UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

SISTEMÁTICA Y EVOLUCIÓN DEL GRUPO DE AVISPAS PARASITOIDES DE LA FAMILIA BRACONIDAE (HYMENOPTERA: ICHNEUMONOIDEA)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS BIOLÓGICAS

PRESENTA:

JOVANA MAGDALENA JASSO MARTÍNEZ

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CIUDAD UNIVERSITARIA, CD. MX., MARZO, 2022

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M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 17 de enero de 2022 se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **JASSO MARTÍNEZ JOVANA MAGDALENA** con número de cuenta **306028645** con la tesis titulada **"SISTEMÁTICA Y EVOLUCIÓN DEL GRUPO DE AVISPAS PARASITOIDES DE LA FAMILIA BRACONIDAE (HYMENOPTERA: ICHNEUMONOIDEA)"**, realizada bajo la dirección **del DR. ALEJANDRO ZALDIVAR RIVERÓN**, quedando integrado de la siguiente manera:

Presidente:	DRA. HELGA OCHOTERENA BOOTH
Vocal:	DR. CARLOS SALVADOR PEDRAZA LARA
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Secretario:	DR. DANIEL IGNACIO PIÑERO DALMAU

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 22 de febrero de 202

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

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ÍNDICE

RESUMEN

La familia Braconidae (Hymenoptera: Ichneumonoidea) es un grupo megadiverso y cosmopolita de avispas en su mayoría parasitoides, característica biológica considerada como una innovación clave en los insectos. Los miembros de esta familia presentan una extraordinaria diversidad de estrategias de parasitoidismo en diferentes y complejas combinaciones. Tal es el caso del ecto y endoparasitoidismo (i.e., los huevos se depositan sobre o dentro del hospedero, respectivamente) y de la idio- y koinobiosis (i.e., el hospedero es paralizado permanentemente o continúa desarrollándose, respectivamente). La clasificación supraespecífica dentro de Braconidae ha cambiado constantemente a lo largo del tiempo, en parte debido a la considerable convergencia morfológica entre sus miembros, el muestreo taxonómico limitado en estudios previos, y a que se han utilizado pocos marcadores moleculares para la reconstrucción filogenética. Una hipótesis filogenética robusta es, por lo tanto, necesaria para proponer una clasificación supraespecífica estable dentro de la familia, y así responder preguntas evolutivas relacionadas con las estrategias de parasitoismo en el grupo. En esta tesis se empleó información a escala genómica (elementos ultraconservados -UCEs-, genomas mitocondriales) para investigar la sistemática de Braconidae en distintos niveles supraespecíficos. En el Capítulo I se investigaron las relaciones filogenéticas en Rogadinae, una subfamilia compuesta por endoparasitoides exclusivos de larvas de Lepidoptera, así como de otras subfamilias cercanamente relacionadas, esto con base en datos de UCEs, y se investigó la evolución de preferencias de hospederos dentro del grupo. La hipótesis filogenética obtenida confirmó la monofilia de Rogadinae con la inclusión de Betylobraconini, y se recuperó a Lysitermiinae y Hormiinae como no reciprocamente monofiléticos, por lo que formalmente se unió a sus miembros dentro de Hormiinae. La preferencia ancestral de hospedero dentro de Rogadinae probablemente fue atacar lepidópteros ocultos, con la subsecuente transición a atacar tanto a hospederos expuestos como ocultos en las tribus Rogadini y Aledioini. En el Capítulo II se empleó el muestreo taxonómico más exhaustivo hasta la fecha para Braconidae (395 taxones) y se generaron datos de UCEs (~104,724 pb) para investigar las relaciones filogenéticas entre sus subfamilias e investigar la evolución de sus estrategias de parasitoidismo. Se obtuvo una filogenia casi completamente resuelta, a partir de la cual se establecieron 41 subfamilias dentro de Braconidae. Además, se confirmaron varias afinidades filogenéticas a nivel supraespecífico y se establecieron algunos cambios taxonómicos, entre ellos

la reubicación de Trachypetinae **stat. rev.** y Masoninae **stat. rev.** y la confirmación de Apozyginae como subfamilias de Braconidae, así como el reconocimiento de Ichneutinae *sensu stricto* y Proteropinae como subfamilias independientes dentro del clado no ciclostomo. La reconstrucción de estados ancestrales sugiere que el ancestro del complejo braconoide y del clado ciclotomo *sensu lato* fue un endoparasitoide koinobionte. Estos resultados proporcionan fuerte evidencia de una transición del endo- al ectoparasitoidismo en Braconidae, así como tres reversiones subsecuentes al endoparasitoidismo en el clado ciclostomo *s.s.* Las transiciones de koino- e idiobiosis fueron idénticas a las inferidas para endo- *versus* ectoparasitoidismo, con excepción de una reversión adicional a la estrategia koinobionte en Rhysipolinae. Por último, en el Capítulo III se llevó a cabo un estudio filogenético para las subfamilias de Braconidae con base en datos de genomas mitocondriales. Las relaciones filogenéticas obtenidas con este locus fueron altamente congruentes con aquellas generadas previamente con UCEs. Además, se demostró que tanto las secuencias de los genes codificantes del genoma mitocondrial así como su organización (genes codificantes, tRNAs, rRNAs) contienen señal filogenética valiosa para la reconstrución de relaciones evolutivas dentro de Braconidae. Los resultados de esta tesis están consistentemente apoyados por diferentes conjuntos de datos genómicos, dando lugar a una clasificación supraespecífica actualizada más estable para Braconidae, que es la segunda familia más diversa de Hymenoptera. Estos resultados también representa una base importante para estudios evolutivos subsecuentes que investiguen el origen y diversificación de los grupos principales de Braconidae, así como una posible relación entre la riqueza diferencial de especies en sus subfamilias y sus distintas estrategias de parasitoidismo, siendo la condición parasitoide una característica clave en la diversificación de Hymenoptera.

ABSTRACT

The cosmopolitan family Braconidae (Hymenoptera: Ichneumonoidea) is a megadiverse group of wasps that are parasitoids mostly, a biological feature largely regarded as a key innovation among insects. Members of this family exhibit an extraordinary diversity of parasitoidism strategies that are present in different and complex combinations, including ecto- and endoparasitoidism (i.e. eggs are laid on or into the host, respectively) and both idio- and koinobiosis (i.e. the host is either paralyzed permanently or continues to develop, respectively). The higher-level classification of Braconidae has constantly changed through time, in part due to the high levels of morphological convergence among its members, limited taxonomic sampling in previous studies and the scarce number of available molecular markers for phylogenetic reconstruction. A robust phylogenetic hypothesis is therefore needed to establish a robust classification within the family in order to address different evolutionary questions related to its parasitoidism strategies. In this thesis I employed genomic-scale information (ultraconserved elements -UCEs-, mitochondrial genomes) to investigate the systematics of Braconidae at different supraspecific levels. In Chapter I, the phylogenetic relationships of Rogadinae, a subfamily composed of exclusive endoparasitoids of Lepidoptera larvae, and other closely related subfamilies, and the evolution of host preferences within the group, were investigated using UCE data. The phylogenetic hypothesis obtained confirmed the monophyly of Rogadinae with the inclusion of Betylobraconini, and Lysitermiinae and Hormiinae were recovered as not reciprocally monophyletic, and thus their members were formally proposed to be united within Hormiinae. The ancestral host preference within Rogadinae was probably attacking concealed lepidopterans, with the subsequent transition for attacking both exposed and concealed hosts in the tribes Rogadini and Aleiodini. In Chapter II, I made use of the most exhaustive taxonomic sampling for Braconidae (395 taxa) carried out to date and used UCE data (~104,724 bp) to investigate the phylogenetic relationships among their subfamilies and to assess the evolution of their parasitoid strategies. An almost-fully resolved phylogeny was obtained, from which I established a total 41 subfamilies within Braconidae. A number of supraspecific phylogenetic relationships were also confirmed, leading to various taxonomic changes among which were the restoration of Trachypetinae **stat. rev.** and Masoninae **stat. rev.** and the confirmation of Apozyginae as subfamilies of Braconidae, as well as the recognition of Ichneutinae *sensu stricto*

and Proteropinae as independent subfamilies within the non-cyclostome clade. The ancestral states reconstruction suggested that the ancestor of the braconoid complex and the cyclotomes *sensu lato* was a koinobiont endoparasitoid. These results provide strong evidence for a transition from endo- to ectoparasitoidism in Braconidae, as well as three subsequent reversions to endoparasitoidism within the cyclostomes *s.s.* The koino- and idiobiosis transitions were identical to those inferred for endo- *versus* ectoparasitoidism, with the exception of an additional reversion to the koinobiont strategy in the Rhysipolinae. Finally, in Chapter III I carried out a phylogenetic study among the subfamilies of Braconidae based on mitochondrial genome data. The phylogenetic relationships obtained with this locus were highly congruent with those previously generated with UCEs. Moreover, it was shown that both the protein coding gene sequence data from the mitochondrial genome as well as its organization (protein coding genes, tRNAs and rRNAs) contain valuable phylogenetic signal for the reconstruction of evolutionary relationships within Braconidae. The results obtained in this thesis are consistently supported by different genomic data sets, allowing to propose an updated, more stable supraspecific classification for Braconidae, which is the second most diverse family of Hymenoptera. These results also represent an important basis for subsequent evolutionary studies that aim to investigate the origin and diversification of the main groups of Braconidae, as well as a possible correlation between the differential species richness in their subfamilies and their different parasitoidism strategies, being the parasitoid condition a key feature in the diversification of Hymenoptera.

INTRODUCCIÓN GENERAL

El estudio de los fenómenos evolutivos responsables del origen de la biodiversidad actual es uno de los grandes retos en biología evolutiva. Los estudios macroevolutivos se concentran en investigar estos fenómenos en grupos supraespecíficos, entre los que destacan las extinciones masivas, estasis y radiaciones adaptativas (Pybus y Harvey, 2000). Asimismo, a través de este tipo de estudios se pueden responder preguntas relacionadas con el origen, y diversificación de distintos linajes (Peterson et al., 2004; Weir y Schluter, 2008). En este sentido, los estudios llevados a cabo en un contexto filogenético han permito un mayor conocimiento para la delimitación de grupos biológicos, así como para generar hipótesis sobre su historia evolutiva (Leys et al., 2002; Zakharov et al., 2004; Verma y Jolivet, 2008).

Aún con la creciente cantidad de hipótesis filogenéticas para distinos linajes, existen todavía muchos grupos biológicos cuyas relaciones evolutivas continúan siendo poco conocidas. Muchos de estos grupos presentan una distribución geográfica muy amplia o son incluso cosmopolitas, y llevan a cabo diversas interacciones de extrema importancia en la naturaleza (Rakhshani et al., 2008; Lin et al., 2017). Una limitación importante para el estudio de muchos grupos de invertebrados megadiversos y de amplia distribución geográfica es la falta de conocimiento sobre su riqueza de especies, ya que en muchos casos presentan características morfológicas conservadas, y/o biologías complejas (Leys et al., 2002; Zakharov et al., 2004). A este respecto, los datos moleculares han resultado ser un importante marco de referencia no solo para la actualización de las clasificaciones taxonómicas en estos grupos, sino también para responder al gran interés en el estudio de la evolución de características morfológicas y/o de aspectos relacionados con la biología (p.ej. Davis et al., 2016), la reconstrucción de historias biogeográficas (p.ej., Zaldívar-Riverón et al., 2017), la estimación del origen y diversificación de distintos taxones (p. ej. Peterson et al., 2004; Weir y Schluter, 2008) e incluso la correlación de ciertos aspectos de historia de vida de algunos taxones con el estado actual de la diversidad de los mismos (Condamine et al., 2016).

Inicialmente, las filogenias moleculares se reconstruían con base en un locus. No obstante, las incorporación de múltiples loci empezó a ser ampliamente utilizada en este campo debido a la consideración emergente de los árboles de especies *vs.* los árboles de genes en las reconstrucciones filogenéticas (Edwards, 2009). Estas aproximaciones multilocus empezaron a

dar cuenta de la variación aleatoria en los patrones de los genes, dando paso a la creciente búsqueda de marcadores moleculares adecuados para sistemas de estudio específicos (McCormack et al., 2013). La tarea de utilizar aproximaciones multilocus sin duda se vio beneficiada con técnicas más recientes de secuenciación de nueva generación (NGS por sus siglas en inglés), las cuales permiten secuenciar una enorme cantidad de datos genéticos, como genomas completos o bien, una representación considerable del genoma de diferentes organismos y grupos de organismos con un costo/tiempo-beneficio significativo (Ekblom y Galindo, 2011).

Métodos de representación reducida del genoma y filogenómica
Los estudios filogenéticos se han beneficiado enormemente en la actualidad con el uso de datos de secuenciación de nueva generación (NGS por sus siglas en inglés), y específicamente con técnicas que obtienen una representación reducida del genoma (McCormack et al., 2013). Estas técnicas generan de cientos a miles de loci, permitiendo en muchos casos obtener hipótesis filogenéticas mucho más robustas en comparación con el uso de marcadores puntuales (Eckblom y Galindo, 2011).

Entre los métodos de representación reducida del genoma más ampliamente utilizados están los marcadores de ADN asociados a sitios de restricción (RADseq por sus siglas en inglés) a través del corte y selección aleatoria de sitios con enzimas de restricción (Davey y Blaxter, 2010) y la captura de secuencias de elementos ultraconservados (UCEs por sus siglas en inglés) (Faircloth et al., 2012). La técninca de RADseq fue originalmente diseñada para estudios de genética de poblaciones, estudios filogeográficos o bien, para el esclareciemiento de relaciones filogenéticas que implican reciente divergencia (Davey y Blaxter, 2010).

Los UCEs son regiones altamente conservadas y homólogas en el genoma que se comparten entre taxones evolutivamente distantes (Bejerano et al., 2004), la cuales son flanqueadas por ADN de alta variabilidad (Faircloth et al., 2012; 2015). Los UCEs pueden ser selectivamente capturados y enriquecidos con sondas de ARN, y sus secuencias pueden ser utilizadas como marcadores para la reconstrucción filogenética (Faircloth et al., 2012). El que los UCEs incluyan sitios altamente conservados, así como regiones flanqueantes variables, es lo que les confiere su principal cualidad, que es su gran utilidad en estudios filogenéticos tanto de grupos de temprana como de reciente divergencia (McCormack et al., 2012).

Recientemente, se ha demostrado que como subproducto de la secuenciación de datos de UCEs pueden recuperarse secuencias de ADN mitocondrial, muchas veces suficientes para la reconstrucción de genomas mitocondriales completos (Raposo do Amaral et al., 2015). Los genomas mitocondriales obtenidos como subproducto de la técnica de UCEs han demostrado ser filogenéticamente informativos, siendo capaces de recuperar relaciones evolutivas a diferentes niveles taxonómicos, icluyendo grupos supraespecíficos (Raposo do Amaral et al., 2015; Meza-Lázaro et al., 2018; Samacá-Sáenz et al., 2019).

Genomas mitocondriales

Las mitocondrias juegan un papel central en el metabolismo celular, proporcionando energía a casi todos los organismos eucariontes vivos (Carlucci et al., 2008; Osellame et al., 2012). Por lo tanto, el estudio del ADN mitocondrial es fundamental en una serie de áreas de investigación, incluidas la fisiología, biología molecular y en estudios evolutivos (Osellame et al., 2012; Ballard y Pichaud, 2014). En los eucariontes existe una amplia variación en longitud y estructura del genoma mitocondrial (Formaggioni et al., 2021), aunque en particular para los metazoarios, éste normalmente consta de 15-18 kilobases, que comprenden 13 genes codificantes, 22 ARN de transferencia (tRNA) y dos ARN ribosomales (rRNA) (Boore, 1999).

El análisis de secuencias mitocondriales ha sido uno de los enfoques más comunes para investigar relaciones evolutivas. A menudo, la información mitocondrial se ha considerado más informativa a escalas evolutivas recientes (Song et al., 2016), aunque se ha demostrado cada vez más que también puede ser informativa para investigar relaciones profundas (Simon y Hadrys, 2013; Tang et al., 2019). Otra fuente importante de información filogenética puede obtenerse de la organización de los genes mitocondriales, que generalmente se conserva en muchos grupos de metazoarios, aunque se ha demostrado que en algunos grupos de insectos, incluidos los himenópteros, existen varios reordenamientos genéticos (Black y Roehrdanz, 1988; Dowton et al., 2002; Cameron, 2014). Dentro de Hymenoptera, las moscas de la sierra y las avispas de la madera suelen tener un orden de sus genes conservado, mientras que para Apocrita han sido reportados varios reordenamientos (Tang et al., 2019), de los cuales, se ha reportado que aportan señal filogenética para el reconocimiento de grupos supraespecíficos (p.ej., Song et al., 2014; Dowton et al., 2009; Tang et al., 2019).

Orden Hymenoptera

El orden Hymenoptera (moscas de sierra, avispas, hormigas y abejas) es un grupo de insectos holometábolos haplodiploides, i.e. los machos son el producto haploide de huevos sin fertilizar, mientras que las hembras son diploides y provienen de huevos fertilizados (Hanson y Gauld, 2006; Sharkey, 2007). Este es probablemente el orden de insectos más rico en especies (Forbes et al., 2018), comprendiendo en la actualidad más de 153 mil especies descritas, las cuales presentan una gran variedad de estrategias de alimentación y desarrollo, incluyendo depredadores, polinizadores y parasitoides (Godfray 1994; Peters et al., 2017). Esta variedad de estrategias de alimetación hace que este orden tenga un papel fundamental en casi todos los ecosistemas terrestres, y que sea uno de los grupos biológicos de mayor importancia económica (Grimaldi y Engel, 2005).

El orden Hymenoptera ha sido tradicionalmente dividido en dos grupos: 1) Symphyta (moscas de la sierra), conformado por aquellos himenópteros que carecen de constricción abdominal (cintura de avispa) y con hábitos de alimentación principalmente fitófagos; y 2) Apocrita, que contiene a los grupos a) Parasitica, himenópteros sin aguijón y principalmente formado por parásitos y parasitoides; y b) Aculeata, conformado por los himenópteros con aguijón (Figura 1; Grimaldi y Engel, 2005). Symphyta se ha reconocido como un grupo parafilético con respecto a Apocrita, mismo que se ha recuperado como monofilético (Rasnitsyn, 1988; Vilhelmsen et al., 2010). Dentro de Apocrita, Aculeata se ha recuperado consistentemente como monofilético con base en datos morfológicos y moleculares (Dowton y Austin, 2001), sugiriendo que la modificación del ovipositor en aguijón se dio una sola vez en la evolución del orden.
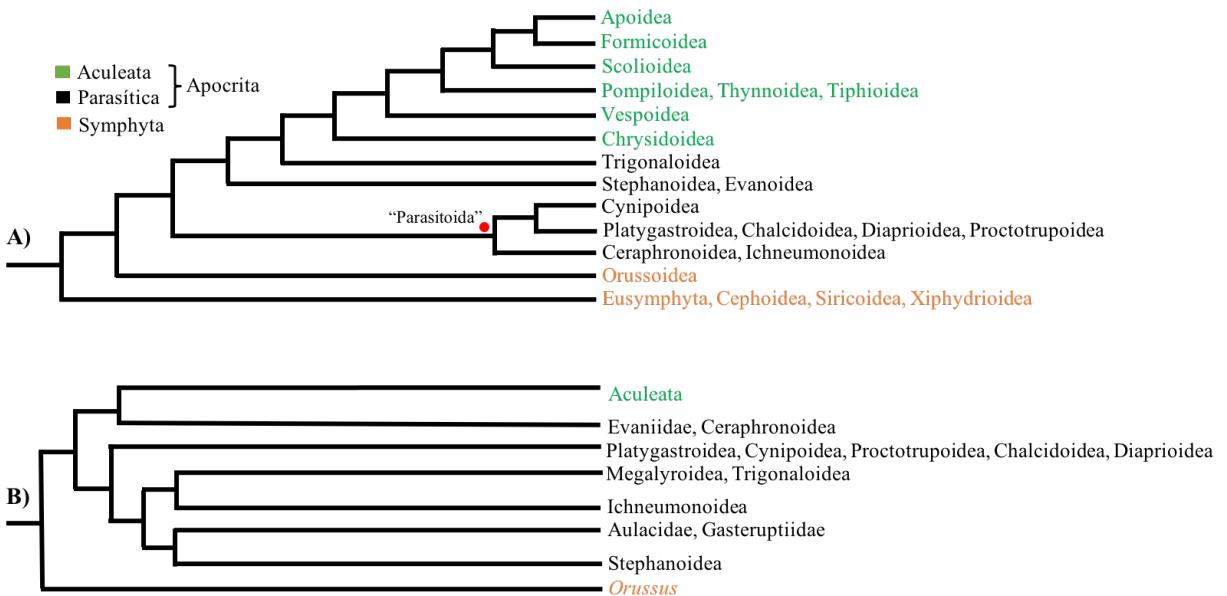
Figura 1. Filogenia de Hymenoptera basada en A) Peters et al. (2017), usando datos transcriptómicos; B) Tang et al. (2019), usando genomas mitocondriales.

De acuerdo con datos transcriptómicos, los himenópteros parasitoides (Ichenumonoidea, Ceraphronoidea, Proctotrupoidea, Diaprioidea, Chalcidoidea, Platygastroidea y Cynipoidea) conforman un grupo monofilético (Parasitoida) descendiente de un ancestro parasitoide endofítico que vivió hace aproximadamenre 247 millones de años, y cuya radiación pudo haberse desencadenado por la optimización del hábito parasitoide (p. ej. endoparasitoidismo, reducción del tamaño corporal), lo que pudo haber permitido atacar con éxito una mayor variedad de hospederos (Peters et al., 2017). Asimismo, se estimó que el origen y temprana diversificación del orden ocurrió hace alrededor de 281 millones de años (Peters et al., 2017). En un trabajo más reciente con base en datos de genomas mitocondriales (Tang et al., 2019), las superfamilias Stephanoidea, Megalyroidea y Trigonaloidea se recuperaron consistentemente como parte del grupo de los parasitoides, mientras que Ceraphronoidea + Evaniidae se recuperó como grupo hermando de Aculeata, por lo que "Parasitoida" *sensu* Peters et al. (2017) no fue monofilético.

Familia Braconidae

Con poco más de 21 mil especies descritas (Yu et al., 2016), distribuídas en 41 subfamilias, Braconidae es la segunda familia más rica en especies en el orden Hymenoptera. Esta riqueza de

especies solamente es antecedida por su grupo hermano, la familia Ichenumonidae, para la que se han descrito más de 25 mil especies (Yu et al., 2016). Los bracónidos son avispas parasitoides (i.e. el hospedero muere después de ser atacado; Quicke, 1997) que atacan en su mayoría a estados larvales de insectos holometábolos (Shaw y Huddleston, 1991; Quicke, 1997).

Los bracónidos se han divido tradicionalmente en dos grupos principales, los ciclostomos y los no ciclostomos (Figura 2; Wharton, 1997). Los primeros se caracterizan por tener la parte ventral del clipeo retraída y labro cóncavo (aunque algunos miembros de este grupo han perdido dicha condición de forma secundaria, i.e.. Alysiinae, Opiinae, Gnamptodontinae, Betylobraconini), mientras que en los no ciclostomos el clipeo cubre completamente el labro (Wharton, 1997). La monofilia de ambos grupos ha sido recuperada en varios trabajos utilizando diferentes fuentes de evidencia (e.g. Belshaw et al., 1998; Dowton et al., 2002; Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011).
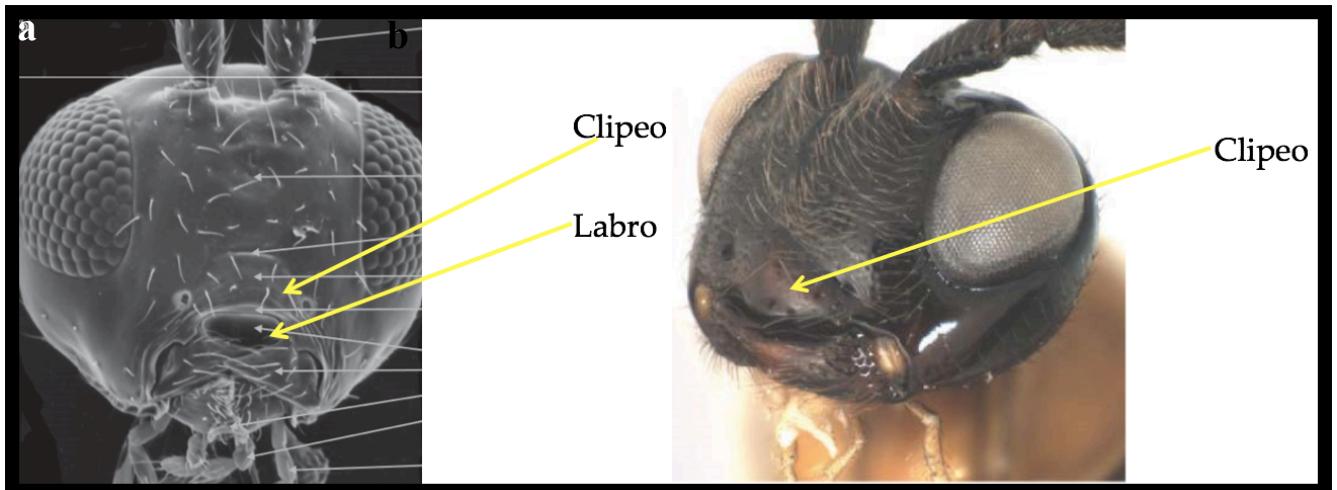
Figura 2. a) Ejemplo de la condición ciclostoma en Braconidae. Las flechas amarillas señalan el clipeo retraído mostrando el labro cóncavo, formándose una paertura oral. b) Condición no ciclostoma. Modificado de Quicke (2015).

Si bien existe evidencia evidencia morfológica y molecular de que tanto el grupo de los ciclostomos como los no ciclostomos son monofiléticos, su número de subfamilias y los límites de varias de ellas ha variado a lo largo del tiempo (Quicke y van Achterberg, 1990; Belshaw et al., 1998; Dowton, 1999; Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011; Chen y van Achterberg, 2019). El gran número de estudios filogenéticos que se ha realizado entre las subfamilias de Braconidae empleando diferentes tipos de información ha llevado a establecer de

manera robusta varias relaciones. Entre las relaciones que se han recuperado consistentemente se encuentran: Alysiinae como grupo hermano de Opiinae, el complejo aphidioide (Mesostoinae, Aphidiinae, Maxfischeriinae) como grupo hermano del clado ciclostomo (Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011), así como la agrupación del grupo no ciclostomo en cuatro complejos de subfamilias, *i.e.* los complejos sigalphoide, microgastroide, euphoroide y helconoide (Sharanowski et al., 2011). Sin embargo, las afinidades y límites de varias subfamilias aún continúan sin ser resueltas.

Rogadinae es una de las subfamilias cuyos límites y relaciones entre sus miembros aún son objeto de intenso debate. Esta subfamilia cosmopolita está exclusivamenente representada por especies endoparasitoides koinobiontes de lepidópteros que se caracterizan por causar la "momificación" de sus hospederos, i.e. el hospedero adquiere un aspecto seco y endurecido, del cual el parasitoide emerge como adulto abriendo un pequeño agujero (Shaw, 2002; 2006; Quicke y Shaw, 2005).

La considerable heterogeneidad morfológica entre los miembros de Rogadinae ha dificultado el descubrimiento de caracteres morfológicos diagnósticos, impidiendo el establecimiento claro de sus límites (Shaw y Huddleston, 1991). En un estudio filogenético para Rogadinae con base en evidencia molecular, Zaldívar-Riverón et al. (2008a) propusieron cinco tribus para esta subfamilia: Aleiodini, Clinocentrini, Stiropiini, Rogadini y Yeliconini. Si bien este estudio esclareció gran parte de la composición de Rogadinae, algunas relaciones permanecieron sin resolver, entre ellas la ubicación de Betylobraconinae dentro de Braconidae, la cual en el estudio antes mencionado, se recuperó como grupo hermano de Rogadinae pero con bajo soporte. Estudios más recientes con base en evidencia morfológica y molecular recuperaron a Betylobraconinae como una tribu de Rogadinae, aunque de forma no consistente (i.e., Quicke y Butcher, 2015). El esclarecimiento de la compoisición de esta subfamilia ayudaría a esclarecer la evolución de su rango de hospederos, los cuales incluyen varios grupos de lepídoperos con larvas tanto ocultas como expuestas.

Evolución de las estrategias de parasitoidismo en Braconidae
Los bracónidos presentan una gran diversidad de estrategias de parasitoidismo, las cuales implican características biológicas y del ciclo de vida diferenciales (Tabla 1). La mayoría de las especies de bracónidos parasitan a etapas inmaduras de otros insectos holometábolos, aunque

hay algunas excepciones, incluído el uso de insectos adultos e insectos hemimetábolos (p.ej., Euphorinae y Aphidiinae; Mackauer et al., 1996; Stigenberg et al., 2015), así como la existencia de algunas especies fitófagas en las subfamilias Doryctinae, Braconinae y Mesostoinae (Infante et al., 1995; Austin y Dangerfield, 1998; Zaldívar-Riverón et al., 2014; Ranjith et al., 2016).

Dentro de Braconidae además hay especies tanto con hábitos solitarios como gregarios (oviposición de uno o más huevos en el hospedero) (Quicke, 2015), ecto y endoparasitoides (oviposición fuera o dentro del hospedero) (Gauld, 1988), koino- (el hospedo puede continuar con su desarrollo después de la oviposición del parasitoide) o idiobiontes (el desarrollo se previene mediante la parálisis permanente del hospedero) (Colinet et al., 2005), así como especies con hábitos especialistas o generalistas, por atacar a pocos o solo uno, o a varias especies de hospederos (Hassell y May, 1986).

Tabla 1. Características biológicas y de ciclo de vida de las estrategias idiobionte y koinobionte en Braconidae (Gauld y Hanson, 1995, Quicke, 1987; Quicke, 2015).

Idiobiontes	Koinobiontes
El hospedero es paralizado de forma permanente, impidiendo su posterior desarrollo luego de la oviposición	El hospedero puede continuar con su desarrollo (movilidad, crecimiento) por un tiempo luego de la oviposición
Ectoparasitoides (excepto Aspidobraconina)	Endoparasitoides (excepto Rhysipolinae)
Atacan a hospederos no expuestos	Atacan a hospederos expuestos
Mayormente generalistas	Mayormente especialistas
Hospedero de mayor talla que estado adulto del parasitoide	Hospedero regularmente más pequeño o de tamaño similar al estado adulto del parasitoide
Desarrollo rápido de la larva parasitoide	El desarrollo de la larva parasitoide suele ser más prolongado, especialmente en el primer estadio
Dimorfismo sexual marcado	Dimorfismo sexual poco pronunciado o ausente
Más longevos	Menos longevos
En su mayoría de hábitos diurnos	Hábitos diurnos y nocturnos

Se ha observado que el rango de hospederos en Braconidae se relaciona con el tipo de estrategia que sus especies llevan a cabo. De forma general, los ectoparasitoides suelen tener un rango de hospederos más amplio en contraste con los endoparasitoides, los cuales parecen tener estrategias más especializadas (Quicke, 2015). El rango de hospederos de las avispas bracónidas,

así como los procesos biológicos y químicos relacionados con dicha elección son características escenciales en su uso potencial como control biológico de plagas de cultivos de importancia comercial (Snyder e Ives, 2001; Quicke, 2015).

Dada la complejidad de las diferentes estrategias de parasitoidismo de la familia Braconidae, el conocer la historia evolutiva de éstas, inlcuyendo la evolución de la preferencia de hospederos, ha sido un tema común en los estudios evolutivos para esta familia de himenópteros (Whitfield, 2002; Zaldívar-Riverón et al., 2006; 2008a; Stigenberg et al., 2015; Sharanowski et al., 2021; Samacá-Saenz et al., 2022). Las primeras hipótesis propusieron que la condición ancestral de Ichneumonoidea fue el ectoparasitoidismo e idiobiosis, con una preferencia de atacar hospederos ocultos (Gauld, 1988; Whitfield, 1992; Vilhelmsen, 1997; Quicke et al., 1999), con una subsecuente transición temprana a avispas endoparasitoides koinobiontes de hospederos tanto ocultos como expuestos (Quicke et al., 1999). Recientemente, con base en una filogenia obtenida a partir de datos a escala genómica y reconstrucción de estados ancestrales, Sharanowski et al. (2021) propusieron que el ancestro de la superfamilia Ichneumonoidea fue ectoparasitoide idiobionte, confirmando así las hipótesis previas. En particular, los resultados de este último estudio sugirieron que la condición ancestral en Braconidae pudo ser endoparasitoide koinobionte; no obstante, los estados ancestrales inferidos variaron ampliamente, por lo que los autores no pudieron apoyar su hipótesis de manera robusta.

JUSTIFICACIÓN

En el presente estudio se investigará la sistemática de las subfamilias de Braconidae, así como la evolución de las estrategias de parasitoidismo de esta familia, empleando para ello datos de UCEs y genomas mitocondriales obtenidos en gran parte como subproducto de la técnica anterior. La generación de estos datos a escala genómica permitirá obtener hipótesis filogenéticas robustas para establecer una clasificación supraespecífica estable para el grupo de estudio. Además, la obtención de una hipótesis de filogenia robusta dentro de Braconidae permitirá poner a prueba en un contexto filogenético la evolución de las estrategias de parasitoidismo en la familia. Si bien para algunas subfamilias hay evidencia robusta tanto de su monofilia como de sus límites (p.ej. Opiinae, Alysiinae, Microgastrinae), las relaciones entre varias subfamilias aún se desconocen o no están fuertemente apoyadas. Tal es el caso de Rogadinae y sus subfamilias

cercanamente relacionadas, Lysiterminae, Hormiinae y Betylobraconinae, en donde sus límites y afinidades filogenéticas necesitan ser evaluados con detalle.

OBJETIVO GENERAL

- Investigar la sistemática de las avispas parasitoides de la familia Braconidae (Hymenoptera: Ichneumonoidea) empleando datos de elementos ultraconservados (UCEs por sus siglas en inglés) y de genomas mitocondriales, e investigar la evolución de las estrategias de parasitoidismo dentro de la familia.

OBJETIVOS PARTICULARES

1. Realizar un estudio filogenómico entre los miembros de Rogadinae y sus subfamilias cercanamente relacionadas empleando datos de UCEs, e investigar la evolución de sus preferencias de hospederos lepidópteros.

2. Investigar la sistemática de las subfamilias de Braconidae empleando datos de UCEs e investigar la evolución de las estrategias de parasitoidismo en la familia.

3. Evaluar la informatividad de los datos de secuenciación de genomas mitocondriales y reordenamientos genéticos para reconstruir las relaciones filogenéticas dentro de Braconidae.

CAPÍTULO I (ARTÍCULO DE REQUISITO): PHYLOGENOMICS OF THE LEPIDOPTERAN ENDOPARASITOID WASP SUBFAMILY ROGADINAE (HYMENOPTERA:BRACONIDAE) AND RELATED SUBFAMILIES

Phylogenomics of the lepidopteran endoparasitoid wasp subfamily Rogadinae (Hymenoptera: Braconidae) and related subfamilies

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Abstract. Rogadinae are a cosmopolitan, species-rich braconid wasp subfamily whose species are endoparasitoids that attack larvae of a number of lepidopteran families. Members of this subfamily are characterized by pupating within the mummified host larval skin. The subfamily contains six tribes whose relationships have only been partially clarified: Aleiodini, Betylobraconini, Clinocentrini, Rogadini, Stiropiini and Yeliconini. The limits and composition of the closely related subfamilies to the Rogadinae, Hormiinae and Lysiterminae, also remain unclear. Here, we generated ultraconserved element data to reconstruct an almost fully resolved phylogeny for the members of Rogadinae and related subfamilies. Based on our best estimate of phylogeny, we confirm the monophyly of Rogadinae including Betylobraconini, synonymize *Xenolobus* Fahringer and *Bequartia* Cameron within the species-rich genus *Aleiodes* Wesmael (**syn.n.**) based on DNA, and synonymize *Promesocentrus* van Achterberg with *Pilichremylus* Belokobylskij (**syn.n.**) based on morphology. We also consistently recovered Hormiinae and Lysiterminae as not reciprocally monophyletic, and thus propose to unite their members under Hormiinae. The ancestral host preference for Rogadinae was probably attacking concealed lepidopteran larvae, with the occurrence of at least two main subsequent transitions to attack both concealed and exposed hosts, one within Rogadini and a second within Aleiodini. We highlight the importance of natural history collections as a source for conducting genomic-based studies using techniques that allow to obtain a substantial amount of data from considerably old preserved insect specimens.

Introduction

Hymenoptera (sawflies, wasps, ants and bees) are one of the four largest insect orders, covering a variety of feeding and developmental modes (Godfray, 1994; Peters *et al.*, 2017). The vast

majority of its species are parasitoids, a strategy in which one or more wasp larvae develop on or in a single host, invariably killing it before it can reproduce (Quicke, 1997). Braconidae are the second most species-rich family within Hymenoptera (Wharton, 1997; Yu *et al.*, 2016), most species being parasitoids on the larvae of various holometabolous insects (Shaw & Huddleston, 1991; Quicke, 1997). However, braconids collectively display a wide range of parasitoid strategies: solitary or gregarious (Quicke, 2015), specialist or generalist (Hassell & May, 1986; Quicke, 2015), idio- or koinobiont (i.e. preventing or

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1

permitting further host development after parasitization, respectively), and endo- or ectoparasitoid (Gauld, 1988; Belshaw *et al.*, 2003).

Braconidae have been traditionally divided into two major groups of subfamilies, the cyclostomes and non-cyclostomes, based on the presence or absence of an oral opening formed by a ventrally concave clypeus exposing a concave labrum (van Achterberg, 1984; Wharton, 1997), though this has been secondarily lost in several 'cyclostome' lineages. Mutual monophyly of the extant members of these two lineages has been supported consistently based on analyses of one or few gene fragments when taxon-sampling has been relatively high (Sharanowski *et al.*, 2011). All members of the non-cyclostome lineage are koinobiont endoparasitoids, whereas cyclostomes are predominantly either idiobiont ectoparasitoids (most groups) or koinobiont endoparasitoids (predominantly the Alysiinae, Opiinae and Rogadinae *s. s.*) (Wharton, 1997; Zaldívar-Riverón *et al.*, 2006, 2008; Quicke, 2015).

Rogadinae *s.s.* is a cosmopolitan, species-rich subfamily with more than 1200 described species grouped in 68 currently recognized genera (Yu *et al.*, 2016). They are characterized by their habit of pupating within the mummified larval cuticle of their lepidopteran host, a feature that makes them excellent for the detailed investigation of host-parasitoid relationships (Shaw, 2002a; Shaw, 2006). In this strategy, the host turns into an often hardened and tanned mummy, within which the parasitoid larva pupates and then emerges as an adult (Quicke & Shaw, 2005). Although Rogadinae biology is well characterized, the considerable morphological heterogeneity among its members has impeded the discovery of consistent diagnostic features both for the group as a whole and its tribes (Shaw & Huddleston, 1991). Even in the restricted sense, the tribe Betylobraconini has until recently been regarded as a distinct subfamily based on morphology (Tobias, 1979; van Achterberg, 1993, 1995), though there is no biological information on any of its species.

Various morphological studies have attempted to clarify the supraspecific classification in Rogadinae with limited success based on external morphology (van Achterberg, 1991, 1993), venom gland apparatus (Zaldívar-Riverón *et al.*, 2004) and combined molecular and morphological data (Chen *et al.*, 2003). The most exhaustive molecular phylogenetic study that focused on Rogadinae was based on two gene fragments and 118 ingroup species (Zaldívar-Riverón *et al.*, 2008). These authors recognized the following tribes: Clinocentrini van Achterberg, Stiropiini van Achterberg, Yeliconini van Achterberg, Rogadini Foerster and the resurrected tribe Aleiodini Muesebeck, which contains the cosmopolitan *Aleiodes* Wesmael, a genus that comprises for more than half of all rogadine species (Yu *et al.*, 2016). According to this classification, members of Rogadinae can be defined by a single biological feature, the mummification of the host caterpillar (van Achterberg, 1995; Quicke & Shaw, 2005; Zaldívar-Riverón *et al.*, 2008). It has been observed that the level of mummy's hardening varies among rogadine tribes, with some inducing hard mummification (e.g. Aleiodini), whereas in others it is weak (e.g. Stiropiini) to moderate (e.g. Yeliconini) (Quicke & Shaw, 2005; Quicke *et al.*, 2006; Zaldívar-Riverón

et al., 2008). The site of emergence of the wasp (anterodorsal, posterodorsal, radial), on the other hand, appears to be more variable (Quicke & Shaw, 2005; Quicke *et al.*, 2006; Zaldívar-Riverón *et al.*, 2008).

Despite the above phylogenetic study, the composition and evolutionary relationships in Rogadinae are still not fully resolved. In particular, some molecular phylogenetic studies have recovered the small subfamily Betylobraconinae as sister to, or nested within Rogadinae, although in both cases with low support (Zaldívar-Riverón *et al.*, 2006; Belokobylskij *et al.*, 2008; Ranjith *et al.*, 2017). These and other studies also found that Betylobraconinae, as interpreted by van Achterberg (1995), was not monophyletic, with only *Betylobracon* Tobias, *Mesocentrus* Szépligeti, *Pilichremylus* Belokobylskij and *Gondwanocentrus* Quicke *et* Butcher being included (Belokobylskij *et al.*, 2008; Zaldívar-Riverón *et al.*, 2008; Butcher *et al.*, 2014). van Achterberg (1995) also included the tribe Facitorini in Betylobraconinae, but this was subsequently shown to form a separate clade more closely related to Yeliconini (Belokobylskij *et al.*, 2008; Zaldívar-Riverón *et al.*, 2008; Butcher & Quicke, 2015). More recently, based on summarized molecular evidence, as well as on multiple putative morphological synapomorphies, Betylobraconinae *s.s.* was formally transferred to Rogadinae and treated as a separate tribe (Quicke & Butcher, 2015).

Lysiterminae and Hormiinae are two relatively small, cosmopolitan subfamilies that have been found to be closely related to Rogadinae (van Achterberg, 1991; Zaldívar-Riverón *et al.*, 2006, 2008), although their respective monophyly and relationships amongst the three subfamilies have not been confirmed. Historically, Hormiinae has been a dumping ground for a heterogeneous set of cyclostome braconids and its limits have varied widely due to its lack of clear diagnostic morphological features (Wharton, 1993; Whitfield & Wharton, 1997). Although a number of genera and tribes previously included in Hormiinae have now been reclassified elsewhere (e.g. *Monitoriella* Hedqvist: Zaldívar-Riverón *et al.*, 2006; *Austrohormius* Belokobylskij: Shimbori *et al.*, 2017), it remains a poorly defined and likely non-monophyletic assemblage. Little is currently known about the biology of hormiines, though species whose biology is known are idiobiont ectoparasitoids of the lepidopteran families Coleophoridae, Depressariidae, Gelechidae, Gracillariidae, Oecophoridae, Pyralidae, Scythrididae and Tortricidae (Shaw & Huddleston, 1991; Belokobylskij, 1993; Whitfield & Wharton, 1997). Many, but not all taxa currently placed in the restricted Hormiinae possess metasomal tergites that are moderately to strongly desclerotized medially, though this is a character also displayed to varying degrees in other groups (Wharton, 1993; van Achterberg, 1995).

van Achterberg (1995) delimited Lysiterminae to contain four tribes: Cedriini Belokobylskij, Lysitermini Tobias, Pentatermini, Belokobylskij and Tetratermini van Achterberg. More recently, Aulosaphobraconini has also been suggested to belong to Lysiterminae (Butcher *et al.*, 2014) rather than as a tribe of Betylobraconinae (Belokobylskij & Long, 2005). The monophyly of Lysiterminae has not been thoroughly tested. The lysitermines have been grouped by a single morphological

synapomorphy, a strongly sclerotized carapace-like metasoma, though the number of tergites involved varies (Wharton, 1993). There are few host records of lysitermines, though they are presumed mainly to be ectoparasitoids of concealed lepidopteran larvae (van Achterberg, 1995; Quicke, 2015; Gupta & Quicke, 2018). For instance, species of *Cedria* Wilkinson (Cedriini) are known to be gregarious ectoparasitoids of concealed Pyralidae caterpillars and at least one species shows parental care (Beeson & Chatterjee, 1935; Mathur, 1959). A few lysitermines have also been reported to have unusual hosts, such as *Katytermus palmicola* van Achterberg (Tetratermini), an endoparasitoid of leaf-rolling cricket nymphs (Orthoptera: Gryllacrididae) (van Achterberg & Steiner, 1996).

