length; small region just posterior to origin of spine lacking serrations; some serrations directed towards origin of spine, perpendicularly directed or directed towards tip of spine; base of branched rays with small laminar expansions on its inner margin, generally more evident on first rays; laminar expansions with irregular margins in some specimens (Figure 5). Anteroventral portion of cleithrum partially exposed: posterolateral portion of scapulocoracoid reduced, externally visible. Pelvic fin oblong, located just below fourth ventrolateral body plate, and at vertical through third or fourth dorsal-fin branched ray. Pelvic-fin rays i,5 (15). Adipose fin roughly triangular, separated from posterior origin of dorsal-fin base by eight or nine dorsolateral body plates. Anal fin somewhat triangular, located just posterior to 12th or 13th ventrolateral body plates, and at vertical through region of preadipose platelets. Anal-fin rays, ii,6 (15). Caudal-fin rays i,12,i (15), generally six dorsal and/or ventral procurrent rays; bilobed; dorsal and ventral lobes generally with similar size.

Two laterosensory canals on trunk; first ossicle tubular and second ossicle laminar. Body plates with conspicuous line of relatively large odontodes confined on posterior margins; dorsolateral body plates 25 (1), 26* (20), 27 (5); ventrolateral body plates 23 (14), 24* (12); dorsolateral body plates along dorsal-fin base 5 (1), 6* (25); dorsolateral body plates between adipose-fin spine and caudal-fin base 7 (1), 8 (19), 9* (6); preadipose platelets 3 (12), 4* (9), 5 (4), 6 (1); small platelets covering base of caudal-fin rays; small platelets disposed dorsally and ventrally between junctions of lateral plates on posterior portion of caudal peduncle. Ventral surface of trunk covered by small irregular or roundish platelets.

Vertebral count 24 (1), 25 (1); ribs 6 (2), first pair conspicuously large; parapophysis of complex vertebra poorly developed.

3.6 | Coloration in alcohol

The overall colour pattern is shown in Figure 1. Ground colour of body light or brownish yellow, with top of head dark brown. Posterodorsal portion of head, region below eye, opercle, branchiostegal membrane and cleithrum with scattered dark-brown or black chromatophores. Snout covered by dark-brown or black chromatophores on its dorsal surface; chromatophores densely disposed in some specimens; generally, forming dark-brown or black rounded, striated or irregular relatively large spots; some specimens with diffuse spots or lacking spots; with darkbrown or black diffuse or conspicuous stripe from anteroventral portion of eye to upper lip lateral area in some specimens; or stripe absent; ventrolateral portion of snout generally with dark-brown or black chromatophores. Upper lip and maxillary barbel with dark-brown or black chromatophores; area of lateral portion of upper lip with conspicuous concentration of dark-brown or black chromatophores in some specimens; outer mental barbel with dark-brown or black chromatophores, generally more evident on its proximal portion; region of isthmus around lower lip generally with dark-brown or black chromatophores.

Dorsal series of three to five dark-brown or black blotches, first on anterior portion of dorsal-fin base, second on posterior portion of dorsal-fin base, third, if present, between dorsal and adipose fins, fourth on adipose-fin base, and fifth, if present, on caudal-fin base; blotches diffuse in some specimens. Dorsal portion of body with conspicuous concentration of dark-brown or black chromatophores between counterparts of dorsolateral body plates in some specimens. Ventral surface of trunk and region posterior to urogenital opening generally with dark-brown or black chromatophores. First dorsolateral body plate with conspicuous concentration of dark-brown or black chromatophores: posterior margin of some dorso- and ventrolateral plates with conspicuous concentration of dark-brown or black chromatophores in some specimens. Midline of flank with longitudinal series of three to four medium- to large-sized conspicuous darkbrown or black blotches; blotches rounded, oblong or irregular. Dorsal half of dorsolateral body plates with dark-brown or black chromatophores; region of anterior and posterior portions of dorsal-fin base, adipose-fin base and base of caudal peduncle with more concentrated chromatophores, generally forming conspicuous blotches fused with flank midline blotches. Ventral half of dorsolateral body plates and dorsal half of ventrolateral body plates with concentration of darkbrown or black chromatophores, forming conspicuous blotches in some specimens; blotches generally more evident on anterior portion of body and on area of flank midline blotches. Mid-ventral portion of ventrolateral body plates on area of flank midline blotches with concentration of dark-brown or black chromatophores, generally forming conspicuous blotches fused to flank midline blotches; generally, blotches more evident posteriorly to pelvic-fin origin; ventral portion of ventrolateral body plates with concentration of dark-brown or black chromatophores, generally more evident posterior to pelvic-fin origin.

Dorsal fin with conspicuous concentration of dark-brown or black chromatophores on some areas of membranes, generally forming large dark-brown or black patches; smaller spots absent; dorsal-fin base with conspicuous concentration of dark-brown or black chromatophores, generally more concentrated on bases of first and last branched rays; spine covered by dark-brown or black chromatophores. Pectoral fin with dark-brown or black chromatophores on its dorsal surface, generally more evident on spine and on its middle portion; spots, when present, generally diffuse; region of body around dorsal portion of pectoral-fin origin generally with concentration of dark-brown or black chromatophores. Pelvic fin with concentration of dark-brown or black chromatophores on its dorsal surface, generally more evident on its middle portion; forming conspicuous dark-brown or black patch in some specimens; region of body around dorsal portion of pelvic-fin origin generally with concentration of dark-brown or black chromatophores. Adipose-fin membrane with dark-brown or black chromatophores; conspicuous concentration of dark-brown or black chromatophores in some areas of membrane, generally more evident close to spine, forming isolated patches in some specimens; adipose-fin spine generally with dark-brown or black chromatophores. Anal fin with conspicuous concentrations of dark-brown or black chromatophores in some areas, generally more evident on its middle portion and bases of last branched rays; forming one or two dark-brown or black blotches in some specimens. Middle portion of caudal-fin base, posteriorly to last flank midline blotch, generally with small- to medium-sized dark-brown or black blotch; blotch diffuse or fused with last midlateral blotch in some specimens. Caudal fin with three to six transversal dark-brown or black slender to thick bars.

