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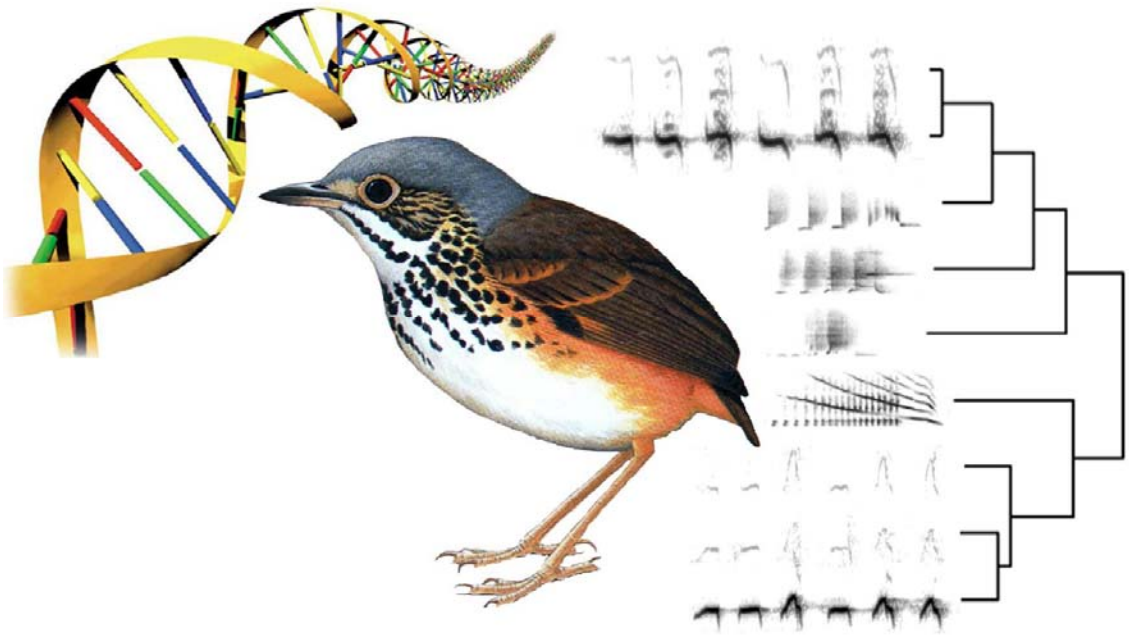
Lincoln Silva Carneiro



BELÉM – PARÁ
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Variação morfológica, vocal e molecular em *Hylopezus macularius* (Temminck, 1830) (Aves, Grallariidae).

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Dissertação de mestrado apresentada ao Programa de Pós-graduação em Zoologia, Curso de Mestrado, do Museu Paraense Emílio Goeldi e Universidade Federal do Pará como requisito para obtenção do grau de mestre em Zoologia.

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SUMÁRIO

1. Introdução Geral

O objetivo do presente projeto foi revisar a taxonomia e os limites interespecíficos da espécie politípica *Hylopezus macularius* (Aves: Grallaridae) baseando-se em caracteres morfológicos, vocais e moleculares, no intuito de delimitar unidades evolutivas independentes que tenham relevância para conservação e que possam fornecer suporte para estudos biogeográficos. Avaliar a validade e os limites interespecíficos de táxons crípticos é de fundamental importância para a conservação desses grupos que dependem, em grande parte, do reconhecimento de suas identidades taxonômicas reais. Além disso, tais estudos fornecem uma avaliação acurada do patrimônio biológico da região Amazônica e, conseqüentemente, corrigem uma possível sub-estimativa da sua biodiversidade. A proposta desenvolvida neste trabalho poderá servir de modelo para outros estudos envolvendo espécies crípticas, que em conjunto contribuirão para um melhor conhecimento da biogeografia da Amazonia.

Na região Neotropical a revisão de espécies politípicas tem sido sugerida para resolver incongruências envolvendo conceitos de espécie, eleger áreas prioritárias para a conservação, identificar corretamente a diversidade dos complexos de espécies e fornecer condições para uma avaliação mais precisa da real historia evolutiva dos grupos (Zimmer 2002, Aleixo 2004). A expansão do conhecimento da avifauna Neotropical tem evidenciado que vários táxons descritos ou atualmente considerados subespécies, podem ser reconhecidos como espécies distintas (Isler *et al*. 1999). Um grande número de estudos revelou que diversas espécies com ampla distribuição na bacia Amazônica são na verdade compostas por um complexo de espécies (Bierregaard *et al.* 1997, Krabbe *et al.*1999, Aleixo 2002, 2004, Zimmer 2002), ou seja, por

populações alopátricas bastante diferenciadas vocal e geneticamente, que se comportam evolutivamente como espécies independentes. Portanto, é certo que novos estudos revelarão uma diversidade de espécies muito maior do que aquela conhecida atualmente para a avifauna Amazônica.

O gênero *Hylopezus* foi descrito por Ridgway em (1909) e tem como espécie-tipo *H. perspicillatus,* coletado no Equador. A espécie *Hylopezus macularius* foi descrita por Temminck em 1823 sob o nome de *Pitta macularia* e tendo como localidade tipo "Brésil". Em 1842 Lafresnaye sugeriu que a espécie mudasse para o gênero *Grallaria,* e em 1848 Cabanis sugeriu a mudança para o gênero *Colobathris*. A maioria dos autores que revisaram o gênero seguiu Lafresnaye e continuou usando o nome de *Grallaria* para o gênero. Hellmayr (1910) sugeriu que a localidade tipo de *H. macularius* seria "Cayenne" na Guiana Francesa e não "Brésil" como dito por Temminck. Cory & Hellmayr (1924), levantou a possibilidade de *Pitta macularia* Temminck ser sinônimo de *Myioturdus ochroleucus* Wied, questão esclarecida através do exame de fotos do tipo coletado por Temminck, que nos foram cedidas pelo Museu de Leiden, ficou claro que tratam-se realmente de dois táxons distintos. Em (1969) Lowery e O'Neill, sugeriram que a espécie mudasse para o gênero *Hylopezus* e passasse a se chamar *Hylopezus macularius,* com base em alguns caracteres morfométricos, como tamanho das asas e do tarso e no padrão de cor das primarias e das coberteiras das asas da espécie-tipo do gênero, *H. perspicillatus.* A sugestão de Lowery e O'Neill (1969), é a mais bem aceita atualmente, sendo aceita inclusive pelo CBRO (2008) e pelo (SACC 2008).

A espécie *H. macularius* é tratada atualmente como espécie politípica com quatro subespécies reconhecidas (Krabbe e Schulenberg 2003): (1) *Hylopezus*

macularius macularius (Temminck, 1830) que tem como localidade tipo "Cayenne" e cujo espécime-tipo está depositado no Museu de Leiden, Holanda. Distribuição: norte da Venezuela (Sierra de Imataca), Guianas, norte do Brasil, a oeste até Manaus; uma população disjunta ao sul do rio Amazonas ocorre entre os rios Xingú e Tocantins. Descrição: Sexos idênticos. O adulto tem o topo da cabeça e a nuca cinza ou verde-oliva acinzentado. O espaço entre os olhos e o bico é amarelo, a região em torno dos olhos é ocre, e a região auricular verde-oliva com listras pretas e amarelas; resto das partes superiores verde-oliva e marrom, pontas das coberteiras amarelas ou avermelhadas, formando barras fracas na asa, coberteiras das primárias pretas, contrastando com manchas avermelhadas pequenas e bem definidas na base das primárias, restante das primárias verde-oliva ou marrom, garganta branca, estria malar preta, peito branco, coberto com amarelo a com estrias pretas curtas, barriga clara, flancos e crisso ocre-amarronzado; íris marrom escuro; mandíbula superior preta, inferior rosa com ponta preta; tarso marrom-rosado. (2) *Hylopezus macularius paraensis* (Snethlage, 1907) Descrito como *Grallaria macularia berlepschi* e tendo como localidade tipo Ourém, Rio Guamá, estado do Pará. Porém, *Grallaria berlepschi* era um nome pré-ocupado descrito por Hellmayr em 1903, obrigando uma mudança do nome do táxon que passou a se chamar *Grallaria macularia paraensis* Snethlage,1910. De acordo com a descrição, o espécime-tipo está depositado no MPEG, mas o mesmo não se encontra na coleção, tendo sido provavelmente transferido para o museu de zoologia Berlin nos anos 1920's como outros espécimes-tipo existentes no MPEG até aquela data. Distribuição: Brasil, ao sul do Rio Amazonas, da margem esquerda do Rio Madeira para o leste até Belém e sul de Rondônia. Supostamente, a população de *H. macularius* distribuída entre a margem oeste do rio Negro e a margem norte do Solimões pertence também a esta

subespécie (Krabbe e Schulenberg 2003). Diagnose em relação à forma nominal: Estrias amarelas do peito bem definidas no preto, flancos amarelo mais pálido (Krabbe e Schulenberg 2003). (3) *Hylopezus macularius diversus* (Zimmer, 1934), cuja localidade tipo é Puerto Indiana, margem norte do rio Amazonas, Peru. O espécime tipo esta depositado no American Museum of Natural History (AMNH) Distribuição: Sul da Venezuela (região em torno do Monte Duida, parte superior do rio Orinoco e Cano Casiquiare), sudeste da Colômbia (Loretoyacu, no extremo sudeste do Amazonas), nordeste do Peru a norte do rio Amazonas (Iquitos, Puerto Indiana) sul do rio Marañon e oeste do rio Ucayali (Pacaya-Samiria), (Zimmer 1934, Krabbe e Schulenberg 2003), (Fig. 1). Diagnose: Mais amarronzado que o *H. m. paraensis* e com as estrias do eixo do manto mais visíveis.

O arranjo taxonômico que criou a espécie politípica *H. macularius* (Zimmer 1934) já se tornou bastante antigo e foi feito com base num número limitado de espécimes. Como verificado em outros estudos, várias espécies biológicas delimitadas desta maneira no início do século 20 foram mais tarde subdivididas com base em estudos que incorporaram caracteres vocais e/ou genéticos nas análises (Aleixo 2004). Nossas análises bioacusticas indicaram uma pronunciada variação vocal entre as subespécies do complexo *H. macularius*, que até agora haviam sido tratada taxonomicamente com base apenas em caracteres de plumagem (Snethlage 1907, Hellmayr 1910, Cory e Hellmayr 1924, Zimmer 1934). Os dados moleculares mostraram que todas as subespécies são reciprocamente monofiléticas. Além da constatação da validade das subespécies analisadas, um novo táxon tratado como espécie contida no complexo foi descoberto e é descrito neste trabalho. Inferências evolutivas acerca dessa nova espécie e a posição dos diferentes táxons do grupo

constituem mais um estudo de caso sobre a estrutura filogeográfica e a biogeografia histórica de um grupo de organismos endêmico da Amazônia.

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THE AUK

SYSTEMATIC REVISION OF THE SPOTTED ANTPITTA *HYLOPEZUS MACULARIUS*, (GRALLARIIDAE), WITH DESCRIPTION OF A CRYPTIC NEW SPECIES FROM BRAZILIAN AMAZONIA

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ABSTRACT.- A systematic revision of the polytypic Spotted Antpitta (*Hylopezus macularius*, Grallariidae) based on morphometric, plumage, vocal, and molecular characters is presented. Morphological and vocal analyses were based, respectively, on 45 specimens and 104 recordings. Molecular phylogenies were inferred based on ca. 1.371 bp of the mitochondrial DNA genes 16S, ND2, and cyt *b* belonging to 26 specimens, including several outgroups. Our results revealed the existence of an undescribed taxon endemic to the Madeira – Xingu interfluve, cryptically similar in morphology to *paraensis*, but vocally and genetically readily distinguished from the latter and any other taxon grouped under *H. macularius*. Molecular trees obtained recovered with strong support the reciprocal monophyly among four main lineages of the Spotted Antpitta, three corresponding to already named taxa (*dilutus, macularius*, and *paraensis*), and one to the unnamed taxon, which is described herein. We show that those four taxa are also mutually diagnosed by a combination of both vocal and morphological features, and therefore recommend treating them as separate species. Dating of the molecular trees indicated that splits among species of the Spotted Antpitta complex took place between 2.92 and 0.78 mya, with the older splits concentrated in northwestern Amazonia (across the Negro and upper Amazon rivers) and the most recent ones in the southeastern part of the basin (across the Xingu river).

Key words: *Hylopezus macularius*, molecular systematics, song evolution, species limits, taxonomy, vocal characters

Resumo. - Uma revisão sistemática da espécie politípica *Hylopezus macularius* (Grallariidae), baseada em caracteres morfométricos, de plumagem, vocais e moleculares, é apresentada. As análises morfológicas e vocais foram baseadas, respectivamente, em 45 espécimes e em 104 gravações. As filogenias moleculares basearam-se em 1.371 pares de bases de ADN dos genes mitocondriais 16S, ND2, e cyt *b* de 26 espécimes, incluindo diversos táxons como grupos externos. Nossos resultados revelaram a existência de um táxon não descrito, endêmico do interflúvio Xingu - Madeira, cripticamente similar morfologicamente ao *paraensis*, mas distinguível vocal e geneticamente do último e de todos os outros táxons agrupados sob *H. macularius*. As árvores moleculares obtiveram forte apoio e monofiletismo recíproco entre as quatro linhagens principais de *H. macularius*, três das quais correspondem aos táxons já nomeados (*dilutus, macularius,* e *paraensis*), e um ao táxon anônimo, que é descrito neste trabalho. Nós mostramos que aqueles quatro táxons são mutuamente diagnosticáveis através de uma combinação de características vocais e morfológicas, portanto recomendamos tratá-los como espécies separadas. Datas das árvores moleculares indicaram que as separações entre espécies do complexo ocorreram entre 2.92 e 0.78 milhões de anos, com as separações mais antigas concentradas no noroeste da Amazônia (através do rio Negro) e as mais recentes na parte sudeste da bacia (através do rio Xingu).

Palavras chaves: *Hylopezus macularius*, sistemática molecular, evolução vocal, limite entre espécies, taxonomia, caracteres vocais

INTRODUCTION

The genus *Hylopezus* was described by Ridgway (1909) and currently includes eight recognized species distributed throughout most of the Neotropics (Honduras to northeastern Argentina): Spotted Antpitta *H. macularius* (Temminck 1830), Streak-chested Antpitta *H. perspicillatus* (Ridgway 1884), White-lored Antpitta *H. fulviventris* (P. L. Sclater 1858), Amazonian Antpitta *H. berlepschi* (Hellmayr 1903), White-browed Antpitta *H. ochroleucus* (Wied 1831), Masked Antpitta *H. auricularis* (Gyldenstolpe 1941), Speckle-breasted Antpitta *H. nattereri* (Pinto 1937), and Thicket Antpitta *H. dives* (Salvin 1865) (Krabbe and Schulenberg 2003 and SACC 2009). The Spotted-Antpitta *H. macularius* is endemic to the Amazon basin in South America, inhabiting both upland and seasonally-flooded lowland humid forests. Currently, *H. macularius* is treated as a polytypic species with three recognized subspecies: *H. m. macularius* (Temminck 1830), *H. m. paraensis* (Snethlage 1910), *H. m. diversus* (Zimmer 1934), (Krabbe and Schulenberg 2003). This taxonomic treatment began with Snethlage (1907) who described *paraensis* as a subspecies of *H. macularius* and was consolidated by Zimmer (1934), who lumped *macularius*, *paraensis*, and the new taxon he described, *diversus,* into a single biological species because plumage differences separating them were very subtle, suggesting only subspecific differentiation. Beyond these taxons currently recognized, has a fourth subspecie, *H. m. dilutus*, described by Hellmayr (1910) and later synonimized in *paraensis* by Cory and Hellmayr (1924) who our analyses indicated as a valid taxon (see below).

