INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ECOLOGIA DE ANUROS DO OESTE DO PARÁ E O USO DE FERRAMENTAS ALTERNATIVAS PARA ECOTURISMO E CONSERVAÇÃO DE ESPÉCIES

Manaus, Amazonas
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INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

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ECOLOGIA DE ANUROS DO OESTE DO PARÁ E O USO DE FERRAMENTAS ALTERNATIVAS PARA ECOTURISMO E CONSERVAÇÃO DE ESPÉCIES

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 08 dias do mês de Março do ano de 2022, às 09h00min, no Auditório dos PPGs ATU/ECO/CFT – INPA, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o Dr. **Igor Luis Kaefer**, da Universidade Federal do Amazonas – UFAM, o Dr. **Emil José Hernández Ruz**, da Universidade Federal do Pará – UFPA, o Dr. **Mario Eric Cohn-Haft**, do Instituto Nacional de Pesquisas da Amazônia – INPA, a Dra. **Fernanda de Pinho Werneck**, do Instituto Nacional de Pesquisas da Amazônia – INPA e o Dr. **Marcelo Gordo**, da Universidade Federal do Amazonas – UFAM, tendo como suplentes o Dr. Fabricio Beggiato Baccaro, da Universidade Federal do Amazonas - UFAM e o Dr. Miquéias Ferrão da Silva Junior, da Universidade Harvard, sob a presidência do orientador, a fim de proceder a arguição pública do trabalho de **TESE DE DOUTORADO** de **KELLY CRISTHYNA TORRALVO**, intitulado: **"ECOLOGIA DE ANUROS DO OESTE DO PARÁ E O USO DE FERRAMENTAS ALTERNATIVAS PARA ECOTURISMO E CONSERVAÇÃO DE ESPÉCIES"**, orientada pelo Dr. William Ernest Magnusson, do Instituto Nacional de Pesquisas da Amazônia – INPA e Co-orientada pelo Dr. Rafael de Fraga, da Universidade do Oste do Pará – UFOPA.

Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

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Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

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O investimento na ciência é a fonte para o futuro!

RESUMO

Informações sobre a biodiversidade e relações entre espécies e ambientes nativos ou antrópicos, são fundamentais para tomadas de decisões sobre políticas e programas de conservação. No Brasil, a conservação da biodiversidade é principalmente viabilizada pela implementação de diferentes tipos de unidades de conservação (UCs). As Florestas Nacionais (FLONAs) são áreas protegidas de uso sustentável, que permitem a permanência de populações humanas, com base em diretrizes estabelecidas pelo plano de gestão. Em contraste, áreas de proteção ambiental (APAs) permitem uma ampla variedade de usos e ocupações do solo, incluindo a expansão de zonas urbanas, agropecuária e outros tipos de indústria, que as tornam muito suscetíveis aos potenciais impactos negativos de ações antrópicas, como o desmatamento. A região oeste do Pará oferece uma grande oportunidade para investigarmos como um gradiente de desmatamento entre uma APA (Alter do Chão) e uma FLONA (Tapajós) afeta a biodiversidade, já que sofre as consequências ambientais do cultivo de soja e da especulação imobiliária. Por sua beleza cênica, essa região também oferece oportunidades para o ecoturismo, embora ainda seja carente de material de apoio para guias e turistas. Uma das razões pela carência pode ser a dificuldade inerente à identificação de espécies, uma vez que a Amazônia abriga a maior diversidade de anfíbios do mundo. Por isso, na primeira parte dessa tese, nós usamos técnicas de espectroscopia de infravermelho próximo (NIR) e análises multivariadas, para obter assinaturas espectrais espécie-específicas, que funcionam como impressões digitais. Nós aplicamos NIR tanto em espécimes depositados em meio líquido (fixadas em formol e mantidas e etanol) em coleções zoológicas (Capítulo 1), quanto em indivíduos vivos (Capítulo 2) para testar a eficiência da técnica para identificar espécies e diferenciar espécies morfologicamente semelhantes. Nós obtivemos taxas de até 100% de eficiência no reconhecimento de espécimes depositados em coleção herpetológica, mesmo que por até 30 anos. Também identificamos que o ventre é a posição para a coleta de dados espectrais que gera os melhores resultados. Para anuros vivos, um equipamento portátil de NIR foi capaz de identificar corretamente 62,5% das espécies testadas, com taxas de acerto acima de 80%. No entanto, detectamos alguns vieses no uso dessa técnica que poderão direcionar novos estudos para otimização das taxas de acerto. Na segunda parte dessa tese (Capítulo 3), usamos anfíbios anuros (sapos, pererecas e rãs) como modelos para investigar os efeitos de desmatamento e outros gradientes ambientais sobre

estimativas de variação na diversidade de espécies e traços funcionais (e.g., características morfológicas, tipo de microhábitat mais frequentemente ocupado, modo reprodutivo). Por meio de amostragem padronizada em 57 parcelas, nós observamos que as assembleias de anuros são localmente estruturadas por substituição de espécies ao longo de gradientes de distância de corpos d'água, estrutura da vegetação, conteúdo de areia e silte do solo e proporção da área desmatada. Além disso, observamos que as espécies de corpo pequeno e aquelas com reprodução direta (sem estágio larval) não estão mais presentes nas áreas mais desmatadas. Esses resultados sugerem a importância da criação de reservas para proteger as florestas e manter a conectividade entre os fragmentos florestais resultantes de desmatamento, os quais estão principalmente concentrados na APA, região menos protegida pela legislação e fiscalização ambiental. Considerando a combinação entre diversidade de anuros dependente da integridade de florestas, e o potencial ecoturístico da área de estudo, na terceira parte dessa tese (Capítulo 4) nós desenvolvemos e disponibilizamos gratuitamente dois produtos de uso popular enfocados no ecoturismo como método de conservação, que são um guia ilustrado de identificação de anuros (Guia de Sapos da Floresta Nacional do Tapajós, disponível em formato impresso e E-book), e um aplicativo digital para celulares e tablets (BioCheck Tapajós), o qual será continuamente atualizado com informações e fotos de vários grupos de animais e plantas. O objetivo de ambos os produtos é fomentar o protagonismo das populações locais nas práticas de ecoturismo, uma vez que são materiais didáticos eficientes e gratuitos para educação ambiental e popularização do conhecimento científico.

ABSTRACT

Information on biodiversity and relationships between species and native or human-altered environments are essential for decision-making on conservation policies and programs. In Brazil, biodiversity conservation is mainly done by the implementation of different types of reserves (UCs - Brazilian acronym for “conservation units”, a term used for environmental reserves). National Forests (FLONAs) are protected areas for sustainable use, which allow populations to remain living within their borders, based on a guidelines established by the management plan. In contrast, environmental protection areas (APAs) allow a wide variety of land uses and occupations, including the expansion of urban areas, agriculture and other types of industry, which make them very susceptible to the potential negative impacts of human actions, such as deforestation. The western region of Pará provides a great opportunity to investigate how a deforestation gradient between an APA (Alter do Chão) and a FLONA (Tapajós) affects biodiversity. Due to its scenic beauty, this region also provides opportunities for ecotourism, although it still lacks support material for guides and tourists. One of the reasons for the lack may be the inherent difficulty in identifying species, since the Amazon has the greatest biodiversity in the world. Therefore, in the first part of this thesis, we used near-infrared (NIR) spectroscopy techniques and multivariate analysis to obtain species-specific spectral signatures, which act as fingerprints. We applied NIR both on specimens preserved in formalin and conservedin ethanol within zoological collections (Chapter 1), and on live individuals (Chapter 2) to test the efficiency of the technique to identify frog species and differentiate morphologically similar species. We have achieved success rates of up to 100%, even for specimens conserved over 30 years. We also identified that the belly is the position for collecting spectral data that generates the best results. For live frogs, a portable NIR device was able to correctly identify 62.5% of the species tested, with success rates above 80%. However, we detected some biases in the use of this technique that may direct further studies to optimize success rates. In the second part of this thesis (Chapter 3), we used frogs as models to investigate the effects of deforestation and other environmental gradients on estimates of variation in species diversity and functional traits (e.g., morphological variables, type of most frequently occupied microhabitat, reproductive mode). Through standardized

sampling in 57 plots, we observed that frog assemblages are locally structured by species turnover along gradients of distance from water bodies, vegetation structure, soil sand and silt content, and proportional deforestation. Furthermore, we observed that small-bodied species and those with direct reproduction (without larval stage) are no longer present in the most deforested areas. These results suggest the importance of creating reserves to protect forests and maintain connectivity between forest fragments, which are mainly concentrated in the APA, a region less protected by legislation and environmental inspection. Considering the combination of frog diversity dependent on the integrity of forests, and the ecotourism potential of the study area, in the third part of this thesis (Chapter 4) we developed two freely distributed products focused on ecotourism as a conservation method, which are an illustrated guide to frog identification (Guide to the Frogs of the Floresta Nacional do Tapajós, available in print and E-book format), and a digital app for mobiles and tablets (BioCheck Tapajós), which will be continually updated with information and photos of many groups of animals and plants. The objective of both products is to promote the role of local people in ecotourism practices, since they are efficient and easy-to-use materials for environmental education and popularization of scientific knowledge.

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INTRODUÇÃO GERAL

Embora investigar o papel de gradientes ambientais naturais e antropogênicos seja uma demanda contínua para a conservação da biodiversidade, esse tipo de estudo é geralmente desafiador em regiões megadiversas como a Amazônia. Uma das principais dificuldades é identificar corretamente as espécies-alvo, uma vez que existem muitas espécies morfologicamente semelhantes para as quais caracteres diagnósticos observáveis não têm sido claramente definidos, e muita diversidade críptica, que pode levar ao negligenciamento de espécies não descritas. Por exemplo, cerca de 329 espécies de anfíbios anuros são conhecidas na Amazônia Brasileira (Hoogmoed 2019), mas essa estimativa deve ser muito enviesada já que novas espécies são descritas todos os anos (e.g., Carvalho et al. 2020, Melo-Sampaio et al. 2021, Moraes & Lima 2021), corroborando com a previsão feita na ultima década de que 40% das espécies neotropicais ainda não são descritas (Giam *et al.* 2012). Portanto, existe uma demanda urgente de se estabelecer métodos seguros e eficientes para a identificação de espécies, uma vez que esse geralmente é o primeiro passo para modelagens ecológicas que são usadas como embasamento técnico para tomadas de decisão sobre conservação.

Classificar um organismo até o nível taxonômico mais específico possível é altamente desejável para que profissionais de diferentes áreas e tomadores de decisões possam se comunicar durante o planejamento e execução de ações de conservação (Gotelli 2004). Erros na identificação de espécimes podem resultar em vieses de amostragem que irão refletir em manejo e tomadas de decisão ineficientes ou mesmo danosas (Bortolus 2008). Porém, identificar espécimes geralmente depende de taxonomistas especialistas (Margules & Pressey 2000, Bortolus 2008), especialmente em regiões megadiversas como a Amazônia (Jenkins *et al.* 2013, Valsecchi *et al.* 2017). No entanto, os números de especialistas em alguns grupos de organismos vêm diminuindo na Amazônia desde a década de 1990, uma vez que a maior concentração de especialistas atua fora desse bioma (Marques & Lamas 2006). Consequentemente, existe muito material com erros de identificação em coleções biológicas de instituições na Amazônia e em bancos de dados globais de biodiversidade, o que obviamente gera desconfiança na qualidade dos dados. Diante disso, o desenvolvimento de novas técnicas e ferramentas para auxiliar a identificação de espécimes é necessário para amenizar um problema que potencialmente afeta todos os níveis no fluxo de trabalho enfocado na conservação da biodiversidade (Dayrat 2005). Adicionalmente, técnicas

eficientes de identificação podem contribuir para o descobrimento de novas espécies ou para solucionar diversidade críptica, integrando a taxonomia que delimita as unidades de diversidade e são fundamentais para ações de conservação (Dayrat 2005, Padial *et al.* 2010).

Uma das técnicas com potencial eficiente para o reconhecimento de indivíduos das espécies, independentemente da disponibilidade de especialistas, é a espectroscopia de infravermelho próximo (NIR). Essa é uma ferramenta que capta as vibrações moleculares de compostos químicos presentes na estrutura de uma amostra por meio de radiação de ondas eletromagnéticas (Pasquini 2003, Pavia *et al.* 2010). Quando aliada a técnicas multivariadas, NIR caracteriza amostras orgânicas, que podem ser organismos inteiros (Vance *et al.* 2016) ou pedaços de organismos (Rigby *et al.* 2014), sendo possível obter uma assinatura molecular (Jouquet *et al.* 2014) atribuída ao reconhecimento refinado de amostras. Essa técnica gera informações análogas a uma impressão digital de cada espécie, de modo que habilita identificações taxonômicas precisas de plantas (Fan *et al.* 2010, Lang *et al.* 2015, 2017, Hadlich *et al.* 2018) e animais (Rodriguez-Fernandez *et al.* 2011, Rigby *et al.* 2014, Vance *et al.* 2014, 2016, Almeida de Azevedo *et al.* 2019), incluindo anuros (Vance *et al.* 2014). Além disso, o uso da técnica pode ser uma alternativa viável ou de caráter complementar às abordagens usadas na taxonomia clássica, pois são altamente econômicas (considerando que a aquisição dos equipamentos ocorre uma única vez), rápidas e não destrutivas, como no caso da aquisição de espectros em animais inteiros (Rodriguez-Fernandez *et al.* 2011, Vance *et al.* 2016).

Nos **capítulos 1 e 2** dessa tese nós testamos a eficiência de técnicas de NIR para distinguir pares de espécies de anuros de diferentes famílias e tamanhos. Nós utilizamos um equipamento de bancada com transformada de Fourier (FT-NIR) em espécimes de anuros de coleções zoológicas (capítulo 1), e um equipamento portátil (VIS-NIR) em indivíduos vivos (capítulo 2). Além dos testes de eficiência, discutimos protocolos de coleta de dados espectrais para guiar o uso dessas técnicas em estudos de biodiversidade e que possam também ser utilizadas em planos de gestões ambientais e estratégias de conservação.

No Brasil, existem tipos de unidades de conservação (UCs) que diferem pelo nível de rigidez no controle do uso e ocupação do solo. As Florestas Nacionais (FLONAs) exigem a desapropriação de áreas particulares, mas permitem a permanência de populações tradicionais e o uso para visitação turística, desde que sejam respeitadas

as diretrizes de zoneamento estabelecidas pelo plano de gestão. Em contraste, áreas de proteção ambiental (APAs) são muito mais flexíveis em relação ao controle do uso e ocupação do solo, e permitem a expansão de zonas urbanas, agropecuárias, e de uso por outras indústrias (ICMBio 2009), o que as tornam muito suscetíveis aos potenciais impactos negativos de ações antrópicas, como o desmatamento.

Com a disponibilidade de um protocolo simples e reproduzível para identificar espécies em uma região tão diversa como a Amazônia, modelos ecológicos concentrados em testar associações entre espécies e hábitats são muito confiáveis e úteis para conservação. Essas associações podem ser testadas pela quantificação da heterogeneidade ambiental gerada tanto por gradientes naturais quanto por distúrbios antropogênicos, como desmatamento (e.g., Benchimol & Peres, 2015; Cintra et al., 2013). De modo geral, é amplamente esperado que processos ecológicos como filtragem ambiental, competição, dispersão reduzida ou bloqueada e intolerância fisiológica limitem a distribuição local de algumas espécies, mesmo que sejam amplamente distribuídas em escalas de paisagem (Azevedo-Ramos & Magnusson, 1999; Braga-Neto et al., 2008; Carr e Fahrig, 2001). No entanto, uma decisão importante na implementação de modelos ecológicos usados para testar associações entre a biodiversidade e gradientes ambientais é sobre o tipo de dados que serão utilizados para quantificar biodiversidade. A maioria dos estudos tem abordado comunidades ou assembleias (*sensu* Stroud *et al.* 2015) caracterizadas por dados de ocorrência ou abundância de espécies, mas essa abordagem potencialmente negligencia a distribuição espacial de espécies mediada pelos hábitats selecionando traços morfológicos ou relativos a aspectos da história natural (e.g., microhábitat mais frequentemente ocupado, modo reprodutivo). Alternativamente, é possível caracterizar comunidades ou assembleias com base em traços funcionais, o que deve gerar respostas complementares da biodiversidade à variação ambiental, em relação às métricas baseadas em ocorrência ou abundância de espécies (e.g., Farneda et al., 2015).

A região oeste do Pará oferece uma ótima oportunidade para investigarmos como gradientes de ocupação humana mediados pelo estabelecimento de diferentes tipos de UCs podem afetar a biodiversidade. Na margem leste do baixo Rio Tapajós está a FLONA do Tapajós que contém grandes extensões de cobertura florestal íntegra e, ao norte da FLONA, está a APA de Alter do Chão que se encontra bastante fragmentada por desmatamento. A região sofre as consequências ambientais do cultivo de soja que há décadas vem sendo alertada como um impulso ao desmatamento na Amazônia

(Fearnside, 2001), além da pressão imobiliária. Juntas as áreas representam um gradiente de desmatamento crescente na direção sul-norte. Diante disso, no **terceiro capítulo**, utilizamos essa região para testar a hipótese de que a composição de assembleias de anuros estimadas pela abundância de espécies e por características funcionais podem ser previstas por processos ecológicos de filtragem ambiental. Nós amostramos anuros em 57 parcelas padronizadas, distribuídas em quatro módulos RAPELD (Magnusson et al., 2005) na FLONA Tapajós e em conjuntos de transectos localizados na APA de Alter do Chão. Especificamente, usamos modelos lineares para testar os efeitos de gradientes de distância de corpos d'água, cobertura vegetal, serapilheira, conteúdo de areia e silte do solo e proporção de desmatamento sobre estimativas de composição taxonômica (baseada em abundância de espécies) e funcional de assembleias de anfíbios anuros.

A FLONA do Tapajós é administrada com base em ações de gestão participativa, pelas quais os próprios moradores monitoram o uso de recursos florestais, como caça, pesca e coleta (ICMBio 2009). O monitoramento participativo também inclui a visitação frequente por turistas, a qual gera uma fonte alternativa de renda para muitas famílias que trabalham como guias e vendedores de artesanato. O protagonismo das comunidades tradicionais no ecoturismo é muito interessante de um ponto de vista da conservação, pois representa uma correlação positiva entre geração de benefícios para a população local e efetividade de estratégias em manter a floresta conservada (Gössling 1999, Stronza 2007, Peralta 2012). Visitas por turistas à FLONA do Tapajós ocorrem frequentemente, já que a área faz parte do circuito de passeios oferecido em municípios da região. Por exemplo, em 2017 foram registrados 42.636 visitantes, dos quais 28,45% (12.130) eram turistas, o que representao potencial da região para o ecoturismo (*dados não publicados* Equipe ICMBio/Santarém). No entanto, o potencial talvez pudesse ser mais bem aproveitado com a disponibilização de material de apoio, enfocado em guiar boas práticas de ecoturismo, e principalmente em criar expectativa e fornecer informações sobre animais e plantas que os turistas podem encontrar durante uma visita. Isso pode ser alcançado por meio de guias de identificação de animais e plantas, que sejam construídos com alta riqueza de imagens, linguagem simples e didática, e preferencialmente multilingues (por ex. português, inglês e línguas indígenas da região).

A disponibilização de guias ilustrados para identificação de espécies pode melhorar a qualidade dos serviços de ecoturismo, porque os usuários têm acesso

imediato a informações sobre biologia e distribuição das espécies que estão observando em hábitat natural. Atualmente, aplicativos digitais representam a modernização dos guias de identificação impressos tradicionais, a fim de facilitar o acesso às informações ecológicas e de biodiversidade e incluir informações coletadas por meio da ciência cidadã (por exemplo, https://mol.org/ e https://www.inaturalist.org/). Essas ferramentas tornam o ecoturismo local mais atrativo, o que é convertido em aumento na captação financeira e o interesse pela conservação da floresta. Portanto, o **quarto capítulo** dessa tese é destinado à apresentação de duas ferramentas de uso por não especialistas, sendo um guia de identificação bilíngue "Guia de Sapos Floresta Nacional do Tapajós", e um aplicativo digital interativo "BioCheck Tapajós". Essas ferramentas foram criadas tanto para simplificar o acesso à informação e direcionar atividades de ecoturismo, quanto para estimular o desenvolvimento da ciência cidadã, uma vez que os usuários do aplicativo poderão registrar animais e plantas com fotos e coordenadas geográficas, representando também a aquisição de novos dados.

REFERÊNCIAS

Almeida de Azevedo, R., J. Wellington de Morais, C. Lang, and C. de Sales Dambros. 2019. Discrimination of termite species using Near-Infrared Spectroscopy (NIRS). Eur. J. Soil Biol. 93: 103084. Available at: https://doi.org/10.1016/j.ejsobi.2019.04.002.

Azevedo-Ramos, C., and W. E. Magnusson. 1999. Tropical tadpole vulnerability to predation: Association between laboratory results and prey distribution in an Amazonian savanna. Copeia 58–67.

Benchimol, M., and C. A. Peres. 2015. Edge-mediated compositional and functional decay of tree assemblages in Amazonian forest islands after 26 years of isolation. J. Ecol. 103: 408–420.

Bortolus, A. 2008. Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. Ambio 37: 114–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18488554.

Braga-Neto, R., R. C. C. Luizão, W. E. Magnusson, G. Zuquim, and C. V. de Castilho. 2008. Leaf litter fungi in a Central Amazonian forest: The influence of rainfall, soil and topography on the distribution of fruiting bodies. Biodivers. Conserv. 17: 2701–2712.

Carr, L. W., and L. Fahrig. 2001. Effect of road traffic on two amphibian species of differing vagility. Conserv. Biol. 15: 1071–1078.

Carvalho, T. R. D., L. J. C. L. Moraes, A. P. Lima, A. Fouquet, P. L. V Peloso, D. Pavan, L. O. Drummond, M. T. Rodrigues, A. A. Giaretta, M. Gordo, S. Neckel-Oliveira, and C. F. B. Haddad. 2020. Systematics and historical biogeography of Neotropical foam-nesting frogs of the Adenomera heyeri clade (Leptodactylidae), with the description of six new Amazonian species. Zool. J. Linn. Soc. 1–39.

Cintra, R., W. E. Magnusson, and A. Albernaz. 2013. Spatial and temporal changes in bird assemblages in forest fragments in an eastern Amazonian savannah. Ecol. Evol. 3: 3249–3262.

Dayrat, B. 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85: 407–415.

Fan, Q., Y. Wang, P. Sun, S. Liu, and Y. Li. 2010. Discrimination of Ephedra plants with diffuse reflectance FT-NIRS and multivariate analysis. Talanta 80: 1245–1250. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0039914009007073.

Farneda, F. Z., R. Rocha, A. López-Baucells, M. Groenenberg, I. Silva, J. M. Palmeirim, P. E. D. Bobrowiec, and C. F. J. Meyer. 2015. Trait-related responses to habitat fragmentation in Amazonian bats. J. Appl. Ecol. 52: 1381–1391.

Fearnside, P. M. (2001). Soybean cultivation as a threat to the environment in Brazil. Environmental Conservation, 28(1), 23-38.

Giam, X., B. R. Scheffers, N. S. Sodhi, D. S. Wilcove, G. Ceballos, and P. R. Ehrlich. 2012. Reservoirs of richness: least disturbed tropical forests are centres of undescribed species diversity. Proc. R. Soc. B Biol. Sci. 279: 67–76. Available at: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2011.0433.

Gössling, S. 1999. Ecotourism: a means to safeguard biodiversity and ecosystem functions? Ecol. Econ. 29: 303–320. Available at: http://www.sciencedirect.com/science/article/pii/S0921800999000129.

Gotelli, N. J. 2004. A taxonomic wish-list for community ecology. Philos. Trans. R. Soc. B Biol. Sci. 359: 585–597.

Hadlich, H. L., F. M. Durgante, J. Dos Santos, N. Higuchi, J. Q. Chambers, and A. Vicentini. 2018. Recognizing Amazonian tree species in the field using bark tissues spectra. For. Ecol. Manage. 427: 296–304. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0378112717320121.

Hoogmoed, M. 2019. Censo da Biodiversidade da Amazônia Brasileira. Mus. Goeldi - Plataforma Digit. Available at: http://www.museu-goeldi.br/censo/.

ICMBio. 2009. Legislação ICMBio - Sistema Nacional de Unidades de Conservação. 1: 69. Available at: http://www.icmbio.gov.br/portal/images/stories/comunicacao/legislacaoambientalvolume1.pdf.

Jenkins, C. N., S. L. Pimm, and L. N. Joppa. 2013. Global patterns of terrestrial vertebrate diversity and conservation. Proc. Natl. Acad. Sci. U. S. A. 110: E2603–E2610.

Jouquet, P., Y. Capowiez, N. Bottinelli, and S. Traoré. 2014. Potential of Near Infrared Refl ectance Spectroscopy (NIRS) for identifying termite species. Eur. J. Soil Biol. 60: 49–52. Available at: http://dx.doi.org/10.1016/j.ejsobi.2013.11.004.

Lang, C., D. R. A. Almeida, and F. R. C. Costa. 2017. Discrimination of taxonomic identity at species, genus and family levels using Fourier Transformed Near-Infrared Spectroscopy (FT-NIR). For. Ecol. Manage. 406: 219–227. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0378112717309672.

Lang, C., F. R. C. Costa, J. L. C. Camargo, F. M. Durgante, and A. Vicentini. 2015. Near Infrared Spectroscopy Facilitates Rapid Identification of Both Young and Mature Amazonian Tree Species D. Gomory (Ed.). PLoS One 10: e0134521. Available at: http://dx.plos.org/10.1371/journal.pone.0134521.

Magnusson, W. E., A. P. Lima, R. Luizão, F. Luizão, F. R. C. Costa, C. V. De Castilho, and V. F. Kinupp. 2005. RAPELD: a modification of the Gentry method for biodiversity surveys in long-term ecological research sites. Biota Neotrop. 5: 21–26.

Margules, C. R., and R. L. Pressey. 2000. Systematic conservation planning. Nature 405: 243–253.

Marques, A. C., and C. J. E. Lamas. 2006. Taxonomia zoológica no Brasil: Estado da arte, expectativas e sugestões de ações futuras. Pap. Avulsos Zool. 46: 139–174. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0031-10492006001300001&lng=en&nrm=iso&tlng=en.

Melo-Sampaio, P. R., M. Ferrão, and L. J. C. de Lima Moraes. 2021. a New Species of Osteocephalus Steindachner, 1862 (Anura, Hylidae), From Brazilian Amazonia. Breviora 572.

Moraes, L. J. C. L., and A. P. Lima. 2021. A New Nurse Frog (Allobates, Aromobatidae) with a Cricket-Like Advertisement Call from Eastern Amazonia. Herpetologica 77: 146–163.

Padial, J. M., A. Miralles, I. D. La Riva, and M. Vences. 2010. The integrative future of taxonomy Review. Front. Zool. 1–14. Available at: http://www.frontiersinzoology.com/content/7/1/16.

Pasquini, C. 2003. Near Infrared Spectroscopy: fundamentals, practical aspects and analytical applications. J. Braz. Chem. Soc. 14: 198–219. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-50532003000200006&lng=en&nrm=iso&tlng=en.

Pavia, D. L., G. M. Lampman, G. S. Kriz, and J. R. Vyvyan. 2010. Introdução à espectroscopia Cengage Learning (Ed.).

Peralta, N. 2012. Ecotourism as an incentive to biodiversity conservation: the case of Uakari Lodge, Amazonas, Brazil. Uakari 8: 75–94. Available at: http://www.uakari.org.br/index.php/UAKARI/article/view/133%5Cnhttp://uakari.org.br/index.php/UAKARI/article/view/133.

Rigby, C. L., B. B. Wedding, S. Grauf, and C. A. Simpfendorfer. 2014. The utility of near infrared spectroscopy for age estimation of deepwater sharks. Deep Sea Res. Part I 94: 184–194. Available at: http://dx.doi.org/10.1016/j.dsr.2014.09.004.

Rodriguez-Fernandez, J. I., C. J. B. De Carvalho, C. Pasquini, K. M. Gomes De Lima, M. O. Moura, and G. G. Carbajal Arizaga. 2011. Barcoding without DNA? Species identification using near infrared spectroscopy. Zootaxa 54: 46–54.

Stronza, A. 2007. The economic promise of ecotourism for conservation. J. Ecotourism 6: 210–230.

Stroud, J. T., M. R. Bush, M. C. Ladd, R. J. Nowicki, A. A. Shantz, and J. Sweatman. 2015. Is a community still a community? Reviewing definitions of key terms in community ecology. Ecol. Evol. 5: 4757–4765.

Valsecchi, J., M. Marmontel, C. L. B. Franco, D. P. Cavalcante, I. V. D. Cobra, I. J. Lima, J. M. Lanna, M. T. M. Ferreira, P. M. Nassar, R. Botero-Arias, and V. Monteiro. 2017. Atualização E Composição Da Lista - Novas Espécies De Vertebrados e Plantas Na Amazônia 2014-2015 Iniciativa. Brasília, DF e Tefé, AM Available at: https://d3nehc6yl9qzo4.cloudfront.net/downloads/relatorio_novas_especies___wwf_brasil_mamiraua_2.pdf.

Vance, C. K., A. J. Kouba, and S. T. Willard. 2014. Near Infrared Spectroscopy Applications in Amphibian Ecology and Conservation: Gender and Species Identification. NIR news 25: 10–15. Available at: http://journals.sagepub.com/doi/10.1255/nirn.1444.

Vance, C. K., D. R. Tolleson, K. Kinoshita, J. Rodriguez, and W. J. Foley. 2016. Near infrared spectroscopy in wildlife and biodiversity. J. Near Infrared Spectrosc. 24: 1–25.

OBJETIVOS

O principal objetivo desta tese foi fornecer informações sobre a comunidade de anuros em um gradiente natural e de perturbações na Amazônia Brasileira e sobre o uso de ferramentas alternativas para estudos e conservação de espécies.

Capítulo 1. Testar a eficiência da espectroscopia FT-NIR no reconhecimento de espécies de anuros da Amazônia, utilizando espécimes fixados com formaldeído e armazenados em álcool.

Capítulo 2. Testar a eficiência da espectroscopia com equipamento portátil no reconhecimento de anuros da Amazônia, utilizando indivíduos vivos coletados *in situ*.

Capítulo 3. Testar os efeitos de gradientes ambientais e de desmatamento sobre estimativas da diversidade taxonômica e funcional de comunidades de anfíbios anuros da Floresta Nacional (FLONA) do Tapajós e região de Alter do Chão - PA.

Capítulo 4. Embasar e apresentar a criação das ferramentas de uso popular para identificação de espécies: guia de identificação bilíngue "Guia de Sapos da Floresta Nacional do Tapajós", e aplicativo digital interativo "BioCheck Tapajós".

CAPÍTULO 1

Effectiveness of Fourier transform near-infrared spectroscopy spectra for species identification of anurans fixed in formaldehyde and conserved in alcohol: A new tool for integrative taxonomy.

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Title: Effectiveness of FT-NIR spectra for species identification of anurans fixed in formaldehyde and conserved in alcohol: a new tool for integrative taxonomy

Short running title: FT-NIR species identification of preserved anurans

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Abstract

Integrative taxonomy takes into account multiple perspectives to delimit the units of diversity, which is fundamental for communication in ecological studies and conservation actions. Near-infrared spectroscopy is efficient for identification of species in many taxa and may be a viable alternative to the descriptions used in classical taxonomy as it is highly cost-effective, rapid and can be non-destructive. Animal specimens are often conserved in alcohol and formaldehyde, which hinders some techniques. In this study, we tested the effectiveness of near-infrared spectroscopy in recognizing species of fixed specimens of closely related anuran species when the raw data were used and after preprocessing to reduce noise due to variation in surface structure. We used individuals of adult frogs belonging to different species, sizes and time conserved in alcohol. The tests were divided into spectral readings of the belly and back of each sample in wet and pre-drying manipulations. The results indicated that the presence of superficial alcohol in individuals does not impede the use of the technique to recognize anuran species. We recognized anuran species using spectra collected on the back or belly with up to 100% correct identification in raw data or preprocessed data. These results open up numerous possibilities for taxonomic investigations using individuals of zoological collections worldwide, but this requires the creation of open-access data banks and availability of spectra of all species.

Introduction

Currently, the number of frog species is estimated at 7248 (Frost 2019) with 329 for the Brasilian Amazon (Hoogmoed 2019). However, it is predicted that around 40% of Neotropical species are yet to be described (Giam *et al.* 2012). This is corroborated by frequent descriptions of new species from the Amazon (e.g., Ferrão *et al.*, 2017, 2018b; Kaefer *et al.*, 2019). "Species-level" identification is critical for communication in many ecological studies and conservation actions (Gotelli 2004), but using fine taxonomic levels to measure and map biodiversity is complex and generally dependent on experts (Margules & Pressey 2000, Bortolus 2008).

Integrative taxonomy (Dayrat 2005, Padial *et al.* 2010) takes into account multiple perspectives (e.g., phylogeography, morphology, genetics and ecology) as complementary to delimit the units of diversity, considering conceptual and technological advances, and has been used frequently in recent studies (e.g., Funk *et al.*, 2012; Moraes *et al.*, 2016; Ferrão *et al.*, 2016; Prata *et al.*, 2018; Kaefer *et al.*, 2019). This approach considers that taxonomic characters can be equivalent and combinable units, discarding the idea of a restrictive taxonomy for biodiversity studies (Padial *et al.* 2010). Mistakes in species identification can affect planning and result in inefficient management and decision making, in some cases even directly linked to human health (Bortolus 2008). Therefore, there is a need for simple and reliable tools suitable for different users, such as pharmacologists, physiologists, biologists, ecologists and biodiversity managers (Dayrat 2005).

Near-infrared spectroscopy (NIR) captures the molecular vibrations of chemical compounds in the structure of a sample (Pasquini 2003, Pavia *et al.* 2010), that may be a whole organism (Vance *et al.* 2016) or a part of an organism (Rigby *et al.* 2014). From the NIR spectra, it is possible to obtain a molecular signature (Jouquet *et al.* 2014) attributed to refined sample recognition that functions as a physicochemical fingerprint. Fourier transform near-infrared spectroscopy (FT- NIR) is used to recover the intensities of individual wavelengths and ensure better accuracy (Pasquini 2003).

Recent studies have shown the effectiveness of FT-NIR spectroscopy in botanical species identifications (Fan *et al.* 2010, Durgante *et al.* 2013, Lang *et al.* 2015, 2017, Hadlich *et al.* 2018, Prata *et al.* 2018). The method has also been used in vertebrate and invertebrate studies (Rodriguez-Fernandez *et al.*, 2011; Vance *et al.*, 2014; Vance *et al.*, 2016; Rigby *et al.*, 2014; Almeida de Azevedo *et al.*, 2019) and may

be a viable alternative to characteristics used in classical taxonomy as it is highly cost-effective, rapid and can be non-destructive (Rodriguez-Fernandez *et al.* 2011, Vance *et al.* 2016).

The data available in collections inform or contribute to the knowledge of species biology, ecology, and biogeography (Margules & Pressey 2000, Meineke *et al.* 2019). However, many species-identification errors or the lack of curation of deposited samples lead to problems in data use. Readings of the spectra in dry plants of herbarium collections brought an alternative to investigate identification problems using specimens already available without the need for new collections (Fan *et al.* 2010, Durgante *et al.* 2013, Lang *et al.* 2015, 2017, Prata *et al.* 2018). The same limitations found in botanical collections apply to zoological collections (Meineke *et al.* 2019). Unlike plants, animal specimens are often conserved in alcohol and formaldehyde, which hinders some techniques that use DNA extraction (Schander & Halanych 2005, Friedman & DeSalle 2008, Jaksch *et al.* 2016) and may also be limiting for using NIR techniques (Pasquini 2003, Almeida de Azevedo *et al.* 2019).

FT-NIR is affected both by the chemical composition of the sample and by the physical structure of the surface (Pasquini 2003, 2018). Therefore, readings may differ between different body regions on the same individual because of differences in rugosity. To reduce the effect of physical differences in samples, FT-NIR spectra can be preprocessed, which sometimes allows better detection of chemical differences (Rinnan *et al.* 2009, Pasquini 2018). Therefore, we analyzed the data before and after preprocessing using the Savitzky-Golay method (Barak 1995, Rinnan *et al.* 2009).

