

A newly invaded eucalypt gall wasp and its parasitoid in China: identification and biology

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Abstract

Gall pests have caused seriously damage to eucalyptus in recent years in China. In this study, we collected and identified the invaded eucalyptus gall wasp *Ophelimus maskelli* and its parasitoid *Closterocerus chamaeleon* for the first time in China. *O. maskelli* induced two types of gall shape, i.e., blister- and neoplastic-shaped galls. The blister-shaped galls were most likely induced on leaves rather on petioles and stems, while the neoplastic-shaped galls were more frequently found on petioles rather than on leaves and stems. We find that of the four solar orientations, *O. maskelli* significantly preferred to colonize *Eucalyptus* trees in the sunnier areas in south over that in west and north with significantly lower gall infestation rate detected in east. The parasitism rate of *C. chamaeleon* increased from 23.4% in February to 97.4% in March 2023 when the population of *O. maskelli* galls was low (236.8–251.4 galls/20 branches); however, when gall population increased to a high level (≈ 450 galls/20 branches), the parasitism rate significantly decreased to 26.5% in April, although it raised to 62.6% again in May. Our findings deliver insigne into development of biological control programs of *O. maskelli* using *C. chamaeleon*.

Introduction

Eucalypts (Myrtales: Myrtaceae: *Eucalyptus*) endemic to Australia were first introduced to China in 1890 (Qi, 2002). They have now consisted of the largest forest plantations in the world because of their fastgrowing nature (Rejmánek & Richardson, 2011) and high economic value for wood and non-wood products (Zheng, 2007). Guangxi Zhuang Autonomous Region is the main production area of eucalypts in China. By the end of 2021, their planting area in this region was about three million hectares (Forestry Bureau of Guangxi Zhuang Autonomous Region, 2022). Compared to other tree species, the planting area and storage volume of eucalyptus are ranked the first in China (Li, 2023). However, with the increase of eucalypt planting area, the frequent outbreaks of insect pests have threatened the industry (Liao, 2019).

In later December 2022, heavy infestation of *Eucalyptus leizhou* trees by the unknown gall wasp(s) was first observed in the fields in Guangxi Zhuang Autonomous Region, China. The wasps and their parasitoids were reared in laboratory and adults emerged from the galls in early February 2023. In China, only two species of eucalyptus gall wasps, i.e., *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) (Wu et al., 2009) and *Ophelimus bipolaris* Chen & Yao (Chen et al., 2021), have been reported, with three hymenopteran parasitoids, the *Quadrastichus mendeli* Kim & La Salle (Eulophidae), *Aprostocetus causalis* La Salle & Wu (Eulophidae) and *Megastigmus sichuanensis* Doğanlar & Zheng (Torymidae) attacking the former (Zheng et al., 2016; Doğanlar et al., 2017) and no parasitoids recorded for the latter. Our preliminary study suggests that the gall wasp and its parasitoid are the new species currently established in China.

The genus *Ophelimus* (Hymenoptera: Eulophidae), originated in Australia, comprises approximately 53 species of gall wasps on *Eucalyptus* (Borowiec, 2019; Molina-Mercader, 2019). However, only four *Ophelimus* species are considered as the major pests of eucalypts, i.e., *O. maskelli* (Ashmead) on

Eucalyptus globulus, E. camaldulensis, and *E. tereticornis* (Withers et al., 2000; Protasov et al., 2007a), O. *mediterraneus* Borowiec & Burks on *E. globulus, E. cinerea, E. gunni,* and *E. parvula* (Borowiec et al., 2019), O. *migdanorum* Molina-Mercade on *E. globulus* (Molina-Mercader et al., 2019)d *bipolaris* Chen & Yao on *E. grandis, E. grandis* × *E. urophylla, E. tereticornis* and *E. urophylla* (Chen et al., 2021). Among these gall wasps, *O. maskelli* is the most widely distributed (Mansfield, 2016) and has established itself as an invasive species in more than 40 countries across Europe, Asia, the Middle East, Mediterranean, Africa and North America (Doğanlar et al., 2007; Protasov et al., 2007a; Branco et al., 2009; Caleca, 2010; Burks et al., 2015a, 2015b; Garcia et al., 2019; BiCEP, 2022; CABI, 2023). This wasp induces the blistershaped galls by laying eggs on the leaves of several *Eucalyptus* species (Protasov et al., 2007a). Heavy leaf galling induced by *O. maskelli* results in widespread defoliation in a very short time, loss of growth and vigor in susceptible trees, and serious economic losses (Mendel et al., 2007; Protasov et al., 2007a). Like other *Ophelimus* species, *O. maskelli* is a thelytokous parthenogenetic species which builds up the population quickly (Protasov et al., 2007a).

