Universidade de Brasília

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Programa de Pós-graduação em Ecologia

FILOGEOGRAFIA DE *MICRABLEPHARUS ATTICOLUS* (SQUAMATA, GYMNOPHTHALMIDAE) NO CERRADO BRASILEIRO

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Marcella Gonçalves Santos

Dissertação apresentada ao Curso de Pós-Graduação em Ecologia
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Orientador: Prof. Dr. Guarino Rinaldi Colli

Co-orientadora: Prof^{a}. Dr^{a}. Lilian Gimenes Giugliano

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SUMÁRIO

RESUMO

A filogeografia é uma disciplina que vem se tornando cada vez mais relevante para a compreensão da história evolutiva no nível intraespecífico, trazendo contribuições valiosas para o conhecimento sobre a biogeografia de uma região. No Cerrado, esta abordagem pode ajudar a resolver questões acerca dos padrões e processos envolvidos na diversificação de sua biota. Neste trabalho, foi realizada uma análise filogeográfica de *Micrablepharus atticolus*, um lagarto endêmico do Cerrado. Foram utilizados dados de distribuição e sequências do citocromo *b* para investigar uma possível estruturação populacional dentro da espécie e confrontá-la com hipóteses relacionadas à diferenciação entre platôs/depressões, áreas de estabilidade/instabilidade histórica derivadas de modelos de distribuição potencial no Pleistoceno e Holoceno e regiões de centro/periferia da distribuição. Os dados de distribuição geográfica confirmaram a forte associação de *M. atticolus* com o Cerrado e foi encontrada alta variação no marcador utilizado, que está geograficamente estruturada e associada à distância entre as localidades amostradas. Foram identificados quatro agrupamentos de populações, separados por barreiras genéticas situadas principalmente na região entre os estados de Goiás e Mato Grosso. Foi estimado que a diversificação entre as linhagens de *M. atticolus* se iniciou há aproximadamente 3 milhões de anos, coincidindo com o soerguimento final do Planalto Central. Os modelos paleoclimáticos indicam grandes mudanças na distribuição da espécie nos últimos 130 mil anos, porém, ao contrário do esperado, as áreas de estabilidade não estão associadas à alta diversidade genética. As predições das hipóteses investigadas não foram suportadas pela topologia da filogenia a pelos índices de diversidade nas localidades bem amostradas, sugerindo uma história biogeográfica complexa na paisagem do Cerrado.

INTRODUÇÃO GERAL

A filogeografia é uma disciplina recente, voltada para o estudo dos padrões e processos relacionados à distribuição de linhagens genealógicas no tempo e no espaço, em especial dentro de espécies ou entre espécies proximamente relacionadas (Avise, 2000). A disciplina se originou a partir do emprego de ferramentas da sistemática filogenética para resolver questões da genética de populações (Avise *et al.*, 1987). No entanto, ela diferencia-se das duas por tratar de questões intraespecíficas (diferentemente da sistemática, preocupada com relações interespecíficas) e de processos genealógicos (diferentemente da genética de populações, preocupada com frequências alélicas). Além da abordagem histórica, a filogeografia apresenta uma abordagem espacialmente explícita, classificando-se como uma sub-área da biogeografia (Avise, 2009).

Boa parte do conhecimento na filogeografia baseia-se na teoria coalescente, derivada da genética de populações. Esta constitui um conjunto de modelos matemáticos que descrevem as genealogias de genes, buscando o ancestral comum mais recente de linhagens presentes ligando as mesmas numa árvore retrospectiva (Wakeley, 2009). Muitas das análises filogeográficas são fundamentadas nessa teoria e têm apresentado grandes avanços, com testes de hipóteses biogeográficas explícitas e métodos estatísticos para inferência de parâmetros demográficos e da confiança nos modelos (Knowles & Maddison, 2002). Ainda, observa-se a tendência em utilizar informações ambientais como fontes independentes de evidências e hipóteses para os testes, como dados paleoecológicos e bioclimáticos (Knowles, 2004; Carstens & Richards, 2007; Richards *et al.*, 2007). Todos esses progressos são viáveis devido ao desenvolvimento de ferramentas computacionais e de métodos de coleta de dados que abrangem crescente número de táxons e marcadores moleculares.

Devido ao seu caráter multidisciplinar as análises filogeográficas têm grande impacto em diversas áreas, sendo ferramentas importantes para o teste de hipóteses biogeográficas (Cabanne *et al.*, 2007; Quijada-Mascareñas *et al.*, 2007) e para a resolução de problemas taxonômicos, identificando linhagens evolutivas distintas (Pellegrino *et al.*, 2005; Martins *et al.*, 2007; Torres & Ribeiro, 2009) e zonas de hibridação (Hewitt, 2001; Zarza *et al.*, 2008). A relevância desses estudos se estende ainda para a biologia da conservação, já que um dos componentes biológicos mais importantes para a preservação de uma espécie é a sua estrutura genética e os processos evolutivos que a sustentam (Moritz, 2002; Telles *et al.*, 2007; Lara-Ruiz *et al.*, 2008).

Apesar do enorme potencial dos estudos em filogeografia, a disciplina ainda é incipiente no Brasil, com viés para estudos na Mata Atlântica (Puorto *et al.*, 2001; Pellegrino *et al.*, 2005; Cabanne *et al.*, 2008; Carnaval *et al.*, 2009; Thomé *et al.*, 2010). Os demais biomas brasileiros foram poucos explorados, dentre eles o Cerrado (para exemplos, vide Collevatti *et al.*, 2003; Ramos *et al.*, 2007; Collevatti *et al.*, 2009; Diniz-Filho *et al.*, 2009; Moraes *et al.*, 2009), apesar da alta riqueza e grande grau de ameaça que o classifica como *hotspot* de biodiversidade (Myers *et al.*, 2000). Até recentemente, os padrões biogeográficos na região eram mal conhecidos, com poucas hipóteses sugerindo mecanismos geradores da diversidade. Este quadro vem se alterando, com a publicação de trabalhos dando enfoque a esta temática. Em relação à herpetofauna, Colli (2005) destacou a importância de eventos no Terciário (surgimento da dicotomia entre formações florestais e abertas na América do Sul, intensificação de um gradiente latitudinal de temperatura no continente a partir do soerguimento dos Andes e eventos de vicariância causados por transgressões marinhas e pelo soerguimento do Planalto Central) para a especiação (corroborado por Garda & Cannatella, 2007; Gamble *et al.*, 2008; Werneck *et al.*, 2009). Por outro lado, a filogeografia de espécies neotropicais tem mostrado que flutuações climáticas no Quaternário podem ter sido importantes para a estruturação genética das populações devido às supostas alterações na distribuição das espécies que seguiram essas mudanças ambientais (Carnaval *et al.*, 2009; Diniz-Filho *et al.*, 2009). Em recente revisão sobre a biogeografia dos biomas abertos ao leste da América do Sul, Werneck (2011) aponta para a existência de padrões complexos na biota do Cerrado, destacando a importância da realização de estudos moleculares para elucidar os processos de diversificação na região. Dentre os fatores a serem investigados, a autora destaca a diferenciação entre platôs e vales e a dinâmica de mudanças na vegetação causadas pelas flutuações climáticas durante o Quaternário. Assim, tendo em vista o debate sobre quais processos moldaram a biota do Cerrado e quando eles ocorreram, a filogeografia é uma abordagem com grande potencial para elucidar a história evolutiva da região. Ela é capaz de gerar conhecimentos a respeito de eventos ocorridos a milhares ou até mesmo a alguns milhões de anos atrás, possibilitando o reconhecimento de divergências datadas no Terciário e Quaternário (Beheregaray, 2008).

Devido a seu endemismo e ampla distribuição no Cerrado, alta abundância e boa representatividade em coleções zoológicas, *Micrablepharus atticolus* é um bom modelo de estudo para investigar a história dessa região. *Micrablepharus* Dunn, 1932 pertence à família Gymnophthalmidae, que é composta por aproximadamente 41 gêneros e 180 espécies de lagartos de pequeno porte, conhecidos como microteídeos (Rodrigues *et al.*, 2007b). A família é exclusivamente neotropical, distribuída do sul da América Central até o sul da América do Sul, a leste dos Andes (Zug *et al.*, 2001). A taxonomia de Gymnophthalmidae é pouco compreendida devido ao pequeno número de coletas de indivíduos pertencentes à maioria das espécies. O fato ainda é agravado pela presença de muitas características morfológicas convergentes, em geral relacionadas ao hábito fossorial, como corpo alongado, redução de membros, perda das pálpebras e das aberturas timpânicas e fusão/fissão de escamas na cabeça (Pellegrino *et al.*, 2001; Rodrigues *et al.*, 2007b). Muitos gêneros e espécies têm sido descritos na família e diversos trabalhos foram realizados recentemente procurando elucidar as relações entre seus membros (Pellegrino *et al.*, 2001; Castoe *et al.*, 2004; Doan & Castoe, 2005; Doan *et al.*, 2005; Rodrigues & Ávila-Pires, 2005; Rodrigues *et al.*, 2007a; Rodrigues *et al.*, 2007b; Kizirian *et al.*, 2008; Siedchlag *et al.*, 2010).

Dentre as diversas radiações de Gymnophthalmidae, o gênero *Micrablepharus* pertence a um grupo que não possui pálpebras, reconhecido por Pellegrino e colaboradores (2001) como Gymnophthalmini. Diversos trabalhos corroboram o monofiletismo desse grupo a partir de dados morfológicos e moleculares (Rodrigues, 1995; Benozzati & Rodrigues, 2003; Castoe *et al.*, 2004). Perda das pálpebras e acentuada redução dos membros escapulares em relação aos pélvicos, entre outras adaptações para o hábito fossorial, são as principais tendências observadas neste grupo (Rodrigues, 1995). *Micrablepharus* é diagnosticado pela redução ou ausência do primeiro dedo da mão, presença de pálpebra discreta, oculta por baixo das supraciliares e circundada por pequenos grânulos, presença de um par de escamas frontoparietais e ausência de escamas pré-frontais (Rodrigues, 1996).

Poucos trabalhos tratam especificamente da ecologia, genética, morfologia ou evolução do gênero (Rodrigues, 1996; Teixeira *et al.*, 1999; Yonenaga-Yassuda & Rodrigues, 1999; Vieira *et al.*, 2000), porém ele está representado nas principais análises filogenéticas recentes da família (Pellegrino *et al.*, 2001; Castoe *et al.*, 2004). Muitos estudos sobre comunidades de lagartos ainda discutem brevemente aspectos da ecologia de *Micrablepharus* (Vitt, 1991; Vitt & Caldwell, 1993; Gainsbury & Colli, 2003; Delfim & Freire, 2007; Mesquita *et al.*, 2007). Os lagartos do gênero são terrestres, têm preferência por ambientes abertos, possuem hábito diurno, estando ativos na parte mais quente do dia, e alimentam-se de artrópodes variados (Vitt, 1991; Rodrigues, 1996; Vieira *et al.*, 2000). *Micrablepharus* é composto por duas espécies, sendo que *M. atticolus* é diferenciado de *M. maximiliani* pela presença de poros femorais em ambos os sexos, 8 em machos e 5-7 em fêmeas, quarta supralabial abaixo do olho e presença de um par de listras brancas laterais (Rodrigues, 1996). Já *M. maximiliani* apresenta de 10 a 15 poros femorais apenas em machos, quinta supralabial abaixo do olho e apenas uma listra branca lateral (Rodrigues, 1996).

Micrablepharus maximiliani é amplamente distribuída pelas vegetações abertas brasileiras, ocorrendo desde a Caatinga, passando pelo Cerrado até o Pantanal e Paraguai (Nogueira, 2006). *Micrablepharus atticolus* é restrita ao Cerrado e ocorre com ampla distribuição no bioma, inclusive em enclaves amazônicos em Rondônia (Gainsbury & Colli, 2003; Nogueira, 2006). Apesar da alta sobreposição na distribuição geográfica na escala continental, localmente as espécies são raramente coletadas no mesmo ponto, sugerindo a existência de algum padrão de segregação em escalas mais refinadas. Nogueira (2006) sugeriu que *M. atticolus* aparentemente está associada a áreas mais elevadas (planaltos em geral acima de 500 m) e *M. maximiliani* está possivelmente associada a áreas mais baixas, nas depressões interplanálticas internas e adjacentes à região do Cerrado, porém sem um teste de hipótese formal. Neste trabalho foi testada a hipótese desta segregação das duas espécies associada à altitude.

Dessa forma, buscando investigar fatores que originaram a diversidade no Cerrado, esta dissertação descreve os padrões de variação geográfica de caracteres genéticos do lagarto *Micrablepharus atticolus* e, a partir destes dados, investiga a presença e possível diversificação histórica das linhagens componentes da espécie. O trabalho está escrito na forma de manuscrito em inglês, a ser submetido à revista Journal of Biogeography.

Materiais e Métodos

Os pontos de ocorrência de *M. atticolus* foram compilados e georreferenciados com base nos registros de literatura e de espécimes e tecidos tombados nas principais coleções herpetológicas do país. As altitudes de ocorrência da espécie foram contrastados com *M.*

maximiliani, para testar a possível segregação das espécies no gradiente altitudinal. Modelos de distribuição potencial de *M. atticolus* nas condições climáticas atuais e no passado (6, 21 e 130 mil anos atrás) foram construídos com o algoritmo Maxent. Para investigar os fatores históricos e ambientais atuais associados à distribuição da espécie foi realizada uma regressão logística.

Um fragmento de 715 pares de base do gene mitocondrial citocromo b foi amplificado e sequenciado para 116 amostras de *M. atticolus*, distribuídas em 30 localidades. Foram obtidos índices de genética de populações para a descrição geral do conjunto de dados. Com o objetivo de elucidar as relações entre os haplótipos, foram obtidas redes de haplótipos, árvore filogenética bayesiana e datação molecular. Ainda, barreiras genéticas foram identificadas pelo algoritmo de Monmonier. Associação entre distâncias genética e geográfica foi avaliada por um teste de Mantel e a hipótese da dos platôs/depressões foi investigado com mapeamento da altitude de ocorrência das amostras na filogenia. Índices de diversidade foram obtidos para cinco localidades bem amostradas, para avaliar as predições das hipóteses dos platôs/ depressões, estabilidade/instabilidade e centro/periferia.

Resultados

Não foi encontrada segregação entre as duas espécies de *Micrablepharus* no gradiente altitudinal. O modelo de distribuição potencial de *M. atticolus* para condições climáticas atuais é coincidente com a distribuição do Cerrado. Os modelos para o passado indicam mudanças relevantes na presença de condições ambientais favoráveis para a ocorrência da espécie nos últimos 130 mil anos. Variáveis selecionadas na regressão logística são associadas à ocorrência do Cerrado.

Foram encontradas alta diversidade e estruturação genética. As redes de haplótipos, árvore filogenética e o algoritmo de Monmonier suportam a existência de quatro grupos geográficos. A divergência de tais grupos é antiga, tendo ocorrido entre 3,5 e 1,5 milhões de anos atrás. Houve associação entre distâncias genéticas e geográficas. O mapeamento dos caracteres de altitude mostrou uma história complexa de ocupação da espécie na paisagem topográfica, com ausência de monofiletismo recíproco em unidades de relevo como seria esperado de acordo com a hipótese dos platôs e depressões. Os resultados dos índices de diversidade para as localidades bem amostradas não estão de acordo com as predições das hipóteses avaliadas, exceto para o caso de um isolado de Cerrado em Rondônia que apresentou baixa diversidade.

