MICHAEL ALEJANDRO CASTRO BONILLA

MORFOMETRIA E MODELAGEM DE NICHO ECOLÓGICO DE Inga subnuda SALZM. EX BENTH. (LEGUMINOSAE, MIMOSOIDEAE), UMA ESPÉCIE DA FLORESTA ATLÂNTICA BRASILEIRA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de Magister Scientiae.

VIÇOSA
MINAS GERAIS – BRASIL
2018

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T

Castro Bonilla, Michael Alejandro, 1991-

C355m
2018

Morfometria e modelagem de nicho ecológico de *Inga subnuda* Salzm. Ex Benth. (Leguminosae, Mimosoideae), uma espécie da Floresta Atlântica brasileira / Michael Alejandro Castro Bonilla. – Viçosa, MG, 2018.
x, 52 f. : il. (algumas color.) ; 29 cm.

Texto em português e inglês.
Inclui apêndices.
Orientador: Flávia Cristina Pinto Garcia.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Nicho (Ecologia). 2. Análise multivariada. 3. Biogeografia. 4. Mata Atlântica . I. Universidade Federal de Viçosa. Departamento de Biologia Vegetal. Mestrado em Botânica. II. Título.

CDD 22. ed. 577.82

MICHAEL ALEJANDRO CASTRO BONILLA

MORFOMETRIA E MODELAGEM DE NICHO ECOLÓGICO DE *Inga subnuda* SALZM. EX BENTH. (LEGUMINOSAE, MIMOSOIDEAE), UMA ESPÉCIE DA FLORESTA ATLÂNTICA BRASILEIRA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Magister Scientiae*.

APROVADA: 23 de agosto de 2018.

Vanessa Pontara

Vanessa Pontara

Jéferson Nunes Fregonezi
(Coorientador)

Flavia Cristina Pinto Garcia
(Orientadora)

Whether then the plant vegetates, blossoms, or bears fruit, it nevertheless is always the same organs, with varying functions and with frequent changes in form, that fulfill the dictates of Nature. The same organ which expanded on the stem as a leaf and assumed a highly diverse form, will contract in the calyx, expand in the petal, contract in the reproductive organs, and expand for the last time as fruit.

Johann Wolfgang von Goethe. The Metamorphosis of Plants.

AGRADECIMENTOS

Ao Brasil pela oportunidade de estudar e conhecer mais de perto a realidade de vocês, alias de me fazer esquecer de ser estrangeiro.

À Universidade Federal de Viçosa pela infraestrutura e boas lembranças.

Ao Departamento de Biologia Vegetal e ao Programa de Pós-Graduação em Botânica pela oportunidade de fazer mestrado no Brasil.

À CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela concessão da bolsa de estudo.

Aos meus pais porque as minhas conquistas são também deles.

Aos meus queridos irmãos José Eduardo e Steven que sempre me apoiaram.

À professora Flavia Cristina Pinto Garcia pela orientação e ensinamentos durante esses dois anos de curso.

Aos meus coorientadores, Jéferson Nunes Fregonezi e Valquiria Ferreira Dutra por disponibilizar e compartilhar seus conhecimentos e pela sua prontidão nas leituras minuciosas e sugestões valiosas na elaboração desta dissertação.

Ao Pedro Romano pela sua valiosa ajuda na confeição e análise dos dados para a análise morfometria e pelas sugestões na elaboração do manuscrito.

Ao Marcelo Bueno, pela sugestão e ajuda na modelagem de nicho ecológico, assim como na elaboração do manuscrito.

Aos funcionários do Departamento de Biologia Vegetal, em especial ao Celso, Ângelo e Fernanda, por serem sempre prestativos e atenciosos.

Aos meus queridos colegas de pós-graduação, especialmente Libia, Genilson, Cristielle, Ronaldo, Nayara e Maiky.

A minha família e amigos pelas boas lembranças e companhia mesmo na distância durante o mestrado.

Aos curadores e funcionários dos herbários ALCB, ASE, BHCB, CEN, CESJ, CVDR, EAN, FLOR, FUEL, FURB, HRCB, HST, HUFES, INPA, MBML, PEUFR, RB, SP, SPF, UEC, UFP, VIC e VIES. Obrigado vocês pelo material disponibilizado.

A FAPEMIG pelo suporte financeiro mediante o projeto: Edital Universal 2017/PROCESSO APQ-01703-1.

A FAPES pelo suporte financeiro para as coletas do Espirito Santo mediante o processo: 61855880/2012.

BIOGRAFIA

Michael Alejandro Castro Bonilla, filho de Blanca Stella Bonilla Ballen e Joselito Castro Cardozo, nasceu em 22 de novembro de 1991, na cidade de Ibagué, Tolima, Colômbia.

Ingressou no curso de Biologia em 2008, na Universidad del Tolima na Colômbia, e gradou-se em 2015.

Iniciou o Mestrado no Programa de Pós-Graduação em Botânica da Universidade Federal de Viçosa em agosto de 2016, concluindo em agosto de 2018.

NOTA BENE

Nesta dissertação é apresentado um status novo [Inga luschnathiana Benth.]. O autor enfatiza que esta dissertação não é considerada local efetivo de publicação de tal nome, pois o mesmo será devidamente validado num artigo científico publicado num periódico específico, atendendo aos requisitos do Código (Art. 29 e 41) após a eventual aprovação desta dissertação. Com esta nota, o autor quer evitar a incorreta publicação para o novo status aqui apresentado.

SUMÁRIO

RESUMO

CASTRO-BONILLA, Michael Alejandro, M.Sc., Universidade Federal de Viçosa, agosto de 2018. **Morfometria e Modelagem de Nicho Ecológico de Inga subnuda Salzm. Ex Benth. (Leguminosae, Mimosoideae), uma Espécie da Floresta Atlântica Brasileira**. Orientadora: Flavia Cristina Pinto Garcia. Coorientadores: Jefferson Nunes Fregonezi e Valquiria Ferreira Dutra.

O gênero neotropical Inga Mill pertence à família Leguminosae compreende aproximadamente 300 spp., destas 48 são endêmicas da floresta Atlântica brasileira e Inga subnuda Salzm. ex Benth é uma delas. O tratamento taxonômico atual considera I. subnuda como uma espécie com duas subespécies; I. subnuda subsp. subnuda e I. subnuda subspp. luschnathiana, que apresentam morfologia sobreposta, e além disso, estão distribuídas ao longo da costa Atlântica brasileira, desde o estado da Paraíba até Santa Catarina. Contudo, as dificuldades em estabelecer os limites morfológicos entre as duas subespécies têm sido discutidas. Este trabalho visa realizar uma abordagem integrativa entre evidencias morfológicas e ecológicas para resolver e esclarecer os limites nas duas subespécies. As variações morfológicas e os dados bioclimáticos das duas subespécies foram avaliados através da morfometria e de modelagem de nicho ecológico. A morfometria foi realizada com base em 613 espécimes depositados em herbários e obtidos após excursões de campo. Foram selecionados 54 espécimes e avaliados 25 caracteres de folha, flor e fruto, incluindo aqueles que se mostraram consistentes na identificação dos táxons. Devido à ausência de materiais com dados completos o estudo foi separado em duas matrizes, uma contendo os caracteres de flor e outra os caracteres de folha. Foram realizadas a análise de componentes principais (PCA) e a análise discriminante. De posse dos caráteres indicados nas análises preliminares foram realizados teste de comparação de média para explorar diferenças. A modelagem de nicho foi realizada em base nos registros dos herbários. A informação foi filtrada e uma tabela final foi elaborada com as identificações e coordenadas verificadas. Um total de 11 variáveis bioclimáticas e a altitude foram utilizadas na modelagem. A construção do modelo foi feita através do software MaxEnt nos cenários passados e atual. Potenciais áreas de estabilidade foram indicadas. A validação do modelo foi realizada mediante TSS (true skill statistics) e AUC (area under curve). A sobreposição de nicho foi analisada mediante índice de Schoener's e testes de similaridade e equivalência. Os resultados da morfometria mediante a análise de componentes principais e a analise discriminante indicaram três caracteres de folha e 3 caracteres de flor como aqueles que mais contribuíram na separação dos grupos. Os caracteres de folha foram: comprimento do pecíolo, largura da ala do ápice e comprimento da estípula. Nesses caracteres o teste indicou diferenças significativas na largura da ala do ápice, mas sem diferenças nos outros dois caracteres. Os caracteres de flor foram: comprimento do pedúnculo, comprimento da raque floral e o comprimento do pedicelo da flor. Nesses caracteres o teste indicou diferenças significativas no comprimento do pedicelo da flor, mas não indicou diferenças nos outros caracteres. Os resultados da modelagem de nicho mostraram que quando a espécie era tradada como táxons diferentes o modelo mostrava-se mais consistente. A modelagem do passado evidenciou mudanças ao longo do Quaternário e indicaram que as duas subespécies foram afetadas de forma diferente. O modelo foi condizente com outros estudos de modelagem para a floresta Atlântica coincidindo em áreas estáveis mencionadas em outros estudos. No que tange à sobreposição do nicho, o índice de Schoener's foi moderado, o espaço climático foi similar, mas não idêntico, o que pode sugerir especiação por divergência de nicho. Os resultados do presente estudo permitem considerar as duas subespécies de I. subnuda como espécies diferentes. É proposto para I. subnuda subsp. luschnathiana um novo status como espécie, são fornecidos uma ilustração, descrição e comentários taxonômicos. A presente dissertação foi organizada no formato de artigo científico intitulado **‘Paleoclimatical Quaternary Cycles and Traditional Morphometrics Unravel Newness in Atlantic Forest Endemic subspecies of Inga subnuda Salzm. ex Benth. (Mimosoideae: Fabaceae)’.**

ABSTRACT

CASTRO-BONILLA, Michael Alejandro, M.Sc., Universidade Federal de Viçosa, August, 2018. **Morphometrics and Niche Ecological Modeling of Inga subnuda Salzm. Ex Benth. (Leguminosae, Mimosoideae), a Brazilian Atlantic Forest Species**. Adviser: Flavia Cristina Pinto Garcia. Co-advisers: Jefferson Nunes Fregonezi and Valquiria Ferreira Dutra.

Inga Mill is a neotropical genus of the legume family with ca. 300 spp. comprising 48 endemic species in the Brazilian Atlantic Forest, and Inga subnuda Salzm. ex Benth is one of these. The current taxonomic treatment considers I. subnuda as one species with two subspecies subnuda and luschnathiana, that presents intermixed morphology in leaf and floral traits, and it is distributed along coastal region of Brazil from the state of Paraiba to the state of Santa Catarina. Notwithstanding the difficulties in disentailing these two subspecies have been discussed. This work aims to perform an integrative approach between morphological and ecological evidences to solve and clarify the limits in the two subspecies. Morphological variation and bioclimatic data of the two subspecies of the complex were assessed by using multivariate morphometric analyses and ecological niche modeling. Multivariate morphometric analyses were carried out based in 613 herbarium specimens. Fifty-four specimens with developed leaves, flowers and legumes were selected and measured for their inclusion in the statistical analyses. Twenty-five vegetative, floral and fruit characters were explored, including those that are known to be consistent for the identification of the two subspecies. Due to the difficult of material with presence of complete characters, the analysis was divided in two groups, one with leaf characters and other with floral characters. Principal component analyses and Discriminant analyses were performed. The characters that more contribute for variance in the two matrices were tested the difference between means (t-test) of both subspecies. Ecological niche modeling (ENM) was performed based on herbarium records. The information was filtered and a final table with the identifications and coordinates verified was performed. A total of 11 bioclimatic variables and altitude were used in the modeling. The construction of the model was done through MaxEnt software for the past and the present conditions. Potential stability areas were indicated. The validation of the model was performed through TSS (true skill statistics) and AUC (area under curve). Niche overlap was analyzed using Schoener's index and similarity and equivalence tests. The results of morphometry using principal component analysis and discriminant analysis indicated three leaf characters and three flower characters as most contributed to the separation of the groups. The leaf characters were: length of petiole, width of apex wing and length of stipules. In these characters the test indicated significant differences in the width of apex wing, but without differences in the other two characters. The flower characters were: length of peduncle, length of floral rachis and length of flower pedicel. In these characters the test indicated significant differences in length of flower pedicel, but did not indicate differences in the other characters. The results of the ENM showed that when the species was regarded as different taxa the model was more consistent. ENM of past conditions revealed changes throughout the quaternary that indicated how the two subspecies were affected differently. The model was consistent with other studies for the Atlantic forest coinciding in its stable areas mentioned in these studies. Regarding the niche overlap, the Schoener's index was moderate, the climatic space was similar, but not identical, which may suggest speciation due to niche divergence. The results of the present study allow to consider the two subspecies of I. subnuda as different species. It is proposed for I. subnuda subsp. luschnathiana a new status as species, provide an illustration, description and taxonomic comments. Thus, the present dissertation was organized in the format of scientific paper with the name ‘**Paleoclimatical Quaternary Cycles and Traditional Morphometrics Unravel Newness in Atlantic Forest Endemic Subspecies of Inga subnuda Salzm. ex Benth. (Mimosoideae: Fabaceae)’.**

INTRODUÇÃO GERAL

O gênero Inga Mill possui cerca de 300 espécies lenhosas com distribuição neotropical e diversificação recente (Pennington, 1997), sustentado como monofilético por dados moleculares e a presença de sarcotesta (polpa branca adocicada que envolve as sementes) (Richardson et al., 2001; Brown, 2008). Algumas espécies de Inga são utilizadas em restauração de solos, como em sombra para cultivos, assim como, em construção e como lenha (Léon, 1966; Garcia, 1998; Paulino et al, 2016).

No Brasil ocorrem 132 espécies de Inga, sendo 48 delas restritas à Floresta Atlântica (BFG, 2015), a segunda floresta tropical na América do Sul, com uma área original de cobertura de mais de um milhão de quilômetros quadrados ao longo da costa leste do Brasil até o oeste de Paraguai e noroeste da Argentina (Oliveira Filho & Fontes, 2000). É um dos biomas mais diversos do mundo, com alta riqueza de espécies e endemismos, porém, considerado ameaçado com uma cobertura atual de aproximadamente 15,2% da sua área original devido a intensas perturbações (Myers et al., 2000; SOS Mata Atlântica, 2018).

Inga subnuda Salzm. ex Benth. tem distribuição restrita à Floresta Atlântica, ocorrendo do estado da Paraíba até Santa Catarina (Pennington, 1997; Garcia, 1998; BFG, 2015). De acordo com Pennington (1997) essa espécie pode ser reconhecida pelas folhas com 4-5 pares de folíolos, raque cilíndrica a alada, lóbulos do cálice pouco desenvolvidos, com 0.5-1.5 mm compr. e tricomas ondulados no ápice da corola. A espécie apresenta duas subespécies: subsp. subnuda e subsp. luschnathiana. Com essa delimitação, a espécie I. subnuda apresenta uma grande variação morfológica chegando a dois extremos: (1) Flores pediceladas, pecíolo e ráquis foliar sem alas, frutos com quilhas, margens estriadas, faces estreitas quando jovens e cilíndricos quando maduros, encontrados no litoral nordeste da costa até o Espírito Santo que corresponde a Inga subnuda subsp. subnuda e (2) Flores sésseis ou curtamente pediceladas, ráquis alada, frutos sem quilha mas com margens estriadas e face aberta mesmo quando maduro, encontrados em Minas Gerais, São Paulo, Paraná, Santa Catarina, que corresponde a Inga subnuda subsp. luschnathiana (Garcia, 1998).

Bentham (1845) considerou originalmente as duas subespécies como sendo espécies diferentes. O material coletado por Salzmann (s.n.) do estado da Bahia foi descrito e publicado como Inga subnuda Salzm. ex Benth. O material coletado por

Luschnath (s.n.) no estado do Rio de Janeiro foi descrito e publicado como sendo Inga luschnathiana Benth (Bentham, 1845). Em 1976, Bentham manteve o status de espécie na obra de Martius, Flora Brasiliensis (Bentham, 1976). No entanto, Pennington (1997) baseado em materiais observados nos estados de Rio de Janeiro, Espirito Santo e Bahia com caracteres de flor e folha entremisturados, considerou I. luschnathiana como sendo subespécie de I. subnuda. No entanto, até o momento, a delimitação morfológica delas ainda não foi estabelecida.

Uma das tarefas importantes da taxonomia vegetal é a delimitação das espécies, um processo que reflete na sociedade, assim como, na demanda de informações taxonômicas confiáveis que permitam conservar, manejar e compreender melhor a biodiversidade, porém, o reconhecimento das espécies pode ser afetado por questões teóricas, metodológicas ou práticas (Wheeler, Raven & Wilson, 2004; Souza, Funch & Queiroz, 2014). De Queiroz (2007) definiu as espécies como linhagens de meta-populações evoluindo isoladamente (o conceito unificado de espécie – USC), e propôs que propriedades anteriormente consideradas nas espécies, como por exemplo: singularidades morfológicas, monofilia recíproca, coalescência genética e diferenciação ecológica, as quais são adquiridas durante a especiação, deveriam se reinterpretar como propriedades contingentes em lugar de definitivas e, portanto, consideradas como diferentes linhas de evidência relevante para acessar a separação das linhagens (Souza, Funch & Queiroz, 2014).

O conceito unificado de espécie permite o uso das propriedades das espécies como critérios secundários na sua delimitação, ao tratar a conceitualização e a delimitação de espécies como assuntos separados, assim, possibilita considerar as espécies atualmente aceitas como hipóteses a serem testadas por meio de qualquer uma das propriedades secundárias, o que serve como evidência para afirmar a existência de uma espécie (De Queiroz, 2007).

A morfometria tradicional é uma ótima ferramenta aplicada à taxonomia para quantificar e indicar as diferenças entre as formas dos seres vivos (Nery, 2016). Devido à plasticidade ou uniformidade de alguns atributos morfológicos, a morfometria tradicional, surge como uma ferramenta útil em determinar com objetividade os limites entre espécies, inclusive em casos de hibridação e poliploidia, por conseguinte, demonstra o valor que possui este método para solucionar diversas situações que a prática taxonômica tradicional resolve com dificuldade (Castillo-Batista, Ponce-Saavedra & Montero-Castro, 2017). Além de determinar com mais critério quais características são fundamentais ou tem maior influência na identificação dos grupos, é possível verificar se a variação morfológica em determinados complexos, quando associada à ausência de descontinuidades evidentes entre grupos propostos, está relacionada com as tendências dos gradientes populacionais ao longo de uma faixa de distribuição geográfica (Watanabe, 2009).

