



Molecular systematics of the neotropical shovelnose catfish genus *Pseudoplatystoma* Bleeker 1862 based on nuclear and mtDNA markers

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ABSTRACT

Pseudoplatystoma is a commercially important genus of Neotropical migratory catfishes widely distributed in all major river basins of South America. Historically, only three species were recognized, but a recent revision proposed eight putative morphospecies for the genus. A molecular study based on mitochondria DNA (mtDNA) provided support for recognition of only some of the species and raised questions about species boundaries in this group. We present a more encompassing analysis based on mtDNA (cytochrome b, 818 bp) and nuclear DNA-based phylogenies (Rag1 intron 1, 664 bp and S7 intron 1, 635 bp) for a more extensive sampling (279 individuals from 42 localities) of all putative species in all major river basins. Patterns generated by individual gene genealogies and a multispecies coalescent analysis provided evidence to suggest recognition of only four distinct species in this genus: *Pseudoplatystoma magdaleniatum*, *Pseudoplatystoma corruscans*, *Pseudoplatystoma tigrinum* (*sensu lato*) and *Pseudoplatystoma fasciatum* (*sensu lato*). The species phylogeny places *P. magdaleniatum* as the sister group to all the other species in the genus, but the relationships among *P. fasciatum* s.l., *P. tigrinum* s.l., and *P. corruscans* could not be resolved with confidence.

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1. Introduction

The Neotropics harbour the most diverse ichthyofauna of the world, representing 46% of all freshwater fish species and 10% of extant vertebrates (Vari and Malabarba, 1998; Lundberg et al., 2000; Reis et al., 2003). Among the freshwater fishes, catfishes form one of the most diverse groups, including the endemic family Pimelodidae, composed by more than 90 species and 30 genera (Lundberg and Littmann, 2003). *Pseudoplatystoma* Bleeker 1862 (Bleeker, 1862) is an economically important pimelodid genus from South America, with species reaching large size. Their piscivorous and migratory habits make them key top-down regulators of the trophic structure of all major river drainages (Burgess, 1989; Reid, 1983; Barthem and Goulding, 1997), but current anthropogenic activities in most of South America, such as damming and

overfishing, are reducing the effective size of their populations and raising conservationist concerns.

A recent revisionary study of *Pseudoplatystoma* (Buitrago-Suárez and Burr, 2007) proposed that eight species should be recognized in this genus. Before this publication, most taxonomists recognized only three, that could be distinguished mainly by coloration pattern (Lundberg and Littmann, 2003; Burgess, 1989): (i) *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829), the spotted surubim or “pintado”, distributed in the São Francisco and Paraná–Paraguay–Uruguay basins; (ii) *Pseudoplatystoma fasciatum* (Linnaeus, 1766), the striped catfish (“bagre rayado” or “surubim/cachara/pintadillo”), is widely distributed in the Amazonas, Magdalena, Orinoco, Paraná–Paraguay, and drainages from Guyana and Suriname and Northeastern Brazil; and (iii) *Pseudoplatystoma tigrinum* (Valenciennes, 1840), the tiger surubim or “caparari”, distributed in both Amazonas and Orinoco basins. External morphological differences among these species involve minor variation in body shape so the species are usually recognized by coloration (dark vertical bars, loops, or spots), as described. In their recent study, Buitrago-Suárez and Burr (2007), recognize eight species based on these characters plus evidence from skeletal anatomy, vertebral numbers, and geographic distribution (namely, river basin). On this basis, the widely distributed striped surubim

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previously assigned to *P. fasciatum* has been split into five species: *P. fasciatum* (*sensu stricto*): restricted to Guyana and Suriname river basins; *Pseudoplatystoma punctifer* (Casteunau, 1855): present in the Amazonas basin and, presumably, in Northeastern Brazil drainages; *Pseudoplatystoma reticulatum* (Eigenmann and Eigenmann, 1889): distributed widely in the Paraná–Paraguay system and presumably also restricted to an area of the central Amazonas river (near Manaus); *Pseudoplatystoma orinocoense*: restricted to the Orinoco basin; and *Pseudoplatystoma magdaleniatum*: distributed only in the Magdalena basin. The tiger catfish (*P. tigrinum*) populations from the Orinoco drainage were assigned to a new species (*Pseudoplatystoma metaense*), restricting the name *P. tigrinum* to the tiger catfish of the Amazonas basin. The species status of *P. corruscans* and its geographic distribution remained unchanged.

Two years later, [Torrice et al. \(2009\)](#) assessed the taxonomy and phylogeny of *Pseudoplatystoma* based on mitochondrial DNA (mtDNA) markers (cytochrome *b* and control region sequences). Their study supported four clades of mitochondrial haplotypes, corresponding to: (i) *P. tigrinum*, (ii) *P. corruscans*, (iii) *P. reticulatum* + *P. punctifer* + *P. fasciatum*, and (iv) *P. magdaleniatum* (following the taxonomy proposed by [Buitrago-Suárez and Burr](#)). In addition, for some of the included species the mtDNA haplotypes did not form monophyletic groups, casting doubt about their taxonomic status; however, many important issues could not be addressed by this study due to incomplete taxonomic sampling and evidence limited to a single molecular locus (mtDNA).

In the present study, we present new evidence to assess the taxonomy and systematics of this group by using two nuclear genes in addition to mtDNA markers. We include specimens obtained from an extensive sampling of all major river basins in South America where *Pseudoplatystoma* is distributed, covering some critical drainages that were not sampled in the previous studies. In addition to analyzing single gene genealogies, we use a recently developed coalescent approach to estimate a species trees from multilocus data ([Heled and Drummond, 2010](#)).

2. Materials and methods

Pseudoplatystoma specimens were sampled from all major river basins where the genus occurs, except the Gurupi and Munin rivers (Northeastern Brazil) and some drainages from Guyana and Suriname ([Table 1, Fig. 1](#)). Samples were identified based on the old taxonomy, the only one available at the time of sampling. Considering that the new taxonomy is generally concordant with species distribution (river basin), we reassigned species names based on their geographic distribution ([Buitrago-Suárez and Burr, 2007](#)). This allowed comparison of the old and new taxonomies in the light of the molecular phylogenies unraveled in our study. Voucher information is provided in [Appendix D](#). DNA was extracted from fin clips or muscle tissues following a rapid salt-extraction protocol ([Aljanabi and Martinez, 1997](#)) or using the DNeasy Blood and Tissue Kit ([QIAGEN Inc.](#)).

The complete mitochondrial Cytochrome *b* gene (Cytb, 1140 bp) and two nuclear gene fragments containing intron sequences were analyzed in this study. The nuclear genes targeted were Rag1 (recombination activating gene intron one; Rag1int1, 800 bp) and S7 ribosomal gene (first intron, S7int1, 1000 bp). All gene fragments were amplified via PCR (polymerase chain reaction), in a final reaction volume of 30 μ l containing 10–50 ng of DNA, 200 μ M of dNTPs (dATP, dGTP, dCTP and dTTP), 1 \times PCR buffer, 0.2 μ M of each primer, 1.5–2.0 mM of MgCl₂ and 1 U of Taq polymerase (Invitrogen Life Technologies). All reactions were conducted in either PTC100 (MJ Research) or Mastercycler (Eppendorf) thermocyclers, following the amplification program: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, annealing temperature ([Table 2](#)) for 35 s,

72 °C for 40 s and a final extension step (72 °C for 5 min). PCR primers for Cytb and S7int1 were available from the literature ([Table 2](#)), while those for Rag1int1 were designed using exons 1 and 2 sequences available on GenBank for other fishes. PCR products were purified (Illustra GFX PCR DNA and Gel band purification, GE HealthCare) and sequenced on a MegaBACE 1000 (GE HealthCare) using EtDye kit (GE HealthCare). All fragments were directly sequenced using forward and reverse primers. Sequences were checked on Codoncode 3.0 (Codoncode Corp.) and aligned using Clustalw ([Thompson et al., 1994](#)) with Bioedit ([Hall, 1999](#)). Heterozygous individuals for a single nucleotide site at nuclear loci were resolved manually. Otherwise, haplotype phases were determined using Phase 2.1 ([Stephens et al., 2001](#); [Stephens and Donnelly, 2003](#)) that implements a Bayesian method for haplotype reconstruction. Posterior probabilities higher than 0.9 were accepted if repeated throughout five runs of 500 iterations each. Possible instances of recombination events on nuclear alleles were assessed with Rdp3 3.34 ([Martin et al., 2005](#)) using the RDP and Bootscan/RECSCAN methods ([Salminen et al., 1995](#); [Padidam et al., 1999](#)), the method applied in the program GENECONV ([Padidam et al., 1999](#); [Sawyer, 1989](#)), the MaxChi method ([Maynard-Smith, 1992](#); [Posada and Crandall, 2001](#)), and the 3SEQ approach ([Posada and Crandall, 2001](#)).

Overall variation among DNA sequences was characterized using Mega 4.0 ([Tamura et al., 2007](#)) and DnaSP 4.10 ([Rozas et al., 2003](#)). Uncorrected *p*-distances were calculated with Mega and neutrality tests (Tajima's *D*, *F*^{*}, and *D*^{*}) ([Tajima, 1989](#); [Fu and Li, 1993](#)) were conducted using DnaSP 4.10. Maximum likelihood (ML) gene genealogies were estimated for each gene separately using Treefinder ([Jobb, 2008](#)). Each data set was reduced to distinct haplotypes or alleles for each gene before phylogenetic analysis (the full list of “collapsed” alleles/haplotypes is shown in [Appendices A–C](#)). The evolutionary model for each gene also was estimated using Treefinder, based on the Akaike Information Criterion (AIC). Cytochrome *b* sequences were partitioned by grouping 1st and 2nd codon positions into one partition and 3rd codon positions into another, each fitted with an independent model. Sequences from *Brachyplatystoma* and *Zungaro* were used as outgroup. Statistical support for nodes was estimated by bootstrap analysis with 1000 pseudoreplicates ([Felsenstein, 1985](#)). Topological tests were conducted for each gene genealogy *a posteriori* to compare the best tree to alternative hypotheses using the Shimodaira and Hasegawa (SH) and the Approximately Unbiased tests (AU) Shimodaira and Hasegawa, 1999; Shimodaira, 2002; both testing procedures are available in Treefinder and use the RELL technique ([Kishino et al., 1990](#)).

The species tree was estimated under a Bayesian Markov chain Monte Carlo method for the multispecies coalescent with the *Beast protocol ([Heled and Drummond, 2010](#)) implemented in the program BEAST v 1.5.3 ([Drummond and Rambaut, 2007a](#)). All sequences obtained in the study for all genes and individuals were used as input for this analysis. This includes redundant haplotypes and alleles. For the nuclear genes, both alleles for each individual were included in the input file, even for homozygous individuals. The species from which each allele/haplotype was obtained was used as the trait value for the species tree and specified using the program BEAUTi, a simple user interface for creating input files to run BEAST. Other parameters for this analysis included specifying a relaxed clock and coding for ploidy types (haploid for mtDNA and diploid autosomal for the nuclear genes). Population sizes were assumed to be constant and all other priors used default conditions. The xml file used as input is available upon request from the corresponding author. Two runs, each of 10 million generations, were conducted and stationarity was checked using the program TRACER ([Drummond and Rambaut, 2007b](#)), a graphical tool for visualization and diagnostics of MCMC output.

Table 1
Species, sample localities and total number of individuals sequenced for each marker.