Phylogenomic studies that make use of next-generation sequencing (NGS) techniques to address the evolution of different biological groups are rapidly increasing. Some of these techniques employ a reduced representation of the genome that is composed of thousands of variable loci, such as the target enrichment of ultraconserved elements (UCEs) (Faircloth *et al.*, 2015). UCEs have proven informative at different taxonomic levels, and particularly for resolving deep evolutionary relationships (Branstetter *et al.*, 2017). Among the main advantages of the UCE technique are that its protocols and bioinformatic pipelines are publicly available, as well as its high sequencing success rate even from low-quality and degraded DNA (Blaimer *et al.*, 2016; Zhang *et al.*, 2019), which is often the case for historical museum specimens (Guschanski *et al.*, 2013; Gauthier *et al.*, 2020; Jin *et al.*, 2020). Museomics, therefore, represents an invaluable resource to obtain large amounts of DNA sequence information for extinct or rare species, or for species that are otherwise difficult to collect (Besnard *et al.*, 2015; Kollias *et al.*, 2015; Anmarkrud & Lifjeld, 2017). However, implementing NGS approaches for insect museum specimens is challenging because many of them are considerably small and their amount of DNA that can be retrieved frequently is considerably low for reliable sequencing (Weirauch *et al.*, 2020).

Here, we generated UCE data to reconstruct an almost fully resolved phylogeny for the members of Rogadinae. For this, we included species belonging to representative genera from the six currently recognized rogadine tribes, as well as members of several genera of Lysiterminae, Hormiinae and other related cyclostome subfamilies. Based on our best estimate of phylogeny, we evaluate the monophyly of Rogadinae, including the Betylobraconini, examine the classification of the subfamilies studied here and assess the evolution of lepidopteran host ranges among the rogadine tribes. We also evaluate the performance of the UCE technique for recovering informative loci from old, pinned museum specimens, and whether there is an impact of including them in our phylogenomic analyses due to their differential amount of missing data.

Materials and methods

Exemplar sampling

UCEs were generated for representatives of 20 of the 68 currently recognized rogadine genera (Yu *et al.*, 2016) belonging to its six tribes, which were collected from all biogeographic regions: Aleiodini (three genera, nine spp.), Yeliconini (three genera, three spp.), Clinocentrini (two genera, eight spp.), Stiropiini (two genera, four spp.), Betylobraconini (two genera, four spp.) and Rogadini (eight genera, eight spp.). We also sampled species that have been placed in three subfamilies that are closely related to Rogadinae: Hormiinae, six genera, 11 species; Lysiterminae, 11 genera, 16 species from its five recognized tribes (Aulosaphobraconini, Cedriini, Lysitermini, Pentatermini and Tetratermini); Rhysipolinae, two genera, two species. The outgroups comprised members of other six cyclostome subfamilies: Alysiinae (one genus, one species), Braconinae (one genus, one species), Doryctinae (two genera, two species), Exothecinae (one genus, one species), Gnamptodontinae (two genera, two species) and Pambolinae (one genus, two species). These subfamilies were selected as outgroups because in previous studies (Zaldívar-Riverón *et al.*, 2006; Sharanowski *et al.*, 2011) they appeared nested together with Rogadinae in a major clade within the cyclostome group, with Doryctinae being sister to all of them. A list with the examined specimens, their taxonomic assignment, locality of provenance and DNA voucher and Gen-Bank accession numbers are given in Table S1.

Eleven of the sequenced specimens were preserved in 96% ethanol and stored at −20°C, whereas 46 were pinned. Collection dates for the ethanol and pinned specimens ranged from 1998 to 2010 and from 1937 to 2016, respectively (Table S1). The year of the collection was not available for 17 samples, and thus these specimens were excluded from statistical analyses related to the performance of the UCE loci recovery. All voucher specimens used for this study is deposited in the Colección Nacional de Insectos at the Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM) and in the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia (ZISP).

DNA extraction, library preparation and target enrichment

Genomic DNA was extracted using a non-destructive technique (Ceccarelli *et al.*, 2012) with the EZ-10 Spin Kit minipreps DNA Genomic Column Kit (BIOBasic, Toronto, Ontario, Canada) following the manufacturer's protocol. DNA fragmentation was performed with the Qsonica Q800R sonicator (Qsonica 457 LLC, Newton, CT, U.S.A.). DNA quantification was performed using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies, CA, U.S.A.). We used from 2 to 100 ng of input DNA in 100 μL of ultrapure water for library preparation. The number of cycles employed (movement for 15 s, static for 90 s) depended on the kind of preservation and the time of collection of the samples. We employed one cycle for pinned and old samples (40 years or older) and two or three for more recent and ethanol preserved samples.

Genomic libraries were prepared following the protocol described by Branstetter *et al.* (2017) using the Kapa Hyper Prep kit (Kapa Biosystems Inc., Wilmington, MA, U.S.A), and the TruSeq-style dual-indexing adapters (Glenn *et al.*, 2016). Most of the library enrichments were performed using the RNA

bait set developed by Faircloth *et al.* (2015), which includes 2749 baits targeting 1510 UCE loci (Hym. v1). Enrichment for most of the outgroup specimens and three rogadine samples (CNIN3040, CNIN3042 and CNIN3052) was performed with the bait set Hym.v2 (Branstetter *et al.*, 2017). The sequencing was conducted in an Illumina HiSeq 2500 instrument (PE125, v4 chemistry) at the Department of Environmental Health Science, University of Georgia, Athens, GA, U.S.A.

UCE data processing and sequence alignment

Raw reads were cleaned and trimmed using Illumiprocessor (Faircloth, 2013), a wrapper around Trimmomatic (Del Fabbro *et al.*, 2013; Bolger *et al.*, 2014) in the Phyluce version 1.6.6 pipeline (Faircloth, 2015). The cleaned reads were subsequently de novo assembled with Trinity (Grabherr *et al.*, 2011; Galaxy v 0.0.1) on the open-source, web-based platform Galaxy (https://usegalaxy.org). UCE loci for each sample were extracted from the contigs generated by Trinity (phyluce_assembly_match_contigs_to_probes and phyluce_assembly_get_fastas_from_match_counts) using the bait set Hym. v.1 (Arbor Biosciences, formerly MYcroarray, Ann Arbor, MI, U.S.A) available at https://www.ultraconserved .org/#software. The extracted UCE loci were aligned with the programme MAFFT version 7 (Katoh & Standley, 2013) implemented in Phyluce, and the resulting alignments then were filtered and trimmed with Gblocks version 0.91b (Castresana, 2000; Talavera & Castresana, 2007) using the following settings: b1 = 0.5, b2 = 0.5, b3 = 12 and b4 = 7 (Branstetter *et al.*, 2017).

We built five UCE matrices (74 terminal taxa) for phylogenomic analyses restricting locus coverage to loci that were recovered for at least 30, 40, 50, 60 and 70% of taxa. We also built a 'reduced' matrix (matrix; 61 terminal taxa) using the original 50% completeness matrix, which was the dataset that retained a higher number of loci for the ingroup, to conduct a coalescent-based species analysis. For the reduced matrix we excised most of the outgroups (members of Exothecinae, Alysiinae, Braconinae, Pambolinae and Doryctinae), the three rogadine specimens that were enriched with the probe set Hym. v.2 and two specimens assigned to *Avga* Nixon, 1940 (see results). *Neognamptodon* sp. (CNIN3882) was included to root the tree.

The above six matrices were partitioned by locus, and their partitioning schemes and evolutionary models were selected with the programme PartitionFinder version 2 (Lanfear *et al.*, 2016) in the web platform CIPRES (https://www.phylo .org/) under the Bayesian Information Criterion and the cluster option employed for large datasets. For the 50% completeness matrix (74 taxa) we also implemented the sliding-window site characteristics-entropy (SWSC-EN) algorithm (Tagliacollo & Lanfear, 2018), which defines partitions that account for rate heterogeneity and patterns of molecular evolution within each UCE locus. The evolutionary models and the best partition scheme that employed the partitions derived from the above algorithm were subsequently selected with PartitionFinder version 2. The 50% completeness matrix and its partition scheme

derived from the SWSC-EN algorithm and the PartitionFinder model selection are shown in File S1.

We investigated the correlation between specimen age (1–82 years old samples), input DNA for library preparation (2–100 ng) and the preservation method of the samples (pinned, preserved in alcohol) with the number of UCE loci recovered. For this, we calculated the Pearson's correlation coefficient for the above combinations of variables with the R package version 3.6.0 2013.

Phylogenetic reconstruction

We performed partitioned Maximum Likelihood (ML) analyses for all matrices with the programme RAxML version 8.2 (Stamatakis, 2014), using the GTRGAMMA substitution model and 1000 bootstrap replicates. We also carried out Bayesian analyses with the programme MrBayes version 3.2.6 (Ronquist *et al.*, 2012). Each Bayesian analysis consisted of two simultaneous runs of 50 million generations each, sampling trees every 1000 generations and using uniform priors. We verified the Effective Sample Size (ESS) values of the tested parameters with the programme Tracer version 1.7 (Rambaut *et al.*, 2018). A burn-in fraction of 25% was established in all cases. The remaining trees were employed to reconstruct a majority rule consensus tree with posterior probabilities (PP) of clades.

For the coalescent-based species analysis based on the 'reduced' matrix, we first estimated a tree for each locus with the programme RAxML version 8.2 (Stamatakis, 2014), using best tree search with the GTRGAMMA model and 200 bootstrap replicates. We used the programme STRAW (http:// bioinformatics.publichealth.uga.edu/SpeciesTreeAnalysis/ index.php) to root the trees and performed the species tree analysis with the programme ASTRAL-III version 5.6.3 (Zhang *et al.*, 2018).

Phylogenetic analyses were conducted in the CIPRES portal (https://www.phylo.org/) and in the Miztli supercomputer from the Dirección General de Cómputo y de Tecnologías de Información y Comunicación, Universidad Nacional Autónoma de México (DGTIC, UNAM).

Results

UCE performance and alignment statistics

The read assembly produced an average of 71 340 (3658–230 479) and 72 259 (4044–230 479) contigs for all examined samples and for Rogadinae, respectively (Table S2). In total, we recovered 898 UCE loci with a mean length of 410.42 bp. Different amounts of UCE loci were obtained depending on the completeness percentage employed (Table 1). Within Rogadinae, the 50% complete matrix had an average of 377 loci, with *Mesocentrus* sp. (CNIN4163) and *Clinocentrus* sp. (CNIN3042) having the lowest and highest number of loci, respectively (283 and 457 loci) (Table S2).

Table 1. Alignments summary for matrices with different completeness percentages and the locus-by-locus partition, the 50% complete matrix containing the loci partition following the SWSC-EN algorithm (swsc) and the matrix with the subset of taxa used to conduct the coalescent-based species analysis (reduced).

Matrix	Number of taxa	Total of UCE loci	Loci mean length (bp) [min−max]	Matrix length (bp)	Informative sites (bp)	No. partitions	Nucleotide positions
30%	74	561	405.00 [199–707]	227 203	112 113	68	8 567 014
40%	74	485	406.51 [199–666]	197 159	99 165	65	7 923 538
50%	74	411	409.39 [199–664]	168 261	86 026	62	7 125 829
60%	74	335	413.50 [241–664]	138 523	71 942	48	6 103 335
70%	74	196	421.02 [241–664]	82 520	42 436	36	3 831 427
Reduced	61	416	400.41 [218–640]	166 569	78 488	48	5 837 795
50% (swsc)	74	411	409.39 [199–664]	168 261	86 026	98	7 125 829

Specimens aged between 1 and 29 years of being collected had an average of 375.08 UCE loci, whereas for older ones (≥30 years) this average was 339.88. For instance, 130 and 384 UCE loci were recovered for the 82 and 1-year-old pinned specimens of *Allobracon scorteus* Clark and *Triraphis* sp. (CNIN4028), respectively (Table S2). There is a significant correlation between the number of recovered loci and the age of the pinned specimens ($P < 0.001$, $R^2 = 0.2644$; Fig. 1A). In contrast, we did not find a significant correlation between the number of recovered loci with respect to both the age of samples that were preserved in alcohol nor the amount of input DNA used to start the library preparation protocol ($P = 0.23$, $R^2 = 0.1554$; $P = 0.36$, $R^2 = 0.0194$, respectively; Fig. 1B, C).

Phylogenetic analysis

All phylograms derived from the ML and Bayesian analyses with the matrices built with different levels of taxa completeness differed only in three relationships, and most clades were strongly supported (Fig. 2, Figs S1, S2).

In the topologies obtained from the 30, 50 and 60% completeness and the locus-by-locus partition, Betylobraconini was recovered as sister to Stiropiini and Clinocentrini sister to Aleiodini + Yeliconini, though with low support in both cases (BS < 70, PP < 0.95). On the other hand, in the topologies obtained with the 40 and 70% completeness matrices, Clinocentrini was recovered as sister to Betylobraconini and Stiropiini was sister to Aleiodini + Yeliconini, both also with low support (Fig S2). In addition, in the 40 and 50% completeness datasets (Figs S1, S2) *Allobracon* Gahan was weakly supported in a clade together with the species of Rhysipolinae, whereas in the remaining topologies it was placed with low support as sister to a Rogadinae + Lysiterminae/Hormiinae clade (BS < 70, PP < 0.95).

The relationships recovered by the analyses that used the 50% completeness matrix both with the loci partition following the SWSC-EN algorithm (Fig. 2) and the locus-by-locus partition (Fig S1) were identical. However, the Bayesian and ML analyses performed with the former partition strategy only had one and two relationships with low support, respectively. Below we only refer to these weakly supported relationships. Rogadini was recovered as sister to the remaining five rogadine tribes. Yeliconini was not recovered as monophyletic. Instead, they

formed a grade with *Conobregma* van Achterberg + *Facitorus* van Achterberg at the base, followed by *Yelicones* Cameron and both being sister to Aleiodini. The Yeliconini + Aleiodini clade was strongly supported as sister to Clinocentrini, whereas Betylobraconini was strongly supported as sister to Stiropiini only in the Bayesian analysis (BS = 69; PP = 0.99).

Members of Lysiterminae were intermingled in a well-supported clade together with various Hormiinae *s. s.*, these collectively being sister to Rogadinae. The Lysiterminae + Hormiinae clade had *Cedria* at its base, followed by *Aulosaphobracon* Belokobylskij *et* Long, a *Parahormius* Nixon + *Pseudohormius* Tobias *et* Alexeev subclade and two inclusive clades with intermingled members of both subfamilies, in that order. The second subclade had *Pentatermus* Hedqvist as sister to the four species of *Hormius* Nees. In the second clade, the two members of Tetratermini (*Platyrmus* Belokobylskij + *Katytermus* van Achterberg) were sister to the eight included genera of Lysitermini. *Lysitermus* Foerster, *Acanthormius* Ashmead and *Afrotritermus* Belokobylskij were not reciprocally monophyletic.

The rhysipolines *Rhysipolis* Foerster and *Pseudavga* Tobias together with *Allobracon* were recovered as sister to Rogadinae + Hormiinae/Lysiterminae but with low support (BS = 61, PP = 0.86). Moreover, the two species of *Avga*, currently placed in the Hormiinae, were nested among the outgroup taxa, being sister to Pambolinae.

The relationships recovered by the ASTRAL-III analysis were highly similar to the ones obtained with the ML and Bayesian inference methods, though seven relationships were not significantly supported (Fig S3). Among these poorly supported relationships are the placement of Clinocentrini as sister to Aleiodini + Yeliconini (local posterior probability, LPB = 0.66), Stiropiini as sister to the latter three tribes (LPB = 0.94) and *Allobracon* as sister to *Pseudoavga*, *Parachremylus* Granger and *Rhysipolis* (LPB = 0.42).

Discussion

Monophyly of Rogadinae

This study yielded for the first time a robust, almost fully resolved phylogenetic hypothesis for Rogadinae and related subfamilies, which serves to revise their limits and tribal
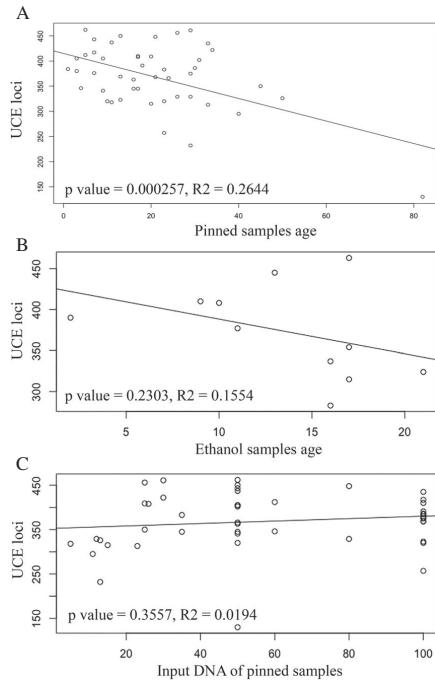
Fig 1. (A) UCE loci recovered with respect to the age of pinned samples. There was a strong correlation between the number of recovered loci and the age of the pinned samples. (B) UCE loci recovered with respect to the age of samples preserved in ethanol. (C) UCE loci recovered with respect to the input DNA used in UCE library preparation. No correlation was found between the latter two factors and the number of recovered loci.

classification and helps to place some taxa of a difficult assignment. All our phylogenetic analyses recovered Rogadinae (including Betylobraconini) with high support (Fig. 2, Figs S1–S3) and composed of six tribes. The sister group to Rogadinae was also strongly supported as comprising the Lysiterminae + Hormiinae and with the inclusion of *Cedria* + *Aulosaphobracon*, whose phylogenetic affinities had not been confirmed.

van Achterberg (1991) had recognized four tribes within Rogadinae: Lysitermini Pentatermini, Clinocentrini and Rogadini, with the first two being wrongly presumed at that time to be represented by koinobiont endoparasitoid species of lepidopteran larvae (van Achterberg, 1995; Quicke, 2015). This treatment is indicative of the overall morphological similarity of the involved groups. However, none of the morphological features that the author mentioned are shared among all members of these taxa. Hormiinae were on the other hand excluded from Rogadinae by van Achterberg (1991), mainly due to their substantially different (highly reduced) metasomal sclerotization and their known ectoparasitoid biology.

*Phylogenetic relationships within Rogadinae*

Our results show clearly, and for the first time with high support, that betylobraconines are members of Rogadinae. This relationship had previously been expected based on analyses using one or two gene fragments (Zaldívar-Riverón *et al.*, 2008), though many such investigations failed to recover Rogadinae as monophyletic to the exclusion of various Lysiterminae (Butcher *et al.*, 2014; Quicke *et al.*, 2014, 2016; Quicke & Butcher, 2015; Ranjith *et al.*, 2017). Although there are still no host records for any betylobraconine species, their protruding faces, robust legs and short ovipositors would be consistent with them locating semi-concealed hosts, by physically forcing themselves into host shelters and parasitizing them directly (Quicke, 2015). Indeed, the type genus, *Betylobracon* has a highly distinctive face and has totally lost the cyclostome condition.

All our analyses recovered Aleiodini, Betylobraconini, Clinocentrini, Rogadini and Stiropiini as monophyletic (Fig. 2, Figs S1–S3). However, Yeliconini (Yeliconina+Facitorina) was consistently rendered paraphyletic by Aleiodini, with Facitorina being sister to a Yeliconina + Aleiodini clade. Previous molecular studies that included members of both Yeliconina and Facitorina recovered them as monophyletic, though without significant support (Zaldívar-Riverón *et al.*, 2008; Butcher *et al.*, 2014). In contrast, all trees from our present study recovered (Facitorina + (Yeliconina, Aleiodini)) with high support. It is important to note that we only had data for a single species of *Yelicones* and no representatives of the two other known genera of Yeliconina, *Pseudoyelicones* van Achterberg, Penteado-Dias *et* Quicke and *Bulborogas* van Achterberg. Further work will, therefore, be needed to resolve this outstanding issue.

Different from Zaldívar-Riverón *et al.* (2008), in which Clinocentrini was recovered as sister to the remaining Rogadinae with the exclusion of Betylobraconinae, all our phylogenomic trees placed Rogadini as sister to the remainder of the subfamily. In agreement with Zaldívar-Riverón *et al.*'s (2008) molecular phylogenetic study of Rogadinae, we recovered *Heterogamus* Wesmael as a sister to *Aleiodes*. This is consistent with the finding that all members of *Aleiodes* exclusive of *Heterogamus* have a derived 28S rDNA motif: AGCGT located at positions 264–268 (stems 3i'-3j of the 28S braconid secondary structure model of Gillespie *et al.*, 2005), whereas virtually all other ichneumonoids have the plesiomorphic sequence of TGCGT (Zaldívar-Riverón *et al.*, 2008). This cosmopolitan genus can sometimes be difficult to distinguish from *Aleiodes* and has not been reared despite extensive Lepidoptera parasitoid rearing efforts in Europe and North America. Our results show that the Afrotropical genus *Xenolobus* Cameron is also a derived member of *Aleiodes* despite their large size and apomorphic venation and propodeum (van Achterberg, 1991).

Phylogenetic relationships among other subfamilies

Our study confirms that Hormiinae and Lysiterminae are not reciprocally monophyletic. The included genera of Hormiinae (i.e. *Hormius*, *Parahormius* and *Pseudohormius*) form a
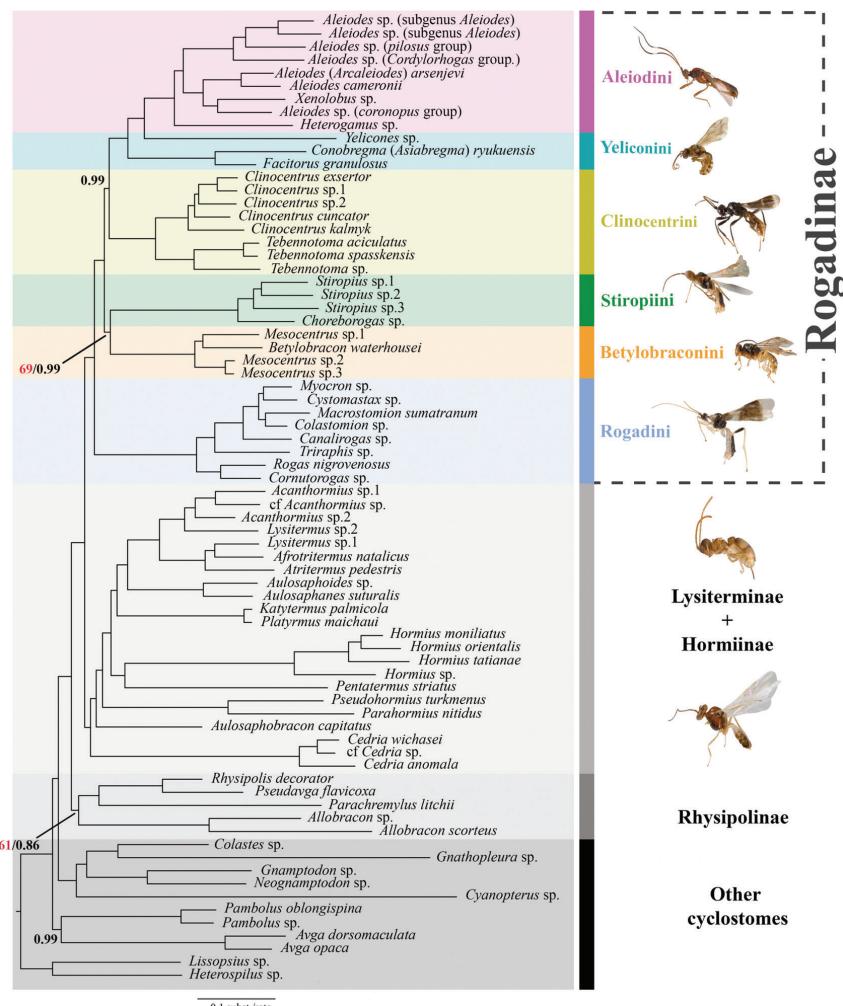
Fig 2. Maximum Likelihood phylogram derived from a 50% complete matrix containing the loci partition following the SWSC-EN algorithm (411 UCE loci, 98 partitions). Identical relationships were obtained in the Bayesian analysis with the same matrix and partition strategy. Numbers near nodes represent Bayesian posterior probabilities (PP; black) and bootstrap (BS; red) support values. Nodes without numbers are supported by PP = 1.0 and BS > 70 values. [Colour figure can be viewed at wileyonlinelibrary.com].

grade leading to Lysitermini and Tetratermini, with *Pentatermus*, recovered between *Hormius* and *Parahormius* and with *Cedria* and then *Aulosaphobracon* being sister to all of them. The main diagnostic feature of Hormiinae is the weakly sclerotized, membranous metasomal tergites, which separates it from Lysiterminae and Rogadinae (van Achterberg, 1991). However, Wharton (1993) proposed that hormiines in a broader sense could also include those with a carapace-like metasoma

(lysitermines) since both share various wing venation, leg and sculpture features. Wharton's (1993) broad concept of Hormiinae is, therefore, congruent with our results.

According to our estimate of phylogeny, a hard, sclerotized metasoma appeared early in the evolution of the group with *Cedria*, and subsequently was lost and gained again at least twice in *Pentatermus* and the Lysitermini + Tetratermini clade. The placement of Cedriini is consistently supported for the

first time. As with Lysitermini, members of Cedriini have a three-segmented metasomal carapace (Belokobylskij, 1993; Wharton, 1993), though they differ from the latter tribe mainly by wing venation features. The placement of *Pentatermus* as sister to *Hormius* is also consistent with previous studies based on molecular analyses with few genes (Zaldívar-Riverón *et al.*, 2008; Quicke & Butcher, 2015; Quicke *et al.*, 2016; Ranjith *et al.*, 2017). In addition, Lysitermini *sensu* Belokobylskij & Quicke (1999) was strongly supported as sister to Tetratermini in all topologies. Although we did not obtain UCEs for *Tetratermus* Wharton, Quicke *et al.* (2016) recovered *Katytermus* as sister to *Tetratermus* with 82% bootstrap ML support.

Aulosaphobracon was originally described as an aberrant member of Betylobraconinae and was placed within its own monotypic tribe (Aulosaphobraconini, Belokobylskij & Long, 2005). It shares with the betylobraconines a considerably narrow hypostomal cavity and an almost flat labrum. However, species of *Aulosaphobracon* also possess morphological features that are absent in betylobraconines, but present in some lysitermines, such as a flat face, considerably large mandibles, short and wide clypeus, distinct areola of propodeum, long tarsal segments and a relatively long ovipositor (Belokobylskij & Long, 2005; Belokobylskij *et al.*, 2008). Subsequently, based on molecular data, Belokobylskij *et al.* (2008) showed that *Aulosaphobracon* does not belong to Betylobraconinae, but instead was recovered as sister to a clade composed of Rogadinae + Betylobraconinae. Aulosaphobraconini was suggested to belong to Lysiterminae by Butcher *et al.* (2014), which is consistent with our results based on UCE data.

Recent molecular phylogenetic studies have supported the monophyly of the Rhysipolinae comprising *Cantharoctonus* Viereck, *Noserus* Foerster, *Pachystigmus* Hellén, *Pseudavga*, *Rhysipolis* and *Troporhysipolis* Quicke, Belokobylskij et Butcher (Quicke *et al.*, 2016, 2019). Although the biology is only known for a few species of this group, they are of great interest because they display the unusual combination of ectoparasitic koinobiosis (Shaw, 1983, 2017). Our phylogenomic trees consistently support the Oriental-Ethiopian genus *Parachremylus* as sister to *Pseudoavga* + *Rhysipolis*, and also place the New World genus *Allobracon* as sister to all three, although with weak support. The biology of *Pseudavga* has only recently been elucidated (Shaw & Sims, 2015; Shaw, 2017) and as with *Rhysipolis* it also displays the uncommon biology of being a koinobiont ectoparasitoid. Regarding the phylogenetic affinities of *Avga*, it was consistently placed in our resulting topologies outside Hormiinae, being more related to the outgroup subfamilies included. Further studies adding representative species of all cyclostome subfamilies will clarify the phylogenetic placement of this enigmatic genus.

Previously, *Allobracon* and *Parachremylus* had both been included within a broader definition of Hormiinae, with which they share a largely desclerotized metasoma. The latter two genera also have the first metasomal tergite with lateral membranous areas, although as Wharton (1993) noted, the pattern of desclerotization is rather different. Wharton (1993) expressed uncertainty as to the relationships of *Allobracon*, which he left in Hormiini, while also suggesting a possible relationship with

Rhysipolinae. *Allobracon* completely lacks an occipital carina, though it is complete in *Parachremylus*, reaching the base of the mandible separate from the hypostomal carina, which is consistent with the condition found in Rhysipolinae (Whitfield, 1988; Whitfield & Wharton, 1997).

Evolution of lepidopteran host preference

Previous studies that attempted to assess the lepidopteran host preference within Rogadinae divided the different families as either micro- or macrolepidopterans (Whitfield & Wagner, 1991; Shaw, 2002a; Zaldívar-Riverón *et al.*, 2008). Based on this, Zaldívar-Riverón *et al.* (2008) observed a general pattern where Aleiodini and Rogadini attack both exposed macrolepidoptera and concealed or semiconcealed microlepidoptera families. The much smaller tribes Clinocentrini, Stiropiini and Yeliconini appear to attack exclusively microlepidopteran larvae (notably Bucculatricidae, Gracillariidae, Lyonetiidae, Pyralidae and Tortricidae) living in somewhat concealed places such as leaf mines, leaf rolls or webs (Whitfield, 1988; Shaw & Huddleston, 1991; van Achterberg, 1991; Quicke *et al.*, 2018). A recent phylogeny of Lepidoptera based on transcriptomic data (Kawahara *et al.*, 2019) recovered various superfamilies that were previously regarded as microlepidopteran intermingled with macrolepidopteran superfamilies. Below we, therefore, discuss the evolution of hosts ranges preferences among the rogadine tribes on the light of this updated lepidopteran classification.

Members of Aleiodini, which is mainly represented by the enormous genus *Aleiodes*, utilize a broad range of host families, mainly attacking species of a variety of apoditrysian superfamilies as Gelechioidea, Zygaenoidea, Lasiocampoidea, Geometroidea and Noctuoidea, among others (Shaw, 2002a; Quicke & Shaw, 2005; Fortier, 2006; Bazinet *et al.*, 2013; Mitter *et al.*, 2017). Members of Rogadini also attack a broad range of lepidopteran groups including several apodytrisian superfamilies, notably Bombycoidea, Drepanoidea, Geometroidea, Noctuoidea, Papilionoidea, Pyraloidea, Sphingoidea and Zygenoidea (Austin, 1987; van Achterberg, 1991; Shaw, 2002b; Quicke & Shaw, 2005; Quicke *et al.*, 2012). The hosts of both Aleiodini and Rogadini are typically fully exposed or poorly concealed (Shaw, 2006; Hrcek *et al.*, 2013). According to Zaldívar-Riverón *et al.* (2008), the high species richness of *Aleiodes* could be a result of successive host recruitments leading to host range expansion followed by speciation.

The mummies produced by species of Aleiodini are specialized in being hard and heavily tanned, with the wasps always egressing from the posterodorsal part of the host remains, whereas members of the Rogadini display a wider variety of endoparasitoidism strategies, for example inducing weak, moderate or hard mummies, and emergence from various radial orientations as well as often from the anterior end of the host mummy (Shaw, 2002b; Maetô & Arakaki, 2005; Quicke & Shaw, 2005). The other tribes for which mummification strategy is known are Clinocentrini (*Clinocentrus* Haliday) (Shaw, 1983), Stiropiini (*Choreborogas* Whitfield, *Stiropius* Cameron) (Whitfield & Wagner, 1991) and

Yeliconiini (*Yelicones*, *Pseudoyelicones*) (Quicke & Kruft, 1995; Janzen, 2019). All these tribes have a considerably narrower host range (Whitfield, 1988; Whitfield & Wagner, 1991; Quicke & Shaw, 2005) and generally produce rather weakly sclerotized, soft mummies (Zaldívar-Riverón *et al.*, 2008), but *Yelicones* includes species forming both mummy types (Zaldívar-Riverón *et al.*, 2008; Quicke *et al.*, 2018).

Based on our phylogenetic hypothesis and the above information, the ancestral host preference within Rogadinae appears to have been to attack weakly concealed lepidopterans, and subsequently, there were two main transitions to attack both concealed and exposed host groups, one within the Rogadini and a second within the Aleiodini. This is supported by the host preference displayed by the sister group of Rogadinae, the Hormiinae + Lysiterminae, which also appears to be restricted to attacking concealed Lepidoptera larval hosts.

Nomenclatural changes

Based on our well-supported phylogeny we synonymize *Bequartia* Fahringer, (type species *B. gigantea* Fahringer, 1936: 537) and *Xenolobus* Cameron (type species *X. rufus* Cameron 1911: 199) with *Aleiodes* (**syn.n.**), and all species currently classified within each of these are hereby transferred to *Aleiodes* (**comb.n.**). These two small genera (three and one known species, respectively, (van Achterberg, 1991) comprise large-bodied, brightly coloured, and highly autapomorphic species. Notably, they both have forewing vein r-rs continuous with the baso-posterior margin of the pterostigma. As with several other large and colourful species that were described in separate genera, molecular data have shown them again to be derived *Aleiodes* species groups, for example *Arcaleiodes* Chen *et* He synonymized by Belokobylskij (2000), *Cordylorhogas* Enderlein synonymized by Zaldívar-Riverón *et al.* (2008), *Eucystomastax* Enderlein synonymized by Shaw (1993), *Hemigyroneuron* Baker synonymized by Zaldívar-Riverón *et al.* (2008) and *Pholichora* van Achterberg synonymized by Zaldívar-Riverón *et al.* (2008). All of these share with *Aleiodes* the hind wing 2RS diverging away from the wing's anterior margin at approximately its midlength, a derived state present in most *Aleiodes* and absent in virtually all other Rogadinae.

The betylobraconine *Promesocentrus* van Achterberg (type species *Pr. tricolor* van Achterberg) and *Pilichremylus* Belokobylskij (type species *Pi. reiki* Belokobylskij) share several characters in the fore and hind wing venation, as well as similarities in other structures as a metasoma with an almost carapace-shaped first-third tergites, structure of the head dorsally and anteriorly (especially the shape and type of face, hypostomal cavity and clypeus), and the presence of relatively long, lamellifrom propodeal apophyses (Belokobylskij, 1992; van Achterberg, 1995). Based on the above morphological features, we synonymize *Promesocentrus* with *Pilichremylus* (**syn.n.**), and accordingly, the single species of *Promesocentrus* is transferred to the latter genus (*Pi. tricolor* (van Achterberg) **comb.n.**).

Aulosaphobraconini, Cedriini, Hormiini, Aulosaphobraconini, Lysitermini and Tetratermini are hereby considered tribes of Hormiinae (*sensu* Wharton, 1993). However, *Pentatermus* rendered Hormiini (*Hormius* and *Parahormius*) paraphyletic; because of the marked morphological differences and our relatively sparse taxon sampling, we choose to leave *Pentatermus incertae sedis* within Hormiini.

The genera *Allobracon* and *Parachremylus* are here transferred to Rhysipolinae, but retained separate as the tribe Leuriniini Belokobylskij (*Leurinion* Muesebeck, being a junior synonym of *Allobracon* Gahan) (Belokobylskij, 1993).

Museum specimens, missing data and phylogenetic signal

Natural history collections currently represent an important source of biological information not only for conducting traditional taxonomic research (i.e. morphology-based) but also for evolutionary, genetic, behavioural, ecological and conservation studies (Lister *et al.*, 2011). In particular, specimens preserved in scientific collections have become the main source to obtain massive quantities of DNA sequence data using various NGS techniques.

The target enrichment approaches such as UCEs have an important advantage relative to other reduced genome representation techniques, such as RAD-based methods or transcriptomic studies, which require the use of fresh samples with a larger amount of good-quality input DNA (Zhang *et al.*, 2019). Various studies have shown that enrichment methods have a higher success rate compared to other methods when sequencing old and/or dried museum specimens with highly degraded DNA (Lim & Braun, 2016; Ruane & Austin, 2017). In insects, this has been corroborated by Blaimer *et al.* (2016), who were able to recover approximately 1000 UCE loci from pinned bees up to 121 years old.

Here, we obtained a relatively high number of informative loci from old insect material (≥30 years old) compared to the average number of UCE loci recovered from more recently collected samples (1–29 years old). Similar to the above studies, we found that the preservation method and the sample age affected the number of recovered loci (as in *A. scorteus*). However, despite their lower number of loci, the gathered data proved to be highly informative, since our considerable old, pinned samples (up to 82 years old) always appeared in congruent parts of the trees and with strong support values in most cases. In the ethanol-preserved samples, however, we did not find this correlation, though most of them were between 1 and 21 years old. Thus, this result suggests that the latter preservation method could be more adequate for molecular studies.

Specimen size is also important for obtaining a good DNA quantity for use in NGS protocols (Cruaud *et al.*, 2018). Most of our braconid samples were small (5 mm or less), and this probably affected the quantity of DNA obtained to use for NGS library preparation. Nevertheless, we successfully recovered informative UCE loci from a range of 2 to 100 ng of input DNA, though we did not find a correlation between the input DNA of pinned specimens used for library preparation against the

number of recovered loci, which indicates that the UCE protocol is suitable for obtaining enough informative data not only from old and dry but also for small specimens.

Another factor that must be considered when working with old museum samples is their high amount of missing data in the UCE matrices (Blaimer *et al.*, 2016). We generally obtained a high number of loci; however, we could not retain a high number of shared loci in the matrices with lower missing data levels (e.g. 70% completeness matrix, 196 loci). This contrasts with recent phylogenomic studies with other hymenopteran taxa, which shared high amounts of UCE loci in more complete matrices (≥90% completeness matrices) using recently collected samples (Blaimer *et al.*, 2015; Samacá-Sáenz *et al.*, 2019). However, it is important to highlight that the differences in the amount of missing data in our datasets did not have a strong negative effect in the phylogenetic reconstruction, since their recovered trees were very similar, only having differences in three poorly supported relationships.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. ML topology derived from the analysis using the 50% complete matrix and the locus-by-locus partition. An identical topology was obtained from the Bayesian analysis with the same dataset and partition strategy. Black and red circles near nodes represent bootstrap values (BS) < 70 and posterior probability values (PP) < 0.95, respectively. Bty. = Betylobraconini; Stp. = Stiropiini; Clino. = Clinocentrini; Yel. = Yeliconini.

Figure S2. ML topologies derived from the matrices at 30, 40, 60 y 70 completeness percentage. We obtained identical topologies from the Bayesian analysis with the same matrices, except for the monophyly of Rhysipolinae in the matrix with a completeness percentage of 60. Black and red circles near nodes represent bootstrap values (BS) < 70 and posterior probability values (PP) < 0.95, respectively.

Figure S3. ASTRAL-III species tree. A monophyletic Rogadinae was recovered, being composed of six tribes with the inclusion of the Betylobraconini. Black circles near nodes represent local posterior probabilities (LPP) < 0.95.

Table S1. List of specimens included in this study. Their taxon ID, locality, the biogeographic region of provenance and GenBank accession number for the data in the SRA database. We included the previous classification for each specimen (at the subfamily and tribe level) as well as the updated classification (in bold).

Table S2. Statistics of the assembling and UCE loci recovery. The number of contigs recovered per sample, average of recovered loci per group, UCE loci recovered per sample (total: 898) and mean, max and min of UCE loci recovered per group.

File S1. 50% complete matrix containing the loci partition following the SWSC-EN algorithm. Nexus format, 74 taxa, 98 partitions, 411 UCE loci, 168 261 bp.

Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

References

van Achterberg, C. (1984) Essay on the phylogeny of Braconidae (Hymenoptera: Ichneumonoidea). *Entomologisk Tidskrift*, **105**, 41–58.

van Achterberg, C. (1991) Revision of the genera of the Afrotropical and W. Palaearctical Rogadinae Foerster (Hymenoptera: Braconidae). *Zoologische Verhandelingen, Leiden*, **273**, 1–102.

van Achterberg, C. (1993) Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). *Zoologische Verhandelingen, Leiden*, **283**, 1–189.

van Achterberg, C. (1995) Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. *Zoologische Verhandelingen*, **298**, 1–242.

van Achterberg, C. & Steiner, H. (1996) A new genus of Tetratermini (Hymenoptera: Braconidae: Lysiterminae) parasitic on grasshoppers (Gryllacrididae). *Zoologische Mededeelingen*, **70**, 249–259.

Anmarkrud, J.A. & Lifjeld, J.T. (2017) Complete mitochondrial genomes of eleven extinct or possibly extinct bird species. *Molecular Ecology Resources*, **17**, 334–341.

Austin, A.D. (1987) A review of the Braconidae (Hymenoptera) that parasitize Limacodidae in south-east Asia, particularly those associated with coconut and oil palm. *Slug and Nettle Caterpillars. The Biology, Taxonomy and Control of the Limacodidae of Economic Importance on Palms in South-East Asia* (ed. by M.J.W. Cock, H.C.J. Godfray and J.D. Holloway), pp. 139–164. CAB International, Wallingford.

Bazinet, A.L., Cummings, M.P., Mitter, K.T. & Mitter, C.W. (2013) Can RNA-Seq resolve the rapid radiation of advanced moths and butterflies (Hexapoda: Lepidoptera: Apoditrysia)? An exploratory study. *PLoS One*, **8**, e82615.

Beeson, C.F.C. & Chatterjee, S.N. (1935) On the biology of the Braconidae (Hymenoptera). *Indian Forest Records*, **1**, 105–138.

Belokobylskij, S.A. (1992) Braconid wasps of the tribe Pambolini (Hymenoptera, Braconidae) of Australia. *Entomologicheskoe Obozrenie*, **71**, 179–198 (in Russian).

Belokobylskij, S.A. (1993) On the classification and phylogeny of the braconid wasps of subfamilies Doryctinae and Exothecinae (Hymenoptera, Braconidae). Part I. On the classification, 2. *Entomologicheskoe Obozrenie*, **72**, 143–164 (in Russian).

Belokobylskij, S.A. (2000) Fam Braconidae. Subfam. Rogadinae, pt. 4. *Key to Insects of the Russian Far East Neuropteroidea, Mecoptera, Hymenoptera*, Vol. **4** (ed. by A.S. Lelej), pp. 15–109. Dal'nauka, P.A.L. Vladivostok.

Belokobylskij, S.A. & Long, K.D. (2005) The braconid wasps of the subfamily Betylobraconinae (Hymenoptera, Braconidae) in Vietnam. *Deutsche Entomologische Zeitschrift*, **52**, 155–164.

Belokobylskij, S. & Quicke, D.L.J. (1999) A new genus and two new species of brachypterous Lysiterminae (Hymenoptera: Braconidae). *Journal of Hymenoptera Research*, **8**, 120–125.

Belokobylskij, S.A., Zaldívar-Riverón, A., Maetô, K. & Sáez, A.G. (2008) Asian Betylobraconinae (Hymenoptera, Braconidae), with description of a new genus and phylogenetic affinities of the tribe Facitorini. *Insect Systematics and Evolution*, **39**, 133–154.

Belshaw, R., Grafen, A. & Quicke, D.L.J. (2003) Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis. *Zoological Journal of the Linnean Society*, **139**, 213–228.

Besnard, G., Bertrand, J.A., Delahaie, B., Bourgeois, Y.X., Lhuillier, E. & Thébaud, C. (2015) Valuing museum specimens: high-throughput DNA sequencing on historical collections of New Guinea crowned pigeons (Goura). *Biological Journal of the Linnean Society*, **117**, 71–82.

Blaimer, B.B., Brady, S.G., Schultz, T.R., Lloyd, M.W., Fisher, B.L. & Ward, P.S. (2015) Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. *BMC Evolutionary Biology*, **15**, 271.

Blaimer, B.B., Lloyd, M.W., Guillory, W.X. & Brady, S.G. (2016) Sequence capture and phylogenetic utility of genomic ultraconserved elements obtained from pinned insect specimens. *PLoS One*, **11**, e0161531.

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114–2120.

Branstetter, M.G., Longino, J.T., Ward, P.S. & Faircloth, B.C. (2017) Enriching the ant tree of life: enhanced UCE bait set for genome–scale phylogenetics of ants and other Hymenoptera. *Methods in Ecology and Evolution*, **8**, 768–776.

Butcher, B.A. & Quicke, D.L. (2015) A remarkable new genus and species of Rogadinae (Hymenoptera: Braconidae) of uncertain tribal placement, from Papua New Guinea, resembling Betylobraconini stat. nov. *Journal of Natural History*, **49**, 2045–2054.

Butcher, B.A., Zaldívar-Riverón, A., Van De Kamp, T., Dos Santos Rolo, T., Baumbach, T. & Quicke, D.L.J. (2014) Extension of historical range of Betylobraconinae (Hymenoptera: Braconidae) into Palaearctic Region based on a Baltic amber fossil, and description of a new species of *Mesocentrus* Szépligeti from Papua New Guinea. *Zootaxa*, **3860**, 449–463.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.

