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

FILOGENÓMICA Y EVOLUCIÓN DE LAS ESTRATEGIAS DE FITOFAGIA EN LAS AVISPAS DEL GÉNERO *ALLORHOGAS* (BRACONIDAE: DORYCTINAE)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS BIOLÓGICAS

PRESENTA:

ERNESTO JOSÉ SAMACÁ SÁENZ

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Ciudad Universitaria, CD. MX., Enero, 2021

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
OFICIO CPCB/057/2021
ASUNTO: Oficio de Jurado

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 30 de noviembre de 2020, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **SAMACÁ SÁENZ ERNESTO JOSÉ** con número de cuenta **514351117** con la tesis titulada: **"Filogenómica y evolución de las estrategias de fitofagia en las avispas del género *Allorhogas* (Braconidae: Doryctinae)",** realizada bajo la dirección del **DR. ALEJANDRO ZALDÍVAR RIVERÓN,** quedando integrado de la siguiente manera:

Presidente:	DR. ANTONIO GONZÁLEZ RODRÍGUEZ
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Suplente:	DR. DANIEL IGNACIO PIÑERO DALMAU
Suplente	DR. DAVID SEBASTIAN GERNANDT

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"
Cd. Universitaria, Cd. Mx., a 25 de enero de 2021

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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Índice

Resumen

Las interacciones biológicas, particularmente las de que ocurren entre plantas e insectos, desempeñan un papel fundamental al dar forma a la biodiversidad del planeta. Entre éstas, la fitofagia ha incrementado de manera considerable la riqueza de especies de muchos órdenes de insectos entre los que destaca Hymenoptera. La subfamilia Doryctinae (Braconidae) es un grupo de avispas principalmente parasitoides, cuya riqueza y diversidad morfológica se concentra en el Neotrópico. Entre los taxones de doryctinos que son endémicos de esta región hay un grupo de géneros asociado con la formación de agallas en varias familias de plantas vasculares. Estudios anteriores con pocos marcadores puntuales han recuperado de forma consistente la monofilia de este grupo y sugieren que la fitofagia se originó a partir del parasitoidismo hacia otros insectos agalladores. No obstante, a la fecha algunas relaciones están sin resolver y se desconoce el número de eventos de transición hacia la fitofagia en la historia evolutiva del grupo, particularmente en *Allorhogas*, género con la mayor riqueza de especies dentro del clado. Para este trabajo se realizaron estudios filogenómicos de los géneros de doryctinos asociados con la formación de agallas empleando la técnica de captura de secuencias de elementos ultraconservados (UCEs), así como con datos de genomas mitocondriales. Se realizaron análisis de estimación de tiempos de divergencia y reconstrucción de estados ancestrales de sus diferentes historias de vida. Por otro lado, se utilizaron varias fuentes de evidencia para descubrir y delimitar especies de *Allorhogas* asociadas con agallas de avispas de la familia Cynipidae en especies del género *Quercus* subsección *Virentes.* Los análisis filogenéticos realizados generaron topologías en su mayoría resueltas y que son parcialmente congruentes con filogenias previas obtenidas a partir de pocos marcadores puntuales. Se

confirma *Allorhogas* como polifilético, con la mayoría de sus especies dentro de un clado asociado con diferentes familias de plantas vasculares y con diferentes estrategias de fitofagia, incluyendo formación de agallas, depredación de semillas e inquilinismo. Los tiempos de divergencia estimados sugieren que la asociación a agallas ocurrió probablemente durante el Oligoceno tardío y el Mioceno temprano, con una subsecuente diversificación de los clados principales durante el Mioceno medio y tardío hasta el Plioceno, lo cual es congruente con los tiempos de origen y de diversificación de algunas de sus plantas hospederas. Se recalca la importancia de los estudios detallados sobre la historia natural de estos organismos y se discute la existencia potencial de especiación ecológica debida a diferenciación asociada con el hospedero (HAD), así como de posibles procesos de codiversificación para algunos linajes de *Allorhogas* y géneros relacionados asociados con la formación de agallas en angiospermas.

Abstract

Biological interactions and particularly plant-insect interactions play a fundamental role in shaping Earth's biodiversity. Phytophagy, for example, has promoted the diversification of many insect orders including Hymenoptera. The subfamily Doryctinae is a group of braconid wasps mainly represented by ectoparasitoid species. However, within this cosmopolitan subfamily there is a group of mainly Neotropical genera whose species are strictly associated with galls of various plant families. Previous studies based on few DNA sequence markers have consistently recovered the monophyly of this group and have supported its origin of phytophagy from parasitoidism. However, various relationships and the number of transitions to phytophagy are still unclear, particularly in *Allorhogas*, which is by far its most speciose genus. We performed several phylogenomic studies among the gall-associated doryctine genera based on ultraconserved element data (UCEs) and mitochondrial genome data. Moreover, we performed macroevolutionary analyses including divergence time estimates and ancestral state reconstructions of the known life histories for the group. On the other hand, we used several sources of evidence including molecular, morphological and biological data to conduct a species delimitation study to discover different lineages of *Allorhogas* associated with cynipid galls on live oaks (*Quercus* subsection *Virentes*). Our phylogenetic analyses yielded almost fully resolved topologies that are partially congruent with the previous estimates of phylogeny based on few gene markers. *Allorhogas* was confirmed as polyphyletic, though most of its members were recovered within a major clade composed of species with different phytophagous strategies in different host plant families including gall formation, seed predation and inquilinism. The origin of the gall-association was estimated to have occurred mainly

during the late Oligocene to early Miocene, with a subsequent diversification of the main clades of the gall-associated genera during the middle to late Miocene and Pliocene and is related with the diversification of the associated host plants. In this study we highlight the importance of conducting detailed studies of the natural history of these group of wasps and discuss the potential existence of ecological speciation due to host-associated differentiation (HAD) and also probable codiversification processes for some *Allorhogas* lineages and other gall-associated doryctinae related genera.

INTRODUCCIÓN GENERAL

Las interacciones ecológicas han desempeñado un papel fundamental al dar forma a la biodiversidad del planeta. La extraordinaria diversidad de los insectos, tal como la conocemos en la actualidad, está estrechamente relacionada con la forma en que éstos se relacionan con las angiospermas (Mayhew 2007, Futuyma y Agrawal 2009), ya que muchos dependen de forma directa o indirecta de las plantas para sobrevivir. Dentro de las interacciones que existen entre ambos grupos, un caso notable es el de la fitofagia o herbivoría, debido a que ha incrementado de manera considerable la riqueza de especies de varios órdenes de insectos (Mitter et al. 1988; Wiens et al. 2015) entre los que se destacan cuatro grandes grupos: Coleoptera, Diptera, Hymenoptera y Lepidoptera.

Doryctinae: una de las subfamilias más diversas de Braconidae

Con aproximadamente 153.000 especies, los himenópteros constituyen uno de los órdenes más diversos que existen (Grimaldi y Engel 2005; Aguiar et al 2013), y sus integrantes (avispas, abejas y hormigas) representan el 8% de todos los animales descritos para el planeta (Davis et al. 2010). El éxito particular del orden Hymenoptera se ha atribuido principalmente al predominante estilo de vida parasitoide (Eggleton y Belshaw 1992; La Salle y Gauld 1992; Forbes et al. 2018), término que se utiliza para distinguir a los organismos que son parásitos únicamente durante sus estados inmaduros y que inevitablemente causan la muerte de sus hospederos (Godfray 1994). Por otra parte, la fitofagia también ha evolucionado de manera secundaria en algunas familias como

Agaonidae (Munro et al. 2011) y Cynipidae (Ronquist et al. 2015) en donde la mayor parte de sus integrantes han adquirido este hábito.

Después de Ichneumonidae, Braconidae es la familia más diversa de Hymenoptera; hasta el momento se han descrito unas 19,500 especies y cerca de 1000 géneros (Yu et al. 2016). Las larvas de estas avispas se alimentan principalmente de estados inmaduros de otros insectos, en particular de especies de varios órdenes de insectos holometábolos (Wharton 1997). Aunque la familia Braconidae está compuesta principalmente por especies con forma de vida parasitoide, se han reportado y confirmado hábitos herbívoros para algunos subclados de algunas de sus subfamilias, incluyendo Braconinae, Doryctinae y Mesostoinae (Infante et al. 1995; Flores et al. 2005; Wharton y Hanson 2005).

La subfamilia Doryctinae es una de las más diversas dentro de Braconidae con casi 200 géneros y aproximadamente 1,700 especies descritas a nivel mundial (Yu et al. 2016), aunque su representación es particularmente alta en el Neotrópico, de donde dos terceras partes de sus géneros han sido descritos (Marsh 1993, 1997). Las especies de este grupo son en la mayoría de los casos parasitoides de larvas de coleópteros xilófagos, aunque algunas especies atacan larvas de otros órdenes de insectos como Hymenoptera y Lepidoptera. A pesar de que el parasitoidismo es el estilo de vida preponderante en esta subfamilia, hay un pequeño grupo de géneros que se distribuye principalmente en las regiones Neotropical y Neártica, cuyas especies parecen estar asociadas exclusivamente con agallas en varias familias de angiospermas, varias de las cuales se ha confirmado o sugerido son fitófagas.

Allorhogas y otros géneros asociados con la formación de agallas en plantas

Las agallas son estructuras vegetales inducidas por varios grupos de organismos, entre ellas insectos de distintos órdenes, las cuales proveen de protección y alimento al organismo inductor (Price, 2005). Aunque la asociación con agallas en Doryctinae se ha reportado en especies de diez géneros (*Allorhogas* Gahan*; Donquickeia* Marsh*; Ficobolus* Martínez, Belokobylskij & Zaldívar-Riverón*; Labania* Hedqvist; *Monitoriella* Hedqvist; *Mononeuron* Fischer*; Percnobracon* Kieffer & Jörgensen*; Plesiopsenobolus* Belokobylskij, Martínez & Zaldívar-Riverón*; Psenobolus* Reinhard y *Sabinita* Belokobylskij, Zaldívar-Riverón & Martínez)*,* la fitofagia solo ha sido corroborada para especies de *Monitoriella* y *Allorhogas* con datos de cría y observación (de Macêdo y Monteiro 1989; Infante et al. 1995). De estos géneros, *Allorhogas* es el que presenta mayor riqueza con 57 especies descritas a la fecha y, por lo que se sabe de su biología hasta el momento, sus integrantes están asociados con varias familias de plantas vasculares con diferentes estrategias de fitofagia, incluyendo la formación de agallas (de Macêdo y Monteiro 1989; Morales-Silva y Modesto-Zampieron 2017; Joele et al. 2019), inquilinismo (Moreira et al. 2017) y depredación de semillas (Zaldívar-Riverón et al. 2018).

Estudios recientes han reportado que los géneros de doryctinos asociados con agallas en plantas forman un grupo monofilético, por lo que la evolución de la fitofagia en la subfamilia al parecer está restringida únicamente a este clado (Zaldívar-Riverón et al. 2007, 2008, 2014). Los resultados del más reciente de estos estudios sugieren que la inducción de agallas en *Allorhogas* ha evolucionado de manera independiente en al menos tres ocasiones a partir del parasitoidismo de huéspedes agalladores de distintas familias de angiospermas. No obstante, el escaso conocimiento de la biología e historia natural de la

mayoría de las especies de *Allorhogas* y de otros géneros relacionados, así como la resolución incompleta de sus relaciones filogenéticas, han impedido conocer con mayor detalle la evolución de la fitofagia dentro del grupo.

Métodos de representación reducida del genoma y filogenómica

Las técnicas de secuenciación de nueva generación están revolucionando la forma en que se obtiene información genómica a partir de organismos no modelo gracias a la eficiencia y magnitud con la que se producen secuencias de DNA. Entre los métodos de representación reducida del genoma frecuentemente utilizados para análisis filogenéticos y poblacionales se destacan la captura de secuencias y la secuenciación asociada con sitios de restricción (RADseq), debido a que resultan útiles para investigar la evolución de los organismos a diferentes escalas de tiempo evolutivo (Leaché et al., 2015). La diferencia entre estas dos metodologías radica en la forma en que se fragmenta el DNA genómico, ya sea empleando enzimas de restricción en el caso la secuenciación asociada con sitios de restricción, o bien mediante sonicación para la captura de secuencias (Baird et al., 2008; Mamanova et al., 2010). Por otra parte, estas dos técnicas difieren en el conocimiento previo requerido del genoma; mientras para la captura de secuencias es necesario emplear sondas previamente diseñadas para hibridar regiones específicas, la secuenciación asociada con sitios de restricción no requiere dicha información (Gnirke et al., 2009; Davey y Blaxter, 2010).

Se ha demostrado la gran utilidad de estas dos herramientas filogenómicas para ampliar el conocimiento sobre la evolución de varios grupos biológicos. Tanto el empleo de captura de secuencias, así como de RADseq son especialmente útiles en sistemática

filogenética y filogeografía, debido a que son aplicables a organismos no modelo (Harvey et al., 2016). Los dos métodos no requieren de un genoma completo secuenciado e incrementan la probabilidad de obtener secuencias de loci ortólogos para varias muestras (Davey y Blaxter, 2010). No obstante, la secuenciación de DNA asociada con sitios de restricción produce miles de polimorfismos nucleotídicos únicos (SNPs) y es útil sino se tiene poca o ninguna información previa del genoma (Peterson et al., 2011). La metodología de RADseq fue diseñada originalmente para descubrir SNPs en estudios de genética de poblaciones, filogeográficos y de especiación reciente (por ejemplo, Baird et al. 2008).

La técnica de captura de UCEs (Faircloth et al. 2012) es parte de la familia de las técnicas de captura de secuencias y ha sido utilizada principalmente para recuperar loci que se encuentran conservados a escalas de tiempo relativamente profundas (por ejemplo, Smith et al. 2014; Faircloth et al. 2015; Branstetter et al. 2017). No obstante, se ha demostrado que esta técnica también resulta útil para estudiar organismos con tiempos de divergencia más recientes y responder preguntas microevolutivas relacionadas con la delimitación de especies y genómica de poblaciones (por ejemplo, Harvey et al. 2016; Manthey et al. 2016; Andermann et al. 2019).

Entre la gran cantidad de información que algunas de las técnicas de secuenciación de nueva generación pueden recuperar, los genomas mitocondriales resultan útiles por ser marcadores frecuentemente usados para estudiar las relaciones filogenéticas a diferentes niveles taxonómicos de varios órdenes de insectos (por ejemplo, López-López y Vogler 2017; Song et al. 2017; Zhang et al. 2019; Aguilera-Uribe et al. 2020). Adicionalmente, el estudio de los mitogenomas y sus marcadores ha permitido abordar preguntas evolutivas y

comparativas dentro de la clase Insecta gracias a la frecuencia y tipo con que se presentan reordenamientos genéticos (Timmermans y Vogler 2012). Las secuencias de genomas mitocondriales son un subproducto frecuente de la implementación de la técnica de captura de secuencias de UCEs y se han recuperado, ensamblado y anotado de esta forma para algunos vertebrados (Raposo do Amaral et al. 2015; Hawkins et al. 2016) y recientemente para dos especies diferentes de hormigas en el orden Hymenoptera (Ströher et al. 2017; Meza-Lázaro et al. 2018).

Objetivos

El objetivo general de esta tesis es investigar la evolución de la fitofagia y la sistemática de *Allorhogas* y géneros asociados con la formación de agallas en plantas vasculares. Los objetivos particulares son: 1) estudiar las relaciones filogenéticas y la evolución del genoma mitocondrial en los géneros asociados con la formación de agallas empleando tanto información de genomas mitocondriales como con datos provenientes de UCEs, 2) actualizar la taxonomía del género *Allorhogas* investigando los límites entre un grupo de especies asociadas con agallas de avispas de la familia Cynipidae en *Quercus* subsección *Virentes* con base en relaciones filogenéticas obtenidas, morfología, historia natural y métodos de delimitación de especies aplicados a marcadores nucleares (UCEs) y mitocondriales (COI), y 3) investigar la evolución de la fitofagia y la sistemática de los géneros de doryctinos asociados con la formación de agallas, con énfasis en el género *Allorhogas*, empleando para ello la técnica captura de secuencias de UCEs.

Capítulo I. Artículo de requisito

Filogenómica y evolución de los genomas mitocondriales del clado de avispas de la subfamilia Doryctinae asociados con la formación de agallas en plantas vasculares (Hymenoptera: Braconidae)

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Research Article

Phylogenomics and mitochondrial genome evolution of the gall-associated doryctine wasp genera (Hymenoptera: Braconidae)

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The braconid subfamily Doryctinae is a cosmopolitan, highly diverse group of wasps mainly represented by parasitoid species. In this subfamily, however, there is a group of mainly Neotropical genera whose species are associated with galls of several vascular plant families. Previous molecular phylogenetic studies based on a few markers consistently recovered the monophyly of this group, though various relationships among genera and the monophyly of its most speciose genus, *Allorhogas* Gahan, remained unresolved. We characterized the mitogenomes of 13 representative species of the gall-associated doryctine clade and performed a phylogenomic analysis with both mitogenome and ultraconserved element (UCE) DNA sequence data to assess the classification of Doryctinae and to examine the evolution of phytophagy within the group. We found different patterns of tRNA gene rearrangements, two of which are present in most of the ingroup taxa. All phylogenetic analyses yielded highly similar, well-supported topologies that are congruent with relationships reported in previous studies. The resulting phylogenies confirmed *Percnobracon* as sister to the remaining genera and supported two separate clades whose genera are exclusively associated with *Ficus* (Moraceae). *Allorhogas* is confirmed to be polyphyletic. The combined evidence also supported a main *Allorhogas* clade composed of multiple phytophagous species that feed on at least five different host plant families. Further rearing records and phylogenetic studies are needed to examine host plant shifts and to broaden our understanding of a poorly studied radiation of gall-associated wasps.

Key words: Ichneumonoidea, mitogenome, Neotropics, phylogenomics, phytophagy, ultraconserved elements

Introduction

The insect order Hymenoptera is mainly represented by parasitoid species. Phytophagy, however, has secondarily evolved in various hymenopteran families, with the trait occurring in most of its members (e.g. Agaonidae, Cynipidae; Munro et al., 2011; Ronquist et al., 2015) or in small subclades within predominantly parasitoid families, such as Braconidae (Quicke, 2015). In the latter family, phytophagy has independently evolved in a few subclades within three different subfamilies: Braconinae, Mesostoinae, and Doryctinae (Flores, Nassar, & Quicke, 2005; Wharton & Hanson, 2005).

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With over 200 genera and 1,700 described species distributed worldwide (Yu, van Achterberg, & Horstmann, 2016), but with an estimate of about 4,000 species (Jones, Purvis, Baumgart, & Quicke, 2009), the Doryctinae represents one of the most speciose braconid subfamilies. This group is mainly represented by ectoparasitoids of xylophagous and bark-boring beetle larvae (Belokobylskij, 1992). However, in the Nearctic and Neotropics there is a group of 10 genera whose species appear to be exclusively associated with galls of various vascular plant families. Although natural history information is sparse, some of the gall-associated species are confirmed or inferred to be phytophagous (Belokobylskij, Solís, Hanson, & Zaldívar-Riverón, 2015; Wharton & Hanson, 2005; Zaldívar-Riverón et al., 2018).

To date, most of the gall-associated doryctine genera are only known from a few described species with the exception of the genus *Allorhogas* Gahan, which is by far the most speciose with 52 currently described species and a large number of undescribed species (Yu et al., 2016; Zaldívar-Riverón et al., 2018). *Allorhogas* is the only genus of the group known to have species with different phytophagous strategies, including inquilinism (Moreira et al., 2017), gall formation on seeds (de Macêdo & Monteiro, 1989; Morales-Silva & Modesto-Zampieron, 2017), and seed predation (Zaldívar-Riverón et al., 2018). Moreover, some species of this genus are presumed to be gall-formers of different host plant organs (Marsh, 2002) or parasitoids of other gall-forming insects (Martínez & Zaldívar-Riverón, 2013), though this has not been corroborated.

Previous molecular phylogenetic studies revealed that the gall-associated doryctine genera form a monophyletic group (Zaldívar-Riverón et al., 2007, 2008, 2014). These studies also suggested that phytophagy in this clade evolved from parasitoidism of gall-formers during the middle Miocene to early Oligocene. Moreover, *Allorhogas* was recovered as polyphyletic, and the gathered rearing records suggested that species diversification in its largest component clade was probably promoted by shifts to different host plant families. In contrast, the remaining genera are associated primarily with *Ficus* (Moraceae) and species diversification is probably linked to shifts to different host species and/or plant organs. Despite these insights, various key relationships remain weakly supported, leaving knowledge of the evolution of phytophagy within the group unclear.

The advent of next-generation sequencing has considerably facilitated the generation and use of genomic information for phylogenetic analyses. One of the most commonly used techniques for obtaining a reduced representation of the genome is the enrichment and sequencing of ultraconserved elements (UCEs). This approach provides thousands of variable markers for conducting phylogenetic studies of non-model organisms at different taxonomic levels (Branstetter, Longino, Ward, & Faircloth, 2017; Faircloth et al., 2012; Faircloth, Branstetter, White, & Brady, 2015; Smith, Harvey, Faircloth, Glenn, & Brumfield, 2014). In addition to UCE data, mitochondrial (mt) sequences are a common byproduct obtained from UCE sequencing and these data have been employed to reconstruct mitogenomes of several animal taxa (e.g. Hawkins et al., 2016; Meza-Lázaro, Poteaux, Bayona-Vásquez, Branstetter, & Zaldívar-Riverón, 2018; Raposo do Amaral et al., 2015; Ströher et al., 2017).

Mitogenomic data are widely employed to reconstruct evolutionary relationships of insect taxa at different evolutionary scales (e.g. López-López & Vogler, 2017; Song, Cai, & Li, 2017; Zhang et al., 2019). The mt genome has features other than DNA sequence data that can be phylogenetically informative, such as gene rearrangements, inversions, translocations, and random insertions (Timmermans & Vogler, 2012; Wang et al., 2017). In Hymenoptera, mitogenomes have been shown to be highly conserved in the suborder Symphyta, whereas in Apocrita rearrangement events are frequent at different taxonomic levels (Dowton, Cameron, Austin, & Whiting, 2009; Song, Tang, Wei, & Chen, 2016; Tang et al., 2019). In Braconidae, the only two phylogenetic studies based on mitogenomic information assessed the evolutionary relationships among its subfamilies. These two studies recovered fully supported topologies (Li et al., 2016; Wei, Shi, Sharkey, van Achterberg, & Chen, 2010), though they assembled mitogenomes for a limited number of taxa, including only one mitogenome for a member of Doryctinae (*Spathius sinicus* Chao (= *S. agrili* Yang); Wei et al., 2010).

Here we have conducted a phylogenomic study of the gall-associated doryctine clade based on 13 representative species belonging to nine of its 10 known genera. For this work, we used both UCE and mitogenomic data generated from UCE sequencing. We analysed the two genomic data sources both separately and in combination. We also described and compared the main features in the assembled mitogenomes. The phylogenomic analyses of UCE and mitogenomic data yielded an almost fully resolved topology that helps to clarify various generic relationships and provide insights into the evolution of phytophagy within the group. The resultant phylogenies confirmed *Perconbracon* as sister-group to the remaining genera and also recovered the polyphyly of *Allorhogas*.

Materials and methods

Taxon sampling

We generated both mt and nuclear genomic data for one representative species belonging to the following eight gall-associated doryctine genera: (1) *Ficobolus* Martínez, Belokobylskij & Zaldívar-Riverón; (2) *Labania* Hedqvist; (3) *Monitoriella* Hedqvist; (4) *Mononeuron* Fischer; (5) *Percnobracon* Kieffer & Jörgensen; (6) *Plesiopsenoblus* Belokobylskij, Martínez & Zaldívar-Riverón; (7) *Psenobolus* Reinhard; and (8) *Sabinita* Belokobylskij, Zaldívar-Riverón & Martínez. We also generated the same genomic data for five

species of *Allorhogas*. One is an undescribed species of *Allorhogas* that is morphologically more similar to the type species of the genus, *A. gallicola* Gahan, and both were reared from cynipid galls on *Quercus* in the eastern USA. Two additional sampled species are known to feed on seeds (*A. mineiro* Zaldívar-Riverón & Martínez and *A. granivorous* Zaldívar-Riverón & Martínez; Zaldívar-Riverón et al., 2018). The last two sampled *Allorhogas* species (*A. crassifemur* Martínez et Zaldívar-Riverón, *A. scotti* Martínez & Zaldívar-Riverón) may belong to a separate genus, because a previous phylogenetic study recovered them in an unrelated *Allorhogas* clade (Zaldívar-Riverón et al. 2014).

We also included one species of the doryctine genera *Stenocorse* (Crawford), *Lissopsius* Marsh, *Rhaconotus* Ruthe, and the highly diverse, polyphagous *Heterospilus* Haliday, with the latter being consistently recovered as sister to the gall-associated doryctine clade (Zaldívar-Riverón et al., 2007, 2008, 2014). We employed one species of the subfamily Pambolinae, *Pambolus oblongispina* Papp, to root the trees. A list with the examined species, their localities, DNA voucher, and NCBI SRA (raw reads) and GenBank (mt genomes) accession numbers is provided in Table S1 (see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2019.1685608).

Library preparation and target enrichment

Genomic DNA was extracted using the Spin Column Genomic DNA Minipreps Kit (Bio Basic). Digestion of protein components was carried out overnight in 300 µL of digestion solution and 20 µL of proteinase K, and the supernatant was subsequently processed following the manufacturer's protocol. The specimens were then removed and subsequently washed in distilled water, mounted and deposited at the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (IB UNAM). All DNA extractions were quantified using a Qubit 2.0 (Life Technologies) and for each sample up to 50 ng of extracted DNA was fragmented to a mean fragment size of 400–600 bp using either a BioRuptor® Pico sonication device or a Qsonica Q800R sonicator.

We obtained sequencing libraries following Branstetter et al.'s (2017) protocol. Libraries were generated using Kapa Hyper Prep kits (Kapa Biosystems Inc., Wilmington, MA) and custom, TruSeq-style dual-indexing barcodes (Glenn et al., 2019). For enrichment, we pooled libraries at equimolar concentrations. We used the two Mybaits® RNA bait libraries developed for Hymenoptera and synthesized by Arbor Biosciences (formerly MYcroarray, Ann Arbor, MI) following the standard enrichment protocol for UCEs (www.ultraconserved.org). For most ingroup taxa we used the first Hymenoptera bait set ('hym-v1'; Faircloth et al., 2015), which includes 2,749 probes and targets 1,510 UCE loci. *Percnobracon* and all outgroup taxa were on the other hand enriched with the second Hymenoptera bait set ('hym-v2'; Branstetter et al., 2017), which includes 31,829 probes and targets 2,590 UCE loci. The final enriched library pool quality was verified using an Agilent TapeSation 2200 (Agilent Tech). A first enriched pool was sent for sequencing to the University of Utah Genomics Core Facility on an Illumina HiSeq 2500 instrument (PE125, v4 chemistry), and a second to the Georgia Genomics Facility, University of Georgia, Athens, USA, on an Illumina NextSeq v2 300 cycle kit. Sequenced libraries produced 125 and 150 nucleotide paired-end reads respectively.

Assembly, annotation, and alignment of mitogenomes

We used FastQC version 0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc; Andrews 2010) to perform quality control of the generated data. We then cleaned and trimmed raw reads with Illumiprocessor (Faircloth, 2013), which utilizes Trimmomatic (Bolger, Lohse, & Usadel, 2014; Del Fabbro, Scalabrin, Morgante, & Giorgi, 2013; Lohse et al., 2012). The cleaned and filtered paired-end reads were employed to assemble contigs with the programme Geneious version 10.0.7 (http://www.geneious.com/; Kearse et al., 2012) using a reference-based assembly approach. We employed the strategy described by Meza-Lázaro et al. (2018) to reconstruct the 18 mitogenomes, where the longest contig generated in an assembly session was used as template for assembling the subsequent contigs. The longest consensus sequence of each specimen was submitted to MITOS (Bernt et al., 2013) for mt annotation. The assembled and annotated mt genomes were compared with the previously published mt sequence of the doryctine wasp *Spathius sinicus* (Wei et al., 2010) and with the hypothetical pancrustacean ancestor (Boore, Collins, Stanton, Daehler, & Brown, 1995; Boore, Lavrov, & Brown, 1998).

We extracted the protein-coding genes from the 18 mitogenomes and aligned and concatenated them using the online version of the programme MAFFT (http://mafft.cbrc.jp/alignment/server/; Katoh & Standley, 2013). We selected the best-fit partitioning scheme of evolutionary models with the programme PartitionFinder version 2 (Lanfear, Frandsen, Wright, Senfeld & Calcott, 2017) based on the Bayesian Information Criterion (BIC) and the greedy algorithm option. In

addition, we made a matrix containing only the 1st and 2nd positions to ensure that heterogeneity of the 3rd position would not affect the derived topologies.

Filtering and alignment of UCE data

We used the software package PHYLUCE version 1.5.0 (Faircloth, 2016) for assembly and alignment of the UCE data. The cleaned and trimmed reads were assembled *de novo* using the programme ABySS version 1.3.6 (Simpson et al., 2009). We extracted UCE contigs and removed putative paralogues by aligning all assembled contigs to the Hymenoptera v1 probe set with LASTZ (Harris, 2007). We then separated the data by locus and aligned each locus with the programme MAFFT version 7.130 b (Katoh & Standley, 2013). The resulting alignments were filtered and trimmed with the programme Gblocks version 0.91 b (Castresana, 2000) using reduced stringency settings of 0.5, 0.5, 12, and 7 for the b1–b4 options, respectively (Branstetter et al., 2017). We then partitioned data by locus and selected the best scheme with the programme PartitionFinder version 2 (Lanfear et al., 2017) based on the Bayesian Information Criterion (BIC) and the rcluster option, which is more appropriate for larger data sets. We built three final matrices based on filtering UCE loci for three differing levels of taxon occupancy (percentage of taxa required to be present in a given locus): 50%, 75%, and 90%.

Phylogenetic analyses

We carried out Maximum likelihood (ML) and Bayesian inference analyses separately on our mitogenome and UCE matrices at nucleotide level. ML analyses were performed using the programme RAxML version 8.2.10 (Stamatakis, 2014) and the GTRGAMMA model of sequence evolution. Non-parametric BTP replicates were performed with the autoMRE function.

Bayesian phylogenetic analyses were performed with two different programmes. We first carried out analyses with the programme MrBayes version 3.2.6 (Ronquist et al., 2012). Each Bayesian analysis included two simultaneous runs of 100 (mitogenome) or 50 (UCE) million generations each with uniform priors and sampling trees every 1,000 generations. We discarded the first 25% sampled trees as burn-in based on the programme Tracer version 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018), and the remaining trees were used to reconstruct a majority rule consensus tree.

We also conducted Bayesian inference analyses for both data sources with the programme PhyloBayes version 4.1 (Lartillot, Lepage, & Blanquart, 2009) using the GTRCAT configuration (Lartillot & Philippe, 2004; 2006; Lartillot, Brinkmann, & Philippe, 2007, Quang, Gascuel, & Lartillot, 2008). This programme has been successfully employed to overcome the long-branch attraction problem observed in phylogenomic analyses of mt genome data in other insect taxa (López-López & Vogler, 2017; Timmermans et al., 2016). We performed an RY-coding analysis with the 90% UCE matrix to reduce possible negative effects caused by base composition heterogeneity (Branstetter et al., 2017). This data set was converted to binary RY-coding and it was subsequently analysed with RAxML using the above parameters.

We carried out concatenated phylogenetic analyses with the 90% of completeness UCE and the complete codon mitogenome matrices with the programmes RAxML and MrBayes, using the same parameters described above. A coalescent-based species tree was also reconstructed. For this, we used RAxML to estimate gene trees for all loci contained in the 90% UCE data set, performing each analysis as a best tree plus rapid bootstrap search (GTRGAMMA model; 200 BT replicates). We used the Species TRee Analysis Web Server (STRAW: http://bioinformatics.publichealth.uga.edu/SpeciesTreeAnalysis/index.php; Shaw, Ruan, Glenn, & Liu, 2013) to root the obtained gene trees with the outgroup species, and performed a species tree analysis with the programme ASTRAL-III version 5.6.3 (Zhang et al., 2018).

We tested the phylogenetic hypotheses derived from the mt, 90% UCE and the concatenated matrix with the latter three data sets with the programme TreePuzzle version 5.3 (Schmidt, Strimmer, Vingron, & Haeseler, 2002), using a Four cluster Likelihood-Mapping (FcLM) analysis with a GTRGAMMA model (Strimmer & von Haeseler, 1997). For this test, all species in each analysis were grouped into four different clusters representing alternative resolutions to estimate the quartet support among relationships. FcLM testing was used to evaluate the two relationships recovered in the analyses performed involving the position of *Monitoriella*, one as sister to *A. crassifemur*, and a second as sister to *Ficobolus* and *Labania*.

Ancestral state reconstruction (ASR) analyses were inferred over the resultant topology with the programme Mesquite version 2.75 (Maddison & Maddison 2011) using a parsimony model to trace the known plant family association over the topology derived from the concatenated analysis. All RAxML, MrBayes and PhyloBayes analyses were performed in the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010) online services. The RAxML analyses that were conducted for each gene tree estimation included in the coalescent-based species tree analysis were carried out on the Miztli supercomputer owned by the Dirección

Table 1. Main features of the 18 mitochondrial genomes assembled for this study.

Taxa	Reads (R1-R2 average)	Length (bp)	Coverage	Genes not found
Allorhogas crassifemur	1,504,158	14,790	117.3	–
Allorhogas granivorus	567,241	15,082	41.9	–
Allorhogas mineiro	623,658	14,818	28.7	*trnQ*
Allorhogas scotti	1,050,456	14,773	97.4	*trnQ*
Allorhogas sp.	602,272	14,995	29.0	–
Ficobolus paniaguai	769,045	14,774	14.1	*trnQ*
Labania ficophaga	801,356	15,820	103.4	–
Monitoriella rufithorax	693,318	14,656	20.4	*trnM*
Mononeuron duguetiae	1,176,375	14,974	49.3	–
Percnobracon stenopterus	2,145,921	15,168	72.4	–
Plsesiopsenobolus mesoamericanus	1,048,558	14,860	74.7	*trnW*
Psenobolus parapygmaeus	1,429,656	15,255	39.7	–
Sabinita sp.	299,588	11,851	36.9	*nad2*, *trnQ*, *trnW*, *trnC*, *trnY*, *trnS2*, *rrnS*, *trnI*, *trnV*, *trnM*
Heterospilus sp.	2,204,393	15,973	48.4	–
Lissopsius sp.	2,649,887	14,867	96.3	–
Rhaconotus sp.	1,182,434	15,485	31.3	*atp8*, *nad6*, *trnC*, *trnF*, *trnI*, *trnL1*, *trnP*, *trnQ*, *trnS1*, *trnT*, *trnY*, *trnV*
Stenocorse sp.	2,220,639	15,313	49.1	*trnV*
Pambolus oblongispina	2,177,153	14,949	78.5	*atp8*, *trnG*

General de Cómputo y de Tecnologías de Información y Comunicación, Universidad Nacional Autónoma de México (DGTIC, UNAM).

Results

Mitogenome assembly and annotation

We obtained an average of 1,285,895 reads for the 18 samples after trimming and cleaning the raw data. All mitogenomes were nearly complete, varying from 11,851 to 15,973 bases. The mean coverage ranged from 14.1X to 117.3X. The mt genomes were composed of 13 protein-coding loci, 22 tRNAs, and two rRNAs. The main features of the assembled mitogenomes are listed in Table 1. The concatenated mitogenome matrix with the 13 protein-coding genes had 10,355 positions.

No gene rearrangements were identified in the protein-coding or rRNA genes for the ingroup (Fig. 1). However, we identified different patterns of gene arrangement in the region surrounding the *nad2* protein-coding gene involving the cluster of the tRNA genes *trnQ*, *trnW*, *trnC*, and *trnY* (Fig. 1). In several ingroup taxa and *Stenocorse*, the *trnW*, *trnY*, and *trnC* genes are located between the *nad2* and *cox1* genes in that order, and the *trnQ* gene is located between the *nad2* and *rrnS* region. In contrast, in *A. crassifemur* and *Psenobolus* the *trnY* and *trnW* genes are translocated to the region between *nad2* and *rrnS* next to the *trnQ* gene, respectively, whereas in *Labania* and the outgroup *Pambolus* there is an interchange of positions between the *trnC* and *trnY* genes. This region has a different structure in the doryctine *Lissopsius*, since the above tRNA cluster is located between the *cox1* and the *trnL2* genes. Moreover, in *Heterospilus* there is a translocation of the *trnQ*, which is located between the *trnC* and *trnW* genes. A reversion in the direction of the *trnW* was also observed in *Psenobolus* and *Heterospilus*.

We identified a translocation event with a reversion of the *trnH* gene in the outgroup *Heterospilus*, *Rhaconotus*, and *Pambolus* with respect to the ingroup taxa and the Pancrustacean hypothetical ancestor. This tRNA gene was placed near the region of the rRNAs and the *nad2* genes in *Heterospilus*, and between the *cox2* and the *atp8* protein-coding genes in *Rhaconotus* and *Pambolus*. In contrast, in all members of the ingroup, *Lissopsius* and *Stenocorse*, it appeared between the *nad5* and the *nad4* protein-coding genes. In addition, there was a reversion in the *trnT* gene between the protein-coding *nad4l* and the *trnP* genes in *Stenocorse* and the ingroup taxa excluding *A. granivorous*, *A. mineiro*, *Allorhogas* sp., and *Percnobracon*.

The examined species of *Stenocorse* have a different structure involving protein-coding genes rearrangements, since the region that comprises the genes that range

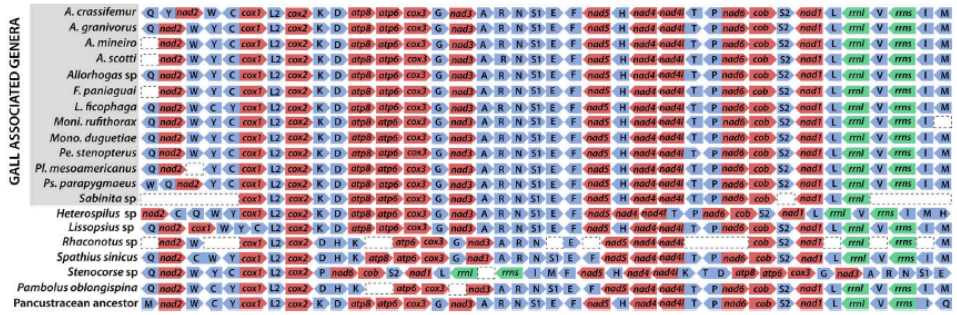

Fig. 1. Schematic representation of the gene order and composition of the mitochondrial genomes included in this study compared with the assembled mitogenome of the doryctine species *Spathius sinicus* (Wei et al., 2010) and the hypothetical pancrustacean ancestor. The protein-coding genes are shown in red, tRNAs in blue, and rRNAs in green. Genes not found are highlighted with dotted lines.

from *trnP* to *trnM* is translocated between the *cox2* and *trnF*, and the gene cluster that ranges from *trnK* to *trnE* is located between *nad4l* and *trnQ*.

UCE enrichment and alignment

The assembled sequence data produced an average of 119,936 contigs per sample (min 25,010 – max 276,425; Table 2). The mean contig coverage of the assemblies was of 5.0X, and the average coverage per UCE contig was of 40.0X. The lowest and highest numbers of UCEs were recovered for *Plesiopsenobolus* and *Rhaconotus* (640 and 902 loci, respectively). The general alignment summary list of the UCE data set is given in Table S2 (see supplemental material online), including the main features for the 50%, 75%, and 90% matrices. We recovered a total of 1,098 out of the 1,510 UCE loci targeted by the Hymenoptera v1 probe set, yielding an incomplete matrix with 640,110 aligned nucleotide positions. For all taxa, the average length of UCE contigs was 582.98 bp.

Phylogenetic analyses

We obtained identical topologies with the RAxML and MrBayes analyses derived from the mitogenome data sets both including all nucleotide positions and excluding the 3rd position. The derived phylogram including all nucleotide positions is shown in Fig. 2.1. *Percnobracon* + *Heterospilus* (BTP = 61; PP = 0.82) was recovered as sister to the remaining ingroup genera, followed by a *Ficobolus* + *Labania* clade. The five species of *Allorhogas* appeared in three separate clades, with *A. scotti* as sister to *Mononeuron*, *A. crassifemur* as sister to *Monitoriella*, though with low support (BTP= 56; PP= 0.89), and the remaining three species being nested in a clade sister to a *Plesiopsenoblus* + *Psenobolus* + *Sabinita* clade. The Bayesian phylogram obtained with PhyloBayes (Fig. S1, see supplemental material online) was mostly congruent with the above topology, though it recovered *Percnobracon* as sister to the remaining ingroup genera (PP = 0.71) and collapsed the remaining relationships that had low support in the above analyses.