Locality ^a	Basin (Tributary)	Species ^b (n)	CytB (n): haplotypes ^c	RAG1 (n): alleles ^d	S7 (n): alleles ^e
1. Santa Helena (Bra)	Turiaçu	pun (10)	(10): H17–18	(10): R5, R7–9	(2): S5–7
2. Pindaré-Mirim (Bra)	Pindare	pun (28)	(28): H19, H21–22, H25–26	(25): R5	(17): S1–2, S4
3. Lago-Açu (Bra)	Mearim	pun (26)	(22): H19–24	(26): R5	(12): S1–3
4. Codó (Bra)	Itapecuru	pun (2)	(2): H27–28	(2): R5	(1): S1
5. Rosário (Bra)	Itapecuru	pun (1)	(1): H28	N/A	N/A
6. Guadalupe (Bra)	Parnayba	pun (3)	(3): H19, H29	(3): R9	N/A
7. Santa Quitéria (Bra)	Parnayba	pun (6)	(1): H29	(6): R9	N/A
8. Cocalinho (Bra)	Amazon (Araguaia)	pun (1)	(1): H13	(1): R4, R10	(1): S8, S12
9. Tucuruí (Bra)	Amazon (Tocantins)	pun (2)	(2): H1, H14	(2): R4, R9, R12	N/A
10. Iriiri (Bra)	Amazon (Xingú)	pun (6)	(6): H11–12	(4): R4, R15	N/A
11. Santarém (Bra)	Amazon	tig (2)	(2): H58	(2): R36–37	N/A
12. Manaus (Bra)	Amazon	pun (3)	(3): H2–3	(2): R4–5, R10	N/A
		tig (2)	(2): H2, H9	(1): R10–11	N/A
13. Tefé (Bra)	Amazon	pun (1)	(1): H1	(1): R10–11	N/A
		tig (2)	(2): H54	(2): R37, R41	N/A
14. Tabatinga (Bra)	Amazon	pun (13)	(13): H1–3, H5–7	(9): R3–5, R10, R12	(6): S5, S8, S13–16
		tig (6)	(6): H54–56	(6): R37–39	(3): S36–38
15. Iquitos (Per)	Amazon	pun (3)	(3): H2, H4, H8	(3): R3–4, R10–11	(2): S5, S8, S17
		tig (8)	(6): H2, H54, H57	(8): R13–14, R37, R39–40	(3): S36–37
16. Boa Vista (Bra)	Amazon (Branco)	pun (3)	(2): H10	(3): R3, R5, R10	N/A
17. Lethem (Tacutu) (Guy)	Amazon (Branco)	pun (1)	(1): H10	(1): R16–17	N/A
18. Yapukarri (Guy)	Essequibo (Rupunini)	fas (2)	(2): H15–16	(2): R5, R10, R18	N/A
19. Paramaribo (Sur)	Suriname	fas (1)	(1): H4	(1): R18–19	(1): S18–19
20. Atabapo (Ven)	Orinoco	met (4)	(4): H10, H38, H54	(2): R25, R36–37	No
21. Parguaza (Ven)	Orinoco	ori (2)	(1): H38	(2): R26	(1): S20
22. Cinaruco (Ven)	Orinoco	ori (3)	(3): H36	N/A	(2): S7–8, S23
23. Caura (Ven)	Orinoco (Caura)	met (2)	N/A	(2): R33–35	N/A
		ori (4)	(4): H37–38	(2): R26	(3): S5, S20
24. Puerto Ayacucho (Ven)	Orinoco	met (2)	(2): H54	(2): R37	(1): S32–33
		ori (5)	(5): H38, H54	(5): R26, R36–37	(1): S20
25. Portuguesa (Ven)	Orinoco (Portuguesa)	met (1)	(1): H54	(1): R37	(1): S32, S35
		ori (4)	(3): H35, H37–38	(3): R26	(4): S20, S22–23
26. Matyure (Ven)	Orinoco (Apure)	met (1)	(1): H54	(1): R36–37	N/A
27. Tucupita (Ven)	Orinoco	met (3)	(3): H54	(3): R36–37	(3): S32–34
		ori (3)	(2): H37–38	(1): R26	(3): S20–21, S24
28. Apure (Ven)	Orinoco (Apure)	met (3)	(3): H54	(1): R37	(2): S32–33, S35
		ori (1)	(1): H37	N/A	(1): S20–21
29. Curamoni (Ven)	Orinoco	ori (3)	(3): H10	(3): R10, R20–24	(2): S7, S13, S21
30. Caicara del Orinoco (Ven)	Orinoco	met (2)	N/A	(2): R36–37	(1): S32–33
		ori (1)	(1): H38	(1): R26	(1): S20, S22
31. Puerto Paez (Ven)	Orinoco	ori (1)	(1): H38	(1): R25	(1): S20
32. Caño La Guardia (Ven)	Orinoco	ori (1)	N/A	N/A	(1): S20
33. Villavicencio (Col)	Orinoco (Meta)	met (4)	(4): H54	(4): R36–37	(3): S32–33
		ori (5)	(5): H38	(4): R5, R26	(5): S5, S20–21, S23
34. Neiva (Col)	Magdalena	mag (12)	(4): H59	(12): R42–43	(6): S39–40
35. Cuiabá River (Bra)	Paraguay	cor (8)	(8): H39, H41, H43	(5): R27–28, R31	(3): S25–27
		ret (13)	(13): H30–31, H33	(4): R1–4	(8): S5, S8–11
36. Taquari River (Bra)	Paraguay	cor (3)	(2): H39, H42	(3): R27–29	N/A
		ret (3)	(2): H30	(3): R1, R3–4	(2): S5, S8–10
37. São Lourenço River (Bra)	Paraguay	cor (6)	(6): H39–40, H44, H46–47	(5): R27–30	(2): S25–26
		ret (1)	(1): H30	(1): R3	N/A
38. Miranda River (Bra)	Paraguay	cor (5)	(2): H45	(5): R27–29	N/A
		ret (6)	(6): H30, H32, H34	(4): R1–4	(4): S5, S8–9, S11–12
39. Grande River (Bra)	Paraná	cor (3)	(3): H39	(2): R27–28	(2): S26–27
40. Corrientes (Arg)	Paraná	cor (2)	(2): H39, H46	(2): R27–29	N/A
		ret (1)	N/A	(1): R3	N/A
41. Itapiranga (Bra)	Uruguay	cor (5)	(5): H39	(5): R27–29	(4): S25–28
42. Três Marias (Bra)	São Francisco	cor (22)	(22): H48–53	(15): R28, R32	(7): S25, S28–31
TOTAL		279	243	227	121

Countries of origin shown in parenthesis: Bra: Brazil; Per: Peru; Guy: Guyana; Sur: Suriname; Ven: Venezuela; Col: Colombia; Arg: Argentina.

^a Localities shown in Fig. 1.

^b Species name according to Buitrago-Suárez and Burr, 2007 (n = sample size); pun = *P. punctifer*; fas = *P. fasciatus*; ori = *P. orinocoense*; met = *P. metaense*; tig = *P. tigrinum*; cor = *P. corruscans*; mag = *P. magdaleniatum*; ret = *P. reticulatum*.

^c mtDNA haplotypes (n = number of individuals sequenced), Haplotype identities as shown in Appendix A.

^d RAG1 alleles (n = number of individuals sequenced), Haplotype identities as shown in Appendix B.

^e S7 alleles (n = number of individuals sequenced), Haplotype identities as shown in Appendix C.

3. Results

A total of 279 individuals were obtained from 42 localities throughout South America from all the major river basins where the genus is distributed (Fig. 1 and Table 1). Most individuals were screened for mtDNA variation first and subsequently nuclear gene

sequences also were obtained. A Cytochrome b gene fragment of 818 bp was sequenced for 243 individuals. Variation among sequences was restricted to 121 variable sites that collectively defined 59 distinct haplotypes (H1–H59, Table A1, Appendix A, GenBank: GU593097–GU593157). The average number of nucleotide differences among sequences (k) was to 25.3. Fu and Li's D^*

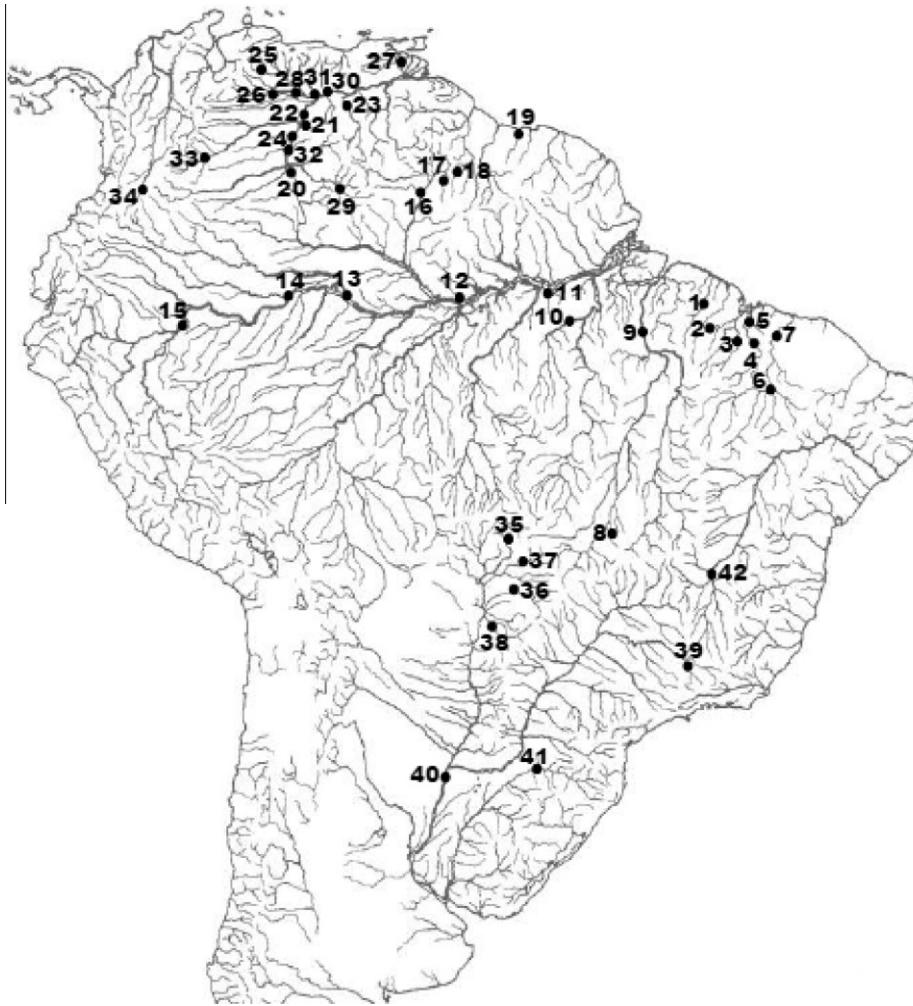


Fig. 1. Sampling sites for *Pseudoplatystoma* species in South American river basins. Species identity, numbers for localities, and sample sizes are indicated in Table 1.

Table 2

PCR and sequencing primers. Ta = annealing temperature; F = forward; R = reverse.

Primer	Sequence (5' → 3')	Ta (°C)	References
Gludgl-F	TGACTTGAARAACCAAYCGTTG	49	Palumbi et al. (2002)
H15915-R	AACTGCAGTCATCTCCGGTTTACAAGAC	49	Irwin et al. (1991)
Rag1PseX1-F	GCTGGCAGACAAGTGGATCT	58	Our study
Rag1PseX2-R	AGCCATCTAGAAGCCTTGC	58	Our study
S7RPEX1-F	TGGCTCTTCCTTGGCCGT	56	Chow and Hazama (1998)
S7RPEX2-R	AACTCGTCTGGCTTTTCGCC	56	Chow and Hazama (1998)

(1.29432, $P > 0.10$), Fu and Li's F^* (1.10681, $P > 0.10$) and Tajima's D (0.53560, $P > 0.10$) tests did not reject the null hypothesis of neutrality. Most haplotypes were exclusive to each species, but in several cases haplotypes were shared among species (Appendix A). For example, haplotype 4 [H4_pun(1)fas(1)] was shared between one specimen each from *P. punctifer* and *P. fasciatum*. Similarly, haplotype 10 [H10_ori(4)pun(3)] was shared among three *P. punctifer* and four *P. orinocoense* specimens. Shared haplotypes among these three putative species suggests either a recent divergence with retention of ancestral haplotypes among species, or that the species boundaries are fuzzy and there is significant gene flow (see below the alleles shared among species for the nuclear genes and Fig. 2a for discussion on the "*P. fasciatum sensu lato*" clade).