Estudos com morfometria tradicional em Leguminosae têm ajudado na delimitação em grupos como: Acacia caven (Mol.) Mol. (Pometti et al., 2007), Chamaecrista sect. Absus subsect. Baseophyllum (Conceição et al., 2008), Daniellia spp. (De la Estrella, Aedo & Velayos, 2009), Cercis canadenses L. (Fritsch, Schilller & Larson, 2009), Hymenaea courbaril L. (Souza, Funch & Queiroz, 2014), Pediomelum megalanthum (Wooton & Standl.) Rydb. (Egan, 2015).

A aplicação de caracteres ecológicos na sistemática e taxonomia tem oferecido informação complementar com potencial para o reconhecimento e diferenciação nos limites das espécies, junto com os caracteres morfológicos, inclusive com espécies morfologicamente similares (Wiens, 2007; Viera-Barreto et al., 2018). Os modelos de nicho ecológico utilizam associações entre variáveis ambientais e localidades de ocorrência da espécie para definir as condições abióticas dentro das quais as populações podem se manter (Guisan & Thuiller, 2005). Wiens & Graham (2005) têm proposto que a identificação de isolamento geográfico entre populações alopátricas, utilizando o modelamento de nicho ecológico, tem importância prática para a delimitação de espécies, estabelecendo que duas populações separadas por uma região fora do nicho climático evita que ocorra fluxo gênico entre as duas populações e, assim, dá suporte para as duas serem consideradas espécies separadas.

Vários processos ecológicos, tais como adequabilidade do habitat local e limitação da dispersão entre populações em ambientes fragmentados, determina a distribuição da diversidade genética sobre grandes áreas geográficas, por conseguinte esse processo influencia as distribuições das espécies sobre o tempo e conduz sua história evolutiva (Poncet et al., 2013).

Os ciclos climáticos do passado têm tido reflexos nos padrões de diversidade e endemismo atuais no neotrópico (Haffer & Prance, 2001). Estudos paleopalinológicos confirmam essas mudanças na vegetação da Floresta Atlântica, relacionadas com as mudanças climáticas do Pleistoceno, com substituição de amplas áreas florestais por pastagens e savanas subtropicais durante condições muito frias e muito secas (Behling 1995, 2002; Behling & Negrelle, 2001). Carnaval & Moritz (2008) baseados em modelos paleoclimáticos previram a presença de áreas historicamente estáveis (refúgios) na Mata Atlântica, durante o quaternário superior: uma ampla área na região central (o refúgio Bahia) e outra pequena área (o refúgio Pernambuco). Além disso, Leite et al., (2015) sugerem um papel importante do nível do mar e da distribuição da placa litorânea exposta na história biogeográfica da Mata Atlântica.

Na parte central da Floresta Atlântica (da Bahia até São Paulo) descontinuidades genéticas foram encontradas em algumas espécies animais (Ribeiro et al., 2011), tais como pequenos mamíferos (Metachirus nudicaudatus; Costa, 2003), lagartos (Gymnodactilus darwin, Pellegrino et al., 2005), preguiça (Bradypus variegates, Moraes-Barros et al., 2006), pássaros (Xiphorhynchus fuscus, Cabanne et al., 2007) e canídeos (Cerdocyon thous, Tchaicka et al., 2007). Esta descontinuidade genética foi localizada na região entre os rios Jequitinhonha e Doce (Ribeiro et al., 2011) e a separação das linhagens foi datada do Pleistoceno (Cabanne et al., 2007; Tchaicka et al., 2007) e anterior ao Pleistoceno (Costa, 2003; Pellegrino et al., 2005). Os escassos estudos realizados em plantas também evidenciaram descontinuidades genéticas na parte central da Mata Atlântica, porém, localizadas mais ao Sudeste, entre o sudeste do Espirito Santo e o nordeste do Rio de Janeiro, como encontrado nas espécies de Leguminosae: Caesalpinia echinata Lam. (Lira et al., 2003) e Hymenaea courbaril L. (Ramos et al., 2009). Contudo, nas coníferas, Podocarpus sellowi Klotzsch ex Endl., P. brasiliensis Laubbenf. e P. lambertii Klotzsch ex Endl., a quebra foi observada em latitudes mais ao sul da Bahia (Ledru et al., 2007).

Neste contexto da história biogeográfica da Floresta Atlântica e considerando-se a necessidade de delimitação de Inga subnuda, foram realizados estudos morfométricos e de modelagem de nicho ecológico nas duas subespécies de I. subnuda, com o objetivo de explorar evidências morfológica e ecológica para elucidar o problema na delimitação da espécie supracitada. A dissertação aqui apresentada, foi organizada no formato de artigo científico, com o nome: **Paleoclimatical Quaternary Cycles and Traditional Morphometrics Unravel Newness in Atlantic Forest Endemic Subspecies of Inga subnuda Salzm. ex Benth. (Mimosoideae: Fabaceae)**, a ser submetido ao jornal Botanical Journal of Linnaean Society (ISSN online: 1095-8339; https://academic.oup.com/botlinnean), segundo o disposto nas normas de redação de teses e dissertações da Universidade Federal de Viçosa.

REFERÊNCIAS BIBLIOGRÁFICAS

Behling H, Negrelle RRB. 2001. Late Quaternary tropical rain forest and climate dynamics from the Atlantic lowland in southern Brazil. Quaternary Research **56:** 383–389.

Behling H. 1995. A high resolution Holocene pollen record from Lago do Pires, SE Brazil: vegetation, climate and fire history. Journal of Paleolimnology **14:** 253–268.

Behling H. 1998. Late Quaternary vegetational and climatic changes in Brazil. Review of Paleobotany and Palynology **99:** 143–156.

Bentham G. 1845. Notes on Mimoseae with a synopsis of species. London Journal of Botany **4:** 613-618.

Bentham G. 1876. Leguminosae II et III. Swartzieae, Cesalpinieae, Mimosoideae. In: Martius C. Flora Brasiliensis, Vol. 15, pt. 2. Monachii, 498-499.

Brown GK. 2008. Systematics of the tribe Ingeae (Leguminosae-Mimosoideae) over the past 25 years. Muelleria **26:** 27-42.

Cabanne GS, Santos FR, Miyaki CY. 2007. Phylogeography of Xiphorhynchus fuscus (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in Southern Atlantic forest. Biological Journal of Linnean Society **91:** 73–84.

Carnaval AC, Moritz C. 2008. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic Forest. Journal of Biogeography **35:** 1187–1201.

Castillo-Batista AP, Ponce-Saavedra J, Montero-Castro JC. 2017. Análisis morfométrico de Cestrum guatemalense, C. mexicanum y C. pacayense (Solanaceae). Revista Mexicana de Biodiversidad **88:** 56–64.

Conceição AS, Queiroz LP, Lambert SM, Pereira ACS, Borba EL. 2008. Biosystematics of Chamaecrista sect. Absus subsect. Baseophyllum (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. Plant Sistematycs and Evolution **270:** 183-207.

Costa LP. 2003. The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. Journal of Biogeography **30:** 71–86.

De la Estrella M, Aedo C, Velayos M. 2009. A morphometric analysis of Daniellia (Fabaceae-Caesalpinioideae). Botanical Journal of the Linnean Society **159:** 268–279.

De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology **56:** 879–886.

Egan AN. 2015. Species delimitation and recognition in the Pediomelum megalanthum complex (Fabaceae) via multivariate morphometrics. PhytoKeys **44:** 65–87.

Fritsch PW, Schiller AM, Larson KW. 2009. Taxonomic Implications of Morphological Variation in Cercis canadensis (Fabaceae) from Mexico and Adjacent Parts of Texas. Systematic Botany **34:** 510–520.

Garcia FCP. 1998. Relacões sistemáticas e fitogeografia de Inga Miller (Leguminosae-Mimosoideae) nas florestas da costa sul e sudeste do Brasil. Tese de Doutorado, Universidade Estadual Paulista, Rio Claro.

Guisan A, Thuiller W. 2005. Predicting species distribution: offering more than simple habitat models. Ecology letters **8:** 993-1009.

Haffer J, Prance GT. 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. Amazoniana-Limnologia Et Oecologia Regionalis Systemae Fluminis Amazonas **16:** 579–605.

Ledru MP, Salatino MLF, Ceccantini G, Salatino A, Pinheiro F, Pintaud JC. 2007. Regional assessment of the impact of climatic change on the distribution of a tropical conifer in the lowlands of South America. Diversity and Distributions **13:** 761–771.

Leite YL, Costa LP, Loss AC, Rocha RG, Batalha-Filho H, Bastos AC, Quaresma VS, Fagundes V, Paresque R, Passamani M, Pardini R. 2016. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. Proceedings of the National Academy of Sciences **113:** 1008-1013.

Léon J. 1966. Central American and West Indian species of Inga (Leguminosae). Annals of the Missouri Botanical Garden **35:** 41–46.

Lira CF, Cardoso SRS, Ferreira PCG, Cardoso MA, Provan H. 2003. Long-term population isolation in the endangered tropical tree species Caesalpinia echinata Lam. revealed by chloroplast microsatellites. Molecular Ecology **12:** 3219–3225.

Moraes-Barros N, Silva JAB, Miyaki CY, Morgante JS. 2006. Comparative phylogeography of the Atlantic forest endemic sloth (Bradypus torquatus) and the widespread three-toed sloth (Bradypus variegatus) (Bradypodidae, Xenarthra). Genetica **126:**189–198.

Myers N, Mittermeier R, Mittermeier C, da Fonseca G, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature **403:** 853-858.

Nery EK. 2016. Morfometria geométrica e morfologia de hydrocotyle quinqueloba ruiz & pav. (araliaceae) e suas implicações taxonômicas. Undergraduate research. Universidade Federal de Santa Catarina. Florianópolis.

Oliveira-Filho A, Fontes MA. 2000. Patterns of floristic differentiation among atlantic forests in southeastern Brazil and the influence of climate. Biotropica **32**: 793-810.

Paulino JV, Mansano VF, Prenner G, Teixeira SP. 2016. High developmental lability in the perianth of Inga (Fabales, Fabaceae): a Neotropical woody rosid with gamopetalous corolla. Botanical Journal of the Linnean Society **183:** 146-161.

Pellegrino KCM, Rodrigues MT, Waite AN, Morando M, Yassuda YY, Sites-Jr JW. 2005. Phylogeography and species limits in the Gymnodactylus darwinii complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic Forest. Biological Journal of Linnean Society **85:** 13–26.

Pennington TD. 1997. The genus Inga: Botany. Royal Kew: Botanic Gardens.

Pometti CL, Cialdella AM, Vilardi JC, Saidman, BO. 2007. Morphometric analysis of varieties of Acacia caven (Leguminosae, Mimosoideae), taxonomic inferences in the context of other Argentinean species. Systematics and Evolution **264:** 239–249.

Poncet V, Munoz F, Munzinger J, Pillon Y, Gomez C, Couderc M, Tranchant-Dubreuil C, Hamon S, Kochko A. 2013. Phylogeography and niche modelling of the relict plant Amborella trichopoda (Amborellaceae) reveal multiple Pleistocene refugia in New Caledonia. Molecular Ecology **22:** 6163–6178.

Ramos ACS, Lemos-Filho JP, Lovato MB. 2009. Phylogeographical structure of the neotropical forest tree Hymenaea courbaril (Leguminosae: Caesalpinioideae) and its relationship with the vicariant Hymenaea stigonocarpa from Cerrado. Journal of Heredity. **100:** 206–216.

Ribeiro RA, Lemos-Filho JP, Ramos A, Lovato MB. 2011. Phylogeography of the endangered rosewood Dalbergia nigra (Fabaceae): insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. Heredity **106:** 46-57.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science **293:** 2242-2245.

SOS Mata Atlântica. 2018. Atlas da evolução dos remanescentes florestais e ecossistemas associados do domínio da Mata Atlântica no período 2016-2017. Fundação SOS Mata Atlântica, São Paulo, Brazil.

Souza IM, Funch L, Queiroz LP. 2014. Morphological analyses suggest a new taxonomic circumscription for Hymenaea courbaril L. (Leguminosae, Caesalpinioideae). PhytoKeys **38:** 101–118.

Tchaicka L, Eizirik E, De Oliveira TG, Candido-Jr JF, Freitas TRO. 2007. Phylogeography and population history of the crabeating fox (Cerdocyon thous). Molecular Ecology **16:** 819–838.

The Brazil Flora Group (BFG). 2015. Growing knowledge: an overview of seed plant diversity in Brazil. Rodriguésia **66:** 1085-1113.

Viera-Barreto JN, Pliscoff P, Donato M, Sancho G. 2018. Disentangling morphologically similar species of the Andean forest: integrating results from multivariate morphometric analyses, niche modelling and climatic space comparison in Kaunia (Eupatorieae: Asteraceae). Botanical Journal of the Linnean Society **186:** 259–272.

Watanabe MTC. 2009. Análise Morfométrica e Variabilidade Morfológica em Populações de Syngonanthus nites (Bong.) Ruhland (Erioucaulaceae). Master research. Universidade de São Paulo. São Paulo.

Wheeler QD, Raven PH, Wilson EO. 2004. Taxonomy: impediment or expedient? Science **303:** 285.

Wiens JJ, Graham C. 2005. Niche Conservatism: Integrating Evolution, Ecology, and Conservation Biology. Annual Review of Ecology, Evolution, and Systematics **36:** 519-539.

Wiens JJ. 2007. Species Delimitation: New Approaches for Discovering Diversity. Systematic Biology **56:** 875-878.

Paleoclimatical Quaternary Cycles and Traditional Morphometrics Unravel Newness in Atlantic Forest Endemic Subspecies of Inga subnuda Salzm. ex Benth (Mimosoideae: Fabaceae).

Normas: Botanical Journal of Linnean Society

Michael A. Castro-Bonilla,[1] Marcelo L. Bueno,[2] Pedro Romano,[3] Valquíria F. Dutra,[4] Jéferson N. Fregonezi,[1] Flavia C. P. Garcia.[1]

1 Universidade Federal de Viçosa, Centro de Ciências Biológicas e da Saúde, Departamento de Biologia Vegetal, Programa de Pós-Graduação em Botânica, CCB2, 3º andar, Avenida P.H. Rolfs s/n, Campus Universitário, CEP 36570-900, Viçosa, MG, Brazil.

2 Universidade Federal de Viçosa, Centro de Ciências Biológicas e da Saúde, Departamento de Biologia Geral, Programa de Pós-Graduação em Ecologia, Avenida P.H. Rolfs s/n, Campus Universitário, CEP 36570-900, Viçosa, MG, Brazil.

3 Universidade Federal de Viçosa, Centro de Ciências Biológicas e da Saúde, Departamento de Biologia Animal, Laboratório de Paleontologia e Osteologia Comparada, Avenida P.H. Rolfs s/n, Campus Universitário, CEP 36570-900, Viçosa, MG, Brazil.

4 Universidade Federal do Espírito Santo, Herbário VIES, Depto. Ciências Biológicas, Av. Fernando Ferrari 514, 29075-910, Vitória, ES, Brasil.

ABSTRACT

Inga is a neotropical genus of the legume family with ca. 300 spp. comprising 48 endemic species in the Brazilian Atlantic Forest and Inga subnuda Salzm. ex Benth. is one of these. The current taxonomic treatment considers I. subnuda as one species with two subspecies subnuda and luschnathiana, that presents morphology with leaf and floral traits intermixed. We aim to explore different lines of evidence under the unified species concept (USC) to disentangle and clarify species boundaries in these two subspecies. Morphological variation and bioclimatic data of the two subspecies of the complex were assessed by using multivariate morphometric analyses and ecological niche modeling. Morphological quantitative characters allowed the recognition of different groups. The climatic space was similar but not identical, and the biogeographic context that could have shaped the current distribution is discussed. The results of our integrative study under the USC suggest regard both subsp. subnuda and subsp. luschnathiana as two different species. We propose to subsp. luschnathiana a new taxonomic treatment.

ADDITIONAL KEYWORDS: Brazilian Atlantic Forest – morphological traits – multivariate analyses – ecological niche modeling (ENM) – biogeography – Quaternary.

INTRODUCTION

Inga Mill is a species-rich tree genus of legume family with ca. 300 spp. and are supported as monophyletic by present fleshy seed coat, despite his infrageneric relationships continue poorly comprehend (Richardson et al., 2001; Brown, 2008). Some Inga spp. are used in soil restoration, agroforestry, timber, firewood and for medicinal purposes (Léon, 1966; Garcia, 1998; Paulino et al., 2016).

The species Inga subnuda Salzm. ex Benth. is one of the 132 Inga species within Brazil, and one of 48 that are endemic of the Brazilian Atlantic Forest (BAF) (Garcia, 1998; BFG, 2015). Bentham (1845, 1876) initially published I. subnuda and Inga luschnathiana Benth as separate species. However, Pennington (1997) considered I. luschnathiana as conspecific of I. subnuda, based in collected specimens from Rio de Janeiro, Espírito Santo and Bahia states that had both leaf and floral traits intermixed. Thereby, with this delimitation, I. subnuda was regarded occupying two extremes of a range of variation: pediceled flowers, terete petiole and rachis, legume margins keeled when immature, faces almost completely covered by the expanded margins and mature legume more or less cylindrical corresponding to I. subnuda Salzm. ex Benth. subspp. subnuda and short pediceled or sessile flowers, winged rachis, legume margins not keeled, faces exposed and mature legume more or less quadrangular corresponding to I. subnuda subspp. luschnathiana (Benth.) T. D. Penn. (Pennington, 1997; Garcia, 1998). Despite of this treatment, difficulty in disentailing these two subspecies have been discussed (Garcia, 1998), and under those circumstances, traditional morphometric can help to identify and indicate with objectivity species delimitation (e.g. Pometti et al., 2007; Conceição et al., 2008; De la Estrella, Aedo & Velayos, 2009; Fritsch, Schiller & Larson, 2009; Souza, Funch & Queiroz, 2014; Egan, 2015).

