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ARTICLE

Systematic review of the Cinnamon-throated Woodcreeper *Dendrexetastes rufigula* (Aves: Dendrocolaptidae) based on a multilocus phylogeography

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ABSTRACT: The Amazon is one of the most speciose regions in the world. Yet there are still undescribed and misidentified species, and scarce information about the biology of the described species in the region. Here, we evaluate for the first time the existence of genetically differentiated lineages within the polytypic species *Dendrexetastes rufigula*, an endemic Amazonian lineage. We identified three major evolutionary independent units using both mitochondrial (Cytb and ND2) and nuclear (G3PDH, BF5 and MUSK) markers that roughly corresponded to currently recognized subspecies. Although we found strong statistical support for the reciprocal monophyly of *D. r. rufigula* and *D. r. devillei*, we did not find reciprocal monophyly between *D. r. moniliger* and *D. r. paraensis,* which were paraphyletic. However, these two taxa grouped together in a clade with Bayesian but not bootstrap support. Moreover, clades *D. r. rufigula*, *D. r. devillei*, and *D. r. moniliger*/*paraensis* differed from each other by much higher mitochondrial genetic distances (between 1 and 2%), than that separating *D. r. paraensis* from *D. r. moniliger* (0.3 ± 0.1%). We add molecular evidence to the morphological data supporting that *D. r. rufigula* and *D. r. devillei* are highly diagnostic taxa that could be regarded as two distinct species. Conversely, although *D. r. moniliger* and *D. r. paraensis* are both genetically and morphologically distinct from either *D. r. rufigula* or *D. r. devillei*, *D. r. moniliger* and *D. r. paraensis* cannot be considered mutually independent evolutionary lineages. This result is particularly important from a conservation perspective, since *D. r. paraensis* is considered threatened in Brazil. Our results support that at least three main evolutionary lineages deserving evolutionary species status exist in the Cinnamon-throated Woodcreeper, and that the endangered lineage in the Belém area of endemism is a morphologically slightly distinct subset of a more widespread lineage endemic to southeastern Amazonia east of the Madeira River.

KEY-WORDS: Amazonia, conservation, *Dendrexetastes rufigula paraensis*, leapfrog pattern, species limits, taxonomy.

INTRODUCTION

Growing evidence highlights that global biodiversity levels are higher within the Amazon region than previously acknowledged (plants: Kier *et al.* 2005; mammals: Ceballos & Ehrlich 2006; birds: Jetz *et al.* 2012; several examples: Jenkins *et al.* 2013). Overall, birds are one of the best known taxonomic groups within the region, with fewer bird species discovered since the 1950s in comparison to mammals, and amphibians (Jenkins *et al.* 2013). Yet, a considerable percentage of the latest "new" bird species described from the Amazon resulted from the recognition of widespread species as species complexes (*e.g.* Carneiro *et al.* 2012, Whitney & Cohn-Haft 2013). Molecular analytical tools have been particularly important in the assessment of species limits for these geographically widespread species complexes in Amazonia (see revision by Bickford *et al.* 2007; and recent examples, such as D'Horta *et al.* 2013, Fernandes *et al.* 2013, Sousa-Neves *et al.* 2013, Thom & Aleixo 2015). This hidden diversity, as described by Bickford *et al.* (2007), results from the inability to distinguish two or more species, cryptic species, due to their morphological similarities, and so they are treated as the same nominal species. Thus, currently, despite being a well-known group, the Amazonian avifauna still suffers from a chronic under-estimation of its diversity, namely needing an accurate assessment of its cryptic diversity (see Bates & Demos 2001, Aleixo 2009, Whitney & Cohn-Haft 2013, Barrowclough *et al.* 2016).

The Cinnamon-throated Woodcreeper *Dendrexetastes rufigula* (Aves: Dendrocolaptidae) is a widespread and polytypic Amazonian endemic species, which occurs in both upland *terra-firme* and seasonally flooded forests, such as *várzea* and *igapó* (Figure 1A). The genus is considered monospecific, and four subspecies are currently recognized (Marantz *et al.* 2003): *D. r. devillei* (occurring west of the Negro River to the west bank of the Madeira River in Brazil, and across southern Colombia, eastern Ecuador, eastern Peru, and northwestern Bolivia); *D. r. moniliger* (found from the east bank of the Madeira River to the west bank of the Tocantins River in Brazil, and northeastern Bolivia); *D. r. paraensis* (found east of the Tocantins River in the Brazilian states of Pará and Maranhão); and *D. r. rufigula* (occurring on the Guiana shield from eastern Venezuela, the Guianas, and Brazil east of the Negro River to Amapá state). Vocalizations are very similar among subspecies, but each is distinguishable by discrete plumage characters, which prompted their recognition as separate taxa (Marantz *et al.* 2003). In fact, *D. r. devillei* had been treated as a separate species by some sources (Hellmayr 1907, Snethlage 1908), but later subsumed under *D. rufigula* as a subspecies (Hellmayr 1910), an arrangement that has been followed ever since (Cory & Hellmayr 1925, Peters 1951, Marantz *et al.* 2003).

