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MAESTRÍA EN RECURSOS NATURALES
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**Molecular species delimitation of leaf beetle species (Coleoptera:
Chrysomelidae) in the cloud forests of southern Ecuador**

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Nombre del autor:

Samara Elizabeth Zeas Bermeo

Nombre de la Directora:

Gissela Nathali De la Cadena Mendoza

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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.

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

Samara E. Zeas-Bermeo ^{1,3}
&
Gissela N. De la Cadena Mendoza ^{2,3}

¹Maestría en Recursos Naturales Renovables, Universidad del Azuay

²Escuela de Biología, Universidad del Azuay

³Laboratorio de Entomología, Museo de Zoología MZUA, Universidad del Azuay

Av. 24 de mayo 7-77 y Hernán Malo, Cuenca, Ecuador.

samarazeas@es.uazuay.edu.ec
gissela.delacadena@uazuay.edu.ec

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.