However, recent fieldwork conducted by us and other colleagues indicated that pronounced vocal variation exists among subspecies of *H. macularius*, and that major vocal patterns conflict strongly with all taxonomic treatments proposed so far based on plumage characters (Snethlage 1910; Cory and Hellmayr 1924; Zimmer 1934; Krabbe and Schulenberg 2003). More recently, Maijer (1998) showed that *H. auricularis* (until then regarded as a subspecies of *H. macularius*) is sufficiently vocally so distinct from any other taxa grouped under the Spotted Antpitta and thus deserved full species status, a recommendation which has been followed ever since (Krabbe and Schulenberg 2003 and SACC 2009). Other similar examples of cryptic undescribed variation still persist in the polytypic *H. macularius* complex,suggesting that further splits are warranted (Krabbe and Schulenberg 2003; SACC 2009 and pers. obs.).

Therefore, a major multi-character taxonomic revision is long due for *H. macularius* to objectively base taxonomic decisions concerning the ranking of its taxa and also to provide an additional framework for the study of Amazonian historical biogeography. Here, we use a combination of morphological, vocal, and molecular characters to review the taxonomy and inter-specific limits in *H. macularius*, and also to elucidate its evolutionary history. Our analyses show that the four main molecular lineages recovered for *H. macularius* are also reciprocally diagnosed by a combination of both vocal and morphological features, and therefore are best treated as separate species, including a new taxon described herein.

TAXONOMIC HISTORY

The Spotted Antpitta was originally described by C J. Temminck as *Pitta macularia* in his book "Nouveaux Recueil de Planches Coloriées d'Oiseaux, pour servir de suite et de complément aux Planches Enluminées de Buffon", published in Paris, France, in several installments between 1820 and 1829. As explained by Dekker (2003), *P. macularia* is not illustrated but only described in the introduction to the genus *Pitta* of which only the first page gives reference to book 85, published in 1830, not 1823 as usually given in the literature for *Pitta macularia* (Cory and Hellmayr 1924). Temminck´s short description of *P. macularia* was based on an adult specimen (mounted skin) currently deposited at RMNH under accession number 88799 and clearly labeled as the type and having only "Le Brésil" as collecting locality (Cory and Hellmayr 1924, Dekker 2003). Subsequently, based on the description given by Temminck, Hellmayr (1910) corrected the type locality to "Cayenne", French Guyana, but later remarked that *Pitta macularia* could in fact be an earlier name for *Myioturdus ochroleucus* Wied, 1831 now *Hylopezus ochroleucus*, whose type was lost until at least the late 20th Century (Cory and Hellmayr 1924, Whitney et al. 1995). Cory and Hellmayr (1924) suspicion was based on a supposed specimen of *Grallaria ochroleuca* (*H. ochroleucus*) also deposited at RMNH and described by Wied (1831) as similar to Temminck´s *Pitta macularia*, but with the sides of breast and flanks only spotted. To settle this question, Cory and Hellmary (1924) argued that a re-examination of Temminck´s type was needed. A good quality picture of *Pitta macularia* holotype can be found in Dekker (2003), where the characteristic ochraceous flanks and sides of breast typical of *H. macularius* can be seen, thus leaving no doubt that Wied (1831) *Grallaria ochroleuca* and Temminck´s bird do not belong to the same taxon. Therefore, the name *macularia* is unequivocally applicable to the Amazonian Spotted Antpitta,

although the exact collecting locality of the holotype will be never known with certainty. Given this uncertainty and the fact that all natural populations of *H. macularius* characterized in this study are very similar morphologically, the best alternative seems to be to agree with Hellmayr (1910) in establishing Cayenne as *Pitta macularia* type locality; this makes sense historically, since Cayenne in French Guyana (where *H. macularius* is common; Restall et al. 2006) was an important trading post in the early 19th Century, from where many bird skins (called "Cayenne skins") studied during this time in Europe originated. Therefore, the name *macularia* is applicable only to natural population D characterized in this study (Figure 1), which is endemic to the Guyana center of endemism, but not to birds distributed between the Xingu and Tocantins rivers as previously suggested (Krabbe and Schulenberg 2003; see below).

The next taxon described for the Spotted Antpitta was *Grallaria macularia berlepschi*, based on a single male specimen collected at Ourém, state of Pará, Brazil (Snethlage 1907); however, the name *berlepschi* was already preoccupied by *Grallaria berlepschi* Hellmayr, 1903 (today *Hylopezus berlepschi*), and thus the new taxon was later named *Grallaria macularia paraensis* (Snethlage 1910). Therefore, the name *paraensis* is unequivocally applicable to population A of *H. macularius* (Figure 1); birds from this population, occurring between the Xingu and Tocantins rivers, were mistakenly called *macularius* by Krabbe and Schulenberg (2003), since they belong vocally, genetically, and morphologically to diagnostic population A along with birds occurring east of the Tocantins, where the *paraensis* type locality is located (Figures 1, 2, 6 and 7).

Hellmayr (1910) described *Grallaria macularia diluta* based on three specimens and chose as the holotype a bird collected by J. Natterer at Tomar on the rigth bank of

the upper Negro river in the Brazilian state of Amazonas. Interestingly, one of the birds studied by Hellmayr (1910) was the holotype of *Grallaria macularia berlepschi* from Ourém, and he concluded that this bird and the holotype of *diluta* were "perfectly identical", but at the time decided to give them a new name because Snethlage´s *Grallaria macularia berlepschi* was preoccupied. However, Snethlage (1910) had already renamed the bird from Ourém as *Grallaria macularia paraensis* in a short communication published just a few days before Hellmayr (1910) description of *diluta* came to light. Accordingly, later Cory and Hellmayr (1924) synonimized *diluta* into *paraensis* and recognized only two subspecies in the Spotted Antpitta: macularius (distributed in the Guyanas) and *paraensis* (occurring from the left bank of the Negro river westward to northern Peru and on the south bank of the Amazon to eastern Pará). Ten years later, Zimmer (1934) described *Grallaria macularia diversa* from Puerto Indiana at the mouth of the Napo river in northern Peru and included in it 12 additional specimens he had at hand from southern Venezuela. Zimmer (1934) thought that the name *diluta* would be available for this population, but upon a consultation of Hellmayr, concluded that the holotype of *diluta* was distinct enough (particularly due to the greenish rather than brownish back, distinct pale shaft lines, wing coverts with well defined ochraceous tips, and primary tips with conspicuous pale outer margins) to be considered a different taxon. although no specimens of *diversa* were analyzed in this study, Zimmer´s (1934) diagnosis fits very well the features of the series of five specimens we examined from the region between the west bank of the Negro and northern bank of the Amazon rivers in Brazil (Figure 1, Appendix 1), thus in the same interfluve as *diluta´*s type locality (Tomar). We also had access to two high quality digitized pictures of the holotype of *diluta* and concluded that it possessed most of the

features diagnosing *diversa* (including the brownish back, *contra* Hellmayr), the notable exception being the conspicuous pale shaft lines on the back, which tend to be absent or obsolete in *diversa* and also in the series of Brazilian specimens mentioned above. An explanation to this apparent contradiction is that pale shaft lines are less numerous or absent on the back surface of the series of Brazilian specimens examined directly by us, but nonetheless present just below the surface as down feathers. Thus, we hypothesize that during collection, preparation, or nearly 180 years of deposition in a collection, the holotype of *diluta* lost some of its outer back feathers making the pale dorsal shaft lines more evident as in other taxa of the Spotted Antpitta, except *diversa*. This line of evidence added to the fact that (1) Zimmer (1934) also included in *diversa* a series of 11 southern Venezuela specimens collected much closer to *diluta´*s type locality in Brazil than that of *diversa* itself in Peru; (2) our vocal samples of Brazilian and Peruvian birds distributed north of the Amazon between the Negro and Napo rivers could not be distinguished from each other, and shared the same pattern exhibited by population C of the Spotted Antpitta, thus prompting us to propose the synonymization of *diversa* into *diluta*, the name with priority. Consequently, the name *paraensis* is no longer applicable to population C of the Spotted Antpitta, as originally put forward by Cory and Hellmayr (1924) when they mistakenly synonymized the latter taxon with *diluta* based on only three specimens from widely separated locations (Hellmayr 1910).

Population B belonging to the new taxon described above was historically treated under *paraensis*, a name in fact applicable to the vocally and genetically distinct population A (Figures 1, 2, 6 and 7). In their review of Spotted Antpitta taxonomy, Krabbe and Schulenberg (2003) noted that two vocal types were present in the traditional *paraensis* area of distribution south of the Amazon (see also Isler and

Whitney 2002, Marantz and Zimmer 2006), but since one of those vocal types (loudsong of population A in Figure 1) was (to audition at least) more similar to that of the nominate population from the Guiana center of endemism, they mistakenly thought that the name *paraensis* would apply to population B (loudsong in Figure 1). Probably because they did not listen to vocal samples from east of the Tocantins river in the Belém center of endemism, they assumed that this population would also have the same vocal as their "*paraensis*", hence creating an unusual distribution pattern in which two populations of "*paraensis*" were separated by a population of "*macularius*". As showed above, populations separated by the Tocantins river share the same vocal type of population A, to which the name *paraensis* is correctly applied (Figures 1, 6 and 7). On the other hand, no name was currently available for the vocally distinct population B distributed between the Madeira and Xingu rivers, until the description of *taxon novum* above.

MATERIAL AND METHODS

MORPHOLOGICAL ANALYZES

We examined 45 specimens of *H. macularius* (25 males, 12 females and 8 specimens of unknown sex) housed in the following Brazilian ornithological collections: Museu Paraense Emílio Goeldi, Belém, (MPEG), Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), and Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ). This material includes specimens of subspecies *H. m. macularius* (n = 13), *H. m. paraensis* (n = 16), *H. m. dilutus* (n = 5), and 11 specimens

attributable to *Taxon nuvum* were represented in morphological analyses. In addition to those specimens, high resolution digital pictures of *H. m. macularius* and *H. m. dilutus* type specimens (deposited, respectively, at the Nationaal Natuurhistorisch Museum, Leiden, The Netherlands [RMNH], and the Naturhistorisches Museum, Vienna, Austria [NHMV]) were examined (Appendix 1).

Measurements of the following characters were taken to the nearest 0.1 mm with an electronic caliper: wing length (**WL)**, tail length (**TL)**, tarsus length (**TSL)**, bill length from the anterior end of nostril to the tip of the culmen (**BL)**, bill depth at the anterior end of the nostrils (**BD)**, bill width at the anterior end of the nostrils (**BW)**, length of clear area of the penalties of the lower breast (**LCA**), width of the clear area of the penalties of the lower breast (**WCA**), and width of black terminal area of the penalties of the lower breast (**WBA)**. All morphological nomenclature follows Proctor and Lynch (1993). We used Smithe (1975, 1981) as a standard color reference when describing plumage features.

Groupings for statistical analysis of morphological data were based on the molecular phylogeny obtained (see below), which recognized four main natural groupings (lineages or populations) in *H. macularius* (Figure 1). We assessed normality of morphometric data with Kolmogorov-Smirnov tests and used Discriminant-Function Analyses (DFA) to test for differences in the morphometric space among groups. We combined both sexes in the analyses since no evidence of sexual dimorphism was found for any character, and also to prevent the exclusion of groups due to small sample size after removal of one sex. Missing morphometric values for some specimens were estimated using a Missing Value Analysis, based on the linear regression of the observed variables (Peloso 2009). All statistical analyses were performed with software

SYSTAT 12 for Windows (Systat Software, San Jose, California, USA). In all tests statistical significance was accepted at $P \leq 0.05$.

VOCAL ANALYZES

We analyzed recordings of 104 different recordings, including more than 310 distinct songs, from 51 localities throughout the Amazon, belonging to all currently recognized subspecies of *H. macularius* (Figure 1). The vocalizations were obtained in the field by us or borrowed from the British Library Sound Archive, London, UK (BLS), the Macaulay Library of Natural Sounds of the Cornell University, Ithaca, NY (MLS), the Xeno-canto America web site (http://www.xeno-canto.org/; XC), and the personal archives of several colleagues (PAC) (Appendix 2).

The vocalizations were categorized as loudsongs and calls through auditory and visual comparisons of spectrograms, Loudsong (*sensu* Willis 1967). On the other hand, alarm calls of *H. macularius* complex usually show a short interval of time (less than 2 seconds) and were structurally simple.

Generally, the *H. macularius* loudsong consists of six clear whistled notes. We measured the duration of each of the six notes, the duration of each of the five intervals between notes, and the interval between loudsongs, thus yielding 12 different time-related characters for each individual song. However, because some individuals, especially those of the new taxon described below, eventually omitted one or two final notes from their loudsongs, only 10 time-related vocal characters present in the majority of vocalizations (94%) were submitted to statistical analyses. Time was measured in seconds to the nearest 0.01 s. These measurements were made in the waveform; when

necessary, background noise was removed through lowpass and highpass filtering. We measured three loudsong repetitions for each recording and used the mean to account for individual variation and minimize measurement errors. Loudsongs of *H. m. dilutus* were insufficient for statistical analyses. We assessed the normality of vocal measurements with Kolmogorov-Smirnov tests and used Discriminant-Function Analyses (DFA) to test for differences in among groups. We used software SYSTAT 12 for Windows (Systat Software, San Jose, California, USA) in all statistical analyses. Statistical significance was accepted at $P \leq 0.05$.

Loudsong and call frequency measurements were made in audiospectrograms and refer to the fundamental harmonic, which in all vocalizations analyzed was also the dominant one. The "max frequency" measurement corresponded to the frequency at which the maximum power occurs within a given time interval (Charif et al. 2004). Thus, values given are the dominant frequency measured while considering the entire duration of each note. The syntax (*sensu* Isler et al 1998) was analyzed qualitatively through of the inspection of audiospectrograms.

Audiospectrograms and all song and call measurements were made with the Raven sound analysis software (version 1.3; Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY) with all vocalizations digitized at a sample rate of 44.1 kHz and 16 bits in the mono pattern.

MOLECULAR ANALYZES

Molecular analyses were based on vouchered muscle tissue samples obtained from the following collections: Field Museum of Natural History, Chicago (FMNH),

Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP), Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG), Laboratório de Genética e Evolução Molecular de Aves, Universidade de São Paulo, São Paulo, Brazil (LGEMA), Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (MCT-PUCRS), Universidade Federal do Piauí, Teresina, Brazil (UFPI), and Laboratório de Genética e Biologia Molecular da Universidade Federal do Pará, Bragança, Brazil (LGBM). We also obtained samples from study skins housed at MPEG through the removal of digital and metatarsal pads.