Closterocerus chamaeleon (Girault) (Hymenoptera: Eulophidae), native to Australia and widely distributing in 34 countries (Noyes, 2023), is a parasitoid with a narrow host range including *O. maskelli* and perhaps some other *Ophelimus* spp. associated with *Eucalyptus* sp. (Protasov et al., 2007b). *C. chamaeleon* is parthenogenetic and can pierce the galls and lay one egg on the *O. maskelli* larva (Protasov et al., 2007b). *C. chamaeleon* adults survive longer than that of *O. maskelli* (21 vs. 10 days at 25°C) (Protasov et al., 2007a, 2017b). Caleca et al. (2011) reported that *C. chamaeleon* was released to control *O. maskelli* in Sicily, Italy in 2006. The parasitism rate reached 62% at the release sites within 5 months after release and 65–100% throughout Sicily and in many surrounding islets by winter 2007–2008 (Caleca et al., 2011). In 2007, *C. chamaeleon* was recorded from Portugal, about 2,700 km of the release sites in Italy (Branco et al., 2009). Due to this its greater dispersal rate and higher capacity of suppressing *O. maskelli* populations, *C. chamaeleon* has been introduced into some countries for example, Israel (Protasov et al., 2007b) and Turkey (Doğanlar & Mendel, 2007), for biological control of *O. maskelli*.

In this study, we identified the invaded gall wasp and its parasitoid, reported its damaged characteristics and parasitism rate of the parasitoid. Our results will provide critical information to evaluate the economic importance of the invaded gall wasp and to evaluate the biological control potential of the established parasitoid.

Materials and methods

Insect sampling

Branches of 2-year-old eucalypt trees (*E. Leizhou* No. 11) infected with galls were collected from Changle Town (21°53'29"N 109°21'46"E), Hepu County, Beihai City, Guangxi Zhuang Autonomous Region, and brought back to the laboratory for examination. Branches were divided into five groups with 20 branches per group. Branches of each group were inserted in a plastic container (15 cm height and 13 cm

diameter) filled with water to keep them fresh and the container was placed in a sealed net cage (40 cm × 40 cm × 80 cm). Branches were maintained at 26 \pm 1°C (the average air temperature of the sampling sites during collection), 70–80% relative humidity with a photoperiod of 13:11 h (light:dark) for adult emergence. The emerged adults landing on the inner wall of the net cage were captured using the 1.5-mL centrifuge tubes. Adults were maintained in centrifuge tubes before used for molecular and morphological identification.

Species identification

Molecular biology identification

Samples of the two different wasp species were killed directly in 95% ethanol. Prior to DNA extraction, the preserved insect specimens were rinsed with sterilized distilled water and DNA was extracted from adult specimens using the TIANamp Genomic DNA Kit DP304-02 (Tiangen Biotech Co. Ltd., Beijing, China) following the manufacturer's protocol.

The 28s gene was amplified using the primers D2-3551F and D2-4057R (5'- TCAAGACGGGTCCTGAAAGT-3') (Gillespie et al., 2005). The reaction volume of PCR was 25 μ L. The PCR reaction solution comprised of 12.5 μ L of Premix Taq TM (Ex Taq TM Version 2.0 plus dye) RR902A (TaKaRa Biotechnology Co. Ltd., Beijing, China), 1 μ L of DNA extract, 0.4 μ L of each primer (10 μ M) and 10.7 μ L of doubledistilled H₂O. The PCR program consisted of 35 cycles at 98°C (30 s) for denaturation, 55°C (30 s) for annealing, 72°C (1 min) for extension, and final holding at 4°C before used for PCR. The PCR products were taken on a 1% (w) agarose gel and electrophoresed at 120 V for 40 min before analysis of the electrophoresis results on a UV gel imaging system. Finally, the products were sent to (Sangon Biotech Co. Ltd., Shanghai, China) for sequencing.