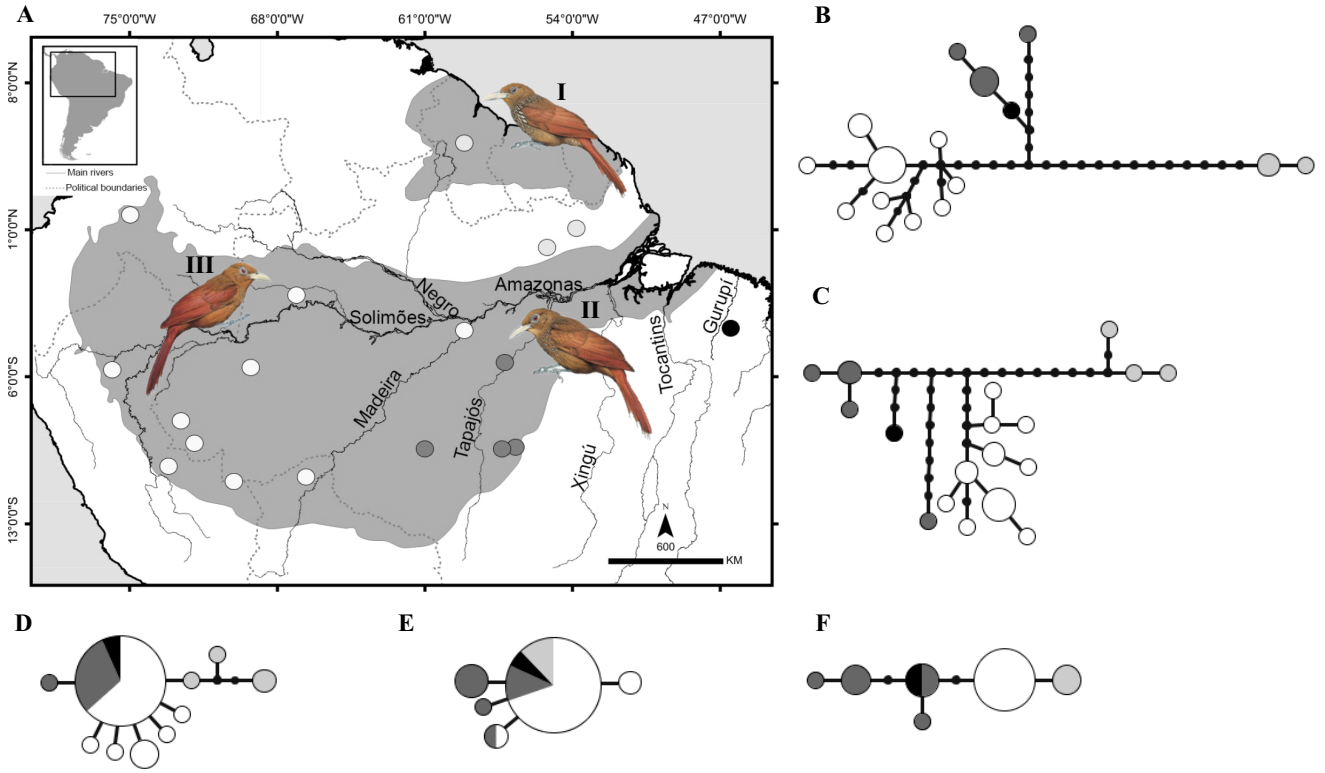

FIGURE 1. Putative distribution range of *Dendrexetastes rufigula* (modified from Marantz *et al.* (2003), sampling localities for each subspecies (**A**); and haplotype networks for NADH dehydrogenase (**B**), cytochrome *b* (**C**), β-fibrinogen intron 5 (**D**), glyceraldehyde-3-phosphate dehydrogenase intron 11 (**E**) muscle, skeletal, receptor tyrosine kinase intron 3 (**F**). In the haplotype networks, circle areas are proportional to haplotype frequencies. I and light grey *D. r. rufigula*, II and dark grey *D. r. moniliger*, III and white *D. r. devillei*, and black *D. r. paraensis*.

Given the species wide range and putative large population size, the Cinnamon-throated Woodcreeper is evaluated as Least Concern by IUCN, yet deforestation might be affecting its populations, and leading to a demographic decrease (Bird *et al.* 2012, BirdLife International 2012). Indeed, the most recent version of the Brazilian list of threatened species included *D. r. paraensis* from the Belém area of endemism (Da Silva *et al.* 2005) under the status "Vulnerable" (MMA 2014). The species is thought to have gone locally extinct in the Belém metropolitan area over the last 70 years, due to habitat destruction and fragmentation (Moura *et al.* 2014).

So far, no phylogeographic study exists for the Cinnamon-throated Woodcreeper, which prevents the assessment of the degree of evolutionary independence among its taxa, including the endangered *D. r. paraensis*. Here, we estimate for the first time the evolutionary history and degree of genetic differentiation among subspecies of the Cinnamon-throated Woodcreeper based on a multilocus approach, and discuss the systematic and taxonomic implications of these data.

METHODS

Specimens analyzed

Tissue samples of 28 specimens of Cinnamon-throated Woodcreeper *D. rufigula* were sequenced (Table 1; Figure

1A), as follows: *D. r. devillei* (n = 17), *D. r. rufigula* (n = 3), *D. r. moniliger* (n = 7), and *D. r. paraensis* (n = 1). A sample from *Nasica longirostris* was used as outgroup following Derryberry *et al.* (2011). For comparative purposes with the genetic data, we inspected plumage variation patterns of 31 Cinnamon-throated Woodcreeper study skins housed at the Museu Paraense Emílio Goeldi (MPEG) bird collection (Appendix I), as follows: *D. r. devillei* (n = 15), *D. r. rufigula* (n = 5), *D. r. moniliger* (n = 8), and *D. r. paraensis* (n = 3); of these, a total of 18 specimens were the same individuals used in the molecular analyses (Appendix I). We searched for any plumage characters diagnosing any taxon or recovered clade of the Cinnamon-throated Woodcreeper. Each specimen examined was scored qualitatively for the color and shape of any marks on the plumage of its different body parts. Alphanumeric color designations were determined through direct comparison with Smithe (1975).

TABLE 1. Voucher information of *Dendrexetastes rufigula* and *Nasica longirostris* tissue samples. M – Male; F – Female.

