

UNIVERSIDADE FEDERAL DE VIÇOSA

**Genetic Diversity and Ecological Modelling of the Invasive Gall Wasp
Ophelimus maskelli (Hymenoptera: Eulophidae) Across Native and Invaded
Ranges**

Paulo Cesar Afonso Neto
Doctor Scientiae

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PAULO CESAR AFONSO NETO

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Thesis submitted to the Ecology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Maykon Passos Cristiano

Co-adviser: Danon Clemes Cardoso

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Dedico a meus avós.

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ABSTRACT

NETO, Paulo Cesar Afonso, D.Sc., Universidade Federal de Viçosa, June, 2026.
Genetic Diversity and Ecological Modelling of the Invasive Gall Wasp *Ophelimus maskelli* (Hymenoptera: Eulophidae) Across Native and Invaded Ranges. Adviser: Maykon Passos Cristiano. Co-adviser: Danon Clemes Cardoso.

Biological invasions represent an increasing threat to biodiversity, forest production, and the stability of managed ecosystems. This thesis investigates the invasion risk, evolutionary history, and phylogeographic structure associated with *Ophelimus maskelli*, an Australian gall-inducing wasp related to damage in *Eucalyptus* species. The study combines potential distribution modelling, molecular, phylogenetic, and phylogeographic analyses to understand the processes that favor the dispersal, establishment, and identification of invasive lineages. Ecological modelling, based on MaxEnt and CLIMEX, evaluated the climatic suitability of *O. maskelli* under current and future conditions, with emphasis on the risk to *Eucalyptus* plantations in Brazil. The results indicate climatically favorable areas for the species and highlight differences between correlative models and climate-matching approaches, allowing a broader assessment of its establishment potential. The integration of climatic distribution, *Eucalyptus* cultivation areas, and possible dispersal routes contributes to the identification of priority regions for phytosanitary monitoring. Subsequently, sequences of the mitochondrial COI gene were used to reconstruct evolutionary relationships and validate diagnostic regions with potential application in molecular identification. The molecular analyses showed that the *Ophelimus* complex includes genetically distinct lineages, some of which may have been previously misidentified as *O. maskelli*, reinforcing the importance of molecular approaches to reduce taxonomic identification errors in occurrence records. This thesis demonstrates that the combination of ecological modelling, phylogeography, and DNA barcoding provides a robust framework for understanding biological invasions in insects associated with planted forests, while also supporting strategies for surveillance, management, and prevention of new introductions.

Keywords: *Ophelimus maskelli*; Biological invasion; Ecological modelling; *Eucalyptus*; Phylogeography; DNA barcoding.

RESUMO

NETO, Paulo Cesar Afonso, D.Sc., Universidade Federal de Viçosa, junho de 2026. **Diversidade Genética e modelagem ecológica da vespa invasora *Ophelimus maskelli* (Hymenoptera: Eulophidae) ao longo de suas áreas nativas e invadidas.** Orientador: Maykon Passos Cristiano. Coorientador: Danon Clemes Cardoso.

As invasões biológicas representam uma ameaça crescente à biodiversidade, à produção florestal e à estabilidade de ecossistemas manejados. Esta tese investiga o risco de invasão, a história evolutiva e a estrutura filogeográfica associada à espécie *Ophelimus maskelli*, uma vespa galhadora de origem australiana relacionada a danos em espécies de *Eucalyptus*. O estudo combina modelagem de distribuição potencial, análises moleculares, filogenéticas e filogeográficas para compreender os processos que favorecem a dispersão, o estabelecimento e a identificação de linhagens invasoras. A modelagem ecológica, baseada em MaxEnt e CLIMEX, avaliou a adequabilidade climática de *O. maskelli* sob condições atuais e futuras, com ênfase no risco para plantações de *Eucalyptus* no Brasil. Os resultados indicam áreas climaticamente favoráveis à espécie e destacam diferenças entre modelos correlativos e de correspondência climática, permitindo uma avaliação mais ampla do potencial de estabelecimento. A integração entre distribuição climática, áreas de cultivo de *Eucalyptus* e possíveis rotas de dispersão contribui para a identificação de regiões prioritárias para monitoramento fitossanitário. Em seguida, sequências do gene mitocondrial COI foram utilizadas para reconstruir relações evolutivas e validar regiões diagnósticas com potencial aplicação em identificação molecular. As análises moleculares mostraram que o complexo *Ophelimus* inclui linhagens geneticamente distintas, algumas das quais podem ter sido erroneamente identificadas como *O. maskelli*, reforçando a importância de abordagens moleculares para reduzir erros de identificação taxonômica em registros de ocorrência. Esta tese propõe que a combinação de modelagem ecológica, filogeografia e DNA barcoding fornece uma estrutura robusta para compreender invasões biológicas em insetos associados a florestas plantadas, além de apoiar estratégias de vigilância, manejo e prevenção de novas introduções.

Palavras-chave: *Ophelimus maskelli*; Invasão biológica; Modelagem ecológica; *Eucalyptus*; Filogeografia; DNA barcoding.;

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Introdução Geral

Invasões biológicas é um dos principais desafios no manejo de plantações florestais (Lanfranco and Dungey, 2001; Floris et al., 2018; Hirsch et al., 2020). O aumento contínuo das introduções de pragas pode levar ao estabelecimento de espécies exóticas em diferentes fitofisionomias ao redor do mundo. Compreender a história evolutiva desses organismos é importante para gerar ações de controle, através da investigação da biologia, ecologia, impactos e estratégias de manejo (Brockerhoff and Liebhold, 2017). A expansão do cultivo de eucaliptos no mundo facilitou a introdução de artrópodes herbívoros australianos, promovendo padrões variados de colonização. Enquanto algumas dessas espécies se estabeleceram sem causar problemas, outras levaram a prejuízos (Hirsch et al., 2020).

A microvespa galhadora australiana *Ophelimus maskelli* (Hymenoptera: Eulophidae), integra um grupo cuja posição taxonômica passou por diversas revisões ao longo das últimas décadas. Inicialmente, o gênero *Ophelimus* foi incluído na tribo Ophelimini, dentro da subfamília Eulophinae (Bouček, 1988). Posteriormente, análises moleculares utilizando o gene 28S demonstraram que apenas os gêneros *Ophelimus* e *Australsecodes* permaneciam em Ophelimini, levando à reorganização do grupo dentro de Eulophidae, fora de Eulophinae (Gauthier et al., 2000). Mais recentemente, evidências morfológicas e filogenéticas resultaram na elevação de Ophelimini ao status de subfamília, Opheliminae, atualmente reconhecida como grupo-irmão de Eulophinae (Burks et al., 2011). Essa vespa destaca-se por sua ampla associação com plantações de eucalipto e seu hábito, que altera diretamente a morfologia foliar da planta hospedeira (Gauthier et al., 2000; Gonzalez and Hernández, 2025). As galhas formadas por essa espécie ocorrem principalmente na lâmina foliar e envolvem processos anatômicos especializados, incluindo hiperplasia, espessamento do mesófilo, diferenciação de tecido nutritivo e formação de uma camada protetora de esclerênquima, podendo comprometer o crescimento e a produtividade das plantas atacadas (Wilson Fernandes e Carlos Santos, 2014; Gonzalez and Hernández, 2025).

Para evitar a introdução e possível estabelecimento de *O. maskelli* em novas regiões, especialmente no Brasil, onde não há registros atuais de sua ocorrência, é fundamental antecipar as áreas com maior risco de invasão, por meio da identificação antecipada das regiões climaticamente adequadas ao estabelecimento da espécie, permitindo direcionar ações de vigilância, inspeção fitossanitária e monitoramento para áreas consideradas de maior risco (Mota et al., 2022). A introdução em longas distâncias pode ocorrer principalmente por meio do transporte de material vegetal de *Eucalyptus* infestado, incluindo mudas, plantas para

plantio, ramos e folhas contendo galhas. Dessa forma, a inspeção fitossanitária de material vegetal importado em portos, aeroportos, postos de fronteira e viveiros constitui uma medida importante para reduzir o risco de entrada e dispersão da praga (Dittrich-Schröder et al., 2020).

Infestações por *O. maskelli* podem gerar danos econômicos na indústria do eucalipto (Sofia Revez dos Santos et al., 2009), somando-se as perdas de 1,2 trilhões de dólares causadas por espécies invasoras ao redor do mundo, entre 1980 e 2019 (Turbelin et al., 2023). Além do impacto financeiro, a invasão biológica dessa vespa pode alterar a dinâmica das comunidades vegetais e favorecer a disseminação de outros organismos associados as galhas e ao material vegetal infestado (Pan et al., 2024).

Entender as diferenças moleculares entre populações de *O. maskelli* encontradas em diferentes habitats e propor mapas de adequabilidade futura para a espécie alvo, se configuram como importantes ferramentas capazes de evitar danos posteriores e contribuir para o bem-estar social e florestal. A fim de contribuir para o maior entendimento da história evolutiva da vespa, este trabalho investigou a distribuição geográfica, padrões de estabelecimento de *O. maskelli* em diferentes zonas ecológicas, perfil filogenético das populações observadas e processos históricos associados a diferenciação das linhagens no complexo. Assim será possível estimar o estabelecimento desta espécie em novas áreas, sob a perspectiva de cenários climáticos atuais e futuros. Este estudo também buscou compreender como eventos históricos e pressões ambientais podem ter influenciado a estruturação genética e os padrões de diversificação observados entre populações distribuídas em diferentes regiões geográficas (Borowiec et. al., 2019, Naves et. al, 2026), contribuindo para um melhor entendimento da dinâmica evolutiva do complexo *Ophelimus*.

Entender as diferenças moleculares entre populações de *Ophelimus maskelli* encontradas em diferentes habitats e propor mapas de adequabilidade futura para a espécie alvo, se configuram como importantes ferramentas capazes de evitar danos posteriores e contribuir para o bem-estar social e florestal das áreas objetivadas.

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Capítulo 1

Climate-Based Invasion Risk Modeling of *Ophelimus maskelli* (Hymenoptera: Eulophidae) in Brazilian *Eucalyptus* Forestry

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1. Abstract

The increasing occurrence of invasive species highlights the importance of developing models to predict their potential spread and harmful impacts. *Ophelimus maskelli*, a gall-inducing eulophid wasp, is a species capable of tolerating a range of environmental conditions and has been invading regions around the globe. This species is endemic to Australia and can now be found in Africa, Asia, Europe, and North America. This study used Species Distribution Modeling to project the future distribution of *O. maskelli* based on current known distributions under a climate change scenario. The modeling used Shared Socioeconomic Pathways (SSP) climate data, specifically, the SSP5-8.5 scenario, representing a pathway of a high-emission trajectory with minimal mitigation efforts. The modeling predicted a wide potential distribution of *O. maskelli* in Brazil in relation to forest plantations under current climatic conditions, followed by a contraction in some areas under this future climate scenario. This study provided early insights into the potential risk of establishment by *O. maskelli* in Brazil, using MaxEnt and two CLIMEX models. By focusing on areas of eucalypt plantations and nearby ports, we integrated correlative and mechanistic modeling to identify and anticipate the risk of pest establishment, which is crucial for implementing measures to mitigate ecological and economic damage.

Keywords: forest health, invasive species, Hymenoptera, climate change, Eulophidae, *Ophelimus maskelli*, *Eucalyptus*, plantations

2. Introduction

The arrival, establishment, and continuous spread of introduced species in new regions can cause economic damage, ecological degradation, and public health concerns (Andersen et al., 2004). These impacts may result from invasive species altering the composition and abundance of native communities and, in some cases, driving native species to extinction (Vitousek et al., 1997; Mooney and Cleland, 2001; Mollot et al., 2017).

The dissemination of *Eucalyptus* plantations worldwide has paved the way for the introduction of Australian-origin insect herbivores, resulting in diverse colonization patterns and posing challenges in multiple regions (Hirsch et al., 2020, Paine et al., 2011). The dispersal of these Australian endemic insects to areas where eucalypts are cultivated has been observed since the 19th century (Withers, 2001). As invasion rates accelerate, the processes underlying the establishment and spread of invasive species have become an increasingly important focus of ecological research. (Mack et al., 2000; Paine et al., 2010; Stazione et al., 2025).

Plantation forestry relies on a limited number of *Eucalyptus* species and/or clones besides being over 700 species of this and related genus, thereby opening new areas where forestry pests can spread (Garnas et al., 2013). Gall-forming wasps can become a particular problem in this scenario, as they include some of the most threatening species to non-native *Eucalyptus* (Paine et al., 2011). Hurley et al. (2016) reported a marked increase in invasive Hymenoptera associated with non-native *Eucalyptus*, making them the third most frequently recorded group of invasive insects on these trees. This trend was further supported by Dittrich-Schröder et al. (2020), who documented at least 16 invasive gall-forming Hymenoptera associated with plantation-grown *Eucalyptus*, most of which became invasive during the preceding two decades.

Australian gall-formers can be divided into groups based on the plant parts they target (seed, stem, or leaf galls) (Dittrich-Schröder et al., 2020). A prominent driver of this recent surge in invasive gall inducers is the eulophid wasp, *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae), which is now established in over 20 countries (Dittrich-Schroder et al. 2020). This native Australian eulophid wasp was first detected in Europe in 2000 (Branco et al., 2016) and rapidly spread to the Mediterranean Basin, Asia, South Africa and North America (Protasov et al., 2007; Branco et al., 2009; Garcia et al., 2019). *Ophelimus maskelli* is

a leaf gall-inducing and a uniparental species that lays its eggs near the petiole, on the leaf blade of young leaves on the lower canopy of the tree (Branco et al., 2016). Galls initiated by *O. maskelli* resemble blisters, with one individual per gall. Leaves may exhibit galls of various ages, with up to three generations per year in temperate climates (Protasov et al., 2007).

Ophelimus maskelli belongs to a group of sixteen gall-forming hymenopterans that have become invasive on Eucalyptus in the last two decades, gaining economic importance as these introductions have led to large economic losses and significant harm to local ecosystems (Dittrich-Schröder et al., 2020). *Ophelimus maskelli* is the second most widely distributed invasive gall-inducer wasp on Eucalyptus, exceeded only by *Leptocybe invasa* (Eulophidae) (Dittrich-Schröder et al., 2020). Other invasive *Ophelimus* species like *O. mediterraneus* (Borowiec et al., 2019), *O. migdanorum* (Molina-Mercader et al., 2019), *O. eucalypti* (Protasov et al., 2007), and more recently, *Ophelimus ramorum* sp. nov. (Naves et al., 2026) have been recognized outside their Oceanian native range reinforcing the worldwide emergence of the genus as an important group of Eucalyptus pests.

In addition to damaging eucalypts, *O. maskelli* can cause health and nuisance problems for nearby residents during adult emergence, when large swarms of the wasp affect residential and industrial areas surrounded by ornamental Eucalyptus trees (Protasov et al., 2007; Quang Thu et al., 2010). So, it is essential to consider that climate change can exacerbate the effects of invasive species in new habitats, as such species tend to tolerate a broad range of conditions, which could make management efforts more challenging (Hellmann et al., 2008).

In Brazil, where Eucalyptus plantations cover approximately 8.1 million hectares (IBÁ, 2025) and represent a key component of the forestry sector, the threat posed by *O. maskelli* is concerning. Extensive monocultures with low genetic diversity can create ideal conditions for the establishment and rapid spread of this exotic pest (Liu et al., 2018; Wondafrash et al., 2020). The absence of natural enemies and favourable climatic conditions can facilitate population outbreaks (Ziesche et al., 2024), which can lead to defoliation, reduced growth, increased costs, and potential quarantine restrictions from international markets (Santos da Mota et al., 2024). This scenario underscores the persistent biosecurity risk associated with gall-inducing wasps in Brazilian Eucalyptus plantations, as recognized by the inclusion of *O. maskelli* on Brazil's list as Category A1 (absent) of quarantine pests in the latest phytosanitary regulations of Brazil (MAPA, 2025).

Increases in global travel and trade have driven a rise in invasive alien insect pests, including gall-forming hymenopterans (Csóka et al., 2017), making it imperative to identify

where species may invade as a biosecurity measure (Sutherst, 2014; Kriticos et al., 2015). We employed complementary modelling approaches to predict areas potentially susceptible to *O. maskelli* invasion within Brazil: maximum entropy modelling (MaxEnt) (Phillips et al., 2006, 2017), and regional climate-matching and climate-based niche modelling (CLIMEX) (Sutherst et al., 2007). Integrating these three methodologies allowed to identify regions at higher risk based on climatic suitability and species occurrence records. Our nationally focused assessment aims to identify areas of eucalypt plantations in Brazil that may be more exposed to this gall wasp under current and climate change scenarios.

3. Materials and Methods

3.1. Spatial modelling

Three ecological model approaches were combined to estimate the invasive potential of the gall-forming wasp *O. maskelli* providing complementary perspectives on climatic suitability. MaxEnt estimates suitability from statistical associations between known occurrences and environmental variables; CLIMEX regional climate matching identifies areas with climates similar to those in the known distribution; and the CLIMEX species model evaluates whether local climates satisfy biologically interpretable requirements for growth, survival, and generation completion. Comparing these outputs allowed us to distinguish areas supported by multiple lines of evidence from areas where predictions depended strongly on model structure or assumptions.

3.1.1. Maxent

MaxEnt (version 3.4.4) was employed to model the potential distribution of *O. maskelli* under current and future climate conditions. Ecological niche modelling (ENM) is a widely used approach for predicting the potential distribution of species based on associations between occurrence records and environmental variables (Elith et al., 2006; Yackulic et al., 2013). Within this framework, MaxEnt is a machine-learning algorithm that estimates species suitability from presence-only occurrence data and environmental predictors (Phillips et al., 2006, 2017). One of its main advantages is its ability to generate robust predictions from presence-only data, and it has been shown to perform comparatively well against other species distribution modelling algorithms (Elith et al., 2006; Kumar and Stohlgren, 2009). In the present study, we developed a novel MaxEnt model for *O. maskelli* specifically tailored to Brazilian eucalypt landscapes.

3.1.2. CLIMEX Regional Climate Matching

CLIMEX simulations (Sutherst et al., 2007) are based on long-term climatic averages from the CliMond dataset (Kriticos et al., 2012). CLIMEX Match Climates (Regional) was used to estimate the climatic similarity between the known native and invaded distribution of *O. maskelli* and the projected areas in Brazil. The model output was expressed as the Composite Match Index (CMI). This index ranges from 0, indicating poor climatic similarity, to 1, indicating a perfect climatic match. Following previous CLIMEX applications, values above 0.7 were considered indicative of a biologically meaningful or good climate match (Kriticos et al., 2015). Therefore, only areas with CMI greater than 0.7 were displayed on the CLIMEX maps.

3.1.3. CLIMEX Compare Locations

A mechanistic climatic suitability model for *O. maskelli* was developed in CLIMEX using Compare Locations. Although a recent CLIMEX model for *O. maskelli* has been published (Cheng et al. 2025), we developed an independent parameterization because our objective was to evaluate climatic suitability specifically in relation to Brazil's eucalypt plantation estate, using a parameter set explicitly calibrated against marginal native and invaded-range records and documented with parameter-level biological rationale. The model was iteratively parameterized to reproduce the known native and invaded distribution of the species while maintaining biologically realistic global predictions. Calibration was guided by occurrence patterns in south-eastern Australia, Mediterranean Europe, California, South Africa, and South-East Asia, including Vietnam and North Sumatra. The species was modelled as a warm-temperate to subtropical gall wasp capable of tolerating Mediterranean summer drought, humid subtropical and monsoonal climates, and mild winter conditions, but limited by sustained cold, extreme aridity, and persistently saturated rainforest climates. Temperature-growth parameters were set to allow development from 9 °C, with optimal growth between 16 and 26 °C and an upper developmental limit of 34 °C. Cold stress was configured to accumulate gradually below 6 °C, while heat stress accumulated above 34 °C. Moisture parameters were selected to permit persistence across seasonally dry Mediterranean climates and humid subtropical or monsoonal regions, while excluding deserts and the wettest equatorial rainforest zones. No light or diapause constraints were included, consistent with the apparent capacity of the species for multiple generations per year under favourable conditions. The final parameter set was visually evaluated across continents to ensure consistent predictions within the known native and invaded ranges

and to provide a biologically plausible basis for assessing climatic suitability in Brazil. See **Table 3** in the **Supplementary Materials Anex III** for the full parameter set.