3.7 | Coloration in life

Similar to that observed in preserved specimens but generally with ground colour of body greyish yellow. Body covered by whitish yellow and green iridescent coloration (Figure 6).

3.8 | Sexual dimorphism

Except for the presence of lanceolate genital papillae in males, presented by all Corydoradinae (see Britto, 2003; Nijssen & Isbrücker, 1980), no other conspicuous sexually dimorphic feature was observed.



FIGURE 6 Live specimen of *A. azaghal*, new species, from a stream tributary to the Igarapé do Pontal, lower rio Xingu basin, Altamira, Pará, Brazil, showing general colour pattern in lateral view. Photograph: Adriano Gambarini

3.9 | Larva description

In the streams at the top of Serra do Pardo there were only two Callichthyidae: Aspidoras (vast majority) and Callichthys (only a young specimen collected). The larval specimen described below can be promptly assigned to the new Aspidoras species by the presence of relatively short maxillary barbel, not surpassing the pelvic girdle, and its overall colour pattern.

Specimen in final flexion stage, with 7.0 mm TL (Figure 7). Head slightly depressed with convex dorsal profile; somewhat rounded in dorsal view. Snout rounded, but not blunt. Head profile convex from tip of snout to dorsal-fin flap. Profile roughly straight to caudal-fin base. Ventral profile of body nearly straight from mouth to fin-fold origin; slightly convex along fin fold up to caudal-fin base. Body roughly dome-like in cross-section at pectoral girdle, gradually becoming compressed towards caudal fin. Eye rounded, located dorsolaterally on head. One long nasal opening, in roughly shallow groove, touching anterodorsal margin of orbit; anterior tip with minute fleshy flap. Mouth subterminal, width slightly larger than bony orbit diameter. Maxillary barbel reaching to pectoral-fin base. Outer mental barbel slightly smaller than maxillary barbel. Inner mental barbel fleshy and flat, with base close to its counterpart. Rhomboid papillae covering entire surface of all barbels, upper and lower lips, snout and isthmus; papillae more evident than in juvenile or adult specimens. One large cranial opening covered by skin almost all-over cranial roof. extending between dorsal margin of orbits and nostrils until region

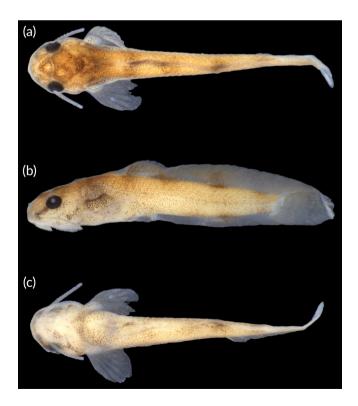


FIGURE 7 Larva of *A. azaghal* in final flexion stage, MNRJ 51792, 7.0 mm TL, showing general morphology and colour pattern in (a) dorsal, (b) lateral and (c) ventral views

just posterior to eyes. Pseudotympanum covered by skin just above pectoral region. Median fin fold present, extending from post-cephalic region to genital opening. Dorsal- and caudal-fin rays distinct, but fins not detached from fin fold. Anal-fin rays not distinct. Dorsal fin with seven rays. Caudal-fin rays 12; asymmetrical, dorsal portion distinctly longer than ventral. Hypural plates visible by transparency. Pectoral fin roughly rounded. Pectoral-fin rays 8. Pelvic fin not formed; no pelvic-fin bud yet. No body plates; 23 myomeres. Ground colour of body light to brownish yellow, with top of head dark brown. Scattered dark-brown or black chromatophores all over head and trunk, mainly on sides of pectoral region. Chromatophores densely disposed on sides of snout, forming roughly dark-brown stripe. Maxillary and outer mental barbels hyaline. Isthmus and ventral area of body with darkbrown or black chromatophores. Fin fold with scattered brown chromatophores, more concentrated on dorsal-fin rays, and on dorsal and ventral areas nearly at middle of fold. Pectoral-fin with brown chromatophores.

3.10 | Genetic diversity

For the mitochondrial COI gene, 609 base pairs of 38 individuals were analysed, of which 19 are from upstream and 19 from downstream of a relatively large waterfall (about 30 m height) at the type-locality (Figure 8a), allowing the identification of three polymorphic sites, three haplotypes and other parameters of genetic diversity (Table 2). The haplotype 1 (H1) was the most frequent in the population, being shared by 31 specimens (16 from upstream and 15 from downstream), H2 was shared by three upstream and one downstream, and H3 was shared exclusively by three downstream specimens (Figure 9a). For the control region, 749 base pairs of 34 specimens (16 from upstream and 18 from downstream) were analysed, allowing the identification of 41 polymorphic sites, 17 haplotypes and other genetic diversity parameters (Table 2). Haplotypes 4, shared by two upstream and three downstream specimens, and 19, shared by three upstream and two downstream specimens, were the most frequent in the population (Figure 9b). In addition to the most frequent haplotypes, H6 was also shared by individuals from both localities (two upstream and two downstream). On the other hand, the haplotypes 12, 16 and 18 were not shared between upstream and downstream specimens, with H12 (two specimens) and H18 (three specimens) exclusive to the downstream region, and H16 (four specimens) exclusive to the upstream region (Figure 9b).

3.11 | Genetic structure within the species

The calculation of the distance P (uncorrected) showed that there is no significant genetic divergence between the individuals sampled in both upstream and downstream locations. For analyses of the control region, the value of P is equal to 0.007 when evaluating intrapopulational genetic divergences for both locations, and when assessed between upstream and downstream, P is also equal to 0.007.

