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Análisis filogenético y filogeográfico de avispas inductoras de agallas asociadas al género
Quercus en bosques fragmentados en México

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RESUMEN GENERAL

Las avispas inductoras de agallas de la familia Cynipidae (Hymenoptera: Cynipidae: Tribu Cynipini) inducen agallas específicamente en el género *Quercus*. En este estudio se analizó aspectos ecológicos, taxonómicos y evolutivos de esta interacción biótica. En el primer y segundo capítulo de la tesis evalué a lo largo de tres años de muestreo de campo el efecto de la fragmentación sobre la diversidad de cinípidos en fragmentos remanentes de bosque en la Cuenca de Cuitzeo, Michoacán. Además, se evaluó la importancia de los encinos como recurso clave para los cinípidos, y se considera la importancia de los procesos “bottom-up” (i.e. vigor de las plantas y disponibilidad de recursos) sobre su riqueza de especies. Los resultados muestran que tanto la riqueza como la abundancia de avispas inductoras de agallas asociadas a los encinos se incrementó en fragmentos de bosque más pequeños, en los bordes de los fragmentos y en los árboles aislados. Las plantas hospederas más vigorosas y la mayor producción de hojas se encontraron en los encinos aislados y los árboles presentes en fragmentos de bosque más pequeños. Otro resultado relevante fue la gran riqueza de especies de cinípidos asociados a tres especies de encino, *Quercus obtusata*, *Quercus castanea* y *Quercus deserticola*, consideradas como “Super-Hospederos”. En el tercer capítulo reconstruí las relaciones filogenéticas de 18 especies de avispas inductoras de agallas asociadas al “Super-Hospedero” *Quercus castanea*. El objetivo fue evaluar posibles explicaciones evolutivas del origen de la asociación de estas avispas con la especie de encino. El análisis filogenético reveló que las avispas asociadas pertenecen a linajes de cinípidos independientes, por lo que no ocurrió especiación *in situ* o radiación adaptativa dentro *Q. castanea*, sino que las especies de cinípidos convergieron en la misma especie de planta hospedera como eventos evolutivos no relacionados. Finalmente, en el cuarto capítulo abordamos un tema de la taxonomía de los cinípidos, que resulta muy problemática por la

presencia de adultos morfológicamente diferentes. Realizé una revisión taxonómica de las especies del género *Amphibolips* de México que no pertenecen al complejo "*niger*". Este género en México presenta caracteres morfológicos muy uniformes entre la mayoría de las especies conocidas. Sin embargo, hay algunas especies de *Amphibolips* que presentan anomalías en algunos de estos caracteres diagnósticos, lo que revela que los límites taxonómicos del género no están todavía claros. Se presenta una nueva clave de identificación para todas las especies del grupo. Siete nuevas especies para la ciencia de *Amphibolips* fueron descritas.

ABSTRACT

Gall wasps of Cynipidae family (Hymenoptera: Cynipidae: Tribe Cynipini) induce galls specifically on *Quercus*. In this study, we analyzed ecological, taxonomic and evolutionary aspects of this biotic interaction. In the first and second chapters of the thesis I evaluated over three years of field sampling the effect of fragmentation on cynipids diversity in remnant forest fragments in Cuitzeo Basin, Michoacán. Moreover, we analyzed the importance of oak trees as "key resource" for cynipids, and considered the importance of the processes "bottom-up" (i.e. vigor and resource availability) on species richness. The results show that gall wasps richness and gall abundance increased in smaller forest fragments, at fragment edges and isolated trees. The most vigorous trees were found in isolated oaks and trees presented in small forest fragments. Another important result was the great gall wasp species richness associated to three oak species, *Quercus obtusata*, *Q. castanea* and *Q. deserticola*, considered "*Super-Hosts*". In the third chapter I analyzed the phylogenetic relationships of 18 gal wasp species associated with the "*Super-Hospedero*" *Quercus castanea*. The objective was to evaluate possible evolutionary explanations of the origin of the association of these wasps with *Q. castanea*. The phylogenetic analysis revealed that the gall wasps associated belong to separate lineages cynipids. It did not occur speciation *in situ* or adaptive radiation within *Q. castanea*, but cynipids species converged on the same host plant species as evolutionary unrelated events. Finally, in the fourth chapter we address a taxonomy topic of cynipids. I conducted a taxonomic revision of the genus *Amphibolips* of Mexico that do not belong to "*niger*" complex. This gender in Mexico has very uniform morphological characters between most known species. However, there are some species that have abnormal diagnostic characters, revealing that taxonomic boundaries of the gender are still unclear. A new key identification for all species group is presented. Seven species new to science were described *Amphibolips*.

INTRODUCCIÓN GENERAL

La fragmentación de bosques es un proceso dinámico en el que el hábitat se reduce progresivamente a parches de bosque más pequeños, aislados y que son afectados por los efectos de borde (Forman y Godron 1986; Echeverría et al. 2007). Características de estos fragmentos de bosque como el tamaño, aislamiento y proporción de bordes pueden modificar la composición, abundancia y distribución de los insectos herbívoros, y por lo tanto, afectar los patrones de herbivoría (Chust et al. 2007; Ruiz-Guerra et al. 2010). En general, el efecto de la fragmentación sobre la diversidad de herbívoros refleja tanto los impactos directos sobre la dinámica de los herbívoros, como los impactos mediados a través de los enemigos naturales “efectos top-down” (Holt 1996; Stone et al. 2002; Askew et al. 2013) y sus plantas hospederas “efectos bottom-up” (Tscharntke et al. 2002). Una de las interacciones bióticas especialistas más susceptibles a la fragmentación del hábitat es la de los insectos inductores de agallas y sus plantas hospederas (Thies y Tscharntke 1999; Kruess y Tscharntke 2000; Chust et al. 2007). Los insectos inductores de agallas (IIA) reciben frecuentemente ataques por numerosos parasitoides e inquilinos (Stone et al. 2002). Los estudios que han analizado los efectos "top-down" en IIA sugieren que la fragmentación de bosques reduce en general la diversidad de insectos parasitoides favoreciendo el incremento de las poblaciones de insectos herbívoros (Hanski 1994; Kruess y Tscharntke 1994; Tscharntke et al. 2007). Los mayores niveles tróficos, como los parasitoides, son más vulnerables a la fragmentación del hábitat (Tscharntke et al. 2007) debido a los requerimientos mayores de energía y área (Holt 1996).

Otro de los principales efectos de la fragmentación de bosques, ocurre particularmente en los bordes de los fragmentos, donde existen cambios ambientales abruptos (e.j. aumento de intensidad de la luz, velocidad del viento, temperatura y disminución de la humedad) (Murcia 1995; Kapos et al. 1997) que pueden afectar los procesos “bottom-up” de las plantas

hospederas (Fernandes y Price 1988). Una característica que se ve afectada es el vigor de la planta, presentando un crecimiento más lento, o una menor producción de hojas o brotes en fragmentos de bosque (Price 1991; Saunders et al. 1991; Prada et al. 1995). La hipótesis del “Vigor de la planta” (Price 1991), ha sido usada para explicar la diversidad y distribución de IIA. Esta hipótesis predice que insectos endófagos como los IIA eligen preferencialmente plantas más vigorosas o módulos de plantas (i.e. hojas o brotes), resultando en un incremento en su desempeño y adecuación “fitness” (Price 1991). Por lo tanto, un posible escenario sugiere una reducción en la diversidad de IIA en fragmentos de bosque que presenten plantas menos vigorosas. Además, el ciclo de vida de los IIA está sincronizado con la producción de órganos de la planta hospedera debido a que la inducción de agallas requiere de la presencia de tejido vegetal indiferenciado (Weis et al. 1988; Stone et al. 2002; Hayward y Stone 2005). Sin embargo, en los fragmentos de bosque, principalmente en los bordes, la disponibilidad de recursos (producción de hojas, flores y frutos) es afectada por las condiciones ambientales presentes en los fragmentos de bosque (Mopper 2005; Karban 2007) afectando la incidencia y preferencia de los IIA (Weis et al. 1988). Como consecuencia, los árboles aislados resultado de la fragmentación, pueden representar recursos clave para la sobrevivencia de las poblaciones de insectos en ambientes fragmentados (Hanski y Gilpin 1997; Fischer et al. 2010), y representar refugios que mantienen la conectividad entre fragmentos de bosque y promueven su regeneración (Manning et al. 2006).

Sistema de estudio

Los bosques de encinos (especies de *Quercus*) son comunidades vegetales características de México, que han sido muy afectados por las actividades humanas (Rzedowski 1994). Grandes extensiones de encinares mexicanos son reducidas debido a la explotación desmedida para la obtención de carbono vegetal (Rzedowski 1994), la agricultura y la

ganadería (Nixon 1993; De Jong et al. 1999). Masera et al. (1997) han estimado que 167,000 hectáreas por año de bosques templados se pierden, lo que resulta en una tasa de deforestación anual de 0.64% en México. Además, la destrucción de los bosques de encino implica la extinción local de las especies de encinos y de docenas de especies de artrópodos (Tovar-Sánchez et al. 2003).

Los encinos son especies dominantes en los bosques templados de México y proveen funciones ecológicas como el ciclaje de nutrientes, el balance hídrico, además de que un gran número de plantas, insectos, aves y mamíferos se encuentran asociados a ellos (Faivre-Rampant et al. 2011). Particularmente, las avispas inductoras de agallas o cinípidos (Hymenoptera: Cynipidae: Tribu Cynipini) están asociadas al género *Quercus* y cuentan con aproximadamente 1000 especies agrupadas en 40 géneros (Ronquist 1999; Nieves-Aldrey 2001). Los cinípidos inducen agallas altamente diferenciadas y morfológicamente muy complejas en todos los órganos de sus plantas hospederas (e.i. flores, bellotas, hojas, ramas, yemas y raíces) (Abrahamson et al. 1998). La mayoría de los ciclos de vida de los cinípidos inductores de agallas asociados a los encinos involucran una estricta alternancia de generaciones: la generación sexual que se desarrolla en la primavera o principios del verano, mientras que la generación asexual se desarrolla durante el verano y el otoño, por lo general durante el mismo año (Stone et al. 2002).

Las avispas inductoras de agallas presentan un alto grado de especificidad a la especie de encino y al órgano atacado (Abrahamson y Weis 1987; Abrahamson et al. 1998). Sin embargo, en algunos casos una especie de *Quercus* puede ser atacada por distintas especies de cinípidos, donde cada especie de cinípido induce agallas morfológicamente diferentes (Shorthouse y Rohfritsch 1992). En la literatura se han descrito solo algunos casos como el de *Quercus turbinella* en Norte América que está asociado a 20 especies de avispas inductoras de agallas (Fernandes y Price 1988) y *Q. robur* y *Q. petraea* en Europa, que soportan más de

70 especies (Fernandes y Price 1988; Csóka et al. 2005). A estas especies de plantas se les han denominado “*Super-Hospederos*” (Araújo et al. 2013, Maldonado-López et al. en prensa) y su abundancia y distribución puede producir cambios en los patrones de riqueza locales y regionales de los IIA. Sin embargo, pocos son los estudios que han explorado las posibles explicaciones evolutivas del origen de los “*Super-Hospederos*” (Joy y Crespi 2007). Una primera hipótesis propone una radiación adaptativa *in situ* dentro de la misma especie de hospedero (Cook et al. 2002; Després et al. 2002; Joy y Crespi 2007). Una segunda hipótesis es la colonización de linajes independientes hacia la misma especie de planta hospedera que representa eventos evolutivos no relacionados, que no implican un proceso de especiación. Debido a que los insectos inductores de agallas controlan el crecimiento, la diferenciación y la fisiología de los tejidos de sus plantas hospederas, los convierte en buenos candidatos para radiar en diferentes nichos o zonas adaptativas (Schluter 2000), ya sea al cambiar de especie de planta hospedera, como a nuevos órganos dentro de la misma planta (Abrahamson y Weis 1997; Cook et al. 2002). En el caso específico de los cinípidos, la evolución de la preferencia por las plantas hospederas, parece estar caracterizada por un alto grado de conservacionismo mezclado con cambios poco frecuentes a hospederos filogenéticamente distantes (Ronquist y Liljeblad 2001; Cook et al. 2002). Sin embargo, se han documentado casos de especiación dentro del hospedero como las avispas del género *Andricus* (Cynipini) (Cook et al. 2002) donde la divergencia fue dirigida principalmente por factores ecológicos que operan a una escala individual de las plantas hospederas, como competencia indirecta por espacios libres de enemigos (Stone et al. 2002) o competencia directa por sitios de oviposición (Hails y Crawley 1991).

Los cinípidos se distribuyen principalmente en las zonas templadas del Hemisferio Norte (región holártica). El patrón de distribución del grupo de avispas inductoras de agallas está determinado por la distribución de los encinos. En Europa se han reportado alrededor de 280

especies de cinípidos en las 25 especies de encinos reportadas para ese continente (Nieves-Aldrey 2001). En México hay reportes de 157 especies (Pujade-Villar et al. 2009). Sin embargo, los valores de diversidad de cinípidos aún son inciertos y algunos autores sugieren que la mayor riqueza de avispas asociadas a los encinos potencialmente se encuentra en México debido a la gran riqueza de especies de *Quercus* (161 especies incluyendo 109 especies endémicas) (Valencia-A 2004).

La taxonomía de los cinípidos es problemática debido tanto a la falta de especialistas en el mundo, como a la presencia de adultos morfológicamente diferentes, resultado de ambas generaciones. Por lo que en muchos estudios, estos insectos adultos han sido descritos como especies diferentes y en ocasiones incluso en géneros separados (Pujade-Villar et al. 2001). Especialmente el género *Amphibolips* Reinhard 1865 es un género distintivo de avispas inductoras de agallas registradas como exclusivas de América. En la actualidad, 45 especies son conocidas en todo el área de distribución de este género, que se extiende desde Canadá hasta Panamá. En México 12 especies de *Amphibolips* son conocidas (Kinsey 1937) y los caracteres morfológicos de los adultos y sus agallas son muy uniformes entre la mayoría de las especies conocidas. Sin embargo, hay algunas especies de *Amphibolips* que presentan anomalías en algunos de estos caracteres diagnósticos, lo que revela que los límites taxonómicos del género no están todavía claros. Particularmente en México las especies de *Amphibolips* fuera del complejo "*niger*" parecen ser un grupo morfológicamente distintivo. Por lo tanto, resulta importante una revisión taxonómica de las especies de *Amphibolips* de México que no pertenecen a este complejo.

Estructura y objetivos del estudio

Esta tesis doctoral está formada por cuatro capítulos. El objetivo del primer capítulo fue evaluar a lo largo de tres años de muestreo de campo, el impacto de la fragmentación de

bosques sobre la interacción de las avispas inductoras de agallas y los encino como recurso clave para los insectos inductores de agallas. Analizamos la importancia de los procesos “bottom-up” (i.e. vigor de las plantas) sobre la comunidad de avispas inductoras de agallas en un paisaje de fragmentación y determinamos la variación temporal y espacial de la diversidad de agallas y vigor de las plantas hospederas, analizando la importancia del tamaño de fragmento, los efectos de borde y la presencia de árboles aislados en la Cuenca de Cuitzeo, Michoacán.

El objetivo del segundo capítulo fue evaluar el efecto de la fragmentación del bosque sobre la disponibilidad de recursos (hojas) para las avispas inductoras de agallas asociadas a los encinos. Debido a que los cinípidos presentan un alto grado de especificidad a la especie de encino y su ciclo de vida debe de estar muy sincronizado con la fenología de la planta hospedera para poder inducir agallas exitosamente. Por lo tanto en este capítulo analizamos durante tres años los cambios en la riqueza y abundancia de avispas inductoras de agallas, asociados a la producción de hojas en fragmentos de bosque de distintos tamaños, borde de los fragmentos y en árboles aislados en la Cuenca de Cuitzeo, Michoacán.

En el tercer capítulo reconstruimos las relaciones filogenéticas de 18 especies de avispas inductoras de agallas asociadas al "*Super-Hospedero*" *Quercus castanea*. Nuestro objetivo fue evaluar dos posibles explicaciones evolutivas del origen de la asociación de estas avispas con la especie de encino. El muestreo de agallas se realizó a lo largo de toda la distribución geográfica de *Q. castanea*. La primera hipótesis propone que los cinípidos radiaron adaptativamente *in situ*, esperando encontrar que las especies de avispas asociadas a esta especie de encino, formen un grupo monofilético con radiación adaptativa a diferentes nichos o zonas adaptativas. La segunda hipótesis postula que la asociación de esta especie de "*Super-Hospedero*" con los cinípidos fue resultado de la convergencia de estas especies de

avispas, hacia la misma especie de planta hospedera. Además evaluamos la relación entre la proximidad filogenética de los cinípidos y la posición y fenología de las agallas.

Finalmente, el objetivo del cuarto capítulo fue realizar una revisión taxonómica de las especies del género *Amphibolips* de México, que no pertenecen al complejo "*niger*". Se realizó una colecta intensiva en varios estados de México. Para una completa revisión del género, se analizaron materiales "tipo" de tres especies de *Amphibolips* descritas por Kinsey en 1937 y se incluyeron dos especies relacionadas de Panamá. Siete nuevas especies para la ciencia de *Amphibolips* fueron descritas. De esta manera se obtuvo una nueva clave de identificación para todas las especies del grupo.

CAPITULO I.

Gall wasp community response to fragmentation of oak tree species: importance of fragment size and isolated trees

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EN PRENSA. ECOSPHERE

Abstract

We explore the impact of habitat fragmentation on interactions between keystone resources of forest trees – oaks, genus *Quercus* (Fagaceae) – and an associated radiation of specialist cynipid gall wasps. Habitat fragmentation is predicted to have bottom-up impacts on cynipid communities through impacts on host plant quality (plant vigour hypothesis). We explored the bottom-up impacts on cynipid communities of habitat fragment size, fragment edge effects and presence of isolated oaks. We quantified temporal and spatial variation of leaves produced in the canopy to quantify plant vigour, and surveyed cynipid gall species abundance and richness over three years using 15 permanent forest patches and 25 isolated oaks in a fragmented oak woodland landscape in central Mexico. Cynipid gall abundance and species richness were higher in isolated oaks and small woodland fragments than in larger ones. Cynipid abundance and species richness were also higher along fragment edges in comparison with fragment interiors. This contrasts with patterns observed in other taxa. In addition, host plant quality was higher in isolated trees, in smaller fragments and along fragment edges. We therefore hypothesize that observed patterns in cynipid abundance and species richness are driven by changes in host plant quality due to forest fragmentation. Our data represent a baseline for longer-term monitoring of fragmentation effects at a landscape scale. Further work is required to explore alternative potential explanations for observed patterns, including the estimation of potential top-down impacts of fragmentation mediated by natural enemies.

Key words: cynipids; edge effect; habitat fragmentation; isolated trees; Lake Cuitzeo basin; oak gall wasps; plant vigour; *Quercus*.

Introduction

Oaks (*Quercus* species) are dominant late successional species in a wide range of habitats and offer key environmental services (i.e., carbon sequestration, energy production and water cycle regulation) (Faivre-Rampant et al. 2011). Oaks support characteristic and species-rich assemblages of associated organisms, particularly herbivorous insects (Tews et al. 2004, Tovar-Sánchez and Oyama 2006*a, b*). Of these, the most specialized include the cynipid gall wasps (Hymenoptera: Cynipidae: Cynipinae). Each gall wasp is specific to a single oak species or higher taxonomic section (Nieves-Aldrey 2001, Stone et al. 2009). For example, very few cynipids that induce galls on white oaks (*Quercus* section *Quercus*) also induce galls in other taxonomic sections (e.g. red oaks, section *Lobatae*) (Abrahamson et al. 2003) and evolutionary shifts of gall wasps between oak sections are extremely rare (Stone et al. 2009). Cynipids are a useful model system in examining patterns of insect herbivore species richness and distribution due to their species richness and host specificity (Hayward and Stone 2005). An important feature of some oak-cynipid systems is the ability of some oak species to support very rich communities, providing considerable resolution for analysis of habitat-associated changes in assemblage structure. Examples include *Quercus turbinella* in North America, which supports 20 species of cynipids wasps and *Quercus robur* and *Quercus petraea* in Europe, which support more than 70 species (Fernandes and Price 1988, Csóka et al. 2005). Such host plant species have been called “*super-hosts*” (Araújo et al. 2013). Here, we analyze oak cynipid assemblages associated to “*super-hosts*” species to examine the impact of habitat fragmentation on Mexican oak communities.

In Mexico, oak forests have been highly fragmented because of the great economic importance of the trees (Valencia-Avalos and Nixon 2004). Masera et al. (1997) have estimated that 167,000 ha/yr of temperate forests are lost, resulting in an annual deforestation rate of 0.64% in Mexico. An extreme consequence of habitat fragmentation is the isolation of

individual trees, which may then represent refuges and keystone resources to herbivorous insects (Hanski and Gilpin 1997, Tews et al. 2004, Manning et al. 2006, Müller and Goßner 2007, Fischer et al. 2010). Such isolated trees enhance the connectivity among forest fragments and aid their regeneration (Manning et al. 2006).

Specialized biotic interactions associated with keystone resources are seriously affected by habitat fragmentation (Tews et al. 2004, Wang et al. 2005, Rodríguez-Cabal et al. 2010). Forest fragmentation can modify the composition, abundance and distribution of herbivores such as gall inducing insects (Didham et al. 1996, Chust et al. 2007, Ruiz-Guevara et al. 2010, Kaartinen and Roselin 2011). In general, habitat fragmentation affects herbivore diversity through their biotic interactions (Tscharntke 1992, Didham et al. 1996, Fagan et al. 1999) with natural enemies (top-down effects) (Holt 1996, Stone et al. 2002, Askew et al. 2013) and host plants (bottom-up effects) (Tscharntke et al. 2002). Holt (1996) developed models showing that higher trophic level species such as parasitoids should be more vulnerable to the effects of habitat fragmentation than herbivorous insects due to their higher requirements of energy and area in the forest (see also Tscharntke et al. 2007). The release of herbivores from top-down control in habitat fragments can thus benefit local populations (Kruess and Tscharntke 1994). Gall wasp populations are strongly influenced by top-down effects (Stone et al. 2002), with most studies showing high mortality through attack by chalcid parasitoids (Moriya et al. 1989, Stone et al. 2002, Askew et al. 2013). If top-down effects have a strong impact on gall wasp community structure, habitat fragments could support relatively enriched gall inducer communities by providing relatively enemy-free space.

Habitat fragmentation may also influence gall inducer population and community dynamics through bottom-up effects on host plant preference (Yamasaki and Kikuzawa 2003, Ruiz-Guerra et al. 2010) and quality. Habitat fragments experience a suit of environmental

changes, which are even stronger along the fragment edges (Saunders et al. 1991, Murcia 1995). Certain plants are adapted to the conditions provided by continuous forest (i.e. higher humidity, lower temperature, photosynthetically active radiation and wind speed) (Young and Mitchell 1994, Chen et al. 1995). However, changes in abiotic conditions due to fragmentation may be stressful for these plants (Fernandes and Price 1988), reducing plant vigour (i.e. reduction in growth rate, and production of leaves, shoots and reproductive structures) (Price 1991, Saunders et al. 1991, Prada et al. 1995). Some studies show that gall inducing insects prefer vigorously growing plants or plant modules (e.g. leaves or shoots) (Price 1991, Preszler and Price 1995, Cornelissen et al. 2008), hence, it is expected that gall inducing insects are less abundant or diverse in small patches if plants are more stressed.

The effects of habitat fragmentation on populations of gall inducing insects are poorly known (but see Chust et al. 2007) and few long-term studies have analyzed gall inducing insect interactions (Santos et al. 2008). Studies to date show no consistent response of gall inducing insects to habitat fragmentation; in some cases, gall abundance increases with habitat fragmentation (Wang et al. 2005, Chust et al. 2007), but in others, no relationship has been found (Julião et al. 2004, Dunley 2009). High regional species richness of oak gall wasps, combined with ease of sampling make gall wasps a suitable taxon for quantifying impacts of habitat fragmentation (Kinsey 1937, Pujade-Villar et al. 2009, Nieves-Aldrey et al. 2012). Our study documents biotic interactions over three years. We studied the spatial and temporal variation of gall wasp diversity and plant vigour across oak fragmented populations, to quantify the importance of fragment size, edge effects and isolated oaks on gall abundance and diversity in a fragmented landscape in Mexico. We hypothesize that habitat fragmentation will have a strong impact on gall wasp community structure, where most fragmented habitats will support enriched gall wasps communities. A second hypothesis proposes that isolated oaks represent key resources for gall wasps, having higher richness and

abundance of gall wasps in isolated trees than in forest fragments. Finally, we expected that plant vigour (canopy cover) will be negatively influenced by habitat fragmentation affecting in turn gall wasps diversity.

Methods

Study location

This study was conducted in the Lake Cuitzeo basin, a hydrological unit with an area of 4026 km² located in Michoacán state, Mexico. It is located in the physiographic province of the Transmexican Volcanic Belt. The basin contains the Lake of Cuitzeo with a wetland of approximately 300 km². The basin is representative of the environmental and socioeconomic conditions of central Mexico and has experienced strong fragmentation resulting in a highly fragmented landscape forming a mosaic of scrubland, forests (mainly pine, oak and mixed forests) and agricultural lands (López et al. 2006). The basin includes Morelia, the state capital of Michoacán state, for which urban area grew six-fold between 1975 and 2000 (López et al. 2001). Land cover and land use change analyses indicate that the period 1986-1996 was characterized by high rates of deforestation and forest degradation throughout the basin (Mendoza et al. 2011) due to strong human pressures including urban growth, expansion of the agricultural frontier and the removal of trees for charcoal production (López et al. 2006, Aguilar et al. 2012, Castillo-Santiago et al. 2013). Consequently, large continuous oak populations have been reduced to a many small patches of variable size.

Fragmentation of oak forests and selection of sampling sites

Michoacán state has a very high deforestation rate of approximately 1.8 % per year over 18 years (Bocco et al. 2001). Remaining oak forests in the Lake Cuitzeo basin have previously been characterized into 1241 fragments of different sizes (López et al. 2001). We

selected 15 permanent forest fragments which were divided equally among three size categories: 1) five small (≤ 4 ha) forest fragments; 2) five medium-sized (4-12 ha) forest fragments; and 3) five large (>12 ha) forest fragments. We also selected 25 individual oak trees isolated by distances of at least 400 m from surrounding forest (see Fig. A.1 in Supporting information). The oak species present at each sampling site are shown in Table A.1.

Study system and sampling

Cynipid gall wasps induce structurally complex galls on various oak tissues (Hayward and Stone 2005). Most oak gall wasp life cycles involve strict alternation between two generations: a sexual generation gall develops in the spring or early summer, while an asexual generation develops during the summer and autumn, usually during the same year (Stone et al. 2002). Oak gall wasp taxonomy is problematic, and adults of the two generations are so different morphologically that they have sometimes been described as different species, occasionally even in separate genera (Pujade-Villar et al. 2001). Nevertheless, galls from each generation can usually be identified unambiguously on the basis of characteristic morphology, location on the tree and oak host taxon (Stone et al. 2002, Stone et al. 2009).

Gall surveys were conducted monthly from September 2007 to October 2010. At each study site, we established two parallel 5m x 50m transects, one located on the fragment edge and one in the fragment interior (average distance of 115 m from forest border). In each transect, we recorded oak density and oak species richness. To determine the effects of habitat fragmentation on these measures, we used a generalized linear model (GLM) analysis for each response variable, using a Poisson error distribution and log link function. An LSMeans test was used for a posteriori comparisons (SAS 2000).

We recorded monthly cynipid gall abundance and species richness on each individual oak tree. Gall sampling incorporated the full height of each canopy, through collection of three branches from each of top, intermediate and bottom strata of each tree, following Cuevas-Reyes et al. (2004). We verified that each distinct gall morphology collected was induced by a different gall wasp taxon by rearing galls in the laboratory and identifying the adult to genus and to morphospecies. For ecological studies of gall inducing insects, morphospecies has become an acceptable substitute for species, assuming that each gall morphospecies is unique to a particular gall inducing insect (Stone and Schönrogge 2003, Cuevas-Reyes et al. 2004, Cuevas Reyes et al. 2011). Gall wasps can then be identified on the basis of their characteristic gall morphology, location on the oak, and oak host taxon (Abrahamson et al. 1998, Stone et al. 2002, Stone et al. 2009). Samples of all gall species collected are preserved in a dry collection at the Museo Nacional de Ciencias Naturales, Madrid (Spain) and the Laboratorio de Ecología Genética y Molecular, CIEco, UNAM (Mexico), awaiting formal taxonomic identification.

The surveys and analyses were separated into spring and autumn generations on the basis of phenology and wasp morphology to reduce issues of non-independence associated with having one or both generations of a single species in the same analysis (Bailey et al. 2009). We used a GLM to test differences in gall richness between all oak species. To determine the effects of fragment size and distance to the edge on gall wasp species richness and abundance, we performed a GLM. The model used a Poisson error distribution and log link function. An LSMMeans test was used for a posteriori comparisons (SAS 2000). We also analyzed overall changes in community composition in response to the fragment size and isolated oaks using a permutational multivariate ANOVA (Permanova; Anderson 2005) for oak and gall wasp community composition using species richness, McIntosh diversity index and oak abundance or gall abundance. To examine whether differences in species richness of

gall wasps between fragment sizes were driven by differences in gall abundance, we constructed rarefaction curves for each fragment size and estimated cumulative species per tree using EstimateS 9.1.0 (Colwell 2011).

Plant vigour

Plant vigour was quantified as numbers of leaves produced in the canopy (Prada et al. 1995, Faria and Fernandes 2001). This is probably an appropriate measure for gall wasps, many of which induce their galls on these organs or associated buds and shoots (Price 1991, Fritz et al. 2003). In each transect, we marked the adult trees of each species and in each survey we classified leaf canopy cover according to the proportion of trees in each of four production categories: 1) 0%; 2) 1-25%; 3) 26-50% and 4) 51-100%, following Williams et al. (1997). We used a GLM to determine the effect of fragment size on plant vigour. The same analysis was conducted to determine the differences in plant vigour between transects. The analysis used a binomial distribution and a logit link function. A linear regression analysis was used to determine the relationship between gall abundance and percentage of canopy cover, for each fragment size and isolated oaks.

Results

General description of the oak-gall community

Over all fragment types (isolated oaks, small, medium-sized and large fragments), we sampled ten *Quercus* species. In total, our surveys incorporated 179 trees of five species in the white oak section *Quercus* (*Q. laeta*, *Q. obtusata*, *Q. deserticola*, *Q. magnoliifolia* and *Q. glaucooides*) and 206 trees of five species in the red oak section *Lobatae* (*Q. castanea*, *Q. scythophylla*, *Q. candicans*, *Q. dysophylla* and *Q. crassifolia*). All except *Q. crassifolia* hosted cynipid galls. Over three years, we sampled a total of 5843 galls, comprising 1336

spring generation galls and 4507 autumn generation galls. We identified 105 distinct gall morphotypes, of which 69 (65.7 %) were induced on leaves (14 spring and 55 autumn generations), 8 (7.6 %) on leaf petioles (6 spring and 2 autumn generations), 20 (19 %) on stems (8 spring and 12 autumn generations), 7 (6.7 %) on buds (all autumn generation) and 1 (1%) on catkins (spring generation).

Oaks species richness and host-plant density

Oak species richness did not differ significantly among forest fragment size categories ($\chi^2 = 0.4$, d.f. = 3, $P = 0.8$) or between edge and internal transects in each fragment ($\chi^2 = 0.5$, d.f. = 1, $P = 0.9$). Similarly, we found no differences in host plant density among forest fragment size categories ($\chi^2 = 0.04$, d.f. = 3, $P = 0.9$) or between edge and internal transects in each fragment ($\chi^2 = 0.08$, d.f. = 1, $P = 0.9$). These results were supported by Permanova analysis of oak communities, which showed diversity, richness and density to be similar between all fragments (Permanova: density: $F = 0.47$, $P = 0.6$; richness: $F = 0.8$, $P = 0.4$; McIntosh diversity index: $F = 0.1$, $P = 0.9$).

Oak gall wasp diversity patterns

Oak gall wasp species richness differed among oak species. *Quercus obtusata*, *Q. castanea* and *Q. deserticola* may be considered "super-hosts" because they supported most of the gall species richness (Table 1) and abundance (Table 2). Oak gall wasp richness was significantly higher in *Q. obtusata* (40 gall species), *Q. deserticola* (25) and *Q. castanea* (33), in comparison with *Q. candicans* (8), *Q. scythophylla* (8), *Q. magnoliifolia* (7), *Q. glaucooides* (7), *Q. dysophylla* (2) and *Q. laeta* (2) ($\chi^2 = 46.1$, d.f. = 8, $P = 0.0001$).

We found a similar pattern of oak gall wasp species richness in both gall generations (Fig. 1). Isolated trees had higher gall wasp species richness (spring: 2.7 ± 0.3 ; autumn: $3.1 \pm$

0.4) than trees in small (spring: 1.7 ± 0.2 ; autumn: 2 ± 0.1), medium-sized (spring: 1.5 ± 0.11 ; autumn: 2.1 ± 0.2) and large (spring: 1.3 ± 0.1 ; autumn: 1.58 ± 0.1) forest fragments (spring: $\chi^2 = 19.8$, d.f. = 3, $P = 0.0002$; autumn: $\chi^2 = 13.3$, d.f. = 3, $P = 0.004$). There were no significant differences in gall species richness between generations in each fragment size ($\chi^2 = 12.7$, d.f. = 1, $P = 0.3$) (Fig. 1A). Gall species richness was higher in fragment edges (spring: 1.6 ± 0.1 ; autumn: 1.73 ± 0.08) than in fragment interiors (spring: 1.9 ± 0.16 ; autumn: 1.43 ± 0.1) (spring: $\chi^2 = 16.43$, d.f. = 1, $P = 0.013$; autumn: $\chi^2 = 11.21$, d.f. = 1, $P = 0.02$). Both gall generations showed similar contrasts between fragment edge and interior ($\chi^2 = 0.98$, d.f. = 1, $P = 0.4$) (Fig. 1B).

Gall abundance (Fig. 2) was higher in isolated oaks (spring: 21.39 ± 25 ; autumn: 41.0 ± 5) than on trees in small (spring: 14.0 ± 6 ; autumn: 7.45 ± 2.6), medium-sized (spring: 7.1 ± 2.0 ; autumn: 14.25 ± 3.5) and large forest fragments (spring: 4.8 ± 2.1 ; autumn: 7.7 ± 2.6) (spring: $\chi^2 = 1567$, d.f. = 3, $P = 0.0001$; autumn: $\chi^2 = 174.6$, d.f. = 3, $P = 0.0001$). Isolated trees also showed higher abundance of autumn generation than spring generation galls ($\chi^2 = 9.4$, d.f. = 1, $P = 0.003$) (Fig. 2A). As for species richness, gall abundance was higher in fragment edges (spring: 10.77 ± 1.7 ; autumn: 13.19 ± 1.6) than in fragment interiors (spring: 6.6 ± 2 ; autumn: 7.8 ± 1.4) (spring: $\chi^2 = 31.6$, d.f. = 1, $P = 0.0001$; autumn: $\chi^2 = 359.3$, d.f. = 1, $P = 0.0001$), again with no difference in pattern between gall generations ($\chi^2 = 5.5$, d.f. = 1, $P = 0.5$) (Fig. 2B).

These results were supported by Permanova analysis, which showed gall community diversity, abundance and richness in each generation to be higher in isolated oaks and small fragments than in larger forest fragments (Abundance: spring: $F = 4.7$ $P = 0.004$, autumn: $F = 8.5$ $P = 0.001$. Richness: spring: $F = 3.2$ $P = 0.05$, autumn: $F = 11.3$ $P = 0.03$. McIntosh diversity index: spring: $F = 6.8$ $P = 0.01$, autumn: $F = 11.6$ $P = 0.001$). Rarefaction curves

showed that the observed differences in cumulative species richness persisted even when samples were rarefied to similar abundances of individuals (Fig. 3).

Plant vigour

We found significant differences in oak tree vigour (as measured by leaf canopy cover) among fragment size classes. Leaf production was significantly higher in isolated oaks (69.7 ± 3.5) in comparison with small (48.3 ± 1.2) medium-sized (45.9 ± 1.4) and large fragments (44.1 ± 1.1) ($F = 21.3$, d.f. = 3, $P = 0.0001$). We also found differences in oak organ production between the interior and the edge of the fragments. Leaf production was higher at fragment edges than interiors ($F = 10.67$, d.f. = 3, $P = 0.001$). Finally, we found a positive relationship between gall abundance and percentage of canopy cover only for isolated trees ($F = 37.4$; $R^2 = 0.61$, $P = 0.0001$). No relationship between gall abundance and percentage of canopy cover was observed in small fragments ($F = 3.2$; $R^2 = 0.12$, $P > 0.05$), medium-sized fragments ($F = 4.8$; $R^2 = 0.04$, $P > 0.05$) and large fragments ($F = 2.2$; $R^2 = 0.06$, $P > 0.05$).

Discussion

We found that both gall wasp species richness and abundance increased with habitat fragmentation. Gall wasp species richness and abundance were also higher along fragment edges than in the interior. Most strikingly, isolated oaks supported high gall wasp richness and abundance, and can thus be seen as key resources for cynipid survival and reproduction in a fragmented landscape (Chust et al. 2007, Müller and Goßner 2007).

Our results are initially counterintuitive, and contrast with the predictions of Holt's (1996) model and patterns observed in other herbivore systems (e.g. Ruiz-Guerra et al. 2010). Landscape fragmentation reduces the connectivity between patches, diminishing richness of

organisms living in smaller patches due to higher rates of extinction, and reducing occupancy in the most isolated sites through low rates of recolonization (Hanski 1999). However, the extent and spatial scale of predicted fragmentation effects depend not only on structural connectivity, but also on the dispersal ability of the study species (Hanski 1999, Driscoll and Weir 2005). Species with low dispersal ability are more severely affected by fragmentation, and are the first to lose the ability to recolonize as habitat fragments become smaller and more isolated (Thomas 1995). Gall wasps, however, include highly dispersing species, and can spread a long way very quickly in air currents, even across unfavourable habitats (Hough 1951, Stone and Sunnucks 1993, Nieves-Aldrey 1995, Schönrogge et al. 2011). In this way, isolated oaks analyzed in our study, can promote the persistence of different gall wasp species along a fragmented landscape. For these reasons, we propose that oak gall wasps may have relatively high probability of locating isolated habitat fragments and isolated trees (Dauber et al. 2005). Higher frequency of encounter may also explain the high gall wasp abundance and richness at fragment edges in comparison with their interiors.

Studies that have analyzed the importance of isolated oaks on insect diversity consider them islands because they increase the survival of herbivore specialists (Chust et al. 2007, Müller and Goßner 2007). In general, isolated trees are considered keystone resources because of their ecological importance relative to their low abundance and the small area occupied (Manning et al. 2006). In tropical and temperate systems, isolated trees provide numerous ecological functions across fragmented landscapes (Ozanne et al. 2000, Manning et al. 2004). For example, canopy invertebrate arthropods, birds and mammals can all depend on isolated trees as a food resource, shelter or nesting site (Tews et al. 2004, Manning et al. 2006). Our study found isolated oaks to maintain the highest gall wasp diversity, suggesting that these trees represent keystone resources for gall wasps that contribute to their survival and reproduction in the fragmented landscape of the Lake Cuitzeo basin.