Museum	Taxa	Sex	Locality
FMNH 395555	*Dendrexetastes rufigula devillei*	M	Brazil, Acre, Reserva Extrativista Alto Juruá, River Tejo
LSUMZ B-1159	*Dendrexetastes rufigula devillei*	M	Bolivia, La Paz Department
LSUMZ B-103621	*Dendrexetastes rufigula devillei*	?	Peru, Loreto Department
LSUMZ B-28077	*Dendrexetastes rufigula devillei*	M	Peru, Loreto Department
LSUMZ B-4329	*Dendrexetastes rufigula devillei*	M	Peru, Loreto Department
LSUMZ B-11084	*Dendrexetastes rufigula devillei*	M	Peru, Ucayali Department
ANSP 183229	*Dendrexetastes rufigula devillei*	M	Equador, Imuya Cocha
ANSP 183230	*Dendrexetastes rufigula devillei*	F	Equador, Imuya Cocha
MPEG 58872	*Dendrexetastes rufigula devillei*	F	Brazil, Acre, ESEC River Acre, Acampamento 2 (11°00'53.4"S; 70°13'02.7"W)
MPEG 58873	*Dendrexetastes rufigula devillei*	M	Brazil, Acre, ESEC River Acre, Acampamento 2 (11°00'53.4"S; 70°13'02.7"W)
MPEG 62041	*Dendrexetastes rufigula devillei*	F	Brazil, Acre, Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08°20'35.7"S; 72°36'19.7"W)
MPEG 62670	*Dendrexetastes rufigula devillei*	M	Brazil, Amazonas, Japurá, River Acanauí (01°56'12.4"S; 66°36'18.8"W)
MPEG 60145	*Dendrexetastes rufigula devillei*	M	Brazil, Amazonas, RDS Cujubim, E bank River Jutaí (05°38'19"S; 69°10'59"W)
MPEG 62669	*Dendrexetastes rufigula devillei*	M	Brazil, Amazonas, Japurá, River Acanauí (01°56'12.4"S; 66°36'18.8"W)
MPEG 73774	*Dendrexetastes rufigula devillei*	F	Brazil, Amazonas, Autazes (03°46'52.8"S; 59°03'23.8"W)
LSUMZ B-39873	*Dendrexetastes rufigula devillei*	M	Peru, Loreto Department
LSUMZ B-35686	*Dendrexetastes rufigula devillei*	M	Peru, Loreto Department
ANSP 187812	*Dendrexetastes rufigula rufigula*	M	Guyana, Iwokrama Reserve Surama, Kurupukari Base Camp
MPEG 65390	*Dendrexetastes rufigula rufigula*	F	Brazil, Pará, Alenquer, ESEC Grão-Pará (00°09'S; 55°11'W)
MPEG 66217	*Dendrexetastes rufigula rufigula*	M	Brazil, Pará, Almeirim, REBIO Maicuru (00°49'N; 53°55'W)
FMNH 389808	*Dendrexetastes rufigula moniliger*	F	Brazil, Rondonia, Waterfall Nazare, W bank River Jiparana
FMNH 389815	*Dendrexetastes rufigula moniliger*	F	Brazil, Rondonia, Waterfall Nazare, W bank River Jiparana
LSUMZ B-35540	*Dendrexetastes rufigula moniliger*	M	Brazil, Mato Grosso
MPEG 69376	*Dendrexetastes rufigula moniliger*	F	Brazil, Mato Grosso, Paranaíta, River Teles Pires, left margin (09°24'51.4"S; 56°33'39.7"W)
MPEG 67351	*Dendrexetastes rufigula moniliger*	M	Brazil, Mato Grosso, Paranaíta, River Teles Pires (09°25'310"S; 56°33'753"W)
MPEG 67350	*Dendrexetastes rufigula moniliger*	F	Brazil, Mato Grosso, Paranaíta, River Teles Pires (09°25'310"S; 56°33'753"W)
MPEG 76624	*Dendrexetastes rufigula moniliger*	M	Brazil, Pará, Itaituba, River Tapajós left margin, Penedo (05°27'21.61"S; 57°04'12"W)
MPEG 76873	*Dendrexetastes rufigula paraensis*	F	Brazil, Maranhão, Centro Novo, REBIO Gurupi (03°42'12.8"S; 46°45'44"W)
MPEG 73862	*Nasica longirostris*	M	Brazil, Amazonas, Autazes, Uricurituba, Ilha (03°35'31.2"S; 58°56'35.6"W)

Institution acronyms: ANSP - Academy of Natural Sciences of Drexel University, Philadelphia, USA; FMNH - Field Museum of Natural History, Chicago, USA; LSUMZ - Louisiana State University Museum of Natural Science, Baton Rouge, USA; MPEG - Museu Paraense Emílio Goeldi, Belém, Brazil.

Genetic analyses

Total genomic DNA was extracted using the Genomic DNA Purification Kit (Promega; Wizard®). Two mitochondrial molecular markers were amplified: cytochrome *b* (Cytb) using primers L14841/H16065 (Kocher *et al.* 1989, Sorenson *et al.* 1999), and NADH Dehydrogenase Subunit 2 (ND2) using primers L5215/H6313 (Hackett 1996, Sorenson *et al.* 1999); two nuclear autosomal markers: β-fibrinogen Intron 5 (BF5) with primers S713/AS767 (Marini & Hackett 2002), and Glyceraldehyde 3-phosphate Dehydrogenase Intron 11 (G3PDH) using primers G3PD-13b/G3PD-14b (Fjeldså *et al.* 2003); and a Z-linked marker Muscle Skeletal Receptor Tyrosine Kinase Intron 3 (MUSK) using primers 13F/13R (Clark & Witt 2006). Polymerase chain reaction (PCR) amplifications were performed using an initial denaturation at 94°C for 5 min, followed by 33 (ND2 and BF5) or 35 cycles (all the other loci) of a denaturation at 94°C for 1 min, annealing for 1 min at temperatures between 50°C and 70°C according to the marker, and an extension at 72°C for 1 min; and the final extension was at 72°C for 5 min for all markers. Master Mix (Promega, Inc.) was used to perform PCR for Cytb and MUSK with the following concentrations: 6.25 µl of Master Mix, 10 pmol of each primer, 50 ng/µl of DNA, in a final volume of 12.5 µl. Taq DNA polymerase recombinant kit (Invitrogen, Inc.) was used to amplify all the other loci using 1× buffer; 0.4 mM DNTP; 10 pmol of each primer, 0.5 U Taq DNA polymerase; 2 mM, 1.4 mM or 1.5 mM $MgCl_2$ (for ND2, BF5 and G3PDH, respectively) and 50 ng/µl of DNA in a final volume of 12.5 µl. PCR products were visually inspected in an 1% agarose gel, after electrophoresis; and positive results were purified using PEG8000 2.5 M (Hawkins *et al.* 1994). After sanger sequencing reactions using the Big Dye Terminator v3.01 kit, sequence products for both strands were electrophoresed on an ABI 3130 automatic sequencer, following the manufacturer's protocol (Applied Biosystems, CA).

Sequences were visually inspected in BioEdit, and aligned using Clustal W (Hall 1999). Sequences from nuclear molecular markers were phased using the PHASE algorithm (Stephens & Scheet 2005), implemented in DnaSP 5.0 (Librado & Rozas 2009). A threshold of 80% was used. For all loci and subspecies, standard genetic diversity indices (*e.g.*, haplotype and nucleotide diversity) were estimated, and mismatch distribution plots were obtained in DnaSP 5.0 (Librado & Rozas 2009). Neutrality (Tajima's D and R_2; Tajima 1989, Ramos-Onsins & Rozas 2002), and recombination tests were also performed (Hudson & Kaplan 1985, Hudson *et al.* 1987, Rozas *et al.* 2001) using the same software, and the coalescent simulation test therein implemented (Rozas 2009). One thousand replicates were run to estimate statistical significance ($P < 0.05$) of the tests. Insertions/deletions detected in nuclear markers were coded as (-) and considered a fifth state.

