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Sistemática molecular e implicações para a conservação de uma linhagem endêmica da Amazônia: o gênero *Hylexetastes* Sclater, 1889 (Aves: Dendrocolaptidae)

Belém, 2017

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Dissertação apresentada ao Programa de Pós-graduação em Zoologia, do Convênio da Universidade Federal do Pará e o Museu Paraense Emílio Goeldi, como requisito parcial para a obtenção do grau de mestre em Zoologia.
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SUMÁRIO

Molecular systematics of the Amazonian endemic genus *Hylexetastes* (AVES: DENDROCOLAPTIDAE): taxonomic and conservation implications

ABSTRACT

The genus *Hylexetastes* is endemic to the Amazon rainforest. Currently, two species are accepted in the genus (*H. perrotti and H. stresemannii*), each one divided into three subspecies. Nevertheless, some authors defend that the subspecies of *H. perrotti* should be considered as full species. In particular, *H. p. brigidai* is an endemic taxon from Pará and Mato Grosso and seems to have the smallest distribution area. This lineage is distributed by the most deforested region within the biome and thus its taxonomic status is of particular concern for conservation. So far, only morphological characters have been evaluated for taxonomic definition of this genus. Therefore, in this study we present a molecular phylogenetic hypothesis to help solve the taxonomic uncertainties within the genus. Fragments of two mitochondrial markers (Cytb and ND2) and three nuclear markers (BF5, G3PDH and MUSK) were sequenced on 58 *Hylexetastes* specimens. In addition, ecological niche modeling was developed for each of the identified strains to evaluate their potential distribution area, climatic requirements and their vulnerability to deforestation. The phylogenetic analyzes support the designation of *H. perrotti*, *H. uniformis* and *H. brigidai* as full species, and *H. perrotti* seems to be a sister species of *H. stresemanni* and not of the other taxa considered co-specific. In addition, it was possible to distinguish the presence of two Significant Evolutionary Units within *H. uniformis*. Each of these taxa are distributed in different interfluvial / endemic areas of the Amazon basin. In particular, it confirms the status of full species for *H. brigidai*, endemic to the second area of Amazonian endemism with greater deforestation. Thus, we suggest the continued in-depth evaluation of its conservation status to promote its preservation.

Keywords: Phylogeography, Ecological niche modeling, Species delimitation, Taxonomy, Significant Evolutionary Units.

Sistemática molecular e implicações na conservação de uma linhagem endêmica da Amazônia: *Hylexetastes* Sclater, 1889 (Aves: Dendrocolaptidae)

RESUMO

O gênero *Hylexetastes* é endêmico da floresta Amazônica. Atualmente, duas espécies são aceitas no gênero (*H. perrotti* e *H. stresemannii*), cada uma dividida em três subespécies. No entanto, alguns autores defendem que as subespécies de *H. perrotti* devem ser consideradas como espécies plenas. Em particular, *H. p. brigidai* é um táxon endêmico do Pará e Mato Grosso e parece ter a menor área de distribuição. Esta linhagem distribui-se pela região mais desmatada dentro do bioma e assim o seu status taxonômico é de particular preocupação para conservação. Até agora, somente caracteres morfológicos foram avaliados para definição taxonômica deste gênero. Portanto, neste estudo apresentamos uma hipótese filogenética molecular para ajudar a resolver as incertezas taxonômicas dentro do gênero. Foram sequenciados fragmentos de dois marcadores mitocondriais (Cytb e ND2) e três marcadores nucleares (BF5, G3PDH e MUSK) em 58 espécimes de *Hylexetastes*. Além disso, foram elaboradas modelagens de nicho ecológico para cada uma das linhagens identificadas, para avaliar sua potencial área de distribuição, requerimentos climáticos e sua a vulnerabilidade ao desmatamento. As análises filogenéticas sustentam a designação de *H. perrotti*, *H. uniformis* e *H. brigidai* como espécies plenas, sendo que *H. perrotti* parece ser espécie irmã de *H. stresemanni* e não dos demais táxons considerados co-específicos. Além disso, foi possível distinguir a presença de duas Unidades Evolutivas Significativas dentro de *H. uniformis*. Cada um destes táxons está distribuído em diferentes interflúvios / áreas de endemismo da bacia Amazônica. Em particular, confirma-se o status de espécie plena para *H. brigidai*, endêmica da segunda área de endemismo Amazônica com maior desmatamento. Assim, sugerimos a continuada avaliação aprofundada do seu status de conservação para promover sua preservação.

Palavras-chave: Filogeografia, Modelagem do nicho ecológico, Delimitação de espécies, Taxonomia, Unidades Evolutivas Significativas.

INTRODUÇÃO GERAL

A Biodiversidade está sendo afetada rapidamente como resultado direto e indireto das atividades humanas. Para contrapor este quadro, a biologia da conservação surgiu como uma disciplina para tentar reduzir as taxas atuais de extinção e promover a preservação das espécies (Frankham *et al*. 2008). Existe uma percepção equivocada de que estudos taxonômicos podem contribuir menos para a conservação das espécies do que aqueles enfocados em monitoramento de espécies ameaçadas em seus hábitats (Mace 2004, Aleixo 2009a). Uma taxonomia que falha em delimitar entidades evolutivas basais, distintas uma das outras, pode ser responsável pelo não reconhecimento de espécies distintas e em perigo, que podem, portanto, ficar sem proteção e tornarem-se extintas (Frankham *et al*. 2008). Além disso, o estabelecimento de áreas prioritárias para a conservação das espécies é altamente sensível ao tratamento taxonômico usado para sua delimitação (Peterson and Navarro-Siguenza 1999). Portanto, não é possível conservar as espécies que não estejam corretamente delimitadas num contexto evolutivo (Mace 2004). Neste sentido, é recomendável que a delimitação de espécies leve em consideração a história evolutiva, morfologia, genética, comportamento e distribuição geográfica dos táxons estudados (De Queiroz 2007, Cartstens *et al*. 2013).

Na história evolutiva de algumas espécies Neotropicais parecem estar envolvidos processos repetidos de isolamento geográfico, diferenciação e expansão populacional. O tempo em que estas linhagens persistiram e sua capacidade de dispersão através da paisagem tiveram influência na diversidade de espécies presentes no Neotrópico (Smith *et al.* 2014). Paralelamente, na Amazônia, os processos de formação da bacia Amazônica teriam sido também uma das principais fontes de diversificação das espécies (Ribas *et al*. 2012). Desta combinação, teria se gerado um mosaico de distintas áreas de endemismo, delimitadas pelos principais rios Amazônicos, cada uma com as suas próprias relações evolutivas e comunidades bióticas. Estas áreas de endemismos variam consideravelmente em tamanho e estado de conservação (Da Silva *et al*. 2005, INPE 2017).

A diversidade genética é um dos componentes principais da Biodiversidade. Assim, é um dos focos fundamentais da biologia da conservação (Heywood e Watson 1995). A manutenção da diversidade genética fornece o potencial adaptativo e evolutivo de uma espécie. Esta é necessária para que as populações evoluam e se adaptem às mudanças ambientais, em um processo contínuo na natureza

(Frankham *et al*. 2008). Além disso, a informação genética permite identificar linhagens significativamente distintas que podem ser consideradas como espécies plenas, segundo o conceito filético geral de espécies (De Queiroz 2005, Aleixo 2007, Aleixo 2009a); criar hipóteses das relações filogenéticas entre os táxons estudados e ainda, estabelecer limites temporais para a diferenciação e diversificação desses táxons (Frankham *et al*. 2008).

Em conservação, a identificação de meta-população ou meta-populações geneticamente diferenciadas dentro de uma mesma espécie contribui para a definição de Unidades Evolutivas Significativas (UES; Moritz 1994). Uma mesma espécie pode ter uma ou mais UES (Hey *et al*. 2003, Frankham *et al*., 2008, Aleixo, 2009a). O conceito de UES foi definido primeiramente por Rider (1986) para refletir adequadamente a diversidade genética presente nas espécies e dar prioridade para a conservação. Posteriormente, este conceito a sofrido algumas modificações ao longo do tempo. Waples (1991) considera que elas consistem em populações que estão reprodutivamente separadas de outras populações e têm adaptações únicas ou diferentes. Crandall *et al*. (2000) indica que as populações podem ter variados níveis de fluxo gênico, envolvendo deriva genética e seleção. Em termos práticos, segundo Moritz (1994), o critério para a identificação das UES faz referência àquelas populações que apresentam monofilia recíproca para alelos mitocondriais e mostram diferenciação significativa nas frequências de alelos nucleares. Esta definição vai ser considerada para a identificação das UES ao longo deste trabalho.

O reconhecimento das UES precisa ser complementado por dados de área de distribuição das próprias UES para que seja possível planejar áreas protegidas e prover um critério para dar prioridade para a conservação dos táxons (Ryder 1986, Moritz 1994). Além disso, uma correta avaliação da área de ocorrência de uma espécie, e da sua diversidade intra-específica, não é só uma informação vital para o planejamento de áreas protegidas, mas também para a realização de estudos ecológicos e evolutivos que nos informem quanto à capacidade adaptativa de suas UES (Crandall *et al*. 2000, Frankham *et al*. 2008, De Barros Ferraz *et al*. 2012, Rangel and Loyola 2012, Hipolito *et al*. 2015).

Modelagens de nicho ecológico permitem identificar a área de ocorrência de uma espécie, mesmo quando os dados de distribuição são escassos (Phillips *et al*. 2006, Frankling 2009). Além disso, o uso de modelagens de nicho ecológico permite uma avaliação da diferenciação geográfica e ambiental entre as espécies, contribuindo ainda mais para a identificação de espécies distintas (Ruedas *et al*. 2017, Tocchio *et al*. 2014, Martinez-Gordillo *et al*. 2010). Estas metodologias precisam de dados climáticos e de registros de ocorrência da espécie-alvo. Estes dados podem ser compilados a partir de publicações científicas, bem como fontes não publicadas, incluindo relatórios, dados de espécimes de museus e arquivos sonoros. Os modelos resultantes devem ser avaliados por especialistas que verificam criticamente a validade dos limites de distribuição das espécies (Aleixo 2009b, Carstens *et al*. 2013, Albernaz 2014). O método de máxima entropia (Maxent) combina registros de ocorrência com variáveis ambientais para gerar estes modelos (Elith *et a*l. 2006, Phillips *et al*. 2006) e tem sido amplamente usado numa variedade de estudos, especialmente aqueles correspondentes a predição de distribuição de espécies raras, ameaçadas de extinção ou com taxonomia controversa (De Marco Jr. e Ferreira De Siqueira 2009, De Barros Ferraz *et al*. 2012, Hipólito *et al*. 2015, Ruedas *et al*. 2017).

A Família Dendrocolaptidae é constituída por 13 gêneros e 50 espécies, agrupando as aves Neotropicais conhecidas como arapaçus (Marantz *et al*. 2016). Para algumas das suas espécies, têm sido realizados estudos de sistemática molecular, com importantes implicações taxonômicas (Aleixo 2002, Sousa-Neves *et al*. 2013, Batista *et al*. 2013, Rocha *et al*. 2015, Ferreira *et al*. 2016). A falta de uma coloração contrastante da plumagem entre suas espécies é um fator que tem contribuído para a dificuldade de identificação das espécies desta família, particularmente em campo (Bickford *et al*. 2007). Os arapaçus vivem em florestas tropicais de diferentes tipos, e se alimentam de insetos e pequenos vertebrados que encontram desde o sub-bosque até sub-dossel. As penas duras e longas na cauda, com espinhas curvadas para baixo nas suas pontas, as garras fortes ajudam no suporte durante a escalada das árvores, mostrando a sua adaptação a habitats florestais. Assim, muitas das espécies da família estão sofrendo com a perda e fragmentação de hábitat (Camfield 2004, Marantz *et al*. 2016, Hilty 2003). Por estes motivos, espécies da família Dendrocolaptidae são consideradas alvos importantes para o monitoramento do efeito de alterações antrópicas no Neotrópico (Marantz *et al*., 2016, Remsen *et al*. 2016), mas de uma maneira geral, esta família é também um dos exemplos Neotropicais da necessidade de estudos mais aprofundados de âmbito taxonômico.

Dentro dos dendrocolaptídeos, o gênero *Hylexetastes* é de particular interesse. O gênero foi criado por Sclater em 1889, que sinonimizou a espécie *Dendrocolaptes perrotii* Lafresnaye (1844) para *Hylexetastes perrotii perrotii*. Posteriormente, *Xiphocolaptes perrotii* Hellmayr (1909) foi incluído no gênero como *Hylexetastes uniformis* Hellmayr (1909). Mais recentemente, foi adicionado um terceiro táxon, *Hylexetastes brigidai*, descrito por Da Silva (1995). Atualmente estes três táxons estão descritos como subespécies de *Hylexetastes perrotii* (Marantz *et al*. 2016). Porém, alguns autores consideram *H. p. uniformis* e *H. p. brigidai* como espécies plenas, baseando-se em diferenças nas cores da plumagem das lores, pescoço e ventre (Da Silva *et. al*. 1995, Piacentini *et al*. 2015). *Hylexetastes perrotti* está distribuída ao leste da Amazônia (Fig. Ia). A outra espécie deste gênero, *Hylexetastes stresemanni* Snethlage (1925), agrupa três subespécies: *Hylexetastes stresemanni stresemanni* Snethlage (1925), *Hylexetastes stresemanni undulatus* Tood (1925) e *Hylexetastes stresemanni insignis* Zimmer (1934). Todas as subespécies de *H. stresemanni* estão distribuídas a oeste da Amazônia (Fig. Ib) (Cory e Helmayr 1925, Zimmer 1934, Peters 1951, Marantz *et al*. 2016, Perlo 2009, Piacentini *et al*. 2015, Remsen *et al*. 2016). Além da controvérsia taxonômica, a relação evolutiva entre todos estes táxons não é conhecida. Os critérios adotados para a identificação das subespécies de *Hylexetastes* correspondem a diferenças morfológicas, vocais e de distribuição geográficas (Marantz *et al*. 2016): *H. p. perrotii* está distribuída ao longo do escudo das Guianas; *H. p uniformis* encontra-se na cabeceira do rio Xingu e no interflúvio Xingu – Tapajos; *H. p. brigidai* provém da porção média e baixa do interflúvio Xingu – Tocantis; *H. s. stresemanni* está distribuída na porção baixa do interflúvio Rio Negro e Rio Solimões; *H. s. undulatus* encontra-se presente ao leste do rio Purus e o rio Madeira e finalmente *H. s. insignis* localiza-se ao Norte do rio Uaupés.

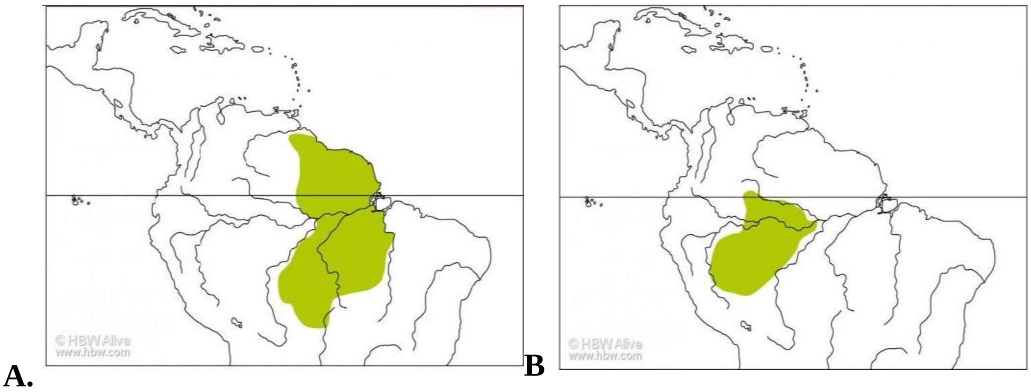

Figura I. Mapa de distribuição das espécies do gênero *Hylexetastes* reconhecidas por Remsen *et al*. (2016); extraído e modificado de Marantz *et al*. (2016): **a.** *Hylexetastes perrotti* e **b.** *Hylexetastes stresemanni*.