The two subspecies of I. subnuda are distributed along coastal region of Brazil from the state of Paraiba to the state of Santa Catarina, furthermore frequently found within Restingas (Garcia, 1998; BFG, 2015) (Pennington, 1997; Garcia, 1998; Garcia & Fernandes, 2015). Nevertheless, one study of its potential distribution range using ecological niche modeling has not been performed for these two subspecies so far, as well as its relationship with the recent biogeographic history of the BAF. Recent studies have explored the role of climatic niche attributes in speciation (e.g. Kozak & Wiens, 2010; Kostikova, Salamin & Pearman, 2014; Viera-Barreto et al., 2018; Jezkova & Wiens, 2018). Ecological process as local habitat suitability can influence species distribution and evolutionary history (Poncet et al., 2013). For this reason, the use of ecological niche modeling is the most direct and operational way of evaluating the effects of climate changes upon the distribution areas changes (contraction and expansion) (Carnaval & Moritz 2008; Buzatti et al., 2017; Bueno et al., 2017). Recent quaternary paleoclimatic cycles has reflected in current distribution and genetic diversity of many neotropical species (Haffer & Prance 2001). Carnaval & Moritz (2008) based in paleoclimatic models predicted the presence of historically stable areas in BAF (Refugia) during late quaternary: a wide central region (Bahia Refugia) and another small area (Pernambuco refugia). Furthermore, Leite et al., (2016) suggest that sea level and land distribution must have been important role in the biogeographic history of BAF.

Ecological niche modeling together with traditional morphometrics are able to circumscribe better the differences between species (Raxworthy et al., 2007; Hawlitscheck et al., 2011; Aguirre-Gutiérrez et al., 2015; Shrestha & Zang, 2015;

Gama, Aguirre-Gutiérrez & Stech, 2017). This approach is in accordance with the conceptual framework of De Queiroz (2007), that is called the unified species concept (USC). USC defined species as separately evolving metapopulation lineages and propose consider the properties of species as phenetic distinctiveness, reciprocal monophyly, genetic coalescence, or ecological distinctiveness, which are acquired during speciation, as secondary rather than critical criteria and therefore considered like different lines of evidence relevant to assessing lineage separation (Souza, Funch & Queiroz, 2014).

Under the USC concept framework and delimitation difficulties in the subspecies of I. subnuda and the biogeographic history of BAF, we aim to explore morphometric, geographical and paleoecological patterns to disentangle and clarify species boundaries in these two subspecies. We assessed morphological and ecological data of the subspecies using multivariate approach and ecological niche modelling of present and past conditions (ENM). Also, we want to address the following questions: What is the potential ecological niche of the two subspecies of I. subnuda? How recent paleoclimatic cycles (Holocene, LGM and LIG) has shaped the current distribution of the two subspecies of I. subnuda? There is overlap niche in the two subspecies of I. subnuda and which ecological consequences?

MATERIAL AND METHODS

STUDY AREA

The definition of BAF choose here was sensu lato (sl comprises a unique series of ecosystems originally extending over 1.5 million km2 on the Brazilian coast and in continental inland areas (Stehmann et al., 2009). Its geographic range covers diverse climatic zones and tropical and subtropical vegetation formations, such as semi-deciduous and Araucaria mixed forests to coastal rain forests (Oliveira-Filho & Fontes, 2000; Tabarelli et al., 2005). For instance, latitude ranges from 58 N to 338 S, longitude from 358 W to 528 W, elevation goes from sea level to approximately 2,200 m; annual precipitation 800 to 4,000 mm and mean temperature vary from 5 to 25 ºC (Stehmann et al., 2009). The study area and recorded specimens are showed in Fig. 1.

MORPHOMETRIC ANALYSES

Plant material and morphological characters

The present work is based on the study of 613 herbarium specimens from the following herbaria (herbarium acronyms according to Thiers, 2013): ALCB, ASE, BHCB, CEN, CESJ, CVDR, EAN, FLOR, FUEL, FURB, HRCB, HST, HUFES, INPA, MBML, PEUFR, RB, SP, SPF, UEC, UFP, VIC and VIES. Pennington (1997) and Garcia (1998) were used for initial determinations. Fifty-four specimens with developed leaves, flowers and legumes were selected and measured for their inclusion in the statistical analyses (Appendix). Twenty-five vegetative, floral and fruit characters were explored (Table 1), including those that are known to be consistent for the identification of the two subspecies (Pennington, 1997; Garcia, 1998). Unvarying characters were excluded from the analyses. Because of lack of sufficient well-developed fruits, legume characters were omitted as well. Due to the difficult of material with presence of complete characters, the analysis were divided in two groups, one with leaf characters and other with floral characters. Thus, eighteen characters were used in the final analyses, nine for both leaf and floral characters (Table 1).

Statistical analyses

Morphological variation within the subspecies was accessed using univariate statistics (mean, SD, variance, min, max) and multivariate morphometric analyses [Principal component analysis (PCA) and discriminant analysis (DA)]. Statistical analyses were performed using PAST v. 3.06 (Hammer, Harper & Ryan, 2001).

We performed PCA based on a variance-covariance matrix, iterative imputation for missing values and the results were plotted in two-dimensional scatter plots. Among a priori groups were determine significant differences through DA with quantitative morphological variables in each of the matrices. Multivariate normality (Mardia, 1970) and equivalence of covariance matrices (Rencher, 2002) were tested for each matrices. Wilk's lambda, Pillai's trace and Hotelling-Lawley trace tests were carried out based on Mahalanobis distances between coordinates in N-dimensional hyperspace and the F statistics on the distances (Cook, Semple & Baum, 2009).

A preliminary two matrices of both leaf and floral traits with each 50 and 31 observations was used in exploratory analyses. Then, within the characters without normal distribution the outliers were excluded. The final analyses were carried out based on 45 specimens and nine variables for leaf matrix, but in the floral matrix final analyses were performed based on 24 specimens and nine variables. DA and MANOVA analysis was performed in both leaf and floral matrices only with the variables that fulfill multivariate normality, equivalence of covariance and without missing data. Preliminary analyses (PCA and DA) based in floral matrix showed low resolution due to scale differences, therefore the variables were standardized with log10 transformation, then was carried out a new multivariate analysis. Finally, with the characters that more contribute for variance in the two matrices were tested the difference between means (t-test) of both I. subnuda subsp. subnuda and I. subnuda subsp. luschnathiana.

ECOLOGICAL NICHE MODELING

Dataset

The preliminary dataset for I. subnuda subspp. subnuda e I. subnuda subspp. luschnathiana were extracted from registers of herbaria and “SpeciesLink” (http://http://www.splink.org.br/). Then, an accurate dataset was performed with the coordinates and taxonomy duly verified. Specimens with dubious taxonomy and coordinates was excluded. Once the dataset was checked, a final dataset of 104 registers was used. To I. subnuda subspp. subnuda were 68 registers and I. subnuda subspp. luschnatiana were 36 registers. For all registers, we obtained the value, at 30 arc-second (approximately 1 km) resolution, of the 19 standard BIOCLIM variables, which reflect various aspects of temperature, precipitation, and seasonality and which are likely to be important in determining species distributions (Hijmans et al., 2005). We cropped the bioclimatic layers to span from 4°50′S to 33°45′S and 57°38′W to 35°00′W (a larger spatial range than compromise the BAF and states distribution of the two subspecies of I. subnuda). After assessing correlations between the bioclimatic variables, we retained 12 of 19 variables, eliminating those with less biological relevance from groups of strongly interrelated variables ($r > 0.9$) (Dormann et al., 2013). This procedure was done to avoid overparametrization of our modelling with redundant variables. The final selected variables were annual mean temperature, mean temperature diurnal range, isothermality, max temperature of warmest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation of warmest quarter, and precipitation of coldest quarter. We also include altitude raster in the analyses.

Model Construction

We modelled the distribution of the two subspecies of I. subnuda (Fig. 1) under four times projections: Current (0 ka pre-industrial), Mid-Holocene (6 ka BP), Last Glacial Maximum (LGM – 21 ka BP), and Last Interglacial (LIG – 130 ka BP) time periods based on climatic simulations (< www.worldclim.org >; Hijmans et al., 2005). For the Last Glacial Maximum (21 ka, LGM), Holocene (6 ka) and Current (0 ka pre-industrial) time periods, we employed the Community Climate System Model – CCSM4 (Gent et al. 2011) and MIROC-ESM, which represents downscaled climate data from simulations with Global Climate Models (GCMs) based on the Coupled Model Intercomparison Project Phase 5 (CMIP5; Taylor, Stouffer & Meehl, 2012). The paleo-climatic model for the Last Interglacial (120 ka, LIG) used the approach of Otto-Bliesner et al. (2006). We performed all geographic information system (GIS) analyses in ArcGIS v.10 (ESRI, 2011). We modelled the ecological niche using Maxent ver. 3.4.1 (Phillips, Anderson & Schapire, 2006). It has been demonstrated that Maxent often outperforms other modelling techniques to identify areas critical to the maintenance of species populations, besides it allowed us to use presence-only species data, which is the more common form biotic data is available (Elith et al., 2006, 2011; Pearson et al., 2007; Phillips & Dudík, 2008; Pena et al., 2014).

To calibrate and evaluate the quality of the models, we divided the data for each species into a training set (75% of occurrences) and a test or validation set (25% of occurrences). We constructed models ten times and averaged the output to produce the final results used in downstream analyses. Next, for each species, we defined a threshold value above which grid cells were considered to have environmental characteristics suitable for the maintenance of viable populations of the species (Pearson et al., 2007). We used the 'minimum training presence' as the threshold selection method because it assumes that the species presence is restricted to sites at least as suitable as those at which the species has been observed so far (Pearson et al., 2007).

To indicate potential areas of climatic stability for the two subspecies of I. subnuda during the whole of the Quaternary, we adopted protocols similar to those used in recent studies for other Neotropical Domains (Carnaval & Moritz, 2008; Werneck, 2011; Werneck et al., 2012; Buzzati et al., 2017; Bueno et al., 2017; Buzzati et al., 2018). Spatial models were converted from continuous outputs into presence/absence maps by applying the lowest presence threshold for each model, thus maximizes agreement between observed and modelled distributions and improve the prediction (Pearson et al., 2007). By summing up the presence/absence maps obtained under Current, Holocene (6 ka BP), LGM (21 ka BP) and LIG (130 ka BP) projections, we generated a map of areas showing historical stability. This combined map depicted areas that were potentially occupied by the subspecies of Inga subnuda during the climatic oscillations of the Quaternary. These historically stable areas, which we considered to be potential refugia, were defined as those grid cells for which the presence of the two subspecies of I. subnuda was inferred across all time projections.

Species distribution modelling validation

We calculated the sensitivity (the proportion of observed presences in relation to those that were predicted, which quantifies omission errors), the specificity (the proportion of observed absences compared to those that were predicted, which quantifies commission errors) and the TSS (true skill statistic), following Allouche, Tsoar & Kadmon (2006). The TSS test corrects the overall accuracy of the model prediction by the accuracy expected by chance and it is a threshold-dependent measure that is appropriate for evaluating predictive accuracy in cases where the model prediction is formulated as a presence–absence map (Jones, Acker & Halpern, 2010). These analyses used the occurrence points of the two subspecies of Inga subnuda and 202 occurrences of Eugenia uruguayensis Cambess. obtained from NeoTropTree (Oliveira-Filho, 2014), which has a restricted niche that differs from the two subspecies of I. subnuda and is ideal for simulating absence points for these two subspecies.

We also used a threshold-independent method of model validation, the receiver operating characteristic (ROC) curve analysis, this analysis characterizes the predictive performance of a model at all possible thresholds by a single number, the area under the curve (AUC) (Fielding & Bell, 1997; Elith, 2002; Fawcett, 2004; Rushton, Ormerod & Kerby, 2004; Phillips, Anderson & Schapire, 2006).

Measuring ecological niche overlap

Niche analyses overlaps between the subspecies of I. subnuda was performed in R (R Core Team, 2017), and we follow the framework of Broenninman et al. (2012). The Schoener's D index and niche similarity and equivalency tests were calculated as well (Di Cola et al., 2017). This framework allows through the availability of bioclimatic variables, the analyses of species-climate relationships within niche space. It has been used to explore niche dynamics, track climatic change and distinctiveness between species (Viera-Barreto et al., 2018).

RESULTS

MORPHOMETRIC ANALYSES

Univariate analysis

The N, min, max, mean, variance, SDs and median of the eighteen traits of both leaf and floral matrices were calculated for each 45 and 24 specimens of the two subspecies of Inga subnuda (Supporting Information, Appendix S1, Table S1). In general, almost in each trait for both matrices an overlap in some extent can be observed, although WAW presented little overlap and LFP did not shows overlap.

Principal component analysis

The results of PCA for leaf matrix with all characters in the scatter plot showing PC1 vs PC2 (Fig. 2A) are distributed in one undifferentiated could. In this PCA the first and second principal components (PCs) accounted for 90.95% of the total variance of the data (73.37% and 17.58%). In decreasing order, PC1 has the highest loadings for length of rachis (LR; eigenvector: 0.62), length of terminal leaflet (LTL; eigenvector: 0.58) and width of terminal leaflet (WTL; eigenvector: 0.36). PC2 has the highest loadings for the same characters of PC1 but with different scores (LR; eigenvector; -0.77, LTL; eigenvector: 0.54, WTL; eigenvector: 0.23). A second PCA was performed using only the characters that fulfill the normal multivariate distribution, equivalence covariance and do not have missing value, therefore only LS, WS, LP and WAW were used. The result in this analysis in the scatter plot showing PC1 and PC2 (Figure 2B) displayed moderate better resolution in relation with first PCA containing all leaf characters. In this PCA first and second principal components (PCs) accounted for 97.92% of total variance of the data (84.49% and 13.43%). In decreasing order, PC1 has the highest loadings for length of petiole (LP; eigenvector: 0.99) and width of apex wing (WAW; eigenvector: 0.11). PC2 has the highest loadings for width of apex wing (WAW; eigenvector: 0.98) and length of stipule (LS; eigenvector: 0.14).

The results of PCA for floral matrix with all characters in the scatter plot showing PC1 vs PC2 (Figure 3A) gives relatively good resolution. In this PCA first and second principal components (PCs) accounted for 85.54% of total variance of the data (48.14% and 37.40%). In decreasing order, PC1 has the highest loadings for length of floral rachis (LFR; eigenvector: 0.80), length of stamens (LST; eigenvector: -0.36), length of peduncle (LOP; eigenvector: 0.35) and length of style (LSTY; eigenvector: -0.34). PC2 has the highest loadings for length of peduncle (LOP; eigenvector: 0.75), length of style (LSTY; eigenvector: 0.48), length of stamens (LST; eigenvector: 0.43) and length of flower pedicel (LFP; eigenvector: 0.11). A second PCA was carried out using only the floral character without missing values. This analysis revealed reasonably resolution as can be observed in Fig 3B. In this PCA first and second principal components (PCs) accounted for 97.33% of total variance of the data (67.43% and 29.90%). In decreasing order, PC1 has the highest loadings for length of floral rachis (LFR; eigenvector: 0.76), length of peduncle (LOP; eigenvector: 0.64) and length of corolla (LC; eigenvector: 0.04). PC2 has the highest loadings for length of peduncle (LOP; eigenvector: 0.75), length of floral rachis (LFR; eigenvector: -0.64) and length of flower pedicel (LFP; eigenvector: 0.14).

The results in the PCA with log transformation using all floral characters was calculated (Supporting Information; Appendix S2, Figure S1.A.). In this PCA first and second principal components (PCs) accounted for 84.90% of total variance of data (59.73% and 25.17%). In decreasing order, PC1 has the highest loadings for length of flower pedicel (LFP; eigenvector: 0.88), length of peduncle (LOP; eigenvector: 0.39) and length of calix (LK; eigenvector: 0.13). PC2 has the highest loadings for length of floral rachis (LFR; eigenvector: 0.74), length of peduncle (LOP; eigenvector: 0.58) and length of bract (LB; eigenvector: 0.23). A second PCA performed with only floral characters without missing data (Supporting Information; Appendix S2, Figure S1.B). In this PCA first and second principal components (PCs) accounted for 88.89% of total variance of data (63.39% and 25.50%). In decreasing order, PC1 has the highest loadings for length of flower pedicel (LFP; eigenvector: 0.89), length of peduncle (LOP; eigenvector: 0.39) and length of calix (LK; eigenvector: 0.74). PC2 has the highest loadings for length of floral rachis (LFR; eigenvector: 0.77), length of peduncle (LOP, eigenvector: 0.6) and length of flower pedicel (LFP; eigenvector: -0.18).

Discriminant analysis

According to our results (in analyses including leaf characters without missing data, and achieve both multivariate normality and equal covariance matrix) the DA more clearly discriminated I. subnuda subspp. than those of the PCA. As first result of DA (Fig. 2C), the eigenvalue for the canonical axis (1.84) accounted for 100% of the variation for total dispersion of the specimens. The most discriminating characters were, in decreasing order: width of apex wing (WAW; loading = 0.99), length of petiole (LP; loading = 0.44) and length of stipule (LS; loading = 0.18). The probabilities for Wilk's lambda [Value (v) = 0.3533, F value (F) = 18.3; d. f. = 40], Pillai's Lawley trace (v = 0.6467; F = 18.3; d.f. = 40) indicated significant difference between the two subspecies of I. subnuda (P < 0.0001).

The second DA with floral characters (analyses including characters without missing data, and achieve both multivariate normality and equal covariance matrix) can be found in Fig. 3C. In this DA the eigenvalue for the canonical axis (3.89) accounted for 100% of the variation for total dispersion of the specimens. The most discriminating characters were, in decreasing order: length of peduncle (LOP; loading = 2.15), length of floral rachis (LFR; loading = -1.95) and length of flower pedicel (LFP; loading = 0.80). The probabilities for Wilk's lambda [Value (v) = 0.2045, F value (F) = 11.2; d. f. = 17], Pillai's trace (v = 0.7955; F = 11.02; d.f. = 17) and Hotelling-Lawley trace (v = 114.57, F = 10.42, d.f. = 6) indicated significant difference between the two subspecies of I. subnuda (P < 0.0001).

The last DA with log transformed floral characters (analyses including characters without missing data, and achieve both multivariate normality and equal covariance matrix) outperformed the previous DA (Supporting information; Appendix S2, Fig. S1.C). The eigenvalue of DA for the canonical axis (5.18) accounted for 100% of the variation for total dispersion of the specimens. The most discriminating characters were, in decreasing order: length of flower pedicel (LFP; loading = 0.16), length of floral rachis (LFR; loading = -0.04) and length of peduncle (LOP; loading = 0.038). The probabilities for Wilk's lambda [Value (v) = 0.1619, F value (F) = 14.67; d. f. = 17], Pillai's trace (v = 0.8381; F = 14.67; d.f. = 17) and Hotelling-Lawley trace (v =

270.89, F = 24.626, d.f. = 6) indicated significant difference between the two subspecies of I. subnuda (P < 0.0001). F values based on Mahalanobis distances indicate significant differences between the groups centroids, therefore separation between the subspecies of I. subnuda were well supported (P<0.001).