Mean uncorrected P-distances, between and within subspecies, were estimated for mitochondrial markers, using concatenated datasets, in MEGA 5.0 (Tamura *et al.* 2011).

Haplotype networks for each molecular marker were constructed in haplotype viewer (Blake *et al.* 2012). Haplotype viewer requires the input of a maximum likelihood tree, which was obtained in raxmlGUI (Silvestro & Michalak 2012, Stamatakis 2014), using the mutation model that best fit the data (GTR-GAMMA) as determined by PartitionFinder (Lanfear *et al.* 2012). A more thorough phylogenetic analysis was obtained using raxmlGUI (Silvestro & Michalak 2012, Stamatakis 2014), by running 10 independent runs with 1000 slow bootstrap pseudo-replicates (Felsenstein 1985).

Species limits in the Cinnamon-throated Woodcreeper were tested using BPP3.2 (Yang 2015). This method considers gene tree/species tree conflicts, and the possible occurrence of incomplete lineage sorting (Yang & Rannala 2010, Rannala & Yang 2013). A joint species delimitation and species tree analysis was conducted (Yang 2015) to test the delimitation of the three clades recovered by ML analyses (*D. r. rufigula*, *D. r. devillei* and *D. r. moniliger*/*D. r. paraensis*; see results section for more details). We ran the reversible-jump Markov Chain Monte Carlo (rjMCMC) analysis, with algorithm 0 and e = 2, for 500,000 generations (sampling interval of five), and a burnin of 100,000 generations. Priors for ancestral population size and divergence times might influence the posterior probability distributions (Yang 2015), so we tested different combinations for these priors, considering relatively large and small ancestral population sizes: θ~G(1,10) and θ~G(2,2000), respectively; and shallow and deep divergence times: τ~G(2,2000) and τ~G(1,10), respectively. The other divergence time parameters were assigned the default Dirichlet prior (Yang & Rannala 2010). A heredity file was input to account for the different inheritance patterns in the dataset. Each analysis was run twice to confirm consistency of results.

RESULTS

We sequenced a total of 3564 base pairs (bp), respectively 1015, 997, 571, 556 and 425 from ND2, Cytb, MUSK, BF5 and G3PDH. All generated sequences have been deposited in GenBank under accession numbers KY510693 to KY510809. Standard sequence summary statistics are presented in Table 2. For *D. r. devillei*, mismatch distributions for G3PDH and BF5 genes fit

well with expected curves of population growth (data not shown). For *D. r. moniliger*, mismatch distribution analyses also detected signs of expansion for BF5 (data not shown). All other mismatch distributions did not support demographic expansions. Recombination tests did not detect recombination events.

Mean uncorrected P-distances within and between subspecies are presented in Table 3. These indices ranged within subspecies between 0.1% (*D. r. rufigula*) and 0.4% (*D. r. moniliger*), and between subspecies from 0.4% (*D. r. moniliger*/*D. r. paraensis*) to 2.0% (*D. r. devillei*/*D. r. rufigula*).

TABLE 2. *Dendrexetastes rufigula* genetic diversity and neutrality tests results for the (A) full dataset and (B) by subspecies for each locus. bp – base pairs, S – number of segregating sites, H – number of haplotypes, Hd – haplotype diversity, π – nucleotide diversity, D – Tajima's D, SD – standard deviation, n – number of sequences analysed, Cytb – cytochrome *b*, ND2 – NADH dehydrogenase 2, BF5 – β-fibrinogen intron 5, G3PDH – Glyceraldehyde-3-phosphate dehydrogenase intron 11, MUSK – muscle, skeletal, receptor tyrosine kinase intron 3.* P < 0.05. *D. r. paraensis* only included in Table A, because only one sample was available.

Locus	bp	S	H	Hd ± SD	∏ ± SD	D	R_2
A							
Cytb	997	43	18	0.967 ± 0.024	0.01001 ± 0.00131	-0.819	0.16
ND2	1015	42	15	0.934 ± 0.030	0.00941 ± 0.00122	-0.765	0.27
G3PDH	425	4	5	0.377 ± 0.092	0.00097 ± 0.00026	-1.319	0.06
BF5	556	10	10	0.490 ± 0.094	0.00165 ± 0.00047	-1.791	0.02*
MUSK	571	6	6	0.714 ± 0.080	0.00321 ± 0.00059	0.426	0.71
B							
D. r. devillei							
Cytb	15	13	10	0.924 ± 0.053	0.00340 ± 0.00054	-0.784	0.04*
ND2	17	19	8	0.912 ± 0.056	0.00357 ± 0.00053	-1.455	0.02*
G3PDH	26	2	3	0.218 ± 0.103	0.00053 ± 0.00026	-1.224	0.11
BF5	26	5	6	0.465 ± 0.116	0.00094 ± 0.00027	-1.709	0.06
MUSK	14	0	1	0.000	0.00000	-	-
D. r. moniliger							
Cytb	5	13	4	0.900 ± 0.161	0.00593 ± 0.00291	-0.978	0.91
ND2	7	7	4	0.810 ± 0.130	0.00391 ± 0.00074	0.952	0.82
G3PDH	10	3	4	0.733 ± 0.101	0.00221 ± 0.00048	-0.431	0.15
BF5	10	1	2	0.200 ± 0.154	0.00036 ± 0.00028	-1.112	0.71
MUSK	7	3	4	0.810 ± 0.130	0.00284 ± 0.00052	1.459	0.83
D. r. rufigula							
Cytb	3	3	3	1.000 ± 0.272	0.00208 ± 0.00073	-	0.27
ND2	3	1	2	0.667 ± 0.314	0.00066 ± 0.00031	-	1.00
G3PDH	4	0	1	0.000	0.00000	-	-
BF5	4	4	3	0.833 ± 0.222	0.00420 ± 0.00109	0.650	0.18
MUSK	2	0	1	0.000	0.00000	-	-

TABLE 3. Uncorrected genetic P-distance (%) between and within *Dendrexetastes rufigula* subspecies estimated using cytochrome *b* and NADH dehydrogenase 2 (Cytb e ND2) sequences.