All phylogenies derived from the UCE matrices with different levels of missing data recovered identical topologies. Below we therefore only refer to the concatenated matrix that included loci present in at least 90% of taxa. We also concatenated this UCE matrix with the mt data set. The final alignment derived from the UCE-mitogenome concatenated matrix contained 236,566 nucleotide positions.

The ML and Bayesian topologies derived from the 90% UCE matrix were identical. The phylogram derived from the 90% UCE matrix is shown in Fig. 2.2. All but six of the relationships recovered with the UCE matrices were congruent with the ones obtained by the mitogenome data set, and most of their clades were significantly supported. However, different from the mitogenome topology, the UCE topology recovered *Percnobracon* as sister to the remaining gall-associated group genera, followed by *A. scotti* + *Mononeuron*, *Monitoriella* as sister to *Ficobolus* + *Labania* (BTP = 87; PP = 0.92), *A. crassifemur* as sister to a clade with the *Allorhogas* species with rearing records and *Plesiopsenoblus* + *Psenobolus* + *Sabinita*, and *Psenobolus* as sister to *Plesiopsenobolus* + *Sabinita*. Identical relationships were recovered by the ASTRAL-III species tree and the 90% UCE analyses (Fig. S2, see supplemental material online).

Table 2. Contigs and UCE loci recovered for the species included in this study.

Taxa	Contigs	Contig coverage	UCE loci	UCE coverage
Allorhogas crassifemur	173,290	4.3	643	40.9
Allorhogas granivorus	67,412	5.1	747	34.2
Allorhogas mineiro	65,608	4.2	759	23.9
Allorhogas scotti	119,689	3.1	795	16.5
Allorhogas sp.	62,779	4.1	689	29.9
Ficobolus paniaguai	83,318	4.6	706	28.1
Labania ficophaga	66,807	3.9	737	19.4
Monitoriella rufithorax	93,746	5.0	750	35.5
Mononeuron duguetiae	129,479	3.7	746	24.7
Percnobracon stenopterus	56,828	13	815	101.8
Plsesiopsenobolus mesoamericanus	129,849	4.4	640	48.8
Psenobolus parapygmaeus	158,200	5.0	710	48.8
Sabinita sp.	25,010	5.7	820	21.2
Heterospilus sp.	52,178	12.5	824	86.2
Lissopsius sp.	276,425	3.5	740	42.5
Rhaconotus sp.	128,746	4.6	902	39.1
Stenocorse sp.	214,787	3.1	682	34.3
Pambolus oblongispina	254,699	3.2	790	50.4

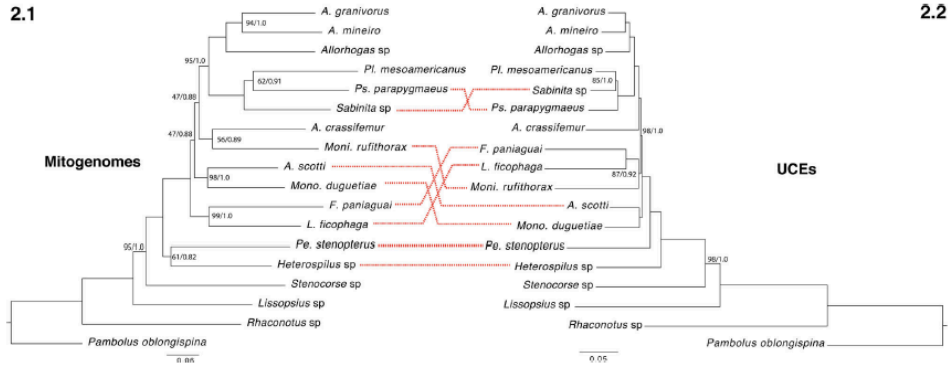

Fig. 2. Phylograms of the gall-associated doryctine genera derived from the RAxML analyses of the mitochondrial (2.1) and the nuclear (2.2) data. Bootstrap values of clades ≤100 are showed next to branches. Posterior probabilities values obtained in the MrBayes analysis for these clades are also shown. Conflicting clades between data sets are highlighted with dotted lines in red.

The UCE topologies derived from the PhyloBayes and the RY-coding analyses (Figs S3–S4, see supplemental material online) were congruent with the trees derived from the above UCE analyses, except for the placement of *Monitoriella*, though it was recovered with low support (PP = 0.51; BT = 37 in RY-coding analysis) as sister to the major clade containing *A. crassifemur*, the three species of *Allorhogas* with rearing records and the *Plesiopsenoblus* + *Psenobolus* + *Sabinita* clade.

The phylogram recovered by the analysis with the concatenated UCE and mitogenome data set including the ASR of plant family association is shown in Fig. 3. The recovered topology was similar to the one obtained in all previous UCE analyses, with the placement of *Monitoriella* as sister to *Ficobolus* + *Labania* (BTP = 67; PP = 1.0), and *Psenobolus* as sister to the *Plesiopsenobolus* + *Sabinita* clade. Association to *Ficus* (Moraceae) was recovered to have evolved in two separate clades, one in *Ficobolus* + *Labania* and the other one in the *Plesiopsenoblus* + *Psenobolus* + *Sabinita*. The main *Allorhogas* clade included four states equally parsimonious at its base and involves three different plant families.

The FcLM analysis showed a better support for the sister-group relationship between *Monitoriella* and *Ficobolus* + *Labania* in the three data bases including the mt, 90% UCE, and concatenated matrices (64.3%, 71.4%, and 64.3%, respectively). In contrast, the relationship that places *Monitoriella* as sister to *A.*

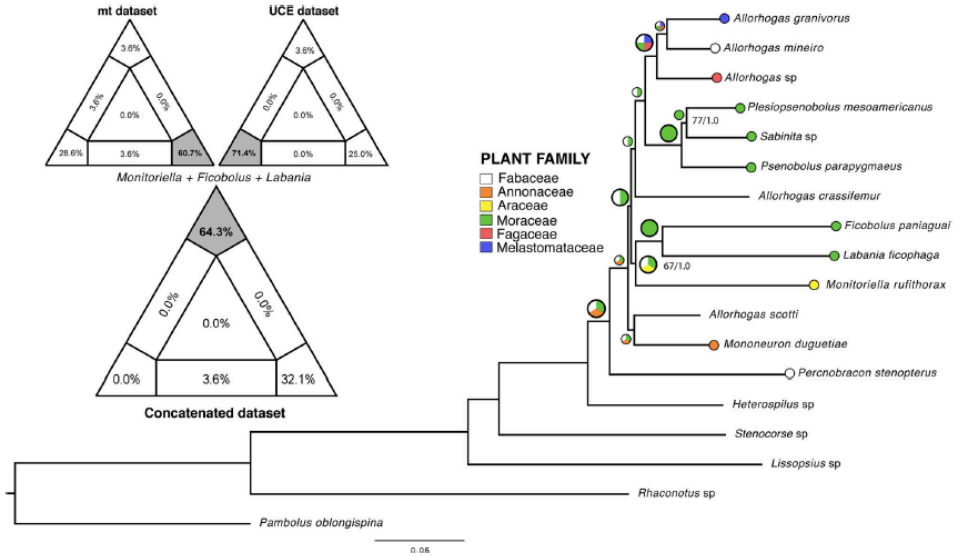

Fig. 3. Phylogram of the gall-associated doryctine genera derived from the RAxML analysis using the concatenated UCE and mitogenome (13 mitochondrial protein-coding genes) data sets. Bootstrap values of clades <100 are shown next to branches. Posterior probabilities values obtained in the MrBayes analysis for these clades are also shown. ASR of the plant family association is shown next to nodes and at the tips of branches. Results from FcLM are also shown at the left of the phylogram. The clusters pointing to the relationship *Monitoriella* + *Ficobolus* + *Labania* obtained the highest probability values for the three data sets.

crassifemur had low percentage values (7.1%, 3.6%, and 3.6%, respectively) (Fig 3).

Discussion

Mitogenome evolution of the gall-associated doryctine genera

The general structure of the assembled mitogenomes for the ingroup and outgroup taxa are similar to the one reported for the doryctine genus *Spathius* (*S. sinicus*) and other braconid genera (Li et al., 2016; Wei et al., 2010). However, we found some tRNA rearrangements and reversion events in the ingroup taxa. The particular position of the *trnC* next to the *cox*1 gene and the change of direction of the *trnT* gene are present in most of the gall-associated doryctine genera, though they also occur in *Stenocorse*. The configuration *trnQ-nad2-trnW-trnC-trnY* observed in *Labania* seems to be a regression to the ancestral condition, since it is also present in the outgroup *Pambolus*, and the ancestral arthropod mitogenome. The direction of the *trnT* gene in the clade with three of the species assigned to *Allorhogas* also appears to represent a regression to the ancestral arrangement, since it is also present in *Percnobracon*, which was sister to the remaining ingroup taxa, *Heterospilus*, *Lissopsius*, *Rhaconotus*, *Spathius*, *Pambolus*, other cyclostome taxa and the ancestral arrangement of Hexapoda (Wei et al., 2010).

Accelerated rates of substitutions and gene rearrangements, including translocation events in protein-coding genes, have been found to occur in members of the families Pteromalidae (Oliveira, Raychoudhury, Lavrov, & Werren, 2008) and Braconidae (Wei et al., 2010). In particular, previous studies have identified hot-spots of tRNA gene rearrangements in three regions within Braconidae: *trnK-trnD*, *trnA-trnR-trnN-trnS1-trnE-trnF*, and *trnT-trnP* (Dowton, 1999; Dowton & Austin, 1999; Li et al., 2016). We observed that the first three of these regions are conserved in the gall-associated genera, whereas a rearrangement event was found in the last region, though it corresponded to a change in the direction of the *trnT* gene instead of a translocation with the *trnP* which seems to be characteristic of members belonging to other braconid subfamilies (Li et al., 2016).

Our results indicate that the gall-associated doryctine genera have in general conserved patterns and few

rearrangement events compared with the assembled mitogenomes observed for the remaining members of the subfamily, the closely related Pambolinae and the hypothetical pancrustacean ancestor. However, it is still necessary to examine additional doryctine taxa to know whether they share the above tRNA arrangements present in most of the gall-associated genera.

Particular attention deserves the mt genome of *Stenocorse*, since it comprises a translocation event involving an interchange of two large clusters of genes, which include the two rRNAs, seven protein-coding and several tRNAs genes. In Hymenoptera, rearrangements of protein-coding genes have been reported for various Chalcidoidea families (Chen et al., 2018; Oliveira et al., 2008; Xiao, Jia, Murphy, & Huang, 2011; Yang, Liu, Li, & Wei, 2019; Zhu et al., 2018), in particular for species of the families Aulacidae (Wei, Wu, van Achterberg, & Chen, 2015), Bethylidae (Wei, Li, van Achterberg, & Chen, 2014), Ichneumonidae (Dowton, Cameron, Dowavic, Austin, & Whiting, 2009) and Trigonalyidae (Wu et al., 2014). In Braconidae, protein-coding gene rearrangements have been reported for the microgastrine wasp *Cotesia vestalis* (Haliday) (Wei et al., 2010).

Phylogenetic relationships

The gall-associated doryctine clade has received special attention in phylogenetic studies during the last few years (Zaldívar-Riverón et al., 2007, 2008, 2014). This is due to the variety of life history strategies that have been discovered in its species, including their association with a number of different vascular plant families and plant organs. These phylogenetic studies, which were based on Sanger sequencing of a few nuclear and mt markers, served as a basis to start understanding the evolution of gall association and phytophagy within the group. However, these studies failed to adequately resolve generic relationships and limits, leaving many unanswered questions. Here we gathered both mt and nuclear genomic data to carry out a phylogenomic study for representative species of most of the known gall-associated doryctine genera. Our analyses yielded an almost fully resolved estimate of phylogeny that was consistent with the well-supported relationships obtained in the previous molecular studies. Below we mention the most relevant confirmed and novel relationships obtained in our study and discuss their implications for the classification and evolution of the group.

Percnobracon is confirmed as sister to the remaining gall-associated doryctine genera. Two separate clades containing genera whose species appear to be exclusively associated with different organs of *Ficus* species were also corroborated. One of these clades includes a sister relationship between *Ficobolus* and *Labania*. These two taxa are considerably different in their external morphology and thus their close relationship was not expected. The other clade associated with *Ficus* species is represented by *Plesipsenobolus*, *Psenobolus*, and *Sabinita*. Members of these genera are morphologically similar, lacking any clear diagnostic features that help to distinguish them from each other (Zaldívar-Riverón et al., 2014).

Allorhogas is confirmed to be polyphyletic. All analyses carried out with both UCE and mitogenome information recovered *A. crassifemur* and *A. scotti* distantly related to the clade with the remaining three species of *Allorhogas*. One member of this clade is morphologically similar to the type species of the genus, and thus the generic name probably should be retained for this group. *Allorhogas crassifemur* and *A. scotti* on the other hand have external morphological features that are absent in most of the remaining described species of *Allorhogas*. These include differences in the first flagellomere length, wing venation, and sculpture of the metasoma (Martínez & Zaldívar-Riverón, 2013). *Allorhogas scotti* is only morphologically similar to *A. coccolobae* Martínez & Zaldívar-Riverón and *A. marshi* Martínez & Zaldívar-Riverón, which were also recovered outside the main *Allorhogas* clade in Zaldívar-Riverón et al.'s (2014) phylogenetic study.

Among the novel relationships revealed by our phylogenomic analyses was the placement of the monotypic *Mononeuron* as sister to the remaining genera except *Percnobracon* and with *A. scotti* as its sister taxon. The only described species of *Mononeuron*, *M. duguetiae* Fischer, was largely suspected to belong to *Allorhogas* since their members appeared to share various external morphological features (Fischer, 1981). For this reason, the phylogenetic affinities of *M. duguetiae* with respect to *Allorhogas* were tested in a previous molecular phylogenetic study (Nunes et al., 2012), where the former species was recovered as the sister of a *Labania* + *Monitoriella* clade, though with low support. This work, however, only included DNA sequence data for three *Allorhogas* species. Our gathered phylogenomic data thus highlight the necessity to re-evaluate the generic composition and morphological diagnostic features of *Mononeuron*.

The placement of *Monitoriella* remains unresolved. This genus was recovered as sister to *A. crassifemur* in the analyses with the mt data set, which is consistent with the previous estimate of phylogeny using a few molecular markers (Zaldívar-Riverón et al., 2014). However, *Monitoriella* appeared with higher support as sister to the *Ficobolus* + *Labania* clade in the UCE and

Table 3. Summary of the plant families, plant organs and confirmed and presumed (?) host associations that have been recorded for the gall-associated doryctine genera.

Genus	Plant family	Plant organ	Trophic interaction
Allorhogas	10 families	Leaf/Stem/Seed	Gall former/Inquiline/Seed predator/Parasitoid?
Ficobolus	Moraceae	Stem	Gall former?
Labania	Moraceae	Aerial root/Leaf	Gall former?
Monitoriella	Araceae	Leaf	Gall former
Mononeuron	Annonaceae	Leaf	–
Percnobracon	Fabaceae	Stem	Inquiline?
Plesiopsenobolus	Moraceae	Stem/Syconia	Inquiline?
Psenobolus	Moraceae	Syconia	–
Donquickeia	Myrtaceae, Asteraceae	Leaf	–
Sabinita	Moraceae	Leaf	Gall former?

concatenated analyses, and this relationship was also preferred in the FcLM analyses. Further studies adding more representative species for the taxa involved will help to confirm this relationship. *Monitoriella* had been previously placed outside the Doryctinae due to its disparate external morphology (Hedqvist, 1963; Wharton, 1993), and its affinity with the remaining gall-associated associated genera was only discovered after its inclusion in molecular phylogenetic studies (Zaldívar-Riverón, Mori, & Quicke, 2006).

Evolution of phytophagy

A list summarizing the host plant association that has been confirmed or presumed for the ingroup genera is given in Table 3. Currently, only *Allorhogas* and *Monitoriella* have species with confirmed rearing records. These species are known to be either gall-formers (e.g. Badenes-Pérez & Johnson, 2007; Infante, Hanson, & Wharton, 1995; de Macêdo & Monteiro, 1989), seed predators (e.g. Zaldívar-Riverón et al., 2018), or inquilines that feed on galls made by other insect species (Moreira et al., 2017). However, observations made for members of other genera suggest that their species could also be phytophagous. This includes species of *Ficobolus*, *Labania*, *Mononeuron*, and *Sabinita*, which also probably are gall-formers based on their high number of reared individuals or because they were the only reared specimens (Belokobylskij et al., 2015; Nunes, Penteado-Dias, Ceccarelli, & Zaldívar-Riverón, 2012; Zaldívar-Riverón et al., 2014; Zaldívar-Riverón, pers. obs.).

Percnobracon, which is sister to the remaining gall-associated genera, was previously suggested to contain parasitoid species of gall-former dipterans (Martínez, 2006). Recent rearing records, however, point out that they might actually be phytophagous inquilines (Martínez, unpubl. data). If this is confirmed, the transition to phytophagy in the gall-associated doryctine clade could have occurred early during its evolution. The remaining unconfirmed records of parasitoidism in the group are for only a few species assigned to *Allorhogas*, most of which are old and/or only based on indirect observations (see Zaldívar-Riverón et al., 2014). Further rearing confirmations will therefore reveal whether phytophagy is the only feeding strategy within *Allorhogas*, or if parasitoidism is present, how the latter two conditions evolved in the species assigned to this genus.

The phylogenetic analyses and the ASR showed that association to *Ficus* species probably evolved in two separate clades. In one of them, species of *Psenobolus* appear to be restricted to syconia (van Achterberg & Marsh, 2002; Ramírez & Marsh, 1996), whereas the only reared species of *Plesiosenobolus* and the only described species of *Sabinita*, *S. mexicana* Belokobylskij, Zaldívar-Riverón & Martínez, were obtained from stem and leaf galls, respectively (Zaldívar-Riverón et al., 2014). In the second clade, *Ficobulus* and *Labania* contain species that have been reared from stem galls and aerial root, stem and leaf galls, respectively (Belokobylskij et al., 2015; Marsh, 2002; Zaldívar-Riverón et al., 2014). The rearing records gathered so far for the above five genera thus suggest that their species diversification was probably restricted to host shifts to distinct *Ficus* species, as well as to shifts to different plant organs. Similarly, *Monitoriella* appeared to have specialized to attack species of a particular host plant family, Araceae (Infante et al., 1995; Shimbori, Penteado-Dias, & Nunes, 2011), though its phylogenetic placement within the gall-associated doryctine clade still remains to be resolved.

The main *Allorhogas* clade recovered in Zaldívar-Riverón et al.'s (2014) phylogenetic study was represented here by three species. Two of these are confirmed to feed on seeds of species of the plant families Melastomataceae and Fabaceae (*A. granivorus* and *A. mineiro*; Zaldívar-Riverón et al., 2018), whereas the third was reared from galls made by the cynipid *Callirhytis quercusbatatoides* (Ashmead, 1881) in the

oak species *Quercus geminata* Small (Fagaceae). Thus, *Allorhogas sensu stricto* is composed of species reared from at least five different host plant families, since in the latter study it also contained species present in the plant families Myrtaceae and Solanaceae. Further phylogenetic studies based on a vast taxon sampling will therefore allow the assessment of the mode and tempo of the shifts to colonize/feed on different plant families in *Allorhogas*, which probably triggered its species diversification.

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No potential conflict of interest was reported by the authors.

Supplemental data

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Capítulo II

Actualización del conocimiento taxonómico de *Allorhogas* Gahan: Delimitación de las especies del clado norteamericano asociadas con agallas de avispas de la familia Cynipidae (Hymenoptera) en especies de *Quercus* (Fagaceae) y redescripción del género

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Research

Taxonomy

Species Diversity in the Braconid Wasp Genus *Allorhogas* (Doryctinae) Associated With Cynipid Galls on Live Oaks (Quercus: Fagaceae) Using Natural History, Phylogenetics, and Morphology

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Abstract

The discovery of new biodiversity, during an age of unprecedented extinction, is vital for all the life sciences and the quality of human life. One ecologically and economically important group that requires attention is the hymenopteran family Braconidae, which is estimated to include thousands of undescribed species. Here we assessed the genetic structure and species diversification in the braconid wasp genus *Allorhogas* Gahan (Doryctinae) that were reared from galls of five cynipid wasp species associated with three live oak species (Fagaceae: *Quercus*: subsection *Virentes*) in the southeastern United States. We explored genetic variation in the single-locus barcoding COI region of the mitochondrial DNA (mtDNA), and conducted analyses with different DNA sequence-based species delimitation approaches both for the above marker and genome-wide nuclear data using ultraconserved elements (UCEs). We found high variation in the mtDNA barcoding region among specimens of *Allorhogas* reared from galls made by different cynipid species in distinct plant organs and among specimens reared from the same type of gall from two separate geographic regions. In addition, our analyses of mtDNA and multilocus nuclear data were concordant in consistently delimiting at least five genetic lineages. We combined this molecular evidence with morphological data to describe four new species and redescribe the type species of the genus, *Allorhogas gallicola* Gahan, which exhibited similar morphological, ecological, and biogeographic characteristics to the four new species. This study highlights the importance of carrying detailed rearing surveys to uncover the intricate species interactions and species diversity that is present in gall-former systems.

Key words: host assosiation, Braconidae, taxonomy, Hymenoptera, species discovery

Ecology can play a fundamental role in the diversification of species (Schluter 2000, 2001). This is particularly true for species-rich herbivorous insects, their host plants, and their natural enemies (e.g., Mayhew 2007, Futuyma and Agrawal 2009, Matsubayashi et al. 2010, Becerra 2015, Wiens et al. 2015), which collectively make up the vast majority of described eukaryotic species (Forbes et al. 2018, Stork 2018). The evolution of this interconnected and grand diversity is, in part, explained by the species interactions within these multitrophic systems at both the level of insects on plants (Mayhew 2007, Futuyma and Agrawal 2009, Wiens et al. 2015) and their associated natural enemy communities, which include predators, parasitoids and other coexisting herbivores (Nyman et al. 2007, Haddad et al. 2009, Scherber et al. 2010, Ebeling et al. 2014).

However, a fundamental problem in biodiversity science exists that precludes detailed scientific exploration of species-rich communities such as these—we still do not know, within an order of magnitude, how many and which species are present. One challenge in determining the number of species in any taxon is the growing awareness that cryptic diversity contributes to this problem (Janzen et al. 2017), even in well-studied groups (e.g., Duran et al. 2019). Moreover, discovering new biodiversity, during an unprecedented rate of global extinction, is vital for all the life sciences and the quality of human life (e.g., Díaz et al. 2006, Garibaldi et al. 2013).

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One important source to gather ecological information from insect–plant interactions is through the direct observation of organisms in their environment (Futuyma 1998). Natural history records are essential to understand biological diversity, serving as a window for a better comprehension of the evolutionary processes on Earth (Holmes et al. 2016). This information represents a rich source of data to conduct studies on different biological disciplines, such as biogeography, systematics, and biological conservation (Suarez and Tsutsui 2004). Moreover, natural history data can be used to address specific ecological questions about interspecific associations, population sizes, distribution, and richness of species, as well as attributes of individual specimens (Pyke and Ehrich 2010). Detailed reports associated with specimens, therefore, add significant value and increase data richness in biological studies, which can be recorded through videos, sound recordings, information on habitat and photographs, among other tools (Bakker et al. 2019).

Wasps of the family Cynipidae (Hymenoptera) represent a diverse group of specialized herbivores that induce gall formation in different plant tissues and organs, mainly in species of *Quercus* L., but also in members of other genera of the families Fagaceae (e.g., *Lithocarpus* Blume, *Chrysolepis* Hjelmq., *Castanea* Mill., and *Prosopis* L.) and Rosaceae (*Rosa* L.) (Stone et al. 2002, Ronquist et al. 2015, Zhang et al. 2019a). Cynipid gall wasps are mainly distributed within temperate areas of the Northern Hemisphere, constituting one the largest radiations of gall-inducing organisms, currently having ca. 1,400 described species (Ronquist et al. 2015, Pénzes et al. 2018). The galls that are induced by these wasps are known to represent an important resource for other arthropods, which structures the communities of their natural enemies, including parasitoids and inquilines (Sanver and Hawkins 2000, Stone and Schönrogge 2003, Hayward and Stone 2005) and has led to galls being identified as important ecosystem engineers (Wetzel et al. 2016, Egan et al. 2018b).

American live oaks (*Quercus* subsection *Virentes*) include seven recognized tree species that are distributed in North and Central America and Cuba (Muller 1961, Cavender-Bares et al. 2015, Hipp et al. 2018). Live oaks that are distributed in southeastern United States, *Q. fusiformis* Small, *Q. geminata* Small, and *Q. virginiana* Mill, harbor a diverse cynipid wasp community whose fauna has been documented and studied (Ashmead 1881, 1885, 1896; Mayr 1881; Lund et al. 1998; Wilson et al. 2000; Melika and Abrahamson 2002, Egan et al. 2013). The different cynipid genera that have been reported to induce galls on these live oak species tend to overlap geographically and ecologically, with species of *Andricus* Hartig, *Bassettia* Ashmead, *Belonocnema* Mayr, *Callirhytis* Förster, *Disholcaspis* Dalla Torre and Kieffer, *Loxaulus* Mayr, *Odontocynips* Kieffer, and *Neuroterus* Hartig being found on the same individual host trees and sometimes sharing the same host organs (Egan et al. 2013, Forbes et al. 2016, Egan et al. unpublished data). In contrast, the information available to date for the cynipid species present in the remaining of live oak species, *Q. brandegeei* Goldman, *Q. minima* (Sarg.) Small, *Q. oleoides* Schltdl. and Cham. and *Q. sagreana* Nutt., is limited (Díaz and Gallardo 1998, Abrahamson et al. 2003, Pujade-Villar et al. 2009).

The communities that comprise live oaks and their associated gall-inducing cynipid species represent an ideal model to explore the extraordinary undocumented insect species diversity present in gall-former systems. The recent finding of a previously unreported interaction in nature that involves the love vine species *Cassytha filiformis* L. (Lauraceae) parasitizing leaf galls induced by cynipid wasps on *Q. geminata* in Florida (Egan et al. 2018a) is just an example of the intricate relationships that are present in this multitrophic system (Egan et al. 2018b). Currently, the invertebrate communities associated with the following three species of cynipids that induce galls on the live oaks occurring along southeastern United States have been characterized: *Disholcaspis quercusvirens* (Ashmead) (Bird et al. 2013), *Belonocnema treatae* Mayr (Forbes et al. 2016), and *Bassettia pallida* Ashmead (Weinersmith et al. 2020). These studies found a high diversity of species in the natural enemy communities, with many undescribed or cryptic species unique to each gall former on the same set of host plants.

Among the arthropods that were reared in the above faunistic studies in the southeastern United States, the presence of specimens belonging to the braconid wasp genus *Allorhogas* Gahan (Doryctinae) was reported on galls made by *Be. treatae* and *Ba. pallida* (Forbes et al. 2016, Weinersmith et al. 2020). This observation was surprising, as previously there had been only two species of *Allorhogas* described from all of North America north of Mexico. Previously, *Allorhogas* was considered more of a Central and South American lineage, with 53 species described to date, making it the most species-rich gall-associated doryctine genus. All species of *Allorhogas* whose biology is known are phytophagous with different feeding habits, including gall formation, inquilinism, and seed predation (de Macêdo and Monteiro 1989, Morales-Silva and Modesto-Zampieron 2017, Moreira et al. 2017, Zaldívar-Riverón et al. 2018, Joele et al. 2019). Interestingly, this genus was originally described based on a single species, *Allorhogas gallicola* Gahan, which was reared in Maryland, United States, from cynipid galls present on the stems of an oak species with an invalid recorded name ('*Q. pinnifolia*'; Gahan 1912). These galls were mentioned by the author to be infested by the inquiline moth *Synanthedon scitula* (Harris) (Sesiidae: Lepidoptera), thus presuming that *A. gallicola* was a parasitoid, though no additional evidence was provided.

Recently, one of the authors of this study (S.P.E.) and his labreared specimens of *Allorhogas* from three additional species of cynipids that make galls on leaves and stems of live oaks in various localities of Florida and Texas, United States. The galls induced by the five cynipid species from which the *Allorhogas* wasps have been reared from live oaks in the southeast United States have different morphologies and are found in two distinct organs from the same host trees (leaves and stems) (Melika and Abrahamson 2007, Bird et al. 2013, Egan et al. 2013, Hood et al. 2018, Weinersmith et al. 2020). The observations that each cynipid species hosting an *Allorhogas* wasp has different gall morphologies and gall development phenologies (Melika and Abrahamson 2007, Bird et al. 2013, Egan et al. 2013, Hood et al. 2018), along with preliminary observations that showed subtle morphological variation in color and ovipositor length in the *Allorhogas* specimens, suggest that this genus in the United States may contain additional cryptic and/or undescribed species.

Herein, we explore the species diversity in this group by combining natural history, morphology, and tests of genetic structure and species delimitation. First, we assess the genetic structure among these newly discovered specimens of *Allorhogas* that were reared from galls made by five cynipid species present on live oaks in the southeastern United States based on a DNA sequence fragment of the cytochrome oxidase I (COI) mitochondrial (mtDNA) gene (the barcoding locus; Hebert et al. 2003a). Second, since the examined specimens exhibited marked genetic structure with respect to the galls from which they were reared, we then investigated the number of species diversification events in the samples conducting DNA sequence-based species delimitation analyses using the mtDNA barcoding region and for phased multilocus nuclear DNA sequences obtained from ultraconserved elements (UCE; Faircloth et al. 2012).

Third, we explored the external morphology in this group of specimens to assess diagnostic features for species delimitation. Collectively, our results consistently suggest the existence of at least five distinct evolutionary lineages of *Allorhogas* supported by molecular, morphological, and ecological data. Thus, we describe here four new species and also redescribe the morphologically similar type species of the genus, *A. gallicola* Gahan, which was originally reared from a species of oak in a different oak lineage collected over 1,500 km away. This study further highlights the importance of combining detailed natural history with systematic rearing surveys to uncover the intricate insect species diversity that is present in gall-former systems.

Materials and Methods

Sample Collection

We reared 24 specimens assigned to *Allorhogas* from five well-characterized cynipid gall morphotypes that specialize on live oaks (Egan et al. 2013) in the monophyletic subsection *Virentes* within the genus *Quercus* (Hipp et al. 2018). The specimens included in this study were collected on *Q. fusiformis*, *Q. geminata* and *Q. virginiana* in various localities across the southeastern United States in the states of Florida and Texas between 2014 and 2019 (Fig. 1A). The *Allorhogas* specimens were reared from galls made by *Be. treatae* and *Ba. pallida* (Fig. 1B and C), whose associated insect communities were reported by Forbes et al. (2016) and Weinersmith et al. (2020), respectively; and from galls made by *Andricus quercuslanigera* (Ashmead), *C. quercusbatatoides* (Ashmead) and *D. quercusvirens* (Ashmead) (Egan et al. unpublished data; Fig. 1D–F). All the entomological material that emerged from the collected galls was stored and refrigerated in 95% ethanol. The examined specimens were sent to the Colección Nacional de Insectos (CNIN), Instituto de Biología, Universidad Nacional Autónoma de México (IB UNAM), in Mexico City, Mexico, for molecular processing and morphological examination. A list with the examined specimens of *Allorhogas*, their associated cynipid and oak species, localities of provenance, DNA voucher and GenBank (COI), and NCBI SRA (raw reads for the UCE generated data) accession numbers is shown in Supp Table S1 (online only).

Mitochondrial and Nuclear UCE Data

Genomic DNA was extracted following a nondestructive technique (Ceccarelli et al. 2012) with the Spin Column Genomic DNA Minipreps Kit (Bio Basic, Toronto, Canada). Samples were incubated overnight and subsequently were removed, washed, mounted, and labeled for morphological descriptions. The supernatant was then processed following the manufacturer's protocol. For this study, we generate sequences for the COI mtDNA gene for 22 specimens using the primers LCO (5′-GTCAACAAATCATAAAGATATTGG-3′) and HCO (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). PCR was performed in 15 µl of total volume, containing 3 µl of 10× PCR Buffer, 0.1 µl of $MgCl_2$ 50 mm, 0.2 µl of each primer 10 µM, 0.2 µl of MyTaq DNA Polymerase (Bioline, London, United Kingdom), 4 µl of DNA template and 7.3 µl of ddH_2O. The PCR temperature conditions were as follows: 3 min of initial denaturation at 95°C, followed by 35 cycles of 94°C for 40 s, 48°C for 50 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. All final PCR products were sent for sequencing at IB UNAM. Sequences were edited and aligned manually with the program Geneious v10.0.7 (https://www.geneious.com; Kearse et al. 2012).

DNA extractions of eight representative specimens (as well as the outgroup, see below) that were selected based on the results obtained from the species delineation analyses performed with the mtDNA data set were quantified in a Qubit 2.0 (Life Technologies). Approximately 50 ng of each DNA template was sonicated in a BioRuptor Pico device, and genomic libraries for these specimens were made following Branstetter and colleagues' (2017) protocol. We used the Kapa Hyper Prep kit (Kapa Biosystems Inc., Wilmington, MA) and the TruSeq-style dual-indexing barcodes (Glenn et al. 2019). The Hymenoptera probe set that targets 1,510 loci (Faircloth et al. 2015) and synthesized by MYcroarray (MYcroarray, Ann Arbor, MI) were employed for library enrichment. Libraries were sequenced on an Illumina NextSeq v2300 using a 150 base paired-end sequencing at the Georgia Genomics Facility, University of Georgia, Athens, United States.

UCE Data Assembly and Allele Phasing

To assemble and phase the UCE data, we followed the suggested workflow from the software package PHYLUCE version 1.6.6 (Faircloth 2016). In addition to the eight representative specimens we chose for UCE processing, we also included the raw data of an *Allorhogas* specimen reared from a cynipid gall on a live oak published by Samacá-Sáenz et al. (2019) and a specimen reared from galls of the cynipid species *Eschatocerus niger* Kieffer and Joergensenon on a *Prosopis* tree (Fabaceae) in Argentina to root the trees. First, we cleaned and trimmed the raw reads with Illumiprocessor (Faircloth 2013), which uses Trimmomatic version 0.39 (Bolger et al. 2014). Clean reads were assembled *de novo* using Trinity version 2.2.0 (Grabherr et al. 2011) in the Galaxy web platform at the public server (https://usegalaxy.org/; Afgan et al. 2018). Alignments were made with MAFFT version 7.407 (Katoh and Standley 2013) and an edge trimmed incomplete matrix was generated. We then exploded the alignments and followed the pipeline developed by Andermann et al. (2019), which utilizes SAMtools version 1.9 (Li et al. 2009) to sort and obtain phased allele sequences. For the subsequent analysis, we did not allow the inclusion of missing data and only employed the phased data of the loci present in all taxa using a complete matrix. SNPs were subsequently extracted directly from allele sequences, also using the python function (https://github.com/tobiashofmann88/snp_extraction_from_alignments/) to call one variable position per locus.

Genetic Variation and Species Delineation Approaches

COI Analyses

A fragment of the COI mtDNA gene was obtained for 24 specimens of *Allorhogas* reared from five different types of galls. All but two of these sequences were generated from this study. Sequences of the remaining two specimens were obtained from two previously published studies (Forbes et al. 2016, Weinersmith et al. 2020).

We first tested for genetic differentiation in the COI data set, calculating corrected pairwise genetic distances among the examined samples using the Kimura 2-parameter (K2P) evolutionary model with the program MEGA version 10.1.7 (Stecher et al. 2020). Since there was clear genetic differentiation among samples, we then performed two DNA sequence-based species delineation approaches for the mtDNA COI data set, the 2% genetic divergence criterion (Hebert et al. 2003b) using the corrected pairwise genetic distances using the K2P evolutionary model and the generalized mixed Yule coalescent (GMYC) model (Pons et al. 2006). We obtained the ultrametric tree required for the latter model with

Fig. 1. (A) *Quercus virginiana* oak tree in Florida. Types of galls from which the *Allorhogas* specimens included in this study were reared; (B) *Belonocnema treatae* leaf gall; (C) *Bassettia pallida* stem gall; (D) *Andricus quercuslanigera* leaf gall; (E) *Callirhytis quercusbatatoides* stem gall; (F) *Disholcaspis quercusvirens* stem gall.

the program BEAST version 1.10.4 (Suchard et al. 2018). We ran the analysis for 100 million generations, sampling trees each 1,000 generations, using a log-normal distribution relaxed clock and a coalescent prior. We inspected convergence and effective sampling sizes (ESS) of all parameters with the program Tracer v1.7.1 (Rambaut et al. 2018); burn-in was established after 25 million generations. We reconstructed a maximum clade credibility tree with the remaining sampled trees and built a GMYC topology using the SPLITS package (https://r-forge.r-project.org/projects/splits; Ezard et al. 2009) with the single-threshold approach implemented in R v3.6.1 (R Core Team 2019).

UCE Analyses

We performed a phylogenetic analysis of the UCE phased data for nine specimens of *Allorhogas* that were reared from cynipid galls on live oaks and for a species assigned to *Al.* cf. *prosopidis*, which was employed as outgroup. The latter species was recovered as sister to a clade with the taxa examined in this study in a phylogenetic study of the genus based on UCE data (Samacá-Sáenz, in prep.). We carried out a maximum likelihood (ML) inference analysis using the program RAxML version 8.2.10 (Stamatakis 2014), the GTRGAMMA model of sequence evolution, and the autoMRE function for non-parametric bootstrap replicates.

Two multispecies coalescent (MSC) model analyses were then implemented for the phased UCE data for the nine individuals of *Allorhogas*. We first used the DISSECT method, which groups specimens in multiple coalescent clusters (Jones et al. 2015), implemented in the package STACEY version 1.2.5 (Jones 2017) and available in the program BEAST2 version 2.6.1 (Bouckaert et al. 2019). We did not use a priori taxon assignments, thus treating each allele as a different terminal. Model selection was performed for each locus using the bModeltest option (Bouckaert and Drummond 2017), which estimates the substitution models simultaneously with the Bayesian tree search. Species trees were estimated using a strict clock at 1.0 under the BirthDeathCollapse model, using a value of 1×10^{-4} for the collapseHeight parameter. Other prior values were set following Anderman et al. (2019): bdcGrowthRate = log-normal (M = 4.6, S = 2); collapseWeight = beta (alpha = 2, beta = 2); popPriorScale = log-normal (M = −7, S = 2); relativeDeathRate = uniform (upper = 1.0). Analyses were run for 200 million generations, sampling trees every 20,000 generations. Sampled species trees were visualized with DensiTree 2.2.7 (Bouckaert 2010). Molecular species delimitation was performed with SpeciesDelimitationAnalizer version 1.8.0 (Jones et al. 2015), discarding the first 20 million generations as burn-in and using a collapse height of 3×10^{-4}.

We also carried out an MSC analysis with the SNPs obtained from the phased UCE data with SNAPP version 1.5.0 (Bryant et al. 2012) implemented in the program BEAST2 version 2.6.1. The Bayes Factor Delimitation (BFD*, Leaché et al. 2014) method for genomic data was employed to compare the following alternative species delimitation hypotheses: 1) a morphospecies assignment (five morphospecies), 2) species delimited both by the 2% divergence criterion and the GMYC model with the COI data set (six spp.), 3) species recovered from the STACEY analysis with UCE data (six spp.), and 4) assignment of two species according to the geographical distribution of the examined specimens (Florida and Texas). All analyses were run for 48 steps and 100,000 generations, with a preburnin of 10,000 following Leaché et al. (2014). We subsequently calculated the Bayes Factors (BF) by subtracting the marginal likelihood estimates (MLE) between two models and then multiplying the difference by two (Leaché et al. 2014). All RAxML, BEAST, and BEAST2 analyses were performed in the CIPRES Science Gateway (Miller et al. 2010) online services.

Morphospecies Assignment

We examined the external morphology of 24 specimens assigned to *Allorhogas* that were reared from five types of galls on live oaks in various localities of Florida and Texas, United States, and assigned them to morphospecies. We followed the terminology of Sharkey and Wharton (1997), Harris (1979), and Marsh (2002) for the external morphological, surface sculpture, and wing venation features, respectively. We also examined the type material of *A. gallicola*, the type species of the genus, which was reared from an oak species in Maryland, United States (Gahan 1912), and is morphologically similar to the specimens examined in this study. Moreover, we examined additional unidentified specimens of *Allorhogas* from various localities in United States that are deposited in the National Museum of Natural History, Washington, DC, United States (NMNH).