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Landscape evolution and the phylogeography of *Micrablepharus atticolus* (Squamata, Gymnophthalmidae), an endemic lizard of the Brazilian Cerrado

Marcella Gonçalves Santos[1], Cristiano Nogueira [2], Lilian Gimenes Giugliano[3], Guarino Rinaldi Colli[2]*

[1] Programa de Pós-Graduação em Ecologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil.

[2] Departamento de Zoologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil.

[3] Departamento de Genética e Morfologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil.

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* Corresponding author: Guarino Rinaldi Colli, Departamento de Zoologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil. Fax number: +55-61-3107-3036. E-mail: grcolli@unb.br

Abstract

The search of an explanation for the origin of high biodiversity in the Neotropics has traditionally focused on forest biomes. Here we investigate the effects of geoclimatic events that putatively shaped the evolution of organisms inhabiting South American open biomes. We conducted a phylogeographic analysis of *Micrablepharus atticolus*, an endemic lizard from the Cerrado of Brazil. We used distributional data and cytochrome *b* sequences to investigate a possible structuring within the species, and confront it with hypotheses related to differentiation on plateaus/depressions, historical stability/instability areas derived from potential distribution models from the Pleistocene and Holocene, and core/periphery regions of the distribution. Geographic distribution data confirm the strong association o *M. atticolus* with Cerrado and we found high variation in cytochrome *b* sequences, which was geographically structured and associated with distance among sampled localities. Four groups of populations were identified, separated by genetic barriers mainly located in the region between Goiás and Mato Grosso states. Diversification of *M. atticolus* lineages was estimated to have started around 3 mya, coinciding with final Centreal Plateau uplift. Paleoclimatic models indicate great shifts on the species distribution during the last 130,000 years, but contrary to our expectation stable areas are not associated with high genetic diversity. Predictions for investigated hypotheses were not supported by phylogeny topology and diversity indexes on well sampled localities, suggesting a complex biogeographical history on the Cerrado landscape.

Introduction

Comprehension of spatial patterns of genetic diversity and mechanisms associated with such patterns is a fundamental biological component of population studies. Integrating these concepts with systematics and biogeography, phylogeography seeks to build genealogical lineages on spatial and temporal scales (Avise *et al.*, 1987). Thus, this discipline has greatly improved knowledge about biogeographic history in many regions (Carstens *et al.*, 2005; Thomé *et al.*, 2010), taxonomy and evolution in diverse groups (Hewitt, 2001; Pellegrino *et al.*, 2005; Martins *et al.*, 2007; Zarza *et al.*, 2008; Torres & Ribeiro, 2009), and conservation of these species and places (Carnaval *et al.*, 2009).

Although being a powerful tool to test biogeographic hypothesis, only few phylogeography studies have been conducted in the Cerrado region (Collevatti *et al.*, 2003; Ramos *et al.*, 2007; Collevatti *et al.*, 2009; Diniz-Filho *et al.*, 2009; Moraes *et al.*, 2009). The Brazilian Cerrado is the largest Neotropical savanna, with high levels of diversity and endemism, especially for some groups like plants and squamates (Oliveira & Marquis, 2002). The region is highly threatened, and is considered one of the world's biodiversity hotspots (Myers *et al.*, 2000). Colli (2005) presented a comprehensive scenario for diversification of Cerrado herpetofauna, pointing out that most extant families already existed at the end of Mesozoic and most lineage diversification took place during Paleogene and Neogene. As South America was isolated during most of the Tertiary, an endemic biota developed in this period, followed by faunal interchange with Central and North America through the Great American Biotic Interchange – GABI in the Pliocene (Webb, 2006; Cody *et al.*, 2010). During the Paleogene, there was a latitudinal differentiation of climates in South America, enhanced by the Andes uplift, accompanied by the formation of a dichotomy between open and forest vegetation (Webb, 1978; Haffer, 1985; Pascual & Jaureguizar, 1990; Clapperton, 1993; Romero, 1993). A marine transgression occurred in the Miocene, causing lineage diversification by vicariance (Räsänen *et al.*, 1995; Hoorn *et al.*, 2010). Additionally, the final uplift of the central Brazilian Plateau and subsidence of depressions that occurred between the Pliocene and Pleistocene also promoted lineage diversification by vicariance (Brasil & Alvarenga, 1989; Del'Arco & Bezerra, 1989). Recent studies on phylogeny and biogeography of Cerrado taxa point to histories well-suited with these predictions (Garda & Cannatella, 2007; Werneck *et al.*, 2009).

After speciation, populations within a species continue differentiating through time, by historical and contemporary processes that shape genetic structure across the species' geographical range. For Cerrado species, these events might have occurred from the Pliocene on. Apparently one of the most important events for the region is the compartmentalization between plateaus (above 500 m) and depressions (below 500 m) (Del'Arco & Bezerra, 1989; Silva, 1997; Werneck, 2011). Thus, a main dynamic of vicariance between high and low compartments must have occurred around 2 – 3 million years ago (mya), herein referred as the plateaus/depressions hypothesis. The plateaus are separated by depressions holding the main rivers of the region, namely Araguaia, Tocantins, São Francisco, Paraguai, and Paraná. This represents subdivisions within plateaus and depressions, which is possibly reflected on the species' genetic structure. Additionally, it is hypothesized that depressions are younger than plateaus, being formed by erosion processes during Quaternary, harboring seasonally dry forests or gallery forests, whereas plateaus are dominated by savannas (Silva, 1997). Silva (1997) investigated this hypothesis with endemic Cerrado's bird species and found out that species derived from more recent lineages (called neoendemics) are associated with depressions, while species of older lineages (called paleoendemics) are associated with plateaus.

An historically important explanation to neotropical diversification is the Pleistocene refuge hypothesis (Haffer, 1969). It points out that during Quaternary climatic cycles there was expansion of open habitats and isolation of Amazon Forest in colder/drier periods, with the opposite occurring in hotter/more humid periods. Supposedly, speciation would have occurred in these isolated areas, and the idea is applicable for other regions besides Amazon. Despite the refuge hypothesis has been questioned as a valid explanation for speciation (Colli, 2005; Hoorn *et al.*, 2010), it recently became evident again, been known as the stability hypothesis (Carnaval *et al.*, 2009). Stable areas (i.e., areas that didn't experience vegetational changes) can now be estimated with paleomodeling, constituting the refugia. Populations within these refugia are expected to have higher genetic diversity in comparison with populations in unstable areas, which should present signature for recent population expansion (e.g., Carnaval *et al.*, 2009). Werneck *et al.* (in review) used paleodistribution modeling to predict suitable areas for the occurrence of Cerrado in the Holocene (6 thousand years before present, kyr BP), Last Glacial Maximum (LGM, 21 kyr BP) and Last Interglacial (LIG, 130 kyr BP), validating their models with fossil pollen records. In the Holocene model, the distribution of suitable conditions for occurrence of Cerrado was similar to the current model, while in LGM there was a contraction of this distribution, and the largest expansion was observed in LIG (Werneck *et al.*). Thus, although the scenario modeled disagrees with the initial idea of expansion of Cerrado during Glacial Maximum (Ledru, 2002), it points to changes in vegetation distribution, highlighting the importance of testing the stability hypothesis. Further work on biogeography of the Cerrado squamate fauna uncovered areas with high endemism, highlighting strong structuring of species distributions and pointing to the importance of vicariance in the evolutionary history of the Cerrado (Nogueira *et al.*, 2011).

Yet, demographic parameters such as effective population size (N_e) and migration (m) affect spatial patterns of genetic diversity (Vucetich & Waite, 2003). A well-known pattern is isolation by distance, in which genetic differentiation is proportional to geographical distance simply due to restricted gene flow between populations far from each other (Wright, 1943). A more complex model is the core/periphery hypothesis, which predicts lower genetic diversity and higher differentiation in populations located at the margins of the species' range, based on the assumption of abundant core leading to low N_e in periphery and isolation causing low gene flow (Eckert *et al.*, 2008). Given that Cerrado covers a large area, it is likely that these major gradients of genetic patterns occur in species with distribution associated with the region.

The gymnophthalmid *Micrablepharus atticolus* is a Cerrado endemic lizard, typical of open areas, broadly distributed and locally abundant (Rodrigues, 1996). These characteristics make the species a good model to investigate patterns resulting from Cerrado's dynamic history. The congeneric *M. maximiliani* has a broader distribution, occurring in Caatinga, Pantanal and Paraguay, besides Cerrado. Evidencing that both species are rarely found in the same locality, Nogueira (2006) hypothesized that *M. atticolus* and *M. maximiliani* are segregated along the altitudinal gradient, the former occurring on plateaus.

Herein we conducted a phylogeographic analysis of *M. atticolus* based on cytochrome *b* and the species distributional records. First, we tested the hypothesis of altitudinal segregation between the two species of *Micrablepharus*. Then, we assessed historical and ecological factors associated with the distribution of *M. atticolus*. Finally, we conducted exploratory phylogenetic and phylogeographic analyses to assess patterns of genetic variation, which were contrasted with altitudinal, geological, and paleodistributional information to identify mechanisms that affected the species diversification.

Materials and Methods

Spatial distribution

We compiled a database of distribution records of *Micrablepharus atticolus* based on literature records, and specimens deposited in major Brazilian herpetological collections (Appendix 1): Coleção de Herpetologia do Museu Paraense Emílio Goeldi (MPEG), Coleção Herpetológica da Universidade de Brasília (CHUNB), Coleção de Tecidos de Vertebrados do Departamento de Zoologia da Universidade de São Paulo, Coleção de Répteis do Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ), Coleção de Répteis do Museu de Zoologia da Universidade de Campinas (ZUEC), Coleção de Répteis do Museu de Zoologia da Universidade de São Paulo (MZUSP), and Coleção Zoológica de Vertebrados da Universidade Federal do Mato Grosso (UFMT-R). Coordinates were obtained directly from collections or collectors. When this information was not available, we georeferenced records based on locality description, and, as the last alternative, in 20 cases we used municipality centroids.

After mapping species occurrences, we tested the postulated topographical segregation between the two *Micrablepharus* species, inferred based on their presence in plateaus (above 500 m) and depressions (below 500 m). In this test, we used occurrence records for *M. maximiliani* in Nogueira (2006). Elevation values were extracted for each locality and tested for equality of variances and normality assumptions. As data violated the normal distribution assumption, we used the non-parametric Wilcoxon test (Quinn & Keough, 2002) to test for differences in elevation between species. Three different tests were performed, with total records of *M. atticolus* versus: (1) total records of *M. maximiliani*; (2) records of *M. maximiliani* outside *M. atticolus* distribution; (3) records of *M. maximiliani* inside *M. atticolus* distribution. This approach was used aiming to distinguish topographical segregation were species distributions overlap from the bias due to differences in extension of geographic distribution. Limits of *M. atticolus* distribution were defined as a polygon described below.

We modeled suitable areas for the potential distribution of *M. atticolus* based on current and past climatic conditions with Maxent v. 3.3 (Phillips & Dudik, 2008). The algorithm is robust to accuracy problems on occurrence records (Elith *et al.*, 2006), been reliable in the use of coordinates derived from municipality centroids. sing the same environmental variables as Werneck *et al.* (in review): altitude, isothermality (BIO3), temperature seasonality (BIO4), temperature annual range (BIO7), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), precipitation of driest month (BIO14), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), and precipitation of driest quarter (BIO17). Other variables were excluded due to high correlation ($r > 0.90$). The current climatic and altitude variables were obtained from WorldClim (www.worldclim.org) on a 30'' resolution and cropped from latitude 12º 47' N to 34º 46' S and longitude 78º 31' W to 35º W. Palaeoclimatic data were obtained for Holocene (6 kyr BP), Last Glacial Maximum (LGM, 21 kyr BP), and Last Interglacial (LIG, 130 kyr BP) from Palaeoclimatic Modelling Intercomparison Project (PMIP; http://pmip.lsce.ipsl.fr/), and cropped as the current variables. Three localities – Alta Floresta (Mato Grosso), Nova Monte Verde (Mato Grosso), and Ourilândia do Norte (Pará) – were excluded from the analysis due to their long distance from the Cerrado range, characterizing isolated open areas within a forest matrix. These points act like outliers, having strong negative influence over models. Thus, we had a total of 108 spatially unique occurrence points, 25% of which were randomly assigned for testing. Based on the results from current modeling, Cerrado limits, known occurrence points for the species, and personal field experience, we drew a polygon characterizing the contemporary distribution of *M. atticolus*. To define refugial areas for the species, all four models were transformed into presence/absence maps using a threshold that equals sensitivity and specificity. The models were then overlaid, defining areas with predicted occurrence of the species in none, one, two, three, or four periods.

To assess the relative importance of historical and ecological predictors of the species distribution, we performed a logistic regression analysis, following Silvano (2011). The ecological factors consisted on all ten current environmental variables included on Maxent modeling. The historical predictors were obtained from Werneck *et al.* (in review) and consisted on the projections of potential distribution of Cerrado under narrow definition during the Holocene (6 kyr BP), Last Glacial Maximum (LGM, 21 kyr BP), and Last Interglacial (LIG, 130 kyr BP) as continuous variables, plus refugial areas defined by the overlap of binary Cerrado models. In other words, we used each Cerrado model in different time frames (three continuous variables), plus the consensus with zero, one, two, three or four overlapping models (refugia, one discrete variable). We randomly sampled 500 points inside and 500 points outside the distribution of *M. atticolus*, using the same latitude and longitude limits described for environmental variables. The values for each variable were extracted for the created points using ArcGIS extension Multiple Raster-Value Extractor (Pérez, 2007). These presence/absence points were used as the dependent variable on the model. Predictors were selected on a stepwise approach, using the Akaike Information Criterion (AIC) to screen for the most informative variables and chi-squared tests to evaluate the significance of coefficients of the increasingly complex models (Tabachnick & Fidell, 2007). Importance values of predictors selected were obtained by model averaging using package MuMin v. 1.0 for R (Burnham & Anderson, 2002). All GIS based analyses were carried under ArcGIS v. 9.3 (ESRI) and statistical analyses were carried under R v. 2.13.0 (R Development Core Team, 2011).

Molecular data

We used 116 tissue samples of *Micrablepharus atticolus* from 30 localities, deposited at CHUNB, Coleção de Tecidos de Vertebrados do Departamento de Zoologia da

Universidade de São Paulo, MZUSP, and UFMT-R (Appendix 2). Samples consisted on liver, muscle, tail, or toes and total DNA was extracted with DNeasy Blood & Tissue Kit (QIAGEN). A fragment of nearly 800bp of mitochondrial gene cytochrome *b* was amplified by PCR using primers CB1-5 (5' CCATCCAACATCTCAGCATGATGAAA 3') and CB3-3 (5' GGCAAATAGGAARTATCATTC 3') described by Palumbi (1996). 50 µl reactions were prepared with PCR buffer (Invitrogen, 20 mM Tris-HCl pH 8.4 and 50 mM KCl), 1.5 mM $MgCl_2$ (Invitrogen), 1.875 U Taq DNA Polymerase, recombinant (Invitrogen), 0.2 mM of each dNTP (Ludwuig Biotec), and 0.375 µM of each primer. Amplifications were performed on Veriti Thermal Cycler (Applied Biosystems) and started with a 3 min step at 95ºC, followed by 40 cycles of denaturation for 1 min at 94ºC, annealing for 1 min at 50ºC and extension for 1 min at 72ºC, ending with a 5 min step at 72ºC. Quality of extractions and PCR reactions were verified with electrophoresis on 1% agarose gels stained with ethidium bromide, using High DNA Mass Ladder or Low DNA Mass Ladder (Invitrogen). Gels were visualized under UV light and samples were quantified with KODAK Molecular Imaging Software (Carestream). Sequences were obtained by standard sequencing service on Macrogen Inc.