Ceccarelli, F.S., Sharkey, M.J. & Zaldívar-Riverón, A. (2012) Species identification in the taxonomically neglected, highly diverse, neotropical parasitoid wasp genus *Notiospathius* (Braconidae: Doryctinae) based on an integrative molecular and morphological approach. *Molecular Phylogenetics and Evolution*, **62**, 485–495.

Chen, X.X., Piao, M.H., Whitfield, J. & He, J.H. (2003) A molecular phylogeny of the subfamily Rogadinae (Hymenoptera: Braconidae) based on the D2 variable region of 28S ribosomal RNA. *Acta Entomologica Sinica*, **46**, 209–217.

Cruaud, A., Groussier, G., Genson, G., Sauné, L., Polaszek, A. & Rasplus, J.Y. (2018) Pushing the limits of whole genome amplification: successful sequencing of RADseq library from a single microhymenopteran (Chalcidoidea, *Trichogramma*). *PeerJ*, **6**, e5640.

Del Fabbro, C., Scalabrin, S., Morgante, M. & Giorgi, F.M. (2013) An extensive evaluation of read trimming effects on Illumina NGS data analysis. *PLoS One*, **8**, e85024.

Faircloth, B.C. (2013) *Illumiprocessor: A Trimmomatic Wrapper for Parallel Adapter and Quality Trimming*. https://doi.org/10.6079/J9ILL.

Faircloth, B.C. (2015) PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics*, **32**, 786–788.

Faircloth, B.C., Branstetter, M.G., White, N.D. & Brady, S.G. (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Molecular Ecology Resources*, **15**, 489–501.

Fortier, J.C. (2006) Twelve new species and a new combination of the *Aleiodes pilosus* species-group (= *Tetrasphaeropyx* Ashmead) (Hymenoptera, Braconidae, Rogadinae) in North America: part 1. *Entomological News*, **117**, 465–485.

Gauld, I.D. (1988) Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biological Journal of the Linnean Society*, **35**, 351–377.

Gauthier, J., Pajkovic, M., Neuenschwander, S., Kaila, L., Schmid, S., Orlando, L. & Alvarez, N. (2020) Museomics identifies genetic erosion in two butterfly species across the 20th century in Finland. *Molecular Ecology Resources* (in press). https://doi.org/10.1111/1755-0998.13167.

Gillespie, J.J., Yoder, M.J. & Wharton, R.A. (2005) Predicted secondary structure for 28S and 18S rRNA from Ichneumonoidea (Insecta: Hymenoptera: Apocrita): impact on sequence alignment and phylogeny estimation. *Journal of Molecular Evolution*, **61**, 114–137.

Glenn, T.C., Nilsen, R., Kieran, T.J., Finger, J.W. Jr, Pierson, T.W., Bentley, K.E., Hoffberg, S., Louha, S., García-De León, F.J, del Río Portilla, M.A., Reed, K.D., Anderson, J.L., Meece, J.K., Aggery, S.E., R. Rekaya, R., Alabady M., Belanger M., Winker, K. & Faircloth, B.C. (2016) Adapterama I: universal stubs and primers for thousands of dual–indexed Illumina libraries (iTru and iNext). *PeerJ*, **7**, e7755.

Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton UniversityPress, Princeton, New Jersey.

Grabherr, M.G., Haas, B.J., Yassour, M. *et al.* (2011) Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, **29**, 644–652.

Gupta, A. & Quicke, D.L.J. (2018) A new species of *Acanthormius* (Braconidae: Lysiterminae) reared as a gregarious parasitoid of

psychid caterpillar (Lepidoptera: Psychidae) from India. *Zootaxa*, **4388**, 425–430.

Guschanski, K., Krause, J., Sawyer, S. *et al.* (2013) Next-generation museomics disentangles one of the largest primate radiations. *Systematic Biology*, **62**, 539–554.

Hassell, M.P. & May, R.M. (1986) Generalist and specialist natural enemies in insect predator-prey interactions. *Journal of Animal Ecology*, **55**, 923–940.

Hrcek, J., Miller, S.E., Whitfield, J.B., Shima, H. & Novotny, V. (2013) Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. *Oecologia*, **173**, 521–532.

Janzen, D.J. (2019) *Chloropaschia mennusalis* (Pyralidae). https://www.acguanacaste.ac.cr/paginas-de-especies/insectos/298-pyralidae/932-i-chloropaschia-mennusalis-i-pyralidae.

Jin, M., Zwick, A., Ślipiński, A., De Keyzer, R. & Pang, H. (2020) Museomics reveals extensive cryptic diversity of Australian prionine longhorn beetles with implications for their classification and conservation. *Systematic Entomology* (in press). https://doi.org/10.1111/syen.12424.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.

Kawahara, A.Y., Plotkin, D., Espeland, M. *et al.* (2019) Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proceedings of the National Academy of Sciences*, **116**, 22657–22663.

Kollias, S., Poortvliet, M., Smolina, I. & Hoarau, G. (2015) Low cost sequencing of mitogenomes from museum samples using baits capture and ion torrent. *Conservation Genetics Resources*, **7**, 345–348.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**, 772–773.

Lim, H.C. & Braun, M.J. (2016) High-throughput SNP genotyping of historical and modern samples of five bird species via sequence capture of ultraconserved elements. *Molecular Ecology Resources*, **16**, 1204–1223.

Lister, A.M., Brooks, S.J., Fenberg, P.B. *et al.* (2011) Natural history collections as sources of long-term datasets. *Trends in Ecology & Evolution*, **26**, 153–154.

Maetô, K. & Arakaki, N. (2005) Gregarious emergence of *Macrostomion sumatranum* (Hymenoptera: Braconidae; Rogadinae) from the mummified, full-grown larvae of *Theretra silhetensis* (Lepidoptera: Sphingidae). *Entomological Science*, **8**, 131–132.

Mathur, R.N. (1959) Breeding technique and utilisation of *Cedria paradoxa* Wilk. (Hym., Braconidae) for biological control of teak defoliator. *Entomologist's Monthly Magazine*, **95**, 248–250.

Mitter, C., Davis, D.R. & Cummings, M.P. (2017) Phylogeny and evolution of Lepidoptera. *Annual Review of Entomology*, **62**, 265–283.

Peters, R.S., Krogmann, L., Mayer, C. *et al.* (2017) Evolutionary history of the Hymenoptera. *Current Biology*, **27**, 1013–1018.

R Core Team (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Quicke, D.L.J. & Kruft, R.A. (1995) Species of Yelicones (Hymenoptera: Braconidae: Rogadinae) in North America with descriptions of two new species. *Annals of the Entomological Society of America*, **88**, 129–138.

Quicke, D.L.J. (1997) *Parasitic Wasps*. Chapman & Hall Ltd, London.

Quicke, D.L.J. (2015) *The Braconid and Ichneumonid Parasitoid Wasps: Biology, Systematics, Evolution and Ecology*. John Wiley & Sons, Ltd, Chichester.

Quicke, D.L.J. & Butcher, B.A. (2015) Description of a new Betylobraconini-like parasitoid wasp genus and species (Hymenoptera: Braconidae: Rogadinae) from Chile. *Zootaxa*, **4021**, 459–466.

Quicke, D.L.J. & Shaw, M.R. (2005) First host records for the rogadine genera *Rogasodes* Chen and He and *Canalirogas* van Achterberg and Chen (Hymenoptera: Braconidae) with description of a new species and survey of mummy types within Rogadinae s. str. *Journal of Natural History*, **39**, 3525–3542.

Quicke, D.L.J., Mori, M., Zaldívar-Riverón, A., Laurenne, N.M. & Shaw, M.R. (2006) Suspended mummies in *Aleiodes* species (Hymenoptera: Braconidae: Rogadinae) with descriptions of six new species from western Uganda based largely on DNA sequence data. *Journal of Natural History*, **40**, 2663–2680.

Quicke, D.L.J., Smith, M.A., Miller, S.E., Hrcek, J. & Butcher, B.A. (2012) *Colastomion* baker (Braconidae, Rogadinae): nine new species from Papua New Guinea reared from Crambidae. *Journal of Hymenoptera Research*, **28**, 85–121.

Quicke, D.L.J., Shaw, M.R., van Achterberg, C., Bland, K.P., Butcher, B.A., Lyszkowski, R. & Zhang, Y.M. (2014) A new Australian genus and five new species of Rogadinae (Hymenoptera: Braconidae), one reared as a gregarious endoparasitoid of an unidentified limacodid (Lepidoptera). *Zootaxa*, **3881**, 237–257.

Quicke, D.L.J., Belokobylskij, S.A., Smith, M.A., Rota, J., Hrcek, J. & Butcher, B.A. (2016) A new genus of rhysipoline wasp (Hymemoptera: Braconidae) with modified wing venation from Africa and Papua New Guinea, parasitoid on Choreutidae (Lepidoptera). *Annales Zoologici*, **66**, 173–192.

Quicke, D.L.J., Kuslitzky, W. & Butcher, B.A. (2018) First host record for Old World *Yelicones* (Hymenoptera: Braconidae: Rogadinae) adds to evidence that they are strictly parasitoids of Pyralidae (Lepidoptera). *Israel Journal of Entomology*, **48**, 33–40.

Quicke, D.L.J., Belokobylskij, S.A., Ward, D.F., Hebert, P.D.N. & Butcher, B.A. (2019) Revision of the endemic New Zealand braconid wasp genus *Metaspathius*: new subfamily placement, and descriptions of four new species including three with fully winged females (Hymenoptera: Braconidae: Mesostoinae). *The New Zealand Entomologist*, **41**, 46–65.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, **67**, 901–904.

Ranjith, A.P., Belokobylskij, S.A., Quicke, D.L.J., Kittel, R.N., Butcher, B.A. & Nasser, M. (2017) An enigmatic new genus of Horminae (Hymenoptera: Braconidae) from South India. *Zootaxa*, **4272**, 371–385.

Ronquist, F., Teslenko, M., Van Der Mark, P. *et al.* (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Ruane, S. & Austin, C.C. (2017) Phylogenomics using formalin-fixed and 100+ year-old intractable natural history specimens. *Molecular Ecology Resources*, **17**, 1003–1008.

Samacá-Sáenz, E., Meza-Lázaro, R.N., Branstetter, M.G. & Zaldívar-Riverón, A. (2019) Phylogenomics and mitochondrial genome evolution of the gall-associated doryctine wasp genera (Hymenoptera: Braconidae). *Systematics and Biodiversity*, **17**, 731–744.

Sharanowski, B.J., Dowling, A.P.G. & Sharkey, M.J. (2011) Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. *Systematic Entomology*, **36**, 549–572.

Shaw, M.R. (1983) On[e] evolution of endoparasitism; the biology of some genera of Rogadinae (Braconidae). *Contributions of the American Entomological Institute*, **20**, 307–328.

Shaw, S.R. (1993) Systematic status of *Eucystomastax* Brues and characterization of the Neotropical *Aleiodes melanopterus* (Erichson) species complex (Hymenoptera: Braconidae: Rogadinae: Rogadini). *Journal of Hymenoptera Research*, **2**, 1–11.

Shaw, M.R. (2002a) Host ranges of *Aleiodes* species (Hymenoptera: Braconidae), and an evolutionary hypothesis. *Parasitic Wasps: Wvolution, Systematics, Biodiversity and Biological Control* (ed. by G. Melika and C. Thuroczy), pp. 321–327. Agroinform, Budapest.

Shaw, M.R. (2002b) A new species of *Macrostomion* Szépligeti (Hymenoptera: Braconidae: Rogadinae) from Papua New Guinea, with notes on the biology of the genus. *Zoologische Mededelingen, Leiden*, **76**, 133–140.

Shaw, S.R. (2006) *Aleiodes* wasps of eastern forests: a guide to parasitoids and associated mummified caterpillars. *Forest Health Technology Enterprise Team Special Publication*, **121**, 2006–2008.

Shaw, M.R. (2017) Further notes on the biology of *Pseudavga flavicoxa* Tobias, 1964 (Hymenoptera, Braconidae, Rhysipolinae). *Journal of Hymenoptera Research*, **54**, 113–128.

Shaw, M.R. & Huddleston, T. (1991) Classification and biology of braconid wasps (Hymenoptera: Braconidae). *Handbooks for the Identification of British Insects*, London: Royal Entomological Society of London, **7**, 1–126.

Shaw, M.R. & Sims, I. (2015) Notes on the biology, morphology, nomenclature and classification of *Pseudavga flavicoxa* Tobias, 1964 (Hymenoptera, Braconidae, Rhysipolinae), a genus and species new to Britain parasitizing *Bucculatrix thoracella* (Thunberg) (Lepidoptera, Bucculatricidae). *Journal of Hymenoptera Research*, **42**, 21–32.

Shimbori, E.M., Souza-Gessner, C.D.S.S., Penteado-Dias, A.M. & Shaw, S.R. (2017) A revision of the genus *Andesipolis* (Hymenoptera: Braconidae: Mesostoinae) and redefinition of the subfamily Mesostoinae. *Zootaxa*, **4216**, 101–152.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.

Tagliacollo, V.A. & Lanfear, R. (2018) Estimating improved partitioning schemes for ultraconserved elements. *Molecular Biology and Evolution*, **35**, 1798–1811.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, **56**, 564–577.

Tobias, V.I. (1979) Two New and Little Known Subfamilies of Braconidae (Hymenoptera) from Australia. *Entomologicheskoe Obozrenie*, **58**, 128–142 (in Russian).

Weirauch, C., Cranston, P.S., Simonsen, T.J. & Winterton, S.L. (2020) New ways for old specimens – museomics is transforming the field of systematic entomology. *Antenna*, **44**, 22–24.

Wharton, R.A. (1993) Review of the Hormiini (Hymenoptera: Braconidae) with a description of new taxa. *Journal of Natural History*, **27**, 107–171.

Wharton, R.A. (1997) Introduction. *Manual of the New World Genera of the Family Braconidae (Hymenoptera)* (ed. by R.A. Wharton, P.M. Marsh and M.J. Sharkey), pp. 1–18. International Society of Hymenopterists, Special Publ. 1, Washington, District of Columbia.

Whitfield, J.B. (1988) Revision of the Nearctic species of the genus *Stiropius* Cameron (= *Bucculatriplex* Auct.) with the description of a new related genus (Hymenoptera: Braconidae). *Systematic Entomology*, **13**, 373–385.

Whitfield, J.B. & Wagner, D.L. (1991) Annotated key to the genera of Braconidae (Hymenoptera) attacking leafmining Lepidoptera in the Holarctic region. *Journal of Natural History*, **25**, 733–754.

Whitfield, J.B. & Wharton, R.A. (1997) Subfamily hormiinae. *Manual of the New World Genera of the Family Braconidae (Hymenoptera)* (ed. by R.A. Wharton, P.M. Marsh and M.J. Sharkey), pp. 285–301. International Society of Hymenopterists, Special Publ. 1, Washington, District of Columbia.

Yu, D.S.K., van Achterberg, C. & Horstmann, K. (2016) *Taxapad 2016, Ichneumonoidea (2015). Database on Flash-drive*. Nepean, Ontario, Canada. www.taxapad.com

Zaldívar-Riverón, A., Areekul, B., Shaw, M.R. & Quicke, D.L.J. (2004) Comparative morphology of the venom apparatus in the braconid wasp subfamily Rogadinae (Insecta, Hymenoptera, Braconidae) and related taxa. *Zoologica Scripta*, **33**, 223–237.

Zaldívar-Riverón, A., Mori, M. & Quicke, D.L.J. (2006) Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. *Molecular Phylogenetics and Evolution*, **38**, 130–145.

Zaldívar-Riverón, A., Shaw, M.R., Sáez, A.G., Mori, M., Belokobylskij, S.A., Shaw, S.R. & Quicke, D.L.J. (2008) Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. *BMC Evolutionary Biology*, **8**, 329.

Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. (2018) ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics*, **19**, 153.

Zhang, Y.M., Williams, J.L. & Lucky, A. (2019) Understanding UCEs: a comprehensive primer on using Ultraconserved elements for arthropod phylogenomics. *Insect Systematics and Diversity*, **3**, 3.

CAPÍTULO II: PHYLOGENOMICS OF BRACONID WASPS (HYMENOPTERA, BRACONIDAE) SHEDS LIGHT ON CLASSIFICATION AND THE EVOLUTION OF PARASITOID LIFE HISTORY TRAITS

Phylogenomics of braconid wasps (Hymenoptera, Braconidae) sheds light on classification and the evolution of parasitoid life history traits

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ABSTRACT

The parasitoid lifestyle is largely regarded as a key innovation that contributed to the evolutionary success and extreme species richness of the order Hymenoptera. Understanding the phylogenetic history of hyperdiverse parasitoid groups is a fundamental step in elucidating the evolution of biological traits linked to parasitoidism. We used a genomic-scale dataset based on ultra-conserved elements and the most comprehensive taxon sampling to date to estimate the evolutionary relationships of Braconidae, the second largest family of Hymenoptera. Based on our results, we propose the existence of 41 braconid subfamilies, confirmed a number of subfamilial placements and proposed subfamily-level taxonomic changes, notably the restoration of Trachypetinae **stat. rev.** and Masoninae **stat. rev.** as subfamilies of Braconidae, confirmation that *Apozyx penyai* Mason belongs in Braconidae placed in the subfamily Apozyginae and the recognition of Ichneutinae *sensu stricto* and Proteropinae as non-cyclostome subfamilies robustly supported in a phylogenetic context. The correlation between koinobiosis with endoparasitoidism and idiobiosis with ectoparasitoidism, long thought to be an important aspect in parasitoid life history, was formally tested and confirmed in a phylogenetic framework. Using ancestral reconstruction methods based on both parsimony and maximum likelihood, we suggest that the ancestor of the braconoid complex was a koinobiont endoparasitoid, as was that of the cyclostome *sensu lato* clade. Our results also provide strong evidence for one transition from endo- to ectoparasitoidism and three reversals back to endoparasitoidism within the cyclostome *sensu stricto* lineage. Transitions of koino- and idiobiosis were identical to that inferred for endo-*versus* ectoparasitoidism, except with one additional reversal back to koinobiosis in the small subfamily Rhysipolinae.

Keywords: Parasitoid wasp, parasitoidism, ultra-conserved elements, cyclostome, non-cyclostome

1. Introduction

It is widely assumed that different attributes such as physiological, behavioral and life history traits may have major impacts on the evolutionary history of organisms and influence their adaptive success and diversification (Mayhew, 2007; Ebel et al., 2015; Condamine et al., 2016). Within insects, the Hymenoptera (ants, bees, wasps, sawflies, parasitoid wasps and woodwasps) are one of the most successful lineages and have recently been suggested as the insect order with the highest species richness, largely due to the extraordinary diversity found among parasitoid wasps (Forbes et al., 2018). The adoption of a parasitoid lifestyle is widely regarded as a key innovation in the evolutionary history of Hymenoptera (Hanson and Gauld, 1995; Grimaldi and Engel, 2005), leading not only to extreme diversity but to ubiquitous niche occupation: almost every insect species is attacked by at least one parasitoid species (Schoenly, 1990; Memmott and Godfray, 1993). As a consequence, parasitoids exert key functional roles in ecosystems and have enormous economic impact by controlling the populations of insect pests (Godfray, 1994).

In light of their extreme diversity and ecological importance, understanding the phylogenetic history of hyperdiverse parasitoid families is a fundamental step in elucidating the evolution of biological traits linked to parasitoidism. Obtaining comprehensive and reliable phylogenies for such broad lineages, however, has been challenging due to their extreme diversity and historical taxonomic neglect. One such highly speciose group is the Braconidae (Fig. 1), the second largest family of Hymenoptera with more than 21,000 described species worldwide (Yu et al., 2016) currently distributed in 41 subfamilies. Nearly all braconids are parasitoids, with a few species reported or being suspected as secondarily phytophagous (Wharton and Hanson, 2005; Ranjith et al., 2016; Maqbool et al., 2018; Zaldívar-Riverón et al., 2014). Braconids exhibit an impressive array of biological strategies, and their hosts collectively span 12 insect orders (Quicke, 2015; Yu et al., 2016), making them an excellent study system to investigate the evolution of biological traits related to parasitoidism.

1.1 *Taxonomic history and phylogeny of Braconidae*

Van Achterberg (1984a) provided the first hypothesis of relationships among braconids based on a Hennigian cladistics approach using morphological characters from adults and larvae, as well as biological data. The first quantitative cladistic analyses for Braconidae (Quicke and van Achterberg 1990), also based on morphological and biological data, shed light on the difficulties of interpreting morphological characters and resulted in subsequent reassessments (Wharton et al., 1992; van Achterberg and Quicke, 1992). These early quantitative cladistic studies (Quicke and van Achterberg, 1990; Wharton et al., 1992; van Achterberg and Quicke, 1992) significantly advanced our understanding of evolutionary relationships among braconids but were also limited due to the use of a relatively small number of characters to infer relationships within a hyperdiverse group.

The advent of DNA sequencing and increased computational capabilities resulted in more extensive and robust phylogenies, and the results of those efforts over the last ~30 years serve as the basis for contemporary classifications of Braconidae (Chen and van Achterberg, 2019). These phylogenetic studies have revealed that some of the morphological traits traditionally employed to define higher-level taxa are actually homoplastic (Zaldívar-Riverón et al., 2007; Quicke, 2015).

Braconidae is considered the sister group to Ichneumonidae (Belshaw et al., 1998; Quicke et al., 1999a; Dowton et al., 2002; Wei et al., 2010; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021), although familial placement is historically uncertain for a few taxa in Braconidae (e.g., Apozyginae, Mason, 1978; Masoninae, Quicke et al., 2020a;

Trachypetinae, Quicke et al., 2020b). Aphidiinae has been treated as a family previously (Mackauer, 1961; Tobias and Kirijak, 1986; Finlayson, 1990; Chen and Shi, 2001; Davidyan, 2007) but clearly belongs in Braconidae (Belshaw et al., 1998; Dowton et al., 1998; Belshaw et al., 2000; Dowton et al., 2002; Belshaw and Quicke, 2002; Pitz et al., 2007; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021).

Braconids are frequently divided into two informal groups: the cyclostomes, characterized by having the lower part of the clypeus sharply recessed exposing a concave labrum and the non-cyclostomes, which have a clypeus that conceals the labrum, or if the labrum is exposed it is flat or convex (Sharkey, 1993; Wharton, 1993a; Wharton et al., 1997) (Fig. 2). Beyond the cyclostome and non-cyclostome groupings, braconids have been arranged into subfamily complexes.

The cyclostome subfamilies have been named as the cyclostome complex *sensu stricto* (Sharanowski et al., 2011). Within this complex, the subfamilies Alysiinae, Opiinae, Exothecinae, Telengaiinae, Gnamptodontinae and Braconinae are grouped within the alysioid subcomplex (Sharanowski et al., 2011; Quicke, 2015). The cyclostome complex *s.s.* has been recovered as sister to the aphidioid complex, a clade that includes the subfamilies Aphidiinae, Maxfischeriinae and Mesostoinae (Dowton et al., 2002; Zaldívar-Riverón et al., 2006; Wei et al., 2010; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021). A clade with the Aphidiinae and the members of the cyclostome complex *s.s.* was named in a previous molecular phylogenetic study as the braconoid complex (Dowton et al., 1998). Some members of the aphidioid complex—*Maxfischeria* (Maxfischeriinae), *Mesostoa* (Mesostoinae) and most aphidiines—lack the cyclostome condition (Quicke, 2015), but the remaining aphidioids have this condition. It has been suggested that the cyclostome feature was secondarily lost in members of the aphidioid complex (Dowton et al., 2002), which is the case for some taxa within the cyclostomes *s. s.*, such as Alysiinae, Opiinae and Betylobraconini (Rogadinae).

Non-cyclostome braconids have been further grouped into the euphoroid, microgastroid, sigalphoid and helconoid complexes (Quicke and van Achterberg, 1990; Wharton, 1993a; Belshaw and Quicke et al., 2002; Sharanowski et al., 2011), the latter containing the macrocentroid subcomplex with Amicrocentrinae, Charmontinae, Homolobinae, Macrocentrinae, Microtypinae, Orgilinae and Xiphozelinae (Sharanowski et al., 2011).

These past efforts have provided strong support for several hypothesized relationships among braconids, but consistent, well-supported definitions remain elusive for certain subfamilies (e.g., Doryctinae, Hormiinae, Ichneutinae, Mesostoinae). In addition, it is still unclear whether some enigmatic taxa belong to Braconidae (e.g., Apozyginae, Masoninae and Trachypetinae) (Schulz, 1911; Tobias, 1979; Mason, 1978; Quicke et al., 2020a; Quicke et al., 2020b), as well as their phylogenetic relationships to other braconids.

1.2 *Evolution of parasitoidism strategies in Braconidae*

The Braconidae undoubtedly represent one of the most diverse of all parasitoid lineages in terms of biological traits and strategies for host use. The majority of species parasitize the immature stages of holometabolous insects, but there are remarkable exceptions, including the use of adult holometabolous and hemimetabolous insects (e.g., Euphorinae and Aphidiinae; Mackauer et al., 1996; Stigenberg et al., 2015), as well as secondary phytophagy (in Doryctinae, Braconinae and Mesostoinae; Infante et al., 1995; Austin and Dangerfield, 1998; Ranjith et al., 2016; Zaldívar-Riverón et al., 2014).

The family includes ecto- and endoparasitoids (i.e. eggs are laid on or into the host, respectively) and both idiobionts and koinobionts (i.e. the host is either paralyzed permanently or

continues to develop, respectively). Non-cyclostome braconids are koinobiont endoparasitoids, whereas an array of life history strategies is found among cyclostome braconids (koinobiont endo- and ectoparasitoids, idiobiont endo- and ectoparasitoids, herbivores). Other remarkable specializations include the use of endogenous viruses to disable the immune system of the host (in the microgastroid complex and opiines; Whitfield, 2002; Burke and Strand, 2012; Whitfield et al., 2018; Burke et al., 2018), host mummification (in Rogadinae and Aphidiinae; Hagvar and Hofsvang, 1991; van Achterberg, 1995; Zaldívar-Riverón et al., 2008a), gregarious parasitoidism (e.g., Microgastrinae; Michel-Salzat and Whitfield, 2004) and polyembryony (*Macrocentrus*, Macrocentrinae; Krugner et al., 2005).

Understanding the evolutionary history of biological traits related to host use has been a common theme in braconid-related research (e.g., Whitfield, 2002; Zaldívar-Riverón et al., 2006; Zaldívar-Riverón et al., 2008a; Stigenberg et al., 2015; Sharanowski et al., 2021; Samacá-Saenz et al., 2022). It has been proposed that parasitoidism in Ichneumonoidea has evolved from idiobiont ectoparasitoid wasps that attacked weakly concealed hosts (Gauld, 1988; Whitfield, 1992; Vilhelmsen, 1997) to koinobiont endoparasitoids of deeply concealed, as well as exposed hosts (Quicke et al., 1999b). More recently, using a phylogeny based on genomic-scale data, Sharanowski et al. (2021) tested this hypothesis and found evidence that the ancestor of Ichneumonoidea was indeed an idiobiont ectoparasitoid, with multiple transitions in mode of parasitoidism occurring within the superfamily. In the case of Braconidae, the results suggested that its ancestor may have been a koinobiont endoparasitoid; however, inferred ancestral states varied widely, leading the authors to refrain from making strong conclusions about the evolution of parasitoidism in the group (Sharanowski et al., 2021).

The use of next-generation sequencing techniques (NGS) is rapidly increasing as an approach for exploring evolutionary questions in entomology (Paula, 2021). In particular, data obtained from targeted enrichment methods such as the capture of ultra-conserved elements (UCEs) (Faircloth et al., 2015) and anchored hybrid enrichment (AHE) (Lemmon et al., 2012) have been used to generate a reliable phylogenetic framework for many groups of Hymenoptera, including Braconidae (Sharanowski et al., 2021 for AHE; Samacá-Saenz et al., 2019, 2022; and Jasso-Martínez et al., 2021 for UCEs). As noted by Zhang et al. (2019), AHE and UCE approaches target different types of loci; AHE recovers fewer loci (300–600) that are longer and exclusively exonic, while UCEs target a larger number of shorter loci (>1,000) that include both coding and non-coding regions. Among the advantages of UCEs in phylogenetics are their performance for obtaining hundreds or thousands of loci even from low-quality and degraded samples, as well as the availability of protocols, bioinformatic pipelines and baits that aid in their reproducibility.

1.3 *Study aims*

This study aims to reconstruct the phylogenetic relationships of Braconidae based on genomic data from ultra-conserved elements (UCEs) and on the most comprehensive taxonomic sampling carried out to date for the family, affording an unprecedented phylogenetic analysis in terms of data volume and robustness of the results. We then use these phylogenies to develop a revised classification for Braconidae, as well as to explore long pursued questions regarding the evolution of life history strategies within the family.

2. Methods
2.1 *Taxon sampling*

We sequenced UCE data for a total of 393 braconid species from 276 genera (Supplementary Table S1), including members of all subfamilies except for three small subfamilies: Amicrocentrinae (5 spp.), Dirrhophinae (5 spp.) and Xiphozelinae (16 spp.). Within our braconid ingroup, 236 species belong to the cyclostomes *s.s.* and aphidioid complex and 156 species to the non-cyclostome group; for the latter, all subfamily complexes are represented (i.e. the helconoid, euphoroid, sigalphoid and micrograstroid complexes; Sharanowski et al., 2011).

      We included species of three taxa for which familial placement is unclear. *Apozyx penyai* Mason is currently considered part of Braconidae within the monotypic subfamily Apozyginae (Quicke and van Achterberg, 1990; Perrichot et al., 2009; Belokobylskij and Jouault, 2021), although it was originally placed in its own family (Apozygidae; Mason, 1978). Also, we included a specimen of *Trachypetus clavatus* Guérin-Meneville for Trachypetinae, a group traditionally treated as a subfamily of Braconidae (Schulz, 1911; Tobias, 1979) that was recently raised to family status based on molecular and morphological evidence (Trachypetidae: Quicke et al., 2020b). Similarly, we included a specimen of *Masona* for Masoninae, a subfamily previously considered within Braconidae (van Achterberg, 1995) that was recently transferred to Ichneumonidae based on molecular evidence (Quicke et al., 2020a). Phenotypic vouchers for all of the sampled species are housed at the Smithsonian Institution National Museum of Natural History, Washington, DC; in the Colección Nacional de Insectos at the Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM); in the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia (ZISP) and at the Canadian National Collection of Insects (CNC), Ottawa, Canada.

      Subfamily and species names are as in Yu et al. (2016). Genus and subgenus names are as in Wharton et al. (1997) except we used Yu et al. (2016) for exclusively Old World taxa. Exceptions to the aforementioned use of names are as follows: Histeromerinae as a junior synonym of Rhyssalinae (Zaldívar-Riverón et al., 2006); Lysiterminae as a junior synonym of Hormiinae, *Parahormius* and *Pseudohormius* as Hormiinae and *Allobracon* and *Parachremylus* as Rhysipolinae (Jasso-Martínez et al., 2021); *Chremylus* as Hormiinae (Gadallah et al., 2021); *Monitoriella* as Doryctinae (Zaldívar-Riverón et al., 2006); *Avga dorsomaculata sensu* Belokobylskij and Tobias (1986); *Tetrasphaeropyx* and *Xenolobus* as junior synonyms of *Aleiodes* (Fortier, 2006; Jasso-Martínez et al., 2021); *Triraphis sensu* van Achterberg (1991) and Valerio and Shaw (2015); Blacinae as a junior synonym of Brachistinae (Sharanowski et al., 2011); *Vadumasonium* for the primary homonym *Vadum* Mason (Kammerer, 2006); *Euphoriella* as a junior synonym of *Leiophron* (Zhang et al., 2018) and Microgastrinae species and genus names as in Fernández-Triana et al. (2020).

      The outgroup includes 11 species of Ichneumonidae from the subfamilies Cremastinae, Ctenopelmatinae, Cryptinae, Ichneumoninae, Labeninae, Orthocentrinae, Pimplinae, Tryphoninae and Xoridinae, representing all major lineages in the family. In order to provide further clarity on the placement of taxa of uncertain familial status (i.e. whether they are closer to Braconidae or Ichneumonidae) and following the observation by Sharanowski et al. (2021) that it may not be appropriate to root braconid or ichneumonid phylogenies with their sister family, we used *Gasteruption floridanum* Bradley (Evanioidea, Gasteruptiidae) to root the trees. A list with details of the taxa examined in this study, subfamily classification and NCBI accession numbers of the UCE raw data analyzed is provided in Supplementary Table S1.

      Terminology of external morphology, including wing venation, follows Sharkey and Wharton (1997).

2.2. *Library preparation, target enrichment and sequencing*

Library preparation and enrichment was conducted in three different facilities (the Laboratories of Analytical Biology at the Smithsonian Institution National Museum of Natural History, the Laboratorio de Biología Molecular de Zoología at the Instituto de Biología, Universidad Nacional Autónoma de Mexico and the Ottawa Research and Development Centre, Agriculture and Agri-Food Canada). Protocols varied slightly across institutions; a detailed overview of methods is provided in Supplementary File S2 but can be summarized as follows.

Genomic DNA was extracted using commercial spin-column based kits by Qiagen (Hilden, Germany). The DNA yield was quantified using a Qubit fluorometer (High sensitivity kit, Life Technologies, Inc., Carlsbad, CA), and an aliquot of < 2 to 150 ng was used as input for library preparation. Samples with high molecular weight DNA were sheared either enzymatically or using a sonicator (Q800, Qsonica Inc., Newtown, CT) to obtain fragments with a size range of approximately 200–600 bp. Library preparation used commercially available kits targeted for Illumina libraries (Kapa Hyper Prep Kit and NEBNext Ultra II FS), with dual-indexing adapter-primers adopted to allow for *in silico* de-multiplexing of each sample. Stub and adaption ligation and PCR were followed by a purification step using SPRI magnetic beads, and the DNA of the resulting libraries was again quantified using a Qubit fluorometer. Samples were pooled at equimolar concentrations in groups of 8–12 libraries for enrichment, with 500 ng of DNA input at each enrichment reaction. UCE enrichment was performed using custom probe libraries for Hymenoptera UCE loci. For the vast majority of samples, we used a probe set targeting 2,590 loci (Hymenoptera v2; Branstetter et al., 2017), but some samples were also enriched using a previous probe set (Hymenoptera v1; Faircloth et al., 2015) that targets 1,510 loci, most of them compatible with the latter probe set (see Supplementary File S2 for details). Enrichment protocols followed the standard MYBaits kit procedure (Blumenstiel et al., 2010) except using a lower concentration for the biotinylated RNA probes. Incubation of 24 hours at 65ºC was followed by a series of cleanups with Streptavidin beads (Thermo Fisher Scientific Inc., Waltham, MA) and a final PCR step using KAPA Hifi HotStart ReadyMix (Roche). Post-enrichment DNA pools were quantified and combined at equimolar ratios. Fragment size distribution and final molarity were checked prior to sequencing with a 4200 TapeStation system (Agilent Technologies, Santa Clara, CA) using a High Sensitivity D1000 ScreenTape Assay. Size-selected pools were sequenced at 4 nM as single lanes on Illumina MiSeq, HiSeq 2500 or HiSeq 4000 platforms. Raw sequence reads for all samples are available from the NCBI Sequence Read Archive under accession no. [Data submission number: SUB10389972. Data will be released after acceptance].

2.3 *UCE data processing*
All informatic processing and analyses were conducted using the Smithsonian's High-Performance Computing cluster (Smithsonian Institution, 2020). Sequencing reads were filtered and trimmed using Illumiprocessor (Faircloth, 2013; Bolger et al., 2014) and assembled using either Trinity v. r2013-02-25 (Grabherr et al., 2011) or SPAdes (Bankevich et al., 2012). The resulting contigs were then processed following the Phyluce v1.5 pipeline (Faircloth, 2016). First, contigs were queried against a FASTA file of all enrichment baits, creating a relational database with the location of the UCE loci. Samples that recovered less than 100 UCE loci were discarded from the pipeline and not used in downstream analyses. Individual loci were then extracted to separate FASTA files, and each locus was aligned using MAFFT v. 7.130b (Katoh et al., 2002) and trimmed with GBLOCKS v. 0.91b (Castresana, 2000; Talavera and Castresana, 2007) with reduced stringency settings (0.5, 0.5, 12, and 7 for b1–b4 settings, respectively). Alignments were filtered with different settings to produce two matrices with different levels of

completeness: one including only loci available for at least 50% of the taxa and one with loci available for at least 25% of the taxa.

2.4 *Phylogenetic reconstruction*

We used the SWSC-EN algorithm (Tagliacollo and Lanfear, 2018) to define partitions within each UCE locus that account for rate heterogeneity and patterns of molecular evolution. The resulting concatenated alignments were then partitioned by schemes defined by PartitionFinder2 (Lanfear et al., 2016). Maximum-likelihood (ML) analyses were run with IQTREE v1.6.12 (Nguyen et al., 2015), with 10,000 rounds of ultra-fast bootstrapping (Hoang et al., 2018) to assess clade support and using ModelFinder (Kalyaanamoorthy et al., 2017) to choose the best model for each partition via the option -MFP. Analyses were run with the safe numerical mode (option -safe) to avoid numerical underflow that can result from large datasets.

2.5 *Ancestral state reconstruction*

Ancestral state reconstruction methods were used to investigate the evolutionary history of two key biological traits in Braconidae: ectoparasitoidism (0) *versus* endoparasitoidism (1) and idiobiosis (0) *versus* koinobiosis (1). Character coding was performed collectively by most authors (RRK, JMJM, AZR, JFT, BJS) based on a comprehensive review of original and compiled literature, notably Wharton et al. (1997) and Yu et al. (2016) (Supplementary Table S1). For the *Masona* and *Trachypetus*, biological traits were inferred following the reasoning and morphological evidence provided by Quicke et al. (2020a) and Belshaw et al. (2003), but coding either as unknown (missing data) had negligible impact in the results (unpublished data). Natural history data are scarce for most parasitoid groups, and host records are missing for many braconid species, but in many cases such records are available for closely related species in Yu et al. (2016), the most complete summary of host use for the family. The examination of the above resource shows that host use is almost always conserved within genera, which means that almost no genera have records of both ectoparasitoids and endoparasitoids or koinobionts and idiobionts. For the purposes of our analyses, biological traits were extrapolated from congeneric species when known for at least one member of the genus and with no conflicting evidence. We did not include *A. penyai* in the ancestral reconstruction analyses since it represents a monotypic taxon, which makes extrapolating its biological traits difficult.

The association between koinobiosis/endoparasitoidism and idiobiosis/ectoparasitoidism is well known based on general observation (Hanson and Gauld, 2006); in order to explicitly test this correlation while accounting for phylogenetic history, we used the 'fitPagel' function in the *phytools* package (Revell, 2012) in R (R Core Team 2021), which fits Pagel's (1994) model for correlated evolution of binary characters.

Reconstructions were performed first by optimizing the characters under parsimony onto the reference tree using the 'change' command in TNT (Goloboff et al., 2008) and obtaining a visual representation of state switches in Winclada (Nixon, 1999). In addition, a maximum-likelihood approach was used to estimate relative probabilities for each state using the 'ray.disc' function of the *corHMM* package (Beaulieu et al., 2013) in R (R Core Team 2021). To that end, phylogenetic trees were ultrametricized using the penalized likelihood criterion under a relaxed clock model as implemented in the function 'chronos' of the *ape* package (Paradis et al., 2004). Both equal (ER, "equal rates") and unequal (ARD, "all rates different") transition rate matrices were tested, and the difference in log likelihoods obtained under the two models were compared against a chi-square distribution to determine whether the gain in likelihood justified the adoption of the more parameterized model.

3.   Results
3.1 *UCE performance and alignment statistics*
We recovered a total of 1,829 UCE loci (mean length prior to trimming = 464.86 bp), with the braconids *Parachremylus* sp. and *Proterops* sp. having the lowest (101) and highest (1,809) number of loci. Matrices with higher levels of locus completeness resulted in a rapid drop in the number of loci (e.g., 0 loci recovered for 90% of the taxa) and in preliminary analyses indicated some clearly artifactual results. The 25% completeness matrix had 1,299 UCE loci with a mean length of 146.16 bp; the 50% matrix, included 780 UCE loci with a mean length of 134.26 (Supplementary Files S1, S3–S5).

3.2 *Phylogenetic relationships*
We recovered highly similar topologies from the two analyzed datasets (matrix completeness of 25% and 50%; Supplementary File S5A–B, Figs 3–8). Most relationships were strongly supported with bootstrap (BTP) values of 100 (average BTP was 98.85 for the 50% completeness tree and 99.01 for the 25% completeness tree). We only found differences in the BTP values of some nodes and in various generic-level relationships. The only topological change at the subfamily level was the placement of *Avga* + *Xenosternum orginis.* This group was recovered as sister to the alysioid subcomplex (Fig. 8) in the 50% matrix but sister to the subfamilies Rogadinae, Hormiinae and Rhysipolinae in the 25% matrix (Supplementary File S5A).

Hereafter, we only describe the relationships obtained in the phylogram derived from the 50% completeness matrix (Figs 4–8) and only mention BTP values < 100. Braconidae was recovered as monophyletic as were all but four subfamilies: Doryctinae, Brachistinae, Ichneutinae and Mesostoinae. Of particular interest was the consistent recovery of several subfamilies of previously contested placement. For instance, Apozyginae, represented by *A. penyai*, was recovered as sister to all remaining braconid subfamilies (Fig. 3). *Meteoridea hutsoni* Nixon, the single representative of Meteorideinae, was sister to all other non-cyclostomes followed by *Trachypetus clavatus* (Trachypetinae). *Masona* sp. was recovered as sister to the aphidioid complex, representing a relationship not previously recovered (Figs 3, 6). Both cyclostomes and non-cyclostomes were recovered as monophyletic and are discussed in detail below along with the aphidioid complex.

3.3 *Non-cyclostomes*
The subfamily Meteorideinae, represented by *Meteoridea hutsoni*, was recovered as sister to the remaining non-cyclostomes. *Trachypetus clavatus* (Trachypetidae *sensu* Quicke et al., 2020b) was sister to all non-cyclostomes other than *M. hutsoni*. The helconoid complex *sensu* Sharanowski et al. (2011) was monophyletic (Fig. 4). Within the helconoid complex, Acampsohelconinae was sister to the remaining helconoid complex *sensu* Sharanowski et al. (2011). Within Brachistinae (excluding *Dyscoletes canadensis* Mason), *Vadumasonium* sp. (Diospilini) was sister to the remaining Brachistinae, recovered as Blacini (Diospilini + Brachistini); thus, Diospilini was paraphyletic. Helconinae was sister to the macrocentroid subcomplex *sensu* Sharanowski et al. (2011), recovered as the (Microtypinae + Homolobinae) Orgilinae + (Charmontinae + Macrocentrinae) clade (note that Xiphozeliinae and Amicrocentrinae were not represented in the present study).

The euphoroid complex *sensu* Sharanowski et al. (2011) (i.e. Cenocoeliinae + Euphorinae) was monophyletic (Fig. 4). Within Euphorinae (*sensu* Stigenberg et al., 2015),

Centistini (*Centistes* sp.) and Meteorini (*Zele* sp., *Meteorus*) were recovered as sister tribes but with low support (BTP = 77). The *Elasmosona* sp. (*Syntretus* sp.+ *Myiocephalus* sp.) clade, representing the tribes Neoneurini, Syntretini and Myiocephalini, respectively, was recovered as sister to Centistini + Meteorini clade. Cosmophorini, represented by *Cosmophorus* sp., was sister to the clade consisting of all the aforementioned euphorine tribes. Pygostolini (*Pygostolus falcatus*) and Perilitini (*Perilitus rutilus*, *Microctonus*) were recovered as sister tribes, whereas Euphorini (*Peristenus* and *Leiophron sensu* Zhang et al., 2018) was sister to Ecnomiini + Helorimorphini (i.e. *Ecnomius* sp. [*Aridelius* sp. + *Wesmaelia petiolata* Wollaston]; BTP = 98).

Ichneutinae was non-monophyletic and divided into two separate clades. One clade consisted of *Ichneutes*, *Oligoneurus* and *Paroligoneurus* and was sister to the sigalphioid complex (i.e. Sigalphinae and Agathidinae, *sensu* Sharanowski et al., 2011) (Fig. 4). The second clade of ichneutines contained *Hebichneutes*, *Masonbeckia* and *Proterops* and was sister to the remaining represented microgastroid subfamilies (Microgastrinae, Cardiochilinae, Miracinae, Khoikhoiinae, Mendesellinae, Cheloninae) (Fig. 5). Mendesellinae, represented by *Epsilogaster bicolor* Whitfield and Mason, was sister to the Khoikhoiinae + Microgastrinae + Miracinae + Cardiochilinae clade; Khoikhoiinae, represented by *Sania browni* Sharkey, was sister to all other taxa in that clade. Within Cheloninae, *Phanerotomella longipes* Szépligeti was sister to Adeliini (*Paradelius* and *Adelius*), rendering Phanerotomini non-monophyletic (Fig. 5). Within Cardiochilinae, *Toxoneuron* was sister to *Retusigaster noguerai* Mercado. In Microgastrinae, the New Zealand genus *Kiwigaster* was sister to the remaining microgastrine taxa, which formed a large clade with intermingled members of the *Microplitis, Cotesia* and *Parapanteles* genus groups, together with five unplaced genera *sensu* Fernández-Triana et al. (2020)—*Miropotes*, *Prasmodon*, *Xanthomicrogaster*, *Neoclarkinella* and *Fornicia*.