Derryberry *et al*. (2011) apresentaram uma filogenia geral para a família Dendrocolaptidae, no qual estão representadas as duas espécies de *Hylexetastes* (Figura II). No entanto, a sistemática dentro do gênero foi estudada até hoje apenas com base em caracteres morfológicos (Cory e Helmayr 1925, Zimmer 1934, Peters 1951, Da Silva *et al.* 1995, Perlo 2009, Piacentini *et al*. 2015, Remsen *et al*. 2016, Marantz *et al*. 2016). Assim, caracteres moleculares podem ser uma importante ferramenta para ajudar na correta delimitação destas subespécies ou espécies dentro do gênero. Além disso, a revisão taxonômica pode ter repercussões relevantes para a conservação. As duas espécies atualmente reconhecidas não estão globalmente ameaçadas (Birdlife International 2016), mas seu verdadeiro estatuto de conservação é pouco conhecido, considerando que são espécies raras (Stotz *et al*. 1996). Além disso, *H. p. brigidai*, hoje em dia, não é reconhecido como espécie plena pela IUCN, BirdLife International nem pelo Handbook of the Birds of the World, mas devido à sua possível área de distribuição restrita, suas populações podem estar seriamente afetadas pelo desmatamento e degradação ambiental causada pela perturbação antropogénica presente na área de ocorrência (Da Silva *et al*. 2005, Marantz *et al*. 2016, Bird *et al*. 2011, Moura *et al*. 2013, Barlow *et al*. 2016, Birdlife 2017, IUCN 2017). Considerando a controvérsia taxonômica envolvendo o gênero *Hylexetastes*, bem como o crescente grau de ameaça à conservação de pelo menos alguns de seus táxons, o presente estudo teve como objetivo geral realizar uma revisão sistemática do gênero *Hylexetastes* usando sequências de DNA de genes mitocondriais e nucleares. Os objetivos específicos foram realizar a reconstrução de hipóteses filogenéticas com base em sequências de DNA para auxílio na distinção das espécies, e avaliar a vulnerabilidade de cada espécie ao desmatamento com base em modelagens de nicho ecológico.

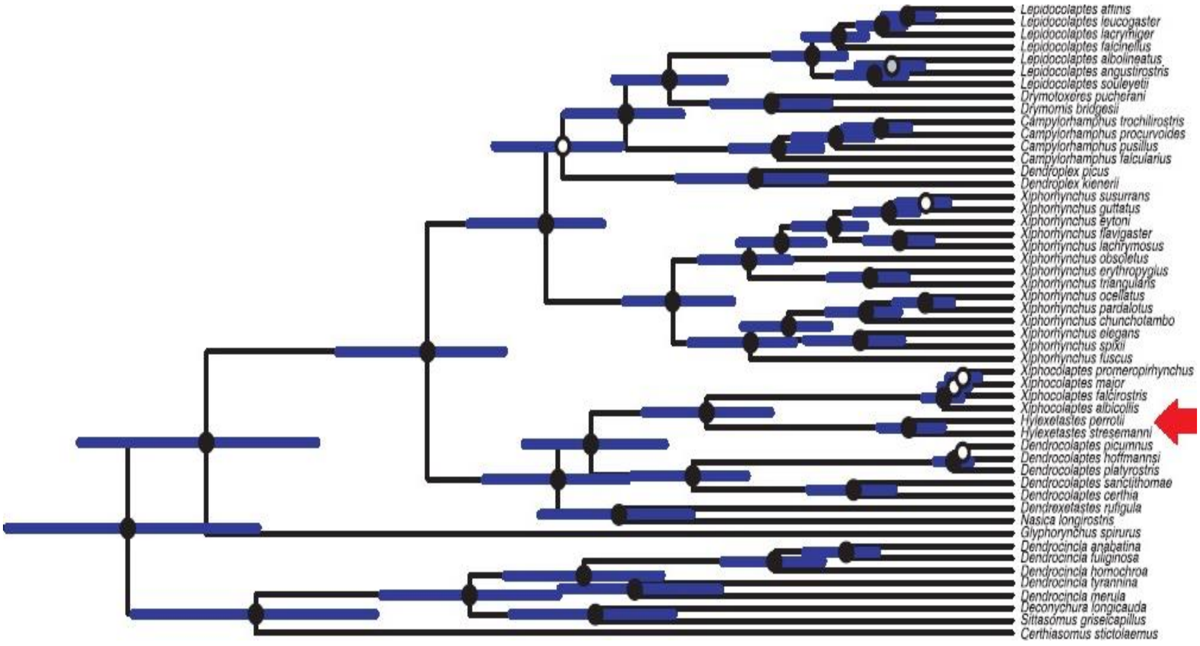

Figura II. Filogenia geral para a família Dendrocolaptidae (modificado de Derryberry *et al*. 2011). A seta vermelha indica a posição do gênero *Hylexetastes* dentro da família.

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Capítulo 1

Sistemática molecular e implicações na conservação de uma linhagem endêmica da Amazônia: *Hylexetastes* Sclater, 1889 (Aves: Dendrocolaptidae)

O capítulo I desta tese/Dissertação foi elaborado e formatado conforme as normas da publicação científica *Birds Conservation International*, as quais se encontram em anexo (Anexo 1)

Molecular systematics of the Amazonian endemic genus *Hylexetastes* (AVES: DENDROCOLAPTIDAE): taxonomic and conservation implications

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Summary

Hylexetastes woodcreepers are endemic to the Terra Firme forest of the Amazon basin. Currently, two species of *Hylexetastes* (*H. perrotti* and *H. stresemanni*) are recognized, each one divided into three subspecies. Nevertheless, some authors maintain that the *H. perrotti* subspecies should be elevated to full species status. In particular, *H. p. brigidai* is endemic to the eastern Amazon, the second area of endemism (Xingú) most affected by deforestation and habitat degradation. Consequently, the taxonomic status of *H. p. brigidai* is of special concern for conservation. Thus far, only morphological characters have been evaluated for the taxonomic delimitation of species and subspecies of *Hylexetastes*. Therefore, in this study we present a molecular phylogenetic analysis to help delimit *Hylexetastes* interspecific limits. Fragments of two mitochondrial (Cytb and ND2) and three nuclear genes (BF5, G3PDH and MUSK) from 58 *Hylexetastes* specimens, including all subspecies, were sequenced. Furthermore, an ecological niche model was estimated to describe more accurately the potential distributions of taxa and to evaluate their vulnerability to ongoing deforestation. Phylogenetic analyses support the paraphyly of *H. perrotti* with respect to *H. stresemanni* and the elevation of *H. p. uniformis* and *H. p. brigidai* to full species rank, as well as the presence of two Evolutionary Significant Units within *H. p uniformis*. Each of these taxa are distributed in different interfluvial areas of the Amazon basin. Due to its vulnerability regular assessments of the conservation status of *H. p. brigidai* are paramount.

Key words*:* Phylogeography, Ecological Niche Models, Species Delimitation, Significant Evolutionary Units, Taxonomy.

Introduction

There is a misconception that taxonomic studies may overly complicate species conservation relative to studies focused on monitoring endangered species in their habitats. (Mace 2004, Aleixo 2009, Garnett and Chistidis 2017). However, taxonomic studies can help delimit distinct basal evolutionary entities and enable us to recognize endangered species which are both critical to preventing extinctions (Kahindo *et al*. 2007, Frankham *et al*. 2008). Furthermore, the establishment of priority areas for species conservation is highly sensitive to the taxonomic treatment used for the species delimitation (Peterson and Navarro - Siguenza 1999). Therefore, conservation of species is inhibited when they are not correctly delimited under an evolutionary context (Mace 2004). Today, species delimitation can take into account a variety of information related to the taxa under study, such as evolutionary history, morphology, genetics, natural history and geographical distribution (e.g., De Queiroz 2007, Carstens *et al*. 2013, Aleixo *et al*. 2013, Portes *et al*. 2013, Engel *et al*. 2014, Halley *et al*. 2017).

In some Neotropical species, speciation resulted from the range expansion followed by isolation that often corresponds to specific geological barriers (Ribas *et al*. 2012, Smith *et al*. 2014, Fabre *et al*. 2017). In particular, changes in river formations are among the main sources of geographic isolation and diversification affecting vertebrate species in Amazonia (Ayres and Clutton 1992, Bates *et al*. 2004, Ribas *et al*. 2012, De Oliveira *et al*. 2016). These processes have generated a mosaic of distinct areas of endemism of different sizes and conservation statuses (Cracraft 1985, Bates and Demos 2001, Da Silva *et al*. 2005, Borges 2007) across the basin.

Genetic data have not only been important in the reconstruction of biogeographic and phylogenetic history, in addition is also useful for detecting cryptic species, which may be prevalent among Amazonian taxonomies (Bickford *et al*. 2007, Batista *et al*. 2013, Rocha *et al*. 2015, Ferreira *et al*. 2017). Therefore, genetic information allows the identification of significantly different lineages that can be considered as full species, according to the general lineage species concept (De Queiroz 2005, Aleixo 2007, Aleixo 2009a). Moreover, genetic diversity is necessary for species to evolve and adapt to environmental changes (Frankham *et al*. 2008) and hence, is one major focus of conservation biology (Heywood and Watson 1995). Furthermore, the description of patterns of genetic diversity also allows the identification of Evolutionary Significant Units (ESUs), which are meta-populations genetically differentiated within what is currently considered a single species, and are also potentially in need of distinct conservation plans. A single species may have one or more ESUs (Ryder 1986, Moritz 1994, Hey *et al*. 2003, Frankham *et al*. 2008, Aleixo 2009a).

The recognition of threatened or endangered ESUs or species needs to be complemented by distributional data to facilitate planning an efficient system of protected areas and generating criterion to prioritize conservation of these taxa (Ryder 1986, Moritz 1994). Ecological niche modelling estimates the geographic distribution of focal species based on occurrence and environmental data (Elith *et* al. 2006, Phillips *et al*. 2006), even not well studied species (Frankly 2010, Carstens *et al*. 2013). These models allow an evaluation of the distributional limits and environmental differentiation of each taxon (Martinez – Gordillo *et al*. 2010, Tocchio *et al*. 2014, Ruedas *et al*. 2017) and add critical data to effective conservation planning.

The woodcreeper genus *Hylexetastes* (Family: Dendrocolaptidae) is endemic to Amazonia with unknown interspecific limits and controversial taxonomy. *Hylexetastes* is a well-supported monophyletic lineage (Derryberry *et al*. 2011), with two species currently recognized: one distributed in western Amazonia (*Hylexetastes stresemanni* Snethlage 1925, with three subspecies described: *H. s. insignis* J. T. Zimmer, 1934, *H. s. stresemanni* Snethlage, 1925 and *H. s. undulatus* Todd, 1925), and another in eastern Amazonia (*Hylexetastes perrotii*, also with three subspecies described: *H. p. perrotii* Lafresnaye, 1844, *H. p. uniformis* Hellmayr, 1909 and *H. p. brigidai* Da Silva *et al*., 1995). However, *H. p. uniformis* and *H. p. brigidai* are considered as full species by some authors, based on slight differences in the colors of the neck and belly plumage (Figure 1) (Da Silva *et al*. 1995, Piacentini *et al*. 2015).

The two currently recognized species are not considered globally threatened (Birdlife International 2017), but their conservation status is uncertain, given these are rare species across their ranges (Stotz *et al*. 1996, Ridgely and Tudor 2004). Moreover, one subspecies, *H. p. brigidai*, is endemic to the Xingu area in eastern Amazonia (Marantz *et al*. 2016, IUCN 2017), a region heavily affected by Amazonia's infamous arc of deforestation and by habitat degradation due to anthropogenic disturbance (Da Silva *et al*. 2005, Soares-Filho *et al*. 2005, Moura *et al*. 2013, Barlow *et al*. 2016, INPE 2017). Therefore, recognition of this taxon as an ESU or a full species would have important implications for its conservation.

So far, only morphological characters have been used to evaluate *Hylexetastes* interspecific limits (Cory and Helmayr 1925, Zimmer 1934, Peters 1951, Da Silva *et al*. 1995, Perlo 2009, Bird *et al*. 2011, Piacentini *et al*. 2015, Marantz *et al*. 2016, Remsen *et al*. 2016). Thus, to independently assess taxonomic uncertainties within *Hylexetastes*, we gathered genetic data to construct a population level phylogenetic hypothesis for the genus. Using our phylogenetic hypothesis, we also estimated ecological niche-based models (ENMs) to distinguish the potential distribution of each identified taxon and evaluate the conservation vulnerability of each species**.**

Materials and methods

Sampling

We sequenced DNA extracted from frozen or ethanol fixed muscle tissue from 58 *Hylexetastes* specimens from all described taxa in the genus (Marantz *et al*. 2016). These samples were deposited in several ornithological collections (Figure 2A and Table S1 in supplementary material). *Xiphocolaptes promeropirhynchus*, which is known to be the sister group to *Hylexetastes* (Derryberry *et al*. 2011), was used as an outgroup.

DNA extraction, amplification and sequencing

Total DNA was extracted from tissue samples using the Genomic DNA Purification Kit (Promega; Wizard®) or Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Fragments of two mitochondrial DNA (mtDNA) genes (NADH dehydrogenase subunit 2 - ND2, and Cytochrome b - Cytb) and three nuclear genes (β-fibrinogen intron 5- BF5, glyceraldehyde-3-phospho-dehydrogenase intron 11 - G3PDH, and muscle specific receptor tyrosine kinase intron 3 - MUSK) were amplified by polymerase chain reaction (PCR) (see Table S2 for primer and PCR conditions). Amplification products were visualized by electrophoresis on a 1% agarose gel containing 10,000x SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA) and purified using ExoSAP or polyethylene glycol (PEG-8000) (Hawkins *et al*., 1994). We conducted cycle sequencing using Big Dye Terminator v.3.01 or v.3.1 kit, following the manufacturer's instructions (Applied Biosystems, CA). After an ethanol precipitation or PureSEQ^{TM} (Aline Biosciences) cleanup, purified sequencing reaction products were run on either an ABI 3130 or ABI 3730xl capillary sequencer. These procedures were performed in the laboratory of molecular biology of the Museu Paraense Emílio Goeldi (MPEG), Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, and the Laboratory of Molecular Systematics and Evolution at the Academy of Natural Sciences of Drexel University (where PCR purification and sequencing was completed by Functional Biosciences).

Data processing and phylogenetics analyses

Nucleotide sequences obtained were manually edited using the program BioEdit (Hall 1999) or Geneious (version 8.1.3 and 8.1.8, Biomatters LTD) and aligned using the default settings of the Clustal W algorithm (Thompson *et al*. 1994) implemented in BioEdit. Heterozygous positions for the nuclear genes were coded according to the IUPAC code. Mesquite v.3.0.4 was used to concatenate mtDNA and nuclear sequences. The codon reading frame was set to translate the mtDNA sequences to amino acid sequences and to check the sequence alignment for inappropriate stop codons, an indication of errors (Maddison and Maddison 2017). Partition Finder v.1.1.1 was used to determine the best evolutionary model for each gene, and best partitioning scheme using the criterion of Bayesian Information (BIC) (Lanfear *et al*. 2012) (Table S3). MrBayes v.3.2 was used to construct phylogenetic trees using the concatenated multilocus alignment under the criterion of Bayesian inference (Ronquist *et al*. 2012) and run for five million generations, using four heated chains, sampled every 500 generations. We discarded 10% of the sampled trees as burn-in.