The difference means results of the traits that more account for variance in both leaf and floral matrix were calculated (Supporting information, Appendix S2, Fig S2; Fig. S3). Three variables were indicated for DA analyses in leaf matrix (width apex wing, length of petiole, and length of stipule). The t-test to width apex wing indicated significant difference between the two subspecies of I. subnuda (P < 0.0001). The t-test to length of stipule were less significant and to length of petiole any difference was obtained. The t-test to length of flower pedicel indicated significant difference between means of the subspecies of I. subnuda (P < 0.0001). To both lengths of floral rachis and peduncle the t-test do not have significant differences between means.

ECOLOGICAL NICHE MODELING

The results of the current ecological niche modeling based in all I. subnuda records (I. subnuda subsp. subnuda + I. subnuda subsp. luschnathiana) carried out with Maxent can be observed in Fig. 4A. Modeling each subspecies as separately results in the overlaid ecological niche model as indicated in Fig 4B. The quality of the model regarding the two subspecies of I. subnuda as separate entities provide AUC value reasonably better than niche model of I. subnuda as a whole. In this scenario the importance of climatic variables used for the definition of potential current distribution model regarded separately (Figure 4B, Supporting Information, Appendix S3, Fig. S1) presents different results, both precipitation of driest month and precipitation of warmest quarter being the most relevant variable for I. subnuda subsp. luschnathiana (hereafter subsp. luschnathiana). In I. subnuda subsp. subnuda (hereafter subsp. subnuda), precipitation of warmest quarter and temperature annual range were relevant in indicating a difference with subsp. luschnathiana. Our results for niche overlap in environmental space, the contribution of the variables and the equivalence and similarity test can be seen from Fig. 5. The results reveals moderate niche overlap (D: 0.40), similar but not equivalent niche space (Fig. 5C, Fig. 5D).

Across the Quaternary significant changes were suggested by ENM in the suitability and distribution ranges of subsp. subnuda and subsp. luschnathiana (Fig. 6). According to AUC (0.92) and TSS (0.88) values, the quality of our models were significant for all, thus the models performed well in relating species occurrences to bioclimatic variables. The two subspecies of Inga subnuda experienced expansion of its suitability areas during the last interglacial (LIG), where the modelled subsp. subnuda spread toward south of BAF as well as in some extant inward northeast Brazil. The modelled subsp. luschnathiana presented suitability areas toward south and northeast Brazil, but without connection between northeast and south suitability areas.

In contrast with LIG, our models suggest a retraction in the distributions of subsp. subnuda during the last glacial maximum (LGM). Notwithstanding, subsp. luschnathiana shows suitability expansion areas during LGM (Fig. 6; Supporting Information, Appendix S3, Figure S2), with remarkable areas in east of Bahia-Brazil and Midwest Brazil. In the Mid-Holocene the modelled subspecies expanded their distributions near those of the current distribution. To subsp. luschnathiana predicted areas of ecological stability are indicated as multiple potential refugia for both species (Fig. 6).

DISCUSION

MORPHOMETRIC ANALYSES

Leaf and floral characters are useful for support differences between species (Cook, Semple & Baum, 2009; Souza, Funch & Queiroz, 2014; Viera-Barreto et al., 2018). Bentham (1845, 1876) qualitatively regarded the following characters as diagnostic for distinguish I. subnuda and I. luschnathiana: leaflets pubescence, pairs of leaflets, leaflet form, peduncle, inflorescence rachis, flower pedicel, pubescence of corolla and calyx proportion. However, Pennington (1997) consider character as leaf rachis, flower pedicel and legume margins and faces to differentiate between subspecies. Yet, according to our results, the following characters are significant for discrimination between taxa with high statistical significance ($p < 0.0001$): width apex wing, length of petiole, length of stipule, length of flower pedicel, length of floral rachis and length of peduncle.

I. luschnathiana and I. subnuda were described by Bentham (1845, 1876) as separate species, based basically in differences in leaf and floral characters. Pennington (1997) highlighted the overlaying of leaf and floral characters, thus regarded I. luschnathiana as subspecies of I. subnuda, although our results point out discontinuity with high statistical support ($p < 0.001$) in width of apex wing (WAW < 3.74 mm in subsp.

subnuda; WAW > 3.38 mm in subsp. luschnathiana) and length of flower pedicel (LFP < 1.65 mm in subsp. luschnathiana; LFP > 1.87 mm in subsp. subnuda), two characters that Pennington (1997) regard as overlaying with intermediate specimens. Therefore, we consider these two character as useful to discrimination between the two taxa. In addition, our results give support to the original view of Bentham (1845) in regarding two species. The DA results more noticeably distinguished the groups than the PCA results (Vieira-Barreto et al., 2018). Together, the two analyses allowed the identification of two distinct groups.

ECOLOGICAL NICHE MODELING

Historical climatic modelling of the two subspecies of Inga subnuda

The speciation in Inga is estimated as concentrated in the past 10 ma, with many species arising recently since 2 ma, corresponding with the more major uplifts of the Andes, the bridging of the Isthmus of Panama, and Quaternary glacial cycles (Richardson et al., 2001). It is estimated that during the past 7.1 to 3.5 ma was characterized by climate oscillations of cold and dry to warm and moist conditions (Li et al., 2014). Records obtainded by measurements of ice cores reports that eight glacial cycles occurred in the last 420 ka, and at least 20 episodes of glacial/interglacial periods cycles occurred in Pleistocene (Augustin et al., 2004; Farmer & Cook, 2013). In this sense, Inga species underwent several glacial cycles in the last 2,3 Ma, and our ENM results can be extended to earlier periods than LGM and LIG scenarios. During the last 120-140 ka, our ENM results distinguished expansion of range in interglacial periods (LIG, Holocene and Current) and retraction of these areas in glacial periods (LGM) for both subspecies. Furthermore, their influence in the distribution of the two subspecies of I. subnuda was consistent with phylogeographic studies in BAF (Lira et al., 2003; Grazziontin et al., 2006; Cabanne, Santos & Miyaki, 2007; Ribeiro et al., 2011).

Despite expansion and contraction range was detected for both subspecies, our ENM results showed remarkable expansion of suitability areas during LGM for subsp. luschnathiana for northeastern. This expansion is corroborated by palynological studies of northeastern of Brazil, demonstrating more humid and rainy periods, which would have favored the expansion of humid forests and mountain forests (Behling et al., 2000). Furthermore, this species has a climatic bias from subtropical occurrence, in a way that enable the expansion related with the colder conditions reaching until the northern region of BAF, now colder. These facts corroborated with Leite et al., (2016) that described predominantly LGM characterized by cooler climate, significantly lower sea levels, and slightly reduced precipitation, but which was still enough to keep large stretches of rainforest instead of small refugia (Leite et al., 2016).

After the glacial period in LGM and with the rise of temperature in the Holocene, both species reach the more favourable climatic areas. Consequently, the subsp. subnuda more related with the higher temperature remained in the northern region, while the subsp. luschnathiana recovering suitability areas more toward south. Thereby, the climatic conditions in the northern of BAF (subsp. subnuda suitability areas) were similarly drier in the LGM relative to today's climate, and generally wetter in the Mid-Holocene, whereas the contrary happened in the southern (subsp. luschnathiana suitability areas) of BAF (Cheng et al., 2013).

The southern coastal plain and northeast area of BAF, especially in Bahia and Pernambuco region was identified as suitable for subsp. subnuda. These areas might have remained stable during climatic fluctuations of recent quaternary cycles and served as glacial refugium, as previously it has been reported in the BAF for vertebrate species (Carnaval & Moritz, 2008; Carnaval et al., 2009), Myrcia spp. (Staggemeier et al., 2015) and Eugenia uniflora (Truchetto-Zolet et al., 2016). In contrast with this pattern, suitable areas for subsp. luschnathiana, was identified throughout the southeast coastal region between Rio de Janeiro to northeast of Santa Catarina states, and, evidence support environmental in this region, also indicated for Eugenia sect. Phyllocalyx (Bünger et al., 2016), palynological studies (Marchant et al., 2002; Behling & Negrelli, 2006) and vertebrates (Carnaval et al., 2014; Leite et al., 2016). This region, especially the south-east coastal region (southern of São Paulo and northern of Paraná states) comprise estuarine and lagoon complexes, with various micro-regions and micro-climates most probably influenced by the uplift of Serra do Mar during the Oligocene (Almeida & Carneiro, 1998). These results are well integrated with Carnaval et al., (2014) that suggest how the climatic variability through the last 250 ka impacted the northern and southern regions of the BAF differently.

Distinctiveness of the two subspecies of I. subnuda in the context of partially overlapping distributions

The values of AUC and TSS of our climatic models of current distribution of the two subspecies of Inga subnuda indicated better performance when regarding as separate entities, that is, the AUC and TSS values were more close to 1 in I. subnuda as separate entities than in I. subnuda as a whole (Fielding & Bell, 1997; Jones, Acker & Halpern, 2010). For subsp. subnuda, precipitation of the coldest quarter and temperature annual range emerges as significant climatic variables distinguishing this species from subsp. luschnathiana, where both altitude and precipitation of the warmest quarter were the most important climatic variable. In this context, our results suggest that subsp. subnuda has high suitability in tropical zone and subsp. luschnathiana in humid subtropical zone. The overlap zone between the species locating between tropical and humid subtropical zone with dry winter (Alvares et al., 2013). The overlap could be owing to a climatic transition between north and southern climatic spaces happen near of the Rio Doce, a known biogeographic divisor (Carnaval et al., 2014). Despite the overlap index was moderate (D: 0.40), the test of similarity and equivalence suggest that the niches of subsp. subnuda and subsp. luschnathiana tend to be less similar, but not identical (Warren, Glor & Turelli, 2008). Hence, could be evidence of parapatric speciation through niche divergence, where species are more climatically similar than expected (Jezkova & Wiens, 2018).

CONCLUSION

The results present here were able to circumscribe the differences between both subspecies of I. subnuda through integrative approaching of traditional morphometrics and ecological niche modeling. Consequently, in agreement with some recent studies in other groups, as Pinus (Aguirre-Gutiérrez et al., 2015), Huperzia (Shrestha & Zhang, 2015) mosses (Gama, Aguirre-Gutiérrez & Stech, 2017) and Kaunia (Viera-Barreto et al., 2018). Therefore, ecological niche approaching can help considerably in establish boundaries in morphologically similar species, it can aid frame cases for species diversification, together with other sources of data (Raxworthy et al., 2007; Hawlitschek et al., 2011; Gama, Aguirre-Gutiérrez & Stech, 2017). We provide so, consistent evidence for discrimination between Inga subnuda subsp. subnuda and Inga subnuda subsp. luschnathiana trough the conceptual framework of USC (Unified Species Concept) (De Queiroz, 2007). Thus, it is preferable regard these two subspecies as separate species.

TAXONOMIC TREATMENT

In accordance with our results, we propose the following taxonomic changes.

Inga luschnathiana Benth. London J. Bot. 4:618. 1845, **st. nov.** – Lectotype (designated by Pennington T.D. in The Genus Inga: Bot. 1997): Brazil, Rio de Janeiro, s.d., Luschnat s.n. (OXF, photo!).

≡ Inga subnuda subsp. luschnathiana (Benth.) T.D. Penn. The Genus Inga: Bot: 753. 1997.

= Inga eriantha Benth. in Hooker London J. Bot. 4: 614. 1845, Lectotype (Designated by Pennington T.D. in The Genus Inga: Bot. 1997): Brazil, fl., 1842, Miers 3869 (K, photo!).

= Feuilleea luschnathiana (Benth.) Kuntze. Rev Gen. 1:188. 1981.

Description. –trees or treelets. Stipules 1.34-3.42 mm long, ovate, tomentose. Petiole 1.1-2.51 cm, terete, densely tomentose; rachis 6.3-12.6 cm long, winged (apex wing 3.38-7.77 mm wide), tomentose, appendix absent. Leaflets (3)4-5 pairs; terminal pair 7.14-13.7 × 1.9-8.1 cm, elliptic, apex acute or acuminate; basal pair 2.4-7.8 × 1.6-4.5 cm, elliptic, apex acute, sometimes retuse. Inflorescence axillary, solitary or paired; peduncle 0.57-3.24 cm long, tomentose; floral rachis 1.5-4.8 cm long (elongating with age); bracts 1.1-4.1 mm long, ovate, pedicel 0.19-1.65 mm long. Calyx open in bud; tube 2.97-5.92 mm long; lobes 0.45-1.15 mm long; tomentose. Corolla tube 1.27-2.13 cm long, villose, indumentum undulate in bud. Androecium, stamens 70-90, staminal tube 1.24-2.5 cm long, equaling the corolla or included, free filaments 2.53-3.69 cm long. Gynoecium, ovary of 1 carpel, glabrous, style exceeding the stamens, style 3.81-5.91 cm long. Legume 14.3-18.1 x 1.9-2.6 x 1.4-2.6 cm, convex when immature, quadrangular when mature, straight, seldom slightly curved, faces moderately covered when immature but exposed when mature, margins striate, moderately expanded (Fig. 7).

Material examined –Brazil. Espirito Santo. Santa Teresa. Reserva Biológica Sta Lúcia, 4 Mar 1997 (fl), F.C.P. Garcia 763 (VIC), Cabeceira do Rio Bonito, 22 Jun 2001 (fr), L. Kullmann et al 3881 (HUEFS), 27 Apr 2008 (fl), D. A. Folli 6049 (VIC); Fundão. Goiapaba-Açu, 6 Aug 1998 (fr), L. Kullmann 308 (VIC); Presidente Kenedy. Praia das Neves, 17 Oct 2008 (fl), Calvancanti 254 (VIC).

Minas Gerais. Araponga. Proximidades do Parque Estadual da Serra do Brigadeiro, 20 Apr 2012 (fl), L.C. Siqueira 744 (VIC); Carandaí. Pedra do Sino Hotel Fazenda, BR 040 km 6, Trilha da Matinha, 2 Oct 2005 (fr), Mota & Stehmann 402 (BHCB); Descoberto. Reserva Biologica Represa da Grama, 2 Sep 2001 (fl), R.C. Forzza 860 (HUEFS); Nova Lima. Reserva Biológica da Mata do Jambreiro, 5 Oct 2001 (fl), L.M. Versieux 3 (BHCB); Canãa. Beira estrada, próximo usina UFV, 12 Aug 2006 (fl), J. M. Fernandes 368 (VIC); Rio Petro. Serra do Funil, 3 Nov 2017 (fl), Garcia, Castro & Alves 1534 (VIC). Rio de Janeiro. Angra dos Reis. Saco de Piracuara de Fora, 8 Sep 1999 (fl), A. E. Oliveira 25 (RB); Cabo Frio. Tamoios, Reserva Biológica do Mico Leão Dourado, 27 Mar 2003 (fl, fr), R. D. Ribeiro 11 (RB); Mangaratiba. Ilha do Mangaratiba, 22 Apr 2005 (fl), D. C. Carvalho 20 (RB); Rio das Ostras. Fazenda Itapebussus, 1 Sep 2004 (fr), Oliveira 878 (RB); Rio das Ostras. Reserva Biológica da União, Trilha 1, 15 Oct 1997 (fr), P.P. Oliveira 44 (BHCB); Santa Catarina. Florianopolis, Ilha de Santa Catarina, 16 Nov 1984 (fr), Silva, Souza & Zanin 227 (FLOR), Cachoeira de Bom Jesus, 31 Mar 1970 (fr), Klein 8646 (FLOR), Lagoinha Pequena, Rio Tavares, 20 Oct 2011 (fl), Mello 628 (FLOR), ibid., 9 Nov 2011 (fl), Nuernberg & Mello 422 (FLOR), Jurerê, 16 Jun 1995 (fl), J. Mattos 32336 (VIC), Parque do Rio Vermelho, final da estrada geral da praia, 28 Dec 1994 (fl), Falkenberg & Souza 6784 (FLOR), Perto da Vila, Ribeirão, 24 Oct 1967 (fl), Klein & Bresolin 7612 (FLOR), Ribeirão da Ilha, 15 Oct 1968 (fl), Klein 7881 (VIC), Rio Vermelho, 31 Aug 1984 (fr), J. S. Oliveira-Filho 51 (FLOR), ibid., 16 Nov 1984 (fr), F. A. Silva Filho et al. 227 (FLOR), ibid., 12 Apr 1994 (fl), Falkenberg & Silva Filho 6674 (FLOR), ibid., 3 May 1994 (fr), Falkenberg & Silva Filho 6685 (FLOR); Itajaí. Cunhas, 29 Oct 1954 (fl), Klein 839 (FLOR); Governador Celso Ramos, Vargem do Macário, 14 Oct 1971 (fl), Bresolin & Klein 372 (FLOR), ibid., 18 Nov 1971 (fl), Klein & Bresolin 9944 (FLOR); Itapoá. Balneario Uirapuru, 17 Nov 2010 (fl) A. Korte 4960 (FURB); São Francisco do Sul. Itapema, 31 Oct 2009 (fl), S. Dreveck 1198 (FURB). São Paulo. Cananeia. Ilha de Cardoso, 12 Oct 1979 (fl), De-Grande et al. 338 (HUEFS), ibid., 20 May 1988 (fl), H. F. Leitão-Filho et al. 20337 (UEC), ibid., 30 Oct 1991 (fl), F. Barros 2327 (HUEFS); Iguape. Praia do Grajau, 10 Dec 1981 (fl), Stubblebine, Shepherd & Tamashiro 13198 (UEC); Parequera-Açu. Estrada para Cananeia, 7 Feb 1995 (fr), H. F. Leitão-Filho et al. 32646 (UEC); Peruíbe. Arredores da Foz do Rio Guaraú, 9 Oct 1995 (fl), V. C. Souza & J. P. Souza 9281 (UEC); São Paulo. Parque Estado de São Paulo, 12 Oct 1944 (fl), Hoehne s.n. (SPF); Ubatuba. Picinguaba, 9 Oct 1988 (fl), Cunha NML 193 (VIC), Trilha do Morro do Corsário em direção ao mangue, 6 Aug 1988 (fr), J.E.L.S. Ribeiro 407 (VIC), Trilha das 3 lagoas ou Picadão da Serra, 12 Jan 1991 (fr), F. C. P. Garcia et al. 600 (VIC), Parque Estadual da Serra. BR-101, margem da rodovia, 10 Oct 1995 (fl), P.F. Assis et al. 316 (VIC).

