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TAXONOMÍA, DESCRIPCIÓN Y DATOS BIOLÓGICOS DE UNA NUEVA ESPECIE DE *OPHELIMUS* HALIDAY (1844) (HYMENOPTERA: EULOPHIDAE)

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DEDICATORIA

A mi amado esposo Miguel.

A mis amados hijos, Micky y Dany.

Y en memoria de mi querida amiga Patricia Fierro y mi amigo Dr. John La Salle

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RESÚMEN

Una especie del género *Ophelimus* fue detectada el año 2003 en la región de Valparaíso-Chile, induciendo agallas sobre *E. globulus*. Entre el 2017 a 2018 se realizó el presente estudio que tuvo como objetivo, validar y describir esta nueva especie, estudiar su distribución-población sobre *E. globulus* ubicados entre Maule a La Araucanía y explorar la presencia del parasitoide *Closterocerus chamaeleon* en esas mismas regiones. La descripción y validación se realizó en forma morfológica y molecular (Genbank), respectivamente, sobre adultos emergidos desde ramillas de *E. globulus* afectadas. La distribución y población de la plaga y *C. chamaeleon*, fueron estimadas por colectas realizadas en 165 predios ubicados entre Maule a La Araucanía. Se verificó que se trata de una nueva especie del género *Ophelimus*, se la denominó *Ophelimus migdanorum* Molina-Mercader, que se distingue de sus congéneres, principalmente por presentar 1, 2 o 3 setas en la vena submarginal (VSM), 2 anillos en las antenas y 3 segmentos foniculares en el flagelo. Se determinó que el número de setas en la VSM incrementa con el tamaño de los individuos, estableciéndose como hipótesis que se trata de un posible caso de polifenismo. Se constató la presencia de *O. migdanorum* y *C. chamaeleon*, emergiendo en conjunto en todas las regiones evaluadas en un 92,1% y 46,7% de los predios muestraedos, respectivamente. Se determinó por primera vez, *C. chamaeleon* en las zonas productivas de *E. globulus* del país y se sugiere por primera vez en el mundo, su rol como posible biocontrolador de *O. migdanorum*.

ABSTRACT

A species of the genus *Ophelimus* (Hymenoptera: Eulophidae) was detected in 2003 in the Valparaiso Region of Chile, inducing galls on *E. globulus*. The present work was carried out between 2017 and 2018 and it was aimed to validate and describe this new species, study its population distribution on *E. globulus* located between Maule and Araucanía Regions and explore the presence of the parasitoid *Closterocerus chamaeleon* in the same regions. Both description and validation were performed through morphological and molecular techniques (Genbank) on adults that emerged from affected *E. globulus* twigs. Distribution and population of the pest and *C. chameleon* were estimated by collections carried out in 165 forest farms located between Maule and Araucanía. It was verified that it a new species of the genus *Ophelimus*, which was named *Opehlimus migdanorum* Molina-Mercader. The species are distinguished from its congeners mainly by presenting 2 or 3 setae in the submarginal vein (SMV), 2 rings in the antennae and 3 funicles fragments in the flagellum. It was possible to determine that the number of setae in the SMV is increased with the size of the individuals, being established as hypothesis that this is a possible case of polyphenism. The presence of *O. migdanorum* and *C. Chamaéleon* was observed emerging together in all regions evaluated, in 92.1% and 46.7% of the sampled farms, respectively. The presence of *C. chameleon* was determined for the first time in all productive areas of *E. globulus* in the country and the role of possible biocontroller of *O. migdanorum* is suggested for the first time in the world.

CAPITULO I. INTRODUCCIÓN GENERAL.

El género *Eucalyptus* (Myrtaceae) es endémico de Australia y de la Región Indomalaya (Withers, 2001), aunque sus especies han sido plantadas en diversas partes de todos los continentes, principalmente en los trópicos y el hemisferio sur; debido a su interés comercial, tanto ornamental como industrial, alcanzando una superficie estimada de 20 millones de hectáreas en el mundo (Hodkinson, 1999, Iglesias Trabado and Wilstermann, 2008; Wingfield et al., 2013; Pujade-Villar & Riba-Flinch, 2004). En Chile, la superficie cultivada con *Eucalyptus* asciende a 860.317 ha, de las cuales 592.136 ha, equivalentes a 67%, corresponden a *E. globulus* Labill, lo que la sitúa como la segunda especie cultivada más relevante para la industria Forestal nacional, después del *Pinus radiata* D. Don (INFOR, 2018).

Los problemas fitosanitarios ocasionados por plagas y enfermedades han ido incrementando en cantidad e intensidad en las plantaciones de *Eucalyptus* ubicadas fuera de su distribución natural; tendencia que se espera se mantenga, dado el creciente movimiento de personas e intercambio comercial en el mundo, por lo que la amenaza de daños por plagas se considera como las limitaciones más importantes para la plantación de *Eucalyptus* a nivel mundial (Wingfield *et al*., 2013). Los insectos propios del lugar de origen de los *Eucalyptus* ejercen una importante y permanente presión en sus nuevos hábitats (Protasov *et al.*, 2007a), encontrándose entre estos los inductores de agallas del género *Ophelimus* (Haliday, 1844) (Eulophidae-Ophemilinae).

El género *Ophelimus,* está constituido por aproximadamente 50 especies de microhimenópteros asociadas con inducción de agallas sobre diferentes especies de *Eucalyptus* (Lasalle, 2005). En general, las especies de este género han sido poco estudiadas, se cuenta con escasa información para distinguirlas (SAG, 2006; La Salle, 2005) y a pesar que diversas especies han sido obtenidas a partir de la crianza de agallas encontradas en *Eucalyptus* (La Salle 2005), sólo dos de ellas cuentan con algún nivel de documentación, *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) (Protasov et al., 2007a) y *Ophelimus eucalypti* Gahan (Hymenoptera: Eulophidae) (Gahan et al., 1922; Withers et al., 2000; La Salle, 2005). En adición a lo anterior, se encuentra en proceso de identificación una nueva especie de *Ophelimus* (Hymenoptera: Eulophidae) detectada el año 2010 en la cuenca mediterránea del sur de Francia (Branco et al., 2016) y de la que a la fecha se cuenta con algunos antecedentes parciales de su biología, que se describen en Branco et al. (2016).

El ataque de estas micro-avispas fitófagas, en base a descripción realizada para *O. eucalypti*, se inicia cuando la hembra deposita los huevos entre las células de empalizada en el limbo foliar, y también en algunos casos bajo la epidermis de peciolos y ramillas (Pujade-Villar & Riba-Flinch, 2004; La salle, 2005), una vez eclosionados los huevos comienzan a aparecer células nutritivas especializadas en la hoja, la larva emergida inicia su alimentación en estos tejidos, manteniéndola por varios meses hasta pupar, acción que ocasiona la degeneración de tejidos nutritivos hasta entonces activos (La Salle, 2005), emergiendo finalmente los adultos desde las agallas. La abundante formación de agallas cerca del peciolo de hojas afectadas puede conducir a intensas defoliaciones y pérdidas de crecimiento y vigor en árboles susceptibles (La Salle, 2005).

Figura 1.1. A: árbol de *E. globulus* afectado en el tercio medio de su copa por *Ophelimus* sp; B: ramilla (tallo-hojas) afectadas por *Opheliumus* sp; C. Tallo afectado por *Ophelimus*; D.E: agallas de *Ophelimus* sp sobre hojas de *E. globulus*.

Después de varios ataques los brotes del nuevo crecimiento adquieren aspecto retorcido, se produce la disminución de la capacidad de crecimiento de las ramas por una pérdida de las hojas de las ramas terminales, la muerte de ramas al ser atacadas también hojas de mayor tamaño y finalmente la muerte del árbol (Bain, 1977, Pujade_Villar & Riba_Flinch, 2004) (Figura 1.2).

Respecto *de O. maskelli*, Protasov et al., (2007a) señalan que una alta ocurrencia de agallas conduce a la muerte de hojas poco tiempo después de iniciado el ataque.

Entre las especies hospederos de *O. maskelli* y *O. eucalypti*, se describe entre otros a: *E. globulus*, *E camaldulensis* Dehnhardt y *E. saligna* Sm. (CABI/EPPO, 2011; Protasov et al*.,* 2007a). Para *O. eucalypti* se señala además como hospederos a *E. botryoides* Sm. (CABI/EPPO, 2011) y en el caso de *O. maskelli* a *E. teriticornis* Smith, *E. grandis* Whill ex. Maiden y *E. gunii,* Hook F., además de otras nueve especies de importancia económica (Protasov *et al*., 2007a*).* Para *Ophelimus* sp., detectado en Francia se indica como hospederos a: *E. cinerea* F Muell ex Benth*, E. cypellocarpa* L.A.S. Johnson*, E. globulus, E. gunni, E. párvula* L.A.S. Johnson & K.D. Hill*, E. bicostata* Maiden, Blakely & Simmons *y E. nitens* H. Deane & Maiden (Borowiec et al. 2012, datos de unp. Branco et al., 2016).

Las especies de *Ophelimus*, representan una importante radiación desde Australia, desde donde son originarios a otras áreas; constituyéndose en importantes especies invasoras (La Salle et al., 2009; La Salle 2005; Austin et al.

2004). *Ophelimus maskelli,* ha sido reportado en Israel, Sud África, Nueva Zelanda, Vietnam, Indonesia (Branco et al., 2016)), Turkía (Doganlar & Mendel, 2007), Italia (Arzone & Alma, 2000), España (Sánchez, 2003), Francia (European and mediterranean plant protection organization, 2006), Portugal (Branco et al., 2009), Argentina (Aquino et al., 2014) y Estados Unidos de Norteamérica (Burks, et al., 2015) (Figura 1.2). *Ophelimus eucalypti* ha sido determinado en Nueva Zelanda (Withers et al., 2000), Irán, Marruecos, Kenia, Uganda (Arzone & Alma, 2000; Maina, 2003; Sánchez, 2003; Pujade_Villar & Riba_Flinch, 2004) y Grecia (Kavaliieratos, 2006) (Figura 1.2).

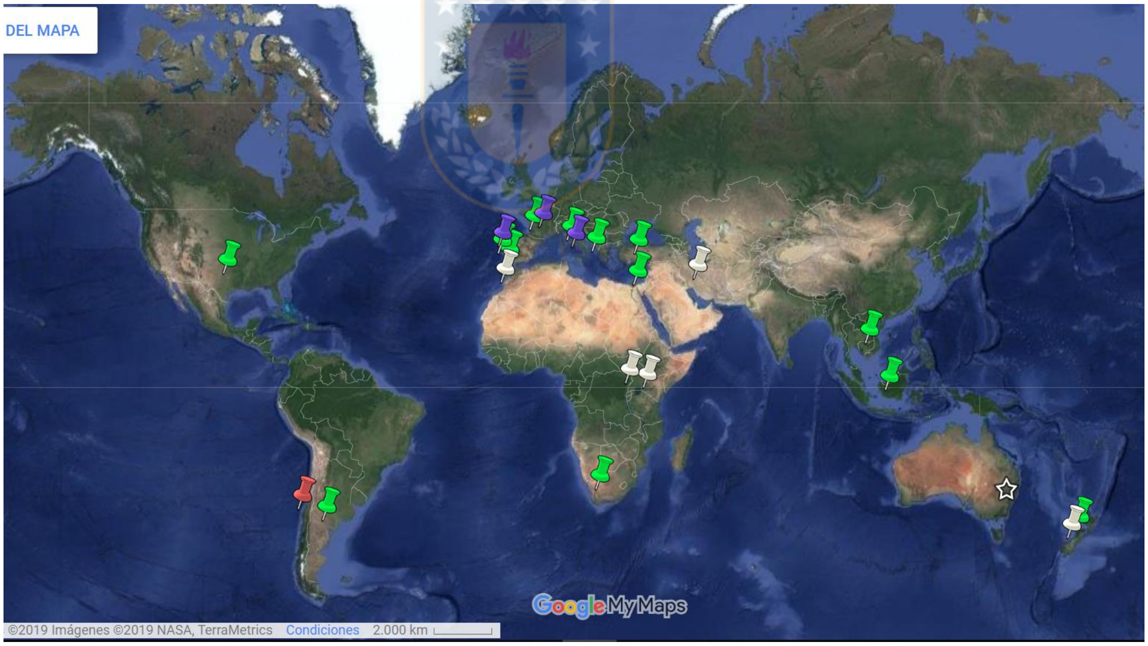

Figura 1.2. distribución de *Ophelimus* en el mundo, modificado de https://www.cabi.org/isc/datasheet/108961. Re confeccionado en Googe Maps, Estrella señala lugar de origen. Chinche verde Detección de *Ophelimus maskelli;* blanco: *O. eucalypti*; morado: *Ophelimus* spp, (franceses).

La nueva especie de *Ophelimus* detectada en Francia, fue reportada también en Italia y Portugal (Branco et al., 2016).

Defoliaciones de significancia económica atribuibles a *Ophelimus* han sido reportadas en diversos países. En Israel, Mendel *et al*., (2007) reportan altas irrupciones poblacionales de *O. maskelli* causando defoliaciones casi totales en árboles adultos de *Eucalyptus*, con densidades suficientemente altas como para interrumpir actividades humanas cotidianas, incluso la cosecha de otros cultivos (Burks et al., 2015). En tanto que *Ophelimus eucalypti* ha sido descrito ocasionando graves daños en, Nueva Zelanda (Withers et al., 2000) sobre *E. globulus,* imposibilitando su cultivo a nivel comercial (Velentine, 1963; Willson, 1963), también, en Irán, Marruecos, Kenia y Uganda (Maina, 2003; Sánchez, 2003) tanto en *E. camaldulensis* como en *E. globulus* (Pujade & Rama, 2004). Además, en Australia, La Salle, (2005) reporta, deformaciones de tallo en plantaciones jóvenes de *E. globulus*, con diámetro de tallo de 5–8 cm, en las que se encontraron dos especies de *Ophelimus.* Adicionalmente, se ha observado en Israel daño sobre la salud de las personas como consecuencia de la emergencia de masas de avispas adultas de *O. maskelli* (Protasov et al., 2007a).

1.1.- *Ophelimus*, antecedentes de morfología, ciclo de vida y agallas.

Las especies del género *Ophelimus*, corresponden a pequeños himenópteros apócritos y de color oscuro con reflejos verde metálicos. Los huevos son ovoides, blancos y semitransparentes, las larvas son pequeñas, blancas y ápodas

(Pujade-Villar & Riba_Flinch, 2004). *Ophelimus maskelli* es una pequeña avispa, uniparental (0.83-1.07 mm), el lóbulo medio del mesosescudo y escutelo con sólo dos pares de setas; antena con cuatro anillos y sólo un único segmento funicular; y el largo combinado del anillo y el funículo menor que el largo de la clava (Protasov et at., 2007 a). *Ophelimus eucalypti*, es una especie biparental, con claro dimorfismo sexual (Raman y Withers 2003), el tamaño de la hembra es de 2 a 2,5 mm de longitud (Withers et al., 2000), las antenas son cortas, articuladas con 11 artejos, funiculo más ancho en el vértice de la primera articulación y disminuyendo gradualmente al ápice; tibias traseras con un espolón (Gahan, 1922) se observa un número mayor o igual a 4 setas[1].

Figura 1.3. *Ophelimus maskelli*, vista lateral de espécimen colectado en Lazio, Italy (UCRCENT161366), (reproducida de Burks et al., 2015).

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[1] John Lasalle, comunicación escrita vía correo electrónico diciembre 2011

Las especies del género *Ophelimus* son holometábolos y su desarrollo desde huevo a adulto ocurre dentro de los tejidos vegetales afectados. El número de generaciones de *Ophelimus eucalypti* en Nueva Zelandia, corresponde a dos, mediando entre la ovipostura y la emergencia de adultos un período de 6 meses en cada generación (Whiters et al., 2000). Esta emergencia de adultos de *O. eucalypti* ocurre, uno en primavera entre agosto a septiembre y otro en verano desde enero a febrero (Whiters et al., 2000), al respecto Clark (1938), indica que estos períodos ocurrirían entre diciembre-enero y otro en junio-Julio en el mismo país (Pujade-Villar & Riba-Flinch, 2004). *O. maskelli* es un insecto partenogenético, con desarrollo multivoltino, en Israel presenta tres generaciones anuales (Aquino et al., 2014); iniciándose la generación de verano en julio y presentando una duración de tres meses (Branco et al., 2016). La especie de *Ophelimus* detectada en Francia, presenta una generación al año, ocurriendo la emergencia de adultos en Portugal entre abril a mayo (Branco et al., 2016).

Ophelimus maskelli y *O. eucalypti*, presentan patrones distinguibles en cuanto a la forma y distribución de agallas sobre hojas. *O. maskelli* ataca principalmente la lámina de la hoja, induciendo formación de numerosas agallas redondas, en forma de ampollas, visibles tanto en el as como en el envés de la hoja (Branco et. al., 2009; Protasov et al. 2007a); de color verde, amarillo verdoso, rosado o púrpura oscuro dependiendo del nivel de exposición a la luz solar directa (Protasov et al. 2007a) (Figura 1.4). Las agallas de *O. eucalypti* son lenticulares, discretas y pequeñas y sobrepasan ambas caras de la hoja se ubican en el peciolo de las hojas, limbo foliar y nervadura, (Pujade_Villar & Riba_Flinch, 2004, Bain, 1977); sin embargo, Sánchez (2003) señala para España, que las agallas de los peciolos o de la nerviación principal correspondería a otra especie agallícola (Sánchez, 2003). Withers (2003) indica que en la sección Transversaria las larvas que dan lugar a hembras inducen agallas circulares y protuberantes en las hojas de eucaliptos, mientras que las larvas que dan lugar a machos inducen "pit galls" en los mismos órganos vegetales (Pujada_Villar & Riba_Flinch, 2004). Las agallas de *Ophelimus* sp dectado en Francia son similares a las de *O. maskelli*, pero difieren en que son más pequeñas que las inducidas por *O. maskelli*, tienen un color café grisáceo, aspecto rugoso y se ubican sólo en el as de la hoja (Branco et al., 2016).

Figura 1.4. Agallas de *Ophelimus maskelli* sobre hojas de *Eucalyptus* sp desde San Diego, CA. (Reproducida de Burks et al., 2015).

1.2.- *Ophelimus*, antecedentes de taxonomía y biología.

El género *Ophelimus* pertenece a la superfamilia de avispas *Chalcidoidea*, la cual incluye 22.000 especies conocidas (de entre 60.000 a 500.000 que se estima deberían existir); agrupadas en 2.000 géneros y 19 familias (La Salle 2005) difíciles de clasificar, dado que se trata de especímenes muy pequeños (usualmente menor a 3 mm), y que cuentan con una alta variabilidad intraespecífica (Gibson et al, 1997). En general, son unas avispitas pequeñas de color oscuro, en algunos casos con brillos metálicos azules o verdes y con una compleja textura de la superficie del cuerpo; también se las reconoce por la reducción de las venas de las alas (Gibson et al, 1997).

Chalcidoidea, está conformada principalmente por especies parasitoides y en menor nivel fitófagas formadoras de agallas, la larva vive dentro del tejido vegetal y se alimenta de semillas, tallos y hojas (La Salle, 2005). Las especies parasíticas afectan a un amplio rango de hospederos entre los que se cuentan algunos insectos agallícolas (La Salle, 2005), pertenecientes a su misma superfamilia. La inducción de agallas la producen principalmente 6 familias: Agaonidae, Eulophidae, Eurytomidae, Pteromalidae, Tanaostigmatidae, Torymidae (La Salle 2005).

Eulophidae es una de las familias más grandes, más diversas y económicamente importantes de los Chalcidoidea, con más de 4.400 especies (Noyes, 2003; Gauthier et al., 2000; Burks et al., 2011; Clausen, 1978, in Burks et al., 2011), constituidas por insectos pequeños que abundan en todas las regiones templadas tropicales y que contemplan una amplia gama de biologías y huéspedes, respecto de lo cual existen sólo revisiones limitadas, (Gauthier et al., 2000; Clausen, 1940; BoucÏek & Askew, 1968; LaSalle & Schauff, 1995; Efremova, 1997; Noyes, 1998). Aunque la mayoría de las especies que componen esta familia son parasitoides, tambien considera algunas fitófagas y otras depredadoras (Gauthier et al., 2000). Las especies parasitoides pueden atacar huevos, larvas, pupas o adultos y pueden ser parasitoides o ectoparasitoides; Idiobiontes o koinobiontes; solitarios o gregarios; parasitoides primarios, hiperparasitoides o hiperparasitoides facultivativos; o especialistas o generalistas (Gauthier et al., 2000). Los eulófidos depredadores muestran una forma especializada de parasitismo donde la larva de la avispa consume muchas presas dentro de un espacio cerrado (como una agalla o un saco de huevos) (Gauthier et al., 2000), como huevos de araña en sacos (LaSalle, 1990a). Las especies fitófagas muestran también una variedad de estilos de vida, y pueden alimentarse al interior de semillas (BoucÏek, 1988; LaSalle, 1994; Gauthier, 2000)), ser inquilinas dentro de las agallas (Sheng & Zhao, 1995; Gauthier, 2000) o bien, formadores de agallas propiamente tal (Somer®eld, 1976; Hawkins & Goeden, 1982; Headrick et al., 1995) en una variedad de plantas, incluido *Eucalyptus* (Boucˇek, 1988; La Salle, 2005, in Burks et al., 2011).

