UNIVERSIDAD DE COSTA RICA

SISTEMA DE ESTUDIOS DE POSGRADO

PATRONES DE COLORACIÓN EN HYMENOPTERA: SU ORIGEN, EVOLUCIÓN Y POSIBLE FUNCIÓN EN MIRAS A LA APLICACIÓN BIOMIMÉTICA

Tesis sometida a la consideración de la Comisión del Programa de Doctorado en Ciencias para optar al grado y título de Doctorado Académico en Ciencias

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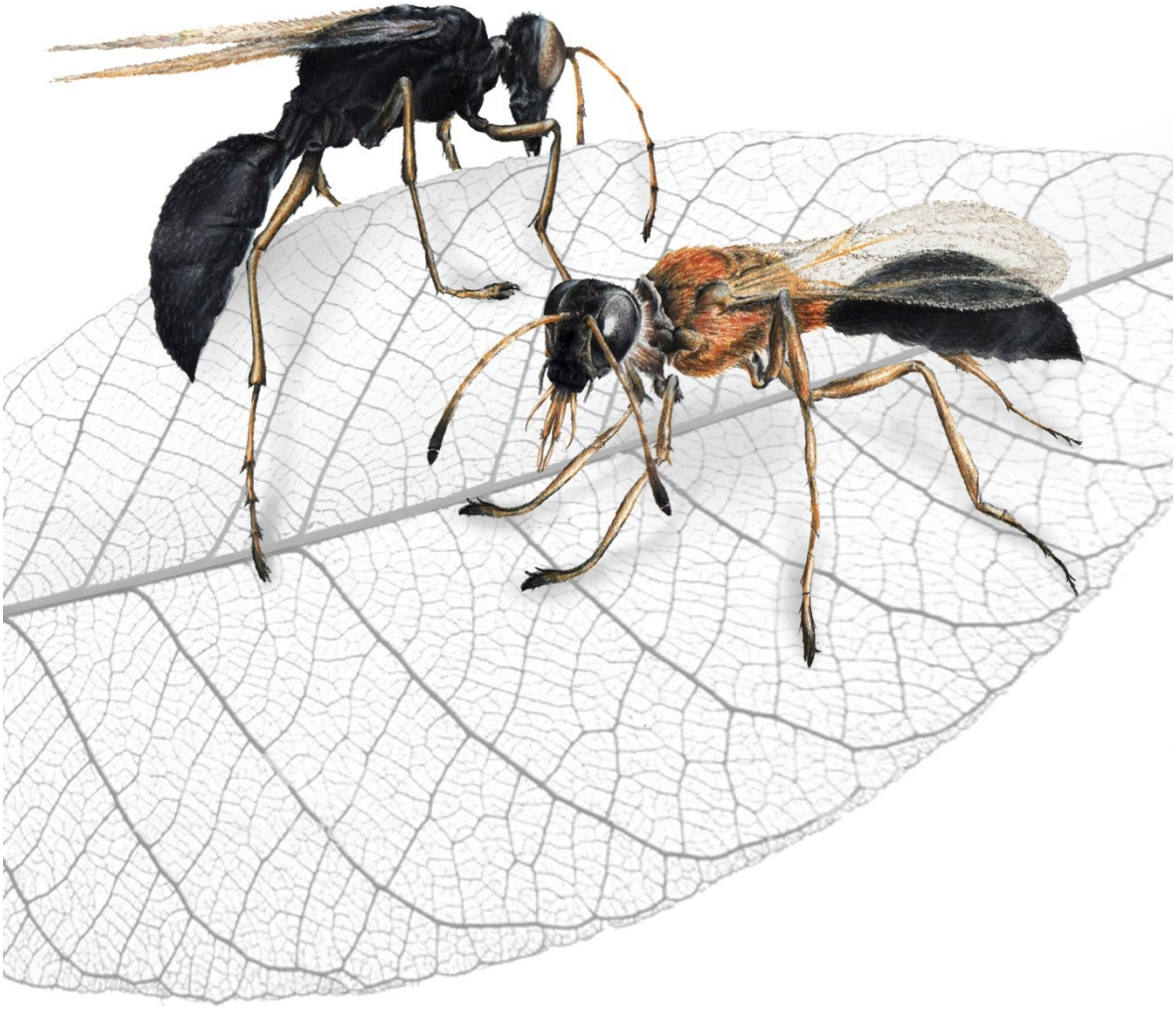

Ilustración1. Jennifer Bejarano. Título: forrageo de microavispa.

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"El mundo necesita de la ciencia y la ciencia necesita de mujeres"

Bokova, directora Unesco.

"Esta tesis fue aceptada por la Comisión del Programa de estudios de Posgrado en Ciencias de la Universidad de Costa Rica, como requisito parcial para optar al grado y título de Doctorado Académico en Ciencias."

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Cuerpo de Trabajo

RESUMEN

El presente estudio proporciona la primera caracterización física y funcional del patrón negro-naranja-negro (BOB) que se encuentra en avispas parasitoides de muy pequeño tamaño (3-10 mm). Aunque se había reportado previamente que la coloración BOB ocurre en himenópteros, especialmente en Scelioninae, los resultados de esta investigación demuestran que este patrón está mucho más extendido de lo que se creía. Este patrón abarca tanto ecto- como endoparasitoides, idiobiontes y koinobiontes, de una diversidad de hospederos, así como también sínfitos fitófagos. Con respecto al tamaño, la coloración BOB parece ser especialmente común (no exclusiva) en himenópteros con una longitud corporal entre 3 y 10 mm. Con respecto a la distribución altitudinal de la coloración BOB, la gran mayoría de especímenes que muestran este patrón de color fueron recolectados debajo de los 2,000 msnm. Finalmente, este patrón de color se encuentra en todas las regiones biogeográficas, aunque con menor frecuencia que en el neotrópico. Para ahondar más en la caracterización de este patrón más allá de la taxonomía y hábitos biológicos, se utilizó la técnica óptica de la microspectrofotometría para generar espectros de reflectancia para ocho géneros de sceliónidos. Estos datos de reflectancia se analizaron mediante análisis univariados y análisis de datos funcionales los cuales demostraron ser superiores al ofrecer una mejor representación de la información física, dado que tienen en cuenta la curva espectrofotométrica completa. Adicionalmente, se implementó el método de componentes espectrales RGB para estudiar el origen del color en los casos en que no es posible dilucidar una composición química directa. En el caso de los géneros estudiados, se descubrió que los componentes espectrales azules del color naranja y negro eran casi idénticos, lo que sugiere que existe un compuesto común para los pigmentos. Se utilizó una correlación entre los valores medios de las características de los componentes de color en un intento de agrupar géneros que muestran valores similares y algunos, pero no todos, son similares a sus posiciones en las filogenias publicadas en la literatura. Profundizando más en la investigación, se propuso realizar análisis nanométricos y micrométricos para investigar las propiedades morfológicas y mecánicas del patrón de color negro-naranja-negro (BOB), lo cual nunca antes había sido estudiado. El objetivo fue explorar las diferencias estructurales y mecánicas en el mesoscoto de cuatro géneros: *Baryconus* con mesosoma naranja (es decir, patrón BOB) y todo negro, así como *Scelio* con un mesosoma naranja (es decir, patrón BOB) y todo negro. Los hallazgos más destacados incluyen la ausencia de estructuras multicapa que generan color estructural, un pigmento concentrado en la superficie superior de la epicutícula y sorprendentes diferencias entre los cuatro géneros. Tres de los cuatro géneros mostraron una estructura similar a acordeón en el surco (notaulus), mientras que el mesoscoto adyacente resultó diferente en cada especie. Además, los espectros de componentes de color normalizados para los colores azul, verde y rojo del mesoscoto negro de cada género mostraron la misma dependencia espectral, mientras que el color naranja manifestó pequeños cambios en la longitud de onda dominante, lo cual correlaciona con tonos anaranjados ligeramente diferentes. Finalmente, con el comprender la función del patrón BOB en la naturaleza, *L. jemineus* (un depredador con una agudeza visual sobresaliente) y 4 géneros Scelionidae (una pequeña avispa conspicua que conforma un estímulo móvil no quiescente) sirvieron como modelos de estudio. Aunque las respuestas de comportamiento fueron bastante variables, dos resultados acuerpan claramente la naturaleza aposemática del patrón de color estudiado: en cinco ocasiones las arañas capturadas en el campo consumieron avispas negras (todas pertenecientes al género *Scelio*), pero ninguna araña consumió una avispa con coloración BOB y en segundo lugar, las pruebas de toxicidad aguda con *Daphnia magna* mostraron que las avispas con coloración BOB causaron una mayor mortalidad que las avispas negras,

lo que sugiere que las avispas BOB pueden contener compuestos defensivos a diferencia de las avispas negras (lo que explicaría por qué solo se consumieron avispas negras).

Lista de Cuadros

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INTRODUCCIÓN

La coloración es un tapiz con el cual un organismo se presenta al mundo; constituye un "lenguaje de silencio" funcional con el que puede comunicar su lugar, en la comunidad donde vive.

Cutícula

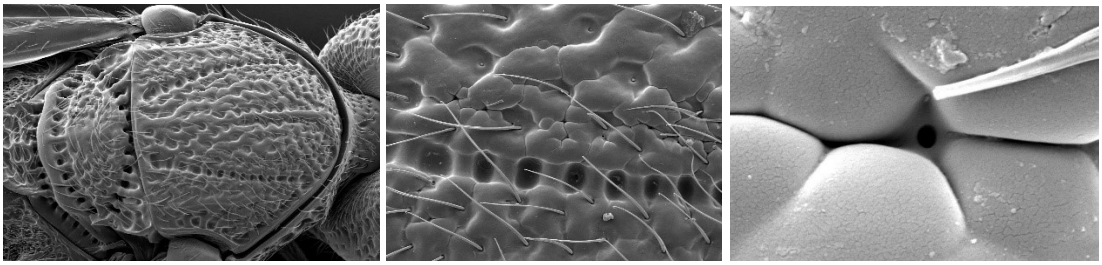

Ilustración fotográfica 2. Fotografía realizada por R. Mora y R. Pereira. Cúticula microavispa. Técnica Microscopio Electrónico de Barrido.

Los insectos son maestros de color, cuya virtuosidad es particularmente evidente en la estructura de la cutícula, el material que forma el exoesqueleto y sirve de límite entre el animal vivo y el mundo exterior (Neville, 1975).

La cutícula, es un compuesto de fibrillas, adaptada para resistencia, rigidez o flexibilidad, permeabilidad, elasticidad, según las necesidades. La cutícula, no solo retiene el insecto, sino que le da su forma, medio de locomoción, impermeabilización y una gama de especializaciones mecánicas localizadas tales como elasticidad, adhesión, resistencia al desgaste y control de difusión. También puede servir para almacenar alimentos temporalmente y es una barrera importante para el parasitismo y las enfermedades. La cutícula deriva sus propiedades de sus componentes como nanofibras de quitina, proteína, contenido de agua y grado de reticulación de la proteína, lípidos, iones metálicos (Zn, Mn, Fe) y carbonato de calcio. Estos componentes pueden variar en orientación y fracción de volumen, lo cual produce la amplia gama de propiedades mecánicas (Artz et al., 2002).

Patrones de color

Tradicionalmente, los estudios en ecología visual se han centrado en señales estáticas de color uniforme. Sin embargo, cada vez más se reconoce que las señales animales son complejas y dinámicas en ambos espacio y tiempo, con patrones espaciales (marcas) y fuertes componentes basados en movimiento. Además, los colores en sí mismos, como la forma en que se organizan en patrones, a menudo varían entre los individuos de una especie.

Se han observado diferencias en las tasas de la capacidad de aprendizaje de algunos organismos. Algunos reconocen color mejor que un patrón y otros patrones mejor que formas. Se discute que las diferencias en cuanto a esta habilidad radican y reflejan las diferentes presiones de selección impuestas sobre las diversas especies por sus necesidades ecológicas (Wackers y Lewis, 1999).

Señales visuales: producción de color y luz

Los insectos poseen una variedad excepcional de colores con patrones complejos y diversos. La coloración puede ser producida por diversas formas de estructuras superficiales y epidérmicas (colores estructurales) o por pigmentos en las capas externas del cuerpo que absorben, reflejan o dispersan selectivamente longitudes de onda específicas de luz o incluso por ambos. Los pigmentos son responsables de la mayoría de los colores naranja, rojo, amarillo y marrón-negro observados en los insectos, mientras que la mayoría de los colores azul o verde son el resultado de características nanoestructurales que reflejan estos colores (Schroeder et al., 2018).

El color es un atributo que depende de la iluminación, reflectancia espectral de un objeto y sus alrededores, así como los tipos de receptores espectrales y el procesamiento neural en el animal que percibe el color (R. F. Chapman, 1988).

Luz, por definición implica longitudes de onda dentro de la parte visible del espectro electromagnético. Para los seres humanos consiste en longitudes de onda que van desde aproximadamente 400 nm (violeta) a 725 nm (rojo). Muchos organismos, incluyendo los insectos, amplían esta gama en el ultravioleta cercano (300 - 100 nm). La luz "blanca" para un organismo particular se compone de todas las longitudes de onda visibles de ese organismo, por otra parte, la luz de color tiene un espectro incompleto en el que están representadas sólo algunas longitudes de onda.

La materia, para producir color, interactúa con la luz de dos maneras. Una manera es por absorción selectiva de longitudes de onda particulares por un producto químico o pigmento

(biocromo). Las longitudes de onda absorbidas (que son determinados por la estructura molecular del pigmento) se restan del espectro total, mientras que el resto se refleja o transmite para producir el color visible. La otra manera es por causa de la naturaleza física de las superficies (colores de interferencia), la cual pueda generar que solo ciertas longitudes de onda sean reflejadas, más específicamente debido a los esquemocromos, nanoestructuras que producen color al interactuar con la luz. Si todas las longitudes de onda son reflejadas igualmente, la superficie reflectante aparece "blanca", si todas son absorbidas la apariencia es "negra."

a. Color estructural

Los colores estructurales llaman la atención debido a su espectacular apariencia, caracterizada por tonos metálicos, brillos e iridiscencias. Se han encontrado en una numerosa cantidad de organismos como alas de mariposa (Kinoshita et al., 2002), élitros de coleópteros (Seago et al., 2009), plumas de aves (Zi et al., 2003), invertebrados marinos (Welch et al., 2005) y materia no viviente como ópalo (Parker et al., 2003). En el orden Hymenoptera, se han reportado pocos colores estructurales en la literatura. Sarrazin y colegas (2008) describieron un reflector de una capa en las alas de *Megascolia procer javanensis*, la abeja *Amegilla* sp. exhibe un cristal fotónico *2D* en sus escamas dorsales. Fung (2005) y Strohm y colegas (2009) reportaron la presencia de una estructura cuticular responsable de la coloración iridiscente de la avispa *"cuckoo" Hedychrum rutilant*. En general, varios esquemocromos o mecanismos de interferencia en la cutícula se han reportado como causantes del color estructural en diversos insectos (Cuadro 1).

Cuadro 1. Lista de esquemocromos presentes en algunos insectos. (basado en Hsiung et al, 2014)

Tipos de nanoestructuras	**Principios ópticos**	**Color**
Árbol de navidad	Interferencia, difracción	Iridiscente
Multicapas	Interferencia	Iridiscente
Cristales fotónicos	Interferencia, dispersión	Iridiscente
Rejilla de difracción	Difracción, Interferencia	Iridiscente
Cristales fotónicos, "quasi" ordenados	Dispersión, Interferencia	No-Iridiscente
Estructura desordenada	Dispersión incoherente	Azul y blanco

b. Biocromos

Los naturalistas han estado siempre fascinados por las variaciones en la coloración de insectos, sin embargo, cerca del año 1900 la química de los pigmentos apenas comenzó a ser entendida. Los pigmentos naturales se producen en cantidades muy pequeñas, razón por la cual era difícil aislarlos previamente. Aunque el aislamiento de pigmentos había sido posible, las muestras puras no podían ser analizadas con precisión. Habría sido necesario un gran número de insectos para generar medidas precisas. El estudio de la química de los pigmentos (biocromos) aumentó significativamente a medida que se desarrolló la cromatografía. Los biocromos que han sido estudiados, se agrupan según su estructura química la cual es resultado de diversas rutas biosintéticas (Cuadro 4). Por ejemplo, los sintetizados por los insectos, que incluyen antraquinonas, afinas, pterinas, tetrapirroles, omocromos, melaninas y papiliocromos, y los secuestrados de sus plantas hospedadoras, los carotenoides antioxidantes y flavonoides solubles en agua. También se pueden clasificar en los que se producen por precursores lineales, por ejemplo, antraquinonas, afinas y tetrapirroles, y los derivados de precursores cíclicos tales como pterinas, omocromos, melaninas y antocianinas (Shamim et al, 2014). En el orden Hymenoptera, se han reportado principalmente derivados de las pterinas y purinas (ácido úrico) (Ikan y Ishay, 1967, Plotkin et al, 2009a) (Cuadro 2).

Cuadro 2. Algunas fuentes del color (biocromos) en diversos insectos. (basado en R. F. Chapman, 1988).

Negro	Afinas, melanina
Rojo	Omocromos, pterinas, carotenoides, antraquinonas, porfirinas
Café	Omocromos, pterinas y melanina
Anaranjado	Pterinas
Amarillo	Carotenoides, flavonoides, pterinas
Verde	Colores de interferencia, insectoverdin
Azul	Colores de interferencia + omocromos, carotenoides
Blanco	Acido úrico, pterinas (dispersión), flavonoides

Significancia de la coloración

La pigmentación de insectos es un rasgo muy variable, con espectaculares diferencias entre especies, entre las poblaciones de una misma especie, y entre individuos dentro de una población. Los pigmentos varían en las diversas etapas de la vida de un individuo y en las partes del cuerpo durante las etapas de vida (Thery y Gomez 2010). Asimismo, las investigaciones generalmente intentan identificar funciones clave únicas de apariencias externas, pero los patrones de color individuales pueden experimentar múltiples, a menudo opuestas, presiones de selección, además de diversos efectos claves que facilitan el mantenimiento de diferentes niveles de variación dentro y entre especies conspicuas (Stevens y Ruxton 2012) (Cuadro 3)

La diversidad que enmarca el término pigmentación se refiere a los colores que se utilizan, así como la forma en que estos colores se organizan en patrones. En la literatura científica, la pigmentación se asocia con pleiotropía, el comportamiento y la inmunidad (Wittkopp y Beldade, 2009). Asimismo, algunos pigmentos de insectos se consideran productos finales de procesos metabólicos y pudieron haber evolucionado originalmente como formas de almacenamiento de excreciones. Las pterinas, por ejemplo, son derivados de purinas como el ácido úrico. Por otra parte, el almacenamiento no es la única, ni la función primaria, de muchos pigmentos en la mayoría de los insectos. Algunos insectos emplean pigmentos derivados de su dieta en sus patrones de color, pero (tal vez) usan más comúnmente pigmentos que sintetizan "*de novo*" (Shamim et al, 2014).

En la mayoría de los casos, los colores de los insectos presentes hoy en día en la naturaleza son de importancia ecológica, relacionados con funciones biológicas importantes como la termorregulación (Stuart-Fox et al., 2017, Chapman, 1988), los caracteres sexuales secundarios (García et al.,2016) y la evitación de depredadores a través del camuflaje (cripsis y mascarada) (Skelhorn y Rowe, 2016), advertencia (coloración aposemática) (Stevens y Ruxton, 2012) o mimetismo (Mallet y Joron, 1999).

Mimetismo

Los depredadores aprenden a evitar los insectos desagradables con colores distintivos. El mimetismo toma dos formas denominadas Mülleriano y Batesiano. Las especies que exhiben mimetismo Mülleriano son desagradables para el depredador (Wallace, 1877). Aquí la ventaja para los

insectos es que la depredación de cualquier especie que se asemeja al patrón se reduce. Por ejemplo, muchas especies sociales de Vespidae tienen el mismo patrón negro y amarillo de base; si el depredador aprende a evitar dicha especie es probable que evite otras con un patrón similar (Marchini et al, 2016). En el mimetismo Batesiano sólo uno de un par de especies es desagradable. Se les llama "modelo" y "imitador". En este caso, las ganancias apuntan hacia las especies palatables que aprovechan su parecido con las especies de mal gusto (Ruxton et al, 2004).

Aposematismo

La coloración notable es a menudo, pero no siempre, indicativo de aposematismo, por el cual los depredadores aprenden a asociar un patrón de color particular con un químico nocivo (defensas), aunque este proceso de aprendizaje es más complejo que simplemente desarrollar una aversión a ciertos tipos de presas (Skelhorn *et al.*, 2016). El aposematismo se refiere a los organismos que utilizan coloraciones brillantes o conspicuos para advertir de sus defensas química a los depredadores (Sherratt y Speed, 2004). La selección por depredadores favorece estas señales de advertencia notables porque los patrones grandes y brillantes son más fáciles de aprender y recordar por los depredadores (Lindstrom *et al.,* 2008).

Sin embargo, la mayoría de investigación sobre aposematismo utiliza depredadores vertebrados, especialmente aves (Ruxton 2004). Esto ignora la gran influencia de los depredadores artrópodos en las comunidades, especialmente hacia pequeñas presas de insectos (Lang et al 1999). Independientemente de si los depredadores artrópodos son capaces de superar las defensas o aprender a evitar presas tóxicas, los depredadores de artrópodos dan forma a la evolución de los patrones de coloración aposemática (Skelhorn, 2016).

En el cuadro 4 se muestra un resumen que responde a la interrogante ¿qué hace una señal de advertencia efectiva?

Cuadro 3. Resumen de factor/efectos claves que facilitan el mantenimiento de diferentes niveles de variación dentro y entre especies aposemáticas (basado en Briolat et al., 2019)

Densidad y agregación	La densidad de especies aposemáticas puede alterar paisajes selectivos, particularmente la influencia de la selección

	dependiente de la frecuencia impuesta por los depredadores. (Briolat et al., 2019).
Señalamiento intraespecífico	Los colores de advertencia también pueden servir como señales sociales, por ejemplo, de calidad o estatus social. (Briolat et al., 2019).
Enfermedad y carga parasitaria	Puede revelar el efecto de una infección en la condición individual, participar en la estimulación de la melanización por infección o compensaciones entre el uso de melanina para la pigmentación o la resistencia a la infección, ser una respuesta de rasgos correlacionadas como la inmunocompetencia o el riesgo de parasitismo. Asimismo, extinciones locales impulsadas por patógenos, o cuellos de botella repetidos, pueden interrumpir la selección de purificación y mantienen la variación de color. (Briolat et al., 2019).
Variación dentro de depredadores	Diferencias a gran escala en fisiología (diferencias en capacidades sensoriales, tolerancia a toxinas y cognición) y comportamiento entre especies y poblaciones de depredadores. Diferencias en la experiencia de los depredadores entre especies, poblaciones y temporalmente dentro de las poblaciones. Diferencias a pequeña escala en fisiología y comportamiento entre individuos, vinculadas a la motivación o la experiencia individual. (Briolat et al., 2019).
Daño por UV	El mayor riesgo de UV favorece los componentes melánicos de las señales de advertencia, mientras que la depredación selecciona los morfos melánicos. (Briolat et al., 2019).

Desecación	El aumento del riesgo de desecación favorece los componentes melánicos de las señales de advertencia, mientras que la depredación selecciona los morfos melánicos. (Briolat et al., 2019).
Disponibilidad de recursos	La disponibilidad de recursos influye en la inversión en la coloración de advertencia, a menudo a través del efecto en la señalización de la honestidad. (Briolat et al., 2019).

Cuadro 4. Resumen de factores que determinan la efectividad de una señal de advertencia.

Conspicuidad	La conspicuidad se ha considerado durante mucho tiempo un aspecto clave del aposematismo, y puede ser una función del contraste interno de las marcas dentro de la coloración del cuerpo y/o contraste de la coloración del animal con el fondo.	Gamberale-Stille, 2001 y Prudic et al, 2007
Patrón	Se ha realizado relativamente poco trabajo para dilucidar si algunos tipos de patrones son mejores que otros para mejorar señales de advertencia. Muchas presas con colores de advertencia tienen marcas que comprenden elementos de patrón repetidos los cuales pueden aumentar la redundancia/maximizar la señal, pero al mismo tiempo mejorar la probabilidad de ser detectado por el receptor. Componentes de patrones simples (como manchas) pueden facilitar la detección y también acelerar la evitación si en el aprendizaje son más fáciles de memorizar.	Aronsson y Gamberale-Stille, 2008, Valkonen et al, 2011, Wuster. et al., 2004 y Kauppinen y Mappes, 2003.

Distinción	Como se discutió anteriormente, alta conspicuidad en presas aposemáticas puede conferir ventajas en términos de explotación de los sistemas sensoriales y cognitivos de los depredadores. Sin embargo, hay una explicación alternativa o adicional: los organismos indefensos son generalmente discretos y, por lo tanto, si un organismo defendido adopta una apariencia que mejora su distinción a los depredadores con respecto a especies indefensas, es probable que la apariencia sea conspicua.	Sherratt y Beatty, 2003 y Merilaita y Ruxton, 2007.

¿Por qué usualmente los colores de advertencia son naranja, amarillo y negro?

a) Proporcionan un alto contraste con el fondo (por ejemplo, rojo / amarillo contra el follaje verde), lo que promueve la detección. (Stevens y Ruxton, 2012)

b) Son resistentes a las sombras (que son ricas en UV azul) y a los cambios de iluminación. Por lo tanto, proporcionan una señal confiable bajo hábitats variados y condiciones de luz (Stevens y Ruxton, 2012).

c) Amarillo / rojo y negro tiene un alto contraste cromático y de luminancia (Stevens y Ruxton, 2012).

d) Estos colores pueden permitir camuflaje-distancia dependiente. Si el amarillo / rojo y el negro se mezclan con un color del fondo (como por ejemplo follaje) esto puede generar que a una determinada distancia, cuando la visión de los depredadores ya no es suficiente, los mismos, no logren discriminar los componentes de marcado individuales (Stevens y Ruxton, 2012).

Algunos estudios de caso:

a. Un caso interesante en aposematismo son algunos patrones con anaranjado. Este color es, un profundo indicador aposemático para aves agresoras, pero también conserva un alto grado de utilidad en términos de confundir la visión de algunos artrópodos como los mántidos (Prete, 1999). Hay que señalar que la mayoría de insectos y arañas carecen de un receptor dedicado al "rojo" (Thery y Gómez, 2010). Esto no indica que los objetos de color rojo y naranja son invisibles para los artrópodos que carecen de un receptor "rojo", pero estos van a aparecer monocromáticamente "verdes", y potencialmente serán difíciles de distinguir cromáticamente de un fondo verde (Chittka y Waser, 1997).

b. Aunque el rojo y naranja se utilizan comúnmente en las señales aposemáticas (Steven y Ruxton, 2012), estos colores es probable que no tengan el mismo valor en la creación de visibilidad para los depredadores de artrópodos. Los artrópodos son capaces de percibir diferencias a lo largo de longitudes de onda vía información acromática o luminancia (Reisenman y Giurfa, 2008). Algunos taxones depredadores como las mantis son monocromáticas y se basan en el contraste de luminancia para la detección visual (Prete, 1999a).

c. Es importante resaltar que a pesar de que las especies que exhiben rasgos conspicuos (colores brillantes o contrastantes) y químicos tóxicos son llamadas aposemáticas, muchas veces esta etiqueta se aplica sin la conducción de ensayos de comportamiento. Estudios previos han demostrado que para que los colores de advertencia sean efectivos, deben promover tanto el aprendizaje de evitación inicial como el de aversión en los depredadores y existe evidencia de que ciertos tipos de color y señales conspicuas son más efectivos para lograr dicha tarea (Talianchich *et al.*, 2003). Los colores más efectivos, producen señales relativamente estables durante todo el día y bajo diferentes condiciones climáticas, lo cual refuerza que las señales aposemáticas han evolucionado bajo selección para ser más efectivas al ser más conspicuas y confiables para el sistema visual de sus posibles depredadores (Arenas, 2015). Por tanto, es muy importante explorar las posibles funciones de la coloración conspicua evaluando diversos depredadores, ya que la eficacia de las defensas químicas y la señal que un organismo despliegue es posible impacte diferente según cada grupo específico de depredador (MacIver y Lattin, 1990).

Modelos de estudio

Cuadro 5. Generalidades de los modelos de estudio.

Scelionidae	Salticidae
En comparación con los lepidópteros y los coleópteros, existen relativamente pocos estudios de patrones de color conspicuos (potencialmente aposemáticos) en himenópteros, y los estudios que existen están restringidos principalmente al patrón amarillo y negro en Vespidae (Vidal-Cordero et al. 2012, Marchini et al. 2016). Sin embargo, durante mucho tiempo se ha reconocido que muchos himenópteros más pequeños, especialmente los sceliónidos, muestran un patrón de color recurrente de cabeza negra, mesosoma naranja y metasoma negro (BOB). Lubomir Masner (1988) fue probablemente el primero en enfatizar la presencia generalizada de este patrón de color en himenópteros, más aún en sceliónidos (refiriéndose a él como negro, rojo, negro). Este patrón BOB parece ocurrir en el 90% de las especies actualmente conocidas del género *Chromoteleia*, 70% de *Acanthoscelio* y *Triteleia*, 50% de *Baryconus*, 40% de *Pseudoheptascelio*, 30% de *Opisthacantha*, *Scelio* y *Sceliomorpha*, y 15% de *Macroteleia* (Valerio *et al.* 2013). También se ha documentado en algunas especies de *Leptoteleia*, *Oethecoctonus* y *Probaryconus* (Masner y Hanson, 2006). Si bien el patrón de color BOB es más conocido en los sceliónidos, también se presenta en muchas otras familias de himenópteros e incluso en especies de otros órdenes (estafilínidos, algunas moscas bibiónidas y estratiomídicas).	La subfamilia Salticidae Lyssomaninae se compone de solo dos géneros, *Lyssomanes* Hentz, 1845 y *Chinoscopus* Simon, 1900, ambos restringidos al Nuevo Mundo (Maddison 2015). Casi cien especies de Lyssomaninae se reconocen actualmente, la mayoría de los cuales se encuentran en los bosques tropicales de la región Amazónica (Logunov 2014; Prószyński 2016; Rubio et al.2017). La familia en general presenta una impresionante agudeza espacial y visual (o resolución espacial), lo cual se refiere a un ángulo visual del ojo, que se define como la mínima separación requerida antes de que los objetos en una escena sean vistos como separados y generalmente es expresado en grados, radianes o minutos de arco. El espacio más alto la resolución encontrada en cualquier saltícido es de 0.04 grados para *Portia fimbriata* (especie atípica) y especies más típicas (*Phidippus johnsoni*) que tiene ojos que apoyan ángulos visuales de alrededor de 0.13 grados (Blest, McIntyre y Carter, 1988, Williams y McIntyre, 1980).

Ilustración fotográfica 3. Fotografía realizada por R. Mora. Lyssomanes, modelo de estudio. Técnica macrofotografía.

Justificación

La mayoría de estudios recientes en coloración y patrones de color han flanqueado la arista biológica de la coloración relacionada con la percepción y comunicación presa/depredador (Eisner et al., 1967, Williams, 2007, Song y Wenzel, 2008, Doucel y Meadows, 2009, Welch et al., 2007 y Seago et al., 2009), así como el análisis objetivo y cuantitativo (que se aleja de mediciones subjetivas desde nuestro espacio visual) de la variabiliadad espectral de la señal emitida. Por tanto, es evidente un comportamiento bimodal de intereses en esta área de investigación que difícilmente intersecta: literatura entomológica sin contenido óptico o literatura física sin un contexto biológico. En el presente trabajo, se propone dilucidar mecanismos causantes de la coloración (BOB, negro-naranja-negro/ black-orange-black) en el orden Hymenoptera y consecuentemente su importancia funcional, sintetizando perspectivas entomológicas y ópticas.

El patrón de color BOB presenta una coloración conspicua con un patrón de tonos muy similar a patrones aposemáticos en otros grupos de seres vivos. Sin embargo, el grado en que cualquier patrón aposemático mejora la condición física es un producto de muchas presiones selectivas diferentes, que van desde interacciones depredador-presa y condiciones ambientales (Lindstrom et al, 2001). Investigaciones anteriores han demostrado que no todas las especies que exhiben coloración aposemática usan esto para defenderse contra los depredadores (Talianchich et al. 2003). Otros usos comunes incluyen exhibiciones sexuales (Metz y Weatherhead 1993), comunicación intraespecífica no sexual (Papaj y Newsom 2005), mimetismo (Brodie y Howard 1973), coloración disruptiva (Stevens et al. 2006). Por lo tanto, es importante explorar las posibles funciones de coloración conspicua en cada organismo de interés, en este caso avispas de la familia Scelionidae, y corroborar efectivamente cuál es su función.

Para evaluar la función del patrón de interés, se realizarán ensayos con un depredador. Se estudiará una posible eficacia aposemática del color usando poblaciones silvestres y criadas en cautiverio del depredador. Esto debido a que el uso de poblaciones silvestres del depredador versus depredadores ingenuos o criados en cautiverio nos permite eliminar la influencia de la generalización, en donde la experiencia previa con presas desagradables visibles resulta en evitación no aprendida por similitud (Hotova Svadova et al. 2013). En condiciones controladas, podemos identificar si cualquier efecto aposemático se debe a la cautela innata, sesgos no aprendidos o evitación aprendida. Asimismo, estos ensayos se realizarán en el campo y emularán las condiciones de luminosidad y entorno con el fin de reproducir las condiciones naturales en que esta señal es percibida. ¿Por qué es esto importante? Quizás de mayor importancia para la señalización aposemática, es tener presente que los artrópodos difieren de los seres humanos en sus capacidades visuales y visión del color. Por lo tanto, es importante considerar el espacio visual del depredador. Por ejemplo, la mayoría de los grupos de insectos y arañas carecen de un receptor "rojo" (Briscoe y Chittka 2001; Thery y Gómez 2010). Asimismo, los artrópodos aún pueden ser capaces de percibir diferencias de longitud de onda larga a través de información acromático o información de luminancia (Chittka y Waser 1997; Reisenman y Giurfa 2008). Las técnicas físicas propuestas nos ayudarán a dilucidar cómo el depredador seleccionado interpreta la variabilidad de la señal y al mismo tiempo el color se estará reportando de una manera cuantitativa y no subjetiva en donde la variabilidad espectral como una medida funcional realmente nos puede dar información objetiva de lo que perciben los depredadores potenciales en el entorno natural.

Objetivos

Objetivo 1. Describir la diversidad y distribución geográfica del patrón de coloración BOB en diversas familias del orden Hymenoptera.

Objetivo 2. Especificar la base física que determina el patrón de coloración BOB en especímenes seleccionados de la familia Scelionidae del orden Hymenoptera.

Objetivo 3. Estudiar cuáles son las posibles funciones del patrón de coloración BOB en especímenes seleccionados de la familia Scelionidae del orden Hymenoptera.

CAPÍTULO 1

Distribución del patrón de color negro-naranja-negro en himenópteros

Mora, R. & Hanson, P. E. (2019). Widespread occurrence of black-orange-black color pattern in Hymenoptera. ***Journal of Insect Science***, 19(2), 13. DOI: 10.1093/jisesa/iez021

RESUMEN

Ciertos patrones de color en los insectos muestran una evolución convergente que refleja funciones biológicas potencialmente importantes, por ejemplo, el aposematismo y la mímica. Estos fenómenos han sido documentados con mayor frecuencia en lepidópteros y coleópteros, siendo poco estudiados en himenópteros. Desde hace tiempo, se reconoce que muchos himenópteros, especialmente los sceliónidos (Platygastridae), muestran un patrón recurrente de cabeza negra, mesosoma naranja / rojo y metasoma negro (coloración BOB, black-orange-black). Sin embargo, la distribución taxonómica de este llamativo patrón de color nunca se ha documentado. El objetivo principal de esta investigación fue proporcionar una tabulación preliminar de este patrón de color en himenópteros, a través del análisis de muestras de museos y literatura relevante. Se incluye 11 variaciones del patrón de color BOB típico, pero no se incluyen todas las variaciones observadas. Estos patrones de color se encontraron en especies pertenecientes a 23 familias de himenópteros, y se observaron con mayor frecuencia en sceliónidos, evánidos y mutílidos, pero fueron relativamente poco frecuentes en cinipoides, diaprioides, chalcidoides y apoides. La ocurrencia generalizada de este patrón de color en himenópteros podría sugerir evolución convergente y una función potencialmente importante. El patrón de color BOB se encontró en especies de todas las regiones biogeográficas y dentro de una especie, generalmente estaba presente en ambos sexos (con algunas excepciones). En regiones tropicales bastante estudiadas, como Costa Rica, este patrón de color fue más común en especies que se encuentran en elevaciones más bajas (por debajo de 2,000 msnm). La biología de los taxones tabulados, y la presencia de BOB, abarca tanto ecto- como endoparasitoides, idiobiontes y koinobiontes, con una diversidad de hospederos, así como sínfitos fitófagos.

CAPÍTULO 2

Fenología de una población urbana costarricense de *Lyssomanes jemineus* Peckham & Wheeler (Araneae, Salticidae) y listado de otras arañas brincadoras del mismo sitio.

Mora-Castro, R., G. B. Edwards & P. Hanson-Snortum. 2019. Phenology of an urban population of *Lyssomanes jemineus* Peckham & Wheeler (Araneae: Salticidae) with a list of other jumping spiders from the same Costa Rican site. ***Peckhamia*** 194.1: 1-6.

RESUMEN

En este capítulo, se tomaron muestras de arañas saltarinas durante doce meses en un entorno urbano costarricense y se observó en detalle una de las especies recolectadas, *Lyssomanes jemineus*, para documentar su fenología en este hábitat perturbado. Otros nueve géneros y especies también fueron identificados en el mismo sitio.

CAPÍTULO 3

Medida espectral de la variación de color del patrón negro - naranja - negro (BOB) en avispas parasitoides pequeñas (Hymenoptera: Scelionidae), un enfoque estadístico.

Mora-Castro, R., Hernández-Jiménez, M., Alfaro-Córdoba, M., Avendaño, E., & Hanson-Snortum, P. (2019). Spectral measure of color variation of black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Scelionidae), a statistical approach. ***PloS one***, 14(10). doi:10.1371/journal.pone.0218061

RESUMEN

Las avispas parasitoides pequeñas son abundantes y extremadamente diversas, sin embargo, sus colores han sido poco estudiados. Uno de los patrones de color más comúnmente observado en estas avispas es un patrón negro-naranja-negro, que es especialmente común entre las especies neotropicales de Scelionidae que varían en tamaño de 2 a 10 mm. Debido a los desafíos metodológicos involucrados en la extracción y análisis de pigmentos de insectos de pequeño tamaño, se deben explorar otros métodos para examinar los colores. En este capítulo, se propone el uso de la microspectrofotometría en combinación con métodos de análisis estadísticos para estudiar las propiedades espectrales en tales casos. Se examinaron 8 géneros sceliónidos y 1 género de una familia lejanamente relacionada (Evaniidae), todos mostrando el patrón negro-naranja-negro. Se propone un análisis funcional de datos y un análisis estadístico de distancias euclidianas para los componentes de color para estudiar las diferencias de color entre y dentro de los géneros. El análisis de datos funcionales demostró ser un mejor método para tratar los datos de reflectancia porque brinda una mejor representación de la información física. Además, los espectros de reflectancia se separaron en contribuciones de componentes de color espectral y cada componente se etiquetó de acuerdo con su propia longitud de onda dominante al máximo del espectro: rojo, verde y azul. Cuando se compararon las curvas de componentes espectrales, los componentes espectrales azules de los colores naranja y negro, independientemente de los géneros que se comparan, resultan casi idénticos, lo cual sugiere que existe un compuesto común para estos pigmentos. Los resultados también sugieren que la cutícula de diferentes géneros, pero con el mismo color, podría tener una composición química similar. Esta es la primera vez que se analizan los colores negro y naranja en avispas parasitoides pequeñas y estos resultados proporcionan una base para futuras investigaciones sobre los patrones de color de un grupo de insectos abundante, pero bastante olvidado.

CAPÍTULO 4

Enlazando la biología, óptica y propiedades nano mecánicas en micro avispas

Mora-Castro, R., Hernández-Jiménez, M., Sáenz-Arce, G., Porras-Peñaranda, J., Hanson-Snortum, P. & Avendaño, E. (2019). Connecting biology, optics and nanomechanical properties in micro-wasps. **Scientific Reports**, 10, 1418 (2020). https://doi.org/10.1038/s41598-020-58301-2

RESUMEN

La coloración en insectos brinda una oportunidad fructífera para la investigación interdisciplinaria que involucra tanto la física como la biología, así como para comprender los principios de diseño de las estructuras biológicas. En esta investigación, se utilizan análisis nanométricos y micrométricos para investigar las propiedades morfológicas y mecánicas del patrón de color negro-naranja-negro (BOB) en sceliónidos, nunca antes estudiadas. El objetivo principal del presente capítulo fue explorar las diferencias estructurales y mecánicas en el mesoscoto de cuatro géneros: *Baryconus* con mesosoma naranja (es decir, patrón BOB) y todo negro y *Scelio* con mesosoma naranja (es decir, patrón BOB) y todo negro. Los hallazgos más destacados incluyen la ausencia de estructuras multicapa que generan color estructural, un pigmento concentrado en la superficie superior de la epicutícula y sorprendentes diferencias estructurales entre los cuatro géneros. Tres de los cuatro géneros mostraron una estructura similar a un acordeón en el surco (notaulus), mientras que el mesoscoto adyacente fue diferente en cada especie. Además, los espectros de componentes de color normalizados para los colores azul, verde y rojo del mesoscoto negro, de cada género, mostraron la misma dependencia espectral, mientras que el color naranja manifestó pequeños cambios en la longitud de onda dominante, lo que resultó en tonos anaranjados ligeramente diferentes.

CAPÍTULO 5

Desentrañando la función del patrón negro-naranja-negro (BOB) en avispas parasitoides pequeñas (Hymenoptera: Platygastridae) a través de los ojos de las arañas brincadoras (Aranae: Salticidae).

**Este artículo será sometido en el mes de abril del año 2020.*

Unraveling the function of the black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Platygastridae) through the eyes of jumping spiders (Aranae: Salticidae).

Investigadores participantes en la investigación: Rebeca Mora-Castro, Marcela Alfaro-Córdoba, Marcela Hernández-Jiménez, Mauricio Fernández-Otárola, Michael Méndez; Didier Ramírez-Morales; Carlos E. Rodríguez-Rodríguez, Andrés Durán-Rodríguez y Paul Hanson-Snortum.

RESUMEN

Muchas avispas parasitoides pequeñas tienen un patrón de color negro-naranja-negro (BOB), que generalmente está presente en ambos sexos. Una función probable de este patrón generalizado es la coloración aposemática (advertencia), pero esto nunca se ha investigado. Para probar esta hipótesis, presentamos a los depredadores araña (*Lyssomanes jemineus*), tanto individuos capturados en el campo como criados en laboratorio, a una especie de avispa BOB y una especie negra de cuatro géneros (*Baryconus, Chromoteleia, Macroteleia y Scelio*). Cada ensayo de araña / avispa se filmó durante 40 minutos en condiciones controladas y las respuestas de comportamiento (detectar, atacar, evitar) se registraron en cada uno de los 136 ensayos, nunca utilizando el mismo depredador y presa más de una vez. En cinco ocasiones, las arañas capturadas en el campo consumieron una avispa negra, pero ninguna araña consumió una avispa con coloración BOB. El componente verde de los espectros de reflexión de los colores negro y naranja se midió en las avispas. Entre las avispas BOB, la mayoría de los eventos de "ataque" ocurrieron con *Baryconus*, que también tuvo el contraste de absorción más bajo, mientras que el mayor número de eventos de "detección" ocurrió con *Macroteleia*, que tuvo el mayor contraste de absorción y la contribución más alta de naranja. A las arañas también se les presentaron presas falsas (granos de arroz pintados con colores que coincidían espectrofotométricamente con el color de las avispas) en una arena especialmente diseñada con un mecanismo automatizado para mover la presa; sin embargo, los resultados no fueron concluyentes. Los ensayos de toxicidad aguda con la pulga de agua, *Daphnia magna*, dieron como resultado una mayor mortalidad cuando se expusieron a extractos de avispas BOB que de avispas negras, lo que sugiere que los primeros contienen compuestos defensivos.

Ilustración fotografica 4. Fotografía realizada por S. Pessoa. Título: Trabajo de campo en el Rodeo. Natalia; Paulina y Geovanna (asistentes y estudiantes del proyecto), tesiaria Rebeca (derecha)

CONSIDERACIONES FINALES

Los estudios han usado el color para entender procesos evolutivos desde la década de 1860 con Bates y posteriormente con Wallace (Wallace, 1877 y Bates, 1862), pero solo recientemente fisiólogos visuales, ecologistas sensoriales, ecologistas conductuales, físicos y biólogos con intereses compartidos en coloración se están uniendo para estudiar los mecanismos de producción y percepción, las complejidades de la función y los patrones de evolución del color (Endler y J. Mappes, 2017). Estamos en el umbral de una nueva era del color, y la naturaleza interdisciplinaria y la ciencia colaborativa como resultado de este tema resulta muy prometedora.

La distribución del patrón de coloración BOB en diversas familias del orden Hymenoptera.

¿Es el patrón de coloración BOB un rasgo que está ampliamente presente y distribuido en las diversas familias del orden Hymenoptera en todas las regiones biogeográficas?

Ilustración 5. Arte por R. Mora. Título: patrón BOB

Como dijo Syr Charles Lyell en una carta a Sir John Herschell "probablemente no haya una pizca de color en un cuerpo o ala de insecto cuya elección sea bastante arbitraria, o que no afecte su duración durante miles de años". Lubo Masner fue una de las primeras personas en llamar la atención

sobre el conspicuo y hermoso patrón de color negro-naranja y negro (denominado BOB en este proyecto), dicho patrón de coloración sirvió de inspiración para esta investigación.

El patrón de coloración BOB fue encontrado en 23 familias de himenópteros, muchas pertenecientes a las subfamilias de Ichneumonidae y Braconidae. Por otra parte, la compilación preliminar realizada en esta investigación sugiere que la coloración BOB es muy poco frecuente en ciertos taxones (por ejemplo, Cynipoideos, Diaprioideos, Chalcidoideos y Apoideos) y bastante común en otros (Scelioninae, Evaniidae y Mutillidae). Con respecto a la proporción de especies que muestran el patrón BOB en sceliónidos, se reporta un 90% en *Chromoteleia* y un 15% en *Macroteleia* (Valerio et al.2013).

La naturaleza preliminar de nuestra descripción, así como la falta de filogenias para la mayoría de los taxones, impiden estimar el número de veces que ha evolucionado la coloración BOB. Sin embargo, la ocurrencia generalizada de este patrón de color podría sugerir que ha surgido en numerosas ocasiones, lo que a su vez podría estar relacionado con alguna función biológica. Parece poco probable que este color se use en la comunicación intersexual, ya que ambos sexos generalmente presentaron el mismo color, y la mayoría de las variaciones intraespecíficas que observamos incluían variaciones de color dentro del mismo sexo. Los pocos casos de variación de color intersexual se observaron en grupos donde las hembras son ápteras y los machos alados (por ejemplo, Mutillidae), en estos casos solo las hembras muestran coloración BOB.

La función más probable de la coloración BOB es el aposematismo (coloración de advertencia), ya que se sabe que los patrones de color naranja y negro contrastantes son aposemáticos en otros insectos, por ejemplo, los escarabajos mariquita (Coleoptera: Coccinellidae) (María Arenas et al.2015). También es posible que al menos algunos de los taxones que muestran la coloración BOB estén simulando hormigas, por ejemplo, la especie, *Pseudomyrmex gracilis*. Si bien esta especie está restringida al Nuevo Mundo, se podría argumentar que otras hormigas sirven como modelos en otras regiones, por ejemplo, especies de *Mymecia* en Australia y *Formica rufa* en la región Paleártica. Sin embargo, hay otros modelos posibles, como los especímenes de Mutillidae femeninos (ver el anillo de imitación de "Timulla de cabeza negra) (Wilson *et al*. 2015). Muchos mutílidos femeninos que muestran el patrón BOB también tienen manchas blancas laterales en el metasoma, un patrón que también ocurre en varios taxones que examinamos, por ejemplo, *Bephrata* e *Isosomodes* (Eurytomidae)

y *Cleptidea* (Chrysididae). En *Leptodrepana* (Braconidae) a menudo hay una mancha blanca central en el primer tergito. Si la coloración BOB en himenópteros no aculeados implica mimetismo, queda por ver qué proporción de estos son imitadores Batesianos versus imitadores Mullerianos.

Cuatro factores importantes que podrían relacionarse con la prevalencia de la coloración BOB son (Masner, 1988, Masner y Hanson, 2006): tamaño del insecto, hábitat, distribución altitudinal y distribución geográfica. Con respecto al tamaño, la coloración BOB parece ser especialmente común en himenópteros con una longitud corporal entre 3 y 10 mm, sin embargo, se requieren análisis cuantitativos para examinar esta pregunta con mayor detalle.

Masner (1988) sugirió que la coloración BOB es más frecuente entre las especies que habitan en vegetación baja, entre 1 y 2 m de altura. En esta investigación parece haber más especímenes de trampas Malaise y barrido de pantalla, y menos de trampas panorámicas y otras técnicas terrestres, pero esto requiere confirmación. La biología de los taxones que muestran la coloración BOB abarca tanto ecto- como endoparasitoides, idiobiontes y koinobiontes, de una diversidad de hospederos, así como fitófagos. Es interesante que los parasitoides de huevo (sceliónidos, evánidos) estén especialmente bien representados, pero se necesita más investigación para determinar si en realidad están proporcionalmente mejor representados que los parasitoides de larvas y pupas.

Con respecto a la distribución altitudinal de la coloración BOB, la gran mayoría de los especímenes que muestran este patrón de color se recolectaron por debajo de los 2.000 msnm (Masner y Hanson 2006). En algunos casos se pudo examinar especímenes recolectados en altitudes que van desde el nivel del mar hasta los 5,000 msnm. Por ejemplo, los especímenes de *Triteleia* con coloración BOB fueron comunes en las tierras bajas, sin embargo, se encontraron especímenes completamente negros en altitudes superiores como Cotopaxi en Ecuador (5,000 msnm), Sierra Nevada en España (3,200 msnm) y Chiapas en México (4,000 msnm) Por otro lado, dos especímenes (menos de 3 mm de longitud) de *Probaryconus* que muestran coloración BOB se recolectaron a altitudes superiores en Ecuador, uno de Napo a 3.000 m y el otro de Oyacachi a 3.190 msnm. En las especies que muestran una variación de color intraespecífica, a menudo hay una tendencia a que los especímenes de

elevaciones más altas sean más oscuros. Por ejemplo, en Costa Rica, una especie no identificada de *Lapitha* muestra una coloración BOB típica en las tierras bajas, pero a mayor altitud (más de aproximadamente 1.300 msnm) los especímenes se vuelven más oscuros, con un propodeo negro y un mesoscutum más oscuro. Aunque estas tendencias altitudinales merecen una mayor investigación con taxones adicionales, la escasez de coloración BOB en altitudes más altas parece ser un patrón real, pero se desconoce la razón. Sería de gran potencial estudiar más a fondo si esta coloración manifiesta alguna función (como termorregulación) dependiente a las temperaturas o a la radiación UV en las diferentes altitudes estudiadas.

Aunque previamente se sugirió que la coloración BOB ocurre principalmente en el Neotrópico (Masner 1988, Masner y Hanson 2006), nuestros resultados muestran que este patrón de color se encuentra en todas las regiones biogeográficas. Aunque es posible que este patrón de color sea más frecuente en el Neotrópico, al menos entre los sceliónidos, nuestros datos son insuficientes para corroborar esta posibilidad.

Ir al campo y conocer el potencial depredador

¿Cuáles especies de saltícidos hay cerca de la Universidad de Costa Rica, cómo se podrían criar bajo condiciones controladas y cuál es su fenología?

Ilustración 6. Macrofotografías M. Valverde. Título: Brincadora del campus UCR.

Personas de todo el mundo estudian las arañas saltarinas por sus simples ojos capaces de hacer "grandes" cosas. “Tigres de la hierba” o “tigres voladores”, así eran llamadas durante la Dinastía de Ming en China. Las arañas brincadoras (Salticidae) miran alrededor, entre hojas y tallos, rocas y grietas. Ven incluso más colores que nosotros los seres humanos, siendo sensibles a la luz ultravioleta.

Ha habido muy pocos estudios de arañas brincadoras en Costa Rica, por tanto, para alentar futuras investigaciones sobre este grupo, y dado que se propuso en esta investigación, este grupo como potencial depredador de las microavispas-sceliónidos, se realizaron observaciones en campo por un año en un parche de bosque urbano ubicado en el campus de la Universidad de Costa Rica. Este período fue crucial para observar con detalle el comportamiento de estos especímenes en Costa Rica y familiarizarnos con el modelo de estudio y posible depredador.

Se ha informado que varios factores afectan la abundancia estacional de arañas, como, la estructura del hábitat (Lubin, 1978; Nentwig, 1993), las lluvias (Wolda 1978), la estructura de las plantas (Raizer y Amaral, 2001; Uetz, 1977), el período reproductivo de cada especie (Sosa-Romero *et al.*, 2016), la humedad (Flórez, 1998), la sincronía de mortalidad de los adultos (Jackson, 1978), los sitios de refugio y la presencia de enemigos naturales (Uetz, 1977). En nuestro estudio, una de las especies más comunes fue *Lyssomanes jemineus*. Observamos que las hembras adultas estuvieron ausentes durante los meses con muy poca lluvia, así como los machos adultos, ambos, más comunes durante los meses lluviosos. Este patrón de machos y hembras que aparecen durante los meses lluviosos podría deberse al hecho de que la cobertura vegetal y las fuentes de agua aumentan, al igual que muchas presas potenciales, durante esos meses (Miyashita et al., 1998). Las plantas son importantes para las arañas por varias razones: como refugio contra la desecación (Riechert y Tracy, 1975), protección contra enemigos naturales (Gunnarsson 1996), oportunidades de forrajeo (Morse y Fritz 1982) y sitios para oviposición y reproducción (Smith 2000). El cortejo de *Lyssomanes jemineus* se observó principalmente durante los meses de abril y mayo. Se encontraron juveniles durante todo el año, pero en mayor número desde julio hasta marzo.

Como se ha informado anteriormente para *Lyssomanes viridis* (Walckenaer), las hembras permanecieron muy cerca de su descendencia durante los primeros dos estadios, pero en el tercer o cuarto instar se observaron más distantes o ausentes (Richman y Whitcomb, 1981). Además, durante el desarrollo de sus masas de huevos, generalmente se encontraba una hembra adulta que los vigilaba

en la parte superior del refugio o cerca, y si la molestaban, la hembra permanecía vigilante y, en algunos casos, reacia a moverse, lo que respalda la opinión de que el cuidado materno es importante (Hallas y Jackson, 1996). Con respecto a los juveniles, las observaciones de este estudio respaldan las de otros autores que informan que los *Lyssomanes* juveniles requieren un año completo para madurar (Richman y Whitcomb, 1981).

Principios de diseño de las estructuras biológicas relacionadas con el patrón BOB.

¿Cuál es la naturaleza física de BOB?

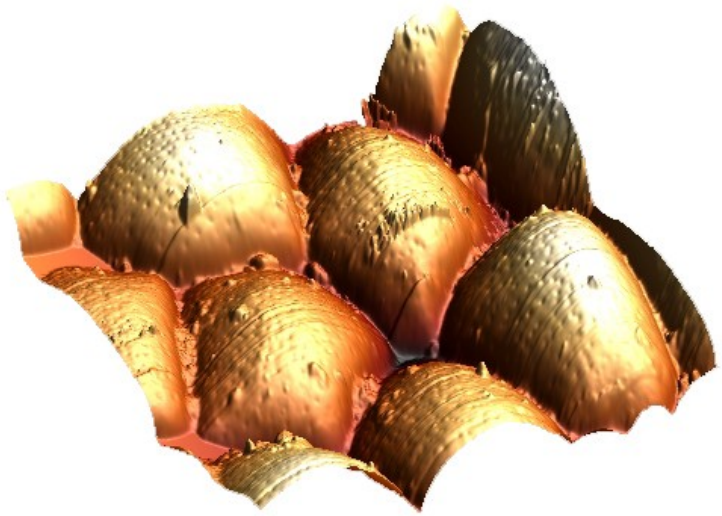

Ilustración 7. Imagen 3D realizada por E. Avendaño, como resultado de la técnica de Microscopía de Fuerza Atómica. Título: Cutícula Baryconus.

La coloración en insectos brinda una oportunidad fructífera para la investigación interdisciplinaria que involucra tanto la física como la biología, y para una mejor comprensión de los principios de diseño de las estructuras biológicas. En esta investigación, se utilizaron análisis nanométricos y micrométricos para investigar las propiedades morfológicas y mecánicas del patrón BOB en micro avispas, nunca antes estudiado.

La estructura cuticular de los insectos muy pequeños, como las micro avispas examinadas aquí, generalmente ha sido un tema poco estudiado. Comparando nuestros resultados con los de otras

investigaciones en el orden Hymenoptera (Pertsis, 2005 y Ishay *et al*, 2002), algunas similitudes son evidentes. Por ejemplo, la cutícula de los especímenes estudiados aquí exhibe un material compuesto en el que se observaron matrices, laminillas y fibras similares a proteínas. Otras características también muestran ciertas similitudes, por ejemplo, el grosor gradualmente decreciente de las láminas, rangos similares de grosor de las capas cuticulares y presencia de poros. Algunas de las características de la superficie están muy extendidas en los insectos. Por ejemplo, se han reportado poros en varias familias de escarabajos (Coccinellidae, Dystiscidae, Lucanidae, Scarabaeidae), pero la microestructura y la densidad de estos poros pueden variar (Dai y Yang, 2010 y Sun *et al.*, 2015). Los colores naranja y negro generalmente se deben a los pigmentos en oposición y no a estructuras de interferencia en la cutícula y los resultados de la presente investigación sugieren claramente que estos colores en los sceliónidos no son de naturaleza estructural. Hasta donde se ha reportado, Mutillidae es el único grupo de himenópteros en el que se han examinado las bases químicas de la coloración negra y naranja, reportándose feomelaninas anaranjadas y eumelaninas negras (Hines, 2017). El hecho de que todos los componentes azules del análisis espectral coincidan, mientras que los componentes verde y rojo difieren entre negro y naranja, sugiere que existe una composición química compartida. Por lo tanto, la información obtenida de los espectros de reflexión sugiere que la coloración se debe a pigmentos que están estrechamente relacionados en términos de composición química, como se discutió en un capítulo anterior, y que dichos pigmentos están encapsulados dentro de la epicutícula.