We estimated a species tree and divergence times from the multilocus tree under a coalescent-based model using the program BEAST v.1.8.0 (Drummond and Rambaut 2007). Prior assignment of individual alleles to a ‘‘species’’ was performed using the well-supported and geographically structured mtDNA concatenated tree (see Results), and we tested these lineages as independent, using BPP v.3.2. (Yang 2015). A joint species delimitation and species tree analysis was conducted to test the delimitation of the clades recovered by the mtDNA concatenated tree. 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 burn-in of 100,000. Priors for ancestral population size and divergence times may influence 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 and Rannala 2010). An initial run was performed to confirm convergence of the run (model A00), and tested in TRACER 1.6 (Drummond and Rambaut 2007). 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. To estimate divergence times we used the CIPRES Science Gateway Portal v.3.1 at the San Diego Supercomputer Center (Miller *et al*. 2010; www.phylo.org/portal/) to run *BEAST (Drummond and Rambaut 2007; Drummond *et al*. 2012) twice with each run consisting of a chain of 10^8 iterations sampled every 10,000 iterations, with 10% burn-in. Convergence was verified in Tracer v.1.6 (Rambaut *et al*. 2007) and the tree topology was summarized in TreeAnnotator v.1.8 to make a consensus tree (Drummond *et al*. 2012) and visualized in FigTree 1.4.2 (Rambaut 2006). Two independent approaches were used to date the diversification history of *Hylexetastes.* The first dating approach, broadly used in avian phylogenetic studies, applies a relaxed molecular clock and a mutation rate of 0.01105 (with a standard deviation of 0.0034) substitution /site / million years (Myr) (Weir and Shluter 2008). For the second approach, we used a more recent approach, which considers the correlations between the substitution rate for the third codon position of Cytb and the body mass of the birds (Nabholz *et al* 2016). *Hylexetastes* body masses were obtained from specimen data in Marantz *et al* (2016).

Summary statistics

We used the Bayesian algorithm PHASE v.2.1 (Stephens *et al*. 2001, Stephens and Donnelly 2003) implemented in DnaSP v.5.10 (Librado and Rozas 2009) to phase haplotypes of nuclear genes. A threshold of 0.9% was used. DnaSP was also used to estimate genetic parameters, such as nucleotide and haplotype diversity, perform neutrality tests such as Tajima's D and R_2, and for recombination tests for the nuclear genes (Hudson and Kaplan 1985, Hudson *et al*. 1987, Rozas *et al.* 2001, Librado and Rozas 2009). The program MEGA v.7.0.2 was used to estimate the genetic distances for the mitochondrial gene, within and between each clade, using 1,000 bootstrap replicates (Kumar *et al*. 2016). Finally, differentiation indices (Fst values) were estimated using Arlequin v.3.5 under default settings (Excoffier and Lischer 2010).

Ecological Niche-Based Models

The study area includes the Amazon basin in South America (latitude from N -77 to S - 47 and longitude from W -17 to E -9; Figure 1A). Two hundred and forty eight occurrence records of *Hylexetastes* were collected (Table S3) from museum specimens using Vertnet (2016), from bird vocalization recordings deposited in Xenocanto (2016), and several other ornithological museum collections: *H. s. undulatus* (n=20), *H. s. stresemanni* (n=22), *H. s. insignis* (n=4), *H. p. perrotii* (n=86), *H. p. uniformis* (n=89) and *H. p. brigidai* (n=26). We used geographical references for localities from the Ornithological Gazetteer of Brazil (Paynter and Traylor 1991) to fill in the incomplete occurrence data. The processing of these data was completed using the Geographical Information System QGIS platform v.2.18 (QGIS Development Team 2016). We obtained environmental layers from Worldclim (Hijmans *et al*. 2005) with a spatial resolution of 30 seg (0.93 x 0.93 = 0.86 Km^2 at the equator) for current conditions (average for 1960-1990). To select uncorrelated environmental variables for the modelling procedure, we performed a Pearson's correlation analysis in RStudio 3.3.1 (Zar 1999, RStudio Team 2015).

Models were developed with the Maximum Entropy approach using Maxent v.3.3.3k (Phillips *et al*., 2006, Phillips and Dudík 2008). This modeling technique requires only presence data as input and consistently performs better than other methods (Elith *et al*. 2006, Hernandez *et al*. 2006, Wisz *et al*. 2008). A total of 15 model replicates were run, allowing for a random 75% training and 25% testing data partition in each run. Each replicate was analyzed using bootstrap, allowing sampling with replacement. Jacknifing was used to measure the importance of environmental variables in the models. Models were run with auto-features (Phillips *et al*. 2006) and the Area under the Curve (AUC) of the receiver-operating characteristics (ROC) plot was taken as a measure of individual model fit (Fielding and Bell 1997). We evaluated the model´s prediction using the Partial ROC approach (Peterson *et al*. 2008) with a proportion omission of 0.05 and 0.15, 50% random points, and 500 bootstrap iterations. To estimate the Partial ROC we used the niche tool box site (http://shiny.conabio.gob.mx:3838/nichetoolb2/). To make the binary prediction and obtain the area of occurrence from the area of suitability for *Hylexetastes* species, we used the linear maximum test of sensitivity and specificity, which is considered to have a better performance than other approaches (Liu *et al*. 2005). Finally, we compared the amount of potential area of distribution of each species of *Hylexetastes* with the amount of native forest under protection and affected by deforestation and other habitat conversions. Accurate data is only available for the Brazilian Amazon Basin, but at least half of the predicted species ranges are within this region (55%-100%; Figure 5; Table 3). We used raster files at a spacial resolution of 30 m (Terraclass 2014: http://www.inpe.br/cra/projetos_pesquisas/terraclass2014.php). The digital data resulting from these maps were divided into their respective Landsat 8 satellite orbits (OLI sensor), Lat / Long Projection System and SAD 69 Geodetic Reference System (Almeida *et al*. 2016).

Results

Phylogenetic analyses

A total of 3474 base pairs (bp) were sequenced: 1013, 1018, 471, 352 and 575 bp for ND2, Cytb, BF5, G3PDH, and MUSK, respectively. The topologies of phylogenetic trees constructed with either mitochondrial or nuclear genes sequences (Figure 2B), or the concatenation of the mitochondrial dataset only (Figure S1) were congruent with each other and analyses of these datasets recovered six statistically well supported clades. Overall, these six clades correspond to the six currently accepted *Hylexetastes* subspecies, except for *H. p. uniformis*, which has two subclades, and *H. s. stresemanni* and *H. s. insignis*, which grouped into a single clade (Figure 2B). Phylogenetic analysis using only nuclear sequences recovered three main clades, not only grouping *H. s. stresemanni* and *H. s. insignis*, but also *H. p. uniformis* and *H. p. brigidai*. In this phylogeny, *H. p. perrotti* was recovered as sister to all the other taxa (Figure S2). The low number of samples available for *H. s. stresemanni* and *H. s. insignis* influenced the types of additional analyses that were possible.

Species delimitation analyses performed in BPP, irrespective of the models of demographic and divergence time considered, also supported the existence of the aforementioned six clades (posterior probability, PP=1.0) (Figure 2C). Nonetheless, both *BEAST species trees, set with distinct calibrations, recovered five clades with high support (>0.98), and low support for the delimitation of the two *H. p. uniformis* (1 and 2) subclades (Figure 2C). Reconstruction of phylogenies based on the concatenation of the genes sequences and the species tree approaches produced different topologies with respect to the *H. p. uniformis* and *H. p. brigidai* clades. The concatenated analysis of mtDNA recovered one subclade of *H. p. uniformis* as sister to *H. p. brigidai* with strong support, whereas the species tree approach joined both subclades of *H. p. uniformis* together, with weak support. Therefore, taken together, all phylogenetic analyses in this study are concordant in recovering reciprocal monophyly of four main clades or species.

Genetic diversity levels varied considerably among genes and the six mtDNA lineages (Table 3). There were too few samples of *H. s. insignis* (n=2) and *H. s. stresemanni* (n=1) for these analyses. Uncorrected p-distance between *H. stresemanni* subspecies was low, ranging from 0 (*H. s. insignis)* to 0.004 ± 0.001 (*H. s. undulatus*) for Cytb, and from 0 (*H. s. insignis*) to 0.008 ± 0.002 (*H. s. undulatus*) for ND2 (Table 1A). Between the clades, the highest uncorrected p-distance for Cytb was between *H. p. uniformis* 1 and *H. p. perrotii* (0.063 ± 0.012), and the lowest uncorrected p-distances were between *H. s. stresemanni* and *H. s. insignis* (0.003 ± 0.002). For ND2 the highest uncorrected p-distance was between *H. s. undulatus* and *H. p. uniformis* 2 (0.059 ± 0.008) and the lowest uncorrected p-distances were between *H. s. stresemanni* and *H. s. insignis* (0.002 ± 0.001) (Table 1B). Most of the well supported clades exhibited relatively high genetic differentiation (Fst) for both the mitochondrial genes (Fst >0.38; p>0; Table S4A) and for the nuclear genes (Fst >0.027; p>0; Table S4B-C). However, the two subclades of *H. p. uniformis* (1 and 2) exhibited statistically significant genetic differentiation for only one nuclear gene (BF5; Fst =0.122; p=0.012).

Although overlapping, time estimates for the diversification within *Hylexetastes* were different for the two calibration approaches used. Avian substitution rate calibration using Weir and Schluter (2008) provided older divergences than the corrected rates method (Table 2; Nabholz *et al*. 2016). The oldest divergence corresponds to the split between the *H. p. perrotii* and *H. stresemanni* clade from the *H. p. uniformis* and *H. p. brigidai* clade around 3.64 or 1.88 Myr (node A: Figure 2C). The younger split corresponds to the split between *H. p. uniformis* clades, around 0.38 or 0.16 Myr (node E: Figure 2C).

Ecological niche-based models

Phylogenetic analyses (Figures 1B-C) suggest, at minimum, four species-level taxa (see discussion below) and therefore we constructed corresponding ecological niche-based models (ENM) for each. In a low proportion, preliminary ENM tests for each of the four species predicted some occurrence in areas beyond known ranges, and demonstrated environmental similarities among the ecological niches of all the *Hylexetastes* species. To avoid these ENM overfits, we treated all four *Hylexetastes* species as a single ecospecies and estimated a single model for all of them (Figures 5A-B). The phylogenetic clades observed were coincident with interfluvial areas, indicating *Hylexetastes* species are not distributed at random across the Amazon basin. For this reason, rivers were superimposed into the potential distribution of the ecospecies to delimit the ranges of distribution of each *Hylexetastes* species (Figure 5C). Eight different uncorrelated environmental variables were chosen after performing a Pearson's correlation analysis ($r < 0.80$). The ROC plots exhibited high average AUCs with low standard deviations (SD) for both training and test datasets in the model replicates for each species (AUC ≥ 0.817; Table S5). The evaluation of the model predictions using the Partial ROC AUC for the two tests gave statistically significant values 1.684 (± 0.03) that support the model selected ($p>0.05$, Table S5). Also the occurrence data adjusted to the predicted areas. We found that the Precipitation of Coldest and Warmest Quarter are the climatic variables with the most contribution to the ENM for the complex as a whole (Table S6). *H. stresemanni* exhibits the largest potential area of distribution with 1,127,350 km^2, whereas *H. p. brigidai* exhibited the smallest distribution area with only 202,015 km^2 (Figure 3A, Table 3). The probability of occurrence for each phylogenetically delimited species-level taxon shows that *H. p. brigidai* and *H. p. uniformis* are coincident with areas in the Brazilian Amazon basin impacted by deforestation and habitat degradation. These species also are predicted to occur in regions with the lowest percentage of protected areas (8 and 22%, respectively) where approximately half of the forest is already lost (56% and 63% of forest remains, respectively).

Discussion

Phylogenetic relationships and taxonomic implications

As Carstens *et al.* (2013) suggested, researchers conducting species delimitation should analyze their data using a wide range of methods and place their trust in the congruence across the results from different methods. The phylogenetic analyses performed in this study were concordant and strongly supported reciprocal monophyly of four main clades within *Hylexetastes*, which are allopatrically distributed in different interfluves and areas of endemism (AOEs) of the Amazon basin (Da Silva *et al*. 2005, Marantz *et al.* 2016). Time estimates based on the species tree indicate that the first differentiation event in *Hylexetastes* likely occurred during the Pliocene, which is congruent with previous temporal inferences for other differentiation events within Dendrocolaptidae (Derryberry *et al*. 2011, Rocha *et al*. 2015). These events are also spatio-temporally congruent with those estimated for other birds such as *Psophia* (Psophiidae), which also has species distributed in each area of endemism of the Amazon basin (Ribas *et al*. 2012).

A clade containing *H. p. perrotii* specimens, distributed in northeastern Amazonia (The Guianan AOE) is most closely related to a clade of *H. stresemanni* specimens rather than other specimens currently assigned to *H. perrotii* (Marantz *et al.* 2016). Furthermore, *H. p. perrotii* exhibits high genetic distance (≥5%) from all the other lineages in the genus. Although some authors (e.g. Halley *et al*. 2017) consider the so-called "yard-stick approach" (Helbig *et al*. 2002, Tobias *et al*. 2010), to comparison of the genetic distances between clades to be subjective for the delimitation of allopatric species, the level of differentiation found between *H. perrotii* and all other *Hylexetastes* lineages is even higher than estimates for other Dendrocolaptidae species, such as *Dendrocolaptes radiolatus* - *Dendrocolaptes certhia* (2%) and *Xiphorhynchus pardalotus* - *Xiphorhynchus ocellatus* (3.3%) (Batista *et al*. 2013, Sousa–Neves *et al*. 2013). Thus, these results support *H. p. perrotii* as an independent species.

Two clades, one with *H. p. brigidai*, and a second with *H. p. uniformis* (*H. p. uniformis* 1 and *H. p. uniformis 2*) specimens are distributed in specific interfluves of the southeastern Amazon basin, and central Amazonia south of the Amazon River. These clades exhibit low pairwise genetic distances (<1.6%), a recent diversification time (<0.49 Mya), and different phylogenetic histories. However, genetic structuring between them is significant. Moreover, besides geographical and genetic differences, morphological differences have also been reported between these clades (Marantz *et al*. 2016, Piacentini *et al*. 2015). For example, *H. p. brigidai* differs from *H. p. uniformis* in the pattern of coloration of the chin, throat and under wing-coverts (Figure 1; Da Silva *et al*. 1995). Thus, combined evidence supports that *H. p. brigidai* and *H. p. uniformis* are best treated as full species.

BPP analyses reported suggest that the subclades *H. p. uniformis* 1 *and H. p. uniformis* 2, themselves were two independent lineages. Both subclades, are reciprocally monophyletic when mtDNA is analyzed, and exhibit significant genetic structure for one nuclear gene (BF5; Fst =0.122; *p*=0.012). However, these lineages have the most recent divergence time within the genus *Hylexetastes* (<0.40 Mya), and the lowest level of genetic differentiation. Furthermore, a lack of phenotypic differences between these clades suggests that they are members of the same species. Therefore, they should be considered potential Evolutionary Significant Units (ESUs) rather than distinct species based on current data (Rider 1986, Moritz 1994).

Time estimates indicate that the diversification of *H. stresemanni,* distributed in the western part of the Amazon basin, is recent (<0.75 Mya). The small sampling of specimens in this clade prevents us from rigorously assessing monophyly of its subspecies, and thus does not allow for a more detailed taxonomic evaluation. Moreover, despite BPP indicated *H. s. undulatus* and *H. s. stresemanni* / *H. s. insignis* as independent lineages, these two subclades have a low uncorrected pairwise genetic distance (0.2 – 1.3%), which suggests that they may be the same species or incipient species, as supported by current taxonomy (Marantz *et al*. 2016).