Digital color multifocal images were taken at the IB UNAM with a Leica Z16 APO-A stereoscopic microscope, a Leica DFC490 camera and the Leica Application Suite (LAS) version 4.3.0. The type material of the newly described species and the additional specimens reared from Florida and Texas are deposited in the Colección Nacional de Insectos (CNIN) of the Instituto de Biología, Universidad Nacional Autónoma de México (IB UNAM). The type material of *A. gallicola* and other examined material is deposited in the NMNH.

Results

Sequence Data Summary

The COI fragment obtained for the 24 specimens of *Allorhogas* had a length of 647 bp, whereas for the UCE data, we recovered a total of 1,019 UCE loci from nine specimens. Alignments from the incomplete edge trimmed matrix were separated in FASTA files for each individual and phased to obtain two allelic sequences per specimen, thus duplicating the original number of sequences to 18. There were 164 shared phased UCE loci among the 18 terminals with an average length of 954.30 bp. The complete matrix, for which we did not allow the inclusion of missing data and only used the phased data of the loci present in all taxa, comprised 156,506 aligned nucleotide positions and 6,833 informative sites. We used the above alignments to summarize the data and call one variant per locus, obtaining a total of 164 SNPs.

Genetic Differentiation and Species Delimitation Analyses

The corrected COI pairwise distances using the K2P evolutionary model and the ultrametric tree reconstructed using this gene marker with the results obtained from the GMYC model are shown in Supp Table S2 (online only) and Fig. 2, respectively. The highest COI divergence values (10.1–15.0%) are those between the specimens reared from galls of *Ba. pallida* and the remaining specimens. Two specimens associated with galls of *Be. treatae* in McAllen, Texas, had considerably high values (6.9–7.1%) with respect to other specimens reared from leaf galls induced by the same species and *An. quercuslanigera*. A specimen reared from stem galls of *C. quercusbatatoides* in Suwannee River, Florida, showed high COI distances (9.9–11.5%) with respect to the remaining specimens associated with galls of *C. quercusbatatoides* and *D. quercusvirens* in Florida, which, on the other hand, had lower values among them (0.7–2.8%).

The 2% genetic divergence criterion and the GMYC model recovered the same six species. One of these species was exclusively reared from stem galls of *Ba. pallida* in Camp Helen, Florida; two had intermingled specimens reared from stem galls of *C. quercusbatatoides* and *D. quercusvirens* in various localities along Florida; one was represented by singleton reared from stem galls of *C. quercusbatatoides* from Suwannee River, Florida; one was associated with leaf galls of *An. quercuslanigera* and *Be. treatae* in Rice University and Devil's Backbone, Texas; and one was found on *Be. treatae* leaf galls from McAllen, Texas.

The phylogram derived from the RAxML analysis using phased UCE data is shown in Supp Fig. S1 (online only). The resultant fully resolved topology (all clades with BTP = 100), recovered the specimen reared from *Ba. pallida* in Florida as sister to the remaining ingroup specimens, followed by two main clades evident geographical structure.

The species tree obtained from the MSC model analysis performed with the program STACEY using the phased UCE data from nine representative specimens of *Allorhogas* is shown in Fig. 3 along with a comparison with the results obtained from the morphospecies assignment, the 2% genetic divergence criterion and the GMYC model. Congruent with the two species delimitation approaches performed with COI, six species clusters were also delineated with

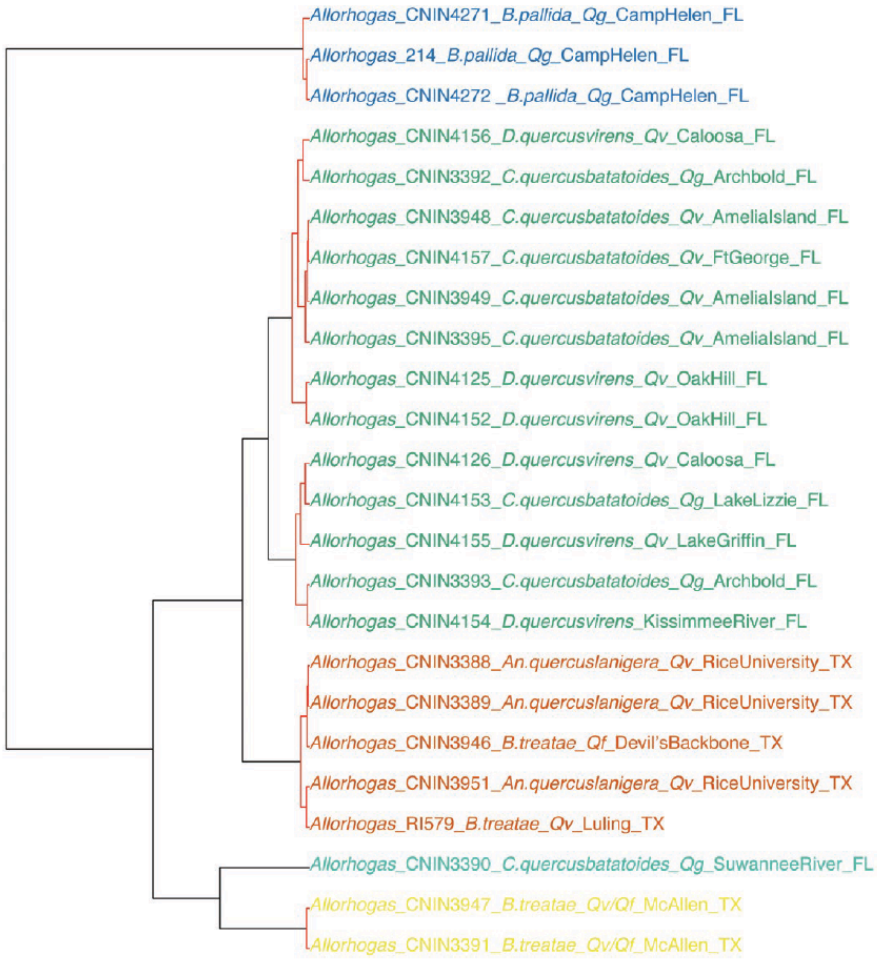

Fig. 2. Species delimited by the GMYC model for 24 specimens of *Allorhogas* using the ultrametric tree reconstructed with the COI gene of the mtDNA. Species containing various specimens are represented in red branches. Singletons are represented by black terminal branches.

highest similarity values in the STACEY analysis based on the 164 SNPs obtained from the UCE data set (Supp Table S3 [online only]). However, the relationships recovered in the latter analysis were more consistent with the phylogenetic analysis and geographic proximity of the three groupings containing the specimens reared from *C. quercusbatatoides* and *D. quercusvirens*, since the species reared from a gall of the former cynipid species in Suwannee River, Florida, was recovered at the base of the remaining two species from various localities of the same state. Similarly, the species reared from galls of *Be. treatae* in McAllen, Texas, closely paired with the two specimens associated with galls of *An. quercuslanigera* from Rice University, in Texas.

On the other hand, the results from the MSC model species delimitation analysis using SNAPP supported the five species model obtained by morphospecies assignment (MLE = −924) as the most likely over the model recovered by the two COI approaches and by the UCE data using STACEY (MLE = −1,170 and −998, respectively) according to the BFD* calculations (Table 1). The five species delimitation hypothesis also was preferred over the additional species model based on geographical distribution (MLE = −1,211).

Morphological Examination

Five morphospecies were discriminated from our morphological examination. Similar to the molecular species delimitation approaches, we identified a well-differentiated morphospecies reared from galls of *Ba. pallida* in Florida, two morphospecies reared from galls from *An. quercuslanigera* and *Be. treatae* in different localities in Texas and one morphospecies associated with *C. quercusbatatoides*

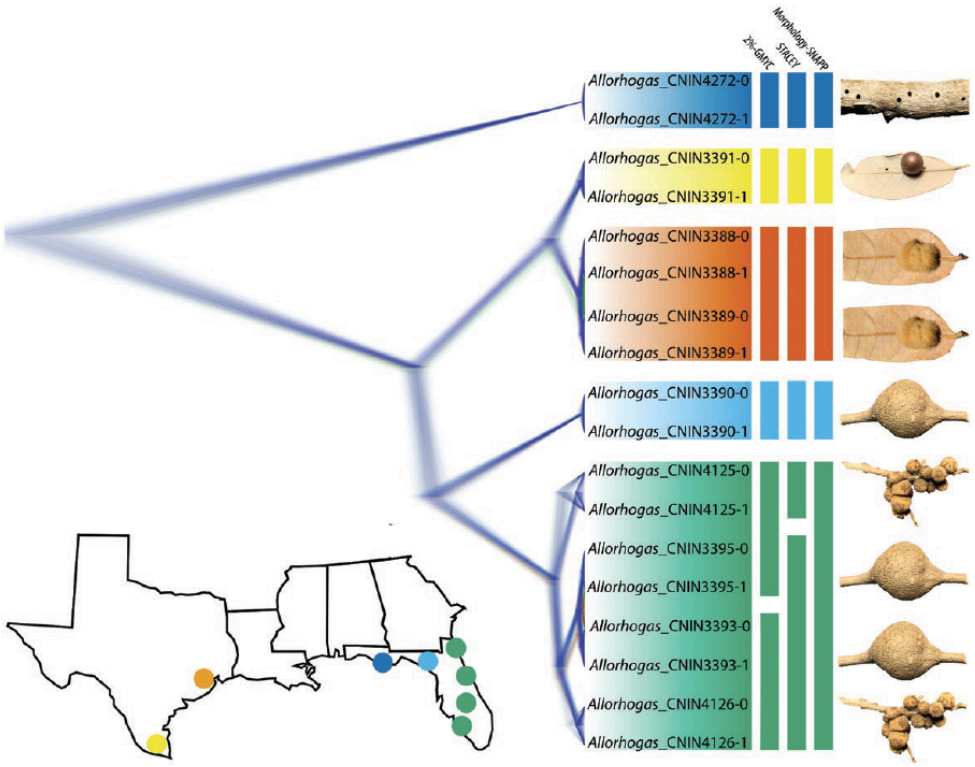

Fig. 3. Cloudogram of species trees derived from the MSC model analysis using STACEY for the phased UCE allele sequences of nine *Allorhogas* specimens. Trees with the highest clade credibility are shown in blue. Bars next to the tip labels summarize the results obtained from species delimitation approaches implemented in this study. A map of collection localities and pictures of the type of gall from which the specimens were reared are also shown.

Table 1. SNAPP results for different species delimitation models for the examined specimens of *Allorhogas*, including the two approaches performed for the COI marker (COI), the UCE phased data (STACEY), the morphospecies assignment (Morphology) and additional hypotheses that consist in the assignment of two species according to the geographical distribution of the examined specimens (Geography)

Model	No. species	MLE	BF	Rank
Morphology	5	−924	-	1
STACEY	6	−998	−148	2
COI	6	−1,170	−492	3
Geography	2	−1,211	−574	4

from Suwannee River, Florida. We did not find morphological features that helped us to differentiate the specimens reared from galls of *C. quercusbatatoides* and *D. quercusvirens* in Florida that were divided into two species by the species delineation approaches carried out with COI and the UCE data with the STACEY analysis.

We followed an integrative taxonomy criterion by congruence (Sensu Padial et al. 2010) and followed the species model that considers the five species identified by the morphospecies assignment, and supported by the MSC model analysis performed with the program SNAPP over the remaining species models. Based on the latter results and on the availability of samples, below, we describe four of these species. We also redescribe the type species of the genus, *A. gallicola*, which was also reared from cynipid galls on a *Quercus* species in the United States and is morphologically similar to the new described species.

Nomenclature

This article and the nomenclatural act(s) it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:116A6713-9B25-467B-8581-B6D1AE33EDAF.

Taxonomy and Species Accounts

Allorhogas caulinarius Samacá-Sáenz, Zaldívar-Riverón et Egan **sp. nov.**

(Fig. 4A–F).

(urn:lsid:zoobank.org:act:28422F42-EB75-4F1F-B66A-353AB16A0A85).

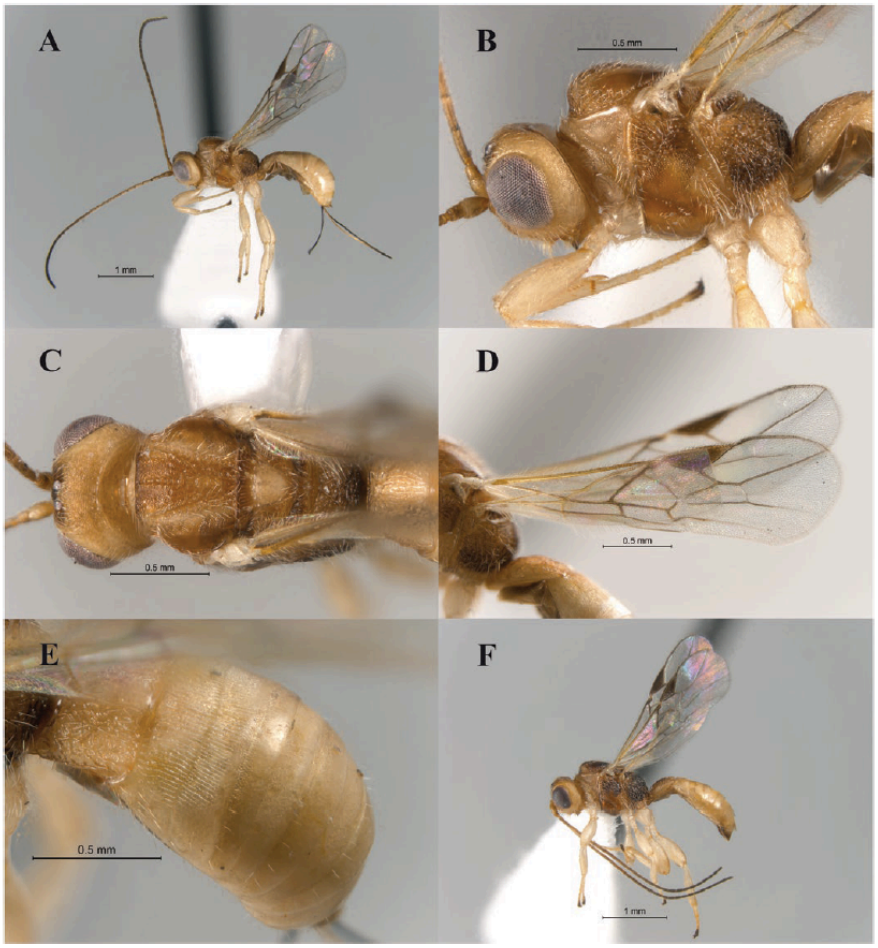

Fig. 4. *Allorhogas caulinarius* **sp.nov.** Female, Holotype: (A) habitus, lateral, view; (B) head and mesosoma, dorsal view; (C) head and mesosoma, lateral view; (D) forewing; (E) metasoma, dorsolateral view. Male, Paratype: (F) habitus, lateral view.

Diagnosis. Al. caulinarius can be distinguished from the remaining described species of *Alllorhogas* that have been reared from cynipid galls on *Quercus* subsection *Virentes* in southeastern United States by having: 1) head, metasoma, and legs whitish-yellow (honey yellow to dark brown in the remaining species), 2) prescutellar furrow with 7–8 transverse carinae (4–6 in the remaining species) and 3) a smooth and polished sculpture on the fifth and subsequent tergites (slightly punctate, slightly costate or slightly coriaceous in the remaining species). Additionally, *Al. caulinarius* differ from the other species by its association with stem galls of *D. quercusvirens* and *C. quercusbatatoides* on *Q. geminata* and *Q. virginiana.*

Female. Body size 3.0 mm (Fig. 4A), forewing 2.6 mm. *Color*: head whitish-yellow to light brown; mesosoma brown to light brown; metasoma and legs whitish-yellow, first metasomal terguite whitish-yellow to light brown; tarsal claws dark brown; wings hyaline, stigma and veins brown; ovipositor sheath dark brown to black, ovipositor honey yellow.

Head: transverse in dorsal view, 1.9 times wider than its median length (dorsal view) (Fig. 4B), and 1.1 times wider than high (lateral view); occipital carina complete and reaching hypostomal carina; post ocellar line (POL) as long than ocellar diameter (OD), 0.5 times ocular ocellar line (OOL); frons, vertex and gena pilose-coriaceous; frons excavation distinct but not defined by sharp lateral margins; eye 1.3 times longer than wide; eye width 2.1 times longer than the temple in dorsal view; malar space 0.2 times eye height and as long as width of hypoclypeal depression; mandibles bidentate; antenna with 31 flagellomeres, first flagellomere about 3.3 times longer than wide, as long as second flagellomere.

Mesosoma: 1.4 times longer than high (Fig. 4C) and 1.3 times longer than wide; pronotal collar short, visible in dorsal view, pronotal furrow scrobiculate; mesoscutum not transverse in dorsal view, its median length 0.8 times its width; median mesoscutal lobe rugose along edges, with a deep median, scrobiculated longitudinal furrow; mesoscutal lobes coriaceous, notauli present, complete and scrobiculate, running along the end of mesoscutum on a strongly rugose area; scutellar disc coriaceous, prescutellar furrow with eight transverse carinae; propodeum strongly rugose-areolate, with two distinct diverging carinae; mesopleuron coriaceous-transversely costated anteriorly, remaining area entirely coriaceous; subalar sulcus scrobiculate; precoxal sulcus wide deep and scrobiculate, running along two-thirds of mesopleuron.

Wings: forewing 3.0 times longer than wide (Fig. 4D). Pterostigma 3.0 times as long as wide and 0.8 times as long as R. Vein r 0.4 times as long as 3RSa, 0.4 times as long as 3RSb, and 0.8 times as long as r-m. Vein 2RS interstitial with m-cu, vein RS+Mb absent. Hind wing vein M + CU 0.9 times as long as 1 M, m-cu slightly curved towards wing apex.

Legs: fore tibia with a row of spines along anterior margin. Hind coxa with distinct basoventral tooth. Hind femur 3.7 times longer than wide.

Metasoma: first tergite 1.6 times wider than long, longitudinally costate-rugose; anteriorly delimited by a transverse carina (Fig. 4E). Second and third tergite entirely costate-slightly coriaceous. Fourth tergite costate-slightly coriaceous. Remaining tergites smooth and polished. Ovipositor about as long as metasoma.

Variation. Body color whitish-yellow to light brown. Body size 2.1–3.0 mm. Antenna with 28–31 flagellomeres. Prescutellar furrow with 7–8 transverse carinae.

Male. Similar to female. Body size 2.1–2.3 mm (Fig. 4F). Antenna with 24–26 flagellomeres. Hind femur swollen, about 3.1 times longer than wide.

Biology. Reared from stem galls of asexual generations of *C. quercusbatatoides* and *D. quercusvirens* on *Q. geminata* and *Q. virginiana* in various localities along Florida. Gall harvest was made between 12-X-2015 to 16-XII-2017; emergence occurred 10 d to approximately 6 mo after the galls were collected, from 21-X-2015 to 22-VII-2018.

Etymology. The name of this species refers to the stem, the plant organ from which it was reared.

Material Examined. HOLOTYPE (IB UNAM): one female, Oak Hill, FL, ex. galls of *Disholcaspis quercusvirens* on *Quercus virgininana,* The Egan Lab at Rice University. PARATYPES (IB UNAM): one female, Archbold, FL, 21-X-2015, ex. galls of *Callirhytis quercusbatatoides* on *Quercus geminata,* The Egan Lab at Rice University, DNA voucher number CNIN3392. Two females, Caloosa Hato, FL, 20-IV-2018 and 18-VII-2018, ex. galls of *Disholcaspis quercusvirens* on *Quercus virgininana,* The Egan Lab at Rice University, DNA voucher number CNIN4156. One female, Amelia Island, FL, 18-X1-2016, ex. galls of *Callirhytis quercusbatatoides* on *Quercus virginiana,* The Egan Lab at Rice University, DNA voucher number CNIN3395. One female, Lake Lizzie, FL 20-IV-2018, ex. galls of *Callirhytis quercusbatatoides* on *Quercus geminata,* The Egan Lab at Rice University, DNA voucher number CNIN4153. One female, Lake Griffin, FL, 18-VII-2018, ex. galls of *Disholcaspis quercusvirens* on *Quercus virgininana,* The Egan Lab at Rice University, DNA voucher number CNIN4155. One female, Ft George, FL, 22-VII-2018, ex. galls of *Callirhytis quercusbatatoides* on *Quercus virginiana,* The Egan Lab at Rice University, DNA voucher number CNIN4157. One male, Amelia Island, FL, 1-XI-2016, ex. galls of *Callirhytis quercusbatatoides* on *Quercus virginiana,* The Egan Lab at Rice University, DNA voucher number CNIN3948. 1 male, Archbold, FL, 22-X-2015, ex. galls of *Callirhytis quercusbatatoides* on *Quercus geminata*, The Egan Lab at Rice University, DNA voucher number CNIN3393.

Allorhogas gallifolia Samacá-Sáenz, Zaldívar-Riverón et Egan **sp. nov.**
(Fig. 5A–F).
(urn:lsid:zoobank.org:act:0E96FE78-47BD-47D5-ADF9-8B5AFCE2E771).

Diagnosis. This species is morphologically similar to *Al. belonocnema* **sp. nov.**, which was also obtained from *Be. treatae* galls, but from a different locality in south Texas over 560km away (McAllen, Texas, United States). Interestingly, this distance crosses a clear break in the genetic structure observed between lineages of the live oak host plants (Cavender-Bares et al. 2015), but not the gall wasp *Belonocnema* (Driscoe et al. 2019). However, it can be distinguished from the latter and the remaining described species associated with cynipid galls in southeastern United States by 1) its smaller body size (1.6–1.9 mm as compared to 2.1–4.1 mm in the other species) and 2) the acinose sculpture of the mesoscutal lobes, which is coriaceus in the remaining species. In addition to its association to the leaf galls of *Be. treatae* on *Q. fusiformis* in central Texas, *Al. gallifolia* was also reared from leaf galls of *An. quercuslanigera* on *Q. virginiana* in southeast Texas.

Female. Body size 1.8 mm (Fig. 5A), forewing 1.5 mm. *Color*: body color honey yellow; legs whitish-yellow; wings hyaline, stigma and veins light brown; ovipositor sheaths dark brown to black, ovipositor honey yellow.

Head: distinctly transverse in dorsal view, 2.0 times wider than its median length (Fig. 5B), and 0.8 times wider than high; occipital carina complete and reaching hypostomal carina; POL as longer than OD, 0.3 times OOL; frons, vertex and gena pilose-coriaceous, frons excavation distinct, without sharp lateral margins; eye 1.2 times longer than wide; eye width 2.0 times longer than temple in dorsal view; malar space 0.5 times eye height and as long as width of hypoclypeal depression; mandibles bidentate; broken antenna with nine flagellomeres, first flagellomere about 5.0 times longer than wide, 1.1 times longer than second one.

Mesosoma: 1.6 times longer than high (Fig. 5C) and 1.7 times longer than wide; pronotal collar very short, visible in dorsal view, pronotal lobe scrobiculate; mesoscutum transverse in dorsal view, its median length 0.6 times its width; median lobe of mesoscutum with a median, rugose furrow; mesoscutal lobes acinose with rugose-areas surrounding notauli; notauli present, complete and rugose-scrobiculate, running along the end of mesoscutum in a posterior rugose median area; scutellum coriaceous; prescutellar furrow with four transverse carinae; propodeum entirely rugose-areolate, including basal areas delimited by distinct carinae; mesopleuron mainly coriaceous; subalar sulcus scrobiculate; precoxal sulcus wide deep and scrobiculate, running along the end of mesopleuron.

Wings: forewing 3.1 times longer than wide (Fig. 5D). Pterostigma 2.6 times as long as wide and 0.8 times as long as R. Vein r 0.8 as

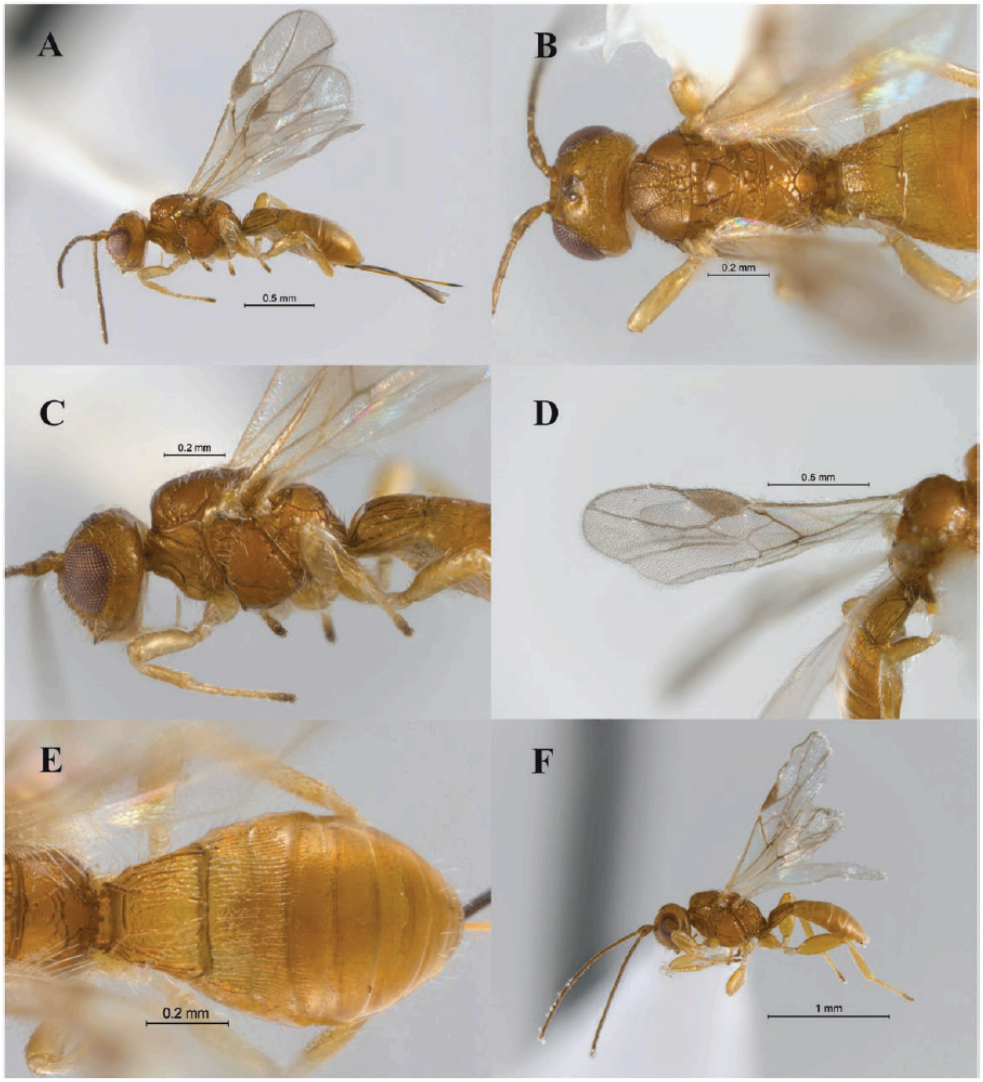

Fig. 5. *Allorhogas gallifolia* **sp.nov.** Female, Holotype: (A) habitus, lateral, view; (B) head and mesosoma, dorsal view; (C) head and mesosoma, lateral view; (D) forewing; (E) metasoma, dorsal view. Male, Paratype: (F) habitus, lateral view.

long as 3RSa, 0.2 times as long as 3RSb, as long as r-m. Vein 2RS interstitial with m-cu, vein RS+Mb not distinguishable. Hind wing vein M + CU 0.7 times as long as 1 M, m-cu slightly curved towards wing apex.

Legs: fore tibia with a row of spines along anterior margin. Hind coxa with a small basoventral tooth. Hind femur 3.2 times longer than wide.

Metasoma: first tergite wider than long, 0.6 times as long as its apical width, longitudinally costate-coriaceous, anterior delimited by a transverse carina (Fig. 5E). Second and third tergite longitudinally costate-coriaceous; suture between second and third tergites distinct and slightly sinuate. Fourth tergite slightly costate-coriaceous basally, remaining area smooth slightly punctate. Remaining tergites slightly punctate. Ovipositor about 0.9 times as long as metasoma.

Variation. Body color honey yellow. Body size 1.6–1.9 mm. Antennae broken. Prescutellar furrow with four transverse carinae.

Male. Similar to female. Body size 1.4–1.8 mm (Fig. 5F). Antennae broken. Hind femur swollen, about 2.4 times longer than wide.

Biology. Reared from leaf galls of the asexual generation of *Be. treatae* and *An. quercuslanigera* on *Q. fusiformis* and *Q. virginiana* in Rice University in southeast Texas and Devil's Backbone in central Texas. Gall harvest was made on 31-X-2014; emergence occurred approximately 10 d after being collected, on 10-XI-2014.

Etymology. The name of this species refers to its association to leaf galls.

Material Examined. HOLOTYPE (IB UNAM): one female, Rice University, TX, 10-XI-2014, ex. galls of *An. quercuslanigera* on *Quercus virginiana*, The Egan Lab at Rice University, DNA voucher number CNIN3389. PARATYPES (IB UNAM): one female, two males, same data, DNA voucher number CNIN3388 and CNIN3950-51. One male, Devil's Backbone, TX, ex. galls of *Belonocnema treatae* on *Quercus fusiformis*, J.R. Ott, DNA voucher number CNIN3946.

Allorhogas belonocnema Samacá-Sáenz, Zaldívar-Riverón et Egan **sp.nov.**
(Fig. 6A–F).
(urn:lsid:zoobank.org:act:6B56FE1E-9A73-4C2F-9424-B38D74963092).

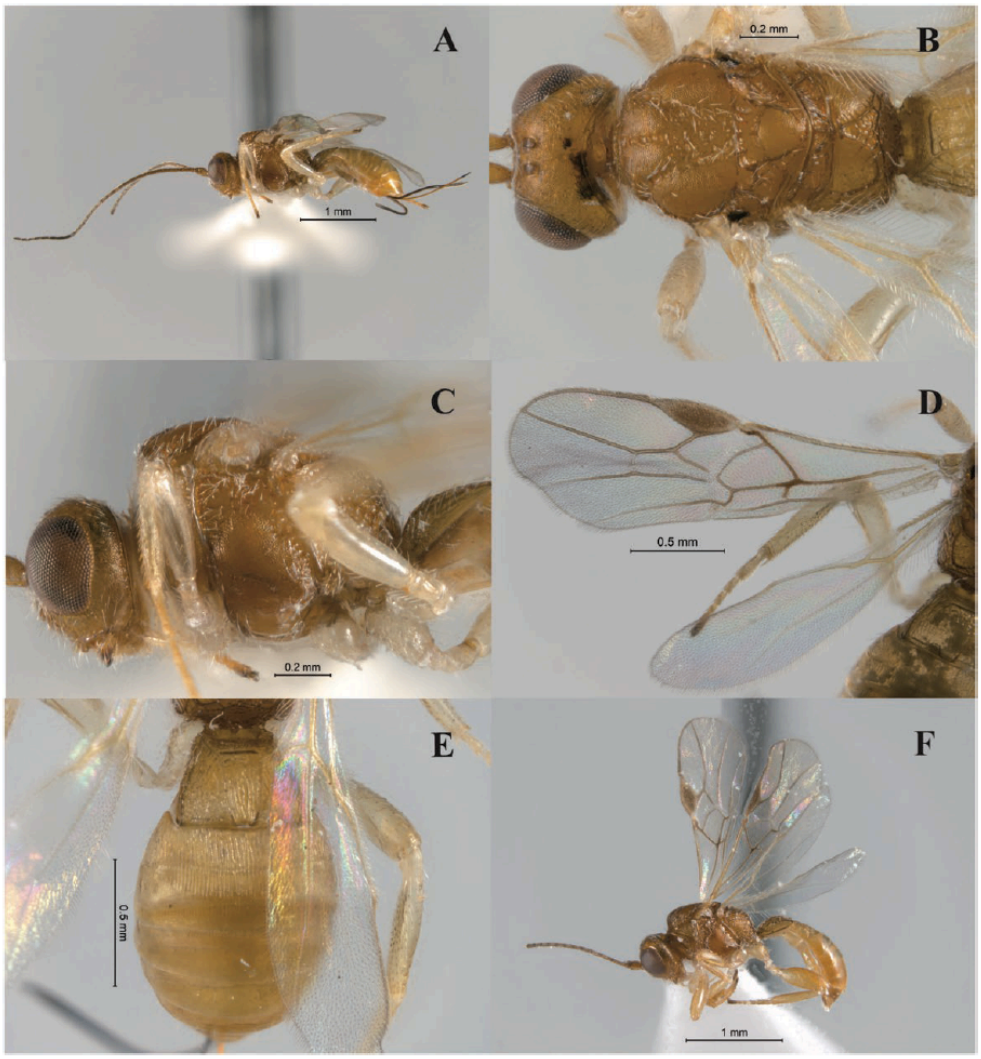

Fig. 6. *Allorhogas belonocnema* **sp.nov.** Female, Holotype: (A) habitus, lateral, view; (B) head and mesosoma, dorsal view; (C) head and mesosoma, lateral view; (D) forewing; (E) metasoma, dorsal view. Male, Paratype: (F) habitus, lateral view.

Diagnosis. This species can be distinguished from the remaining described species of the genus that are associated with cynipid galls in southeastern United States by having the fifth metasomal tergite slightly longitudinally costate (smooth and polished slightly coriaceous or smooth slightly punctate in the remaining species). *Allorhogas belonocnema* is similar to *Al. gallifolia*, but can be distinguished from the latter species by 1) its larger body size (2.2–2.5 mm; 1.6–1.9 mm in *Al. gallifolia*), 2) coriaceous sculpture of the mesoscutal lobes (acinose in *Al. gallifolia*), 3) smooth to coriaceous-rugulose propodeum (entirely rugose-areolate in *Al. gallifolia*), and 4) legs with whitish-yellow paler color. Similar to *Al. gallifolia*, this species was reared from leaf galls of *Be. treatae* on *Q. virginiana* and *Q. fusiformis*, although from a different geographical location (McAllen, Texas, United States), which is 560 km away and spans an important break in host oak population genetic structure (Cavender-Bares et al. 2015).

Female. Body size 2.5 mm (Fig. 6A), forewing 2.4 mm. *Color*: head and mesosoma honey yellow; metasoma and legs yellow to whitish-yellow; wings hyaline, stigma and veins brown to honey yellow; ovipositor sheaths brown turning black to apex, ovipositor honey yellow strongly sclerotized.

Head: transverse in dorsal view, 2.5 times wider than its median length (Fig. 6B), and 1.2 times wider than high; occipital carina complete and reaching hypostomal carina before the mandible; POL 0.7 times OD, 0.6 times OOL; face pilose, slightly coriaceous-transversally rugose, frons excavation distinctively coriaceous, without sharp lateral margins; vertex, temple and gena coriaceous; eye 1.4 times longer than wide; eye width 3.6 times longer than temple in dorsal view; malar space 0.2 times eye height and as long as width of hypoclypeal depression; mandibles bidentate; antenna with 24 flagellomeres, first flagellomere about 3.5 times longer than wide, 1.2 times longer than second one.

Mesosoma: 1.3 times longer than high (Fig. 6C) and 1.6 times longer than wide; pronotal collar very short, slightly visible in dorsal view, pronotal lobe scrobiculate-slightly coriaceous, mesoscutum slightly transverse in dorsal view, its median length 1.5 times its width; medial mesoscutal lobe with a wide, deep, scrobiculate longitudinal furrow, mesoscutal lobes coriaceus; notauli distinct deep, and scrobiculate, running along the end of mesoscutum in a posterior longitudinal rugose median area; scutellum coriaceous; prescutellar furrow with four transverse carinae; propodeum smooth to slightly coriaceous-rugulose, basal areas delimited by distinct carinae; mesopleuron coriaceous; subalar sulcus scrobiculate-slightly coriaceus; precoxal sulcus wide deep, slightly scrobiculate running along two-thirds mesopleuron.

Wings: forewing 3.0 times longer than wide (Fig. 6D). Pterostigma 2.0 times as long as wide and 0.5 times as long as R. Vein r 0.8 as long as 3RSa, 0.6 times as long as 3RSb, 1.7 as long as r-m. Vein 2RS interstitial with m-cu, vein RS+Mb absent. Hind wing vein M + CU 0.7 times as long as 1 M, m-cu slightly curved towards wing apex.
Legs: fore tibia with a row of spines along anterior margin. Hind coxa with a small basoventral tooth. Hind femur 3.1 times longer than wide.

Metasoma: first tergite wider than long, 0.6 times as long as its apical width, longitudinally costate coriaceous with two longitudinal carinae, anterior delimited by a transverse carina (Fig. 6E). Second and third tergite longitudinally costate-coriaceous; suture between second and third tergites slightly sinuate. Fourth and fifth tergite mostly longitudinally slightly costate, remaining area smooth and polished. Ovipositor as long as metasoma.

Male. Similar to female. Body size 2.2 mm (Fig. 6F). Antenna with 24 flagellomeres. Hind femur swollen, about 2.8 times longer than wide.

Biology. Reared from leaf galls of asexual generations of *Be. treatae* on *Q. fusiformis*/*Q. virginiana* in McAllen, Texas.

Etymology. The name of this species refers to the cynipid host genus that forms the gall from which it was reared.

Material Examined. HOLOTYPE (IB UNAM): one female, McAllen, TX, ex. galls of *Belonocnema treatae* on *Quercus virginiana* - *Quercus fusiformis*, J.R. Ott, DNA voucher number CNIN3391. PARATYPE (IB UNAM): one male, same data, DNA voucher number CNIN3947.

Allorhogas bassettia Samacá-Sáenz, Zaldívar-Riverón et Egan **sp. nov.**
(Fig. 7A–D).
(urn:lsid:zoobank.org:act:FBA497BD-9654-4581-A8ED-DEE5093ED0CE).

Diagnosis. This species can be distinguished from the remaining described species of the genus associated with cynipid galls in southeastern United States by having: 1) darker background color, mainly in the metanotum and in the area surrounding the scutellar disk (whitish-yellow to honey yellow in the remaining species), 2) absence of a medial longitudinal furrow in the medial mesoscutal lobe (wide, deep and scrobiculate in the remaining species), and 3) propodeum area enclosed by carinae distinctive coriaceous-transversally rugulose (rugose-areolate, slightly coriaceous-rugulose or smooth and polished in the remaining species). *Al. bassettia* can also be distinguished by its association to stem galls made by *Ba. pallida* on *Q. geminata*.

Female. Body size 2.4 mm (Fig. 7A), forewing 1.9 mm. *Color*: body color honey yellow; metanotum and area surrounding scutellar disk dark brown to black; wings hyaline, stigma, and veins brown.; ovipositor sheaths brown turning black to apex, ovipositor brown.

Head: slightly transverse in dorsal view, 2.1 times wider than its median length (Fig. 7B), and 1.3 times wider than high; occipital carina complete and reaching hypostomal carina before the mandible; POL as longer than OD, 0.2 times OOL; face coriaceous-transversally rugose, frons excavation distinct coriaceous-transversally rugose, without sharp lateral margins; vertex, temple and gena coriaceous; eye 1.3 times longer than wide; eye width 2.3 times longer than the temple in dorsal view; malar space 0.3 times eye height and as long as the width of hypoclypeal depression; mandibles bidentate; antenna with 22 flagellomeres, first flagellomere about 3.5 times longer than wide, 1.2 times longer than the second one.

