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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

INSTITUTO DE BIOCIÊNCIAS – CÂMPUS BOTUCATU

PÓS – GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

Desvendando o mundo oculto de espécies da subfamília Conoderinae (Coleoptera: Curculionidae): história natural, aspectos morfológicos e filogeografia

Priscila Andre Sanz Veiga

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Orientador: Prof. Dr. Felipe W. Amorim

Coorientador: Prof. Dr. Alberto Soares Corrêa

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CAPÍTULO IV – Natural history and description of a new species of *Eulechriops* Faust, 1896 (Coleoptera: Curculionidae) associated with fruits of *Ouratea* spp. (Ochnaceae) in the Brazilian Cerrado

SANZ-VEIGA, P.A. **DESVENDANDO O MUNDO OCULTO DE ESPÉCIES DA SUBFAMÍLIA CONODERINAE (COLEOPTERA: CURCULIONIDAE): HISTÓRIA NATURAL, ASPECTOS MORFOLÓGICOS E FILOGEOGRAFIA**. 2022. 159p. TESE (DOUTORADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU, SÃO PAULO, BRASIL.

RESUMO – Dentre os fatores relacionados à radiação e evolução dos insetos fitófagos destacam-se os eventos históricos, características ambientais e a interação com as plantas hospedeiras. Assim, para compreender os mecanismos envolvidos na história evolutiva desses insetos precisamos adotar abordagens integrativas e multidisciplinares que envolvam os aspectos da dinâmica das interações com os hospedeiros em um contexto espaço-temporal. Neste trabalho, utilizamos uma abordagem integrativa, mediante o estudo de história natural, taxonomia, ecologia e filogeografia, com o objetivo de elucidar os principais fatores envolvidos na distribuição e diversificação de espécies de Conoderinae (Coleoptera: Curculionidae). No primeiro capítulo mostramos que o ciclo de vida e reprodução do predador de sementes *Hemicolpus abdominalis* Hustache, 1938 estão finamente sincronizados à fenologia reprodutiva da planta hospedeira, *Tocoyena formosa* (Rubiaceae). Embora a distribuição geográfica desse curculionídeo seja congruente com a ampla distribuição da planta no Cerrado, mudanças nas condições ambientais podem levar a desajustes fenológicos e afetar a sua distribuição. No segundo capítulo, integramos o estudo dos aspectos biológicos, morfológicos e genéticos para a delimitação de duas espécies de *Hemicolpus*. Espécimes amostrados no sul do Brasil, anteriormente classificados como *H. abdominalis*, representam uma nova espécie com distribuição restrita à porção sul da Mata Atlântica, cujas larvas se desenvolvem em frutos de outra espécie de Rubiaceae, *Randia ferox*. No terceiro capítulo, utilizamos uma abordagem filogeográfica para explorar o papel de mudanças ambientais históricas sobre a diversificação das duas espécies de *Hemicolpus* com distribuição contrastante. A diferenciação entre as espécies do Cerrado e Mata Atlântica foi datada durante o Mioceno. A espécie da Mata Atlântica apresentou menor diversidade genética e estabilidade populacional frente às condições climáticas pretéritas. Enquanto a espécie amplamente distribuída no Cerrado apresentou maior diversidade genética, estrutura filogeográfica marcada, com diferenciação entre as linhagens do Cerrado e da Caatinga estimada no pleistoceno. No Cerrado detectamos estrutura filogeográfica moderada em quatro províncias fitogeográficas, e expansão demográfica durante o período interglacial no pleistoceno. Sugerimos que as diferenças observadas refletem histórias divergentes moldadas por fatores abióticos e bióticos em conjunto. Destacamos a influência da distribuição da planta hospedeira e o bioma. No quarto capítulo descrevemos a história natural, e uma nova espécie do gênero *Eulechriops* Faust cujas larvas se desenvolvem dentro das estruturas reprodutivas de duas espécies de *Ouratea* (Ochnaceae) no Cerrado. Finalmente, destacamos a importância e necessidade de ampliarmos estudos sobre a distribuição e interação com as plantas hospedeiras, principalmente em gêneros de Conoderinae, a fim de compreendermos a história evolutiva dos curculionídeos.