Lastly, our analyses of concatenated data, genetic distances, Fst, and species trees demonstrate comparatively high levels of divergence and reciprocal monophyly among alloptric lineages within the genus *Hylexetastes,* therefore, we suggest a new taxonomic arrangement for *Hylexetastes,* with recognition of at least four independent species (De Queiroz 2007): *H. stresemanni, H. p. perrotii, H. p. brigidai,* and *H. p. uniformis.* It is possible that more detailed sampling of *H. p. uniformis* may reveal that the two shallowly diverged and reciprocally monophyletic subclades within this taxon are in fact two separate species. However, for now, we have chosen to be conservative with regard to these two subclades.

Species distribution and conservation

Effective conservation policies must be based on a variety of data related to a given species and its distribution. For this reason, scientists have employed novel approaches such as ENMs to predict the effects of anthropogenic alterations of the landscape on the distribution of species. This task requires models with reliable levels of predictive power, as well as minimum risk and low uncertainty (De Barros Ferraz *et al*. 2012, Rangel and Loyola 2012, Hipólito *et al*. 2015).

In this study, the ecological niche-based model identifies areas of occurrence that follow the general distribution patterns previously documented for each of the four species of *Hylexetastes* recognized herein (Piacentini *et al*. 2015, Marantz *et al.* 2016). The strong association of these species with *terra-firme* forest is indicated in Figures 2C and 2D by the congruence of the areas of potential distribution of each species with the forested area. Besides climatic factors, historical and ecological factors (such as vicariant barriers maintained by Amazonian rivers and interspecific competition, respectively), currently define the distribution patterns of these species. These factors were not taken into consideration by this ENM (Frankling 2009, Rangel and Loyola 2012, Ribas *et al*. 2012). The geographic distribution of *Hylexetastes* species might constitute a case of parapatric ranges of closely related taxa delimited by the major Amazonian rivers (see Ribas *et al*. 2012 and Rocha *et al*. 2015 for other examples). River barriers appear to be effective for lineages of birds restricted to the upland *terra-firme* forest, away from rivers and flooded forests (Da Silva *et al*. 1995, Marantz *et al*. 2016). However, Amazonian rivers differ in length and width and underlying geologic history, and some, such as the Teles Pires River, may represent comparatively weaker faunal barriers for some species of birds (Bates *et al*. 2004, Weir *et al*. 2015). This could explain the low and recent genetic divergence between *H. p. uniformis* 1 and *H. p. uniformis* 2, which are distributed on opposite banks of the Tapajos and Teles Pires River headwaters. This is the typical pattern for most Terra Firme species (e.g. Psophia; Ribas *et al*. 2012).

Our analyses confirm that the area of occurrence of *H. p. brigidai* is restricted to the Xingu area of endemism, which is the second most deforested AOE of Amazonia (Da Silva *et al*. 2005, Moura *et al*. 2013, Barlow *et al*. 2016, INPE 2017). Approximately 20% of the forest remaining in the Xingu area is under protection and 27% of forest in this area is already lost (Da Silva *et al.* 2005). However, only 56% of the predicted area of occurrence of *H. p. brigidai* is forest, 8% of this area is under protection, and more than 7% is affected by deforestation and other habitat conversion. Currently, *H. p. brigidai* is not recognised as a species by BirdLife International or the IUCN, but our results support the need to evaluate the degree of vulnerability of this taxon, which constitutes a distinctive species endemic to the Brazilian Amazon Basin.

Finally, both *H. p. uniformis 1* and *H. p. uniformis 2* are confined to the Tapajós and Rondônia AOEs to the west of *H. brigidai*, of which at least 9-12% is now deforested (Da Silva *et al*. 2005). About 95% of the potential area of occurrence of *H. p. uniformis* is within the Brazilian Amazon Basin and thus only 63% of its predicted area is likely habitable, 22% is under protection, and more than 4% is affected by habitat conversion. Deforestation and expansion of plantations in the Amazon basin are continuously increasing (INPE 2017), and the region presents high number of bird species and populations loss (Ceballos *et al*. 2017). Since the distributions of both *H. p. uniformis* clades overlap with some of the most deforested regions in the Amazon, and avian species have a high global extinction rate due to anthropogenic actions (Pimm *et al*. 2014), the conservation status of both ESUs should be monitored separately and continuously (Rider 1986, Moritz 1994, Haig *et al*. 2006), maximizing the likelihood that the species and its meta-populations will persist into the future (Hey *et al*. 2003).

Conclusion

We demonstrate the importance of the integration of several methods to provide an accurate identification of ESUs and species (De Queiroz 2007, Carstens *et al.* 2013) for the woodcreeper genus *Hylexetastes*. The correct delimitation of these units more accurately reflects patterns of biotic diversity and provides critical data for informed conservation planning. Our genetic data and ENMs (both potential parapatric geographic distributions and distinct climatic variables influencing these distributions) coincide with previously published morphological descriptions to support the recognition of four species within *Hylexetastes* (Da Silva *et al.* 1995, Piacentini *et al*. 2015). The evidence presented herein supports a new taxonomic arrangement for this genus, elevating the taxa *H. p. perrotii*, *H. p. uniformis* and *H. p. brigidai* to full species status. Therefore, new conservation status assessments are needed for each recognized species, particularly for *H. p. brigidai* and *H. p. uniformis*, whose distributions overlap with deforested areas in eastern Amazonia.

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Figure 1. Ventral views of representative specimens illustrating plumage differences among *Hylexetastes* subspecies, as recognized in the current taxonomy (Marantz *et al*. 2016). From left to right: **A.** *Hylexetastes stresemanni stresemanni*, **B.** *Hylexetastes stresemanni insignis*, **C.** *Hylexetastes stresemanni undulatus*, **D.** *Hylexetastes perrotii perrotii*, **E.** *Hylexetastes perrotii uniformis* and **F**. *Hylexetastes perrotii brigidai*. Note the differences in the coloration of the plumage of the neck and belly between each subspecies. *Position: Between page 1 and 2 of the section introduction

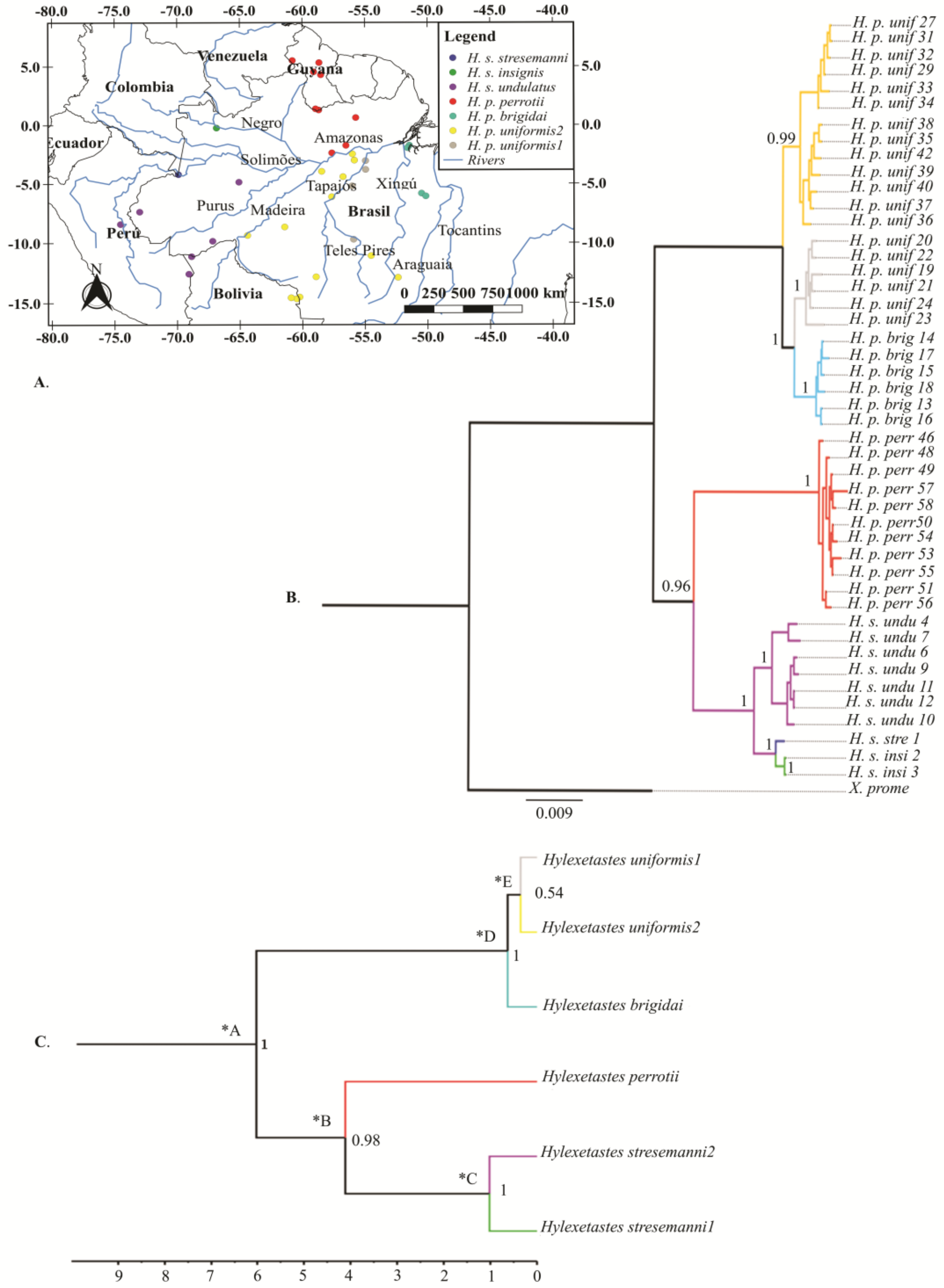

Figure 2. Location map and phylogenetic trees for the specimens of *Hylexetastes* sequenced for this study. **A.** Map of locations where 58 specimens were collected. Different Clades were assigned to different taxa based on their respective type localities (Peters 1951). **B.** Phylogenetic tree obtained by Bayesian Inference of the concatenated mitochondrial (Cytb and ND2) and nuclear genes (BF5, G3PDH, MUSK) alignment of 46 terminal *Hylexetastes* taxa. **C.** Bayesian inference of species tree generated using *BEAST with data from mtDNA (Cytb and ND2) and nuclear genes (BF5,

G3PDH, MUSK) for 46 terminal *Hylexetastes* taxa. Node labels indicate posterior probability values. Letters labeling each node indicate estimates of the divergence times from Table 1. Asterisks labelling nodes indicates clades supported by the species delimitation analysis performed in BPP for all demographic and divergence time models considered. Colors of clades indicate terminal taxa and match the distribution map legend. *Position: Between page 1 and 2 of the section results

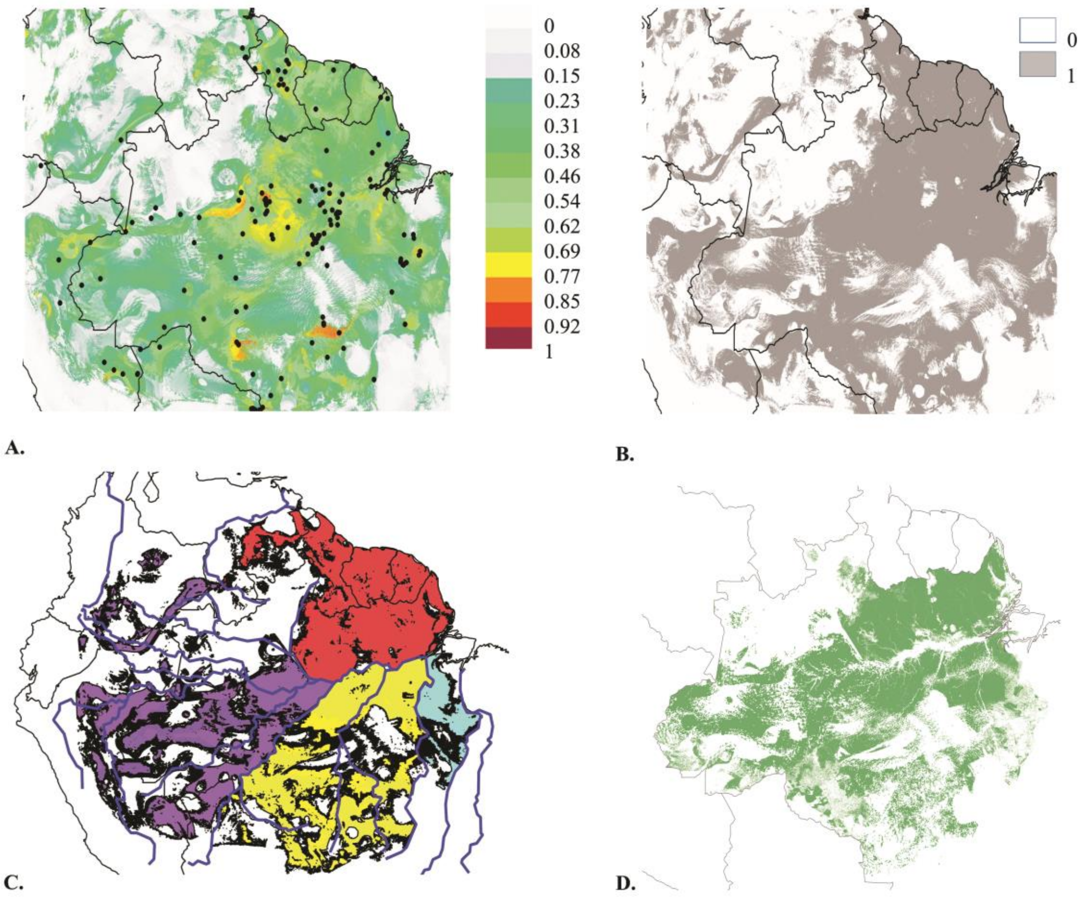

Figure 3. Maps of potential distribution of *Hylexetastes*. **A.** Predicted suitability areas for the *Hylexetastes* complex derived from 15 model replicates run in Maxent. Black points correspond to the occurrence records used. Colours indicate the suitability of areas with warmer colors indicating higher suitability for the complex. **B.** Potential distribution of *Hylexetastes* complex ignoring deforestation. **C.** Potential distribution of each lineage of the *Hylexetastes* complex: *H. stresemanni* (purple), *H. p. perrotii* (red), *H. p. uniformis* (yellow) and *H. p. brigidai* (light blue). Main Amazonian rivers are depicted in dark blue. **D.** Current distribution of Terra Firme forested areas within the Brazilian Amazon basin. *Position: After Page 3 of the section results

Table 1. Uncorrected p-distance (± standard deviation) **A.** within and **B.** between *Hylexetastes* clades for both mitochondrial genes analysed, Cytb (lower diagonal) and ND2 (upper diagonal). *Position: Between page 1 and 2 of the section results

A	**Cytb**	**ND2**
H. s. undulatus	0.004 ± 0.001	0.008 ± 0.002
H. s. insignis	0.000 ± 0000	0.000 ± 0.000
H. p. perrotii	0.001 ± 0.000	0.003 ± 0.001
H. p. brigidai	0.001 ± 0.001	0.002 ± 0.001
H. p. uniformis 1	0.001 ± 0.001	0.001 ± 0.001
H. p. uniformis 2	0.003 ± 0.001	0.005 ± 0.001