FIGURE 8 Type-locality of A. azaghal, new species, unnamed stream tributary to the Igarapé do Pontal, lower rio Xingu basin, Terra do Meio Region, Parque Nacional da Serra do Pardo, Altamira, Pará, Brazil, showing the approximately 30 m high waterfall (a), a general view of its downstream stretch, with large waterfall in the background (b), and a detail of its margin with accumulation of sand, foraging sites of the new species

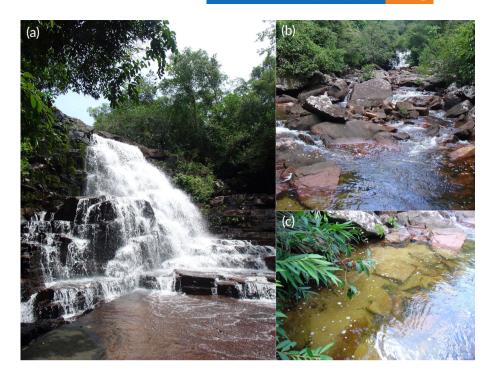


TABLE 2 Genetic diversity for each marker and sampled locality in relation to a large waterfall (~30 m high)

	N		н		h		S		π	
	CR	COI	CR	COI	CR	COI	CR	COI	CR	COI
Upstream waterfall	16	19	9	2	0.90	0.28	20	1	0.0084	0.0004
Downstream waterfall	18	19	11	3	0.94	0.36	21	2	0.0082	0.0006

Note. CR, control region; h, haplotype diversity; H, haplotypes; N, individuals; S, polymorphic sites; π , nucleotide diversity.

For COI analyses, the P value is lower (0.001 downstream and upstream), showing that there is no genetic divergence within the groups, and, when evaluated between groups, the P value is also equal to 0.001, showing no genetic divergence between them.

3.12 | Gene flow

For the COI marker FST = 0.05249, P > 0.05, and for the control region FST = -0.01466, P > 0.05, which demonstrates the existence of gene flow between the upstream and downstream regions of the waterfall. Additionally, our results show a unidirectional gene flow pattern, similar to the pattern observed by Reis *et al.* (2015), in which genetic diversity increases in the downstream direction (Table 2).

3.13 | Genetic difference among congeners

With the aid of the BOLD database, the new species was compared with nine congeners, revealing a significant genetic difference

(Table 3). The highest genetic similarity was detected between A. *albater* and A. *raimundi*, which shared 93.47% of this gene region (COI), nevertheless, these species still have 6.53% of genetic differentiation, which is a significant value to distinguish A. *azaghal* from A. *albater* and A. *raimundi*.

3.14 | Geographic distribution

A. azaghal is currently known only from two tributaries to the Igarapé do Pontal basin, itself a tributary to the rio Xingu basin in Pará, Brazil (Figure 10).

3.15 | Natural history notes

A general view of the type-locality of A. azaghal is shown in Figure 8b. The streams where A. azaghal was found were narrow (less than 2.0 m width) to moderately large (up to 20 m width), with low riffles alternating with smoothly running stretches. The substrate was

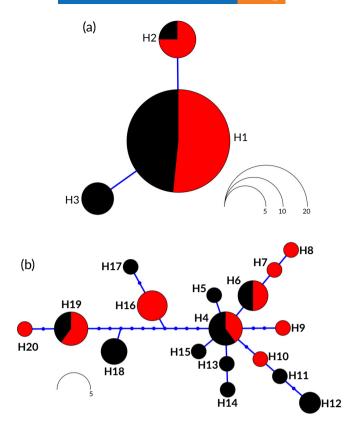


FIGURE 9 Haplotype network of *A. azaghal* n. sp. for the COI marker (a) and control region (b). The size of the circles is proportional to the number of individuals who have a particular haplotype and the colours refer to the location. Blue dots represent the number of mutations. Upstream, downstream

composed by white silica sand stretches mixed with pebbles, rock slabs and ledges, as well as large rocks of up to 2.0 m diameter; some coarse litter patches and submerged macrophyte banks were found close to the margins and in backwaters. The banks were covered by thick riparian vegetation composed by trees, palms (mostly the buriti *Mauritia flexuosa*, Arecaceae) and herbaceous plants dominated by *Spatiphyllum* sp. (Araceae) in some stretches. The water was clear to yellowish and relatively cold (24–25°C). The new species was found in abundance in the main streams of the upper area of Serra do Pardo ridge, occupying moderate- to fast-flowing and shallow (less than 1 m deep) stretches. Groups of up to a dozen individuals were observed actively foraging during the day by sifting food particles amidst the upper layer of the sand substrate in shallow areas close to the margins (Figure 8c).

Few other fishes were found sharing the streams with A. azaghal, namely Callichthys callichthys and one species each of Laimosemion, Synbranchus, Astyanax and Characidium, all still unidentified and possibly new to science. The presence of a large waterfall of nearly 30 m height in the type-locality (Figure 8a) may constitute a barrier to the fish fauna inhabiting the lower portions of the Igarapé do Pontal basin, to which the streams of the upper portions of the Serra do Pardo are connected, which may explain the apparently depauperate local fish fauna.

TABLE 3 Genetic similarity and difference between A. azaghal and its congeners

Species name	Genetic similarity (%)	Genetic difference (%)
A. albater	93,47	6,53
A. raimundi	93,47	6,53
A. psammatides	93,3	6,7
A. psammatides	93,13	6,87
A. fuscogutatus	92,96	7,04
A. fuscogutatus	92,63	7,37
A. belenos	92,46	7,54
A. belenos	92,29	7,71
A. poecilus	92,29	7,71
A. poecilus	92,13	7,87
A. taurus	92,13	7,87
A. taurus	91,96	8,04
A. belenos	91,79	8,21
A. eurycephalus	91,67	8,33
A. poecilus	91,47	8,53
A. poecilus	90,79	9,21
A. pauciradiatus	88,61	11,39
A. pauciradiatus	88,44	11,56

Note. Values for congeners were obtained from the Barcoding of Life Database (BOLD).

3.16 | Etymology

The epithet 'azaghal' refers to a fictional character within the fantastic universe created by the South African/British writer, poet, philologist and academic, John Ronald Reuel Tolkien (1892–1973). Azaghâl was the king of the Broadbeam Dwarves, one of the seven dwarf clans, and Lord of the dwarven realm of Belegost in the Blue Mountains during Middle Earth's First Age. The name comes from a double allusion, first about the region where the species was found, Terra do Meio, freely translated as 'Middle Earth' in English, name of the fictional world of Tolkien's legendarium, and second by the fact that the new species occurs in a mountainous region and presents a relatively small size, which are both typical features of the fictional dwarves. A noun in the nominative case used in apposition.