The resulting sequences were verified by NCBI Nucleotide Blast tool

(https://blast.ncbi.nlm.nih.gov/Blast.cgi), sequences were aligned using DNAMAN. MEGA 7.0 software was subsequently used to align sequences and construct the phylogenetic tree (Kumar et al., 2016). Neighbor-joining (NJ) trees (Saitou & Nei, 1987) were reconstructed with 1000 bootstrap replications (Felsenstein, 1985).

Morphological identification

Terminology of morphological characters follows Protasov et al. (2007a, b). A digital Microscope VHX-6000 (Keyence, Osaka, Japan) was used to examine the morphology both species. Scanning Electron Microscopy FEI Quattro S (Thermo Fisher Scientific, Czech Republic) was used to record the morphological characteristics of mesosoma and antenna. For scanning electron microscopy, 20 adults of each species were immersed in 70% ethanol. An ultrasonic bath JP-010 T (Skymen Cleaning Equipment Co., Ltd., Shenzhen, China) was used to clean specimens once for 180 s at 250 W. Washed samples were then fixed in 2.5% glutaraldehyde at 4°C for 24 h, and then in a phosphate buffer (0.01 mol/L, pH = 7.0) for 30 min. Samples were dehydrated through an ascending ethanol series (70, 75, 80, 85, 90, and 95% ethanol; 10 min for each) and then fully dehydrated twice in 100% ethanol (15 min for each). Samples were placed in a clean environment and dried naturally. Finally, specimens were coated with gold, then were observed and photographed with an FEI Quattro S at 10.0 kV.

Gall infestation, shape, and distribution Gall infestation at different orientations

The percentage of leaves infected by the gall wasps was examined on two hills (88 and 93 m above sea level, respectively) on the same sample collection site (see Section 2.1) on 24 March and 27 April 2023, respectively. On each hill, we randomly selected 60 trees in each of the four orientations (east, south, west, and north facing). One branch with 30-40 leaves from each tree was randomly selected to record the galled leaves (GL) and total number of leaves (TL). The percentages of galled leaves (PL) were calculated as: PL = (GL/TL) × 100%.

Gall shape and distribution on different positions of host plant branches

To determine the different shapes and their population on different positions on branches, we randomly collected 100 leaves, 100 petioles and 100 stems from 2-year-old *E. Leizhou* in the study site. The shapes locating on leaves, petioles and stems and their number were counted.

Gall population size and parasitism in different months

To determine the species of parasitic natural enemy and parasitism rate, 100 branches with gall-infected leaves were collected at the end of each of four successive months from February 2023 to May 2023. Branches were reared and wasps were collected as described in Section 2.1. Newly emerged adults of gall wasps and their parasitoids were individually checked using Digital Microscope VHX-6000. The number of adult wasps of both species was recorded. The parasitism rate was calculated as: $P = CC / (CC + OM) \times 100\%$.

Statistical analysis

All data were analyzed using SAS 9.13 (SAS Institute Inc, USA) with a rejection level set at *P* = 0.05. Data on the proportion of galled leaves (Fig. 5) were analyzed using a generalized linear model (GLIMMIX procedure) with tree orientation as fixed factor and 'tree' nested within 'hill' as random factor in the model followed by a Gamma distribution and a Log function. The same model was also used to analyse data on the number of different gall shape with a Poisson distribution and a Log function (Table 1). We applied a Tukey-Kramer test for multiple comparisons of proportion of galled leaves between orientations and of gall shapes between locations (Fig. 4, Table 1). A Wilcoxon Signed-Rank test (a nonparametric paired-t test) was applied to compare the difference in the number of blister-shaped and neoplastic-shaped galls on leaves, perioles or stems (Table 1). Data on the gall population size and parasitism rate in different months (Fig. 6) were normally distributed (Shapiro-Wilk test; UNIVARIATE procedure) and thus

analyzed using ANOVA (GLM procedure) with Tukey's Studentized Range (HSD) test for multiple comparisons between months.