	D. r. devillei	***D. r. moniliger***	***D. r. rufigula***
D. r. devillei	0.4 ± 0.1		
D. r. moniliger	1.2 ± 0.3	0.4 ± 0.1	
D. r. rufigula	2.0 ± 0.5	1.8 ± 0.4	0.1 ± 0.1
D. r. paraensis	1.0 ± 0.3	0.3 ± 0.1	1.6 ± 0.4

Haplotype networks for both mtDNA gene fragments (Cytb and ND2) recovered three totally distinct haplogroups, corresponding to *D. r. devillei*, *D. r. rufigula*, and *D. r. moniliger* plus *D. r. paraensis* (Figures 1B–C). These three haplogroups are also depicted in nDNA networks (although some haplotype sharing among subspecies is present; Figures 1D–F); and in both maximum likelihood (ML) trees obtained (Figure 2). In the ML trees, *D. r. devillei* and *D. r. rufigula* clades were recovered with high bootstrap values (≥ 94%). The mtDNA tree was obtained with the full sampling (n = 28; Figure 2B), whereas the combined mtDNA and nDNA inference was obtained with 21 *D. rufigula* samples, including all subspecies, from which all the molecular markers could be amplified (Figure 2A).

All species delimitation and species tree tests, irrespective of the demographic and divergence time model considered, supported the existence of three reciprocally monophyletic clades (posterior probability, PP = 1.0), and the following species tree (*D. r. rufigula*, (*D. r. devillei*, *D. r. moniliger*/*D. r. paraensis*)), 0.986 > PP > 0.577. The second most likely species tree was (*D. r. moniliger*/*D. r. paraensis*, (*D. r. rufigula*, *D. r. devillei*), 0.224 > PP > 0.00828.

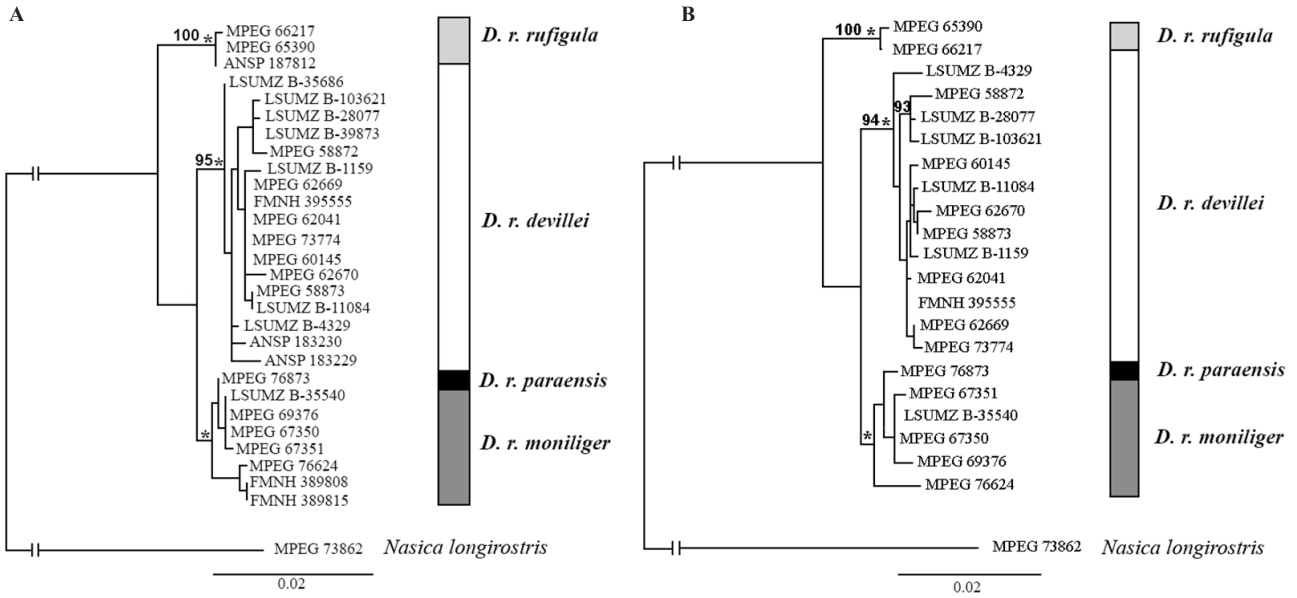

FIGURE 2. Maximum likelihood phylogenetic trees for *Dendrexetastes rufigula* inferred from mitochondrial (**A**) and both mitochondrial and nuclear molecular markers (**B**). Only bootstrap values above 90% are represented. * Clades supported by BPP analysis (PP = 1.0).

Our plumage analyses did not detect any sexual dimorphism and consistently confirmed the diagnoses of all currently recognized subspecies of the Cinnamon-throated Woodcreeper (Figure 3). With no exception, all examined specimens from each subspecies differed consistently from those of the other subspecies based on the following features: a) throat color; b) size and shape of pectoral stripes; c) size and shape of nuchal and upper dorsal stripes; and d) presence or absence of a superciliary stripe (Figure 3). *Dendrexetastes rufigula rufigula*, with its Robin Rufous (#340) colored throat with few markings is readily distinguished from the remaining subspecies. *D. r. moniliger* and *D. r. paraensis* share a brown (Buff #124) spotted throat, and *D. r. devillei* has a lower throat colored True Cinnamon (#139), which is barely marked by thin light brown stripes (Figure 3A). In contrast, the size and shape of pectoral stripes appear to follow a "leapfrog" pattern (*sensu* Remsen, 1984) whereby the more boldly patterned *D. r. rufigula* from the Guianan shield approaches the distantly related and allopatrically distributed *D. r. paraensis* from the Belém area of endemism, with the geographically intermediate and more closely related *D. r. devillei* and *D. r. moniliger* distinguishing themselves by narrower stripes, which are much narrower in *D. r. devillei* (Figure 3A). With respect to the dorsal stripes, the same "leapfrog" pattern is observed, with the nominate form from the Guiana shield approaching *D. r. paraensis* in having wider and longer stripes than *D. r. devillei* and *D. r. moniliger*, with *D. r. devillei* having nearly unmarked upperparts (Figure 3B). Finally, *D. r. paraensis* distinguishes itself from all remaining taxa by the presence of a faint and interrupted superciliary stripe.