4 | DISCUSSION

A. *azaghal* is the second species within Corydoradinae recognized by lacking dorsal-fin spinelet (Figure 3), a condition reported heretofore only for A. *velites* (see Tencatt & Bichuette, 2017: 7, fig. 4b). Although these two species share this peculiar feature, the morphologies of their dorsal-fin spines conspicuously differ. It is widely known that most Siluriformes primitively presents two dorsal-fin spines, the first element (spinelet) is conspicuously reduced and often forms a complex friction-locking mechanism with the second spine, which is

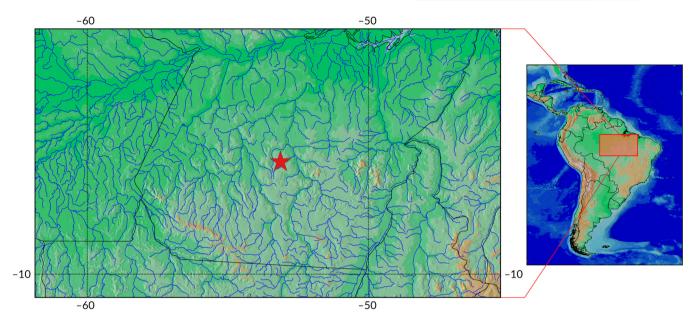


FIGURE 10 Map showing the geographic distribution of *A. azaghal* n. sp., with the red star representing its type-locality and an additional record, both unnamed streams tributaries to the Igarapé do Pontal, lower rio Xingu basin, Pará, Brazil

generally well developed and the only one to be referred as a spine in the literature (see Halstead *et al.*, 1953; Alexander, 1965; Howes, 1985; Nelson *et al.*, 2016). Additionally, in *A. velites* and remaining congeners, it is possible to observe a small rounded perforation at dorsal-fin spine base, the median foramen (Figure 2b), as illustrated for other catfishes in previous studies (*e.g.*, Halstead *et al.*, 1953: 303, fig. 1g; Alexander, 1965: 113, fig. 9). Interestingly, *A. azaghal* lacks this foramen (Figure 2a), which represents the first report of such condition for Siluriformes.

Detailed anatomical descriptions of the locking mechanisms of both dorsal- and pectoral-fin spines in catfishes were provided by Halstead et al. (1953) and Alexander (1965), and Royero (1987) just for dorsal fin, which basically works through a combination of friction between roughened bony surfaces, including one on posterior surface of the spinelet, with contractions of erector and/or depressor muscles associated with the spinelet, which is attached to dorsal-fin spine (= second dorsal-fin element) by a strong ligament. Despite the fact that it was not possible to undoubtedly verify the functionality of the dorsal-fin locking mechanism in both A. azaghal and A. velites, all attempts to manually lock their dorsal-fin spine, which were done in the same way that generally works in Corydoras Lacépède, 1803 species (L. Tencatt, personal observation), were unsuccessful. Considering the absence of a spinelet, one of the main components of the locking mechanism, in both species, it seems reasonable to infer that such mechanism is not functional in these congeners. Generally, the Siluriformes presents three condyles at the basis of the dorsal-fin spine, one on the middle and two laterally disposed (Halstead et al., 1953: 303, fig. 1G; Alexander, 1965: 113, fig. 9). Most species of Aspidoras present conspicuous grooves on these condyles (Figure 2b), contrary to the conditions observed in A. azaghal and A. velites. Although it is possible to observe the presence of the three typical condyles in A. azaghal, their grooves are clearly less developed

(Figure 2a). In the case of A. *velites*, the condyles are strongly reduced, mainly the median one, which generally seems to be completely absent, and no evident grooves were observed.

Currently, there are at least three other congeners known from the rio Xingu basin, A. marianae, A. microgalaeus and A. poecilus, and possibly A. brunneus, which presents an unclear type-locality regarding its river drainage. In addition to the absence of the dorsal-fin spinelet, other features can also be useful to distinguish the new species from its sympatric congeners. In the case of A. brunneus, its typical colour pattern promptly differs it from the new species (see Diagnosis). Besides frontal bone morphology (see Diagnosis), A. azaghal can be distinguished from A. marianae, A. microgalaeus and A. poecilus by having a nuchal plate entirely covered by a thick layer of skin (vs. only small areas covered by thick layer of skin). Considering the general morphological pattern, the most similar congeners to A. azaghal are A. albater and A. gabrieli. When compared to A. albater, the new species can be recognized by the presence of anterior portion of frontal bone with relatively large areas covered by a thick layer of skin (vs. small areas). Besides the differences in the infraorbital morphology (see Diagnosis), the new species can be distinguished from A. gabrieli by having dorsolateral body plates on the predorsal region closer from each of their counterparts (vs. more distant) and strongly forked caudal fin (vs. moderately forked).

Considering that the two closest congeneric species, $A.\ albater$ and $A.\ raimundi$, presented a genetic difference of 6.53%, the molecular evidence raised herein clearly reinforces $A.\ azaghal$ as a new species. Moreover, population analyses of $A.\ azaghal$ show that the 30 m waterfall at its type-locality acts as a barrier for the downstream specimens, allowing the isolation of the upstream specimens. However, this isolation is not a reason for genetic differentiation of the groups, since the intrapopulational distance for both groups (P = 0.007) is equal when compared between groups (P = 0.007). Genetic data also revealed that gene flow occurs unidirectionally (upstream-

downstream), explaining the isolation of A. *azaghal* specimens located upstream. Additionally, H1, H4 and H19 were the most common and shared haplotypes between upstream and downstream individuals (Figure 9), confirming that the analysed specimens belong to the same population.