Results

Species identification

Molecular biology identification

The amplification was performed successfully for the samples of two species. We obtained matrices of 587 bp and 604 bp for 28S. The DNA sequences were submitted to the National Center for Biotechnology Information (NCBI) database and assigned accession numbers OQ654036 and OQ654046. Results of the phylogenetic analysis show that the two species were identified as *O. maskelli* and *C. chamaeleon*, respectively (Fig. 1).

Morphological identification

For *O. maskelli*, body length 0.9–1.2 mm. Body brown to black with green metallic luster. Eyes black. Mesosoma with distinct and uniform reticulation. Pronotum short, arched, mesoscutum with three pairs of short setae, anterior first pairs of setae relatively shorter; notauli distinct and deep; scutellum approximately elliptical, with two pairs of setae, length slightly longer than setae which born on the mesoscutum; metanotum short but wider than scutellum (Fig. 2a). Antenna brown to black, with four ring-like anelli, and only fourth segment had the sensillum chaetica, only one funicle (Fig. 2b). Wings hyaline, stigmal vein and stigma pale brown to brown. Coxae balck. The end of femora and tibia were pale brown, tarsomeres brown, remainders of femora and tibia brown to black. The end of tarsus dark brown, remainders of it hyaline to pale brown. Metasoma darker dorsally (Fig. 2c). Wings surface cilium medium density. Only one single seta on the submarginal vein of *O. maskelli* was found (Fig. 2d, arrow), premarginal vein and marginal vein were discontinuous in color; marginal vein with four to six short setae, stigma and marginal vein were discontinuous in color (Fig. 2d). Legs had tiny cilium; coxae reticulate, femur and tibia imbricate, all legs had a spur seta on the medial end of the tibiae (Fig. 2c, arrow).

For *C. chamaeleon*, body length 0.7–1.1 mm. Body brown with metallic green and orange. Antenna scape and its basal part hyaline to pale gray, pedicel and flagellum pale brown to brown. Eyes from the upper part of the red transition to the lower part of the blue-violet. Mesosoma distinct and uniform reticulation, mesoscutum with 2 pairs of short setae. Scutellum just with 1 seta, much longer than setae which born on the mesoscutum (Fig. 3a). Antennae geniculate, flagellum nearly fus iform, two funicles, first funicular segment smaller than another one, clava with first segment stout, and clava tapering toward the end (Fig. 3b). Wings hyaline. Legs hyaline, each femur, tibia, tarsus tip is pale gray. Metasoma darker brown (Fig. 3c). Wings uniformly ciliated, submarginal vein 2–3 setae (Fig. 3d, arrow), postmarginal vein shorter

than stigmal vein (Fig. 3d). Legs full of cilium, each legs had 1 spur seta at the medial end of the tibia segment (Fig. 3c, arrow).

Percentage of galled leaves, gall shapes and distribution of galls on the host trees

Percentage of galled leaves at different foliage orientations

Our results show that O. maskelli adults significantly preferred leaves in South over that in West and North for oviposition with significantly lower infestation rate detected in East (Tukey-Kramer test: F_{3,357} = 23.79, P<0.0001) (Fig. 4).

Gall shape and distribution on different positions of host plant branches

Two gall shapes, i.e., blister- and neoplastic-shaped galls were observed on leaves, petioles and stems of *E. leizhou* in this study (Fig. 5).

The number of blister-shaped galls on leaves was significantly higher than that on stems, and that on stems was significantly higher than those on petiole (Tukey-Kramer test: $F_{2,297}$ = 2433.89, P<0.0001) (Table 1). While the number of neoplastic-shaped galls on petioles was significantly higher than that on leaves and stems (Tukey-Kramer test: F_{2.297} = 32.19, P < 0.0001) (Table 1). Furthermore, the number of blister-shaped galls was significantly higher than that of neoplastic-shaped galls on leaves or stems (Wilcoxon signed-rank test: S = 2525 and 2517 for leaf and stem, respectively, P < 0.0001), but the number of blister-shaped galls was significantly lower than that of neoplastic-shaped galls on petioles (Wilcoxon signed-rank test: S = -576, P = 0.280) (Table 1).