Sequences were edited on gap4 and pregap4 of Staden Package (Staden *et al.*, 2003b, a) installed through eBioX v. 1.5.1 (Lagercrantz, 2008). Multiple alignment was performed with ClustalW (Thompson *et al.*, 1994) through web service of Jalview v. 2.6.1 (Waterhouse *et al.*, 2009). Descriptive population genetics indexes such as number of polymorphic sites (S), number of parsimony informative sites, number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π), and F_{st} were estimated on DnaSP v. 5.10.01 (Librado & Rozas, 2009). For F_{st} estimation, populations represented by a single sample were excluded. DnaSP does not deal appropriately with missing values, excluding sites where any absent data occur, otherwise treating missing values as a fifth state (beside the four nucleotides). As we had 13 incomplete sequences, we decided to run analyses with two distinct datasets to avoid information loss. The first dataset included only complete sequences (DS1, n = 103, 715bp) and the second included all sequences, excluding sites with missing occurrence (DS2, n = 116, 661bp).

Relationships among haplotypes were reconstructed with median-joining algorithm on Network software v. 4.6.0 (www.fluxus-engineering.com, Bandelt *et al.*, 1999). Again, analyses were run for DS1 and DS2 separately. Default epsilon and weighting parameters were used and networks were cleared with MP post-processing to eliminate unnecessary median vectors (Polzin & Daneshmand, 2003). A sample from Sapezal was excluded from the analysis, due to its long distance from other *Micrablepharus atticolus* sequences (see Results).

To assess historical relationships among unique haplotypes, we conducted a Bayesian phylogenetic analysis on MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). All samples and sites were included, since the software handles missing data appropriately. Sequences for the same locus of *Vanzosaura rubricauda* and *M. maximiliani* supplied by Werneck (in prep) were used as outgroups. The best-fit nucleotide substitution model was selected based on the Akaike Information Criterion (AIC) on jModelTest v. 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008). As a transitional model with proportion of invariable sites and gamma-distributed rate variation (TIM2+I+G) was selected, we changed default settings for the structure of model parameters on MrBayes to nst = 6 and rates = invgamma. No priors were fixed, leaving model parameters to be estimated during analysis. Two independent runs were carried for 5×10^6 generations with chain sampling every 100th generation, producing a total of 50,001 trees. All other settings were maintained as default, implicating that each run used one cold and three hot chains on a MC3 approach. For tree construction, we calculated a 50% majority-rule consensus excluding the first 200 generated trees that didn't achieve stationary log-likelihood on the cold chain. Branches with posterior clade probability values equal or greater than 0.95 are considered well supported.

We estimated divergence times among lineages with BEAST v. 1.6.1 (Drummond & Rambaut, 2007). As no gymnophthalmid fossils are available, we used a standard 2% per million years divergence rate for lizard mitochondrial DNA with 1% standard deviation with a relaxed molecular clock (Thorpe *et al.*, 2005; Corl *et al.*, 2010). This is a rough approximation, but has been a useful tool to situate divergence events through time. We used a general time reversible + gamma + invariable sites model and priors estimated by the jModelTest analysis. Markov chain Monte Carlo analysis was ran with 3×10^7 generations sampled every 1,000th generation and repeated three times to avoid sticking on local optima. Tracer v. 1.5 was used to analyze BEAST output and define burn-in for tree construction. We calculated the 50% majority-rule consensus excluding the first 5,000 generated trees.

Aiming to identify genetic barriers, Monmonier's maximum-difference algorithm (Monmonier, 1973) was implemented on Alleles in Space (AIS) v. 1.0 (Miller, 2005). The method defines genetic boundaries delineating barriers where there is maximum genetic differentiation between sample localities (Dupanloup *et al.*, 2002; Manni *et al.*, 2004; Miller, 2005). The analysis was run with raw genetic distances estimated by the AIS software.

To investigate if isolation by distance is a relevant factor in determining genetic structure, we performed a Mantel test, evaluating the association between genetic distance and geographical distance matrices. Geographical distances between populations were extracted with ArcGIS extension Hawth's Tools (Beyer, 2004). Mean genetic distance between sampling points were obtained with MEGA v. 5.05. This distance was calculated with pairwise deletion of missing sites and based on a maximum composite likelihood model with gamma parameter equal to 1.31, according to the model selected on jModelTest. The

Mantel test was run on ape package v. 2.7.2 for R software (Paradis, 2006) with 10,000 permutations.

We traced the altitudinal occurrence of samples on the Bayesian phylogenetic tree, seeking evidence for the effect of distribution on plateaus vs. depressions in shaping genetic and geographical patterns in *Micrablepharus atticolus*. Altitude was traced both as a binary (above and bellow 500 m) and as a continuous character, using maximum likelihood and maximum parsimony methods, respectively, for ancestral state estimation on Mesquite v. 2.74 (Maddison & Maddison, 2010).

Using well-sampled localities (14 to16 samples), we calculated genetic diversity indexes for Vilhena, Pimenta Bueno, Brasília, Rancho Ponte de Pedra, and Pium, to investigate predictions of plateus/depression, central/periphery, and stable/unstable hypotheses. As sites with missing values were excluded, a total of 674bp were used to estimate number of sequences (n), number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (H_d), and nucleotide diversity (π) using DnaSP. If plateaus are more ancient areas, we expect that genetic diversity is higher in these areas compared to depressions with recent colonization. It is expected that isolated populations suffered a decrease in diversity due to reduction of population size and gene flow (migration), leading to increased endogamy. Similarly, if stable areas as modeled for the species distribution really served as refuges during climate cycles, they should harbor higher genetic diversity than unstable areas.

Results

Spatial distribution

Micrablepharus atticolus occurred at 577.6 ± 309.1 m. There is significant difference on altitudinal occurrence between *Micrablepharus* species when considering total distribution of *M. maximiliani* (*M. maximiliani*: 389.0 ± 324.4 m; $W_{165} = 4082.5$, $p < 0.001$; Fig. 1) and with records of *M. maximiliani* outside *M. atticolus* distribution (*M. maximiliani*: 254.2 ± 258.2 m; $W_{138} = 2265.5$, $p < 0.001$; Fig. 1). However, when considering only the area where the species distributions overlap, that is, using records of *M. maximiliani* inside *M. atticolus* distribution, there is no segregation between the species along an altitudinal gradient (*M. maximiliani*: 508.8 ± 333.5 m; $W_{141} = 1817$; $p = 0.196$; Fig. 1). Thus, *M. atticolus* is on average found on higher elevations than its congeneric species, but occurrence points of *M. maximiliani* in low areas are located outside *M. atticolus* distribution. If the region without occurrence of *M. atticolus* is unconsidered, there is no support for the hypothesis of topographical segregation between the two *Micrablepharus* species.

The compiled occurrence points for *M. atticolus* are presented on Fig. 2A. They are primarily inside Cerrado boundaries, although there are some records from isolated open areas within forest formations, particularly in Amazonian Forest. The receiver operating characteristic (ROC) curve presented a high area under the curve value (AUC = 0.96), indicating that the modeled potential distribution is better than the random prediction. This is confirmed by the correct assignment of testing data under the model, which performed significantly better than the null hypothesis. The prediction of suitable areas for the potential occurrence of *Micrablepharus atticolus* is highly coincident with Cerrado, except for the low probability on northeast and some overprediction towards southeast and northwest Cerrado (Fig. 2A). Paleomodels suggested noticeable changes on suitable areas for the occurrence of the species through the last 130 kyr BP (Fig. 2A - D). On LIG, these suitable areas are concentrated northwest of current distribution, in the region correspondent with current Amazon Forest. The smallest predicted range was observed on LGM, when the model shows two major disjunct areas, one in southeast and another in northwest, divided by the Tocantins and Araguaia Basins. During the Holocene, there is lower probability for the occurrence of the species in northern Cerrado in comparison with the current prediction. Stability areas defined by presence/absence models overlap are mainly located in high elevation regions of the current distribution in Guimarães Plateau, Caiapônia Plateau, Central Plateau, and Canastra Plateau (Fig. 2E).

The selected logistic regression model included the following predictors in increasing order of importance: LIG, Holocene, precipitation on driest month, precipitation on wettest quarter, temperature seasonality, precipitation seasonality, and isothermality. Importance indexes, coefficients, and significance of predictors are presented in Table 1.

Molecular data

As expected for protein-coding mitochondrial genes, there were no gaps on sequences alignment. Results of population genetics statistics for DS1 and DS2 are presented in Table 2. Cytochrome *b* gene is highly diverse in populations of *M. atticolus*, with many unique haplotypes. F_{st} values indicate high inter-population structuring.

Median-joining networks presented a highly structured pattern (Figures 3 – 4), as expected from F_{st} results. There is no haplotype sharing between locations sampled, except for Ilha do Bananal, Parque Nacional do Araguaia and Novo Santo Antônio. Networks produced with DS1 and DS2 are similar to each other.

In general, Bayesian phylogenetic analysis presented well-supported clades and both runs achieved the same topology, highly congruent with haplotype networks (Fig. 5). A single sample of *Micrablepharus atticolus* from Sapezal, Mato Grosso, clustered with *M. maximiliani*, indicating a possible identification error. Except for this case, all other *M. atticolus* formed a monophyletic group. Haplotypes from PCH Toricoejo were distantly placed on the tree. One of them grouped with haplotypes from Rancho Ponte de Pedra, while the other two formed a basal group jointly with samples from Cuiabá, separated from all other *M. atticolus*. This constituted the first deep split within the species. A second split occurred, separating the southern samples (Águas de Santa Bárbara, Bataguassu, Serranópolis, Santa Rita do Araguaia, Arinos and Curvelo) from remaining sequences. Subsequently, haplotypes from the northwestern part of the species' distribution diverged. Within this clade, Rondônia samples formed a monophyletic branch, which is the sister group of Alta Floresta and Nova Monte Verde. This constitutes a group of Cerrado isolates within Amazonia, which is closely related to haplotypes from Gaúcha do Norte. The remaining samples are structured with a central clade nested within haplotypes from southeast Mato Grosso. The central clade is constituted by a basal group of haplotypes from Arinos, Caldas Novas and Paracatu, and a derived group of samples from Peixe, Brasília, and Pium plus Novo Santo Antônio. Samples from southeastern Mato Grosso are complexly structured. All but one haplotype from UNEMAT, only 30 km apart from Rancho Ponte de Pedra, are closely related to the central clade.

The tree constructed for divergence time estimation had the same topology on all three runs (Fig. 7). This topology was very similar to the MrBayes tree, except for the position of Peixe, which grouped with Paracatu and Caldas Novas (BEAST) instead of Brasília and Pium (MrBayes). Minor differences on relationships among haplotypes from the same localities were also observed, but they do not interfere with our conclusions. Divergence between the two species of *Micrablepharus* was estimated around 6.5 – 11 mya. The first divergence within *M. atticolus* took place at approximately 2.3 – 3.5 mya, at the end of Pliocene and beginning of Pleistocene. The second split, separating southern haplotypes, occurred around 1.8 – 2.8 mya. The northwestern samples, including the Cerrado isolates haplotypes, diverged from other samples about 1.3 – 2.2 mya.

Monmonier's algorithm detected three main genetic barriers across sampled region, defining four groups of sampling localities (Fig. 7A). The first barrier delineates group 1, including Cuiabá and PCH Toricoejo. The second genetic barrier separated southern sampled localities constituting group 2. Isolated Cerrado points formed group 3, isolated from remaining localities (group 4) by the third barrier detected.

The Mantel test indicated a significant association between genetic and geographical distances ($z = 82.8$, $p = 0.024$). Tracing elevation either as binary (Fig. 8A) or continuous (Fig. 8B) character reveals a complex history of *M. atticolus* occupation across the topographical landscape. For the binary model, most ancestral proportional likelihoods for assignment to a particular state are inconclusive on basal nodes. Distribution of the species across altitudinal landscape is given in Fig. 7B.

Genetic diversity indexes for well-sampled localities are presented in Table 3. Only Pimenta Bueno showed low values of haplotype and nucleotide diversities, as expected for isolated areas in relation to core areas. We found no evidence for higher genetic diversity on plateaus or stable areas.

Discussion

Since *Micrablepharus atticolus* description, it is suggested that it is associated with Cerrado (Rodrigues, 1996), a result also found in a broader review by Nogueira (2006). The addition of records on the present work agrees with the general picture previously described, supporting the status of *M. atticolus* as endemic of the biome. In accordance, the result of the logistic regression reflect the strong association of this species with Cerrado and its open formation characteristics. The environmental variables selected in logistic regression determine the markedly seasonal pattern in the Cerrado (Nimmer, 1989), and the historical variables selected have the most similar distribution in comparison with current Cerrado configuration (Werneck *et al.*).

The lack of altitudinal segregation between *M. atticolus* and *M. maximiliani* does not support the idea of an influence of topography in the diversification of the Cerrado fauna (Silva, 1997; Colli, 2005; Werneck, 2011). Observed elevation values largely overlapped between the species, specially when considering exclusively the region where both species occur. Additionally, the tracing of elevation by maximum likelihood failed to resolve the condition (plateau or depression) of the common ancestral of the species. Nevertheless, Nogueira (2006) suggested a pattern of local segregation between *Micrablepharus* species, claiming that records of localities where both species were collected are rare. If this is true, smaller scale processes may be driving this segregation. As congeneric species tend to be ecologically similar, competition may impede *M. atticolus* and *M. maximiliani* coexistence. Alternatively, different ecological requirements other then altitude may define the species distributions. *Micrablepharus maximiliani* is more abundant in typical Cerrado areas, a savannic phytophysiognomy with moderate vegetation coverage, opposing to *M. atticolus* that is found majorly in open areas (Vieira *et al.*, 2000; Gainsbury & Colli, 2003; Nogueira, 2006).

We found high levels of *cyt b* diversity within *M. atticolus*. Isolation by distance seems to play an important role in defining diversity patterns, as indicated by a positive relation between genetic and geographical distances, which is expected given the broad distribution of the species. Yet, genetic barriers were identified between neighbor localities, mainly situated around Mato Grosso and Goiás states frontier (Fig. 7A). Thus, based these genetic barriers defined by AIS analysis, four groups were found, and supported by Bayesian phylogenies and haplotype networks.

The more ancient divergence times within the species are coincident with the final uplift of the Central Brazilian Plateau (Silva, 1997). Thus, if *M. atticolus* is associated with topography, it is expected that this distribution scenario determined the population genetic divergence. However, the phylogenetic tree topology isn't congruent with this picture, at least in the way hypothesized by Werneck (2011), i.e., a pattern of older lineages on plateaus, with signs of recent population expansion towards lower and supposedly younger areas. Instead, it appears that *M. atticolus* repeatedly colonized plateaus and depressions, and that geographical distance played an important role on population differentiation. Further, there was no association between genetic diversity and elevation.