B	***H. s. undulatus***	***H. s. insignis***	***H. s. stresemanni***	***H. p. perrotii***	***H. p. brigidai***	***H. p. uniformis 1***	***H. p. uniformis 2***
H. s. undulatus	------	0.013 ± 0.003	0.013 ± 0.005	0.046 ± 0.007	0.053 ± 0.008	0.051 ± 0.007	0.059 ± 0.008
H. s. insignis	0.013± 0.004	-------	0.002 ± 0.001	0.040 ± 0.006	0.048 ± 0.007	0.046 ± 0.007	0.053 ± 0.008
H. s. stresemanni	0.013 ± 0.004	0.003 ± 0.002	-------	0.040 ± 0.006	0.048 ± 0.007	0.046 ± 0.007	0.053 ± 0.008
H. p. perrotii	0.043 ± 0.008	0.043 ± 0.009	0.041 ± 0.008	------	0.049 ± 0.008	0.047 ± 0.007	0.053 ± 0.008
H. p. brigidai	0.056 ± 0.011	0.059 ± 0.011	0.057 ± 0.011	0.060 ± 0.011	------	0.005 ± 0.002	0.016 ± 0.004
H. p. uniformis 1	0.055± 0.011	0.059 ± 0.011	0.057 ± 0.011	0.063 ± 0.012	0.011 ± 0.003	------	0.015 ± 0.003
H. p. uniformis 2	0.052 ± 0.010	0.054 ± 0.010	0.052 ± 0.010	0.059 ± 0.011	0.013 ± 0.004	0.009 ± 0.003	-----

Table 2. Divergence time estimates and confidence intervals (95% HPD) obtained with *BEAST analyses. Calibration 1 is based on a substitution rate of 0.01105 substitution/site/lineage/million years (Myr) for Cytb (Weir and Schluter 2008), and Calibration 2 is based on a corrected rate accounting for body mass (Nabholz *et al*. 2016). *Position: Page 2 of the section results

Node	**Calibration 1**		**Calibration 2**	
	Mya	**95% HPD**	**Mya**	**95% HPD**
A	3.641	4.479 – 2.704	1.876	2.518 – 1.075
B	2.654	3.429 – 1.761	1.410	1.927 – 0.808
C	0.753	1.056 – 0.421	0.432	0.642 - 0.233
D	0.486	0.759 - 0.259	0.265	0.422 – 0.137
E	0.384	0.602 – 0.150	0.155	0.281 – 0.054

Table 3. Potential area of occurrence (km^2) for each *Hylexetastes* species compared to the land use amounts within the Brazilian Amazon basin. *Position: Page 3 of the section results

	H. stresemanni	***H. p. perrotii***	***H. p. uniformis***	***H. p. brigidai***
Total potential distribution	1127350	1054230	1047330	202015
Area within the Brazilian Amazon Basin	640020	580606	990337	202015
Forest	566271 (89%)	488114 (84%)	620567 (63%)	112298 (56%)
Deforestation	290 (0.05%)	217 (0.04%)	1456 (0.15%)	313 (0.15%)
Urbanized	674 (0.11%)	370 (0.06%)	1198 (0.12%)	360 (0.18%)
Reforestation	0	743 (0.13%)	55 (0.01%)	88 (0.04%)
Secondary vegetation	7044 (1%)	9400 (1.62%)	35083 (4%)	13193 (7%)
Under protection	158576 (24%)	255002 (44%)	221658 (22%)	16021 (8%)

Supplementary material

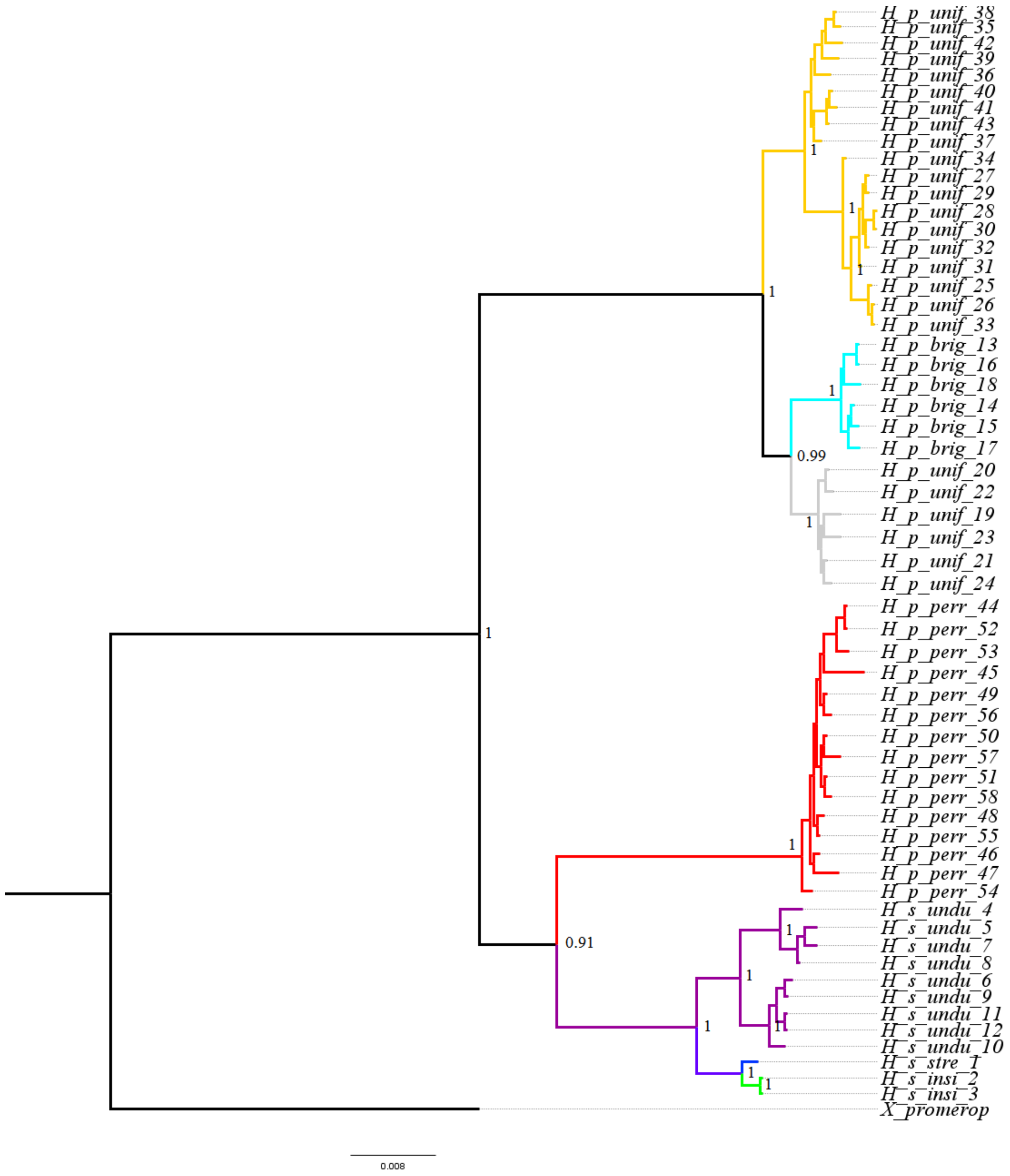

Figure S1. Phylogenetic tree of the genus *Hylexetastes*, constructed using Bayesian Inference of concatenated mtDNA genes (Cytb and ND2) for 58 terminal taxa. Numbers labelling nodes indicate Bayesian posterior probabilities. Colours of clades indicate terminal taxa and match the map legend on Figure 1: Yellow: *H. perrotti uniformis* 2; Grey: *H. p. uniformis* 1; Light blue: *H. p. brigidai*; Red: *H. p. perrotti*; Purple: *H. stresemanni undulatus*; Dark blue: *H. s. stresemanni*; Green: *H. s. insignis*.

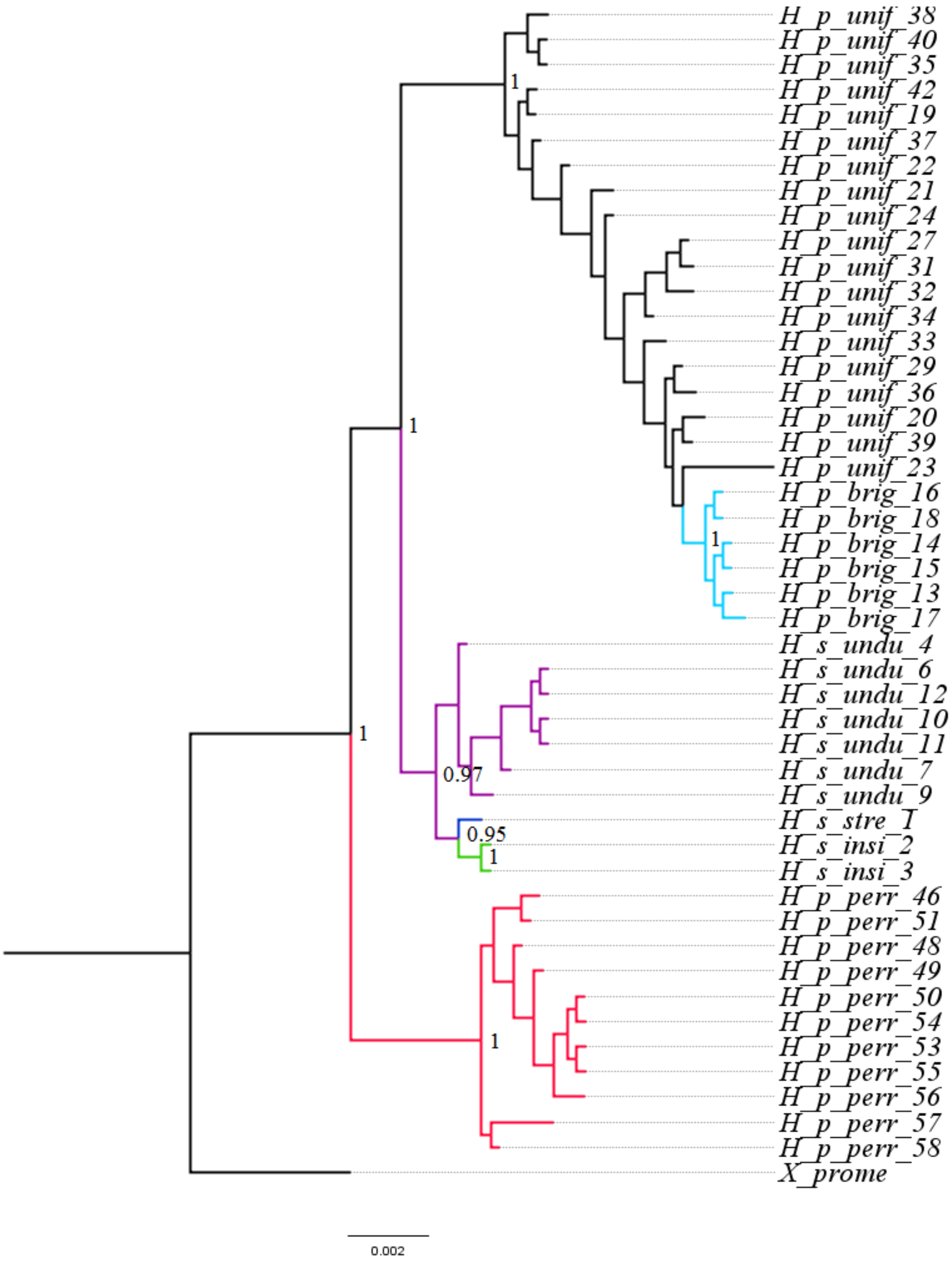

Figure S2. Phylogenetic tree of the genus *Hylexetastes*, constructed using Bayesian Inference of three concatenated nuclear genes (BF5, G3PDH and MUSK) for 46 terminal taxa. Numbers labelling nodes indicate Bayesian posterior probabilities. Colours of clades indicate terminal taxa and match the map legend on Figure 1: Black: *H. perrotti uniformis*; Light blue: *H. p. brigidai*; Red: *H. p. perrotti*; Purple: *H. stresemanni undulatus*; Dark blue: *H. s. stresemanni*; Green: *H. s. insignis*.

Table S1. Summary of records of *Hylexetastes* specimen occurrence used in this study to perform ecological niche modelling analyses are marked by an *. Samples used for DNA analyses are also included and check boxes indicate genes successfully amplified and sequenced for these individuals. The records are organized by number and identified by subspecies under the current taxonomy (Marantz *et al*. 2016). Acronyms in the table correspond to following institutions: Museum Paraense Emílio Goeldi (MPEG), Academy of Natural Sciences of Drexel University (ANSP), Louisiana State University Museum of Natural Science (LSU), Smithsonian Institution National Museum of Natural History (USNM), University of Kansas Natural History Museum (KU), Field Museum of Natural History (FMNH) and Museum of Zoology of the University of São Paulo (MZUSP).

N °	**Code**	**Taxon**	**Longitude**	**Latitude**	**Locality**	**State**	**Country**	**ND2**	**Cytb**	**Bf5**	**G3PDH**	**MUSK**
1	MPEG AMA 027*	*H. s. stresemanni*	-69.89	-4.19	Tabatinga, entry of INCRA	Amazonas	Brazil	x	x	x	x	x
2	MPEG SGC 442*	*H. s.insignis*	-66.85	-0.23	São Gabriel da Cachoeira, middle portion of Negro river, Arabo	Amazonas	Brazil	x	x	x	x	x
3	MPEG SGC 443*	*H. s.insignis*	-66.85	-0.23	São Gabriel da Cachoeira, middle portion of Negro river, Arabo	Amazonas	Brazil	x	x	x	x	x
4	MPEG 57058*	*H. s.undulatus*	-65.06	-4.85	Tefé, Petrobras - Urucu, Papagaio	Amazonas	Brazil	x	x	x	x	x
5	MPEG 57481*	*H. s.undulatus*	-73.00	-7.37	Guajará, 35 km NW of Cruzeiro do Sul	Acre	Brazil	x	x	x	x	
6	MPEG 64374*	*H. s.undulatus*	-67.17	-9.84	Senador Guiomard, Br 364 km 80, Ramal Oco of Mundo, km 16	Acre	Brazil	x	x	x	x	x
7	LSU B11097*	*H. s.undulatus*	-74.53	-8.41	SE slope Cerro Tahuayo, ca km ENE Pucallpa	Ucayali Department	Peru	x	x	x	x	x
8	LSU B11134*	*H. s.undulatus*	-74.53	-8.41	SE slope Cerro Tahuayo, ca km ENE Pucallpa	Ucayali Department	Peru	x	x	x	x	
9	LSU B11137*	*H. s.undulatus*	-74.53	-8.41	SE slope Cerro Tahuayo, ca km ENE Pucallpa	Ucayali Department	Peru	x	x	x	x	x
10	KU B643*	*H. s.undulatus*	-69.08	-12.59	14 km E Puerto Maldonado	Madre de dios	Peru	x	x	x	x	x
11	LSU B9111*	*H. s.undulatus*	-68.86	-11.13	Nicolás Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden	Pando Department	Bolivia	x	x	x	x	x