The efficient species identification of anuran species in biological collections by FT-NIR spectra is a promising new taxonomic method that may allow the construction of a reference database for species recognition. However, first it is necessary to determine if the method can distinguish closely related species based on alcohol- and formaldehyde-fixed specimens.

Considering the potential of FT-NIR spectroscopy and the need to develop tools for integrative anuran taxonomy, our principal objective was to test the effectiveness of the technique in recognizing anuran species with specimens that had been fixed with formaldehyde and stored in alcohol. We also considered two methodological questions: (1) is it necessary to dry the alcohol on the surface of samples and (2) what position on the body of individuals (back or belly) gives a more efficient spectral reading to distinguish anuran species?

Material and methods

Sample collection and FT-NIR spectroscopy readings

All anurans used were in the Collection of Amphibians and Reptiles of the National Institute of Amazonian Research – INPA-H, and had been fixed in formaldehyde and maintained in 70% alcohol for periods ranging from three to 28 years. Pairs (and one trio) of closely-related and sympatric species from four families were used. We used sympatric individuals because practical questions are often about species assemblages and to reduce the probability of including cryptic species. Many species of Amazonian frogs that previously were thought to have wide distributions have recently been separated into geographically separated species (e.g., *Boana semilineata* Species Group - Peloso *et al.*, 2018; and *Adenomera heyeri* clade - Carvalho *et al.*, 2020).

Two sets of readings were used in this study, one for each series of tests undertaken. Some species differed between the two series because some specimens were not available for loan on both occasions, but 53 individuals were used in both series. The first series of tests evaluated the influence of alcohol drying on species identification through the FT-NIR spectrum. For this series, a total of 89 anuran individuals belonging to eight species were used. Species of different body sizes (average 1.4 to 10.3 cm long and 0.5 to 3.2 cm wide) and storage time in alcohol (3 to 28 years) were selected (Table 1) to maximize variation and test the tool on realitistic samples from collections. Measurements in this dataset were divided into spectral readings of the belly and back of each sample (Figure 1) in two manipulations:
Wet - excess alcohol dried with an absorbent cloth (reusable cloth - Scott Duramax®) and spectral reading;

Dry - The same animals were used as in the wet manipulation, but a second reading was taken after the specimen had been drying at 24 ° C for 20 to 40 minutes (Table 1).

The drying manipulation did not eliminate the presence of formaldehyde or alcohol in the samples and was used to evaluate the need for removal of liquid preservative from the surfaces of specimens to use the technique.

Table 1. Characteristics of samples used to test the pre-reading drying effect on species identification.

Species	***N°***	***Body length****	***Body width****	***Age *****	***Drying minutes***
Adenomera andreae (Müller, 1923)	13	1.93	0.68	6 and 3	30-40
Adenomera hylaedactyla (Cope, 1868)	11	2.30	0.86	27 and 12	20-30
Leptodactylus fuscus (Schneider, 1799)	17	4.40	2.0	26	25-35
Leptodactylus longirostris Boulenger, 1882	10	3.6	1.4	25 and 13	20-30
Callimedusa tomopterna (Cope, 1868)	10	4.20	0.9	23 and 7	20-30
Phyllomedusa bicolor (Boddaert, 1772)	9	10.3	3.2	28 and 24	20-30
Amazophrynella minuta (Melin, 1941)	9	1.40	0.5	15	20-30
Amazophrynella manaos Rojas-Zamora, Carvalho, Ávila, Farias, and Hrbek, 2014	10	2.20	0.7	Unknown	20-30

*mean snout-urostyle length of individuals in cm, **storage time in alcohol in years

For the second data series, used to identify the position on the body of the frogs with the most effective spectrum for recognizing species we used a set of 100 samples of adult frogs belonging to nine species with average lengths ranging from 1.4 to 10.3 cm and storage time in alcohol from 8 to 28 years (Table 2), with no predrying. Spectral readings were taken at different sites on the belly and back of each specimen (Figure 1).

The spectral readings were taken with an Antaris II FT-NIR Analyzer provided by the botany spectroscopy laboratory of the National Institute of Amazonian Research. The equipment was programmed to near-infrared spectral readings of 16, with 8 cm^{-1} resolution and wavenumber in the range of 10000 to 4000cm^{-1}, which corresponds to the electromagnetic spectrum in wavelengths from 1000 to 2500nm. Each spectrum consisted of 1557 individual absorbance values. A black rubbery plate (E.V.A. - Ethylene Vinyl Acetate) was placed on the smaller individuals to prevent light scattering since these samples did not cover the entire reading area. The background was calibrated automatically by the equipment every four hours. The spectra of each individual were checked in order to detect anomalies that would be excluded in the reading procedure before analysis (see Supporting Information – Figure S1).

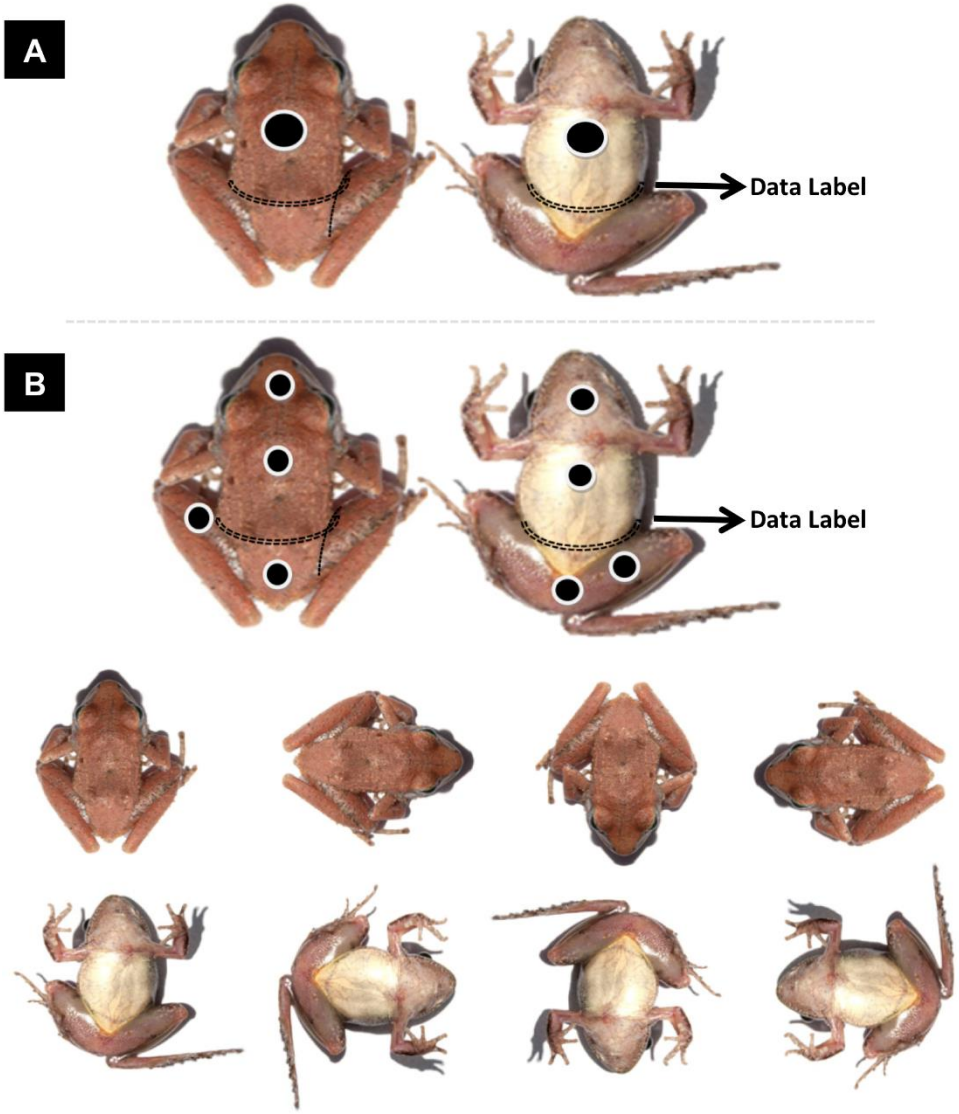

Figure 1. FT-NIR reading sites on the body of anurans. **(A)** Readings at central points on the back and belly, assigned to all samples for series one; **(B)** Readings at eight different sites on the back and belly, depending on the sizes of frogs for series two. For larger-sized frogs: **on the back -** 1. front, close to the snout and between the eyes; 2. central, between the arms; 3. posterior, between the legs; 4. outer thigh; **on the belly -** 1. front, on the throat; 2. central, between the arms; 3. posterior, between the legs; 4. inner thigh. For smaller individuals, eight different readings were taken from center of the body, with 90 ° turns of the sample on the reader in back and belly positions.

Data analysis

We used the first data series in different combinations between reading position (back and belly - Figure 1A) and alcohol drying time. In the second series, we used the mean of the spectral values for dorsal and ventral position and original spectra values at

randomly-selected sites on the back and belly (Figure 1B). Different combinations of data were used to test different reading models (Table 3).

Table 2. Characteristics of the samples used to test the best spectral-reading site for species identification.

Species	***N°***	***Body length****	***Body width****	***Age*****
Amazophrynella manaos Rojas-Zamora, Carvalho, Ávila, Farias, and Hrbek, 2014	12	2.2	0.7	Unknown
Amazophrynella bilinguis Kaefer, Rojas-Zamora, Ferrão, Farias, and Lima, 2019	12	1.9	0.5	8
Leptodactylus fuscus (Schneider, 1799)	12	4.7	2.1	26
Leptodactylus longirostris Boulenger, 1882	10	3.6	1.4	25 and 13
Callimedusa tomopterna (Cope, 1868)	10	4.2	0.9	23 and 7
Phyllomedusa bicolor (Boddaert, 1772)	9	10.3	3.2	28 and 24
Allobates caeruleodactylus (Lima and Caldwell, 2001)	9	1.6	0.4	22
Allobates magnussoni Lima, Simões, and Kaefer, 2014	13	1.8	0.5	12
Allobates tapajos Lima, Simões, and Kaefer, 2015	13	1.6	0.5	12

*mean snout-urostyle length of individuals in cm, **storage time in alcohol in years

Table 3. Description of spectral models tested for anuran species identification.

MODELS FOR SERIES 1	
Combinations	**Descriptions**
MODEL 1.1: Back - wet	Spectra collected on the back without alcohol drying of samples.
MODEL 2.1: Back - dry	Spectra collected on the back with alcohol drying of samples.
MODEL 3.1: Belly - wet	Spectra collected on the belly without alcohol drying of samples.
MODEL 4.1: Belly - dry	Spectra collected on the belly with alcohol drying of samples.
MODELS FOR SERIES 2 (all wet)	
MODEL 1.2: Mean Back	Mean spectra collected on the back of individuals.
MODEL 2.2: Mean Belly	Mean spectra collected on the belly of individuals.
MODEL 3.2: Mean Back and Belly	Mean spectra collected on the back and belly of individuals.
MODEL 4.2: Back - random all spots	All spectra collected at randomly-selected points on the back of individuals.
MODEL 5.2: Belly - random all spots	All spectra collected on the at randomly selected points on the belly of individuals.

MODEL 6.2: Random all data	All spectra collected at randomly selected points on both the back and belly of individuals.

For a preview of the spectral behavior, the raw and preprocessed data were subjected to a principal component analysis - PCA. To test the potential of the technique to recognize anuran species in different data series, the models were subjected to discriminant analysis (LDA) and evaluated through two forms of cross-validation: holdout method (70-30) with 100 randomizations that uses 70% of the data to calibrate the model and 30% (independent group) to test the model; and the leave-one-out (LOO) method, which tests sample by sample by removing one of the n specimens at a time (independent group) and testing its identification with the model generated with n-1 specimens.

The statistical analyses were done in the R statistical program (R Development Core Team 2019) with the support of the MASS package (Venables & Ripley 2002) and the klaR package (Weihs *et al.* 2005), with adaptations of command scripts already used in data processing and spectral analysis of botanical samples (available in: *http://www.botanicaamazonica.wiki.br/labotam/doku.php?id=analises:nir:inicio*).

We used the Savitzky-Golay filter to pre-process the spectra through the *savitzkyGolay* function available in the PROSPECTR package (Stevens *et al.* 2020). This function requires the provision of three arguments: polynomial order (p), differentiation order (m) and window size (w). We created combinations of these values (p from 2 to 10; m from 0 to 2; w from 3 to 101, in regular intervals of 2 units, and with the value of w always greater than the order of the polynomial within the combination) and executed for each combination of filtering of the raw spectra followed by an LDA holdout, in which we reserved 70% of the data for training the model and 30% for testing the model. The data was scaled and centralized before executing each LDA. At the end of the execution, we calculated the performance of each filtered set by combining the values of p, m, and w with the *MulticlassSummary* function of the CARET package (Kuhn 2012). We selected the best Savitzky-Golay filter for the four models of the first series of tests and for the three models of spectral-mean of the second series of tests, considering the sensitivity values returned by the function and we analyzed preprocessed data using holdout method (70-30) with 100 randomizations and the leave-one-out (LOO) method to calculate the species prediction-accuracy rates (%).

For the raw and preprocessed spectral-mean data of the back and belly, used in the second series, we also tested the species prediction-accuracy rates with a reduction

in the number of spectral absorbance values (stepwise - most informative regions of the spectrum), through LDA holdout. The data were scaled and centralized before executing each LDA.

We also analyzed the influence of the size and age of the samples on species-identification errors of the raw and preprocessed data for all condensed models, using a Generalized Linear Model (GLM), with binomial family.

Results

Influence of alcohol drying on species identification

Using the preprocessed spectra, the maximum sensitivity value for the first series of tests was achieved with the Savitzky-Golay filter in the first derivative, with orders of polynomials 2 and 3 and windows between 25 and 97 (Table 4).

Table 4. Species prediction-accuracy rates measured in discriminant analysis with holdout and LOO validations, in different combinations of position and drying for raw and preprocessed data in (test series 1).

	Raw data					Preprocessed data							
	Holdout (%)				**LOO**	**Savitzky-Golay Filter**			**Holdout**				**LOO**
Combinations	**Mean**	**Min**	**Max**	**CI (99%)**	**(%)**	**P**	**M**	**W**	**Mean**	**Min**	**Max**	**CI (99%)**	**(%)**
MODEL 1.1: Back - wet	**93.9**	83.3	100	92.63 - 95.28	**98.9**	3	1	33	**92.1**	75	100	90.6 – 93.0	**98.9**
MODEL 2.1: Back - dry	**95.5**	87.5	100	94.50 - 96.49	**96.6**	2	1	97	**98.3**	87.5	100	97.6 - 99.1	**100**
MODEL 3.1: Belly - wet	**98.9**	87.5	100	98.32 - 99.51	**100**	2	1	33	**98.7**	87.5	100	98.0 - 99.3	**100**
MODEL 4.1: Belly - dry	**98.0**	87.5	100	97.39 - 98.76	**98.9**	2	1	25	**96.1**	87.5	100	95.2 - 96.9	**96.6**

Principal components analysis (PCA) was used for an initial visual inspection of the data. The first axes of the PCAs on preprocessed data explained between 37.4% and 42.8% and the second axes explained 24.6% to 30.1% of the total variability. Similar results were obtained for the raw data, 57% and 89% in first axes, and the second axes

explained 8% to 41% of the total variability. The ordinations grouped measures taken on the same part of the body (back or belly), but individuals of both manipulations (dry and wet) were dispersed throughout the ordination space (Supporting Information - Figure S2 and S3).

To estimate the error rate in classification we used linear discriminant analysis (LDA). Species were more than 92% correctly identified in all datasets testing the influence of excess alcohol. The belly-wet model achieved better recognition (LOO – 100%) with both raw data and preprocessed data. Preprocessing resulted in few changes, sometimes increasing and sometimes decreasing the discrimination power of the model (Table 4), but overall species discrimination was similar for raw and preprocessed data. The LOO validation results for raw data in the back-wet model readings misidentified an individual of *Leptodactylus longirostris* as *Leptodactylus fuscus* and for preprocessed data a *Adenomera hylaedactyla* was identified as a *A. andreae*. The same species were confused with each other in the back-dry model, but with different individuals and no errors for preprocessed data. In the belly-dry model for raw and preprocessed data there were errors in the identification for the species *A. andrea*e, *A. hylaedactyla* and *Amazophrynella minuta* (Figure 2).

Effect of body site on species identification

The preprocessed spectra of the three mean models in the second test series also returned the maximum sensitivity value through the Savitzky-Golay filter in the first derivative, with orders of polynomials 2 and windows between 9 and 39 (Table 5).

The first axes of the PCAs based on preprocessed data explained 36.4% to 41.7% and the second axes explaining 26.1% to 34.3% of the total variance. Similar results were obtained with the raw data with 66% to 95% of explained variance in the first axes, and the second axes explaining 5% to 32% of the total variability. Most individuals of each species were grouped for the readings with mean spectra of positions (back and belly) tested (Supporting Information - Figure S4 and S5).

Results from the second series based on raw data also had high hit rates for the three spectral-mean models tested (mean rates between 94.4% and 98.8% with holdout, and 97% to 100% with LOO - Table 5). As with data from the first series, the model constructed with the means of the belly spectral readings resulted in 100% correct prediction of the species. Preprocessing did not increase the ability of the LDA to

distinguish species in models of the second data series; the rates remained high and similar (Table 5).

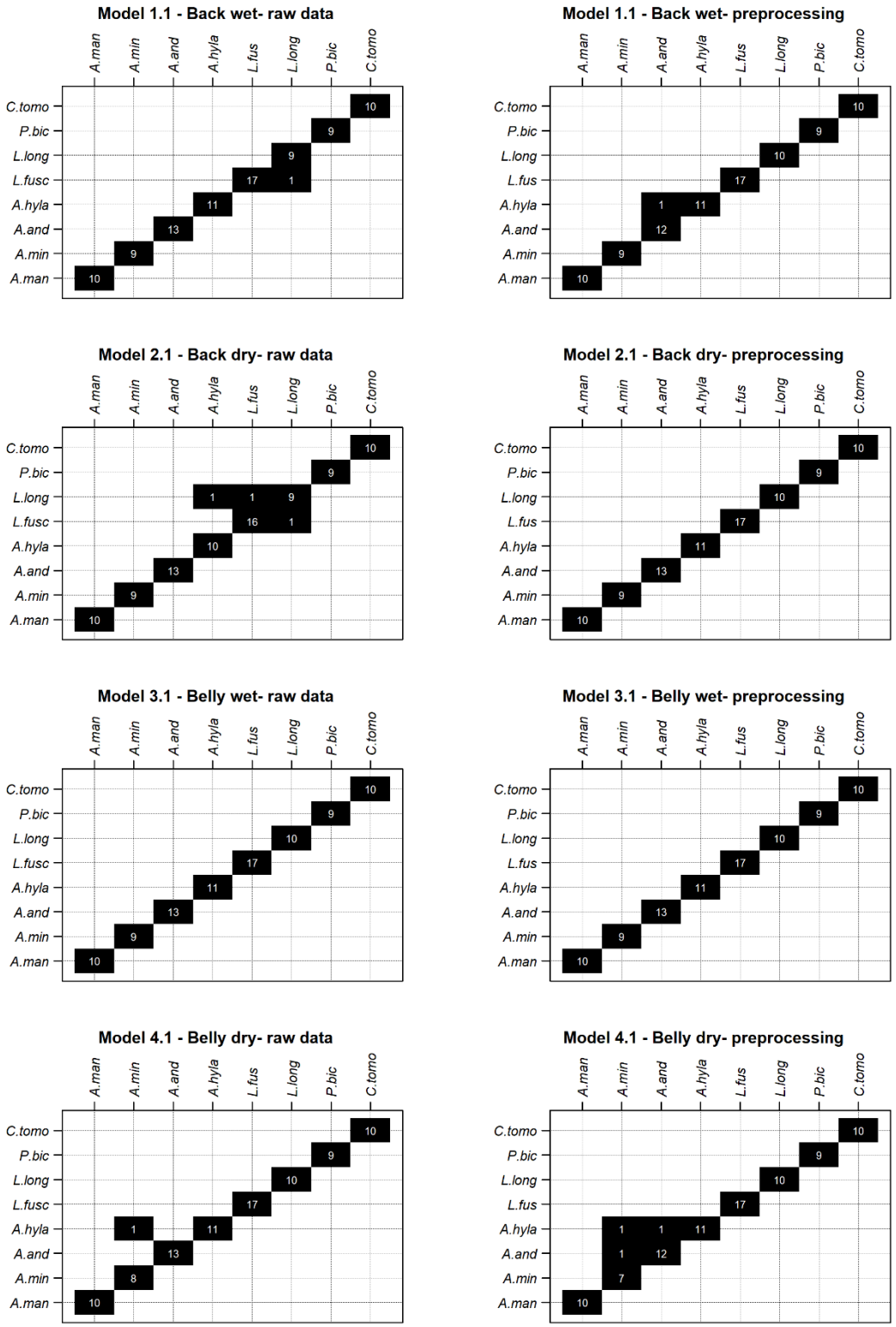

Figure 2. LDA-LOO results matrix (test series 1) **for raw and preprocessed data**. The species names in the calibration are given in rows, while predicted names are given in columns. Diagonal values are correct predictions and off-diagonal values are incorrect predictions. The

number within the squares refers to the number of samples used in the models for each species. **A.and**= *Adenomera andreae*, **A.hyla**= *Adenomera hylaedactyla*, **L.fusc**= *Leptodactylus fuscus*, **L.long**= *Leptodactylus longirostris*, **C.tomo**= *Callimedusa tomopterna*, **P.bic**= *Phyllomedusa bicolor*, **A.min**= *Amazophrynella minuta*, **A.man**= *Amazophrynella manaos*.

Table 5. Species prediction-accuracy rates measured in discriminant analysis with holdout and LOO validations, in different combinations of position for raw and preprocessed data (test series 2).

	Raw data					Preprocessed data							
	Holdout (%)				**LOO**	**Savitzky-Golay Filter**			**Holdout**				**LOO**
Combinations	**Mean**	**Min**	**Max**	**CI (99%)**	**(%)**	**P**	**M**	**W**	**Mean**	**Min**	**Max**	**CI (99%)**	**(%)**
MODEL 1.2: Mean Back	**95.6**	84	100	94.5 - 96.7	**97**	2	1	39	**96.0**	84	100	95.0 - 96.9	**97**
MODEL 2.2: Mean Belly	**98.8**	96	100	98.3 - 99.2	**100**	2	1	11	**96.6**	84	100	95.7 - 97.5	**99**
MODEL 3.2: Mean Back and Belly	**94.4**	84	100	93.2 - 95.7	**99**	2	1	9	**97.2**	88	100	96.5 - 97.9	**98**
MODEL 4.2: Back - random all spots	**81.4**	60	96	79.6 - 83.2	**85**	-	-	-	-	-	-	-	-
MODEL 5.2: Belly - random all spots	**88.5**	76	100	87.2 - 89.8	**93**	-	-	-	-	-	-	-	-
MODEL 6.2: Random all data	**69.3**	48	96	67.1 - 71.5	**73**	4	2	91	**78.4**	60	92	76.5 - 80.3	**83**

The LDA-LOO result matrices with back spectral means resulted in an error for two individuals of *L. fuscus* identified as *L. longirostris* for raw and preprocessed data, and errors in the identification of *A. bilinguis* and *A. tapajos*. There were no errors when using the mean-belly model for raw data, but for preprocessed data one individual of *A. bilinguis* was identified as *Allobates caeruleodactylus*. With mean spectra of back and belly of raw data, an *L. fuscus* individual was identified as *L. longirostris* and an *A.*

tapajos individual was identified as *A. bilinguis*. For the preprocessed data, two individuals of *L. fuscus* was identified as *L. longirostris* (Figure 3).

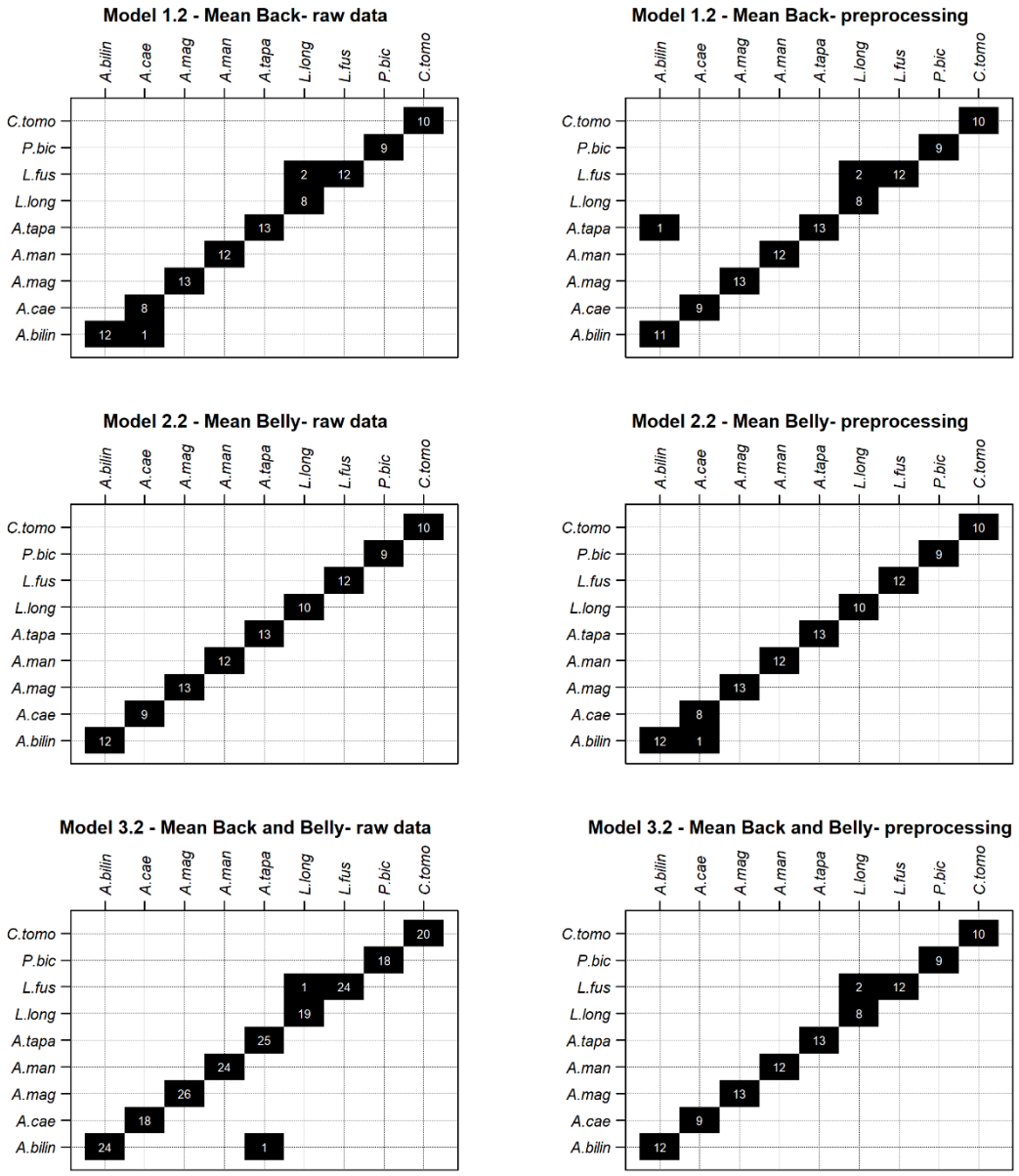

Figure 3. LDA-LOO results matrix for the tests with spectral-mean models (test series 2) **for raw and preprocessed data**. The species names in the calibration are given in rows, while predicted names are given in columns. Diagonal values are correct predictions and off-diagonal values are incorrect predictions. The number within the squares refers to the number of samples used in the models for each species. **A.bilin**= *Amazophrynella bilinguis*, **A.cae**= *Allobates caeruleodactylus*, **A.mag=** *Allobates magnussoni*, **A.man=** *Amazophrynella manaos*, **A.tapa=** *Allobates tapajos*, **L.long**= *Leptodactylus longirostris*, **L.fus**= *Leptodactylus fuscus*, **P.bic**= *Phyllomedusa bicolor*, **C.tomo**= *Callimedusa tomopterna*.

Low prediction success occurred in the models with a randomly-chosen single raw spectrum from the back (81.4%) and belly (88.5%) analyzed separately, that we tested to understand the performance with different body sites. For the model tested with the single raw spectrum randomly selected from all readings (back and belly together) at different body sites (eight sites- Figure 1), the mean correct-prediction rates were even lower. With these data, preprocessing resulted in an increase of about 10 % in the correct-prediction rates (raw data 69.2% in holdout and 73% in LOO / preprocessed data 78.4% in holdout and 83% in LOO). For this analysis, the Savitzky-Golay filter in the second derivative, with polynomial order 4 and window 91 was selected as the best combination (Table 5).

Variable-selection (stepwise) analysis in unpreprocessed mean back and belly models (models 1.2 and 2.2) resulted in 33 spectral regions that best distinguished species. By reducing the number of variables from 1,557 to 33 the predictive power decreased, but was still high. The holdout validation results using the selected variables from the models with spectral means were 88.3% (CI 86.8 - 89.8) of hits for the back model and 90.1% (CI 88.6 - 91.6) for the belly model. The most informative spectral regions to recognize the species were distinct between the back and the belly and were mainly distributed in the spectral range of 4000 to 7100 cm^{-1} for both back and belly and additionally in regions 9000 to 10000 cm^{-1} for the belly (Figure 4).

For the preprocessed mean back and belly models (models 1.2 and 2.2), stepwise selection of variables resulted in 33 more informative areas of the spectrum for both models. The most informative regions were in the spectral range between 4072 and 8554 cm^{-1} with several points between 4072 and 6444 cm^{-1} and in the mean-belly model a distribution between 4018 and 8543 cm^{-1} with several points between 4018 and 4674 cm^{-1} (Figure 5). With reduction in the number of variables, the predictive power was lower for both models, but results were similar for raw and preprocessed data. The results of the holdout validation using the variables selected from the models with preprocessed mean spectra were 94.2% (CI 92.9 - 95.5) of prediction accuracy in the dorsal model and 93% (CI 91.7 - 94.3) in the belly model. The complete list of selected variables for each model for raw and preprocessed data is given in the Supporting Information (Table S1 and Table S2).

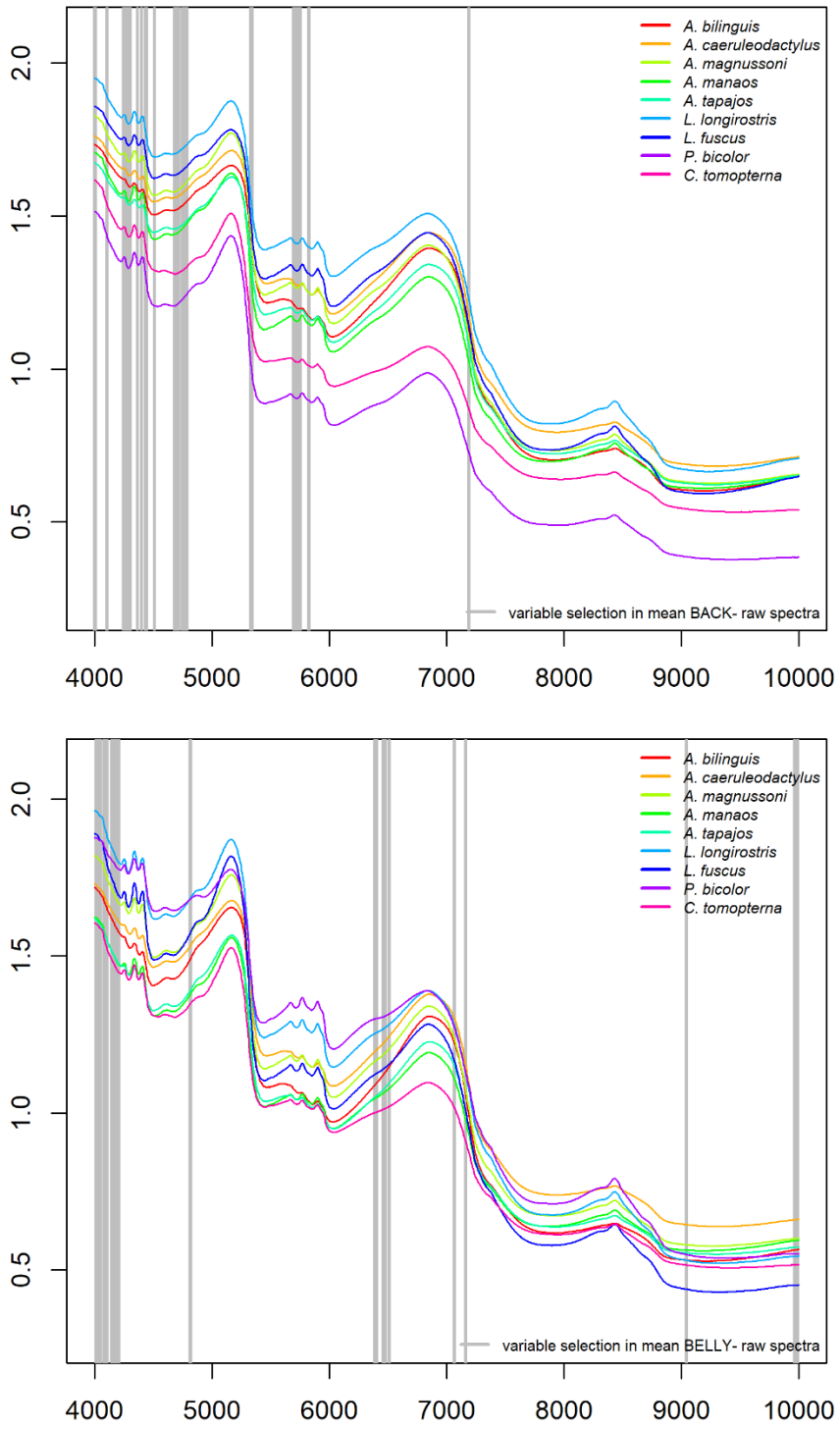

Figure 4. Spectra with the most informative regions selected by the stepwise method for the back and belly spectral-mean models with raw data - test series 2.

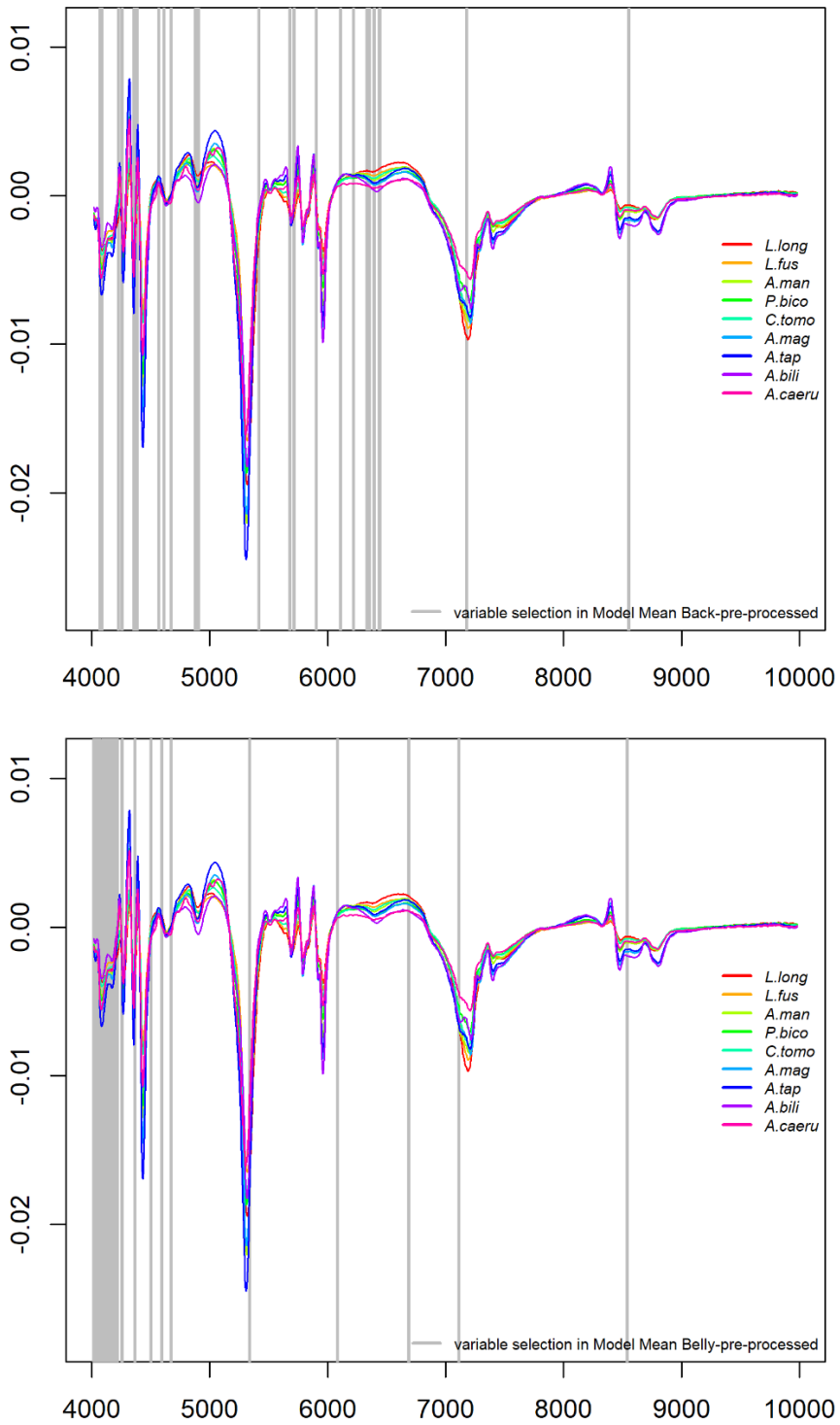

Figure 5. Spectra with the most informative regions selected by the stepwise method for the back and belly spectral-mean models with preprocessing - test series 2.