Sin embargo, las micrografías ópticas de la superficie cuticular del mesoscoto correspondiente muestran que la coloración no es necesariamente homogénea. Esto es especialmente evidente para el mesoscoto naranja de *Scelio* y *Baryconus*, y este efecto también podría explicar las similitudes encontradas entre los espectros de reflexión naranja y negro. Uno de los objetivos de este estudio fue determinar cómo las propiedades de la cutícula negra difieren de las de la cutícula naranja. Al comparar las cuatro combinaciones posibles (naranja vs negro y *Baryconus* vs *Scelio*), el resultado, sorpresivamente, fue que cada combinación es bastante diferente. El mesoscoto naranja en *Baryconus* está formado por estructuras granulares, cada estructura redondeada consiste en capas flexibles y rígidas alternas, mientras que el surco (notualus) parece ser una estructura de conexión más suave. El mesoscoto negro en *Baryconus* tiene una estructura similar a un acordeón tanto en el surco como en el mesoscoto adyacente, siendo este último más rígido. El surco tiene capas intercaladas de material rígido y blando, más o menos paralelas entre sí, y casi el doble de grosor con respecto al mesoscoto adyacente. El mesoscoto naranja de *Scelio*, tiene una estructura similar a una esponja, con un compuesto de

materiales blandos y duros, el surco tiene una estructura similar a un acordeón. Finalmente, el mesoscoto negro de *Scelio* consiste en capas horizontales de láminas, con capas intercaladas de material rígido y blando, el surco tiene una estructura similar a un acordeón similar a las dos combinaciones anteriores. Presumiblemente, las cuatro combinaciones discutidas anteriormente permiten la flexibilidad requerida a medida que el mesotórax se deforma durante el vuelo (debido a la acción de los músculos de vuelo indirectos), pero se requiere más investigación para mejorar el entendimiento de las notables diferencias observadas. Dentro del surco mesoscotal de la estructura tipo fibra de *Baryconus* se encontró una dirección preferencial en el orden de las fibras que muestran un ángulo de 47°. El valor del ángulo entre fibras se ha informado previamente para indicar una mayor ductilidad del material (Wang et al., 2014 y Mortazavian.y Fatemi, 2014), lo que significa que esta característica permite una mayor deformación con respecto a otras estructuras.

Funciones del patrón de coloración BOB en especímenes seleccionados de la familia Scelionidae del orden Hymenoptera.

¿Es el patrón de coloración BOB aposemático?

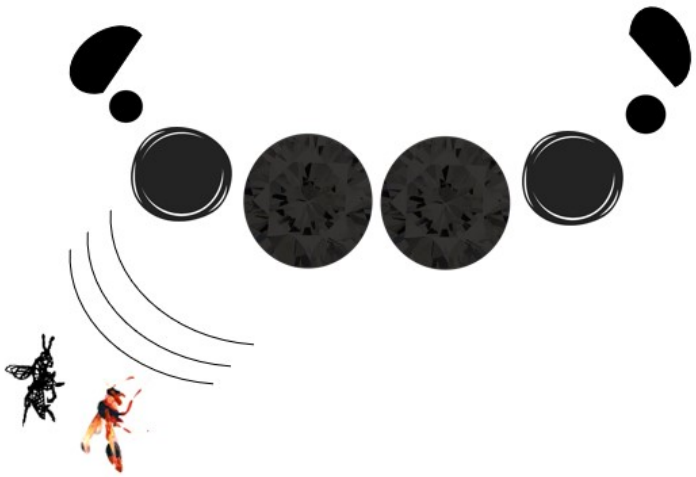

Ilustración 8. R. Mora. Título: Agudeza visual y presa.

El objetivo específico de esta publicación, capítulo 5 (en proceso de aceptación) fue comprender la función de la coloración BOB en la naturaleza y en dos modelos de estudio: *L. jemineus* como

depredador y avispas de 4 géneros de sceliónidos como presa. Específicamente, se evaluó la eficacia de BOB y el patrón de color negro en los sceliónidos para inducir diferentes respuestas de comportamiento, como la aversión, en el saltícido *L. jemineus*.

El aposematismo de la coloración BOB

Un estudio anterior mostró que el patrón de color negro-naranja-negro (BOB) está muy extendido entre las avispas parasitoides pequeñas y que generalmente está presente en ambos sexos (Mora y Hanson, 2019), lo que sugiere que este patrón de color no está relacionado con dimorfismo sexual. La hipótesis principal con respecto a la función de este patrón de color generalizado es el aposematismo y / o la mímica, y según nuestro conocimiento, la presente investigación es la primera en probar esta hipótesis. Aunque los resultados fueron mixtos, al menos dos de nuestros hallazgos proporcionan evidencia de que la coloración BOB es de hecho aposemática.

Primero, aunque las respuestas de comportamiento fueron bastante variables, en cinco ocasiones las arañas capturadas en el campo consumieron avispas negras (todas pertenecientes al género *Scelio*), pero ninguna araña consumió una avispa con coloración BOB. En segundo lugar, las pruebas de toxicidad aguda con *Daphnia magna* mostraron que las avispas con coloración BOB causaron una mayor mortalidad que las avispas negras, lo que sugiere que las avispas BOB pueden contener compuestos defensivos a diferencia de las avispas negras (lo que explicaría por qué solo se consumieron avispas negras).

Los ensayos de toxicidad mostraron que los extractos de avispas eran más tóxicos para las bacterias (*V. fischeri*) que para los crustáceos (*D. magna*). Desafortunadamente, aunque en el mismo rango de toxicidad, no se observaron diferencias claras para las muestras de extracto que presentaron toxicidad en los ensayos de *V. fischeri*, debido a la superposición de los intervalos de confianza. No obstante, los resultados de esta prueba deben tenerse en cuenta para estimar la posible presencia de componentes tóxicos en las avispas para bacterias. Un resultado consistente para ambos bioindicadores es que el *Scelio* negro no mostró toxicidad, y podría considerarse el menos tóxico de los cuatro géneros. Los resultados para *D. magna* son más relevantes para este estudio, ya que se pueden observar diferencias entre los géneros y también porque es un artrópodo al igual que las arañas de *Lyssomanes*. Además, los extractos de las avispas BOB mostraron toxicidad, mientras que solo uno de cada cuatro extractos de avispa negra lo hizo. Asimismo, los valores de CE50 para los extractos de avispa BOB

fueron más bajos, es decir, tuvieron una mayor toxicidad. Se necesita más investigación para verificar que un compuesto defensivo esté presente en las avispas BOB (y ausente en las avispas negras) y, si está presente, sería muy interesante proceder a identificarlo y caracterizarlo.

Respuestas de depredadores hacia las avispas BOB

Debe enfatizarse que se necesita un esfuerzo considerable para obtener avispas sceliónidas vivas; se requirió un total de aproximadamente 540 horas para recolectar las avispas utilizadas en nuestros experimentos e incluso con este esfuerzo hubo números insuficientes para algunas de las pruebas. Esta limitación fue una de las motivaciones para realizar ensayos con presas falsas (señuelos). A pesar de un cuidado considerable al proporcionar señuelos que combinaran estrechamente con los espectros de los colores negro y naranja de las avispas y una arena en la que los movimientos de los señuelos se controlaron cuidadosamente, los resultados no fueron concluyentes. La explicación probable es que los señuelos carecían de señales visuales adicionales (por ejemplo, patas y ojos) y / o señales químicas necesarias para provocar una respuesta depredadora, que se ha demostrado en otras arañas saltarinas (Harland y Jackson, 2000). En futuras investigaciones, sería interesante utilizar avispas muertas como señuelos, en lugar de granos de arroz pintados.

En nuestros experimentos con los depredadores, intentamos tener en cuenta las recomendaciones de otros investigadores trabajando en el tema como: el contraste de fondo, la visión de los depredadores, la etapa de vida de los depredadores, los tamaños de depredadores y presas, y las oportunidades previas de aprendizaje del depredador (Stevens y Ruxton, 2012, Arenas et al, 2015). Se utilizaron tres tipos de arañas en nuestros experimentos: adultos recolectados en el campo, juveniles recolectados en el campo y adultos criados en laboratorio. Si el aprendizaje juega un papel en evitar la presa aposemática, uno esperaría que el primer grupo muestre la mayor discriminación entre las avispas negras y las avispas BOB, ya que el uso de especímenes criados elimina el papel de la generalización aprendida (Fabricant y Smith, 2014) y los juveniles recolectados han tenido menos tiempo para aprender. Los resultados de nuestros experimentos proporcionan evidencia parcial de esto. Los únicos casos de arañas que consumieron avispas (avispas negras no aposemáticas) fueron los de *Lyssomanes* que habían sido capturados en el campo. Además, los adultos recolectados en el campo tenían más probabilidades de detectar o evitar que atacar, en comparación con los adultos criados en cautiverio, los cuales parecían preferir atacar. Por lo tanto, los adultos del campo, que han tenido más experiencia, son más selectivos.

Al analizar el origen de la araña en sus respuestas a las avispas a lo largo del tiempo, existe un efecto de falta de aprendizaje en las arañas criadas en cautiverio, es decir, una menor capacidad de detección y ataque que las arañas recolectadas en el campo. Esto se espera, dada la complejidad de los factores y las diferentes presas que se encuentran en condiciones naturales en comparación con un entorno de reproducción artificial y una dieta que consistía en solo dos tipos de presas. Sin embargo, un posible efecto innato es evidente en la clara aversión de las arañas criadas en cautiverio a las avispas con la coloración BOB; se detectaron ambos colores (BOB y negro), así como ataques principalmente a avispas negras. Además, para las arañas de condiciones naturales, la aversión aumentó con el tiempo, y esta aversión coincide con los análisis de toxicidad y el patrón aposemático de las avispas. Estos resultados merecen más investigación ya que generalmente se supone que se aprende una respuesta de comportamiento adversa a la coloración aposemática, a pesar de la presencia generalizada de coloración BOB en varios grupos taxonómicos.

Componentes espectrales del patrón BOB

Aunque el color naranja generalmente está presente en la coloración aposemática y la señalización de advertencia (Thery y Gomez, 2010; Stevens y Ruxton, 2012), nuestros resultados sugieren que no todos los especímenes BOB fueron tratados de manera similar por los depredadores-arañas. La coloración naranja en las avispas BOB varía entre géneros, a pesar de parecer similar al ojo humano. Es posible que la diferencia entre los dos elementos absorbentes de luz (pigmentos negros y naranjas) dentro de cada género de avispas sea importante para afectar las respuestas de los depredadores. Dichas diferencias podrían favorecer el aumento de la visibilidad y, en consecuencia, intervenir en la evitación inicial (Stevens et al., 2010; Fabricant y Herberstein, 2014). *Baryconus* tuvo el contraste de absorción más bajo entre negro y naranja y, curiosamente, este género recibió la mayoría de los eventos de "ataque". En el otro extremo, *Macroteleia* tuvo el mayor contraste de absorción, así como la mayor contribución de naranja, lo que quizás explica por qué este género tuvo el mayor número de eventos de "detección". Los eventos de "detección" parecen estar asociados con la contribución del negro: los valores más altos de la contribución del negro, como en *Macroteleia* y *Chromateleia*, produjeron un mayor porcentaje de eventos de "detección", mientras que los valores más bajos de la contribución del negro, como en *Scelio* y *Baryconus*, produjo un menor porcentaje de eventos de "detección". Esto refuerza el hecho de que los artrópodos aún pueden percibir una diferencia de

longitud de onda larga a través de información acromática o de luminancia (Reisenman y Giurfa, 2008) y que algunos patrones de color pueden funcionar como señales multicomponentes (Grether et al., 2004).

También es importante tener en cuenta el espacio visual del depredador. Se ha informado que las arañas saltarinas son menos sensibles al extremo naranja del espectro y, por lo tanto, perciben la cutícula anaranjada como más acromática que las estructuras con relativamente más reflectancia en la porción verde del espectro. Lo mencionado, más la ausencia de un receptor rojo en muchas arañas podría causar que los objetos naranjas sean percibidos como objetos monocromáticos "verdes" difíciles de distinguir cromáticamente del follaje verde (Fabricant y Herberstein, 2014; Chittka y Waser, 1997; Briscoe y Chittka, 2001). Además, dado que los depredadores tienen diversos sistemas visuales y cognitivos, una señal de advertencia que mejora el reconocimiento de las presas y el aprendizaje de la aversión para un depredador podría ser ineficaz para otro (Speed, 2000).

La mayoría de los estudios de aposematismo han utilizado depredadores vertebrados y presas más grandes, mientras que los depredadores artrópodos de presas más pequeñas generalmente se han descuidado (Lang et al, 1999). El patrón negro-naranja-negro (BOB) está extremadamente extendido en pequeños himenópteros (así como en algunos otros insectos) y evidentemente ha evolucionado independientemente en numerosas ocasiones (Mora y Hanson, 2019). Hasta donde sabemos, el presente estudio proporciona la primera evidencia de que este patrón de color común tiene una función aposemática. Sin embargo, todavía hay muchas preguntas sin respuesta. Por ejemplo, sería interesante evaluar las respuestas de comportamiento de otros depredadores cuando se enfrentan con avispas sceliónidas que tienen el patrón BOB. La identidad de los pigmentos y los supuestos compuestos nocivos aún se desconocen. Finalmente, sería instructivo examinar las propiedades espectrales de otras avispas con este patrón de color, así como la capacidad visual de otros posibles depredadores.

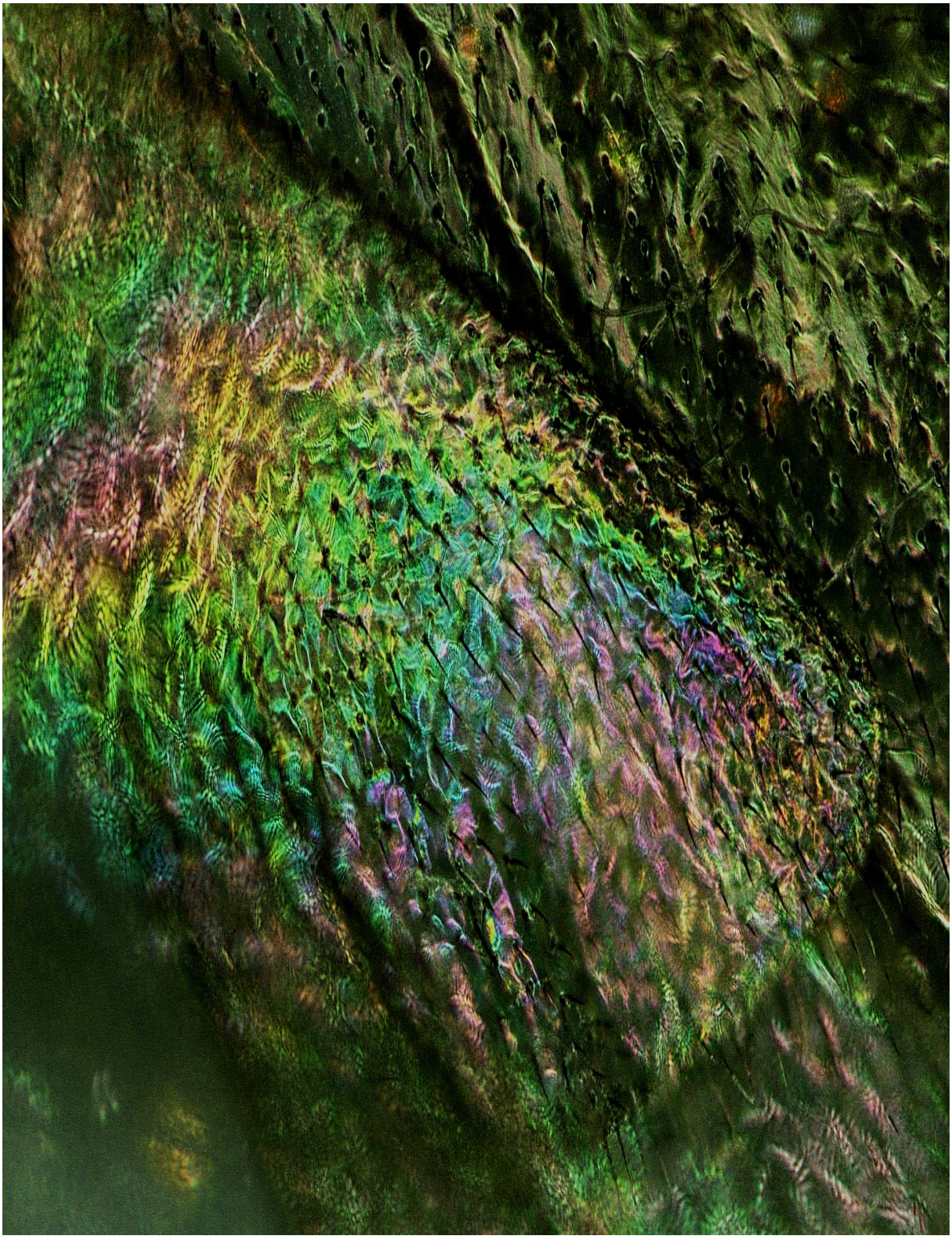

Ilustración 9. Fotografía R. Mora. Título: Irisdiscencia en alas de microavispa (Scelionidae).Técnica macrofotografía a travez de microespectrofotómetro.

ANEXOS

Anexo 1. Un método adecuado para criar juveniles de *Lyssomanes jemineus* Peckham & Wheeler (Araneae: Salticidae: Lyssomaninae). En este anexo se proponen varios diseños de jaulas enriquecidas en condiciones controladas para evaluar la influencia de las dietas en la supervivencia de *Lyssomanes* Nuestros resultados indican que una dieta politípica (mezcla de dos grupos de insectos) de acuerdo con la madurez de la muestra, así como la crianza en la planta donde se recogieron los sacos o muestras de huevos, minimiza el alto pico de mortalidad observado en la segunda semana de vida de la muestra. De arañas. Un 53% de supervivencia se logró con éxito al ingerir una dieta de dos presas, *Bemissia tabaci* MED (Biotipo Q) para juveniles con menos de 200 días y luego hacer la transición a *Drosophila melanogaster* hasta la edad adulta. Este método de crianza de un año para *Lyssomanes* se complementó con dos años de observaciones de campo, lo que concluye que el éxito de un sistema de cría implica una serie de circuitos de retroalimentación entre lo que se observa en el campo y lo que se ha aprendido dentro del sistema. El sistema también es económico, fácil de implementar y no requiere mucha mano de obra, ya que no separamos las crías individualmente. Es particularmente adecuado para realizar experimentos en condiciones de laboratorio controladas y para establecer colonias de *Lyssomanes* de un año.

Anexo 2. Artículo *Messua sp*. (Salticidae), the host spider of the polysphinctine *Inbioia pivai* (Ichneumonidae: Pimplinae). Informamos el primer registro de huésped para el género monotípico *Inbioia*, el artrópodo de la familia Salticidae.

Anexo 3. Patente Arena de depredación automatizada y programable. Una arena para observar arañas con presas vivas (avispas) y con presas móviles falsas (señuelos) la cual consiste en una caja acrílica transparente cerrada con un volumen interno de 1550 cm^3. Las placas acrílicas se hicieron en una máquina de corte CNC, modelo Redsail CM1690, con un tubo láser rojo de 100 vatios de potencia. Para proporcionar una mayor complejidad estructural y rutas adicionales para acercarse a la presa, se colocó una estructura acrílica en forma de árbol en el centro del piso (Hill, 1979; Tarsitano y Andrew, 1999). En los experimentos con señuelos falsos, la caja de acrílico se colocó dentro de un marco hecho de madera prensada que contenía un mecanismo automatizado, dependiente de un Arduino, para mover el señuelo.

Anexo 4. Divulgación de artículos. Periodismo Nacional e Internacional.

REFERENCIAS

Arenas M., Walter D., Stevens M. 2015. Signal honesty and predation risk among a closely related group of aposematic species. Scientific Reports. 5:11021. doi:10.1038/srep11021.

Arzt E., Enders S., Gorb S. 2002. Towards a micromechanical understanding of biological surface devices. Zeitschrift fur Metallkunde 93(5), 345– 351.

Aronsson, M. y Gamberale-Stille, G. 2008 Domestic chicks primarily attend to colour, not pattern, when learning an aposematic coloration. Anim. Behav. 75, 417–423. (doi: 10.1016/j.anbehav.2007.05.006).

Bates H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Trans. Linn. Soc. Lond. 23, 495–566. doi: 10.1111/j.1096-3642. 1860.tb00146.x

Blest, A. D., McIntyre, P., Carter, M. 1988. A re-examination of the principal retinae of *Phidippus johnsoni* and *Plexippus validus* (Araneae: Salticidae): Implications for optical modelling. Journal of Comparative Physiology A, 162, 47–56.

Briscoe A.D. y Chittka, L. 2001. The evolution of color vision in insects. Annual review of entomology, 46(1), pp.471-510.

Briolat E. S., Burdfield-Steel E. R., Paul S. C., Rönkä K. H., Seymoure B. M., Stankowich T.y Stuckert A. M. 2019. Diversity in warning coloration: selective paradox or the norm? Biological Review 94.2: 388-414.

Brodie Jr E. D.y Howard R. R. 1973. Experimental study of Batesian mimicry in the salamanders *Plethodon jordan*i and *Desmognathus ochrophaeus*. American Midland Naturalist, 38-46.

Chapman R.F. 1988. The insects: Structure and Function. Cambridge University Press, Cambridge.

Chittka L.y Waser N. 1997. Why red flowers are not invisible to bees. Isr J Plant Sci. 45:169–183.

Dai Z. y Yang Z. 2010. Macro-micro-structures of elytra, mechanical properties of the biomaterial and the coupling strength between elytra in beetles. J. Bionic Eng.7, 6–12.

Doucet S.M. y Meadows M.G. 2009. Iridescense: a functional perspective. J.R. Soc. Interfase. 6, S115-S132.

Eisner T., Van Tassell E.y Carrell J.E. 1967. Defensive use of a "fecal shield" by a beetle larva. Science. 158, 1471-1473.

Endler J. A y Mappes J. 2017. The current and future state of animal coloration research. Phil. Trans. R. Soc. B 372, 20160352. doi: 10.1098/rstb.2016.0352; pmid: 28533467.

Fabricant, S.A. y Herberstein, M.E. 2014. Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. Behavioral Ecology, 26 (1), pp.38-44.

Fabricant S. A. y Smith C. L. 2014. Is the hibiscus harlequin bug aposematic? The importance of testing multiple predators. Ecology and evolution, 4(2), 113-120.

Fung K. 2005. Photonic iridescence of a blue-banded bee. Microsc. Microanal. 11(2): 1202–1203

García J., Polidori A. C., Nieves-Aldrey J. L. 2016. Pheomelanin in the secondary sexual characters of male parasitoid wasps (Hymenoptera: Pteromalidae). Arthropod Struct. Dev. 45: 311–319.

Gamberale-Stille, G. 2001 Benefit by contrast: an experiment with live aposematic prey. Behav. Ecol. 12, 768–772. (doi:10.1093/beheco/12.6.768)

Grether G. F., Kolluru G. R. y Nersissian, K. 2004. Individual colour patches as multicomponent signals. Biological Reviews, 79(3), 583-610.

Gunnarsson B. 1996. Bird predation and vegetation structure affecting spruce-living arthropods in a temperate forest. Journal of Animal Ecology 65 (3): 389-397.

Hallas, S. E. y Jackson R. R. 1986. A comparative study of Old and New World lyssomanines (Araneae, Salticidae): utilisation of silk and predatory behaviour of *Asemonea tenuipes* and *Lyssomanes viridis*. New Zealand Journal of Zoology 13 (4): 543-551.

Harland D. P., Li D.y Jackson, R. R. 2012. How jumping spiders see the world.Cambridge, MA: the MIT Press.

Hotová Svádová K., Exnerová A., Kopečková M.y Štys, P. 2013. How do predators learn to recognize a mimetic complex: experiments with naïve great tits and aposematic Heteroptera? Ethology, 119(10), 814-830.

Ikan R y Ishay J.1967.Pteridines and purines of the queen of the Oriental hornet *Vespa orientalis*. Journal of lnsect Physiology.13:159-169.

Ishay, J. S. y Pertsis, V. 2002. The specific heat of the cuticle and the morphological differences between the brown and yellow cuticles of hornets. J. Electron Microsc. 51, 401–411.

Ishay J. S., Josep Z., Galushko, D. V., Bergman D. J. 2005. The nanostructure of the oriental hornet (Hymenoptera, Vespinae) cuticle and silk and some of their biophysical properties.Curr. Nanosci.1, 125–156.

Jackson R. R. 1978. Life history of *Phidippus johnsoni* (Araneae, Salticidae). Journal of Arachnology 6 (1): 1-29.

Kauppinen J. y Mappes J. 2003 Why are wasps so intimidating: field experiments on hunting dragonflies (Odonata: Aeshna grandis). Anim. Behav. 66, 505-511.

Kinoshita S., Yoshioka S., Kawagoe K. 2002. Mechanisms of structural colour in the Morpho butterfly: cooperation of regularity and irregularity in an iridescent scale. Proc. Biol. Sci. 269 (1499), 1417-1421.

Lang A., Filser J., Henschel J.R. 1999. Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. Agric Ecosyst Environ 72:189–199.

Lindstrom L., Alatalo R.V., Lyytinen A., Mappes J. 2001. Strong antiapostatic selection against novel rare aposematic prey. Proc. Natl. Acad. Sci USA. 98: 9181-9184.

Logunov D. V. 2014. New species and records of *Lyssomanes* Hentz, 1845 from Central and South America (Araneae: Salticidae). Arthropoda Selecta 23 (1): 57–56.

Lubin Y. D. 1978. Seasonal abundance and diversity of web-building spiders in relation to habitat structure on Barro Colorado Island, Panama. Journal of Arachnology 6 (1): 31-51.

Maddison W. P. 2015. A phylogenetic classification of jumping spiders (Araneae: Salticidae). Journal of Arachnology 43(3): 231-292

Mallet J. y Joron M. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Ann. Rev. Ecol. Syst. 30: 201–233.

Marchini M., Sommaggio D., Minelli. A. 2016. Playing with black and yellow: the evolvability of a Batesian mimicry. Evol. Biol. 44: 100–112.

Masner L. 1988. Convergent chromatic mimicry among some Neotropical Hymenoptera: a search for the model, pp. 12. In Proceedings XVIII International Congress of Entomology, 3–9 July 1988, Vancouver, BC, Canada. Abstracts.

Masner L. y Hanson P. E. 2006. Familia Scelionidae. Mem. Am. Entomol. Inst. 77: 254–265.

McIver J. D. y Lattin J. D. 1990. Evidence for aposematism in the plant bug *Lopidea nigridea* Uhler (Hemiptera: Miridae: Orthotylinae). Biol. J. Linn. Soc. 40:99–112.

Miyashita T., A. Shinkai, T. Chida. 1998. The effects of forest fragmentation on web spider communities in urban areas. Biological Conservation 86 (3): 357-364.

Mora R. y Hanson P.E. 2019. Widespread occurrence of black-orange-black color pattern in Hymenoptera. Journal of Insect Science.; 19 (2). pmid:30851035

Morse D. H. y R. S. Fritz. 1982. Experimental and observational studies of patch choice at different scales by the crab spider *Misumena vatia*. Ecology 63 (1): 172-182.

Mortazavian S. y Fatemi. 2015. Effects of fiber orientation and anisotropy on tensile strength and elastic modulus of short fiber reinforced polymer composites. Compos. part B: engineering 72, 116–129 (2015). 16.

Nentwig W., Cutler B., Heimer S. 1993. Spiders of Panama: biogeography, investigation, phenology, check list, key and bibliography of a tropical spider fauna. Flora and Fauna Handbook No. 12. Sandhill Crane Press.

Neville A.C. 1975. Biology of the Arthropod Cuticle. Springer, Berlin.

Parker A.R., Welch V.L., Driver D., Martini N. 2003. Structural colour: opal analogue discovered in a weevil. Nature. 426, 786.

Papaj, Daniel R.y Ginny M. Newsom. 2005. "A within-species warning function for an aposematic signal. “Proceedings of the Royal Society B: Biological Sciences: 272.1580 2519-2523.

Plotkin M., Volynchik S., Ermakov N.Y., Benyamini A., Boiko Y., Bergman D.J. y Ishay J.S. 2009 a. Xanthopterin in the Oriental hornet (*Vespa orientalis*): Light absorbance is increased with

maturation of yellow pigment granules. Journal of Photochemistry and Photobiology. 85, 955–961.

Prete F. R. 1999 a. Prey recognition. In the Praying Mantids (ed. F. R. Prete, H. Wells, P. Wells and L. E. Hurd). pp. 141-179. Baltimore, MD: Johns Hopkins University Press.

Prószyński J. 2016. Monograph of Salticidae (Araneae) of the World 1995-2015. Part II: Global Species Database of Salticidae (Araneae). Version May, 5, 2016.

Prudic K. L., Skemp A. K. y Papaj, D. R. 2007 Aposematic coloration, luminance contrast, and the benefits of conspicuousness. Behav. Ecol. 18, 41–46. (doi:10.1093/ beheco/arl046)

Raizer J. y Amaral M. E. C. 2001. Does the structural complexity of aquatic macrophytes explain the diversity of associated spider assemblages? Journal of Arachnology 29 (2): 227-237.

Reisenman C.E. y Giurfa M. 2008. Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee *Apis mellifera*. Arthropod-Plant Interactions. 2: 137-146.

Richman D. B. y Whitcomb W. H. 1981. The Ontogeny of *Lyssomanes viridis* (Walckenaer) (Araneae: Salticidae) on *Magnolia grandiflora* L. Psyche 88(1-2):127-133, 1981.

Riechert S. E. y Tracy C. R. 1975. Thermal balance and prey availability: bases for a model relating web-site characteristics to spider reproductive success. Ecology 56 (2): 265-284.

Rowe C. y Halpin C. 2013. Why are warning displays multimodal? Behavioral Ecology and Sociobiology,67(9), 1425-1439.

Rubio G. D., Galvis W., Nadal M. F. 2017. Description of the female of *Lyssomanes miniaceus*, with a new distribution record for *L. belgranoi* (Araneae: Salticidae). Caldasia 39 (2): 239-246.

Ruxton G.D., Sherratt T.N., Speed M.P. 2004. Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry. Oxford: Oxford University Press.

Sarrazin M., Vigneron J.P., Welch V., Rassart M. 2008. Nanomorphology of the blue iridescent wings of a giant tropical wasp *Megascolia procer* javanensis (Hymenoptera). Physical Review E. 78(051902): 1-5.

Schroeder T. B., Houghtaling J., Wilts B. D., Mayer, M. 2018. It's not a bug, it's a feature: functional materials in insects. Advanced Materials, 30(19), 1705322.

Seago A.E., Brady P., Vigneron J.P., Schultz T.D. 2009. Gold bugs and beyond: A review of iridescence and structural colour mechanisms in beetles (Coleoptera). J R Soc Interface. (Suppl 2): S165–S184

Shamim G., Sanjeev K. R., Dev M. P., Ranganathan. 2014. Biochemistry and biosynthesis of insect pigments. R. Eur. J. Entomol. 111 (2): 149-164.

Sherratt T.N., Speed M.P., Ruxton G.D. 2004. Natural selection on unpalatable species imposed by state-dependent foraging behaviour. Journal of Theoretical Biology. 228, 217-226.

Skelhorn J. y Rowe C. 2016. Cognition and the evolution of camouflage. Proc. R. Soc. Lond. B Biol. Sci. 283: 20152890.

Skelhorn J., Halpin C. G., Rowe C. 2016. Learning about aposematic prey. Behav. Ecol. 27: 955–964.

Smith H. 2000. The status and conservation of the fen raft spider (*Dolomedes plantarius*) at Redgrave and Lopham Fen National Nature Reserve, England. Biological Conservation 95 (2): 153-164.

Song H. y Wenzel J.W. 2008. Phylogeny of bird-grasshopper subfamily *Cyrtacanthacridinae* (Orthoptera: Acrididae) and the evolution of locust phase polyphenism. Cladistics. 24, 515-542.

Sosa-Romero, M., Menéndez-Acuña M. y Burgos-Solorio A. 2016. Fenología y estacionalidad del género *Mexigonus* Edwards, 2002 (Araneae: Salticidae) en un bosque templado al norte de Cuernavaca, Morelos, México. Entomología Mexicana 3: 919-923.

Speed M.P., Ruxton G.D., Stephens P.A. y Blount J.D. 2009. Warning displays may function as honest signals of toxicity. Proceedings of the Royal Society B: Biological Sciences, 276(1658), 871-877.

Stevens M. y Ruxton G.D. 2012. Linking the evolution and form of warning coloration in nature. Proceeding of the Royal Society of London B. 279, 417–426.

Stevens M., Cuthill I. C., Windsor A. M. y Walker H. J. 2006. Disruptive contrast in animal camouflage. Proceedings of the Royal Society B: Biological Sciences, 273(1600), 2433-2438.

Stevens M., Mappes J. y Sandre S. L. 2010. The effect of predator appetite, prey warning coloration and luminance on predator foraging decisions. Behaviour, 147(9), 1121-1143.

Strohm E., Kroiss J., Herzner G., Laurien-Kehnen C., Boland W., Schreier P., Schmitt T. 2008. A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). Front Zool. 5:2.

Stuart-Fox D., Newton E., Clusella-Trullas S. 2017. Thermal consequences of colour and near infrared reflectance. Phil. Trans. R. Soc Lond. B Biol Sci. 372: 20160345.

Sun, J., Wu, W., Xue, W., Akhtar, R., Ren, L. y Tong, J. 2015. Quantitative nanomechanical properties of the cuticle of the multicolored asian lady beetle using the modulus mapping technique.Current Nanoscience,11(2), pp.245-252.

Talianchich A., Bailey W.J., Ghisalberti E.L. 2003. Palatability and defense in the aposematic diurnal whistling moth, *Hecatesia exultans* Walker (Lepidoptera: Noctuidae: Agaristinae). Aust. J. Entomol. 42:276–280.

Thery M. y Gomez D. 2010. Insect colours and visual appearance in the eyes of their predators. Adv Insect Physiol. 38:267-353

Uetz, G. W. 1977. Coexistence in a guild of wandering spiders. The Journal of Animal Ecology 46 (2): 531-541.

Valerio A. A., Musetti L., Johnson N.F. 2013. Poster at the Entomological Society of America (Austin, TX, 10–13 November). Poster entitled "Species of the colorful genus Chromoteleia Ashmead (Hymenoptera: Platygastroidea, Platygastridae s.l.)". (Poster #78056).

Valkonen J., Niskanen M., Bjorklund M. y Mappes J. 2011 Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. Evol. Ecol. 25, 1047–1106. (doi:10.1007/s10682-011-9463-0) 18

Vidal-Cordero J.M., Moreno-Rueda G., López-Orta A., Marfil-Daza C., Ros-Santaella J.L., Ortiz-Sánchez F.J. 2012. Brighter-colored paper wasps (*Polistes dominula*) have larger poison glands. Frontiers in Zoology. 9: 20.

Wallace R. 1877. The colors of animals and plants. Part I. Am. Nat. 11, 641–662. doi: 10.1086/271979

Wäckers F. L. y Lewis W. J. 1999. A comparison of color-, shape-and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. Journal of Comparative Physiology A, 184(4), 387-393.

Wang H., Zhou H., Gui L., Ji H. y Zhang X. 2014. Analysis of effect of fiber orientation on young's modulus for unidirectional fiber reinforced composites. Compos. part B: engineering 56, 733–739.

Weatherhead Patrick J. 1993."Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds."Behavioral Ecology and Sociobiology33.1: 13-23.

Welch V.L., Vigneron J.P., Parker A.R. 2005. The cause of colouration in the ctenophore *Beroe cucumis*. Curr Biol. 15: R985–R986.

Welch V., Lousse V., Deparis O., Parker A., Vigneron J. 2007. Orange reflection from a three-dimensional photonic crystal in the scales of the weevil *Pachyrrhynchus congestus* pavonius (Curculionidae). Phys. Rev. E. 75:41919-1–41919-9.

Williams P. 2007. The distribution of bumble bee colour patterns worldwide: posible significance for thermoregulation, crypsis, and warning mimicry. Biol. J. Linnean Soc. 92,97-118.

Williams D. S. y McIntyr, P. 1980. Th e principal eyes of a jumping spider have a telephoto component. Nature, 288, 578 – 580.

Wittkopp P.J. y Beldade P. 2009. Development and evolution of insect pigmentation: genetic mechanisms and the potential consequences of pleitropy. Seminars in Cell and Development Biology. 20, 65-71.

Wolda H. 1978. Seasonal fluctuations in rainfall, food and abundance of tropical insects. Journal of Animal Ecology 47 (2): 369-381.

Wüster W., Allum C.S., Bjargardóttir I.B., Bailey K.L., Dawson K.J., Guenioui J., Lewis J., McGurk J., Moore A.G., Niskanen M. y Pollard C.P. 2004 Do aposematism and Batesian mimicry require bright markings? A test, using European viper markings. Proc. R. Soc. Lond. B 271, 2495–2499. (doi:10.1098/rspb.2004.2894)

Zi J., Yu X. D., Li Y. Z., Hu X. H., Xu C., Wang X. J., Liu X. H., Fu. R. T. 2003. Coloration strategies in peacock feathers. Proc. Natl. Acad. Sci. U.S.A. 100(22), 12576–12578.

Capítulo 1

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Research

Widespread Occurrence of Black-Orange-Black Color Pattern in Hymenoptera

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Abstract

Certain color patterns in insects show convergent evolution reflecting potentially important biological functions, for example, aposematism and mimicry. This phenomenon has been most frequently documented in Lepidoptera and Coleoptera, but has been less well investigated in Hymenoptera. It has long been recognized that many hymenopterans, especially scelionids (Platygastridae), show a recurring pattern of black head, orange/red mesosoma, and black metasoma (BOB coloration). However, the taxonomic distribution of this striking color pattern has never been documented across the entire order. The main objective of our research was to provide a preliminary tabulation of this color pattern in Hymenoptera, through examination of museum specimens and relevant literature. We included 11 variations of the typical BOB color pattern but did not include all possible variations. These color patterns were found in species belonging to 23 families of Hymenoptera, and was most frequently observed in scelionids, evaniids, and mutillids, but was relatively infrequent in Cynipoids, Diaprioids, Chalcidoids, and Apoids. The widespread occurrence of this color pattern in Hymenoptera strongly suggests convergent evolution and a potentially important function. The BOB color pattern was found in species from all biogeographic regions and within a species it was usually present in both sexes (with a few notable exceptions). In better studied tropical regions, such as Costa Rica, this color pattern was more common in species occurring at lower elevations (below 2,000 m). The biology of the tabulated taxa encompasses both ecto- and endoparasitoids, idiobionts and koinobionts, from a diversity of hosts, as well as phytophagous sawflies.

Key words: aposematism, Braconidae, Evaniidae, Ichneumonidae, Platygastridae, Scelioninae

Insects show an exceptional variety of colors with complex and diverse patterns. Coloration can be produced by diverse forms of surface and epidermal structures (structural colors) or by pigments in the outer body layers that selectively absorb, reflect, or scatter specific wavelengths of light. Pigments are responsible for most of the orange, red, yellow, and brown-black colors observed in insects while most blue or green colors result from nanostructural features that reflect these colors (Schroeder et al. 2018). Both the colors themselves and the way they are arranged into patterns often vary among individuals of a species.

Insect colors can have important biological functions such as thermoregulation (Stuart-Fox et al. 2017), secondary sexual characters (Jorge García et al. 2016), and predator avoidance via camouflage (crypsis and masquerade) (Skelhorn and Rowe 2016), warning (aposematic) coloration (Stevens and Ruxton 2012), or mimicry (Mallet and Joron 1999). Conspicuous coloration is often, but not always, indicative of aposematism, whereby predators learn to associate a particular color pattern with noxious chemical defenses, although this learning process is more complex than simply developing an aversion to certain types of prey (Skelhorn et al. 2016). Moreover, the visual and cognitive capacities of predators vary; for example, orange shield-back stinkbugs (Hemiptera: Scutelleridae) on a green background are probably conspicuous to birds but much less so to mantids (Fabricant and Herberstein 2015).

Compared with Lepidoptera and Coleoptera there are relatively few studies of conspicuous (potentially aposematic) color patterns in Hymenoptera, and what studies exist are mostly restricted to the yellow and black pattern in Vespidae (Vidal-Cordero et al. 2012, Marchini et al. 2016). However, it has long been recognized that many smaller hymenopterans, especially scelionids, show a recurring color pattern of black head, orange mesosoma, and black metasoma (BOB). (While we recognize that scelionids are currently placed in Platygastridae (Murphy et al. 2007, Sharkey 2007), we shall use the informal term 'scelionid' in the traditional sense.) Lubomir Masner (1988) was probably the first to emphasize the widespread occurrence of this color pattern in Hymenoptera (referring to it as a black, red, black). This BOB pattern appears to occur in 90% of the currently known species of *Chromoteleia*,

70% of *Acanthoscelio* and *Triteleia*, 50% of *Baryconus*, 40% of *Pseudoheptascelio*, 30% of *Opisthacantha*, *Scelio*, and *Sceliomorpha*, and 15% of *Macroteleia* (Valerio et al. 2013). It has also been documented in some species of *Lapitha*, *Leptoteleia*, *Oethecoctonus*, and *Probaryconus* (Masner and Hanson 2006). While the BOB color pattern is best known in scelionids, it also occurs in many other hymenopteran families, and even in species of other orders (we have observed it, for example, in some paederine staphylinids, and a few bibionid and stratiomyiid flies).

To the best of our knowledge, the taxonomic distribution of this color pattern in Hymenoptera has never been tabulated, except at the generic level in scelionids as noted above and in some mutillids (Wilson et al. 2015). Thus, the primary objective of the present investigation was to provide a compilation of hymenopteran taxa showing a black-orange-black color pattern in order to draw attention to this widespread phenomenon and to provide an initial framework for future studies.

Materials and Methods

This investigation was primarily restricted to hymenopteran specimens within the general size range of scelionids showing BOB coloration, approximately 3–20 mm in body length. Thus, some groups (e.g., Ceraphronoidea, Aphelinidae, Encyrtidae, Mymaridae) were excluded as being too small while others (many aculeates) were excluded as being too large. However, within the focal size range, all hymenopteran specimens were examined, independently of whether the taxa were previously known to include species with BOB colorations. R.M. examined ca. 418,000 hymenopteran specimens in the collections of the Museo Nacional of Costa Rica (MNCR, formerly the Instituto Nacional de Biodiversidad) from August to December 2015, and ca. 783,000 specimens in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) in Ottawa from August to December 2016. P.E.H. examined ca. 81,000 hymenopteran specimens in the Museo de Zoología at the Universidad de Costa Rica (MZUCR) from 2016 to 2017, and reviewed the relevant taxonomic literature to supplement museum records since the majority of microhymenopteran specimens in museums are not identified to species level.

Observations were made with various stereomicroscopes using magnifications ranging from 10× to 30×. Color patterns were recorded in dorsal view since the pattern often differed in lateral view. Even when restricting the observations to dorsal view, there was considerable variation. Eleven of the observed variations of the BOB pattern were used for coding the included species (Fig. 1). We did not include tricolored patterns that deviated from head black, mesosoma at least partially orange, and metasoma mostly black. Bicolored patterns were also excluded, for example, orange head and mesosoma, with black metasoma, or black head and mesosoma with orange metasoma. However, the diversity of black-orange patterns of all the observed specimens was documented.

For each species showing BOB coloration we recorded the exact morphological location of the black and orange colors (as coded in Fig. 1), the geographic distribution of the species and the source of these data (acronyms for museums mentioned above and literature references). Unidentified species were only included for genera where there were no identified species showing BOB coloration. For higher level classification of Hymenoptera, we follow Branstetter et al. (2017) and Peters et al. (2017).

A chi-square test was used to evaluate the distribution of color (orange and black, vs all black) with respect to altitude (greater than 2,000 m and less than 2,000 m). Using a contingency table, the number of insects found within each combination was counted. Then a comparison of the expected value of each combination with the observed value was divided by the expected value. The probability value was obtained with the sum of these values and a chi-square distribution with 1 df.

Three limitations in our approach should be mentioned. First, determining what constitutes orange versus, for example, reddish brown, was sometimes difficult. We attempted to include only the orange or reddish orange color as exemplified by numerous scelionids, but other, similar colors are mentioned in the text for some taxa. Second, due to the breadth of this study, it was not possible to exhaustively record all intraspecific color variation, but some examples of such variation are noted. Third, this compilation is geographically biased toward the Neotropical and Neartic regions, and is certainly incomplete even for these regions.

Results

In total, 66 orange and black color patterns were observed in Hymenoptera (Figs. 2 and 3), and of these 66 patterns, four patterns in braconids, one in chrysidids, and five in mutillids also showed whitish markings, mainly on the metasoma. The results for species showing one or more of the patterns illustrated in Fig. 1 are presented in Tables 1–7. The BOB 0 and BOB 9 patterns were found in all taxa (though sawflies lack a propodeum so what appears as BOB 0 is actually BOB 9). Other BOB variations were found in only one taxon, for example, such BOB 3 and BOB 4 (Platygastroidea), BOB 8 (Proctotrupomorpha), BOB 10 (Braconidae), and BOB 7 and BOB 11 (Ichneumonidae). Other patterns were found in two or more groups: BOB 1 (Braconidae and Platygastriodea), BOB 2 (Evanioidea and Aculeata), and BOB 5 (Braconidae, Platygastriodea, and Ichneumonidae).

In terms of geographical distribution, the most widespread patterns worldwide were BOB 0 and 9. Among scelionids BOB 0 was very well represented in the Neotropics but was also found in the other biogeographical regions (Table 1). The same was generally true for Braconidae and Ichneumonidae (Tables 3 and 4). BOB 2, BOB 5, and BOB 9 were found in three to five geographical regions, and the remaining patterns in just one region. The vast majority of specimens showing a black-orange-black pattern were collected below 2,000 m. In a statistical analysis (n = 100), altitude was categorized in two groups, greater than 2,000 m and less than 2,000 m, and an association was found ($P < 0.0001$) between all black coloration and an altitude greater than 2,000 m.

As in the BOB patterns, several other black-orange dorsal patterns were found in all or most biogeographical regions. Some examples include the following. 1) Black head and mesosoma, with orange metasoma, was observed in all regions except the Indo-Malayan realm. 2) Black head and mesosoma, with metasoma orange anteriorly (the first tergites) and black posteriorly (the remaining tergites), was observed in all regions except the Afrotropical and Australasian realms. 3) Black head, with mesosoma and metasoma orange except last tergite(s) black, was observed in all regions.

On the other hand, the majority of unique patterns (found only in a specific region and within a particular taxon) were found predominantly in three realms: Afrotropical, Indo-Malayan, and Australasian. These unique patterns were characterized in the Indo-Malayan and Afrotropical realms by black or whitish markings on the mesosoma or metasoma, embedded in an orange background, as in some *Macroteleia* (Scelioninae), *Therophilus* (Braconidae), and *Trogaspidia* (Mutillidae). In the Australasian realm, some *Orgilus* and *Syngaster* (Braconidae) had a black or orange mesosoma, and a combination of orange and black on the metasoma.

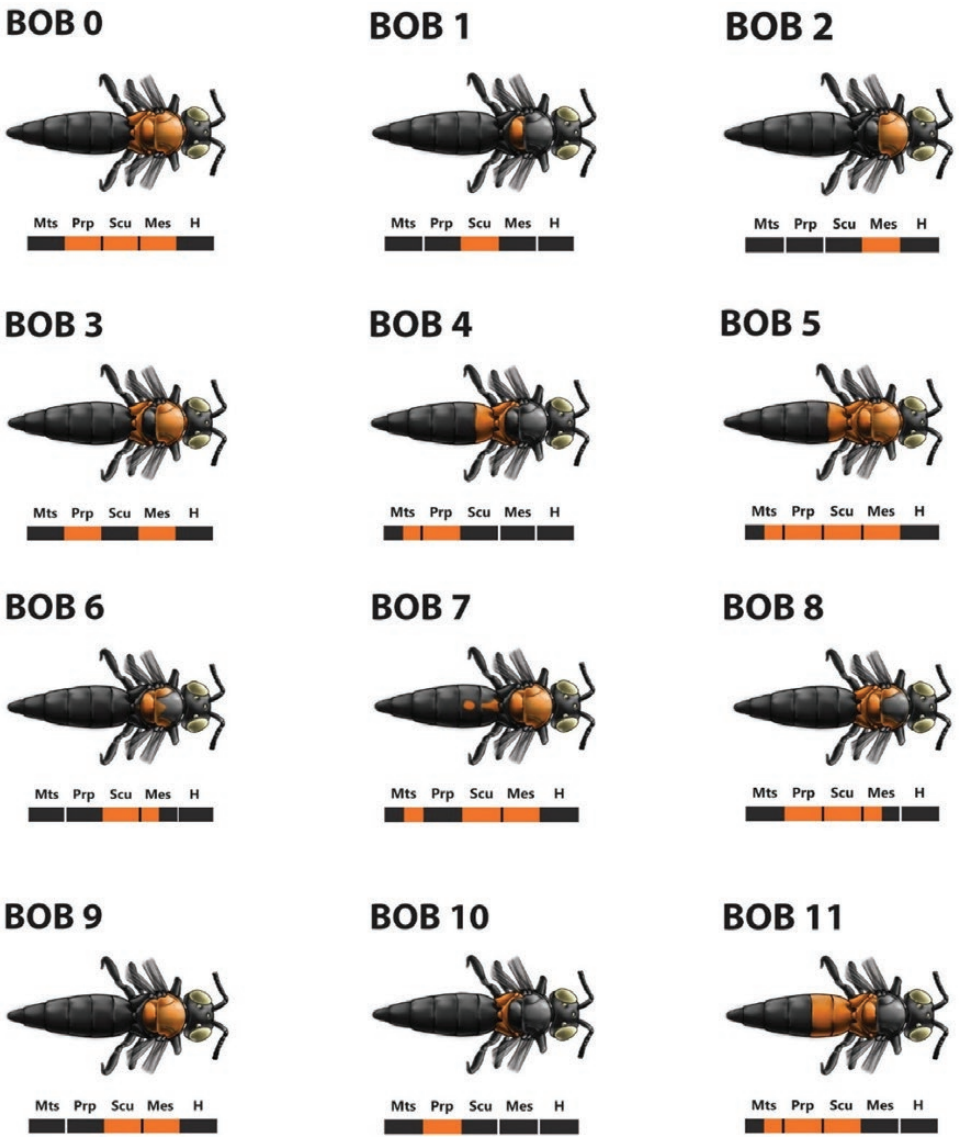

Fig. 1. Eleven variations of the black head, orange mesosoma, black metasoma (BOB pattern) in Hymenoptera. Mts = metasoma, Prp = propodeum, Scu = scutellum, Mes = mesoscutum, H = head. This simplified color code does not include the pronotum and metanotum.

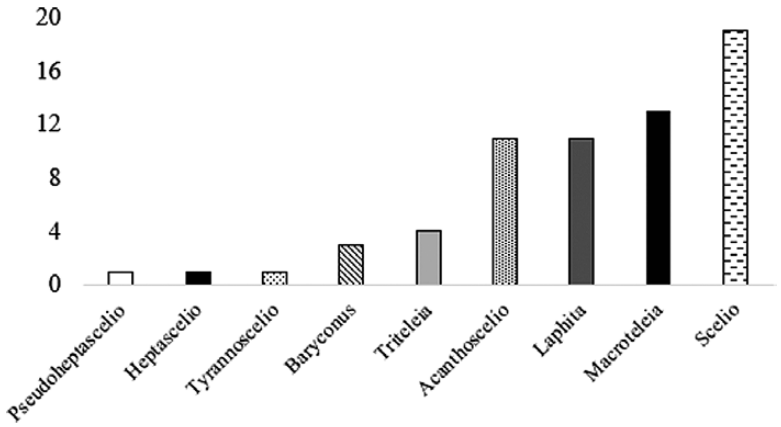

Fig. 2. Total number of observed black-orange patterns in some genera of Platygastridae, including those illustrated in Fig. 1 plus additional patterns not illustrated (for example, bicolored patterns and tricolored patterns that did not follow the sequence of head black, mesosoma at least partially orange, metasoma mostly black).

Platygastroidea

Most 'platygastrids' (in the traditional sense) and Telenominae are below the size range included in our survey, but BOB coloration was not encountered in either of these groups. In contrast, this color pattern was found in 14 genera of Scelioninae and one Teleasinae (*Trimorus*) (Table 1). In *Chromoteleia*, a neotropical genus except for one African species, 13 species show the BOB 0 pattern, four the BOB 9 pattern, three the BOB 2 pattern, one the BOB 5 pattern, and one has just the pronotum orange (based on images in Chen et al. 2018). Intraspecific variation in color appears to be quite common in Scelioninae, including variation between individuals of the same sex. For example, in several species of *Acanthoscelio* (Dotseth and Johnson 2001) and in *Pseudoheptascelio rex* (Johnson and Musetti 2011) the mesosoma varies from orange to entirely black. In addition to the typical BOB 0 pattern and the 11 variations illustrated in Fig. 1, numerous other black-orange combinations are present, including a total of at least 19 combinations in species of *Scelio* (Fig. 2).

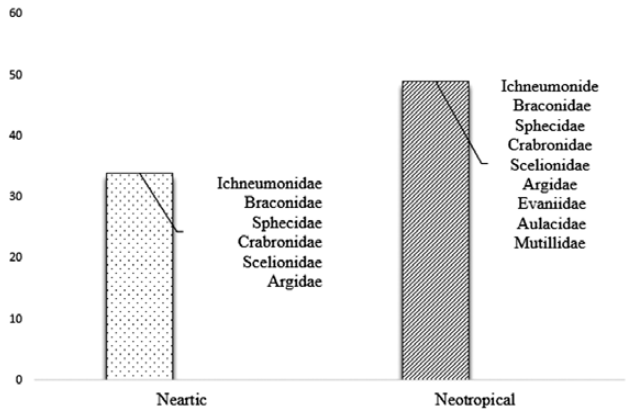

Fig. 3. Total number of observed black-orange patterns in all Neotropical and Neartic specimens examined, which are the geographical areas best represented in our survey. These include the patterns illustrated in Fig. 1 as well as others not illustrated (for example, bicolored patterns and tricolored patterns that did not follow the sequence of head black, mesosoma at least partially orange, metasoma mostly black).

Other Proctotrupomorpha (Cynipoids, Proctotrupoidea, Diaprioids, Chalcidoids)

Given the prevalence of BOB coloration in Platygastridae it is curious how infrequent this pattern is in the other Proctotrupomorpha (Table 2). Several species of Cynipoids have a black head and mesosoma with an orange metasoma, but BOB colorations seems to be very rare and when present (a couple of *Callaspidia* species) individuals often vary in color. In Proctotrupoidea, some Proctotrupidae and Roproniidae have an orangish metasoma but no examples of BOB coloration were found. In Diaprioids a few *Trichopria* approach a BOB coloration and among larger-sized Chalcidoids (>3 mm) the color pattern occurs primarily in a few species of Chalcididae and Eurytomidae. *Bephrata* and *Isosomodes* show considerable variation in color between species, and species with BOB coloration often have light colored markings on the sides of the metasoma; a few species show extreme variations of BOB not included in our color codes (Fig. 1). Some pteromalids (e.g., *Epistenia* and *Neocatolaccus*) superficially fit the BOB 9 pattern, but the mesosoma is metallic bronze instead of orange. Eulophidae is probably the most speciose chalcidoid family, yet the BOB pattern appears to be virtually absent, at least in the specimens we examined. A few Tetrastichinae and Eulophinae approach the BOB pattern, although they are more yellowish, as opposed to orange.

Ichneumonidae

The BOB color pattern was found in species belonging to six subfamilies of Ichneumonidae (Table 3). Some of these species show intraspecific variation, for example, in females of *Glypta*

Table 1. Species of Platygastroidea (Platygastridae) with BOB color patterns

Species	Color	Distributión	Reference
Acanthoscelio acutus Dotseth & Johnson	BOB 0, 2	Neotropical	CNC
A. radiatus Dotseth & Johnson	BOB 2, 4	Neotropical	CNC
Baryconus sp.	BOB 0	Neotropical	MZUCR
Chromoteleia: 22/27 spp.	BOB 0, 2, 5, 9	Neotropical	Chen et al. (2018)
Lapitha sp.	BOB 0	Neotropical	MZUCR
Leptoteleia majkae Masner	BOB 0	Neotropical	CNC
Macroteleia eximia Muesebeck	BOB 0	Neotropical	CNC
M. insignis Muesebeck	BOB 0	Neotropical	CNC
M. simulans Muesebeck	BOB 0	Neotropical	CNC
Oethecoctonus sp.	BOB 0	Neotropical	MZUCR
Parascelio sp.	BOB 0	Neotropical	CNC
Probaryconus sp.	BOB 0, 9	Neotropical	CNC
Pseudoheptascelio rex Johnson & Musetti	BOB 0	Neotropical	CNC
P. tico Johnson & Musetti	BOB 0	Neotropical	CNC
Scelio fulvithorax Dodd	BOB 0	Australasian	CNC
S. schmelio Dangerfield & Austin	BOB 1	Australasian	CNC
S. semisanguineus Girault	BOB 3	Australasian	CNC
S. variegatus Kozlov & Kononova	BOB 5	Palearctic	CNC
Sceliomorpha rufithorax Kieffer	BOB 0	Neotropical	CNC
Triteleia sp.	BOB 0	Neotropical	MZUCR
Tyrannoscelio genieri Masner & Johnson	BOB 0	Neotropical	CNC
Trimorus sp.	BOB 0	Neotropical	MZUCR

Valerio et al. (2013) also mention *Opisthacantha*.

Table 2. Species of Proctotrupomorpha (excluding Platygastroidea) with BOB color patterns

Species	Color	Distribution	Reference
Figitidae			
Callaspidia notata (Fonscolombe)	BOB 8	Palearctic	Ros-Farré and Pujade-Villar (2009)
Heloridae			
Helorus brethesi Ogloblin	BOB 0	Neotropical	CNC, MZUCR
Chalcididae			
Brachymeria sp.		Neotropical	MZUCR
Stypiura dentipes (Fabricius)	BOB 0	Neotropical	MZUCR
Eupelmidae			
Brasema sp.	BOB 0	Neotropical	MZUCR
Eurytomidae			
Aximopsis masneri Gates	BOB 9	Neotropical	MZUCR
Bephrata flava Gates & Hanson	BOB 0	Neotropical	MZUCR
B. ticos Gates & Hanson	BOB 2	Neotropical	MZUCR
Isosomodes azofiefai Gates & Hanson	BOB 0	Neotropical	CNC, MZUCR
I. colombia Gates & Hanson	BOB 9	Neotropical	Gates and Hanson (2009)
Rileya tricolor Gates	BOB 9	Neotropical	MZUCR
Pteromalidae			
Lelaps sp.	BOB 0	Neotropical	MZUCR

metadecoris and *Zaglyptomorpha cornuta* where the mesoscutum varies from entirely orange to almost entirely black (Godoy and Gauld 2002). Many Banchinae, Pimplinae, and Tryphoninae show a BOB-like pattern but the mesosoma is reddish brown instead of orange and these are not included in the table. Ten Neotropical species of *Stethantyx* (Tersilochinae) (Khalaim and Broad 2013) were also excluded for this reason. Some ichneumonids (e.g., several *Xiphosomella*, Cremastinae) have a BOB pattern in lateral view but not in dorsal view. Also excluded are numerous species having a black-orange-black sequence but where only the anterior part of the metasoma is orange. Just a few of the many examples of this color pattern include some species in the following subfamilies and genera: Banchinae (*Cryptopimpla*, *Glypta*), Campopleginae (*Casinaria*), Cryptinae (*Agrothereutes*, *Aritranis*, *Atractodes*, *Ceratophygadeuon*, *Gambrus*, *Idiolispa*, *Mastrus*, *Mesoleptus*, *Phygadeuon*, *Rhembobius*, *Sphecophaga*, *Theroscopus*, *Thrybius*), and Tersilochinae (*Barycnemis*).