La diversa familia Eulophidae, incluye 5 subfamilias; *Eulophinae*, *Euderinae*, *Entedoninae* y *Tetrastichinae* (Gauthier et al., 2000) y *Opheliminae* (Burks et al., 2011). Se los distingue de otros Chalcidoidea porque las patas tienen sólo 4 segmentos o tarsos y un pequeño espolón protibial en vez de uno largo y curvo. Las antenas tienen 2 a 4 segmentos intermedios (en casos excepcionales 5), entre la base y el final ensanchado, llamados funículo (Gibson et al, 1997).

Sin embargo, se debe hacer notar que las características morfológicas definitorias de Eulophidae se comparten con otros calcidoides y en general, corresponden a reducciones de los estados calcidoides habituales, por lo que se sospecha que son potencialmente convergentes (Burks et al, 2011; Gauthier et al., 2000; La Salle et al., 1997), de ahí que hoy se esté usando como herramienta taxonómica la combinación de morfología y características moleculares de las especies. Los autores, Gauthier et al., (2000) y Burks et al., (2011), han trabajado en esta línea, generando importantes cambios a través de la incorporación aspectos morfológicos y moleculares en el análisis de la clasificación de los Eulophidae.

La subfamilia *Opheliminae*, deriva de la ex tribu *Ophelimini*, incluida previamente en *Eulophinae* (Bou´cek, 1988), desde donde fue removida por Gauthier et al., (2000) y ubicada como Incertae Sedis en *Eulophidae*; siendo posteriormente elevada a la categoría de subfamilia por Burks et al., (2011).

Ophemilinae, contiene dos géneros, *Ophelimus* (probablemente el con mayor cantidad de especies inductoras de agallas) y *Australsecodes* (Burks et al., 2011; Gauthier et al., 2000; La Salle 2005) y se caracteriza morfológicamente por presentar, cara con surco transverso ausente, ala anterior con vena marginal débilmente engrosada, mesonoto con notaulos profundos en todo el largo, escutelo con estrías sublaterals ausentes (http://ponent.atspace.org/fauna/ins/fam/eulophidae/ophelimus_oph.htm).

Las especies del género *Ophelimus* se caracterizan morfológicamente por presentar, antena femenina claviforme, con 0-4 anillos funiculares, nudillos anulares presentes o no. Ala anterior con vena marginal engrosada, vena postmarginal presente. Mesonoto con notaulos profundos en todo el largo, estría medial ausente. Escutelo sin estrías sublaterales. (http://ponent.atspace.org/fauna/ins/fam/eulophidae/ophelimus_oph.htm).

La sistemática de este inusual género australiano, *Ophelimus*, (La Salle, 2009) es muy poco conocida; la única clave existente para distinguir entre las más de 50 especies reportadas se encuentra en un manuscrito inconcluso elaborado por Girault (Protasov et al., 2007a). Entre las especies de *Ophelimus* inductores de agallas, sólo dos se encuentran con un nivel de documentación respecto de su biología y morfología, principalmente *Ophelimus maskelli* (Ashmead) (Protasov et al., 2007a) y en menor nivel *Ophelimus eucalypti* Gahan (Somerfield 1976, Bain 1977, McLaren 1989, Withers et al 2000, Raman and Withers 2003, in Lasalle, 2005). A estos se le debe agregar la nueva especie de *Ophelimus* detectada en Francia, en proceso de identificación y generación de antecedentes (Branco et al., 2016).

La característica morfológica fundamental que ha permitido diferenciar las especies del género *Ophelimus*, es principalmente la presencia de setas en la vena submarginal del ala, pues la diferencia en tamaño y color son casi imperceptibles (La Salle 2005), esta característica no fue considerada en la clave de Girault y es desconocida en las otras especies de *Ophelimus*, pero claramente permite distinguir entre *O. maskelli y O. eucalypti* (Ptrotasov et al., 2007a). La característica más distintiva de *O. maskelli* es la presencia de sólo una única seta en la vena submarginal, (Protasov et al., 2007a), en tanto que en *O. eucalypti* se observa un número mayor o igual a 4 setas[2] en la vena submarginal. El Ophelimus detectacto en Francia, presenta 2, 3 o 4 setas en la vena submarginal (Branco et al., 2016).

1.3.- Parasitoide, *Closterocerus chamaeleon*.

Las especies del género *Closterocerus* muestran una amplia gama de biologías, la mayoría de las especies son parasitoides de huevos o larvas jóvenes, que a menudo son minadores de hojas u otros insectos escondidos dentro del tejido vegetal, y algunas especies son conocidas como parasitoides dentro de las agallas (Boucˇek, 1988; Hansson, 1990, 1994; Noyes, 2003 en Protasov et al., 2007 b).

El año 2004, fue detectado en Australia el himenóptero *Closterocerus chamaeleon* Girault (Eulophidae), parasitando *O. maskelli* (Mendel

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[2] John Lasalle, comunicación escrita vía correo electrónico diciembre 2011

et al., 2007, Borrajo, 2008). Esta microavispa parasitoide de avispas parece estar especializado en *Ophelimus maskelli*, o al menos en huéspedes en agallas pequeñas (Protasov et al., 2007b). No ataca ni a los mineros de hojas ni a sus parasitoides, ni a Leptocybe invasa Fisher y La Salle (Hymenoptera; Eulophidae), que induce agallas en las hojas de las mismas plantas hospedadoras (Mendel et al. al., informe inédito) (Protasov et al., 2007b).

Closterocerus chamaeleon, tiene un tamaño en base a dimensión de hembra de 0.6- 0.9 mm.; la cabeza, cuerpo y coxas son de color azul o verde metálico; mesopleurón dorsalmente naranja amarillento, escapo basalmente blanco, tercio apical fusco; el resto de los artejos antenales castaño a negro (Protasov et al., 2007b en Aquino, 2014). Patas blancas, fémur con in- fuscación media; tibia con una mancha basal en la superficie externa; alas hialinas, usualmente con una mancha posterior a la vena estigmal; antena con los artejos funiculares más anchos que largos, F1 más corto que F2, clava con el primer artejo ancho. Cabeza con reticulación uniforme al igual que el dorso del mesosoma. Gaster con el primer segmento liso, el resto con reticulación (Protasov et al., 2007b en Aquino, 2014).

Closterocerus chamaeleon es partenogenético, de ciclo de vida corto (tres semanas a 25° C) en comparación con su huésped, alta fecundidad, posee actividad invernal y una gran capacidad de dispersión, atributos que le confieren un alto potencial como biocontrolador (Borrajo et al*.,* 2008, Protasov et al., 2007b). El parasitoide fue introducido desde Australia, para ser usado en programas de control biológico clásico de *O. maskelli* en Israel (2005-2006) e Italia (2006), en ambos países con resultados exitosos de control (Protasov et al*.,* 2007b; Mendel et al., 2007; Caleca et al*.,* 2011; Laudonia et al., 2006).

Closterocerus chamaeleon es ampliamente descrito como ectoparasitoide (Borrajo et al*.,* 2008), condición que podría no ser aplicable a todos sus estadios de desarrollo, ya que ha sido determinado en estado de larva madura en tejido pupal de *O. maskelli* (Protasov et al*.,* 2007b). Se desconoce si el comportamiento parasitoide de *C. chamaeleon* corresponde a un koinobionte o idiobionte, estimándose que, por su amplio rango de tamaño corporal, pero estrecha variación en tiempo de desarrollo correspondería más bien al tipo idiobionte, lo que sumado a su condición de ectoparasitoide, podría reflejar un estrecho grupo de hospederos para esta especie (Protasov et al., 2007b).

1.4.- Detección *Ophelimus* sp. en Chile

El año 2003, una especie del género *Ophelimus* fue detectado en Chile, en la región de Valparaíso, induciendo formación de agallas sobre *E. globulus* y *E. camaldulensis* (Servicio Agrícola y Ganadero de Chile, 2006), siendo más tarde reportado en la Provincia de Cauquenes, Región del Maule y en la región de Biobío (Molina-Mercader et al., 2019).

El año 2010, en un trabajo conjunto, entre los autores, el Dr. La Salle (CSIRO-Australia/en memoria), el Servicio Agrícola y Ganadero de Chile y la Universidad de Concepción, se determinó, preliminarmente en base a morfología, que la avispa agallícola detectada en el país, correspondía a una especie distinta a *O. maskelli* y *O. eucalypti* (La Salle 2010, comunicación personal).

En este contexto, y conocida la relevancia de *E. globulus* para la industria Forestal Nacional, se plantea el siguiente estudio, cuyo objetivo es describir morfológicamente la especie de *Ophelimus* detectada en Chile, validar su condición de nueva especie usando herramientas morfológicas y moleculares, estudiar su distribución-población sobre *E. globulus* ubicados entre Maule a La Araucanía y explorar la presencia del parasitoide *Closterocerus chamaeleon* en esas mismas regiones del país.

1.5.- HIPÓTESIS.

Los especímenes de *Ophelimus* colectados en Chile, corresponde a una nueva especie inductora de agallas y se encuentra presente entre las regiones de Valparaíso y Los Lagos sobre *Eucalyptus globulus* Labill.

1.6.- OBJETIVO GENERAL.

Definir el estatus sistemático de los especímenes del género *Ophelimus* colectados en Chile y su distribución sobre *Eucalyptus globulus* establecidos entre las Regiones de Maule a La Araucanía.

1.5.1.- Objetivos específicos

1. Contrastar morfológicamente los individuos de *Ophelimus* spp. detectada en Chile, con *O. maskelli*.

2. Categorizar filogenéticamente los individuos de *Ophelimus* colectados en Chile.
3. Determinar distribución de *Ophelimus migdanorum* sp. nov., en las zonas forestalmente más productivas de *Eucalyptus globulus* entre las regiones de Maule y La Araucanía.

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CAPÍTULO II, *Ophelimus migdanorum* Molina-Mercader sp. nov (Hymenoptera: Eulophidae): Testing Integrative Taxonomy and disentangling polyphenism cases in Chile.

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Capítulo en revision en

2.1.-ABSTRACT.

In 2003, a new gall-inducing wasp of the genus *Ophelimus* was detected in Valparaíso region (Chile), affecting tree plantations of *Eucalyptus globulus Labill* and *Eucalyptus camaldulensis.* Later, between 2009 and 2017, was detected in other localities of the country. In a preliminary work between the first author, the Dr. John La Salle of the Scientific and Industrial Research Organization (SCIRO)(in memory), the Servicio Agrícola y Ganadero (SAG) of Chile and the University of Concepción, it was found that the micro-wasp detected in Chile correspond to a new *Ophelimus* species to science. Thus, the aim of this study was to describe at a morphological and molecular level this new species. The description of the new species was made based on adult specimens, obtained from breeding carried out in the laboratory of *MI*Plagas Ltda., from twigs of *E. globulus* infested by the pest, which were collected between the regions of Valparaíso and Los Lagos. The morphological structures were described according to Protasov *et al* (2007a); Burks *et al*., (2011) and Gibson *et al* (1997) and the molecular characterization based on 648 bp specific fragment of the mitochondrial gene COI (COI 5 region), sequenced in Macrogen. It was found that the new species is close to *Ophelimus maskelli* Ashmead, differing with the new species by few morphological characters. Among these characters, the number of setae (1, 2 or 3 setae) in the submarginal vein showed a highly variable behavior among specimens of the new species. In addition, it showed a high correlation with the size of the adult, which could represent a new case of

polyphenic species. The new species, named *Ophelimus migdanorum* Molina-Mercader, is currently present between the Valparaíso Region and the Los Lagos Region in Chile.

Key words: New Species, Eulophidae, Wasp Galls, Chile, *Eucalyptus sp.*, Polyphenism.

2.2.-INTRODUCTION.

Species of the genus *Eucalyptus* L'Hér. (Myrtaceae) are native to Australia and the Indomalaya Realm [1] (Withers, 2001) and has been planted worldwide due to their commercial interest, both ornamental and industrial (Hodkinson, 1999; Pujade-Villar & Riba_Flinch, 2004) [2, 3]. The phytosanitary problems caused by pests and diseases have been increasing in the plantations of *Eucalyptus* spp. outside its natural distribution, threatening its sustainability (Wingfield, 2013) [4]. The insects of their place of origin represent permanent pressure in the new habitats (Wold, 2004; Protasov, 2007a) [5, 6]. The gall inducers of the *Eulophidae* family, which includes the micro wasp Hymenoptera of the genus *Ophelimus* represent an important radiation from Australia, constituting one of the most important invasive species of these forest crops (la Salle et al., 2009; La Salle, 2005; Austin et al., 2004) [7, 8, 9].

The *Eulophidae* family is one of the largest, most diverse and economically important of the super family Chalcidoidea (Burks et al., 2011) [10]. This family is mainly composed of parasitoid species, which also considers predators and phytophages, and includes the galls formers that affect several hosts, including *Eucalyptus* (La Salle, 2005; Burks et al., 2011; Gauthier et al, 2000; Boucek, 1988) [8, 10, 11, 12]. This family has more than 4,472 species of hymenopterans, grouped in 297 genera (La Salle, 2005; Noyes, 2018) [8, 13] and 5 subfamilies: Eulophinae, Entedoninae, Tetrastichinae (Gauthier, 2000) [11], Entiinae and Opheliminae (Burks, 2011) [10]. Four main features have been described to support the monophyly of Eulophidae; small, straight and simple protibial spur, 4 tarsal segments, marginal vein is usually long, antenna with a maximum of 10 segments with one to four or rarely five funicular segments (la sale et al., 1997; Gibson et al., 1999) [14, 15]. Of these, *Opheliminae* stands out because it contains the genera *Ophelimus* and *Australsecodes*, the first being probably the largest number of gall-inducing species (La Salle, 2005; Burks et al., 2011; Gauthier et al, 2000) [8, 10, 11].

The genus *Ophelimus Haliday* (1844) includes species of micro hymenoptera (Chalcidoidea: Eulophidae, Opheliminae) that develop galls in different species of *Eucalyptus*, (Pujade-Villar & Riba-Flinch, 2004; La Salle, 2005; Boucek, 1988) 3, 8, 12]. These are morphologically characterized for presenting, female, claviform antenna with 0-4 funicular rings, anterior wing with thickened marginal vein, marginal post vein present, mesonotum with deep notches throughout the length, absent medial striae, scutellum without sub-lateral striae (http://ponent.atspace.org/fauna/ins/fam/eulophidae/ophelimus_oph.htm, 2018) [16]. The systematics of the genus *Ophelimus* is very little known, more than 50 species have been partially described, however, the only existing key for such species is contained in an unfinished manuscript prepared by the American entomologist, specialized in the study of wasps of the super family Chalcidoidea Alexandre Arsene Girault (1884 - 1941) (Protasov et al 2007a) [6].

The origin of the genus *Ophelimus* is Australian (La sale et al., 2009) [7] and from there some of its species have been dispersed to different regions of the world. *Ophelimus maskelli,* has been reported in Italy (Arzone & Alma, 2000) [23], Spain Sánchez et al., 2003) [24], France (European and mediterranean Plan protection Organization, 2006) [25], Turkey (Dolangar and Mendel [26], Portugal (Branco et al., 2009) [27], Argentina (2013) (Aquino et al., 2014) [28], United States of America (2014)(Burks et al., 2015)(Burks et al., 2015) [22], Israel, South Africa, New Zealand, Vietnam, Indonesia (Branco et al., 2016) [29] and Greece (kavallieratos et al., 2006) [32]. *O. eucalypti* has been reported in New Zealand [30], Iran, Morocco, Kenya, Uganda; (Arzone & Alma, 2000; Sánchez, 2003; Maina, 2003) [23, 24, 31]

Among the hosts of *O. maskelli* and *O. eucalypti* has been described the tree species *E. globulus* Labill*, E. camaldulensis* Dehnhardt and *E. saligna* Sm., as well as other species of economic importance (Protasov et al., 2007a; CAB/EPPO, 2011) [6, 33]. Strong attacks and gall production lead to intense defoliation and therefore to a decrease in the growth, development and vigor of susceptible trees ((la sale et al., 2009) [7]. Thus, its presence has reported significant economic losses in several countries, among others, in Israel, where high population disruptions of *O. maskelli* were reported causing almost total defoliation in adult trees of *Eucalyptus* (Mendel et al., 2007) [34]. In New Zealand, defoliation caused by *O. eucalypti* on *E. globulus* is indicated, making it impossible to grow commercially (Withers et al., 2000; Willson et al, 1963) [30, 35]. In addition, La Salle (2005) accounts for stem deformations in young plantations of *E. globulus*, with stem diameter of 5-8 cm, in which two *Ophelimus* species were found.

Given that the defining morphological characters of Eulophidae are widely shared with other chalcidoids, being reductions from the usual chalcidoid character states, suspicion has been cast on their potentially convergent origin (Burks et al, 2011; Gauthier et al., 2000; La Salle et al., 1997) [10, 11, 14]. As a result, issues remain in Eulophid taxonomy, particularly in the delimitation of groups at intra-family levels. Morphological characters not necessarily represent evolutionary polarity, because of the effect of reversals and parallel evolution, little understanding exists about the stability of diagnostic characters in current taxonomic treatments of Eulophidae (Burks et al, 2011) [10]. Recent studies have attempted to improve this limitation, demonstrating the relevance of employing multifaceted approaches (morphological and molecular data) to generate significant improvement in classifications of this group (Burks et al, 2011; Gauthier et al., 2000) [10, 11]. Yet, pending status remains in several understudied genera and species of Eulophidae, including *Ophelimus* (Burks et al., 2011) [10].

A recurrent issue in the use of diagnostic morphological characters in insects is their lability given their susceptibility to a response driven by adaptive change. Among the possible mechanisms involved in this response, polyphenism is one of the most frequent, which is defined by the generation of multiple different phenotypes produced by the same genotype. For example, many insect species exhibit facultative wing growth, which reflects a physiological and evolutionary compromise between dispersal and reproduction, triggered by environmental conditions. Environmental conditions can alter the shape, function and behavior of organisms on short and long-time scales, and even for generations. Polyphenisms are an important reason for the success of insects, which allows them to divide the stages of the history of life (with larvae dedicated to feeding and growth, and adults dedicated to reproduction and dispersal), to adopt different phenotypes that better adapt them to predictable environmental changes (seasonal morphs), to cope with temporally heterogeneous environments (dispersal morphs), and to divide work within social groups (the castes of eusocial insects) (Lin et al., 2017; Simpson et al., 2015; Cridge et al., 2015; Srinivasan & Brisson, 2015) [17, 18, 19, 20].

In Chile, the culture of *Eucalyptus* occurs in the Mediterranean region were dealing with a growing species of pest. A species of the genus *Ophelimus* was detected for the first time in Chile in 2003, associated with galls on *E. globulus* and *E. camaldulensis* (SAG, 2006) [36] in the town of Los Andes, Valparaíso region and at the end of 2009 its presence was detected in two towns, located in the Biobío and Maule regions, respectively (Molina - Mercader, 2019 unpublished data). In a preliminary work carried out by Molina-Mercader (first autors of this paper) with the support of Dr. John La Salle of the Commonwealth Scientific and Industrial Research Organization (SCIRO) (in memory), University of Concepción, in coordination with Servicio Agrícola y Ganadero of Chile (SAG), it was determined that *Ophelimus* specimens registered in the country correspond to a species not yet described, given that the specimens found at that time had two setae in the submarginal vein, in addition to being larger and of a light metallic green color, when compared to the description of *O. maskelli* (La Salle 2010, personal communication). Subsequently, individuals with one, two and three setae were found in the submarginal vein, which led to believe that the first was *O. maskelli* and the other two other unknown species.

In this context, the present study was proposed with the objective of carrying out the description (morphological and molecular) of the specimens found in Chile and their diagnosis. What allowed to demonstrate that these specimens belong to a single taxon that is different from *O. maskelli,* discover and discuss the causes of the phenotypic variation that initially made its diagnosis difficult in Chile and the consequences in the handling of this pest that it behaves differently to *O. maskelli*.

2.3-MATERIALS AND METHODS

2.3.1.- Field Sampling

Between the months of July to September 2017, samples were collected from stands of *E. globulus* that presented evidence of *Ophelimus* attack, located between the Valparaíso region in the north, to the Los Lagos Region in the south part of Chile (Table 2.1). The sample consisted of 9 twigs per sampling points, of approximately 50 cm in length, which contained galls, petioles and main veins of the leaves forming galls. The samples were placed in polystyrene bags of 35 cm x 40 cm that contained absorbent paper, labeled, and sent to the laboratory of *MIP*lagas Ltda., located in San Pedro de La Paz city (Biobío region, Chile), where they were processed and analyzed. Each sampling point was georeferenced.

Table 2.1. Location of the plantations of *Eucalyptus globulus* where samples were collected to obtain *Ophelimus* sp. nov.