A total of 227 individuals were sequenced for the nuclear locus Rag1int1 (664 bp). Among the sequences we detected 50

polymorphic sites that defined 45 distinct alleles (R1–R45, Table B1, Appendix B, GenBank: GU593198–GU593242). Only 72 individuals were heterozygous for this locus and in 24 cases the allelic phases were resolved using the program Phase, the rest were trivial to solve because they implied a single polymorphism. Average number of nucleotide differences was $k = 7.2$. All neutrality tests (Fu and Li's $F^* = 1.07503$, $P > 0.10$; Tajima's $D = -0.11878$, $P > 0.10$), except Fu and Li's D^* (1.69581*, $P < 0.05$), were not significant. Three indels were found: a 15 bp deletion (not present in outgroup) in all *P. punctifer*, in most samples of *P. reticulatum*, *P. fasciatum* and few *P. orinocoense* samples; a 12 bp deletion in two *P. metaense* samples; and a different 12 bp deletion in one sample of *P. corruscans* from São Francisco. There was extensive haplotype sharing between species for this locus (Appendix B, Fig. 2b), especially among the species included in the "*P. fasciatum sensu lato*" clade [e.g.

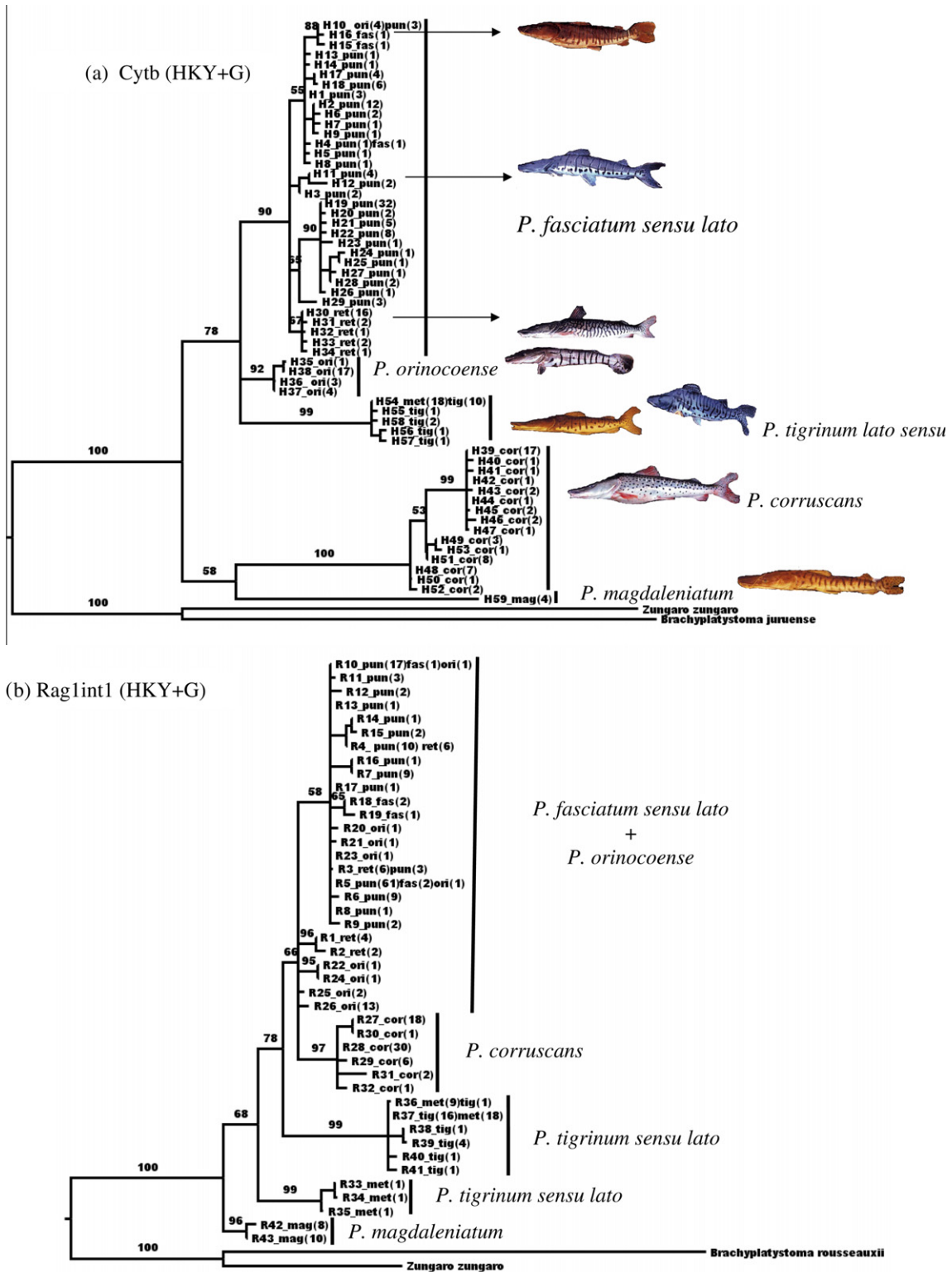


Fig. 2. Gene genealogies for unique haplotypes/alleles obtained by maximum likelihood for the three independent genes sampled from *Pseudoplatystoma* species. (a) Cytochrome b; (b) Rag1 intron 1; (c) S7 intron 1. Terminal labels are (a) distinct haplotypes for cyt b (H1–H59), followed by species (abbreviated as in Table 1) and number of individuals found carrying the haplotype (in parenthesis following species name). Likewise, for nuclear genes distinct alleles are shown (b) R1–R45 for Rag1 intron, and (c) S1–S41 for S7 intron. Geographic distribution of alleles and species names are in Table 1. See the Appendix for a complete list of individuals found carrying the same haplotype/genotype.

R5_pun(61)fas(2)ori(1) and R5_pun(61)fas(2)ori(1)] and between *P. tigrinum* and *P. metaense* [e.g., R36_met(9)tig(1) and R37_tig(16)met(18)].

A total of 121 samples were sequenced for S7int1 (635 bp, 49 variable sites, 41 distinct alleles, S1–S41, Table C1, Appendix C, GenBank: GU593158–GU593197), where 68 were heterozygous

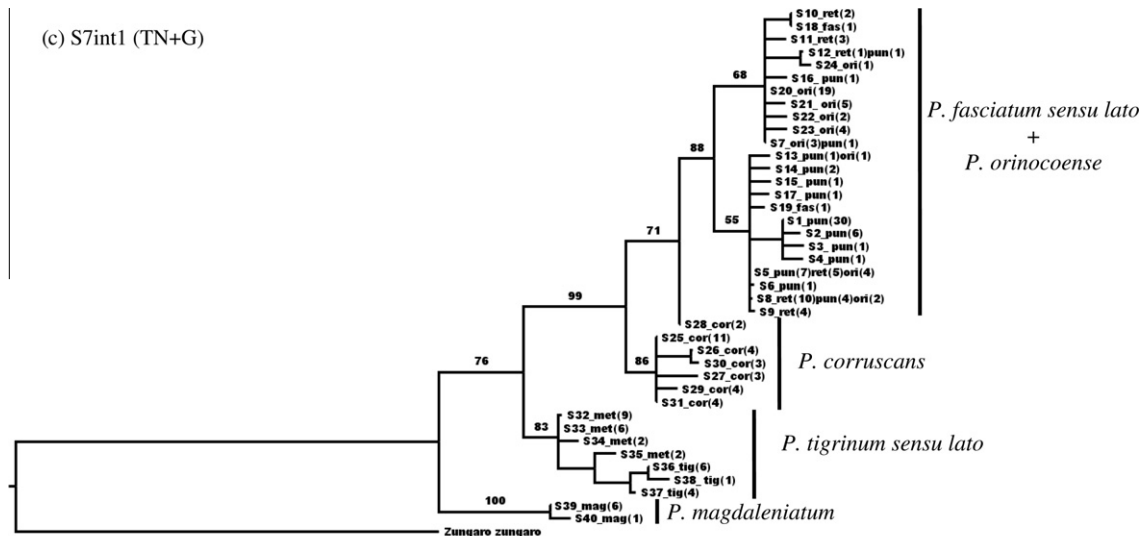


Fig. 2 (continued)

(25 were resolved by Phase, the rest were trivial to solve because they implied a single polymorphism). Average number of nucleotide differences was $k = 6.7$, and no significant departure from neutrality was observed (Fu and Li's $F^* = -0.04091$, $P > 0.10$; Fu and Li's $D^* = 0.39149$, $P > 0.10$; Tajima's $D = -0.58284$, $P > 0.10$). Two separate indels were observed: a two bp deletion present in *P. corruscans*, *P. magdaleniatum* and *P. metaense*; and a four bp deletion (the first two bp seemed to be homologous with the two bp found in *P. metaense*) only found in *P. tigrinum*. Shared haplotypes between different species also were observed (Fig. 2c, Appendix C), again especially among specimens in the "*P. fasciatum s.l.*" clade [e.g., S5_pun(7)ret(5)ori(4) and S8_ret(10)pun(4)ori(2)].

No signal of recombination was detected in both intron fragments for any of the tests performed (data not shown). Within and between species distances (uncorrected "*p*-distances") are shown in Table 3, where Rag1int1 presents the smallest values (most conserved marker). Comparisons of genetic divergence between species did not take into account those haplotypes shared between different species. *P. magdaleniatum* and *P. corruscans* have the greatest distances to the other species (1–6%). *P. tigrinum* differs less than 0.3% from *P. metaense*, and *P. reticulatum* weakly diverged from *P. punctifer* (0.6–0.8%) and *P. fasciatum* (0.5–0.7%). *P. fasciatum* *p*-distances ranged from 0.4–0.8% in relation to *P. punctifer*. *P. orinocoense* showed large values in Cytb (2–2.3%) from *P. punctifer*, *P. fasciatum* and *P. reticulatum*, while it presented

2.1–3.3% in sequence divergence from its co-occurring species in the Orinoco basin (*P. metaense*).

Fig. 2 displays the inferred evolutionary relationships among haplotypes/alleles from *Pseudoplatystoma* based on each of three markers. The "*P. fasciatum* clade" (*P. fasciatum*, *P. punctifer*, *P. reticulatum*, *P. orinocoense*, *P. magdaleniatum* and *P. corruscans*) suggested by morphological characters in Buitrago-Suárez (2006) was not recovered. However, a *P. tigrinum* clade was observed. The mtDNA haplotype phylogeny (Fig. 2a) is resolved into five different clades: (i) *P. magdaleniatum*, (ii) *P. corruscans*, (iii) *P. tigrinum* + *P. metaense* (herein defined as *P. tigrinum s.l.*), (iv) *P. orinocoense*, and (v) *P. reticulatum* + *P. fasciatum* + *P. punctifer* (named *P. fasciatum s.l.*). The latter included 4 individuals from the Orinoco (putatively *P. orinocoense*) that carried haplotype H10. Also based on the Cytb analysis, we found support for clades composed of samples of *P. punctifer* from the Maranhão basins, excepting Turiaçu, and Xingú. In addition, *P. corruscans* populations from São Francisco and Paraná–Paraguay–Uruguay basins also were well differentiated from each other for this marker (*p*-distance = 1.5%).