Fragmentation and host plant quality

An important finding of our study is that habitat fragmentation strongly influences host plant quality. Host quality, in terms of abundance of gall induction sites (leaves, and inflorescence associated buds and petioles), was highest in isolated trees and in smaller forest fragments relative to larger fragments and along fragment edges relative to their interiors. We observed a similar pattern, though unquantified, for oak stems – which are also gall induction sites. We also found a positive relationship between gall abundance and canopy cover in isolated oaks. These patterns in host plant quality are entirely concordant with the observed patterns in gall wasp abundance and species richness. Even though we have not demonstrated the cause and effect relationship, the patterns observed across this set of fragments are consistent with strong bottom-up effects of host plant quality on cynipid wasp abundance and species richness (Price 1991, Preszler and Price 1995). One possible explanation for the observed variation in plant quality may be the effect of environmental stress resulting from habitat fragmentation (Fernandes and Price 1988). Changes in abiotic conditions in forest fragments are particularly pronounced at forest edges (i.e. increased solar radiation, increased air temperature, decreased air humidity and increased wind speed) (Young and Mitchell 1994, Chen et al. 1995, Kapos et al. 1997). These changes could physiologically stress arboreal species in fragmented habitats, and cause trees to promote more frequent flowering, fruiting (Aldrich and Hamrick 1998), leaf production (Lovejoy et al. 1986, Sundarapandian and Swamy 1999) and leaf shedding (Sizer and Tanner 1999). For example, Magrath et al. (2014) indicated that some plant species show compensatory responses to habitat fragmentation involving demographic effects, and changes in plant defenses and regrowth rates. Alternatively, fragmentation may confer benefits; for example, isolated trees are free of competition from neighboring conspecifics or other species. Plant vigour may increase after

disturbance because the timing of leaf abscission, leaf expansion and production is altered in these environmental conditions (Trombulak and Frissell 2000, Karban 2007).

Taken together, these host plant changes result in a greater abundance of gall induction sites for oak cynipids in isolated oaks, small fragments and forest edges. In addition, gall inducing insect radiation is expected to happen in harsh environments (e.g. xeric conditions) (Price et al. 1998, Cuevas-Reyes et al. 2004) since dry condition is known to be associated with gall richness (Price et al. 1998). Specifically, edge effect can reproduce similar conditions to those found in more xeric habitats (Fernandes and Price 1988) explaining the higher gall inducing insect richness (Murcia 1995, Araùjo et al. 2011). Stressed plants can also produce higher concentrations of chemical defense (e.g. tannins) (Müller et al. 1987, Stone et al. 2002), which decreases the frequency of other herbivores, predators and fungi and creates an enemy-free space for gall inducing insects (Fernandes and Price 1988, Fleck and Fonseca 2007).

Therefore, habitat fragmentation can favor the colonization and maintenance of gall inducing insects in comparison with other insect guilds (Ruiz-Guerra et al. 2010, Kaartinen and Roselin 2011). Some studies have shown a “crowding effect” that is a relatively positive effect of fragmentation on insect population density (Debinski and Holt 2000). After habitat fragmentation, insect populations may disperse to adjacent fragments, resulting in a local increase in population density (Debinski and Holt 2000, Grez et al. 2010) in small fragments that have a larger edge proportion (Fagan et al. 1999, Grez et al. 2010). Our results suggest a "crowding effect" of gall wasp community, in the remaining habitat as small fragments and isolated oaks.

Two further points need to be made. First, alternative correlated factors may have caused the observed patterns in gall communities. For example, habitat fragmentation may have influenced cynipid diversity through top-down effects mediated by natural enemies such

as parasitoids or lethal inquilines, which can inflict high mortality on gall wasp populations (Stone et al. 2002). In small or isolated habitat fragments, phytophagous insects increase population densities when they are released from top-down control (Kruess and Tscharntke 1994, Roland and Taylor 1997). High trophic levels such as parasitoids are more affected by habitat fragmentation than herbivores (Kruess and Tscharntke 1994, Davies et al. 2000). According to theoretical (Holt et al. 1999) and empirical studies (Kruess and Tscharntke 1994, Thies and Tscharntke 1999), the main factors are that: 1) Parasitoids are more sensitive to habitat fragmentation than their hosts, because they can only colonize patches already occupied by their hosts (Weisser 2000, van Nouhuys 2005); 2) Parasitoids have smaller population sizes and depend more on recolonization processes because they suffer more from frequent disturbances and their populations are more likely to become extinct (Pimm 1991, Lawton 1995, Holt et al. 1999); 3) Habitat isolation will negatively affect parasitoids even on small spatial scales because they can disperse less well than second-trophic-level insects (Roland 1993). In this way, enemy-imposed mortality falls in smaller or more isolated fragments, resulting in ecological release of oak gall wasp populations (Holt 1996, Chust et al. 2007). If top-down forces have a strong impact on gall wasp community structure, habitat fragments could support relatively enriched gall inducer communities by providing relatively enemy-free space. One testable explanation for our results is that habitat fragmentation indirectly affects cynipid diversity due to the decrease or disappearance of natural enemy populations (such as parasitoids and inquilines). Second, the effects of habitat fragmentation on local patterns of cynipid communities are likely to be independent of patterns at larger regional scales (e.g. Cornell 1985), paralleling patterns that have been seen in other galling herbivore communities (Araùjo et al. 2013). It remains to be seen whether the patterns described for gall inducers differ from those observed in other herbivore guilds because of specific properties of gall-associated communities (Ruiz-Guerra et al. 2010).

Oaks species as "Super-hosts"

In the literature, there is a lack of studies showing that oak species can support many gall wasp species (Fernandes and Price 1988, Csóka et al. 2005). In our study, we found that three oak species (*Quercus obtusata*, *Quercus castanea* and *Quercus deserticola*) hosted 80 oak gall wasp species between them, which have been called “*super-hosts*” (Araújo et al. 2013). The difference in species richness among oak species is striking, and the cause(s) of this variation require further study. It remains to be seen whether high diversity in these Mexican species reflects high net rates of speciation by specialist lineages associated with each oak, high rates of colonization by independent lineages, or a combination of both of these (Cook et al. 2002, Joy and Crespi 2007).

Given ongoing deforestation in the Lake Cuitzeo basin, it is therefore crucial to determine the critical fragmentation level (minimum fragment size, maximum fragment separation) beyond which populations of component species will collapse (Olson and Andow 2008). In recently fragmented habitats, local diversity may remain higher than the sustainable equilibrium value (supersaturation) (Boudjemadi et al. 1999), showing gradual decline in species richness with fragment age until this equilibrium is reached (Eliason and Potter 2000, Ribas et al. 2005). Our study provides a 3-year baseline of data collected using systematic and standardized sampling, on which future sampling can build to explore longer term changes in the oak gall wasp community.

Our study shows the variety of effects that habitat fragmentation exerts on remnant native populations of oak gall wasp and their host plants. We concluded that habitat fragmentation affects gall wasp diversity and plant vigour, with higher species richness, abundance and canopy cover in small fragments and isolated oaks of the Lake Cuitzeo basin, in comparison with larger fragments. Similarly, we found a positive effect of forest edge on

gall richness and abundance and plant vigour. Isolated oaks can be considered keystone resources for maintaining of gall wasp species diversity in a fragmented landscape in Mexican temperate forests. Finally, in future studies, it will be important to evaluate the impact of “*super-hosts*” species in the insect community associated in fragmented landscapes.

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Appendix

Table A.1. Oak species present at each sampling site; Figure A.1. Field sampling sites.

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Table 1. Differences in oak gall wasp species richness in oak “*super-hosts*” species. GENMOD procedure (SAS 2000) was applied for modelling log function to each plant species. Different superscript letters after values indicate significantly different means. Values in bold show the maximum for each oak species and metric. Values shown are means \pm 1 standard error.

Host plant	Isolated trees	Small fragments	Medium-sized fragments	Larger fragments	χ^2	$P <$
<i>Q. obtusata</i>	13.3^A (± 0.8)	5.1 ^B (± 0.9)	2.4 ^C (± 0.9)	1.6 ^C (± 0.7)	8.1	0.04
<i>Q. castanea</i>	12.7^A (± 1.2)	5.2 ^B (± 0.5)	3.5 ^C (± 0.5)	1.1 ^C (± 0.5)	28.3	0.001
<i>Q. deserticola</i>	10.8^A (± 0.8)	4.8 ^B (± 0.6)	4.1 ^B (± 0.7)	1.8 ^C (± 0.5)	11.9	0.007

Table 2. Differences in oak gall wasp abundance in oak “*super-hosts*” species. GENMOD procedure (SAS 2000) was applied for modelling log function to each plant species. Different superscript letters after values indicate significantly different means. Values in bold show the maximum for each oak species and metric. Values shown are means \pm 1 standard error.

Host plant	Isolated trees	Small fragments	Medium-sized fragments	Larger fragments	χ^2	$P <$
<i>Q. obtusata</i>	26.8^A (\pm 4)	8.1 ^B (\pm 1.3)	8.1 ^B (\pm 1.3)	5.1 ^C (\pm 1.6)	16.2	0.005
<i>Q. castanea</i>	21.3^A (\pm 5)	17.1 ^B (\pm 3.0)	5.1 ^C (\pm 4.0)	4.9 ^C (\pm 3.0)	21.1	0.0001
<i>Q. deserticola</i>	23.3 ^C (\pm 9)	45.3^B (\pm 6.0)	9.8 ^C (\pm 3.0)	5.3 ^D (\pm 7.0)	4.2	0.03

Figures

Fig. 1. Impacts of fragment size and interior/exterior transect location on gall species richness, by generation. A) Effects of fragment size, including isolated oaks. B) Comparison between interior and edge transects in each fragment. Untransformed data are shown.

Fig. 2. Impacts of fragment size and interior/exterior transect location on gall abundance, by generation. A) Effects of fragment size, including isolated oaks. B) Comparison between interior and edge transects in each fragment. Untransformed data are shown.

Fig. 3. Rarefaction curves plotting the number of species of gall wasps *vs.* the number of galls sampled.

Fig. 1

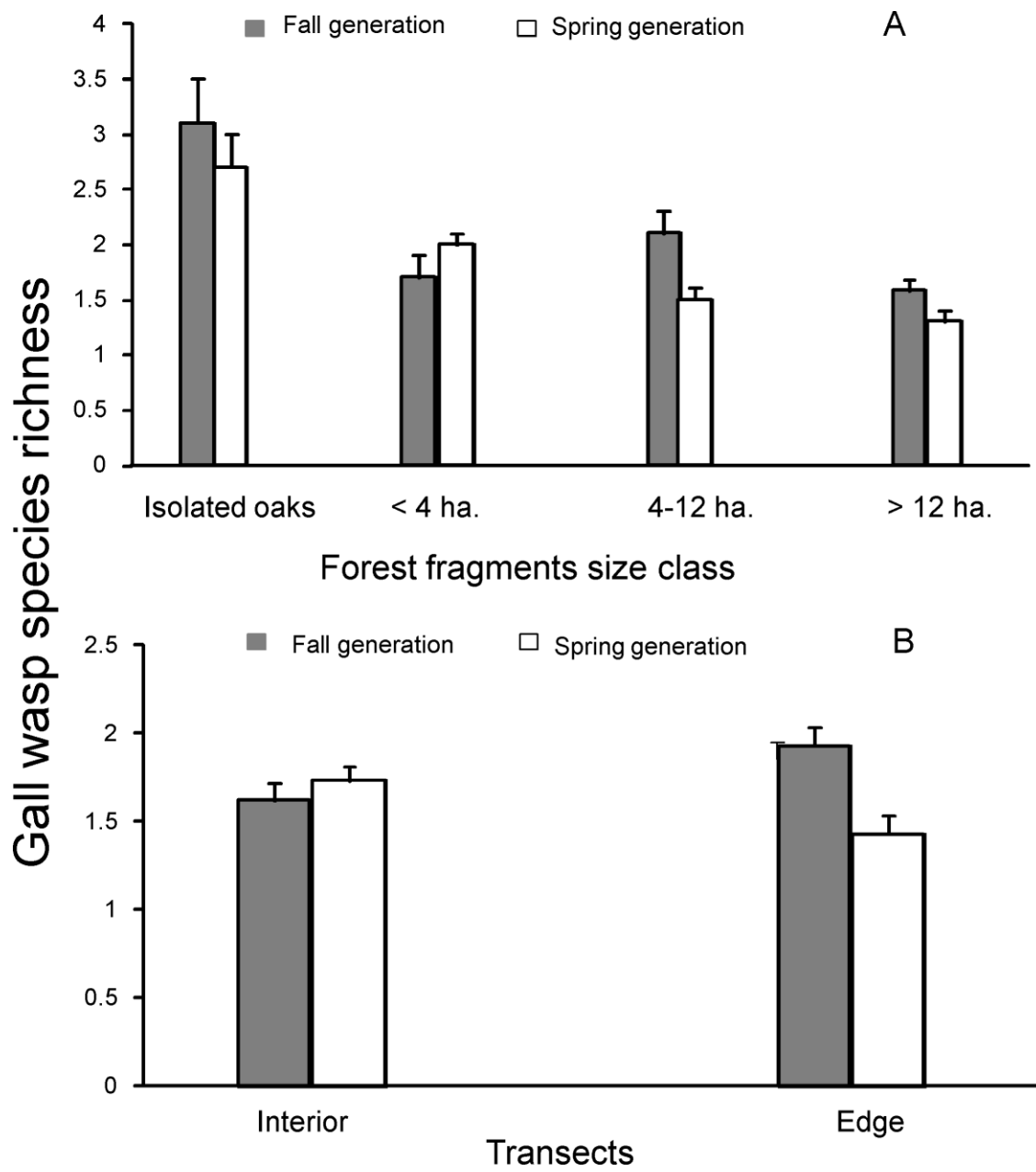


Fig. 2

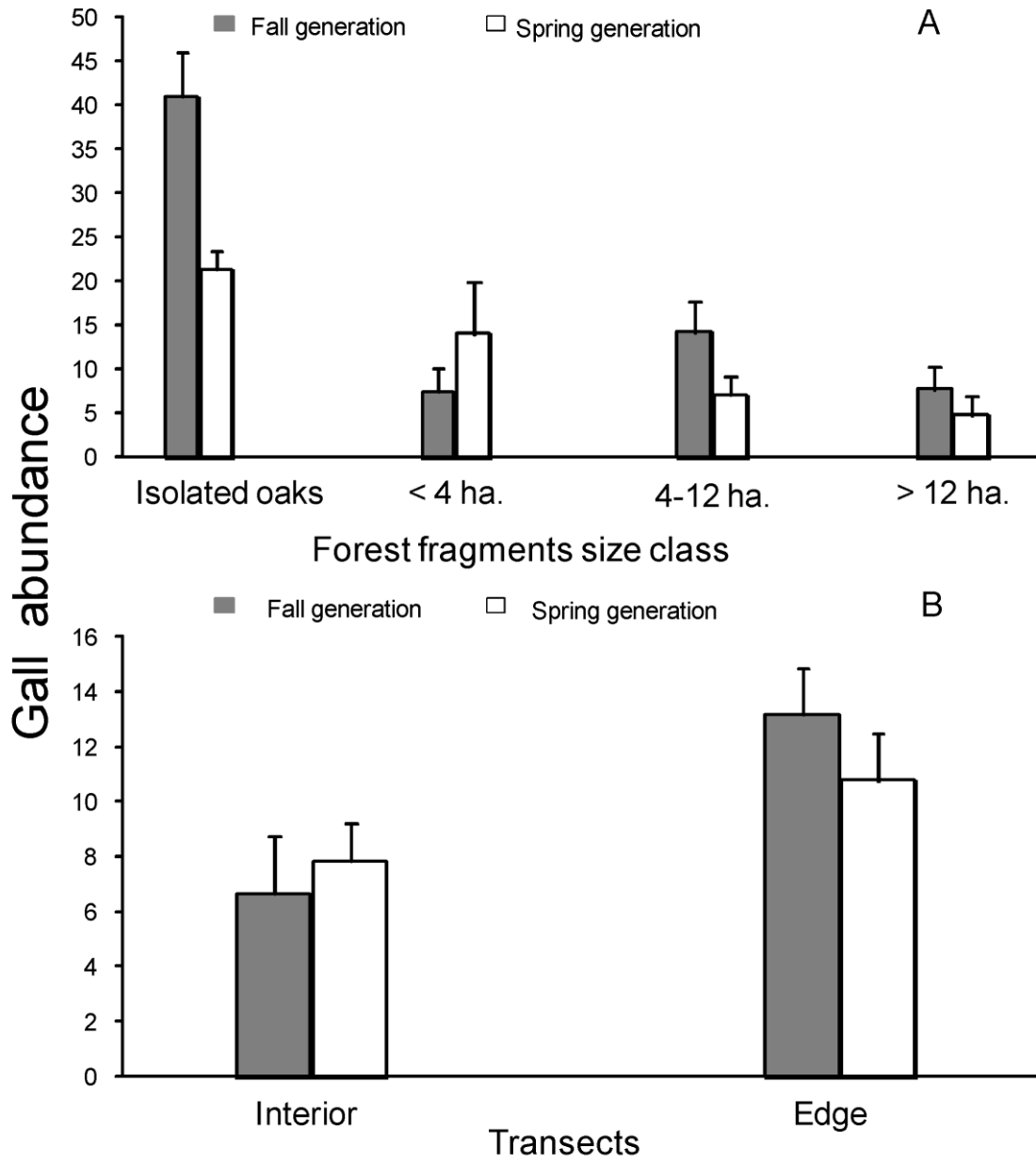
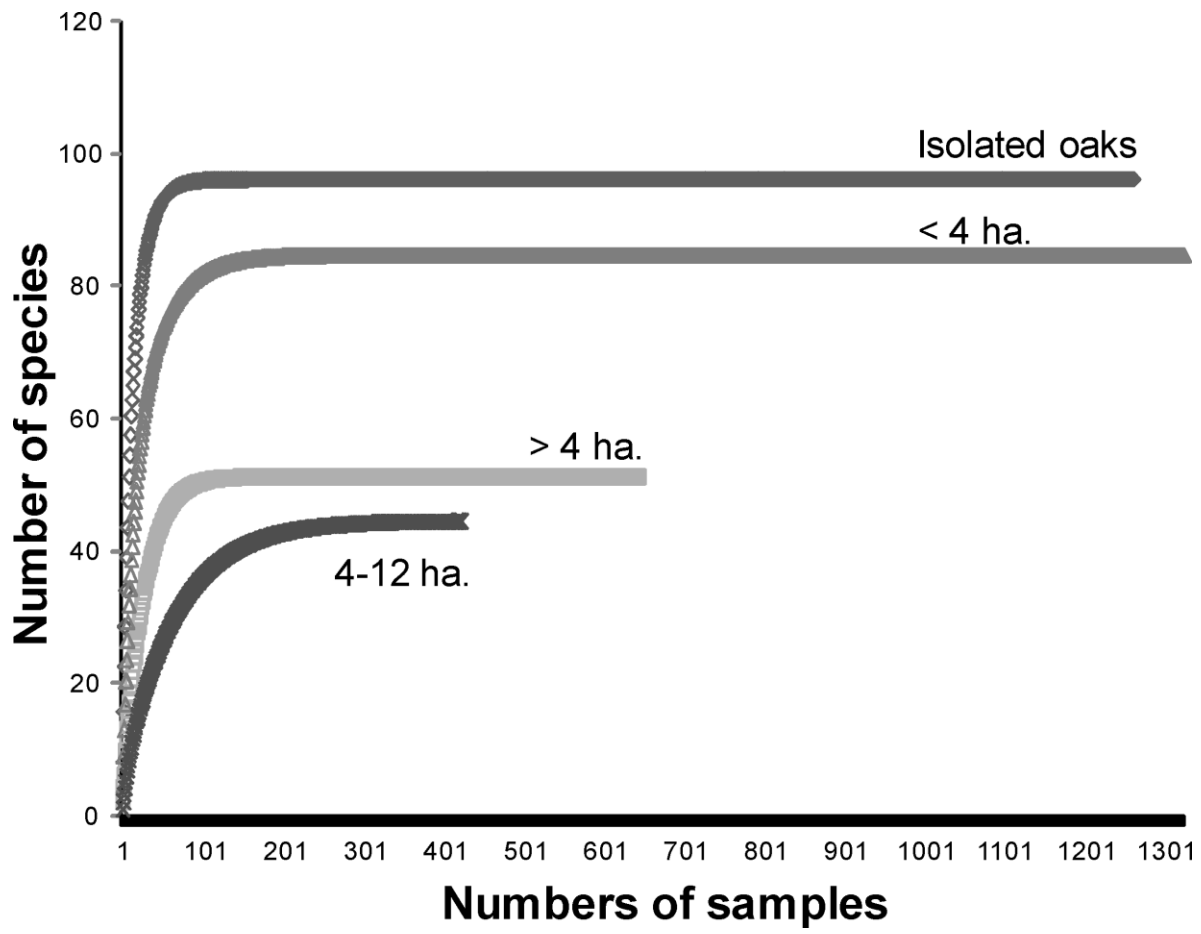


Fig. 3



Appendix.

Table A.1. Oak species present at each sampling site.

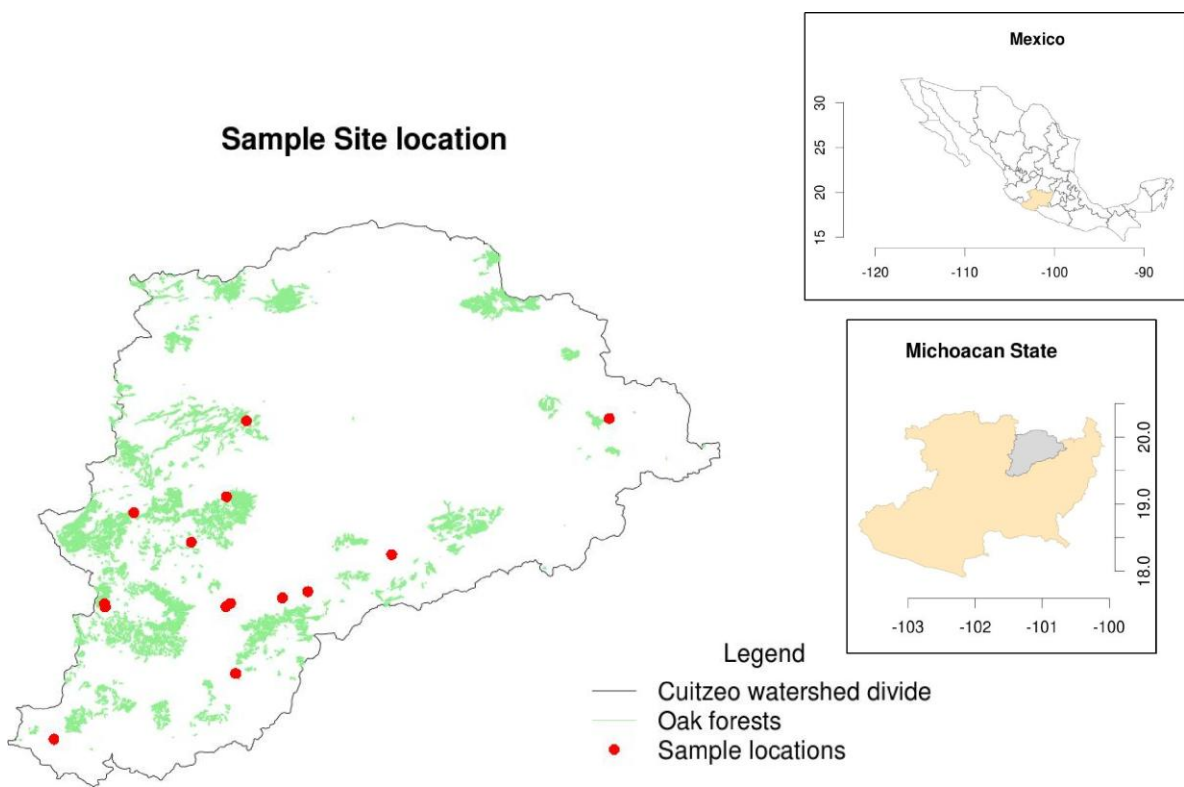
Size fragments	Site	Oak species
Smaller fragments (≤ 4 ha)	San José de las Torres	<i>Q. castanea</i>
	La Concepción	<i>Q. deserticola</i> , <i>Q. obtusata</i> , <i>Q. castanea</i>
	Jesús del Monte	<i>Q. obtusata</i> , <i>Q. deserticola</i> , <i>Q. laeta</i>
	Cuanajo	<i>Q. castanea</i> , <i>Q. deserticola</i>
	Autopista	<i>Q. castanea</i> , <i>Q. magnoliifolia</i>
Medium-sized fragments (4-12 ha)	Canoas	<i>Q. deserticola</i> , <i>Q. castanea</i>
	San José del Rincón	<i>Q. castanea</i> , <i>Q. deserticola</i>
	Cepamisa	<i>Q. castanea</i> , <i>Q. obtusata</i> , <i>Q. deserticola</i>
	Teremendo	<i>Q. castanea</i> , <i>Q. deserticola</i>
	Acuitzio	<i>Q. castanea</i> , <i>Q. obtusata</i> , <i>Q. deserticola</i>
Continuous forests (>12 ha)	Umécuaro	<i>Q. castanea</i> , <i>Q. glaucoides</i>
	Atécuaro	<i>Q. castanea</i> , <i>Q. candicans</i> , <i>Q. magnoliifolia</i> .
	Lagunillas	<i>Q. castanea</i> , <i>Q. deserticola</i> , <i>Q. laeta</i>
	San Miguel del Monte	<i>Q. laeta</i> , <i>Q. crassifolia</i> , <i>Q. scythophylla</i> , <i>Q. castanea</i> , <i>Q. obtusata</i>
	Chiquimitio	<i>Q. castanea</i> , <i>Q. obtusata</i> , <i>Q. deserticola</i>
Isolated oaks	Isolated oaks	<i>Q. deserticola</i> , <i>Q. castanea</i> , <i>Q. obtusata</i> , <i>Q. magnoliifolia</i> , <i>Q. dysophylla</i>

Figure A.1

Field sampling sites. The study was conducted at 15 permanent sites within the Cuitzeo basin.

We selected the study sites accord to size fragments: i) five smaller fragments (≤ 4 ha): San José de las Torres (19.69745, -101.060133), La Concepción (19.70943, -101.320383), Jesús del Monte (19.6506, -101.168267), Cuanajo (19.464917, -101.494733), Autopista (19.63082,

-101.274083); ii) five medium-sized fragments (between 4-12 ha): Canoas (19.8593055, -101.2508333), San José del Rincón (19.867617, -100.779417), Cepamisa (19.63487, -101.2683), Teremendo (19.74455, -101.395217), Acuitzio (19.498786, -101.343967) and iii) five continuous forests (>12 ha): Umécuaro (19.54872, -101.260333), Atécuaro (19.6423, -101.2012), Lagunillas (19.62823, -101.430917), San Miguel del Monte (19.63211, -101.431752) Chiquimitio (19.76583, -101.27531).



CAPÍTULO II

Diversity of gall wasps (Hymenoptera: Cynipidae) associated to oak trees (Fagaceae: *Quercus*) in a fragmented landscape in Mexico

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Sometido: Arthropod-Plant Interactions

Abstract

The life cycle of gall-inducing insects must be synchronized with the phenology of host plants because they require undifferentiated tissue to initiate gall induction. However, forest fragmentation modifies the microenvironment conditions of remaining forest patches and isolated trees that may alter the availability of resources (i.e. leaves) that determine the patterns of gall-inducing insects in a fragmented landscape. Oak gall wasps are closely associated with oak trees and the time of gall induction is specific for each gall wasp species. In Mexico oak forests have been highly fragmented resulting in small fragments of variable size and isolated trees. We conducted a three-year study to test if the species richness of oak gall wasps associated with oaks, decreased in function of the degree of forest fragmentation assuming a modification of leaf production and resource availability patterns of different oak species in forest fragments. Our results showed that oak canopy cover was significantly higher in isolated oaks and small fragments, affecting the spatial and temporal variation of gall wasps and increasing the abundance and species richness of oak gall wasp species in most fragmented habitats. Oaks canopy cover is altered by forest fragmentation having higher production of leaves on trees that are more exposed to fragmentation and can provide important resources for maintain gall wasp species diversity in a fragmented landscape. Species diversity of gall wasps was primarily maintained by isolated oaks that could be considered “*super-hosts*” because they hosted approximately 80 oak gall wasp species.

Key words: canopy cover, oak gall wasp, gall abundance, gall richness, *Quercus*, habitat fragmentation.

Introduction

Gall-inducing insect populations are regulated by both top-down (i.e. parasitoids and inquilines) and bottom-up forces (i.e. plant traits) (Taper et al. 1986; Stone et al. 2002; Askew et al. 2013). Specifically, temporal and spatial variation in resource availability such as quantity of leaves is one of the most important factors that affect the abundance and distribution of this insect guild since their life cycle is synchronized with the production of host plant organs and because they require undifferentiated tissues to initiate gall induction (Weis et al. 1988; Yukawa 2000; Stone et al. 2002; Hayward and Stone 2005). Consequently, the timing and site of oviposition are crucial to successfully complete gall development in many gall-inducing insects (Stone et al. 2002; Hayward and Stone 2005), representing a “window of opportunity” because they attack the first leaf flush of their host plants (Yukawa 2000; Hayward and Stone 2005).

In fragmented forests, abiotic conditions are modified increasing light intensity, wind speed and temperature and decreasing humidity and soil fertility (Kapos et al. 1997; Silva and Simonetti 2009). As a consequence, the timing and frequency of leaf abscission and the new events of leaf production can be indirectly altered (Lovejoy et al. 1986, Aldrich and Hamrick 1998) affecting the preference and the incidence of gall-inducing insects (Weis et al., 1988). A good estimator of plant resource availability is to measure the percentage of canopy cover by leaves (percentage of sky covered by trees) (Tyas et al. 1998; Klein et al. 2002; Rautiainen et al. 2005).

Cynipids or gall wasps (Hymenoptera: Cynipidae: Cynipinae) are closely associated with oak trees (Fagaceae: *Quercus*). Each wasp species is specific to a particular host oak species or species group (Nieves-Aldrey 2001; Stone et al. 2009). The cynipid life cycle progresses through two generations, a sexual generation in the spring or early summer and an asexual generation during the summer and autumn of the same year (Stone et al. 2002). The

time of gall induction is species-specific (Abrahamson et al. 2003). The galls occur for a short period of the season, and the adult stage of gall wasps lasts only a few weeks (Wiebes-Rijks and Shorthouse, 1992). Therefore, host plant resource availability is crucial for host use, outbreaks, spatial structure and seasonal variation for gall-inducing insects (Yukawa 2000).

In Mexico, oak forests have been highly fragmented because of agriculture, grazing and deforestation (Arredondo-León et al. 2008). In a three-year study, we determined oak gall wasps species diversity associated to temporal and spatial variation of canopy cover of different oak species that occur in a fragmented landscape in Mexico. We hypothesized that a greater gall wasps abundance and richness is associated to a higher canopy cover of leaves in most fragmented habitats (isolated trees, smaller fragments and fragment edges). Our expectations were supported by positive responses. The following questions were addressed:

- i) Does forest fragmentation affect gall abundance and species richness in a temperate forest?
- (ii) Are oak canopy cover patterns modified by habitat fragmentation and how affect gall richness and abundance?
- (iii) Does seasonal variation in canopy cover affect gall richness and abundance?

Materials and Methods

Study site

This study was conducted in the Lake Cuitzeo Basin, a hydrological basin with an area of 4026 km² located in Michoacán state, in western Mexico between 19° 30' - 20° 05' (N) and 100° 35' - 101° 30' (W) and at an altitude of 2000 m. The climate is temperate with a summer rainy season. The average annual temperature is 17 °C and the annual rainfall is *ca.* 800 mm (Carlón-Allende et al. 2009). The site is located in the physiographic province of the Transmexican Volcanic Belt. The basin contains the Lake of Cuitzeo, with a wetland area of

approximately 300 km². The basin is representative of the environmental and socioeconomic conditions of central Mexico and has experienced strong fragmentation resulting in a highly fragmented landscape forming a mosaic of scrubland, forests (mainly pine, oak and mixed forests) and agricultural lands (López et al. 2006). The basin includes Morelia, the state capital of Michoacán state, which urban area grew six fold between 1975 and 2000 (López et al. 2001). Land cover and land use change analyses indicate that the period 1986-1996 was characterized by high rates of deforestation and forest degradation throughout the basin (Mendoza et al. 2011). Consequently, large continuous oak populations have been reduced to a large number of small patches of variable size due to strong human pressures including urban growth, expansion of the agricultural frontier and the removal of trees for charcoal production (López et al. 2006; Aguilar et al. 2012; Castillo et al. 2013).

Study system

Cynipids induce galls on various oak tissues (i.e. buds, stems, catkins, leaves and roots) (Hayward and Stone 2005). Each species of cynipid usually induce a morphologically distinct gall (Abrahamson et al. 1998; Nieves-Aldrey 2001; Stone et al. 2009). Oak gall wasps are obligated to oviposit inside the tissues of their vegetal hosts, and the larvae develop in galls induced on specific plant tissues (Stone and Schönrogge 2003).

Oak forests offer important environmental services (Faivre-Rampant et al. 2011), and support the maintenance of biological diversity (Müller and Goßner 2007). The genus *Quercus* has a wide distribution, abundance and biomass and supports a high number of arthropod species (Tovar-Sánchez and Oyama 2006a, b). Oak forests have been highly fragmented in Mexico because of the great economic importance of the trees (Valencia-Avalos and Nixon 2004). Masera et al. (1997) have estimated that 167,000 ha/yr of temperate

forests are lost, resulting in an annual deforestation rate of 0.64% in Mexico. In particular, Michoacán state has a very high deforestation rate of approximately 1.8% per year (Bocco et al. 2001).

Fragmentation of oak forests and selection of sampling sites

Surveys were conducted from September 2007 to October 2010. The remaining oak forests in the Lake Cuitzeo Basin have previously been divided into 1241 fragments of different sizes and shapes covering approximately 20% of the basin (López et al. 2006). Fifteen permanent forest fragments were selected and classified into the following three categories according to their size: (i) 5 small forest fragments (≤ 4 ha); (ii) 5 medium-sized forest fragments (4-12 ha); and (iii) 5 large forest fragments (>12 ha) (Fig. 1). In each forest fragment, two parallel transects of 5 x 50 m were established as permanent sampling sites, one located on the fragment edge and one in the fragment interior at a distance of more than 100 m from the forest border. Twenty-five isolated oak trees were selected at distances of at least 400 m from the surrounding forest. We sampled nine oak species: *Q. laeta*, *Q. obtusata*, *Q. deserticola*, *Q. magnoliifolia*, *Q. glaucoides*, *Q. castanea*, *Q. scythophylla*, *Q. candicans* and *Q. dysophylla*.

Oaks and gall wasp diversity

All fragments were sampled every month to record oak gall wasp morphospecies. At each site, oak species were identified and their abundances recorded. Stratified samples of galls were collected from the lower branches to the top of the canopy. Three branches were collected from each of three layers into which the canopy was divided (i.e., top, intermediate and bottom) (Cuevas-Reyes et al. 2004a). The galls were identified on the basis of gall morphology and host plant species. The galls were reared in the laboratory, and the adult

insects that emerged were identified to confirm that each gall morphospecies was induced by a particular oak gall wasp species. Gall richness and gall abundance were recorded, and we included galls from both generations (spring and autumn). Taxonomic identification of the gall wasps is in progress.

Canopy cover

All trees of each oak species were marked in all permanent study sites including isolated trees. For each individual tree, the canopy cover was recorded every month over the course of three years. The canopy cover of leaves was measured as a proportion of the potentially complete cover (Basset 1991; Ranius and Jansson 2000) using a 3m pole as a vertical sighting instrument (>5 m). We used the following classification: 1) 0% of canopy cover; 2) 1-25% of canopy cover; 3) 26-50% of canopy cover; and 4) 51-100% of canopy cover (Williams et al. 1997; Ranius and Jansson 2000). In addition, we measured the tree height and diameter at breast height (DBH) of each tree sampled as estimators of plant size (Cuevas-Reyes et al. 2004a, b).

Statistical analysis

To analyze the differences in tree abundance between oak species, a logistic regression analysis was applied using oak species as the independent variable and abundance as the response variable. The model used oak species as a fixed effect, with a Poisson distribution and log link function. A generalized linear model was applied to identify the differences in gall richness (response variable) between oak species and host plant organs (independent variables, fixed effects).

We used multivariate analyses of variance (MANOVA) to determine the effects of forest fragmentation conditions (isolated trees, small fragments, intermediate fragments and

larger fragments) on diameter at breast height (DBH) and tree height (estimators of plant size), canopy cover, gall wasp richness and abundance. When significant effects were detected by MANOVA, we followed up with a one-way ANOVA test for each variable (SAS 2000). Similarly, we performed analyses of variance (MANOVA) to evaluate the effects of interior and edge of fragments on the same response variables. In all cases, the normality was tested after suitable transformations. Canopy cover data was transformed to arc-sine square root values (Ranius and Jansson 2000).

To determine differences in seasonal gall variation, a logistic regression analysis was applied using gall generation (autumn and spring) and host plant organ (leaf, stem, petiole, bud) as the independent variables and gall wasp richness as the response variable. The model used gall generation and host plant organ as fixed effects, with a Poisson distribution and log link function. A multivariate analysis (MANOVA) was performed to determine the effects of season (spring vs. autumn) on diameter at breast height and tree height, canopy cover and gall wasp richness and abundance (SAS 2000). When significant effects were detected by MANOVA, we applied a one-way ANOVA test for each variable. In all cases, the normality was tested after suitable transformations. Canopy cover data was transformed to arc-sine square root values (Ranius and Jansson 2000).

To determine the temporal differences in oak canopy cover between all oak species along three years, a generalized linear model was used for repeated measurements, using trees as the repeated factor in the model. The model used oak species as independent variables, and the percentage of canopy cover as response variables. The analysis used a binomial distribution and a log link function. An LSMeans test was used for a posteriori comparisons (SAS 2000).

In our study, we detected three oak species that support a great number of gall wasp species (*Q. obtusata*, *Q. castanea* and *Q. deserticola*). Therefore, to determine the differences

in canopy cover and gall abundance between seasons, in each oak species we applied a generalized linear model of repeated measurements, using trees as the repeated factor in the model. The model use generation and plant organ as the independent variables and canopy cover and gall abundance as the response variables respectively.

Results

General description of the oak-gall wasp community

Nine oak species were found in all the fragmented landscape. *Quercus obtusata*, *Q. castanea* and *Q. deserticola* occurred in all the fragments and were the most abundant oak species in the basin ($\chi^2 = 79.21$, d.f. = 8, $P < 0.0001$). In total, 105 gall wasp species were recorded. Of these, 29 (27.6%) were of the spring and 76 (72.4%) species of the autumn generation. Most of the galls were found in leaves and stems rather than in petioles and buds. Oak gall wasp richness differed among oak species ($\chi^2 = 46.1$, d.f. = 8, $P < 0.0001$) and between host plant organs ($\chi^2 = 54.9$, d.f. = 3, $P < 0.0001$). Gall wasp richness was significantly higher in *Q. obtusata* (40 species), *Q. castanea* (33) and *Q. deserticola* (25) than in *Q. candicans* Née (8), *Q. scytophylla* Liebm. (8), *Q. magnoliifolia* Née (7), *Q. glaucoides* Humb. (7), *Q. dysophylla* Benth. (2) and *Q. laeta* Liebm. (2) (Fig. 2).