3.2. Climatic variables and spatial resolution

Environmental data were selected according to the requirements of each modeling approach. For MaxEnt, we used bioclimatic predictor variables from WorldClim, which provide biologically meaningful summaries of temperature and precipitation seasonality and are widely used in species distribution modelling (Hijmans et al., 2005; Adhikari et al., 2012; Yang et al., 2013; Fick and Hijmans, 2017). Nineteen bioclimatic variables were initially considered as potential predictors of *O. maskelli* habitat suitability (**Table 1**). Current WorldClim layers were downloaded at 30 arc-second spatial resolution, approximately 1 km at the equator. Because MaxEnt is sensitive to collinearity among predictor variables, multicollinearity was assessed before model fitting. Raster values were extracted using the raster package in R, and pairwise correlations among candidate variables were calculated. Strongly correlated variables were removed progressively before final model calibration. The R script used for variable screening and model calibration is provided in Supplementary Material Annex 1, together with details of the ENMeval, dismo, raster and sp packages used in the analysis.

CLIMEX analyses used the CliMond climate dataset, which provides the direct climatic inputs required by CLIMEX, including temperature, rainfall and relative humidity variables used to calculate growth and stress indices. Unlike MaxEnt, the CLIMEX species model is parameterized mechanistically using biologically interpretable temperature, moisture, and stress parameters. Therefore, correlation screening of bioclimatic predictor variables was not required for CLIMEX. The CLIMEX species model and regional climate-matching analyses were run using CliMond climate layers at 10 arc-minute spatial resolution, approximately 18.5 km at the equator. Consequently, CLIMEX outputs were interpreted as broad climatic suitability surfaces rather than fine-scale local predictions. This distinction is important in coastal, mountainous or topographically complex regions, where 10 arc-minute climate grids may smooth local climatic variation

Future climatic suitability was assessed using projections centered on 2050, represented by 2041–2060 climate averages. For MaxEnt, future bioclimatic layers were obtained from WorldClim using ACCESS-CM2 under the SSP5-8.5 scenario. For CLIMEX, corresponding ACCESS-based future climate layers were obtained from CliMond. ACCESS-CM2 is a global climate model used within the CMIP6 framework and assessed in relation to the IPCC Sixth

Assessment Report (Bi et al., 2020; Eyring et al., 2016; Firpo et al., 2022). The SSP5-8.5 scenario represents a high-emissions, fossil-fuel-intensive pathway and was used here as a conservative high-risk scenario for evaluating potential shifts in climatic suitability. Baseline climatic conditions were represented using climate data centred on 1995, corresponding to long-term climate averages for the late twentieth to early twenty-first century, depending on the source dataset.

For MaxEnt, polar regions were excluded from the analysis's spatial extent. Unlike Santos da Mota et al. (2024), who applied a 10 km spatial filtering procedure using climatic layers at 5 arc-minute resolution, no additional distance-based thinning was applied here because the WorldClim layers used for MaxEnt were available at a finer spatial resolution. Applying a coarse filtering distance could remove records located in distinct environmental cells. Instead, duplicate presence records occurring within the same environmental raster cell were removed during MaxEnt processing, and records lacking valid environmental data in any predictor layer were excluded before model calibration. A bias file was also incorporated to account for uneven sampling effort during background point selection. From an initial dataset of 445 occurrence records, 210 valid, non-duplicated records were retained for the final cross-validated MaxEnt model.

All model outputs were imported into QGIS for mapping and comparison. Model predictions for Brazil were overlaid with mapped plantation areas to identify where climatic suitability coincided with host availability and plantation exposure. Suitability outputs were therefore interpreted as potential climatic suitability, not as predicted realized distribution, because establishment risk also depends on host presence, propagule pressure, nursery and planting-stock movement, local management, and surveillance.

Table 1. Environmental variables used to predict *Ophelimus maskelli* occurrence. The table includes all 19 bioclimatic variables initially considered. Variables selected for the final model after multicollinearity analysis are highlighted.

Code	Climatic variables	Unit
BIO 01	Annual mean temperature	°C
BIO 02	Mean diurnal range (mean of monthly (max temp - min temp)	°C
BIO 03	Isothermality (BIO2/BIO7) ($\times 100$)	
BIO 04	Temperature seasonality (standard deviation $\times 100$)	°C of V

BIO 05	Max temperature of warmest month	°C
BIO 06	Min temperature of coldest month	°C
BIO 07	Temperature annual range (BIO5-BIO6)	°C
BIO 08	Mean temperature of wettest quarter	°C
BIO 09	Mean temperature of driest quarter	°C
BIO 10	Mean temperature of warmest quarter	°C
BIO 11	Mean temperature of coldest quarter	°C
BIO 12	Annual precipitation	mm
BIO 13	Precipitation of wettest month	mm
BIO 14	Precipitation of driest month	mm
BIO 15	Precipitation seasonality (coefficient of variation)	CV %
BIO 16	Precipitation of wettest quarter	mm
BIO 17	Precipitation of driest quarter	mm
BIO 18	Precipitation of warmest quarter	mm
BIO 19	Precipitation of coldest quarter	mm

3.3. Occurrence data

Occurrence records for *O. maskelli* were compiled from the Global Biodiversity Information Facility (GBIF) and supplemented with records reported in the scientific literature (Figure 1). Records were screened for obviously erroneous localities before use in the modelling. The final occurrence dataset included records from the native range in Australia and from invaded regions, including Mediterranean Europe, California, South Africa, and South-East Asia. These records were used both to train the MaxEnt model and to evaluate the biological plausibility of the CLIMEX outputs.

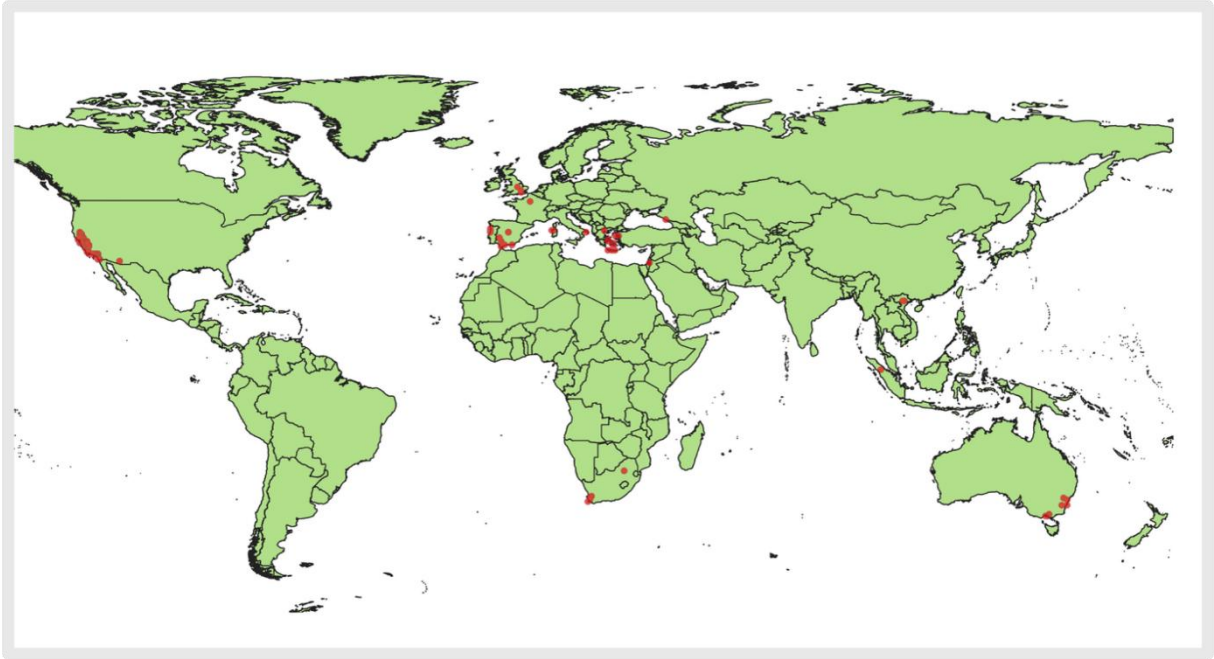


Figure 1. Global distribution of the eulophid wasp *Ophelimus maskelli* (Hymenoptera: Eulophidae) based on Global Biodiversity Information Facility (GBIF) and literature records.

3.4. Detection of Eucalyptus Cultivation Areas with Invasion Potential

Data on areas in Brazil engaged in plantation forestry were retrieved from the Ministry of Agriculture, Livestock and Supply (MAPA), available at MapBiomas, (2024), based on records from the year 2025. The main plantation areas are concentrated in the states of Minas Gerais (2,210,212 ha), São Paulo (1,002,923 ha), Mato Grosso do Sul (1,515,250 ha), Bahia (639,480 ha), Paraná (459,590 ha), and Rio Grande do Sul (631,805 ha), forming connected *Eucalyptus* forestry production landscapes associated with timber, charcoal, cellulose, and bioenergy industries.

To assess which of these areas may be susceptible to invasion, we overlaid the habitat suitability maps generated with MaxEnt, the Composite Match Index (CMI), and the Ecoclimatic Index (EI) generated by CLIMEX with the distribution of forest plantations in Brazil. This visual spatial intersection allowed us to identify regions where current forest plantations coincide with predicted suitable habitats. All spatial analyses and map processing were carried out using QGIS (QGIS Development Team, 2024).

3.5. Potential Invasion Pathways in Eucalyptus Cultivation Areas

To identify areas with potential for introduction followed by secondary spread, the habitat suitability output EI in CLIMEX was overlaid with major transport infrastructure layers (Ministry

of Transport, Brazil; online sources of city locations). This included major cities, large maritime ports, federal highways, railway networks, and international airports.

4. Results

For the MaxEnt model, the multicollinearity analysis resulted in a reduced set of bioclimatic variables with correlation values below 0.8, which were retained for model calibration (Figure 2). These selected variables provided the framework for evaluating each predictor's contribution to the MaxEnt model. The MaxEnt model calibration test for *O. maskelli* yielded consistent performance metrics, with AUCtrain = 0.9605 and AUCtest = 0.9599 ± 0.018. According to the input variables, precipitation of warmest quarter (BIO18) contributed 47.18%, mean temperature of warmest quarter (BIO10) 26.32%, and isothermality (BIO3) 22.91%, respectively (Table 2). Precipitation seasonality was the lowest contributing climatic variable (0.00%) and did not contribute to the model when the variables were analysed together but showed moderate importance (training gain = 0.1940) when analysed alone in the jackknife test. The jackknife test (**Figure 3**) showed that precipitation of warmest quarter (BIO18) had the highest training gain (0.8369) when considered alone (Table 2).

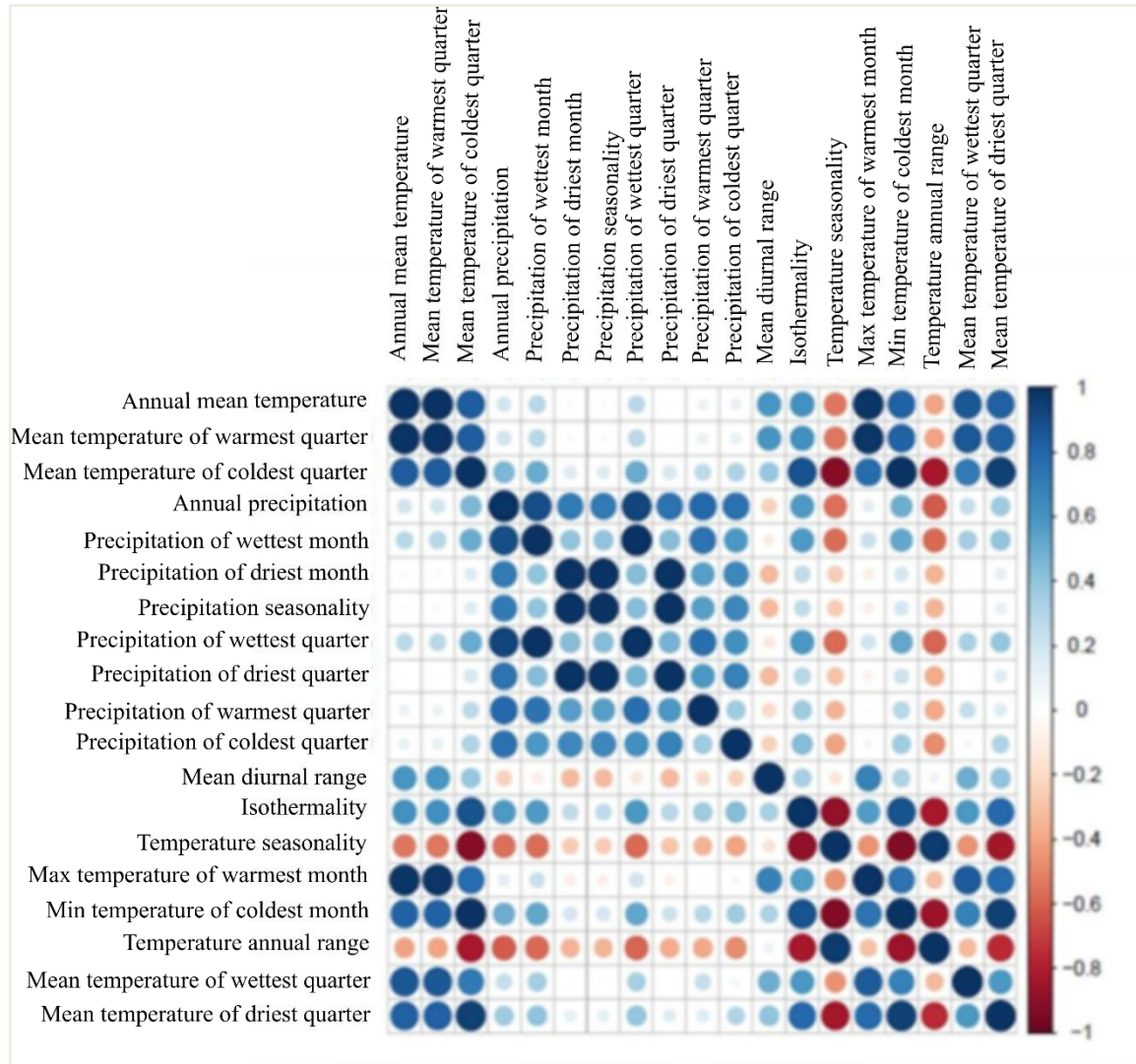


Figure 2. Point size and color intensity indicate the strength of the correlation. Cells with non-significant correlations ($p > 0.05$) were left blank to ensure robust variable selection.

Table 2. MaxEnt model performance and variable importance. AUC values and regularized training gain describe model accuracy. Percent contributions indicate the relative influence of each variable. Jackknife training gains show the explanatory power of each variable.

Parameter	Value	Training Gain
Training AUC	0.9605	
Test AUC	0.9599 ± 0.018	
Regularized Training Gain	1.9765	
BIO18 (Precip. Warmest Quarter)	47.18%	0.8369
BIO10 (Mean Temp. Warmest Quarter)	26.32%	0.6252

BIO3 (Isothermality)	22.91%	0.669
BIO2 (Mean Diurnal Range)	2.56%	0.0564
BIO5 (Max Temp. Warmest Month)	1.02%	0.3307
BIO15 (Precip. Seasonality)	0.00%	0.194

The configuration that presented the best performance was characterized by linear and quadratic feature classes (LQ) combined with a regularization multiplier (RM) of 2. While simpler linear features were initially considered, the LQ approach was selected because it better captures unimodal response curves commonly observed in species-environment relationships (Elith et al., 2011; Merow et al., 2013). This is particularly relevant for *O. maskelli*, where temperature and precipitation variables likely exhibit non-linear biological thresholds (Austin, 2007), meaning that organismal performance and survival may decline abruptly once critical physiological limits are exceeded, rather than decreasing gradually across environmental gradients.

Following this setup the model achieved a good balance between model fit and complexity, as evidenced by an AICc of 1529.72 (lowest among all models) and delta AICc of 0, confirming its position as the best supported model under information theoretic criteria (Burnham & Anderson, 2002). The quadratic terms added necessary flexibility to represent the ecological niche without overcomplicating the model.

In terms of performance metrics, this model achieved an AUC of 0.961 in training and 0.960 in validation, indicating exceptional discriminatory power with minimal overfitting (AUC difference = 0.001).

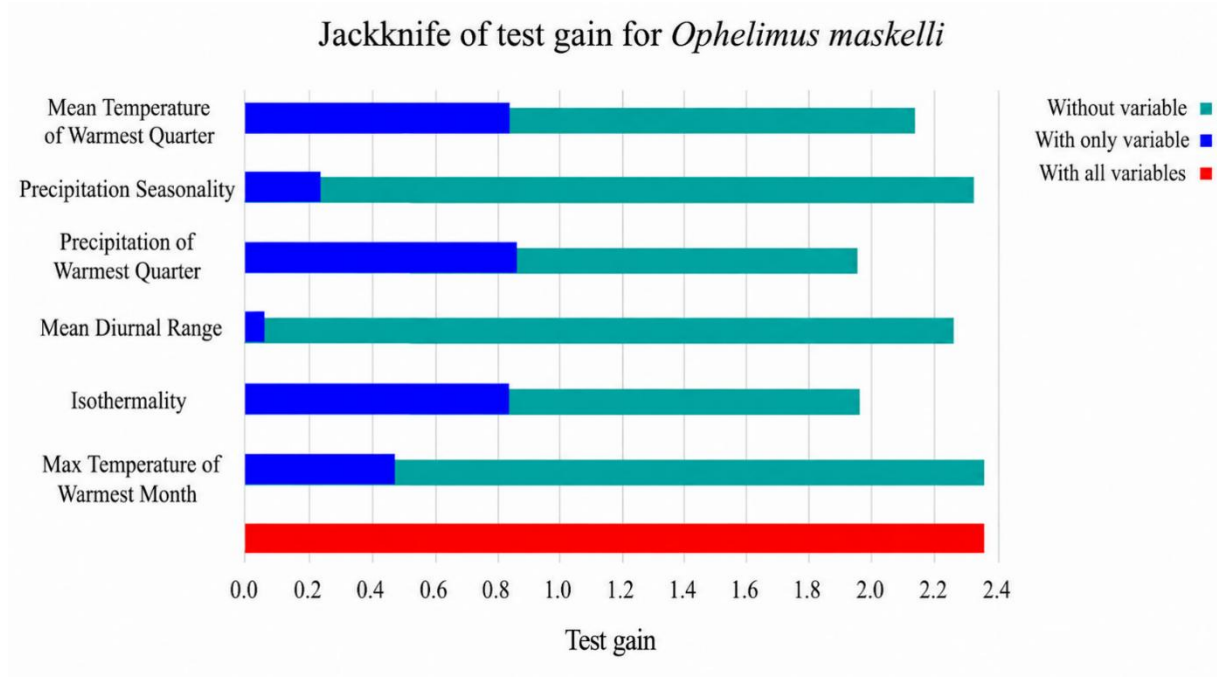


Figure 3. The Jackknife test was used to evaluate the relative importance of environmental variables.

