

**FONDO DE INVESTIGACIONES DE LA  
UNIVERSIDAD DEL ROSARIO (FONDO CONCURSABLE)**

**INFORME FINAL PROYECTOS DE INVESTIGACIÓN**

**27 de noviembre 2023.**

**ESTRUCTURA DEL INFORME**

**1. Información General del Proyecto**

- Nombre del Proyecto: ¿Representa la diversidad de colores en las mariposas una señal honesta de su toxicidad?
- Grupo(s) de Investigación: Filoevómica
- Línea de Investigación: Genómica y Genética de Poblaciones y filogeografía del género *Heliconius*.
- No. del proyecto: IV-FPD004/IV-FGV021
- Investigador principal:** Camilo Andrés Salazar Clavijo
- Correo electrónico:** camilo.salazar@urosario.edu.co
- Unidad académica a la que pertenece:** Facultad de Ciencias Naturales

## **Resumen**

A continuación, se presenta el informe final del proyecto de investigación “¿Representa la diversidad de colores en las mariposas una señal honesta de su toxicidad?”. Las actividades descritas corresponden a (1) adquisición de los datos de toxicidad de 9 anillos miméticos en distintas zonas geográficas de Colombia, (2) análisis e interpretación de resultados del espectro de toxicidad (3) toma de medidas de coloración de 418 pares aleatorios que contrastan los diferentes elementos del patrón de coloración de 58 individuos para 11 anillos miméticos bajo varios tratamientos (4) resultados de la evaluación de los contrastes de coloración y percepción de señales visuales por depredadores y (5) Evaluación de la premisa de señal honesta del patrón respecto al grado de toxicidad para los casos estudiados en (2), (3) y (4). Se adjunta un primer manuscrito a ser sometido a revista internacional. Se encontró que la variación del perfil cianogénico de *Heliconius* no está explicada por el anillo mimético en el que las especies participan, tampoco por su ubicación geográfica. En lugar de esto la variación en toxicidad es explicada por cercanía filogenética (ancestría común) y seguramente por factores ecológicos tales como especialización en plantas hospederas, diversidad de estas, presencia de precursores para la biosíntesis de compuestos cianogénicos en polen y variación en la comunidad de predadores.

- Palabras claves: *Heliconius*, mimétismo, diversificación, glicósidos cianogénicos, coloración.
- Fecha de iniciación efectiva: 1/12/2022.

## **2. Objetivos del proyecto**

### **Objetivo general:**

Cuantificar la toxicidad y coloración de diferentes complejos miméticos/aposemáticos en *Heliconius* para estudiar cómo las señales visuales de las mariposas reflejan dicha toxicidad y cómo estos dos elementos contribuyen a la evolución del mimetismo.

### **Objetivos específicos**

1. Cuantificar los patrones de toxicidad de los cuatro principales anillos miméticos en *Heliconius* a saber, postman, dennis/ray, blanco/amarillo y atigrado, evaluando si existen diferencias intra e interespecíficas.

2. Establecer, mediante modelación de visión específica de un depredador, cuál de los anillos miméticos y de las especies que los conforman son más conspicuos para las aves.
3. Evaluar la relación entre la conspicuidad y la toxicidad de cada uno de los anillos miméticos y, determinar si la señal de coloración es cuantitativamente honesta.

### 3. Cronograma de actividades

Cronograma				
Número	Actividad	Desde	Hasta	Tiempo
1	Cuantificación del color	1 agosto 2022	30 octubre 2022	3 meses
2	Mapeo de las imágenes a los modelos de visión	1 noviembre 2022	30 enero 2023	3 meses
3	Análisis estadísticos	1 febrero 2023	30 abril 2023	3 meses
4	Redacción de manuscrito	1 mayo 2023	30 julio 2023	3 meses

**Tabla 1.** Cronograma sometido en la propuesta, en donde se expone los tiempos que se estimaron para cumplir con un trabajo de esta envergadura, a pesar de que se hubiera iniciado tarde (ver fecha de inicio) y de que se esté pidiendo informe final para el 1/12/2022.

### 4. Resultados obtenidos

Indique los resultados alcanzados, justificándolos o explicando las razones por las cuales los resultados son o no satisfactorios, de acuerdo con los objetivos planteados.

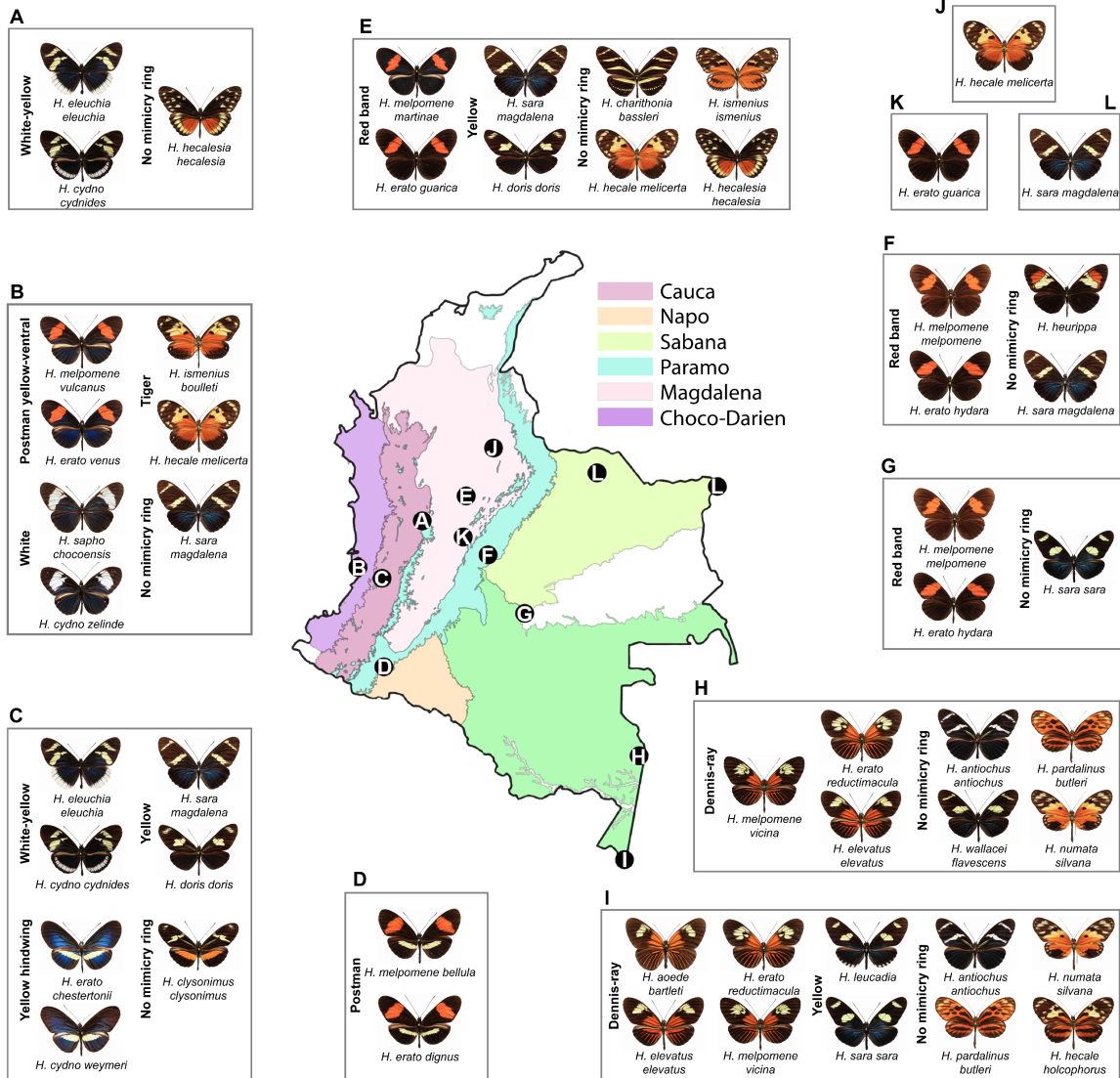
Resultados esperados	Resultados entregados
Productos de nuevo conocimiento científico o desarrollos tecnológico esperados	
<b>Compromiso:</b> un artículo científico	<p><b>Resultado:</b> N. Rueda-M. et al. (2024). Chemical defense variation in Heliconius butterflies: testing the role of mimicry rings and ecoregions (<b>Anexo 1</b>).</p> <p><b>Estado:</b> satisfactorio. Se generó el manuscrito a ser sometido a publicación, una vez las revisiones de los coinvestigadores del presente proyecto (Mónica Arias y Erika de Castro) sean incorporadas al mismo (<b>Anexo 1</b>). El set de datos da para más publicaciones y estas se irán sacando a medida que se terminen los análisis de datos y se escriban los manuscritos.</p>

Conducentes al fortalecimiento de la capacidad científica nacional	
<p><b>Compromiso:</b> una tesis de pregrado</p>	<p><b>Resultado:</b> se entregó una tesis de doctorado, en la cual los resultados del presente proyecto constituyen el capítulo 3 de dicha tesis. Documento en CRAI:  <a href="https://repository.urosario.edu.co/handle/10336/40996">https://repository.urosario.edu.co/handle/10336/40996</a>  <a href="https://doi.org/10.48713/10336_40996">https://doi.org/10.48713/10336_40996</a></p> <p><b>Estado:</b> satisfactorio, los resultados obtenidos acá, permitieron aportar a un nivel más alto de formación.</p>
Dirigidos a la apropiación social del conocimiento	
<p><b>Compromiso 1:</b> Una presentación oral o poster en congresos científicos</p> <p><b>Compromiso 2:</b> Informes técnicos y/o de investigación</p> <p><b>Compromiso 3:</b> Publicación editorial no especializada. Por ejemplo Boletín de la sociedad colombiana (COLEVOL) y Nova et Vetera.</p>	<p><b>Resultados para compromiso 1:</b></p> <ol style="list-style-type: none"> <li>1. Presentación de parte del proyecto en la conferencia internacional BG22 (<b>Anexo 2</b>).</li> <li>2. Presentación en el congreso nacional: VI congreso colombiano de zoología. (<b>Anexo 3</b>).</li> <li>3. Presentación de parte del Proyecto en el Congress of the European Society for Evolutionary Biology (<b>Anexo 4</b>).</li> <li>4. XXIII International congress of genetics and genomics. Magistral presentada por Dr. Carolina Pardo, del grupo de investigación (<b>Anexo 5</b>).</li> </ol> <p><b>Estado:</b> satisfactorio, los resultados obtenidos acá en distintos estados de desarrollo han sido incluidos/presentados en cada una de estas conferencias.</p> <p><b>Resultados para compromiso 2:</b></p> <p>El presente informe final, se constituye en el resultado de este compromiso.</p> <p><b>Estado:</b> satisfactorio, este informe está siendo entregado conforme a lo requerido.</p> <p><b>Resultados para compromiso 3:</b></p> <p>En lugar de métodos de divulgación escritos de menor difusión se optó por divulgación masiva en medios digitales. De esta forma se tiene:</p> <ol style="list-style-type: none"> <li>1. Espacio televisivo Ruta país Rosario: emitido en el canal 1 y disponible en Youtube a partir del minuto 13:  <a href="https://www.youtube.com/watch?v=-1vrHmxMWQk&amp;t=588s">https://www.youtube.com/watch?v=-1vrHmxMWQk&amp;t=588s</a></li> </ol>

	<p>2. Defensa de doctorado de Nicol Rueda (personal vinculada al proyecto y al grupo de investigación) en donde se expone los resultados de este proyecto en su capítulo 3:  <a href="https://www.youtube.com/watch?v=KgPoxxmsYYI">https://www.youtube.com/watch?v=KgPoxxmsYYI</a></p> <p>3. Presentación en el seminario internacional de lepidópteros (Lepinar) de parte de los resultados encontrados acá en la conferencia de la Dr. Erika de Castro titulada : The relationships between butterflies and their food plants: from coevolutionary enemies to best friends. Segunda en el listado de charlas 2023 en : <a href="https://www.heliconius.org/lockdown-seminars/">https://www.heliconius.org/lockdown-seminars/</a></p> <p><b>Estado:</b> satisfactorio, en los tres casos se tuvieron más de 200 vistas o asistentes que pudieron ver la investigación que hace el grupo y en particular resultados asociados/obtenidos en el presente proyecto.</p>
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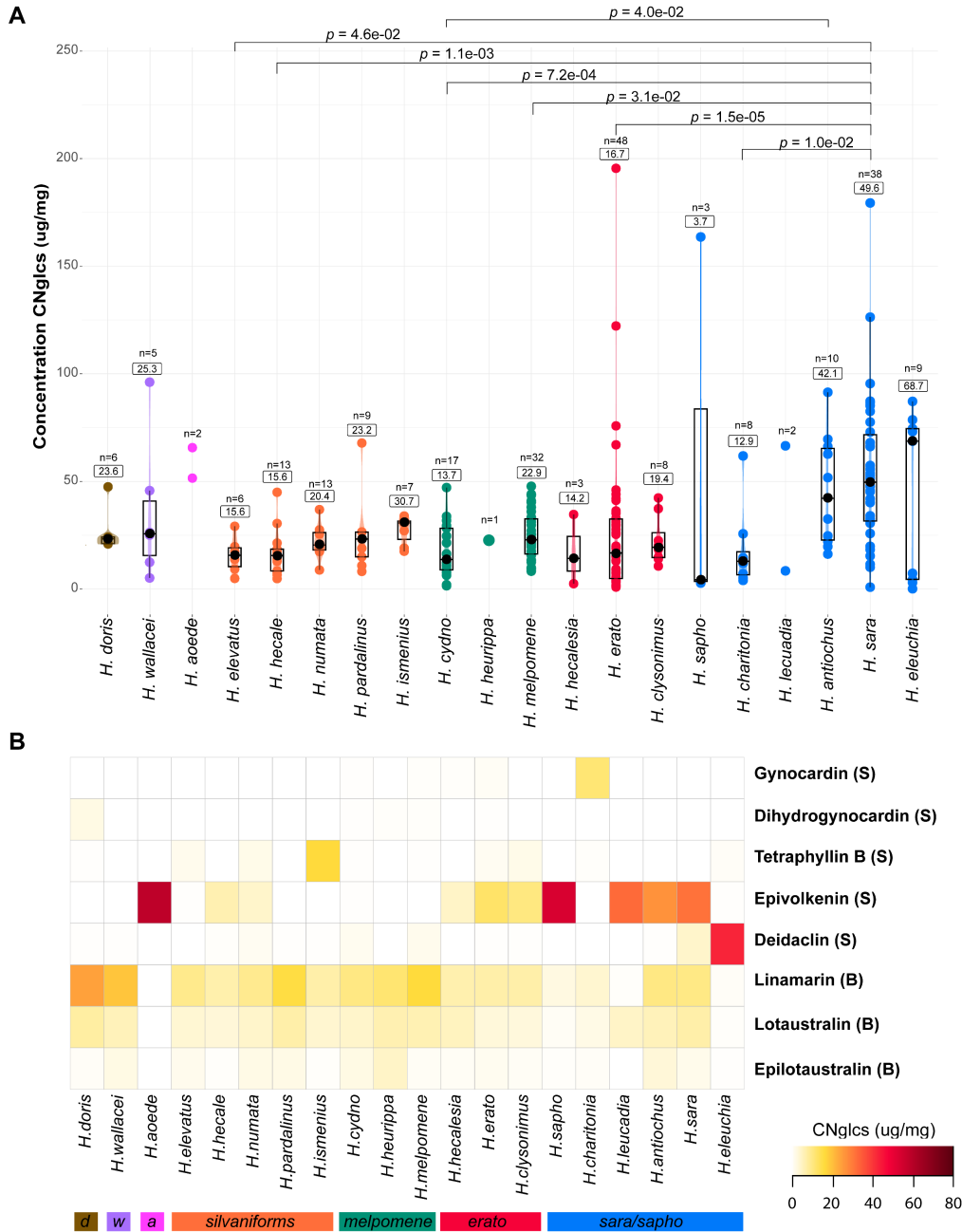
**Breve descripción de resultados científicos asociados al cumplimiento de cada objetivo.**

**Objetivo 1, numerales 1 y 2 en el resumen:** se procesaron 240 individuos de 20 especies de mariposas *Heliconius* en Colombia (**Figura 1**). Estos extractos fueron corridos en espectrometría de masas y se usaron estándares de glicósidos cianogénicos conocidos para identificar la composición y concentración de estos compuestos en las muestras.



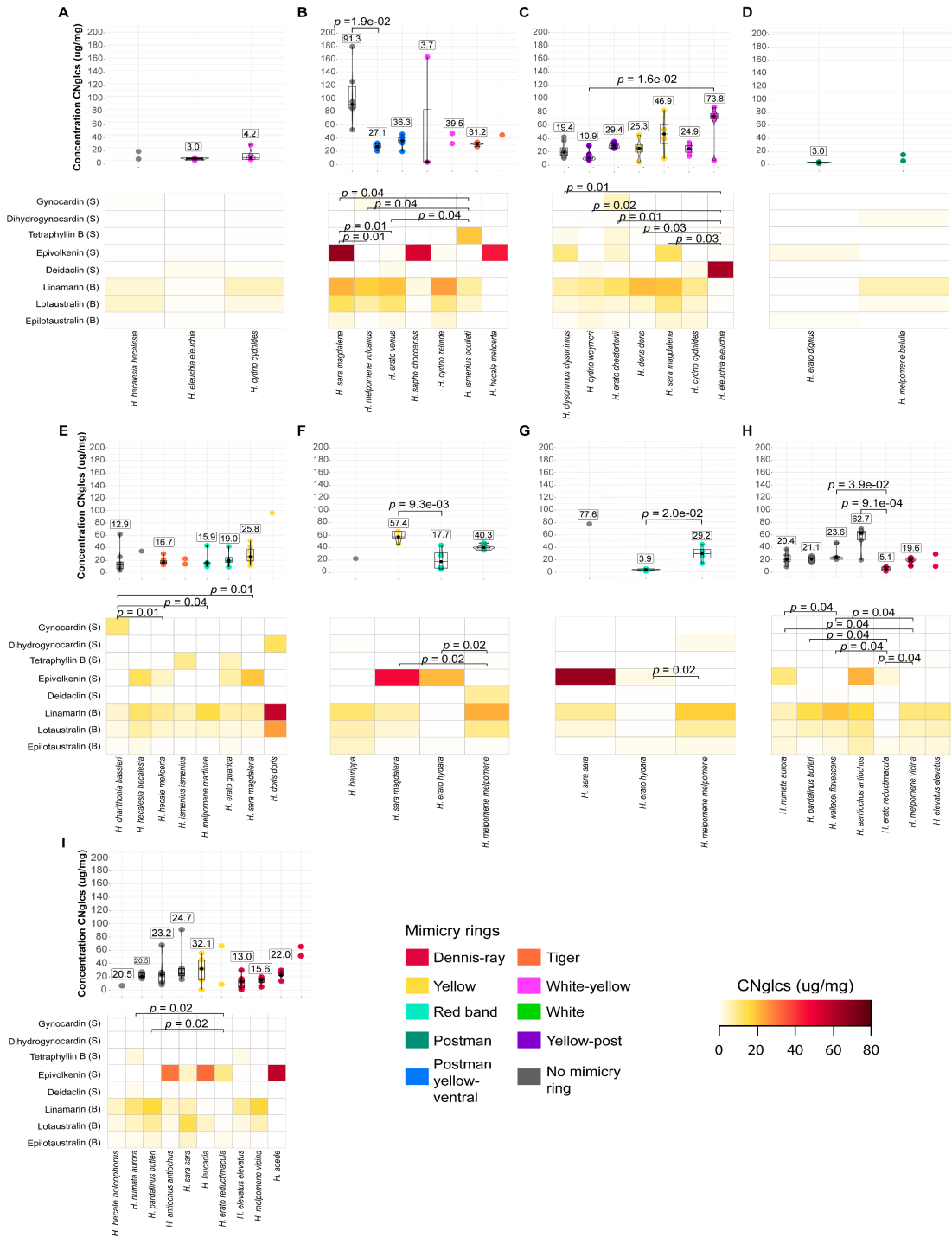
**Figura 1.** Resume las biorregiones estudiadas y las especies/anillos que se encuentran allí.

Se encontró variación en el extracto total (A) y en el perfil promedio cianogénico (B) entre especies y clados (**Figura 2**). Con mayor concentración en secuestración que en biosíntesis, siendo las especies blanco/amarillas las más tóxicas (por ejemplo *H. sara*).



**Figura 2. (A)** concentración total de cianógeno por especie, **(B)** perfil promedio cianogénico entre especies. **(S)** compuestos secuestrados a partir de precursores, **(B)** compuestos biosintetizados. Barras de color abajo denotan los clados filogenéticos.

La concentración total de glicósidos cianogénicos no presenta gran variación por especie (y por ende en anillos miméticos) con la geografía (excepto casos particulares, los significativos en la figura), aunque su composición si presenta variación (**Figura 3**).

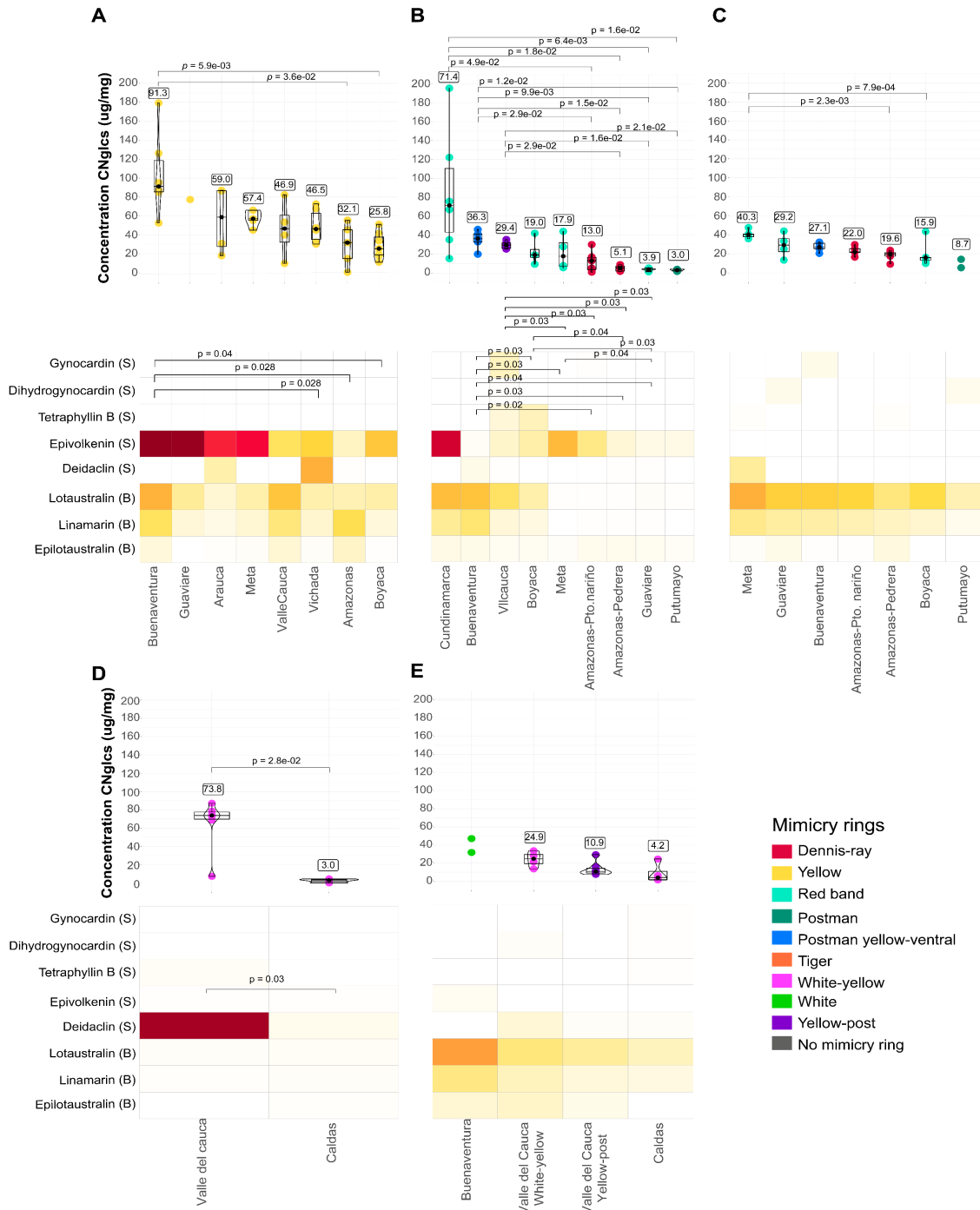


**Figura 3.** Variación en la concentración de glicósidos cianogénicos total (panel superior) y en composición promedio (panel inferior) por especie por localidad. (A) Caldas, (B) Buenaventura, (C) Cauca Valley, (D) Putumayo, (E) Boyacá, (F) Meta, (G) Guaviare, (H) Amazonas-Pedraera, (I) Amazonas-Puerto Nariño. B y S como en la figura 2.

Al revisar la variación en el perfil de glicósidos cianogénicos de manera intra-específica por geografía se encontró una gran variación dentro de cada especie, al grado que se observaron individuos con distintos niveles de toxicidad y en algunos casos individuos de

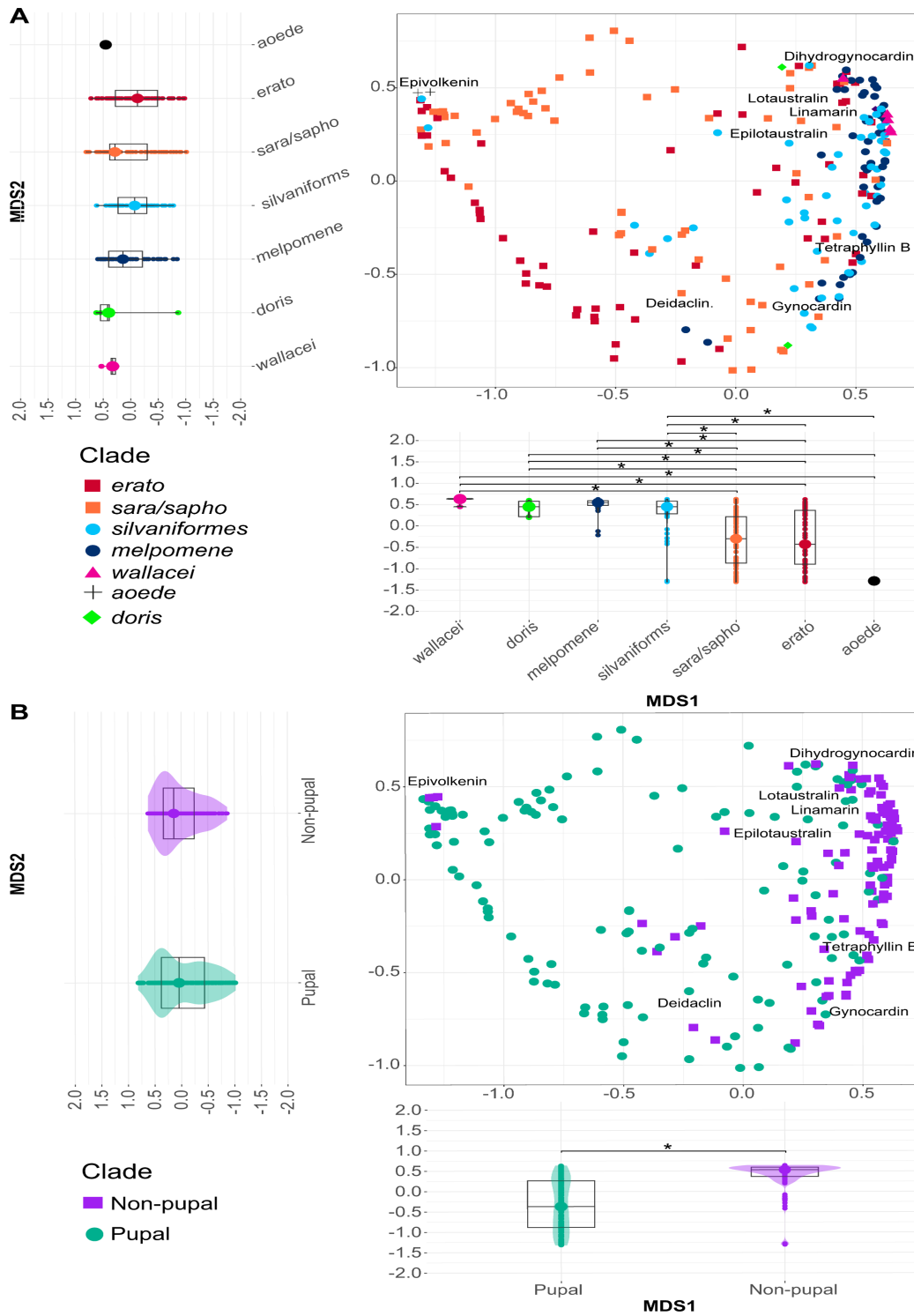


un linaje que previamente se consideraba que solo sintetizaban compuestos, presentan biosíntesis y viceversa (Figura 4).



**Figura 4.** Variación intra-específica en los glicósidos cianogénicos. (A) *H. sara*, (B) *H. erato*, (C) *H. melpomene*, (D) *H. eleuchia*, (E) *H. cydno*. Abreviaciones en la figura como en las figuras anteriores.

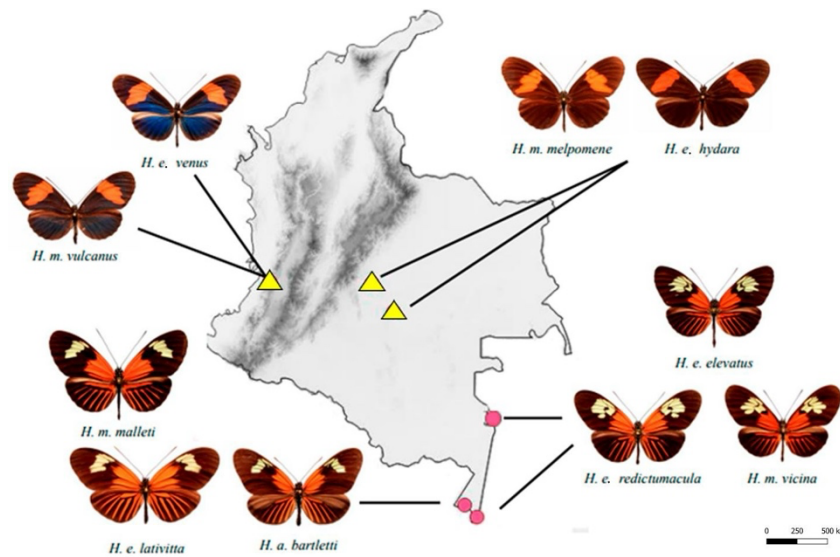
Las diferencias presentadas en las figuras anteriores no se mantienen cuando se tiene en cuenta la filogenia (**Figura 5**). Indicando que la ancestría influye en gran medida la composición cianogénica de una especie determinada.



**Figura 5.** Señal filogenética y composición cinogénica. Se presenta la distribución de los compuestos cianogénicos, coloreando los individuos por clado (símbolos y color). Los clados melpomene y sivariformes que son principalmente sintetizadores jalonan la señal filogenética. Está también se refleja en el rasgo de vida de apareamiento en adulto o en pupa.

**Objetivo 2, numerales 3 y 4 en el resumen:**

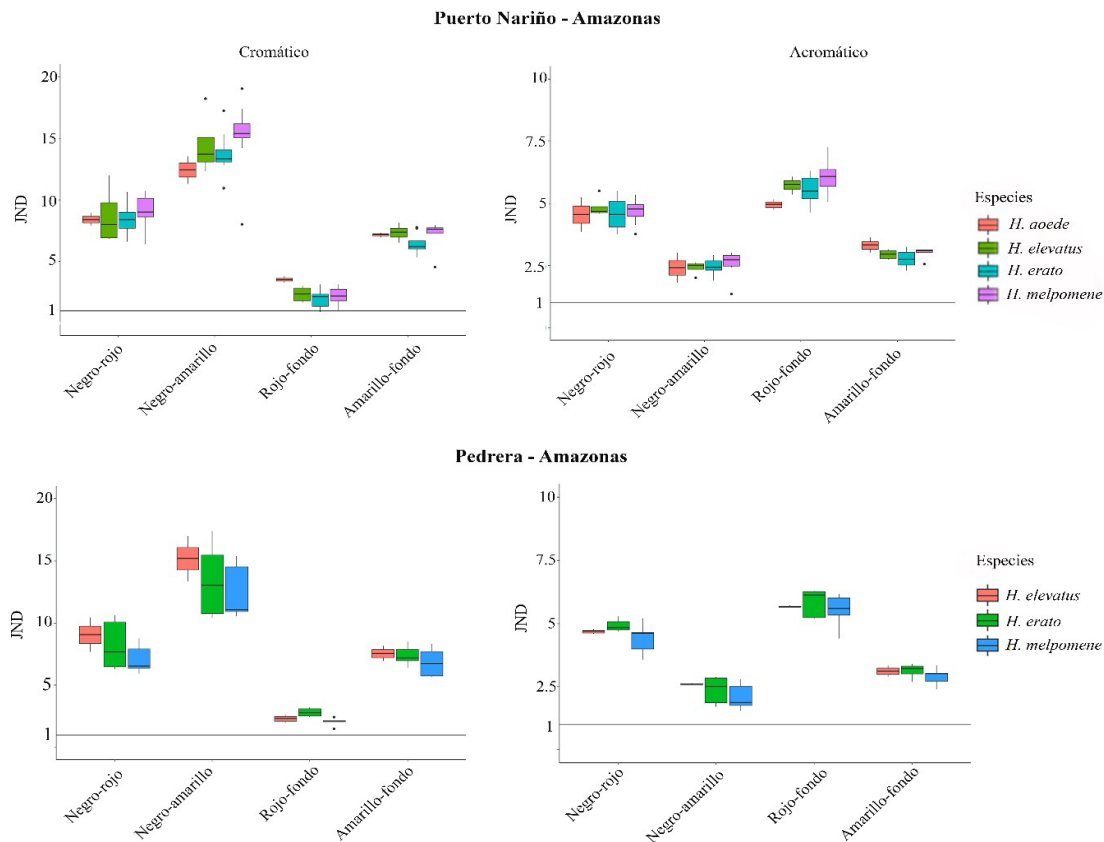
Los análisis de los contrastes de coloración (calculados a partir de espectrometría: reflectancia) para los distintos anillos miméticos (**Tabla suplementaria 1**) se tabularon en hojas de cálculo (**Tabla suplementaria 2**). Se tienen alrededor de 40 tablas de este estilo, presentando solo la anteriormente mencionada a manera de ejemplo. Estas tablas siguen la nomenclatura que se presenta a continuación, usando pares de letras en ese orden: primera letra; alas anteriores (A) o posteriores (P), segunda letra; superficie dorsal (D) o ventral (V), tercera letra; parche de color; Orange (O), Black (B), yellow (Y), white (W) y blue (Bl), Brown (Br), cuarta letra. Las coloraciones fueron evaluadas bajo dos modelos de visión de depredadores: *Cyanistes caeruleus* (sensible a la luz UV) y *Pavo cristatus* (sensible a luz violeta). Abreviados UVS y VS respectivamente. También, se evaluó el contraste de los parches de color y diferentes tratamientos de luz ambiental: fs= Bosque ws= Bosque en transición, sg= Claro pequeño y lg= Claro grande. En general el contraste entre parches de coloración se nombró como cromático (dS) y entre el parche de color y el ambiente como acromático (dL). Estos contrastes se obtienen siempre entre pares de individuos de cada anillo mimético y para cada localidad. Como ejemplo, acá se presentan los resultados a manera demostrativa de los anillos miméticos de la **figura 6**.



**Figura 6.** Anillos miméticos para los que se presentan resultados de la evaluación de los contrastes de coloración entre parches de color y de tipos de ambiente para dos modelos de visión de aves. Esto a manera de ejemplo de lo que se hizo con todas las comparaciones de la **Tabla suplementaria 1**. Fenotipos rayados: Dennis-ray, aquellos con banda roja: Postman.

En los dos anillos miméticos, independientemente de la superficie alar evaluada y el modelo de visión implementado, los valores de JNDs fueron mayores a 1 en todas las especies, lo cual indica que las aves logran distinguir entre estos dos colores (**Figura 7,8**) y

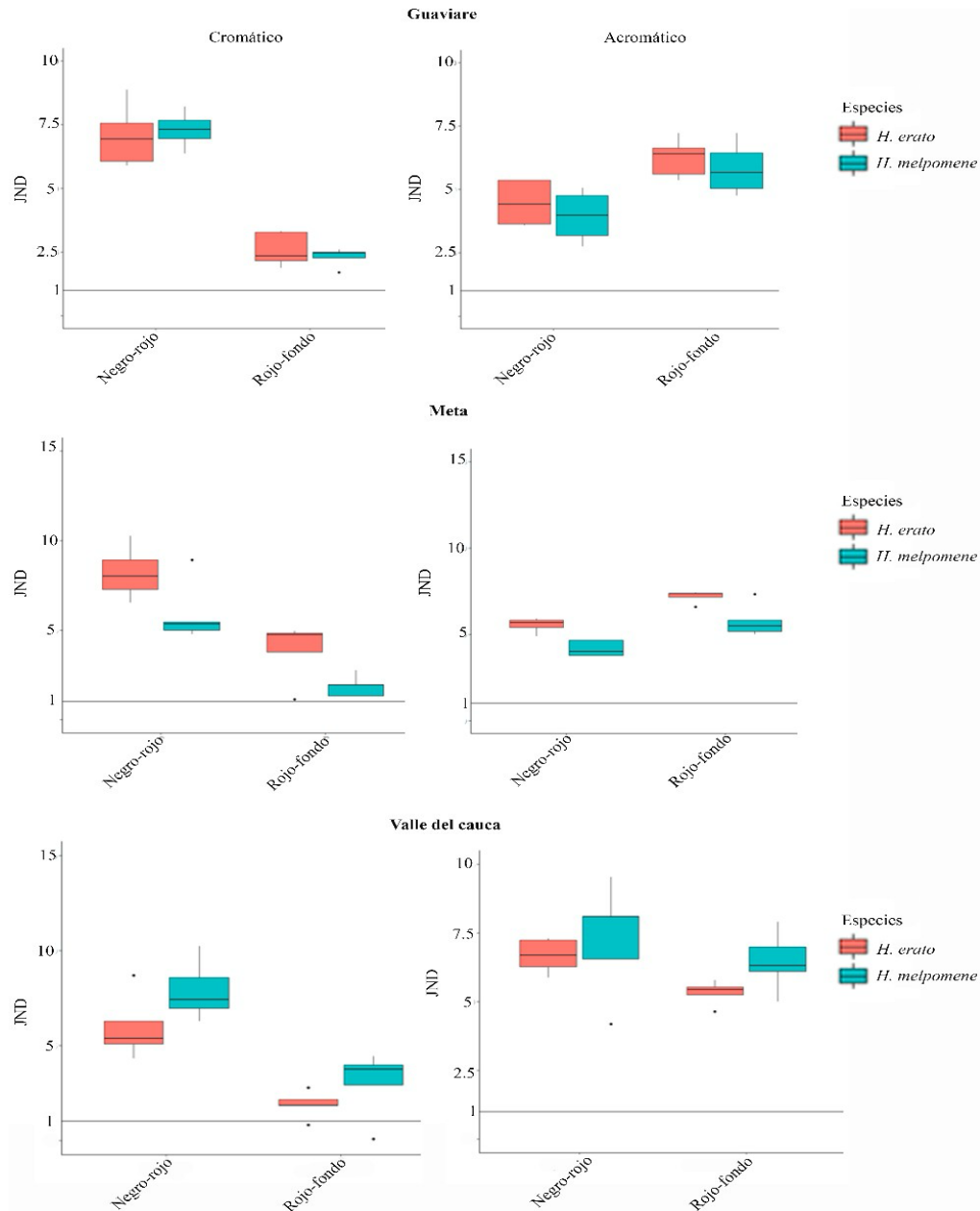
suplementario **Figura S1-S30**). Aunque esto no se cumple bajo todos los tipos de luz y de contrastes (cromáticos y acromáticos). En general, los valores de los contrastes disminuyen a medida que la cantidad de luz ambiental se reduce en todas las especies afectando la capacidad discriminatoria de las aves. En específico, en los contrastes cromáticos el ave no logra distinguir entre el rojo y el fondo del bosque (**Suplementario Figura S1-S18 y S23-S26**) y en los contrastes acromáticos no distingue el amarillo contra el negro (**Suplementario Figura S1-S7 y S10-S15**).



**Figura 7.** JNDs entre los parches de color para cada especie para los diferentes contrastes. Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión aviar UVS, vista dorsal del ala y la luz ambiental claro grande.

En el anillo *dennis-ray* los parches de color amarillo contra el negro interno del ala obtuvieron el mayor contraste cromático en todas las especies. Sin embargo, el patrón cambia en el contraste acromático ya que los parches de color rojo contra el fondo verde del bosque son los que obtienen el mayor valor (**Figura 7**) y **suplementarios (Figura S1-S3 y S8-S11)**. Este patrón es el mismo para las dos localidades en estudio y no se encontraron diferencias entre los JNDs entre las especies (**Figura 7**) y **suplementarios (Figura S1-S15)**. En el anillo *postman* en todas las localidades se encontró que las bandas de color rojo contra el negro obtuvieron el mayor contraste cromático en las dos especies. Sin embargo, en las localidades de Guaviare y Meta el patrón cambia en el contraste acromático ya que el color rojo contra el fondo verde del bosque son los que obtienen el mayor valor de contraste. En la localidad del Valle del Cauca, el contraste entre el parche

de color rojo contra negro del ala vuelve a ser ligeramente mayor que el contraste entre el rojo y el fondo del bosque (**Figura 8**).



**Figura 8. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión aviar UVS, vista dorsal del ala y la luz ambiental claro grande.

**Objetivo 3, numeral 5 en el resumen:**

Con las medidas de toxicidad y coloración obtenidas en los objetivos anteriores se procedió a establecer la relación entre ellos. Para esto se corrieron distintos modelos lineales (**Tabla 1**).