Nuclear gene markers were able to distinguish only monophyletic groups comprising alleles obtained from *P. magdaleniatum* and *P. corruscans* (Fig. 2b and c). *P. tigrinum s.l.* was a distinct clade on the intron phylogenies, but for Rag1 this group was subdivided into two highly supported clades (2.9% in sequence divergence);

Table 3

Uncorrected average *p*-distances for Cytb (first value), Rag1int1 (second value) and S7int1 (third value). Short names follow Table 1.

	pun (0.007/0.005/ 0.005)	ret (0.002/0.006/ 0.005)	fas (0/0.008/ 0.005)	cor (0.009/0.003/ 0.003)	ori (0.002/0.003/ 0.002)	met (0/0.003/ 0.0019)	tig (0.003/0.002/ 0.003)
ret	0.008/0.006/0.006						
fas	0.008/0.006/0.004	0.007/0.005/0.005					
cor	0.057/0.012/0.014	0.056/0.013/0.015	0.052/0.012/ 0.013				
ori	0.022/0.008/0.011	0.020/0.007/0.010	0.023/0.005/ 0.010	0.053/0.014/0.015			
met	0.036/0.018/0.024	0.036/0.019/0.023	0.032/0.019/ 0.023	0.061/0.017/0.027	0.033/0.021/0.025		
tig	0.038/0.019/0.025	0.038/0.020/0.025	0.034/0.019/ 0.024	0.063/0.017/0.028	0.035/0.021/0.026	0.002/0.003/ 0.002	
mag (0/0.002/ 0.002)	0.061/0.027/0.019	0.057/0.027/0.020	0.056/0.027/ 0.018	0.067/0.025/0.020	0.059/0.029/0.020	0.067/0.019/ 0.026	0.069/0.019/0.027

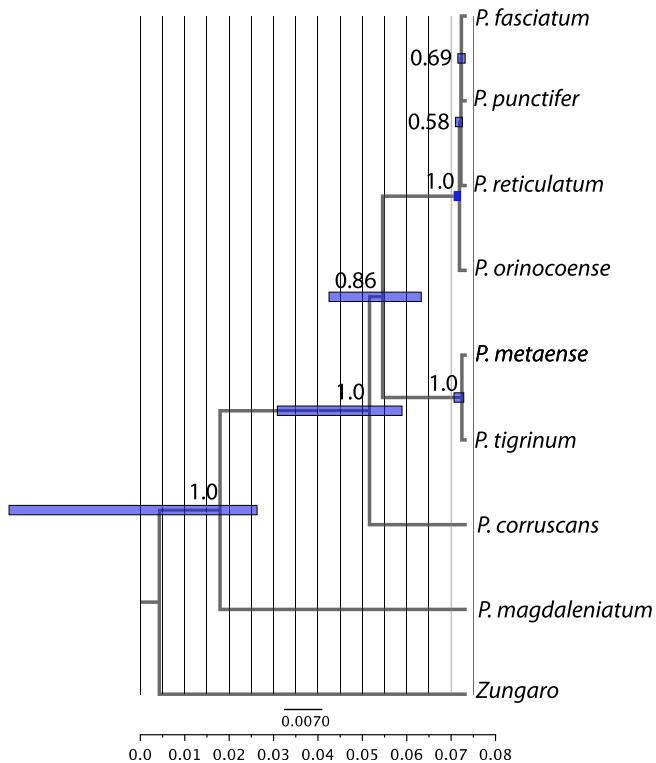


Fig. 3. Maximum clade credibility species tree produced by *Beast. Posterior probabilities and 95% node high HPD are graphed at each node.

one containing all *P. tigrinum* samples and most *P. metaense* and the other one composed of few *P. metaense* sequences (R33, R34, and R3, with a 12 bp deletion and many fixed substitutions, but two of them inferred by PHASE) (Fig. 2b). *P. orinocoense* alleles mixed extensively with those from *P. fasciatum sensu lato* (including a diagnostic 15 bp deletion). *P. fasciatum s.l.* was not well delimited in Rag1 and S7, since many alleles from *P. orinocoense* were included or had close affinities with this clade, suggesting that this species also should be included in this species complex (Fig. 2b and c).

The mtDNA and nuclear gene trees disagree about the position of *P. corruscans* and *P. tigrinum sensu lato*. For the intron trees, *P. corruscans* alleles have closer affinities to the *P. fasciatum s.l. lato* + *P. orinocoense* clade, whereas in the mtDNA tree *P. tigrinum s.l.* holds this position to the exclusion of *P. tigrinum*. The topology tests shown no significant differences (*P* values between 0.96 and 1.0) when the *P. corruscans* clade was constrained to be sister to the *P. fasciatum s.l.* + *P. orinocoense* clade for Cytb or when it was placed in a clade with *P. tigrinum s.l.* for the nuclear genealogies.

P. reticulatum haplotypes H30–H34 formed a moderately supported clade (bootstrap value = 70%) within the “*P. fasciatum s.l.*” clade on the Cytb genealogy (Fig. 2a). For Rag1int1, *P. reticulatum* shared haplotypes with *P. punctifer* (Amazonas), although a highly supported clade (R1 and R2) also was observed (Fig. 2b). The same reticulated pattern holds for *P. fasciatum* (Guyana and Suriname), whose samples shared haplotypes with *P. punctifer* for Cytb (Fig. 2a) and with *P. punctifer* and *P. orinocoense* in Rag1int1 (Fig. 2b). Nevertheless, *P. fasciatum* (Guyana and Suriname) also presented exclusive haplotypes in all markers (Fig. 2).

Due to the complex pattern of haplotype and allele sharing among putative species, a concatenated analysis of DNA sequences from each individual is not simple or desirable. Instead, a multispecies coalescent approach was taken to infer the most likely species tree on the basis of gene tree topologies estimated for each molec-

ular marker. The species tree obtained with *BEAST is shown in Fig. 3. The “*P. fasciatum s.l.*” clade is well supported in this tree, with very shallow divergences among the putative species included (*P. fasciatum*, *P. punctifer*, *P. reticulatum* and *P. orinocoense*). Based on the posterior probabilities obtained for each of the putative species in this clade (0.58–0.69), this approach fails to provide support for the recognition of barriers to gene flow or lack of interbreeding among populations of these fishes. The same lack of evident species boundary is observed for the two taxa in the clade that includes *P. tigrinum* and *P. metaense* (*P. tigrinum s.l.*). Therefore, this analysis supports the distinction of four well-supported groups in the genus (that may correspond to four species), given the high posterior probabilities (*P* = 1) supporting *P. magdaleniatum* and *P. corruscans*. The relationship among *P. corruscans*, *P. tigrinum s.l.*, and *P. fasciatum s.l.* is not well resolved, as implied by the overlapping confidence intervals for speciation times separating these three clades (Fig. 3).

4. Discussion

Complex gene genealogies have emerged for the three markers used in this study, which sampled more than 2000 bp of both nuclear and mitochondrial genomes of *Pseudoplatystoma*. As expected, the cytochrome b genealogy shows more resolution in terms of well-supported clades, while the S7-based evolutionary history was more resolved than Rag1, which seemed to have a slower rate of molecular evolution. Only five clades were recovered with the molecular markers used: *P. magdaleniatum*, *P. corruscans*, *P. tigrinum s.l.*, *P. fasciatum s.l.*, and *P. orinocoense*. The latter however, is seen to be part of the *P. fasciatum sensu lato* group, especially in light of results of the species tree analysis.

Although some issues from previous studies were confirmed, others remain to be discussed. First of all, we have detected topological incongruence concerning the *P. corruscans*/*P. tigrinum s.l.* branch placement among mitochondrial and nuclear DNA genealogies. For mtDNA, Torrico et al. (2009) also reported the same evolutionary relationship that we obtained for the cytb gene. On the other hand, a morphology-based phylogeny (Buitrago-Suárez, 2006) agrees with the nuclear trees. Nonetheless, topology tests performed here show no significant differences when alternative topologies for those taxa were constrained on each marker. The different mutation rates between nuclear and mitochondrial genomes might produce such discrepancy between the gene trees used to infer species trees (Zhang and Hewitt, 2003). However, as nuclear loci tend to be less affected by the effect of homoplasy (Avice, 2004) the well resolved relationships of *P. corruscans* and *P. tigrinum* in the nuclear markers could be a good hint of the reality of the deep relationships in *Pseudoplatystoma*. However, lineage sorting at this level also may explain the discrepancy, as suggested by the species tree result (Fig. 3). Therefore, the issue remains poorly resolved with the evidence at hand and more markers might be necessary to solve this question.

The results of our study show geographically distinct clades defined by the mtDNA analysis, Cytb for *P. corruscans*, clearly separating fish from the São Francisco and Paraná–Paraguay–Uruguay drainages. Buitrago-Suárez (2006) found no differences between those populations, but the genetic differences suggest a substantial time of divergence. Cytogenetic differences between these populations have also been reported (Swarça et al., 2005). Such distinctness, however, was not observed in the nuclear DNA markers, perhaps due to the longer time required for lineages to be completely sorted for autosomal loci (Avice, 2000). Torrico et al. (2009) did not address this issue since no samples from the São Francisco basin were included in their phylogeny. Whether or not this evolutionary divergence is related to isolating

reproductive barriers is yet to be determined in future studies, since the geographical separation of these populations for extended periods of time could be a sufficient explanation.

Our results are congruent with those from Buitrago-Suárez and Burr (2007) and Torrico et al. (2009) regarding the evolutionary uniqueness of *P. magdaleniatum*, which used to be considered a geographically isolated population of *P. fasciatum* inhabiting the Magdalena basin (Colombia). This is not surprising since this clade has been probably isolated since the uplift of eastern Andean Cordillera about 13–11.5 million years ago, which explains the higher sequence divergence observed for this species. Furthermore, the morphological study by Buitrago-Suárez and Burr (2007) report strong and reliable anatomical characters supporting it.

Our study also conveyed information about the lack of distinction of *P. metaense* and *P. tigrinum* samples, which clustered together in all DNA genealogies. We refer to this clade as *P. tigrinum s.l.*, which seems to represent what Buitrago-Suárez and Burr (2007) called the “*P. tigrinum* clade”. The genetic cohesiveness between of these putatively different species might invalidate the status of *P. metaense* as a separate species from *P. tigrinum* populations in the Orinoco basin. One single species of *Pseudoplatystoma* (*P. tigrinum*) seems to be widely distributed in the Orinoco and Amazonas basins, given that inter-basin dispersal is feasible via the physical connection through the Casiquiare river (Armbruster and Provenzano, 2000). Nonetheless, a 2-bp deletion in S7int1 found only in *P. metaense* and exclusive haplotypes in each river reveal some level of population differentiation at this macro geographical level. It is worth noticing that the diagnosis of *P. metaense* given by Buitrago-Suárez and Burr (2007) is mostly based on body shape and color pattern, which, according to the authors, might be variable and therefore does not provide unambiguous support for the differentiation of these species. The highly supported clade in Rag1int1 for few *P. metaense* haplotypes might be due to an idiosyncratic evolutionary history for these intron lineages during their lineage sorting process, or due to artificially divergent alleles reconstructed by the program PHASE.

The phylogenies show a discrete clade of *P. orinocoense* samples (formerly known as a *P. fasciatum* population from the Orinoco) only on Cytb and Rag1int1, but some samples shared haplotypes found within the *P. fasciatum s.l.* clade. That is the case of four samples (three from the region next to the Casiquiare) that presented the same haplotype (H10) found in *P. fasciatum s.l.* in cytb (Fig. 2a). These same individuals also carried shared haplotypes with *P. fasciatum s.l.* on Rag1 intron, including a 15 bp deletion and many substitutions (Fig. 2b). Misidentification of samples cannot explain this observation because *P. orinocoense* is endemic to Orinoco. Ruling out ancestral polymorphism in cytb for *P. orinocoense*, given well-delimited genetic populations, these fish might have migrated from Amazonas to Orinoco, which means that the *P. fasciatum s.l.* clade might have representatives in Orinoco. Past dispersal between Amazonas and Orinoco basins already have been reported for other fishes (Lovejoy and Araújo, 2000; Hubert et al., 2007; Willis et al., 2010). Our results provide evidence for introgression of both mitochondrial and nuclear DNA genes from the Amazonas to the Orinoco. However, theoretical expectations and empirical evidence suggest that mitochondrially inherited genes are more likely to undergo introgression than their counterparts in nuclear autosomal DNA (Matos and Schaal, 2000). Genes from nuclear genome are usually responsible for interspecific incompatibility, unless cytonuclear incompatibilities exist or if incompatibility is linked to females (Abe et al., 2005). Although sequences from some of these samples are available for S7, the boundary between *P. orinocoense* and *P. fasciatum s.l.* is not clear with this marker. Ancestral polymorphism might explain the existence of a Rag1 haplotype from the Meta River (tributary of Orinoco) that is identical to the one found in fish from Maranhão

and Amazonas (Fig. 2b). Different effective population sizes of some loci (particularly those from mtDNA) or different patterns of selection might lead some loci to reach reciprocal monophyly sooner than others (Di Candia and Routman, 2007), probably explaining why Cytb delimited species better than the autosomal loci, even differentiating conspecific populations of *Pseudoplatystoma*. The species tree analysis, however, provides no support for the recognition of *P. orinocoense* as a divergent set of populations in the *P. fasciatum s.l.* clade (Fig. 3).