Location	**Region**	**Farm**	**Coordinates S**	**Coordinates O**
Casa Blanca	Valparaíso	Santa Rita	33°18’57,82”	71°28’15,68”
Litueche	O’Higgins	Alborada 2	34°04’33,8”	71°48’12,2”
Cauquenes	Maule	Unicaven	36°04’27,9”	72°00’31,0”
Chillán Viejo	Biobío	San Vicente	36°39’21,4”	72°23’27,2”
Nueva Imperial	La Araucanía	Huerto Semillero	38°43’18,8”	72°54’47,3”
Valdivia	Los Ríos	Pichoy	39°42’56,8”	73°09’50,8”
Fresia	Los Lagos	Loma Verde	41°15’36,0”	73°31’51,0”

2.3.2.- Laboratory Breeding.

The collected branches were disposed, individually, in breeding chambers; which consisted of transparent plastic boxes with lid, whose dimensions correspond to 20 x 30 x 40 cm, based on the camera 2 sheets of absorbent paper were installed. Each breeding chamber was sealed with plastic tape, labeled to maintain traceability and kept in the laboratory until adult emergence, at a temperature that oscillated between 20 ± 2° C, with a relative humidity of approximately 60% and a photoperiod of 16 hours of light and 8 hours of darkness. The breeding chambers were checked every day and the absorbent paper changed every other day to avoid contamination.

The specimens of *Ophelimus* emerged from the breeding chambers, were fixed, individually, in 70% ethanol or in isopropyl alcohol and placed in cryopreservation bottles with screw cap. The individuals maintained in 70% alcohol, were used for morphological description and assembly of the type material; while those fixed in isopropyl alcohol were used in molecular characterization.

Adicionally, in the laboratory 294 galls from sampled farm, 2 of each part: leaf blade, midrib, secondary rib, petiole, branch, twigs and stem, were randomly selected, which were kept in breeding until adult emergence in the same conditions described above. The galls were placed in breeding chambers according to their location in the plant tissue.

2.3.3.- Morphological Description.

The description of the new species was based on adult females and males emerged from the breeding chamber. Terminology of the morphological structures followed Protasov *et al.* (2007a), Burks *et al.* (2011), and Gibson *et al.*, (1997).

As a measurement and observation instrument a trinocular flat chromatic stereo mic magnifying glass BEL model Solaris-T-Led and a Microscope OPTIKAL B - 1000PH were used. For the photographs and measurements, the program OPTIKALS view version 3.9.0.602 was utilized.

The specimens used to make microphotographs were taken to the CMA Biobío Advanced Microscopy Center of the University of Concepción, where the Scanning Electron Microscope (SEM) Tescan Vega 3 SBU Easy Probe was used. The equipment was emptied in "high vacuum" mode, using a secondary electron detector. Since the samples were insects and contained a chitin exoskeleton, this facilitated the emission of electrons on the sample. The parameters used come with the processed images. As the samples were unmetallized, they were placed directly in a sample holder covered with a carbon sheet.

A comparative table was made between the morphological characters presented by the individuals obtained from the samples collected in the field and those described for *Ophelimus maskelli* by Burks *et al.*, (2011) (Table 2.3; Table S2.1).

2.3.4. Molecular Protocols and Sequence Editing.

Genomic DNA was extracted using extraction methods DNeasy Qiagen Plant Mini Kit. PCR amplification, and sequencing of the COI barcode region was performed following standard protocols (Hebert et al., 2004) [37]. PCR and sequencing used a single pair of primers:

LepF1 (ATTCAACCAATCATAAAGATATTGG) and

LepR1 (TAAACTTCTGGATGTCCAAAA AATCA) [34]

which recovers a 658 bp region near the 5´ ends of COI including the 648 bp barcode region for the animal kingdom (Nguyen et al, 2015) [39]. Sequence editing and alignment were automatically done using “map Sanger sequencing reads to a reference” using Unipro UGENE (Okonechnikov et al., 2012) [41] and manually corrected. DNA sequences have been submitted to GenBank (see Table 4 for accession numbers) and BoldSystem. DNA voucher specimens were deposited at the Museo de Zoología, Universidad de Concepción, Chile.

Additional DNA sequences from generous *Ophelimus* and outgroup members were downloaded from GenBank, (Benson et al., 2014) [6] and BoldSystem. This sampling was complemented with the inclusion of COI sequences belonging to the Entiineae subtribe: *Astichus, Bellerus, Beornia and Euderus* (Burks et al, 2011) [10]. *Hubbardiella* did not have COI sequences at the time of this sampling. Only sequences from species of well-known distribution and identification have been downloaded. Finally, a data matrix with 97 entries (Table 2.2) was built for subsequent analyzes.

2.3.5.- Identity of obtained COI sequences.

The identity of obtained COI sequences was determined using a phylogenetic approach. To achieve this, all sequences were aligned with MAFFT v1.3.7 [41] as implemented in Geneious R11 (www.geneious.com), to subsequently analyze the resulting dataset employing both Maximum Likelihood (ML) and Bayesian Inference (BI) criteria.

The ML analysis was conducted with the program iqtree v1.6.8 (Nguyen, 2013) [42], for which a TPM3u + F + I + G4 nucleotide substitution model was estimated with the built-in program ModelFinder (Kalyaanamoorthy et al., 2015) [43] under a Bayesian Information Criterion (BIC). ML analyses were conducted in iqtree using default settings and search parameters. Branch support values were calculated using a non-parametric bootstrap (BS) of 1000 pseudoreplicates. All branches with BS values over 70 % (BS > 70) were considered well supported.

BI analysis was conducted with Mr. Bayes v.3.2.6 (Ronquist et al., 2012) [44]. Given that the Bayesian approaches can integrate accounted uncertainty in phylogenetic trees and nucleotide substitution models simultaneously (Huelsenbeck, 2004) [45], a reversible jump MCMC search approach (rjMCMC) was employed with two independent runs of 100.000 iterations, each of 4 chains (3 cold and 1 hot) and using default priors. All resulting trees were summarized in a consensus tree, after discarding 20% of trees as burn-in, retaining all compatible clade. All clades with a posterior probability over 95% were considered as well supported (PP > 0.95). Levels of mixing and convergence on splits were scrutinized with Tracer v1.7 (Rambaut et al., 2018) [46] and the R package *rwty* (warren et al., 2017) [47].

For the estimation of species delimitation with COI sequences (single locus), two analyses were conducted. First, both ML and BI summary trees were used to delimit entities based on a multi rate Poisson Tree Process (mPTP, (Kapli et al., 2017) [48]). This method is a technical improvement compared to the Poisson Tree Process (PTP; (Zhang et al., 2013) [49]), which involves the modeling of the branching process on the number of accumulated expected substitutions between speciation events but assuming different rates of speciation events among lineages (Kapli et al., 2017) [48].

Alternatively, an analysis of rates of speciation and neutral coalescence thresholds via GMYC algorithm was employed for delimitation (Pons et al., 2006) [50]. As this approach requires an ultrametric tree, a Bayesian tree search and sampling were conducted with a subset of COI of *Ophelimus* sequences with BEAST 10.0.0 (Suchard et al., 2018) [51]. Following a molecular substitution model predefined with ModelFinder (GTR + G + I), a MCMC search was conducted on two runs with 10 million of iterations, discarding a burn-in of 10%. In this case, a strict molecular clock and a coalescent model of fixed population size was assumed for this analysis. These models were used under the assumption that a constant process of generation in populations is being produced among the targeted lineages, which are currently in a continuous process of speciation and differentiation. Mixing and convergence of splits were equally controlled using the same programs and protocols employed for BI phylogenetic analyses. All resulting trees were summarized in a Maximum Clade Credibility (MCC) tree on median clade heights, which was calculated with TreeAnotator v1.10.0 (available in http://beast.community/treeannotator).

For the estimation of species limits, a Bayesian-based approach of GMYC was implemented (bGMYC), which ponders and identifies possible effects of phylogenetic uncertainty in the placement of thresholds along branch lengths for species delimitation (Reids & Castens, 2013) [52]. This analysis was conducted over the last 100 MCMC ultrametric trees obtained from each BEAST run (50 trees per-run) and pondered over the previously obtained MCC tree. In this case, the analysis was run with 1 million of iterations and a discarded burn-in of 5000 iterations. Since the choice of molecular clock can also alter the inference of branch lengths among delimited groups, we also performed a bGMYC analysis with a relaxed log-normal clock for comparative purposes. In this case, both the inference of MCC trees and bGMYC were conducted following same parameters and setting than those with strict clock.

2.4.-RESULTS.

In this study, a total of 4,632 individuals of *Ophelimus* were obtained, which corresponds exclusively to the new *Ophelimus* species detected in Chile, of this total, 58.9% corresponded to females. The emergence of this individuals occurred from each of the parenting chambers that represented each of the 7 regions sampled.

2.4.1.-Morphology.

2.4.1.1.-New Ophelimus species Diagnosis

Ophelimus migdanorum Molina - Mercader sp. nov. (Figures 1a, 1b and 1c).

Diagnosis: Imeasures between 0.7 and 1.4 mm long (Figures 1a, 1b and 1c), from head to metasoma. Head and thorax of light metallic green color, brown abdomen, light brown antennae, dark brown and light chestnut femur, warm and light brown tars and dark brown color nail, hyaline wings, with submarginal, marginal and stigmal veins of light brown color (Figures 1a, 1b and 1c). In submarginal vein presents 1, 2 and 3 satae of uniform size according to the size of everyone (details on point 2.1.3 of this paper) (Figures 1a, 1b and 1c; Table 3).

The antenna presents the escape with cellular crosslinking, elongated on the longitudinal axis; smooth pedicel, 1.5x shorter than the scape; funiculus with 3 rings and two joints that increase in width towards the club, (Figures 2a). The club is 2 ± 0.5 times longer than the funiculus, is globose and has 3 parts, the last (third) is not easily seen, is curved very little and in the center has a long seta (terminal spine) (Figure 2b). Around the second and protruding part of the third layer, 4 to 6 longitudinal sencilles are observed (Figure 2b) (Table 2.4; Figure S2).

Male: No difference with respect to the female, only can be differentiated under stereoscopic magnifying glass, the most representative and visual characteristic under this instrument is the curvature present in the thorax and the position of the head, finding the head of the male in a straight line with the thorax (Verbal communication Dr. Andrés Angulo) (Figures 2c, 2d).

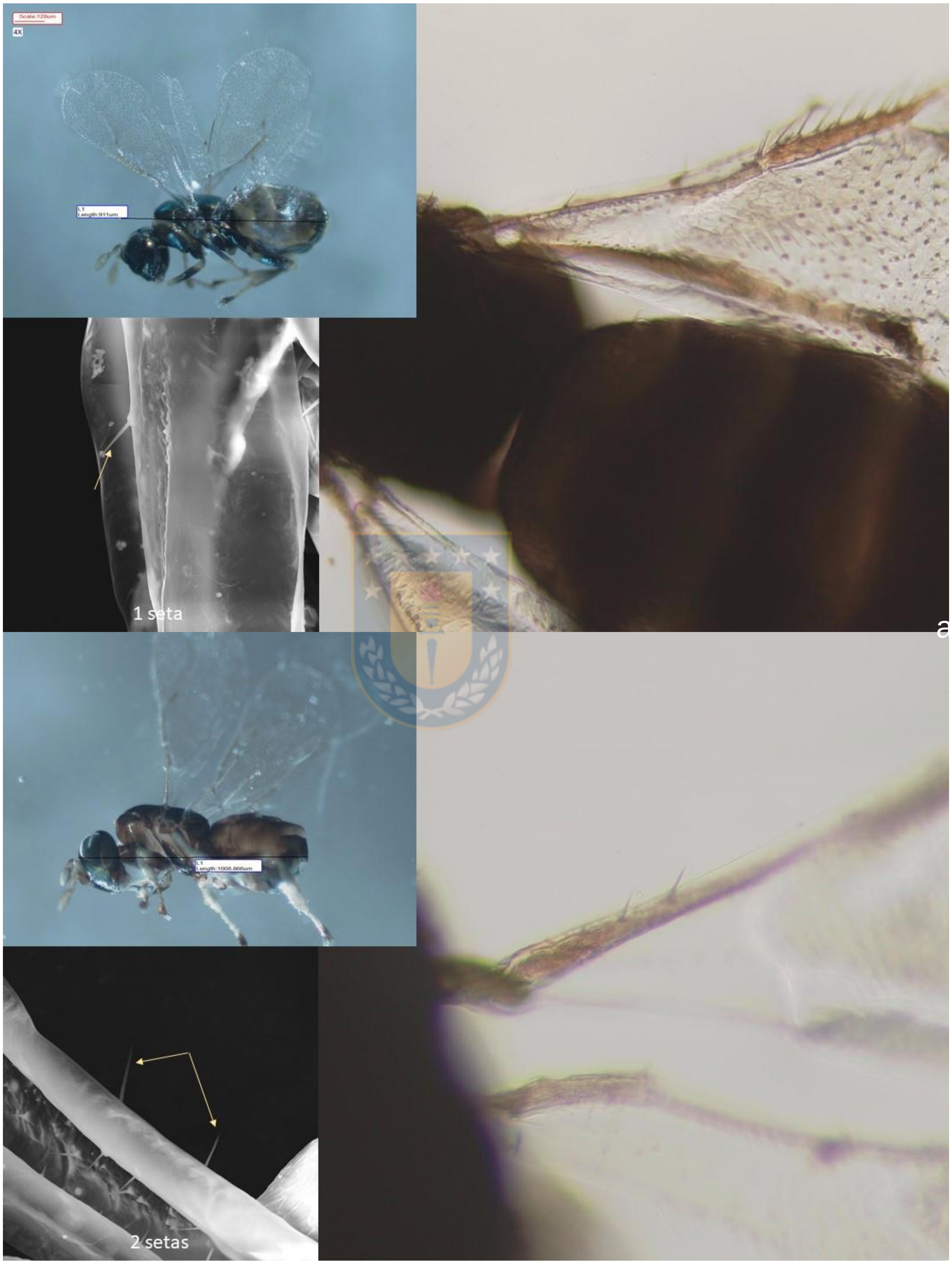
Distribution: Chile sampled regions Valparaiso to Los Lagos.

Hosts: *Eucalyptus globulus* corresponding to this study.

Etymology: the name designated to this species corresponds to the abbreviation of the first names of the children of the author Miguel and Daniel.

Provision of the Holotype, alotype and paratypes (Table S4).

(Other measures of this wasp in Table S2, S3).


Scale:120um
4X
L1
Length:911um
1 seta
a
L1
Length:1008.866um
2 setas

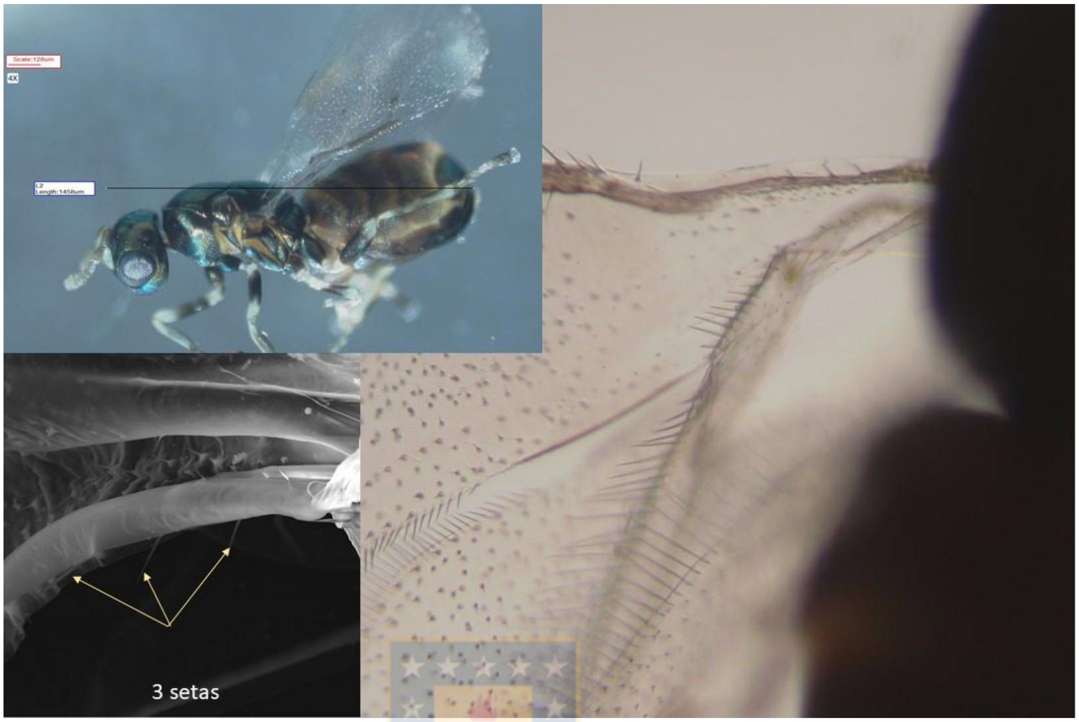


Figure 2.1. *Ophelimus migdanorum* nov sp., a) 1 setae on sub marginal vein, b) 2 setaes on sub marginal vein, c) 3 setaes on submarginal vein.

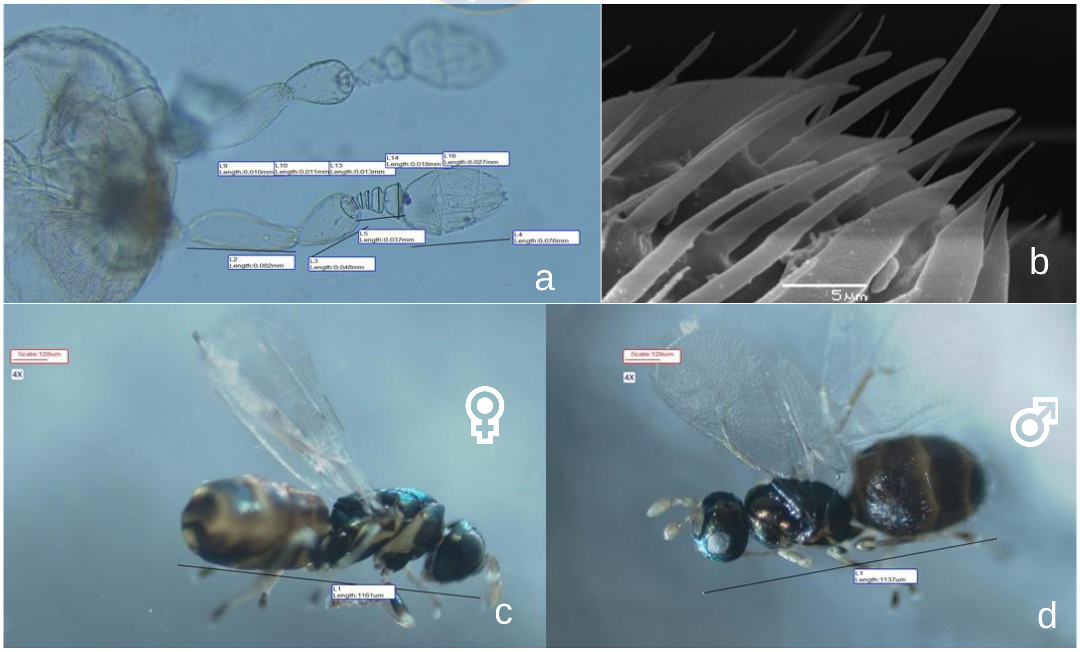


Figure 2.2. *Ophelimus migdanorum nov sp*., a) antenna, b) Top club, c) famale, d) male.

2.4.1.2.-Comparative diagnosis.

The specimens detected in Chile form a taxa (genus) morphologically like *O. maskelli*, according to Burks *et al. (*2011) and Protasov *et al*., (2007a), but are differentiated by the number of setae present in the submarginal vein (SVS) (Figure 2.1; Table 2.3, 2.4). Furthemore, based on description of *O. maskelli* by Protasov et al (2007a), chilean *Ophelimus* differs from this in the following characteristics: adult body color-tone, antenna color, number of rings in antennae and funicular segment in the flagellum, and break of wing venation (Tabla 2.4) and morphology and location of the galls (details in point 2.4.2). These characteristics are shared by specimens that have 1, 2 and 3 setae.

Table 2.3. Result of the morphological characterization *Ophelimus migdanorum* nov sp. Chile according to Burks and comparison with other members of *Ophelimini* and Entiinae.