The new Aspidoras seems to be endemic from the headwater of the rio Pardo system, at the hills of the Serra do Pardo, further increasing the already known endemism in the rio Xingu basin (see Dagosta & De Pinna, 2019). The Serra do Pardo National Park is a protected area of about 445,000 ha formally under a regime of strict protection and is managed by an autarchy of the Brazilian Ministry of the Environment, the ICMBio. The park occupies the south-eastern region of the municipality of Altamira but the nearest city is actually São Félix do Xingu, Southern Pará state. The Brazilian National Biodiversity Programme (Pronabio) classifies it as a top priority area for biodiversity conservation in the State of Pará (MMA, 2007). Refined knowledge of the diversity and distribution/endemism of fish fauna is a priority in this region, since it contains a rich and fragile sociobiodiversity, with several indigenous territories and protected federal and state natural reserves that are under pressure from various anthropic impacts related mainly to hydroelectric dams, mining, deforestation, expansion of monocultures and paving of roads.

The ichthyofauna from the Terra do Meio region seems to be adapted to moments of strong environmental stress generated by seasonal drought, which is a positive point in terms of resilience to negative anthropogenic impacts (i.e., the ichthyofauna would be adapted to periodic disturbances due to extreme droughts, and there would be a high probability of natural recomposition of the ichthyofauna after spatial and temporal localized environmental impacts). However, this ability to recompose fish assemblages depends on the maintenance of water quality and riparian forest integrity. Without these conditions, recruitment failures may occur, greatly increasing local extinctions risk.

The possible occurrence of endemic species in these hilly streams makes the possibility of localized extinctions quite worrying. In this sense, it is imperative to address the integrity of the landscape in the area of the mountain range, especially the water quality of the streams, as a way to guarantee the survival and perpetuation of the local ichthyofauna. The activities of high environmental impact, such as increasing the regional deforestation and mining of different types of ores, represents a threat for the biodiversity. The proximity of the National Park to the city of São Félix do Xingu, as well as the presence of a consolidated road south of the park, indicate the need to protect the boundaries of the conservation unit. The implementation of a buffer zone for this Conservation Unity becomes urgent, since invasions can generate serious and very rapid environmental disturbances. The physiognomy of the high mountain vegetation of the predominantly rupestrian type presents low resilience to environmental disturbances and its loss can completely compromise the local ecosystem.

In spite of the excellent state of environmental integrity of the aquatic environments studied during the expedition in the Serra do Pardo, it is extremely important to implement activities to prevent disturbances through effective supervision of the area. The Serra do Pardo region is one of the most important areas for the recharge of

aquifers and maintenance of water resources in the National Park region. The streams that drain the mountain range constitute the source of the Igarapé do Pontal watershed, one of the main local water bodies, so any interference in these stretches may reflect negatively on the rest of the basin. From the point of view of the ichthyofauna, they are extremely fragile areas, since a disturbance, even if temporary and of moderate intensity, can lead to local extinctions motivated by factors such as small population size, low local gene diversity (reduced heterozygosity), difficulty or even impossibility of recolonization and geographic isolation.

ADDITIONAL SPECIMENS EXAMINED

All from Brazil. Aspidoras albater: LBP 15302, 2, 28.9-33.9 mm L_S, Goiás State, rio da Lapa; MZUSP 12991, holotype, 34.2 mm L_S, Goiás State, rio Tocantinzinha near São João da Aliança; MZUSP 12992, 4, paratypes, 25.2-31.0 mm L_S, same data as holotype; MZUSP 114401, 20 of 21, 20.3-35.9 mm L_S, 1 c&s of 21, 30.1 mm L_S, Goiás State, stream tributary to the rio Parana. Aspidoras belenos: MNRJ 12433, holotype, 27.8 mm L_S, Mato Grosso State, stream at the road Primavera do Leste - Paranatinga, 82 km N from Primavera do Leste; MZUSP 51208, 3, paratypes, 15.0-26.6 mm L_S, same data as holotype. Aspidoras brunneus: USNM 213569, 1, paratype, 17.9 mm L_S, Mato Grosso State, Serra do Roncador, kilometre 125 of the road Chavantina - Casximba. Aspidoras carvalhoi: MNRJ 5230, holotype, 25.4 mm L_S, Ceará State, Canabrava Reservoir. Aspidoras depinnai: MZUSP 56214, holotype, 32.5 mm L_S, Pernambuco State, stream at the road Amaraji - Primavera. UFPB 6194, 6 of 7, 21.8-35.5 mm L_s, 1 c&s of 7, 28.0 mm L_S, Pernambuco State, do Meio Creek. Aspidoras eurycephalus: MNRJ 13080, 8 of 9, 10.8-34.5 mm L_S, 1 c&s of 9, 32.2 mm L_s, Goiás State, São Bento Creek. Aspidoras fuscoguttatus: MZUSP 8573, holotype, 29.5 mm L_S, Mato Grosso do Sul, Corguinho Creek, road of Três Lagoas. NUP 11397, 2 c&s, 30.6-30.7 mm Ls, Goiás, rio dos Bois. Aspidoras gabrieli: MPEG 17394, 5 of 139, paratypes, 16.2-26.3 mm L_s, Pará, unnamed tributary to the left bank of the rio Parauapebas; MZUSP 87674, 12, 12.7-32.9 mm L_S, 1 c&s, 31.4 mm L_s, Pará State, Igarapé Jacaré. Aspidoras kiriri: MNRJ 47400, holotype, 30.6 mm L_S, Bahia State, Cai-Camarão Stream; NUP 18245, 3, paratypes, 12.9-30.0 mm L_{S_1} same data as holotype; NUP 18246, 1 c&s, paratype, 30.4 mm L_s, same data as holotype. Aspidoras lakoi: MNRJ 5292, holotype, 33.4 mm Ls, Minas Gerais, stream at the Floresta do Grotão, Fazenda da Cachoeira; MNRJ 5293, 18 of 21, 13.7-32.8 mm L_S, 3 c&s of 21, disarticulated, indeterminate size, same data as holotype. Aspidoras maculosus: MZUSP 88170, 9, 15.4-30.8 mm L_S, rio Paiaiá. UFBA 3291, 2 of 5, 24.7-26.9 mm L_S, 1 c&s of 5, 30.7 mm L_S, rio Paiaiá. Aspidoras marianae: CPUFMT 2060, 5 of 6, 12.1-27.7 mm L_S , 1 c&s of 6, 27.6 mm L_S , Pará, unnamed creek. Aspidoras menezesi: MCP 47303, 9 of 16, 23.2-28.8 mm L_S, 1 c&s of 16, 28.4 mm L_s, Ceará, rio Batateiras. Aspidoras mephisto: MNRJ 48268, holotype, 45.6 mm L_S, Goiás State, Anésio III Cave; NUP 18760, 2 c&s, paratypes, 29.5-31.7 mm L_s, Russão II Cave. Aspidoras microgalaeus: LBP 15895, 31 of 32, 15.5-30.2 mm L_S, 1 c&s