P. reticulatum was not supported as a monophyletic group, except with moderate support in the mtDNA genealogy, but showing low genetic distance from *P. fasciatum s.l.* A recent speciation event among these putative species could lead to insufficient time for the polymorphism to be completely sorted, albeit reproductive barriers might be already present. Nevertheless, Torrico et al. (2009) argue that this species represents a highly supported clade, but support from nuclear genes is lacking. Among our samples from Maranhão, we observed 1.1% sequence divergence in cytb from the remaining haplotypes in the *P. fasciatum s.l.* clade, and *P. corruscans* haplotypes from the São Francisco are on average 1.5% divergent from the Paraná–Paraguay–Uruguay populations. Therefore, unless the status of these other conspecific populations is reviewed, the species status of *P. reticulatum* seems doubtful. Buitrago-Suárez and Burr (2007) describe *P. reticulatum* as inhabiting the Central Amazonas and the Paraná–Paraguay basin, even though no sample from Amazonas was examined other than the holotype. This diagnosis is mainly on the basis of color pattern, which seems to be very polymorphic in *Pseudoplatystoma*. Thus, it may be more appropriate to regard this species as a natural variation of *P. fasciatum s.l.*

Likewise, no differences were found supporting the distinct status of *P. fasciatum* (to the exclusion of all other taxa in the *fasciatum s.l.* group). Although we studied few samples from only two localities in Guyana and Suriname, these results would, at minimum, indicate incomplete lineage sorting. Samples presented unique haplotypes but also shared haplotypes with *P. fasciatum s.l.* (Fig. 2). If *P. fasciatum* is not different from the species occurring in the Amazonas, then ancestral polymorphism or current gene flow can explain the shared haplotypes.

Unless one shows that the non-monophyletic *Pseudoplatystoma* species are recently split taxa and are effectively isolated meta populations, our study suggests that the group of nominal species that includes *P. punctifer* (Amazonas, Maranhão basins, Tocantins–Araguaia), *P. reticulatum* (Paraná–Paraguay), *P. fasciatum* (Guyana and Suriname), and *P. orinocoense* may in fact constitute a single widespread taxon as was considered in the older taxonomy (*P. fasciatum*). Only *P. magdaleniatum* is supported as a completely distinct species from the former *P. fasciatum* terminology as was proposed by Buitrago-Suárez and Burr (2007). The same rationale holds for *P. tigrinum* and *P. metaense*, where *P. tigrinum* may have to be revalidated for the Orinoco basin.

5. Final considerations

In conclusion, we were able to confirm some results presented by a previous revisionary study of this group by Buitrago-Suárez and Burr (2007) and a partial analysis of mtDNA by Torrico et al. (2009), such as the phylogenetic distinctness of *P. magdaleniatum*, and *P. corruscans*, but also instances of non-monophyletic species (*P. punctifer*, *P. metaense*, *P. tigrinum*, *P. fasciatum*, *P. orinocoense*, and *P. reticulatum*). Given the relevant implications of this information for conservation and fishery purposes, since *Pseudoplatystoma* is one of the most important resources in inland fishery of South America, we strongly suggest that a critical reevaluation of the morphological characters used for separating these species – mostly supported by color pattern – be reconsidered. Morpholog-

ical characters and additional genetic information will help efforts to delineate units for the sustainable management of this resource.

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Appendix A

Table A1. Cytochrome b haplotypes (H1–H59) are listed next to the individual samples from which they were isolated. These labels are at the tips of the tree in Fig 2a. Species names are abbreviated as in Table 1 and the locality codes follow the specimen number. The haplotype label contains information of the species of origin and (in parenthesis) of the number of individuals from that species where the haplotype was found. A complete list of specimens with their cytochrome b haplotype and their linked nuclear genotypes is presented in Appendix D.

H1_pun(3)	[pun10AMTa, pun7122AMTe, pun02TO]
H2_pun(12)	[pun31AMTa, pun2AMTa, pun04AMTa, pun30AMTa, punAM07Ta, puno8AMTa, pun09AMTa, pun751AMIk, tig633AMIk, pun2548AMMa, pun927AMMa, pun979AMMan]
H3_pun(2)	[pun28AMTa, pun994AMMa]
H4_pun(1)fas(1)	[pun750AMIk, fas1312RU]
H5_pun(1)	[pun3AMTa]
H6_pun(2)	[pun05AMTa, pun29AMTa]
H7_pun(1)	[pun06AMTa]
H8_pun(1)	[pun753AMIk]
H9_pun(1)	[pun980AMMa]
H10_ori(4)pun(3)	[ori11ORCu, ori12ORCu, ori13ORCu, ori865ORAt, pun5156AMRB, pun5158AMRB, pun1313AMRB]
H11_pun(4)	[pun1382AMXi, pun1433AmXi, pun1537AMXi, pun1541AMXi]
H12_pun(2)	[pun1539AMXi, pun1540AMXi]
H13_pun(1)	[pun01AR]
H14_pun(1)	[pun01TO]
H15_fas(1)	[fas60RU]
H16_fas(1)	[fas61RU]
H17_pun(4)	[pun38TU, pun65TU, pun41TU, pun36TU]
H18_pun(6)	[pun01TU, pun33TU, pun40TU, punsp5TU, pun56TU, pun39TU]
H19_pun(32)	[punA03ME, punA04ME, pun45ME, pun69ME, pun27ME, pun47ME, pun55ME, punA07ME, pun39ME, punE10ME, punD08ME, punD10ME, pun11ME, pun51ME, punC06ME,

Appendix A (continued)

	punSP8ME, punB09PM, punB11PM, punC02PM, punA06PM, punC09PM, punC10PM, punC12PM, punD01PM, punD02PM, punD05PM, punSP7PM, punSP9PM, punE11PM, pun58PM, punF04PM, pun268PBSJ]
H20_pun(2)	[pun67ME, punAC10ME]
H21_pun(5)	[punC07ME, punC11PM, punD09PM, punF05PM, pun53PM]
H22_pun(8)	[punC08ME, punC03PM, punC04PM, punC05PM, pun59PM, pun57PM, punE12PM, pun12PM]
H23_pun(1)	[punSP4ME]
H24_pun(1)	[punD07ME]
H25_pun(1)	[punD03PM]
H26_pun(1)	[punD04PM]
H27_pun(1)	[punB07ITCo]
H28_pun(2)	[punC01ITCo, pun216ITRo]
H29_pun(3)	[pun2611PBSJ, pun212PBGd, pun218PBSQ]
H30_ret(16)	[retA08PG, retB03PG, retB02PG, retA10PG, ret127PG, ret139PG, ret135PG, retA11PG, ret138PG, ret128PG, ret137PG, ret140PG, ret124PG, retA12PG, retF09PG, retSP1PG]
H31_ret(2)	[ret107PG, ret136PG]
H32_ret(1)	[ret97PG]
H33_ret(2)	[ret132PG, ret123PG]
H34_ret(1)	[ret133PG]
H35_ori(1)	[ori373ORPo]
H36_ori(3)	[ori446ORCi, ori445ORCi, ori447ORCi]
H37_ori(4)	[ori482ORTc, ori371ORPo, ori547ORAp, ori614ORCa]
H38_ori(17)	[ori478ORPP, ori466ORCr, ori122ORMt, ori120ORMt, ori121ORMt, ori115ORMt, ori114ORMt, ori481ORTc, ori376ORPo, ori414ORPA, ori483ORPA, ori502ORPz, ori604ORCa, ori612ORCa, ori613ORCa, ori687OrAt, ori869ORAt]
H39_cor(17)	[corB04PG, cor145PG, cor147PG, cor158PG, cor160PG, cor156PG, cor169PG, cor144PG, cor01PNRG, cor02PNRG, cor03PNRG, cor1316PNCr, cor212UR, cor213UR, cor214UR, cor215UR, cor216UR]
H40_cor(1)	[corD12PG]
H41_cor(1)	[cor143PG]
H42_cor(1)	[corF06PG]
H43_cor(2)	[cor148PG, cor155PG]
H44_cor(1)	[cor149PG]
H45_cor(2)	[cor150PG, cor146PG]
H46_cor(2)	[cor232PG, cor1315PNCr]
H47_cor(1)	[corB05PG]
H48_cor(7)	[corD11SF, corF10SF, cor191SF, cor199SF, corE01SF, corE04SF, corAC1SF]
H49_cor(3)	[corF11SF, corH02SF, corSP3SF]
H50_cor(1)	[corF12SF]
H51_cor(8)	[cor170SF, cor171SF, cor172SF, cor174SF, cor175SF, cor176SF, cor192SF, corE03SF]

(continued on next page)

Appendix A (continued)

H52_cor(2)	[cor173SF, corAC2SF]
H53_cor(1)	[corSP2SF]
H54_met(18)tig(10)	[met116ORMt, met117ORMt, met118ORMt, met119ORMt, met02ORAY, met03ORAY, met04ORAY, met526ORAp, met527ORAp, met528ORAp, met866ORAt, met411ORPA, met413ORPA, met433ORMr, met375ORPo, met486ORTc, met488ORTc, met489ORTc, tig22AMTa, tig24AMTa, tig25AMTa, tig27AMTa, tig675AMIk, tig701AMIk, tig755AMIk, tig756AMIk, tig7129AMTe, tig7132AMTe]
H55_tig(1)	[tig23AMTa]
H56_tig(1)	[tig26AMTa]
H57_tig(1)	[tig671AMIk]
H58_tig(2)	[tig4501AMSt, tig4502AMSt]
H59_mag(4)	[mag168MD, mag167MD, mag150MD, mag158MD]

Appendix B

Table B1. Rag1 intron 1 alleles (R1–R45) are listed next to the individual samples from which they were isolated. These labels are at the tips of the tree in Fig 2b. Species names are abbreviated as in Table 1 and the locality codes follow the specimen number. The haplotype label contains information of the species of origin and (in parenthesis) of the number of individuals from that species where the allele was found. For 24 individuals the allelic phases were resolved using the program Phase. Alleles reconstructed by this approach are marked with an asterisk. A complete list of specimens with their rag1 genotype and their linked nuclear and mitochondrial genotypes is presented in Appendix D.