3.4 *Aphidioid complex and cyclostomes sensu stricto*
Members of the aphidioid complex (*sensu* Sharanowski et al., 2011), Aphidiinae, Mesostoinae and Maxfischeriinae, formed a clade with *Masona* sp. as its sister-group (Fig. 6). Mesostoinae was recovered as non-monophyletic due to the inclusion of *Maxfischeria tricolor* Papp as sister to *Austrohormius* sp. (Fig. 6) and the placement of *Avga* + *Xenosternum* as sister to the alysioid subcomplex (Fig. 8). The clade of mesostoines including *M. tricolor* was recovered as sister to Aphidiinae. Within Aphidiinae, the only included member of Ephedrini, a species of *Ephedrus*, was sister to the representatives of the remaining aphiidine tribes (Praiini + Aphidiini), although Praiini was represented only by *Praon*. The aphidioid complex + *Masona* sp. were sister to the cyclostomes *s.s.*

Rhyssalinae was sister to the remaining cyclostomes *s.s.*, being composed of two main clades with *Histeromerus* as sister to both (Fig. 6). One of those clades had *Pseudobathystomus* (*Pseudobathystomus*) *vernalis* Belokobylskij sister to *Lysitermoides* + *Oncophanes*; the other clade had species of *Dolopsidea* as sister to *Acrisis* sp. + *Proacrisis* sp. Doryctinae was polyphyletic and recovered in two main clades. The more basal clade was mainly composed of Neotropical genera ("South American" major clade *sensu* Zaldívar-Riverón et al., 2008b), and a more derived and less supported clade (BTP = 79) mostly consisted of Old World genera ("Holarctic-African-Madagascan" major clade *sensu* Zaldívar-Riverón et al., 2008b) (Figs 6, 8). The latter also contained Pambolinae deeply nested within the clade and sister to *Spathius*, although with low support (BTP = 77 (Fig. 8).

Rhysipolinae (with the inclusion of *Allobracon* and *Parachremylus sensu* Jasso-Martínez et al. 2021) was recovered as sister to Hormiinae + Rogadinae (Fig. 7). Hormiinae (*sensu* Jasso-Martínez et al., 2021) had *Aulosaphobracon capitatus* Belokobylskij and Long, of the tribe

Aulosaphobraconini, as sister to the remaining hormiines followed in a nested configuration by the representatives of Cedriini, Hormiini (including *Pentatermus striatus* Szépligeti of Pentatermini) and Lysitermini + Tetratermini, in that order (Fig. 7), although Hormiini was paraphyletic. Within Rogadinae, a clade with the included members of Rogadini was sister to the remaining rogadine tribes. The monotypic Telengaiinae, represented by *Telengaia ventralis* Tobias, was sister to Gnamptodontinae and they in turn were sister to Braconinae, albeit with lower support (BTP = 72) (Fig. 8). The latter subfamily had a species of *Tropobracon* as sister to the remaining genera, and *Digonogastra* was non-monophyletic.

The clade (Braconinae (Telengaiinae + Gnamptodontinae)) was sister to the clade (Exothecinae (Opiinae + Alysiinae)), and thus, the alysioid subcomplex *sensu* Sharanowski et al. (2011) was not recovered as monophyletic (Fig. 8). Two main clades were recovered within Alysiinae—one with most members of the tribe Alysiini, and the other clade containing species of Dacnusini along with four taxa placed historically in Alysiini (Fig. 8). One species of Alysiini in *Glyphogaster* was recovered as sister to Dacnusini; the other three species of Alysiini formed the clade *Oenonogastra* (*Dapsilarthra* sp. + *Pseudopezomachus masii* Nixon) that was sister to all other dacnusines + *Glyphogaster* sp. Within Opiinae, members of the tribes Opiini and Biosterini were not sister taxa, *Diachasma muliebris* (Muesebeck) was sister to *Diachasmimorpha* (*Diachasmimorpha*) *longicaudata* (Ashmead) within the Opiini clade, and *Biosteres* (*Biosteres*) *spinaciae* (Thomson) was sister to the remaining opiines (Fig. 8). Within the Exothecinae, the genera *Colastes* and *Xenarcha* were non-monophyletic.

3.5 *Ancestral states reconstructions*

Explicit testing for correlation between the koinobiosis/endoparasitoidism and idiobiosis/ectoparasitoidism characters showed that a model of dependent trait evolution was a significantly better fit (AIC=90.86) than a model of independent evolution (AIC=119.90) (P<0.0001; Supplementary Fig. S6). The only two cases in which both traits are decoupled in Braconidae occurs in Rhysipolinae and Aspidobraconina (Braconinae), which are koinobiont ectoparasitoids and idiobiont endoparasitoids, respectively.

The ARD model was significantly better for endoparasitoidism *vs*. ectoparasitoidism (P=0.0099) with an estimated rate of change from idiobiont to koinobiont about 17 times higher than the opposite. For idiobiosis *vs.* koinobiosis the difference in rate was non-significant (P= 0.0654), hence the ER model was marginally better. Both ER and ARD models suggest that the ancestor of all braconids except *A. penyai* was a koinobiont endoparasitoid (Fig. 9; Supplementary Fig. S6). Inferred proportional likelihoods (PL) for koinobiosis were 0.877 and for endoparasitoidism 0.961, using the best-fit model for each trait. The inferred biology at the most ancestral node of the non-cyclostome clade was also koinobiont (PL=0.961) endoparasitoid (PL=0.994). Cyclostomes *s.s.* + aphidioid complex + Masoninae were also inferred as most likely to have been ancestrally koinobionts but with much lower proportional likelihood (PL=0.911 and 0.759 for endoparasitoidism and koinobiosis, respectively). Within this clade, many transitions in biological traits were inferred both by the proportional likelihoods observed at the nodes and by parsimony optimization. An ACCTRAN optimization (*sensu* Farris, 1970) is most consistent with the results of the ML analyses and suggests one transition from endo- to ectoparasitoidism in the cyclostomes *s.s*. Within this broad clade, three reversals back to endoparasitoidism were identified: one in *Katytermus palmicola* van Achterberg (Hormiinae), one at the node leading to Rogadinae and one in the Alysiinae+Opiinae clade. For idiobiosis *vs.* koinobiosis, inferred transitions were identical except for one additional switch from idio- to koinobiosis in Rhysipolinae. Most changes in biological traits were largely unambiguous across

the braconid tree, with over 90% of the internal nodes showing over 0.99 proportional likelihood towards one state or another.

4. Discussion

4.1 *Family-level classification in Ichneumonoidea*

The superfamily Ichneumonoidea currently includes the extant families Braconidae and Ichneumonidae, as well as the extinct families Eoichneumonidae (Jell and Duncan, 1986) and Praeichneumonidae (Rasnitsyn, 1983). Species of the ichneumonid subfamily Tanychorinae, known only from fossils, clearly belong in Ichneumonoidea. While tanychorines have been placed within Ichneumonidae (Quicke, 2015; Yu et al., 2016), their phylogenetic affinities with other ichneumonoids remain uncertain (Spasojevic et al., 2021). More recently, Trachypetidae, which contains extant species, has been treated as a family of Ichneumonoidea (Quicke et al., 2020b). The placement of Trachypetidae has historically been uncertain. One of its three recognized genera, *Megalohelcon*, has been included in Helconinae (Turner, 1918). Decades later, its three genera were split into two separate subfamilies, one containing *Megalohelcon* and *Cercobarcon* (Cercobarconinae; Tobias, 1979) and the other containing *Trachypetus* (Trachypetinae) (Schulz, 1911; Tobias, 1979). The monophyly of the aforementioned three genera was first proposed on the basis of one morphological trait—the presence of a glandular structure at the base of the mandible (Austin et al., 1993). More recently, a phylogenetic analysis of molecular and morphological data including members of the three aforementioned genera (Quicke et al., 2020b) recovered the group as robustly monophyletic and sister to all other braconids, although cyclostomes and non-cyclostomes were not recovered as sister taxa in that study, unlike almost all previous studies. Based on multiple morphological character states found in Trachypetinae that are atypical for Braconidae, as well as a number of molecular diagnostic features such as specific indels in the 18S and 16S rRNA loci, those authors decided to raise Trachypetinae to family level.

Our analyses consistently recovered the monophyly of Braconidae with the inclusion of *T. clavatus* as sister to all non-cyclostome subfamilies except Meteorideinae and not as a separate family as proposed recently (Quicke et al., 2020b). The placement of *T. clavatus* within Braconidae was also obtained in a phylogenetic study based on mitochondrial genome sequence data but in that case as sister to the euphoroid complex (Jasso-Martínez et al., submitted). Trachypetines possess a well-developed hind wing vein 2-CU and a distinctly small open fore wing costal cell, both present in some non-cyclostome lineages such as Agathidinae, Sigalphinae, Acampsohelconinae and Meteorideinae (Sharkey and Wahl, 1992; Quicke et al., 2020b). Given our results based on nuclear genome-scale and mitogenomic data (Jasso-Martínez et al., submitted), as well as the above morphological information, we restore Trachypetinae **stat. rev**. as a non-cyclostome braconid subfamily.

The monotypic Apozyginae, with its single species *A. penyai*, was originally described as a separate family (Apozygidae) within Ichneumonoidea (Mason, 1978). Subsequent studies based on morphological characters placed this taxon as a cyclostome subfamily within Braconidae (Quicke and van Achterberg, 1990; Sharkey and Wahl, 1992; Quicke et al., 1999a). Our study represents the first phylogenetic analysis based on nuclear DNA sequence data that includes *A. penyai*. All our analyses consistently place this species as sister to all extant braconid subfamilies, and the same relationship was found with mitochondrial genome sequence data (Jasso-Martínez et al., submitted). *Apozyx penyai* shares with Ichneumonidae the presence of fore vein 2m-cu, which is absent in all braconids with the rare exception of some rhyssalines and doryctines (van Achterberg, 1993; Sharkey, 1993; Quicke et al., 2020c). Nevertheless, *Apozyx*

shares morphological features with Braconidae, including fusion of second and third metasomal terga, hind wing vein 1r-m basal to the separation of veins R1 and Rs, and the presence of a hypoclypeal depression that characterizes the members of most cyclostome *s.s.* subfamilies (Sharkey and Wahl, 1992). We thus confirm for the first time based on molecular data a clade consisting of *A. penyai* + Braconidae and consider *A. penyai* a braconid in the monotypic subfamily Apozyginae.

4.2 *Relationships and taxonomic inferences within Braconidae*
Based on our estimate of phylogeny, previous classifications and the diagnostic morphological features of the included taxa, we propose a total of 41 subfamilies within Braconidae, of which 25 are included within the non-cyclostome group, three within the aphidioid complex with Masoninae as its sister taxon, and 11 within the cyclostomes *s.s.* We consider the cyclostomes *s.l.* as a lineage consisting of the cyclostomes *s.s.* + aphidioid complex + Masoninae, and we refer to the cyclostome *s.l.* + non-cyclostome lineage as the braconoid complex, with Apozyginae as its sister subfamily (Table 1). Below we discuss the most relevant relationships obtained and the main taxonomic implications derived from this study.

4.3 *Non-cyclostome braconids*
We recovered the four monophyletic non-cyclostome complexes mentioned by Sharanowski et al. (2011), although the relationships among them were different than those recovered in the latter work and also in Jasso-Martínez et al. (submitted) (Fig. 10). Meteorideinae was previously found closely related to the sigalphioid complex based on both morphological and molecular data (e.g., Quicke and van Achterberg, 1990; Belshaw et al., 2003; Belshaw and Quicke, 2002) and also as sister to the sigalphoid + microgastroid complexes but with low support (Sharanowski et al., 2011). Members of Meteorideinae have the CUb vein present in the hind wing as in Agathidinae and some sigalphines (Sharkey, 1997; Sharkey et al., 2021), although this trait is also present in *A. penyai* (Apozyginae). Therefore, this trait is likely a symplesiomorphy, as indicated by the position of Meteorideinae in our tree.

The helconoid complex was recovered here as sister to the remaining non-cyclostomes. *Dyscoletes canadensis* (Brachistinae) was sister to the rest of the helconoid complex, and Acampsohelconinae was sister to the clade containing all the helconoid subfamilies as recovered in Sharanowski et al. (2011). The three genera that comprise Acampsohelconinae have been recovered both as monophyletic (Quicke et al., 2002) and non-monophyletic (Quicke et al., 2008). We did not include species of *Canalicephalus*, but we consistently recovered *Urosigalphus* and *Afrocampsis* as monophyletic. All the helconoid complex subfamilies were recovered as monophyletic except Brachistinae due to the position of *D. canadensis* as sister to all helconoid subfamilies. Species of *Dyscoletes* have been placed in Diospilini of Helconinae (Mason, 1976) and in Blacinae within the tribe Dyscoletini (van Achterberg, 1988) but were further moved to Brachistinae with other Blacines (Sharanowski et al., 2011). Here our results support a basal placement of *Dyscoletes* relative to other members of the helconoid complex. Given that it is not recovered near any other Brachistinae, it may warrant its own subfamily status. This is further supported by its unique biology as parasitoids of larval Mecoptera (Mason, 1976). However, because we did not include either the type species, *Dyscoletes lancifer* (Haliday), or species of other putative closely related taxa (e.g., *Hellenius*, also placed in Dyscoletini), we consider the genus *Dyscoletes* as *incertae sedis* within Braconidae pending further studies to confirm its taxonomic status.

A close relationship between Cenocoeliinae and Euphorinae has been obtained in previous phylogenetic studies using molecular data (Belshaw and Quicke, 2002; Sharanowski et al., 2011). The limits of Euphorinae with respect to other closely related groups (i.e. Neoneurinae, Ecnomiinae and Meteorinae) have been unclear (Sharanowski et al., 2011). Stigenberg et al. (2015) recently recovered the latter three groups within Euphorinae and proposed to treat them as its tribes, and our results support that classification. Thus, we consider the euphoroid complex to contain only two subfamilies, Cenocoeliinae and Euphorinae, with the latter including the tribes Neoneurini, Ecnomiini and Meteorini. Meteorini was not sister to all other euphorines as was recovered in Stigenberg et al. (2015). Rather, euphorines consisted of two major lineages—one with Cosmophorini, Neoneurini, Syntretini Myiocephalini, Centistini and Meteorini, and the other with Pygostolini, Perilitini, Ecnomiini, Helorimorphini and Euphorini. Whether meteorines are a derived group within Euphorinae could change the interpretation regarding the evolution of host use in Euphorinae, suggesting a potential reversion in the Meteorini clade from attacking adults to attack larvae of Coleoptera. The sister relationship between Agathidinae and Sigalphinae has also been consistently recovered in several studies (Belshaw et al., 1998; Belshaw and Quicke, 2002; Dowton et al., 2002; Pitz et al., 2007; Sharanowski et al., 2011; Jasso Martínez et al., submitted). Both subfamilies (and Ichneutinae *s. s.*, see below) comprise the sigalphoid complex (Belshaw and Quicke, 2002; Sharanowski et al., 2011), which has been recovered as sister to either the euphoroid complex (Sharanowski et al., 2011; Jasso-Martínez et al., submitted) or the microgastroid complex (Sharanowski et al., 2011) depending on analysis used.

The Ichneutinae *s. l.* has been proposed as closely related to either the sigalphoid complex (Sharkey and Wharton, 1994) or the microgastroid complex (e.g., Quicke and van Achterberg, 1990; Dowton et al., 2002; Shi et al., 2005; Pitz et al., 2007; Sharanowski et al., 2011). Similar to Quicke and van Achterberg (1990) and Jasso-Martínez et al. (submitted), in this work we recovered a non-monophyletic Ichneutinae, with *Ichneutes*, *Oligoneurus* and *Paroligoneurus* sister to the sigalphoid complex, whereas *Hebichneutes*, *Masonbeckia* and *Proterops* were sister to the microgastroid subfamilies.

Ichneutinae and Agathidinae share the presence of spines in the fore tibia, although in the latter the spines are not restricted to the apex; ichneutines have subpronopes as in Agathidinae and Sigalphinae, although these are absent in the ichneutine genera *Oligoneurus*, *Paroligoneurus* and *Lispixys*; both ichneutines and sigalphines share short ovipositors and Ichneutinae, Sigalphinae, Agathidinae and Cheloninae (the last belonging to the microgastroid complex) have a derived position of the last abscissa of Rs of the fore wing (Sharkey and Wharton, 1994). Given that we recovered *Ichneutes* (*Oligoneurus* + *Paroligoneurus*) as sister to Sigalphinae + Agathidinae, along with the morphological evidence described above, we propose to expand the sigalphoid complex to include Ichneutinae *s.s*. Five genera that were previously in Ichneutinae *s.l.* are now placed in a different subfamily (Sharkey et al., 2021, see further discussion below); thus, Ichneutinae *s.s.* currently consists of *Ichneutes*, *Lispixys*, *Oligoneurus*, *Paroligoneurus* and *Pseudichneutes*. On the other hand, *Hebichneutes*, *Masonbeckia* and *Proterops* were recovered as sister to the microgastroid complex. These genera were previously within Ichneutinae *s.l.* but were recognized recently as members of the subfamily Proteropinae (Chen and van Achterberg, 2019; Sharkey et al., 2021) given that previous phylogenetic analyses did not recover Ichneutinae *s.l.* as monophyletic (e.g., Sharanowski et al., 2011). Sharkey et al. (2021) provided a diagnosis for Proteropinae, with the subfamily consisting of *Hebichneutes*, *Helconichia*, *Masonbeckia*, *Michener*, *Muesonia* and *Proterops.* This is the first phylogenetic study that recovered, with strong support, Proteropinae and Ichneutinae as separate lineages. Therefore, we

support the recognition of Proteropinae within the non-cyclostomes as sister to the microgastroid complex.

The relationships within the microgastroid complex are mostly in agreement with previous works. We do not consider Proteropinae as part of the microgastroid complex, as members of that subfamily utilize sawfly larvae as hosts (van Achterberg, 1976; Sharkey et al., 2021). Rather, we regard Proteropinae as sister to the microgastroid complex, as members of the latter utilize, or in the case of Khoikhoiinae likely utilize (Sharkey et al., 2009), Lepidoptera larvae as hosts (Quicke and van Achterberg, 1990; Whitfield, 1997; Murphy et al., 2008; Whitfield et al., 2018; Fernandez-Triana et al., 2020). The relationships between Mendesellinae and Khoikhoiinae with the rest of the complex have varied slightly among authors (Mason, 1983; Whitfield and Mason, 1994; Whitfield, 1997; Belshaw et al., 1998; Banks and Whitfield, 2006; Murphy et al., 2008; Sharanowski et al., 2011) but in all cases, including our present work, Cheloninae has been recovered as sister to all microgastroids and Microgastrinae as sister to Cardiochilinae + Miracinae.

4.4 *Cyclostomes sensu lato*

The aphidioid complex, which currently comprises the subfamilies Aphidiinae, Mesostoinae and Maxfischeriinae, has been consistently recovered as sister to the cyclostomes *s.s.* in the latest molecular phylogenetic studies (Zaldívar-Riverón et al., 2006, Wei et al., 2010, Sharanowski et al., 2011; Sharanowski et al., 2021; Jasso-Martínez et al., submitted). The composition of the aphidioid complex was again supported in our study but with *Maxfischeria* nested within Mesostoinae and *Masona* as sister to all aphidioids.

The composition of Mesostoinae is still unclear, with various genera being recently transferred to either this subfamily (e.g., *Metaspathius*, Quicke et al., 2019; *Austrohormius* and *Neptihormius,* Shimbori et al., 2017) or from Mesostoinae to other groups (e.g., *Parachremylus* and *Allobracon* to Rhysipolinae, Jasso-Martínez et al., 2021). However, the type genus of Mesostoinae, *Mesostoa*, has been recovered previously as part of the aphidioid complex in a clade with *Andesipolis*, *Aspilodemon* and *Hydrangeocola* (Zaldívar-Riverón et al., 2006) thus supporting our treatment of *Andesipolis, Hydrangeocola, Austrohormius* and *Neptihormius* as Mesostoinae. We also recovered the *Avga + Xenosternum* clade as sister to the alysioid subcomplex, whereas in Jasso-Martínez et al. (submitted) *Avga* was sister to a large clade comprising Rogadinae, Hormiinae, Rhysipolinae, the alysioid subcomplex and the Holarctic-African-Madagascan doryctines + Pambolinae. *Avga* and *Xenosternum* were proposed to comprise the tribe Avgini together with *Parachremylus, Pseudohormius* and *Parahormius* (Ranjith et al., 2017). The placement of these genera, however, has varied considerably, as they have been placed either within Exothecinae, Mesostoinae or Hormiinae (Nixon, 1940; Belokobylskij, 1993a; Belokobylskij, 1993b; Wharton, 1993b; Ranjith et al., 2017; Quicke et al., 2019; Quicke et al., 2020c). Our best phylogenetic estimate confirms the placement of *Parachremylus* within Rhysipolinae and *Pseudohormius* and *Parahormius* within Hormiinae. Moreover, we confirm that *Avga* and *Xenosternum* do not belong to Mesostoinae, although given their poorly supported relationships, we suggest maintaining them as *incertae sedis* within Braconidae pending further studies to definitively discern their phylogenetic affinities.

In our study, *Maxfischeria* was found nested within Mesostoinae and not as sister to Aphidiinae as found by Sharanowski et al. (2011). Our results are congruent with those recently obtained with mitogenome sequence data (Jasso-Martínez et al., submitted) suggesting that *Maxfischeria* actually belongs to the Mesostoinae. However, we recommend the continued

treatment of Maxfischeriinae as a subfamily within the aphidioid complex pending analyses that include *Mesostoa* and more extensive sampling of taxa historically placed in Mesostoinae.

We recovered *Masona* as sister to the aphidioid complex, indicating its clear placement in Braconidae. *Masona* was originally placed in its own subfamily within Braconidae (Masoninae) based on fusion of the second and third metasomal terga and reduced fore wing venation of males (van Achterberg, 1995), although Quicke et al. (2020a) interpreted a small separation of the second and third terga laterally in two species of *Masona* (cf. Quicke et al., 2020a: Fig. 1e—f). Belshaw and Quicke (2002) recovered this genus within Braconidae based on molecular data, although they could not confirm its phylogenetic affinity since the relationships obtained were sensitive to the phylogenetic method employed. More recently, Quicke et al. (2020a) transferred Masoninae within Ichneumonidae based on a phylogenetic analysis with Sanger sequence markers, as well as on the absence of fore wing vein RS+M and interpretation that the second and third terga are separated laterally in *M. popeye* and *M. similis*. However, more extensive examination of *Masona* species via scanning electron microscopy would help facilitate interpretation of the latter morphological feature. Metasomal terga 2 and 3 are fused in all braconids, although in aphidiines terga 2 and 3 are flexible at the groove between them (van Achterberg, 1997). Furthermore, the absence of RS+M occurs not only in multiple genera of Aphidiinae but is also observed in a broad phylogenetic spectrum of Braconidae (Wharton et al., 1997). Given the placement of *Masona* as sister to the aphidioid complex with the highest support and the uncertain morphological support for placing masonines within Ichneumonidae, we restore Masoninae **stat. rev**. as a subfamily of Braconidae. It is worth noting, however, that the reduction of anatomical features in masonines, due to allometry given their diminutive size, hinders the discovery and interpretation of morphological synapomorphies that support their phylogenetic placement and whether it represents a monophyletic group. Thus, like other subfamilies of Braconidae, morphological support for Masoninae may rely on the absence of features present in other braconids.

We recovered Rhyssalinae as sister to the remaining cyclostome subfamilies and confirm the inclusion of *Histeromerus* as a tribe within Rhyssalinae (Histeromerini) as proposed by Zaldívar-Riverón et al. (2006). The highly diverse, morphologically heterogeneous subfamily Doryctinae, on the other hand, was non-monophyletic, being divided into two clades that are similar in composition to the "South American" and the "Holarctic-African-Madagascan" major clades recovered in Zaldívar-Riverón et al. (2007; 2008b). This division of Doryctinae in two separate clades had not been strongly supported in any of these previous studies, but it emerges very clearly from our trees. Doryctinae also fell into two separate clades in Sharanowski et al. (2011), but the composition of those clades as "South American" and the "Holarctic-African-Madagascan" is uncertain due to limited taxon sampling. Among the morphological synapomorphies that have been proposed for Doryctinae are the presence of two secondary ducts in the venom apparatus, the presence of a series of pegs on the fore tibia, ovipositor structure and microsculpture of the egg canal (Quicke et al., 1992a; Quicke et al., 1992b; Belokobylskij et al., 2004). However, none of these features are shared by all members included in this group. Further assessment of the taxonomic status of Doryctinae is necessary and requires more extensive taxon sampling, particularly pantropical taxa.

The relationship of Pambolinae with the members of Doryctinae needs to be further assessed, as the former taxon was recovered deeply nested within the clade comprising Holarctic-African-Madagascan doryctine genera. Pambolinae is a small subfamily with species distributed on all continents, with some of them being reported as ectoparasitoids of coleopteran and lepidopteran larvae (Belokobylskij, 1986; Quicke, 2015). The main diagnostic

morphological feature for this group is the presence of a pair of lateral spines on the propodeum; however, this condition also occurs in doryctines of the Neotropical and Australasian genera *Notiopambolus* and *Equinodoryctes,* respectively (Belokobylskij et al., 2004).

Our results confirmed the placement of *Allobracon* within Rhysipolinae as in Jasso-Martínez et al. (2021; submitted). Both *Parachremylus* and *Allobracon* share various morphological features, including the first metasomal tergum with membranous postero-lateral parts (Ranjith et al., 2017), dorsope absent and a median carina of the petiole present (Wharton, 1993b), which support their close relationship within Rhysipolinae. Rhysipolines are the only known members of Braconidae that display the unusual combination of ectoparasitoid koinobiosis (Shaw, 1983; Shaw, 2017). The biology of *Allobracon* and *Parachremylus* is unknown; thus, additional studies are needed to confirm whether both genera are also ectoparasitoid koinobionts.

Hormiinae was for a long time a heterogeneous assemblage of taxa, although phylogenetic studies carried out in the last 15 years have transferred a number of its genera to other subfamilies (e.g., *Monitoriella*: Zaldívar-Riverón et al., 2006). The main diagnostic morphological feature used to characterize Hormiinae was their moderately to strongly desclerotized metasomal terga (Wharton, 1993b; van Achterberg, 1995). However, Wharton (1993b) suggested that the subfamily also could include genera with a carapacelike metasoma, such as those placed historically in Lysiterminae, given their similarity in various wing venation, leg and body sculpture features. This latter suggestion was confirmed by Jasso-Martínez et al. (2021) in a phylogenetic study based on UCE data, where they formally synonymized Lysiterminae with Hormiinae. This synonymy was supported in Quicke et al.'s (2021) phylogenetic study of Rogadinae and related subfamilies using a vast taxon sampling. Our results also confirm this concept of Hormiinae, although here with Aulosaphobraconini as sister to the remaining hormiines rather than Cedriini, as recovered by Jasso-Martínez et al. (2021; submitted). Both Aulosaphobraconini and Cedriini have strongly sclerotized metasomal terga, and whether any of them are sister to the remaining hormiines, they support an early appearance of the sclerotized metasoma within the Hormiinae lineage with subsequent transitions to desclerotization.

Rogadinae is a subfamily exclusively composed of koinobiont endoparasitoids of lepidopteran larvae, whose diagnostic synapomorphy is the mummification of the host within which the parasitoid larva pupates and then emerges as an adult (Quicke and Shaw, 2005). Previous concepts of Rogadinae had been generally broader, including genera currently placed within other subfamilies such as Rhysipolinae (Shaw and Huddleston, 1991) and Hormiinae (van Achterberg, 1991). This subfamily has been recently confirmed as monophyletic with the inclusion of Betylobraconini using different nuclear and mitogenomic data (Jasso-Martínez et al., 2021; Quicke et al., 2021; Jasso-Martínez et al., submitted). Our results confirm this composition, with Rogadini being sister to the remaining tribes, and also support the close relationship between Rogadinae and Hormiinae, with the latter also attacking concealed lepidopterans, although its species mostly are ectoparasitoid idiobionts. This reinforces Jasso-Martínez et al.'s (2021) hypothesis that the ancestral host preference of Rogadinae was attacking weakly concealed lepidopterans with subsequent transitions to concealed and exposed host larvae. Further discovery of host preferences for members of Betylobraconini and some of Hormiinae will help to confirm this hypothesis.

The alysioid subcomplex (Sharanowski et al., 2011) was proposed to include the subfamilies Alysiinae, Opiinae, Exothecinae, Gnamptodontinae and Telengaiinae, with Braconinae as its sister group. The close relationship of braconines to the members of the

alysioid subcomplex has been recovered in other studies (Belshaw et al., 1998; Dowton et al., 2002; Zaldívar-Riverón et al., 2006); based on that Quicke (2015) proposed to expand this complex to include braconines. Here we did not recover Braconinae as sister to the remaining alysioid subfamilies but as sister to Telengaiinae + Gnamptodontinae. In Jasso-Martínez et al. (submitted.), braconines were also recovered as part of the alysioid subcomplex but as sister to Exothecinae (Opiinae + Alysiinae). Braconines share various morphological features with some members of the alysioid subfamilies, including a distinct pair of diagonal grooves near the anterior corners of the third metasomal tergum (shared with telengaiines and gnamptodontines) and a complete loss of both occipital and epicnemial carinae (as in most alysiines, opiines, telengaiines and gnamptodontines) (Wharton et al., 2006; Quicke, 2015). We therefore confirm the expansion of this subcomplex to include braconines, and we update its name to the "braconoid subcomplex" (Braconinae Nees, 1811; Alysiinae Leach, 1815). The monotypic genus *Vaepellis* is currently placed within Braconinae (Tobias, 1988), although it was originally described in its own subfamily, Vaepellinae (Quicke, 1987). The known species, *Vaepellis varica* Quicke, has not been assessed in a phylogenetic context, in part due to its rarity in insect collections. Thus, further studies are needed to elucidate the placement of this taxon within Braconidae.

A close relationship between the monotypic Telengaiinae and the Gnamptodontinae has been recovered by our analyses, as well as in other studies using both Sanger markers and mitogenome sequence data (Zaldívar-Riverón et al., 2006; Jasso-Martínez et al., submitted). Species of *Telengaia* do not possess the transverse rectangular area at the base of the second metasomal tergum as in gnamptodontines (Quicke, 2015). However, the former taxon shares with the gnamptodontines a distinct pair of diagonal grooves near the anterior corners of the third metasomal tergum (Quicke, 2015), as well as similarities in the venom apparatus (Zaldívar-Riverón et al., 2004). Considering the close relationship between both taxa recovered in previous studies (i.e. Zaldívar-Riverón et al., 2006), Chen and van Achterberg (2019) treated Telengaiinae as a tribe within Gnamptodontinae. Given our results and considering morphological similarities between *Telengaia* and gnamptodontines, we agree in treating them as a single subfamily, although following the principle of priority, gnamptodontines should treated as a tribe (Gnamptodontini **stat. rev.**) within Telengaiinae (Telengaiinae: Tobias, 1962; Gnamptodontini: Fischer, 1970, the latter elevated to subfamily in van Achterberg, 1983). The tribe Exodontiellini, comprising the exodont genus *Exodontiella*, was transferred from Opiinae to the Gnamptodontinae based on both molecular and morphological data (Wharton et al., 2006). In this study we did not include members of *Exodontiella*, and thus, we have decided to maintain it as the tribe Exodontiellini following Wharton et al.'s (2006) study. The subfamily Telegaiinae, therefore, is regarded as consisting of the tribes Telengaiini, Exodontiellini and Gnamptodontini pending further assessment to discern the placement of *Exodontiella* within Braconidae.

Alysiinae and Opiinae were recovered as sister taxa, as has been the case in previous analyses based on morphological characters and Sanger sequencing (Quicke and van Achterberg, 1990; Gimeno et al., 1997; Belshaw et al., 1998; Dowton et al., 1998; Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011). However, within Alysiinae, the tribes Alysiini and Dacnusini were not monophyletic. One species of Alysiini was recovered as sister to Dacnusini, and a clade of three other species of Alysiini was sister to that clade. Dacnusines are parasitoids of plant-feeding flies, almost exclusively Agromyzidae, Chloropidae, and Ephydridae, with most species parasitic on agromyzids. Nearly all species of Alysiini are parasitoids of saprophagous flies (Shaw and Huddleston, 1991; Wharton, 1997; Yu et al., 2016). The four species of Alysiini that formed a clade with the dacnusines in this study are all parasitoids of leaf-mining Agromyzidae

(Wharton, 1997; Yu et al., 2016). These four species belong to *Glyphogaster*, *Dapsilarthra*, *Pseudopezomachus* and *Oenonogastra*; Quicke et al. (1997) found that like most or perhaps all dacnusines, species of those four genera have an unsculptured anterior bulbous swelling on the venom reservoir. Thus, the monophyly of Dacnusini as defined currently, based on the absence of forewing vein r-m, is questionable, but host use and morphology of the venom apparatus might give biological and morphological character support for a reconfigured Dacnusini that includes parasitoids of plant-feeding flies currently in Alysiini. More extensive taxon sampling, as well as more complete data on host use and morphology of the venom apparatus, are necessary for determining the utility of those features for establishing monophyletic tribes within Alysiinae.

The subfamily Opiinae is a group for which limited molecular-based phylogenetic studies have been carried out (i.e. Gimeno et al., 1997; Li et al., 2013). We did not recover the tribes Opiini and Biosterini as monophyletic similar to Li et al. (2013; when using both nuclear and mitochondrial data and Bayesian phylogenetic reconstruction), with *Biosteres* being sister to all other opiines, as well as *Diachasma* as sister to *Diachasmimorpha* and nested within the clade containing all Opiini species included in this study. *Biosteres* (Biosterini) has been characterized by the presence of a short second submarginal cell of the fore wing (Fischer, 1972); however, this is not exclusive of this genus but also present in other opiines, including *Fopius* (Opiini). Given the extraordinary species richness of this subfamily, further phylogenetic studies are needed to delimit tribes within Opiinae.

4.5 *Transitions in the mode of parasitoidism*
The koinobiont-idiobiont distinction has long been thought to be one of the most important in the evolution of parasitoid wasps. Whether or not wasps interrupt the development of the host during/after oviposition is thought to be linked to a number of other important biological distinctions, from the degree of host specificity to the size of the eggs laid (Gauld, 2006; Quicke, 2015). Although much sensible reasoning has been used to draw conclusions regarding the biological and evolutionary implications of this single trait, few studies have explicitly tested for correlation between idiobiosis/koinobiosis and other biological traits, particularly using a phylogenetic framework Mayhew and Blackburn (1999) attempted such an investigation, but in that study taxonomy was used as a proxy for phylogeny across parasitoid wasps as a whole. In that sense, our study helps formally establish the link between koinobiosis-endoparasitoidism and idiobiosis-ectoparasitoidism in Braconidae.

As expected in traits subject to interdependent evolution, we recovered an almost identical character history for both idiobiosis-ectoparasitoidism and koinobiosis-endoparasitoidism. In fact, most of the differences in proportional likelihoods observed between the two traits arise from the difference in the evolutionary model adopted for each trait (ER for ectoparasitoidism/endoparasitoidism, ARD for idiobiosis/koinobiosis). Rhysipolines are braconids in which the association between the respective states at each trait is broken and whose species for which biological data are known are koinobiont ectoparasitoids. For instance, species of *Rhysipolis* attack leaf-mining caterpillars and lay their eggs onto the host's intersegmental membranes (Shaw, 1983). While the host continues feeding, it ceases molting (thus preventing the dislodging of the parasitoid from its external surface) and usually enters a prepupal state prematurely. This interesting interaction with host development led Shaw (1983) to hypothesize that the biology of *Rhysipolis* represented an intermediate state towards "true" koinobiont endoparasitoidism, but our topology suggests it is better understood as an independent offshoot from a clade with ancestrally idiobiont ectoparasitoid biology. Species of Aspidobraconina, a

subtribe of Braconini (van Achterberg, 1984b), are another example in which both traits are decoupled, being idiobiont endoparasitoids (Quicke, 1989; Quicke, 1997; Quicke, 2015). We did not include any member of this group in our analyses; however, their biology could potentially represent another independent origin of endoparasitism as recovered by Zaldívar-Riverón et al. (2006) in a clade composed of idiobiont ectoparasitoids.

Although there have been many studies investigating the phylogeny of braconid wasps at many levels, there have been relatively few efforts to reconstruct ancestral states for biological characters across the whole family. Former evolutionary studies have sought to understand a diverse array of biological traits but focused on more limited subgroups; for example, Belshaw and Quicke (2002) analyzed the transition between the use of exposed versus concealed hosts among braconid koinobiont lineages; Zaldívar-Riverón et al. (2008a) reconstructed the evolution of lepidopteran host ranges and mummy characteristics in Rogadinae; and Samacá-Saenz et al. (2022) investigated the evolution of gall-association in Doryctinae.

Meanwhile, Sharanowski et al. (2021) performed an ancestral state reconstruction for ecto- *versus* endoparasitoidism and idio- *versus* koinobiosis across the whole of Ichneumonoidea using a phylogeny based on anchored hybrid enrichment (Lemmon et al., 2012). Their results were variable according to the analytical framework and whether traits were reconstructed individually for Braconidae and Ichneumonidae or using both families together. Specifically, analyses including all Ichneumonoidea recovered the ancestor of all Braconidae as likely to be an idiobiont ectoparasitoid, whereas the analysis of the family on its own suggested a koinobiont endoparasitoid state. Our study with a much deeper taxon sampling within Braconidae strongly suggests that the ancestral states for the braconid complex are koinobiosis and endoparasitoidism. Note, however, that while our selected outgroup taxa represented all major lineages of Ichneumonidae, a more thorough sampling may be necessary to draw stronger conclusions regarding these biological traits for the braconoid complex. Also, unraveling biological information for *Apozyx* has the potential to change our interpretations, or to greatly improve confidence in the current results.

Regardless of the specific ancestral state, our tree topology implies that multiple transitions across states must have happened across the evolution of Braconidae. Our topology suggests that the most parsimonious character history include either one transition from koinobiosis to idiobiosis and four reversals back to koinobiosis (under ACCTRAN optimization) or two transitions from koinobiosis to idiobiosis and three reversals back to koinobiosis (under DELTRAN). It is noteworthy that these particular reconstructions are recovered in parsimony when state changes are defined *a priori* as symmetrical, and the inferred character histories could change under different cost regimes. However, the results from ACCTRAN are supported by the probabilities inferred by ML at each node where the state transitions were inferred to have occurred (Fig. 8).

One important caveat to these results is that there are no empirical data to suggest the comparative probability of changing from one state to another or *vice versa;* therefore, cost matrices used in any set of analyses can be seen as arbitrary. It has been suggested that transitions in host use would logically occur from a supposedly less specialized state–idiobiosis– to a more specialized strategy–koinobiosis (Gauld, 1988; Bennett et al., 2019). The reasoning is that koinobiosis, and especially the endoparasitoidism that seems to accompany it, requires deep changes to adult and larval morphology, venom properties and oviposition behavior; hence a reversion back to idiobiosis/ectoparasitoidism would be unlikely. However, one could argue that the repeated evolution of such specialized characters is also unlikely and that reversals back to a less specialized state are also plausible; for example, a recent phylogeny of Ichneumonidae, the

sister group to Braconidae, recovered more transitions from koinobiosis to idiobiosis than the opposite (Bennett et al., 2019). It is important to note, however, that while koinobiont endoparasitism has been considered historically a more specialized life history strategy than idiobiont ectoparasitism, koinobiosis/idiobiosis and endoparasitoidism/ectoparasitoidism involve the evolution of an array of associated traits that could be misinterpreted as more or less specialized relative to one another.

Recent research has shown that a number of apparently complex biological traits can undergo multiple transitions in parasitoid lineages. For instance, switching between larval and pupal hosts seems to be common in the evolution of Ichneumoninae (Santos et al., 2021), transitioning into the use of deeply concealed hosts has happened many times in Cryptinae (Santos and Perrard, 2018) and the use of endogenous viral elements to overcome the immune defenses of lepidopteran hosts has occurred multiple times in Ichneumonoidea (Sharanowski et al., 2021; Santos et al., 2022). We are clearly only beginning to understand the complex evolutionary pathways for host use in parasitoid wasps and building robust phylogenies with deep taxonomic sampling will be an essential step to investigate these complex interactions.

5. Acknowledgments

6. References

van Achterberg, C., 1976. A preliminary key to the subfamilies of the Braconidae (Hymenoptera). *Tijdschr. Ent.* 119, 33–78.

van Achterberg, C., 1983. Revisionary notes on the subfamily Gnaptodontinae, with description of eleven new species (Hymenoptera, Braconidae). *Tijdschr. Entomol.* 126, 25–57.

van Achterberg, C., 1984a. Essay on the phylogeny of Braconidae (Hymenoptera: Ichneumonoidea). *Ent. Tidskr.* 105, 41–58.

van Achterberg, C., 1984b. Revision of the genera of Braconini with first and second metasomal tergites immovable joined (Hymenoptera, Braconidae, Braconinae). *Tijdschr Entomol.* 127, 137–164.

van Achterberg, C., 1988. Revision of the subfamily Blacinae Foerster (Hymenoptera, Braconidae). *Zool. Verh.* 249, 1–324.

van Achterberg, C., 1991. Revision of the genera of the Afrotropical and W. Palaearctical Rogadinae Foerster (Hymenoptera: Braconidae). *Zool. Verh.* 273, 1–102.

van Achterberg, C., 1993. Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). *Zool. Verh.* 283, 1–189.

van Achterberg, C., 1995. Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. *Zool. Verh.* 298, 1–242.

van Achterberg, C., 1997. Subfamily Aphidiinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the Family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 119–32.

van Achterberg, C. and Quicke, D.L.J., 1992. Phylogeny of the subfamilies of the family Braconidae: a reassessment assessed. *Cladistics* 8, 237–264.

Austin, A.D. and Dangerfield P.C., 1998. Biology of the *Mesostoa kerri* Austin and Wharton (Insecta: Hymenoptera: Braconidae: Mesostoinae), an endemic Australian wasp that causes stem galls on *Banksia marginata* Cav. *Aust. J. Bot.* 46, 559–569.

Austin, A.D., Wharton, R.A. and Dangerfield, P.C., 1993. Revision of the endemic Australian subfamily Trachypetinae Schulz *s.l.* (including Cercobarconinae Tobias) (Hymenoptera: Braconidae). *Syst. Entomol.* 18, 97–119.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M. … and Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477.

Banks, J.C. and Whitfield, J.B., 2006. Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. *Mol. Phylogenet. Evol.* 41, 690–703.

Beaulieu, J.M., O'Meara, B.C. and Donoghue, M.J., 2013. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Syst Biol.* 62, 725–737.

Belokobylskij, S.A., 1986. A review of the Palaearctic species of the genera *Pambolus* Hal. and *Dimerus* Ruthe (Hymenoptera: Braconidae). *Tr. Zool. Inst.* 159, 18–37. [in Russian].

Belokobylskij, S.A., 1993a. On the classification and phylogeny of the braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera, Braconidae). Part I. On the classification. *Entomol. Obozr.* 71, 900–928.

Belokobylskij, S.A., 1993b. On the classification and phylogeny of the braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera: Braconidae). Part II. Phylogeny. *Entomol. Obozr.* 72, 891–914.

Belokobylskij, S.A. and Tobias, V.I., 1986. Doryctinae. In: Medvedev GS (Ed.) Opredelitel Nasekomych Evrospeiskoi Tsasti SSSR 3, Peredpontdatokrylye 4. Opr. Faune SSSR. 145: 1–501. pp. 21–72.

Belokobylskij, S.A. and Jouault, C., 2021. Two new striking braconid genera (Hymenoptera: Braconidae) from the mid-Cretaceous Burmese amber. *Proc. Geologists' Assoc.* 132, 426–437.

Belokobylskij, S.A., Zaldívar-Riverón, A. and Quicke, D.L.J., 2004. Phylogeny of the genera of the parasitic wasps subfamily Doryctinae (Hymenoptera: Braconidae) based on morphological evidence. *Zool. J. Linnean Soc.* 142, 369–404.