Braconidae

The BOB color pattern was found in species belonging to 13 subfamilies of Braconidae (Table 4). Some species of Agathidinae (*Alabagrus*, *Bassus*, *Pharpa*), Meteorinae, and Rogadinae have a black-orange-black sequence, but the orange is restricted to the anterior segments of the metasoma and often the propodeum as well. Most species of *Alabagrus* have bright color patterns (Leathers and Sharkey 2003), but the majority do not fit the BOB pattern. Intraspecific color variation is present in several braconids. For example, most *Alabagrus ixtilton* Sharkey from Mexico are all black but a few (8%) have an orange mesoscutum (BOB 2 pattern). *Odontobracon janzeni* females vary in coloration, with the mesoscutum usually orange but occasionally partially to entirely black (Marsh 2002).

Evanioidea

Within the superfamily Evanioidea, Aulacidae and Gasteruptiidae were not extensively examined since most are larger than our focal size range. A cursory examination of these two families revealed no BOB coloration as defined here, although some have the base of the metasoma orange with the rest of the body dark colored. On the other hand, BOB coloration was common in Evaniidae, being present in nearly half of the extant genera (Table 5). All genera with species showing this coloration also have entirely dark-colored species, and often species with some other type of black-orange combination.

Aculeata

The BOB color pattern was found in species belonging to 10 families of aculeates (Table 6). Many aculeates are larger than the size range included in this study. Nonetheless, BOB coloration appears to be scarce in groups such as Scoliidae and Vespidae. Although many Pompilidae are also larger than our focal size range, it is notable that when orange coloration is present it is often just on the anterior part of the metasoma, which results in a black-orange-black sequence but with the mesosoma mostly to entirely black. A similar pattern can be seen in a few other groups: some *Ammophila* and *Podalonia* (Sphecidae); a few Crabronidae, such as some *Mimesa* (Pemphredoninae), *Miscophus*, *Tachysphex* (Larrinae), *Didineis* and *Harpactus* (Nyssoninae); a few *Andrena* (Andrenidae); and most *Sphecodes* (Halictidae) (BWARS 2016).

In the superfamily Chrysidoidea the greatest number of species showing BOB coloration was found in chrysidids belonging to the subfamilies Cleptinae and Amiseginae (Table 6), although the black coloration in these species often includes some metallic reflections. It appears that a majority of *Cleptidea* species have some form of BOB coloration (Kimsey 1986; only a few examples are included in Table 6) and their color pattern is often complemented by banded wings. We observed several dryinids with orange coloration, but relatively few conformed to the BOB pattern. Orange coloration appears to be extremely scarce in Bethylidae although some apterous females of *Sclerodermus domesticus* approach the BOB pattern.

Orange to red coloration is very common in female Mutillidae although there are a diversity of patterns and their size ranges from 2 to 25 mm. BOB coloration is found in several species of *Timulla*, as well as some *Ephuta*, *Darditilla*, *Dasymutilla*, *Hoplocrates*, *Horcomutilla*, *Lynchiatilla*, *Pertyella*, *Pseudomethoca*, *Ptilomutilla*, and *Xystromutilla*. Species in these genera showing a BOB pattern usually have pubescent or tegumentary markings (white, yellow, orange-red) on the metasoma, especially the second tergite. BOB coloration appears to be less common in male Mutillidae although it is found, for example, in both sexes of the Palearctic *Mutilla europaea* and *Smicromyrme rufipes* (BWARS 2016).

Table 3. Species of Ichneumonidae with BOB color patterns

Species	Color	Distribution	Reference
Banchinae			
Apophua schoutedeni (Benoit)	BOB 0	Afrotropical	Van Noort (2017)
Cryptopimpla hantami[a]	BOB 0	Afrotropical	Reynolds Berry and van Noort (2016)
C. rubrithorax[a]	BOB 0	Afrotropical	Reynolds Berry and van Noort (2016)
C. zwarti[a]	BOB 0	Afrotropical	Reynolds Berry and van Noort (2016)
Glypta cuericiensis[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
G. geoginensis[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
G. metadecoris[a]	BOB 0	Neotropical	Godoy and Gauld (2002)
G. punctata[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
G. tumifrons[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Zaglyptomorpha bella[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Z. cornuta[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Z. gabrieli[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Z. hebeae[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Z. pediflava[a]	BOB 9	Neotropical	Godoy and Gauld (2002)
Cremastinae			
Pristomerus mexicanus Cresson	BOB 0	Neotropical	Gauld (2000)
Trathala flaca[a]	BOB 0	Neotropical	CNC, Gauld (2000)
T. gifa[a]	BOB 0	Neotropical	Gauld (2000)
T. henryi[a]	BOB 9	Neotropical	Gauld (2000)
T. hora[a]	BOB 9	Neotropical	Gauld (2000)
T. paula[a]	BOB 9	Neotropical	Gauld (2000)
Cryptinae			
Apotemnus truncatus Cushman	BOB 9	Neotropical	CNC
Aritranis sp.	BOB 0	Palearctic	CNC
Astomaspis violaceipennis (Cameron)	BOB 9	Afrotropical	Van Noort (2017)
Bathythrix sp.	BOB 2	Nearctic	CNC
Diapetimorpha sp.	BOB 11	Neotropical	CNC
Diracela latifasciata (Cameron)	BOB 0	Afrotropical	Van Noort (2017)
Gelis apterus (Pontoppidan) female	BOB 0	Palearctic	Korenko et al. (2013)
Madastenus nigrinotus Seyrig	BOB 0	Afrotropical	CNC
Polycyrtus condylobus[a]	BOB 5	Neotropical	Zúñiga Ramírez (2004)
P. duplaris[a]	BOB 5	Neotropical	Zúñiga Ramírez 2004
P. latigulus[a]	BOB 5	Neotropical	Zúñiga Ramírez (2004)
P. luisi[a]	BOB 5	Neotropical	Zúñiga Ramírez (2004)
P. nanii[a]	BOB 5	Neotropical	Zúñiga Ramírez (2004)
Ichneumoninae			
Jacotitypus sp.	BOB 0	Afrotropical	CNC
Joppa sp.	BOB 0, 7	Neotropical	CNC
Pimplinae			
Acrotaphus chedelae Gauld	BOB 5	Neotropical	MNCR
A. fasciatus (Brullé)	BOB 5	Neotropical	Gauld (1991)
A. franklini Gauld	BOB 11	Neotropical	MNCR
A. latifasciatus (Cameron)	BOB 5	Neotropical	MNCR
A. tibialis (Cameron)	BOB 5	Neotropical	CNC, MNCR
Calliephialtes grapholithae (Cresson)	BOB 9	Nearctic	CNC
C. guevarae[a]	BOB 11	Neotropical	Gauld (1991)
C. ledezmae[a]	BOB 0	Neotropical	Gauld et al. (1998)
Clydonium moragai[a]	BOB 9	Neotropical	Gauld et al. (1998)
Polysphincta janzeni[a]	BOB 9	Neotropical	Gauld (1991)
Tryphoninae			
Boethus taeniatus Townes & Gupta	BOB 0	Neotropical	Gauld (1997)
Oedemopsis cyranoi[a]	BOB 9	Neotropical	Gauld (1997)
O. dentipara[a]	BOB 9	Neotropical	Gauld (1997)
O. noyesi[a]	BOB 9	Neotropical	Gauld (1997)
O. ojoa[a]	BOB 0	Neotropical	Gauld (1997)
O. quemadoi[a]	BOB 9	Neotropical	CNC, Gauld (1997)
O. riyitoi[a]	BOB 0, 9	Neotropical	Gauld (1997)

[a]Author names same as reference.

Table 4. Species of Braconidae with BOB color patterns

Subfamily, species	Color	Distribution	Reference
Agathidinae			
Aerophilus vaughntani (Sharkey)	BOB 2	Neotropical	Sharkey et al. (2011)
Agathacrista depressifera (van Achterberg & Long)	BOB 2	Indo-Malayan	Sharkey and Stoelb (2013)
Bassus calculator (Fabricius)	BOB 9	Palearctic	CNC
B. ebulus (Nixon)	BOB 9	Indo-Malayan	CNC
Braunsia fumipennis (Cameron)	BOB 9	Indo-Malayan	Sharkey and Clutts (2011)
Cremnops violaceipennis (Cameron)	BOB 10	Neotropical	Tucker et al. (2015)
Euagathis ophippium (Cameron)	BOB 0	Indo-Malayan	van Achterberg and Long (2010)
Zelodia anginota[a]	BOB 9	Indo-Malayan	van Achterberg and Long (2010)
Zelomorpha similis (Szépligeti)	BOB 5	Neotropical	Sarmiento-Monroy (2006)
Alysiinae			
Gnathopleura sp.	BOB 5	Neotropical	MNCR
Phaenocarpa sp.	BOB 0	Neotropical	CNC
Brachistinae			
Eubazus sp.	BOB 0	Nearctic	CNC
Eubazus sp.	BOB 9	Neotropical	MNCR
Nealiolus sp.	BOB 0	Neotropical	CNC
Braconinae			
Aphrastobracon biroi (Szépligeti)	BOB 0	Australasian	CNC
Bracon campyloneurus Szépligeti	BOB 0	Afrotopical y Australasian	CNC
Calobracon sp.	BOB 0	Nearctic	CNC
Compsobracon sp.	BOB 9	Neotropical	MZUCR
Cyanopterus sp.	BOB 0	Neotropical	CNC
Digonogastra sp.	BOB 0	Neotropical	CNC
Gracilibracon sp.	BOB 0, 1	Neotropical	MZUCR
Megabracon sp.	BOB 5	Neotropical	MNCR
Pycnobraconoides mutator (Fabricius)	BOB 0	Australasian	CNC
Cardiochilinae			
Cardiochiles fallax Kokujev	BOB 9	Palearctic	Farahani et al. (2015)
Cardiochiles sp.	BOB 2	Neotropical	MZUCR
Toxoneuron leve (Mao)	BOB 9	Nearctic	CNC
Cenocoelinae			
Capitonius pulcher (Cameron)	BOB 5	Neotropical	CNC, MNCR
C. tricolorvalvus Ent	BOB 0	Neotropical	MNCR, MZUCR
Cenocoelius sp.	BOB 0	Neotropical	CNC
Charmontinae			
Charmon cruentatus Haliday	BOB 0	Holarctic	CNC
C. extensor (Linnaeus)	BOB 0	Holarctic	CNC
Cheloninae			
Leptodrepana atalanta Dadelahi & Shaw	BOB 0	Neotropical	Dadelahi et al. (2018)
L. conda Dadelahi & Shaw	BOB 9	Neotropical	Dadelahi et al. (2018)
L. conleyae Dadelahi & Shaw	BOB 0	Neotropical	Dadelahi et al. (2018)
L. demeter Dadelahi & Shaw	BOB 0	Neotropical	Dadelahi et al. (2018)
L. lorenae Dadelahi & Shaw	BOB 9	Neotropical	Dadelahi et al. (2018)
L. munjuanae Dadelahi & Shaw	BOB 9	Neotropical	Dadelahi et al. (2018)
L. ninae Dadelahi & Shaw	BOB 9	Neotropical	Dadelahi et al. (2018)
L. schuttei Dadelahi & Shaw	BOB 9	Neotropical	Dadelahi et al. (2018)
L. scottshawi Dadelahi	BOB 0	Neotropical	Dadelahi et al. (2018)
L. stasia Dadelahi & Shaw	BOB 0	Neotropical	Dadelahi et al. (2018)
Microchelonus rubescens[a]	BOB 0	Neotropical	Papp (2010)
M. ruficollis Viereck	BOB 9	Neotropical	Papp (2010)
Doryctinae			
Gymnobracon megistus Marsh	BOB 0	Neotropical	CNC, MNCR
Megaloproctus strongylogaster (Cameron)	BOB 5	Neotropical	MNCR
Odontobracon batesi Roman	BOB 0	Neotropical	CNC
O. janzeni Marsh	BOB 5	Neotropical	MNCR
Pedinotus columbianus Enderlein	BOB 0	Neotropical	CNC
Macrocentrinae			
Macrocentrus bicolor Curtis	BOB 0	Palearctic	van Achterberg (1993)
Meteorinae			
Meteorus sp.	BOB 0	Nearctic	CNC
Orgilinae			
Orgilus sp.	BOB 9	Neotropical	MZUCR
Rogadinae			
Aleiodes lucidus (Szépligeti)	BOB 0	Neotropical	Shimbori and Penteado-Dias (2011)
A. melanopterus (Erichson)	BOB 0	Neotropical	CNC
A. shaworum[a]	BOB 10	Neotropical	Shimbori and Penteado-Dias (2011)

[a]Author names same as reference.

Table 5. Species of Evaniidae with BOB color patterns

Species	Color	Distribution	Reference
Acanthinevania clavaticornis (Kieffer)	BOB 0	Australasian	Deans et al. (2017)
Evania stenochela Kieffer	BOB 0	Palearctic	Deans et al. (2017)
Evaniella erythraspis (Cameron)	BOB 6	Neotropical	Deans et al. (2017)
E. nana (Schletterer)	BOB 9	Neotropical	Deans et al. (2017)
E. nobilis (Westwood)	BOB 0	Neotropical	Deans et al. (2017)
E. ruficornis (Fabricius)	BOB 0	Neotropical	Deans et al. (2017)
E. rufosparsa (Kieffer)	BOB 9	Neotropical	Deans et al. (2017)
E. semaeoda (Bradley)	BOB 9	Nearctic	CNC
Evaniscus rufithorax Enderlein	BOB 9	Neotropical	Mullins et al. (2012)
Hyptia chalcidipennis (Enderlein)	BOB 0, 9	Neotropical	Deans et al. (2017)
H. peruanus (Enderlein)	BOB 9	Neotropical	Deans et al. (2017)
H. reticulata (Say)	BOB 0	Nearctic	CNC
H. rufipectus Dewitz	BOB 0, 9	Neotropical	Deans et al. (2017)
H. rufipes (Fabricius)	BOB 9	Neotropical	Deans et al. (2017)
H. stimulata (Schletterer)	BOB 0	Neotropical	Deans et al. (2017)
Parevania kriegeriana (Enderlein)	BOB 0	Indo-Malayan	Deans et al. (2017)
P. micholitzi (Enderlein)	BOB 9	Indo-Malayan	Deans et al. (2017)
Prosevania erythrosoma (Schletterer)	BOB 0	Afrotropical	Ramage and Martiré (2016)
P. lombokiensis (Szépligeti)	BOB 9	Indo-Malayan	Deans et al. (2017)
P. rufoniger (Enderlein)	BOB 0, 9	Indo-Malayan	Deans et al. (2017)
P. sauteri (Enderlein)	BOB 0	Indo-Malayan	Deans et al. (2017)
P. tricolor (Szépligeti)	BOB 0	Indo-Malayan	Deans et al. (2017)
Semaeomyia magnus (Enderlein)	BOB 9	Neotropical	Deans et al. (2017)
S. pygmaea (Fabricius)	BOB 9	Neotropical	Deans et al. (2017)
S. reticulifer (Enderlein)	BOB 9	Neotropical	Deans et al. (2017)
Szepligetella formosa (Kieffer)	BOB 0	Australasian	Deans et al. (2017)
Zeuxevania lamellata Benoit	BOB 9	Afrotropical	Deans et al. (2017)

Among ants (Formicidae), BOB coloration is relatively uncommon (Table 6), occurring primarily in Myrmeciinae (*Myrmecia*) and Formicinae (a few *Camponotus* and *Formica*). Examples of *Myrmecia* species showing BOB coloration include *M. aberrans*, *M. cephalotes*, *M. desertorum*, *M. fuscipes*, *M. nigriceps*, *M. nigrocincta*, *M. nobilis*, and *M. swalei*. In addition to an orange mesosoma, many of these ants often have the petiole and postpetiole orange colored as well, and some (e.g., *M. nigrocincta*) have black in the middle of the mesosoma, resulting in a BOBOB pattern. In the Neotropical region the most common ant showing BOB coloration is *Pseudomyrmex gracilis* (Pseudomyrmecinae), although the coloration is highly variable, ranging from all black to predominantly orange.

There are very few examples of BOB coloration in Apoids, a group that includes Heterogynaeidae, Ampulicidae, Sphecidae, Crabronidae, and bees. Moreover, there is often intraspecific variation in those that do have this color pattern (Table 6). Most *Incastigmus* are predominantly black but a few have BOB coloration: some females of *I. hexagonalis*, and females and some males of *I. pyrrhopyris*. Two other species, *I. ignithorax* Finnamore and *I. thoracicus* Finnamore, show similar intraspecific variation but are yellow-orange instead of red-orange (Finnamore 2002). A few bees, for example, *Andrena clarkella* (Kirby) and *A. thoracica* (Fabricius), have reddish hairs on the mesosoma (BWARS 2016), but these were not included.

Sawflies

Our examination of sawflies was less thorough than in other groups and was limited to three families in the New World (Table 7). Intraspecific variation occurs in at least some species, sometimes with males being all black (e.g., *Scobina dorsalis*), and in some cases females vary in color, as in *Scobina lepida* (Klug) and *S. melanocephala* (Lepeletier) (Smith 1992). In *Perreya tropica* some males have just the mesoscutum orange (especially at higher elevations) while in others the entire thorax and abdomen is orange; females have both the thorax and abdomen orange, but the dark wings cover the abdomen.

Discussion

We found BOB coloration in 23 families of Hymenoptera, and in many of the subfamilies of Ichneumonidae and Braconidae. Due to lack of revisionary taxonomic studies, quantification of the proportion of species having this coloration in each family is not currently possible. Nonetheless, our preliminary compilation suggests that BOB coloration is very infrequent in certain taxa (e.g., Cynipoids, Diaprioids, Chalcidoids, and Apoids) and quite common in others (Scelioninae, Evaniidae, and female Mutillidae). As noted in the introduction, the proportion of species showing a BOB pattern, in scelionid genera where this color is present, ranges from about 90% in *Chromoteleia* to 15% in *Macroteleia* (Valerio et al. 2013). As more taxonomic revisions become available it will become possible to expand these data; for example, 10 of the 24 Costa Rican species of *Leptodrepana* (Braconidae) (Dadelahi et al. 2018).

The preliminary nature of our survey as well as the lack of phylogenies for most of the taxa preclude estimating the number of times BOB coloration has evolved. Nonetheless, the widespread occurrence of this color pattern strongly suggests that it has arisen on numerous occasions, which in turn suggests that it has some biological function. It seems unlikely that this color is used in intersexual communication since both sexes usually had the same color, and most of the intraspecific variation we observed included color

Table 6. Species of Aculeata (excluding Mutillidae) with BOB color patterns

Species	Color	Distribution	Reference
Chrysididae			
Cleptidea balboana Kimsey	BOB 9	Neotropical	MZUCR
C. janzeni Kimsey	BOB 9	Neotropical	MZUCR
C. panamensis Kimsey	BOB 9	Neotropical	MZUCR
Adelphe masneri Kimsey	BOB 0	Neotropical	CNC
Alieniscus: both of the two spp.	BOB 0	Afrotropical	Van Noort (2017)
Anadelphe alvarengai[a]	BOB 9	Neotropical	Kimsey (1987)
Atoposega: all six spp.	BOB 9	Indo-Malayan	Kimsey (2014)
Mahinda sulawesiensis[a]	BOB 0	Indo-Malayan	Kimsey et al. (2016)
Sclerogibbidae			
Sclerogibba talpiformis Benoit female	BOB 0	Afrotropical	Van Noort (2017)
Dryinidae			
Dryinus collaris (Linnaeus)	BOB 9	Palearctic	BWARS (2016)
Rhopalosomatidae			
Olixon myrmosaeforme (Arnold)	BOB 0	Afrotropical	Van Noort (2017)
Thynnidae			
Methocha articulata Latreille female	BOB 0	Palearctic	Agnoli (2011)
Pompilidae			
Aegeniella sp.	BOB 0	Neotropical	CNC
Agenioideus rubicundus Evans	BOB 0	Nearctic	CNC
Balboana sp.	BOB 0	Neotropical	CNC
Dipogon iracundus Townes	BOB 0	Nearctic	CNC
Epipompilus aztecus (Cresson)	BOB 2	Neotropical	MZUCR
E. delicatus Turner	BOB 0	Neotropical	MZUCR
Bradynobaenidae			
Gynecaptera bimaculata (André) female	BOB 0	Palearctic	Romano (2011)
Formicidae			
Camponotus nigriceps (Smith)	BOB 5	Australasian	AntWeb (2018)
C. vicinus Mayr	BOB 5	Nearctic	AntWeb (2018)
Formica rufa Linnaeus	BOB 5	Palearctic	AntWeb (2018)
Dilobocondyla fouqueti Santschi	BOB 5	Indo-Malayan	AntWeb (2018)
Myrmecia spp.	BOB 5	Australasian	AntWeb (2018)
Psudomyrmex gracilis (Fabricius)	BOB 0	Neotropical	MZUCR
Temnothorax isabellae (Wheeler)	BOB 5	Neotropical	AntWeb (2018)
Heterogynaidae			
Heterogyna saudita Gadallah & Soliman female	BOB 0	Palearctic	Van Noort (2017)
Crabronidae			
Alysson tricolor Lepeletier & Serville female	BOB 0	Palearctic	CNC
Incastigmus hexagonalis (Fox) female	BOB 9	Neotropical	Finnamore (2002)
I. pyrrhopyris[a]	BOB 0	Neotropical	Finnamore (2002)
Stigmus sp.	BOB 9	Neotropical	CNC, MZUCR
Trypoxylon sp.	BOB 9	Neotropical	MZUCR

For Mutillidae and *Myrmecia* (Formicidae) see text.
[a]Author names same as reference.

variation within the same sex. Among the few cases of intersexual color variation were in groups where females are apterous and males are winged (e.g., Mutillidae and *Methocha*), and in these cases only females show BOB coloration.

The most likely function of BOB coloration is aposematism (warning coloration) since contrasting orange and black color patterns are known to be aposematic in other insects, for example, ladybird beetles (Coleoptera: Coccinellidae) (María Arenas et al. 2015). It is also possible that at least some of the taxa showing BOB coloration are mimicking ants, for example, *P. gracilis*. While this species is restricted to the New World, it could be argued that other ants serve as models in other regions (Table 6), for example, *Mymecia* species in Australia and *Formica rufa* in the Palearctic region. There are, however, other possible models, namely female Mutillidae (see 'black-headed *Timulla*' mimicry ring in Wilson et al. 2015). Many female mutillids showing the BOB pattern also have lateral white spots on the metasoma, a pattern that also occurs in several other taxa we examined, for example, *Bephrata* and *Isosomodes* (Eurytomidae), and *Cleptidea* (Chrysididae); in *Leptodrepana* (Braconidae) there is often a central white spot on the first tergite. If BOB coloration in nonaculeate hymenopterans involves mimicry, it remains to be seen what proportion of these are Batesian mimics (only the model is distasteful) versus Mullerian mimics (both model and mimic are distasteful).

Four factors have been speculated to be correlated with the prevalence of BOB coloration (Masner 1988, Masner and Hanson 2006): insect size, habitat, altitudinal distribution, and geographic distribution. With respect to size, BOB coloration does indeed appear to be especially common in hymenopterans with a body length between 3 and 10 mm, although we included species up to 20 mm in length. Outside the 3–20 mm size range, there were examples of BOB coloration in both smaller specimens (among neotropical scelionids:

Table 7. Species of sawflies with BOB color patterns

Species	Color	Distribution	Reference
Argidae-Arginae			
Arge pectoralis (Leach)	BOB 9	Neartic	CNC
A. quidia Smith	BOB 9	Neartic	CNC
A. scapularis Klug	BOB 9	Neartic	CNC
Scobina dorsalis (Klug) female	BOB 9	Neotropical	MZUCR
Argidae-Atomacerinae			
Atomacera decepta Rohwer	BOB 9	Neartic	CNC
A. debilis Say	BOB 2	Neartic	CNC
A. ebena Smith	BOB 9	Neotropical	MZUCR
A. lepidula (Konow)	BOB 9	Neotropical	MZUCR
Argidae-Erigleninae			
Sericoceros gibbus (Klug)	BOB 9	Neotropical	MNCR
Argidae-Sterictiphorinae			
Acrogymnia palama Smith	BOB 9	Neotropical	MZUCR
Durgoa sp.	BOB 9	Neotropical	CNC
Hemidianeura leucopoda Smith	BOB 9	Neotropical	CNC, MZUCR
Neoptilia malvacearum (Cockerell)	BOB 9	Neartic	CNC
N. xicana Smith	BOB 9	Neotropical	CNC
Pergidae-Perreyiinae			
Decameria similis (Enderlein)	BOB 9	Neotropical	MZUCR
D. varipes Cameron	BOB 9	Neotropical	MZUCR
Perreya tropica (Norton)	BOB 9	Neotropical	CNC, MZUCR
Tenthredinidae-Allantinae			
Eriocampa ovata Linnaeus	BOB 2	Nearctic	Smith (1979)
Phrontosoma brocca Smith	BOB 2	Nearctic	CNC
P. usta Smith	BOB 9	Nearctic	CNC
Tenthredinidae-Blennocampinae			
Waldheimia amazonica (Kirby)	BOB 9	Neotropical	MZUCR
Tenthredinidae-Selandrinae			
Dolerus rufilobus Ross	BOB 9	Neartic	CNC

The BOB 0 pattern is not possible in sawflies since they lack a propodeum; what appears as BOB 0 is actually BOB 9.

Laphita, *Macroteleia*, *Tyrannoscelio*, *Probaryconus*) and larger specimens (several Mutillidae and Ichneumonidae), but our impression is that BOB coloration is most prevalent in the size range mentioned above. However, quantitative analyses are required to examine this question in greater detail.

Masner (1988) suggested that BOB coloration is most prevalent among species that inhabit low vegetation, between 1 and 2 m high. Although data on collecting techniques were generally not available and we did not quantify what little was available, there did appear to be more specimens from Malaise traps and screen-sweeping, and fewer from pan traps and other ground-based techniques, but this requires confirmation. The biology of the taxa showing BOB coloration (Tables 1–7) encompasses both ecto- and endoparasitoids, idiobionts and koinobionts, from a diversity of hosts, as well as phytophagous sawflies. It is interesting that egg parasitoids (scelionids, evaniids, amisegine chrysidids) are especially well represented, but more research is needed to determine whether they are in fact proportionately better represented than parasitoids of larvae and pupae.

With regard to the altitudinal distribution of BOB coloration, the vast majority of specimens showing this color pattern were collected below 2,000 m, as has been previously observed (Masner and Hanson 2006). In a few cases we were able to examine specimens collected at altitudes ranging from sea level to 5,000 m. For example, *Triteleia* specimens with BOB coloration were common in the lowlands, however, entirely black specimens were found in higher altitudes such as Cotopaxi in Ecuador (5,000 m), Sierra Nevada in Spain (3,200 m), and Chiapas in Mexico (4,000 m). On the other hand, two specimens (less than 3 mm in length) of *Probaryconus* showing BOB coloration (one with BOB 9) were collected at higher altitudes in Ecuador, one from Napo at 3,000 m and the other from Oyacachi at 3,190 m. In species showing intraspecific color variation there is often a tendency for specimens from higher elevations to be darker. For example, in Costa Rica an unidentified species of *Lapitha* shows typical BOB coloration in the lowlands, but at higher altitudes (above about 1,300 m) specimens become darker, with a black propodeum (BOB 10) and a darker mesoscutum. Although these altitudinal trends merit further investigation with additional taxa, the scarcity of BOB coloration at higher altitudes appears to be a real pattern, but the reason (e.g., temperature, UV radiation, predators) for this pattern is unknown.

Although it has previously been suggested that BOB coloration occurs mostly in the Neotropics (Masner 1988, Masner and Hanson 2006), our results show that this color pattern is found in all biogeographic regions. Although it is possible that this color pattern is more frequent in the Neotropics, at least among scelionids, our data are insufficient to substantiate this possibility.

While BOB coloration was previously known to occur in Hymenoptera, especially in Scelioninae, our results demonstrate that it is much more widespread than previously realized. Although this color pattern occurs in other insects, we are not aware of any systematic surveys. In addition to extending the survey to other groups of insects, potential research questions for the future include the following. First, the fact that some observers see the mesosoma as orange, while others see it as red, demonstrates the need for spectrophotometric analyses. Moreover, it would be useful to compare scelionids with taxa such as agathidine braconids, where the orange color appears to be slightly different. Second, to the best of our knowledge,

Mutillidae is the only group in which the physical/chemical basis of BOB coloration has been examined, namely orange pheomelanins and black eumelanins (Hines et al. 2017); similar studies are needed in the other groups, especially scelionids. Third, future studies should include other black and orange patterns, for example, where only the base of the metasoma is orange, and bicolored species. In some cases wing coloration contributes to the color pattern; for example, in *Cardiochiles nigriceps* Viereck (Braconidae) both the mesosoma and metasoma are reddish, but when the black wings cover the metasoma it has a black appearance. Fourth, it would be useful to examine in greater detail species that show intraspecific variation in color. Finally, and perhaps most importantly, in order to address the question of the function of this widespread color pattern, feeding trials with potential predators of species listed in Tables 1–7 are needed to determine whether this color pattern is indeed aposematic. Similarly, the possible presence of repugnatorial glands in species showing BOB coloration needs to be examined, and compared with closely related species that lack this color pattern (for example, completely black species).

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References Cited

van Achterberg, C. 1993. Revision of the subfamily Macrocentrinae Foerster (Hymenoptera: Braconidae) from the Palaearctic region. Zool. Verhand. Leiden. 286: 1–110.

van Achterberg, C., and K. D. Long. 2010. Revision of the Agathidinae (Hymenoptera, Braconidae) of Vietnam, with the description of forty-two new species and three new genera. ZooKeys. 54: 1–184.

Agnoli, G. L. 2011. *Methocha*, interim version. http://www.chrysis.net/methocha/ (accessed 30 August 2018).

AntWeb. 2018. Available from:https://www.antweb.org/description.do?-family=formicidae&rank=family (accessed 30 August 2018).

Branstetter, M., B. Danforth, J. Pitts, B. Faircloth, P. Ward, M. Buffington, M. Gates, R. Kula, and S. Brady. 2017. Phylogenomic analysis of ants, bees and stinging wasps: improved taxon sampling enhances understanding of hymenopteran evolution. Curr. Biol. 27: 1019–1025.

BWARS. 2016. Bees, wasps & ants recording society. www.bwars.com (accessed 3 November 2017).

Chen, H., E. J. Talamas, A. A. Valerio, L. Masner, and N. F. Johnson. 2018. Revision of the world species of the genus *Chromoteleia* Ashmead (Hymenoptera, Platygastridae, Scelioninae). ZooKeys. 778: 1–95.

Dadelahi, S. D., S. R. Shaw, H. Aguirre, and L. F. V. Almeida. 2018. A taxonomic study of Costa Rican *Leptodrepana* with the description of twenty-four new species (Hymenoptera, Braconidae, Cheloninae). ZooKeys. 750: 59–130.

Deans, A. R., M. J. Yoder, and K. Dole. 2017. Evanioidea online catalog of information about evanioid wasps (Hymenoptera). http://evanioidea.info (accessed 28 October 2017).

Dotseth, E. J., and N. F. Johnson. 2001. Revision of the Neotropical genus *Acanthoscelio* (Hymenoptera: Scelionidae). Can. Entomol. 133: 487–507.

Fabricant, S. A., and M. E. Herberstein. 2015. Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. Behav. Ecol. 26: 38–44.

Farahani, S., A. A. Talebi, and E. Rakhshani. 2015. Study of the genus Cardiochiles (Hymenoptera: Braconidae: Cardiochilinae) in Tehran and Alborz provinces, with one new record from Iran. Proceedings of 1st Iranian International Congress of Entomology. 19–25.

Finnamore, A. T. 2002. Revision of the world genera of tribe Stigmini (Hymenoptera: Apoidea: Crabronidae: Pemphredonnae), Part 2. Species of *Incastigmus* Finnamore. J. Hymenopt. Res. 11: 12–71.

Gates, M. W., and P. E. Hanson. 2009. A revision of *Bephrata* and *Isosomodes* (Hymenoptera: Eurytomidae). J. Hymenopt. Res. 18: 25–73.

Gauld, I. D. 1991. Subfamily Pimplinae. Mem. Am. Entomol. Inst. 47: 135–530.

Gauld, I. D. 1997. Subfamily Tryphoninae. Mem. Am. Entomol. Inst. 57: 330–428.

Gauld, I. D. 2000. Subfamily Cremastinae. Mem. Am. Entomol. Inst. 63: 35–315.

Gauld, I. D., G. Ugalde, and P. E. Hanson. 1998. Guía de los Pimplinae de Costa Rica (Hymenoptera: Ichneumonidae). Rev. Biol. Trop. 46(Suppl. 1): 1–189.

Godoy, C., and I. D. Gauld. 2002. Tribe Glyptini. Mem. Am. Entomol. Inst. 66: 666–743.

Hines, H. M., P. Witkowski, J. S. Wilson, and K. Wakamatsu. 2017. Melanic variation underlies aposematic color variation in two hymenopteran mimicry systems. PLoS One. 12: e0182135.

Johnson, N. F., and L. Musetti. 2011. Redescription and revision of the Neotropical genus *Pseudoheptascelio* Szabó (Hymenoptera, Plattygastridae, Scelioninae), parasitoids of eggs of short-horned grasshoppers (Orthoptera, Acrididae). ZooKeys. 136: 93–112.

Jorge García, A., C. Polidori, and J. L. Nieves-Aldrey. 2016. Pheomelanin in the secondary sexual characters of male parasitoid wasps (Hymenoptera: Pteromalidae). Arthropod Struct. Dev. 45: 311–319.

Khalaim, A. I., and G. R. Broad. 2013. Tersilochinae (Hymenoptera: Ichneumonidae) of Costa Rica, part 2. Genera *Megalochus* gen. nov. and *Stethantyx* townes. Zootaxa. 3693: 221–266.

Kimsey, L. S. 1986. *Cleptidea* revisited (Hymenoptera, Chrysididae). J. Kans. Entomol. Soc. 59: 314–324.

Kimsey, L. S. 1987. New genera and species of neotropical Amiseginae (Hymenoptera, Chrysididae). Psyche. 94: 57–76.

Kimsey, L. S. 2014. Reevaluation of the odd chrysidid genus *Atoposega* Krombein (Hymenoptera, Chrysididae, Amiseginae). ZooKeys. 409: 35–47.

Kimsey, L. S., T. Mita, and H. T. Pham. 2016. New species of the genus *Mahinda* Krombein, 1983 (Hymenoptera, Chrysididae, Amiseginae). ZooKeys. 551: 145–154.

Korenko, S., S. Schmidt, M. Schwarz, G. A. P. Gibson, and S. Pekar. 2013. Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae, Zodariidae). Zookeys. 262: 1–15.

Leathers, J. W., and M. J. Sharkey. 2003. Taxonomy and life history of Costa Rican *Alabagrus* (Hymenoptera: Braconidae), with a key to world species. Contr. Sci. Nat. Hist. Mus. Los Angeles County. 497: 1–82.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Ann. Rev. Ecol. Syst. 30: 201–233.

Marchini, M., D. Sommaggio, and A. Minelli. 2016. Playing with black and yellow: the evolvability of a Batesian mimicry. Evol. Biol. 44: 100–112.

María Arenas, L., D. Walter, and M. Stevens. 2015. Signal honesty and predation risk among a closely related group of aposematic species. Sci. Rep. 5: 11021.

Marsh, P. M. 2002. The Doryctinae of Costa Rica (excluding the genus *Heterospilus*). Mem. Am. Entomol. Inst. 70: 1–319.

Masner, L. 1988. Convergent chromatic mimicry among some Neotropical Hymenoptera: a search for the model, pp. 12. *In* Proceedings XVIII International Congress of Entomology, 3–9 July 1988, Vancouver, BC, Canada. Abstracts.

Masner, L., and P. E. Hanson. 2006. Familia Scelionidae. Mem. Am. Entomol. Inst. 77: 254–265.

Mullins, P. L., R. Kawada, J. P. Balhoff, and A. R. Deans. 2012. A revision of *Evaniscus* (Hymenoptera, Evaniidae) using ontology-based sematic pehnotype annotation. ZooKeys. 223: 1–38.

Murphy, N. P., D. Carey, L. R. Castro, M. Dowton, and A. D. Austin. 2007. Phylogeny of the platygastroid wasps (Hymenoptera) based on sequences

from the 18S rRNA, 28S rRNA and cytochrome oxidase I genes: implications for the evolution of the ovipositor system and host relationships. Bio. J. Linnean Soc. 91: 653–669.

Papp, J. 2010. Ten new *Microchelonus* Szépligeti species from the Neotropical region (Hymenoptera, Braconidae: Cheloninae). Ann. Hist.-Nat. Mus. Natl. Hung. 102: 155–191.

Peters, R. S., L. Krogmann, C. Mayer, A. Donath, S. Gunkel, K. Meusemann, A. Kozlov, L. Podsiadlowski, M. Petersen, R. Lanfear, et al. **2017**. Evolutionary history of the Hymenoptera. Curr. Biol. 27: 1013–1018.

Ramage, T., and D. Martiré. 2016. A new evaniid wasp for Reunion Island (Hymenoptera, Evaniidae). Cah. Sci. Océan Indien Occident. 7: 15–18.

Reynolds Berry, T., and S. van Noort. 2016. Review of Afrotropical *Cryptopimpla* Taschenberg (Hymenoptera, Ichneumonidae, Banchinae), with description of nine new species. ZooKeys. 640: 103–137.

Romano, M. 2011. *Gynecaptera bimaculata* (André, 1898), ♀ - Bradynobaenidae Apterogyninae. http://www.entomologiitaliani.net/public/forum/phpBB3/viewtopic.php?f=11&t=25664 (accessed 30 August 2018).

Ros-Farré, P., and J. Pujade-Villar. 2009. Revision of the genus *Callaspidia* Dahlbom, 1842 (Hym: Figitidae: Aspicerinae). Zootaxa. 2105: 1–31.

Sarmiento-Monroy, C. E. 2006. Taxonomic revision of *Zelomorpha* Ashmead, 1900 and *Hemichoma* Enderein, 1920 (Hymenoptera: Braconidae: Agathidinae) with a phylogenetic analysis of color patterns. Ph.D. dissertation, University of Lexington, Lexington, KY.

Schroeder, T. B. H., J. Houghtaling, B. D. Wilts, and M. Mayer. 2018. It's not a bug, it's a feature: functional materials in insects. Adv. Mater. 30: e1705322.

Sharkey, M. J. 2007. Phylogeny and classification of Hymenoptera. Zootaxa. 1668: 521–548.

Sharkey, M. J., and S. A. Clutts. 2011. A revision of Thai Agathidinae (Hymenoptera: Braconidae), with descriptions of six new species. J. Hymenopt. Res. 22: 69–132.

Sharkey, M. J., and S. A. C. Stoelb. 2013. Revision of *Agathacrista* new genus (Hymenoptera, Braconidae, Agathidinae, Agathidini). J. Hymenopt. Res. 33: 99–112.

Sharkey, M. J., S. Clutts, E. M. Tucker, D. Janzen, W. Hallwachs, T. Dapkey, and M. A. Smith. 2011. *Lytopylus* Förster (Hymenoptera, Braconidae, Agathidinae) species from Costa Rica, with an emphasis on specimens reared from caterpillars in Area de Conservación Guanacaste. ZooKeys. 130: 379–419.

Shimbori, E. M., and A. M. Penteado-Dias. 2011. Taxonomic contribution to the *Aleiodes melanopterus* (Erichson) species-group (Hymenoptera, Braconidae, Rogadinae) from Brazil. ZooKeys. 142: 15–25.

Skelhorn, J., and C. Rowe. 2016. Cognition and the evolution of camouflage. Proc. R. Soc. Lond. B Biol. Sci. 283: 20152890.

Skelhorn, J., C. G. Halpin, and C. Rowe. 2016. Learning about aposematic prey. Behav. Ecol. 27: 955–964.

Smith, D. R. 1979. Nearctic sawflies IV. Allantinae: adults and larvae (Hymenoptera: Tenthredinidae). U.S. Dept. Agr. Tech. Bull. 1595: 1–172.

Smith, D. R. 1992. A synopsis of the sawflies (Hymenoptera: Symphyta) of America south of the United States: Argidae. Mem. Am. Entomol. Soc. 39: 1–201.

Stevens, M., and G. D. Ruxton. 2012. Linking the evolution and form of warning coloration in nature. Proc. R. Soc. Lond. B Biol. Sci. 279: 417–426.

Stuart-Fox, D., E. Newton, and S. Clusella-Trullas. 2017. Thermal consequences of colour and near infrared reflectance. Phil. Trans. R. Soc Lond. B Biol Sci. 372: 20160345.

Tucker, E. M., E. G. Chapman, and M. J. Sharkey. 2015. A revision of the New World species of *Cremnops* Förster (Hymenoptera: Braconidae: Agathidinae). Zootaxa. 3916: 1–83.

Valerio, A. A., L. Musetti, and N. F. Johnson. 2013. Poster at the Entomological Society of America (Austin, TX, 10–13 November). Poster entitled "Species of the colorful genus *Chromoteleia* Ashmead (Hymenoptera: Platygastroidea, Platygastridae s.l.)". (Poster #78056).

Van Noort, S. 2017. Wasp web: hymenoptera of the Afrotropical region. www.waspweb.org (accessed 27 October 2017).

Vidal-Cordero, J. M., G. Moreno-Rueda, A. López-Orta, C. Marfil-Daza, J. L. Ros-Santaella, and F. J. Ortiz-Sánchez. 2012. Brighter-colored paper wasps (*Polistes dominula*) have larger poison glands. Front. Zool. 9: 20.

Wilson, J. S., J. P. Jahner, M. L. Forister, E. S. Sheehan, K. A. Williams, and J. P. Pitts. 2015. North American velvet ants form one of the world's largest known Müllerian mimicry complexes. Curr. Biol. 25: R704–R706.

Zúñiga Ramírez, R. J. 2004. The taxonomy and biology of the *Polycyrtus* species (Hymenoptera: Ichneumonidae, Cryptinae) of Costa Rica. Contr. Am. Entomol. Inst. 33: 1–159.

Capítulo 2

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Phenology of an urban population of *Lyssomanes jemineus* Peckham & Wheeler (Araneae: Salticidae) with a list of other jumping spiders from the same Costa Rican site

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Abstract. Jumping spiders were sampled for 24 months in a Costa Rican urban environment and one of the species collected, *Lyssomanes jemineus*, was observed in detail in order to document its phenology in this disturbed habitat. Nine other species of salticids were also identified from the same site.

Keywords. *Bagheera*, *Balmaceda*, *Colonus*, *Corythalia*, *Messua*, *Mexigonus*, *Nagaina*, *Paraphidippus*, San Pedro de Montes de Oca , urban ecosystems

The salticid subfamily Lyssomaninae is comprised of just two genera, *Lyssomanes* Hentz, 1845 and *Chinoscopus* Simon, 1900, both restricted to the New World (Maddison 2015). Nearly a hundred species of Lyssomaninae are currently recognized, most of which occur in tropical forests of the Amazonian region (Logunov 2014; Prószyński 2016; Rubio et al. 2017). There have been relatively few studies of the jumping spider fauna of Costa Rica. To encourage future research on this group, we carried out a year-long inventory in a patch of urban forest located on the campus of the University of Costa Rica. The most abundant species collected, *Lyssomanes jemineus*, was observed in greater detail to determine its phenology in this disturbed habitat.

Methods. We collected salticid adults and juveniles monthly from January 2017 to December 2018. For *L. jemineus*, egg sacs were also collected. Collecting was done on Finca 2 of the University of Costa Rica, San Pedro Montes de Oca, San José, Costa Rica (N9°56'07'', W84°03'04'') (Figure 1). This site is located in a disturbed urban environment that is now covered with semi-woodland vegetation including both native and introduced herbaceous plants (Biamonte et al. 2011). Spiders were collected from 0 to 1.5 m above ground level. Since many of the plants were quite widely spaced, the transect consisted of 15 to 30 places where diverse herbaceous plants were haphazardly searched. Adults were collected individually using 15 ml Falcon tubes. Each tube had an opening for aeration, a small fragment of the plant from which the specimen was collected, and a small piece of damp cotton. Specimens were kept individually in these tubes for no more than two hours, and then quickly transferred to the laboratory. Specimens were identified by G.B. Edwards. Some of the specimens collected for identification were either undescribed or juveniles and therefore could not be identified to species level.

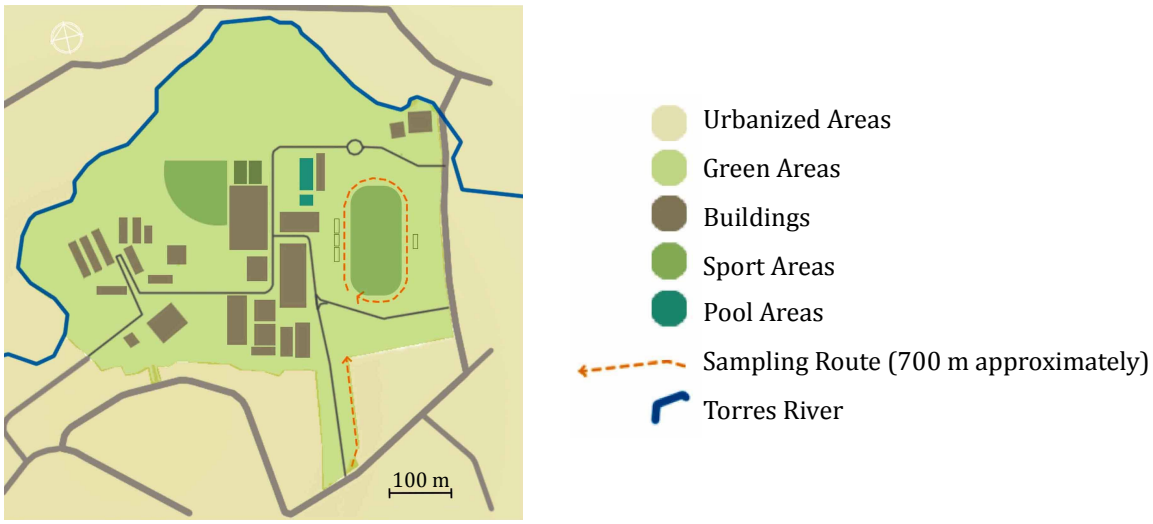

Figure 1. Study area at the University of Costa Rica in San Pedro de Montes de Oca, northwest of the central campus, usually referred to as Instalaciones Deportivas, Finca 3. The dashed orange line shows the transect used to sample specimens.

Climate. The mean temperature in the study site generally varied from 19.1° to 20.8°, and rarely dropped below 15° or rose above 29°C. The warmest months were April to November with a mean temperature of 20.6°C. The amount of rainfall per month varied considerably throughout the year, whereas the temperature was quite constant (Figure 2).

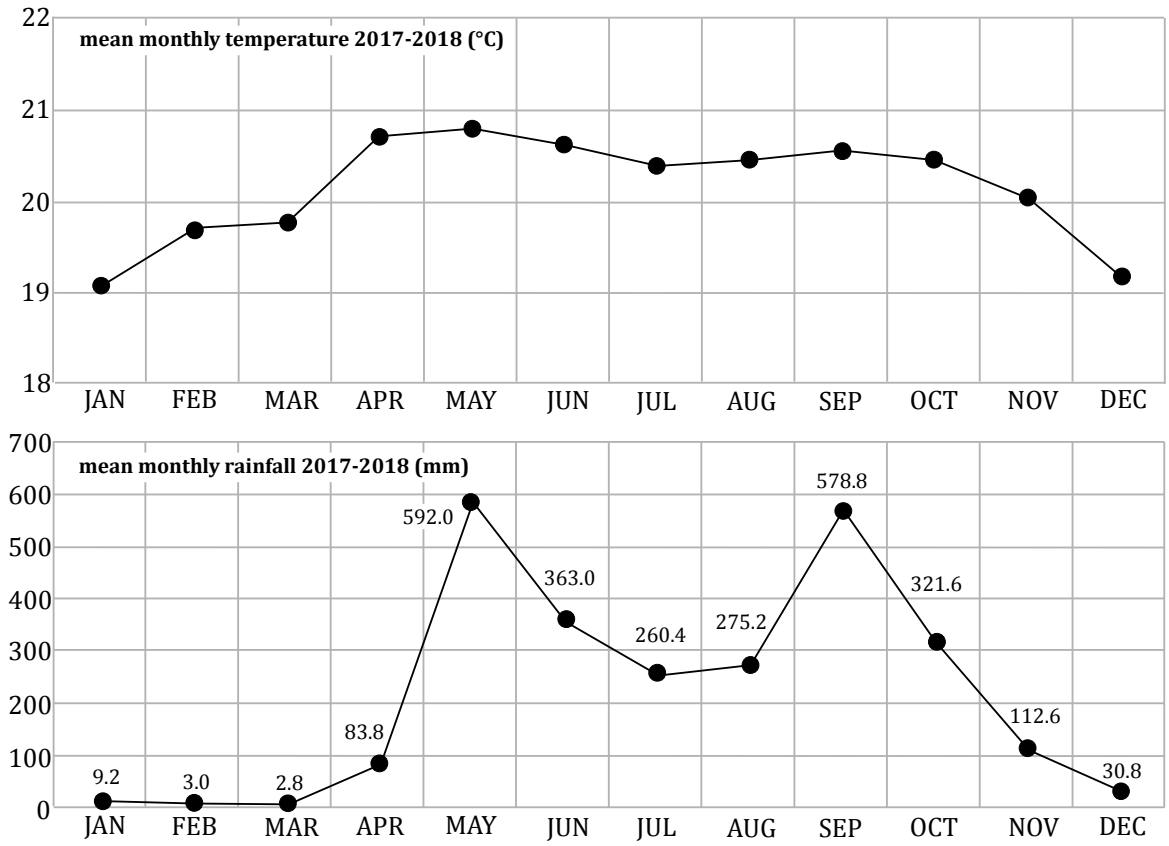

Figure 2. Average month temperature and rainfall during two years of field sampling at the study site. Compiled from data posted by Instituto Metereológico Nacional de Costa Rica (IMNCR 2019).

Phenology (Figure 3). Immatures were found throughout the year, but in greatest numbers from July through March. Most penultimates were observed from November through March. After temperatures rose a few degrees and rainy days became more frequent in April penultimate spiders developed into adults. Adult females were present for a longer period in the field (9 months) than were males (5 months), and they were detected during months with different amounts of average rainfall, from months with abundant precipitation (May, 592 mm) to months with very moderate rainfall (November, 112 mm). They were abundant from May through November. However, during months with very little rain, such as February and March (mean 5 mm), the population of females was drastically reduced and it became very difficult to find specimens. The presence of adult males coincided with the rainy season from April to June. Adult males were scarce and even disappeared from September through the first part of the year, only appearing once again in April.

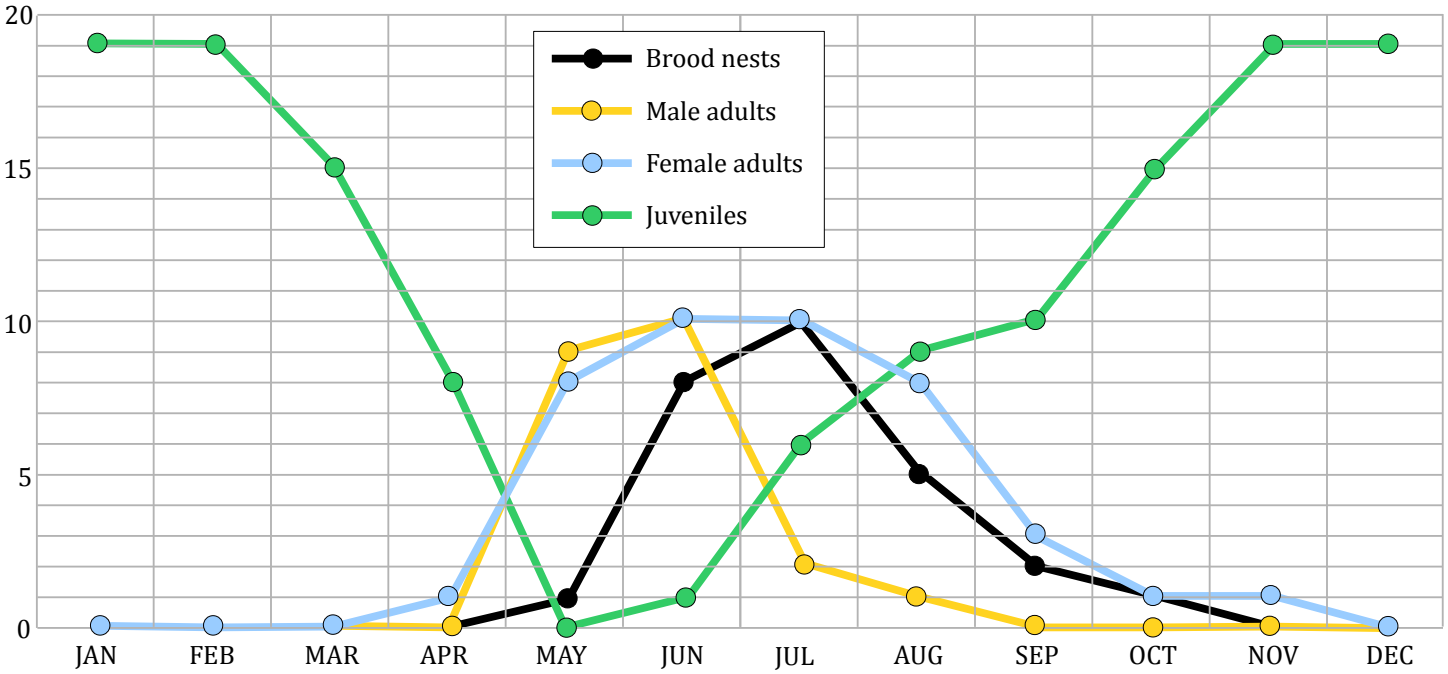

Figure 3. Count of brood nests, adult males, adult females, and juveniles of *Lyssomanes jemineus* by month, based on sampling from 1 JAN 2017 to 12 DEC 2018.

Courtship of *Lyssomanes jemineus* was observed mostly during the months of April and May. Females laid approximately 50 to 190 eggs in nests covered with a loose silk on the undersides of leaves (Figure 4C), mostly from May to July. The presence of brood nests (nests with eggs or young) coincided with months with moderate rainfall (June to August), slightly less rainy that either the previous month, May, or the subsequent month, September (Figure 2).

We found both adult and juvenile *Lyssomanes jemineus* (Figure 4) on their silk nests on the undersides of green leaves. We also observed them foraging on both stems and leaves of various plant species. Penultimate juveniles and adults were sometimes observed hunting or feeding on soft-bodied insects such as dipterans, small hemipterans, and chalcidoid wasps (Figure 4:A,F). In the majority of observations they were feeding on acalypterate flies and Chironomidae. Cannibalism of large juveniles on small juveniles was observed, and one case of a mature *Lyssomanes jemineus* feeding on another spider, *Leucauge mariana* Keyserling (Tetragnathidae) was also observed. In addition to *Lyssomanes jemineus*, 10 other salticid species were found at the study site (Table 1).

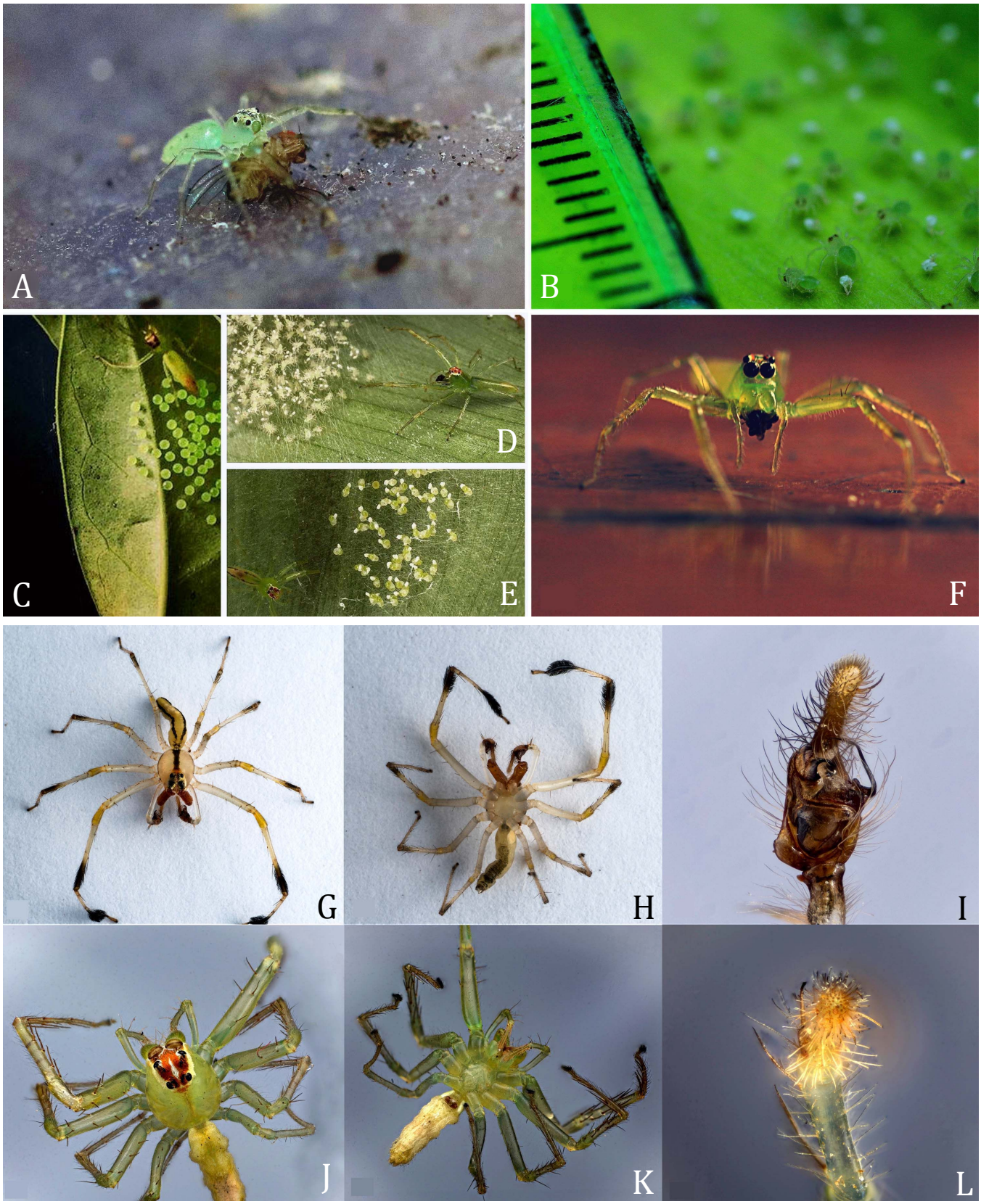

Figure 4. *Lyssomanes jemineus* from study site in Costa Rica. **A,** Spiderling, 18 weeks old, feeding on *Drosophila melanogaster*. **B,** Spiderlings, 2 weeks old, with a mm scale at the left. **C,** Oviposition by adult female. **D-E,** Maternal care of hatching spiderlings by female during the month of May. **F,** Adult female feeding on a microhymenopteran. G.-I, Adult male. **G,** Dorsal view. **H,** Ventral view. **I,** Ventral view of right pedipalp. **J-L,** Adult female. **J,** Dorsal view. **K,** Ventral view. **L,** Ventral view of right pedipalp. Macrophotography with Reflex Camera 850, 20X microscope lenses and focus stacking of 180 images.