Samples of the currently recognized subspecies *H. m. macularius* (n = 6), *H. m. dilutus* (n = 2), *H. m. paraensis* (n = 4), and 3 specimens attributable to *Taxon nuvum* were included in the molecular analyses (Table 1). Representatives of other four *Hylopezus* species (*H. auricularis, H. berlepschi, H. nattereri,* and *H. ochroleucus*) and another Grallariidae genus (Thrush-like Antpitta *Myrmothera campanisona* Hermann, 1783) were also sequenced as outgroups (Table 1).

Genetic samples obtained from skins were extracted from digital and metatarsal pads using DNeasy kits (Qiagen Inc., Valencia, CA), whereas those obtained from muscle tissue were extracted following a phenol-clorophorm method as described in Sambrook et al. (1989). The material was digested by ribonuclease for 1 h at 37°C, followed by 2–4 h of treatment with proteinase K at 55ºC. The product was washed with phenol–chloroform and precipitated in alcohol. The Polymerase Chain Reaction (PCR) technique was used for the amplification of a segment of ~510 base pairs of the rRNA 16S mitochondrial gene (16s rRNA) with the following primers as described by Palumbi et al. (1991): 5′-GCCTCGCCTGTTTACCAAAAAC-3′ (L-1987) and 5′-CGGTCTGAACTCAGATCACGT-3′ (H-2609). For the segment of ~480 base pairs of

the *cytochrome b* (cytb) gene the following primers developed by Sorenson et al. (1999) were used: 5′-CCGATAAAATCCCATTCCACCC-3′ (L-15560) and 5′-CTTCGATCTTTGGCTTACAAGACC-3` (H-16064), whereas for the segment of ~380 base pairs of the NADH dehydrogenase subunit 2 (ND2) the following primers developed by Hackett (1996) were used : 5′-TATCGGGCCCATACCCCGAAAAT-3′ (L-5215) and 5′-CCTTGAAGCACTTCTGGGAATCAGA-3` (H-5578). Each reaction was based on a final volume of 50 µL, containing 8 µL of the nucleotide mixture (1.25 mM), 5µL of the buffer (10X), 2 µL of MgCl2 (25 mM), 1 µL of each primer (200 ng/µL), ~150 ng of the extracted total DNA, 0.5 µL of the Taq polymerase enzyme (5 U/µL — Invitrogen, NY, USA) and sterile distilled water to complete the final reaction volume.

Amplification conditions for the segment of 16S rRNA gene consisted of initial denaturation at 94°C for 3 min, followed by 35 cycles of 0:30 s at 94°C, 1 min at 50° C and 2 min at 72° C. Final extension was at 72° C for 7 min. Amplification of the cytb and ND2 segments had the following steps: 35 cycles of 30 s at 94°C, 1 min at 55°C and 2 min at 72°C, followed by a final step of 7 min at 72°C. All PCR products were purified using the ExoSAP-IT (Amersham Pharmacia Biotech. Inc., UK) kit, with 2 µL of the enzymatic solution being added to each aliquot of 5 µL, and incubated at a temperature of 37°C for 15 min, and then for 15 min at 80°C. These purified PCR products were cycle sequenced using a Big Dye reagent kit (ABI PrismTM Dye Terminator Cycle Sequencing Ready Reaction — Applied Biosystems, USA), according to the manufacturer's instructions. The reactions were carried out to a final volume of 10 µL, containing 1 µL of the purified PCR product, 0.5 µL of primer (200 ng/L concentration), 3 µL of buffer (Tris 0.4 M, pH 9.0/MgCl2 25 mM), 1

µL of Big Dye mix and 4.5µL of distilled water. The amplification protocol consisted of 25 cycles of 50 s at 96°C, 5 s at 50°C and 4 min at 60°C. Reagents not incorporated during the cycle sequencing reaction were eliminated by washing with isopropanol and products were run on an ABI Prism 3700 sequencer.

The sequences of each gene region were aligned using the CLUSTALW application (Thompson et al.1994), with the penalty parameters suggested by Schneider (2003). The resulting file was converted into the Fasta format and transferred to the BIOEDIT software (Hall 1999) sequence editor for the visual inspection of the initial alignment and to check protein coding sequences for erroneous indels and proper coding. The following measures were taken to ensure that the DNA fragments sequenced were accurate and of mitochondrial origin (not pseudogenes): (1) both DNA strands were sequenced; (2) sequences were inspected using BioEdit (Hall 1999) for insertions, deletions, and stop codons that would result in a nonfunctional protein; and (3) sequences were expected to exhibit high transition to transversion substitution ratios characteristic of mitochondrial, not nuclear substitution patterns. The composition of the bases, transition and transversion rates, and the percentage of variable and informative sites were calculated using MEGA, version 3.1 (Kumar et al. 2004). The presence of saturated sequences was established by plotting nucleotide divergence (model TN93) against transversions using version 4.2.13 of the DAMBE program (Xia & Xie 2001). The evolutionary model selected by the MODELTEST program (version 3.07, Posada & Crandall 1998) was based on the minimum theoretical information criterion, AIC (Akaike 1974), which minimizes the expected distance between the true model and the estimated sample. The AIC penalizes for the increasing number of

parameters in the model, taking into account not only the goodness-of-fit but also the variance of the parameter estimates.

Phylogenetic analyses were performed on the concatenated 16S/cyt *b*/ND2 dataset, after a partition-homogeneity test as implemented in PAUP* 4.0b10 (Swofford 2002) failed to detect significantly different phylogenetic signals among the three genes analyzed ($P > 0.05$). Phylogenetic trees were constructed with Maximum likelihood (ML) and Maximum parsimony (MP) procedures, using PAUP (version 4.10b, Swofford 2002). Bootstrap support values for MP and ML trees were based on 1000 nonparametric replications (Felsenstein 2004). Using the software MrBayes 3.1.2, we also estimated phylogenies with a site-partitioned Bayesian analysis (BA) with the following specifications (Ronquist and Huelsenbeck 2003): (1) assuming separate optimal ML models selected for each gene partition by Modeltest 3.7; (2) running the Markov chain for 5,000,000 generations and sampling 1 tree every 1000 generations. We discarded the first 1250 trees sampled (25% of the sample) as "burn-in", and used the remaining 3750 trees to estimate posterior probabilities of the trees' topologies.

MOLECULAR DATING

To estimate divergence dataes for the relevant ancestral nodes in the *H. macularius* phylogeny, we used the cyt *b* data partition and assumed an average calibration of 2.1% nucleotide substitutions per million years (Weir and Schluter 2008). The software BEAST v 1.4.7 (Bayesian Evolutionary Analysis Sampling Trees) was used for the inference of divergence times through a Bayesian approach (Drummond et al. 2007). On a tree composed of the taxa in Figure 2, (excluding the outgroups, i.e. *H. ochroleucus, H. auricularis, H. nattereri, H. berlepschi*, and *M.*

campanisona), and with the same topology, parameters in a K81uf+I model of molecular evolution selected by Modeltest (rate matrix = 1,0000 21,0961 $1x10^{-4}$ $1x10^{-4}$ 21,0961; base frequencies = 0,3028 0,3150 0,1043, and proportion of invariant sites = 0,6432) were used to estimate divergence times with the "Relaxed clock: uncorrelated Lognormal" option of BEAST, which assumes independent rates of molecular evolution on different branches, with one or two parameters defining the distribution of rates across branches (Drummond et al. 2007). As BEAST does not accept values of rate matrix =0, we substituted the zeros in this parameter for a low value ($1x10^{-4}$). Three independent BEAST runs were carried out and combined with the LogCombiner software v1.4.8 to obtain values of Effective Sample Size (ESS) greater than 200, as recommended by Drummond *et al.* (2007).

RESULTS

Molecular phylogenetics

We obtained 1371 base pairs (bp) from segments of the 16S rRNA (505 bp), ND2 (389 bp), and cytochrome *b* (477 bp) mitochondrial genes for 15 specimens of *H. macularius* and 11 outgroup taxa (Table 1), 311 bp (ca. 23%) of which were phylogenetically informative. No insertions, deletions or stop codons were observed. Transition versus transversion plots do not indicate saturation among ingroup. As a partition-homogeneity test did not detect significant differences in the phylogenetic signal of the three genes sequenced (P = 0.73), they were combined in phylogenetic analyses. The model of sequence evolution selected for the combined dataset and used

in ML phylogeny estimates was GTR + I + G, with estimated base frequencies (A = 0.3117, C = 0.3258, G = 0.1429, T = 0.2296), substitution model (A-C = 17935, A-G = 6.5052, A-T = 0.8704, C-G = 0.3454, C-T = 14.6513, G-T = 1), proportion of invariable sites (I = 0.5762), and rates for variable sites following a gamma distribution (α = 3.1099). For the gene-partitioned BA, the following general model parameters were chosen for the 16S rRNA (GTR+G), ND2 (K81uf+1), and cytochrome *b* (HKY+G) data sets.

All phylogenetic trees obtained by MP, ML, and BA grouped all *H. macularius* samples in a strongly supported clade, but the node connecting it to its sister group (*H. ochroleucus*) was highly supported only in BA (Figure 2). Nevertheless, MP, ML, and BA trees ruled out the possibility that *H. auricularis* (once regarded as a subspecies of *H. macularius*) is nested in the Spotted Antpitta clade (Figure 2). The reciprocal monophyly of subspecies *H. m. macularius* and *H. m. paraensis*, as currently defined, was statistically strongly rejected in MP, ML, and BA trees, where those taxa were paraphyletic (Figure 2). Instead, trees recovered four separate major clades grouping taxa of *H. macularius*, as follows (Figures 1 and 2; Amazonian centers of endemism are those defined by Silva et al. 2005): (1) birds occurring south of the Amazon and east of the Xingú river in the Xingú and Belém centers of endemism (corresponding to population / clade A); (2) birds found south of the Amazon and between the Madeira and Xingú rivers in the Madeira and Tapajós centers of endemism (corresponding to population / clade B); (3) birds found north of the Amazon and west of the Negro rivers in the Napo and Imeri centers of endemism (corresponding to population / clade C), and (4) birds found north of the Amazon and east of the Negro rivers in the Guiana center of endemism (corresponding to population / clade D). The phylogenetic position of

subspecies *H. m. diversus* was not assessed due to lack of tissues, but strong vocal evidence suggests that this taxon is included in clade C (see below). Because the molecular phylogenies produced conflicted strongly with subspecies limits of *H. macularius*, clades / populations A – D (Figures 1 and 2) will be used as natural evolutionary units for a taxonomic reassessment of the entire group based on morphological and bioacoustical characters.

Timing of divergences

Molecular dating analyses indicated that on average the older splits among populations of *H. macularius* occurred in northwestern Amazonia between 5.54 and 1.15 mya (across the Negro and upper Amazon rivers, respectively), whereas the more recent splits took place in southeastern Amazonia starting at ca. 0.780 mya (Figure 3). The confidence level intervals for those estimates were broad and thus must be interpreted with caution (Table 2).

Morphological characters

DFA results for the morphometric characters sampled did not show strong differentiation among natural populations A, B, and D of *H. macularius*, but indicated that population C is set apart from the others in the morphometric space (Figure 4). Because of the small sample size of population C, for which only five specimens were measured, and the assumptions of DFA, which stipulates that the number of variables should not exceed the sample size for any particular group, a DFA analysis was carried out including only the four most important characters, chosen by stepwise analysis, for separating populations of *H. macularius*. The stepwise analysis indicated that tarsus length, bill depth, bill width, and the extent of white on the pectoral spots were the characters that best discriminating populations A – D. When a new DFA was performed with only these four characters, significant differences were found among populations A – D in the morphometric space (Willk's lambda 0.323, $P = 0.000$), with all specimens of population C classified correctly (Table 3). Population C is

distinguished from others by significantly shorter tarsi and greater bill depth and width values, whereas population B is only partially differentiated from populations A and D by the highest bill width values in the entire sampling; on the other hand, populations A and D overlap broadly in the morphometric space (Figure 4, Table 3).

The four natural populations of *H. macularius* are also very similar in plumage, although population C is distinguished from the others by more olivaceous (color #29), and less brownish upperparts, less ochraceous subterminal band of pectoral feathers, wingbars extended only to the middle rather than the tips of the wing, shaft-streaks on the mantle absent or obsolete, and black terminal band of pectoral spots more pronounced. Population D on average paler buff (color #123B) flanks and more perceptible shaft-streaks on the mantle than the remaining populations. Populations A and B could not be mutually differentiated by any plumage character.

Vocal characters

A DFA based on the vocal characters measured showed a strong differentiation between population B and populations A and D, which overlap partially (Figure 5). The DFA showed significant differences among populations (Willk's lambda = 0.167, P = 0.000), and classified correctly among populations most vocal samples, including all those of population B (Table 4). Due to the lack of sufficient vocal samples, population C was not included in the DFA analysis, but song structure in this population resembled closely those of populations A and D (Tables 5 and 6; see below). The duration of the second note and the duration of the second interval (between the second and third notes) were the characters best discriminating populations A - D. Furthermore, the number of

loudsong notes was an important character setting apart birds of population B (in which over 90,9% of the recordings sampled uttered songs with five or four notes) from those of populations A, C, and D (in which birds normally uttered songs with six notes; Tables 5 and 6). As for the quantitative vocal characters analyzed, the loudsongs and calls of populations A – D can be mutually diagnosed as follows:

Population A

The loudsong of this population is characterized by notes of nearly identical shape and dominant frequency (861.30 Hz), although occasionally some notes (especially the fourth) exhibited a slightly lower frequency around 689.10 Hz (Figure 7A). Unlike in the other populations, the third, fifth and sixth notes show a richer harmonic structure, which gives them a distinct raspy quality. For loudsong analysis, 81 vocalization sections belonging to 27 recordings were analyzed. Calls (Figure 6A) distinguished in two characters: (1) number of notes (7 – 8); and (2) duration of notes and interval between notes, which are longer than in any other population; furthermore, the first call note is very different in shape from the remaining ones. For this population 6 calls belonging to 2 individuals were analyzed.

Taxon novum

Population B

Three characters readily distinguished the loudsong in this population: (1) number of notes, which in 90,9% of the samples consisted of only four or (more often) five, and less often (9,1% of the samples) six notes, as in the remaining populations; (2) interval between the second and third notes, which was longer than in any other population and

longer than those between the other notes of the song (mean 0.41 ± SD of 0.09 s; Table 5; Figure 7B). For loudsong analysis, 84 vocalization sections belonging to 28 recordings of this population were analyzed. Calls differed only in number of notes (five) (Figure 6B). For this population 3 calls belonging to 1 recording were analyzed.