Mesosoma: 1.5 times longer than high and 1.9 times longer than wide; pronotal collar very short, visible in dorsal view, pronotal furrow scrobiculate; mesoscutum slightly transverse in dorsal view, its median length 1.3 times its width; mesoscutal lobes coriaceus; notauli distinct deep, and scrobiculate, running along the end of mesoscutum in a posterior longitudinal scrobicutalete median area; scutellum coriaceous; prescutellar furrow with five transverse carinae; propodeum coriaceous-slightly rugulose basally, remaining area slightly coriaceus-transversally rugulose, basal areas delimited

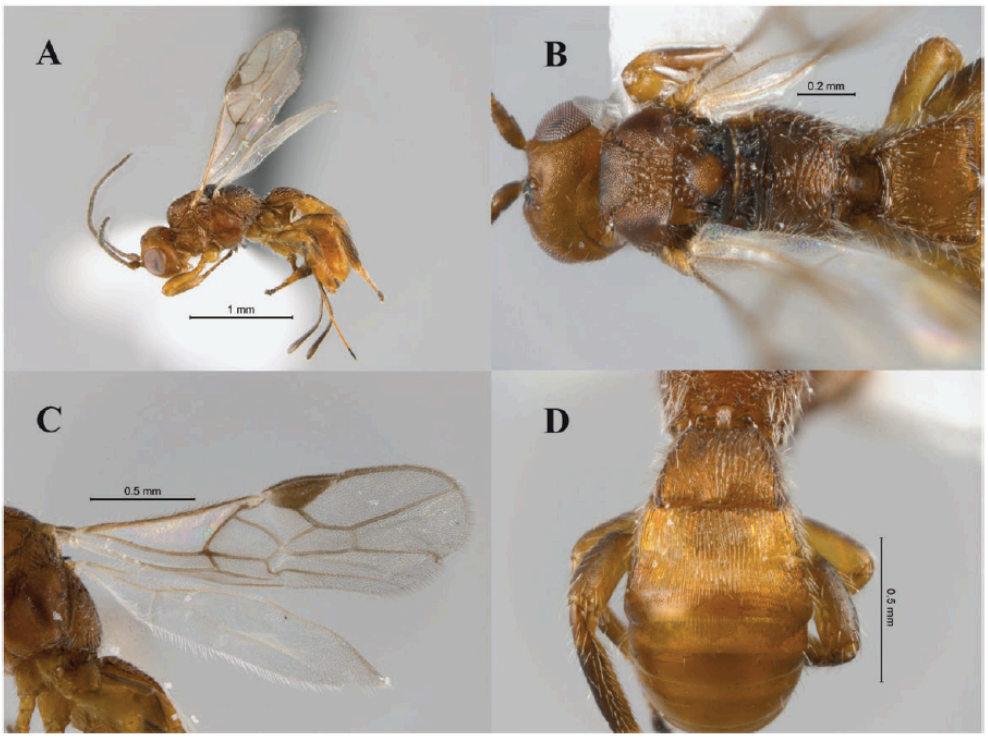

Fig. 7. *Allorhogas bassettia* **sp.nov.** Female, Holotype: (A) habitus, lateral, view; (B) head and mesosoma, dorsal view; (C) forewing; (D) metasoma, dorsal view.

by distinct carinae; mesopleuron mainly coriaceous; subalar sulcus scrobiculate-slightly coriaceus; precoxal sulcus wide deep, wide, slightly scrobiculate running along most part mesopleuron.

Wings: forewing 3.4 times longer than wide (Fig. 7C). Pterostigma 2.9 times as long as wide and 0.7 times as long as R. Vein r 0.8 as long as 3RSa, 0.2 times as long as 3RSb, 1.5 as long as r-m. Vein 2RS interstitial with m-cu, vein RS+Mb absent. Hind wing vein M + CU 0.6 times as long as 1 M, m-cu slightly curved towards wing apex.

Legs: fore tibia with a row of spines along anterior margin. Hind coxa with a small basoventral tooth. Hind femur 3.4 times longer than wide.

Metasoma: first tergite wider than long, 0.7 times as long as its apical width, longitudinally costate-slightly coriaceous, anterior delimited by a transverse carina (Fig. 7D). Second and third tergite longitudinally costate-coriaceous; suture between second and third tergites distinct and slightly sinuate; fourth tergite mostly costate-coriaceous, slightly punctate apically. Remaining tergites slightly punctate. Ovipositor about 0.8 times as long as metasoma.

Variation. Body color whitish-yellow to light brown. Body size 2.2–2.8 mm. Antenna with 19–23 flagellomeres. Prescutellar furrow with four transverse carinae.

Male. Unknown.

Biology. Reared from stem galls of asexual generations of *Ba. pallida* on *Q. geminata* in Camp Helen, Florida. Gall harvest was made on 18-III-2019; emergence occurred approximately 1 mo after the galls were collected, on 24-IV-2019.

Etymology. The name of this species refers to the cynipid host genus that induces the gall from which it was reared.

Material Examined. HOLOTYPE (IB UNAM): one female, Camp Helen, FL, 24-IV-2019, ex. galls of *Bassettia pallida* on *Quercus geminata*, K. Weinersmith, DNA voucher number CNIN4272. PARATYPES (IB UNAM): two females, same data, DNA voucher number CNIN4271.

Allorhogas gallicola Gahan, 1912
(Fig. 8A–F).

Diagnosis. Allorhogas gallicola can be distinguished from the described species of *Allorhogas* associated with cynipid galls on live oaks mainly by 1) its larger size (3.0–4.2 mm; 1.4–3.0 mm in the remaining species), 2) propodeum area enclosed by carinae smooth and polished (rugose-areolate, slightly coriaceous-rugulose or coriaceous-transversally rugulose in the remaining species) and 3) its distinctive longer ovipositor (2.2 times as long as metasoma; approximately as long as metasoma in the remaining species). *Allorhogas gallicola* can be also differentiated because it was reared from a cynipid twig galls in '*Quercus pinifolia*' (Gahan 1912) near College Park, Maryland, United States.

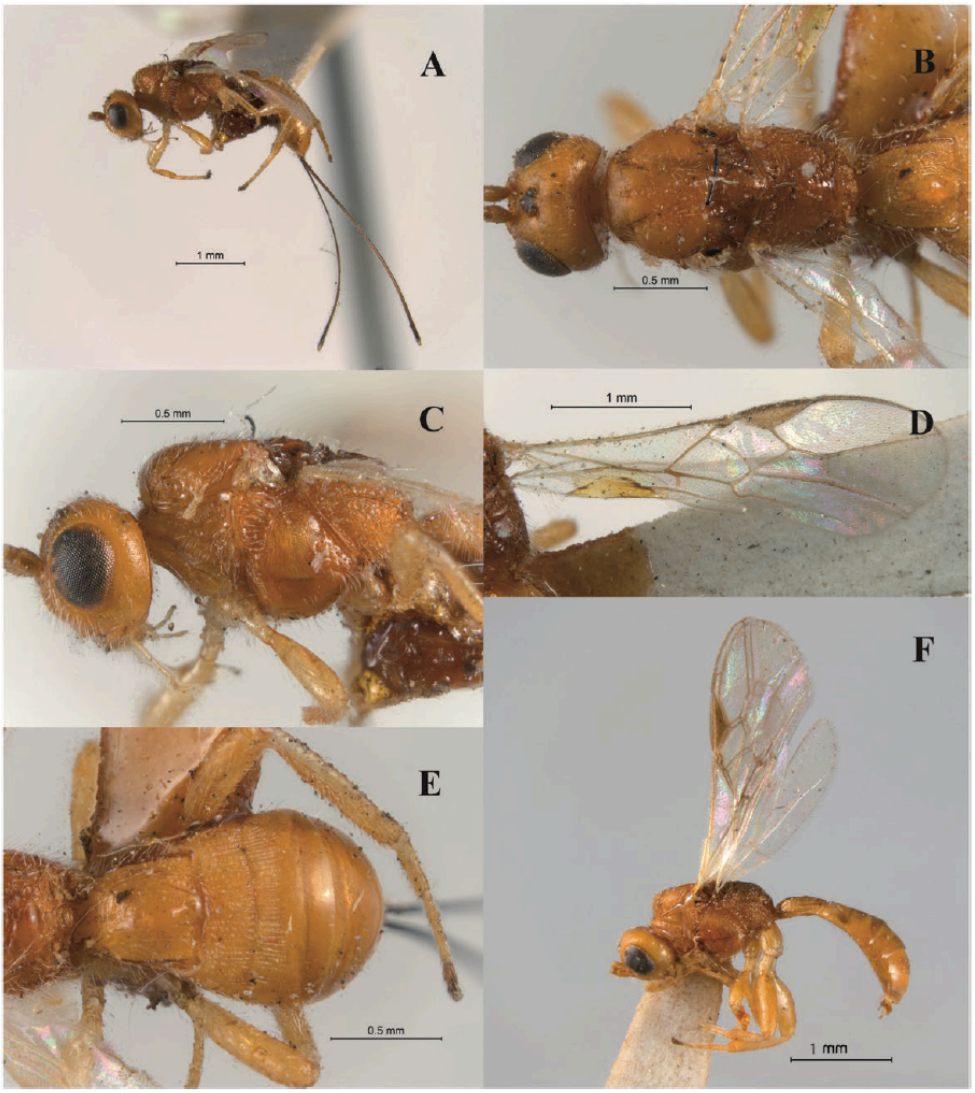

Fig. 8. *Allorhogas gallicola.* Female, Holotype: (A) habitus, lateral, view; (B) head and mesosoma, dorsal view; (C) head and mesosoma, lateral view; (D) forewing; (E) metasoma, dorsal view. Male, Paratype: (F) habitus, lateral view.

Female. Body size 4.2 mm (Fig. 8A), forewing 4.0 mm. *Color*: metasoma and head honey yellow; mesosoma and legs honey yellow to yellow; scape honey yellow to light brown at the base, antennae broken; wings hyaline, stigma brown, veins brown to honey yellow; ovipositor sheaths dark brown.

Head: transverse in dorsal view, 1.9 times wider than its median length (Fig. 8B), and 1.5 times wider than high; occipital carina complete, reaching hypostomal carina before base of mandible; POL 1.2 times OD, 0.5 times OOL; frons coriaceous-slightly rugose, vertex, temple and gena coriaceous, face coriaceous-transversally rugose; frons excavated, not delimited by sharp lateral margins; eye 1.6 times longer than wide; eye width 1.75 times longer than the temple in dorsal view; malar space 0.4 times eye height slightly shorter than the width of hypoclypeal depression; mandibles apparently bidentate; antennae broken.

Mesosoma: 1.4 times longer than high (Fig. 8C), and 1.5 times longer than wide; pronotal collar short but visible in dorsal view,

pronotal lobe scrobiculate; mesoscutum slightly transverse in dorsal view, its median length 0.8 times its width; median mesoscutal lobe coriaceous with a median, scrobiculate longitudinal furrow; lateral mesoscutal lobes coriaceous; notauli wide deep and scrobiculate, not joining, finishing at the base of mesoscutom in longitudinal rugose area, prescutellar furrow with six transverse carinae; propodeum rugose-areolate, with two distinct diverging carinae, area between carinae smooth and polished; mesopleuron ventrally coriaceous-rugose remaining area rugose; subalar sulcus scrobiculate; precoxal sulcus wide, deep, smooth, slightly scrobiculate running along two thirds of mesopleuron.

Wings: forewing 3.1 times longer than wide (Fig. 8D). Pterostigma 3.4 as long as wide and 0.7 times as long as R. Vein r as long as 3RSa, 0.3 times as long as 3RSb, and 1.3 times as long as r-m. Vein 2RS interstitial with m-cu, vein RS+Mb absent. Hind wing vein M + CU 0.6 times as long as 1 M, m-cu slightly curved towards wing apex.
Legs: fore tibia with a row of spines along anterior margin. Hind coxa with distinct basoventral tooth. Hind femur 2.5 times longer than wide.

Metasoma: first tergite, slightly longer than its apical width, coriaceous longitudinally rugose, with two longitudinal, subparallel carinae running along the entire length of tergite (Fig. 8E). Second tergite costate-coriaceous; third tergite costate-coriaceous on basal half, coriaceous apical in the half; suture between second and third tergite distinct and almost straight. Remaining tergites smooth and polished slightly coriaceous. Ovipositor about 4.9 mm, 2.2 times as long as metasoma.

Variation. Body color honey yellow to yellow. Body size 3.0–3.7 mm. Antenna with 28 flagellomeres. Prescutellar furrow with six transverse carinae.

Male. Similar to female. Body size 2.7–3.4 mm (Fig. 8F). Antenna with 28 flagellomeres. Hind femur swollen, 2.5 times longer than wide.

Biology. Gahan (1921) mentioned that the type specimens of *A. gallicola* were reared from galls of a cynipid wasp in '*Quercus pinifolia*'; however, this species name is not valid. It is, therefore, possible that the valid name for the aforementioned species is *Q. palustris* Münchh, since this oak species is distributed in the eastern and central United States and its common name is pin oak, which probably led to the confusion in the original description.

Material Examined. HOLOTYPE (NMNH): one female, Prince George, University of Maryland Campus, ML, 20-IV-1911, ex. galls on *Quercus pinifolia*, A.B. Gahan. PARATYPES (NMNH): two males, same data as Holotype except on collection dates, 27-IV-1911 and 19-V-1911. Other examined material (NMNH): two females, Stamford, CT, 27-VI-1930 and summer1931, ex. galls on *Quercus palustris*. One female, one male, Washington Co., AR, 4-VI-1965, ex. galls on *Quercus rubra*. One male, East Fall Church, VA, ex. galls of *Callirhytis seminosa* on *Quercus rubra*.

A list with the remaining examined specimens of *Allorhogas* from United States that are deposited in the NMNH and that do not belong to the species included in this study is shown in Supp Table S4 (online only).

Key to the species of *Allorhogas* associated with cynipid galls on species of *Quercus* in eastern United States.

1. Mesoscutum with a medial scrobiculate longitudinal furrow in its medial lobe, metanotum and the area surrounding scutellar disk honey yellow to brown; propedeum rugose-areolate, slightly coriaceous-rugulose or smooth and polished 2

- Mesoscutum without medial longitudinal furrow in its medial lobe; metanotum and area surrounding scutellar disk dark brown to black; propodeum coriaceous-transversally rugulose .... ***Al. bassettia*** Samacá-Sáenz, Zaldívar-Riverón et Egan sp. nov.

2 (1). Body size 1.6–3.0 mm; propodeum rugose-areolate or slightly coriaceous-rugulose; ovipositor as long, or near as long as metasoma 3

- Body size 3.0–4.2 mm; propodeum smooth and polished; ovipositor considerably longer than metasoma, about twice its size ..... ***A. gallicola*** Gahan

3 (2). Head, legs and metasoma honey yellow; prescutellar furrow with 4–5 transverse carinae; fifth tergite smooth slightly punctate or slightly costate 4

- Head, legs and metasoma whitish-yellow to whitish-brown; prescutellar furrow with 7–8 transverse carinae, fifth tergite smooth and polished ..... ***Al. caulinarius*** Samacá-Sáenz, Zaldívar-Riverón et Egan sp. nov.

4 (3). Body size 2.2–2.5 mm; mesoscutal lobes coriaceus; propodeum smooth to slightly coriaceous-rugulose; fifth tergite slightly longitudinally costate..... ***Al. belonocnema*** Samacá-Sáenz, Zaldívar-Riverón et Egan sp. nov.

- Body size 1.6–1.9 mm; mesoscutal lobes acinose; propodeum entirely rugose-areolate; fifth tergite smooth slightly punctate..... ..... ***Al. gallifolia*** Samacá-Sáenz, Zaldívar-Riverón et Egan sp. nov.

Discussion

The Genus Allorhogas Sensu Stricto

Here we report a previously unknown diversity of species of the braconid genus *Allorhogas* that are part of the insect communities associated with cynipid galls present on species of *Quercus* subsection *Virentes* in the southeastern United States. Our study only included taxa from a restricted geographic region of this country. However, according to our examination of specimens of *Allorhogas* collected several decades ago in a number of localities in the United States (Supp Table S4 [online only]), we found that there are various species that remain to be described, and only a few have host plant and associated cynipid records. The only other species of *Allorhogas* previously described from United States is *Allorhogas arizonensis* (Ashmead). This species was also reared from a cynipid gall on an unknown oak species in Arizona, United States (Ashmead 1889). An undescribed species of *Allorhogas* from Argentina has recently been reared from galls made by a cynipid species on leaf buds of *Prosopis* sp. (Fabaceae) (J. J. Martínez, in preparation), and preliminary phylogenetic analyses based on UCE data have confirmed that is closely related to the species described here (Samacá-Sáenz, unpublished data).

Based on the wide geographic distribution of cynipid species in the Nearctic and Neotropical regions, their host plant associations, and the museum material mentioned above, we believe that the species of *Allorhogas* described here only represent the 'tip of the

iceberg' of a much higher species richness of this genus that is associated with gall wasps. However, discovering this species richness will not be an easy task, since our rearing records and previous collection efforts suggest that the *Allorhogas* species that are associated with cynipid galls on live oaks are rare, unlike most other hymenopteran parasitoid and inquiline taxa that also belong to the same communities of natural enemies (Forbes et al. 2016, Weinersmith et al. 2020, Egan et al. unpublished data). This study thus highlights the importance of conducting more detailed, systematic field work to obtain reliable host associations and natural history information that help to disentangle the systematics and evolutionary history of the insect taxa that are part of gall-former communities.

Allorhogas has been of particular interest in recent phylogenetic studies along with other doryctine genera that apparently are exclusively composed of gall-associated species since they have shown to have various phytophagous strategies and are associated with several plant families. Previous phylogenetic studies based on few molecular markers (Zalívar-Riverón et al. 2014) and mitogenomic and UCE data (Samacá-Sáenz et al. 2019) have confirmed the monophyly of the gall-associated doryctine genera, but have found that *Allorhogas* as a whole is polyphyletic. In particular, Samacá-Sáenz et al. (2019) recovered a main *Allorhogas* clade composed of species that are reported to be phytophagous together with the undescribed species delimited here that was reared from stem galls of *C. quercusbatatoides*. Since the type species of *Allorhogas*, *A. gallicola*, is morphologically similar to all the species described here, it is highly plausible that this generic name should be restricted to the members of the above main *Allorhogas* clade. However, additional taxonomic and phylogenetic studies are needed to clarify the composition and limits of this genus. Among the morphological features that are shared by the examined species of the main *Allorhogas* clade are an excavated frons and a slightly curved vein m-cu in the hind wing.

Species of *Allorhogas* Sensu Zaldívar-Riverón et al. (2014) with reliable biological records have been currently associated with 11 different plant families (Moreira et al. 2017, Zaldívar-Riverón et al. 2018). Most of the described *Allorhogas* species have been confirmed or observed to be phytophagous with different feeding habits, including gall formers on seeds and ovules on floral buds (de Macêdo and Monteiro 1989, Morales-Silva and Modesto-Zampieron 2017, Joele et al. 2019), seed predators (Zaldívar-Riverón et al. 2018) or phytophagous inquilines of other gall-forming insects (Moreira et al. 2017). It has been presumed that some species of *Allorhogas* may be parasitoids of other gall-forming insects (Martínez and Zaldívar-Riverón 2013), though this was not based on direct observations. Regarding the species of *Allorhogas* examined in this study, some of the specimens associated with the gall wasp *C. quercusbatatoides* were reared from stem galls that also had larvae of the lepidopteran genus *Synanthedon* (Sesiidae) (Egan et al., unpublished data). These observations are concordant with Gahan's (1912) records of the type species, *A. gallicola*, associated with *Synanthedon*, and with Forbes and colleagues' (2016) report of moths of the genus *Sinoe* (Gelechiidae) coexisting in the leaf galls induced by *Be. treatae*. Therefore, whether the species of *Allorhogas* reared from cynipid galls are parasitoids of the associated lepidopterans or inquilines of the gall-inducer cynipids still needs to be clarified.

Potential Host-Associated Differentiation in Allorhogas

In phytophagous insects, evolution of reproductive isolation and sympatric speciation is sometimes related to host shifts, and therefore host-associated differentiation (HAD) represents a form of isolation by environment, the latter being represented by the host plant (Matsubayashi et al. 2010, Antwi et al. 2015, Driscoe et al. 2019). This process also contributes to the consequent cascading HAD of the arthropod communities that are associated with the herbivores, where sequential speciation could occur across higher trophic levels (Stireman et al. 2006, Forbes et al. 2009, Feder and Forbes 2010, Hood et al. 2015, Zhang et al. 2019b). At both levels (herbivore and parasitoid), new barriers to gene flow emerge in natural populations as a consequence of ecologically based divergent selection between environments, which is consistent with ecological speciation (Nosil 2012).

Few studies have addressed the existence of HAD in members of Cynipidae, and less have explored this phenomenon within their associated insect communities. These studies, however, have revealed the existence of HAD both for the gall wasps and their parasitoids. For instance, the alternative use of the host oaks *Q. virginiana* and *Q. geminata* by the gall wasp *Be. treatae* has been revealed to act as a source of divergent natural selection and genetic differentiation, promoting speciation among gall wasp populations (Egan et al. 2012a, b; Zhang et al. 2017; Driscoe et al. 2019; Hood et al. 2019). Egan et al. (2013) also showed the existence of parallel patterns of morphological and behavioral variation among host-associated populations of *Be. treatae* and *D. quercusvirens*, whereas Zhang et al. (2019b) reported parallel patterns of temporal differences in five gall wasp species and a gall-forming fly on the same set of host plants. In addition, this same study showed that host plant use was associated with phenological differences in parasitoid species attacking three gall wasp species (Zhang et al. 2019b), whereas significant host-associated genetic structuring was observed in *Megastigmus dorsalis* (Fabricius) (Torymidae), a parasitoid wasp complex that attacks different oak galls (Nicholls et al. 2018).

Our host association records and the analyses performed suggest that diversification in some of the delimited species of *Allorhogas* may have occurred due to HAD, since they were associated with an exclusive type of gall or to galls made by two cynipid species in the same organ. *Al. gallifolia* emerged from leaf galls made by *An. quercuslanigera* and *Be. treatae*. The former cynipid species induces fuzzy single-chambered leaf galls located on the underside of new leaves on the midvein alone or in close proximity to other galls (Hood et al. 2018), whereas *Be. treatae* develops within single-chambered spherical leaf galls on the underside of newly flushed leaves (Egan et al. 2013). On the other hand, *Al. caulinarius* was reared from stem galls of *C. quercusbatatoides* and *D. quercusvirens*, the former producing stem swelling-like galls, and the second developing individually or in clusters of detachable, spherical branch woody galls (Bird et al. 2013). In addition, specimens of *Al. bassettia* were exclusively reared from galls of *Ba. pallida*, which forms concealed galls composed of compartments that run parallel to the bark of twigs (Melika and Abrahamson 2007, Weinersmith et al. 2020). One new species described here, however, could be explained by allopatric speciation. *Al. belonocnema* was associated with leaf galls made by *Be. treatae* in oaks from a locality that is 560km distant from the *Al. gallifolia* populations in Texas, which represents a break in the genetic structure of the host oak populations (Cavender-Bares et al. 2015). Further studies comprising a broad sampling of *Allorhogas* specimens along south and southeast United States is required to further address HAD and allopatric speciation events in this system.

Performance of DNA Sequence-Based Species Delimitation Approaches

Molecular species delimitation methods emerged to deal with some of the problems related with species assignment based on morphological features (Hebert et al. 2004, Bickford et al. 2007). Various

DNA sequence-based approaches using single-locus data, mainly based on sequence divergence and coalescence criteria, have been developed during the last two decades, and some of them have successfully been implemented to delimit species boundaries among a vast number of animal groups (Luo et al. 2018, DeSalle and Goldstein 2019). These methods, however, have been criticized because of their problems to discriminate recently diverged taxa (van Velzen et al. 2012) and their tendency to species overestimation (Lohse 2009, Talavera et al. 2013). The MSC model represents a useful alternative for delimiting species based on multilocus DNA sequence data since it allows gene trees to have different evolutionary histories, thus being more able to deal with long-branch attraction and incomplete lineage sorting effects, and it is also apparently more robust to limited taxon sampling (Liu et al. 2015, Bravo et al. 2019).

In this study, we conducted various DNA sequence-based approaches using a fragment of the mtDNA COI gene and nuclear UCE data to assess the species boundaries among a group of probably recently diverged braconid wasps that are present on galls of five cynipid species in south and southeast United States. We obtained mostly congruent results between the species delineation approaches used for the COI marker and the two MSC model methods employed for the UCE, with six species being discriminated by three of the four species delineation approaches performed. The approach developed by Andermann et al. (2019) to obtain SNPs from UCE allele phased sequences was the only one that recovered five species, fusing two of the species reared from *quercusbatoides* and *D. quercusvirens*. The result obtained by the latter approach, however, was concordant with the morphological evidence. We, therefore, followed a conservative approach and only recognized five species, though further studies based on more samples, additional morphological and genomic information are needed to clarify whether *Al. caulinarius* actually comprises more than evolutionary lineage.

Our study confirms the utility of allele phasing and SNPs obtained from UCE data for sequence-based species delimitation studies, particularly when allele phased sequences and SNPs are used under the MSC model. To date, only few studies that have incorporated allele phasing and SNPs derived from UCEs (Manthey et al. 2016, McCormack et al. 2016, Zarza et al. 2018, Anderman et al. 2019, Derkarabetian et al. 2019, French et al. 2019). Among the reported advantages of this procedure are the improvement of tree inference under the MSC model, a better estimation of divergence times and the additional increase of sample size when using the phased allele sequences (Andermann et al. 2019, Zhang et al. 2019c). On the other hand, the presumably low variation of the UCE loci in species delimitation analyses and possible sequencing errors have been mentioned that could negatively affect phylogenetic inference and consequently cause mistakes in gene tree reconciliation for species tree estimation (Hosner et al. 2016, Manthey et al. 2016).

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

Supp. Table S1. List of *Allorhogas* specimens included in the study, their voucher numbers, cynipid host, host plant, localities, gall harvest, emergence and NCBI GenBank and SRA accession numbers for COI and raw data for the individuals processed for UCEs. *Qv* = *Quercus virginiana*, *Qg* = *Quercus geminata*, *Qf* = *Quercus fusiformis*.

Supp. Table S2. Corrected pairwise genetic distances for COI using the K2P evolutionary model.

Supp. Table S3. Species clusters obtained from the SpeciesDelimitationAnalizer analysis with the UCE phased data for nine representative specimens of *Allorhogas*. A = *Allorhogas*_CNIN3388, B = *Allorhogas*_CNIN3389, C = *Allorhogas*_CNIN3391, D = *Allorhogas*_CNIN3390, E = *Allorhogas*_CNIN4126, F = *Allorhogas*_CNIN3393, G = *Allorhogas*_CNIN3395, H = *Allorhogas*_CNIN4125, I = *Allorhogas*_CNIN4272.

Supp. Table S4. List of the *Allorhogas* specimens from U.S. deposited in the National Museum of Natural History (NMNH).

Supp. Figure S1. Phylogram derived from the RAxML analysis using the phased UCE allele sequences of nine *Allorhogas* specimens and an outgroup. Bootstrap values of clades are shown next to nodes.

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Capítulo III

Evolución de las diferentes estrategias de fitofagia y sistemática de las avispas del género *Allorhogas* Gahan (Braconidae: Doryctinae)

Host-plant family shift evolution drives species diversification within a predominantly parasitoid wasp family

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Keywords: Braconidae, Doryctinae, Hymenoptera, phylogenomics, ultraconserved elements.

ABSTRACT

Phytophagy has promoted species diversification in many insect groups, including Hymenoptera, one of the most diverse animal orders on earth. Although species richness in this order has been mainly attributed to a parasitoid lifestyle, its interactions with plants and herbivorous feeding habits have also contributed to its current known diversity. In the predominant parasitoid family Braconidae, gall-association with angiosperms has been reported in three subfamilies, but in particular in the Doryctinae, where it has been recorded to occur in species of 10 genera. *Allorhogas* Gahan is by far the most speciose of these genera, with its species having three different phytophagous strategies, inquilinism, gall formation and seed predation. Previous studies recovered the monophyly of the gall-associated group and the polyphyly of *Allorghogas*, though various relationships and the tempo and mode of the evolution of herbivorous strategies are still unclear. Here we conducted a comprehensive phylogenomic study for the doryctine gall-associated genera, with an emphasis in *Allorhogas*, using ultraconserved elements (UCE). Based on our results we: 1) updated their taxonomic classification, 2) estimated the timing of origin of the gall-associated clade and divergence of its main subclades, and 3) performed ancestral state reconstruction (ASR) analyses for three life history features: feeding strategy, plant family and plant organ association. Our almost fully resolved estimate of phylogeny confirmed *Allorhogas* as polyphyletic, with most of its members being nested in a main clade composed of species with different herbivorous feeding habits. The origin of gall-association was estimated to have occurred during the late Oligocene to early Miocene, with a subsequent diversification during the middle to late Miocene and Pliocene. Some codiversification processes presumably occurred between some taxa and their host-

associated plant lineages. Evolution of the examined feeding strategies in the group show "inquilinism-feeding" as the ancestral state, with gall-formation and seed predation having independently evolved on multiple occasions.

INTRODUCTION

Insect richness has been explained by the great diversity of phytophagous species that interact with plants, particularly angiosperms (Mayhew 2007, Futuyma and Agrawal 2009). The evolution of phytophagy probably promoted the diversification of organisms with this feeding strategy since herbivorous clades generally are significantly more diverse than non-herbivorous related clades (Mitter et al. 1988; Wiens et al. 2015). The extraordinary richness of Coleoptera, for example, is the result, among other factors, of its codiversification with flowering plants (Hunt et al. 2007) and by the evolution of specialized plant cell wall-degrading enzymes (McKenna et al. 2019).

With more than 153,000 described species, Hymenoptera is one of the most diverse animal orders (Grimaldi and Engel 2005; Aguiar et al. 2013, Peters et al. 2017). Members of this insect order, which is represented by ants, bees, sawflies and wasps, approximately represent 8% of all described species of animals on Earth (Davis et al. 2010), with its current diversity being mainly attributed to a parasitoid lifestyle (Forbes et al. 2018). The Braconidae is the second family in terms of number of described and estimated species within the Hymenoptera, with most of its species mainly being parasitoids of various holometabolous insect orders (Wharton 1997; Yu et al. 2016).

Interaction with plants has also played a fundamental role in the evolutionary history of various hymenopteran families. For instance, species diversification of ants has been attributed to their coevolution with angiosperms (e.g. Moreau et al. 2006; Nelsen et al. 2018), whereas strong cospeciation apparently occurred between fig wasps (Agaonidae) and their host plants (Cruaud et al. 2012; Satler et al. 2019). Despite Braconidae is

predominantly represented by parasitoid species, phytophagy has been reported and confirmed in some species of the subfamilies Braconinae, Doryctinae and Mesostoinae (de Macêdo and Monteiro 1989; Infante et al. 1995; Flores et al. 2005; Wharton and Hanson 2005). However, whether this plant-herbivore interaction has contributed to the diversification of the lineages involved is still unknown.

In the subfamily Doryctinae, gall-association in different plant organs (leaves, stems, fruits, aerial roots) has been reported for species belonging to 10 Neotropical genera (Zaldívar-Riverón et al. 2007, 2008, 2014). Of these genera, *Allorhogas* Gahan is by far the most speciose with 57 currently described species (Yu et al. 2016; Zaldívar-Riverón et al. 2018; Samacá-Sáenz et al. 2020). Various phytophagous strategies have been confirmed or reported within *Allorhogas*, including inquilinism on galls made by other insect species (Moreira et al. 2017), gall formation on leaves, seeds and fruits (de Macêdo and Monteiro 1989, Morales-Silva and Modesto-Zampieron 2017, Joele et al. 2019) and seed predation without gall formation (Zadívar-Riverón et al. 2018). Moreover, members of this genus have been associated with 11 flowering plant families, among which Fabaceae, Fagaceae and Melastomataceae are the ones with more recorded species (Zadívar-Riverón et al. 2018; Joele et al., 2019).

Various recent phylogenetic studies have consistently recovered *Allorhogas* as polyphyletic based on both a few molecular markers (Zaldívar-Riverón et al. 2014; Joele et al., 2019) and ultraconserved elements (UCE; Faircloth et al. 2012) and mitochondrial genome data (Samacá-Sáenz et al. 2019). These studies recovered a main *Allorhogas* clade composed of phytophagous species associated with five different plant families though they had a limited taxon sampling and incomplete biological information. In particular, the study

conducted by Samacá-Sáenz et al. (2020) recovered an almost fully supported estimate of phylogeny, but it only included 13 ingroup taxa, five of which were assigned to *Allorhogas*. That study also failed to confirm the phylogenetic affinity of the genus *Monitoriella* and did not include the enigmatic genus *Donquickeia*, whose species are known to be gall-associated (Penteado-Dias, 2000). It is therefore necessary to investigate the phylogenetic relationships among the plant-associated doryctine genera, with an emphasis in *Allorhogas*, based on a dense taxon sampling. This will also help to assess whether shifts to different plant families, plant organs and feeding strategies (inquilinism, gall-formation, seed predation) have triggered the species diversification in the group.

Here we conducted a phylogenomic analysis of the plant-associated doryctine genera based on UCE data and a vast taxon sampling that focused on members of *Allorhogas*, for which we included more than half of its currently described species and various undescribed species with biological data. We also included representative species of the remaining nine gall-associated genera. Most of our examined species have host-plant records and display different life history strategies, including inquilinism, gall-formation and seed predation on several plant families and plant organs. Our analyses yielded an almost fully resolved topology that helped to confirm unclear relationships. We also generated a time-calibrated phylogenetic tree to investigate the divergence times among the gall-associated genera and within the main *Allorhogas* clade. Moreover, through ancestral state reconstruction (ASR) analyses, we assessed the evolution of different life history strategies of plant association in the group, including feeding strategy (inquilinism, gall-formation, seed predation) and plant family and plant organ association.

MATERIALS AND METHODS

Taxon selection

We sampled a total of 86 taxa, of which 71 belong to the 10 currently known gall-associated doryctine genera, including 19 species that were examined in two previous phylogenomic studies (Samacá-Sáenz et al. 2019, 2020). We included 44 species of *Allorhogas*, 31 of which are described and 13 undescribed. The number of described species that were included in the latter genus represents 54% of its currently known species diversity. For the remaining gall-associated genera we included two species of *Donquickea* Marsh, one of *Ficobolus* Martínez, Belokobylskij & Zaldívar-Riverón, seven of *Labania* Hedqvist, five of *Monitoriella* Hedqvist, one of the monotypic *Monoeuron* Fischer, three of *Percnobracon* Kieffer & Jörgensen, two of *Plesipsenobolus* Belokobylskij, Martínez & Zaldívar-Riverón, four of *Psenobolus* Reinhard, and two of *Sabinita* Belokobylskij, Zaldívar-Riverón & Martínez.

We included three species of the polyphagous genus *Heterospilus* Haliday, since this has been consistently recovered as sister to the gall-associated clade in previous works (Zaldívar-Riverón et al. 2007, 2008, 2014). We also included species of the following doryctine genera, which were recovered in a main "South American clade" together with the members of the gall-associated clade (Zaldívar-Riverón et al., 2008): *Leluthia* Cameron, *Parallorhogas* Marsh, *Polystenus* Förster and *Stenocorse* (Crawford). Moreover, we included species of *Lissopsius* Marsh, *Hecabalodes* Wilkinson and *Pareucorystes* Tobias, which presumably belong to the above-mentioned main clade. We also included species of the genera *Eodendrus* Belokobylskij, *Dendrosotinus* Telenga, *Euscelinus* Westwood and

Rhaconotus Ruthe to root the trees. The latter two genera (*Euscelinus* and *Rhaconotus*) were nested in a main Old-World clade in the aforementioned phylogenetic study. A list with all the examined species, their locality and year of collection, DNA voucher and SRA Gen-Bank accession numbers and biological information is given in Table S1.

The specimens examined in this study are deposited in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM) and in the Insect Museum, Department of Renewable Resources, University of Wyoming, Laramie, USA (ESUW).

DNA extraction, UCE data collection and sequencing

All DNA extractions were conducted in the Laboratorio de Biología Molecular at IB-UNAM. DNA was extracted from voucher specimens following a nondestructive technique (Ceccarelli et al. 2012) using the EZ-10 Spin Mini preps Kit (Bio Basic, Toronto, Canada). Library preparation and target enrichment were conducted in the Laboratorio de Biología Molecular at IB-UNAM and in the Laboratories of Analytical Biology (LAB) at the National Museum of Natural History (NMNH) in Washington D.C., USA. We followed Branstetter et al.'s (2017) protocol for library preparation and capture and enrichment of UCE loci. All DNA extractions were quantified using Qubit 2.0 (Life Technologies). For each sample, up to 50 ng of extracted DNA was fragmented to an average fragment distribution of 400–600 bp using either a Bioruptor® Pico sonication device (Diagenode Inc., Danville, NJ, USA) or a Qsonica Q800R sonicator (Qsonica LLC, Newton, CT, USA).

Genomic libraries were constructed using the Kapa Hyper Prep library preparation kit (Kapa Biosystems Inc., Wilmington, MA, USA) and TruSeq-style dual-indexing barcodes (Glenn et al. 2019).

Target enrichment of the DNA libraries was performed both at IB-UNAM and LAB-NMNH using the two enhanced commercial myBaits UCE sets (1.5Kv1 and 2.5Kv2; ArborBiosciences, Ann Arbor, MI, USA), which were designed specifically for the order Hymenoptera and developed by Faircloth et al. (2015) and Branstetter et al. (2017). Pooled enriched libraries were sent to two different locations for sequencing services. A first pool was sent to the Georgia Genomics Facility, University of Georgia, Athens, USA, and sequencing was performed with an Illumina NextSeq v2 300 cycle kit (150-bp paired-end, Illumina Inc., San Diego, CA, USA). The remaining enriched libraries were sent to Novogene Corporation Inc. (Sacramento, CA, USA) for sequencing using an Illumina NovaSeq 6000 (150-bp paired-end, Illumina Inc., San Diego, CA, USA).

UCE data processing

Quality control of all sequences was first performed in FastQC v0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc; Andrews 2010). Raw reads were then cleaned and trimmed using Illumiprocessor (Faircloth 2013), which uses Trimmomatic v0.39 (Bolger et al. 2014) available in the software package PHYLUCE v1.6.6 (Faircloth, 2016). Trimmed and cleaned sequences were uploaded to the Galaxy web platform (https://usegalaxy.org/; Afgan et al. 2018). We assembled all data with SPAdes v3.14.0 (Bankevich et al. 2012). Assembled contigs were downloaded from the public

server to reincorporate them to the PHYLUCE pipeline, and UCE loci were extracted and subsequently aligned using MAFFT v7.471 (Katoh and Standley 2013). We filtered and trimmed the resulting alignments using a wrapper script in Gblocks v0.91b (Castresana, 2000) and the following reduced stringency settings: b1 = 0.5, b2 = 0.5, b3 = 12 and b4 = 7 (Branstetter et al. 2017).

We first generated a 60% completeness matrix (loci present in 51 of the 86 terminal taxa), to identify and remove loci that significantly deviated ($p < 0.05$) from base composition homogeneity among taxa using the program BaCoCa v1.105 (Kück and Struck 2014). As a result, we discarded 47 problematic loci from 1,082 shared alignments (Table S2). The filtered 60% completeness matrix (1,035 loci) was then employed to build matrices at 70% (≥60 of 86 taxa, 674 loci), 80% (≥68 of 86 taxa, 562 loci) and 90% (≥77 of 86 taxa, 343 loci) of completeness for subsequent analyses.

Phylogenomic inferences

We partitioned the four filtered data sets using the Sliding-Window Site Characteristics of size entropy algorithm (SWSC-EN; Tagliacollo and Lanfear 2018), and then selected the best scheme with PartitionFinder v2 (Lanfear et al., 2017) based on the Bayesian Information Criterion (BIC) and the rcluster option. A maximum likelihood (ML) phylogenomic inference was conducted for each matrix with the program IQ-TREE v2.1.1 (Nguyen et al. 2015). We assessed support performing 1,000 ultrafast bootstrap (BTP) replicates.

The 80% completeness matrix (562 loci), which was selected as the best locus set based on topological consistency and BTP support values (see below), was employed for gene tree estimation for each UCE independent locus with the program RAxML. Each analysis implemented a best tree plus rapid BTP search (GTRGAMMA model; 200 BT replicates). We then calculated the average BTP support (Table S2) for each gene tree using a script modified from Borowiec et al. (2015) implemented in R v3.6.1 (R Core Team 2019). We retained the 500 loci that produced the best supported gene trees (500-loci matrix). The above partition and ML phylogenomic analyses were performed for the latter matrix, and the program ASTRAL-III v5.6.3 (Zhang et al. 2018) was used to build a species tree.

Divergence times estimate

We used the "clocklikeness" values obtained from the script developed by Borowiec et al. (2015) (Table S2) to sample the 100 UCE loci with lowest scores to generate a time-calibrated phylogenetic tree with the program BEAST v1.10.4 (Suchard et al. 2018). Divergence time estimation is particularly difficult in this group due to the lack of a fossil record. We therefore employed two calibration points derived from two previous studies. The first calibrated node was the most recent common ancestor (MRCA) of the main "South American" clade containing all the gall-associated group and remaining Neotropical parasitoid genera (Zaldívar-Riverón et al. 2008). We used for this node a normal distribution, which allows a bidirectional uncertainty, with a mean age of 44.26 Myr ± 2.99 SD. For the second calibration point, we used the MRCA for the gall-associated +

Heterospilus clade, using a normal distribution and a mean age of 23.44 ± 4.32 SD (Zaldívar-Riverón et al. 2014).