Table 1. Other salticid species observed at the study site. In addition to these spiders there were some juvenile dendryphantines, most likely *Messua* sp. Peckham & Peckham.

Bagheera sp. Peckham & Peckham	*Colonus* sp. (1) F. O. Pickard-Cambridge	*Mexigonus* sp. Edwards
Balmaceda picta Peckham & Peckham	*Colonus* sp. (2) F. O. Pickard-Cambridge	*Nagaina incunda* Peckham & Peckham
Balmaceda sp. Peckham & Peckham	*Corythalia* sp. Koch	*Paraphidippus funebris* Banks

Discussion. Several factors have been reported to affect the seasonal abundance of spiders (Table 2). In our study of *Lyssomanes jemineus* we observed that adult females were absent during months with very little rainfall and, like adult males, more common during the rainy months. This pattern of males and females appearing during the rainy months could be due to the fact that plant cover and water sources increase, as do many potential prey, during those months (Miyashita et al. 1998). Plants are important to spiders for a number of reasons (Table 3).

Table 2. Factors that may affect the seasonal abundance of spiders.

Factor	References
habitat structure	Lubin 1978; Nentwig 1993
rainfall	Wolda 1978
plant structure	Raizer & Amaral 2001; Uetz 1977
reproductive period of individual species	Sosa-Romero et al. 2016
humidity	Flórez 1998
synchrony of adult mortality	Jackson 1978
refuge sites and the presence of natural enemies	Uetz 1977

Table 3. Important functions of plants with respect to spiders.

Function	References
shelter from dessication	Riechert & Tracy 1975
protection from natural enemies	Gunnarsson 1996
foraging opportunities	Morse & Fritz 1982; Romero & Vasconcellos-Neto 2003
sites for mating and oviposition	Rossa-Feres et al. 2000; Smith 2000

As has been reported previously for *Lyssomanes viridis* (Walckenaer), females remained very close to their offspring during the first two instars, but in the third or fourth instar they were more distant or absent (Richman & Whitcomb 1981). Also, during the development of her eggs, an adult female was usually found guarding them on top of the brood nest or nearby, and if she was disturbed the female remained vigilant and in some cases was reluctant to move, supporting the view that maternal care is important (Hallas and Jackson 1996). With respect to juveniles, our observations support those of other authors who report that juvenile *Lyssomanes* require an entire year to mature (Richman & Whitcomb 1981).

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References

Biamonte, E., L. Sandoval, E. Chacon and G. Barrantes. 2011. Effect of urbanization on the avifauna in a tropical metropolitan area. Landscape Ecology 26 (2): 183-194.

Gunnarsson, B. 1996. Bird predation and vegetation structure affecting spruce-living arthropods in a temperate forest. Journal of Animal Ecology 65 (3): 389-397.

Hallas, S. E. and R. R. Jackson. 1986. A comparative study of Old and New World lyssomanines (Araneae, Salticidae): utilisation of silk and predatory behaviour of *Asemonea tenuipes* and *Lyssomanes viridis*. New Zealand Journal of Zoology 13 (4): 543-551.

IMNCR. 2019. Instituto Metereológico Nacional de Costa Rica, Monthly Bulletin, *online at:* https://www.imn.ac.cr/en/boletin-meteorológico, accessed 2019.

Jackson, R. R. 1978. Life history of *Phidippus johnsoni* (Araneae, Salticidae). Journal of Arachnology 6 (1): 1-29.

Logunov, D. V. 2014. New species and records of *Lyssomanes* Hentz, 1845 from Central and South America (Araneae: Salticidae). Arthropoda Selecta 23 (1): 57–56.

Lubin, Y. D. 1978. Seasonal abundance and diversity of web-building spiders in relation to habitat structure on Barro Colorado Island, Panama. Journal of Arachnology 6 (1): 31-51.

Maddison, W. P. 2015. A phylogenetic classification of jumping spiders (Araneae: Salticidae). Journal of Arachnology 43(3): 231-292.

Miyashita, T., A. Shinkai and T. Chida. 1998. The effects of forest fragmentation on web spider communities in urban areas. Biological Conservation 86 (3): 357-364.

Morse, D. H. and R. S. Fritz. 1982. Experimental and observational studies of patch choice at different scales by the crab spider *Misumena vatia*. Ecology 63 (1): 172-182.

Nentwig, W., B. Cutler and S. Heimer. 1993. Spiders of Panama: biogeography, investigation, phenology, check list, key and bibliography of a tropical spider fauna. Flora and Fauna Handbook No. 12. Sandhill Crane Press.

Prószyński, J. 2016. Monograph of Salticidae (Araneae) of the World 1995-2015. Part II: Global Species Database of Salticidae (Araneae). Version May, 5, 2016.

Raizer, J., and M. E. C. Amaral. 2001. Does the structural complexity of aquatic macrophytes explain the diversity of associated spider assemblages? Journal of Arachnology 29 (2): 227-237.

Richman, D. B. and W. H. Whitcomb. 1981. The Ontogeny of *Lyssomanes viridis* (Walckenaer) (Araneae: Salticidae) on *Magnolia grandiflora* L. Psyche 88(1-2):127-133, 1981.

Riechert, S. E. and C. R. Tracy. 1975. Thermal balance and prey availability: bases for a model relating web-site characteristics to spider reproductive success. Ecology 56 (2): 265-284.

Romero, G. Q. and J. Vasconcellos-Neto. 2003. Natural history of *Misumenops argenteus* (Thomisidae): seasonality and diet on *Trichogoniopsis adenantha* (Asteraceae). Journal of Arachnology 31 (2): 297-304.

1. **Rossa-Feres, D. D. C., G. Q. Romero, E. Gonçalves-de-Freitas and R. J. F. Feres. 2000.** Reproductive behavior and seasonal occurrence of *Psecas viridipurpureus* (Salticidae, Araneae). Revista Brasileira de Biologia 60 (2): 221-228.

Rubio, G. D., W. Galvis and M. F. Nadal. 2017. Description of the female of *Lyssomanes miniaceus*, with a new distribution record for *L. belgranoi* (Araneae: Salticidae). Caldasia 39 (2): 239-246.

Smith, H. 2000. The status and conservation of the fen raft spider (*Dolomedes plantarius*) at Redgrave and Lopham Fen National Nature Reserve, England. Biological Conservation 95 (2): 153-164.

Sosa-Romero, M., M. Menéndez-Acuña and A. Burgos-Solorio. 2016. Fenología y estacionalidad del género *Mexigonus* Edwards, 2002 (Araneae: Salticidae) en un bosque templado al norte de Cuernavaca, Morelos, México. Entomología Mexicana 3: 919-923.

Uetz, G. W. 1977. Coexistence in a guild of wandering spiders. The Journal of Animal Ecology 46 (2): 531-541.

Wolda, H. 1978. Seasonal fluctuations in rainfall, food and abundance of tropical insects. Journal of Animal Ecology 47 (2): 369-381.

Capítulo 3

PLOS ONE

RESEARCH ARTICLE

Spectral measure of color variation of black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Scelionidae), a statistical approach

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Data Availability Statement: All data and code files are available from the public repo https://github.com/malfaro2/Mora_et_al. Following the

Abstract

Small parasitoid wasps are abundant and extremely diverse, yet their colors have not been analyzed. One of the more common color patterns observed in these wasps is a black-orange-black pattern, which is especially common among neotropical species of Scelionidae ranging in size from 2 to 10 mm. Due to the methodological challenges involved in extracting and analyzing pigments from small-sized insects, other methods for examining colors need to be explored. In this work, we propose the use of microspectrophotometry in combination with statistical analysis methods in order to 8 study the spectral properties in such cases. We examined 8 scelionid genera and 1 genus from a distantly related family (Evaniidae), all showing the black-orange-black pattern. Functional Data Analysis and statistical analysis of Euclidean distances for color components were applied to study color differences both between and within genera. The Functional Data Analysis proved to be a better method for treating the reflectance data because it gave a better representation of the physical information. Also, the reflectance spectra were separated into spectral color component contributions and each component was labeled according to its own dominant wavelength at the maximum of the spectrum: Red, Green and Blue. When comparing spectral components curves, the spectral blue components of the orange and black colors, independent of the genera being compared, result almost identical, suggesting that there is a common compound for the pigments. The results also suggest that cuticle from different genera, but with the same color might have a similar chemical composition. This is the first time that the black and orange colors in small parasitoid wasps has been analyzed and our results provide a basis for future research on the color patterns of an abundant but neglected group of insects.

suggestion from one reviewer we tried to upload the repository contents in a zip file to the submission system, but it seems that the file size exceeds the limit (zip file is 86 MB). The GitHub repository is updated.

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Competing interests: The authors have declared that no competing interests exist.

Introduction

The reflection, absorption or scattering of specific wavelengths of light by surface structures, the deposition of different chemical pigments in the outer body layers, or the interaction between various mechanisms such as pigments amplifying iridescence in butterflies [1], produce a remarkable variety of colors with complex and diverse patterns in insects. These color patterns play important roles in the biology of insects, for example as secondary sexual characters [2], in aposematism and mimicry [3, 4], and crypsis [5].

Compared with Lepidoptera and Coleoptera, there are relatively few studies of color patterns in the order Hymenoptera. Studies are mostly related to the yellow and black patterns in Vespidae [6, 7] and the variation in color patterns in *Bombus* Latreille [8]. In Hymenoptera there are also few studies regarding the chemical and physical nature of the color patterns exhibited, some of the pigments reported being pheomelanins (black, orange-red, yellow variations in velvet ants and bumble bees) [3], eumelanin (brown-colored cuticle) and xanthopterin (yellow-colored cuticle) in *Vespa orientalis* Linnaeus [9]. Various scelionid wasps [10], principally neotropical species ranging from 2 to 10 mm in length, show a recurring color pattern of black head, orange mesosoma, and black metasoma (BOB pattern; Fig 1), which has also been documented in species of numerous other families of Hymenoptera [11]. Several lines of evidence suggest that this color pattern has evolved many times independently within scelionids: the phylogenetically most basal scelionids [12] do not show this pattern; though quite common in the Neotropics only a minority of species show this pattern; species showing the BOB pattern occur in genera that are widely scattered in the phylogeny and not all species in these genera have this pattern [11]. This in turn strongly suggests that the BOB color pattern has some biological function but there are currently no studies addressing this question. Nor

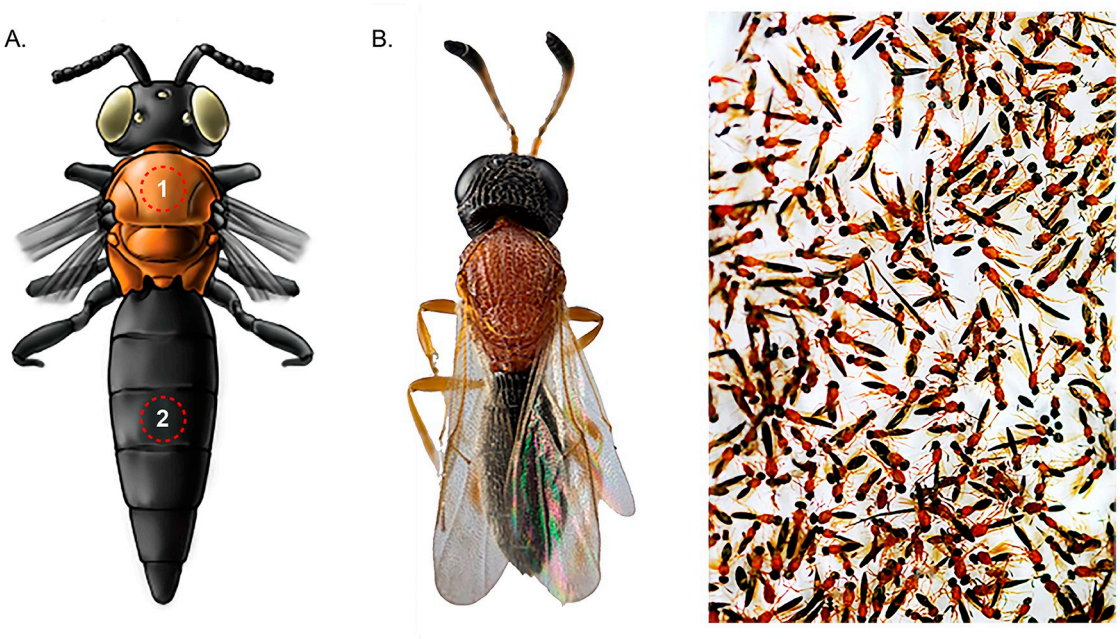

Fig 1. BOB pattern illustration. (A) Schematic detail of the area (dotted circles) used for measurements of both colors, zone of 1 mm^2 black of mesoscutum (1) and third tergite of metasoma (2). (B) Focus-stacked macro photography of a scelionid wasp with blak-orange-black color pattern obtained with a Reflex 850 camera coupled to a 20x microscope lens. The photograph at the far right, shows the striking black-orange-black color pattern of a group of scelionid specimens recently collected from the field; it was obtained with a Canon SX10 IS camera and a 105 mm macro lens.

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have the orange and black colors present in these wasps been analyzed, which is the primary objective of the present investigation.

Color characterization of specimens, especially in taxonomy, has been done mostly subjectively, although more quantitative methods have been proposed [13]. There are few reports treating the measurement of color rigorously or analyzing spectral data and its variability as a functional measurement, which potentially can show differences detected by insects, and shed a light on their biology.

Among many studies that measure and compare colors in insects, the methods vary widely [7, 14, 15]. Endler et al. [16] took into account the continuous nature of the wavelengths as measures of color and used Principal Component Analysis (PCA) on the radiance spectra to analyze patterns of color, while recognizing its limitations, specifically, those related to the violation of assumptions for statistical tests to discriminate between colors. Functional Data Analysis (FDA) is a powerful method to study spectral data since it assumes that the underlying process generating the data is smooth, and hence provides tools to analyse a spectral measurement as a function and not as a series of data points [17]. For example, Rivas et al. [18] proposed the use of FDA techniques to test color changes in stone, and present a comparison with a multivariate method.

Materials and methods

All experimental protocols were carried out in accordance with the involved institution's guidelines and regulations, thus, a scientific-academic license was obtained by the Ministry of Environment and Energy in relation to the National System of Conservation Areas, document N° ACTo-050-18, for the sampling and collection of the material under study.

A statistical approach based upon reflectance measurements of the dorsal surface of eight scelionid genera and 1 genus from a distantly related family (Evaniidae) was used in order to study the BOB color pattern. The reflectance spectra were measured by means of a microspectrophotometer. A statistical description of the BOB color pattern was obtained by means of different statistical approaches, applied to the spectral information as a function of genus, color, spots of measurement and specimens. This approach was used due to the small size of these wasps and the difficulty involved in obtaining sufficient fresh material for chemical analysis of the pigments.

Microspectrophotometry

The reflectance spectra of 8 genera belonging to the hymenopteran family Scelionidae were measured with a 508 PV UV-Visible-NIR Microscope Spectrophotometer (CRAIC, Los Angeles, USA). The spectrophotometer is coupled to an Eclipse LV100 ND Microscope using episcopic illumination (Nikon, Tokyo, Japan). A spot size area of 200μm x 200μm was measured. A spectralon standard was used as a white reference because of the diffuse reflection produced by the roughness of the surface of the cuticle. Table 1 includes further information regarding the measurement settings.

Specimens and sampling for optical measurements

Recently collected specimens are crucial to ensure the good condition of the external cuticle structures. Since Scelionidae are small in size and their hosts (insect eggs) are difficult to find, collecting specimens requires considerable effort.

Specimens of 8 genera were collected: *Acanthoscelio* Ashmead, *Baryconus* Foerster, *Chromoteleia* Ashmead, *Macroteleia* Westwood, *Opisthacantha* Ashmead (*Lapitha* Ashmead is currently considered to be a junior synonym [19]), *Scelio* Latreille, *Sceliomorpha* Ashmead,

Table 1. Information about the analysis of color data.

Microspectrophotometry technical details	Information
Light source	12 V-100 W halogen lamp
Magnification	5X, CFI60 2 TU Plan Fluor BD, N.A 0.15, WD 18.0 mm, (Nikon,Tokyo, Japan)
White reference	Spectralon Diffuse Reflectance Standard USRS-00-010(Labsphere, North Sutton,NH, USA)
Dark reference	Internal attenuators
Software for spectral capture	Lambda Fire (CRAIC,Los Angeles,USA)
Integration time	150 ms
Number of spectra averaged	100

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Triteleia Kieffer. They were collected with entomological nets over a period of 12 months. Additionally some specimens of the genus *Evaniella* Bradley (Evaniidae) were included, because they exhibit a similar BOB pattern, but are quite far removed from scelionids in the phylogeny of Hymenoptera [20].

The collection site was a patch within the protected zone of El Rodeo, which is located in the Colon district of the San José province, between the geographical coordinates 9° 52′–9° 56′ N and 84° 14′–84° 20′ W (Abra 3345 I and Río Grande 3345 IV cartographic sheets, National Geographic Institute 1989), at a distance of 18 km in a straight line from the capital city. The natural boundaries of the area are the Virilla River to the north, the Jaris river to the southeast and the Quebrada Honda river to the northeast. This natural reserve is composed of a secondary forest and a remnant of primary forest (approximately 200 ha). Specimens were identified by R.M. and P.H. using the keys to the scelionid genera [19] and voucher specimens were deposited in the Museum of Zoology of the University of Costa Rica. Due to the lack of taxonomic studies for six of the eight genera, species level identifications were not carried out. A previous study demonstrated that the vast majority hymenopterans with the BOB pattern, and those that are all black, do not show sexual dimorphism in color, at least in specimens that have been identified to species level in museum collections [11].

The specimens were killed by freezing and then, using a needle cut and polished on both ends, one square millimeter or a small notch of area was dissected in the mesoscutum (orange) and in the third tergite of the metasoma (black) (Fig 1). The orange and black samples of 4 specimens of each of the 8 genera were analyzed. For each specimen three samples were taken from the mesosoma (orange) and three from the metasoma (black).

Colorimetric differences

Spectral reflectance curves can be studied in terms of colors using a geometrical representation or color space. The CIE color spaces are recognized to have important characteristics such as being device-independent and having a perceptual linearity [21]. Nevertheless, not all color spaces are suitable for comparing colors because distances between coordinates are not easily quantifiable due to the lack of uniformity of the space. For these effects, the CIE recommends using CIELAB color space due to its uniformity [22]. The corresponding coordinates are calculated using the procedure described in S1 Appendix.

The CIELAB color space is Euclidean, therefore distances between points can be used to represent approximately the perceived magnitude of color differences between object color

stimuli of the same size and shape, viewed under similar conditions. The CIE 1976 L, a, b (CIELAB) color difference definition was adopted [22]:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Here, the quantities ΔL, Δa, Δb, represent the subtraction of the corresponding coordinates of the two colors being compared and ΔE represents the distance between the two colors within the CIELAB space. There are other CIE definitions for color differences, see for example the ones explained in reference [22]. Nevertheless, as discussed by Melgosa [23], the definition in Eq 1 yields results that are not very different from more recent and complex definitions. Since the purpose of this work is not to evaluate the color space metrics, but to consolidate a quantitative framework to compare colors using the information available from microspectrophotometry, the CIE 1976 definition (Eq 1) was chosen for the sake of simplicity. Regarding the definition of a color difference threshold, it can be argued that CIE color spaces are based on human vision sensibility, and the color difference threshold will depend not only on the spectral region but also on the observer. Nevertheless, in order to establish a mean threshold value to define whether two colors are different, we used the value 2.3 as reported by [24] specifically for CIELAB. In this sense, when the color difference ΔE is less than this value, colors are considered as equal. Otherwise, colors are said to be different.

RGB spectral components. When calculating ΔE, all the spectral information is condensed into a single number and it is not clear whether the difference comes from shorter or longer wavelengths. In this sense, the use of a spectral space instead of a color space would provide a more objective description of the physical variation among stimuli and also allows the identification of different physical mechanisms producing the same perception of color but having different spectral behavior [25]. Therefore, in order to visualize the characteristics of the the reflectance curves in different regions of the electromagnetic spectrum, we propose a trichromatic spectral space defined as follows using the CIE color matching functions (CMFs):

$$R(\lambda) = \frac{\bar{x}(\lambda)Z(\lambda)}{\int \bar{x}(\lambda)Z(\lambda)d\lambda}, \quad (2)$$

$$G(\lambda) = \frac{\bar{y}(\lambda)Z(\lambda)}{\int \bar{y}(\lambda)Z(\lambda)d\lambda} \quad (3)$$

and

$$B(\lambda) = \frac{\bar{z}(\lambda)Z(\lambda)}{\int \bar{z}(\lambda)Z(\lambda)d\lambda}, \quad (4)$$

where $Z(\lambda)$ is the measured reflectance, $\bar{x}(\lambda)$, $\bar{y}(\lambda)$ and $\bar{z}(\lambda)$ are the CIE 1976 CMF's [22].

We label these components as Red, Green and Blue (RGB) because their maximum amplitudes occur at 600 nm, 555 nm and 446 nm respectively.

It is recognized that spectral spaces have the advantage of providing information about the different processes generating phenotypic color diversity [25], and therefore we propose this RGB spectral component method as an alternative for the case when a direct chemical characterization can not be performed.

Statistical analysis

Data sets. Each of the observations has 1185 recorded points: the reflectance (Y) as a function of wavelength value (λ), where λ is a sequence of 1185 equidistant points from 420.2 nm to 919.7 nm. The use of (λ) instead of the usual (s) in FDA, is done to match the representation of a wavelength in the physics literature. For the remainder of this paper, the 1185 points will be called reflectance curves, which were recorded in the following way: each curve was constructed with the reflectance of color in mesoscutum and metasoma spots per colored area, and was measured three times in each specimen, in different spots. The observations correspond to the reflectance measured in the orange mesoscutum areas versus the black metasoma areas for 36 specimens: 4 specimens for each of the 8 genera, and 4 specimens for *Evaniella*, which was observed as a control (the latter genus belongs to a different family, which is not closely related to scelionids). The response variable is either a difference of reflectance or a measure of reflectance, and it can be measured in three different levels:

- The univariate mean measure: m_{ijk} where m is the mean difference between black and orange measures for all possible wavelengths in curve ijk, where k represents repetitions, j specimens, and i genera. This is is used as a naive base comparison.
- The functional measure: $Y_{ijk}(\lambda)$ where the difference in reflectance between black and orange curves $Z(\lambda)$ is represented by Y, and was measured for each wavelength λ, and repeated k times in each specimen j, and genera i. In this case, $Y_{ijk}(\lambda)$ describes a reflectance difference curve.
- The multivariate distance measure: a Euclidean distance ΔE as defined in 1 for each pair of curves being compared.

A table was created relating the different factors (spots, specimen, and genera) with the response in the univariate and functional case. In total, 96 combinations were recorded. In the multivariate distance case, a total of $N = C_2^{192} = 18336$ comparisons were used as observations, and then it was recorded whether or not each pair had a difference: genus, color, spot and specimen.

Data analysis. Prior to the analysis, descriptive statistics were generated for each genus and color section (black and orange). The objective of the data analysis is to contrast the null hypothesis of no difference between color reflectance means of each combination of genus and color area, and for that, univariate and functional ANOVA were performed. The multivariate distance case was also analyzed to match the physics literature on color differences. The following methods were used in each case:

- Univariate method: A one-way ANOVA was used to compare the mean difference between black and orange for all possible bandwidths m_{ijk} with genus as the factor and controlling for specimen and spot.
- Functional method: A FANOVA [17] was used to compare the difference curves $Y_{ijk}(\lambda)$ with genus as the factor, and controlling for specimen and spot. The statistical F tests were included to interpret the contrast results for the mean difference curves. R packages `erpFtest` [26], and `fdANOVA` [27] were used.
- Multivariate distance method: A two-way ANOVA was used to compare the Euclidean distance ΔE between each combination of color and genus, using dichotomous variables as factors: color as 0 if the pair is from the same color, 1 if it differs; genus as 0 if the pair is from the same genus and 1 if it differs. Here also, specimen and spot were included as factors in the linear equation.

The objective is to illustrate the functional method compared to a univariate analysis approach, and with a multivariate distance alternative, to compare the results when taking into account all the information from the difference curves. More specifically, for the univariate and functional responses, the ANOVA model can be written as the following:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}, \tag{5}$$

for $i = 1, 2, \ldots, 8$ genera, $j = 1, 2, \ldots, 4$ specimens, and $k = 1, 2, 3$ sampled spots in each colored area from each of the 4 specimens. Usual restrictions and assumptions for the ANOVA model apply:

$$\sum_{i=1}^{8}\alpha_i = 0, \qquad \sum_{j=1}^{4}\beta_j = 0, \qquad \epsilon_{ijk} \sim N(0, \sigma^2). \tag{6}$$

In the model, the global mean $\mu = 0$ given that we are working with difference curves, and α_i is the genera effect of level i, and β_j is a block effect to explain the intra-genus variance. To account for the dependence between spots from the same specimens (in the two colored areas), a random effect η_k was tested, and different covariance structures for Σ_η were fitted (assuming dependence $\eta_k \sim N(0, \Sigma_\eta)$ or independence between spots). Lastly, ϵ_{ijk} is the residual that accounts for the unexplained variation specific to the kth observation, ith genus and jth specimen. Depending on the response Y_{ijk}, the residuals ϵ_{ijk} are a number (univariate) or a curve (functional). Assumptions were tested for all the model options.

For the multivariate distances, the ANOVA model differed in the factors:

$$\Delta E_i = \mu + \gamma_i + \eta_i + \omega_i + \rho_i + \gamma_i * \eta_i + \epsilon_i, \tag{7}$$

where the global mean μ represents the average change for the N comparisons, and then each of the factors add the change given that the pair has different genera (γ_i), color (η_i), spots (ω_i) or specimens (ρ_i) for each pair i. Finally, ϵ_i is the error modeled as white noise. Assumptions were tested for this model as well.

Statistical analysis was performed using the computing environment R [33]. The code and data to perform each of the tests mentioned in this paper are available for download in https://github.com/malfaro2/Mora_et_al. Data formatting and figures were prepared using the collection of R packages `Tidyverse` [28].

Results

Reflectance curves in Fig 2 show differences both when comparing curves measured for the same genus and curves representing the reflectance of different genera. At the same time, such differences present variability within the group of measurements performed. For both between and within genus cases, these reflectance differences are statistically compared to zero in both the univariate data and the functional data (FDA method) (p-value < 0.00001 in all cases). The result is supported by the multivariate distance test, where there is evidence to say that overall change μ is greater than zero (p-value < 0.00001). Details about the significant factors that explain that change are summarized in the following sections.

Overall differences

Fig 2 presents descriptive statistics for measured spectra for each genus and each of the spectral colors. There are some genera with more variability, and there is a tendency to have low reflectance at lower wavelengths for both black and orange spots. It is important to point out that the measurements were performed grouping genera and not species, a factor that may account

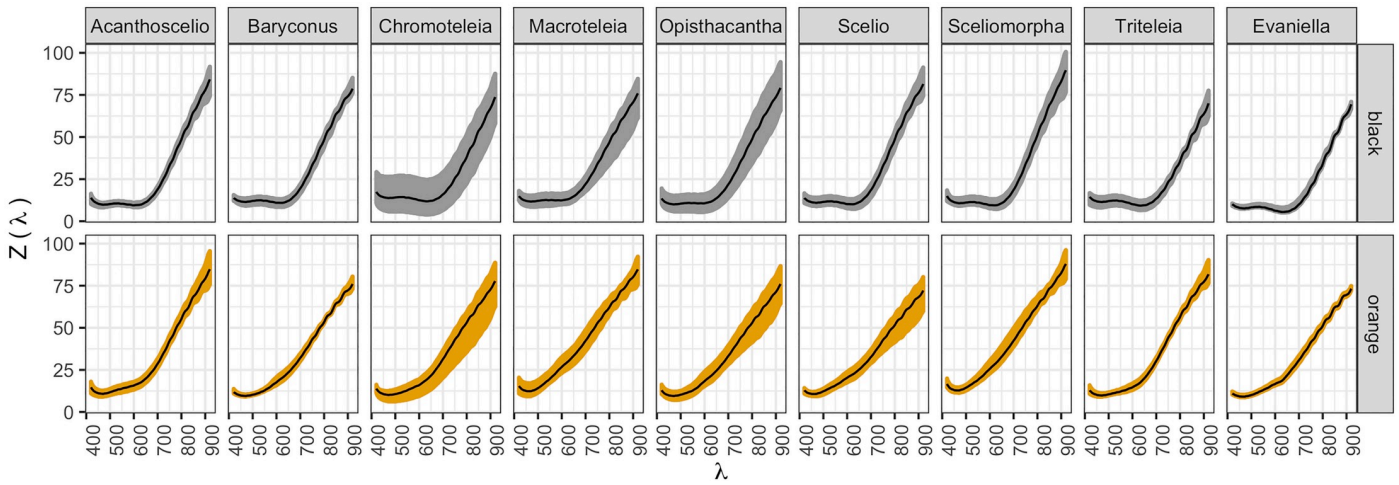

Fig 2. Reflection functions. Functions $Z(\lambda)$ in y axis, for each color within each genus. Black lines show the functional mean per case.

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for the variability for some genera such as *Chromoteleia*. Because the spot size of 200 μm x 200 μm used in the reflectance measurements is more than 20 times larger than the features that comprise the mesosoma, the effect of topography of the cuticle and homogeneity of the pigment distribution (see S1 Fig), can partly explain the data dispersion in some of the genera.

The difference curves for reflectance spectra, defined as $Y_{ijk}(\lambda)$, are plotted in Fig 3. The patterns from Fig 2 appear more evident. For each genus, the behavior of the difference curves between black and orange can be described according to the wavelength in the following way. Curves above the black line mean that the black curves have higher reflectance values than the orange ones. Before 550 nm, differences are around zero, meaning that the reflectance values

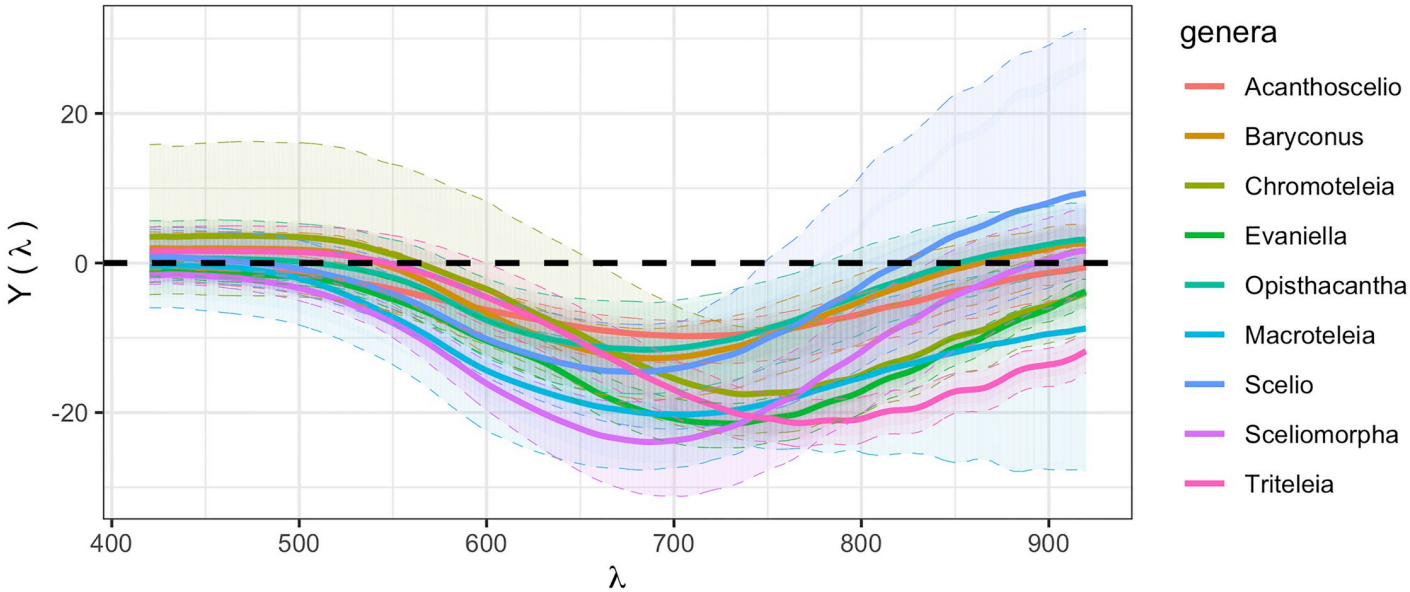

Fig 3. Difference (black-orange) of mean reflectance functions in each genus. Solid lines represent functional means, colored areas represent the area where difference functions in each genus are observed. A black dashed line represents the null hypothesis of no difference between colors in each genus. Curves above the black line mean that the black curves have higher reflectance values than the orange ones.

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Table 2. Mean reflectance m_i for all possible bandwidths, by genus. Genera are ordered according to the difference magnitude. ** The univariate difference is not significantly different from zero. [a,b,c] Represent a group different from the rest, when doing univariate multiple comparisons. All analyses use $\alpha = 0.05$. Color representation corresponds to the CIE 1976 (L^* a^* b^*) color space coordinates calculated from the mean reflectance curves from Fig 1.

Genera	Black	Orange	Absolute Difference
Baryconus	29.5307	33.692	4.1613**,a
Opisthacantha	29.5492	33.7317	4.1825**,a
Scelio	29.3678	33.8015	4.4337**,a
Acanthoscelio	29.2331	34.3834	5.1503[a]
Chromoteleia	26.5133	33.3825	6.8692[a,c]
Triteleia	24.0089	34.1785	10.1696[b,c]
Sceliomorpha	30.683	41.7377	11.0547[b]
Macroteleia	29.5722	41.3887	11.8165[b]
Evaniella	21.2445	32.3144	11.0699[b]

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are similar between orange and black in this region. Between 550 nm and 750 nm, the differences are below zero and are similar between genera. And finally, for wavelengths greater than 750 nm differences are both above and below zero and are dispersed with respect to genus. This analysis cannot be done if only the univariate measures are studied, given the information reduction as presented in S2 Fig.

In order to test the null hypothesis of H_0: $\alpha_i = 0$, whether or not the difference in reflectance between black and orange differs among genera, models were used to fit the data for each response. The best fit in all cases was from the models that assume independence between spots on the same specimen, so linear models were used subsequently. The univariate (p-value < 0.0001) and functional (p-value < 0.0001) results coincide in that in at least one genus examined, the mean reflectance of the orange spot is significantly different from the mean reflectance of the black spot, controlling for specimen and spot.

The statistical tests confirm what the descriptive plots showed. For each genus, the difference between black and orange is significantly different from zero for both the univariate and functional (FDA) case. Results from the functional ANOVA and a permutation test based on a Fourier basis function representation (`fdANOVA`), to test whether there is a mean difference between colors and genera, show that the calculated statistic is $T = 18.66961$ [26] with an associated p-value <0.0001, which means that the mean difference between the black and orange spots in at least one genus is different from the mean difference between the black and orange spots in the other genera, as shown in Fig 3. The univariate differences between genera shown in Table 2 only take into account the aggregated data collected at all wavelengths considered. The results for univariate comparison between genera can be found in S2 Appendix.

Multivariate distance analysis

ANOVA results for the model of Eq 7 presented in Table 3, show how the overall average change in all comparisons is 5.0535 units with a standard error of 0.2642, which makes the average statistically greater than the 2.3 threshold value. The estimate of η is 26.2081, which means that keeping all the other factors constant, the average ΔE (the distance between the two colors within the CIELAB space) is 26 units greater than the overall mean when the curves differ in color. Also, if the curves differ in both color and genus, the overall mean ΔE is 5.0535 + 4.3540 + 26.2081 + −4.3814 = 31.2342. The differences due to specimens are statistically not significant and the mean reflectance difference between measurements done in the same specimen but in different spots are on average 0.85, which makes it statistically significant but negligible on average, compared to the threshold.

Table 3. ANOVA Results for model 7.

	Estimate	Std. Error	t value	p-value
μ	5.0535	0.2642	19.12	0.0000
γ	4.3540	0.2420	17.99	0.0000
η	26.2081	0.3149	83.21	0.0000
ω	0.8524	0.1268	6.72	0.0000
ρ	-0.0425	0.1161	-0.37	0.7142
γ:η	-4.3814	0.3358	-13.05	0.0000

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Given that it has been established that there is evidence of a difference between colors and genera in all analyses, there is a need to clarify which genera and which colors are making the difference. For that, a comparison matrix for ΔE was constructed and presented in two ways: comparisons between genera per color difference and comparisons between colors per genus difference. Such results are presented in Figs 4 and 5 respectively. As an example, Fig 4 depicts how the distance ΔE between *Triteleia* and *Acanthoscelio* curves presents two distributions: one when both have the same color (in vermilion) and another when they have different colors (in bluish green). Both distributions present relatively low variability, and the first distribution is closer to zero, although its box— 50% of its data— is not close to zero or the threshold. In contrast, the distance ΔE between *Acanthoscelio* curves for the same color and different specimens (in vermilion) clearly include the threshold and can be taken as not significantly different.

The difference between distributions is unclear when comparing colors. Fig 5 presents how in the case in which the colors differ (upper right corner) both distributions for ΔE are both clearly different from zero, while the distributions for ΔE of the curves with the same color are more difficult to distinguish from zero, especially if they are from the same genus (in vermilion).

The differences between curves can be described as an average of all ΔE, for each genus and color. By that metric, both the maximum mean difference and the minimum mean difference can be established, as described in Table 4 for comparisons between curves of different colors and Table 5 for comparisons between curves of the same color, pointing to the top ten comparisons with wider differences. Similarly, top ten comparisons of different genera and the same genus are presented in S1 and S2 Tables.

RGB spectral components

The comparison of the color component curves red ($R(\lambda)$), green ($G(\lambda)$) and blue ($B(\lambda)$), was done in terms of distance:

$$Distance = \sum_{\lambda} |R_c(\lambda) - Rc\prime(\lambda)|$$

for each pair of comparisons (c, c'). The results in Fig 6 show how the same differences depicted when using ΔE are very clear for the green and red components, but not for the blue component.

Panel (D) of Fig 6 shows the color component contributions for the *Acanthoscelio* and *Triteleia* genera for the black and orange measurements, respectively. The spectra were normalised by their own areas to illustrate the similarity of the contributions that are reflected in all data taken and presented in Figs 4 and 6 in panels A, B and C. Apart from the small changes in intensity for the normalised spectra, the maxima of each curve have a different dominant

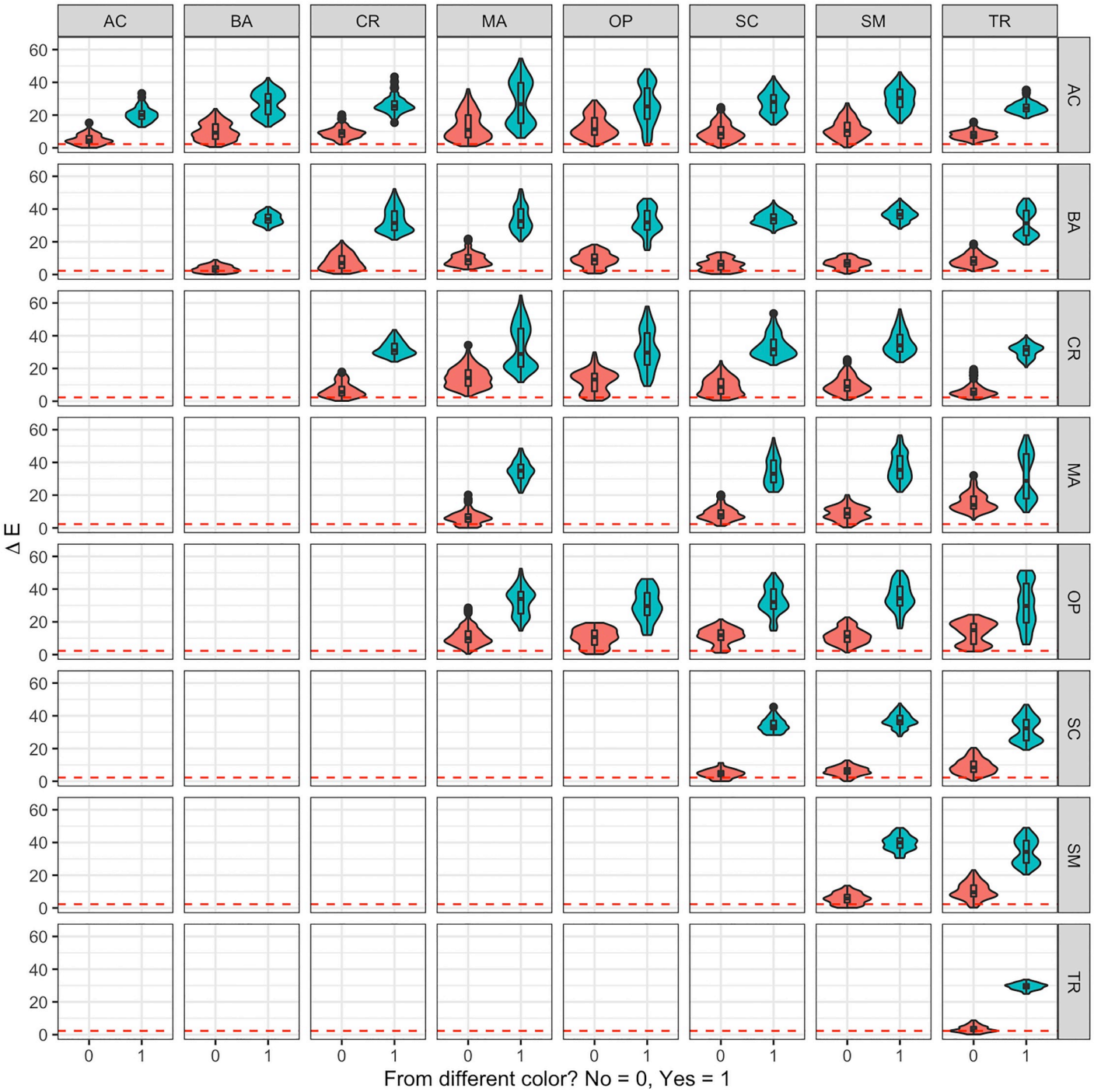

Fig 4. Violin and box plot comparison per genus. Distributions of Δ*E* calculated for pairs of curves of eight different genera: *Acanthoscelio* (AC), *Baryconus* (BA), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP), *Scelio* (SC), *Sceliomorpha* (SM) and *Triteleia* (TR) and two scenarios: when they have the same color (in vermilion) and when their colors differ (bluish green). The dashed vermilion line represents the threshold of 2.3.

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wavelength between genera, but the shape of the curves was very similar. The main changes are the differences in area when not normalised.

Mapping color variants in a spectral space can yield information about the relationship between genotypic space and evolution [29]. In this context, in order to visualize the data

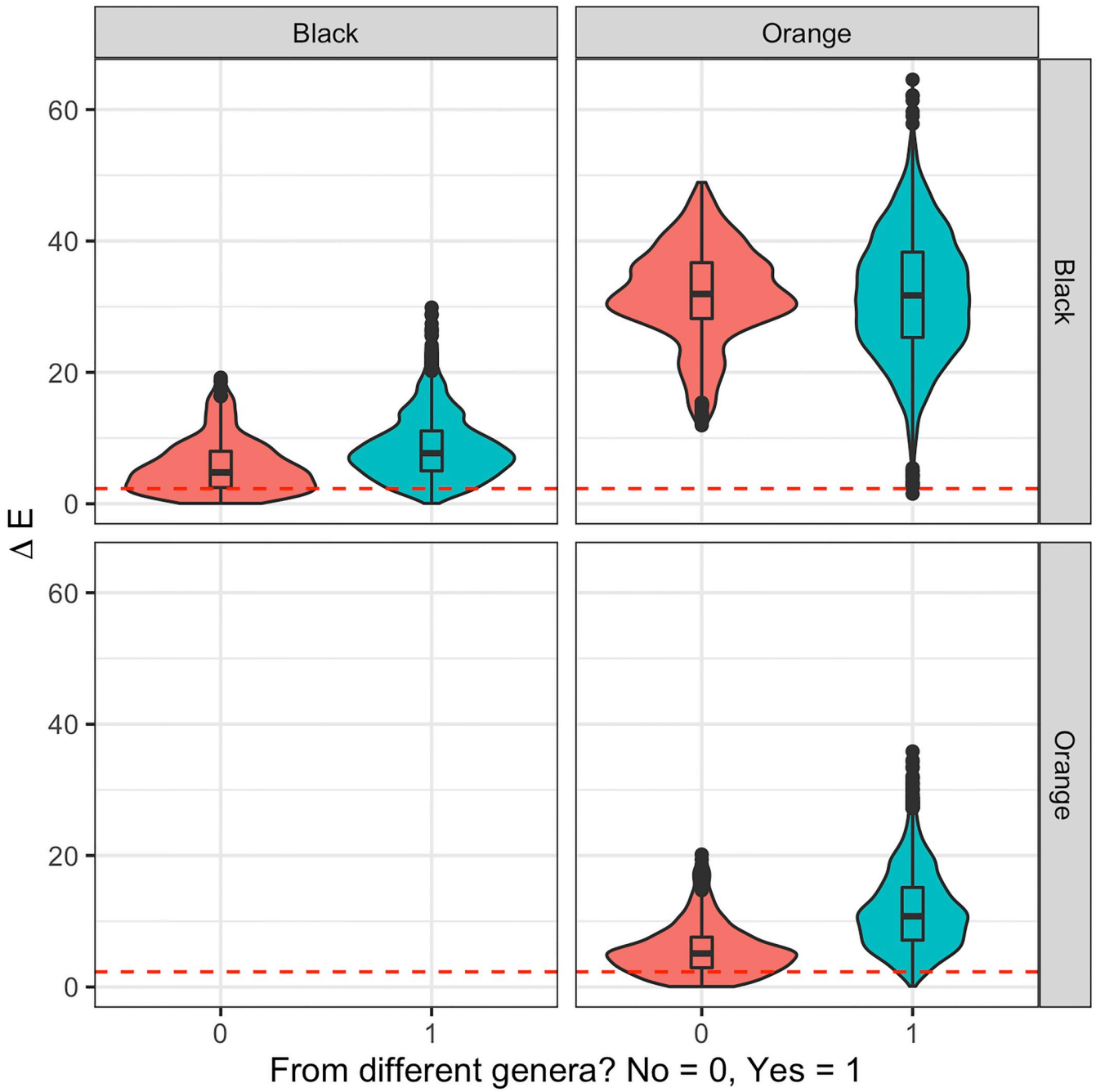

Fig 5. Violin and box plot comparison per color. Distributions of Δ*E* calculated for pairs of two different colors, black and orange, and two scenarios: when they are the same genus (in vermilion) and when the genus differs (bluish green). The dashed vermilion line represents the threshold of 2.3.

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provided by the spectral component analysis as a function of the genus, Fig 7 is presented. It shows the scatter plots for the black and orange measurements for all data, representing the logarithm of the area of each color component versus the dominant wavelength at the maxima of each contribution. The mean is plotted as a solid circle of the same color for each set of faded data points.

Table 4. Top 10 according to $\overline{\Delta E}$ for comparisons of curves of different color. The top 10 was extracted from a sample with overall minimum mean equal to 2.96 and maximum mean to 45.08. *Baryconus* (BA), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP) and *Sceliomorpha* (SM).

	Genus 1	Genus 2	Color 1	Color 2	$\overline{\Delta E}$
1	CR	MA	BL	OR	45.08
2	MA	TR	OR	BL	44.79
3	MA	SM	OR	BL	43.59
4	OP	TR	OR	BL	41.51
5	CR	SM	BL	OR	41.25
6	SM	TR	OR	BL	41.22
7	MA	SC	OR	BL	41.02
8	CR	OP	BL	OR	40.84
9	SM	SM	BL	OR	39.71
10	BA	TR	OR	BL	39.69

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The range of wavelength values at the maxima for each color component contribution is interesting, being around 5 nm for the blue contribution in both black and orange measurements. For the green (black measurement) and red (orange measurement) components it is about 5 nm, but for the red (black measurement) and green (orange measurement) it is approximately 20 nm.

The genus *Evaniella* appears to separate from the group in the black measurements, but it is not that obvious for the orange measurements. Meanwhile, *Sceliomorpha* and *Macroteleia* group together for the green and red orange observations. The genus *Chromoteleia* is outside the main group in all three color components for the black observations and in the blue component of the orange observations. *Chromoteleia* and *Baryconus* also pair in the green and red components of the black and orange observations. Additionally *Sceliomorpha* and *Acanthoscelio* present a mean that is very close in the black measurements for the blue, green and red contributions. The genera *Opisthacantha* and *Triteleia* group together in the blue and green components for the orange contribution.

Discussion

The following discussion is focused mainly on the contribution of the different statistical approaches to the physical and biological interpretation of the data obtained following the

Table 5. Top 10 according to $\overline{\Delta E}$ for comparisons of curves of the same color. The top 10 was extracted from a sample with overall minimum mean equal to 2.96 and maximum mean to 45.08. *Acanthoscelio* (AC), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP) and *Sceliomorpha* (SM).

	Genus 1	Genus 2	Color 1	Color 2	$\overline{\Delta E}$
1	AC	MA	OR	OR	19.64
2	MA	TR	OR	OR	18.26
3	CR	MA	OR	OR	17.02
4	AC	OP	OR	OR	16.95
5	AC	SM	OR	OR	15.77
6	AC	BA	OR	OR	14.35
7	OP	TR	BL	BL	14.16
8	SM	TR	OR	OR	13.69
9	OP	MA	OR	OR	13.61
10	CR	OP	BL	BL	13.32

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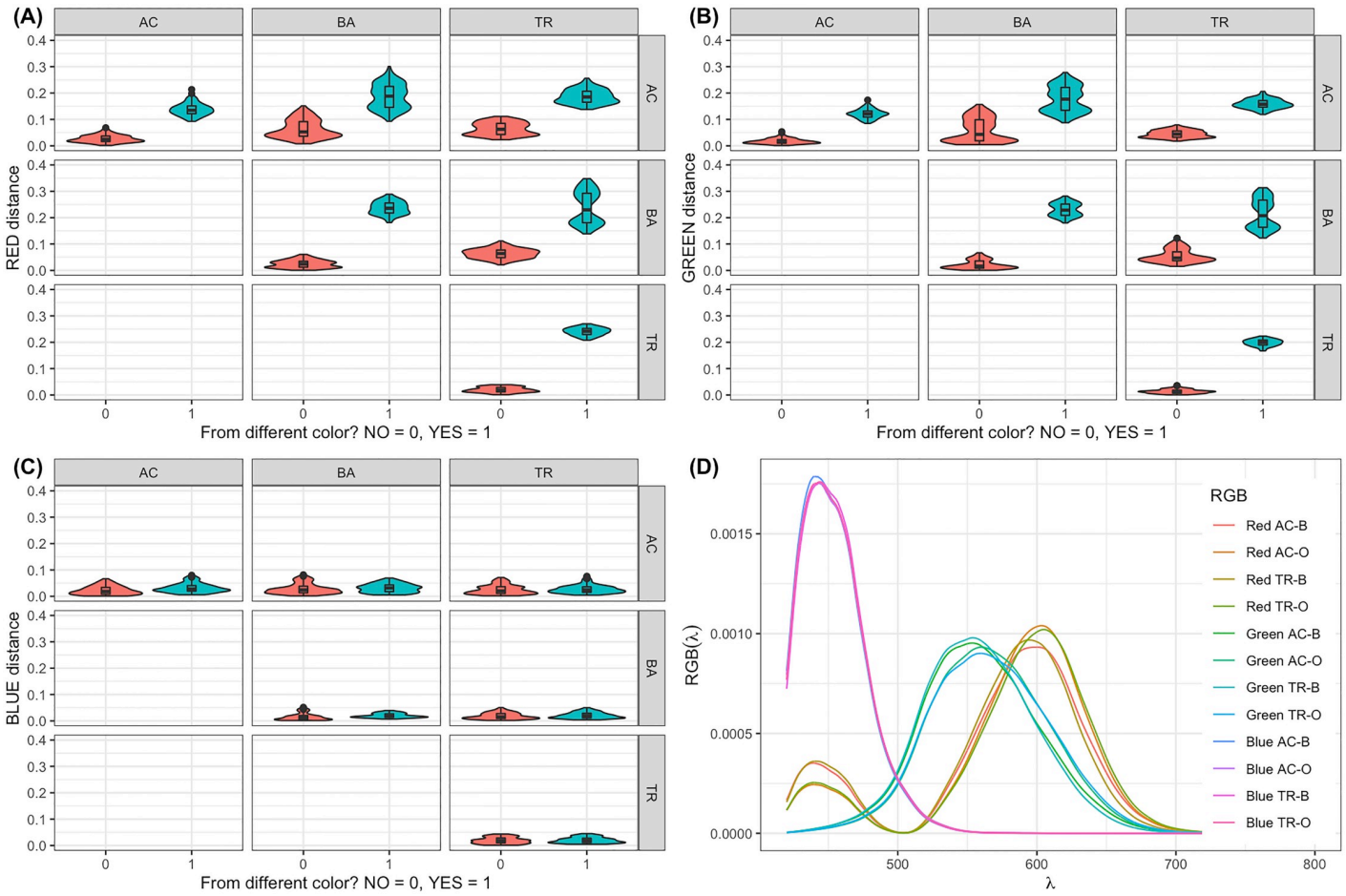

Fig 6. Examples of violin and box plot comparison per genus and color component. Distributions of *Distance* for (A) green, (B) red and (C) blue components, calculated for pairs of curves of three different genera: *Acanthoscelio* (AC), *Baryconus* (BA) and *Triteleia* (TR), and two scenarios: when they have the same color (in vermilion) and when their colors differ (bluish green). Plot (D) is an example of how the R, G, and B curves look for *Acanthoscelio* (AC) and *Triteleia* (TR) in orange and black spots.

https://doi.org/10.1371/journal.pone.0218061.g006

procedures proposed, with the aim of establishing a quantitative analysis method for the study of cuticle color of insects. This section has two main parts: discussion about differences and suitability of the statistical methods and the interpretation of the physical results within a biological context.

Suitability of the statistical methods

Regarding the suitability of the statistical methods applied, the present study highlights the differences that could arise using univariate methods, which ignore physical information contained in the reflectance measurement as a function of wavelength, versus analyzing data using FDA techniques.

The difference in intrageneric variances, confirms that the analysis of reflectance differences should not be done using univariate statistics. This variability is due to the difference in measurements across wavelengths: some sections are equal to zero, others are statistically different from zero, as described in the previous section. This can yield misleading results, finding univariate groups statistically similar, when the functional results are describing something different. In contrast, FDA provides methods for analyzing data that are believed to arise from

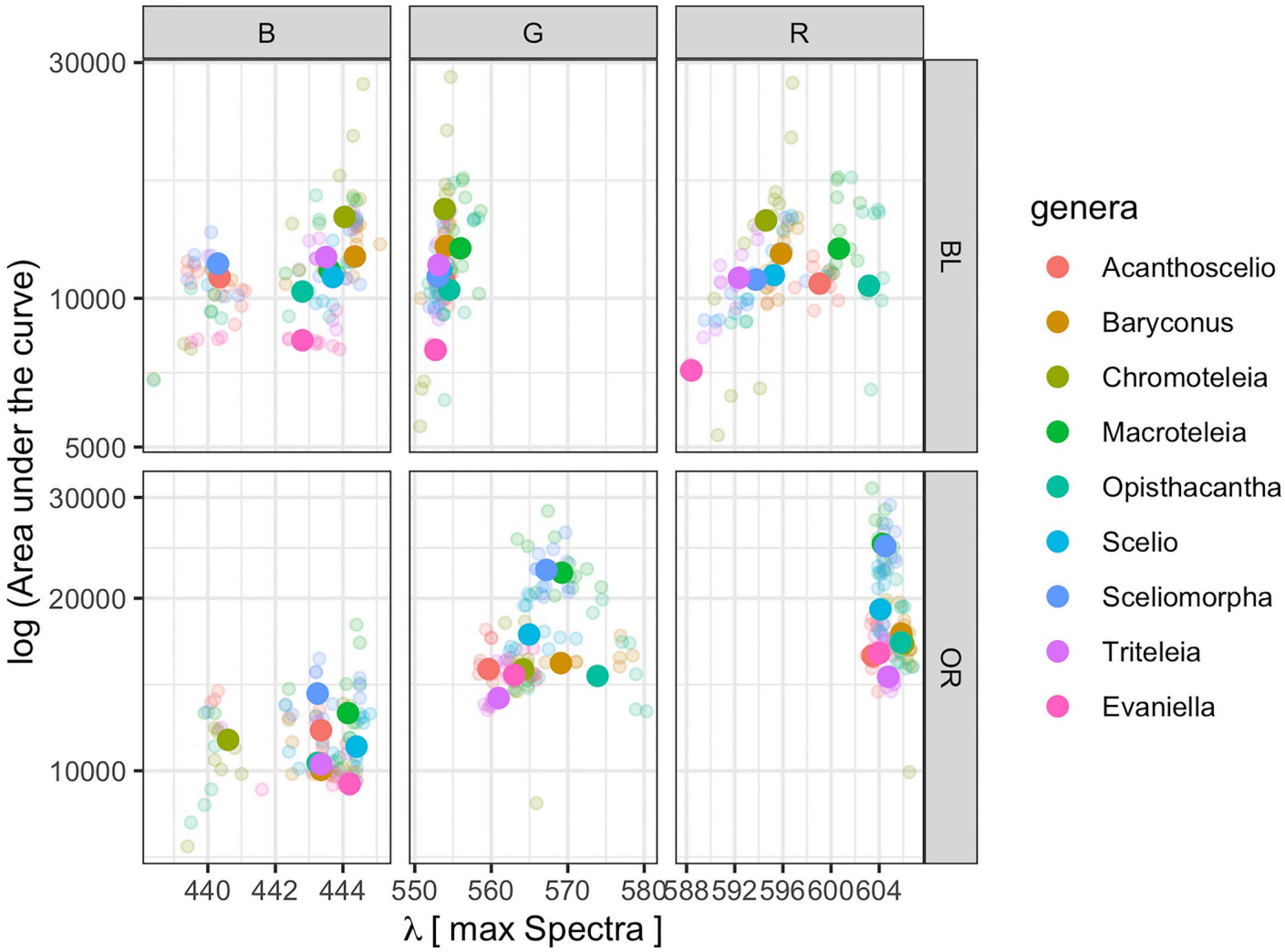

Fig 7. Scatter plot for color components per genus. Logarithm of the area under the curve, per component (red, green or blue) versus the corresponding wavelength in nm where the maximum occurs. Solid color points represent means for each genus and faded points correspond to independent observations. Plot (A) and (B) represent the components for the black and orange colors respectively.