Population C

Loudsongs were readily distinguished from those of the other populations by the shorter duration of all note intervals (ranging from 0.10 to 0.16 s), thus also yielding an overall shorter loudsong (lasting approximated 2 s) than in any other population and notes delivered at an overall slightly higher frequency. Furthermore, the syntax of the loudsong in this population differs markedly from that of populations A and B by having two different types of notes, following the pattern A A B A B B, which is found also in population D. The notes A are more flat in frequency, while notes B show an strongly ascending/descending pattern of frequency modulation (Figure 7C,D). The dominant frequency of the six notes of this population was (861.30 Hz), even though occasionally the notes exhibited two different frequencies (689.10 Hz and 1033.60 Hz) (Table 6). Unfortunately, only 12 loudsongs from 4 recordings from 4 locations were analyzed, and no calls for population C were sampled. Of the four individuals of population C sampled, two referred to the taxon *H. m. dilutus* (Brazil: Amazonas, Japurá (A. Aleixo – 1 PAC); Jau National Park (S. Borges – 1 PAC)), and two to *H. m. diversus* (Peru: Sabalillo, Loreto (D. Edwards – XC 20058); Quebrada sucusari, Loreto (B. Whitney – 1 PAC)), according to the current taxonomy. Despite the small sample sizes, song variation among those individuals was minimal and they all conformed to the pattern described above for population C.

Population D

The syntax of loudsong in this population follows the same pattern as that observed in population C, in which the first, second, and fourth notes and the third, fifth and sixth notes formed two groups of notes very similar in shape (Figure 7D). The dominant frequency of the third note was different of all the others: the third with the frequency around 1033.60 Hz and the others with frequency around 861.30 Hz (Table 6). Although the frequency varied in some cases, this pattern was observed in 83 loudsongs belonging to 30 recordings (61,8 %, respectively, of song sections and individuals analyzed). Call structure (Figure 6) was distinguished by three characters: (1) number of notes (n = 12); (2) note structure, in which the first note is different in shape and duration from the remaining eleven notes, and (3) dominant frequency, which is roughly the same for all notes (1033.60 Hz). For call analyzes, only six calls from 2 recordings were sampled.

Taxonomy

Our results indicated that each natural population of *H. macularius* recognized in this study (Figure 1) can be diagnosed through a combination of vocal, molecular, and morphological features and thus can be interpreted as basal taxa deserving formal taxonomic recognition. As we discuss in detail below, already existing names are applicable to populations A, C, and D, but no available name exists for population B, which we propose to name as follows:

***Hylopezus oreni* sp. nov.**

Alta Floresta Antpitta

Holotype.- Museu Paraense Emilio Goeldi (MPEG) 56099, Adult male (skull 100% ossified). Collected on 23 July 2002 by W. Figueiredo in Belterrra, Floresta Nacional do Tapajós, Sucupira base, Km 117 of BR-163 highway, state of Pará, Brazil (03° 21' 22''S, 54° 56' 57'' W). Tissues samples sequenced in this study and deposited at LGBM-UFPA under accession number WN 350.

Designation of paratypes.- MPEG 58757 (skin and tissue): Adult male, skull ossified, testes 7 x 5 mm; MPEG 38808 (skin): adult male, skull ossified, testes 7 x 4 mm; MPEG 39820 (skin): adult male, skull ossified, testes 6 x 3 mm; MPEG 39821 (skin): female, skull 40% ossified, ovarium 4 x 8 mm; and MPEG 34420 (skin): Adult female, skull ossified; MNRJ 21895 (skin); MZUSP 58838 (skin): male; FMNH 389869 (tissue subsample).

Diagnosis.- Plumage coloration similar to other taxa in the *H. macularius* complex. Separable from population C (to which the name *H. m. dilutus* applies; see Discussion below) by more conspicuous mantle shaft-streaks, upperparts surface less brownish, subterminal band of pectoral spots less ochraceous, and wingbars occupying the entire length of the wing. Distinguished from population D (to which the name *H. m. macularius* is applicable) by paler flanks and more conspicuous mantle shaft-streaks. Diagnosis from population A (to which the name *H. m. paraensis* applies) is attained only through vocal and genetic characters (see below). The loudsong of *taxon novum* is readily distinguished from that of any other taxa in the *H. macularius* complex by an

unusually longer interval between the second and third notes, and by a normal series of four to five flatter shaped, unmodulated notes, resulting in an overall slower paced song, with a "hesitating" quality. Calls are also distinguished from those of any other taxa in the *H. macularius* complex by a shorter series of five or six notes, with distinct higher frequencies, (Figure 6 and 7, Tables 5 and 6).

Vocalizations.- The loudsong in general comprises five clear whistled notes, although occasionally the final note is omitted or an additional sixth note may appear. The full song (5 notes) lasts about 2.8 s; the interval between the first and second notes is shorter (0.2 s) than that between second and third (0.5 s). The three last notes are separated by regular intervals (0.2−0.3 s). The duration of notes vary slightly (0.16−0.19 s), but the two first notes are usually slightly longer than the three remaining ones. All five notes are similar in the dominant frequency (861.30 Hz or occasionally 689.10 Hz). Calls consist of a succession of six notes similar in shape but different in frequency, which varied among notes but was usually 1216.6, 1022.8, 1033.6, 1033.6, 1044.4, 969.0 Hz, respectively.

Description of holotype.- (Color names and numbers follow Smithe 1975, 1981): top of head and nape blackish neutral gray (82); lores buff–yellow (53); broad eyering yellow-ocher (123c) bordered by a continuous narrow blackish line; auricular region with distinct black and buff streaks; malar region crossed by a conspicuous black streak, contrasting with the whitish throat and center of chin. Upperparts olive (30) with conspicuous pale shaft-lines on the central portions of mantle feathers. Breast strongly marked with mixed black and buff-yellow (53) spots, with black usually restricted to the

v-shaped tips feathers. Flanks conspicuously buff (24); belly white; wing-coverts tipped yellow-ocher (123c), faint cinnamon (123a) wing bars occupying the entire length of wing; primary coverts blackish, contrasting with a well-defined tawny (38) patch at the base of primaries; remainder of the primaries olive-brown (28).

Measurements of holotype.- Bill width at the anterior end of nostrils 5.4 mm; bill depth at the anterior end of nostrils 6.7 mm; culmen length from anterior end of nostril to the tip 13.7 mm; wing length 83.1 mm; tail length 39.6 mm; tarsus length 33.8 mm; body mass 40.0 g, and testes 4 x 2 mm.

Remarks

Variation in the type series.- The type series includes seven specimens: five males and two females. No sexual dimorphism was detected for any of the measurements taken (Table 7) and characters studied. Two specimens (MPEG 39820 and 39821) present more visible yellowish mantle shaft-streaks on the back surface than the holotype, but this could be due to differences in specimen preparation and conservation. Some specimens (MZUSP 58838 and MPEG 38808) lack the discrete black line that subdivides the wingbar as present in holotype and other specimens in the type series. Back color varies in several specimens, going from black olive (29) (MZUSP 58838) to olive-brown (28) (MNRJ 21895) similarly, under wing coverts vary from clay color (123b) (MNRJ 21895) to cinnamon (39) (MPEG 58838).

Habitat.- Ground and dense undergrowth of humid lowland forest (sea level to about 500 m), with an apparent preference for wet or flooded areas in upland *terra firme* forest. The species seems also more commonly found around treefall gaps and streams, rarely in more open and disturbed areas.

Behavior.- As other *Hylopezus* species, *taxon novum* is difficult to observe due to its secretive habits and preference for the lower strata of dense and often tangled vegetation. It generally responds well to play-back, but due to its ventriloqual song, it is often difficult to locate even when singing close to the observer. When not attracted with play-back, individuals are observed perched or flying short distances between fallen logs at 1–1.5 m above the ground. Frequently, several territories are located nearby, where individuals counter sing and are apparently stimulated by their neighbor vocalization, especially at dawn, early morning, and towards dusk. At a few sites sampled more frequently, such as the Cristalino Lodge in Alta Floresta, neighboring territories appear to be stable through time.

Etymology. The specific epithet of this new species honors Dr. David Oren, researcher at the Museu Paraense Emílio Goeldi, in recognition of his contributions to the study of Brazilian birds, for over 20 years. As common names for this species we propose Alta floresta antpitta (English) and Torom de alta floresta (Portuguese), referring to the type locality region, the Alta floresta in the Mato grosso.

DISCUSSION

SYSTEMATICS AND INTRA-GENERIC RELATIONSHIPS

The phylogenetic trees obtained supported the first split of a taxon formerly grouped in the Spotted Antpitta as a subspecies: *H. auricularis* (Maijer 1998; Figure 2). Originally described as a full species by Gyldenstolpe (1941), *H. auricularis* was subsequently lumped with *H. macularius* based on the assessment of Zimmer and Mayr (1943). Many years later, Maijer (1998) showed that the loudsong of *auricularis* was very distinct from those of other taxa grouped under the Spotted Antpitta, and proposed to treat it as a separate biological species, a proposal followed thereafter (Krabbe and Schulenberg 2003, Remsen et al. 2009). All molecular trees obtained placed *H. auricularis* neither within the strongly supported *H. macularius* clade nor as its sister taxon, a position occupied by *H. ochroleucus* instead (Figure 2). Even though three *Hylopezus* species were not sampled in the molecular analyses (*H. perspicillatus, H. fulviventris,* and *H. dives*), the strongly supported nodes joining (1) all taxa currently grouped under the Spotted Antpitta, and (2) *H. ochroleucus* as their sister group, both to the exclusion of *H. auricularis*, indicate that the latter species is not even closely related to the Spotted Antpitta. In fact, all molecular trees obtained favored a topology where *H. auricularis* and *H. nattereri* from southeastern South America were sister species; this interesting finding must be confirmed by future analyses with more robust taxa and sequence sampling regimes, but agrees with the recent split between *H. ochroleucus* and the *H. macularius* clade (which used to be lumped into a single biological species; Peters 1951) also based on vocal characters (Whitney et al. 1995). Even though all

molecular trees obtained in this study placed the northeastern Brazilian *H. ochroleucus* as the sister species of the Amazonian *H. macularius* clade, the node joining these taxa was statistically strongly supported only in Bayesian analyses (Figure 2). Superficial vocal similarities indicate that among all *Hylopezus* species, *H. macularius* and *H. ochroleucus* are more similar to each other than to any other species (Isler and Whitney 2002), which is in agreement with the tree topologies obtained in this study (Figure 2). Nevertheless, this sister-relationship should be regarded only as speculative, pending a more complete phylogeny estimate that includes the additional three species of *Hylopezus* missing from our sample.

SPECIES LIMITS AND CHARACTER EVOLUTION

We demonstrated that the current taxonomy of the Spotted Antpitta contrasts strongly with its evolutionary history (Figure 1). Four main reciprocally monophyletic and statistically well-supported lineages of the Spotted Antpitta were recovered by phylogenetic analyses of mtDNA sequences. These lineages were also readily diagnosed by calls and loudsongs and to a lesser extent by plumage and morphometric characters (Figures 2, 3, 4, 5, 6 and 7). When interpreted together, these findings allow a redefinition of the Spotted Antpitta inter-specific limits, whose taxa have been treated as subspecies (Cory and Hellmayr 1924, Peters 1951, Krabbe and Schulenberg 2003).

Each of the four lineages of the polytypic *H. macularius* occupies one or more Amazonian areas of endemism, thought to be areas of common historical diversification for several animal and plant species (Silva et al. 2005; Figure 1). After splitting from its sister group (inferred as being *H. ochroleucus*, endemic to northeastern Brazil; Figure 2), the first and main split in the *H. macularius* clade occurred between the population

endemic to the Guyana area of endemism (*H. macularius*) and all remaining populations at an estimated date of ca. 2.92 mya (Figures 1 and 3). Average pair-wise uncorrected *p*-distances between *H. macularius* and other lineages were as follows: 3.7% (*H. dilutus*), 3% (*taxon novum*), and 2.8% (*H. paraensis*) (Table 8). On the other hand, pair-wise *p* distances within *H. macularius* ranged from 0 to 0.4%. As shown earlier, there are several diagnostic loudsong and call features distinguishing *H. macularius* (population D) from any other Spotted Antpitta lineage (Figure 6 and 7, Tables 5 and 6), even though its loudsong is more similar in structure to *H. dilutus* (population C). Morphologically, only minor differences in flank color and the intensity of pale shaft streaking on the back distinguish *H. macularius* from the other populations.

The second main split in the Spotted Antpitta was tentatively dated to about 1.54 mya and occurred between *H. dilutus* (population C) from the Imeri and Napo areas of endemism north of the Amazon river and populations south of this river (Figures 1, 2, and 3). Average pair-wise uncorrected *p* genetic distances between *H. dilutus* and other lineages were as follows: 3.7% (*H. macularius*), 2.5% (*taxon novum*), and 2.3% (*H. paraensis*) (Table 8), whereas no genetic divergence was detected between the only two individuals of *H. dilutus* sequenced. Vocally, *H. dilutus* is distinguished from all other lineages by a significantly shorter loudsong lasting around 2s (in other lineages loudsong lasts usually between 2.5 and 3s), and notes delivered at an overall slightly higher frequency, whereas morphologically birds from this group can be considered the most distinct among all Spotted Antpitta lineages, due to significantly shorter tarsi, greater bill depth and width values, and a brownish rather than greenish-olivaceous back with little or no pale-shaft streaking.

Finally, the last major split in the Spotted Antpitta occurred between the two lineages distributed south of the Amazon river and was tentatively dated to about 0.780 mya (Figures 1 and 3): *taxon novum* and *H. paraensis*. Average pair-wise uncorrected *p* genetic distances between *taxon novum* and *H. paraensis* was only 0.75%, and within those lineages 0.2%. When compared to *H. macularius* and *H. dilutus*, *taxon novum* diverged by an average *p* distance of 3% and 2.5%, whereas *H. paraensis* diverged from those two lineages by 2.8% and 2.3%, respectively (Table 8). Vocally, *taxon novum* is the most distinct lineage in the Spotted Antpitta, as revealed by several loudsong and call parameters (Figures 5, 6 and 7, Table 4), whereas morphologically it can only be distinguished from the cryptically similar *H. macularius* and *H. paraensis* by average greater bill width measurements. The latter taxon can also be diagnosed by its unique loudsong and call parameters, and the distinct harmonic structure of some of its loudsong notes. Morphologically, *taxon novum*, *H. paraensis*, and *H. macularius* can be hardly distinguished from each other, overlapping broadly in the morphometric space.

The statistically well supported reciprocal monophyly recovered for the four lineages of the Spotted Antpitta identified in this study, added to their vocal mutual diagnoses, which remain constant within each clade, are indicative of species level status either under the Phylogenetic Species Concept (PSC) or the Biological Species Concept (BSC). Under the PSC, their reciprocal molecular, vocal, and morphological diagnoses provide the basis for considering *H. macularius*, *H. dilutus*, *taxon novum*, and *H. paraensis* as separate species, whereas under the BSC the concerted evolution between genetic and vocal characters, added to the absence of genetically / vocally intermediate individuals in our sampling, are also indicative of species level status for

these lineages. The importance of vocal characters as a pre-mating isolating mechanism in Grallariidae was recently underscored by a study showing that loudsong variation appears to have a strong genetic basis in this family (Cadena et al. 2007), which agrees with the pattern documented herein for the Spotted Antpitta.