The Continuous Boyce Index (CBI) values (0.961 training, 0.753 validation) confirm reliable ecological signal transfer to independent data. The LQ model provides biologically realistic response curves while maintaining exceptional predictive performance ($AUC > 0.96$).

To establish a baseline, we modelled the potential distribution under current climatic conditions (Maxent 1971–2000, CLIMEX 1981 – 2010) (**Figure 4 and 5**). Based on our model, Australia, where the wasp is native, is highly suitable for the maintenance of the species (**Figure 8**).

To assess potential effects of climate change on range, we applied both Maxent and CLIMEX models to current and independent future climate datasets based on the ACCESS-CP SSP5-8.5 scenario (Figure 4 and 5). Details on the grid setup and script used are provided in the Supplementary Materials, Annex II, and the corresponding global prediction outputs are presented in Annex IV, Figures 9 and 10.

The MaxEnt model for the current climate indicated moderate to high suitability in the northeast region of Brazil and lower in the south. CLIMEX CMI showed moderate climatic suitability for the current climate in Brazil, with a CMI range of 0.3 to 0.9 and mean 0.55 (Figure 5), with CMIs above 0.7 in the States of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, northern Minas Gerais, southern Bahia and central Espírito Santo. For predicted future climates, CLIMEX showed a strong contraction of areas exceeding CMI 0.7 (range 0 to 0.8) to the

central-southern part of Bahia, the southern parts of Brazil and in northwestern and southern Brazil (Figure 5). These results broadly align with MaxEnt predictions, although CLIMEX suggests higher suitability values for these regions.

The calibrated CLIMEX species model predicted climatic suitability for *O. maskelli* across Brazil, with the strongest suitability concentrated in the south-eastern and southern regions. Under the 1995 baseline climate, areas of non-zero Ecoclimatic Index were widespread across the principal plantation belt, particularly in São Paulo, Minas Gerais, Espírito Santo, Paraná, Santa Catarina and Rio Grande do Sul. Suitability was not restricted to coastal areas, with substantial inland areas also predicted to be climatically permissive. The ACCESS-CM2 SSP5-8.5 2050 projection retained broad suitability across the plantation estate, indicating that future warming under this scenario is unlikely to remove risk for the industry according to this model. Rather, the model suggests continued or increased exposure of key plantation regions to climates capable of supporting establishment and population persistence. Predicted suitability in regions without extensive eucalypt plantings should be interpreted as climatic permissiveness rather than realised invasion risk; operational risk is greatest where positive Ecoclimatic Index values coincide with host availability, plantation concentration and movement pathways.

For future scenarios from 2061 to 2080, MaxEnt results indicate a lower level of climatic suitability across northeastern and southern coastal Brazil compared to present conditions (Figure 4). The suitability presented at RCP 5.85 suggests that future climate conditions may reduce the establishment and spread of the species.

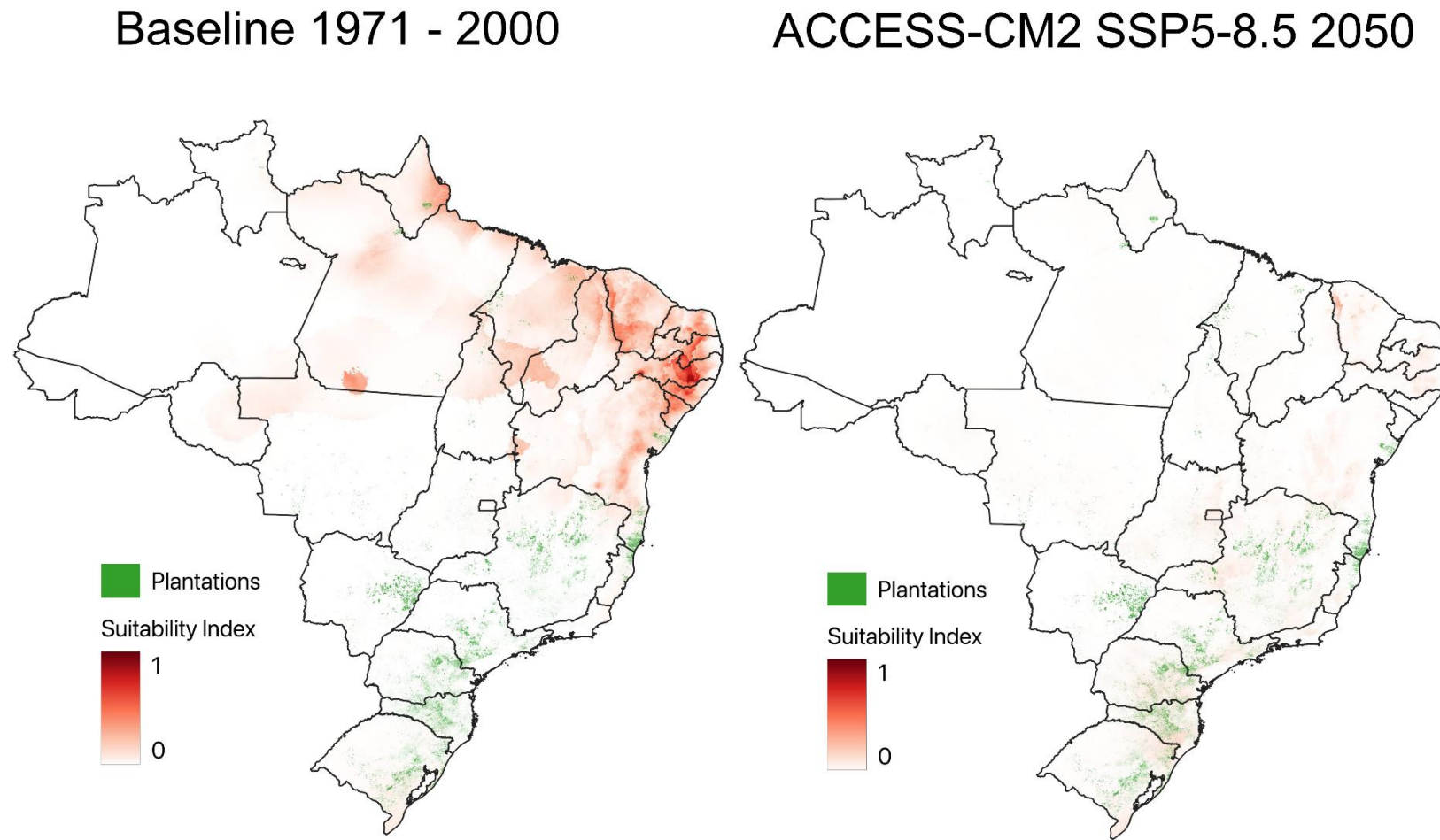
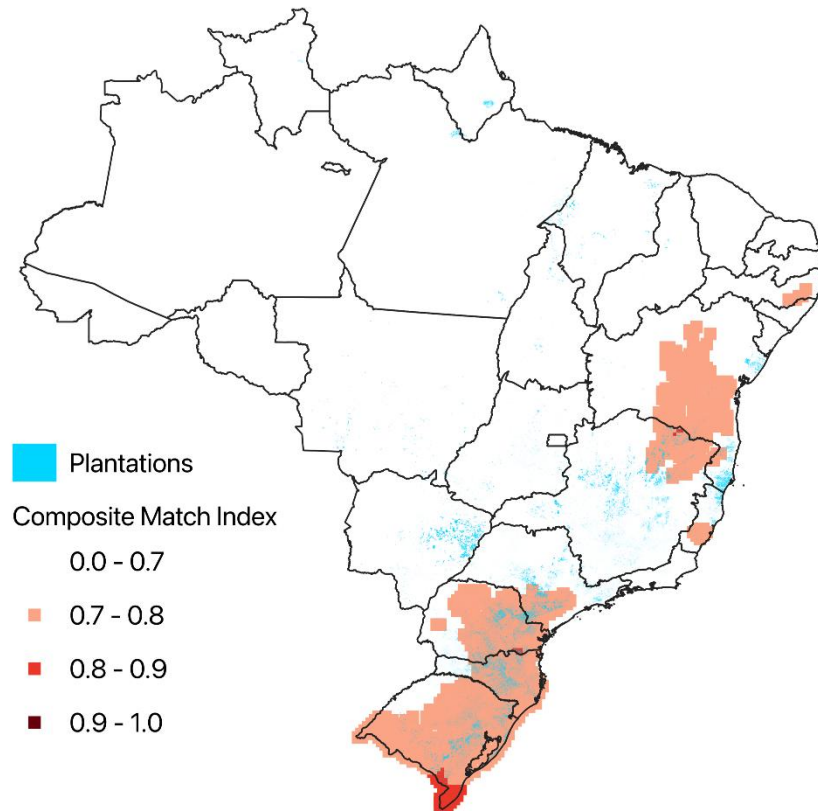


Figure 4. Predicted potential distribution of *Ophelimus maskelli* under present and future bioclimatic conditions based on MaxEnt modelling. Left - present climatic scenario (1971 – 2000); Right - Access CM-2 SSP5-8.5 for 2050, overlaid (green) with the 2024 distribution of forest plantations in Brazil.

Baseline 1995



ACCESS-CM2 SSP5-8.5 2050

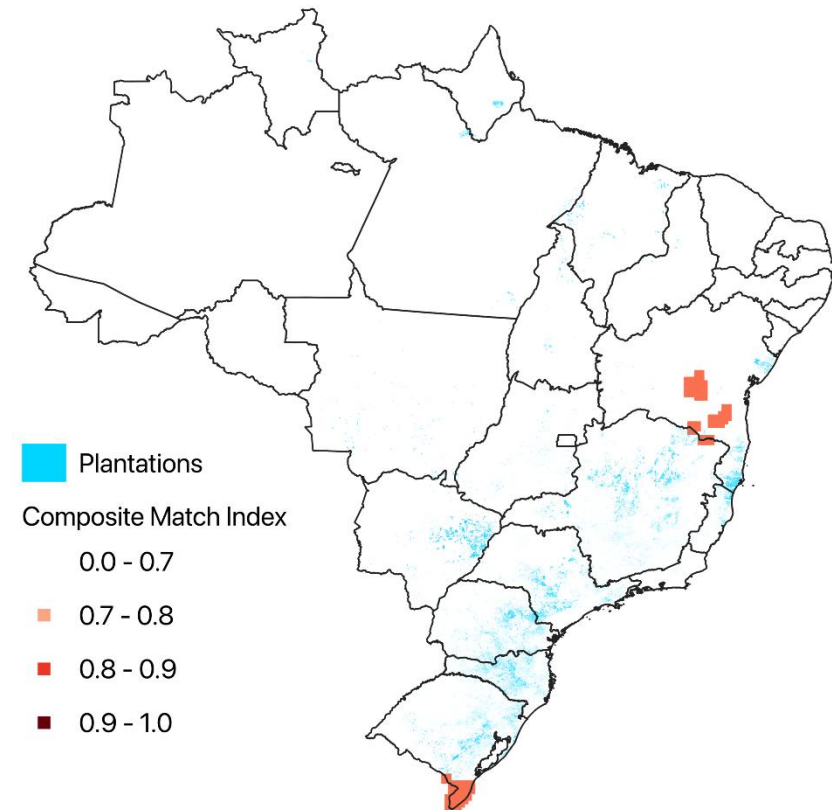


Figure 5.. Predicted climate match for *Ophelimus maskelli* in Brazil under current and future climatic conditions, based on CLIMEX Match Climates (Regional). Left - current climate scenario centred on 1995; Right- Access CM-2 SSP5-8.5 centred on 2050, Climate match is expressed as the Composite Match Index (CMI), ranging from 0 to 1, with higher values indicating greater climatic similarity to the known distribution of *O. maskelli*. Only areas with CMI > 0.7 are displayed, representing what is considered a good climatic match. Blue areas indicate the distribution of forest plantations in Brazil.

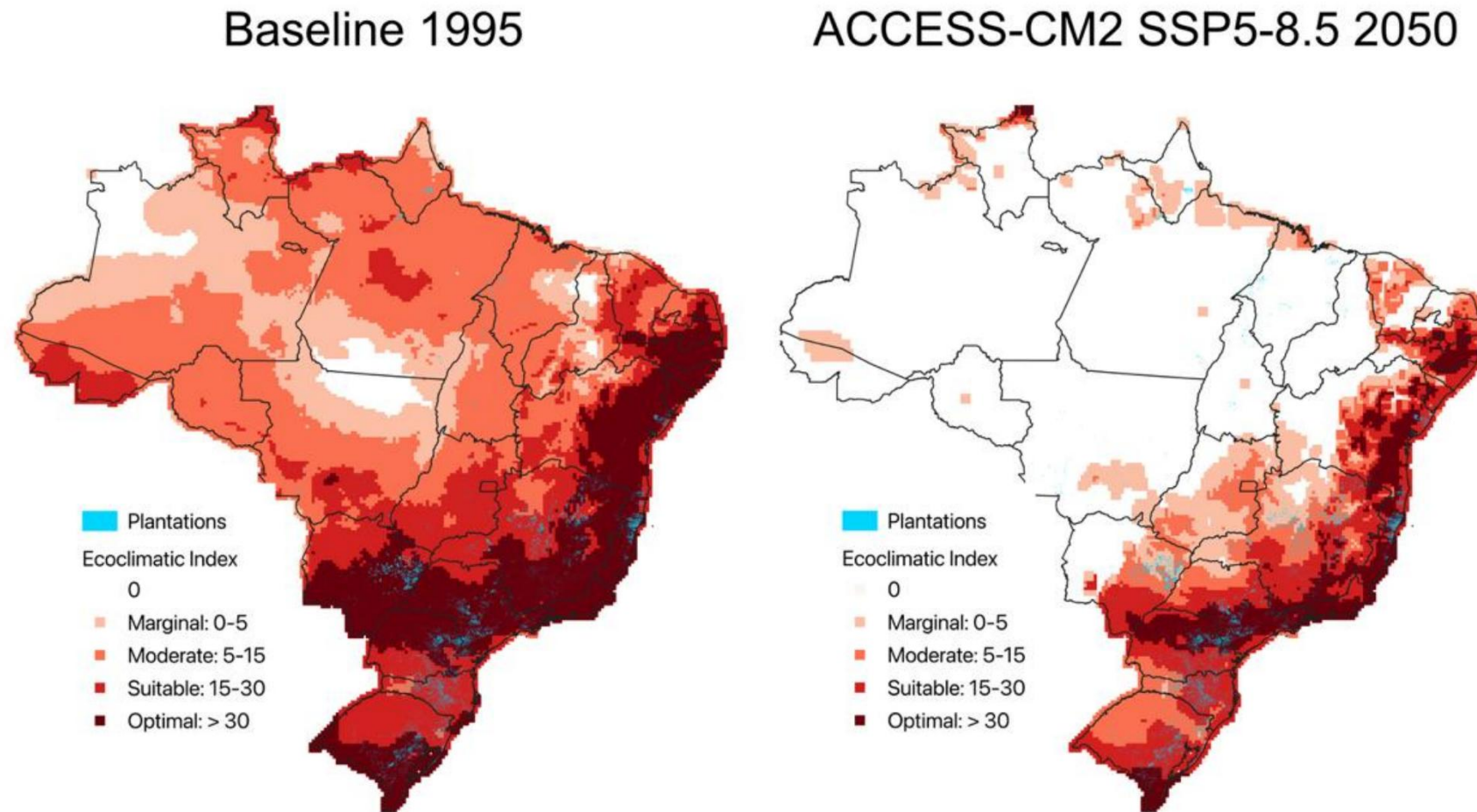


Figure 6. Predicted climatic suitability for *Ophelimus maskelli* in Brazil under current and future climatic conditions based on the Ecoclimatic Index (EI) generated by CLIMEX Compare Locations (1 species). Left - baseline climate scenario centred on 1995; Right - future projection for 2061–2080 under the ACCESS-CM2 SSP5-8.5 scenario. Climatic suitability is expressed as the Ecoclimatic Index (EI), ranging from 0 to 100: 0 = unsuitable; 1–10 = marginal; 10–20 = low; 20–40 = moderate; 40–80 = high; 80–100 = very high.

The current predicted CLIMEX EI map revealed a spatial overlap between climatically suitable areas and Brazilian plantation regions, federal highways, railway lines, airports, major cities, and ports (**Figure 7**). These overlaps were in regions where highly favorable conditions coincided with dense transportation infrastructure and logistics networks. Ports identified from data provided by the Brazilian National Waterway Transportation Agency (ANTAQ, 2025) were located near several of these areas, together with major federal highways, railway corridors, international airports, and maritime ports associated with commercial trade and forestry activities. This spatial pattern highlights the coincidence between predicted climatic suitability, plantation areas, and potential pathways of entry or internal spread.

Ports such as Suape (Pernambuco), Cabedelo (Paraíba), and Salvador (Bahia) were located within or adjacent to areas of high climatic suitability and nearby coastal export corridors. Similarly, major ports in southeastern and southern Brazil, including Santos (São Paulo), Paranaguá (Paraná), and Rio Grande (Rio Grande do Sul), were associated with suitable areas and important commercial routes. The most suitable areas in the Northwest were found alongside key transportation networks connecting northeastern and southeastern Brazil. Likewise, major international airports, including Recife, Fortaleza, Salvador, and São Paulo–Guarulhos, the largest international airport in Brazil, were located near highly suitable areas, highlighting potential pathways for long-distance dispersal and establishment. Many of the best-suited areas are located along key transportation routes connecting northeastern and southeastern Brazil. These include the country's major federal highways and railway lines, which help move products from plantations to ports for export. International airports—like those in Recife, Salvador, Fortaleza, and other northeastern cities are also conveniently located near places with favorable climates, making travel and trade easier.

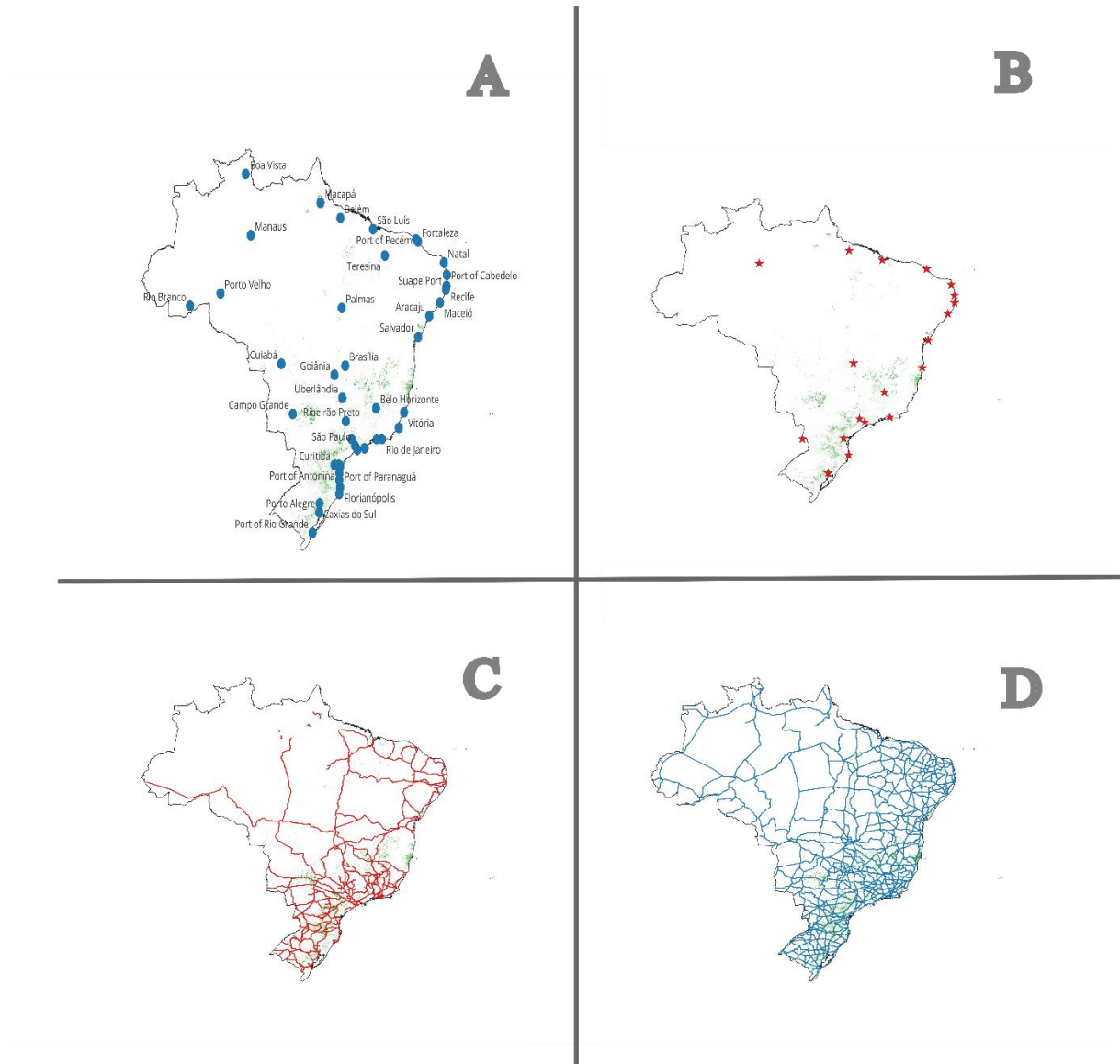


Figure 7. Current Ecoclimatic Index generated by CLIMEX showing favourability for *Ophelimus maskelli* establishment in Brazil, overlaid with Brazilian *Eucalyptus* plantations and: (A) major cities and ports; (B) international airports; (C) railway lines; and (D) federal highways, illustrating the complexity of potential areas and pathways associated with invasion risk.