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curves evaluated at a finite grid of points allowing the incorporation of spectral information from the reflectance curves [17].

In addition, a multivariate Euclidean distance defined in terms of a quantity with physical meaning such as ΔE, provides more evidence for analysis, besides confirming the FDA results. The details about multiple comparisons can be even more specific, if the color components are separated for each curve and the analysis is repeated.

The RGB component analysis showed that the nature of the differences found using the FDA and ΔE methods are related to the green and red color components, but no correlation was found for the blue component. This is consistent with results obtained qualitatively using the difference curves $Y_{ijk}(\lambda)$ shown in Fig 3. Moreover, ANOVA results for model 7 (Table 3) confirm the reproducibility of the experimental protocol and, for most of the cases studied, the results suggest that there is a low variability in the sample spectral measurements even though the taxonomic identification to just the genus level means that at least some of the genera could consist of more than one species.

The methodology described in this paper for statistically testing for color differences differs from those in the literature due to the level of scrutiny. It goes from the general question: "Is there a difference between colors?" to more specific contrasts between genera and color, using both the functional ANOVA and multivariate methods to compare color components. In this way, we are statistically comparing each pair of colors and genera, controlling for the other experimental factors.

Physical results within a biological context

In general, the results of the spectral decomposition, together with the color differences as analyzed in Fig 5, suggest that the cuticle segments from different genera, but with the same color (black vs. black, orange vs. orange) might have a similar chemical composition or an identical chemical composition with different concentrations. Tonal variations could be related to different amounts of an individual pigment or a combination of similar polymers distributed in the cuticle.

Recent studies report that the color phenotype is associated with the concentration and ratio of pigment forms [3, 30], but other factors such as nanostrucutres of the integument can affect the expression of color [31]. In the case of pigments of different color (black vs. orange), the spectral blue components remain almost identical in terms of shape and characteristic wavelength (where the maximum occurs), suggesting that there is a common compound for the pigments. Notice that the normalization of the spectral components, Eqs (2), (3) and (4) allows the use of these features for comparison instead of intensity, which is related to the quantity of red, green or blue present in each color. The information given by the spectral components is important because optical properties are related to energetic processes due to electronic transitions when the pigment molecules interact with electromagnetic radiation. The shorter the wavelength, the more energetic the process is and therefore differences can be interpreted in terms of differences of electronic structure, which is related to the chemical composition of the pigments. A chemical characterization using infrared spectroscopy was attempted directly on the cuticle and results showed subtle differences suggesting a similar nature of the functional group attached to the molecule. Such results are not shown here because they are not conclusive due to the fact that the exact chemical composition of the cuticle for each color is unknown. A definitive identification of the pigment requires chemical extraction which at the moment could not be done because of the small amount of cuticle available.

Evaniella belongs to a completely different family (Evaniidae) and might therefore be expected to differ markedly from all the other genera. This indeed appears to be the case for its black color, but not its orange color (Fig 7). Nonetheless, within Scelionidae there are more differences in the orange color than in the black (Table 5). Comparing the results shown in Fig 7 with a recent phylogenetic reconstruction of scelionid genera [12], a few other relationships between spectral properties and phylogeny appear to be present. For example, *Baryconus* and *Chromoteleia* are paired in the green and red components for both black and orange, and these two genera are hypothesized to be closely related. *Opisthacantha* and *Triteleia* group together in the blue and green components of the orange coloration, and these two genera are also closely related. On the other hand, the orange color of *Macroteleia* and *Sceliomorpha* group together for the green and red components (Fig 7B) despite these genera not being closely related. These results are intriguing and merit further investigation to determine whether certain spectral properties parallel the phylogeny.

In some other insects, contrasting black and orange color patterns are known to serve as aposematic (warning) coloration for potential predators [32] and it is possible that the same is

true of the BOB pattern, although this has not yet been tested. However, the orange mesosoma becomes less common at higher elevations [11], suggesting that other factors such as thermoregulation (black absorbs more sunlight) might play a role at these elevations; it is also possible that predators or aposematic models vary with elevation.

There is obviously much more to be learned about the black and orange colors present in numerous parasitoid wasps, but it is hoped that the results of the present spectral analyses provide a framework for future research, especially when a direct chemical characterization of the pigments is not possible and the optical and statistical methods proposed in this work can be applied.

Conclusions

The present study provides the first analysis of the common black-orange-black pattern that is found in small (2-10 mm) parasitoid wasps. Because of their small size and difficulty in obtaining sufficient numbers of fresh specimens for pigment extraction, microspectrophotometry was used to obtain reflectance spectra for eight genera of scelionids. These reflectance data were analyzed by means of univariate analyses and by Functional Data Analysis, the latter proving to be superior because it gave a better representation of the physical information, given that it takes into account complete curves and not just summaries.

The RGB spectral components method is proposed for studying the origin of the color in the cases when a direct chemical composition is not possible. In the case of the genera studied, the spectral blue components of the orange and black color were found to be almost identical, suggesting that there is a common compound for the pigments. This result holds even when different genera were considered for comparison. Also spectral measures can and should be used for quantifying differences in biological color patterns since such analysis provides information that is not tinted by the illuminant.

A correlation between the mean values of characteristics of the color components was used in an attempt to group genera that show similar values and some, but not all, of these groupings are similar to their positions in a previously published phylogeny of the genera.

Further study, including a chemical analysis of the pigments and classification of specimens into species should be performed in order to obtain validation of these results.

Supporting information

S1 Appendix. Color coordinates calculation.
(PDF)

S2 Appendix. Univariate comparison for the inter-genera case.
(PDF)

S3 Appendix. Eosin hematoxylin technique.
(PDF)

S1 Fig. Eosin hematoxylin technique. Cross section of the cuticle shows the pigment concentrated in the epicuticle, of a specimen of *Baryconus* with black-orange-black color (a) and black color (b). The variation in the distribution of the pigment together with the roughness of the cuticle surface can contribute to the reflection of light in different directions and therefore to the dispersion of the data.
(PDF)

S2 Fig. Box plots for univariate mean differences per genus. Black dashed line represents the null hypothesis of no difference between colors per genus. *Acanthoscelio* (AC), *Baryconus*

(BA), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP), *Scelio* (SC), *Sceliomorpha* (SM), *Triteleia* (TR) and *Evaniella* (EV).
(PDF)

S1 Table. Top 10 $\overline{\Delta E}$ differences for comparisons of curves of different genera. The top 10 was extracted from a sample with overall minimum mean equal to 2.96 and maximum mean to 45.08 *Acanthoscelio* (AC), *Baryconus* (BA), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP) and *Sceliomorpha* (SM).
(PDF)

S2 Table. Top 10 $\overline{\Delta E}$ differences for comparisons of curves of the same genera. The top 10 was extracted from a sample with overall minimum mean equal to 2.96 and maximum mean to 45.08. *Acanthoscelio* (AC), *Baryconus* (BA), *Chromoteleia* (CR), *Macroteleia* (MA), *Opisthacantha* (OP), *Scelio* (SC), *Sceliomorpha* (SM) and *Triteleia* (TR).
(PDF)

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References

1. Rutowski RL, Macedonia JM, Morehouse N, Taylor-Taft L. Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, Colias eurytheme. Proceedings of the Royal Society B 2005, 272: 2329-2335.
2. Garcia JA, Polidori C, Nieves-Aldrey JL. Pheomelanin in the secondary sexual characters of male parasitoid wasps (Hymenoptera: Pteromalidae). Arthropod Structure & Development. 2016; 45(4):311–319. https://doi.org/10.1016/j.asd.2016.05.001
3. Hines HM, Witkowski P, Wilson JS, Wakamatsu K. Melanic variation underlies aposematic color variation in two hymenopteran mimicry systems. PLOS ONE. 2017; 12(7):e0182135. https://doi.org/10.1371/journal.pone.0182135 PMID: 28753659
4. Mallet J, Joron M. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annual Review of Ecology and Systematics. 1999; 30(1):201–233. https://doi.org/10.1146/annurev.ecolsys.30.1.201
5. Brakefield PM. Industrial melanism: Do we have the answers? Trends in Ecology & Evolution. 1987; 2 (5):117–122. https://doi.org/10.1016/0169-5347(87)90051-6 PMID: 21227832
6. Marchini M, Sommaggio D, Minelli A. Playing with black and yellow: the evolvability a Batesian mimicry. Evolutionary Biology. 2017; 44(1):100–112. https://doi.org/10.1007/s11692-016-9397-0
7. Vidal-Cordero JM, Moreno-Rueda G, López-Orta A, Marfil-Daza C, Ros-Santaella JL, Ortiz-Sánchez FJ. Brighter-colored paper wasps (*Polistes dominula*) have larger poison glands. Frontiers in zoology. 2012; 9(1):20. https://doi.org/10.1186/1742-9994-9-20 PMID: 22901602
8. Rapti Z, Duennes MA, Cameron SA. Defining the colour pattern phenotype in bumble bees(*Bombus*): a new model for evo devo. Biological Journal of the Linnean Society. 2014; 113(2):384–404. https://doi.org/10.1111/bij.12356
9. Plotkin M, Hod I, Zaban A, Boden SA, Bagnall DM, Galushko D. et al. Solar energy harvesting in the epicuticle of the oriental hornet (*Vespa orientalis*). Naturwissenschaften. 2010; 97(12):1067–1076. https://doi.org/10.1007/s00114-010-0728-1 PMID: 21052618
10. Austin AD, Johnson NF, Dowton M. Systematics, evolution, and biology of scelionid and platygastrid wasps. Annu. Rev. Entomol.2005; 50:553–582. https://doi.org/10.1146/annurev.ento.50.071803.130500 PMID: 15450001
11. Mora R, Hanson PE. Widespread occurrence of black-orange-black color pattern in Hymenoptera. Journal of Insect Science. 2019; 19(2). https://doi.org/10.1093/jisesa/iez021 PMID: 30851035
12. Murphy NP, Carey D, Castro LR, Dowton M, Austin A.D. Phylogeny of the platygastroid wasps (Hymenoptera) based on sequences from the 18S rRNA, 28S rRNA and cytochrome oxidase I genes: implications for the evolution of the ovipositor system and host relationships. Biological Journal of the Linnean Society.2007; 91(4):653–669. https://doi.org/10.1111/j.1095-8312.2007.00825.x
13. Aguiar A. An accurate procedure to describe colors in taxonomic works, with an example from Ichneumonidae (Hymenoptera). Zootaxa. 2005; 1008(1):30–38. https://doi.org/10.11646/zootaxa.1008.1.4
14. Stavenga DG, Leertouwer HL, Wilts BD. Quantifying the refractive index dispersion of a pigmented biological tissue using Jamin–Lebedeff interference microscopy. Light: Science & Applications. 2013; 2(9): e100–e100. https://doi.org/10.1038/lsa.2013.56
15. Skaldina O, Sorvari J. Not simply red: colouration of red wood ant *Formica rufa*(Hymenoptera: Formicidae) is polymorphic, modular and size-dependent. European Journal of Entomology. 2017; 114:317–324. https://doi.org/10.14411/eje.2017.039
16. Endler JA, Mielke PW. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society. 2005; 86(4):405–431. https://doi.org/10.1111/j.1095-8312.2005.00540.x
17. Ramsay J, Silverman BW. Functional data analysis. 2nd ed. Springer Series in Statistics. New York: Springer-Verlag; 2005. Available from: www.springer.com/gp/book/9780387400808.
18. Rivas T, Matías JM, Taboada J, Ordóñez C. Functional experiment design for the analysis of colour changes in granite using new L*a*b* functional colour coordinates. Journal of Computational and Applied Mathematics. 2011; 235(16):4701–4716. https://doi.org/10.1016/j.cam.2010.08.005
19. Masner L. Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). The Memoirs of the Entomological Society of Canada.1976; 108(S97):1–97 https://doi.org/10.4039/entm10897fv
20. Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, et al. Evolutionary History of the Hymenoptera. Current Biology. 2017; 27(7):1013–1018. https://doi.org/10.1016/j.cub.2017.01.027 PMID: 28343967
21. Tkalcic M, Tasic JF. Colour spaces: perceptual, historical and applicational background. vol. 1. IEEE;2003

22. Schanda J.(Ed). Colorimetry: understanding the CIE system. New Jersey: John Wiley & Sons; 2007.
23. Melgosa M, Trémeau A, Cui G. Colour difference evaluation. Advanced color image processing and analysis New York: Springer New York; 2013. p. 59–79.
24. Mahy M, Van Eycken L, Oosterlinck A. Evaluation of uniform color spaces developed after the adoption of CIELAB and CIELUV. Color Research & Application. 1994; 19(2):105–121.
25. Renoult JP, Kelber A, Schaefer HM. Colour spaces in ecology and evolutionary biology Biological Reviews. 2017; 92(1):291–315.
26. Causeur D, Sheu CF, Chu MC, Rufini F. ERP: Significance analysis of event-related potentials data; 2018. Available from: https://CRAN.R-project.org/package=ERP.
27. Gorecki T, Smaga L. fdANOVA: Analysis of variance for univariate and multivariate functional data; 2018. Available from: https://CRAN.R-project.org/package=fdANOVA.
28. Wickham H. tidyverse: Easily Install and Load the'Tidyverse' R package version 1.2.1. 2017. url:https://CRAN.R-project.org/package=tidyverse
29. Whibley AC, Langlade NB, Andalo C, Hanna AI, Bangham A, Thébaud C, Coen E. Evolutionary paths underlying flower color variation in *Antirrhinum*. Science. 2006; 313(5789):963–966. https://doi.org/10.1126/science.1129161 PMID: 16917061
30. Galván I, Wakamatsu K. Color measurement of the animal integument predicts the content of specific melanin forms. RSC Advances.2016; 6(82):79135–79142. https://doi.org/10.1039/C6RA17463A
31. Shawkey MD, D'Alba L. Interactions between colour-producing mechanisms and their effects on the integumentary colour palette. Philosophical Transactions of the Royal Society B: Biological Sciences. 2017; 372(1724):20160536. https://doi.org/10.1098/rstb.2016.0536
32. Arenas LM, Walter D, Stevens M. Signal honesty and predation risk among a closely related group of aposematic species. Scientific Reports. 2015; 5:11021. https://doi.org/10.1038/srep11021
33. R Core Team RFfSC. R: A Language and Environment for Statistical Computing; 2018. Available from: http://www.R-project.org/.

Capítulo 4

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Connecting biology, optics and nanomechanical properties in micro-wasps

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Coloration in insects provides a fruitful opportunity for interdisciplinary research involving both physics and biology, and for a better understanding of the design principles of biological structures. In this research we used nanometric and micrometric analyses to investigate the morphological and mechanical properties of the black-orange-black (BOB) color pattern in scelionid wasps, which has never been studied. The primary objective of the present investigation was to explore the structural and mechanical differences in the mesoscutum of four species: *Baryconus* with an orange mesosoma (i.e. BOB pattern), all black *Baryconus*, *Scelio* with an orange mesosoma (i.e. BOB pattern), and all black *Scelio*. The most outstanding findings include the absence of multilayer structures that generate structural color, a pigment concentrated in the upper surface of the epicuticle, and surprising differences between the four species. Three of the four species showed an accordion-like structure in the furrow (notaulus), whereas the adjacent mesoscutum was different in each species. Moreover, the normalized color component spectra for blue, green and red colors of the black mesoscutum of each genus showed the same spectral dependence while the orange color manifested small changes in the dominant wavelength, resulting in slightly different orange tones.

Biological systems on virtually any scale provide real opportunities for advancing our understanding of both the physics and biology of organisms, serving as models and trusted sources of inspiration. Within this wide range of biological examples, insects stand out. They are some of the oldest animals on the planet, having survived a diverse range of environmental conditions. Among the insect features allowing for fruitful interdisciplinary research are their colors.

Coloration in insects can be generated by numerous forms of surface and epidermal structures (structural colors), by the deposition of different chemical pigments in the outer body layers that selectively reflect, absorb or scatter specific wavelengths of light or by a combination of both mechanisms. Pigments are responsible for most of the yellow, orange, red, and brown-black colors observed in insects while most green or blue colors result from nanostructured features that reflect these colors[1]. To accomplish these intricate designs, cuticle must be an adaptable and versatile material, being light, tough, rigid, flexible, elastic, rubbery, solid or porous, isotropic or anisotropic, as the needs required[2].

Compared with Lepidoptera and Coleoptera, there are relatively few studies of morphological and mechanical properties of cuticle color in Hymenoptera, and they are mostly restricted to Vespinae[3–6]. However, it has long been recognized that many hymenopterans, especially scelionids, show a recurring color pattern of black head, orange mesosoma, and black metasoma (BOB) (Fig. 1)[7] and appears to occur in 90% of the currently known species of *Chromoteleia*, 70% of *Acanthoscelio* and *Triteleia*, 50% of *Baryconus*, 40% of *Pseudoheptascelio*, 30% of *Opisthacantha*, *Scelio* and *Sceliomorpha*, and 15% of *Macroteleia*[8]. However, no final conclusions about the function and mechanism of this color pattern have been drawn so far. It seems unlikely that this color is used in inter-sexual communication since both sexes usually have the same color, and most of the intraspecific variation reported included color variation within the same sex[7]. The BOB color pattern has been documented to occur in at least 23 families of Hymenoptera; while scelionid larvae are parasitoids of insect eggs, this color pattern occurs

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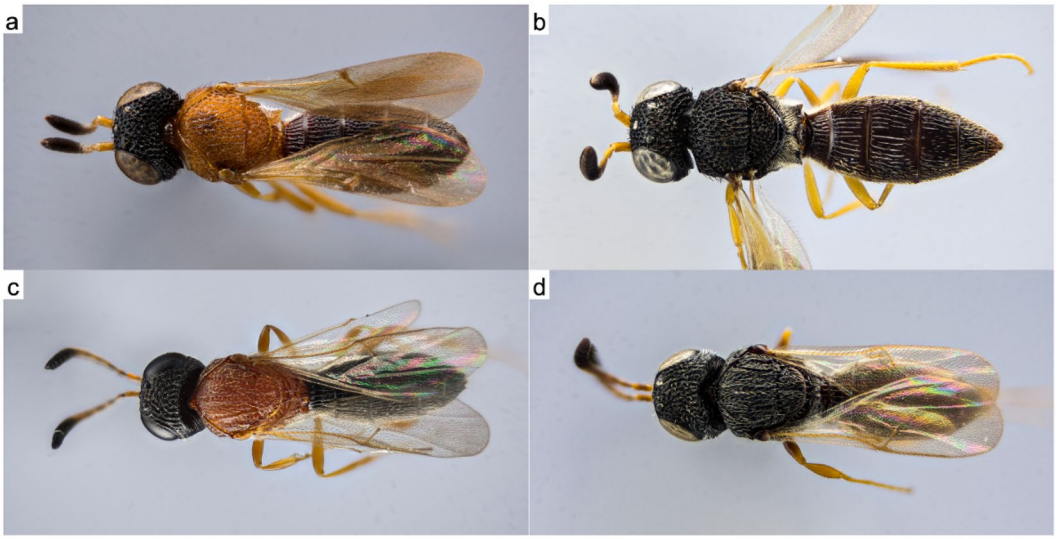

Figure 1. Macrophotography of dorsal color patterns of two scelionid genera. Macrophotography of dorsal color patterns of two scelionid genera obtained with a Reflex Camera 850, 20X microscope lenses and results of focus stacking of 180 captures. Genus *Scelio* (**a**) orange morph (SO), (**b**) black morph (SB) and genus *Baryconus* (**c**) orange morph (BO), (**d**) black morph (BB).

even in some sawflies, which have phytophagous larvae. Hymenopterans with the BOB color pattern are mostly small in size (between 3 mm and 10 mm) and are especially common in the Neotropics where they occur primarily at lower elevations; as far as known they are diurnal[7,9].

Obtaining fresh specimens of scelionid wasps with BOB coloration for chemical analysis is extremely difficult since the hosts (eggs of crickets and related insects) are nearly impossible to find in the field and no laboratory cultures are currently available. At the present time fresh specimens can only be obtained by sweeping the vegetation with an insect net, which requires many hours to obtain just a few specimens. For this reason a previous study used microspectrophotometry to study the spectral properties of the orange and black colors, and the results suggested that there is a common compound for the pigments[10]. The primary objective of the present investigation was to further explore the physical basis of these colors; more specifically, our aim was to investigate the structural and mechanical differences between orange and black mesosoma in each of two genera, *Scelio* and *Baryconus*. In addition, an understanding of the nanomechanical properties of the cuticle of these micro-wasps may provide valuable background information and suggest directions for future research.

Results

Scanning electron microscopy, cryofracture and eosin hematoxylin. Different magnifications of the SEM images show the main features of the morphology of the specimens coated with a thin layer of gold to avoid charging. The cuticle of all specimens of both morphs is quite intricate and sclerotized (Fig. 2a,f,k,p) as reported previously[9]. In the small fragment that was analyzed, one main structure stands out in both genera and both morphs (black and orange): pentagonal and/or hexagonal shaped structures. Observing in more detail shows either rosette-shaped structures with six pentagonal and/or hexagonal projections as in *Scelio* (Fig. 2a–c,f–h) or a repeatable and symmetrical pentagonal and/or hexagonal covering along the entire surface as in *Baryconus* (Fig. 2k–m,p–r). Several of these rosettes or repeatable structures showed a central pore, some of them featuring setae. Another evident structure in SB and BO is a furrow, known in the entomological literature as the notaulus (Fig. 2g,l). However, the cuticle as a whole in BB seems to be less flat exhibiting an undulating surface (Fig. 2r).

As mentioned before, the cuticle of these specimens is far from being flat and simple, even the thickness varies in the different parts of the mesoscutum (part of interest). It presents many irregularities and the subsidences, hooks, peaks and curvatures are manifested in the internal architecture of all layers of the cuticle (Fig. 3a–f). Cryofractures of the dorsal thorax (mesonotum) cuticle revealed a total thickness of about ≈28 μm–50 μm (BO) (Fig. 3h) and ≈15 μm–40 μm (SB) (Fig. 3g). The cuticle of BO is composed of approximately 15–19 lamellae (Fig. 3h) whose thickness diminishes as one proceeds from the outer covering inwards while the cuticle of SB consists of 17–23 lamellae (Fig. 3g). The outermost part of the cuticle, the epicuticle, appears as a compact and homogenous layer of ≈0, 5–1 μm in BO and SB. Multilayer structures or other interference structures were not observed in this area, neither in the black or orange morphs. It is important to mention that in this layer clearly deposited black and orange pigments were observed (Fig. 3a,b). Interestingly, in SB a very subtle orange color was also observed embedded in the lower layers of the epicuticle, as if it was embedded below the black pigment in lower amounts (Fig. 3a). With respect to the procuticle there are two conformations clearly present, the lamellae mentioned before (lower layers of the procuticle) and another ordering, just below the epicuticle: in SB it appears to be composed of an intralayer microstructure of irregular compartments (Fig. 3g), differing moderately from what was observed in BO, which appears more like a longitudinal columnar pattern (Fig. 3h). In both genera, in the deepest part of the procuticle, what some authors previously called the endocuticle, a stack-like arrangement

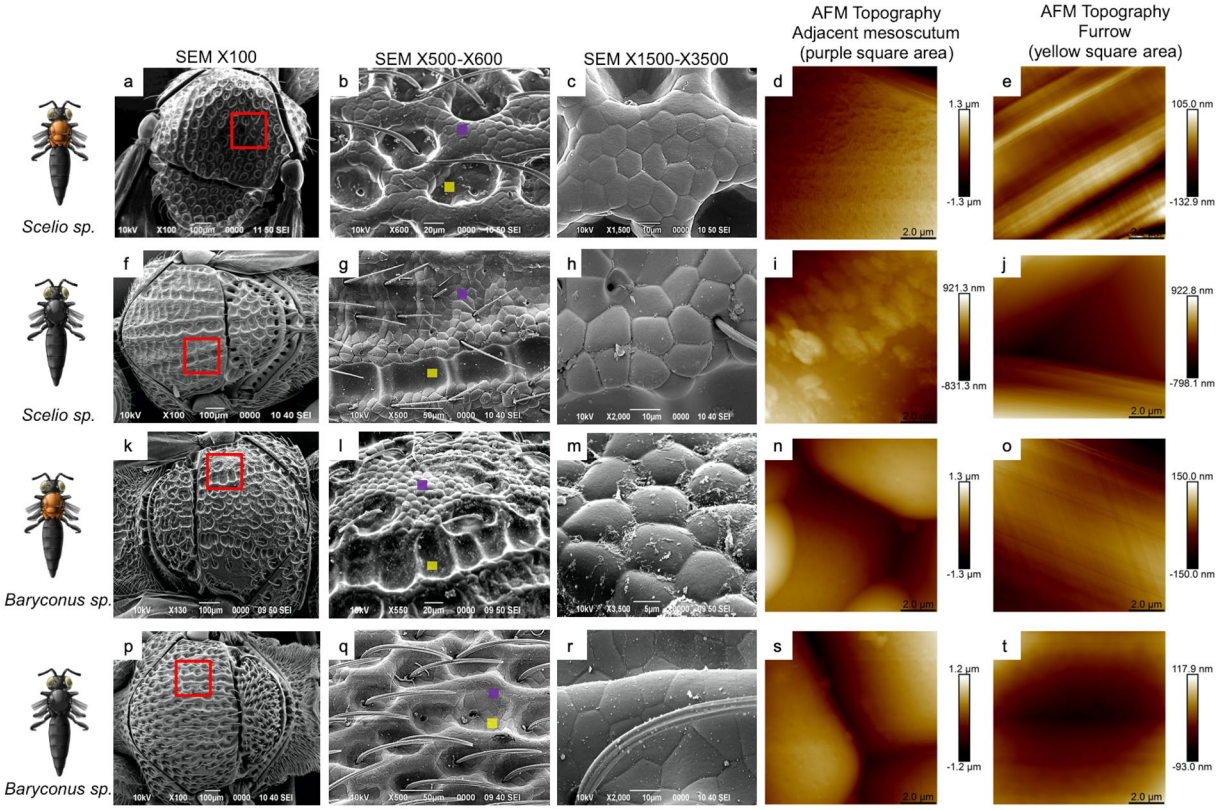

Figure 2. SEM and AFM topography of *Scelio* and *Baryconus*. SEM and AFM topography for the mesosoma of: *Scelio* orange (SO) (**a–e**), *Scelio* black (SB) (**f–j**), *Baryconus* orange (BO) (**k–o**) and *Baryconus* black (BB) (**p–t**). The red box points out the area studied at different scales in SEM and the small purple and yellow filled squares indicate the precise spot, from the red marked area, analyzed with AFM.

of lamellae (Fig. 3i) is observed, layers appearing to be of equal thickness, but the space between the individual layers varies.These layers appeared chitin based and it seems that between those layers a filling, perhaps proteinaceous, could be present. Another noticeable detail is that the layers seem divided by frequent ringed partitions. According to Fig. 3j, which is a magnification of the possible filling material mentioned before, it appears to contain fibers, of possible protein origin, with mesh-like and irregular configurations.

Reflectance spectra and spectral components. Reflectance spectra for black and orange segments are shown in Fig. 3k. As in cryofracture, the eosin hematoxylin technique revealed features of the internal structure of the cuticle. In those structures, the black pigment seems to have a more compact and localized distribution at the upper edge of the epicuticle (Fig. 3a) than the orange pigment (Fig. 3b). Because of the relatively low reflectance values, it can be inferred that most of the light is dispersed within the cuticular structure, and that the spectral characteristics are generated mainly by the pigment in the epicuticle. Therefore the reflectance spectra correspond primarily to information obtained from epicuticular structures within a thickness of no more than 10 μm.

For short wavelengths, the curves have a similar behavior up to 520 nm approximately. For longer wavelengths, there is a clear difference between orange and black, the two black samples being very similar. In terms of spectral components (Fig. 3l), there is no difference between the blue components even when considering different genera (*Baryconus* vs. *Scelio*) or even different colors (black vs. orange). Differences between curves become appreciable in the green and red components. In such cases orange and black reflectance curves are different, but are the same in terms of genus, i.e., *Scelio* orange (black) has the same spectral behavior as *Baryconus* orange (black). In a prevous work[10] a statistical analysis showed that these observed differences and similarities are statistically significant even when considering factors such as different specimens and measurement spots. Thus, the normalized color component spectra for the blue, green and red colors, of the black mesoscutum on each genus, show the same spectral dependence. This means that both blacks are the same with the only variation being the hue of the color. Irrespective of the genus or the orange vs black mesoscutum, the blue component had the same spectral dependence, with only differences in the area when not normalized. In the case of the color component for the orange variation in both genera, similar behavior is observed, but small changes in the dominant wavelength are observed, resulting in slightly different orange tones. Further analyses, which also include other genera of scelionid wasps, can be found in a previous publication[10].

Atomic force microscopy. Figure 2 shows the morphology measured by means of two techniques: scanning electron microscopy (explained above) and atomic force microscopy. For the latter, a peak force method was used in

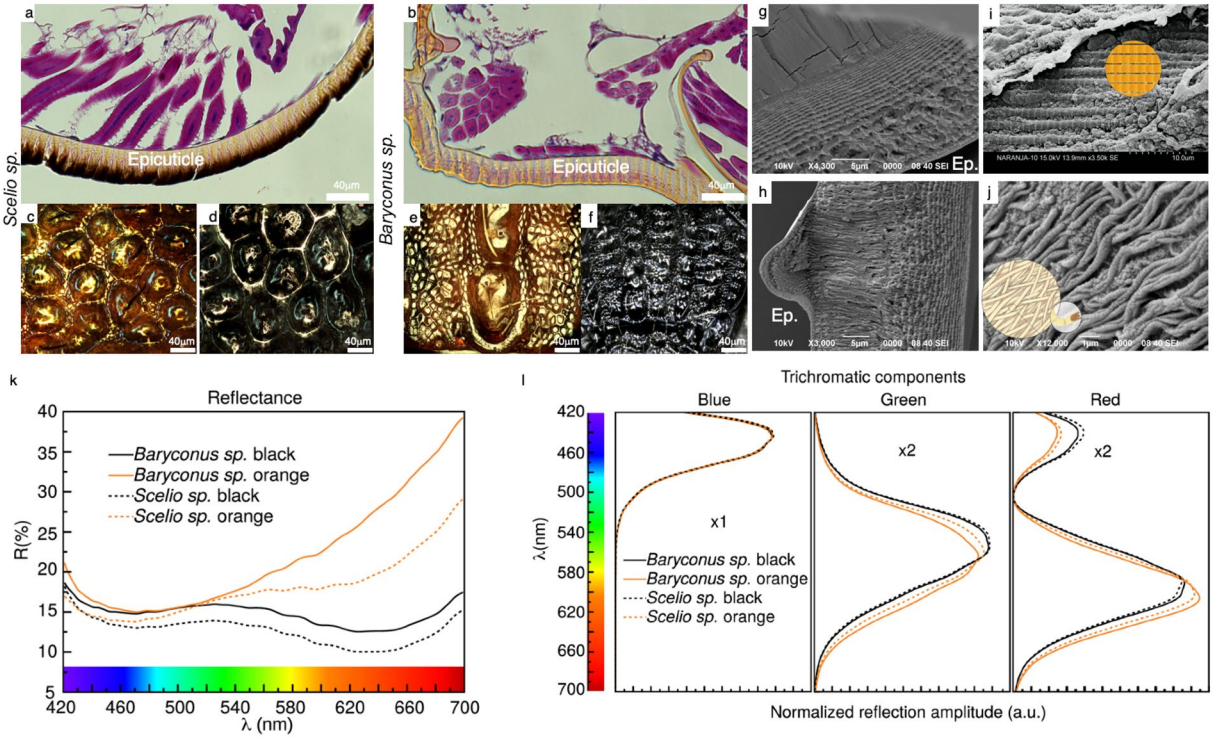

Figure 3. Optical image of *Scelio* and *Baryconus*. As a result of eosin hematoxylin technique, the mesosoma of: *Scelio* black (**a**), orange (**c**), black (**d**) and *Baryconus* orange (**b**), orange (**e**), black (f) is observed. Ep: epicuticle. Cryofractures of *Scelio* black (**g**) and *Baryconus* orange (**h**), highlighting details: *Scelio* showing lamellae (**i**) and *Baryconus* showing fibers (**j**). Reflectance spectra for black and orange mesosoma are shown in inset (**k**), while inset (**l**) shows the corresponding spectral components.

order to generate the image. Two distinct regions and their features were measured, inside the furrow (notaulus) and adjacent mesoscotum, which corresponds mostly to the hexagonal and/or pentagonal structures mentioned before. The AFM images show the same morphological features as in SEM but with better detail of the surface characteristics, because there is no need for a gold coating or exposure of the sample to vacuum as in the SEM preparation. The peak force method allows the tip to be submerged into the water layer covering the sample without affecting the topographic information. The surfaces in general appear to be very smooth in the nanometric scale. The roughness inside the furrow is approximately: 24.5 nm (SO), 23.9 nm (BO), 31.5 nm (SB) and 25.9 nm (BB). For the adjacent mesoscotum, the roughness values are 181 nm (SO), 211 nm (BO), 362 nm (SB) and 323 nm (BB). The hexagonal and/or pentagonal structures in *Scelio* (approx. 12 μm each) are more flat and regular with respect to *Baryconus* (approx.10 μm each), in which those features are more rounded. Figure 4 shows the detail of the *Baryconus* orange morphology. The hexagonal and/or pentagonal features outside the furrows show a surface densely covered with pores, with an outermost diameter of approximately 0.5 μm. It is not possible to quantify the depth of these pores because of the limitation of the aspect ratio of the tip with respect to dimensions of the pores. The roughness between the pores is 1.3 nm. Inside the furrow, a fiber like structure is shown. The Fast Fourier Transform analysis shows a preferential direction in the ordering of the fibers, in which an angle of 47° was measured.

Figures 5 and 6 show the morphological and nanomechanical images of the BOB pattern for the two genera. The figures include the Height, DMT-modulus and Adhesion information. Figure 7 is a graphical representation in 3 D modeling of the six major structures that were observed by means of peak force AFM in Figs. 5 and 6. In the case of the furrows for the two genera, there are two variations, an accordion-like or soft connecting structures. For the adjacent mesoscutum there are four types: granulated stacked accordions, sponge-like, lamellae or accordion-like structures.

The *Baryconus* orange mesoscutum presents two distinct structures, the granulated agglomerations and the furrow between them. The furrow is a soft connecting structure with a DMT-modulus on the order of 50 MPa. The granulated structure that comprises the mesoscutum has rounded structures (more hexagonal/polygonal-like in SEM) approximately 12 um of diameter and presents a DMT-modulus on average 10 times higher. Each grain shows a layered structure that resembles a laminated or stacked spring. All structures together form a resonant like chamber.

The *Baryconus* black presents two similar structures for the furrow and adjacent mesoscutum, and both structures resemble an accordion. The furrow has intercalated layers of stiff and soft material more or less parallel and regular with respect to each other that lie between the furrow structures with a thinner intercalated layered structure with undulated shapes. The furrow layers are almost double in thickness with respect to that of the adjacent mesoscutum.

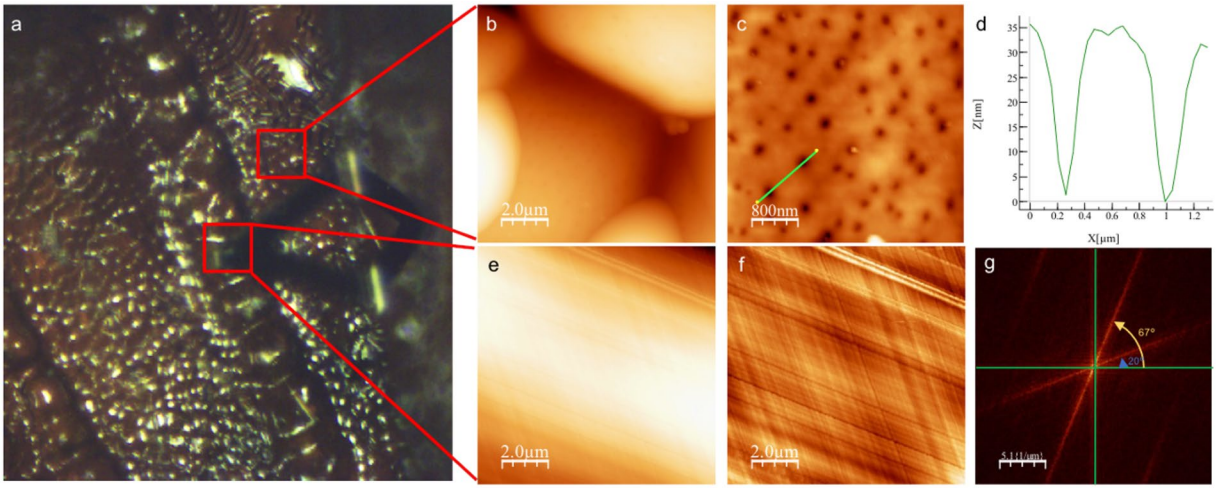

Figure 4. *Baryconus* orange AFM topography. (**a**) Optical image of *Baryconus* orange mesosoma. (**b**) Adjacent mesoscotum, which corresponds mostly to the hexagonal and/or pentagonal structures. (**c**) High resolution of the surface of a granule. (**d**) Profile of two pores. (**e**) Inside the furrow. (**f**) Image **e** with a second order filter where a fibrillar structure is shown. (**g**) Fast Fourier Transform of image **f**.

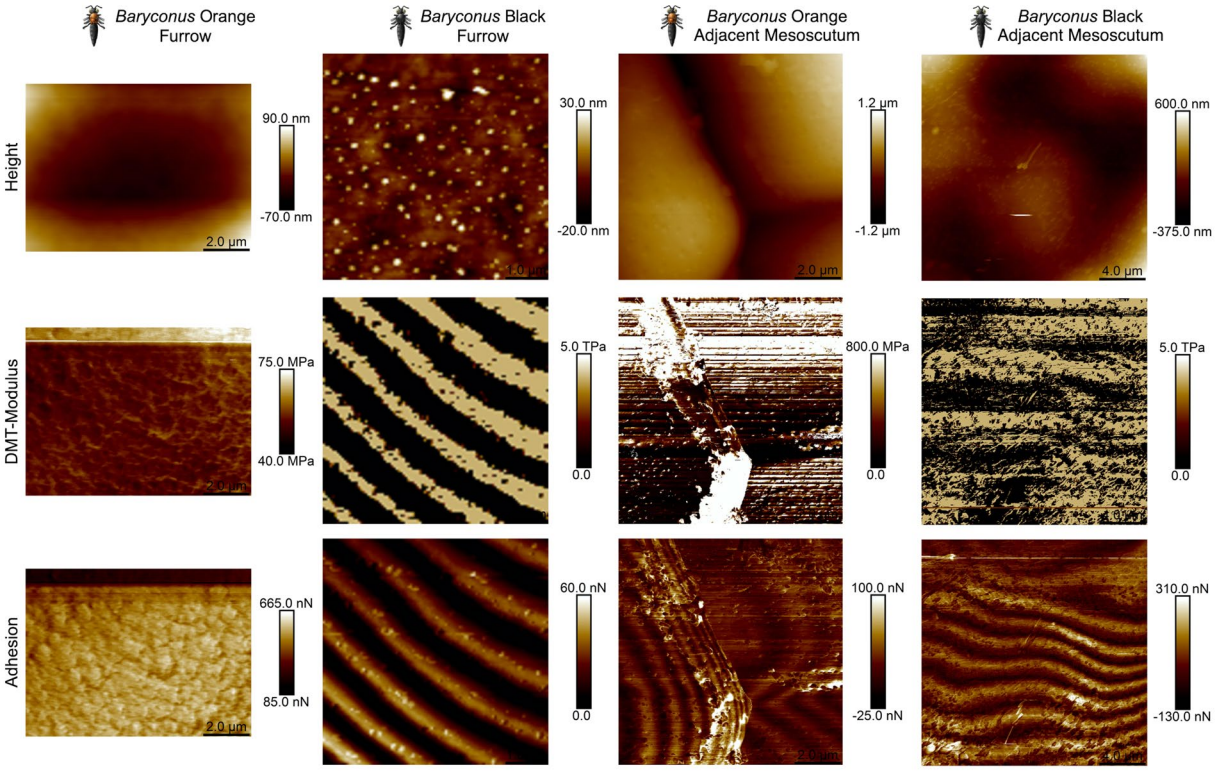

Figure 5. Peak Force AFM images for *Baryconus* orange and black mesosomas. Peak Force AFM images for the *Baryconus* orange and black mesosomas where the rows are ordered from top to the bottom as follows: Height, DMT-modulus and Adhesion. The columns are grouped in pairs as furrow (notaulus) and adjacent mesoscutum for the orange (BO) and black (BB) variations of *Baryconus*.

The *Scelio* orange has a similar furrow structure as the *Baryconus* black, but the furrow resemble a sponge-like structure, with a composite of soft and hard materials. Finally, the *Scelio* black presents the same accordion-like furrow, but the adjacent mesoscutum structure is composed of large lamellae with intercalated layers of stiff and soft material. There are much larger structures than those found in the *Baryconus* orange furrows.

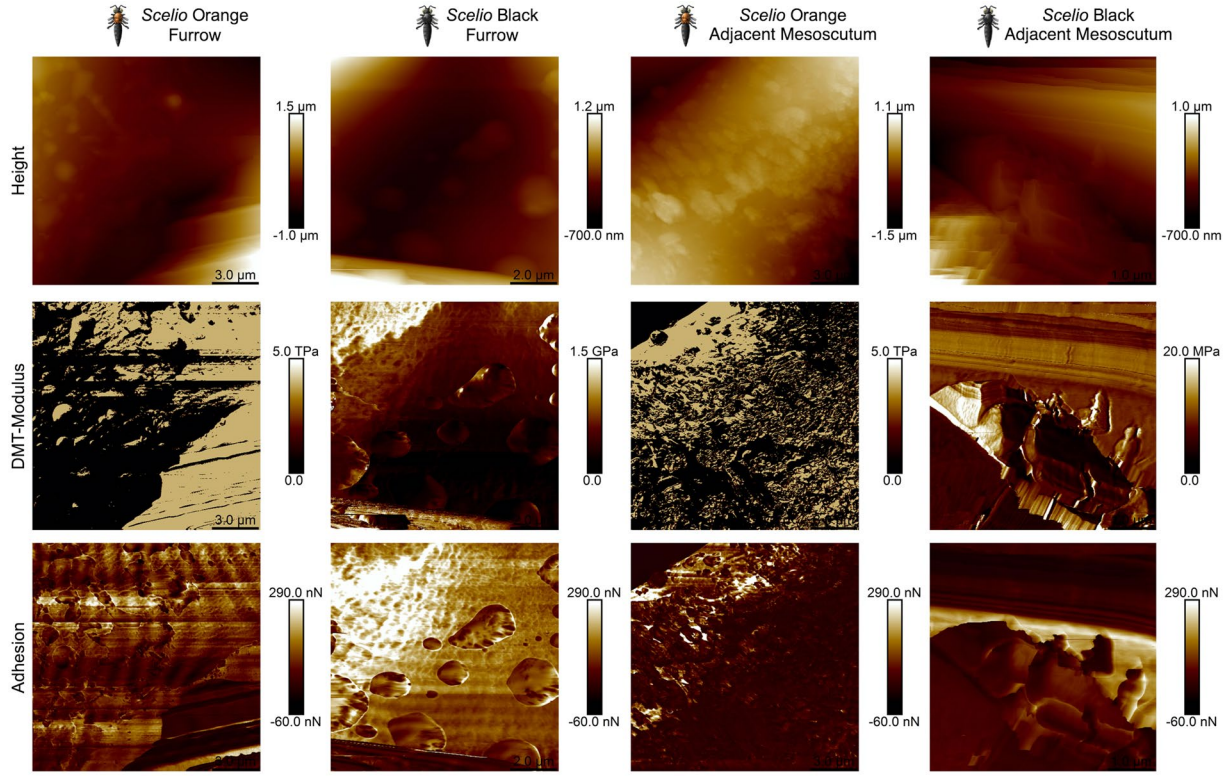

Figure 6. Peak Force AFM images for *Scelio* orange and black mesosomas. Peak Force AFM images for the *Scelio* orange and black mesosomas where the rows are ordered from top to bottom as follows: Height, DMT-modulus and Adhesion. The columns are grouped in pairs as furrow (notaulus) and adjacent mesoscutum for the orange (SO) and black (SB) variations of *Scelio*.

Discussion

The cuticular structure of very small insects, such as the micro-wasps examined here, has generally been neglected. Comparing our results with those of a much larger wasp (*Vespa orientalis*)[3,4,11] some similarities are evident. For example, the cuticle of the specimens studied here exhibit a composite material in which protein-like matrices, lamellae and fibers were observed. Other characteristics also show certain similarities, for example the gradually decreasing thickness of lamellae, similar ranges of thickness of cuticular layers and the presence of pores. Some of the surface characteristics are widespread in insects; for example, pores have been reported in various beetle families (Coccinellidae, Dystiscidae, Lucanidae, Scarabaeidae) but the microstructure and the density of these pores can vary[12,13]. Orange and black colors are generally due to pigments as opposed to interference structures in the cuticle[1] and the results of the present investigation clearly suggest that these colors in scelionid wasps are not structural in nature. To the best of our knowledge, Mutillidae is the only group of Hymenoptera in which the chemical basis of BOB coloration has been examined, namely orange pheomelanins and black eumelanins[14]. The fact that all blue components of the spectral analysis coincide, while the green and red components differ between black and orange, suggests that there is a shared chemical composition. Thus, information obtained from the reflectance spectra suggests that the coloration is due to pigments that are closely related in terms of chemical composition, as discussed in a previous work[10], and that such pigments are encapsulated within the epicuticle (Fig. 3a,b).

Nonetheless, optical micrographs of the cuticular surface of the corresponding mesoscutum (Fig. 3c–f), show that the coloration is not necessarily homogeneous. This is especially evident for the orange mesoscutum of both *Scelio* and *Baryconus*, and this effect could also account for the similarities found between orange and black reflectance spectra.

One of our objectives was to ascertain how the properties of black cuticle differ from those of orange cuticle. However, it should be noted that the results from the Peak Force AFM technique are composed of different contributions (structure, topography and chemical composition) and should be interpreted within this holistic context. In order to clarify whether the color has an influence on the nanomechanical properties, the chemical composition of the pigments needs to be determined and then more experiments should be carried out. In fact, when comparing the four possible combinations (orange vs black and *Baryconus* vs *Scelio* the rather surprising result was that each combination is quite different (Fig. 7). The orange mesoscutum in *Baryconus* (Fig. 7A), is made up of granular structures, each rounded structure consisting of alternating pliable and rigid layers, while the furrow (notaulus) appears to be a softer connecting structure. The black mesoscutum in *Baryconus* (Fig. 7B) has an accordion-like structure in both the furrow and the adjacent mesoscutum, the latter being more rigid. The furrow has intercalated layers of stiff and soft material, more or less parallel to each other, and almost double in

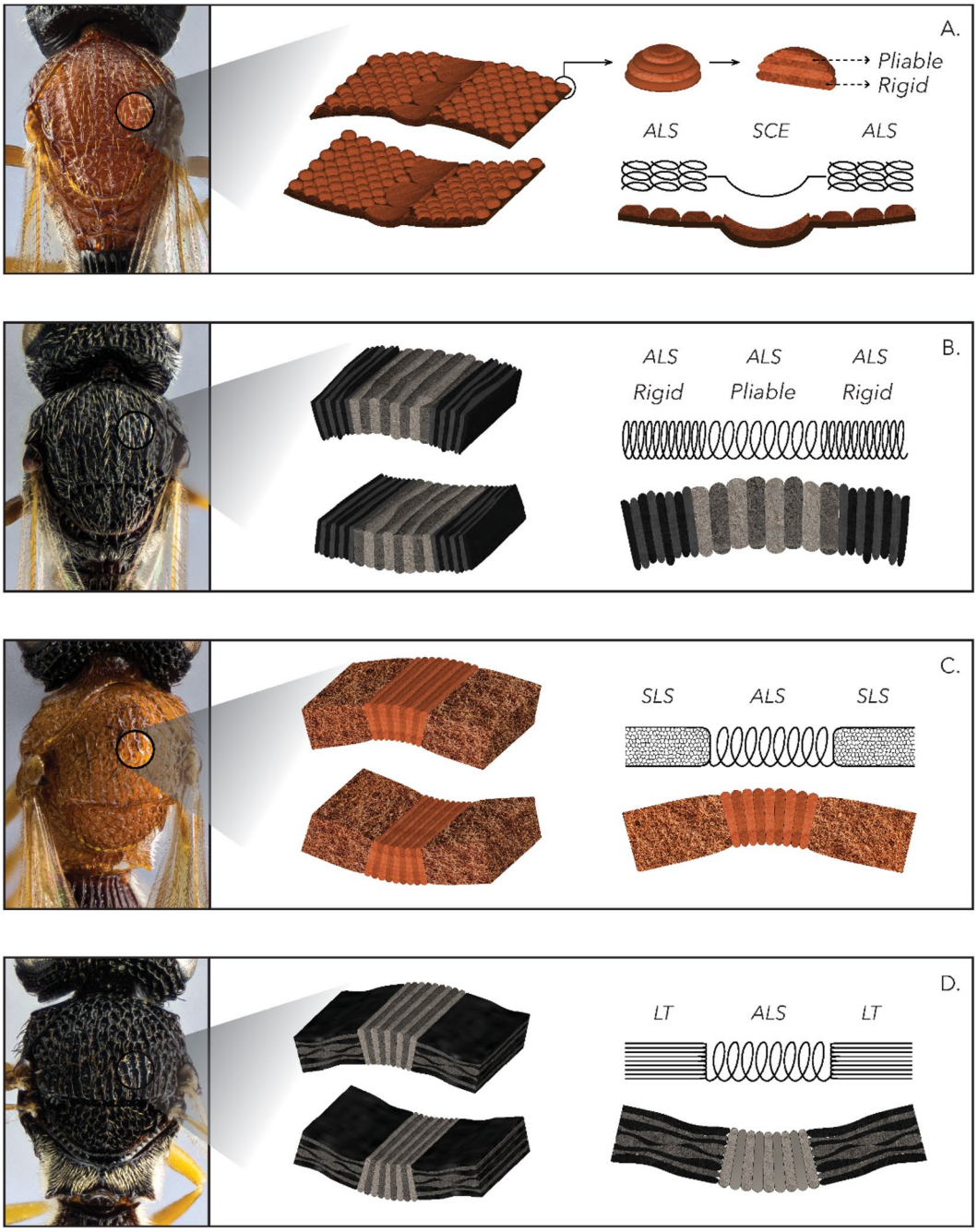

Figure 7. Graphic representation of structures observed by means of AFM. Graphic representation in 3 D modeling of the six major distinct structures that were observed by means of peak force AFM in the orange and black specimens of *Baryconus* (**A,B**) and *Scelio* (**C,D**). In the case of the furrows for the two genera, there are two variations, accordion-like structures (ALS) (**B–D**) or soft connecting structure (SCE) (**A**). For the adjacent mesoscotum there are four variations, granulated stacked accordion within an accordion-like structure (ALS) (**A**), accordion-like structures (ALS) (**B**), sponge-like structures (SLS) (**C**) and lamellae terraces (LT) (**D**).

thickness with respect to the adjacent mesoscutum. The orange mesoscutum of *Scelio* (Fig. 7C), has a sponge-like structure, with a composite of soft and hard materials; the furrow has an accordion-like structure. Finally, the black mesoscutum of *Scelio* (Fig. 7D), consists of horizonal layers of lamellae, with intercalated layers of stiff and soft material; the furrow has an accordion-like structure similar to the previous two combinations.

Presumably all four combinations discussed above permit the flexibility required as the mesothorax becomes deformed during flight (due to the action of the indirect flight muscles), but further research is required to better understand the notable differences observed in Fig. 7. Within the mesoscutal furrow of *Baryconus* a fiber-like structure was found with a preferential direction in the ordering of the fibers showing an angle of 47°, Fig. 4f,g.

Microspectrophotometry technical details	Information
Light source	12 V–100 W halogen lamp
Magnification	5X, CFI60 2 TU Plan Fluor BD,
	N.A 0.15, WD 18.0 mm, (Nikon,Tokyo,Japan)
White reference	Spectralon Diffuse Reflectance Standard
	USRS-00-010 (Labsphere, North Sutton,NH, USA)
Dark reference	Internal attenuators
Software for spectral capture	Lambda Fire (CRAIC,Los Angeles,USA)
Integration time	150 ms
Number of spectra averaged	100

Table 1. Information about the analysis of color data.

The value of the angle between fibers has been reported to indicate higher ductility of the material[15] meaning that this characteristic allows greater deformation with respect to other structures[15,16].

Biological studies are required to determine whether the BOB pattern serves as a warning (aposematic) coloration for potential predators and whether the black colors play a role in thermoregulation. Addressing these questions would be greatly facilitated by being able to rear scelionids in the laboratory, which will require rearing of their hosts, namely the eggs of crickets or katydids. Lab rearing of scelionids would also allow studies of cuticle development, for example cuticle from adults recently emerged from the pupa versus older wasps. Moreover, phylogenetic studies of scelionids as a whole, and of individual genera, would allow us to address the question of how many times this coloration has evolved.

Many additional questions remain regarding the BOB color pattern that is so prevalent among scelionids. How do the contrasting black and orange colors interact in the visual system of potential predators or other receivers of these visual signals? How do the superficial and internal structures reported here interact? What are the pigments producing the orange and black, to what degree do pigments vary among scelionids (and other wasps showing the BOB pattern), and to what extent do the structures in which the pigments are deposited affect the color as viewed by signal receivers. A few species show color polymorphism[7], a phenomenom that poses numerous additional questions. We obviously have much more to learn from these micro-wasps.

Methods

Specimens and samplig. Live specimens of Scelionidae are extremely difficult to find in the field because of their small size and concealed nature of their hosts (insect eggs). In order to obtain fresh specimens with internal and external structures in good condition, our field collecting focused on two common scelionid genera, *Scelio* and *Baryconus*, which were captured with fine entomological nets. All experimental protocols were carried out in accordance with the institutions' guidelines and regulations. The taxonomic classification of the specimens was carried out in the laboratory on the same day of the collection. Collecting was done in El Rodeo, which is part of the Mora County (Municipality), located 30 Km southwest of San Jose, Costa Rica, within a natural reserve of a secondary forest and a remnant of primary forest (200 ha) in the Central Valley of Costa Rica. This site provided the greatest abundance (1 to 3 specimens collected per hour) and diversity of the group (eight Scelionidae genera were found with BOB color pattern), which is why it was chosen as the definitive field site for this study. The specimens were freeze-killed and immediately analyzed with the techniques mentioned below.

Five complementary techniques were used in this study and were intended to provide the data and information needed to generate a design principle that may elucidate the nature of the orange and black colors in Scelionidae: micro spectrophotometry, eosin hematoxylin, scanning electron microscopy, cryofracture and atomic force microscopy. A minimum of three specimens of each genus were analyzed with each technique. The techniques analyzed fragments of no more than 3 mm extracted with entomological microneedles from the mesosoma, directly in the mesoscutum of the specimens.

Microspectrophotometry and spectral components. In order to study the spectral characteristics of the cuticle of these insects, we used a microspectrophotometer (508PV, CRAIC), in the visible - near infrared portion of the electromagnetic spectrum, coupled to a trinocular microscope (Eclipse LV200ND, Nikon) in episcopic illumination mode. Reflectance spectra were normalized using a Spectralon white reference (see Table 1). Rather than color coordinates in a predetermined color space, we used the CIE 1931 color-matching functions (CMFs) to obtain three components for each reflectance spectrum in a RGB manner. Further description of this analysis can be found in ref. [10]. Components were defined in the following way:

$$R(\lambda) = \frac{Y(\lambda)\overline{x}(\lambda)}{\int Y(\lambda)\overline{x}(\lambda)d\lambda},\ G(\lambda) = \frac{Y(\lambda)\overline{y}(\lambda)}{\int Y(\lambda)\overline{y}(\lambda)d\lambda},\ \text{and}\ B(\lambda) = \frac{Y(\lambda)\overline{z}(\lambda)}{\int Y(\lambda)\overline{z}(\lambda)d\lambda}, \quad (1)$$

where $Y(\lambda)$ is the reflectance spectrum as a function of wavelength λ and $\overline{x}(\lambda)$, $\overline{y}(\lambda)$ $\overline{z}(\lambda)$ are CMFs regarding the CIE 1931 standard (2°) colorimetric observer.

Even though the CMFs are related to human visual sensibility, the aim of studying the reflectance spectra by components is to access information about differences in the chemical composition of the pigments involved in

coloration and that might be inferred from the spectral characteristics of the reflectance. As discussed in a previous work by our group[10], the study of optical properties is related to the interaction of the electronic structure of the material with electromagnetic radiation, and therefore is an indirect chemical characterization. Morever, this procedure can be eventually adapted to study the relevance of spectral characteristics when considering visual sensibilities of a given insect.

Eosin hematoxylin. Each of the specimens was fixed in 10% formalin, where they were preserved until they were processed. Subsequently, a post fixation in formaldehyde, acetic acid, ethanol solution was carried out for two hours. Dehydration was carried out in alcohols with increasing concentration (70%, 95%, 100%). Clarification was implemented with xylol and later the sample was impregnated with paraffin. Once the specimens were processed, they were included in paraffin blocks. Continuous cuts were made at 4 microns in thickness and 3 or 4 cuts were placed per slide, using the entire mesoscutum. Hematoxylin and eosin (VENTANA HE 600 system) stains were performed on all sections. The final cuts were observed in an Olympus bx51 microscope and the photographs obtained with an Olympus Dp72 camera.

Scanning electron microscopy. The samples were analyzed using a SEM JSM-5900 LV (JEOL, Tokyo, Japan), voltage 10–20 kV, pressure of 1×10^{-4} Pa. The biological samples were fixed with glutaraldehyde 2%, paraformaldehyde 2% in phosphate buffer (PB) 0.05 M pH 7.2 at 4 °C for 48 hours, washed in PB 0.05 M pH 7.2, post-fixed in OsO4 2% in PB 0.05 M pH 7.2 and dehydrated in a serial gradient of acetone solutions, being finally dried with carbon dioxide in a critical point dryer LEICA EM CPD 300. Lastly, the samples were coated with a 20 nm gold layer in a sputter coater EMS 550X. The images were analyzed at 200 to 2500X magnifications. A carbon tape or freshly cleaved mica on top of a SEM sample holder was used to support the samples.