When compared to other lineages in Grallariidae, our results indicate that significant vocal variation can occur even between lineages separated by small uncorrected molecular distances, such as that separating *taxon novum* and *H. paraensis*, i.e. 0.75%. Other proven or likely sister species pairs in Grallariidae for which pair-wise *p* distances are available are *Myrmothera campanisona* x *M. simplex* (4.8%) and *Hylopezus berlepschi* x *H. fulviventris* (5.8%; Rice 2005), and vocal variation between those taxa are at least comparable or less evident than that between *taxon novum* and *H. paraensis* (Isler and Whitney 2002). When mapped onto the molecular tree obtained (Figure 2), it appears that loudsong evolved in most Spotted Antpitta lineages through a combination of rearrangements in note shape and frequency, syntax, and intervals between notes, but in one instance (*taxon novum*) the number of notes was also affected, thus creating a different loudsong (Figure 7, Table 5 and 6). Thus, we hypothesize that while those major loudsong differences were readily fixed in *taxon novum*, its sister species (*paraensis*) conserved most of the same loudsong overall structure present in other more ancestral lineages. Therefore, while different vocal types can distinguish sister species in Grallariidae, non-sister taxa can share more of the same overall loudsong structure as documented herein. Therefore, we predict that other instances of cryptic species in *Hylopezus* will come to light as more taxonomic studies including vocal and molecular characters become available, as this and other recent studies have shown (Whitney et al. 1995, Maijer 1998).

CONCLUSIONS

Based on the combined character analyses presented and discussed herein, we recommend the splitting of the Spotted Antpitta complex into four distinct biological and phylogenetic species, as follows:

Spotted Antpitta - *Hylopezus macularius* (Temminck, 1830) – Distributed on the Guyanan shield from the eastern bank of the Negro river in Brazil eastward through eastern Venezuela (Bolívar), Guyana, French Guyana, and state of Amapá in Brazil (Figure 1; see also Hilty 2003, Restall et al. 2006). Based on southern Venezuelan records attributed to the species below (Zimmer 1934; see above), we postulate that the Branco river separates *H. macularius* from the species below north of the Negro – Branco confluence in the Brazilian states of Amazonas and Roraima.

Zimmer´s Antpitta – *Hylopezus dilutus* (Hellmayr, 1910) – Distributed north of the Amazon from the western banks of the Negro and Branco rivers in the Brazilian state of Amazonas through southern Venezuela (Amazonas), southern Colombia (Amazonas), eastern Ecuador, and northern Peru west of the Ucayali river (Hilty and Brown 1986, Ridgely and Greenfield 2001, Schulenberg et al. 2007).

Alta Floresta Antpitta – *Hylopezus oreni*, Carneiro et al. 2009 – Distributed south of the Amazon between the Madeira and Xingu rivers in the Brazilian states of Amazonas, Rondônia, Mato Grosso, and Pará (Snethlage 1914, Stotz et al. 1997, Zimmer et al. 1997, Whittaker 2009). There are no confirmed records of this species west of the Madeira river (Whittaker et al. 2009).

Snethlage´s Antpitta – *Hylopezus paraensis* (Snethlage, 1910) – Distributed south of the Amazon in Brazil from the Xingu river eastward in the state of Pará to the

western part of the state of Maranhão, and southward to southern Pará (Snethlage 1914, Pacheco et al. 2007).

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APPENDIX 1. Inventory of specimens examined. The following list identifies specimens analyzed in this study by taxon, country, and location. Acronym for specimens deposited in the following ornithological collections: MPEG = Museu Paraense Emílio Goeldi; MZUSP = Museu de Zoologia da Universidade de São Paulo; MNRJ = Museu Nacional, Universidade Federal do Rio de Janeiro; RMNH = Nationaal Natuurhistorisch Museum, Leiden, The Netherlands; NHMV = Naturhistorisches Museum, Vienna, Austria.

***Hylopezus macularius macularius*:** 14 specimens. **BRAZIL:** (RMNH 88799, holotype, examined from digital photographs); **Amapá**: Serra do navio, rio Amapari (MNRJ 29429); Macapá, Alto rio Araguari (MPEG 21181); Mazagão, Igarapé novo (MPEG 29257); Rio Araguari, areia vermelha (MPEG 20427); Foz do rio Cacouí, afluente esquerdo do rio Araguarí (MPEG 21235); Macapá, Alto Rio Araguari, margem direita (MPEG 21172); Mazagão, cachoeira Amapá (MPEG 28744); P**ará:** Faro, Floresta Estadual de Faro, 01° 42's, 57° 12'w (MPEG 64739); Óbidos, Esec Grão-Pará (MPEG 66675, 66676, 66677, 66678); Alenquer, Esec Grão - Pará. 00° 09's, 55° 11'w (MPEG, not catalogued; field number cn417).

Hylopezus macularius paraensis: 16 specimens. **BRAZIL: Pará**: Ourém, Fazenda Reunida (MPEG 32407, 32408); Senador José Porfírio, margem direita do Rio Xingu (MPEG 55691); Rodovia Belém-Brasilia Km 307 (MPEG 18127); Rodovia Belém-Brasilia-Km 92 (MPEG 15940); Paragominas, Fazenda Rio Capim (MPEG 58982); Rio Acará (MPEG 1670); Altamira, Rio Xingu (MPEG, not catalogued; field number BmP74); Altamira, Rio Xingu, Caracol (MPEG 64919); Utinga (MZUSP 36544);

Capim, Belém-Brasilia Km 93 (MZUSP 45208, 45205, 45206, 45207); Belém, Utinga (MZUSP 36543); **Maranhão**: Carutapera, Rio Gurupi (MPEG 36922).

***Hylopezus macularius dilutus*:** 5 specimens. **BRAZIL:** (NHMV 16440, holotype, examined from digital photographs). **Amazonas**: Maraã, Rio Japurá (MPEG 42749, 42750); Maraã, Lago Cumapi (MPEG 62966); Codajás, Rio Solimões (MZUSP 16062, 16063).

Taxon novum: 11 specimens. **BRAZIL: Pará**: Rio Jamary (MNRJ 21895); Fordlândia, Rio Tapajós (MZUSP 58838); Ruropolís, Santarém-Cuiabá Km 84 (MPEG 47847); Belterra, Flona do Tapajós, Km 117 da BR-163 (MPEG 56099); **Mato Grosso**: Dardanelos, Rio Aripuanã (MPEG 34420); Dardanelos, Rio Aripuanã, nucleo pioneiro Humboldt (MPEG 45606); **Rondônia**: Alvorada D'oeste, BR 429 Km 87 (MPEG 38808); Cachoeira Nazaré, West bank of rio Jiparaná (MPEG 39819, 39820, 39821); **Amazonas**: Humaitá, T. I. Parintintin; aldeia Traíra-Chororó (MPEG 58757).

APPENDIX 2. Inventory of vocalization recordings examined. The following list identifies recordings used in this study by taxon, country, location, and recordist names. Acronym for recording archive: MLS = Macaulay Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, NY; BLS = British Library Sound Archive, London, UK; XC = Xeno-canto America web site (http://www.xeno-canto.org/); (PAC) personal archives of several colleagues.

***Hylopezus macularius macularius*:** 45 recordings. **BRAZIL: Amazonas:** Manaus (R. O. Bierregaard – MLS 42818, 31469, 39417); (P. Stouffer – MLS 74395, 74397, 74427); Br-174 on zf-2 (C. A. Marantz – MLS 127371); BR-174 km 51, INPA tower zf 2 km 14 (A. Whittaker – 4 PAC); Dimona (A. Whittaker – 2 PAC); Manaus (A.

Whittaker – 1 PAC); Marupiara (A. Whittaker – 2 PAC); Presidente figueiredo (A. Whittaker – 1 PAC); **Amapá**: Serra do Navio (T. Gullick – BLS 56573; A. Whittaker – 1 PAC); Porto Grande (A. Whittaker – 2 PAC) **Pará**: Rebio Maecuru (A. Aleixo – 3 PAC); **GUYANA:** Kanuku, Nappi Village (J. D. Kerr – MLS 90692); **Rupununi**: Kanuku, Nappi village (T. A. Parker III – MLS 70096); Maipaima camp (T. A. Parker III – MLS 73054); Mango Landing, West Side of Essequibo River (W. D. Finch – MLS 68507); Kassikaityu River (W. D. Finch – MLS 117947); **VENEZUELA**: **Bolívar**: Rio Grande, El Palmar (P. A. Schwartz – MLS 62469, 62470, 62471, 62472, 62473, 62474); Campamento Rio Grande (T. A. Parker III – MLS 34472); Rio Grande, Sierra de Imabaca (P. Coopmans – MLS 40466); Km 63, trail east to Guiana (L. R. Macaulay – MLS 60952); Km 88 trails (R. Innes – BLS 1418, 1419, 1432); Km 66, La Escalera (D. Willis – BLS 68694, 68695, 6871); 5 km west of San Isidro (N. Athanas – XC 3577); road to Cuyuni east of Tumeremo (N. Pieplow – XC 11648); Imataca Forest Reserve, East of El Palmar (D. Fisher – BLS 47453); **SURINAM**: **Surinam (**P. K Donahue – BLS 31909).

Hylopezus macularius paraensis: 27 recordings. **BRAZIL: Pará**: Redenção, reserva indigena Gorotire (A. Aleixo – MLS 94535); Floresta Nacional de Caxiuanã, Estação Cientifica Ferreira Penna (C. A. Marantz – MLS 113105, 113117, 113118, 127437, 127440, 127444, 127445); Rio Xingu - margem direita, Caracol (L. Carneiro – 2 PAC); Altamira, rio Xingu, Itapuama (S. Dantas – 1 PAC); Tucuruí, rio Tocantins, margem direita, Tucuruí (S. Dantas – 1 PAC); Parauapebas, Serra de Carajás (S. Dantas – 1 PAC); Parauapebas, Serra de Carajás (A. Whittaker – 1 PAC); Caxiuanã (A. Whittaker – 1 PAC); Caxiuanã, canopy tower (A. Whittaker – 2 PAC); Fazenda Rio Capim (A. Whittaker – 8 PAC); Fazenda Rio Capim (A. Aleixo – 1 PAC).

***Hylopezus macularius dilutus*:** four recordings. **BRAZIL: Amazonas**: Japurá, Rio Mapari (A. Aleixo – 1 PAC); Jau National Park (S. Borges – 1 PAC); **Peru**: Sabalillo, Loreto, Left bank of the Rio Napo (D. Edwards – XC 20058); Quebrada sucusari, Loreto (B. Withney – 1 PAC).

Taxon novum: 28 recordings. **BRAZIL: Mato Grosso**: Alta Floresta, Floresta Amazonica Hotel (C. A. Marantz – MLS 88357, 88985); Alta Floresta, 16 km, West of the Teles Pires (A. Lees – 2 PAC); Alta Floresta (M. L. Isler – MLS 48111, 48137, 48138); (P. R. Isler – MLS 48068); (A. Whittaker – 2 PAC); Rio Cristalino, reserva Ecológica Cristalino (C. A. Marantz – MLS 88478, 88479, 88592, 89010, 88889); Rio Cristalino – right bank, Alta Floresta (J. Minns – BLS 170); Cristalino Jungle (N. Athanas – XC 7224); Rio Teles Pires (T. A. Parker III – MLS 52216, 52318); **Rondônia**: Cachoeira Nazare; west bank of Rio Jiparana (T. S. Schulenberg – MLS 40236, 43308, 43315, 43316); **Pará**: Base de Sucupira, Br-163, Km 117 (C. A. Marantz – MLS 115081); Apa Tapajós (S. Dantas – 1 PAC); Amazonia National Park (A. Whittaker – 1 PAC); **Amazonas**: Pousada rio Roosevelt (A. Whittaker – 1 PAC); Rio Roosevelt (A. Whittaker – 1 PAC).

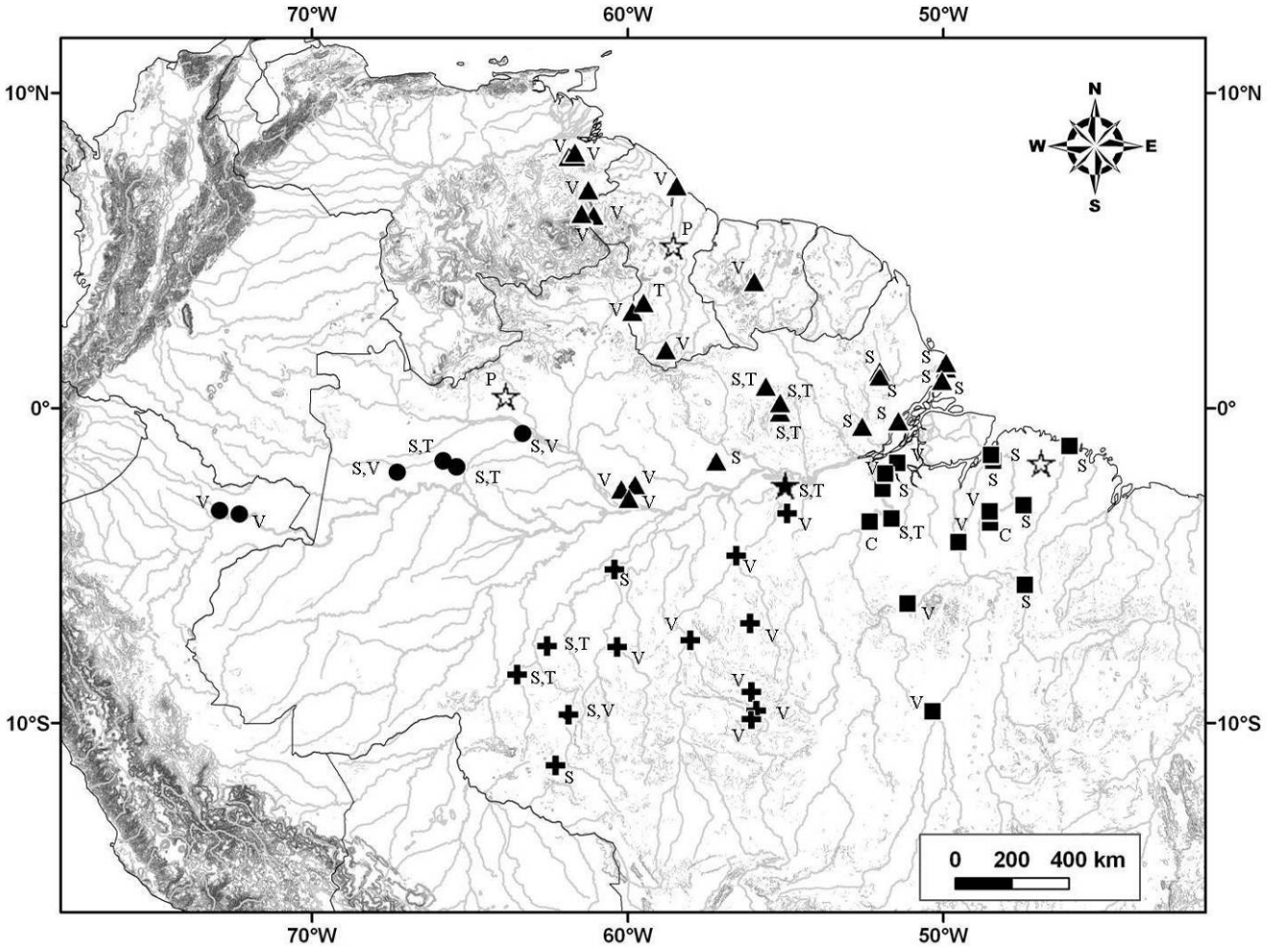

Figure 1. Geographic distribution of specimens, vocalizations, and tissues of the Spotted-Antpitta (*Hylopezus macularius*) complex sampled in this study. Symbols denote samples of each of the four main lineages recovered by a molecular phylogeny and here interpreted as natural populations (labeled A-D; see text for details). Currently defined subspecies (Krabbe and Schulenberg 2003): Squares = Population A (*H. m. paraensis*); Crosses = Population B (*Taxon novum*); Circles = Population C (*H. m. dilutus*); Triangles = Population D (*H. m. macularius*); Open stars = Indicate the type locality for each taxon; Black star = Indicates the type locality defined by us for new taxon. Letters next to a symbol denote locations for which the following types of samples were analyzed: "P" = photographs only; "S" = specimens only; "V" = tape-recordings only; "T" tissues only; "S,V" = specimens and vocalizations only; "S,T" = specimens and tissues only; "C" = tape-recordings, specimens, and tissues.