We concatenated the 100 selected loci and analyzed the matrix without partitioning to reduce computational time. Four independent runs were performed, each one consisting of 300 million generations and sampling every 10,000 generations. The analyses used an uncorrelated lognormal clock, a GTRGAMMA substitution model and a Yule model prior. We used the program Tracer v1.7.1 (Rambaut et al. 2018) to determine chain convergence and burn-in. After discarding a burn-in of 25% of the sampled trees, the maximum clade credibility tree was summarized with the programs LogCombiner v1.10.4 and TreeAnnotator v1.10.4 both available from the BEAST package, using the common ancestor heights (CAH) option to obtain the clade ages.

Ancestral state reconstruction analyses

ASR analyses were performed using a ML approach and the following character coding related with life history strategies: A) feeding strategy: 0) parasitoid; 1) inquiline; 2) gall former; 3) seed predator without gall formation; B) plant family association: 0) Anacardiaceae, 1) Annonaceae, 2) Araceae, 3) Asteraceae, 4) Fabaceae, 5) Fagaceae, 6) Malvaceae, 7) Melastomataceae, 8) Moraceae, 9) Myrtaceae, 10) Primulaceae, 11) Polygonaceae, 12) Rubiaceae, 13) Solanaceae; and C) plant organ association: 0) syconia, 1) leaf, 2) stem, 3) floral bud, 4) root, 5) fruit/seed.

We used the ultrametric tree obtained by our divergence times estimate analysis to assess the ancestral states of the above biological features. For the feeding strategy

character, we included all ingroup and the 15 outgroup taxa, whereas for the remaining two characters we pruned the outgroup taxa and rooted the trees using *Percnobracon* as sister to the remaining gall associated genera according to our recovered topologies.

The ML approach used for the ARS analyses was implemented using the rayDISC command contained in the package corHMM (Beaulieu et al. 2013) in R. This method allows for multistate characters, unresolved nodes and also considers ambiguities for polymorphic taxa or missing data. The three available models of character evolution implemented in this package were evaluated under the ML method: equal rates (ER), symmetrical (SYM) and all rates different (ARD). We selected the best model for each character performing a likelihood ratio test using the −lnL scores.

RESULTS

UCE data summary statistics

After adapter and quality trimming of raw sequences, an average of 178,313 contigs was assembled with SPAdes (Table S3). We recovered a total of 2,352 UCE loci with an average of 1,016 loci per sample and a mean length of 364.19 bp from all the assembled contigs. Final filtering and concatenation of UCE loci generated matrices of 497,270 (60%), 349,634 (70%), 300,066 (80%) and 185,122 bp (90%). The 80% completeness matrix (562 loci) was selected as the data set with the best analysis performance (see below) and recovered UCE contigs with a mean length of 533.93 bp and 133,073 informative sites.

Phylogenomic analyses

The topologies derived from the ML analyses for each of the matrices with different completeness percentages yielded well-supported relationships and were mostly concordant (Figs. S1-S4). The topology derived from the 80% completeness matrix recovered the highest support values for the relationships that had BTP values < 100. The topology derived from the 500-loci matrix obtained after filtering the loci that produced independent gene tree topologies with the highest average BTP support values is shown in Fig. 1.

The gall-associated genera were recovered as monophyletic (BTP=100) with the exclusion of *Donquickeia*, which was nested outside in a clade together with *Hecabalodes*, *Leluthia*, *Pareucorystes, Polystenus* and *Stenocorse.* Within the clade with the gall-associated genera, *Percnobracon* was recovered at the base, followed by a clade that contained the monotypic genus *Mononeuron* and seven *Allorhogas* species, including *A. ardisia* Marsh*, A. lacuna* Centrella & Shaw*, A. scotti* Martínez & Zaldívar-Riverón and *A. coccolobae* Martínez & Zaldívar-Riverón (*Allorhogas+Mononeuron* clade). A clade with *A. gauldi* Marsh, *Ficobolus, Labania* and *Monitoriella* was recovered above as sister to the remaining ingroup taxa (*A. gauldi+Ficobolus+Labania+Monitoriella* clade), followed by a clade (BT=100) with *A. crassifermur* Martínez & Zaldívar-Riverón at its base and by three species assigned to *Allorhogas* together with a non-monophyletic *Plesiobsenobolus*, a monophyletic *Psenobolus* (BT=100) and *Sabinita* (*Allorhogas+Plesiopsenobolus+Psenobolus+Sabinita* clade). The latter clade was sister to a clade that comprised most of the species of *Allorhogas* included in this study (the main *Allorhogas* clade).

The main *Allorhogas* clade was divided into two subclades. One of them had a subclade with species associated to *Quercus* (Fagaceae), and another one with species associated to Rubiaceae and Fabaceae. The second subclade, on the other hand, had a subclade with *A. mendocinus* (Kieffer & Jörgensen), reared from Solanaceae, and two undescribed species reared from Anacardiaceae, which was sister to two subclades, one whose species with biological data were reared from Fabaceae, and a second that had species reared from Melastomataceae and Myrtaceae and some species without biological information.

The species tree estimated with ASTRAL-III had lower support values in some of its clades compared to the concatenated analyses, though the same major clades were recovered with local posterior probability (LPP) values of 1.0 (Fig. S5).

Divergence time estimates

The ultrametric tree recovered by the Bayesian relaxed molecular clock analysis performed with the 100 UCE loci with the best "clocklikeness" scores extracted from the 80% completeness matrix is shown in Fig. 2. The gall-associated clade was estimated to have diverged from *Heterospilus* 29.41-18.95 Ma during the middle Oligocene to early Miocene. A subsequent radiation of this group probably occurred during the Miocene and Pliocene. Among the groups whose MRCA diverged during the Early Miocene are the *Allorhogas+Mononeuron* (21.68–13.16 Ma) and the *Ficobolus+Labania+Monitoriella* clades (21.22–13.11 Ma). The MRCA of the *Allorhogas+Plesiopsenobolus+Psenobolus+Sabinita* clade was on the other hand

estimated to have diverged 16.95–10.09 Ma during the middle Miocene, whereas the MRCA of *Perconobracon* diverged 12.89-3.74 Ma during the late Miocene to early Pliocene.

Within the main *Allorhogas* clade, its two subclades were estimated to have diverged 14.05-7.64 Ma during the middle to late Miocene. Within one of these subclades, the groups associated to Fagaceae and Rubiaceae probably diverged 11.48-5.76 Ma during the late Miocene. Within the second *Allorhogas* subclade, the Anacardiaceae-associated group apparently diverged from the Fabaceae+Melastomataceae/Myrtaceae subclades 10.37-5.84 Ma during the late Miocene. The latter two subclades, on the other hand, were estimated to have diverged 9.87-5.52 Ma, during the late Miocene.

Ancestral state reconstruction

The SYM and ARD were selected as the best fitting models by the likelihood ratio tests for the ASR analyses performed for the feeding strategy and for the plant family and plant organ associations, respectively.

The ancestral condition for the feeding strategy characters is likely to be parasitoid, with a transition to inquilinism in the MRCA of the gall-associated clade (Fig. 3). Inquilinism is the dominant lifestyle during the evolution of the clade. Gall formation, on the other hand, appeared to have evolved twice, once in the MRCA of the *A. gauldi+Ficobolus+Labania+Monitoriella* clade and another in the main *Allorhogas* clade, particularly with the MRCA of the Melastomataceae/Myrtaceae associated clade.

There are several transitions to different plant families within the gall-associated clade, with its MRCA recovered to be associated with plants of the family Moraceae. Four main shifts from Moraceae to other plant families were observed, particularly to Fabaceae in the MRCA of *Percnobracon*; to Malvaceae in the MRCA of the *Allorhogas+Mononeuron* clade; to Araceae in *Monitoriella*; and to Anacardiaceae in the MRCA of the main *Allorhogas* clade. Independent shifts from Moraceae to Malvaceae and Fabaceae were also observed within *Labania* and *A. ingavera*, respectively.

Fabaceae is a dominant plant associated family represented in the evolutionary history of the group, and the association to various genera of legumes occurred multiple times during the evolution of the gall-associated clade, particularly in the MRCA of *Percnobracon* and in the Fabaceae-associated clade, which apparently evolved from an ancestor associated to Solanaceae. Moreover, a separate association to Fabaceae is observed outside the main *Allorhogas* clade in *A. ingavera*. The plant association to Melastomataceae is also represented by independent origins, one in *A. lacuna* and a second in the Melastomataceae-associated clade. Some plant families are only represented by associations that occurred just once during the evolution of the group, including the association to Rubiaceae with a consequent transition to Fagaceae, as well as a shift from an ancestor associated to Solanaceae to Anacardaceae. Individual shifts are represented by the plant families Primulaceae and Asteraceae, the first in *A. ardisia* and the second in *A. punctatus.*

The results of the ASR with the plant organ association feature recovered leaves as the ancestral state for the gall-associated clade (Fig. 5). This condition predominated in the early evolution of the group, with a probable transition to stems in the MRCA of

Percnobracon and in the MRCA of the main *Allorhogas* clade. A single shift to aerial roots and syconia was recovered in species associated to *Ficus,* particularly in the MRCA of *Labania* and within the *Allorhogas+Plesiopsenobolus+Psenobolus+Sabinita* clade. Transition to fruits/seeds occurred three times, the fist probably evolving in the MRCA of the Rubiaceae-associated clade, the second in the MRCA of the Fabaceae-associated clade, and the third in the MRCA of the Melastomataceae/Myrtaceae-associated clade. Finally, a single shift from fruit/seeds to floral buds was observed in *A. uberlandiensis* within the latter clade.

DISCUSSION

Systematics of the gall-associated doryctine genera

Previous phylogenetic works have studied the systematics of the gall-associated doryctine clade; however, they have failed to completely resolve its generic relationships and to establish its generic limits (Zaldívar-Riverón et al. 2007, 2008, 2014). These phylogenetic studies included a reduced number of taxa with incomplete biological information and an underrepresentation of described species for all genera (Samacá-Sáenz et al. 2019). Here, we conducted a phylogenomic study based on UCE data with a broad taxonomic sampling, with most species having biological records and including several described species. The resultant topologies yielded the most complete and comprehensive estimate of phylogeny for the gall-associated doryctine clade obtained to date. Below we summarize the confirmed and novel relationships derived from our study and compare our results with

those obtained in previous morphological and molecular phylogenetic studies that have been performed for the group.

Monophyly of the gall-associated doryctine genera was recovered with the exclusion of the two species assigned to *Donquickeia*. Members of this genus, which were included for the first time in a phylogenetic study, were nested in a clade as sister to *Stenocorse*, *Hecabalodes*, *Pareucorystes* and *Leluthia*. *Donquickea* is morphologically different from all the gall-associated genera, being more similar to species of the doryctine genus *Semirhytus* Szepligeti (Marsh, 1993). Although we confirmed the identification of the specimens of *Donquickeia* processed for this study (Marsh, 1997), it is necessary to include the two described species of the genus (Marsh, 1993), as well as the undescribed species that Penteado-Dias (2000) reported as presumably phytophagous to confirm whether gall-association has evolved twice in the Doryctinae.

Among the confirmed relationships, *Percnobracon* was sister to the remaining gall-associated genera. Moreover, like previous studies, we recovered two different clades associated with different organs of *Ficus*, the *Ficobolus+Labania* and the *Plesiopsenobolus+Psenobolus+Sabinta* clades. *Monitoriella* is also confirmed as sister to the *Ficobolus+Labania* clade. These relationships were obtained in Samacá-Sáenz et al's (2019) study but with low support. A *Plesiopsenobolus+Psenobolus+Sabinta* was also recovered here, with *Plesiopsenobolus* being non-monophyletic. *Allorhogas* was again recovered as polyphyletic being divided into two major clades, the *Allorhogas+Mononeuron* clade and the main *Allorhogas* clade. Among the *Allorhogas* species that were nested together with the monotypic *Mononeuron* were *A. ardisia, A. coccolobae* Martínez & Zaldivar-Riverón, *A. lacuna* Centrella & Shaw and *A. scotti*

Martínez & Zaldivar-Riverón along with two undescribed species. Some of these species share the absence of the hind wing vein cu-a, a character previously proposed as diagnostic for *Mononeuron* (Nunes et al. 2012). On the other hand, *Allorhogas coccolobae* is similar to *A. scotti*. It also resembles morphologically a species not included in this study, *A. shawi* Marsh, but differs from it by having a longer second submarginal cell (Martínez and Zaldívar-Riverón 2013). *Allorhogas ardisia* is distinguished from other *Allorhogas* species by its laterally coriaceous propodeum and the enlargement of the second and third metasomal tergites. Although it is similar to *A. lacuna, A. ardisia* distinguishes from the latter species by its sternaulus pit-like (Centrella and Shaw 2013). A detailed taxonomic study will help to establish the actual taxonomic status of the *Allorhogas* species that were recovered in the above group.

Some species of *Allorhogas* that also are morphologically different of those recovered in the main *Allorhogas* clade appear to be more related to other genera. *Allorhogas gauldi* was closely related to the *Monitoriella* clade, *A. crassifemur* was confirmed as sister to the *Plesiopsenobolus+Psenobolus+Sabinta* and the main *Allorhogas* clades, whereas *A. ingavera* and *A. costaricensis* Marsh were placed within the *Allorhogas+Plesiopsenobolus+Psenobolus+Sabinita* clade. Among the morphological differences that have been observed for *A. gauldi* are its fore wing vein m-cu distinctly distal to vein 2RS, a characteristic shared with *A. shawi* and *A. sulcatus* Marsh (Marsh 2002). *Allorhogas crassifemur* on the other hand can be distinguished from all the other species of the genus by its vein 2RS meeting RS+M anterior to m-cu, long first flagellomere, short ovipositor sheaths and convergent striations on the apical area of the first metasomal tergite (Martínez and Zaldívar-Riverón 2013).

Various novel relationships were recovered for the first time within the main *Allorhogas* clade. We recovered a subclade with species reared from *Psychotria* and *Quercus*. Moreover, the relationships of the Fagaceae-associated clade are the same as those previously reported by Samacá-Sáenz et al. (2020), with the inclusion of an undescribed species from Costa Rica that was recovered as sister to *A. bassettia.* Although *A. psychotria* was recovered here to be closely related to the species from the Fagaceae-associated clade, it can be differentiated from the latter group and other *Allorhogas* species by its considerably larger size and long ovipositor (Zaldívar-Riverón et al. 2018). This last characteristic, however, is also present and notorious in the type species *A. gallicola* Gahan, which probably belongs to this group since it shares morphological characters with those of the Fagaceae-associated clade (Samacá-Sáenz et al. 2020) and have been reared from a cynipid gall present on an oak species (Gahan 1912).

The second subclade within the main *Allorhogas* clade was divided into three monophyletic groups. The first include an Anacardaceae-associated clade that was sister to *A. mendocinus,* a species that differs from the remaining *Allorhogas* by its considerably rugose head and mesoscutum and by its obscured, weakly impressed notauli (Martínez et al., 2008)*,* The second clade groups the Fabaceae-associated clade together with *A.cordobensis* and an undescribed species.

Species belonging to the Fabaceae-associated clade share various morphological features, and all were reared from seeds of legume species, including a generally uniformly yellow body color (Marsh 2002; Martínez and Zaldivar-Riverón 2013).

The last subclade recovered within the main *Allorhogas* group contains the species associated with Melastomataceae plant species (*A.conostegia*, *A. minimus, A. uberlandiensis* and *A. granivorus*), as well as *A. brevithorax, A. jaliscoensis, A. laselva, A. punctatus* and *A. tico.* The species recovered in this group share a compressed metasoma, short pronotal collar and sharp bidentate mandibles (Chavarría et al. 2008; Centrella and Shaw 2010; Zaldívar-Riverón et al. 2018). These characteristics are shared with *A. brevithorax,* which was reared from an undetermined species of the Myrtaceae genus *Myrcia* DC. and was recovered as sister of the Melastomataceae-associated clade.

Origin of gall-association and host codiversification

We calculated the divergence times of several clades within the group of gall-associated genera using two secondary calibration points. Although this practice has been frequently criticized (Graur and Martin 2004; Sauquet et al. 2012; Wheat and Wahlberg 2013; Shenk 2016), sometimes is the only source of information for many groups, particularly for those in which the fossil record is sparse or non-existent (Forest 2009). Our divergence times estimated that gall association had its origin during the late Oligocene to early Miocene, approximately 20.8 Ma, followed by a radiation of its major clades during the middle to late Miocene, about 18.07-10.76 Ma. Within the main *Allorhogas* clade, its main groups diverged during the Pliocene and more recently during the Pleistocene, which is the case of some of the members of the Anacardiaceae, Fabaceae, Fagaceae and Melastomataceae associated clades.

Previous studies have proposed that the main diversification events of the Neotropical biota are related with two events that occurred mainly during the Miocene and extending to the Pliocene, the continuous uplift of the Andes and the retreatment of the Pebas system (Wesselingh et al. 2002; Wesselingh and Salo 2006; Antonelli et al. 2009; Antonelli and Sanmartín 2011; Hoorn et al. 2010,). These events provided new opportunities for lineages to radiate and allowed several taxa to disperse, which supports the proposal of the Amazonia as the main source of Neotropical diversity (Antonelli et al. 2018). Subsequent climatic fluctuations have also been proposed as drivers of diversification during the Pleistocene (Haffer 1969). The divergence times that we recovered are congruent with those of other Neotropical insect lineages that also had their species diversification after the latter climatic events (e.g. Chazot et al. 2019; Menezes et al. 2020). However, further studies of historical biogeography and ancestral range reconstruction in the gall-associated clade are still necessary to confirm whether it had an Amazonian origin, and that its current species diversity can be explained by the aforementioned climatic Pleistocene events.

Some of the plant lineages with Neotropical distribution that are hosts of the gall-associated species examined here also diversified during the middle Miocene to Pliocene, suggesting the existence of codiversification processes. The age of the MRCA of *Percnobracon* is congruent with the high diversification rate of the American species of the genus *Prosopis* L. during the late Miocene, which is correlated with the spreading of arid environments in this continent (Catalano et al. 2008). A high diversification rate was also detected approximately 12 Ma in Neotropical species of *Philodendron* Schott (Canal et al. 2018), which is concordant with our estimated ages of the MRCA of *Monitoriella*.

Similarly, the plant genus *Ficus*, started its species diversification in the Neotropics between the Oligocene and Miocene, with two posterior bursts of diversification during the middle Miocene and Pliocene (Machado et al. 2018). The above times are concordant with the estimated ages of the MRCAs of the two clades within the ingroup whose species are associated with the latter plant genus, the *Ficobolus*+*Labania* and *Plesiopsenobolus*+*Psenobolus*+*Sabinita* clades.

Our results also show evidence of codiversification in the Americas between the members of the main *Allorhogas* clade and their host plant genera. The Rubiaceae-associated clade includes two species from Costa Rica reared from *Psychotria,* whose MRCA was estimated to diversify during the late Miocene to Pliocene, 7.95-2.80 Ma. *Psychotria* primarily diversified about 16 Ma in Mesoamerica, with most of its lineages diversifying within the last 12 Myr (Paul et al. 2009). On the other hand, American live oaks (*Quercus* subsection *Virentes*) originated ca. 11 Ma, with subsequent speciation events occurring during the Pliocene, ca. 5 Ma (Cavender-Bares et al. 2015). This last age is concordant with the more recent divergence time between the *Allorhogas* species associated to the *Quercus* subsection *Virentes*.

Our estimated ages of 3.30-0.61 Myr for the MRCA of the *Allorhogas* species associated with the plant family Anacardiaceae are concordant with the times of diversification of the genus *Schinus,* which was estimated to have diverged from other Anacardiaceae genera during the middle Miocene (Muellner-Riehl et al. 2016). The members of *Allorhogas* reared from Fabaceae and Melastomataceae are associated to different plant genera. One of these genera, *Inga*, experienced an accelerated radiation in Neotropical rain forests within the last 10 Myr (Richardson et al., 2001). On the other hand,

Miconia Ruiz & Pav. and *Clidemia* D. Don, two closely related genera well represented in the Melastomataceae-associated clade, probably diverged from other genera during the middle to late Miocene (Berger et al. 2016). Although the diversification times of the species of these two genera are unknown, it could also be concordant with estimated age for the MRCA of the Melastomataceae associated clade, estimated here to occur during the late Miocene to Pliocene, 6.11-2.82 Ma.

Evolution of life history features

Our ancestral state reconstruction analyses of life history features strongly suggest an scenario where gall-association in the Doryctinae first evolved from parasitoidism to subsequent phytophagous inquilinism. Transition from parasitoidism to gall-induction in Hymenoptera has been previously proposed (Hanson 1995; Ronquist 1995; Quicke 1997). These authors suggested that gall-formation in Hymenoptera originated from ectoparasitoids of hosts that lived inside plants or galls with a consequent facultative herbivory of the parasitoid larvae due to the competition for resources. Therefore, inquilinism has been explained as an intermediate physiological trait between entomophagy and phytophagy, the first represented by parasitoidism and the second by phytophagy (Blaimer et al., 2020). Our results confirm that gall-association diverged from the MRCA of the ingroup and the cosmopolitan, polyphagous genus *Heterospilus*, whose species with known biology are parasitoids of wood-boring beetle larvae, but also stem-boring sawflies and moths (Marsh et al. 2013). Considering this, it is probable that inquilinism in the gall-

associated clade evolved from parasitoidism of immature states of insects of these orders living inside plants.

The above "inquiline first" hypothesis has been recently suggested for several cynipid lineages (Ronquist et al. 2015; Blaimer et al. 2020). In these studies, gall wasps were suggested to be originally inquilines, with gall formation independently occurring several times in different lineages. Our results suggest that, although inquilinism is the predominant lifestyle in the evolutionary history of the gall-associated clade, gall formation and seed predation also independently evolved in specific clades of the doryctine gall-associated genera. For instance, gall induction appeared once in the MRCA of the *A. gauldi*+*Ficobolus*+*Labania*+*Monitoriella* clade and another in the MRCA of the Melastomataceae/Myrtaceae-associated clade, whereas seed predation, evolved once in the Fabaceae-associated clade.

The evolution of plant family and plant organ association from which the examined species were reared increases the complexity of this model. Our ASR results show that the MRCA of the gall-associated clade was inquiline of leaf galls in plants of the family Moraceae. Two independent shifts to *Ficus* were previously proposed by Samacá-Sáenz et al. (2019); however, we recovered here a probable single association event to this plant, although with the MRCA of the *Ficobolus*+*Labania* clade being gall-former and the MRCA of the *Plesiopsenobolus*+*Psenobolus*+*Sabinita* being inquiline. Species diversification within the above two clades are thus apparently restricted to transitions to different *Ficus* species and different plant organs, instead of shifts to other plant genera or families. A shift from inquilinism of leaf galls to stem galls in Anacardiaceae within the main *Allorhogas* clade probably triggered the multiple phytophagous strategies in various

plant families that are observed in this group, including gall formation on seeds/fruits and floral buds and seed predation.

Galls are structured by communities of several arthropod species that act as parasitoids or inquilines of the gall inducer and that simultaneously interact with each other (Sanver and Hawkins 2000). Some of the doryctine species included in this study are known to belong to the natural communities of enemies that are associated with the galls from which they were reared. For example, the *Allorhogas* species belonging to the Fagaceae-associated clade are part of the invertebrate communities of galls induced by different cynipid species that occur on live oaks (Forbes et al. 2016; Weinersmith et al. 2020). Similarly, the Anacardiaceae-associated clade is composed by some undescribed inquiline species that attack galls induced by moths of the family Cecidosideae (Lepidoptera) (Martínez unpublished data). Host plant shift and host plant organ shift are thought to represent an evolutionary strategy to escape from natural enemies (Hayward and Stone 2005; Bailey et al., 2009), and this probably has led to speciation and diversification of the gall-associated clade with its various life histories, particularly in *Allorhogas* and its multiple phytophagous strategies.

Diversification of the ingroup alternatively could be explained from an "escape and radiate" scenario (Ehrlich and Raven 1964, Thompson 1989), in which the acquisition of the ability to overcome the chemical plant defenses promotes the rapid diversification of phytophagous insects. Under this hypothesis, adaptation towards host plants is the driving force of insect adaptive radiations (Wheat et al. 2007; Janz 2011); and this probably accelerated the diversification in the main *Allorhogas* clade, for which some of its species are associated to known toxic plant families, such as Anacardiaceae and Solanaceae (e.g.

Mitchel 1990; Griffin and Lin 2000; Wink 2003; Aguilar-Ortigoza et al. 2003). Associations with these plants were recovered as the ancestral states in the early evolutionary history of the main *Allorhogas* clade, and the evolution of adaptations to overcome their defenses could explain the multiple shifts to other plant families and the incorporation of other phytophagous strategies.

There is still a need to clarify the biology of many of the species included in this study. Of all gall-associated genera, only *Allorhogas* and *Monitoriella* have species for which herbivory has been confirmed experimentally or from reliable rearing records (e.g. de Macêdo and Monteiro 1989; Infante et al. 1995). However, detailed observations conducted by several authors indicate that it is very likely that species of other genera including *Ficobolus, Labania, Mononeuron Sabinita, Percnobracon* and *Sabinita* have also phytophagous habits, with some of them being potential gall-inducers (Nunes et al. 2012; Zaldívar-Riverón et al. 2014; Belokobylskij et al. 2015; Martínez unpublished data). Some of the species of *Allorhogas* included here were originally presumed to be parasitoids of other gall-forming insects (Martínez and Zaldívar-Riverón 2013; Forbes et al. 2016; Weinersmith et al. 2020). However, this information needs to be confirmed based on direct observations.

Considering the uncertainty of natural history for some of the species included here, we employed methods for ASR that allow missing data (Beaulieu et al. 2013), assigning an equal probability to be parasitoids, inquilines gall formers, or seed predators for the some of the specimens for which we still do not have biological information. Our results suggest that it is more likely that parasitoidism have been lost in the gall-associated clade, but it is still necessary to confirm whether this condition could be definitively discarded for some

species of group. Future studies should include detailed descriptions on the life histories of these organisms to support the scenario that we obtained here. Also, it is necessary to carry out further investigations on the evolution of phytophagy in the predominant parasitoid, extraordinarily diverse subfamily Doryctinae.

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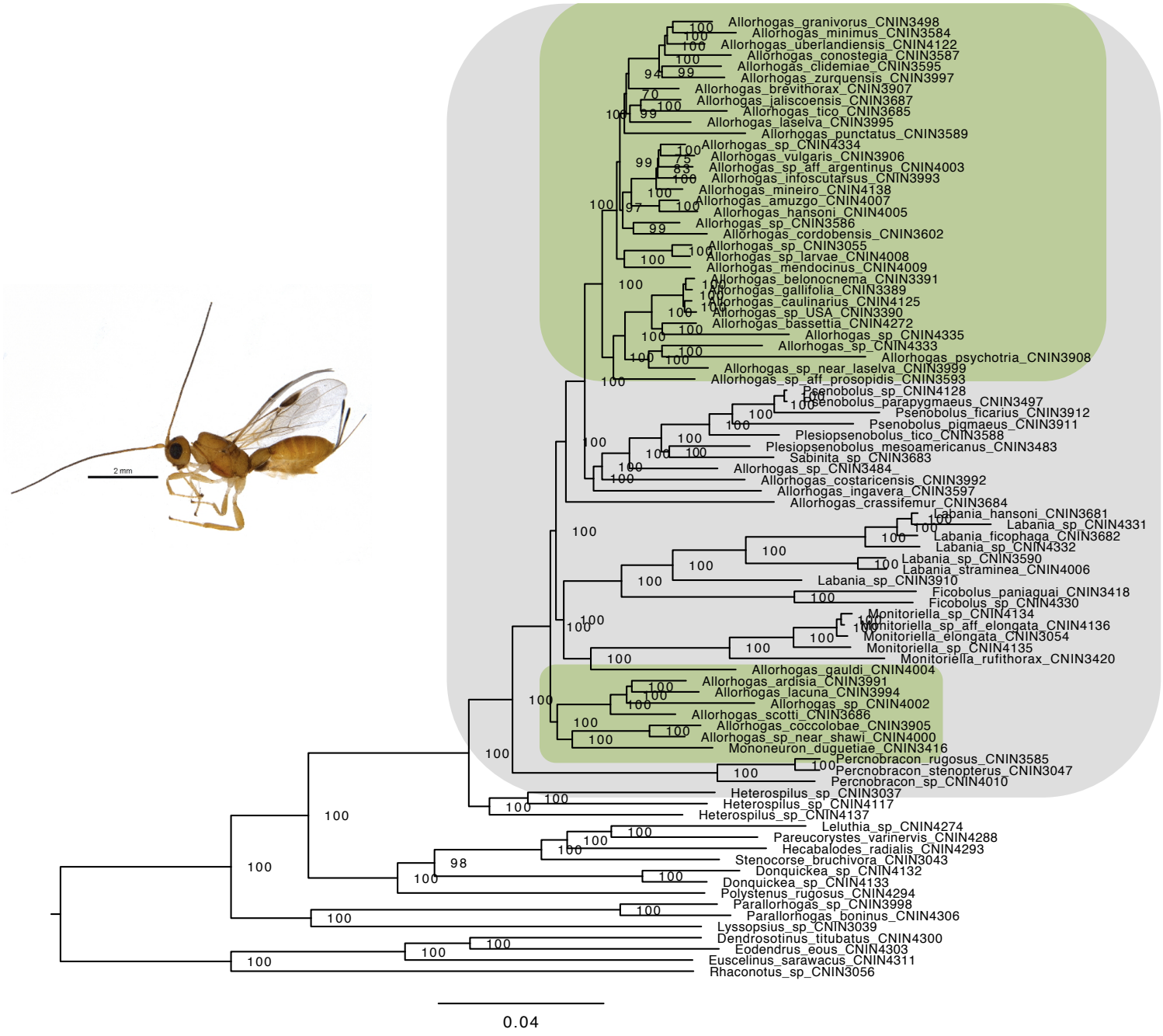

Figure 1. Maximum Likelihood tree resulting from IQ-TREE analysis of the 500 best alignments using the SWSC-EN partitioning scheme. This data set was obtained by filtering the loci that produce independent gene tree topologies with the highest average bootstrap support values from the 80% matrix. Bootstrap support values are shown next to nodes. The gall-associated clade is colored in grey, whereas the two main clades recovered for *Allorhogas* are shown in green.

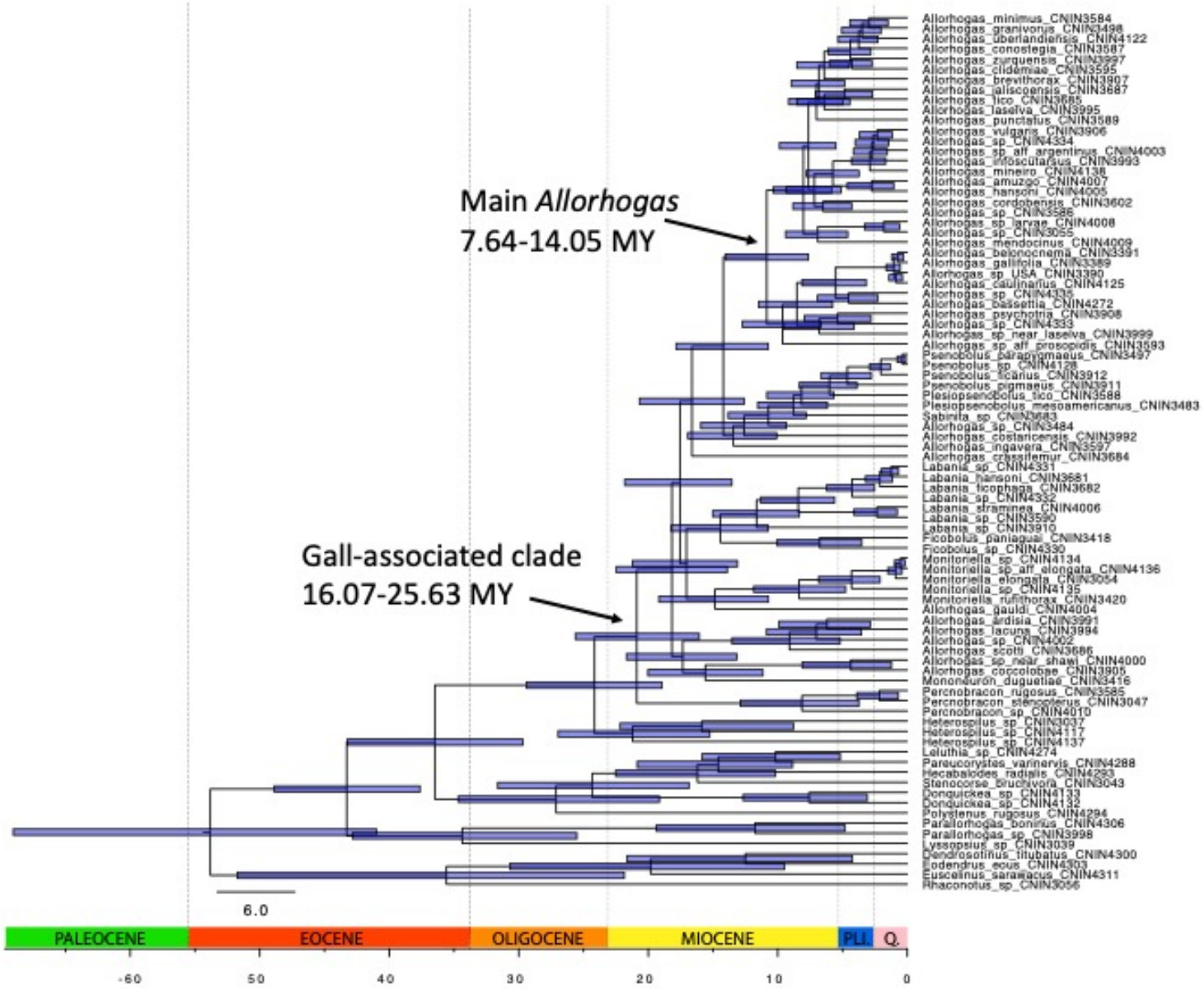

Figure 2. Time-calibrated phylogeny of the gall-associated genera recovered from the BEAST analysis performed with the 100 loci of the 80% data set with best "clocklikeness" scores. Divergence time estimates for two selected clades are highlighted.

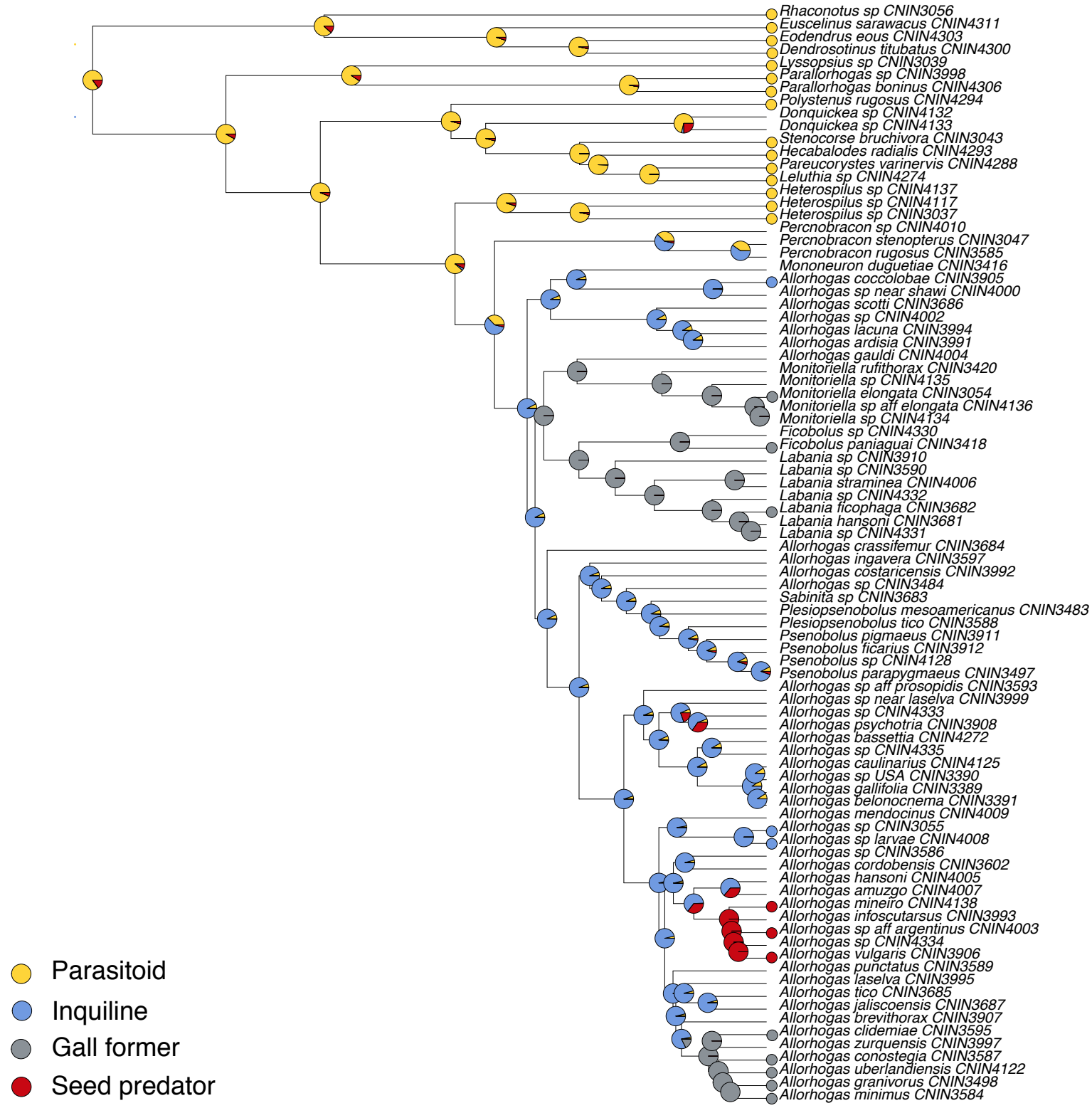

Figure 3. Ancestral state reconstruction for the gall-associated life histories of the four character state system (parasitoid, inquiline, gall former and seed predator) using the SYM model, a maximum likelihood approach and the rayDISC function in the R package corHMM.

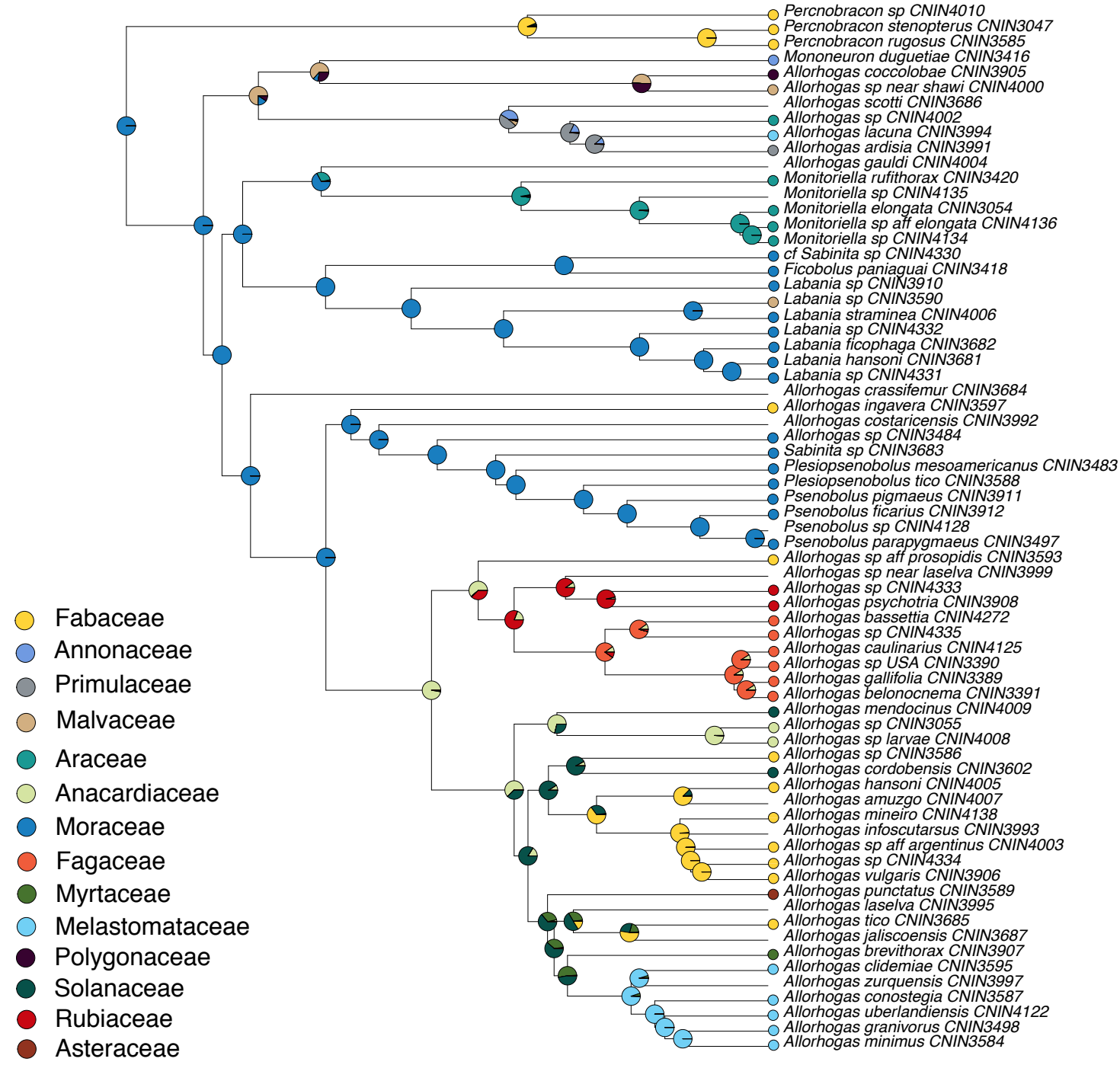

Figure 4. Ancestral state reconstruction for the gall-associated life histories of the associated plant family using the ARD model, a maximum likelihood approach and the rayDISC function in the R package corHMM.