Habitat fragmentation effects in oak gall wasp diversity

We found that means for gall abundance and richness varied among forest fragment conditions (Table 1A) as indicated by overall MANOVA effects (Table 1B). Gall wasp richness and abundance were higher in isolated oaks and small fragments (Table 1C). In addition, means of gall abundance and richness differed among edge and interior of fragments (Table 2A) as indicated by overall MANOVA test (Table 2B). Edge fragments had

higher gall abundance and richness than interior of fragments according to the one-way ANOVA analysis (Table 2C).

Oak gall wasp seasonal variation

Galls were present throughout the year, but they showed seasonal variation. Mean of gall wasp richness and abundance were higher in autumn than in the spring (Table 3A, 3C) as indicated by overall MANOVA effects (Table 3B). Particularly, the species richness of wasps that induce galls on leaves and stems were higher in the autumn than in spring generation (Leaf: $\chi^2 = 6.5$, d.f. = 1, $P > 0.008$; Stem: $\chi^2 = 9.5$, d.f. = 1, $P > 0.01$) (Fig. 3a and 3b). Wasp species that induce gall on buds were only present in the autumn (Fig. 3c), whereas the species richness of wasps that induce galls on petioles were higher in the spring generation ($\chi^2 = 13.5$, d.f. = 1, $P > 0.001$) (Fig. 3d).

Oaks canopy cover and plant size

We found that means for canopy cover, DBH and tree height varied among conditions of forest fragmentation (i.e. isolated trees, small, intermediate and large fragments) (Table 1A) as indicated by overall MANOVA effects (Table 1B). These response variables were higher on isolated oaks and small fragments (Table 1C). Similarly, means of the each variable analyzed (i.e. canopy cover, DBH and tree height) varied between edge and interior of fragments (Table 2A), according to MANOVA test (Table 2B). All response variables were higher in the edge than in the interior of fragments (Table 2C). Means of DBH and tree height did not differ significantly between seasons. Only canopy cover varied between spring and autumn (Table 3A) as indicated by overall MANOVA effects for seasons (Table 3B). Canopy cover was higher in autumn in comparison with spring (Table 3c).

The results of repeated measurements analysis show that canopy cover patterns along three years differed among oak species ($F = 4.9$, d.f. = 8, $P < 0.0001$). *Quercus obtusata*, *Q. castanea* and *Q. magnoliifolia* showed the higher leaf canopy cover, *Q. glaucooides* and *Q.*

deserticola had medium leaf canopy cover, and *Q. scytophylla* and *Q. laeta* had low leaf canopy cover (Fig. 4).

Finally, we found a general pattern in the three “*super-hosts*” species analyzed in this study. The canopy cover of *Q. obtusata* ($F = 3.3$, d.f. = 1, $P < 0.03$), *Q. castanea* ($F = 6.5$, d.f. = 1, $P = 0.004$) and *Q. deserticola* ($F = 12.9$, d.f. = 1, $P < 0.0001$) was higher in the autumn than in the spring. Similarly, gall abundance on leaves, stems and petioles in *Q. obtusata* ($F = 3.5$, d.f. = 1, $P = 0.03$), *Q. castanea* ($F = 4.3$, d.f. = 1, $P = 0.002$) and *Q. deserticola* ($F = 2.9$, d.f. = 1, $P = 0.04$) was higher in the autumn generation for each year (Fig. 5).

Discussion

Ecological interactions among species affect plant growth, survival and reproduction of many species (i.e. mutualisms and antagonisms) (Bascompte et al. 2006; Ives and Carpenter 2007). Recent studies suggest that mutualistic interactions are often more affected by forest fragmentation compared to antagonistic interactions such as herbivory that can be unaffected or may even be favored by increasing the levels of herbivory and species diversity (Chalfoun et al. 2001; Ruiz-Guerra et al. 2010). These differences between mutualistic and antagonistic interactions may be explained by the degree of specialization, which generally is lower in antagonistic interactions (Poisot et al. 2011). However, specialist interactions between plants and herbivores have been little studied in fragmented landscape (Magrach et al. 2014).

Habitat fragmentation may modify insect-plant interactions via bottom-up forces (Tschardt et al. 2002; Chust et al. 2007) because plant traits such as chemical defenses, phenology and plant growth are directly affected in fragmented habitats, influencing insect herbivore diversity (Wettstein and Schmid 1999; Floren and Linsenmair 2001; Tschardt et

al. 2002) principally in forest edges and in small fragments (Yamasaki and Kikuzawa 2003; Ruiz-Guerra et al. 2010). Therefore, under fragmented conditions, is probably expected a decrement of plant nutritional quality and changes of resource availability to herbivores, which in turn can affect the interactions with others trophic levels and modify the structure and composition of the insect community (Didham et al. 1996; Tschardtke and Kruess 1999; Chust et al. 2007). However, some empirical studies show opposite effects of forest fragmentation on abundance or distribution of some insect species (Kremsater and Bunnell 1999; Carlson and Hartman 2001; Fahrig 2003). Specifically, Chust et al. (2007) show that overall abundance of gall wasps was affected by forest fragmentation, increasing in small or scattered patches of oak forests in comparison with continuous forests. These differences may be explained because temporal availability resources (leaves) potentially may be modified in this environment conditions.

In our study, we found that oak gall wasps diversity is associated with an increment of resource availability (leaves) in most fragmented habitats (isolated trees, small fragments and fragment edges). More leaves may represent more potential sites for gall induction (Weis et al. 1988), considering that their life cycle are synchronized with the production of host plant organs and because they require undifferentiated tissues to initiate gall induction (Yukawa 2000; Stone et al. 2002; Hayward and Stone 2005). In addition, we found that plant size (DBH and tree height) was higher in isolated trees, small fragments and the edge of fragments. Similarly, canopy cover was higher in these fragmented conditions for all oak species studied. The relationship between resource availability and herbivore incidence may become even more important in fragmented environments where microclimate changes alter the timing of leaf abscission, new leaf expansion and production of some plant species (Karban 2007). One possible explanation for these results is that abiotic conditions in small fragments and the edge of fragments change more abruptly than in larger fragments and

continuous forest (Trombulak and Frissell 2000; Silva and Simonetti 2009), affecting the patterns of canopy cover of plants increasing the leaf production (Lovejoy et al. 1986), which in turn can affect the incidence of insect herbivores such as gall-inducing insects (Mopper 2005; Karban 2007).

Despite that negative effects on plant growth has been observed in forest fragments (Harrington 1986; Marchand et al. 1986; Miller et al. 1987), some authors suggest that an increment of light incidence in small fragments and forest edges promotes plant growth in some woody plant species (Williams-Linera 1990; Sizer and Tanner 1999) and therefore the canopy cover (leaf production) may be modified in these conditions. For example, a meta-analysis of the effects of forest fragmentation on interspecific interactions (Magrath et al. 2014) indicated the presence of compensatory responses of plants as result of habitat fragmentation such as demographic effects (Bruna and Ribeiro 2005; Beckman et al. 2012), changes in plant defenses and regrowth rates (Agrawal 2000), or cascading effects on predatory pressure (e.g., carnivore abundance and mobility) may counterbalance the changes in the abundance and mobility of herbivores (Williams-Linera 1990; Murcia 1995; Hagen et al. 2012). Besides, because isolated trees occurs in open areas, is probably expected that they not experience an intra and interspecific competition in their neighborhood, and therefore tend to have higher plant size with larger canopies covered (Fuchs et al. 2003; Herrerías-Diego et al. 2006).

Oak gall wasp species composition differed among oak species and several hypotheses have been proposed to explain why some plants may be more infested by galling insects than others (Eliason and Potter 2000). Differences in host-plant physiology, chemistry, development and temporal and spatial resource availability have been suggested as possible causes for these differences in cynipids (Abrahamson et al. 1998). Particularly, in our study, isolated trees of *Q. obtusata*, *Q. castanea* and *Q. deserticola* hosted approximately

80 oak gall wasp species, establishing them as “*super-hosts*” (Fernandes and Price 1988; Veldtman and McGeoch 2003; Araùjo et al. 2013). These oak species show a high canopy cover, coinciding the peak canopy cover patterns with higher abundance of galls. These gall wasps were associated with a specific organ, and their appearance occurs at a specific time during the season. If gall-inducing insects on a given host plant organ show differences in seasonal patterns, we could expect a niche divergence in species that coexistence in sympatry, and avoidance of competition resulting from niche overlap (Tokeshi 1999; Schluter 2000). Ecologically, species can coexist for long periods if this species differ on one niche axis alone may be sufficient to reduce competition under stable conditions (Webb et al. 2002). For example, for gall wasps, the oak species galled, gall location and gall seasonality are traits that describe the cynipid niche (Bailey et al. 2009). We found that part of oak gall wasp species are associated with a specific organ, but show differences in seasonal patterns, suggesting a partition of resource (Abrahamson et al. 1998, 2003).

We also found that multiple galls were developing on the same plant part at the same time (specially leaf galls). Therefore, we proposed that our results may be explain by the "hypothesis of the coexistence" that predicted that certain oak gall wasp species occurs in a particular host organ at the same time (Chesson 2000). Sympatric coexistence of high several oak gall wasps species on a single host tree may be regulated by partitioning of generalist parasitoids between different gall phenotypes to avoid gall mortality and reduced the parasitoid efficiencies resulting in a enemy-free space to gall wasp species (Price et al. 1987; Stone and Cook 1998; Stone and Schönrogge 2003).

We conclude from our study that habitat fragmentation increases the temporal and spatial resource availability (canopy cover) for gall-inducing insects, with important implications on oak gall wasps diversity and their conservation. Canopy cover is altered by forest fragmentation having higher production of leaves on trees that are more exposed to

fragment edges or are isolated. Therefore, larger plants that occur in most fragmented habitats as isolated oaks, can provide important resources for maintain gall wasp species diversity in a fragmented landscape in Mexican temperate forests. In addition, habitat fragmentation affects gall diversity, increasing the abundance and species richness of oak gall wasp species in isolated oaks, small fragments and along fragment edges. Finally, the coexistence of many gall-inducing insects associated to a "*super-host*" plants species, allow to test ecological and evolutive hypotheses.

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Table 1. Differences in plant traits and gall wasp diversity between forest fragmentation conditions according to MANOVA test

(A) Mean \pm standard error

Response variables	Isolated trees	Small fragments (< 4 ha)	Intermediate fragments (4-12 ha)	Large fragments (> 12 ha)
DBH	144.1 \pm 17.3	87.1 \pm 7.8	104.3 \pm 10.5	69.4 \pm 7.1
Tree height	20.4 \pm 2.4	8.8 \pm 1.67	10.4 \pm 1.0	13.1 \pm 0.6
Canopy cover	78.3 \pm 5.3	63.7 \pm 1.6	47.7 \pm 2.3	46.6 \pm 1.6
Gall wasp richness	15.4 \pm 0.8	5.2 \pm 0.2	2.7 \pm 0.3	1.6 \pm 0.5
Gall wasp abundance	172.7 \pm 6.3	22.1 \pm 2.7	6.8 \pm 3.7	4.6 \pm 2.0

(B) MANOVA test for overall effects

Source	Pillai trace	<i>F</i>	d.f.	<i>P</i>
Intercept	0.711	384.7	249	< 0.0001
Forest fragmentation	0.972	158.6	5, 245	< 0.0001

(C) MANOVA tests of between-subjects effects

Source	Response variable	d.f.	<i>F</i>	<i>P</i>
Forest fragmentation	DBH	3	8.6	0.003
	Tree height	3	39.2	0.0001
	Canopy cover	3	37.3	< 0.0001
	Gall-wasp richness	3	123.5	< 0.0001
	Gall-wasp abundance	3	119.4	< 0.0001

Table 2. Differences in plant traits and gall wasp diversity between interior and edge fragments according to MANOVA test.

(A) Mean \pm standard error

Response variables	Interior of fragments	Edge of fragments
DBH	80.1 \pm 4.8	100.4 \pm 5.9
Tree height	11.1 \pm 0.9	16.4 \pm 1.0
Canopy cover	41.1 \pm 2.1	66.9 \pm 1.2
Gall wasp richness	1.6 \pm 0.1	3.8 \pm 0.2
Gall wasp abundance	10.6 \pm 2.3	36.8 \pm 1.5

(B) MANOVA test for overall effects

Source	Pillai trace	<i>F</i>	d.f.	<i>P</i>
Intercept	0.411	368.4	229	0.001
Transects	0.523	464.4	5, 225	0.0001

(C) MANOVA tests of between-subjects effects

Source	Response variable	d.f.	<i>F</i>	<i>P</i>
Transects	DBH	1	14.3	0.01
	Tree height	1	29.2	0.0001
	Canopy cover	1	88.1	0.0001
	Gall wasp richness	1	66.7	0.0001
	Gall wasp abundance	1	89.8	0.0001

Table 3. Comparison of plant traits and gall wasp diversity between seasons according to MANOVA test

(A) Mean \pm standard error

Response variables	Spring	Autumn
DBH	89.9 \pm 9.3	96.2 \pm 9.8
Tree height	12.6 \pm 0.8	12.9 \pm 0.5
Canopy cover	45.4 \pm 2.1	59.7 \pm 1.2
Gall wasp richness	3.6 \pm 0.2	6.8 \pm 0.2
Gall wasp abundance	27.4 \pm 2.1	69.8 \pm 2.6

(B) MANOVA test for overall effects

Source	Pillai trace	<i>F</i>	df	<i>P</i>
Intercept	0.435	432.4	249	< 0.0001
Season	0.689	527.5	5, 245	< 0.0001

(C) MANOVA tests of between-subjects effects

Source	Response variable	d.f.	<i>F</i>	<i>P</i>
Season	DBH	1	2.6	n.s.
	Tree height	1	3.4	n.s.
	Canopy cover	1	34.1	< 0.0001
	Gall wasp richness	1	22.8	< 0.0001
	Gall wasp abundance	1	24.4	< 0.0005

Figure legends

Fig. 1 Map of oak forest fragments in Lake Cuitzeo Basin, showing the permanent sites of sampling.

Fig. 2 Differences in gall species richness between oak species and host plant organs.

Fig. 3 Oak gall wasps seasonal variation: (a) leaf galls; (b) stem galls; (c) bud galls; and (d) petiole galls.

Fig. 4 Temporal variation of canopy cover between oak species studied.

Fig. 5 Oak canopy cover and gall abundance of three "Super-Host" species: (a) *Q. obtusata*; (b) *Q. castanea*; and (c) *Q. deserticola*.

Fig. 1

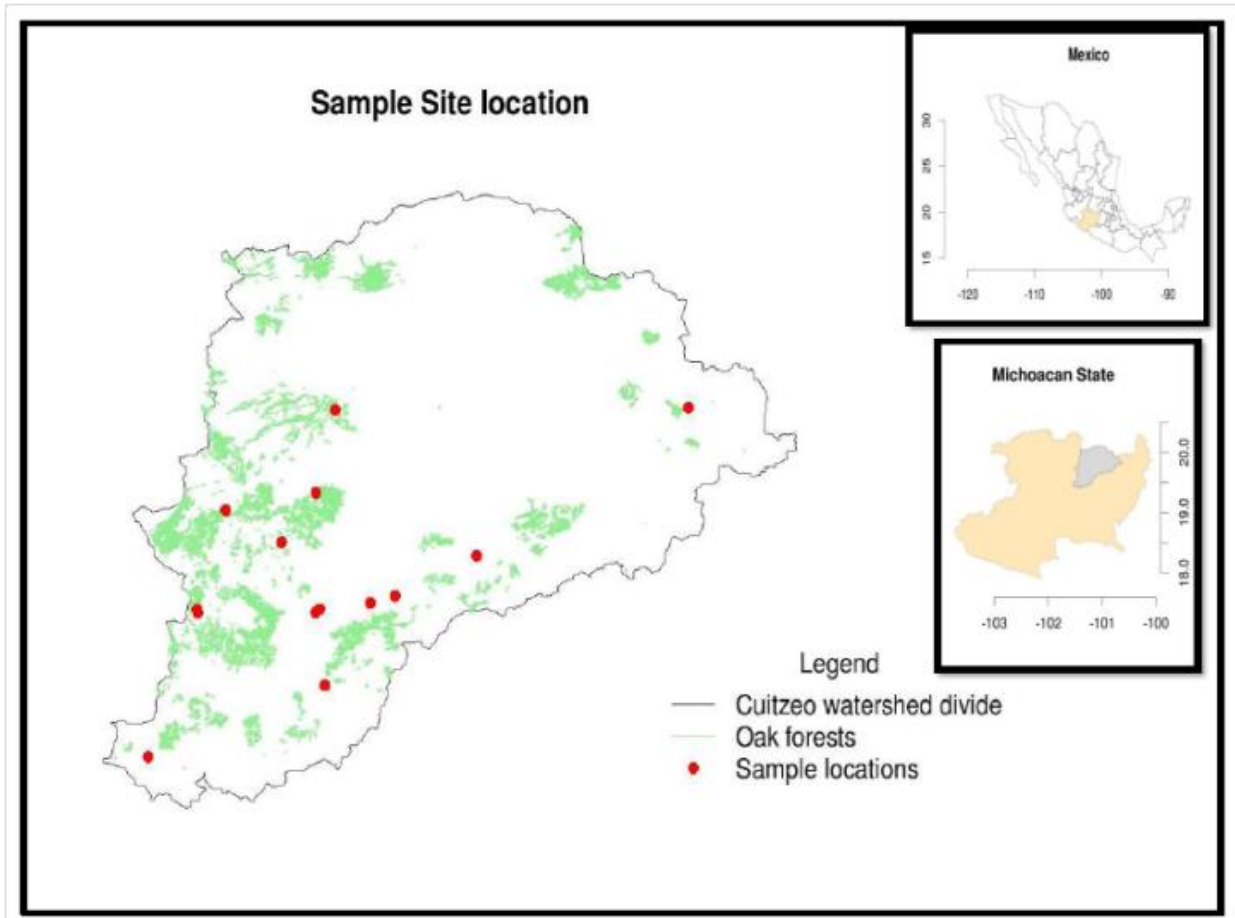


Fig. 2

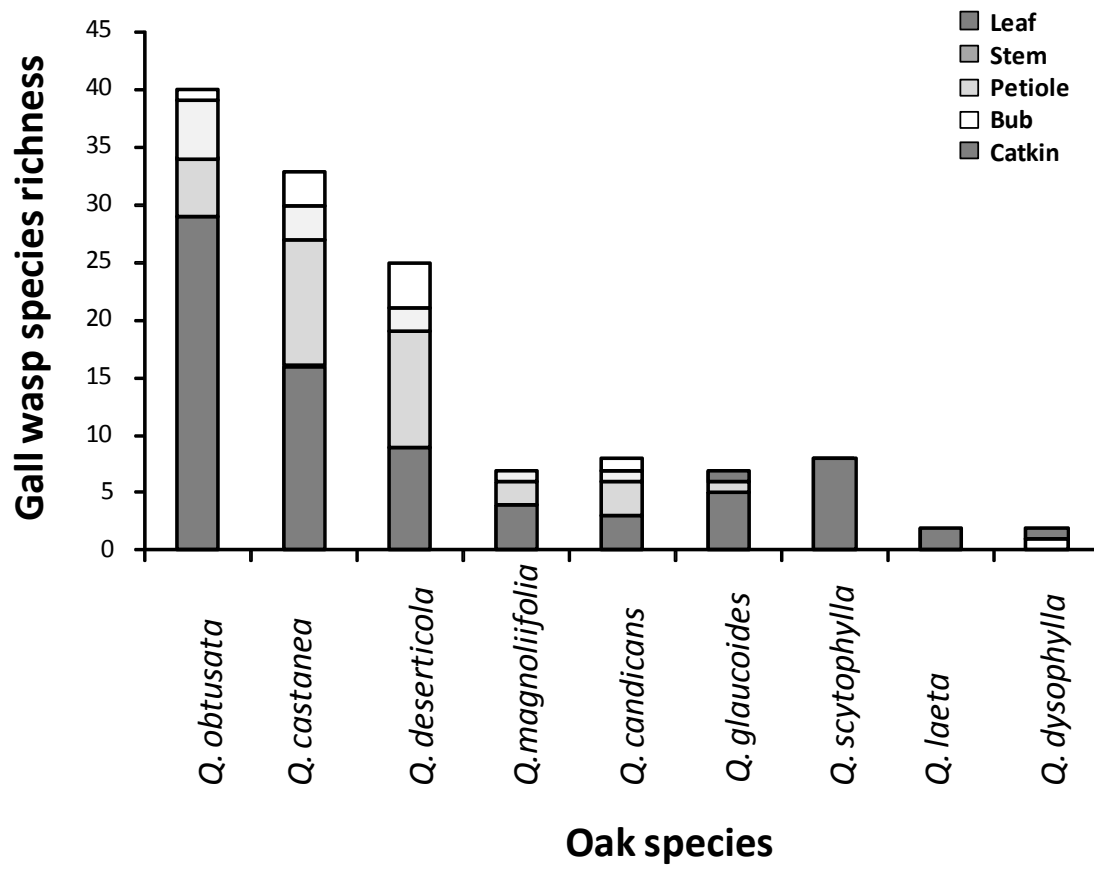
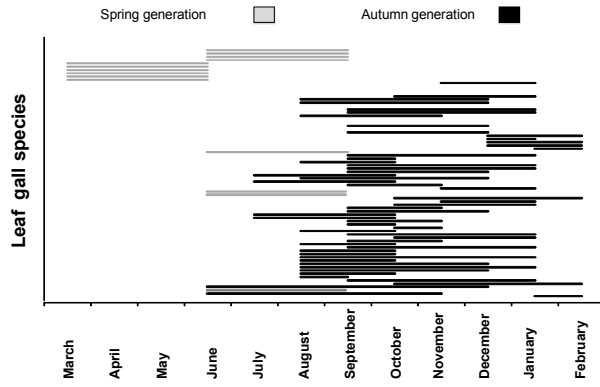
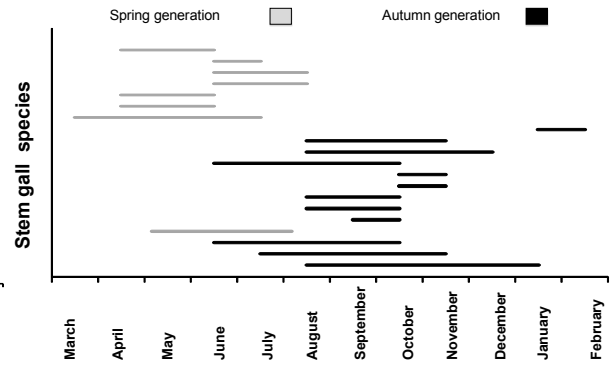


Fig. 3

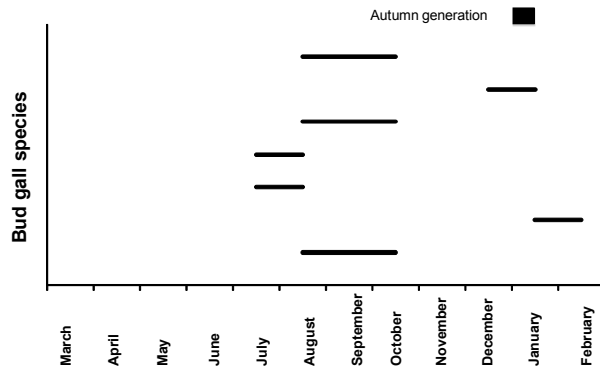
(a)



(b)



(c)



(d)

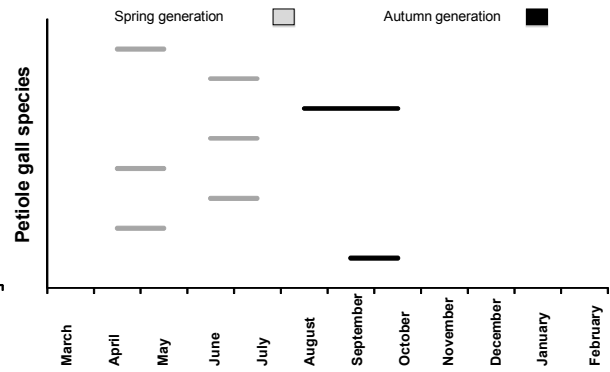


Fig. 4

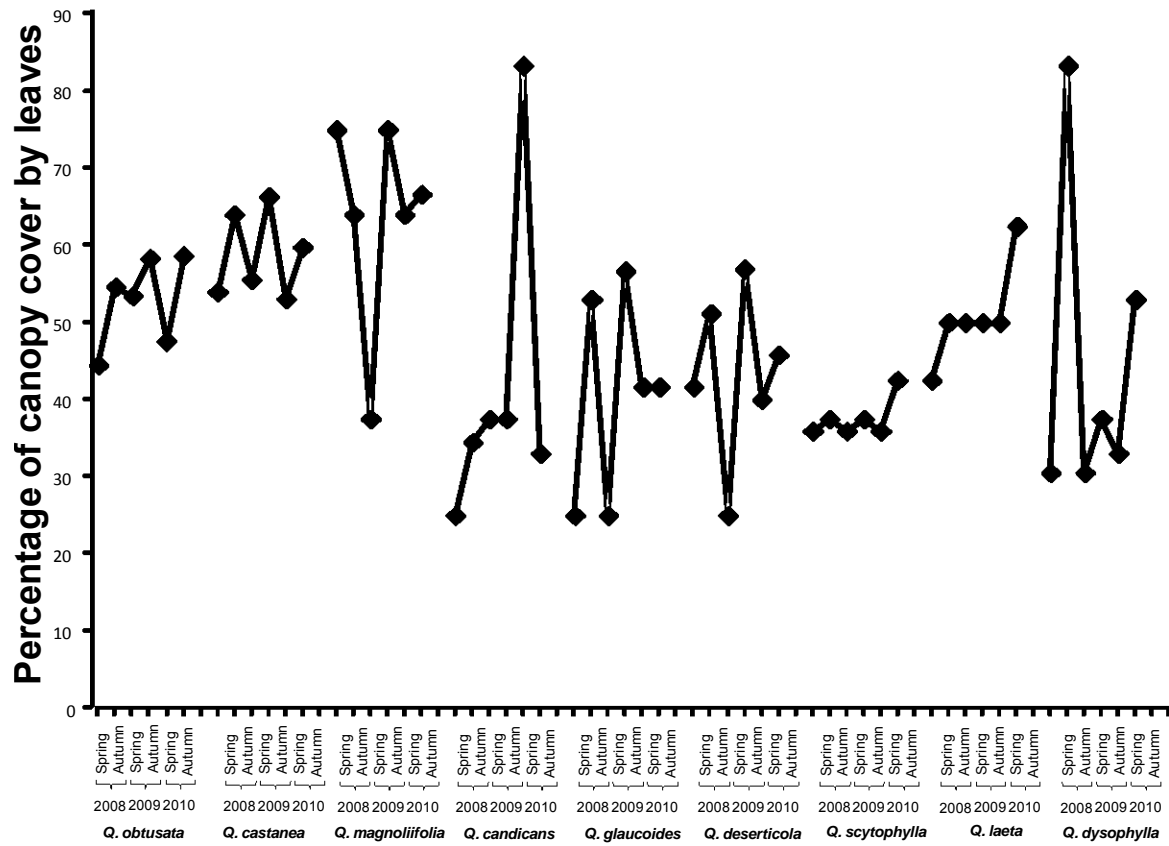
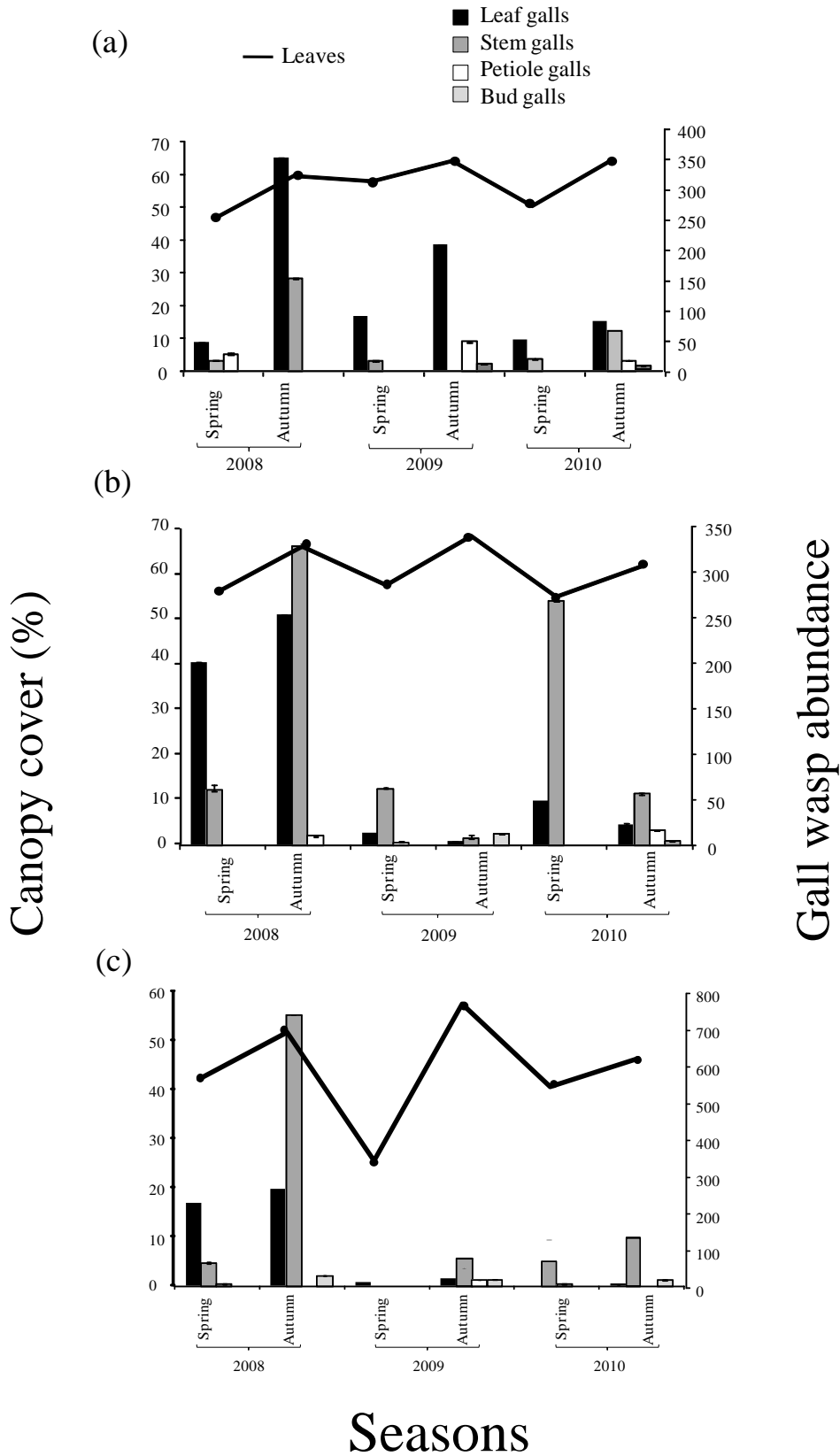


Fig. 5



CAPÍTULO III.

Multiple colonization events of gall wasps in a widespread endemic oak tree in Mexico

Running Title: Evolutive convergence of cynipids in a oak species

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Abstract

Plant species that are highly susceptible to gall induction have been called "*super-host*". This is the case of some oak species (Fagaceae: *Quercus*) that are attacked by many species of cynipids or gall wasps (Hymenoptera: Cynipidae: Tribu Cynipini). Specifically, *Quercus castanea* supports many gall wasp species along its geographic distribution. We analyzed alternative explanations of the association of *Quercus castanea* with numerous gall wasp species. We used DNA sequence data from two mitochondrial genes to analyze the phylogenetic relationships of gall wasps associated with different organs of *Quercus castanea* and gall position (organ) and gall phenology were mapped on the phylogeny to analyze the habitat use of gall wasps. Our results show that gall wasp species convergence in *Q. castanea*, as unrelated evolutionary events, which shows none process of speciation or adaptive radiation.

KEY WORDS: Cynipid, *Q. castanea*, gall phenology, gall position, evolutive convergence.

Introduction

Plant species are consumed by a variety of herbivores that range from specialist to generalist (Rasmann and Agrawal 2011; Barret and Heil 2012; Castillo et al. 2013). In particular, specialist herbivores are adapted to a limited spectrum of potential resources (Barret and Heil 2012) and with the loss of ability to use many host plants, they can manipulate hosts to their benefit and reduce predation and parasitism (Ali and Agrawal 2012). This is the case of gall inducing insects, that are usually specific to a single plant species and the organ attacked (Abrahamson et al. 1998; Cuevas-Reyes et al. 2004). A consequence of their high specificity is that these guild insect and their life cycle has to be synchronized with host plant phenology to successful induce galls (Weis et al. 1988; Stone et al. 2002). However, numerous gall inducing insects species can induce galls in the same plant species, that are highly susceptible to gall induction (Fernandes and Price 1988). These plant species have being called "*super-host*" (Fernandes and Price 1988; Araùjo et al. 2013; Maldonado-López et al. *in press*).

Clear examples of "*super-host*" are some oak species (Fagaceae: *Quercus*) that are attacked by many species of cynipids or gall wasps (Hymenoptera: Cynipidae: Tribu Cynipini) (Shorthouse and Rohfritsch 1992). For example, *Quercus turbinella* in North America supports 20 species and *Q. robur* and *Q. petraea* in Europe are associated to more than 70 species (Fernandes and Price 1988; Csóka et al. 2005). According to the authors, the high tannin diversity and sclerophylly on *Quercus turbinella* may enhance cynipid galls richness and abundance (Fernandes and Price 1988). Cynipids attacking the same plant species and organ often partition resources by attacking in different phenological times and such partitioning of resources likely has been driven by selection acting to reduce competition among these gall inducers (Abrahamson et al. 1998).

Only few studies have explored evolutive explanations of how these host plants were colonized by a high number of gall inducing insects (Joy and Crespi 2007). A first hypothesis proposes an adaptive radiation within the same host species (*in situ*) (Cook et al. 2002; Després et al. 2002; Joy and Crespi 2007). Evolutive changes to new host plants are frequently associated to adaptations to differences in morphology, phenology and chemical defense (Becerra and Venable 1999; Cook et al. 2002). However, the speciation *in situ* in the same host plant species (Cook et al. 2002; Després et al. 2002; Joy and Crespi 2007) may not require substantial evolutionary changes, while other barriers such as high rates of gene flow, have probably inhibited the process of speciation via ecological changes within a host species (Ferdy et al. 2002). The evolution of host plant preference of cynipids seems to be characterized by a high degree of conservatism mixed with infrequent changes to phylogenetically distant hosts (Ronquist and Liljeblad 2001; Cook et al. 2002). Cook et al. (2002) describe an *in situ* speciation of the genus *Andricus* associated with shifts in gall location more often than shifts in host oak section. The authors suggested that a similar host gene-for-gene correspondance systems could be operating, as in other galler systems (Zantoko and Shukle 1999). Since speciation *in situ* was observed within *Andricus* clades that gall a given host oak section, a possible explanation is that speciation might be driven by ecological factors operating at the scale of individual host plants, such as direct competition for oviposition sites (e.g. in *A. quercuscalicis*; Hails and Crawley 1991; Atkinson et al. 2002) or indirect competition for enemy free space (Stone et al. 2002).

An alternative hypothesis is the colonization of independent lineages of cynipids on the same host plant species as unrelated evolutionary events that not involve a process of speciation. Gall wasps that have repeatedly converged in the same plant species involve inquiline tracking

their host among the woody rosid galls (Ronquist and Liljeblad 2001). For example, galls complexes of cynipids *Phanacis-Timaspis* and *Aulacidea-Isocolus* that are distantly related, attack the same plant genera with a remarkable degree of overlap. This convergence appears to represent an independent evolutionary event, because the attacked plants don't present obvious similarity in secondary compounds, morphology, plant community or phytophagous insect community. The only mechanism that could explain this extreme convergence, is the parasitism among cynipids involved in many changes of host plants shifts (Ronquist and Liljeblad 2001).

Considering the host use by cynipids, they represent an excellent study model for the evaluation of evolutionary processes of speciation and adaptive radiation (Abrahamson and Weis 1997). In this way, we analyzed alternative explanations of the association of *Quercus castanea*, an endemic oak in Mexico, with numerous gall wasps species (Maldonado-López et al. *in press*). We hypothesized that if a radiation *in situ* occurred within *Q. castanea*, it is expected that associated gall wasps species form a well supported monophyletic group with adaptive radiation to different niches or adaptive zones. A second hypothesis is that if the convergence of cynipids were unrelated evolutionary events, we expected to find none process of speciation or adaptive radiation. We used DNA sequence data from two mitochondrial genes. Gall position (organ) and gall phenology were mapped on the phylogeny to analyze the habitat use of gall wasps. The objectives of this study were: 1) to analyze the phylogenetic relationships of gall wasps associated with different organs of *Quercus castanea*; and 2) to determine the relationship between phylogenetic proximity of gall wasps and gall position and phenology (time of adult emergence).

Materials and Methods

Study species

Cynipids (Hymenoptera: Cynipidae: Cynipinae) are gall wasps associated with genus *Quercus*. They produce complex galls on various oak tissues (buds, stems, catkins, leaves and roots) (Hayward and Stone 2005). The specificity of cynipids to oaks demonstrates their degree of monophagy (Abrahamson and Weis 1987). Oak gall wasps are obligated to oviposit inside the tissues of their vegetal host organ and the larvae develop in galls induced on specific plant tissues (Stone and Schönrogge 2003). The cynipid life cycle progresses through two generations: a sexual generation gall develops in the spring or early summer, while an asexual generation occurs during the summer and autumn of the same year (Stone et al. 2002). The wasps of the two generations are often very different, but each can be identified on the basis of its characteristic morphology, location on the tree and oak host taxon (Stone et al. 2002).

Quercus castanea Née is a red oak (section *Lobatae*) (Nixon 1993). It is a moderately large forest tree 10-18 m in height and a trunk diameter up to 0.4m. Their populations are found between 1100 and 2600 m of elevation in oak forest and pine-oak forests. It grows in warm temperate to humid climates, with average annual temperatures ranging from 10 to 26 °C, but more often from 12 to 20 °C (Kappelle 2006). The acorns mature from July to November with a flowering period in April and May (Valencia 1995).

Study techniques

Sampling

Gall samples were collected in 23 different populations of *Q. castanea* over all its distribution in Mexico (Figure 1). Galls were kept in rearing cages until the emergence of

insects. Each species of insect was stored in 70% ethanol for taxonomic identification. Voucher adult specimens and their galls were deposited in the entomological collection of the Museo Nacional de Ciencias Naturales, Madrid (Spain) and the Laboratorio de Ecología Genética y Molecular, CIEco, UNAM (Mexico).

Specimen preparation

Specimens were dissected into parts under stereomicroscope, *air dried, mounted on a stub and coated with gold*. From these preparations, micrographs were obtained with an FEI QUANTA 200, FEI Company (Oregon, USA), operating with high vacuum technique, from several standardized views. Forewings were mounted on slides in Euparal and later examined under a Wild MZ8 stereo microscope. Representatives of some species that were poorly represented in the samples were not dissected but were instead directly observed with the same SEM using a low vacuum (voltage) technique, without coating.

Classification of gall position and phenology

We recorded the host plant organ where galls were found (i.e., stem or twig, inflorescence, acorn, leaf, and petiole). The gall phenology was also recorded as the time of adult emergence of each gall wasp species.