Palavras-chave: Cerrados, história natural, *Eulechriops*, *Hemicolpus*, frutos, taxonomia

SANZ-VEIGA, P.A. **UNRAVELING THE HIDDEN WORLD OF SPECIES OF THE SUBFAMILY CONODERINAE (COLEOPTERA: CURCULIONIDAE): NATURAL HISTORY, MORPHOLOGICAL ASPECTS AND PHYLOGEOGRAPHY**. 2022. 159p. TESE (DOUTORADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU, SÃO PAULO, BRASIL.

ABSTRACT – Among the factors related to the radiation and evolution of phytophagous insects, historical events, environmental characteristics, and interaction with host plants stand out. Thus, to understand the mechanisms involved in the evolutionary history of such insects, we must adopt pluralistic, multidisciplinary and integrative approaches considering the dynamics of the interactions with their host plants in a spatio-temporal context. In this work we used an integrative approach, through the study of natural history, taxonomy, ecology and phytogeography to elucidate the main factors related to the distribution and diversification of species of Conoderinae (Coleoptera: Curculionidae). In the first chapter we highlight the importance of studying the insect life cycle and its association with the host plant. The life cycle and reproduction of the pre-dispersal seed-feeding weevil *Hemicolpus abdominalis* Hustache, 1938 is synchronized with the reproductive phenology of its host plant, *Tocoyena formosa* (Rubiaceae). Even though the insect's geographic distribution is largely congruent with the plant distribution in the Cerrado, changes in the environmental conditions can lead to phenological mismatch and, therefore, affect the weevil distribution. In the second chapter, we integrated the study of biological, morphological, and genetic aspects for the delimitation of two species within *Hemicolpus*. Specimens sampled in southern Brazil, previously classified as *H. abdominalis*, represent a new species of *Hemicolpus* restricted to the southern portion of the Atlantic Forest, associated with the fruits of another Rubiaceae, *Randia ferox*. In the third chapter, we followed a phylogeographic approach to explore the role of historical environmental changes on the diversification of the two species with contrasting distribution. The differentiation between the Atlantic Forest and the Cerrado species was dated to the Miocene. The Atlantic Forest species showed lower genetic diversity and population stability under past climate. The widely distributed species presented greater genetic diversity, and deep phylogeographic structure, with differentiation between the Cerrado and the northwest of the Caatinga estimated in the Pleistocene. In the Cerrado we detected moderate phylogeographic structure according to four phytogeographic provinces, and demographic expansion during the interglacial period in the Pleistocene. We suggest that the observed differences reflect divergent histories shaped by abiotic and biotic factors, highlighting the influence of host plant distributions and biome shifts. In the fourth chapter we explored the natural history and describe a new species of *Eulechriops* Faust whose larvae develop within the reproductive structures of two species of *Ouratea* (Ochnaceae) in the Cerrado. Finally, we highlight the importance and need to expand studies on the distribution and interaction with host plants, specifically within Conoderinae, to understand the evolutionary history of Curculionids.

Key words: Cerrados, natural history, *Eulechriops*, *Hemicolpus*, fruits, taxonomy