**Tabla 1.** Selección del mejor modelo lineal para las distintas variables explicativas y su interacción. Valores del Akaike Information Criterion (AIC) para los modelos con y sin la interacción (contraste-especie) para los anillos miméticos dennis ray y postman. Para el anillo postman se adiciona la localidad como una variable explicativa. Los mejores modelos según este criterio se señalan en negrilla.

Anillo mimético	Contraste	Variable dependiente	Variables explicativas	VALOR AIC
DENNIS-RAY	CROMÁTICO	TOXICIDAD	Negro-Amarillo	<b>104.314</b>
			Interacción	108.477
			Negro-Rojo	<b>104.610</b>
			Interacción	109.224
			Amarillo-Fondo	<b>105.066</b>
			Interacción	110.244
			Rojo-Fondo	100.686
	Interacción	<b>99.736</b>		
	ACROMÁTICO	TOXICIDAD	Negro-Amarillo	<b>103.365</b>
			Interacción	103.795
			Negro-Rojo	<b>104.558</b>
			Interacción	109.806
			Amarillo-Fondo	<b>104.729</b>
			Interacción	110.424
Rojo-Fondo			<b>102.807</b>	
Interacción	107.205			
POSTMAN	CROMÁTICO	TOXICIDAD	Negro-Rojo	231.922
			Negro-Rojo + Localidad	<b>225.990</b>
			Interacción	227.885
			Rojo-Fondo	230.679
			Rojo-Fondo + Localidad	<b>223.235</b>
	Interacción	224.60		
	ACROMÁTICO	TOXICIDAD	Negro-Rojo	230.590
			Negro-Rojo + Localidad	225.850
			Interacción	<b>222.658</b>
			Rojo-Fondo	231.199
Rojo-Fondo + Localidad			224.246	
Interacción	<b>222.096</b>			

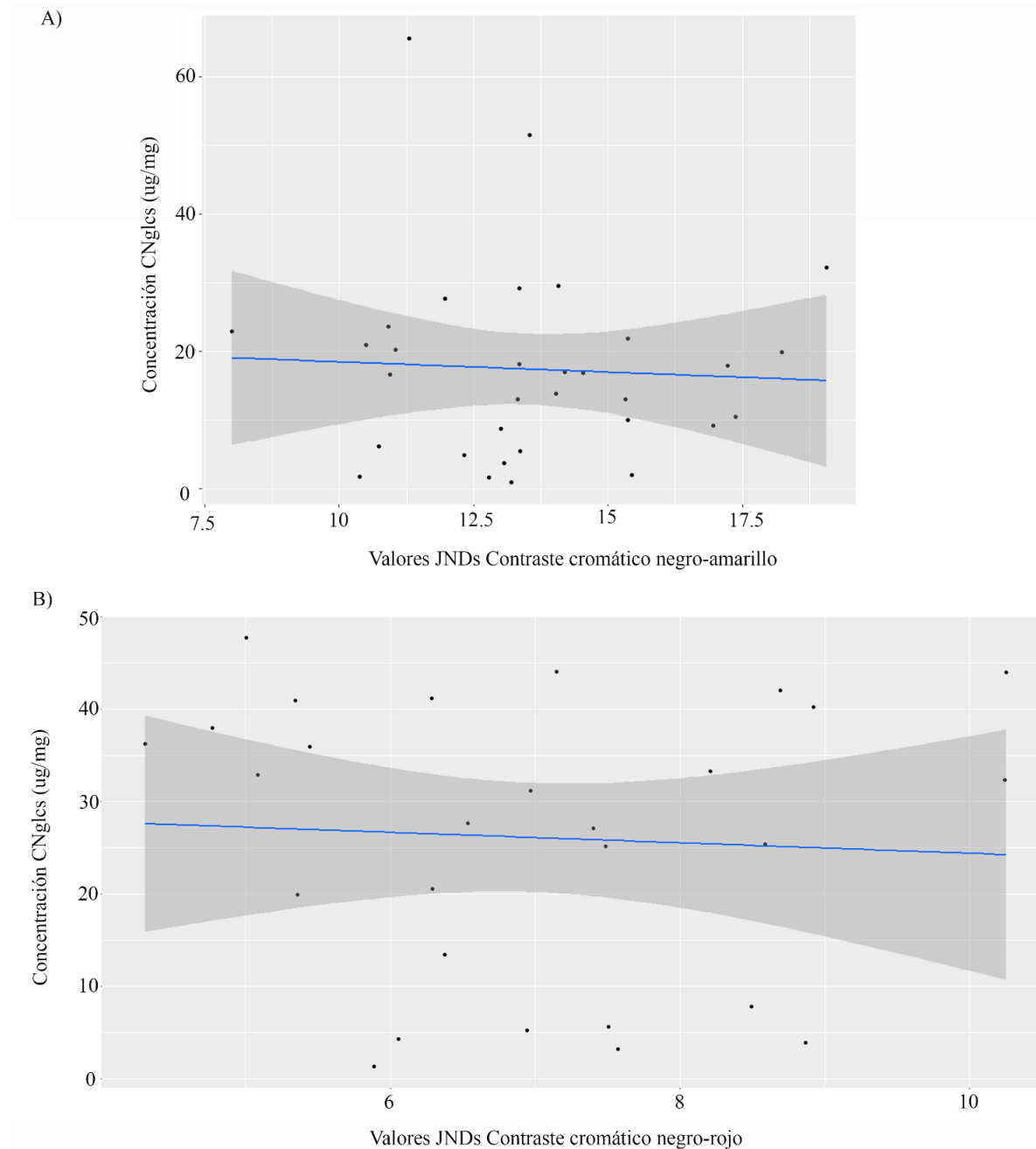
Para los dos anillos miméticos se encontró que en la mayoría de los casos el mejor modelo lineal (lm) que explica la relación entre la toxicidad, los contrastes de color, y las especies

es aquel donde no se incluye la interacción entre las variables explicativas (**Tabla 1**). Ninguno de los 28 modelos indico que haya una relación significativa entre la variable de la toxicidad total y la coloración, tanto en los contrastes cromáticos como acromáticos (**Tabla 2**).

**Tabla 2.** Modelos lineales evaluados en este estudio entre toxicidad, coloración y especie. La variable dependiente es la toxicidad y las variables explicativas evaluadas fueron coloración, especie para el anillo dennis-ray. Adicionando como variable explicativa la localidad para el anillo postman. La varianza explicada en todos los modelos esta entre el 50 y 70%. Los valores en negrilla fueron significativos al 0.05.

Anillo mimético	Contraste	Variable dependiente	Variables explicativas	Pr(> t )
DENNIS-RAY	Cromático	Toxicidad	Negro-Amarillo	0.425
			Especie	<b>1.25E-05</b>
			Negro-Rojo	0.5318
			Especie	<b>1.42E-05</b>
			Amarillo-Fondo	0.9072
			Especie	<b>1.89E-05</b>
			Rojo-Fondo	0.05632
	Especie	<b>7.51E-06</b>		
	Acromático	Toxicidad	Negro-Amarillo	0.233
			Especie	<b>9.89E-06</b>
			Negro-Rojo	0.5099
			Especie	<b>1.39E-05</b>
			Amarillo-Fondo	0.5884
			Especie	<b>4.42E-05</b>
Rojo-Fondo			0.1694	
Especie	<b>6.70E-06</b>			
POSTMAN	CROMÁTICO	Toxicidad	Negro-Rojo	0.861397
			Especie	<b>1.11E-02</b>
			Localidad	<b>7.47E-03</b>
			Rojo-Fondo	0.120438
			Especie	<b>0.015054</b>
	Localidad	<b>3.01E-03</b>		
	ACROMÁTICO	Toxicidad	Negro-Rojo	0.7027
			Especie	<b>1.48E-02</b>
			Localidad	<b>1.48E-02</b>
			Rojo-Fondo	0.21913
Especie			<b>1.29E-02</b>	
Localidad	<b>4.07E-03</b>			

En general no se encontró que los elementos del patrón de coloración sean señales honestas de la toxicidad (i.e. una relación directa creciente) en ninguno de los tratamientos de iluminación (**Figura 9, Figura 31S-40S**). Tener presente algo de toxicidad y una señal conspicua es suficiente para estar bien protegido de depredadores, la ecología propia del lugar (por ejemplo planta huésped) donde se encuentren las especies puede influir en la generación del espectro de toxicidad.



**Figura 9. Ejemplos de la no correlación entre coloración-toxicidad para los anillos *dennis-ray* y *postman*.** A) Eje X: Valores JNDs para contraste acromático evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNglcs por cada individuo perteneciente al anillo *dennis-ray* B) Eje X: Valores JNDs para contraste cromático evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro



grande para el anillo *postman*. Eje Y: Concentración total de CNGlcs por cada individuo perteneciente al anillo *postman*.

#### **5. Consideraciones éticas y de integridad científica**

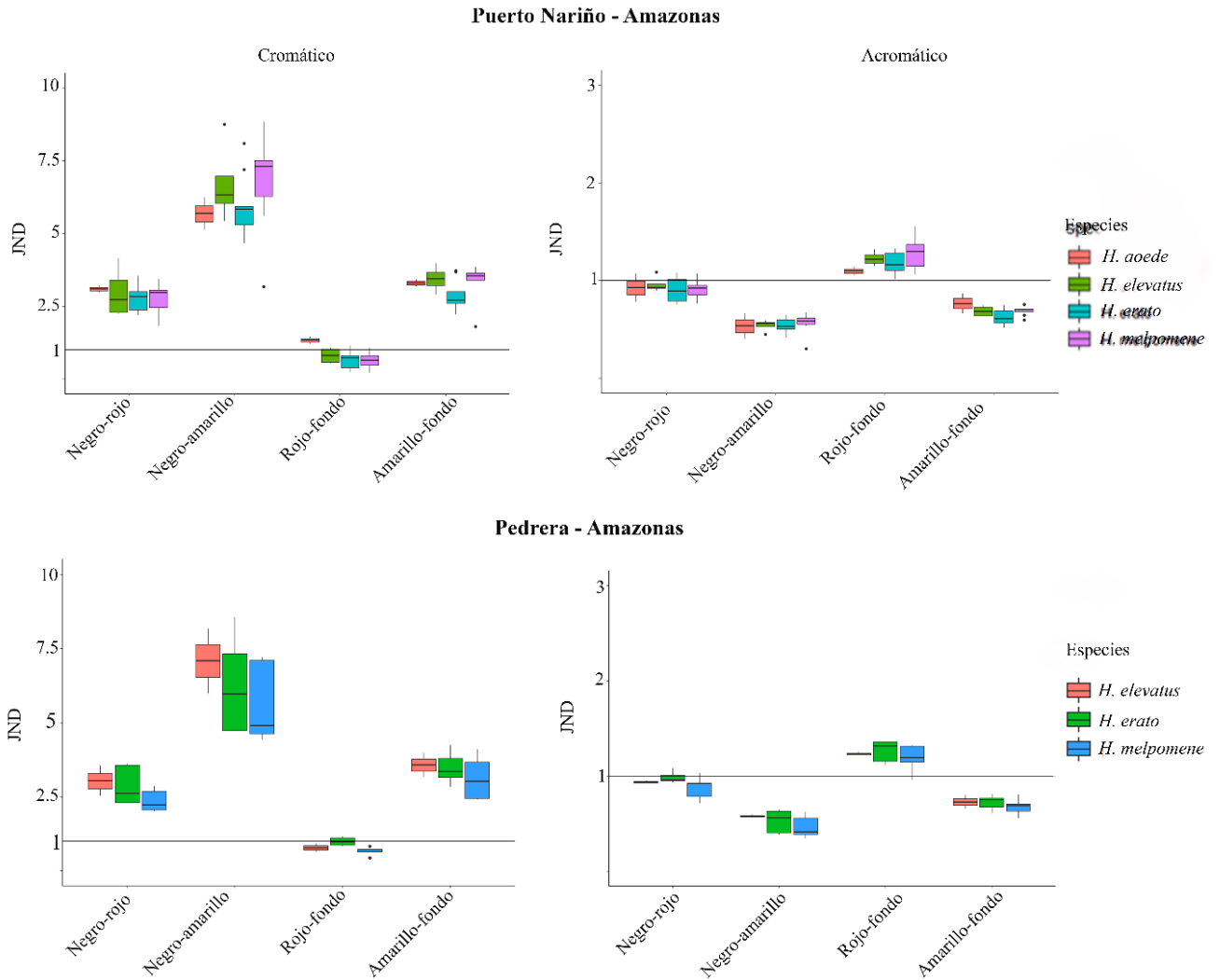
El número de participantes del proyecto son: Camilo Salazar, Nicol Rueda, Erika de Castro, Mónica Arias. No se han presentado situaciones relacionadas con los derechos de los participantes (personas, animales o plantas). Ningún participante se ha retirado y la información se encuentra en condiciones de buena confidencialidad. No hay ningún cambio en los acuerdos de autoría de los productos de investigación o en los responsables de la propuesta.

#### **6. Ejecución del Presupuesto**

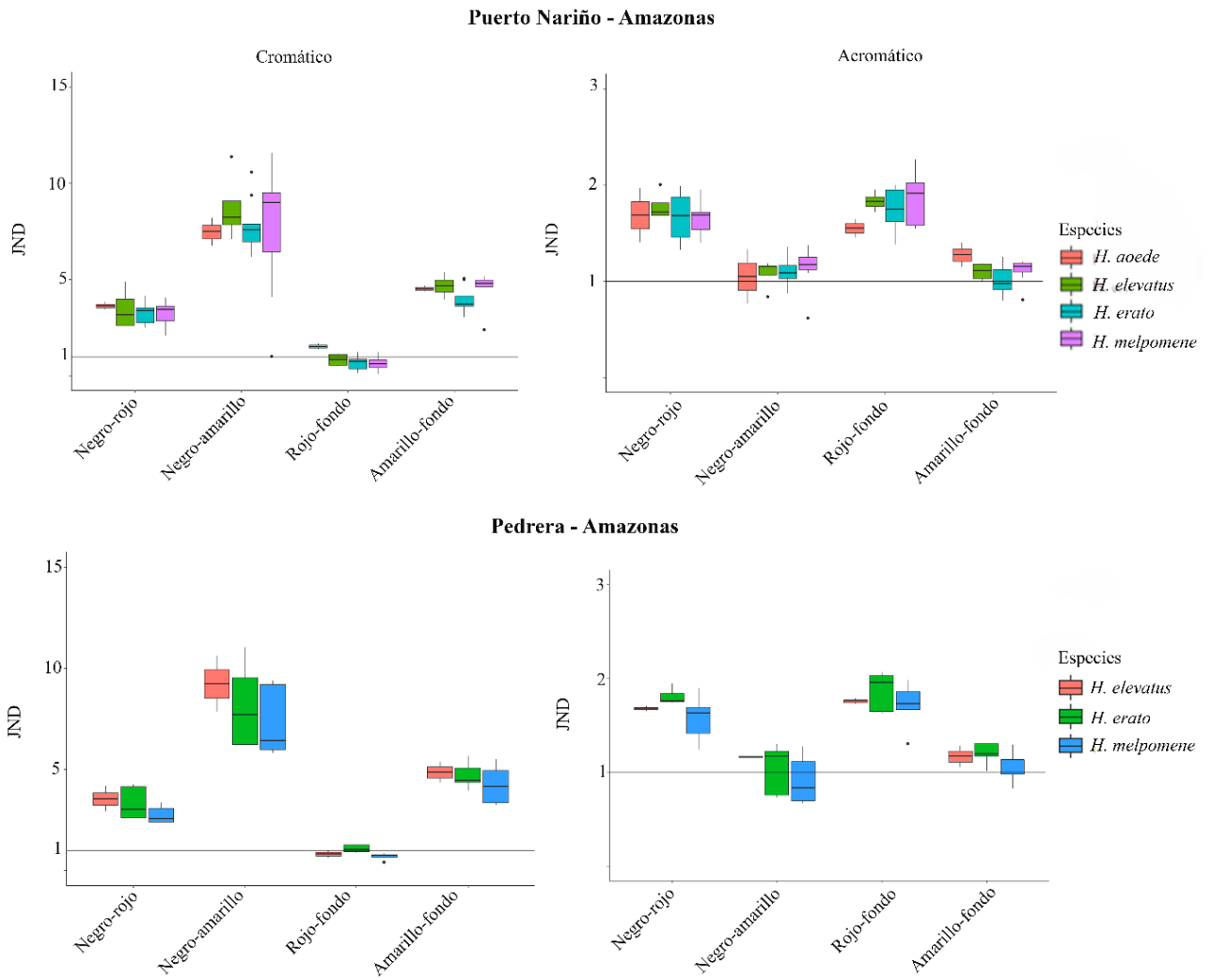
Ver el informe financiero adjuntado en Pure por la DAF de FCN.

## FIGURAS SUPLEMENTARIAS

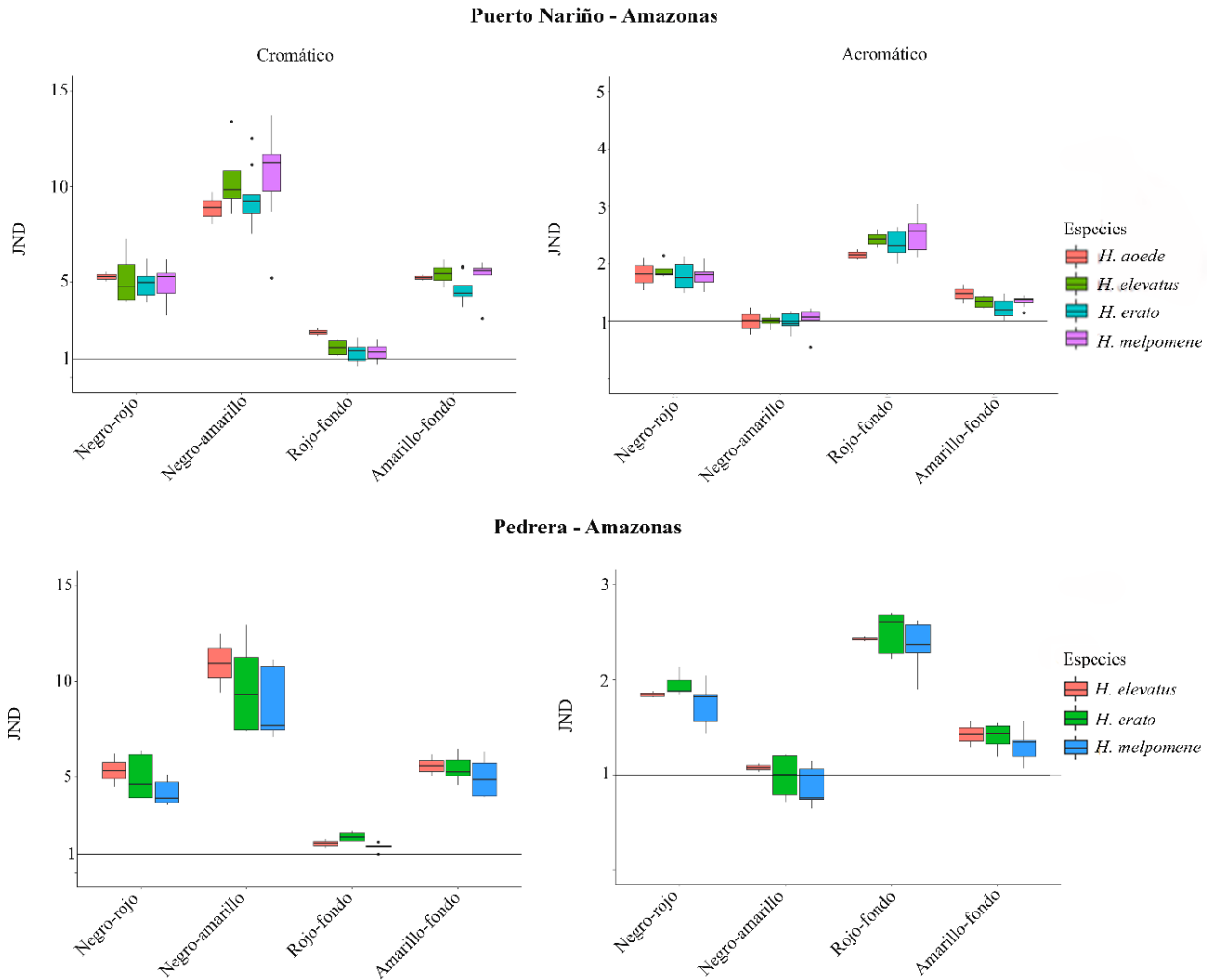
### *Dennis ray*



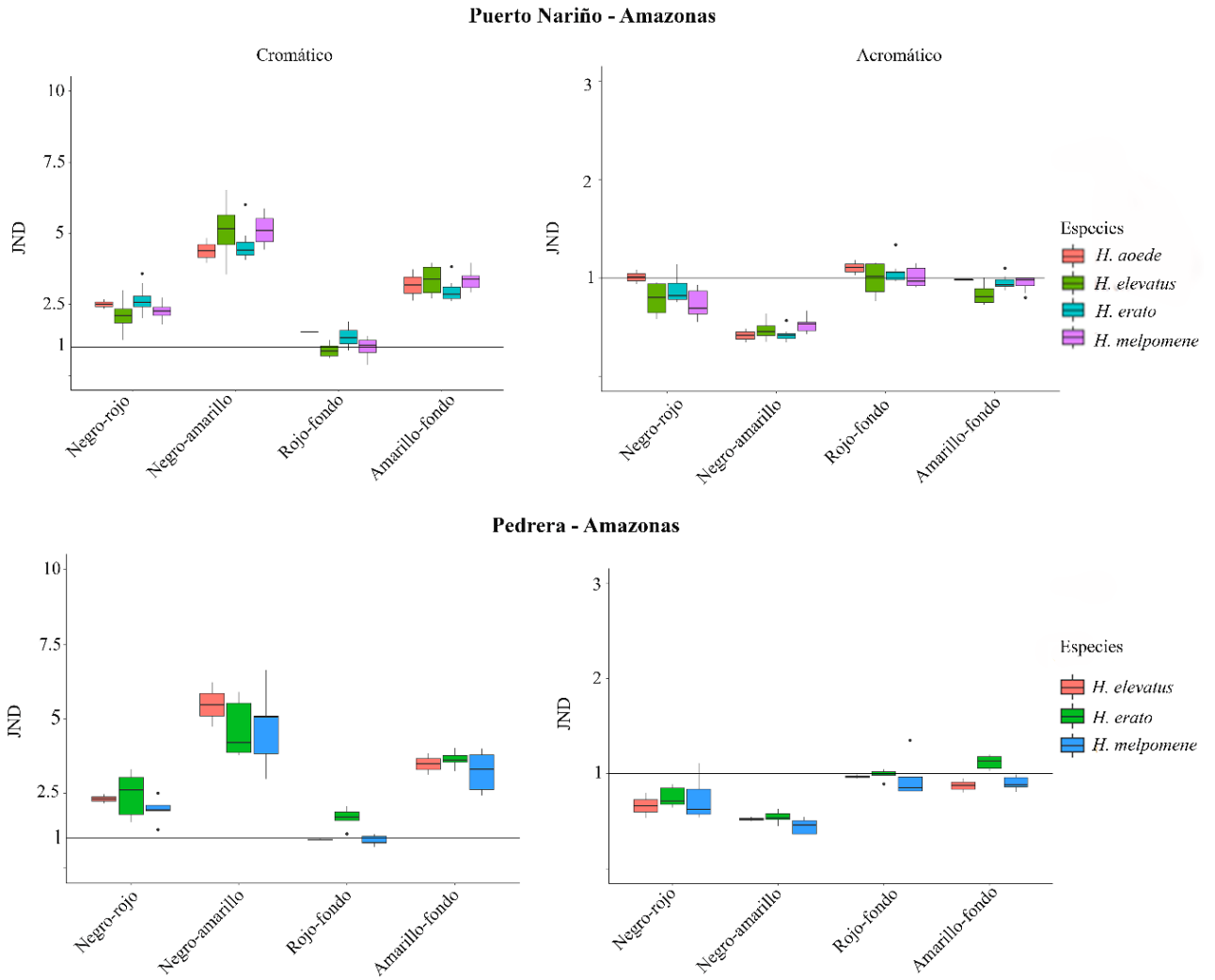
**Figura S1. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental bosque.



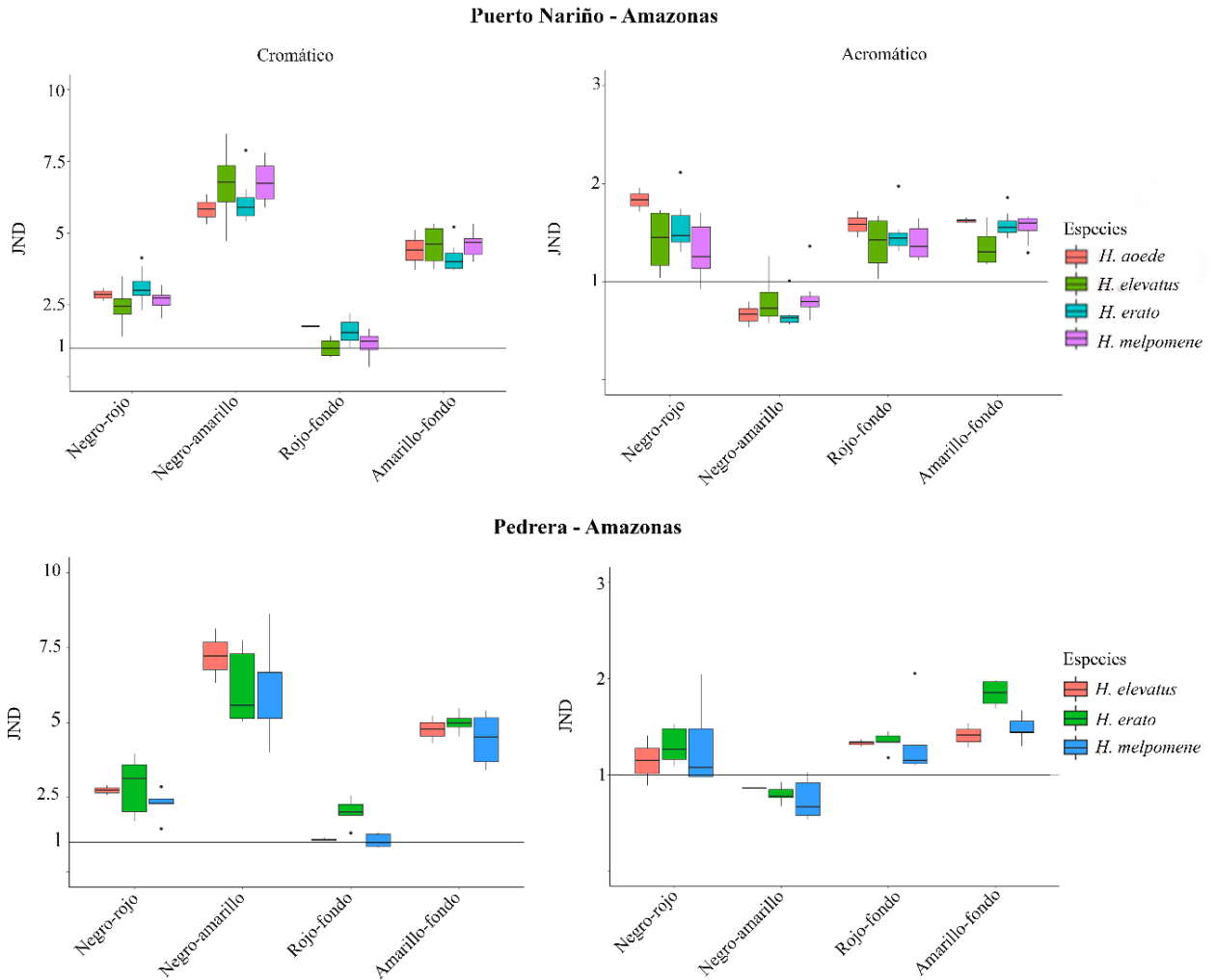
**Figura S2. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental bosque en transición.



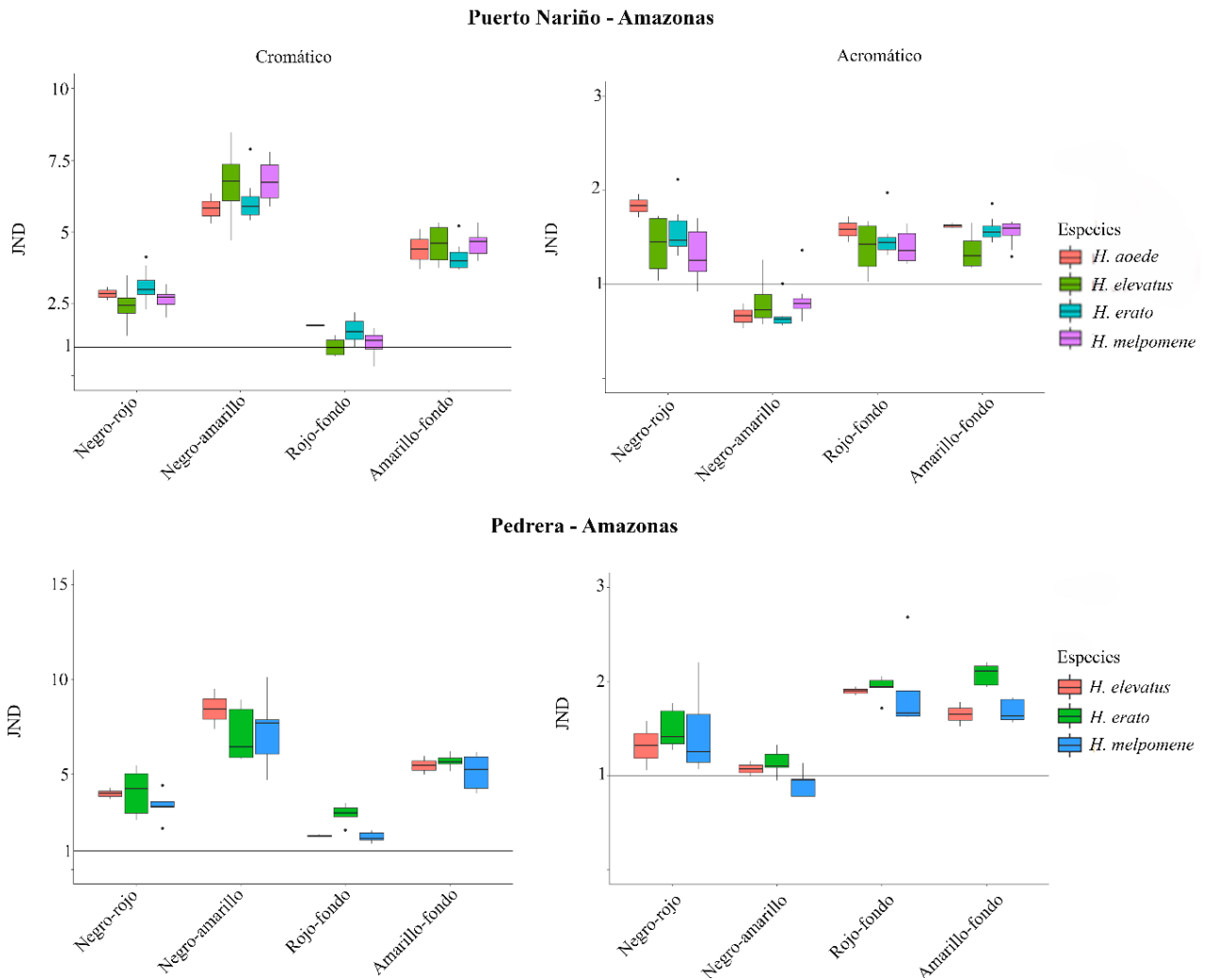
**Figura S3. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental claro pequeño.



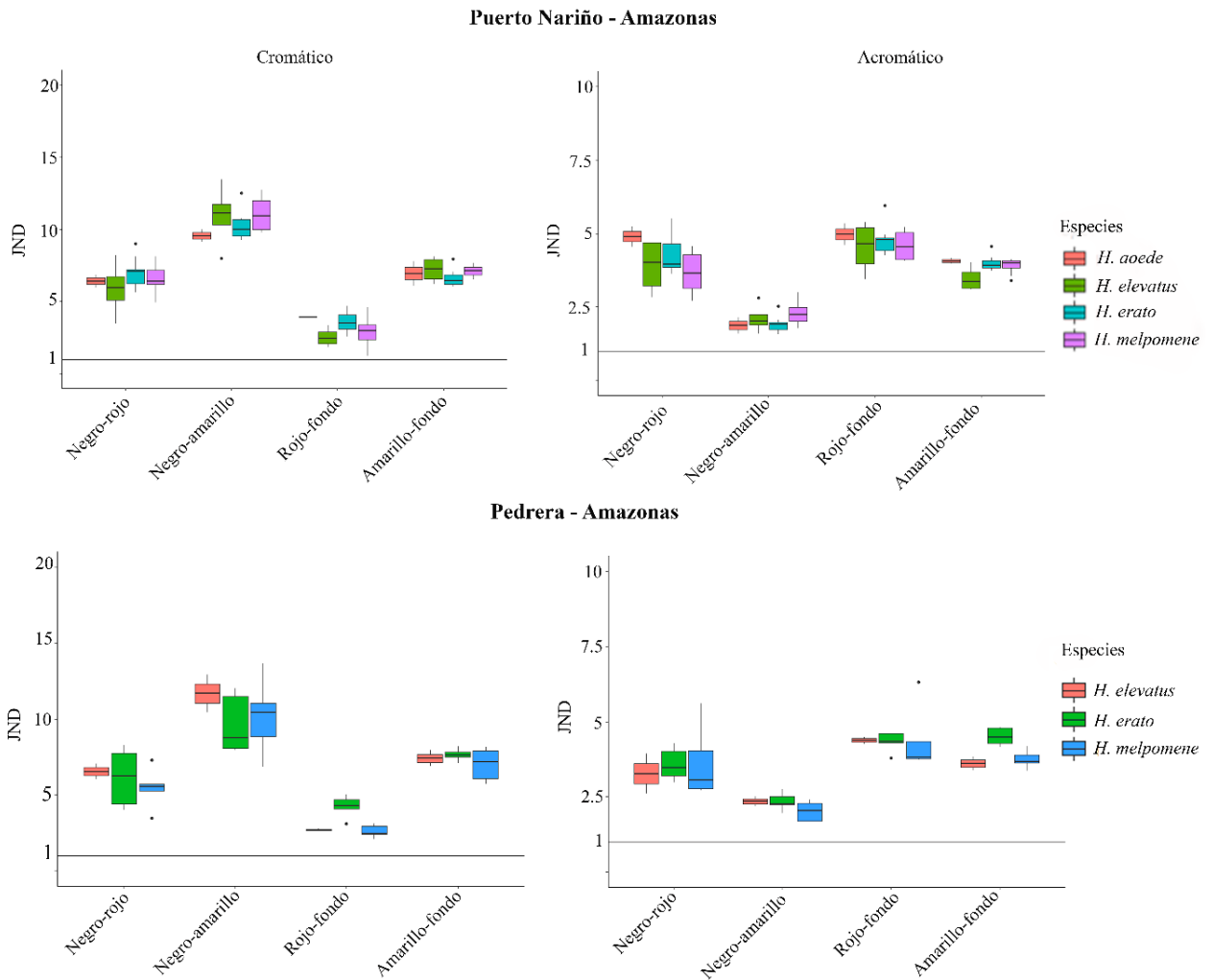
**Figura S4. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental bosque.