DNA amplification and sequencing

DNA was extracted from wasp adults using the QIAamp DNA Mini Kit (QIAGEN). A fragment of the mitochondrial Cytochrome Oxidase I gene (COI; primers lco and hcoexternb, Rokas et al. 2002) and Cytochrome B (CB; primers CB1 and CB2, Rokas et al. 2002) were

amplified by polymerase chain reaction (PCR). The thermal cycling conditions consisted of 35 cycles, each at 95°C for 1 min, annealing at 51.1°C for 1 min, and extension at 72°C for 2 min. A final extension at 72 °C for 7 min was included. PCRs were carried out in a Eppendorf Thermal Cycler. Twenty-five microlitre PCRs were carried out using 1 mL Taq polymerase (Invitrogen or Promega), 0.5 mL primers, 1.0 mL template DNA and 20.5 mL dH₂O, 0.5 mL, 0.5 Taq buffer, 0.5 mL MgCl₂ (25 mM), 0.5 mL dNTPs (10 mM). PCR products were purified using the QIAquick PCR Purification Kit (QUIAGEN) and sequenced directly using ABI Prim 3130xl Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis

Phylogenetic reconstruction of 18 species of cynipids associated to *Quercus castanea* was performed. Phylogeny were rooted using five oak gall wasps as outgroups: *Andricus curvator*, *Andricus kollari*, *Biorhiza pallida*, *Diplolepis rosae*, and *Barbotinia oraniensis*. We used a combined mitochondrial (COI and CB) databaset for Bayesian reconstruction (see below) (Ronquist and Huelsenbeck 2003). ClustalW v1.83 (Thompson et al. 1994) was used to aligned sequences and checked by eye. We selected suitable models of molecular evolution for phylogeny reconstruction using the program MODELTEST 3.7 (Posada and Crandall 1998) in conjunction with PAUP* 4.0 (Swofford 2002). For COI and CB fragments was supported the general time reversible model with a gamma distribution of rate variation across sites (GTR+G). Because the gall wasps species included multiple gall location and phenology, we reported these traits in the phylogeny.

Results

The phylogeny of oak gall wasps reconstructed with the combined mitochondrial dataset and the BPP values are shown in Figure 2. The phylogeny identified several robustly supported clades, with a good resolution of lineages, with the exception of some clades with low support values (see below), which findings should be taken with caution.

Support for the node indicating monophyly of this entire group was 100 (BPP), however, it involve multiple lineages of different gall wasps species, which indicated that there is no evidence of host-plant speciation within a single genus. We identified three principal lineages. The clade with lower support values (44; BPP) is the *Zapatella* group that includes an unidentified species, *Amphibolips michoacaensis*, a species of a new genus (sp.1) and *Zapatella* sp.2. The second lineage is the *Cynips* group, that includes two species of a new genus and two *Cynips* species, coming out as sister groups of a clade that includes *Andricus* sp.2 and *Discholcaspis* sp. (70; BPP). As a sister species of *Zapatella* and *Cynips* groups, there is an *Andricus* species (sp.1) (86; BPP). The third principal lineage is the *Neuroterous* group that includes an unidentified species, the genus *Andricus* (sp.3), a *Loxaulus* species (sp.1) and two *Neuroterus* species (52; BPP). Two gall wasp species were found outside of all the above-mentioned genera; *Zapatella* sp.1 (72; BPP) and *Loxaulus* sp.2 (100; BPP). Our phylogenetic analysis show some paraphyletic species that forms part of monophyletic clade (*Zapatella*, *Loxaulus*, *Andricus*, *Cynips* and a new genus) (Figure 2).

Gall position

In the *Zapatella* group we found differences between species in gall position. *Zapatella* sp.2, as well as *Zapatella* sp.1, induced galls in acorns, an unidentified species and *Amphibolips*

michoacaensis induced galls on stems or twigs. All species of the new genus induced galls in leaves. In the *Cynips* group, *Cynips* sp.1 induce galls on leaves and *Cynips* sp.2 induce galls on petioles. *Andricus* sp.2 and *Discholcaspis* sp. induce galls on stems or twigs. *Neuroterus* species and an unidentified species induced galls on leaves, meanwhile *Andricus* sp.3 and *Loxaulus* sp.1 induce galls on stems or twigs (Figure 3).

Gall Phenology

We found differences in gall phenology periods between all gall wasp species analyzed. *Zapatella* sp.1 as well as *Zapatella* sp.2 have a time of adult emergence between October and November, an unidentified species (sp.1) is found in August and September, wasp adults of *Amphibolips michoacaensis* emerge in March and April, and all species of the new genus have a time of emergence between February and March. In the *Cynips* group, all species have different phenological times (*Cynips* sp.1, October-November; *Cynips* sp.2, February-March; *Discholcaspis* sp., September- October; *Andricus* sp.2, May-June). *Neuroterus* species and *Loxaulus* species have a similar time of emergence between May and June, while adults of an unidentified species (sp.2) are found in March and April and *Andricus* sp.3 between February and March (Figure 3).

Discussion

Oak gall wasps relationships

We identified three principal lineages in the gall wasp phylogeny. The *Neuroterous* group, the *Cynips* group and the *Zapatella* group. Few studies have analyzed the oak gall wasps relationships of the tribe Cynipini, for species in the Palearctic, based on morphological

(Liljeblad 2002; Liljeblad et al. 2008) and molecular analysis (Rokas et al. 2003). These studies support the monophyly of the group (Liljeblad 2002; Rokas et al. 2003) and show the presence of three different lineages: the *Neuroterus* group, the *Cynip* group and a third group that includes the paraphyletic genus *Andricus* and related taxa (Ronquist and Liljeblad 2001). Although we have only few cynipid genera in common with these studies, we found some similarities: the genus *Andricus* is paraphyletic, as well *Zapatella*, *Loxaulus*, *Cynips* and a new genus. Anterior studies describe *Disholcaspis spectabilis* and *Loxaulus* sp. as basal genera. We also found the genus *Loxaulus* and *Zapatella*, in a basal position. For the contrary, in our phylogenetic analysis, *Neuroterus* is not a primitive genus as was suggested by Kinsey (1923).

Multiple colonization of gall wasps species on the "Super-host" Quercus castanea

Our results revealed that gall wasps associated to the "super-host" *Quercus castanea* belong to independent cynipid lineages. Some authors have suggested that gall inducing insects are good candidates for adaptive radiation into different niches or adaptive zones (Cook et al. 2002; Joy and Crespi 2007), because they have the ability to manipulate the growth, and development of plant tissue, modifying host nutritional quality and plant secondary metabolites (Pascual-Alvarado et al. 2008). However, we found no evidence of speciation *in situ* or adaptive radiation within *Q. castanea*. Our results show a phylogeny that comprises multiple lineages of different gall wasps genera with different life histories, which indicated that there is no evidence of speciation within a single host plant species. The phylogenetic study shows gall wasp species convergence to the same oak species as unrelated evolutionary events. Another reason why we argue that no event of speciation *in situ* or adaptive radiation occurred in *Q. castanea* is because this gall wasps species are not exclusive for *Q. castanea*. In a recent study, we found that *Q.*

castanea shared oak gall wasp species with other red oaks (*Q. dysophylla*, *Q. candicans* and *Q. scythophylla*) (Maldonado-López et al. *in press*). Despite oak gall wasps genera are usually specific to a single oak species, they frequently can gall multiple oaks in the same section or species group (Nieves-Aldrey 2001; Cook et al. 2002; Stone et al. 2008).

The presence of “*super-host*” species produce differences in local and regional patterns of gall inducing richness because represent keystone resources (Veldtman and McGeoch 2003; Araùjo et al. 2013; Maldonado-López et al. *in press*). An ecological hypothesis proposes that “*super-host*” plant species support richer cynipid communities because they present a wide geographic distribution (Cornell and Washburn 1979). *Quercus castanea* is associated to many oak gall wasps along its wide distribution that includes 18 states of México (Valencia 2004). A second ecological hypothesis proposes that cynipid richness is higher in oak species with higher tannins concentrations, perhaps because high-tannin hosts concentration reduces mortality inflicted by natural enemies (Taper and Case 1987; Stone et al. 2002). Unfortunately, we are not able to determine if tannins concentrations in *Q. castanea* have an important role in gall wasps richness, because there are no available studies that analyze secondary metabolites and nutritional quality in comparison with other oak species. In this way, we suggest as future studies to compare the secondary metabolites profile of “*super-host*” with no “*super-host*” plant species.

Coexistence of oak gall wasps on a single host tree

A main hypothesis that have been proposed to explain the sympatric coexistence of high diversity of oak gall wasps on a single host tree, states that the high oak gall wasps richness could be maintained by partitioning of generalist parasitoids among different gall phenotypes. In

this way, cynipids can avoid mortality reducing parasitoid efficiencies and acquire a measure of enemy-free space (Price et al. 1987; Stone and Cook 1998; Stone and Schönrogge 2003). The Janzen-Connell hypothesis states that predators may promote species coexistence when each species has its own specialist natural enemy that hold down the density of each species independently (Armstrong 1989). Cynipids could be exposed to an apparent competition (indirect competition) by shared natural enemies that could drive all but one of its hosts to extinction (Holt 1977; Holt and Lawton 1993). Although the natural history of most gall inducers and parasitoids of Nearctic cynipid species are not well described (Stone et al. 2002), we suggested that oak gall wasps community associated to *Q. castanea* could be regulated by their parasitoids community. For example, Espinosa-Olvera (unpublished data) found that gall wasp community associated with *Q. castanea* is regulated by 32 parasitoid species in Mexico. In the same way, Quesada-Béjar (unpublished data) show that 25 parasitoids species regulated the population size of gall wasps species associated to Mexican hybrid complex of *Q. affinis* x *Q. laurina*.

A second hypothesis is a direct competition inter and intraspecific in cynipids communities that could occurred for limiting resources, especially in a particular host organ where multiple galls develop at the same time (Whitham 1986; Atkinson et al. 2003). Niche divergence can be promoted in species that coexistence in sympatry, and avoid competition resulting from niche overlap (Tokeshi 1999; Schluter 2000). Species can coexist for long periods if they occupy different niches. Ecologically, if this species differ on one niche axis alone may be sufficient to reduce competition under stable conditions (Webb et al. 2002) or show ecological differences between species that may distinguish their niches (Chesson 2000; Emerson and Gillespie 2008). For oak gall wasps the oak species galled, gall location and gall

phenology are traits that describe the cynipids niche (Bailey et al. 2009). Although we analyzed the same oak species, we found that oak gall wasp species that are close phylogenetically, showed ecological differences in some niche traits (gall position or gall phenology), suggesting that their coexistence could be due a niche divergence. If ecologically similar species occupy the same niche, the realized niche/habitat distribution of taxa is divided in subniches within the fundamental niche or may lead to classic competitive exclusion process (Webb et al. 2002). Besides, two competing species may coexist if one of them is more strongly limited by the resource and the other is more strongly limited by the predator (Grover and Holt 1999).

We concluded that oak gall wasps species convergence in *Q. castanea*, as unrelated evolutionary events, originating a "*super-host*" species. For future studies of the analysis of the maintenance of diversity in communities, will be important to highlight the similarities and differences of closely related, ecologically similar species occupying the same niche and the mechanisms that permit the co-existence of similar organisms.

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Figures Legends

Figure 1. Map showing sampling localities over the potential distribution for *Quercus castanea* in Mexico.

Figure 2. Phylogenetic relationships between oak gall wasps species associated with *Q. castanea*. For each node Bayesian support values are shown.

Figure 3. Gall position and gall phenology are reported for each species in the oak gall wasps phylogeny based on a combined database. The presence of a triangle of different colors between the branch tip and a species name on the tree, indicates the gall position (Green: leaf; yellow: petiole; brown: stem or twig; blue: acorn). Adult emergence time is indicated for each species.

Figure 1.

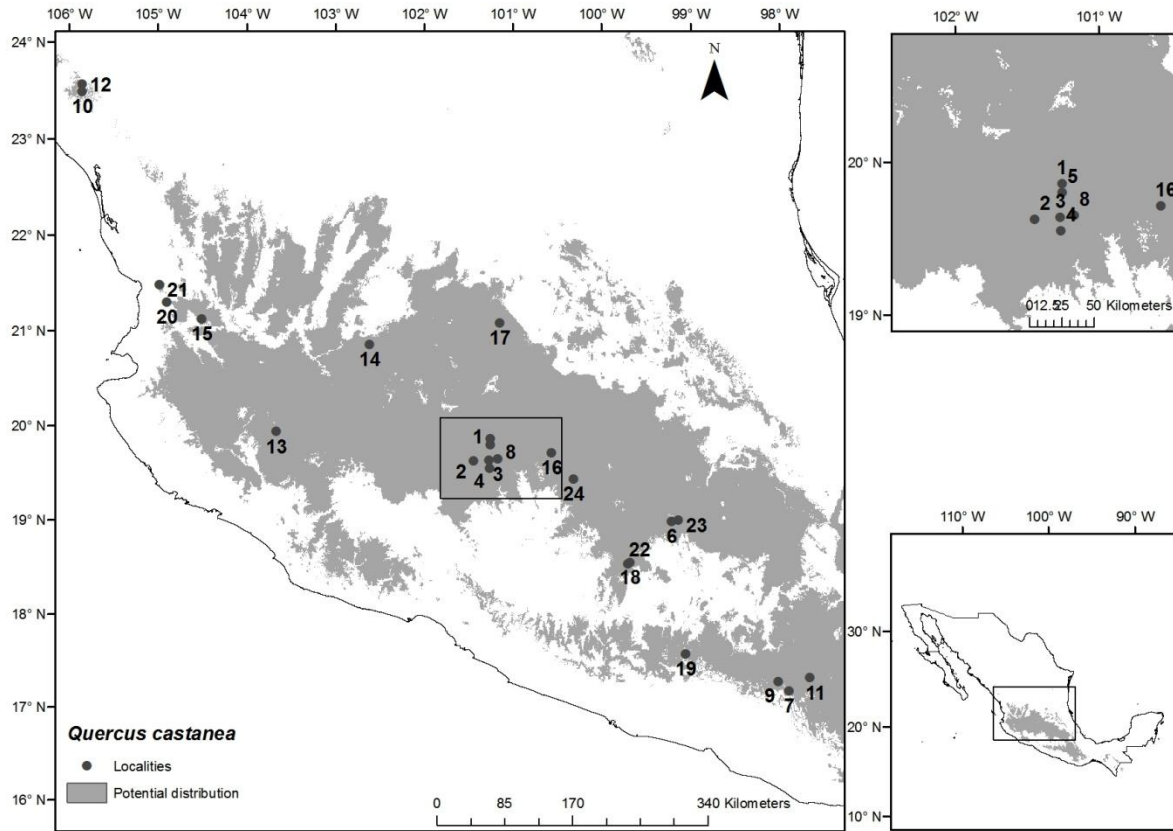


Fig. 2

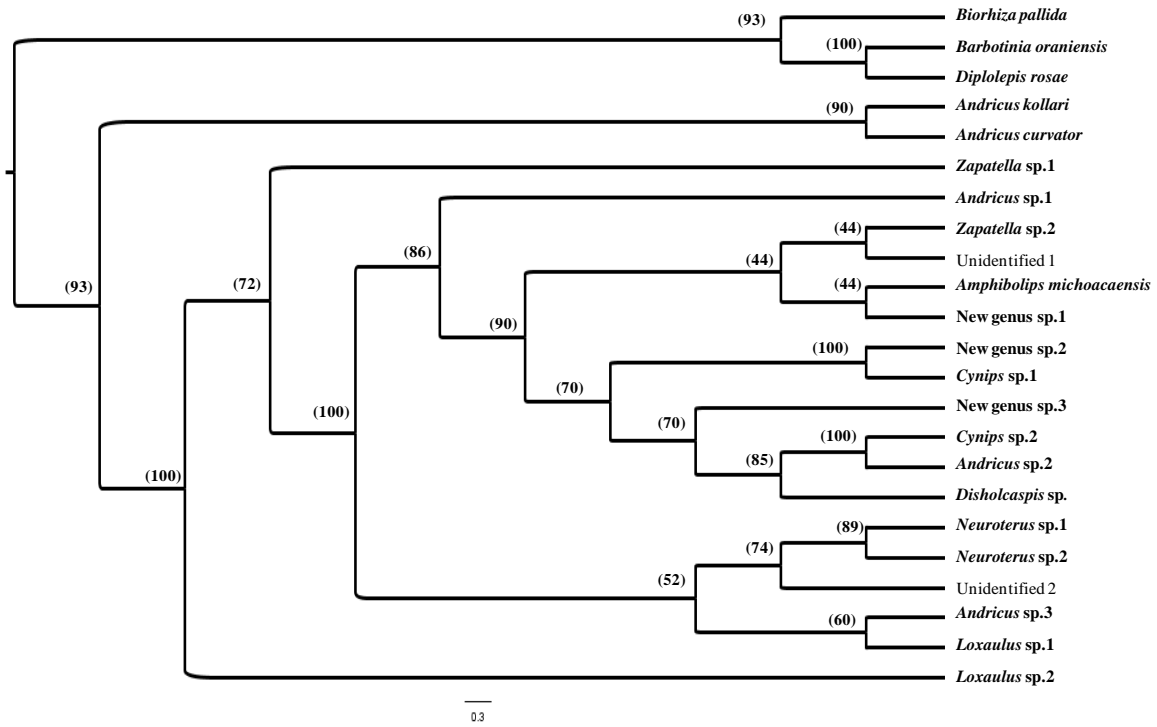
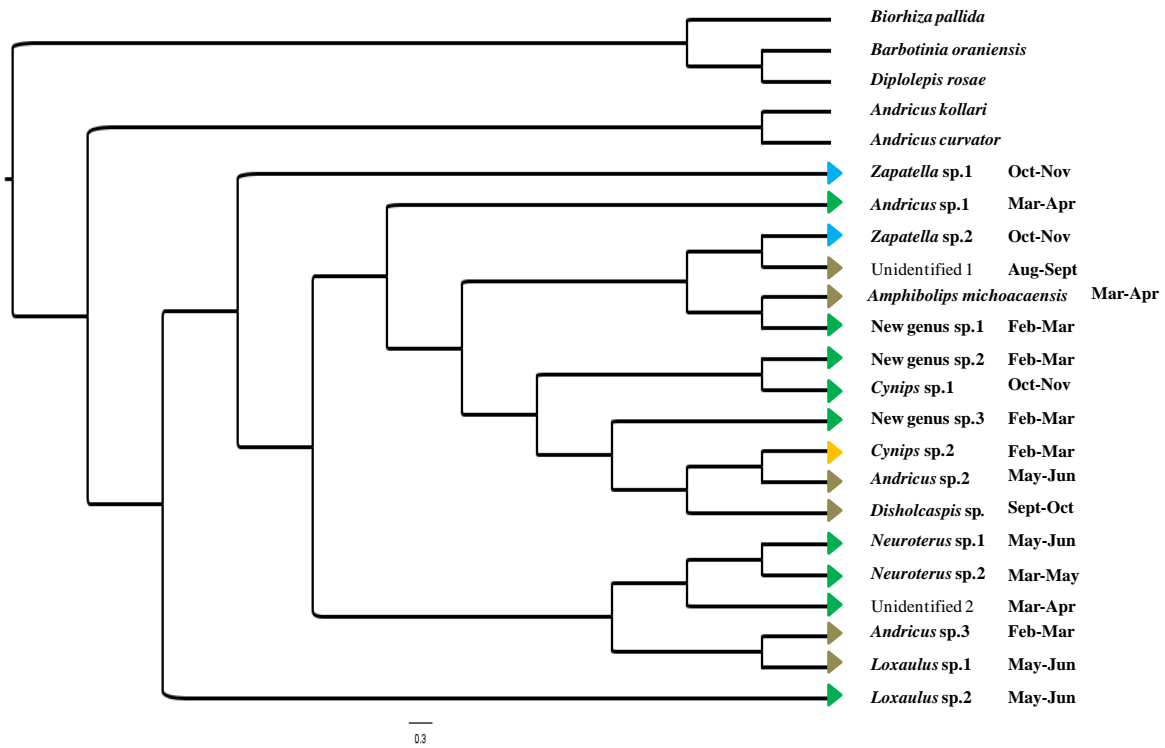


Fig. 3



Capítulo IV.

Revision of the *Amphibolips* species of Mexico excluding the “niger complex” Kinsey (Hymenoptera: Cynipidae), with description of seven new species. *Zootaxa* 3545:1–40.

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Article

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Revision of the *Amphibolips* species of Mexico excluding the “*niger* complex” Kinsey (Hymenoptera: Cynipidae), with description of seven new species

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Abstract

The species of *Amphibolips* Reinhard 1865 (Hymenoptera: Cynipidae: Cynipini) of Mexico not included in the “*niger* complex” Kinsey 1937 are revised. The “*nassa* complex” recently created by Melika *et al.* (2011) for these species is questioned. As a result of faunistic surveys of oak gall wasps in Mexico, seven new species of *Amphibolips* are described: *Amphibolips durangensis* Nieves-Aldrey & Maldonado, *A. malinche* Nieves-Aldrey & Pascual, *A. jaliscensis* Nieves-Aldrey & Pascual, *A. oaxaca* Nieves-Aldrey & Pascual, *A. nevadensis* Nieves-Aldrey & Pascual, *A. tarasco* Nieves-Aldrey & Pascual and *A. michoacaensis* Nieves-Aldrey & Maldonado. The types of *A. dampfi* Kinsey 1937, *A. nebris* Kinsey 1937 and *A. fusus* Kinsey 1937 are revised, redescribed and compared with the new species described herein. A new key for the identification of adults of all species not included in the “*niger* complex” from Mexico, including the related species *A. castroviejo* Medianero & Nieves-Aldrey and *A. aliciae* Medianero & Nieves-Aldrey from Panama, is presented. Diagnostic characters, distribution data, host plants and species biology are provided for of all of the studied species. The richness and distribution of *Amphibolips* in Mexico and the Neotropical region, their known biological data, and the taxonomic limits of this genus are also discussed.

Key words: Cynipidae, oak gall wasps, *Quercus*, Mexico

Introduction

The cynipids of the tribe Cynipini (Hymenoptera: Cynipidae), known as the oak gall wasps, form the largest group of gall wasps, including more than 1000 described species, all of which are gall inducers exclusively on plants of the Fagaceae family (Nieves-Aldrey 2001; Liljeblad *et al.* 2008). The oak gall wasp fauna of Mexico has not been well studied and remains poorly known. However, it is estimated that this fauna is potentially very rich because of the great diversity of their host *Quercus* species, 161 species of which are found in México, including 109 endemic species (Valencia-A 2004). A recent inventory of the Cynipidae of Mexico recorded 157 cynipid species (Pujade-Villar *et al.* 2009). However, more recent and ongoing studies on Mexican oak gall wasps are rapidly increasing the number of recorded species.

Amphibolips Reinhard 1865 is a distinctive genus of oak gall wasps (Cynipidae: Cynipini) recorded as being confined to America. The known range of this genus, which was assumed to extend only in the Nearctic, was recently enlarged to the Neotropical region, with three species described from Panama (Medianero & Nieves Aldrey 2010).

At present, forty-five species are known throughout the distribution area of this genus, extending from Canada to Panama. There are 42 species recorded in the Nearctic region, and three are known from Panama: *A. castroviejo* Medianero & Nieves-Aldrey, *A. aliciae* Medianero & Nieves-Aldrey and *A. salicifoliae* Medianero & Nieves-Aldrey (Medianero & Nieves-Aldrey 2010). Of the 42 Nearctic species, 30 have been recorded from the USA and Canada,

and 11 species are known only from Mexico (Burks 1979; Melika & Abrahamson 2002; Melika *et al.* 2011). Additionally, one species, *A. niger* Beutenmüller, is known from Mexico and southern Arizona (Burks 1979).

Twelve species are known from Mexico, of which seven species belongs to the “*niger* complex” of species (Kinsey 1937; Melika *et al.* 2011): *A. niger* Beutenmüller 1911, *A. gumia* Kinsey 1937, *A. jubatus* Kinsey 1937, *A. elatus* Kinsey 1937, *A. maturus* Kinsey 1937, *A. nebris* Kinsey 1937 and *A. pistrinx* Kinsey 1937. The remaining six species were recently included into the “*nassa* complex” (Melika *et al.* 2011); these include *Amphibolips palmeri* Bassett 1890; three species described by Kinsey, *A. dampfi* Kinsey 1937, *A. nassa* Kinsey 1937 and *A. fusus* Kinsey 1937; and two recently described species, *A. zacatecaensis* Melika & Pujade-Villar 2011 and *A. hidalgoensis* Pujade-Villar & Melika 2011.

The morphological characters of adults and their galls are very uniform among the majority of the known species of *Amphibolips*. These characters include 12–14 segmented antennae in females and 15–16 segmented antennae in males; a robust body with a strong coarsely sculptured head and mesosoma; notauli often obscured by the rugose sculpture; a mesoscutellum often more or less emarginated posteriorly; tarsal claws with a large acute basal lobe or tooth; metasomal tergites usually micropunctate from the posterior part of the second metasomal tergite; forewings usually more or less smoked, often very heavily infuscate, especially in males, with dark bands or spots and a long, narrow radial cell, opened on the wing margin; and the projected part of hypopygium very long and robust (Melika & Abrahamson 2002; Medianero & Nieves-Aldrey 2010; Melika *et al.* 2011). However, there are some *Amphibolips* species that present anomalies in some of these diagnostic characters, thus revealing that the taxonomic limits of the genus are not still clear. This is the case for *A. salicifoliae* Medianero & Nieves-Aldrey from Panama, a species that while presenting most of the diagnostic characters of the genus, exhibits simple metatarsal claws. Another example provided by the species of the “*niger* complex” (Kinsey, 1937) from Mexico, which present 16–17 segmented antennae as well as other morphological and biological characters, such as the sculpture of the mesoscutum and the morphology of their galls, that differ from the typical *Amphibolips* species.

The known *Amphibolips* species from Mexico, out of the “*niger* complex”, appears to be a group morphologically distinctive, easily differentiate by their darkest forewing color pattern and emarginated mesoscutellum, from the *Amphibolips* species from USA and Canada.

The understanding of the phylogenetic relationships of *Amphibolips*, both within the genus and with other related Cynipini genera, is still poor. In a recent morphological phylogenetic analysis performed by Liljeblad *et al.* (2008), one *Amphibolips* species was included, but the results were not conclusive with regard to its phylogenetic relationships with other genera. Ongoing molecular studies will hopefully contribute to filling this gap in knowledge.

The life cycles of *Amphibolips* species are heterogonic, and both bisexual and asexual forms are known. However, for a great majority of these species, including all of the Mexican and Panamanian species, the complete life cycle is not still known. It is therefore likely that some species described separately based on only either the bisexual or asexual generation could belong to a single species.

All known *Amphibolips* species are associated with *Quercus* species of the section Lobatae (red oaks). The galls induced by species of this genus develops mainly in buds, stems or leaves, rarely also in acorns. They are usually globose to spindle shaped and detachable, with a spongy parenchyma surrounding a central larval cell, sometimes supported by radiating filaments (Beutenmüller 1909; Kinsey 1937; Melika & Abrahamson 2002).

In a recent paper by Melika *et al.* (2011), current knowledge of the Mexican species of *Amphibolips* was updated, and two species new were described: *A. zacatacaensis* Melika & Pujade-Villar and *A. hidalgoensis* Pujade-Villar & Melika. Symmetric to the “*niger* complex” proposed by Kinsey (1937), a second group, the “*nassa* complex” of species, was proposed, and a key for identification of the species of this group was provided. However, this key is not entirely satisfactory in our opinion because the important diagnostic character of forewing colour pattern was neglected or misunderstood. This shortcoming is because the forewing was not examined in the types of some key species described by Kinsey as *A. dampfi*, *A. fusus* and *A. nassa*. As a result, the interpretation of the affinities between species may be incorrect, and their taxonomic separation was not clear in the key.

The objective of this study is to present a revision of the species of *Amphibolips* from Mexico not belonging to the “*niger* complex”. As a result of several years of oak gall wasp sampling efforts in a number of states, mainly in the centre of Mexico, new materials were collected containing representatives of seven species new to science, including males of three species, which are described herein. Based on these new materials and examination of Kinsey types, we revise the entire “*nassa* complex” (*sensu* Melika *et al.* 2011), including the related species from

Panama *A. castroviejoi* and *A. aliciae*. A new identification key is provided for all of the species in the group as considered herein. And finally, in the light of the new data and understandings provided in this study, the actual and expected species richness of this genus in Mexico are discussed as well as the taxonomic problems still existing within the group.

Material and methods

Study material. Samplings of *Quercus* species were performed in several states of Mexico from 2007 to 2011, with more intensive samplings of *Quercus castanea* being carried out in selected localities of the state of Michoacan. Galls were collected on the sampled oaks and stored in rearing cages until the emergence of insects. The adult insects emerged from the galls under laboratory conditions. Voucher adult specimens and their galls were deposited in the entomology collections of the Museo Nacional de Ciencias Naturales, Madrid (Spain) and the Laboratorio de Ecología Genética y Molecular, CIEco, UNAM (Mexico). The identification of the *Quercus* species was based on several key references (Govaerts & Frodin, 1998; Valencia-A 2004).

Examination of types

Type materials of three species described by Kinsey from Mexico were examined: *Amphibolips fusus* Kinsey 1937; *Amphibolips nebris* Kinsey 1937; and *Amphibolips dampfi* Kinsey 1937. Kinsey's type materials were borrowed from the American Museum of Natural History, New York (AMNH) (James Carpenter). Additionally, two paratypes of *A. hidalgoensis* Pujade-Villar & Melika and galls of *A. hidalgoensis* and *A. zacatacaensis* Melika & Pujade-Villar given by the University of Barcelona (Juli Pujade-Villar) were also examined. We could not examine materials of two other species, *Amphibolips palmeri* Basset and *A. nassa* Kinsey 1937. However, some notes on the latter were provided in Melika *et al.* (2011) and have been used here.

Specimen preparation. For observation under a scanning electron microscope (SEM), adult cynipids were dissected in 70% ethanol, air dried, mounted on a stub and coated with gold. Micrographs were obtained with an FEI QUANTA 200, FEI Company (Oregon, USA), operating with high vacuum technique, from several standardised views. Forewings were mounted on slides in Euparal and later examined under a Wild MZ8 stereo microscope. Representatives of some species that were poorly represented in the samples were not dissected but were instead directly observed with the same SEM using a low vacuum (voltage) technique, without coating. Images of the adult habitus and gall dissections were obtained with a NIKON Coolpix 4500 digital camera attached to a Wild MZ8 stereo microscope. Measurements were performed using a calibrated micrometre scale attached to an ocular of the light microscope.

Morphological terms

The terminology of morphological structures and abbreviations follow Ronquist & Nordlander (1989), Nieves-Aldrey (2001) and Liljebblad *et al.* (2008).

Results

Studied species

“*niger* complex” Kinsey, 1937

Amphibolips nebris Kinsey 1937

Figs 1A–B &H, 13C, 14F

Amphibolips nebris Kinsey. *Rev. Entom.*, 7(4): 442

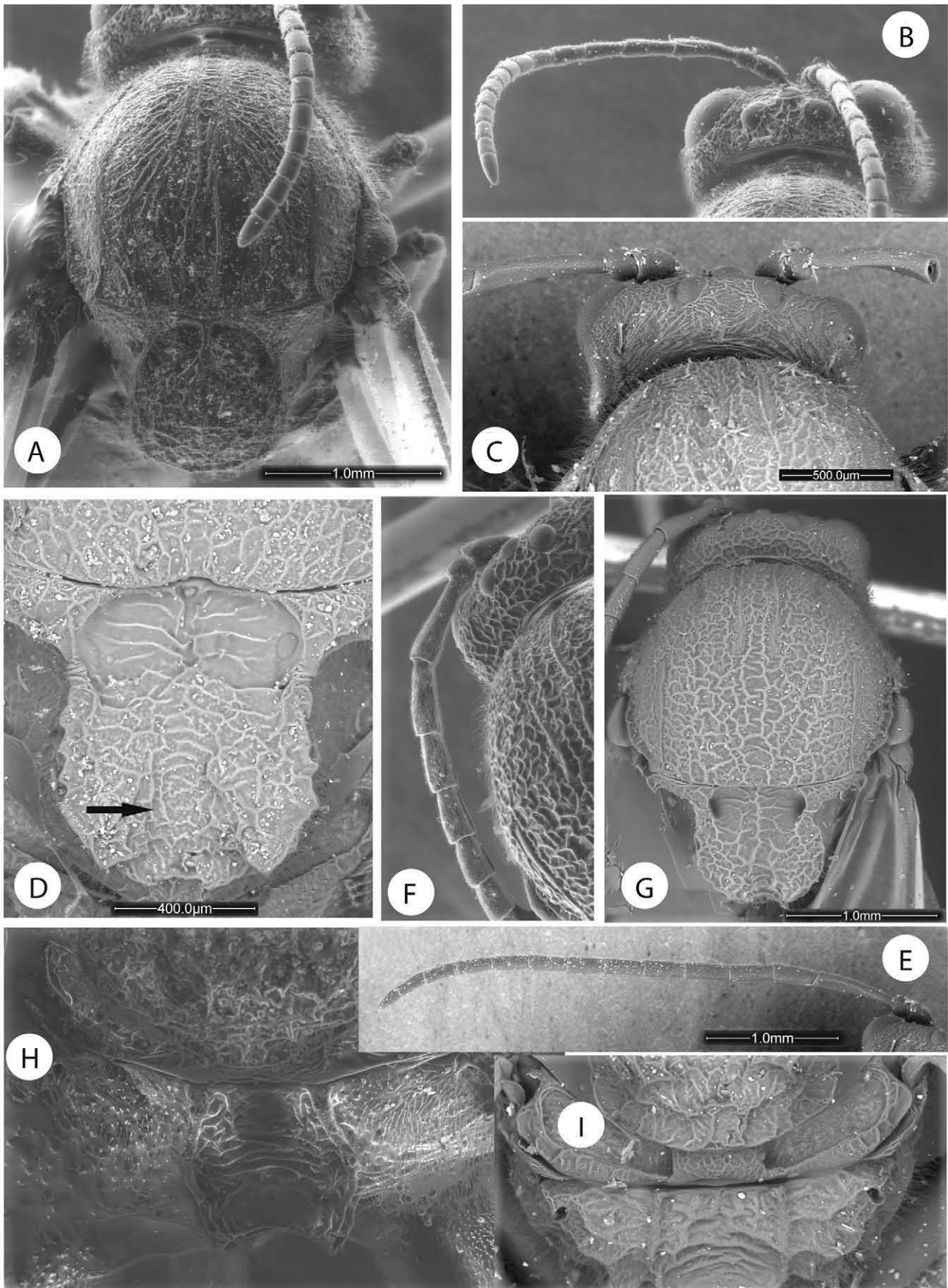


FIGURE 1. *Amphibolips nebris* (A) mesosoma dorsal view; (B) head and antennae dorsal view; and (H) propodeum. *Amphibolips dampfi* (C) head dorsal view; (D) mesoscutellum dorsal view; (E) antenna; and (I) propodeum. *Amphibolips fusus* (F) antenna; and (G) head and mesosoma dorsal view.

Material examined: Holotype female (AMNH).

The holotype, glued to a card point, is in poor condition. The metasoma is missing.

Labels: red Holotype, Parral /2W; Chi 6800; 28–31; 1 ♀, 4.22.32

Quercus eduardi, Kinsey coll.

Here, we present and illustrate some diagnostic characters in addition to those provided in the original description.

Head, dorsal view, 2.7 as wide as long. POL 4 times OOL: OOL shorter than diameter of a lateral ocellus. Antennae with 16 antennomeres (Fig. 1B). Pedicel 1.4 as long as wide. F1 1.3 as long as F2. Mesoscutum with longitudinal carinate sculpture, more radiate anteriorly (Fig. 1A). Scutellar foveae ellipsoidal, separated medially by a ridge. Mesoscutellum moderately rugose, rounded posteriorly, not emarginated at its posterior margin (Fig. 1A). Median area of propodeum only slightly rugose laterally; lateral propodeal carina distinct, slightly divergent (Fig. 1H). Forewing only clouded near anterior margin; first abscissa of radius distinctly projected into radial cell; areolet conspicuously large (Fig. 13C).

Distribution: Chihuahua, Parral

Host: *Quercus eduardi*

Gall: Of the type of galls of the “*niger* complex”: a large densely wooly mass containing a hard woody core in which the larval cell is located (Kinsey 1937; Melika *et al.* 2011). In *A. nebris* the gall is large, up to 70 mm; color yellow tan and rosy russet (Kinsey 1937).

Comments

The characters of this species are representative of those shared by the species of the “*niger* complex” (Kinsey 1937), being the most important the 16 segmented antennae, mesoscutellum not emarginated posteriorly, sculpture on mesoscutum not coarsely rugose and forewing less heavily infuscate, which together with its morphologically different galls readily differentiates this species from the remaining Mexican species studied herein.

Species included in the “*nassa* complex” (Melika *et al.* 2011)

The species of *Amphibolips* from Mexico not included into the “*niger* complex” present the following diagnostic characters:

Diagnosis

Head and mesosoma coarsely rugose-reticulate sculptured. Antennae of 13–14 antennomeres in females, 15 segmented in male. Mesoscutellum emarginated in its posterior margin, continued into a submedian impression more or less deep and wide. Forewing moderately to heavily infuscate in females, more entirely in males; with a pattern of clear spots and bands variable extended. Metatarsal claws with a large acute triangular basal lobe or tooth. Sexual forms. Galls from spherical or globose, pointed or not at apex, to more or less fusiform or spindle-shaped.

Comments

The “*nassa* complex” of species was implicitly created by Melika *et al.* (2011), who used this name in a key for the identification of the Mexican species of *Amphibolips*. This convention was confusing because the grouping was not formally described in the referenced paper, as was performed by Kinsey for the “*niger* complex”. The only diagnostic character provided for the “*nassa* complex” was a mesoscutellum with posteromedian emargination. However, as we will discuss later, this main character is not discriminant because it is also present in other species from the USA and Panama. Consequently, the “*nassa*” complex should no longer be maintained.

Amphibolips fusus Kinsey 1937

Figs 1F–G, 14C–E

Amphibolips fusus Kinsey. *Rev. entom.*, 7(4): 430

Material examined: Holotype female (AMNH)

The Kinsey description of this species is clear and provided the main diagnostic characters. Some of these characters, such as the forewing colour pattern and the shape of the mesoscutellum, are now illustrated.

Diagnosis

F1 1.3 as long as F2 (Fig. 1F). Mesoscutellum slightly emarginated posteriorly; posteromedian impression shallow and not reaching posterior margins of scutellar foveae (Fig. 1G). Scutellar foveae rounded, with some irregular rugae, the intervals smooth and shining. Forewing (Fig. 14E) with basal half only slightly infusate; apical half heavily infusate from the radial cell, which is interrupted by a broad clear cross band extended from antero-apical area of radial cell, to posterior area, into the discoidal cells.

Distribution: Zacatecas: Cantuna

Host: *Quercus eduardi*

Gall. A slender to globose, spindle-shaped oak apple, with the tip and base quite fine. A photograph of the gall was provided in Melika *et al.* (2011).

Life history. Unknown; only females reared from galls in November.

Comments

This species was regarded by Melika *et al.* (2011) as very similar to *A. zacatecaensis*. However, the forewing patterns of the two species are quite different, which is a character that seems to be mistaken or overlooked by that authors. According to the coloration pattern of the forewing and shape of the gall, we consider this species to be more closely allied with the Panamanian species *A. aliciae* Medianero & Nieves-Aldrey 2010. One female that we reared from a globose, apically pointed gall collected in Nuevo León presented a similar forewing pattern (Fig. 21B) and could be identified as *A. fusus*, or more likely, a different closely allied species because of slight differences in the forewing patterns (Fig. 12F). However, other morphological characters are not well differentiated in this specimen, and it is not possible to come to a conclusion regarding its taxonomic status. We also collected galls similar to those of *A. fusus* in Monterrey and Nuevo León (Figs. 20E, 20F), but we unfortunately did not rear any adults from these galls.