5. Discussion

Here we provide insight into the potential current and future distribution and spatial patterns in Brazil of the invasive species *O. maskelli* under climate change, as well as its establishment risk within the Brazilian forestry sector. The current predictions of habitat favourability (Maxent) and climate and ecoclimatic match (CLIMEX) for *O. maskelli* indicate decreased suitability under the SSP 5-8.5 scenario compared to the current scenario. In Australia, where this wasp is native, a significant loss in favorable distribution is also predicted in the future under that scenario (**Figure 8**).

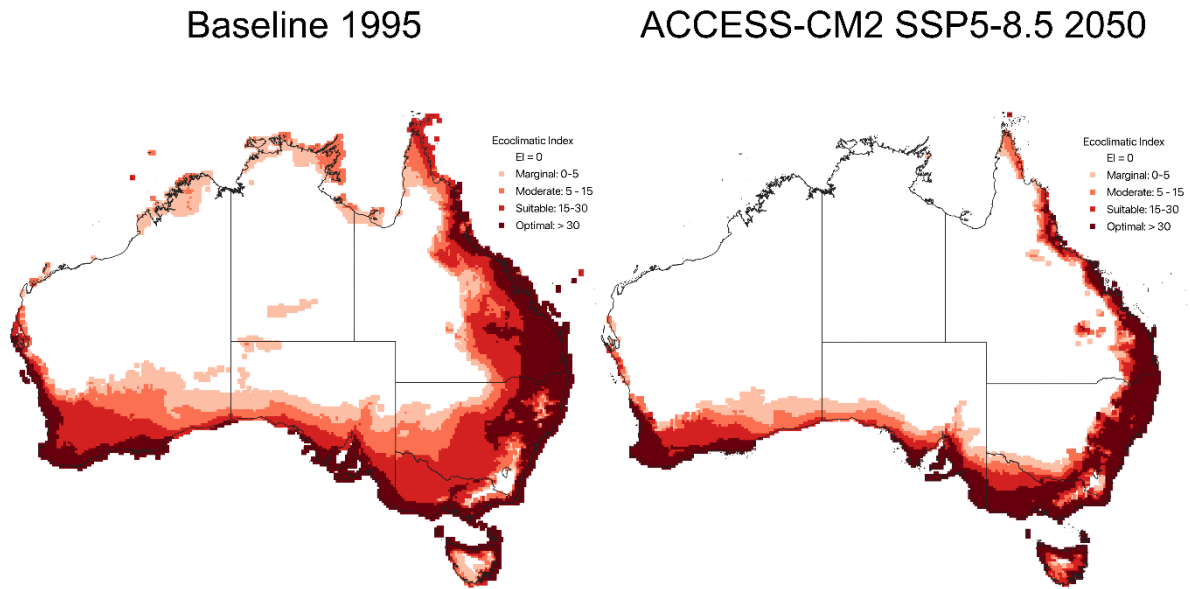


Figure 8. Predicted climate match for *Ophelimus maskelli* in Australia under current and future climatic conditions, based on CLIMEX Match Climates (Regional). From left to right, panels represent the current climate scenario and future climate projections for 2061–2080 under two climate-change scenarios. Climate match is expressed as the Composite Match Index (CMI), ranging from 0 to 1, with higher values indicating greater climatic similarity to the known distribution of *O. maskelli*.

Ophelimus maskelli is not currently established in South America. Although originally thought to be established in Argentina and Chile (Dittrich-Schröder et al., 2020; CABI, 2025), these records are likely *O. migdanorum*, which is also present in Colombia (Pinzon-Florian, 2020). It is not included in the official list of forest pests in Brazil (MAPA, 2025), but current and future climate conditions appear suitable for this species to establish, highlighting the urgent need for enhanced phytosanitary measures to lower the risk of a potential future invasion. Brazil has high potential for the establishment of this gall wasp due to favourable climatic conditions. Our results can be compared with Santos da Mota et al. (2024), who also modelled *O. maskelli* in Brazil using MaxEnt and reported generally low suitability based on 60 spatially filtered occurrence records. They identified BIO1, BIO12 and elevation as the main predictors and estimated 24% overlap with eucalypt plantations, whereas our model, refined from 445 to 210 valid non-duplicated records, highlighted BIO18, BIO10 and BIO3 and resulted in 0.15% overlap based on plantation occurrence points. Cheng et al. (2025) also modelled *O. maskelli* using MaxEnt and CLIMEX and identified isothermality as an important predictor, partially agreeing with our result, in which BIO3 also contributed strongly to the model.

According to previous studies on the presence of *O. maskelli* in the Mediterranean basin, warmer temperatures and lower precipitation levels are more suitable for the persistence of the species (Floris et al., 2018). This aligns with our findings, which in Maxent identify Mean

Temperature of Warmest Quarter (BIO10) and Precipitation of Warmest Quarter (BIO18) as the primary drivers of our model.

One limitation of our MaxEnt model was that, when projected globally, it did not adequately capture several known invaded localities in humid tropical and monsoonal South-East Asia, including Vietnam and northern Sumatra. This suggests that this model may under-represent part of the species' realised climatic tolerance, particularly in regions where occurrence records are sparse, geographically uneven, or climatically distinct from the main body of training data.

For the Maxent model, in Brazil, the Northeast region emerges as a particularly suitable area for a potential invasion and establishment by *O. maskelli*. Based on the findings of Cheng et al. (2025), localized areas in this region were also identified as suitable for the pest, aligning with our predictions. Both CLIMEX models (CMI and EI) expand the areas of potential suitability further south and north, indicating a broader potential distribution under the evaluated climatic conditions. This vulnerability is primarily linked to regional climatic conditions characterized by high temperatures and precipitation during the warmest quarter, as identified by the model. Additionally, the proximity of ports to eucalypt plantations in these areas increases the risk of infestation, as maritime trade can facilitate the unintentional introduction of insect pests through wood and plant materials (Greenwood et al., 2023).

Following establishment, the spread phase of invasive forest insects is often intensified by anthropogenic dispersal mechanisms, in which long-distance transport events create isolated populations that subsequently expand and coalesce through a stratified dispersal process (Liebhold and Tobin, 2008; Brockerhoff and Liebhold, 2017). Within this framework, internal movement via highways, railway networks, air transport, and the movement of nursery stock may facilitate secondary spread across climatically suitable regions. Favourable areas identified in our models coincide with highly connected transport corridors and logistics hubs in northeastern Brazil, reinforcing the importance of these regions as priority areas for monitoring and early detection. Transport infrastructure and trade-related pathways have been recognised as important drivers of invasive eucalypt pest dispersal, particularly in regions associated with intensive forestry activity and international commerce (Huang et al., 2018; Huang et al., 2019; Wingfield et al., 2015).

In addition, future projections from both CLIMEX and MaxEnt identified parts of southern Brazil as climatically suitable under future climate scenarios (**Figures 4 and 5**). This pattern is particularly relevant because southern and southeastern Brazil contain some of the

largest and most economically important Eucalyptus plantation areas in the country and account for a substantial proportion of national forestry production (IBÁ, 2025; MAPA, 2025).

In this context, Branco et al. (2016) and Dittrich-Schröder et al. (2020) emphasized that invasive pests such as *Leptocybe invasa* and *O. maskelli* have frequently been introduced to various parts of the world through commercial trade routes. These introductions are often linked to the international movement of seedlings, and such pathways facilitate the unintentional spread of gall-inducing wasps, especially in regions with expanding eucalypt cultivation and insufficient phytosanitary controls. Their findings highlight the critical need for stricter biosecurity measures and monitoring protocols at points of entry, particularly in countries with high volumes of forestry-related imports and exports. Furthermore, Dittrich-Schröder et al. (2020) suggest that invasive Australian gall-forming species could alter their feeding preferences, further intensifying the threats posed by alien species invasions.

Using MaxEnt, CLIMEX regional climate matching, and a calibrated CLIMEX species model together provided a more robust assessment of *O. maskelli* risk in Brazil than any single modeling approach could provide alone. In the Brazilian context, the broad overlap between predicted climatic suitability and the eucalypt plantation estate indicates that surveillance and biosecurity planning should treat much of the current plantation area as climatically exposed, while recognizing that realized establishment risk will also depend on the presence of suitable hosts and their distribution, pathways, and local management.

Rapid responses to invasions by gall-forming hymenopterans are urgent and essential for maintaining healthy and sustainable eucalyptus plantations. This includes monitoring plans focused on early detection and rapid taxonomic assessment. However, species identification within the *Ophelimus* complex can be particularly challenging because of morphologically similar cryptic species and unresolved taxonomic relationships. Consequently, integrative approaches that combine morphological and molecular analyses are essential for accurate species delimitation and for supporting effective management strategies. Once detected and properly identified, targeted control actions and phytosanitary measures can be implemented.

6. References

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7. Supplementary Material

Annex I. R script for environmental suitability modeling of *Ophelimus maskelli*

Including climate data processing, occurrence data integration, Maxent modeling configuration, and correlation analysis of environmental layers.

```
# Install required packages
install.packages(c("ENMeval", "dismo", "raster", "sp", "ecospat"))

# Load packages

library(ENMeval)

library(dismo)

library(raster)

library(sp)

library(ecospat)

# Define the directory where the layer files are stored

climate_data_dir <- "PASTE_YOUR_PATH_HERE"
```

```

# List TIFF files in the directory

files <- list.files(climate_data_dir, pattern = "\\\\.tif$", full.names = TRUE)

if (length(files) == 0) stop("No .tif files found in the specified directory.")

print(files)

# Load all layers into a stack

climate_layers <- stack(files)

print(climate_layers)

# Correlation among layers before running the model.

layer_values <- na.omit(values(climate_layers))

cor_matrix <- cor(layer_values, use = "pairwise.complete.obs")

print(cor_matrix)

# Load occurrence data

occurrence_data <-

read.csv("PASTE_YOUR_OCCURRENCE_DATA_PATH_HERE")

head(occurrence_data)

# Convert occurrence data to a spatial points object

coordinates(occurrence_data) <- ~longitude + latitude

print(occurrence_data)

# Define Maxent model settings

tune.args <- list(

  rm = 1, # Regularization multiplier

  fc = "l" # Feature class: Linear

)

# Run ENMevaluate with block cross-validation

eval_results <- ENMevaluate(

  occs = occurrence_data,

```

```

envs = climate_layers,

method = "block",

tune.args = tune.args,

algorithm = "maxent.jar"

)

```

Annex II. R script for comparing MaxEnt (Cloglog) and CLIMEX (CMI) suitability predictions

This script imports environmental suitability datasets, normalizes values, merges data, and generates a scatter plot to evaluate the correlation between models.

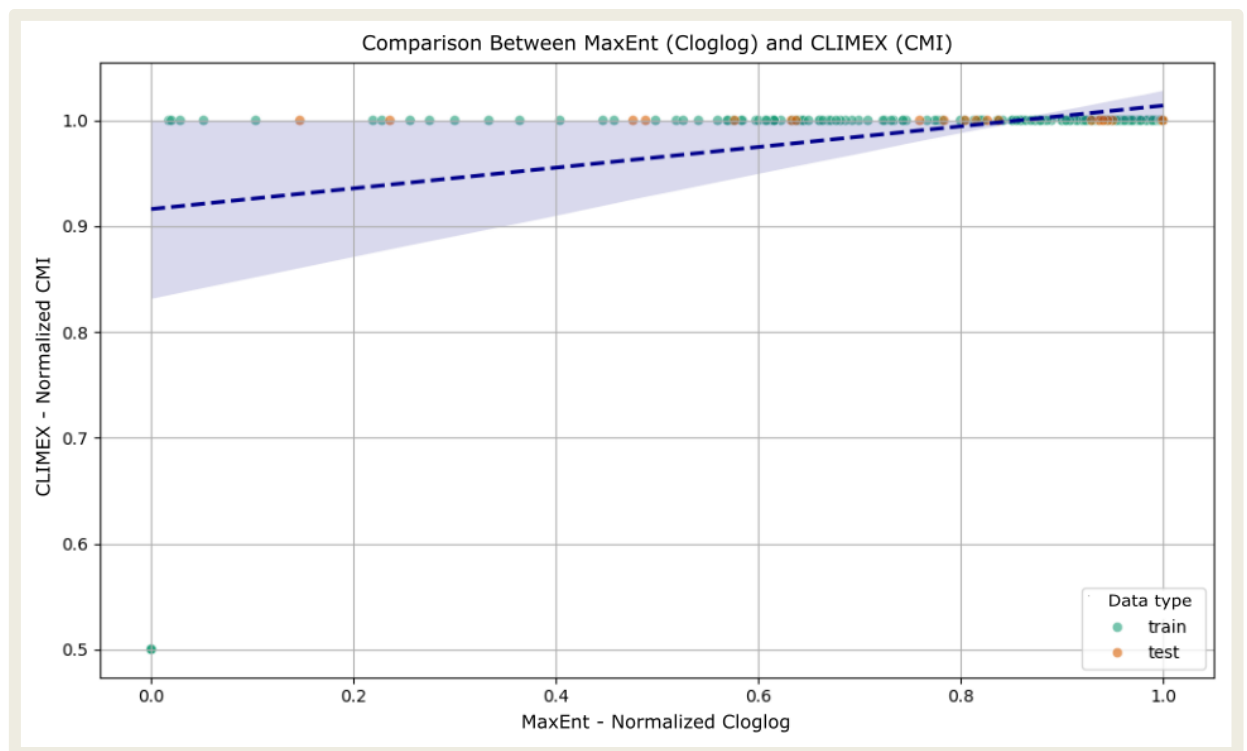


Figure 9. Correlation between environmental suitability predictions generated by different ecological models. Suitability values were normalized and plotted to evaluate the degree of agreement between model outputs.

```

# Install and load necessary packages

install.packages("ggplot2")

install.packages("dplyr")

install.packages("readr") # For reading CSV files

library(ggplot2)

```

```

library(dplyr)
library(readr)

# 1. Load CSV files (Replace with your actual file paths)
maxent_data <- read_csv("PASTE_YOUR_MAXENT_CSV_PATH_HERE.csv")
climex_data <- read_csv("PASTE_YOUR_CLIMEX_CSV_PATH_HERE.csv")

# 2. Ensure correct column selection
# Replace 'Suitability' with the actual column containing suitability values
maxent_data <- data.frame(
  LocationID = maxent_data$PASTE_LOCATION_COLUMN_HERE, # Ensure both
  datasets have a common location identifier

# 3. Merge datasets by LocationID
data <- merge(maxent_data, climex_data, by = "LocationID")

# 4. Normalize the data using Min-Max scaling
data$MaxEnt <- (data$MaxEnt - min(data$MaxEnt)) / (max(data$MaxEnt) -
min(data$MaxEnt))
data$CLIMEX <- (data$CLIMEX - min(data$CLIMEX)) / (max(data$CLIMEX) -
min(data$CLIMEX))

# 5. Generate scatter plot with trend line
ggplot(data, aes(x = MaxEnt, y = CLIMEX)) +
  geom_point(alpha = 0.7, size = 3, color = "blue") + # Data points
  geom_smooth(method = "lm", linetype = "dashed", color = "red", se = TRUE) + #
  Trend line
  labs(title = "Comparison Between MaxEnt (Cloglog) and CLIMEX (CMI)", x =
"MaxEnt - Normalized loglog",
  y = "CLIMEX - Normalized CMI"
) + theme_minimal()

```

Annex III. CLIMEX parameter values used for the calibrated *Ophelimus maskelli* species model

Table 3. CLIMEX parameter values used for the calibrated *Ophelimus maskelli* species model

Parameter group	CLIMEX parameter	Value	Biological / modelling justification
Soil moisture	Soil moisture capacity	100	Standard soil moisture capacity used in the CLIMEX Compare Locations framework, allowing consistent comparison across baseline and future climate scenarios.
	Evapotranspiration coefficient	0.8	Standard evapotranspiration setting to represent the relationship between temperature-driven water loss and soil moisture availability.
Temperature Index	Limiting low temperature	9 °C	Lower threshold for population growth. Allows low-level development in mild coastal, maritime and elevated environments while still excluding genuinely cold temperate climates. It reflects the observed ability of <i>O. maskelli</i> to persist in cooler marginal regions without treating cool climates as optimal.
	Lower optimal temperature	16 °C	Represents the onset of favourable growth under mild spring and early summer conditions. Supports suitability in warm-temperate, Mediterranean and mild coastal climates.
	Upper optimal temperature	26 °C	Represents favourable growth under warm-temperate to subtropical conditions. Maintains high suitability across much of the known invaded range while avoiding unrealistic optimisation of very hot lowland tropical or continental climates.
	Limiting high temperature	34 °C	Upper developmental limit. Permits persistence in warm humid subtropical and monsoonal regions, including South-East Asia, while preventing excessive expansion into very hot interiors.
Moisture Index	Limiting low moisture	0.05	Allows persistence in seasonally dry environments while excluding deserts and extremely dry interiors. Consistent with <i>O. maskelli</i> 's occurrence in Mediterranean climates subject to summer moisture limitation.
	Lower optimal moisture	0.25	Represents moderately dry but productive climates, including Mediterranean Europe, southern Australia and South African regions.