Mean (± SE) number of different shapes of galls on different positions of host plant branches		
Location	Blister-shaped	Neoplastic-shaped
Leaf	68.70 ± 5.74 a	0.01 ± 0.01 b
Petiole	1.00 ± 0.14 c	1.68±0.18 a
Stem	5.64 ± 0.41 b	0.02±0.01 b

T I I 4

Different letters in each column indicate significant difference (P < 0.05)

Gall population size and parasitism in different months

The number of galls or oviposition of O. maskelli was significantly higher in April and May than in February and March (Tukey's Studentized Range test: $F_{3,16} = 14.22$, P<0.0001) (Fig. 6A). The parasitism rate of C. chamaeleon on O. maskelli was significantly higher in March than in May, with significantly

lower parasitism rate detected in February and April (Tukey's Studentized Range test: $F_{3,16}$ = 1228.51, P< 0.0001) (Fig. 6B).

Discussion

Species identification

The number of setae on the submarginal vein of the fore wing is a key diagnostic characteristic for identifying the *Ophelimus* species (Protasov et al., 2007a; Burks et al., 2015b). For example, *O. bipolaris* has 3–5 of dorsal setae on the submarginal vein (Chen et al., 2021) with 2–4 for *O. mediterraneus* (Borowiec et al., 2019), 1–3 for *O. migdnorum* (Molina-Mercader et al., 2019). However, Molina-Mercader et al. (2019) report that the number of submarginal vein setae is related to body size, and larger specimens may have more submarginal vein setae than the smaller ones. We show that regardless of the body size, our target gall wasp had only one single seta on the submarginal vein (Fig. 3d), suggesting that it could be another *Ophelimus* species differing from *O. bipolaris, O. mediterraneus*, and *O. migdnorum*. Results of the phylogenetic analysis also confirmed that the species collected in this study was *O. maskelli*.

Three parasitoids of eucalyptus gall wasp, the *Q. mendeli, A. causalis* and *M. sichuanensis* have established in China (Zheng et al., 2016; Doğanlar et al., 2017). Unlike the *M. sichuanensis* whose ovipositor sheath extremely elongates 1.6 times as long as metasoma in dorsal view (Doğanlar et al., 2017), Q. *mendeli, A. causalis* (Kim et al., 2008; Yang et al., 2014) and out target species have slightly protruding ovipositor sheath which is very short in dorsal view. Further, there is only 1 seta observed on the submarginal vein in *Q. mendeli* (Kim et al., 2008), 3–4 setae in *A. causalis* (Yang et al., 2014), and 2–4 setae in our species. Therefore, the parasitoid specie of *O. maskelli* collected in this study differed from that have already established previously. Basing on the results of the phylogenetic analysis, the parasitoid of *O. maskelli* in this study was actually the *C. chamaeleon*.

Percentage of galled leaves, and gall shapes and their distribution on host branch of O. maskelli

Ophelimus maskelli reproduce by thelytokous parthenogenesis (Protasov et al., 2007a), and a successful oviposition results in full development of the gall, holding a single egg. In this study, the average percentage of galled leaves was relatively higher, ranging between 46.89% and 58.5% (Fig. 5). In Portugal, the average percentages of infested leaves caused by *O. maskelli* is about 18% and 40% on *E. globulus* and *E. camaldulensis*, respectively (Branco et al., 2009) and 37% on 25 *Eucalyptus* sp. in Choucha, Tunisia (Branco et al., 2014). The higher infestation rate of *O. maskelli* galls detected in this study may associate with the generally higher air temperature in the south subtropical regions in China, but this assumption remains further investigations.

Previous research indicates that insect pests tend to colonize trees planted the sunnier areas in south due to the thermophilic nature, for example the beetle *Agrilus biguttatus* Fabricius (Moraal & Hilszczanski, 2000). Agreeing with the work of Moraal and Hilszczanski (2000), we find that of the four solar

orientations, *O. maskelli* preferred eucalyptus trees growing in south over that in west and north for oviposition with significantly lower infestation rate detected in the east (Fig. 5). Therefore, thermal requirement relevant to solar orientation is critical to build up to the populations of *O. maskelli* especially in early spring when the temperature is still low. Our results suggest that to improve the biological control efficiency of *C. chamaeleon* on *O. maskelli*, augmentative release of mass-reared parasitoids will be conducted or more parasitoids released in southern and/or western and northern areas in fields.