	Species	**Character (*)**															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Ophelimini	*Ophelimus maskelli*	8	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0
	Ophelimus nov. sp. 1 SVS	8	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0
	Ophelimus nov. sp. 2 SVS	8	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0
	Ophelimus nov. sp. 3 SVS	8	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Entinae	*Astichus n. sp*	8	3	2	0	0	0	0	2	0	1	0	0	0	0	2	0
	A. mirissimus	8	3	2	0	0	0	0	2	0	1	0	0	0	0	3	0
	Bellerus sp.	8	3	2	0	0	0	0	0	0	1	0	0	0	0	1	0
	Euderus sp.	8	3	2	0	0	0	0	2	0	0	0	0	0	0	3	0

	Species	**Character (*)**														
		17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
Ophelimini	*Ophelimus maskelli*	2	0	0	0	0	0	0	0	1	4	0	1	0	1	2
	Ophelimus nov. sp. 1 SVS	2	0	0	0	0	0	0	0	1	4	0	1	0	1	2
	Ophelimus nov. sp. 2 SVS	2	0	0	0	0	0	0	0	1	4	0	2	0	1	2
	Ophelimus nov. sp. 3 SVS	2	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Entinae	*Astichus n. sp*	2	0	0	0	0	1	0	0	1	4	0	3	0	0	2
	A. mirissimus	2	0	0	0	0	1	0	0	1	4	0	3	0	0	2
	Bellerus sp.	2	0	0	0	0	0	0	0	1	4	0	3	0	0	2
	Euderus sp.	2	0	0	0	0	0	0	0	1	4	0	3	0	0	2

In table, carácter number 28, indicated N° setaes in the submarginal vein. (*) Name of the other characters in table S1

In a dendrogram made from the morphological data (Figure S1; Table 2.3), it can be observed that *Ophelimus* morphologically deferred less from the *Belerus* than from the *Euderus* or *Astichus* genera. Being between these two only the character 3 and 30 different.

Table 2.4. Comparison of *Ophelimus migdanorum* with *Ophelimus maskelli* based on Protasov *et al.* (2007a) and La Salle (2008).

Character	**Zoom**	***O. maskelli* (um)**	***O. migdanorum (um)***					
			1 seta		**2 setas**		**3 setas**	
			♀ (n)	♂ (n)	♀ (n)	♂ (n)	♀ (n)	♂ (n)
Long of the adult	4X	1.026 (*)	865 (±26,0)	923 (±27,9)	1.059 (±30,6)	1.130 (±33,1)	1.156 (±28,9)	1.221 (±46,7)
Width of the adult	4X	234 (*)	249 (±06,2)	250 (±06,2)	276 (±06,8)	285 (±07,6)	295 (±05,2)	319 (±08,0)
Long of the Wing	10X	900 (*)	768 (±19,2)	788 (±10,6)	875 (±17,0)	900 (±25,7)	934 (±26,6)	1.075 (±49,5)
Width of the wing	10X	306 (*)	347 (±11,4)	348 (±10,8)	297 (±10,5)	413 (±12,6)	422 (±19,4)	494 (±25,2)
Submarginal	10X	304 (*)	213 (±06,4)	226 (±04,4)	241 (±12,7)	245 (±09,0)	245 (±13,3)	300 (±14,7)
Marginal	10X	153 (*)	157 (±10,1)	176 (±07,5)	190 (±11,5)	207 (±12,7)	220 (±21,8)	255 (±21,2)
Postmarginal	10X	210 (*)	209 (±06,1)	221 (±05,4)	261 (±09,5)	274 (±11,4)	283 (±13,3)	315 (±16,6)
Stigma	10X	99 (*)	64 (±02,2)	64,6 (±01,7)	73 (±01,9)	74 (±02,1)	74 (±01,9)	90 (±04,1)
Uncus	10X	0,02 (*)	18 (±00,7)	19,6 (±00,7)	32 (±11,9)	22 (±00,9)	22 (±00,8)	24 (±01,6)
Width/Long of the adult		4,4x (*)	3,5 x	3,7 x	3,8 x	4,0 x	3,9 x	3,8 x
Width/Long of the wing		2,9x (*)	2,2 x	2,3 x	2,9 x	2,2 x	2,2 x	2,2 x
Submarginal/marginal		2,0x (*)	1,4 x	1,3 x	1,3 x	1,2 x	1,1 x	1,2 x
Marginal/postmarginal		0,7x (*)	0,7 x	0,8 x	0,7 x	0,8 x	0,8 x	0,8 x
Abdominal long	10X	512 (*)	409 (±14,8)	430 (±11,7)	512 (±15,2)	547 (±17,1)	568 (±21,2)	627 (±19,5)
Color	4X	Clear metal green	Dark metalic green					
Number of setaes in SV	10X	1	1 a 3					
Break in wing Venation	4X	Yes	No					
Antennae color	10X	Dark yellow	light brown					
N° midlobe sctellum	10X	2 setaes	2 setaes					
N° of rings in the funicle	10X	4	3					
N° artejos in funicle	10X	1	2					
Galls	4X	Leaf blade (Figs. 3a)	Leaf blade, midrib, secondary rib, petiole, twigs, flower cones (Figs. 3b and 3c)					

In table, (*) indicated measurements provided by Dr. Daniel Aquino, wich were obtained from measurements made on photography of *O. maskelli* in paper Protasov *et al*., (2007a); (n) indicated measurements made on 30 individuals. Values in parentheses indicate standard error.

2.4.1.3.- Relationship between *O. migdanorum* size and number of setae in submarginal vein.

In this study, was observed individuals of *O. migdanorum* with 1, 2 and 3 setae in the submarginal vein; determining a group of smaller individuals (females 0.712 - 1.105 mm and males 0.781 - 1.144 mm, long) who had a seta in the submarginal vein, another group larger (female 0,846 - 1,253 mm and males 0,945 - 1,346 mm long) that had two setae in the submarginal vein and finally another group of largest individuals (females 0,930 - 1,413 mm and males 1,118 - 1,841 mm long) with three setae in the submarginal vein (Table 2.5, S2, S3). Significant difference was determined, between the size of adults for 1, 2 and 3 setae respectively and in the size of the submarginal vein (Table 2.5).

Table 2.5. Length of the insects *Ophelimus migdanorum* nov sp, both of 1, 2 and 3 setae in the Submarginal veins.

O. migdanorum **(N° setae)**	**Gender**	**Long individuals**		**n**
3	male	1,331.7 (±46.7)	a	30
3	female	1,156.9 (±28.9)	b	30
2	male	1,130.9 (±33.1)	bc	30
2	female	1,059.9 (±30.6)	c	30
1	male	922.9 (±27.9)	d	30
1	female	865.5 (±26.0)	d	30
Media	**both**	**1,078.0** (±32.2)		**180**

Values in parentheses indicate standard error. The different letters indicate statistically significant differences between the mean from each for males and females (rows), with 3, 2 and 1 setae (p≤0.05).

2.4.2.-Galls of *Ophelimus migdanorum* nov sp., and comparation.

The galls of *Ophelimus migdanorum nov sp.* Chile, *O. maskelli* and *O. eucalypti* are completely different. In the case of *O. maskelli* they are located only on the sheet and they have a perfect circle shape (Figure 2.3a) and in the case of *O. eucalypti*, the galls are on the petiole and the blade, forming a flat gall in the beam of the leaf and a mound on the underside (Figure 2.3b) (La sale, 2005) [8]. In contrast, *Ophelimus migdanorum nov sp.* of Chile, forms galls on the blade, the central vein, the petiole, the twig and the stem of the trees (Table 4), which is amorphous and for the adjacent position in some cases elongated (Figure 2.3c, Table 2.6). From the 294 galls used in this study, the only ophelimus species that emerged, corresponded in its entirety to *Ophelimus migdanorum* nov sp.

Table 2.6. Emergence of adults of *Ophelimus migdanorum*, from 294 galls located in midrib, petiole, leaf blade and stem, wich were taken from different localities, randomly. Compared with *Ophelimus maskelli* according to Branco *et al*., (2009) and Protasov et al, (2007a)

	O. maskelli	***O. migdanorum***		
		1 seta	**2 setae**	**3 setae**
Midrib			√	√
Petiole		√	√	√
Leaf Blade	√	√	√	√
Stems			√	√

√: ticket indicated from the place from where the individuals emerged.

Figure 2.3.- Galls formed on *Eucalyptus* sp.: a) *Ophelimus maskelli* galls on *Eucalyptus camaldulensis* leaves (Photo Garden, gentleness Dra. Paula Borrajo, Huelva, Spain); b) *Ophelimus eucalypti* galls on *Eucalyptus saligna* leaves (Photo gentleness Dr. John La Salle, in memoriam); c) *Ophelimus nov* sp. Chile, on *Eucalyptus globulus*, leaves and branches.

2.4.3. Molecular analysis.

2.4.3.1. Sequence Identity COI

The analyzes performed with ML and BI reveal that *Ophelimus* COI sequences would not be recovered in a monophyletic group (Figures 2.4 and 2.5). In both cases, the sequences of *Ophelimus migdanorum* nov sp., Chile appear separated from the rest of the *Ophelimus* sequences, being significantly grouped with the sequences of Australian origin (Figures 2.4 and 2.5). The rest of the Ophelimus sequences and those registered as *O. maskelli* and *Ophelimus sp. nov.* France are grouped monophilically, although only with significant support in the analyzes conducted with BI. According to other previously published results (Burks et al, 2011) [10], the monophyly of the sequences belonging to the Eutiineae subtribe and the basal position of Astichus are confirmed (Figures 2.4 and 2.5). The analyzes carried out with both criteria were mostly concordant in topology and support, detecting significant differences only in the length of branches and in the support of certain individual groups (eg, Clade *Ophelimus* sp. nov. France France + *O. maskelli*).

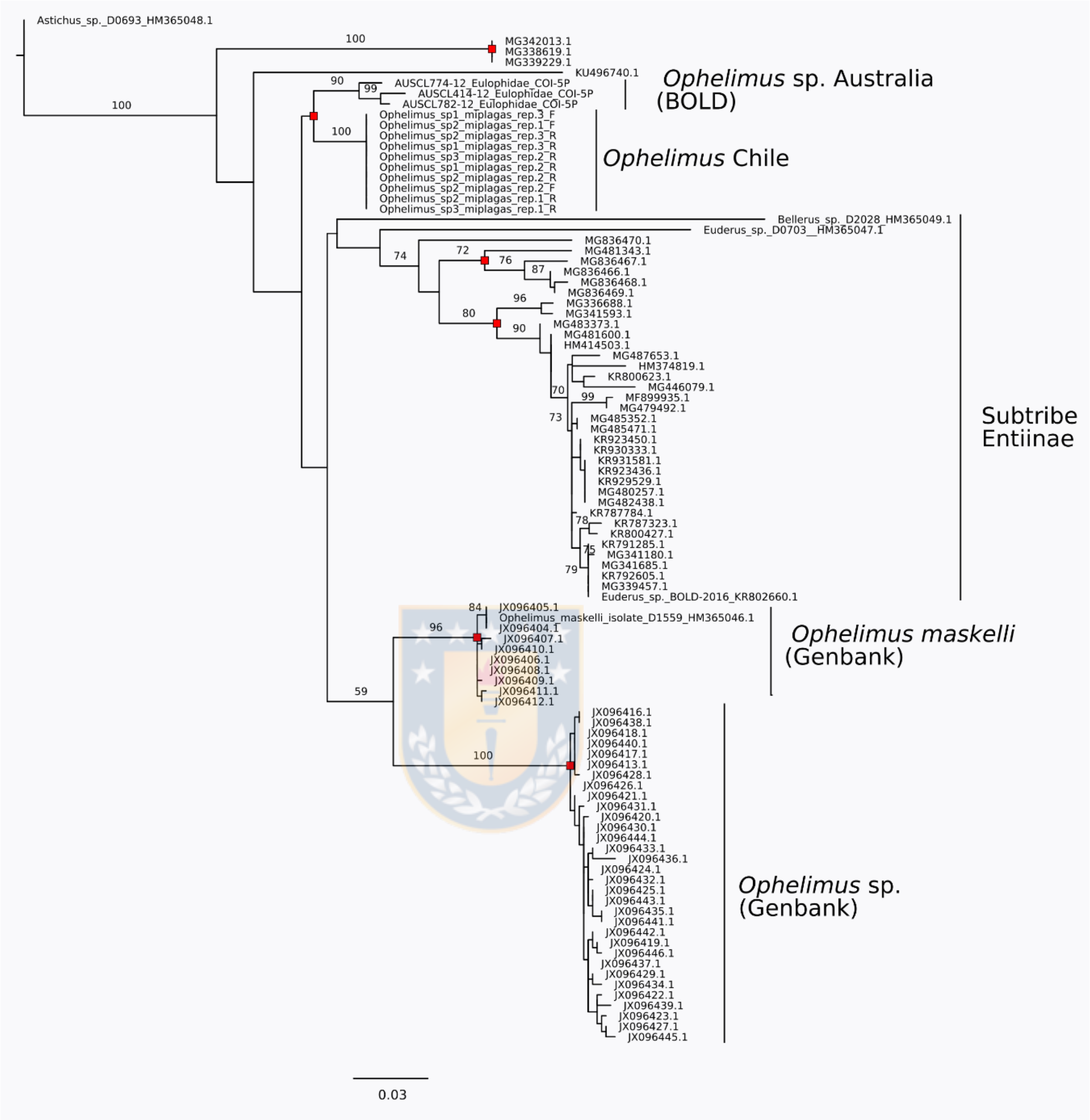


Figure 2.4. ML tree (log-likehood= -3745.5244) obtained with iqtree. Red squares represent proposed groups made by mPTP. Significant bootstrap values (BP > 70) are shown above branches.

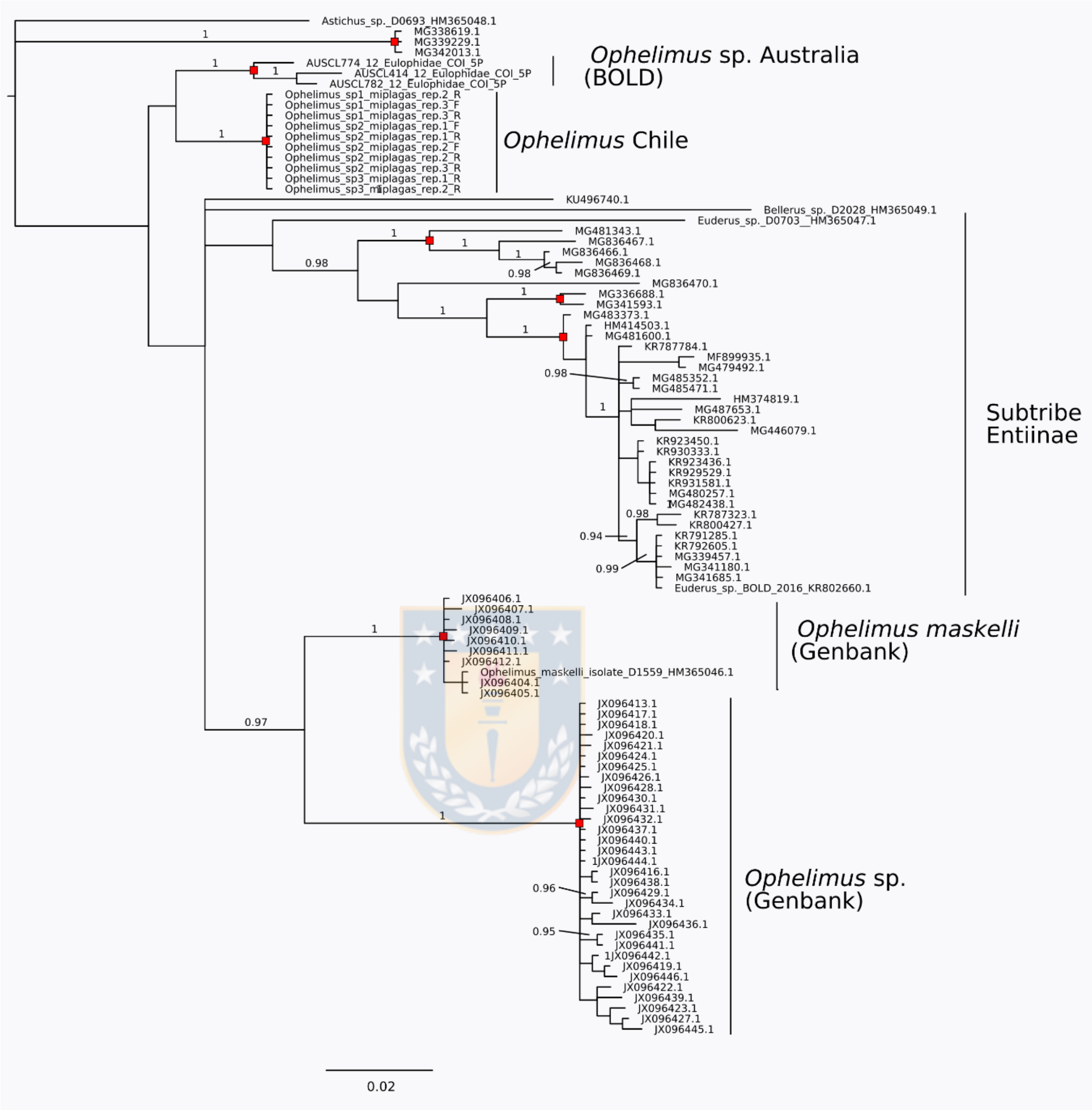


Figure 2.5. BI consensus tree obtained with MrBayes. Red squares represent proposed groups made by mPTP. Significant posterior probability values (pp > 0.95) are shown above branches.

2.4.3.2. Sequence Delimitation COI

The analysis carried out on the trees obtained with ML and BI criteria revealed very similar delimitation proposals. In the case of the Chilean sequences, these were detected forming a discriminable group together with the Australian sequences in the ML analysis (Figure 2.4). In contrast, analyzes with BI suggest both groups as different entities (Figure 2.5). In the case of the rest of the registered sequences, *O. maskelli* was detected as different from *Ophelimus* sp. nov. France (Figures 2.4 and 2.5). For the rest of the groups, delimitable associations can be differentiated for *Astichus* sp. and within Eutiineae, being in this last variable the number of groups depending on the analysis criteria used (ML or BI, Figures 2.4 and 2.5).

In the case of analysis of exclusive *Ophelimus* sequences, four differentiable groups were detected, which received different levels of grouping support and delimitation depending on the choice of molecular clock used. In the case of strict clock, a very significant grouping was detected between the Chilean and Australian sequences of *Ophelimus* (PP> 0.99, Figure 2.6), which also showed high levels of significance in delimitation in the bGMYC (PP> 0.99, Figure 2.6). In the same analysis, the recorded sequences of *O. maskelli* and *Ophelimus* nov. sp. France were not recovered as monophyletic and each group demonstrated a high level of individual significance in their discrimination (PP> 0.99, Figure 2.6). For the lognormal flexible clock, the grouping of Chilean and Australian sequences was maintained with high levels of significance (PP> 0.99, Figure 2.7), although only the Chilean sequences showed a distinguishable level of discrimination support (0.9> PP <0.95, Figure 2.7). In the case of the other sequences, a group that is not significantly supported is distinguished (PP <0.95, Figure 2.7) and it is individually possible to discriminate entities, but with a low level of intergroup delimitation (0.5> PP <0.9, Figure 2.7).

