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Molecular species delimitation of leaf beetle species (Coleoptera: Chrysomelidae) in the cloud forests of southern Ecuador

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Dedication

I dedicate this work to my family who have been a constant source of support and motivation in helping me discover at its core, something that often goes unnoticed at first glance: the leaf beetles.

I also dedicate this work to all those, from various fields, who are committed to the conservation of the diverse ecosystems of our country, Ecuador.

Acknowledgments

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Finally, I extend a special thank you to my family and friends, who were my main source of motivation throughout this process. Their love, patience, and sincere support were always present, even during the most challenging moments, reminding me that this achievement is also the result of a shared effort.

Contents

List of Tables

Figure legends

Figure 1. Study area and sampling localities in the southern Andes of Ecuador. The map displays the distribution of the Tropical Montane Cloud Forest (TMCF) along the eastern mountain range. Sampling points for the four localities are illustrated: (i) Sumak Kawsay In Situ Reserve (SKIS) (blue dot); (ii) Sangay National Park (PNS) (green dot); (iii) Río Negro-Sopladora National Park (PNRNS) (black dot); and (iv) Tinajillas-Río Gualaceño Municipal Conservation Area (TJ) (orange dot). The TMCF shapefile was obtained from the Ministerio del Ambiente y Energía (http://ide.ambiente.gob.ec:8080/mapainteractivo/). The location of Ecuador within South America is highlighted in yellow..47

Figure 2. Nucleotide composition of the cox1 gene across Chrysomelidae subfamilies. The bar chart illustrates the relative composition (%) of Thymine (green), Cytosine (blue), Adenine (red), and Guanine (yellow) for each taxonomic group. The "general" profile represents the global mean calculated from the 194 sequences used for phylogenetic reconstruction. A marked A + T bias (~66.2% on average) is evident across all subfamilies, with Thymine being the most frequent nucleotide (range: 33.5%–38.9%), a characteristic feature of mitochondrial markers in Coleoptera..48

Figure 3. Bayesian phylogenetic tree of Ecuadorian Chrysomelidae and comparative species delimitation. The tree was inferred using *cox1* mitochondrial sequences in BEAST. Phylogenetic relationships with node labels representing Bayesian posterior probabilities (>0.80). To the right, vertical bars represent the results of four delimitation approaches: Column 1 (MSP) shows preliminary morphospecies assignments, where shared colors highlight cases of split lineages (potential cryptic diversity). Columns 2-4 depict Molecular Operational Taxonomic Units (MOTUs) inferred by PTP, bPTP, and GMYC models, respectively, with each individual block representing a delimited unit. Yellow blocks indicate incongruences between the phylogenetic topology and the molecular delimitation results. Representative thumbnail photos illustrate the phenotypic diversity of the subfamilies identified across the study sites. Due to the tree's extent, the phylogeny is presented in sequential sections.. 49

Figure 4. Individual-based rarefaction and extrapolation curves for Chrysomelidae MOTUs across the four study localities. Solid lines represent the observed species accumulation (rarefaction), while dashed lines indicate the extrapolation up to double the size of the smallest sample. Shaded areas denote the 95% confidence intervals derived from 1,000 bootstrap replicates. The end points for each curve correspond to the observed richness (S_{obs}) for Sumak Kawsay In Situ (SKIS), Sangay National Park (PNS), Tinajillas-Río Gualaceño (TJ), and Río Negro-Sopladora (PNRNS). The lack of an asymptotic plateau across all sites indicates that the sampling effort has not yet fully captured the total molecular diversity, highlighting a significant proportion of rare or undetected genetic lineages in these Andean cloud forest assemblages..52

Figure 5. Spatial patterns and beta diversity partitioning of Chrysomelidae assemblages. a) Map of the four study localities in the southern Ecuadorian Andes, including the pairwise linear distances (km). b) Hierarchical clustering dendrogram based on the Sørensen dissimilarity index (β_{SOR}). The total beta diversity is partitioned into its two components: species turnover (β_{SIM}) and nestedness-resultant dissimilarity (β_{SNE}). The high total