R1_ret(4)	[ret95APG, ret106PG, ret126APG, ret133PG]
R2_ret(2)	[ret95BPG, ret126BPG]
R3_ret(6)pun(3)	[ret124PG, ret98PG, ret100APG*, ret94APG*, ret123APG*, ret1314PNCr, pun77AAMTa*, pun750AAMIk*, pun5155AMRB]
R4_pun(10)ret(6)	[ret96PG, ret108PG, ret130PG, ret100BPG*, ret94BPG*, ret123BPG*, pun109BAR, pun05BAMTa, pun750BAMIk*, pun997BAMMa, pun1433BAMXi, pun1534AAMXi, pun1535AMXi, pun1541AAMXi, pun01BTO*, pun02BTOB*]
R5_pun(61)fas(2)ori(1)	[pun27ME, pun43ME, pun28ME, pun62ME, pun44ME, pun68ME, pun67ME, pun45ME, pun55ME, pun05ME, pun61ME, pun47ME, pun31ME, pun46ME, pun24ME, pun29ME, pun66ME, pun8ME, pun69ME, pun51ME, pun60ME, pun11ME, pun49ME, pun52ME, pun10ME, pun54ME, pun21PM, pun20PM, pun17PM, pun23PM,

Appendix B (continued)

	pun25PM, pun35PM, pun73PM, pun13PM, pun16PM, pun63PM, pun26PM, pun18PM, pun72PM, pun71PM, pun30PM, pun6PM, pun22PM, pun70PM, pun15PM, pun58PM, pun57PM, pun53PM, pun59PM, pun09PM, pun12PM, pun03IT, pun04IT, pun40BTU, pun2BTU*, pun39BTU*, pun06BAMTa, pun31BAMTa, pun75BAMTa*, pun994BAMMa, pun5158BAMRB, ori121ORMt, fas60BRU, fas61ARU]
R6_pun(9)	[pun217PBSQ, pun218PBSQ, pun219PBSQ, pun2110PBSQ, pun2111PBSQ, pun2112PBSQ, pun2610PBSJ, pun2611PBSJ, pun2612PBSJ]
R7_pun(9)	[pun34TU, pun38TU, pun56TU, pun41TU, pun65TU, pun33TU, pun36BTU*, pun2ATU*, pun39ATU*]
R8_pun(1)	[pun36ATU*]
R9_pun(2)	[pun40ATU, pun01ATO*]
R10_pun(17)fas(1)ori(1)	[pun109AAR, pun06AAMTa, pun05AAMTa, pun753AAMIk, pun09AMTa, pun07AMTa, pun03AMTa, pun748AMIk, pun31AAMTa, pun77BAMTa*, pun7122BAMTe, pun994AAMMa, pun997AAMMa, pun5156AMRB, pun5158AAMRB, pun980BAMMa, pun1433AAMXi, fas61BRU, ori11AORCi]
R11_pun(3)	[pun753BAMIk, pun7122AAMTe, pun980AAMMa]
R12_pun(2)	[pun75AAMTa*, pun02ATO*]
R13_pun(1)	[pun633AAMIk*]
R14_pun(1)	[pun633BAMIk*]
R15_pun(2)	[pun1534BAMXi, pun1541BAMXi]
R16_pun(1)	[pun1313AAMRB*]
R17_pun(1)	[pun1313BAMRB*]
R18_fas(2)	[fas1312ARU, fas60ARU]
R19_fas(1)	[fas1312BRU]
R20_ori(1)	[ori11BORCu]
R21_ori(1)	[ori12AORCu*]
R22_ori(1)	[ori12BORCu*]
R23_ori(1)	[ori13AORCu*]
R24_ori(1)	[ori13BORCu*]
R25_ori(2)	[ori869ORAt, ori478ORPP]
R26_ori(13)	[ori485ORPz, ori122ORMt, ori614ORCa, ori114ORMt, ori502ORPz, ori612ORCa, ori120ORMt, ori483ORTc, ori371ORPo, ori372ORPo, ori373ORPo, ori412ORPA, ori466ORCr]
R27_cor(18)	[cor148APG, cor150APG, cor169PG, cor157PG, cor231APG, cor227PG, cor160PG, cor221APG*, cor146APG*, cor147APG*, cor153APG*, cor02PNRG,

Appendix B (continued)

R28_cor(30)	cor1316APNRG, cor212UR, cor213AUR, cor216AUR, cor215AUR, cor214AUR*] [cor148BPG, cor150BPG, cor231BPG, cor222PG, cor223BPG, cor217PG, cor226PG, cor228PG, cor155APG*, cor01PNRG, cor1315BPNCr, cor1316BPNRG, cor213BUR, cor216BUR, cor215BUR, cor171SF, cor205BSF, cor208SF, cor209SF, cor210SF, cor172SF, cor174SF, cor176SF, cor177SF, cor182SF, cor187SF, cor190SF, cor199SF, cor191SF, cor192SF]
R29_cor(6)	[cor223APG, cor221BPG*, cor146BPG*, cor147BPG*, cor1315APNCr, cor214BUR*]
R30_cor(1)	[cor232PG]
R31_cor(2)	[cor155BPG*, cor153BPG*]
R32_cor(1)	[cor205ASF]
R33_met(1)	[met581ORCa]
R34_met(1)	[met731AORCa*]
R35_met(1)	[met731BORCa*]
R36_met(9)tig(1)	[met117BORMt, met04BORPA, met119AORMt, met01BORPA, met488BORTc, met866BORAt, met433BORMr, met487AORTc, tig4501AAMSt, met469ORCr]
R37_tig(16)met(18)	[met116ORMt, met117AORMt, met02ORPA, met04AORPA, met03ORPA, met118ORMt, met119BORMt, met01AORPA, met488AORTc, met866AORAt, met375ORPo, met411ORPA, met413ORPA, met433AORMr, met453ORCr, met486ORTc, met487BORTc, met527ORAp, tig755AMiK, tig756AMiK, tig671AMiK, tig21AMTa, tig27AMTa, tig22AMTa, tig676AMiK, tig692AMiK, tig701AAMiK, tig25AAMTa*, tig26AAMTa*, tig4792AMSt, tig7132AAMTe, tig7129AMTe, tig4501BAMSt, tig4502AMSt]
R38_tig(1)	[tig24AAMTa]
R39_tig(4)	[tig24BAMTa, tig675AMiK, tig25BAMTa*, tig26BAMTa*]
R40_tig(1)	[tig701BAMiK]
R41_tig(1)	[tig7132BAMTe]
R42_mag(8)	[mag164AMD, mag168AMD, mag13AMD, mag14AMD, mag150AMD, mag151AMD, mag152AMD, mag159AMD]
R43_mag(10)	[mag168BMD, mag10MD, mag11MD, mag12MD, mag13BMD, mag14BMD, mag151BMD, mag152BMD, mag158MD, mag159BMD]
R44_Zungaro(1)	[Zungaro]
R45_	[Brachyplatystoma]
Brachyplatystoma(1)	

Appendix C

Table C1. S7 intron 1 alleles (S1–S41) are listed next to the individual samples from which they were isolated. These labels are at the tips of the tree in Fig 2c. Species names are abbreviated as in Table 1 and the locality codes follow the specimen number. The haplotype label contains information of the species of origin and (in parenthesis) of the number of individuals from that species where the allele was found. For 25 individuals the allelic phases were resolved using the program Phase. Alleles reconstructed by this approach are marked with an asterisk. A complete list of specimens with their S7int1 genotype and their linked nuclear and mitochondrial genotypes is presented in Appendix D.

S1_pun(30)	[pun5AME, pun8BME, pun44BME, pun27ME, pun29ME, pun31ME, pun32ME, pun42ME, pun45ME, pun54ME, pun55ME, pun66ME, pun06PM, pun07PM, pun13PM, pun16PM, pun17APM, pun18BPM, pun19PM, pun20PM, pun21BPM, pun22PM, pun23PM, pun25PM, pun30APM, pun35APM, pun72PM, pun73PM, pun09PM, pun03IT]
S2_pun(6)	[pun5BME, pun8AME, pun18APM, pun21APM, pun30BPM, pun35BPM]
S3_pun(1)	[pun44AME]
S4_pun(1)	[pun17BPM]
S5_pun(7)ret(5)ori(4)	[pun1ATU, pun02AAMTa, pun03AAMTa, pun05AAMTa, pun08BAMTa, pun750AAMiK, pun751AAMiK, ret106APGTq, ret127APGCb, ret135BPGCb, ret133APGMI*, ret137APGCb*, ori604AORCa*, ori612AORCa*, ori613AORCa*, ori113AORMt*]
S6_pun(1)	[pun1BTU]
S7_ori(3)pun(1)	[pun2TU, ori11BORCu*, ori13AORCu*, ori445AORCi*]
S8_ret(10)pun(4)ori(2)	[ret95APGMI, ret105PGMI, ret106BPGTq, ret107APGCb, ret108APGCb, ret127BPGCb, ret135APGCb, ret125PGCb, ret128BPGCb*, ret97BPGMI*, pun109BAR*, pun28BAMTa*, pun05BAMTa, pun750BAMiK, ori445BORCi*, ori447AORCi*]
S9_ret(4)	[ret95BPGMI, ret107BPGCb, ret108BPGCb, ret142BPGTq*]
S10_ret(2)	[ret126PGCb, ret142APGTq*]
S11_ret(3)	[ret128APGCb*, ret133BPGMI*, ret137BPGCb*]
S12_ret(1)pun(1)	[ret97APGMI*, pun109AAR*]
S13_pun(1)ori(1)	[pun28AAMTa*, ori11AORCu*]
S14_pun(2)	[pun02BAMTa, pun08AAMTa]
S15_pun(1)	[pun03BAMTa]
S16_pun(1)	[pun06AMTa]
S17_pun(1)	[pun751BAMiK]
S18_fas(1)	[fas1312AAMRB]
S19_fas(1)	[fas1312BAMRB]
S20_ori(19)	[ori376ORPo, ori120BORMt, ori1306ORCC, ori373ORPo,

(continued on next page)

Appendix C (continued)

	ori484ORTc, ori122BORMt, ori485ORPz, ori371AORPo, ori483BORTc, ori372BORPo, ori466AORCr, ori482BORTc, ori478ORPP, ori412ORPA, ori113BORMt*, ori547BORAp, ori604BORCa*, ori612BORCa*, ori613BORCa*]
S21_ori(5)	[ori120AORMt, ori122AORMt, ori483AORTc, ori13BORCu*, ori547AORAp]
S22_ori(2)	[ori371BORPo, ori466BORCr]
S23_ori(4)	[ori372AORPo, ori115ORMt, ori121ORMt, ori447BORCi*]
S24_ori(1)	[ori482AORTc]
S25_cor(11)	[cor149APGSL, cor153PGCb, cor160APGcb, cor232PGSL, cor169APGcb*, cor212UR, cor213UR, cor211BUR*, cor02PNRG, cor191BSF*, cor206ASF*]
S26_cor(4)	[cor149BPGSL, cor160BPGCb, cor216AUR*, cor01APNRG*]
S27_cor(3)	[cor169BPGCb*, cor216BUR*, cor01BPNRG*]
S28_cor(2)	[cor211AUR*, cor191ASF*]

Appendix C (continued)

S29_cor(4)	[cor187ASF*, cor206BSF*, cor208BSF, cor210ASF]
S30_cor(3)	[cor187BSF*, cor199BSF, cor205BSF]
S31_cor(4)	[cor199ASF, cor205ASF, cor208ASF, cor210BSF]
S32_met(9)	[met413BORPA, met489BORTc, met526ORAp, met375AORPo*, met488AORTc*, met469AORCr, met117ORMt, met118BORMt, met119ORMt]
S33_met(6)	[met469BORCr, met413AORPA, met486BORTc, met489AORTc, met118AORMt, met527AORAp*]
S34_met(2)	[met486AORTc, met488BORTc*]
S35_met(2)	[met375BORPo*, met527BORAp*]
S36_tig(6)	[tig676AAMik, tig755AAMik, tig756AAMik, tig24AMTa, tig26AAMTa, tig27BAMTa]
S37_tig(4)	[tig676BAMik, tig755BAMik, tig756BAMik, tig26BAMTa]
S38_tig(1)	[tig27AAMTa]
S39_mag(6)	[mag164MD, mag08MD, mag09MD, mag12MD, mag13MD, mag14AMD]
S40_mag(1)	[mag14BMD]
S41_Zungaro(1)	[Zungaro]

Appendix D

Table D1. List of individuals sequenced for this study, with their associated genotypes for the three molecular markers used (CITB, RAG1, and S7), voucher availability, and collector information.