The size and age of the samples had no significant influence on species identification errors with the preprocessed (Body length – p = 0.11; Body width – p =

0.11; Age – p = 0.58) or raw data (Body length – p = 0.11; Body width – p = 0.15; Age – p = 0.08).

Discussion

The different models tested indicate that it is possible to generate anuran spectral models to recognize species even in alcohol-conserved samples of zoological collections. The presence of residual alcohol on individuals does not impede the efficient use of the technique to recognize anuran species, as predicted by other studies (Pasquini 2018, Almeida de Azevedo *et al.* 2019). All individuals used in this study contained traces of the alcohol in which they are preserved. All or most of the samples coming from collections will contain traces of formaldehyde and alcohol because specimens are almost always conserved in alcohol after being fixed in formaldehyde before they are deposited. Therefore, we believe that the presence of alcohol and formaldehyde did not affect discrimination because they were common to all individuals in the collection (Simmons 2002). In addition, most anurans included in the tests were correctly identified, and errors were independent of fixation time, which varied from three to 28 years, and independent of individual size, which average varied from 1.4 to 10.3 cm long and 0.5 to 3.2 cm wide.

Both series of tests we carried out with spectra that were not preprocessed indicated higher effectiveness when the spectral reading was done on the belly. Models with the mean belly spectra always had higher predictive power for raw data. Belly readings in live anurans have already been used to identify species and sex (Vance *et al.* 2014), but no test of the reading position on the anuran's body was presented. We believe that the greater efficiency in species identification with the belly spectrum is because it is a more uniform location and the contact with the equipment reading area was more regular than for the back spectrum (see Supporting Information- Figure S1). Another explanation may be related to the minor variation in color, roughness and environmental factors (e.g., fungi) that may be present on the back of individuals. Preprocessing data is suggested to smooth the sample physical variation on the spectra and was expected to improve LDA model performance. However, in this study, preprocessing smoothed the differences among readings from different parts of the body (Supporting Information – separate file: Raw_vs_Preprocessed_Spectra_plot.pdf), but it did not improve the performance of most models and it was not necessary to obtain high hit rates in species identification.

We recommend the identification of the position on the anuran body where the NIR spectral reading was made to ensure a standard method for rapid and practical species identification. However, tests with the back spectrum also showed relatively high rates of species identification accuracy, indicating that it is possible to capture important information also at this body position and these could be used if the specimens had damaged bellies. As studies with FT-NIR may include individuals with only some body parts (e.g., Rigby et al., 2014), we suggest that the spectral readings be collected at different back and belly sites to construct a more complete spectral-reference bank.

In this study, pre-processing increased correct-prediction rates over raw spectra when samples were from random body sites. This is probably related to the efficiency of smoothing physical differences from the readings that were made in very different sites (e.g., on the throat and inner thigh - Figure 1) used in the same model. Therefore we recommend preprocessing in similar cases, to obtain maximum accuracy when it is not possible to standardize body sites.

The high level of accuracy maintained even with the reduction in the number of preprocessed spectral variables from 1557 to 33 in the tests indicates the robustness of the spectral data in recognizing anuran species with few spectral bands. This selection in addition to reducing the number of variables to less than 1/3 the number of individuals, as recommended by Williams & Titus, 1988, decreases a possible collinearity interference of spectral data in discriminant analysis. The selection of variables also helps to recognize the regions of the electromagnetic spectrum that are important in the identification of anuran species and this may be important when data are analyzed with spectrophotometers with different spectral ranges.

For raw data, spectral regions selected as the most informative to distinguish species across the back spectrum occurred from 3999 to 7169 cm^{-1} with several points ranging from 4246 to 4786 cm^{-1}. In this region, there are bands that express signals of functional groups, such as methylenes (4261 cm^{-1}), aromatics (4246 cm^{-1}), proteins (4265 cm^{-1}) and polyamides (4701 cm^{-1}) (Workman Jr & Weyer 2008). On the belly, the most informative spectral regions occurred in a wider region of the electromagnetic spectrum, occurring from 4011 to 9993 cm^{-1}. Nitrogen functional groups characterized as starches, proteins and hydrocarbons are recognized among the selected spectral bands (4049 cm^{-1}), as well as alkanes and cycloalkanes (7065 and 7162 cm^{-1}) and O-H bonded chemical compounds (4184 cm^{-1}) (Workman Jr & Weyer 2008). For preprocessed data,

the spectral regions selected as the most informative to distinguish species in both mean-spectra models ranged between 4018 and 8554 cm^{-1} with several points between 4072 and 6444 cm^{-1} in the back mean model and 4018 and 4674 cm^{-1} in the belly mean model. In this region, there are bands that express signals of functional groups, such as methylenes (4261, 5708, 5731 and 5754 cm^{-1}), aromatics (4080 and 4246 cm^{-1}), proteins (4265 cm^{-1}) and polyamides (4701 cm^{-1}) (Workman Jr & Weyer, 2008). In addition, nitrogen functional groups characterized as starches, proteins, and hydrocarbons are recognized among the selected spectral bands (4049 cm^{-1}), as well as alkanes and cycloalkanes (7065 and 7162 cm^{-1}) and O-H bonded chemical compounds (4184 cm^{-1}) (Workman Jr & Weyer, 2008). The region of O-H bonds of alcohol with a peak at 7090 cm^{-1} was not selected as the most informative to recognize species, corroborating the conclusion that alcohol presence in individuals does not impede the use of the technique. The regions between 4760 to 4445cm^{-1} contain the aldehyde groups in which formaldehyde is expressed (Workman Jr & Weyer 2008), and these were selected as informative in some selections in the back model raw data and in both models with preprocessing. Possibly, fixation produces useful chemicals to distinguish species, but this should not be a problem unless analyses mix fixed and fresh specimens.

The species prediction-accuracy rates obtained with the both raw and preprocessed data spectra reinforces the efficiency of the method in the identification of species, even with the presence of alcohol and formaldehyde in individuals. Preprocessing may control noise related to physical differences among samples that were captured by the spectrum and an adjustment of the baseline (Rinnan et al., 2009; Pasquini, 2018), though this had little effect on the ability to correctly identify specimens in this study. In addition, due to the high species prediction-accuracy rates in the spectral mean models of the back and belly, we suggest that both anuran body positions can be used successfully in species identification. However, the reduced accuracy when random positions were used indicates that body position should be controlled or that body position should be included as a variable in the LDAs using the mean of all body positions.

In general, the results presented for both series of tests indicate the effectiveness of the use of FT-NIR spectra in the identification of anuran species fixed in formaldehyde and conserved in alcohol, reaching up to 100% accuracy. These results open up numerous possibilities for investigations using samples that are in zoological collections worldwide and are not restricted to species identification. The identification

of caste, sex and age has already been tested using spectral data in anurans and other animal groups (e.g., Vance *et al.*, 2014; Rigby *et al.*, 2014; Almeida de Azevedo *et al.*, 2019).

We only investigated the efficiency of the FT-NIR technique for the identification of anuran species from the zoological collections into species that have already been described. However, the technique can also be used to identify groups of individuals that do not conform to an already determined group/class (e.g., soft independent modeling of class analogy- SIMCA) (Pasquini 2018), and FT-NIR could also be used to identify the presence of undescribed species.

The number of samples/individuals of each group/species will be decisive for the effectiveness of the analysis. The FT-NIR technique returns predictions based on the reported reference group (Pasquini 2003, 2018, Burns & Ciurczak 2007), so the greater the number and diversity of samples, the greater the representativeness of each group (here species) and the chance of correct prediction. We have shown that the technique can distinguish closely-related species in sympatry. However, within-species geographic variation results in phenotypical variation due to the interaction between individuals and the environment, and these signals may be captured by spectra. Therefore, the technique will be most effective for species identification if the discriminant functions are based on specimens from across the species range.

Considering that spectroscopy returns a unique physicochemical fingerprint for each sample, the technique represents further evidence for taxonomic classification. This integration of spectra into taxonomy has recently been used in botanical studies (Prata *et al.* 2018). However, for the efficient use of FT-NIR spectra in species identification, there is a need for reference spectral data that can calibrate the models that will be used to classify new samples (Pasquini 2003). Additionally, to expand the use of the technique by different research groups, it is important to understand the spectra generated by different instruments when defining the inclusion of reference data that will be made available. This will only be possible when the spectra are linked to standardized taxonomic databases and made available with open access (Miralles *et al.* 2020). The spectra collected in this study are available at: https://ppbiodata.inpa.gov.br/metacatui/view/PPBioAmOc.592.

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References

Almeida de Azevedo, R., Wellington de Morais, J., Lang, C., & de Sales, D. C. (2019). Discrimination of termite species using Near-Infrared Spectroscopy (NIRS). *European Journal of Soil Biology*, *93*, 103084.

Barak, P. (1995). Smoothing and Differentiation by an Adaptive-Degree Polynomial Filter. *Analytical Chemistry*, *67*, 2758–2762.

Bortolus, A. (2008). Error cascades in the biological sciences: The unwanted consequences of using bad taxonomy in ecology. *Ambio*, *37*, 114–118.

Burns, D. A., & Ciurczak, E. W. (Eds.). (2007). *Handbook of near-infrared analysis*. CRC press.

Carvalho, T. R., Moraes, L. J., Lima, A. P., Fouquet, A., Peloso, P. L., Pavan, D., Drummond, L. O., Rodrigues, M. T., Giaretta, A. A., Gordo, M., Neckel-Oliveira, S., & Haddad, C. F. B. (2020). Systematics and historical biogeography of Neotropical foam-nesting frogs of the *Adenomera heyeri* clade (Leptodactylidae), with the description of six new Amazonian species. *Zoological Journal of the Linnean Society*, 1–39. [Epub ahead of print].

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*, 407–415.

Durgante, F. M., Higuchi, N., Almeida, A., & Vicentini, A. (2013). Species spectral signature : discriminating closely related plant species in the amazon with near-infrared leaf-spectroscopy. *Forest Ecology and Management*, *291*, 240–248.

Fan, Q., Wang, Y., Sun, P., Liu, S., & Li, Y. (2010). Discrimination of Ephedra plants with diffuse reflectance FT-NIRS and multivariate analysis. *Talanta*, *80*, 1245–1250.

Ferrão, M., Colatreli, O., de Fraga, R., Kaefer, I. L., Moravec, J., & Lima,A. P. (2016). High species richness of scinax treefrogs (Hylidae) in a threatened Amazonian landscape revealed by an integrative approach (WJ Etges, Ed.). *PLoS One*, *11*, e0165679.

Ferrão, M., Moravec, J., de Fraga, R., de Almeida, A. P., Kaefer, I. L., & Lima, A. P. (2017). A new species of Scinax from the Purus-Madeira interfluve, Brazilian Amazonia (Anura, Hylidae). *ZooKeys*, *706*, 137–162.

Ferrão, M., Moravec, J., Kaefer, I. L., de Fraga, R., & Lima, A. P. (2018). New species of scinax (Anura: Hylidae) with red-striped eyes from Brazilian Amazonia. *Journal of Herpetology*, *52*, 472–488.

Friedman, M., & DeSalle, R. (2008). Mitochondrial DNA extraction and sequencing of formalin-fixed archival snake tissue. *Mitochondrial DNA*, *19*, 433–437.

Frost, D. R. (2020). *Amphibian species of the world: an online reference* Version 6. (pp. 1–23). Electronic Database. American Museum of Natural History. http://resea rch.amnh.org/herpe tolog y/amphi bia/index.html

Funk, W. C., Caminer, M., & Ron, S. R. (2012). High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 1806–1814.

Giam, X., Scheffers, B. R., Sodhi, N. S., Wilcove, D. S., Ceballos, G., & Ehrlich, P. R. (2012). Reservoirs of richness: Least disturbed tropical forests are centres of undescribed species diversity. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 67–76.

Gotelli, N. J. (2004). A taxonomic wish-list for community ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *359*, 585–597.

Hadlich, H. L., Durgante, F. M., dos Santos, J., Higuchi, N., Chambers, J. Q., & Vicentini, A. (2018). Recognizing Amazonian tree species in the field using bark tissues spectra. *Forest Ecology and Management*, *427*, 296–304.

Hoogmoed, M. (2019). *Censo da Biodiversidade da Amazônia Brasileira*. Museu Goeldi -Plataforma digital. http://censo.museu -goeldi. br:8080/museu goeld i-web-1.2.0/pagin as/espec ie_consu ltar.xhtml

Jaksch, K., Eschner, A., Rintelen, T. V., & Haring, E. (2016). DNA analysis of molluscs from a museum wet collection: A comparison of diferente extraction methods. *BMC Research Notes*, *9*, 348.

Jouquet, P., Capowiez, Y., Bottinelli, N., & Traoré, S. (2014). Potential of Near Infrared Refl ectance Spectroscopy (NIRS) for identifying térmite species. *European Journal of Soil Biology*, *60*, 49–52.

Kaefer, I. L., Rojas, R. R., Ferrão, M., Farias, I. P., & Lima, A. P. (2019). A new species of Amazophrynella (Anura: Bufonidae) with two distinct advertisement calls. *Zootaxa*, *4577*, 316–334.

Kuhn, M. (2012). *The caret package*. Vienna, Austria: R Foundation for Statistical Computing. https://cran.r-proje ct.org/packa ge=caret

Lang, C., Almeida, D. R. A., & Costa, F. R. C. (2017). Discrimination of taxonomic identity at species, genus and family levels using Fourier Transformed Near-Infrared Spectroscopy (FT-NIR). *Forest Ecology and Management*, *406*, 219–227.

Lang, C., Costa, F. R. C., Camargo, J. L. C., Durgante, F. M., & Vicentini, A. (2015). Near infrared spectroscopy facilitates rapid identification of both young and mature amazonian tree species (D Gomory, Ed.). *PLoS One*, *10*: e0134521.

Margules, C. R., & Pressey, R. L. (2000). Systematic conservation planning. *Nature*, *405*, 243–253.

Meineke, E. K., Davies, T. J., Daru, B. H., & Davis, C. C. (2019). Biological collections for understanding biodiversity in the Anthropocene. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*, 20170386.

Miralles, A., Bruy, T., Wolcott, K., Scherz, M. D., Begerow, D., Beszteri, B., Bonkowski, M., Felden, J., Gemeinholzer, B., Glaw, F., Glöckner, F. O., Hawlitschek, O., Kostadinov, I., Nattkemper, T. W., Printzen, C., Renz, J., Rybalka, N., Stadler, M., Weibulat, T., … Vences, M. (2020). Repositories for taxonomic data: Where we are and what is missing. *Systematic Biology*, *69*(6), 1231–1253.

Moraes, L. J. C. L., Pavan, D., Barros, M. C., & Ribas, C. C. (2016). The combined influence of riverine barriers and flooding gradients on biogeographical patterns for amphibians and squamates in south-eastern Amazonia. *Journal of Biogeography*, *43*, 2113–2124.

Padial, J. M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy review. *Frontiers in Zoology*, *7*(1), 1–14.

Pasquini, C. (2003). Near Infrared Spectroscopy: Fundamentals, practical aspects and analytical applications. *Journal of the Brazilian Chemical Society*, *14*, 198–219.

Pasquini, C. (2018). Near infrared spectroscopy: A mature analytical technique with new perspectives – A review. *Analytica Chimica Acta*, *1026*, 8–36.

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2010). *Introdução à espectroscopia*. Cengage Learning.

Peloso, P. L. V., De, O. R. M., Sturaro, M. J., Rodrigues, M. T., Lima-Filho, G. R., Bitar, Y. O. C., Wheeler, W. C., & Aleixo, A. (2018). Phylogeny of map tree frogs, *Boana semilineata* Species Group, with a New Amazonian Species (Anura: Hylidae). *South American Journal of Herpetology*, *13*, 150.

Prata, E. M., Sass, C., Rodrigues, D. P., Domingos, F. M., Specht, C. D., Damasco, G., Ribas, C. C., Fine, P. V. A., & Vicentini, A. (2018). Towards integrative taxonomy in Neotropical botany: disentangling the Pagamea guianensis species complex (Rubiaceae). *Botanical Journal of the Linnean Society*, *188*(2), 213–231.

R Core Team (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http:// www.R.proje ct.org/

Rigby, C. L., Wedding, B. B., Grauf, S., & Simpfendorfer, C. A. (2014). The utility of near infrared spectroscopy for age estimation of deepwater sharks. *Deep Sea Research Part I*, *94*, 184–194.

Rinnan, Å., van den Berg, F., & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *TrAC - Trends in Analytical Chemistry*, *28*, 1201–1222.

Rodriguez-Fernandez, J. I., De Carvalho, C. J. B., Pasquini, C., Gomes De Lima, K. M., Moura, M. O., & Carbajal Arizaga, G. G. (2011). Barcoding without DNA? Species identification using near infrared spectroscopy. *Zootaxa*, *54*, 46–54.

Schander, C., & Halanych, K. M. (2005). DNA, PCR and formalinized animal tissue ? A short review and protocols. *Organisms Diversity and Evolution*, *3*, 195–205.

Simmons, J. E. (2002). Herpetological Collecting and Collections Management. *Herpetological Circular*.

Stevens, A., Ramirez-Lopez, L., Stevens, M. A., & Rcpp, L. (2020). *Package 'prospectr'*. R Package Version, (2).

Vance, C. K., Kouba, A. J., & Willard, S. T. (2014). Near infrared spectroscopy applications in amphibian ecology and conservation: Gender and species identification. *NIR News*, *25*, 10–15.

Vance, C. K., Tolleson, D. R., Kinoshita, K., Rodriguez, J., & Foley, W. J. (2016). Near infrared spectroscopy in wildlife and biodiversity. *Journal of Near Infrared Spectroscopy*, *24*, 1–25.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. Springer.

Weihs, C., Ligges, U., Luebke, K., & Raabe, N. (2005). *klaR analyzing German business cycles. In Data analysis and decision support* (pp. 335–343). Berlin, Heidelberg: Springer.

Williams, B. K., & Titus, K. (1988). Assessment of sampling stability in ecological applications of discriminant analysis. *Ecology*, *69*, 1275–1285.

Workman, J. Jr, & Weyer, L. (2008). *Practical guide and spectral atlas for interpretive near-infrared spectroscopy*. CRC Press.

Supporting Information

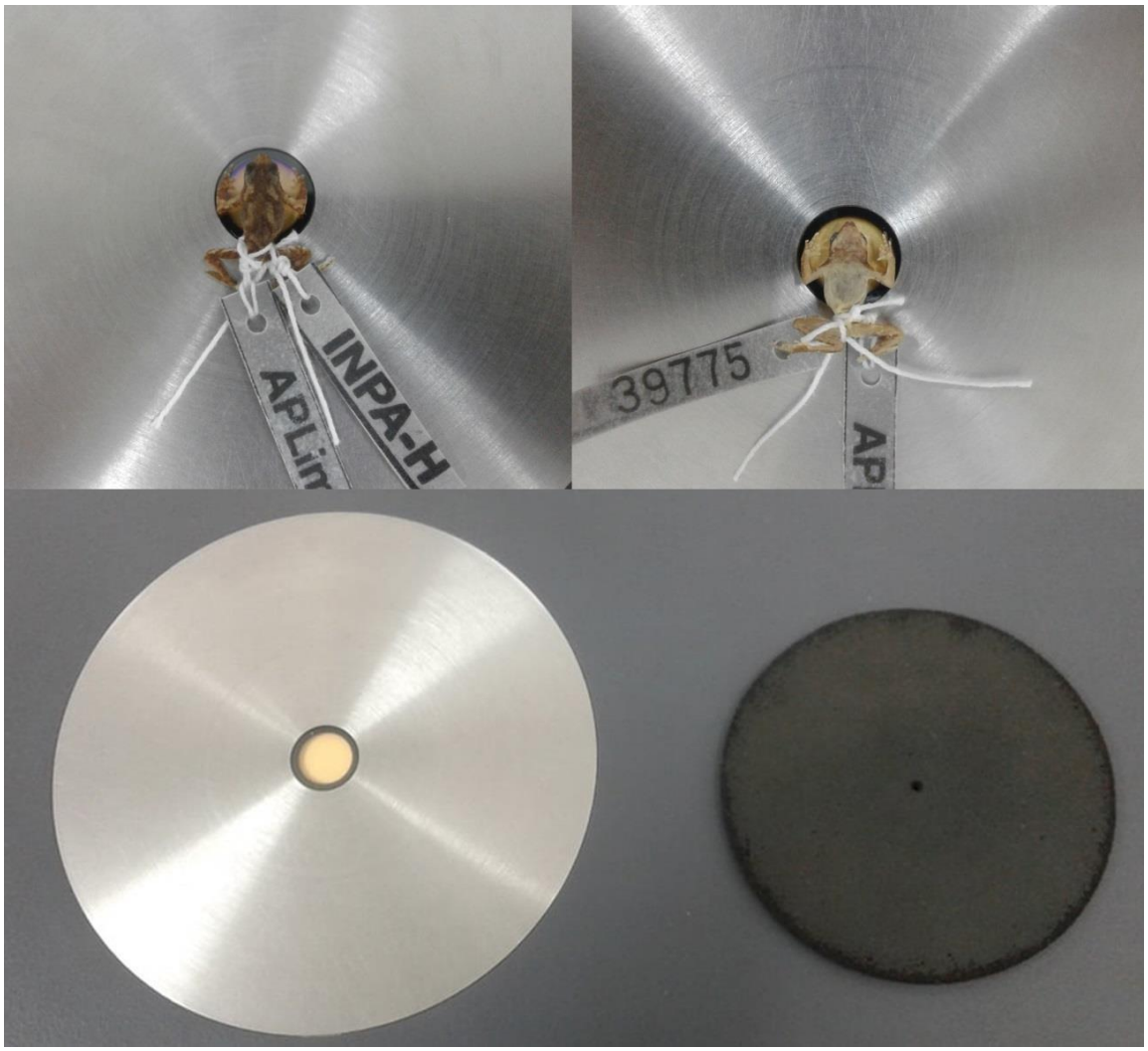

Figure S1. Understanding of the method. Above: an example of a smaller individual upon the reading area of the Antaris II equipment (back and belly). Below: the black rubber plate used on top of these small individuals at the time of reading to prevent light scattering.

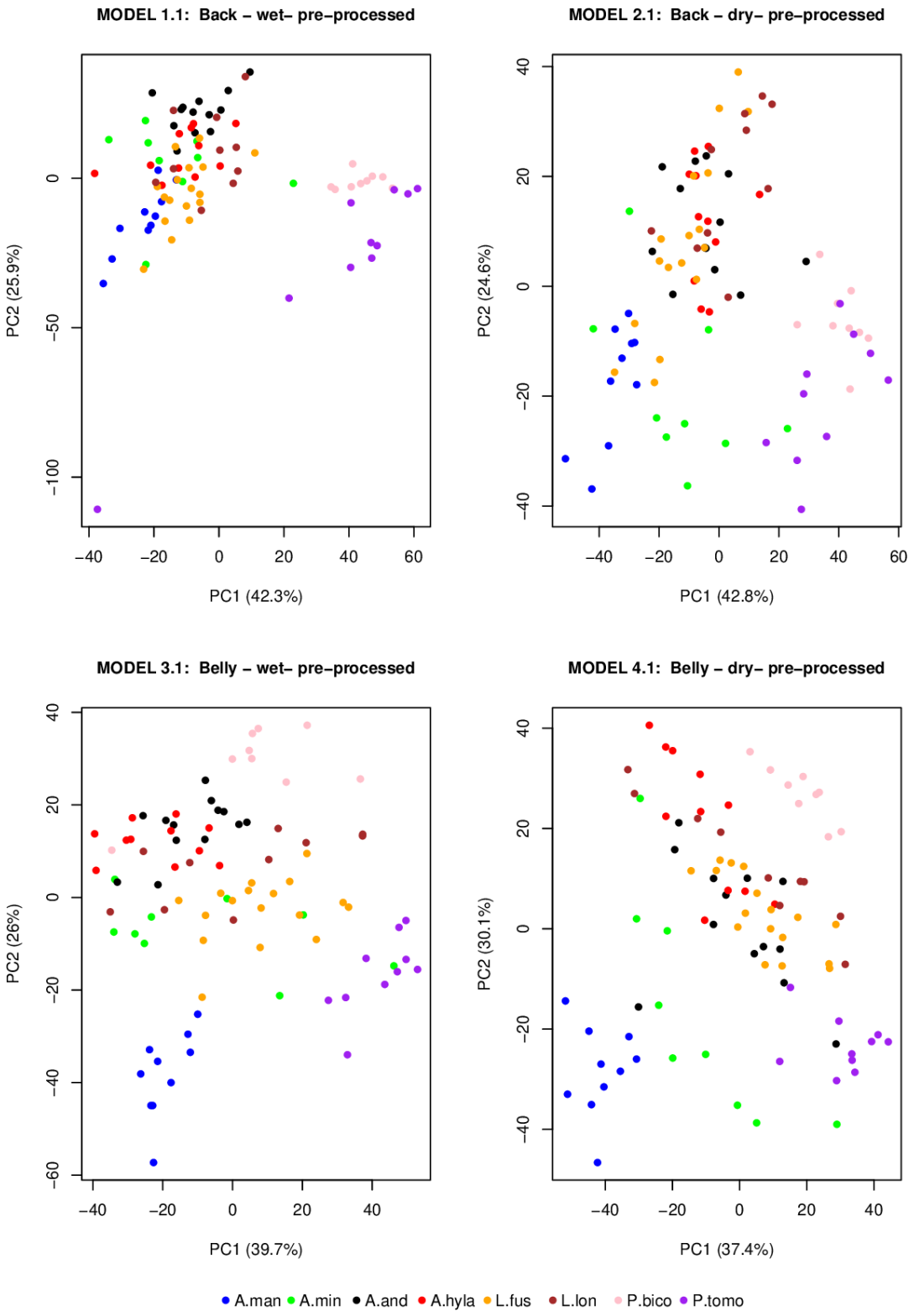

Figure S2: Principal components analysis (PCA) of **preprocessed data** including samples of series 1, indicating the grouping of individuals of each species in different combinations of position (belly and back) and manipulations (dry and wet) and on two main axes.

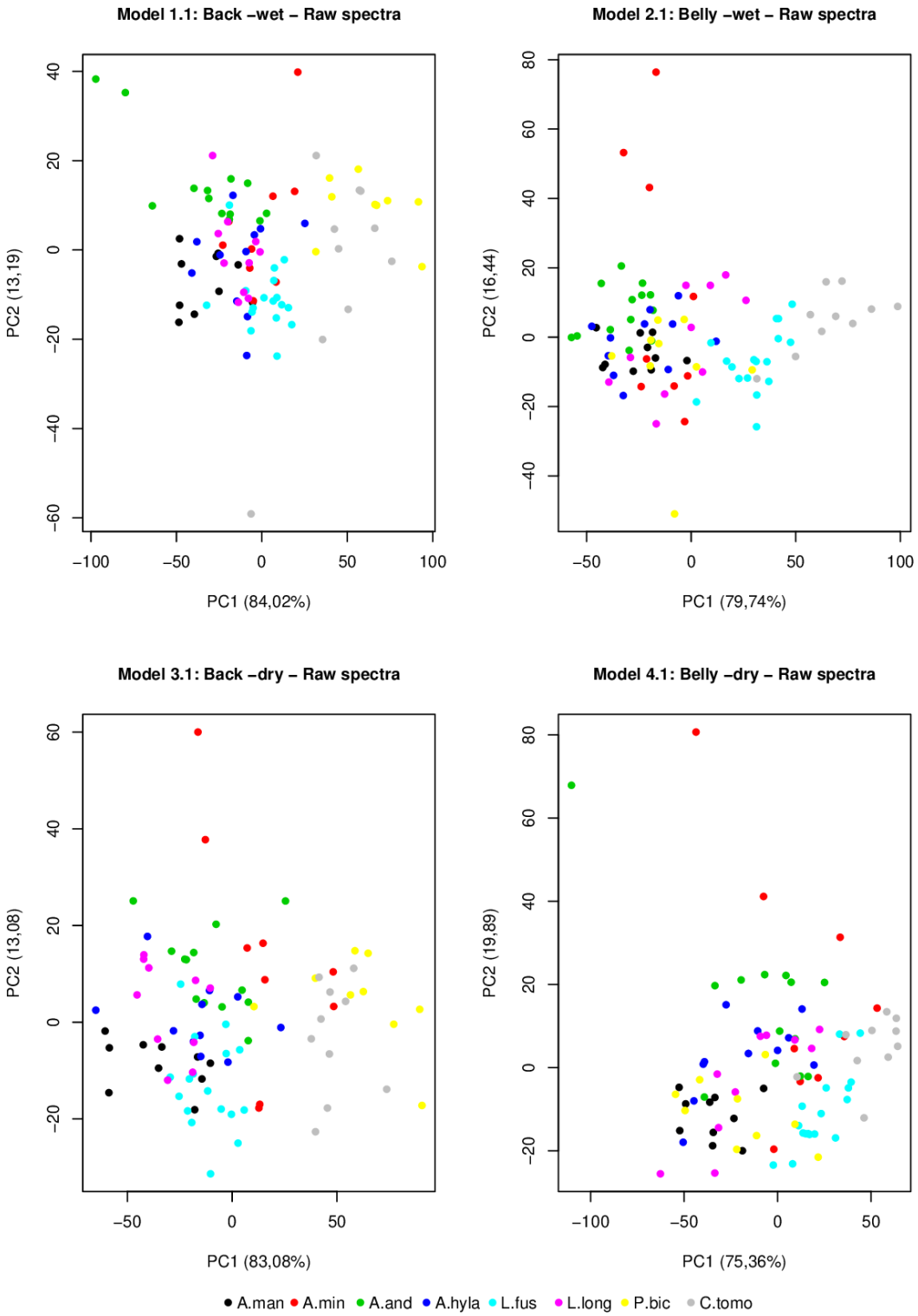

Figure S3: Principal components analysis (PCA) of **raw data** including samples of series 1, indicating the grouping of individuals of each species in different combinations of position (belly and back) and manipulations (dry and wet) and in two main axes.

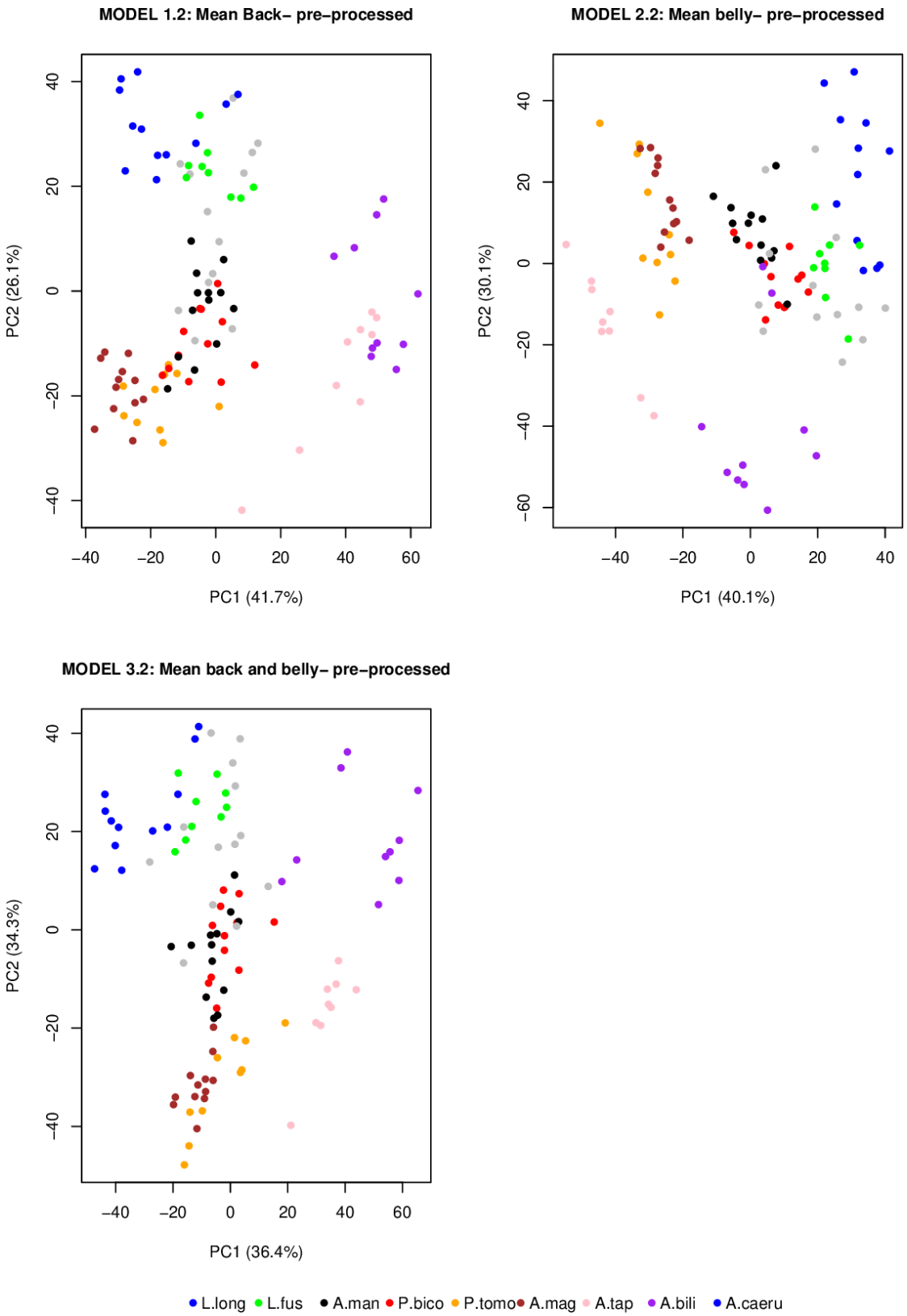

Figure S4: Principal components analysis (PCA) of **preprocessed data** including spectral-mean models (series 2), indicating the grouping of individuals of each species in different combinations of position (belly and back).