Distribution and Habitat –The species has been recorded only in the BAF in the states of Espírito Santo, Rio de Janeiro, Minas Gerais, São Paulo, Parana and Santa Catarina (Fig. 1), in agreement with our ecological niche modeling for current distribution that can be seen in Fig. 4B. The specimen Lewis & Carvalho 1070 was identified as I. subnuda, thus the distribution of the specie was restricted to states cited above, yet the ENM shows suitability areas southward in the state of Bahia.

Phenology –Flowering specimens were collected in March, April, May, June, August, September, October, November and December, and fruiting specimens in January, February, March, May, June, August, September, October and November.

Taxonomic comments –the leaf and floral characters distinguishing I. luschnathiana from I. subnuda are discussed above in the discussion section, however it was possible observe some variation in the length of flower pedicel in one population of I luschnathiana found in Pareriquera-Açu (State of São Paulo), this population has length of flower pedicel a little longer. I. luschnathiana is readily distinguishing from I. subnuda by its faces moderately covered at first, becoming exposed at maturity, margins striate moderately expanded, and legume transversal section convex at first, becoming quadrangular at maturity (Table 2). I. luschnathiana is frequently confused with I. vera subsp. affinis (DC.) T.D.Penn., although it is possible differentiate because I. luschnathiana has stipules ovate, 4-5 pairs of leaflets, larger elliptic terminal pair leaflets, larger and terete petiole, calix open in bud, narrower and smaller tube calix and shorter lobes calyx.

Table 2. Morphological comparison between Inga luschnathiana and Inga subnuda.

	I. luschnathiana	**I. subnuda**
Length of stipules (mm)	1.34–3.42	1.12–3.01
Length of petiole (cm)	1.1–2.51	1.02–2.5
Width apex wing (mm)	3.38–7.77	0–3.74
Length of Peduncle (cm)	0.57–3.24	0.65–5.02
Length of floral rachis (cm)	1.47–4.79	0.51–5.28
Length of flower pedicel (mm)	0.19-1.65	1.87-7.09
Legume shape	Straight	Curve, slightly curve
Transversal section of legume	Convex at first, becoming quadrangular at maturity	Convex when young, becoming cylindrical when mature
Margin of Legume	Moderately expanded	Completely expanded
Faces of Legume	Moderately covered at first, becoming exposed at maturity	Covered

Figure 7. Inga luschnathiana. A, habit; B, apex wing; C, undulate indumentum in bud; D, flower (D. C. Carvalho 20); E, Immature legume; F, transversal section (Oliveira & Oliveira 878); G, Legume; H, transversal section (Leitão-Filho et al. 3246).

ACKNOWLEDGEMENTS

We are grateful to the curators and staff of the herbaria mentioned in ‘material and methods’ for use and loan of specimens examined. Funding for this research was supplied by FAPEMIG Edital Universal 2017/PROCESSO APQ-01703-17 and FAPES Processo nº 61855880/2012.

REFERENCES

Aguirre-Gutiérrez J, Serna-Chavez HM, Villalobos-Arámbula AR, Pérez de la Rosa JA, Raes N. 2015. Similar but not equivalent: Ecological niche comparison across closely related Mexican white pines. Diversity and Distributions **21:** 245–257.

Allouche O, Tsoar A, Kadmon R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). Journal of applied ecology **43:** 1223-1232.

Almeida FFM, Carneiro CDR. 1998. Origem e evolução da Serra do Mar. Revista Brasileira de Geociências **28:** 135-150.

Alvares CA, Stape JL, Sentelhas PC, Moraes G, Leonardo J, Sparovek G. 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift **22:** 711-728.

Augustin L, Barbante C, Barnes PR, Barnola JM, Bigler M, Castellano E, Cattani O, Chappellaz J, Dahl-Jensen D, Delmonte B, Dreyfus G. 2004. Eight glacial cycles from an Antarctic ice core. Nature **429:** 623-628.

Behling H, Arz HW, Pätzold J, Wefer G. 2000. Late Quaternary vegetational and climate dynamics in northeastern Brazil, inferences from marine core GeoB 3104-1. Quaternary Science Reviews **19:** 981-994.

Behling H, Negrelle RR. 2006. Vegetation and pollen rain relationship from the tropical Atlantic rain forest in southern Brazil. Brazilian archives of biology and technology **49:** 631-642.

Bentham G. 1845. Notes on Mimoseae with a synopsis of species. London Journal of Botany **4:** 613-618.

Bentham G. 1876. Leguminosae II et III. Swartzieae, Cesalpinieae, Mimosoideae. In: Martius C. Flora Brasiliensis, Vol. 15, pt. 2. Monachii, 498-499.

Broennimann O, Fitzpatrick MC, Pearman PB, Petitpierre B, Pellissier L, Yoccoz NG, Thuiller W, Fortin MJ, Randin C, Zimmermann NE, Graham CH, Guisan A. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecology and Biogeography **21:** 481–497.

Brown GK. 2008. Systematics of the tribe Ingeae (Leguminosae-Mimosoideae) over the past 25 years. Muelleria **26:** 27-42.

Bueno ML, Pennington RT, Dexter KG, Kamino LHY, Pontara V, Neves DM, Ratter JA, Oliveira-Filho AT. 2017. Effects of Quaternary climatic fluctuations on the distribution of Neotropical savanna tree species. Ecography **40:** 403-414.

Bünger MO, Mazine FF, Forest F, Bueno ML, Stehmann JR, Lucas EJ. 2016. The evolutionary history of Eugenia sect. Phyllocalyx (Myrtaceae) corroborates historically stable areas in the southern Atlantic forests. Annals of botany **118:** 1209-1223.

Buzatti RSDO, Lemos-Filho JP, Bueno ML, Lovato MB. 2017. Multiple Pleistocene refugia in the Brazilian cerrado: evidence from phylogeography and climatic nichemodelling of two Qualea species (Vochysiaceae). Botanical Journal of the Linnean Society **185:** 307-320.

Buzatti RSDO, Pfeilsticker TR, Magalhães RF, Bueno ML, Lemos-Filho JP, Lovato MB. 2018. Genetic and Historical Colonization Analyses of an Endemic Savanna Tree, Qualea grandiflora, Reveal Ancient Connections between Amazonian Savannas and Cerrado Core. Frontiers in Plant Science **9:** 981.

Cabanne GS, Santos FR, Miyaki CY. 2007. Phylogeography of Xiphorhynchus fuscus (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in Southern Atlantic forest. Biological Journal of Linnean Society **91**: 73–84.

Carnaval AC, Hickerson MJ, Haddad CF, Rodrigues MT, Moritz C. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science **323:** 785-789.

Carnaval AC, Moritz C. 2008. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic Forest. Journal of Biogeography **35:** 1187–1201.

Carnaval AC, Waltari E, Rodrigues MT, Rosauer D, VanDerWal J, Damasceno R, Prates I, Strangas M, Spanos Z, Rivera D, Pie MR. 2014. Prediction of phylogeographic endemism in an environmentally complex biome. Proceedings of the Royal Society B **281:** 20141461.

Cheng H, Sinha A, Cruz FW, Wang X, Edwards RL, d'Horta FM, Ribas CC, Vuille M, Stott LD, Auler AS. 2013. Climate change patterns in Amazonia and biodiversity. Nature communications **4:** 1411.

Conceição AS, Queiroz LP, Lambert SM, Pereira ACS, Borba EL. 2008. Biosystematics of Chamaecrista sect. Absus subsect. Baseophyllum (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. Plant Sistematycs and Evolution **270:** 183-207.

Cook RE, Semple JC, Baum BR. 2009. A multivariate morphometric analysis of Solidago subsect. Glomeruliflorae (Asteraceae: Astereae). Botany **87:** 97–111.

De la Estrella M, Aedo C, Velayos M. 2009. A morphometric analysis of Daniellia (Fabaceae-Caesalpinioideae). Botanical Journal of the Linnean Society **159:** 268–279.

De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology **56**: 879–886.

Di Cola V, Broennimann O, Petitpierre B, Breiner FT, D'amen M, Randin C, Engler R, Pottier J, Pio D, Dubuis A, Pellissier L. 2017. ecospat: An R package to support spatial analyses and modeling of species niches and distributions. Ecography **40:** 774-787.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ, Münkemüller T. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography **36:** 27-46.

Egan AN. 2015. Species delimitation and recognition in the Pediomelum megalanthum complex (Fabaceae) via multivariate morphometrics. PhytoKeys **44:** 65–87.

Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JMcC, Peterson AT, Phillips SJ, Richardson KS, ScachettiPereira R, Schapire RE, Soberon J, Williams S, Wisz MS, Zimmermann NE. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography **29:** 129–151.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and distributions **17:** 43-57.

Elith J. 2002. Quantitative methods for modeling species habitat: Comparative performance and an application to Australian plants. In: Ferson S, Burgman M, eds. Quantitative methods for conservation biology. Springer Science & Business Media, 39–58.

ESRI. 2011. ArcGIS Desktop: release 10. Environmental Systems Research Inst., Redlands, CA.

Framer GT, Cook J. 2013. Pleistocene Glaciations. In: Framer GT, Cook J, eds. Climate Change Science: A Modern Synthesis: Volume 1 - The Physical Climate. Dordrecht: Springer, 407-427.

Fawcett T. 2004. ROC graphs: Notes and practical considerations for researchers. Machine learning **31:** 1-38.

Fielding AH, Bell JF. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental conservation **24:** 38-49.

Fritsch PW, Schiller AM, Larson KW. 2009. Taxonomic Implications of Morphological Variation in Cercis canadensis (Fabaceae) from Mexico and Adjacent Parts of Texas. Systematic Botany **34:** 510–520.

Gama R, Aguirre-Gutiérrez J, Stech M. 2017. Ecological niche comparison and molecular phylogeny segregate the invasive moss species Campylopus introflexus (Leucobryaceae, Bryophyta) from its closest relatives. Ecology and evolution **7:** 8017-8031.

Garcia FCP. 1998. Relacões sistemáticas e fitogeografia de Inga Miller (Leguminosae-Mimosoideae) nas florestas da costa sul e sudeste do Brasil. Thesis, Universidade Estadual Paulista, Rio Claro.

Gent PR, Danabasoglu G, Donner LJ, Holland MM, Hunke EC, Jayne SR, Lawrence DM, Neale RB, Rasch PJ, Vertenstein M, Worley PH. 2011. The community climate system model version 4. Journal of Climate **24:** 4973-4991.

Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL. 2006. Phylogeography of the Bothrops jararaca complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. Molecular Ecology **15:** 3969-3982.

Haffer J, Prance GT. 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. Amazoniana-Limnologia Et Oecologia Regionalis Systemae Fluminis Amazonas **16:** 579–605.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica **4:** 9. Available at: http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hawlitschek O, Porch N, Hendrich L, Balke M. 2011. Ecological niche modelling and nDNA sequencing support a new, morphologically cryptic beetle species unveiled by DNA barcoding. PLoS One **6:** e16662.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology **25:** 1965–1978.

Jezkova T, Wiens JJ. 2018. Testing the role of climate in speciation: new methods and applications to squamate reptiles (lizards and snakes). Molecular ecology **00:** 1–16.

Jones CC, Acker SA, Halpern CB. 2010. Combining local-and large-scale models to predict the distributions of invasive plant species. Ecological Applications **20:** 311-326.

Kostikova A, Salamin N, Pearman PB. 2014. The role of climatic tolerances and seed traits in reduced extinction rates of temperate polygonaceae. Evolution **68:** 1856-1870.

Kozak KH, Wiens JJ. 2010. Accelerated rates of climatic niche evolution underlie rapid species diversification. Ecology Letters **13:** 1378–1389.

Leite YL, Costa LP, Loss AC, Rocha RG, Batalha-Filho H, Bastos AC, Quaresma VS, Fagundes V, Paresque R, Passamani M, Pardini R. 2016. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. Proceedings of the National Academy of Sciences **113:** 1008-1013.

Léon J. 1966. Central American and West Indian species of Inga (Leguminosae). Annals of the Missouri Botanical Garden **35:** 41–46.

Li F, Wu N, Rousseau DD, Dong Y, Zhang D, Pei Y. 2014. Late Miocene–Pliocene paleoclimatic evolution documented by terrestrial mollusk populations in the Western Chinese Loess Plateau. PloS one **9:** p.e95754.

Lira CF, Cardoso SRS, Ferreira PCG, Cardoso MA, Provan H. 2003. Long-term population isolation in the endangered tropical tree species Caesalpinia echinata Lam. revealed by chloroplast microsatellites. Molecular Ecology **12:** 3219–3225.

Marchant R, Almeida L, Behling H, Berrio JC, Bush M, Cleef A, Duivenvoorden J, Kappelle M, De Oliveira P, Oliveira-Filho, AT, Lozano-Garćia S. 2002. Distribution and ecology of parent taxa of pollen lodged within the Latin American Pollen Database. Review of Palaeobotany and Palynology **121:** 1-75.

Mardia KV. 1970. Measures of multivariate skewness and kurtosis with applications. Biometrika **57:** 519-530.

Oliveira-Filho A, Fontes MA. 2000. Patterns of floristic differentiation among atlantic forests in southeastern Brazil and the influence of climate. Biotropica **32:** 793-810.

Oliveira-Filho AT. 2014. NeoTropTree, Flora arbórea da Região Neotropical: um banco de dados envolvendo biogeografia, diversidade e conservação. – <www.icb.ufmg.br/treeatlan/>

Otto-Bliesner BL, Marshall SJ, Overpeck JT, Miller GH, Hu A. 2006. Simulating Arctic climate warmth and icefield retreat in the last interglaciation. Science **311:** 1751-1753.

Paulino JV, Mansano VF, Prenner G, Teixeira SP. 2016. High developmental lability in the perianth of Inga (Fabales, Fabaceae): a Neotropical woody rosid with gamopetalous corolla. Botanical Journal of the Linnean Society **183:** 146-161.

Pearson RG, Raxworthy CJ, Nakamura M, Peterson AT. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of biogeography **34:** 102-117.

Pena JCC, Kamino LHY, Rodrigues M, Neto EM, Siqueira MF. 2014. Assessing the conservation status of species with limited available data and disjunct distribution. Biological Conservation, **170:** 130-136.

Pennington TD. 1997. The genus Inga: Botany. Royal Kew: Botanic Gardens.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecological modelling **190:** 231-259.

Phillips SJ, Dudík M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography **31:** 161-175.

Pometti CL, Cialdella AM, Vilardi JC, Saidman, BO. 2007. Morphometric analysis of varieties of Acacia caven (Leguminosae, Mimosoideae), taxonomic inferences in the context of other Argentinean species. Systematics and Evolution **264:** 239–249.

Poncet V, Munoz F, Munzinger J, Pillon Y, Gomez C, Couderc M, Tranchant-Dubreuil C, Hamon S, Kochko A. 2013. Phylogeography and niche modelling of the relict plant Amborella trichopoda (Amborellaceae) reveal multiple Pleistocene refugia in New Caledonia. Molecular Ecology **22:** 6163–6178.

R Core Team. 2017. R: A language and environment for statistical computing, version 3.3.3. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org.

Raxworthy CJ, Ingram CM, Rabibisoa N, Pearson RG. 2007. Applications of ecological niche modeling for species delimitation: A review and empirical evaluation using day geckos (Phelsuma) from Madagascar. Systematic Biology **56:** 907–923.

Rencher AC. 2002. Methods of multivariate analysis. John Wiley & Sons.

Ribeiro RA, Lemos-Filho JP, Ramos ACS, Lovato MB. 2011. Phylogeography of the endangered rosewood Dalbergia nigra (Fabaceae): insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. Heredity **106:** 46-57.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science **293:** 2242-2245.

Rushton SP, Ormerod SJ, Kerby G. 2004. New paradigms for modelling species distributions? Journal of applied ecology **41:** 193-200.

Shrestha N, Zhang X. 2015. Recircumscription of Huperzia serrata complex in China using morphological and climatic data. Journal of Systematics and Evolution **53:** 88-103.

Souza IM, Funch L, Queiroz LP. 2014. Morphological analyses suggest a new taxonomic circumscription for Hymenaea courbaril L. (Leguminosae, Caesalpinioideae). PhytoKeys **38:** 101–118.

Staggemeier VG, Diniz-Filho JAF, Forest F, Lucas E. 2015. Phylogenetic analysis in Myrcia section Aulomyrcia and inferences on plant diversity in the Atlantic rainforest. Annals of Botany **115:** 747-761.

Stehmann JR, Forzza RC, Salino A, Sobral M, Costa DP, Kamino LHY. 2009. Plantas da Floresta Atlântica. Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

Tabarelli M, Pinto LP, Silva JMC, Hirota MM, Bedê LC. 2005. Desafios e oportunidades para a conservação da biodiversidade na Mata Atlântica brasileira. Megadiversidade **1:** 132-138.

Taylor KE, Stouffer RJ, Meehl GA. 2012. An overview of CMIP5 and the experiment design. Bulletin of the American Meteorological Society **93:** 485-498.

The Brazil Flora Group (BFG). 2015. Growing knowledge: an overview of seed plant diversity in Brazil. Rodriguésia **66:** 1085-1113.

Thiers B. 2013. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. Available at: http://sweetgum.nybg.org/ih/

Turchetto-Zolet AC, Salgueiro F, Turchetto C, Cruz F, Veto NM, Barros MJ, Segatto AL, Freitas LB, Margis R. 2016. Phylogeography and ecological niche modelling in Eugenia uniflora (Myrtaceae) suggest distinct vegetational responses to climate change between the southern and the northern Atlantic Forest. Botanical Journal of the Linnean Society **182:** 670-688.

Viera-Barreto JN, Pliscoff P, Donato M, Sancho G. 2018. Disentangling morphologically similar species of the Andean forest: integrating results from multivariate morphometric analyses, niche modelling and climatic space comparison in Kaunia (Eupatorieae: Asteraceae). Botanical Journal of the Linnean Society **186:** 259–272.