Belshaw, R. and Quicke, D.L.J., 2002. Robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. *Syst. Biol.* 51, 450–477.

Belshaw, R., Herniou, E., Gimeno, C., Fitton, M.G. and Quicke, D.L.J., 1998. Molecular phylogeny of the Ichneumonoidea (Hymenoptera) based on D2 expansion region of 28S rDNA. *Syst. Entomol.* 23, 109–123.

Belshaw, R., Dowton, M., Quicke, D.L.J. and Austin, A.D., 2000. Estimating ancestral geographical distributions: a Gondwanan origin for aphid parasitoids? *Proc. R. Soc. Lon. B* 267, 491–496.

Belshaw, R., Grafen, A. and Quicke, D.L.J., 2003. Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis. *Zool. J. Linn. Soc. 139*, 213–228.

Bennett, A.M.R., Cardinal, S., Gauld, I.D. and Wahl, D.B., 2019. Phylogeny of the subfamilies of Ichneumonidae (Hymenoptera). *J. Hym. Res*. 71, 1–156.

Blumenstiel, B., Cibulskis, K., Fisher, S., DeFelice, M., Barry, A. … and Gabriel, S., 2010. Targeted exon sequencing by in-solution hybrid selection. *Curr. Protoc. in Hum. Genet*. 66, 18.4.1–18.4.24.

Bolger, A.M., Lohse, M. and Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120.

Branstetter, M.G., Longino, J.T., Ward, P.S. and Faircloth, B.C., 2017. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods Ecol. Evol.* 8, 768–776.

Burke, G.R. and Strand, M.R., 2012. Deep sequencing identifies viral and wasp genes with potential roles in replication of *Microplitis demolitor* bracovirus. *J. Virol.* 86, 3293–3306.

Burke, G.R., Simmonds, T.J., Sharanowski, B.J. and Geib, S.M., 2018. Rapid viral symbiogenesis via changes in parasitoid wasp genome architecture. *Mol. Biol. Evol*. 35, 2463–2474.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol*. 17, 540–552.

Chen, J. and Shi, Q., 2001. Systematic Studies on Aphidiidae of China (Hymenoptera: Aphidiidae). Fujian Science and Technology Press, Fuhzou, China. 273 pp.

Chen, X.-X. and van Achterberg, C., 2019. Systematics, phylogeny, and evolution of braconid wasps: 30 years of progress. *Annu. Rev. Entomol*. 64, 335–358.

Condamine, F.L., Clapham, M.E. and Kergoat, G.J., 2016. Global patterns of insect diversification: towards a reconciliation of fossil and molecular evidence?. *Sci. Rep.* 6, 19208.

Davidyan, E.M., 2007. Fam. Aphidiidae. In: Leley, A.S., (Ed.), Keys to the Insects of Russian Far East. Vladivostok, Dal'nauka. pp. 192–254. [in Russian]

Dowton, M., Austin, A.D. and Antolin, M.F., 1998. Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. *Insect Mol. Biol.* 7, 129– 50.

Dowton, M., Belshaw, R., Austin, A.D. and Quicke, D.L.J., 2002. Simultaneous molecular and morphological analysis of braconid relationships (Insecta: Hymenoptera: Braconidae)

indicates independent mt-tRNA gene inversions within a single wasp family. *J. Mol. Evol.* 54, 210–226.

Ebel, E.R., DaCosta, J.M., Sorenson, M.D., Hill, R.I., Briscoe, A.D., Willmott, K.R. and Mullen, S.P., 2015. Rapid diversification associated with ecological specialization in Neotropical *Adelpha* butterflies. *Mol. Ecol.* 24, 2392–2405.

Faircloth, B.C., 2013. Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. Available at: https://github.com/faircloth-lab/illumiprocessor

Faircloth, B.C., 2015. PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics* 32, 786–788.

Faircloth, B.C., 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics* 32, 786–788.

Farris, J.S., 1970. Methods for computing Wagner trees. *Syst. Zool.* 19, 83–92.

Fernández-Triana, J., Shaw, M.R., Boudreault, C., Beaudin, M. and Broad, G.R., 2020. Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *ZooKeys* 920, 1–1089.

Finlayson, T., 1990. The systematics and taxonomy of final-instar larvae of the family Aphidiidae (Hymenoptera). *Mem. Ent. Soc. Can*. 122, 3–74.

Fischer, M., 1970. Probleme der Systematik bei den Opiinae (Hym,. Braconidae). *Z. Arbeitsgem. Österr. Entomol.* 22, 81–88.

Fischer, M., 1972. Hymenoptera: Braconidae (Opiinae I). *Das Tierreich* 91, 1–260.

Forbes, A.A., Bagley, R.K., Beer, M.A., Hippee, A.C. and Widmayer, H.A., 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecol.* 18, 1–11.

Fortier, J.C., 2006. The first host association for *Aleiodes pilosus* (=*Tetrasphaeropyx* Ashmead) (Hymenoptera: Bracondiae: Rogadinae). *Entomol. News* 117, 457–459.

Gadallah, N.S., Ghahari, H., and Quicke, D.L.J., 2021. Further addition to the braconid fauna of Iran (Hymenoptera: Braconidae). *Egyp. J. Biol. Pest Control* 31, 32.

Gauld, I.D., 1988. Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biol. J. Linn. Soc*. 35, 351–377.

Gauld, I.D., 2006. Family Ichneumonidae. In: Hanson P.E. and Gauld I.D. (Eds.) Hymenoptera de La Región Neotropical. *Mem. Am. Entomol. Inst*. 77, 446–487.

Gimeno, C., Belshaw R. and Quicke D.L.J., 1997. Phylogenetic relationships of the Alysiinae/Opiinae (Hymenoptera: Braconidae) and the utility of cytochrome b, 16S and 28S D2 rRNA. *Insect Mol. Biol.* 6, 273–284.

Godfray, H.C., 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, 488 pp.

Goloboff, P.A., Farris, J.S. and Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson D.A. … and Regev, A., 2011. Full–length transcriptome assembly from RNA–Seq data without a reference genome. *Nat. Biotechnol*. 29, 644–652.

Grimaldi, D. and Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press. New York, USA.

Hagvar, E.B. and Hofsvang, T., 1991. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol news and Information* 12, 13-42.

Hanson, P.E. and Gauld I.D., 1995. The Hymenoptera of Costa Rica. Oxford University Press, Oxford, UK.

Hanson, P.E. and Gauld, I.D., 2006. Hymenoptera de la región Neotropical. *Mem. Am. Entomol. Inst.* 77, 1–994.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. and Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35, 518–522.

Infante, F., Hanson, P. and Wharton, R., 1995. Phytophagy in the genus *Monitoriella* (Hymenoptera: Braconidae) with description of new species. *Ann. Entomol. Soc. Am.* 88, 406–415.

Jasso-Martínez, J.M., Quicke, D.L.J., Belokobylskij, S.A., Meza-Lázaro, R.N. and Zaldívar-Riverón, A., 2021. Phylogenomics of the lepidopteran endoparasitoid wasp subfamily Rogadinae (Hymenoptera: Braconidae) and related subfamilies. *Syst. Entomol*. 46, 83–95.

Jasso-Martínez, J.M., Quicke, D.L.J., Belokobylskij, S.A., Santos, B.F., Fernández-Triana, J.L., Kula, R.R., Zaldívar-Riverón, A. submitted. Mitochondrial phylogenomics and mitogenome organization in the parasitoid wasp family Braconidae (Hymenoptera: Ichneumonoidea). *BMC Ecol. Evol.*

Jell, P.A. and Duncan, P.M., 1986. Invertebrates, mainly insects, from the freshwater, Lower Cretaceous, Koonwarra fossil bed, (Korumburra Group), South Gippsland, Victoria. In P. W. Jell and J. Roberts (Eds.) *Plants and invertebrates from the Lower Cretaceous Koonwarra fossil bed, South Gippsland, Victoria.* Memoirs of the Association of Australasian Palaeontologists 3, 111–205.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A. and Jermiin L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589.

Kammerer, C.F., 2006. Notes on some preoccupied names in Arthropoda. *Acta Zootaxonomica Sinica* 31, 269–271.

Katoh, K., Misawa, K., Kuma, K. and Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. 30, 3059–3066.

Krugner, R., Daane, K. M., Lawson, A.B. and Yokota, G.Y., 2005. Biology of *Macrocentrus iridescens* (Hymenoptera: Braconidae): a parasitoid of the Obliquebanded Leafroller (Lepidoptera: Tortricidae). *Environ. Entomol.* 34, 336-343.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. and Calcott, B., 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* 34, 772–773.

Lemmon, A.R., Emme, S.A. and Lemmon, E.M., 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. *Syst. Biol*. 61, 727–744.

Li, X.-Y., van Achterberg, C. and Tan, J.-C., 2013. Revision of the subfamily Opiinae (Hymenoptera, Braconidae) from Hunan (China), including thirty-six new species and two new genera. *ZooKeys* 268, 1–186.

Li, Q., Wei, S.J., Tang, P., Wu, Q., Shi, M., Sharkey, M.J. and Chen, X.-X., 2016. Multiple lines of evidence from mitochondrial genomes resolve phylogenetic relationships of parasitic wasps in Braconidae. *Genome Biol. Evol.* 8, 2651–2662.

Mackauer, M., 1961. Die Gattungen der Familie Aphidiidae und ihre verwandtschaftliche Zuordnung (Hymenoptera: Ichneumonoidea). *Beitr. Entomol.* 11, 792–803.

Mackauer, M., Michaud, J.P. and Völkl, W., 1996. Invitation paper: CP Alexander Fund: Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality, and host value. *Can. Entomol.* 128, 959–980.

Mason, W.R.M., 1976. A revision of *Dyscoletes* Haliday (Hymenoptera: Braconidae). *Can. Entomol.* 108, 855-858.

Mason, W.R.M., 1978. A new genus, species and family of Hymenoptera (Ichneumonidae) from Chile. *Proc. of the Entomol. Soc. Wash.* 80, 606–610.

Mason, W.R.M., 1983. A new South African subfamily related to Cardiochilinae (Hymenoptera: Braconidae). *Contrib. Am. Entomol. Inst.* 20, 49–62.

Maqbool, A., Akbar, S.A. and Wachkoo, A.A., 2018. First record of the genus *Ficobracon* (Hymenoptera: Braconidae) from India, with description of new species. *Zootaxa* 4379, 421–428.

Mayhew, P.J., 2007. Why are there so many insect species? Perspectives from fossils and phylogenies. *Biol. Rev.* 82, 425–454

Mayhew, P.J. and Blackburn, T.M., 1999. Does development mode organize life-history traits in the parasitoid Hymenoptera?. *J. Anim. Ecol.* 68, 906–916.

Memmott, J. and Godfray, H.C.J., 1993. Parasitoid webs. In: Lasalle, J. and Gauld, I.D. (Eds.) Hymenoptera and Biodiversity. Wallingford: CAB International, pp. 217–34.

Michel-Salzat, A. and Whitfield, J.B., 2004. Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Syst. Entomol.* 29, 371–382.

Murphy, N., Banks, J., Whitfield, J.B. and Austin, A., 2008. Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage. *Mol. Phylogenet. Evol*. 47, 378–395.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A. and Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274.

Nixon, G.E.J., 1940. New genera and species of Hormiinae, with a note on *Hormiopterus* Giraud (Hymenoptera: Braconidae). *Ann. Mag. Nat. Hist.* 5, 473–493.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15, 407–414.

Pagel, M., 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lon. B* 255, 37–45.

Paradis, E., Claude, J. and Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290.

Paula, D.P., 2021. Next-Generation Sequencing and Its Impacts on Entomological Research in Ecology and Evolution. *Neotrop. Entomol.* 50, 679-696.

Perrichot, V., Nel, A. and Quicke, D.L.J., 2009. New braconid wasps from French Cretaceous amber (Hymenoptera, Braconidae): synonymisation with Eoichneumonidae and implications for the phylogeny of Ichneumonoidea. *Zool. Scripta* 38, 79–88.

Pitz, K., Dowling, A.P.G., Sharanowski, B.J., Boring, C.A.B., Seltmann, K.C. and Sharkey, M.J., 2007. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) as proposed by Shi et al.: a reassessment. *Mol. Phylogenet. Evol*. 43, 338–343.

Quicke, D.L.J., 1987. A new subfamily of Braconidae, the Vaepellinae, based on a new genus and species from Ghana (Insecta, Hymenoptera). *Zool. Scr.* 16, 73–77.

Quicke, D.L.J., 1989. Further new host records for genera and species of Braconinae (Hym., Braconidae). *Entomol. Mon. Mag.* 125, 199-205.

Quicke, D.L.J., 1997. Subfamily Braconinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the Family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 149-174.

Quicke, D.L.J., 2015. The Braconid and Ichneumonid Parasitoid Wasps: Biology, Systematics, Evolution and Ecology. John Wiley & Sons, Chichester, U.K.

Quicke, D.L.J. and van Achterberg, C., 1990. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera). *Zool. Verh. Leide.* 258, 1–95.

Quicke, D.L.J. and Shaw, M.R., 2005. First host records for the rogadine genera *Rogasodes* Chen and He and *Canalirogas* van Achterberg and Chen (Hymenoptera: Braconidae) with description of a new species and survey of mummy types within Rogadinae *s. str*. *J. Nat. Hist.* 39, 3525–3542.

Quicke, D.L.J., Ficken, L. and Fitton, M.G., 1992a. New diagnostic ovipositor characters for doryctine wasps (Hymenoptera, Braconidae). *J. Nat. Hist.* 26, 1035–1046.

Quicke, D.L.J., Tunstead, J., Falco, J.V. and Marsh, P.M., 1992b. Venom gland and reservoir morphology in the Doryctinae and related braconid wasps (Insecta, Hymenoptera, Braconidae). *Zool. Scripta* 21, 403–416.

Quicke, D.L.J., van Achterberg, K. and Godfray, H.C.J., 1997. Comparative morphology of the venom gland and reservoir in opiine and alysiine braconid wasps (Insecta, Hymenoptera, Braconidae). *Zool. Scr.* 21, 23–50.

Quicke, D.L.J., Basibuyuk, H.H. and Rasnitsyn, A.P., 1999a. Morphological, palaeontological and molecular aspects of ichneumonoid phylogeny (Hymenoptera, Insecta). *Zool. Scr.* 28, 175–202.

Quicke, D.L.J., LeRalec, A. and Vilhelmsen, L., 1999b. Ovipositor structure and function in the parasitic Hymenoptera. *Atti Acad. Naz. Ital. Entomol. Rc.* 47, 197–239.

Quicke, D.L.J., Manzari, S. and van Achterberg, C., 2002. The systematic placement of Afrocampsis van Achterberg & Quicke (Hymenoptera: Braconidae): molecular and morphological evidence indicate that it belongs to Helconinae *s.l.* not Sigalphinae. *Zool. Meded.* 76, 443–450.

Quicke, D.L.J., Sharkey, M.J., Laurenne, N.M. and Dowling, A., 2008. A preliminary molecular phylogeny of the Sigalphinae (Hymenoptera: Braconidae), including *Pselaphanus* Szépligeti, based on 28S rDNA, with descriptions of new Afrotropical and Madagascan *Minanga* and *Malasigalphus* species. *J. Nat. Hist.* 42, 2703–2719.

Quicke, D.L.J., Belokobylskij, S.A., Ward, D.F., Hebert, P.D.N. and Butcher, B.A., 2018. Revision of the endemic New Zealand braconid wasp genus *Metaspathius*: new subfamily placement, and descriptions of four new species including three with fully winged females (Hymenoptera: Braconidae: Mesostoinae). *N. Z. Entomol.* 41, 46–65.

Quicke, D.L.J., Belokobylskij, S.A., Ward, D.F., Hebert, P.D.N. and Butcher, B.A., 2019. Revision of the endemic New Zealand braconid wasp genus *Metaspathius*: new subfamily placement, and descriptions of four new species including three with fully winged females (Hymenoptera: Braconidae: Mesostoinae). *N. Z. Entomol.* 41, 46–65.

Quicke, D.L.J., Austin, A.D., Fagan-Jeffries, E.P., Hebert, P.D.N. and Butcher, B.A., 2020a. Molecular phylogeny places the enigmatic subfamily Masoninae within the Ichneumonidae, not the Braconidae. *Zool. Scr.* 49, 64–71.

Quicke, D.L.J., Austin, A.D., Fagan-Jeffries, E.P., Hebert, P.D. and Butcher, B.A., 2020b. Recognition of the Trachypetidae stat. n. as a new extant family of Ichneumonoidea

(Hymenoptera), based on molecular and morphological evidence. *Syst. Entomol.* 45, 771–782.

Quicke, D.L.J., Belokobylskij, S.A., Braet, Y., van Achterberg, C., Hebert, P.D.N. … and Butcher, B.A., 2020c. Phylogenetic reassignment of basal cyclostome braconid parasitoid wasps (Hymenoptera) with description of a new, enigmatic Afrotropical tribe with a highly anomalous 28S D2 secondary structure. *Zool. J. Linn. Soc.* 190, 1002–1019.

Quicke, D.L.J., Fagan-Jeffries, E., Jasso-Martínez, J.M., Zaldívar-Riverón, A., Shaw, M.R. … and Butcher, B.A., 2021. A molecular phylogeny of the parasitoid wasp subfamily Rogadinae (Hymenoptera: Ichneumonoidea: Braconidae) with descriptions of three new genera. *Syst. Entomol.* 46, 1019–1044.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ranjith, A.P., Quicke, D.L.J., Saleem, U.K.A., Butcher, B.A., Zaldívar-Riverón, A. and Nasser, M., 2016. Entomophytophagy ('Sequential predatory, then phytophagous behaviour') in an Indian braconid 'parasitoid' wasp (Hymenoptera): Specialized larval morphology, biology and description of a new species. *PLoS One* 11, e0156997.

Ranjith, A.P., Belokobylskij, S.A., Quicke, D.L.J., Kittel, R.N., Butcher, B.A. and Nasser, M., 2017. An enigmatic new genus of Hormiinae (Hymenoptera: Braconidae) from South India. *Zootaxa* 4272, 371–385.

Rasnitsyn, A.P., 1983. Ichneumonoidea (Hymenoptera) from the lower cretaceous of Mongolia. *Contrib. Am. Entomol. Inst.* 20, 259–265.

Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223.

Santos, B.F. and Perrard, A., 2018. Testing the Dutilleul syndrome: host use drives the convergent evolution of multiple traits in parasitic wasps. *J. Evol. Biol.* 31, 1430–1439.

Santos, B.F., Wahl, D.B., Rousse, P., Bennett, A.M.R., Kula, R. and Brady, S.G., 2021. Phylogenomics of Ichneumoninae (Hymenoptera, Ichneumonidae) reveals pervasive morphological convergence and the shortcomings of previous classifications. *Syst. Entomol.* 46, 704–724.

Santos, B.F., Klopfstein, S., Whitfield, J.B. and Sharanowski, B. 2022. Many evolutionary roads led to virus domestication in ichneumonoid parasitoid wasps. *Curr. Opin. Insect Sci.* 50, 100861.

Samacá-Saenz, E., Meza-Lázaro, R., Branstetter, M.G. and Zaldívar-Riverón, A. 2019. Phylogenomics and mitochondrial genome evolution of the gall-associated doryctine wasp genera (Hymenoptera: Braconidae). *Syst. Biodiv.* 17, 731–744.

Samacá-Sáenz, E., Santos, B.F., Martínez, J.J., Egan, S.P., Shaw, S.R., Hanson, P.E. and Zaldívar-Riverón, A., 2022. Ultraconserved elements-based systematics reveals evolutionary patterns of host-plant family shifts and phytophagy within the predominantly parasitoid braconid wasp subfamily Doryctinae. *Mol. Phylogenet. Evol.* 107319.

Schoenly, K., 1990. The predators of insects. *Ecol. Entomol.* 15, 333–345.

Schulz, W.A., 1911. Zweihundert alte Hymenopteren. *Zool. Annln.* 4, 1–220.

Sharanowski, B.J., Dowling, A.P. and Sharkey, M.J., 2011. Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. *Syst. Entomol.* 36, 549–572.

Sharanowski, B.J., Ridenbaugh, R.D., Piekarski, P.K., Broad, G.R., Burke, G.R. … and Hines, H.M., 2021. Phylogenomics of Ichneumonoidea (Hymenoptera) and implications for

evolution of mode of parasitism and viral endogenization. *Mol. Phylogenet. Evol*. 156, 107023.

Sharkey, M.J., 1993. Family Braconidae. In: Goulet, H. and Huber, J.T. (Eds.). Hymenoptera of the World: An Identification Guide to Families, Research Branch Agriculture Canada Publication, Ottawa, Canada, pp. 362–395.

Sharkey, M.J., 1997. Subfamily Agathidinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the Family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 69-83.

Sharkey, M.J. and Wahl D.B., 1992. Cladistics of the Ichneumonoidea (Hymenoptera). *J. Hym. Res*. 1, 15–61.

Sharkey, M.J. and Wharton, R.A., 1994. A revision of the genera of the world Ichneutinae (Hymenoptera: Braconidae). *J. Nat. Hist.* 28, 873–912.

Sharkey, M.J. and Wharton, R.A., 1997. Morphology and terminology. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 21–40.

Sharkey, M.J., van Noort, S. and Whitfield, J., 2009. Revision of Khoikhoiinae (Hymenoptera, Braconidae). *ZooKeys* 20, 299–348

Sharkey, M.J., Janzen, D.H., Hallwachs, W., Chapman, E.G., Smith. … and Burns, J.M., 2021. Minimalist revision and description of 411 new species in 11 subfamilies of Costa Rican braconid parasitic wasps, including host records. *Zootaxa* 1013, 1–665.

Shaw, M.R., 1983. On[e] evolution of endoparasitism; the biology of some genera of Rogadinae (Braconidae). *Contrib. Am. Entomol. Inst.* 20, 307–328.

Shaw, M.R., 2017. Further notes on the biology of *Pseudavga flavicoxa* Tobias, 1964 (Hymenoptera, Braconidae, Rhysipolinae). *J. Hym. Res.* 54, 113–128.

Shaw, M.R. and Huddleston, T., 1991. Classification and biology of braconid wasps (Hymenoptera: Braconidae). Handbooks for the Identification of British Insects, London: Royal Entomological Society of London, 7, 1–126.

Shi, M., Chen, X.-X. and van Achterberg, C., 2005. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA, 28S rDNA D2, 18S rDNA gene sequences and morphological characters. *Mol. Phylogenet. Evol.* 37, 104-116.

Shimbori, E.M., Souza-Gessner, C.D.S.S., Penteado-Dias, A.M. and Shaw, S.R., 2017. A revision of the genus *Andesipolis* (Hymenoptera: Braconidae: Mesostoinae) and redefinition of the subfamily Mesostoinae. *Zootaxa* 4216, 101–152.

Smithsonian Institution, 2020. *High Performance Computing Cluster*. URL: https://doi.org/10.25572/SIHPC

Spasojevic, T., Broad, G.R., Sääksjärvi, I.E., Schwarz, M., Ito, M., Korenko, S. and Klopfstein, S., 2021. Mind the Outgroup and Bare Branches in Total-Evidence Dating: a Case Study of Pimpliform Darwin Wasps (Hymenoptera, Ichneumonidae). *Syst. Biol.* 70, 322–339.

Stigenberg, J., Boring, C.A. and Ronquist, F., 2015. Phylogeny of the parasitic wasp subfamily Euphorinae (Braconidae) and evolution of its host preferences. *Syst. Entomol.* 40, 570–591.

Tagliacollo, V.A. and Lanfear, R., 2018. Estimating improved partitioning schemes for ultraconserved elements. *Mol. Biol. Evol.* 35, 1798–1811.

Talavera, G. and Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol*. 56, 564–577.

Tobias, V.I., 1962. A new subfamily of braconids (Hymenoptera, Braconidae) from Central Asia. *Trudy Zool. Inst. Akad. Nauk SSSR* 30, 268–270.

Tobias, V.I., 1979. Two new and one little known subfamilies of Braconidae (Hymenoptera) from Australia. *Entomol. Obozr.* 58, 128–142 (in Russian).

Tobias, V.I. and Kirijak, I.G., 1986. Aphidiidae. In: Medvedev, G. S. (Ed.), Opredelitel′ nasekomykh Evropeiskoi chasti SSSR. v.3 pt. 5. Academia Nauk, Leningrad. pp. 232–307.

Tobias, V.I., 1988. A new subfamily of braconids (Hymenoptera, Braconidae) from Australia. *Ent. Obozr.* 67, 644–648.

Turner, R.E., 1918. Notes on the Braconidae in the British museum. IV. On new Helconinae, mostly Australian. *Ann. Mag. Nat. Hist.,* 9, 163–173.

Valerio, A.A. and S.R. Shaw., 2015. Thirteen new Costa Rican species belonging to the genus *Triraphis* Ruthe (Braconidae: Rogadinae) with their host records. *Zootaxa* 3904, 501–540.

Vilhelmsen, L., 1997. The phylogeny of lower Hymenoptera (Insecta), with a summary of the early evolutionary history of the order. *J. Zool. Syst. Evol. Res.* 35, 49-70.

Wei, S-J., Shi, M., Sharkey, M.J., van Achterberg, C. and Chen, X.-X., 2010. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. *BMC Genomics* 11, 1–16.

Wharton, R.A., 1993a. Bionomics of the Braconidae. *Annu. Rev. Entomol.* 38, 121–143.

Wharton, R.A., 1993b. Review of the Hormiini (Hymenoptera: Braconidae) with a description of new taxa. *J. Nat. Hist.* 27, 107–171.

Wharton, R.A., 1997. Subfamily Alysiinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 84–116.

Wharton, R.A. and Hanson, P.E., 2005. Biology and evolution of braconid gall wasps (Hymenoptera). In: Raman, A., Schaefer, C.W. and Withers, T.M (Eds.). Biology, Ecology, and Evolution of Gall-inducing Arthropods. Science Publishers, Enfield, New Hampshire, pp. 495–505.

Wharton, R.A., Shaw, S.R., Sharkey, M.J., Wahl, D.B., Woolley, J.B., Whitfield, J.B., Marsh, P. M. and Johnson, W., 1992. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea): a reassessment. *Cladistics* 8, 199–235.

Wharton, R.A., Marsh, P.M. and Sharkey, M.J., 1997. Manual of the New World Genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, 439 pp.

Wharton, R.A., Yoder, M.J., Gillespie, J.J., Patton, J.C. and Honeycutt, R.L., 2006. Relationships of *Exodontiella*, a non-alysiine, exodont member of the family Braconidae (Insecta, Hymenoptera). *Zool. Scr.* 35, 323–340.

Whitfield, J.B., 1992. The polyphyletic origin of endoparasitism in the cyclostome lineages of Braconidae (Hymenoptera). *Syst. Entomol.* 17, 273–286.

Whitfield, J.B., 1997. Subfamily Microgastrinae. In: Wharton, R.A., Marsh, P.M., Sharkey, M.J. (Eds.). Manual of the New World Genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 333–364.

Whitfield, J.B., 2002. Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 99, 7508–7513.

Whitfield, J.B. and Mason, W.R., 1994. Mendesellinae, a new subfamily of braconid wasps (Hymenoptera, Braconidae) with a review of relationships within the microgastroid assemblage. *Syst. Entomol.* 19, 61–76.

Whitfield, J.B., Austin A., Fernandez-Triana J.L., 2018. Systematics, Biology, and Evolution of Microgastrine Parasitoid Wasps. *Annu. Rev. Entomol.* 63, 389–406.

Yu, D.S., van Achterberg, C. and Horstmann, K., 2016. Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive (CD).

Zaldívar-Riverón, A., Areekul, B., Shaw, M.R. and Quicke, D.L.J., 2004. Comparative morphology of the venom apparatus in the braconid wasp subfamily Rogadinae (Insecta, Hymenoptera, Braconidae) and related taxa. *Zool. Scr.* 33, 223–237.

Zaldívar-Riverón, A., Mori, M. and Quicke, D.L.J., 2006. Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. *Mol. Phylogenet. Evol.* 38, 130–145.

Zaldívar-Riverón, A., Belokobylskij, S.A., León-Regagnon, V., Martínez, J.J., Briceno, R. and Quicke, D.L.J., 2007. A single origin of gall association in a group of parasitic wasps with disparate morphologies. *Mol. Phylogenet. Evol.* 44, 981–992.

Zaldívar-Riverón, A., Shaw, M.R., Sáez, A.G., Mori, M., Belokoblylskij, S.A., Shaw, S.R. and Quicke, D.L.J., 2008a. Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. *BMC Evol. Biol.* 8, 1–20.

Zaldívar-Riverón, A., Belokobylskij, S.A., León-Regagnon, V., Briceño-G., R. and Quicke, D.L.J., 2008b. Molecular phylogeny and historical biogeography of the cosmopolitan parasitic wasp subfamily Doryctinae (Hymenoptera: Braconidae). *Invertebr. Syst.* 22, 345–363.

Zaldívar-Riverón, A., Martínez, J.J., Belokobylskij, S.A., Pedraza-Lara, C.S., Shaw, S.R., Hanson, P.E. and Varela-Hernández, F., 2014. Systematics and evolution of gall formation in the plant-associated genera of the wasp subfamily Doryctinae (Hymenoptera: Braconidae). *Syst. Entomol.* 39, 633–659.

Zhang, Y.M., Stigenberg, J., Meyer, J.H. and Sharanowski, B.J., 2018. Multilocus phylogeny of the parasitic wasps in the tribe Euphorini (Hymenoptera: Braconidae) with revised generic classifications. *PeerJ* 6, e4783.

Zhang, Y.M., Williams, J.L. and Lucky, A., 2019. Understanding UCEs: a comprehensive primer on using Ultraconserved elements for arthropod phylogenomics. *Insect Syst. Divers.* 3, 1-12.

TABLES

Table 1. List of braconid subfamilies prior to and after this study. Former classification of subfamilies and complex composition mainly follow Zaldívar-Riverón et al. (2006), Sharanowski et al. (2011), Yu et al. (2016), Quicke (2015) and Jasso-Martínez et al. (2021). *Avga*, *Xenosternum* and *Dyscoletes canadensis* are treated here as *incertae sedis*.

CLASSIFICATION PRIOR TO THIS STUDY			UPDATED CLASSIFICATION				
ICHNEUMONIDAE	Masoninae		BRACONIDAE				
TRACHYPETIDAE	*Trachypetus* Guérin de Meneville, *Megalohelcon* Turner, *Cercobracon* Tobias (Quicke et al., 2020b)		BRACONIDAE				
BRACONIDAE			BRACONIDAE				
Unplaced		Apozyginae			**Not sampled**		Amicrocentrinae
Unplaced		Meteorideinae			**Not sampled**		Dirrhophinae
Cyclostomes *sensu stricto*	**alysioid subcomplex**	Alysiinae			**Not sampled**		Xiphozelinae
		Braconinae	**braconoid complex**	**Cyclostomes *sensu lato***	**Cyclostomes *sensu stricto***	**braconoid subcomplex**	Alysiinae
		Exothecinae					Braconinae
		Gnamptodontinae					Exothecinae
		Opiinae					Opiinae
		Telengaiinae					Telengaiinae
		Doryctinae					Doryctinae
		Hormiinae					Hormiinae
		Pambolinae					Pambolinae
		Rhysipolinae					Rhysipolinae
		Rhyssalinae					Rhyssalinae
		Rogadinae					Rogadinae
aphidioid complex		Aphidiinae			Masoninae		
		Masxfischeriinae			**aphidioid complex**		Aphidiinae
		Mesostoinae					Masxfischeriinae
Non-cyclostomes	**euphoroid complex**	Cenocoeliinae					Mesostoinae
		Euphorinae		**Non-cyclostomes**	Meteorideinae		
		Meteorinae			Trachypetinae		
	helconoid complex	Acampsohelconinae			**euphoroid complex**		Cenocoeliinae
		Amicrocentrinae					Euphorinae
		Brachistinae			**helconoid complex**		Acampsohelconinae
		Charmontinae					Brachistinae
		Helconinae					Charmontinae
		Homolobinae					Helconinae
		Macrocentrinae					Homolobinae
		Microtypinae					Macrocentrinae
		Orgilinae					Microtypinae
		Xiphozeliinae					Orgilinae
		Cardiochilinae			Proteropinae		

61

	microgastroid complex	Cheloninae				**microgastroid complex**	Cardiochilinae
		Dirropinae					Cheloninae
		Ichneutinae					Khoikhoiinae
		Khoikhoiinae					Mendesellinae
		Mendesellinae					Microgastrinae
		Microgastrinae					Miracinae
		Miracinae				**sigalphoid complex**	Agathidinae
	sigalphoid complex	Agathidinae					Ichneutinae
		Sigalphinae					Sigalphinae
				Apozyginae (*Apozyx penyai* Mason)			