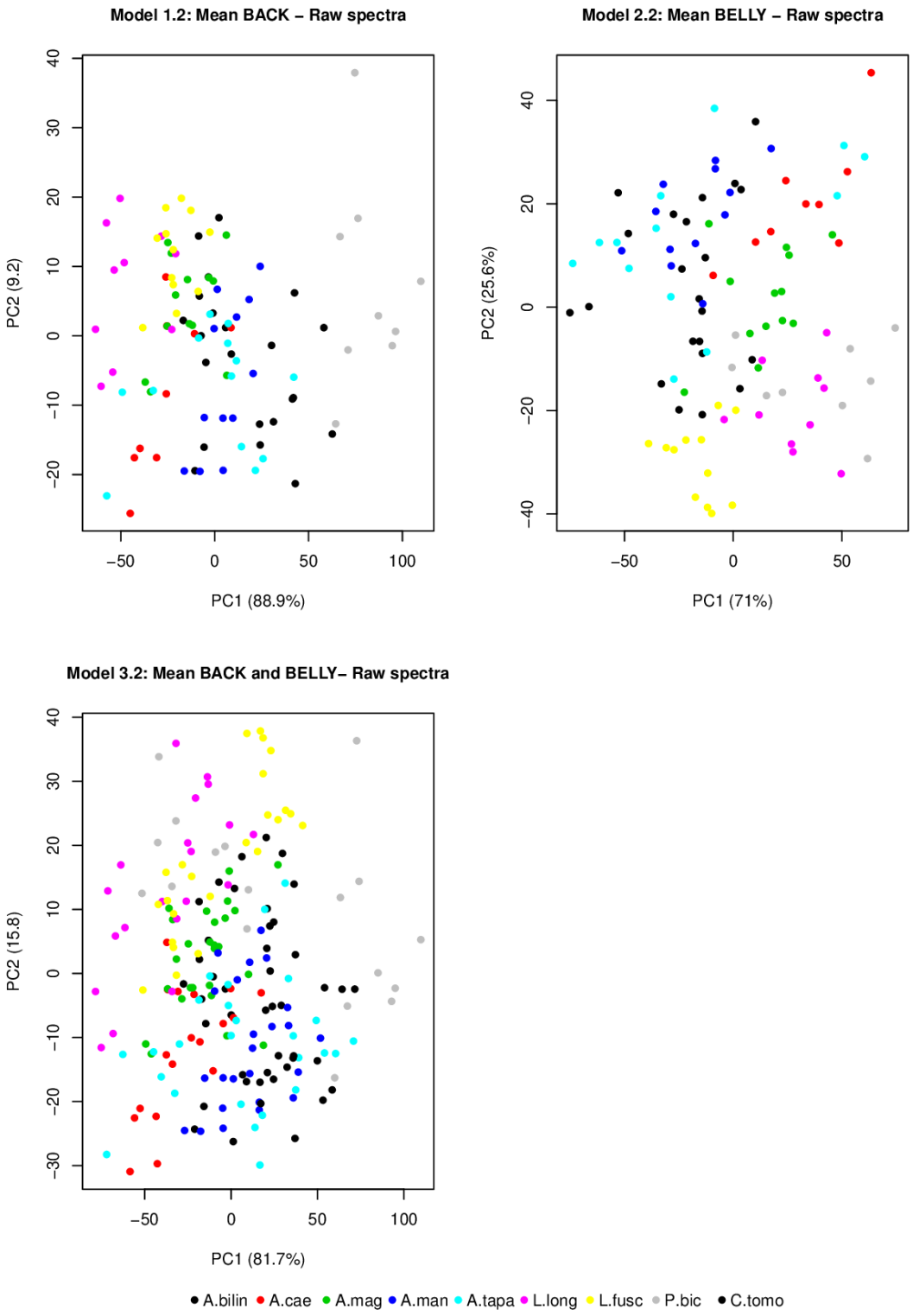

Figure S5: Principal components analysis (PCA) of **raw data** including spectral-mean models (series 2), indicating the grouping of individuals of each species in different combinations of position (belly and back).

Table S1: Complete list of selected variables for each body position of the individuals and the recognized functional group when known for mean models for back and belly **with raw data**.

Position	Spectral region	Recognized functional group
Back	3999.64	NA
Back	4007.354	NA
Back	4103.777	NA
Back	4246.483	Aromatic compounds
Back	4261.911	Methylene groups
Back	4265.768	C-H methylene C-H, associated with ovalbumin protein side
Back	4281.196	CHO — classic filter instrument
Back	4285.053	NA
Back	4292.767	C-H e CH2 Polyssacarideos
Back	4296.624	NA
Back	4304.337	NA
Back	4366.048	NA
Back	4400.761	OˇH/CˇO from glucose
Back	4431.616	NA
Back	4443.187	NA
Back	4508.755	NA
Back	4682.317	NA
Back	4697.745	NA
Back	4701.602	NˇH/C=O combination from polyamide 11
Back	4717.029	NA
Back	4747.885	NA
Back	4751.742	NA
Back	4767.169	NA

Back	4786.454	NA
Back	5330.282	NA
Back	5341.853	NA
Back	5696.69	NA
Back	5708.261	Methylene groups
Back	5731.403	Methylene groups
Back	5754.544	Methylene groups
Back	5823.969	Hydrocarbons
Back	5827.826	Hydrocarbons
Back	7189.324	NA
Belly	4011.211	NA
Belly	4015.068	NA
Belly	4022.781	NA
Belly	4026.638	NA
Belly	4042.066	NA
Belly	4049.78	C- N-C amide: (.CˇNˇC) e CˇH (.CH2)
Belly	4080.635	Aromatic compounds
Belly	4084.492	NA
Belly	4096.063	NA
Belly	4103.777	NA
Belly	4107.634	NA
Belly	4146.203	NA
Belly	4150.06	NA
Belly	4165.488	NA
Belly	4169.345	NA
Belly	4180.916	NA
Belly	4184.772	C-H e COH

Belly	4188.629	NA
Belly	4192.486	NA
Belly	4200.2	NA
Belly	4207.914	NA
Belly	4813.453	NA
Belly	4817.31	NA
Belly	6387.082	NA
Belly	6406.366	NA
Belly	6460.363	NA
Belly	6479.648	NA
Belly	6510.503	NA
Belly	7065.902	C-H alkanes and cycloalkanes
Belly	7162.325	C-H alkanes and cycloalkanes
Belly	9044.509	NA
Belly	9966.315	NA
Belly	9993.314	NA

Table S2. Complete list of selected variables for each body position of the individuals and the recognized functional group when known for mean models for back and belly **with preprocessing**.

Position	Spectral region (cm^{-1})	Recognized functional group
Back	4072.922	NA
Back	4080.635	Multiple methyl groups (CH_3 groups)
Back	4084.492	NA
Back	4088.349	NA
Back	4227.199	NA
Back	4258.054	NA
Back	4362.191	NA
Back	4373.762	NA
Back	4377.619	NA
Back	4381.476	NA
Back	4385.333	NA
Back	4570.466	C˘H aromatic C˘H (aryl)
Back	4612.892	NA
Back	4674.603	NA
Back	4882.877	NA
Back	4886.734	NA
Back	4890.591	NA
Back	4894.448	NA
Back	4906.019	NA
Back	4909.876	NA
Back	5418.991	NA
Back	5681.263	NA
Back	5715.975	NA

Back	5904.965	NA
Back	6113.239	NA
Back	6221.233	NA
Back	6336.941	NA
Back	6356.226	NA
Back	6394.795	NA
Back	6441.079	NA
Back	6444.936	NA
Back	7181.61	NA
Back	8554.678	NA
Belly	4018.925	Poly(acrylic acid)
Belly	4022.781	NA
Belly	4038.209	NA
Belly	4042.066	NA
Belly	4053.637	NA
Belly	4069.065	NA
Belly	4072.922	NA
Belly	4096.063	NA
Belly	4103.777	NA
Belly	4107.634	NA
Belly	4123.062	NA
Belly	4126.918	NA
Belly	4130.775	NA
Belly	4134.632	NA
Belly	4138.489	NA
Belly	4142.346	NA
Belly	4153.917	NA

Belly	4161.631	NA
Belly	4177.059	NA
Belly	4188.629	NA
Belly	4211.771	Methylene Groups in CH_2Cl_2
Belly	4215.628	NA
Belly	4219.485	NA
Belly	4261.911	CˇH methylene CˇH, associated with linear aliphatic R(CH2) NRC
Belly	4369.905	NA
Belly	4504.898	C–H stretch + C=O stretch combination band (aliphatic aldehyde)
Belly	4597.464	Vinyl group in polybutadienes
Belly	4674.603	NA
Belly	5341.853	NA
Belly	6086.241	NA
Belly	6687.922	NA
Belly	7112.185	NA
Belly	8543.107	NA

Appendix

Additional information specimen list.

Test	Species	Sample	Site	Colletion year
Dry	*Allobates tapajos*	INPA_H34402	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34403	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34404	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34405	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34406	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34407	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34408	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34409	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34410	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34411	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34412	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34413	PARNA da Amazonia, Pará	2007
Dry	*Allobates tapajos*	INPA_H34414	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32961	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32962	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32963	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32964	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32965	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32966	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32967	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32968	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32969	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32970	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32971	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32972	PARNA da Amazonia, Pará	2007
Dry	*Allobates magnussoni*	INPA_H32973	PARNA da Amazonia, Pará	2007
Dry	*Allobates caeruleodactylus*	INPA_H7229	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7230	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7231	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7232	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7234	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7235	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7236	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7237	Careiro	1995
Dry	*Allobates caeruleodactylus*	INPA_H7238	Careiro	1995

Dry	*Amazophrynella bilinguis*	INPA_H39774	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39775	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39776	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39777	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39778	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39779	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39780	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39781	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39782	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39783	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39784	Faz. Taperinha, Santarem, PA	2011
Dry	*Amazophrynella bilinguis*	INPA_H39785	Faz. Taperinha, Santarem, PA	2011
Wet	*Adenomera andreae*	INPA-H036705	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036706	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036776	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036777	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036778	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036781	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036784	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036788	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036789	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H036797	Resex Tapajós-Arapiuns	2013
Wet	*Adenomera andreae*	INPA-H037396	Presidente Figueiredo	2016
Wet	*Adenomera andreae*	INPA-H037397	Presidente Figueiredo	2016
Wet	*Adenomera andreae*	INPA-H037398	Presidente Figueiredo	2016
Wet	*Adenomera andreae*	INPA-H037399	Presidente Figueiredo	2016
Wet	*Adenomera andreae*	INPA-H037400	Presidente Figueiredo	2016
Wet	*Leptodactylus fuscus*	INPA-H001938	Lago Puraquequara	1993
Wet	*Leptodactylus fuscus*	INPA-H001939	Lago Puraquequara	1993
Wet	*Leptodactylus fuscus*	INPA-H001940	Lago Puraquequara	1993
Wet	*Leptodactylus fuscus*	INPA-H001941	Lago Puraquequara	1993
Wet	*Leptodactylus fuscus*	INPA-H001942	Lago Puraquequara	1993
Wet	*Adenomera hylaedatyla*	INPA_H002421	Rio Juruá, left bank	1992
Wet	*Adenomera hylaedatyla*	INPA_H005754	Rio Juruá, left bank	1992
Wet	*Adenomera hylaedatyla*	INPA_H005180	Rio Juruá, left bank	1992
Wet	*Adenomera hylaedatyla*	INPA_H005625	Rio Juruá, left bank	1992
Wet	*Adenomera hylaedatyla*	INPA_H019787	UFAM, Manaus	2007
Wet	*Adenomera hylaedatyla*	INPA_H019788	UFAM, Manaus	2007
Wet	*Adenomera hylaedatyla*	INPA_H019789	UFAM, Manaus	2007
Wet	*Adenomera hylaedatyla*	INPA_H019790	UFAM, Manaus	2007

Wet	*Adenomera hylaedatyla*	INPA_H019791	UFAM, Manaus	2007
Wet	*Adenomera hylaedatyla*	INPA_H034546	UFAM, Manaus	2007
Wet	*Adenomera hylaedatyla*	INPA_H034547	UFAM, Manaus	2007
Wet	*Amazophrynella minuta*	INPA-H12235	R.Madeira, Rondonia	2004
Wet	*Amazophrynella minuta*	INPA-H12236	R.Madeira, Rondonia	2004
Wet	*Amazophrynella minuta*	INPA-H12237	R.Madeira, Rondonia	2004
Wet	*Amazophrynella minuta*	INPA-H12248	R.Madeira, Rondonia	2004
Wet	*Amazophrynella minuta*	INPA-H12249	R.Madeira, Rondonia	2004
Wet	*Amazophrynella minuta*	INPA-H12324	R.Aripuanã,Amazonas	2004
Wet	*Amazophrynella minuta*	INPA-H12325	R.Aripuanã,Amazonas	2004
Wet	*Amazophrynella minuta*	INPA-H12327	R.Aripuanã,Amazonas	2004
Wet	*Amazophrynella minuta*	INPA-H12329	R.Aripuanã,Amazonas	2004
Wet and Dry	*Leptodactylus fuscus*	INPA-H007601	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007602	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007603	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007604	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007605	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007606	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007607	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007608	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007609	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007610	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007611	UHE Serra da mesa- Goias	1993
Wet and Dry	*Leptodactylus fuscus*	INPA-H007612	UHE Serra da mesa- Goias	1993
Wet and Dry	*Amazophrynella manaos*	INPA_H21864	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21868	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21869	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21877	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21884	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21886	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21891	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21893	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21897	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21901	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21903	ZF2, Região de Manaus	Unknown
Wet and Dry	*Amazophrynella manaos*	INPA_H21914	ZF2, Região de Manaus	Unknown
Wet and Dry	*Leptodactylus longirostris*	INPA-H019191	Parque Nacional do Viruá	2002
Wet and Dry	*Leptodactylus longirostris*	INPA-H019192	Parque Nacional do Viruá	2002
Wet and Dry	*Leptodactylus longirostris*	INPA-H019193	Parque Nacional do Viruá	2002
Wet and Dry	*Leptodactylus longirostris*	INPA-H019195	Parque Nacional do Viruá	2002

Wet and Dry	*Leptodactylus longirostris*	INPA-H019196	Parque Nacional do Viruá	2002
Wet and Dry	*Leptodactylus longirostris*	INPA-H001807	Reserva Ducke - Manaus	1994
Wet and Dry	*Leptodactylus longirostris*	INPA-H001808	Reserva Ducke - Manaus	1994
Wet and Dry	*Leptodactylus longirostris*	INPA-H001809	Reserva Ducke - Manaus	1994
Wet and Dry	*Leptodactylus longirostris*	INPA-H001810	Reserva Ducke - Manaus	1994
Wet and Dry	*Leptodactylus longirostris*	INPA-H001811	Reserva Ducke - Manaus	1994
Wet and Dry	*Phyllomedusa bicolor*	INPA-H2234	R.Jurua, Amazonas	1991
Wet and Dry	*Phyllomedusa bicolor*	INPA-H2964	R.Jurua, Amazonas	1991
Wet and Dry	*Phyllomedusa bicolor*	INPA-H3574	R.Jurua, Amazonas	1991
Wet and Dry	*Phyllomedusa bicolor*	INPA-H3675	R.Jurua, Amazonas	1991
Wet and Dry	*Phyllomedusa bicolor*	INPA-H8437	Res.Gavião, Amazonas	1995
Wet and Dry	*Phyllomedusa bicolor*	INPA-H8438	Res.Gavião, Amazonas	1995
Wet and Dry	*Phyllomedusa bicolor*	INPA-H8439	Res.Gavião, Amazonas	1995
Wet and Dry	*Phyllomedusa bicolor*	INPA-H8440	Res.Gavião, Amazonas	1995
Wet and Dry	*Phyllomedusa bicolor*	INPA-H8441	Res.Gavião, Amazonas	1995
Wet and Dry	*Callimedusa tomopterna*	INPA-H6802	Anavilhanas, Amazonas	1996
Wet and Dry	*Callimedusa tomopterna*	INPA-H6815	Anavilhanas, Amazonas	1996
Wet and Dry	*Callimedusa tomopterna*	INPA-H6817	Anavilhanas, Amazonas	1996
Wet and Dry	*Callimedusa tomopterna*	INPA-H6819	Anavilhanas, Amazonas	1996
Wet and Dry	*Callimedusa tomopterna*	INPA-H30959	M.Taboca, Amazonas	2012
Wet and Dry	*Callimedusa tomopterna*	INPA-H30960	M.Taboca, Amazonas	2012
Wet and Dry	*Callimedusa tomopterna*	INPA-H30961	M.Taboca, Amazonas	2012
Wet and Dry	*Callimedusa tomopterna*	INPA-H30962	M.Taboca, Amazonas	2012
Wet and Dry	*Callimedusa tomopterna*	INPA-H30963	M.Taboca, Amazonas	2012
Wet and Dry	*Callimedusa tomopterna*	INPA-H30964	M.Taboca, Amazonas	2012

CAPÍTULO 2

Near infrared (NIR) spectroscopy for the identification of live anurans: towards rapid and automated identification of species in the field.

Manuscrito a ser submetido para a revista **Journal of Near Infrared Spectroscopy** e apresentado segundo suas normas.

Near infrared (NIR) spectroscopy for the identification of live anurans: towards rapid and automated identification of species in the field.

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Abstract

In megadiverse regions, such as the Amazon, the identification of species generally requires specialists that are often not available. Therefore, the use of new species-recognition tools is necessary to streamline surveys and avoid errors in species identification that lead to ineffective decision-making. Near infrared spectroscopy is a quick and non-destructive tool that has been widely used in the recognition of biodiversity. In addition to being used as a bioindicator group, anurans have species with high morphological diversity, which make them the focus for studies and application of new tools that help in the identification and recognition at the species level. In this study, we present tests for the recognition of species of live Amazonian frogs under field conditions using the NIR technique and portable equipment. We analyzed the performance of a model built with spectra obtained from the back and a model with spectra from the belly of four pairs of phylogenetically close and morphologically similar species. It was possible to identify indivuals of live anurans in five of the eight species tested with hit rates above 80% when using only one spectral reading per individual. However, the overall mean of correct prediction of the models was below that of other studies that tested the method with anurans, which we attribute to particularities in the acquisition of spectra under field conditions and live species. We therefore suggest measures to improve the predictive capacity of the techniques.

Key-words: Amazon, Diversity, Frogs, Species recognition, Tools.

Introduction

"Know to conserve" has been the most used justification for studies dealing with biodiversity[1]. Classifying and naming an organism are the most efficient ways to communicate between different study areas and plan conservation actions[2]. Mistakes in species identification can result in sampling errors that can cause inefficient, and even harmful, management and decision-making[3]. However, in megadiverse regions, such as the Amazon[4,5], the identification and recognition of species are challenges that generally require the action of specialists.

As a result of the lack of vacancies to absorb professionals specialized in taxonomy, their number has been decreasing since the 1990s. In addition, specialists tend to work in regions outside the Amazon biome[6]. Added to the already known megadiversity of the biome, a large number of new species are being discovered every year[5]. Therefore, the use of new techniques and tools and the formation of more taxonomists across the tree of life is necessary to carry out surveys and recognize species[7] by non-specialists working in the Amazon.

Near infrared spectroscopy is a fast and non-destructive tool, which allows accessing the composition of a sample based on the interaction of the vibrations of the bonds of atoms or groups of atoms, which constitute the chemical compounds present in a sample, with electromagnetic radiation[8,9]. When combined with multivariate data analysis, the technique allows the characterization of organic samples, which can be from whole organisms[10] or from parts of these organisms[11]. The technique produces spectra with reflectance or absorbance values according to the type of sample and equipment used[12], being possible to obtain a molecular signature[13].

NIR spectroscopy has been relatively efficient in species identification and recognition tests for plant organisms[14–18] and animals[10,11,19], including frogs[20,21]. These results are promising, but not yet consolidated, generate expectations for future spectral and taxonomic databases, standardized and made available in open-access data banks[22].

Given the various applications and advantages related to the technique, many types of equipment are on the market today. Portable standalone spectrometers have the advantage of obtaining data at collection sites[17,20]. However, the design and technological peculiarities of the different models imply different performances in each application, mainly due to the narrower spectral regions and lower spectral resolution of the compact instruments[23].

Amphibians, especially anurans, are organisms commonly used as environmental bioindicators, due to their sensitivity to the different environments they occupy during their life stages (egg stage, larval stage and post-metamorphosis)[24–28]. However, because it is a diverse group in number (estimated at 7354 species for the world[29], 329 for the Brasilian Amazon[30]), anurans have species with high morphological diversity, including similarities attributed to complexes of cryptic species[31], making the group a challenge in fauna studies.

Therefore, anurans have been the focus for proposals for new tools that help in the identification and recognition at the species level. In a previous study, a high efficiency of the use of bench-top near infrared spectroscopy with fourier transform (FT-NIR) was found for anurans fixed in formalin and kept in ethyl alcohol that had been housed in a zoological collection[21]. These results indicated a new field of study that has a high potential to help resolve numerous taxonomic problems found in collections and which are sources for reviewing species around the world[32].

The use of spectroscopy in studies and conservation of anurans, especially in situ, would be useful for several reasons. Environmental surveys and licensing require data from organisms sampled and identified in the field (e.g., environmental impacts studies), with little or no participation by specialists, often resulting in inaccurate and/or contradictory information[33,34]. Difficulties of identifying young individuals or individuals with similar characteristics to another species (cryptic species), can be solved through genetic or molecular tests, which are expensive. NIR spectroscopy technique can potentially complement[21] or a replace[19] the usual methods at lower cost. However, knowledge about the use of spectroscopy in the recognition of biodiversity is limited[10] and different applications need to be tested. The present study aimed at evaluating the performance of spectroscopy using portable equipment in the identification of live anurans collected in situ in the Amazon. To meet this objective, discriminant analyses were carried out to better understand the spectral responses of live anurans from four species pairs.

Developing high-tech tools that help to recognize Amazonian biodiversity is extremely important to improve species recognition, communication between researchers and zoological collections, monitoring and management actions, and the development of public policies for the conservation of biodiversity.

Methods

In this study, spectra of live whole anuran specimens collected by the ASD Field Spec 3 portable spectrophotometer were evaluated. The equipment is hyperspectral, capable of monitoring from the visible region to the near infrared (VIS-NIR). Each spectrum obtained consists of 2151 reflectance values measured in the wavelength region of 350–2500 nm. The nominal spectral resolution is 3 nm for the visible region (350 –700 nm) and 10 nm for the NIR and SWIR regions (700-2100 nm)[35].

Four pairs of phylogenetically close anuran species were selected to test the equipment on species complexes in which morphological-similar species could be misidentified (Figure 1). This allowed classification models based on spectral data to be evaluated under realistic conditions considering the similarities between the studied species. We used common species with well-defined taxonomic identification to test the method. Classification models based on spectral data for each species were constructed with a minimum of 9 specimens (Table 1).

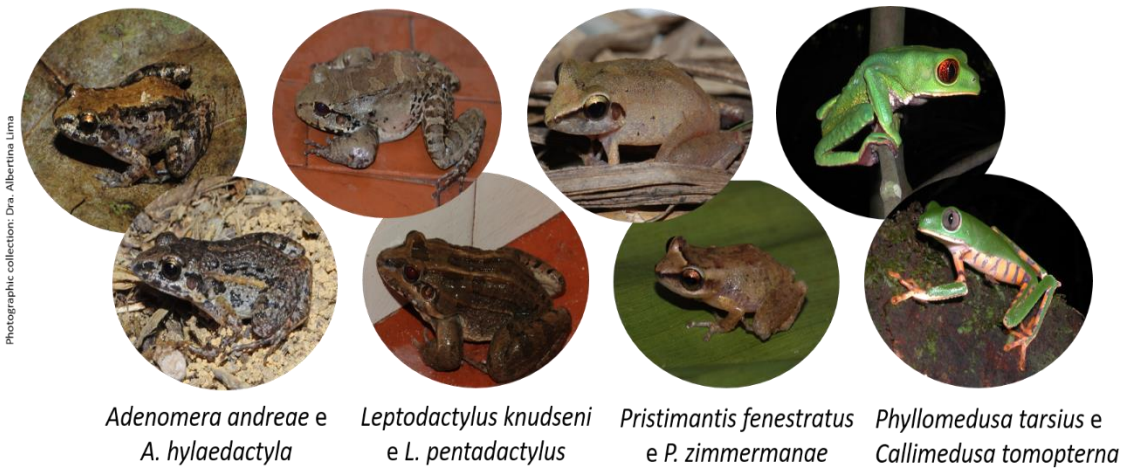

Figure 1. Four pairs of phylogenetically-close and morphologically-similar anuran species used for NIR spectral collection.

Table 1. Number of live specimens of each species collected in the field for spectra acquisition.

	Samples
Species	
Adenomera andreae (Müller, 1923)	11
Adenomera hylaedactyla (Cope, 1868)	11
Callimedusa tomopterna (Cope, 1868)	11
Leptodactylus knudseni Heyer, 1972	9
Leptodactylus pentadactylus (Laurenti, 1768)	13
Phyllomedusa tarsius (Cope, 1868)	9
Pristimantis fenestratus (Steindachner, 1864)	10
Pristimantis zimmermanae (Heyer and Hardy, 1991)	11
Total samples	**85**

Obtaining spectra of live anurans

Live anurans were captured manually between January and May 2020 in four tropical forest areas in the state of Amazonas, Brazil (Table S1 - Supplementary Information). Of these areas, three correspond to fragments of native forest in the middle of the urban center of the municipality of Manaus, mainly composed of little disturbed and secondary forest, with a few open areas on the Campus of the University of Amazonas (Campus UFAM)[36,37], the Adolpho Ducke Reserve (Ducke) and its forest in the Amazon Museum (MUSA)[38]. We also sampled individuals at the UFAM Experimental Farm (FEX-UFAM) which corresponds to an area of continuous dryland forest, with a mostly closed canopy and a low understory[39,40] (Figure S1 – Supplementary Information).

A total of 85 adult anuran specimens belonging to 8 species were captured (Table 1). The frogs were cleaned with water and an absorption cloth (reusable cloth - Scott Duramax®) to remove soil, excreta and urine that could be present after capture. Then, a spectral reading was taken on the back and one on the belly of each specimen[21] with the portable equipment in the field. To protect the equipment and avoid possible contamination between the frogs used in the readings, the reading window was wrapped with insulfilm® plastic made of PVC polymer[20]. For each specimen read (Figures 2a and 2b) the plastic was replaced and the reference reading, which is equivalent to 100% reflectance in the entire spectral region monitored by the equipment, was taken. For species smaller than the equipment's circular reading window, the reading area was

restricted with a black rubber disk (ethyl, vinyl, acetate - E.V.A) containing a small central hole (Figure 2c), simulating an accessory known as an iris or fish eye.

About three specimens of each species were killed after the reading with the use of topical lidocaine, fixed by injection of 10% formalin and preserved in 70% ethanol. These specimens were deposited in the Collection of Amphibians and Reptiles of the National Institute of Amazonian Research – INPA-H. The rest of the specimens were released in their same capture site. All procedures followed the capture, collection and release protocols approved under license SISBIO 70834-3 and ethics committee - nº 004/2020, SEI 01280.000146/2020-71.

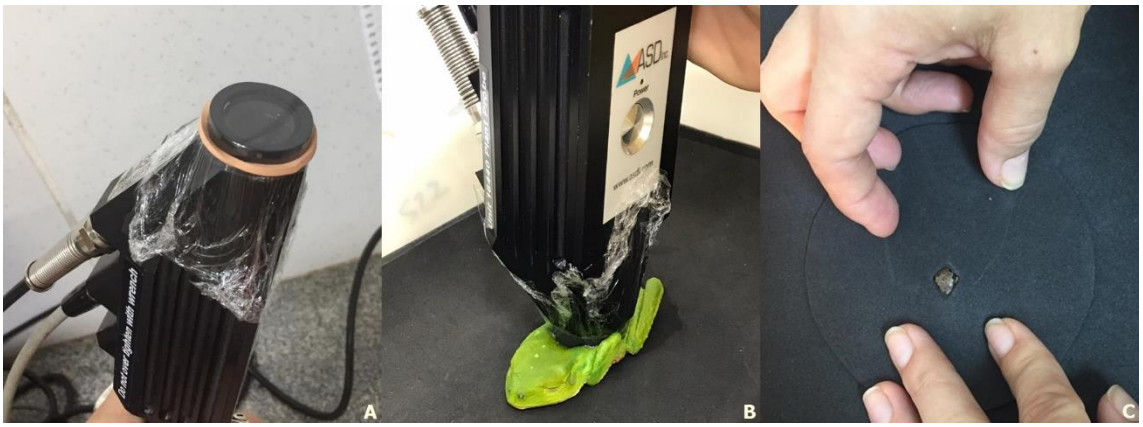

Figure 2. Detailed images of the method used for spectral readings with the VIS-NIR equipment. A) Reading window wrapped in PVC film; B) Spectral collection of a live anuran specimen; C) Spectral collection of live small anuran specimen with the help of accessory to restrict the reading area.

Data analysis

The collected spectra were initially observed visually to detect anomalies. The spectra collected in reflectance mode were transformed into absorbance by the formula - $\log_{10}(1/R)$, where R is the reflectance. Two transients (or breaks) resulting from the technology employed by the equipment were detected, in addition to spectral regions that presented noise normally found from readings at both ends of the monitored spectral range (Figure 3a). Therefore, only the central part of the spectrum between 1002 and 1825cm^{-1} (Figure 3b) was employed. Additionally, the Savitzky-Golay filter algorithm was used to pre-process the spectra with the second derivative, 2nd degree polynomial and a 31-point window (15 points for each side), in order to highlight the information present in the spectra and simultaneously reduce non-informative sources

of variability, such as radiation scattering and noise. These procedures were undertaken with the Unscrambler® program, version 11.0[41].

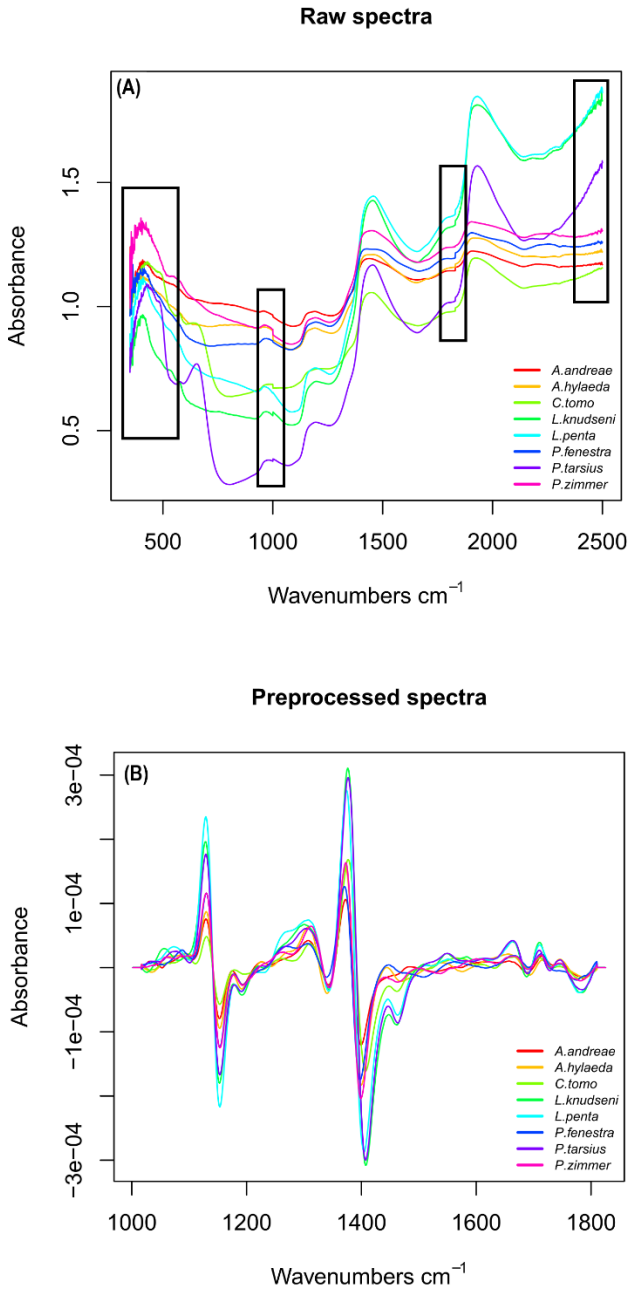

Figure 3. Species spectra collected from the back and belly of live anurans: (A) original VIS-NIR spectra with emphasis (rectangles) indicating the regions with noise and transients; (B) spectra pre-processed by the 2nd derivative, using the region without noise or transients (between 1002 and 1825cm^{-1}) and with Savitzky-Golay filter. In the caption: **A.andreae=** *Adenomera andreae*, **A.hylaeda=** *Adenomera hylaedactyla*, **C.tomo=** *Callimedusa tomopterna*, **L.knudseni=** *Leptodactylus knudseni*, **L.penta=**

Leptodactylus pentadactylus, **P.fenestra=** *Pristimantis fenestratus,* **P.tarsius=** *Phyllomedusa tarsius,* **P.zimmer=** *Pristimantis zimmermanae.*

The performance of two types of main models used for the identification and species recognition of live anurans was analyzed: a model built with spectra obtained from the back and a model with spectra from the belly. Principal component analysis (PCA) was used to reduce the dimensionality of the spectral data and illustrate the occurrence of groups of samples according to species. The analysis considered the 6 main components, observing the values of scores of the spectra of the samples generated by the PCA. Thus, the multicollinearity among the spectral variables were eliminated. The score values were used to construct classification models based on linear discriminant analysis (LDA) using holdout cross-validation, which returns prediction values in percentage. The models were built using 70% of the data for calibration and 30% (independent group) to test the models. Probabilities were based on with 100 randomizations. We also used cross-validation with the leave-one-out (LOO) method, which tests sample by sample, removing one at a time (independent group) and testing its classification with the model generated with n-1 samples, where n is the total number samples used in the study. The test group predictions were shown in a confusion matrix (NIRtools R-package)[42]. Statistical analyzes were undertaken using the R statistical program[43] with the support of the packages MASS[44], klaR[45], caret[46], dplyr[47], lattice[48] e ggpubr[49], with adaptations of command scripts already used in data processing and spectral analysis of botanical samples (available at: http://www.botanicaamazonica.wiki.br/labotam/doku.php?id=analises:nir:inicio).

Results

The graphs of PCA scores indicated the group of spectral data according to some species in the first two main components (e.g., *C. tomopterna*, *L. knudseni* and *P. fenestratus*), with these components capturing 76% of the spectral variance existing in the sets of pretreated spectra obtained from the back and belly of the specimens, respectively (Figure 4). The distribution of scores for other species (e.g., *L. pentadactylus* e *P. zimmermanae*) were more distinct in other main components (Figure S2 - Supplementary Information). Six principal components resulting from the analysis

of the entire spectral dataset captured 89% of the original variance for the data collected on the back and 92% on the belly.

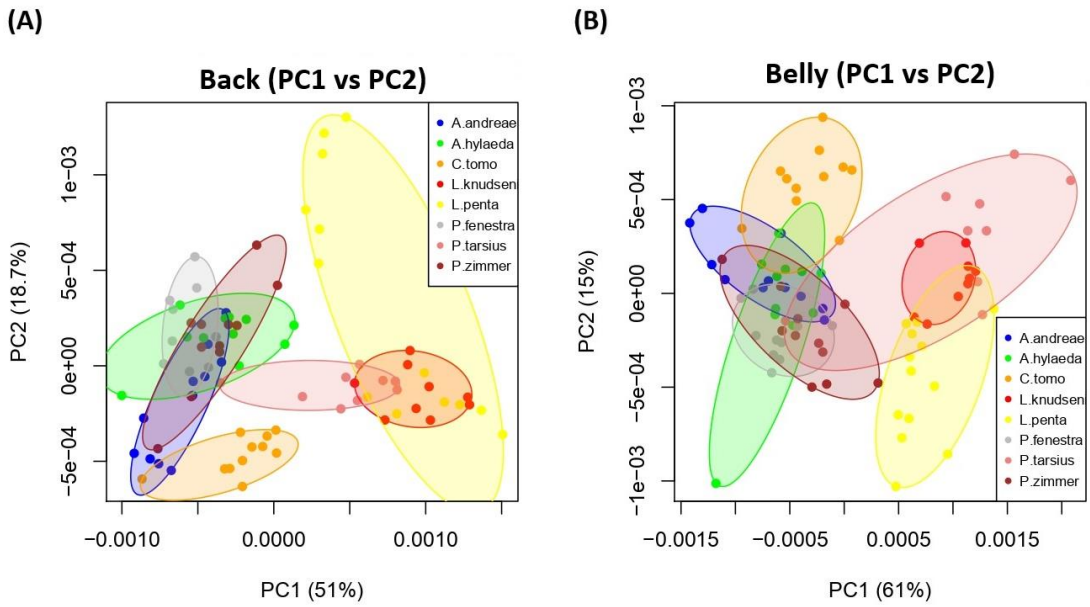

Figure 4. Two-dimensional plots of first two principal components (PCA) scores showing spectral groupings of live anuran species measured on (a) back and (b) belly. In the caption: **A.andreae=** *Adenomera andreae*, **A.hylaeda=** *Adenomera hylaedactyla*, **C.tomo=** *Callimedusa tomopterna*, **L.knudseni=** *Leptodactylus knudseni*, **L.penta=** *Leptodactylus pentadactylus*, **P.fenestra=** *Pristimantis fenestratus*, **P.tarsius=** *Phyllomedusa tarsius*, **P.zimmer=** *Pristimantis zimmermanae*.