Suitable areas for the potential occurrence of *M. atticolus* on Holocene, LGM and LIG differed from models constructed for Cerrado by Werneck *et al.* (in review). Areas with suitable conditions for the species shift between periods, along a northwest/southeast diagonal. Stability areas are also different from the models for Cerrado, with four main stable areas, coincident with high altitudes. Despite these differences, paleomodels for Cerrado and for *M. atticolus* agree that suitable areas are more broadly distributed in LIG than in LGM, challenging evidences for open formations expansion during drier and colder periods (Ledru, 2002; Mayle & Beerling, 2004; Mayle *et al.*, 2004). Unfortunately, we couldn't test predictions proposed by Carnaval *et al.* (2009), due to lack of appropriate sampling in predicted refugia. The only locality with large sample size in a stable area was Brasília, which didn't present higher genetic diversity then localities in unstable areas. Nevertheless, divergence times between haplotypes from different localities seem to be more ancient then the time scale covered by paleomodels. In other words, most populations were already differentiated by the LIG, possibly having persisted even during periods of unfavorable climatic conditions. A recent phylogeography of Brazilian Atlantic Forest endemic toads also found no congruence of genetic patterns with predicted stable areas (Thomé *et al.*, 2010).

Molecular dating indicated that lineages from Cerrado peripheral isolates were already differentiated at least 500 kyr BP. This estimation is much older than presumed savanna expansion towards Amazonia border around 3 – 9 kyr BP (Freitas *et al.*, 2001).

Thus, our results indicate that open formations occupied the northwestern contact zone between Cerrado and Amazonia around the middle Pleistocene. Pimenta Bueno presented the lowest haplotype and nucleotide diversities among all large sampled populations. Pimenta Bueno is more distant from core Cerrado than Vilhena, presenting low lizard richness (Gainsbury & Colli, 2003), which is consistent with our genetic data. In contrast, Vilhena shows diversity levels equivalent to core Cerrado areas, indicating longer periods of connection with core Cerrado.

Conclusion

The cytochrome *b* data from *M. atticolus* exhibited high diversity, revealing strong structuring among populations. Most within-species divergence occurred from the late Pliocene on. We found low support for the plateaus/depressions hypothesis based on tree topology. The Pimenta Bueno enclave showed low diversity levels indicating long-term isolation. Stability areas predicted by paleomodeling apparently had low importance in determining diversification among populations due to low congruence in time scale. Better sampling in terms of more individuals within the species, more genetic markers, and other Cerrado species can improve this picture, allowing more rigorous and refined hypothesis testing. Nevertheless, in agreement with other recent work, our results support the existence of a complex history of the Cerrado region.

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Table 1. Results of logistic regression relating the distribution of *Micrablepharus atticolus* with environmental and historical variables. Importance of variables was assessed using model averaging.

Variable	Coefficient	*z*	*p*	Importance
Last Interglacial (LIG, 130kyr BP)	2.01	8.58	<0.001	1.00
Holocene (6kyr BP)	2.19	10.06	<0.001	1.00
Precipitation of driest month (BIO14)	-4.51	-4.48	<0.001	1.00
Precipitation of wettest quarter (BIO16)	1.54	4.82	<0.001	1.00
Temperature seasonality (BIO4)	1.76	3.52	<0.001	0.98
Precipitation seasonality (BIO15)	-1.33	-2.70	0.007	0.88
Isothermality (BIO3)	0.91	2.04	0.042	0.71
Intercept	-1.27	-4.45	< 0.001	NA

Table 2. Statistics for cytochrome *b* sequence data of the Cerrado endemic lizard, *Micrablepharus atticolus*. DS1: dataset excluding incomplete sequences; DS2: dataset including incomplete sequences.

Parameter	DS1	DS2
Nº of sequences	103	116
Nº of included sites	715	661
Polymorphic sites (S)	195	183
Parsimony informative sites	132	131
Nº of haplotypes (h)	57	64
Haplotype diversity (H_d)	0.974	0.976
Nucleotide diversity (π)	0.042	0.043
Nº of populations included on F_{st} estimation	12	15
F_{st}	0.69	0.76

Table 3. Genetic diversity parameters of samples of *Micrablepharus atticolus* from the Brazilian Cerrado biome. Location refers to the position of the site relative to the biome; stability refers to ; *n*: number of sequences; *h*: number of haplotypes; H_d: haplotype diversity; *S*: number of polymorphic sites; π: nucleotide diversity.

Site	Location	Stability	Altitude (m)	*n*	*h*	H_d	*S*	π
Pimenta Bueno, RO	periphery	unstable	211	15	2	0.34	1	0.0005
Vilhena, RO	periphery	unstable	512	14	9	0.92	9	0.0029
Brasília, DF	core	stable	1170	16	5	0.67	4	0.0012
Nova Xavantina, MT (Rancho Ponte de Pedra)	core	unstable	304	14	6	0.75	17	0.0079
Pium, MT	core	unstable	216	14	8	0.90	13	0.0053

Figure Legends

Figure 1. Box-plots depicting elevation of locality records of *Micrablepharus atticolus* and *M. maximiliani* (total records, records of *M. maximiliani* outside *M. atticolus* distribution, and records of *M. maximiliani* inside *M. atticolus* distribution) from open biomes in Brazil. The bold horizontal line is the median; the box is bounded by the 25% and 75% percentiles (interquartile range-IQR), and the whiskers extend to 1.5 times the IQR.

Figure 2. Predictions of suitable areas for *Micrablepharus atticolus* occurrence under past and current environmental conditions. A) Map with *M. atticolus* occurrence points used on Maxent modeling (black dots), current prediction for areas suitable for the species occurrence (gradient colors) and distributional range (pink). Stars correspond to localities excluded from modeling – Alta Floresta (MT), Nova Monte Verde (MT), and Ourilândia do Norte (PA) – due to their long distance from Cerrado (grey). Small pink circles indicate occurrence records isolated from core distribution of the species. B – D) Prediction of suitable areas for *M. atticolus* occurrence on Holocene (B; 6 ky BP), Last Glacial Maximum (C; LGM, 21 ky BP) and Last Interglacial (D; LIG, 130 ky BP). E) Stable areas and unstable areas for the occurrence of the species defined by overlapping of current, Holocene, LGM and LIG models: (1) Planalto dos Guimarães; (2) Planalto da Caiapônia; (3) Planalto Central; (4) Planalto da Canastra.

Figure 3. Median-joining network for 102 cytochrome *b* sequences (complete sequences only – DS1) of *Micrablepharus atticolus* from the Brazilian Cerrado. Circles represent haplotypes and their sizes are proportional to sample frequency. Branch lengths represent number of mutations between nodes, except when crossed. Colors correspond to groups defined by Monmonier's algorithm (see Fig. 7A).

Figure 4. Median-joining network for 115 cytochrome *b* sequences (including incomplete sequences – DS2) *Micrablepharus atticolus* from the Brazilian Cerrado. Circles represent haplotypes and their sizes are proportional to sample frequency. Branch lengths represent number of mutations between nodes, except when crossed. Colors correspond to groups defined by Monmonier's algorithm (see Fig. 7A).

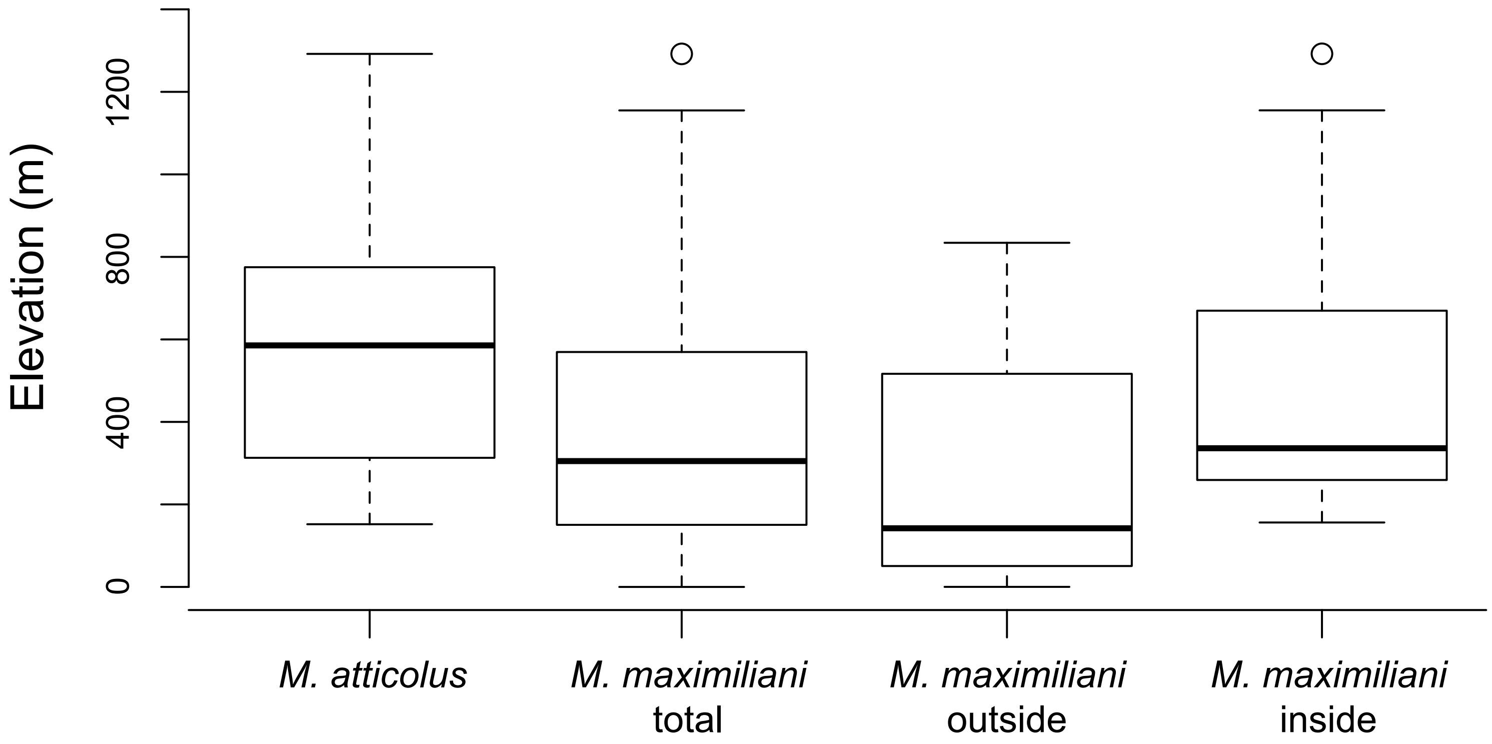
Figure 5. Bayesian phylogenetic tree for *Micrablepharus atticolus*, based on a cytochrome *b* fragment. *Vanzosaura rubricauda* and *M. maximiliani* samples were used as outgroups. Numbers indicate posterior probability for branches. When an haplotype is present in more then one sample, terminal name is the haplotype number plus number of samples in which the haplotype is present. Otherwise, terminal name is specimen's tag. Colors correspond to groups defined by Monmonier's algorithm (see Fig. 7A). Locality abbreviations: (BA) Co - Cocos; (DF) Br - Brasília; (GO) CaN - Caldas Novas, SRA - Santa Rita do Araguaia, Se - Serranópolis; (MG) Ar - Arinos, Cur - Curvelo, Pa - Paracatu, AlNo - Alvorada do Norte; (MS) Bat - Bataguassu, Aqu - Aquidauana; (MT) AlF - Alta Floresta, Tor - Toricoejo, BaG - Barra do Garças, Coc - Cocalinho, Cu - Cuiabá, Gau - Gaúcha do Norte, NMVer – Nova Monte Verde, Pin - Pindaíba, Ran - Rancho Ponte de Pedra, UNE - UNEMAT, NSAn - Novo Santo Antonio, Sap - Sapezal; (RO) PiB - Pimenta Bueno, Vi – Vilhena, GuMi - Guajará-Mirim; (SP) AgSB - Águas de Santa Bárbara; (TO) Pei - Peixe, IBan - Ilha do Bananal, PNA - Parque Nacional do Araguaia, Ma - Mateiros.

Figure 6. Divergence time estimation for *Micrablepharus atticolus* based on relaxed molecular clock. Boxes indicate 95% confidence interval divergence time. Taxon names are given according to Figure 6.

Figure 7. A) Genetic barriers defined by Monmonier's algorithm for cytochrome *b* sequences of *Micrablepharus atticolus* from Brazilian Cerrado (shown in gray). Dots define sampling points, letters indicate localities while colors correspond to groups defined by the genetic barriers: (DF) A - Brasília; (GO) B - Caldas Novas, C - Santa Rita do Araguaia, D - Serranópolis; (MG) E - Arinos, F - Curvelo, G - Paracatu; (MS) H - Bataguassu,; (MT) I - Alta Floresta, J - Toricoejo, K - Barra do Garças, L - Cocalinho, M - Cuiabá, N - Gaúcha do Norte, O – Nova Monte Verde, P - Pindaíba, Q - Rancho Ponte de Pedra, R - UNEMAT, S - Novo Santo Antonio; (RO) T - Pimenta Bueno, U – Vilhena; (SP) V - Águas de Santa Bárbara; (TO) W - Peixe, X - Ilha do Bananal, Y - Parque Nacional do Araguaia; pink - group 1, blue - group 2, green - group 3, red - group 4. B) Altitude map. Coloured dots are the same as in A.

Figure 8. A) Trace of locality records on plateus (above 500 m) or depressions (below 500 m) for *Micrablepharus atticolus*' haplotypes with ancestral state reconstruction based on maximum likelihood. Values indicate proportional likelihood for ancestral occurrence on depressions. Asterisks specify significant difference between likelihoods for occurrence on depressions or plateaus. B) Trace of altitude occurrence records (continuous variable) for *Micrablepharus atticolus*' haplotypes with ancestral state reconstruction based on maximum parcimony. Taxon names are given according to Figure 6.