FIGURE CAPTIONS

Fig. 1. Morphological variation in Braconidae. (A–C) Live specimens in the field; photos taken by Steve Marshall in Canada (University of Guelph), used with permission. (A) *Bracon* sp. (Braconinae); (B) *Meteorus* sp. (Euphorinae); (C) *Spathius* sp. (Doryctinae). (D–L) Habitus images of pinned specimens. (D) *Aphaereta genevensis* (Alysiinae); (E) *Aphidius ohioensis* (Aphidiinae); (F) *Rhaconotus fasciatus* (Doryctinae); (G) *Chelonus* sp. (Cheloninae); (H) *Toxoneuron viator* (Cardiochilinae); (I) *Ontsira mellipes* (Rhyssalinae); (J) *Alabagrus texanus* (Agathidinae); (K) *Proterops abdominalis* (Proteropinae); (L) *Paroligoneurus newharti* (Ichneutinae).
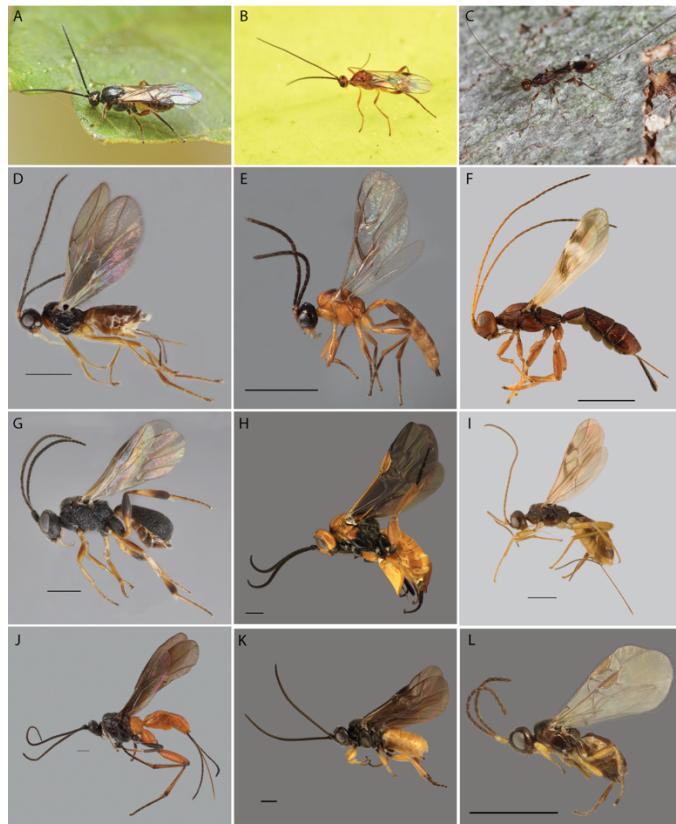
Fig. 2. Anterior view of head. (A) *Orgilius* sp. (Orgilinae), a non-cyclostome braconid with a convex clypeus that mostly conceals the labrum. (B) *Doryctes erythromelas* (Doryctine), a cyclostome braconid with a hypoclypeal depression exposing a concave labrum.
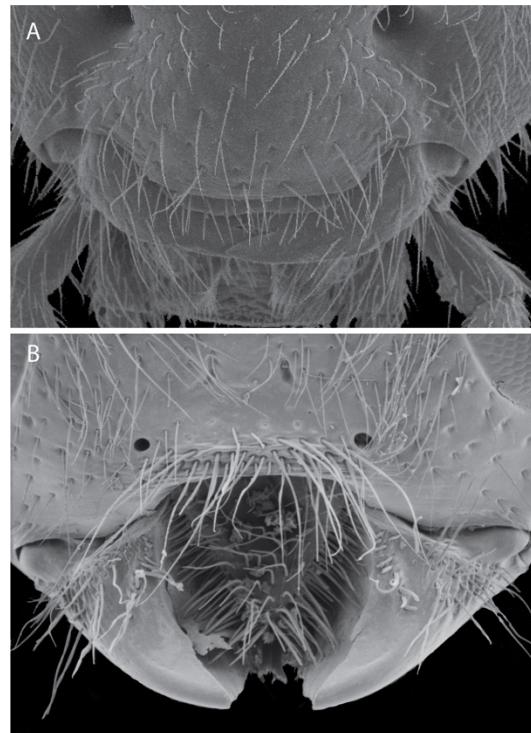
Fig. 3. Summary of phylogenetic relationships recovered in this study. Within Braconidae *Apozyx penyai* (Apozyginae) is in blue text, non-cyclostome braconids are in orange text and cyclostome braconids *sensu lato* are in green text.
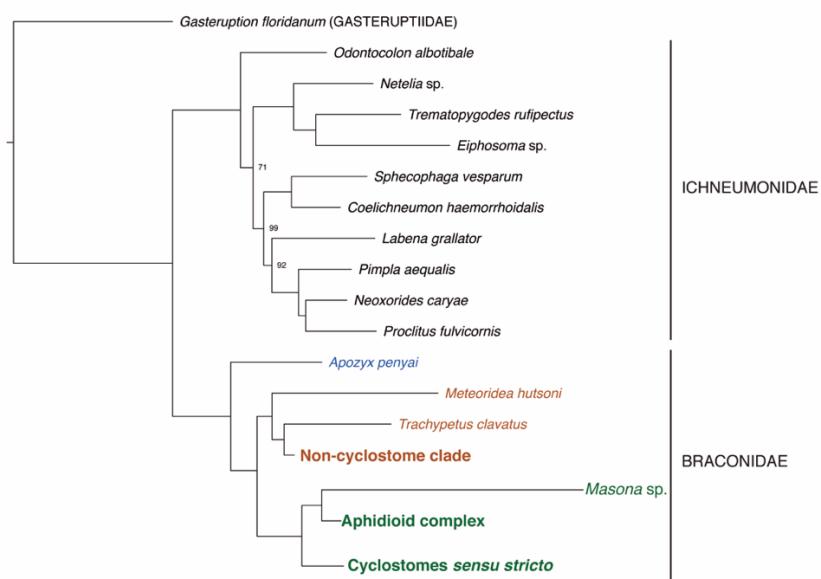
Fig. 4. Section of the ML phylogram derived from the 50% completeness matrix showing the non-cyclostome complexes helconoid, euphoroid and sigalphoid. Family and subfamily names in parenthesis correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without a number are supported with BTP = 100. Each subfamily within each complex is highlighted in different shades of orange.
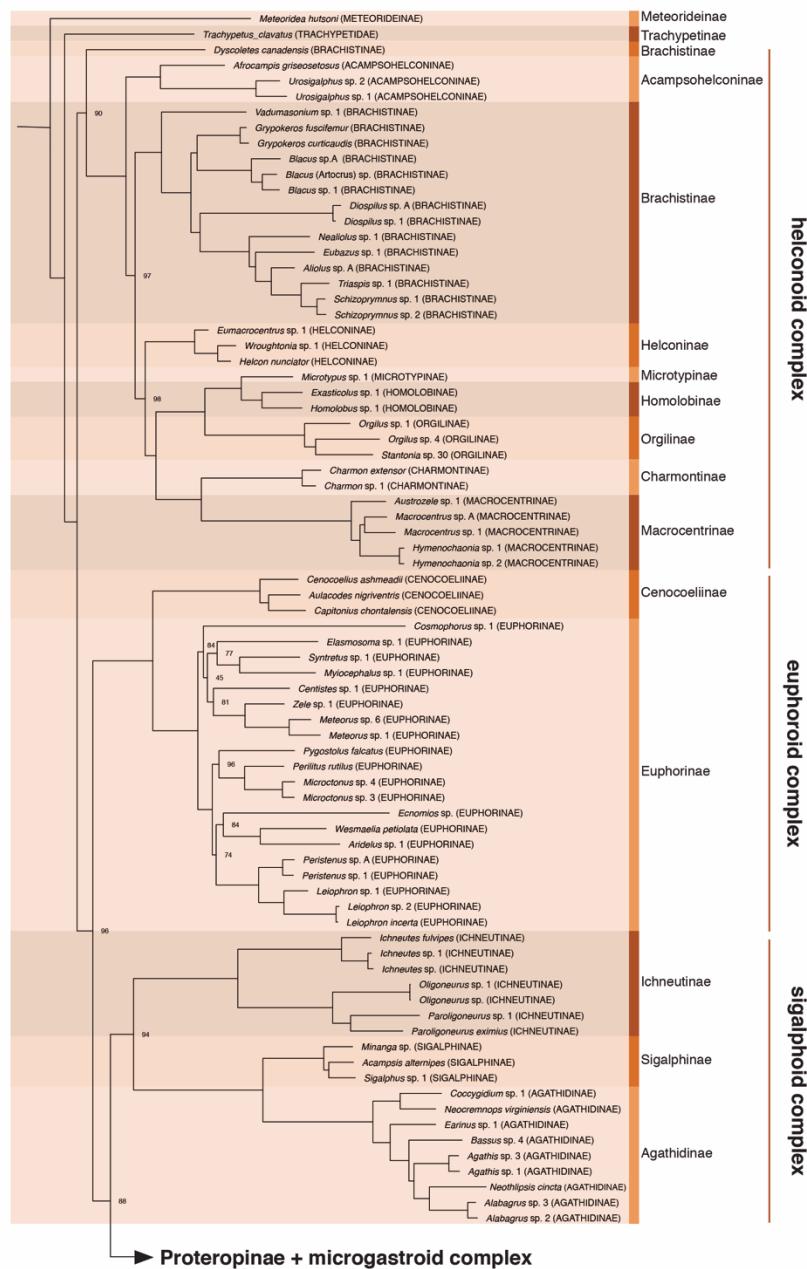
Fig. 5. Section of the ML phylogram derived from the 50% completeness matrix showing the non-cyclostome microgastroid complex and Proteropinae. Subfamily names in parenthesis correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without a number are supported with BTP = 100. Each subfamily within the complex is highlighted in different shades of orange.
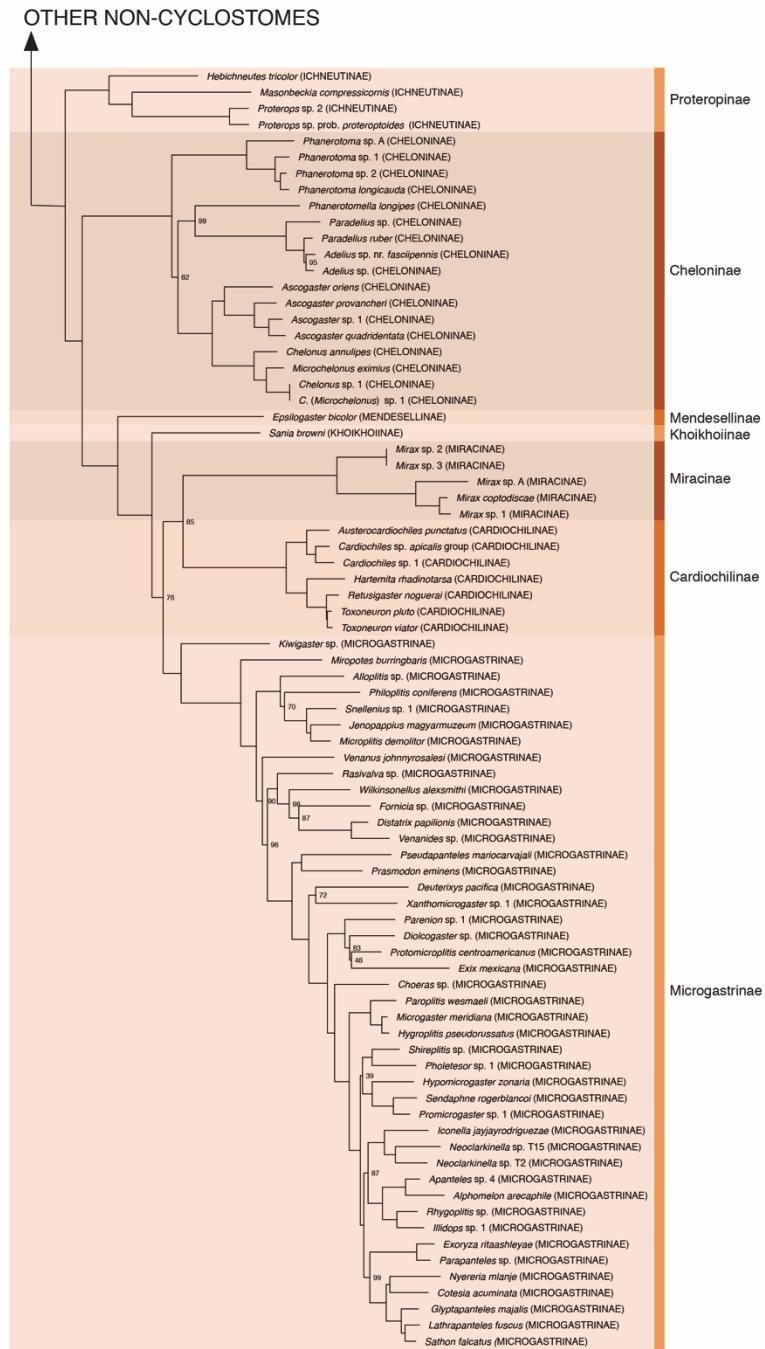
Fig. 6. Section of the ML phylogram derived from the 50% completeness matrix showing Masoninae, the three aphidioid subfamilies, Rhyssalinae and the "South American" doryctine clade. Family and subfamily names in parenthesis correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without a number are supported with BTP = 100.
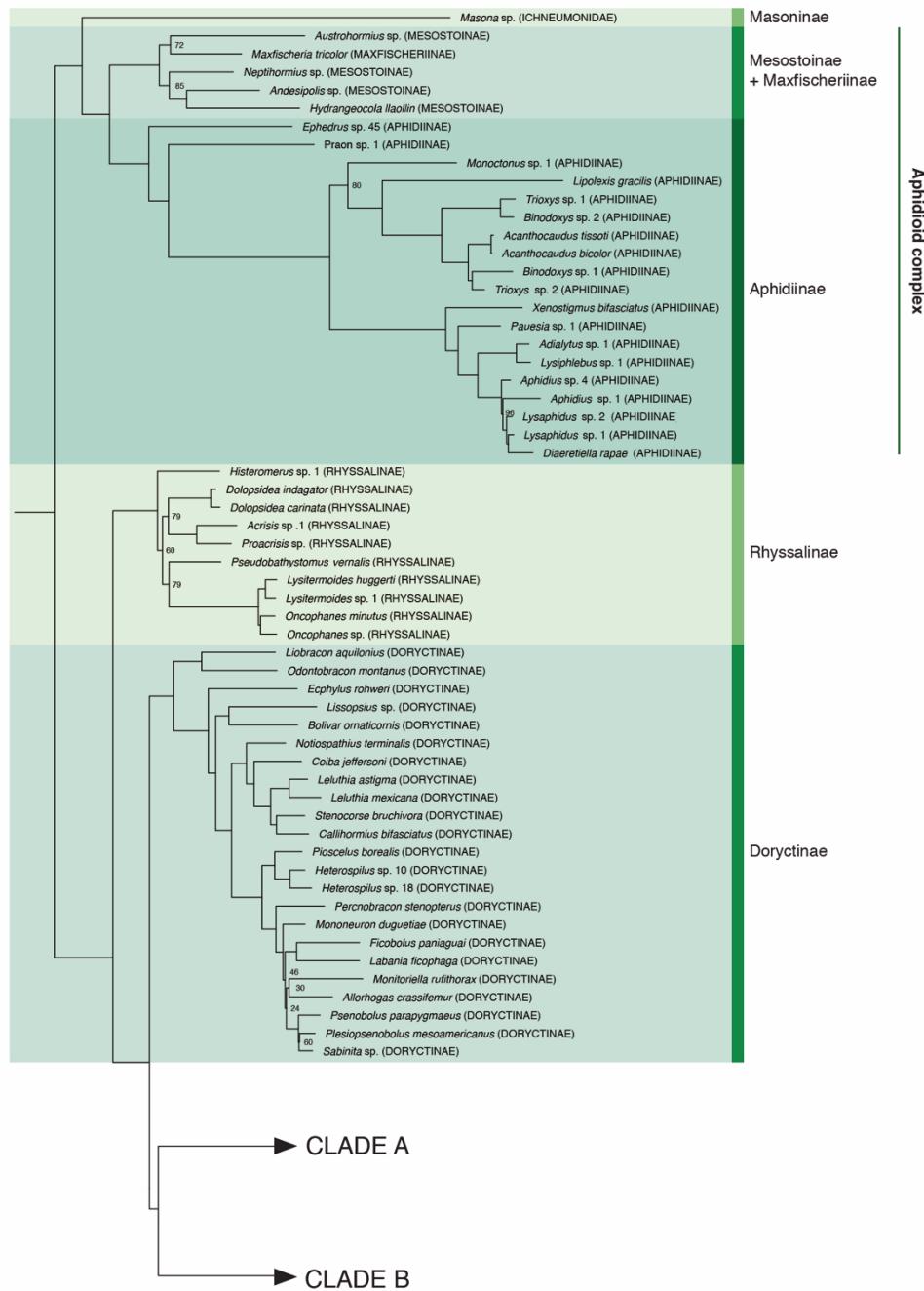
Fig. 7. Section of the ML phylogram derived from the 50% completeness matrix showing the subfamilies Rhysipolinae, Hormiinae and Rogadinae. Subfamily names in parenthesis correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without number are supported with BTP = 100.
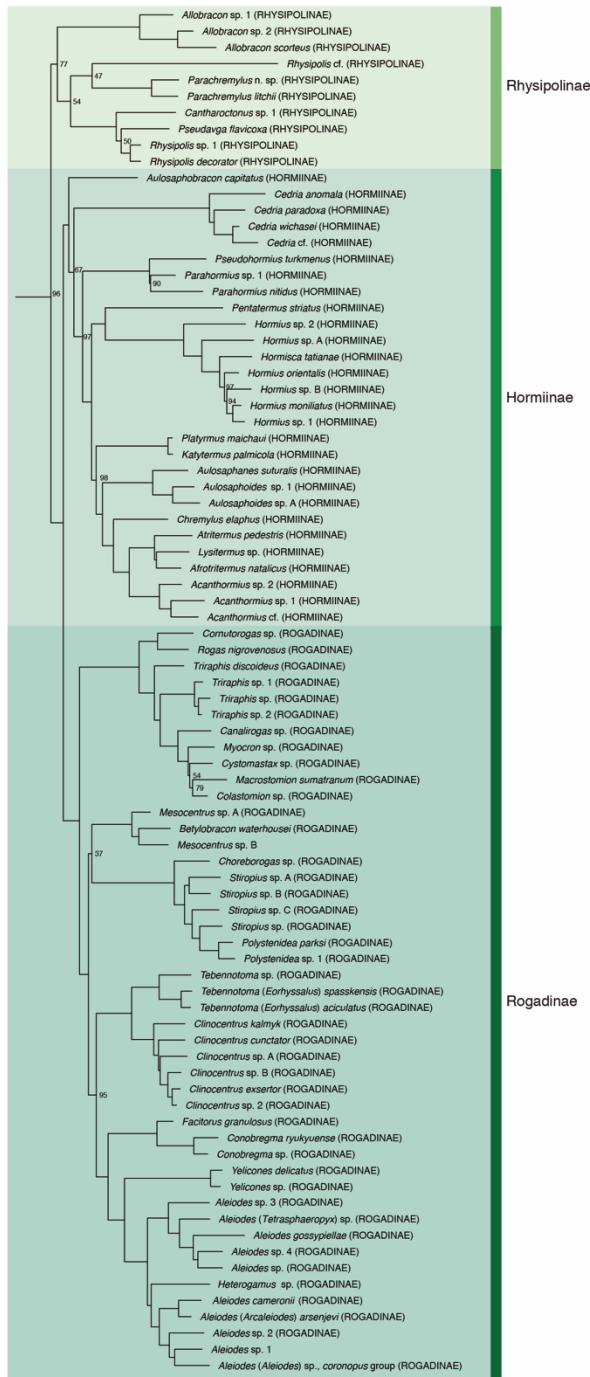
Fig. 8. Section of the ML phylogram derived from the 50% completeness matrix showing the African-Holarctic-Madagascan doryctines + Pambolinae, *Xenosternum* + *Avga* and the subfamilies that comprise the braconoid subcomplex. Subfamily names in parenthesis correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without number are supported with BTP = 100.
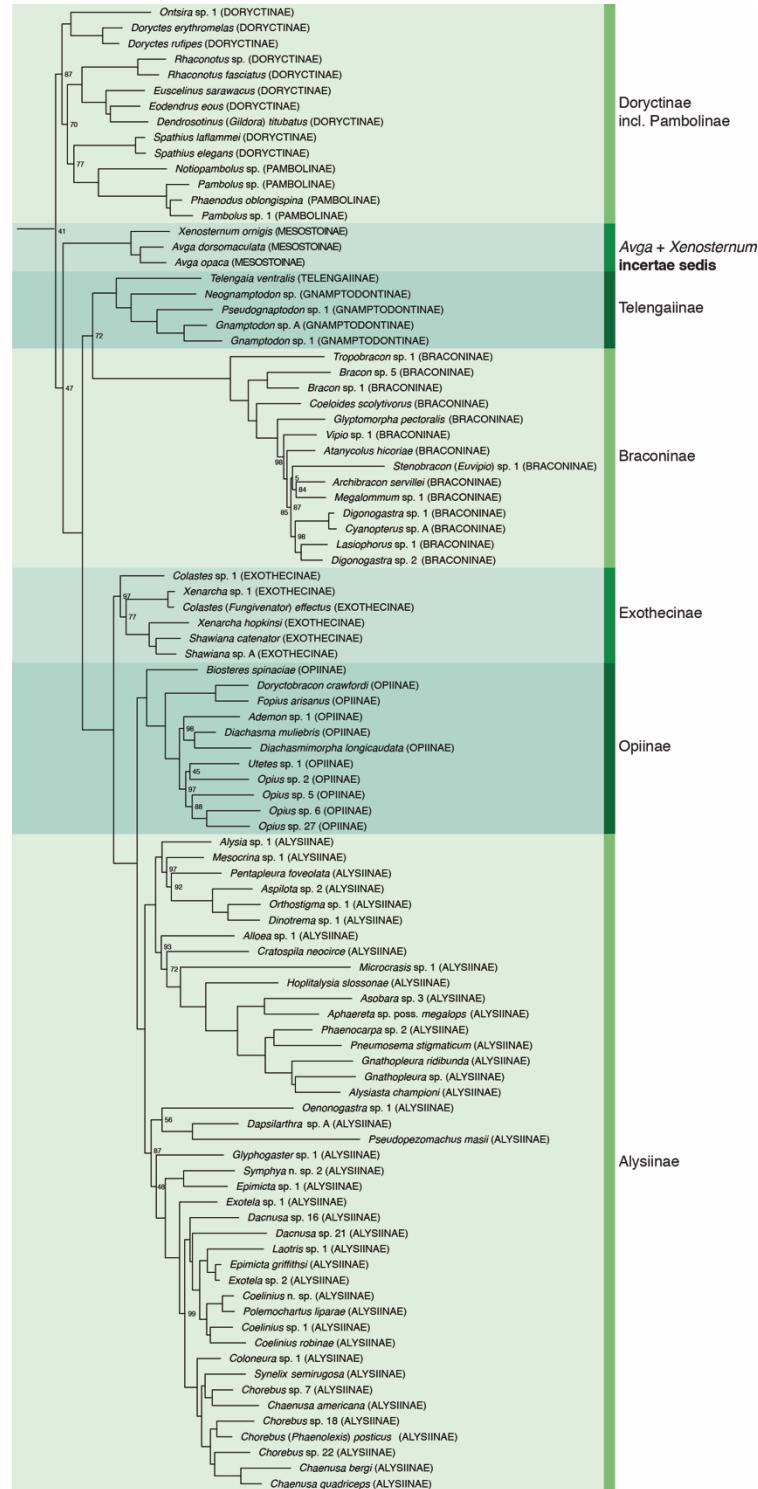
Fig. 9. Ancestral state reconstruction for idiobiosis (green) vs. koinobiosis (blue). Pie charts show proportional likelihoods as inferred under an unequal rate transition model; branch colors represent state transitions as inferred from ACCTRAN parsimony optimization. Branches in lighter shades and dashed lines represent terminals for which biological traits are unknown, colored according to the inferred state suggested by the reconstruction analyses. Reconstructed states for ectoparasitoidism and endoparasitoidism match those of idiobiosis and koinobiosis, except for Rhysipolinae, which are koinobiont ectoparasitoids.
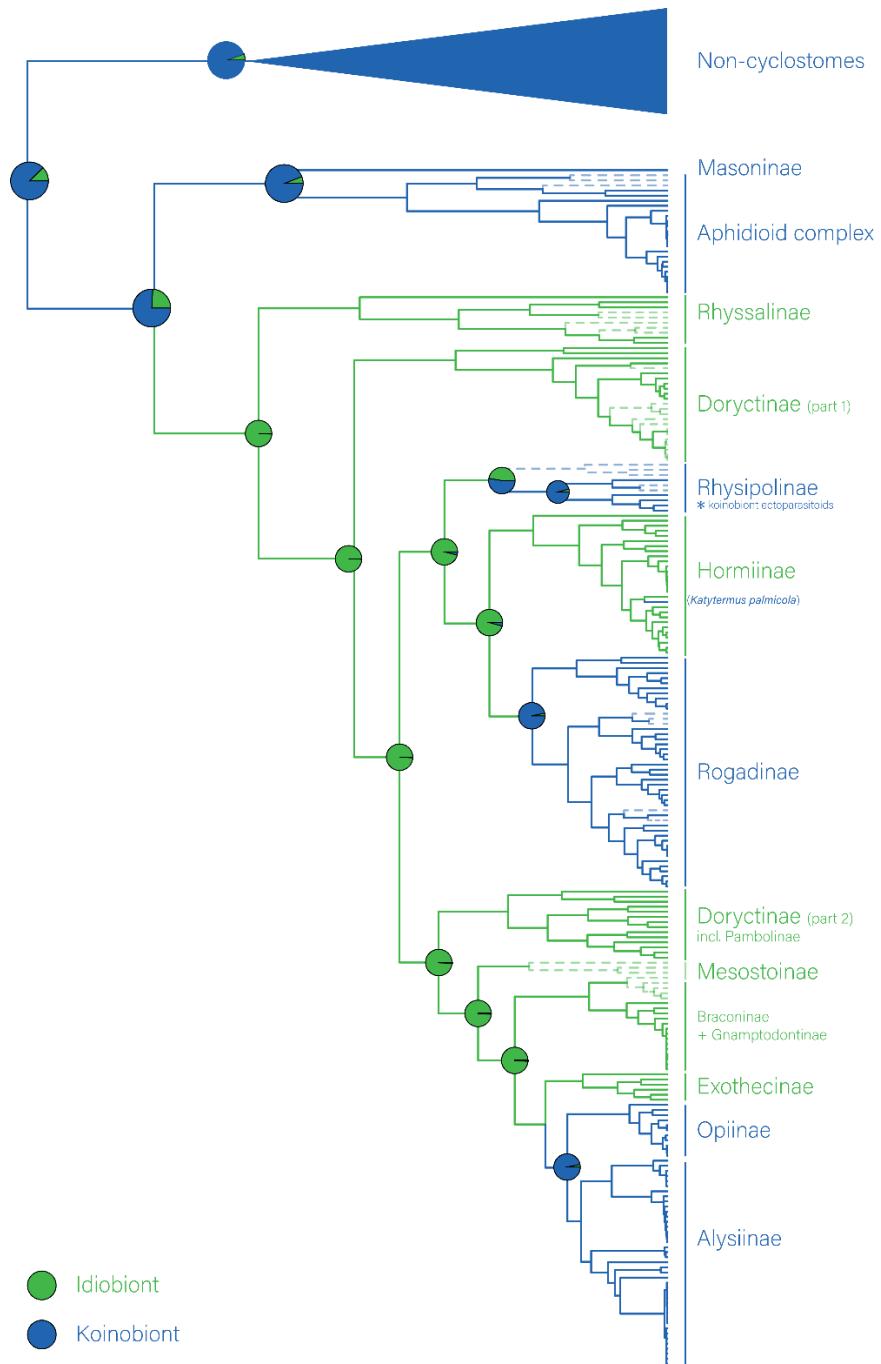
Fig. 10. Summary of relationships among braconids in the non-cyclostome subfamily complexes from this and other studies. (A) Sharanowski et al. (2011); (B) Jasso-Martínez et al. (submitted); (C) This study.
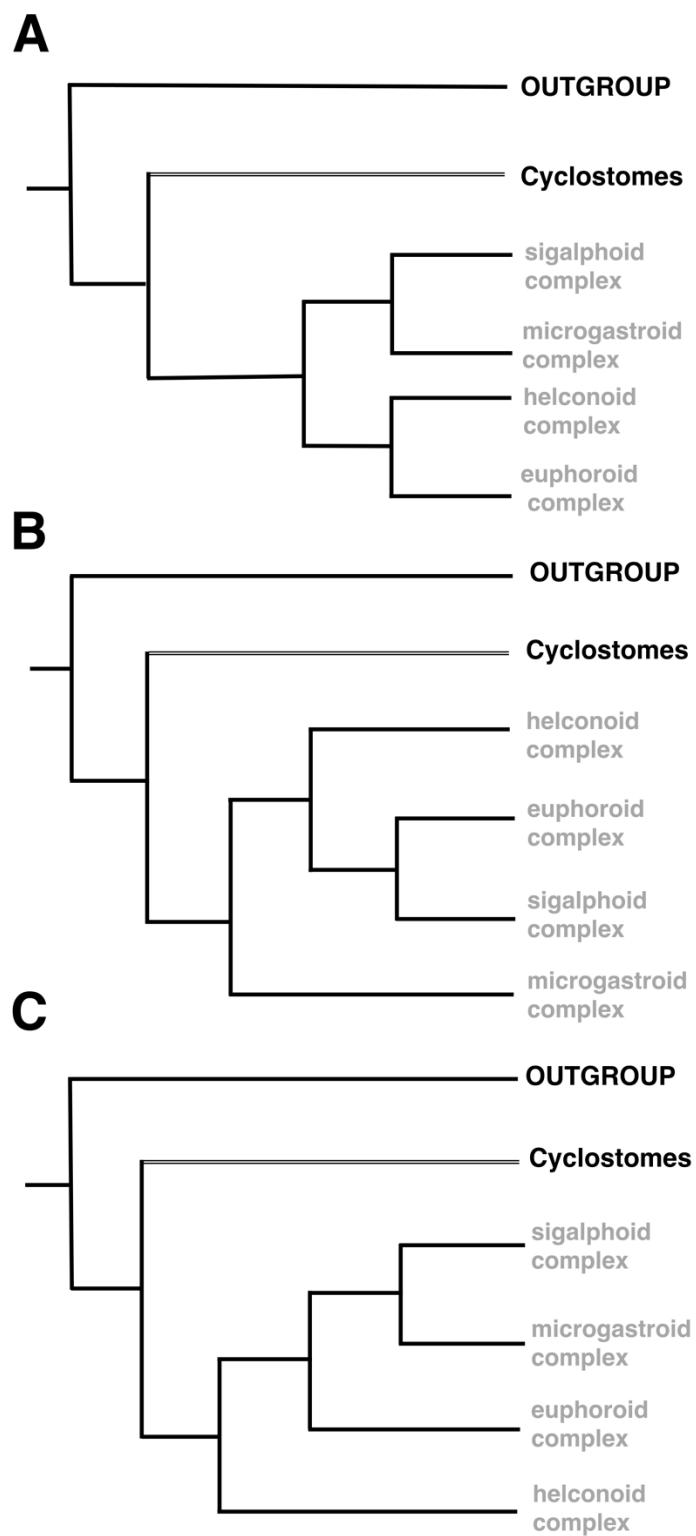
SUPPLEMENTARY MATERIAL

Supplementary data 1. Master spreadsheet including list of taxa with specimen information, lab protocols data, sequencing and assembly data, and SRA codes.

Supplementary data 2. Compilation of lab protocols and methods used across all three sequencing institutions.

Supplementary data 3. 25% matrix in nexus format

Supplementary data 4. 50% matrix in nexus format.

Supplementary data 5. (A) Maximum likelihood trees resulting from the analyses of the matrices with 25% and (B) 50% completeness, both in editable format (.tre).

Supplementary data 6. Results of ancestral states reconstruction and phylogenetic correlation analyses: graphs show proportional likelihoods in each node and basic statistics of each analysis.

CAPÍTULO III: MITOCHONDRIAL PHYLOGENOMICS AND MITOGENOME ORGANIZATION IN THE PARASITOID WASP FAMILY BRACONIDAE (HYMENOPTERA: ICHNEUMONOIDEA)

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Mitochondrial phylogenomics and mitogenome organization in the parasitoid wasp family Braconidae (Hymenoptera: Ichneumonoidea)

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Abstract
Background: Mitochondrial (mt) nucleotide sequence data has been by far the most common tool employed to investigate evolutionary relationships. While often considered to be more useful for shallow evolutionary scales, mt genomes have been increasingly shown also to contain valuable phylogenetic information about deep relationships. Further, mt genome organization provides another important source of phylogenetic information and gene reorganizations which are known to be relatively frequent within the insect order Hymenoptera. Here we used a dense taxon sampling comprising 148 mt genomes (132 newly generated) collectively representing members of most of the currently recognised subfamilies of the parasitoid wasp family Braconidae, which is one of the largest radiations of hymenopterans. We employed this data to investigate the evolutionary relationships within the family and to assess the phylogenetic informativeness of previously known and newly discovered mt gene rearrangements.
Results: Most subfamilial relationships and their composition obtained were similar to those recovered in a previous phylogenomic study, such as the restoration of Trachypetinae and the recognition of Apozyginae and Proteropinae as valid braconid subfamilies. We confirmed and detected phylogenetic signal in previously known as well as novel mt gene rearrangements, including mt rearrangements within the cyclostome subfamilies Doryctinae and Rogadinae.
Conclusions:
Our results showed that both the mt genome DNA sequence data and gene organization contain valuable phylogenetic signal to elucidate the evolution within Braconidae at different taxonomic levels. This study serves as a basis for further investigation of mt gene rearrangements at different taxonomic scales within the family.

Keywords
gene rearrangements, mitochondrial genome, phylogenomics, cyclostome, non-cyclostome

Background
Mitochondria play a central role in cellular metabolism, providing energy to nearly all living eukaryotic organisms [1,2]. Study of mitochondrial (mt) DNA therefore is fundamental in a number of areas of research, including physiology, molecular biology and evolution [2,3]. The metazoan mt genome typically consists of 15-18 kilobases, comprising 13 protein-coding genes, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs) [4], and this composition is generally conserved across bilaterian metazoans [5,6].

The analysis of mt nucleotide sequence data is one of the most common approaches to investigate evolutionary relationships. While often considered more useful for shallow evolutionary scales [7], it has been increasingly shown that mtDNA can also be informative to investigate deeper relationships [8,9]. Another important source of phylogenetic information can be obtained from the mt genome organization, which is generally conserved in many groups of Metazoans but has been shown to be relatively plastic in some insect orders, including Hymenoptera [5,10,11].

With more than 21,000 described species [12] distributed in 41 subfamilies [13], Braconidae (Hymenoptera: Ichneumonoidea) represents one of the largest radiations in Hymenoptera [14]. The vast majority of braconid species are either ecto- or endoparasitoids of juvenile stages of other holometabolous insects [14,15]. Members of this family are divided into two main groups, the cyclostomes *sensu lato,* which are characterized by having the lower part of the clypeus sharply recessed exposing a concave, smooth glabrous labrum [16], and the non-cyclostomes, which do not have the clypeus sharply recessed and with the labrum flat and setiferous [16]. Cyclostomes *s.l.* include the aphidioid subfamilies and other groups that have secondarily lost the cyclostome condition such as Alysiinae, Opiinae, some Betylobraconini within the subfamily Rogadinae and some Gnamptodontini within Telengaiinae. Cyclostomes *s.l.* and non-cyclostomes together comprise the braconoid complex [13].

The higher-level classification of Braconidae has changed considerably through time, in part because of the challenges posed by high levels of morphological convergence among its members [14], limited taxonomic sampling and/or limited number of available molecular markers [e.g., 11,17,18]. For example, some taxa treated as subfamilies within Braconidae (i.e. Apozyginae, Trachypetinae, Masoninae) have been either considered or recovered as separate lineages outside of the family [19,20,21] but also as part Braconidae based on different sources of evidence [11,13,17,22].

There is a general consensus for several subfamily-level relationships both for the cyclostome and non-cyclostome groups using different sources of data [e.g., 11,23,24,25,26]. In particular, two key molecular phylogenetic studies [24,26] confirmed a number of subfamilial relationships with strong support, as well as confidently placing a number of genera whose affinities had previously been rather doubtful. However, the placement and relationships of some other genera and subfamilies has remained unclear; for example, relationships of various genera within Hormiinae and Rhysipolinae and also whether some groups (e.g. Doryctinae and Mesostoinae) are actually monophyletic.

Mt gene rearrangements have been used for inferring evolutionary relationships among the braconid subfamilies since the pioneering study of [27], which found a clear pattern of gene rearrangement between the cyclostome and non-cyclostome groups. For the non-cyclostomes,

the block of tRNAs located between the protein-coding genes *COX2* and *ATP8* was recovered with a *trnK-trnD* pattern [27]. On the other hand, for the cyclostomes this tRNAs block was recovered with three different arrangements: *trnK-trnD*, *trnD-trnK* and *trnK-trnD-trnH* [27]. Subsequent studies have confirmed the above tRNAs arrangements and phylogenetic relationships among subfamilies that have been recovered in previous studies using protein-coding gene sequence data for phylogenetic reconstruction [25,28,29]. Although these studies represent an important basis for investigating the higher-level relationships of Braconidae, their taxonomic sampling was limited, with most of the subfamilies being represented only by one species or not represented at all.

Here, we provide the most comprehensive comparative mitogenomic study of Braconidae carried out to date, using 148 mt genomes (132 newly generated) including species belonging to all cyclostome *s.l.* and most non-cyclostome subfamilies, as well as a number of outgroup taxa. We used protein-coding and rRNA gene sequence data to reconstruct the phylogenetic relationships of Braconidae. We also characterized the organization of the protein-coding, tRNA and rRNA genes at subfamily and tribal levels. We found important variation in the mt gene organization within Braconidae, revealing that this source of data is informative to recognize groups within Braconidae.

Results

Mitogenome assembly and annotation

We generated and annotated a total of 132 complete and partial ichneumonoid mt genomes (Additional file: Table S1). All but 21 mt genomes were recovered in a single contig in the *de novo* assembly (Additional file: Table S1). For cyclostome *s.l.* braconids, we assembled and annotated 29 complete and 58 partial mitogenomes, ranging from 2,730 to 19,429 bp with a mean read depth from 8.8 to 870.9. For the non-cyclostome braconids, we generated four complete and 36 partial mitogenomes, ranging from 4,364 to 16,033 bp with a mean read depth from 12.1 to 1996.0. For the ichneumonid taxa, we obtained one complete and three partial mitogenomes that ranged from 7,924 to 18,583 pb, with a mean read depth from 44.8 to 105.8, whereas we recovered a partial mt genome for *Apozyx penyai* (Apozyginae) that comprised 12,332 bp with a mean read depth = 60.2 (Additional file: Table S1). We found a significant correlation between the mt genome assembly size and age of the specimens (p = 0.0020, R^2 = 0.0946) (Fig. 1A), with specimens between 1-28 and ≥30 years having an average of 13,246.45 and 11,174.42 bp assemblies, respectively.

For 66 out of the 122 mt genomes that were assembled as a secondary product of UCE libraries we recovered the two *rRNAs*, for 28 we only recovered the *rrnL*, for three only the *rrnS* and for 25 both rRNAs were missing (Additional file: Table S1). The protein coding gene that was recovered for most mt genomes was *COX1* (121 mt genomes), whereas NAD2 was missing for 37 (Fig. 1B, Additional file: Table S1). Similarly, for the tRNAs both *trnL2* and *trnA* were recovered for 113 and trnQ was recovered for 55 mt genomes (Fig. 1C, Additional file: Table S1). Within the tRNA blocks *trnA-trnR-trnN-trnS1-trnE-tnrF*, *trnI-trnM-trnQ*, *trnW-trnY-trnC* and *trnK-trnD-trnH*, the tRNAs *trnA*, *trnI*, *trnY* and *trnD* were recovered in most of the assembled mt genomes, respectively (Fig. 1C).

Phylogenetic relationships

The phylograms derived from the two ML analyses (predefining partitions and best-fit partitioning scheme) recovered the same topology, with few differences in bootstrap (BTP) values for some nodes (Fig. 2, Additional file: Fig. S1). The phylogram derived from the

Bayesian analysis recovered almost the same topology obtained in the ML analyses, with most nodes having significantly supported posterior probability (PP) values. Few exceptions were in the placement of the two representatives of *Avga*, which were recovered in the Bayesian phylogram as sister to the clade comprising the Holartic-African-Madagascan (HAM) doryctines + Pambolinae but with low support (PP = 0.52) (Additional file: Fig. S2), whereas in the ML topology *Avga* was sister to a large clade including HAM doryctines + Pambolinae but also Rogadinae, Hormiinae, Rhysipolinae and the braconoid subcomplex (Fig. 2, Additional file: Fig. S2). Other exceptions were the tribal relationships at the interior of Rogadinae, with some nodes recovered with low support, and the placement of *Spathius elegans* Matthews (Doryctinae) as sister to the braconoid subcomplex, but with low support (PP = 0.40).

The topologies obtained from the analyses with distinct levels of missing data were mostly congruent with the phylograms recovered in the ML and Bayesian analyses using the complete matrix (Additional file: Fig. S3). Examples of the same recovered relationships are the monophyly of both cyclostomes and non-cyclostomes, monophyly of most subfamilies (except Doryctinae and Mesostoinae), monophyly of the braconoid subcomplex, sister relationship of the non-cyclostomes with the subfamilies that comprise the aphidioid complex, and the monophyly of the non-cyclostome complexes in the datasets that had taxa representing all four complexes. Finally, in the cases where the only member of Apozyginae was included, it was always recovered as sister to the remaining braconid subfamilies.

Hereafter we refer to the results obtained in the ML analysis conducted using the complete matrix with the best-fit partitioning scheme (Fig. 2), only mentioning the BTP values < 100. The Braconidae was recovered as monophyletic and included *T. clavatus* within the non-cyclostome clade. Both cyclostomes and non-cyclostomes were recovered as reciprocally monophyletic. *Apozyx penyai* was recovered as sister to Braconidae, whereas Ichneumonidae was sister to Braconidae + *A. penyai*.

Cyclostome s.l. clade
Most cyclostome subfamilies were recovered as monophyletic with strong support with the exception of Doryctinae and Mesostoinae (with respect to *Maxfischeria tricolor* Papp).

Rogadinae was recovered as sister to Hormiinae. Within Rogadinae, Rogadini was sister to Stiropiini and both were sister to the remaining tribes. Within Hormiinae, Cedriini was strongly supported as sister to the remaining hormiine tribes (BTP = 99). Hormiini and Pentatermini were sister tribes, but Lysitermini was non-monophyletic, with *Aulosaphoides* sister to *Aulosaphobracon capitatus* Belokobylskij and Long (Aulosaphobraconini) but with low support (BTP = 65). Rhysipolinae appeared as sister to Rogadinae + Hormiinae (BTP = 83), and it was divided into two clades, one containing *Pseudavga* and *Rhysipolis bicarinator* Belokobylskij and the other *Allobracon* and *Parachremylus litchii* Belokobylskij and Maeto.

The Alysiinae and Opiinae were sister groups. Within Alysiinae, most representatives of Dacnusini were nested in a single clade except for *Symphya*, which was sister to *Asyntactus rhogaleus* Marshall (Alysiini) (BTP = 91). The opiines *Diachasma muliebre* Muesebeck (Biosterini) and *Diachasmimorpha longicaudata* Ashmead, on the other hand, were recovered as sister taxa, rendering the Opiini as non-monophyletic. Exothecinae was sister to Alysiinae + Opiinae; *Colastes* was recovered as non-monophyletic with *Colastinus crustatus* Belokobylskij as sister to the remaining exothecines. Braconinae was recovered as sister to the Alysiinae + Opiinae + Exothecinae clade with high support (BTP = 93). Telengaiinae was sister to a clade comprising Braconinae, Exothecinae, Opiinae and Alysiinae.

The included members of the Doryctinae were divided into two separate clades. One contained *Eodendrus*, *Euscelinus*, *Rhaconotus* and *Spathius*, together with the members of Pambolinae. The second doryctine clade mainly included Neotropical genera and was sister to all the aforementioned cyclostome braconid subfamilies. The two representative species of *Avga* (Avgini Belokobylskij) were recovered a sister to all the above subfamilies. Rhyssalinae was sister to all aforementioned cyclostome subfamilies, with the acrisidines *Acrisis brevicornis* Hellén and *Proacrisis orientalis* Tobias as sister taxa with strong support (BTP = 96). However, the placement of *Rhyssalus clavator* Haliday as sister to the remaining rhyssalines rendered the tribe Rhyssalini non-monophyletic.

Aphidioid subfamilies were recovered as sister to remaining cyclostomes *s.s*. For instance, *Maxfischeria tricolor* was recovered within Mesostoinae as sister to *Andesipolis* sp., *Hydrangeocola llaollin* Martínez and *Austrohormius* sp. The subfamily Aphidiinae was recovered as sister to Mesostoinae + *M. tricolor*.

Non-cyclostome clade
The non-cyclostome clade was recovered as sister to the cyclostome *s.l.* subfamilies. Within the helconoid complex, Brachistinae was sister to Acampsohelconinae + Helconinae (BTP = 92). Homolobinae, Microtypinae and Orgilinae were all nested in a single clade. The Macrocentrinae + Charmontinae clade was recovered as sister to all helconoid subfamilies. Within the euphoroid complex, the relationship Euphorinae + Cenocoellinae was recovered, with *T. clavatus* as its sister taxon with a relatively low support (BTP = 85). The sigalphoid complex, composed of the Agathidinae and Sigalphinae, was sister to the euphoroid complex + *T. clavatus* with low support (BTP = 71).

Two main clades were recovered within the microgastroid complex. One had Cardiochilinae at the base followed by Miracinae + Khoikhoinae + Microgastrinae. In the second clade, Cheloninae was sister to *Hebicheutes tricolor* Sharkey and Wharton (Proteropinae) (BTP = 92), whereas *Paroligoneurus* sp. (Ichneutinae) was sister to the microgastroid subfamilies (BTP = 99).

Mitochondrial gene patterns
The 13 protein-coding genes were recovered in the same order in the mt genome of all ichneumonoids, with the following two exceptions. In *Chelonus* sp. (Cheloninae), there was an inversion of the *ATP6* and *ATP8* genes, whereas *Stenocorse bruchivora* Crawford (Doryctinae) displayed various translocations (Additional file: Table S2).

We found several tRNAs rearrangements. For the tRNAs block surrounding the *NAD2* protein-coding gene we found three different rearrangements for the non-cyclostome subfamilies: (1) *trnW-trnY-trnC,* (2) *trnY-trnC-trnW,* (3) *trnW-trnC-trnY* (Fig. 3, Additional file: Table S2). For the cyclostomes *s.l.* (including Aphidiinae, Mesostoinae and Maxfischeriinae), we found five general rearrangements: (1) *trnW-trnY-trnC*, (2) *trnC-trnW-trnY*, (3) *trnW-trnC-trnY*, (4) *trnY-trnC-trnW* and (5) *trnC-trnY-trnW* (Fig. 3, Additional file: Table S2).

The tRNA block located between the protein-coding genes *COX2* and *ATP8* showed a *trnK-trnD* pattern for some cyclostome subfamilies (Mesostoinae, Maxfischeriinae, Aphidiinae and neotropical doryctines) and *trnD-trnK* in Rhyssalinae. The *trnK-trnD* pattern was also found for most non-cyclostomes, except for *Meteorus* sp. (Euphorinae) and for the Microgastrinae taxa, which had the *trnD-trnK* and *trnH-trnD-trnK* orders, respectively (Fig. 3, Additional file: Table S2). For most remaining cyclostome subfamilies there was a *trnD-trnH-trnK* order, although the orders *trnK-trnH-trnD* and *trnD-trnK-trnH* were also recovered. We found different

rearrangements for the tRNA block located between the protein-coding genes *NAD3* and *NAD5*, although for most cyclostome and non-cyclostome subfamilies the *trnA-trnR-trnN-trnS1-trnE-trnF* order was the most common (Additional file: Table S2).

We observed tRNAs rearrangement patterns within subfamilies with a better taxon representation. For instance, within Rogadinae we observed rearrangements that were congruent with its tribal classification. The *trnG* was mostly found between the protein-coding genes *COX3* and *NAD3*, although for members of the tribe Rogadini it was found as part of the *trnI, trnM, trnQ* block, located between the rRNAs and the protein-coding gene *NAD2* (Fig. 3, Additional file: Table S2). For the clade including Pambolinae + the doryctine genera *Eodendrus*, *Euscelinus*, *Rhaconotus* and *Spathius*, we recovered three different patterns of the tRNAs block between the protein coding genes *COX2* and *ATP8*: (1) *trnD-trnK-trnH*, (2) *trnD-trnH-trnK* and (3) *trnD-trnH* (with the *trnK* located together with *trnI-trnM-trnA-trnQ,* near to the protein coding gene *NAD2*) (Fig. 3, Additional file: Table S2). On the other hand, for the Neotropical Doryctinae clade, this tRNA block followed a *trnK-trnD* order, except for *S. bruchivora*, with this block located between the protein coding genes *NAD4* and *ATP8* including the *trnT* (Fig. 3, Additional file: Table S2).

Discussion

Here we have generated and assembled a large number of mitogenomes for representative species belonging to most subfamilies of Braconidae based on both recently collected and older museum specimens. Our analyses yielded a robustly supported phylogeny that was generally concordant with a recent estimate based on nuclear UCE data [13] thus supporting previous results that mt genome DNA sequence data contain considerable phylogenetic signal at deep-level relationships in insects [8,9,30]. Moreover, our comprehensive taxon sampling helped confirm previously known and discover novel gene rearrangements, respectively, which contain phylogenetic signal that correspond with recognized taxa within Braconidae. Below we discuss the most relevant relationships that were supported both by the mitogenome sequence data and gene rearrangements and also highlight the importance that gene reorganization has to unveil the evolutionary history of this megadiverse family.

Phylogenetic relationships and subfamily level classification in Braconidae

Jasso-Martínez et al. [13] recently proposed 41 braconid subfamilies based on a phylogenomic study with UCE data. Although our study lacked representatives of six subfamilies, the well-supported relationships that were obtained in our best estimate of phylogeny are mostly concordant with those found in the aforementioned study and thus confirmed most of their subfamilial limits and composition.

Our analyses consistently recovered *A. penyai* as sister to the remaining braconid subfamilies. This sister group relationship was also recovered in an ultra-conserved elements (UCE) data study [13]. *Apozyx penyai* possess some morphological features that are absent in nearly all extant braconids but present in Ichneumonidae and some extinct Braconidae [31], such as the presence of fore wing vein 2m-cu (although also occurring occasionally as an atavism in some Rhyssalinae and Doryctinae) [32,33,34]. However, it also shares several morphological features with braconids, including the cyclostome condition, fusion of second and third metasomal terga and various venation features [35], thus supporting its placement within the family as the subfamily Apozyginae.

Trachypetidae, consisting of the genera *Trachypetus*, *Megalohelcon* and *Cercobarcon*, was recently elevated to family level based on a phylogenetic study that employed five gene

sequence markers and external morphological features [20]. Similar to the Jasso-Martínez et al. [13] study, here we recovered a monophyletic Braconidae with the inclusion of *T. clavatus*. However, we recovered this species as sister to the euphoroid complex without strong support, whereas in the latter nuclear phylogenomic study it was consistently placed as sister to all non-cyclostome subfamilies except Meteorideinae. Trachypetines possess morphological features that are absent in all braconids but are typical of ichneumonids, such as a separation of hind wing veins C and SC+R and the presence of a wing flexion line anterior to hind wing vein M. It also has a small and open fore wing costal cell as in many Cretaceous braconids. Trachypetines also have a well-developed hind wing vein 2-CU typical of ichneumonids but also present in Apozyginae and in the non-cyclostome braconid subfamilies Agathidinae, Sigalphinae, Acampsohelconinae and Meteorideinae [20]. Further studies including members of the two remaining trachypetine genera are necessary to definitively discern the placement of trachypetines within the non-cyclostome clade.

Most of the subfamilial relationships that were strongly supported within Braconidae were concordant with those obtained in other molecular studies [13,24,26,34,36,37,38]. Among these are the placement and composition of the aphidioid complex, which we recovered as sister to all other cyclostomes *sensu stricto* and containing Aphidiinae, Mesostoinae and Maxfischeriinae, with the only member of Maxfischeriinae, *M. tricolor*, deeply nested within Mesostoinae (both cyclostome *s.s.* and aphidioid complex comprising the cyclostome *s.l.* group [13]). Other subfamilial relationships, such as the placement of Rhyssalinae as sister to the remaining cyclostomes *s.s.,* the close relationship and composition of Rhysipolinae, Hormiinae and Rogadinae, and composition of the non-cyclostome subfamily complexes were also confirmed by our mt genome data.

Non-monophyly of the highly diverse subfamily Doryctinae has been recovered both with Sanger sequence and genomic-scale data but with low support [13,39,40]. Here we again recovered a non-monophyletic Doryctinae, being divided into two main non-sister clades but with the implied relationships having low support. One of the large main clades included members of the "South American" and the other of the "Holarctic-African-Madagascan" clades that were obtained in Zaldívar-Riverón et al. [40] phylogenetic study of the subfamily, although here the latter clade included the species of the small subfamily Pambolinae. Comprehensive taxon sampling will be needed to elucidate whether Doryctinae as defined traditionally is monophyletic. This subfamily has long been considered hard to diagnose based on derived characters, the row of pegs on the fore tibia often used in subfamily keys being a homoplastic character associated with egress from concealed pupation sites in wood [14]. However, its monophyly is suggested by a small suite of ovipositor tip characters [41] and separate insertions of venom ducts onto the venom reservoir [42].

As has been revealed by numerous previous studies, the Hormiinae *sensu lato* and the Exothecinae are not closely related, even though they had often been treated as synonymous [e.g., 43]. The genus *Avga* was proposed, together with other genera, to comprise the tribe Avgini, and subsequently it has been placed within Exothecinae, Mesostoinae or Hormiinae [34,44,45,46,47,48,49]. Here we recovered *Avga* as sister to a clade comprising Rogadinae, Hormiinae, Rhysipolinae, the braconoid subcomplex and the Holarctic-African-Madagascan (HAM) doryctines + Pambolinae, whereas in the UCE study by Jasso-Martínez et al. [13] it was nested together with *Xenosternum* at the base of the Rhysipolinae + Hormiinae + Rogadinae clade. Additional studies will thus reveal the phylogenetic affinities of *Avga*, which currently is considered as *incertae sedis* within Braconidae [13].

Based on Sanger sequence data, Sharanowski et al. [26] recovered a clade with intermingled species of Alysiinae, Opiinae, Exothecinae and Telengaiinae (previously Gnamptodontinae), naming it as the alysioid subcomplex. In our analyses, this clade consistently had Braconinae as sister to the former three subfamilies, a relationship that has been recovered in other studies based on analyses of Sanger-sequenced genes [48]. Jasso-Martínez et al. [13] recovered the same taxon composition but with Braconinae as sister to Telengaiinae and renamed it as the braconoid subcomplex.

Four subfamily complexes were considered by Sharanowski et al. [26] within the non-cyclostome lineage—the euphoroid, helconoid, microgastroid and sigalphoid complexes. Our phylogenetic estimates recovered a mainly similar subfamily grouping composition but with different relationships among the complexes in comparison with the Sharanowski et al. [26] and Jasso-Martínez et al. [13] phylogenies. We obtained the microgastroids as sister to the remaining complexes, followed by the helconoids, sigalphoids and the euphoroids + *T. clavatus*. In contrast, in the above two studies the sigalphoids were sister to the microgastroids, and particularly for Jasso-Martínez et al. [13] the only examined member of Meteorideinae was sister to all the non-cyclostomes followed by *T. clavatus* (Trachypetinae), the helconoids and then the euphoroids.