**Figura S5. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental bosque en transición.

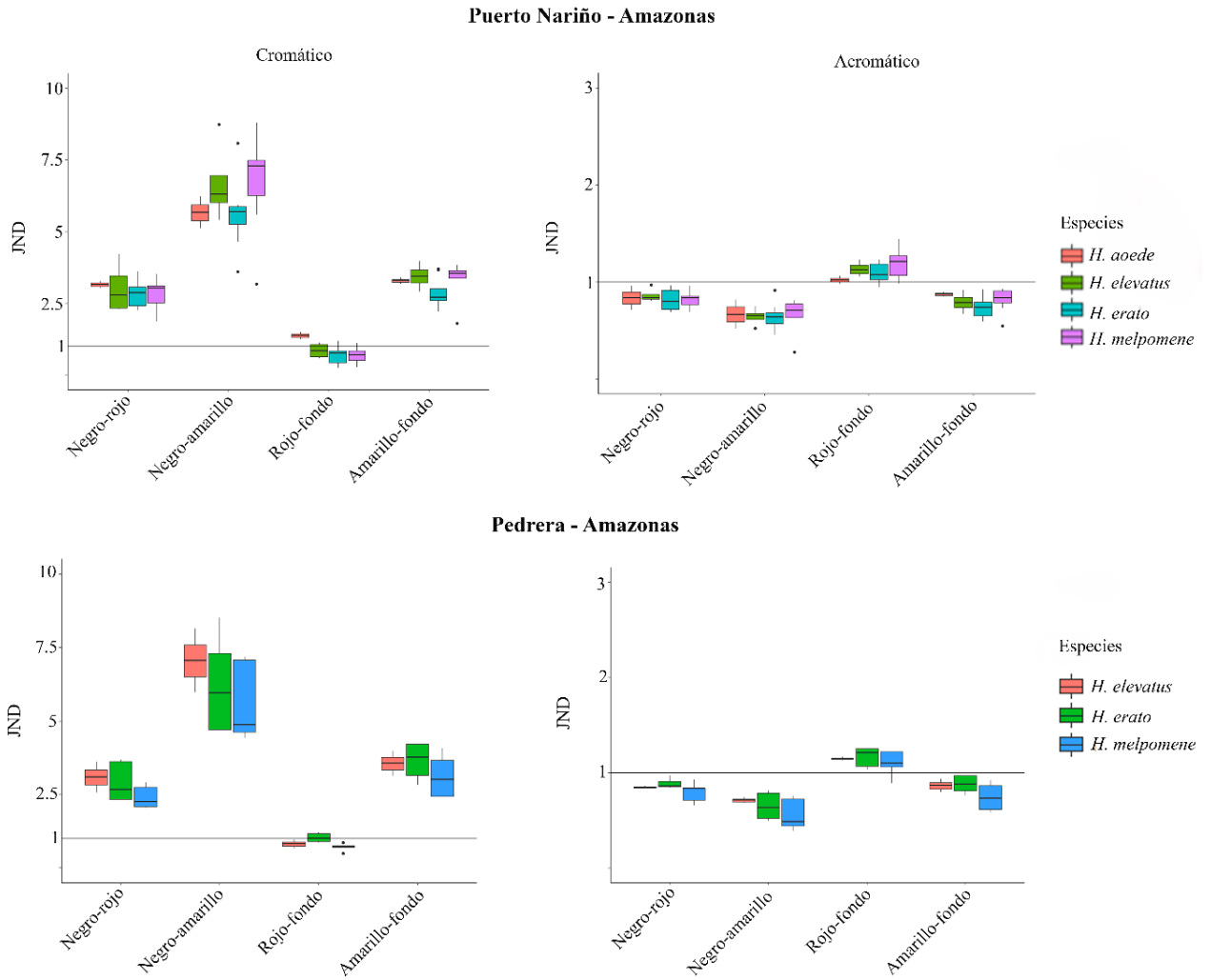


**Figura S6. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental claro pequeño.

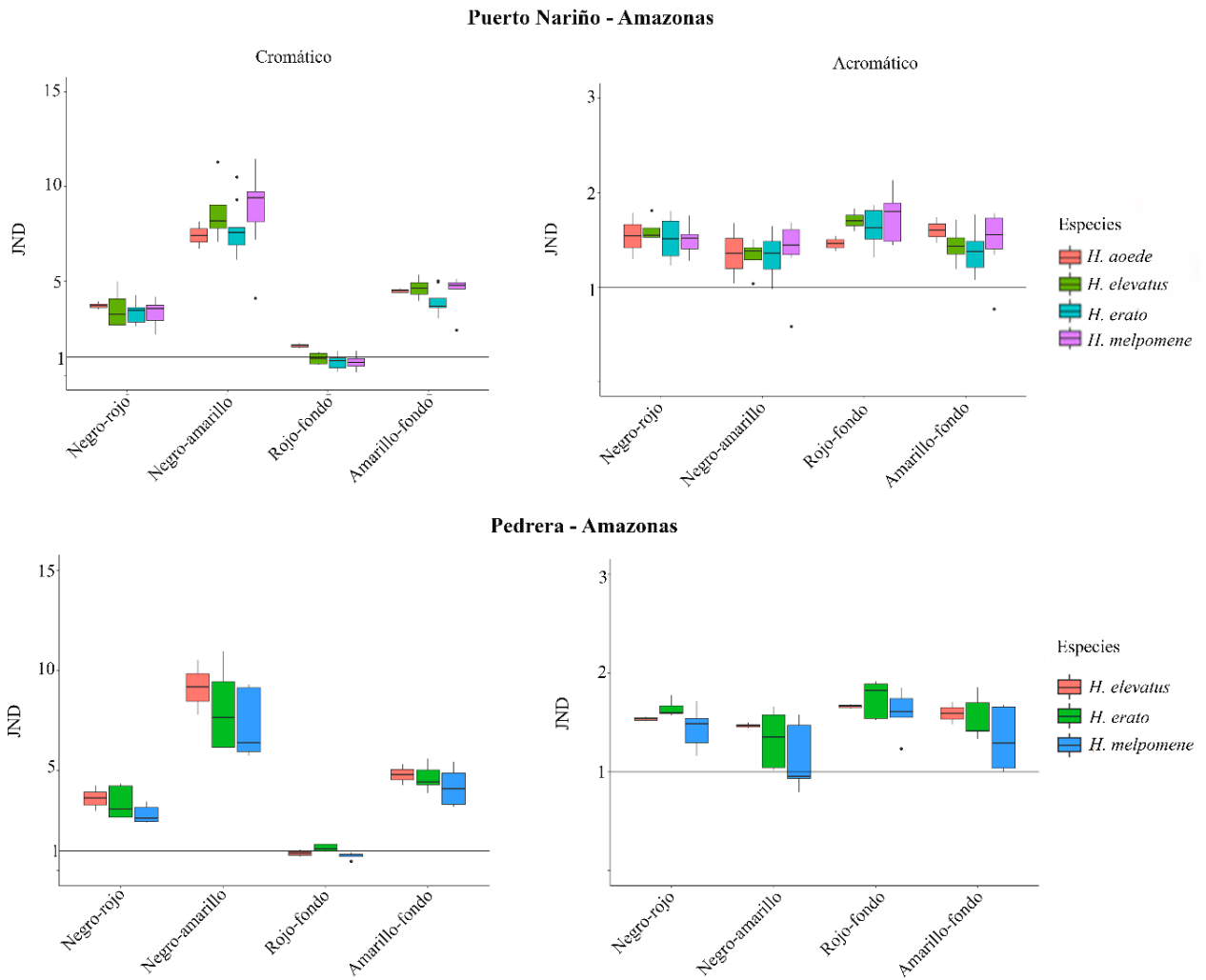


**Figura S7. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental claro grande.

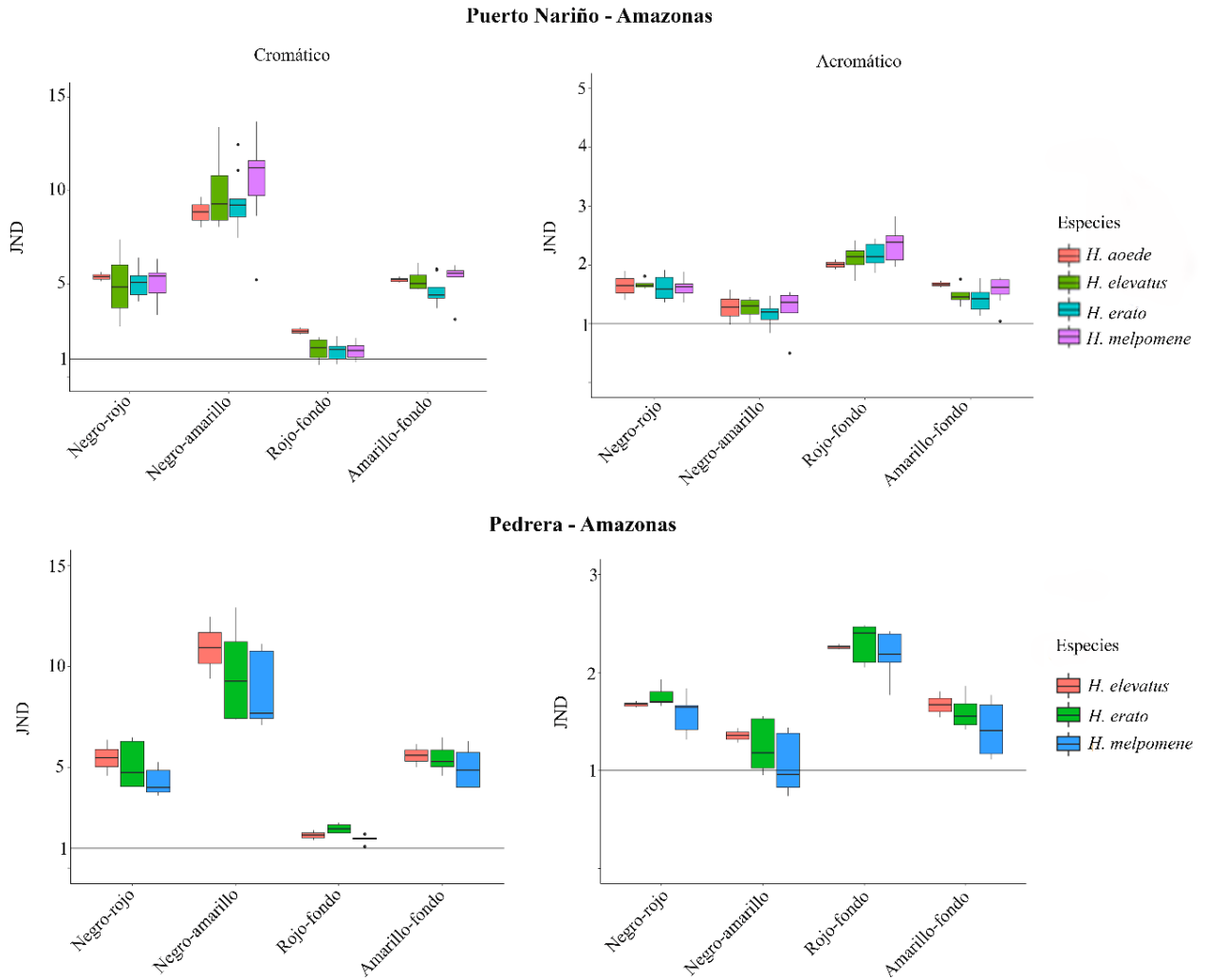




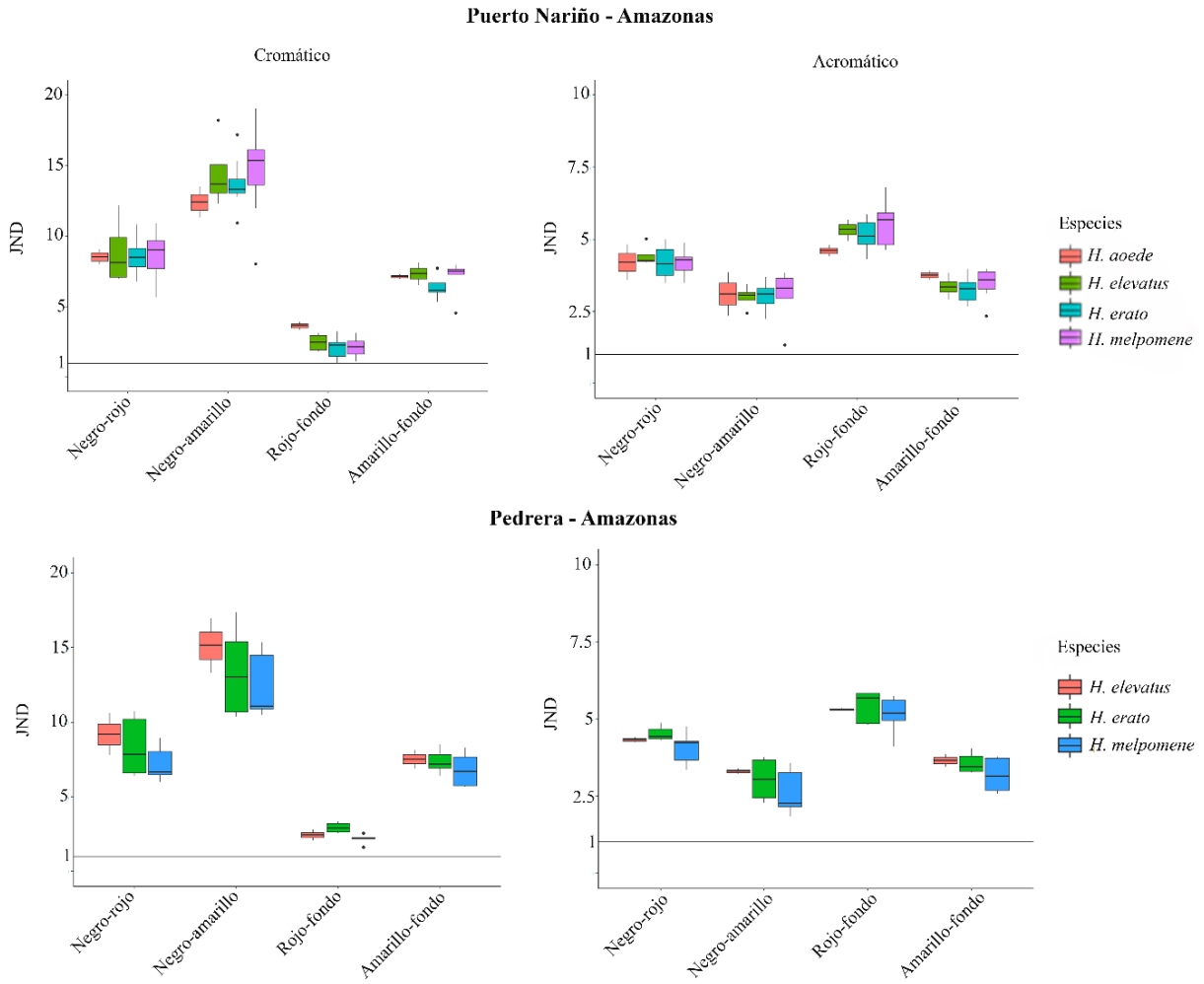
**Figura S8. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental bosque.



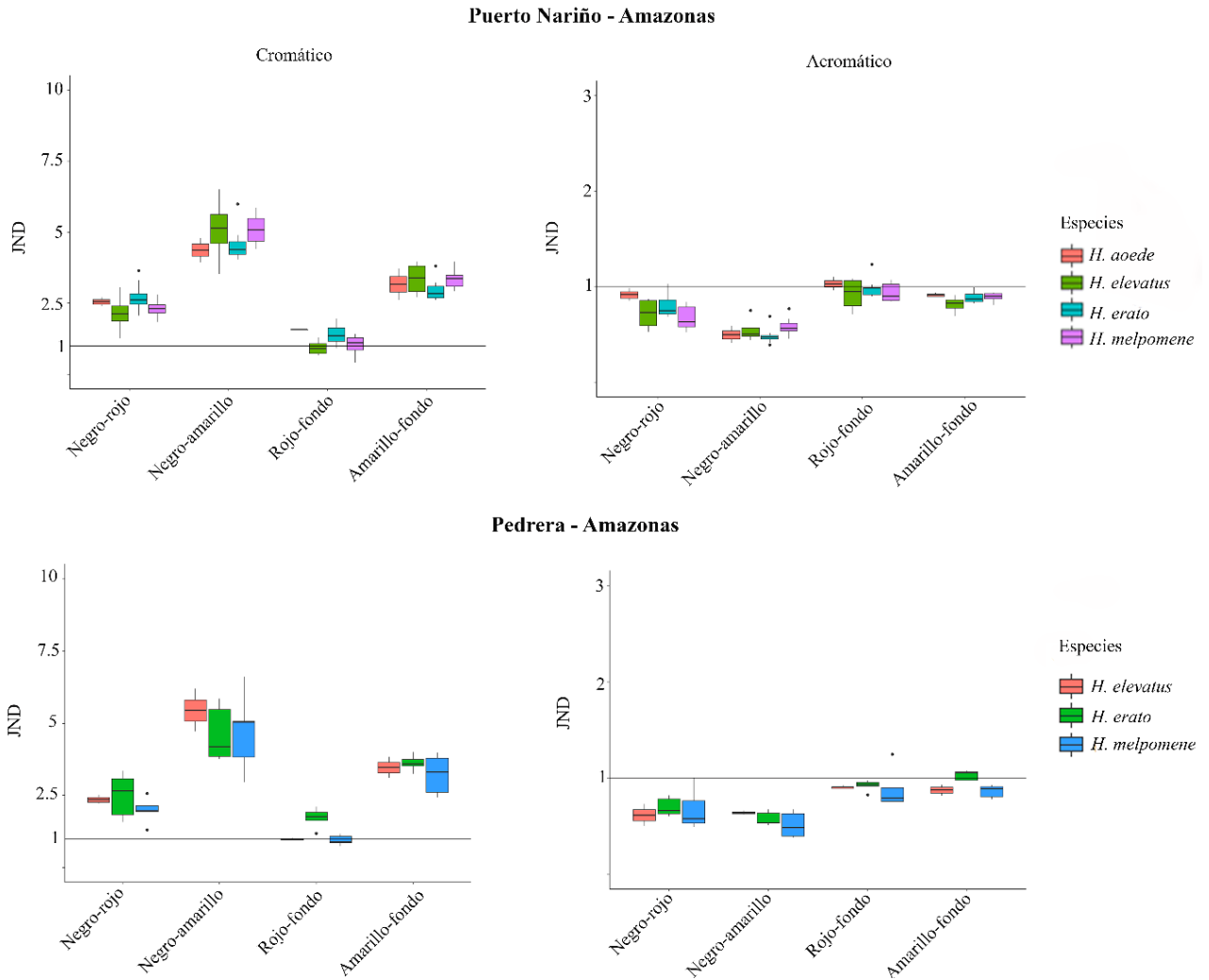
**Figura S9. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental bosque en transición.



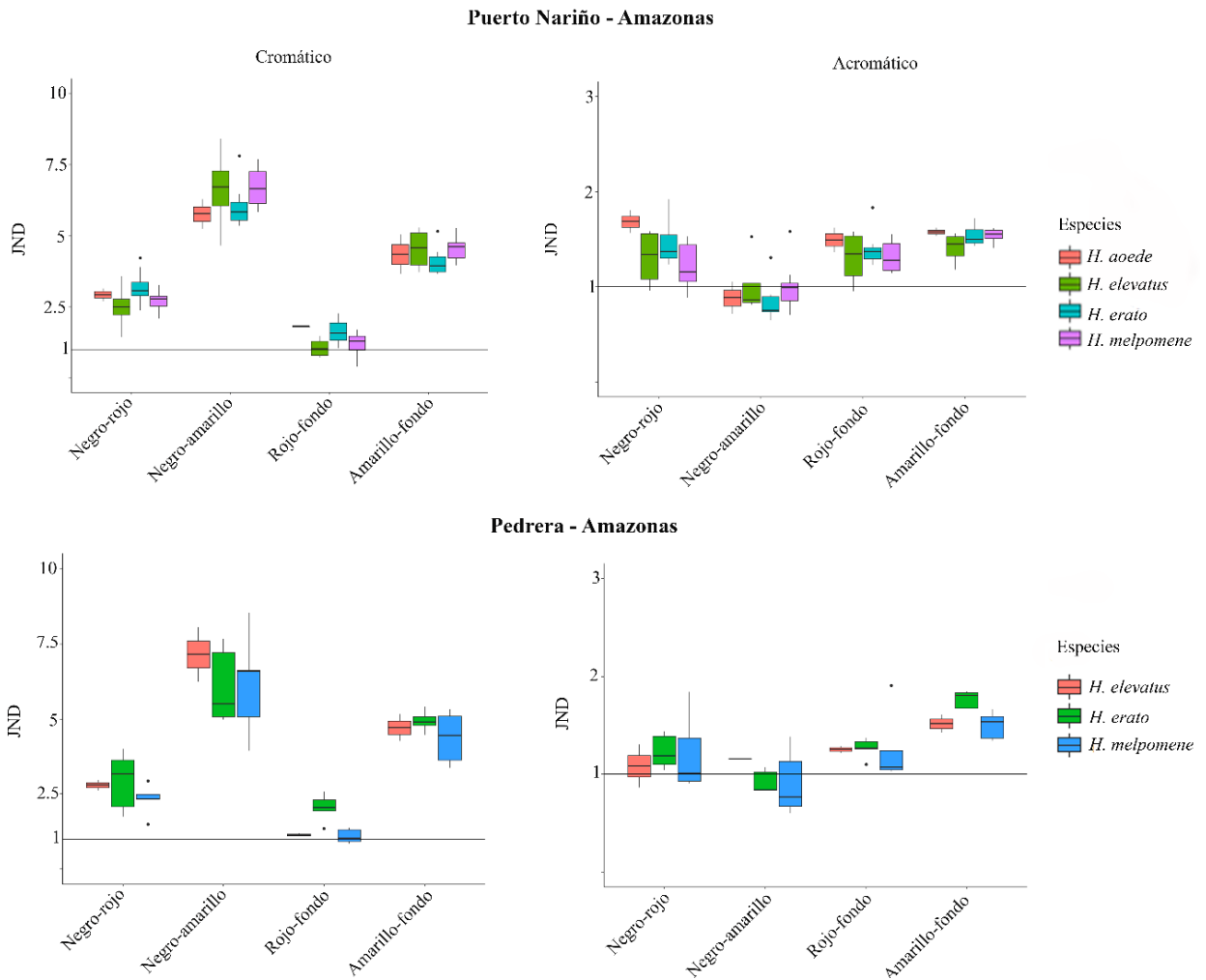
**Figura S10. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental claro pequeño.



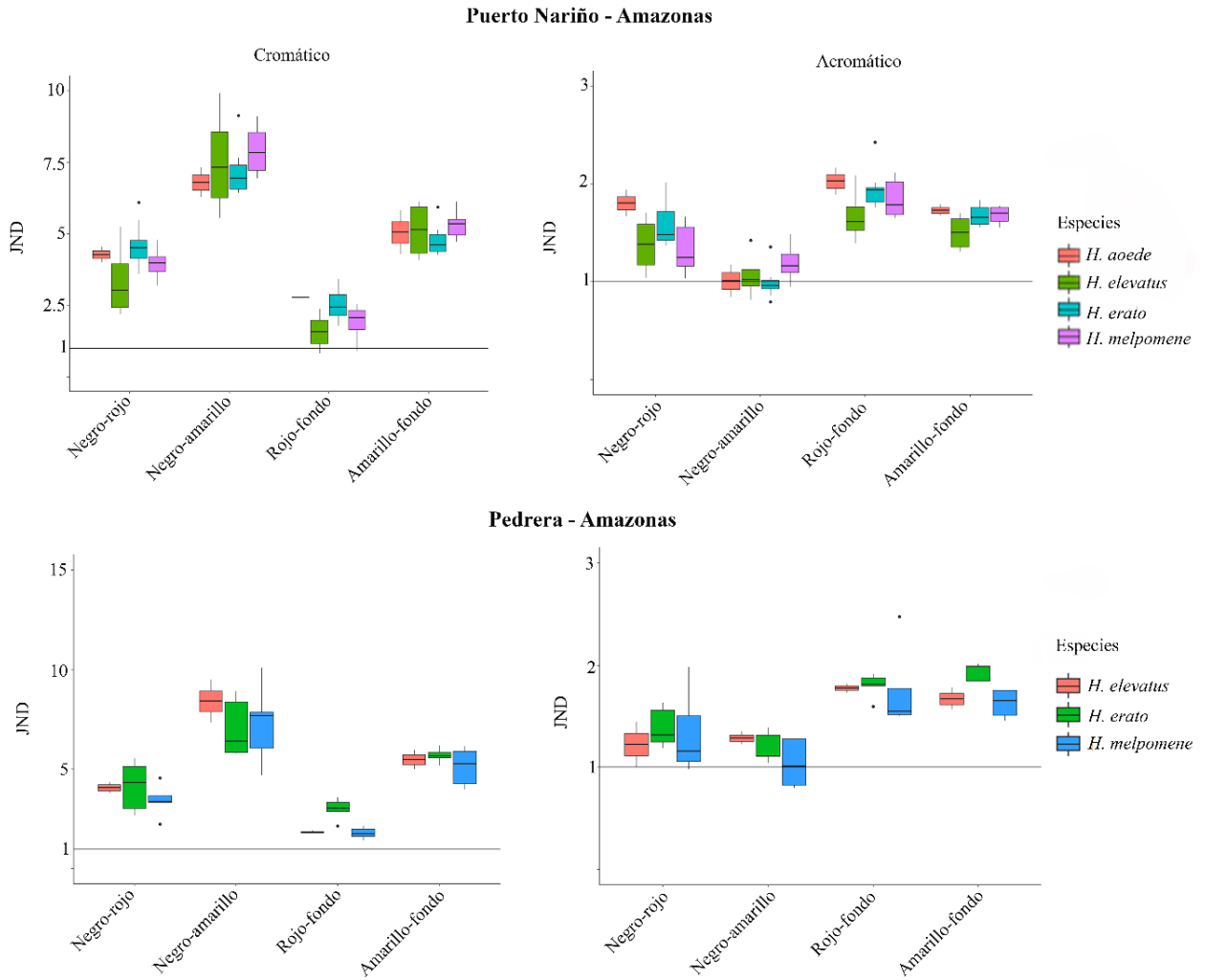
**Figura S11. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental claro grande.



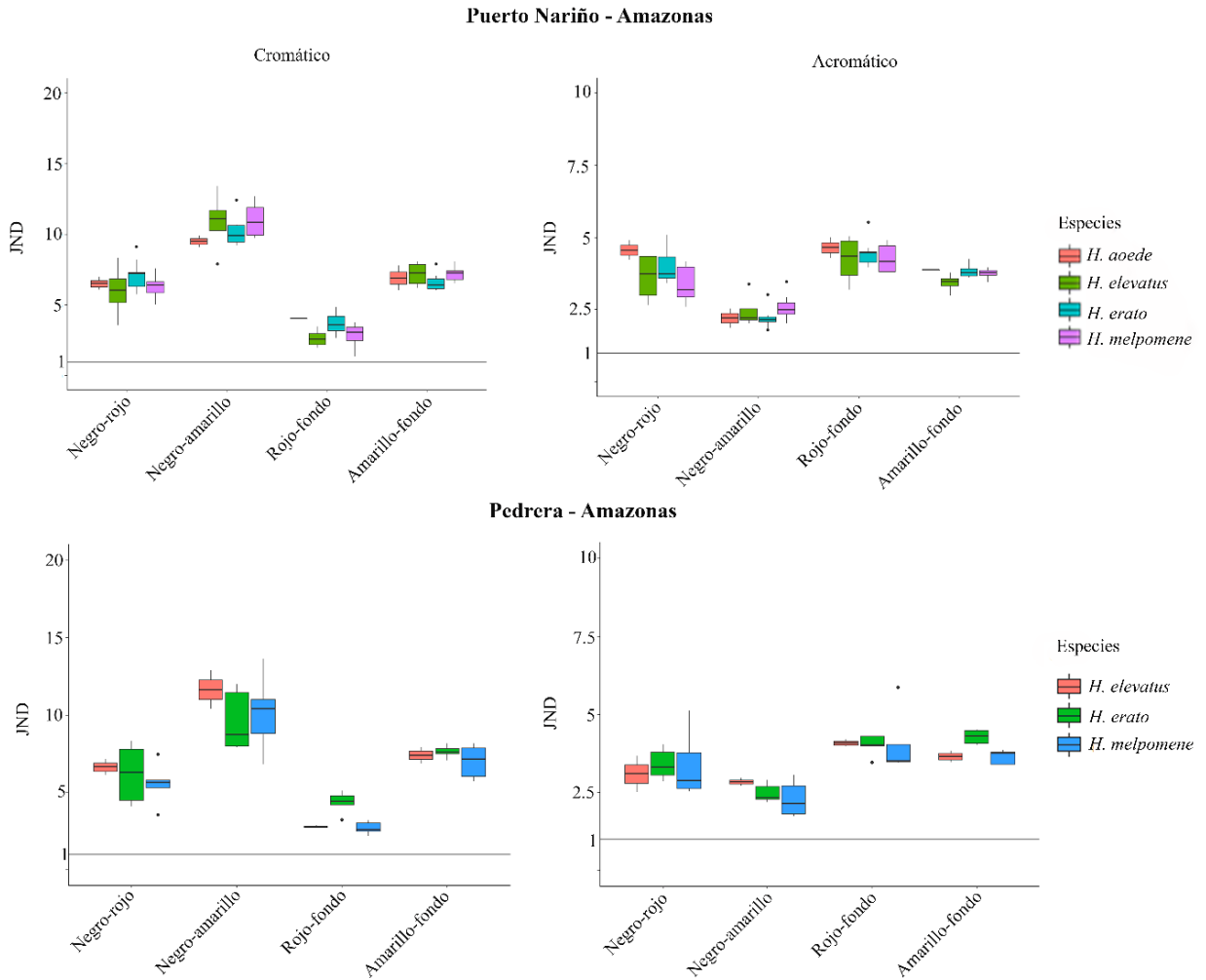
**Figura S12. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental bosque.



**Figura S13. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental bosque en transición.



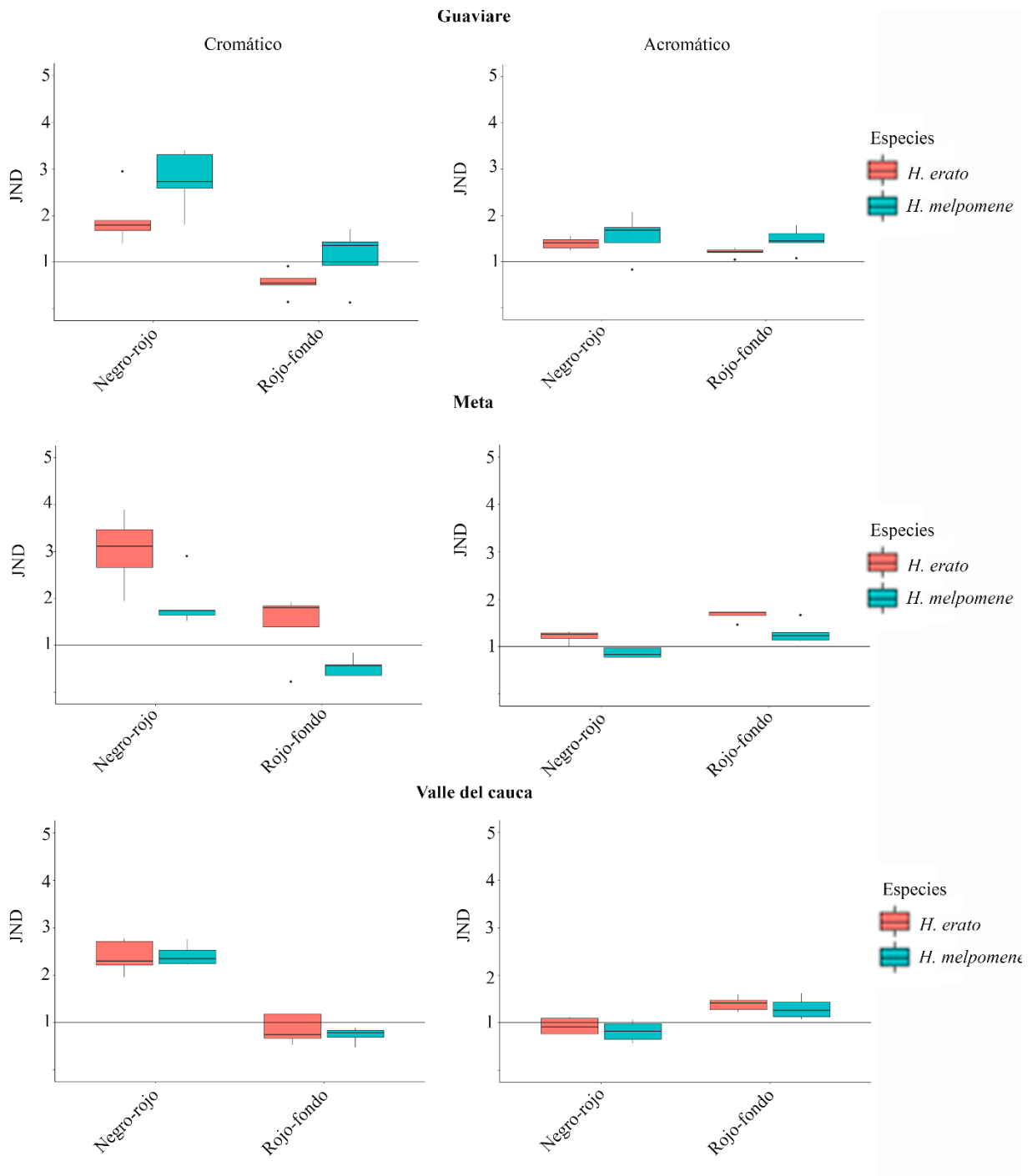
**Figura S14. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental claro pequeño.



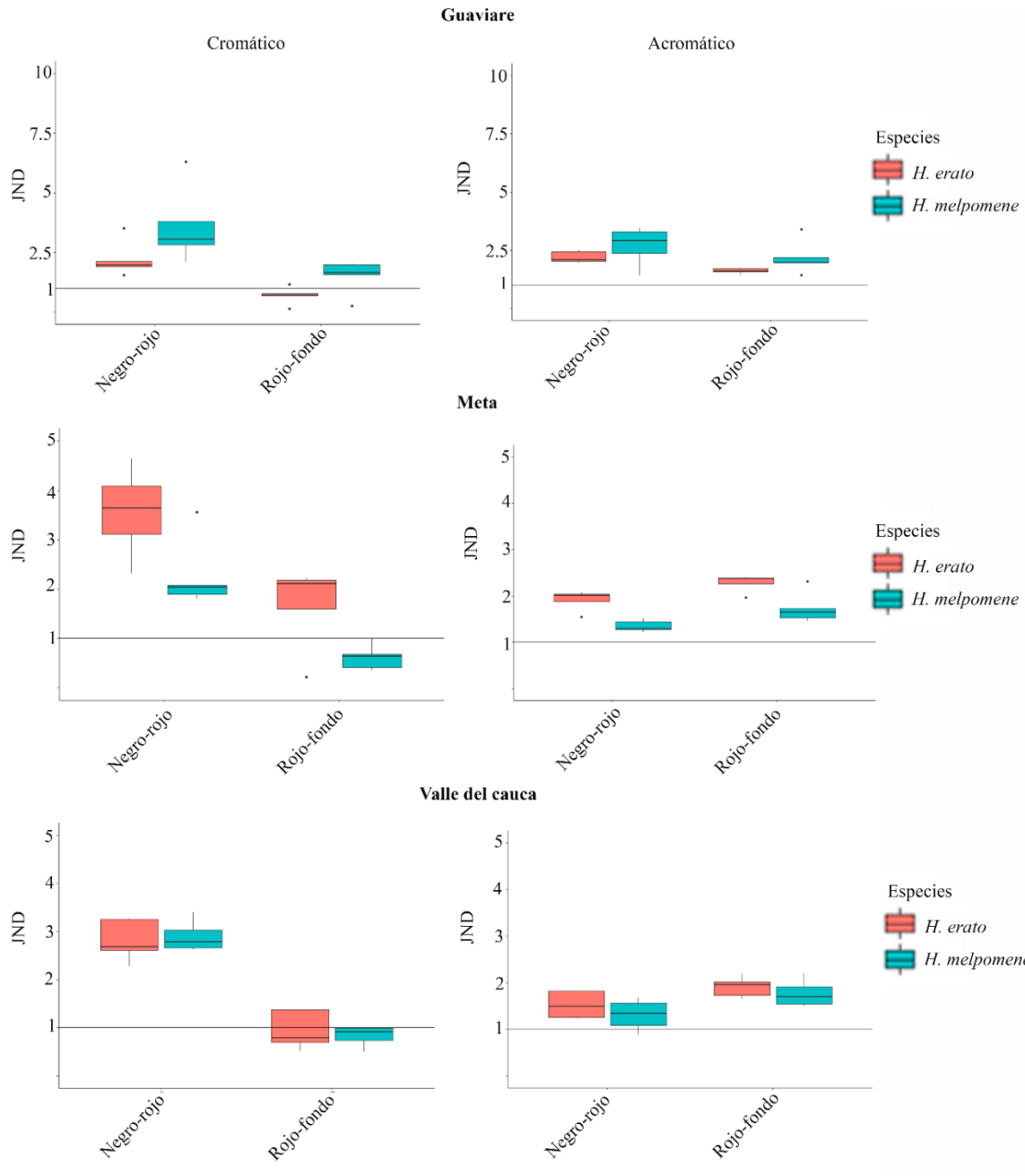
**Figura S15. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *dennis-ray* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental claro grande.



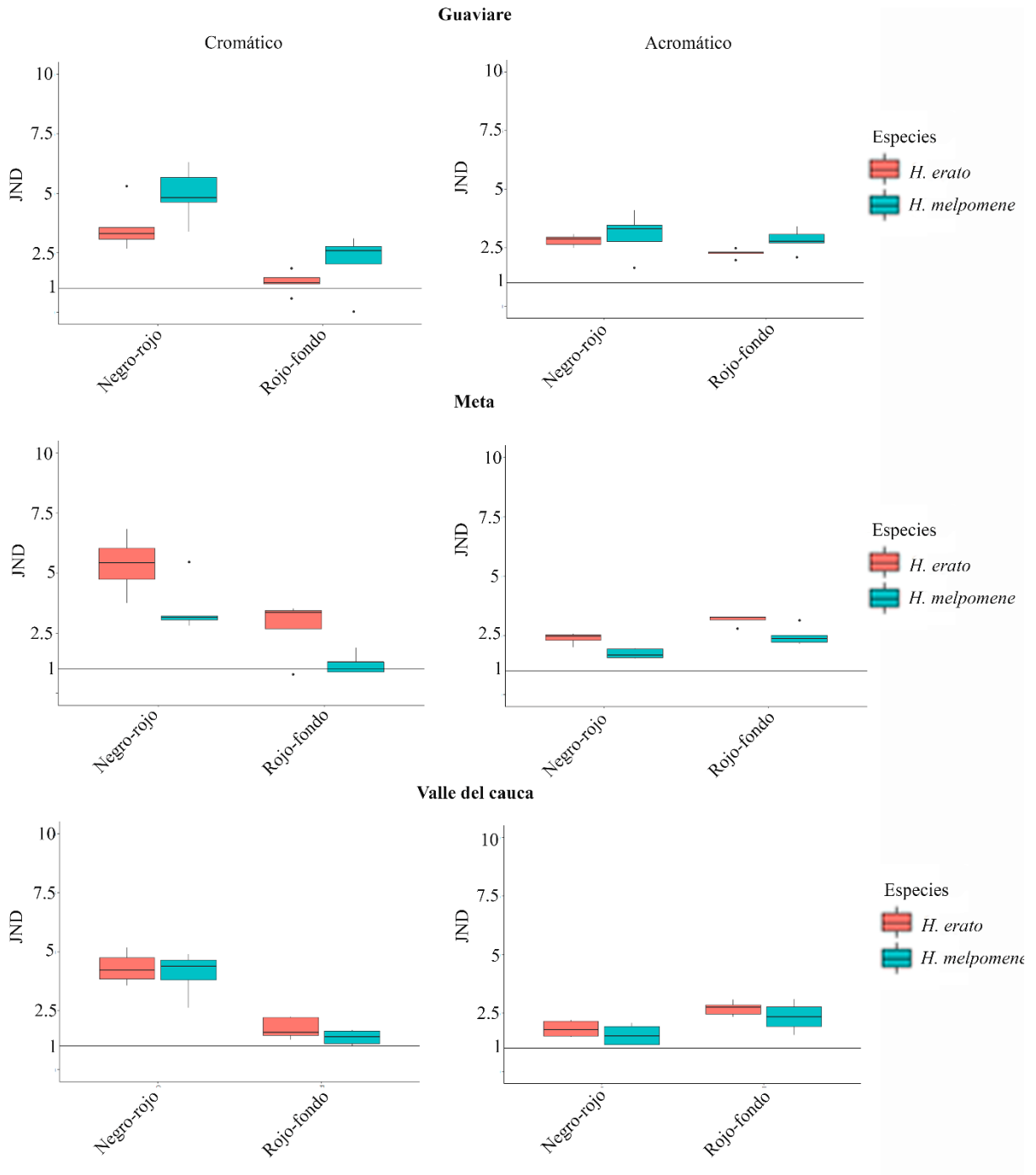
## Postman



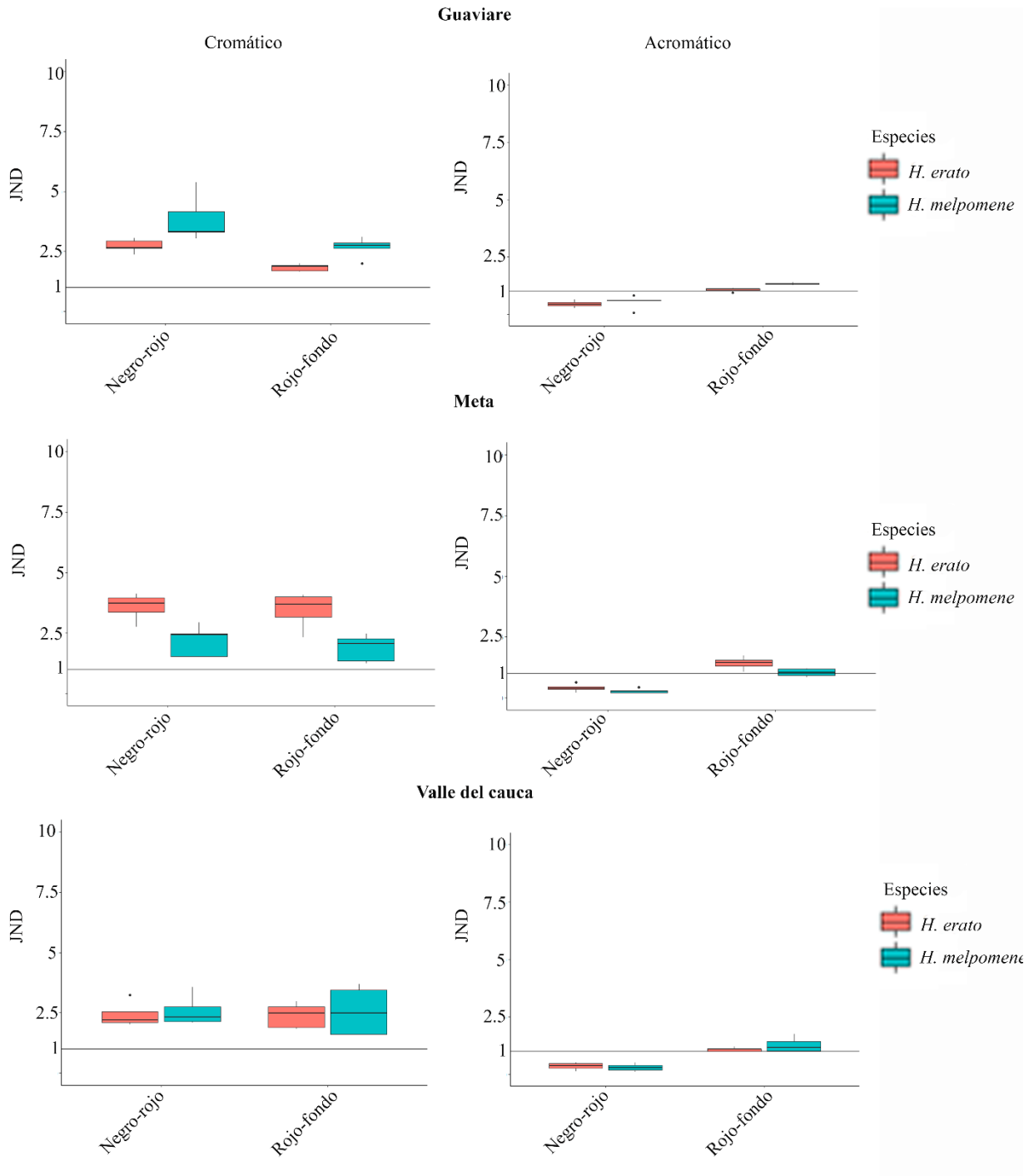
**Figura S16. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental bosque.



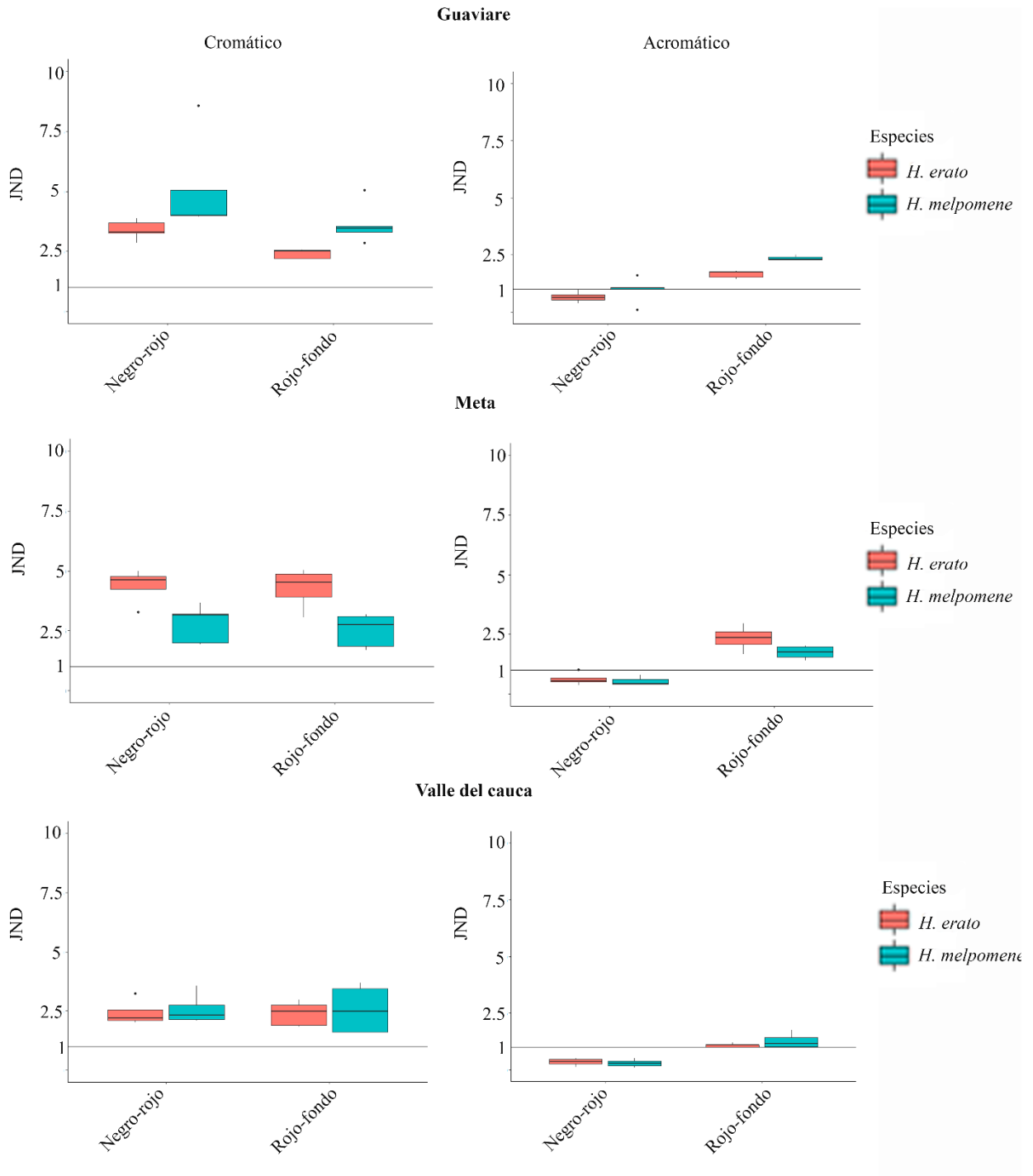
**Figura S17. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental bosque en transición.