***Amphibolips dampfi* Kinsey 1937**

Figs 1C–E & I, 13A, 14A–B

Amphibolips dampfi Kinsey. *Rev. entom.*, 7(4): 429

Material examined. Holotype male (AMNH)

The holotype is in bad condition. Glued to a card; right antenna and left forewings missing.

Labels: Holotype. *Amphibolips dampfi*. Sierra Juarez between Villa Juarez and Tepan Zacoalco 2.24.32/ 8700 *Quercus ocoteaefolia* Dampf coll.

We present and illustrate some diagnostic characters in addition to those provided in the original description.

Diagnosis

Head, dorsal view 3 as wide as long; with wrinkled reticulate sculpture (Fig. 1C). Antennae with 15 antennomeres (Fig. 1E): Relative lengths of antennal segments as: 10:7:30:16:16:16:16:15:14:14:13:13:12:10:10; F1 1.8 as long as F2, flattened ventrally and slightly enlarged apically. Placodeal sensillae visible in all flagellomeres. Notauli indistinct, obscured by coarse rugose reticulate sculpture of mesoscutum. F1 Scutellar foveae 0.4 as long as mesoscutellum; rounded, with defined margins, medially not well separated by a carina; with some strong transversal carinae, the intervals smooth and shining. Mesoscutellum strongly and widely emarginated posteriorly, V shaped in dorsal view (Fig. 1D); the posteromedial impression deeply and widely extended anteriorly to reach near posterior margins of scutellar foveae. In lateral view with sharp horn projection. Metascutellum reticulate rugose; metanotal trough smooth and pubescent. Median propodeal area coarsely and irregularly carinate, and pubescent; lateral propodeal carinae distinct, subparallel (Fig. 1I). Nucha smooth medially, laterally carinate. Forewing (Fig. 13A) heavily infusate, with a clear cross band from apical area of radial cell which is extended widening below the radial cell, crossing the Medial vein, but not reaching posterior margin of wing. Radial cell 3.7 as long as wide; veins being visible, R1 and radius not quite reaching anterior margin of wing; Rs1 projected into the radial cell; Rs2 slightly curved.

Distribution: Oaxaca, Sierra Juarez

Host: *Quercus ocoteifolia*, a synonym of *Q. laurina*

Gall. A spongy oak apple similar to that of the American *A. confluentus* (Harris). More or less spherical, thin-shelled, spongy interior dense though soft, larvae central or nearer the base of the gall, galls up to 48 mm in diameter, detachable, on twigs.

Biology

Bisexual; galls mature by February, and adults emerge between February and April

***Amphibolips nassa* Kinsey 1937**

Amphibolips nassa Kinsey. *Rev. entom.*, 7(4): 432

Type material: This species was described by Kinsey from a single female and a gall. The female holotype has not been located in the Kinsey collection, actually at the AMNH. A photograph of the gall was provided in Melika *et al.* (2011), but these researchers did not examine the adult type specimen (Melika pers comm.).

Kinsey's description of the species, with reference to the diagnostic characters of the forewing colour pattern and scutellum, was as follows: wings smoky yellow all over, with a heavy brown cloud on the anterior margin covering most of the basal, first cubital, radial cells and the anterior portion of the third cubital cell, without the clear break in this band found in some other Mexican species of *Amphibolips*.

Scutellum: mesoscutellum broad, square, deeply depressed anteriorly to form a wide, nearly smooth, and almost undivided foveal groove, median longitudinal depression of the mesoscutellum narrow and shallow, except posteriorly, where it cuts a wide, deep notch into the posterior edge of the mesoscutellum.

Gall: A rather large, globose but slightly spindle-shaped oak apple with a fine tip and base. Body of gall quite globose. A photography of the gall was provided by Melika *et al.* (2011).

Distribution: Michoacán, Purépero on *Quercus mexicana* and *Q. castanea* (= *Q. serrulata*).

Comments

Based on its forewing pattern and gall, *A. nassa* is very similar to the recently described *Amphibolips zacatecaensis* Melika & Pujade-Villar 2011. However, according to the descriptions of the two species, the mesoscutellum seems to be more deeply and widely emarginated in *A. nassa*. The galls of *A. nassa* and *A. zacatecaensis* are quite similar, but Melika *et al.* (2011) noted that the galls of *A. nassa* are more elongate and fusiform, with a hard lignified parenchyma, while the galls of *A. zacatecaensis* are globose, with a soft spongy parenchyma. In his key for the identification of the “*nassa* complex”, Melika *et al.* (2011) mistakes the forewing pattern of *A. nassa*, in which, according to Kinsey's description and illustration of the forewing, the heavy dark stripe or band along the anterior margin of the wing is not interrupted by any clear cross band. After our analysis, we consider *A. nassa* to be more similar to the new species *A. oaxacae* described here, with the two species being differentiated by their coloration, relative emargination of the mesoscutellum and shape of the gall.

***Amphibolips palmeri* Basset 1890**

Amphibolips palmeri Basset 1890. *Tran. Am. Ent. Soc.*, 17:86

This species is cited as the largest known species of gall fly and “oak apple gall” (Beutenmüller 1909). It was described from materials collected in Sierra Madre, Chihuahua, and Guadalajara, Jalisco, Mexico. This species was apparently overlooked by Kinsey in his work addressing the *Amphibolips* of Mexico (Kinsey 1937).

It was not possible to find the type material. However, we reproduce the description provided by Beutenmüller (1909), which is diagnostic with regard to the forewing colour pattern.

Wings dark, smoky brown, with a very dark brown cloud covering the areolet and the lower half of the radial area; beyond this and extending across the radial area to almost the posterior margin is a light colourless spot, and the anterior margin from the dark, broad, first cross-vein to a short distance beyond the second cross-vein is of the same light colour; tip of wing beyond the pale spot is dark smoky brown, as below this spot. Areolet very small but well defined.

Gall. Produced on twigs of an unidentified *Quercus* species. Spherical, with a wrinkly surface with a few scattered very short projections. Internally, the gall is of a spongy consistency. A central rounded larval cell is embedded in the soft internal tissue.

Comments. According to Melika *et al.* (2011), this species may be a synonym of *A. dampfi*. In our opinion, however, although the galls of the two species are similar, the descriptions of the forewing colour patterns of the two species are not in accordance. Thus, we maintain the status of *A. palmeri* as a good species.

***Amphibolips hidalgoensis* Pujade-Villar & Melika 2011**

Amphibolips hidalgoensis Pujade-Villar & Melika. *Zootaxa*, 3105: 53

Material examined. Two female paratypes (MNCN) sent by UB (J. Pujade-Villar).

Diagnosis

Characterised by the forewing colour pattern, presenting a dark infuscate cloud on the anterior margin of the wing, extending from the radial cell to the apical margin. The rest of forewing surface is much clearer, yellowish and slightly infuscate, including the costal cell, first cubital cell and basal cell. Mesoscutellum moderately emarginated posteriorly. F3 as long as F4.

Distribution: Hidalgo state

Gall (Figs 18A, 18B). Spherical, diameter up to 6.5 cm, thin walled, with a smooth and naked surface with soft, spongy parenchyma and radiating filaments supporting the single central larval cell (Melika *et al.* 2011).

Host: *Quercus crassipes* and *Q. crassifolia*

Life history. Only females are known. According Melika *et al.* (2011), they may represent the asexual generation.

***Amphibolips zacatecaensis* Melika & Pujade-Villar 2011**

Amphibolips zacatecaensis Melika & Pujade-Villar. *Zootaxa*, 3105: 48.

Material studied. Two females. Zacatecas, Tlaltenango, 2400 m. ex gall *Quercus sp.* aff *conzatii* (06/08/2005), adults emerged 09/2005, E. Pascual leg.

Diagnosis and comments. *A. zacatecaensis* has been recently described from a single female reared from galls collected on *Q. eduardi* in Zacatecas. The species is characterised by a combination of characters, including the presence of a dark heavily infuscated band along the anterior area of the forewing, which is not interrupted in the first cubital cell, and the mesoscutellum only slightly emarginated posteriorly. The authors outlined the resemblance of this species with the species *A. fusus* and *A. nassa* described by Kinsey. With regard to the resemblance to *A. fusus*, they indicated that *A. zacatecaensis* and *A. fusus*, both reared from *Q. eduardi*, exhibit a similar pattern of forewing pigmentation. However, that affirmation is erroneous, as we demonstrated by examination of the holotype of *A. fusus*. *A. fusus* presents a quite distinct forewing colour pattern, with a colourless cross band, as is found in *A. dampfi*, *A. durangensis* and *A. castroviejoi*, which is not present in *A. nassa* and *A. zacatecaensis*. Furthermore, that *A. fusus* and *A. nassa* do not closely resemble each other was previously correctly outlined by Kinsey (1937). With regard to the differences from *A. nassa*, in the cited paper, it is affirmed that *A. zacatecaensis* differs from *A. nassa* in its forewing colour pattern, but that affirmation is not correct according the original description and the figure (the types of *A. nassa* could not be found when we requested Kinsey types from the AMNH for direct comparison). According to the original description, the forewing is “everywhere smoky-yellow, with a heavy brown cloud on anterior margin covering most of basal, first cubital, and radial cell and anterior portion of third cubital cell; without the clear break in this band found in some other Mexican species of *Amphibolips*” (it is clear that Kinsey is referring to *A. dampfi* and *A. fusus*, as stated in another paragraph on pp. 434). The above description is coincident with the forewing pattern of *A. zacatecaensis*, except that the forewing drawing of *A. nassa* shows the costal cell as infuscate as the basal cell, differing from *A. zacatecaensis*. In conclusion, it is true that *A. zacatecaensis* closely resembles *A. nassa* (not also *A. fusus*), but it could not be differentiated based on the forewing pattern from *A. nassa*. Because the two

species share morphologically similar galls, the only difference between them may be the inner consistency of the gall, which presents a soft, spongy parenchyma, whereas it is hard and lignified in *A. nassa* (Melika pers. comm.). The forewing colour pattern and the moderately emarginated mesoscutellum assigns the materials we studied to *A. zacatacaensis*/*A. nassa*. However, our specimens differ in that the dark smoky band is less heavily infuscate, especially in the first cubital cell, and that veins Sc+R, R1 and Rs are distinct and more visible than in the nominal typical form.

Distribution: Zacatecas state

Gall. Large, globose to slightly spindle shaped, with a nipple at the top (Fig. 18E). The gall is thin walled, very lightweight, internally showing a soft spongy consistency. Internally, the gall exhibits a single hard-walled central larval cell (Melika *et al.* 2011).

Host. *Quercus eduardi*

Life History. Unknown.

New species

Amphibolips durangensis Nieves-Aldrey & Maldonado sp. nov.

(Figs. 2A–G, 11C, 15A)

Type material. Holotype female (Fig. 15A). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), dissected and mounted on a stub. Cat. n° 2246. MEXICO, Durango, Palmito, N 23° 33' 49.4" W 105° 51' 08.8", 1977 m; ex gall *Quercus scytophylla* (Fagaceae), gall collected 08/07/2008, insect emerged 10/2008. Y. Maldonado leg. Paratypes: 1 female Durango, Loberas, 1948 m a.s.l ex gall *Quercus scytophylla*, gall collected 10/07/2008, insect emerged 10/2008, Y. Maldonado leg., dissected and mounted on a stub. In MNCN.

Etymology. Named after the Mexican state, Durango, where the materials were collected.

Diagnosis and comments. Closely related to *Amphibolips castroviejoi* Medianero & Nieves-Aldrey from Panama, being similar in colour and a majority of morphological characters. The two species share a similar forewing coloration pattern, which is predominantly infuscate, with a clear crossing band extending from the radial cell to the discoidal cell. The new species differ from *A. castroviejoi* in the clear crossing band being narrower as well as the costal cell and discoidal cell being less heavily infuscate. Both *A. durangensis* and *A. castroviejoi* differ from *A. dampfi* in the wide clear band extending across the forewing from the tip of the radial cell to the posterior part of the apical margin (Figs 11A, 11C), whereas the band is smaller and does not extend as far across the ventral margin of the wing in *A. dampfi* (Fig. 13A). Additionally, *A. durangensis* and *A. dampfi* differ from *A. castroviejoi* in exhibiting indistinct notauli, nearly lost in the sculpture, while the last species has a complete notauli, only lost on the coarse surface in the anterior one-third. *Amphibolips dampfi* exhibit the mesoscutellum strongly emarginated posteriorly, with a sharp horn projection in lateral view (Fig. 1D), while in *A. durangensis*, the mesoscutellum is only moderately emarginated posteriorly (Fig. 2E).

Description. Body length 5.8 mm (N = 2) for females. Head, mesosoma, antenna and legs black. Metasoma chestnut blackish, hipopygium reddish. Forewing predominantly dark infuscate, excepting costal cell and the areas below cubital vein and between medial and cubital veins, which are lighter; an irregular wide clear cross band is present, extending transversally across wing from one third apical of radial cell to posterior margin of wing (Fig 11C).

Female. Head, coarsely rugose, pubescent; in dorsal view about 2 times wider than long. POL about as long as OOL, posterior ocellus separated from inner orbit of eye by 2.5 times its longest diameter. Head in anterior view (Fig. 2A) 1.2 times wider than high, gena slightly broadened behind eye. Vertex, frons, lower face, gena, and occiput with strong reticulate-rugose sculpture, irradiating carinae from clypeus not discernible; head moderately pubescent, with relatively long setae, except vertex and frons with sparse and shorter setae. Clypeus trapezoid, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits well visible; epistomal sulcus and clypeo-pleurostomal lines slightly discernible. Malar space 0.6 times height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye 1.1 times width of antennal socket including rim. Ocellar plate slightly raised.

Mouthparts (Fig. 2A): mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 2C–D), of moderate length, as long as 1/2 body length; with 13 antennomeres; last flagellar segment partially divided into two segments; flagellum not broadening towards apex; with relatively long, erect setae, and elongate placodeal sensilla (Fig. 2D). Relative lengths of antennal segments: 15:7:29:19:15:13:11:11:11:9:8:8:20. Pedicel (Fig. 2C), short, small, 0.5 as long as scape; F1 1.5 times as long as F2. F6–F10 longer than wide, F11 2.7 times longer than wide, 2.1 times as long as F10 (Fig. 2D). Placodeal sensillae on F5–F11, disposed in dense rows of 6–8 sensillae, only in half dorsal area of each flagellomere.

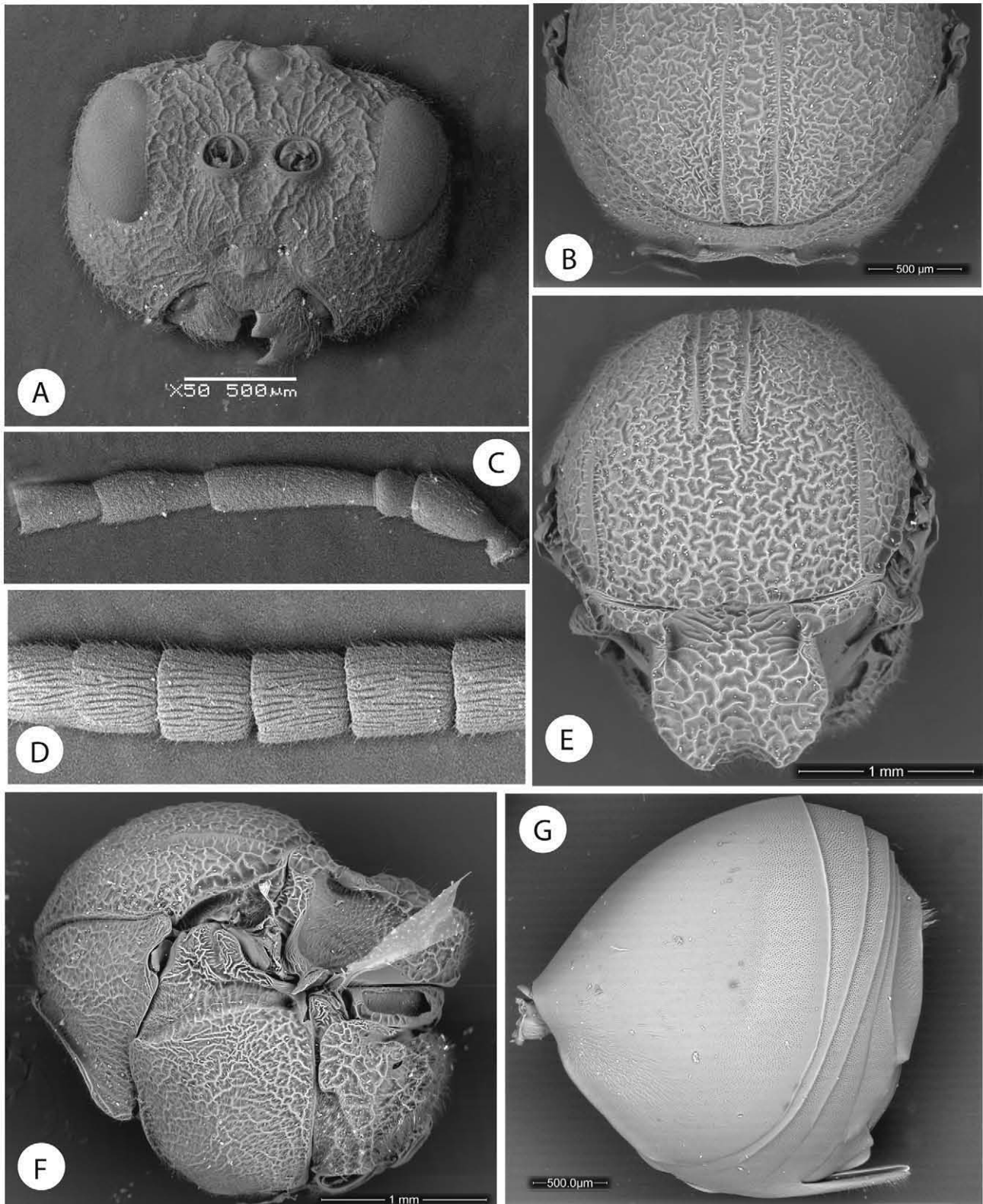


FIGURE 2. *Amphibolips durangensis* sp. nov., female. (A) head anterior view; (B) pronotum anterior view; (C) detail of basal antennomeres; (D) detail of apical flagellomeres; (E) mesosoma dorsal view; (F) mesosoma lateral view; (G) metasoma lateral view.

Mesosoma. Coarsely reticulate rugose, in lateral view as high as long. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture. Pronotum medially short (Fig. 2B); ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally (Fig. 2B).

Mesonotum. Mesoscutum (Fig. 2E) barely pubescent and with strong rugose-reticulate sculpture. Notauli indistinct. An irregular, shallow, longitudinal median impression, crossed by transversal rugae, visible from anterior to posterior part of mesoscutum. Anteroadmedian signa quite visible, extended back to near one half of mesoscutum; parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum subquadrate, about 0.6 as long as mesoscutum. Scutellar foveae ellipsoidal about 0.3 as long as mesoscutellum, indistinctly separated medially, deep, crossed by irregular longitudinal rugae, the intervals smooth, posterior margins indistinct. Mesoscutellum strongly reticulate-rugose, with a median longitudinal impression and moderately emarginated at posterior margin (Fig. 2E); the emargination reaching posterior about one sixth of scutellum length. Axillula moderately pubescent, their anterior and posterior margins marked. Mesopleuron coarsely reticulate rugose, the rugae not as strong as mesoscutum. (Fig. 2F).

Metanotum. Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex (Fig. 2F). Metascutellum rugose; metanotal trough smooth and pubescent. Median propodeal area reticulate rugose and densely pubescent; lateral propodeal carinae indistinct. Nucha smooth medially.

Legs. Densely pubescent; femora and tibiae robust; metafemur 2.6 as long as wide, strongly curved ventrally. Metatibia about as long as metatarsus; apical margin of metatarsomeres 1–4, with long strong erect setae. Metatarsal claws with strong triangular basal lobe or teeth.

Forewing (Fig. 11C): 1.15 as long as body, radial cell 3.5 times longer than wide; open along anterior margin; areolet small, ovoid, obscured by infuscation. R1, Rs and M nearly straight, not reaching wing margin. Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) and 2r-m curved. Apical margin with obsolete hair fringe.

Metasoma (Fig. 2G), as long as head and mesosoma combined, in lateral view as high as wide. Second metasomal tergite covering about two third of metasoma, with band of micropunctures clearly visible in posterior one third; punctures visible on subsequent tergites; ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine long (Fig. 2G); about 5 times as long as wide in lateral view; laterally with long setae, longer than spine width but not forming an apical patch.

Male. Unknown

Gall (Figs. 18C, 18D). A spindle-shaped gall with an elongated and narrow tip and base. Longitudinally, the surface of the gall is crossed by longitudinal ridges. The gall is monothalamic; the outer shell is thin but firm; internally, it has a spongy consistency, filling the entire gall (Fig. 18D). The larval cell is rounded and is embedded in the soft internal substance. Diameter of 54 x 43 mm on average). Forms on twigs of *Quercus scytophylla*. The gall closely resembles that of *Amphibolips fusus* Kinsey 1937, also described from Mexico, and was illustrated recently by Melike *et al.* (2012). However, the gall of *A. duranguensis* differs from the gall of *A. fusus* due to its much more elongated and narrow points, both at the tip and at base. Another difference is the gall surface, which is smooth in the gall of *A. fusus*, without longitudinal ridges.

Distribution. *A. duranguensis* was found at 1900 m a.s.l. in Durango state, Mexico.

Biology. Only females of the presumably sexual generation are known. The galls were collected in July, and the insects emerged in October.

Comment: We examined one female reared from a gall also developing on *Quercus scytophylla*, but collected in Durango, Loberas, on 18/07/2008, which is very similar to *A. duranguensis* and could be conspecific with this species. However, this specimen differs from *A. duranguensis* in the following ways: the clear crossing band is slightly different, being narrower between the radial and the medial veins and wider and regular between the medial and the cubital. The infuscate area on the apical margin is not as uniform as in the holotype.

Amphibolips jaliscensis Nieves-Aldrey & Pascual sp. nov.

Figs 3A–H, 11D, 13B, 17A–B.

Type material. Holotype female (Fig. 17A). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card Cat. n° 2247. MEXICO, Jalisco, Nevado de Colima, 19° 37' 408" N, 103° 33' 216" W,

2048 m; ex gall *Quercus* aff. *candicans*, gall collected 31/03/2009; insect emerged 04/2009, E. Pascual leg. Paratypes: One male, presenting the same data as the holotype (Fig. 17B). In the MNCN.

Etymology. Named after the Mexican state, Jalisco, where the materials were collected.

Diagnosis and comments. The main diagnostic character of this species is the clear, less infuscate spot on the apical one-third of the radial cell. In most remaining morphological characters, this species is very similar to *A. hidalgoensis* Melika & Pujade-Villar, and the new species is described here as *A. malinche* sp. nov. It further differs from *A. hidalgoensis* in its basal cell, which is more infuscate, and in that due to being a bisexual generation, females and males are both known, while only females are known in *A. hidalgoensis*, and it is presumably an asexual form (Melika *et al.* 2011). From *A. malinche*, the new species differs in the clear spot on the radial cell, which is relatively larger and more extended, and in F1 being 1.4 as long as F2 (1.6 as long as F2 in *A. malinche*).

Description. Body length 7 mm (N = 1) for females; 5.5 mm (N=1) for males. Head, and mesosoma black, excepting base of mandibles and clypeus anteriorly chestnut. Antennae black dorsally, chestnut brown ventrally. Legs black, excepting apical tarsomeres brown. Metasoma red brown in half basal, black in posterior half; hypopigium red brown. Forewing infuscate, with a darker band extended on anterior margin along basal cell, first cubital cell (lightly here), radial cell (heavily in anterior one half), and beyond to posterior margin of wing. The entire costal cell as well as posterior one third of radial cell is colorless.

Female. Head, in dorsal view coarsely rugose, about 2.3 times wider than long, narrower than mesosoma. POL 1.8 OOL, posterior ocellus separated from inner orbit of eye by 1.5 times its longest diameter. Genae strongly broadened behind eye. Head in anterior view 1.2 as wide as high. Vertex, frons, lower face, gena, and occiput with strong reticulate-rugose sculpture; some incomplete and irregular irradiating carinae from clypeus visible. Head moderately pubescent, except vertex and frons with sparse and shorter setae. Genae quite broadened, visible behind eye. Clypeus trapezoid, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits well visible; epistomal sulcus and clypeo-pleurostomal lines slightly visible. Malar space about half height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye as width of antennal socket including rim. Ocellar plate slightly raised.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 3A), of moderate length, as long as 1/2 body length; with 13 antennomeres; last flagellar segment partially divided into two segments; flagellum not broadening towards apex; with relatively long, erect setae, and elongate placodeal sensilla. Relative lengths of antennal segments: 18:12:42:30:22:20:17:15:12:12:10:10:22. Pedicel short, small, as long as wide; 0.6 as long as scape; F1 1.4 times as long as F2. F7–F10 about as long as wide, F11 2 times longer than wide, 2 times as long as F10. Placodeal sensillae on F5–F11, disposed in rows of 6–8 sensillae, only in half dorsal area of each flagellomere.

Mesosoma. Coarsely reticulate rugose, in lateral view as high as long. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture. Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally.

Mesonotum. Mesoscutum (Fig. 3B) barely pubescent and with coarse rugose-reticulate sculpture. Notauli indistinct anteriorly, posteriorly visible by indistinct, obscured by the irregular sculpture; longitudinal median impression indistinct. Anteroadmedian signa and parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum squared (Fig. 3B), about 0.6 as long as mesoscutum. Scutellar foveae large, rounded with distinct margins; smooth, excepting by some transversal carinae, and shining. Mesoscutellum roughly reticulate-rugose, with a shallow median longitudinal impression, and deeply emarginated at posterior margin, the incision reaching deeply only one third of distance to posterior margin of scutellar foveae. Axillula moderately pubescent, their anterior and posterior margins marked. Mesopleuron irregularly reticulate rugose, the rugae not as strong as mesoscutum. (Fig. 3C).

Metanotum (Fig. 3F). Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex. Metascutellum rugose; metanotal trough smooth and pubescent. Median propodeal area reticulate rugose and densely pubescent; lateral propodeal carinae indistinct, obscured by coarse sculpture. Nucha smooth medially.

Legs. Densely pubescent; femora and tibiae robust. Tarsal claws with strong triangular basal lobe or teeth.

Forewing (Fig. 13B). Slightly longer than body, radial cell 4 times longer than wide; open along anterior margin; areolet distinct. All the veins heavily infuscated; Color as described before. R1, Rs and M nearly straight, not reaching wing margin. Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) and 2r-m curved. Apical margin with very short hair fringe.

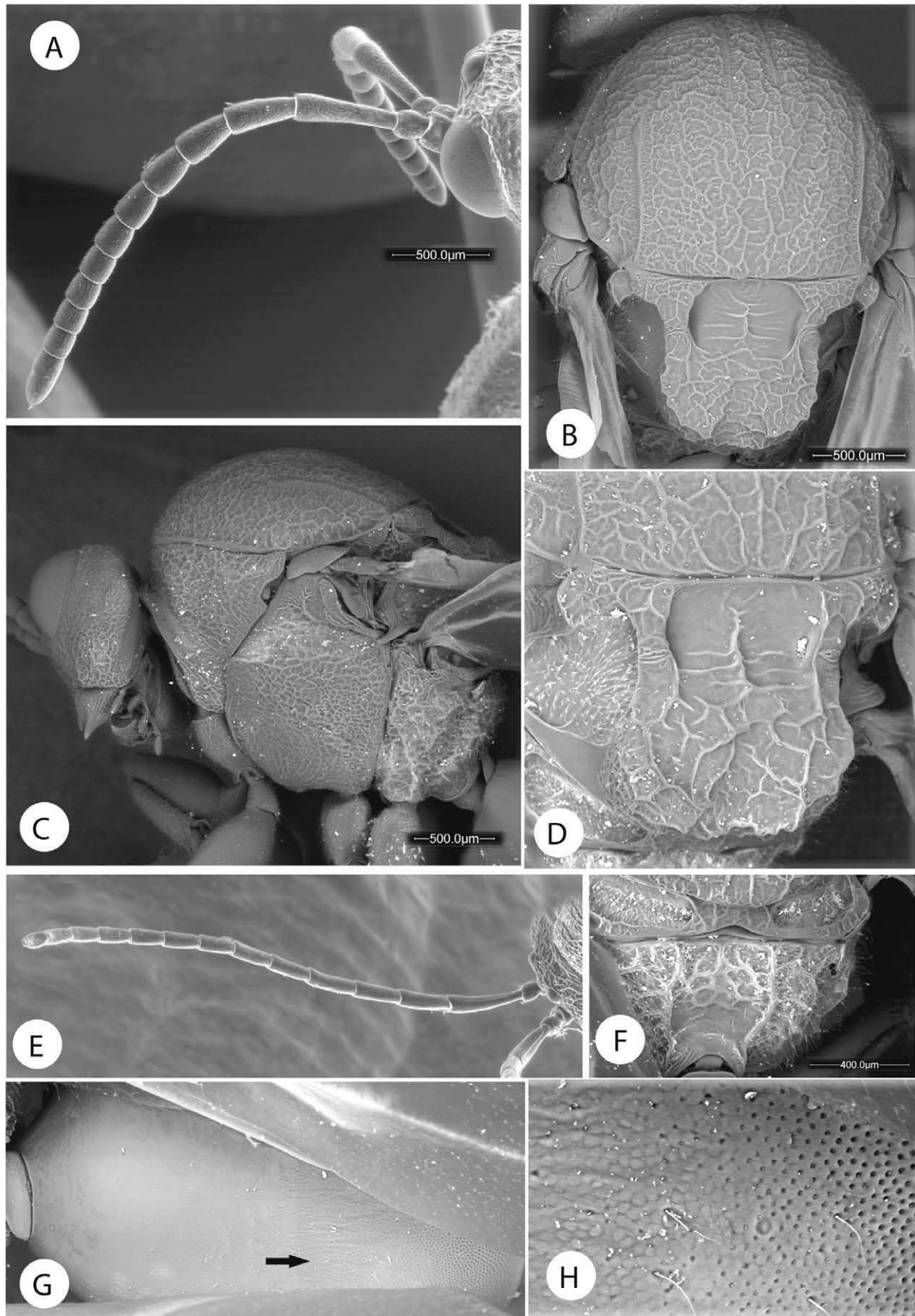


FIGURE 3. *Amphibolips jaliscensis* sp. nov. (A) female, head and antenna; (B) female, mesosoma dorsal view; (C) female, mesosoma lateral view; (D) male, mesoscutellum; (E) male antenna; (F) male propodeum; (G) female, metasoma dorsal view; (H) detail of the sculpture of metasoma in dorsal view.

Metasoma. About as long as head and mesosoma combined; in lateral view 1.2 as long as high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one fourth of the large metasomal tergite; anteriorly to the area of micropunctures there is a broad area with weak coriaceous- alutaceous sculpture, after the anteriormost smooth area of MT2 (Figs 3G–H); micropunctures extended on subsequent tergites; ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine long; about 4.5 times as long as wide in ventral view; laterally with long setae, longer than spine width but not forming an apical patch.

Male (Fig. 17B). Differs from female as follows. Coloration darker, antenna and metasoma entirely black. Forewing much more strongly infuscate; infuscation extended also on costal cell, and much strongly than in female below the dorsal darker band extended on veins and closed cells (Fig. 13B). Antennae (Fig. 3E) of 14 antennomeres; F1 long, slightly modified, flattened ventrally and slightly broadened toward apex; lateral propodeal carinae distinct.

Gall (Figs 19A, 19B). A globular oak apple gall. Monothalamic; outer shell thin and internally showing a spongy consistency, filling the entire gall. Grows on twigs of *Quercus sp. aff. candicans*.

Distribution. *A. jaliscensis* was found at 2370 m a.s.l. at Jalisco state, Mexico.

Biology. Exhibits a sexual generation; both males and females are known. The galls were collected in late March, and the insects emerged in April.

Amphibolips oaxacae Nieves-Aldrey & Pascual sp. nov.

Figs 4A–F, 11F, 17D, 18F

Type material. Holotype female (Fig. 17D). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card. Cat. n° 2249. MEXICO, Oaxaca, S. Pedro Tapanatepec, 16° 22' 34.40" N, 94° 04' 43 11" W, 1300 m; ex gall *Quercus sp.*, gall collected 29/03/2006, insect emerged 03/2006, E. Pascual leg.

Etymology. Named after the Mexican state Oaxaca, where the material was collected.

Diagnosis and comments. The forewing colour pattern distinguishes this new species well from other Mexican or Neotropical *Amphibolips* species. The infuscation along the veins is much less heavy than in other Mexican species of *Amphibolips*, with the veins being discernible (Fig. 11F). A slightly darker, more infuscate area extending along the dorsal margin of the wing across the radial cell and reaching the posterior margin of the wing. The infuscate area extends across the costal cell, first cubital cell and basal cell and to the anterior area below the M+Cu1 vein (Fig. 11F). In this forewing colour pattern, the new species resembles *A. nassa* Kinsey, but its coloration, the posterior emargination of the mesoscutellum and the gall are different in these two species. In exhibiting a reddish body and mesoscutellum deeply emarginated and in the shape and size of its gall, this new species also resembles *A. dampfi* and *A. palmeri*. However, the pattern of forewing infuscation distinguishes these species well from *A. oaxacae*; *A. dampfi* exhibits a clear cross-band near the tip of the wing, while *A. oaxacae* does not present this cross-band. The gall is similar in size to that of *A. palmeri*, also described from Mexico and measuring 35–70 mm in diameter. However, the surface of the gall of *A. palmeri* was described and illustrated as uneven, or somewhat wrinkly, sometimes with a few, scattered very short projections, while the surface of the gall of *A. oaxacae* is uniformly smooth.

Description. Female. Body length 6.5 mm (N = 1). Head, and mesosoma black; clypeus, mandibles and areas of mesosoma around tegulae reddish brown. Antennae light brown more light distally and ventrally. Legs and metasoma reddish brown, darker in posterior half; hypopygium red brown. Forewing lightly but entirely infuscate, the veins being clearly visible. A slightly darker band extended dorsally along the costal and basal cells, first cubital cell, and radial cell to postero dorsal area of forewing. The area below basal cell is also more darkened as the anterior band.

Female. Head, in dorsal view strongly reticulate rugose, about 2 times as wide as long. POL as long as OOL, posterior ocellus separated from inner orbit of eye by 1.5 times its longest diameter. Head in anterior view (Fig. 4A) about 1.3 as wide as high, gena broadened behind eye. Vertex, frons, lower face, gena, and occiput with strong irregular reticulate-rugose sculpture, without irradiating carinae from clypeus. Head moderately pubescent. Clypeus trapezoidal, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits conspicuous; epistomal sulcus and clypeo-pleurostomal lines discernible. Malar space 0.5 times height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye as wide as antennal socket including rim. Ocellar plate slightly raised.

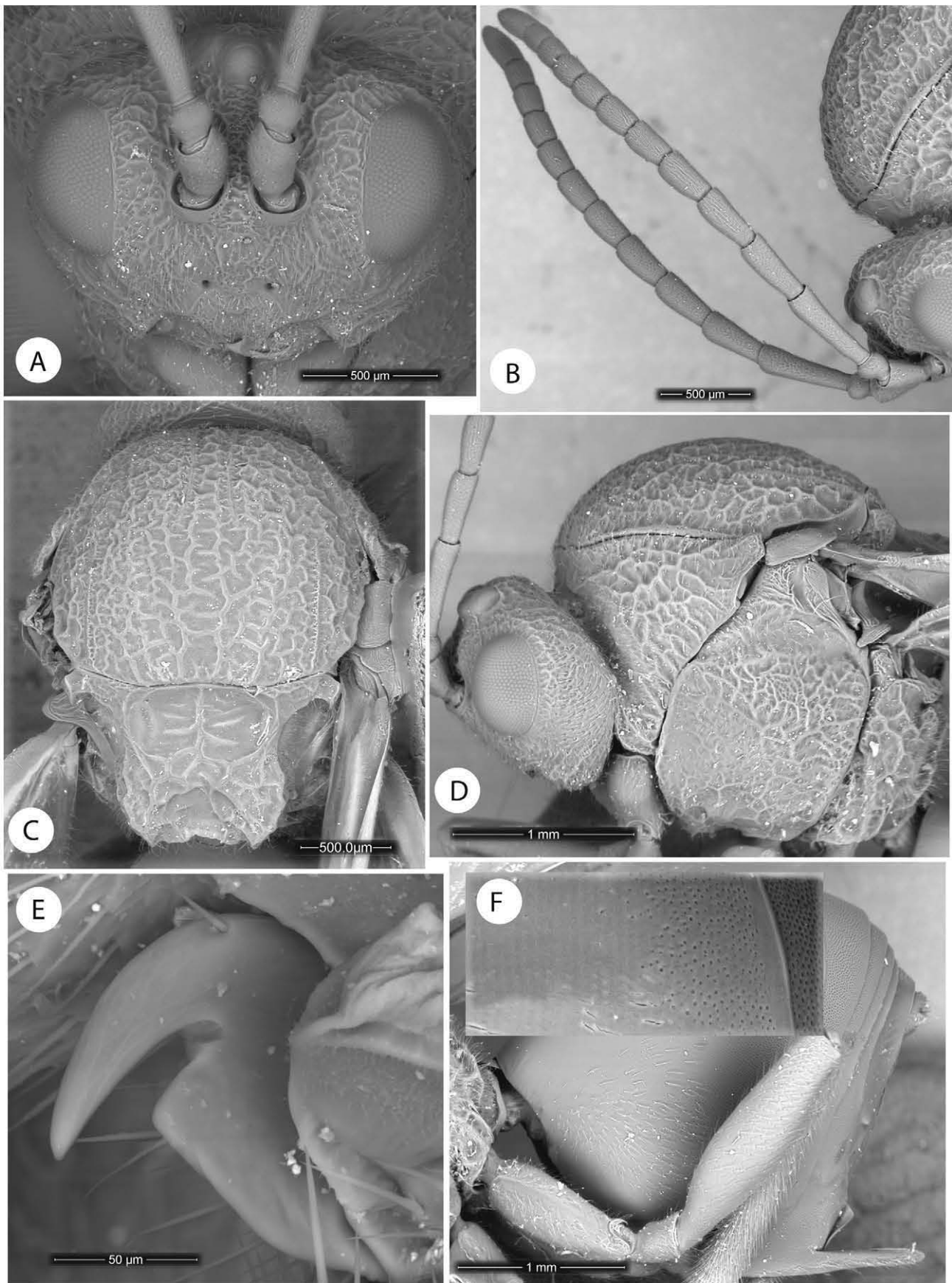


FIGURE 4. *Amphibolips oaxacae* sp. nov., female. (A) head anterior view; (B) antennae; (C) mesosoma dorsal view; (D) mesosoma lateral view; (E) metatarsal claw; (E) male antenna; (F) metasoma lateral view and detail of sculpture.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 4B), of moderate length, 0.6 as long as body; with 13 antennomeres; flagellum not broadening towards apex; with relatively long, erect setae, and elongate placodeal sensilla in flagellomeres F3–F11 (Fig. 4B). Relative lengths of antennal segments: 25:10:42:32:30:25:20:18:16:15:12:11:20. Pedicel (Fig. 2C), short, globose, 0.5 as long as scape; F1 1.3 times as long as F2. F6–F10 longer than wide, F11 2 times as long as F10 (Fig. 4B). Placodeal sensillae on F3–F11, disposed in rows of 6–8 sensillae, only in half dorsal area of each flagellomere.