Parameter group	CLIMEX parameter	Value	Biological / modelling justification
	Upper optimal moisture	0.90	Allows strong suitability in humid subtropical and monsoonal climates, including southern China, Vietnam and other humid invaded or climatically analogous regions.
	Limiting high moisture	1.50	Permits persistence in humid tropical climates while still restricting the wettest rainforest regions. This parameter works with wet stress to retain suitability in monsoonal South-East Asia while suppressing suitability in the Amazon and Congo rainforest cores.
Cold Stress	Cold Stress Temperature Threshold	6 °C	Temperature below which cold stress begins to accumulate. This allows persistence in mild-winter coastal, Mediterranean and inland-margin climates while continuing to exclude colder continental and high-latitude regions.
	Cold Stress Temperature Rate	−0.0008 week ^{−1}	Represents gradual accumulation of cold stress. This allows short or mild cold periods to be tolerated without eliminating marginal populations, while still limiting persistence in climates with sustained winter cold.
	Cold Stress Degree-day Threshold	0	Not used. Temperature-threshold cold stress was sufficient to capture the cold limitation of the species.
	Cold Stress Degree-day Rate	0	Not used.
Heat Stress	Heat Stress Temperature Threshold	34 °C	Aligned with the upper developmental limit. This constrains suitability in hot continental and arid regions such as central Australia, the Sahara, the Arabian Peninsula and other hot interiors, while permitting suitability in warm subtropical and coastal regions.
	Heat Stress Temperature Rate	0.01 week ^{−1}	Provides gradual but biologically meaningful heat-stress accumulation in persistently hot climates.
	Heat Stress Degree-day Threshold	0	Not used.
	Heat Stress Degree-day Rate	0	Not used.

Parameter group	CLIMEX parameter	Value	Biological / modelling justification
Dry Stress	Dry Stress Threshold	0.05	Matches the lower soil moisture limit and prevents persistence in strongly arid environments.
	Dry Stress Rate	−0.005 week ^{−1}	Allows Mediterranean and seasonally dry plantation climates to remain suitable while excluding deserts and very dry interiors.
Wet Stress	Wet Stress Threshold	1.40	Allows suitability in monsoonal and humid subtropical climates while limiting excessively wet climates.
	Wet Stress Rate	0.001 week ^{−1}	Applies a low rate of wet-stress accumulation. This prevents catastrophic exclusion of humid tropical and monsoonal regions while still suppressing suitability in persistently saturated rainforest climates.
Stress interactions	Cold-Dry Stress	Not used	No combined cold-dry stress term was required.
	Cold-Wet Stress	Not used	No combined cold-wet stress term was required.
	Hot-Dry Stress	Not used	No combined hot-dry stress term was required.
	Hot-Wet Stress	Not used	No combined hot-wet stress term was required.
Light and diapause	Light Index	Not used	Photoperiod was not included as a limiting factor.
	Diapause Index	Not used	No obligate diapause requirement was included, consistent with a species capable of multiple generations per year in favourable climates.
Degree-day and generation settings	Cold Stress DD threshold temperature	16 °C	Retained as exported by CLIMEX for the degree-day stress settings.
	Heat Stress DD threshold temperature	43 °C	Retained as exported by CLIMEX for the degree-day stress settings.
	Model time step	7 days	Standard weekly time step used by CLIMEX for growth and stress accumulation.
	Degree-days per generation	210 degree-days	Calibrated value representing the thermal accumulation required to complete one generation. This value retained good fit to known occurrences while avoiding over-

Parameter group	CLIMEX parameter	Value	Biological / modelling justification
			interpretation of high potential generation numbers in warm regions. Modelled generation output was treated as an index of thermal opportunity rather than a direct prediction of realised annual voltinism.

Annex IV.

Global ecological suitability projections for *Ophelimus maskelli* using MaxEnt and CLIMEX models

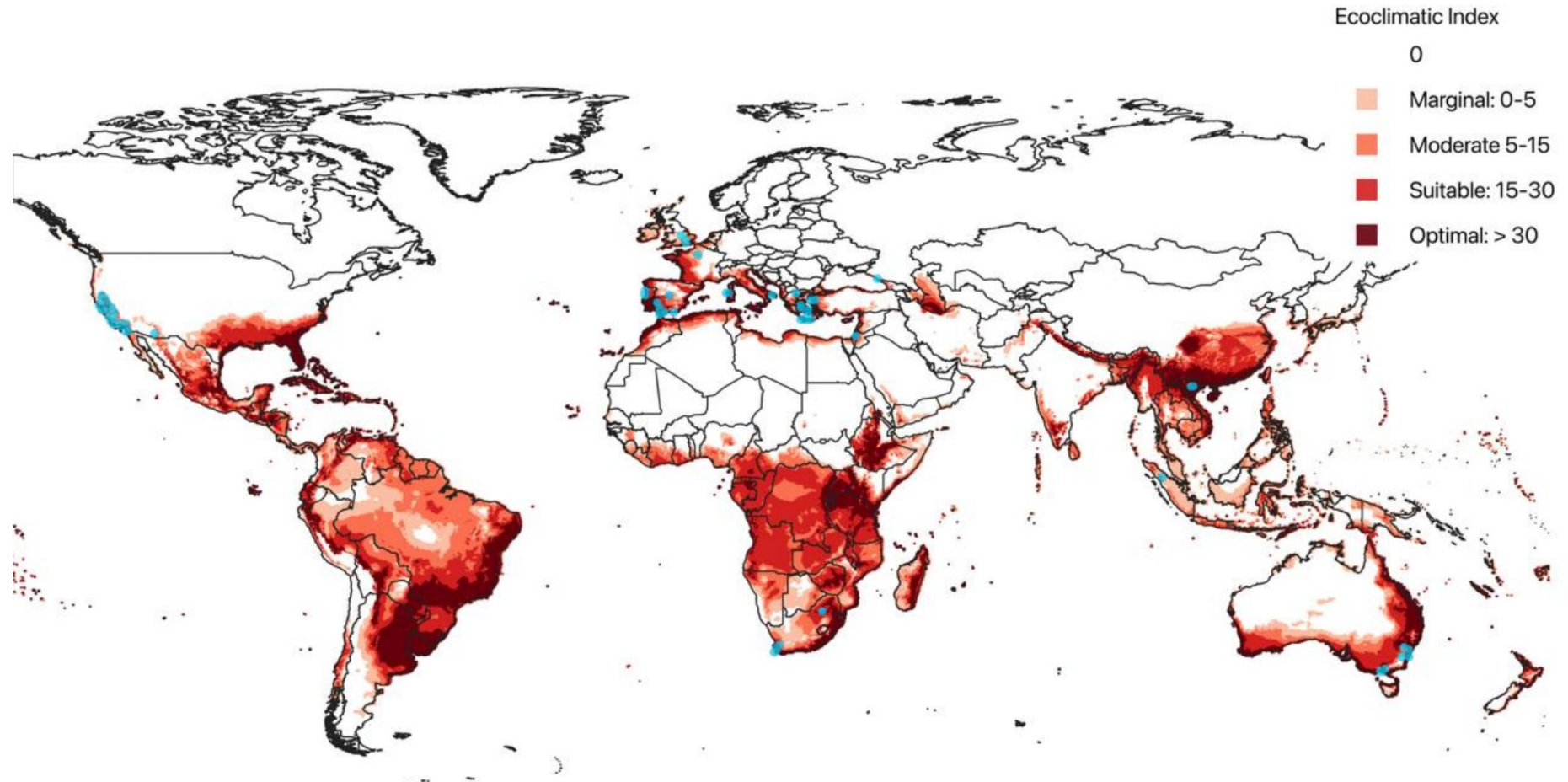


Figure 10. Global habitat suitability for *Ophelimus maskelli* estimated using CLIMEX. Suitability classes are represented according to the Ecoclimatic Index (EI), ranging from marginal to optimal conditions. Darker red tones indicate areas with higher ecoclimatic suitability, while blue points represent known occurrence records.

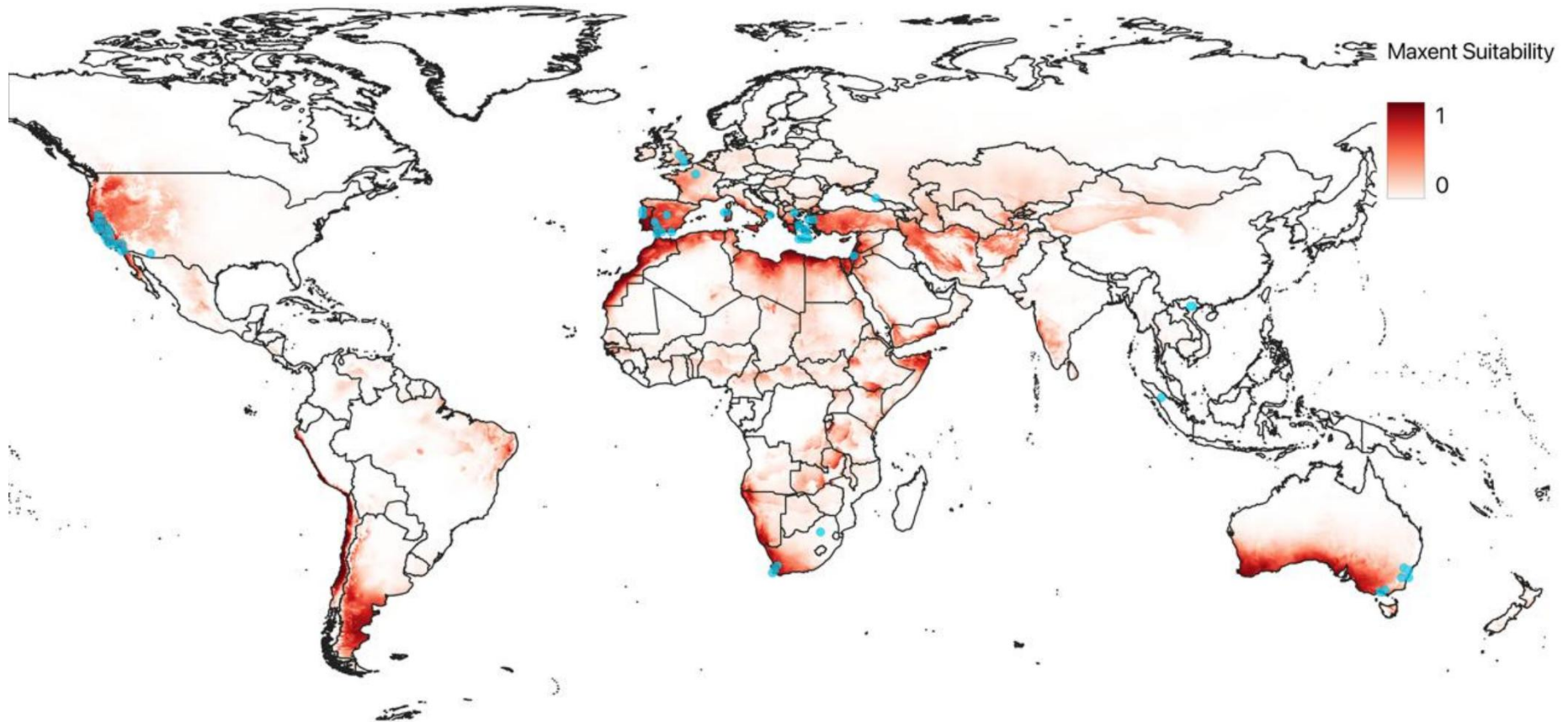


Figure 11. Global ecoclimatic suitability for *Ophelimus maskelli* estimated using Maxent.. Warmer red tones indicate areas with higher predicted suitability, while lighter tones indicate lower suitability. Blue points represent known occurrence records used to support model calibration and interpretation.

Capítulo 2

Late Pleistocene Microrefugia Persistence and Evolutionary Divergence in the *Ophelimus* Complex

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1. Abstract

The gall-inducing wasp *Ophelimus maskelli* Ashmed 1900 represents a invasive threat to global Eucalyptus forestry, yet its effective management is hampered by taxonomic ambiguities within the genus. In this study, we employed an integrative approach using Bayesian Inference and Maximum Likelihood to reconstruct the evolutionary history of the *Ophelimus* cluster. Our findings reinforce the monophyletic position of *O. maskelli* and its sister group *O. mediterraneus* with maximum statistical support (PP = 1.0; BS = 100). Molecular clock analysis estimates that these lineages diverged approximately 32,000 to 24,000 years ago during the Late Pleistocene. This divergence timeframe coincides with the onset of the Last Glacial Maximum (LGM) in Australia, where significant environmental transitions acted as a selective filter, forcing ancestral populations into isolated microrefugia. To resolve identification challenges, we propose a diagnostic DNA barcode (GenBank PZ286359) with a highly conserved 50 bp molecular signature within the COI gene (positions 134-183) that aligns exclusively with *O. maskelli*, providing a molecular tool for rapid species determination and targeted biosecurity responses.

Keywords: *Ophelimus maskelli*; Phylogenetics; Last Glacial Maximum; DNA Barcoding; Invasive Species; Eucalyptus.

2. Introduction

Gall-inducing wasp *Ophelimus maskelli* (Ashmead, 1900) (Eulophidae), is recognized as the second most widely distributed invasive species and has been detected outside Australia in several countries, including Iran, Iraq, Portugal, Spain, Greece, South Africa, Canada, and the United States (Protasov et al., 2007; Molina-Mercader et al., 2019). Each gall is a single-chamber structure that contains one larva of the wasp, which emerges as an adult by breaking through the gall surface. The shape of *O. maskelli* galls can vary from circular to lenticular, with green or reddish coloration, measuring between 0.9 mm and 1.2 mm (Gonzalez and Hernández, 2025).

Ophelimus maskelli main host is *Eucalyptus* trees, with a preference for species such as *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* (Gauthier et al., 2000; Branco et al., 2009). Populations can rapidly reach high densities, leading to severe defoliation, decline of aboveground parts, mortality of young trees, and reduced growth rates in mature stands (Dittrich-Schröder et al., 2020). Large swarms of emerging wasps may also cause health problems in human communities living near infested forests and disrupt industrial operations, as occurred in Israel (Protasov et al., 2007; Quang Thu et al., 2010).

This invasive micro-wasp was first described as *Eriophyes maskelli* by Ashmead (1900) and later misidentified as *Ophelimus eucalypti* in Europe (Branco et al., 2009). *Ophelimus maskelli* was initially identified as *O. eucalypti*, but the galls generated by the first are and found only on the leaf blade, close to the petiole, whereas those of *O. eucalypti* occur near the blade margin (Protasov et al., 2007). Subsequent morphological and phylogenetic analyses confirmed that specimens corresponded to *O. maskelli* (Protasov et al., 2007).

To improve the control of this invasive species and provide a foundation for future studies targeting management strategies for different lineages, this research aims to investigate evolutionary relationships within the *O. maskelli* complex, improving the understanding of how populations have evolved across different geographic regions, especially the phylogenetic boundaries between *O. maskelli* and its sister group, *Ophelimus mediterraneus* (Hymenoptera: Eulophidae) (Borowiec et al., 2019). Furthermore, we infer that their speciation was driven by significant environmental transitions during the Late Pleistocene under the Last Glacial Maximum (LGM) in the Australian Alps. To address the identification problems involving *O. maskelli* specimens, we also propose a conserved 50 bp molecular marker within the COI gene as a high-resolution tool for rapid identification.

3. Material and Methods

3.1. Sample Collection

A total 82 *O. maskelli* specimens were obtained from three sources. Field-collected individuals were sampled in Victoria, Australia. Preserved material was accessed from institutional collections, including the University of the Sunshine Coast (Australia) and Scion Institute (New Zealand). In addition, cytochrome c oxidase subunit I (COI) sequences available in GenBank were included to broaden the geographic coverage of populations analysed. Sample details, including collection site, DNA extraction method, gene fragment amplified, primers used, and geographic coordinates, are summarized in Table 1.

From the total pool of specimens subjected to PCR amplification, a subset was selected for the final analysis based on the attainment of high-quality, unambiguous consensus sequences, with a minimum Phred quality score threshold of Q30 following bidirectional sequencing and manual editing in Geneious Prime 2025.0.2. This filtering process ensured that all analysed sequences met strict quality thresholds for assembly and alignment, prioritizing individuals that provided the most reliable molecular data for phylogenetic inference (Burks et al., 2011). Comprehensive metadata for these selected vouchers, including collection sites, DNA extraction methods, primer sets, and geographic coordinates, are summarized in **Table 1**. A map with collection sites from our samples originated from Australia in New Zeland is better observed in **Figure 1**.

Table 1. Individuals of *Ophelimus maskelli* (Hymenoptera: Eulophidae) sent for COI sequencing, with corresponding sample ID, collection locality, DNA extraction method, PCR primers used, and geographic coordinates.

PCR ID	Location	Gene Amplified	PCR Primers	Latitude	Longitude
CJ08	Braidwood, NSW, Australia	COI	LCO1490 / HCO2198	-35.462.861	149.784.722
LP103-1	Logan City, QLD, Australia	COI	LCO1490 / HCO2198	-27.537.957	152.968.687
CJ43-3	Traralgon, VIC, Australia	COI	LCO1490 / HCO2198	-38.163.694	146.943.889

CJ41-3	Sale / Gippsland, VIC, Australia	COI	LCO1490 / HCO2198	-38.001.500	147.318.861
CJ12-2	Canberra, ACT, Australia	COI	LCO1490 / HCO2198	-35.288.139	149.065.111
CJ21-1	Snowy Mountains, NSW, Australia	COI	LCO1490 / HCO2198	-36.414.278	148.624.972
CJ09	Braidwood region, NSW, Australia	COI	LCO1490 / HCO2198	-35.496.944	149.724.083
QLD103-1	Ipswich, QLD, Australia	COI	LCO1490 / HCO2198	-27.860.242	152.934.725
2 +	Footscray, VIC, Australia	COI	LCO1490 / HCO2198	-37.796	144.899
CJ12-6 (1)	Tomerong, NSW, Australia	COI	LCO1490 / HCO2198	-35.072.756	150.578.181
CJ65-3 (2)	Kosciuszko National Park, NSW, Australia	COI	LCO1490 / HCO2198	-35.364.570	150.472.934
Q2.6	Riccarton, Christchurch, New Zealand	COI	LCO1490 / HCO2198	-35.904.500	148.966.472
Q2.7	Kosciuszko National Park, NSW, Australia	COI	LCO1490 / HCO2198	-35.904.500	148.966.472

A1	Riccarton, Christchurch, New Zealand	COI	LCO1490 / HCO2198	-43.529.98	172.597.44
A2	Wellington, New Zealand	COI	LCO1490 / HCO2198	-41.302.297	174.768.392
A6	Fiordland National Park, New Zealand	COI	LCO1490 / HCO2198	-45.422.206	167.716.328

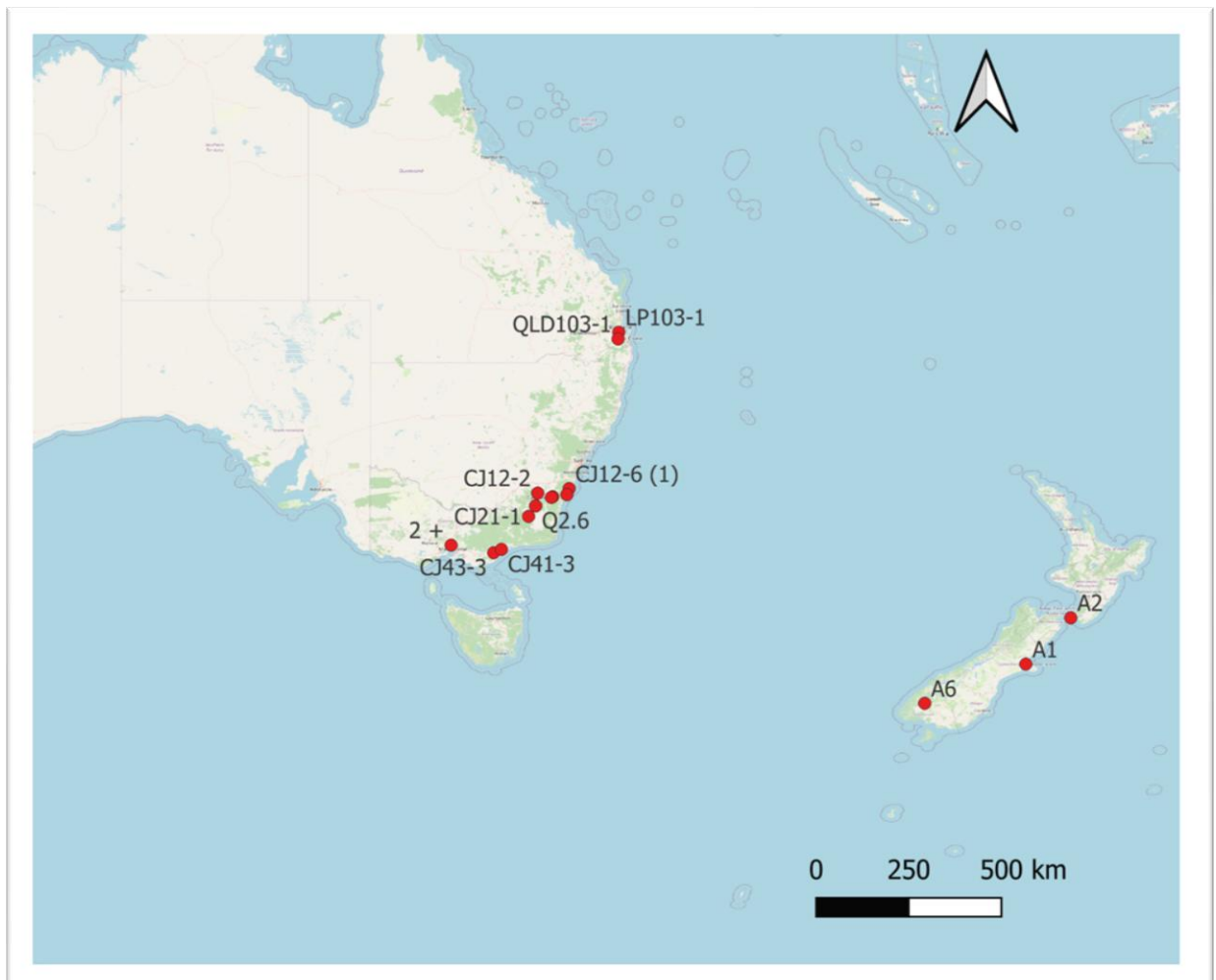


Figure 1. Geographic distribution of *Ophelimus* samples across Australia and New Zealand. Red dots indicate collection sites.