Recently, Jasso-Martínez et al. [13] expanded the composition of the sigalphoid complex to contain Ichneutinae in a restricted sense, including the genera *Ichneutes*, *Oligoneurus* and *Paroligoneurus*, whereas the genera *Hebichneutes*, *Masonbeckia* and *Proterops*, previously placed within Ichneutinae, were included in the subfamily Proteropinae, with the latter being sister to the microgastroid complex. The Proteropinae had also been treated as a subfamily by Chen and van Achterberg [50] and Sharkey et al. [51] based on evidence of previous phylogenetic studies that failed to recover Ichneutinae as monophyletic [e.g. 26]. Nevertheless, Jasso-Martínez et al. [13] phylogenomic study is the only one that has separately recovered both lineages with high support, and therefore, they confirmed Proteropinae as a subfamily. Despite that we had a limited taxon sampling for the non-cyclostome taxa, our results are partially in agreement with the above study, since *Hebichneutes* (Proteropinae) was nested within the microgastroid complex, although *Paroligoneurus* (Ichneutinae) was sister to all microgastroid subfamilies. This contrasts with Jasso-Martínez et al. [13], where Ichneutinae was placed within the sigalphoid group of subfamilies.

Mt gene rearrangement evolution within Braconidae
Mt gene rearrangements have been shown to be phylogenetically informative at different evolutionary scales in various insect orders, recovering particular patterns for specific lineages [7,25,29,52,53,54,55]. In Hymenoptera, sawflies and woodwasps (previously known as Symphyta) usually have a conserved mt gene order, whereas various gene rearrangements have been reported for Apocrita [7,9,56].

Previous studies have reported the existence of particular mt gene rearrangements within Braconidae, although taxon sampling in these works was rather limited, only including part of the currently recognised subfamilies and only one or few of their species [11,25,27,28,29]. These studies showed that mt protein-coding gene organization in Braconidae is not substantially different from the putative ancestral Pancrustacean mt genome or among members of this family. Our results confirm this conservative mt protein-coding gene order, as we only found a novel inversion of the *ATP6* and *ATP8* genes in one member of Cheloninae and confirmed previously reported translocations in the doryctine *S. bruchivora* [57]. A conserved protein-coding gene order is also present in other hymenopteran families [7,56].

In contrast with the protein-coding genes, it has been shown that there are some differences in the tRNAs order pattern between the cyclostome and non-cyclostome subfamilies [11,25,27,28,29]. However, the existence of additional phylogenetically informative tRNAs rearrangements at different levels of divergence was unknown. Our comprehensive taxonomic sampling not only helped confirm the above tRNAs rearrangements but also found that tRNAs reorganizations appear to be consistent with tribes recognized in the two subfamilies with highest species representation, Rogadinae and Doryctinae.

We corroborated the previously observed rearrangements in three main tRNA clusters between members of the cyclostome and non-cyclostome subfamilies, with the non-cyclostomes and the earliest diverging cyclostomes having a more conserved mt gene organization [25,27,28]. These tRNAs rearrangements involve the blocks comprising *trnK-trnD-trnH* located between the protein-coding genes *COX2* and *ATP8* [25,27,28], the block comprising *trnW-trnC-trnY* near to the protein-coding gene *NAD2* [25] and the block comprising *trnA-trnR-trnN-trnS1-trnE-trnF* located between the protein-coding genes *NAD3* and *NAD5* [58].

We found two novel patterns of tRNA rearrangements that appear to be phylogenetically informative and correspond to tribes within Rogadinae. In one of them, the included species of the tribe Rogadini had a translocation of the *trnG*, which was flanked by the ribosomal *rrnS* locus as part of the tRNAs cluster *trn*I-*trn*M-*trn*Q. In contrast, the remaining tribes had the putative ancestral condition, where the *trnG* was located between the protein-coding genes *COX3* and *NAD3*. The second rearrangement was detected in the tRNA block situated between the protein coding genes *NAD2* and *COX1*. Within Aleiodini we recovered the *trnY-trnC-trnW* order; we found *trnW-trnC-trnY* for Yeliconini and *trnW-trnY-trnC* for Stiropiini and Betylobraconini. For Rogadini there were three different orders—*trnY-trnW-trnC, trnW-trnC-trnY* and *trnW-trnY-trnC*.

Similar to other phylogenetic studies [13,40], we recovered the Doryctinae as non-monophyletic, being divided into two separate main clades that were each mainly composed of "South American" (SA) and "Holartic-African-Madagascan" (HAM) genera, respectively. We found two clear differential patterns of tRNAs among these clades between the protein-coding genes *COX2* and *ATP8*. In the HAM doryctine clade, which also included Pambolinae, this tRNAs cluster included *trn*D*, trn*H and *trn*K. On the other hand, similar to the results obtained in Samacá-Sáenz et al. [57], in the SA clade this tRNAs cluster was generally composed of *trnD* and *trnD* except for *S. bruchivora*, whose translocation was located between the protein-coding genes *NAD4L* and *ATP8* with a *trnK-trnT-trnD* order. The *trnK-trnD* order observed in the Neotropical doryctine clade was similar to the one found here in the cyclostome *s.l.* subfamilies Rhyssalinae, Aphidiinae, Mesostoinae and Maxfisherinae, as well as in all non-cyclostomes.

Recovering mt genomes from UCE libraries
The analysis of mt nucleotide sequence data is one of the most common approaches to investigate evolutionary relationships. Generation of mt DNA was until the last decade generally obtained using Sanger sequencing; however, with the advent of next-generation sequencing (NGS), the generation of complete mt genomes has become relatively simple to obtain due the considerably higher efficiency of NGS technologies [30].

In recent years, the sequence capture of UCEs has become one of the most used methods for obtaining genomic-scale data to investigate evolutionary relationships of several animal taxa, including insects [e.g. 59,60,61]. Regardless of the targeted nature of this technique, raw UCEs datasets can be harvested to recover off-target sequences such as mt DNA, with the possibility of

assembled complete mt genomes [62]; thus, the recovery of mt genomes from UCE libraries is currently increasing in phylogenomic studies [e.g., 57,63].

In this study, we have shown the efficiency that the raw UCE data have to obtain mt genome sequence data for phylogenomic reconstruction, even when using old and dry museum specimens, since target enrichment methods have shown a higher success rate when working with old museum specimens over other techniques such as RADseq [64]. Here, we recovered shorter assemblies from older samples compared to recently collected samples whose mt genomes were extracted as a secondary product of the UCE data. However, despite the direct relationship between sample age and size of mt assembly, the assembled mitogenomes contained considerable phylogenetic information. As a result we were able to recover a robust estimate of phylogeny, even with a high amount of missing data, that was mostly congruent with a phylogeny obtained using targeted UCE regions [i.e., 13].

Conclusions

This comprehensive mt phylogenomic study of Braconidae showed that both the mt genome DNA sequence data and gene organization contain valuable phylogenetic signal that can be employed to elucidate the evolution of this megadiverse group of hymenopterans at different levels of divergence, including deep relationships. This is supported by our phylogenetic reconstruction, which was mostly consistent with previous phylogenetic hypotheses, particularly the one based on nuclear-genome scale data [13]. Moreover, the gene rearrangements discovered in our study can be used as diagnostic features for tribal delimitation within Rogadinae and Doryctinae. Future studies should be carried out with more extensive taxon sampling to discern the existence of phylogenetically informative variation within other braconid subfamilies.

Methods

Taxonomic sampling

Our taxon sampling comprised 128 and 143 ingroup genera and species, respectively, covering all biogeographic regions and belonging to most of the extant currently recognized braconid subfamilies [see 13,14,37,50]. We included 102 species from all cyclostome *s.l.* subfamilies and 40 non-cyclostome species comprising the helconoid, euphoroid, sigalphoid and microgastroid complexes (*sensu* Sharanowski et al., [26]), representing most of the non-cyclostome subfamilies except for Amicrocentrinae, Dirrhopinae, Masoninae, Mendesellinae, Meteorideinae, and Xiphozelinae. We also included a specimen of *Apozyx penyai* Mason. This enigmatic taxon has been placed in its own family, Apozygidae [19] or within Braconidae [13,17,31,65]. We also included a specimen of *Trachypetus clavatus* Guérin-Meneville, which has been placed within Braconidae [66,67] or elevated as the family Trachypetidae [20], although it was recently returned to Braconidae based on genomic-scale data [13].

We included four species of the family Ichneumonidae as outgroup taxa: *Vulgichneumon* sp. (Ichneumoninae), *Pimpla aequalis* Provancher (Pimplinae), *Netelia* sp. (Tryphoninae) and *Odontocolon albotibiale* Bradley (Xoridinae). We used data from a species of Megaspilidae, of the superfamily Ceraphronoidea, to root the trees. This superfamily was found to be sister to Ichneumonoidea in a recent study based on transcriptomic data [68]. Voucher specimens are housed in the Colección Nacional de Insectos at the Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM); at the Smithsonian Institution National Museum of Natural History, Washington, DC (USNM); in the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia (ZISP) and at the Canadian National Collection of Insects (CNC), Ottawa, Canada. A list with GenBank accession numbers of the mitogenomes assemblies

and further details of all the taxa examined in this study are provided in Additional file: Table S3. [DATA WILL BE RELEASED AFTER ACCEPTANCE]

Assembly and annotation of mt genomes
The mt genomes of 122 samples were extracted in silico from raw reads generated from libraries that were originally prepared for obtaining ultra-conserved element (UCE) loci. Details of genomic DNA extraction and library prep protocols are given by Jasso-Martínez et al. [13,37]. For 10 additional samples (Additional files: Tables S1, S3), we used data generated by whole-genome shotgun sequencing. Shotgun libraries were prepared using the Kapa Hyper Prep kit (Kapa Biosystems Inc. Wilmington, MA, U.S.A) and the TruSeq-style dual-indexing adapters [69]. Sequencing was performed in an Illumina HiSeq 2500 instrument (PE125, v4 chemistry) at the Department of Environmental Health Science, University of Georgia, Athens, GA, U.S.A.

Raw reads from the UCEs libraries were trimmed and filtered using Illumiprocessor [70], a wrapper around Trimmomatic [71,72] in the pipeline Phyluce version 1.6.6 [73]. Raw reads from the shotgun sequencing were filtered using Geneious 10.2.6 [74]. Cleaned reads were *de novo* assembled into the mt genome sequence with the GetOrganelle toolkit [75] using the default database 'animal_mt'. For the datasets from which we did not recover the complete mitogenome or obtained more than one contig in the *de novo* assembly, we used a combination of assembly approaches in order to obtain longer contigs as follows. For a given sample, the contig(s) obtained in GetOrganelle were used as template to obtain a unique and longest contig using by-reference assembly in the program Geneious 10.2.6 [74]. We avoided using as template the assembled mitogenome from a different sample, even if closely related, so as to not bias the specific gene order of each individual.

The mt sequences of 14 doryctines and *Pambolus oblongispina* (Pambolinae) that were generated in Samacá-Sáenz et al. [57] study (Additional file: Table S3) were downloaded from GenBank and annotated together with the assemblies obtained in this study in the MITOS 2 webserver [76] using the invertebrate genetic code. We verified the protein-coding genes signal from the "protein plots" generated by MITOS. Finally, we used the program Geneious version 10.2.6 [74] to confirm the accuracy of our assemblies and annotations. We registered the order of the protein-coding genes, tRNAs and rRNAs to identify patterns of gene rearrangements using as reference the Pancrustacea ground pattern, which is the proposed Crustacea/Hexapoda common ancestor [77,78].

Several of the museum specimens employed in this study were of considerable age. We therefore investigated the correlation between specimen's age (0 – 91 years old) with the mt genomes assembly size calculating the Pearson's correlation coefficient of these variables with R version 3.6.0 [79]. We also used R to plot the number of mt genomes for which each protein-coding and *tRNA* genes were recovered. For both the statistical tests and plots, we excluded the mt genomes of samples that did not have a collection date, that we did not assemble in this study (i.e., most doryctines and *Pambolus oblongispina* [57], as well as those that were assembled from shotgun libraries.

Matrix alignment and phylogenetic analyses
We extracted for all samples the 13 protein-coding and the two ribosomal RNAs (rRNAs) sequences. The alignments of the protein-coding genes were performed independently (13 alignments) with the program MAFFT version 7 [80]. We verified the protein-coding gene alignments with respect to the reading frame (invertebrate mt genetic code). Some regions of the translated alignments had unalignable regions. These ambiguities were delimited by identifying

the conserved flanking regions and removed. The mt rRNA gene regions were aligned according to Wu et al. [81] model with additional reference to Buckley et al. [82]. The 16S gene was aligned between the core I region and five bases after H2675, a length comprising approximately 1140 bases of which 763 were considered reliably alignable. For 12S, we considered approximately 620 bases between H500 until 7 bases following the H1506 helix. Of these, 340 bases were reliably alignable. In both cases, the analyzed reliably alignable positions included a mix of base-pairing helix stems, as well as length conserved loops, expansion regions and stretches of core sequence. The alignable bases of the ribosomal genes and the 13 protein-coding gene alignments, a total of 15 genes for the complete matrix, were concatenated in the program Geneious version 10.2.6 [74].

We predefined 41 partitions for the concatenated matrix: three partitions based on codon position for each of the 13 protein-coding genes and one partition each for the two rRNA genes. We selected the best-fit partitioning scheme and substitution model with ModelFinder [83] in the program IQTREE version 2 [84] according to the Bayesian information criterion, obtaining 17 subsets of partitions. We conducted two Maximum Likelihood (ML) analyses in IQTREE version 2 [84] with 1000 ultra-fast bootstrap replicates using 1) the matrix with the 41 predefined partitions based on codon position and rRNAs, and 2) the matrix with the best-fit partitioning scheme. The concatenated alignment consisted of 148 terminal taxa and 11,717 base pairs. For the matrix with the best-fit partitioned scheme we also conducted a Bayesian analysis with the program Mr. Bayes version 3.2.7 [85], which consisted of two simultaneous runs of 50 million generation each, sampling trees every 5000 generations and a burn-in fraction of 0.25. The concatenated alignment including partition sets and the annotated alignments of the used ribosomal genes 16S and 12S are available as Additional files: S1, S2 and S3, respectively.

We evaluated whether different levels of missing data and number of taxa had an effect on our phylogenetic inferences. For this, we generated four additional datasets considering the number of missing genes as follows: 1) dataset including taxa with no missing genes, 2) dataset including taxa with 0 – 2 missing genes, 3) dataset including taxa with 0 – 6 missing genes and 4) dataset including taxa with 0 – 9 missing genes. Therefore, for each dataset, we included 70, 105, 128 and 142 taxa including the outgroup, respectively. For each dataset we selected the best-fit partitioning scheme and substitution model with ModelFinder [83] and performed ML analyses in IQTREE version 2 [84] with 1000 ultra-fast bootstrap replicates. All four matrices and their included partition sets are available in a single file as Additional file: File S4.

Abbreviations

mt; mitochondrial; mtDNA: mitochondrial DNA; mt genome(s): mitochondrial genome(s); tRNAs: transfer RNAs; rRNAs: ribosomal RNAs; *trn*: a particular tRNA; *ATP6*: mitochondrial synthase membrane subunit 6; *ATP8*: mitochondrial synthase membrane subunit 8; *COX1*: cytochrome c oxidase subunit I; *COX2*: cytochrome c oxidase subunit II; *COX3*: cytochrome c oxidase subunit III; *NAD2*: NADH dehydrogenase 2; *NAD3*: NADH dehydrogenase 3; *NAD4*: NADH-ubiquinone oxidoreductase chain 4; *NAD4L*: NADH-ubiquinone oxidoreductase chain 4L; *NAD5*: NADH-ubiquinone oxidoreductase chain 5; *rrnS*: mitochondrial small subunit ribosomal RNA (12S); *rrnL*: mitochondrial large subunit ribosomal RNA (16S); *s.s.*: *sensu stricto*; *s.l.*: *sensu lato*; bp: base pairs; BTP: bootstrap; UCE(s): ultra-conserved element(s); SA: South American; HAM: Holartic-African-Madagascan; NGS: next-generation sequencing.

Declarations
Ethics approval and consent to participate

Not applicable
Consent for publication
Not applicable
Competing interests
The authors declare that they have not competing interests.

Authors' contributions
JMJM and AZR conceived and designed the study. JMJM performed lab work, data curation, carried out computational analyses and, together with AZR, coordinated the original draft of this manuscript. DLJQ provided samples, performed the alignment of the mt rRNAs and revised and edited previous and final version of this manuscript. SAB, BFS, JFT and RRK provided samples and revised and edited previous and final version of this manuscript. All authors approved the final manuscript.

Availability of data and materials
All examined datasets are available as supplementary materials, raw data is available for download in the Sequence Read Archive of the National Center for Biotechnology Information (SRA-NCBI) under the BioProject accession number [WILL BE PROVIDED AFTER ACCEPTANCE] and the annotated mt genomes are also available in NCBI.

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References

1. Carlucci A, Lignitto L, Feliciello A. Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. Trends Cell Biol. 2008;18:604–613.
2. Osellame LD, Blacker TS, Duchen MR. Cellular and molecular mechanisms of mitochondrial function. Best Pract Res Clin Endocrinol Metab. 2012;26:711-723.
3. Ballard JWO, Pichaud N. Mitochondrial DNA: more than an evolutionary bystander. Funct Ecol. 2014;28:218-231.
4. Boore JL. Animal mitochondrial genomes. Nucleic Acids Res. 1999;27:1767–1780.
5. Cameron SL. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu Rev Entomol. 2014;59:95–117.
6. Shen KN, Yen TC, Chen CH, Li HY, Chen PL, Hsiao CD. Next generation sequencing yields the complete mitochondrial genome of the flathead mullet, *Mugil cephalus* cryptic species NWP2 (Teleostei: Mugilidae). Mitochondrial DNA Part A 2016;27:1758–1759.
7. Song F, Li H, Jiang P, Zhou X, Liu J, Sun C, et al. Capturing the phylogeny of Holometabola with mitochondrial genome data and Bayesian site-heterogeneous mixture models. Genome Biol Evol. 2016;8:1411–1426.
8. Simon S, Hadrys H. A comparative analysis of complete mitochondrial genomes among Hexapoda. Mol Phylogenet Evol. 2013;69:393-403.
9. Tang P, Zhu JC, Zheng BY, Wei SJ, Sharkey M, Chen XX, et al. Mitochondrial phylogenomics of the Hymenoptera. Mol Phylogenet Evol. 2019;131:8-18.
10. Moreno-Carmona M, Cameron SL, Quiroga CFP. How are the mitochondrial genomes reorganized in Hexapoda? Differential evolution and the first report of convergences within Hexapoda. Gene 2021;791:145719.
11. Dowton M, Belshaw R, Austin AD, Quicke DLJ. Simultaneous molecular and morphological analysis of braconid relationships (Insecta: Hymenoptera: Braconidae) indicates independent mt-tRNA gene inversions within a single wasp family. J Mol Evol. 2002;54:210–226.
12. Yu DS, van Achterberg C, Horstmann K. Taxapad 2016, Ichneumonoidea 2015. 2016;Database on flash-drive (CD).
13. Jasso-Martínez JM, Santos BF, Zaldívar-Riverón A, Fernandez-Triana J, Sharanowski BJ, Richter R, et al. Phylogenomics of braconid wasps (Hymenoptera, Braconidae) sheds light on classification and evolution of parasitoid life history traits. Mol Phylogenet Evol. submitted.
14. DLJ. *The braconid and ichneumonid parasitoid wasps: biology, systematics, evolution and ecology*. Chichester: John Wiley & Sons; 2015.
15. Gauld ID. Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). Bio J Linn Soc. 1988;35:351–377.
16. Wharton RA. Introduction. In: Wharton RA, Marsh PM, Sharkey MJ. (eds.) *Manual of the New World genera of the family Braconidae (Hymenoptera)*. Washington DC, USA: International Society of Hymenopterists, Special Publ. 1; 1997. p. 1–18.

17. Quicke DLJ, van Achterberg C. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera). Zool Verh Leide. 1990;258:1–95.
18. Pitz K, Dowling APG, Sharanowski BJ, Boring CAB, Seltmann KC, Sharkey MJ. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) as proposed by Shi et al.: a reassessment. Mol Phylogenet Evol. 2007;43:338–343.
19. Mason WRM. A new genus, species and family of Hymenoptera (Ichneumonidae) from Chile. Proc of the Entomol Soc Wash. 1978;80:606-610.
20. Quicke DLJ, Austin AD, Fagan-Jeffries EP, Hebert PDN, Butcher BA. Recognition of the Trachypetidae stat. n. as a new extant family of Ichneumonoidea (Hymenoptera), based on molecular and morphological evidence. Syst Entomol. 2020a;45:771–782.
21. Quicke DLJ, Austin AD, Fagan-Jeffries EP, Hebert PDN, Butcher BA. Molecular phylogeny places the enigmatic subfamily Masoninae within the Ichneumonidae, not the Braconidae. Zool Scripta 2020b;49:64–71.
22. van Achterberg C. Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. Zool Verh. 1995;298:1–242.
23. Belshaw R, Herniou E, Gimeno C, Fitton MG, Quicke DLJ. Molecular phylogeny of the Ichneumonoidea (Hymenoptera) based on D2 expansion region of 28S rDNA. Syst Entomol. 1998;23:109–123.
24. Zaldívar-Riverón A, Mori M, Quicke DLJ. Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. Mol Phylogenet Evol. 2006;38:130–145.
25. Wei SJ, Shi M, Sharkey MJ, van Achterberg C, Chen XX. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. BMC Genomics 2010;11:1–16.
26. Sharanowski BJ, Dowling AP, Sharkey MJ. Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. Syst Entomol. 2011;36:549–572.
27. Dowton M. Relationships among the cyclostome braconid (Hymenoptera: Braconidae) subfamilies inferred from a mitochondrial tRNA gene rearrangement. Mol Phylogenet Evol. 1999;11:283–287.
28. Li Q, Wei SJ, Tang P, Wu Q, Shi M, Sharkey, et al. Multiple lines of evidence from mitochondrial genomes resolve phylogenetic relationships of parasitic wasps in Braconidae. Genome Biol Evol. 2016;8:2651-2662.
29. Feng Z, Wu Y, Yang C, Gu X, Wilson JJ, Li H. et al. Evolution of tRNA gene rearrangement in the mitochondrial genome of ichneumonoid wasps (Hymenoptera: Ichneumonoidea). Int J Bio. Macromol. 2020;164:540–547.
30. Song N, Cai W, Li H. Deep-level phylogeny of Cicadomorpha inferred from mitochondrial genomes sequenced by NGS. Sci. Rep. 2017;7:1-11.
31. Belokobylskij SA, Jouault C. Two new striking braconid genera (Hymenoptera: Braconidae) from the mid-Cretaceous Burmese amber. Proc Geologists' Assoc. 2021;132:426-437.
32. van Achterberg C. Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). Zool Verh. 1993;283:1–189.
33. Sharkey MJ. Family Braconidae. In: Goulet H, Huber JT (eds.) *Hymenoptera of the World: An Identification Guide to Families*. Ottawa, Canada: Research Branch Agriculture Canada Publication; 1993. p. 362–395.

34. Quicke DLJ, Belokobylskij SA, Braet Y, van Achterberg C, Hebert PDN, Prosser SWJ, et al. Phylogenetic reassignment of basal cyclostome braconid parasitoid wasps (Hymenoptera) with description of a new, enigmatic Afrotropical tribe with a highly anomalous 28S D2 secondary structure. Zool J Linn Soc. 2020;190:1002–1019.

35. Sharkey MJ, Wahl DB. Cladistics of the Ichneumonoidea (Hymenoptera). J Hym Res 1992;1:15–61.

36. Sharanowski BJ, Ridenbaugh RD, Piekarski PK, Broad GR, Burke GR, Deans AR, et al. Phylogenomics of Ichneumonoidea (Hymenoptera) and implications for evolution of mode of parasitism and viral endogenization. Mol Phylogenet Evol. 2021;156:107023.

37. Jasso-Martínez JM, Quicke DLJ, Belokobylskij SA, Meza-Lázaro RN, Zaldívar-Riverón, A. Phylogenomics of the lepidopteran endoparasitoid wasp subfamily Rogadinae (Hymenoptera: Braconidae) and related subfamilies. Syst Entomol. 2021;46:83–95.

38. Quicke DLJ, Fagan-Jeffries E, Jasso-Martínez JM, Zaldívar-Riverón A, Shaw MR, Janzen DH, et al. A molecular phylogeny of the parasitoid wasp subfamily Rogadinae (Hymenoptera: Ichneumonoidea: Braconidae) with descriptions of three new genera. Syst Entomol. 2021;46:1019-1044.

39. Zaldívar-Riverón A, Belokobylskij SA, León-Regagnon V, Martínez JJ, Briceno R, Quicke DLJ. A single origin of gall association in a group of parasitic wasps with disparate morphologies. Mol Phylogenet Evol. 2007;44:981-992.

40. Zaldívar-Riverón A, Belokobylskij SA, León-Regagnon V, Briceño-G R, Quicke DLJ. Molecular phylogeny and historical biogeography of the cosmopolitan parasitic wasp subfamily Doryctinae (Hymenoptera: Braconidae). Invertebr Syst. 2008;22:345-363.

41. Quicke DLJ, Ficken L, Fitton MG. New diagnostic ovipositor characters for doryctine wasps (Hymenoptera, Braconidae). J Nat Hist. 1992;26:1035–1046.

42. Quicke DLJ, Tunstead J, Falco JV, Marsh PM. Venom gland and reservoir morphology in the Doryctinae and related braconid wasps (Insecta, Hymenoptera, Braconidae). Zool. Scr. 1992;21:403–416.

43. Whitfield JB, Wharton RA. Subfamily Hormiinae. In: Wharton RA, Marsh PM, Sharkey MJ. (eds.) *Manual of the New World genera of the family Braconidae (Hymenoptera)*. Washington DC, USA: International Society of Hymenopterists, Special Publ. 1; 1997. p. 285–301.

44. Nixon GEJ. New genera and species of Hormiinae, with a note on *Hormiopterus* Giraud (Hymenoptera: Braconidae). Ann Mag Nat Hist. 1940;5:473–493.

45. Belokobylskij SA. On the classification and phylogeny of the braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera, Braconidae). Part I. On the classification. Entomol Obozr. 1993;71:900–928.

46. Belokobylskij SA. On the classification and phylogeny of the braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera: Braconidae). Part II. Phylogeny. Entomol Obozr. 1993;72:891–914.

47. Wharton RA. Review of the Hormiini (Hymenoptera: Braconidae) with a description of new taxa. J Nat Hist. 1993;27:107–171.

48. Ranjith AP, Belokobylskij SA, Quicke DLJ, Kittel RN, Butcher BA, Nasser M. An enigmatic new genus of Hormiinae (Hymenoptera: Braconidae) from South India. Zootaxa 2017;4272:371-385.

49. Quicke DLJ, Belokobylskij SA, Ward DF, Hebert PDN, Butcher BA. Revision of the endemic New Zealand braconid wasp genus *Metaspathius*: new subfamily placement,

and descriptions of four new species including three with fully winged females (Hymenoptera: Braconidae: Mesostoinae). N Z Entomol. 2019;41:46–65.

50. Chen XX, van Achterberg C. Systematics, phylogeny, and evolution of braconid wasps: 30 years of progress. Annu Rev Entomol. 2019;64:335–358.
51. Sharkey MJ, Janzen DH, Hallwachs W, Chapman EG, Smith MA, Dapkey T, et al. Minimalist revision and description of 411 new species in 11 subfamilies of Costa Rican braconid parasitic wasps, including host records. Zootaxa 2021;1013:1–665.
52. Dowton M, Castro LR, Austin AD. Mitochondrial gene rearrangements as phylogenetic characters in the invertebrates: the examination of genome 'morphology'. Invertebr Syst. 2002b;16:345-356.
53. Timmermans MJ, Vogler AP. Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). Mol Phylogenet Evol. 2012;63:299-304.
54. Mao M, Gibson T, Dowton M. Higher-level phylogeny of the Hymenoptera inferred from mitochondrial genomes. Mol Phylogenet Evol. 2015;84:34-43.
55. Saenz-Manchola OF, Virrueta Herrera S, D'Alessio LM, Yoshizawa K, García Aldrete AN, Johnson KP. Mitochondrial genomes within bark lice (Insecta: Psocodea: Psocomorpha) reveal novel gene rearrangements containing phylogenetic signal. Syst Entomol. 2021;46:938-951.
56. Dowton M, Cameron SL, Austin AD, Whiting MF. Phylogenetic approaches for the analysis of mitochondrial genome sequence data in the Hymenoptera–a lineage with both rapidly and slowly evolving mitochondrial genomes. Mol Phylogenet Evol. 2009;52:512–519.
57. Samacá-Sáenz E, Meza-Lázaro RN, Branstetter MG, Zaldívar-Riverón, A. Phylogenomics and mitochondrial genome evolution of the gall-associated doryctine wasp genera (Hymenoptera: Braconidae). Syst Biodivers. 2019;17:731–744.
58. Dowton M, Castro LR, Campbell SL, Bargon, SD, Austin AD. Frequent mitochondrial gene rearrangements at the hymenopteran nad3–nad5 junction. J Mol Evol. 2003;56:517-526.
59. Branstetter MG, Longino JT, Ward PS, Faircloth BC. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. Methods Ecol Evol. 2017;8:768–776.
60. Rhodén C, Wahlberg E. The phylogeny of *Empis* and *Rhamphomyia* (Diptera, Empididae) investigated using UCEs including an over 150 years old museum specimen. Evol Syst. 2020;4:21-33.
61. Kobayashi T, Sota, T. Evolution of host use in fungivorous ciid beetles (Coleoptera: Ciidae): Molecular phylogeny focusing on Japanese taxa. Mol Phylogenet Evol. 2021;162:107197.
62. Raposo do Amaral F, Neves LG, Resende Jr MF, Mobili F, Miyaki CY, Pellegrino KC, et al. Ultraconserved elements sequencing as a low-cost source of complete mitochondrial genomes and microsatellite markers in non-model amniotes. PloS One 2015;10:e0138446.
63. Meza-Lázaro RN, Poteaux C, Bayona-Vásquez NJ, Branstetter MG, Zaldívar-Riverón A. Extensive mitochondrial heteroplasmy in the Neotropical ants of the *Ectatomma ruidum* complex (Formicidae: Ectatomminae). Mitochondrial DNA Part A 2018;29:1203-1214.
64. Zhang YM, Williams JL, Lucky A. Understanding UCEs: a comprehensive primer on using Ultraconserved elements for arthropod phylogenomics. ISD 2019;3:3.

65. Perrichot V, Nel A, Quicke DLJ. New braconid wasps from French Cretaceous amber (Hymenoptera, Braconidae): synonymisation with Eoichneumonidae and implications for the phylogeny of Ichneumonoidea. Zool Scr. 2009;38:79–88.
66. Schulz WA. Zweihundert alte Hymenopteren. Zool Annln. 1911;41–220.
67. Tobias VI. Two new and one little known subfamilies of Braconidae (Hymenoptera) from Australia. Entomol Obozr. 1979;58:128–142.
68. Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, et al. Evolutionary history of the Hymenoptera. Curr Biol. 2017;27:1013–1018.
69. Glenn TC, Nilsen RA, Kieran TJ, Finger JW Jr, Pierson TW, Bentley KE, et al. Adapterama I: universal stubs and primers for thousands of dual–indexed Illumina libraries (iTru and iNext). PeerJ 2016;7:e7755.
70. Faircloth BC. Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. 2013. Available at: https://github.com/faircloth-lab/illumiprocessor
71. Del Fabbro C, Scalabrin S, Morgante M, Giorgi FM. An extensive evaluation of read trimming effects on Illumina NGS data analysis. PLoS One 2013;8:e85024.
72. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–2120.
73. Faircloth BC. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 2015;32:786–788.
74. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012;28:1647–1649.
75. Jin JJ, Yu WB, Yang JB, Song Y, Depamphilis CW, Yi TS, et al. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 2020;21:1–31.
76. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013;69:313–319.
77. Boore JL, Collins TM, Stanton D, Daehler LL, Brown WM. Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature 1995;376:163–165.
78. Boore JL, Lavrov DV, Brown WM. Gene translocation links insects and crustaceans. Nature 1998;392:667–668.
79. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013; http://www.R- project.org/.
80. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–780.
81. Wu QL, Li Q, Gu Y, Shi BC, van Achterberg C, Wei SJ, et al. The complete mitochondrial genome of *Taeniogonalos taihorina* (Bischoff) (Hymenoptera: Trigonalyidae) reveals a novel gene rearrangement pattern in the Hymenoptera. Gene 2014;543:76–84.
82. Buckley TR, Simon C, Flook PK, Misof B. Secondary structure and conserved motifs of the frequently sequenced domains IV and V of the insect mitochondrial large subunit rRNA gene. Insect Mol Biol. 2000;9:565–580.
83. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 2017;14:587–589.

84. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–1534.

85. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–542.

Figures

Fig. 1. (A) Statistical correlation between the mt genomes assembly size and the age of the examined specimens. (B) Number of mt genomes (y-axis) for which each protein-coding gene was recovered (x-axis). (C) Number of mt genomes (y-axis) for which each tRNA was recovered (x-axis).
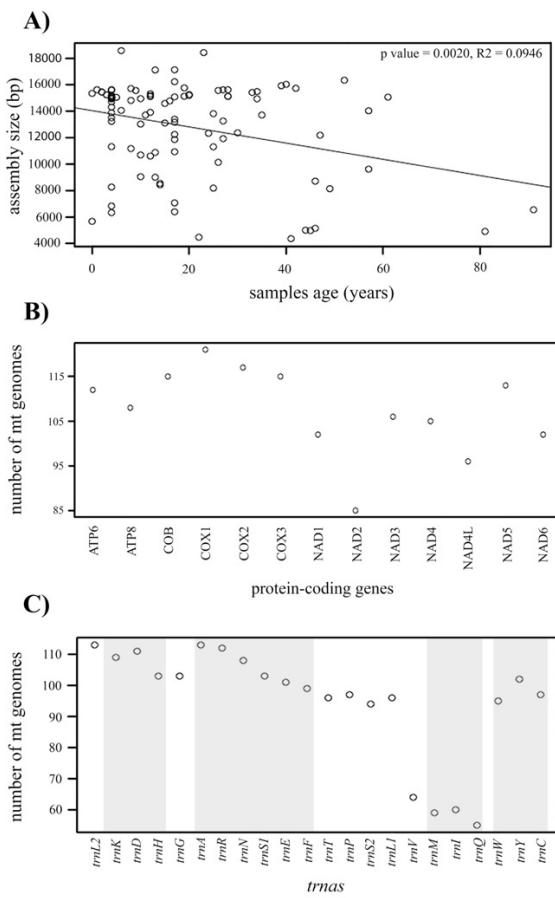
Fig. 2. Maximum likelihood phylogram of Braconidae derived from the concatenated matrix with the best-fit partition model. Green = cyclostome *s.l.* subfamilies, orange = non-cyclostomes subfamilies, blue = *Apozyx penyai* (Apozyginae), grey = Ichneumonidae, light grey = Megaspilidae (outgroup). Numbers near nodes are bootstrap values.

Rogadinae

Hormiinae

Rhysipolinae

Alysiinae

Opiinae

Exothecinae

Braconinae

Telengaiinae

Holartic-African-Madagascan
Doryctinae + Pambolinae

Avga

South American Doryctinae

Rhyssalinae

Mesostoinae +
Maxfischerinae

Aphidiinae

aphidioid
complex

Brachistinae

Acampsohelconinae
Helconinae
Homolobinae

Microtypinae

Orgilinae

Macrocentrinae
Charmontinae

Cenocoeliinae

Euphorinae

Trachypetinae

Agathidinae

Sigalphinae

Microgastrinae
Khoikhoinae
Miracinae
Cardiochilinae

Cheloninae

Proteropinae
Ichneutinae

Apozyginae

Ichneumonidae

Megaspilidae

helconoid
complex

euphoroid
complex

sigalphoid
complex

microgastroid
complex

Cyclostomes *sensu lato*

Non-cyclostomes

Braconidae

Ichneumonoidea

Megaspilidae

Fig. 3. Gene order patterns found for tRNAs clusters *KDH*, *WYC* and *IMQ*. Terminal taxa: green = cyclostome *s.l.* subfamilies, orange = non-cyclostomes subfamilies, blue = *Apozyx penyai* (Apozyginae), black = Ichneumonidae. tRNAs clusters: blue, pink, green = *YCW*, purple, red, yellow = *DHK*, blue, grey, orange = *IMQ*. White squares correspond to other genes (tRNAs or protein coding genes) recovered as part of the *YCW*, *DHK* and *IMQ* clusters. For full results or gene rearrangements, please refers to Additional file: Table S2.
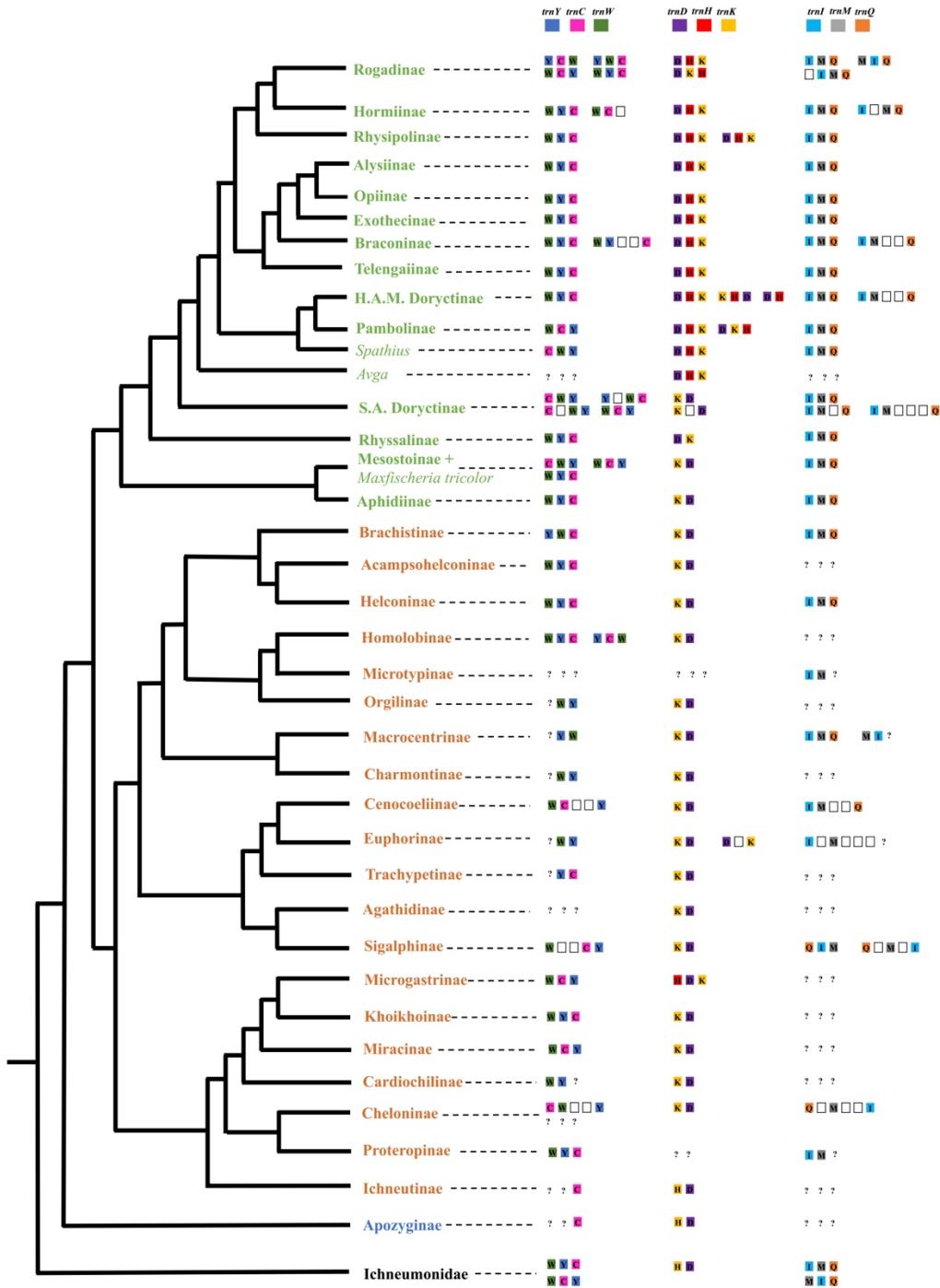
Supplementary material legends

Additional file: Table S1. Main features of the assembled mitochondrial genomes in this study: assembly sizes (pb), mean read depth, number of contigs obtained in GetOrganelle (GO) and not-found genes. Samples with an asterisk (*) correspond to the samples from which the mt assembly was obtained from shotgun libraries.

Additional file: Table S2. Gene order of the mt genomes assembled in this study. The protein-coding genes, tRNAs and rRNAs are highlighted in blue, white and purple respectively. Grey cells correspond to not-recovered genes. Samples with asterisks (*) were sequenced and assembled by Samacá-Sáenz et al. (2019).

Additional file: Table S3. List of specimens included in this study. Their taxon ID, locality, biogeographic region, raw UCEs data and mitogenomes GenBank accession numbers. SRA accession numbers marked with asterisks (*) correspond to shotgun data.

Additional file: Figure S1. Maximum likelihood phylogram of Braconidae derived from the concatenated matrix with the predefined partitions. Green = cyclostome *s.l.* subfamilies, orange = non-cyclostomes subfamilies, blue = *Apozyx penyai* (Apozyginae), grey = non-braconids. Numbers near nodes are bootstrap values.

Additional file: Figure S2. Bayesian phylogram of Braconidae derived from the concatenated matrix with the predefined partitions. Green = cyclostome *s.l.* subfamilies, orange = non-cyclostomes subfamilies, blue = *Apozyx penyai* (Apozyginae), grey = non-braconids. Numbers near nodes are posterior probability (PP) values ≤ 0.95; nodes with no value have PP ≥ 0.95.

Additional file: Figure S3. Maximum likelihood phylograms of Braconidae derived from four dataset to test the level of missing data. A) 0 – 9 missing genes, 142 taxa, B) 0 – 6 missing genes, 128 taxa, C) 0 – 2 missing genes, 105 taxa and D) no missing genes, 70 taxa.

Additional file: File S1. Concatenated matrix including the sequences of the 13 protein-coding genes + the sequences of the two ribosomal RNAs (11,717 pb, 148 terminals), as well as the block of the estimated partitions.

Additional file: File S2. Annotated 16S matrix.

Additional file: File S3. Annotated 12S matrix.

Additional file: File S4. Datasets used for missing data evaluation.

DISCUSIÓN GENERAL

Clasificación actualizada de Braconidae

Recientemente se han llevando a cabo investigación con base en datos a escala genómica para elucidar las relaciones filogenéticas de diferentes grupos de Braconidae mediante el uso de UCEs (Samacá-Sáenz et al., 2019; 2022), genomas mitocondriales (Wei et al., 2010; Li et al., 2016; Samacá-Sáenz et al., 2019) y datos de RADseq (Delgado-Machuca et al., 2020). El empleo de datos a escala genómica sin duda está contribuyendo a la generación de hipótesis filogenéticas más robustas dentro de esta familia de himenópteros predominantemente parasitoides, aunque hasta antes de la presente tesis estos trabajos se habían concentrado principalmente en algunas subfamilias como Doryctinae (p. ej. Samacá-Sáenz et al., 2019; 2022; Delgado-Machuca et al., 2020), o bien emplearon un muestreo taxónomico muy reducido (p. ej. Feng et al., 2020; Sharanowski et al., 2021). Además, la reconstrucción de hipótesis de filogenia robusta y con un muestreo taxonómico exhaustivo son escenciales para poder responder preguntas de tipo evolutivo en la familia, como por ejemplo la evolución de sus estrategias de parasitoidismo.

En el presente trabajo de tesis se generó una cantidad considerable de datos a nivel genómico (UCEs y mitogenomas) y se empleó el muestreo taxonómico más amplio utilizado hasta la fecha para Braconidae para llevar a cabo tres estudios que derivaron en una propuesta de clasificación robusta y actualizada para la familia. Como resultado principal de estos tres estudios, se propuso que Braconidae está compuesta por un total de 41 subfamilias (Tabla 1), de las cuales 25 integran al clado no ciclostomo, tres al complejo aphidioide (i.e. Aphidinae, Maxsfisheriinae y Mesostoinae) con la subfamilia Masoninae como su grupo hermano, y 11 al clado ciclostomo *sensu stricto*. Además, se considera los ciclosomos *s.s.* + el complejo aphidioide + Masoninae como los ciclostomos *sensu lato*, y al grupo ciclostomo *s.l.* + el grupo no ciclostomo como el complejo braconoide. Finalmente, la enigmática subfamilia Apozyginae, representada por una sola especie (*Apozyx penai*), fue incluida por primera vez en un análisis filogenético a nivel molecular para la familia, siendo recuperada como grupo hermano del resto de los bracóidos (Tabla 1). A continuación se discuten algunos casos particulares sobre esta clasificación actualizada.

Tabla 1. Clasificación actualizada de la familia Braconidae. La clasificación previa de la familia principalmente se basa en Zaldívar-Riverón et al. (2006), Sharanowski et al. (2011), Quicke et al. (2015) y Yu et al. (2016).