Mesosoma. Short, in lateral view slightly higher than long. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture (Fig. 4D). Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally (Fig. 4C).

Mesonotum. Mesoscutum (Fig. 4C) barely pubescent and with strong rugose-reticulate sculpture. Notauli distinct in posterior 2/3 of mesoscutum, crossed by transversal rugae. longitudinal median impression indistinct. Anteroadmedian signa well visible, extended back to near one half of mesoscutum; parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum subquadrate, about 0.6 as long as mesoscutum. Scutellar foveae rounded, deep, with distinct margins; with some transversal rugae, the intervals smooth and shining; about 0.5 as long as mesoscutellum. Mesoscutellum strongly reticulate-rugose, deeply and widely emarginated at posterior margin, the incision reaching close posterior margins of scutellar foveae (Fig. 4C). Mesopleuron coarsely reticulate rugose, the rugae not as strong as mesoscutum (Fig. 4D).

Metanotum. Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex. Metascutellum weakly rugose; metanotal trough smooth and pubescent. Median propodeal area shining reticulate-rugose and densely pubescent; lateral propodeal carinae indistinct. Nucha smooth medially.

Legs. Densely pubescent; femora and tibiae robust. Metatarsal claws with strong triangular basal lobe or teeth (Fig. 4F).

Forewing (Fig. 11F): As long as body, radial cell 3.6 times longer than wide; open widely along dorsal margin; areolet small. R1 straight, not reaching wing margin; Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) angulated and radius curved. Apical margin with short hair fringe.

Metasoma (Fig. 4F), slightly shorter as head and mesosoma combined, in lateral view about as long as high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one fourth; punctures visible on subsequent tergites; dorsally the surface of the second metasomal tergite before the band of micropunctures is smooth (Fig. 4F); latero ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine quite long, in lateral view about 6 times as long as wide; laterally with long setae which not form an apical patch.

Male. Unknown.

Gall (Fig. 18F). A regularly spherical, large oak apple gall measuring approximately 40 mm in diameter. Pale green with darker green spots when fresh. The gall is monothalamic; the outer shell is thin but firm; with a spongy consistency internally, filling the entire gall. The larval cell is rounded and is embedded in the soft internal substance. Forming on twigs of *Quercus sp. aff. sapotifolia*. The gall closely resembles that of *Amphibolips palmeri* Basset 1890, also described from Mexico, but for which the host is unknown.

Distribution. *A. oaxacae* was found at 1180 m a.s.l. in Oaxaca State, Mexico.

Biology. Only females of the presumably sexual generation are known. The galls were collected in March, and the insects emerged in the same month.

***Amphibolips malinche* Nieves-Aldrey & Pascual sp. nov.**

(Figs 5A–F, 11E, 15C)

Type material. Holotype. Female (Fig. 15C). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card. Cat. n° 2248. MEXICO, Tlaxcala, La Malinche, 19° 12' 29 49" N, 98° 0' 44 47" W, 2800 m; ex gall *Quercus mexicana*, gall collected 16/05/2006, insect emerged 30/05/2006, E. Pascual leg. Paratype, 1 female, with the same data as for the holotype, except that the gall was collected 15/05/2005, and the insect emerged 7/11/2005, E. Pascual leg. In MNCN.

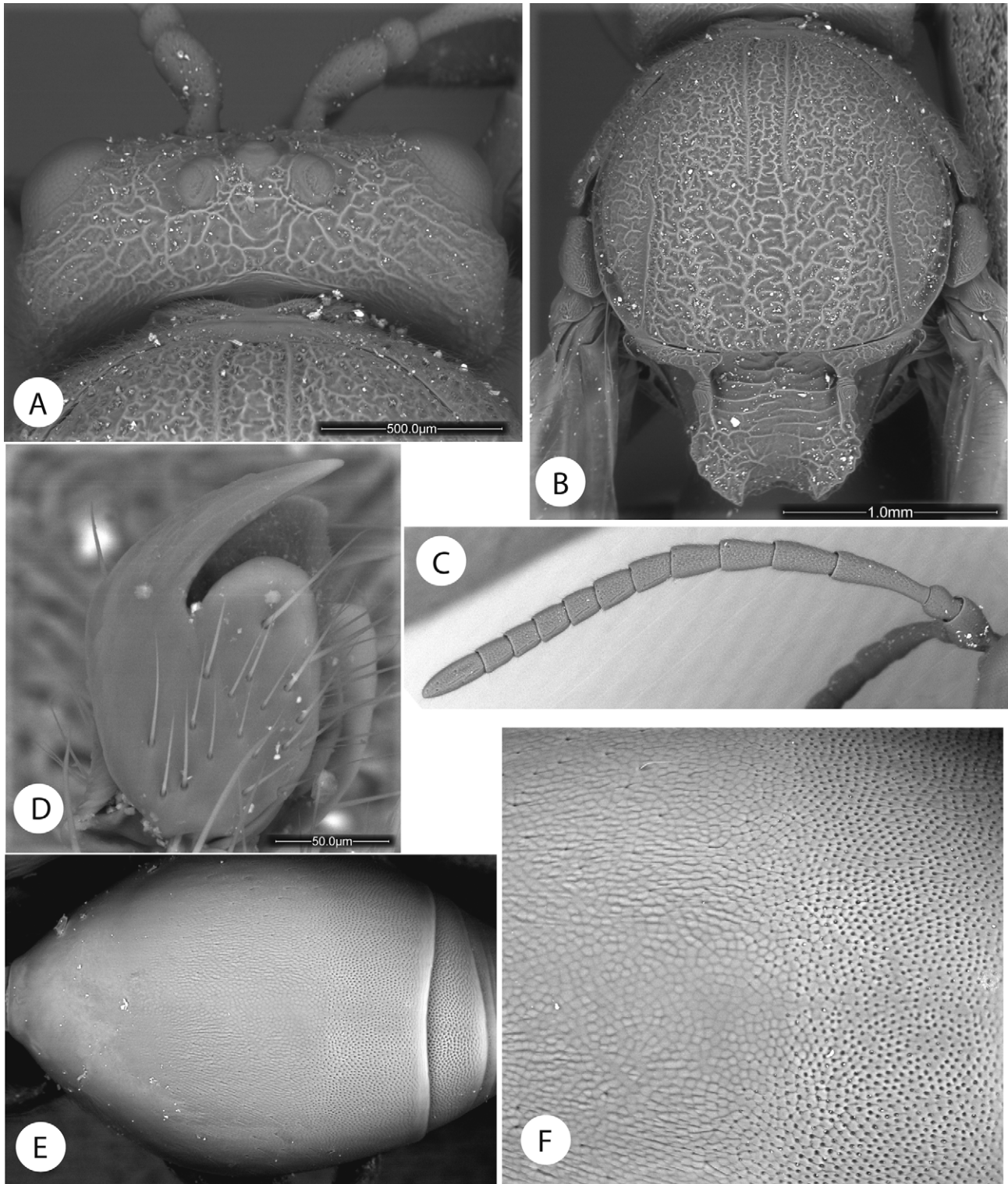


FIGURE 5. *Amphibolips malinche* sp. nov., female. (A) head dorsal view; (B) mesosoma dorsal view; (C) antenna; (D) metatarsal claw; (E) metasoma dorsal view; (F) detail of sculpture.

Etymology. Named after the collection site, referring to Dña Marina, called Malinche, compaign of Hernán Cortés, two key figures in the birth of modern Mexico.

Diagnosis and comments. Closely resembles *A. jaliscensis* Nieves-Aldrey & Pascual, in its forewing pattern based on the colourless, less infuscate spot situated apically on radial cell. However, this clear spot in *A. malinche* is smaller, only extending to the apex of the radial cell, and the first cubital cell is as colourless as the costal cell (Fig. 11E). Additionally *A. malinche* differs from *A. jaliscensis* in its antennal F1 being 1.6 as long as F2 (Fig. 5C)

[F1 is 1.4 as long as F2 in *A. jaliscensis*], and the general coloration is darker in *A. malinche*, with the antenna and metasoma being black.

Description. Body length 7 mm (N = 2) for females. Head, mesosoma, antennae and legs black. Metasoma in great part black, ventral sides and hypopygium dark chestnut. Forewing infusate, with a darker band extended dorsally along basal cell, radial cell, and beyond to posterior margin of wing. The entire costal cell as well as first cubital and the apex of radial cell is colorless, not as heavily infusate.

Female. Head, in dorsal view (Fig. 5A) coarsely rugose, about 2.4 times wider than long, narrower than mesosoma. POL 1.7 OOL, posterior ocellus separated from inner orbit of eye by 1.8 times its longest diameter. Gena strongly broadened behind eye. Head in anterior view 1.2 as wide as high. Vertex, frons, lower face, gena, and occiput with strong reticulate-rugose sculpture; some incomplete and irregular irradiating carinae from clypeus visible. Head moderately pubescent, except vertex and frons with sparse and shorter setae. Genae quite broadened, visible behind eye. Clypeus trapezoid, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits well visible; epistomal sulcus and clypeo-pleurostomal lines slightly visible. Malar space about half height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye slightly shorter as width of antennal socket including rim. Ocellar plate slightly raised.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 5C), 0.4 as long as body length; with 13 antennomeres; flagellum not broadening towards apex. Relative lengths of antennal segments: 20:10:40:25:22:20:17:15:13:12:12:13:27. Pedicel (Fig. 2C), short, small, as long as wide; 0.6 as long as scape; F1 1.6 times as long as F2. F7–F10 about slightly longer than wide, F11 2.7 times longer than wide, 2 times as long as F10. Placodeal sensillae on F5–F11, disposed in rows of 6–8 sensillae, only in half dorsal area of each flagellomere.

Mesosoma. Coarsely reticulate rugose, in lateral view 1.1 as long as high. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture. Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally.

Mesonotum. Mesoscutum barely pubescent and with coarse rugose-reticulate sculpture (Fig. 5B). Notauli only posteriorly visible but indistinct, obscured by the irregular sculpture; a longitudinal median impression obscurely indicated. Anteromedian signa and parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum squared, about 0.4 as long as mesoscutum. Scutellar foveae (Fig. 5B) squared, with distinct lateral margins; smooth, excepting by some transversal carinae, and shining. Mesoscutellum roughly reticulate-rugose, with a shallow median longitudinal impression, and deeply emarginated at posterior margin, the incision reaching deeply about one half of distance to posterior margin of scutellar foveae (Fig. 5B). Axillula moderately pubescent, their anterior and posterior margins marked. Mesopleuron irregularly reticulate rugose, the rugae not as strong as mesoscutum.

Metanotum. Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex. Metascutellum rugose; metanotal trough smooth and pubescent. Median propodeal area reticulate rugose and densely pubescent; lateral propodeal carinae obscured by coarse sculpture but visible. Nucha smooth medially.

Legs. Densely pubescent; femora and tibiae robust. Tarsal claws with strong triangular basal lobe or teeth, apically obtuse (Fig. 5D).

Forewing (Fig. 11E): 1.1 as long as body, radial cell about 4 times longer than wide; open along anterior margin; areolet very small but distinct. Color as described before. R1 and Rs not reaching wing margin; Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) and Rs curved. Apical margin with very short hair fringe.

Metasoma (Fig. 5E), About as long as head and mesosoma combined; in lateral view 1.2 as long as high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one fourth of the large metasomal tergite; anteriorly to the area of micropunctures there is a broad area reaching anterior one half of MT2 with coriaceous sculpture, after the anterior smooth area of the metasomal tergite (Fig. 5F); micropunctures extended on subsequent tergites; ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine long; about 6 times as long as wide in ventral view; laterally with long setae, longer than spine width but not forming an apical patch.

Male. Unknown.

Gall (Figs 19C, 19D). A globular, irregular oak apple gall. Monothalamic; outer shell thin and internally exhibiting a spongy consistency, filling the entire gall. Found on twigs of *Quercus mexicana*.

Distribution. *A. malinche* was found at 2800 m a.s.l. at La Malinche volcano, Tlaxcala state, Mexico.

Biology. Presumably a sexual generation, but males were not reared. The galls were collected in late June, and the insects emerged in July.

***Amphibolips nevadensis* Nieves-Aldrey & Pascual sp. nov.**

Figs 6A–F, 7AE, 12B, 13F, 16A–B

Type material. Holotype. Female (Fig. 16A). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card. Cat. n° 2252. MEXICO, Jalisco, Nevado Colima, Ciudad Guzmán, 2300 m; ex gall *Quercus candicans*, gall collected 20/08/2009, E. Pascual leg. Paratypes, 2 males, Nevado Colima, 2370 m, ex gall *Q. candicans* collected 31/03/2009, insect emerged 04/2009, E. Pascual leg. In the MNCN.

Etymology. Named after the collection site, Nevado Colima

Diagnosis and comments. In its forewing pattern, this new species resembles *A. zacatecaensis* and *A. hidalgoensis*. Compared to the former, *A. nevadensis* differs in its mesoscutellum being more deeply emarginated posteriorly; compared to *A. hidalgoensis*, the new species differs in the basal cell of its forewing, which is infuscate, while it is colourless in *A. hidalgoensis*.

Description. Female. Body length 6.5 mm (N = 1). Head and mesosoma black; clypeus, mandibles, antennae anteroventrally, and metasoma ventrally, dark brown. Legs black. Forewing dark infuscate, all the veins obscured by infuscation. A dark heavily infuscate band extended anteriorly along the basal cell, first cubital cell, and radial cell to antero apical of forewing. Costal cell and the area below the described heavily infuscate band is colorless, only slightly infuscate.

Female. Head, in dorsal view strongly reticulate rugose, about 2.4 times as wide as long (Fig. 6A). POL as long as OOL, posterior ocellus separated from inner orbit of eye by 1.5 times its longest diameter. Head in anterior view about 1.3 as wide as high, gena broadened behind eye. Vertex, frons, lower face, gena, and occiput with strong irregular reticulate-rugose sculpture, some irradiating carinae from clypeus visible. Head moderately pubescent. Clypeus trapezoidal, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits conspicuous; epistomal sulcus and clypeo-pleurostomal lines discernible. Malar space 0.5 times height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye as wide as antennal socket including rim. Ocellar plate slightly raised.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 6B), relatively short, as long as head and mesosoma combined; with 13 antennomeres; flagellum not broadening towards apex; with relatively long, erect setae, and placodeal sensilla in ventral area of flagellomeres F4–F11. Relative lengths of antennal segments: 26:10:52:33:27:22:20:15:14:14:12:12:21. Pedicel short, globose, shorter than long and 0.5 as long as scape; F1 1.6 times as long as F2. F6–F10 longer than wide, F11 about 2 times as long as F10.

Mesosoma. Coarsely reticulate rugose, short, in lateral view slightly longer than high (Fig. 6C). Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture. Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally.

Mesonotum. Mesoscutum barely pubescent and with strong rugose-reticulate sculpture (Fig. 6D). Notauli visible but shallow and indistinct obscured by crossing coarse sculpture. Anteroadmedian signa well visible and parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum subquadrate, about 0.6 as long as mesoscutum. Scutellar foveae rounded, deep, with distinct margins; with some transversal rugae, the intervals smooth and shining; about 0.5 as long as mesoscutellum (Fig. 6D). Mesoscutellum strongly reticulate-rugose, emarginated at posterior margin, the incision being only moderately deep and wide and not reaching posterior margins of scutellar foveae (Fig. 6D). Mesopleuron coarsely reticulate rugose, the rugae not as strong as mesoscutum. (Fig. 6C).

Metanotum. Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex. Metascutellum weakly rugose; metanotal trough smooth and pubescent. Median propodeal area reticulate shining rugose and densely pubescent; lateral propodeal carinae discernible. Nucha rugose medially.

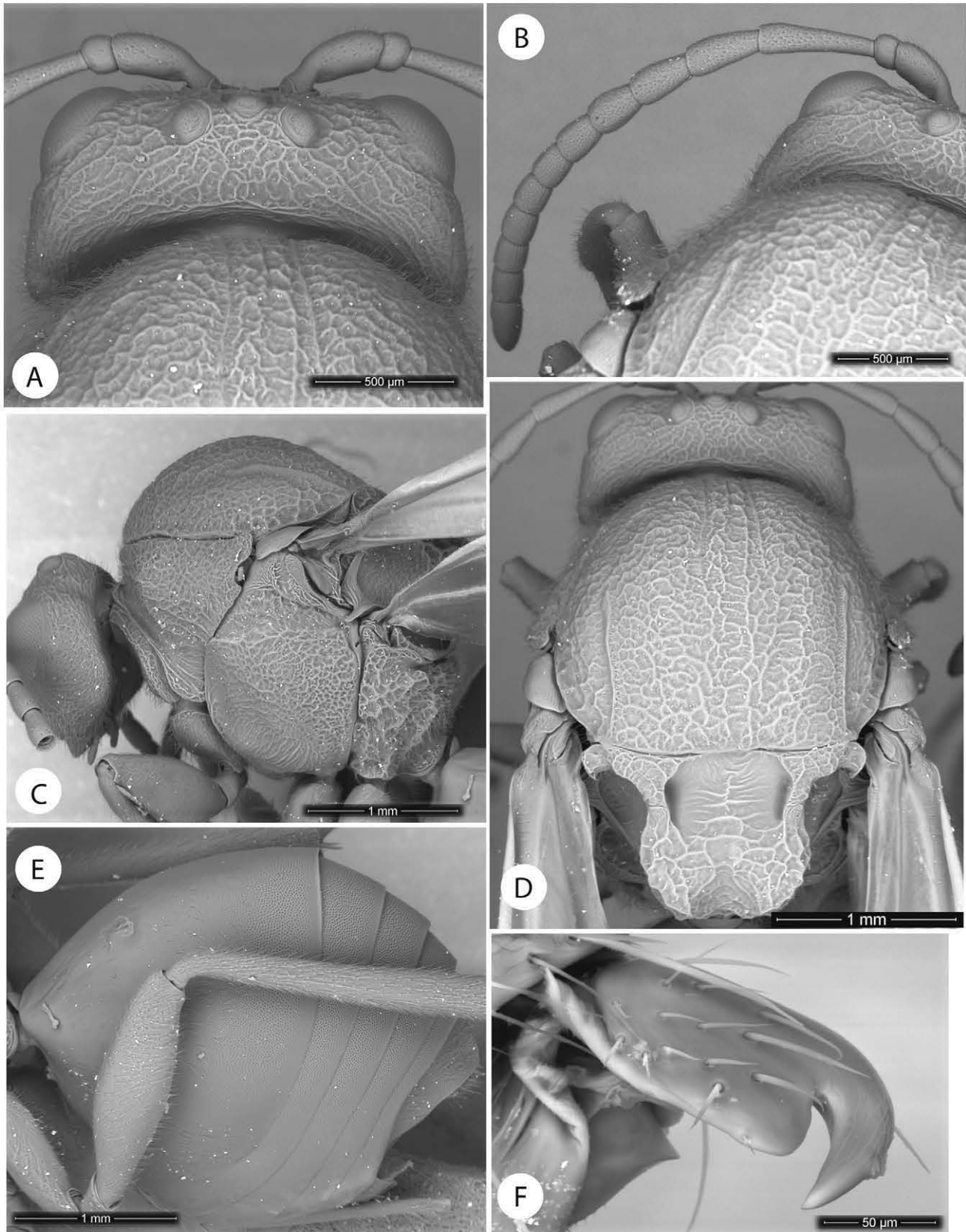


FIGURE 6. *Amphibolips nevadensis* sp. nov., female. (A) head dorsal view; (B) antenna; (C) mesosoma lateral view; (D) mesosoma dorsal view; (E) metasoma lateral view; (F) metatarsal claw.

Legs. Densely pubescent; femora and tibiae robust. Metatarsal claws with strong triangular basal lobe or teeth (Fig. 6F).

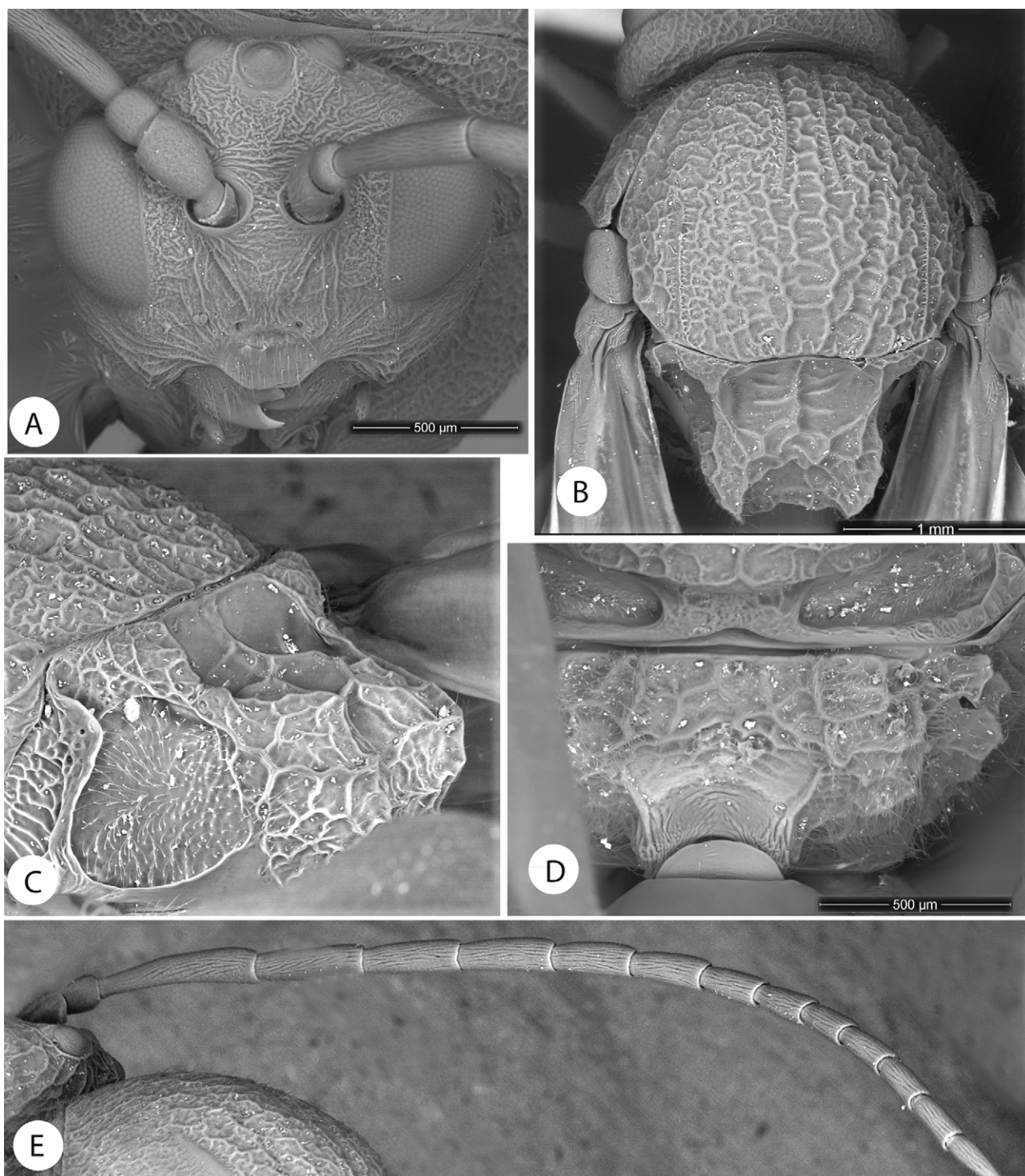


FIGURE 7. *Amphibolips nevadensis* sp. nov., male. (A) head anterior view; (B) mesosoma dorsal view; (C) mesoscutellum lateral view; (D) propodeum. (E) antenna.

Forewing (Fig. 12B): Slightly longer than body; radial cell 4 times longer than wide; open widely along dorsal margin; areolet conspicuous, triangular. R1 straight, not reaching wing margin; Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) obscured by infuscation and radius only slightly curved. Apical margin with short hair fringe.

Metasoma (Fig. 6E), slightly shorter as head and mesosoma combined, in lateral view about slightly longer than high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one third; punctures visible on subsequent tergites; dorsally the surface of the second

metasomal tergite before the band of micropunctures has coriaceous sculpture; latero ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine long, in lateral view (Fig. 6E) about 5 times as long as wide; laterally with long setae which not form an apical patch.

Male. Differs from female as follows: antennae with 14 antennomeres (Fig. 7E); F1 slightly modified, flattened on ventral side, elongate placodeal sensillae visible in all flagellomeres. Mesoscutellum more widely and deeply emarginated in posterior margin (Fig. 7B). Forewings more heavily infuscate in all its surface, but the darker anterior band being still visible (Fig. 13F).

Gall (Figs 19E, 19F). A globular gall ending in a obtuse apical point. Monothalamic. Grows on twigs of *Quercus candicans*.

Distribution. *A. nevadensis* was found at 2300 m a.s.l. at Nevado Colima, Jalisco state, Mexico.

Biology. Exhibits a sexual generation. The galls were collected in March, and the male adults emerged in April. The female was collected in August.

***Amphibolips tarasco* Nieves-Aldrey & Pascual sp. nov.**

Figs 8A–H, 12C, 13E, 16E–F

Type material. Holotype. Female (Fig. 16E). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card. Cat. n° 2250. MEXICO, Michoacán, Santa María, 2000 m; ex gall *Quercus castanea*, gall collected 12/03/2005, emerged, 22/03/2005, E. Pascual leg. Paratypes, 1 female and 2 males presenting the same data as the holotype. In MNCN.

Etymology. Named after the tarasco Indians, an outstanding indigenous people inhabiting Michoacan prior to Spanish conquest.

Diagnosis and comments. In its lightly infuscate forewing, the new species resembles *A. hidalgoensis* and the new species *A. oaxacae* described herein, but the patterns of infuscation are different, being absent in the costal cell and the basal area below the basal cell in *A. tarasco*, whereas these areas are infuscate in *A. oaxacae*. In *A. hidalgoensis*, the basal cell is not infuscate, whereas infuscation is present on the forewing of *A. tarasco*. This forewing pattern of the new species also resembles *A. nevadensis* and *A. michoacaensis*, but in these species, the infuscation is much heavier than in *A. tarasco*. The mesoscutellum in the new species is deeply, but not widely emarginated posteriorly, especially in males. The gall is a typical large oak apple type, regularly spherical with a smooth surface, which is tinted darker green in spots when fresh.

Description. Female (Fig. 16E). Body length 7 mm (N = 2). Head and mesosoma black; clypeus and mandibles chestnut. Antennae brown with last seven apical flagellomeres chestnut. Metasoma and legs reddish chestnut. Forewing lightly infuscate along a band extended in basal cell, first cubital (lighter here), radial cell and beyond to the antero apical margin of wing. Costal cell and the area below the described infuscate band is colorless, only slightly infuscate.

Female. Head, in dorsal view strongly reticulate rugose, 2.4 times as wide as long (Fig. 8B). POL as long as OOL, posterior ocellus separated from inner orbit of eye by 1.5 times its longest diameter. Head in anterior view (Fig. 8A) 1.3 as wide as high, gena broadened behind eye. Vertex, frons, lower face, gena, and occiput with strong irregular reticulate-rugose sculpture, irradiating carinae from clypeus indistinct. Head moderately pubescent. Clypeus trapezoidal, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits, epistomal sulcus and clypeo-pleurostomal lines discernible. Malar space 0.7 times height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye as wide as antennal socket including rim. Ocellar plate slightly raised.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 8D) As long as head and mesosoma combined; with 13 antennomeres; flagellum not broadening towards apex; with relatively long, erect setae, and placodeal sensilla in ventral area of flagellomeres F4–F11; placodeal sensillae on flagellomeres F8–F11 arranged in rows of 7–9 sensillae on ventral area of flagellomere. Relative lengths of first antennal segments: 20:10:36:23:19. Pedicel, short, globose, shorter than long and 0.5 as long as scape; F1 1.5 times as long as F2; F11 about 2 times as long as F10.

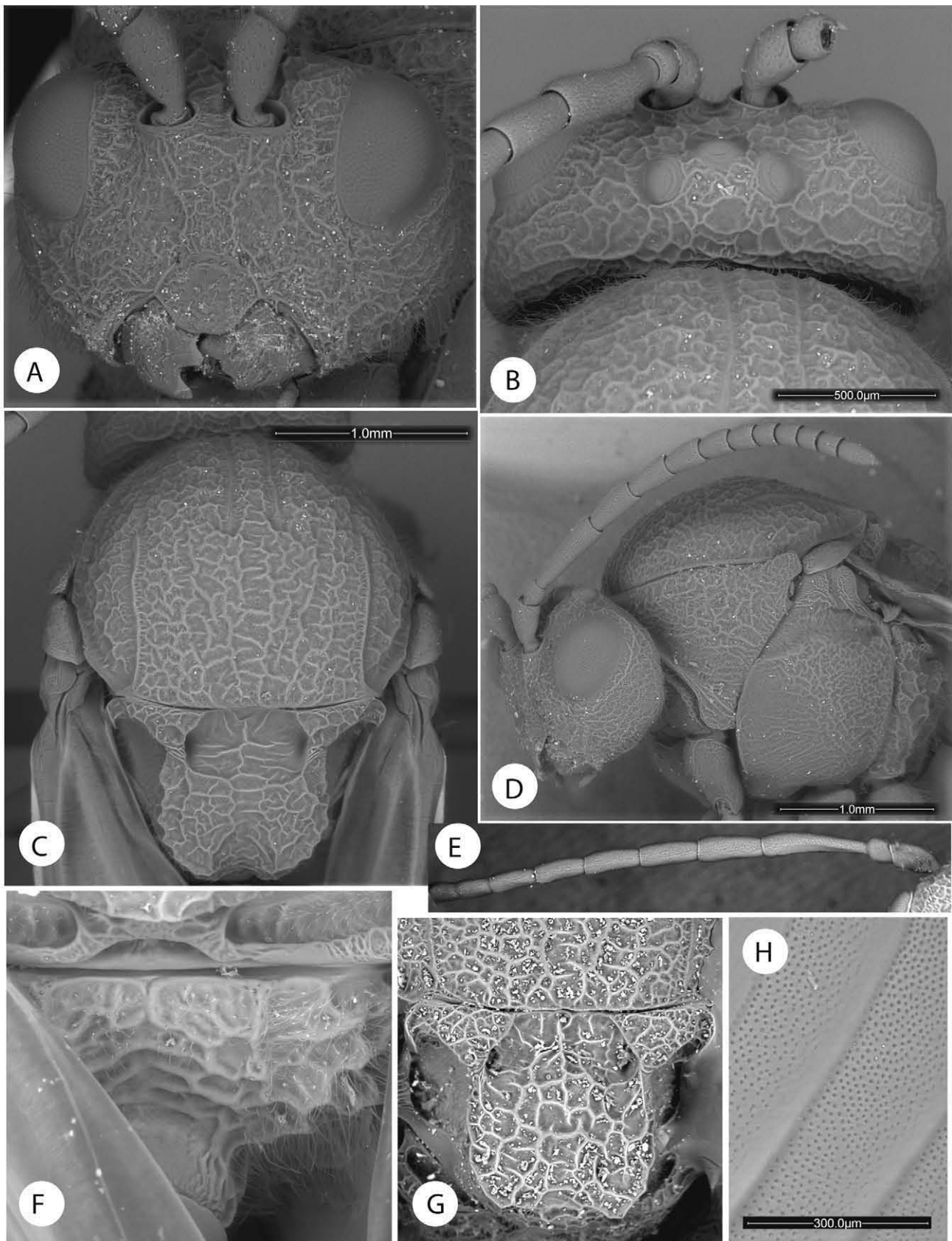


FIGURE 8. *Amphibolips tarasco* sp. nov. (A) female, head anterior view; (B) female, head dorsal view; (C) female, mesosoma dorsal view; (D) female, head and mesosoma lateral view; (E) male antenna; (F) male propodeum; (G) male, mesoscutellum dorsal view; (H) female, metasoma sculpture.

Mesosoma. Coarsely reticulate rugose, short, in lateral view slightly longer than high. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture (Fig. 8D). Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally.

Mesonotum. Mesoscutum sparsely pubescent and with strong reticulate rugose- sculpture (Fig. 8C). Notauli almost invisible obscured by crossing coarse sculpture. Anteromedian signa well visible and parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum subquadrate, about 0.6 as long as mesoscutum. Scutellar foveae rounded, deep, with distinct margins; with some transversal and oblique rugae, the intervals smooth and shining; about 0.4 as long as mesoscutellum (Fig. 8C). Mesoscutellum strongly reticulate-rugose, deeply emarginated at posterior margin, the incision being 0.3 as wide as scutellum width, extending longitudinally across median area of scutellum to reach, although more shallowly, posterior margins of scutellar foveae (Fig. 8C). Mesopleuron coarsely reticulate rugose, the rugae not as strong as mesoscutum. (Fig. 8D).

Metanotum (Fig. 8F). Metapectal-propodeal complex similar to the precedent species. Median propodeal area coarsely rugose and densely pubescent; lateral propodeal carinae discernible. Nucha rugose medially.

Legs. Densely pubescent; femora and tibiae robust. Metatarsal claws with strong triangular basal lobe or teeth.

Forewing (Fig. 12C): Slightly longer than body; radial cell about 4 times longer than wide; open widely along dorsal margin; areolet conspicuous, triangular. All veins well visible. R1 straight, not reaching wing margin; Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) angulated; radius only weakly curved. Apical margin with short hair fringe.

Metasoma. Slightly shorter as head and mesosoma combined, in lateral view about slightly longer than high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one half of metasomal large tergite; punctures visible on subsequent tergites (Fig. 8H); dorsally the surface of the second metasomal tergite before the band of micropunctures without sculpture, smooth and shining; latero ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine long, in lateral view about 4.5 times as long as wide; laterally with long setae which not form an apical patch. *Male*. Differs from female as follows: antennae with 14 antennomeres; F1 slightly modified, weakly twisted, flattened on ventral side, and slightly widened towards apex; 1.6 as long as F2; elongate placodeal sensillae visible in all flagellomeres. Mesoscutellum only slightly and shallowly emarginated in posterior margin (Fig. 8G). Forewings completely and more heavily infuscate, the darker anterior band being still visible (Fig. 13E).

Gall (Figs 20C, 20D). A large, regularly spherical, oak apple gall. The surface of gall is smooth, green with darker green spots when fresh. Monothalamic. Grows on twigs of *Quercus castanea*.

Distribution. *A. tarasco* was found at 2000 m a.s.l. at Santa María, Michoacán state, Mexico.

Biology. Includes a sexual generation. The galls were collected in March, and the adults emerged shortly after the galls were collected.

Amphibolips michoacaensis Nieves-Aldrey & Maldonado sp. nov.

Figs 9A–F, 10A–D, 12D, 13D, 16C–D.

Type material. Holotype female (Fig. 16C). In the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), mounted on a card. Cat. n° 2251. MEXICO, Michoacán, Cuenca del Cuitzeo, Umécuaro, N 19° 32' 55.4'' W 101° 15' 37.2''. 2140 m; ex gall *Quercus castanea*, gall collected 10/04/2009, emerged 10/2009, Y. Maldonado leg. Paratypes, 2 males and 3 females: 2 males showing the same data as the holotype; 1 female with the same data as the holotype, but gall collected 4/06/2008; 2 females found at Jesús del Monte, 2133 m, ex gall *Q. castanea*, collected 21/04/2009, insects emerged 05/2009, Y. Maldonado leg. In MNCN.

Etymology. Named after the Mexican state including the collection sites.

Diagnosis and comments. In its forewing pattern, this species closely resembles *A. nevadensis* and *A. tarasco*. Compared to *A. nevadensis*, it differs in the first cubital cell of the forewing not being as infuscate and compared to *A. tarasco*, in the dark band being much more heavily infuscate, with all veins obscured by infuscation. The mesoscutellum is moderately emarginated, with the incision not quite reaching the posterior margins of the scutellar foveae. MT3 smooth or only weakly coriaceous prior to the band of micropunctures, which are extended laterally over the posterior 1/3 to 1/2 of the length of the metasomal tergite. The intervals between micropunctures are strongly coriaceous.

Description. Female. Body length 5.1 mm (N = 4) (range 4.7–5.4). Head and mesosoma black; clypeus, mandibles and antennae anteroventrally chestnut brown. Metasoma blackish to dark chestnut, lighter ventrally. Legs black. Forewing dark infuscate, all the veins obscured by infuscation. A dark heavily infuscate band extended anteriorly along the basal cell, first cubital cell, and radial cell to antero apical of forewing. Costal cell, first cubital cell and the area below the more heavily infuscate band is colorless, only slightly infuscate.

Female. Head, in dorsal view strongly reticulate rugose, 2.3 times as wide as long (Fig. 9B). POL as long as OOL, posterior ocellus separated from inner orbit of eye by 1.5 times its longest diameter. Head in anterior view (Fig. 9A) about 1.3 as wide as high, gena broadened behind eye. Vertex, frons, lower face, gena, and occiput with strong irregular reticulate-rugose sculpture. A pair of irregular carinae running from ventral margin of antennal sockets to anterior tentorial pits and some irradiating carinae from clypeus visible. Head moderately pubescent. Clypeus trapezoidal, ventral margin strongly projecting over mandibles and slightly sinuate. Anterior tentorial pits conspicuous; epistomal sulcus and clypeo-pleurostomal lines discernible. Malar space 0.5 times height of compound eye. Toruli situated mid-height of compound eye; distance between antennal rim and compound eye as wide as antennal socket including rim. Ocellar plate slightly raised.

Mouthparts: mandibles strong, exposed; with dense setae in base, right mandible with three teeth; left with two teeth.

Antenna (Fig. 9C) as long as head and mesosoma combined; with 13 antennomeres; flagellum not broadening towards apex; with relatively long, erect setae, and placodeal sensilla in ventral area of distal flagellomeres. Scape 2 times as long as pedicel; pedicel, as long as wide; F1 1.5 times as long as F2. F9–F10 as long as wide, F11 about 2.5 times as long as wide; 2.5 as long as F10.

Mesosoma. Coarsely reticulate rugose, short, in lateral view 1.2 as long as high. Pronotum, moderately pubescent; lateral surface of pronotum with strong irregular reticulate rugose sculpture (Fig. 9E). Pronotum medially short; ratio of length of pronotum medially/laterally = 0.20. Pronotal plate indistinct dorsally.

Mesonotum. Mesoscutum barely pubescent and with strong rugose-reticulate sculpture (Fig. 9D). Notauli visible but shallow and indistinct anteriorly, obscured by crossing coarse sculpture. Anteroadmedian signa well visible and parascutal carinae distinct. Transscutal fissure narrow. Mesoscutellum subquadrate, about 0.6 as long as mesoscutum (Fig. 9D). Scutellar foveae rounded, deep, with distinct margins; smooth and shining; 0.3 as long as mesoscutellum. Mesoscutellum strongly reticulate-rugose, emarginated at posterior margin, the incision being only moderately deep and wide and not reaching posterior margins of scutellar foveae (Fig. 9D). Mesopleuron coarsely reticulate rugose, the rugae not as strong as mesoscutum. (Fig. 9E).

Metanotum. Metapectal-propodeal complex. Metapleural sulcus reaching posterior margin of mesopectus at about mid-height of metapectal-propodeal complex. Metascutellum weakly rugose; metanotal trough smooth and pubescent. Median propodeal area reticulate shining rugose and densely pubescent; lateral propodeal carinae discernible. Nucha rugose medially.

Legs. Densely pubescent; femora and tibiae robust. Metatarsal claws with strong triangular basal lobe or teeth (Fig. 10A).