Warren DL, Glor RE, Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution **62:** 2868-2883.

Werneck FP, Nogueira C, Colli GR, Sites JW, Costa GC. 2012. Climatic stability in the Brazilian Cerrado: implications for biogeographical connections of South American savannas, species richness and conservation in a biodiversity hotspot. Journal of Biogeography **39:** 1695-1706.

Werneck FP. 2011. The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quaternary Science Reviews **30:** 1630-1648.

APPENDIX

VOUCHER INFORMATION OF THE SPECIMENS USED IN THE MORPHOMETRIC ANALYSES

Inga subnuda Salzm. ex Benth subsp. subnuda. BRASIL. Alagoas. Coruripe. Sitio Pau Brasil, Machado MABL 510 (HUEFS); São Miguel dos Campos. Povoado Bernardo Lopes, Paiva F. 3340 (HST). Bahia. Camacã. Reserva Serra Bonita, Amorim AM 5450 (HUEFS); Camamu. Povoado de Barcelos do Sul, Miranda AM 5113 (HST); Entre Rios. Algodão, Popovkin AV 1800 (HUEFS); Igrapiúna. Reserva Ecológica Michelin, Queiroz LP 15672 (HUEFS); Ilhéus. Estrada Parque Ilhéus-Tcaré, Goes 5 (HUEFS); Ituberá. APA Cachoeira Pancada Grande, Queiroz LP 15701 (HUEFS); Maraú. Litoral sul, Ponto nº 11, Guedes ML 22038 (ALCB); Santa Cruz de Cabrália. Restinga alta, Silva BM 23 (HUEFS); Santa Terezinha. Serra da Jibóia, Guedes ML 17797 (ALCB); Una. Assentamento Vitoròpolis, Loureiro DM 590 (ALCB); Wenceslau Guimarães. Estação Ecológica, Milliken W 5016 (HUEFS). Espírito Santo. Águia Branca. Rochedo, afloramento rochoso, Magnago 1328 (MBML); Aracruz. Coqueiral a Jacaraipe, de Souza 174 (VIC); Baixo Guandu. Mata de Montanha, Folli 7111 (VIC); Conceição da Barra. Área 135 da Araracruz, Pereira, Gomes & Coelho 4870 (VIES); Conceição da Barra. Barreiras, Margem Rio Cricarè, Lima HC 6651 (RB); Guarapari. Parque Estadual Paulo Cesar Valente, Peterle PL s.n. (VIC); Linhares. Reserva do Vale do Rio Doce, Pinho-Ferreira 642 (VIC); Santa Teresa. Estrada para Caravagio, Dutra & Chagas 860 (VIES); Santa Teresa. Reserva Biol. Augusto Ruschi, Vervloet RR 804 (MBML). Minas Gerais. Guanhães. Parque Estadual Serra da Candonga, Fernandes 949 (VIC); Itamarandiba. PE Serra Negra, Fernandes 1135 (VIC). Santa Rita do Durão. Floresta Estacional Semidecidual, Fernandes 1081 (VIC); Santa Rita do Itueto. Parque Estadual dos Sete Salões, Fernandes 1156 (VIC); Santo Antônio do Itambé. Parque Estadual do Itambé, Fernandes 582 (VIC); São Gonçalo do Rio Abaixo. Estação Ambiental de Peti, Área de Reservatório, Ferreira, França & Martins 69 (BHCB). Pernambuco. Caruaru. Brejo dos Cavalos, Locatelli e Medeiros s.n. (HUFP). Rio de Janeiro. Búzios. Restinga, Garcia FCP & Lima 742 (VIC); Cabo Frio. Restinga, Garcia FCP 1118 (VIC). Sergipe. Santa Luzia do Itanhy. Mata do Crasto, Souza MFL 353 (ASE).

Inga subnuda subsp. luschnathiana (Benth.) T.D. Penn. BRASIL. Espírito Santo. Presidente Kenedy. Praia das Neves, Calvancanti 254 (VIC); Santa Teresa. Reserva Biológica Sta Lúcia, Garcia FCP 763 (VIC). Minas Gerais. Araponga. Proximidades do Parque Estadual da Serra do Brigadeiro, Siqueira LC 744 (VIC); Canãa. Beira estrada, proximo usina UFV, Fernandes 368 (VIC); Descoberto. Reserva Biologica Represa da Grama, Forzza RC 1860 (HUEFS); Nova Lima. Reserva Biologica da Mata do Jambreiro, Versieux LM 3 (BHCB); Rio Petro. Serra do Funil, Garcia, Castro & Alves 1534 (VIC). Rio de Janeiro. Angra dos Reis. Saco de piracuara de fora, Oliveira AE 25 (RB); Cabo Frio. Tamoios, Reserva Biologica do Mico Leão Dourado, Ribeiro RD 11 (RB); Mangaratiba. Ilha do Mangaratiba, Carvalho DC 20 (RB); Paraty. Pedras de Cairuçu, Nadruz M 667 (RB). Santa Catarina. Florianopolis, Ilha de Santa Catarina, Silva, Souza & Zanin 227 (FLOR); Florianopolis, Ribeirão da Ilha, Klein 7881 (VIC); Itapoá. Balneario Uirapuru, Korte 4960 (FURB); São Francisco do Sul. Itapema, Dreveck & Carneiro 1198 (FURB). São Paulo. Cananeia. Ilha de Cardoso, Barros 2327 (HUEFS); Cananeia. Ilha de Cardoso, Leitão-Filho 20337 (UEC); Descalvado. Fazenda Santa Ana do Monte Alegre, Bacia do ribeirão pântano, Oliveira, Accacio & Roxo 3261 (SPF); Iguape. Praia do Grajau, Stubblebine, Shepherd & Tamashiro 13198 (UEC); Parequera-Acu. Estrada para Cananeia, Leitão-Filho 32646 (UEC); Peruíbe. Arredores da Foz do Rio Guaraú, Souza VC & Souza JP 9281 (UEC); São Paulo. Parque Estado de São Paulo, Hoehne s.n. (SPF); Ubatuba. Picinguaba, Cunha NML 193 (VIC).

SUPPORTING INFORMATION

Addition Supporting Information may be found in the online version of this article:

Appendix S1. Morphological analyses. Table S1: Univariate analyses of both leaf and floral matrices for the 18 characters studied from 54 analyzed specimens of the two subspecies of Inga subnuda.

Appendix S2. Multivariate analyses and comparison of means. Figure S1: Scattergrams for the PCA and DA carried out on floral characters with log transformation of the subspecies of Inga subnuda. Figure S2: Boxplot and results of t-test to the three leaf traits. Figure S3: Boxplot and results of t-test to the three floral traits.

Appendix S3. Prediction of Suitable Areas and importance of bioclimatic variables. Figure S1: Predictions of suitable areas of occurrence of the two subspecies of I. subnuda under past and current environmental conditions. Figure S2: Importance of altitude and climatic variables used for the definition of current potential distribution model of the two subspecies of I. subnuda.

FIGURE LEGENDS

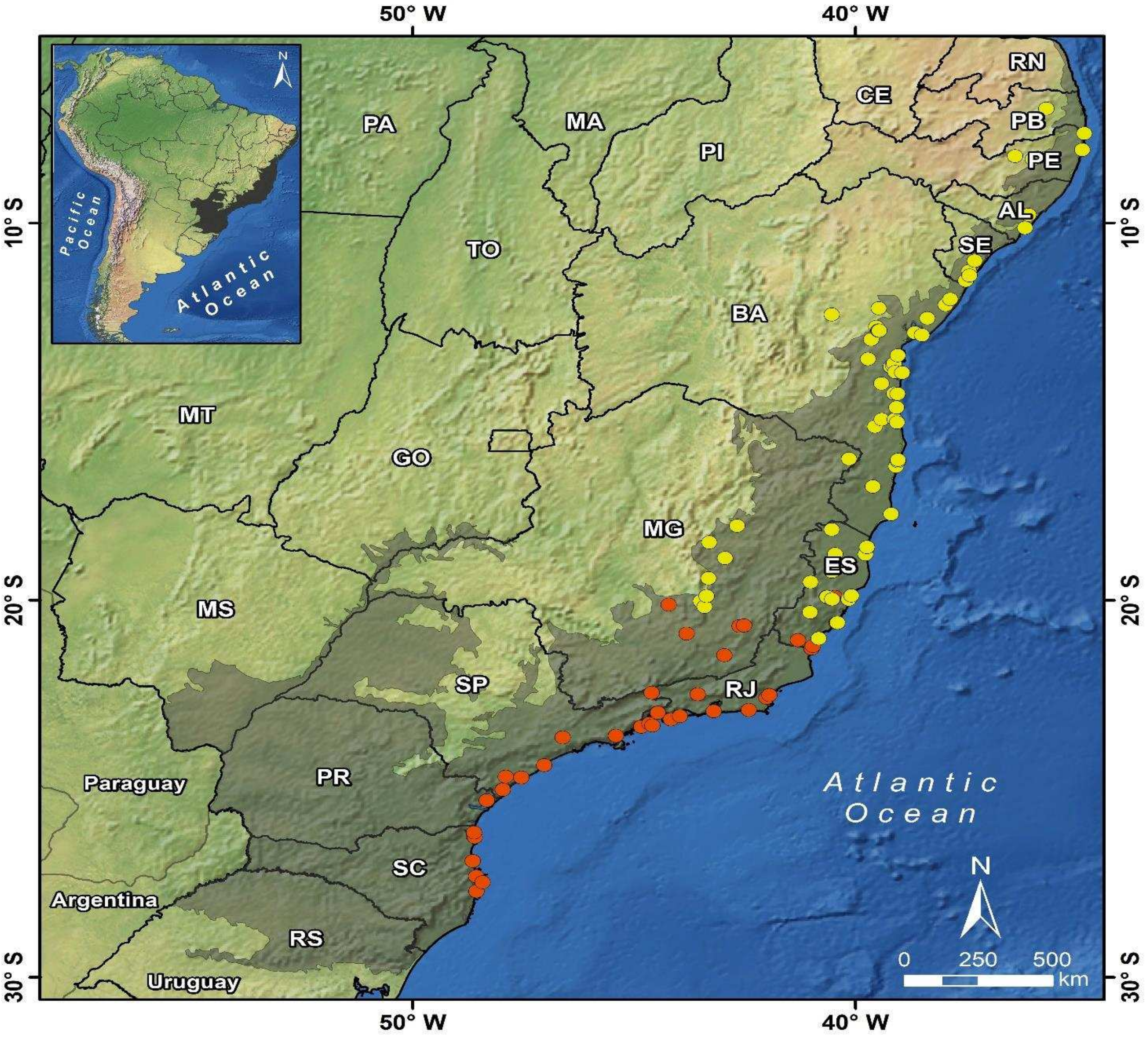


Figure 1. Geographic distribution of the BAF domain, with the location of I. subnuda subspp. oranges dots = I. subnuda subsp. luschnathiana. Yellow dots = I. subnuda subsp. subnuda. Brazilian states are labelled as follows: Alagoas (AL), Amazonas (AM), Bahia (BA), Ceará (CE), Distro Federal (DF), Espírito Santo (ES), Goiás (GO), Maranhão (MA), Minas Gerias (MG), Mato Grosso (MT), Mato Grosso do Sul (MS), Pará (PA), Paraíba (PB), Paraná (PR), Pernambuco (PE), Rio de Janeiro (RJ), Rio Grande do Norte (RN), Rondônia (RO), São Paulo (SP), Sergipe (SE), Tocantins (TO).

Figure 2. Scattergrams for the principal component (PCA) and discriminant (DA) analyses carried out on leaf characters of the subspecies of Inga subnuda. A, first two components of the PCA with overall characters (PCA 1 vs PCA 2); B, first two components of the PCA with only LS, WS, LP and WAW (PCA 1 vs PCA2); C, First axis from DA. The eigenvalue of each axis are provided in parentheses in the three cases. PCA symbols: ▲ subsp. subnuda (red); ◆subsp. luschnathiana (blue).

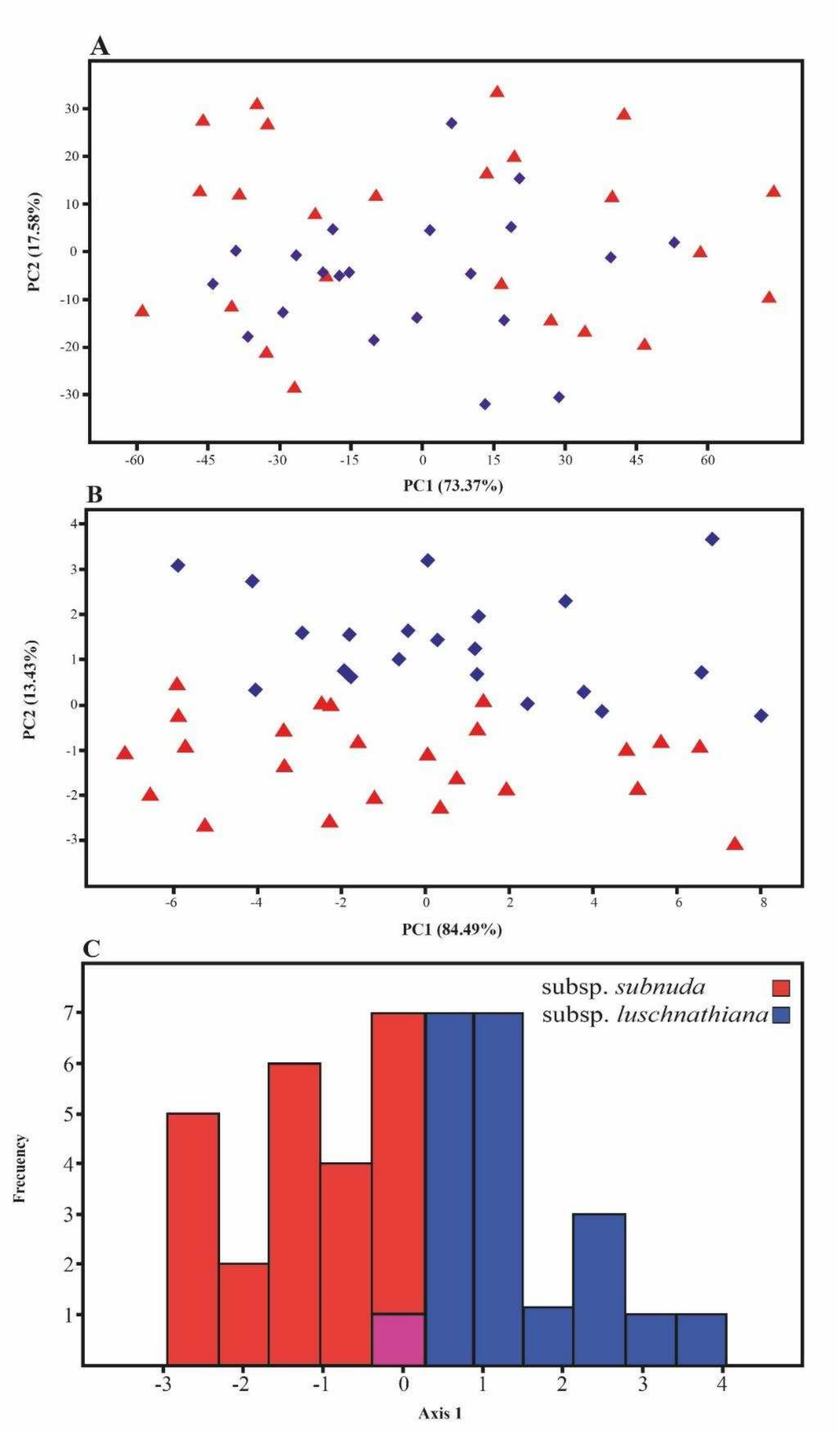
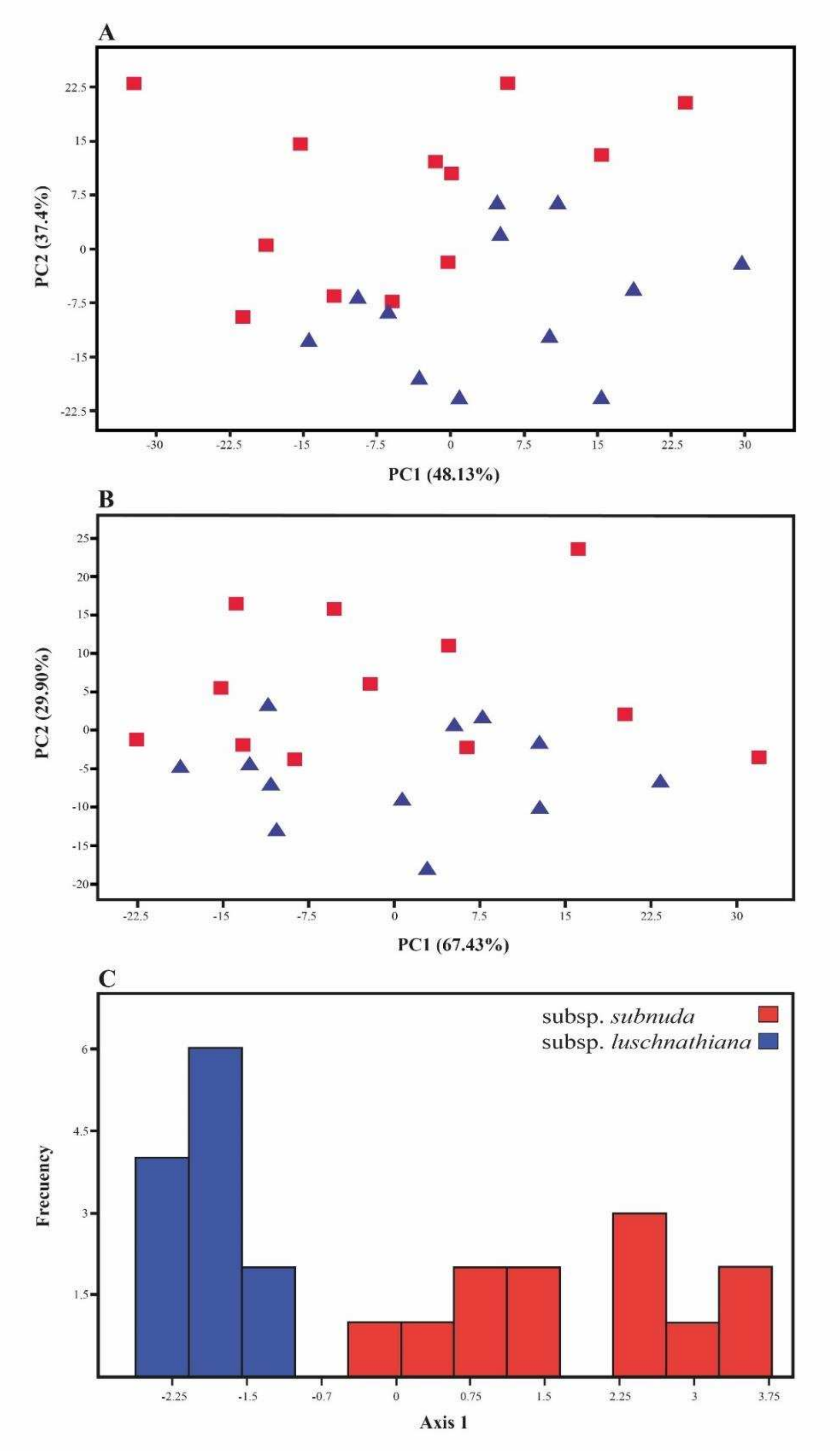


Figure 3. Scattergrams for the principal component (PCA) and discriminant (DA) analyses carried out on floral characters of the two subspecies of Inga subnuda. A, first two components of the PCA with overall characters (PCA 1 vs PCA 2); B, first two components of the PCA with only LOP, LFR, LFP, LK, WK and LC (PCA 1 vs PCA2); C, First axis from DA. The eigenvalue of each axis are provided in parentheses in the three cases. PCA symbols: 🟥 subsp. subnuda (red); ▲ subsp. luschnathiana (blue).