3.2. Specimen Identification

Specimens were morphologically examined and identified by comparison with specimens deposited in institutional collections, using expert knowledge, since no formal identification key is currently available for *O. maskelli*. The taxonomic delimitation within the

genus *Ophelimus* remains particularly challenging due to the high morphological similarity among closely related species and the presence of cryptic lineages, which frequently complicates reliable species identification based on morphology (Protasov et al., 2007; Naves et al., 2026). In particular, the presence of a single submarginal seta on the forewing was used as an important diagnostic feature during specimen evaluation.

Morphological identifications were complemented with molecular confirmation, sequences obtained in this study were compared against public databases using the BLAST function (Basic Local Alignment Search Tool), which provided additional support for species level identification. This integrative approach, combining diagnostic morphology with sequence similarity searches, ensured robust and consistent specimen determination across all samples analysed.

3.3. DNA Processing and Data Accessibility

Genomic DNA was isolated from *O. maskelli* individuals through two different kits, based on their freshness or condition after preservation. For newly collected samples, the PrepGEM® Blood & Tissue Kit kit was used, where the extraction mixture (20 µL) was prepared by combining 7.5 µL of MiliQ water, 2 µL of Black Buffer, and 0.5 µL of prepGEM, which was incubated at 75 °C for 3 h. Lossless enzymatic method is known to provide adequate results in genomic DNA isolation from gall wasps of the genus *Ophelimus* (Borowiec et al., 2019; Garcia et al., 2019). For older or poorly preserved samples, the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used, where the incubation was carried out by mixing 180 µL of Buffer ATL with 20 µL of proteinase K, incubated at 55°C for 24 h, followed by the final elution of DNA in 30 µL of Buffer AE.

The mitochondrial cytochrome c oxidase subunit I (COI) fragment was amplified using specific primers (see Table 1) in a 25 µL reaction volume consisting of 5 µL Taq Buffer, 1.25 µL of each forward and reverse primer, 15.75 µL of water, 0.5 µL of Taq polymerase, and 1.25 µL of DNA template. PCR was performed under a thermal profile of 35 cycles consisting of initial denaturation at 95 °C for 5 minutes, denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72°C for 60 s, concluded by a final extension at 72°C for 10 min. Amplification was verified via 1% agarose gel electrophoresis (prepared with 40 mL TBE and 0.4 g agarose and stained with Ethidium Bromide) run for 60 minutes at 100V, loading 5 µL of PCR product with 1 µL of loading dye. PCR products were enzymatically purified and sequenced bidirectionally using Sanger technology at a commercial sequencing facility. The resulting chromatograms were assembled, trimmed, and manually edited in Geneious Prime

v.2023.0.1 (Biomatters Ltd., Auckland, New Zealand) to generate high-quality consensus sequences. Sequence alignments were performed using the CLUSTALW algorithm implemented in MEGA 11 (Tamura et al., 2021).

3.4. Phylogenetic and demographic analyses

For the mitochondrial COI dataset, the best-fit nucleotide substitution model was selected using the ModelFinder algorithm implemented in IQ-TREE (Nguyen et al., 2015) under standard parameters. Subsequent phylogenetic relationships were inferred within a Bayesian framework implemented in MrBayes version 3.2.1 (Ronquist and Huelsenbeck, 2003). The COI alignment was partitioned by codon position (1st, 2nd, and 3rd codons), and analyses were conducted under the General Time Reversible model with a proportion of invariant sites and gamma-distributed rate variation among sites (GTR + I + G), using six gamma categories. Model parameters for among-site rate heterogeneity and proportion of invariant sites were unlinked across codon partitions, whereas topology, branch lengths, substitution rates, and state frequencies were linked across all partitions. Two independent Markov Chain Monte Carlo (MCMC) runs, each consisting of four chains, were executed for 50 million generations with a heating temperature of 0.10, sampling every 5,000 generations. Convergence and the adequacy of effective sample sizes ($ESS > 200$) were evaluated using Tracer v1.7 (Rambaut et al., 2018). The initial 25% of sampled trees were discarded as burn-in, and the final consensus topology was summarized using the all-compatible consensus criterion. The tree was rooted using the outgroup *Ophelimus ramorum* (deposited in Genbank as PV851504).

Additionally a Maximum Likelihood phylogenetic inference was conducted using RAxML version 8.2.12 (Stamatakis, 2014) under the General Time Reversible model with gamma-distributed rate heterogeneity among sites (GTR + G). All free model parameters were estimated during the analysis, and tree searching was performed using the rapid hill-climbing algorithm. Nodal support was assessed through 1,000 bootstrap replicates, from which an extended consensus tree was generated (Better seen in **Supplementar Material Appendix I**).

Following the initial phylogenetic reconstruction, demographic history was reconstructed in BEAST v.2.7. A coalescent Bayesian Skyline tree and a relaxed log normal molecular clock were used to estimate population size changes over time using a coalescent constant size tree prior and an uncorrelated lognormal relaxed molecular clock, with a lognormal prior applied to the mean substitution rate (ucl.d.mean, mean = 0.0115 substitutions per site per million years, stdev = 0.005), to estimate divergence times (Bouckaert et al., 2019).

The analysis was run for 50 million generations, with parameters sampled every 1,000 states. TreeAnnotator was employed to summarize the posterior distribution into maximum clade credibility (MCC) trees using mean node heights after discarding 25% burn-in, which were visualized in FigTree v.1.4.4.

3.5. Species Delimitation

Molecular species delimitation was performed using the multi-rate Poisson Tree Processes (mPTP) method (Kapli et al., 2017), following the standardized framework established for *Ophelimus* species by Naves et al., 2026. In this analysis, the Bayesian tree produced in MrBayes was employed as the input topology. The tree was analyzed on the mPTP web server (<https://mcmc-mptp.h-its.org>) using a Bayesian MCMC approach. Following the established parameters for the genus, the MCMC chain was run for 100,000 generations, with a burn-in of 1,000 steps, a sampling interval of 1,000 steps, and a fixed random seed of 12345. This process provided a robust probabilistic assessment of species boundaries, allowing for a comparative evaluation of genetic divergence between our specimens and congeneric lineages to confirm phylogenetic limits.

A Haplotype network was constructed to visualize genetic diversity within *Ophelimus maskelli* species. Haplotype data were compiled using DnaSP v.6.12 (Rozas et al., 2017) from the aligned mitochondrial COI dataset. Thereafter a median-joining network was subsequently generated in PopART v.1.7 (Leigh and Bryant, 2015) under default settings. In the resulting topology, node sizes represent haplotype frequencies, while connecting branches indicate the number of mutational steps between lineages.

3.6. Diagnostic COI Barcode and Species-Specific Molecular Signature

To improve the accuracy and reliability of species identification, a DNA barcode region based on the mitochondrial cytochrome c oxidase subunit I (COI) gene was constructed within the alignment, followed by the selection and evaluation of a 50 bp fragment as a potential diagnostic molecular marker for *O. maskelli*. The selected region was extracted from positions 134–183 of the aligned dataset and subsequently used for comparative analyses among the examined haplotypes and related *Ophelimus* taxa.

4. Results

The DnaSP analysis identified 21 polymorphic sites and 21 total mutations across the 568 bp COI alignment. A total of 11 distinct haplotypes were recovered, with a haplotype

diversity (Hd) of 1.000, indicating that each analysed sequence corresponded to a unique haplotype. The nucleotide diversity (Pi) was estimated at 0.00982, while the average number of nucleotide differences (k) reached 5.018. Additionally, Theta-W per site was estimated at 0.01403, further supporting the presence of notable molecular diversity within *O. maskelli*.

The haplotype network analysis identified a total of 11 distinct haplotypes of *O. maskelli* (as showed in **Figure 2** and **Table 2**) distributed between Oceania and Mediterranean (**Figure 3**). The European samples comprised eight haplotypes (Hap1 – Hap8), with a strong predominance of a single high-frequency haplotype (Hap1) and several closely related low frequency variants differing by one to three mutational steps, resulting in a star topology and reduced overall divergence within this group. In contrast, the Oceania samples, despite being fewer, included three haplotypes (Hap9 – Hap11) that formed a distinct cluster separated from the European haplotypes by up to eight mutational steps and exhibiting greater genetic differentiation relative to the European cluster. No haplotypes were shared between continents, and the overall network structure showed clear geographic segregation, with lower haplotype diversity within Europe compared to Oceania.

The phylogenetic analyses using both Bayesian Inference and Maximum Likelihood yielded highly congruent results, recovering a distinct and well-supported clade for *O. maskelli* alongside *Ophelimus mediterraneus* as a sister lineage. Both evolutionary groups were recovered with maximum statistical confidence, showing Bayesian posterior probabilities of 1.0 and Maximum Likelihood bootstrap values of 100. Among our sampled material, four specimens were definitively confirmed as *O. maskelli* through the application of the diagnostic 50 bp DNA barcode motif (positions from 134 to 183) identified as TAATAATAGGAGGATTTGGAAATTTTAAATTCCTATATTTTGGGAATA, which was conserved in a subset of haplotypes associated with *O. maskelli* and consistent phylogenetic placement. The remaining specimens were identified as belonging to other *Ophelimus* groups based on COI BLAST analyses and integrative molecular identification, as presented in **Figure 4**.

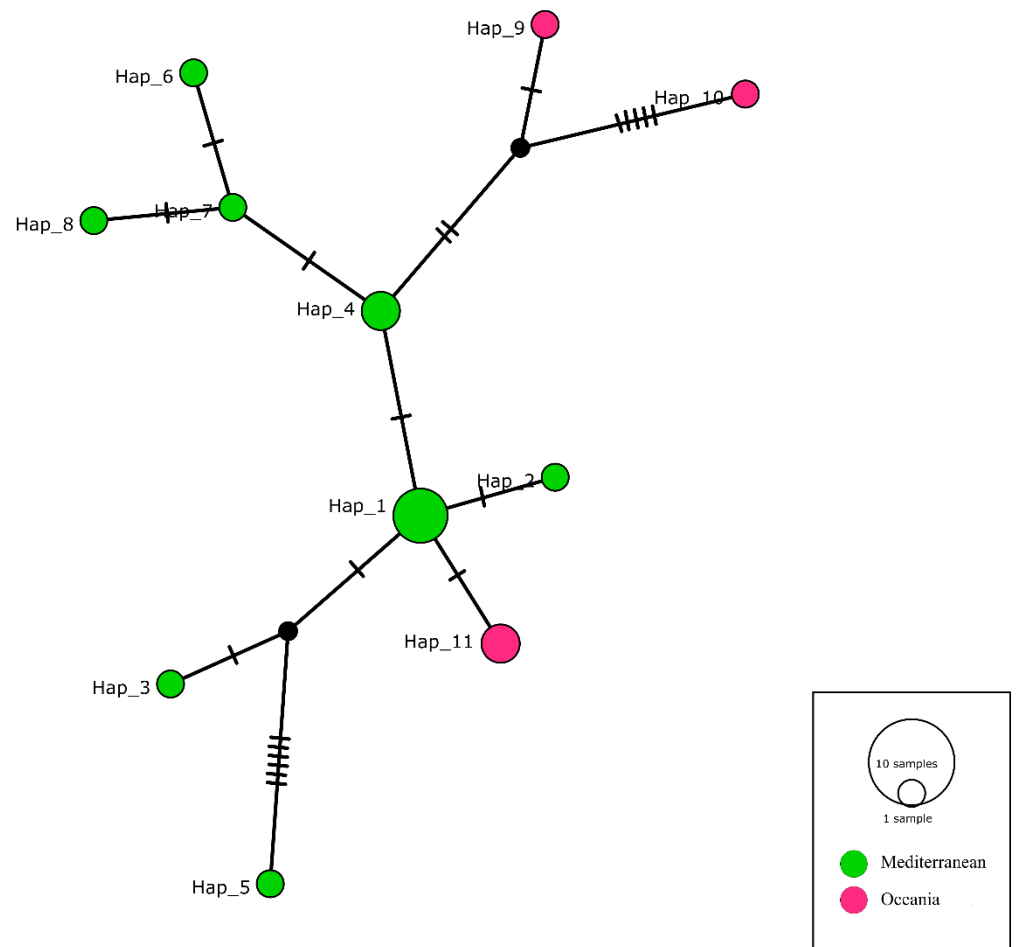


Figure 2. Median-joining haplotype network based on mitochondrial COI sequences of *Ophelimus maskelli* generated in PopART. Circle sizes are proportional to haplotype frequency, and colors indicate the geographic origin of samples from the Mediterranean region (green) and Oceania (pink). Each connection represents a mutational step between haplotypes.

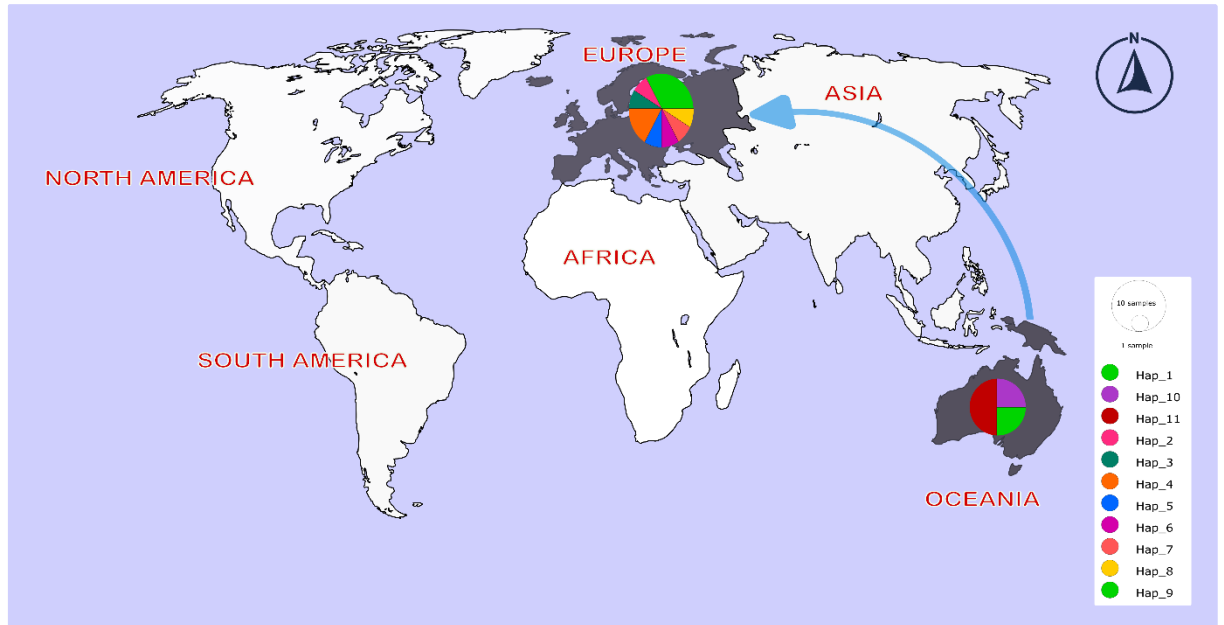


Figure 3. Global geographic distribution of mitochondrial COI haplotypes identified in *Ophelimus maskelli*. Pie charts represent the relative frequency of each haplotype within sampled regions, with colors corresponding to distinct haplotypes recovered from the haplotype network analysis. Circle sizes are proportional to sample size, and arrows indicate inferred migration patterns among geographic regions.

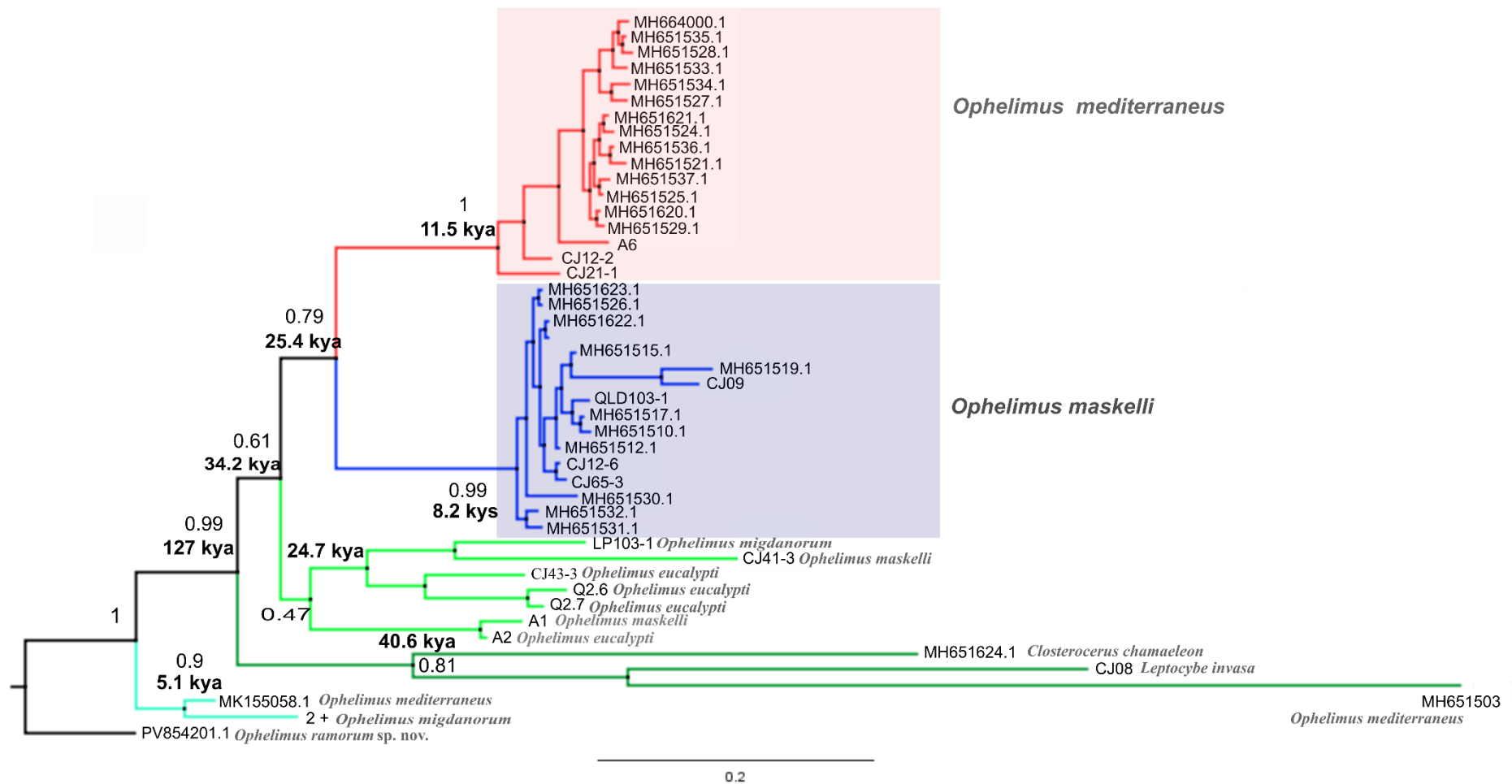


Figure 4. Bayesian phylogenetic reconstruction based in mitochondrial COI sequences of *Ophelimus* specimens. Number associated with branches indicate Bayesian posterior probability and Beas calculated time of divergence. The tree was rooted using PV854201.1 as outgroup.