Table 1. General information on the tissue samples sequenced in this study. Voucher numbers refer to study skins deposited at ornithological collections.

Taxon	Locality	Population[a]	Collection[b]	Voucher number
H. m. macularius	Brazil – Pará. Óbidos, esec Grão-Pará (00° 37' 50N 55° 43' 40W)	D	MPEG	66675
H. m. macularius	Brazil – Pará. Óbidos, esec Grão-Pará (00° 37' 50N 55° 43' 40W)	D	MPEG	66676
H. m. macularius	Brazil – Pará. Óbidos, esec Grão-Pará (00° 37' 50N 55° 43' 40W)	D	MPEG	66677
H. m. macularius	Brazil - Pará. Faro, Flota de Faro (01° 42'S 57° 12'W)	D	MPEG	64739
H. m. macularius	Brazil – Pará. Alenquer. Esec Grão-Pará (00° 09'S 55° 11'W)	D	MPEG	66053
H. m. macularius	Guyana - Iwokrama reserve; kobacalli landing	D	ANSP	21224
H. m. paraensis	Brazil - Amazonas. Maraã, Lago Cumapi (01°43'48,6"S;65°52'45,5"W)	C	MPEG	62966
H. m. paraensis	Brazil – Amazonas – Maraã, rio Japurá, Maguari (01°52'S;65°25'W)	C	MPEG	42750
H. m. paraensis	Brazil – Amazonas. Humaitá. T. I. Parintintin (07°33'S;62°33'W)	B	MPEG	58757
H. m. paraensis	Brazil-Rondônia	B	FMNH	389869
H. m. paraensis	Brazil – Pará.Belterrra, Floresta Nacional do Tapajós	B	MPEG	56099
H. m. paraensis	Brazil - Pará. rio Xingu, Caracol (03°31'S 51°38'32.7"W)	A	MPEG	65326
H. m. paraensis	Brazil - Pará. rio Xingu, Caracol (03°31'S 51°38'32.7"W)	A	MPEG	64919
H. m. paraensis	Brazil - Pará. Senador José Porfirio. Rio Xingu (02°34'S 51°56'W)	A	MPEG	55691
H. m. paraensis	Brazil - Pará. Paragomimas (03°38'37.6'S 48°31'10.3"W)	A	MPEG	58982
Hylopezus auricularis	Bolívia – Beni	_	FMNH	391156
Hylopezus auricularis	Bolívia – Beni	_	FMNH	391157
Hylopezus auricularis	Bolívia – Beni	_	FMNH	391158
Hylopezus berlepschi	Peru – Madre Dios	_	FMNH	322345
Hylopezus berlepschi	Brazil - Pará: rio Xingu, Itapuama	_	MPEG	63449
Hylopezus berlepschi	Peru – Madre Dios	_	FMNH	433523
Hylopezus nattereri	Brazil - Paraná: Quatro Barras	_	MPEG	64834
Hylopezus ocrholeucus	Brazil – Minas Gerais. Mocambinho, Jaíba	_	LGEMA	2036
Hylopezus ocrholeucus	Brazil – Piauí. Curimatá, Serra vermelha	_	MPEG	MG68135
Myrmothera campanisona	Brazil - Pará: rio Xingu	_	MPEG	65325
Myrmothera campanisona	Brazil – Pará. Faro, Flota de Faro	_	MPEG	64781

[a]Natural population of polytypic *H. macularius* as recovered by a molecular phylogeny (see Figure 1).

[b]Collection acronyms are listed in material and methods.

*Specimen not catalogued

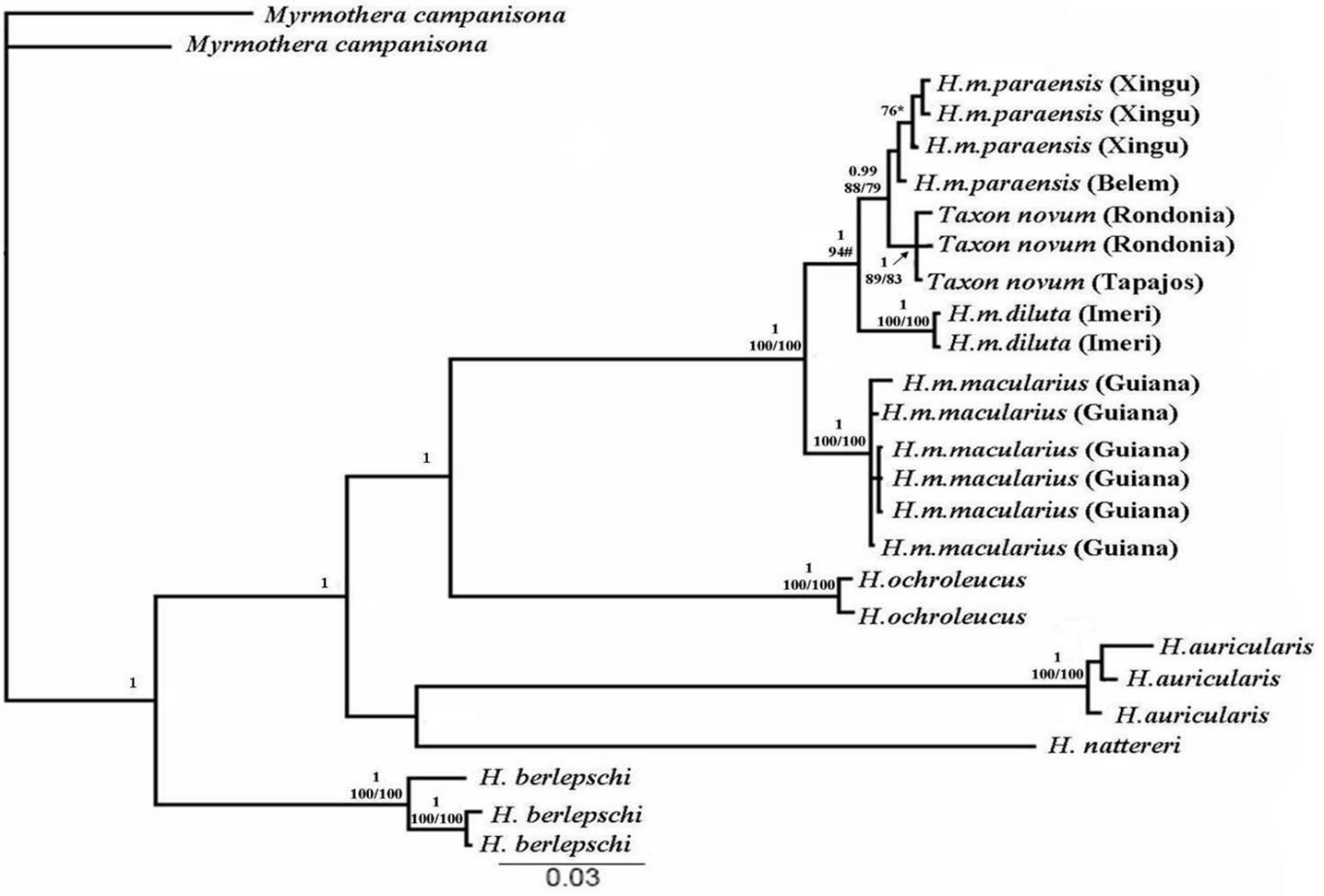

Figure 2. Bayesian posterior probabilities from data combined *cyt b, 16s* and *ND2* data. Bootstrap support values obtained by Bayesian (above), MP (before slash) and ML (after slash), are shown. Nodes poorly supported, as indicated by bootstrap values < 75% for (MP and ML) and < 95% (Bayesian) do not shown. Asterisk (*) and Number sign (#) denote nodes with bootstrap values ≥ 75% only for ML or only for MP respectively. In parenthesis endemism areas by (Silva *et. al* 2005).

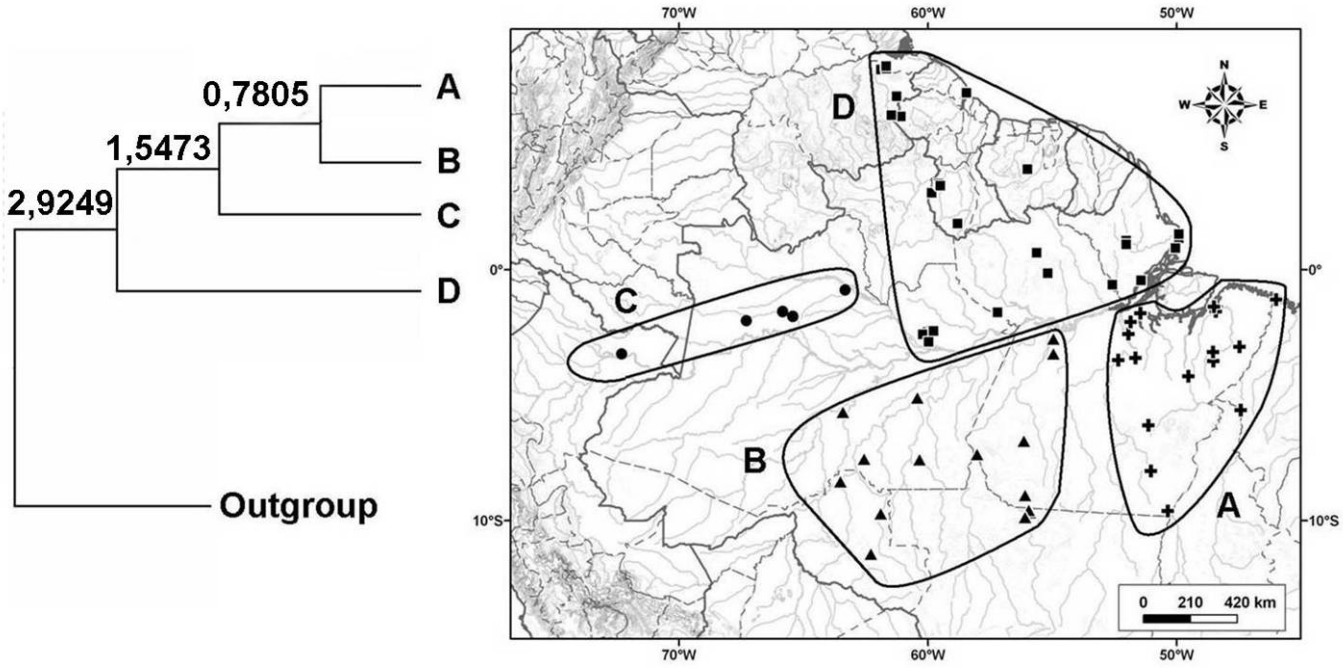

Figure 3. Mean of estimative of time in millions of years calculated by BEAST for diversification of populations A - D.

Table 2. Estimative of time in millions of years calculated by BEAST for diversification of populations A - D.

Estimate in millions of years			
Populations	**95% HPD lower**	**Mean ± SD**	**95% HPD upper**
A	0.1151	0.465 ± 2.9745E-3	0.9069
B	2.2471E-2	0.2317 ± 1.8459E-3	0.5222
C	4.5077E-7	8.2633E-2 ± 1.7421E-3	0.2721
D	7.8446E-2	0.4607 ± 6.2925E-3	1.0155
A – B	0.2514	0.7805 ± 5.6025E-3	1.3942
A.B – C	0.5978	1.5473 ± 1.2363E-2	2.6937
A.B.C – D	1.1544	2.9249 ± 3.3004E-2	5.54

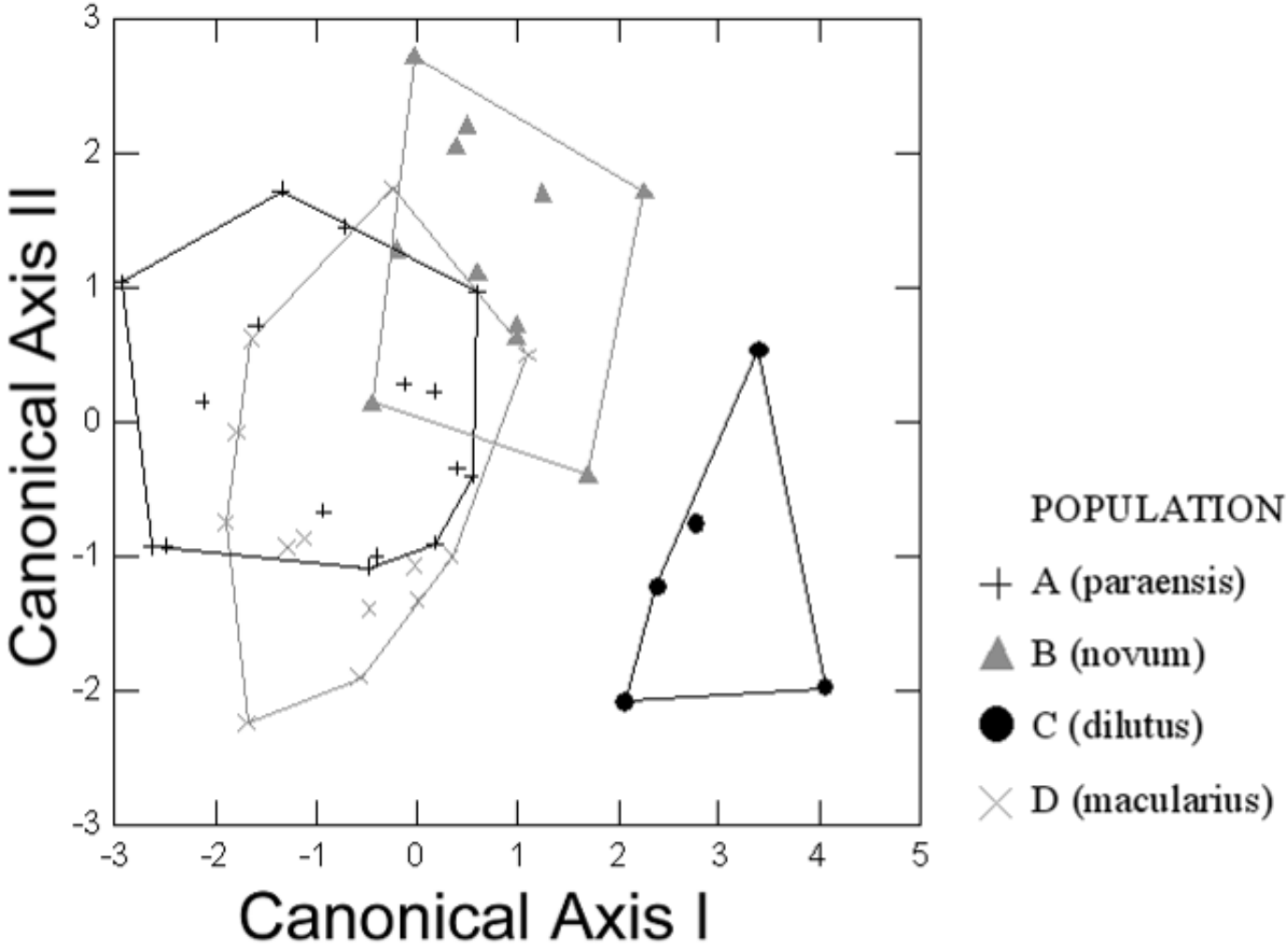

Figure 4. Graphical representation of scores of the first two axes of the Discriminant Functions Analysis performed with four most important morphometric characters, chosen by stepwise analysis, as best discriminating populations A – D (tarsus length, bill depth, bill width, and the extent of white on the pectoral spots).