Supplementary Index

MOLECULAR DELIMITATION OF LEAF BEETLE SPECIES (COLEOPTERA: CHRYSOMELIDAE) IN THE CLOUD FORESTS OF SOUTHERN ECUADOR

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Abstract

Biodiversity in the tropics exceeds that of any other region due to complex biotic and abiotic interactions that drive diversification. In Ecuador, this hyper-diversity -particularly among insects- poses significant taxonomic and ecological challenges, highlighting the need to apply rapid assessment methods that complement traditional frameworks. This study provides the first molecular assessment of leaf beetles (Coleoptera: Chrysomelidae) in the Tropical Montane Cloud Forests (TMCFs) in southern Ecuador. By integrating morphological and molecular approaches, we characterized assemblages across four key localities: Río Negro-Sopladora National Park, Sangay National Park, Sumak Kawsay In Situ Reserve, and Tinajillas–Río Gualaceño Municipal Conservation Area. A total of 824 individuals (212 morphospecies) were recorded, yielding 194 *cox1* sequences analyzed through standardized DNA barcoding protocols. Molecular species delimitation (PTP, bPTP, and GMYC) identified between 137 and 170 molecular operational taxonomic units (MOTUs), consistently exceeding morphological estimates and evidencing significant cryptic diversity, especially within the subfamily Galerucinae. Alpha diversity analyses revealed the highest observed richness in Sumak Kawsay and Sangay, while non-parametric estimators suggest a high proportion of undetected taxa across all sites. Regional beta diversity was overwhelmingly driven by species turnover ($\beta_{SIM} \approx 0.97$), indicating minimal species sharing even between geographically proximate localities. This pattern of extreme spatial heterogeneity suggests that topographic barriers in the Andes promote high local endemism. This work established the first DNA barcode library for Chrysomelidae in the cloud forests of southern Ecuador, providing a fundamental tool for monitoring and conserving these fragile ecosystems. Our results demonstrate that integrating molecular tools with traditional taxonomy is essential for uncovering hidden biodiversity and informs a "complementary-based" conservation strategy for the Andes region.

Key words: Chrysomelidae, cryptic diversity, Tropical Montane Cloud Forest, species turnover, DNA barcoding

Resumen

La biodiversidad en los países tropicales supera ampliamente a la de otras regiones del mundo debido a la interacción de múltiples factores bióticos y abióticos que generan condiciones excepcionales para la diversificación. En Ecuador, esta riqueza -particularmente notable en insectos- presenta desafíos importantes para comprender su biología, taxonomía, ecología e interacciones, lo que evidencia la necesidad de aplicar métodos de evaluación rápidos que complementen a la taxonomía tradicional. En este contexto, este estudio constituye la primera evaluación molecular de los escarabajos de las hojas (Coleoptera: Chrysomelidae) en remanentes de Bosques Nublados Montanos Tropicales (BNTMs) del sur de Ecuador. Se integraron enfoques morfológicos y moleculares para caracterizar su biodiversidad mediante la colecta de individuos en cuatro localidades clave: Parque Nacional Río Negro-Sopladora, Parque Nacional Sangay, Reserva Sumak Kawsay In Situ y el Área de Conservación Municipal Tinajillas–Río Gualaceño. Se registraron en total 824 individuos correspondientes a 212 morfoespecies, lo que permitió obtener 194 secuencias *cox1* siguiendo protocolos estandarizados de código de barras de ADN. La delimitación molecular de especies (PTP, bPTP y GMYC) infirió entre 137 y 170 unidades taxonómicas operativas moleculares (MOTUs), superando las estimaciones morfológicas y evidenciando diversidad críptica, especialmente en la subfamilia Galerucinae. Los análisis de diversidad alfa mostraron mayor riqueza en Sangay y Río Negro-Sopladora, mientras que las estimaciones de riqueza potencial sugieren que aún queda un número importante de especies por detectar en todas las localidades. La diversidad beta regional se vio determinada mayoritariamente por el recambio de especies ($\beta_{SIM} \approx 0.97$), lo que indica que existiría un intercambio mínimo de especies incluso entre localidades geográficamente próximas. Este patrón de extrema heterogeneidad espacial sugiere que las barreras topográficas de los Andes favorecen un alto nivel de endemismo local. Este trabajo ha creado la primera biblioteca de códigos de barras de ADN para los Chrysomelidae de los bosques nubosos del sur de Ecuador, lo que proporciona una herramienta fundamental para el seguimiento y la conservación de estos frágiles ecosistemas. Nuestros resultados demuestran que la integración de herramientas moleculares con la taxonomía tradicional es esencial para descubrir la biodiversidad oculta y sirve de base para una estrategia de conservación complementaria para la región andina.

Palabras clave: Chrysomelidae, biodiversidad, Coleoptera, métodos moleculares, códigos de barras de ADN.

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