Our molecular clock analysis estimated that *O. maskelli* and *O. mediterraneus* group diverged approximately 32,000 to 24,000 years ago (kya). This divergence timeframe coincides with the Late Pleistocene, specifically the period associated with the onset of the Last Glacial Maximum (LGM) (Nistelberger et al., 2014; Dew et al., 2016; Marske and Boyer, 2024).

Tabela 2. Haplotype frequencies and specimen associations for mitochondrial COI sequences within the confirmed *Ophelimus maskelli* clade.

Haplotype	Frequency	Sample IDs
Hap_1	4	MH651623.1, MH651622.1, MH651526.1, MH651515.1
Hap_2	1	MH651532.1
Hap_3	1	MH651531.1
Hap_4	2	MH651519.1, MH651514.1
Hap_5	1	MH651530.1
Hap_6	1	MH651517.1
Hap_7	1	MH651512.1
Hap_8	1	MH651510.1
Hap_9	1	CJ09
Hap_10	1	QLD103-1
Hap_11	2	CJ12-6 / CJ65-3

Our mPTP species delimitation results (**Figure 5**) are highly congruent with the recovered phylogenetic topologies, successfully identifying the confirmed *O. maskelli* clade and its sister lineage *O. mediterraneus*, as distinct species. This molecular separation is strongly supported by a clear barcoding gap, with intraspecific genetic variation within the true *O. maskelli* cluster ranging from only 0.0% to 1.7%. Additionally, the analysis correctly delimited the divergent Australian lineages of the Green Clade as separate evolutionary units, which exhibit high interspecific distances of 9.0% or greater relative to the primary *Ophelimus* group.

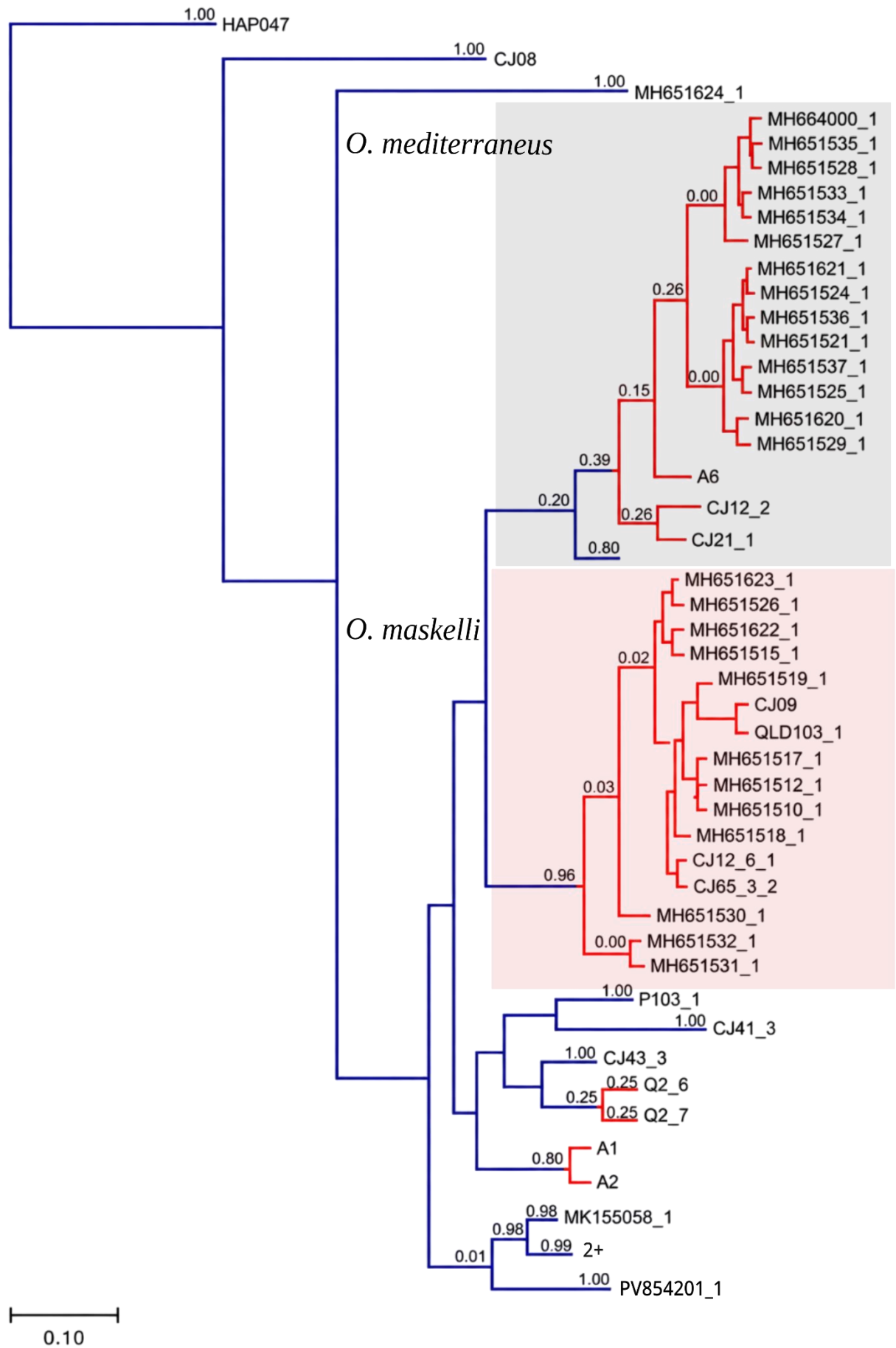


Figure 5. Species delimitation analysis inferred using the multi-rate Poisson Tree Processes (mPTP) based on COI sequences of *Ophelimus* specimens. Blue branches represent the Bayesian phylogenetic topology, whereas red branches indicate delimited species level entities recovered by the mPTP analysis.

5. Discussion

Our studies recovered the well-established monophyletic status of the *O. maskelli* clade, consistent with previous findings (Borowiec et al., 2019; Naves et al., 2026). As previously proposed, *O. mediterraneus* was inferred as the sister group, a relationship that frequently results in misidentification between the two species. This taxonomic challenge was further better elucidated in the work of Naves et al., 2026, which provides a critical framework for differentiating between *Ophelimus* species of forestry importance. OTU (Operational Taxonomic Unit) names in the phylogenetic trees were assigned based on GenBank BLAST matches; however, some OTUs identified as *O. maskelli* and *O. mediterraneus* were recovered outside their expected clades, highlighting the taxonomic and identification difficulties within the genus *Ophelimus*.

Distinguished primarily by the presence of a single submarginal seta on the forewing, this trait may be a weak morphological signal for species delimitation, particularly because *Ophelimus migdanorum* can also exhibit only one seta (Naves et al., 2026). Furthermore, these diagnostic setae are frequently damaged, missing, or difficult to observe in many specimens. Given these significant recognition challenges, combined with the absence of a formal identification key for most of the genus, we can begin to understand why biological control measures are not always effective (Borowiec et al., 2019; Garcia et al., 2019). To better comprehend the relationship between the genus *Ophelimus* and its associated parasitoids, we correlated our time-calibrated analysis, which indicates that the *O. maskelli* lineage diverged from its sister group, *O. mediterraneus*, approximately 32,000 to 24,000 kya, in the late Pleistocene, with the environmental shifts of the Last Glacial Maximum (LGM) in Australia and New zeland (Nistelberger et al., 2014). The recovery of samples CJ12 and CJ21 within the *O. mediterraneus* cluster suggests that this lineage may have persisted in refugial areas associated with the Australian Alps during glacial periods, contributing to its genetic differentiation from other lineages (Slatyer et al., 2014). Our time-calibrated analysis, coincides with the snow line in the Australian Alps was 800 meters lower than it is today, creating a physical ice wedge in the very regions where these specimens were collected (Canberra) (Endo et al., 2015). This finding aligns with the Southern Hemisphere model of glacial survival, which stands in contrast to the patterns observed in the Northern Hemisphere (Hewitt, 2000, 2004)

Conversely Northern Hemisphere models often characterized by massive extirpations and subsequent "genetic purity," the Last Glacial Maximum (LGM) in Australasia supported a model of persistence *in situ* within multiple distinct microrefugia (Wallis and Trewick, 2009; Marske and Boyer, 2024). As pointed out by Ashcroft et al., 2012 a refugia is able to keep species alive in the face of environmental changes during this period. Environmental stressors, such as the predicted ice wedge in the Australian Alps, acted as a selective filter, isolating ancestral populations (Slatyer et al., 2014). This isolation allowed populations to survive the LGM period, during which they could accumulate the mutations responsible for the rich phylogeographic patterns that we observe nowadays (Endo et al., 2015).

While the *O. maskelli* lineage likely maintained a multivoltine cycle in more stable refugia, the *O. mediterraneus* lineage was driven toward a univoltine life cycle to endure harsher, more seasonal conditions (Mendel et al., 2017; Garcia et al., 2019; Numata and Shintani, 2023). This deep Pleistocene divergence may have contributed to established the asynchronous phenological barrier observed today, potentially helping to explaining why the biological control agent *Closterocerus chamaeleon*, has shown limited effectiveness against this sister species in unsuccessful treatment attempts (Borowiec et al., 2019; Garcia et al., 2019). These findings also raise the possibility that *C. chamaeleon* co-evolved more specifically with multivoltine lineages. However, additional ecological and evolutionary studies are still necessary to better understand the mechanisms underlying these interactions.

The haplotype star-form graph, and mtpt distance analyses, together indicates that, despite a lower number of haplotypes in Oceania, these lineages are more divergent from one another than those found in Europe. This pattern suggests that in a new environment, once released from the natural barriers and specialist enemies that regulate populations in the native environmental , this genus undergoes rapid growth and subtle differentiation, exhibiting the behaviour of an introduced invasive species (Withers, 2001; Torchin et al., 2003; Branco et al., 2014). As proposed in studies regarding the unidirectional trajectory of Australian insect pests (Withers, 2001; Hirsch et al., 2020), these migratory movements proceed exclusively from Oceania to the introduced range, with no evidence of back migration, leading the organisms back to Australia. This lack of return movement, combined with the Enemy Release Hypothesis (ERH) (Keane and Crawley, 2002; Liu and Stiling, 2006), may help explain the high densities and rapid expansion of *Ophelimus maskelli* populations in the Mediterranean Basin compared to the more genetically diverse and regulated populations in its native Australian habitat .

Our findings, along to better understanding of the relations within the *Ophelimus* cluster, lead us to another problem that relies on the challenges of species identification. Until this

present work, morphological differentiation between *Ophelimus maskelli* and other species from the group, as *O. eucalypti*, *O. mediterraneus*, and *O. ramorum*—was based on the position of galls in petioles and subtle morphological signals (Garcia et al., 2019; Naves et al., 2026). These can easily be misunderstood on high infestation densities on a single leaf or even simultaneous infection with two species. Even in our dataset, some specimens were initially misidentified due to the absence of an identification key and morphological traits that are very hard to distinguish, such as wing setae.

To solve this, we propose a diagnostic DNA barcode for *Ophelimus maskelli*, deposited in GenBank under accession PZ286359. Inside the COI gene, we observed a molecular signature composed of 50 bp, located at positions 134 to 183 of the mitochondrial cytochrome c oxidase subunit I (COI) gene alignment, that aligns only with *O. maskelli* and no other species: TAATAATAGGAGGATTTGGAAATTTTAAATTCCTATATTTTGGGAATA.

An efficiency of this motif was confirmed by our BLAST analysis, which pointed that 50 bp molecular signature aligns with 100% identity exclusively to *Ophelimus maskelli* accessions, effectively distinguishing it from sister species like *O. mediterraneus* and *O. migdanorum*, which exhibit significantly lower identities of approximately 95–96% (see **Supplementar Material Appendix II**).

Assuming the challenges to properly identify this invasive species, also aligned with its particularly evolutionary history, makes *Ophelimus maskelli* a species that should be treated as a threat to forest health and eucalypty production. Overall, this work improved the understanding of diversification patterns within *Ophelimus* and demonstrated the effectiveness of molecular tools for distinguishing *O. maskelli* from closely related taxa. The identification of a diagnostic barcode region provides a useful tool for rapid species recognition, which may contribute to earlier detection and more effective management of this invasive gall wasp.

6. References

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7. Suplementar Material

Appendix I.

Maximum likelihood phylogenetic reconstructions of *Ophelimus* specimens

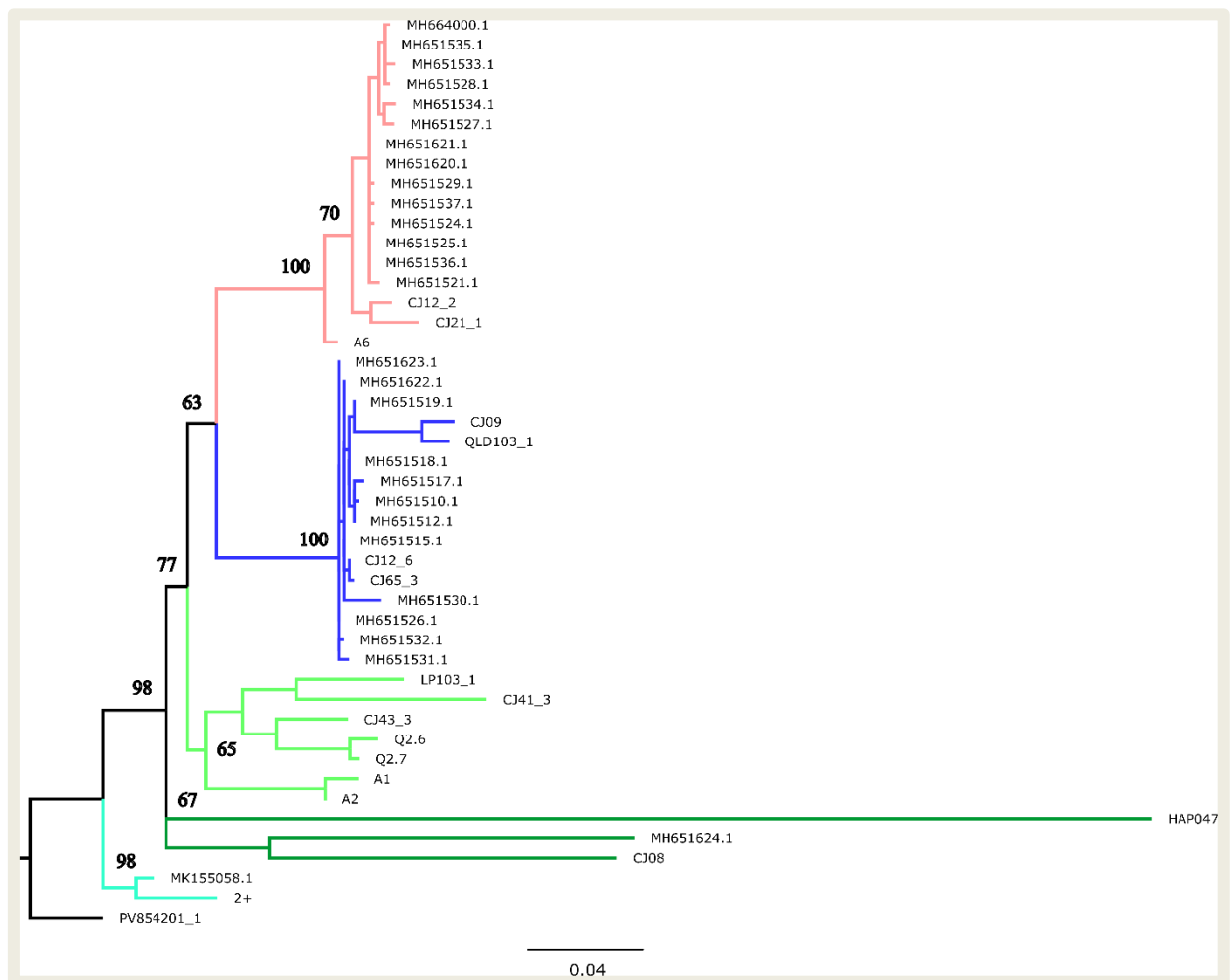


Figure 6. Maximum Likelihood phylogenetic reconstruction based on mitochondrial COI sequences of *Ophelimus* specimens inferred using RAxML under the GTR + G model. Numbers associated with branches indicate bootstrap support values obtained from 1,000 replicates. The topology recovered the principal evolutionary lineages within the *Ophelimus maskelli* complex, including the confirmed *O. maskelli* clade and divergent sister lineages. The tree was rooted using PV as outgroup.

Appendix II.