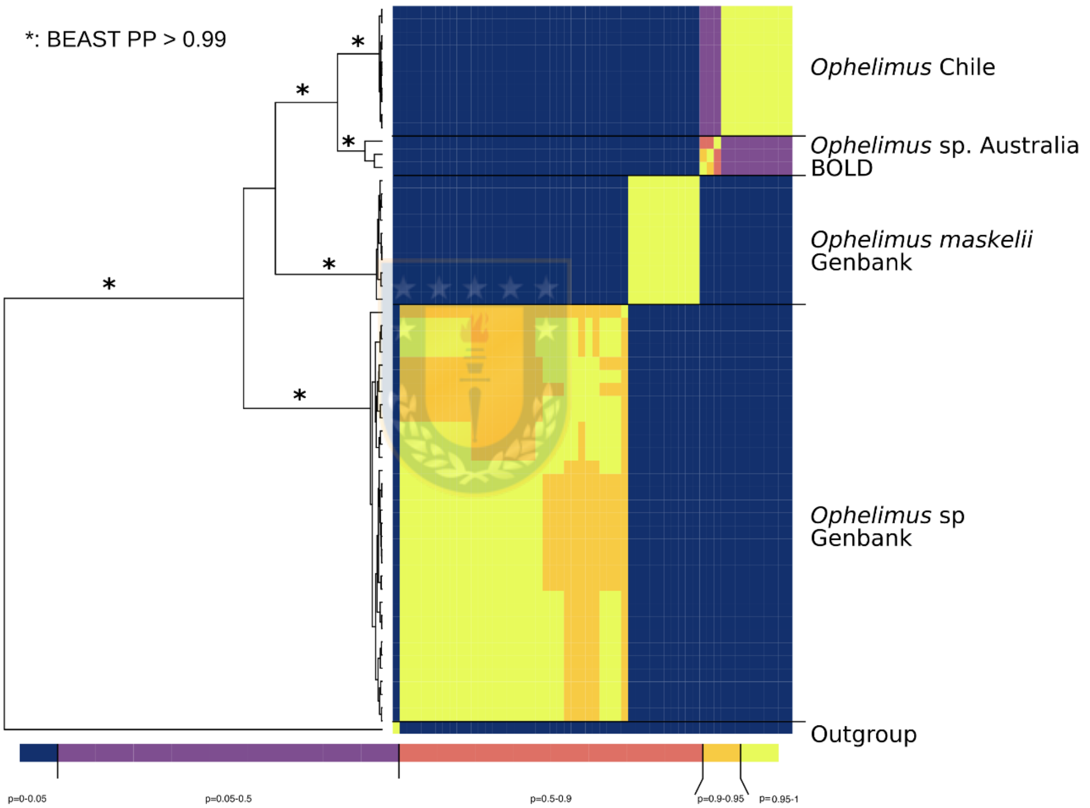


Figure 2.6. Ultrametric tree of inferred genealogical relationships with COI. For this tree a strict molecular clock was used in BEAST. Only those groupings with a very high significance are highlighted (PP > 0.99)

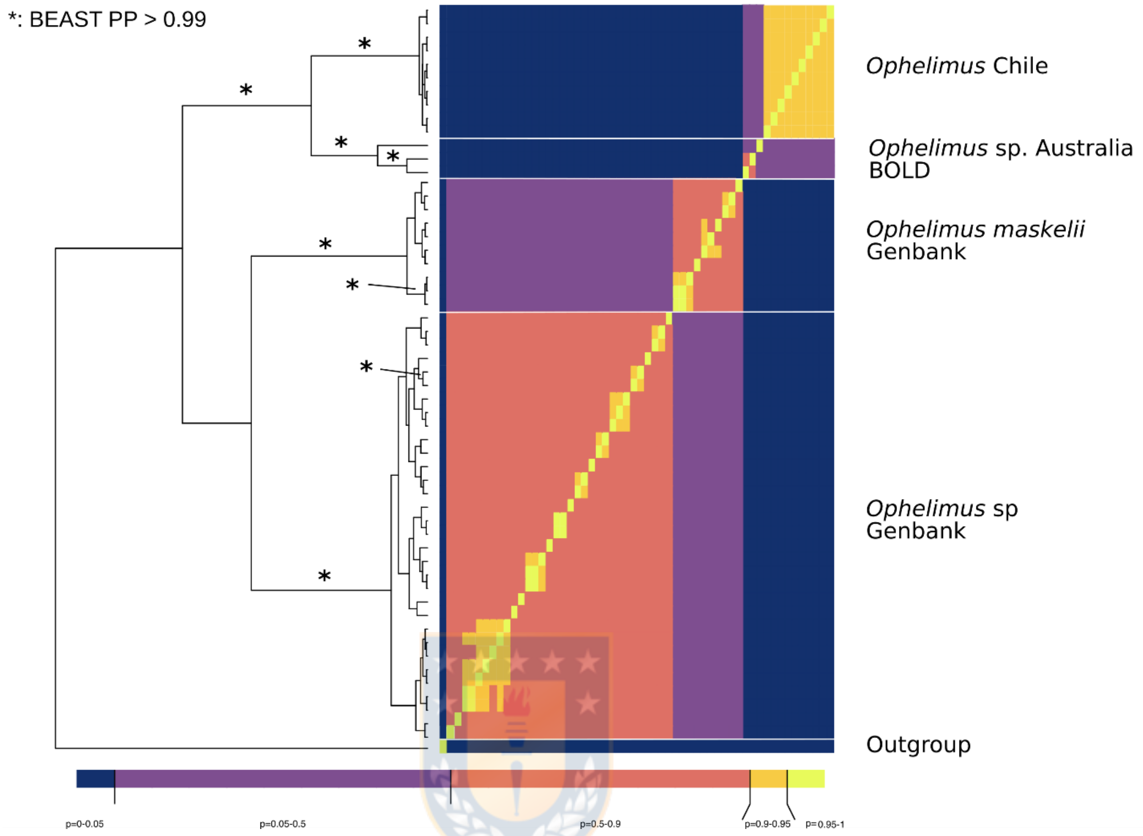


Figure 7. Ultrametric tree of inferred genealogical relationships with COI. For this tree a strict molecular clock was used in BEAST. Only those groupings with a very high significance are highlighted (PP > 0.99)

2.5.-DISCUSSIONS

Ophelimus migdanorum sp. nov., induces galls on stems, petiole, lamina and leaf venation of *E. globulus* and *E camaldulensis* (SAG, 2006) [36]. Its origin is unknown, it is assumed, however, that it is a species from Australia, since it is an insect that attacks *Eucalyptus* species (La sale et al., 2009; La sale et al., 2005) [7, 8] (La Salle, com. pers.). The morphological description of this species made in this study, from individuals obtained from galls developed on *E. globulus* from the 7 sampling areas, located between the regions of Valparaíso and Los Lagos, allow to locate it taxonomically within the genus *Ophelimus* and distinguish it of other congeners, confirming its morphological status of undescribed species.

Until the present study, the fundamental morphological characteristic that had made it possible to differentiate the agallicolous species of the genus *Ophelimus*, is the presence of setae in the submarginal vein, since the difference in size and color are almost imperceptible (La Salle, 2005) [8]. In this work, we observed individuals of *O. migdanorum*, with 1, 2 and 3 setae in the submarginail vein, depending on the size of the micro wasp. Based on this and considering that *O. maskelli* presents only one seta in the submarginal vein (Protasov et al., 2007a) [6], it was initially estimated that the first group could correspond to *O. maskelli,* not reported in Chile and the second and third group to other *Ophelimus* gall species not described. Discarding in all cases to *O. eucalypti* for presenting 4 or more setae in the submarginal vein (La Salle, 2005) [8]. However, our subsequent descriptive results would indicate that, consistently, the presence of setae in the submarginal vein would not be a morphological feature that alone would distinguish between *O. maskelli* and *O. migdanorum* this situation confirms the scarce information available for the species of the genus *Ophelimus* (Protasov et al., 2007a) [6] and the limited available taxonomic information of *O. eucalypti* (Gahan, 1922) [21].

The result of the analysis of the molecular sequencing was mostly consistent with the morphological results, showing that the three groups of insects detected in Chile, with one, two and three setae correspond to the same phylogenetic unit, or that they correspond to the same species of the genus *Ophelimus* and that these differ from *O. maskelli* and other *Ophelimus* sp described in the genetic data bank. Also, the recovery of a monophyletic group with high support between delimitation approximations would confirm that the COI sequences belonging to Chile would correspond to a new species operationally differentiable in a phylogenetic context. Interestingly, the results were less clear to suggest the monophyly of *Ophelimus* (Figures 2.6; 2.7). This result could be explained by the lack of phylogenetic information observable from the COI marker, which requires the inclusion of additional regions to improve its resolution at genus and tribe scales (Burks, 2011) [10]. Despite, this result, it is very probable that the COI region will serve as an effective vessel for the search and identification of new entities in *Ophelimus* and other related genres, particularly for its notorious resolution at interspecific scales.

In addition, we can affirm with the phylogenetic analysis that Ophelimus that present one seta in the SVS is not necessarily *Ophelimus maskelli* and than this specie is not present in the samples taken in Chile. In addition, the morphological characteristics of a single seta in the submarginal vein, that described them as such, indicate that it could be a sister species of *O. migdanorum* nov sp (Angulo, Comunicación Verbal, May 2016), because its genetic allows us to infer that it is a different species from *O. maskelli.* Previously in France, specimens with two, three or four SVS were detected that do not correspond to *O. maskelli* [53]. However, in this study it is detected specimens with 1 SVS that could easily be confused with *O. maskelli.* It should be mentioned that only the SVS number is part of one of the most current keys for the Eulophidae family (Burks et al, 2011) [10], so it is proposed to incorporate several rings and funiculae that had already been proposed by Protasov et al., 2007a for *O. maskelli* (Table 2.3; 2.4). *Ophelimus migdanorum* nov sp belongs to the same taxonomic group at the genus level that *O. maskelli*, but are differentiated, based Protasov et al (2007a), by the number of setae present in the submarginal vein (SVS), adult body color-tone, antenna color, number of rings in antennae and number of funicular segment in the flagellum (Tabla 2.4) and morphology and location of the galls (Table 2.6)(details in point 2.4.2).

Ophelimus migdanorum Molina - Mercader sp. nov. a possible case a polyphenic species. In Figure 2.1, it can be seen the presence of 1, 2 and 3 setae in the submarginal vein of *O. migdanorum,* it is clearly observed that adults emerge with the number of certain setae, since no vestigial setae are observed, that is to say have not developed from gall post-emergence. The correlation between the final size of adults emerging from the galls and the number of setae in the submarginal vein (1, 2 and 3 setae) is high for the individuals belonging to each group (Table 2.5). This may be since the greater number of setae in the submarginal vein, the greater the size of the individual (Table 2.5). This phenomenon can occur because we are facing a possible case of polyphenism. In order to determine this phenomenon briefly, galls present in lamina, central rib, petiole and stem were isolated, in the laboratory 294 galls were taken from different sectors, randomly, corroborating in 100% of the cases that *O. migdanorum* nov. sp, emerged in these and induces galls in petiole, leaf blade and central rib obtaining that adults emerging from the greater nutritional zone (central rib, petiole and stems), are those that possess 2 to 3 setae and those of 1 leaf and petiole seta (Table 5, Tabla 6), those that appear from sheet have a similar behavior to that of *O. maskelli* that induces gall formation in the leaf (Branco et al., 2009; Protasov et al., 2007b) [27, 54] and *O. eucalypti* in the petiole of the leaves, leaf limb and rib, (Bain, 2003) [55]. However, Sánchez (2003) points out for Spain, that the galls of the petioles or of the main nerve, corresponds to another agallicolous species other than *O. eucalypti* (Sánchez, 2003) [24].

The determination of *O. migdanorum* nov. sp in the 7 sampling zones, account for its wide dispersion in the country, between the regions of Valparaíso in the north and the Los Lagos in the south (SAG, 2006); which coincides with Molina-Mercader et al., (2019), who had reported this wasp between the region of Maule and La Araucanía. The wide dispersion could be explained by the high elapsed time, 14 years since its detection as *Ophelimus*. sp (SAG, 2006) [36] and for the continuity of the plantations of *E. globulus* present in the country that reach an estimated area of 563,000 ha in the study area, equivalent to 95% of the country surface covered with this species (INFOR, 2018; Molina-Mercader et al., 2019) [57,58]. This biotic agent represents a new threat to the sustainability of *E. globulus* cultivation in the country, added to the effect of drought and to the damage caused by *Gonipterus platensis* (Molina-Mercader, 2019; Beeche, 1999) 58,59].

The *Ophelimus* species are all associated with eucalyptus galls and in general all are considered as gall-inducing (Boucek, 1988) [12]. In this study, during the farm sampling, gall formation was observed in stems, petioles and in the leaf rib and less frequently in the leaf (Molina-Mercader et al., 2019) (58) This is associated with mortality of leaves and branches mainly in the middle third of the height of the trees and even their mortality (Molina-Mercader et al., 2019) (58). This information, both location of galls and damage, is consistent with the observations made by Molina-Mercader et al., (2019) in Chile on *E. globulus*, and Bain (1977) in New Zealand for the damages caused by *O. eucalypti* on *E. globulus.* The level of damage was not evaluated in this study.

The results constitute a concrete contribution to the knowledge of the *Ophelimus* genus, both in morphological and molecular aspects. In the case of morphology only *O. maskelli* is described with available information. In the Genbank there were only 46 sequences of mitochondrial gene cytochrome oxidase subunit I for the genus Ophelimus. Finally, BOLDSYSTEMS recorded only 7 specimens with Barcodes.

2.6.-CONCLUSION AND FINAL CONSIDERATION.

Specimens of *Ophelimus* detected in Chile determine a new species or taxa that had not previously been described. Both the morphological and molecular analyzes allowed to demonstrate that it is a new species that does not group with other analyzed species but presents intraspecific variations that could correspond to polyphenism determined by feeding sites. It was also found that the galls produced by different species are variable in shape and are in different parts of the plant. An important element is the lack of information about the taxonomic integrity of other *Ophelimus* species, which mostly do not have comprehensive systematic studies. Considering the importance of this and other genera as invasive species with high economic impact for forestry and agricultural activities, it is necessary to encourage a better and more taxonomic treatment of these groups. The present work based on molecular markers proves that it is possible to distinguish and discover entities using complementary characters and support those generated by traditional morphology. Therefore, it is expected that this work will serve as an example of the need to continue deepening the entomological study of Ophelimus and the improvement in the development of the systematics of these study groups.

Table 2.2. Analyzed COI sequences.

Matrix	**Genbank Signs**	**Identity**
AUSCL414-12|Eulophidae|COI-5P	AUSCL414-12|Eulophidae|COI-5P	Ophelimus Australia
AUSCL774-12|Eulophidae|COI-5P	AUSCL774-12|Eulophidae|COI-5P	Ophelimus Australia
AUSCL782-12|Eulophidae|COI-5P	AUSCL782-12|Eulophidae|COI-5P	Ophelimus Australia
Astichus sp. D0693 HM365048.1	HM365048.1 Astichus sp. D0693	Outgroup
Bellerus sp. D2028 HM365049.1	HM365049.1 Bellerus sp. D2028	Subtribu Eutiineae
Euderus sp. BOLD-2016 KR802660.1	KR802660.1 Euderus sp. BOLD-2016 voucher BIOUG01330-F01	Subtribu Eutiineae
Euderus sp. D0703 HM365047.1	HM365047.1 Euderus sp. D0703	Subtribu Eutiineae
HM374819.1	HM374819.1 Euderus sp. BBHYM381-09	Subtribu Eutiineae
HM414503.1	HM414503.1 Euderus sp. BBHEC936-10	Subtribu Eutiineae
JX096404.1	JX096404.1 Ophelimus maskelli isolate 3755	
JX096405.1	JX096405.1 Ophelimus maskelli isolate 3756	O. maskelli Genbank
JX096406.1	JX096406.1 Ophelimus maskelli isolate 6552	O. maskelli Genbank
JX096407.1	JX096407.1 Ophelimus maskelli isolate 6553	O. maskelli Genbank
JX096408.1	JX096408.1 Ophelimus maskelli isolate 6554	O. maskelli Genbank
JX096409.1	JX096409.1 Ophelimus maskelli isolate 6555	O. maskelli Genbank
JX096410.1	JX096410.1 Ophelimus maskelli isolate 6557	O. maskelli Genbank
JX096411.1	JX096411.1 Ophelimus maskelli isolate 6558	O. maskelli Genbank
JX096412.1	JX096412.1 Ophelimus maskelli isolate 6560	O. maskelli Genbank
JX096413.1	JX096413.1 Ophelimus sp. NR-2012 isolate 3739	*Ophelimus nov. sp. Francia*
JX096416.1	JX096416.1 Ophelimus sp. NR-2012 isolate 3745	*Ophelimus nov. sp. Francia*

Matrix	**Genbank Signs**	**Identity**
JX096417.1	JX096417.1 Ophelimus sp. NR-2012 isolate 3746	*Ophelimus nov. sp. Francia*
JX096418.1	JX096418.1 Ophelimus sp. NR-2012 isolate 6533	*Ophelimus nov. sp. Francia*
JX096419.1	JX096419.1 Ophelimus sp. NR-2012 isolate 6534	*Ophelimus nov. sp. Francia*
JX096420.1	JX096420.1 Ophelimus sp. NR-2012 isolate 6535	*Ophelimus nov. sp. Francia*
JX096421.1	JX096421.1 Ophelimus sp. NR-2012 isolate 6536	*Ophelimus nov. sp. Francia*
JX096422.1	JX096422.1 Ophelimus sp. NR-2012 isolate 6537	*Ophelimus nov. sp. Francia*
JX096423.1	JX096423.1 Ophelimus sp. NR-2012 isolate 6538	*Ophelimus nov. sp. Francia*
JX096424.1	JX096424.1 Ophelimus sp. NR-2012 isolate 6540	*Ophelimus nov. sp. Francia*
JX096425.1	JX096425.1 Ophelimus sp. NR-2012 isolate 6541	*Ophelimus nov. sp. Francia*
JX096426.1	JX096426.1 Ophelimus sp. NR-2012 isolate 6542	*Ophelimus nov. sp. Francia*
JX096427.1	JX096427.1 Ophelimus sp. NR-2012 isolate 6544	*Ophelimus nov. sp. Francia*
JX096428.1	JX096428.1 Ophelimus sp. NR-2012 isolate 6545	*Ophelimus nov. sp. Francia*
JX096429.1	JX096429.1 Ophelimus sp. NR-2012 isolate 6546	*Ophelimus nov. sp. Francia*
JX096430.1	JX096430.1 Ophelimus sp. NR-2012 isolate 6547	*Ophelimus nov. sp. Francia*
JX096431.1	JX096431.1 Ophelimus sp. NR-2012 isolate 6549	*Ophelimus nov. sp. Francia*
JX096432.1	JX096432.1 Ophelimus sp. NR-2012 isolate 6551	*Ophelimus nov. sp. Francia*
JX096433.1	JX096433.1 Ophelimus sp. NR-2012 isolate 6562	*Ophelimus nov. sp. Francia*
JX096434.1	JX096434.1 Ophelimus sp. NR-2012 isolate 6564	*Ophelimus nov. sp. Francia*
JX096435.1	JX096435.1 Ophelimus sp. NR-2012 isolate 6565	*Ophelimus nov. sp. Francia*
JX096436.1	JX096436.1 Ophelimus sp. NR-2012 isolate 6566	*Ophelimus nov. sp. Francia*
JX096437.1	JX096437.1 Ophelimus sp. NR-2012 isolate 6569	*Ophelimus nov. sp. Francia*

Matrix	**Genbank Signs**	**Identity**
JX096438.1	JX096438.1 Ophelimus sp. NR-2012 isolate 6569	*Ophelimus nov. sp. Francia*
JX096439.1	JX096439.1 Ophelimus sp. NR-2012 isolate 6571	*Ophelimus nov. sp. Francia*
JX096440.1	JX096440.1 Ophelimus sp. NR-2012 isolate 6572	*Ophelimus nov. sp. Francia*
JX096441.1	JX096441.1 Ophelimus sp. NR-2012 isolate 6573	*Ophelimus nov. sp. Francia*
JX096442.1	JX096442.1 Ophelimus sp. NR-2012 isolate 6574	*Ophelimus nov. sp. Francia*
JX096443.1	JX096443.1 Ophelimus sp. NR-2012 isolate 6575	*Ophelimus nov. sp. Francia*
JX096444.1	JX096444.1 Ophelimus sp. NR-2012 isolate 6577	*Ophelimus nov. sp. Francia*
JX096445.1	JX096445.1 Ophelimus sp. NR-2012 isolate 6578	*Ophelimus nov. sp. Francia*
JX096446.1	JX096446.1 Ophelimus sp. NR-2012 isolate 6579	*Ophelimus nov. sp. Francia*
KR787323.1	KR787323.1 Euderus sp. BOLD-2016 voucher 08BBHYM-1515	Subtribu Eutiineae
KR787784.1	KR787784.1 Euderus sp. BOLD-2016 voucher BIOUG16937-G07	Subtribu Eutiineae
KR791285.1	KR791285.1 Euderus sp. BOLD-2016 voucher BIOUG04552-E05	Subtribu Eutiineae
KR792605.1	KR792605.1 Euderus sp. BOLD-2016 voucher BIOUG01330-B02	Subtribu Eutiineae
KR800427.1	KR800427.1 Euderus sp. BOLD-2016 voucher BIOUG17368-D06	Subtribu Eutiineae
KR800623.1	KR800623.1 Euderus sp. BOLD-2016 voucher BIOUG04324-B05	Subtribu Eutiineae
KR923436.1	KR923436.1 Euderus sp. BOLD-2016 voucher BIOUG19267-C05	Subtribu Eutiineae
KR923450.1	KR923450.1 Euderus sp. BOLD-2016 voucher BIOUG19347-F04	Subtribu Eutiineae
KR929529.1	KR929529.1 Euderus sp. BOLD-2016 voucher BIOUG19992-F01	Subtribu Eutiineae
KR930333.1	KR930333.1 Euderus sp. BOLD-2016 voucher BIOUG19267-G08	Subtribu Eutiineae
KR931581.1	KR931581.1 Euderus sp. BOLD-2016 voucher BIOUG19345-F08	Subtribu Eutiineae
KU496740.1	KU496740.1 Euderus sp. KNWR 5141	Subtribu Eutiineae
MF899935.1	MF899935.1 Euderus sp. BIOUG12155-B04	Subtribu Eutiineae