Table 3. Summary of classification accuracy of morphological data used in the Discriminant function analysis.

Population	*Correctness%*	*Jacknifed %*
A (n = 16)	44	31
B (n = 11)	73	64
C (n = 5)	100	80
D (n = 13)	62	46
Total (n = 45)	62	49

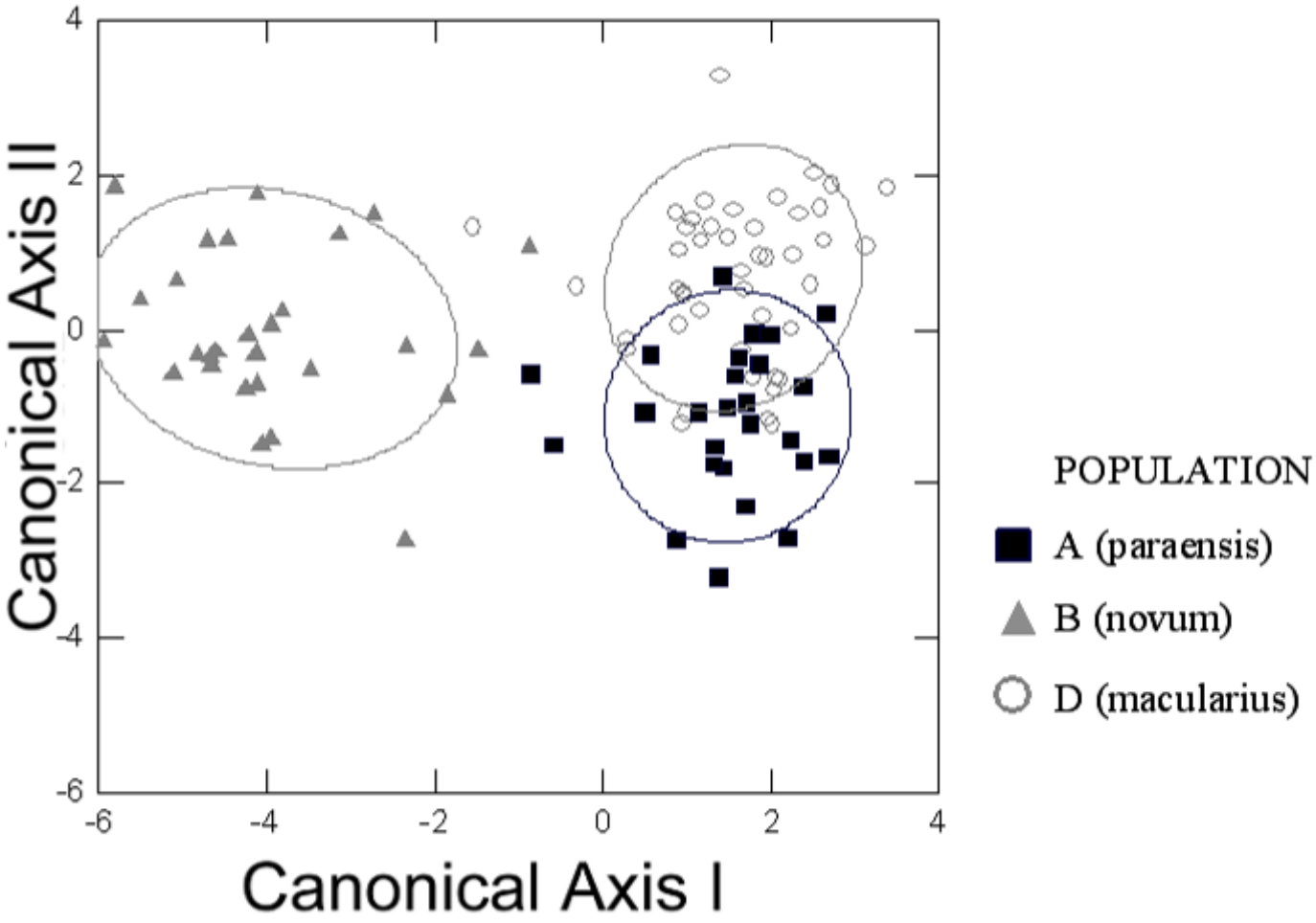

Figure 5. Graphical representation of scores of the first two axes of the Discriminant Functions Analysis performed with vocal characters for the four populations.

Table 4. Summary of classification accuracy of vocal data used in the Discriminant Function analysis.

Population	*Correctness%*	*Jacknifed %*
A (n = 27)	60	45
B (n = 28)	100	96
D (n = 45)	75	60
Total (n = 100)	86	70

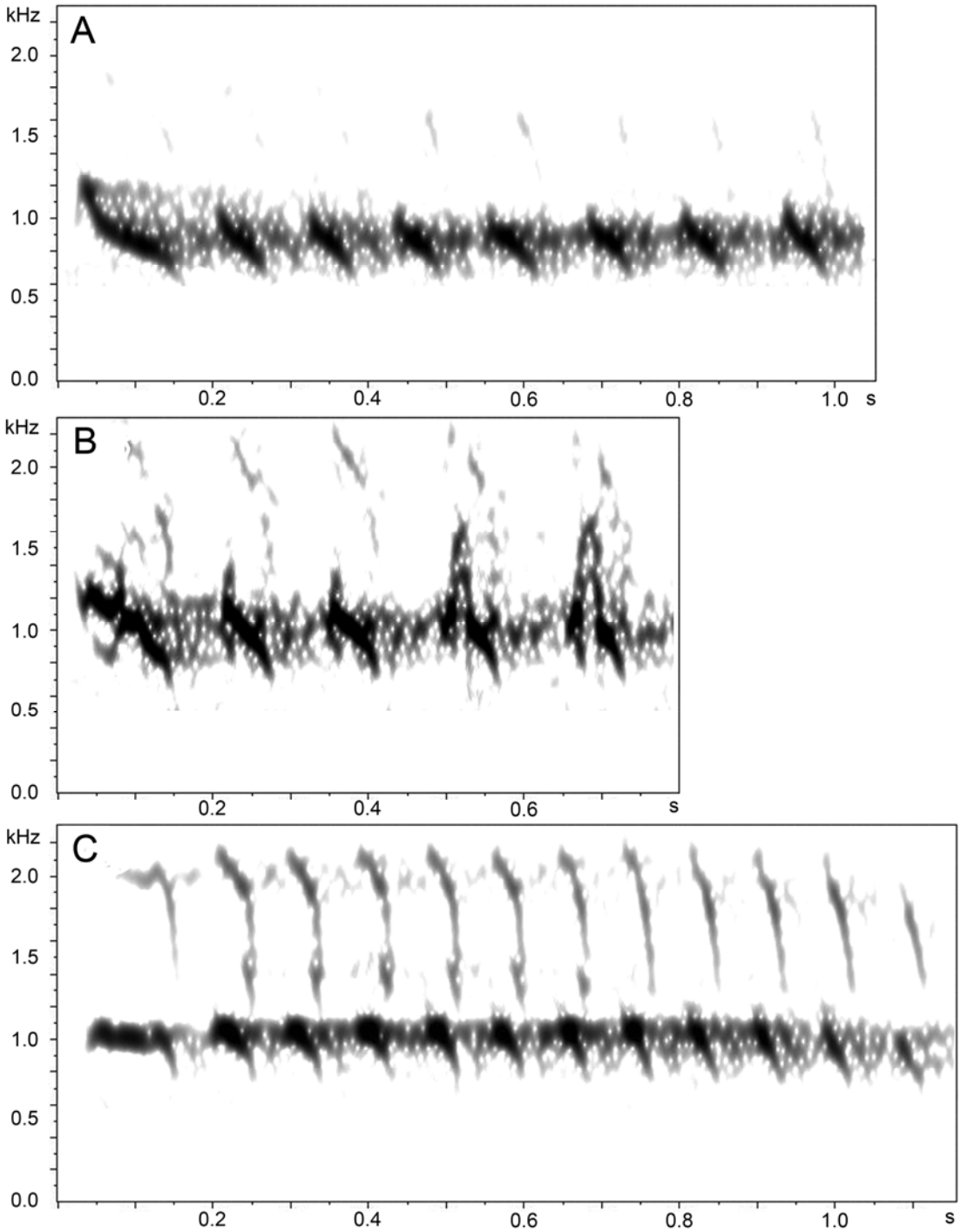

Figure 6. Calls audiospectrograms of populations A–D of *Hylopezus macularius*. (window type Hamming, window size 800 samples, time grid 90% overlap, DFT size 16384 samples) (A) Population A multisyllabic call, Caxiuanã, Pará, Brazil (A. Whittaker – 1 PAC). (B) Population B multisyllabic call, Rondônia, Brazil (A. Whittaker – 1 PAC). (C) Population D multisyllabic call, Bolívar, Venezuela (T. A. Parker III – MLS 34472).

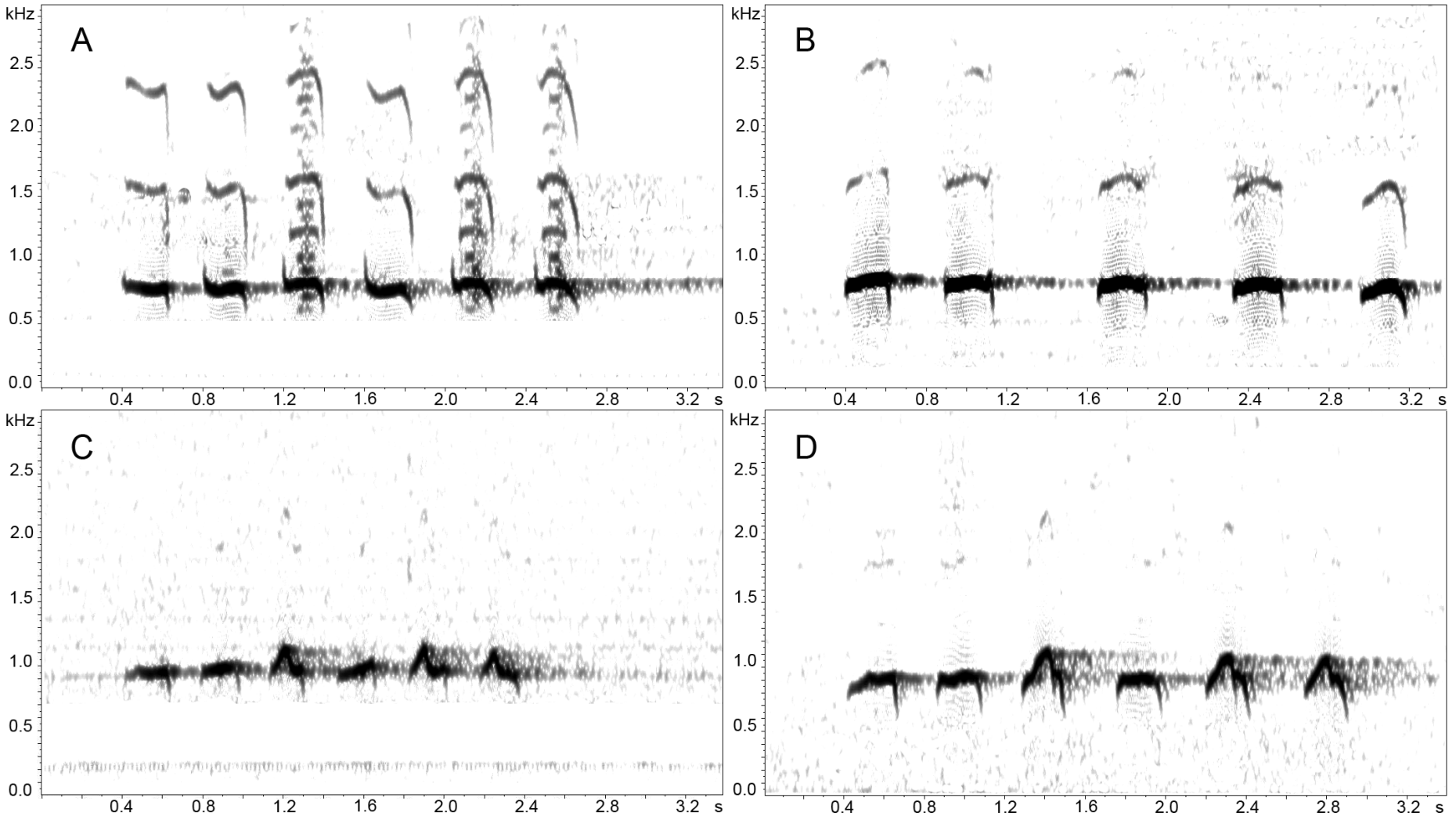

Figure 7. Loudsongs audiospectrograms of populations A– D of *Hylopezus macularius*. (window type Hamming, window size 1300 samples, time grid 90% overlap, DFT size 16384 samples) (A) Population A loudsong, Caxiuanã, Pará, Brazil (C. A. Marantz – MLS 127444). (B) Population B loudsong, Alta Floresta, Mato grosso, Brazil (P. R. Isler – MLS 48068). (C) Population C, Japurá, Amazonas, Brazil (A. Aleixo – 1 PAC). (D) Population D loudsong, Rupununi, Guyana (T. A. Parker III – MLS 73054).

Table 5. Vocal characters and measurements[a] of four populations of the *H. macularius* complex, including the new taxon. Values are mean ± SD, with ranges in parentheses.