With linear discriminant analysis using cross-validation Holdout, mean correctness values of 67.5% (C.I (0.96%) = 65.7 – 69.2) and 68.3% (C.I (0.96%) = 50.0 – 90.9) were obtained for readings on the back and belly, respectively. With the analyzes using the leave one out validation, 47% of mean correctness was registered for the back and 60% for the belly. Both validations indicate a better performance of the technique with spectra collected from the bellies of the specimens. The probability of correct specimen identification in the confusion matrices, generated from the leave-one out validation, obtained higher rates of correct answers in both positions, back and belly (Figure 5). *Callimedusa tomopterna*, *L. knudseni* and *L. pentadactylus* with belly reading obtained correct predictions in 82, 89 and 92% of cases, respectively, while *P. tarsius* had 89% of correct answers in both reading positions (both back and belly) and *P. zimmermanae* showed 82% success on the back. *Adenomera andreae* and *A. hylaedactyla* also showed the highest correct predictions with the back readings;

between 64 and 73%. *Pristimantis fenestratus* had intermediate success in the two spectral reading points (60% on the back and 40% on the belly). The individual errors of each sample (off-diagonal values) did not show a specific pattern of errors among most of the phylogenetically close pairs.

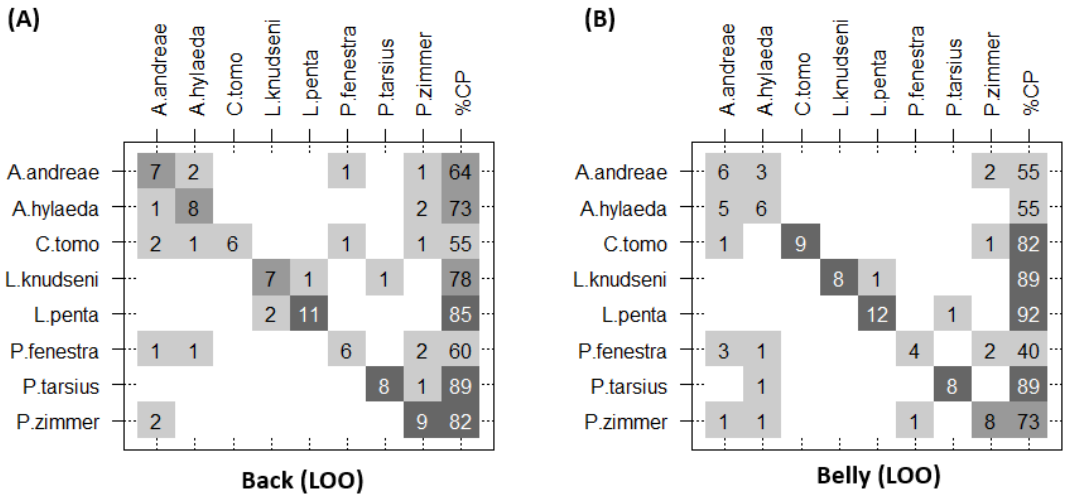

Figure 5. Confusion matrix resulting from LDA-LOO classification for recognition of live anuran samples with NIR spectra collected on (A) back and (B) belly. Diagonal values are correct predictions and off-diagonal values are incorrect predictions. The number inside the squares refers to the number of samples used in the models for each species. The last column shows the correct prediction (CP) rate by species with percentage values. **A.andreae=** *Adenomera andreae*, **A.hylaeda=** *Adenomera hylaedactyla*, **C.tomo=** *Callimedusa tomopterna*, **L.knudseni=** *Leptodactylus knudseni*, **L.penta=** *Leptodactylus pentadactylus*, **P.fenestra=** *Pristimantis fenestratus*, **P.tarsius=** *Phyllomedusa tarsius*, **P.zimmer=** *Pristimantis zimmermanae*.

Discussion

It was possible to recognize the species of live anurans in five of the eight species tested with hit rates above 80% when using only one spectral reading per individual. The overall mean of correct prediction of the models was below that of other studies that tested the method with anurans[20,21]. However, we believe that these data do not necessarily reflect the efficiency of the technique that can be obtained. The low hit percentages (below 73%) obtained for three species in each model for back and belly

are likely significantly affecting the overall hit means, generating the low overall values observed. This is shown in the values of the confidence interval obtained in the hold-out cross-validation, where the belly models reached up to 90% accuracy. The grouping of species observed through the main components (PCA – Figure 4) is also a strong indication that the NIR technique with portable equipment is promising for the recognition of live anuran species.

The analysis distinguished the phylogenetically closely related species in the genus *Leptodactylus*. The results for the pair of species *L. knudseni* and *L. pentadactylus* indicate that, for this genus, with belly reading, it is possible to identify individuals species using spectra of living organisms (only one individual incorrectly predicted). However, for *Adenomera andreae* and *A. hylaedactyla*, which in the two positions maintained intermediate hit rates, eight of the 10 individuals were assigned wrongly in the belly model and three of the seven individuals assigned wrongly in the dorsum model. Other prediction errors were between non congener species, indicating that the prediction errors were not determined only by the predicted similarities between pairs. Also, unlike a previous study with preserved specimens[21], live anuran specimens obtained with field spectra did not show a pattern on the best reading position (belly or back) to recognize the species. This result reinforces the importance of always obtaining spectral readings at both positions to generate spectral models, especially in exploratory studies.

The low rate of correct recognition of some species using models obtained with live anurans was not expected in the present study, as the reading protocol tested in this study was based on the results of previous studies[20,21]. However, the construction of spectral models with data obtained in the field presents some important differences, such as (i) the difference in the quality of bench and field equipment; (ii) the number of spectral readings needed per position (back and belly); (iii) the need to protect the reading window with plastic film (PVC) to avoid direct contact between the equipment and the fluids produced by the animals during the reading; (iv) the need to use E.V.A to reduce the reading window for small anurans and; (v) the depth of reach that radiation penetrates into the animal's tissue, considering that in some animals light can pass through the tissue and capture information from internal organs, stomach contents and intestine due to the transparency of the tissue. These limitations should be addressed, and hopefully circumvented in future studies.

Benchtop NIRs equipment generally has greater stability, requires air humidity control, and is fixed to ensure more consistent spectral readings. The Antaris II FT-NIR Analyzer from Thermo Nicolet (www.thermofisher.com) used in previous study of Amazonian anurans [21]allows a 16-scan scan on each reading and delivers a spectral response already processed by a Fourier transform[8,50]. Different makes of portable equipment have a wide range[23,51], with different resolutions and spectral quality. While the benchtop equipment performs the spectral reading in total stability, the optical reader of the field equipment moves. The ASD Field Spec 3 equipment used in this work has an optical reader that at the time of reading is manually controlled, which can result in instability[35]. For the type of acquisition of spectra in living organisms, the reader can move with the anuran individual breathing during reading in addition to movement when trying to escape manipulation. Because of these movements, the spectral readings may capture some light scattering noise, among other influences that affect the spectral quality[8,50]. The spectral rereading in the same position and a subsequent calculation of the average of these repetitions can minimize noise at the time of collection. We suggest that future studies compare equipment with standard samples and determine how many spectral readings at each position are needed to have similar results on different equipment.

The number of spectral readings required to represent an individual needs to be determined. The spectral mean always presented better results for recognizing plant species, both in studies carried out with bench equipment[16,18] and with portable equipment[17]. In a study with dry leaves[18], the species recognition power decreased when the number of readings to represent the individual was reduced. The decision to collection only one reading per position was based on the results of previous studies[20,21], but without taking into account changes in equipment and variations generated by readings in living organisms. For future studies, we recommend more readings in each position, even if they are made at the same point.

Spectral studies to recognize biodiversity need to use equipment that was not designed for this purpose. The use of plastic in the reading window was used in collection of North American frogs[20] to avoid the transmission of pathogens from one animal to another. Here we are also concerned about damage to the equipment due to the fluids produced by different species of frogs. Even including the calibration with a white plate (which represents 100% reflectance)[50] with the plastic present in the reader

to reduce the material's effect on the spectral response, the plastic can reflect light and the spectral reading has a lower quality when recording the individual's information.

Another adaptation that was necessary in the present field work was the reduction of the reading window as a black E.V.A (see methods). Many species of anurans used in this study (e.g., *Adenomera* and *Pristimantis* genus), are smaller than the reading window. On benchtop equipment, when it is necessary to scan material smaller than the reading window, a black metallic accessory called Iris or Fish Eye allows reduction in the reading area[52]. For the identification of species of a given genus of plant, although the behavior of the spectrum is very different from the others with the use of this factory accessory, with the use of mathematical models it was possible to efficiently access the individual's spectral response[53]. However, the use of any accessory represents the addition of new variables in the spectrum, which can compromise tissue recognition in the sample, as these materials have chemical components and bring extra information not related to the spectral signatures of the species. In the confusion matrices, the species with the highest correct prediction rates were the larger species (genus *Leptodactylus* – Figure 5) for which the spectra could be obtained without the use of E.V.A (see the usage for each species in the Supplementary Material). In contrast to the previous study[21] in which the E.V.A accessory was used to prevent light scattering in small samples without direct contact with the reading sphere, in this study the use of this accessory compromised the acquisition of spectra caused difficulties in centralizing the radiation emitted by the equipment. New tests and standardization in the use of accessories is extremely important in defining an effective protocol for spectral collections in the field.

The depth of penetration of NIR radiation is a function (inverse) of the amount of radiation absorbed and the amount of radiation scattered by the sample[54]. Therefore, the reading can go beyond the superficial biological tissue when the material is transparent, capturing information from parts below the animal's skin. The belly spectra read in individuals of small and medium-sized live anurans may have captured information from internal organs, such as stomach and intestines. Furthermore, the presence of residues, such as soil particles or even the presence of fungi can be expected in acquired spectra of specimens collected in situ. All these sources of variability define the spectral signatures of each individual and can influence the efficiency of the model generated for the species recognition. A greater number of readings at the same position

or at different points using the average of readings can increase the spectral representation of the individual[16,18].

Intraspecific differences are common in anurans in different aspects such as skin irregularities, coloration and secretions. Some species of frogs secrete a variety of chemicals against predators (or other "threats", such as manipulation) and pathogens, with alkaloids being the best studied[55–58]. The composition of alkaloids can vary greatly, even between individuals of the same species, if they are of different size, age or sex[55,58]. Another feature that reflects intraspecific differences is polymorphism. In this case, the same species may present more than one coloring morphotype or with the presence/absence of spots[59,60]. Spectral-response studies of polymorphic individuals and different sexes are needed to understand the quality of spectral responses within these groups. It is necessary to understand, for example, if the spectrum captures the information of the species regardless of sex or if it is necessary to separate sexes within species. The variability of the whole group (in our case species) captured by the spectra is a fundamental point in the creation of reference models that will be used in the recognition of new samples.

Among so many variations that were captured with the spectra of live anurans collected in the field, in the present study we used several tools available in spectral processing to extract relevant information from individuals and minimize noise. The use of derivatives is important to eliminate unimportant baseline signals and filters are used to reduce instrument noise [8,51]. Also, selecting spectral bands that have more consistent information excluding light scattering and instrumental noise are appropriate analytical procedures to capture information from NIRS spectra. Therefore, the pre-processing steps were valuable in order to try to eliminate noise and non-informative sources of variability, making the results more consistent[8].

The results obtained in the present study were consistent and gave promising results for most species studied, considering the inherent difficulty of performing spectral collections of live anurans and in the field with high quality. As this is the first study to evaluate the capacity of NIRs to differentiate live individuals of different species of Amazonian anurans under field conditions with NIR spectra, it can be concluded that new measures to improve the predictive capacity of the models would be the following: I. use average spectra of individuals with a greater number of readings collected in the same place (back and belly); II. increase the number of specimens to compose the spectral datasets for each species; III. Test other materials that allow

reduction in the reading area and capture the spectral response with better quality. With these adjustments it may be possible to build effective spectral models to recognize anurans in the field. Finally, we hope that NIR equipment manufacturers will begin to consider application of the technique in biodiversity recognition and was develop instrumental solutions that address the particularities of this area.

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References

1. Margules CR, Pressey RL. Systematic conservation planning. Nature 2000; 405: 243–253.

2. Gotelli NJ. A taxonomic wish-list for community ecology. Philosophical Transactions of the Royal Society B: Biological Sciences 2004; 359: 585–597.

3. Bortolus A. Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. Ambio 2008; 37: 114–8.

4. Jenkins CN, Pimm SL, Joppa LN. Global patterns of terrestrial vertebrate diversity and conservation. Proc Natl Acad Sci U S A 2013; 110: E2603–E2610.

5. Valsecchi J, Marmontel M, Franco CLB, et al. Atualização E Composição Da Lista - Novas Espécies De Vertebrados e Plantas Na Amazônia 2014-2015. Iniciativa. Brasília, DF e Tefé, AM, https://d3nehc6yl9qzo4.cloudfront.net/downloads/relatorio_novas_especies___wwf_brasil_mamiraua_2.pdf (2017).

6. Marques AC, Lamas CJE. Taxonomia zoológica no Brasil: Estado da arte, expectativas e sugestões de ações futuras. Pap Avulsos Zool 2006; 46: 139–174.

7. Dayrat B. Towards integrative taxonomy. Biol J Linn Soc 2005; 85: 407–415.

8. Pasquini C. Near Infrared Spectroscopy: fundamentals, practical aspects and analytical applications. J Braz Chem Soc 2003; 14: 198–219.

9. Pavia DL, Lampman GM, Kriz GS, et al. Introdução à espectroscopia. 2010.

10. Vance CK, Tolleson DR, Kinoshita K, et al. Near infrared spectroscopy in wildlife and biodiversity. Journal of Near Infrared Spectroscopy, 2016, pp. 1–25.

11. Rigby CL, Wedding BB, Grauf S, et al. The utility of near infrared spectroscopy for age estimation of deepwater sharks. Deep Sea Res Part I 2014; 94: 184–194.

12. Holler FJ, Skoog DA, Crouch SR. Princípios de análise instrumental. Bookman, 2009.

13. Jouquet P, Capowiez Y, Bottinelli N, et al. Potential of Near Infrared Refl ectance Spectroscopy (NIRS) for identifying termite species. Eur J Soil Biol 2014; 60: 49–52.

14. Fan Q, Wang Y, Sun P, et al. Discrimination of Ephedra plants with diffuse reflectance FT-NIRS and multivariate analysis. Talanta 2010; 80: 1245–1250.

15. Lang C, Costa FRC, Camargo JLC, et al. Near Infrared Spectroscopy Facilitates Rapid Identification of Both Young and Mature Amazonian Tree Species. PLoS One 2015; 10: e0134521.

16. Lang C, Almeida DRA, Costa FRC. Discrimination of taxonomic identity at species, genus and family levels using Fourier Transformed Near-Infrared Spectroscopy (FT-NIR). For Ecol Manage 2017; 406: 219–227.

17. Hadlich HL, Durgante FM, dos Santos J, et al. Recognizing Amazonian tree species in the field using bark tissues spectra. For Ecol Manage 2018; 427: 296–304.

18. Durgante FM, Higuchi N, Almeida A, et al. Forest Ecology and Management Species Spectral Signature : Discriminating closely related plant species in the Amazon with Near-Infrared Leaf-Spectroscopy. 2013; 291: 240–248.

19. Rodriguez-Fernandez JI, De Carvalho CJB, Pasquini C, et al. Barcoding without DNA? Species identification using near infrared spectroscopy. Zootaxa 2011; 54: 46–54.

20. Vance CK, Kouba AJ, Willard ST. Near Infrared Spectroscopy Applications in Amphibian Ecology and Conservation: Gender and Species Identification. NIR news 2014; 25: 10–15.

21. Torralvo K, Magnusson WE, Durgante F. Effectiveness of Fourier transform near-infrared spectroscopy spectra for species identification of anurans fixed in formaldehyde and conserved in alcohol: A new tool for integrative taxonomy. J Zool Syst Evol Res 2020; 1–14.

22. Miralles A, Bruy T, Wolcott K, et al. Repositories for Taxonomic Data: Where We Are and What is Missing. Syst Biol. Epub ahead of print 2020. DOI: 10.1093/sysbio/syaa026.

23. Beć KB, Grabska J, Huck CW. Principles and Applications of Miniaturized Near-Infrared (NIR) Spectrometers. Chem - A Eur J. Epub ahead of print 2020. DOI: 10.1002/chem.202002838.

24. Prestes RM, Vincenci KL. BIOINDICADORES COMO AVALIAÇÃO DE IMPACTO AMBIENTAL. Brazilian J Anim Environ Res 2019; 2: 1473–1493.

25. Parmar TK, Rawtani D, Agrawal YK. Bioindicators: the natural indicator of environmental pollution. Front Life Sci 2016; 9: 110–118.

26. Degarady CJ, Halbrook RS. Using Anurans as Bioindicators of PCB Contaminated Streams Published By : The Society for the Study of Amphibians and Reptiles Your use of this PDF , the BioOne Web site , and all posted and associated content. J Herpetol 2006; 40: 127–130.

27. Burger J, Gochfeld M, Powers CW, et al. Habitat protection for sensitive species: Balancing species requirements and human constraints using bioindicators as examples. Nat Sci 2013; 05: 50–62.

28. Blaustein AR, Bancroft BA. Amphibian Population Declines: Evolutionary Considerations. Bioscience 2007; 57: 437–444.

29. Frost DR. Amphibian Species of the World: an Online Reference. Electron Database Access http//research.amnh.org/herpetology/amphibia/index.html Am Museum Nat Hist New York, USA 2021; Version 6.: 1–23.

30. Hoogmoed M. Censo da Biodiversidade da Amazônia Brasileira. Museu Goeldi -Plataforma digital, http://www.museu-goeldi.br/censo/ (2019).

31. Bickford D, Lohman DJ, Sodhi NS, et al. Cryptic species as a window on diversity and conservation. Trends Ecol Evol 2007; 22: 148–155.

32. Meineke EK, Davies TJ, Daru BH, et al. Biological collections for understanding biodiversity in the Anthropocene. Philos Trans R Soc B Biol Sci 2019; 374: 20170386.

33. MPU. Deficiências em Estudos de Impacto Ambiental - Síntese de uma Experiência. Brasília: Ministério Público Federal, 4a Câmara de Coordenação e Revisão: Escola Superior do Ministério Público da União, (2004).

34. Faria ID. Ambiente e energia: crença e ciência no licenciamento ambiental. Parte III: sobre alguns dos problemas que dificultam o licenciamento ambiental no Brasil. Textos para Discussão 2011; 33.

35. ASD Inc. FieldSpec ® 3 User Manual, http://www.geo-informatie.nl/ (2010).

36. Marcon JL, Cruz J, Menin M, et al. Biodiversidade fragmentada na floresta do campus da Universidade Federal do Amazonas: conhecimento atual e desafios para a conservação. JL Marcon, M Menin, MGP Araújo, T Hrbek Biodiversidade Amaz Caracter Ecol e Conserv Manaus, Bras Ed da Univ Fed do Amaz.

37. Almeida-Corrêa T, Frazão L, Costa DM, et al. Effect of environmental parameters on squamate reptiles in an urban forest fragment in central amazonia. Acta Amaz 2020; 50: 239–245.

38. Lima A, Magnusson W, Menin M, et al. Guide to the frogs of Reserva Adolpho Ducke, Central Amazonia, https://repositorio.inpa.gov.br/handle/1/36309 (2006).

39. Faria AS, Menin M, Kaefer IL. Riparian zone as a main determinant of the structure of lizard assemblages in upland Amazonian forests. Austral Ecol 2019; 44: 850–858.

40. Rojas-Ahumada DP, Landeiro VL, Menin M. Role of environmental and spatial processes in structuring anuran communities across a tropical rain forest. Austral Ecol 2012; 37: 865–873.

41. Helland K. UNSCRAMBLER 11, version 3.10: A program for multivariate analysis with PLS and PCA/PCR. Available from CAMO AS, Jarlevn. 4, Trondheim, Norway. Price $3000; academic discount price $1500. J Chemom 1991; 5: 413–415.

42. Perdiz R de O. NIRtools: Tools to deal with near infrared (NIR) spectrocospy data. 2021; 1–2.

43. R Development Core Team. R: A Language and Environment for Statistical Computing, https://www.r-project.org/ (2019).

44. Venables WN, Ripley BD. Modern Applied Statistics With S. New York, 2002.

45. Weihs C, Ligges U, Luebke K, et al. klaR analyzing German business cycles. In: Data analysis and decision support. Springer, 2005, pp. 335–343.

46. Kuhn M. The caret package. R Found Stat Comput Vienna, Austria URL https//cran r-project org/package= caret.

47. Wickman H, François R, Henry L, et al. dlpyr: A Grammar of Data Manipulation. CRAN Repos 2021; 1–2.

48. Sarkar D. Lattice: Multivariate Data Visualization with R, http://lmdvr.r-forge.r-project.org/ (2008).

49. Kassambara A. ggplot2' Based Publication Ready Plots Version, https://cran.r-project.org/package=ggpubr (2020).

50. Pasquini C. Near infrared spectroscopy: A mature analytical technique with new perspectives – A review. Anal Chim Acta 2018; 1026: 8–36.

51. Teixeira Dos Santos CA, Lopo M, Páscoa RNMJ, et al. A review on the applications of portable near-infrared spectrometers in the agro-food industry. Appl Spectrosc 2013; 67: 1215–1233.

52. Gold TB. Applications of NIR spectroscopy in assessing raw materials and solid dosage forms. Tablets Capsul 2015; 215–227.

53. Silva GS da. Contribuição na delimitação de espécies do gênero neotropical Dimorphandra Schott (Fabaceae-Caesalpinioideae). Instituto Nacional de Pesquisas da Amazônia- INPA, Manaus, Amazonas, https://w1files.solucaoatrio.net.br/atrio/inpa-ppgbot_upl/THESIS/235/dissertao_dimorphandra_oficial_20190411122141473.pdf (2019).

54. Schwanninger M, Rodrigues JC, Fackler K. A review of band assignments in near infrared spectra of wood and wood components. J Near Infrared Spectrosc 2011; 19: 287–308.

55. Jeckel AM, Saporito RA, Grant T. The relationship between poison frog chemical defenses and age, body size, and sex. Front Zool; 12. Epub ahead of print 2015. DOI: 10.1186/s12983-015-0120-2.

56. Jeckel AM, Kocheff S, Saporito RA, et al. Geographically separated orange and blue populations of the Amazonian poison frog Adelphobates galactonotus (Anura, Dendrobatidae) do not differ in alkaloid composition or palatability. Chemoecology 2019; 29: 225–234.

57. Hantak MM, Paluh DJ, Saporito RA. Bufadienolide and alkaloid-based chemical defences in two different species of neotropical anurans are equally effective against the same arthropod predators. J Trop Ecol 2016; 32: 165–169.

58. Saporito RA, Donnelly MA, Madden AA, et al. Sex-related differences in alkaloid chemical defenses of the dendrobatid frog oophaga pumilio from cayo Nancy, bocas del Toro, Panama. J Nat Prod 2010; 73: 317–321.

59. Hoffman EA, Blouin MS. A review of colour and pattern polymorphisms in anurans. Biol J Linn Soc 2000; 70: 633–665.

60. Kakazu S, Toledo LF, Haddad CFB. Color polymorphism in Leptodactylus fuscus (Anura, Leptodactylidae): A defensive strategy against predators? Herpetol Notes 2010; 3: 69–72.

Supplementary Information

Table

Table S1. Data from live anuran samples collected in the field.

Family	Specie	E.V.A	ID	Site	Date
Leptodactylidae	*Adenomera andreae*	SIM	KT05	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	KT06	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	KT07	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	KT08	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	KT09	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S04	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S05	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S15	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S21	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S22	Ducke	feb/20
Leptodactylidae	*Adenomera andreae*	SIM	S32	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	KT10	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	KT14	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	KT15	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	KT16	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S06	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S07	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S08	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S09	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S10	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S11	Ducke	feb/20
Leptodactylidae	*Adenomera hylaedactyla*	SIM	S12	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	KT19	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	KT20	Ducke	feb/20

Phyllomedusidae	*Callimedusa tomopterna*	SIM	KT21	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	KT27	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S13	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S14	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S19	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S33	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S34	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S35	Ducke	feb/20
Phyllomedusidae	*Callimedusa tomopterna*	SIM	S36	Ducke	feb/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	KT31	Ducke	feb/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S47	Ducke	feb/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S48	Ducke	feb/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S50	Campus UFAM	mar/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S51	Campus UFAM	mar/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S52	Campus UFAM	mar/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S53	Campus UFAM	mar/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S56	Campus UFAM	mar/20
Leptodactylidae	*Leptodactylus knudseni*	NÃO	S65	FEX- UFAM	dez/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	KT03	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	KT04	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	KT13	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	KT29	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S03	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S16	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S17	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S18	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S23	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S24	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S37	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S38	Ducke	feb/20
Leptodactylidae	*Leptodactylus pentadactylus*	NÃO	S55	Campus UFAM	mar/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	KT17	Ducke	feb/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	KT18	Ducke	feb/20

Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	KT28	Ducke	feb/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	S43	Ducke	feb/20
Phyllomedusidae	*Phyllomedusa tarsius*	SIM	S57	FEX- UFAM	dez/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	S58	FEX- UFAM	dez/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	S59	FEX- UFAM	dez/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	S60	FEX- UFAM	dez/20
Phyllomedusidae	*Phyllomedusa tarsius*	NÃO	S64	FEX- UFAM	dez/20
Craugastoridae	*Pristimantis fenestratus*	SIM	KT24	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	KT25	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	KT26	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S26	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S27	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S28	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S29	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S30	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S31	Ducke	feb/20
Craugastoridae	*Pristimantis fenestratus*	SIM	S39	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	KT22	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	KT23	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	KT30	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S40	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S41	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S42	Ducke	feb/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S66	MUSA	dez/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S67	Ducke	dez/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S68	Ducke	dez/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S69	Ducke	dez/20
Craugastoridae	*Pristimantis zimmermmani*	SIM	S70	Ducke	dez/20

Figures

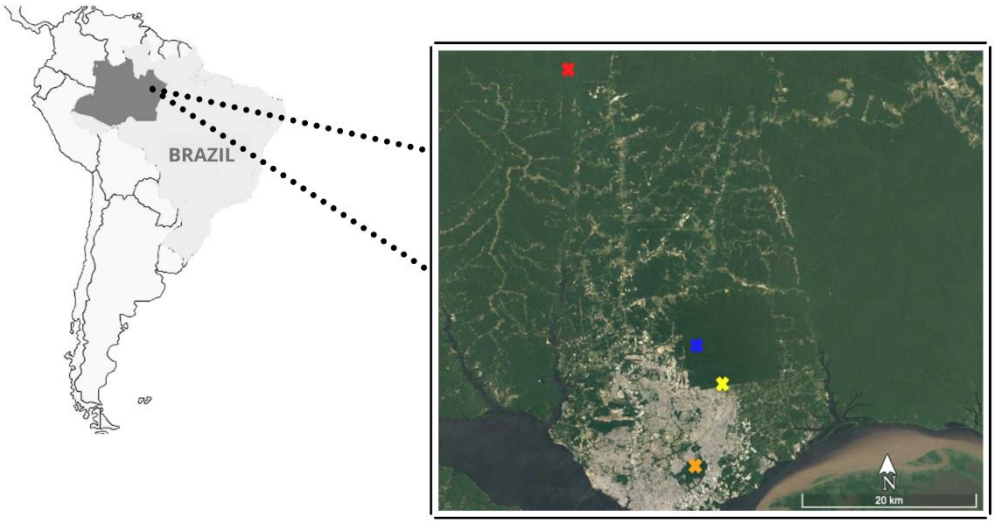

Figure S1. Areas for the collection and acquisition of live anuran spectra, located in the state of Amazonas, Brazil: FEX-UFAM (red), Reserva Ducke (blue), MUSA (yellow), Campus UFAM (orange).

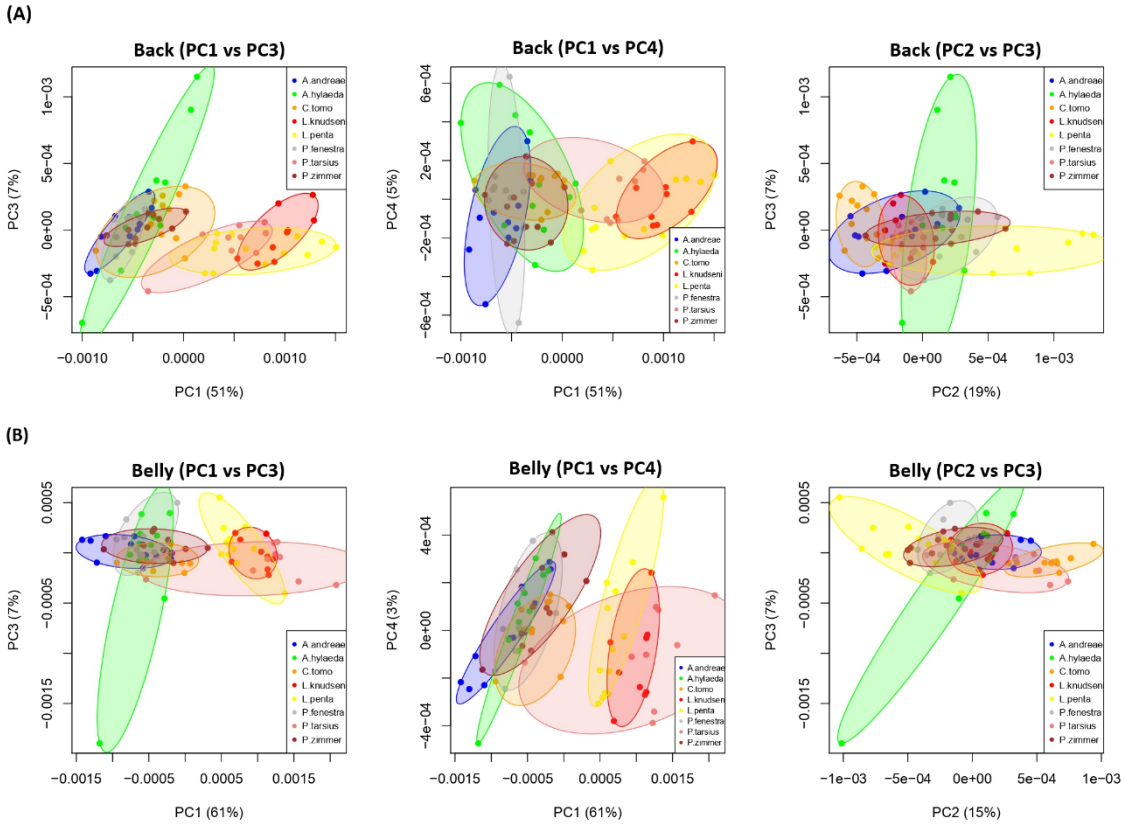

Figure S2. Two-dimensional plots in different combinations of principal components (PCA) scores showing spectral groupings of live anuran species measured on (a) back

and (b) belly. In the caption: **A.andreae=** *Adenomera andreae*, **A.hylaeda=** *Adenomera hylaedactyla*, **C.tomo=** *Callimedusa tomopterna*, **L.knudseni=** *Leptodactylus knudseni*, **L.penta=** *Leptodactylus pentadactylus*, **P.fenestra=** *Pristimantis fenestratus*, **P.tarsius=** *Phyllomedusa tarsius*, **P.zimmer=** *Pristimantis zimmermanae*.

CAPÍTULO 3

Environmental filtering and deforestation shape frog assemblages in Amazonia: an empirical approach assessing species abundances and functional traits.

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Environmental filtering and deforestation shape frog assemblages in Amazonia: an empirical approach assessing species abundances and functional traits.

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ABSTRACT

Biological assemblages are often predictable from knowledge of natural environmental heterogeneity and change in response to anthropogenic disturbances, such as deforestation, so understanding ecological mechanisms and processes mediating assemblages is essential to direct conservation actions. We sampled frogs along an edaphic and vegetation-structure gradient in the Brazilian Amazon to test the hypothesis that assemblages change in species composition and functional-trait characteristics across landscapes due to environmental filtering. Our study area covered a gradient of forest fragmentation and we hypothesized that assemblages would change in response to both natural gradients and deforestation. We found that frog assemblages are locally structured by species turnover along gradients in distance to water bodies, vegetation structure, soil sand and silt content, and proportion of the area deforested. Additionally, we found that small-bodied species and those with direct breeding (no larval stage) were no longer present in these areas. We conclude that frog assemblages are not randomly distributed across forests, but trait filtering has resulted in different species subsets from the regional pool, which change among sites with different environmental conditions and disturbance levels. Our findings highlight the importance of creating reserves to effectively protect forests and maintain connectivity among forest fragments resulting from deforestation.

Key-words: Alter do Chão, Anthropogenic disturbance, Community structure, Tapajós FLONA, Functional diversity, Taxonomic composition.

Introduction

Biological assemblages (*sensu* Stroud *et al.* 2015) are likely to be affected by multiple variables measured at interacting spatial scales, such as biogeographic barriers and climatic clines limiting dispersal across landscapes, or the environmental heterogeneity at borders of regional habitat patches (Forman 1995, Rahel 2007, Travis *et al.* 2013, Robillard *et al.* 2015). At local scales (e.g., samples taken a few kilometers apart), even species widely distributed across landscapes may have their distributions limited by combinations of environmental filtering, competition, reduced or blocked dispersal, and physiological intolerance (Azevedo-Ramos & Magnusson 1999, Carr & Fahrig 2001, Braga-Neto *et al.* 2008). Despite local environmental conditions being largely dependent on broader scales, investigating the role of environmental gradients shaping local assemblages may reveal biotic complementarities and redundancies among sites (Díaz & Cabido 2001, McGill *et al.* 2006). This approach provides an empirical basis for assessing the efficiency of reserves and environmental legislation at a scale compatible with most management operations (Fraga *et al.* 2011, Bueno *et al.* 2012).

In Amazonian tropical forests, local assemblage turnover along environmental gradients has been found for a wide variety of organisms, such as plants (e.g., Costa *et al.* 2008), fungi (e.g., Braga-Neto *et al.* 2008), vertebrates (e.g., Keller *et al.* 2009, Fraga *et al.* 2011, Oliveira *et al.* 2019) and invertebrates (e.g., Franklin *et al.* 2013, Torres *et al.* 2020). Overall, these studies showed that assemblages differ between sites because environmental gradients select different species subsets from the regional pool. However, few studies have investigated how Amazonian assemblages respond to anthropogenic changes in habitat quality, such as deforestation leading to forest fragmentation (e.g., Cintra *et al.* 2013, Benchimol & Peres 2015). Forest fragmentation is one of the main anthropogenic disturbances affecting ecosystem functioning, and has eroded biodiversity globally (Haddad *et al.* 2015, Fahrig 2017). A key-difference between continuous and fragmented forests is that assemblage resilience to fragmentation depends on a balance between the size and degree of isolation of habitat patches, matrix quality, colonization capacity (Murcia 1995, Gustafson & Gardner 1996, Ricketts 2001, Laurance *et al.* 2011), and level of forest dependence (Palmeirim *et al.* 2018). Additionally, since edge effects can penetrate habitat patches, especially if they are small, local assemblages are likely to be affected by environmental conditions within patches (Schlaepfer & Gavin 2001).