of 32, 27.1 mm L_s, Mato Grosso State, unnamed stream tributary to the rio Tanguro; MZUSP 51209, holotype, 25.7 mm L_S, Mato Grosso State, small tributary of the rio Culuene, km 86 of the road Paranatinga - Canarana; MZUSP 51210, 4, paratypes, 19.2-23.6 mm L_S, same data as holotype. Aspidoras pauciradiatus: INPA 34595, 5 of 22, 18.9-21.9 mm L_S, Roraima State, uncertain locality, close to Dona community. Aspidoras poecilus: UNT 6249, 29 of 46, 20.0-32.8 mm L_S, 1 c&s of 46, 29.3 mm L_S, Tocantins State, Manduca Stream. Aspidoras psammatides: MNRJ 28407, holotype, 25.7 mm L_S, Bahia State, rio Caldeirão; UNT 9604, 18 of 40, 16.4-24.2 mm L_S, 2 c&s of 40, 22.9-26.2 mm L_S, Bahia State, rio Roncador. Aspidoras raimundi: UFPB 9418, 43 of 51, 16.0-24.2 mm L_S , 2 c&s of 51, 21.0–23.0 mm L_S , Piauí State, stream under the bridge on the road between São Miguel da Baixa Grande-São Félix do Piauí. Aspidoras rochai: MZUSP 2195, lectotype, 38.7 mm L_S, Ceará State, Fortaleza; MZUSP 2195, 1, paralectotype, 35.5 mm L_S, Ceará, Fortaleza. Aspidoras spilotus: UFPB 9247, 3 of 7, 35.3-40.3 mm L_s, 2 c&s of 7, 27.4-30.5 mm L_S, Ceará State, rio das Minas. Aspidoras taurus: MZUSP 57154, holotype, 52.1 mm L_S, Mato Grosso State, rio Itiquira; LBP 1427, 29 of 31, 17.3-40.3 mm L_S , 2 c&s of 31, 25.3-36.1 mm L_S , Mato Grosso State, rio Itiquira. Aspidoras velites: MZUSP 74447, holotype, 23.6 mm L_s, Mato Grosso State, córrego Boiadeiro; LIRP 4435, 14 of 16, 20.7-26.6 mm L_S, 2 c&s of 16, 23.0-23.7 mm L_S, Mato Grosso State, córrego do Sapo; LIRP 4479, 11 of 12, 18.6-29.0 mm L_S, 1 c&s of 12, 22.0 mm L_S, Goiás State, rio Araguaia. Aspidoras virgulatus: MBML 6750, 6 of 9, 23.5-30.2 mm L_s, 2 c&s, 32.2-33.0 mm L_S, Bahia State, córrego Manoelzinho.

ACKNOWLEDGEMENTS

The third scientific expedition to the Terra do Meio region was organized by the ICMBio and WWF-Brazil, with support of the Brazilian Army through the use of a Cougar helicopter, which allowed fieldwork at the top of the Serra do Pardo. The Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) of the Universidade Estadual de Maringá, The Museu Nacional, Universidade Federal do Rio de Janeiro, the Universidade Federal de Mato Grosso do Sul, Universidade Estadual de Mato Grosso do Sul, INPA, and UFPA provided logistical support. The authors are grateful to Mário de Pinna, Aléssio Datovo and Osvaldo Oyakawa (MZUSP), Cláudio Oliveira (LBP), Flávio Bockmann (LIRP) and Alexandre Ribeiro (CPUFMT) for hosting museum visits and loan of material. We also thank Angela Zanata (UFBA), Francisco Langeani (DZSJRP), Paulo Lucinda (UNT), Carlos Lucena and Margarete Lucena (MCP), Ronaldo Pinheiro and Luisa Soares (MBLM), Telton Ramos (UFPB), Lucia Rapp (INPA) and Wolmar Wosiacki (MPEG) for the loan of several specimens analysed in this paper. We thank Héctor Vera-Alcaraz (MCP), Cláudio Oliveira, Ricardo Britzke, Fábio Roxo, Bruno Melo and Gabriel Silva (LBP), Willian Ohara, Vinícius Espíndola and Túlio Teixeira (MZUSP), Andressa Oliveira, Francisco Severo-Neto and Thomaz Sinani (ZUFMS), Fábio Mise, Murilo Pastana, Thiago Pereira and Pedro Rizzato (LIRP), and Kris Murphy, Sandra Raredon and Jeff Clayton (USNM) for generously welcoming LFCT during museum visits; Robert 'Rob' McLure for the pleasant late-night talks on Corydoradinae and for kindly reviewing the English language of this paper; Alêny Francisco and Alan Eriksson for assistance with the image capture laboratory of the Laboratório de Ecologia da Universidade Federal de Mato Grosso do Sul; Victor de Brito for the photographs of the holotype (Figure 14), and Adriano Gambarini for the photograph of the live specimen (Figure 6); Roberto Antonelli Filho and the ICMBio team for planning special collection location in the Terra do Meio region; Gustavo Gutembergue for field work assistance. We thank Grazielle Gomes for support, and Raimundo Silva and Luciana Watanabe for assistance with genetic data and analyses (IECOS/UFPA). We also thank the staff of Setor de Herpetologia at MNRJ for use of the auto-stacking multifocus stereomicroscope facilities. The authors are supported by personal grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (LFCT: processes #141061/2014-6 and #304997/ 2016-1; MRB: process #309285/2018-6; JZ: process #313183/ 2014-7). JMC was supported by a research project for the Fundação Amazônia de Amparo a Estudos e Pesquisas (process #184/2009). MRB was supported by grants from Edital Programa Institucional de Pesquisa nos Acervos da USP and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grant #200.103/2019). This study is a partial result of the PhD project of LFCT, and an undergraduate project from the Programa de Bolsa de Iniciação Científica (PIBIC/UFPA) of MFCF.