Character	*Population A*	*Population B*	*Population C*	*Population D*
Loudsongs	***N = 27 recordings and 81 songs***	***N = 28 recordings and 84 songs***	***N = 4 recordings and 12 songs***	***N = 45 recordings and***
Number of notes	5.96 ± 0.19 (5-6)	4.93 ± 0.19 (4-6)	6	5.97 ± 0.26 (5-
Duration of first note(s)	0.24 ± 0.03 (0.18-0.33)	0.24 ± 0.03 (0.18-0.29)	0.25 ± 0.06 (0.16-0.31)	0.24 ± 0.03 (0.18-
Duration of second note(s)	0.22 ± 0.02 (0.17-0.27)	0.25 ± 0.03 (0.18-0.29)	0.23 ± 0.01 (0.21-0.24)	0.21 ± 0.03 (0.14-
Duration of third note(s)	0.23 ± 0.03 (0.19-0.30)	0.26 ± 0.03 (0.19-0.31)	0.21 ± 0.03 (0.18-0.24)	0.21 ± 0.04 (0.12-
Duration of fourth note(s)	0.24 ± 0.03 (0.14-0.31)	0.26 ± 0.03 (0.21-0.34)	0.24 ± 0.02 (0.22-0.26)	0.21 ± 0.03 (0.16-
Duration of fifth note(s)	0.22 ± 0.03 (0.17-0.31)	0.24 ± 0.07 (0.15-0.50)	0.21 ± 0.02 (0.17-0.22)	0.21 ± 0.03 (0.13-
Duration of sixth note(s)	0.22 ± 0.04 (0.09-0.30)	0.22 ± 0.06 (0.13-0.27)	0.21 ± 0.05 (0.16-0.27)	0.20 ± 0.03 (0.15-
Duration of first interval(s)	0.16 ± 0.03 (0.10-0.24)	0.27 ± 0.05 (0.16-0.37)	0.13 ± 0.02 (0.12-0.17)	0.19 ± 0.04 (0.09-
Duration of second interval(s)	0.19 ± 0.04 (0.13-0.27)	0.41 ± 0.09 (0.25-0.58)	0.14 ± 0.03 (0.10-0.17)	0.21 ± 0.04 (0.14-
Duration of third interval(s)	0.20 ± 0.04 (0.11-0.28)	0.39 ± 0.06 (0.26-0.54)	0.13 ± 0.02 (0.10-0.15)	0.24 ± 0.04 (0.15-
Duration of fourth interval(s)	0.21 ± 0.04 (0.15-0.31)	0.40 ± 0.08 (0.26-0.59)	0.14 ± 0.02 (0.12-0.16)	0.21 ± 0.05 (0.11-
Duration of fifth interval(s)	0.22 ± 0.05 (0.13-0.33)	0.29 ± 0.13 (0.08-0.43)	0.16 ± 0.02 (0.14-0.19)	0.24 ± 0.05 (0.15-
Interval between loudsongs	22.14 ± 11.93 (8.40-50.99)	12.43 ± 3.18 (6.39-19.76)	10.32 ± 8.16 (8.24-19.04)	17.64 ± 6.13 (9.37-
Calls	***N = 2 individuals and 6 calls***	***N = 1 individuals and 3 calls***	*	***N = 1 individuals and***
Number of notes	7-8	5	-	11-12

[a] Terminology and methods see text.

[*] No call sample available for this taxon.

Table 6. Percentage of emission of each dominant frequency of four populations of the *H. macularius* complex. including the new taxon.

Character	***Population A***	***Population B***	***Population C***	***Population D***
Max frequency	***N = 27 Recordings and 81 songs***	***N = 28 Recordings and 84 songs***	***N = 4 Recordings and 12 songs***	***N = 45 Recordings and 135 songs***
first note	861.30 = 82.8% 689.10 = 17.1% -	861.30 = 88.3% 689.10 = 10.4% 1033.60 = 1.3%	861.30 = 58.1% 689.10 = 33.2% 1033.60 = 8.3%	861.30 = 91.5% 937.50 = 5.7% 1033.60 = 2.43%
second note	861.30 = 78.9% 689.10 = 21.1% -	861.30 = 90.9% 689.10 = 9.1% -	861.30 = 66.4% 1033.60 = 33.2% -	861.30 = 91.5% 937.50 = 5.7% 1033.60 = 2.43%
third note	861.30 = 100% - -	861.30 = 85.7% 689.10 = 10.4% 1033.60 = 3.9%	861.30 = 49.8% 689.10 = 24.9% 1033.60 = 24.9%	1033.60 = 61.8% 861.30 = 30.7% 937.50 = 7.3%
fourth note	861.30 = 56.5% 689.10 = 43.4% -	861.30 = 79.2% 689.10 = 18.2% 1033.60 = 2.6%	861.30 = 49.8% 689.10 = 24.9% 1033.60 = 24.9%	861.30 = 89.9% 937.50 = 7.3% 1033.60 = 2.4%
fifth note	861.30 = 94.7% 689.10 = 3.9% Omitted = 1.3%	861.30 = 40.2% 689.10 = 20.8% Omitted = 39%	861.30 = 66.4% 689.10 = 16.6% 1033.60 = 16.6%	861.30 = 57.5% 1033.60 = 34.0% 937.50 = 7.3% Omitted = 0.8%
sixth note	861.30 = 88.1% 689.10 = 5.2% Omitted = 6.6%	861.30 = 9.1% Omitted = 90.9%	861.30 = 49.8% 689.10 = 33.2% 1033.60 = 16.6%	861.30 = 64.0% 1033.60 = 20.2% Omitted = 10.5% 937.50 = 4.9%

Table 7. Morphometrics (details of measurements in the text) and weight data of four populations of the *H. macularius* complex. including the new taxon.

Values are mean with ranges in parentheses.

Population	Sex	Sample size	Wing	Tail	Tarsus	Culmen	Bill-width	Bill-depth	Body mass (g)[a]
	Male	9	83.6 (81.1-86.8)	37.3 (34.1-39.2)	35.6 (34.3-37.2)	13.0 (12.2-14.0)	5.6 (5.0-6.0)	5.5 (5.1-5.8)	43g (42-44)
A	Female	6	84.6 (80.8-87.0)	36.6 (34.9-40.8)	36.2 (33.8-38.5)	13.6 (12.3-14.2)	5.9 (5.2-6.4)	5.6 (5.1-5.9)	45.20g (42-48.4)
	Sex unknown	1	83.5	34.8	33.5	-	-	-	-
	Total	16	83.9	36.2	35.1	13.3	5.7	5.5	44.1g
	Male	6	85.3 (82.0-89.6)	37.5 (36.4-39.6)	34.8 (33.3-37.6)	13.6 (13.1-14.0)	6.2 (5.6-6.7)	5.8 (5.3-6.5)	44.1g (40-47)
B	Female	2	84.4 (84.2-84.6)	36.0 (36.0-36.0)	34.6 (33.7-35.4)	13.1 (12.7-13.5)	6.2 (6.1-6.2)	5.3 (5.1-5.4)	42.4g
	Sex unknown	3	87.5 (84.8-90.1)	36.9 (33.9-39.0)	35.7 (34.8-37.2)	13.4 (12.8-13.8)	6.3 (6.0-6.4)	6.3(5.6-7.7)	-
	Total	11	85.7	36.8	35	13.4	6.2	5.8	43.2g
	Male	4	82.5 (79.0-84.0)	34.3 (31.0-37.0)	33.4 (30.9-34.4)	13.0 (12.7-13.3)	5.9 (5.7-6.1)	6.3 (5.7-7.0)	42.8g (39-45.5)
C	Female	1	85.4	37.8	31.8	13.8	6.1	6.0	-
	Total	5	83.9	36.0	32.6	13.4	6	6.15	42.8g
	Male	7	84.9 (82.0-88.9)	37.2 (35.2-41.3)	36.5 (35.3-38.6)	13.5 (13.2-13.9)	5.6 (5.1-6.3)	5.9 (5.2-6.8)	42g(41-43)
D	Female	5	79.1 (78.2-80.3)	34.8 (33.9-35.5)	32.9 (32.2-33.7)	13.4 (13.2-13.5)	5.8 (5.6-6.2)	5.7	39.5g
	Sex unknown	1	87.9	34.8	35.5	12.4	5.2	5.8	39g
	Total	13	83.9	35.6	34.9	13.1	5.5	5.8	40.1g

[a] Body mass information not available for some specimens.

Table 8 Matrix of genetic divergence among taxa from combined data (uncorrected (below diagonal) and corrected by model (above diagonal)).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
(1) MPEG 65326 (Xingu)	-	0.002	0.001	0.003	0.03	0.031	0.032	0.033	0.032	0.032	0.008	0.011	0.007	0.023	0.03	0.18	0.189	0.186	0.234	0.214	0.203	0.159	0.16	0.193	0.189	0.205
(2) MPEG 64919 (Xingu)	-	-	0.001	0.003	0.03	0.031	0.032	0.033	0.032	0.032	0.008	0.011	0.007	0.023	0.03	0.18	0.189	0.186	0.239	0.219	0.208	0.159	0.16	0.197	0.189	0.206
(3) MPEG 55691 (Xingu)	-	0.001	-	-	0.028	0.029	0.03	0.031	0.03	0.03	0.006	0.006	0.004	0.02	0.025	0.173	0.18	0.178	0.227	0.207	0.195	0.153	0.153	0.179	0.167	0.202
(4) MPEG 58982 (Belém)	-	0.003	-	-	0.03	0.027	0.029	0.029	0.029	0.028	0.005	0.009	0.005	0.02	0.026	0.178	0.187	0.185	0.253	0.232	0.223	0.161	0.162	0.207	0.184	0.204
(5) ANSP 21224 (Guiana)	0.03	0.028	0.026	0.028	-	0.004	0.004	0.004	0.004	0.003	0.033	0.037	0.033	0.038	0.052	0.171	0.184	0.181	0.272	0.247	0.238	0.165	0.163	0.201	0.186	0.203
(6) MPEG 64739 (Guiana)	0.03	0.028	0.027	0.025	0.003	-	0.002	0.002	0.002	0.001	0.032	0.033	0.029	0.035	0.049	0.172	0.187	0.184	0.244	0.223	0.218	0.152	0.151	0.2	0.178	0.192
(7) MPEG 66675 (Guiana)	0.03	0.029	0.028	0.026	0.004	0.002	-	-	-	0.001	0.033	0.034	0.03	0.036	0.049	0.174	0.185	0.183	0.253	0.234	0.222	0.158	0.157	0.204	0.185	0.202
(8) MPEG 66676 (Guiana)	0.03	0.03	0.029	0.027	0.004	0.002	-	-	-	0.001	0.034	0.034	0.031	0.037	0.049	0.174	0.183	0.181	0.256	0.234	0.222	0.157	0.156	0.202	0.185	0.203
(9) MPEG 66677 (Guiana)	0.03	0.029	0.028	0.026	0.004	0.002	-	-	-	0.001	0.033	0.034	0.03	0.036	0.049	0.173	0.185	0.183	0.253	0.234	0.221	0.158	0.157	0.204	0.185	0.202
(10) MPEG 66053 (Guiana)	0.03	0.029	0.028	0.026	0.003	0.001	0.001	0.001	0.001	-	0.033	0.033	0.03	0.036	0.046	0.172	0.184	0.181	0.262	0.239	0.227	0.157	0.155	0.202	0.183	0.208
(11)FMNH 389869 (Rondônia)	0.01	0.008	0.006	0.005	0.03	0.029	0.03	0.031	0.03	0.03	-	0.003	0.002	0.023	0.034	0.172	0.177	0.176	0.234	0.214	0.202	0.153	0.156	0.177	0.162	0.205
(12)MPEG 58757 (Rondônia)	0.01	0.01	0.006	0.009	0.034	0.031	0.031	0.031	0.031	0.03	0.003	-	0.002	0.024	0.032	0.185	0.185	0.183	0.241	0.222	0.21	0.155	0.157	0.202	0.183	0.209
(13) MPEG 56099 (Tapajós)	0.01	0.007	0.004	0.005	0.03	0.027	0.028	0.029	0.028	0.028	0.002	0.002	-	0.021	0.029	0.175	0.184	0.181	0.247	0.226	0.217	0.158	0.161	0.2	0.178	0.21
(14) MPEG 62966 (Imeri)	0.02	0.021	0.019	0.019	0.034	0.032	0.033	0.034	0.033	0.033	0.022	0.023	0.02	-	-	0.179	0.188	0.185	0.24	0.22	0.215	0.163	0.164	0.205	0.186	0.206
(15) MPEG 42750 (Imeri)	0.03	0.028	0.024	0.025	0.045	0.043	0.042	0.042	0.042	0.04	0.031	0.029	0.027	-	-	0.274	0.254	0.249	0.329	0.329	0.322	0.226	0.229	0.294	0.302	0.306
(16) MPEG 63449	0.11	0.114	0.111	0.113	0.109	0.109	0.11	0.11	0.11	0.109	0.111	0.116	0.111	0.114	0.15	-	0.023	0.02	0.252	0.23	0.214	0.198	0.202	0.136	0.111	0.205
(17) FMNH 322345	0.12	0.119	0.116	0.119	0.117	0.116	0.116	0.115	0.116	0.116	0.115	0.117	0.117	0.119	0.145	0.022	-	0.002	0.261	0.238	0.223	0.182	0.183	0.135	0.121	0.206
(18) FMNH 433523	0.12	0.118	0.115	0.118	0.115	0.115	0.115	0.114	0.115	0.115	0.115	0.117	0.116	0.118	0.143	0.019	0.002	-	0.257	0.235	0.22	0.182	0.184	0.131	0.116	0.205
(19) FMNH 391156	0.13	0.134	0.13	0.138	0.144	0.135	0.137	0.138	0.137	0.14	0.132	0.133	0.136	0.135	0.162	0.135	0.141	0.14	-	0.01	0.012	0.244	0.25	0.253	0.244	0.271
(20) FMNH 391157	0.13	0.128	0.124	0.132	0.137	0.129	0.132	0.132	0.132	0.134	0.126	0.127	0.13	0.129	0.162	0.129	0.135	0.134	0.01	-	0.005	0.222	0.226	0.232	0.224	0.253
(21) FMNH 391158	0.12	0.123	0.118	0.128	0.134	0.126	0.128	0.128	0.127	0.13	0.121	0.122	0.126	0.128	0.161	0.123	0.129	0.128	0.012	0.005	-	0.214	0.218	0.228	0.226	0.236
(22) LGEMA 2036	0.11	0.107	0.105	0.108	0.11	0.104	0.106	0.106	0.106	0.106	0.105	0.104	0.106	0.11	0.136	0.121	0.115	0.116	0.133	0.126	0.124	-	0.004	0.204	0.181	0.202
(23) MPEG MG68135	0.11	0.108	0.105	0.109	0.109	0.104	0.106	0.105	0.106	0.106	0.106	0.106	0.108	0.11	0.138	0.123	0.116	0.117	0.136	0.128	0.126	0.004	-	0.204	0.182	0.203
(24) MPEG 65325	0.12	0.126	0.118	0.13	0.127	0.125	0.127	0.126	0.127	0.126	0.116	0.127	0.127	0.129	0.165	0.098	0.097	0.095	0.143	0.136	0.134	0.126	0.126	-	0.074	0.217
(25) MPEG 64781	0.12	0.12	0.111	0.118	0.117	0.114	0.116	0.116	0.116	0.115	0.108	0.116	0.114	0.118	0.163	0.083	0.089	0.086	0.139	0.133	0.134	0.115	0.115	0.06	-	0.186
(26) MPEG 64834	0.13	0.125	0.123	0.125	0.124	0.119	0.123	0.123	0.123	0.125	0.125	0.124	0.126	0.125	0.155	0.123	0.125	0.125	0.146	0.141	0.134	0.122	0.123	0.128	0.116	-