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ABSTRACT

The genus *Hemicolpus* Heller, 1895 (Curculionidae: Conoderinae) currently includes six species: *H. cubicus* (Lacordaire) (Brazil); *H. heteromorphus* Hustache (Brazil); *H. abdominalis* Hustache (Bolivia, Brazil, and Paraguay); *H. costaricensis* Hespenheide (Costa Rica); *H. randiae* Hespenheide (El Salvador and Mexico) and *H. prenai* Hespenheide (El Salvador and Mexico). The known species are predispersal seed predators whose larvae feed and develop within fruits of the Rubiaceae family. Species from Central America have been reared from the fruits of *Randia* L. (Rubiaceae). In contrast, the only host plant known for the South American species, *H. abdominalis*, is *Tocoyena formosa* (Cham. & Schltdl.) K. Schum. (Rubiaceae), a plant species widely distributed in the Cerrado biome, occurring from southeast to north and northeast of Brazil. Here, we describe a seventh species of *Hemicolpus*, *Hemicolpus* sp. 1, morphologically close to *H. abdominalis*, associated with fruits of *Randia ferox* (Cham. & Schltdl.) DC. in the south of Brazil. Furthermore, we designate a lectotype and provide a redescription of *H. abdominalis*, including additional characters to differentiate it from *Hemicolpus* sp. 1. For both species, we provide morphological descriptions of external and internal characters, including male and female genitalia illustrations, distribution data, and notes on the biology and host plant. A barcode region of the mitochondrial DNA is also included for both species adding genetic information to the species characterization and differentiation. We also provide an identification key for the species of the genus.

Keywords: Conoderinae, host plants, integrative taxonomy, America, *Randia ferox*, Rubiaceae, *Tocoyena formosa*, weevil.

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important contribution to the understanding of the evolutive aspects of Curculionids, one of the most diverse, though highly neglected insect group. Finally, we highlight the importance of considering biotic and ecological factors when studying the evolutionary history of specialized insects. Major historical shifts, such as between hosts and/ or biomes, can lead to lineage diversification and speciation, while regional factors, such as the phytogeographical provinces, can influence the intraspecific genetic diversity. Thus, by considering the biological and distributional aspects of host plants, one can better understand the phylogeographical patterns and demographic history of specialized insects. Furthermore, a next step would be to include a niche modeling approach to understand whether and how host plant demographical changes over time could have influenced the phylogeographic pattern and historical demography of these seed-feeding weevils.

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CONSIDERAÇÕES FINAIS

Embora a presente tese tenha abordado uma porção ínfima da biodiversidade, nossos resultados apresentam implicações interessantes para diversas áreas do conhecimento, como taxonomia, ecologia, evolução e conservação de insetos. Cabe lembrar que o atual trabalho surgiu como extensão de um projeto iniciado em 2014, quando buscávamos entender o efeito das interações multitróficas de insetos antagonistas e mutualistas sobre o êxito reprodutivo feminino (i.e. formação de frutos e sementes) de *Tocoyena formosa*. Além dos resultados inerentes ao objetivo do estudo, a informação gerada foi relevante para suscitar novas questões taxonômicas e evolutivas acerca do gênero *Hemicolpus* (Coleoptera: Curculionidae), abordadas na presente tese.

Assim, no primeiro capítulo buscamos conhecer os principais aspectos do ciclo de vida de *H. abdominalis* a fim de entender os principais fatores e mecanismos determinantes de sua distribuição espacial e temporal. Demonstramos que o ciclo de vida dessa espécie predadora de sementes pré-dispersão está sincronizado à fenologia da planta hospedeira de tal forma que a maturação reprodutiva dos adultos ocorre em sincronia ao início da floração e frutificação da planta, o desenvolvimento das formas imaturas ocorre durante o desenvolvimento e maturação dos frutos, e os adultos emergem dos frutos ao final da época de frutificação permanecendo em diapausa reprodutiva durante o período em que não há frutos disponíveis para sua reprodução.

Posteriormente, confirmamos que a ampla distribuição de *H. abdominalis* é congruente com a distribuição da planta hospedeira no bioma Cerrado. Apesar disso, os resultados da modelagem de nicho apontaram menor adequabilidade ambiental para sua ocorrência em regiões no interior da Caatinga. Sugerimos que questões climáticas, como elevadas temperaturas e início tardio da estação chuvosa, poderiam ocasionar desajuste fenológico entre o inseto e planta e, consequentemente, dificultar ou impedir o estabelecimento do inseto em certas regiões. Finalmente, destacamos a importância em considerar outros fatores bióticos, como os polinizadores, uma vez que a polinização e produção dos frutos em *T. formosa* é completamente dependente de mariposas da família Sphingidae (Lepidoptera), que por sua vez, também estão sujeitas a variações espaço-temporais.