Ophelimus maskelli usually induce single-blister like galls on both sides of host leaves (Protasov et al., 2007a). However, we find that *O. maskelli* also induced neoplastic-shaped galls especially on the petioles (Table 1). It is known that species richness of galling taxa inducing the dramatic diversity of galls on host plants can be linked causally to the feeding behaviours of gallers (Stone & Schönrogge, 2003). Although host-plant genotype may have a significant impact on gall phenotype (Whitham, 1992), the available evidence suggests that gall morphology should be regarded as the extended phenotype of galler genes (Dawkins, 1982). Therefore, a galler should induce structurally similar galls on different host plant species. The morphological diversity of galls induced by *O. maskelli* in this study may attribute to the differences in the strength of mechanical tissue among plant organs, resulting in a variety of gall morphotypes (Espírito-Santo et al., 2007). It is expected that compared to the blister-shaped galls on leaves, neoplastic-shaped galls of a high density on petioles may cause more serious damage on the *Eucalyptus* trees especially the seedlings.

Parasitism of C. chamaeleon on O. maskelli

Closterocerus chamaeleon was the only parasitoid of *O. maskelli* found during the study. *C. chamaeleon* is an ectoparasite with a narrow range of host species among eulophids forming galls on eucalypts (Protasov et al., 2007a), and regarded as the greater potential agent for biological control of *O. maskelli* due to its thelytokous nature (Doğanla & Mendel, 2007; Protasov et al., 2007b), longer longevity and higher survival rate than its hosts (Sinulingga et al., 2021), and high parasitism rate (Caleca et al., 2011). Here, we show that the parasitism rate of *C. chamaeleon* increased from 23.4% in February to 97.4% in March 2023 when the population of *O. maskelli* galls was low (236.8–251.4 galls/20 branches), suggesting that *C. chamaeleon* may efficiently suppress the population of overwintering *O. maskelli* in early spring. However, when gall population increased to a high level (\approx 450 galls/20 branches) in April, the parasitism rate significantly decreased to 26.5%, although it raised to 62.6% again in May. The mechanisms leading to this phenomenon is not clear. Garcia et al. (2019) also detect a lack of parasitism in *Ophelimus* sp., which is attributed to the seasonal asynchrony between *C. chamaeleon* and the gall-wasp phenology, as reported in many parasitoid-host systems (e.g., Godfray et al., 1994; Van Nouhuys & Lei, 2004; Shaw et al., 2005). Our results suggest that augmentative release of mass-reared parasitoids is required to suppress the field populations of *O. maskelli* galls in mid- and late-spring.

Conclusions

The eucalyptus gall wasp *O. maskelli* and its parasitoid *C. chamaeleon* are collected and identified for the first time in China. *O. maskelli* prefer to colonize *Eucalyptus* trees planted in the sunnier areas in south, and induce blister-shaped and neoplastic-shaped galls with the former mainly on the leaves and the latter on the petioles. The parasitism rate of *C. chamaeleon* on *O. maskelli* varies significantly over the growing seasons. Our findings may help improve the biological control programs of *O. maskelli* using *C. chamaeleon*.

Declarations

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Author contributions X.-L. Zheng and X.-Z. He designed the research. Y.-H. Li, J.-H. Su and X.-Y Wang conducted the experiments. Y.-H. Li and J.-H. Su collected the raw data. Y.-H. Li, X.-Z. He and X.-L. Zheng analyzed the data. Y.-H. Li and X.-L. Zheng wrote the first draft of the manuscript. X.-Z. He, Q. Wang and X.-L. Zheng revised the final draft of the manuscript. S.-Y. Chen assisted in sample collection. All authors have read and agreed to the published version of the manuscript.

Data availability The data that are presented in this study are available in the article.

Ethics approval and Consent to participate Not applicable.

Consent for publication Not applicable.

Conficts of interest The authors declare no conficts of interest.