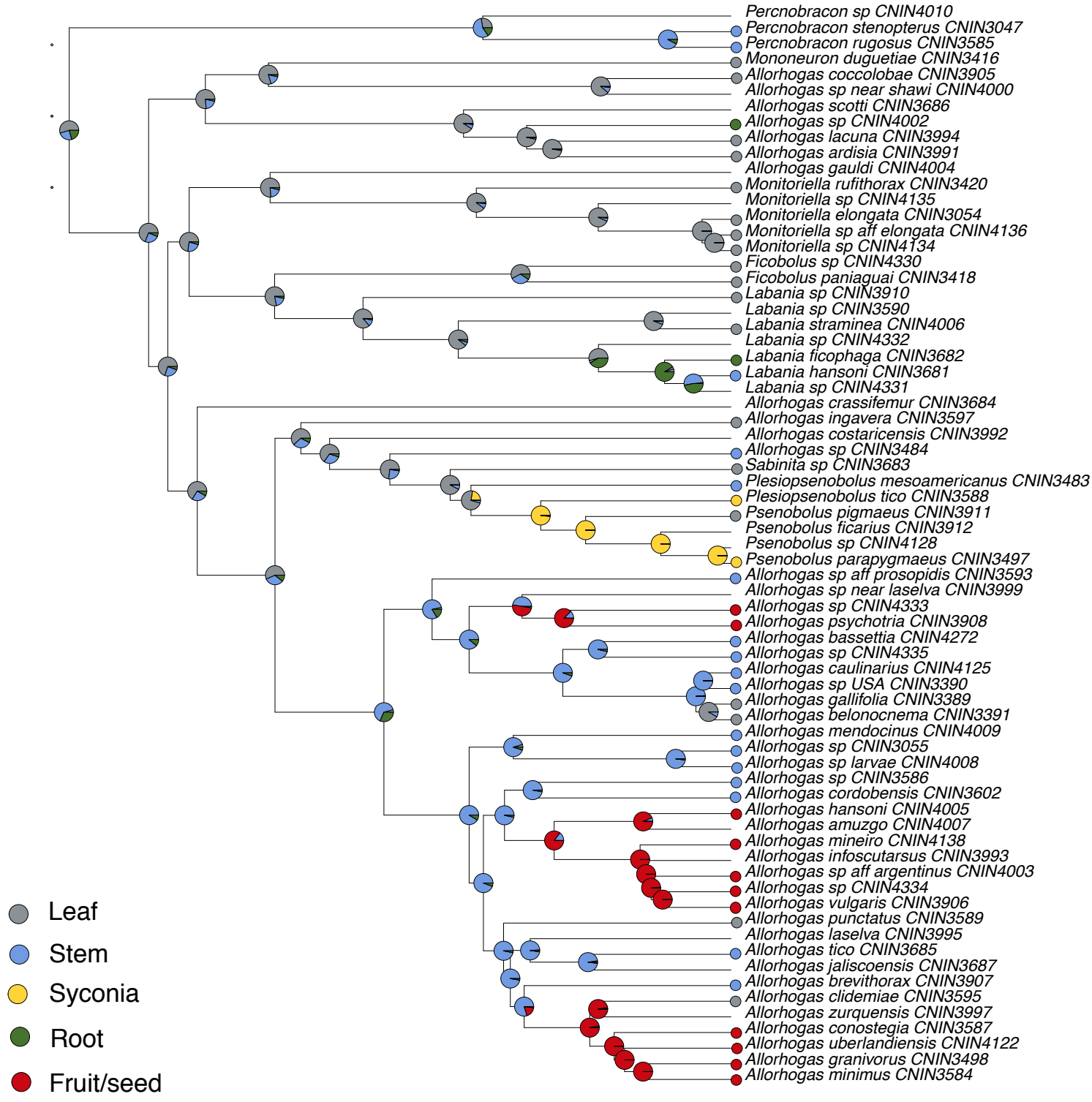

Figure 5. Ancestral state reconstruction for the gall-associated life histories of the plant organ using the ARD model, a maximum likelihood approach and the rayDISC function in the R package corHMM.

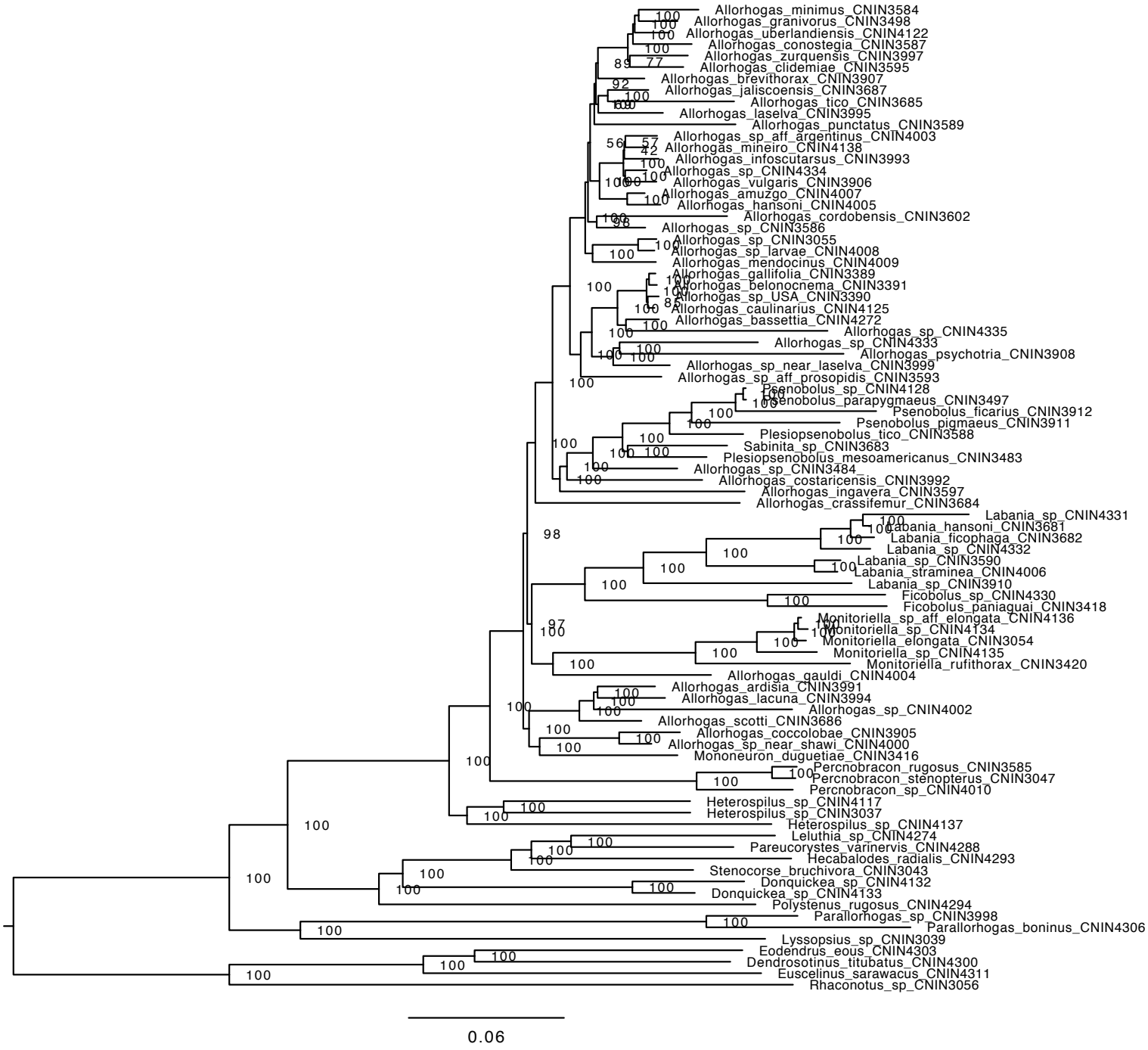

Figure S1. Maximum likelihood tree resulting from IQ-TREE analysis of the 60% completeness matrix using SWSC-EN partitioning scheme. Bootstrap support values are shown next to nodes.

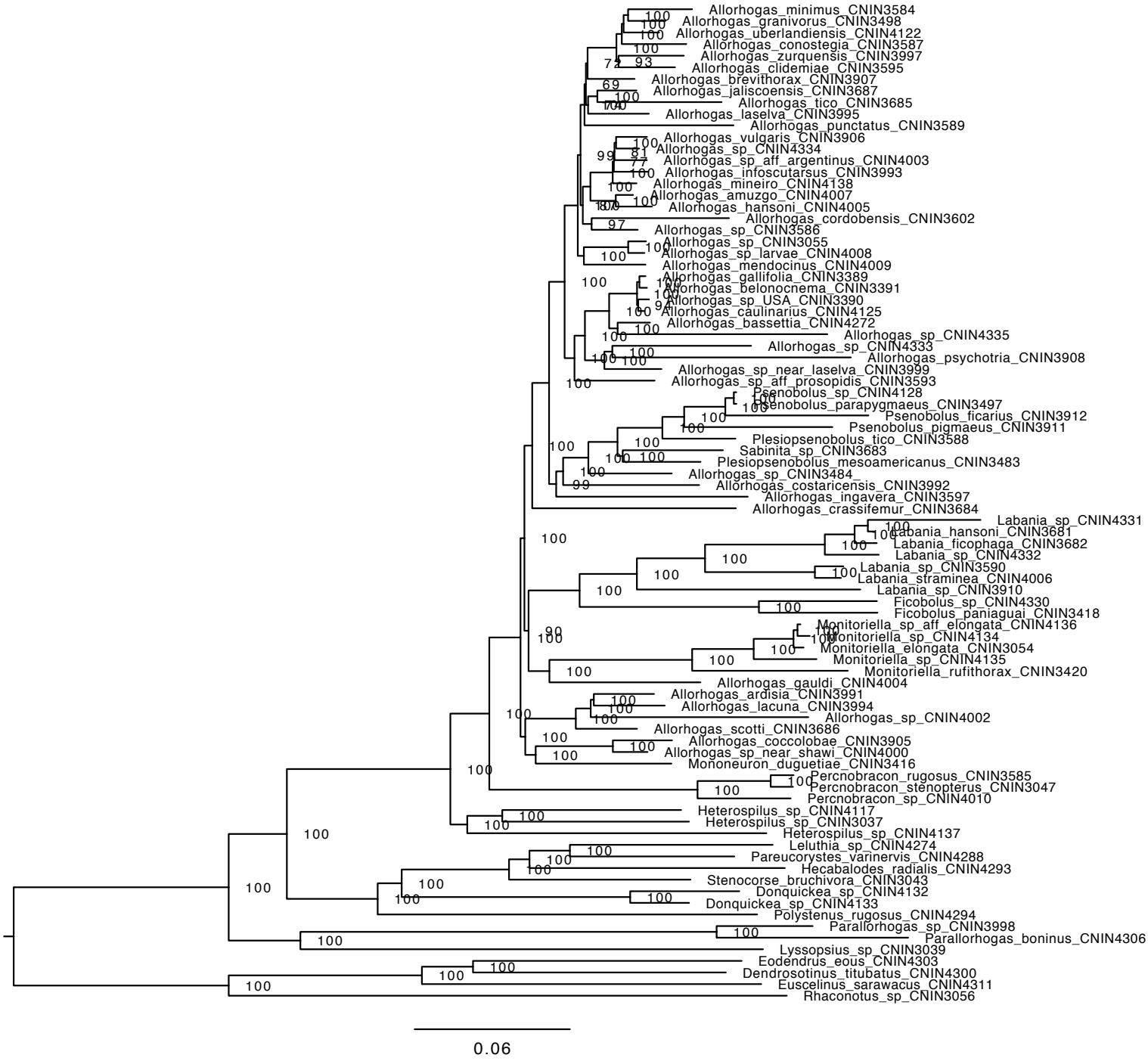

Figure S2. Maximum likelihood tree resulting from IQ-TREE analysis of the 70% completeness matrix using SWSC-EN partitioning scheme. Bootstrap support values are shown next to nodes.

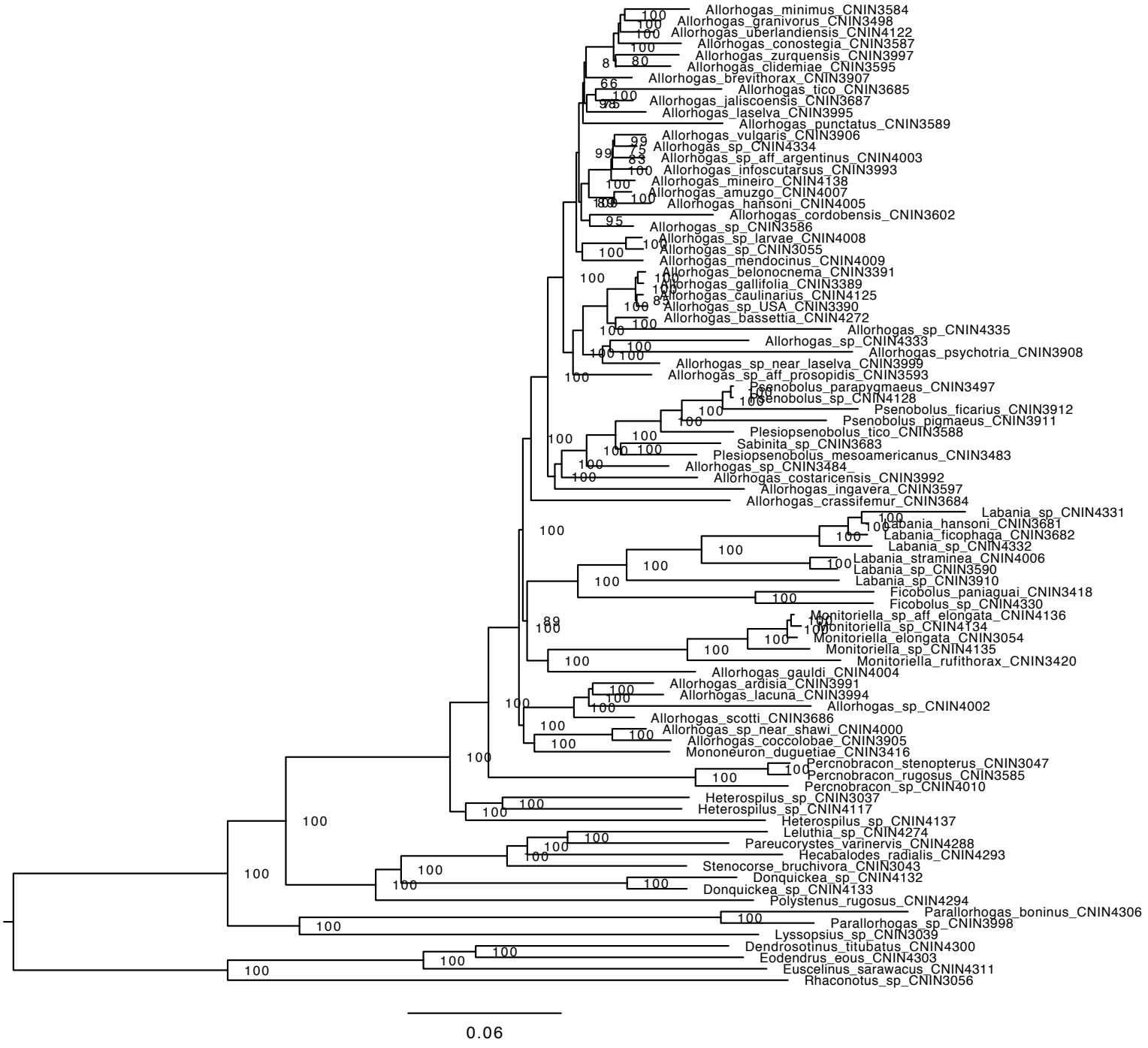

Figure S3. Maximum likelihood tree resulting from IQ-TREE analysis of the 80% completeness matrix using SWSC-EN partitioning scheme. Bootstrap support values are shown next to nodes.

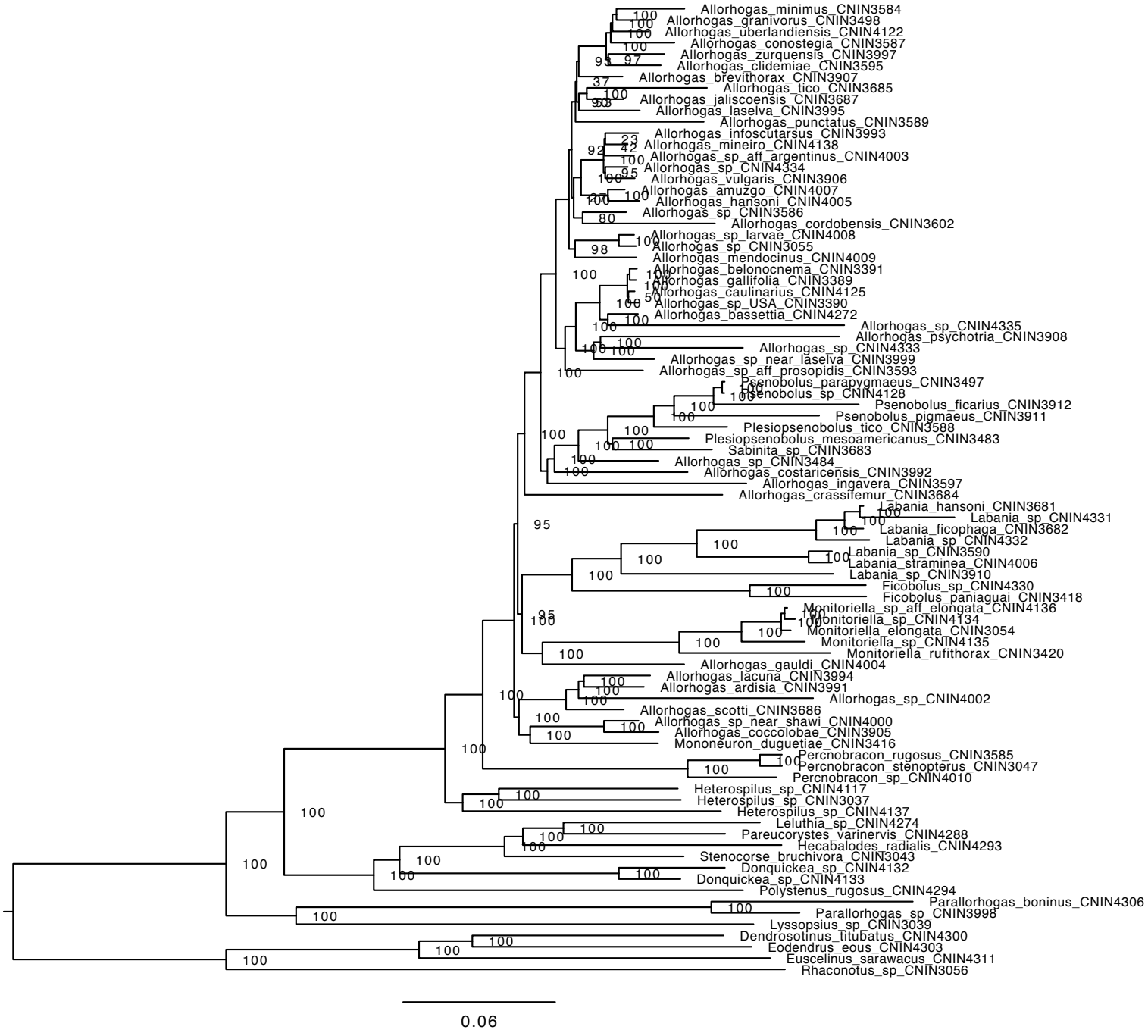

Figure S4. Maximum likelihood tree resulting from IQ-TREE analysis of the 90% completeness matrix using SWSC-EN partitioning scheme. Bootstrap support values are shown next to nodes.

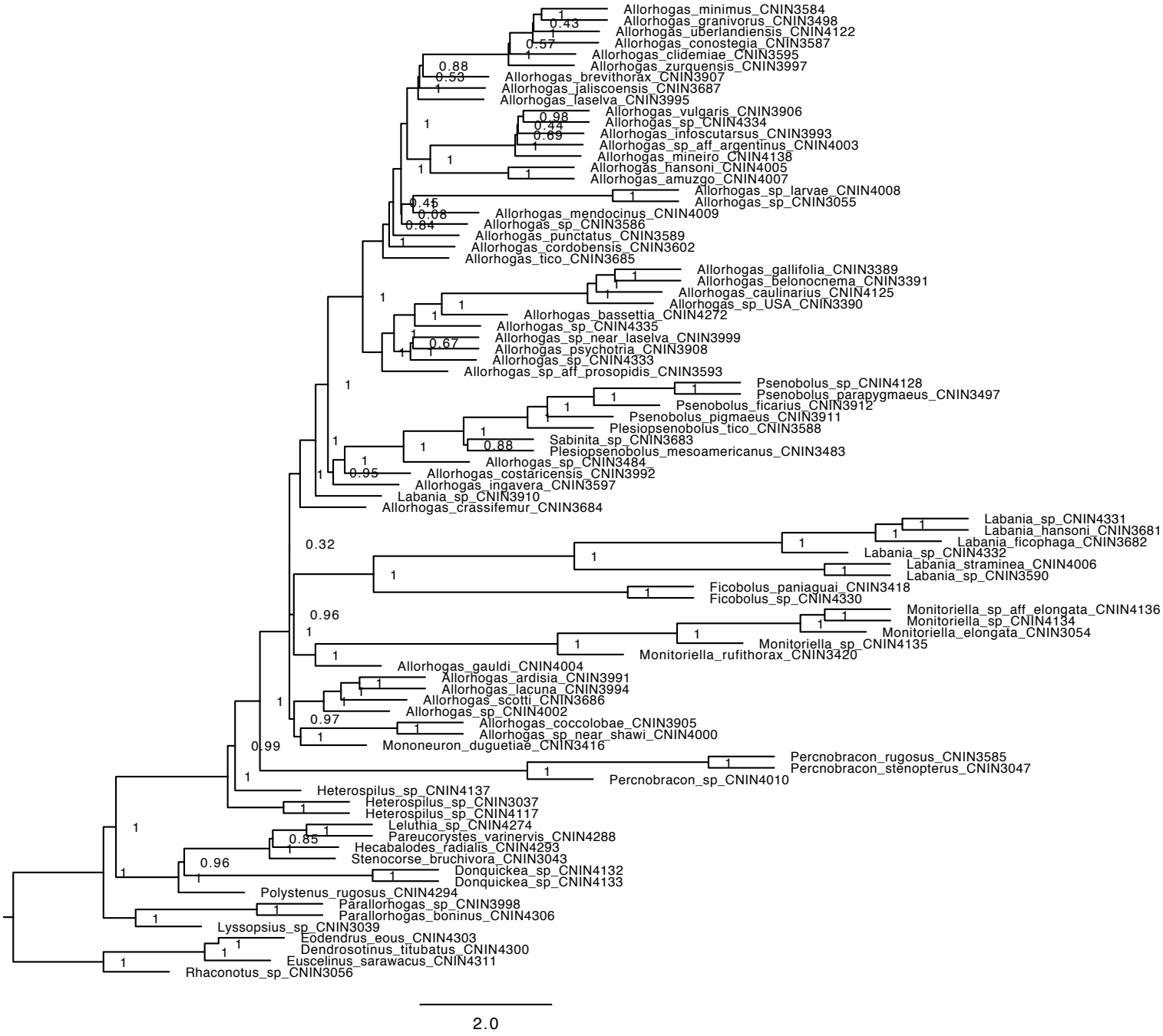

Figure S5. Species tree estimated with ASTRAL-III from 500 UCE gene trees obtained from the 80% matrix.

Local posterior probabilities values are shown next to nodes.

Table S1. List of specimens included in this study including their taxon ID, author, DNA voucher, locality, biological information, and GenBank SRA accession numbers.

Genus	species	Author	Voucher	Location	Date	Plant Family	Plant genus	Plant species	Plant organ	Biology	Notes	SRA accession
Allorhogas	*amuzgo*	Martínez and Zaldívar-Riverón	CNIN4007	Mexico, Oaxaca, Municipio de Pinotepa Nacional	06/25/10	?	?	?	?	?		
Allorhogas	*ardisia*	Marsh	CNIN3991	Costa Rica, Heredia, Puerto Viejo, La Selva	26/01/91	Primulaceae	*Ardisia*	sp.	Leaf	Inquiline/parasitoid?		
Allorhogas	*bassettia*	Samacá-Sáenz et al.	CNIN4272	USA, Florida, Camp Helen	03/18/19 - 04/24/19	Fagaceae	*Quercus*	*geminata*	Stem	Inquiline/parasitoid?	Galls of *Bassettia pallida* (Hymenoptera: Cynipidae)	
Allorhogas	*belonocnema*	Samacá-Sáenz et al.	CNIN3391	USA, Texas, McAllen		Fagaceae	*Quercus*	*virginiana/fusiformis*	Leaf	Inquiline/parasitoid?	Galls of *Belonocnema treatae* (Hymenoptera: Cynipidae)	
Allorhogas	*brevithorax*	Zaldívar-Riverón and Martínez	CNIN3907	Costa Rica, Puntarenas, Monteverde	jul-ago-07	Myrtaceae	*Myrcia*	sp.	Stem	?		
Allorhogas	*caulinarius*	Samacá-Sáenz et al.	CNIN3393	USA, Florida, Archbold	10/12/15 - 10/22/15	Fagaceae	*Quercus*	*geminata*	Stem	Inquiline/parasitoid?	Galls of *Callirhytis quercusbatatoides* (Hymenoptera: Cynipidae)	
Allorhogas	*clidemiae*	Martínez and Zaldívar-Riverón	CNIN3595	Brazil, Minas Gerais, Guaraciaba	08/21/15	Melastomataceae	*Clidemia*	*hirta*	Fruit/seed	Gall former?		
Allorhogas	*coccolobae*	Martínez and Zaldívar-Riverón	CNIN3905	Mexico , Jalisco, Estación de Biología UNAM, Chamela	jun-17	Polygonaceae	*Coccoloba*	*barbadensis*	Leaf	Inquiline/parasitoid?	Galls of Cecidomyiidae (Diptera)	
Allorhogas	*conostegia*	Marsh and Shaw	CNIN3587	Costa Rica, Cartago, Cachí	oct-01	Melastomataceae	*Conostegia*	*xalapensis*	Fruit/seed	Gall former		
Allorhogas	*cordobensis*	Martínez et al	CNIN3602	Argentina, Prov. Bs. As, San Nicolás	2010	Solanaceae	*Lycium*	*vimineum*	Stem	Gall former?		
Allorhogas	*costaricensis*	Marsh	CNIN3992	Costa Rica, Cartago	jun-jul-92	?	?	?	?	?		
Allorhogas	*crassifemur*	Martínez and Zaldívar-Riverón	CNIN3684	Mexico, Jalisco, Estción de Biología UNAM, Chamela, 19.49814, -105.0444	06/23-24/09	?	?	?	?	?		
Allorhogas	*gallifolia*	Samacá-Sáenz et al.	CNIN3388	USA, Texas, Rice University	10/31/14 - 11/10/14	Fagaceae	*Quercus*	*virginiana*	Leaf	Inquiline/parasitoid?	Galls of *Andricus quercuslanigera* (Hymenoptera: Cynipidae)	
Allorhogas	*gauldi*	Marsh	CNIN4004	Costa Rica, Guanacaste, Santa Rosa Nat Park	11/8-29/86	?	?	?	?	?		
Allorhogas	*granivorus*	Zaldívar-Riverón and Martínez	CNIN3498	Brazil, Minas Gerais	8/28/13 - 9/5/13	Melastomataceae	*Miconia*	*calvescens*	Fruit/seed	Gall former		

Allorhogas	*hansoni*	Marsh	CNIN4005	Costa Rica, Alajuela, Palmares		Fabaceae	*Inga*	*vera*	Fruit/seed	Gall former/inquiline?	
Allorhogas	*infuscotarsus*	Marsh	CNIN3993	Costa Rica, Guanacaste, Santa Rosa Nat Park	01/10-31/82	?	?	?	?	?	
Allorhogas	*ingavera*	Marsh	CNIN3597	Costa Rica, San José, UCR campus	sep-09	Fabaceae	*Inga*	*vera*	Leaf	Inquiline/parasitoid?	Galls of Cecidomyiidae (Diptera)
Allorhogas	*jaliscoensis*	Martínez and Zaldívar-Riverón	CNIN3687	Mexico, Jalisco, Estación de Biología UNAM, Chamela, camino Calandria, 19.50496,-105.0377	09/03/09	?	?	?	?	?	
Allorhogas	*lacuna*	Centrella and Shaw	CNIN3994	Costa Rica, Alajuela, San Ramón, Río San Lorencito	jun-88	Melastomataceae	?	?	Leaf	Inquiline/parasitoid?	Not primary gall former (Hansdon pers. comm.).
Allorhogas	*laselva*	Marsh	CNIN3995	Costa Rica, Heredia, La Selva, 10.26, 84.01		?	?	?	?	?	
Allorhogas	*mendocinus*	(Kieffer and Jörgensen)	CNIN4009	Argentina, Provincia de La Pampa, Toay, Lowo Che, -36.654.431, -64.353.256	feb-18	Solanaceae	*Lycium*	*chilense*	Leaf bud	Inquiline/parasitoid?	Galls probably induced by Cecydomiidae (Diptera)
Allorhogas	*mineiro*	Zaldívar-Riverón and Martínez	CNIN3419	Brazil, Minas Gerais, Itumirim, 21.14982, 44.494329	2015	Fabaceae	*Stryphnodendron*	*adstrigens*	Fruit/seed	Phytophagous	
Allorhogas	*minimus*	Centrella and Shaw	CNIN3594	Costa Rica, Heredia, La Selva	12/01/05	Melastomataceae	*Miconia*	*longifolia*	Fruit	Gall former	
Allorhogas	*psychotria*	Zaldívar-Riverón and Martínez	CNIN3908	Costa Rica, Puntarenas, Corcovado, Los Patos	02/05/00	Rubiaceae	*Psychotria*	sp.	Fruit/seed	Phytophagous?	
Allorhogas	*punctatus*	Martínez and Zaldívar-Riverón	CNIN3589	Costa Rica, Cartago, Bartilla NP	feb-01	Asteraceae	*Piptocarpha*	*poeppiggiana*	Leaf	Inquiline/parasitoid?	Galls of Cecidomyiidae (Diptera)
Allorhogas	*scotti*	Martínez and Zaldívar-Riverón	CNIN3686	Mexico, Jalisco, Chamela, camino Buho-Chachalaca, 19.49786, -105.04217	02/25/10	?	?	?	?	?	
Allorhogas	*tico*	Martínez and Zaldívar-Riverón	CNIN3685	Costa Rica, Guanacaste, Palo Verde	01/11/01	Fabaceae	*Coursetia*	*caribea*	Stem	Inquiline/parasitoid?	Galls of Cecidomyiidae (Diptera) or Tanaostigmatidae (Hymenoptera)
Allorhogas	*uberlandiensis*	Joele and Zaldívar-Riverón	CNIN4122	Brazil, Minas Gerais, Uberlandia, Natural Reserve of the Clube Caça e Pesca Itororó,	08/09/18	Melastomataceae	*Miconia*	*chamissois*	Floral bud	Gall former	

				18.5859, -48.1744								
Allorhogas	*vulgaris*	Zaldívar-Riverón and Martínez	CNIN3906	Brazil, Minas Gerais, Luminarias, 21.313, 44.52384	08/07/15	Fabaceae	*Senegalia*	*tenuifolia*	Fruit/seed	Phytophagous		
Allorhogas	*zurquensis*	Marsh	CNIN3997	Costa Rica, San José, Zurquí de Moravia	jul-91	?	?	?	?	?		
Allorhogas	*sp. aff. argentinus*	(Brèthes)	CNIN4003	Costa Rica, Heredia, Prov., Chilamate	10/26/90	Fabaceae	*Pithecellobium*	*macradenium*	Fruit/seed	Phytophagous		
Allorhogas	sp. aff. *prosopidis*		CNIN3593	Argentina, Mendoza, Las Heras, Can. Chacra, -32.5857, 17.6853	01/20-28/13	Fabaceae	*Prosopis*	sp.	Leaf bud	Inquiline/parasitoid?	Galls of *Eschatocerus niger* (Hymenoptera: Cynipidae)	
Allorhogas	sp. near laselva		CNIN3999	Costa Rica, Guanacaste, Santa Rosa Nat Park	10/06/86	?	?	?	?	?		
Allorhogas	sp. near shawi		CNIN4000	Costa Rica, Guanacaste, La Selva	may-94	Malvaceae	*Luehea*	*seemanii*	?	?		
Allorhogas	sp.		CNIN4002	Costa Rica, Limón, PN Braulio Carrillo, Quebrada Gozales	06/09/03	Araceae	*Philodendron*	sp.	Root	?		
Allorhogas	sp.		CNIN4008	Argentina, La Pampa, Sta Rosa, Campo de Enseñanza Universidad de La Pampa, -36.556995, -64.299539	2018	Anacardiaceae	*Schinus*	*fasciculatus*	Stem	Inquiline	Galls *of Cecidoses eremita* (Lepidoptera: Cecidosidae), larvae	
Allorhogas	sp.		CNIN4333	Costa Rica, Heredia, 3 kms de Puerto Viejo		Rubiaceae	*Psychotria*	*bachatria*	Fruit/seed	?		
Allorhogas	sp.		CNIN4334	Costa Rica, Puntarena		Fabaceae	*Inga*	*densifolia*	Fruit/seed	?		
Allorhogas	sp.		CNIN4335	Costa Rica, Cartago		Fagaceae	*Quercus*	*copeyensis*	Stem	?		
Allorhogas	sp.		CNIN3055	Argentina, Mendoza, Villaviciencio, Ruta Provincial Nro 52, -32.525573, -69.011313	2017	Anacardiaceae	*Schinus*	sp.	Stem	Inquiline	Galls of *Eucecidoses* sp. (Lepidoptera: Cecidosidae)	
Allorhogas	sp.		CNIN3390	USA, Florida, Suwannee River		Fagaceae	*Quercus*	*geminata*	Stem	Inquiline/parasitoid?	Galls of *Callirhytis quercusbatatoides* (Hymenoptera: Cynipidae)	
Allorhogas	sp.		CNIN3484	Consta Rica, San José, Zurqí de Moravia	feb-14	Moraceae	*Ficus*	*hartwegii*	Stem	?		
Allorhogas	sp.		CNIN3586	Argentina, La Pampa, Santa Rosa, -36.617515, -64.323929	12/27/10	Fabaceae	*Prosopis*	*caldenia*	Stem	Inquiline/parasitoid?	Galls of *Tetradiplosis panghitruz* (Diptera: Cecidomyiidae)	

			CNIN4132	Colombia, Amazonas, PNN Amacayacu, Matamata		?	?	?	?	?
Donquickeia	sp.				2000					
			CNIN4133	Colombia, Nariño, La Planada Centro Cientifico		?	?	?	?	?
Donquickeia	sp.				2004					
Ficobolus	*paniaguai*	Martínez et al	CNIN3418	Costa Rica, Alajuela	aug-08	Moraceae	*Ficus*	*perforata*	Stem	Gall former?
	sp.		CNIN4330	Costa Rica, Heredia, Estación biologica La Timbirina		Moraceae	*Ficus*	*richteri*	Leaf	?
Ficobolus										
Labania	*ficophaga*	Belokobylskij et al	CNIN3682	Costa Rica, San José, Reserva El Rodeo	apr-11	Moraceae	*Ficus*	*obtusifolia*	Root	Gall former?
Labania	*hansoni*	Marsh	CNIN3681	Costa Rica, Cartago, Tapantí National Refuge	aug-sep-99	Moraceae	*Ficus*	*citricola*	Stem	Gall former?
Labania	*straminea*	Hedqvist	CNIN4006	Costa Rica, San José,Zurquí de Moravia	may-92	Moraceae	*Ficus*	*colubrinae*	Leaf	Gall former?
Labania	sp.		CNIN3590	Mexico , Veracruz, Tantoyuca, Comunidad Tametate	04/19/16	Malvaceae	?	?	?	?
Labania	sp.		CNIN4331	Costa Rica, Puntarenas, Corcovado, Sirena	07/31/00	Moraceae	*Ficus*	sp.		?
Labania	sp.		CNIN4332	Costa Rica, San José, Zurquí de Moravia	nov-13	Moraceae	*Ficus*	*hartwegii*		?
Labania	sp.		CNIN3910	Costa Rica, Turrialtico	sep-13	Moraceae	*Ficus*	*colubrinae*	Leaf	?
Monitoriella	*elongata*	Hedqvist	CNIN3054	Mexico, Chiapas, near Tapachula	2017	Araceae	*Philodendron*	sp.	Leaf	Gall former
Monitoriella	*rufithorax*	Hedqvist	CNIN3420	Mexico, Veracruz, ESTACIÓN DE BIOLOGÍA UNAM, Los Tuxtlas, 18.5824, -95.075	11-31-16	Araceae	*Philodendron*	sp.	Leaf	Gall former?
Monitoriella	*sp. aff. elongata*	Hedqvist	CNIN4136	Costa Rica, Moredia, Pto. Viejo OET La Selva	2002	Araceae	*Philodendron*	*radiatum*	Leaf	Gall former
Monitoriella	sp.		CNIN4134	Costa Rica, Heredia, La Selva	1988	Araceae	*Monstera*	sp.	Leaf	?
Monitoriella	sp.		CNIN4135	French Guyana, Kaw Mountains	2006	?	*?*	?	?	?
Mononeuron	*duguetiae*	Fischer	CNIN3416	Brazil, Sao Paulo, UFSCAR, 21.58796, 47.53968	1-26-11	Annonaceae	*Duguetia*	*furfuraceae*	Leaf	?