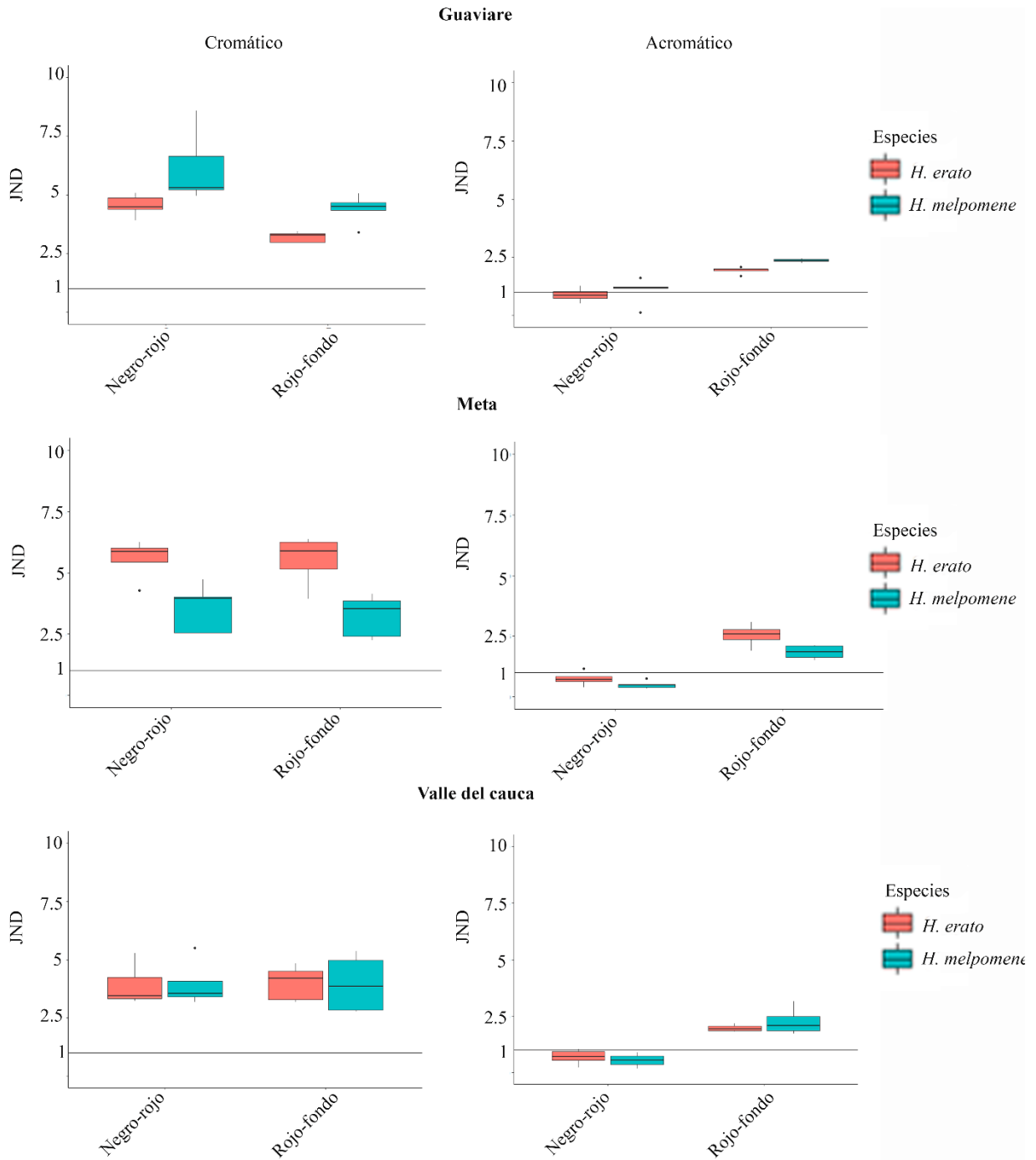
**Figura S18. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista dorsal del ala y la luz ambiental claro pequeño.



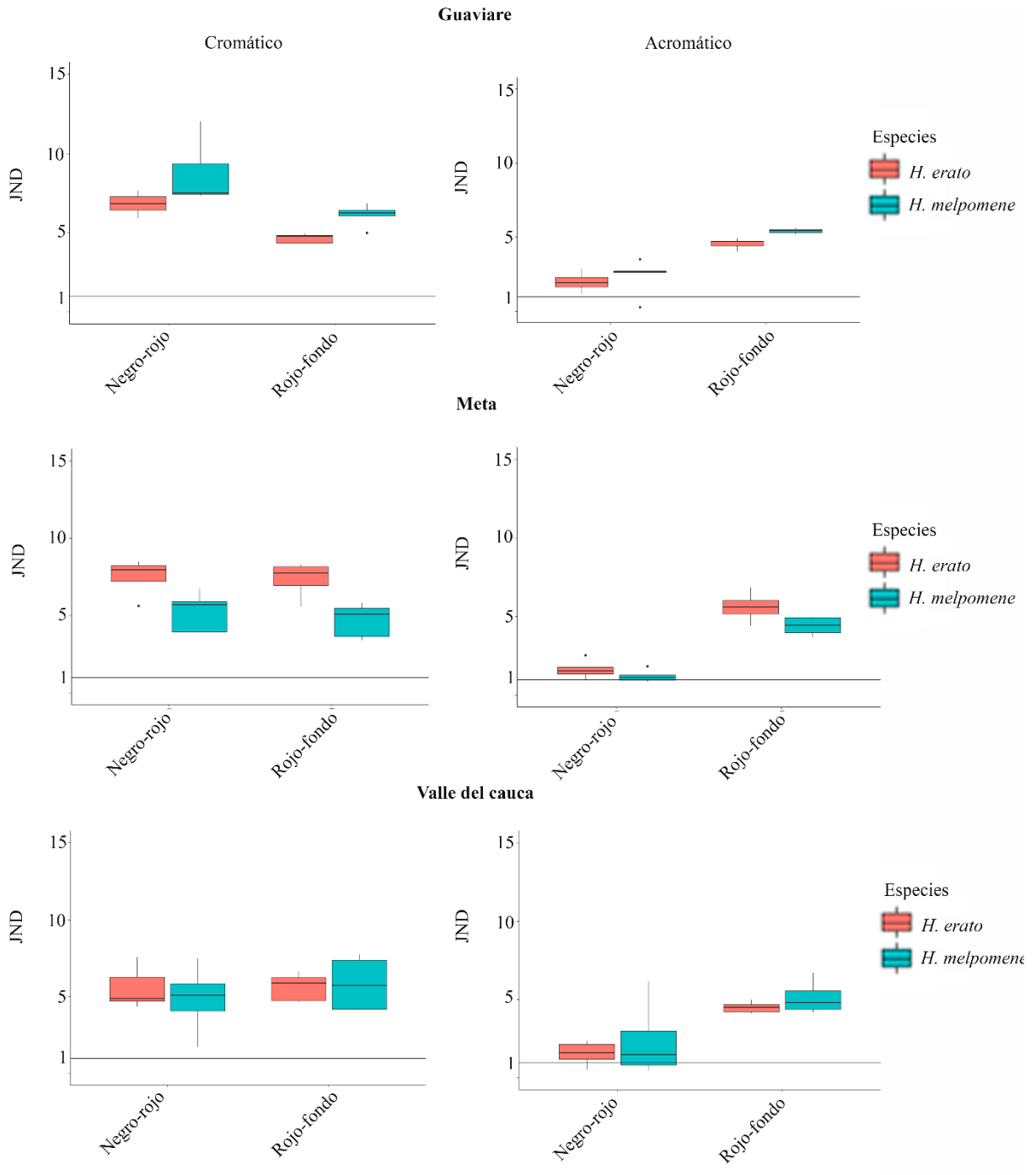
**Figura S19. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental bosque.



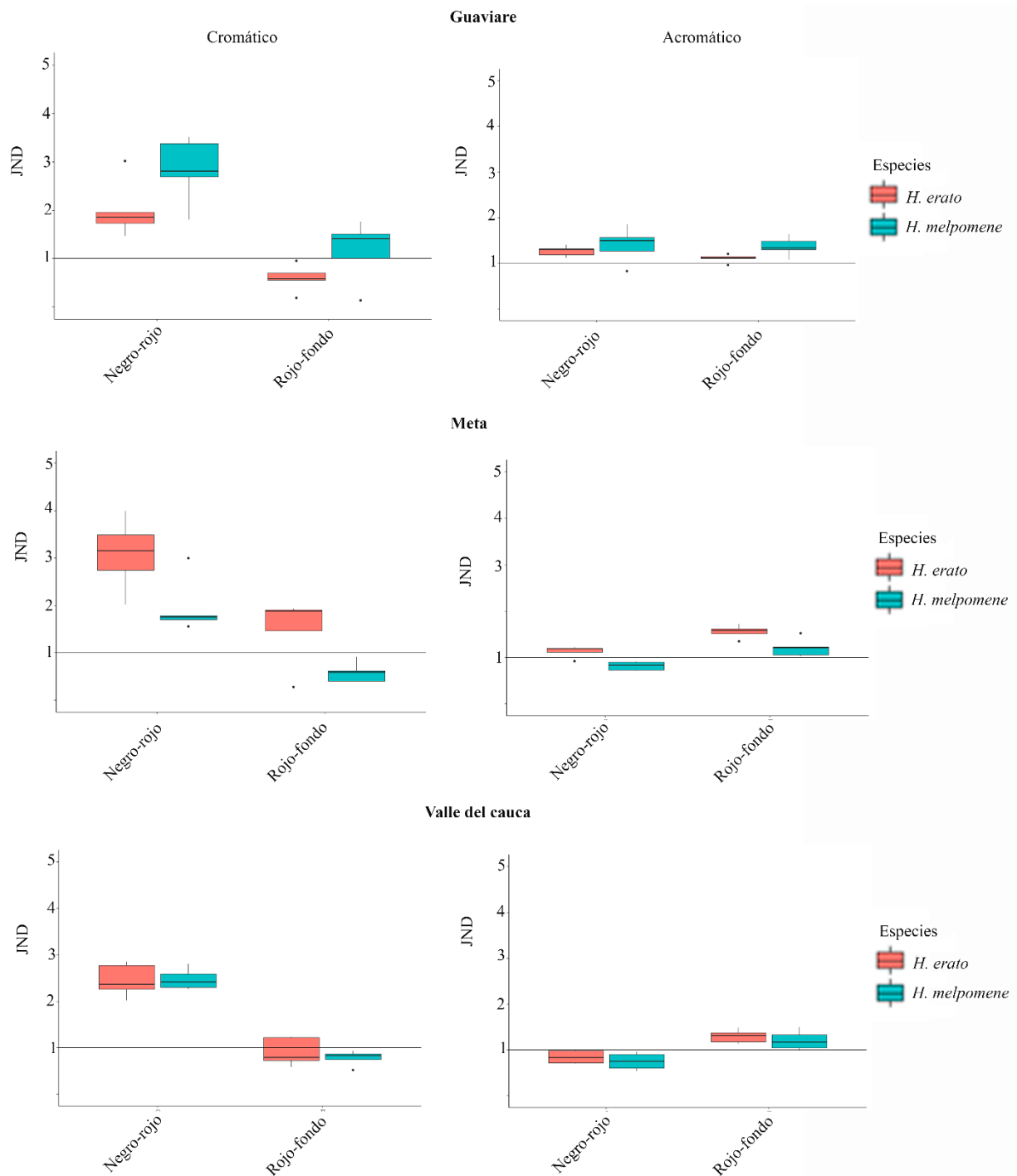
**Figura S20. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental bosque en transición.



**Figura S21. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental claro pequeño.

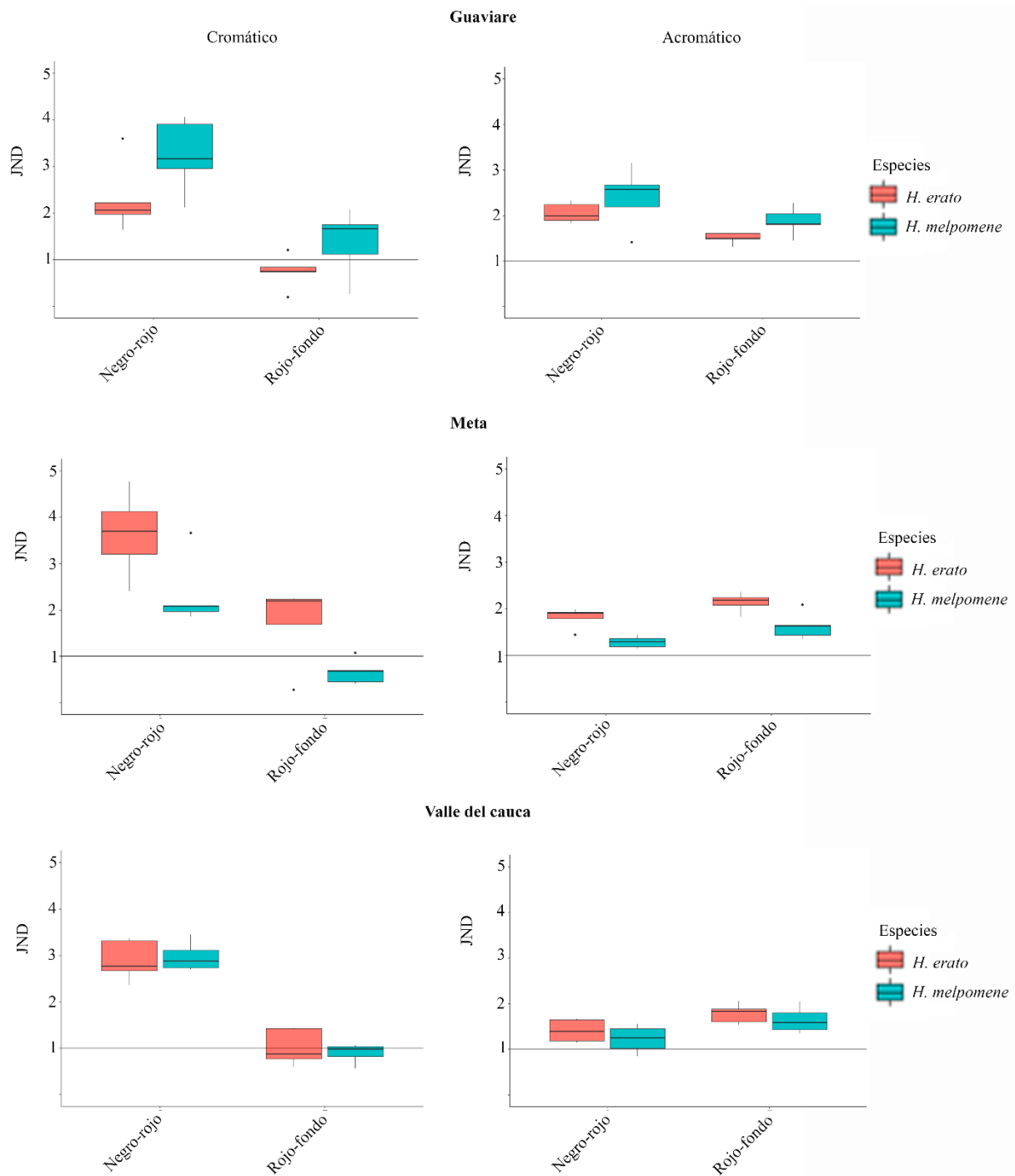


**Figura S22. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave UVS, vista ventral del ala y la luz ambiental claro grande.

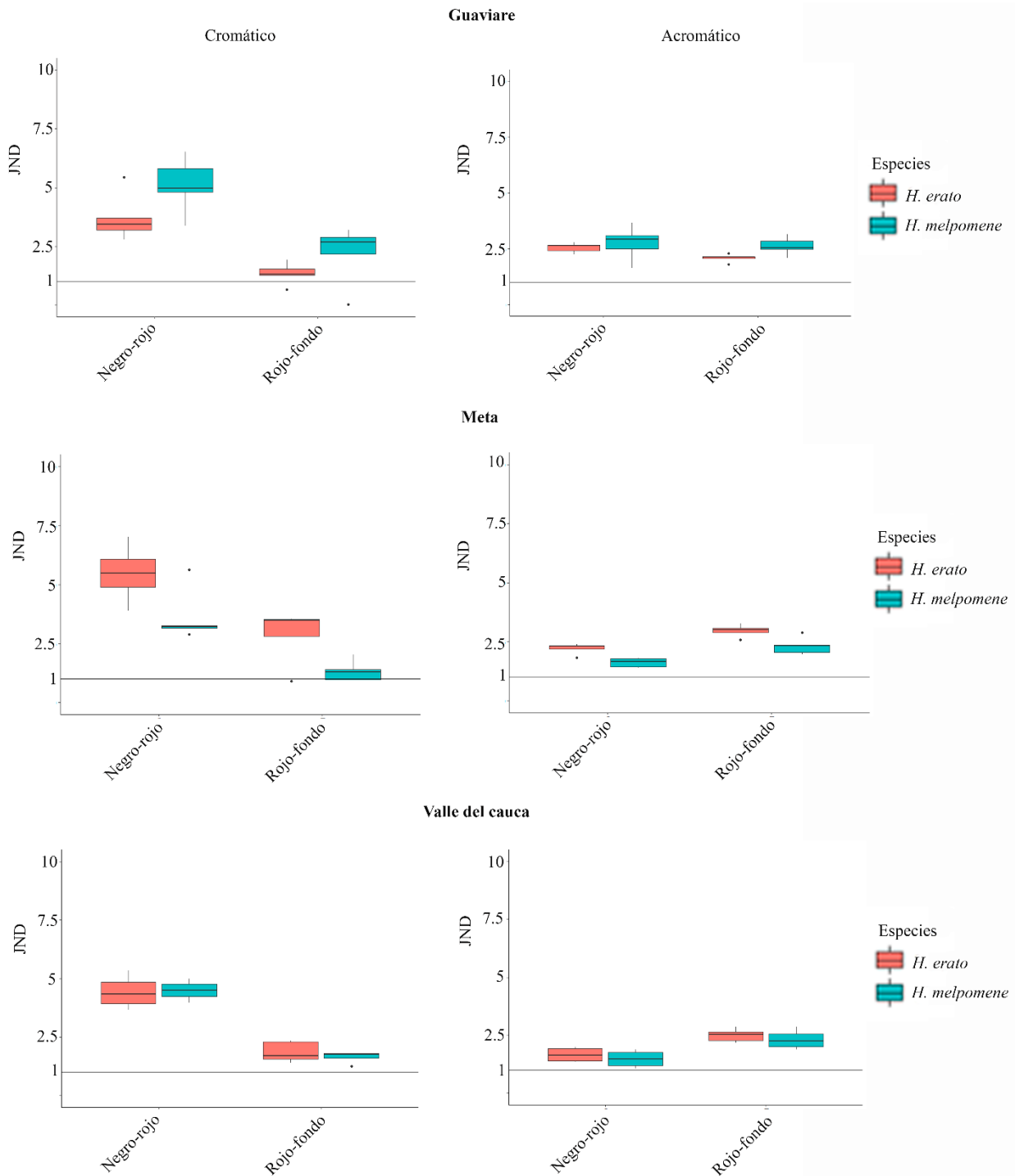


**Figura S23. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental bosque.

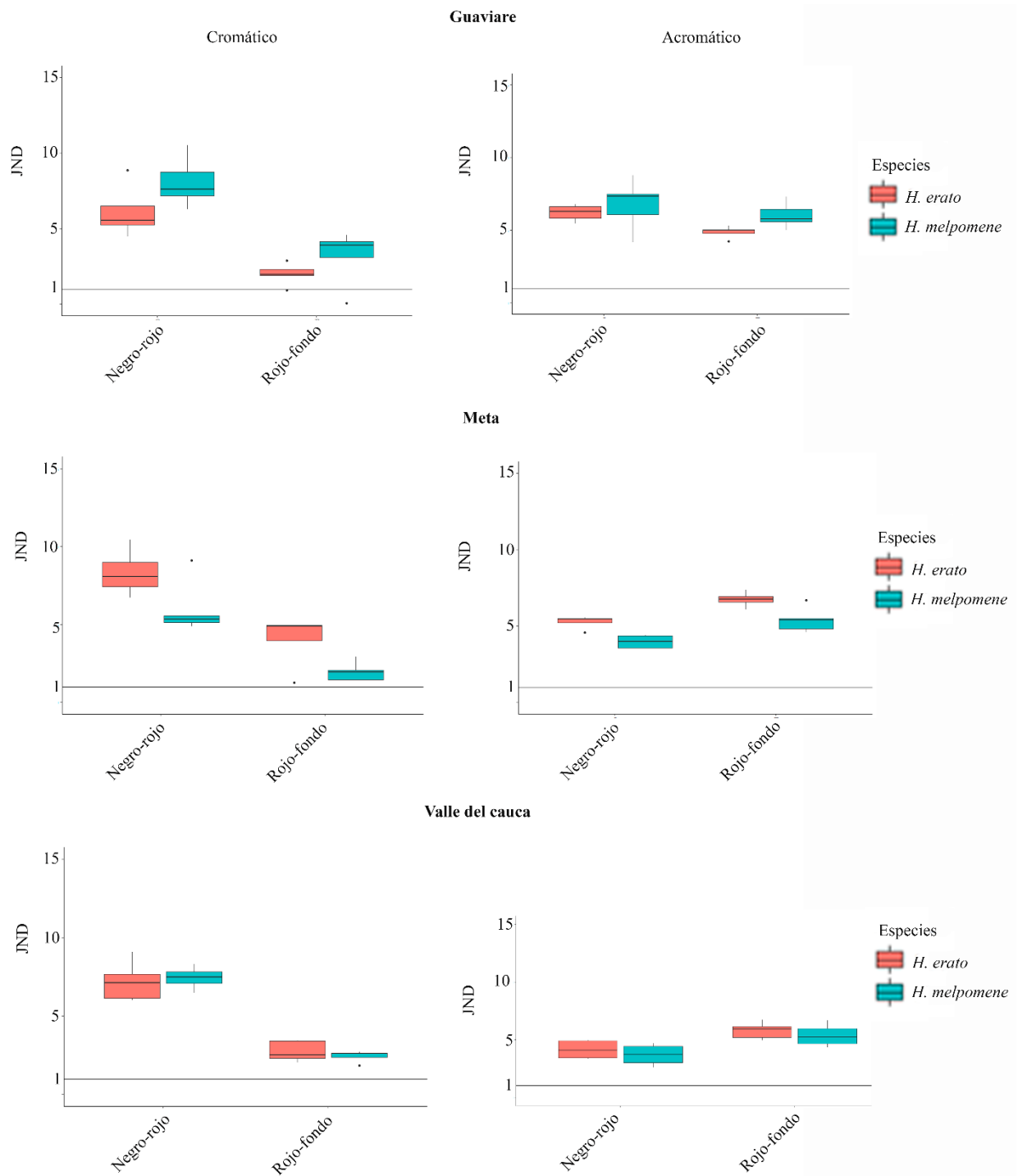




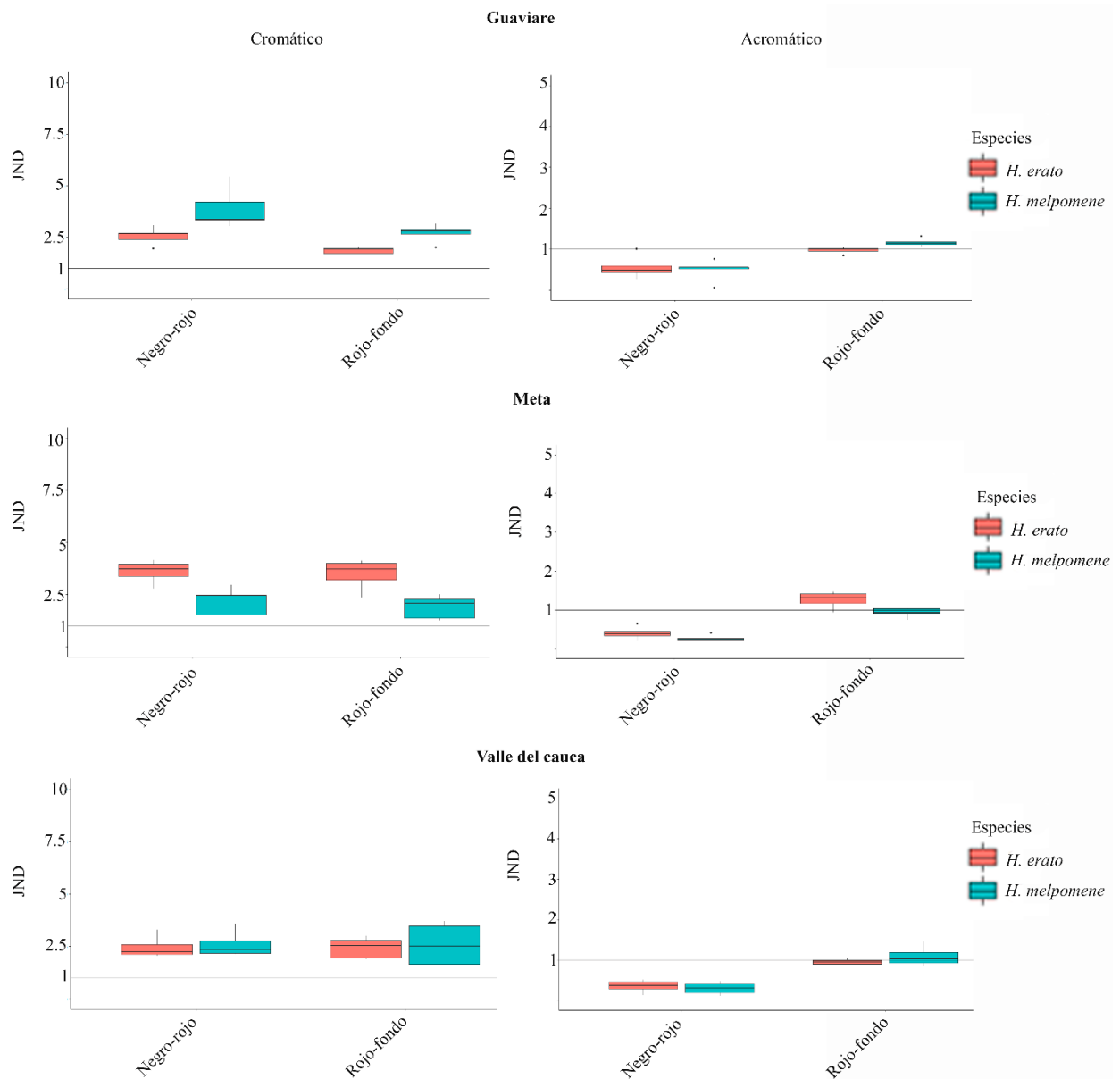
**Figura S24. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental bosque en transición.



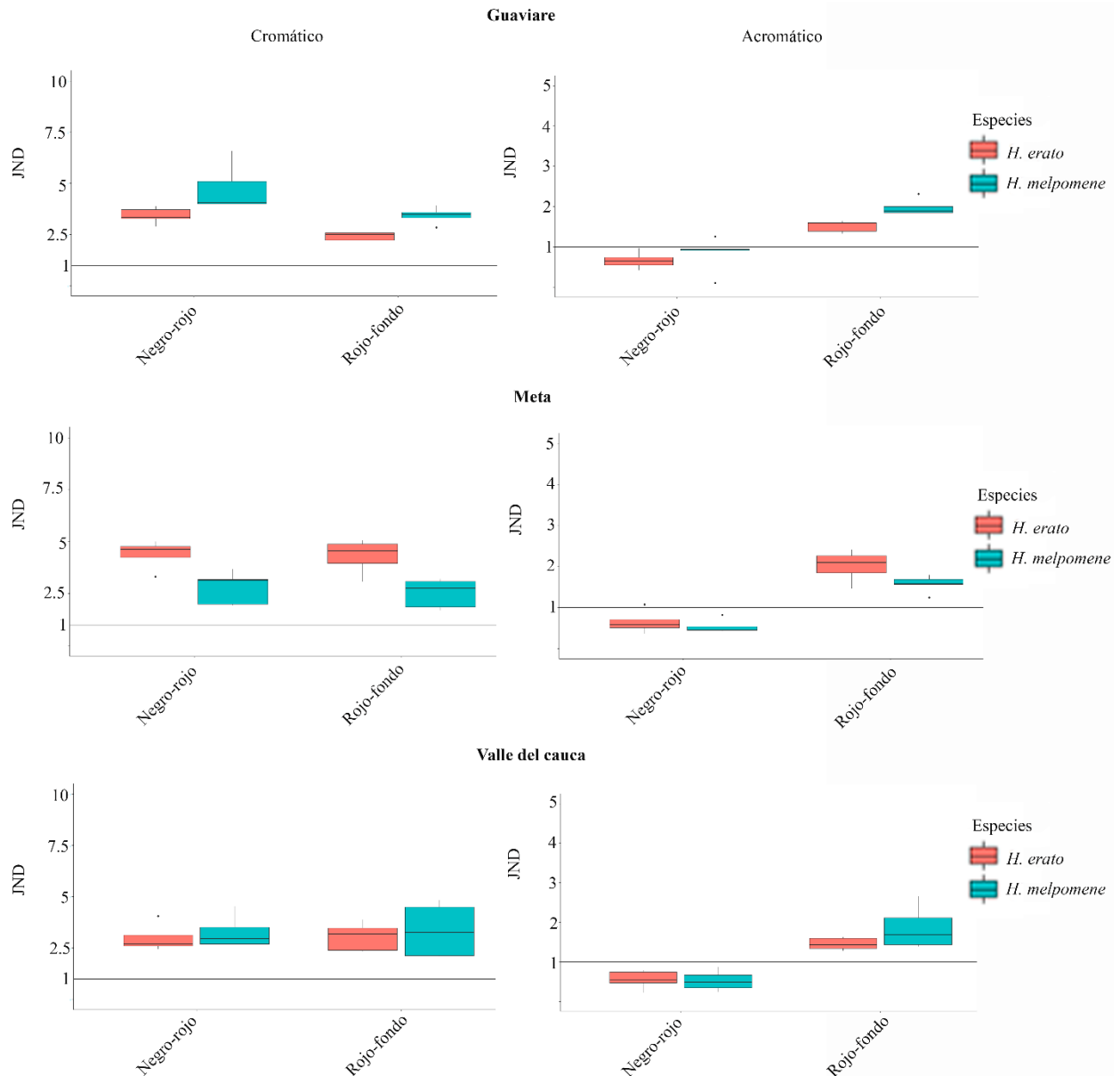
**Figura S25. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental claro pequeño.



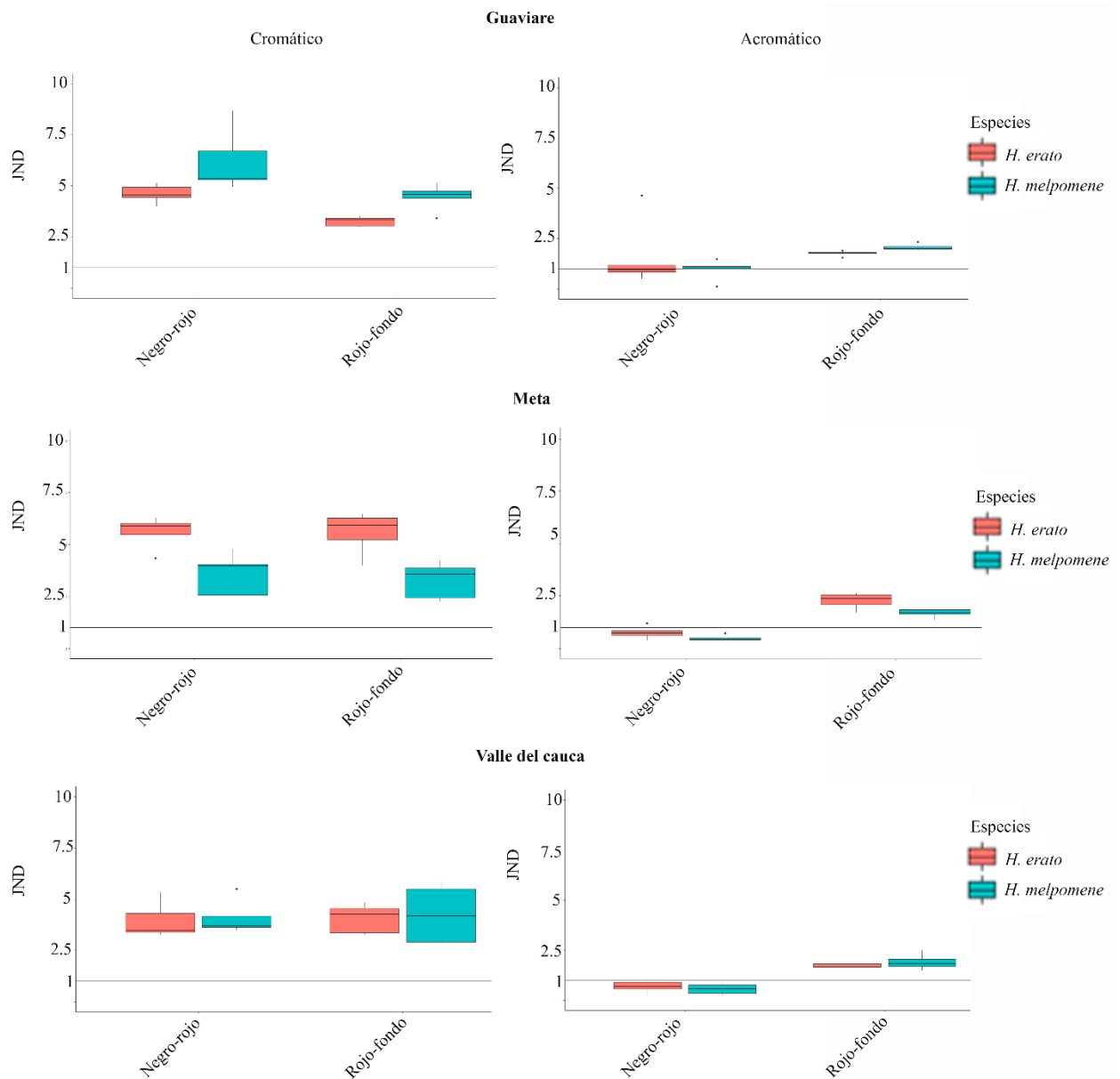
**Figura S26. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista dorsal del ala y la luz ambiental claro grande.



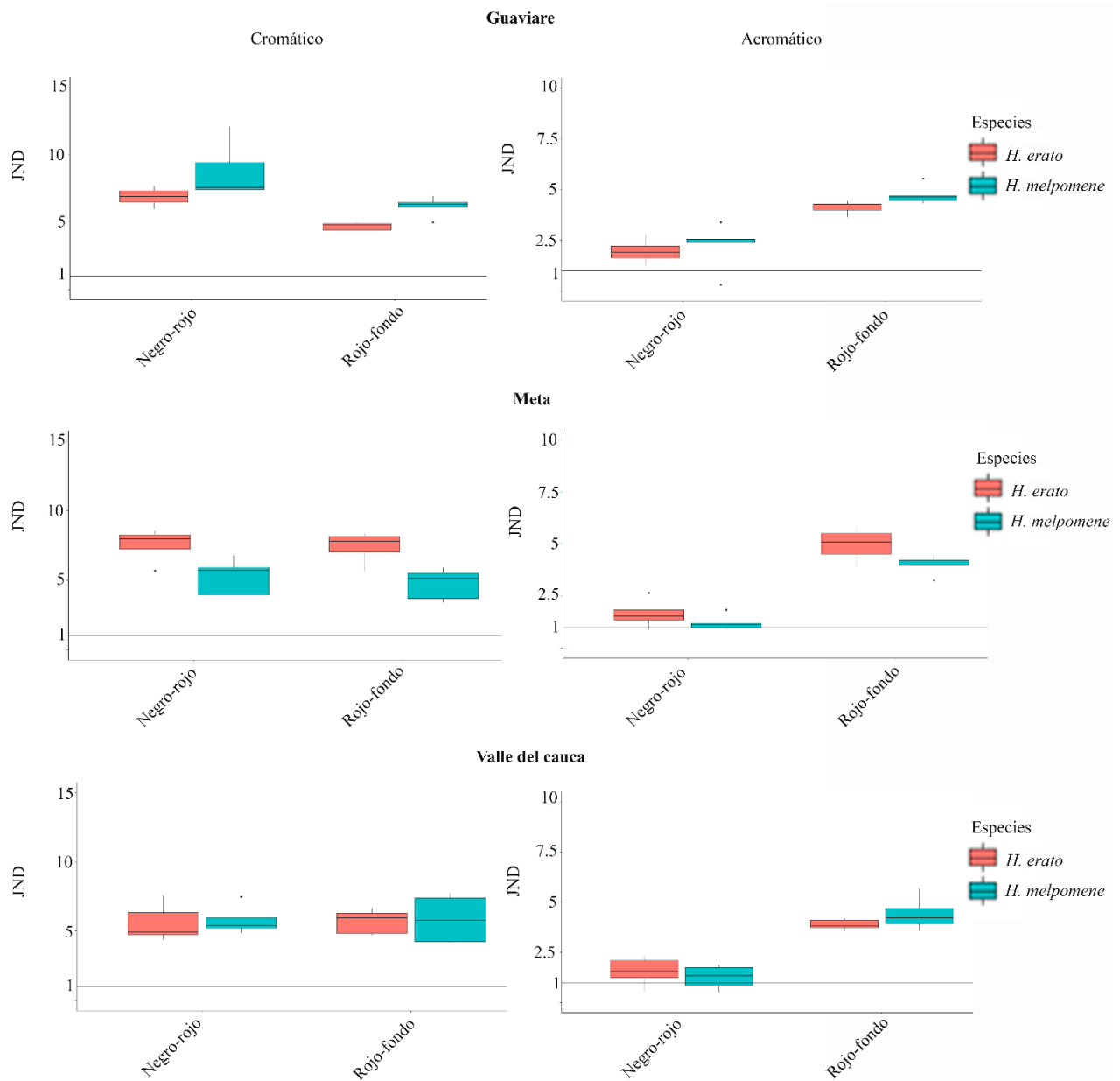
**Figura S27. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental bosque.



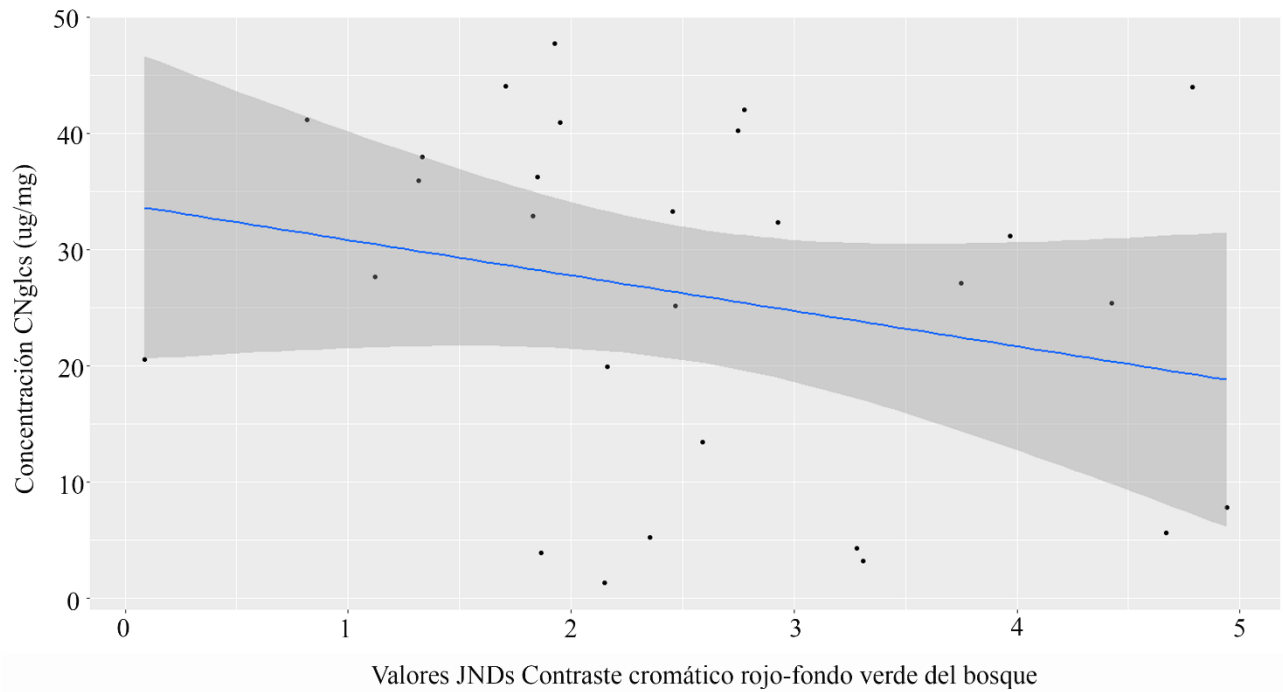
**Figura S28. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental bosque en transición.