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
cor 01PNRG	H39	R28	S26,S27	NO	FURNAS
cor 02PNRG	H39	R27	S25	NO	FURNAS
cor 03PNRG	H39	NO	FURNAS		
cor 1315PNCco	H46	R29,R28	SPECIMEN	M. SABAJ	
cor 1316PNCco	H39	R27,R28	SPECIMEN	M.SABAJ	
cor 143PG	H41	NO	E.K. RESENDE		
cor 144PG	H39	NO	E.K. RESENDE		
cor 145PG	H39	NO	E.K. RESENDE		
cor 146PG	H45	R27,R29	NO	E.K. RESENDE	
cor 147PG	H39	R27,R29	NO	E.K. RESENDE	
cor 148PG	H43	R27,R28	NO	E.K. RESENDE	
cor 149PG	H44	S25,S26	NO	E.K. RESENDE	
cor 150PG	H45	R27,R28	NO	E.K. RESENDE	
cor 153PG	R27,R31	S25	NO	E.K. RESENDE	
cor 155PG	H43	R28,R31	NO	E.K. RESENDE	
cor 156PG	H39	NO	E.K. RESENDE		
cor 157PG	R27	NO	E.K. RESENDE		
cor 158PG	H39	NO	E.K. RESENDE		
cor 160PG	H39	R27	S25,S26	NO	E.K. RESENDE
cor 169PG	H39	R27	S25,S27	NO	E.K. RESENDE
cor 217PG	R28	NO	E.K. RESENDE		
cor 221PG	R27,R29	NO	E.K. RESENDE		
cor 222PG	R28	NO	E.K. RESENDE		
cor 223PG	R29,R28	NO	E.K. RESENDE		
cor 226PG	R28	NO	E.K. RESENDE		
cor 227PG	R27	NO	E.K. RESENDE		
cor 228PG	R28	NO	E.K. RESENDE		
cor 231PG	R27,R28	NO	E.K. RESENDE		
cor 232PG	H46	NO	E.K. RESENDE		

Appendix D (continued)

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
cor 232PG	R30	S25	NO	E.K. RESENDE	
cor B04PG	H39	NO	E.K. RESENDE		
cor B05PG	H47	NO	E.K. RESENDE		
cor F06PG	H42	NO	E.K. RESENDE		
cor D12PG	H40	NO	E.K. RESENDE		
cor 170SF	H51	NO	A. B. SOUSA		
cor 171SF	H51	R28	NO	A. B. SOUSA	
cor 172SF	H51	R28	NO	A. B. SOUSA	
cor 173SF	H52	NO	A. B. SOUSA		
cor 174SF	H51	R28	NO	A. B. SOUSA	
cor 175SF	H51	NO	A. B. SOUSA		
cor 176SF	H51	R28	NO	A. B. SOUSA	
cor 177SF	R28	NO	A. B. SOUSA		
cor 182SF	R28	NO	A. B. SOUSA		
cor 187SF	R28	S29,S30	NO	A. B. SOUSA	
cor 190SF	R28	NO	A. B. SOUSA		
cor 191SF	H48	R28	S28,S25	NO	A. B. SOUSA
cor 192SF	H51	R28	NO	A. B. SOUSA	
cor 199SF	H48	R28	S31,S30	NO	A. B. SOUSA
cor 205SF	H49	R32 R28	S31,S30	NO	A. B. SOUSA
cor 208SF	H48	R28	S31 S29	NO	A. B. SOUSA
cor 209SF	H49	R28	NO	A. B. SOUSA	
cor 210SF	H50	R28	S31 S29	NO	A. B. SOUSA
cor E01SF	H48	NO	A. B. SOUSA		
cor E03SF	H51	NO	A. B. SOUSA		
cor E04SF	H48	NO	A. B. SOUSA		
cor SP2SF	H53	NO	A. B. SOUSA		
cor SP3SF	H49	NO	A. B. SOUSA		
cor AC1SF	H48	NO	A. B. SOUSA		
cor AC2SF	H52	NO	A. B. SOUSA		
cor D11SF	H48	S25 S29	NO	A. B. SOUSA	
cor 211UR	S28 25	NO	E. ZANIBONI-FILHO		
cor 212UR	H39	R27	S25	NO	E. ZANIBONI-FILHO
cor 213UR	H39	R27 R28	S25	NO	E. ZANIBONI-FILHO
cor 214UR	H39	R27 R29	NO	E. ZANIBONI-FILHO	
cor 215UR	H39	R27 R28	NO	E. ZANIBONI-FILHO	
cor 216UR	H39	R27 R28	S26 S27	NO	E. ZANIBONI-FILHO
fas 1312RU	H4	R18 R19	S18 S19	SPECIMEN	M. SABAJ
fas 60RU	H15	R18 R5	PHOTO	G. ORTÍ	
fas 61RU	H16	R5 R10	PHOTO	G. ORTÍ	
mag 08MD	S39	NO	G. ORTÍ		
mag 09MD	S39	NO	G. ORTÍ		
mag 10MD	R43	NO	G. ORTÍ		
mag 11MD	R43	NO	G. ORTÍ		
mag 12MD	R43	S39	NO	G. ORTÍ	
mag 13MD	R42 R43	S39	NO	G. ORTÍ	
mag 14MD	R42 R43	S39 S40	NO	G. ORTÍ	
mag 150MD	H59	R42	NO	G. ORTÍ	
mag 151MD	R42 R43	NO	G. ORTÍ		
mag 152MD	R42 R43	NO	G. ORTÍ		
mag 158MD	H59	R43	NO	G. ORTÍ	
mag 159MD	R42 R43	NO	G. ORTÍ		
mag 164MD	R42	S39	NO	M.C. ÁVILA	
mag 167MD	H59	NO	M.C. ÁVILA		
mag 168MD	H59	R42 R43	NO	M.C. ÁVILA	
met 01ORPA	R37 R36	NO	S.C. WILLIS		
met 02ORPA	H54	R37	NO	S.C. WILLIS	
met 03ORPA	H54	R37	NO	S.C. WILLIS	
met 04ORPA	H54	R37 R36	NO	S.C. WILLIS	
met 116ORMt	H54	R37	NO	M.C. ÁVILA	
met 117ORMt	H54	R37 R36	S32	NO	M.C. ÁVILA

(continued on next page)

Appendix D (continued)

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
met 118ORMt	H54	R37	S32 S33	NO	M.C. ÁVILA
met 119ORMt	H54	R36 R37	S32	NO	M.C. ÁVILA
met 375ORPo	H54	R37	S32 S35	PHOTO	N. LOVEJOY
met 411ORPA	H54	R37	PHOTO	N. LOVEJOY	
met 413ORPA	H54	R37	S33 S32	PHOTO	N. LOVEJOY
met 433ORMr	H54	R37 R36	PHOTO	N. LOVEJOY	
met 453ORCr	R36	PHOTO	N. LOVEJOY		
met 469ORCr	R36	S32 S33	PHOTO	N. LOVEJOY	
met 486ORTc	H54	R37	S34 S33	PHOTO	N. LOVEJOY
met 487ORTc	R36 R37	PHOTO	N. LOVEJOY		
met 488ORTc	H54	R37 R36	S32 S34	PHOTO	N. LOVEJOY
met 489ORTc	H54	S33 S32	PHOTO	N. LOVEJOY	
met 526ORAp	H54	S32	PHOTO	S.C. WILLIS	
met 527ORAp	H54	R37	S33 S35	PHOTO	S.C. WILLIS
met 528ORAp	H54	PHOTO	S.C. WILLIS		
met 581ORCa	R33	PHOTO	S.C. WILLIS		
met 731ORCa	R34 R35	PHOTO	S.C. WILLIS		
met 866ORAt	H54	R37 R36	PHOTO	S.C. WILLIS	
ori 113ORMt	S5 S20	NO	M.C. ÁVILA		
ori 114ORMt	H38	R26	NO	M.C. ÁVILA	
ori 115ORMt	H38	S23	NO	M.C. ÁVILA	
ori 110RCu	H10	R10 R20	S13 S7	NO	M.C. ÁVILA
ori 120ORMt	H38	R26	S21 S20	NO	M.C. ÁVILA
ori 121ORMt	H38	R5	S23	NO	M.C. ÁVILA
ori 122ORMt	H38	R26	S21 S20	NO	M.C. ÁVILA
ori 120RCu	H10	R21 R22	NO	M.C. ÁVILA	
ori 1306ORCG	S20	NO	S.C. WILLIS		
ori 130RCu	H10	R23 R24	S7 S21	NO	S.C. WILLIS
ori 371ORPo	H37	R26	S20 S22	NO	N. LOVEJOY
ori 372ORPo	R26	S23 S20	NO	N. LOVEJOY	
ori 373ORPo	H35	R26	S20	NO	N. LOVEJOY
ori 376ORPo	H38	S20	NO	N. LOVEJOY	
ori 412ORPA	R26	S20	PHOTO	N. LOVEJOY	
ori 414ORPA	H38	PHOTO	N. LOVEJOY		
ori 445ORCi	H36	S7 S8	PHOTO	S.C. WILLIS	
ori 446ORCi	H36	PHOTO	S.C. WILLIS		
ori 447ORCi	H36	S8 S23	PHOTO	S.C. WILLIS	
ori 466ORCr	H38	R26	S20 S22	PHOTO	N. LOVEJOY
ori 478ORPP	H38	R25	S20	PHOTO	S.C. WILLIS
ori 481ORTc	H38	PHOTO	N. LOVEJOY		
ori 482ORTc	H37	S24 S20	PHOTO	N. LOVEJOY	
ori 483ORPA	H38	R26	S21 S20	PHOTO	S.C. WILLIS
ori 484ORTc	S20	PHOTO	N. LOVEJOY		
ori 485ORPz	R26	S20	PHOTO	S.C. WILLIS	
ori 502ORPz	H38	R26	NO	S.C. WILLIS	
ori 547ORAp	H37	S21 S20	PHOTO	S.C. WILLIS	
ori 604ORCa	H38	S5 S20	NO	S.C. WILLIS	
ori 612ORCa	H38	R26	S5 S20	NO	S.C. WILLIS
ori 613ORCa	H38	S5 S20	NO	S.C. WILLIS	
ori 614ORCa	H37	R26	NO	S.C. WILLIS	
ori 687OrAt	H38	PHOTO	S.C. WILLIS		
ori 865ORAt	H10	PHOTO	S.C. WILLIS		
ori 869ORAt	H38	R25	PHOTO	S.C. WILLIS	
pun 01TO	H14	R9 R4	NO	N.M. PIORSKI	
pun 02TO	H1	R12 R4	NO	N.M. PIORSKI	
pun 109AR	H13	R10 R4	S12 S8	PHOTO	W. PEREZ
pun 04AMTa	H2	R3 R10	NO	M.D. LIZARAZO	
pun 05AMTa	H6	R10 R4	S5 S8	NO	M.D. LIZARAZO
pun 06AMTa	H7	R10 R5	S16	NO	M.D. LIZARAZO
pun 07AMTa	H2	R10	NO	M.D. LIZARAZO	
pun 08AMTa	H2	S14 S5	NO	M.D. LIZARAZO	

Appendix D (continued)