ORCID

Luiz F. C. Tencatt https://orcid.org/0000-0002-8437-4354

REFERENCES

Alexander, R. McN. (1965). Structure and function in the catfish. *Journal of Zoology*, 148, 88–152. http://dx.doi.org/10.1111/j.1469-7998.1966. tb02943.x.

Alexandrou, M. A., Oliveira, C., Maillard, M., McGill, R. A. R., Newton, J., Creer, S., & Taylor, M. I. (2011). Competition and phylogeny determine community structure in Mullerian co-mimics. *Nature*, 469, 84–89. https://doi.org/10.1038/nature09660.

Aquino, A. E., & Schaefer, S. A. (2002). The temporal region of the cranium of loricarioid catfishes (Teleostei: Siluriformes): Morphological diversity and phylogenetic significance. *Zoologischer Anzeiger*, 241, 223–244. https://doi.org/10.1078/0044-5231-00071.

Arratia, G., & Gayet, M. (1995). Sensory canals and related bones of Tertiary siluriform crania from Bolivia and North America and comparison with recent forms. *Journal of Vertebrate Paleontology*, 15, 482–505.

Britto, M. R. (1998). Two new species of the genus Aspidoras (Siluriformes: Callichthyidae) from Central Brazil. *Ichthyological Exploration of Freshwaters*, 8, 359–368.

Britto, M. R. (2000). *Aspidoras depinnai* (Siluriformes: Callichthyidae): A new species from Northeastern Brazil. *Copeia*, 2000, 1048–1055.

Britto, M. R. (2003). Phylogeny of the subfamily Corydoradinae Hoedeman, 1952 (Siluriformes: Callichthyidae), with a definition of its genera. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 153, 119–154. https://doi.org/10.1635/0097-3157(2003)153(0119: POTSCH)2.0.CO;2.

Britto, M. R., & Lima, F. C. T. (2003). Corydoras tukano, a new species of corydoradine catfish from the rio Tiquié, upper rio Negro basin, Brazil (Ostariophysi: Siluriformes: Callichthyidae). Neotropical Ichthyology, 1, 83–91. https://doi.org/10.1590/S1679-62252003000200002.

Britto, M. R., Lima, F. C. T., & Moreira, C. R. (2002). Aspidoras velites, a new catfish from the upper rio Araguaia basin, Brazil (Teleostei:

- Siluriformes: Callichthyidae). Proceedings of the Biological Society of Washington, 115, 727–736.
- Britto, M. R., Lima, F. C. T., & Santos, A. C. A. (2005). A new *Aspidoras* (Siluriformes: Callichthyldae) from rio Paraguaçu basin, Chapada Diamantina, Bahia, Brazil. *Neotropical Ichthyology*, *3*, 473–479.
- Dagosta, F. C., & De Pinna, M. (2019). The fishes of the Amazon: Distribution and biogeographical patterns, with a comprehensive list of species. Bulletin of the American Museum of Natural History, 431, 1-163.
- Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10, 564–567.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5 c, Seattle, WA: Joseph Felsenstein.
- Gosline, W. A. (1940). A revision of the Neotropical catfishes of the family Callichthyidae. *Stanford Ichthyological Bulletin*, 2, 1–36.
- Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.
- Halstead, B. W., Kuninobu, L. S., & Hebard, H. G. (1953). Catfish stings and the venom apparatus of the Mexican catfish, Galeichthys felis (Linnaeus). Transactions of the American Microscopical Society, 72, 297–314.
- Howes, G. J. (1985). The phylogenetic relationships of the electric catfish family Malapteruridae (Teleostei: Siluroidei). *Journal of Natural History*, 19, 37–67. http://dx.doi.org/10.1080/00222938500770031.
- Huysentruyt, F., & Adriaens, D. (2005). Descriptive osteology of *Corydoras aeneus* (Siluriformes: Callichthyidae). *Cybium*, 29, 261–273.
- Lee, W. J., Conroy, J., Howell, W. H., & Kocher, T. D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, 41, 54–66.
- Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.
- Lima, F. C. T., & Britto, M. R. (2001). New catfish of the genus *Aspidoras* (Siluriformes: Callichthyidae) from the upper Rio Paraguai system in Brazil. *Copeia*, 2001, 1010–1016.
- Leão, M. D. V., Britto, M. R., & Wosiacki, W. B. (2015). A new species of Aspidoras Ihering (Siluriformes: Callichthyidae: Corydoradinae) from the Rio Xingu Basin, Pará, Brazil. Zootaxa, 3986, 577–587. http://dx. doi.org/10.11646/zootaxa.3986.5.5.
- Lundberg, J. G. (1970). The evolutionary history of North American catfishes, family Ictaluridae. (PhD thesis). University of Michigan, MI.
- Ministério do Meio Ambiente (2007). Áreas Prioritárias para Conservação, Uso Sustentável e Repartição de Benefícios da Biodiversidade Brasileira: Atualização - Portaria MMA n°9, de 23 de janeiro de 2007. Brasília, DF: Ministério do Meio Ambiente, Secretaria de Biodiversidade e Florestas.
- Miranda Ribeiro, P. (1949). Duas novas espécies de peixes na coleção ictiológica do Museu Nacional (Pisces, Callichthyidae et Pygidiidae). Revista Brasileira de Biologia, 9, 143–145.
- Nakatani, K., Agostinho, A. A., Baumgartner, G., Bialetzki, A., Sanches, P. V., Makrakis, M. C., & Pavanelli, C. S. (2001). Ovos e larvas de peixes de água doce: desenvolvimento e manual de identificação, Maringá, PR: Eletrobrás/UEM.
- Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016). Fishes of the World, New Jersey: John Wiley & Sons.
- Nijssen, H., & Isbrücker, I. J. H. (1976). The South American plated catfish genus Aspidoras R. von Ihering, 1907, with descriptions of nine new species from Brazil (Pisces, Siluriformes, Callichthyidae). Bijdragen tot de Dierkund, 46, 107–131.
- Nijssen, H., & Isbrücker, I. J. H. (1980). Aspidoras virgulatus n. sp., a plated catfish from Espírito Santo, Brazil (Pisces, Siluriformes, Callichthyidae). Bulletin Zoölogisch Museum, Universiteit van Amsterdam, 7, 133-139.