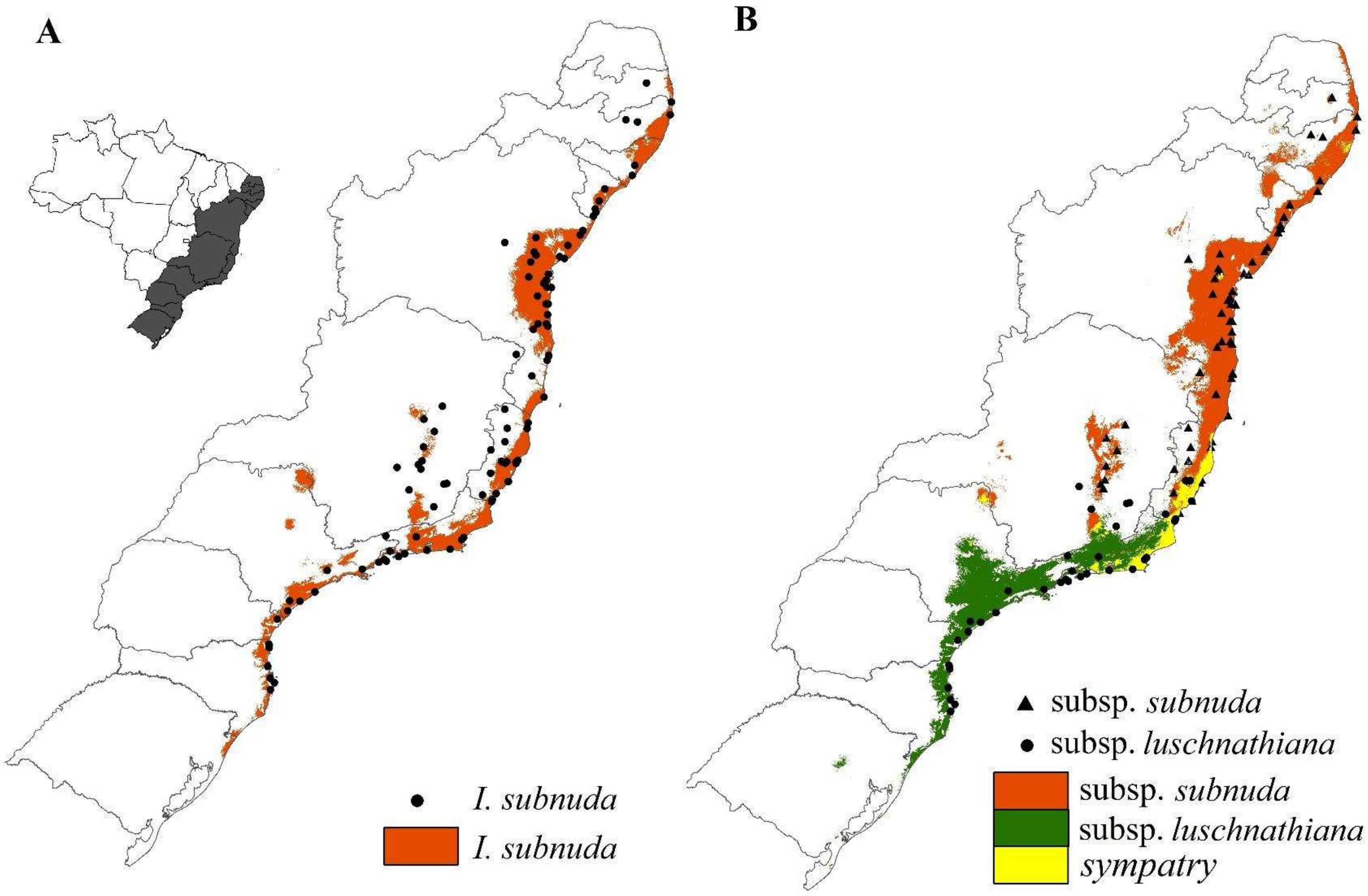


Figure 4. Ecological current niche modeling of the two subspecies of I. subnuda. A, the single ecological niche model based on all localities, when treating the I. subnuda subspp. group as consisting of a single species (AUC = 0.923). B, the two overlaid ecological niche models, when treating each subspecies as separates entities. (I. subnuda subsp. subnuda: AUC = 0.945 and I. subnuda subsp. luschnathiana: AUC = 0.932)

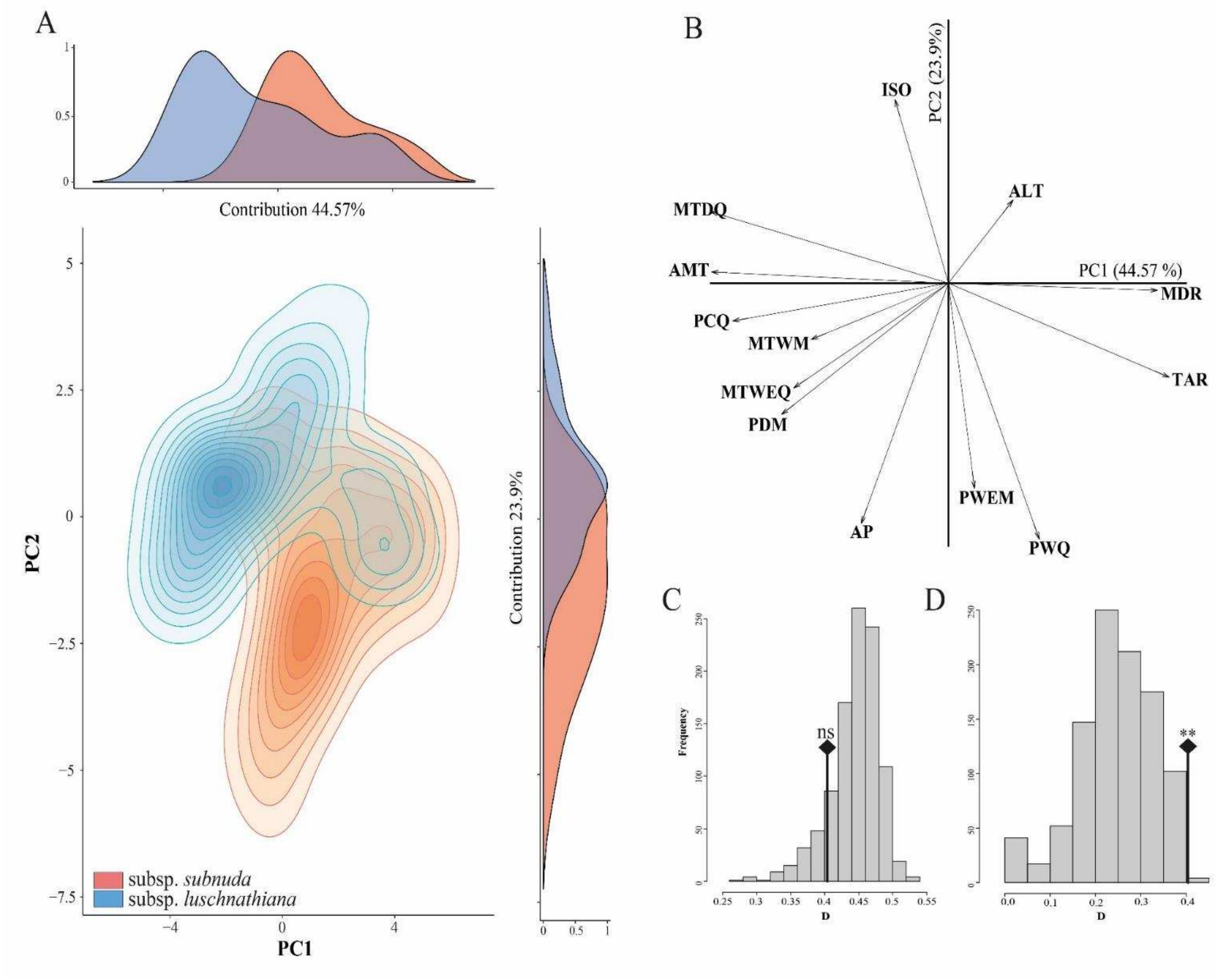


Figure 5. Niche overlap of the two subspecies of I. subnuda in the climatic space available in the BAF. A, Niche overlap of the two subspecies of I. subnuda along the two first axes of the PCA. B, Contribution of the climatic variables on the two axes of the PCA, ALT, altitude; AMT, annual mean temperature; MDR, mean diurnal range; ISO, isothermality; MTWM, max temperature of warmest month; TAR, temperature annual range; MTWEQ, mean temperature of wettest quarter; MTDQ, mean temperature of driest quarter; AP, annual precipitation; PWEM, precipitation of wettest month; PDM, precipitation of driest month; PWQ, precipitation of warmest quarter; PCQ, precipitation of coldest quarter. C, Test of niche equivalency calculated from 100 iterations. D, Test of niche similarity calculated from 100 iterations. The significant test is shown (ns, non-significant; **, $P < 0.01$).

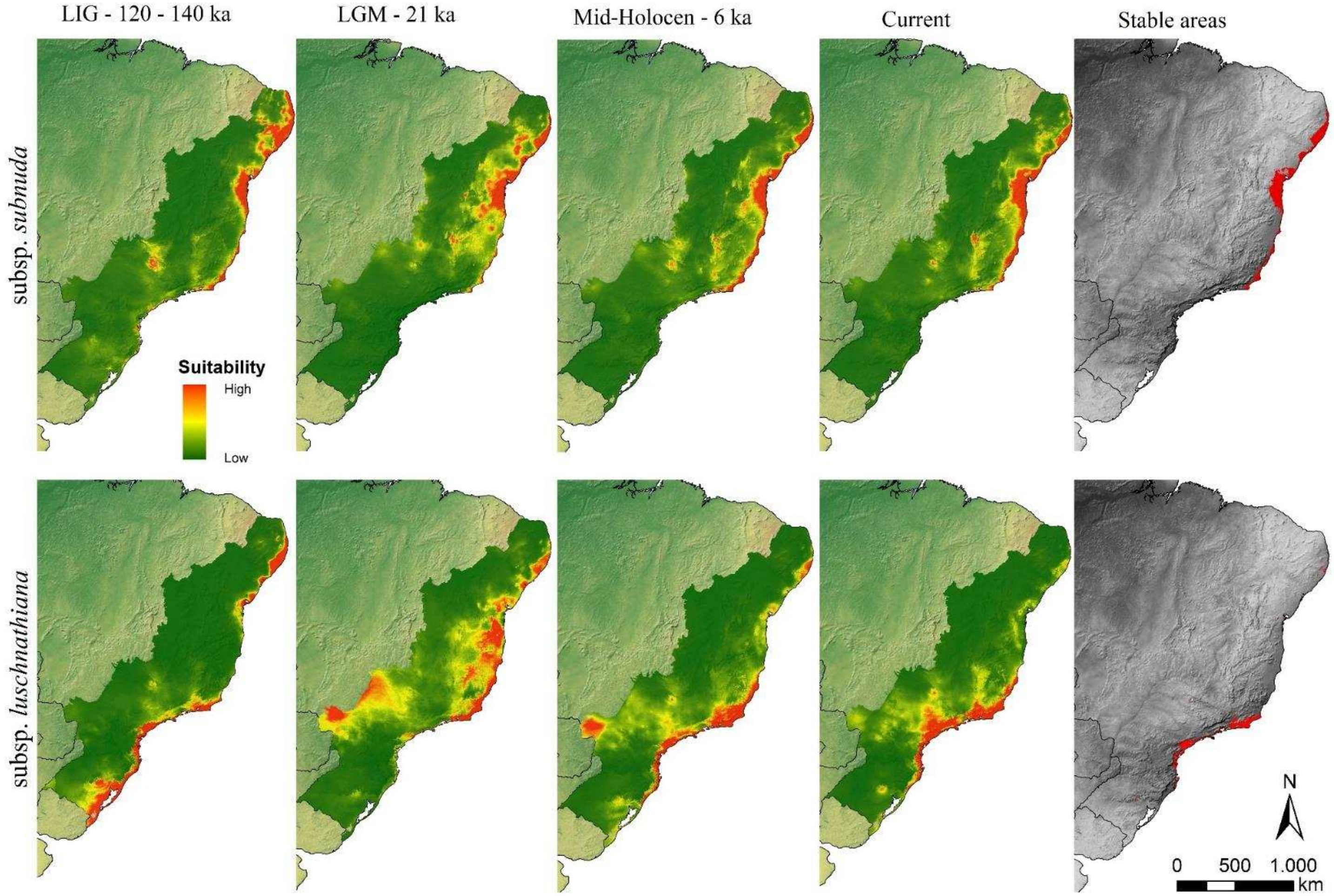


Figure 6. Predictions of suitable areas of occurrence of the two subspecies of I. subnuda under past and current environmental conditions [Last Interglacial (LIG, 120–140 kya), Last Glacial Maximum (LGM, 21 kya), Holocen (6 ka) and current (0 kya, pre-industrial)] and stable areas.

Table 1. Table of morphological traits measured for morphological analyses of the two subspecies of Inga subnuda Salzm. ex Benth.

Character	**Description**
LS	Length of stipules (mm)
WS	Width of stipules (mm)
LP	Length of petiole (mm)
LR	Length of rachis (mm)
WAW	Width apex wing (mm)
LPAIR*	Leaflets pairs
LTL	Length of terminal leaflet (mm)
WTL	Width of terminal leaflet (mm)
LBL	Length of basal leaflet (mm)
WBL	Width of basal leaflet (mm)
NSV*	Number of terminal leaflet secondary veins
NIA*	Number of inflorescence per axil
LOP	Length of peduncle (mm)
LFR	Length of floral rachis (mm)
LB	Length of bract (mm)
LFP	Length of flower pedicel (mm)
LK	Length of calix (mm)
LKL*	Length of calix lobes (mm)
WK	Width of calix (mm)
LC	Length of corolla (mm)
LCL*	Length of corolla lobes (mm)
LST	Length of stamens (mm)
LSTU*	Length of staminal tube (mm)
LSTY	Length of style (mm)
WLF*	Width of legume face (mm)

Traits indicated with * were not included in analyses because measurements were unvarying or scare.

Appendix S1

SUPPORTING INFORMATION

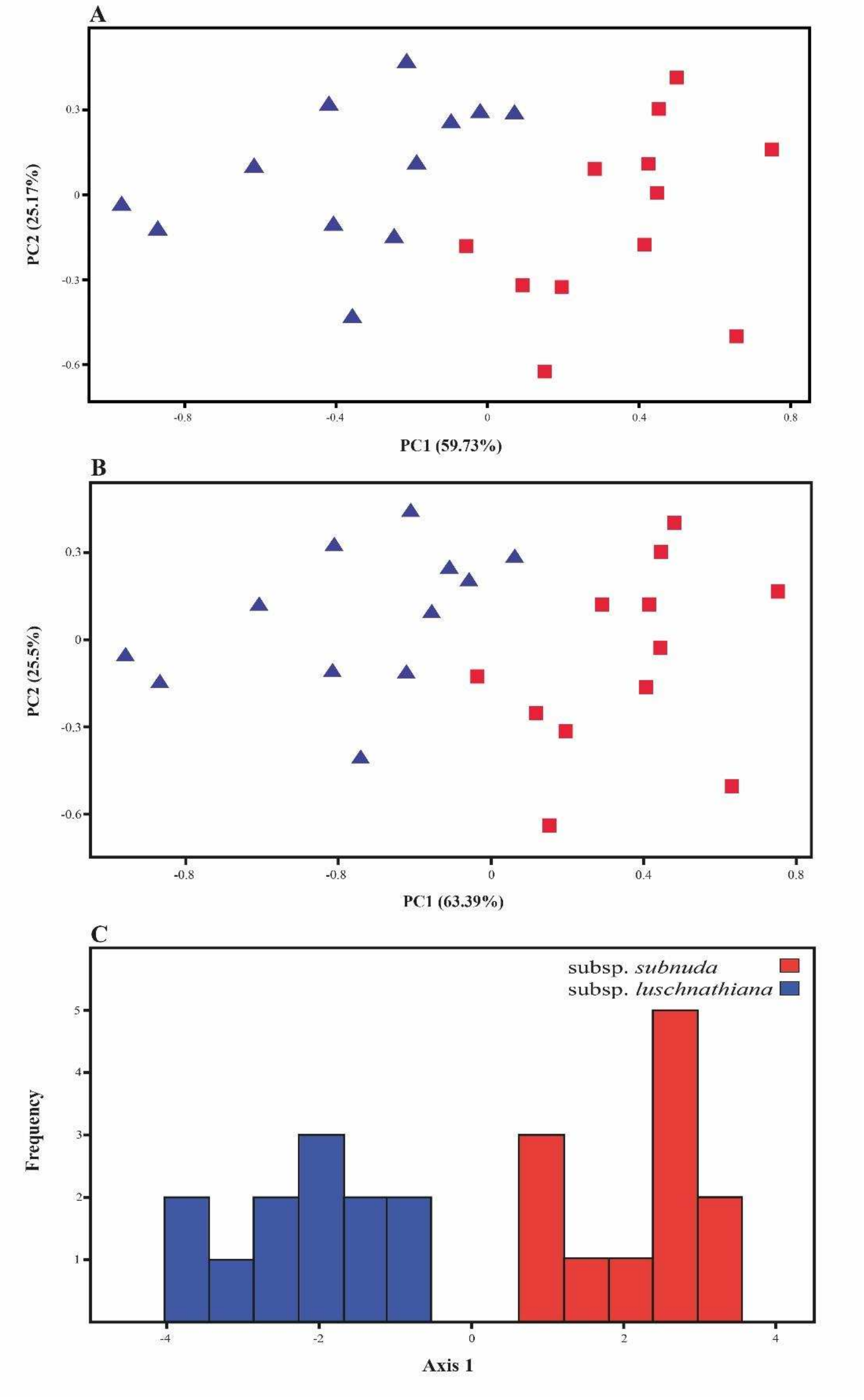
Table S1. Univariate analyses of both leaf and floral matrices for the 18 characters studied from 54 analysed specimens of the two subspecies of Inga subnuda. Leaf matrix: LS, WS, LP, WAW, LR, LTL, WTL, LBL and WBL. Floral Matrix: LOP, LFR, LB, LFP, LK, WK, LC, LST and LSTY. sub = subsp. subnuda. lus = subsp. luschnathiana.