Matrix	Genbank Signs	Identity
MG336688.1	MG336688.1 Euderus sp. BIOUG25051-C12	Subtribu Eutiineae
MG338619.1	MG338619.1 Astichus arithmeticus voucher BIOUG31095-B03	Subtribu Eutiineae
MG339229.1	MG339229.1 Astichus arithmeticus voucher BIOUG05705-F02	Subtribu Eutiineae
MG339457.1	MG339457.1 Euderus sp. BIOUG25593-F05	Subtribu Eutiineae
MG341180.1	MG341180.1 Euderus sp. BIOUG25593-E02	Subtribu Eutiineae
MG341593.1	MG341593.1 Euderus sp. BIOUG26216-E03	Subtribu Eutiineae
MG341685.1	MG341685.1 Euderus sp. BIOUG25599-C01	Subtribu Eutiineae
MG342013.1	MG342013.1 Astichus arithmeticus voucher BIOUG21505-B04	Subtribu Eutiineae
MG446079.1	MG446079.1 Euderus sp. BIOUG25484-E09	Subtribu Eutiineae
MG479492.1	MG479492.1 Euderus sp. BIOUG12942-B10	Subtribu Eutiineae
MG480257.1	MG480257.1 Euderus sp. BIOUG17999-F11	Subtribu Eutiineae
MG481343.1	MG481343.1 Euderus sp. BIOUG21221-B02	Subtribu Eutiineae
MG481600.1	MG481600.1 Euderus sp. BIOUG20087-H02	Subtribu Eutiineae
MG482438.1	MG482438.1 Euderus sp. BIOUG17995-C03	Subtribu Eutiineae
MG483373.1	MG483373.1 Euderus sp. BIOUG20564-B11	Subtribu Eutiineae
MG485352.1	MG485352.1 Euderus sp. BIOUG04072-C07	Subtribu Eutiineae
MG485471.1	MG485471.1 Euderus sp. BIOUG04127-B04	Subtribu Eutiineae
MG487653.1	MG487653.1 Euderus sp. BIOUG20669-D05	Subtribu Eutiineae
MG836466.1	MG836466.1 Euderus albitarsis isolate H26P2	Subtribu Eutiineae
MG836467.1	MG836467.1 Euderus albitarsis isolate H26P3	Subtribu Eutiineae
MG836468.1	MG836468.1 Euderus albitarsis isolate H26P4	Subtribu Eutiineae

Matrix	**Genbank Signs**	**Identity**
MG836469.1	MG836469.1 Euderus albitarsis isolate HE26P4	Subtribu Eutiineae
MG836470.1	MG836470.1 Euderus cushmani isolate HE26P10	Subtribu Eutiineae
Ophelimus maskelli isolate D1559 HM365046.1	HM365046.1 Ophelimus maskelli isolate D1559	O. maskelli Genbank
Ophelimus sp1 Chile rep.2 R	Ophelimus sp1 Chile rep.2 R	*Ophelimus nov. sp.* Chile
Ophelimus sp1 Chile rep.3 F	Ophelimus sp1 Chile rep.3 F	*Ophelimus nov. sp.* Chile
Ophelimus sp1 Chile rep.3 R	Ophelimus sp1 Chile rep.3 R	*Ophelimus nov. sp.* Chile
Ophelimus sp2 Chile rep.1 F	Ophelimus sp2 Chile rep.1 F	*Ophelimus nov. sp.* Chile
Ophelimus sp2 Chile rep.1 R	Ophelimus sp2 Chile rep.1 R	*Ophelimus nov. sp.* Chile
Ophelimus sp2 Chile rep.2 F	Ophelimus sp2 Chile rep.2 F	*Ophelimus nov. sp.* Chile
Ophelimus sp2 Chile rep.2 R	Ophelimus sp2 Chile rep.2 R	*Ophelimus nov. sp.* Chile
Ophelimus sp2 Chile rep.3 R	Ophelimus sp2 Chile rep.3 R	*Ophelimus nov. sp.* Chile
Ophelimus sp3 Chile rep.1 R	Ophelimus sp3 Chile rep.1 R	*Ophelimus nov. sp.* Chile
Ophelimus sp3 Chile rep.2 R	Ophelimus sp3 Chile rep.2 R	*Ophelimus nov. sp.* Chile

Basic data matrix used for the analysis with the methods of maximum probability (ML) and Bayesian Inference (BI).

2.7.- SUPLEMENTARY FILE 1

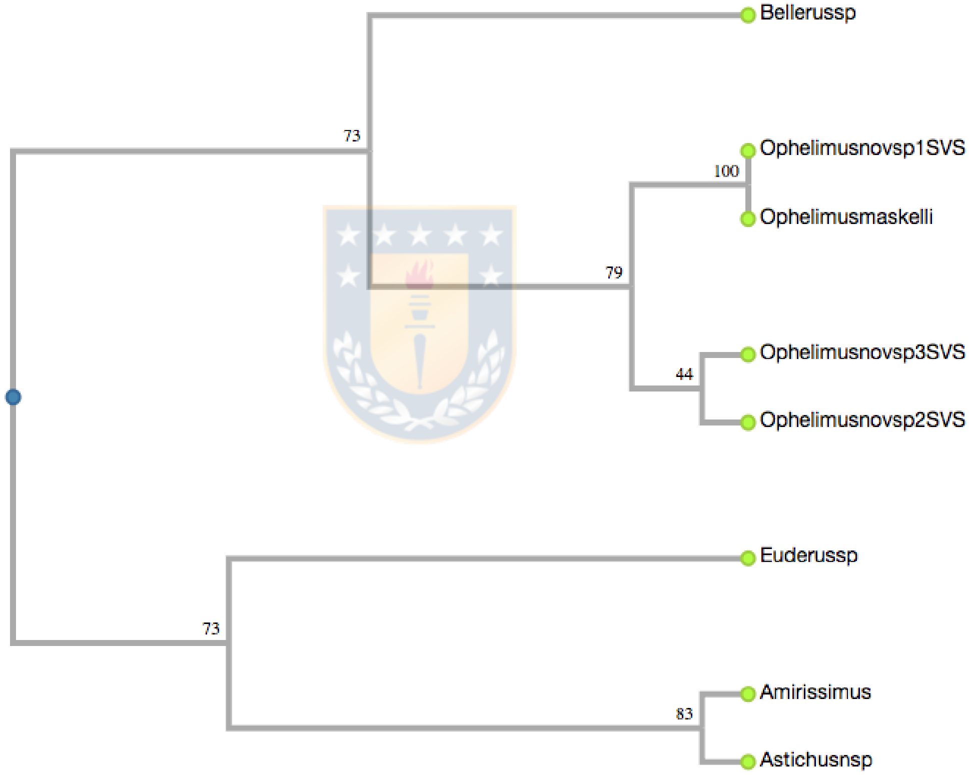
Ophelimus migdanorum Molina - Mercader sp. nov (Hymenoptera: Eulophidae): Testing Integrative Taxonomy and disentangling polyphenism cases in Chile.

Tabla S2.1. Characters used for the morphological description of Ophelimus detected in Chile, according to Burks et al. (2011),

N°	Character	Code
1	Number of flagellomeres	Number or a letter from 6 to C (=12)
2	Number of separate claval segments in females	1 to 4
3	Shape of flagellomeres in males	0 = cylindrical and without branches
		1 = nodose, with a rounded expanded section bearing elongate setae
		2 = bearing two or three branches
		3 = cylindrical apically but with a slight basal expansión.
4	Shape of basiconic peg sensilla of flagellum	0 = symmetrical
		1 = slightly asymmetrical, angular
		2 = strongly asymmetrical, spearshaped
5	Carinae of pedicel:	0 = absent
		1 = present
6	Sulcus across vertex between median and lateral ocelli	0 = absent
		1 = present
7	Vertex posterior carina	0 = absent
		1 = present
8	Transverse facial sulcus	0 = absent
		1 = present and adjacent to the median ocellus
		2 = separated from the median ocellus by at least the diameter of the median ocellus
9	Subtorular grooves	0 = absent
		1 = present, extending from ventral edge of torulus
		2 = present, extending from lateral edge of torulus
10	Delimitation of clypeus:	0 = delimited at least by lateral grooves
		1 = not delimited
11	Clypeus width:	0 = not enlarged, width less than malar space (Fig. 20: cly); 1 enlarged, width greater than or equal to malar space (Fig. 21: cly).

N°	Character	Code
12	Pronotal collar carina:	0 = absent
		1 = present
13	Semicircular ridge of pronotum laterally:	0 = absent
		1 = present
14	External completeness of notauli posteriorly	0 = reaching trans-scutal articulation
		1 = not reaching trans-scutal articulation, essentially absent
15	Pairs of mesoscutal midlobe bristles	Coded using the actual number, from 0 to 3 except that 3 includes counts of 3 or greater
16	Advancement of axillae	0 = dorsal axillar surface not completely advanced beyond anterior margin of scutellar disc
		1 = dorsal axillar surface completely advanced beyond anterior margin of scutellar disc
17	Pairs of scutellar disc setae	From 1 to 3 except that 3 includes counts of 3 or greater.
18	Scutellar grooves	0 = absent
		1 = present as a U-shaped groove open anteriorly
		2 = present as parallel grooves open both anteriorly and posteriorly
19	Pit along scuto-scutellar sulcus between axilla and scutellar disc	0 = absent.
		1 = present.
20	Propleura	0 = posterior margins diverging angularly along prosternum
		1 = posterior margins transverse, diverging at right angles at prosternum.
21	Mesepisternal projection over posterior margin of prepectus	0 = absent
		1 = present
22	Expansion of mesepimeron over metapleuron	0 = mesepimeron not expanded over metapleuron
		1 = mesepimeron expanded, overlapping metapleuron
23	Median carina of propodeum	0 = not flattened dorsally
		1 = flattened dorsally
24	Setae of propodeal disc	0 = not curving mesad
		1 = curving mesad
25	Protibial spur (=calcar)	0 = stout and curved
		1 = slender and straight
26	Number of tarsomeres in fore leg	from 3 to 5.
27	Number of tarsomeres in mid leg compared with fore leg in males	0 = same;
		1 = one less

N°	Character	Code
28	Submarginal vein setae	where 3 includes counts of 3 or greater
29	Postmarginal vein length	0 = more than onethird stigmal vein length
		1 = less than or equal to one-third stigmal vein length
30	Epipygium (Mt9) in females	0 = separate from Mt8
		1 = fused with Mt8, forming a syntergum
31	Number of volsellar digital spines	1 = 1 volsellar spine;
		2 = 2 or more volsellar spines



Numbers at each node represent bootstrap values (over 100)

Figure S2.1. Location of *Ophelimus nov sp*, Chile, in dendrogram made from the characters according to Burks 2011.

TABLE S2.2. Length of the sub marginal vein of *Ophelimus migdanorum* nov sp, both of 1, 2 and 3 SVS.

Ophelimus	**Genero**	**Media_Submarg_VenaAla**	
3_Seta	Male	300,1 (±14,7)	a
3_Seta	Female	245,6 (±13,3)	b
2_Seta	Female	245,3 (±09,0)	b
2_Seta	Male	241,2 (±12,7)	bc
1_Seta	Male	225,8 (±04,4)	bc
1_Seta	Female	213,5 (±06,4)	c
Media	**General**	**245,2** (±10,1)	

Values in parentheses indicate standard error. The different letters indicate statistically significant differences between mean parasitism levels per locality within the same year of evaluation (p≤0.05). In table Note the difference in size according to the amount of SVS

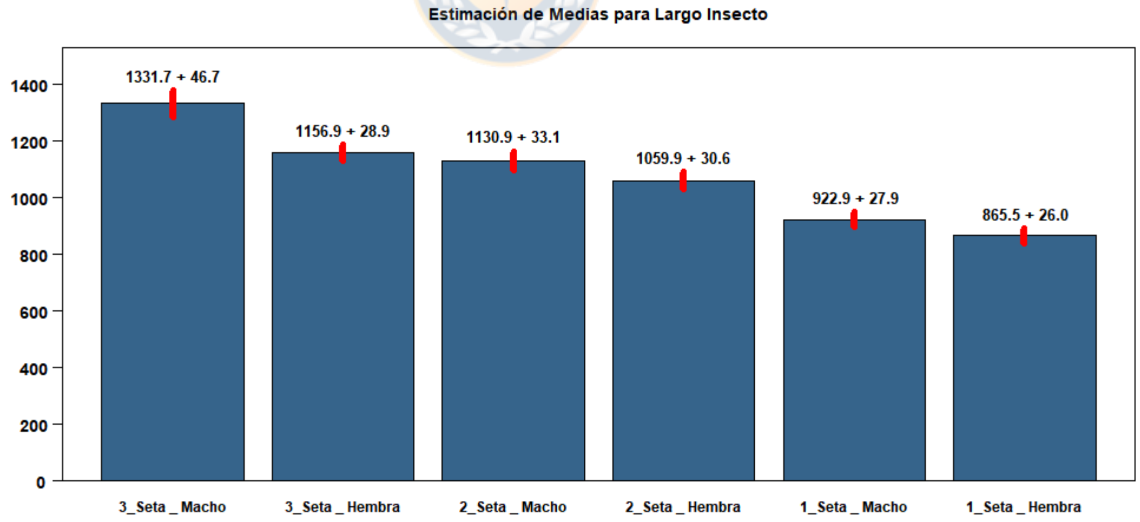


Figure S2.2. Length of insects for males and females with 1, 2 and 3 mushrooms in the SVS.

Table S2.3. Dimensions of morphological structures for individuals of *Ophelimus migdanurum* nov sp, of 1, 2 and 3 setaes.

	Body Length		**Weing length**		**Submarginal vein length**		**Abdomen length**		**Flagelo length**	
Resumen 1 setae	**Female**	**Macle**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Average	865,47	922,93	768,52	787,47	213,46	225,79	409,53	430,07	205,79	169,58
Desviation	100,87	108,20	74,24	41,02	24,76	16,86	57,23	45,25	75,56	12,71
Coef	0,12	0,12	0,10	0,05	0,12	0,07	0,14	0,11	0,37	0,07
Máximum	1105,00	1124,00	888,58	859,78	264,38	250,00	548,00	503,00	363,07	185,07
Mínimum	712,00	781,00	641,00	690,45	166,00	191,00	331,00	345,00	142,00	146,89
Moda	845,00	878,00	888,58	815,00	222,14	228,84	363,00	408,00	363,07	146,89
Median	857,00	878,00	792,00	794,00	214,00	225,14	415,00	427,00	174,00	172,00
Resumen 2 setaes	Female	Macle	Female	Male	Female	Male	Female	Male	Female	Male
Average	1059,93	1130,93	874,95	900,46	245,28	241,20	512,00	547,07	188,17	194,33
Desviation	118,45	128,06	65,67	99,59	34,88	49,04	58,94	66,40	15,88	20,04
Coef	0,11	0,11	0,08	0,11	0,14	0,20	0,12	0,12	0,08	0,10
Máximum	1253,00	1346,00	998,00	1.046,98	294,60	311,00	583,00	672,00	217,00	237,09
Mínimum	846,00	945,00	791,00	713,00	190,00	161,00	383,00	456,00	167,00	160,00
Moda	1016,00	1028,00	806,00	957,19	294,60	238,82	554,00	473,00	200,00	194,71
Median	1018,00	1140,00	871,00	911,00	240,00	257,00	523,00	536,00	186,00	189,50
3 setaes	Female	Macle	Female	Male	Female	Male	Female	Male	Female	Male
Average	1156,93	1331,67	933,97	1.075,01	245,63	300,10	568,67	627,07	181,82	221,16
Desviation	111,96	180,78	102,87	191,83	51,34	56,84	82,18	75,37	8,35	38,10
Coef	0,10	0,14	0,11	0,18	0,21	0,19	0,14	0,12	0,05	0,17
Máximum	1413,00	1841,00	1.089,00	1.513,00	373,32	384,00	700,00	780,32	195,00	336,10
Mínimum	930,00	1118,00	755,00	837,00	189,00	217,00	389,00	509,00	162,00	184,00
Moda	1211,00	1339,00	984,67	1.120,60	189,00	322,57	677,00	702,67	181,00	213,51
Median	1134,00	1318,00	911,00	1.017,59	238,00	316,35	564,00	631,79	182,00	217,26

Tabla S2.4.- Provision of the Holotype, alotype and paratypes, of *Ophelimus migdanorum* nov sp.

Serie Tipo	**N°**	**Género**	**Región**	**Provincia**	**Comuna**	**Predio**	**Fecha**	**Montaje**	**Museo**
Holotipo	1	Hembra	Biobío	Ñuble	Chillán Viejo	San Vicente	09.2017	Puntilla	UCCC
Alotipo	1	Macho	Biobío	Ñuble	Chillán Viejo	San Vicente	09.2017	Puntilla	UCCC
Paratipo	3	Hembras	Valparaíso	Valparaíso	Casa Blanca	Rosario	10.2017	1 Puntilla	MNHN
								2 Glicerina	*MIP*lagas
	2	Machos						Puntilla	UCCC
	2	Machos	O’Higgins	Cardenal Caro	Litueche	Sierramar	12.2017	Puntilla	UCCC
	3	Hembras	Maule	Cauquenes	Cauquenes	San Eduardo	11.2017	Glicerina	*MIP*lagas
	1	Macho						Puntillas	MNHN
	2	Hembras	Biobío	Ñuble	Chillán Viejo	Los Crisoles	03.2017	Glicerina	MNHN
	2	Machos							
	3	Hembras	Araucanía	Cautín	Nueva Imperial	San Carlos	11.2017	Glicerina	MNHN

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CAPÍTULO III. DETECTION AND DISTRIBUTION OF *OPHELIMUS MIGDANORUM* AND ITS POSSIBLE BIOCONTROLLER *CLOSTEROCERUS CHAMAELEON* IN PRODUCTIVE AREAS OF *EUCALYPTUS GLOBULUS* IN CHILE

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3.1.-ABSTRACT.

Wasps that belong to the genus *Ophelimus* develop galls on *Eucalyptus* spp., and *E. globulus* is considered among the most susceptible species. In 2003, a new species of *Ophelimus* was detected in Chile. This species forms galls on *E. globulus* and *E. camaldulensis*, and it was recently considered as *Ophelimus migdanorum nov* sp. The present study was carried out 16 year after its detection and it was aimed to determine the presence of *Ophelimus* in plantations of *E. globulus* located in Maule, Biobío and La Araucanía Regions, which represent 76% of the area covered with this species in the country. Between August and September 2017, a total of 165 sampling forest farms were established in stands of *E. globulus*, located in these regions of the country. In each farm, twigs were collected that represented formation of galls and maintained in laboratory conditions until the emergence of adults. These were identified according to their external morphological characteristics. The presence of *O. migdanorum* and *Closterocerus chamaeleon* was determined. Both species emerged together in all the regions considered in the study, 92.1% and 46.7% of the evaluated forest farms, respectively. Then *Ophelimus migdanorum* is widely distributed in Maule, Biobío and La Araucanía Regions, in Chile

Key words: Biocontroller, *Eucalyptus* spp., gall-maker, galls, *Ophelimus*.

3.2. INTRODUCTION

The cultivation of species of the genus *Eucalyptus* has increased strongly in the world due to its economic, industrial and ornamental interest, reaching an approximate area of 20 million hectares by 2013 (Wingfield et al., 2013). In Chile, the area planted with *Eucalyptus* spp. amounts 860 317 ha, in which *E. globulus* participates in 68.8% (INFOR, 2018).

The genus *Ophelimus* (Haliday, 1844) has approximately 50 species associated to the production of galls on different species of *Eucalyptus* (La Salle et al., 2009). Among these, only two species have been documented: *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) (Protasov et al., 2007a) and *Ophelimus eucalypti* Gahan (Hymenoptera: Eulophidae) (Gahan, 1922; Withers et al., 2000; La Salle, 2005). Both species are native to Australia (La Salle, 2005) and have been dispersed to different countries outside their natural distribution. *Ophelimus maskelli* has been reported in Israel (Protasov et al. 2007a), South Africa, New Zealand (Branco et al., 2016), Vietnam, Indonesia (Lawson et al, 2012); Tunisia (Dhahri et al., 2010) South Africa (Hurley 2014), Turkey (Doganlar and Mendel, 2007), Italy (Arzone and Alma, 2000), Spain (Sánchez, 2003), France (European and Mediterranean Plant Protection Organization, 2006), Portugal (Branco et al., 2009), Greece (Kavallieratos et al., 2006; Branco et al., 2108), Argentina (Aquino et al., 2014), USA (Burks et al., 2015) and Crinea (Nikulina and Martynov, 2018). On the other hand, *Ophelimus eucalypti* has been determined in New Zealand (Withers et al., 2000), Iran, Morocco, Kenya, Uganda

(Arzone and Alma, 2000; Maina, 2003; Sánchez, 2003; Pujade-Villar and Riba-Flinch, 2004).

Among the host species of *O. maskelli* and *O. eucalypti*, *E. globulus*, *E camaldulensis* Dehnh. and *E. saligna* Sm., are described (Protasov et al., 2007a; CABI/EPPO, 2011). For *O. eucalypti*, *E. botryoides* Sm., has been indicated as host (CABI/EPPO, 2011) and in the case of *O. maskelli*, *E. tereticornis* Sm., *E. grandis* W. Hill ex. Maiden and *E. gunnii*, Hook., have been mentioned in addition to other nine species of economic importance (Protasov et al., 2007a).

Defoliations of economic significance attributable to *Ophelimus* have been reported in several countries. In Israel, Mendel et al. (2007) reported high population irruptions of *O. maskelli* causing almost total defoliations in adult *Eucalyptus* trees with densities high enough as to interrupt daily human activities and even, harvesting other crops (Burks et al., 2015). On the other hand, *O. eucalypti* has been described causing serious damages in New Zealand (Withers et al., 2000) on *E. globulus*, making it impossible to grow commercially (Valentine, 1963; Wilson, 1963). In addition, this has also been observed in Iran, Morocco, Kenya and Uganda (Maina, 2003; Sánchez, 2003) in both *E. camaldulensis* and *E. globulus* (Pujade-Villar and Riba-Flinch, 2004).