12	LSU B9114*	*H. s.undulatus*	-68.86	-11.13	Nicolás Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden	Pando Department	Bolivia	x	x	x	x	x
13	MPEG 65981*	*H. p. brigidai*	-50.48	-5.82	Carajás, Flona Tapirapé-Aquiri	Pará	Brazil	x	x	x	x	
14	LSU B25539*	*H. p. brigidai*	-51.45	-1.73	National Forest of Caxiuana, Ferreira Penna Scientific Station	Pará	Brazil	x	x	x	x	x
15	FMNH 456812*	*H. p. brigidai*	-51.60	-1.95	Portel, Flona of Caxiuanã, Plot PPBIO	Pará	Brazil	x	x	x	x	x
16	PPBIO - 186*	*H. p. brigidai*	-51.60	-1.95	Portel, Flona of Caxiuanã, Plot PPBIO	Pará	Brazil	x	x	x	x	x
17	FMNH 456814*	*H. p. brigidai*	-51.60	-1.95	Portel, Flona of Caxiuanã, Plot PPBIO	Pará	Brazil	x	x	x	x	x
18	MPEG CRJ037*	*H. p. brigidai*	-50.14	-6.05	Parauapebas, Flona of Carajás, N4	Pará	Brazil	x	x	x	x	x
19	MPEG67665 *	*H. p. uniformis*	-54.95	-3.79	Rurópolis	Pará	Brazil	x	x	x	x	x
20	FMNH 392022*	*H. p. uniformis*	-55.92	-9.70	Alta Floresta	Mato Grosso	Brazil	x	x	x	x	x
21	MPEG67666 *	*H. p. uniformis*	-54.95	-3.79	Belterra municipality	Pará	Brazil	x	x	x	x	x
22	MPEG 59211*	*H. p. uniformis*	-55.69	-6.20	Itaituba, 7 km NW Moraes of Almeida	Pará	Brazil	x	x	x	x	x
23	MPEG 66117*	*H. p. uniformis*	-54.95	-3.04	Belterra, Flona of Tapajós, Br 163 km 083	Pará	Brazil	x	x	x	x	x
24	MPEG BROJ4*	*H. p. uniformis*	-56.02	-5.18	Trairão, Trilha 2	Pará	Brazil	x	x	x	x	x
25	MPEG 60297*	*H. p. uniformis*	-52.37	-12.89	Canarana, Tanguro Farmhouse	Mato Grosso	Brazil	x	x	x	x	
26	MPEG 60299*	*H. p. uniformis*	-52.37	-12.89	Canarana, Tanguro Farmhouse	Mato Grosso	Brazil	x	x			
27	MZUSP P196*	*H. p. uniformis*	-58.92	-12.83	Juruena	Mato Grosso	Brazil	x	x	x	x	x
28	LSU B100030*	*H. p. uniformis*	-60.46	-14.65	Serrania of Huanchaca, 25 km SE Arco Iris waterfall	Santa Cruz deparment	Bolivia	x	x	x	x	
29	LSU B12310*	*H. p. uniformis*	-60.91	-14.57	Velasco, Santa Cruz department, 32 km E Aserraderos, Nacional Park Noel	Santa Cruz deparment	Bolivia	x	x	x	x	x

					Kempff Mercado							
30	LSU B13841*	*H. p. uniformis*	-60.23	-14.51	Serrania of Huanchaca; 45 km E Florida	Santa Cruz deparment	Bolivia	x	x		x	
31	LSU B14520*	*H. p. uniformis*	-60.46	-14.65	Serrania of Huanchaca, 21 km SE Arco Iris waterfall	Santa Cruz deparment	Bolivia	x	x	x	x	x
32	LSU B15321*	*H. p. uniformis*	-60.91	-14.57	Velasco, Nacional Park Noel Kempff Mercado, 30 km E Aserradero Moira	Santa Cruz deparment	Bolivia	x	x	x	x	x
33	MPEG JTW1432*	*H. p. uniformis*	-54.52	-11.07	Marcelândia, Bom Sucesso Farmhouse	Mato Grosso	Brazil	x	x	x	x	x
34	MPEG MSF449*	*H. p. uniformis*	-64.39	-9.36	Jaci-Paraná, Jaci river	Rondonia	Brazil	x	x	x	x	x
35	MZUSP P338*	*H. p. uniformis*	-55.84	-2.99	Alto Arapiuns, ca 139 km SSW Santarém, W of Tapajós river	Pará	Brazil	x	x	x	x	x
36	MPEG 57573*	*H. p. uniformis*	-61.42	-8.65	Manicoré, highway of Estanho, km 126	Amazonas	Brazil	x	x	x	x	x
37	MPEG 58270*	*H. p. uniformis*	-56.00	-2.47	Municipality of Juruti, Barroso Base	Pará	Brazil	x	x	x	x	x
38	LSU B35607*	*H. p. uniformis*	-55.85	-2.99	Alto Arapiuns, ca 139 km SSW Santarém, W of Tapajós river	Pará	Brazil	x	x	x	x	x
39	MPEG 56623*	*H. p. uniformis*	56.18	2.60	Juruti, Igarapé Mutum	Pará	Brazil	x	x	x	x	x
40	MPEG67026 *	*H. p. uniformis*	-58.46	-3.95	Nova Olinda	Amazonas	Brazil	x	x	x	x	x
41	MPEG67191 *	*H. p. uniformis*	-56.75	-4.40	Itaituba, Tapajo river, left bank	Pará	Brazil	x	x	x	x	
42	MPEG67192 *	*H. p. uniformis*	-56.75	-4.40	Itaituba, Tapajo river, left bank	Pará	Brazil	x	x	x	x	x
43	MPEG JAT(A) 460*	*H. p. uniformis*	-57.66	-6.10	Jacareacanga, Vila São Martins, left bank Rio Tapajós	Pará	Brazil	x	x	x		x
44	ANSP 5659*	*H.p.perrotii*	-58.65	5.32	Ca. 5 km NW Mabura Hill, between Essequibo and Demerara Rivers, 100m;	Upper Demerara Berbice	Guyana	x	x		x	
45	ANSP 5715*	*H.p.perrotii*	-58.65	5.32	Ca. 5 km NW Mabura Hill, between Essequibo and Demerara Rivers, 100m;	Upper Demerara Berbice	Guyana	x	x			

46	MPEG 65844*	*H.p.perrotii*	-58.68	1.28	Oriximiná, ESEC Grão Pará	Pará	Brasil	x	x	x	x	x
47	MPEG 65989*	*H.p.perrotii*	-56.51	-1.74	Porto Trombetas	Pará	Brasil	x	x	x	x	
48	MPEG 66641*	*H.p.perrotii*	-55.73	0.63	Óbidos, ESEC Grão-Pará	Pará	Brasil	x	x	x	x	x
49	ASNP 8208*	*H.p.perrotii*	-58.52	4.28	Iwokrama Reserve, Kabocalli Landing, W bank Essequibo River, ca. 45 river miles SE Kurupukari	Potaro-Siparuni	Guyana	x	x	x	x	x
50	ASNP 8209*	*H.p.perrotii*	-58.52	4.28	Iwokrama Reserve, Kabocalli Landing, W bank Essequibo River, ca. 45 river miles SE Kurupukari	Potaro-Siparuni	Guyana	x	x	x	x	x
51	ANSP 8446*	*H.p.perrotii*	-59.08	4.53	Iwokrama Reserve, "S" Falls, Siparuni River	Potaro-Siparuni	Guyana	x	x	x	x	x
52	MPEG A08083*	*H.p.perrotii*	52.57	1.52	Municipality of Almerim, Monte Dourado, Pacanari Reserve.	Pará	Brasil	x	x			
53	USNM B10735*	*H.p.perrotii*	-58.93	1.38	North Side, Acari Mountains	Upper Takutu-Upper Esequibo	Guyana	x	x	x	x	x
54	USNM B10736*	*H.p.perrotii*	-58.93	1.38	North Side, Acari Mountains	Upper Takutu-Upper Esequibo	Guyana	x	x	x	x	x
55	USNM B10741*	*H.p.perrotii*	-58.93	1.38	North Side, Acari Mountains	Upper Takutu-Upper Esequibo	Guyana	x	x	x	x	x
56	LSU B20373*	*H.p.perrotii*	60.00	2.50	Municipality of Manaus; km 34 ZF-3, Esteio farmhouse, ca 80 km N Manaus.	Amazonas	Brasil	x	x	x	x	x
57	USNM B5215*	*H.p.perrotii*	-60.78	5.50	Waruma River, E Bank, ca 15 river km s Kako River	Esequibo	Guyana	x	x	x	x	x
58	MPEG ORX 242*	*H.p.perrotii*	-57.64	-2.37	Urucará, Marajatuba lake	Amazonas	Brasil	x	x	x	x	x
59	AMNH BIRDS Skin-309338	*H. s. stresemanni*	-64.71	-3.35	Santo Isidoro, Tefe	Amazonas	Brazil					
60	AMNH BIRDS Skin-309341	*H. s. stresemanni*	-64.71	-3.35	Santo Isidoro, Tefe	Amazonas	Brazil					

61	CM BIRDS P96616	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
62	CM BIRDS P97235	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
63	CM BIRDS P97595	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
64	AMNH BIRDS Skin-309339	*H. s. stresemanni*	-64.71	-3.35	Santo Isidoro, Tefe	Amazonas	Brazil					
65	AMNH BIRDS Skin-309340	*H. s. stresemanni*	-64.71	-3.35	Santo Isidoro, Tefe	Amazonas	Brazil					
66	CM BIRDS P97493	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
67	CM BIRDS P97712	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
68	CM BIRDS P99717	*H. s. stresemanni*	-60.99	-2.87	Manacapuru, Solimoes river, left bank	Amazonas	Brazil					
69	CM BIRDS P99718	*H. s. stresemanni*	-60.99	-2.87	Manacapuru, Solimoes river, left bank	Amazonas	Brazil					
70	FMNH BIRDS 310494	*H. s. stresemanni*	-68.09	-3.39	Tonantins, Solimoes river	Amazonas	Brazil					
71	MPEG 49907	*H. s. stresemanni*	-66.03	-3.17	Solimões river, right bank, Caitaú, Uará	Amazonas	Brazil					
72	MPEG 50548	*H. s. stresemanni*	-61.75	-1.87	Nacional Park of Jaú, Igarapé Patauá, Novo Airão, Jaú river, left bank	Amazonas	Brazil					
73	YPM ORN 029286	*H. s. stresemanni*	-67.78	-2.78	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
74	CM BIRDS P96366	*H. s. stresemanni*	-69.44	-3.65	Sao Paulo de Olivencia, Solimoes river, right bank	Amazonas	Brazil					
75	XC62911	*H. s. stresemanni*	-72.39	-4.83	Loreto departament, Yavari Camp 2	Amazonas	Peru					
76	CM BIRDS P97451	*H. s. stresemanni*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
77	AMNH BIRDS	*H. s. stresemanni*	-61.92	-2.33	Igarapé Brabo, Tapajoz river	Amazonas	Brazil					

	Skin-286933											
78	AMA029	*H. s. stresemanni*	-69.89	-4.19	Tabatinga, entry of INCRA	Amazonas	Brazil					
79	AMNH BIRDS Skin-239375	*H. s. stresemanni*	-74.62	-5.87	Lagarto, Alto Ucayali	Ucayali Department	Peru					
80	XC80970	*H. s.insignis*	-70.24	1.26	Mitu	Vaepes Departament	Colombia					
81	XC258282	*H. s.insignis*	-75.88	-0.27	Cuyabeno Faunistic Reserve	Sucumbios	Ecuador					
82	CM BIRDS P91843	*H. s.undulatus*	-61.64	-4.33	Nova Olinda, Purus river, left bank	Amazonas	Brazil					
83	CM BIRDS P99323	*H. s.undulatus*	-60.76	-3.59	Caviana, Solimoes river, right bank	Amazonas	Brazil					
84	CM BIRDS P86821	*H. s.undulatus*	-65.77	-7.67	Hyutanahan, Purus river, right bank	Amazonas	Brazil					
85	MPEG 48153	*H. s.undulatus*	-71.68	-6.98	Seringal Penedo, Ipixuna, Juruá river, right bank	Amazonas	Brazil					
86	KU BIRDS 84194	*H. s.undulatus*	-69.05	12.55	Cuzco Amazonico Reserve, 14 km E Puerto Maldonado	Madre de Dios	Peru					
87	CLO ML 80095	*H. s.undulatus*	-67.50	-11.00	Pando	Pando	Bolivia					
88	UF AUDIO 1546	*H. s.undulatus*	-71.41	-11.89	Manu National Park, Cocha Cashu	Madre de Dios	Peru					
89	CM BIRDS P97912	*H. s.undulatus*	-68.09	-3.39	Tonantis, Solimoes river, left bank	Amazonas	Brazil					
90	XC117998	*H. s.undulatus*	-63.21	-5.69	Tapauá	Amazonas	Brazil					
91	XC73640	*H. s.undulatus*	-70.10	-12.57	CICRA	Madre de Dios	Peru					
92	XC87850	*H. s.undulatus*	-70.73	-12.34	Amazon Manu Lodge	Madre de Dios	Peru					
93	MPEG 52830	*H. p. brigidai*	-51.45	-1.73	Melgaço, Caxiuanã, Scientífic station Ferreira Penna	Pará	Brazil					
94	MPEG 60298	*H. p. brigidai*	-52.37	-12.89	Canarana, Tanguro Farmhouse	Mato Grosso	Brazil					

95	CLO ML 127442	*H. p. brigidai*	-51.46	-1.74	National Forest of Caxiuana; Science Station Ferreira Penna	Pará	Brazil					
96	MPEG 36066	*H. p. brigidai*	*-49.57*	*-4.58*	Tocantins river, left bank, Tucuruí, Jacundá	Pará	Brazil					
97	MPEG 36272	*H. p. brigidai*	*-50.25*	*-4.58*	Tocantins river, Tucuruí, Vale of Caraipé	Pará	Brazil					
98	MPEG 37060	*H. p. brigidai*	*-51.12*	*-7.76*	Fresco river, affluent Xingu River, São Félix do Xingu, Gorotire	Pará	Brazil					
99	MPEG 37992	*H. p. brigidai*	*-49.20*	*-5.58*	Reserve of Companhia Vale do Rio Doce, Marabá, Sororó river	Pará	Brazil					
100	MPEG 37993	*H. p. brigidai*	*-49.20*	*-5.58*	Reserve of Companhia Vale do Rio Doce, Marabá, Sororó river	Pará	Brazil					
101	MPEG 40596	*H. p. brigidai*	*-49.12*	*-5.36*	Marabá/Bacabal, Transamazônica km 12	Pará	Brazil					
102	MPEG 48668	*H. p. brigidai*	*-50.18*	*-9.66*	Santana of Araguaia, Barra das Princesas farmhouse	Pará	Brazil					
103	MPEG 48669	*H. p. brigidai*	*-50.18*	*-9.66*	Santana of Araguaia, Barra das Princesas farmhouse	Pará	Brazil					
104	MPEG 48670	*H. p. brigidai*	*-50.18*	*-9.66*	Santana of Araguaia, Barra das Princesas farmhouse	Pará	Brazil					
105	XC66610	*H. p. brigidai*	-51.46	-1.74	Caxiuanã Scientific Station	Pará	Brazil					
106	XC5030	*H. p. brigidai*	-50.35	-6.16	Serra dos Carajás	Pará	Brazil					
107	XC303304	*H. p. brigidai*	-50.09	-6.03	Parauapebas	Pará	Brazil					
108	XC118968	*H. p. brigidai*	-50.29	-6.18	Parauapebas	Pará	Brazil					
109	XC91220	*H. p. brigidai*	-51.46	-1.74	Caxiuanã Scientific Station	Pará	Brazil					
110	XC18597	*H. p. brigidai*	-49.60	-4.37	Tucuruí dam, left margin of the lake	Pará	Brazil					
111	XC224259	*H. p. brigidai*	-50.50	-5.92	Carajás, Salobo area	Pará	Brazil					
112	XC224260	*H. p. brigidai*	-50.50	-6.00	Carajás, Aguas Claras area	Pará	Brazil					