Atomic force microscopy. Atomic force microscopy images and nanomechanical data were collected using a Multimode 8 microscope (Bruker, USA) operating in PeakForce Tapping mode. The specimens were imaged in room condition using ScanAsyst-Air probes (Bruker) (nominal length 115 μm, tip radius 2 nm, spring constant 0.4 N m^{-1}) for topography and RTESP-300 probes (Brukes) (nominal length 125 μm, tip radius 12 nm, spring constant 40 N m^{-1}). Prior to imaging, the probes were calibrated according to the manufacturer's protocol using the Bruker calibration kit. Images were collected in height sensor, peak force error, Young modulus (DMT model) and adhesion channels. The raw AFM data obtained were processed using Nanoscope Analysis v.1.7. software (Bruker).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Schroeder, T. B., Houghtaling, J., Wilts, B. D. & Mayer, M. It's Not a Bug, It's a Feature: Functional Materials in Insects. *Adv. Mater.* **30**, 1705322 (2018).
2. Andersen, S. O. Cuticle. In *Encyclopedia of insects*, 245–246 (Elsevier, 2009).
3. Ishay, J. S. & Pertsis, V. The specific heat of the cuticle and the morphological differences between the brown and yellow cuticles of hornets. *J. Electron Microsc.* **51**, 401–411 (2002).
4. Ishay, J. S., Joseph, Z., Galushko, D. V. & Bergman, D. J. The nanostructure of the oriental hornet (Hymenoptera, Vespinae) cuticle and silk and some of their biophysical properties. *Curr. Nanosci.* **1**, 125–156 (2005).
5. Plotkin, M., Volynchik, S., Barkay, Z. & Bergman, D. J. Micromorphology and maturation of the yellow granules in the hornet gastral cuticle. *Zool. Res.* **30**, 65–73 (2009).
6. Plotkin, M. *et al*. Solar energy harvesting in the epicuticle of the oriental hornet (vespa orientalis). *Naturwissenschaften* **97**, 1067–1076 (2010).
7. Mora, R. & Hanson, P. Widespread occurrence of black-orange-black color pattern in hymenoptera. *J. Insect Sci.* **19**, 13 (2019).
8. Valerio, A.A. & Johnson, N. F. Poster entitled Species of the colorful genus Chromoteleia Ashmead (Hymenoptera: Platygastroidea, Platygastridae s.l.). In *Entomological Society of America* (2013).
9. Masner, L. & Hanson, P. Familia Scelionidae. *Mem. Am. Entomol. Inst.* **77**, 254–265 (2006).
10. Mora-Castro, R., Hernández-Jiménez, M., Alfaro-Cordoba, M., Avendano, E. & Hanson-Snortum, P. Spectral measure of color variation of black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Scelionidae), a statistical approach. *PloS one* **14**, 10 (2019).
11. Ishay, J. S., Kirshboim, S., Steinberg, D., Kalicharan, D. & Jongebloed, W. L. Hornet cuticle—a composite structure comprised of a series of duplex lamellae attenuating toward the interior of the body. *Comp. Biochem. Physiol. Part A: Mol. & Integr. Physiol.* **120**, 661–670 (1998).
12. Sun, J. *et al*. Quantitative nanomechanical properties of the cuticle of the multicolored asian lady beetle using the modulus mapping technique. *Curr. Nanosci.* **11**, 245–252 (2015).
13. Dai, Z. & Yang, Z. Macro-/micro-structures of elytra, mechanical properties of the biomaterial and the coupling strength between elytra in beetles. *J. Bionic Eng.* **7**, 6–12 (2010).
14. Hines, H. M., Witkowski, P., Wilson, J. S. & Wakamatsu, K. Melanic variation underlies aposematic color variation in two hymenopteran mimicry systems. *PloS one* **12**, e0182135 (2017).
15. Mortazavian, S. & Fatemi, A. Effects of fiber orientation and anisotropy on tensile strength and elastic modulus of short fiber reinforced polymer composites. *Compos. part B: engineering* **72**, 116–129 (2015).
16. Wang, H., Zhou, H., Gui, L., Ji, H. & Zhang, X. Analysis of effect of fiber orientation on young's modulus for unidirectional fiber reinforced composites. *Compos. part B: engineering* **56**, 733–739 (2014).

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Author contributions
R.M.-C. coordinated the field trips, collected the samples and prepared them for the five techniques used, performed the scanning electron microscopy analysis in collaboration with Reinaldo Pereira, cryofracture analysis in collaboration with Cynthia Barboza and micro spectrophotometry with advice from M.H.-J. and E.A.-S., participated in the writing of the manuscript and co-design the 3D modelling graphic representations. G.S.-A. and E.A.-S. performed the experiments, analysis and provided the AFM expertise and participated in the writing of the manuscript, E.A.-S. co-design the 3D modelling graphic representations, M.H.-J. participated in the supervision of optical measurements, calculated the spectral components and contributed to the writing of the manuscript, P.H.-S. suggested potential field trips sites, participated in the taxonomic identification of genera and in the writing of the manuscript, J.P.-P. performed the eosin hematoxylin analysis in collaboration with R.M.-C.

Competing interests
The authors declare no competing interests.

Additional information
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Capítulo 5

Unraveling the function of the black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Platygastridae) through the eyes of jumping spiders (Araneae: Salticidae).

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ABSTRACT

Many small parasitoid wasps have a black-orange-black (BOB) color pattern, which is usually present in both sexes. A likely function of this widespread pattern is aposematic (warning) coloration, but this has never been investigated. To test this hypothesis, we presented spider predators (*Lyssomanes jemineus*), both field-captured and lab-reared individuals, to a BOB species and a black species in four wasp genera (*Baryconus*, *Chromoteleia*, *Macroteleia* and *Scelio*). Each spider/wasp trial was filmed for 40 minutes under controlled conditions and the behavioral responses (detect, attack, avoid) were recorded in each of 136 trials, never using the same predator and prey more than once. On five occasions field-captured spiders consumed a black wasp, but no spider consumed a wasp with BOB coloration. The green component of the reflection spectra of both black and orange colors were measured in the wasps. Among BOB wasps, most "attack" events occurred with *Baryconus*, which also had the lowest absorption contrast, while the greatest number of "detect" events occurred with *Macroteleia*, which had the highest absorption contrast and the highest contribution of orange.

Spiders were also presented with false prey (rice grains painted to match the color of the wasps) in a specially designed arena with an automated mechanism for moving the prey; however, the results were inconclusive. Acute toxicity trials with the water flea, *Daphnia magna*, resulted in a higher mortality when exposed to extracts from BOB wasps than from black wasps, suggesting that the former contain defensive compounds.

KEY WORDS

Scelionid wasps, *Lyssomanes*, black-orange-black coloration, aposematism, reflectance, toxicity tests, predator-prey interaction.

SUMMARY STATEMENT

We provide the first evidence that the widespread black-orange-black pattern in small parasitoid wasps is aposematic, based on behavioral responses of a spider predator and toxicity tests with *Daphnia magna*.

INTRODUCTION

Many small (3-10 mm) parasitoid wasps have a black head, an orange (or reddish orange) mesosoma and a black metasoma. This color pattern has been found in species belonging to 23 families of Hymenoptera (including even phytophagous sawflies) and is especially common in neotropical scelionid wasps (Platygastridae; formerly Scelionidae), but is also common in evaniid wasps from diverse biogeographic regions; moreover, this color pattern is usually present in both sexes (Mora and Hanson, 2019). Within wasp genera showing the black-orange-black (BOB) pattern, only some species have this pattern, the other species usually being totally black (Fig. 1). In previous research it was found that the spectral blue components of the orange and black color in scelionid wasps are almost identical, suggesting that there is a common compound for the pigments (Mora et al., 2019b), but the identity of the pigment remains unknown.

Conspicuous coloration such as the BOB pattern is often, but not always, indicative of aposematism (Skelhorn et al., 2016), whereby predators learn to associate particular color

patterns with noxious chemical defenses, although this learning process is much more complex than simply developing an aversion to specific types of prey (Mora and Hanson, 2019). In some larger insects contrasting black and orange color patterns are known to serve as aposematic (warning) coloration for potential predators, mostly vertebrates (Arenas et al., 2015), and it is possible that the BOB pattern serves as a warning pattern for smaller predators, although this has not yet been tested.

To test whether the BOB pattern serves as aposematic coloration, we chose a common jumping spider (Salticidae) as the predator for our laboratory trials. Jumping spiders have eight eyes functionally adapted as six "secondary eyes" around the carapace that focus large images, relative to photoreceptor spacing, on the fovea and two forward facing "principal eyes" which are camera-type receptors for perceiving shape and pattern. Molecular and electrophysiological data suggest that color vision in the principal eyes of most jumping spiders is based on only two types of photosensitive pigments, one sensitive to ultraviolet (UV) light, the other to green light, although a few of them also exhibit filter-based trichromacy (Foelix, 1996, Harland and Jackson, 2012; Nagata, 2012). These characteristics have allowed salticids to achieve the highest visual acuity thus far measured in any arthropod, with the ability to classify prey into categories, even if they rely solely on vision (Harland et al., 2012; Williams and McIntyre, 1980; Nelson and Jackson, 2012).

Among the various jumping spiders present in our collecting sites, we chose to use *Lyssomanes jemineus* Peckham & Wheeler because it was one of the most common species. *Lyssomanes*, like most salticids, are hunting spiders, primarily insectivorous, and behavioral observations suggest a strong role for vision in predation, although visual acuity in *Lyssomanes* may be somewhat lower than that of other salticids, such as *Portia* (Blest and Sigmund, 1984). *Lyssomanes* species are diurnal foliage dwellers and two hunting behaviors predominate: a sit-wait strategy followed by springing from the underside of the leaf to the upper surface (they usually sit on leaves that are exposed to the sun waiting to ambush prey on the upper surface), and a hunting behavior that consists of exploring both sides of the leaf, actively searching for passing insects (Mora-Castro et al., 2019a). Thus, *Lyssomanes*, like most salticids, can be characterized as a hunting spider that does not use webs, and that executes behavioral responses such as; prey detection by sight, stalking until proximity, and attacking by jumping towards the prey (Forster, 1982; Herberstein, 2011).

Fig. 1. Two scelionid wasp color patterns within the same genus. Color patterns in the genus *Scelio*: black (A), and BOB (B). The focus-stacked macro photography was obtained with a Reflex 850 camera coupled to a 20x microscope lens.

Our specific aim was to understand the function of BOB coloration in nature and for this *L. jemineus* was used as a visual predator and four genera of scelionid wasps were used as prey. Specifically, we tested the behavioural responses of *L. jemineus* to a species with the BOB pattern and a completely black species, in each of the four scelionid genera. From these tests we hoped to address the following questions.

1) Do adult and juvenile spiders collected from the field differ in their predatory behavior when confronted with BOB versus black prey?
2) Is there an innate preference (or avoidance) for BOB versus black prey by adult spiders reared from egg sacs in the laboratory? Such spiders have never encountered

scelionid wasps, thereby minimizing the effects of learning (Nelson and Jackson, 2012).

3) Are there any other behavioral differences when the three types of spiders are confronted with the two-color patterns in their wasp prey?
4) Do false prey items (lures) consisting of moving rice grains (which minimize confounding chemical cues, lack detailed features such as eyes or legs, but which display the relevant color patterns) elicit predatory behavior in the spiders? In other words, are color and movement alone sufficient to elicit predatory behavior in the spiders, and if so, does the response differ when confronted with BOB versus black lures?
5) How stringent are the adult and juvenile spider's visual recognition templates for wasp colors?
6) Do bioassays suggest a difference between BOB wasps and black wasps with respect to potential chemical defences?

MATERIALS AND METHODS

Scelionid collection

The collection site was in the Central Valley of Costa Rica, in a forest patch within the protected zone of El Rodeo, which is located near Ciudad Colon, San Jose (9°52'–9°56'N, 84°14'–84°20'W). The area is composed of secondary forest and a remnant of primary forest (approximately 200 ha). Because the hosts of scelionid wasps (insect eggs) are very difficult to locate, live wasps were obtained via sweeping with insect nets. Collecting samples of wild specimens requires considerable time and effort. Between March 2017 and November 2018 thirty trips were made to the site, and on each visit three people, working for about six hours, obtained four to six scelionid wasps. The latter were promptly used in trials (see below) carried out in a makeshift laboratory at the same site. The wasps were identified by R.M. and P.H. using keys to scelionid genera (Masner, 1976); voucher specimens were deposited in the Museum of Zoology of the University of Costa Rica. Four genera were collected from this site and used in the experiments: *Baryconus* Foerster, *Chromoteleia* Ashmead, *Macroteleia*

Westwood and *Scelio* Latreille. Due to the lack of taxonomic studies for three of the four genera, species level identifications were not carried out.

Salticid collection

Lyssomanes jemineus egg sacs (from which adults in captivity were reared), female adults, and juveniles were collected weekly from January 2017 to January 2019. Collecting was done in Finca 3 of the University of Costa Rica, San Pedro Montes de Oca, San José, Costa Rica (9°56'07''N, 84°03'04''W). This site is located in a disturbed urban environment which exhibits mainly semi-woodland vegetation of native and introduced herbaceous plants (Biamonte et al., 2011). This locality has an average annual precipitation of 1200-1500 mm and an average temperature of approximately 21.0°C. Spiders and egg sacs were collected from 0 to 1.5 m above ground level. Since many of the plants were quite widely spaced, the transect consisted of 25 to 30 locations where diverse herbaceous plants were haphazardly searched. For the collection of egg sacs, weekly samplings of approximately three hours each, were carried out in the months of May to September, according to the reported phenology for this species (Mora-Castro et al., 2019a). Each egg sac (usually located on the underside of the leaf) was collected manually without being detached from the leaf and was quickly transferred to the laboratory using 15 ml sterile Falcon tubes with a humid cotton ball. For the collection of adults the same methodology was applied, but mainly in the months of April to October. Specimens were identified by G.B. Edwards.

Salticid rearing

Several cage designs were tested, but the most successful (in terms of preventing the escape of spiderlings, optimizing environmental conditions, and facilitating the filming of spider behavior) was one made of acrylic, 14 cm x 14 cm and 18 cm high, with two lateral circular areas (10 cm diameter) with small holes for aeration covered with an ultra-fine mesh. Including vegetation in the cages helps mitigate the effects of captivity (Carducci and Jakob 2000), and we found that the best results were obtained by using the same plant species that harbored the egg sac in the field; water was provided in test tubes plugged with cotton. We occasionally observed advanced stage juveniles preying on younger stages, but this behavior was sufficiently uncommon that it was deemed unnecessary to separate specimens individually during the life cycle. However, cannibalism increased as the number of

individuals per cage increased and therefore no more than 10 individuals were maintained in a single cage; they were generally separated during the fourth week after hatching, which is when they usually begin to disperse.

A survival rate of 53% was successfully achieved by providing young spiderlings and juveniles (less than 200 days old) with whiteflies, *Bemisia tabaci* Gennadius, and then transitioning to *Drosophila melanogaster* Meigen; older spiders fed with *Drosophila* showed a survival rate of 94%. Whiteflies were reared on young eggplants (*Solanum melongena*) planted in pots inside aluminum cages that were hermetically closed with anti-aphid fabric. Weekly plant watering and room temperature near 24 º C were crucial. In some cases when populations of *B. tabaci* on eggplant were low we used an undetermined species of *Aleyrodes* found on *Emilia fosbergii, Sonchus oleraceus* and *Sonchus asper*. For *Drosophila* rearing, glass vials with autoclaved *Drosophila* diet (2 bananas, 50 g of oatmeal, 175 ml water and 1.75 ml of propionic acid) were used for rearing at room temperature protected from drafts and direct sunlight.

Observations of spiders with live prey

Field-collected juvenile and adult spiders, as well as adults reared from egg sacs in the laboratory (for about a year), were observed one at a time in an experimental arena (see below). Adult spiders reared in the laboratory had no prior contact with the wasps. Six types of behavior were recorded: 1) prey detection as indicated by swivelling the cephalothorax; 2) following or stalking prey; 3) crouching, jumping and contacting the prey; 4) piercing and ingesting the prey; 5) prey detection followed by withdrawal from the prey; 6) prey undetected or ignored. In the results the first two were combined as “detect” (although detection was not always followed by stalking), three and four were combined as “attack” (although jumping was rarely followed by ingestion), and five and six were combined as “avoid”.

To facilitate observations of such small specimens we used a white background on the floor, but natural light was allowed to enter the remaining walls of the acrylic box. In order to emulate the natural environment as much as possible, all observations were carried out in a makeshift kiosk embedded in the forest in which the wasps were collected. Behavior was filmed with a Sony RX100 IV camera and was done in FullHD 1080 x 1920 in 60fps. Editing was done in Adobe Premiere, the animations and layout in Adobe After Effects.

In order to minimize potential confounding factors, we used the following standardized conditions for all experiments with spiders.

1) We standardized spider sex (adult females only), age (all juveniles were more than 200 days old) and body size (considered in the statistical analysis).
2) To ensure that juvenile and adult female spiders collected from the field would be motivated to feed during testing, we fed the spider and then held it without prey for 8 days before subjecting it to testing. Eight days also ensured that specimens were not weak, unresponsive or otherwise stressed. A faded green body color was sometimes observed in spiders that had fasted for 11 to 15 days and appeared to indicate a suboptimal condition.
3) We used each prey and predator for just a single trial.
4) Every time a test spider entered the arena it remained alone for 50 s before introducing the prey, thereby allowing for a short "acclimatization".
5) Between tests the chamber was washed and cleaned with 80% ethanol, then allowed to dry before beginning the next trial, in order to eliminate any chemical trail of predator or prey since female salticids produce contact and airborne pheromones (Jackson and Cross, 2011; Nelson et al, 2012; Clark and Jackson, 1995).
6) Each trial finished when the prey was eaten or after 40 minutes of observation. Preliminary observations prior to the trials revealed that this was a sufficient amount of time, since in the majority of observations all behaviors were usually manifested (even repeatedly) during this interval.

Experimental Arena

The arena for observing spiders with live prey (scelionid wasps) and with false movable prey (lures) consisted of a closed transparent acrylic box with an internal volume of 1550 cm^3. The acrylic plates were made in a CNC cutting machine, model Redsail CM1690, with a red laser tube of 100 watts of power. To provide greater structural complexity and additional routes for approaching the prey, an acrylic tree-like structure was placed in the center of the floor (Hill, 1979; Tarsitano and Andrew, 1999). For the experiments with false movable prey (lures) we used two types of background: one acrylic box had white-colored walls and the other had black-colored walls.

In the experiments with false lures the acrylic box was placed inside a frame made of pressed wood and containing an automated mechanism for moving the lure (Fig. 2). The lures were moved by a motor mechanism consisting of four guide blocks and two pairs of independent rails allowing for two-dimensional movements. A guide block was moved in each direction by two electric stepper motors connected by a toothed belt to the guide block, which in turn is connected to a busbar that moves the other guide block located parallel to the first one. Each pair of parallel guide blocks was connected to a moving part through a nylon thread, generating movement along two axes. This mobile part consists of a magnet on the outside of the acrylic box which in turn moves a magnet on the inside of the box. The control unit has programming functions that allow one to execute different patterns of movement and comprises an interaction interface with the user. This system has three buttons, each of which enables a different pattern of movements by the lure, but we used only horizontal movements along the side walls of the box (attempting to simulate the movements of live prey that we previously observed to be attractive to the spiders). For the programming of this movement pattern the Arduino platform was used. The positions of the moving lure were coded as coordinates in a plane, where position $x = 0$ and $y = 0$ represents the left rear corner of the box; each desired pattern was programmed as a mathematical function according to this reference. It is important to highlight that gradual acceleration and deceleration systems were incorporated into the engines, as well as flexible plastic couplings (TPU) to absorb the vibrations from the motors so as not to transmit them to the main structure, and to reduce sounds that might distract or stress the spider.

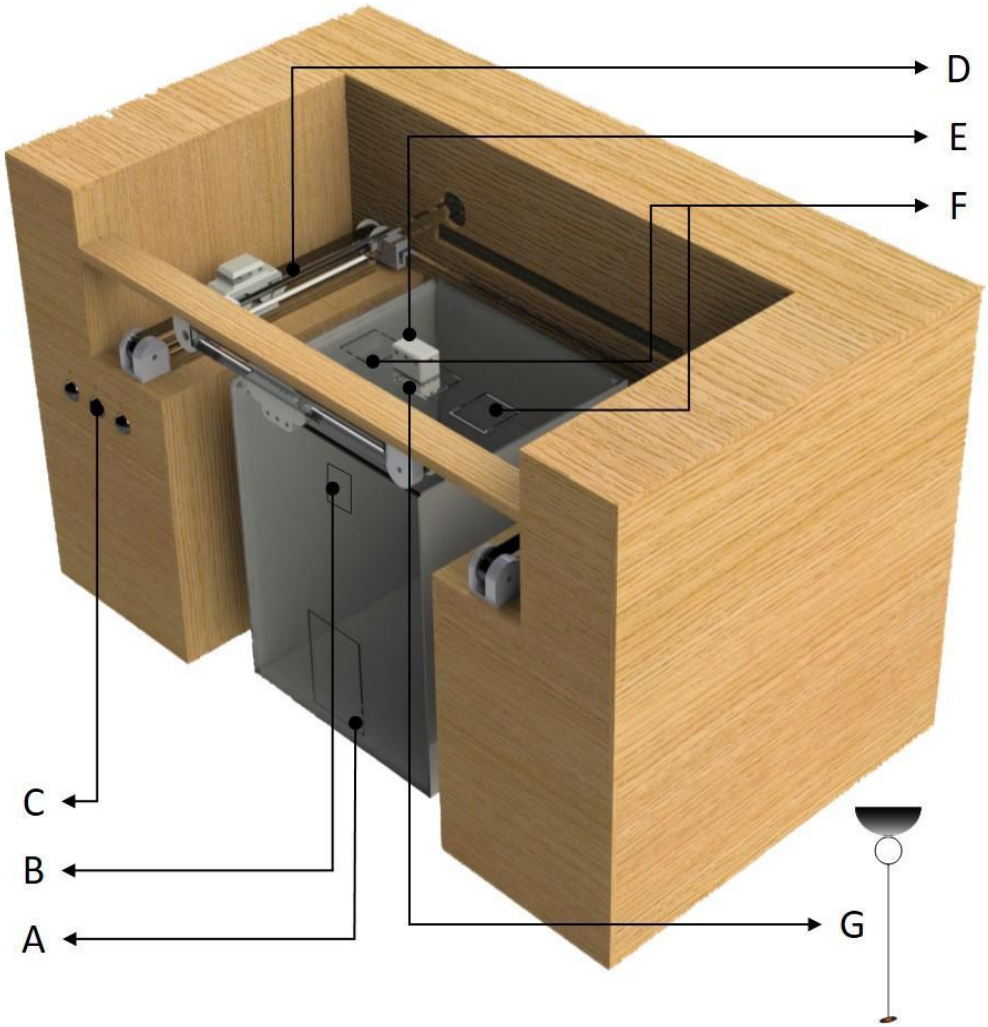


Fig. 2. Components of the automated arena. Aperture for introducing the spider (A). Aperture for introducing live prey (B). Buttons controlling the moving lure (C). Motor mechanism consisting of 4 guide blocks, 4 rails and 2 means of movement that uses two pairs of independent rails; each electric motor is connected by a toothed belt to a guide block, which in turn is connected to a busbar that moves the remaining guide block located in parallel; each pair of guide blocks located in parallel is connected to a moving part through a nylon thread, generating movement along an axis (D). Upper magnet (E). Aperture for introducing false prey (lure) (F). Lower magnet from which the lure (painted rice grain) is suspended by a transparent thread inside the acrylic box (G).

Observations of spiders with false prey in automated cage

During each trial an adult spider had simultaneous access to two types of lures (painted grains of rice), one painted in the BOB pattern and the other painted all black. The lure moved continuously in the chosen pattern explained above, one on each lateral wall of the arena, emulating as much as possible a live wasp. All lures were 4 mm in length, similar to that of the scelionid wasps. Data collection was carried out for 40 minutes, similar to the time used for spiders with live prey. We recorded the following behaviors: a) spider detects prey, as indicated by swivelling its cephalothorax; b) prey stalked and/or contacted; c) prey undetected or ignored; d) prey detected and spider withdraws; e) the color pattern (BOB o black) that is detected first. We also measured the body length of each spider and noted whether a silk dragline was produced.

In order to compare spectral characteristics of BOB colors with paints used to color the lures, as well as a possible interaction of these spectral features with the visual system of the jumping spiders, the reflectance spectra of both wasps and paints were considered. With respect to the four scelionid genera included here, we chose to use the previously reported mean reflectance curves for *Baryconus* because this data set showed low dispersion compared with those for other scelionid genera (Mora et al., 2019b). Six mixtures of different water soluble, solvent-free oil paints (Gamblin®) for the black and orange colors were prepared and tested; the mixtures consisted of combinations between permanent orange, Van Dyke brown, ivory black and Venetian red. Their reflectance spectra were measured using a spectrophotometer (508 PV Craic) coupled to a microscope (Eclipse LV100ND, Nikon). A spectralon standard was used as a diffuse white reference and the experimental setup parameters were the same as in a previous study (Mora et al 2019b).

Two methods were used for choosing the paint mixture for the lures:

1) Calculating CIELAB color coordinates according to the CIE standard using the reflectance spectra, and then calculating the color differences. In general, color coordinates allow the geometrical representation of colors. In particular, the CIE color spaces are recognized as having important characteristics such as being device-independent and having a perceptual linearity (Tkalcic, 2003). The CIELAB is a uniform Euclidean space and therefore distances between points can be used to represent approximately the perceived magnitude of color differences between object

color stimuli viewed under similar conditions. For the sake of simplicity, in the present work the CIE 1976 L, a, b (CIELAB) color difference definition was adopted (see references in Schanda, 2007 and Mora et al., 2019b for details).

2) Using a color space such as CIELAB implies that colors refer to human visual sensibility, and thus it would be preferable to use information about the visual spectral sensibility of the spider. Nevertheless, to the best of our knowledge, such information has not yet been recorded for the jumping spider used in this work, *L. jemineus*. Preliminary studies of other salticid spiders suggest the presence of photopigments with absorption bands in the UV (ca. 360nm) and the green (ca. 520nm) (Kelber et al, 2010). Since the spectral information available to us through the use of microspectrophotometry is restricted to the visible wavelengths, we limited our analysis to the use of the nomogram proposed by Govardovskii et al. (2000) in order to obtain the normalized absorption spectrum of the $\boldsymbol{\alpha}$ band of A1 type pigment with λmax=520nm. This curve was used to calculate the spectral component of each reflectance curve by multiplying the value of the reflectance curve at each point by the corresponding value of the normalized absorption as described in a previous work (Mora et al., 2019b). The comparison of the curves obtained in this way for both the reflectance of the BOB pattern of each genus and the reflectance of the paints, allowed us to hypothesize which mixtures would most resemble the wasp cuticle.

Green spectral components of the BOB pattern

In order to elucidate whether the spider can distinguish between orange (BOB) and black, and to determine whether the orange color varies between genera, the method described above in item b) was also used to obtain spectral green components of the reflectance spectra of the black and orange color of the BOB pattern in the four wasp genera, as was previously reported (Mora et al, 2019b). Nevertheless, this description is not complete since it lacks information about the UV visual sensibility.

After calculating the spectral green components, the areas under each curve were obtained numerically. This quantity represents how much of the light reflected by the cuticle of the wasp can be absorbed by the photosensitive pigment of the spider and therefore can be processed by its visual system. According to this criterium, the absolute value of the arithmetic difference between areas should be proportional to a higher or lower capability of

detecting a difference between colors. The difference was calculated as the area for the green component of the black color minus the area of the green component of the orange color. Negative values therefore indicate a major absorption of electromagnetic input corresponding to orange, whereas positive values indicate a major contribution to the absorbed light coming from the black color. We refer to these differences as "absorption contrasts", in the sense that the only assumption we are making about the physiology of the visual system of the spider is the type of photosensitive pigment present and its maximum absorption wavelength (as described above).

Statistical Analysis

A total of 136 trials with three groups of spiders (68 field-juveniles, 51 field-adults and 17 captivity-adults) and live prey were carried out during which the following data were recorded: spider type (juvenile, adult, reared), wasp type (BOB or black), spider behavior (behavioural scale explained before), time (0-5, 5-10, 10-20, 20-30, 30-40 minutes), the distance between the spider and the prey during each behavior, presence/absence of a silk dragline, and wasp and spider body length. A multinomial logistic regression (Venables et al, 2002) was fitted to the data, where the behavioral responses were grouped in three general categories in order to correct for zero or near zero counts in each response category: a. Follow-stalk prey (Detect); b. Crouch, jump and contact prey (Attack); c. Detect prey and withdraw (Avoid). The remaining variables recorded were included as covariates. Additionally, the descriptive statistics were calculated according to time and behavior, in order to define a clear timeline of spider behavior in the controlled experiments using mosaic plots.

The experiment with false prey (lures) in the automated cage included 30 trials, during which the following variables were recorded: presence/absence of silk dragline, spider size, background experimental arena color (black or white) and color of false prey (BOB vs black) that first attracted the spider.

The response variable in this case was constructed following the algorithm:

- *L. jemineus* responded similarly to black and BOB lures = 1
- *L. jemineus* responded differently to black and BOB lures = 2

Contingency tables were calculated, along with a Chi Square independence test for the response versus each of the variables: presence/absence of silk dragline, background color and prey color that first attracted the spider.

Statistical analysis was performed using the computing environment R (R Core Team, 2019). Data formatting and figures were prepared using the Tidyverse packages (Wickham, 2017). Multinomial logistic regression was done using the nnet package (Venables et al, 2002).

Wasp extract preparation for toxicity tests

The wasp extracts were done according to Arenas et al. (2015). Pools of three organisms from the same genus and with same color were placed in 1.5 ml centrifuge tubes; the initial weight of each pool was taken with a Mettler Toledo XP 205 analytical balance. Extraction was done by adding 0.5 ml of methanol (99.8 % purity) to each pool of individuals and subsequent maceration was done with a glass pestle for 5 minutes. After maceration, the tubes were centrifuged in a Minispin plus Eppendorf centrifuge at 14500 rpm for 10 minutes. The supernatant was transferred into a 6 ml glass vial and the pellet was discarded. The methanol from the supernatant was evaporated to dryness with an Organomation brand nitrogen concentrator, model MULTIVAPTM (flow of 7 l/min at 28 °C). For testing, 1400 µl of reconstituted water was added to the concentrate and homogenized using a vortex; this extract was considered as 100 %, then dilutions of 75 %, 50 % and 25 % from the original extract were prepared with reconstituted water to a final volume of 0.5 ml. A negative control of the extract, lacking the wasp pool, was prepared following the same procedure.

Acute toxicity test with *Daphnia magna*

The toxicity of wasp extracts was measured by an acute toxicity test with the water flea *Daphnia magna* Straus, based on the method described by Arenas et al. (2015). This organism is frequently used for toxicity tests due to its sensitivity to xenobiotics, wide distribution, short life cycle and ease of culturing in the laboratory. The water fleas were kept in reconstituted water ($MgSO_4$, $NaHCO_3$, KCl, $CaSO_4$ x 2 H_2O) (OECD, 2004), at 21 ± 2° C, with a photoperiod of 12:12, and fed with the green alga, *Pseudokirchneriella subcapitata*. The culture medium was changed every week, and after five-weeks adult individuals were discarded and a new culture started from neonates. For the acute toxicity test, ten 24-hour neonates were exposed to 0.5 ml of the different dilutions of the wasp extract during a 48 hour

period in a dark room at a constant temperature of 22 °C. The test end point was mortality (immobilization), which was recorded at 24 h and 48 h. The data were used to calculate the mean lethal concentration (LC50), using the R software, version 3.5.3 (R Development Core Team 2014) and using the "drc" package (Ritz et al. 2015).

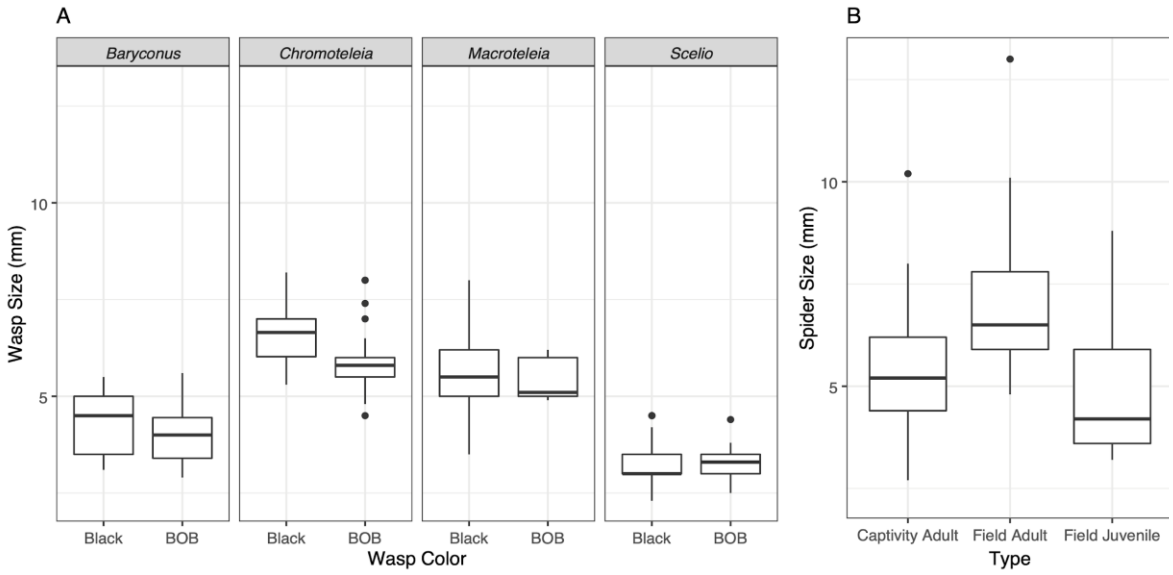
Acute toxicity test with *Vibrio fischeri*

The Microtox test detects inhibition of bioluminescence in the bacterium, *Vibrio fischeri*, using the Microtox® 500 Analyzer. For the analysis, the 2 % Basic Test method recommended by the Microtox® software was employed. Briefly, the lyophilized bacteria (Microtox Acute Reagent) was reconstituted and exposed to four dilutions from the original 100 % wasp extract, prepared with Modern Water Microtox Diluent®. Light emission by the bacteria in contact with the extract dilutions was measured at 0, 5, and 15 minutes; data analysis by the Microtox® software was employed to determine the dilution of the extract that produces a 50% loss of bioluminescence, i.e. the mean effect concentration (EC50).

RESULTS

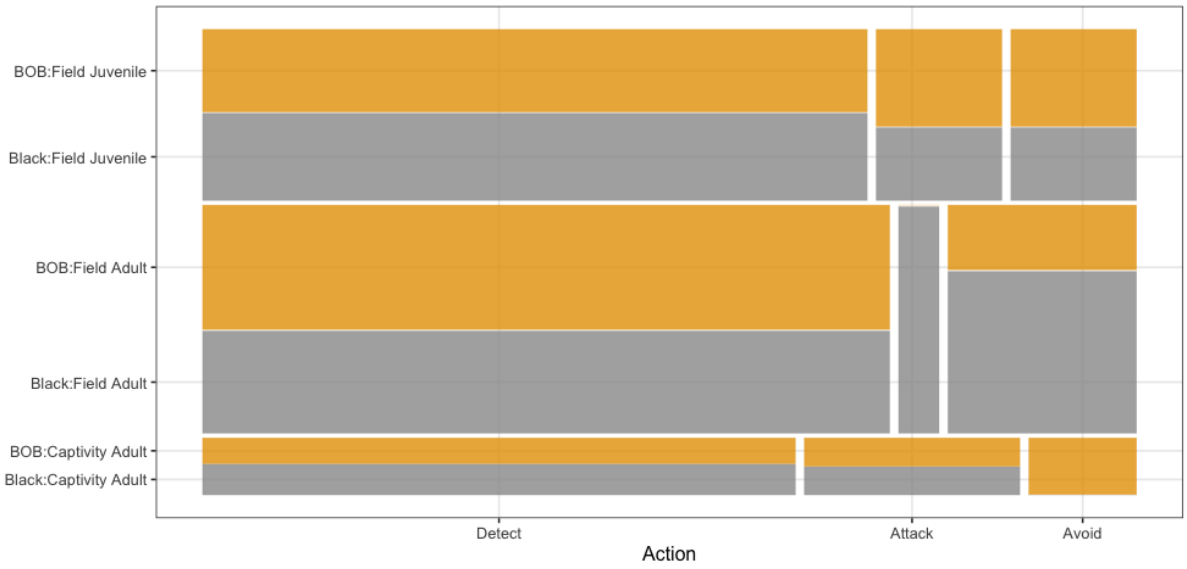
Experiments of spiders with live prey

For each of the 136 trials consisting of a wasp with a spider, the size of each was measured (Fig. 3 A, B). Although there is size variation within each group, predator (spiders) and prey (wasps) fall within the same general range, with field-captured adult spiders being the largest.



388 **Fig. 3. Body length for the organisms involved in this study.** A. wasps according to their
389 genus and color, B. spiders according to their origin and ontogenetic stage.

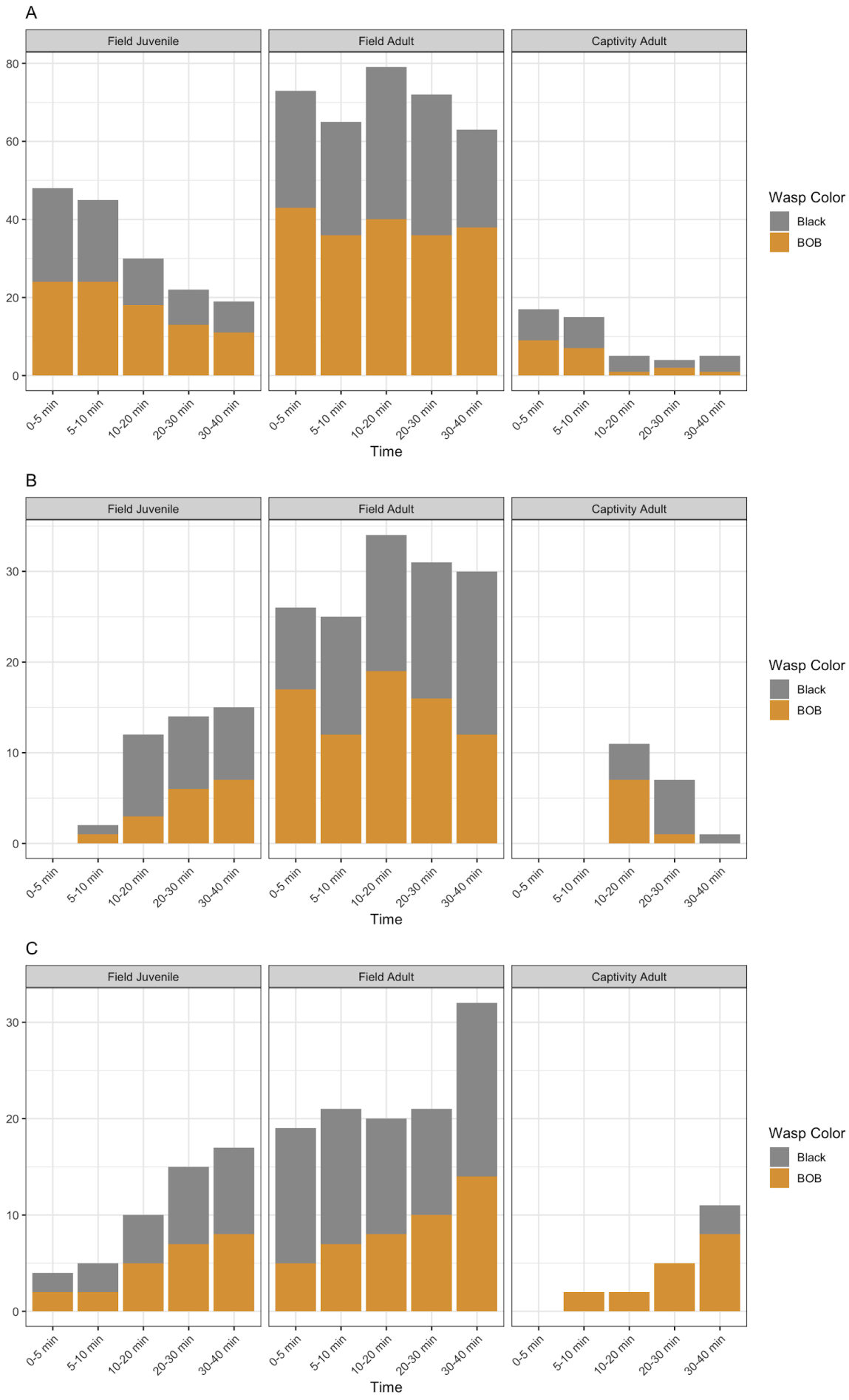
390 The multinomial logistic regression was fitted to a simplified response, where the most
391 common activity for each spider was classified as “detect”, “attack” or “avoid”. A summary
392 of the data included in the final model is presented in Figure 4. Note that in this figure the
393 number of spiders differs between groups, which is why the bar for lab-reared (captivity)
394 adults is thinner than the ones for field adults and field juveniles. The full model includes the
395 following covariates: wasp color, spider type, wasp size, spider size, wasp genus and
396 presence/absence of silk dragline. This model was compared with simplified versions, with
397 the result being that the model with wasp color and spider type was the model with the best
398 fit, according to an ANOVA test.



399

400 **Fig. 4. Most common actions of *L. jemineus* in trials with different combinations of wasp**
401 **color (BOB vs black) and spider type.** Each bar represents the proportion of spiders per type
402 and wasp color, according to the most common action taken by the spider in a total of 136
403 trials.

404 According to the aforementioned model, being a field adult is a significant factor for
405 increasing the odds of detecting versus attacking (p-value = 0.0108), and for avoiding versus
406 attacking (p-value = 0.0189). This shows that field adults are more likely to detect or avoid
407 than to attack, compared to adults reared in captivity which appear to prefer attacking.
408 Similarly, not using silk is associated with an increased chance of detecting versus attacking
409 (p-value = 0.0160), which makes sense since these spiders use silk only when attacking. Being
410 a field-caught juvenile versus an adult reared in captivity did not make a difference in the
411 most common spider actions. Other factors such as wasp color, wasp size, spider size, and
412 wasp genus did not make a statistically significant difference when included in the full model.
413 It should however be noted that field-caught adults only attacked black wasps, and adults
414 reared in captivity avoided only BOB wasps.


A
Field Juvenile
Field Adult
Captivity Adult
80
60
40
20
0
0-5 min
5-10 min
10-20 min
20-30 min
30-40 min
Time
Wasp Color
Black
BOB
B
Field Juvenile
Field Adult
Captivity Adult
30
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10
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0-5 min
5-10 min
10-20 min
20-30 min
30-40 min
Time
Wasp Color
Black
BOB
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Field Juvenile
Field Adult
Captivity Adult
30
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0-5 min
5-10 min
10-20 min
20-30 min
30-40 min
Time
Wasp Color
Black
BOB

Fig. 5. Plots for each behavior of *L. jemineus* when confronted with live wasps. All bar plots are represented according to its behavior per time (0-5 min, 5-10 min, 10-20 min, 20-30 min, 30-40 min), spider type (field-adult, field-juvenile, captivity-adult) and prey color (black, BOB). Behaviors are: detect (A), attack (B), avoid (C). Time slots without bars indicate that none of the spiders performed that action during that time slot.

If the spider's behaviors are analyzed during the 40 min and in the different observation tracts (0-5 min, 5-10, and so on) a greater complexity is observed, depending on the type of spider, where the field adult usually differs from the adult raised in captivity. There is a clear effect of learning, with captive adults showing a lower detection and attack capacity than field spiders (including juveniles). This may be due to a lack of learning since captive spiders were reared on a simple diet and were thus responding an unfamiliar prey. However, there is evidence of a capacity that seems to be innate, in delaying attack responses in the presence of wasps and in an aversion to BOB wasps. In total there were 954 observations.

Figure 5 presents the timeline bar plots, which are a way to summarize the actions taken by each spider throughout the timeline of the experiment. The statistic illustrated in these bars are the number of spiders that performed an action (detect, attack or avoid) per time slot and type of spider, and using color in the bars to represent the proportion of each wasp color (black and BOB). There are some time slots without observations: 0-5 mins in field adults, as well as 0-5 mins and 5-10 mins for captivity adults in plot B, and 0-5 mins for captivity adult in plot C. Thus, the number of spiders for each category changes according to each combination. In the bar plot A it can be seen that adults raised in captivity detect/stalk wasps of both color patterns, but during certain time periods their behaviour is directed slightly more toward the black wasps than the BOB wasps (10-20 min and 30-40 min), a behavior not observed in field-adults or field-juveniles. In plot B it is worth noting that adults raised in captivity attack only black wasps during the last time period, unlike the first 30 min during which both colors are attacked, as in field juveniles and adults. In plot C captive adults avoid wasps with the BOB pattern during most time periods, which differs from the behavior of field-caught spiders, which showed equal avoidance of both colors of wasps.

Green spectral components of the BOB pattern

The green spectral components of the black and orange colors of the BOB pattern in the four wasp genera are presented in Figure 6. In general, the spectral components for black and orange colors differ in the position of the maximum wavelength and the height of the peak, indicating different interaction of the reflected light with the photosensitive pigment. The exception is *Baryconus*, for which the spectral components of black and orange colors are similar. In order to provide a quantitative assessment of these differences or similarities, the area under the curve for each spectral component was calculated and the difference (subtraction) between the areas was used as comparison parameter which was called absorption contrast, as described in the methods. This information, together with the results of the predation experiments, is presented in Table 1. The four genera were ranked from the highest negative value to the highest positive value of the absorption contrast between black and orange spectral components. The information in Table 1 shows that for the wasps with a BOB color pattern, most of the "attack" events occurred with BOB-colored *Baryconus*, which also has the lowest absorption contrast. On the other hand, the greatest number of "detect" events occurred with BOB-colored *Macroteleia*, which has the highest absorption contrast and the highest contribution of orange. The greatest number of "avoid" events occurred with BOB-colored *Scelio*, which has the second highest absorption contrast. Also, the "detect" events seem to be associated with the contribution of black: higher values of black contribution, as in *Macroteleia* and *Chromateleia*, yielded a higher percentage of "detect" events, while lower values of black contribution, as in *Scelio* and *Baryconus*, yielded a lower percentage of "detect" events.

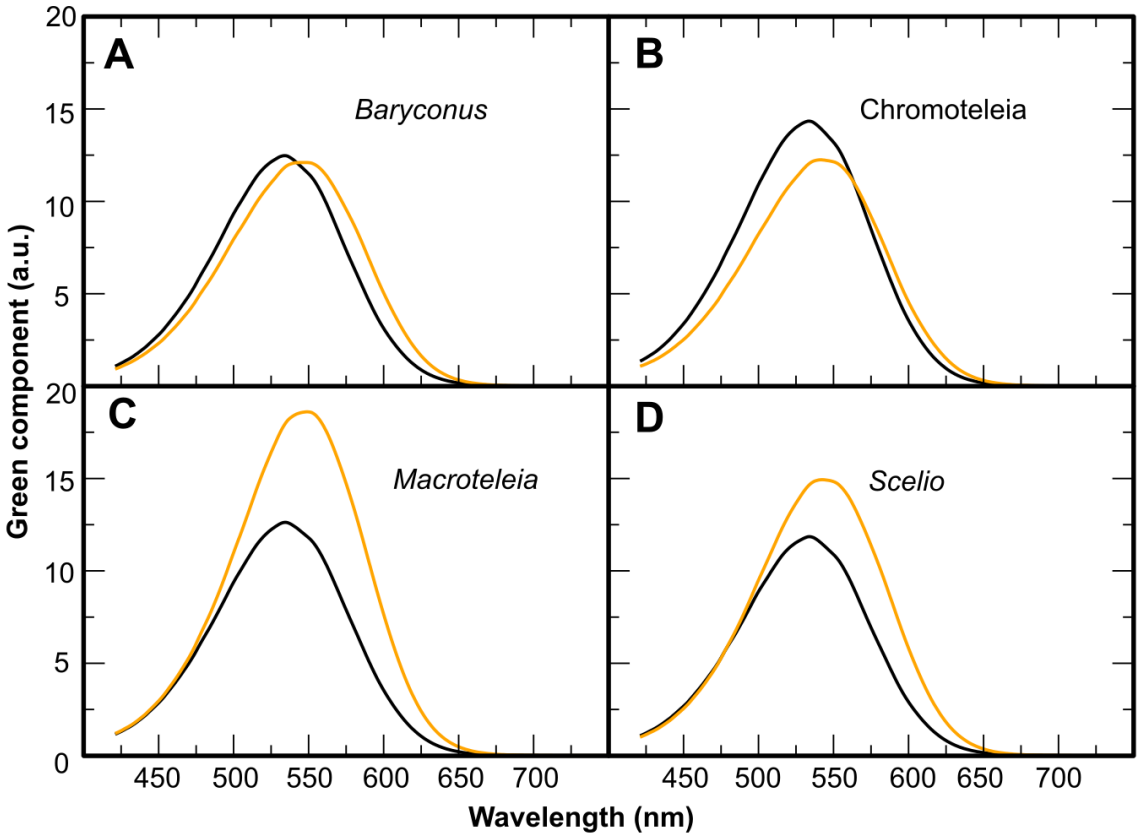

Fig. 6. Green component of the reflection spectra, in arbitrary units (a.u.), of both black (black line) and orange (orange line) colors. The BOB color pattern of 4 genera was analyzed, *Baryconus* (A), *Chromateleia* (B), *Macroteleia* (C) and *Scelio* (D).

Table 1. Summary of information about the relationship between the green components of the BOB colors of each genus and the percentage of events observed and grouped in the categories "detect", "avoid" and "attack". AB and AO, in arbitrary units, represent the area under the curve for the green component of the black and orange colors, respectively, of the BOB coloration.

	Macroteleia (BOB)	*Scelio* (BOB)	*Baryconus* (BOB)	*Chromoteleia* (BOB)	Black (all genera)
AO	2035	1654	1388	1392	---
AB-AO	-644	-377	-42	172	---
	more orange			more black	
AB	1392	1277	1346	1564	---
Detect	100%	60%	60%	84%	69%
Avoid	0	30%	7%	16%	19%
Attack	0	10%	33%	0	12%

476

477 **Experiment of spiders with false prey in automated cage**

478 The reflectance spectra of six blends of black paint and six blends of orange paint were
479 compared with that of the black and orange color, respectively, of BOB-colored *Baryconus*
480 (Fig. 7.). The best matches in terms of similarity to the black and orange colors of actual wasp
481 cuticle were, respectively, our blend #3 (ivory black plus Van Dyke brown) and blend #5
482 (orange plus Venetian red). For black, the difference between wasp cuticle and paint blend
483 was less than 4.5 points, whereas for orange it was 13 points. For reference, 2.3 is the mean
484 threshold for color differentiation by the human eye (Mahy 1994).

485

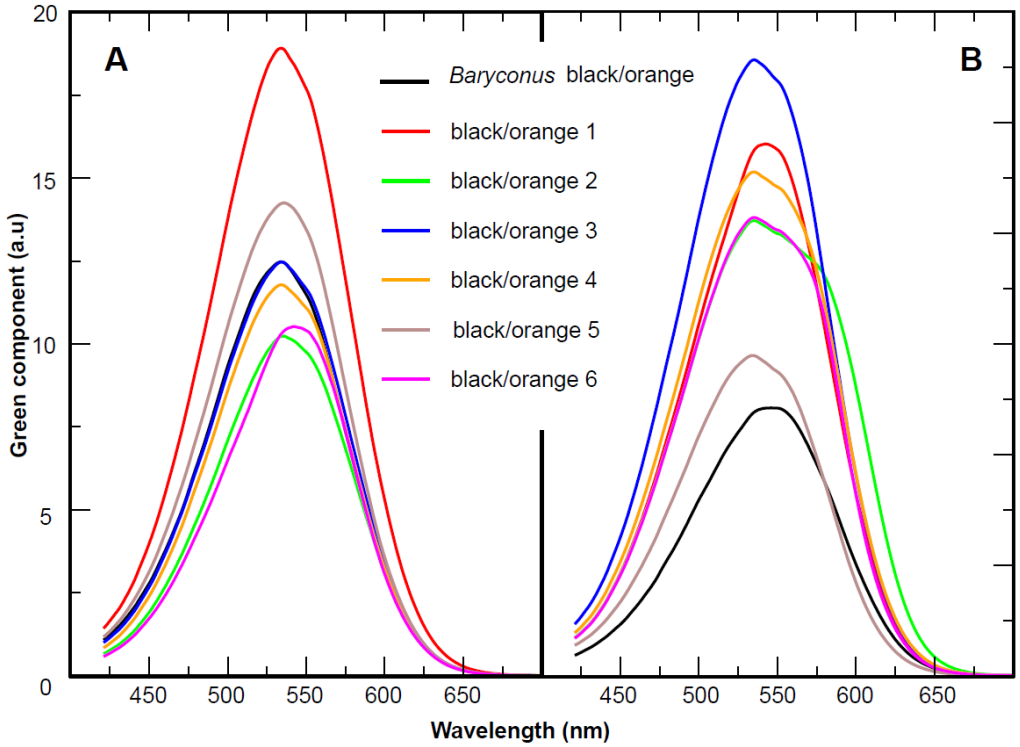

Fig. 7. Spectral components, in arbitrary units (a.u.), of the reflectance curves for black-based blends (A) and orange-based blends (B) of paints compared to black and orange colors in *Baryconus*. Note that in (A) the black line for *Baryconus* completely overlaps that for paint #3.

Table A1 presents the results from the experiment with false prey. Each contingency table was tested for independence, and there was no evidence of dependence in any of the cases. In other words, spider behaviors are not associated with background color, the color of lure that was detected first, the use or non-use of silk, or predator size.

Acute toxicity tests with *Daphnia magna* and *Vibrio fischeri*

During the exposure period, acute toxicity trials with *D. magna* resulted in a higher mortality (LC50 < 65.2%) of water fleas when exposed to the extracts from the BOB wasps, which employed *Chromoteleia* and *Baryconus* (Table 2). In the case of the black wasps, three out of four extract samples did not show toxic effects (LC50 > 100 %) on *D. magna*; the one sample of black *Macroteleia* that showed toxicity had a lower mortality than with the BOB wasps. No mortality of water fleas was observed with the negative control, thus suggesting that the toxic effects may be ascribed to the components of the wasps. In the toxicity tests with *V.*

fischeri only black *Macroteleia* and BOB *Chromoteleia* resulted in high bioluminescence inhibition; the results for the two genera have overlapping confidence intervals, so no difference can be inferred; on the contrary, there were no inhibitory effects with BOB *Baryconus* or black *Scelio* (Table 2). Overall, the wasp extracts presented a much higher toxicity for the bacteria bioindicator (*V. fischeri*) than for the arthropods (*D. magna*).

Table 2. Toxicity (± I.C.) of extracts of scelionid wasps measured with the bioindicators *Daphnia magna* and *Vibrio fischeri*.

Genus/Phenotype	Total mass (mg)	LC50 (*Daphnia magna*)	EC50 15 minutes (*Vibrio fischeri*)
Baryconus (BOB)	3,41	59.6 % (50.2, 68.9)	> 100 %
Chromoteleia (BOB)	3,46	60.6 % (51.5, 69.7)	1.7 % (0.9, 3.0)
Chromoteleia (BOB)	3,75	65.2 % (54.4, 75.9)	5.2 % (0.8, 32)
Macroteleia (black)	1,34	85.9 % (73.5, 98.3)	1.0 % (0.4, 2.0)
Macroteleia (black)	1,42	> 100 %	2.1 % (1.1, 4.1)
Scelio (black)	0,66	>100 %	> 100 %
Scelio (black)	0,86	>100 %	> 100 %

DISCUSSION

The aposematism of the BOB coloration

A previous study showed that the black-orange-black (BOB) color pattern is very widespread among small parasitoid wasps and that it is usually present in both sexes (Mora and Hanson, 2019), suggesting that this color pattern is not related to sexual behavior. The principal hypothesis regarding the function of this widespread color pattern is aposematism and/or mimicry, and to the best of our knowledge, the present research is the first to test this hypothesis. Although the results were mixed, at least two of our findings provide evidence that BOB coloration is indeed aposematic. First, although the behavioral responses were quite variable, on five occasions field-captured *Lyssomanes* spiders consumed black wasps (all belonging to the genus *Scelio*), but no spider consumed a wasp with BOB coloration. Second, the acute toxicity tests with *Daphnia magna* showed that wasps with BOB coloration caused greater mortality than did the black wasps, suggesting that BOB wasps may contain defensive compounds unlike black wasps (which would explain why only black wasps were consumed).

The toxicity assays showed that the wasp extracts were more toxic for the bacteria (*V. fischeri*) than for the crustacean (*D. magna*). Unfortunately, although in the same toxicity range, no clear differences were observed for the extract samples that presented toxicity in the *V. fischeri* assays, due to overlapping confidence intervals. Nonetheless results from this test should be taken into account to estimate the potential presence of toxic components in the wasps. A consistent result for both bioindicators is that the black *Scelio* did not show toxicity, and might be considered the least toxic of the four genera. The results for *D. magna* are more relevant for this study since differences between the genera can be observed and because it is an arthropod as are the *Lyssomanes* spiders. Moreover, the extracts from the BOB wasps all showed toxicity while just one out of four black wasp extracts did so; moreover, the EC50 values for the BOB wasp extracts were lower, i.e. had higher toxicity. Further research is needed to verify that a defensive compound is indeed present in BOB wasps (and absent in black wasps), and if present, to identify and characterize it.

Predator responses towards BOB wasps

It should be emphasized that considerable effort is needed to obtain live scelionid wasps; a total of about 540 hours were required to collect the wasps used in our experiments and even with this effort there were insufficient numbers for some of the trials. This limitation was one

of the motivations for carrying out trials with false prey (lures). Despite considerable care taken in providing lures that closely matched the black and orange colors of the wasps (Fig. 7) and an arena in which the movements of the lures were carefully controlled (Fig. 2), the results were inconclusive. The likely explanation is that the lures lacked additional visual cues (e.g. legs and eyes) and/or chemical cues necessary to elicit a predatory response, which has been shown in other jumping spiders (Harland and Jackson, 2000). In future research it would be interesting to use dead scelionid wasps as lures, instead of painted rice grains.

In our experiments with predators we attempted to take into account previous recommendations such as background contrast, predator vision, predator life stage, predator and prey sizes, and prior opportunities for learning by the predator (Stevens and Ruxton, 2012, Arenas et al, 2015). Three types of *Lyssomanes* spiders were used in our experiments: field-collected adults, field-collected juveniles, and lab-reared adults. If learning plays a role in avoiding aposematic prey, one would expect the first group to show the greatest discrimination between black wasps and BOB wasps, since the use of reared specimens eliminates the role of learned generalization (Fabricant and Smith, 2014) and field-collected juveniles have had less time to learn. The results of our experiments provide partial evidence for this. The only instances of spiders consuming wasps (non-aposematic, black wasps) were of *Lyssomanes* that had been captured in the field. Moreover, field-collected adults were more likely to detect or avoid than to attack, compared to adults reared in captivity which appeared to prefer to attack. Thus, adults from the field, which have had more experience, are more selective.