Figure 1



Elevation (m)
0
400
800
1200
M. atticolus
M. maximiliani total
M. maximiliani outside
M. maximiliani inside

Figure 2

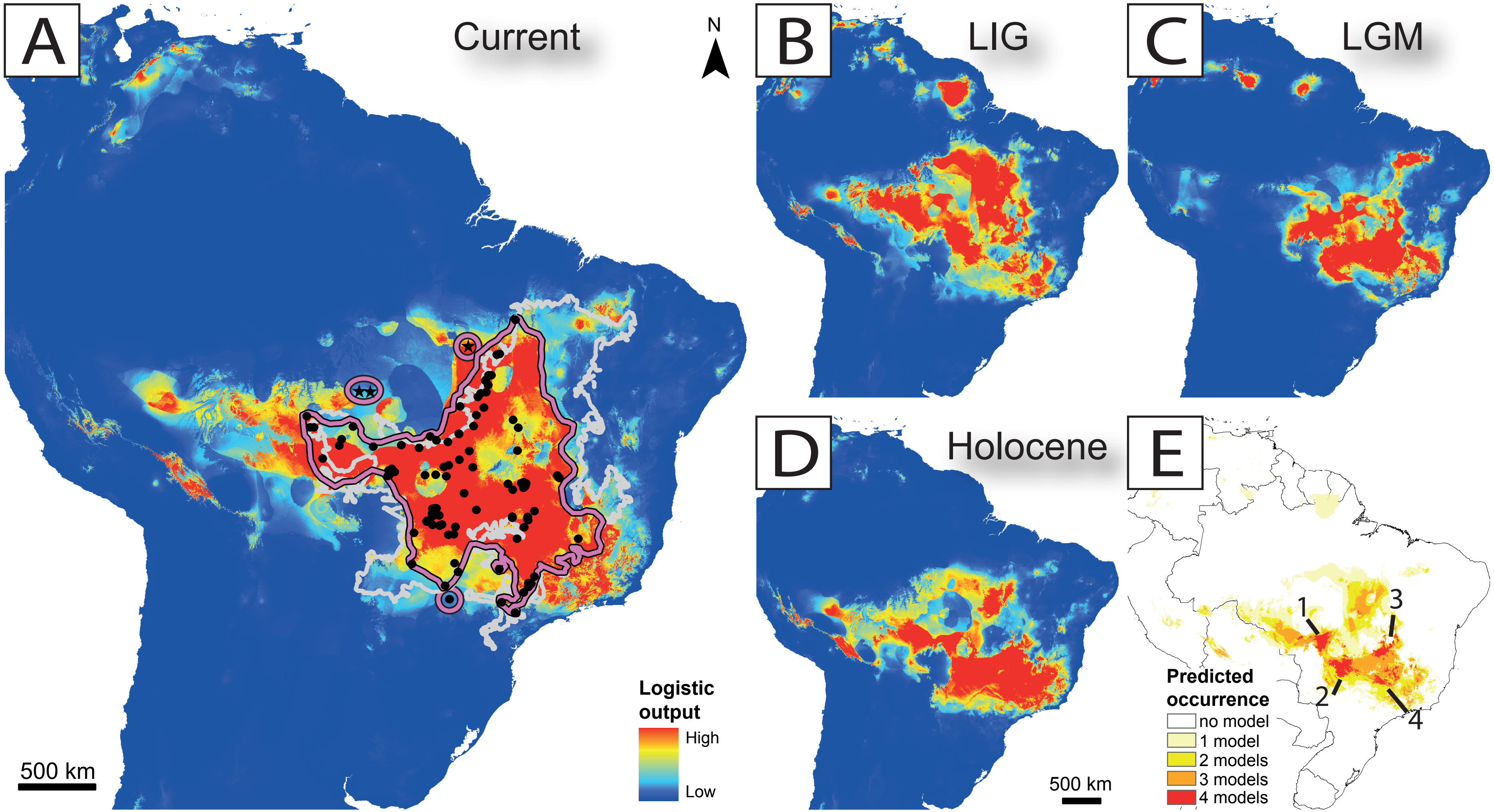
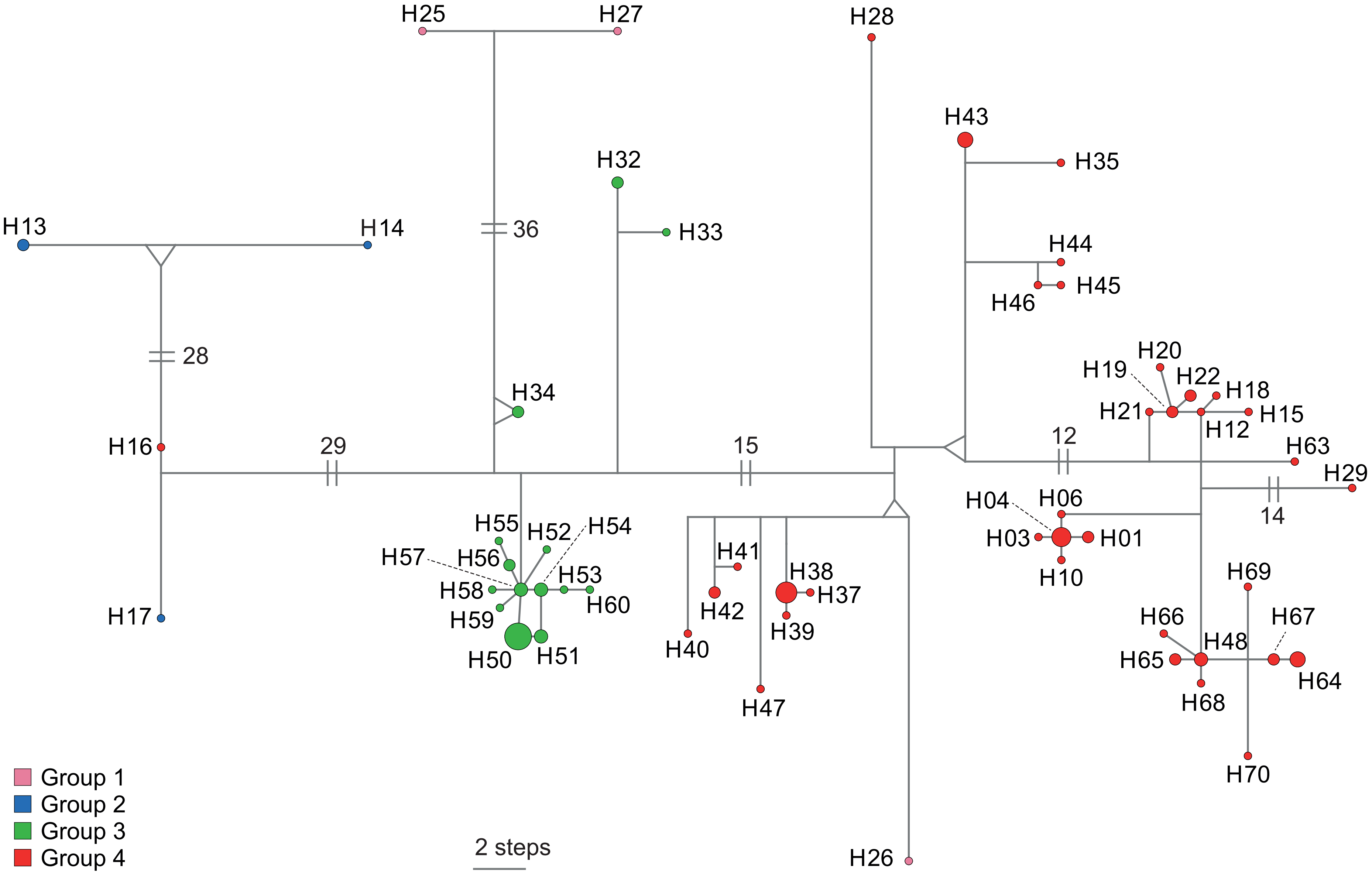

A
Current
N
B
LIG
C
LGM
D
Holocene
E
500 km
Logistic output
High
Low
500 km
1
2
3
4
Predicted occurrence
no model
1 model
2 models
3 models
4 models

Figure 3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H27
H28
H43
H35
H32
H33
H13
H14
36
H44
H45
H46
28
H34
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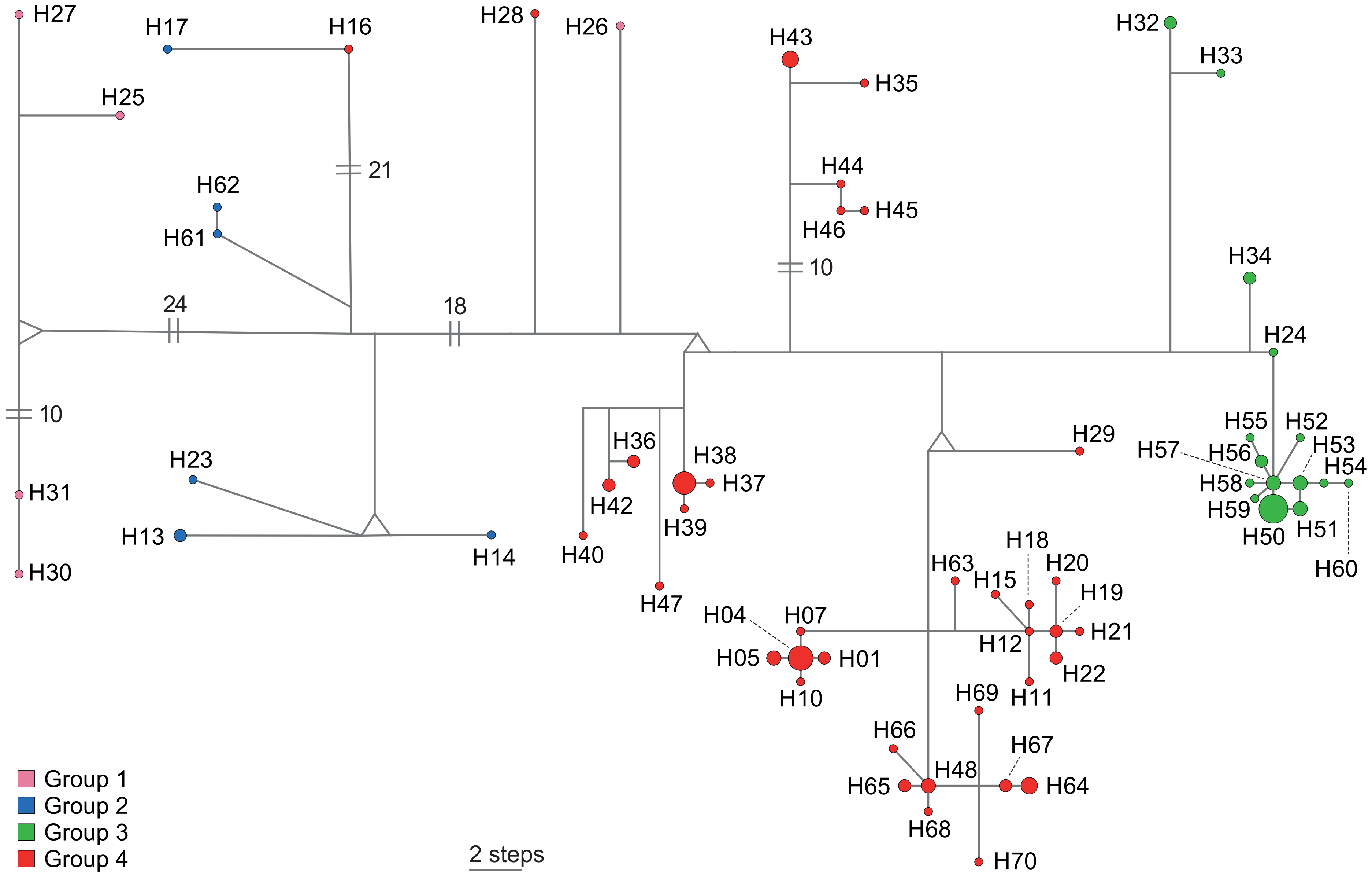
H19
H22
H18
H21
H15
H12
H16
29
15
12
H63
H29
H04
H06
14
H55
H52
H54
H03
H01
H57
H56
H53
H41
H38
H10
H58
H37
H60
H69
H42
H59
H39
H66
H67
H17
H48
H50
H51
H40
H65
H64
H68
H47
H70
🟥 Group 1
🟦 Group 2
🟩 Group 3
🟥 Group 4
2 steps
H26

Figure 4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H35
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H45
H46
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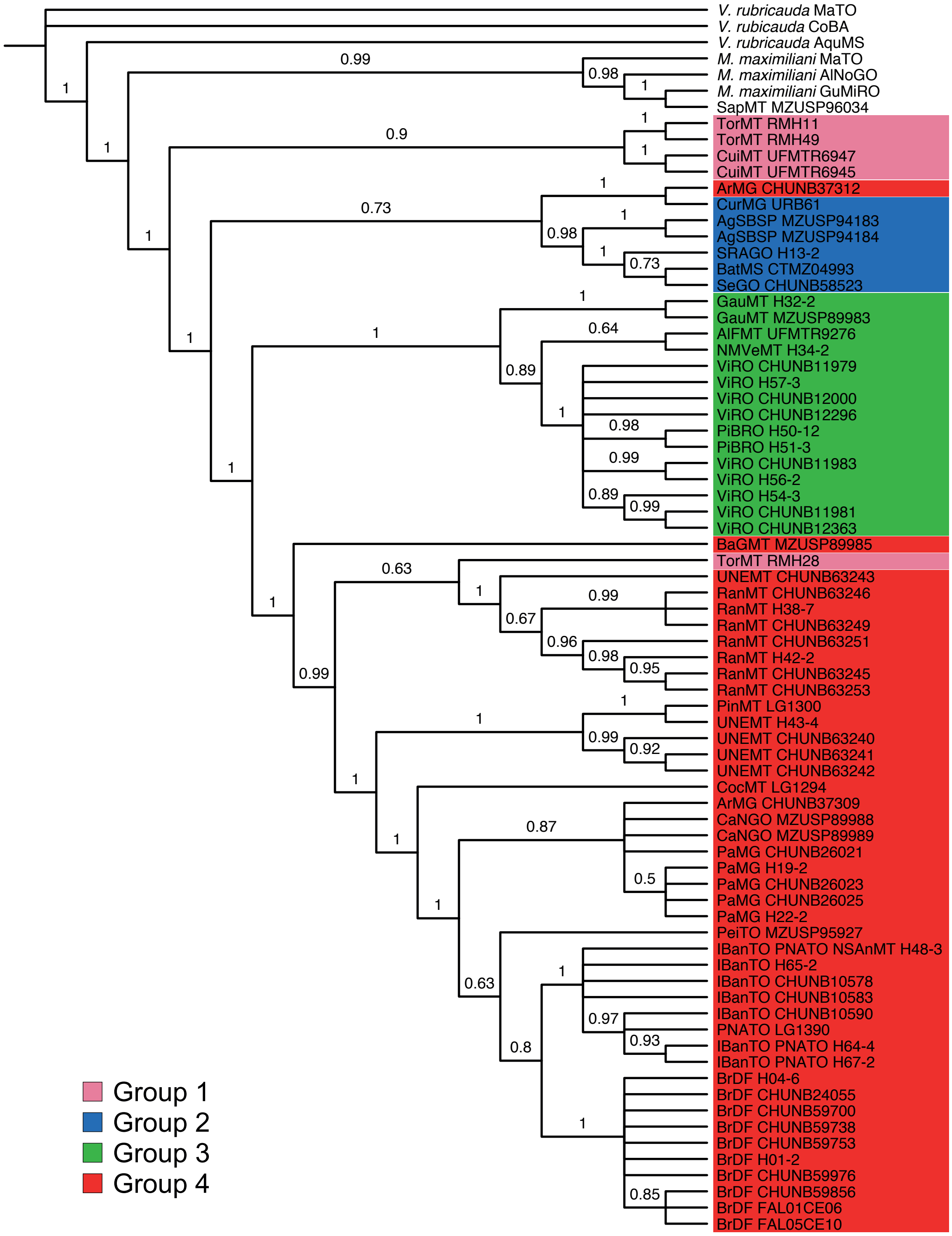
H61
10
H34
24
18
H24
10
H31
H23
H13
H14
H36
H38
H37
H42
H39
H40
H47
H29
H55
H52
H57
H56
H53
H54
H58
H59
H50
H51
H60
H30
H63
H18
H20
H15
H19
H04
H07
H12
H21
H05
H01
H22
H10
H69
H11
H66
H67
H48
H65
H64
H68
H70
Group 1
Group 2
Group 3
Group 4
2 steps