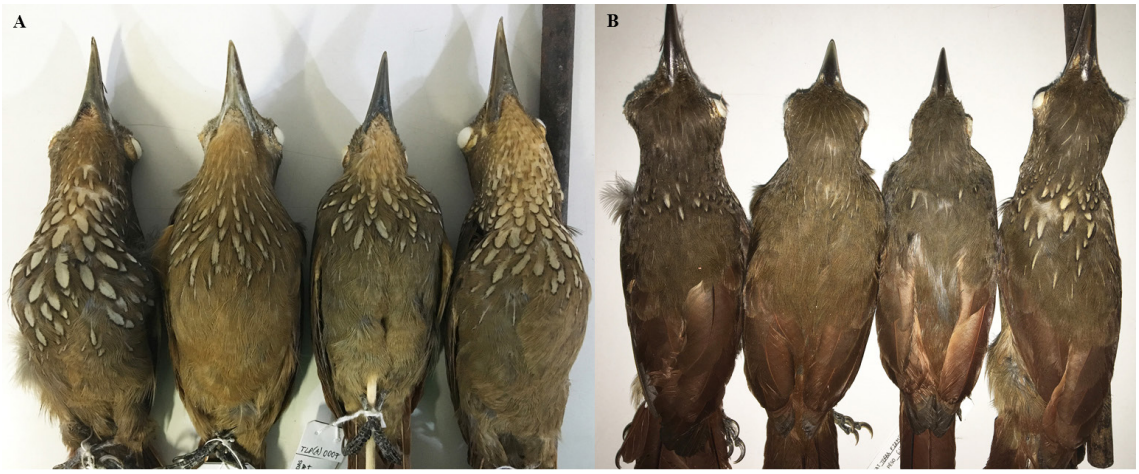

FIGURE 3. Ventral (**A**) and dorsal (**B**) views of representative specimens illustrating plumage diagnoses among *Dendrexetastes rufigula* subspecies, as recognized in Marantz *et al.* (2003). From left to right: *D. r. rufigula* (MPEG 65390), *D. r. devillei* (MPEG 58872), *D. r. moniliger* (MPEG 67350), and *D. r. paraensis* (MPEG 76873). Note the three characteristic distinct throat color patterns diagnosing unequivocally *D. r. rufigula*, *D. r. devillei*, and *D. r. moniliger/paraensis*, which correspond to the main evolutionary lineages in *Dendrexetastes*. The size and shape of pectoral, nuchal, and upper dorsal stripes follow a "leapfrog" pattern whereby the more boldly patterned *D. r. rufigula* approaches the distantly related and allopatrically distributed *D. r. paraensis*, with the geographically intermediate and more closely related *D. r. devillei* and *D. r. moniliger* distinguishing themselves by smaller markings.

DISCUSSION

Species limits and taxonomy

Our phylogeographic analyses identified three major evolutionary independent units in the Cinnamon-throated Woodcreeper that roughly corresponded to currently recognized subspecies. Although we found support for the reciprocal monophyly and consequent evolutionary independence of *D. r. rufigula* and *D. r. devillei*, the same did not occur between *D. r. moniliger* and *D. r. paraensis,* which were paraphyletic, but grouped together in a single clade without significant statistical support in ML analyses. In this clade, our lone sample of *D. r. paraensis* was nested within *D. r. moniliger* and their pairwise mitochondrial distance (0.3 ± 0.1%) indicates a level of differentiation slightly lower than that verified within other subspecies, such as *D. r. devillei* (*i.e.*, 0.4 ± 0.1%; see Table 3). This suggests that *D. r. paraensis* is a morphologically slightly distinct subset of a more widespread lineage endemic to southeastern Amazonia east of the Madeira River that includes *D. r. moniliger* (Figures 3 & 4).

FIGURE 4. Ventral (**A**) and dorsal (**B**) views of specimens showing plumage variation within *Dendrexetastes paraensis*, as defined herein (grouping *D. r. paraensis stricto sensu* and *"D. r. moniliger*"). From left to right (specimens were organized from west to east): "*D. r. moniliger*" (MPEG 39641, 39640, 76624, 69376, 67350, 67351, 51404, 54679), and *D. r. paraensis stricto sensu* (MPEG 26817, 17214, 76873). Note the trend of *D. r. paraensis stricto sensu* specimens to have broader lower throat and pectoral spots (**A**) as well as wider and longer nuchal and dorsal stripes (**B**) than those of "*D. r. moniliger*". The faint and interrupted superciliary stripe distinguishing all *D. r. paraensis stricto sensu* specimens examined cannot be seen in these pictures.

The species delimitation analysis indicated with high support that clades *D. r. rufigula*, *D. r. devillei*, and *D. r. moniliger*/*paraensis* are separate evolutionary species (de Queiroz 2007) and that gene flow, even if present, has not affected their mutual diagnoses. The same could be inferred from the plumage data based on the specimens analyzed, whereby each clade was characterized by a unique combination of characters, and no intermediate specimens were found. Despite these findings, the relatively small sampling of specimens and molecular markers screened by the present study did not allow for a more detailed evaluation of levels of gene flow among the three main evolutionary lineages of the Cinnamon-throated Woodcreeper, preventing an assessment of whether they are separate biological species. Nevertheless, Gill (2014) points out, based on recent advances on the genetics of speciation, reproductive isolation, directional selection, and hybridization dynamics, that "distinct and reciprocally monophyletic sister populations of birds exhibit essential reproductive isolation and would not

interbreed freely if they were to occur in sympatry". In other words, in instances such as documented herein for the Cinnamon-throated Woodcreeper, the burden of proof should now stand in demonstrating that levels of gene flow are actually high enough among its three main linages so that they cannot be regarded as reproductively isolated from each other, in strong contrast with the data shown herein. Given the apparent allopatry of these three divergent Cinnamon-throated Woodcreeper clades, this approach to assessing species limits seems appropriate. Similarly, our study provides the first assessment ever of the evolutionary history within this lineage, which had otherwise been treated as a polytypic species based on the purported morphological intermediacy of the population later named *D. r. moniliger* between *D. r. rufigula* and *D. r. devillei* (Hellmayr 1910, Zimmer 1934). As we discuss below, plumage evolution in the Cinnamon-throated Woodcreeper complex has involved some degree of convergence, which can obscure the inference of true evolutionary relationships and species limits.