When analyzing the origin of the spider in its responses to wasps over time, there is an effect of lack of learning in captive-bred spiders, namely a lower capacity for detection and attack than spiders collected in the field. This is expected, given the complexity of factors and different prey found in natural conditions compared to an artificial breeding environment and a diet that consisted of only two types of prey. However, a possible innate effect is evident in the clear aversion of spiders bred in captivity to wasps with the BOB coloration; there was detection of both colors (BOB and black) as well as attacks mainly on black wasps. Furthermore, for spiders from natural conditions the aversion increased over time, and this aversion coincides with the toxicity analyses and the aposematic pattern of the wasps. These results merit further investigation since it is generally assumed that an adverse behavioral

response to aposematic coloration is learned, despite the widespread presence of BOB coloration in various taxonomic groups.

Spectral components of the BOB pattern

Although the orange color is usually present in aposematic coloration and warning signaling (Thery and Gomez, 2010; Stevens and Ruxton, 2012), our results suggest that not all BOB specimens were treated similarly by the spider predators. The orange coloration in BOB wasps varies between genera, despite appearing similar to the human eye (Fig. 6). It is possible that the difference between the two light absorbing elements (black and orange pigments) within each genus of wasps is important in affecting predator responses. Such differences could favor the increase of conspicuity and consequently intervene in initial avoidance (Stevens et al., 2010; Fabricant and Herberstein, 2014). *Baryconus* had the lowest absorption contrast between black and orange (Fig. 6) and, interestingly, this genus received the most "attack" events (Table 1). At the other extreme, *Macroteleia* had the highest absorption contrast, as well as the greatest contribution of orange, which perhaps explains why this genus had the greatest number of "detect" events. The "detect" events seem to be associated with the contribution of black: higher values of black contribution, as in *Macroteleia* and *Chromateleia*, yielded a higher percentage of "detect" events, while lower values of black contribution, as in *Scelio* and *Baryconus*, yielded a lower percentage of "detect" events. This reinforces the fact that arthropods may still be able to perceive long wavelength difference via achromatic or luminance information (Reisenman and Giurfa, 2008) and that some color patterns may perform as multicomponent signals (Grether et al., 2004).

It is also important to consider the visual space of the predator. It has been reported that jumping spiders are less sensitive to the orange end of the spectrum and thus perceive orange cuticle as more achromatic than structures with relatively more reflectance in the green portion of the spectrum. The aforementioned plus the absence of a red receptor in many spiders could cause orange objects to be perceived as monochromatically "green" objects difficult to distinguish chromatically from green foliage. (Fabricant and Herberstein, 2014; Chittka and Waser, 1997; Briscoe and Chittka, 2001). Additionally, since predators have diverse visual and cognitive systems, a warning signal that enhances prey recognition and aversion learning for one predator might be ineffectivek for another (Speed, 2000).

Most studies of aposematism have used vertebrate predators and larger prey, whereas arthropod predators of smaller prey have generally been neglected (Lang et al, 1999). The black-orange-black (BOB) pattern is extremely widespread in small hymenopterans (as well as a few other insects) and has evidently evolved independently on numerous occasions (Mora and Hanson, 2019). To the best of our knowledge, the present study provides the first evidence that this common color pattern has an aposematic function. However, there are still many unanswered questions. For example, it would be interesting to evaluate the behavioral responses of other predators when confronted with scelionid wasps having the BOB pattern. The identity of the pigments and the putative noxious compounds are still unknown. Finally, it would be instructive to examine the spectral properties of other wasps with this color pattern, as well as the visual capacity of other potential predators.

Data Availability

All data used to support the findings of this study are available in this public repository: https://malfaro2.github.io/Mora_et_al2/

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Author contribution statement

R.M.C coordinated field trips and design the research methodology, collected the data, the samples, prepared them for the techniques used and participated in the writing of the manuscript and co-design of the graphic representations. M.A.C executed the statistical analysis and graphic representations and contributed to the writing of the manuscript. M.H.J participated in the supervision of optical measurements executed by R.M.C., calculated the spectral components and contributed to the writing of the manuscript. M.F.O and P.H.S contributed to the writing of the manuscript and co-design of the graphic representations. A.D.R developed the Arduino programming and co-design the automated arena with R.M.C and M.M.R, D.R.M. and C.E.R.R. executed the toxicity essays and contributed to the writing of the manuscript.

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References

Arenas LM, Walter D and Stevens M. (2015). Signal honesty and predation risk among a closely related group of aposematic species. *Scientific Reports* **5**:11021.

Biamonte, E., Sandoval, L., Chacón, E. and Barrantes, G. (2011). Effect of urbanization on the avifauna in a tropical metropolitan area. Landscape Ecology, **26**(2), 183-194.

Blest, A. D. and Sigmund, C. (1984). Retinal mosaics of the principal eyes of two primitive jumping spiders, *Yaginumanis* and *Lyssomanes*: clues to the evolution of salticid vision. Proceedings of the Royal Society of London. Series B. Biological sciences, **221**(1222), 111-125.

Briscoe, A.D. and Chittka, L. (2001). The evolution of color vision in insects. Annual review of entomology, **46**(1), pp.471-510.

Carducci, J. P. and Jakob, E. M. (2000). Rearing environment affects behavior of jumping spiders. Animal Behaviour, **59**(1), 39-46.

Chittka, L. and Waser, N.M. (1997). Why red flowers are not invisible to bees. Israel Journal of Plant Sciences, **45**(2-3), pp.169-183.

Clark, R. J. and Jackson, R. R. (1995). Dragline-mediated sex recognition in two species of jumping spiders (Araneae Salticidae), Portia labiata and P. fimbriata. Ethology ecology & evolution, **7**(1), 73-77.

Fabricant, S. A., & Smith, C. L. (2014). Is the hibiscus harlequin bug aposematic? The importance of testing multiple predators. Ecology and evolution, **4**(2), 113-120.

Fabricant, S.A. and Herberstein, M.E. (2014). Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. *Behavioral Ecology*, **26** (1), pp.38-44.

Foelix, R. (2011). Biology of spiders. OUP USA.

Forster, L. (1982). Vision and prey-catching strategies in jumping spiders. American Scientist.

Govardovskii, V. I., Fyhrquist, N., Reuter, T. O. M., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. Visual Neuroscience, **17**(4), 509-528.

Grether, G. F., Kolluru, G. R., & Nersissian, K. (2004). Individual colour patches as multicomponent signals. Biological Reviews, **79**(3), 583-610.

D. P. and Jackson, R. R. (2000). Eight-legged cats and how they see: A review of recent research on jumping spiders (Araneae: Salticidae). Cimbebasia, **16**, 231-240.

Harland, D. P., Li, D., and Jackson, R. R. (2012). How jumping spiders see the world.Cambridge, MA: the MIT Press.

Herberstein, M. E. (Ed.). (2011). Spider behaviour: flexibility and versatility. Cambridge University Press.

Jackson, R. R. and Cross, F. R. (2011). Olfaction-based mate-odor identification by jumping spiders from the genus *Portia*. Journal of Arachnology, **39**(3), 439-444.

Lang, A., Filser, J. and Henschel, J.R. (1999). Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. Agriculture, Ecosystems & Environment, ***72***(2), pp.189-199.

Mahy M., Van Eycken L. and Oosterlinck A. (1994). Evaluation of uniform color spaces developed after the adoption of CIELAB and CIELUV. Color Research & Application. 1994;**19**(2):105–121.

Masner L. (1976). Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). The Memoirs of the Entomological Society of Canada. **108**(S97):1–97

Mora R. and Hanson P.E. (2019) Widespread occurrence of black-orange-black color pattern in Hymenoptera. *Journal of Insect Science*.; **19** (2). pmid:30851035

Mora-Castro, R., Edwards, G.B. and Hanson Snortum, P. (2019a). Phenology of an urban population of *Lyssomanes jemineus* Peckham & Wheeler (Araneae: Salticidae) with a list of other jumping spiders from the same Costa Rican site. *Peckhamia* **194**.1.

Mora-Castro, R., Hernández-Jiménez, M., Alfaro-Cordoba, M., Avendano, E. and Hanson-Snortum, P. (2019b). Spectral measure of color variation of black-orange-black (BOB) pattern in small parasitoid wasps (Hymenoptera: Scelionidae), a statistical approach. *PloS one*, **14** (10).

Nagata, T., Koyanagi, M., Tsukamoto, H., Saeki, S., Isono, K., Shichida, Y., Tokunaga,F., Kinoshita, M., Arikawa, K., and Terakita, A. (2012). Depth perception from image defocus in a jumping spider. Science **335**, 469–471

Nelson, X. J. and Jackson, R. R. (2012). The discerning predator: decision rules underlying prey classification by a mosquito-eating jumping spider. Journal of Experimental Biology, **215** (13), 2255-2261.

Nelson, X. J., Warui, C. M. and Jackson, R. R. (2012). Widespread reliance on olfactory sex and species identification by lyssomanine and spartaeine jumping spiders. Biological Journal of the Linnean Society, **107**(3), 664-677.

OECD. (2004). Guidelines for the testing of chemicals. Guideline **202**: *Daphnia* sp., Acute immobilisation test, adopted April 2004.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reisenman, C. E. and Giurfa, M. (2008). Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee Apis mellifera. Arthropod-Plant Interactions, **2**(3), 137.

Ritz, C., Baty, F., Streibig, J. C. and Gerhard, D. (2015). Dose-Response Analysis Using R. PLoS ONE, **10** (12): e0146021.

Skelhorn, J., Halpin, C. G. and Rowe, C. (2016). Learning about aposematic prey. Behavioral Ecology, **27** (4), 955-964.

Speed, M.P., Ruxton, G.D., Stephens, P.A. and Blount, J.D. (2009). Warning displays may function as honest signals of toxicity. Proceedings of the Royal Society B: Biological Sciences, 2**76**(1658), 871-877.

Stevens, M. and Ruxton, G. D. (2011). Linking the evolution and form of warning coloration in nature. Proceedings of the Royal Society B: Biological Sciences, **279** (1728), 417-426.

Stevens, M., Mappes, J. and Sandre, S. L. (2010). The effect of predator appetite, prey warning coloration and luminance on predator foraging decisions. Behaviour, **147**(9), 1121-1143.

Thery, M. and Gomez, D. (2010). Insect colours and visual appearance in the eyes of their predators. In Advances in Insect Physiology. Vol. 38, pp. 267-353. Academic Press.

Tkalcic M. and Tasic J.F. (2003). Colour spaces: perceptual, historical and applicational background. Vol. 1. IEEE.

Venables, W. N. and Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Wickham, H. (2017). tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse

Williams, D. S. and McIntyre, P. (1980). The principal eyes of a jumping spider have a telephoto component. Nature, **288** (5791), 578.

Legends to figures

Fig. 1. Two scelionid wasp color patterns within the same genus. Color patterns in the genus *Scelio*: black (A), and BOB (B). The focus-stacked macro photography was obtained with a Reflex 850 camera coupled to a 20x microscope lens.

Fig. 2. Components of the automated arena. Aperture for introducing the spider (A). Aperture for introducing live prey (B). Buttons controlling the moving lure (C). Motor mechanism consisting of 4 guide blocks, 4 rails and 2 means of movement that uses two pairs of independent rails; each electric motor is connected by a toothed belt to a guide block, which in turn is connected to a busbar that moves the remaining guide block located in parallel; each pair of guide blocks located in parallel is connected to a moving part through a nylon thread, generating movement along an axis (D). Upper magnet (E). Aperture for introducing false prey (lure) (F). Lower magnet from which the lure (painted rice grain) is suspended by a transparent thread inside the acrylic box (G).

Fig. 3. Body length for the organisms involved in this study. A. wasps according to their genus and color, B. spiders according to their origin and ontogenetic stage.

Fig. 4. Most common actions of *L. jemineus* in trials with different combinations of wasp color (BOB vs black) and spider type. Each bar represents the proportion of spiders per type and wasp color, according to the most common action taken by the spider in a total of 136 trials.

Fig. 5. Plots for each behavior of *L. jemineus* when confronted with live wasps. All bar plots are represented according to its behavior per time (0-5 min, 5-10 min, 10-20 min, 20-30 min, 30-40 min), spider type (field-adult, field-juvenile, captivity-adult) and prey color (black, BOB). Behaviors are: detect (A), attack (B), avoid (C). Time slots without bars indicate that none of the spiders performed that action during that time slot.

Fig. 6. Green component of the reflection spectra, in arbitrary units (a.u.), of both black (black line) and orange (orange line) colors. The BOB color pattern of 4 genera was analyzed, *Baryconus* (A), *Chromateleia* (B), *Macroteleia* (C) and *Scelio* (D).

Table 1. Summary of information about the relationship between the green components of the BOB colors of each genus and the percentage of events observed and grouped in the categories "detect", "avoid" and "attack". AB and AO, in arbitrary units, represent the area under the curve for the green component of the black and orange colors, respectively, of the BOB coloration.

Fig. 7. Spectral components, in arbitrary units (a.u.), of the reflectance curves for black-based blends (A) and orange-based blends (B) of paints compared to black and orange colors in *Baryconus*. Note that in (A) the black line for *Baryconus* completely overlaps that for paint #3.

Table 2. Toxicity (± I.C.) of extracts of scelionid wasps measured with the bioindicators *Daphnia magna* and *Vibrio fischeri*.

Appendix

Table A1. Results from experiment with false prey in three contingency tables. All 30 observations were aggregated according to the response variable (same or different actions) and three variables: A) background color (white or black), B) color (black or BOB) that first attracted the spider, C) use of silk dragline (yes or no).

Behavioural responses	(A) Background		(B)		(C) Use of silk	
	White	Black	Black	BOB	No	Yes
L. jemineus took the same actions with both black and BOB wasps	13	8	15	6	16	5

L. jemineus took different actions with black and BOB	3	6	6	3	7	2
TOTAL (30)	16	14	21	9	23	7

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ANEXOS

A suitable method for rearing *Lyssomanes jemineus* Peckham & Wheeler juveniles (Araneae: Salticidae: Lyssomaninae)

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Abstract. In this study we examined several cage designs and diets in order to assess their effects on the survivorship of *Lyssomanes jemineus* juveniles. Our results indicate that using the same plant species as that on which the egg sacs or adults were collected minimized the high peak of mortality that is observed during the second week of life of the spiderlings. A survival rate of 53% was successfully achieved by providing young juveniles (less than 200 days old) with whiteflies, and then transitioning to *Drosophila melanogaster* Meigen for older juveniles and adults. The rearing system reported here is inexpensive, easy to implement, and not labour intensive since we did not separate hatchlings individually. It is particularly well suited for conducting experiments under controlled laboratory conditions and for establishing year-long *Lyssomanes jemineus* colonies.

Keywords. Rearing, *Lyssomanes jemineus*, juveniles, egg sacs, hatchlings, diet.

The lyssomanine jumping spiders include two described genera, *Lyssomanes* Hentz, 1845 and *Chinoscopus* Simon, 1900, both neotropical (Logunov & Marusik 2003, Galiano 1980; Wanless 1980, 1984). They are somewhat translucent, with greenish and light-yellow tones (Galiano 1980, 1996, 1998; Logunov & Marusik 2003; Logunov 2014, 2015), and dwell on foliage, especially large leaves. Their behaviour is of interest due to the basal position of this subfamily within the Salticidae (Richman & Jackson 1992; Maddison & Needham 2006).

Lyssomanine rearing systems have not been widely explored (Jackson 1974) even though prey preferences of adults have been quite well studied (Li & Jackson 1997). In contrast, studies of juveniles are quite scarce. The information presented here is drawn from two sources: two years of observation of this group in the field, and one year of experience attempting to develop a suitable rearing system. We tested the effect of different rearing conditions on the mortality of *Lyssomanes jemineus*. New information presented here focuses mainly on juvenile rearing, especially with respect to a) optimal cage design, b) adequate controlled conditions, c) food sources to achieve survival of juveniles to adulthood. Suitable prey for adults, mainly dipterans, has been previously reported (Jackson 1974).

Materials and Methods

Collection of *Lyssomanes jemineus* egg sacs. Weekly samplings of approximately two hours each were carried out during 12 months, from August 2016 to July 2017. Egg sacs were obtained on the campus of the University of Costa Rica (Finca 3), San Pedro Montes de Oca, San José, Costa Rica (9°56'07"N, 84°03'04"W). This locality has an average annual precipitation of 1200-1500 mm and a temperature of approximately 21.0 °C. The vegetation corresponds to that of a humid pre-montane forest and can be characterized as an urban forest patch (Di Stefano *et al*., 1995). Because the site is quite altered the plants are somewhat dispersed. For this reason, about 15 to 30 spots with diverse herbaceous plants were sampled haphazardly. Each egg sac (usually located on the underside of the leaf) was collected manually without being detached from its host plant/leaf and was quickly transferred to the laboratory using 15 ml sterile Falcon tubes with a humid cotton ball. The plant species that harbored the egg sac was recorded.

Collection of *Lyssomanes jemineus* adults. Weekly samplings of approximately two hours each were carried out during 12 months, from January 2016 to January 2017, in the aforementioned locality. Adults were visually detected and were caught manually using small plastic bags or 15 ml Falcon tubes. They were kept individually and then quickly transferred to the laboratory. The plant species that harbored the specimen was recorded.

Alternative diets. We based our choice of alternative prey for adults and juveniles on soft-bodied species, which are preferred by the predator, and were easy to obtain and rear in laboratory under controlled conditions (Table 1).

Container design. Our goal was to achieve a cage design that fulfilled five conditions: a) exclusion of possible predators and parasitoids, b) temperature, humidity and other environmental conditions suitable for the development of the plant and spiders, c) retention of smaller stages to avoid their escape, d) appropriate size and other specifications adequate for courtship and reproduction, e) clear surfaces for filming behavior (avoiding hidden parts or blind spots). Three designs (positioning of openings and vents), dimensions (ideal size for courtship and hunting) and materials (aluminum, sieves, wood) were evaluated (Table 2). To enrich the system, we placed a healthy plant that occupied, with its foliage, the entire height and width of the cage in order to favor ambush hunting, mobility, shelter and oviposition. We also provided a reservoir of water and air inlets, attempting to maintain 60-80% relative humidity, a temperature between 20 °C and 28 ° C, and some degree of natural sunlight.

Results

Cage design and rearing conditions. Of the five cage designs several factors contributed negatively to the rearing of *Lyssomanes jemineus*: wood as a material favors high humidity which affected the spiders and the condition of the plants; some closures were not completely hermetic which resulted in the appearance of predators and the escape of juvenile spiders due

to their extremely small (< 2 mm) size. Likewise, aluminum and wood did not allow for proper observation of the spiders. Juvenile counts were very difficult in these containers as they hid in corners, died attached to water droplets as a result of excess moisture, or escaped. The most appropriate design (design 3, Figure 1) was characterized by plants in excellent condition, no excess humidity accumulation on the walls, no escapes by juvenile spiders, observation without hindrances (e.g. corners that interfere with the counts), and environmental conditions appropriate for development of immature and adult stages.

It is also worth mentioning an aspect that is often omitted from discussions of rearing systems, namely spider density. Although araneophagy was observed observed in the field (*Lyssomanes jemineus* adults preying on *Leucage mariana* Keyserling) very little araneophagy was detected in the laboratory due to strict cage cleaning and the invader-proof design of the cage; however, we did occasionally observe cannibalism whereby advanced stage juveniles preyed on younger stages. This behavior was sufficiently uncommon that it was deemed unnecessary to separate specimens individually during the life cycle. However, cannibalism increased as the number of individuals per cage increased and was observed mainly in cages with high densities, resulting from masses of 50 to 150 eggs hatching in the cages. Therefore, in order to facilitate cleaning, observation, and counts, no more than 10 specimens were maintained in a single cage, which also minimized cannibalism. Spiders were generally separated during the fourth week after hatching, which is when they usually begin to disperse.

We initially reared *Lyssomanes jemineus* on various types of broadleaf foliage. However, during the first two weeks after hatching there was an extremely high peak of mortality (98%) in the hatchlings from 8 egg sacs containing 42 to 150 eggs each. These egg sacs were collected in the field with the leaf fragment to which they were attached and were placed at the base of the caged plant near a leaf blade. However, due to the negative results, we began rearing newly collected egg sacs on a new plant of same species on which the egg masses were found (Table 3). This strategy improved the results significantly, so that the 96% mortality observed at day 15 was reduced to 42% (Figure 2).

During the one year collecting period 27 egg masses were obtained. Despite the fact that the leaf fragment tended to shrivel and lose its green coloration, 90% of all eggs hatched (eggs were observed through a stereoscope to determine if each of them had hatched). Moreover, 16 pairs of females and males mated, and from these pairs, each in a separate cage, 9 egg sacs were obtained (courtship and copulation has been described by Tedore and Johnsen, 2013). Another 9 egg sacs were obtained by collecting 13 gravid females in the field (these were detected by their distended, almost spherical abdomens), and 6 of these females successfully managed to establish a refuge for the egg sac and juveniles emerged in the laboratory. (Figure 3). Eggs and adults of *Lyssomanes jemineus* and Dendryphantinae (another group found) were regularly found on *Chamaedorea costaricana* Oerst, the plant on which most *Lyssomanes jemineus* egg sacs were found (Figure 4).

Feeding behavior and appropriate prey. Prey for spiderlings was provided in the cages three times a week. *Lyssomanes jemineus* showed the following predatory sequence: a. visually detecting and turning to face a prey item that was active from two body lengths away, approximately 5 mm to 12 cm; b. walking slowly toward the prey and if the prey

remained stationary the spider paused when it was close and its body became more compact; c. lunging at the prey, mostly from one to three body lengths away; d. at the end of a leap or lunge the spider contacted the prey with its palps and chelicerae, paralyzing it, and then feeding on it. In some cases, after a slight contact of 1 or 2 seconds with the prey, the latter was released or escaped; in other cases, it completely avoided the prey or even fled when it was near (Hallas & Jackson 1986).

To evaluate diets a total of approximately 30 hatchlings (low density egg masses were chosen to facilitate observation) were used for each diet and observed for up to 6 months. *Drosophila melanogaster* was an excellent prey for spiders that were 17 weeks old and older. For younger spiders *D. melanogaster* was too large and difficult to handle, although some juveniles (less than 17 weeks old) did show an initial interest in hunting, with leaps and unsuccessful attacks. Most of them approached, but when just a few millimeters away from the prey they quickly moved away (Fig. 5). Adults fed with *D. melangogaster* showed constant activity and foraging as previously reported in literature (Jackson 1974).

Juvenile *Lyssomanes jemineus* were observed preying on mites, but after 4 weeks spider mortality reached 100%. Nor was this type of prey appropriate for adults since, although feeding occurred, it was not possible to obtain a second generation with this diet. This type of prey was inappropriate or insufficient for juveniles in their first stage of feeding (between day 15 and 32 after hatching), when they move away from the remains of the egg sacs and begin to slowly forage around their old refuge. Some successful hunting by juveniles was observed, but only 20% were observed hunting mites. In contrast, 66% of the adults showed interest in this prey. The results obtained with using *Aphis citricidus* Kirkaldy (Hemiptera: Apididae) as prey were similar to those observed with mites, death of all *Lyssomanes jemineus* juveniles during the first four weeks. Both juveniles and adults moved quickly away from *Aedes aegypti* (Diptera: Culicidae). In contrast, whiteflies (Hemiptera: Aleyrodidae) proved to be an excellent prey item for both juvenile and adult spiders. Three species of whiteflies were used: *Bemissia tabaci* Gennadius (Biotype Q), *Aleyrodes* sp., and *Trialeurodes* sp. Juveniles moved away from their old refuge and began to take an interest in this prey between week 3 and 4 after hatching. The prey is active enough to be visually detected, but is smaller than *Drosophila*, which allowed for its manipulation. Nonetheless, only 38% to 53% of the juveniles reached adulthood when fed on whiteflies.

In summary, cage design 3 and the diet mentioned above (whiteflies for juveniles and *Drosophila* for adults) proved to be an adequate rearing system for *Lyssomanes jemineus*. After week 17 of the spiderlings, the most suitable setup was a modified environment with two plants, one plant with eggs and adults of *Bemissia* already established and the other the plant species where the eggs hatched. This greatly facilitated maintenance. On fast growing plants such as *Solanum lycopersicum* Karst, *S. melongena* L., *Phaseolus vulgaris* L. and *Lantana camara* L. the whiteflies reproduced very well. By having these plants mixed in the same environment we managed to avoid the scarcity of prey and it worked very well during holiday periods when inoculating the prey every week became difficult. In this way, the system was self-sustaining for the juveniles. In the case of colonies with adults, the same

could be done by introducing flasks with small openings for the controlled weekly exit of *Drosophila* similar to what is reported in Jackson 1974.

Other biological observations. Figure 6 provides a synthesis of our behavioral observations utilizing the most appropriate diets mentioned above. Egg sacs were derived from two sources, gravid females collected in the field and offspring reared in the lab (not all female-male pairs were successful). *Lyssomanes jemineus* males usually exhibit a more yellow-brown coloration with distinct markings on the legs and body, large chelicerae with long fangs, and enlarged pedipalps. The objective of this research was not to document copulation in detail but to rear the spiders under controlled conditions. However, stereotypical male displays were observed: extension of the forelegs and chelicerae, a rapid flicking of fore metatarsi, with the pedipalps extended over the head as it slowly steps over the female, and stroking of the female abdomen by the male's forelegs. The male did not appear to hold the female, but instead the male's legs were outstretched during copulation.

Females were usually observed above or quite near the silken egg sac, except for one female that seemed weak and thin, which we later verified with a microbiological culture to contain *Bacillus*. Most juveniles spent 1-2 weeks in the refuge before moulting to fully developed spiderlings. Instar duration was greater in later than in earlier instars. Generally, the spiderlings dispersed from the egg sac 12 to 22 days after the first moult. The exact number of moults was difficult to define but we managed to observe an average of 6 to 8.

Discussion

Rearing and maintaining spiders in captivity is often a rather laborious task. Four basic problems must be dealt with: a) providing food and water, b) minimizing factors that cause mortality c) minimizing maintenance time, and d) providing an adequate design of the enclosure. The optimal rearing method will depend on the spider taxon and so the exact methodology varies from case to case, depending on the interests of the researcher. New methods will undoubtedly be continually designed.

Mortality in our study decreased when using the same plant species on which the spider (whatever its stage) was found, which may suggest some kind of dependence and/or preference for chemical properties of certain plants (Tedore & Johnsen 2015). Adult *Lyssomanes* were successfully maintained, with relatively little mortality, on a diet of *Drosophila*, which is not surprising since flies are known to be an important part of the diet of salticids (Jackson 1978). Another obvious advantage of using *Drosophila* is that they are easily produced in the laboratory.

Observations from other studies have indicated that spiders often die before maturing when they lack critical nutritional requirements and usually do not survive the first five instars (Miyashita 1968). Thus, it was not surprising that the main challenge in rearing *Lyssomanes jemineus* was to ensure the survival of juveniles. From the first trials it was clear that young *Lyssomanes jemineus* rejected and even showed escape behavior after detecting the *Drosophila* prey that was provided. Very few attempted to hunt the flies and even for more mature juveniles they appeared to be an extremely challenging prey to manipulate. We

thus had to evaluate new and previously unreported diets, including whiteflies. This latter diet facilitated the survival of juveniles that were approximately 200 days old or younger. Beyond this age it proved feasible to begin using *Drosophila* as prey. Although we managed to rear juveniles to adulthood, mortality of juveniles was still greater than that observed in adults. In future research it would be worthwhile considering specific nutritional requirements of juveniles versus adults (Slansky & Rodriguez 1987).

The rearing system presented here provided a useful observation arena to confirm previously known aspects of salticid biology, such as 1-3 egg sacs per female and successive egg sacs with fewer eggs, as shown in *Astia*, *Cyrba*, *Holoplatys*, *Plexippus* (Jackson 1978; Zhao & Chen 1991). The total number of days required for development from first instar to adult was in excess of 150 days, with more than 6 moulting events, as has been shown previously in *Lyssomanes* (Richman & Whitcomb 1981). Field observations demonstrated that the life cycle encompasses an entire year in this tropical environment. Courtship and reproductive behavior observed in our rearing system coincides well with that observed in the field. This suggests that the negative effects of laboratory rearing may be reduced by careful design of the enclosure conditions, and providing a suitable diet for both juveniles and adults.

Acknowlegments

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References

Di Stéfano, J. F., V. Nielsen, J. Hoomans and L. A. Fournier. 1996. Regeneration of tree cover in a small urban forest reserve from the moist Premontane region. Revista de Biología Tropical 44: 575-580

Galiano, M. E. 1980. Catálogo de los especímenes típicos de Salticidae (Araneae) descriptos por Cândido F. de Mello-Leitão. Primera parte. Physis B. Aires (C) 39: 31-40.

Galiano, M. E. 1996. Descripción de tres nuevas especies de *Lyssomanes* de Brasil (Araneae, Salticidae) Iheringia, Série Zoologia 81: 23–30.

Galiano, M. E. 1998. Revision of the genus *Chinoscopu*s (Araneae, Salticidae, Lyssomanidae). Bulletin of the British Arachnological Society 11: 1–9.

Guevara-Coto, J. A., N. Barboza-Vargas, E. Hernandez-Jimenez, R. W. Hammond and P. Ramirez-Fonseca. 2011. *Bemisia tabaci* Biotype Q is present in Costa Rica. European Journal of Plant Pathology 131: 167.

Jackson, R. R. 1974. Rearing methods for spiders. Journal of Arachnology 2: 53-56.

Jackson, R. R. 1978. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae) I. Pursuit time and persistence. Behavioural Ecology and Sociobiology 4: 123-132.

Li, D. and R. R. Jackson. 1997. Influence of diet on survivorship and growth in *Portia fimbriata*, an araneophagic jumping spider (Araneae: Salticidae). Canadian Journal of Zoology 75: 1652-1658.

Logunov, D. V. 2014. New species and records of *Lyssomanes* Hentz, 1845 from Central and South America (Aranei: Salticidae). Arthropoda Selecta 23: 57–76.

Logunov, D. V. 2015. Taxonomic notes on the genus *Lyssomanes* Hentz 1845 (Araneae: Salticidae) from French Guiana. Acta Arachnologica 64: 39–44.

Logunov, D. V. and Y. M. Marusik. 2003. Taxonomic and faunistic notes on *Chinoscopus* Simon, 1900 and *Lyssomanes* Hentz, 1845 from the Neotropical Region (Araneae, Salticidae). Bulletin of the British Arachnological Society 12: 415–424.

Maddison, W. P. and K. M. Needham. 2006. *Lapsiines* and *Hisponines* as phylogenetically basal salticid spiders (Araneae: Salticidae). Zootaxa 1255: 37-55.

Miyashita, K. 1968. Changes of the daily food consumption during adult stage of *Lycosa pseudoannulate* BOES. et STR. (Araneae; Lycosidae). Applied Entomology and Zoology 3: 203-204.

Richman, D. B. and R. R. Jackson. 1992. A review of the ethology of jumping spiders (Araneae, Saticidae). Bulletin of the British Arachnological Society 9: 33–37.

Richman, D. B. and W. H. Whitcomb. 1981. The ontogeny of *Lyssomanes viridis* (Walckenaer) (Araneae: Salticidae) on *Magnolia grandiflora* L. Psyche 88: 127-133.

Slansky, F. and J. G. Rodriguez. 1987. Nutritional ecology of insects, mites, spiders, and related invertebrates. John Wiley & Sons, New York. 1016 pp.

Tedore C. and S. Johnsen. 2015. Immunological dependence of plant-dwelling animals on the medicinal properties of their plant substrates: a preliminary test of a novel evolutionary hypothesis. Arthropod-Plant Interactions 9: 437-466.

Wanless, F. R. 1980. A revision of the spider genera *Asemonea* and *Pandisus* (Araneae: Salticidae). Bulletin of the British Museum of Natural History (Zoology) 39: 213-257.

Wanless, F. R. 1984. A review of the spider subfamily Spartaeinae nom. n. (Araneae: Salticidae) with descriptions of six new genera. Bulletin of the British Museum of Natural History (Zoology) 46: 135-205.

Zhao J. Z. and W. H. Chen. 1991. The effects of temperature on development and fecundity in *Tetragnatha vermiformis* Emerton (Araneae: Tetragnathidae). Chinese Journal of Ecology 10: 11-15.

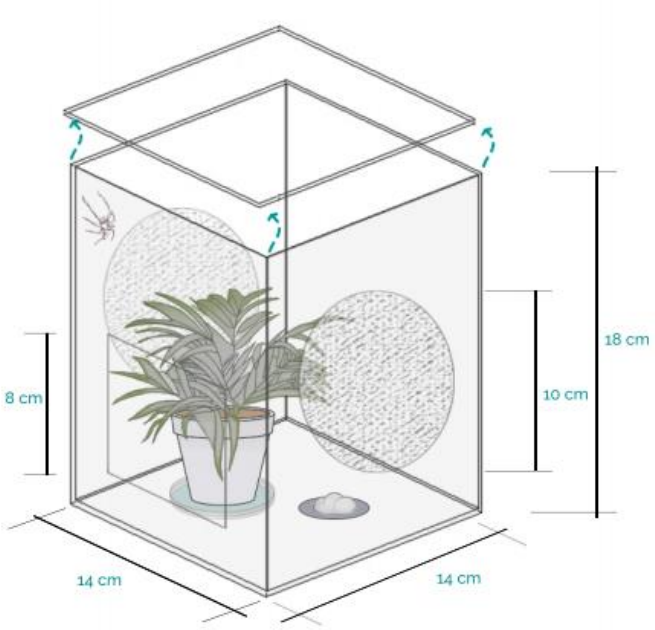

Figure 1. Container design 3. Dimensions: 18 cm high, 14 cm wide and 14 cm deep. Openings and vents: one 8 cm frontal opening, two lateral openings 10 cm in diameter for ventilation and sealed with an ultra-thin screen, even finer than the anti-aphid type.

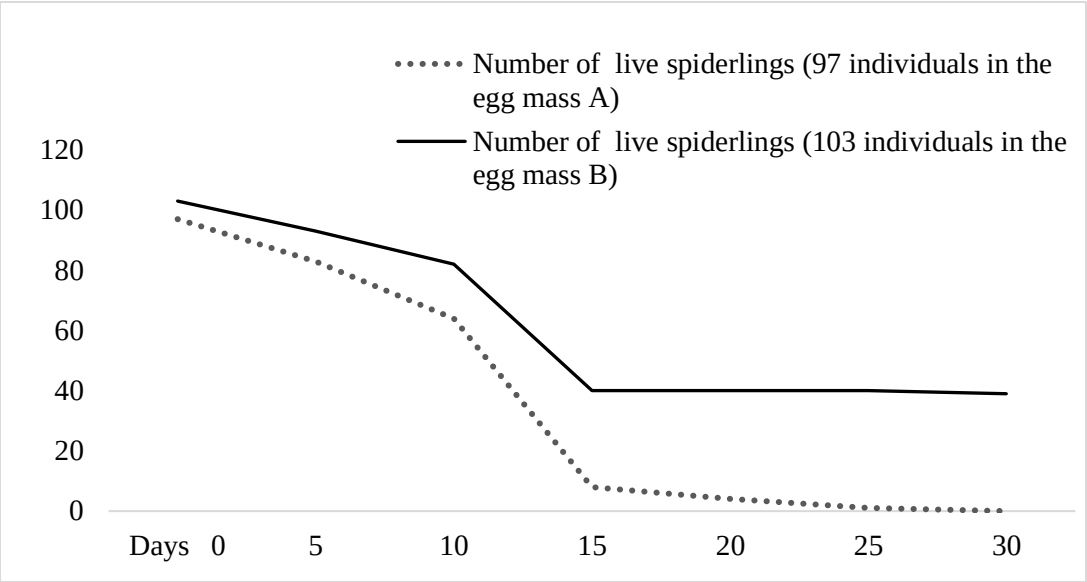

Figure 2. Egg mass A. Mortality of spiderlings reared with whiteflies as prey on foliage of various plant species. Egg mass B. Mortality of spiderlings reared with whiteflies as prey on the same plant species as where the egg sac was found in field.

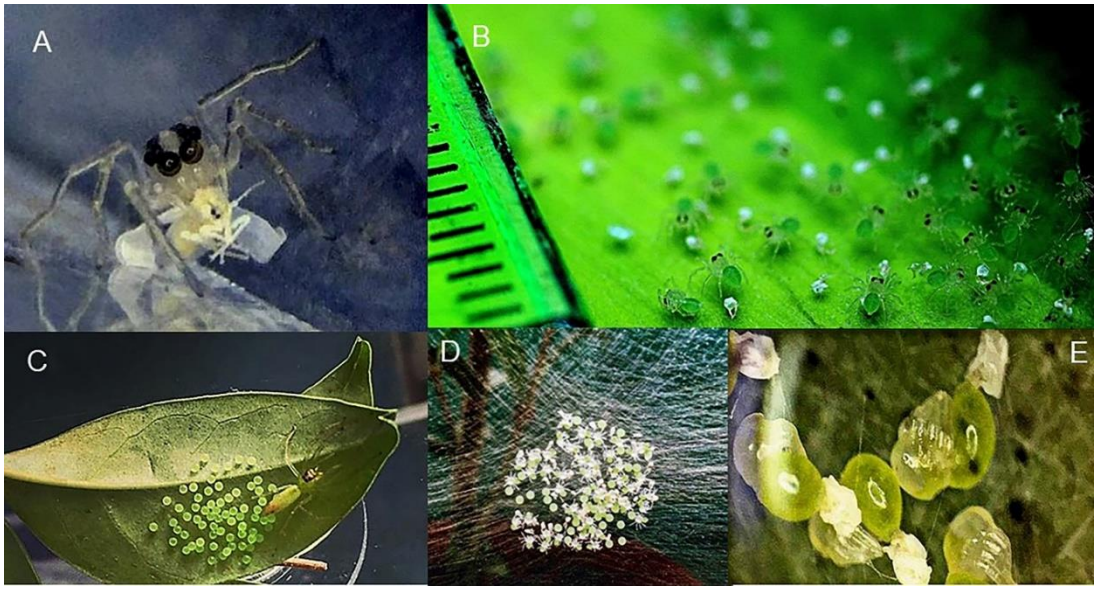

Figure 3. A. Spiderling of 22 days ingesting *B. tabaci* in captivity. B. 13-day spiderlings C. Female spider oviposition of her first egg mass observed in captivity. D. Silk shelter and hatchlings from field. E. Silk shelter and eggs the moment they hatched in captivity.

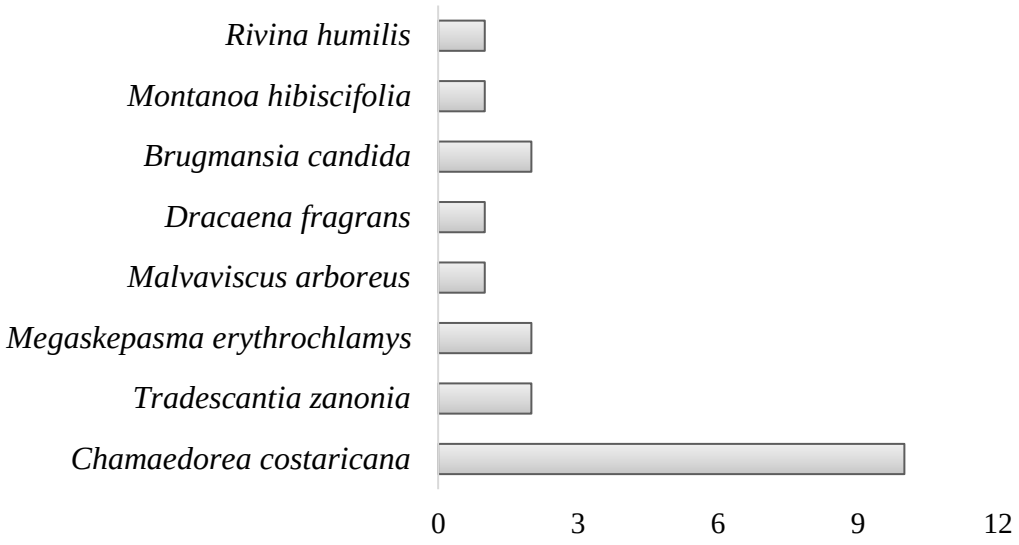

Figure 4. Number of egg sacs of *Lyssomanes jemineus* collected on diverse plant species along the transect.

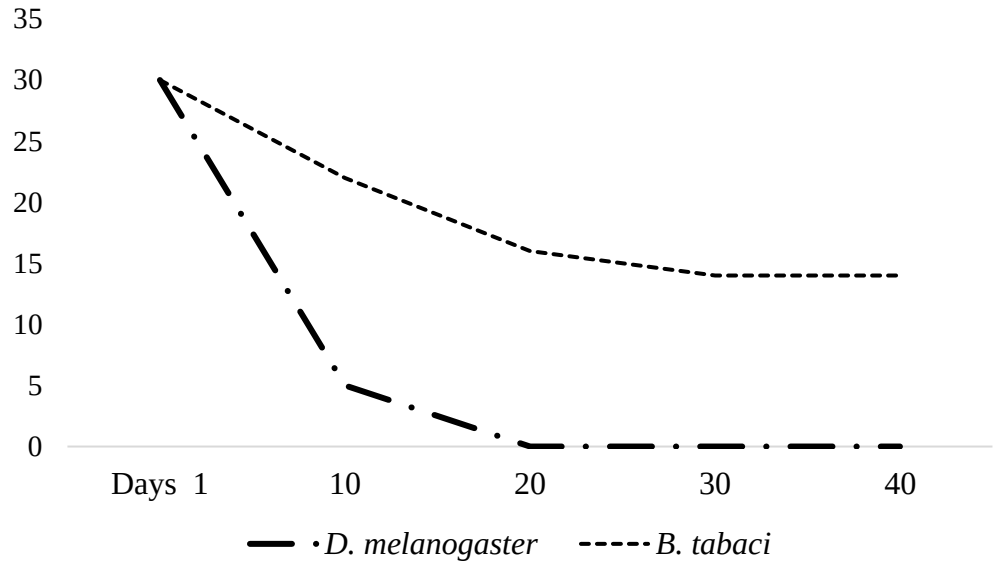

Figure 5. Survival of 30 hatchlings from day 1 to day 40 under two types of diet.

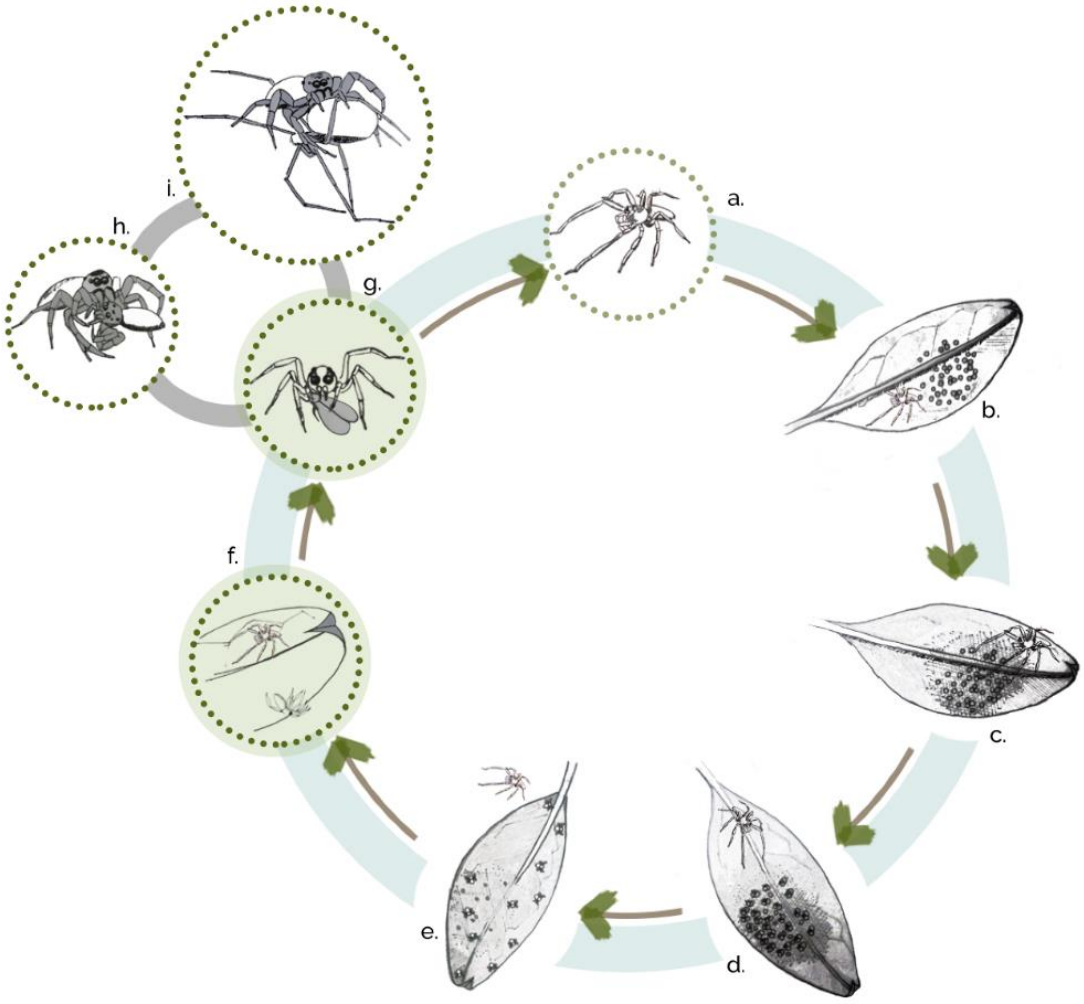

Fig. 6. Synthesis of biological observations of *Lyssomanes jemineus*. A. Adult female. B. Adult female ovipositing. C. Adult female caring for her egg mass. D. More mature eggs with more distant female. E. Female remains alert but more distant a few days before and during hatching of juveniles; 15 days later the mother is usually not present or dies. F. Juvenile and its moult. G, H, I. Predation behavior with various prey. Cannibalism was not observed at low densities.

Diet	Conditions	Rearing
Drosophila melanogaster	Reared in laboratory	Glass vials were used and their bottom (2 cm) filled with autoclaved *Drosophila* diet, which is what the flies lay their eggs in. Batches of fly food were prepared every month and consisted of a blend of water, banana, oats and

		propionic acid. Flies were reared at room temperature (15 days from egg to adult), protected from drafts and direct sunlight or heat sources. Every 3 weeks the medium was changed.
Arachnida: Acari	Reared in laboratory	*Coffea arabica* residues were maintained humid (sprinkled with water weekly) but avoiding excess water. Aeration was crucial to avoid hardening of the substrate and providing better conditions for reproduction, which was done by stirring the substrate every week.
Aphis citricidus	Reared in laboratory	Citrus shoots planted in pots under the same conditions as *B. tabaci* (see below)
Bemissia tabaci MED (*B. tabaci* Biotype Q)	Reared in laboratory and collected near campus	Provided by Natalia Barboza Vargas from University of Costa Rica (Guevara-Coto *et al*. 2011). Whiteflies were reared on young eggplants (*Solanum melongena*) planted in pots inside aluminum cages and hermetically closed with anti-aphid fabric. Weekly plant watering and room temperature near 24 º C were crucial. In some cases when populations of *B. tabaci* on eggplant were low we used a population of *Aleyrodes* sp. This population was located in the outside areas of the insectarium on *Emilia fosbergii*, *Sonchus oleraceus* and *Sonchus asper*.
Aedes aegypti	Reared in laboratory	Provided by Adriana Troyo Rodríguez from University of Costa Rica. We used males fed with sugars.

Table 1. Alternative diets and their rearing conditions.

Design	Materials	Dimensions	Openings and vents	Plants and water
1	Wooden frame and fine saran	50 cm high, 40 cm wide and 40 cm deep	Frontal part opens completely and is secured with clamp (this is the only entry to incorporate plants or specimens)	One plant was placed in plastic pot with water reservoir plate.
2	Polyurethane and aluminum	40 cm high, 30 cm wide and 30 cm deep	Frontal part opens completely and is secured with clamp (this is the only entry to	One plant was placed in plastic pot with water reservoir plate.

			incorporate plants or specimens)	
3	Acrylic	18 cm high, 14 cm wide and 14 cm deep	One 8 cm frontal opening, another two lateral openings of 10 cm in diameter for ventilation and sealed with an ultra-thin sieve, even finer than the anti-aphid type	One plant was placed in plastic pot with a water reservoir plate, using the top container cover

Table 2**.** Cage designs and specifications.

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Messua sp. (Salticidae), the host spider of the polysphinctine *Inbioia pivai* (Ichneumonidae: Pimplinae)

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Abstract

The koinobiont ectoparasitoids of spiders in the *Polysphincta* genus group are divided into two well-supported clades. Species in one clade parasitize spiders in the higher araneoid group, and the behaviour of these ectoparasitoids and the parasitized host spiders is relatively well known. On the contrary, wasp species in the other clade parasitize spiders in the RTA group and their behaviour is fragmentary, being known from just a few Palaeartic species. With respect to the latter clade, we report the first host record for the monotypic genus *Inbioia*, the salticid *Messua*. The larva of *I. pivai* was attached to the posterior edge of the dorsal cephalothorax of *Messua*. The larva apparently induces the host spider to construct a final retreat, much denser than the spider's typical retreat. In the retreat, the larva kills the spider, sucks out its tissue, and then builds a thin cocoon, tightly attached to the spider retreat.

Keywords: host parasitoid interaction • koinobiont parasitoid • larval cocoon

Introduction

Wasps in the *Polysphincta* genus group are obligate koinobiont ectoparasitoids that lay a single egg on spiders of various families (Nielsen 1923; Korenko *et al*. 2014). The *Polysphincta* group was recently divided into two well-supported clades based on molecular data (Matsumoto 2016) as well as ovipositor morphology and oviposition behaviour (Takasuka *et al*. 2018): Clade I includes wasp species that exclusively attack spiders in the RTA group, whereas wasps in Clade II attack spiders in the higher araneoid group (Garrison *et al*. 2016). The wasp's attack behaviour, site of egg deposition on the host spider, larval behaviour, and cocoon construction are stereotypic and vary little within each clade, but vary markedly between clades in response to differences in behaviour of the spider hosts (Takasuka *et al*. 2018).

Polysphinctine wasps in Clade II attack aerial cob-web and orb-web spiders in their webs, temporarily paralysing the spider and laying an egg on its antero-lateral or dorso-lateral section of its abdomen (Nielsen 1923; Gonzaga *et al*. 2010, 2016; Barrantes *et al*. 2018; Takasuka *et al*. 2018). The spider apparently continues its normal life while the larva feeds on the spider's haemolymph by biting through its cuticle. During the last hours of the penultimate instar larva, it induces the host spider to construct a cocoon web which is finely adjusted to provide protection to the larva and pupa from environmental conditions and predation (Eberhard 2001; Weng & Barrantes 2007; Sobczak *et al*. 2009). The larva then kills the host spider, sucks out its tissues while hanging from the cocoon web threads, where it later spins its cocoon (Eberhard 2001; Barrantes *et al*. 2018). In contrast, wasps in Clade I attack cursorial spiders (e.g. Salticidae, Clubionidae), funnel web spiders (e.g. Agelenidae), and some aerial-mesh web spiders (e.g. Dictynidae) (Matsumoto 2009, 2016; Korenko 2017). These wasps lay their eggs on the cephalothorax rather than the abdomen, and the larvae construct their cocoons inside the spider retreat, rather than hanging from a cocoon web as in species of Clade II (Takasuka *et al*. 2018). An exception to this pattern is *Zatypota anomala* (Holmgren, 1860), a wasp species in Clade II that attacks the dictynid *Dictyna pusilla* Thorell, 1856, which constructs an aerial-mesh web (Korenko 2017).

Information on the behaviour of Clade I polysphinctines is fragmentary and extremely scarce. In the European salticid *Salticus cingulatus* (Panzer, 1797) and other ground-dwelling RTA spiders, wasps of clade I deposit their eggs on the spider's cephalothorax, but other behaviours are basically unknown. This report documents *Inbioia pivai* Gauld & Ugalde, 2002 parasitizing the salticid *Messua* sp. and describes the larval cocoon and host spider behaviour. This is the first report of the host spider for the monotypic genus *Inbioia* and the first confirmed record (see Gauld & Debois 2006) of a spider parasitized by a wasp species of Clade I in the New World.

Materials and methods

We collected two immature female spiders in the genus *Messua* (Salticidae) with larvae, along a trail in the sports facilities of the University of Costa Rica, San José, Costa Rica (9°54′N 84°03′W, 1200 m a.s.l.). The site is located in the western section of the Costa Rican Central Valley which is characterized climatically by an annual precipitation of 2500 mm, mainly from May through December, and a mean annual temperature of 21°C. We found one spider in March 2017 and the other in September 2018, inside their retreats constructed in folded leaves of *Chamaedorea costaricana* (Arecaceae) and *Bauhinia purpurea* (Fabaceae), respectively, at about 1.5 m above the ground, in a patch of scrub vegetation in an urban environment (Biamonte *et al*. 2011).

Each spider with the larva attached was maintained alive inside a plastic container (10 cm × 10 cm × 8 cm height) in the laboratory. We attached a dry leaf from the lid of the container for the spider to build its retreat, and maintained a piece of water-soaked cork inside the container. Spiders were fed with *Drosophila* flies three times a week while in the container.

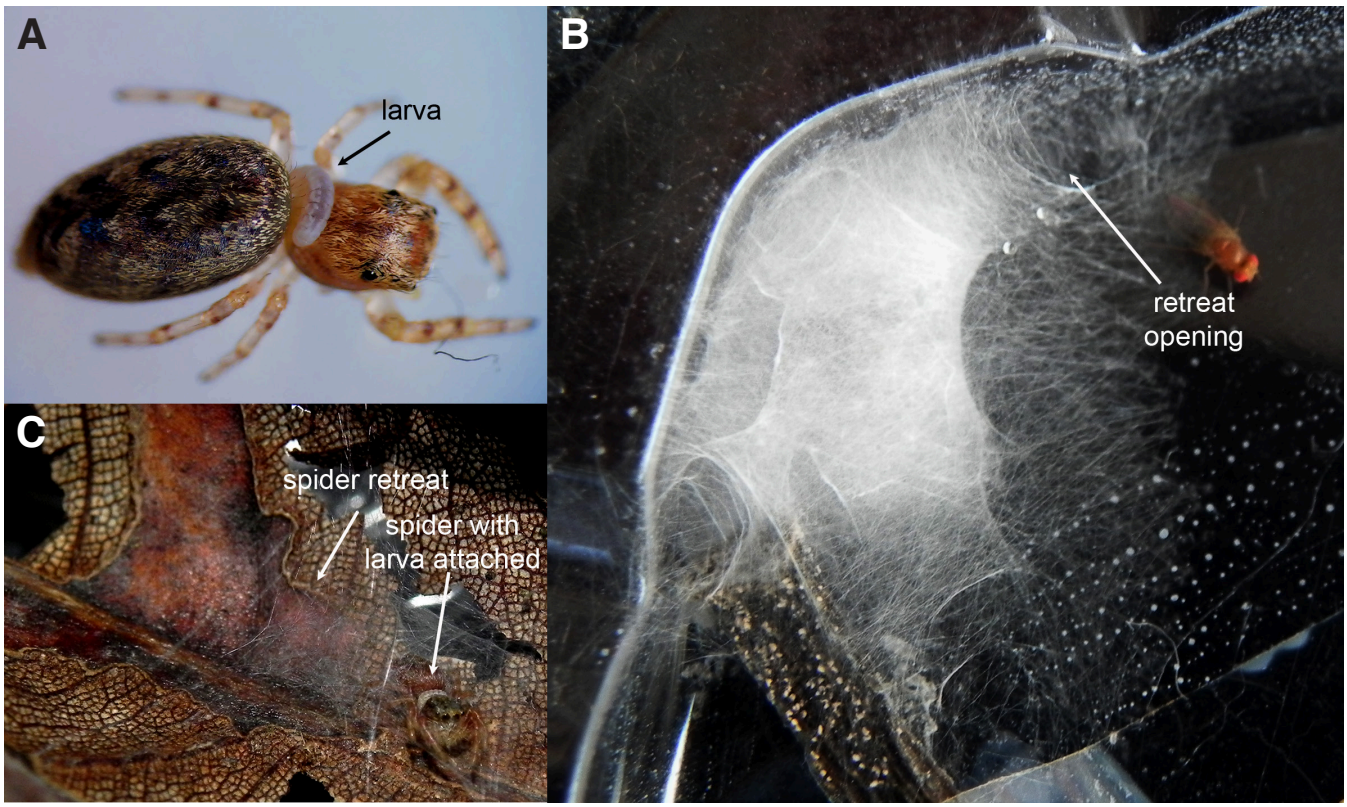

Fig. 1: Spider with attached larva and retreats. **A** *Messua* sp. with the larva of *Inbioia pivai* attached to edge of the cephalothorax; **B** typical spider retreat; **C** spider retreat where the larva killed the spider.

We registered the behaviour of the spider and the larva photographically, and recorded the time required for the larva to pupate and emerge. Adult female wasps were photographed under a dissecting microscope (Nikon SMZ645) and, for some details of the wasp morphology, we used a DS-Fi3 microscope Nikon camera coupled to an Eclipse LV100 ND Nikon Microscope, with a 5× magnification objective (Nikon CFI60 2 TU Plan Fluor BD 5×, NA 0.15, WD 18.0 mm), and an episcopic light illumination, with a 12V-100W halogen lamp. We took multiple photos at different focus levels of the wasp abdomen and then merged the multiple images to produce a single image with greater depth of field, using Adobe Creative Cloud Photoshop v. 4.7.

The adult wasps that emerged from the cocoons were preserved in 70% ethanol and identified by comparing them with the original description of the genus and species (Gauld & Ugalde 2002). Wasp specimens are deposited in the Museo de Zoología, Universidad de Costa Rica.

Results

The *I. pivai* larva was positioned across the posterior edge of the dorsal plane of the spider cephalothorax (Fig. 1A), and apparently did not affect the spider's activity since it was capable of feeding and constructing retreats (Fig. 1B). One spider constructed a retreat on the leaf we provided, but three days later she constructed another, much denser retreat with an opening (Fig. 1C), on the lid of the container (the second spider constructed a retreat equally dense). During five days it was possible, using a magnifying glass, to see the spider inside the retreat, but not the larva because of the density of the retreat threads; nor was it possible to discern whether or not the spider was alive. At day seven the larva discarded the spider's carcass, and the larval meconium was observed after seven additional days (Fig. 3B). The adult females emerged 21 and 22 days, respectively, after the larva built the cocoon.