113	CM BIRDS P73540	*H. p. uniformis*	-54.81	-2.42	Santarem, Tapajos river, right bank	Pará	Brazil					
114	CM BIRDS P78202	*H. p. uniformis*	-54.81	-2.42	Santarem, Tapajos river, right bank	Pará	Brazil					
115	CM BIRDS P72068	*H. p. uniformis*	-54.81	-2.42	Santarem, Tapajos river, right bank	Pará	Brazil					
116	CM BIRDS P78133	*H. p. uniformis*	-54.81	-2.42	Santarem, Tapajos river, right bank	Pará	Brazil					
117	CLO ML 117106	*H. p. uniformis*	-55.05	-3.14	Floodplain forest below Belterra	Pará	Brazil					
118	CLO ML 88413	*H. p. uniformis*	-55.93	-9.60	Ecological Reserve Cristalino, Cristalino river	Mato Grosso	Brazil					
119	CLO ML 89117	*H. p. uniformis*	-55.93	-9.60	Ecological Reserve Cristalino, Trilha da Serra	Mato Grosso	Brazil					
120	MPEG 47700	*H. p. uniformis*	-55.00	-3.10	Santarém/Rurópolis, estrada Santarém/Cuiabá km 84	Pará	Brazil					
121	XC121134	*H. p. uniformis*	-56.69	-4.93	Itaituba	Pará	Brazil					
122	AMNH BIRDS Skin-128602	*H. p. uniformis*	-56.45	-4.83	Jamanxim, Tamauchim river	Pará	Brazil					
123	AMNH BIRDS Skin-286932	*H. p. uniformis*	-55.41	-3.78	Caxiricatuba, Tapajós river	Pará	Brazil					
124	XC90740	*H. p. uniformis*	-54.80	-3.06	Bacia 269, Santarem	Pará	Brazil					
125	CLO ML 115129	*H. p. uniformis*	-54.95	-3.36	National forest of Tapajós; Sucupira base, BR-163, Santarém highway - Cuiabá, km. 117	Pará	Brazil					
126	MPEG 34623	*H. p. uniformis*	-55.90	-4.30	Tapacurazinho river, Miritituba/Rurópolis, Transamazônica km 25	Pará	Brazil					
127	MPEG 36469	*H. p. uniformis*	-55.90	-4.30	Tapajós river, right bank, National forest of Tapajós, Santarém/Cuiabá km 67	Pará	Brazil					
128	MPEG 40543	*H. p. uniformis*	-55.90	-4.30	Tapacurazinho river, Miritituba/Rurópolis, Transamazônica	Pará	Brazil					

					km 25							
129	MPEG 47701	*H. p. uniformis*	-55.90	-4.30	Tapacurazinho river, Miritituba/Rurópolis, Transamazônica km 25	Pará	Brazil					
130	MPEG A00807	*H. p. uniformis*	-57.05	-5.52	Itaituba-Jacareacanga km 212, Flexal	Pará	Brazil					
131	MPEG A00809	*H. p. uniformis*	-57.05	-5.52	Itaituba-Jacareacanga km 212, Flexal	Pará	Brazil					
132	MCZ Orn 174236	*H. p. uniformis*	-55.41	-3.78	Caxiricatuba	Pará	Brazil					
133	MPEG 34119	*H. p. uniformis*	-56.60	-4.62	National park of Tapajós, Itaituba	Pará	Brazil					
134	MPEG 39637	*H. p. uniformis*	-61.93	-10.85	Nazaré rainfall, west bank Rio Ji-paraná	Rondonia	Brazil					
135	MPEG 39638	*H. p. uniformis*	-61.93	-10.85	Nazaré rainfall, west bank Rio Ji-paraná	Rondonia	Brazil					
136	MPEG 39639	*H. p. uniformis*	-61.93	-10.85	Nazaré rainfall, west bank Rio Ji-paraná	Rondonia	Brazil					
137	MPEG 51001	*H. p. uniformis*	-56.60	-4.62	Itaituba, Nacional park of Tapajós	Pará	Brazil					
138	MPEG 53856	*H. p. uniformis*	-56.60	-4.62	National forest of Tapajós, Estrada Santarém/Cuiabá km 117	Pará	Brazil					
139	MPEG 53857	*H. p. uniformis*	-56.60	-4.62	National forest of Tapajós, Estrada Santarém/Cuiabá km 117	Pará	Brazil					
140	MPEG 56624	*H. p. uniformis*	-56.18	-2.60	Juruti, Igarapé Mutum	Pará	Brazil					
141	MPEG 56623	*H. p. uniformis*	-56.18	-2.60	Juruti, Igarapé Mutum	Pará	Brazil					
142	AMNH BIRDS Skin-524608	*H. p. uniformis*	-62.13	-6.94	Calama, Madeira river	Rondonia	Brazil					
143	CM BIRDS P75528	*H. p. uniformis*	-56.23	-4.42	Vila Braga, Tapajos river, left bank	Pará	Brazil					
144	USNM BIRDS 304083.4318	*H. p. uniformis*	-56.23	-4.42	Villa Braga, Tapajos River	Pará	Brazil					

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145	CM BIRDS P75129	*H. p. uniformis*	-56.31	-4.43	Vila Braga, Tapajos river, left bank	Pará	Brazil					
146	CM BIRDS P77648	*H. p. uniformis*	-55.46	-3.51	Apacy , Tapajos river, left bank	Pará	Brazil					
147	FMNH BIRDS 343852	*H. p. uniformis*	-60.92	-12.59	Pedra Branca, Anari river	Rondonia	Brazil					
148	XC91221	*H. p. uniformis*	-65.20	-10.97	Guajará-Mirim, RO. c.20 km SE of Pakaas Palafitas Lodge	Rondonia	Brazil					
149	XC38685	*H. p. uniformis*	-59.59	-4.39	Borba	Amazonas	Brazil					
150	XC5089	*H. p. uniformis*	-59.51	-4.62	TI Coatá-Laranjal, south of Borba, AM	Amazonas	Brazil					
151	XC171006	*H. p. uniformis*	-59.66	-13.82	Comodoro	Mato Grosso	Brazil					
152	XC167307	*H. p. uniformis*	-62.08	-8.92	State Forest Rio Preto-Jacundá, Machadinho d'Oeste	Rondonia	Brazil					
153	XC91218	*H. p. uniformis*	-59.59	-4.39	Borba	Amazonas	Brazil					
154	XC63508	*H. p. uniformis*	-60.77	-14.64	Noel Kempff Mercado National Park, Huanchaca Dos	Santa Cruz deparment	Bolivia					
155	XC63507	*H. p. uniformis*	-61.03	-14.59	Noel Kempff Mercado National Park, El Encanto	Santa Cruz deparment	Bolivia					
156	MPEG 36560	*H. p. uniformis*	-62.07	-10.72	Paraíso river, Ouro Preto do Oeste, line 62 km 16	Rondônia	Brazil					
157	MPEG 36561	*H. p. uniformis*	-61.93	-10.87	Ji-Paraná, Sítio Novo Tupassi, line 94 km 7	Rondônia	Brazil					
158	FMNH BIRDS 330372	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
159	FMNH BIRDS 330369	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
160	FMNH BIRDS	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					

	330370											
161	FMNH BIRDS 330371	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
162	FMNH BIRDS 330373	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
163	FMNH BIRDS 330374	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
164	FMNH BIRDS 330375	*H. p. uniformis*	-61.93	-10.85	Nazare waterfall, W bank Ji parana river	Rondonia	Brazil					
165	MPEG 33610	*H. p. uniformis*	-54.83	-10.17	Rio Peixoto de Azevedo, São José farmhouse	Mato Grosso	Brazil					
166	DEDp030	*H. p. uniformis*	-55.67	-11.60	Missioneira farmhouse, Sinop municipality, right bank Teles Pires river	Mato Grosso	Brazil					
167	XC115396	*H. p. uniformis*	-55.93	-9.59	Cristalino Jungle Lodge	Mato Grosso	Brazil					
168	XC108062	*H. p. uniformis*	-55.93	-9.59	Cristalino Jungle Lodge	Mato Grosso	Brazil					
169	XC39441	*H. p. uniformis*	-55.93	-9.59	Cristalino Jungle Lodge	Mato Grosso	Brazil					
170	XC9420	*H. p. uniformis*	-56.75	-10.75	Serra dos Caiabis, Alta Floresta - MT	Mato Grosso	Brazil					
171	XC14777	*H. p. uniformis*	-55.99	-9.25	Rio Azul hostel	Pará	Brazil					
172	XC119574	*H. p. uniformis*	-56.33	-4.58	Itaituba	Pará	Brazil					
173	XC119451	*H. p. uniformis*	-56.41	-4.66	Itaituba	Pará	Brazil					
174	AMNH BIRDS Skin-288683	*H. p. uniformis*	-55.32	-2.91	Igarapé Amorin, Tapajos river	Pará	Brazil					

175	AMNH BIRDS Skin-288684	*H. p. uniformis*	-55.32	-2.91	Igarapé Amorin, Tapajos river	Pará	Brazil					
176	AMNH BIRDS Skin-288685	*H. p. uniformis*	-55.32	-2.91	Igarapé Amorin, Tapajos river	Pará	Brazil					
177	MPEG 20438	*H. p. perrotii*	-51.38	1.67	Macapá, Amapari river	Amapá	Brazil					
178	MPEG 34539	*H. p. perrotii*	-56.75	-1.50	Cruz Alta, Trombetas river	Pará	Brazil					
179	MPEG 34540	*H. p. perrotii*	-56.75	-1.50	Cruz Alta, Trombetas river	Pará	Brazil					
180	MPEG 55971	*H. p. perrotii*	-54.61	-1.97	Alenquer, Capintuba farmhouse	Pará	Brazil					
181	ANSP ORN 186690	*H. p. perrotii*	-58.65	5.32	5 km NW Mabura Hill; Between Essequibo and Demerara R.	Upper Demerara Berbice	Guyana					
182	CLO ML 195607	*H. p. perrotii*	-59.84	5.30	Ayanganna airstrip	Potaro-Siparuni	Guyana					
183	CLO ML 39350	*H. p. perrotii*	-60.08	-2.32	Manaus	Amazonas	Brazil					
184	CLO ML 39418	*H. p. perrotii*	-59.75	-2.42	Manaus	Amazonas	Brazil					
185	CLO ML 74331	*H. p. perrotii*	-60.00	-2.50	Manaus	Amazonas	Brazil					
186	CLO ML 74412	*H. p. perrotii*	-60.00	-2.50	Manaus	Amazonas	Brazil					
187	LACM BIRDS 59712	*H. p. perrotii*	-52.05	0.98	Serra do Navio	Amapá	Brazil					
188	UMMZ BIRDS 134562	*H. p. perrotii*	-55.52	-1.91	Obidos	Pará	Brazil					
189	YPM ORN 029285	*H. p. perrotii*	-55.52	-1.92	Obidos	Pará	Brazil					
190	ANSP ORN 186688	*H. p. perrotii*	-58.65	5.32	5 km NW Mabura Hill; Between Essequibo and Demerara R.	Upper Demerara	Guyana					

						Berbice						
191	ASNP ORN 186689	*H. p. perrotii*	-58.65	5.32	5 km NW Mabura Hill; Between Essequibo and Demerara R.	Upper Demerara Berbice	Guyana					
192	ASNP ORN 187813	*H. p. perrotii*	-58.77	4.55	Iwokrama Reserve; Camp 24	Potaro-Siparuni	Guyana					
193	CLO ML 74341	*H. p. perrotii*	-60.00	-2.50	Manaus	Amazonas	Brazil					
194	KU BIRDS 86506	*H. p. perrotii*	-60.77	5.47	Waruma River, E bank, ca 15 river km S Kako River	Esequibo	Guyana					
195	KU BIRS 89743	*H. p. perrotii*	-58.93	1.38	North Side Acari Mountains	Upper Takutu-Upper Esequibo	Guyana					
196	USNM BIRDS 621755.4357686	*H. p. perrotii*	-58.93	1.38	North Side Acari Mountains	Upper Takutu-Upper Esequibo	Guyana					
197	ROM BIRDS 147668	*H. p. perrotii*	-58.94	4.87	Potaro-Siparuni	Potaro-Siparuni	Guyana					
198	AMNH BIRDS Skin-125792	*H. p. perrotii*	-59.13	5.41	Potaro Landing	Potaro-Siparuni	Guyana					
199	AMNH BIRDS Skin-125793	*H. p. perrotii*	-59.13	5.41	Potaro Landing	Potaro-Siparuni	Guyana					
200	AMNH BIRDS Skin-125794	*H. p. perrotii*	-59.13	5.41	Potaro Landing	Potaro-Siparuni	Guyana					
201	AMNH BIRDS Skin-461853	*H. p. perrotii*	-55.23	5.42	Zanderij	Para Distric	Suriname					
202	CM BIRDS P68434	*H. p. perrotii*	-51.42	3.72	Cajary island, Uaca river	Amapá	Brazil					
203	CM BIRDS P82764	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					

204	CM BIRDS P83039	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
205	CM BIRDS P83100	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
206	CM BIRDS P83154	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
207	CM BIRDS P83221	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
208	CM BIRDS P83522	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
209	CM BIRDS P83811	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
210	CM BIRDS P83936	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
211	USNM BIRDS 304084.4318265	*H. p. perrotii*	-55.49	-1.41	Obidos	Pará	Brazil					
212	ANSP ORN 162599	*H. p. perrotii*	-55.49	-1.41	Obidos	Pará	Brazil					
213	CM BIRDS P68389	*H. p. perrotii*	-51.42	3.72	Cajary island, Uaca river	Amapá	Brazil					
214	CM BIRDS P82630	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
215	CM BIRDS P82701	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
216	CM BIRDS P82969	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
217	CM BIRDS P83155	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
218	CM BIRDS P83432	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
219	CM BIRDS P83550	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					
220	CM BIRDS P83870	*H. p. perrotii*	-55.49	-1.41	Obidos, Amazonas river, left bank	Pará	Brazil					

221	FMNH BIRDS 106112	*H. p. perrotii*	-58.52	5.98	Rockstone	E Demerara-W Coast Berbice	Guyana					
222	FMNH BIRDS 108305	*H. p. perrotii*	-58.55	5.98	Rockstone, Essequibo River	E Demerara-W Coast Berbice	Guyana					
223	MLZ BIRD 65974	*H. p. perrotii*	-55.49	-1.41	Obidos	Parana	Brazil					
224	UF AUDIO 15839	*H. p. perrotii*	-59.92	-2.98	Reserva Forestal Adolfo Ducke, Manaus	Amazonas	Brazil					
225	FMNH BIRDS 32354	*H. p. perrotii*	-58.39	5.78	Demerara River	Upper Demerara Berbice	Guyana					
226	MCZ orn 141096	*H. p. perrotii*	-55.49	-1.41	Obidos	Pará	Brazil					
227	MCZ Orn 77104	*H. p. perrotii*	-52.31	4.92	Cayenne	Cayenne	French Guiana					
228	CM BIRDS P33919	*H. p. perrotii*	-61.66	6.73	Yuruan river	Bolivar	Venezuela					
229	CM BIRDS P68190	*H. p. perrotii*	-51.91	3.79	Pied Saut, fleuve Oyapock	Cayenne	French Guiana					
230	CM BIRDS P65266	*H. p. perrotii*	-51.91	3.79	Pied Saut, fleuve Oyapock	Cayenne	French Guiana					
231	FMNH BIRDS 260354	*H. p. perrotii*	-56.48	3.09	Kaiserberg Airstrip, Zuid River	Nickerie	Surinam					
232	FMNH BIRDS 260355	*H. p. perrotii*	-56.48	3.09	Kaiserberg Airstrip, Zuid River	Nickerie	Surinam					
233	UF AUDIO 25830	*H. p. perrotii*	-61.48	6.15	Calf Bird Trail, Km. 88, unknown5 Km. W of San Isidro	Bolivar	Venezuela					
234	UF AUDIO 25831	*H. p. perrotii*	-61.48	6.15	Calf Bird Trail, Km. 88, unknown5 Km. W of San Isidro	Bolivar	Venezuela					
235	MPEG 16492	*H. p. perrotii*	-52.50	0.50	Igarapé Novo, Igarapé Amazonas, left bank, Iratapuru river, left bank	Amapá	Brazil					
236	MPEG 20437	*H. p. perrotii*	-51.47	-0.20	Maracá river, left bank, Mazagão, Prosperidade	Amapá	Brazil					

237	MPEG 20439	*H. p. perrotii*	-51.45	-0.20	Maracá river, left bank, Mazagão, Prosperidade	Amapá	Brazil					
238	MPEG 30121	*H. p. perrotii*	-60.03	-3.13	Manaus, Reserva Ducke	Amazonas	Brazil					
239	MPEG 43663	*H. p. perrotii*	-59.63	-1.48	Uatumã river, right bank, 5 km SW foz do Rio Pitinga	Amazonas	Brazil					
240	MPEG 43895	*H. p. perrotii*	-60.28	-1.90	Manaus, BR 174 km 137	Amazonas	Brazil					
241	XC293857	*H. p. perrotii*	-59.94	-3.01	Musa, Manaus	Amazonas	Brazil					
242	XC44062	*H. p. perrotii*	-53.82	5.65	Mana, track ONF Dardanelles	Saint-Laurent-du-Maroni	French Guiana					
243	XC21167	*H. p. perrotii*	-56.01	-1.67	Saracá-Taquera National Forest, Porto Trombetas	Pará	Brazil					
244	XC9162	*H. p. perrotii*	-61.41	6.19	La Barquilla de Fresa Lodge, Las Claritas	Bolivar	Venezuela					
245	XC119141	*H. p. perrotii*	-59.98	-2.60	Manaus	Amazonas	Brazil					
246	XC5086	*H. p. perrotii*	-60.02	-2.23	Km85 road off BR-174 north of Manaus, AM	Amazonas	Brazil					
247	XC126454	*H. p. perrotii*	-52.65	-1.10	Almeirim	Pará	Brazil					

Table S2. List of primers and general PCR conditions used for each gene amplified and sequenced.