Dispersal ability and physiological tolerance are key elements determining the effects of environmental gradients on assemblages. Thus, assemblages are likely to be affected by the ability of individual species to survive and thrive under a given local environmental condition. Such ability is often defined by their morphological or physiological traits that directly influence individual performance via effects on growth, reproduction and survival, addressed as functional traits (Violle *et al.* 2007). The taxonomic and functional components of biodiversity may respond differently to anthropogenically altered habitats, and although these components may be partially related to each other, they cover widely different facets of biodiversity, which is usually ideal for integrating biodiversity monitoring (Vandewalle *et al.* 2010). The role of traits in determining assemblages has been investigated traditionally through measures of functional diversity, although this term covers a variety of concepts and applications at both trophic and evolutionary levels (see Laureto *et al.* 2015, Rosado *et al.* 2016). Here we adopt the classic assumption that assemblages are largely affected by how species use available resources (Elton 1927), and that specific traits that are selected by the environment vary along gradients according to their fitness (Violle *et al.* 2007, Bradshaw *et al.* 2009). We rely on this theoretical background to assess the importance of trait diversity in the variation in frog-assemblage composition along a gradient of continuous to fragmented forests in eastern Amazonia.

Amazonian frog assemblages vary along environmental gradients in both pristine (Menin *et al.* 2007, 2011, Rojas-Ahumada *et al.* 2012) and human-modified landscapes (Tsuji-Nishikido & Menin 2011, Díaz-Ricaurte *et al.* 2020). Assemblages based on species abundances can change spatially in response to levels of competition changing along environmental gradients, or to multiple evolutionary and ecological processes that have shaped species distributions at different spatial scales (Menin *et al.* 2008, Dias-Terceiro *et al.* 2015, Ramalho *et al.* 2018). Additionally, the wide variety of body sizes and other traits which allow frogs to explore different vertical and horizontal strata generate different levels of specificity in the use of habitats (Ernst & Rödel 2006, Bolochio *et al.* 2020, Ganança *et al.* 2021). Fragmentation may affect the ability of species with certain traits to persist. For instance, small-bodied species may find it difficult to colonize isolated patches of forest, as crossing open corridors can lead to death from desiccation or exposure to predators, arboreal species obviously lose habitats if forests are cleared, and species with aquatic eggs or larvae are unlikely to thrive if fragmentation disrupts the connection between aquatic and terrestrial habitats. Therefore,

quantifying both species abundances and traits are potentially complementary approaches to understand the ecological processes shaping assemblages. However, these two approaches may interact, such as when traits suitable for dispersal and shelter under certain local environmental conditions generate high local species abundances (Ganança *et al.* 2021).

In this study, we sampled 57 plots distributed over about 100 km of forest in eastern Amazonia to identify predictor variables for frog assemblages. We used a generalized linear model fitted to high-dimensional data to test the hypothesis that species abundances change along gradients of distance from water bodies, forest structure, soil sand and silt content, and proportional deforestation. This hypothesis is largely based on the premise that environmental gradients form habitat-quality mosaics, which can reduce local abundance, or even preclude the occurrence of poorly tolerant species, weak competitors, or those that cannot reach a fraction of a gradient via dispersal. We hypothesized that the high forest fragmentation concentrated in the north of our study area has driven assemblages to be predominantly composed of species that have evolved to occupy open areas or forest edges. We used a combination of double inertia RLQ and fourth-corner analysis (Dray *et al.* 2014) to quantify relationships between the occurrence of traits and the measured environmental gradients. We expected local frog assemblages to change spatially as multiple traits are selected from segments of environmental gradients, which defines the subsets of species with common traits that can share habitats. In both well-conserved and fragmented areas, assemblages are subject to filtering effects, competition, and dispersal limitation, but we expect traits to be associated with intolerance to edge effects to be infrequent or absent from highly fragmented areas.

Methods

Study area

The Tapajós National Forest (hereafter FLONA) covers 527,319 ha of mainly old-growth forests near the Tapajós River (Figure 1). The region is predominantly covered by dense rainforest, with trees over 40 m tall and woody lianas, palms and epiphytes (ICMBio 2019). The floristic composition and canopy opening vary in response to topographic and edaphic changes along a gradient of distance from the Tapajós River, and along the north-south axis of the reserve (Espírito-Santo *et al.* 2005). The climate is

marked by seasonally greater rainfall between January and May, and annual average precipitation (1971–2016) of 1,892 mm (ICMBio 2019). The average annual temperature between 1981 and 2010 was 25.6 ºC (INMET 2020).

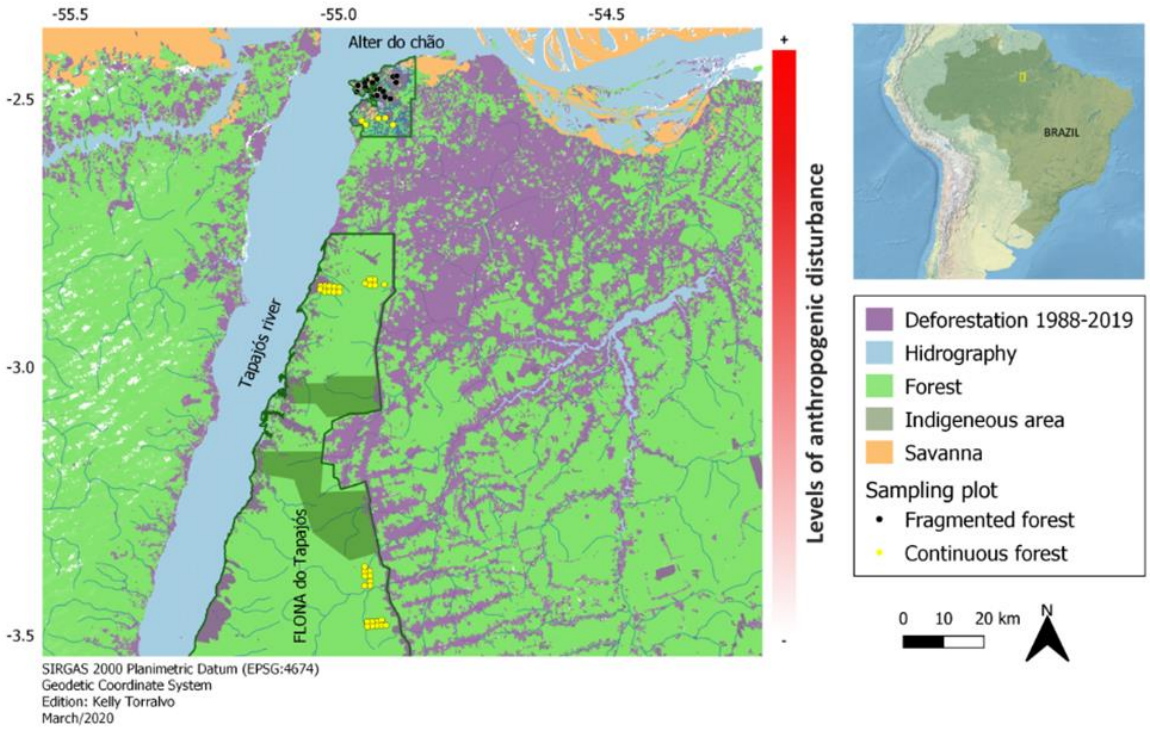

FIGURE 1. Study area in an eastern-Amazon region (western Pará) showing the location of 57 sampling plots, each 250 m long and 20 m wide. Note that the gradient in anthropogenic deforestation generally increased in the south-north direction.

To the north of the study area is Alter do Chão (Figure 1), an originally indigenous village colonized by the Portuguese, which has been converted into a tourist attraction that is important for the local economy. This region is mainly covered by secondary continuous or fragmented forests that are distinct from the mostly old-growth forests covering the southern portion of the study area. Forest fragments have often been isolated by savannas for at least the past 150 years (Bates 1863) , which possibly originated by ancestral peoples cutting and burning vegetation over thousands of years (Clayton 1961). Such habitat mosaics induce turnover in some animal assemblages, such as small mammals (Borges-Matos *et al.* 2016).

Sampling design

In the southern portion of the study area, our sampling design followed the RAPELD method (Magnusson *et al.* 2005, 2013), which is based on 250 m long and 40 m wide plots (width may vary depending on the taxonomic group being sampled). We sampled 33 plots organized in four modules (with 10, 6, 8 and 9 plots) composed of two 5 km parallel trails, 1 km apart, with a minimum distance of 1 km between neighboring plots. In the northern portion of the study area, we sampled 24 plots; 19 in forest fragments, and five in continuous forests. The plots did not follow the RAPELD design, though they were 250 m long, because they did not follow the terrain contours. However, the ground was comparatively flat in these areas, so this caused negligible differences in within-plot variability. The minimum distance between neighboring plots was 723 m.

We sampled all plots using visual and acoustic search from day to twilight (16h30min–18h00min), and night (19h00min–23h00min pm). Acoustic search was particularly useful for small, diurnal, litter species, which are usually difficult to detect (e.g., *Adenomera* spp., *Allobates* spp., *Pristimantis* spp.). Frogs detected within 20 m of the plot center line were recorded. To reduce potential detectability effects on assemblage-level data, we sampled each plot three times. We surveyed all plots during the rainy season (January–March) in 2018 and 2019, because this covers the peak of reproductive activity of most frog species. Our dataset consists of counts of frogs found by two simultaneous observers per plot. The average observation time per plot per survey was 22.8 minutes (SD = 8.45) during the day and twilight, and 61.4 minutes at night (SD = 11.8).

We identified species based on the literature (e.g., Lima *et al.* 2006, Oliveira *et al.* 2017, Carvalho *et al.* 2020) and comparisons with specimens deposited in the herpetological section of the Collection of Amphibians and Reptiles of the National Institute of Amazonian Research – INPA-H, Manaus, Brazil. We collected a maximum of five voucher specimens per species, killed them using 2% topical benzocaine, fixed them in 10% formaldehyde, and preserved them in 70% ethanol. All protocols for collecting and preserving specimens were authorized by ICMBio/SISBIO license nº 60193-2. No regionally, nationally or globally endangered species were collected.

Environmental variables

We measured litter depth with a Marimon-Hay litter collector (Marimon-Junior & Hay 2008), because this variable determines the availability of refuge, breeding and

foraging sites for terrestrial species (Fauth *et al.* 1989, Van Sluys *et al.* 2007). We measured litter depth at six points separated by 50-m intervals in each plot and used mean values in the analyses.

We measured a set of variables quantifying forest structure (see Torralvo *et al.* 2020), and summarized them into a variable which potentially affects the overall habitat availability and quality, because it is related to levels of vertical stratification, incidence of sunlight and moonlight, microclimate, and primary productivity (Parker *et al.* 2004). In addition, forest structure as measured here may affect frogs indirectly, because primary productivity affects the overall availability or turnover of invertebrate food resources (e.g., Death & Zimmermann 2005). We used data collected by a portable Light Detection and Ranging (LIDAR) device, which scans the environment with a laser for rapid measurement of multiple variables quantifying forest structure (Parker *et al.* 2004). We measured average and maximum vegetation height, leaf density and area between 0 and 15 m high, and above 15 m, canopy opening and clearing fractions at 5, 10 and 15 m above ground. To represent forest structure in the inferential models, we summarized all LIDAR variables as the first axis of a principal-component analysis (PCA), which captured 59% of the variance in the original variables (Supplementary information - Table S1). We also tested the original variables with the harmonicmeanp R-package, a statistical technique used for addressing the multiple comparisons problem that controls the probability of making one or more false discoveries, or type I errors when performing multiple hypotheses tests (Wilson 2019). In our case, the PCA axis had a stronger relationship with the response variables than using all the variables, which indicates that it captures most of the useful information about forest structure.

Temperature potentially limits frog species abundances and trait occurrence because it affects metabolic and physiological processes linked to survival and reproductive success (Ståhlberg *et al.* 2001, von May *et al.* 2017) of frogs themselves or interacting animals , such as predators (Greenwald 1974) and prey (Gilbert & Raworth 1996). We measured temperature at the beginning of day and night sampling, using AK172-AKSO® data loggers, and used mean values for each plot in the inferential models.

We measured distance from the nearest water body, because it differentiates assemblages between riparian and non-riparian zones (Fraga *et al.* 2011, Bueno *et al.* 2012, Rojas-Ahumada *et al.* 2012). We measured the distances from the coordinates of the beginning of each plot to the nearest water body using the linear-distance matrix and

Qchainage tools of the QGis 3.16.2 software (QGIS Development Team 2020), applied over combined hydrography shapefiles from public repositories (MMA 2006), following Venticinque *et al.* (2016).

We measured soil texture because this variable potentially affects frog diversity by determining moisture retention and availability of invertebrates that are part of the diet of most frog species (Menin *et al.* 2007, Nielsen *et al.* 2010). We used SoilGrids (Arrouays *et al.* 2014) to obtain sand and silt content 0–5 m deep, assuming that this superficial layer is more likely to affect frogs than deeper strata. We extracted sand- and silt-content values from centroid coordinates of each plot using the raster R-package (Hijmans *et al.* 2020).

We measured deforestation because habitat loss and fragmentation due to the expansion of human occupation is one of the main causes of the local and global amphibian decline (Sodhi *et al.* 2008, Collins *et al.* 2009, Menin *et al.* 2019). Although our sampling scheme was designed at a local scale, assemblage responses to environmental conditions are usually hierarchically dependent (Vellend 2010, Peixoto *et al.* 2020). We used a 30 x 30 m² satellite-based raster layer summarizing deforestation until 2019 from the MapBiomas project (Souza *et al.* 2020, see full description in http://mapbiomas.org) to extract proportions of deforested areas in 500 m-buffers using the raster R-package (Hijmans *et al.* 2020). The size of the buffers was defined to avoid overlap, so deforestation values are spatially independent.

Taxonomic composition

We applied generalized linear models to high-dimensional data to test the effects of environmental variables on frog abundances. We used the manyglm function of the mvabund R-package (Wang *et al.* 2020), recommended for model-based analysis of multivariate abundance data (Wang *et al.* 2012). This analysis allows for hypothesis testing, and unlike distance-based methods, does not confound node and link effects due to the misspecification of the mean-variance relationship (Warton *et al.* 2012). We evaluated levels of multicollinearity among environmental variables based on the Variance Inflation Factor (VIF), which we calculated using the car R-package (Fox *et al.* 2012). This approach indicated that there was little redundancy in the predictor variables (VIF < 1.91 in all cases). We assessed the significance of the final model using Wald's test and PIT-trap resampling with 999 iterations (anova.manyglm function) and plotted diagnostic residual distributions in Figure S1. To visualize the

variation in species abundance along the environmental gradients and deforestation, we sorted the plots in ascending order of values relative to the gradients, and plotted their positions with symbol sizes encoding abundances (Figure S2).

Functional composition

We measured species traits which are potentially mediators of environmental filtering, based on the assumption that traits poorly fitted to local environmental conditions tend to generate low abundance or absence of species. Relating these traits to the predictive variables of taxonomic diversity can provide a clear link between spatial variation in species and trait selection due to environmental heterogeneity. We selected traits that could potentially be selected by the environmental heterogeneity as they determine species fitness and dispersal ability under local environmental conditions (Table 1).

TABLE 1. Species traits and underlying ecological processes potentially mediating relationships between frog functional composition and environmental gradients. Trait abbreviations (Abbr) are used in some of the graphs shown in the results.

Trait	Abbr	Type of data	Underlying ecological process	References
SVL	SVL	Continuous	Ability to disperse, forage and escape predators	Altwegg & Reyer 2003, Buckley *et al.*
SVL relative to leg length	legs	Continuous	Ability to disperse, forage and escape predators	Altwegg & Reyer 2003, Buckley *et al.*
Arboreal or terrestrial habit	arbo; ter	Binary	Direct interactions between frogs and habitats	Duellman & Trueb 1994
Direct or larval breeding	breed_dir; breed_lar	Binary	Mediates reproductive-migration behavior	Lutz 1948, Sinsch 1990
Terrestrial or water eggs and terrestrial or larval tadpoles	eggs_ter; eggs_ter; tad_ter; tad_wat	Binary	Exposure of eggs and metamorphs to predation	Magnusson & Hero 1991, Azevedo-Ramos & Magnusson

Diurnal, crepuscular or nocturnal activity	act_diur; act_crep; act_noc	Binary	Circadian patterns of foraging, reproduction and resting-site occupancy	Duellman & Trueb 1994

We measured snout-vent length (SVL) and leg length relative to SVL in five adult males, and used mean values per plot in the inferential analyses. For those species for which no voucher specimens were collected, we obtained mean-SVL and leg-length values for adult males from the literature (e.g., Lima *et al.* 2006, Oliveira *et al.* 2017, Carvalho *et al.* 2020). This does not include species identified as aff. or gr., for which traits were measured or observed in the field. Additionally, we evaluated the effects of environmental variables on thirteen binary traits (presence or absence). We evaluated the effects of terrestrial and arboreal habits, expecting that plots where the forest structure is relatively open and little vertically stratified, or the vegetation density is relatively low because of high deforestation, would have less availability of foraging and reproduction sites for arboreal species. Consequently, the occurrence frequency of arboreal species should be higher in plots with denser and taller vegetation, and plots with less deforestation.

We hypothesized that species with direct breeding should be able to occur at greater distances from the water, and in sites with greater litter depth in the case of species that build foam nests. Additionally, proportion of deforestation may affect breeding mode by selecting species more sensitive to edge effects. We expected that species with terrestrial eggs and tadpoles would be affected by litter depth and soil water-retention capacity associated with sand content. These variables may be altered by deforestation, so we expected effects of deforestation on the type of substrate for eggs and tadpoles. Diurnal, crepuscular and nocturnal activity are also likely to be affected by deforestation, because forest fragmentation increases light incidence and temperature in the hottest hours of the day, which potentially leads to dehydration.

To test the effects of environmental gradients and deforestation on species traits, we used a combination of RLQ and fourthcorner analysis (Dray *et al.* 2014). This approach performs a double inertia analysis of environmental data (R) and functional traits (Q) with a link expressed by a contingency table composed of species relative abundances (L). We undertook RLQ using the ade4 R-package (Dray & Dufour 2007), which applies a correspondence analysis (CA) on the L array, and evaluates covariance

between the R and Q arrays trough Hill-Smith and PCA (Principal Components Analysis) respectively. We set up the model with environmental variables scaled to have mean approximately zero and standard deviation one, and species abundances standardized by the maximum abundance (*decostand* function of the vegan R-package, Oksanen *et al.* 2013). We tested a combination of null hypotheses assuming that (i) the distribution of species with fixed traits (site-independent) is not influenced by environmental variables, and (ii) the distribution of species along fixed environmental conditions is not influenced by traits (trait-independent). This configuration corresponds to the model 6 available in the ade4 R-package, for which we assessed coefficients based on 49999 permutations (following Farneda *et al.* 2015, Núñez *et al.* 2019). The predictive power of this combination of null hypotheses depends on well-adjusted relationships between the RLQ arrays, but an exploratory analysis showed that to achieve this relationship two outlier species (*Adenomera* gr. *hylaedactyla* and *Leptodactylus* gr. *petersii*) had to be removed (Figure S3).

The fourthcorner analysis tests correlations between environmental variables and species traits, and we combined it with the first two axes produced by the RLQ, summarizing environmental variables and species traits. This approach was advantageous for producing correlation matrices, which is a simplified way of showing the results.

Spatial autocorrelation

Considering that plots within highly deforested areas were concentrated in the north of our study area, spatial autocorrelation effects could confound the results of both the linear model to predict species abundance, and the RLQ + fourthcorner applied to predict species traits. To quantify the effects of spatial autocorrelation we applied Moran´s *I* tests on the summed residuals derived from the linear model, and the scores produced by the RLQ analysis. We used the ape R-package (Paradis *et al.* 2004) for global spatial autocorrelation and the pgirmess R-package (Giraudoux 2013) for spatial autocorrelation in seven distance classes. Additionally, we partitioned the variances explained by the RLQ + fourthcorner between the RLQ axis 1 and 2 separately and the geographic distance, which we represented by the axis of a Principal Coordinates of the Neighborhood Matrix (PCNM) applied on the Euclidean distances among paired plots that was more positively correlated with the RLQ axis (Borcard & Legendre 2002) (see Figure S4).

Results

Sampling overview

We found 1221 individuals classified in 26 species and seven families (Table 2). Some of the frogs sampled were in unsolved species complexes or were undescribed species. For those we assigned gr., aff. or sp. The leptodactylids *Leptodactylus paraensis* and *Adenomera amicorum* were the most frequently sampled species, being found in 84.2 and 64.9% of the plots, respectively. In contrast, the leptodactylid *Leptodactylus* gr. *petersii* and the treefrog *Boana* gr. *geographica* (Hylidae) were recorded in only 3.5 and 1.7% of the plots, respectively.

TABLE 2. Frog species found in 57 sampling plots in the Tapajós National Forest (southern portion of the study area) and around the village of Alter do Chão (northern portion of the study area), western Pará, Brazil. Species abbreviations (Abbr) are used in some of the graphs shown in the results. Occurrence frequencies are relative to the number of plots.

Taxa	Abbr	Frequency
Aromobatidae		
Allobates femoralis (Boulenger, 1884)	A.fem	42.10
Allobates magnussoni Lima, Simões & Kaefer, 2014	A.ma	5.26
Bufonidae		
Rhinella castaneotica (Caldwell, 1991)	R.cas	15.78
Rhinella magnussoni Lima, Menin & Araújo, 2007	R.ma	35.08
Rhinella major (Muller & Helmich, 1936)	R.maj	8.77
Rhinella marina (Linnaeus, 1758)	R.mar	35.08
Craugastoridae		
Pristimantis aff. *fenestratus* (Steindachner, 1864)	P.fen	7.01
Pristimantis latro Oliveira, Rodrigues, Kaefer, Pinto & Hernández-Ruz, 2017	P.lat	59.64
Pristimantis ockendeni (Boulenger, 1912)	P.ock	15.78
Dendrobatidae		
Adelphobates castaneoticus (Caldwell & Myers, 1990)	A.cas	7.01
Hylidae		
Boana gr. *geographica* (Spix, 1824)	B.geo	1.75
Boana wavrini (Parker, 1936)	B.wa	8.77

Osteocephalus aff. *oophagus* Jungfer & Schiesari, 1995	O.oop	33.33
Osteocephalus aff. *taurinus* Steindachner, 1862	O.tau	33.33
Trachycephalus helioi Nunes, Suárez, Gordo, and Pombal, 2013	T.hel	8.77
Trachycephalus typhonius (Linnaeus, 1758)	T.typ	7.01
Trachycephalus resinifictrix (Goeldi, 1907)	T.res	57.89
Leptodactylidae		
Adenomera gr. *andreae* (Müller, 1923)	A.and	63.15
Adenomera gr. *hylaedactyla* (Cope, 1868)	A.hyl	49.12
Adenomera amicorum Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2020	A.ami	64.91
Leptodactylus gr. *petersii* (Steindachner, 1864)	L.pet	3.50
Leptodactylus mystaceus (Spix, 1824)	L.mys	12.28
Leptodactylus paraensis Heyer, 2005	L.par	84.21
Leptodactylus pentadactylus (Laurenti, 1768)	L.pen	12.28
Phyllomedusidae		
Phyllomedusa vaillantii Boulenger, 1882	P.vai	5.26
Pithecopus hypochondrialis (Daudin, 1800)	P.hyp	7.01

Taxonomic- and functional-assemblage composition

Assemblage composition was significantly (P = 0.001) affected by deforestation (Wald = 6.96, P = 0.002), distance from nearest water body (Wald = 7.11, P = 0.003), forest structure (Wald = 6.78, P = 0.005), soil sand (Wald = 7.32, P = 0.001) and silt (Wald = 6.76, P = 0.004) contents. These results suggest that distinct local assemblages have been generated by multiple environmental variables filtering species across landscapes. Furthermore, they show that deforestation has altered frog-assemblage composition by causing changes in the occurrence or abundance of some species, particularly in the north of the study area, where forests have been more intensely fragmented (see Figure 1).

We investigated the outputs from the linear model by ordering the species abundances along the measured environmental gradients. We found that most of the sampled species had a locally restricted distribution or noticeably higher abundances at one of the ends of the environmental gradients, while smaller proportions of species occurred with approximately homogeneous abundances along the gradients (Figure S2).

Overall, these findings suggest that frog assemblages are structured by species turnover along environmental gradients, since random assemblage composition would be shown in these graphs by a rectangular pattern of multiple species distribution (most species occurring along large portions of the gradients), and nested assemblages would form triangles (most species more common at one extreme of the gradient). Furthermore, 46% of the sampled species (e.g., *Adelphobates castaneoticus*, *Boana* gr. *geographica*, *Allobates magnussoni*, *Pristimantis ockendeni*, *Osteocephalus* aff. *oophagus*) occurred exclusively in plots with no deforestation, while 19% of the species (e.g., *Boana wavrini*, *Leptodactylus* gr. *petersii*, *Rhinella major*, *Adenomera* gr. *hylaedactyla*) occurred exclusively or with higher abundances in plots with high proportional deforestation. The other species occurred with similar abundances throughout the deforestation gradient, indicating that the sampled species have different levels of tolerance to deforestation, ranging from low tolerance leading to local exclusion to high tolerance permitting wide distributions. Similar associations occurred for the soil silt-content gradient.

The first two axes produced by the RLQ analysis, which inform the inertia derived from the relationship between species traits and environmental variables, captured an accumulated variance of 96.81% (92.56% axis 1, and 4.25% axis 2). The Hill-Smith PCA summarizing the R table captured 32.9% of the variance in environmental variables along axis 1, which was associated with clustered deforestation plus soil silt content and forest structure plus distance from water body at opposite ends (Figure 2-A). PCA axis 2 captured 22.4% of the variance in environmental variables and was associated with litter depth, soil sand content and temperature clustered at its lower end. The correspondence analysis (CA) summarizing table L captured 19.4% of the variance in species abundance along its axis 1, and 9.1% along its axis 2. In summary, this analysis graphically revealed at least four clusters, which are composed of (i) Hylid treefrogs that live close to water bodies (e.g., *Boana wavrini*, *Boana* gr. *geographica*, *Leptodactylus pentadactylus*), (ii) large-bodied species plus *Rhinella major*, which typically occupy open areas and forest edges, (iii) forest-dweller species such as Phyllomedusid treefrogs (e.g., *Phyllomedusa vaillanti*, *Phyllomedusa hypochondrialis*) and small-bodied litter frogs (e.g., *Allobates femoralis*, *Adenomera amicorum*, *Adelphobates castaneoticus*), and (iv) direct-breeding forested welling Craugastorid species (Figure 2-B). The PCA summarizing the Q table captured 38.4% of the variance in species traits along its axis 1, which mainly separated into opposite

ends spawning and larval-breeding site (aquatic or terrestrial), and period of activity (night, and diurnal plus crepuscular). Axis 2 captured 27.1% of the variance in the traits, and more prominently separated species with larvae from those with direct development of juveniles (Figure 2-C). Complete results from the RLQ can be found in Table 3.

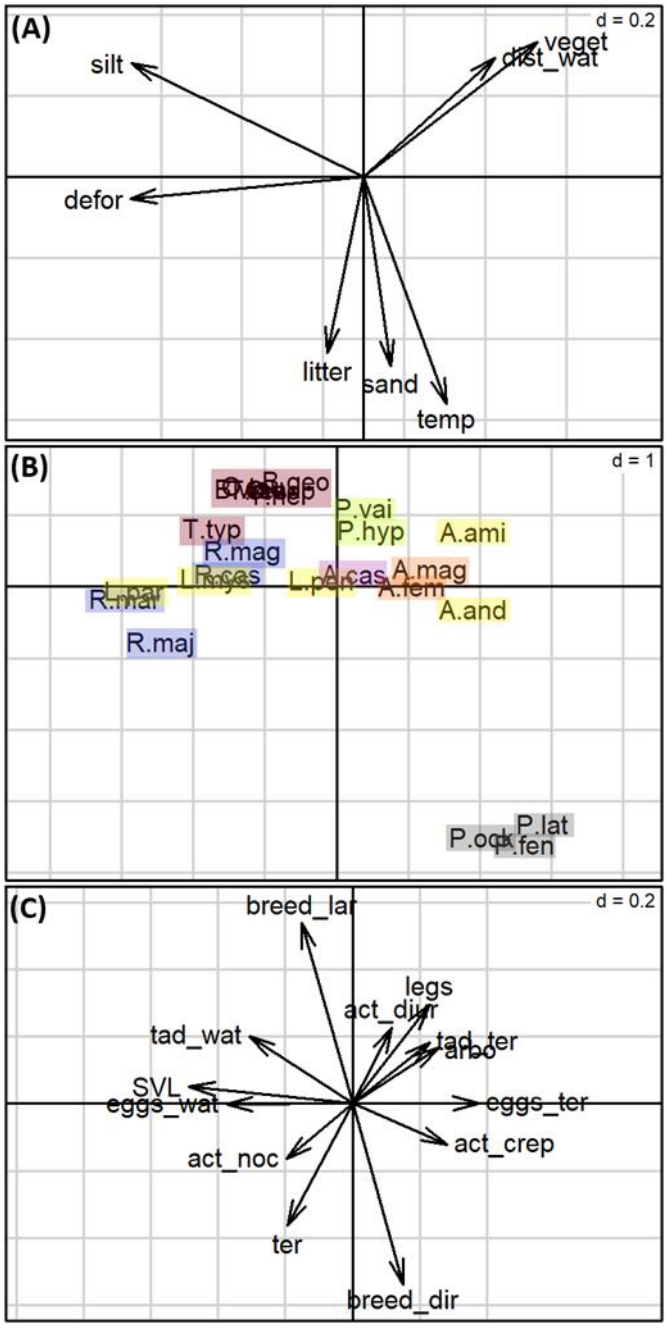

FIGURE 2. Biplots derived from a RLQ analysis applied to estimate the effects of environmental variables on frog species traits. (A) Hill-Smith PCA (Principal Component Analysis) summarizing environmental gradients, where vectors denote distances and directions from the centroid, (B) row CA scores summarizing species,

where colors distinguish frog families (dark red = Hylidae, blue = Bufonidae, yellow = Leptodactylidae, orange = Aromobatidae, green = Phyllomedusidae, purple= Dendrobatidae, gray = Craugastoridae. (C) PCA loadings summarizing species traits. Environmental variables are abbreviated as defor = deforestation, litter = litter depth, dist_wat = distance from water bodies, temp = temperature, sand = soil sand content, silt = soil silt content, veget = forest structure). For species and trait abbreviations see tables 1 and 2, respectively.

TABLE 3. Summary of a RLQ analysis applied to estimate the effects of environmental variables on frog species traits. Percentages denote total co-inertias explained by the first two RLQ axes. The R table summarizes environmental variables, the L table summarizes species abundances, and the Q table summarizes species traits. Traces are shown when a coefficient does not apply to RLQ-derived attributes. PCA is Principal Component Analysis, and CA is Correspondence Analysis.

	RLQ 1		**RLQ 2**	
	Eigenvalues	%	Eigenvalues	%
RLQ ordination	1.57	92.56	0.07	4.25
Correlation: L	0.45	56	0.15	28
Covariance	1.25	-	0.26	-
Projected variance: Q	3.59	72	5.5	64
Projected variance: R	2.15	93	3.76	97
R table (Hill-Smith PCA)	2.3	32.98	1.56	22.4
L table (CA)	0.63	19.41	0.3	9.15
Q table (PCA)	4.99	38.4	3.53	27.15

Combining the RLQ analysis with the fourthcorner approach (Figure 3) revealed that the axis relative to the species traits (table Q) was significantly affected by deforestation ($P = 0.002$), distance from the water body ($P = 0.02$), temperature ($P = 0.03$), soil silt content ($P < 0.001$) and forest structure ($P = 0.01$). Overall, these findings were consistent with the linear model predicting species abundances, suggesting that spatial variation in abundances is mediated by the environment selecting species traits. Specifically, these variables negatively affected SVL ($P = 0.008$) and the occurrence of species which lay eggs on land ($P = 0.04$), while positively affecting the occurrence of

species which lay eggs in water (P = 0.04). Considering the distribution of values related to environmental gradients measured along the axes derived from the Hill-Smith PCA (Figure S5), these findings indicate that (i) small-bodied species have been filtered from plots located in heavily-deforested areas, with silt-rich soils and low and sparse vegetation. Additionally, small-bodied species have been filtered from plots close to water bodies, but this finding reflects the fact that mean distances to water bodies were higher in the north of the study area (ANOVA $F_{1–51} = 15.08$, $P < 0.0001$), where deforestation levels are lower. Exceptionally, the medium-sized bufonid toad *Rhinella major* (55 mm on average) clustered with large-bodied species under the same environmental conditions, but this species typically occupies open areas and forest edges, and is unlikely to be found in the interior of forests; (ii) species that lay eggs on land or have direct juvenile development (without larval stage) have been filtered from plots in heavily-deforested sites. Exceptionally, *Leptodactylus paraensis* (Leptodactylidae) is not water-dependent for breeding because it lays eggs in foam nests, suggesting that the greater availability of breeding sites in the north of the study area is not the only factor mediating the local species distribution via trait selection.

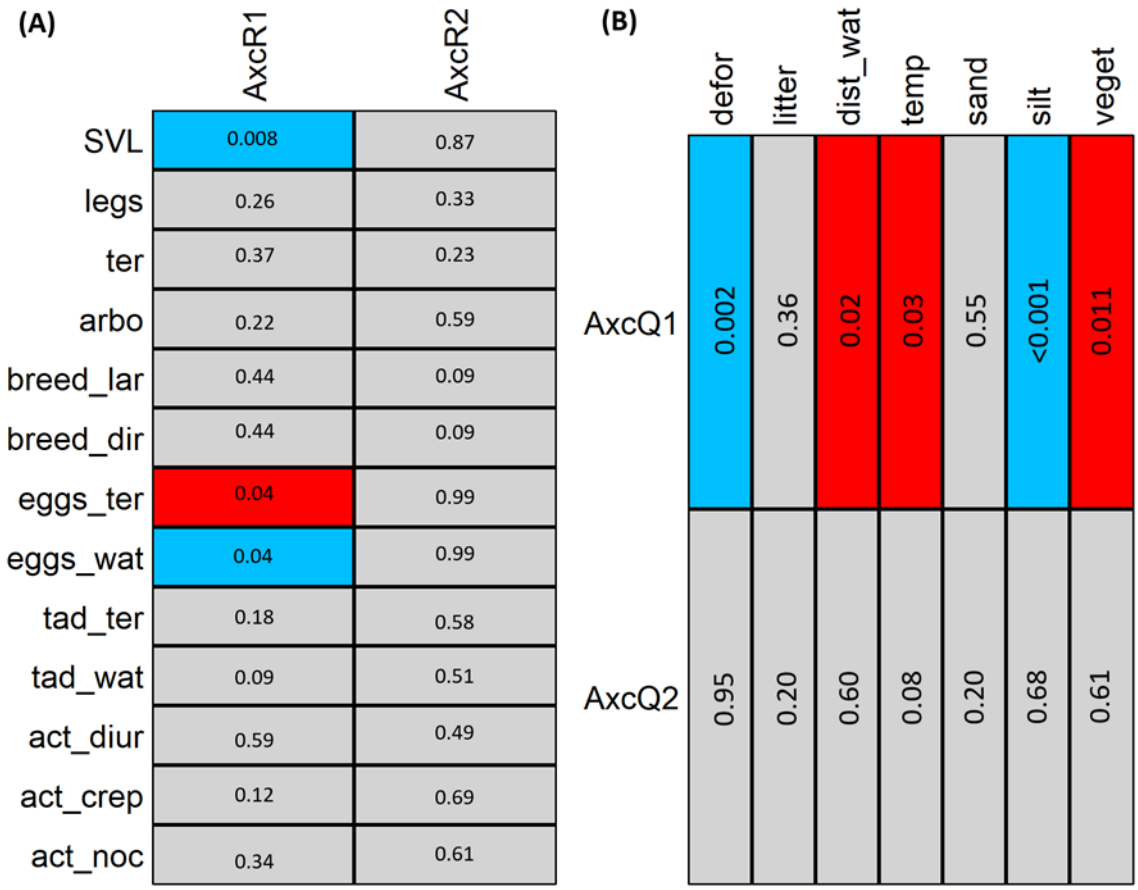

FIGURE 3. Summarized coefficients derived from a combined RLQ and fourthcorner analysis used to infer the effects of environmental variables on frog species traits. (A) correlations between the first two RLQ axes summarizing environmental variables (AxcR1 and AxcR2) and species traits. (B) Correlations between the first two RLQ axes summarizing species traits (AxcQ1 and AxcQ2) and environmental variables. Red cells are positive correlations, blue cells are negative correlations, and gray cells show non-significant correlations (P > 0.05). Environmental variables are abbreviated as defor = deforestation, litter = litter depth, dist_wat = distance from water bodies, temp = temperature, sand = soil sand content, silt = soil silt content, veget = forest structure). For traits abbreviations see Table 2.