Forewing (Fig. 12D). Slightly longer than body; radial cell 4 times longer than wide; open widely along dorsal margin; areolet inconspicuous, small. R1 straight, not reaching wing margin; Rs+M reaching basalis at its mid-height. First abscissa of radius (2r) obscured by infuscation and radius only slightly curved. Apical margin with short hair fringe.

Metasoma. Slightly shorter as head and mesosoma combined, in lateral view about slightly longer than high. Second metasomal tergite covering about two third of metasoma, with a band of micropunctures clearly visible in posterior one third; dorsolaterally the punctures being strong with coriaceous sculpture in the intervals. Punctures present on subsequent tergites; dorsally the surface of the second metasomal tergite, before the band of micropunctures, has weak coriaceous sculpture (Fig. 10B); latero ventral area of second metasomal tergite moderately pubescent. Projecting part of hypopygial spine very long, in lateral view about 6.5 times as long as wide; laterally with long setae, more dense apically but not forming an apical patch.

Male (Fig. 16D). Differs from female as follows: antennae with 14 antennomeres; F1 slightly modified, flattened on ventral side, elongate placodeal sensillae visible in all flagellomeres (Fig. 10C). Mesoscutellum only slightly emarginated in posterior margin (Fig. 10D). Forewings more heavily infuscate in its entire surface, but the darker anterior band being still visible (Fig. 13D).

Gall (Figs 20A, 20B). A globular more or less regular gall with spongy interior Monothalamic. Grows on twigs of *Quercus castanea* or nearer the base of the gall, galls up to 48 mm in diameter, detachable, on twigs.

Distribution. *A. michoacaensis* was found at Umécuaro and other sites of Cuenca of Cuitzeo at 2100 m at Michoacán state, Mexico.

Biology. Exhibits a sexual generation. The galls were collected in April, and the adults emerged in May.

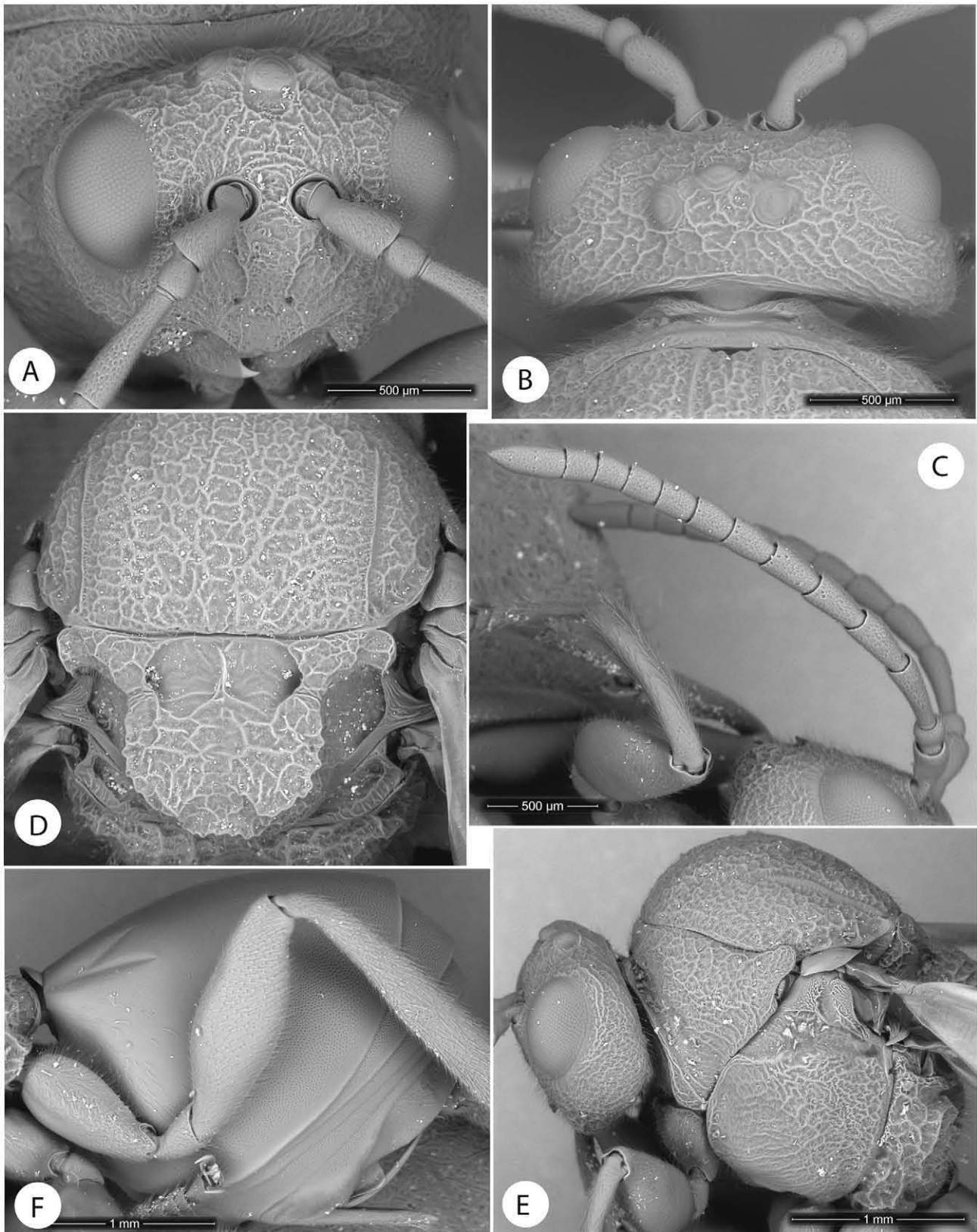


FIGURE 9. *Amphibolips michoacaensis* sp. nov., female. (A) head anterior view; (B) head dorsal view; (C) antennae; (D) mesosoma dorsal view; (E) mesosoma lateral view; (F) metasoma lateral view.

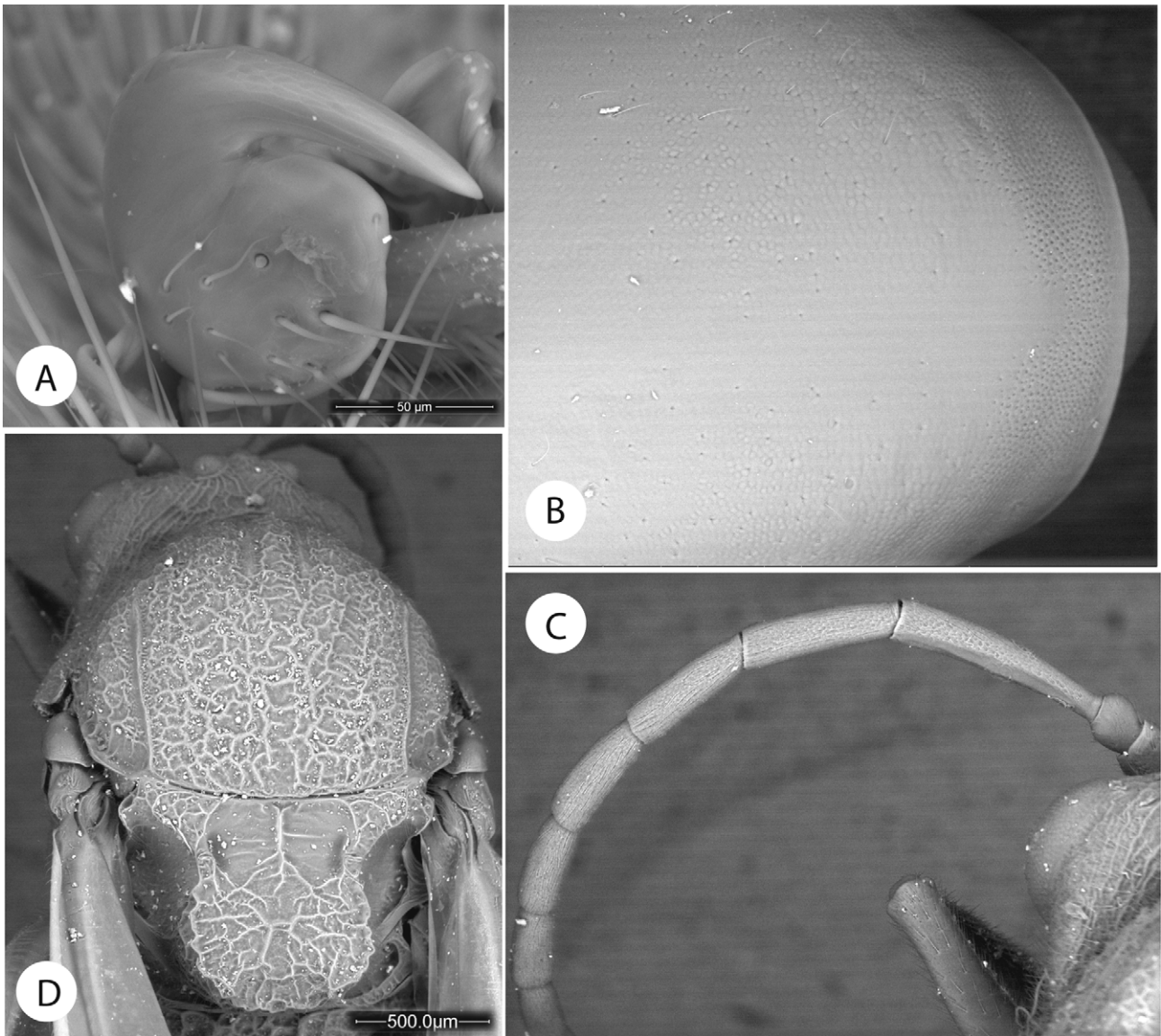


FIGURE 10. *Amphibolips michoacaensis* sp. nov. (A) female, metatarsal claw; (B) female, metasoma dorsal view; (C). male antenna; (D) male, mesosoma dorsal view.

Key to adult *Amphibolips* species of Mexico and related species of Panama (species of “niger complex” excluded)

1. Antenna with 16–17 antennomeres. Mesoscutellum rounded posteriorly. Mesoscutum sometimes with carinate longitudinal sculpture (Figs 1A–B). Asexual forms. Galls rounded with a woolly surface and a central hard woody cell “niger complex” Kinsey
- Antenna with 13–14 antennomeres (females) or 15 antennomeres (males) (Fig. 3A). Mesoscutellum emarginated posteriorly, with the emargination being more or less deep (Figs 7B, 10D). Mesoscutum always with coarse rugose reticulate sculpture (Figs 2E, 6D). Sexual forms. Gall form spherical or globose to spindle shaped. Surface smooth or rugose, never woolly 2
2. Females 3
- Males 16
3. Forewing with a heavily infuscate spot on the basal area of radial cell; remainder of the forewing hyaline to only slightly infuscate (Fig. 11B). *aliciae*
- Forewing entirely infuscate, more heavily along a band on anterior margin of wing (Figs. 11C–F). 4
4. More heavily infuscate band along anterior margin of forewing with a clear cross-band on one-third apical part of radial cell which is more or less extended towards posterior margin of wing (Figs 11A, 11C, 13A, 14E). 5
- More heavily infuscate band along the anterior margin of the forewing, without a clear cross-band on apical part of radial cell extended towards posterior margin of wing. If there is a clear colourless spot apically on the radial cell, it does not extend below the radial cell (Figs 12A–E) 8

5. Basal and first cubital cells colourless or only weakly infuscate prior to the heavily infuscate basal half of the radial cell (Fig. 14E). Mesoscutellum weakly emarginate posteriorly. F1 1.2 as long as F2 (Fig. 1F) *fusus*
- Basal and first cubital cells as heavily infuscate as basal half of radial cell. F1 1.4–1.5 as long as F2 (Figs 11A, 11C, 13A) 6
6. Clear cross-band narrow, extended below radial cell, but not reaching posterior margin of wing (Fig. 13A). Mesoscutellum strongly emarginated posteriorly (V-shaped in dorsal view (Fig. 1D), with a sharp horn projection in lateral view) *dampfii*
- Clear cross-band wide and extended posteriorly to reach posterior margin of wing (Fig. 11A, 11C). Mesoscutellum only moderately emarginated posteriorly (Fig. 2E) 7
7. Forewing heavily and entirely infuscate outside of the clear cross-band in the anterior area of the radial cell, which is relatively wider; costa cell infuscate (Fig. 11A). Notauli visible; scutellar foveae smooth *castroviejoii*
- Forewing not entirely infuscate outside of the clear cross-band in the anterior area of the radial cell, which is relatively narrower; costal cell and posterior half of wing colourless, only weakly infuscate (Fig. 11C). Notauli almost invisible; scutellar foveae with carinate sculpture (Fig. 2E) *durangensis*
8. Basal half of forewing uniformly infuscate; the costal cell and the area below basal cell as infuscate as the basal, first cubital and radial cells. 9
- Costal cell and the area below basal cell and beyond colourless, much less infuscate than the basal and radial cells. 10
9. Antenna and metasoma predominantly black. Mesoscutellum moderately emarginated posteriorly. F3 1.3 times as long as F4; gall elongate, spindle shaped. *nassa*
- Antenna and metasoma predominantly reddish. Mesoscutellum strongly emarginated posteriorly. Gall regularly spherical *oaxacae*
10. Heavily infuscate band along the anterior margin of the forewing extended uniformly from basal cell to apical margin of wing, not interrupted in the first cubital cell 11
- Heavily infuscate band along the anterior margin of the forewing, interrupted in the first cubital cell, sometimes also in the basal cell 12
11. Mesoscutellum slightly emarginated posteriorly. Heavily infuscate anterior band of forewing quite dark (Fig. 12E). Galls with an acute point. Male unknown *zacatecaensis*
- Mesoscutellum strongly emarginated posteriorly (Fig. 7B). Heavily infuscate anterior band of forewing not as dark (Fig. 12B). Galls end in an obtuse point. Bisexual form; male known *nevadensis*
12. Colourless, less infuscate spot present on apical area of radial cell (Figs 11D, 11E) 13
- Colourless, less infuscate spot absent on apical area of radial cell; radial cell uniformly and heavily infuscate (Figs 12A, 12C, 12D). 14
13. All the veins strongly infuscate, basally on the radial cell not being visible. Clear, colourless area on radial cell relatively more extended on one half apical area of radial cell; first cubital cell more heavily infuscate (Fig. 11D). F1 1.4 as long as F2 (Fig. 3A). *jaliscensis*
- All the veins less strongly infuscate, all being visible. Clear, colourless area on the radial cell relatively small, extending only on more apical area of the radial cell; first cubital cell only slightly infuscate (Fig. 11E). F1 1.6 as long as F2 (Fig. 5C). *malinche*
14. Basal cell not infuscate (Fig. 12A) *hidalgoensis*
- Basal cell infuscate (Figs 12C, 12D). 15
15. Forewing weakly infuscate, all veins visible (Fig. 12C). Mesoscutellum only slightly emarginated posteriorly (Fig. 8C). *tarasco*
- Forewing more heavily infuscate, veins on anterior half of radial cell not visible (Fig. 12D) *michoacaensis*
- Males**
16. Forewing with a heavily infuscate spot in the basal area of the radial cell; rest of the forewing only slightly infuscate. *aliciae*
- Forewing entirely and heavily infuscate, more so along a band on the anterior margin of the wing (Figs. 13A–F) 17
17. More heavily infuscate band along the anterior margin of the forewing, with a clear cross-band on one-third of the apical part of the radial cell, which is more or less extended towards the posterior margin of the wing (Fig. 13A) 18
- More heavily infuscate band along the anterior margin of the forewing, without a clear cross-band on the apical part of the radial cell, extended towards the posterior margin of the wing. If there is a clear colourless spot apically on the radial cell, it does not extend below the radial cell (Figs 13B, 13D) 19
18. Clear cross-band wide and extended posteriorly to reach margin of the wing. Mesoscutellum only moderately emarginated posteriorly. *castroviejoii*
- Clear cross-band narrow, extended below the radial cell, but not reaching the posterior margin of the wing (Fig. 13A). Mesoscutellum strongly emarginated posteriorly (V-shaped in dorsal view) (Fig. 1D), with a sharp horn projection observed in lateral view *dampfii*
19. Mesoscutellum widely and deeply emarginated posteriorly (Fig. 3D). Radial cell with a clear spot apically (Fig. 13B). *jaliscensis*
- Scutellum slightly or moderately emarginated posteriorly (Figs 8G, 10D). Radial cell without a clear spot apically (Figs 13D, 13E) 20
20. Mesoscutellum only slightly emarginated posteriorly (Fig. 8G); scutellar foveae ellipsoidal. F1 1.6 as long as F2 (Fig. 8E). Costal cell of the forewing relatively clearer, less infuscate than the rest of the forewing (Fig. 13E) *tarasco*
- Mesoscutellum moderately to deeply emarginated posteriorly; scutellar foveae rounded. F1 1.4–1.5 as long as F2 (Fig. 10C). Costal cell relatively darker; as infuscate as the remaining forewing surface (Fig. 13D) 21
21. Mesoscutellum moderately emarginated posteriorly (Fig. 10D). F1 1.4 as long as F2 (Fig. 10C). Radial cell 3.3 as long as wide, second abscissa of radius slightly curved (Fig. 13D) *michoacaensis*
- Mesoscutellum deeply emarginated posteriorly (Figs 7B–C). F1 1.5 as long as F2 (Fig. 7E). Radial cell 3.7 as long as wide, second abscissa of the radius only slightly curved (Fig. 13F) *nevadensis*

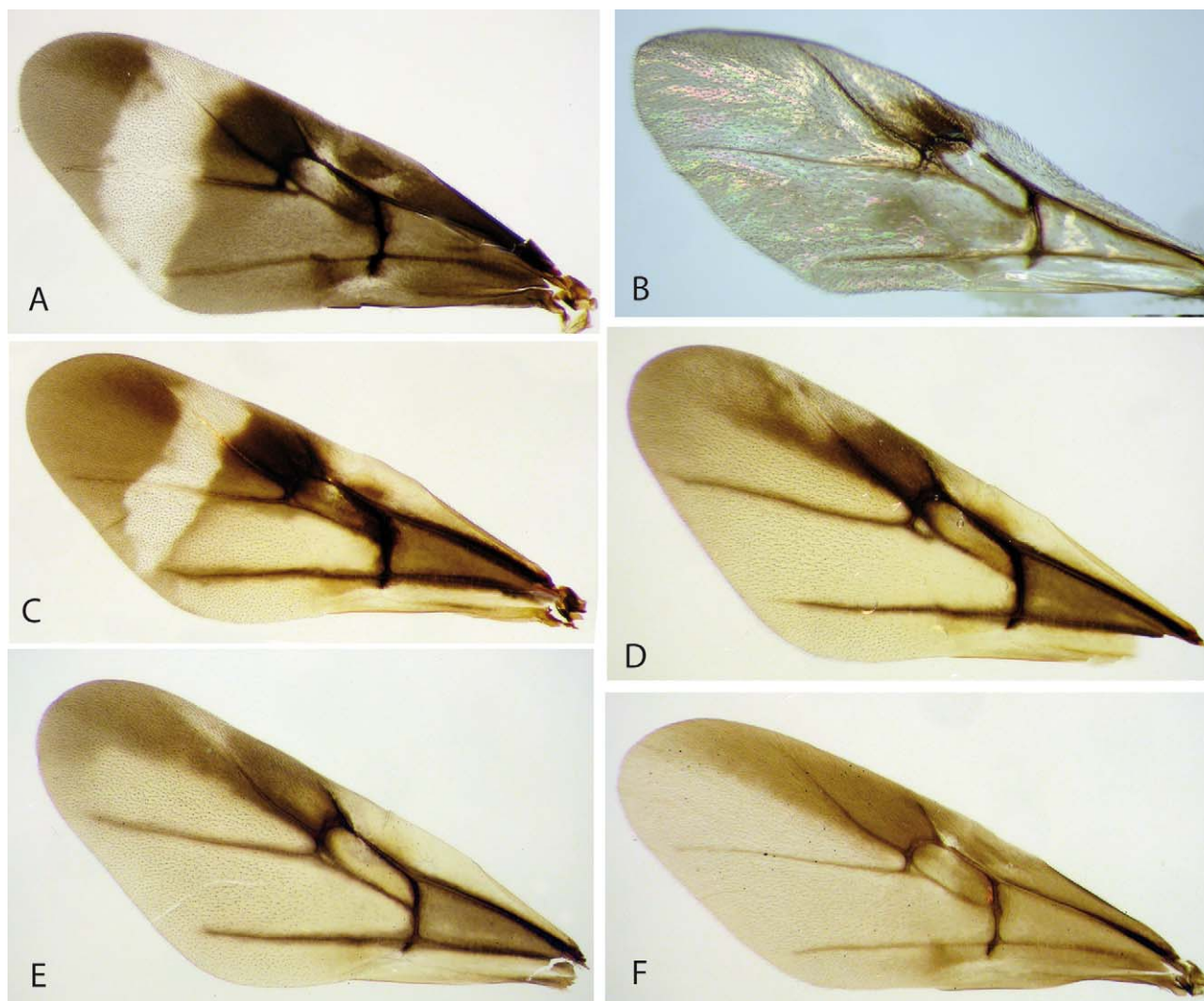


FIGURE 11. Forewings of species of *Amphibolips*, females; (A) *Amphibolips castroviejoii*, from Panama; (B) *A. aliciae*, from Panama; (C) *A. durangensis* sp. nov. (D) *A. jaliscensis* sp. nov. (E) *A. malinche* sp. nov. (F) *A. oaxacae* sp. nov.

Discussion

In his pioneer study of the *Amphibolips* of Mexico, Kinsey (1937) separated the Mexican species of this genus into two groups. He described the new species *A. dampfi*, *A. fusus* and *A. nassa*, but without constructing any formal or informal taxonomic grouping for them. Of these three species, only *A. dampfi* exhibits a demonstrated bisexual generation, as both males and females have been described. The other two species, *A. fusus* and *A. nassa*, have been described based only on females, and Kinsey doubted whether they presented bisexual or agamic generations. For instance, he recorded the life history of *A. nassa* as unknown. However, he formally proposed the group “*niger* complex” (asexual generations) for six new species he described from Mexico, including the species *Amphibolips nigra* Beutenmüller 1911, which he included in the “*niger* complex” under the name *Amphibolips (niger) niger* Beutenmüller. One last Mexican *Amphibolips* species, *A. palmeri* Basset 1890, which was in fact the first *Amphibolips* species described from Mexico, was not mentioned in Kinsey (1937) and was not formally included in any group.

The species of the “*niger* complex” are well characterised according to Kinsey (1937) based on their morphology and biology. With regard to morphology, the main diagnostic character separating the species of this group from other *Amphibolips* species is their 16–17 segmented antennae, which typically exhibit 13–14 antennomeres in the majority of species of this genus (Medianero & Nieves-Aldrey 2010; Melika *et al.* 2011).

Other morphological characters are less clear because they present mixed character states and are shared by other *Amphibolips* species, such as heavily infuscate wings, the sculpture of the mesoscutum and mesoscutellum and the shape and sculpture of the scutellar foveae. Melika *et al.* (2011) provided some additional diagnostic characters of the “*niger* complex” as follows: the surface sculpture of the head and mesosoma is less rugose and smoother; the mesoscutellum is rounded posteriorly, without a median posterior depression; all metasomal tergites with white setae posterolaterally; and the projecting part of the ventral spine of the hypopygium shorter and thinner. However, some of these diagnostic characters, especially the mesoscutellum character, which is supposedly rounded posteriorly in species of the “*niger* complex”, were not mentioned in Kinsey’s original description, and we ignore whether or not these additional diagnostic characters were examined in all of the species of the “*niger* complex”. The character of the mesoscutellum was examined and illustrated in at least two *Amphibolips* species: one of the “*niger* complex”, *Amphibolips pistrix* Kinsey, 1937 and another outside of this group, *A. quercuscinereae* (Ashmead, 1881), both of which present a posteriorly rounded mesoscutellum. We have examined this character in one additional species of the “*niger* complex”, *Amphibolips nebris* Kinsey, whose mesoscutellum is also rounded posteriorly (Fig. 1A), as in the cases illustrated by Melika *et al.* (2011).

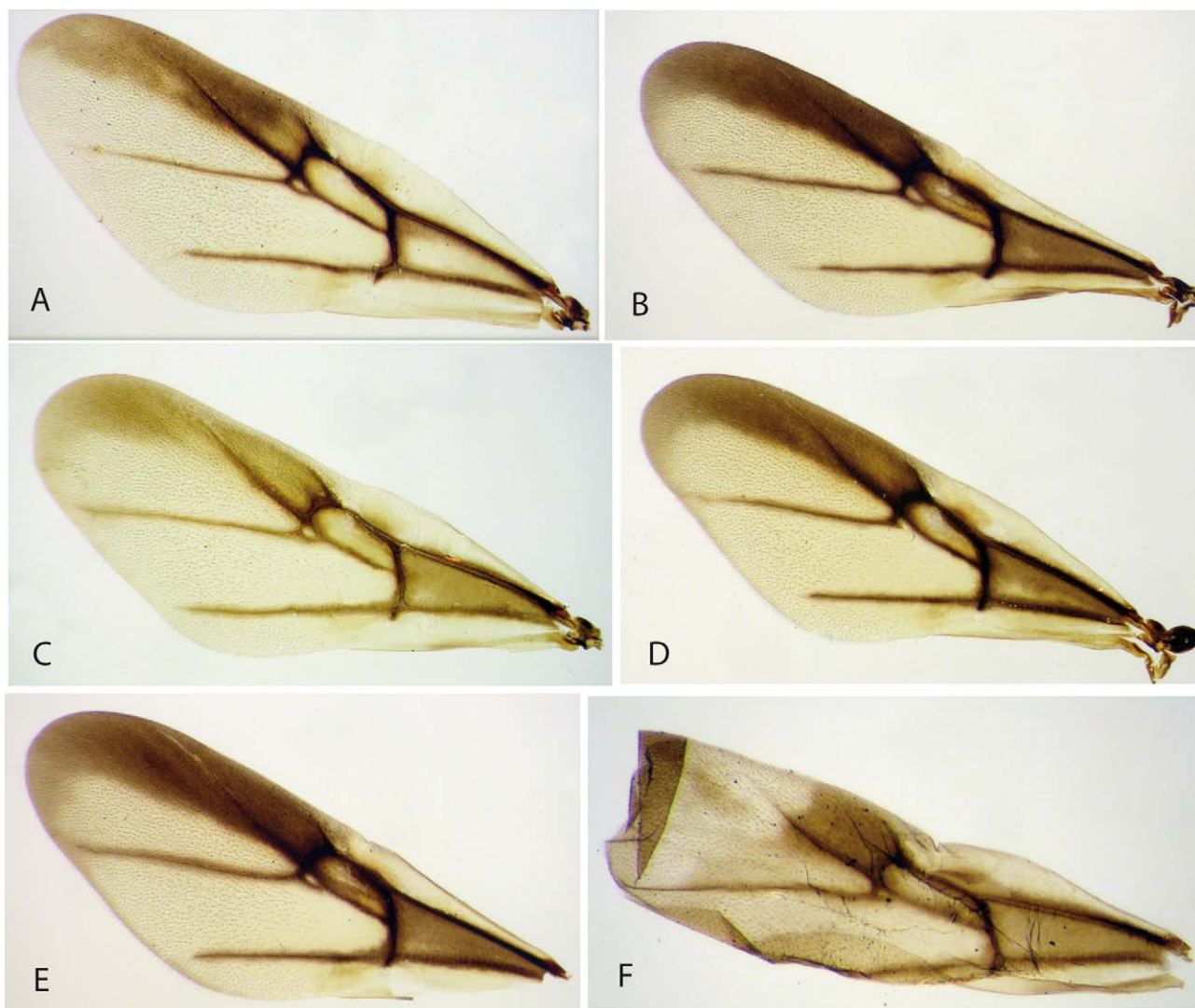


FIGURE 12. Forewings of species of *Amphibolips*, females; (A) *Amphibolips hidalgoensis*; (B) *A. nevadensis* sp. nov. (C) *A. tarasco* sp. nov. (D) *A. michoacaensis* sp. nov. (E) *A. zacatecaensis*; (F) *Amphibolips* sp., from Nuevo León.

However, Melika *et al.* (2011) omitted the most important diagnostic character of the “*niger* complex” mentioned in Kinsey’s original description, that is, the 16–17 segmented antennae. We have examined this character in the female holotype of a species of this group, *A. nebris* Kinsey. This specimen in fact exhibits a 16 segmented antenna (Fig. 1B). The cited authors questioned whether all seven species of the “*niger* complex” are distinct biological

entities or are simply varieties based entirely on different collection localities. After this study, we will go further, asking whether the entire “*niger* complex” should be excluded from *Amphibolips* because its members differ strongly, both morphologically and biologically, from the core species of this genus. A revision of the entire complex, as well as biological and molecular analyses, should provide us with initial crucial information for solving this problem.

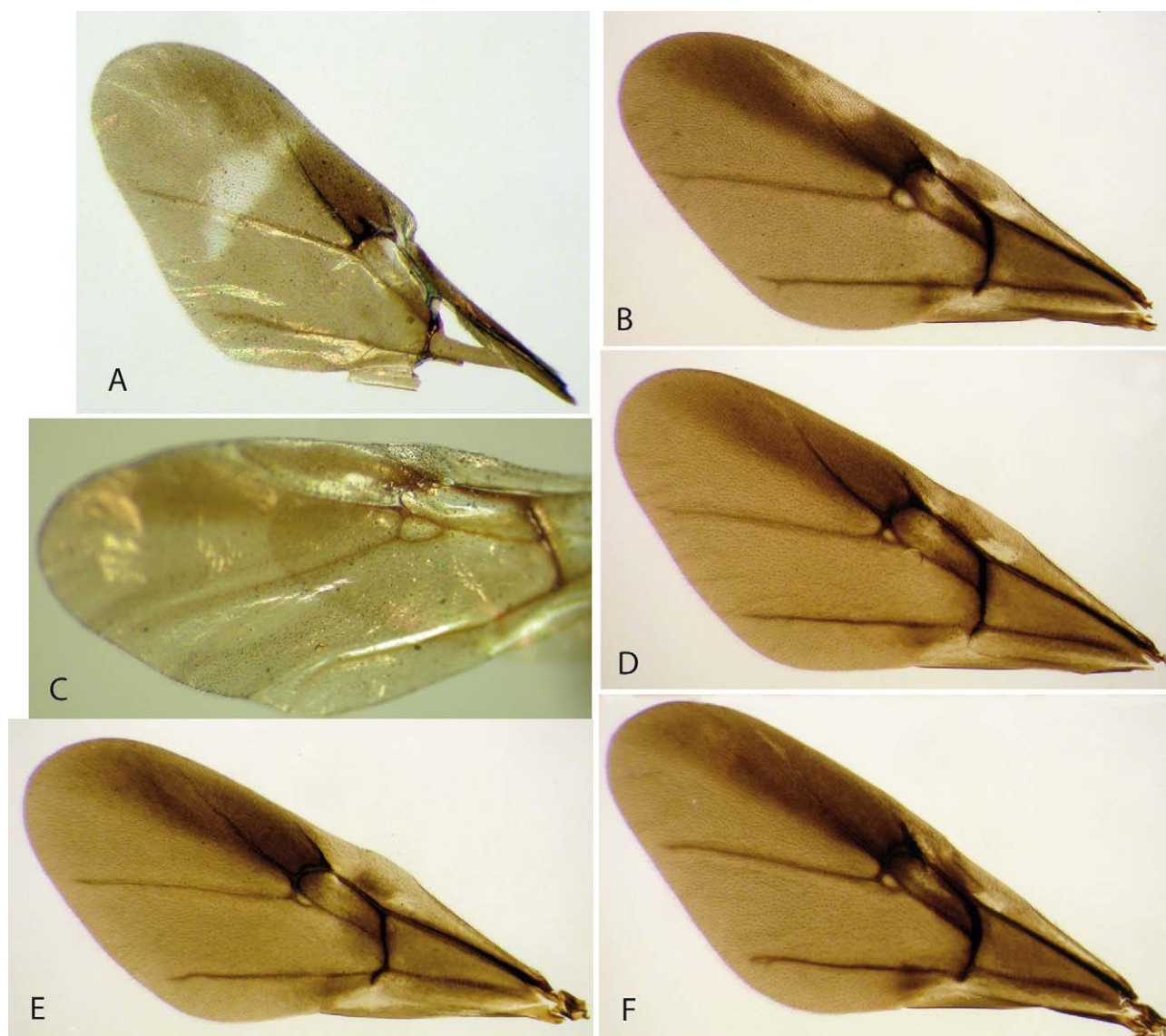


FIGURE 13. Forewings of species of *Amphibolips*, males except as indicated; (A) *Amphibolips dampfi*; (B) *A. jaliscensis* sp. nov. (C) *A. nebris*, female; (D) *A. michoacaensis* sp. nov. (E) *A. tarasco* sp. nov. (F) *Amphibolips nevadensis* sp. nov.

Melika *et al.* (2011) erected a new group, the “*nassa* complex”, for the six Mexican species not included in the “*niger* complex”, including the three described by Kinsey plus *A. palmeri* and two species described as new. However, they did not formally describe the “*nassa* complex” in the same way that was used by Kinsey, despite providing a key for the identification of these species. We do not follow this informal taxonomic proposal here due to the reasons presented below.

The species included by Melika *et al.* (2011) in the “*nassa* complex” are characterised, according the identification key provided by the above-cited authors, by presenting a mesoscutellum with a posteromedian emargination. The other couplet included the species of the “*niger* complex” and all known *Amphibolips* species from the USA and Panama. However, this couplet is clearly erroneous because at least three species of *Amphibolips* from the USA (*A. confluentus* (Harris, 1841), *A. quercusinanis* (Osten Sacken, 1861) and *A. melanocerus* (Ashmead, 1885)) and two from Panama (*A. castroviejoi* Medianero & Nieves-Aldrey, 2010 and *A. aliciae* Medianero & Nieves-Aldrey, 2010) also present a mesoscutellum emarginated posteriorly (Beutenmüller 1909; Medianero & Nieves-

AldreY 2010). This morphological character cannot then be used as diagnostic for defining the “*nassa* complex”. Other characters shared by the species included in the “*nassa* complex” are as follows:

- Antennae with 13–14 antennomeres in females; 14–15 in males.
- Forewings always more or less deeply infusate, with bands or spots variably extended to the anterior margin of the forewing or/and to the radial and cubital cells.
- Ventral projection of the spine of the hypopygium quite long, more than five times as long as wide in lateral view.
- Head and mesoscutum with a strong reticulate rugose sculpture.
- Second metasomal tergite covering approximately two-thirds of the metasoma, ventrolaterally pubescent and with a band of micropunctures posteriorly.
- Galls of the “oak-apple” type, spherical or globose, pointed or not, to more or less fusiform or spindle shaped.

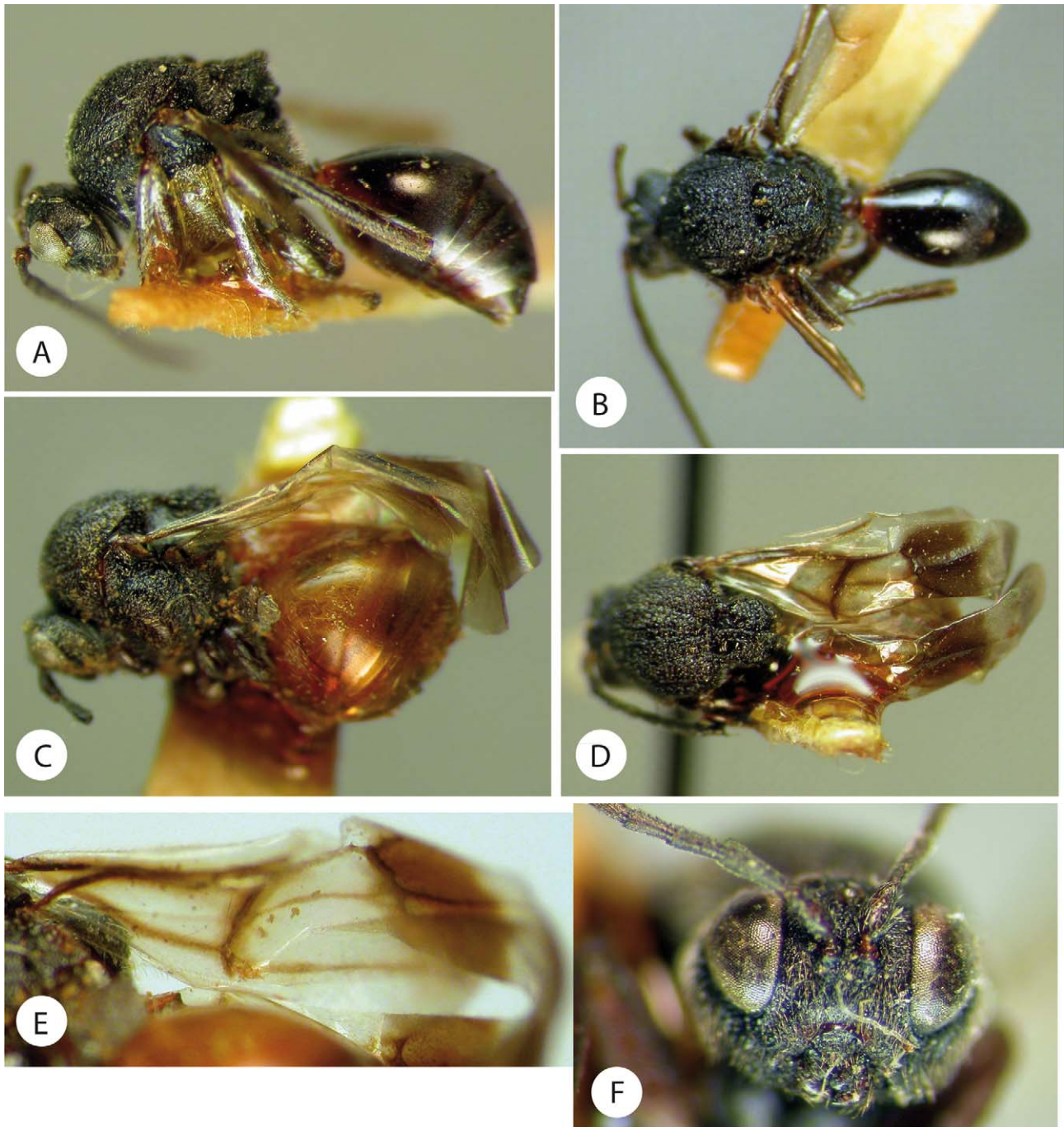


FIGURE 14. Type material examined of species of *Amphibolips* (A) *Amphibolips dampfi*, holotype male, lateral view; (B) *Amphibolips dampfi*, holotype male, dorsal view; (C) *Amphibolips fusus*, holotype female, lateral view; (D) *Amphibolips fusus*, holotype female, dorsal view; (E) *A. fusus*, forewing; (F) *Amphibolips nebris*, head anterior view.