	Primers	**Sequence (5' – 3')**	**Size (bp)**	**Tm (°C)**	**References**
ND2	ND2-L – 5216 ND2-H – 6313	GGC CCA TAC CCC GRA AAT G ACT CTT RTT TAA GGC TTT GAA GGC	1045	55	Sorenson *et al.*, 1999
Cytb	L 14841 H16065	GCT TCC ATC CAA CAT CTC AGC ATG ATG AAC TGC AGT CAT CTC CGG TTT ACA AGA C	1016	56	Sorenson *et al.* 1999 Brumfield *et al.*, 2007
BF5	FIB5L FIB5H	CGC CAT ACA GAG TAT ACT GTG ACA T GCC ATC CTG GCG ATC TGA A	563	54	Brumfield *et al.*, 2007
G3PDH	13b 14b	TCC ACC TTT GAT GCG GGT GCT GGCAT AAG TCC ACA ACA CGG TTG CTG TA	438	61	Fjeldsa *et al.*, 2003
MUSK	13 F 13 R	CTT CCA TGC ACT ACA ATG GGA AA CTC TGA ACA TTG TGA TCC TCA A	600	49	Kimball *et al.*, 2009

Table S3. Best partitions and models of nucleotide substitution for mitochondrial and nuclear genes based on the Bayesian information criterion (BIC) and analysis in PartitionFinder.

Gene	**Best partitions**	**Best models for MrBayes**	**Best models for Beast**
ND2	ND2_codon2	HKY+I	HKY+I
	ND2_codon3	HKY	TrN
	ND2_codon1	HKY+G	HKY+G
Cytb	Cytb_codon3	HKY	TrN
	Cytb_codon1	K80+I	K80+I
	Cytb_codon2	HKY+I	HKY+I
BF5	---	F81+I	HKY+I
G3PDH	---	K80	K80
MUSK	---	F81	HKY

Table S4. Summary statistics of the genes sequenced for all \ *Hylexetastes* taxa: N = number of sequences, S = number of variable sites, H = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity, D = Tajima's D, R_2 = Ramos-Onsins and Rozas neutrality test, * $p < 0.05$. Due to the low number of samples obtained for *H. stresemanni insignis* (n=2) and *H. s. stresemanni* (n=1), it was not possible to perform some analyses.

Gene	Taxon	N	S	h	Hd	π	D	R_2
Cytb	***H. s. undulatus***	9	14	7	0.917	0.050	0.221	0.152
	H. s. insignis	2	0	1	0	0	---	---
	H. s. stresemanni	1	---	---	---	---	---	---
	H. p. perrotii	15	9	7	0.724	0.001	-1.966*	0.099
	H. p. brigidai	6	4	3	0.733	0.001	-0.676	0.287
	H. p. uniformis 1	6	5	4	0.867	0.002	-0.825	0.196
	H. p. uniformis 2	19	16	10	0.883	0.004	-0.619	0.1062
	Total	58	130	33	0.964	0.041	1.614	0.156*
ND2	***H. s. undulatus***	9	18	7	0.944	0.008	0.811	0.187*
	H. s. insignis	2	0	1	0	0	---	---
	H. s. stresemanni	1	---	---	---	---	---	---
	H. p. perrotii	15	18	10	0.895	0.003	-1.992*	0.175
	H. p. brigidai	6	5	5	0.933	0.002	-0.144	0.190
	H. p. uniformis 1	6	4	5	0.933	0.001	-1.295	0.118*
	H. p. uniformis 2	19	13	8	0.807	0.005	1.736	0.936
	Total	58	120	37	0.970	0.033	1.225	0.889
BF5	***H. s. undulatus***	18	0	1	0	0	---	---
	H. s. insignis	4	1	2	0.667	0.001	1.632	0.333
	H. s. stresemanni	2	1	2	1.000	0.002	---	0.500
	H. p. perrotii	24	7	7	0.783	0.0043	0.263	0.145
	H. p. brigidai	12	1	2	0.167	0.0003	-1.140	0.276*
	H. p. uniformis 1	12	8	4	0.561	0.0052	-0.270	0.148*
	H. p. uniformis 2	32	3	6	0.702	0.0020	0.616	0.157
	Total	104	18	18	0.858	0.007	-0.255	0.914

Gene	**Taxon**	**N**	**S**	**h**	**Hd**	**π**	**D**	**R_2**
G3PDH	***H. s. undulatus***	18	3	4	0.529	0.003	0.738	0.184
	H. s. insignis	4	2	2	0.500	0.002	-0.709	0.433
	H. s. stresemanni	2	0	1	0	0	---	---
	H. p. perrotii	26	0	1	0	0	---	---
	H. p. brigidai	12	2	3	0.318	0.0013	-0.849	0.157
	H. p . uniformis 1	12	5	6	0.848	0.0047	0.024	0.159
	H. p. uniformis 2	34	18	8	0.765	0.0102	-0.657	0.097
	Total	108	25	19	0.843	0.020	1.420	0.140
MUSK	***H. s. undulatus***	14	0	1	0	0	----	----
	H. s. insignis	4	0	1	0	0	----	----
	H. s. stresemanni	2	0	1	0	0	----	----
	H. p. perrotii	22	1	2	0.173	0.0003	-0.641	0.086
	H. p. brigidai	12	0	1	0	0	----	----
	H. p. uniformis 1	12	1	2	0.167	0.0002	-1.140	0.276
	H. p. uniformis 2	26	4	4	0.483	0.0012	-0.962	0.074
	Total	92	11	10	0.748	0.003	-0.642	0.0743

Table S5. Genetic structure (Fst) between *Hylexetates* clades. **a.** values for mitochondrial genes (upper row ND2, lower row Cytb) and **b-c.** Nuclear genes (upper row G3PDH, lower row BF5, and MUSK). P-values are presented in brackets.

a.	***H. s. undulatus***	***H. s. insignis***	***H.s. stresemanni***	***H. p. perrotii***	***H. p. brigidai***	***H. p. uniformis 1***	***H. p. uniformis 2***
H. s. undulatus	------	0.505 (0.019)	0.384 (0.099)	0.894 (0.000)	0.891 (0.000)	0.894 (0.000)	0.898 (0.000)
H. s. insignis	0.713 (0.03)	------	1.000 (0.999)	0.933 (0.007)	0.961 (0.034)	0.975 (0.037)	0.914 (0.005)
H. s. stresemanni	0.658 (0.999)	1.000 (0.999)	------	0.928 (0.999)	0.953 (0.999)	0.970 (0.151)	0.908 (0.048)
H. p. perrotii	0.942 (0.000)	0.975 (0.008)	0.972 (0.999)	------	0.944 (0.000)	0.946 (0.000)	0.924 (0.000)

H. p. brigidai	0.938 (0.000)	0.981 (0.038)	0.976 (0.999)	0.980 (0.000)	-----	0.653 (0.002)	0.751 (0.000)
H. p. uniformis 1	0.936 (0.000)	0.977 (0.038)	0.971 (0.999)	0.980 (0.000)	0.877 (0.002)	-----	0.747 (0.000)
H.p. uniformis 2	0.928 (0.000)	0.943 (0.004)	0.936 (0.999)	0.960 (0.000)	0.789 (0.000)	0.717 (0.000)	-----

b.	***H. s. undulatus***	***H. s. insignis***	***H. s. stresemanni***	***H. p. perrotii***	***H. p. brigidai***	***H. p. uniformis 1***	***H. p. uniformis 2***
H. s. undultus	------	0.462 (0.008)	0.397 (0.112)	0.939 (0.000)	0.805 (0.000)	0.749 (0.000)	0.444 (0.000)
H. s. insignis	0.970 (0.000)	-----	0.263 (0.999)	0.939 (0.000)	0.728 (0.001)	0.627 (0.001)	0.232 (0.053)
H. s. stresemanni	0.983 (0.005)	0.225 (0.276)	-----	0.941 (0.003)	0.747 (0.249)	0.632 (0.011)	0.160 (0.248)
H. p. perrotii	0.710 (0.000)	0.529 (0.000)	0.479 (0.008)	------	0.945 (0.000)	0.936 (0.000)	0.781 (0.000)
H. p. brigidai	0.989 (0.000)	0.950 (0.000)	0.956 (0.009)	0.526 (0.000)	-----	0.129 (0.008)	0.118 (0.020)
H. p. uniformis 1	0.858 (0.000)	0.692 (0.000)	0.649 (0.023)	0.437 (0.000)	0.234 (0.005)	-----	0.027 (0.172)
H. p. uniformis 2	0.910 (0.000)	0.853 (0.000)	0.846 (0.002)	0.579 (0.000)	0.141 (0.023)	0.122 (0.012)	-----

c.	***H. s. undulatus***	***H. s. insignis***	***H. s. stresemanni***	***H. p. perrotii***	***H. p. brigidai***	***H. p. uniformis 1***	***H. p. uniformis 2***
H. s. undultus	------						
H. s. insignis	1.000 (0.000)	-----					
H. s. stresemanni	1.000 (0.007)	1.000 (0.067)	-----				
H. p. perrotii	0.903 (0.000)	0.929 (0.000)	0.922 (0.004)	------			
H. p. brigidai	1.000 (0.000)	1.000 (0.001)	1.000 (0.016)	0.970 (0.000)	-----		
H. p. uniformis 1	0.963 (0.000)	0.957 (0.000)	0.950 (0.011)	0.946 (0.000)	0.915 (0.000)	-----	
H.p. uniformis 2	0.271 (0.001)	0.208 (0.052)	0.100 (0.042)	0.365 (0.000)	0.163 (0.031)	0.109 (0.056)	-----

Table S6. Average and standard deviation of training and test AUC of each Maxent model for *Hylexetastes*.

AUC				**Partial AUC (0.05)**			**Partial AUC (0.15)**		
Best model Training	**Best model Test**	**Mean Training**	**Mean Test**	**Mean (SD)**	**Max**	**Min**	**Mean (SD)**	**Max**	**Min**
0.904	0.824	0.905 (± 0.006)	0.817 (± 0.04)	1.684 (± 0.03)	1.78	1.59	1.684 (± 0.03)	1.77	1.57

Table S7. Contribution of each environmental variable to the distribution models for the *Hylexetastes* complex. **A** percent contribution and **B** permutation importance of each variable to the model.

Variable	***Hylexetastes***	
	A	**B**
Isothermality	19	9.3
Annual Mean Temperature	18.2	9.5
Precipitation during Coldest Quarter	15.9	18.8
Precipitation during Warmest Quarter	13.5	17.7
Annual Precipitation	11.5	16.8
Temperature Seasonality	7.9	12
Precipitation during Driest Month	7.1	8.9
Mean Diurnal Range	6.9	7.1

CONCLUSÕES GERAIS

Demonstamos a importância da integração de vários métodos para fornecer uma identificação precisa de ESUs e espécies (De Queiroz 2007, Carstens et al., 2013) para o gênero *Hylexeastes*. A delimitação correta dessas unidades reflete mais precisamente os padrões de diversidade biótica e fornece dados críticos para um planejamento de conservação informado. Nossos dados genéticos e ENMs (distribuições geográficas parapáricas potenciais e variáveis climáticas distintas que influenciam essas distribuições) coincidem com descrições morfológicas previamente publicadas para apoiar o reconhecimento de quatro espécies dentro de *Hylexetastes* (Da Silva et al., 1995, Piacentini et al., 2015). A evidência aqui apresentada apoia um novo arranjo taxonômico para este gênero, elevando os taxa *H. p. perrotii*, *H. p. uniformis e H. p. brigidai* para o estado completo das espécies. Portanto, são necessárias novas avaliações do estado de conservação para cada espécie reconhecida, particularmente para *H. p. brigidai e H. p. uniformis*, cujas distribuições se sobrepõem com áreas desmatadas no leste da Amazônia.

ANEXOS

Anexo 1 - Normas da revista Birds Conservation Internatioanl, na qual foi publicado/encaminhado para publicação o capítulo I dessa Tese/Dissertação

CAMBRIDGE | Instructions for Contributors

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Papers should be concise and factual, taking proper account of previous relevant literature; opinions expressed should be based on adequate evidence. Whilst there is no formal restriction on length, authors planning to submit a paper which is likely to exceed 15 printed pages should discuss the work with the Editor at an early stage in its preparation. BCI now supports on-line supplementary materials on the journal webpages. Wherever possible lengthy appendices and graphics, and other supplementary materials such as detailed methods, calculations, site-by-site bird lists, sound files and photographs should make use of this facility.

Titles of papers must be accurate and concise, and (for the benefit of abstraction services) include any relevant scientific (taxonomic) name; a running head is needed (the editors will provide this if authors do not). A full-length paper must include a proper summary. We welcome word-for-word translations of summaries where appropriate.

Papers should be double-spaced throughout, with positions of figures and tables indicated in the margin. We prefer Microsoft Word or a compatible format. If in doubt please supply your paper in rich text format. and give details of the word processing software used.

Guidelines for submitting figures can be found by accessing the Cambridge Journals Artwork Guide here.

Conventions

Whenever possible, authors should consult an issue of *BCI* for style and layout. Spelling generally follows *The shorter Oxford English dictionary*, supplemented by various standard references such as 'Topography' in *A dictionary of birds* (1985) and the most recent edition of *The Times atlas of the world.* Localities with well-known other spellings or older names should have these placed in parentheses after first mention, while localities too small to be in the *Times atlas* should be given their precise geographical co-ordinates (preferably with some evidence of source).

Authors are encouraged to follow BirdLife International's taxonomy (the latest Checklist can be downloaded from http://www.birdlife.org/datazone/species/taxonomy.html) and to provide explanations of any deviation, if they choose not to. On first mention of a bird both English and scientific name should be given, thereafter only one, preferably the English. Scientific trinomials need be used only if sub specific nomenclature is relevant to the topic under discussion. These recommendations also apply for any other animal or plant species mentioned.

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