**Figura S29. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental claro pequeño.

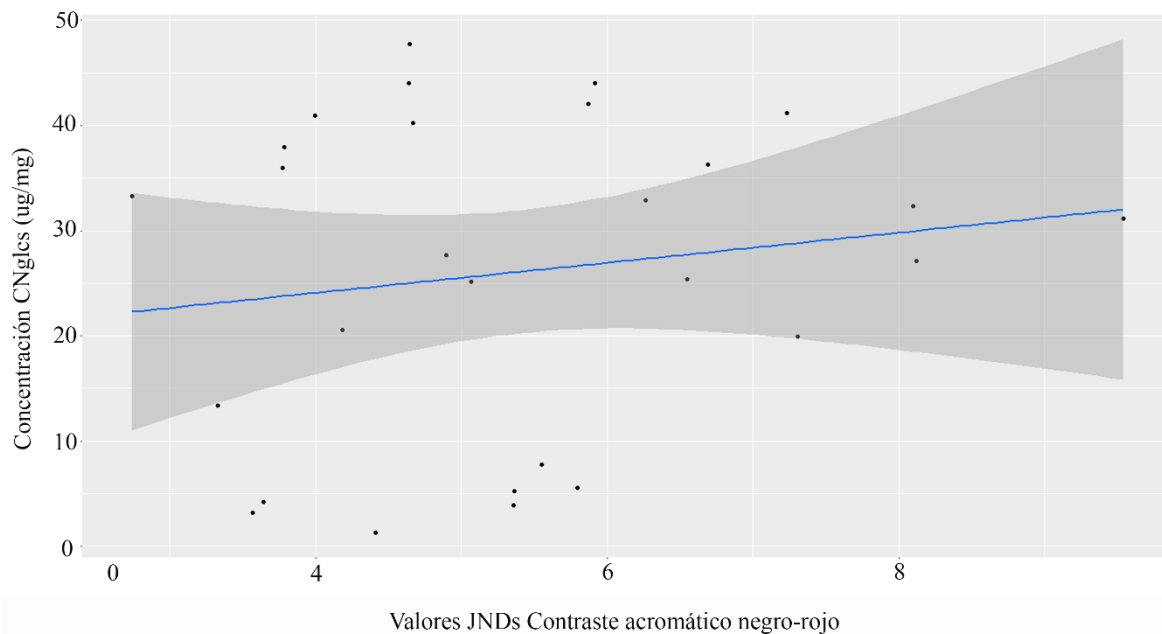


**Figura S30. JNDs entre los parches de color para cada especie para los diferentes contrastes.** Contraste cromáticos y acromáticos entre las diferentes bandas de color contra el negro y el fondo del bosque para el anillo *postman* en las diferentes localidades. Evaluados bajo el tipo de visión del ave VS, vista ventral del ala y la luz ambiental claro grande.

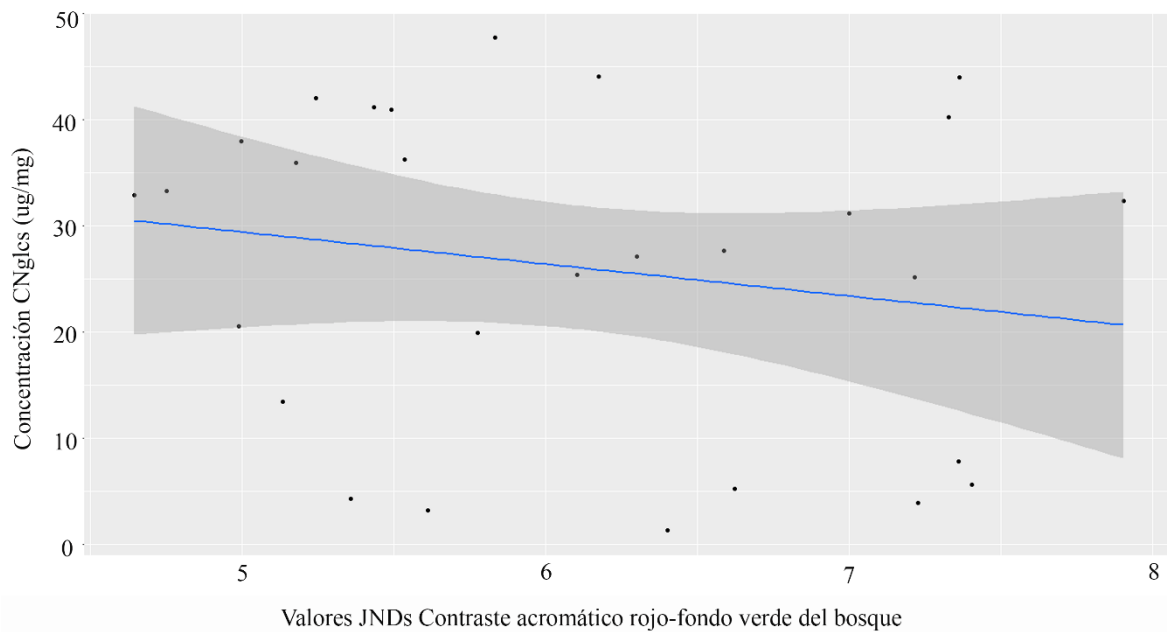


**Figura S31. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *postman*.** A) Eje X: Valores JNDs para contraste cromático rojo contra el fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *postman*. Eje Y: Concentración total de CNglcs por cada individuo perteneciente al anillo *postman*.

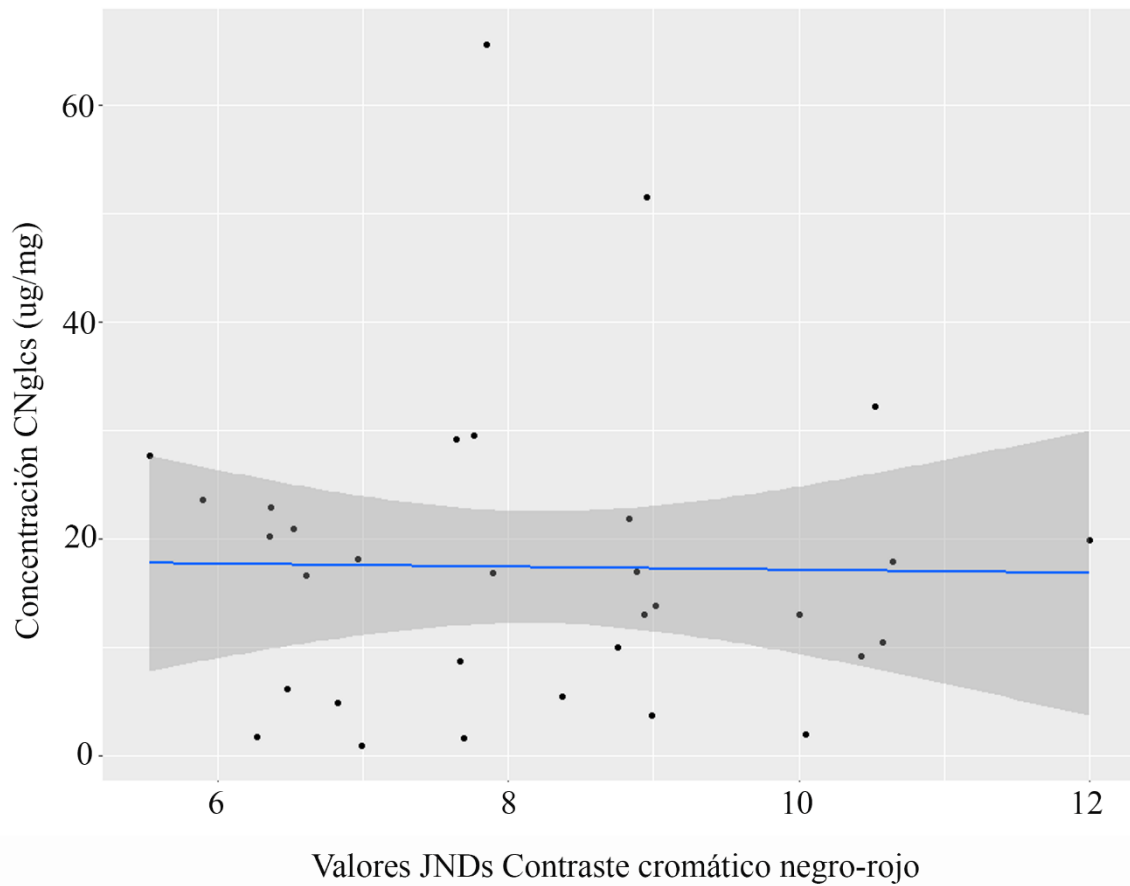




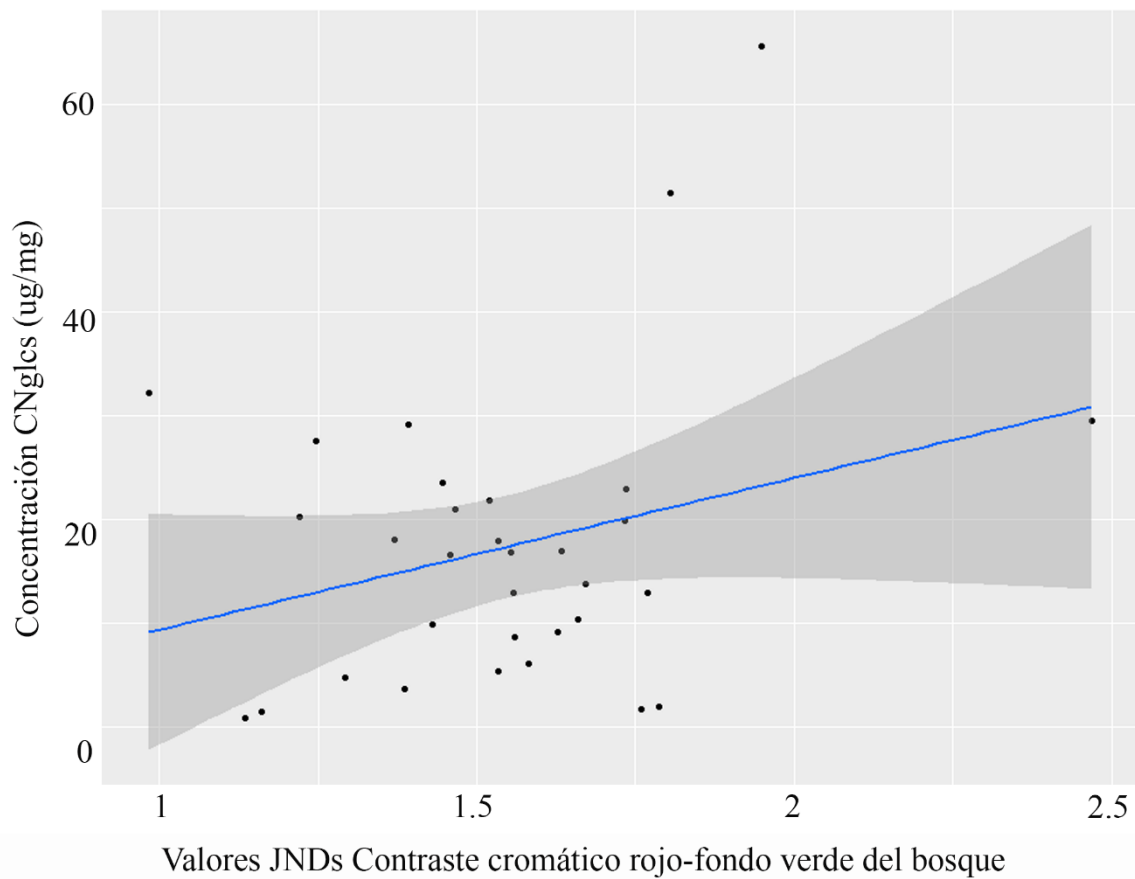
**Figura S32. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *postman*.** A) Eje X: Valores JNDs para contraste acromático negro interno del ala contra rojo, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *postman*. Eje Y: Concentración total de CNglcs por cada individuo perteneciente al anillo *postman*.



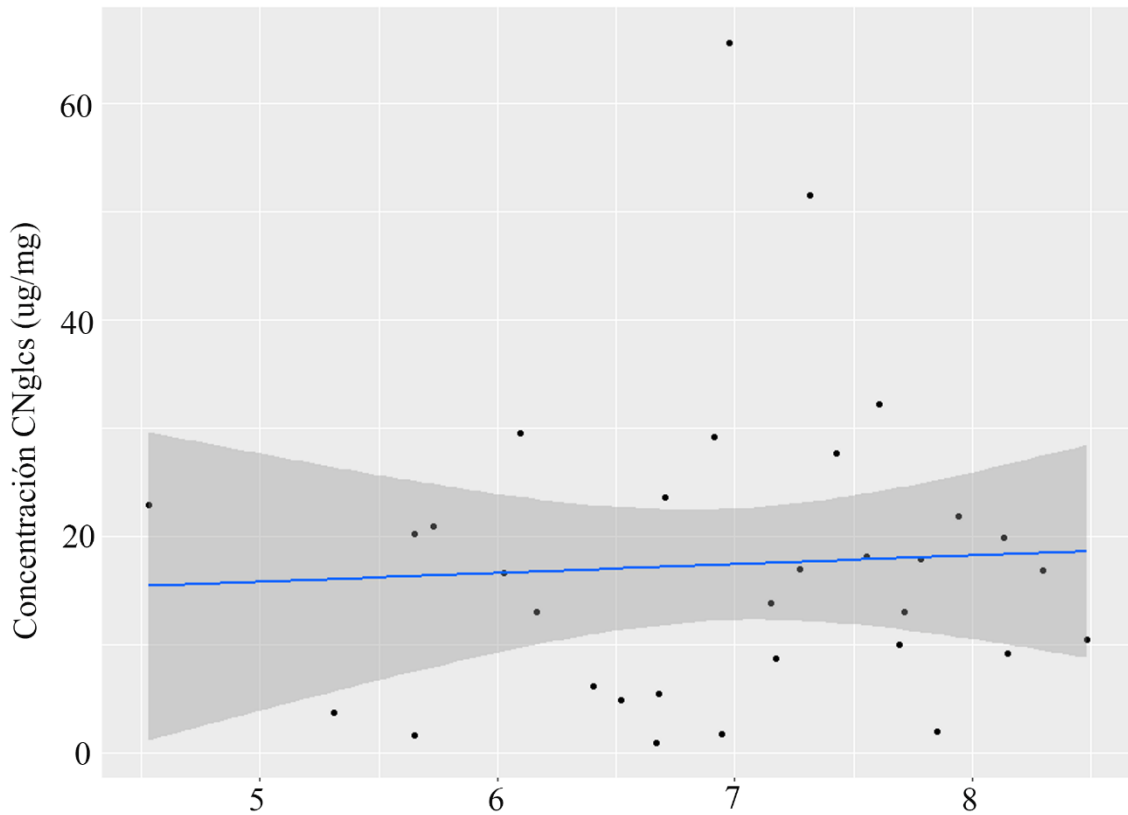
**Figura S33. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *postman*.** A) Eje X: Valores JNDs para contraste acromático rojo contra fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *postman*. Eje Y: Concentración total de CNglcs por cada individuo perteneciente al anillo *postman*.



**Figura S34. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste cromático negro interno del ala contra rojo, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNgles por cada individuo perteneciente al anillo *dennis-ray*

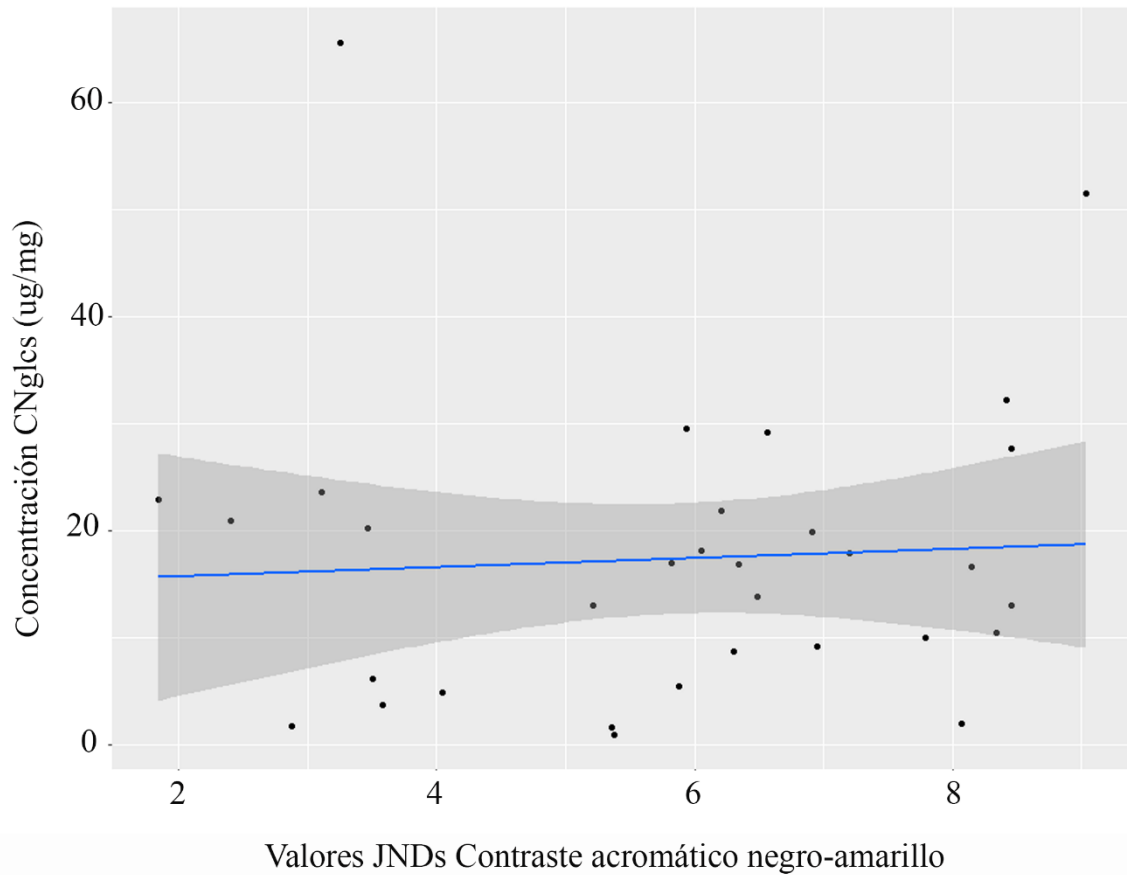


**Figura S35. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste cromático rojo contra fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNgles por cada individuo perteneciente al anillo *dennis-ray*

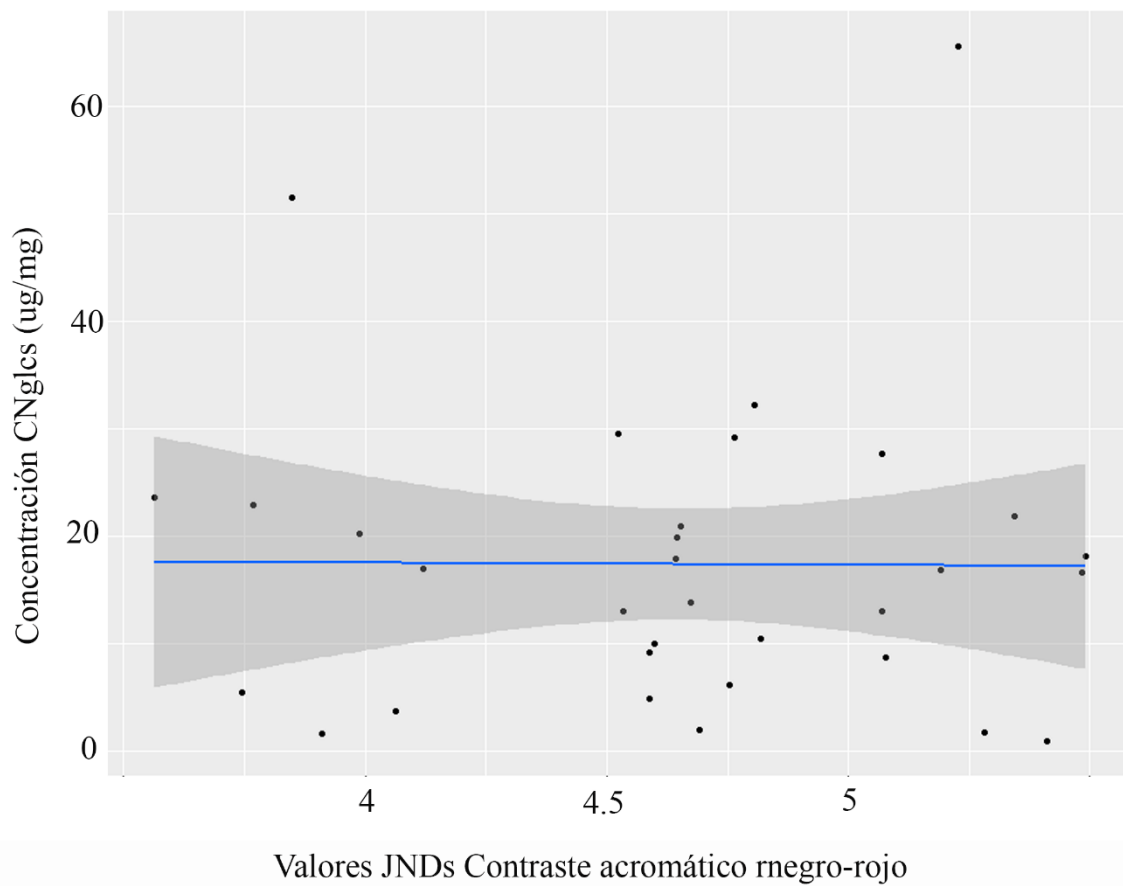


Valores JNDs Contraste cromático amarillo-fondo verde del bosque

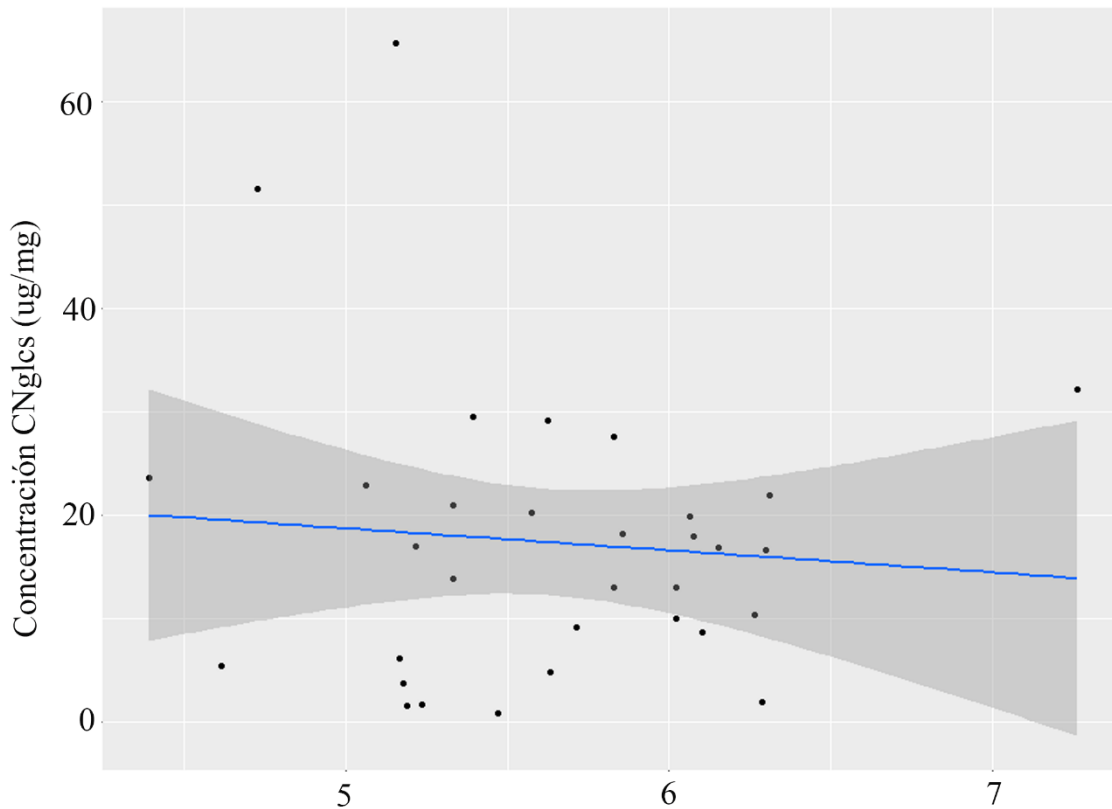
**Figura S36. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste cromático amarillo contra fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNgles por cada individuo perteneciente al anillo *dennis-ray*



**Figura S37. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste acromático negro interno del ala contra el amarillo, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNgles por cada individuo perteneciente al anillo *dennis-ray*.



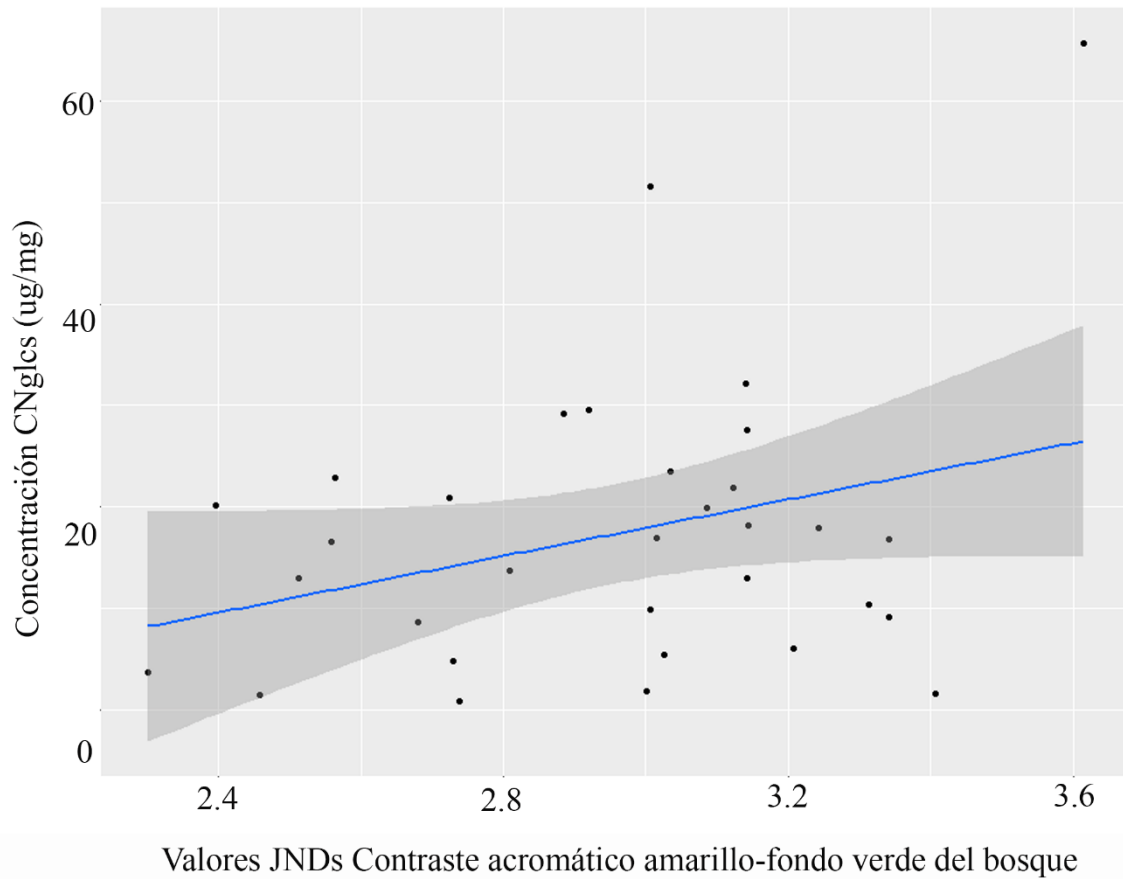
**Figura S38. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste acromático negro interno del ala contra el rojo, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNgles por cada individuo perteneciente al anillo *dennis-ray*.



Valores JNDs Contraste acromático rojo-fondo verde del bosque

**Figura S39. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste acromático rojo contra fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNGlcs por cada individuo perteneciente al anillo *dennis-ray*.





**Figura S40. Comportamiento y visualización de la no correlación entre coloración-toxicidad para el anillo *dennis-ray*.** A) Eje X: Valores JNDs para contraste acromático amarillo contra fondo verde del bosque, evaluado bajo el tipo de visión aviar UVS, vista dorsal del ala y luz ambiental claro grande para el anillo *dennis-ray*. Eje Y: Concentración total de CNglcs por cada individuo perteneciente al anillo *dennis-ray*.

1 **Chemical defense variation in *Heliconius***  
2 **butterflies: testing the role of mimicry rings and**  
3 **ecoregions**

4  
5 Nicol Rueda - M<sup>1</sup>, Érika C. Pinheiro de Castro<sup>2</sup>, Carolina Pardo-Diaz<sup>1</sup>, Frasella de  
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34 **Abstract**

35

36 Chemically defended animals often exhibit conspicuous colour patterns to  
37 warn and teach predators about their unpalatability and thus avoid attacks. This  
38 predator learning is fuelled when multiple defended species share a common  
39 warning signal, which promotes convergence between phylogenetically distant  
40 species in sympatry (i.e. Müllerian mimicry). *Heliconius* butterflies are chemically  
41 defended by cyanogenic glucosides (CNglcs), exhibit warning colour patterns, and  
42 most species in the genus participate in local Müllerian mimicry rings. While  
43 previous studies suggest that local comimics vary in terms of identity and  
44 concentration of CNglcs, no study has yet tested whether this chemical variation  
45 holds true when analysing multiple species and mimicry rings across multiple  
46 Neotropical ecoregions. Here, we study variation in the CNglc profile of nine  
47 mimicry rings in *Heliconius* across six Neotropical ecoregions. We found that  
48 variation in the cyanogenic profile of *Heliconius* is not explained by the mimicry ring  
49 that a species belongs to or its locality. Instead, cyanogenic variation is the result  
50 of phylogenetic closeness and, likely, ecological factors, such as host plant  
51 specialization, diversity and abundance of local hostplants, availability of  
52 precursors for biosynthesis of cyanogenic compounds in pollen-source plants, as  
53 well as the local predator community.

## 54 **Introduction**

55

56           Cyanogenic glucosides (CNgls) are secondary metabolites found in many  
57 plants where they usually play a role of protection from herbivores (Nahrstedt,  
58 1996). The presence of CNgls in animals seems to be restricted to arthropods  
59 (Zagobelny et al., 2007), particularly to Coleoptera, Heteroptera and Lepidoptera  
60 (Davis & Nahrstedt, 1987; Zagobelny et al., 2018) which obtain them via *de novo*  
61 synthesis, or more commonly, via sequestration from host plants (Mattila et al.,  
62 2021; Opitz & Müller, 2009). Insects usually use CNgls as deterrents due to their  
63 bitterness and as defence due to the release of hydrogen cyanide (HCN) (Pentzold  
64 et al., 2016; Zagobelny et al., 2018). These defences are usually coupled with  
65 bright colour patterns (warning signals) that local predators learn to avoid, which is  
66 known as aposematism (Ruxton et al., 2018). Because benefits of aposematism to  
67 preys increase with population size, multiple prey species within an area may  
68 evolve towards a single local phenotype, thereby leading to local Müllerian mimicry  
69 rings (Müller, 1879). The resulting convergence in colour patterns allows co-  
70 mimics to share the cost of teaching predators and lower the *per capita* predation  
71 risk (Joron & Mallet, 1998; Mallet, 2010). Despite the theoretical premise that  
72 convergence should favour the establishment of a single local Müllerian ring, there  
73 is an immense mimetic diversity in nature even at the population level, which leads  
74 to local polymorphisms. However, the latter is possible if toxic compounds are  
75 deterrent enough to predators (Kuo, 2023).

76           Variation in chemical defences within and between prey populations both in  
77 terms of chemical composition and concentration has been widely documented  
78 (Arias et al., 2016; Eisner, 1967; Goodger et al., 2002; Speed et al., 2012).  
79 Although the drivers of this variation are still unknown, frequency dependent  
80 selection and genetic variability may broadly contribute to the toxicity spectrum  
81 (Skelhorn & Rowe, 2005; Yezerski et al., 2004). In species that only sequester  
82 CNgls, toxicity may be constrained by geographic and temporal availability of host  
83 plants (Fahey & Garson, 2002; Saporito et al., 2006), variation in the concentration  
84 and/or availability of CNgls in the host plant (Camara, 1997; Hay-Roe & Nation,

85 2007), and detoxification capacity of the prey to overcome the chemical arsenal of  
86 the host plant (Reudler et al., 2015). In contrast, in prey species that *de novo*  
87 synthesise, variation in the cyanogenic profile may be affected by age,  
88 reproductive state, resource optimisation, as well as availability of precursors for  
89 CNgls biosynthesis (Bowers, 1992; Smilanich et al., 2009). Also, because the  
90 protection that CNgls provide to a prey does not increase with toxin concentration  
91 (Chouteau et al., 2019; Kuo, 2023), variation in toxicity may be the result of simple  
92 drift due to cyanogenic profiles being selectively neutral (Briolat et al., 2019; Speed  
93 et al., 2012). In this case, the effectiveness of the warning signal and/or the  
94 mimicry dynamics would rely upon the frequency of the signal rather than the level  
95 of toxicity (Joron & Mallet, 1998). Therefore, it is important to determine whether  
96 variation in toxicity is ubiquitous in communities of mimetic species that are  
97 chemically defended, and if so, investigate the causes behind such variation and  
98 its effects on the mimicry dynamics.

99         Mimetic butterflies of the genus *Heliconius* are chemically defended by  
100 cyanogens that they obtain either via *de novo* synthesis or sequestration from their  
101 host plants (de Castro et al., 2019). Biosynthesis is the most used strategy by  
102 these butterflies (Wray et al., 1983), and it seems to be more important in  
103 generalist (de Castro et al., 2019). In contrast, monophagous specialists mostly  
104 sequester (de Castro et al., 2019; Engler-Chaouat & Gilbert, 2007). For example,  
105 some species in the *sara/sapho* clade exclusively feed on plants of the subgenus  
106 *Astrophea* from each they obtain CNgls, and consequently, their cyanogenic  
107 profile contains virtually no biosynthesised CNgls (de Castro et al., 2019). Multiple  
108 studies have addressed cyanogenic variation in *Heliconius*. One compares  
109 sympatric but non-mimetic species, co-mimics, and mimicry rings in a single  
110 locality in Peru (Arias et al., 2016). Another compares between colour patterns and  
111 subspecies in different countries (Sculfort et al., 2020). A third one focuses on the  
112 species *H. erato* and compares between geographic populations and  
113 environmental gradients (Mattila et al., 2021). However, to date, no study has  
114 tested the generality of the patterns described in those studies, especially in

115 mimetic communities that occur in different Neotropical ecoregions (Morrone et al.,  
116 2022).

117 In this study, we investigated whether there is variation in the cyanogenic  
118 profile and concentration in *Heliconius* from multiple localities across Colombia that  
119 encompass six out of the seven Neotropical ecoregions. We compared the CNGlc  
120 of *Heliconius* butterflies : (i) between phylogenetic clades, (ii) between and within  
121 species, (iii) between sympatric but non mimetic species, and (iv) between and  
122 within mimicry rings. We also investigated whether cyanogenic variation was  
123 explained by phylogenetic relationships, host plant specialization, ability to  
124 biosynthesize or sequester CNGlcs, or the mimicry ring to which a species belongs.

125

## 126 **Materials and methods**

127

### 128 **Sample collection and metabolites extraction**

129

130 We collected 240 individuals of *Heliconius* from 20 species across Colombia (Table  
131 S1 and Figure 1). We immediately preserved the right wing, half of the thorax, and  
132 the abdomen of each individual in a tube containing ~1 mL of 100% methanol to  
133 preserve cyanogenic glucosides (CNGlcs). The remaining tissue was preserved in  
134 DMSO, and left wings were stored in glassine envelopes. Long term storage was  
135 done keeping the tubes at -80°C until further processing. All individuals were  
136 deposited in the 'Colección de Artrópodos de la Universidad del Rosario – CAUR  
137 229'.

138 Samples in methanol were evaporated at room temperature using the  
139 Savant Automatic Environmental SpeedVac System AES1010 until the tissue was  
140 fully dried. Then, the tissue was homogenised using a porcelain mortar and a  
141 pestle, the resulting powder was added into a previously weighed Eppendorf tube  
142 filled with 800 µl of 80% methanol, and the mix was vigorously vortexed. Each  
143 extract was centrifuged at 14,000 g for 5 min and the supernatant was collected in  
144 a glass vial. We then filtered 45 µl of the supernatant and made a 50X (v/v) dilution  
145 in ultra-pure water. Individual extracts were analysed by Liquid Chromatography -

146 Mass Spectrometry (LC-MS) that was conducted either in a LC-Orbitrap (Bruker  
147 Daltonics, Bremen, Germany) or a LC-qToF (Bruker Daltonics, Bremen, Germany)  
148 mass spectrometer.

149

## 150 **Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and chemical** 151 **analyses**

152

153 LC-MS was conducted as previously described for both the LC-Orbitrap-MS (de  
154 Castro et al., 2019) and for the LC-qToF-MS (Thodberg et al., 2020). In both  
155 cases, LC-MS data was analysed using the Bruker Compass DataAnalysis 4.3  
156 software (Bruker Daltonics, Bremen, Germany). CNglcs were identified as formic  
157 adducts  $[M + CH_2O_2]$  in samples analysed by LC-qToF-MS, whereas in those  
158 analysed by LC-Orbitrap-MS, CNglcs were identified as sodium adducts  $[M + Na^+]$   
159 (Table S2). In all cases, the concentration of each CNglc was calculated using the  
160 peak area ratio of the analyte to the internal standard (amygdalin) using a  
161 regression equation generated from a five-point calibration curve for the standard  
162 (concentrations of 0.5, 1.0, 2.0, 5.0 and 20.0 ng/mL). The concentration of each  
163 CNglc is reported in  $\mu\text{g}$  of CNglc/mg of dry butterfly weight. To make sure our data  
164 was comparable despite it being generated in two different MS, we tested whether  
165 the amount of CNglcs resulting from the LC-qToF-MS were different from those  
166 from the LC-Orbitrap-MS with a Mann-Whitney U-test (as data was not normal and  
167 not suitable for transformation).

168 We organised the results per individual into two sets: (i) concentration of all  
169 CNglcs in the sample - referred to as '*CNglcs total*', and (ii) concentration of each  
170 of the CNglcs identified in the sample - referred to as '*CNglcs profile*'.

171

## 172 **Statistical analyses**

173

174 We first tested for differences in mean '*CNglcs total*' between species with a non-  
175 parametric Kruskal-Wallis followed by a post-hoc Dunn test. We adjusted  $p$ -values  
176 for multiple comparisons using the method false discovery rate (FDR). We then

177 compared between sympatric species and between mimetic pairs from the same  
178 location using a Kruskal-Wallis. Additionally, we tested for intraspecific differences  
179 within each species. We conducted our analyses and created figures using the  
180 ggstatsplot package in R software (Patil, 2021).

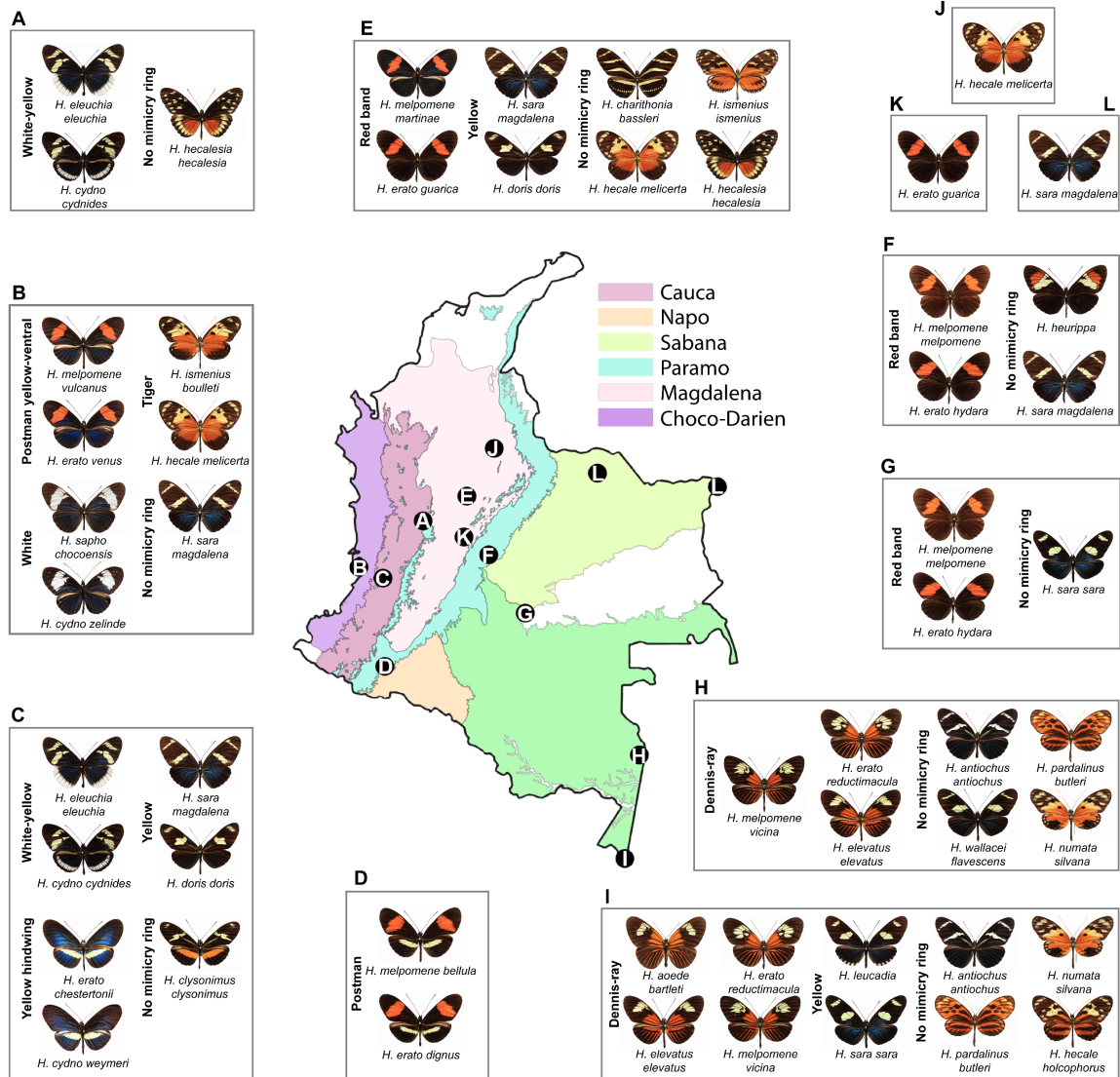
181 We later performed a multivariate analysis of variance (PERMANOVA) in R  
182 to test for differences in 'CNGlc profile' between species, sympatric species and  
183 mimetic pairs from the same location, and for intraspecific differences within each  
184 species. The latter were set as predictor variables whilst 'CNGlc profile' data was  
185 transformed into a Euclidean dissimilarity matrix and used as the dependent  
186 variable. The analysis was carried out with the *adonis2* function in the R package  
187 Vegan (Oksanen, 2017), with 1000 permutations. The post-hoc analysis was  
188 conducted using the *pairwise.adonis* function and the FDR method also in Vegan  
189 (Oksanen, 2017). As before, we compared between sympatric species and  
190 between mimetic pairs from the same location, as well as within species, using  
191 PERMANOVA. Heatmaps were plotted using the function *heatmap.2* in the R  
192 package gplots (Warnes et al., 2022).