Figure 5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V. rubricauda CoBA
V. rubricauda AquMS
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M. maximiliani MaTO
0.98
M. maximiliani AlNoGO
1
1
M. maximiliani GuMiRO
SapMT MZUSP96034
1
TorMT RMH11
0.9
TorMT RMH49
1
1
CuiMT UFMTR6947
CuiMT UFMTR6945
1
ArMG CHUNB37312
0.73
CurMG URB61
1
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0.98
1
AgSBSP MZUSP94184
1
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SeGO CHUNB58523
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GauMT MZUSP89983
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1
AlFMT UFMTR9276
1
NMVeMT H34-2
ViRO CHUNB11979
0.89
ViRO H57-3
ViRO CHUNB12000
1
ViRO CHUNB12296
0.98
PiBRO H50-12
PiBRO H51-3
0.99
ViRO CHUNB11983
1
ViRO H56-2
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ViRO H54-3
0.99
ViRO CHUNB11981
ViRO CHUNB12363
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0.63
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0.99
RanMT CHUNB63246
1
RanMT H38-7
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RanMT CHUNB63249
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RanMT CHUNB63251
0.98
RanMT H42-2
0.95
0.99
RanMT CHUNB63245
RanMT CHUNB63253
1
PinMT LG1300
1
UNEMT H43-4
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UNEMT CHUNB63240
0.92
UNEMT CHUNB63241
1
UNEMT CHUNB63242
CocMT LG1294
ArMG CHUNB37309
CaNGO MZUSP89988
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CaNGO MZUSP89989
1
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PaMG H19-2
0.5
PaMG CHUNB26023
PaMG CHUNB26025
1
PaMG H22-2
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IBanTO H65-2
1
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IBanTO CHUNB10583
IBanTO CHUNB10590
0.97
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0.8
IBanTO PNATO H67-2
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BrDF CHUNB24055
BrDF CHUNB59700
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BrDF CHUNB59738
BrDF CHUNB59753
BrDF H01-2
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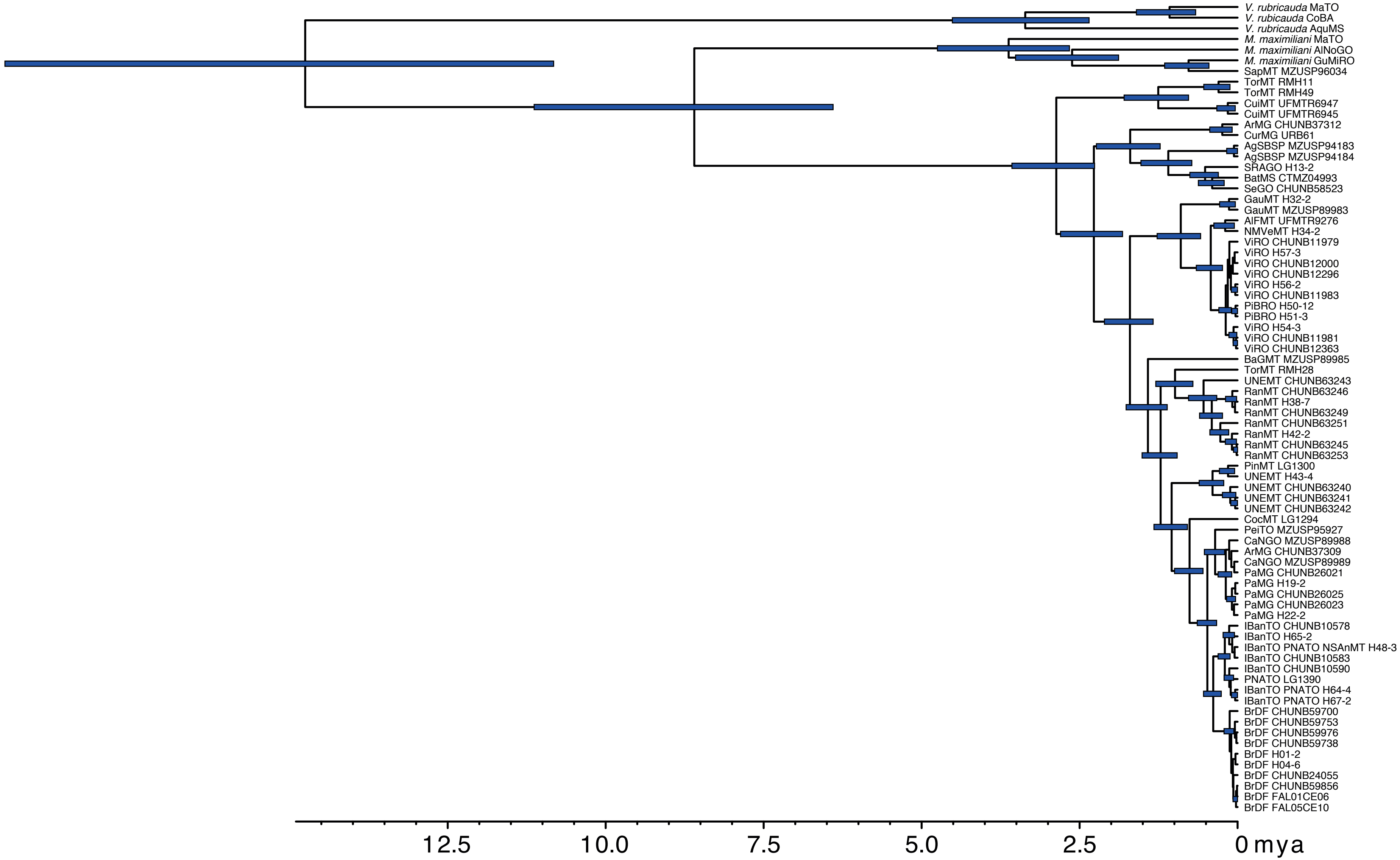
BrDF FAL05CE10
Group 1
Group 2
Group 3
Group 4

Figure 6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V. rubricauda AquMS
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M. maximiliani AlNoGO
M. maximiliani GuMiRO
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TorMT RMH11
TorMT RMH49
CuiMT UFMTR6947
CuiMT UFMTR6945
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CurMG URB61
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AgSBSP MZUSP94184
SRAGO H13-2
BatMS CTMZ04993
SeGO CHUNB58523
GauMT H32-2
GauMT MZUSP89983
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ViRO H57-3
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ViRO H56-2
ViRO CHUNB11983
PiBRO H50-12
PiBRO H51-3
ViRO H54-3
ViRO CHUNB11981
ViRO CHUNB12363
BaGMT MZUSP89985
TorMT RMH28
UNEMT CHUNB63243
RanMT CHUNB63246
RanMT H38-7
RanMT CHUNB63249
RanMT CHUNB63251
RanMT H42-2
RanMT CHUNB63245
RanMT CHUNB63253
PinMT LG1300
UNEMT H43-4
UNEMT CHUNB63240
UNEMT CHUNB63241
UNEMT CHUNB63242
CocMT LG1294
PeiTO MZUSP95927
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ArMG CHUNB37309
CaNGO MZUSP89989
PaMG CHUNB26021
PaMG H19-2
PaMG CHUNB26025
PaMG CHUNB26023
PaMG H22-2
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IBanTO H65-2
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IBanTO CHUNB10590
PNATO LG1390
IBanTO PNATO H64-4
IBanTO PNATO H67-2
BrDF CHUNB59700
BrDF CHUNB59753
BrDF CHUNB59976
BrDF CHUNB59738
BrDF H01-2
BrDF H04-6
BrDF CHUNB24055
BrDF CHUNB59856
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BrDF FAL05CE10
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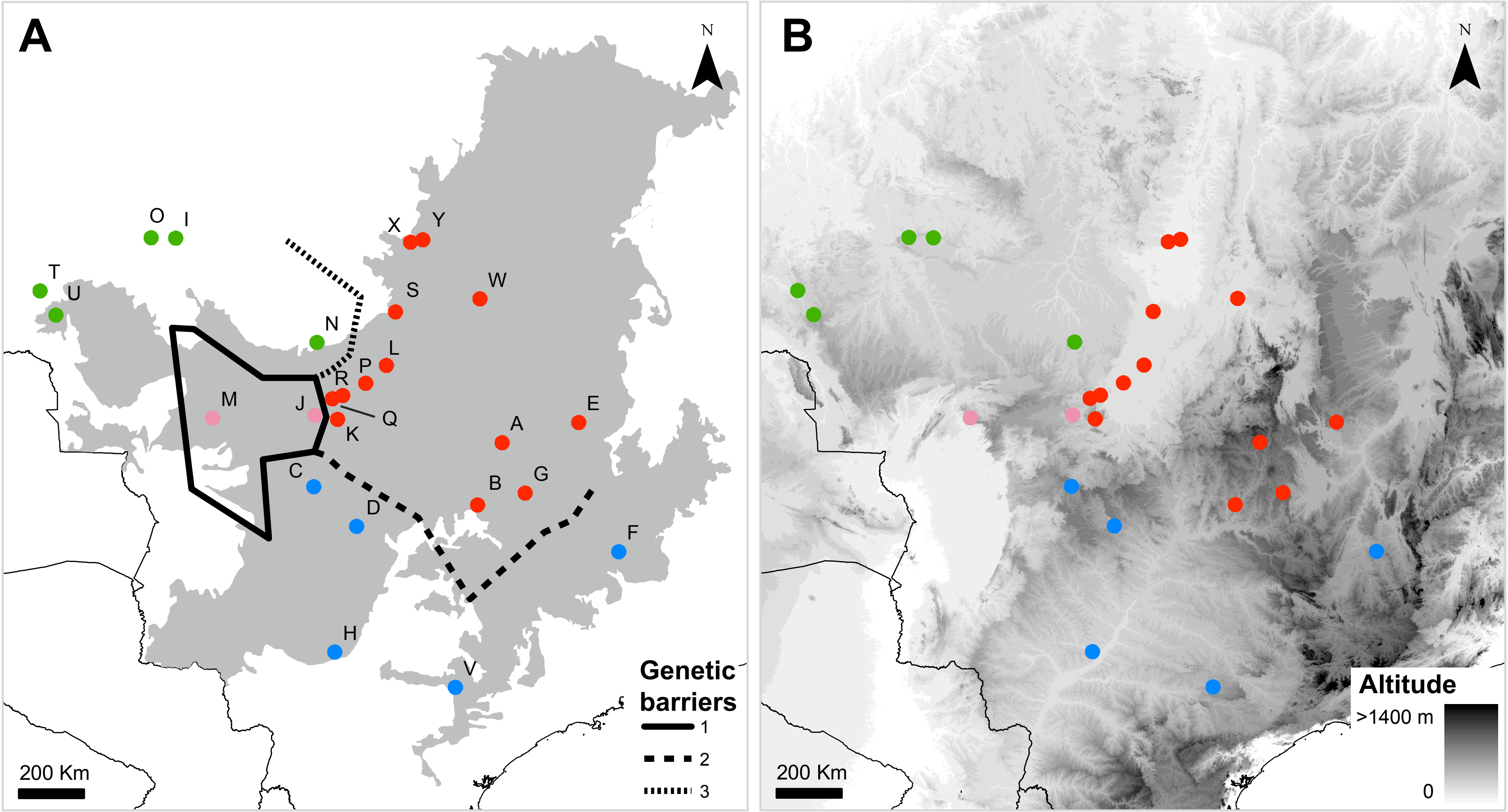
10.0
7.5
5.0
2.5
0 mya

Figure 7


A
N
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X
Y
S
W
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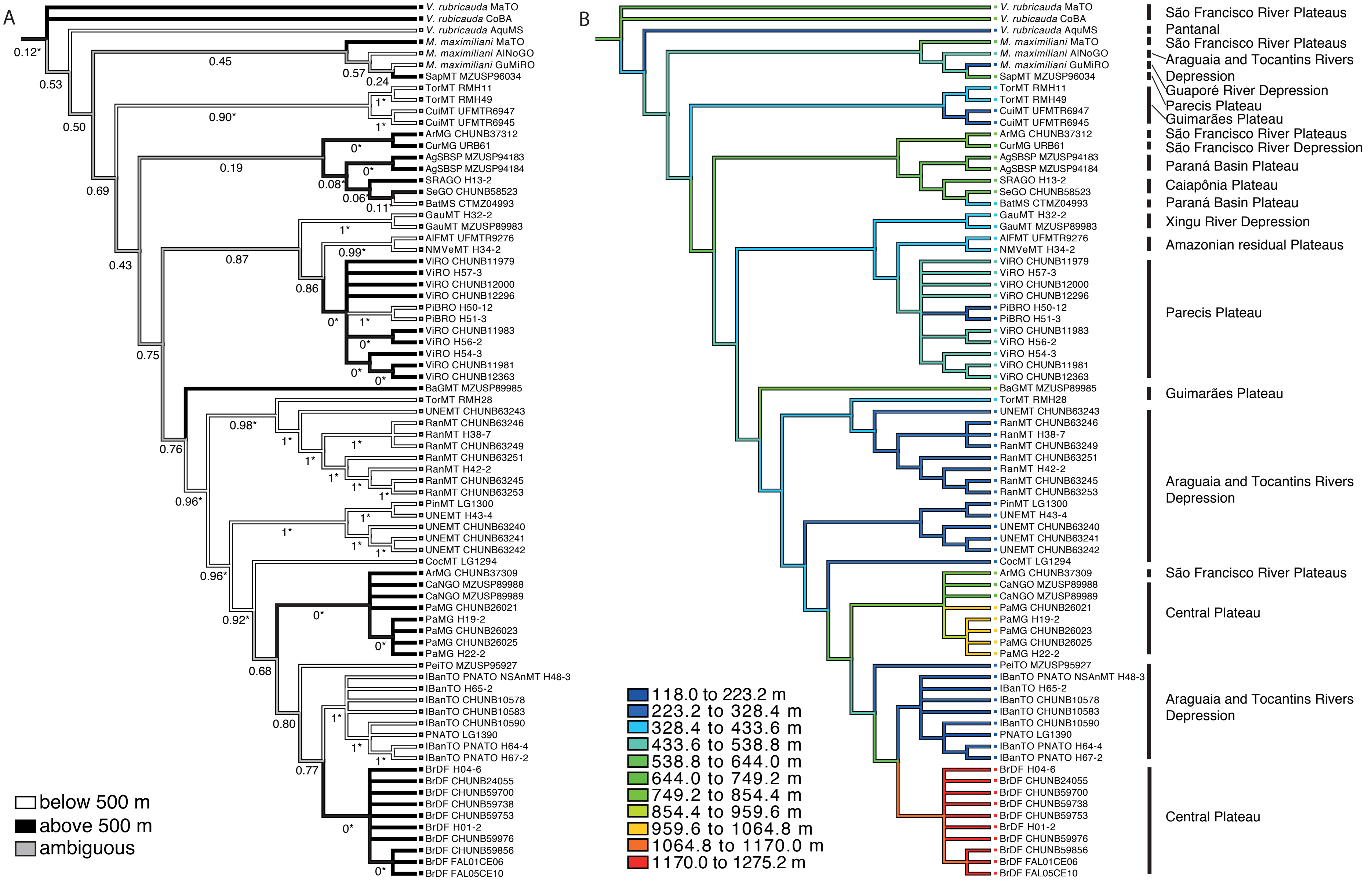
L
P
R
M
J
Q
K
A
E
C
B
G
D
F
H
V
Genetic barriers
1
2
3
200 Km
B
N
Altitude
>1400 m
0
200 Km