Discussion

Our results indicate that a combination of environmental variables naturally changing over space and deforestation determine the local frog composition in an eastern Amazonian region. Overall, the sampled forests are not homogeneously suitable for all species, but habitat suitability mosaics have spatially structured assemblages

through species turnover. This is consistent with the general premises of environmental filtering, which predict that since species have different environmental requirements, local species subsets from the regional pool differ across heterogeneous landscapes (Leibold & McPeek 2006, Chase & Myers 2011). Additionally, considering that forest structure was one of the main predictors of the variation in the species abundances and trait occurrence, it is evident that deforestation has driven local assemblage composition across the study area. Deforestation has been shown to be an important predictor of frog abundance, as it negatively affects forest-dwelling species and favors the establishment of generalist species (Furlani *et al.* 2009), and potentially increases the risk of lethal-disease spread (Becker *et al.* 2016). Here, deforestation was associated with assemblage turnover along the south-north axis of the study area, with the north being mainly occupied by species relatively more tolerant to open areas and edge effects.

We found robust evidence that abundance-based frog assemblages are spatially heterogeneous across the study area because species are filtered from heavily deforested areas through ecological relationships mediated by some of their functional traits. For instance, assemblages occupying heavily deforested areas were mainly dominated by large-bodied species such as *Leptodactylus paraensis* (Leptodactylidae) and *Rhinella marina* (Bufonidae) (Figure S2), which may be related to the fact that small-bodied species have relatively low dispersal capacity, and depend on forest corridors to disperse, or have a higher risk of death when crossing open environments to reach nearby forest fragments (Gibbs 1998, Carr & Fahrig 2001). Exceptionally, the medium-bodied *Rhinella major* (Bufonidae) shares habitats with large-bodied species in deforested sites, but this species is part of a clade formed by typically open-habitat dwellers, which reproduce near forest edges and river beaches without vegetation cover (Silva *et al.* 2017). Additionally, the thick skin of Bufonid toads usually provides higher resistance to cutaneous dehydration than the thin skins of Hylid frogs, which likely enables them to occupy warm, dry habitats (Young *et al.* 2005). Therefore, our results suggest that frog assemblages in areas fragmented by deforestation are structured by environmental conditions limiting the local species distribution through a combination of dispersal capacity and dehydration tolerance.

We also found evidence that species with direct breeding and terrestrial oviposition (e.g., *Pristimantis* spp., *Allobates* spp. and *Adenomera andreae*) were less abundant in deforested sites. These species were most abundant in the well-conserved forests of the FLONA. Therefore, the availability of egg-deposition and tadpole-

development sites seems to be a limiting factor for regional frog diversity, which has been considerably reduced in fragmented forests. These findings may simply reflect the general expectation that forests tend to have more frog species that do not depend on water to reproduce than non-forest habitats (Höld 1990, Duellman 1992). However, these findings may also be biased by the inequity in the distribution of water bodies throughout the study area as a confounding factor. Although our analyses did not detect any effect of spatial autocorrelation in the predictive models, the higher concentration of water bodies in the most deforested sites does not allow us to precisely distinguish whether species that lay eggs on land have been filtered from these sites by competition with species that lay eggs in water (e.g., by space or food), or if they do not tolerate the edge effects caused by forest fragmentation. Future studies should focus on a deeper understanding of the role of distance from water bodies on frog assemblages, through sampling in the southern portion of the Tapajós FLONA, where the density of forest streams and ponds is higher.

Species turnover along environmental gradients per se (regardless of deforestation) is usually relevant for biodiversity conservation because it indicates biotic complementarities among sites, which can be used as a criterion to define priority areas (Margules *et al.* 1994, Rodrigues *et al.* 2000, Cabeza & Moilanen 2001), or to assess the efficiency of conservation techniques and policies (Fraga *et al.* 2011, Bueno *et al.* 2012). Although our models have identified species turnover mainly associated with anthropogenic deforestation, we have also detected turnover along gradients that do not necessarily reflect human activities, such as distance from water body and soil sand content. Distance from water is commonly attributed as a significant variable in studies of frog assemblages in the Amazon, as it determines the presence or absence of species with specialized reproductive modes (Menin *et al.* 2011, Ribeiro *et al.* 2012, Rojas-Ahumada *et al.* 2012). Soil texture determines soil potential to retain moisture (e.g., Woinarski *et al.* 1999, Menin *et al.* 2007) and the composition of soil fauna (Anderson 1977, Nielsen *et al.* 2008, 2010) that are prey for frogs. The most deforested areas were also the areas with the highest silt concentrations (Figure S2). This may be an effect of deforestation, or human activities may have been directed towards areas with more silt. The species most common to these areas were terrestrial species known to be associated with deforestation in other areas in the Amazon (e.g., Tsuji-Nishikido & Menin 2011, Menin *et al.* 2019), so it is unlikely that they were more common there only because of the greater silt availability. Additionally, some species that did not

occur in these areas are found in areas with high silt content (e.g., *Allobates femoralis*, Ferreira *et al.* 2018), so it is unlikely that their absence from the most disturbed areas was because of excess silt. Future studies should focus on investigating the effects of deforestation on soil structure, especially in regard to silt content, which has been little discussed in studies of Amazonian frogs.

We conclude that frog assemblages in an eastern-Amazonia are not randomly distributed across forests but represent local species subsets from a regional pool. Many of the species we sampled are widely distributed across eastern Amazonia, or cover several interfluves in the Amazon Basin (Moraes *et al.* 2016, Frost 2019). However, these species have found barriers to dispersal and survival, which have limited their distribution on a local scale. Specifically, small bodied-species and those that lay eggs on land have been filtered from deforested sites, probably because they do not tolerate edge effects, while species that do not depend on water for reproduction and those with direct development of juveniles have shared habitats in continuous forests. Therefore, the barriers to the local species distribution are mainly derived from the deforestation in the north of the study area (Alter do Chão), a particularly sensitive site for biodiversity conservation in the region we sampled. Although this area is classified as an APA (acronym in Portuguese for environmental protection area), it has been under strong pressure from real-estate speculation because, in practice, APAs have little legal protection in Brazil (ICMBio 2009).

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References

Altwegg, R., & Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. Evolution, 57, 872–882. https://doi. org/10.1111/j.0014-3820.2003.tb00298.x

Anderson, J. M. (1977). The organization of soil animal communities. Components of Ecosystems Ecological Bulletins, 25, 15–23.

Arrouays, D., Grundy, M. G., Hartemink, A. E., Hempel, J. W., Heuvelink, G. B., Hong, S. Y., Lagacherie, P., Lelyk, G., McBratney, A. B., McKenzie, N. J., Mendonca-Santos, M. L., Minasny, B., Montanarella, L., Odeh, I. O. A., Sanchez, P. A., Thompson, J. A., & Zhang, G. L. (2014). GlobalSoilMap: Toward a fine-resolution global grid of soil proper- ties. Advances in Agronomy, 125, 93–134.

Azevedo-Ramos, C., & Magnusson, W. E. (1999). Tropical tadpole vul- nerability to predation: Association between laboratory results and prey distribution in an Amazonian savanna. Copeia, 1999(1), 58–67. https://doi.org/10.2307/1447385

Becker, C. G., Rodriguez, D., Longo, A. V., Toledo, L. F., Lambertini, C., Leite, D. S., Haddad, C. F. B., & Zamudio, K. R. (2016). Deforestation, host community structure, and amphibian disease risk. Basic and Applied Ecology, 17(1), 72–80. https://doi.org/10.1016/j.baae.2015.08.004

Benchimol, M., & Peres, C. A. (2015). Edge-mediated compositional and functional decay of tree assemblages in Amazonian forest islands after 26 years of isolation. Journal of Ecology, 103, 408–420.

Bolochio, B. E., Lescano, J. N., Cordier, J. M., Loyola, R., & Nori, J. (2020). A functional perspective for global amphibian conservation. Biological Conservation, 245, 108572. https://doi.org/10.1016/j. biocon.2020.108572

Borcard, D., & Legendre, P. (2002). All-scale spatial analysis of ecologi- cal data by means of principal coordinates of neighbour matrices. Ecological Modelling, 153, 51–68. https://doi.org/10.1016/S0304-3800(01)00501-4

Borges-Matos, C., Aragón, S., da Silva, M. N. F., Fortin, M., & Magnusson, W. E. (2016). Importance of the matrix in determining small- mammal assemblages in an Amazonian forest-savanna mosaic. Biological Conservation, 204, 417–425. https://doi.org/10.1016/j. biocon.2016.10.037

Bradshaw, C. J. A., Sodhi, N. S., & Brook, B. W. (2009). Tropical turmoil: A biodiversity tragedy in progress. Frontiers in Ecology and the Environment, 7, 79–87. https://doi.org/10.1890/070193

Braga-Neto, R., Luizão, R. C. C., Magnusson, W. E., Zuquim, G., & De Castilho, C. V. (2008). Leaf litter fungi in a Central Amazonian for- est: The influence of rainfall, soil and topography on the distribu- tion of fruiting bodies. Biodiversity and Conservation, 17, 2701–2712.

Buckley, C. R., Michael, S. F., & Irschick, D. J. (2005). Early hatch- ing decreases jumping performance in a direct-developing frog, Eleutherodactylus coqui. Functional Ecology, 19, 67–72.

Bueno, A. S., Bruno, R. S., Pimentel, T. P., Sanaiotti, T. M., & Magnusson, W. E. (2012). The width of riparian habitats for understory birds in an Amazonian forest. Ecological Applications, 22, 722–734. https:// doi.org/10.1890/11-0789.1

Cabeza, M., & Moilanen, A. (2001). Design of reserve networks and the persistence of biodiversity. Trends in Ecology & Evolution, 16, 242– 248. https://doi.org/10.1016/S0169-5347(01)02125-5

Carr, L. W., & Fahrig, L. (2001). Effect of road traffic on two amphibian species of differing vagility. Conservation Biology, 15, 1071–1078. https://doi.org/10.1046/j.1523-1739.2001.0150041071.x

Carvalho, T. R., Moraes, L. J., Lima, A. P., Fouquet, A., Peloso, P. L., Pavan, D., Drummond, L. O., Rodrigues, M. T., Giaretta, A. A., Gordo, M., Neckel-Oliveira, S., & Haddad, C. F. B. (2021). Systematics and his- torical biogeography of Neotropical foam-nesting frogs of the Adenomera heyeri clade (Leptodactylidae), with the

description of six new Amazonian species. Zoological Journal of the Linnean Society, 191, 395–433. https://doi.org/10.1093/zoolinnean/zlaa051/5880331

Chase, J. M., & Myers, J. A. (2011). Disentangling the importance of eco- logical niches from stochastic processes across scales. Philosophical Transactions of the Royal Society B: Biological Sciences, 366, 2351– 2363. https://doi.org/10.1098/rstb.2011.0063

Cintra, R., Magnusson, W. E., & Albernaz, A. (2013). Spatial and tempo- ral changes in bird assemblages in forest fragments in an eastern Amazonian savannah. Ecology and Evolution, 3, 3249–3262. https:// doi.org/10.1002/ece3.700

Collins, J. P., Crump, M. L., & Lovejoy, T. E. III (2009). Extinction in our times: Global amphibian decline. Oxford University Press.

Costa, F. R., Guillaumet, J. L., Lima, A. P., & Pereira, O. S. (2009). Gradients within gradients: The mesoscale distribution patterns of palms in a central amazonian forest. Journal of Vegetation Science, 20, 69–78. https://doi.org/10.3170/2008-8-18478

da Silva, M. I., Schiesari, L. C., & Menin, M. (2017). The egg clutch and tadpole of Rhinella merianae (Gallardo, 1965) (Anura: Bufonidae) from Central Amazonia, Brazil. Zootaxa, 4294, 145. https://doi. org/10.11646/zootaxa.4294.1.12

de Fraga, R., Lima, A. P., & Magnusson, W. E. (2011). Mesoscale spatial ecology of a tropical snake assemblage: The width of riparian corri- dors in central Amazonia. Herpetological Journal, 21, 51–57.

de Oliveira, E. A., Rodrigues, L. R., Kaefer, I. L., Pinto, K. C., & Hernández- Ruz, E. J. (2017). A new species of Pristimantis from eastern Brazilian Amazonia (Anura, Craugastoridae). ZooKeys, 2017, 101– 129. https://doi.org/10.3897/zookeys.687.13221

Death, R. G., & Zimmermann, E. M. (2005). Interaction between disturbance and primary productivity in determining stream invertebrate diversity. Oikos, 111, 392–402. https://doi. org/10.1111/j.0030-1299.2005.13799.x

Dias-Terceiro, R. G., Kaefer, I. L., de Fraga, R., de Araújo, M. C., Simões, P. I., & Lima, A. P. (2015). A matter of scale: Historical and envi- ronmental factors structure

anuran assemblages from the Upper Madeira River, Amazonia. Biotropica, 47, 259–266. https://doi. org/10.1111/btp.12197

Díaz, S., & Cabido, M. (2001). Vive la différence: Plant functional diver- sity matters to ecosystem processes. Trends in Ecology & Evolution, 16, 646–655. https://doi.org/10.1016/S0169-5347(01)02283-2

Díaz-Ricaurte, J. C., Arriaga Villegas, N. C., López Coronado, J. D., Macias Garzón, G. X., & Fiorillo, B. F. (2020). Effects of agricultural sys- tems on the anuran diversity in the Colombian Amazon. Studies on Neotropical Fauna and Environment. https://doi.org/10.1080/01650 521.2020.1809334

Dray, S., Choler, P., Dolédec, S., Peres-Neto, P. R., Thuiller, W., Pavoine, S., & Ter Braak, C. J. F. (2014). Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental varia- tion. Ecology, 95, 14–21. https://doi.org/10.1890/13-0196.1

Dray, S., & Dufour, A.-B. (2007). The ade4 Package: Implementing the duality diagram for ecologists. Journal of Statistical Software, 22, 1–20.

Duellman, W. E. (1992). Reproductive strategies of frogs. Scientific American, 267, 80–87. https://doi.org/10.1038/scientificameri can0792-80

Duellman, W. E., & Trueb, L. (1994). Biology of amphibians. JHU Press. Elton, C. (1927). Animal ecology, 1927 (p. 56). Sidgwick Jackson, LTD.

Ernst, R., & Rödel, M. (2006). Community assembly and structure of tropical leaf-litter Anurans. Ecotropica, 12, 113–129.

Fahrig, L. (2017). Ecological responses to habitat fragmentation per se. Annual Review of Ecology Evolution and Systematics, 48, 1–23. https://doi.org/10.1146/annurev-ecolsys-110316-022612

Farneda, F. Z., Rocha, R., López-Baucells, A., Groenenberg, M., Silva, I., Palmeirim, J. M., Bobrowiec, P. E. D., & Meyer, C. F. J. (2015). Trait-related responses to habitat fragmentation in Amazonian bats. Journal of Applied Ecology, 52, 1381–1391. https://doi. org/10.1111/1365-2664.12490

Fauth, J. E., Crother, B. I., & Slowinski, J. B. (1989). Elevational pat- terns of species richness, evenness, and abundance of the costa rican leaf-litter Herpetofauna. Biotropica, 21, 178. https://doi. org/10.2307/2388708

Ferreira, A. S., Jehle, R., Stow, A. J., & Lima, A. P. (2018). Soil and for- est structure predicts large-scale patterns of occurrence and local abundance of a widespread Amazonian frog. PeerJ, 6, 1–26. https:// doi.org/10.7717/peerj.5424

Forman, R. T. T. (1995). Some general principles of landscape and regional ecology. Landscape Ecology, 10, 133–142. https://doi.org/10.1007/ BF00133027

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., & Graves, S. (2012). Package 'car'. R Found. Stat. Comput.

Frost, D. R. (2019). Amphibian Species of the World: an Online Reference. Electron. Database Access. Am. Museum Nat. Hist. New York, USA. Version 6: 1–23. http://research.amnh.org/herpetology/amphibia/ index.html

Furlani, D., Francesco Ficetola, G., Colombo, G., Ugurlucan, M., & De Bernardi, F. (2009). Deforestation and the Structure of Frog Communities in the Humedale Terraba-Sierpe, Costa Rica. Zoological Science, 26, 197–202. https://doi.org/10.2108/zsj.26.197

Ganança, P. H. S., Santos, A. P., Kawashita-Ribeiro, R. A., de Vasconcelos Neto, L. B., dos Santos Júnior, I. A., Guedes, D. S., & Fraga, R. (2021). Habitats determining local frog assemblages within aquatic macrophyte meadows in Amazonia, through species traits filtering. Austral Ecology, 46, 574–587.

Gibbs, J. P. (1998). Amphibian movements in response to forest edges, roads, and streambeds in Southern New England. Journal of Wildlife Management, 62, 584.

Gilbert, N., & Raworth, D. A. (1996). Forum: Insects and temperature—a general theory. Canadian Entomologist, 128, 1–14. https://doi. org/10.4039/Ent1281-1

Giraudoux, P. (2018). pgirmess: Spatial Analysis and Data Mining for Field Ecologists. R package version 1.6.9. https://CRAN.R-project.org/ package=pgirmess

Greenwald, O. E. (1974). Thermal dependence of striking and prey capture by Gopher Snakes. Copeia, 1974, 141. https://doi. org/10.2307/1443016

Gustafson, E. J., & Gardner, R. H. (1996). The effect of landscape hetero- geneity on the probability of patch colonization. Ecology, 77, 94– 107. https://doi.org/10.2307/2265659

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., Lovejoy, T. E., Sexton, J. O., Austin, M. P., Collins, C. D., Cook, W. M., Damschen, E. I., Ewers, R. M., Foster, B. L., Jenkins, C. N., King, A. J., Laurance, W. F., Levey, D. J., Margules, C. R., … Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances, 1, 1–10. https://doi. org/10.1126/sciadv.1500052

Hijmans, R. J. (2020). raster: Geographic Data Analysis and Modeling. R package version 3.4-5, 1–249. https://CRAN.R-project.org/packa ge=raster

Höld, W. (1990). Reproductive diversity in Amazonian lowland frogs. Fortschritte Der Zoologie, 38, 40–60.

ICMBio. (2009). Legislação ICMBio - Sistema Nacional De Unidades De Conservação, 1, 69. http://www.icmbio.gov.br/portal/images/stori es/comunicacao/legislacaoambientalvolume1.pdf

ICMBio. (2019). Plano De Manejo - Floresta Nacional do Tapajós. Volume I - Diagnóstico I, 2–165. http://www.icmbio.gov.br/portal/image s/stories/plano -de-manejo/plano_de_manejo_flona_do_tapaj ós_2019_vol1.pdf

INMET (2020). Instituto Nacional de Meteorologia-Monitoramento da Estação Meteorológica de Belterra-PA. https://portal.inmet.gov.br/

Laurance, W. F., Camargo, J. L. C., Luizão, R. C. C., Laurance, S. G., Pimm, S. L., Bruna, E. M., Stouffer, P. C., Bruce Williamson, G., Benítez- Malvido, J., Vasconcelos, H. L., Van Houtan, K. S., Zartman, C. E., Boyle, S. A., Didham, R. K., Andrade, A., & Lovejoy, T. E. (2011). The fate of Amazonian forest fragments: A 32-year investigation. Biological Conservation, 144(1), 56–67. https://doi.org/10.1016/j. biocon.2010.09.021

Laureto, L. M. O., Cianciaruso, M. V., & Samia, D. S. M. (2015). Functional diversity: an overview of its history and applicability. Natureza & Conservação, 13(2), 112–116. https://doi.org/10.1016/j. ncon.2015.11.001

Leibold, M. A., & McPeek, M. A. (2006). Coexistence of the niche and neutral perspectives in community ecology. Ecology, 87, 1399– 1410. https://doi.org/10.1890/00129658(2006)87[1399:COTNA N]2.0.CO;2

Lima, A., Magnusson, W., Menin, M., Erdtmann, L., Rodrigues, D., Keller, C., & Hodl, W. (2006). Guide to the frogs of Reserva Adolpho Ducke. Central Amazonia. https://repositorio.inpa.gov.br/handle/1/36309

Lutz, B. (1948). Ontogenetic evolution in frogs. Evolution, 2, 29–39. https://doi.org/10.1111/j.1558-5646.1948.tb02729.x

Magnusson, W., Braga-Neto, R., Pezzini, F., Baccaro, F., Bergallo, H., Penha, J., Rodrigues, D., Verdade, L. M., Lima, A., Albernaz, A. L., Hero, J.-M., Lawson, B., Castilho, C., Drucker, D., Franklin, E., Mendonça, F., Costa, F., Galdino, G., Castley, G., … Pontes, A. R. M. (2013). Biodiversidade e Monitoramento Ambiental Integrado PPBio INPA. https://repositorio.inpa.gov.br/bitstream/1/36304/1/Biodi versidade_e_monitoramento_ambiental_integrado.pdf

Magnusson, W. E., & Hero, J. M. (1991). Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. Oecologia, 86, 310–318. https://doi.org/10.1007/BF00317595

Magnusson, W. E., Lima, A. P., Luizão, R., Luizão, F., Costa, F. R. C., de Castilho, C. V., & Kinupp, V. F. (2005). RAPELD: A modification of the Gentry method for biodiversity surveys in long-term ecological research sites. Biota Neotropica, 5, 21–26. https://doi.org/10.1590/ S1676-06032005000300002

Margules, C. R., Nicholls, A. O., & Usher, M. B. (1994). Apparent species turnover, probability of extinction and the selection of nature reserves: A case study of the Ingleborough limestone pavements. Conservation Biology, 8, 398–409.

Marimon-Junior, B. H., & Hay, J. D. (2008). A new instrument for mea- surement and collection of quantitative samples of the litter layer in forests. Forest Ecology and Management, 255, 2244–2250. https:// doi.org/10.1016/j.foreco.2008.01.037

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. Trends in Ecology & Evolution, 21, 178–185. https://doi.org/10.1016/j.tree.2006.02.002

Menin, M., Ferreira, R. F. B., Melo, I. B., Gordo, M., Hattori, G. Y., & Sant'anna, B. S. (2019). Anuran diversity in urban and rural zones of the Itacoatiara municipality, central Amazonia, Brazil. Acta Amazonica, 49, 122–130. https://doi.org/10.1590/1809-4392201800284

Menin, M., Lima, A. P., Magnusson, W. E., & Waldez, F. (2007). Topographic and edaphic effects on the distribution of terrestrially reproducing anurans in Central Amazonia: Mesoscale spatial patterns. Journal of Tropical Ecology, 23(5), 539–547. https://doi.org/10.1017/S0266467407004269

Menin, M., Waldez, F., & Lima, A. P. (2008). Temporal variation in the abundance and number of species of frogs in 10,000 ha of a forest in central Amazonia, Brazil. South American Journal of Herpetology, 3, 68–81. https://doi.org/10.2994/1808-9798(2008)3[68:TVITA A]2.0.CO;2

Menin, M., Waldez, F., & Lima, A. P. (2011). Effects of environmental and spatial factors on the distribution of anuran species with aquatic re- production in central Amazonia. Herpetology Journal, 21, 255–261.

MMA (2006). Mapas de Cobertura Vegetal dos Biomas Brasileiros. Minist. do Meio Ambient. Retrieved from: http://mapas.mma.gov.br/ mapas/aplic/probio/datadownload.htm?/ [Accessed November 14, 2016].

Moraes, L. J. C. L., Pavan, D., Barros, M. C., & Ribas, C. C. (2016). The combined influence of riverine barriers and flooding gradients on biogeographical patterns for amphibians and squamates in south- eastern Amazonia. Journal of Biogeography, 43, 2113–2124. https:// doi.org/10.1111/jbi.12756

Murcia, C. (1995). Edge effects in fragmented forests: Implications for conservation. Trends in Ecology & Evolution, 10, 58–62. https://doi. org/10.1016/S0169-5347(00)88977-6

Nielsen, U. N., Osler, G. H. R., Campbell, C. D., Neilson, R., Burslem, D. F. R. P., & van der Wal, R. (2010). The enigma of soil animal species diversity revisited: The role of small-scale heterogeneity. PLoS One, 5, 26–28. https://doi.org/10.1371/journal.pone.0011567

Nielsen, U. N., Osler, G. H. R., van der Wal, R., Campbell, C. D., & Burslem,D.F.R.P. (2008). Soil pore volume and the abundance of soil mites in two contrasting habitats. Soil Biology & Biochemistry, 40, 1538– 1541. https://doi.org/10.1016/j.soilbio.2007.12.029

Núñez, S. F., López-Baucells, A., Rocha, R., Farneda, F. Z., Bobrowiec, P.E. D., Palmeirim, J. M., & Meyer, C. F. J. (2019). Echolocation and stratum preference: Key trait correlates of vulnerability of insectiv- orous bats to tropical forest fragmentation. Frontiers in Ecology and Evolution, 7, 1–12. https://doi.org/10.3389/fevo.2019.00373

Palmeirim, A. F., Benchimol, M., Vieira, M. V., & Peres, C. A. (2018). Small mammal responses to Amazonian forest islands are modulated by their forest dependence. Oecologia, 187(1), 191–204. https://doi. org/10.1007/s00442-018-4114-6

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Parker, G. G., Harding, D. J., & Berger, M. L. (2004). A portable LIDAR system for rapid determination of forest canopy structure. Journal of Applied Ecology, 41, 755–767. https://doi. org/10.1111/j.0021-8901.2004.00925.x

Peixoto, G. M., de Fraga, R., Araújo, M. C., Kaefer, I. L., & Lima, A. P. (2020). Hierarchical effects of historical and environmental factors on lizard assemblages in the upper Madeira River, Brazilian Amazonia. PLoS One, 15, 1–19.

QGIS Development Team (2020). QGIS Geographic Information System. Proj: Open Source Geospatial Found. http://qgis.osgeo.org

Rahel, F. J. (2007). Biogeographic barriers, connectivity and homogenization of freshwater faunas: It’s a small world after all. Freshwater Biology, 52, 696–710.

Ramalho, W. P., Machado, I. F., & Vieira, L. J. S. (2018). Do flood pulses structure amphibian communities in floodplain environments? Biotropica, 50, 338–345. https://doi.org/10.1111/btp.12523

Ribeiro, J. W., Lima, A. P., & Magnusson, W. E. (2012). The effect of Riparian Zones on species diversity of frogs in Amazonian forests. Copeia, 2012, 375–381. https://doi.org/10.1643/CE-11-117

Ricketts, T. H. (2001). The matrix matters: Effective isolation in fragmented landscapes. American Naturalist, 158, 87. https://doi. org/10.1086/320863

Robillard, C. M., Coristine, L. E., Soares, R. N., & Kerr, J. T. (2015). Facilitating climate-change-induced range shifts across continental land-use barriers. Conservation Biology, 29, 1586–1595. https://doi. org/10.1111/cobi.12556

Rodrigues, A. S. L. L., Gregory, R. D., & Gaston, K. J. (2000). Robustness of reserve selection procedures under temporal species turnover. Proceedings of the Royal Society B-Biological Sciences, 267, 49–55. https://doi.org/10.1098/rspb.2000.0965

Rojas-Ahumada, D. P., Landeiro, V. L., & Menin, M. (2012). Role of envi- ronmental and spatial processes in structuring anuran communities across a tropical rain forest. Austral Ecology, 37, 865–873. https:// doi.org/10.1111/j.1442-9993.2011.02330.x

Rosado, B. H. P., Figueiredo, M. S. L., de Mattos, E. A., & Grelle, C. E. V. (2016). Eltonian shortfall due to the Grinnellian view: functional ecology between the mismatch of niche concepts. Ecography, 39, 1034–1041. https://doi.org/10.1111/ecog.01678

Schlaepfer, M. A., & Gavin, T. A. (2001). Edge effects on lizards and frogs in tropical forest fragments. Conservation Biology, 15, 1079–1090. https://doi.org/10.1046/j.1523-1739.2001.0150041079.x

Sinsch, U. (1990). Migration and orientation in anuran amphibians. Ethology Ecology & Evolution, 2, 65–79. https://doi.org/10.1080/08927014.1990.9525494

Sodhi, N. S., Bickford, D., Diesmos, A. C., Lee, T. M., Koh, L. P., Brook, B. W., Sekercioglu, C. H., & Bradshaw, C. J. A. (2008). Measuring the meltdown: Drivers of global amphibian extinction and decline. PLoS One, 3, 1–8. https://doi.org/10.1371/journal.pone.0001636

Souza, C. M., Z. Shimbo, J., Rosa, M. R., Parente, L. L., A. Alencar, A., Rudorff, B. F. T., Hasenack, H., Matsumoto, M., G. Ferreira, L., Souza-Filho, P. W. M., de Oliveira, S. W., Rocha, W. F., Fonseca, A. V., Marques, C. B., Diniz, C. G., Costa, D., Monteiro,

D., Rosa, E. R., Vélez-Martin, E., … Azevedo, T. (2020). Reconstructing three decades of land use and land cover changes in Brazilian biomes with landsat archive and earth engine. Remote Sensing, 12, 2735. https:// doi.org/10.3390/rs12172735

Ståhlberg, F., Olsson, M., & Uller, T. (2001). Population divergence of developmental thermal optima in Swedish common frogs, Rana temporaria. Journal of Evolutionary Biology, 14, 755–762.

Stroud, J. T., Bush, M. R., Ladd, M. C., Nowicki, R. J., Shantz, A. A., & Sweatman, J. (2015). Is a community still a community? Reviewing definitions of key terms in community ecology. Ecology and Evolution, 5, 4757–4765. https://doi.org/10.1002/ece3.1651

Torralvo, K., de Fraga, R., Lima, A. P., Dayrell, J., & Magnusson, W. E. (2021). Data from: Environmental filtering and deforestation shape frog assemblages in Amazonia: an empirical approach assessing species abundances and functional traits. Dryad Digital Repository, https://doi.org/10.5061/dryad.gb5mkkwqv

Torralvo, K., Lima, A., Rosa, C. A., & Magnusson, W. (2020). Dados de estrutura da vegetação obtidos com LIDAR portátil de chão em parcelas permanentes de módulos RAPELD na Floresta Nacional (FLONA) do Tapajós- PA. PPBio Repos. -ID PPBioAmOc.577.2. https://search. dataone.org/view/PPBioAmOc.577.2

Travis, J. M. J., Delgado, M., Bocedi, G., Baguette, M., Bartoń, K., Bonte, D., Boulangeat, I., Hodgson, J. A., Kubisch, A., Penteriani, V., Saastamoinen, M., Stevens, V. M., & Bullock, J. M. (2013). Dispersal and species' responses to climate change. Oikos, 122, 1532–1540. https://doi.org/10.1111/j.1600-0706.2013.00399.x

Tsuji-Nishikido, B. M., & Menin, M. (2011). Distribution of frogs in riparian areas of an urban forest fragment in Central Amazonia. Biota Neotropica, 11, 63–70. https://doi.org/10.1590/S1676-06032011000200007

Van Sluys, M., Vrcibradic, D., Alves, M. A. S., Bergallo, H. G., & Rocha, C. F. D. (2007). Ecological parameters of the leaf-litter frog community of an Atlantic Rainforest area at Ilha Grande, Rio de Janeiro state, Brazil. Austral Ecology, 32, 254–260.

Vandewalle, M., de Bello, F., Berg, M. P., Bolger, T., Dolédec, S., Dubs, F., Feld, C. K., Harrington, R., Harrison, P. A., Lavorel, S., da Silva, P. M., Moretti, M., Niemelä, J., Santos, P., Sattler, T., Sousa, J. P., Sykes, M. T., Vanbergen, A. J., & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation, 19, 2921– 2947. https://doi.org/10.1007/s10531-010-9798-9

Vellend, M. (2010). Conceptual synthesis in community ecology. The Quarterly Review of Biology, 85, 183–206. https://doi. org/10.1086/652373

Venticinque, E., Forsberg, B., Barthem, R., Petry, P., Hess, L., Mercado, A., Cañas, C., Montoya, M., Durigan, C., & Goulding, M. (2016). An explicit GIS-based river basin framework for aquatic ecosystem conservation in the Amazon. Earth System Science Data, 8, 651–661. https://doi.org/10.5194/essd-8-651-2016

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! Oikos, 116, 882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

von May, R., Catenazzi, A., Corl, A., Santa-Cruz, R., Carnaval, A. C., & Moritz, C. (2017). Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. Ecology and Evolution, 7, 3257–3267. https://doi.org/10.1002/ece3.2929

Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., & Warton, D. (2020). mvabund: Statistical Methods for Analysing Multivariate Abundance Data. R package version 4.1.3. https://CRAN.Rproject. org/package=mvabund

Warton, D. I., Wright, S. T., & Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution, 3, 89–101. https://doi. org/10.1111/j.2041-210X.2011.00127.x

Wilson, D. J. (2019). The harmonic mean p-value for combining depen- dent tests. Proceedings of the National Academy of Sciences of the United States of America, 116, 1195–1200.

Woinarski, J. C. Z., Fisher, A., & Milne, D. (1999). Distribution patterns of vertebrates in relation to an extensive rainfall gradient and variation in soil texture in the tropical

savannas of the Northern Territory, Australia. Journal of Tropical Ecology, 15, 381–398. https:// doi.org/10.1017/S0266467499000905

Young, J. E., Christian, K. A., Donnellan, S., Tracy, C. R., & Parry, D. (2005). Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. Physiological and Biochemical Zoology, 78, 847–856. https://doi.org/10.1086/432152

Supplementary information

TABLES

Table S1. Loadings derived from a principal component analysis (PC) summarizing vegetation-cover variables measured by a Light Detection and Ranging (LiDAR) device.

Variable	PC1	PC2
Maximum forest height	0.7372553	0.008774
Average forest height	0.9144892	0.098861
Canopy opening ratio	-0.512449	0.783561
Canopy gaps computed at 5 m	-0.615662	0.026319
Canopy gaps computed at 10 m	-0.876835	-0.25571
Canopy gaps computed at 15 m	-0.890656	-0.343
Leaf area index in the vertical profile	0.4980229	-0.84293
Leaf area index in the vertical profile 0–15 m	-0.780405	-0.57461
Leaf area index in the vertical profile above 15 m	0.9567454	-0.24822
Variance captured	**0.59**	**0.21**

FIGURES

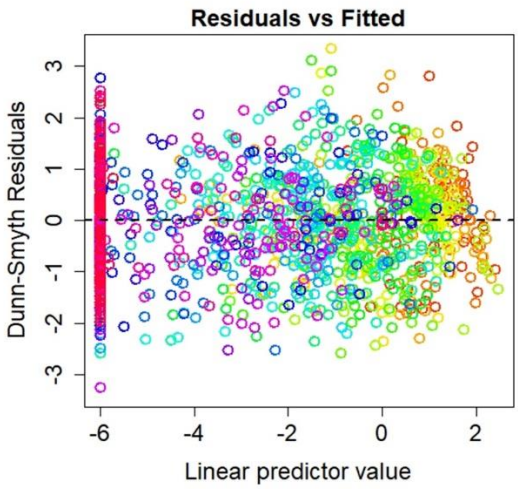


Figure S1. Residual distribution derived from a generalized linear model fitted to high-dimensional data used to test the effects of environmental variables on frog abundance in eastern Amazonia. Colors represent different species.

Figure S2. Direct ordination of plots along environmental gradients measured in an eastern Amazon region. The height of the black rectangles denotes species relative abundances, and blank spaces indicate species absence.