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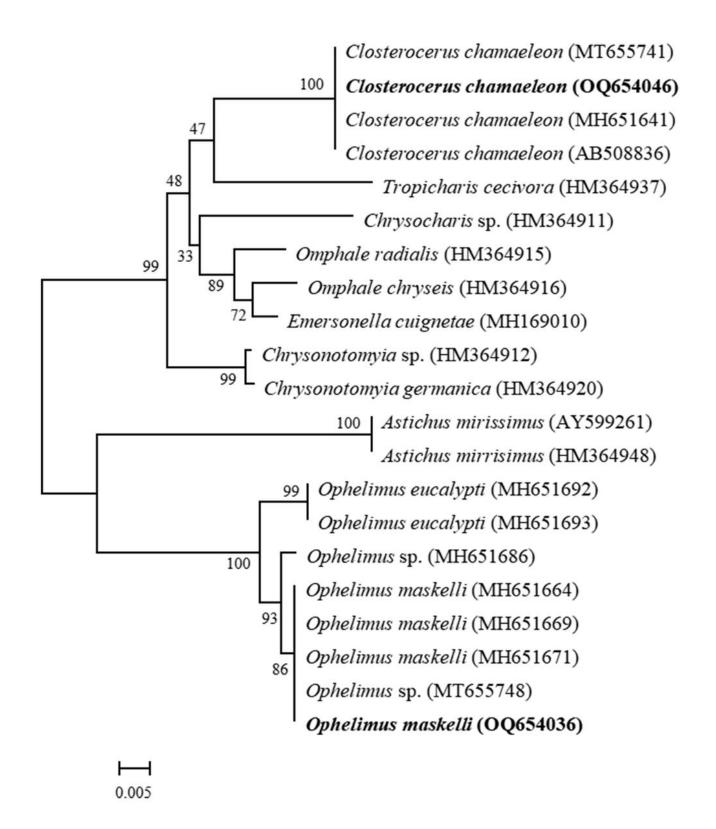
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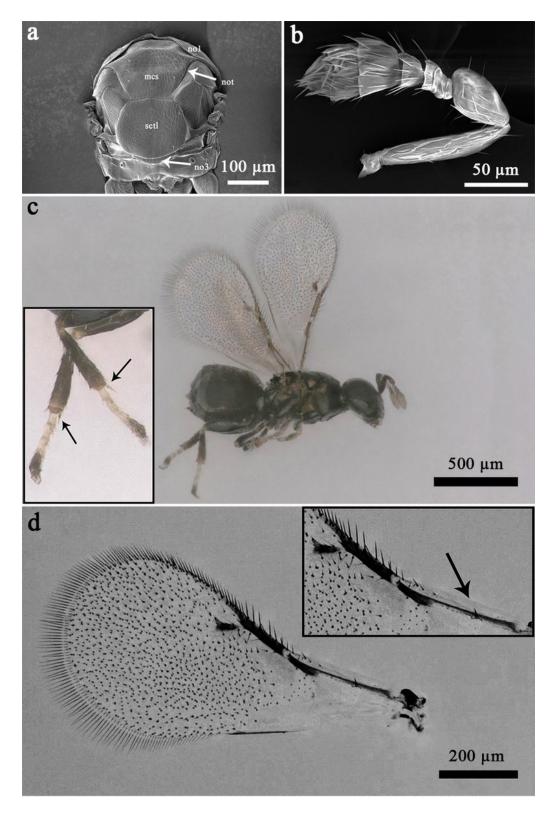
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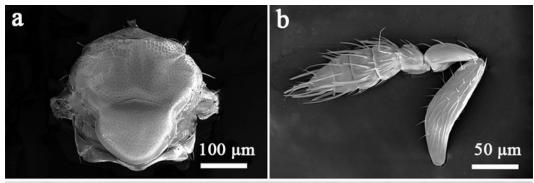
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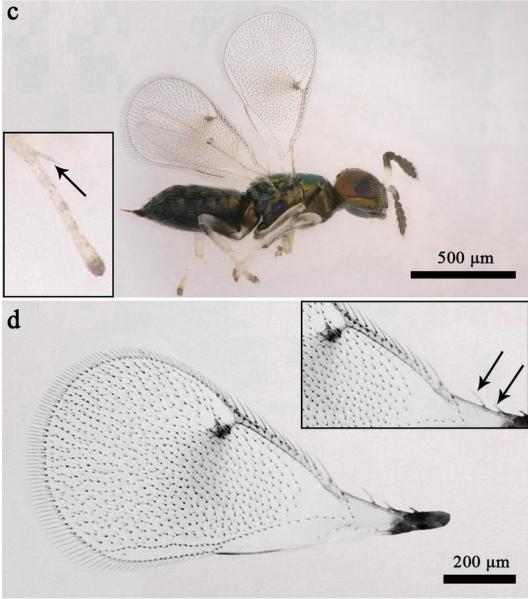