Rather than recognizing a single polytypic species, the results obtained in this study support the following three taxa should be treated as species diagnosable by molecular and plumage characters (Figures 2 & 3) – a possibility already indicated by Piacentini *et al*. (2015): 1) Cinnamon-throated Woodcreeper - *Dendrexetastes rufigula* (Lesson, 1844). Unequivocally diagnosable from all remaining *Dendrexetastes* taxa by a Robin Rufous (#340) colored and nearly unmarked throat (Figure 3); it is distributed on the Guianan shield north of the Amazon River and east of the Negro River in Venezuela, Brazil, Guyana, Surinam, and French Guiana (Marantz *et al*. 2003); 2) Devillei's Woodcreeper - *Dendrexetastes devillei* (Lafresnaye, 1850). Distinguished from the remaining species by a dark True Cinnamon (#139) throat and much shorter and narrower pectoral and nuchal stripes, resulting in nearly unmarked upperparts (Figure 3); found west of the Negro River in Amazonian Brazil westward towards the base of the Andes in Colombia, Ecuador, and Peru both north and south of the Amazon River, northern Bolivia and east to the west bank of the Madeira River (Borges *et al.* 2001, Marantz *et al.* 2003); 3) Pará Woodcreeper - *Dendrexetastes paraensis* Lorenz, 1895. Told apart from the other species by a Buff (#124) and heavily spotted throat, which has an overall squamate appearance (Figure 3); it occurs from the east bank of the Madeira River to easternmost Amazonia in the Belém area of endemism. The name *D. r. paraensis* Lorenz, 1895 has priority over *D. r. moniliger* (Zimmer, 1934), and thus should be used to identify the clade grouping specimens of these taxa (Figure 2). Some variation in plumage within *D. paraensis* has been detected (see Figure 4, as well as Cory & Hellmayr 1925) and is discussed in more detail below.

Plumage evolution

When contrasted with the molecular phylogeny estimated for the *Dendrexetastes* taxa, plumage patterns such as the size and length of pectoral, nuchal, and upper dorsal spots followed a "leapfrog" pattern (Remsen 1984), whereby the more boldly patterned and allopatrically distributed *D. paraensis* and *D. rufigula* approached each other despite their more distant phylogenetic affinities, to the exclusion of the overall concolor and more closely related *D. devillei*, which is in contact via parapatry with both of these taxa along the middle-upper courses of the Negro (*D. rufigula*) and Madeira Rivers (*D. devillei*). Despite the fact that the sister relationship between *D. paraensis* and *D. devillei* is poorly supported in our estimated phylogeny, it nevertheless suggests that they are sister taxa and hence that plumage characters may not have evolved in concert with the history of diversification in this group (Figures 2 & 3). This conclusion is reinforced by the observed differentiation in plumage between the taxa *paraensis* (*stricto sensu*) and *moniliger* (Figure 4), despite their little genetic divergence, which is even lower than that found within *moniliger* alone (see above; Table 3), and the fact that they are nested within the same clade. Our results are then consistent with either a scenario of convergent phenotypic change or retention of ancestral traits among geographically distant lineages, that is typically associated with a "leapfrog" pattern (Remsen 1984). Understanding the underlying causes of "leapfrog" patterns of geographic variation are difficult, but several studies documented similar scenarios of parallel evolution or retention of ancestral traits in geographically and phylogenetically distant tropical and temperate avian lineages, with differentiation of geographically intermediate populations (Norman *et al.* 2002, Pavlova *et al.* 2005, Cadena *et al.* 2011). In the case of *Dendrexetastes*, both parallel evolution and retention of ancestral traits remain valid hypotheses behind the documented "leapfrog" pattern of plumage variation. The first split in the *Dendrexetastes* tree involves the separation between the more boldly patterned *D. rufigula* and the more concolor-like *D. devillei* plus *D. paraensis*, so it can be assumed that bigger pectoral, nuchal, and upper-dorsal stripes represent more ancestral rather than derived character states, which appeared more conspicuously in the easternmost *D. paraensis* population. Alternatively, as supported by Cadena *et al.* (2011), selection could produce convergent or parallel evolution in plumage characters to maximize the fitness of local populations. Both boldly patterned *Dendrexetastes* taxa are found in eastern Amazonia, which is significantly drier and more seasonal than western Amazonia, where the least marked species *D. devillei* occurs (Davidson *et al*. 2012, Cheng *et al*. 2013); therefore, selection along an environmental gradient running from western to eastern

Amazonia could influence convergent plumage types in *Dendrexetastes*. Future studies with more powerful datasets, both in terms of specimens and number of loci, could test between these two hypotheses.

Conservation implications

Our study did not support a separate evolutionary species status for the "Endangered" *Dendrexetastes paraensis stricto sensu* (MMA 2014), since it failed to uncover significant genetic differentiation between this population and "*D. r. moniliger*". This contrasts with previous taxonomy and patterns of plumage variation, which allow for the distinction of a more boldly patterned population (to which the name *paraensis* originally applies) distributed east of the Tocantins River and a less marked group found between the Madeira and the west bank of the Tocantins River (to which the name *moniliger* applies; Zimmer 1934; Figure 4). The phylogenies obtained showed that these populations are paraphyletic, and hence that they cannot be treated as independent evolutionary lineages, despite some morphological differentiation (Figure 4). Despite this mismatch between plumage patterns and the phylogeny, three geographically structured sub-clades were recovered within *D. paraensis*, each associated with a major Amazonian interfluve, as follows: a) Madeira-Tapajós (grouping samples MPEG 76624, FMNH 389808 and 389815); Tapajós-Xingu (grouping specimens LSUMZ 35540, MPEG 67351, 67350 and 67351); and east of the Tocantins (MPEG 76873). Interestingly, this same degree of geographic structure is not observed in *D. devillei*, whose populations north and south of the Amazon were not recovered as reciprocally monophyletic (Figure 2), although their degree of genetic differentiation is comparable to that found in the clade joining *D. paraensis stricto sensu* and "*D. r. moniliger*" (Table 3). This demonstrates that important phylogeographic structure exists in *D. paraensis*, yet to a smaller extent than that verified among the three main *Dendrexetastes* lineages. Unfortunately, our small sampling of specimens and molecular markers does not allow for a more in-depth phylogeographic analysis of *D. paraensis*, which includes three sub-clades apparently endemic to the most deforested sectors of Amazonia (Da Silva *et al.* 2005, Bird *et al*. 2012). Therefore, while we recommend that *D. paraensis* as defined herein is treated as an independent species whose conservation status should be evaluated separately from other *Dendrexetastes* species, we also stress the importance of evaluating threat levels for each of its three sub-clades, particularly during national and regional conservation assessments. This cautious approach is justified from a conservation standpoint, and we suggest that Next Generation Sequencing methodologies be used in the future as a stronger test of the genetic distinctiveness among *D. paraensis* sub-clades. Unfortunately, only a few specimens of *D. paraensis* exist in collections worldwide, and the great advantage of these methods is that they work well for suboptimal samples, such as study skins collected dozens and even hundreds of years ago (McCormack *et al*. 2015).