193 To investigate whether the 'CNGlc profile' of *Heliconius* is explained by their  
194 phylogeny, we assessed the phylogenetic signal with a phylogenetically controlled  
195 MANOVA (PhyloMANOVA) using the *aov.phylo* function from the package *geiger*  
196 (Pennell et al., 2014) with 10,000 randomizations. Then, we examined the  
197 variation of CNGlcs in a non-metric multidimensional scaling (NMDS) calculating a  
198 Bray-Curtis dissimilarity index between individuals using *metaMDS* function in the  
199 *vegan* (Oksanen, 2017). We calculated NMDS for dimensions ranging from 1 to 10  
200 in order to find the best number of dimensions, and used the *NMDS.scree()*  
201 function to check the associated stress values as a measure of goodness-of-fit  
202 (<https://ourcodingclub.github.io/tutorials/ordination/#section6>). We next used the  
203 *envfit* function of *vegan* to overlap pupal and non-pupal mating clades and  
204 phylogenetic clades onto the NMDS plots without disrupting the original ordinations  
205 (999 random permutations). This generates  $R^2$  and significance values that reflect  
206 whether these clades are associated with the 'CNGlcs profile'. We tested clade  
207 differences in the two main NMDS axes with a non-parametric Kruskal-Wallis



208 followed by a post-hoc Dunn test. False discovery rate (FDR) correction for  
209 multiple testing was used to adjust *p*-values for multiple comparisons.

210 We also examined whether the '*CNglcs profile*' of *Heliconius* is influenced  
211 by: (i) ability to biosynthesize or sequester CNglcs, (ii) host plant specialization,  
212 and (iii) mimicry ring. To test whether the ability to biosynthesize or sequester  
213 CNglcs is associated with the '*CNglcs profile*' we first quantified biosynthesized  
214 compounds (linamarin, lotaustralin, and epilotaustralin) and sequestered  
215 compounds (gynocardin, dihydrogynocardin, tetraphyllin B, epivolkenin, and  
216 deidaclin) per individual. We then categorized each individual as "synthesizer"  
217 when biosynthesized compounds were >50%, "catcher" when sequestered  
218 compounds were >50%, or "synthesizer + catcher" when biosynthesized and  
219 sequestered compounds were in similar proportion. To test whether host plant  
220 specialization is associated with '*CNglcs profile*' we classified individuals based on  
221 their specialization in the following host plants: *Decaloba*, *Astrophea*, or *Passiflora*.  
222



223

224 **Figure 1. Geographic distribution of individuals included in this study.** We collected butterflies  
 225 in 12 locations across Colombia: (A) Caldas, (B) Buenaventura, (C) Cauca Valley, (D) Putumayo,  
 226 (E) Boyacá, (F) Meta, (G) Guaviare, (H) Amazonas-Pedrera, (I) Amazonas-Puerto Nariño, (J)  
 227 Santander, (K) Cundinamarca, and (L) Arauca and Vichada. The species collected in each location  
 228 are shown, as well as the mimicry ring they participate in (unless otherwise indicated).  
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236 **Results**

237

238 **Sample collection**

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240 We collected 240 individuals from 13 localities throughout Colombia, which led to  
241 include nine *Heliconius* mimicry rings: (i) dennis-ray, (ii) yellow, (iii) red band, (iv)  
242 postman, (v) tiger, (vi) white-yellow, (vii) white, (viii) postman yellow-ventral, and  
243 (ix) yellow hindwing (Figure 1 and Table S1). Although we sampled some  
244 *Heliconius* that participate in mimicry rings with members of other tribes or even  
245 moths, these non-*Heliconius* mimics were not included in our analyses (Figure 1).  
246 Also, in some localities we sampled a single species of *Heliconius* either because it  
247 does not participate in a mimicry ring or because we did not collect the mimic  
248 (Figure 1).

249

250 **Quantification of all CNglcs combined ('CNglcs total')**

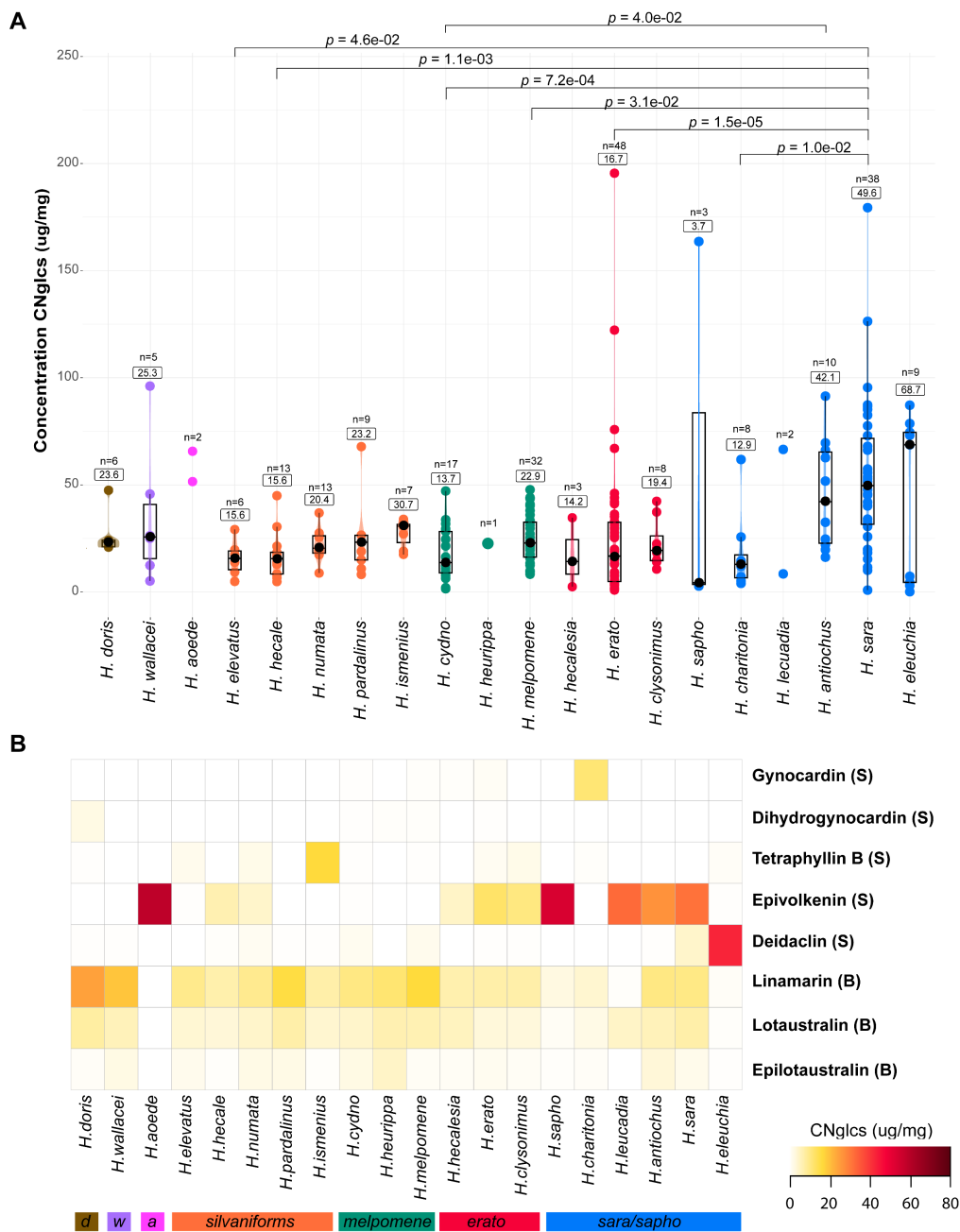
251

252 Levels of CNglcs obtained by LC-qToF-MS and LC-Orbitrap-MS were not different  
253 (Mann-Whitney U-test,  $p > 0.05$ ; Figure S1), and thus, we proceeded to combine  
254 the data. We found that only a few pairs species of *Heliconius* differ in their 'CNglcs  
255 total' (out of 190 comparisons, 7 exhibited differences), with *H. sara* vs. *H.*  
256 *charithonia*, *H. sara* vs. *H. erato*, *H. sara* vs. *H. melpomene*, *H. sara* vs. *H. cydno*,  
257 *H. sara* vs. *H. hecale*, *H. sara* vs. *H. elevatus* and *H. antiochus* vs. *H. cydno* being  
258 the most different (Dunn post-hoc test;  $p < 0.05$ , Figure 2A). *H. eleuchia* was the  
259 species with the highest median concentration of CNglcs, followed by *H. sara* and  
260 *H. antiochus* (Figure 2A), meaning that the most toxic species in our sampling are  
261 in the *sara/sapho* clade.

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**Figure 2. Quantification of CNgls in *Heliconius*.** (A) Quantification of all CNgls combined or ‘CNgls total’. Phylogenetic clades are colour coded, from left to right, as follows: *doris* (brown; 1 species), *wallacei* (purple; 1 species), *aeode* (pink; 1 species), *silvaniforms* (orange; 5 species), *melpomene* (green; 3 species), *erato* (red; 3 species), and *sara/sapho* (blue; 6 species). The number of individuals included per species (n) is indicated on top of each box, as well as the mean concentration of CNgls total per species (numbers in squares), except when the number of individuals was less than 3. Significantly different comparisons ( $\alpha < 0.05$ ) are shown. (B) Quantification of each CNgls or ‘CNgls profile’ per species, where the mean concentration (ug/mg) of each compound is colour coded. The phylogenetic clade to which each species belongs is indicated at the bottom following the same colour code as in A. The CNgls quantified are shown at the right, indicating whether they are biosynthesized (B) or sequestered (S).

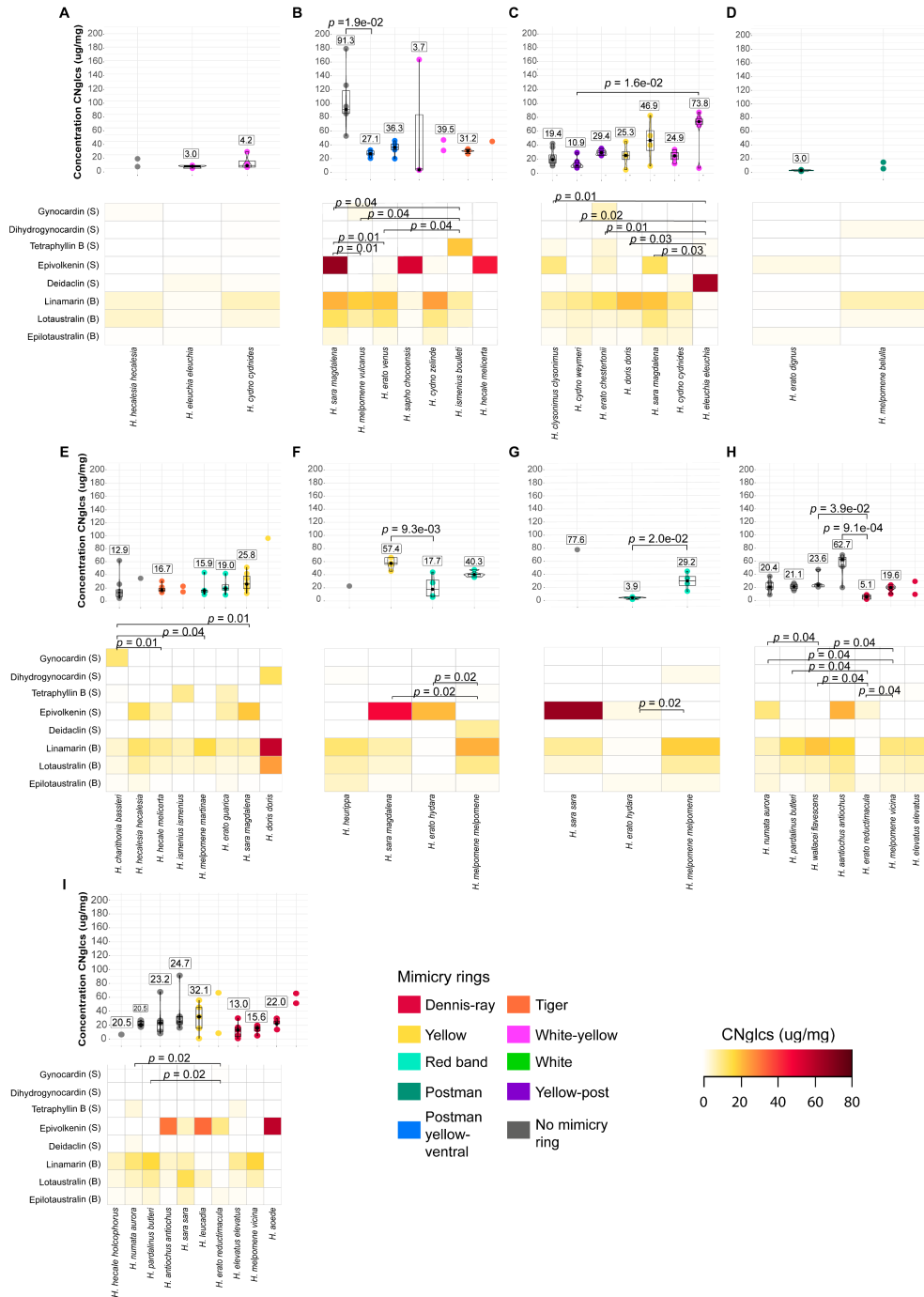
277 Overall, we found sympatric species being similar in overall toxicity. First,  
278 sympatric but non-mimetic species were similar in toxicity except for *H. m.*  
279 *vulcanus* vs. *H. a. antiochus* (Buenaventura, Figure 3B top panel), *H. e. eleuchia*  
280 vs. *H. c. weymeri* (Cauca Valley, Figure 3C top panel), *H. e. hy dara* vs. *H. s.*  
281 *magdalena* (Meta, Figure 3F top panel), and *H. e. reductimacula* vs. *H. a.*  
282 *antiochus* and *H. e. reductimacula* vs. *H. w. flavescens* (Pedrera-Amazonas,  
283 Figure 3H top panel). Second, most mimetic pairs do not differ in their overall  
284 toxicity except for *H. e. hy dara* and *H. m. melpomene* in Guaviare (Figure 3G top  
285 panel). In contrast, we observed that the overall toxicity of two mimicry rings varied  
286 geographically (Figure S2). Specifically, the *white-yellow* mimicry ring was 17X  
287 more toxic in Cauca Valley than in Caldas (Figure S2A), while the *red-band* ring  
288 was more toxic in Cundinamarca than in any other locality (Figure S2B).

289 We also observed geographic variation in toxicity in *H. sara*, *H. erato*, and *H.*  
290 *melpomene* (Figure 4 and Figure S3). In *H. sara*, for example, individuals from  
291 Buenaventura were the most toxic, while those from Puerto Nariño-Amazonas and  
292 Boyacá were the least (Figure 4A top panel). In *H. erato*, populations from  
293 Cundinamarca, Buenaventura and the Cauca Valley were significantly more toxic  
294 than populations from the jungle (Amazonas, Guaviare and Putumayo; Figure 4B  
295 top panel). In *H. melpomene*, individuals from Meta had the highest toxicity, which  
296 differed from those from Pedrera-Amazonas and Boyacá (Figure 4C top panel). In  
297 those species where we only sampled two populations, we observed significant  
298 differences in toxicity only for *H. eleuchia*, with individuals from Caldas being less  
299 toxic than those from the Cauca Valley (Figure 4D top panel and Figure S3).  
300 Interestingly, *H. cydno* did not exhibit significant geographic variation in toxicity  
301 despite this species participates in several mimicry rings (Figure 4E top panel).

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306 **Figure 3. Quantification of cyanogenic glucosides per species and locality.** (A) Caldas, (B)  
 307 Buenaventura, (C) Cauca Valley, (D) Putumayo, (E) Boyacá, (F) Meta, (G) Guaviare, (H)  
 308 Amazonas-Pedrera, (I) Amazonas-Puerto Nariño. Boxplots on the top of each panel show the mean  
 309 concentration (numbers in squares) of all CNGLcs combined or 'CNGLcs total' per species. The  
 310 mimicry ring to which each species belongs is also indicated at the bottom using a colour code.  
 311 Matrices on the bottom of each panel show the quantification of each CNGLcs or 'CNGLcs profile'  
 312 per species, where the mean concentration (ug/mg) of each compound is colour coded from pale  
 313 yellow to dark red. The CNGLcs quantified are shown at the right, indicating whether they are  
 314 biosynthesized (B) or sequestered (S). Significantly different comparisons ( $\alpha < 0.05$ ) are shown.

### 315 **Quantification of each CNgls ('CNgls profile')**

316

317 When we compared each of the nine CNgls between the 20 species sampled  
318 (190 comparisons) we found 81 comparisons as significantly different  
319 (PERMANOVA  $R^2 = 0.283$ ,  $p = 0.001$ , Table S3; pairwise adonis  $p > 0.05$ , Table  
320 S4). Interestingly, 66% of these significant comparisons included species of the  
321 *sara/sapho* clade, which characterised by having a higher concentrations of  
322 sequestered CNgls (gynocardin, dihydrogynocardin, tetraphyllin B, epivolkenin,  
323 and deidaclin; Figure 2B) compared to biosynthesized CNgls (linamarin,  
324 lotaustralin and epilotaustalin). Within this clade we found *H. sara*, *H. antiochus*,  
325 *H. leucadia* and *H. sapho* having larger amounts of epivolkenin, while *H. eleuchia*  
326 contained more deidaclin, and *H. charithonia* contained more gynocardin (Figure  
327 2B). Additionally, although all members of the clade contained biosynthesized  
328 CNgls, *H. eleuchia* and *H. sapho* showed only traces of these compounds (Figure  
329 2B). In contrast, species of the *erato* clade exhibited a more uniform distribution of  
330 sequestered and biosynthesized CNgls (Figure 2B), while species of the  
331 *melpomene*, *silvaniform* and *wallacei* clades contained mostly biosynthesized  
332 CNgls (Figure 2B). Species from the *silvaniform* clade had higher levels of  
333 linamarin except for *H. ismenius*, which contained more Tetraphyllin B (Figure 2B).  
334 We unexpectedly observed *H. numata* and *H. hecale* having traces of the  
335 sequestered CNgc epivolkenin (Figure 2B). Finally, like most of the species in the  
336 *sara/sapho* clade, *H. aoede* had high concentrations of epivolkenin (Figure 2B).

337 Our results show that, in general, sympatric species are similar in terms of  
338 individual CNgc profiles (Figure 3 bottom panels and Table S3). However, there  
339 were some exceptions to this pattern. First, the only mimetic pair that significantly  
340 differed in CNgls was *H. erato* and *H. melpomene* in Meta, Guaviare, and  
341 Amazonas (PERMANOVA  $p < 0.05$ , Figure 3F-3H, bottom panel). Second, when  
342 comparing between sympatric but non-mimetic species we found some CNgc  
343 being specific to a given species or in concentrations higher than in any other co-  
344 occurring species ( $p < 0.05$ , Figure 3 bottom panels). For example, in  
345 Buenaventura tetraphyllin B was exclusive to *H. ismenius* while epivolkenin was  
346 exceptionally more abundant in *H. sara* (Figure 3B bottom panel). In the Cauca

347 Valley, epivolkenin was highly abundant only in *H. eleuchia* (Figure 3C bottom  
348 panel). In Boyacá, gynocardin was exclusive to *H. charithonia* (Figure 3E bottom  
349 panel). In Meta, deidaclin was only found in *H. melpomene* while Linamarin was  
350 also highly abundant in this species (Figure 3F bottom panel). In Amazonas-  
351 Pedrera, the concentration of Linamarin was high only in *H. wallacei*, while *H. erato*  
352 contained negligible amounts of any CNglc (Figure 3H-3I bottom panel). We  
353 consistently observed across localities that the most toxic species were those with  
354 high concentrations of epivolkenin: (i) *H. sara* in Buenaventura, Meta, and  
355 Guaviare (Figure 3B, 3F and 3G bottom panel), (ii) *H. aoede*, *H. leucadia*, and *H.*  
356 *antiochus* in the Amazonas (Figure 3I bottom panel), and (iii) *H. hecale* and *H.*  
357 *sapho* in Buenaventura (Figure 3B bottom panel). There were only two exceptions  
358 to this general observation. The first, *H. doris* in Boyacá, that was highly toxic due  
359 to high concentrations of Linamarin (Figure 3E bottom panel), and *H. eleuchia* in  
360 the Cauca Valley, whose high toxicity was due to high concentrations of deidaclin  
361 (Figure 3G bottom panel).

362 CNglc profiles also varied geographically in *H. sara*, *H. erato* and *H.*  
363 *eleuchia* ( $p < 0.05$ , Table S3). First, epivolkenin was highly abundant in *H. sara*  
364 from Buenaventura, Guaviare, Arauca, and Meta whereas Deidaclin was more  
365 abundant in populations from Vichada and Arauca (Figure 4A bottom panel).  
366 Second, *H. erato* showed a remarkable geographic variation in its toxicity.  
367 Populations from Cundinamarca and Meta had more epivolkenin (sequestered),  
368 while populations from Buenaventura had more biosynthesized CNglcs. In  
369 contrast, populations from the Cauca Valley and Boyacá had uniform amounts of  
370 sequestered and biosynthesized CNglcs. Interestingly, populations from  
371 Amazonas, Guaviare and Putumayo were much less toxic than any other  
372 population ( $p < 0.05$ , Figure 4B bottom panel). Third, *H. eleuchia* from the Cauca  
373 Valley had high levels of deidaclin whereas populations from Caldas had low  
374 amounts of any CNglc ( $p > 0.05$ , figure 4D bottom panel). Finally, no significant  
375 differences in individual CNglc profiles were found between populations of *H.*  
376 *melpomene* or between populations of *H. cydno* ( $p > 0.05$ , Figure 4C and 4D  
377 bottom panels).



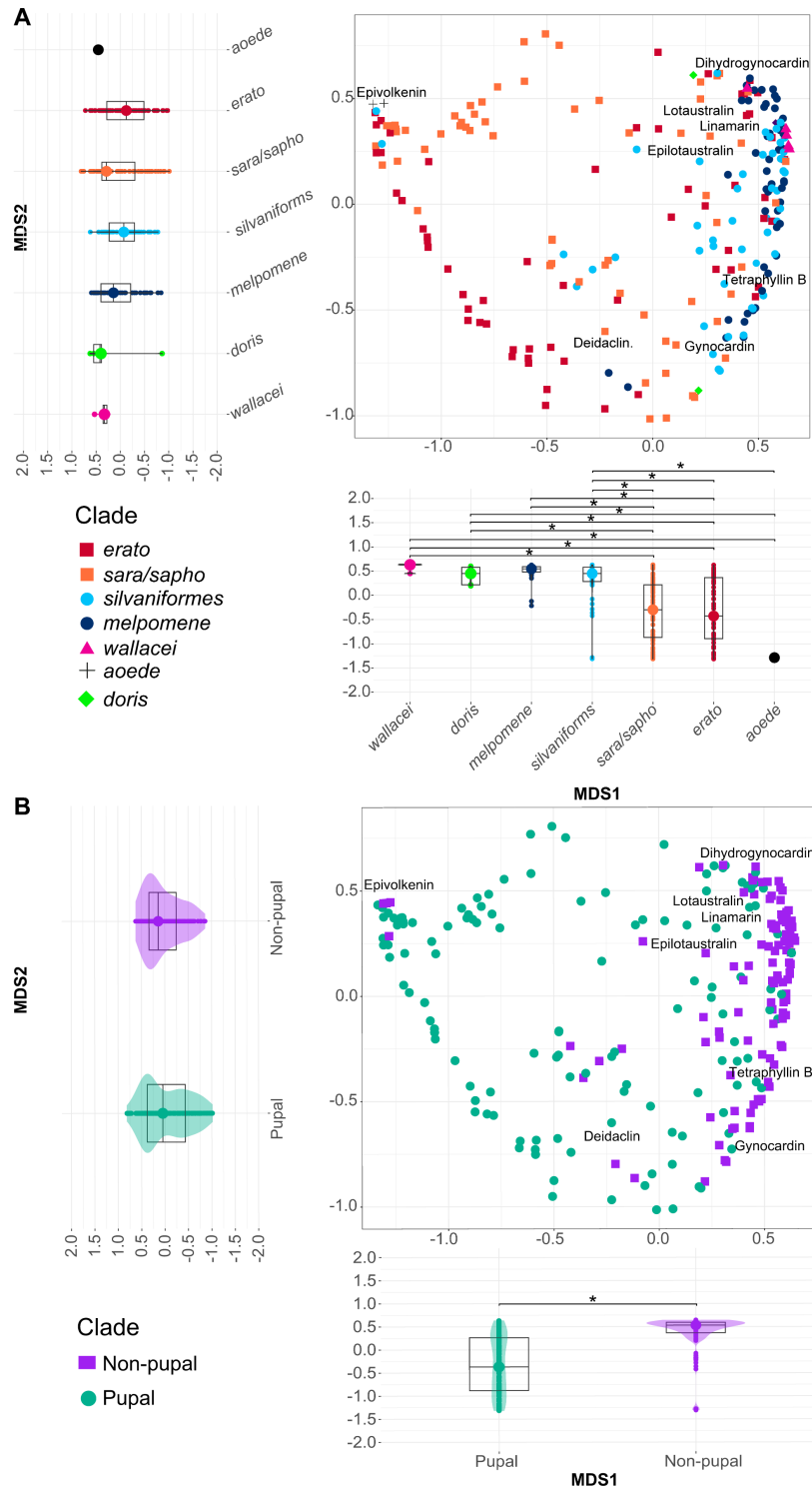


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380 **Figure 4. Intraspecific variation in the concentrations of cyanogenic glycosides.** (A) *H. sara*,  
 381 (B) *H. erato*, (C) *H. melpomene*, (D) *H. eleuchia*, (E) *H. cydno*. Boxplots on top of each panel show  
 382 the mean concentration (numbers in squares) of all CNGlcs combined or ‘CNGlcs total’ per locality  
 383 (in  $\mu\text{g}/\text{mg}$ ). The mimicry ring to which each species belongs is also indicated at the bottom using a  
 384 colour code. Matrices on the bottom of each panel show the quantification of each CNGlcs or  
 385 ‘CNGlcs profile’ per locality, where the mean concentration ( $\text{ug}/\text{mg}$ ) of each compound is colour  
 386 coded from pale yellow to dark red. The CNGlcs quantified are shown at the right, indicating  
 387 whether they are biosynthesized (B) or sequestered (S). Significantly different comparisons ( $\alpha < 0.05$ ) are shown.

388           The above differences did not hold when phylogeny was considered,  
389 suggesting that phylogenetic relationships influence the '*CNglcs profile*'  
390 (PhyloMANOVA  $F = 1573.4$ ,  $df = 18$ ,  $p = 0.000381$ ,  $p$  given phy = 1). Consistently,  
391 we found that pupal, non-pupal, and phylogenetic clades are associated with the  
392 *CNglcs profile* ( $p < 0.05$ ). However, most of the variation in *CNglcs* is due to  
393 differences between ability to biosynthesize or sequester cyanogenic glucosides of  
394 the individuals, followed by phylogenetic, pupal/non-pupal clades and host plant  
395 specialization ( $R^2 = 0.39$ ,  $R^2 = 0.28$ ,  $R^2 = 0.22$ ,  $R^2 = 0.21$  respectively; Figure 5 and  
396 6A), rather than to differences among mimicry rings ( $R^2 = 0.14$ ; Figure 6B and S4).  
397 The composition of *CNglcs* of the clades silvaniforms, melpomene, wallacei, and  
398 doris was different from that of the clades erato, sara/sapho, and aoede ( $p < 0.05$ ;  
399 Figure 5A). Only two individuals (one *H. hecale* and one *H. numata*) were different  
400 from other silvaniforms as they contained high amounts of epivolkenin (Figure 5A).  
401 We also found high variation among the pupal mating clades with several of these  
402 individuals falling into the non-pupal mating clades ( $p < 0.05$ ; Figure 5B). In contrast,  
403 only four individuals from non-pupal mating clades were similar to the pupal mating  
404 clades. Furthermore, pupal mating species (which feed on the subgenera  
405 *Astrophea* and *Decaloba*) have a more similar *CNglcs* profile among them in  
406 contrast to non-pupal mating species (which feed on the subgenus *Passiflora*;  
407 Figure 6A). Consistently, both axes of the NMDs revealed significant differences  
408 between these two groups (Figure 6A). The majority of individuals from the  
409 silvaniforms, melpomene, wallacei, and doris clades were primarily synthesizers. In  
410 contrast, just over half of the individuals from the sara/sapho and erato clades were  
411 primarily catchers, while the rest were synthesizers. Only a handful of individuals  
412 from different species were equally synthesizers and catchers (Figure 6B).  
413 Significant differences were only found in NMD1 between synthesizers and  
414 catchers, as well as between synthesizers and individuals that performed both  
415 process equally (Figure 6B). Finally, we found a small but significant association  
416 between mimetic rings with the *CNglcs* profile (Figure S4).



417

418 **Figure 5. Non-metric multidimensional scaling (NMDS) of cyanogenic glycosides in *Heliconius***

419 **comparing between different clades. (A) Comparison between phylogenetic clades, and (B)**

420 **comparison between pupal and non-pupal mating clades. The distance between two symbols is**

421 **indicative of how different two individuals are in the composition of CNgles. Boxplots for both**

422 **NMDS1 and NMDS2 show differences between species in each axis. Asterisk (\*) symbolises a *p*-**

423 **value < 0.05.**

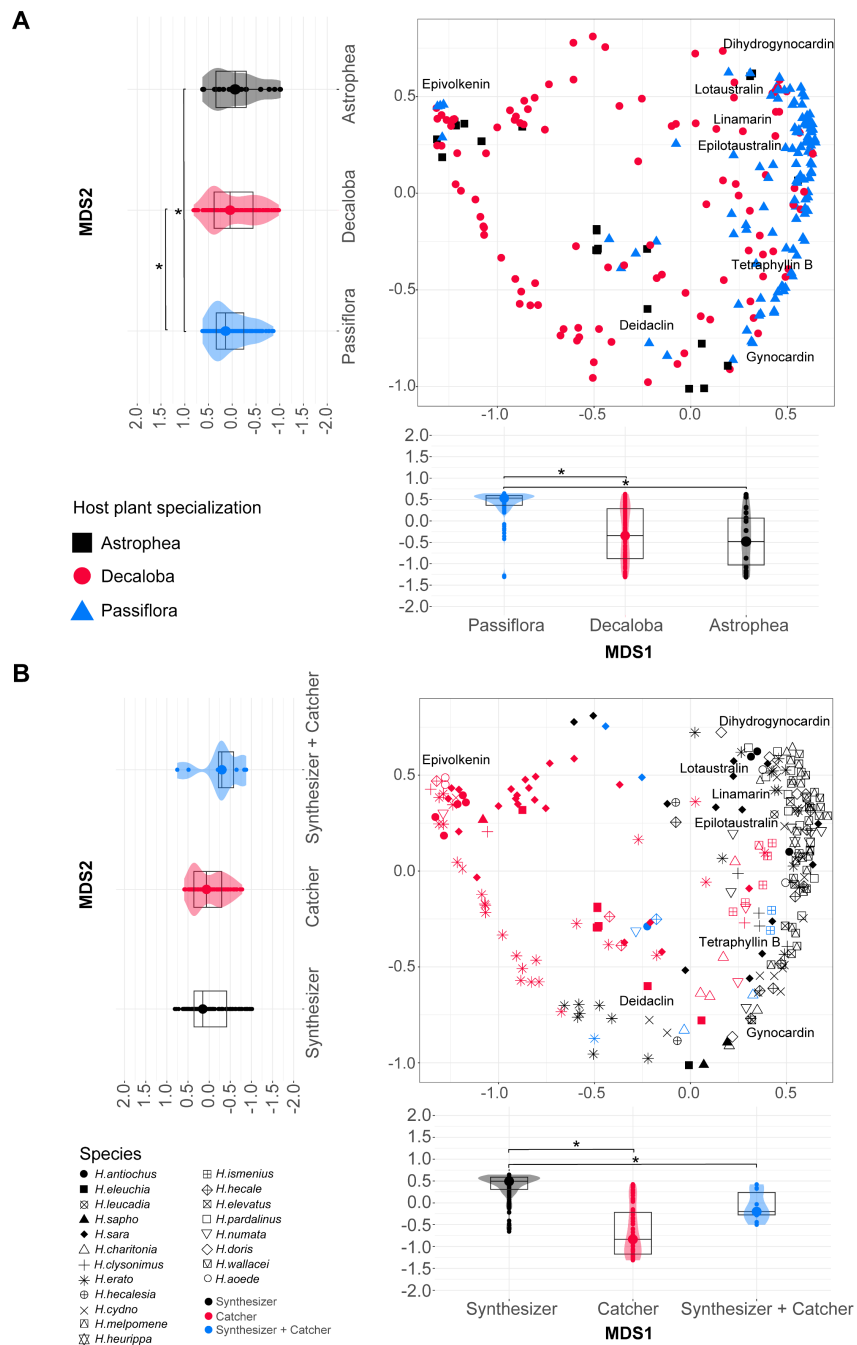
424 **Discussion**

425

426 Our findings suggest that variation in the presence and concentration of  
427 cyanogenic glucosides in individuals of *Heliconius* is mostly due to the ability of a  
428 species to synthesize or sequester these compounds, phylogenetic relationships  
429 and host plant specialization. In contrast, variation in cyanogenic glucosides in  
430 these butterflies is less explained by the mimicry ring to which a species belongs.  
431 While the effect of phylogeny on the presence and concentration of cyanogenic  
432 glucosides in *Heliconius* has been previously reported (de Castro et al., 2019;  
433 Sculfort et al., 2020), it was due to species of *sara/sapho* having a unique  
434 cyanogenic profile defined by the exclusive presence of the sequestered  
435 epivolkenin and deidaclin. Contrary to these reports, we detected the presence of  
436 *de novo* synthesised cyanogenic glucosides in the *sara/sapho* clade, and our  
437 phylogenetic signal was the result of the clades *silvaniform*, *melpomene*, *wallacei*  
438 and *doris* having similar cyanogenic profiles, where the sequestered epivolkenin  
439 and deidaclin were also present but in small amounts. Therefore, the phylogenetic  
440 signal we observed is likely the result of the association between mating clades of  
441 *Heliconius* with host plants of subgenera of *Passiflora*, where pupal mating species  
442 are usually specialists and feed on *Astrophea* and *Decaloba* while non-pupal  
443 mating species are more generalist and feed on *Passiflora* (Arias et al., 2016;  
444 Engler-Chaouat & Gilbert, 2007).

445         These dietary restrictions in *Heliconius* has led to monophagous species, in  
446 particular *sara/sapho* clade species, virtually losing their ability to *de novo*  
447 synthesize cyanogen compounds making them entirely dependent on cyanogen  
448 sequestration from their host plant (de Castro et al., 2019; Engler-Chaouat &  
449 Gilbert, 2007). However, here we found *de novo*-synthesized aliphatic cyanogens  
450 such as linamarin and lotaustralin in individuals of the *sara/sapho* clade. This  
451 finding has two possible explanations: either species of the *sara/sapho* clade did  
452 not lose their ability to *de novo* synthesise cyanogenic glucosides, or they  
453 sequester linamarin and lotaustralin from their host plant. The latter is supported by  
454 the fact that both compounds are known to be sequestered by Lepidoptera such as

455 the moth *Z. filipendulae* (Fürstenberg-Hägg et al., 2014) and the butterfly *H.*  
 456 *melpomene* (Pinheiro de Castro et al., 2020), and also by the fact that the host  
 457 plants *Astrophea* and *Decaloba* contain linamarin and lotaustralin (de Castro et al.,  
 458 2019).



459 **Figure 6. Non-metric multidimensional scaling (NMDS) of cyanogenic glycosides in *Heliconius***  
 460 **comparing between feeding and metabolic strategies. (A) Comparison based on host plant**  
 461 **specialization, (B) comparison based on CNgles acquisition. The distance between two symbols is**  
 462 **indicative of how different two individuals are in the composition of CNgles. Boxplots for both**  
 463

464 NMDS1 and NMDS2 show differences between individuals in each axis. Asterisk (\*) symbolises a  
465  $p$ -value  $< 0.05$

466  
467

468 We did not find evidence of species that either performed biosynthesis only  
469 or sequestration only (de Castro et al., 2019; Engler-Chaouat & Gilbert, 2007). In  
470 contrast, our results point to all species of *Heliconius* using both strategies, with  
471 30% of individuals prioritising sequestration, 60% prioritising biosynthesis, and only  
472 10% equally using both. This is indicative of a potential trade-off between  
473 biosynthesis and sequestration at the individual level but not at the species level. It  
474 was interesting to observe that *de novo* synthesis is a widely used strategy across  
475 species and individuals despite being metabolically costlier (de Castro et al., 2019),  
476 which suggests that other factors, such as local host plant availability may be more  
477 decisive for an individual to determine which strategy to apply.

478 Although variation in cyanogenic profiles of *Heliconius* from different  
479 countries had been previously reported (Sculfort et al., 2020), here we found such  
480 variation at a finer geographic scale, specifically between biogeographic regions  
481 *sensu* Morrone et al. 2022 (Morrone et al., 2022). For example, *H. sara* and *H.*  
482 *erato* are much more toxic in Choco-Darien (Pacific coast) than in Imeri  
483 (Amazonia), while *H. melpomene* is more toxic in Sabana than in Magdalena. We  
484 even detected these variations within a single biogeographic region and mimicry  
485 ring. For instance, although *H. eleuchia eleuchia* and *H. cydno cydnides* both  
486 participate in the white-yellow mimicry ring and occur in the Cauca province, the  
487 concentration of CNglcs drastically varies between populations that are  $<250\text{Km}$   
488 apart. Overall, these long and short range geographic variations in CNglcs may be  
489 the result of differences in the availability of host and pollen plants across localities  
490 (Jeckel et al., 2015; Saporito et al., 2006; Speed et al., 2012) as well as to local  
491 predator communities being differentially sensitive to detect toxicity (Chouteau et  
492 al., 2019). Furthermore, contrary to previous evidence that showed different  
493 mimicry rings differing in cyanogenic profiles (Arias et al., 2016; Sculfort et al.,  
494 2020), with for example ‘blue/yellow’ being more toxic than ‘postman’ (Arias et al.,  
495 2016), we did not observe this pattern for either individual or combined CNglcs.  
496 The most notable example is *H. cydno*, a species that participates in three mimicry

497 rings across its distribution but does not vary in its cyanogenic profile. Similarly,  
498 although *H. melpomene* participates in four mimicry rings, individuals from different  
499 rings do not differ in the composition or concentration CNgls. We believe our  
500 conclusions on the relation between cyanogenic profiles and mimicry rings are  
501 more reliable than those from previous studies (Sculfort et al., 2020) since they  
502 analysed phenotypically similar species as part of the same mimicry ring even if  
503 they were not strict mimetic pairs.

504 Our findings support the existence of mutualistic relationships between both  
505 mimics and sympatric *Heliconius* since we observed sympatric species having  
506 similar cyanogenic concentrations regardless of them being mimics or non-mimics.  
507 Therefore, local communities of *Heliconius* seem to equally contribute to  
508 associative learning of predators (Speed, 1993). The only mimetic pair that did not  
509 follow this pattern was *H. erato* and *H. melpomene*: in all localities where they  
510 occur, we found one species being more toxic than the other. However,  
511 experimental evidence from domestic birds indicate that these cyanogenic  
512 variations between mimics should not impede local predator learning as long as  
513 each species has a toxin content  $>2 \mu\text{g}/\text{mg}$  (Chouteau et al., 2019). In line with the  
514 latter, 96% of the individuals we tested in this study had cyanogenic concentrations  
515 above  $2 \mu\text{g}/\text{mg}$ , which should be enough to ensure their individual protection.  
516 Interestingly, species in the *sara/sapho* clade were consistently found as the most  
517 toxic within their local communities, and although protection from predators does  
518 not increase with cyanogenic concentration, the high toxicity of this clade may  
519 confer these species some protection from toxin-resistant predators (Trigo, 2011;  
520 Williams & Brodie, 2003). Alternatively, the high cyanogenic content in *sara/sapho*  
521 clade species may be a byproduct of their monophagy in *Astrophea* and *Decaloba*  
522 as previously suggested (de Castro et al., 2019; Engler-Chaouat & Gilbert, 2007).

523 In summary, variation in cyanogenic patterns in *Heliconius* is not associated  
524 with mimicry rings as the same wing colour pattern exhibits different cyanogenic  
525 concentrations in different localities. The observed variation is rather explained by  
526 phylogenetic relationships and ecological adaptations such as host plant  
527 specialization, diversity of hostplants locally available, availability of precursors for

528 biosynthesis of cyanogenic compounds in pollen-source plants, as well as the local  
529 predator community. This agrees with recent modelling and meta-analyses that  
530 showed that increased toxicity of preys does not translate into increased predator  
531 learning or generation of mimetic diversity (Chouteau et al., 2019).