No segundo Capítulo, com base no conhecimento da biologia e distribuição de *H. abdominalis* e em conjunto com o estudo detalhado das características morfológicas dos espécimes, averiguamos que exemplares de *Hemicolpus* amostrados na Mata Atlântica diferem quanto a morfologia e constituição genética da espécie do Cerrado. Além de utilizarem hospedeiros diferentes, as duas espécies de *Hemicolpus* também ocorrem em biomas diferentes. Logo, destacamos a importância em se conhecer aspectos da história natural, os hospedeiros, assim como sua distribuição geográfica a fim de garantir maior robustez na delimitação das espécies. Finalmente, integramos os aspectos da biologia, ecologia, caracterização genética e morfológica na descrição de uma nova espécie de *Hemicolpus* para o Brasil. Complementarmente, uma chave taxonômica incluindo as principais características que auxiliam na diferenciação das espécies de *Hemicolpus* descritas até o presente.

No terceiro capítulo exploramos, por meio de uma abordagem filogeográfica, a diversidade genética e a demografia histórica das duas espécies de *Hemicolpus* estudadas. Destacamos a relação entre a diversificação inter e intraespecífica e o bioma de origem, os fatores ecológicos e os eventos ambientais históricos. As espécies teriam se diferenciado durante o Mioceno, possivelmente a partir de um ancestral comum na Mata Atlântica. A espécie com maior distribuição, *H. abdominalis*, está subdividida em duas linhagens, Cerrado e Caatinga, cuja diversificação teria ocorrido cerca de um a dois milhões de anos atrás durante o pleistoceno. Essa quebra genética estaria relacionada ao isolamento geográfico entre populações e, possivelmente, a mudança de biomas. Inclusive, levantando a possibilidade da presença de um ecótipo na Caatinga. Dentro do Cerrado, parte da diversidade genética de *H. abdominalis* está estruturada de acordo com as quatro províncias fitogeográficas, evidenciado certa limitação ao fluxo gênico, embora menos expressiva, também em um contexto regional. Ainda no Cerrado, o período de expansão demográfica de *H. abdominalis* foi congruente com o período de maior expansão da vegetação do Cerrado. Para a espécie da Mata Atlântica os padrões de diversidade genética parecem refletir o menor tamanho populacional ou distribuição restrita da população do inseto e da planta hospedeira. Finalmente, concluímos que as diferenças observadas para as duas espécies refletem histórias divergentes, moldadas pelo conjunto de fatores abióticos e bióticos, eventos históricos, distribuição da planta hospedeira, e fatores ecológicos relacionados ao bioma.

No quarto Capítulo descrevemos uma nova espécie do gênero *Eulechriops* para o Brasil. Estudamos os aspectos morfológicos e biológicos de espécimes cujas larvas se desenvolvem em frutos de *Ouratea* spp. (Ochnaceae) no Cerrado. Apesar da associação com os frutos, as larvas se desenvolvem em uma região espacialmente separada das sementes e não causam danos visíveis ao embrião. Destacamos a necessidade e a importância de expandir as amostragens para outras espécies de *Ouratea* em diferentes localidades geográficas a fim de ampliarmos o conhecimento acerca desse gênero de curculionídeo.

Esse estudo representa um exemplo prático de como a elucidação da associação com os hospedeiros pode fornecer material para outras áreas de estudo, proporcionando o preenchimento de lacunas acerca da diversificação, relações filogenéticas e evolução dos insetos. Finalmente, destacamos a importância do conhecimento dos aspectos básicos da história de vida e ecologia dos organismos, o que nos permite fazer inferências mais amplas e profundas sobre outros aspectos, como distribuição e evolução. Em grupos altamente especializados, cuja reprodução depende exclusivamente da associação com plantas, a compreensão dos mecanismos envolvidos na sua evolução e diversificação requer abordagens plurais, integradas e mutildisciplinares que envolvam diferentes aspectos da dinâmica das interações com os hospedeiros em um contexto espacial e temporal.