Both wasps that emerged were females, which have a distinctive series of characters, such as legs covered by brownish speckles, the last five abdominal segments with a black crown-shaped design on the dorsal section of the most anterior of these segments (Fig. 2A–B). However, the most striking feature is a finger-like projection that emerges ventrally at the posterior border of the penultimate abdominal segment, at each side of the ovipositor (Fig. 2C–D). Each of these structures emerges as a single stem that then branches, producing two lateral shorter segments, and one central longer segment (Fig. 2D). The wasp also possesses two other elongate projections, emerging ventrally at the posterior border of the last abdominal segment, at each side of the ovipositor (Fig. 2D).

The larva constructed a thin-walled cocoon inside the spider retreat (Fig. 3A). The larva tightly attached its cocoon to the internal wall of the spider retreat with abundant, thin threads (Fig. 3B).

Discussion

In many respects, the larval behaviour of *I. pivai* resembles that of other Group I polysphinctines that parasitize spiders in the RTA group (Matsumoto 2016; Takasuka *et al*. 2018). It is attached at the posterior-dorsal edge of the cephalothorax, and spins its cocoon and develops inside the

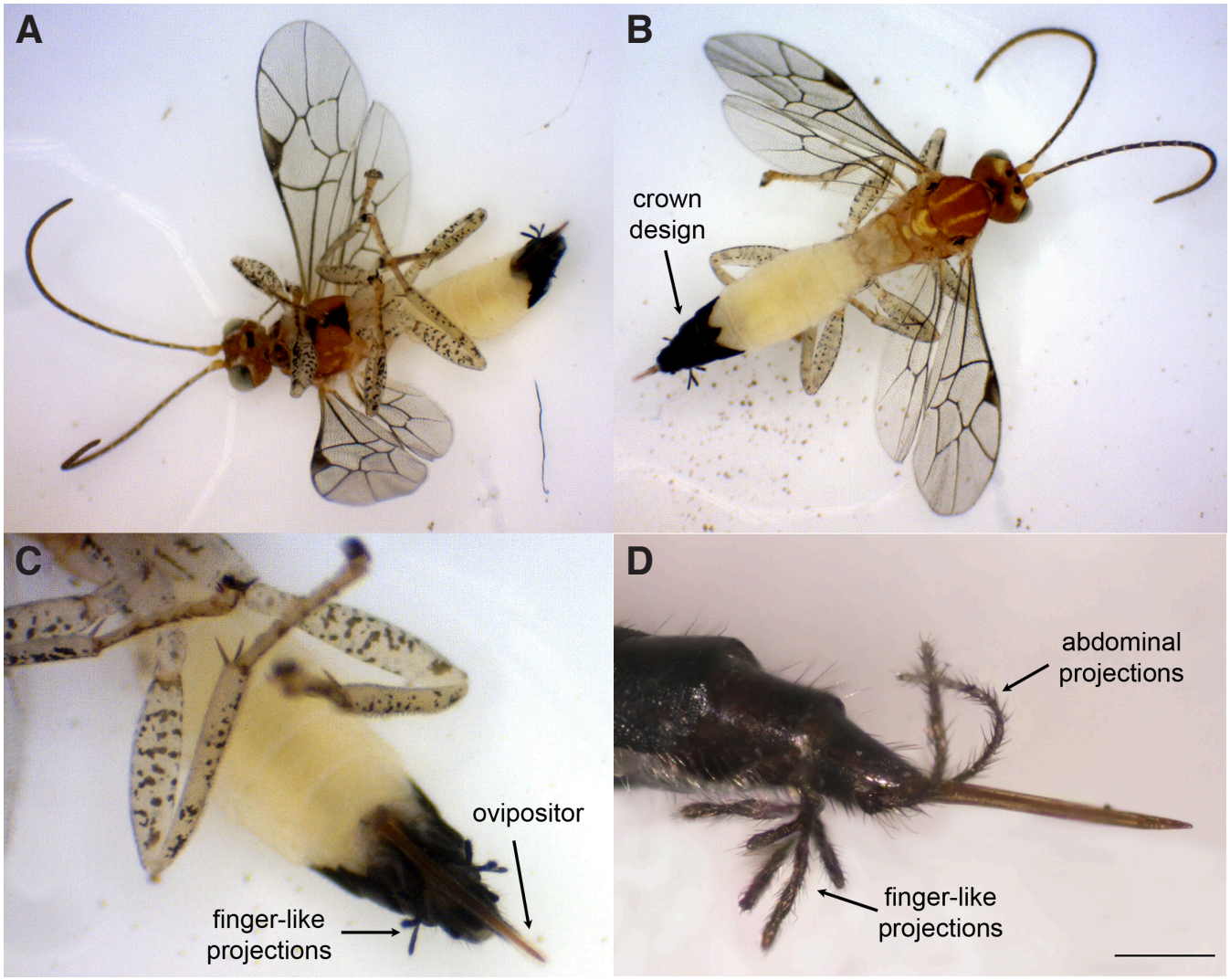

Fig. 2: Morphological features of *I. pivai* female. **A** ventral view; **B** dorsal view, showing the black crown design at the end of the abdomen; **C** ventral view of ovipositor and projections; **D** detail of the abdominal projections.

spider retreat. For the most part, the larva did not appear to change the spider's behaviour, since *Messua* fed on the prey provided and constructed its retreats. However, the final retreat the spider constructed, in which the larva killed the spider and sucked out its tissue, was much denser than the typical retreat the spiders build. This suggests that the larva is capable of manipulating the spider's behaviour, probably to reduce predation and adverse effects of the environment (Sobczak *et al*. 2012; Barrantes *et al*. 2017). However, quantitative information on the retreat density of parasitized and non-parasitized *Messua* spiders and other spiders parasitized by this group of wasps is required to support this hypothesis.

The wall of the *I. pivai* cocoon is thinner than that of polysphinctines that build their cocoons more directly exposed to the environment, such as those that parasitize araneoids (e.g. *Theridion evexum*, *Leucauge* spp., *Nephila clavipes*, *Kapogea cyrtophoroides*; Eberhard 2001; Weng & Barrantes 2007; Gonzaga *et al*. 2012; Barrantes *et al*. 2018), but apparently as thin as those cocoons constructed by some wasps that parasitize other spiders in the RTA group (e.g. *Agelena silvatica*, *Clubiona rostrata*; Matsumoto 2016; Takasuka *et al*. 2018). The dense silk retreat that the larva of *I. pivai* apparently induces *Messua* sp. to build may have influenced the thickness of the wall of its own cocoon.

The impressive abdominal projections in the adult female of *I. pivai* are unique within Ichneumonidae and their function is not yet understood. Because males of *Inbioia* have never been collected, it is not yet known whether these structures are found only in females. If they are, at least two possible functions can be suggested: as sex pheromone dispersing organs, or as organs involved in attacking the host. The first hypothesis, though possible, is unlikely since a large number of insects disperse sex pheromones without such complex organs (Chapman & Simpson 2013). Alternatively, these elongate and complex projections could help the wasp to sense the host spider during the attack, or to liberate an allomone used to subdue the host. Ultrastructural studies and observations of the behaviour during oviposition are needed to understand the function of these abdominal projections.

Similar to other species in Clade I (Matsumoto 2016; Takasuka *et al*. 2018), the larva of *I. pivai* is attached to the cephalothorax of *Messua* sp. rather than to the abdomen as in Clade II. However, the position on the cephalothorax apparently varies among species (Matsumoto 2016; Takasuka *et al*. 2018) as does the position of the larva on the spider abdomen in Clade II, but this aspect of the biology has not received much attention in either group. Information on the behaviour of Clade I larvae and their RTA host spiders

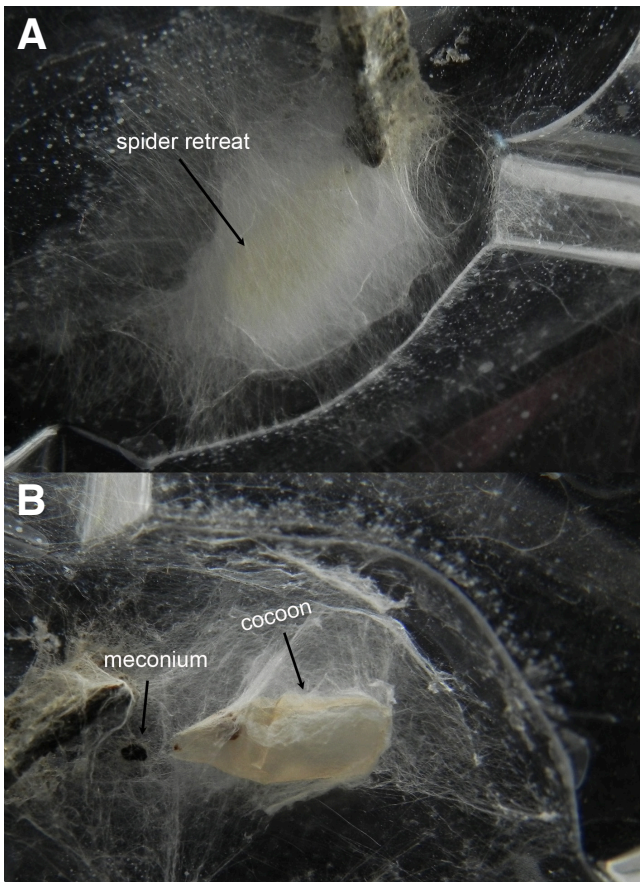

Fig. 3: Spider retreat and larval cocoon of wasp. **A** spider retreat with the larval cocoon inside; **B** larval cocoon exposed. Note the layer of thin threads that attach the larval cocoon to the spider retreat and the pupal meconium.

is scarce, but our results suggest that the larva induces the spider host to construct an extremely dense retreat, probably to enhance larval and pupal survivorship.

Acknowledgments

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References

BARRANTES, G., SEGURA-HERNÁNDEZ, L., SOLANO-BRENES, D. & HANSON, P. 2018: When a little is enough: cocoon web of *Kapogea cyrtophoroides* (Araneae: Araneidae) induced by *Hymenoepimecis heidyae* (Ichneumonidae: Pimplinae). *Arachnologische Mitteilungen* **55**: 30–35.

BIAMONTE, E., SANDOVAL, L., CHACÓN, E. & G. BARRANTES. 2011: Effect of urbanization on the avifauna in a tropical metropolitan area. *Landscape Ecology* **26**: 183–194.

CHAPMAN, R. F. & SIMPSON, S. J. 2013: *The insects. Structure and function*. Cambridge: Cambridge University Press.

EBERHARD, W. G. 2001: Under the influence: webs and building behavior of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenoptera, Ichneumonidae). *Journal of Arachnology* **29**: 354–366.

GARRISON, N. L., RODRIGUEZ, J., AGNARSSON, I., CODDINGTON, J. A., GRISWOLD, C. E., HAMILTON, C. A., HEDIN, M., KOCOT, K. M., LEDFORD, J. M. & BOND, J. E. 2016: Spider phylogenomics: untangling the Spider Tree of Life. *PeerJ* **4**: e1719.

GAULD, I. & UGALDE, J. 2002: Subfamily Pimplinae. Further supplement to Volume 1. *Memoirs of the American Entomological Institute* **66**: 744–746.

GAULD, I. and DEBOIS, J. 2006: Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. *Systematic Entomology* **31**: 529–564.

GONZAGA, M. O., LOFFREDO, A. P., PENTEADO-DIAS, A. M. & CARDOSO, J. C. F. 2016: Host behavior modification of *Achaearanea tingo* (Araneae: Theridiidae) induced by the parasitoid wasp *Zatypota alborhombarta* (Hymenoptera: Ichneumonidae). *Entomological Science* **19**: 133–137.

GONZAGA, M. O., SOBCZAK, J. F., PENTEADO-DIAS, A. M. & EBERHARD, W. G. 2010: Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). *Ethology Ecology and Evolution* **22**: 151–165.

KORENKO, S. 2017: First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pusilla* (Araneae, Dictynidae). *Arachnologische Mitteilungen* **54**: 1–4.

KORENKO, S., ISAIA, M., SATRAPOVA, J. & PEKAR, S. 2014: Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). *Ecological Entomology* **39**: 30–38.

MATSUMOTO, R. 2009: "Veils" against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). *Journal of Insect Behavior* **22**: 39–48.

MATSUMOTO, R. 2016: Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae). *Systematic Entomology* **41**: 854–864.

NIELSEN, E. 1923: Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*). *Entomologiske Meddelelser* **14**: 137–205.

SOBCZAK, J. F., LOFFREDO, A. P. S., PENTEADO-DIAS, A. M., & GONZAGA, M. O. 2009: Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. *Journal of Natural History* **43**: 2691–2699.

SOBCZAK, J. F., LOFFREDO, A. P. D. S., CAMARGO, L. F. & PENTEADO-DIAS, A. M. 2012: *Hymenoepimecis neotropica* (Brues & Richardson) (Hymenoptera, Ichneumonidae, Pimplinae) parasitoid of *Araneus omnicolor* (Keyserling) (Araneae, Araneidae): first host record and new occurrence to Brazil. *Revista Brasileira de Entomologia* **56**: 390–392.

TAKASUKA, K., FRITZÉN, N. R., TANAKA, Y., MATSUMOTO, R., MAETO, K. & SHAW, M. R. 2018: The changing use of the ovipositor in host shifts by ichneumonid ectoparasitoids of spiders (Hymenoptera, Ichneumonidae, Pimplinae). *Parasite* 25: 2018011.

WENG, J. L. & BARRANTES, G. 2007: Natural history and larval behavior of the parasitoid *Zatypota petronae* Hymenoptera: Ichneumonidae). *Journal of Hymenoptera Research* **16**: 327–336.

MÓDULO DE CONTENCIÓN AUTOMATIZADO Y PROGRAMABLE

CAMPO TÉCNICO DE LA INVENCIÓN

La presente invención se relaciona con el campo del albergue de seres vivos. Particularmente con el campo de los equipos para el albergue de animales; aves, peces, insectos, entre otros. En específico provee un módulo de contención automatizado y programable para artrópodos, insectos u otros animales de tamaño similar.

ANTECEDENTES DE LA INVENCIÓN

La presente invención se refiere a módulos de contención automatizados y programables para la observación de especímenes y generación de datos científicos en base al comportamiento de los especímenes que alberga.

En el ámbito del albergue de animales, existe una amplia variedad de terrarios utilizados para alojar y mantener reptiles, anfibios y peces, así como otros animales y plantas. Los terrarios convencionales están constituidos principalmente para que los usuarios puedan observar dichos animales o plantas desde afuera. La gran mayoría se construyen en forma de caja o prisma rectangular para simplificar su fabricación. Por ejemplo, algunos se forman a partir de cuatro paredes de vidrio o plástico transparente, dispuestas perpendicularmente entre sí para formar una caja.

Las paredes se sujetan a un fondo y una cubierta rectangular descansa sobre las paredes para evitar que los animales se escapen. Por lo general, la cubierta está totalmente apoyada sobre las paredes y es removible para poder acceder al interior del recipiente para la limpieza y mantenimiento de rutina. Esto puede ser útil, sin embargo, en muchos casos el acceso al interior desde la parte superior del terrario puede ser limitado, especialmente para los terrarios de paredes altas.

Muchos terrarios se utilizan para alojar animales que requieren ciertas condiciones de humedad, ventilación y térmicas. Si bien algunos fabricantes intentan satisfacer estos requisitos, agregando ventiladores y/o humidificadores especiales, generalmente no logran los valores requeridos debido al tamaño y forma de los recipientes convencionales. Adicionalmente, los terrarios convencionales pueden

equiparse con una pluralidad de accesorios, dependiendo del espécimen que albergue y su hábitat nativo.

Por otra parte, algunos terrarios pueden tener luces dispuestas en la cubierta. Para alimentar dichas luces, se requiere de un cable eléctrico que se extiende desde la cubierta hasta una pared lateral del terrario. Recurrentemente, dicho cable eléctrico se deja sin tapar, lo que resulta antiestético e inseguro.

Dentro de las soluciones existentes en el estado de la técnica se encuentran, por ejemplo, lo propuesto en el documento US 2018/288948, el cual describe un terrario automatizado que comprende una carcasa que tiene una base y una tapa, en la que la tapa está soportada por al menos un pilar de soporte; al menos dos paneles laterales transparentes soportados por al menos un pilar de soporte y colocados entre la base y la tapa; un sistema de riego dentro de la base; un sistema de iluminación dentro de la tapa; y una unidad de control, en donde la unidad de control está adaptada para monitorear el crecimiento de una planta y ajustar tanto el sistema de irrigación como el sistema de iluminación.

Por otra parte el documento US 2017/251642 proporciona un terrario que comprende una base y un segundo canal de base; una columna de soporte que incluye un extremo superior y un extremo inferior asegurado a la base; una parte superior que incluye un primer canal superior y un segundo canal superior, la parte superior está totalmente soportada por la columna de soporte a una primera distancia preseleccionada de la base; un primer panel arqueado que se extiende entre la parte superior y la base, estando dispuesto el primer panel arqueado en el primer canal superior y el primer canal de base, formando el primer panel arqueado una abertura entre la parte superior y la base; un segundo panel arqueado que se extiende entre la parte superior y la base, estando dispuesto el segundo panel arqueado en el segundo canal superior y el segundo canal de base, formando el segundo panel arqueado un cierre de la abertura, en donde al menos uno de los primeros y segundos arqueados los paneles son un panel transparente, en donde los paneles arqueados primero y segundo cooperan en la parte superior y la base para definir un compartimento interno, en donde la columna de soporte se extiende dentro del compartimento interno, en el que el cierre es deslizable selectivamente

dentro del segundo canal superior y el segundo canal de la base para proporcionar acceso al compartimento interno entre la parte superior y la base.

A su vez el documento US 2009/050068 describe un terrario que comprende una pared inferior; al menos una pared lateral transparente, en donde dicha pared inferior y dicha al menos una pared lateral están acopladas entre sí para formar un recinto en su interior; y un sistema de ventilación dispuesto en una parte de al menos una pared lateral, el sistema de ventilación configurado para permitir que entre aire al recinto, el sistema de ventilación que incluye al menos una abertura posicionada para comunicarse entre el interior y el exterior del terrario, en donde el sistema de ventilación incluye un perfil interno colocado dentro del terrario y, en donde al menos una porción de al menos una abertura está posicionada dentro del perfil interno. El perfil interno incluye una proyección que se extiende hacia arriba para guiar el aire en dicha dirección, de manera que el aire que ingresa al recinto a través del sistema de ventilación se dirige hacia la superficie interior de al menos una pared lateral adyacente al sistema de ventilación.

Sin embargo, se han identificado falencias presentes en las soluciones del estado de la técnica, debido a que no controlan gran variedad de factores relevantes para el albergue de determinados especímenes; por ejemplo, la implementación de dietas adecuadas, el control biológico de plagas, la cría de parasitoides, y enemigos naturales.

Por otra parte, para cautiverio se encontraron jaulas de diversos materiales y formas, sin embargo, los únicos elementos automatizados fueron las luces y/o los dispositivos de medición de temperatura. En los documentos encontrados los equipos no presentan automatizaciones de los movimientos de presas, ni pretenden la alimentación artificial con una programación que simule el movimiento de las presas, factor de gran importancia para la investigación y generación de datos.

En consecuencia, se requiere de un sistema automatizado y personalizado enfocado en la observación científica, que permita simular condiciones para optimizar la observación del comportamiento del espécimen que alberga. Principalmente encauzada en la eliminación de factores externos que influyan en el comportamiento del organismo vivo de interés, y en específico enfocado en el sistema de alimentación de los mismos.

SUMARIO DE LA INVENCIÓN

La presente invención provee un módulo de contención automatizado y programable para la observación de especímenes que se caracteriza porque comprende un armazón cerrado que define un volumen interior; un elemento móvil posicionado en dicho volumen interior de dicho armazón cerrado; un mecanismo motor conectado operativamente a dicho elemento móvil, dicho mecanismo que comprende un carro, una vía y medios de traslación de dicho carro, donde dicho carro se configura para desplazarse a lo largo de dicha vía; y una unidad de control conectada operativamente a dicho mecanismo motor, donde dicha unidad de control está configurada para accionar dichos medios de traslación.

En una realización preferida, el módulo de contención automatizado y programable se caracteriza porque dicho armazón cerrado comprende una base, una pluralidad de paredes laterales y una cubierta.

En una realización preferida, el módulo de contención automatizado y programable se caracteriza porque dicha base, dicha pluralidad de paredes laterales y dicha cubierta son de un material transparente.

En otra realización preferida, el módulo de contención automatizado y programable se caracteriza porque dicha cubierta y al menos una de dichas paredes laterales poseen una abertura.

En una realización preferida adicional, el módulo de contención automatizado y programable se caracteriza porque dicha cubierta posee una pluralidad de aberturas.

En una realización preferida, el módulo de contención automatizado y programable se caracteriza porque dicho mecanismo motor comprende una pluralidad de carros, una pluralidad de vías y una pluralidad de medios de traslación.

En una realización más preferida, el módulo de contención automatizado y programable se caracteriza porque dicho mecanismo motor comprende 4 carros, 4 vías y 2 medios de traslación.

En una realización preferida adicional, el módulo de contención automatizado y programable se caracteriza porque dichos 4 carros y 4 vías se posicionan de manera enfrentada 2 a 2.

En una realización preferida, el módulo de contención automatizado y programable se caracteriza porque dichos medios de traslación son un actuador lineal.

En una realización más preferida, el módulo de contención automatizado y programable se caracteriza porque dichos medios de traslación son un motorreductor eléctrico conectado operativamente a un elemento de transformación de movimiento rotativo a lineal.

En otra realización preferida, el módulo de contención automatizado y programable se caracteriza porque dicha unidad de control comprende una interfaz de interacción con un usuario.

En una realización preferida, el módulo de contención automatizado y programable se caracteriza porque comprende una pieza móvil posicionada sobre dicha cubierta y conectada operativamente a dicho mecanismo motor.

En una realización preferida adicional, el módulo de contención automatizado y programable se caracteriza porque dicha pieza móvil se conecta magnéticamente a dicho elemento móvil.

En otra realización preferida, el módulo de contención automatizado y programable se caracteriza porque comprende una pluralidad de piezas móviles conectadas magnéticamente a una pluralidad de elementos móviles.

BREVE DESCRIPCIÓN DE LAS FIGURAS

La Figura 1 muestra una vista isométrica en perspectiva en un diseño 3D de una primera realización del módulo de contención automatizado y programable que es objeto de la presente invención.

La Figura 2 muestra otra vista isométrica en perspectiva en un diseño 3D de una primera realización del módulo de contención automatizado y programable que es objeto de la presente invención.

La Figura 3 muestra una vista isométrica en perspectiva en un diseño 3D de una primera realización del armazón cerrado que forma parte del módulo de contención automatizado y programable que es objeto de la presente invención.

La Figura 4 muestra una vista isométrica en perspectiva con un corte lateral de un despiece de una primera realización de los medios de traslación que forman parte del módulo de contención automatizada y programable que es objeto de la presente invención.

La Figura 5 muestra una primera realización del algoritmo de programación de la unidad de control encargada de regular el funcionamiento de dicho mecanismo motor del módulo de contención automatizado y programable que es objeto de la presente invención.

La figura 6 muestra una vista isométrica en perspectiva en un diseño 3D de una primera realización del conjunto formado por dicho mecanismo motor en conexión con dicha pieza móvil y conectada magnéticamente a dicho elemento móvil.

La figura 7 muestra una vista isométrica en perspectiva en un diseño 3D de una primera realización del conjunto formado por dicho mecanismo motor en conexión con dicha pieza móvil y conectada magnéticamente a dicho elemento móvil.

La figura 8 muestra una vista superior en un diseño 3D de una primera realización del conjunto formado por dicho mecanismo motor en conexión con dicha pieza móvil y conectada magnéticamente a dicho elemento móvil.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

De manera esencial la presente invención proporciona una módulo de contención automatizado y programable (1) para artrópodos, insectos, u otros animales de tamaño similar con simulación de presas similares a la naturaleza, que comprende un armazón cerrado (12) que define un volumen interior; un elemento móvil (13) posicionado en el interior de dicho armazón cerrado (12); un mecanismo motor (14) conectado operativamente a dicho elemento móvil (13), que comprende un carro (141), una vía (142) y medios de traslación (143) de dicho carro (141), donde dicho carro (141) se configura para desplazarse a lo largo de dicha vía (142); y una unidad de control conectada operativamente a dicho mecanismo motor (14), donde dicha unidad de control está configurada para accionar dichos medios de traslación (143).

En el contexto de la presente invención se entenderá como un elemento móvil a un elemento ubicado en el interior de dicho armazón cerrado (12), y que puede ser cualquier elemento de estudio capaz de causar una posible reacción en el espécimen de interés contenido. Pudiendo ser, por ejemplo, un señuelo, sebo, atrayente, repelente, etc. En una realización preferida y sin que esto limite el alcance de la protección, dicho elemento móvil (13) es un señuelo que simula el vuelo de una mosca.

Con respecto a la figura 1 y 2, se observa un esquema con los componentes más importantes y un diseño 3D de una realización preferida del módulo de contención automatizado y programable (1).

Dicho armazón cerrado (12) comprende una base, una pluralidad de paredes laterales y una cubierta. Adicionalmente, dicha base, dicha pluralidad de paredes laterales y dicha cubierta son de un material transparente. Por otra parte, dicha cubierta y al menos una de dichas paredes laterales poseen una abertura. En donde dicha abertura (121) que posee dicha cubierta de dicho armazón cerrado (12) permite al usuario la manipulación de dicho elemento móvil (13). Y en donde dicha abertura (122) que posee al menos una de dichas paredes laterales de dicho armazón cerrado (12) corresponde a una puerta que permite la entrada y salida del espécimen de interés contenido.

En una realización preferida, y sin que esto limite el alcance de la protección, dicha cubierta de dicho armazón cerrado (12) posee una pluralidad de aberturas(121a,

141b). En una realización aún más preferida, dicha cubierta de dicho armazón cerrado (12) posee 3 aberturas (121a, 121b, 121c) para una mejor manipulación de dicho elemento móvil (13).

Adicionalmente dicha cubierta o dichas paredes laterales pueden poseer pequeñas aberturas permanentes para la respiración del espécimen de interés contenido.

En el contexto de la presente invención, se entenderá como armazón cerrado a una pieza o conjunto de piezas unidas que presta estructura o sostén a un volumen cerrado. La cantidad de piezas, dimensiones y forma del volumen que define dicho armazón cerrado, así como también el sistema de fabricación y de unión entre las partes que la conforman no limitan el alcance de la presente invención.

Adicionalmente, en el contexto de la presente invención, se entenderá como volumen interior a aquel espacio de tres dimensiones que ocupa dicho armazón cerrado (12). Asimismo, dicho volumen interior corresponde al espacio limitado como hábitat para el espécimen de interés contenido. La forma y dimensiones de dicho volumen interior no limitan el alcance de la presente invención.

En una realización preferida, y sin que esto limite el alcance de la protección, dicho armazón cerrado (12) es un cubo formado por 6 placas de acrílico cuadradas idénticas correspondientes a la base, paredes laterales y cubierta. En donde dichas placas son fabricadas en una máquina de corte CNC y unidas entre sí con un adhesivo transparente.

Una realización preferida que resulta particularmente ventajosa es aquella en donde el armazón cerrado (12) define un volumen interior prismático, con una base y cubierta de forma idéntica y paredes laterales rectangulares idénticas entre sí; como se ilustra en la figura 3. Con el objetivo de crear un volumen con más altura que anchura, y determinar una distancia más larga entre el organismo vivo de interés y el elemento móvil (13).

En el contexto de la presente invención se entenderá como mecanismo motor a una serie de elementos mecánicos acoplados operativamente entre sí y que permiten transmitir movimiento, generando que dichos carros (141) puedan desplazarse a lo largo de dichas vías (142). De manera tal de proporcionar un conjunto que sea

capaz de moverse a través de toda la superficie de la cubierta de dicho armazón cerrado (12).

En una realización preferida, y sin que esto limite el alcance de la protección, dicho mecanismo motor (14) comprende una pluralidad de carros (141a 141b), una pluralidad de vías (142a, 142b) y una pluralidad de medios de traslación (143a, 143b). En una realización aún más preferida, dicho mecanismo motor (14) comprende 4 carros (141a, 141b, 141c, 141d), 4 vías (142a, 142b, 142c, 142d) y 2 medios de traslación (143a, 143b) conectados operativamente entre sí, tal como se observa en las figuras 6, 7 y 8.

En una realización preferida, y sin que esto limite el alcance de la protección, dichos medios de traslación (143) corresponden a un motorreductor eléctrico conectado operativamente a un elemento de transformación de movimiento rotativo a lineal, como se observa en el despiece presentado en la figura 4.

Dicho mecanismo motor (14), se conecta operativamente a dicho elemento móvil (13) de manera tal que el movimiento de dichos carros (141) a lo largo de dichas vías (142) se traduce en el movimiento de dicho elemento móvil (13) sobre la superficie de la cubierta, en el interior de dicho armazón cerrado (12).

En una realización preferida, sin que esto limite el alcance de la protección, dicho mecanismo motor (14) utiliza dos pares de vías (142) independientes que permiten generar movimientos en dos dimensiones, por cada vía (142) se moviliza un carro (141) debido a la acción de dos motores eléctricos (143). Cada uno de dichos dos motores eléctricos (143a, 143b) se conectan mediante una faja dentada a uno de dichos carros, a su vez se conectan a una barra de distribución que mueven el otro carro restante ubicado en paralelo. Cada par de carros ubicados en paralelo se conectan a una pieza móvil a través de un hilo de nylon, generando el movimiento en un eje. Dicha pieza móvil entra en contacto con la superficie superior de la cubierta de dicho armazón cerrado (12). Adicionalmente dicha pieza móvil se conecta magnéticamente a dicho elemento móvil (13) ubicado en el interior de la cubierta de dicho armazón cerrado (12), permitiendo un movimiento bidimensional del elemento móvil (13) sobre toda la superficie de la cubierta. Tal como se ilustra en la figura 8.

Adicionalmente, dicho módulo de contención automatizado y programable puede comprender una pluralidad de piezas móviles conectadas magnéticamente a una pluralidad de elementos móviles (13a, 13b), de manera tal de simular una multiplicidad de factores naturales relevantes para el espécimen, lo cual sería sumamente interesante para efectos de la observación. Dichos elementos móviles (13a, 13b) no se ilustran en las figuras. Sin embargo, la notación corresponde a una multiplicación de la singularidad (13).

En dicha realización preferida, la disposición de dichas 4 vías (142a, 142b, 142c, 142d) y dichos 4 carros (141a, 141b, 141c, 141d) enfrentadas 2 a 2, conectados operativamente a dichos 2 motorreductores eléctricos corresponden a una configuración que permite un movimiento alineado entre todos los frentes encargados del movimiento en cada uno de los ejes que conforman el movimiento bidimensional. De manera tal, que el movimiento generado por la acción de un motorreductor eléctrico y transmitido hacia la parte enfrentada sea exactamente el mismo, generando un movimiento coordinado en ambos frentes. Dicha disposición se extiende para el movimiento generado por la acción del otro motorreductor eléctrico.

En el contexto de la presente invención, se entenderá como unidad de control al dispositivo configurado para controlar el funcionamiento de dichos medios de traslación (143), específicamente el giro de los motores eléctricos, lo que se traduce finalmente en el control del desplazamiento bidimensional de dicho elemento móvil (13) sobre dicho armazón cerrado (12). Adicionalmente, dicha unidad de control comprende funciones de programación que permiten ejecutar diferentes patrones de movimiento.

Dicha unidad de control comprende, adicionalmente, una interfaz de interacción con un usuario, que cumple la función de accionar dicha unidad de control y/o seleccionar algún modo de movimiento específico del elemento móvil (13). Dicha interfaz de interacción con un usuario que puede ser, y sin limitarse a estos, física, como un botón, una palanca, una pantalla táctil o una unidad de control por voz, o un comando virtual no presencial.

En una realización preferida, y sin que esto limite el alcance de la protección, dicha interfaz de interacción con un usuario es una pluralidad de botones ubicados en

alguna porción exterior a dicho armazón cerrado (12), que necesariamente no sea visible desde el interior del mismo, para no perturbar al organismo vivo de interés que habita en dicho volumen interior. En una realización aún más preferida, dicha interfaz de interacción con un usuario son 3 botones (un botón A, un botón B y un botón C) ubicados contiguamente entre sí.

La figura 5 muestra una primera realización del algoritmo de programación de la unidad de control encargada de regular el funcionamiento de dicho mecanismo motor (14) del módulo de contención automatizado y programable (1) que es objeto de la presente invención.

REIVINDICACIONES

1. Un módulo de contención automatizado y programable (1) para la observación de especímenes, CARACTERIZADO porque comprende:
   - un armazón cerrado (12) que define un volumen interior;
   - un elemento móvil (13) posicionado en dicho volumen interior de dicho armazón cerrado (12);
   - un mecanismo motor (14) conectado operativamente a dicho elemento móvil (13), dicho mecanismo que comprende un carro (141), una vía (142) y medios de traslación (143) de dicho carro (141), donde dicho carro (141) se configura para desplazarse a lo largo de dicha vía (142); y
   - una unidad de control conectada operativamente a dicho mecanismo motor (14), donde dicha unidad de control está configurada para accionar dichos medios de traslación (143).
2. El módulo de contención (1) según la reivindicación 1 CARACTERIZADO porque dicho armazón cerrado (12) comprende una base, una pluralidad de paredes laterales y una cubierta.
3. El módulo de contención (1) según la reivindicación 2 CARACTERIZADO porque dicha base, dicha pluralidad de paredes laterales y dicha cubierta son de un material transparente.
4. El módulo de contención (1) según la reivindicación 2 CARACTERIZADO porque dicha cubierta y al menos una de dichas paredes laterales poseen una abertura.
5. El módulo de contención (1) según la reivindicación 4 CARACTERIZADO porque dicha cubierta posee una pluralidad de aberturas (121a, 121b).
6. El módulo de contención (1) según la reivindicación 1 CARACTERIZADO porque dicho mecanismo motor (14) comprende una pluralidad de carros (141a, 141b), una pluralidad de vías (142a, 142b) y una pluralidad de medios de traslación (143a, 143b).
7. El módulo de contención (1) según la reivindicación 6 CARACTERIZADO porque dicho mecanismo motor (14) comprende 4 carros (141a, 141b, 141c,

141d), 4 vías (142a, 142b, 142c, 142d) y 2 medios de traslación (143a, 143b).

8. El módulo de contención (1) según la reivindicación 7 CARACTERIZADO porque dichos 4 carros (141a, 141b, 141c, 141d) y 4 vías (142a, 142b, 142c, 142d) se posicionan de manera enfrentada 2 a 2.
9. El módulo de contención (1) según la reivindicación 1 CARACTERIZADO porque dichos medios de traslación (143) son un actuador lineal.
10. El módulo de contención (1) según la reivindicación 1 CARACTERIZADO porque dichos medios de traslación (143) son un motorreductor eléctrico conectado operativamente a un elemento de transformación de movimiento rotativo a lineal.
11. El módulo de contención (1) según la reivindicación 1 CARACTERIZADO porque dicha unidad de control comprende una interfaz de interacción con un usuario.
12. El módulo de contención (1) según la reivindicación 2 CARACTERIZADO porque comprende una pieza móvil posicionada sobre dicha cubierta y conectada operativamente a dicho mecanismo motor (14).
13. El módulo de contención (1) según la reivindicación 12 CARACTERIZADO porque dicha pieza móvil se conecta magnéticamente a dicho elemento móvil (13).
14. El módulo de contención (1) según la reivindicación 13 CARACTERIZADO porque comprende una pluralidad de piezas móviles conectadas magnéticamente a una pluralidad de elementos móviles (13a, 13b).

RESUMEN

La presente invención se relaciona con el campo del albergue de seres vivos. Particularmente con el campo de los equipos para el albergue de animales; aves, peces, insectos, entre otros. En específico provee un módulo de contención automatizado y programable para la observación de especímenes, tales como artrópodos, insectos u otros animales de tamaño similar.

La presente invención provee un módulo de contención automatizado y programable para la observación de especímenes que se caracteriza porque comprende un armazón cerrado que define un volumen interior; un elemento móvil posicionado en dicho volumen interior de dicho armazón cerrado; un mecanismo motor conectado operativamente a dicho elemento móvil , dicho mecanismo que comprende un carro, una vía y medios de traslación de dicho carro, donde dicho carro se configura para desplazarse a lo largo de dicha vía; y una unidad de control conectada operativamente a dicho mecanismo motor, donde dicha unidad de control está configurada para accionar dichos medios de traslación.

De acuerdo con la descripción previamente detallada es posible obtener un módulo de contención automatizado y programable para especímenes, con simulación de presas similares a la naturaleza y eliminación de factores externos que pueden influir su comportamiento.

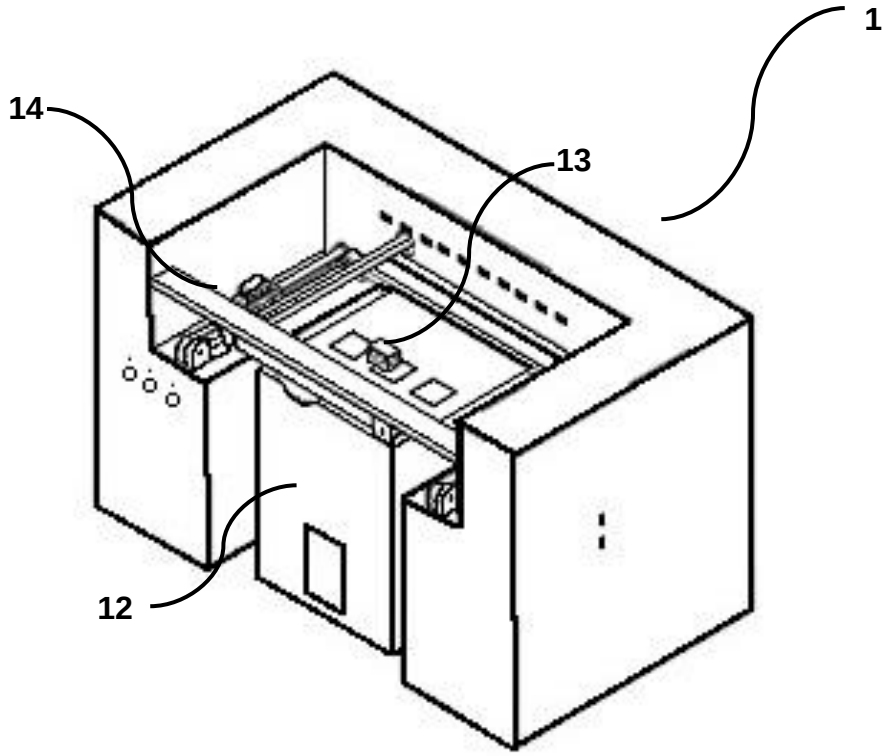

FIG.1

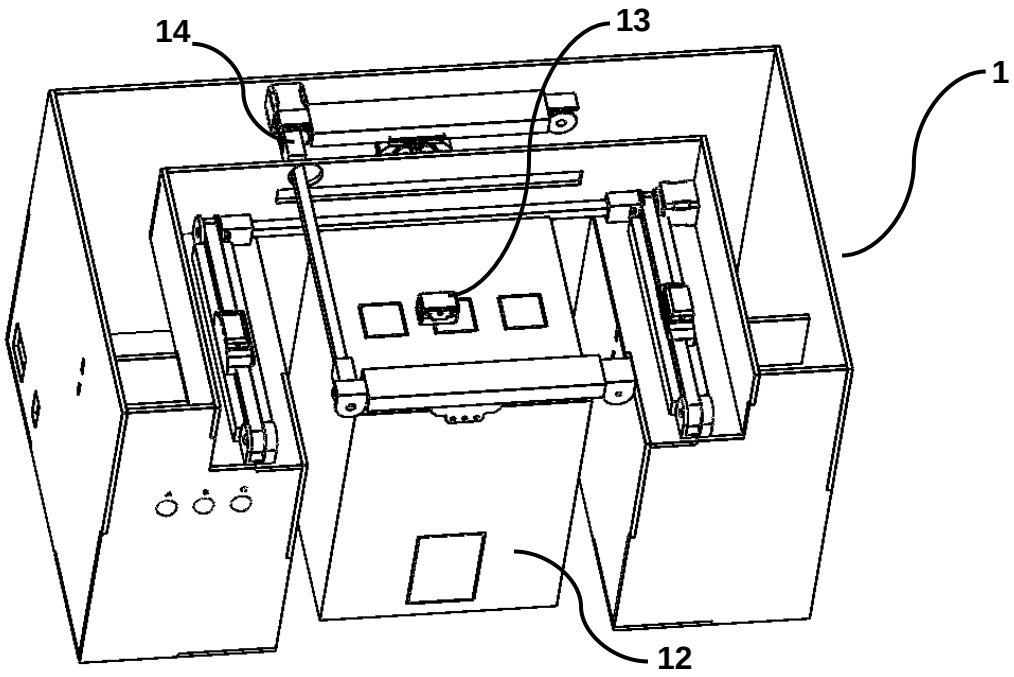

FIG. 2

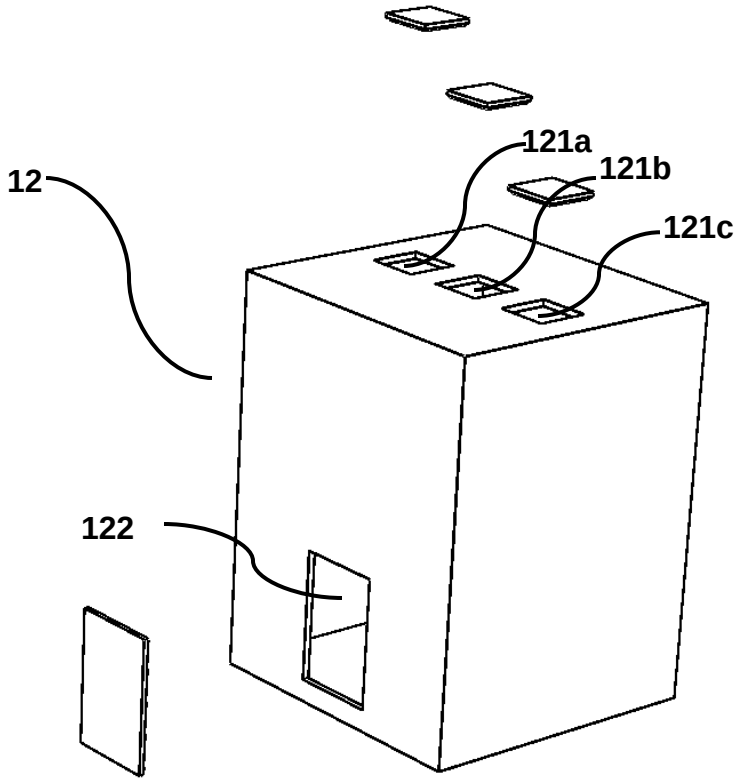

FIG. 3

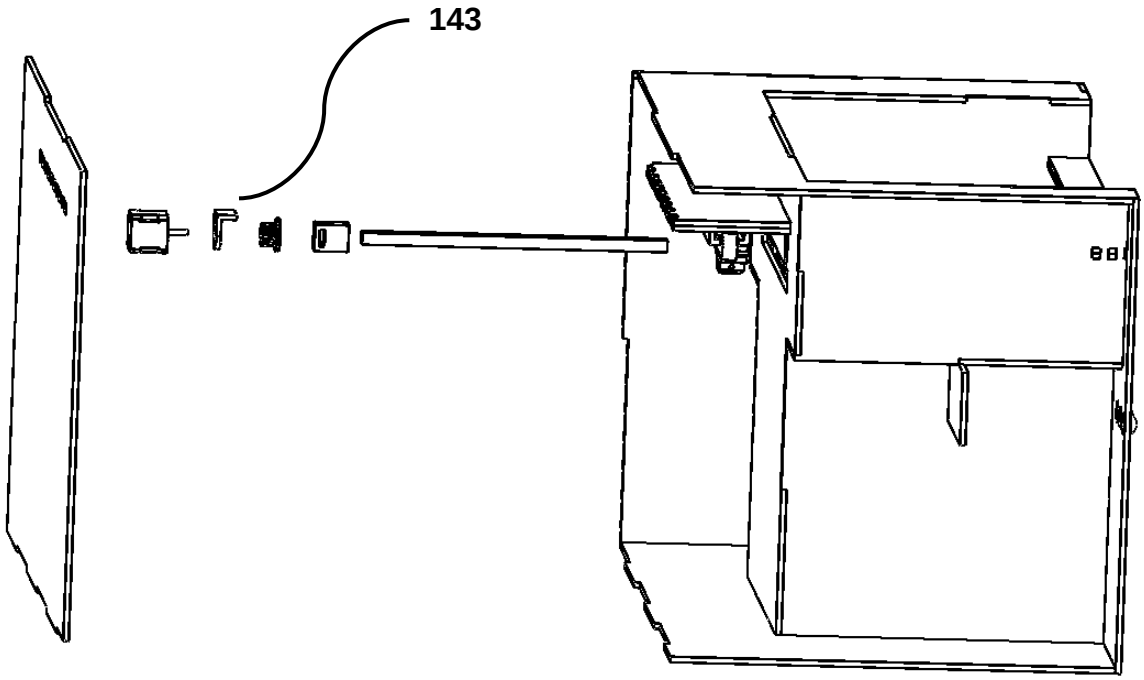

FIG. 4

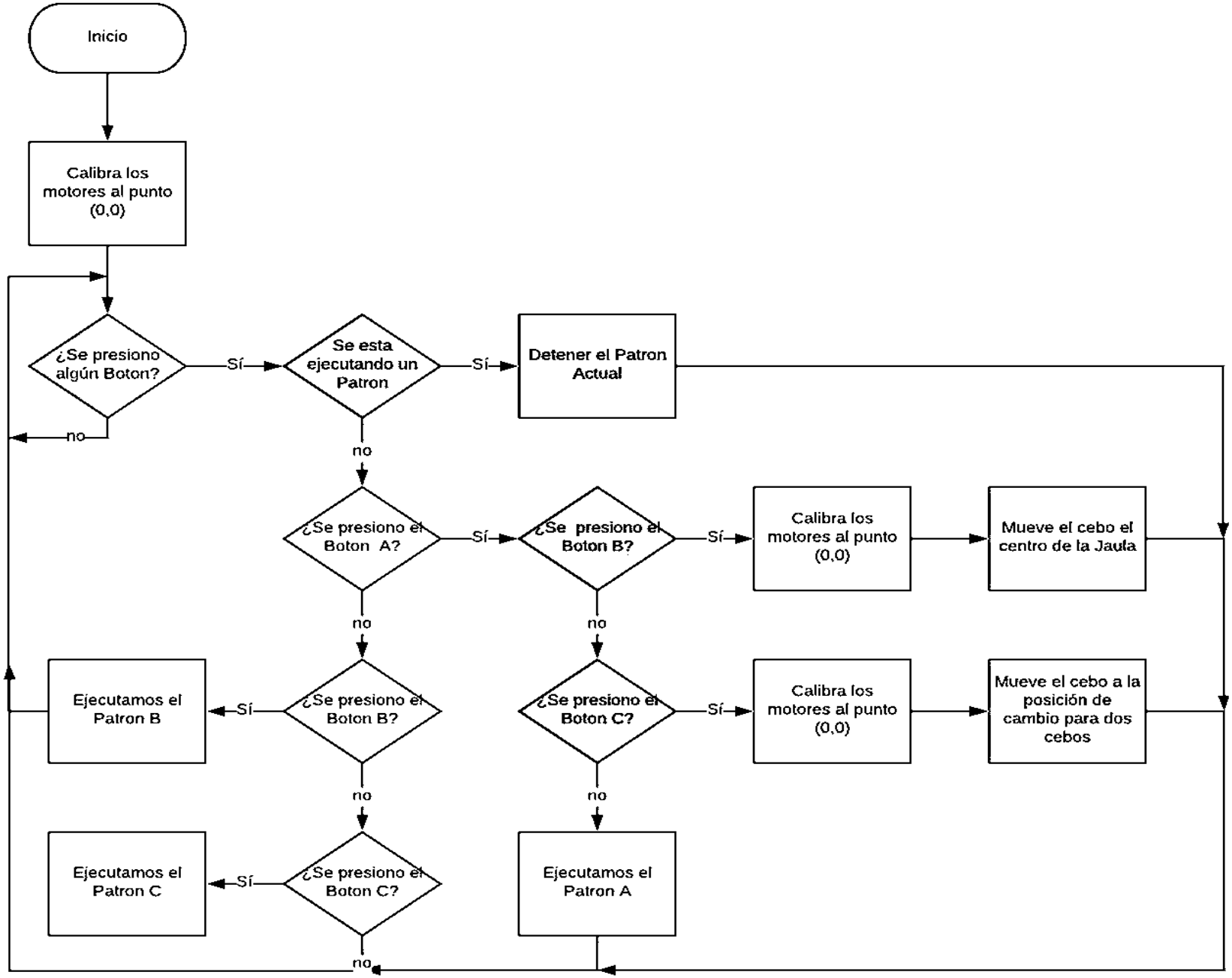
Inicio
Calibra los motores al punto (0,0)
¿Se presiono algún Boton?
Sí
Se esta ejecutando un Patron
Sí
Detener el Patron Actual
no
no
¿Se presiono el Boton A?
Sí
¿Se presiono el Boton B?
Sí
Calibra los motores al punto (0,0)
Mueve el cebo el centro de la Jaula
no
no
Ejecutamos el Patron B
Sí
¿Se presiono el Boton B?
¿Se presiono el Boton C?
Sí
Calibra los motores al punto (0,0)
Mueve el cebo a la posición de cambio para dos cebos
no
no
Ejecutamos el Patron C
Sí
¿Se presiono el Boton C?
Ejecutamos el Patron A
no

FIG. 5

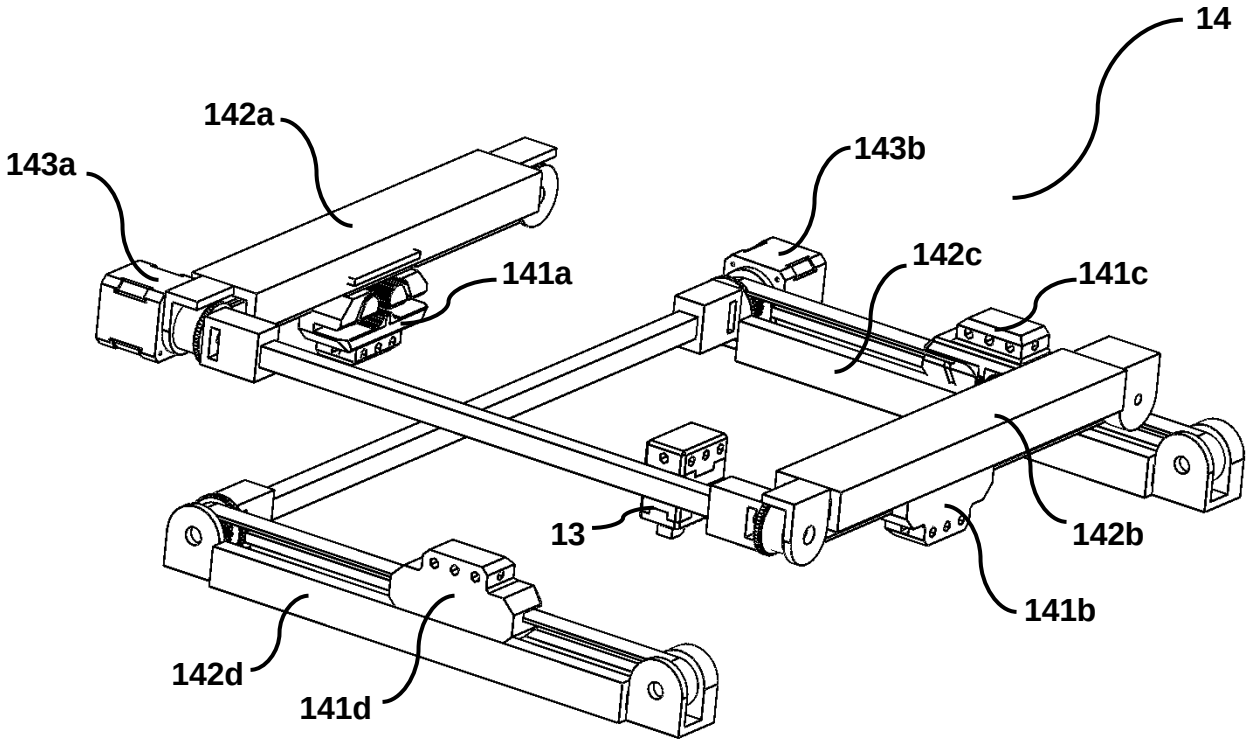
14
142a
143a
143b
141a
142c
141c
13
142b
141b
142d
141d

FIG. 6

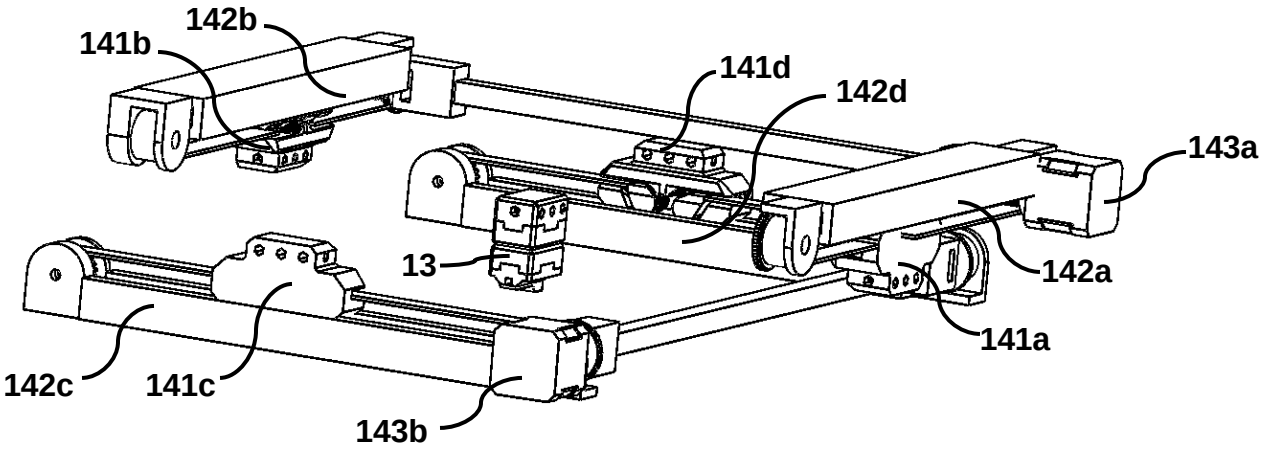
142b
141b
141d
142d
143a
13
142a
141a
142c
141c
143b

FIG. 7

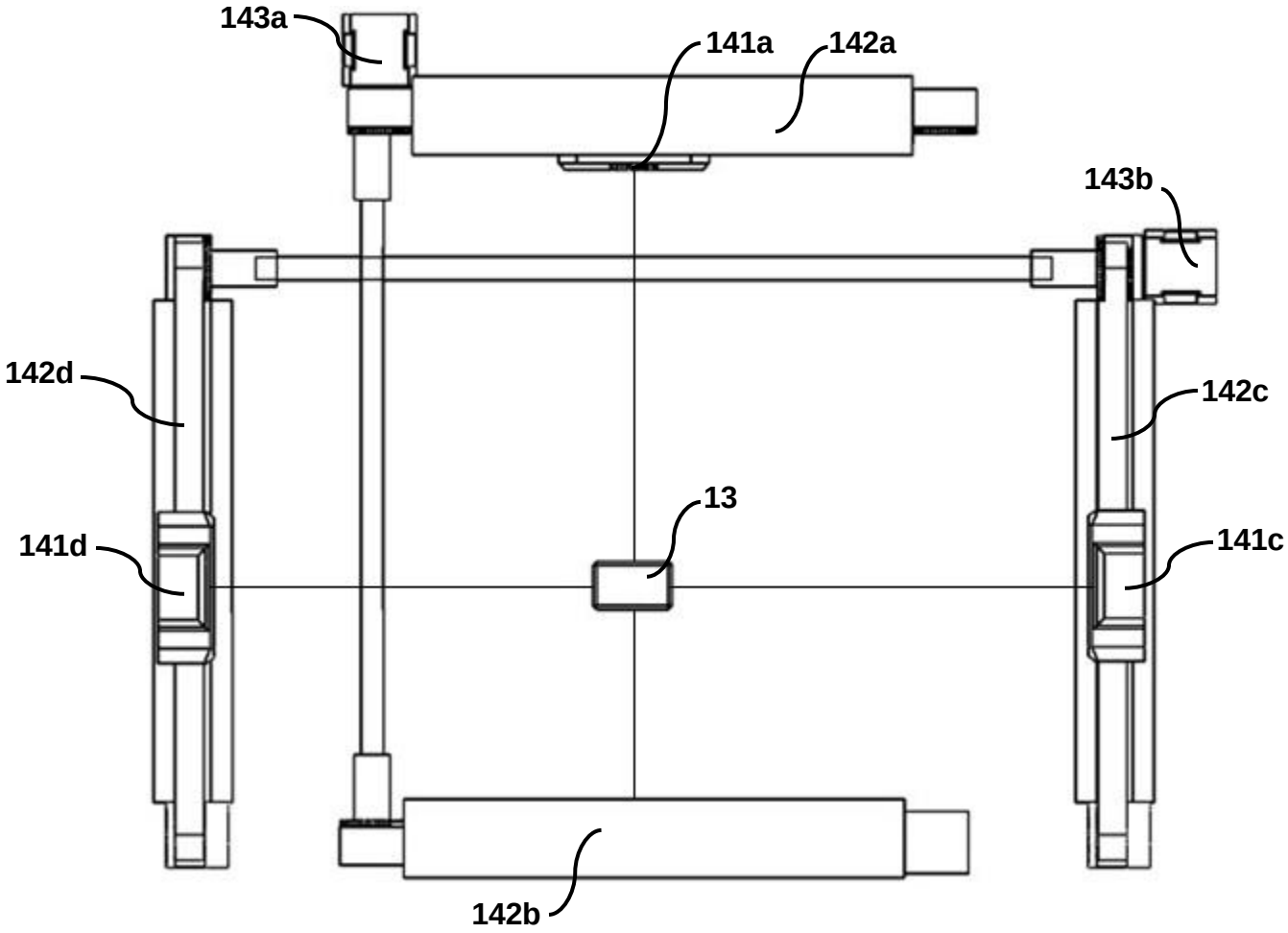

FIG. 8

Divulgación científica

Universidad de Costa Rica:

https://www.ucr.ac.cr/noticias/2019/01/23/la-ciencia-detras-de-los-materiales.html

Entomology Today article:

https://entomologytoday.org/2019/03/22/black-orange-black-color-pattern-23-families-wasps-bees-ants-hymenoptera/

Boletín Ciencia en todo:

https://issuu.com/ct.ucr/docs/c_t_52?fbclid=IwAR1Wo7i1b0n6qt1W6_kyS-fp9aw7ZVPYCvc05FOvjBXa9Ud9xu6zyDGestk