BLAST similarity results for mitochondrial COI sequences obtained from *Ophelimus* specimens

Tabela 3. BLASTn similarity results for mitochondrial COI sequences obtained from *Ophelimus* specimens analyzed in this study. The table presents the closest matches retrieved from GenBank, including scientific identification, sequence similarity metrics, query coverage and E-values.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	GenBank Accession
<i>Ophelimus maskelli</i> isolate								
1288 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	<i>Ophelimus maskelli</i>	93.5	93.5	100%	1,00E-15	100.00	618	MW441534.1
<i>Ophelimus maskelli</i> isolate								
6558 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	<i>Ophelimus maskelli</i>	93.5	93.5	100%	1,00E-15	100.00	583	MH651531.1
<i>Ophelimus maskelli</i> isolate								
D1559 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	<i>Ophelimus maskelli</i>	93.5	93.5	100%	1,00E-15	100.00	755	HM365046.1

Ophelimus maskelli isolate

6555 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096409.1

Ophelimus maskelli isolate

11011 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651511.1

Ophelimus maskelli isolate

6557 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096410.1

Ophelimus maskelli isolate

3760 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

571

MH651623.1

Ophelimus maskelli isolate

11015 cytochrome oxidase

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651515.1

subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli isolate

11016 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651516.1

Ophelimus maskelli isolate

3755 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096404.1

Ophelimus maskelli isolate

11012 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651512.1

Ophelimus maskelli isolate

3747 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

573

JX096401.1

Ophelimus maskelli isolate

3759 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651526.1

Ophelimus maskelli isolate

6552 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096406.1

Ophelimus maskelli isolate

3748 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

550

MH651622.1

Ophelimus maskelli isolate

3753 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

588

JX096402.1

Ophelimus maskelli isolate

6561 cytochrome oxidase

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651532.1

subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli isolate

11019 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651519.1

Ophelimus maskelli isolate

6558 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096411.1

Ophelimus maskelli voucher

maskelli COI cytochrome c
oxidase subunit I (COX1) gene,
partial cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

631

PV763040.1

Ophelimus maskelli isolate

11014 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

583

MH651514.1

Ophelimus maskelli isolate

6560 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096412.1

Ophelimus maskelli isolate

6553 cytochrome oxidase
subunit I gene, partial cds;
mitochondrial

Ophelimus maskelli

93.5

93.5

100%

1,00E-15

100.00

597

JX096407.1

Eulophidae sp. BOLD-2016

voucher BIOUG16743-E10
cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Eulophidae sp. BOLD-
2016

82.4

82.4

94%

3,00E-12

97.87

582

KR800964.1

Ophelimus maskelli isolate

6556 cytochrome oxidase
subunit I (COI) gene, partial
cds; mitochondrial

Ophelimus maskelli

82.4

82.4

100%

3,00E-12

96.00

583

MH651530.1

Closterocerus tau isolate D1568

cytochrome oxidase subunit I

Closterocerus tau

82.4

82.4

94%

3,00E-12

97.87

755

HM365035.1

(COI) gene, partial cds; mitochondrial								
<i>Eulophidae</i> sp. BIOUG22424-								
A03 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG22424-A03	82.4	82.4	94%	3,00E-12	97.87	652	MF908074.1
<i>Aphelinidae</i> sp. BOLD:ACN5665 voucher								
BIOUG13638-C07 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Aphelinidae</i> sp. BOLD:ACN5665	80.5	80.5	98%	1,00E-11	95.92	582	KR418765.1
<i>Eurytoma</i> sp. 13 YMZ-2025a voucher USNMENT01525842								
cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	<i>Eurytoma</i> sp. 13 YMZ- 2025a	80.5	80.5	98%	1,00E-11	95.92	675	PV031799.1
<i>Aphelinidae</i> sp. BOLD:ACN5665 voucher	<i>Aphelinidae</i> sp. BOLD:ACN5665	80.5	80.5	98%	1,00E-11	95.92	564	KR404214.1
BIOUG13642-H11 cytochrome								

oxidase subunit 1 (COI) gene,
partial cds; mitochondrial
Aphelinidae sp.

BOLD:ACN5665 voucher

BIOUG14198-H04 cytochrome
oxidase subunit 1 (COI) gene,
partial cds; mitochondrial

Aphelinidae sp.

BOLD:ACN5665

80.5	80.5	98%	1,00E-11	95.92	591	KR416683.1
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Eulophidae sp.

BOLD:ACM0763 voucher

BIOUG10919-E08 cytochrome
oxidase subunit 1 (COI) gene,
partial cds; mitochondrial

Eulophidae sp.

BOLD:ACM0763

80.5	80.5	86%	1,00E-11	100.00	546	KR421433.1
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Eulophidae sp. BOLD-2016

voucher BIOUG19191-G11

cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Eulophidae sp. BOLD-
2016

80.5	80.5	92%	1,00E-11	97.87	576	KR925886.1
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Pteromalidae sp. BIOUG02547-
E08 cytochrome oxidase

Pteromalidae sp.

BIOUG02547-E08

80.5	80.5	98%	1,00E-11	95.92	624	KY837858.1
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subunit 1 (COI) gene, partial

cds; mitochondrial

Eurytoma genale voucher

USNMENT01525803

cytochrome c oxidase subunit I	Eurytoma genale	80.5	80.5	98%	1,00E-11	95.92	675	PV031787.1
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(COX1) gene, partial cds;

mitochondrial

Aphelinidae sp.

BOLD:ACN5665 voucher

BIOUG13649-A07 cytochrome

Aphelinidae sp.

oxidase subunit 1 (COI) gene,

BOLD:ACN5665

partial cds; mitochondrial

Eulophidae sp. BIOUG07644-

D06 cytochrome oxidase

Eulophidae sp.

subunit 1 (COI) gene, partial

BIOUG07644-D06

cds; mitochondrial

Eurytoma asphodeli isolate

Eurytoma asphodeli

GDEL1415 cytochrome oxidase

subunit 1 (COI) gene, partial

cds; mitochondrial

Pteromalidae sp. BIOUG02543-

C11 cytochrome oxidase	Pteromalidae sp.	80.5	80.5	98%	1,00E-11	95.92	568	KY834200.1
subunit 1 (COI) gene, partial	BIOUG02543-C11							
cds; mitochondrial								

Eurytoma asphodeli isolate

GDEL1477 cytochrome oxidase	Eurytoma asphodeli	80.5	80.5	98%	1,00E-11	95.92	651	MH878891.1
subunit 1 (COI) gene, partial								
cds; mitochondrial								

Aphelinidae sp.

BOLD:ACN5665 voucher	Aphelinidae sp.							
BIOUG13731-A06 cytochrome	BOLD:ACN5665	80.5	80.5	98%	1,00E-11	95.92	573	KR415995.1
oxidase subunit 1 (COI) gene,								
partial cds; mitochondrial								

Aphelinidae sp.

BOLD:ACN5665 voucher	Aphelinidae sp.	80.5	80.5	98%	1,00E-11	95.92	573	KR416004.1
BIOUG13731-E12 cytochrome	BOLD:ACN5665							

oxidase subunit 1 (COI) gene,

partial cds; mitochondrial

Asaphes sp. BIOUG05940-D10

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Asaphes</i> sp. BIOUG05940-D10	78.7	78.7	96%	4,00E-11	95.83	473	MG497033.1
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Eulophidae sp. BIOUG21792-

E05 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG21792-E05	78.7	78.7	90%	4,00E-11	97.78	459	MG480214.1
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Eulophidae sp. BIOUG24653-

B06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG24653-B06	78.7	78.7	90%	4,00E-11	97.78	462	MG338042.1
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Asaphes sp. Asp03 cytochrome

oxidase subunit I (COI) gene, partial cds; mitochondrial	<i>Asaphes</i> sp. Asp03	78.7	78.7	96%	4,00E-11	95.83	617	MF850291.1
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Omphale sp. BIOUG22674-F08

cytochrome oxidase subunit 1	Omphale sp. BIOUG22674-F08	78.7	78.7	94%	4,00E-11	95.74	435	MG339725.1
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(COI) gene, partial cds;

mitochondrial

Eulophidae sp. BIOUG25702-

C08 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG25702-C08	78.7	78.7	90%	4,00E-11	97.78	594	MG338719.1
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Ophelimus sp. 1 RJH-2019

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Ophelimus</i> sp. 1 RJH-2019	76.8	76.8	94%	1,00E-10	95.74	672	MK155058.1
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Eulophidae sp. BIOUG12004-

G06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG12004-G06	76.8	76.8	94%	1,00E-10	95.74	432	MG483654.1
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Eulophidae sp. BIOUG24554-

E06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG24554-E06	76.8	76.8	94%	1,00E-10	95.74	453	MG341903.1
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Ophelimus migdanorum isolate

2_rep.1_F cytochrome oxidase

subunit 1 gene, partial cds;

mitochondrial

Ophelimus migdanorum

76.8

76.8

94%

1,00E-10

95.74

588

MN336339.1

Eulophidae sp. BIOUG25127-

E04 cytochrome oxidase

subunit 1 (COI) gene, partial

cds; mitochondrial

Eulophidae sp.

BIOUG25127-E04

76.8

76.8

94%

1,00E-10

95.74

395

MG343336.1

Ophelimus migdanorum isolate

3_rep.2_R cytochrome oxidase

subunit 1 gene, partial cds;

mitochondrial

Ophelimus migdanorum

76.8

76.8

94%

1,00E-10

95.74

594

MN336333.1

Eulophidae sp. BIOUG13404-

B05 cytochrome oxidase

subunit 1 (COI) gene, partial

cds; mitochondrial

Eulophidae sp.

BIOUG13404-B05

76.8

76.8

94%

1,00E-10

95.74

479

MG483643.1

Eulophidae sp. BIOUG01115-

D02 cytochrome oxidase

Eulophidae sp.

BIOUG01115-D02

76.8

76.8

94%

1,00E-10

95.74

502

MG340774.1

subunit 1 (COI) gene, partial

cds; mitochondrial

Eulophidae sp. BIOUG01049-

A03 cytochrome oxidase	<i>Eulophidae</i> sp.	76.8	76.8	94%	1,00E-10	95.74	502	MG336115.1
subunit 1 (COI) gene, partial	BIOUG01049-A03							
cds; mitochondrial								

Omphale sp. BIOUG21223-B09

cytochrome oxidase subunit 1	<i>Omphale</i> sp.	76.8	76.8	94%	1,00E-10	95.74	462	MG447089.1
(COI) gene, partial cds;	BIOUG21223-B09							
mitochondrial								

Eulophidae sp. BIOUG12750-

E07 cytochrome oxidase	<i>Eulophidae</i> sp.	76.8	76.8	94%	1,00E-10	95.74	475	MG483145.1
subunit 1 (COI) gene, partial	BIOUG12750-E07							
cds; mitochondrial								

Eulophidae sp. BIOUG05120-

B06 cytochrome oxidase	<i>Eulophidae</i> sp.	76.8	76.8	94%	1,00E-10	95.74	441	MG487800.1
subunit 1 (COI) gene, partial	BIOUG05120-B06							
cds; mitochondrial								

Pteromalus sp. BOLD-2016

voucher BIOUG16111-C10

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Pteromalus</i> sp. BOLD- 2016	76.8	76.8	94%	1,00E-10	95.74	616	KR890781.1
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Eulophidae sp. BIOUG21687-

E12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG21687-E12	76.8	76.8	94%	1,00E-10	95.74	477	MG484859.1
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Eulophidae sp. BIOUG13404-

B07 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG13404-B07	76.8	76.8	94%	1,00E-10	95.74	477	MG486715.1
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Eulophidae sp. BIOUG21792-

E11 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eulophidae</i> sp. BIOUG21792-E11	76.8	76.8	94%	1,00E-10	95.74	453	MG482873.1
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Eulophidae sp. BIOUG08245-

E09 cytochrome oxidase	<i>Eulophidae</i> sp. BIOUG08245-E09	76.8	76.8	94%	1,00E-10	95.74	456	MG487752.1
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subunit 1 (COI) gene, partial

cds; mitochondrial

Eulophidae sp. BIOUG12451-

H06 cytochrome oxidase

Eulophidae sp.

76.8

76.8

94%

1,00E-10

95.74

492

MG482690.1

subunit 1 (COI) gene, partial

BIOUG12451-H06

cds; mitochondrial

Eulophidae sp. BIOUG03395-

C10 cytochrome oxidase

Eulophidae sp.

76.8

76.8

94%

1,00E-10

95.74

473

MG480150.1

subunit 1 (COI) gene, partial

BIOUG03395-C10

cds; mitochondrial

Eulophidae sp. BOLD-2016

voucher BIOUG01601-H11

Eulophidae sp. BOLD-
2016

cytochrome oxidase subunit 1

76.8

76.8

94%

1,00E-10

95.74

595

KR799067.1

(COI) gene, partial cds;

mitochondrial

Eulophidae sp. BIOUG25127-

E03 cytochrome oxidase

Eulophidae sp.

76.8

76.8

94%

1,00E-10

95.74

475

MG335119.1

subunit 1 (COI) gene, partial

BIOUG25127-E03

cds; mitochondrial

Ophelimus migdanorum isolate

3_rep.1_R cytochrome oxidase
subunit 1 gene, partial cds;
mitochondrial

Ophelimus migdanorum

76.8

76.8

94%

1,00E-10

95.74

597

MN336334.1

Eulophidae sp. BOLD-2016

voucher 10BBCHY-1132
cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Eulophidae sp. BOLD-
2016

76.8

76.8

94%

1,00E-10

95.74

564

KR806811.1

Torymidae gen. n. cf.

Pseudotorymus sp. PJAN1221
cytochrome oxidase subunit I
gene, partial cds; mitochondrial

Torymidae gen. n. cf.*Pseudotorymus* sp.
PJAN1221

76.8

76.8

94%

1,00E-10

95.74

1404

MF956394.1

Entedoninae sp.

BOLD:ACL5296 voucher
BIOUG10410-B06 cytochrome
oxidase subunit 1 (COI) gene,
partial cds; mitochondrial

Entedoninae sp.
BOLD:ACL5296

76.8

76.8

94%

1,00E-10

95.74

527

KR406478.1

Eurytomidae sp. BOLD-2016

voucher BIOUG03240-F07

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eurytomidae</i> sp. BOLD- 2016	75.0	75.0	98%	5,00E-10	93.88	627	KR795727.1
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Eurytomidae sp. BIOUG31808-

G11 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eurytomidae</i> sp. BIOUG31808-G11	75.0	75.0	98%	5,00E-10	93.88	471	MG338362.1
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Orasema costaricensis voucher

UCRC_ENT 00248188

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Orasema costaricensis</i>	75.0	75.0	98%	5,00E-10	93.88	207	MW983205.1
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Eurytomidae sp. BIOUG21902-

E08 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Eurytomidae</i> sp. BIOUG21902-E08	75.0	75.0	98%	5,00E-10	93.88	537	MG342026.1
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Orasema aenea group sp. 2

JH-2016 isolate D3932

cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Orasema aenea group sp. 2 JH-2016	75.0	75.0	98%	5,00E-10	93.88	651	KR733124.1
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Eulophidae sp. BIOUG24066-

H10 cytochrome oxidase

subunit 1 (COI) gene, partial cds; mitochondrial	Eulophidae sp. BIOUG24066-H10	75.0	75.0	86%	5,00E-10	97.73	453	MG339667.1
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Orasema beameri voucher

USA_CO_D4346 cytochrome c

oxidase subunit I gene, partial cds; mitochondrial	Orasema beameri	75.0	75.0	98%	5,00E-10	93.88	482	MH247586.1
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Eurytomidae sp. BOLD-2016

voucher BIOUG08412-E09

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Eurytomidae sp. BOLD- 2016	75.0	75.0	98%	5,00E-10	93.88	626	KR784912.1
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Orasema erwini voucher

ECU_D1280 cytochrome c
oxidase subunit I gene, partial
cds; mitochondrial

Orasema erwini

75.0

75.0

98%

5,00E-10

93.88

561

MH247601.1

Tetramesa sp. 1 CVS-2023a

voucher LMY_2022_00108
cytochrome c oxidase subunit I
(COX1) gene, partial cds;
mitochondrial

Tetramesa sp. 1 CVS-
2023a

75.0

75.0

98%

5,00E-10

93.88

432

OQ302603.1

Encarsia sp. BIOUG19667-B09

cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Encarsia sp.
BIOUG19667-B09

75.0

75.0

98%

5,00E-10

93.88

599

MF930466.1

Encarsia sp. BOLD-2016

voucher BIOUG06003-D09

cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Encarsia sp. BOLD-2016

75.0

75.0

98%

5,00E-10

93.88

542

KR801376.1

Aphelinidae sp. BIOUG11775-

E04 cytochrome oxidase	Aphelinidae sp.	75.0	75.0	98%	5,00E-10	93.88	481	MG487411.1
subunit 1 (COI) gene, partial	BIOUG11775-E04							
cds; mitochondrial								

Eulophidae sp. BIOUG03596-

G02 cytochrome oxidase	Eulophidae sp.	75.0	75.0	92%	5,00E-10	95.74	469	MG481049.1
subunit 1 (COI) gene, partial	BIOUG03596-G02							
cds; mitochondrial								

Eurytomidae sp. BOLD-2016

voucher BIOUG01018-C07								
cytochrome oxidase subunit 1	Eurytomidae sp. BOLD-	75.0	75.0	98%	5,00E-10	93.88	626	KR786918.1
(COI) gene, partial cds;	2016							
mitochondrial								

Orasema costaricensis voucher

UCRC_ENT 00248202								
cytochrome oxidase subunit 1	Orasema costaricensis	75.0	75.0	98%	5,00E-10	93.88	325	MW983479.1
(COI) gene, partial cds;								
mitochondrial								

Eurytomidae sp. BOLD-2016

voucher BIOUG08412-G07

<i>Eurytomidae</i> sp. BOLD-2016	75.0	75.0	98%	5,00E-10	93.88	609	KR784995.1
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cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Orasema sp. AM1 CRI D3962

voucher AM1_CRI_D3962

<i>Orasema</i> sp. AM1 CRI D3962	75.0	75.0	98%	5,00E-10	93.88	482	MH247605.1
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cytochrome c oxidase subunit I
gene, partial cds; mitochondrial

Eurytomidae sp. BOLD-2016

voucher BIOUG03240-D04

<i>Eurytomidae</i> sp. BOLD-2016	75.0	75.0	98%	5,00E-10	93.88	469	KR783191.1
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cytochrome oxidase subunit 1
(COI) gene, partial cds;
mitochondrial

Tetramesa sp. 1 CVS-2023a

voucher LMY_2022_00008

<i>Tetramesa</i> sp. 1 CVS-2023a	75.0	75.0	98%	5,00E-10	93.88	415	OQ302600.1
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cytochrome c oxidase subunit I
(COX1) gene, partial cds;
mitochondrial

Entedoninae sp. BIOUG25556-

E02 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Entedoninae sp. BIOUG25556-E02	75.0	75.0	86%	5,00E-10	97.67	546	MG338755.1
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Eurytomidae sp. BOLD-2016

voucher BIOUG08412-E01

cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Eurytomidae sp. BOLD-2016	75.0	75.0	98%	5,00E-10	93.88	537	KR796302.1
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Pediobius metallicus isolate

J7P3 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

Pediobius metallicus	75.0	75.0	92%	5,00E-10	95.74	625	MG836440.1
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Eurytomidae sp. BIOUG31799-

D04 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Eurytomidae sp. BIOUG31799-D04	75.0	75.0	98%	5,00E-10	93.88	584	MG342123.1
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Oraema rikra voucher

PER_D4125 cytochrome c	Oraema rikra	75.0	75.0	98%	5,00E-10	93.88	651	MH247618.1
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oxidase subunit I gene, partial

cds; mitochondrial

Orasema sp. n. 2 JH-2016

isolate D4139 cytochrome

Orasema sp. n. 2 JH-

75.0

75.0

98%

5,00E-10

93.88

651

KR733120.1

oxidase subunit I (COI) gene,

2016

partial cds; mitochondrial

Conclusão

O presente trabalho apresenta os resultados da modelagem de nicho da microvespa *Ophelimus maskelli*, bem como sua história evolutiva, além de propor uma ferramenta molecular capaz de distinguir esta espécie das demais linhagens do gênero.

Os locais com maiores índices de adequabilidade para a espécie, tanto no cenário atual quanto em projeções futuras no Brasil, concentram-se principalmente nas regiões Nordeste e Sul do país. Dado o intenso fluxo de material florestal transportado entre essas localidades por redes ferroviárias, rodoviárias, marítimas e aéreas, o país encontra-se sob risco considerável de estabelecimento dessa espécie de microvespa australiana. Além disso, as inúmeras vias que conectam as principais áreas de plantio de eucalipto podem atuar como corredores capazes de favorecer a dispersão de *O. maskelli* para novas plantações.

Ao observarmos a história evolutiva desta vespa e cruzarmos essas informações com os padrões biogeográficos apresentados neste trabalho, torna-se possível compreender o contexto de diferenciação dessa espécie em relação às demais linhagens do gênero. A partir disso, propomos a utilização do DNA barcode e da assinatura molecular identificada como ferramentas auxiliares para a identificação mais precisa de *O. maskelli*, contribuindo potencialmente para o monitoramento e controle da entrada dessa praga nas florestas plantadas do país.