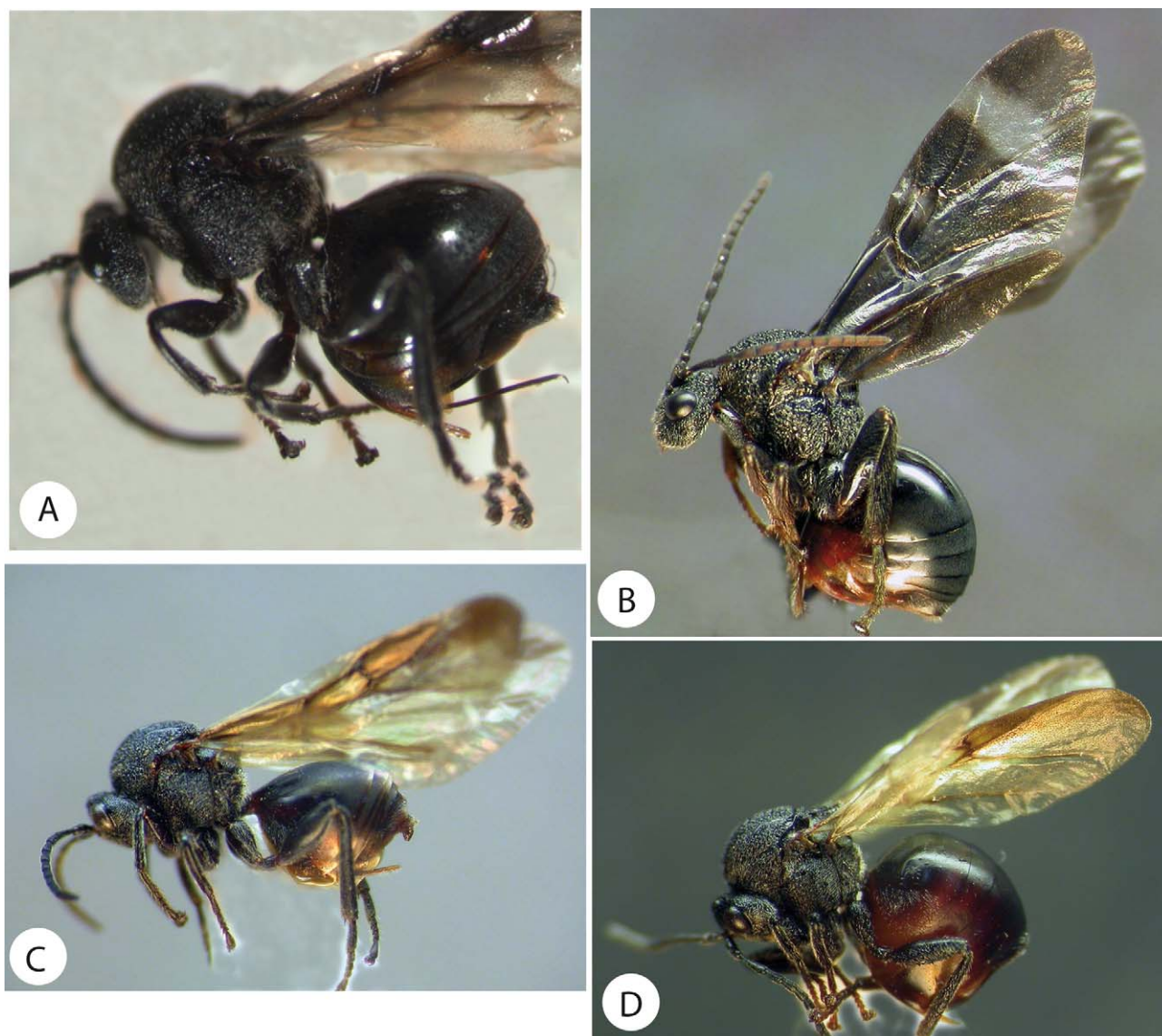


FIGURE 15. Habitus of species of *Amphibolips*, females. (A) *Amphibolips durangensis* sp. nov. (B) *A. castroviejoii*. (C) *Amphibolips malinche* sp. nov. (D) *Amphibolips hidalgoensis*.

Nevertheless, all of these characters are also shared, at least in part, by *Amphibolips* species from the USA and Panama and may not be used to readily define the “*nassa* complex” in the sense used by Melika *et al.* 2011. By example darker clouds or smoky brown transverse bands on the forewings are also present in the North American species *Amphibolips gainesi* Basset, 1900 and *A. trizonata* Ashmead, 1896 (Beutenmüller 1909).

In conclusion, the “*nassa* complex” appears to us to be an useless taxonomic group that is not supported and not well defined with regard to morphology and should no longer be maintained. Further morphological and molecular studies will elucidate whether this hypothesis is or is not supported by additional data.

The Mexican *Amphibolips* species not belonging to the “*niger* complex” comprise a group of species that are closely related and are weakly differentiated morphologically. Gall morphology, which is a distinctive feature used for differentiation of these species as an extended phenotype in many oak gall wasps, appears to be not as discriminant in this group of *Amphibolips* species. There are two main types of gall morphologies, ranging from spherical or globose, pointed at the end or not, to fusiform or more or less spindle shaped. However, morphologically different insects, supposedly belonging to different species, sometimes emerge from similar galls. This fact, together with the extreme morphological similarity within the group, poses the taxonomic problem of the limits of the extant species with respect to whether they represent widely variable single species or closely related different species. The species analysed in the present study, including the species described as new, are all only

slightly differentiated with regard to the morphology of adults and galls. They appear to form a group of closely related sibling species. Ongoing molecular phylogeographic studies may eventually elucidate the real, objective limits of the geographic variability within a given species or between two different species.

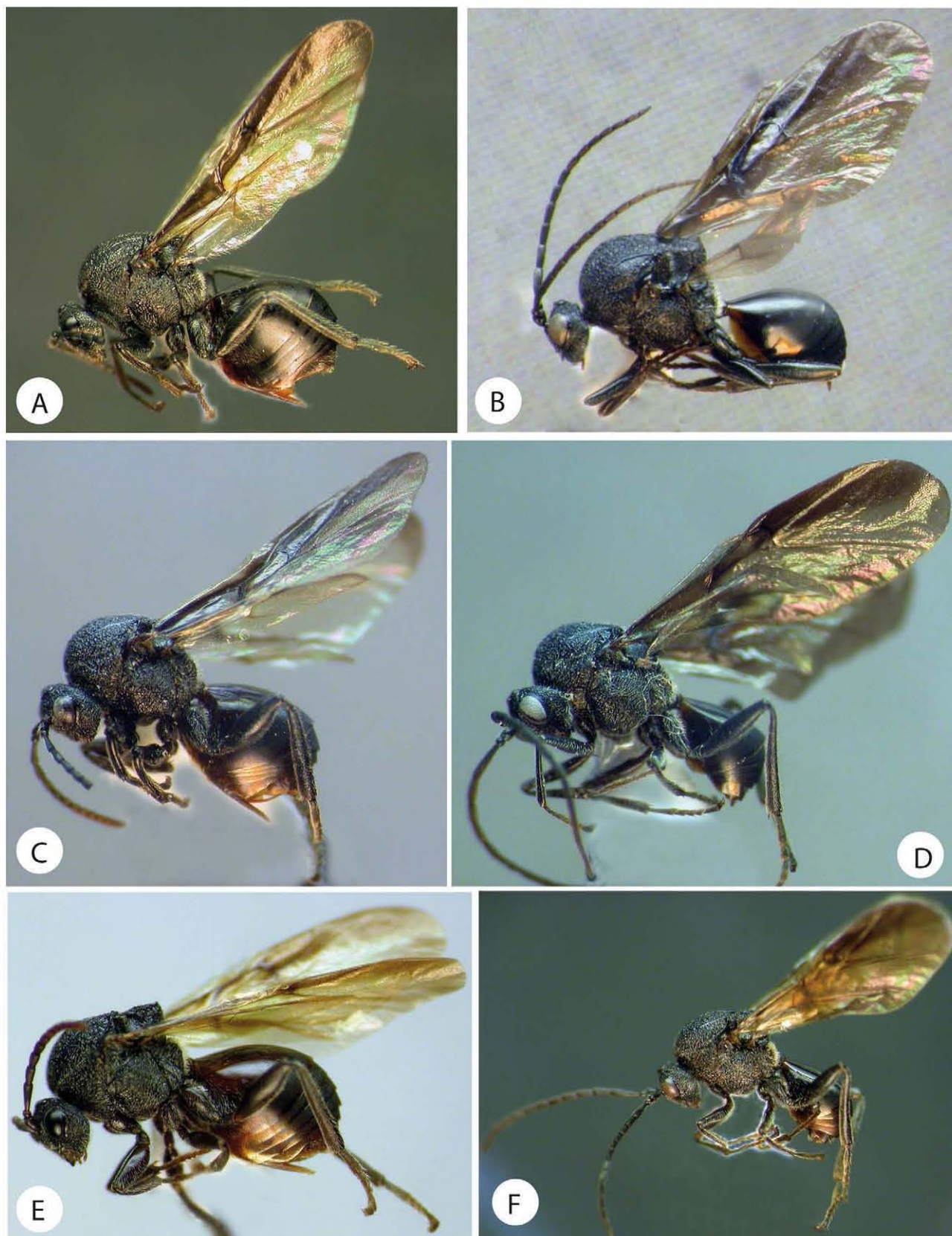


FIGURE 16. Habitus of species of *Amphibolips*. (A) *Amphibolips nevadensis* sp. nov., female. (B) *A. nevadensis*, male. (C) *Amphibolips michoacaensis* sp. nov., female. (D) *A. michoacaensis*, male. (E) *Amphibolips tarasco* sp. nov., female. (F) *A. tarasco*, male.

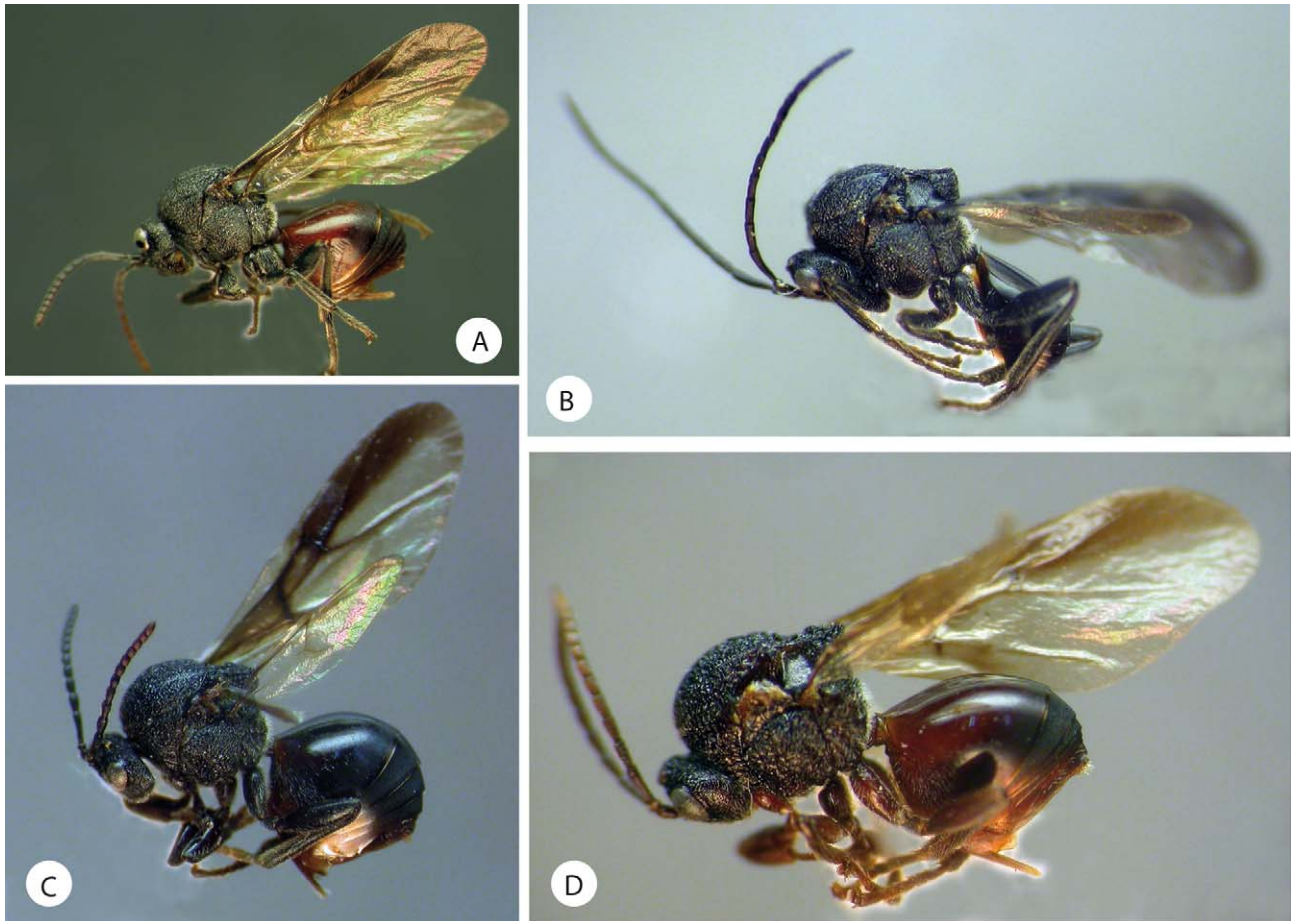


FIGURE 17. Habitus of species of *Amphibolips*. (A) *Amphibolips jaliscensis* sp. nov., female; (B) *A. jaliscensis*, male; (C) *Amphibolips zacatecaensis*, female; (D) *Amphibolips oaxacae* sp. nov., female.

In this work, a far from exhaustive sampling revealed a great number of unknown species of *Amphibolips* in Mexico. However, these findings were not surprising, given the great number of potential host species present in the study area and the limited studies performed on this gall wasp fauna to date, which referred only to the expeditions of Kinsey in the past century.

We collected galls of unknown/unidentified species of *Amphibolips* at several sites in Mexico on several *Quercus* species (Figs 21A–H), though unfortunately, no adults were reared to allow identifications to be made. However, this gall diversity reveals that the fauna of *Amphibolips* species may be quite rich in Mexico and that many other species could remain to be described. It is clear that a much greater sampling effort is still necessary to understand this fauna, as is the case for the Cynipidae of Mexico in general.

Acknowledgements

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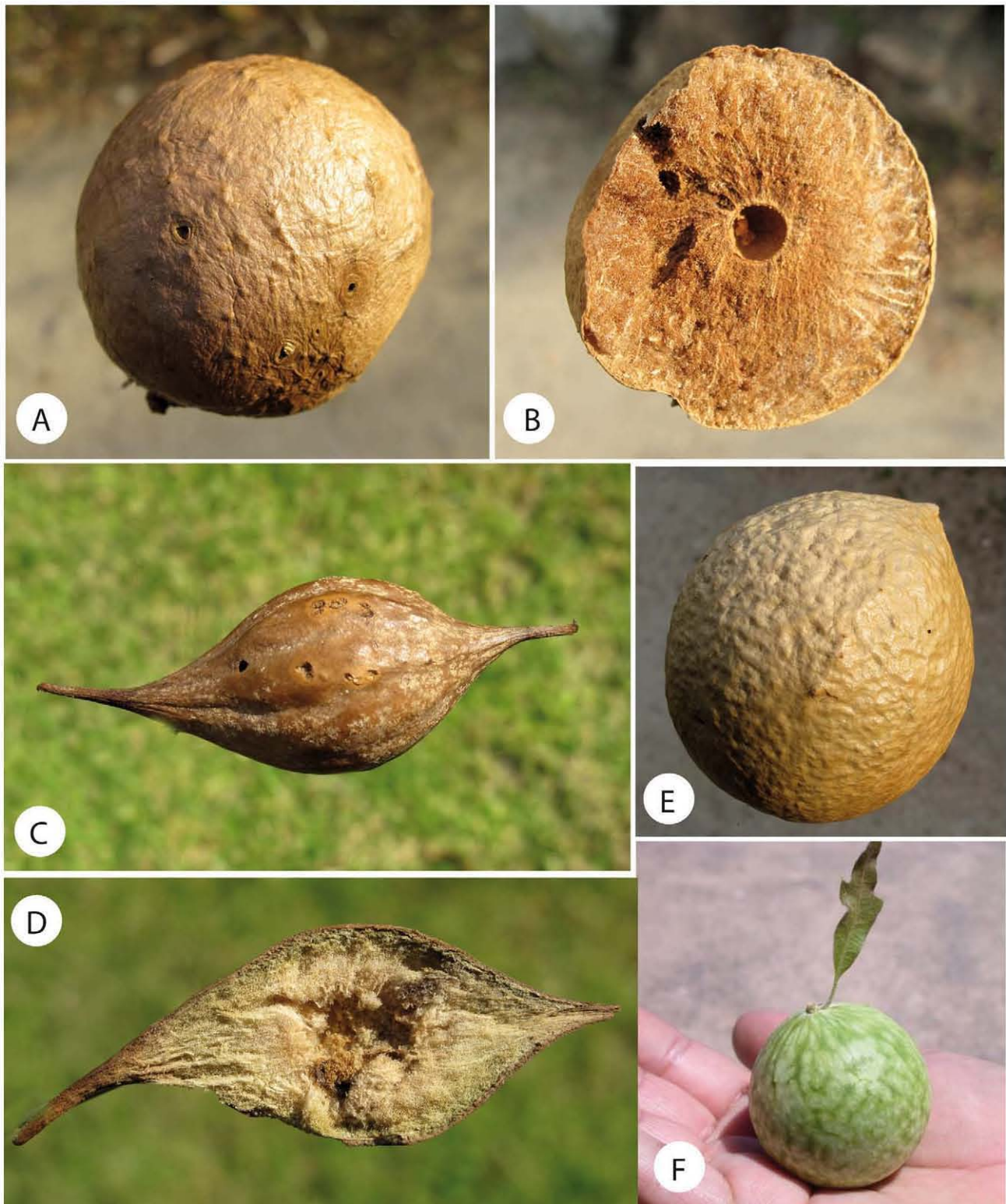


FIGURE 18. Galls of species of *Amphibolips*. (A) *Amphibolips hidalgoensis*; (B) section of a gall; (C) *A. durangensis* sp. nov. (D) section of a gall; (E) *A. zacatecaensis*; (F) *A. oaxacae* sp. nov.

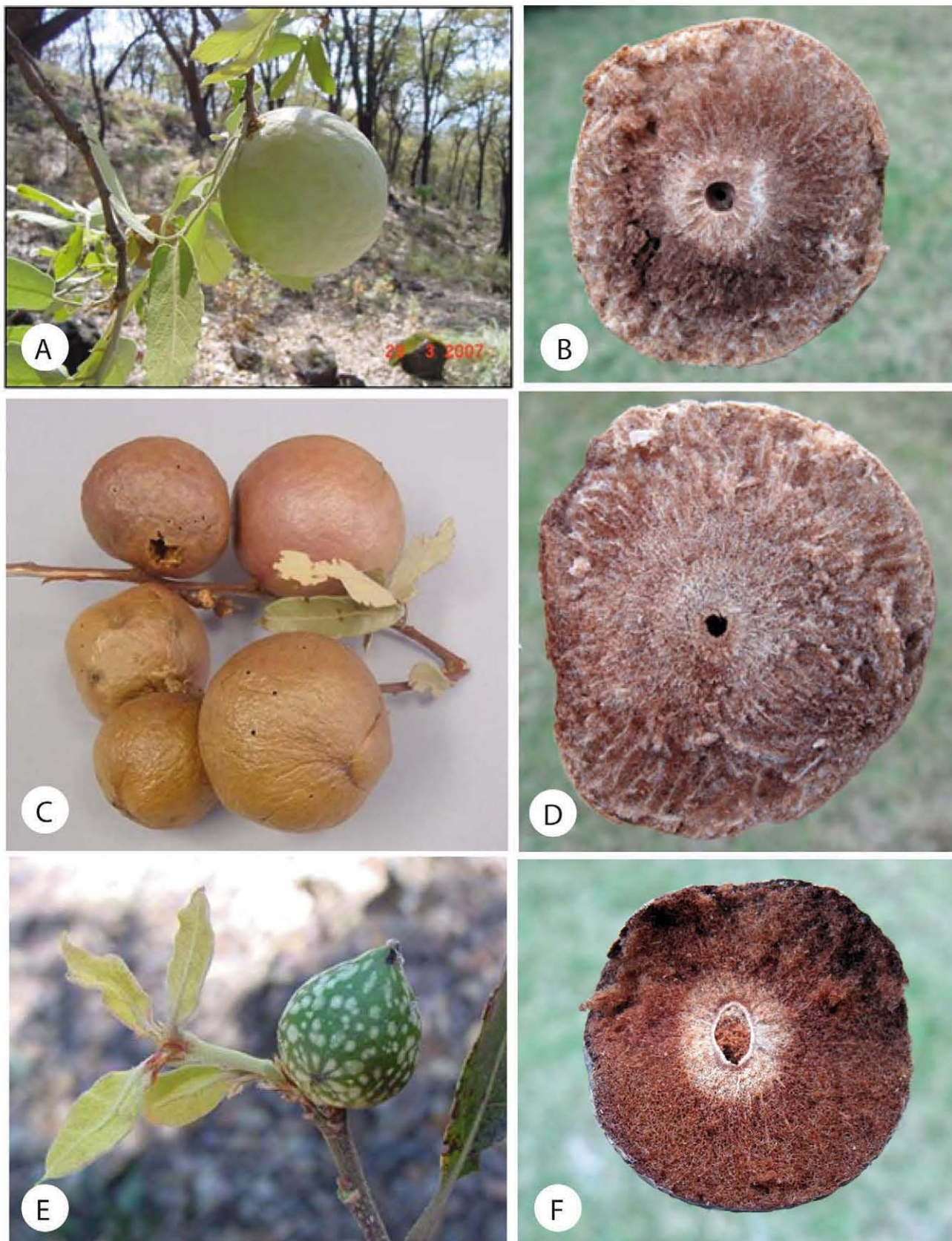


FIGURE 19. Galls and sections of galls of species of *Amphibolips*. (A–B) *Amphibolips jaliscensis* sp. nov. (C–D) *A. malinche* sp. nov. (E–F) *A. nevadensis* sp. nov.

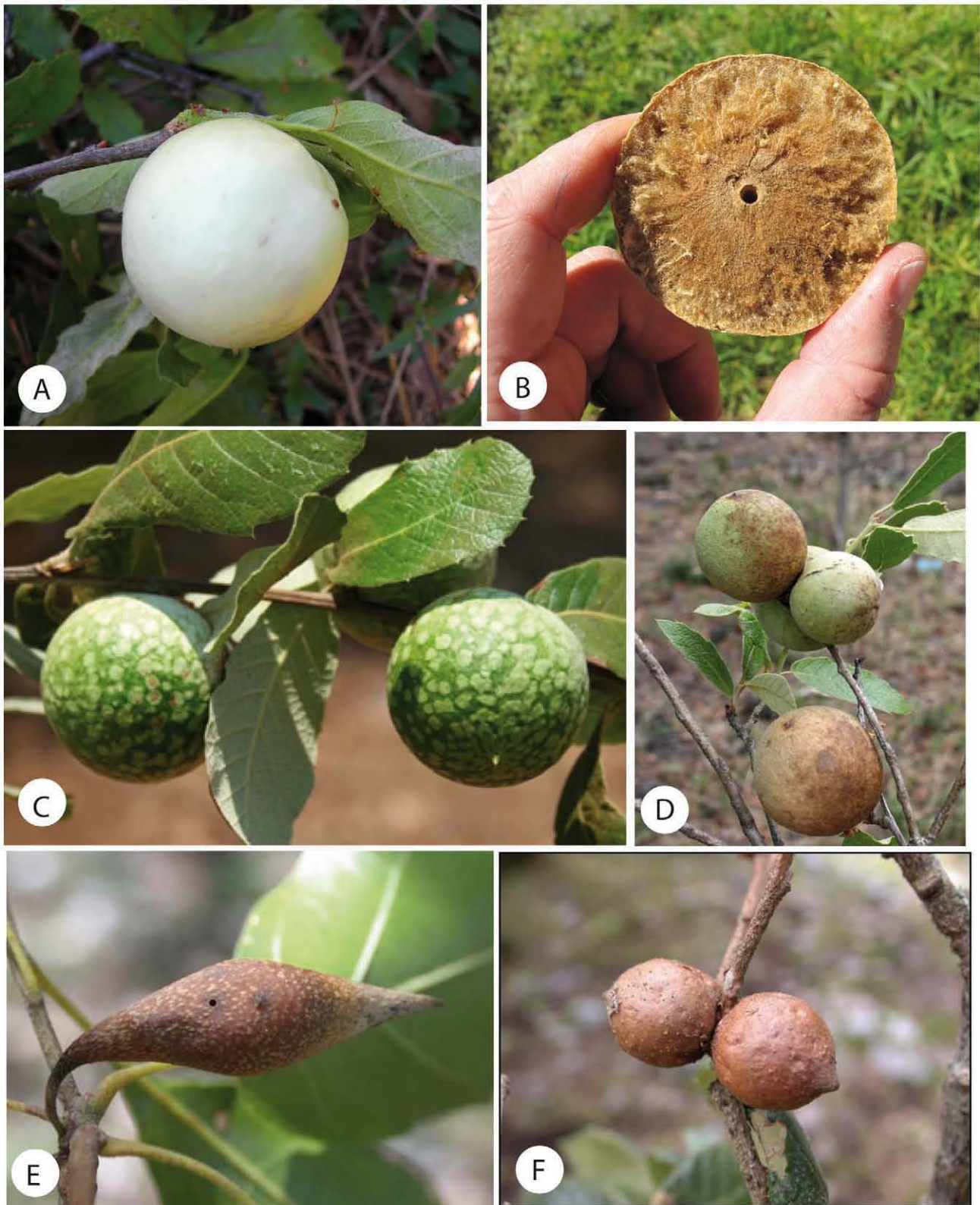


FIGURE 20. Galls of species of *Amphibolips*. (A) *Amphibolips michoacaensis* sp. nov. (B) section of a gall. (C–D) *A. tarasco* sp. nov. (E–F) *Amphibolips* sp., Nuevo León.

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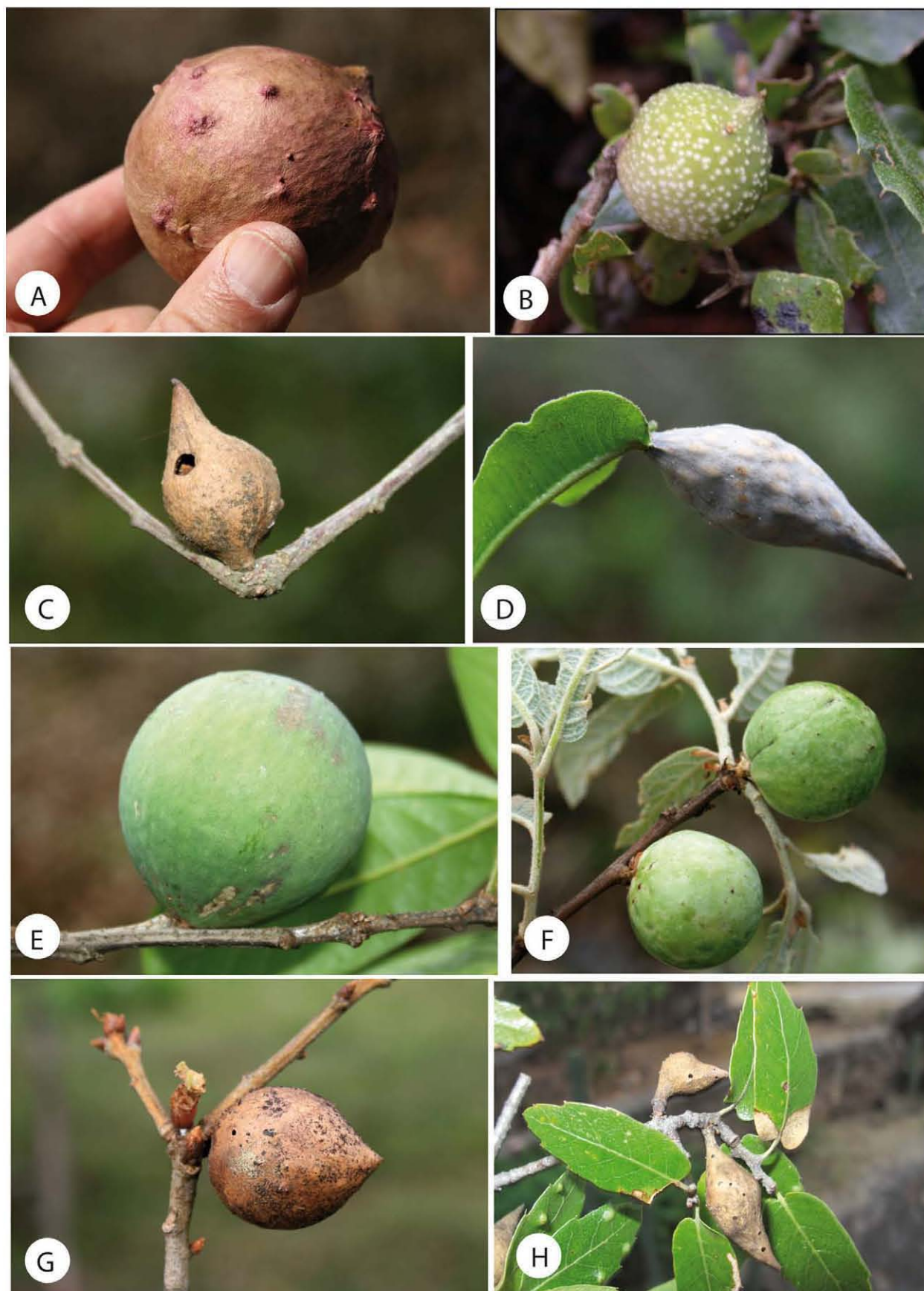


FIGURE 21. Galls of undetermined species of *Amphibolips* from Mexico (collected on 23–28 April, 2008 and 27–28 November, 2010, Pascual & Nieves leg.). (A) Umécuaro on *Q. castanea*. (B) Nuevo León on *Quercus* sp. (C) Xico, on *Quercus* sp. (D) Xalapa, on *Quercus* sp. (E) Xico, on *Q.* sp. (F) Xalapa on *Quercus glaucoides*. (G) Xalapa on *Quercus* sp. (H) Guanajuato, on *Q. microphylla*.

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DISCUSIÓN GENERAL

Los resultados de esta tesis muestran que tanto la riqueza como la abundancia de avispa inductoras de agallas asociadas a los encinos, se incrementa en fragmentos de bosque más pequeños y en los bordes de los fragmentos. Particularmente, un resultado de gran importancia fue la alta riqueza y abundancia de cinípidos presentes en los árboles aislados, lo cual sugiere que estos árboles representan recursos clave para la sobrevivencia y la reproducción de las avispas en un paisaje fragmentado (Chust et al. 2007; Müller y Goßner 2007). La capacidad de dispersión de los insectos es un parámetro de gran importancia para predecir los efectos de la fragmentación de bosque sobre el tamaño de las poblaciones (Hanski 1999; Driscoll y Weir 2005).

Particularmente, las avispas inductoras de agallas que incluyen especies con una alta capacidad de dispersión ya que pueden propagarse en corrientes de aire muy rápidamente, incluso a través de hábitats desfavorables (Hough 1951; Nieves-Aldrey 1995; Schönrogge et al. 2011), y este mecanismo puede favorecer la conexión de poblaciones entre diferentes fragmentos de bosque. De esta manera, es posible que los encinos aislados puedan promover la persistencia de diferentes especies de avispas inductoras de agallas a lo largo de un paisaje fragmentado, ya que representan recursos clave al proveer numerosas funciones ecológicas en bosques fragmentados (Ozanne et al. 2000; Manning et al. 2004).

Otros resultados de gran importancia, muestran que la fragmentación del bosque influye fuertemente sobre la calidad de la planta hospedera en términos del vigor de la planta y la disponibilidad de recursos para las avispas inductoras de agallas. Este resultado es consistente con los efectos "bottom-up" de la calidad de la planta hospedera

sobre la abundancia y la riqueza de cinípidos (Price 1991; Preszler y Price 1995). Las plantas hospederas más vigorosas fueron los encinos aislados y los árboles presentes en fragmentos de bosque más pequeños y en los bordes de fragmento. Los cambios en la disponibilidad de recursos para los herbívoros, afectan las interacciones con otros niveles tróficos y modifican la estructura y composición de la comunidad de insectos (Didham et al. 1996; Chust et al. 2007). Más hojas disponibles para los insectos herbívoros pueden representar más sitios potenciales para la inducción de agallas (Weis et al. 1988), teniendo en cuenta que su ciclo de vida debe sincronizarse con la producción de órganos de la planta hospedera ya que requieren de tejido indiferenciado para iniciar la inducción de agallas (Stone et al. 2002; Hayward y Stone 2005).

La variación en la calidad de la planta hospedera puede estar explicada por los cambios en las condiciones abióticas en fragmentos de bosque, particularmente en los bordes donde existe un aumento de la radiación solar, temperatura, velocidad del aire, y una disminución de la humedad y fertilidad del suelo (Young y Mitchell 1994; Chen et al. 1995; Kapos et al. 1997). Estos cambios ambientales potencialmente representan un agente de estrés fisiológico para las especies arbóreas y pueden promover cambios en los patrones de producción foliar, afectando indirectamente la preferencia e incidencia de insectos herbívoros (Lovejoy et al. 1986; Sundarapandian y Swamy 1999). Incluso algunos autores como Magrach et al. (2014) han mostrado que algunas especies de plantas pueden presentar respuestas compensatorias a la fragmentación del hábitat que involucran efectos demográficos y cambios en las defensas químicas de las plantas y las tasas de rebrote.

Por otro lado, es importante considerar que la fragmentación del bosque puede influir sobre la diversidad de cinípidos a través de los efectos "top-down" mediados por los enemigos naturales como los parasitoides, que pueden determinar una alta mortalidad en las poblaciones de avispas inductoras de agallas (Stone et al. 2002). Se ha reportado que en fragmentos de bosque pequeños o aislados, las poblaciones de insectos fitófagos aumentan su tamaño debido a la reducción en el tamaño poblacional de parasitoides como resultado de la fragmentación de bosque (Kruess y Tscharntke 1994) ya que los niveles tróficos superiores como los parasitoides son más sensibles a las nuevas condiciones ambientales generadas por la fragmentación del hábitat (Kruess y Tscharntke 1994; Davies et al. 2000). Las principales razones son que los parasitoides son más sensibles a la fragmentación del hábitat ya que sólo pueden colonizar los parches ya ocupados por sus hospederos (Weisser 2000; van Nouhuys 2005), tienen tamaños de población más pequeños y dependen más de los procesos de recolonización (Pimm 1991; Lawton 1995; Holt et al. 1999). De esta manera, la mortalidad impuesta por los enemigos naturales disminuye en fragmentos más pequeños o más aislados, lo que resulta en la liberación ecológica de las poblaciones de avispa asociadas a los encinos (Holt 1996; Chust et al. 2007).

En nuestro estudio, encontramos que tres especies de encino, *Quercus obtusata*, *Quercus castanea* y *Quercus deserticola*, están asociadas a 80 especies de avispas inductoras de agallas, por lo cual pueden ser considerados como "Super-Hospederos" (Araújo et al. 2013). Estas especies de encino mostraron una mayor disponibilidad de recursos cuando la abundancia de agallas fue mayor. En algunos casos las agallas de un mismo órgano aparecieron en distintos tiempos fenológicos, sugiriendo una partición de

recursos (Abrahamson et al. 1998, 2003). Sin embargo, en muchos casos, múltiples especies de agallas se desarrollaron en el mismo órgano de la planta al mismo tiempo. La hipótesis de la Coexistencia propuesta por Chesson (2000) propone que la coexistencia de los cinípidos está regulada por los parasitoides. Es posible que la coexistencia simpátrica de la alta diversidad de avispas inductoras de agallas en una sola especie de planta hospedera pueda mantenerse por partición de parasitoides generalistas entre los diferentes fenotipos de agallas. De esta manera, los cinípidos pueden evitar la mortalidad reduciendo la eficiencia de parasitoides y adquiriendo un espacio libre de enemigos naturales (Price et al 1987). Otro estudio en *Q. castanea* ha mostrado que la comunidad avispas asociadas está regulada por una comunidad diversa de parasitoides. Espinosa-Olvera (datos no publicados) encontró que la comunidad de avispas inductoras de agallas asociada a *Q. castanea* tiene 32 especies de parasitoides en México. Si bien la riqueza de cinípidos y sus parasitoides asociados ha sido poco estudiado en el Neártico, algunos autores sugieren que la mayor riqueza de avispas asociadas a los encinos potencialmente se encuentra en México (Pujade-Villar et al. 2009). Un resultado importante de este trabajo, fue el reporte de un gran número de especies nuevas de *Amphibolips* en México. El grupo de especies de *Amphibolips* presentes en México, que no pertenecen al complejo "*niger*" comprende especies cercanamente relacionadas y con diferencias morfológicas muy sutiles. Por ejemplo, la morfología de las agallas de cinípidos es con frecuencia un factor de diferenciación entre especies. Sin embargo, en el caso del género *Amphibolips* parece no ser una característica que no permite discriminar taxonómicamente. Esto reafirma la idea de que la taxonomía de los cinípidos es

problemática, y en lugares como México aún hay muchas especies de cinípidos sin describir (Pujade-Villar et al. 2001).

En el caso específico del "*Super-Hospedero*" *Quercus castanea*, el análisis filogenético reveló que las avispas asociadas pertenecen a linajes de cinípidos independientes, lo que indica que no ocurrió especiación *in situ* o radiación adaptativa dentro *Q. castanea*, sino que las especies de avispas inductoras de agallas convergieron en la misma especie de planta hospedera como eventos evolutivos no relacionados. La competencia directa inter e intraespecífica en la comunidad de cinípidos podrían ser resultado de la limitación de recursos, principalmente cuando múltiples agallas se desarrollan en un mismo órgano al mismo tiempo (Whitham 1986; Atkinson et al. 2003). Sin embargo, especies simpátricas pueden coexistir durante largos períodos si ocupan diferentes nichos. Ecológicamente, si estas especies se diferencian en un eje del nicho, puede ser suficiente para reducir la competencia en condiciones estables (Webb et al. 2002) o mostrar las diferencias ecológicas entre especies que pueden distinguir sus nichos (Chesson 2000; Emerson y Gillespie 2008). Para las avispas inductoras de agallas la especie de encino, la posición de la agalla y la fenología de las agallas son rasgos que describen el nicho de los cinípidos (Bailey et al. 2009). Los resultados del análisis filogenético muestran que las especies de avispas que están filogenéticamente cercanas, presentan diferencias ecológicas en algunas características del nicho (posición de la agalla o fenología de la agalla), lo que sugiere que su coexistencia podría ser debido a la divergencia de nicho.

Nuestro estudio proporciona datos de 3 años colectados mediante muestreo sistemático y estandarizado, proporcionando una base para que en futuros estudios se

pueda explorar cambios a largo plazo en la comunidad avispas asociadas a los encinos. Podemos concluir que la fragmentación del bosque afecta la diversidad de avispas, el vigor de la planta, y la disponibilidad de recursos para las avispas inductoras de agallas. Una mayor riqueza de especies, abundancia y cobertura del dosel está asociada a fragmentos pequeños y en encinos aislados de la Cuenca del lago de Cuitzeo. Del mismo modo, encontramos en los bordes de los fragmentos de bosque que la riqueza, la abundancia de agallas y el vigor de las plantas se incremento en estos sitios. Los encinos aislados pueden ser considerados como recursos clave para el mantenimiento de la comunidad de agallas en bosques templados de México. Por último, la coexistencia de diversas especies de avispas inductoras de agallas asociados a *Quercus castanea*, permite evaluar hipótesis evolutivas, como el origen de esta asociación. Nuestro estudio sugiere que la convergencia de especies avispas asociadas a *Q. castanea*, es resultado de eventos evolutivos no relacionados, y para futuros estudios será importante analizar las similitudes y diferencias de especies filogenéticamente cercanas que ocupan el mismo nicho y los mecanismos que permiten su coexistencia.

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