Characters	Taxon	N	Min	Max	Mean	Variance	SD	Median
LS	sub	24	1.12	3.01	1.885417	0.2315824	0.4812301	1.835
	lus	21	1.34	3.42	2.36	0.40177	0.6338533	2.34
WS	sub	24	1.07	3.08	2.109167	0.2125471	0.4610283	2.05
	lus	21	1.62	3.09	2.438095	0.1467962	0.3831399	2.49
LP	sub	24	10.18	24.83	16.53583	18.93374	4.351292	15.93
	lus	21	10.97	25.07	17.69619	14.08684	3.753245	17.22
WAW	sub	24	0	3.74	2.173333	0.9355449	0.9672357	2.3
	lus	21	3.38	7.77	4.817619	1.200069	1.095477	4.7
LR	sub	24	36.07	139.77	84.51542	895.5264	29.92535	82.035
	lus	21	60.26	126.11	88.94381	365.645	19.12185	83.86
LTL	sub	24	66.5	153.44	106.8104	655.1781	25.59645	107.2
	lus	20	71.38	137.45	98.4525	350.791	18.72942	95.585
WTL	sub	24	19.37	80.64	51.51583	249.0476	15.78124	52.99
	lus	20	29.66	76.47	50.2315	162.8038	12.75946	48.155
LBL	sub	23	30.42	82.26	55.56478	227.1096	15.07016	55.73
	lus	20	24.11	78.45	50.8555	187.145	13.68009	51.785
WBL	sub	23	12.71	49.09	28.77	89.08329	9.438395	30.19
	lus	20	15.96	44.72	27.6725	56.49189	7.516109	27.62

Characters	Taxon	N	Min	Max	Mean	Variance	SD	Median
LOP	sub	12	6.45	50.23	26.08917	163.6646	12.79315	24.895
	lus	12	5.72	32.4	17.9425	89.81284	9.476964	16.775
LFR	sub	12	5.13	52.82	22.39917	188.834	13.74169	21.32
	lus	12	14.74	47.93	29.78417	114.2106	10.68694	30.155
LB	sub	11	0.95	2.72	1.856364	0.2958455	0.5439168	1.99
	lus	12	1.11	4.12	1.888333	0.6207424	0.7878721	1.685
LFP	sub	12	1.87	7.09	3.898333	2.053506	1.433006	3.87
	lus	12	0.19	1.65	0.8641667	0.175572	0.4190131	0.89
LK	sub	12	2.6	6.66	4.855	1.590227	1.261042	5.01
	lus	12	2.97	5.92	4.475	0.9864273	0.9931905	4.315
WK	sub	12	3.86	7.23	5.316667	0.9041152	0.9508497	5.17
	lus	12	3.71	5.95	4.630833	0.3768629	0.6138916	4.455
LC	sub	12	13.57	20.93	17.86	4.435636	2.106095	17.96
	lus	12	12.75	21.34	17.33	7.8098	2.794602	16.715
LST	sub	12	53.9	79.01	61.51583	44.50677	6.67134	59.09
	lus	11	37.73	61.99	51.94273	53.13632	7.289466	53.69
LSTY	sub	10	48.86	80.05	61.025	88.56512	9.410904	58.83
	lus	11	38.19	59.11	49.33	46.77568	6.839275	51.87

Appendix S2

Figure S1. Scattergrams for the principal component (PCA) and discriminant (DA) analyses carried out on floral characters with log transformation of the two subspecies of Inga subnuda. A, first two components of the PCA with overall characters (PCA 1 vs PCA 2); B, first two components of the PCA with only LOP, LFR, LFP, LK, WK and LC (PCA 1 vs PCA2); C, First axis from DA. The eigenvalue of each axis are provided in parentheses in the three cases. PCA symbols: 🟥 subsp. subnuda (red); ▲ subsp. luschnathiana (blue).



Appendix S2

Figure S2. Boxplot and results of t-test to the three leaf traits. Leaf traits: WAW = width apex wing; LP = length of petiole; LS = length of stipule.

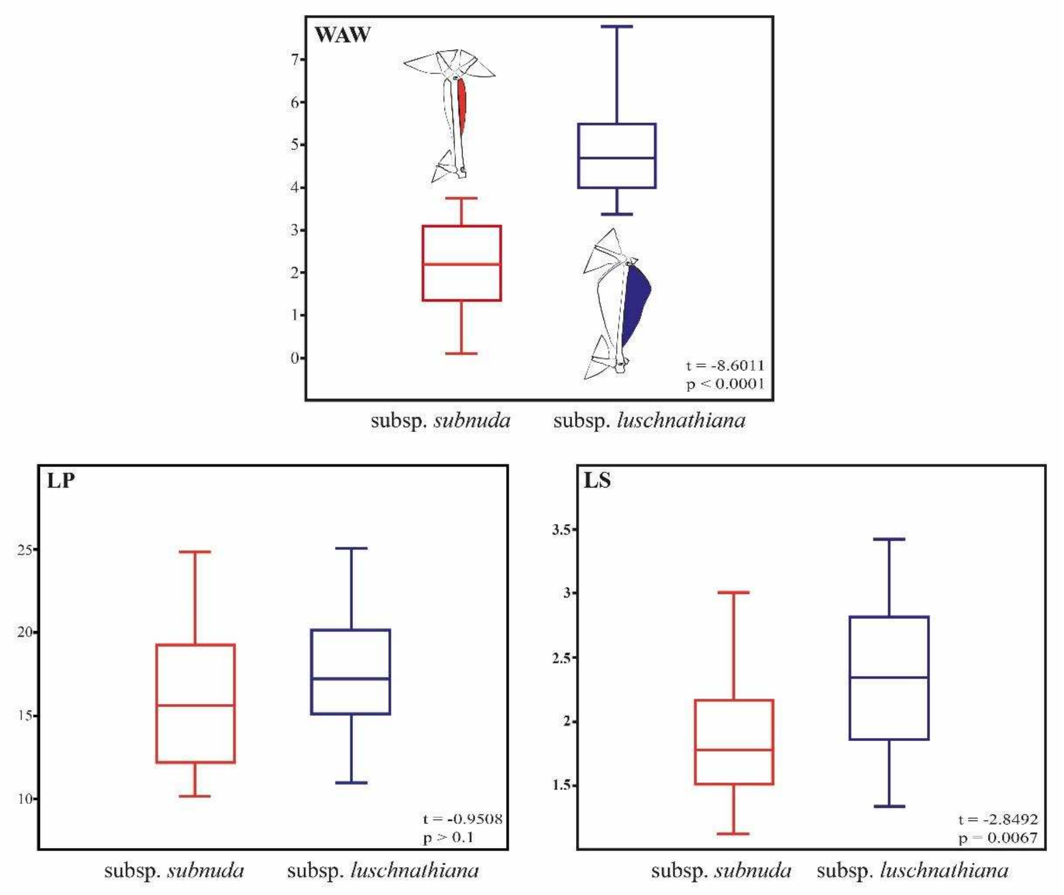
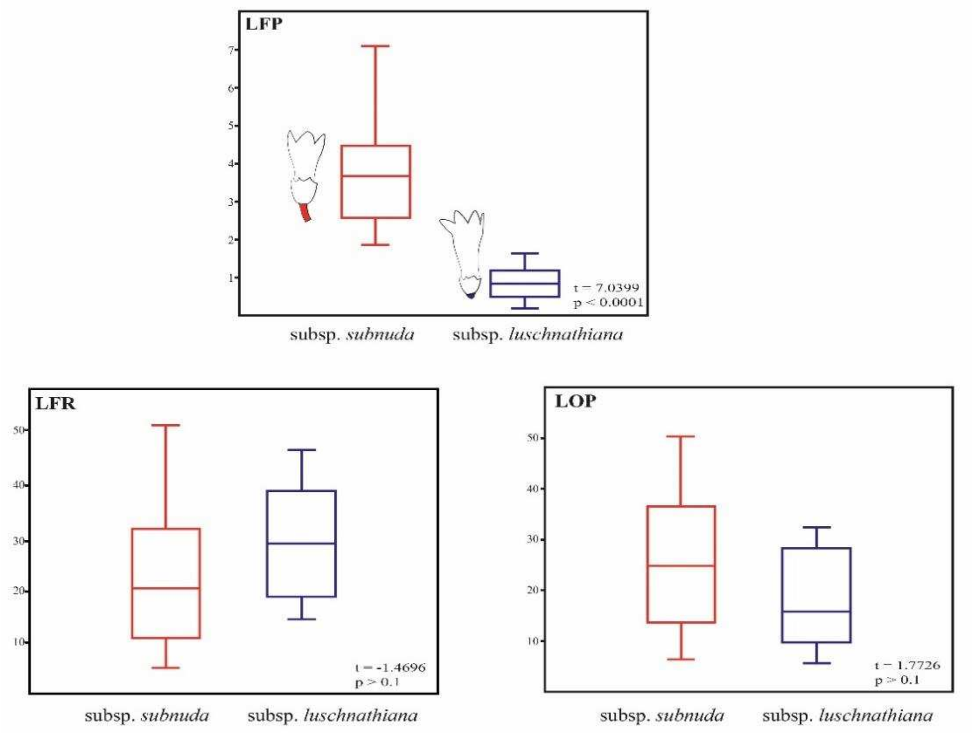


Figure S3. Boxplot and results of t-test to the three floral traits. Floral traits: LFP = length of flower pedicel; LFR = length of floral rachis; LOP = length of peduncle.



Appendix S3

Figure S1. Importance of altitude and climatic variables used for the definition of current potential distribution model of the two subspecies of I. subnuda; ALT, altitude; AMT, annual mean temperature; MDR, mean diurnal range; ISO, isothermality; MTWM, max temperature of warmest month; TAR, temperature annual range; MTWEQ, mean temperature of wettest quarter; MTDQ, mean temperature of driest quarter; AP, annual precipitation; PWEM, precipitation of wettest month; PDM, precipitation of driest month; PWQ, precipitation of warmest quarter; PCQ, precipitation of coldest quarter.

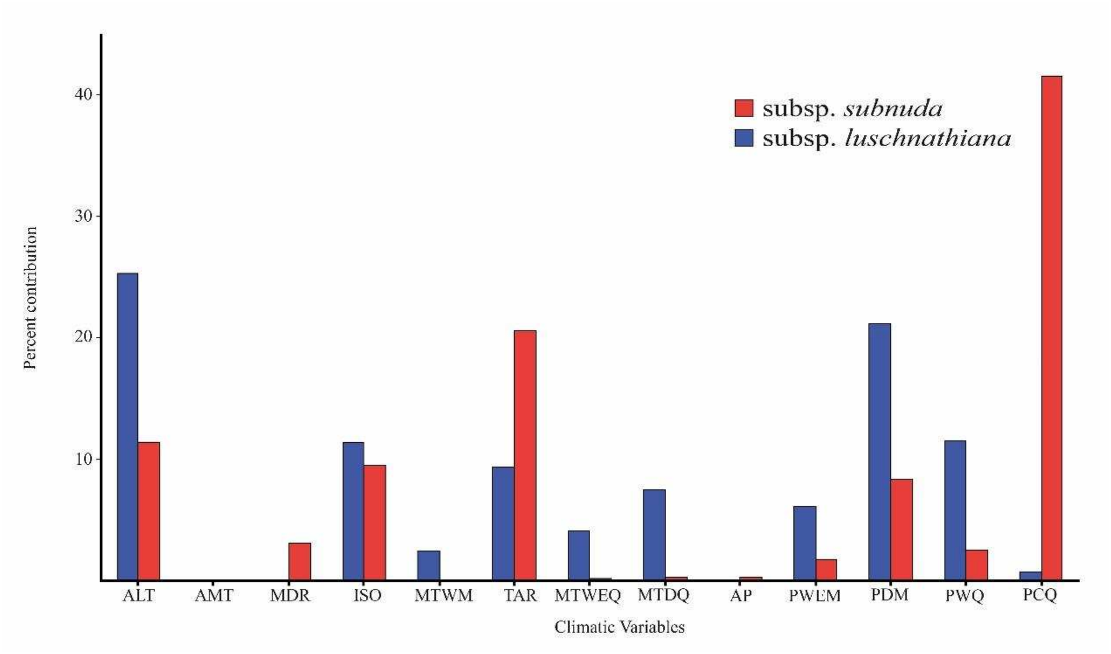
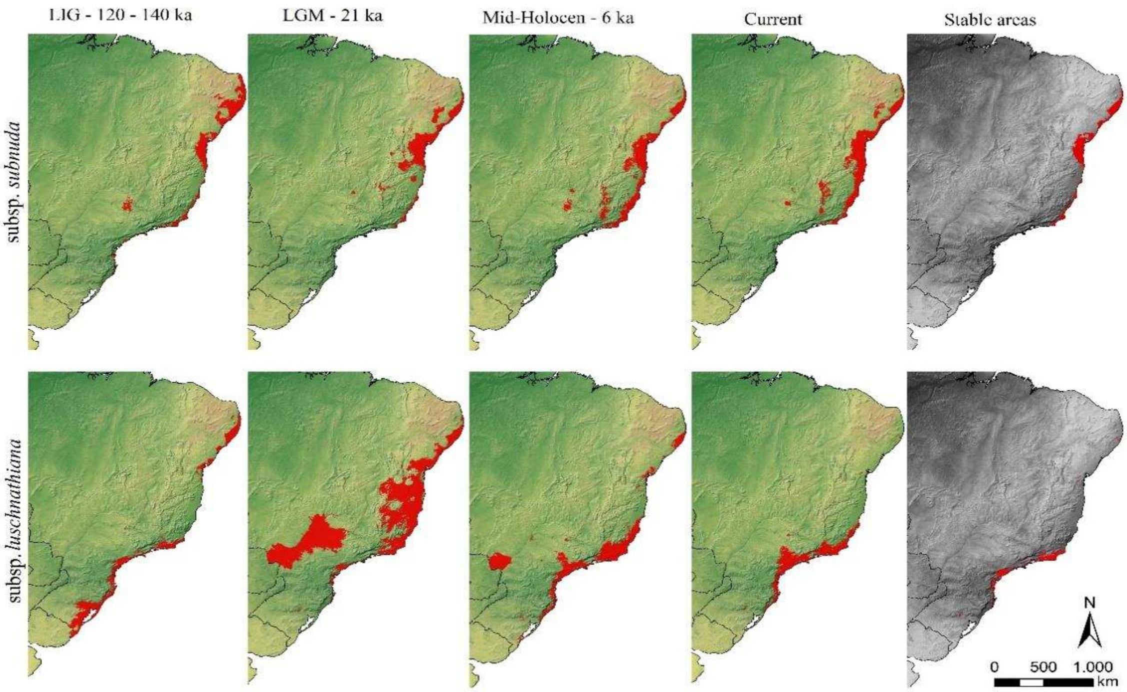


Figure S2. Predictions of suitable areas of occurrence of the two subspecies of I. subnuda under past and current environmental conditions [Last Interglacial (LIG, 120–140 kya), Last Glacial Maximum (LGM, 21 kya), Holocen (6 ka) and current (0 kya, pre-industrial)] and stable areas. Red areas show the final binary consensus model obtained from Maxent.



CONCLUSÕES GERAIS

Os estudos morfométricos e de modelagem de nicho ecológico em Inga subnuda resultantes desta dissertação revelaram novidades no seu tratamento taxonômico e ampliaram o conhecimento da sua distribuição atual e a sua relação com os eventos paleoclimáticos do quaternário na floresta atlântica.

Neste estudo, por meio da morfometria tradicional dois caracteres emergiram como estatisticamente significativos; o comprimento da ala do ápice e o comprimento do pedicelo da flor. Este é o primeiro estudo baseado em morfometria para avaliar os caracteres utilizados nos tratamentos taxonômicos anteriores, difere dos últimos tratamentos realizados por Peninngton e Garcia, mas corrobora o status inicial dado por Bentham.

Com o estudo de modelagem de nicho ecológico aqui conduzido, foi possível reconhecer divergência de nicho entre as duas subespécies de Inga subnuda, por conseguinte junto com os resultados da morfometria, resgatar o status de espécie para I. subnuda subsp. luschnathiana.

O presente estudo apresenta o novo tratamento taxonômico de Inga luschnathiana Benth. Assim como uma descrição taxonômica, dados de distribuição, fenologia e comentários adicionais.

Com o novo tratamento taxonômico, reconhecendo duas espécies, o presente estudo acrescentou o conhecimento da distribuição delas, assim como a sua relação com a dinâmica dos eventos climáticos do quaternário na floresta atlântica.

Os resultados de modelagem de nicho em relação ao passado, confirmam os estudos que mostram a dinâmica da floresta atlântica durante o quaternário e como tem influenciado na distribuição da flora e fauna presente nela, por isso um estudo filogeográfico para as duas espécies ajudaria a elucidar melhor a história das espécies assim como complementar o estudo realizado na presente dissertação.