Figure 8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V. rubricauda AquMS
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M. maximiliani AlNoGO
M. maximiliani GuMiRO
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TorMT RMH11
TorMT RMH49
CuiMT UFMTR6947
CuiMT UFMTR6945
ArMG CHUNB37312
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SeGO CHUNB58523
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NMVeMT H34-2
ViRO CHUNB11979
ViRO H57-3
ViRO CHUNB12000
ViRO CHUNB12296
PiBRO H50-12
PiBRO H51-3
ViRO CHUNB11983
ViRO H56-2
ViRO H54-3
ViRO CHUNB11981
ViRO CHUNB12363
BaGMT MZUSP89985
TorMT RMH28
UNEMT CHUNB63243
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RanMT H42-2
RanMT CHUNB63245
RanMT CHUNB63253
PinMT LG1300
UNEMT H43-4
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UNEMT CHUNB63241
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CocMT LG1294
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CaNGO MZUSP89989
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BrDF H01-2
BrDF CHUNB59976
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BrDF FAL05CE10
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0.45
0.57
0.24
0.53
1*
0.90*
1*
0.50
0*
0.19
0*
0.08*
0.06*
0.11*
0.69
1*
0.99*
0.87
0.86
0*
1*
0*
0.43
0*
0*
0.75
0.98*
1*
1*
1*
1*
1*
1*
0.76
0.96*
1*
1*
1*
1*
0.96*
0*
0.92*
0*
0.68
1*
0.80
1*
1*
0.77
0*
0*
below 500 m
above 500 m
ambiguous
B
V. rubricauda MaTO
V. rubicauda CoBA
V. rubricauda AquMS
M. maximiliani MaTO
M. maximiliani AlNoGO
M. maximiliani GuMiRO
SapMT MZUSP96034
TorMT RMH11
TorMT RMH49
CuiMT UFMTR6947
CuiMT UFMTR6945
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GauMT MZUSP89983
AlFMT UFMTR9276
NMVeMT H34-2
ViRO CHUNB11979
ViRO H57-3
ViRO CHUNB12000
ViRO CHUNB12296
PiBRO H50-12
PiBRO H51-3
ViRO CHUNB11983
ViRO H56-2
ViRO H54-3
ViRO CHUNB11981
ViRO CHUNB12363
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BrDF H01-2
BrDF CHUNB59976
BrDF CHUNB59856
BrDF FAL01CE06
BrDF FAL05CE10
118.0 to 223.2 m
223.2 to 328.4 m
328.4 to 433.6 m
433.6 to 538.8 m
538.8 to 644.0 m
644.0 to 749.2 m
749.2 to 854.4 m
854.4 to 959.6 m
959.6 to 1064.8 m
1064.8 to 1170.0 m
1170.0 to 1275.2 m
São Francisco River Plateaus
Pantanal
São Francisco River Plateaus
Araguaia and Tocantins Rivers Depression
Guaporé River Depression
Parecis Plateau
Guimarães Plateau
São Francisco River Plateaus
São Francisco River Depression
Paraná Basin Plateau
Caiapônia Plateau
Paraná Basin Plateau
Xingu River Depression
Amazonian residual Plateaus
Parecis Plateau
Guimarães Plateau
Araguaia and Tocantins Rivers Depression
São Francisco River Plateaus
Central Plateau
Araguaia and Tocantins Rivers Depression
Central Plateau

Appendix 1. Locality records of *Micrablepharus atticolus* from the Brazilian Cerrado. CHUNB – Coleção Herpetológica da Universidade de Brasília; CTMZ – Coleção de Tecidos do Museu de Zoologia da Universidade de São Paulo; MNRJ – Museu Nacional da Universidade Federal do Rio de Janeiro; MPEG – Museu Paraense Emilio Goeldi; MZUSP – Museu de Zoologia da Universidade de São Paulo; UFMT-R – Coleção Zoológica de Vertebrados da Universidade Federal do Mato Grosso; ZUEC – Museu de Zoologia da Universidade de Campinas; other acronyms correspond to collector's code.

Municipality	Locality	State	Latitude	Longitude	Coordinate source	Specimen's code
Brasília	Água Limpa Farm	DF	15º58'21" S	47º54'35" W	Collector	CHUNB 59024, 59852, 59856, 59961, 59976, 59987; FAL01CE06, FAL05CE10
Brasília	Airport	DF	15º52'55" S	47º55'26" W	Locality description	CHUNB 30865, 30866
Brasília	Área Alfa (CIAB) - CAE a	DF	16º00'43" S	47º56'39" W	Collector	CHUNB 21821, 21830; MZUSP 91876, 91885
Brasília	Área Alfa (CIAB) - Campo Vochysia	DF	15º58'59" S	47º54'22" W	Collector	CHUNB 21824
Brasília	Área Alfa (CIAB) - CAW b	DF	16º00'57" S	47º57'20" W	Collector	CHUNB 21831, 21832; MZUSP 91875
Brasília	Área Alfa (CIAB) - CEE c	DF	16º00'36" S	47º56'30" W	Collector	CHUNB 21822, 21823, 21825, 21826, 21828, 24522, 24526; MZUSP 91878, 91879, 91881-91884, 91887
Brasília	Área Alfa (CIAB) - CEW	DF	16º00'21" S	47º57'42" W	Collector	CHUNB 21827, 21829, 24524, 30961; MZUSP

	d					91877, 91880, 91886, 91888-91895
Brasília	Floresta Nacional de Brasília	DF	15º45'28" S	48º03'35" W	Collector	CHUNB 60072
Brasília	IBGE - Mata de Galeria	DF	15º55'52" S	47º52'58" W	Collector	CHUNB 38480, 43777
Brasília	IBGE - Projeto Fogo	DF	15º57'01" S	47º52'04" W	Collector	CHUNB 44130, 47166, 48021, 49653, 50787, 61951 - 61953
Brasília	Jardim Botânico de Brasília - Mata de Galeria	DF	15º52'54" S	47º50'29" W	Collector	CHUNB 40298; MZUSP 79366
Brasília	Parque Nacional de Brasília	DF	15º41'48" S	47º55'29" W	Collector	CHUNB59030, 59693, 59700, 59738, 59753, 59761, 60071
Brasília		DF	16º00'32" S	47º56'58" W	Collector	MZUSP 91456, 91457
Brasília		DF	15º46'51" S	47º47'49" W	Municipality centroid	CHUNB 08193, 09474, 09482, 09485, 09486, 09594, 13479, 23827, 24055; MPEG 04554, 12891; MZUSP 90084, 90085
Alexânia	Cafundó Farm	GO	16º08'52" S	48º34'45" W	Tomatieli *et al.* (2004)	CHUNB 39046, 39048
Aporé	PCH Planalto	GO	18º47'18" S	52º22'30" W	Locality description (http://www.aneel.gov.br/cedoc/dsp20092237.pdf)	CHUNB 58990 - 58992

Aporé		GO	18º46'04" S	52º02'43" W	Municipality centroid	MNRJ 14113
Arenópolis	PCH Mosquitão - Caiapó River	GO	16º20'30" S	51º26'15" W	Locality description	MZUSP 98285 - 98289
Aruanã		GO	14º48'20" S	50º56'13" W	Municipality centroid	MZUSP 2623 - 2629
Caldas Novas	UHE Corumbá	GO	17º47'23" S	48º36'00" W	Locality description	MZUSP 81363 - 81379
Caldas Novas		GO	17º44'00" S	48º37'00" W	Yonenaga-Yassuda & Rodrigues (1999)	MZUSP 89986 - 89990
Campo Alegre de Goiás	AHE Serra do Facão	GO	17º44'56" S	47º42'11" W	Collection data	CHUNB 62048, 62051, 62053
Campo Alegre de Goiás	AHE Serra do Facão	GO	17º44'54" S	47º42'10" W	Collection data	CHUNB 62047, 62050, 62054
Campo Alegre de Goiás	AHE Serra do Facão	GO	17º44'54" S	47º42'20" W	Collection data	CHUNB 62049, 62052
Catalão	AHE Serra do Facão	GO	17º55'06" S	47º42'30" W	Collection data	CHUNB 62046
Catalão	LT Serra da Mesa	GO	18º21'47" S	47º54'31" W	Collector	CHUNB 44753
Catalão		GO	17º55'49" S	47º39'56" W	Municipality centroid	MNRJ 7431, 7432, 7437
Chapadão do Céu	Parque Nacional das Emas - Água Ruim	GO	18º10'59" S	52º44'36" W	Collector	CHUNB 26590
Minaçu	Serra da Mesa	GO	13º49'50" S	48º17'35" W	Locality description	MZUSP 81197 - 81202

Mineiros	Assentamento Nascentes do Araguaia	GO	17º39'51" S	53º13'02" W	Valdujo *et al.* (2009)	CHUNB 23744 - 23750, 23756
Mineiros	Babilônia Farm	GO	17º39'22" S	52º54'34" W	Valdujo *et al.* (2009)	CHUNB 24520, 24521, 24523, 24525, 24527
Mineiros	Parque Nacional das Emas	GO	18º15'15" S	52º53'11" W	Collector	CHUNB 25650, 25752, 25753
Mineiros	Parque Nacional das Emas	GO	18º15'00" S	52º52'59" W	Collector	CHUNB 11581, 11583; MZUSP87664, 87665
Pirenópolis		GO	15º48'00" S	48º51'35" W	Locality description	MZUSP 79364, 79365
Rio Verde		GO	17º20'19" S	50º42'54" W	Collector	CHUNB 49684
Santa Rita do Araguaia		GO	17º13'37" S	53º09'28" W	Collector	PHV 2846, 2847
Santa Rita do Araguaia		GO	17º14'40" S	53º04'09" W	Municipality centroid	MPEG 16886; MZUSP 79566 - 79568
Santa Rita do Araguaia		GO	17º19'00" S	53º12'00" W	Yonenaga-Yassuda & Rodrigues (1999)	MZUSP 78877 - 78894
Santa Rita do Araguaia		GO	17º18'00" S	53º12'00" W	Yonenaga-Yassuda & Rodrigues (1999)	MZUSP 78870 -78872
Serranópolis	Casarão de Pedra	GO	18º19'50" S	51º57'59" W	Collector	CHUNB 58523
Arinos	RPPN Arara Vermelha and Vereda do Pacari	MG	15º26'46" S	45º49'30" W	Collector	CHUNB 37308 - 37314
Curvelo	Curvelo/Pompéu	MG	19º01'33" S	44º42'32" W	Locality description	URB 61

Formoso	PARNA Grande Sertão Veredas	MG	15º18'25" S	45º56'29" W	Collector	MZUSP 94179
Paracatu		MG	17º23'59" S	47º17'59" W	Mesquita *et al.* (2007)	CHUNB 26018 - 26026, 26201 - 26207
Uberlândia		MG	19º01'41" S	48º19'54" W	Municipality centroid	MNRJ 13298
Alcinópolis	Vista Bonita Farm - Line 1	MS	17º59'21" S	53º37'39" W	Collector	CHUNB 27759 - 27763, 27765, 27767, 27768, 27770 - 27781, 27783, 27784, 27786, 27787 - 27792, 27794 - 27798, 27800 - 27808, 27810 - 27817, 27819 - 27840, 27842 - 27857, 27859, 27860, 30883
Alcinópolis	Vista Bonita Farm - Line 2	MS	17º59'42" S	53º38'00" W	Collector	CHUNB 27799
Alcinópolis	Vista Bonita Farm - Line 3	MS	18º01'34" S	53º38'29" W	Collector	CHUNB 27764, 27766, 27782, 27793, 27858
Alcinópolis	Vista Bonita Farm - Line 4	MS	18º01'02" S	53º39'14" W	Collector	CHUNB 27769, 27841
Bataguassu		MS	21º48'29" S	52º34'14" W	Municipality centroid	CTMZ 04993
Campo Grande	Estância Santa Maria	MS	20º30'36" S	54º31'33" W	Mori *et al.* (Mori *et al.*, 2009)	
Costa Rica	PE Nascentes do Taquari - Mutum Farm	MS	18º12'53" S	53º18'43" W	Collector	CHUNB 24468
Coxim	Diamante Farm - Jauru district	MS	18º41'29" S	54º24'39" W	Giraldelli (2007)	

Três Lagoas	Barra da Moeda Farm	MS	20º58'58" S	51º47'20" W	Collection data	ZUEC 3482 - 3488
Três Lagoas	Canaã Farm	MS	20º28'11" S	52º00'00" W	geoLoc	MZUSP 14369
Alta Floresta	PCH Apiacás	MT	10º21'05" S	56º58'35" W	Collection data	UFMT-R 9276
Alto Araguaia	Bacuri, Bálsamo, and Córrego Fundo Farms	MT	17º15'36" S	53º18'00" W	Locality description (Vitt, 1991)	MZUSP 69790, 69791
Alto Araguaia	Córrego do Sapo region	MT	17º32'49" S	53º19'15" W	Collector	CHUNB 32819 - 32830
Alto Araguaia	Saramandaia Farm - Line 10	MT	17º54'15" S	53º27'58" W	Collector	CHUNB 27809, 27818
Alto Araguaia	Saramandaia Farm - Line 9	MT	17º54'15" S	53º27'58" W	Collector	CHUNB 27785
Barra do Garças	PCH Toricoejo	MT	15º15'12" S	53º07'26" W	Locality description (www.epe.gov.br)	RMH 11, 28, 29, 49
Barra do Garças		MT	15º21'34" S	52º29'49" W	Municipality centroid	MZUSP 89985
Brasnorte		MT	12º25'31" S	57º59'43" W	Municipality centroid	UFMT-R 8117, 8118
Canabrava do Norte	Region of direct influence of BR 158	MT	11º14'09" S	51º41'11" W	Collector	CHUNB 47844 - 47847
Canarana	Peixe Boi Farm	MT	13º19'52" S	52º23'55" W	Municipality centroid	MZUSP 88867 - 88870
Chapada dos Guimarães	APM Manso	MT	14º54'36" S	55º42'00" W	Locality description	UFMT-R 389 - 392, 1075 - 1099, 1143, 2417, 2419 - 2423, 2426, 2427; MZUSP 88247 - 88252, 92044, 92060, 92061