532

533

### References

- 534 Arias, M., Meichanetzoglou, A., Elias, M., Rosser, N., de-Silva, D. L., Nay, B., &  
535 Llaurens, V. (2016). Variation in cyanogenic compounds concentration within  
536 a Heliconius butterfly community: Does mimicry explain everything? *BMC*  
537 *Evolutionary Biology*, 16(1), 272. <https://doi.org/10.1186/s12862-016-0843-5>
- 538 Bowers, M. (1992). The evolution of unpalatability and the cost of chemical defense  
539 in insects. In *Insect chemical ecology: An evolutionary approach*. (pp. 216–  
540 244). Chapman & Hall.
- 541 Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Rönkä, K. H., Seymoure, B. M.,  
542 Stankowich, T., & Stuckert, A. M. M. (2019). Diversity in warning coloration:  
543 Selective paradox or the norm? *Biological Reviews of the Cambridge*  
544 *Philosophical Society*, 94(2), 388–414. <https://doi.org/10.1111/brv.12460>
- 545 Camara, M. D. (1997). Predator Responses to Sequestered Plant Toxins in Buckeye  
546 Caterpillars: Are Tritrophic Interactions Locally Variable? *Journal of Chemical*  
547 *Ecology*, 23(9), 2093–2106.  
548 <https://doi.org/10.1023/B:JOEC.0000006431.34359.c2>
- 549 Chouteau, M., Dezeure, J., Sherratt, T. N., Llaurens, V., & Joron, M. (2019). Similar  
550 predator aversion for natural prey with diverse toxicity levels. *Animal*  
551 *Behaviour*, 153, 49–59. <https://doi.org/10.1016/j.anbehav.2019.04.017>



552 Davis, R. H., & Nahrstedt, A. (1987). Biosynthesis of cyanogenic glucosides in  
553 butterflies and moths: Effective incorporation of 2-methylpropanenitrile and 2-  
554 methylbutanenitrile into linamarin and lotaustralin by *Zygaena* and *Heliconius*  
555 species (Lepidoptera). *Insect Biochemistry*, 17(5), 689–693.  
556 [https://doi.org/10.1016/0020-1790\(87\)90037-0](https://doi.org/10.1016/0020-1790(87)90037-0)

557 de Castro, É. C. P., Zagrobelny, M., Zurano, J. P., Cardoso, M. Z., Feyereisen, R.,  
558 & Bak, S. (2019). Sequestration and biosynthesis of cyanogenic glucosides  
559 in passion vine butterflies and consequences for the diversification of their  
560 host plants. *Ecology and Evolution*, 9(9), 5079–5093.  
561 <https://doi.org/10.1002/ece3.5062>

562 Eisner, H. E. (1967). Defense mechanisms of arthropods. XX. Quantitative  
563 assessment of hydrogen cyanide production in two species of millipedes.  
564 *Psyche*, 74(2), 107–117.

565 Engler-Chaouat, H. S., & Gilbert, L. E. (2007). De novo synthesis vs. sequestration:  
566 Negatively correlated metabolic traits and the evolution of host plant  
567 specialization in cyanogenic butterflies. *Journal of Chemical Ecology*, 33(1),  
568 25–42. <https://doi.org/10.1007/s10886-006-9207-8>

569 Fahey, S. J., & Garson, M. J. (2002). Geographic variation of natural products of  
570 tropical nudibranch *Asteronotus cespitosus*. *Journal of Chemical Ecology*,  
571 28(9), 1773–1785. <https://doi.org/10.1023/a:1020509117545>

572 Fürstenberg-Hägg, J., Zagrobelny, M., Jørgensen, K., Vogel, H., Møller, B. L., & Bak,  
573 S. (2014). Chemical Defense Balanced by Sequestration and De Novo  
574 Biosynthesis in a Lepidopteran Specialist. *PLOS ONE*, 9(10), e108745.  
575 <https://doi.org/10.1371/journal.pone.0108745>

576 Goodger, J. Q. D., Capon, R. J., & Woodrow, I. E. (2002). Cyanogenic polymorphism  
577 in *Eucalyptus polyanthemus* Schauer subsp. *Vestita* L. Johnson and K. Hill  
578 (Myrtaceae). *Biochemical Systematics and Ecology*, 30(7), 617–630.  
579 [https://doi.org/10.1016/S0305-1978\(01\)00141-7](https://doi.org/10.1016/S0305-1978(01)00141-7)

580 Hay-Roe, M. M., & Nation, J. (2007). Spectrum of Cyanide Toxicity and Allocation in  
581 *Heliconius erato* and *Passiflora* Host Plants. *Journal of Chemical Ecology*,  
582 33(2), 319–329. <https://doi.org/10.1007/s10886-006-9234-5>

583 Jeckel, A. M., Grant, T., & Saporito, R. A. (2015). Sequestered and Synthesized  
584 Chemical Defenses in the Poison Frog *Melanophryniscus moreirae*. *Journal*  
585 *of Chemical Ecology*, 41(5), 505–512. [https://doi.org/10.1007/s10886-015-](https://doi.org/10.1007/s10886-015-0578-6)  
586 0578-6

587 Joron, M., & Mallet, J. L. (1998). Diversity in mimicry: Paradox or paradigm? *Trends*  
588 *in Ecology & Evolution*, 13(11), 461–466. [https://doi.org/10.1016/s0169-](https://doi.org/10.1016/s0169-5347(98)01483-9)  
589 5347(98)01483-9

590 Kuo, C.-Y. (2023). *Predator learning can resolve the paradox of local warning signal*  
591 *diversity* (p. 2023.05.04.539348). bioRxiv.  
592 <https://doi.org/10.1101/2023.05.04.539348>

593 Mallet, J. (2010). Shift happens! Shifting balance and the evolution of diversity in  
594 warning colour and mimicry. *Ecological Entomology*, 35(s1), 90–104.  
595 <https://doi.org/10.1111/j.1365-2311.2009.01137.x>

596 Mattila, A. L. K., Jiggins, C. D., Opedal, Ø. H., Montejo-Kovacevich, G., Castro, É.  
597 C. P. de, McMillan, W. O., Bacquet, C., & Saastamoinen, M. (2021).  
598 Evolutionary and ecological processes influencing chemical defense variation

599 in an aposematic and mimetic Heliconius butterfly. *PeerJ*, 9, e11523.  
600 <https://doi.org/10.7717/peerj.11523>

601 Morrone, J. J., Escalante, T., Rodríguez-Tapia, G., Carmona, A., Arana, M., &  
602 Mercado-Gómez, J. D. (2022). Biogeographic regionalization of the  
603 Neotropical region: New map and shapefile. *Anais Da Academia Brasileira de*  
604 *Ciências*, 94, e20211167. <https://doi.org/10.1590/0001-3765202220211167>

605 Müller, F. (1879). Ituna and Thyridia; a remarkable case of mimicry in butterflies.  
606 *Transactions of the Entomological Society of London*, 1879, Xx-Xxix.

607 Nahrstedt, A. (1996). Relationships between the Defense Systems of Plants and  
608 Insects. In J. T. Romeo, J. A. Saunders, & P. Barbosa (Eds.), *Phytochemical*  
609 *Diversity and Redundancy in Ecological Interactions* (pp. 217–230). Springer  
610 US. [https://doi.org/10.1007/978-1-4899-1754-6\\_8](https://doi.org/10.1007/978-1-4899-1754-6_8)

611 Oksanen, F. J. (2017). *Vegan: Community Ecology Package. R package Version*  
612 *2.4-3*.

613 Opitz, S. E. W., & Müller, C. (2009). Plant chemistry and insect sequestration.  
614 *Chemoecology*, 19(3), 117–154. <https://doi.org/10.1007/s00049-009-0018-6>

615 Patil, I. (2021). Visualizations with statistical details: The ‘ggstatsplot’ approach.  
616 *Journal of Open Source Software*, 6(61), 3167.  
617 <https://doi.org/10.21105/joss.03167>

618 Pennell, M. W., Eastman, J. M., Slater, G. J., Brown, J. W., Uyeda, J. C., FitzJohn,  
619 R. G., Alfaro, M. E., & Harmon, L. J. (2014). geiger v2.0: An expanded suite  
620 of methods for fitting macroevolutionary models to phylogenetic trees.  
621 *Bioinformatics*, 30(15), 2216–2218.  
622 <https://doi.org/10.1093/bioinformatics/btu181>

623 Pentzold, S., Zagrobelny, M., Khakimov, B., Engelsen, S. B., Clausen, H., Petersen,  
624 B. L., Borch, J., Møller, B. L., & Bak, S. (2016). Lepidopteran defence  
625 droplets—A composite physical and chemical weapon against potential  
626 predators. *Scientific Reports*, 6(1), Article 1.  
627 <https://doi.org/10.1038/srep22407>

628 Pinheiro de Castro, É. C., Demirtas, R., Orteu, A., Olsen, C. E., Motawie, M. S.,  
629 Zikan Cardoso, M., Zagrobelny, M., & Bak, S. (2020). The dynamics of  
630 cyanide defences in the life cycle of an aposematic butterfly: Biosynthesis  
631 versus sequestration. *Insect Biochemistry and Molecular Biology*, 116,  
632 103259. <https://doi.org/10.1016/j.ibmb.2019.103259>

633 Reudler, J. H., Lindstedt, C., Pakkanen, H., Lehtinen, I., & Mappes, J. (2015). Costs  
634 and benefits of plant allelochemicals in herbivore diet in a multi enemy world.  
635 *Oecologia*, 179(4), 1147–1158. <https://doi.org/10.1007/s00442-015-3425-0>

636 Ruxton, G. D., Allen, W. L., Sherratt, T. N., & Speed, M. P. (2018). *Avoiding Attack:  
637 The Evolutionary Ecology of Crypsis, Aposematism, and Mimicry*. (2nd edn).  
638 Oxford: Oxford University Press.

639 Saporito, R. A., Donnelly, M. A., Garraffo, H. M., Spande, T. F., & Daly, J. W. (2006).  
640 Geographic and Seasonal Variation in Alkaloid-Based Chemical Defenses of  
641 *Dendrobates pumilio* from Bocas del Toro, Panama. *Journal of Chemical  
642 Ecology*, 32(4), 795–814. <https://doi.org/10.1007/s10886-006-9034-y>

643 Sculfort, O., de Castro, E. C. P., Kozak, K. M., Bak, S., Elias, M., Nay, B., & Llaurens,  
644 V. (2020). Variation of chemical compounds in wild Heliconiini reveals  
645 ecological factors involved in the evolution of chemical defenses in mimetic

646 butterflies. *Ecology and Evolution*, 10(5), 2677–2694.  
647 <https://doi.org/10.1002/ece3.6044>

648 Skelhorn, J., & Rowe, C. (2005). Frequency-dependent taste-rejection by avian  
649 predation may select for defence chemical polymorphisms in aposematic  
650 prey. *Biology Letters*, 1(4), 500–503. <https://doi.org/10.1098/rsbl.2005.0359>

651 Smilanich, A. M., Dyer, L. A., Chambers, J. Q., & Bowers, M. D. (2009).  
652 Immunological cost of chemical defence and the evolution of herbivore diet  
653 breadth. *Ecology Letters*, 12(7), 612–621. <https://doi.org/10.1111/j.1461-0248.2009.01309.x>

654

655 Speed, M. P. (1993). Muellierian mimicry and the psychology of predation. *Animal*  
656 *Behaviour*, 45(3), 571–580. <https://doi.org/10.1006/anbe.1993.1067>

657 Speed, M. P., Ruxton, G. D., Mappes, J., & Sherratt, T. N. (2012). Why are defensive  
658 toxins so variable? An evolutionary perspective. *Biological Reviews of the*  
659 *Cambridge Philosophical Society*, 87(4), 874–884.  
660 <https://doi.org/10.1111/j.1469-185X.2012.00228.x>

661 Thodberg, S., Sørensen, M., Bellucci, M., Crocoll, C., Bendtsen, A. K., Nelson, D.  
662 R., Motawia, M. S., Møller, B. L., & Neilson, E. H. J. (2020). A flavin-  
663 dependent monooxygenase catalyzes the initial step in cyanogenic glycoside  
664 synthesis in ferns. *Communications Biology*, 3(1), Article 1.  
665 <https://doi.org/10.1038/s42003-020-01224-5>

666 Trigo, J. R. (2011). Effects of pyrrolizidine alkaloids through different trophic levels.  
667 *Phytochemistry Reviews*, 10(1), 83–98. <https://doi.org/10.1007/s11101-010-9191-z>

668

669 Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A.,  
670 Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M.,  
671 Venables, B., & Galili, T. (2022). *gplots: Various R Programming Tools for*  
672 *Plotting Data* (3.1.3). [https://cran.r-](https://cran.r-project.org/web/packages/gplots/index.html)  
673 [project.org/web/packages/gplots/index.html](https://cran.r-project.org/web/packages/gplots/index.html)

674 Williams, B. L., & Brodie, E. D. (2003). Coevolution of Deadly Toxins and Predator  
675 Resistance: Self-Assessment of Resistance by Garter Snakes Leads to  
676 Behavioral Rejection of Toxic Newt Prey. *Herpetologica*, 59(2), 155–163.

677 Wray, V., Davis, R. H., & Nahrstedt, A. (1983). Biosynthesis of Cyanogenic  
678 Glycosides in Butterflies and Moths: Incorporation of Valine and Isoleucine  
679 into Linamarin and Lotaustralin by *Zygaena* and *Heliconius* Species  
680 (Lepidoptera). *Zeitschrift Für Naturforschung C*, 38(7–8), 583–588.  
681 <https://doi.org/10.1515/znc-1983-7-815>

682 Yezerski, A., Gilmor, T. P., & Stevens, L. (2004). Genetic Analysis of Benzoquinone  
683 Production in *Tribolium confusum*. *Journal of Chemical Ecology*, 30(5), 1035–  
684 1044. <https://doi.org/10.1023/B:JOEC.0000028465.37658.ae>

685 Zagrobelny, M., Bak, S., Thorn Ekstrøm, C., Erik Olsen, C., & Lindberg Møller, B.  
686 (2007). The cyanogenic glucoside composition of *Zygaena filipendulae*  
687 (Lepidoptera: Zygaenidae) as effected by feeding on wild-type and transgenic  
688 lotus populations with variable cyanogenic glucoside profiles. *Insect*  
689 *Biochemistry and Molecular Biology*, 37(1), 10–18.  
690 <https://doi.org/10.1016/j.ibmb.2006.09.008>

691 Zagrobelny, M., De Castro, É. C. P., Møller, B. L., & Bak, S. (2018). Cyanogenesis  
692 in Arthropods: From Chemical Warfare to Nuptial Gifts. *Insects*, 9(2), Article  
693 2. <https://doi.org/10.3390/insects9020051>  
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**Table S1.** Sample information and concentration of each CNgIc per sample (in ug/mg). Gyn.: Gynocardin, Dih.: Dihydrogynocardin, Tet.: Tetraphyllin B, Epi.: Epivolkenin, Dei.: Deidaclin, Lin.: Linamarin, Lot.: Lotaustralin, Epil.: Epilotaustralin. Total: concentration of all CNgIcs combined.

ID	Ring	Species	Subspecies	Locality	Gyn.	Dih.	Tet.	Epi.	Dei.	Lin.	Lot.	Epil.	Total
4128	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas	0.00	0.00	0.00	8.02	0.00	4.77	2.99	0.44	16.23
4129	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas	0.00	0.00	0.00	24.29	0.00	0.25	0.11	0.00	24.66
4130	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas	0.00	0.00	0.00	32.40	0.00	0.09	0.03	0.00	32.52
5029	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	15.42	6.69	0.00	22.11
5030	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas	0.00	0.00	0.00	90.61	0.04	0.68	0.07	0.00	91.41
5341	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	11.31	5.35	3.06	19.72
5363	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	1.34	31.65	19.73	16.85	69.58
5364	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas-Pedreira	0.00	0.00	0.00	60.04	0.00	1.78	0.85	0.03	62.69
5383	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	35.77	19.49	10.91	66.17
5384	No mimicry ring	<i>H. antiochus</i>	<i>antiochus</i>	Amazonas-Pedreira	0.00	0.00	0.00	49.85	0.00	0.87	1.02	0.01	51.75
5021	Dennis-ray	<i>H. aoede</i>	<i>bartleti</i>	Amazonas	0.00	0.00	0.00	65.68	0.00	0.00	0.00	0.00	65.68
5022	Dennis-ray	<i>H. aoede</i>	<i>bartleti</i>	Amazonas	0.00	0.00	0.00	51.52	0.00	0.00	0.00	0.00	51.52
4208	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	10.26	0.00	0.00	0.00	0.00	1.89	1.61	0.07	13.84
4209	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	44.75	0.00	1.87	0.00	0.00	9.17	5.08	0.97	61.84
4211	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	5.54	0.00	0.00	0.00	0.00	4.00	2.05	0.40	11.98
4212	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	19.16	0.00	0.00	0.00	0.00	4.54	1.80	0.17	25.66
4213	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	10.33	0.00	0.00	0.00	0.00	2.75	1.24	0.16	14.49
5588	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	4.30	1.83	0.95	7.08
5589	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	2.07	0.00	0.00	0.00	0.00	0.98	0.94	0.00	3.99
5590	No mimicry ring	<i>H. charithonia</i>	<i>bassleri</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	3.01	1.13	0.96	5.11
4645	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca valley	0.00	0.00	0.00	2.06	0.36	8.63	3.32	0.46	14.82



4646	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	1.07	0.00	7.28	3.63	2.02	14.00
4664	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	3.82	0.00	10.07	4.67	1.71	20.26
4665	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	14.16	0.00	0.00	6.15	1.96	0.17	22.44
4666	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	33.69	0.00	3.23	0.40	0.00	37.31
5674	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	13.27	5.19	0.00	18.46
5675	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	6.94	2.76	0.94	10.64
5676	No mimicry ring	<i>H. clysonimus</i>	<i>clysonimus</i>	Cauca Valley	0.00	0.00	0.00	42.33	0.00	0.00	0.00	0.00	42.33
5007	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Caldas	0.00	0.00	0.72	0.00	1.84	14.35	7.70	0.00	24.61
5008	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Caldas	0.00	0.00	0.00	0.00	0.00	5.26	1.11	0.00	6.37
5010	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Caldas	0.30	0.31	0.00	0.00	0.00	1.20	0.14	0.00	1.94
5011	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Caldas	0.24	0.30	0.00	0.00	0.00	0.84	0.10	0.00	1.49
5485	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Cauca Valley	0.00	0.30	0.00	0.00	0.00	14.51	7.87	5.57	28.25
5486	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Cauca Valley	0.00	0.00	0.00	0.00	6.00	4.96	2.15	0.58	13.69
5487	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Cauca Valley	0.00	1.68	0.00	0.00	0.00	10.91	4.72	4.15	21.46
5488	White_yellow	<i>H. cydno</i>	<i>cydnides</i>	Cauca Valley	0.00	0.00	0.00	0.00	7.58	12.96	6.50	6.70	33.73
4792	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	4.76	2.03	2.64	9.42
4793	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	2.78	9.57	3.97	0.00	16.32
4794	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	6.26	2.15	0.50	8.92
4795	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	4.72	2.69	0.22	7.63
4796	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.90	6.66	2.12	1.20	10.88
5666	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	9.25	2.29	0.65	12.18
5667	Yellow_hindwing	<i>H. cydno</i>	<i>weymeri</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	16.65	5.80	6.94	29.40
5780	White	<i>H. cydno</i>	<i>zelinde</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	27.57	12.45	7.11	47.13
5789	White	<i>H. cydno</i>	<i>zelinde</i>	Buenaventura	0.00	0.00	0.00	1.95	0.00	21.89	7.99	0.00	31.83
4622	Yellow	<i>H. doris</i>	<i>doris</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	29.98	13.12	2.31	45.41
4663	Yellow	<i>H. doris</i>	<i>doris</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.25	23.79	0.70	0.00	24.74

4669	Yellow	<i>H. doris</i>	<i>doris</i>	Cauca Valley	0.00	0.00	0.00	0.00	0.00	24.76	1.12	0.06	25.95
5537	Yellow	<i>H. doris</i>	<i>doris</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	7.28	4.61	0.24	12.12
5636	Yellow	<i>H. doris</i>	<i>doris</i>	Boyaca	0.00	13.29	0.00	0.00	0.00	57.60	24.93	0.00	95.82
5657	Yellow	<i>H. doris</i>	<i>doris</i>	Cauca Valley	0.00	0.00	1.10	0.00	0.00	2.57	1.13	0.00	4.81
5012	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Caldas	0.00	0.00	0.00	0.00	0.00	1.37	0.95	0.68	2.99
5013	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Caldas	0.00	0.00	0.00	0.00	3.68	0.51	0.27	0.00	4.46
5014	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Caldas	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.11
5499	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	4.44	0.00	0.10	2.35	0.38	0.00	7.27
5500	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	0.00	0.15	72.69	0.00	0.30	0.00	73.14
5501	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	0.00	0.70	67.64	0.00	0.35	0.00	68.68
5502	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	0.00	0.09	86.58	0.11	0.40	0.00	87.18
5673	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	0.00	0.00	74.51	0.00	0.00	0.00	74.51
5754	White_yellow	<i>H. eleuchia</i>	<i>eleuchia</i>	Cauca Valley	0.00	0.00	0.00	0.00	78.61	0.00	0.00	0.00	78.61
4007	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	3.31	1.58	0.00	4.89
4008	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas	0.00	0.00	1.32	0.00	0.00	9.61	4.69	1.88	17.50
5023	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas	0.00	0.00	0.91	0.53	0.00	9.58	1.67	1.11	13.80
5329	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas- Pedrera	0.00	0.00	0.51	0.00	0.00	14.36	9.31	4.96	29.14
5389	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.00	0.00	6.85	2.09	0.33	9.27
5514	Dennis-ray	<i>H. elevatus</i>	<i>elevatus</i>	Amazonas	0.00	0.00	5.41	0.00	0.00	12.61	1.63	0.00	19.65
5490	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	9.03	0.00	0.00	5.00	0.00	10.09	1.25	0.00	25.37
5491	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	21.98	0.68	0.00	2.13	0.00	3.60	1.05	0.00	29.43
5492	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	3.49	0.00	0.00	0.00	0.00	18.32	6.92	3.63	32.36
5493	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	1.88	0.00	0.00	5.30	0.49	11.16	4.20	2.99	26.01
5494	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	0.00	0.00	21.59	0.00	0.47	11.43	1.87	0.00	35.35
5715	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	0.00	0.00	0.00	1.92	0.00	25.16	4.03	0.00	31.12
5716	Yellow_hindwing	<i>H. erato</i>	<i>chestertonii</i>	Cauca Valley	0.00	0.00	0.00	14.54	0.00	9.53	2.07	0.00	26.14
4774	Postman	<i>H. erato</i>	<i>dignus</i>	Putumayo	0.00	0.00	0.00	3.73	0.00	0.12	0.00	0.00	3.85
4775	Postman	<i>H. erato</i>	<i>dignus</i>	Putumayo	0.00	0.00	0.00	1.24	0.00	0.31	0.00	0.15	1.70
4777	Postman	<i>H. erato</i>	<i>dignus</i>	Putumayo	0.00	0.00	0.00	0.97	0.00	0.03	0.00	1.99	3.00

5599	Red band	<i>H. erato</i>	<i>guarica</i>	Boyaca	0.00	0.00	14.41	9.12	0.00	13.97	4.53	0.00	42.04
5600	Red band	<i>H. erato</i>	<i>guarica</i>	Boyaca	0.00	0.00	0.00	11.57	0.00	3.28	1.23	3.05	19.13
5601	Red band	<i>H. erato</i>	<i>guarica</i>	Boyaca	0.00	0.00	7.58	6.94	0.00	3.94	0.49	0.00	18.95
5626	Red band	<i>H. erato</i>	<i>guarica</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	6.00	2.81	0.41	9.22
5940	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	122.24	0.00	0.00	0.00	0.00	122.24
5941	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	195.50	0.00	0.00	0.00	0.00	195.50
5942	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	0.00	0.00	9.54	4.53	1.15	15.22
5949	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	0.00	0.00	48.14	17.03	10.64	75.81
5950	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	1.94	0.00	24.83	8.48	0.00	35.24
5951	Red band	<i>H. erato</i>	<i>guarica</i>	Cundinamarca	0.00	0.00	0.00	8.01	0.00	37.99	21.01	0.00	67.01
4887	Red band	<i>H. erato</i>	<i>hydara</i>	Meta	0.00	0.00	0.00	27.42	0.00	0.01	0.00	0.24	27.66
4889	Red band	<i>H. erato</i>	<i>hydara</i>	Meta	0.00	0.00	0.00	4.22	0.00	0.00	0.00	1.37	5.58
4890	Red band	<i>H. erato</i>	<i>hydara</i>	Meta	0.00	0.00	0.00	43.64	0.00	0.00	0.00	0.37	44.01
4891	Red band	<i>H. erato</i>	<i>hydara</i>	Meta	0.00	0.00	0.00	7.80	0.00	0.00	0.00	0.00	7.80
4899	Red band	<i>H. erato</i>	<i>hydara</i>	Guaviare	0.00	0.00	0.00	0.00	0.00	0.06	0.00	1.20	1.27
4902	Red band	<i>H. erato</i>	<i>hydara</i>	Guaviare	0.00	0.00	0.00	2.29	0.00	0.75	0.00	2.18	5.22
4904	Red band	<i>H. erato</i>	<i>hydara</i>	Guaviare	0.00	0.00	0.00	2.81	0.04	0.30	0.00	0.00	3.16
4910	Red band	<i>H. erato</i>	<i>hydara</i>	Guaviare	0.00	0.00	0.00	0.39	0.00	1.10	0.54	1.85	3.88
4911	Red band	<i>H. erato</i>	<i>hydara</i>	Guaviare	0.00	0.00	0.00	4.28	0.00	0.00	0.00	0.00	4.23
5020	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.54	0.87
5025	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	10.83	0.00	0.09	0.00	2.05	12.97
5031	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	15.28	0.00	0.00	0.00	1.29	16.57
5303	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.67	0.14	0.37	0.26	0.50	1.94
5305	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.40	0.00	0.00	0.00	1.37	1.76
5306	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas- Pedrera	0.00	0.00	0.00	4.49	0.00	0.21	0.00	2.60	7.31
5307	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas- Pedrera	0.00	0.00	0.00	8.19	0.13	0.00	0.00	0.43	8.75
5322	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas- Pedrera	0.00	0.00	0.00	3.27	0.00	0.05	0.00	1.78	5.09

5508	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.37	0.00	0.00	2.41	0.00	0.00	1.45	0.91	5.15
5515	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	16.02	0.00	0.00	0.00	0.87	16.89
5516	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	3.13	0.00	0.00	0.00	0.52	3.65
5526	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	28.38	0.00	0.00	0.00	1.69	30.07
5530	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	1.46	0.00	0.00	0.00	1.83	3.29
5536	Dennis-ray	<i>H. erato</i>	<i>reductimacula</i>	Amazonas	0.00	0.00	0.00	11.40	0.00	0.00	0.00	1.90	13.30
4728	Postman yellow_ventral	<i>H. erato</i>	<i>venus</i>	Buenaventura	0.00	0.00	0.00	0.65	1.26	24.24	15.01	0.00	41.17
4729	Postman yellow_ventral	<i>H. erato</i>	<i>venus</i>	Buenaventura	0.00	0.00	0.00	1.04	0.68	19.82	11.36	0.00	32.91
4731	Postman yellow_ventral	<i>H. erato</i>	<i>venus</i>	Buenaventura	0.00	0.00	0.00	0.00	0.66	9.97	5.89	3.39	19.91
4732	Postman yellow_ventral	<i>H. erato</i>	<i>venus</i>	Buenaventura	0.00	0.00	0.00	1.04	3.75	18.24	12.07	1.18	36.28
4733	Postman yellow_ventral	<i>H. erato</i>	<i>venus</i>	Buenaventura	0.00	0.00	0.00	0.64	1.91	25.64	14.10	3.72	46.02
4122	No mimicry ring	<i>H. hecale</i>	<i>holcophorus</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	5.13	1.47	0.00	6.60
5557	No mimicry ring	<i>H. hecale</i>	<i>holcophorus</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	4.18	2.82	1.40	8.41
4011	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Santander	0.00	0.00	0.00	0.00	0.00	5.42	2.45	0.00	7.87
4012	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Santander	0.00	0.00	0.00	0.00	0.00	2.94	1.87	0.00	4.81
4207	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	10.80	0.00	4.47	2.09	0.00	17.36
4238	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	7.84	0.00	5.75	2.49	0.00	16.09
4239	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	8.05	0.00	4.50	1.31	0.00	13.86
4241	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	0.05	0.00	12.82	5.62	0.00	18.49
4242	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	8.56	4.32	0.00	12.89
5615	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	15.47	5.87	0.00	21.34
5616	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	12.12	0.00	10.73	6.12	1.48	30.44
5617	No mimicry ring	<i>H. hecale</i>	<i>melicerta</i>	Boyaca	0.00	0.00	0.00	0.00	3.74	7.38	4.43	0.00	15.55
5787	Tiger	<i>H. hecale</i>	<i>melicerta</i>	Buenaventura	0.00	0.00	0.00	44.94	0.00	0.00	0.00	0.00	44.94
5016	No mimicry ring	<i>H. hecalesia</i>	<i>hecalesia</i>	Caldas	0.82	0.00	0.00	0.00	0.00	1.42	0.21	0.00	2.45
5017	No mimicry ring	<i>H. hecalesia</i>	<i>hecalesia</i>	Caldas	0.00	0.00	0.00	0.00	0.00	5.64	8.25	0.32	14.21
5637	No mimicry ring	<i>H. hecalesia</i>	<i>hecalesia</i>	Boyaca	0.00	0.00	0.00	13.39	0.00	12.36	7.20	1.69	34.64

5479	No mimicry ring	<i>H. heurippa</i>	<i>heurippa</i>	Meta	0.00	0.24	0.00	0.00	0.00	11.64	6.31	4.47	22.65
4236	No mimicry ring	<i>H. ismenius</i>	<i>ismenius</i>	Boyaca	0.00	0.00	9.56	0.00	0.00	5.11	2.85	0.00	17.52
4237	No mimicry ring	<i>H. ismenius</i>	<i>ismenius</i>	Boyaca	0.00	0.00	8.94	0.00	0.00	6.42	3.63	0.00	18.98
4575	Tiger	<i>H. ismenius</i>	<i>boulleti</i>	Buenaventura	0.00	0.00	18.29	0.00	0.00	8.40	5.23	0.00	31.91
4578	Tiger	<i>H. ismenius</i>	<i>boulleti</i>	Buenaventura	0.00	0.00	15.90	0.00	0.00	7.29	3.87	0.00	27.06
4579	Tiger	<i>H. ismenius</i>	<i>boulleti</i>	Buenaventura	0.00	0.00	22.40	0.00	0.00	5.51	2.77	0.00	30.68
4580	Tiger	<i>H. ismenius</i>	<i>boulleti</i>	Buenaventura	0.00	0.00	18.94	0.00	0.00	10.19	4.79	0.00	33.92
4581	Tiger	<i>H. ismenius</i>	<i>boulleti</i>	Buenaventura	0.00	0.00	19.62	0.00	0.17	6.52	3.41	1.45	31.16
5532	Yellow	<i>H. leucadia</i>	<i>leucadia</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	0.00	8.43	0.00	8.43
5544	Yellow	<i>H. leucadia</i>	<i>leucadia</i>	Amazonas	0.00	0.00	0.00	65.01	0.00	0.72	0.80	0.00	66.53
5609	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	6.16	3.70	0.00	9.86
5610	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	10.77	4.91	0.00	15.68
5611	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	11.76	4.52	0.00	16.28
5633	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	11.74	4.45	0.00	16.19
5634	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	8.51	3.98	0.00	12.49
5635	Red band	<i>H. melpomene</i>	<i>martinae</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	42.13	1.44	0.00	43.57
4888	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Meta	0.00	0.00	0.00	0.00	24.11	11.48	4.68	0.00	40.27
4892	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Meta	0.00	0.00	0.00	0.00	5.26	26.80	8.85	0.00	40.92
4900	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Guaviare	0.00	2.48	0.00	0.00	0.00	16.84	5.28	0.58	25.18
4903	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Guaviare	0.00	0.97	0.00	0.00	0.00	21.52	10.83	0.00	33.32
4905	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Guaviare	0.00	0.00	0.00	0.00	0.00	9.78	3.42	0.21	13.41
4906	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Guaviare	0.00	2.72	0.00	0.00	0.00	23.29	11.80	6.24	44.05
4996	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Meta	0.00	0.00	0.00	0.00	2.03	21.64	11.02	1.28	35.97
4997	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Meta	0.00	0.00	0.00	0.00	5.73	22.73	8.74	0.79	37.99
4998	Red band	<i>H. melpomene</i>	<i>melpomene</i>	Meta	0.00	0.00	1.39	0.00	3.56	27.83	14.94	0.00	47.72
4039	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	11.93	3.76	0.93	16.62
5027	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	15.25	6.62	0.00	21.87
5321	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.00	0.00	9.24	5.66	2.67	17.57
5338	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.00	0.00	7.13	2.64	0.00	9.77

5339	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas-Pedreira	0.00	0.00	1.38	0.00	0.00	12.59	4.54	2.65	21.17
5340	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	11.00	5.32	3.26	19.58
5374	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	12.92	6.75	4.04	23.70
5533	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	16.61	5.39	0.00	22.00
5534	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	20.01	9.73	0.00	29.74
5561	Dennis-ray	<i>H. melpomene</i>	<i>vicina</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	20.07	5.46	0.00	25.53
4727	Postman yellow_ventral	<i>H. melpomene</i>	<i>vulcanus</i>	Buenaventura	3.09	0.00	0.00	0.00	0.00	22.03	7.25	0.00	32.37
4730	Postman yellow_ventral	<i>H. melpomene</i>	<i>vulcanus</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	21.75	9.39	0.00	31.14
4741	Postman yellow_ventral	<i>H. melpomene</i>	<i>vulcanus</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	14.20	6.11	0.23	20.54
4743	Postman yellow_ventral	<i>H. melpomene</i>	<i>vulcanus</i>	Buenaventura	5.72	0.00	0.00	0.00	0.00	14.19	6.88	0.31	27.09
4744	Postman yellow_ventral	<i>H. melpomene</i>	<i>vulcanus</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	17.34	7.63	0.41	25.38
4778	Postman	<i>H. melpomene</i>	<i>bellula</i>	Putumayo	0.00	1.43	0.00	0.00	0.00	5.56	1.29	0.00	8.27
4779	Postman	<i>H. melpomene</i>	<i>bellula</i>	Putumayo	0.00	1.28	0.00	0.00	0.00	6.70	1.23	0.00	9.21
5388	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas-Pedreira	0.00	0.00	0.00	8.48	0.00	4.64	2.49	1.85	17.47
5392	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas-Pedreira	0.00	0.00	0.00	3.33	0.00	7.48	5.21	4.37	20.39
5422	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas-Pedreira	0.00	0.00	0.00	4.93	0.00	12.06	5.99	4.50	27.47
5423	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas-Pedreira	0.00	0.00	0.00	36.10	0.00	0.65	0.15	0.00	36.90
5424	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	3.88	1.76	3.17	8.81
5509	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	16.95	0.00	0.00	7.52	0.77	0.24	25.48
5510	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	5.04	0.00	0.00	10.21	4.84	0.15	20.24
5511	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.49	0.00	10.09	4.46	1.66	0.69	17.39
5512	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	15.08	5.32	0.47	20.86

5513	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	14.59	4.62	0.32	19.53
5519	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	12.94	4.90	0.32	18.16
5523	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.00	0.00	0.66	11.76	8.59	5.21	26.22
5548	No mimicry ring	<i>H. numata</i>	<i>silvana</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	15.35	8.27	3.48	27.10
4006	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	8.21	2.64	0.00	10.85
5330	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	15.91	6.41	0.89	23.20
5331	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	14.11	4.20	0.59	18.91
5332	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	10.59	3.95	0.41	14.96
5333	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.93	18.01	7.14	0.37	26.44
5520	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	17.51	7.83	1.20	26.53
5550	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	13.81	7.44	2.00	23.25
5551	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	35.71	21.11	11.02	67.84
5552	No mimicry ring	<i>H. pardalinus</i>	<i>butleri</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	5.61	2.38	0.15	8.15
5786	White	<i>H. sapho</i>	<i>chocoensis</i>	Buenaventura	0.00	0.00	0.00	160.80	0.00	2.43	0.38	0.00	163.61
5788	White	<i>H. sapho</i>	<i>chocoensis</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	2.21	0.67	0.82	3.70
5790	White	<i>H. sapho</i>	<i>chocoensis</i>	Buenaventura	0.00	0.00	0.00	0.00	0.00	2.52	0.80	0.00	3.32
4183	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	25.43	0.00	10.96	5.50	0.00	41.89
4187	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	5.29	0.00	14.72	5.77	0.00	25.79
4231	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	32.24	0.00	1.07	0.68	0.00	33.99
4232	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	50.60	0.00	0.49	0.39	0.00	51.47
4233	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	18.20	0.00	1.38	0.22	0.00	19.81
4374	Yellow	<i>H. sara</i>	<i>magdalena</i>	Arauca	0.00	0.00	0.00	0.00	7.09	6.18	3.93	1.11	18.31
4375	Yellow	<i>H. sara</i>	<i>magdalena</i>	Arauca	0.00	0.00	0.00	87.13	0.09	0.01	0.04	0.00	87.28
4376	Yellow	<i>H. sara</i>	<i>magdalena</i>	Arauca	0.00	0.00	0.00	0.01	20.05	7.75	3.61	0.00	31.42
4377	Yellow	<i>H. sara</i>	<i>magdalena</i>	Arauca	0.00	0.00	0.00	86.56	0.00	0.05	0.00	0.00	86.61
4673	Yellow	<i>H. sara</i>	<i>magdalena</i>	Cauca Valley	0.00	0.00	0.00	42.68	0.00	26.44	13.45	0.00	82.57
4674	Yellow	<i>H. sara</i>	<i>magdalena</i>	Cauca Valley	0.00	0.00	0.00	5.50	0.00	21.64	10.86	2.17	40.16
4683	Yellow	<i>H. sara</i>	<i>magdalena</i>	Cauca Valley	0.00	0.00	0.00	3.91	0.00	23.92	15.55	10.36	53.73

4684	Yellow	<i>H. sara</i>	<i>magdalena</i>	Cauca Valley	0.00	0.00	0.00	0.73	0.00	4.91	3.15	1.33	10.12
5571	Yellow	<i>H. sara</i>	<i>magdalena</i>	Meta	0.00	0.00	0.00	49.94	0.00	3.30	2.35	0.00	55.60
5572	Yellow	<i>H. sara</i>	<i>magdalena</i>	Meta	0.00	0.00	0.00	56.44	0.00	5.91	3.85	0.00	66.20
5573	Yellow	<i>H. sara</i>	<i>magdalena</i>	Meta	0.00	0.00	0.00	49.74	0.00	4.12	3.56	0.00	57.39
5574	Yellow	<i>H. sara</i>	<i>magdalena</i>	Meta	0.00	0.00	0.00	58.21	0.00	6.08	2.02	0.00	66.32
5575	Yellow	<i>H. sara</i>	<i>magdalena</i>	Meta	0.00	0.00	0.00	22.36	0.00	14.80	5.55	2.62	45.33
5650	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	0.75	0.00	5.98	3.55	1.22	11.49
5651	Yellow	<i>H. sara</i>	<i>magdalena</i>	Boyaca	0.00	0.00	0.00	0.00	0.00	13.80	4.59	0.00	18.39
5762	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	47.99	0.00	44.93	25.21	8.21	126.33
5763	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	70.49	0.00	8.92	6.55	1.27	87.22
5764	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	75.79	0.00	59.24	36.43	7.95	179.41
5774	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	84.54	0.00	7.25	3.67	0.00	95.46
5775	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	44.69	0.00	4.71	3.39	0.00	52.79
5776	Yellow	<i>H. sara</i>	<i>magdalena</i>	Buenaventura	0.00	0.00	0.00	76.94	0.00	4.71	3.59	0.00	85.24
4347	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	37.97	0.00	5.48	3.84	0.42	47.72
4348	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	53.28	0.00	11.86	7.11	0.69	72.94
4349	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	0.65	42.06	1.53	0.91	0.07	45.21
4350	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	7.63	0.00	13.67	7.99	1.30	30.59
4351	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	0.00	29.26	2.46	0.84	0.09	32.64
4352	Yellow	<i>H. sara</i>	<i>magdalena</i>	Vichada	0.00	0.00	0.00	0.00	61.68	3.91	2.27	0.12	67.98
4947	Yellow	<i>H. sara</i>	<i>sara</i>	Guaviare	0.00	0.00	0.00	65.96	0.00	8.47	3.14	0.00	77.57
5527	Yellow	<i>H. sara</i>	<i>sara</i>	Amazonas	0.00	0.00	0.00	24.53	0.00	0.00	6.13	1.46	32.12
5538	Yellow	<i>H. sara</i>	<i>sara</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	9.09	5.84	0.42	15.35
5540	Yellow	<i>H. sara</i>	<i>sara</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	0.00	39.56	16.26	55.81
5541	Yellow	<i>H. sara</i>	<i>sara</i>	Amazonas	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.00	0.85
5542	Yellow	<i>H. sara</i>	<i>sara</i>	Amazonas	0.00	0.00	0.00	0.00	0.73	21.41	19.25	4.25	45.64
5308	No mimicry ring	<i>H. wallacei</i>	<i>flavescens</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	16.86	3.92	0.00	20.77
5310	No mimicry ring	<i>H. wallacei</i>	<i>flavescens</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	20.97	2.60	0.00	23.57
5320	No mimicry ring	<i>H. wallacei</i>	<i>flavescens</i>	Amazonas-Pedreira	0.00	0.00	0.00	0.00	0.00	17.21	3.39	0.00	20.60



5342	No mimicry ring	<i>H. wallacei</i>	<i>flavescens</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.00	0.00	19.17	4.65	0.00	23.82
5343	No mimicry ring	<i>H. wallacei</i>	<i>flavescens</i>	Amazonas- Pedrera	0.00	0.00	0.00	0.00	0.93	22.54	12.76	10.94	47.17

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**Table S2** CNglcs identified in this study.

<b>Cyanogenic glucoside</b>	<b>Source</b>	<b>Retention time (min)</b>	<b>Sodium adducts (m/z)</b>	<b>Formic adducts (m/z)</b>
Linamarin	Biosynthesized	2.8	270	292
Lotaustralin	Biosynthesized	4	284	306
Epilotasutralin	Biosynthesized	4.3	284	
Deidaclin/Tetraphylin A	Sequestered	4	290	316
Tetraphylin B	Sequestered	1.4	310	332
Epivolkenin	Sequestered	2	310	332
Gynocardin	Sequestered	1	326	348
Dihydrogynocardin	Sequestered	1.8	328	350
Amygdalin	External Standard	5.3	480	502

**Table S3.** CNGlcs profiles compared among all species tested, between all species from the same locality, and between populations of the same species (PERMANOVA).