Percnobracon	*rugosus*	Martínez	CNIN3585	Argentina, La Pampa, Santa Rosa	10/02/04	Fabaceae	*Prosopis*	*caldenia*	Stem	Inquiline/parasitoid?	Thorn galls of Eurytomidae (Hymenoptera)
Percnobracon	*stenopterus*	Kieffer and Jörgensen	CNIN3047	Argentina, Mendoza, near Gral Alvear	2017	Fabaceae	*Prosopis*	*strombulifera*	Stem	Inquiline/parasitoid?	Galls of Cecidomyiidae (Diptera)
Percnobracon	sp.		CNIN4010	Argentina, La Pampa, Santa Rosa, -36.3892289, -64.19291	27/12/10	Fabaceae	*Prosopis*	*caldenia*		?	
Plesiopsenobolus	*tico*	Belokobylskij et al	CNIN3588	Costa Rica, Alajuela, San Ramón, 10.06 -84.32	01/05/08	Moraceae	*Ficus*	*hemsleyana*	Syconia	Inquiline?	
Plesiopsenobolus	*mesoamericanus*	Belokobylskij et al	CNIN3483	Costa Rica, Alajuela, Sarchí Norte	06/15/08	Moraceae	*Ficus*	*perforata*	Stem	Inquiline??	Collected in the same galls together with *Ficobolus paniaguai*
Psenobolus	*parapygmaeus*	Ramirez and. Marsh	CNIN3497	Mexico, Guerrero, Ceiba mocha	11/24/13	Moraceae	*Ficus*	sp.	Syconia	?	
Psenobolus	*ficarius*	Ramirez and. Marsh	CNIN3912	Costa Rica, San José, Geronimo Moravia, El Tornillal	1992	Moraceae	*Ficus*	*velutina*		?	
Psenobolus	*pygmaeus*	Reinhard	CNIN3911	Costa Rica, Punta, Osa, Rancho Quemado	1994	Moraceae	*Ficus*	sp.	Syconia	?	
Psenobolus	sp.		CNIN4128	Mexico, Oaxaca, Cerca Pinotepa Nacional, Carr 200, 16.37195, 98.177102	11/08/18	?	?	?	?	?	
Sabinita	sp.		CNIN3683	Mexico, Jalisco, Estación de Biología UNAM, Chamela	2009	Moraceae	*Ficus*	sp.	Leaf	?	
OUTGROUP											
Heterospilus	sp.		CNIN3037	Mexico, Jalisco, Estación de Biología UNAM, Chamela	2017						
Heterospilus	sp.		CNIN4117	Brazil, Sao Paulo, Cerrado UFSCAR	27/08/18						
Heterospilus	sp.		CNIN4137	Mexico, Veracruz, Municipio de Actopan, Los Otates	oct-18						
Heterospilus	sp.		CNIN3998	Brazil, Minas Gerais, Sao Carlos, Cerrado UFSCAR	10-29-89						Ex Cerambicidae
Lissopsius	sp.		CNIN3039	Mexico, Oaxaca, Candelaria, Loxicha, Agencia Santiago La	06/19-21/10						

Rhaconotus	sp.		CNIN3056	Galera, 15.96841, -96.47208 Mexico, Jalisco, Chamela, Camino de Terraceria de la estación U de G, a un lado del río Chamela, 19.52307, -105.05093	04/03/08
Stenocorse	bruchivora	(Crawford)	CNIN3043	Mexico, Estado de Mexico, entre Almoloya y La Cumbre, cerca de km 134 sobre 27 de septiembre, 18.945207, 100.13396	2017
Leluthia	sp.		CNIN4274	Mexico, Coahuila, El Campo, Parque la Mora	15/10/09
Pareucorystes	*varinervis*	Tobias	CNIN4288	Russia, Primorskiy Territory, 20 km E of Ussuriysk, Gornotayozhnoe	26/08/99
Hecabalodes	*radialis*	Tobias	CNIN4293	Turkmenistan, Repetek Settlement	
Polystenus	*rugosus*	Foerster	CNIN4294	Russia, Primorskiy Territory, 20 km SE of Ussuriysk, forest, clearing	04/08/91
Dendrosotinus	*titubatus*	Papp	CNIN4300	Tunisia, Hammamet Mrezgue	18/10/07
Eodendrus	*eous*	Belokobylskij	CNIN4303	Japan, Honshu, Hyogo Pr. Kobe, Rokko Mys. Msya Mt. Forest	28/08/05
Parallorhogas	*boninus*	Belokobylskij & Maeto	CNIN4306	Japan, Ogasawara ls. Hahajima L., Sekimon	21/07/98
Euscelinus	*sarawacus*	Westwood	CNIN4311	Mexico, Jalisco, Chamela, Camino de Terraceria de la estación U de G, a un lado del río Chamela, 19.52307, -105.05093	03/04/18

Table S2. Results extracted from the summarized frequencies file of BaCoCa test performed for the 60% (1,082 loci) data set. First 47 loci deviating significantly ($p < 0.05$) from base composition homogeneity were discarded for the consequent analyses.

UCE locus	Chi-square value	Degrees of freedom	p-value
uce10457	484.45166	228	2.17E-20
uce976	369.70093	156	3.7553E-19
uce1503	459.21815	234	1.1254E-16
uce1442	440.43824	231	3.9502E-15
uce1257	405.41838	222	8.4233E-13
uce239	396.66243	222	6.0834E-12
uce399	389.6409	219	1.2053E-11
uce573	288.83563	165	9.121E-09
uce1009	328.11553	204	8.3019E-08
uce12633	279.13497	168	1.6385E-07
uce1048	269.67185	162	2.3755E-07
uce1240	322.52325	210	9.7222E-07
uce642	335.96109	231	7.8458E-06
uce1244	319.43046	228	0.000061119
uce844	316.34915	228	0.00009728
uce680	305.10813	225	0.00030012
uce950	257.35172	186	0.00041654
uce139	312.59152	234	0.00045135
uce10145	222.57138	159	0.00066162
uce147	255.86636	189	0.00086608
uce936	307.27606	234	0.00091821
uce855	267.78125	207	0.0028234
uce1386	233.36321	177	0.0028929
uce1317	283.2851	222	0.0033925
uce1032	282.00127	222	0.0039613
uce333	278.21276	219	0.0041614
uce216	246.71843	192	0.0047092
uce11407	212.0155	165	0.0079374
uce1034	277.67969	225	0.0095696

uce872	283.44195	231	0.010542
uce1156	257.08972	210	0.014734
uce11963	205.49218	165	0.017655
uce4	280.67406	234	0.019751
uce959	256.8769	213	0.021337
uce11691	210.34047	171	0.021779
uce13	280.55519	237	0.027443
uce577	283.525	240	0.02821
uce10936	207.79585	171	0.02885
uce12301	201.07651	165	0.029161
uce618	254.98221	216	0.035546
uce1170	274.43688	234	0.035803
uce12910	198.14589	165	0.039957
uce1366	269.33881	231	0.042334
uce11629	197.43094	165	0.043053
uce10528	216.05598	183	0.047658
uce1331	258.06942	222	0.048632
uce200	257.94251	222	0.049184
uce985	262.19058	228	0.059572
uce141	176.63851	150	0.067658
uce478	227.46971	198	0.074023
uce1175	210.97822	183	0.076572
uce12178	191.14631	165	0.079856
uce427	257.66389	228	0.086264
uce11397	186.89764	162	0.087729
uce1459	246.57297	219	0.097209
uce11658	188.639	165	0.10021
uce1441	264.89741	237	0.10298
uce12369	178.39646	156	0.10575
uce1304	245.47195	219	0.10584
uce11916	190.67585	168	0.11093
uce823	253.85487	228	0.11528
uce10	257.02185	231	0.11529
uce1099	259.82389	234	0.11838
uce1236	230.86279	207	0.12238
uce922	170.04916	150	0.12549
uce11717	201.921	180	0.12579

uce868	185.52755	165	0.13075
uce10847	184.9405	165	0.13722
uce1026	262.65181	240	0.1507
uce10554	186.82147	168	0.1522
uce10424	186.60095	168	0.15486
uce12956	182.97878	165	0.16048
uce10926	179.79763	162	0.16069
uce607	239.3314	219	0.16485
uce1063	229.64563	210	0.16785
uce10339	169.06959	153	0.17713
uce550	249.9599	231	0.18673
uce12491	163.7096	150	0.20982
uce291	247.42541	231	0.21835
uce12886	183.94286	171	0.23608
uce1353	251.49911	237	0.24716
uce10093	166.99835	156	0.25904
uce1167	235.01157	222	0.26191
uce686	249.75917	237	0.27211
uce654	240.41721	228	0.27343
uce1093	242.48395	231	0.28887
uce1046	235.96574	225	0.29454
uce189	247.55265	237	0.30562
uce10607	176.04115	168	0.31981
uce860	243.10236	234	0.32771
uce10464	178.42553	171	0.33291
uce800	239.12757	231	0.34281
uce194	229.76109	222	0.34614
uce1055	238.49328	231	0.35348
uce11177	192.11222	186	0.3639
uce12954	158.30707	153	0.36766
uce1288	240.52219	234	0.37087
uce330	230.90737	225	0.37923
uce595	227.52839	222	0.3852
uce1443	242.52999	237	0.38871
uce1134	223.98377	219	0.39427
uce473	235.85319	231	0.39927
uce382	241.89624	237	0.39975

uce688	232.31175	228	0.40832
uce10061	152.89592	150	0.41893
uce11425	170.3236	168	0.43544
uce1172	233.74192	231	0.4372
uce363	230.51337	228	0.44098
uce10068	173.05953	171	0.44158
uce851	193.80025	192	0.45002
uce336	235.97493	234	0.45151
uce812	235.92091	234	0.45249
uce1291	208.71233	207	0.45355
uce697	232.76246	231	0.45509
uce963	202.4609	201	0.45782
uce1143	232.45343	231	0.46077
uce497	204.37614	204	0.47942
uce1391	224.89226	225	0.48949
uce11666	173.78354	174	0.49038
uce10394	159.70805	162	0.53621
uce881	197.85762	201	0.54946
uce634	218.50113	222	0.55381
uce809	215.34494	219	0.55716
uce761	146.64153	150	0.56233
uce584	217.52052	222	0.57236
uce441	223.82465	231	0.62032
uce683	226.63517	234	0.62296
uce10074	164.27041	171	0.63033
uce12635	193.64859	201	0.63231
uce741	231.2801	240	0.64524
uce643	219.18694	228	0.65059
uce1253	224.867	234	0.65431
uce150	205.68039	216	0.68173
uce448	143.99052	153	0.68701
uce197	207.849	219	0.69507
uce10470	157.78478	168	0.70285
uce1111	180.96154	192	0.70555
uce152	206.82259	219	0.71265
uce158	171.45186	183	0.7197
uce1482	203.38173	216	0.72148

uce857	182.88966	195	0.72328
uce641	223.50016	237	0.72628
uce10719	159.32965	171	0.72905
uce64	225.9621	240	0.73343
uce400	211.08618	225	0.73842
uce974	196.13916	210	0.74514
uce693	198.65287	213	0.75144
uce1171	221.60061	237	0.75572
uce163	154.46027	168	0.76518
uce1061	188.75523	204	0.77076
uce12608	162.67412	177	0.77262
uce1282	214.20228	231	0.77942
uce1190	219.96899	237	0.77969
uce188	208.17628	225	0.78298
uce46	231.2715	249	0.78354
uce12358	139.02092	153	0.78426
uce236	225.32082	243	0.78581
uce1496	199.29078	216	0.78626
uce463	210.06084	228	0.79727
uce12179	138.26966	153	0.79745
uce1207	212.85859	231	0.79845
uce1080	192.19718	210	0.80564
uce10756	137.03666	153	0.81811
uce598	222.27626	243	0.82585
uce10595	162.02386	180	0.82785
uce876	193.16069	213	0.83157
uce10100	138.97462	156	0.83235
uce1067	218.65162	240	0.83507
uce789	215.34621	237	0.84035
uce10974	137.97074	156	0.84732
uce1064	208.68938	231	0.85136
uce1420	185.78927	207	0.85256
uce11633	146.05508	165	0.85288
uce1027	214.21404	237	0.85347
uce10942	157.16606	177	0.8556
uce10821	154.31564	174	0.85587
uce213	159.94091	180	0.85633

uce11746	162.72531	183	0.85694
uce449	148.51487	168	0.85783
uce1015	190.96257	213	0.8587
uce672	224.21367	249	0.86861
uce10801	153.20302	174	0.87007
uce345	177.80214	201	0.87927
uce11925	129.7053	150	0.88306
uce626	188.42668	213	0.88633
uce747	207.80329	234	0.89034
uce11672	162.58795	186	0.89142
uce10366	128.82855	150	0.89368
uce356	139.63884	162	0.89747
uce880	201.21514	228	0.89897
uce1033	195.54312	222	0.89927
uce1144	206.79391	234	0.89954
uce11415	142.21977	165	0.89971
uce12966	155.81766	180	0.9034
uce12	186.53242	213	0.90442
uce531	200.50728	228	0.9052
uce1094	203.23143	231	0.90598
uce11441	132.70035	156	0.91198
uce562	208.13408	237	0.91198
uce10073	152.11935	177	0.91217
uce1213	188.35425	216	0.91296
uce430	199.54805	228	0.91321
uce1124	196.67429	225	0.91374
uce366	190.98749	219	0.91437
uce11716	160.19639	186	0.91472
uce10928	129.64342	153	0.91488
uce620	196.50395	225	0.91512
uce11311	140.10476	165	0.9206
uce346	201.11208	231	0.9228
uce10660	158.76058	186	0.92687
uce340	155.84057	183	0.92806
uce1216	200.24946	231	0.92898
uce1096	136.14758	162	0.93099
uce958	185.63453	216	0.93361

uce1377	202.03837	234	0.93568
uce928	138.10101	165	0.93732
uce10175	148.92866	177	0.93861
uce945	190.12762	222	0.94059
uce1246	195.46241	228	0.94195
uce67	211.88312	246	0.94354
uce927	197.85556	231	0.94422
uce885	203.35745	237	0.94455
uce265	199.7146	234	0.94933
uce12664	130.93251	159	0.94944
uce864	204.97118	240	0.95085
uce253	204.00252	240	0.95566
uce1095	198.30191	234	0.95647
uce11784	126.97246	156	0.95725
uce57	162.29033	195	0.95773
uce997	194.63801	231	0.96064
uce11722	153.21919	186	0.96213
uce10177	147.74745	180	0.96228
uce11592	123.35682	153	0.96242
uce901	204.44133	243	0.96566
uce1440	168.53088	204	0.96681
uce135	184.7838	222	0.96738
uce1238	198.43523	237	0.96759
uce143	198.09854	237	0.96884
uce11376	118.86337	150	0.9713
uce511	191.80911	231	0.9717
uce12860	120.91392	153	0.97392
uce313	193.59205	234	0.97478
uce11345	144.60137	180	0.97548
uce12176	125.47295	159	0.97687
uce1510	146.82387	183	0.97706
uce10201	146.29724	183	0.97874
uce12326	143.38178	180	0.97947
uce144	121.88451	156	0.98004
uce288	161.8873	201	0.98029
uce650	172.43702	213	0.98091
uce33	166.98	207	0.98108

uce10818	116.12963	150	0.98151
uce409	116.04581	150	0.98177
uce12979	118.46411	153	0.98243
uce12989	126.27205	162	0.98278
uce10831	115.28143	150	0.98399
uce362	120.17476	156	0.98497
uce947	183.89994	228	0.9855
uce185	119.9394	156	0.98556
uce96	183.66779	228	0.98596
uce1287	175.52615	219	0.98611
uce1395	186.06978	231	0.98651
uce132	191.34219	237	0.98671
uce11275	140.50516	180	0.98681
uce48	164.44867	207	0.98682
uce1098	169.55946	213	0.98727
uce12407	124.45402	162	0.98728
uce624	185.41367	231	0.98768
uce10412	132.02605	171	0.98795
uce1400	128.93081	168	0.98886
uce544	176.53542	222	0.98907
uce1283	149.88241	192	0.98913
uce10283	128.75443	168	0.98919
uce11763	115.49489	153	0.98955
uce1315	149.3661	192	0.98999
uce293	186.54289	234	0.99011
uce1237	165.18639	210	0.99013
uce12003	122.90616	162	0.9903
uce416	135.98086	177	0.99033
uce35	173.00074	219	0.99038
uce1308	162.33553	207	0.99042
uce12889	140.71804	183	0.99114
uce879	148.56094	192	0.99122
uce11115	140.58886	183	0.99133
uce10663	135.16615	177	0.99159
uce172	184.486	234	0.99269
uce12126	121.11378	162	0.99301
uce37	160.2736	207	0.99309

uce1286	176.0768	225	0.99316
uce1421	188.35182	240	0.99413
uce335	185.17448	237	0.99458
uce12766	122.36132	165	0.99458
uce10447	130.09016	174	0.99461
uce993	119.60995	162	0.99476
uce905	192.84254	246	0.99482
uce1416	187.45654	240	0.99488
uce12857	142.74113	189	0.99493
uce74	181.98831	234	0.99502
uce12680	127.00142	171	0.9951
uce1159	184.43042	237	0.99518
uce1235	186.99024	240	0.99524
uce455	165.48298	216	0.99557
uce1476	178.3501	231	0.99576
uce205	175.48443	228	0.99592
uce917	185.92754	240	0.99598
uce170	188.52589	243	0.99601
uce10646	112.81991	156	0.99627
uce10930	110.21922	153	0.99631
uce12603	122.95623	168	0.99633
uce12717	112.43931	156	0.99655
uce412	147.83145	198	0.9969
uce1052	184.19329	240	0.99697
uce271	127.12499	174	0.99697
uce1497	162.95754	216	0.99714
uce916	137.00643	186	0.99718
uce1199	128.7624	177	0.99748
uce12193	120.74747	168	0.99767
uce10099	110.45403	156	0.99776
uce11766	112.81155	159	0.99784
uce10828	117.81789	165	0.99786
uce783	140.2951	192	0.99804
uce12171	109.6185	156	0.99815
uce12123	129.73906	180	0.99818
uce521	104.46779	150	0.99821
uce632	165.46839	222	0.99824

uce12938	114.1913	162	0.99833
uce588	172.6893	231	0.9984
uce3	123.49914	174	0.99859
uce459	166.83331	225	0.9986
uce1352	171.76638	231	0.99865
uce1274	176.95826	237	0.99865
uce10633	112.95829	162	0.99874
uce11605	110.37911	159	0.99876
uce187	127.40115	180	0.9989
uce59	170.43443	231	0.99895
uce1004	175.47651	237	0.99897
uce1016	164.96403	225	0.99902
uce1325	147.08916	204	0.99902
uce11683	109.34859	159	0.99903
uce114	154.43468	213	0.99908
uce11634	116.56519	168	0.99909
uce1481	159.37499	219	0.99911
uce1191	177.12559	240	0.99914
uce11091	123.49826	177	0.9992
uce10955	113.34586	165	0.99924
uce10616	120.65794	174	0.99926
uce785	173.63552	237	0.99928
uce1211	175.9846	240	0.99931
uce1023	173.35802	237	0.99932
uce1150	152.92998	213	0.99932
uce10849	127.41479	183	0.99938
uce11487	107.58417	159	0.99938
uce12757	105.04752	156	0.99939
uce820	134.52869	192	0.99943
uce733	157.06017	219	0.99945
uce149	172.07395	237	0.99947
uce990	149.17739	210	0.99948
uce12245	104.35765	156	0.99949
uce1206	181.92841	249	0.99951
uce10743	101.64196	153	0.99953
uce710	148.61838	210	0.99954
uce869	171.22129	237	0.99956

uce10190	101.28982	153	0.99957
uce12217	108.59302	162	0.99957
uce780	108.51588	162	0.99958
uce12512	110.43597	165	0.99963
uce10141	110.48432	165	0.99963
uce168	162.71194	228	0.99963
uce12137	107.95636	162	0.99964
uce1031	167.47057	234	0.99965
uce435	154.43295	219	0.99969
uce1217	158.89863	225	0.99972
uce446	136.44662	198	0.99972
uce1128	163.7636	231	0.99973
uce1029	158.83055	225	0.99973
uce609	168.6701	237	0.99974
uce10356	106.72966	162	0.99974
uce728	145.3947	210	0.99978
uce594	170.47656	240	0.99978
uce10819	108.28116	165	0.99979
uce858	108.41296	165	0.99979
uce453	167.27724	237	0.99981
uce1194	174.66799	246	0.99981
uce1227	158.81561	228	0.99984
uce1484	116.74034	177	0.99985
uce1430	166.11523	237	0.99985
uce799	163.62498	234	0.99985
uce11144	102.40357	159	0.99985
uce228	116.5185	177	0.99986
uce625	150.68972	219	0.99987
uce10482	101.73304	159	0.99987
uce1301	175.3872	249	0.99987
uce1181	145.74537	213	0.99987
uce10304	103.84403	162	0.99988
uce979	94.45084	150	0.99988
uce907	157.14247	228	0.99989
uce1070	115.53429	177	0.99989
uce904	147.45786	216	0.99989
uce12735	108.17533	168	0.9999

uce10288	110.61343	171	0.9999
uce1429	146.6826	216	0.99991
uce38	139.2509	207	0.99991
uce877	124.15649	189	0.99992
uce11264	107.00316	168	0.99993
uce11326	92.76785	150	0.99993
uce689	155.38902	228	0.99993
uce999	165.39788	240	0.99993
uce530	116.00497	180	0.99994
uce11936	96.84471	156	0.99994
uce11224	92.0405	150	0.99994
uce1359	152.67964	225	0.99994
uce12661	92.08811	150	0.99994
uce797	149.93494	222	0.99994
uce86	142.59314	213	0.99994
uce11027	108.33301	171	0.99995
uce10202	98.54221	159	0.99995
uce1490	166.54611	243	0.99995
uce815	113.08372	177	0.99995
uce1342	161.15935	237	0.99995
uce1380	154.35084	228	0.99995
uce871	154.28437	228	0.99995
uce10033	98.39636	159	0.99996
uce639	136.451	207	0.99996
uce11399	105.31745	168	0.99996
uce791	143.15393	216	0.99996
uce914	168.38125	246	0.99996
uce1097	160.42937	237	0.99996
uce553	154.38036	231	0.99997
uce10053	109.02019	174	0.99997
uce862	161.39347	240	0.99997
uce631	111.4448	177	0.99997
uce568	140.23279	213	0.99997
uce11685	93.55194	156	0.99998
uce1305	98.12567	162	0.99998
uce11631	102.07677	168	0.99998
uce10414	102.86895	168	0.99998

uce796	142.44409	219	0.99998
uce1079	162.7038	243	0.99998
uce11220	107.14362	174	0.99998
uce1260	145.75357	222	0.99998
uce9	162.1625	243	0.99998
uce365	155.4363	234	0.99998
uce1403	155.78041	234	0.99998
uce10054	94.73065	162	0.99999
uce12554	99.01308	168	0.99999
uce10891	87.37749	150	0.99999
uce10029	98.01762	165	0.99999
uce10442	105.87519	174	0.99999
uce657	156.51766	237	0.99999
uce1279	140.42098	219	0.99999
uce242	153.44147	234	0.99999
uce162	106.06973	177	0.99999
uce10704	103.20498	171	0.99999
uce10502	100.44233	168	0.99999
uce537	150.33822	231	0.99999
uce12578	100.41315	168	0.99999
uce10216	110.73109	180	0.99999
uce835	129.69994	207	0.99999
uce251	100.81279	168	0.99999
uce10701	87.12272	150	0.99999
uce36	129.18355	207	0.99999
uce12992	108.82123	180	0.99999
uce295	146.22435	228	0.99999
uce681	150.6749	231	0.99999
uce1268	144.20193	222	0.99999
uce10008	104.55579	174	0.99999
uce1019	142.03193	219	0.99999
uce10413	107.61803	177	0.99999
uce181	163.98934	246	0.99999
uce263	160.59844	246	0.99999
uce11744	116.52887	189	0.99999
uce653	122.58249	198	0.99999
uce11535	107.204	177	0.99999

uce1062	143.39181	225	0.99999
uce700	143.4727	222	0.99999
uce23	140.70091	219	0.99999
uce100	131.41559	231	1
uce1269	121.82126	234	1
uce604	96.28929	198	1
uce10429	76.2859	162	1
uce10529	62.18701	165	1
uce613	104.52679	240	1
uce192	91.92581	225	1
uce275	120.01082	234	1
uce10725	76.02289	174	1
uce171	80.72967	234	1
uce11123	55.91838	150	1
uce106	109.33838	222	1
uce71	89.60681	240	1
uce1102	126.78789	216	1
uce12062	62.47721	174	1
uce177	78.01743	186	1
uce1071	117.21134	225	1
uce11461	66.99243	177	1
uce12373	87.57136	177	1
uce956	107.32704	240	1
uce12623	62.48849	153	1
uce11465	96.44137	165	1
uce12100	58.12273	168	1
uce902	123.81796	234	1
uce10199	59.81463	162	1
uce803	140.97974	243	1
uce11831	58.85866	150	1
uce12983	85.76421	168	1
uce11197	46.2262	159	1
uce404	130.39524	228	1
uce1125	63.08837	183	1
uce11914	68.17213	162	1
uce442	119.37219	213	1
uce674	95.88356	195	1

uce546	112.77758	219	1
uce12085	42.25378	174	1
uce12319	68.08738	150	1
uce500	135.22195	222	1
uce11296	51.7625	153	1
uce12300	59.84828	168	1
uce1133	161.01995	252	1
uce434	131.32836	237	1
uce1330	141.20212	225	1
uce10392	37.25559	156	1
uce11474	46.10192	165	1
uce645	83.21131	237	1
uce12935	63.49915	165	1
uce260	116.18923	213	1
uce12229	82.87796	165	1
uce1177	118.68563	198	1
uce11329	54.75975	159	1
uce10775	74.58379	171	1
uce658	66.30168	228	1
uce1078	113.1379	222	1
uce541	117.3718	237	1
uce1038	102.91822	243	1
uce12457	68.86918	156	1
uce1020	129.82734	234	1
uce11395	89.34076	159	1
uce11525	95.4673	180	1
uce12529	77.87639	150	1
uce1234	93.44467	228	1
uce870	115.42054	228	1
uce559	120.94518	225	1
uce11714	90.82604	159	1
uce10767	64.23546	156	1
uce1047	100.55885	210	1
uce12875	83.74558	177	1
uce32	141.70657	249	1
uce784	45.05432	150	1
uce1138	92.54978	204	1

uce10468	80.97426	159	1
uce669	75.9053	168	1
uce11752	25.27547	153	1
uce122	97.06095	210	1
uce495	51.46692	207	1
uce660	128.87162	222	1
uce1418	109.71487	234	1
uce11470	78.58326	156	1
uce557	109.70421	225	1
uce69	138.71424	222	1
uce12297	85.30647	153	1
uce169	99.421	234	1
uce212	115.60912	234	1
uce1193	135.45668	234	1
uce1241	127.26215	237	1
uce10727	42.57922	162	1
uce11965	83.03759	159	1
uce1035	95.17879	231	1
uce11971	71.05211	150	1
uce98	105.15754	240	1
uce1337	94.528	234	1
uce695	133.53944	225	1
uce1050	127.18037	222	1
uce793	108.03179	219	1
uce11648	41.25256	153	1
uce1340	109.37576	243	1
uce1139	107.65159	192	1
uce635	110.17515	222	1
uce1278	143.11091	228	1
uce12117	76.53747	162	1
uce11421	54.33856	162	1
uce706	129.11492	231	1
uce915	131.94581	240	1
uce513	116.94149	237	1
uce525	133.91655	234	1
uce12812	89.6532	168	1
uce1306	81.49	186	1

uce11410	46.78265	150	1
uce10794	48.88517	162	1
uce11303	59.45967	156	1
uce10742	74.13765	177	1
uce234	89.8799	222	1
uce1303	113.71915	219	1
uce11740	78.08154	168	1
uce12553	70.99971	156	1
uce12408	83.24846	150	1
uce1392	63.29075	222	1
uce270	97.54619	228	1
uce11045	70.00204	153	1
uce443	75.11354	219	1
uce241	123.05392	234	1
uce10991	63.27082	168	1
uce10063	70.52663	150	1
uce1076	133.76388	234	1
uce47	100.97559	216	1
uce331	86.67062	198	1
uce845	103.47527	225	1
uce34	131.23765	231	1
uce468	107.85782	213	1
uce1013	116.48826	231	1
uce243	73.17336	225	1
uce555	79.50852	168	1
uce10791	64.68382	168	1
uce1379	78.15192	195	1
uce1285	151.34417	237	1
uce11741	98.1258	180	1
uce519	94.35047	237	1
uce1393	119.08521	231	1
uce277	67.04604	171	1
uce1292	104.53455	219	1
uce501	80.23213	234	1
uce276	139.46215	222	1
uce10184	107.58974	198	1
uce1239	126.99889	234	1

uce1014	115.68694	216	1
uce570	79.53841	192	1
uce1092	76.17701	204	1
uce24	98.2418	240	1
uce805	66.13972	222	1
uce252	142.36352	225	1
uce1348	101.7836	204	1
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uce12470	50.00595	165	1
uce39	148.2605	234	1
uce208	74.41814	186	1
uce1202	104.14146	213	1
uce10432	87.43564	162	1
uce882	132.37692	228	1
uce485	104.43484	213	1
uce153	74.58503	198	1
uce520	108.21305	234	1
uce1104	108.9071	189	1
uce161	107.94234	207	1
uce1259	122.46677	213	1
uce758	134.99111	234	1
uce1140	104.45594	186	1
uce10901	78.2546	177	1
uce20	89.39339	204	1
uce44	94.79537	234	1
uce1447	121.97497	234	1
uce1109	127.9302	234	1
uce10913	67.3689	153	1
uce1209	57.23258	240	1
uce10411	69.62689	168	1
uce861	116.10159	231	1
uce68	81.20911	204	1
uce707	137.44505	225	1
uce12111	86.69586	162	1
uce843	135.0422	219	1
uce10360	96.99203	174	1
uce10459	81.63758	165	1

uce125	108.73399	192	1
uce350	87.02567	207	1
uce12970	53.15238	153	1
uce1024	156.10932	243	1
uce240	96.08901	201	1
uce12996	83.62149	150	1
uce82	125.11796	243	1
uce721	87.08264	219	1
uce965	68.80453	240	1
uce437	90.80428	183	1
uce12282	54.93797	168	1
uce1463	67.53215	225	1
uce1495	35.2571	150	1
uce1499	144.55371	231	1
uce565	45.26932	222	1
uce278	102.40308	228	1
uce1419	38.26211	222	1
uce560	100.92173	234	1
uce1010	89.33304	165	1
uce587	129.03192	231	1
uce348	124.23705	207	1
uce633	87.60413	222	1
uce11787	68.54548	186	1
uce676	99.92144	213	1
uce1037	104.41667	222	1
uce1335	115.93715	228	1
uce1122	128.92583	225	1
uce1173	133.06892	231	1
uce12509	54.26593	153	1
uce483	85.56764	177	1
uce1127	136.29863	234	1
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uce1176	72.09564	198	1
uce508	129.78353	237	1
uce1204	111.02388	195	1
uce1281	90.38336	159	1
uce10598	32.49538	156	1

uce11442	74.97062	159	1
uce816	97.32852	207	1
uce847	139.2432	231	1
uce475	63.70722	216	1
uce913	107.1118	240	1
uce617	117.46246	225	1
uce237	141.02535	228	1
uce113	133.19302	213	1
uce65	60.57538	237	1
uce11418	70.60731	156	1
uce10559	31.70719	174	1
uce702	131.85661	225	1
uce10225	44.10992	156	1
uce12614	42.43266	168	1
uce12294	83.35871	168	1
uce554	63.46619	240	1
uce10357	53.57974	156	1
uce11238	85.91665	159	1
uce698	140.23284	240	1
uce518	129.89171	225	1
uce12636	74.80356	156	1
uce506	88.07672	192	1
uce505	116.92211	234	1
uce10403	74.0019	162	1
uce1466	85.94718	171	1
uce610	134.06514	240	1
uce552	109.91185	234	1
uce1011	58.99801	240	1
uce438	97.57149	219	1
uce487	140.59927	243	1
uce95	119.2271	231	1
uce1168	146.70767	231	1
uce694	100.35319	186	1
uce10254	72.39186	165	1
uce1434	79.30067	237	1
uce1145	125.47676	213	1
uce7	92.36696	240	1

uce12733	62.42062	150	1
uce10758	53.25715	162	1
uce516	78.10093	243	1
uce734	92.96214	243	1
uce600	100.48629	234	1
uce11526	72.34553	165	1
uce12290	66.70986	150	1
uce10182	39.56388	165	1
uce978	82.77996	186	1
uce11085	66.64295	162	1
uce50	106.30524	234	1
uce12669	58.20814	171	1
uce649	145.61005	243	1
uce12580	74.63157	159	1
uce12943	59.313	165	1
uce30	159.05277	246	1
uce1232	156.12532	249	1
uce1415	95.80326	210	1
uce1339	78.37877	204	1
uce1462	45.1733	219	1
uce10330	73.80772	168	1
uce819	142.38958	237	1
uce12565	70.21438	162	1
uce10352	63.16957	156	1
uce12052	84.19965	168	1
uce1374	100.18253	240	1
uce1245	102.2608	213	1
uce701	121.15784	240	1
uce12238	79.57287	153	1
uce12545	121.43861	234	1
uce1008	85.08127	216	1
uce842	127.55116	234	1
uce233	126.78527	234	1
uce1112	124.34114	225	1
uce12033	82.30174	150	1
uce11944	46.7169	150	1
uce12793	79.18789	150	1

uce1174	98.44139	231	1
uce10579	50.13455	156	1
uce10803	65.3047	153	1
uce1258	91.40291	234	1
uce145	111.14974	219	1
uce491	39.51956	153	1
uce12679	67.89424	159	1
uce746	118.88787	228	1
uce10185	81.59737	156	1
uce1414	115.41507	198	1
uce58	90.29389	213	1
uce349	135.31971	237	1
uce949	104.88467	234	1
uce12790	59.0427	153	1
uce10089	78.0819	153	1
uce27	129.69055	228	1
uce1091	76.99762	156	1
uce507	46.2132	174	1
uce391	122.51312	240	1
uce646	130.43035	237	1
uce11382	37.30597	153	1
uce381	139.61789	225	1
uce10235	31.8507	162	1
uce316	82.57098	222	1
uce1296	123.06951	201	1
uce1384	82.35585	228	1
uce11909	82.65536	156	1
uce1297	140.42885	222	1
uce12228	55.84445	153	1
uce484	124.01364	213	1
uce88	120.74765	231	1
uce11687	87.14317	162	1
uce402	103.87272	201	1
uce558	89.89787	225	1
uce929	103.52518	216	1
uce987	132.92473	231	1
uce255	140.7679	240	1

uce1464	132.59278	234	1
uce1082	77.74421	162	1
uce42	60.46473	195	1
uce130	132.80538	252	1
uce12384	39.07342	150	1
uce1431	112.18072	216	1
uce12736	70.12244	162	1
uce1457	78.17138	249	1
uce10345	69.73763	150	1
uce1065	143.71505	228	1
uce12535	76.27893	168	1
uce244	88.74642	237	1
uce806	109.85233	213	1
uce723	107.94833	228	1
uce129	83.02541	162	1
uce137	97.31064	213	1
uce115	108.41244	231	1
uce11585	72.67263	165	1
uce326	117.5038	216	1
uce12656	67.51291	156	1
uce10231	77.06613	168	1
uce621	98.49101	228	1
uce11454	96.30164	165	1
uce848	132.44043	234	1
uce12589	44.69332	156	1
uce18	129.27502	225	1
uce1338	83.86108	183	1
uce12275	86.7151	195	1
uce269	127.86929	237	1
uce946	127.00899	225	1
uce873	109.34294	240	1
uce547	95.40418	222	1
uce768	84.67132	231	1
uce12540	100.3232	174	1
uce968	107.64169	198	1
uce529	52.05144	207	1
uce11070	91.34766	159	1

uce11087	69.59272	165	1
uce509	98.07808	234	1
uce1438	98.08229	210	1
uce11083	58.99477	156	1
uce743	65.87366	162	1
uce388	95.23714	168	1
uce301	112.64847	210	1
uce12514	42.84643	153	1
uce11695	51.85815	162	1
uce1075	120.18461	216	1
uce1006	126.5727	234	1
uce390	131.24766	240	1
uce1208	115.15045	219	1
uce10469	47.42952	153	1
uce10340	90.96934	174	1
uce1452	135.9463	234	1
uce10896	77.84757	162	1
uce910	131.36683	243	1
uce12108	54.5241	168	1
uce822	135.8636	228	1
uce11922	90.63469	159	1
uce11489	83.11811	174	1
uce691	133.66879	216	1
uce99	114.01902	234	1
uce11617	48.39577	165	1
uce1263	76.65375	162	1
uce347	138.72915	228	1
uce888	118.65807	201	1
uce220	98.04975	228	1
uce157	95.38128	168	1
uce10344	29.79968	165	1
uce31	85.46987	204	1
uce517	93.44135	165	1
uce1272	105.55379	192	1
uce1314	137.72632	234	1
uce297	127.08309	231	1
uce1131	101.44738	210	1

uce12713	67.94604	153	1
uce1012	146.21678	234	1
uce12571	74.65256	156	1
uce10009	81.32167	174	1
uce11869	75.94608	156	1
uce12225	93.84554	168	1
uce10139	27.39368	156	1
uce310	138.61227	240	1
uce11932	59.9537	174	1
uce989	69.52213	204	1
uce159	107.4549	234	1
uce983	62.7013	234	1
uce12894	80.38329	165	1
uce935	79.92165	240	1
uce1085	156.50296	243	1
uce777	88.22511	222	1
uce829	88.61171	231	1
uce975	123.21778	228	1
uce1425	92.74502	228	1
uce54	115.22892	237	1
uce235	111.35048	210	1
uce12409	105.75509	216	1
uce11046	39.04326	162	1
uce11359	35.47761	162	1
uce988	83.56642	240	1
uce542	113.29638	234	1
uce912	121.2949	198	1
uce10423	60.32695	168	1
uce12167	51.9513	159	1
uce640	146.72274	234	1
uce10988	81.66414	162	1
uce104	120.62841	243	1
uce580	126.62152	240	1
uce121	127.48825	213	1
uce454	110.91549	222	1
uce373	118.97344	228	1
uce12005	56.29052	159	1

uce458	47.20074	198	1
uce837	108.96353	228	1
uce12464	55.53279	159	1
uce11073	55.03236	150	1
uce19	97.32456	207	1
uce699	110.85693	234	1
uce1195	104.23573	225	1
uce11388	74.33268	165	1
uce12747	60.70425	168	1
uce424	101.95856	213	1
uce1327	136.55884	225	1
uce1324	98.5547	216	1
uce12913	78.17142	159	1
uce193	133.62733	240	1
uce415	112.80746	228	1
uce11393	62.60755	156	1
uce1219	59.52124	228	1
uce666	97.256	186	1
uce11457	73.67786	150	1
uce647	115.27514	234	1
uce10286	69.00788	159	1
uce10187	53.26477	159	1
uce962	94.77601	180	1
uce10977	67.43667	159	1
uce11785	52.06521	153	1
uce217	79.53478	228	1
uce12650	64.8327	168	1
uce12063	75.75678	162	1
uce401	143.31099	243	1
uce12672	51.26989	162	1
uce12050	49.69109	156	1
uce1028	117.43801	243	1
uce97	143.6652	237	1
uce984	127.30023	237	1
uce11626	95.24779	168	1
uce1477	112.95715	231	1
uce10329	58.37343	168	1

uce196	144.14828	240	1
uce760	142.16739	231	1
uce908	91.10167	189	1
uce73	149.30909	246	1
uce488	150.23624	234	1
uce850	143.1402	237	1
uce12021	71.26325	171	1
uce10049	91.66827	159	1
uce662	145.06489	237	1
uce231	140.6572	234	1
uce1083	135.76284	246	1
uce12901	47.27074	153	1
uce12399	56.42225	150	1
uce360	107.62041	231	1
uce406	94.6889	237	1
uce11055	58.72139	156	1
uce370	78.36262	234	1
uce566	75.43575	231	1
uce10667	81.19739	156	1
uce11823	44.59321	171	1
uce12133	78.97276	156	1
uce543	69.3465	222	1
uce1142	92.38716	195	1
uce12858	78.45338	162	1
uce60	127.75858	240	1
uce11534	66.76088	159	1
uce10218	94.64476	171	1
uce148	111.69554	219	1
uce12973	59.10479	168	1
uce10451	76.02562	165	1
uce12960	68.31149	183	1
uce43	117.20354	231	1
uce11809	61.30847	165	1
uce10822	66.35194	183	1
uce498	86.24774	231	1
uce12809	59.59548	165	1
uce411	85.42871	198	1

uce120	134.11992	246	1
uce739	98.32526	246	1
uce583	59.73223	186	1
uce918	85.24102	228	1
uce1358	89.81543	243	1
uce55	115.51897	243	1
uce1225	72.74851	243	1
uce94	129.6099	237	1
uce794	88.53093	207	1
uce10604	68.69898	153	1
uce659	68.25399	228	1
uce10548	34.70349	162	1
uce10776	72.67298	162	1
uce11439	69.02866	162	1
uce767	140.58262	228	1
uce528	69.95189	210	1
uce257	147.51376	237	1
uce923	61.63086	219	1
uce10178	84.3612	153	1
uce787	76.49099	174	1
uce8	133.79089	240	1
uce563	107.27168	231	1
uce510	65.18132	243	1
uce11940	69.12406	153	1
uce12558	73.30774	150	1
uce1007	159.51149	246	1
uce1446	82.26436	165	1
uce11775	69.53377	171	1
uce749	88.95466	231	1
uce576	72.63818	165	1
uce211	112.04729	225	1
uce764	95.8367	234	1
uce6	109.81498	234	1
uce753	27.93193	150	1
uce1394	132.71972	243	1
uce225	91.30395	222	1
uce5	122.77072	222	1

uce12709	69.41503	165	1
uce682	147.6159	234	1
uce12762	52.54168	150	1
uce134	93.10781	210	1
uce12059	49.50057	171	1
uce12388	72.35127	171	1
uce173	120.71248	228	1
uce11745	75.55195	156	1
uce272	70.00822	225	1
uce1081	68.69171	177	1
uce589	90.74338	234	1
uce1243	129.3809	213	1
uce991	127.414	231	1
uce479	73.91911	213	1
uce900	79.66411	225	1
uce11826	79.93608	156	1
uce11357	80.18793	153	1
uce11081	44.8127	159	1
uce11060	75.06766	162	1
uce616	112.39909	219	1
uce11394	40.89383	156	1
uce735	94.89137	237	1
uce1073	74.76552	171	1
uce10126	100.87019	171	1
uce1378	122.37745	213	1
uce11720	60.26543	159	1
uce204	78.23074	228	1
uce12854	76.82614	162	1
uce12962	82.05484	174	1
uce12076	60.16004	156	1
uce103	129.45741	219	1
uce436	69.07503	234	1
uce1479	123.71303	231	1
uce10327	103.33441	180	1
uce1424	115.97521	234	1
uce87	130.54671	252	1
uce752	114.02205	228	1

uce1196	114.41684	231	1
uce11905	89.15829	159	1
uce11791	56.89963	165	1
uce279	126.01631	237	1
uce1103	89.3918	222	1
uce955	110.10167	228	1
uce10402	67.12471	177	1
uce423	138.96002	231	1
uce12874	42.26561	180	1
uce472	41.47357	231	1
uce1166	106.31209	195	1
uce1432	73.23849	165	1
uce12852	90.45627	165	1
uce389	110.52774	231	1
uce11490	90.39956	162	1
uce11698	92.25665	165	1
uce713	79.12278	234	1

Table S3. Results extracted from the Borowieck et al (2015) script implemented in R for the 80% matrix, including the values of average bootstrap for each gen tree and "clocklikeness" scores for each locus.