Chapada dos Guimarães		MT	15º06'25" S	55º32'22" W	Collection data	M 1230
Chapada dos Guimarães		MT	14º59'36" S	55º53'11" W	Collector	CHUNB 09477 - 09481, 09483, 09484
Chapada dos Guimarães		MT	15º16'11" S	55º50'24" W	Locality description	MZUSP 79358 - 79363
Cocalinho		MT	13º52'07" S	51º08'50" W	Municipality centroid	LG 1294; MZUSP 83079 - 83093
Cuiabá	Ribeirão do Forte	MT	15º20'02" S	55º56'49" W	Locality description	UFMT-R 6945, 6947, 6983, 6990, 7045, 7046
Gaúcha do Norte		MT	13º14'00" S	53º04'00" W	Locality description	MZUSP 89982 - 89984
Gaúcha do Norte		MT	12º59'46" S	53º27'21" W	Municipality centroid	MZUSP 81829 - 81840
Nova Lacerda		MT	14º18'01" S	59º46'16" W	Municipality centroid	UFMT-R 7911
Nova Monte Verde	Paranorte district - left margin of São João da Barra River	MT	10º19'56" S	57º39'02" W	Collection data	UFMT-R 5965, 5966, 5991, 6758, 6873, 6874
Nova Nazaré	Pindaíba	MT	14º22'00" S	51º43'00" W	Yonenaga-Yassuda & Rodrigues (1999)	LG 1300
Nova Xavantina	Rancho Ponte de Pedra Farm	MT	14º47'42" S	52º38'30" W	Collector	CHUNB 63245 - 63390, 63829
Nova Xavantina	UNEMAT	MT	14º41'55" S	52º21'03" W	Collector	CHUNB 63238 - 63244, 63814

Novo Santo Antônio	Parque Estadual do Araguaia	MT	12º23'05" S	50º53'36" W	Collector	CHUNB 57786
Novo São Joaquim	AHE Agua Limpa	MT	15º17'34" S	53º45'39" W	Locality description	MCP 18029, P18030
Paranatinga		MT	13º40'36" S	54º06'31" W	Municipality centroid	UFMT-R 7280
Ribeirão Cascalheira	Region of direct influence of BR 158	MT	12º50'55" S	51º44'45" W	Collector	CHUNB 47972 - 47979
Ribeirão Cascalheira	Serra do Roncador - Aldeia de Caça	MT	12º51'00" S	51º45'00" W	geoLoc	MZUSP 4741, 4742
Santa Terezinha	Tapirapé River	MT	10º41'00" S	50º38'00" W	Locality description	MZUSP 9745 - 9756, 13970 - 13981; MNRJ 2070, 4535 - 4609
São Félix do Araguaia	São Domingos - das Mortes River	MT	11º45'00" S	50º44'00" W	USBGN	MZUSP 2642
São José do Rio Claro		MT	13º35'46" S	56º47'40" W	Municipality centroid	UFMT-R 5844
Sapezal	UHE Cachoeirão	MT	13º32'00" S	58º47'59" W	Locality description	MZUSP 96034
Sapezal		MT	13º09'07" S	58º39'12" W	Municipality centroid	UFMT-R 7453
Sorriso	Boa Esperança	MT	13º30'32" S	55º08'32" W	Locality description	MTR 15561
Conceição do Araguaia	São José da Fortaleza Farm - Mata do Buraco	PA	08º08'33" S	49º20'20" W	Collection data	CHUNB 58850
Conceição do		PA	08º10'57" S	49º30'45" W	Municipality centroid	MPEG 149

Araguaia						
Ourilândia do Norte	Vermelho River - Kayapó's tribe	PA	07º40'25" S	51º13'11" W	Collector	MZUSP 78398
Palestina do Pará	UHE Santa Isabel - Pedral do Araguaia	PA	06º07'24" S	48º24'45" W	Collector	CHUNB 47245
Santana do Araguaia		PA	09º43'37" S	50º10'52" W	Collection data	CHUNB 58075, 58078, 58085
Santana do Araguaia		PA	09º40'52" S	50º09'29" W	Collection data	CHUNB 58074
Pimenta Bueno		RO	11º48'31" S	60º43'22" W	Collector	CHUNB 18050, 18051 - 18110, 18112 - 18162
Vilhena	BR-399 km 21-23/km 53-55	RO	12º28'12" S	60º31'12" W	Locality description	MZUSP 64613, 64614
Vilhena		RO	12º28'24" S	60º17'22" W	Collector	CHUNB 11973 - 12000, 12281 - 12300, 12361 - 12379
Águas de Santa Bárbara	Estação Ecológica Águas de Santa Bárbara	SP	22º47'23" S	49º14'35" W	Collector	MZUSP 94181
Águas de Santa Bárbara	Estação Ecológica Águas de Santa Bárbara	SP	22º47'13" S	49º14'04" W	Collector	MZUSP 94180, 94182 - 94184
Angatuba	Três Lagoas Farm	SP	23º22'59" S	48º27'13" W	Collection data	MZUSP 99399, 99400
Brotas	EE Itirapina	SP	22º12'25" S	47º56'14" W	Collector	CN 00232
Cajuru		SP	21º16'32" S	47º18'36" W	Municipality centroid	MZUSP 10299

Descalvado		SP	21º52'51" S	47º39'14" W	Municipality centroid	ZUEC 2913
Santa Rita do Passa Quatro	ARIE Pé do Gigante	SP	21º38'21" S	47º38'36" W	Collector	CN 00344
Santa Rita do Passa Quatro		SP	21º41'00" S	47º29'00" W	Collection data	MZUSP 89981
São José do Rio Preto		SP	20º49'12" S	49º22'44" W	Collection data	MZUSP 75189, 75219 - 75221
Teodoro Sampaio	Parque Estadual do Morro do Diabo	SP	22º34'59" S	52º17'59" W	Collection data	MZUSP 95248 - 95267, 95844
Caseara		TO	09º24'01" S	49º50'52" W	Municipality centroid	CHUNB 13894
Lagoa da Confusão	Ilha do Bananal	TO	11º18'00" S	50º16'48" W	Locality description	MZUSP 4743 - 4745
Peixe	UHE Peixe Angical	TO	12º01'47" S	48º32'59" W	Locality description	MZUSP 95927
Pium	Ilha do Bananal	TO	10º27'14" S	50º28'20" W	Collector	CHUNB 10461 - 10466, 10574 - 10591
Pium	Parque Estadual do Cantão	TO	09º58'44" S	50º02'13" W	Collection data	CHUNB 58083
Pium	Parque Estadual do Cantão	TO	09º23'42" S	50º00'05" W	Collection data	CHUNB 58086
Pium	Parque Nacional do Araguaia	TO	10º23'09" S	50º07'58" W	Locality description	LG 1390 - 1393
São Salvador do Tocantins		TO	12º29'08" S	48º15'54" W	Pavan (2007)	MZUSP 89151

Appendix 2 . List of samples of *Micrablepharus atticolus* used for amplification of a cytochrome *b* fragment and corresponding haplotype number. Total sequence length excluding primer regions is 715 bp. Samples were provided by Coleção Herpetológica da Universidade de Brasília (CHUNB), Coleção de Tecidos de Vertebrados do Departamento de Zoologia da Universidade de São Paulo (CTZOO-USP), Coleção de Répteis do Museu de Zoologia da Universidade de São Paulo (MZUSP), and Coleção Zoológica de Vertebrados da Universidade Federal do Mato Grosso (UFMT-R). Additionally, specimens of *M. maximiliani* and *Vanzosaura rubricauda* used as outgroups are listed.

Species	Collection	Specimen's code	Haplotype	Municipality	Locality	State	Length
M. atticolus	CHUNB	CHUNB 59852	H01	Brasília	Água Limpa Farm	DF	715 bp
M. atticolus	CHUNB	CHUNB 59856	H02	Brasília	Água Limpa Farm	DF	682 bp
M. atticolus	CHUNB	CHUNB 59961	H01	Brasília	Água Limpa Farm	DF	715 bp
M. atticolus	CHUNB	CHUNB 59976	H03	Brasília	Água Limpa Farm	DF	715 bp
M. atticolus	CHUNB	CHUNB 59987	H04	Brasília	Água Limpa Farm	DF	715 bp
M. atticolus	CHUNB	FAL01CE06	H05	Brasília	Água Limpa Farm	DF	688 bp
M. atticolus	CHUNB	FAL05CE10	H06	Brasília	Água Limpa Farm	DF	715 bp
M. atticolus	CHUNB	CHUNB 21822	H04	Brasília	Área Alfa (CIAB) - CEE c	DF	715 bp
M. atticolus	CHUNB	CHUNB 38480	H04	Brasília	IBGE - Mata de Galeria	DF	715 bp
M. atticolus	CHUNB	CHUNB 59700	H07	Brasília	Parque Nacional de Brasília	DF	684 bp
M. atticolus	CHUNB	CHUNB 59738	H08	Brasília	Parque Nacional de Brasília	DF	674 bp
M. atticolus	CHUNB	CHUNB 59753	H09	Brasília	Parque Nacional de Brasília	DF	683 bp

M. atticolus	CHUNB	CHUNB 59761	H04	Brasília	Parque Nacional de Brasília	DF	715 bp
M. atticolus	CHUNB	CHUNB 60071	H04	Brasília	Parque Nacional de Brasília	DF	715 bp
M. atticolus	CHUNB	CHUNB 23827	H04	Brasília		DF	715 bp
M. atticolus	CHUNB	CHUNB 24055	H10	Brasília		DF	715 bp
M. atticolus	CTZOO-USP	MZUSP 89988	H11	Caldas Novas		GO	679 bp
M. atticolus	CTZOO-USP	MZUSP 89989	H12	Caldas Novas		GO	715 bp
M. atticolus	CTZOO-USP	PHV 2846	H13	Santa Rita do Araguaia		GO	715 bp
M. atticolus	CTZOO-USP	PHV 2847	H13	Santa Rita do Araguaia		GO	715 bp
M. atticolus	CHUNB	CHUNB 58523	H14	Serranópolis	Casarão de Pedra	GO	715 bp
M. atticolus	CHUNB	CHUNB 37309	H15	Arinos	RPPN Arara Vermelha and Vereda do Pacari	MG	715 bp
M. atticolus	CHUNB	CHUNB 37312	H16	Arinos	RPPN Arara Vermelha and Vereda do Pacari	MG	715 bp
M. atticolus	CTZOO-USP	URB 61	H17	Curvelo	Curvelo/Pompéu	MG	715 bp
M. atticolus	CHUNB	CHUNB 26021	H18	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26022	H19	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26023	H20	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26025	H21	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26201	H22	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26202	H22	Paracatu		MG	715 bp
M. atticolus	CHUNB	CHUNB 26204	H19	Paracatu		MG	715 bp

M. atticolus	MZUSP	CTMZ 04993	H23	Bataguassu		MS	661 bp
M. atticolus	UFMT-R	UFMT-R 9276	H24	Alta Floresta	PCH Apiacás	MT	683 bp
M. atticolus	MZUSP	RMH 11	H25	Barra do Garças	PCH Toricoejo	MT	715 bp
M. atticolus	MZUSP	RMH 28	H26	Barra do Garças	PCH Toricoejo	MT	715 bp
M. atticolus	MZUSP	RMH 49	H27	Barra do Garças	PCH Toricoejo	MT	715 bp
M. atticolus	CTZOO-USP	MZUSP 89985	H28	Barra do Garças		MT	715 bp
M. atticolus	CTZOO-USP	LG 1294	H29	Cocalinho		MT	715 bp
M. atticolus	UFMT-R	UFMT-R 6945	H30	Cuiabá	Ribeirão do Forte	MT	681 bp
M. atticolus	UFMT-R	UFMT-R 6947	H31	Cuiabá	Ribeirão do Forte	MT	684 bp
M. atticolus	CTZOO-USP	MZUSP 89982	H32	Gaúcha do Norte		MT	715 bp
M. atticolus	CTZOO-USP	MZUSP 89983	H33	Gaúcha do Norte		MT	715 bp
M. atticolus	CTZOO-USP	MZUSP 89984	H32	Gaúcha do Norte		MT	715 bp
M. atticolus	UFMT-R	UFMT-R 5966	H34	Nova Monte Verde	Paranorte district - left margin of São João da Barra River	MT	715 bp
M. atticolus	UFMT-R	UFMT-R 6874	H34	Nova Monte Verde	Paranorte district - left margin of São João da Barra River	MT	715 bp
M. atticolus	CTZOO-USP	LG 1300	H35	Nova Nazaré	Pindaíba	MT	715 bp
M. atticolus	CHUNB	CHUNB63245	H36	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	684 bp
M. atticolus	CHUNB	CHUNB63246	H37	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63247	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63248	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp

M. atticolus	CHUNB	CHUNB63249	H39	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63250	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63251	H40	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63252	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63253	H41	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63254	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63256	H42	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63257	H42	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63258	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63260	H38	Nova Xavantina	Rancho Ponte de Pedra Farm	MT	715 bp
M. atticolus	CHUNB	CHUNB63238	H43	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63239	H43	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63240	H44	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63814	H43	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63241	H45	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63242	H46	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63243	H47	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB63244	H43	Nova Xavantina	UNEMAT	MT	715 bp
M. atticolus	CHUNB	CHUNB 57786	H48	Novo Santo Antônio	Parque Estadual do Araguaia	MT	715 bp

M. atticolus	MZUSP	MZUSP 96034	H49	Sapezal	UHE Cachoeirão	MT	715 bp
M. atticolus	CHUNB	CHUNB 18050	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18051	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18052	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18056	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18073	H51	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18077	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18092	H51	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18093	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18095	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18096	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18099	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18108	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18112	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18117	H51	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 18118	H50	Pimenta Bueno		RO	715 bp
M. atticolus	CHUNB	CHUNB 11979	H52	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 11981	H53	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 11982	H54	Vilhena		RO	715 bp

M. atticolus	CHUNB	CHUNB 11983	H55	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 11990	H56	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 11996	H57	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 11999	H56	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12000	H58	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12283	H54	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12295	H54	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12296	H59	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12363	H60	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12366	H57	Vilhena		RO	715 bp
M. atticolus	CHUNB	CHUNB 12368	H57	Vilhena		RO	715 bp
M. atticolus	MZUSP	MZUSP 94183	H61	Águas de Santa Bárbara	Estação Ecológica Águas de Santa Bárbara	SP	684 bp
M. atticolus	MZUSP	MZUSP 94184	H62	Águas de Santa Bárbara	Estação Ecológica Águas de Santa Bárbara	SP	677 bp
M. atticolus	MZUSP	MZUSP 95927	H63	Peixe	UHE Peixe Angical	TO	715 bp
M. atticolus	CHUNB	CHUNB 10461	H64	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10462	H48	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10574	H64	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10575	H65	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10577	H65	Pium	Ilha do Bananal	TO	715 bp

M. atticolus	CHUNB	CHUNB 10578	H66	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10581	H67	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10583	H68	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10584	H64	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CHUNB	CHUNB 10590	H69	Pium	Ilha do Bananal	TO	715 bp
M. atticolus	CTZOO-USP	LG 1390	H70	Pium	Parque Nacional do Araguaia	TO	715 bp
M. atticolus	CTZOO-USP	LG 1391	H64	Pium	Parque Nacional do Araguaia	TO	715 bp
M. atticolus	CTZOO-USP	LG 1392	H48	Pium	Parque Nacional do Araguaia	TO	715 bp
M. atticolus	CTZOO-USP	LG 1393	H67	Pium	Parque Nacional do Araguaia	TO	715 bp
M. maximiliani	CHUNB	CHUNB 33085		Alvorada do Norte		GO	715 bp
M. maximiliani	CHUNB	CHUNB 22676		Guajará-Mirim		RO	715 bp
M. maximiliani	CHUNB	CHUNB 27274		Mateiros		TO	715 bp
V. rubricauda	CHUNB	CHUNB 51300		Cocos		BA	715 bp
V. rubricauda	CHUNB	CHUNB 58572		Aquidauana		MS	715 bp
V. rubricauda	CHUNB	CHUNB 28174		Mateiros		TO	715 bp