CLASIFICACIÓN PREVIA

ICHNEUMONIDAE	Masoninae	
TRACHYPETIDAE	*Trachypetus* Guérin de Meneville, *Megalohelcon* Turner, *Cercobracon* Tobias (Quicke et al., 2020b)	
BRACONIDAE		
afnidades no confirmadas		Apozyginae
		Meteorideinae
ciclostomos *sensu stricto*	**subcomplejo alysioide**	Alysiinae
		Braconinae
		Exothecinae
		Gnamptodontinae
		Opiinae
		Telengaiinae
		Doryctinae
		Hormiinae
		Pambolinae
		Rhysipolinae
		Rhyssalinae
		Rogadinae
complejo aphidioide		Aphidiinae
		Masxfischeriinae
		Mesostoinae
no ciclostomos	**complejo euphoroide**	Cenocoeliinae
		Euphorinae
		Meteorinae
	complejo helconoide	Acampsohelconinae
		Amicrocentrinae
		Brachistinae
		Charmontinae
		Helconinae
		Homolobinae
		Macrocentrinae
		Microtypinae
		Orgilinae
		Xiphozeliinae
	complejo sigalphoide	Cardiochilinae
		Cheloninae
		Dirropinae
		Ichneutinae
		Khoikhoinae

CLASIFICACIÓN ACTUALIZADA

BRACONIDAE

subfamilias no muestreadas			Amicrocentrinae
			Dirrhophinae
			Xiphozelinae
ciclostomos *sensu lato*	**ciclostomos *sensu stricto***	**complejo braconoide**	Alysiinae
			Braconinae
			Exothecinae
			Opiinae
			Telengaiinae
			Doryctinae
			Hormiinae
			Pambolinae
			Rhysipolinae
			Rhyssalinae
			Rogadinae
		Masoninae	
	complejo aphidioide		Aphidiinae
			Masxfischeriinae
			Mesostoinae
complejo braconoide	**no ciclostomos**	Meteorideinae	
		Trachypetinae	
		complejo euphoride	Cenocoeliinae
			Euphorinae
		complejo helconoide	Acampsohelconinae
			Brachistinae
			Charmontinae
			Helconinae
			Homolobinae
			Macrocentrinae
			Microtypinae
			Orgilinae
		Proteropinae	
		complejo sigalphoide	Cardiochilinae
			Cheloninae
			Khoikhoinae
			Mendesellinae

		Mendesellinae				Microgastrinae
		Microgastrinae				Miracinae
		Miracinae				Agathidinae
		Agathidinae			**complejo sigalphoide**	Ichneutinae
	complejo sigalphoide	Sigalphinae				Sigalphinae
		Apozyginae (*Apozyx penyai* Mason)				

La superfamilia Ichneumonoidea ha comprendido tradicionalmente a dos familias, Ichneumonidae y Braconidae, aunque algunos taxones han estado en controversia sobre si representan o no familias independientes. Dos de estos grupos son Apozyginae y Trachypetinae, las cuales habían sido anteriormente consideradas como familias, Apozygidae (Mason, 1978) y Trachypetidae (Quicke et al., 2020a), o bien como subfamilias de Braconidae (Apozyginae: Quicke y van Achterberg, 1990; Belokobylskij y Jouault, 2021; Trachypetinae: Schulz, 1911; Tobias, 1979). Los resultados presentados en el presente trabajo de tesis empleando datos a escala genómica apoyan de forma robusta que ambos taxones se mantengan como subfamilias de Braconidae, lo que en el caso de Trachypetinae implicó un cambio taxonómico que fue establecido de manera formal (Capítulo II).

La mayoría de las relaciones obtenidas entre las subfamilias de Braconidae fueron recuperadas con alto soporte y varias de ellas son congruentes con estudios previos (p. ej., Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011; 2021; Quicke et al., 2020b; 2021). Ejemplos de ello son la composición del complejo aphidioide, así como su relación hermana con los ciclostomos *s.s.*, la obtención de un clado compuesto por las subfamilias Rogadinae, Hormiinae y Rhysipolinae, la composición del subcomplejo braconoide, así como la composisión general de los cuatro complejos no ciclostomos, i.e. los complejos euphoroide, helconoide, microgastroide y sigalphoide. Sin embargo, otras relaciones fueron recuperadas por primera vez con alto soporte y derivaron en actualizaciones en la clasificación supraespecífica dentro de Braconidae. A continuación se mencionan con detalle estos casos.

Rogadinae (incl. Betylobraconini) y Hormiinae (incl. Lysitermiini). En el estudio de Zaldívar-Riverón y colaboradores (2008a), se propuso con base en evidencia molecular que la subfamilia Rogadinae estaba compuesta por cinco tribus: Aleiodini, Clinocentrini, Stiropiini, Rogadini y Yeliconini, mientras que Betylobraconinae fue recuperada como la subfamilia hermana de Rogadinae con bajo soporte. Recientemente, Quicke y Butcher (2015) recuperaron a los betylobraconinos como una tribu dentro de Rogadinae, aunque también con bajo soporte. Los

resultados derivados de este trabajo de tesis (Capítulo I) confirman por primera vez con alto soporte a Betylobraconini como una tribu de Rogadinae, con la tribu Rogadini recuperada como hermana de las tribus restantes. Estos resultados permitieron responder preguntas sobre la evolución del uso de hospederos dentro de la subfamilia, sugiriendo que la condición ancestral fue atacar lepidópteros ocultos, con dos posteriores transiciones a atacar tanto a hospederos ocultos como expuestos.

Hormiinae fue durante mucho tiempo fue un grupo muy heterogéneo en donde eran colocados aquellos taxones con ninguna aparente afinidad con otros bracónidos (Quicke et al., 2021). A partir de estudios filogenéticos llevados a cabo en los últimos 15 años, han sido transferido varios géneros de hormiinos a otras subfamilias (p. ej., *Monitoriella*: Zaldívar-Riverón et al., 2006). Sin bien éste era un grupo morfológicamente heterogéneo, una característica morfológica que se sugirió como diagnóstica para la subfamilia fue la desclerotización del metasoma (Wharton, 1993; van Achterberg, 1995). Sin embargo, Wharton (1993) sugirió que la subfamilia también podría incluir géneros con metasomas fuertemente esclerotizados similares a un caparazón, en particular a especies incluídas dentro de la subfamilia Lysitermiinae. Esta sugerencia se sustentó en la similitud de varias características de la venación alar, las patas y la esculturación del cuerpo. En este trabajo de tesis (Capítulo I), se confirma la sugerencia de Wharton (1993), en donde tanto los géneros de Hormiinae como de Lysistermiinae se recuperaron como no recíprocamente monofiléticos con base en datos de UCEs, formando un clado, hermano de Rogadinae. Siguiendo el criterio de prioridad, ambos grupos fueron sinonimizados en la sufamilia Hormiinae, incluyendo a las diferentes tribus de lysiterminos. Estos resultados fueron también apoyados por los resultados obtenidos en los Capítulos II y III, y recientemente por Quicke et al. (2021) usando marcadores puntuales y un muestreo taxonómico considerablemente extenso.

Ichneutinae y Proteropinae. La subfamilia Ichneutinae había sido propuesta en estudios previos como parte tanto del complejo sigalphoide (Sharkey y Wharton, 1994), así como del complejo microgastroide (Quicke y van Achterberg, 1990; Dowton et al., 2002; Shi et al., 2005; Pitz et al., 2007; Sharanowski et al., 2011). Al igual que en el estudio de Quicke y van Achterberg (1990), en este trabajo de tesis (Capítulo II) se recuperó a la subfamilia Ichneutinae como no monofilética, con los géneros *Ichneutes*, *Oligoneurus* y *Paroligoneurus* formando un clado que fue recuperado como grupo hermano de las subfamilias Agathidinae y Sigalphinae,

quienes son representantes del complejo sigalphoide. Por otra parte, los géneros *Hebichneutes*, *Masonbeckia* y *Proterops*, los cuales anteriormente se consideraban como parte de Ichneutinae, se recuperaron en un clado que fue grupo hermano del complejo microgastroide.

Los géneros de Ichneutinae que con base en evidencia a nivel genómico fueron recuperados cercanamente relacionados a Agathidinae y Sigalphinae, *Ichneutes*, *Oligoneurus* y *Paroligoneurus*, comparten características morfológicas con estas subfamilias (presencia de espinas en las tibias anteriores, presencia de subpronopos, ovipositor corto). Por lo tanto, en este trabajo se restringió a Ichneutinae para incluir a los tres géneros mencionados arriba, así como a *Lispixys* y *Pseudichneutes* de acuerdo con Sharkey et al. (2021). Además, se expandió la composición del complejo sigalphoide para contener ahora a tres subfamilias, Agathidinae, Sigalphinae e Ichneutinae.

Por otro lado, los géneros restantes que anteriormente estaban en Ichneutinae y que fueron muestreados en el presente estudio, *Hebichneutes*, *Masonbeckia* y *Proterops*, ahora se proponen para representar a una subfamilia independiente, Proteropinae. Si bien éstos géneros habían sido considerados dentro de Ichneutinae, fueron recientemente reconocidos como miembros de Proteropinae (Chen y van Achterberg, 2019; Sharkey et al., 2021), esto con base en un estudio filogenético previo que tampoco recuperó a Ichneutinae como monofilética (i.e., Sharanowski et al., 2011). Sharkey y colaboradores (2021) proporcionaron además una diagnosis de Proteropinae, la cual se propuso estar conformada por *Hebichneutes*, *Masonbeckia*, *Proterops*, pero incluyendo también a *Helconichia*, *Michener* y *Muesonia*. Sin embargo, el estudio correspondiente al Capítulo II es el primero en recuperar de manera robusta a Proteropinae e Ichneutinae como linajes independientes.

Masoninae. El género *Masona* originalmente fue ubiccado en su propia subfamilia, Masoninae, dentro de Braconidae, esto debido a la fusión de los tergitos metasomales 2 y 3, la cual es una característica distintiva de la familia (van Achterberg, 1995). Si bien con base en marcadores moleculares *Masona* se ha recuperado al interior de Braconidae (Belshaw y Quicke, 2002), recientemente Quicke et al. (2020c) en un estudio con base en marcadores moleculares puntuales posicionó a *Masona* dentro de la familia Ichneumonidae. Además de la evidencia molecular, Quicke y colaboradores (2020c) dieron soporte a este cambio taxonómico basados en la interpretación de una pequeña separación de los tergos metasomales 2 y 3 en dos especies de *Masona*, la cual es una característica diagnóstica de Ichneumonidae.

En el Capítulo II de esta tesis, *Masona* (Masoninae) fue recuparada con alto soporte como grupo hermano del complejo aphidioide con base en datos de UCEs. Sin bien la interpretación de una separación tan sutil en los tergos 2 y 3 reportada por Quicke et al. (2020c) necesita de confirmación adicional, los aphidiinos, quienes son bracónidos que forman parte del complejo aphidioide, son el único taxón de la familia cuya característica distintiva es una sutura flexible entre dichos tergos metasomales (van Achterberg, 1997). Por lo tanto, dada la ubicación de *Masona* como grupo hermano del complejo aphidioide con base en datos a escala genómica, y considerando la condición de los tergos metasomales 2 y 3, en el Capítulo II se restauró a Masoninae como una subfamilia dentro de Braconidae.

Telengaiinae (incluyendo a la tribu Gnamptodontini). En este trabajo se recuperó una relación cercana entre las subfamilias ciclostomas Telengaiinae y Gnamptodontinae. Ambos grupos de Braconidae comparten caracteristicas morfológicas, incluyendo similitudes en el aparato del veneno (Zaldívar-Riverón et al., 2004; Quicke, 2015). Al igual que en este estudio, ambas subfamilias se han recuperado previamente como estrechamente relacionadas (Zaldívar-Riverón et al., 2006), y con base en ello, Chen y van Achterberg (2019) trataron a Telengaiinae como una tribu dentro de Gnamptodontinae. Los resultados obtenidos en el Capítulos II, y considerando las similitudes morfológicas entre Telengaiinae y Gnamptodontinae, apoyan el trabajo de Chen y van Achterberg (2019) en tratar a ambos taxones como una sola subfamilia. Sin embargo, siguiendo el principio de prioridad, Gnamptodontinae pasa a ser tratada como una tribu (Gnamptodontini **stat. rev.)** dentro de Telengaiinae (i.e. Telengaiinae Tobias, 1962; Gnamptodontini Fischer, 1970).

Uso de reordenamientos genéticos mitocondriales como caracteres diagnósticos en Braconidae
Los reordenamientos mitocondriales son filogenéticamente informativos a diferentes escalas evolutivas en varios órdenes de insectos, mostrando patrones particulares para linajes específicos (p.ej., Dowton et al., 2002; Wei et al., 2010; Song et al., 2016; Feng et al., 2020). Los himenópteros suelen tener un orden de genes mitocondriales conservado, aunque en Apócrita (grupo que incluye a Ichneumonoidea) se han rerportado varios reordenamientos de los tRNAs (Dowton et al., 2009; Song et al., 2016; Tang et al., 2019).

De forma particular para Braconidae, si bien el orden de los genes codificantes es conservado, se han reportado varios reordenamientos de los tRNAs que coincidieron con los dos

grandes grupos al interior de la familia, los ciclostomos *s.l.* y los no ciclostomos (Dowton, 1999; Dowton et al., 2002; Wei et al., 2010; Li et al., 2016; Feng et al., 2020). Sin embargo, la existencia de reordamientos particulares para varias subfamilias permaneció desconocido dado que los anteriores estudios contaron con muestreos taxonómicos bastante limitados.

En el Capítulo II de esta tesis se confirma un orden conservado de genes codificantes tanto para el grupo ciclostomo *s.l.* como para el clado de no ciclostomos, así como la existencia de varios reordenamientos de tRNAs que comprenden el *bloque trnK-trnD-trnH* ubicados entre los genes codificadores de proteínas *COX2* y *ATP8*, el bloque *trnW-trnC-trnY* cerca del gen codificante *NAD2* y el bloque *trnA-trnR-trnN-trnS1-trnE-trnF* ubicado entre los genes codificantes *NAD3* y *NAD5*. Es importante mencionar que si bien los no ciclostomos presentan una estrategia koinobionte-endoparasitoide y los ciclostomos tienen un rango amplio de estrategias, los reordenamientos encontrados en éstos últimos no reflejan alguna estrategia particular, ya que tanto koinobiontes-endoparasitoides, idiobiontes-ectoparasitoides, koinobiontes-ectoparasitoides (Rhysipolinae) y especies fitófagas (en Doryctinae) comparten los mismos ordenamientos en los bloques de tRNAs arriba mencionados.

Además, se encontraron patrones distintos al interior de Rogadinae y entre los dos clados de Doryctinae, los cuales representan reportes nuevos y tienen potencial para ser utilizados como caracteres que ayuden a separar otros grupos menos inclusivos (i.e. tribus) al interior de la familia. En particular, para la subfamilia Rogadinae los ordenamientos de los genes mitocondriales coinciden con las tribus propuesta para dicha subfamilia en el Capítulo I. Por un lado, en Rogadini hay una translocación de *trnG*, encontrándose como parte del bloque comprendido por *trnI-trnM-trnQ*. En contraste, las tribus restantes de Rogadinae presentan el *trnG* ubicado entre los genes codificadores de proteínas *COX3* y *NAD3*. El segundo reordenamiento se detectó en el bloque situado entre los genes codificadores de proteínas *NAD2* y *COX1*. Dentro de la tribu Aleiodini, se recuperó el patrón *trnY-trnC-trnW*, mientras que para Yeliconini se obtuvo el patrón *trnW-trnC-trnY* y para Stiropiini y Betylobraconini el patrón *trnW-trnY-trnC*. Además, para la tribu Rogadini se encontraron tres patrones diferentes, *trnY-trnW-trnC*, *trnW-trnC-trnY* y *trnW-trnY-trnC*.

Al igual que en estudios filogenéticos previos (Zaldívar-Riverón et al., 2007; 2008b; Sharanowski et al., 2011), en este estudio se recuperó a Doryctinae como no monofilética usando tanto UCEs como mitogenomas. Además, Doryctinae se dividió en dos clados distantemente

relacionados, el clado "Sudamericano" (SA) y el clado "Holartico-Africano-de Madagascar" (HAM), que además incluyó representantes de la subfamilia Pambolinae. Para cada grupo se encontraron patrones distintos en los tRNAs que se encuentran entre los genes codificantes *COX2* y *ATP8*. Por un lado para el grupo HAM, se encontró que dicho bloque incuye tres tRNAs, i.e. trnD, *trnH* y *trnK*, mientras que para el grupo SA se confirmó que este bloque de tRNAs sólo incluye *trnK* y *trnD*, tal como encontró Samacá-Sáenz et al. (2019).

Evolución de las estrategias de parasitoidismo en Braconidae

El parasitoidismo es una estrategia de vida compleja, aunque muy común entre los insectos. Luego de que las hembras ovipositan sus huevecillos en el hospedero, este muere una vez que el parasitoide completa su desarrollo y emerge como adulto (Godfray, 1994; Quicke, 2015). Durante mucho tiempo la forma principal para separar o clasificar a los parasitoides fue de acuerdo a si éstos llevaban a cabo la oviposición dentro o fuera del hospedero (i.e. endo- y ectoparasitoidismo; Belshaw et al., 2003). Otras características en el modo de parasitoidismo tienen también implicaciones en el desarrollo del parasitoide. Por ejemplo, si son solitarios o gregarios (Quicke, 2015), especialistas o generalistas (Hassell y May, 1986; Quicke, 2015), o bien si llevan a cabo una estrategia idio- o koinobionte, causando la parálisis permanente o temporal del hospedero, respectivamente (Gauld, 1988; Quicke, 2015).

Si bien el conocimiento de las estrategias idio- koinobiosis así como del endo-ectoparasitoidismo ha tenido gran relevancia en el entendimiento de la biología no sólo de Braconidae, sino de Ichneumonoidea en general, en ningún estudio fueron probados la correlación entre ambos tipos de estrategias, particularmente utilizando un marco filogenético. En ese sentido, el presente estudio ayuda a establecer formalmente el vínculo entre koinobiosis-endoparasitoidismo e idiobiosis-ectoparasitoidismo en Braconidae.

En este trabajo de tesis, se recuperó una historia de carácter casi idéntica tanto para idiobiosis-ectoparasitoidismo como para koinobiosis-endoparasitoidismo. El único grupo de Braconidae incluído en el presente estudio en el que se rompe la asociación entre los estados respectivos en cada rasgo es Rhysipolinae, cuyas especies de las que se conocen datos biológicos son ectoparasitoides koinobiontes. De hecho, Shaw (1983) sugirió que la biología de *Rhysipolis* representaba un estado intermedio entre el ectoparasitoidismo-idiobiosis y el endoparasitoidismo

koinobiosis. Sin embargo, nuestros resultados sugieren que el estado de Rhysipolinae se explica mejor como un surgimiento independiente de esta inusual combinación en esta subfamilia.

De forma particular sobre la investigaión de la estrategia de parasitoidismo ancestral en Braconidae, relativamente pocos esfuerzos se han realizado para reconstruir dicha historia. Sharanowski et al. (2021) realizaron recientemente una reconstrucción de estados ancestrales del ecto- *versus* endoparasitoidismo así como de idio- *versus* koinobiosis en todo Ichneumonoidea usando una filogenía basada en datos genómicos. Sus resultados variaron según el marco analítico, i.e., al incluir a representantes de Ichneumonoidea recuperaron el antepasado de Braconidae como un ectoparasitoide idiobionte, mientras que el análisis sólo de la familia sugirió un estado endoparasitoide koinobionte. Nuestro estudio con un muestreo taxonómico mucho más profundo dentro de Braconidae sugiere fuertemente que los estados ancestrales del complejo braconide (i.e. todos los bracónidos excepto *Apozyx penyai* Mason) fueron koinobiosis y endoparasitoidismo con múltiples transiciones subsecuentes.

Es importante tener en cuenta que una evaluación más completa de otros taxones ichneumonoideos también puede ser informativa para resolver esta pregunta. En particular, conocer la información biológica de *A. penyai* tiene potencial tanto para cambiar nuestra interpretación, o bien, para dar mayor apoyo a los resultados obtenidos.

Los resultados obtenidos en los tres capítulos de esta tesis están consistentemente apoyados por diferentes conjuntos de datos genómicos, dando lugar a una clasificación actualizada y más estable dentro de Braconidae, uno de los grupos más diversos de Hymenoptera. Estos resultados representan una base muy importante para futuros estudios que respondan distintas preguntas de tipo evolutivo, como el origen y la diversificación de especies en los grupos principales de esta familia, así como la posible relación entre la diversificación diferencial de especies en las subfamilias de bracónidos y sus diferentes estrategias de parasitoidismo, siendo la condición parasitoide una característica clave en la diversificaicón de Hymenoptera.

CONCLUSIONES GENERALES

-   La clasificación actualizada para Braconidae propuesta en el presente estudio está fuertemente apoyada por diferentes conjuntos de datos genómicos. Se propone el reconocimiento de un total de 41 subfamilias dentro de esta familia.

-   Trachypetinae y Masoninae son restauradas como subfamilias dentro de Braconidae. Apozyginae se confirma como grupo hermano del resto de Braconidae.

-   Rogadinae y Hormiinae son subfamilias hermanas, y esta útlima subfamilia incluye a los miembros que anteriormente conformaban a Lysitermiinae.

-   Los reordenamientos genéticos mitocondriales poseen señal filogenética importante a diferentes niveles taxonómicos dentro de Braconidae.

-   Los resultados de este trabajo indican que la condición ancestral de la estrategia parasitoide en el complejo braconoide fue koinobiosis-endoparasitoidismo, con una subsecuente transición del endo- al ectoparasitoidismo, así como tres reversiones posteriores al endoparasitoidismo.

-   Las transiciones de la koino- a la idiobiosis fueron idénticas a las recuperadas para ecto- y endoparasitoidismo, con una reversión adicional a la koinobiosis en la subfamilia Rhysipolinae.

PERSPECTIVAS

Una característica evidente dentro de Braconidae es la marcada riqueza diferencial de especies entre sus diferentes subfamilias. Por ejemplo, algunas subfamilias cuentan con menos de 50 especies descritas (p. ej., Charmontinae, Rhysipolinae), mientras que otras tienen desde unos pocos cientos (p.ej., Agathidinae, Rogadinae) hasta más de 2000 especies (p. ej., Doryctinae, Microgastrinae). Sin bien esta riqueza diferencial de especies entre los diferentes grupos al interior de Braconidae podría explicarse ya sea por un esfuerzo taxonómico sesgado o por

distinta edades de origen de sus linajes principales, también podría deberse a tasas de diversificación diferenciales en estos grupos, las cuales podrían estas influenciadas por cambios en las estrategias de parasitoidismo.

Si bien algunos estudios evolutivos en Braconidae, incluído el presente trabajo de tesis, han tenido como objetivo dilucidar la evolución de las estrategias de parasitoidismo tanto de Braconidae como de Ichneumonoidea, ninguno se ha enfocado en estudiar de manera detallada si las diferentes estrategias de parasitoidismo podrían influir en las tasas de diversificación entre los diferentes grupos principales de Braconidae. Una línea de investigación muy atractiva para la familia por lo tanto sería el estudio de los tiempos de origen y divergencia de sus subfamilias con el fin de evaluar si su riqueza diferencial de especies es el producto de cambios en sus tasas de diversificación. De ser así, es necesario investigar si estos cambios podrían estar vinculados a sus diferentes estrategias de parasitoidismo.

REFERENCIAS BIBLIOGRÁFICAS

van Achterberg, C., 1995. Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. *Zool. Verh.* 298, 1–242.

van Achterberg, C., 1997. Subfamily Aphidiinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the Family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 119–32.

Austin, A.D. and Dangerfield P.C., 1998. Biology of the *Mesostoa kerri* Austin and Wharton (Insecta: Hymenoptera: Braconidae: Mesostoinae), an endemic Australian wasp that causes stem galls on *Banksia marginata* Cav. *Aust. J. Bot.* 46, 559–569.

Ballard, J.W.O. and Pichaud N., 2014. Mitochondrial DNA: more than an evolutionary bystander. *Funct. Ecol.* 28:218-231.

Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W. J., Mattick, J. S., and Haussler, D., 2004. Ultraconserved elements in the human genome. *Science* 304, 1321-1325.

Belokobylskij, S.A. and Jouault, C., 2021. Two new striking braconid genera (Hymenoptera: Braconidae) from the mid-Cretaceous Burmese amber, *Proc. Geologists' Assoc.* 132, 426-437.

Belshaw, R. and Quicke, D.L.J., 2002. Robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. *Syst. Biol.* 51, 450–477.

Belshaw, R., Herniou, E., Gimeno, C., Fitton, M.G. and Quicke, D.L.J., 1998. Molecular phylogeny of the Ichneumonoidea (Hymenoptera) based on D2 expansion region of 28S rDNA. *Syst. Entomol*. 23, 109–123.

Belshaw, R., Grafen, A. and Quicke, D.L.J., 2003. Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis. *Zool. J. Linn. Soc. 139*, 213–228.

Black 4th, W.C. and Roehrdanz R.L., 1998. Mitochondrial gene order is not conserved in arthropods: prostriate and metastriate tick mitochondrial genomes. *Mol. Biol. Evol.* 15:1772–1785.

Boore, J.L., 1999. Animal mitochondrial genomes. *Nucleic. Acids Res*. 27, 1767–1780.

Cameron, S.L., 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annu. Rev. Entomol.* 59:95–117.

Carlucci, A., Lignitto L. and Feliciello A., 2008. Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. *Trends Cell. Biol.* 18,604–613.

Chen, X.-X. and van Achterberg, C., 2019. Systematics, phylogeny, and evolution of braconid wasps: 30 years of progress. *Annu. Rev. Entomol*. 64, 335–358.

Colinet, H., Salin, C., Boivin, G., and Hance, T.H., 2005. Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecol. Entomol.* 30, 473-479.

Condamine, F.L., Clapham, M.E., and Kergoat, G.J., 2016. Global patterns of insect diversification: towards a reconciliation of fossil and molecular evidence?. *Sci. Rep*. 6, 19208.

Davey, J.W. and Blaxter, M.L., 2010. RADSeq: next-generation population genetics. *Brief. Funct. Genomics* 9, 416-423.

Davis, R.B., Javois, J., Kaasik, A., Õunap, E., Tammaru, T., 2016. An ordination of life histories using morphological proxies: capital vs. income breeding in insects. *Ecology* 97, 2112e2124.

Delgado-Machuca, N., Meza-Lázaro, R.N., Romero-Nápoles, J., Sarmiento-Monroy, C.E., Amarillo- Suárez, Á.R., Bayona-Vásquez, N.J., and Zaldívar-Riverón, A., 2020. Genetic structure, species limits and evolution of the parasitoid wasp genus *Stenocorse* (Braconidae: Doryctinae) based on nuclear 3RAD and mitochondrial data. *Syst. Entomol*. 45, 33-47

Dowton, M., 1999. Relationships among the cyclostome braconid (Hymenoptera: Braconidae) subfamilies inferred from a mitochondrial tRNA gene rearrangement. *Mol. Phylogenet. Evol*. 11, 283–287.

Dowton, M. and Austin, A.D., 2001. Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: apocrita-evolutionary transitions among parasitic wasps. *Biological J. of Linn. Soc.* 78, 87-11.

Dowton, M., Belshaw, R., Austin, A.D., and Quicke, D.L.J., 2002. Simultaneous molecular and morphological analysis of braconid relationships (Insecta: Hymenoptera: Braconidae) indicates independent mt-tRNA gene inversions within a single wasp family. *J. Mol. Evol.* 54, 210-226.

Dowton, M., Cameron, S.L., Austin, A.D. and Whiting, M.F., 2009. Phylogenetic approaches for the analysis of mitochondrial genome sequence data in the Hymenoptera–a lineage with

both rapidly and slowly evolving mitochondrial genomes. *Mol. Phylogenet. Evol.* 52, 512–519.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging?. *Evolution* 63, 1-19.

Ekblom, R., and Galindo, J. 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity* 107, 1.

Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., and Glenn, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* 61, 717-726.

Faircloth, B.C., 2015. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32, 786–788.

Feng, Z., Wu, Y., Yang, C., Gu, X., Wilson, J.J., Li, H., Li, H., Cai., W., Yang, H. and Song, F., 2020. Evolution of tRNA gene rearrangement in the mitochondrial genome of ichneumonoid wasps (Hymenoptera: Ichneumonoidea). *Int. J. Biol. Macromol.* 164, 540–547.

Forbes, A.A., Bagley, R.K., Beer, M.A., Hippee, A.C. and Widmayer, H.A., 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecol.* 18, 1–11.

Formaggioni, A., Luchetti, A. and Plazzi, F., 2021. Mitochondrial genomic landscape: a portrait of the mitochondrial genome 40 years after the first complete sequence. *Life* 11, 663.

Gauld, I.D., 1988. Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biol. J. Linn. Soc*. 35, 351–377.

Gauld, I.D. and Hanson P.E., 1995. Carnivory in the larval Hymenoptera. En: Hanson P.E. and Gauld, I.D. (Eds.). The Hymenoptera of Costa Rica, Oxford University Press, Oxford, pp.40–5.

Godfray, H.C., 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press.

Grimaldi, D. and Engel, M.S., 2005. *Evolution of the Insects*. Cambridge University Press. New York, USA.

Hanson, P.E. and Gauld, I.D., 2006. Hymenoptera de la región Neotropical. *Mem. Am. Entomol. Inst.* 77, 1–994.

Hassell, M.P., and May, R.M., 1986. Generalist and specialist natural enemies in insect predator-prey interactions. *J. Anim. Ecol.* 923-940.

Infante, F., Hanson, P. and Wharton, R., 1995. Phytophagy in the genus *Monitoriella* (Hymenoptera: Braconidae) with description of new species. *Ann. Entomol. Soc. Am.* 88, 406–415.

Leys, R., Cooper, S.J., Schwarz, M.P., 2002. Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). *Biol. J. Linn. Soc*. 77, 249-266

Li, Q., Wei, S.J., Tang, P., Wu, Q., Shi, M., Sharkey, M.J. and Chen, X.-X., 2016. Multiple lines of evidence from mitochondrial genomes resolve phylogenetic relationships of parasitic wasps in Braconidae. *Genome Biol. Evol.* 8, 2651-2662.

Lin, Y. P., Edwards, R.D., Kondo, T., Semple, T.L., Cook, L. G., 2017. Species delimitation in asexual insects of economic importance: The case of black scale (*Parasaissetia nigra*), a cosmopolitan parthenogenetic pest scale insect. *PloS One* 12, e0175889

Mackauer, M., Michaud, J.P. and Völkl, W., 1996. Invitation paper: CP Alexander Fund: Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality, and host value. *Can. Entomol.* 128, 959–980.

Mason, W.R.M., 1978. A new genus, species and family of Hymenoptera (Ichneumonidae) from Chile. *Proc. of the Entomol. Soc. Wash.* 80, 606–610.

McCormack, J.E., Faircloth, B.C., Crawford, N.G., Gowaty, P.A., Brumfield R.T., and Glenn, T.C., 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Res.* 22, 746–754.

McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C., and Brumfield, R.T., 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. *Mol. Phylogenet. Evol.* 66, 526-538.

Meza-Lázaro, R.N., Poteaux, C., Bayona-Vásquez, N.J., Branstetter, M.G. and Zaldívar-Riverón, A., 2018. Extensive mitochondrial heteroplasmy in the Neotropical ants of the *Ectatomma ruidum* complex (Formicidae: Ectatomminae). *Mitochondrial DNA Part A* 29, 1203-1214.

Osellame, L.D., Blacker, T.S. and Duchen, M.R., 2012. Cellular and molecular mechanisms of mitochondrial function. *Best Pract. Res. Clin. Endocrinol. Metab*. 26:711-723.

Peters, R.S., Krogmann, L., Mayer, C., Donath, A., Gunkel, S., Meusemann, K. … and Niehuis, O., 2017. Evolutionary history of the Hymenoptera. *Curr. Biol.* 27, 1013–1018.

Peterson, K.J., Lyons, J.B., Nowak, K.S., Takacs, C.M., Wargo, M.J., and McPeek, M.A., 2004. Estimating metazoan divergence times with a molecular clock. *PNAS* 101, 6536-6541.

Pitz, K., Dowling, A.P.G., Sharanowski, B.J., Boring, C.A.B., Seltmann, K.C. and Sharkey, M.J., 2007. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) as proposed by Shi et al.: a reassessment. *Mol. Phylogenet. Evol*. 43, 338–343.

Pybus, O.G., and Harvey, P.H., 2000. Testing macro–evolutionary models using incomplete molecular phylogenies. *Proc. Royal Soc. B.* 267, 2267- 2272.

Quicke, D.L.J., 1987. The Old World genera of braconine wasps (Hymenoptera: Braconidae). *J. Nat. Hist.* 21, 43–157.

Quicke, D.L.J. 1997. *Parasitic wasps.* Cambridge University Press. London, England.

Quicke, D.L.J., 2015. *The Braconid and Ichneumonid Parasitoid Wasps: Biology, Systematics, Evolution and Ecology.* John Wiley & Sons, Chichester, U.K.

Quicke, D.L.J. and van Achterberg, C., 1990. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera). *Zool. Verh. Leide.* 258, 1–95.

Quicke, D.L.J. and Shaw, M.R., 2005. First host records for the rogadine genera *Rogasodes* Chen and He and *Canalirogas* van Achterberg and Chen (Hymenoptera: Braconidae) with description of a new species and survey of mummy types within Rogadinae *s. str. J. Nat. Hist.* 39, 3525–3542.

Quicke, D.L.J. and Butcher, B.A., 2015. Description of a new Betylobraconini-like parasitoid wasp genus and species (Hymenoptera: Braconidae: Rogadinae) from Chile. *Zootaxa* 4021, 459–466.

Quicke, D.L.J., LeRalec, A. and Vilhelmsen, L., 1999. Ovipositor structure and function in the parasitic Hymenoptera. *Atti Acad. Naz. Ital. Entomol. Rc.* 47, 197–239.

Quicke, D.L.J., Austin, A.D., Fagan-Jeffries, E.P., Hebert, P.D. and Butcher, B.A., 2020a. Recognition of the Trachypetidae stat. n. as a new extant family of Ichneumonoidea (Hymenoptera), based on molecular and morphological evidence. *Syst. Entomol.* 45, 771–782.

Quicke, D.L.J., Belokobylskij, S.A., Braet, Y., van Achterberg, C., Hebert, P.D.N. … and Butcher, B.A., 2020b. Phylogenetic reassignment of basal cyclostome braconid parasitoid wasps (Hymenoptera) with description of a new, enigmatic Afrotropical tribe with a highly anomalous 28S D2 secondary structure. *Zool. J. Linn. Soc.* 190, 1002–1019.

Quicke, D.L.J., Austin, A.D., Fagan-Jeffries, E.P., Hebert, P.D.N. and Butcher, B.A., 2020c. Molecular phylogeny places the enigmatic subfamily Masoninae within the Ichneumonidae, not the Braconidae. *Zool. Scr.* 49, 64–71.

Quicke, D.L.J., Fagan-Jeffries, E., Jasso-Martínez, J.M., Zaldívar-Riverón, A., Shaw, M.R. … and Butcher, B.A., 2021. A molecular phylogeny of the parasitoid wasp subfamily Rogadinae (Hymenoptera: Ichneumonoidea: Braconidae) with descriptions of three new genera. *Syst. Entomol.* 46, 1019–1044.

Rakhshani, E., Tomanovic, Z., Starý, P., Talebi, A.A., Kavallieratos, N.G., Zamani, A.A., Stamenkovic, S., 2008. Distribution and diversity of wheat aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) in Iran. *Eur. J. Entomol.* 105, 863

Ranjith, A.P., Quicke, D.L.J., Saleem, U.K.A., Butcher, B.A., Zaldívar-Riverón, A. and Nasser, M., 2016. Entomophytophagy ('Sequential predatory, then phytophagous behaviour') in an Indian braconid 'parasitoid' wasp (Hymenoptera): Specialized larval morphology, biology and description of a new species. *PLoS One* 11, e0156997

Raposo do Amaral, F., Neves, L.G., Resende Jr, M.F., Mobili, F., Miyaki, C.Y., Pellegrino, K.C. and Biondo, C., 2015. Ultraconserved elements sequencing as a low-cost source of complete mitochondrial genomes and microsatellite markers in non-model amniotes. *PloS One* 10, e0138446.

Rasnitsyn, A.P., 1988. An outline of evolution of hymenopterous insects (order Vespida). *Orient. Insects* 22, 115–145

Samacá-Sáenz, E., Meza-Lázaro, R.N., Branstetter, M.G. and Zaldívar-Riverón, A., 2019. Phylogenomics and mitochondrial genome evolution of the gall-associated doryctine wasp genera (Hymenoptera: Braconidae). *Syst. Biodivers.* 17, 731–744.

Samacá-Sáenz, E., Santos, B.F., Martínez, J.J., Egan, S.P., Shaw, S.R., Hanson, P.E. and Zaldívar-Riverón, A. 2022. Ultraconserved elements-based systematics reveals evolutionary patterns of host-plant family shifts and phytophagy within the predominantly parasitoid braconid wasp subfamily Doryctinae. *Mol. Phylogenet. Evol.* 107319.

Schulz, W.A., 1911. Zweihundert alte Hymenopteren. *Zool. Annln.* 4, 1–220.

Sharanowski, B.J., Dowling, A.P. and Sharkey, M.J., 2011. Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. *Syst. Entomol.* 36, 549–572.

Sharanowski, B.J., Ridenbaugh, R.D., Piekarski, P.K., Broad, G.R., Burke, G.R. … and Hines, H.M., 2021. Phylogenomics of Ichneumonoidea (Hymenoptera) and implications for evolution of mode of parasitism and viral endogenization. *Mol. Phylogenet. Evol.* 156, 107023.

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

Sarkey, M.J. and Wharton, R.A., 1994. A revision of the genera of the world Ichneutinae (Hymenoptera: Braconidae). *J. Nat. Hist.* 28, 873–912.

Sharkey, M.J., Janzen, D.H., Hallwachs, W., Chapman, E.G., Smith. … and Burns, J.M., 2021. Minimalist revision and description of 411 new species in 11 subfamilies of Costa Rican braconid parasitic wasps, including host records. *Zootaxa* 1013, 1–665.

Shaw, M.R., 1983. On[e] evolution of endoparasitism; the biology of some genera of Rogadinae (Braconidae). *Contrib. Am. Entomol. Inst.* 20, 307–328.

Shaw, M.R., 2002. Host ranges of *Aleiodes* species (Hymenoptera: Braconidae), and an evolutionary hypothesis. *Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control* (ed. by G. Melika and C. Thuroczy), pp. 321–327. Agroinform, Budapest.

Shaw, S.R., 2006. *Aleiodes* wasps of eastern forests: a guide to parasitoids and associated mummi ed caterpillars. *Forest Health Technology Enterprise Team Special Publication*, **121**, 2006–2008.

Shaw, M.R. and Huddleston, T., 1991. *Classification and biology of braconid wasps (Hymenoptera: Braconidae).* Handbooks for the Identification of British Insects, London: Royal Entomological Society of London, 7, 1–126.

Shi, M., Chen, X.-X. and van Achterberg, C., 2005. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA, 28S rDNA D2, 18S rDNA gene sequences and morphological characters. *Mol. Phylogenet. Evol.* 37, 104-116.

Simon, S. and Hadrys H., 2013. A comparative analysis of complete mitochondrial genomes among Hexapoda. *Mol Phylogenet Evol.* 69:393-403.

Snyder, W.E. and Ives, A.R., 2001. Generalist predators disrupt biological control by a specialistparasitoid. *Ecology* 82, 705-716.

Song, F., Li, H., Jiang, P., Zhou, X., Liu, J., Sun, C., Vogler, A. and Cai, W., 2016. Capturing the phylogeny of Holometabola with mitochondrial genome data and Bayesian site-heterogeneous mixture models. *Genome Biol. Evol.* 8, 1411–1426.

Stigenberg, J., Boring, C.A. and Ronquist, F., 2015. Phylogeny of the parasitic wasp subfamily Euphorinae (Braconidae) and evolution of its host preferences. *Syst. Entomol.* 40, 570–591.

Tang, P., Zhu, J.C., Zheng, B.Y., Wei, S.J., Sharkey, M., Chen, X.-X. and Vogler, A.P., 2019. Mitochondrial phylogenomics of the Hymenoptera. *Mol. Phylogenet. Evol.* 131, 8-18.

Tobias, V.I., 1979. Two new and one little known subfamilies of Braconidae (Hymenoptera) from Australia. *Entomol. Obozr.* 58, 128–142

Verma, K. and Jolivet, P., 2008. Eumolpinae - a widely distributed and much diversified subfamily of leaf beetles (Coleoptera, Chrysomelidae). *Terr. Arthropod Rev.* 1, 3e37.

Vilhelmsen, L., 1997. The phylogeny of lower Hymenoptera (Insecta), with a summary of the early evolutionary history of the order. *J. Zool. Syst. Evol. Res.* 35, 49-70.

Vilhelmsen, L., Mikó, I. y Krogmann, L., 2010. Beyond the wasp-waist: structural diversity and phylogenetic significance of the mesosoma in apocritan wasps (Insecta: Hymenoptera). *Zool. J. Linn. Soc.* 159, 22–194.

Wei, S-J., Shi, M., Sharkey, M.J., van Achterberg, C. and Chen, X.-X., 2010. Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. *BMC Genomics* 11, 1–16.

Weir, J. T., and Schluter, D., 2008. Calibrating the avian molecular clock. *Mol. Ecol.* 17, 2321-2328.

Wharton, R.A., 1993 Review of the Hormiini (Hymenoptera: Braconidae) with a description of new taxa. *J. Nat. Hist.* 27, 107–171.

Wharton, R.A., 1997. Subfamily Alysiinae. In: Wharton, R.A., Marsh, P.M. and Sharkey, M.J. (Eds.). Manual of the New World Genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists, Special Publ. 1, Washington, DC, pp. 84–116.

Whitfield, J.B., 1992. The polyphyletic origin of endoparasitism in the cyclostome lineages of Braconidae (Hymenoptera). *Syst. Entomol.* 17, 273–286.

Whitfield, J.B., 2002. Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 99, 7508–7513.

Yu, D.S., van Achterberg, C. and Horstmann, K., 2016. Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive (CD).

Zakharov, E.V., Caterino, M.S., Sperling, F.A., 2004. Molecular phylogeny, historical biogeography, and divergence time estimates for swallowtail butterflies of the genus *Papilio* (Lepidoptera: Papilionidae). *Syst. Biol.* 53, 193-215.

Zaldívar-Riverón, A., Areekul, B., Shaw, M.R. and Quicke, D.L.J., 2004. Comparative morphology of the venom apparatus in the braconid wasp subfamily Rogadinae (Insecta, Hymenoptera, Braconidae) and related taxa. *Zool. Scr.* 33, 223–237.

Zaldívar-Riverón, A., Mori, M. and Quicke, D.L.J., 2006. Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. *Mol. Phylogenet. Evol.* 38, 130–145.

Zaldívar-Riverón, A., Belokobylskij, S.A., León-Regagnon, V., Martínez, J.J., Briceno, R. and Quicke, D.L.J., 2007. A single origin of gall association in a group of parasitic wasps with disparate morphologies. *Mol. Phylogenet. Evol.* 44, 981–992.

Zaldívar-Riverón, A., Shaw, M.R., Sáez, A.G., Mori, M., Belokoblylskij, S.A., Shaw, S.R. and Quicke, D.L.J., 2008a. Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. *BMC Evol. Biol.* 8, 1–20.

Zaldívar-Riverón, A., Belokobylskij, S.A., León-Regagnon, V., Briceño-G., R. and Quicke, D.L.J., 2008b. Molecular phylogeny and historical biogeography of the cosmopolitan parasitic wasp subfamily Doryctinae (Hymenoptera: Braconidae). *Invertebr. Syst.* 22, 345-363.

Zaldívar-Riverón, A., Martínez, J.J., Belokobylskij, S.A., Pedraza-Lara, C.S., Shaw, S.R., Hanson, P.E. and Varela-Hernández, F., 2014. Systematics and evolution of gall formation in the plant-associated genera of the wasp subfamily Doryctinae (Hymenoptera: Braconidae). *Syst. Entomol.* 39, 633–659.

Zaldívar-Riverón, A., Belokobylskij, S.A., Meza-Lázaro, R., Pedraza-Lara, C., García-París, M., Meseguer, A.S., 2017. Species delimitation, global phylogeny and historical biogeography of the parasitoid wasp genus *Spathius* (Braconidae: Doryctinae) reveal multiple Oligocene–Miocene intercontinental dispersal events. *Zool. J. Linn. Soc. Lond.* 182, 723-734.