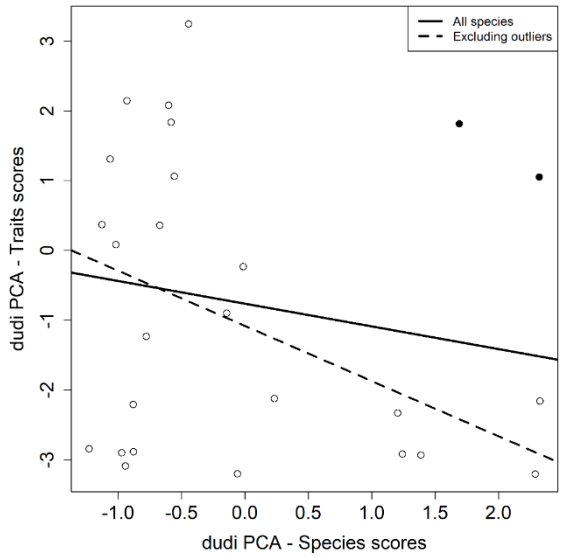


Figure S3. Relationship between scores produced by Principal Component Analyses (PCA) summarizing frog species abundance and traits. This is an exploratory analysis applied to assess a priori quality of inferential models quantifying the effects of environmental variables on the spatial variation in trait occurrence. Note the improved fit by removing two outlier species (black circles).

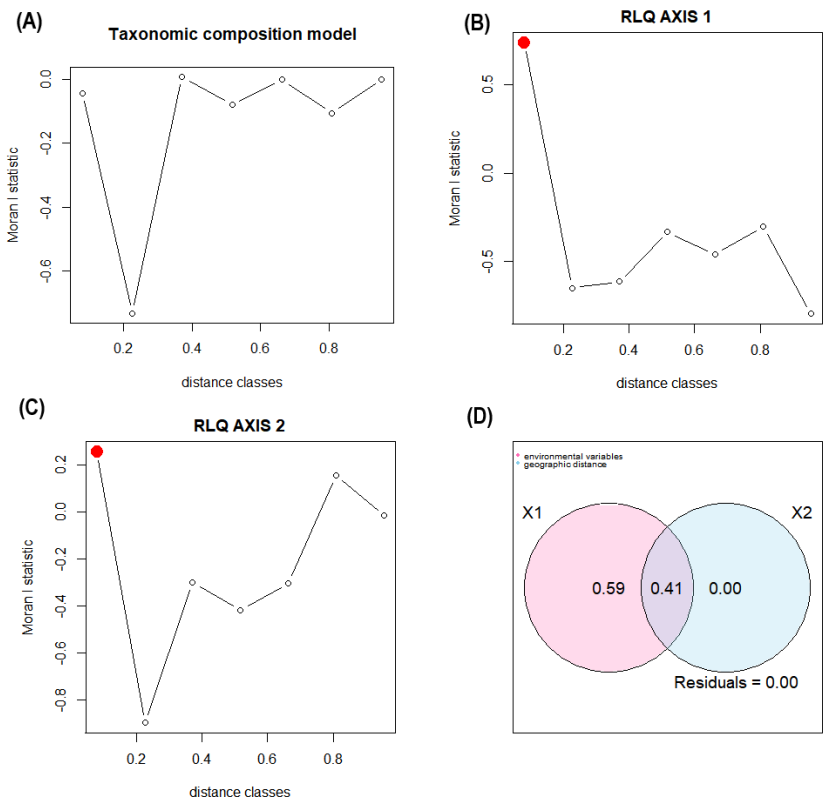


Figure S4. Spatial autocorrelation analysis. (A) Moran's I-based spatial correlograms applied on the sum of residuals derived from a manyglm model constructed with taxonomic composition; (B) and (C) Moran's I-based spatial correlograms applied on RLQ axis 1 and 2, constructed with trait data; (D) partition of variances between predictor variables that were significant for RLQ axis 1.

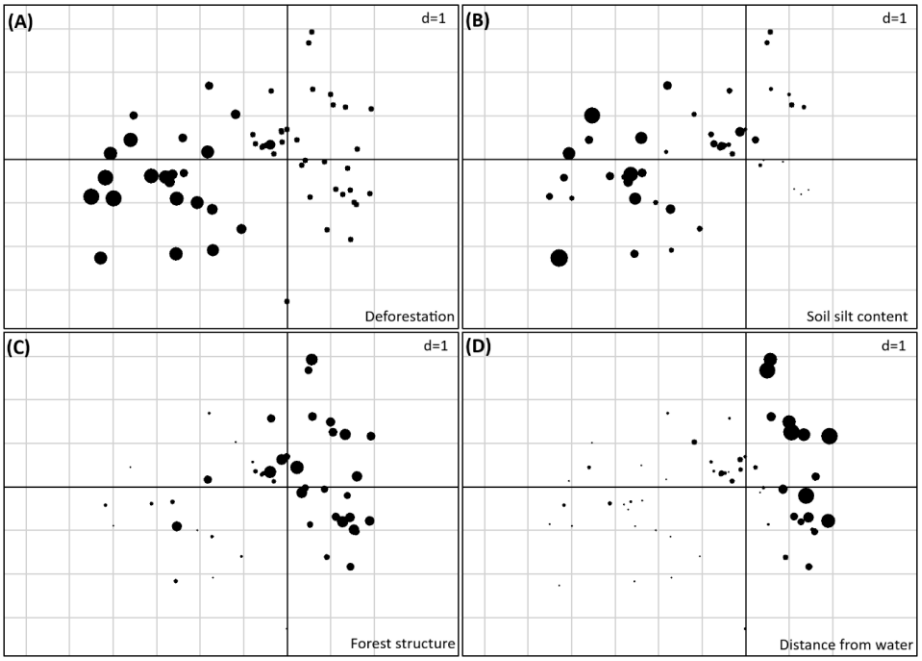


Figure S5. Supplementary biplots from the RLQ analysis. Row scores of the (A) proportion of deforestation in 500 m buffers, (B) soil silt content, (C) forest structure, (D) distance from water. The point sizes are proportional to the value of each variable.

CAPÍTULO 4

Guia impresso e aplicativo digital interativo de anuros da Floresta Nacional do Tapajós para fins de ecoturismo e ciência cidadã.

Guia disponível em formato E-book em:
https://repositorio.inpa.gov.br/handle/1/36351 **e aplicativo digital:** em construção.

Citação: Guia de sapos da floresta nacional do tapajós = Guide to the frogs of the tapajós national forest / Kelly Torralvo, Albertina Pimentel Lima, Rafael de Fraga, William E. Magnusson. - Manaus: Editora INPA, 2021. ISBN : 978-65-5633-019-8

Introdução

Planos de manejo de Unidades de Conservação vêm sendo baseados na gestão participativa, que envolve o conhecimento tradicional dos moradores locais (Lei 9.985/2000 - ICMBio 2009). Bekers (1993) apresentou uma definição para o Conhecimento Ecológico Local como a generalização progressiva das observações dos usuários locais em contextos socioecológicos específicos. O autor afirma que esse conhecimento é relevante para a compreensão dos processos ecológicos, manejo dos recursos naturais, conservação de áreas protegidas, conservação da biodiversidade, avaliação ambiental, desenvolvimento social e ética ambiental. Alguns estudos que se referem ao conhecimento ecológico local (ou tradicional) demonstraram a maneira como os povos tradicionais usam e se apropriam de recursos naturais, por meio de manejo legal ou ilegal, além de crenças, conhecimentos, percepções e comportamentos (Begossi 1993; Bekers 1993; Henriques and Pereira 2009). Além disso, estudos sugerem que o envolvimento de comunidades locais como estratégia de uso de recursos manejados de maneira sustentável poderia resultar em bons resultados (e.g., Campos-Silva and Peres 2016).

O termo Ciência Cidadã pode ser entendido como a participação de amadores, voluntários e entusiastas em projetos científicos gerando experiência, compreensão e novos conhecimentos tanto para a ciência quanto para o cidadão participante (Riesch & Potter 2014, Comandulli *et al.* 2015). Apesar das inúmeras discussões sobre o termo (Cohn 2008), na prática tem sido um meio de engajar as populações locais em iniciativas de conservação da biodiversidade (Riesch & Potter 2014, Comandulli *et al.* 2015).

As atividades de extração de recursos florestais e turismo na Floresta Nacional (FLONA) do Tapajós, localizada no oeste do Pará, são condicionadas por um plano de manejo elaborado por uma equipe multidisciplinar. A elaboração do Plano de Manejo da FLONA do Tapajós considerou o artigo 5° inciso III que estabeleceu que o Sistema Nacional de Unidades de Conservação deverá ser regido por diretrizes que “assegurem a participação efetiva das populações locais na criação, implantação e gestão das unidades de conservação” (ICMBio 2009). Em resumo, o Plano de Manejo contém informações advindas de amostragens de campo, consultas aos moradores locais, e propostas de manejo para o uso de recursos e conservação (ICMBio 2019b, a).

Além da inclusão da comunidade local nos planejamentos de manejo das áreas em que vivem, dados coletados por moradores diminuem a necessidade de recursos financeiros e tempo para amostragens em campo por pesquisadores profissionais. No entanto, é preciso refinar as informações para grupos específicos de organismos. Para anuros, a alta diversidade de espécies na Amazônia (Segalla *et al.* 2016) dificulta a identificação taxonômica, mesmo para especialistas. Essa limitação pode ser consideravelmente reduzida por meio de guias ilustrados de identificação de espécies em áreas específicas, os quais são acessíveis para um público diverso, desde observadores de fauna, até pesquisadores. Essas ferramentas reúnem, além de informações ecológicas, recursos visuais que facilitam o reconhecimento de espécimes observados e podem ser criados com linguagens simples para também facilitar o acesso e uso de qualquer público.

Outra atividade que envolve diretamente os moradores locais e funciona como uma fonte alternativa de recurso é o ecoturismo. O ecoturismo tem servido como uma ferramenta eficaz para a conservação (Gössling 1999, Stronza 2007, Peralta 2012). O uso da fauna em atividades de ecoturismo se destaca pelo mundo todo, incluindo a Amazônia (por ex. o Galo da Serra – *Rupicola rupícola* – em Presidente Figueiredo-AM). Porém, o que se espera do ecoturismo é uma correlação positiva entre geração de benefícios para as populações locais e efetividade de estratégias de conservação (Romagnoli *et al.* 2011, Peralta 2012).

Na Reserva de Desenvolvimento Sustentável Mamirauá, região do médio Solimões, o ecoturismo é realizado pelas comunidades locais em um sistema conhecido como turismo de base comunitária (Peralta 2012). Em 2013, um estudo investigou a viabilidade de um pacote de ecoturismo científico envolvendo a onça-pintada (*Panthera onca*), que visava a união entre a pesquisa científica e a contemplação da espécie (Nassar et al. 2013). Atualmente, as atividades são realizadas e geram recursos que são compartilhados entre os comunitários locais e o projeto de conservação da espécie. No entanto, apesar do aparente sucesso de alguns projetos, a falta de habilidades e experiência em planejamento de ecoturismo impede a autonomia dos moradores locais nas atividades propostas (Coria & Calfucura 2012), sendo necessária uma intervenção inicial de instituições científicas.

Visitas por turistas à FLONA do Tapajós ocorrem frequentemente e a área faz parte do circuito de passeios oferecido em municípios da região. Em 2017 foram registrados 42.636 visitantes na FLONA Tapajós, sendo 12.130 turistas (que pernoitam

na UC) e 30.506 não turistas (pesquisadores, estudantes, familiares dos comunitários, etc) (*dados não publicados* Equipe ICMBio/Santarém). O ecoturismo é composto por diversas atividades, como atividades nas praias, caminhadas, e passeios de barco, pelas quais uma enorme diversidade de animais e plantas pode ser observada (http://www.icmbio.gov.br/flonatapajos/guia-do-visitante.html).

A disponibilização de guias ilustrados para identificação de espécies pode melhorar a qualidade dos serviços, porque os usuários têm acesso imediato a informações sobre biologia e distribuição das espécies que estão observando em hábitat natural. Informações sobre a ocorrência de espécies em locais da FLONA Tapajós pode atrair observadores especificamente interessados em uma ou algumas espécies. Adicionalmente, guias de identificação publicados em formato bilíngue (Português e Inglês) direciona o interesse e o uso por turistas estrangeiros. Portanto, a disponibilização de guias de identificação potencialmente torna o ecoturismo mais atrativo, o que é convertido em aumento na captação financeira.

Atualmente, aplicativos digitais representam a modernização dos guias de identificação impressos tradicionais, a fim de facilitar o acesso às informações ecológicas e de biodiversidade. Existem aplicativos direcionados a informações sobre a distribuição de espécies, como o *Map of Life* (https://mol.org/); aplicativos de utilidade pública, direcionados a saúde associada à vida silvestre, como o SISS Geo (Sistema de Informação em Saúde Silvestre – http://www.biodiversidade.ciss.fiocruz.br); e aplicativos direcionados à conservação, que auxiliam programas de monitoramento de fauna atropelada (http://sistemaurubu.com.br/pt/) e focos de incêndio (DF100Fogo - http://www.jardimbotanico.df.gov.br/). Na maioria dos casos, as ferramentas possibilitam a integração de informações oriundas da sociedade científica e não científica, o que torna o método uma ponte de informação e conscientização ambiental.

Em síntese, a geração de conhecimento e a divulgação da ciência é um caminho eficiente para ações duradouras de conservação. A gestão das florestas habitadas por pessoas precisa ser formulada com a participação e a tomada de decisões que inclui os povos que procuram seu sustento a partir da floresta. Atividades de ecoturismo representa uma nova perspectiva sobre os serviços ambientais oferecidos pela floresta para as comunidades locais. Com isso, o comportamento exploratório das pessoas pode ser substituído por atividades de exploração econômica sustentável que tenha menor impacto na floresta e a inclusão da comunidade na gestão participativa da FLONA Tapajós possa ser firmada, como é proposto na legislação (ICMBio 2009).

Anuros são animais atrativos para o ecoturismo na Amazônia, devido à enorme variedade de formas, tamanho e cores (e.g., Dendrobatídeos) e pelas lendas indígenas conhecidas envolvendo certas espécies em rituais, como as do gênero *Phyllomedusa* (Lima & Labate 2007). Guias de identificação de anuros geralmente abrangem regiões pequenas em escala de Bacia Amazônica (e.g., Lima *et al.* 2006). Os objetivos desse capítulo são embasar e apresentar a criação de ferramentas de uso popular que possam simplificar o acesso à informação e direcionar atividades de ecoturismo e ciência cidadã na FLONA Tapajós.

Guia de sapos da FLONA do Tapajós – E-book e impresso

O guia foi elaborado com linguagem simples, nos idiomas português e inglês, afim de possibilitar o uso tanto pelos comunitários locais quanto pelos diversos visitantes da FLONA do Tapajós. Além das sessões de apresentação e introdução sobre o guia, o local de estudo e informações gerais sobre o grupo dos anuros, o guia conta com pranchas de identificação para cada espécie. Essas pranchas apresentam quatro fotografias, provenientes do acervo dos autores ou cedida por outros pesquisadores e informações morfológicas, biológicas e ecológicas disponíveis na literatura sobre as espécies. No total, são apresentadas imagens e informações de 47 espécies pertencentes a oito famílias.

Nós optamos por incluir informações que auxiliem na identificação das espécies pelo leitor, mas também que despertem o interesse pelas espécies como no item "você sabia". Adicionalmente, o nome da família, nome científico e autoridade da espécie e as legendas das figuras completam a primeira parte de cada prancha (Figura 1). Sete itens de informação compõem cada prancha, sendo: (1) "você sabia? " – com curiosidades sobre a espécie; (2) "como reconhecer" – com informações morfológicas de tamanho, formas e cores; (3) "distribuição" – com informações sobre a distribuição conhecida da espécie; (4) "reprodução" – com informações sobre o processo biológico e ecológico da reprodução da espécie; (5) "atividade" – informações sobre os períodos de atividade da espécie; (6) "onde vive" – informações sobre o local onde as espécies são encontradas naturalmente; (7) "espécie similar" – informações sobre diferenças morfológicas ou sonoras que possam auxiliar na diferenciação de espécies semelhantes.

HYLIDAE

Dendropsophus leucophyllatus

(Beireis, 1783)

VOCÊ SABIA

Essa espécie é popularmente conhecida como perereca-palhaço, devido às manchas semelhantes a bolas nas patas.

DID YOU KNOW

This species is popularly known as clown treefrog, due to its spots similar a balls in the arms and legs.

Legenda: A- adulto em vista dorsolateral; B- adulto em vista dorsal; C- macho vocalizando, vista ventral – ventre mais claro; D- adulto em vista ventral - ventre avermelhado.

Caption: A- adult in dorsolateral view; B- adult in dorsal view; C- male calling, ventral view- lighter belly; D- adult inventral view - reddish belly.

52 Guia de sapos da FLONA do Tapajós

Figura 1. Composição visual e informativa da primeira parte das pranchas de identificação de espécies do Guia de sapos da FLONA do Tapajós.

COMO RECONHECER

TAMANHO ADULTO - 27 a 32 mm nos machos e 36 a 40 mm nas fêmeas;

CORES - tons contrastantes de marrom e amarelo;

DETALHES NO DORSO - o formato das manchas coloridas podem variar, mas o padrão mais comum é uma mancha marrom triangular sobre o dorso contrastando com o amarelo vivo nas laterais do corpo, cabeça e região da cloaca;

DETALHES NO VENTRE - branco imaculado ou avermelhado, levemente mais escuro na região posterior do corpo;

DETALHE NAS PATAS - manchas amarelas arredondadas sobre um fundo marrom escuro;

DETALHES NOS OLHOS - íris bronze.

HOW TO RECOGNIZE

ADULT SIZE - 27 to 32 mm in males and 36 to 40 mm in females;

COLORS - contrasting shades of brown and yellow;

BACK - the shape and colors of the colored blotches may vary, but the most common pattern is a large brown triangular blotch, which contrasts with the bright yellow ofthe body sides, head and cloaca;

BELLY - white, slightly darker in the posterior region of the body;

LIMBS - bright yellow rounded blotches on a dark brown background;

EYES - bronze iris.

DISTRIBUIÇÃO

Bolívia, Brasil, Colômbia, Equador, Guiana Francesa, Guiana, Peru, Suriname.

DISTRIBUTION

Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname.

REPRODUÇÃO

Os ovos são colocados fora da água, nas folhas, e os girinos se desenvolvem na água.

REPRODUCTION

Eggs are laid out of water, on leaves, and tadpoles develop in water.

ATIVIDADE

Noturna

ACTIVITY

Nocturnal

ONDE VIVE

É arborícola. Pode ser encontrada em poças grandes em floresta ou áreas abertas.

WHERE IT LIVES

Arboreal species. It is found on big pools in forest or open areas.

ESPÉCIE SIMILAR

O formato de ampulheta marrom sobre o dorso associado às laterais amarelas torna essa espécie muito diferente de todos os outros sapos que ocorrem na FLONA do Tapajós.

SIMILAR SPECIES

The shape and colors of the body blotches make this species very different from all the other frogs that occur in the FLONA do Tapajós.

Figura 2. Composição visual e informativa da segunda parte das pranchas de identificação de espécies do Guia de sapos da FLONA do Tapajós.

Finalizamos o conteúdo do guia com uma lista completa de espécies registradas e previstas para a área da FLONA do Tapajós- PA e a bibliografia consultada na inclusão das informações. O guia é de autoria de Kelly Torralvo, Rafael de Fraga, Albertina Lima e William Magnusson, foi revisado e editado pela Editora INPA e está disponível nas versões impressa (com exemplares limitados distribuídos gratuítamente) e digital/E-Book (com download gratuíto através do link: https://repositorio.inpa.gov.br/handle/1/36351).

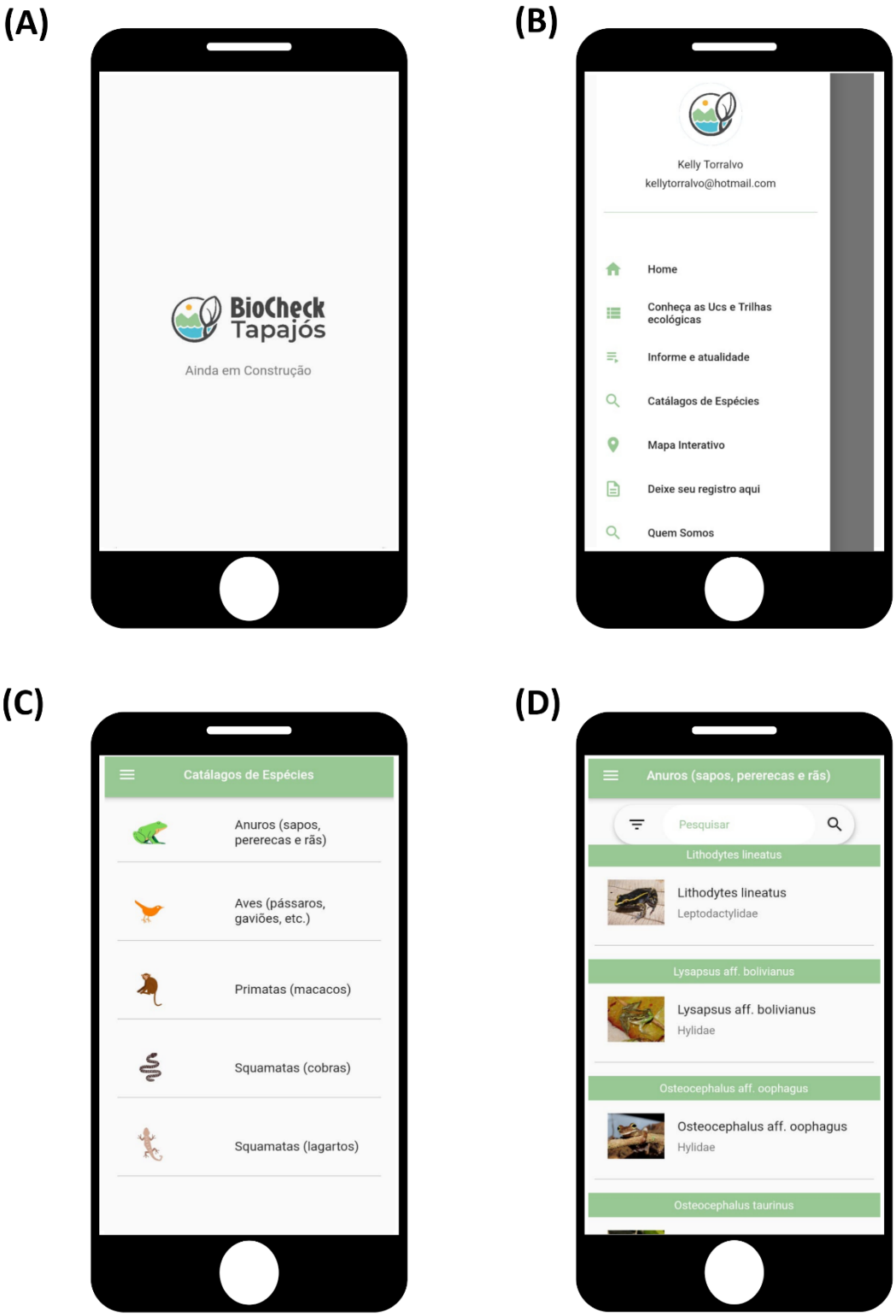
BioCheck Tapajós- Guia Interativo da Biodiversidade do baixo Tapajós – aplicativo digital

Diferente do guia impresso elaborado e voltado especificamente para as espécies de anuros da FLONA Tapajós- PA, a proposta do aplicativo abrange mais ambientes e organismos. O projeto do Bio Tapajós se estendeu a outras parcerias e atualmente é voltado para a biodiversidade de forma geral de toda região do baixo Tapajós. No entanto, inicialmente somente o conteúdo de anuros está disponível (Figura 3a).

A interface do aplicativo conta com diferentes menus, que direcionam o usuário para diferentes opções e informações como informações sobre as principais trilhas das UCs, atualidades locais, a história e a composição das equipes (Figura 3b). Um desses menus nos leva diretamente aos guias da biodiverdade local (Figura 3c). Especificamente, o conteúdo do catálogo de espécies de sapos (Figura 3d) no aplicativo segue a mesma linha visual e informativa proposta para o guia impresso, sendo o seu diferencial a opção de cantos disponíveis para reprodução que possibilita o comparativo em tempo real (Figura 3e-f). Outro menu de destaque é o “deixe seu registro” que será util para atividades direcionadas à pratica da ciência cidadã, onde o usuário registra novas fotografias, cantos e anotações sobre atividades das espécies (direcionados por um questionário), assim como as coordenadas geográficas do local (Figura 3g-h). As informações enviadas pelos usuários serão futuramente triadas e farão parte de um banco de dados da diversidade de anuros que ocorrem no local, contendo informações espaciais de distribuição das espécies e de história natural. Assim, além de sustentar o ecoturismo o aplicativo também será útil no registro constante de novas informações.

A criação do aplicativo ocorre em parceria com o Instituto Aquífero de Alter (http://institutoaquifero.org.br/), através de uma empresa de desenvolvimento. O conteúdo apresentado no grupo de anuros é uma extensão do apresentado no guia impresso e conta com os mesmos autores. O aplicativo segue em construção, sendo

alimentado com informações e conteúdos audiovisuais sobre outros organismos que compõem a biodiversidade local por outros autores, parte da parceria, e em breve estará disponível para downloads através das plataformas de compras buscando pelo nome BioTapajós.



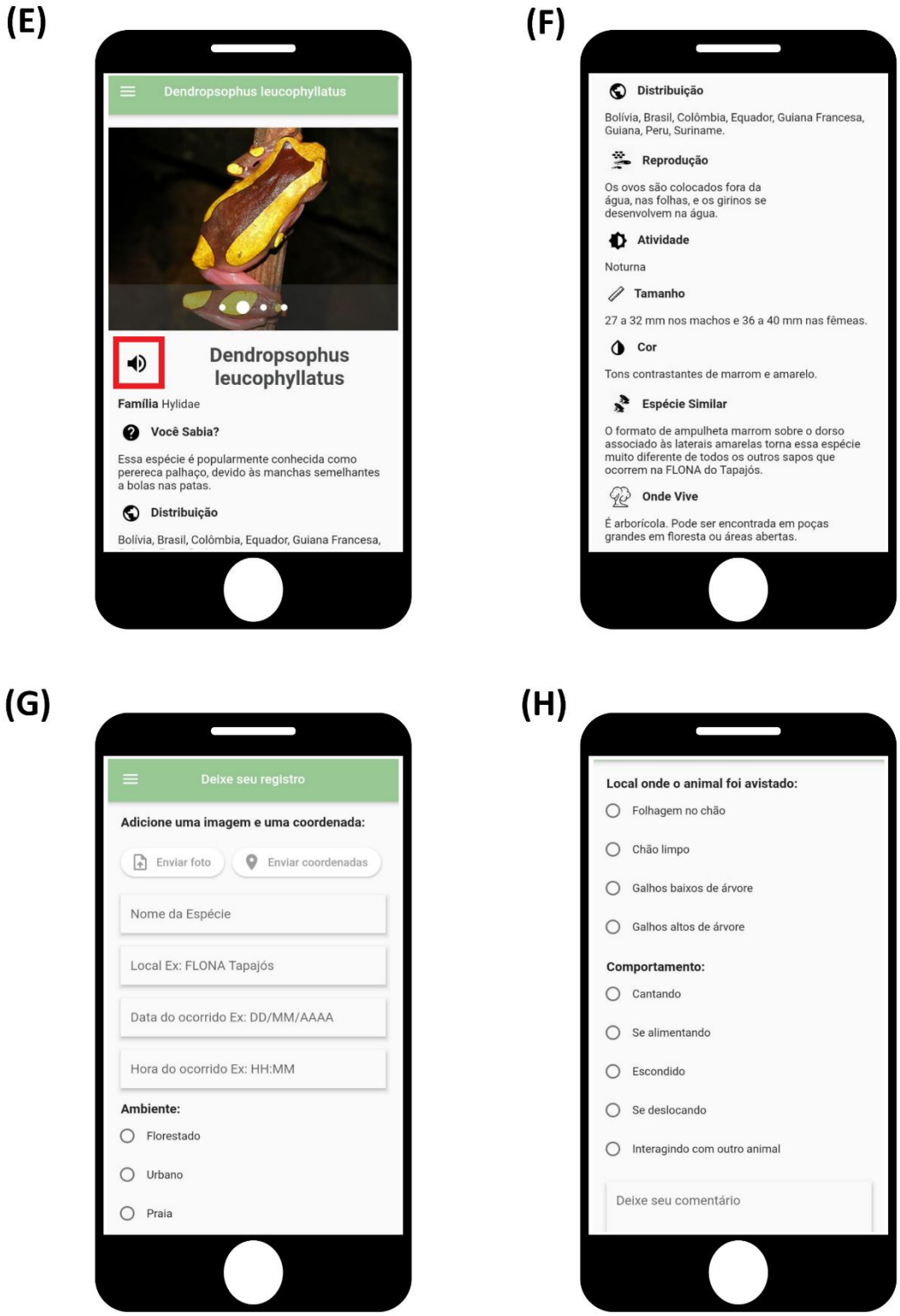


Figura 3. Composição e conteúdo do aplicativo digital BioCheck Tapajós. (a) interface inicial; (b) menus principais; (c) conteúdo sobre biodiversidade no menu catálogo de espécies; (d) catálogo de espécies de anuros; (f-g) conteúdo sobre as espécies, destaque para a opção de reprodução de cantos; (g-h) conteúdo do menu deixe seu registro.

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Referências

Begossi, A. 1993. Ecologia Humana: Um Enfoque Relações Homem-Ambiente. Interciência 18: 121–132. Available at: http://www.interciencia.org/v18_03/art01/index.html.

Bekers, F. 1993. Traditional Ecological Knowledge in Perspective.

Campos-Silva, J. V., and C. A. Peres. 2016. Community-based management induces rapid recovery of a high-value tropical freshwater fishery. Sci. Rep. 6: 34745. Available at: http://www.nature.com/articles/srep34745.

Cohn, J. P. 2008. Citizen science: Can volunteers do real research? Bioscience 58: 192–197.

Comandulli, C., M. Vitos, G. Conquest, J. Altenbuchner, and M. Stevens. 2015. 529-2485-1-Pb. 34–47.

Coria, J., and E. Calfucura. 2012. Ecotourism and the development of indigenous communities: The good, the bad, and the ugly. Ecol. Econ. 73: 47–55. Available at: http://dx.doi.org/10.1016/j.ecolecon.2011.10.024.

Gössling, S. 1999. Ecotourism: a means to safeguard biodiversity and ecosystem functions? Ecol. Econ. 29: 303–320. Available at: http://www.sciencedirect.com/science/article/pii/S0921800999000129.

ICMBIO. 2009. Legislação ICMBio - Sistema Nacional de Unidades de Conservação. 1: 69. Available at: http://www.icmbio.gov.br/portal/images/stories/comunicacao/legislacaoambientalvolume1.pdf.

ICMBIO. 2019a. Plano de Manejo- Floresta Nacional do Tapajós. Volume II - Planejamento. II: 453. Available at: http://www.icmbio.gov.br/portal/images/stories/plano-de-manejo/plano_de_manejo_flona_do_tapajós_2019_vol1.pdf.

ICMBIO. 2019b. Plano de Manejo - Floresta Nacional do Tapajós. Volume I - Diagnóstico. I: 2–165. Available at: http://www.icmbio.gov.br/portal/images/stories/plano-de-manejo/plano_de_manejo_flona_do_tapajós_2019_vol1.pdf.

Lima, A., W. Magnusson, M. Menin, L. Erdtmann, D. Rodrigues, C. Keller, and W. Hodl. 2006. Guide to the frogs of Reserva Adolpho Ducke, Central Amazonia Available at: https://repositorio.inpa.gov.br/handle/1/36309.

Lima, E. C. de, and B. C. Labate. 2007. “Remédio da Ciência” e “Remédio da Alma”: os usos da secreção do kambô (Phyllomedusa bicolor) nas cidades. Campos - Rev. Antropol. Soc. 8: 71–90. Available at: http://ojs.c3sl.ufpr.br/ojs/index.php/campos/article/viewFile/9553/6626.

Machado, I. C., N. Nordi, M. B. Henriques, T. A. Cardoso, and O. M. Pereira. 2011. A Integração da Pesquisa ao Conhecimento Ecológico Local no Subsídio ao Manejo: variações no estoque natural da ostra de mangue Crassostrea spp. na Reserva Extrativista do Mandira, Cananéia-SP, Brasil. Ambient. Soc. 14: 1–22.

Nassar, P. M., E. E. Ramalho, and R. Da Silveira. 2013. Economic and market viability of scientific ecotourism related to the jaguar in a várzea area in Central Amazonia. Uakari 9: 21–32.

Peralta, N. 2012. Ecotourism as an incentive to biodiversity conservation: the case of Uakari Lodge, Amazonas, Brazil. Uakari 8: 75–94. Available at: http://www.uakari.org.br/index.php/UAKARI/article/view/133%5Cnhttp://uakari.org.br/index.php/UAKARI/article/view/133.

Riesch, H., and C. Potter. 2014. Citizen science as seen by scientists: Methodological, epistemological and ethical dimensions. Public Underst. Sci. 23: 107–120.

Romagnoli, F. C., A. Scabin, and F. Pegas. 2011. Boto-vermelho (Inia geofrensis): espécie bandeira para promoção do ecoturismo na Amazônia Central, Brasil. Rev. Bras. Ecoturismo 4: 518.

Segalla, M. V., U. Caramaschi, C. A. G. Cruz, T. Grant, C. F. B. Haddad, P. C. de A. Garcia, B. V. M. Berneck, and J. A. Langone. 2016. Brazilian Amphibians: List of Species. Herpetol. Bras. 5: 34–46.

Stronza, A. 2007. The economic promise of ecotourism for conservation. J. Ecotourism 6: 210–230. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:The+economic+promise+of+ecotourism+for+conservation#0.

SÍNTESE

No primeiro capítulo dessa tese, mostramos a eficiência da técnica de espectroscopia FT-NIR no reconhecimento de espécies de anuros, utilizando amostras de coleção zoológica -fixadas em álcool e formol. Mostramos que componentes utilizados na fixação da amostra não são um impedimento para uma alta taxa de precisão, assim como o tamanho das amostras e tempo em que foram fixadas (idades das amostras). Tanto espectros coletados no dorso, quanto no ventre chegaram a alcançar 100% de acerto nos modelos testados, firmando o sucesso da técnica e abrindo um leque de inúmeras utilidades para seu uso.

No segundo capítulo, apresentamos testes de reconhecimento de espécies de anuros vivos em condições de campo com o uso da técnica NIR e equipamento portátil. Apresentamos alguns vieses que precisam ser contornados em novos estudos. E apesar de taxas mais baixasde precisão no reconhecimento de espécies, quando comparadas com o estudo anterior, consideramos o trabalho como um passo inicial para uma técnica promissora e útil na prática de identificação rápida e automatizada de espécies em campo.

No terceiro capítulo, mostramos como as composições de espécies e traços funcionais variam em resposta à heterogeneidade espacial nas características do habitat em uma região da Amazônia Brasileira coberta por um gradiente de desmatamento. As assembléias de sapos foram localmente estruturadas por substituição de espécies ao longo de gradientes de distância aos corpos d'água, estrutura da vegetação, areia do solo e conteúdo de silte e proporção da área desmatada e as espécies de corpo pequeno e aquelas com reprodução direta (sem estágio larval) não estavam mais presentes nessas áreas. Nossas descobertas destacam a importância de criar reservas para proteger as florestas de forma eficaz e manter a conectividade entre os fragmentos florestais resultantes do desmatamento.

No quarto capítulo, entregamos ao leitor dois produtos de uso popular para difundir o conhecimento das espécies de anuros de ocorrência conhecida na Floresta Nacional do Tapajós- PA. Apresentamos o guia ilustrado de sapos da FLONA do Tapajós, que poderá auxiliar observações de campo guiando a identificação de espécies, além de informações sobre as mesmas. Também apresentamos o aplicativo digital BioCheck Tapajós, que cumpre objetivos semelhantes ao Guia de Sapos, porém de uma

forma mais moderna e interativa, que futuramente estará disponível para mais organismos.

De modo geral, os resultados apresentados nesta tese somaram diferentes abordagens em estudos ecológicos ligados pelos objetivos de geração de conhecimento e aplicabilidade à conservação. Com o avanço da humanidade passamos por mudanças negativas como desmatamentos e fragmentação de habitat, justificadas por um desenvolvimento social e econômico mal planejado e ilegal, e por mudanças promissoras como a disponibilidade de novas tecnologias. Para ambas, precisamos direcionar o olhar científico em prol de um desenvolvimento sustentável, útil a todos e que garanta a floresta em pé.