Comparison between	Sum of squares	R <sup>2</sup>	F-value	p (>F)
All Species of <i>Heliconius</i>	66818	0.28	4.57	0.001***
Species occurring in Puerto Nariño-Amazonas	13289	0.53	4.17	0.003***
Species occurring in Pedrera-Amazonas	3584.2	0.35	2.14	0.034*
Species occurring in Boyaca	6482.6	0.53	4.84	0.001***
Species occurring in Buenaventura	27181	0.54	3.94	0.012*
Species occurring in Caldas	59.486	0.21	0.83	0.541
Species occurring in Guaviare	4611.6	0.95	76.99	0.001***
Species occurring in Meta	7751.1	0.75	11.36	0.002***
Species occurring in Putumayo	52.288	0.87	20.13	0.100
Species occurring in Valle del Cauca	23116	0.67	13.98	0.001***
Populations of <i>H. sara</i>	22826	0.46	3.69	0.001***
Populations of <i>H. erato</i>	17981	0.3	2.11	0.028*
Populations of <i>H. melpomene</i>	1066.1	0.36	2.3962	0.015*
Populations of <i>H. eleuchia</i>	7721.2	0.6	10.73	0.026*
Populations of <i>H. cydno</i>	655.34	0.63	8.76	0.007**

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table S4** CNglcs profiles compared between species pairs (PERMANOVA).

<b>Species pairs</b>	<b>Sum of squares</b>	<b>F-values</b>	<b>R2</b>	<b>p-value</b>	<b>p-adjusted</b>
<i>H. antiochus</i> vs. <i>H. cydno</i>	4415.52	8.71	0.26	0	0.01*
<i>H. antiochus</i> vs. <i>H. eleuchia</i>	12494.56	8.78	0.34	0	0.01*
<i>H. antiochus</i> vs. <i>H. melpomene</i>	5665.73	15.78	0.28	0	0.01*
<i>H. charitonia</i> vs. <i>H. clysonimus</i>	1011.41	3.52	0.2	0	0.01*
<i>H. charitonia</i> vs. <i>H. cydno</i>	983.88	7.95	0.26	0	0.01*
<i>H. charitonia</i> vs. <i>H. hecale</i>	911.54	4.6	0.2	0	0.01*
<i>H. charitonia</i> vs. <i>H. melpomene</i>	1880.01	15.75	0.29	0	0.01*
<i>H. charitonia</i> vs. <i>H. numata</i>	934.96	4.95	0.21	0	0.01*
<i>H. charitonia</i> vs. <i>H. pardalinus</i>	1254.42	7.32	0.33	0	0.01*
<i>H. clysonimus</i> vs. <i>H. melpomene</i>	1316.28	9.39	0.2	0	0.01*
<i>H. cydno</i> vs. <i>H. ismenius</i>	1372.52	21.34	0.49	0	0.01*
<i>H. doris</i> vs. <i>H. eleuchia</i>	8792.45	7.48	0.37	0	0.01*
<i>H. eleuchia</i> vs. <i>H. erato</i>	15183.96	11.57	0.17	0	0.01*
<i>H. eleuchia</i> vs. <i>H. melpomene</i>	13962.38	34.74	0.47	0	0.01*
<i>H. eleuchia</i> vs. <i>H. pardalinus</i>	9377.04	10.94	0.41	0	0.01*
<i>H. eleuchia</i> vs. <i>H. sara</i>	18780.07	13.62	0.23	0	0.01*
<i>H. elevatus</i> vs. <i>H. ismenius</i>	737.29	23.17	0.68	0	0.01*
<i>H. erato</i> vs. <i>H. melpomene</i>	4701.77	5.88	0.07	0	0.01*
<i>H. hecale</i> vs. <i>H. ismenius</i>	1391.21	10.75	0.37	0	0.01*
<i>H. hecale</i> vs. <i>H. melpomene</i>	1276.65	10.84	0.2	0	0.01*
<i>H. ismenius</i> vs. <i>H. melpomene</i>	1999.6	23.83	0.39	0	0.01*
<i>H. ismenius</i> vs. <i>H. numata</i>	1071.8	8.93	0.33	0	0.01*
<i>H. ismenius</i> vs. <i>H. pardalinus</i>	1369.39	16.82	0.55	0	0.01*
<i>H. ismenius</i> vs. <i>H. sara</i>	7546.49	6.56	0.13	0	0.01*
<i>H. melpomene</i> vs. <i>H. sapho</i>	8478.06	13.88	0.3	0	0.01*
<i>H. melpomene</i> vs. <i>H. sara</i>	17721.89	23.08	0.25	0	0.01*
<i>H. antiochus</i> vs. <i>H. charitonia</i>	3990.91	4.89	0.23	0.01	0.02*
<i>H. antiochus</i> vs. <i>H. ismenius</i>	4072.84	5.25	0.26	0.01	0.04*
<i>H. antiochus</i> vs. <i>H. pardalinus</i>	3478.46	4.77	0.22	0.02	0.04*
<i>H. aoede</i> vs. <i>H. cydno</i>	6347.16	81.08	0.83	0.01	0.03*
<i>H. aoede</i> vs. <i>H. eleuchia</i>	8573.75	6	0.4	0.02	0.05*
<i>H. aoede</i> vs. <i>H. hecale</i>	4810.32	27.84	0.68	0.01	0.03*
<i>H. aoede</i> vs. <i>H. ismenius</i>	5851.8	143.88	0.95	0.02	0.04*
<i>H. aoede</i> vs. <i>H. melpomene</i>	7021.45	74.39	0.7	0	0.01*
<i>H. aoede</i> vs. <i>H. numata</i>	5347.55	33.47	0.72	0.01	0.03*
<i>H. aoede</i> vs. <i>H. pardalinus</i>	6098.22	52.01	0.85	0.02	0.04*
<i>H. charitonia</i> vs. <i>H. doris</i>	2021.33	5.85	0.33	0	0.01*
<i>H. charitonia</i> vs. <i>H. eleuchia</i>	8324.13	8.69	0.37	0.01	0.03*
<i>H. charitonia</i> vs. <i>H. elevatus</i>	575.72	3.88	0.24	0.01	0.02*
<i>H. charitonia</i> vs. <i>H. ismenius</i>	1502.68	10.85	0.45	0	0.01*
<i>H. charitonia</i> vs. <i>H. sara</i>	7863.8	6.8	0.13	0.01	0.02*
<i>H. charitonia</i> vs. <i>H. wallacei</i>	1196.28	7.29	0.4	0	0.01*
<i>H. clysonimus</i> vs. <i>H. cydno</i>	674.03	4.26	0.16	0.01	0.04*
<i>H. clysonimus</i> vs. <i>H. doris</i>	1511.51	3.68	0.23	0.01	0.04*

<i>H. clysonimus</i> vs. <i>H. eleuchia</i>	8342.3	8.25	0.35	0.01	0.04*
<i>H. clysonimus</i> vs. <i>H. ismenius</i>	1187.75	5.96	0.31	0	0.01*
<i>H. clysonimus</i> vs. <i>H. pardalinus</i>	861.65	3.85	0.2	0	0.01*
<i>H. cydno</i> vs. <i>H. doris</i>	981.05	5.48	0.21	0.02	0.04*
<i>H. cydno</i> vs. <i>H. eleuchia</i>	10811.05	18.55	0.44	0	0.01*
<i>H. cydno</i> vs. <i>H. hecale</i>	422.17	3.5	0.11	0.01	0.04*
<i>H. cydno</i> vs. <i>H. leucadia</i>	2059.15	10.38	0.38	0.02	0.04*
<i>H. cydno</i> vs. <i>H. melpomene</i>	445.69	5.05	0.1	0.02	0.05*
<i>H. cydno</i> vs. <i>H. sara</i>	11567.34	12.13	0.19	0	0.01*
<i>H. doris</i> vs. <i>H. hecale</i>	1546.11	5.62	0.25	0.01	0.02*
<i>H. doris</i> vs. <i>H. ismenius</i>	1858.66	7.54	0.41	0	0.01*
<i>H. doris</i> vs. <i>H. numata</i>	1086.79	4.1	0.19	0.01	0.04*
<i>H. doris</i> vs. <i>H. sapho</i>	6816.46	2.41	0.26	0.02	0.04*
<i>H. doris</i> vs. <i>H. sara</i>	6234.68	5.05	0.11	0.01	0.02*
<i>H. eleuchia</i> vs. <i>H. elevatus</i>	6878.26	6.92	0.35	0.02	0.04*
<i>H. eleuchia</i> vs. <i>H. hecale</i>	10006.32	13.43	0.4	0	0.01*
<i>H. eleuchia</i> vs. <i>H. ismenius</i>	8347.38	9.03	0.39	0.01	0.02*
<i>H. eleuchia</i> vs. <i>H. numata</i>	9901.76	13.44	0.4	0	0.01*
<i>H. eleuchia</i> vs. <i>H. wallacei</i>	7040.09	6.53	0.35	0.02	0.05*
<i>H. elevatus</i> vs. <i>H. sapho</i>	5847.71	2.35	0.25	0.01	0.04*
<i>H. elevatus</i> vs. <i>H. wallacei</i>	288.23	7.32	0.45	0.01	0.02*
<i>H. erato</i> vs. <i>H. sara</i>	8465.54	6.54	0.07	0.01	0.02*
<i>H. hecale</i> vs. <i>H. pardalinus</i>	724.53	4.67	0.19	0	0.02*
<i>H. hecale</i> vs. <i>H. sara</i>	6361.06	6.06	0.11	0.01	0.03*
<i>H. hecale</i> vs. <i>H. wallacei</i>	759.61	5.21	0.25	0.01	0.02*
<i>H. hecalesia</i> vs. <i>H. ismenius</i>	601.01	11.87	0.6	0.01	0.02*
<i>H. hecalesia</i> vs. <i>H. wallacei</i>	352.88	5.17	0.46	0.02	0.04*
<i>H. ismenius</i> vs. <i>H. sapho</i>	6653.89	3.06	0.28	0.01	0.03*
<i>H. ismenius</i> vs. <i>H. wallacei</i>	1228.84	32.92	0.77	0	0.01*
<i>H. leucadia</i> vs. <i>H. melpomene</i>	2457.43	15.53	0.33	0	0.01*
<i>H. leucadia</i> vs. <i>H. pardalinus</i>	2118.86	6.16	0.41	0.02	0.05*
<i>H. melpomene</i> vs. <i>H. numata</i>	646.08	5.67	0.12	0	0.02*
<i>H. numata</i> vs. <i>H. sara</i>	7363.05	7.04	0.13	0	0.02*
<i>H. numata</i> vs. <i>H. wallacei</i>	444.32	3.28	0.17	0.02	0.05*
<i>H. pardalinus</i> vs. <i>H. sapho</i>	6948.47	3.82	0.28	0.01	0.02*
<i>H. pardalinus</i> vs. <i>H. sara</i>	7432.37	6.65	0.13	0	0.01*
<i>H. sapho</i> vs. <i>H. wallacei</i>	5977.33	2.06	0.26	0.02	0.05*
<i>H. antiochus</i> vs. <i>H. aede</i>	1959.76	1.7	0.15	0.2	0.28
<i>H. antiochus</i> vs. <i>H. clysonimus</i>	1286.14	1.49	0.08	0.23	0.31
<i>H. antiochus</i> vs. <i>H. doris</i>	3440.42	3.45	0.2	0.06	0.11
<i>H. antiochus</i> vs. <i>H. elevatus</i>	2658.54	3.21	0.19	0.08	0.13
<i>H. antiochus</i> vs. <i>H. erato</i>	1850.86	1.46	0.03	0.19	0.27
<i>H. antiochus</i> vs. <i>H. hecale</i>	2431.63	3.76	0.15	0.05	0.09
<i>H. antiochus</i> vs. <i>H. hecalesia</i>	1170.43	1.1	0.09	0.32	0.41
<i>H. antiochus</i> vs. <i>H. heurippa</i>	643.27	0.51	0.05	0.63	0.71
<i>H. antiochus</i> vs. <i>H. leucadia</i>	241.08	0.18	0.02	0.86	0.87
<i>H. antiochus</i> vs. <i>H. numata</i>	2895.47	4.53	0.18	0.03	0.05*
<i>H. antiochus</i> vs. <i>H. sapho</i>	1912.13	0.73	0.06	0.48	0.57

<i>H. antiochus</i> vs. <i>H. sara</i>	340.34	0.26	0.01	0.83	0.86
<i>H. antiochus</i> vs. <i>H. wallacei</i>	2623.09	2.93	0.18	0.11	0.17
<i>H. aoede</i> vs. <i>H. charitonia</i>	5736.49	26.75	0.77	0.03	0.06
<i>H. aoede</i> vs. <i>H. clysonimus</i>	3816.56	12.18	0.6	0.02	0.05*
<i>H. aoede</i> vs. <i>H. doris</i>	6133.24	14.01	0.7	0.03	0.05*
<i>H. aoede</i> vs. <i>H. elevatus</i>	5291.53	119.43	0.95	0.04	0.08
<i>H. aoede</i> vs. <i>H. erato</i>	4226.71	3.41	0.07	0.07	0.13
<i>H. aoede</i> vs. <i>H. hecalesia</i>	3600.55	33.66	0.92	0.1	0.16
<i>H. aoede</i> vs. <i>H. heurippa</i>	2419.54	24.13	0.96	0.33	0.42
<i>H. aoede</i> vs. <i>H. leucadia</i>	702.38	0.63	0.24	0.67	0.74
<i>H. aoede</i> vs. <i>H. sapho</i>	37.38	0.01	0	1	1
<i>H. aoede</i> vs. <i>H. sara</i>	1747.58	1.34	0.03	0.32	0.41
<i>H. aoede</i> vs. <i>H. wallacei</i>	5490.09	94.96	0.95	0.05	0.09
<i>H. charitonia</i> vs. <i>H. erato</i>	1921.42	1.7	0.03	0.15	0.23
<i>H. charitonia</i> vs. <i>H. hecalesia</i>	357.78	1.75	0.16	0.19	0.27
<i>H. charitonia</i> vs. <i>H. heurippa</i>	203.27	0.88	0.11	0.21	0.29
<i>H. charitonia</i> vs. <i>H. leucadia</i>	1933.6	4.12	0.34	0.04	0.09
<i>H. charitonia</i> vs. <i>H. sapho</i>	6566.19	3.13	0.26	0.04	0.07
<i>H. clysonimus</i> vs. <i>H. elevatus</i>	387.25	1.81	0.13	0.24	0.32
<i>H. clysonimus</i> vs. <i>H. erato</i>	38.28	0.03	0	0.99	1
<i>H. clysonimus</i> vs. <i>H. hecale</i>	94.12	0.39	0.02	0.53	0.62
<i>H. clysonimus</i> vs. <i>H. hecalesia</i>	97.05	0.33	0.04	0.78	0.83
<i>H. clysonimus</i> vs. <i>H. heurippa</i>	142.24	0.41	0.06	0.57	0.66
<i>H. clysonimus</i> vs. <i>H. leucadia</i>	864.59	1.52	0.16	0.16	0.24
<i>H. clysonimus</i> vs. <i>H. numata</i>	245.1	1.06	0.05	0.35	0.44
<i>H. clysonimus</i> vs. <i>H. sapho</i>	4139.6	1.9	0.17	0.22	0.31
<i>H. clysonimus</i> vs. <i>H. sara</i>	3195.57	2.72	0.06	0.09	0.15
<i>H. clysonimus</i> vs. <i>H. wallacei</i>	844.03	3.58	0.25	0.05	0.1
<i>H. cydno</i> vs. <i>H. elevatus</i>	20.74	0.31	0.01	0.71	0.78
<i>H. cydno</i> vs. <i>H. erato</i>	2053.62	2.13	0.03	0.1	0.16
<i>H. cydno</i> vs. <i>H. hecalesia</i>	93.87	1.16	0.06	0.31	0.4
<i>H. cydno</i> vs. <i>H. heurippa</i>	12.62	0.16	0.01	0.84	0.86
<i>H. cydno</i> vs. <i>H. numata</i>	142.53	1.24	0.04	0.25	0.33
<i>H. cydno</i> vs. <i>H. pardalinus</i>	221.67	2.43	0.09	0.12	0.19
<i>H. cydno</i> vs. <i>H. sapho</i>	7493.13	7.3	0.29	0.02	0.05*
<i>H. cydno</i> vs. <i>H. wallacei</i>	337.5	4.76	0.19	0.03	0.06
<i>H. doris</i> vs. <i>H. elevatus</i>	742.07	2.76	0.22	0.09	0.15
<i>H. doris</i> vs. <i>H. erato</i>	2554.54	2.14	0.04	0.15	0.23
<i>H. doris</i> vs. <i>H. hecalesia</i>	699.05	1.78	0.2	0.18	0.26
<i>H. doris</i> vs. <i>H. heurippa</i>	156.79	0.31	0.06	0.6	0.68
<i>H. doris</i> vs. <i>H. leucadia</i>	2467.78	3.17	0.35	0.03	0.06
<i>H. doris</i> vs. <i>H. melpomene</i>	389.43	2.57	0.07	0.09	0.15
<i>H. doris</i> vs. <i>H. pardalinus</i>	307.14	1.15	0.08	0.35	0.44
<i>H. doris</i> vs. <i>H. wallacei</i>	102.01	0.34	0.04	0.74	0.8
<i>H. eleuchia</i> vs. <i>H. hecalesia</i>	4271.32	3.29	0.25	0.07	0.12
<i>H. eleuchia</i> vs. <i>H. heurippa</i>	1799.06	1.13	0.12	0.28	0.37
<i>H. eleuchia</i> vs. <i>H. leucadia</i>	4723.49	2.85	0.24	0.08	0.13
<i>H. eleuchia</i> vs. <i>H. sapho</i>	10537.03	3.51	0.26	0.03	0.06

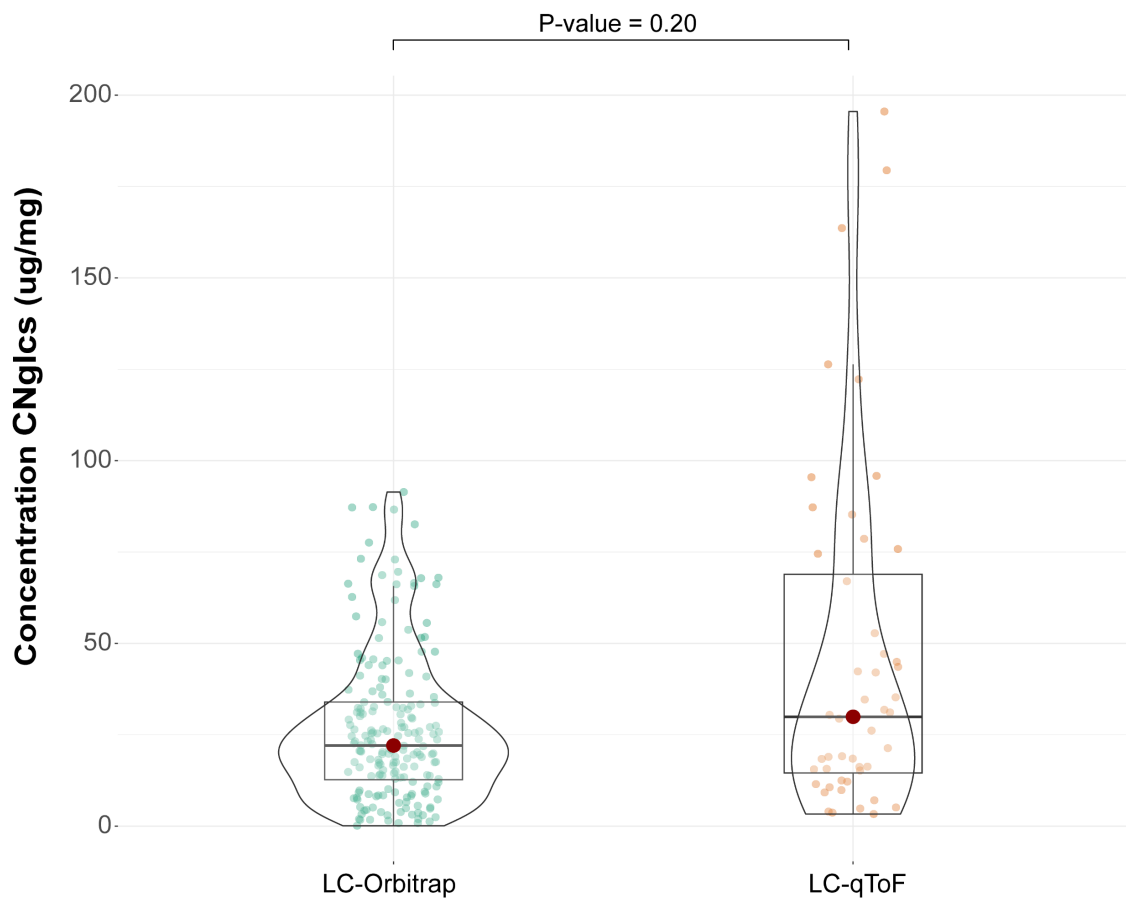
<i>H. elevatus</i> vs. <i>H. erato</i>	835.73	0.73	0.01	0.28	0.36
<i>H. elevatus</i> vs. <i>H. hecale</i>	209.08	1.54	0.08	0.23	0.31
<i>H. elevatus</i> vs. <i>H. hecalesia</i>	66.06	1.2	0.15	0.27	0.35
<i>H. elevatus</i> vs. <i>H. heurippa</i>	20.97	0.63	0.11	0.55	0.63
<i>H. elevatus</i> vs. <i>H. leucadia</i>	1705.99	4.43	0.43	0.03	0.07
<i>H. elevatus</i> vs. <i>H. melpomene</i>	275.99	3.22	0.08	0.05	0.09
<i>H. elevatus</i> vs. <i>H. numata</i>	71.49	0.57	0.03	0.72	0.78
<i>H. elevatus</i> vs. <i>H. pardalinus</i>	186.4	2.16	0.14	0.13	0.21
<i>H. elevatus</i> vs. <i>H. sara</i>	5188.44	4.41	0.09	0.02	0.05*
<i>H. erato</i> vs. <i>H. hecale</i>	381.1	0.37	0.01	0.72	0.78
<i>H. erato</i> vs. <i>H. hecalesia</i>	194.33	0.16	0	0.78	0.83
<i>H. erato</i> vs. <i>H. heurippa</i>	192.67	0.15	0	0.46	0.56
<i>H. erato</i> vs. <i>H. ismenius</i>	2379.47	2.12	0.04	0.14	0.21
<i>H. erato</i> vs. <i>H. leucadia</i>	877.4	0.68	0.01	0.16	0.24
<i>H. erato</i> vs. <i>H. numata</i>	788.79	0.76	0.01	0.45	0.55
<i>H. erato</i> vs. <i>H. pardalinus</i>	1832.77	1.67	0.03	0.17	0.25
<i>H. erato</i> vs. <i>H. sapho</i>	4890.34	3.13	0.06	0.14	0.22
<i>H. erato</i> vs. <i>H. wallacei</i>	1413.81	1.21	0.02	0.16	0.24
<i>H. hecale</i> vs. <i>H. hecalesia</i>	21.12	0.12	0.01	0.85	0.87
<i>H. hecale</i> vs. <i>H. heurippa</i>	87.27	0.49	0.04	0.2	0.28
<i>H. hecale</i> vs. <i>H. leucadia</i>	1251.12	3.79	0.23	0.05	0.1
<i>H. hecale</i> vs. <i>H. numata</i>	126.42	0.74	0.03	0.46	0.56
<i>H. hecale</i> vs. <i>H. sapho</i>	5481.27	3.96	0.22	0.11	0.17
<i>H. hecalesia</i> vs. <i>H. heurippa</i>	46.78	0.42	0.17	0.75	0.81
<i>H. hecalesia</i> vs. <i>H. leucadia</i>	989.52	1.26	0.3	0.3	0.39
<i>H. hecalesia</i> vs. <i>H. melpomene</i>	310.04	3.26	0.09	0.05	0.09
<i>H. hecalesia</i> vs. <i>H. numata</i>	34.97	0.22	0.02	0.9	0.9
<i>H. hecalesia</i> vs. <i>H. pardalinus</i>	238.55	2.03	0.17	0.17	0.25
<i>H. hecalesia</i> vs. <i>H. sapho</i>	3678.8	0.84	0.17	0.7	0.77
<i>H. hecalesia</i> vs. <i>H. sara</i>	2086.25	1.64	0.04	0.21	0.29
<i>H. heurippa</i> vs. <i>H. ismenius</i>	270.48	8.8	0.59	0.11	0.18
<i>H. heurippa</i> vs. <i>H. leucadia</i>	804.48	0.38	0.27	0.67	0.74
<i>H. heurippa</i> vs. <i>H. melpomene</i>	33.47	0.36	0.01	0.6	0.68
<i>H. heurippa</i> vs. <i>H. numata</i>	34.24	0.21	0.02	0.79	0.83
<i>H. heurippa</i> vs. <i>H. pardalinus</i>	20.08	0.17	0.02	0.5	0.59
<i>H. heurippa</i> vs. <i>H. sapho</i>	2256.5	0.26	0.12	0.5	0.59
<i>H. heurippa</i> vs. <i>H. sara</i>	977.86	0.73	0.02	0.48	0.57
<i>H. heurippa</i> vs. <i>H. wallacei</i>	54.55	1.16	0.22	0.33	0.42
<i>H. ismenius</i> vs. <i>H. leucadia</i>	2124.61	6.39	0.48	0.03	0.05*
<i>H. leucadia</i> vs. <i>H. numata</i>	1552.85	4.9	0.27	0.05	0.09
<i>H. leucadia</i> vs. <i>H. sapho</i>	558.2	0.09	0.03	0.8	0.84
<i>H. leucadia</i> vs. <i>H. sara</i>	230.49	0.17	0	0.82	0.85
<i>H. leucadia</i> vs. <i>H. wallacei</i>	2032.48	4.36	0.47	0.05	0.09
<i>H. melpomene</i> vs. <i>H. pardalinus</i>	25.73	0.26	0.01	0.8	0.84
<i>H. melpomene</i> vs. <i>H. wallacei</i>	66.18	0.75	0.02	0.47	0.57
<i>H. numata</i> vs. <i>H. pardalinus</i>	354.3	2.42	0.11	0.05	0.09
<i>H. numata</i> vs. <i>H. sapho</i>	6143.51	4.48	0.24	0.1	0.16
<i>H. pardalinus</i> vs. <i>H. wallacei</i>	55.81	0.59	0.05	0.54	0.62

<i>H. sapho</i> vs. <i>H. sara</i>	1723.11	1.01	0.03	0.36	0.44
<i>H. sara</i> vs. <i>H. wallacei</i>	4773.31	3.95	0.09	0.03	0.05*

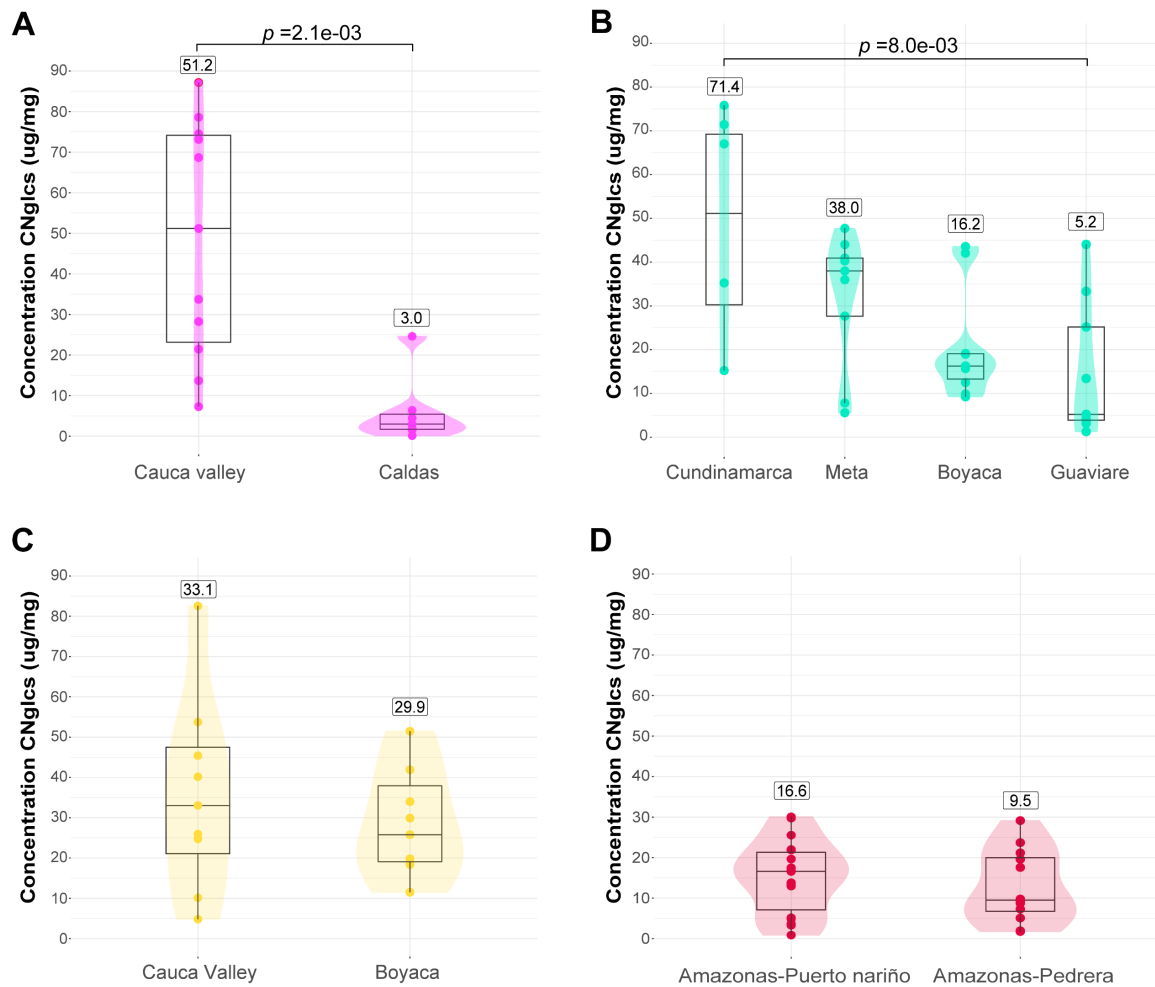
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Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

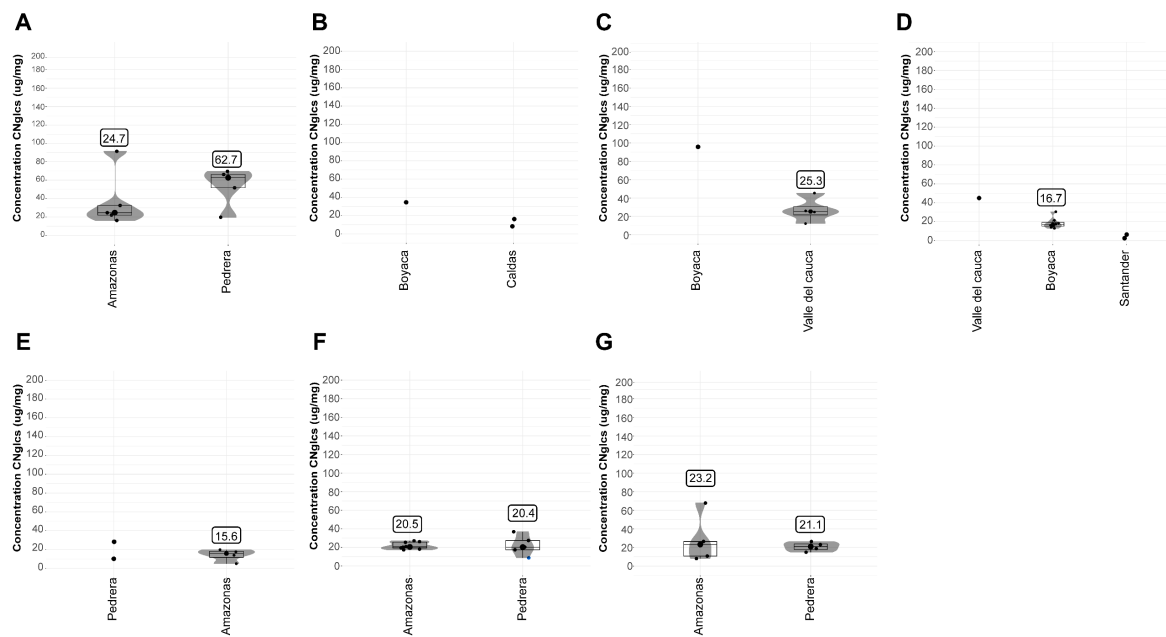




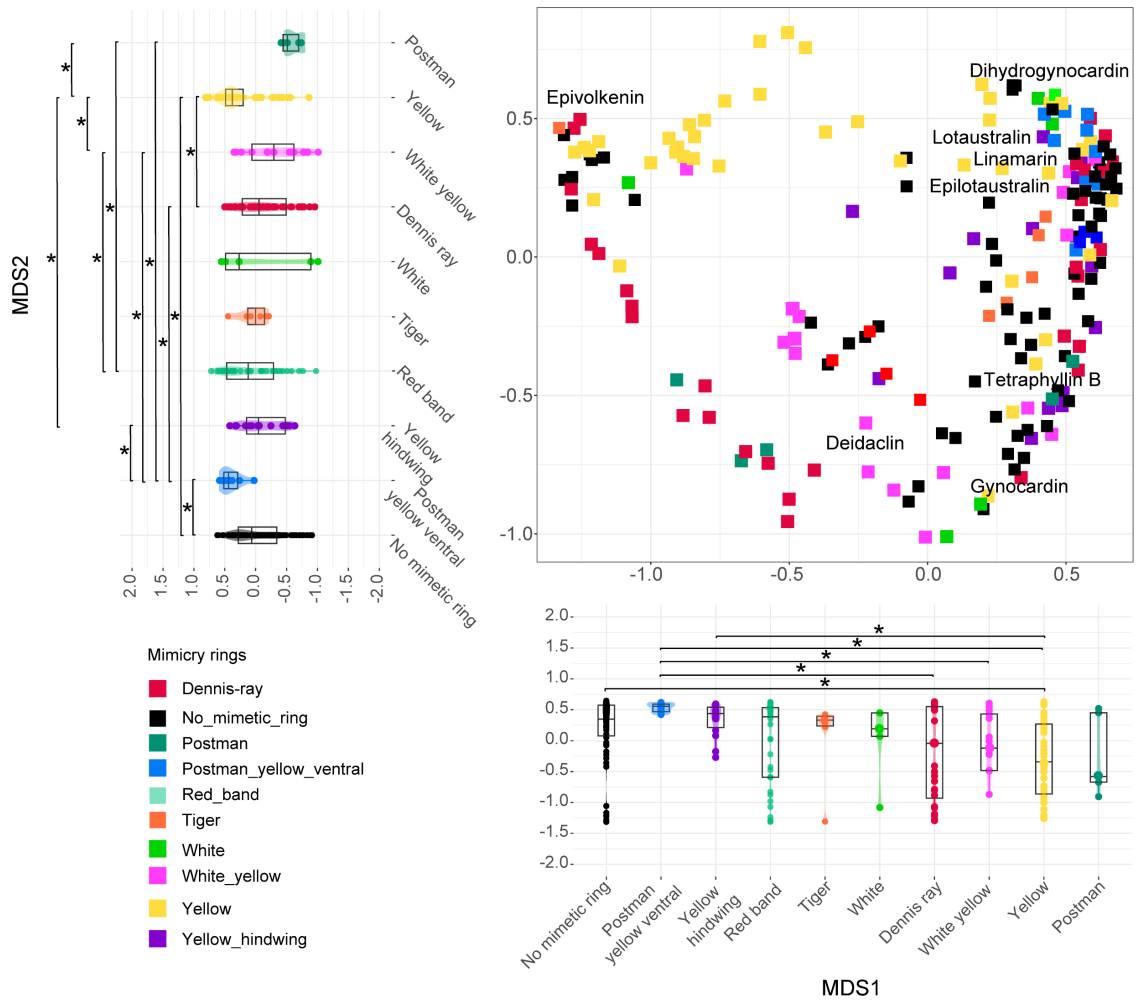
**Figure S1** Differences between the quantification of all CNGlcs combined ('CNGlcs total') obtained from LC-qToF-MS and LC-Orbitrap-MS.



**Figure S2 Quantification of all CNglcs combined ('CNglcs total') per mimicry ring and locality.** (A) white yellow, (B) red band, (C) dennis-ray and (D) yellow. Mean concentration of 'CNglcs total' is shown on top of each box (numbers on squares). Significantly different comparisons ( $\alpha < 0.05$ ) are shown.



**Figure S3 Variation in the concentrations of all CNGlcs combined ('CNGlcs total') within a species occurring in different localities. (A) *H. antiochus*, (B) *H. hecalesia*, (C) *H. doris*, (D) *H. hecale*, (E) *H. elevatus*, (F) *H. numata*, and (G) *H. pardalinus*. Mean concentration of 'CNGlcs total' is shown on top of each box (numbers on squares).**



**Figure S4. Non-metric multidimensional scaling (NMDS) of cyanogenic glycosides in *Heliconius* comparing mimicry rings.** The distance between two symbols is indicative of how different two individuals are in the composition of CNgIcs. Boxplots for both NMDS1 and NMDS2 show differences between mimicry rings in each axis. Asterisk (\*) symbolises a  $p$ -value < 0.05.



BIODIVERSITY GENOMICS  
2022

## Certificate of attendance

This is to certify that: **Nicol Rueda**  
Has attended **Biodiversity Genomics 2022**  
During the dates **3rd - 7th October 2022**  
Held at **Virtual Conference**  
**Hinxton**  
**Cambridge**  
**UK**

Authorised by: A handwritten signature in black ink that reads 'BG' above '2022'.

The Biodiversity Organisers and  
Committee 2022

# CRONOGRAMA OFICIAL

## VI CONGRESO COLOMBIANO DE ZOOLOGÍA

2023

### La Asociación Colombiana de Zoología

se complace en presentar el  
cronograma oficial del Congreso  
de Zoología en su VI versión



Congreso  
Colombiano  
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Montería // 27-31 mar 2023

El presente documento interactivo, contiene la programación completa del evento. En la página de "contenido" podrá acceder a los simposios de su interés y ver las charlas disponibles según el horario y salón.



# Diversificación y estructura genética en *Heliconius timareta*: contrastando historia demográfica y mimetismo

## CATEGORIA DEL TRABAJO

Biogeografía y Filogeografía en el estudio de la diversidad Neotropical

## AUTORES

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PARDO DIAZ, Carolina, U Rosario  
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MONTEJO KOVACEVICH, Gabriela, U Cambridge  
JIGGINS, Chris, U Cambridge  
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## MODALIDAD DE PRESENTACION APROBADA

Oral

---

# Sex chromosome – autosome fusions in *Heliconius* butterflies

## CATEGORIA DEL TRABAJO

Biogeografía y Filogeografía en el estudio de la diversidad Neotropical

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## MODALIDAD DE PRESENTACION APROBADA

Oral

# CRONOGRAMA OFICIAL

## VI CONGRESO COLOMBIANO DE ZOOLOGÍA

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Congreso  
Colombiano  
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**Zoología**

Montería // 27-31 mar 2023

Para conocer cada trabajo presentado en este cronograma, con sus respectivos autores y la descripción específica, dé click en el siguiente enlace:

<https://accolombia.org/wp-content/uploads/2023/03/Informacion-detallada-autores-trabajos-VI-CCZ-23-03-2023-1.pdf>







2022 Prague – Czech Republic

# Congress of the European Society for Evolutionary Biology

on August 14–19, 2022, Prague Congress Centre

The contribution

## **Sex-specific ancestral fusions between autosomes and W chromosome in *Heliconius* butterflies**

by

**Nicol Magaly Rueda Munoz**

has been recognized as

## **Outstanding Student Poster**

**Prof. Adam Petrusek**  
Chair of the Student Awards Committee

**Prof. Lukáš Kratochvíl**  
Conference Chair



*This scientific program is subject to change and is current at 17 July 2023*

## SUNDAY 16 JULY 2023

0900-1700	<b>Optional Satellite Meeting</b>
Room 219	<b>Biodiversity Genomics - A Global Perspective</b> Melbourne Convention & Exhibition Centre
1700 - 1745	<b>Optional Session</b>
Hospitality Room 6 – Upper level	Congress tips for HDR Students and Early Career Researchers
1200-1930	<b>Registration Open</b> Melbourne Convention & Exhibition Centre Foyer
1800-2100 Plenary Room 2	<b>Opening Ceremony &amp; Welcome Reception</b> Melbourne Convention & Exhibition Centre

## MONDAY 17 JULY 2023

0700-1730	<b>Registration Open</b> Melbourne Convention & Exhibition Centre Foyer
0830-0930	<b>Plenary Session 1</b>
Plenary Room 2	<b>Mark Blaxter - Wellcome Sanger Institute, UK</b> New biology from new genomes: The Tree of Life project <i>Chair: Jenny Graves, Australia</i> <i>Session supported by BGI/MGI</i>
1000-1200	<b>Symposia Sessions A</b>
Room 203	<b>A1: Genomes &amp; Biodiversity (Evolutionary Genetics)</b> <i>Session supported by Bioplatforms Australia</i>
Convenors	<i>Tammy Steeves, New Zealand &amp; Claire Mérot, France</i>
1000-1030	<b>Claire Mérot, CNRS ECOBIO / Université de Rennes, France</b> The role of structural genomic variants in the evolution of biodiversity
1030-1100	<b>Carolina Pardo-Diaz, Universidad del Rosario, Colombia</b> <u>Drivers of diversification in a neotropical biodiversity hotspot: lessons from spiders, birds, and butterflies</u>
1100-1115	<b>Scott Edwards, Harvard University, USA</b> Pangenomes of North American Scrub-Jays ( <i>Aphelocoma</i> ) reveal abundant structural variation and rapid shifts in genome size
1115-1130	<b>Jonathan Sandoval Castillo, Flinders University, Australia</b> Genomic basis of body miniaturization in Southern pygmy perch ( <i>Nannoperca australis</i> ; Teleostei)
1130-1145	<b>Carolyn Hogg, University of Sydney, Australia</b> Integrating genomics, conservation, and Indigenous knowledge to protect a unique marsupial
1145-1200	<b>Philipp Schiffer, University of Cologne, Germany</b> Biodiversity genomics of asexual and anhydrobiotic nematodes in extreme environments