In 2004, the Hymenoptera *Closterocerus chamaeleon* Girault (Eulophidae) was detected parasitizing *O. maskelli* (Mendel et al., 2007; Borrajo et al., 2008).

Closterocerus chamaeleon is widely described as ectoparasitoid (Borrajo et al., 2008), condition that may not be applicable to all its stages of development, since has been determined in mature larval stage and in pupal tissue from *O. maskelli* (Protasov et al., 2007b). It is unknown if the parasitic behavior of *C. chamaeleon* corresponds to a koinobiont or idiobiont. However, it has been estimated that due to its wide range of body size, but narrow variation in development time, this parasitoid would correspond rather to the idiobiont type. This added to the ectoparasitoid condition could reflect a narrow group of hosts for this species (Protasov et al., 2007b). The known host range of this parasitoid wasp is limited to *O. maskelli*, but it is possible that other gall-inducing species in *Eucalyptus* leaves also serve as hosts (Protasov et al., 2007b; Burks et al., 2015).

This parasitoid micro-wasp has been detected in Algeria, Australia, France, Israel, Italy, Portugal, Spain, Tunisia, Turkey (Noyes, 2013) and Argentina (Aquino et al., 2014). *Closterocerus chamaeleon* is parthenogenetic, with a short lifecycle (3 week at 25 °C), has winter activity and a high dispersion capacity. These features provide the species a high potential as biocontroller (Borrajo et al., 2008). This parasitoid was introduced from Australia to be used in classical biological control programs of *O. maskelli* in Israel (2005-2006) and Italy (2006). In both countries, the control results were successful (Laudonia et al., 2006; Protasov et al., 2007b; Mendel et al., 2007; Caleca et al., 2011; Mendel et al., 2017; Suma et al., 2018).

Ophelimus sp. was detected for the first time in Chile in 2003 in the Valparaiso Region, forming galls on *E. globulus* and *E. camaldulensis* (SAG, 2006), being later reported in 2009 in the Province of Cauquenes, Maule Region (Molina - Mercader, 2019 unpublished data). In 2010, in a joint work between Molina-Mercader, Dr. John La Salle (CSIRO-Australia) and the Agricultural and Livestock Service of Chile (SAG) it was confirmed that the gall wasp detected in the country corresponded to a species of the genus *Ophelimus*, different from *O. maskelli* and *O. eucalypti* (La Salle 2010, personal communication), being identified through *O. migdanorum* (Molina-Mercader, 2019 unpublished data).

In this context and due the significant economic burden *E. globulus* represent for the Chilean forest industry, we aimed to determine the presence of *Ophelimus migdanorum* in plantations of *E. globulus* located between the Maule, Biobío and La Araucanía Regions, Chile.

3.3.-MATERIALS AND METHODS

The study was carried out between July and August 2017 in plantations of *E. globulus* located between the Regions of Maule (35°25’36” S, 71°40’18” W) and La Araucanía (38°54’00” S, 72°40’00” W). These regions cover 76% of the area established with this forest species in the country (INFOR, 2018) (Table 3.1).

Table 3.1. Forest farms in the Maule, Biobío and La Araucanía Regions, in which samples of *Eucalyptus globulus* twigs with galls were detected.

Region	Sampling farm (N°)	Area of *Eucalyptus globulus* (ha)
Maule	26	45 420
Biobío	93	247 967
La Araucanía	46	156 487
Total	**165**	**449 874**

3.3.1. Field sampling

The sampling was carried out in stands of *E. globulus* older than 2 year. The selection of the forest farms was performed considering the distribution range of *E. globulus* in the regions under study. To this end, a network of points was arranged on a plane that contained the distribution of the target plantations, on which the farms to be sampled were selected, considering an average intensity of sampling of one stand every 2,726 ha. In those cases, in which there were no plantations older than 2 year in the selected farm, it was replaced by the nearest farm. In total, 165 farms were selected (Figure 3.4, Table 3.1). Sampled farms were georeferenced and indicated in a map made using Google Earth (Figures 3.3 and 4, Table 3.1).

A total of three trees that presented formation of gall were selected in each farm, considering a separation of five trees between each one. From each selected tree, a twig of approximately 50 cm length measured from the distal part of the twig towards the stem was extracted. On the other hand, the samples were taken from the lower third of the tree. These samples were placed in polyethylene bags of 30 × 40 cm, with absorbent paper, labeled and then sent to the laboratory of *MIP*lagas Ltda., located in the commune of San Pedro de La Paz, Concepción, in the Biobío Region, Chile, to be analyzed.

3.3.3.-Laboratory breeding, adult emergence and species identification

The collected twigs were placed in breeding chambers, one per chamber, with a total of 495 chambers. The chamber consisted of a transparent plastic box, with cover, of 20 × 30 × 40 cm, on whose base, two sheets of absorbent paper were installed. Each breeding chamber was sealed with film paper and kept in the laboratory until the emergence of adults, at a temperature that ranged between 18 and 22 °C, with a RH approximately 60% and a photoperiod 16:8 h. Breeding chambers were revised every day and the absorbent paper was changed every other day to avoid contamination (Figure 3.1a).

The insects that emerged from the breeding chambers were collected in Petri dishes and subsequently fixed in 96% ethanol, in screw-cap tubes for cryo-preservation (Figure 3.1b).

The identification of each of the individuals was performed based on morphological characteristics and using descriptions available for *O. maskelli* (Protasov et al., 2007a), *C. chamaeleon* (Protasov et al., 2007b) and for *O. migdanorum* (Molina-Mercader et al., 2019 unpublished data).

The specimens identified were counted and registered according to the breeding chamber and sample farm, to be then fixed in 90% ethanol in cryopreservation tubes. Photograph taken with Optima Microscopes Italy B-1000PH, 10X. *MIP*lagas Laboratory.

Figure 3.1. (a) Breeding chambers with leaves of *Eucalyptus globulus*, and their disposition in the laboratory of *MIP*lagas Ltda. (b) Tube with screw cap for storage

of specimens of *Ophelimus migdanorum*. (c) Adults of *Ophelimus migdanorum* in Petri dishes.

3.3.4.-Indicators of dispersion and abundance of species.

The frequency of each species was determined, considering the relative participation of the positive farms to the presence of a certain species of insect, and respect to the total quantity of farms sampled in each region, expressed as a percentage in the following expression:

$$Frequency\ of\ farms\ (\%) = \left(\frac{N°\ of\ positive\ farms\ in\ region}{N°\ of\ sampled\ farms\ in\ the\ regions}\right) \times 100$$

Additionally, the population density at species and region level was estimated, considering the individuals of each species collected in the region and the totality of positive farms to the presence of each species, using the following expression:

$$Individuos\ by\ farms\ (N°) = \left(\frac{N°\ individuals\ collected\ inthe\ region}{N°\ of\ positive\ farms\ in\ the\ region}\right)$$

Both standard error and significant significance in each region was estimated, using the MIXED procedure to means of incidence of pests ($p \leq 0.05$) (1996, SAS-Institute, Cary, North Carolina, USA).

3.4.-RESULTS

The presence of the species *O. migdanorum* and the parasitoid *C. chamaeleon* was determined in each of the three regions evaluated. The individuals that emerged from the breeding chambers during the study period corresponded to 14152 y 927 individuals of *O. migdanorum* y *C. chamaeleon*, respectively (Table 3.2).

Table 3.2. Individuals of *Ophelimus migdanorum* and *Closterocerus chamaeleon* emerged by region**.**

Region	**Individuals (N°)**	
	Ophelimus migdanorium	***Closterocerus chamaeleon***
Maule	872	21
Biobío	7.955	487
La Araucanía	5.325	419
Total	**14.152**	**927**

During the identification procedure of the species it was determined that the eye color and the shape of the abdomen were an effective tool to distinguish between individuals of *O. migdanorum* and *C. chamaeleon* (Figure 3.2, Table 3.3). This preliminary distinction coincided in 100% of the cases with the complete morphological identification for everyone.

Table 3.3. Main characteristics for visual identification of specimens of *Ophelimus migdanorum* and *Closterocerus chamaeleon*.

Characteristic	***Ophelimus migdanorum***	***Closterocerus chamaeleon***
Eyes	The same tone as the body (Figure 2b)	Red (Figure 2a)
Abdomen	Rounded (Figure 2b)	Lanceolate (Figure 2a)

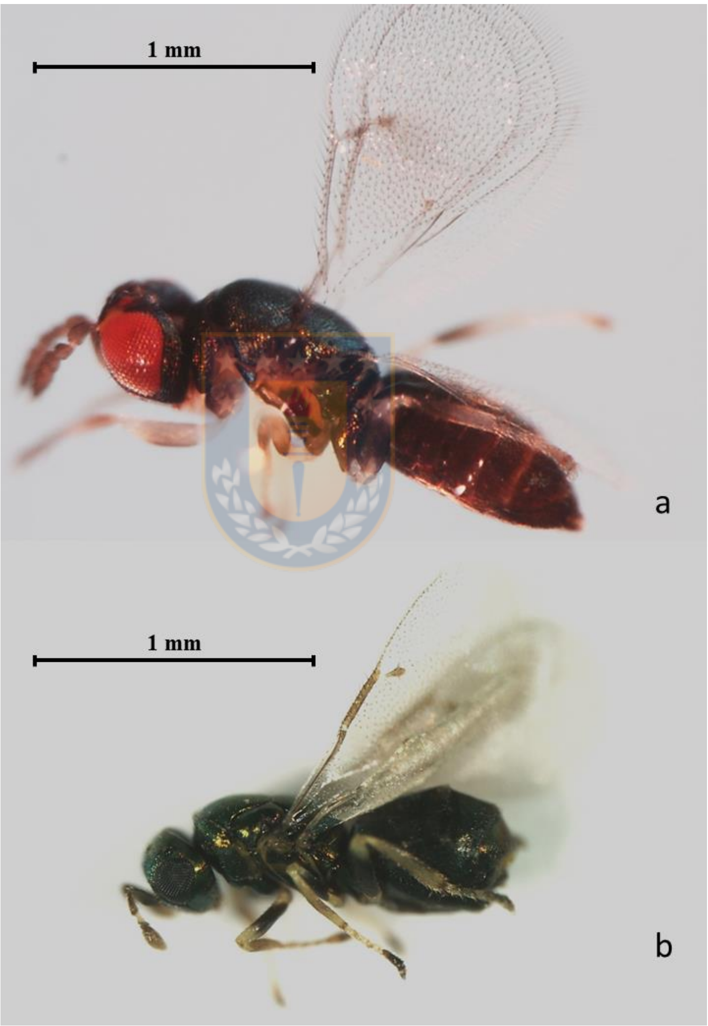

Figure 3.2. Morphological differences between *Closterocerus chamaeleon* and *Ophelimus migdanorum*: (a) Red eyes and lanceolate abdomen in

Closterocerus chamaeleon (b), black eyes and rounded abdomen in *Ophelimus migdanorum*.

Ophelimus migdanorum was determined in 152 farms, equivalent to 92.1% of the total farms sampled in the present study. On the other hand, *C. chamaeleon* was found in 46.7% of the farms (77 farms), coinciding in all cases with those farms in which *O. migdanorum* was detected. The emergence of *C. chamaeleon* together with the gall wasp was observed in 99.1% of the breeding chambers. Only in one chamber in La Araucanía Region and another in the Biobío Region there was the emergence of only specimens of *C. chamaeleon* (Figure 3.4). In both cases, from the other three breeding chambers that completed the sample of each standing each of these regions, the emergence of both species was obtained.

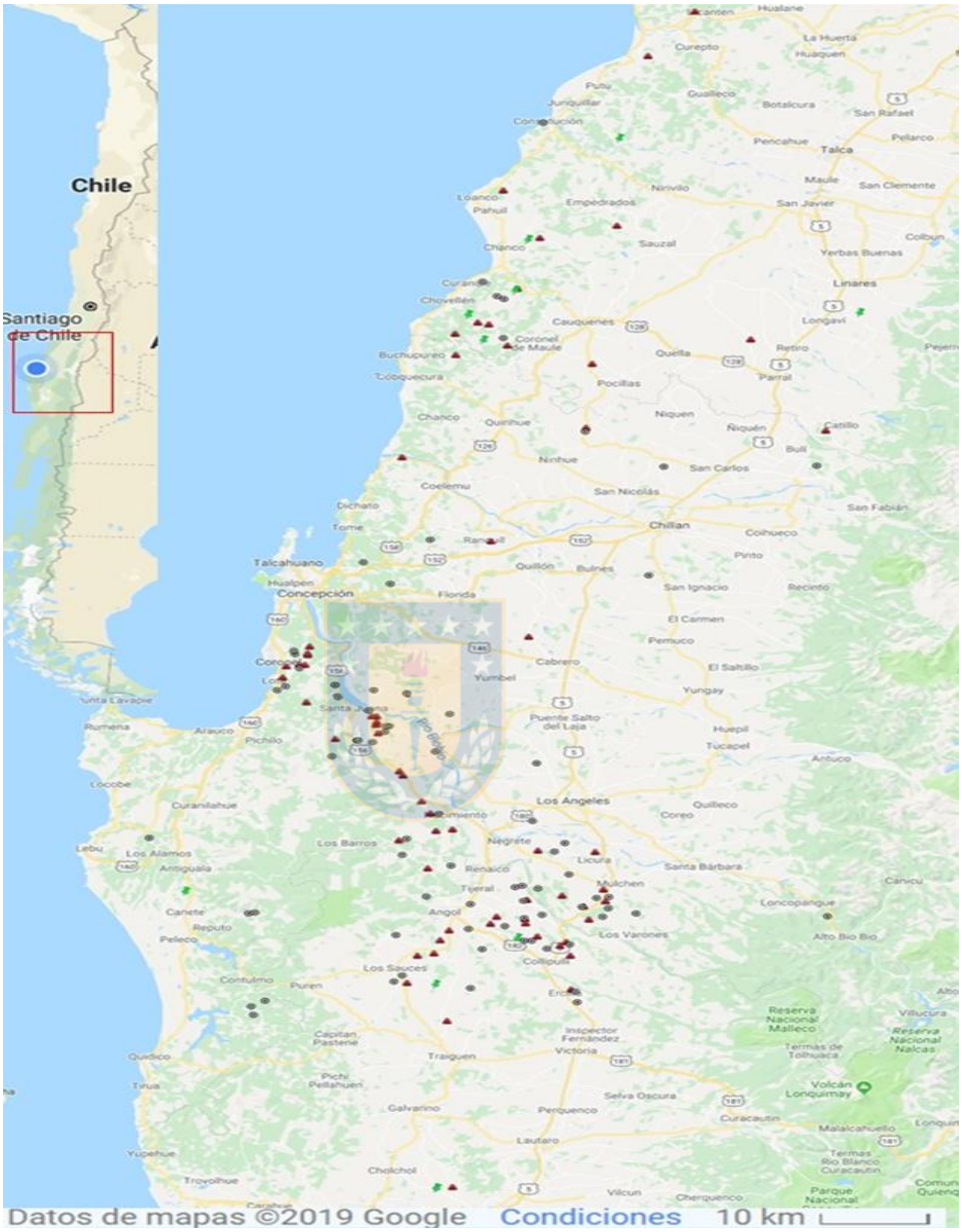

Figure 3.4. Distribution of sampling farms in the Maule, Biobío and La Araucanía Regions. Triangle indicates the presence of *Ophelimus migdanorum*. Circle indicates detection of *O. migdanorum and Closterocerus chamaeleon*. Pushpin indicates no emergence. This map was elaborated in Google Maps.

The highest frequency of farms positive to *O. migdanorum* was observed in the Biobío Region and corresponded to 97.8% and only in two farms located in the north coast this region, the *O. migdanorum* was not detected. The parasitoid *C. chamaeleon* was determined at lower levels than the gall wasp, ranging between 47.9% and 53.8%, in Biobío and La Araucanía Regions, respectively. On the other hand, in the Maule Region the lowest frequency was registered, being observed its presence in 19.2% of the farms evaluated (Figure 3.3).

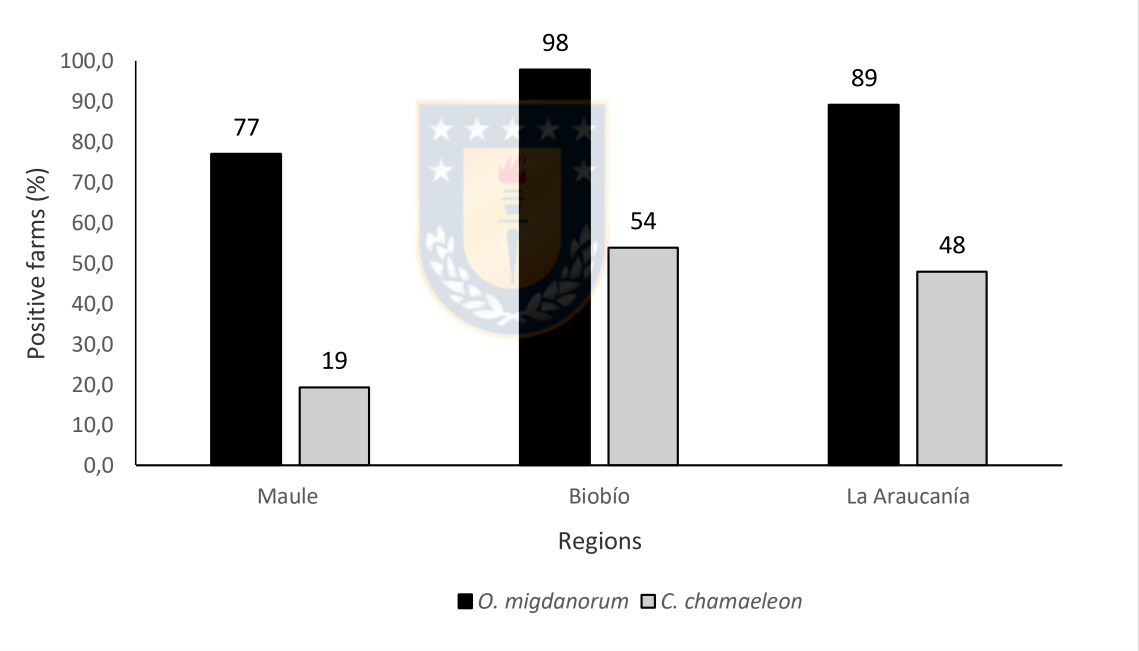

Figure 3.3. Frequency of farms positive (presence/absence) to *Ophelimus migdanorum* and *Closterocerus chamaeleon* per region.

The average population level of *O. migdanorum* for the three regions was 87.0 (individuals in 3 twigs per farm), whereas in the case of *C. chamaeleon* was 11.1. The population levels of both species varied between regions, being increased from north to south (with significant differences only for *O. migdanorum* in La Araucanía Region). The lowest population values were found in the Maule Region, located in the north of the study area and the highest values were found in La Araucanía Region, located further south (Figure 3.3, Table3. 4). Differences between population levels and the estimations of the north respect to the south were 33.5% and 22.1% for *O. migdanorum* and *C. chamaeleon*, respectively.

Table 3.4. Average population of *Ophelimus migdanorum* and *Closterocerus chamaeleon* in farms per region, obtained from three twigs per farm.

Region	**Individuals (N°)**	
	Ophelimus migdanorum	***Closterocerus chamaeleon***
Maule	43.6 ± 8.4 b	4.2 ± 1.2 a
Biobío	87.4 ± 9.8 b	9.9 ± 2.3 a
La Araucanía	129.9 ± 23.2 a	19.0 ± 6.4 a
Total	87.0 ± 24.9	11.1 ± 4.3

Values in parentheses indicate standard error. Different letters indicate significant differences between regions (p ≤ 0.05).

3.5.-DISCUSSION

The presence of *O. migdanorum* and *C. chamaeleon* were determined in Chile in the three regions evaluated in the study, Maule, Biobío and La Araucanía, emerging from galls formed in juvenile and adult twigs of *E. globulus*. *Ophelimus migdanorum* was found in the three regions evaluated, in 92.1% of the farms, with a population level of 87.0 individuals, in 3 twigs per stand (Figure 3.3, Table 3.3). This would indicate its establishment in localities of the country where the plantations of *E. globulus* are concentrated. This represents a phytosanitary threat to the sustainability of the cultivation of this species, together with the effects of *Gonipterus platensis*, detected in the country in 1998 (Beéche, 1999).

Both level and type of damage was not evaluated in this study. However, during the field sampling, gall formation was observed in succulent stems, petiole, blade and midribs associated with death of leaves, twigs and even complete trees. This coincides with observations carried out by Bain (1977) in New Zealand for damage caused by *O. eucalypti* on *E. globulus* (Figure 3.5).