UCE locus	Average_bootstrap	Clocklikeness
uce1006	65.60526316	11.13453753
uce610	50.8974359	11.7824887
uce415	47.93243243	11.8463886
uce1285	45.46753247	12.56500258
uce1211	51.64102564	12.71684547
uce1131	49.57352941	12.88141578
uce1335	43.56756757	12.97257158
uce1027	40.15584416	13.09525034
uce1033	38.75	13.13359108
uce95	41.74666667	13.15664465
uce721	40.71830986	13.27091826
uce257	44.74025974	13.27632864
uce984	49.12987013	13.49427117
uce487	43.51898734	13.52068226
uce733	45.01408451	13.91890559
uce1207	55.64	14.13154399
uce241	45.92105263	14.34740921
uce50	49.77631579	14.4520345
uce914	47.5625	14.54938008
uce557	42.15068493	14.55785547
uce244	47.62337662	14.65093423
uce800	58.33333333	14.7062943
uce1308	51.02985075	14.89067479
uce65	47.80519481	14.95134327
uce565	36.84722222	15.21130935
uce1167	49.98611111	15.27686742
uce659	44.55405405	15.64925939
uce819	63.96103896	15.68380198
uce660	43.75	15.70378002

uce1274	56.22077922	15.7454539
uce239	48.56944444	15.83529015
uce544	50.63888889	15.95614722
uce734	51.69620253	15.96920444
uce1282	48.78666667	16.00337461
uce634	49.02777778	16.0251427
uce882	53.78378378	16.05141947
uce1012	57.11842105	16.05604437
uce103	45.84507042	16.09500221
uce97	53.20779221	16.09667483
uce5	49.98611111	16.13493339
uce1014	49.3	16.2103931
uce785	42.63636364	16.32425373
uce633	38.88888889	16.37877231
uce60	48.61538462	16.39403398
uce1013	43.08	16.44704269
uce542	31.97368421	16.46355145
uce871	50.48648649	16.53070461
uce1016	49.19178082	16.54527963
uce1434	46.44155844	16.59159526
uce217	48.66216216	16.61482431
uce236	35.69620253	16.61886844
uce1479	40.36	16.62682337
uce475	36.42857143	16.63467631
uce1419	49.91666667	16.79061766
uce68	47.98484848	16.79709767
uce1195	68.97260274	16.86846578
uce1094	51.58666667	16.91235328
uce1418	45.73684211	16.95128038
uce269	51.97402597	16.95758735
uce279	58	16.98653973
uce172	51.34210526	16.99747465
uce401	50.59493671	17.00148918
uce1134	49.05633803	17.04403998
uce438	53.14084507	17.05819626
uce913	40.42307692	17.117514
uce525	53.36842105	17.14132834

uce983	25.52631579	17.14590005
uce595	45.90277778	17.16241896
uce316	41.45833333	17.20929127
uce159	37.56578947	17.26624211
uce505	50.22368421	17.28164847
uce835	40.01492537	17.29721244
uce793	37.04225352	17.31695999
uce706	55.49333333	17.31844543
uce64	47.61538462	17.35525871
uce777	45.41666667	17.36215209
uce99	44.96052632	17.40238187
uce390	42.92307692	17.40450426
uce1366	42.65333333	17.45230725
uce488	42.03947368	17.45237423
uce1076	41.36842105	17.45609343
uce1109	45.63157895	17.45945012
uce1394	57.08860759	17.48245214
uce88	53.26666667	17.5695695
uce609	51.07792208	17.65916424
uce333	49.97183099	17.69383667
uce529	31.92537313	17.74119792
uce12409	38.98571429	17.75337714
uce640	40.65789474	17.84192744
uce1124	49.71232877	17.86170214
uce1244	39.52702703	17.8902112
uce1377	53.59210526	17.89363234
uce728	53.66176471	17.90532695
uce313	39.28947368	17.92977109
uce1425	47.95945946	17.9334268
uce96	46.05405405	17.94057129
uce805	29.02777778	17.95379277
uce905	40.9125	17.96021011
uce272	40.7260274	18.00305164
uce552	53.72368421	18.00679625
uce1024	43.70886076	18.05992695
uce1430	52.61038961	18.06574653
uce38	52.46268657	18.0753425

uce366	52.42253521	18.08344013
uce1026	39.78205128	18.09809901
uce519	57.38961039	18.17138964
uce907	39.83783784	18.23746436
uce1331	35.26388889	18.24970503
uce537	44.64	18.25168337
uce181	41.3125	18.28441542
uce1462	45.36619718	18.30187286
uce8	44	18.34827125
uce809	49.25352113	18.36837057
uce59	52.06666667	18.41798528
uce1374	48.97435897	18.45993201
uce1337	47.71052632	18.47666404
uce735	46.57142857	18.48424669
uce168	51.2972973	18.50235719
uce350	44.41791045	18.50960593
uce1327	53.94520548	18.54502523
uce688	53.54054054	18.54685595
uce796	58.53521127	18.58456798
uce1288	52.23684211	18.5858907
uce1227	36.7027027	18.59011915
uce310	51.97435897	18.61992372
uce1171	52.05194805	18.62791643
uce559	35.90410959	18.64203077
uce1342	41.5974026	18.68884549
uce423	53.94666667	18.69581918
uce1138	42.28787879	18.75083766
uce650	27.92753623	18.76430865
uce1097	53.11688312	18.81892168
uce335	46.71428571	18.8252882
uce233	48.44736842	18.87601844
uce10457	36.54054054	18.88819489
uce1007	26.2875	18.89541648
uce1004	33.33766234	18.89677024
uce1194	47.7625	18.9285532
uce370	48.69736842	18.93896237
uce1020	40.52631579	18.94866784

uce562	42.22077922	18.98077328
uce1098	40.92753623	19.02690618
uce87	36.15853659	19.0422401
uce1217	64.30136986	19.04830069
uce1497	55.78571429	19.0509134
uce330	43.20547945	19.07932176
uce658	41.77027027	19.10678645
uce649	49.10126582	19.14041944
uce498	46.42666667	19.14239452
uce1099	45.34210526	19.15553755
uce252	43.71232877	19.21110416
uce702	62.8630137	19.24902291
uce955	40.18918919	19.30056802
uce1352	42.73333333	19.31540995
uce1481	48.49295775	19.35256965
uce1085	52.44303797	19.37092482
uce402	44.24615385	19.38480195
uce424	44.01449275	19.38757536
uce618	55.6	19.41392131
uce873	39.55128205	19.42058293
uce1292	40.05633803	19.44813087
uce55	47.2278481	19.46504697
uce1174	50.50666667	19.46957937
uce139	42.17105263	19.55134427
uce301	47.42647059	19.56086197
uce270	44.67567568	19.61893422
uce1287	47.63380282	19.63901271
uce541	50.15584416	19.63990537
uce956	37.97435897	19.65814596
uce463	39.48648649	19.6756916
uce1112	48	19.69797635
uce442	39.50724638	19.74559773
uce32	49.60493827	19.75365587
uce625	51.38028169	19.76381723
uce1241	41.11688312	19.76882675
uce485	62.52173913	19.78188515
uce27	38.35135135	19.79350612

uce1216	48.32	19.8748201
uce406	47.57142857	19.87784545
uce106	44.81944444	19.90037852
uce170	44.37974684	19.91476631
uce132	41.62337662	19.92110309
uce999	39.23076923	19.92846169
uce69	52.05555556	19.98792976
uce1095	43.77631579	20.00925628
uce1278	33.68918919	20.01311149
uce1067	41.46153846	20.02800682
uce516	35.08860759	20.05205035
uce1340	48.17721519	20.05457256
uce1144	49.60526316	20.09928704
uce1037	50.86111111	20.11386122
uce959	53.15942029	20.14062918
uce1128	44.08	20.15376596
uce855	39.65671642	20.19143409
uce1452	41.30263158	20.20631781
uce1075	49.1	20.21119788
uce18	52.08219178	20.22433893
uce546	30.52112676	20.29790603
uce1102	56	20.30092369
uce150	44.32857143	20.30654384
uce1225	54.81012658	20.31992387
uce707	56.17808219	20.33303881
uce120	45.525	20.37479151
uce862	41.26923077	20.46376522
uce975	44.01351351	20.47858855
uce1259	42.14492754	20.51431486
uce1232	43.50617284	20.52954831
uce1052	41.8974359	20.60442959
uce435	56.32394366	20.61579053
uce918	47.01351351	20.62584257
uce1029	41.75342466	20.62639542
uce791	51.11428571	20.63666155
uce1477	31.06666667	20.65749117
uce1301	48.45679012	20.68136936

uce657	47.14285714	20.68715803
uce363	47.91891892	20.68952875
uce947	58.14864865	20.69744065
uce500	39.86111111	20.75945323
uce1268	50	20.78089551
uce326	45	20.7832553
uce114	55.28985507	20.80604613
uce453	55.31168831	20.86266452
uce104	57.10126582	20.93212599
uce1415	33.63235294	20.93749329
uce749	42.61333333	20.97724627
uce1440	44.03030303	20.97883619
uce253	53.46153846	21.06014211
uce192	33.31506849	21.09996536
uce1403	46.32894737	21.13732092
uce220	46.33783784	21.1763022
uce626	58.63768116	21.21991459
uce1303	51.15492958	21.2356082
uce794	51.1641791	21.25510942
uce98	53.23076923	21.28397372
uce746	39.89189189	21.30352688
uce531	47.24324324	21.32889412
uce806	27.01449275	21.33019448
uce260	50.20289855	21.33188094
uce682	41.42105263	21.35398366
uce1246	41.44594595	21.37295957
uce1441	48.01298701	21.39829463
uce584	49.31944444	21.44040618
uce991	35.05333333	21.46941771
uce1202	47.15942029	21.47074465
uce695	41.53424658	21.5555742
uce196	37.17948718	21.55669921
uce204	37.51351351	21.58225215
uce641	45.93506494	21.59351958
uce161	59.52238806	21.60135964
uce240	48.46153846	21.60699643
uce404	44.09459459	21.61359945

uce613	56.28205128	21.66662084
uce580	53.6025641	21.68680387
uce816	46.76119403	21.72415184
uce1173	44.2	21.75287081
uce1393	66.61333333	21.7540894
uce1443	44.74025974	21.77233585
uce797	53.72222222	21.77459038
uce1476	47.13333333	21.77501603
uce1050	41.79166667	21.79308287
uce936	46.03947368	21.80887914
uce1011	39.94871795	21.83822326
uce263	48.525	21.90754303
uce1503	44.21052632	21.91734932
uce812	40.11842105	21.92233401
uce212	44.86842105	21.9484438
uce9	39.01265823	21.99402296
uce495	34.52238806	21.99660562
uce699	37.67105263	22.03886569
uce1496	46.02857143	22.07038251
uce46	42.80246914	22.07060459
uce1243	49.56521739	22.08996414
uce1447	33.68421053	22.11485673
uce47	40.94285714	22.13996303
uce509	36.94736842	22.14824812
uce672	45.58024691	22.17381058
uce501	32.38157895	22.18706336
uce234	43.86111111	22.19914827
uce1168	60.42666667	22.2096589
uce607	52.08450704	22.21567488
uce621	47.64864865	22.22225358
uce1046	47.84931507	22.27525154
uce829	43.69333333	22.2770905
uce1240	39.08823529	22.2991046
uce1296	46.56923077	22.31164833
uce145	48.57746479	22.322697
uce560	42.18421053	22.36936292
uce37	58.80597015	22.37488669

uce297	42.24	22.40401612
uce24	42.76923077	22.4061721
uce554	28.51282051	22.41234852
uce135	32.97222222	22.41982045
uce876	49.84057971	22.43598402
uce843	50.8028169	22.43735612
uce205	32.94594595	22.44398129
uce173	48.66216216	22.4461424
uce508	42.58441558	22.4870356
uce642	47.29333333	22.51227597
uce1239	36.56578947	22.52268384
uce1482	41.8	22.53071607
uce1031	38.36842105	22.54337504
uce225	37.43055556	22.5467656
uce1038	35.08860759	22.56747589
uce1047	41.80882353	22.58146439
uce7	45.11538462	22.58866695
uce1083	45.3375	22.60993024
uce1429	44.84285714	22.61022734
uce1143	53.29333333	22.61339403
uce698	45.71794872	22.62613951
uce349	46.27272727	22.65017236
uce188	41.68493151	22.65331918
uce336	49.75	22.66495255
uce1172	43.52	22.66758866
uce752	46.05405405	22.69896173
uce4	42.27631579	22.72866315
uce1304	43.83098592	22.74922417
uce454	44.56944444	22.79049306
uce869	41.93506494	22.79332745
uce23	37.22535211	22.80106051
uce115	42.93333333	22.80740405
uce1279	40.18309859	22.82924356
uce1079	44.43037975	22.85265109
uce391	52.14102564	22.86949581
uce1078	53.625	22.90131216
uce265	49.18421053	22.91273309

uce518	37.82191781	22.95937967
uce382	39.96103896	22.98059498
uce345	43.21538462	22.98137273
uce71	44.37179487	22.98850531
uce1213	60.32857143	23.01627963
uce1324	42.14285714	23.02997817
uce799	45.14473684	23.04902368
uce764	57.63157895	23.08758218
uce58	56.11594203	23.09695754
uce701	33.66666667	23.09846137
uce1062	43.15068493	23.13461681
uce1348	42.10606061	23.14636057
uce645	35.68831169	23.1618016
uce620	36.71232877	23.17617388
uce122	22.17647059	23.19037327
uce34	48.73333333	23.24852632
uce723	37.17567568	23.25375531
uce1238	44.38961039	23.2553171
uce1378	52.65217391	23.28150978
uce484	47.28985507	23.29124698
uce479	46.46376812	23.29510601
uce400	48.97260274	23.31629998
uce747	35.94736842	23.32567179
uce1395	43.48	23.33174516
uce1391	48.53424658	23.34456996
uce1380	45.13513514	23.40159517
uce73	46.075	23.40644226
uce430	23.91891892	23.41835776
uce520	38.36842105	23.43282536
uce511	49.08	23.45563457
uce43	41.06666667	23.45790127
uce963	36.55384615	23.47162646
uce904	48.34285714	23.49262365
uce1150	60.5942029	23.54384156
uce550	36.64	23.54978912
uce1071	45.20547945	23.58155521
uce389	44.12	23.59371018

uce850	39.22077922	23.64703756
uce789	50.67532468	23.71170417
uce949	31.97368421	23.71580878
uce365	52.60526316	23.74691677
uce295	44.59459459	23.75170301
uce1237	45.20588235	23.77580237
uce870	43.01351351	23.78107897
uce427	43.59459459	23.80360282
uce1258	56.97368421	23.8405536
uce929	43.61428571	23.90450961
uce1032	40.19444444	23.92948302
uce923	28.1971831	23.97500699
uce276	42.54166667	23.97783884
uce255	39.80769231	24.01617714
uce1196	43.76	24.05483613
uce1170	38.07894737	24.05623087
uce888	47.16923077	24.06880093
uce1317	36.04166667	24.09835742
uce1421	50.69230769	24.11660704
uce639	26.20895522	24.18913454
uce741	44.80769231	24.19006916
uce1286	41.45205479	24.21010873
uce845	38	24.21242298
uce767	56.67567568	24.39518918
uce1008	49.3	24.39667395
uce900	45.28767123	24.44882987
uce1019	48.8028169	24.4735218
uce1127	57.84210526	24.48142826
uce686	31.98701299	24.49696205
uce1156	39.05882353	24.525724
uce872	37.89333333	24.54140422
uce1122	53.36986301	24.54244949
uce152	47.67605634	24.55203416
uce1314	44.36842105	24.55905775
uce558	37.16438356	24.68251628
uce1133	41.41463415	24.69023285
uce1257	55.38888889	24.69570684

uce864	38.70512821	24.7403459
uce990	37.51470588	24.75267735
uce1209	39.12820513	24.76278444
uce94	45.66233766	24.80883633
uce1253	36.03947368	24.81990924
uce989	36.53030303	24.83491103
uce288	39.27692308	24.83918275
uce758	31.84210526	24.86125845
uce293	38.27631579	24.96261435
uce1092	35.22727273	24.99742564
uce20	36.16666667	25.02065973
uce1208	60.28169014	25.04322215
uce1103	43.72222222	25.05312677
uce1291	41.07462687	25.10364978
uce803	37.87341772	25.1061834
uce646	56.44155844	25.11778117
uce381	49.65753425	25.14059378
uce235	41.57352941	25.203865
uce171	35.97368421	25.32309321
uce48	41.67164179	25.38060379
uce143	51.98701299	25.38110683
uce1080	40.30882353	25.41747909
uce510	40.70886076	25.5092024
uce113	34.5942029	25.5264007
uce547	34.5	25.53226056
uce880	32.10810811	25.5434671
uce1064	40.13333333	25.55613754
uce1055	40.13333333	25.60122789
uce566	51.33333333	25.60516093
uce543	40.76388889	25.63087829
uce1297	52.77777778	25.69068242
uce997	39.73333333	25.69645307
uce654	44.83783784	25.80093455
uce134	37.54411765	25.80537146
uce681	48.4	25.84150749
uce74	46.34210526	25.84507075
uce189	48.38961039	25.8455858

uce885	55.72727273	25.9099822
uce441	37.17333333	25.92473261
uce935	45.83333333	25.93067363
uce1028	45.93670886	25.99011207
uce459	42.57534247	26.08003426
uce13	51.23376623	26.10624389
uce563	53.25333333	26.15005683
uce1463	41.17808219	26.24078974
uce12	47.8115942	26.28798503
uce237	47.59459459	26.31770567
uce242	52.73684211	26.4290338
uce1359	41.32876712	26.44186473
uce577	42.62820513	26.45716142
uce823	46.09459459	26.48775288
uce985	33.13513514	26.57096749
uce1464	41.56578947	26.57517721
uce193	42.78205128	26.59189041
uce689	42.63513514	26.59686616
uce275	44.36842105	26.67277561
uce691	48.05714286	26.75484121
uce1035	38.68	26.77168828
uce12635	29.43076923	26.7947172
uce67	42.7	26.83630294
uce1034	44.65753425	26.84420855
uce1442	33.21333333	26.89896658
uce647	40.28947368	26.90644387
uce137	43.49275362	27.03229003
uce1499	43.57333333	27.07117542
uce513	35.41558442	27.11155137
uce243	31.35616438	27.11720998
uce1490	41.82278481	27.31920434
uce231	42.94736842	27.3294311
uce197	35.46478873	27.3485419
uce36	35.1641791	27.40714791
uce291	36.33333333	27.49649348
uce710	34.36764706	27.55629288
uce200	38.125	27.62635985

uce194	51.70833333	27.73694152
uce1260	46.84722222	27.7810621
uce434	44.05194805	27.82253424
uce130	46.42682927	27.90720834
uce860	44.96052632	27.93049326
uce881	39.29230769	27.94679343
uce847	39.28	28.1144433
uce1325	29.46969697	28.19396844
uce1193	44.71052632	28.22291927
uce676	33.75362319	28.25844739
uce1339	33.48484848	28.30499437
uce360	53.30666667	28.3119101
uce347	41.67567568	28.36836064
uce12545	36.84210526	28.37017015
uce455	40.07142857	28.41875614
uce917	39.1025641	28.47537685
uce693	36.68115942	28.57375919
uce1234	48.55405405	28.61426324
uce31	34.81818182	28.62301142
uce902	37.35526316	28.70292193
uce915	44.26923077	28.73164849
uce148	47.09859155	28.78628881
uce697	53.17333333	28.88706587
uce1438	53.42647059	28.96529677
uce598	50.81012658	28.96847458
uce528	33.55882353	29.02535295
uce10	45.85333333	29.06177617
uce600	41.48684211	29.19984727
uce1330	30.56164384	29.34227815
uce680	47.5890411	29.55990819
uce958	40.58571429	29.58925623
uce739	38.2625	29.70496991
uce662	41.75324675	29.76249949
uce837	44.60810811	29.86266707
uce965	37.65384615	29.93921439
uce39	44	29.9776605
uce1093	45.17333333	30.00913722

uce472	29.28	30.10198549
uce1159	48.74025974	30.2370371
uce1457	37.88888889	30.44660146
uce768	39.89333333	30.48040906
uce468	46.52173913	30.64382111
uce594	47.8974359	30.67059162
uce624	40.08	30.9513556
uce1190	35.83116883	31.19587398
uce1061	60.78787879	31.20495133
uce1358	31.08860759	31.2761607
uce1023	34.62337662	31.5184969
uce33	29.17910448	31.53967946
uce1235	41.42307692	31.75029748
uce1384	38.7027027	31.8189496
uce35	56.74647887	31.87494938
uce587	36.50666667	32.37479754
uce589	41.02631579	32.4217596
uce683	52.59210526	32.51234611
uce713	38.15789474	32.76228873
uce568	42.84057971	33.09621041
uce616	40.42253521	33.19831491
uce635	30.83333333	33.19958312
uce1181	47.47826087	33.20351712
uce1065	57.87837838	33.27794042
uce278	37.71621622	33.27870182
uce1236	40.01492537	33.32312741
uce211	47.56164384	33.3684872
uce700	42.27777778	33.47185899
uce121	47.13043478	33.74039169
uce1459	41.23943662	33.80978094
uce149	41.27272727	33.85735166
uce553	38.92	34.34548417
uce910	35.81012658	34.42228598
uce822	38.25675676	34.61863526
uce760	45.8	34.80334941
uce987	36.30666667	35.56937267
uce373	37.40540541	35.60893783

uce1063	49.82352941	35.68011732
uce617	50.02739726	36.21763033
uce169	36	36.53099368
uce945	50.02777778	36.55633444
uce1353	53.4025974	36.63740851
uce946	42.35616438	36.69061726
uce1145	46.07246377	36.75295149
uce1392	45.48611111	37.4115895
uce901	39.2278481	37.66788815
uce842	35.76315789	38.74374945
uce1424	45.28947368	39.0683342
uce844	43.05405405	40.22784382
uce44	32.90789474	40.32332111
uce443	34.90140845	40.41087422
uce100	47.6	40.5804006
uce1206	42.71604938	42.42893578
uce54	51.07792208	43.74128348
uce861	35.56	43.9651851
uce473	56.08	44.52919704
uce436	32.73684211	46.83704381
uce643	46.93243243	46.87105916
uce346	43.45333333	46.96043952
uce19	38.7761194	47.08468486
uce1431	38.67142857	48.39020477
uce1015	49.5942029	48.66966833
uce974	41.73529412	48.89378812
uce86	34.84057971	49.33072388
uce6	50	49.5956407
uce1009	39.62121212	50.37758256
uce1416	39.56410256	51.32174513
uce927	42.68	52.43852584
uce588	38.88	53.38436096
uce988	21.47435897	54.52222596
uce348	49.34328358	56.23879261
uce1191	39.57692308	58.56416863
uce399	44.46478873	62.53181226
uce82	32.2278481	64.58019229

uce1269	28.97368421	64.97227503
uce30	39.15	68.10394579
uce1420	41.47761194	79.26944065
uce1219	19.7027027	83.52380184
uce848	26.94736842	97.64261421
uce497	38.06060606	121.1381223
uce1245	29.2173913	126.8516088
uce632	39.375	229.7751569

Table S4. Summary statistics of the contigs assembly and UCE loci recovery, including the probe kit version used for the enrichment of libraries.

Genus	Species	Voucher	Probe set Version	Contigs	UCE loci
Allorhogas	*amuzgo*	CNIN4007	V2	257,389	1,731
Allorhogas	*ardisia*	CNIN3991	V2	46,029	1,042
Allorhogas	*bassettia*	CNIN4272	V1	183,960	1,129
Allorhogas	*belonocnema*	CNIN3391	V1	214,896	1,296
Allorhogas	*brevithorax*	CNIN3907	V2	325,376	1,566
Allorhogas	*caulinarius*	CNIN4125	V1	231,778	1,731
Allorhogas	*clidemiae*	CNIN3595	V2	630,281	1,764
Allorhogas	*coccolobae*	CNIN3905	V2	63,629	1,188
Allorhogas	*conostegia*	CNIN3587	V2	225,200	1,215
Allorhogas	*cordobensis*	CNIN3602	V2	13,331	544
Allorhogas	*costaricensis*	CNIN3992	V2	153,315	1,582
Allorhogas	*crassifemur*	CNIN3684	V1	229,472	1,190
Allorhogas	*gallifolia*	CNIN3389	V1	233,250	1,814
Allorhogas	*gauldi*	CNIN4004	V2	147,235	1,407
Allorhogas	*granivorus*	CNIN3498	V1	71,481	786
Allorhogas	*hansoni*	CNIN4005	V2	133,744	1,471
Allorhogas	*infuscotarsus*	CNIN3993	V2	137,800	1,443
Allorhogas	*ingavera*	CNIN3597	V2	129,424	1,255
Allorhogas	*jaliscoensis*	CNIN3687	V1	261,146	1,254
Allorhogas	*lacuna*	CNIN3994	V2	74,129	1,247
Allorhogas	*laselva*	CNIN3995	V2	106,683	1,428
Allorhogas	*mendocinus*	CNIN4009	V2	356,911	1,758
Allorhogas	*mineiro*	CNIN4138	V2	202,347	1,637
Allorhogas	*minimus*	CNIN3594	V2	140,423	1,325
Allorhogas	*psychotria*	CNIN3908	V2	220,538	1,161
Allorhogas	*punctatus*	CNIN3589	V2	171,896	1,000
Allorhogas	*scotti*	CNIN3686	V1	158,147	1,063
Allorhogas	*tico*	CNIN3685	V2	66,567	1,032
Allorhogas	*uberlandiensis*	CNIN4122	V2	214,607	1,551
Allorhogas	*vulgaris*	CNIN3906	V2	391,123	1,580
Allorhogas	*zurquensis*	CNIN3997	V2	93,756	1,348

Allorhogas	sp. aff. *argentinus*	CNIN4003	V2	148,318	1,509
Allorhogas	sp. aff. *prosopidis*	CNIN3593	V2	282,242	1,691
Allorhogas	sp. near laselva	CNIN3999	V2	163,183	1,557
Allorhogas	sp. near shawi	CNIN4000	V2	109,844	1,388
Allorhogas	sp.	CNIN4002	V2	29,816	555
Allorhogas	sp.	CNIN4008	V2	355,056	1,844
Allorhogas	sp.	CNIN4333	V2	129,296	1,062
Allorhogas	sp.	CNIN4334	V2	229,501	1,613
Allorhogas	sp.	CNIN4335	V2	39,886	618
Allorhogas	sp.	CNIN3055	V2	239,034	1,269
Allorhogas	sp.	CNIN3390	V1	113,933	929
Allorhogas	sp.	CNIN3484	V1	95,294	842
Allorhogas	sp.	CNIN3586	V2	520,843	1,748
Donquickeia	sp.	CNIN4132	V2	129,506	1,478
Donquickeia	sp.	CNIN4133	V2	131,696	1,574
Ficobolus	*paniaguai*	CNIN3418	V2	97,336	949
Labania	*ficophaga*	CNIN3682	V1	148,391	919
Labania	*hansoni*	CNIN3681	V1	188,748	1,120
Labania	*straminea*	CNIN4006	V2	182,652	1,559
Labania	sp.	CNIN3590	V2	631,573	1,587
Labania	sp.	CNIN4331	V2	80,861	979
Labania	sp.	CNIN4332	V2	195,626	1,477
Labania	sp.	CNIN3910	V2	4,488	404
Monitoriella	*elongata*	CNIN3054	V2	175,795	1,060
Monitoriella	*rufithorax*	CNIN3420	V1	103,796	799
Monitoriella	*sp. aff. elongata*	CNIN4136	V2	193,921	1,554
Monitoriella	sp.	CNIN4134	V2	239,368	1,538
Monitoriella	sp.	CNIN4135	V2	103,796	1,357
Mononeuron	*duguetiae*	CNIN3416	V1	143,820	1,069
Percnobracon	*rugosus*	CNIN3585	V2	607,400	1,794
Percnobracon	*stenopterus*	CNIN3047	V2	200,746	1,194
Percnobracon	sp.	CNIN4010	V2	192,247	1,662
Plesiopsenobolus	*tico*	CNIN3588	V2	176,042	1,250
Plesiopsenobolus	*mesoamericanus*	CNIN3483	V1	157,337	1,095
Psenobolus	*parapygmaeus*	CNIN3497	V1	166,049	1,193
Psenobolus	*ficarius*	CNIN3912	V2	44,212	975
Psenobolus	*pygmaeus*	CNIN3911	V2	102,755	1,143

Psenobolus	sp.	CNIN4128	V2	183,706	1,740
Sabinita	sp.	CNIN3683	V1	49,479	774
cf. *Sabinita*	sp.	CNIN4330	V2	116,356	1,520
OUTGROUP					
Heterospilus	sp.	CNIN3037	V2	224,862	1,139
Heterospilus	sp.	CNIN4117	V2	366,890	1,777
Heterospilus	sp.	CNIN4137	V2	303,062	950
Hetrospilus	sp.	CNIN3998	V2	195,509	1,542
Lissopsius	sp.	CNIN3039	V2	273,970	1,198
Rhaconotus	sp.	CNIN3056	V2	114,079	789
Stenocorse	bruchivora	CNIN3043	V2	173,537	1,004
Leluthia	sp.	CNIN4274	V1	266,898	1,327
Pareucorystes	*varinervis*	CNIN4288	V1	51,493	746
Hecabalodes	*radialis*	CNIN4293	V1	103,521	742
Polystenus	*rugosus*	CNIN4294	V1	121,913	843
Dendrosotinus	*titubatus*	CNIN4300	V1	147,025	1,053
Eodendrus	*eous*	CNIN4303	V1	228,069	1,133
Parallorhogas	*boninus*	CNIN4306	V1	50,529	267
Euscelinus	*sarawacus*	CNIN4311	V1	53,343	735

DISCUSIÓN GENERAL Y CONCLUSIONES

En esta tesis doctoral se utilizó la técnica de captura de secuencias de UCEs, así como marcadores obtenidos a partir del minado de genomas mitocondriales para estudiar la sistemática de un grupo de avispas asociadas con agallas que probablemente tienen hábitos fitófagos dentro de un grupo predominantemente parasitoide. Se pone a prueba el desempeño de estos datos para responder preguntas a diferentes escalas de tiempo evolutivo; incluyendo la resolución de relaciones filogenéticas profundas, así como métodos de delimitación de especies. Además, en este trabajo se recalca la importancia de realizar trabajos taxonómicos integradores empleando fuentes de evidencia tanto moleculares como morfológicas, teniendo siempre presente la historia natural de las especies para el descubrimiento y descripción de la diversidad biológica del planeta.

El primer capítulo de este trabajo, el cual se encuentra publicado (Samacá Sáenz et al., 2019), incluyó una primera hipótesis filogenómica del clado asociado con la formación de agallas de la subfamilia Doryctinae, junto con una descripción del orden y estructura de los genomas mitocondriales de algunas especies representantes de los géneros incluidos. En esta primera aproximación se confirmaron algunas relaciones previamente obtenidas como *Percnobracon* como grupo hermano de los géneros restantes asociados con la formación de agallas restantes y *Allorhogas* como grupo polifilético. También se realizó un análisis preliminar de reconstrucción de estados ancestrales de manera preliminar para rastrear la familia de plantas asociada con los géneros dentro de la historia evolutiva del grupo.

El segundo capítulo de la tesis, también ya publicado (Samacá-Sáenz et al., 2020), correspondió a un estudio de caso de descubrimiento de nuevas especies del género *Allorhogas* asociadas con agallas de avispas de la familia Cynipidae (Hymenoptera), en donde se emplearon varios métodos para delimitar especies con base en marcadores mitocondriales (COI), nucleares (UCEs) y características morfológicas. En este segundo trabajo se identificaron al menos cinco linajes diferentes de *Allorhogas* y se describieron cuatro especies nuevas para la ciencia.

El tercer capítulo comprendió un análisis filogenómico de los géneros de doryctinos asociados con la formación de agallas, el cual incluyó el muestreo más completo hecho hasta la fecha para este grupo e hizo énfasis en *Allorhogas* al incluir más de la mitad de sus especies descritas. Esta última parte incluye además análisis macroevolutivos como la estimación de tiempos de divergencia y reconstrucción de estados ancestrales utilizando varios aspectos de las historias de vida de los géneros asociados con la formación de agallas para investigar más a fondo la evolución de la fitofagia y su origen.

Entre los resultados más importantes obtenidos en este trabajo de tesis doctoral destaca la caracterización de los genomas mitocondriales para 17 especies de la familia Doryctinae, de los cuales 13 corresponden a especies representantes del clado asociado con la formación de agallas. Antes de la publicación de este capítulo, tan solo dos estudios filogenéticos con base en genomas mitocondriales se habían realizado para la familia Braconidae (Wei et al. 2010; Li et al. 2016). Igualmente, se reportan patrones de reordenamiento para algunos tRNAs en el grupo interno y se utilizan las secuencias de los

genes codificantes para proteína para realizar análisis filogenéticos, utilizándolos por separado y en conjunción con los datos de UCEs, los cuales produjeron topologías mejor soportadas. Tanto las secuencias mitocondriales como los reordenamientos genéticos son considerados filogenéticamente informativos y han sido utilizados para estudiar las relaciones de varios grupos de insectos (por ejemplo, Timmermans y Vogler 2012; López-López y Vogler; Aguilera-Uribe et al. 2020), por lo que la información generada en este estudio puede ser implementada para el desarrollo de futuras investigaciones.

Otro resultado destacable corresponde a la posible existencia de diferenciación asociada con el hospedero (HAD por sus siglas en inglés), la cual representa en términos microevolutivos una forma de aislamiento reproductivo y de especiación ecológica en simpatría determinada por los cambios en el tipo de huésped (Nosil 2012; Matsubayashi et al. 2010, Antwi et al. 2015, Driscoe et al. 2019). En este trabajo se propone que las especies de *Allorhogas* asociadas con agallas de la familia Cynipidae en *Quercus* subsección *Virentes* representan un caso potencial de especiación ecológica debido a HAD y que merece atención, ya que se encuentran asociadas con un tipo exclusivo de agalla o con agallas inducidas por especies diferentes de cinípidos en el mismo órgano de la planta. Adicionalmente, se sugiere un posible caso de especiación alopátrica entre dos especies las cuales fueron obtenidas de agallas de cinípidos en poblaciones de encinos que se encentran diferenciadas genética y espacialmente (Cavender-Bares et al. 2015). Para el descubrimiento de la biodiversidad y de especies nuevas, se reitera la importancia de

realizar trabajos taxonómicos bajo un concepto de taxonomía integral (Padial et al. 2010), en donde se utilice más de una fuente de evidencia para la detección de taxones.

Por otra parte, los procesos de codiversificación a nivel macroevolutivo encontrados en los análisis que incluyen un muestreo exhaustivo de las especies de doryctinos del clado asociado con la formación de agallas sugieren tiempos de divergencia concordantes con radiaciones similares para algunos de los subclados y sus plantas hospedadoras. Lo anterior corresponde a las radiaciones aceleradas que se dieron desde el Mioceno medio hasta el Plioceno para algunos linajes neotropicales de plantas representadas en el muestreo. Radiaciones similares entre insectos fitófagos y sus plantas hospedadoras se han demostrado para otros grupos de himenópteros como la familia Agaonidae, en donde ciertos géneros de avispas de esta familia han codiversificado con algunas secciones correspondientes de *Ficus* con quienes mantienen un mutualismo obligado (Jousselin et al. 2003; Cruaud et al. 2012; Satler et al. 2019). No obstante, es importante confirmar mediante muestreos exhaustivos en intervalos de tiempos mayores si ocurre HAD y codiversificación en este grupo de avispas cuya historia evolutiva esta evidentemente ligada a la de las plantas que actúan como sus hospederos.

Aún es necesario confirmar y conocer las estrategias de alimentación y la asociación de plantas para muchas de las especies incluidas en este trabajo. Para esto, la implementación de metodologías de cría que permitan la observación directa de los hábitos alimenticios de los estados inmaduros sigue siendo la fuente de evidencia más confiable. Otras técnicas, como los estudios detallados de la morfología de las mandíbulas en

diferentes estados del desarrollo han sido utilizadas dentro de Braconidae para sugerir el tipo de tejido del que estos organismos se pueden alimentar (Flores et al. 2005; Zaldívar-Riverón et al., 2018) y no deben ser descartadas, ya que pueden proporcionar información útil sobre su biología.

La presente tesis aporta información valiosa y significativa para futuros estudios de evolución molecular, filogenómica, delimitación de especies, así como del amplio campo de las interacciones entre plantas e insectos. Las metodologías, estandarización de protocolos de laboratorio y conductos bioinformáticos utilizados en el desarrollo de este trabajo quedan disponibles para ser aplicados en proyectos próximos que busquen responder preguntas tanto macro como microevolutivas en varios grupos de organismos.

CONCLUSIONES GENERALES

- Los datos de UCEs resultan útiles para resolver la mayor parte de las relaciones filogenéticas de *Allorhogas* y de los géneros de doryctinos asociados con la formación de agallas a diferentes escalas de tiempo evolutivo.
- Los genomas mitocondriales también son informativos para resolver relaciones filogenéticas en el grupo y sus resultados son concordantes con los de estudios previos basados en marcadores puntuales. Igualmente, el marcador COI demostró ser de gran utilidad para estudios de identificación rápida y delimitación de especies en el grupo de estudio.

- Se confirma *Allorhogas* como grupo polifilético, con la mayor parte de sus especies recuperadas en un clado principal, así como en otro clado menor que incluye al género monotípico *Mononeuron*. Aún es necesario estudiar con detalle la morfología de las especies que fueron recuperadas fuera del clado principal de *Allorhogas* para actualizar su clasificación taxonómica.
- *Allorhogas* es un grupo muy diverso, con muchas especies que aún se encuentran sin describir y con diferentes estrategias de fitofagia, incluyendo la formación de agallas, inquilinismo y depredación de semillas en diferentes familias de angiospermas. El descubrimiento de estas especies depende de múltiples fuentes de evidencia, incluyendo datos moleculares, morfológicos y ecológicos.
- La riqueza de especies de los géneros asociados con la formación de agallas de la subfamilia Doryctinae, incluyendo *Allorhogas* está fuertemente ligada con la planta hospedera y a sus eventos y procesos de diversificación. Gracias a esto se discute una potencial especiación ecológica o de HAD para un subclado asociado con agallas de avispas Cynipidae en un linaje particular de *Quercus.*
- Es probable que existan procesos de codiversificación entre varios subclados y linajes de plantas vasculares que actúan como hospederos, debido a tiempos de divergencia concordantes y a las subsecuentes radiaciones aceleradas que parecen haberse dado durante el Mioceno y Pleistoceno en la región Neotropical.
- La asociación con agallas ocurrió probablemente una sola vez en la historia evolutiva de la subfamilia Doryctinae con el inquilinismo de agallas formadas en hojas como forma de vida ancestral. Otro tipo de estrategias de herbivoría como la

inducción a agallas en varios órganos de plantas y la depredación de semillas evolucionaron independientemente en los géneros de doryctinos asociados con formación de agallas y en *Allorhogas* a partir de un probable ancestro inquilino.

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