Phylogenetic tree based on 28S rDNA sequences of Ophelimus maskelli and Closterocerus chamaeleon

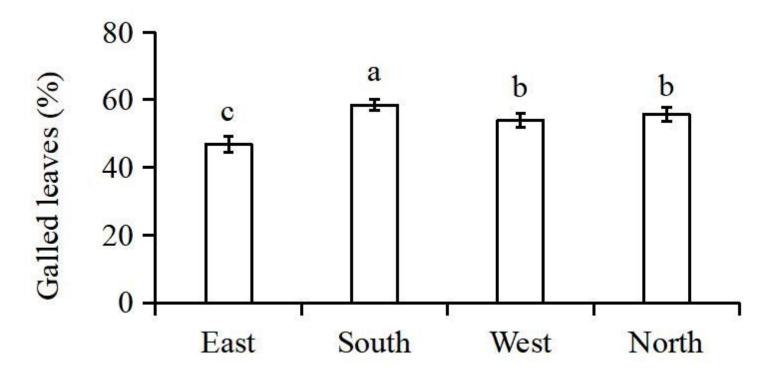


Morphological characteristics of *Ophelimus maskelli*: mesosoma, dorsal view (**a**); antenna (**b**); habitus, lateral view (**c**); forewing (**d**). no1, pronotum; no3, metanotum; msc, mesoscutum; sctl, scutellum; not, notauli

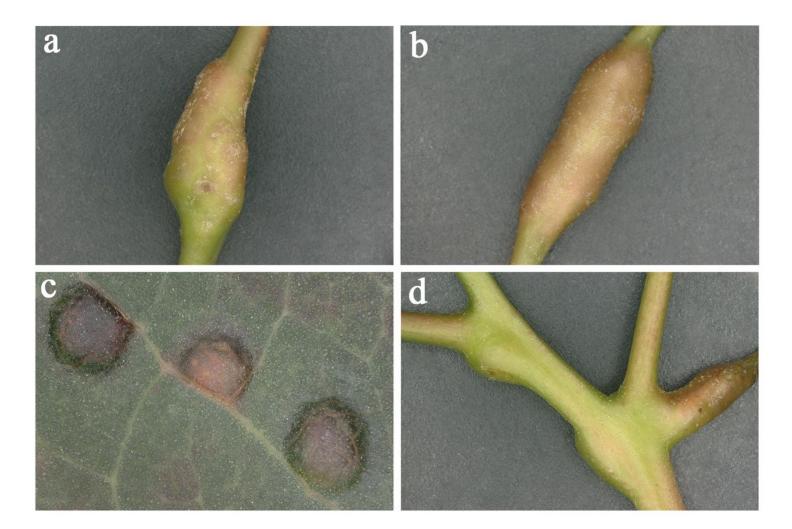




Morphological characteristics of *Closterocerus chamaeleon*: mesosoma, dorsal view (**a**);antenna (**b**); habitus, lateral view (**c**);forewing (**d**)



Mean proportion (± SE) of leaves infested with Ophelimus maskelli galls at different orientations in fields



Shapes of galls induced by *Ophelimus maskelli* on *E. leizhou*: neoplastic-shaped galls on a petiole (**a**-**b**); blister-shaped galls on a leaf (**c**); neoplastic-shaped galls on a stem (**d**)

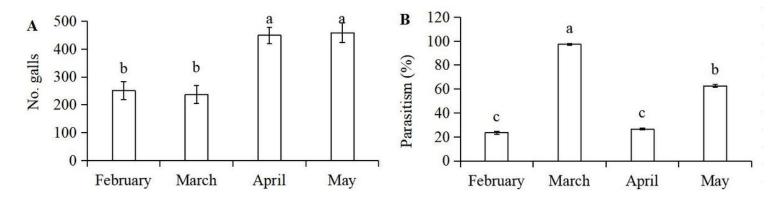


Figure 6

Gall population of *Ophelimus maskelli* (**A**) and parasitism rate of *Closterocerus chamaeleon* on *Ophelimus maskelli* (**B**) in different months