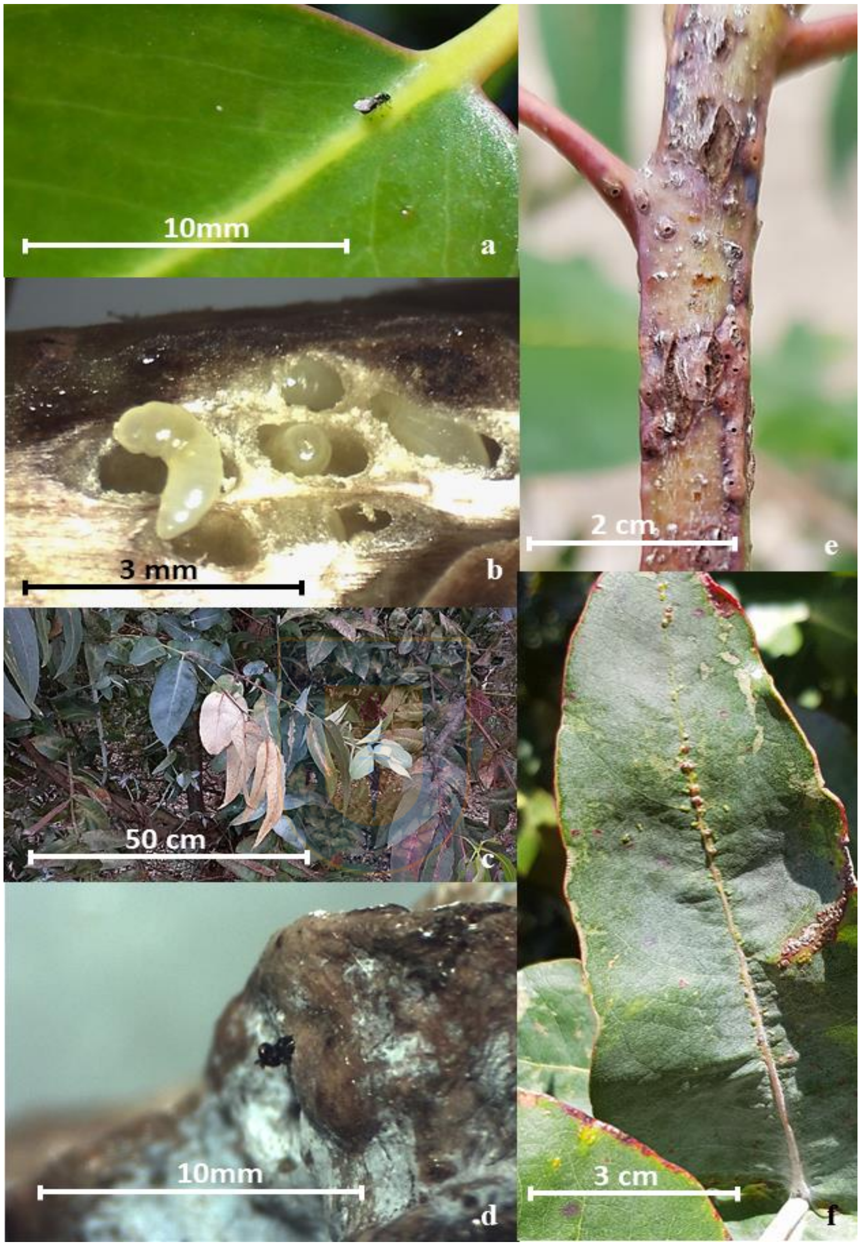

Figure 3.5. (a) *Ophelimus migdanorum* on petiolate spring leaf; (b) cut in gall twig showing 6 larvae/5 mm gall, 40X; (c) dead branch; (d) adult insect emerging from *Eucalyptus globulus* floral capsule, 40X; (e) twig with galls and adult emergency orifice of *Ophelimus migdanorum*; (f) sessile leaf with galls and emergency holes.

The formation of galls in petioles or twigs in repetitive attacks would lead to a decrease in the growth capacity of the twigs by a loss of leaves of terminal twigs, death of the twigs when larger twigs are attacked, decreased growth of adult and young trees and finally the death of the tree (Bain, 1977; Pujade-Villar and Riba-Flinch, 2004; Suma et al., 2018). In Chile, in a seed orchard of *E. globulus* it was observed during 2017 the formation of galls on capsules, associated with *Botryosphaeria* sp., which induces the abortion of such capsules, being estimated loss of up to 50% in the seed production (Molina – Mercader, 2019 unpublished data). According to Branco et al., (2016), regarding the adult of *O. maskelli*, it increases its survival when it feeds on eucalyptus flowers compared to those cases in which only drinking water.

The regions with the highest frequency and population density of *O. migdanorum* corresponded to Biobío and La Araucanía, which concentrated 68.3% (404 454 ha) of the area planted with *E. globulus* established in the country (INFOR, 2018).

The determination of *C. chamaeleon* in the farms evaluated at a presence level of 46.7% would indicate its establishment in Chile, in the three regions prospected. *Closterocerus chamaeleon* has not been introduced in Chile to be used in biological control programs, so its dispersion could have occurred naturally. Among the capabilities described for the wasp, its dispersion capacity can be highlighted. This capacity is favored by the wind, given its reduced size can travel between 100 to 120 km per day in favorable wind conditions (Protasov et al., 2007b). In Israel, it was estimated that the wasp travelled 120 km in a period of 10 to 12 month (Protasov et al., 2007b). On the other hand, in Turkey in 2007 it was determined its transfer from Israel, at 1500 km (Doganlar and Mendel, 2007). Branco et al. (2009) pointed out that the dispersion of this insect in the southern area of the Mediterranean is favored by the high density of its host *O. maskelli* on *E. camaldulensis*. In this context and considering that in his study the highest levels of *C. chamaeleon* coincide with the region with the largest population of *O. migdanorum* (Figure 3, Table 4), the hypothesis that the parasitoid wasp is advancing in its colonization process of the study area from south to north cannot be ruled out. This is supported by the fact of the continuity of the cultivation of *E. globulus* and the dispersion of *O. migdanorum*.

In Chile, pests such as *Rhyacionia buoliana* (Schiff) and *Sirex noctilio* F., have moved forward from south to north, affecting *Pinus radiata* D. Don. *Rhyacionia buoliana* was detected in Los Ríos Region in 1985 and in the Biobío Region in 1991, located 500 km to the north (Lanfranco et al., 1991).

The frequency at standlevel and population density of *C. chamaeleon* estimated in this study were lower than those of *O. migdanorum*. Once the high dispersion potential of *C. chamaeleon* has been known, this could indicate that the natural introduction to the country would be more recent than *O. migdanorum*, which was reported in 2003 as *Ophelimus* sp. (SAG, 2006).

In this work it was not possible to accurately determine the parasitism of *C. chamaeleon* on *O. migdanorum*. However, since this species was determined emerging with *O. migdanorum* in 100% of the cases from the same farms and from the same twig in 99.1% of the breeding chambers, it is possible to propose the hypothesis that *C. chamaeleon* could be performing a parasitic role on *O. migdanorum*. It reinforces the previous approach, the occurrence in collected leaves, of galls with necrosis and others without necrosis, in the first case as action of the phytophagous.

Closterocerus chamaeleon is a recognized parasitoid of *O. maskelli* (Floris et al., 2018), though Protasov et al. (2007b) indicate that it is possible that other species of the gall - inducing *Ophelimus* in leaves of *Eucalyptus* can also serve as hosts. In this study, the emergence of *O. maskelli* was not obtained from the breeding chambers, nor its presence been reported in Chile (Aquino et al., 2015). Further studies are required around this matter, working for instance at the level of gall dissection to identify the species present.

The morphological characteristics of eyes and abdomen used to differentiate *O. migdanorum* from *C. chamaeleon* was effective in 100%, which represents a valuable practical tool in the laboratory, particularly considering the abundance of the populations of these insects. Additionally, the reflected color was used, showing a light metallic green color for *C. chamaeleon* and its way of walking different from that of *O. migdanorum.*

The determination of *C. chamaeleon* in this study constitutes the first reference for the country, in productive areas of *E. globulus*. Its finding could represent, in the case of being found its parasitic condition on *O. migdanorum*, a contribution to the sanitary sustainability of the cultivation of *E. globulus* in the country. Likewise, this could be the first determination *C. chamaeleon*, like possible biocontroller of *O. migdanorum* in the world.

3.6.-CONCLUSIONS

Ophelimus migdanorum is widely distributed in Maule, Biobío and La Araucanía Regions, in Chile.

3.7.-ACKNOWLEDGEMENTS

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DISCUSIÓN GENERAL.

Después de 16 años de la detección de *Ophelimus* en Chile (SAG 2006), en el presente estudio, se determinó mediante el apoyo de herramientas morfológicas y moleculares que los especímenes agallícolas de este género presentes en el país entre las regiones de Valparaíso a Los Lagos configuran o determinan una única y nueva taxa para la ciencia, que no se había descrito previamente; siendo nombrada esta nueva especie como *Ophelimus migdanorum* nov sp., Molina-Mercader (Molina et al., 2019 en prensa) (Diagnósis punto 2.4.1.1 de esta tésis).

Ophelimus migdanorum nov *sp*., se distingue de la especie más cercana descrita, *O. maskelli*, en base a Protasov et al (2007a), por presentar una, dos o tres setas en la vena submarginal (SVS) (Tabla 2.4), por el color del cuerpo del adulto, tono-color y número de anillos de las antenas y número de segmentos funiculares en el flagelo (Figura 2.1; Tabla 2.4). Los cuatro últimos caracteres morfológicos, se mantienen para individuos de *O. migdanorum* que presentan 1, 2 y 3 setas en la vena submarginal. Adicionalmente, difiere en morfología y ubicación de las agallas que induce *O. maskelli* (Aquino et al., 2014) (detalles en el punto 2.2.4).

Consistente con los resultados morfológicos, el análisis de la secuenciación molecular ratificó que los tres grupos de insectos detectados en Chile, con una, dos y tres setas corresponden a la misma especie del género *Ophelimus* y que difieren de *O maskelli* y otros *Ophelimus* spp descritos en el banco de datos genéticos (Tabla 2.2). Además, la recuperación de un grupo monofilético con alto apoyo entre aproximaciones de delimitación confirmaría que las secuencias COI que pertenecen a Chile corresponderían a una nueva especie diferenciable operativamente en un contexto filogenético.

La determinación, en nuestro estudio, de individuos que presentaban 1, 2 y 3 setas pertenecientes a la misma especie de *Ophelimus* (*O. migdanorum* nov sp.), implicó un resultado inesperado, cuyo alcance representa un aporte al conocimiento morfológico del género *Ophelimus*, asociado a su taxonomía. Hasta ahora, la característica morfológica fundamental que había permitido diferenciar entre las especies agallícolas del género *Ophelimus*, era precisamente el número de setas presentes en la vena submarginal (La Salle 2005), así *O. maskelli* que presenta sólo una seta (Protasov *et al*., 2007a), se distingue de *O. eucalypti* que presenta 4 o más setas en la vena submarginal (La Salle, 2005). En nuestro estudio, se determinó individuos de *O. migdanorum* nov. sp de menor tamaño (0,712 – 1,124 mm de largo) que presentaban una seta en la vena submarginal, otro grupo de mayor tamaño (0,846 – 1,253 mm de largo) que poseía dos setas en la vena submarginal y por último otro grupo de individuos más grandes (0,930 – 1,413 mm de largo) (Tabla S2.3) con tres setas en la vena submarginal. De este modo, en nuestro caso, inicialmente habíamos hipotizado que el primer grupo constituído por individuos con una seta podría corresponder a *Ophelimus maskelli*, no reportado en Chile y el segundo y tercer grupo a otras especies de *Ophelimus* agallícolas no descritas. Sin embargo, nuestros resultados descriptivos posteriores, (tanto morfológicos como moleculares) indicarían que, consistentemente, la presencia de setas en la vena submarginal, no sería una característica morfológica que por si sola permitiese distinguir entre a lo menos *O. maskelli* y *O. migdanorum* y que la morfología de antenas y flagelo complenetarían el distingo entre ambas especies, incluso entre individuos que presenten una única seta en la vena submarginal. En la línea de lo anterior en Francia, se detectó individuos de *Ophelimus* con dos, tres o cuatro SVS que no corresponden a *O. maskelli* (Borowiec et al., 2012; Burks et al 2016) y de acuerdo con nuestro análisis molecular tampoco a *O. migdanorum* nov sp. Esta situación confirma la escasa e incompleta información existente para las especies del género *Ophelimus*, (Protasov *et al*. 2007; La Salle, 2005).

El número de setas en la vena submarginal (1, 2 y 3 setas), según nuestro estudio, no se vincula a especies distintas, si no que más bien guarda una estrecha relación con el tamaño de los adultos de la misma especie (*O. migdanorum* nov sp) (Tabla 2.5), cuanto mayor es el número de setas en la vena submarginal, mayor es el tamaño del individuo (Tabla 2.5); situación que al relacionarla con la ubicación de las agallas nos sitúa frete a un potencial caso de polifenismo, en el que los adultos que emergen de las zonas más nutritivas (nervadura central, pecíolo y tallos), son aquellos que poseen de 2 a 3 setas, en tanto que los que emergen de peciolo y lámina de la hoja poseen sólo una seta (Tabla 5, Tabla6). Estos últimos tienen un comportamiento similar al de *O. maskelli* que induce la formación de agallas en la lámina de la hoja (Branco et al., 2009; Protasov et al., 2007b; *O. eucalypti* en tanto se relaciona con el pecíolo de las hojas, margen de la hoja y nervadura, (Bain, 2003), aunque Sánchez

(2003) señala para España que las agallas de los pecíolos o del nervio principal corresponden a otra especie agallicola distinta de *O. eucalypti* (Sánchez, 2003).

La determinación de *O. migdanorum* nov. sp en las 7 zonas de muestreo consideradas para identificación, da cuenta de su dispersión entre las regiones de Valparaíso en el norte (SAG, 2006) y Los Lagos en el sur y su constatación a altos niveles poblaciones en las tres regiones evaluadas, a niveles de 92,1% de los predios evaluados y un nivel poblacional promedio de 87,0 (individuos en tres ramillas/predio) (Figura 3.3, Tabla 3.3), estarían indicando su establecimiento en localidades del país donde se concentra las plantaciones de *E. globulus*, lo que representa una nueva amenaza fitosanitaria para la sustentabilidad del cultivo de esta especie en el país, junto a los efectos de sequía y *Gonipterus platensis* detectado en el país el año 1998 (Béeche, 1999; Molina-Mercader et al., 2019). En este trabajo se observó formación de agallas en tallos suculentos, pecíolo, nervadura central y lámina de hojas, asociados con muerte de hojas, ramas, e incluso árboles completos, coincidiendo con las observaciones realizadas por Bain (1977) en Nueva Zelanda para los daños ocasionados por *O. eucalypti* sobre *E. globulus,* lo cual representa una alerta para el daño potencial de la plaga en el país. Respecto de agallas cerca del peciolo de hojas afectadas La Salle (2005), señala que su abundante formación puede conducir a intensas defoliaciones y vigor en árboles susceptibles, a lo que Bain (1977) y Pujade-Villar & Riba-Flinch (2004) añaden que ataques repetitivos, producirían muerte de ramas y finalmente la muerte de individuos.

Junto a *O. migdanorum nov sp.,* en este estudio *se* determinó por primera vez la presencia de *Closterocerus chamaeleon,* reconocido parasitoide de *O. maskelli* en las tres regiones evaluadas, correspondiente a Maule, Biobío y La Araucanía, Si bien no fue posible determinar con precisión por el diseño de nuestro estudio Considerando que *C. chamaeleon* se determinó emergiendo junto a *O. migdanorum* en 100% de los casos desde los mismos predios y desde una misma ramilla en 99,1% de las cámaras de crianza, permite plantear la hipótesis que *C. chamaeleon* podría estar ejerciendo un rol parasítico sobre *O. migdanorum,* lo que representa aporte a la sustentabilidad del cultivo del *E. globulus* en las regiones de Maule, Biobío y La Araucanía,que concentran el 76% de la superficie cubierta con esta especie en el país (INFOR, 2018). Alineado con lo anterior se debe indicar que en este estudio no se obtuvo la emergencia de *O. maskelli* desde las cámaras de crianza, tampoco ha sido reportada su presencia en Chile (Aquino et al., 2015), por lo que en el alcance de este trabajo se descarta asociación de *C. chamaeleon* con *O. maskelli*. La posibilidad de que *C. chamaeleon*, parasitara también a otros inductoras de agallas de la hoja en *Eucalyptus* distintos a *O. maskelli,* fue observada por Protasov et al*.,* (2007b).

Closterocerus chamaeleon no ha sido introducido al país para ser usado en programas de control biológico, por lo que su introducción y posterior dispersión por el territorio nacional podría haber ocurrido naturalmente. Su introducción también podría haber ocurrido como consecuencia del intenso intercambio comercial con otros países (Castillo et al., 2018). En cualquier caso, la menor frecuencia a nivel predial y densidad poblacional de *C. chamaeleon* estimadas en este estudio (Figuras 3.3, 3.4; Tablas 3.2, 3,4) considerando el alto potencial de dispersión de *C. chamaeleon* ((Protasov et al., 2007b, Farrow, 1981), podría indicar que su introducción natural al país sería más reciente que *O. migdanorum*, que fue reportado el año 2003 como *Ophelimus* sp. (SAG; 2006). La dispersión, de la microavispa *C. chamaeleon*, se ve favorecida por el viento, dado a su reducido tamaño, llegando a desplazarse entre 100 a 120 km por día en condiciones de viento dominante favorable (Protasov et al., 2007b, Farrow, 1981), Branco et al., (2009), agrega que la dispersión de este insecto en la zona sur del Mediterráneo es favorecida por la alta densidad poblacional de su hospedero *O. maskelli* sobre *E. camaldulensis.* En este contexto y considerando que en este estudio los mayores niveles de *C. chamaeleon* coinciden con las regiones con mayor población de *O. migdanorum* (Figura 3.3, Tabla 3.4), no se puede descartar la hipótesis que la avispa parasitoide se encuentre avanzando en su proceso de colonización del área de estudio desde sur hacia el norte, favorecida por la continuidad del cultivo de *E. globulus* y la dispersión de *O. migdanorum.* En Chile, plagas como *Rhyacionia buoliana (*Schiff*)* *Sirex noctilio* F., han avanzado de sur a norte, en este caso afectando a *Pinus radiata* D Don. *R. buoliana* fue detectada en la región de Los Ríos en 1985 y en Biobío el año 1991 (Lanfranco et al., 1991), situada 500 kilómetros al norte.

Este estudio constituye una contribución al conocimiento del género *Ophelimus*, tanto en aspectos morfológicos como moleculares. Un elemento importante para considerar es la falta de información sobre la integridad taxonómica de otras especies de *Ophelimus*, que en su mayoría no cuentan con estudios sistemáticos exhaustivos. (SAG, 2006; La Salle, 2005); encontrándose solo *O. maskelli* con un mayor grado de información disponible (Protasov et al., 2007ª) y en proceso una nueva especie de *Ophelimus* detectada en Francia (Branco et al., 2016). En el Genbank había solo 46 secuencias del gen mitocondrial citocromo oxidasa subunidad I para el género Ophelimus. Finalmente, BOLDSYSTEMS registró solo 7 especímenes con códigos de barras. El presente trabajo basado en marcadores moleculares demuestra que es posible distinguir y descubrir entidades utilizando caracteres complementarios y respalda los generados por la morfología tradicional.

La determinación de *C. chamaeleon* en este estudio constituye la primera para el país en áreas productivas de *E. globulus* y su hallazgo podría representar, en el caso de constatarse su condición parasitaria sobre *O. migdanorum,* la primera determinación sobre otra especie distinta de *O. maskelli* en el mundo y junto con ello un aporte a la sustentabilidad sanitaria del cultivo de *E. globulus* en el país.

CONCLUSIONES GENERALES.

- La especie de *Ophelimus*, presente en Chile desde el año 2003, denominada *O. migdanorum* Molina_Mercader, correponde a una nueva especie de este género para la ciencia.
- El número de setas en la vena submarginal se relaciona directamente con el tamaño de los individuos de *Ophelimus migdanorum* y no representa un carácter válido para distinguir entre especies de Ophelimus.
- *Ophelimus migdanorum* se encuentra presente entre las regiones de Valparaíso a los lagos; encontrándose ampliamente distribuido entre las regiones de maule a la araucanía en un 92,1% de los predios evaluados.
- *Closterocerus chamaeleon* se encuentra presente en el área de estudio (Regiones de Maule, Biobío y Araucania), a nivel de 46,7% de los predios evaluados, ememrgiendo en un 99,1% de los casos junto a *Ophelimus migdanorum*.

APORTES TÉSIS

Taxonomía, Descripción y datos biológicos de una nueva especie de *Ophelimus* Haliday (1844) (Hymenoptera: Eulophidae)

- ✓ Conocimiento de una nueva especie de Ophelimus en Chile y mundo
- ✓ El número de setas en la vena submarginal no es carácter suficiente para distinguir entre especies y podría definitivamente ser polifenismo
- ✓ Estamos frente a un nuevo caso de Polifenismo para la ciencia
- ✓ Distribución en Chile de esta nueva especie.
- ✓ Distribución de su posible controlador biológico *Closterocerus chamaeleon,* siendo en si un Potencial controlador.
- ✓ Nuevas Propuestas de trabajo para profundizar en esta especie relacionadas tanto con Ophelimus como con Closterocerus

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