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
pun 09AMTa	H2	R10	NO	M.D. LIZARAZO	
pun 10AMTa	H1	NO	M.D. LIZARAZO		
pun 28AMTa	H3	S13 S8	NO	M.D. LIZARAZO	
pun 29AMTa	H6	NO	M.D. LIZARAZO		
pun 2AMTa	H2	R12 R5	S5 S14	NO	M.D. LIZARAZO
pun 30AMTa	H2	NO	M.D. LIZARAZO		
pun 31AMTa	H2	R10 R5	NO	M.D. LIZARAZO	
pun 3AMTa	H5	R10	S5 S15	NO	M.D. LIZARAZO
pun 2548AMMa	H2	NO	I.P. FARIAS		
pun 927AMMa	H2	NO	I.P. FARIAS		
pun 979AMMan	H2	NO	I.P. FARIAS		
pun 980AMMa	H9	R11 R10	NO	I.P. FARIAS	
pun 994AMMa	H3	R10 R5	NO	I.P. FARIAS	
pun 997AMMa	R10 R4	NO	I.P. FARIAS		
pun 7122AMTe	H1	R11 R10	NO	I.P. FARIAS	
pun 633AMik	H2	R13 R14	PHOTO	N. LOVEJOY	
pun 748AMik	R10	PHOTO	N. LOVEJOY		
pun 750AMik	H4	R3 R4	S5 S8	PHOTO	N. LOVEJOY
pun 751AMik	H2	S5 S17	PHOTO	N. LOVEJOY	
pun 753AMik	H8	R10 R11	PHOTO	N. LOVEJOY	
pun 1313AMRB	H10	R16 R17	SPECIMEN	M. SABAJ	
pun 5155AMRB	R3	NO	I.P. FARIAS		
pun 5156AMRB	H10	R10	NO	I.P. FARIAS	
pun 5158AMRB	H10	R10 R5	NO	I.P. FARIAS	
pun 1382AMXi	H11	NO	I.P. FARIAS		
pun 1433AMXi	H11	R10 R4	NO	I.P. FARIAS	
pun 1534AMXi	R4 R15	NO	I.P. FARIAS		
pun 1535AMXi	R4	NO	I.P. FARIAS		
pun 1537AMXi	H11	NO	I.P. FARIAS		
pun 1539AMXi	H12	NO	I.P. FARIAS		
pun 1540AMXi	H12	NO	I.P. FARIAS		
pun 1541AMXi	H11	R4 R15	NO	I.P. FARIAS	
pun 03IT	H27	R5	S1	NO	N.M. PIORSKI
pun 04IT	H28	R5	NO	N.M. PIORSKI	
pun 216ITRo	H28	NO	N.M. PIORSKI		
pun 2110PBSQ	R6	NO	N.M. PIORSKI		
pun 2111PBSQ	R6	NO	N.M. PIORSKI		
pun 2112PBSQ	R6	NO	N.M. PIORSKI		
pun 217PBSQ	R6	NO	N.M. PIORSKI		
pun 218PBSQ	H29	R6	NO	N.M. PIORSKI	
pun 218PBSQ	R6	NO	N.M. PIORSKI		
pun 219PBSQ	R6	NO	N.M. PIORSKI		
pun 212PBGd	H29	NO	N.M. PIORSKI		
pun 2610PBGd	R6	NO	N.M. PIORSKI		
pun 2611PBGd	H29	NO	N.M. PIORSKI		
pun 2611PBGd	R6	NO	N.M. PIORSKI		
pun 2612PBGd	H30	R3 R4	NO	N.M. PIORSKI	
pun 268PBGd	H19	NO	N.M. PIORSKI		
pun 09PM	H22	R5	S1	NO	N.M. PIORSKI
pun 13PM	H19	R5	S1	NO	N.M. PIORSKI
pun 12PM	H22	R5	NO	N.M. PIORSKI	
pun 15PM	H21	R5	NO	N.M. PIORSKI	
pun 16PM	R5	S1	NO	N.M. PIORSKI	
pun 17PM	H19	R5	S1 S4	NO	N.M. PIORSKI
pun 18PM	R5	S2 S1	NO	N.M. PIORSKI	
pun 20PM	H22	R5	S1	NO	N.M. PIORSKI
pun 21PM	H19	R5	S2 S1	NO	N.M. PIORSKI
pun 22PM	H19	R5	NO	N.M. PIORSKI	
pun 23PM	H21	R5	S1	NO	N.M. PIORSKI
pun 25PM	H19	R5	S1	NO	N.M. PIORSKI
pun 26PM	H19	R5	NO	N.M. PIORSKI	

(continued on next page)

Appendix D (continued)

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
pun 30PM	H25	R5	S1 S2	NO	N.M. PIORSKI
pun 35PM	H19	R5	S1 S2	NO	N.M. PIORSKI
pun 53PM	H21	R5	NO	N.M. PIORSKI	
pun 57PM	H22	R5	NO	N.M. PIORSKI	
pun 58PM	H19	R5	NO	N.M. PIORSKI	
pun 59PM	H22	R5	NO	N.M. PIORSKI	
pun 6PM	H19	R5	S1	NO	N.M. PIORSKI
pun 70PM	R5	NO	N.M. PIORSKI		
pun 73PM	R5	S1	NO	N.M. PIORSKI	
pun 71PM	H19	R5	NO	N.M. PIORSKI	
pun 72PM	H19	R5	S1	NO	N.M. PIORSKI
pun 63PM	H26	R5	NO	N.M. PIORSKI	
pun C05PM	H22	NO	N.M. PIORSKI		
pun C11PM	H21	S1	NO	N.M. PIORSKI	
pun SP9PM	H19	NO	N.M. PIORSKI		
pun SP7PM	H19	NO	N.M. PIORSKI		
pun D05PM	H19	S1	NO	N.M. PIORSKI	
pun E12PM	H22	NO	N.M. PIORSKI		
pun F04PM	H19	NO	N.M. PIORSKI		
pun 10ME	R5	NO	N.M. PIORSKI		
pun 11ME	H19	R5	NO	N.M. PIORSKI	
pun 05ME	R5	S1 S2	NO	N.M. PIORSKI	
pun 24ME	R5	NO	N.M. PIORSKI		
pun 27ME	H19	R5	S1	NO	N.M. PIORSKI
pun 28ME	H19	R5	NO	N.M. PIORSKI	
pun 66ME	R5	S1	NO	N.M. PIORSKI	
pun 67ME	H20	R5	NO	N.M. PIORSKI	
pun 69ME	H19	R5	NO	N.M. PIORSKI	
pun A07ME	H19	NO	N.M. PIORSKI		
pun AC10ME	H20	NO	N.M. PIORSKI		
pun C06ME	H19	NO	N.M. PIORSKI		
pun C07ME	H21	R5	S1	NO	N.M. PIORSKI
pun C08ME	H22	R5	S2 S1	NO	N.M. PIORSKI
pun D07ME	H24	R5	NO	N.M. PIORSKI	
pun D08ME	H19	NO	N.M. PIORSKI		
pun D10ME	H19	NO	N.M. PIORSKI		
pun E10ME	H19	R5	NO	N.M. PIORSKI	
pun SP4ME	H23	NO	N.M. PIORSKI		
pun SP8ME	H19	NO	N.M. PIORSKI		
pun 31ME	H19	R5	S1	NO	N.M. PIORSKI
pun 32ME	S1	NO	N.M. PIORSKI		
pun 39ME	H19	NO	N.M. PIORSKI		
pun 42ME	S1	NO	N.M. PIORSKI		
pun 43ME	R5	NO	N.M. PIORSKI		
pun 44ME	R5	S3 S1	NO	N.M. PIORSKI	
pun 45ME	H19	R5	S1	NO	N.M. PIORSKI
pun 47ME	H19	R5	NO	N.M. PIORSKI	
pun 49ME	R5	NO	N.M. PIORSKI		
pun 51ME	H19	R5	NO	N.M. PIORSKI	
pun 52ME	R5	NO	N.M. PIORSKI		
pun 54ME	R5	S1	NO	N.M. PIORSKI	
pun 55ME	H19	R5	S1	NO	N.M. PIORSKI
pun 60ME	R5	NO	N.M. PIORSKI		
pun 61ME	R5	NO	N.M. PIORSKI		
pun 62ME	R5	NO	N.M. PIORSKI		
pun 2TU	R7 R5	S7	NO	N.M. PIORSKI	
pun 33TU	H18	R7	NO	N.M. PIORSKI	
pun 34TU	R7	NO	N.M. PIORSKI		
pun 01TU	H18	S5 S6	NO	N.M. PIORSKI	
pun 36TU	H17	R8 R7	NO	N.M. PIORSKI	
pun 38TU	H17	R7	NO	N.M. PIORSKI	

Appendix D (continued)

SAMPLE	CITB	RAG1	S7	VOUCHER	COLECTOR
pun 39TU	H18	R7 R5	NO	N.M. PIORSKI	
pun 40TU	H18	R9 R5	NO	N.M. PIORSKI	
pun 41TU	H17	R7	NO	N.M. PIORSKI	
pun 56TU	H18	R7	NO	N.M. PIORSKI	
pun 65TU	H17	R7	NO	N.M. PIORSKI	
pun sp5TU	H18	NO	N.M. PIORSKI		
ret 100PG	H30	R4	NO	E.K. RESENDE	
ret 105PG	S8	NO	E.K. RESENDE		
ret 106PG	R1	S8	NO	E.K. RESENDE	
ret 107PG	H31	S8 S9	NO	E.K. RESENDE	
ret 108PG	H30	R4	S8 S9	NO	E.K. RESENDE
ret 123PG	H33	R3 R4	NO	E.K. RESENDE	
ret 124PG	H30	R3	NO	E.K. RESENDE	
ret 125PG	S8	NO	E.K. RESENDE		
ret 126PG	R1 R2	S10 S5	NO	E.K. RESENDE	
ret 127PG	H30	S5 S8	NO	E.K. RESENDE	
ret 128PG	H30	S11 S8	NO	E.K. RESENDE	
ret 130PG	R4	NO	E.K. RESENDE		
ret 1314PNCr	R3	SPECIMEN	M. SABAJ		
ret 132PG	H33	NO	E.K. RESENDE		
ret 133PG	H34	R1	S5 S11	NO	E.K. RESENDE
ret 135PG	H30	S8 S5	NO	E.K. RESENDE	
ret 136PG	H31	NO	E.K. RESENDE		
ret 137PG	H30	S5 S11	NO	E.K. RESENDE	
ret 138PG	H30	NO	E.K. RESENDE		
ret 139PG	H30	NO	E.K. RESENDE		
ret 140PG	H30	NO	E.K. RESENDE		
ret 142PG	S10 S9	NO	E.K. RESENDE		
ret 94PG	R3 R4	NO	E.K. RESENDE		
ret 95PG	H30	R1 R2	S8 S9	NO	E.K. RESENDE
ret 96PG	H30	R3	NO	E.K. RESENDE	
ret 97PG	H32	S12 S8	NO	E.K. RESENDE	
ret 98PG	H30	NO	E.K. RESENDE		
ret A11PG	H30	NO	E.K. RESENDE		
ret SP1PG	H30	NO	E.K. RESENDE		
tig 21AMTa	R37	NO	M.D. LIZARAZO		
tig 22AMTa	H54	R37	NO	M.D. LIZARAZO	
tig 23AMTa	H55	NO	M.D. LIZARAZO		
tig 24AMTa	H54	R38 R39	S36	NO	M.D. LIZARAZO
tig 25AMTa	H54	R37 R39	NO	M.D. LIZARAZO	
tig 26AMTa	H56	R37 R39	S36 S37	NO	M.D. LIZARAZO
tig 27AMTa	H54	R37	S38 S36	NO	M.D. LIZARAZO
tig 4501AMSt	H58	R36 R37	NO	I.P. FARIAS	
tig 4502AMSt	H58	R37	NO	I.P. FARIAS	
tig 4792AMSt	R37	NO	I.P. FARIAS		
tig 671AMIk	H57	R37	PHOTO	N. LOVEJOY	
tig 675AMIk	H54	R39	PHOTO	N. LOVEJOY	
tig 676AMIk	R37	S36 S37	PHOTO	N. LOVEJOY	
tig 692AMIk	R37	PHOTO	N. LOVEJOY		
tig 701AMIk	H54	R37 R40	PHOTO	N. LOVEJOY	
tig 755AMIk	H54	R37	S36 S37	PHOTO	N. LOVEJOY
tig 756AMIk	H54	R37	S36 S37	PHOTO	N. LOVEJOY
tig 7129AMTe	H54	R37	NO	I.P. FARIAS	
tig 7132AMTe	H54	R37 R41	NO	I.P. FARIASSSS	

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