- Oliveira, L., Zanata, A. M., Tencatt, L. F. C., & Britto, M. R. (2017). A new species of *Aspidoras* (Siluriformes: Callichthyidae) from a small coastal drainage in northeastern Brazil. *Neotropical Ichthyology*, 15, e160118.
- Reis, K. V., Venere, P. C., Sampaio, I., Rêgo, P. S., Vallinoto, M., & Souza, I. L. (2015). Downstairs gene flow: The effects of a linear sequence of waterfalls on the only population of the endangered minnow Astyanax xavante. Journal of Fish Biology, 87, 754–762.
- Reis, R. E. (1997). Revision of the Neotropical catfish genus Hoplosternum (Ostariophysi: Siluriformes: Callichthyidae), with the description of two new genera and three new species. Ichthyological Exploration of Freshwaters, 7, 299–326.
- Reis, R. E. (1998). Anatomy and phylogenetic analysis of the neotropical callichthyid catfishes (Ostariophysi, Siluriformes). Zoological Journal of the Linnean Society, 124, 105–168.
- Reis, R. E. (2003). Family Callichthyidae (Armored catfishes). In R. E. Reis, S. O. Kullander, & C. J. Ferraris, Jr. (Eds.), Check list of the freshwater fishes of South and Central America (pp. 291–309). Porto Alegre: Edipucrs.
- Royero, R. (1987). Morfologia de la aleta dorsal en los bagres (Teleostei: Siluriformes), con especial referencia a las familias americanas. Unpublished monograph, Universidad Central de Venezuela, Caracas.
- Sabaj, M. H. (2016). Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An Online Reference. Version 6.5 (16 August 2016). American Society of Ichthyologists and Herpetologists, Washington, DC (Internet). Retrieved from http://www.asih.org/sites/default/files/documents/symbolic_codes_for_collections_v6.5.pdf.
- Salzburger, W., Ewing, G. B., & Von Haeseler, A. (2011). The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, 20, 1952–1963.
- Schaefer, S. A. (1988). Homology and evolution of the opercular series in the loricarioid catfishes (Pisces: Siluroidei). *Journal of Zoology*, 214, 81–93. https://doi.org/10.1111/j.1469-7998.1988.tb04988.x.
- Schaefer, S. A., & Aquino, A. (2000). Postotic laterosensory canal and pterotic branch homology in catfishes. *Journal of Morphology*, 246, 212–227. https://doi.org/10.1002/1097-4687(200012)246:3<212:: AID-JMOR5>3.0.CO;2-S.
- Shimabukuro-Dias, C. K., Oliveira, C., Reis, R. E., & Foresti, F. (2004). Molecular phylogeny of the armored catfish family Callichthyidae (Ostariophysi, Siluriformes). *Molecular Phylogenetics and Evolution*, 32, 152–163.
- Steindachner, F. (1907). Über eine neue *Coridoras*-Art aus dem Rio Preto, einem sekundären Nebenflusse des Rio San Francisco, und eine *Xenocara*-Art aus dem Parnahyba bei Victoria und Sa. Filomena. *Anzeiger der Akademie der Wissenschaften in Wien*, 44, 290–293.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30, 2725–2729.
- Taylor, W. R., & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, *9*, 107–119.
- Tencatt, L. F. C., & Bichuette, M. E. (2017). Aspidoras mephisto, new species: The first troglobitic Callichthyidae (Teleostei: Siluriformes) from South America. PLoS One, 12, 1–24.
- Tencatt, L. F. C., Vera-Alcaraz, H. S., Britto, M. R., & Pavanelli, C. S. (2013). A new *Corydoras* Lacépède, 1803 (Siluriformes: Callichthyidae) from the rio São Francisco basin, Brazil. *Neotropical Ichthyology*, 11, 257–264. https://doi.org/10.1590/S1679-62252013000200003.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
- Vera-Alcaraz H. S. (2013). Relações filogenéticas das espécies da família Callichthyidae (Ostariophysi, Siluriformes). (PhD thesis). Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil. Retrieved from http://repositorio.pucrs.br/dspace/bitstream/10923/5538/1/000451889-Texto%2bCompleto-0.pdf.

- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005).DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1847–1857.
- Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.
- Weitzman, S. H., & Nijssen, H. (1970). Four new species and one new subspecies of the catfish genus *Corydoras* from Ecuador, Colombia and Brazil (Pisces, Siluriformes, Callichthyidae). *Beaufortia*, 18, 119–132.
- Wosiacki, W. B., Pereira, T. G., & Reis, R. E. (2014). Description of a new species of *Aspidoras* (Siluriformes, Callichthyidae) from the Serra dos Carajás, Lower Tocantins River basin, Brazil. *Copeia*, 2014, 309–316.

How to cite this article: Tencatt LFC, Muriel-Cunha J, Zuanon J, Ferreira MFC, Britto MR. A journey through the Amazon Middle Earth reveals *Aspidoras azaghal* (Siluriformes: Callichthyidae), a new species of armoured catfish from the rio Xingu basin, Brazil. *J Fish Biol*. 2020;1–15. https://doi.org/10.1111/jfb.14467