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APPENDIX I

Study skins of *Dendrexetastes rufigula* analyzed in this study. All specimens are deposited at the Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG). Specimens marked with asterisks (*) were also included in the molecular analyzes.

Specimen number	Taxon	Locality, in Brazil	State	Sex
03034	*Dendrexetastes rufigula devillei*	Boca do Acre, Rio Purus, Bom Lugar (08°43'S; 67°20'W)	Amazonas	F
03589	*Dendrexetastes rufigula devillei*	Boca do Acre, Rio Purus, Ponto Alegre (08°57'S; 67°50'W)	Amazonas	F
18310	*Dendrexetastes rufigula devillei*	Estirão do Equador, Rio Javari (04°27'S; 71°30'W)	Amazonas	M
43099	*Dendrexetastes rufigula devillei*	Maraã, Lago Paricá, Santa Rita (02°23'S; 66°10'W)	Amazonas	M
43100	*Dendrexetastes rufigula devillei*	Maraã, Rio Japurá, right bank, opposite to Maguari (02°30'S; 65°40'W)	Amazonas	F
52093*[2]	*Dendrexetastes rufigula devillei*	Marechal Thaumaturgo, Rio Tejo, *c.* 5 km from mouth (09°00'S; 72°42'W)	Acre	M
55213	*Dendrexetastes rufigula devillei*	4,5 km NE São Paulo de Olivença, Rio Solimões, north bank (03°25'S; 68°57'W)	Amazonas	M
58872*	*Dendrexetastes rufigula devillei*	Assis Brasil, ESEC Rio Acre, Acampamento 2 (11°00'53.4"S; 70°13'02.7"W)	Acre	F
58873*	*Dendrexetastes rufigula devillei*	Assis Brasil, ESEC Rio Acre, Acampamento 2 (11°00'53.4"S; 70°13'02.7"W)	Acre	M
60145*	*Dendrexetastes rufigula devillei*	RDS Cujubim, margem E Rio Jutaí (05°38'19"S; 69°10'59"W)	Amazonas	F
62041*	*Dendrexetastes rufigula devillei*	Porto Walter, Igarapé Cruzeiro do Vale, Colônia Dois Portos (08°20'35.7"S; 72°36'19.7"W)	Acre	F
62669*	*Dendrexetastes rufigula devillei*	Japurá, Rio Acanauí (01°56'12.4"S; 66°36'18.8"W)	Amazonas	F
62670	*Dendrexetastes rufigula devillei*	Japurá, Rio Acanauí (01°56'12.4"S; 66°36'18.8"W)	Amazonas	M
73774*	*Dendrexetastes rufigula devillei*	Autazes (03°46'52.8"S; 59°03'23.8"W)	Amazonas	M
79707*	*Dendrexetastes rufigula devillei*	Jutaí, ESEC Jutaí/Solimões, Capivara (03°10'56.3"S; 67°22'47.1"W)	Amazonas	M
39640*	*Dendrexetastes rufigula moniliger*	Cachoeira Nazaré, margem oeste do Rio Ji-paraná (10°13'S; 62°28'W)	Rondônia	F
39641*	*Dendrexetastes rufigula moniliger*	Cachoeira Nazaré, margem oeste do Rio Ji-paraná (10°13'S; 62°28'W)	Rondônia	F
51404	*Dendrexetastes rufigula moniliger*	Rio Teles Pires, Alta Floresta, Reserva Florestal Cristalino (09°42'S; 55°55'W)	Mato Grosso	F
54679*	*Dendrexetastes rufigula moniliger*	32 km NE Alta Floresta, margem W Rio Teles Pires (09°38'01"S; 55°56'21"W)	Mato Grosso	M
67350*	*Dendrexetastes rufigula moniliger*	Paranaíta, Rio Teles Pires (09°24'S; 56°33'W)	Mato Grosso	M
67351*	*Dendrexetastes rufigula moniliger*	Paranaíta, Rio Teles Pires (09°24'S; 56°33'W)	Mato Grosso	F
69376*	*Dendrexetastes rufigula moniliger*	Paranaíta, Rio Teles Pires, margem esquerda (09°24'51.4"S; 56°33'39.7"W)	Mato Grosso	M
76624*	*Dendrexetastes rufigula moniliger*	Itaituba, margem esquerda Rio Tapajós, Penedo (05°27'21.61"S; 57°04'12"W)	Pará	F
17214	*Dendrexetastes rufigula paraensis*	São Miguel do Pará, Rodovia Belém-Brasília km 36 (01°37'S; 47°29'W)	Pará	M
26817	*Dendrexetastes rufigula paraensis*	Belém (01°27'S; 48°29'W)	Pará	?
76873*	*Dendrexetastes rufigula paraensis*	Centro Novo Maranhão, REBIO Gurupi (03°42'12.8"S; 46°45'44"W)	Maranhão	M
30118	*Dendrexetastes rufigula rufigula*	Manaus, Reserva Ducke (03°08'S; 60°02'W)	Amazonas	M
53020	*Dendrexetastes rufigula rufigula*	Manaus, km 24 ZF-3, *c.* 80 km N de Manaus, Fazenda Esteio (02°30'S; 60°00'W)	Amazonas	M
53021	*Dendrexetastes rufigula rufigula*	Manaus, km 24 ZF-3, *c.* 80 km N de Manaus, Fazenda Esteio (02°30'S; 60°00'W)	Amazonas	F
65390*	*Dendrexetastes rufigula rufigula*	Alenquer, ESEC Grão-Pará (0°09'S; 55°11'W)	Pará	M
66217*	*Dendrexetastes rufigula rufigula*	Almeirim, REBIO Maicuru (0°49'S; 53°55'W)	Pará	M