

# New Record of *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae) on the White Gum in Timor Tengah Selatan District, East Nusa Tenggara Province, Indonesia

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## ABSTRACT

The eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) was identified for the first time on the white gum *Eucalyptus alba* Reinw. ex Blume (Myrtaceae) or known as *ampupu* in the Timor Tengah Selatan District, East Nusa Tenggara Province, Indonesia. Identification is based on the characteristics of the gall morphotype and the morphological character of the insects that emerge from the eucalyptus gall. Morphologically identified insects were confirmed based on molecular characters. Identification based on molecular characteristics using 28S DNA sequence shows 100% homology with *L. invasa* DNA sequences deposited in GenBank (accession # KP143987.1) The eucalyptus gall wasp, *L. invasa*, causes gall formation on the twigs, young branches and stems on the white gum trees. *L. invasa* is considered one of the most dangerous gall inducer on eucalyptus. This finding contributes important data for the implementation of pest management in the eucalyptus forest industry in Indonesia.

**Keywords:** *gall wasp, invasive insects, Leptocybe invasa, Myrtaceae, lesser sunda*

## 1. INTRODUCTION

Eucalyptus is one of the priority plant species for Industrial Plantations because of its adaptability to tropical and subtropical climates. This plant is generally native to Australia, the Philippines, Papua New Guinea and Indonesia [1]. Attacks from gall-forming insects are one of the problems in eucalyptus plants. These pests damage plants by forming a distinctive lump called a gall. Gall is an abnormal form of swelling that causes enlargement of the cells or plant tissue beyond its normal size. The blue gum chalcid, *Leptocybe invasa* Fischer and La Salle 2004 (Hymenoptera: Eulophidae), is a gall-forming insect that originated from Australia and was first noticed as a pest on eucalyptus in the Mediterranean and Middle East in 2000 [2]. This pest has spread to many countries including Cambodia, China, India, Iran, Malaysia, Sri Lanka, Taiwan, Thailand, Turkey and Vietnam [3]. This insect has become a relevant pest for eucalypt forestry worldwide. Various eucalypt species such as *E. camaldulensis* (var. *Camaldulensis* and *obtusa*), *E. grandis*, *E. robusta* and *E. tereticornis* show very high susceptibility to blue gum chalcid [3–5] and have been

confirmed to be the most susceptible to *L. invasa* [6]. *L. invasa* are well known for its aggressiveness in attacks and rapid dispersion [7]. The objective of this work is to report the presence of *L. invasa* in Indonesia and the first note of *Eucalyptus alba* as its host.

### 1.1. Related Work

Eucalyptus galls are collected from *E. alba* plantation in Soe reforestation area, Timor Tengah Selatan District, East Nusa Tenggara Province (9° 50'59''S; 124°16'20''E) (Figure 1) from October 2017 to June 2018. The shape, colour, and gall location on the plant was observed and documented using camera Fuji XA3. Galls were removed from the host plant and transported to the laboratory. Observation was done under the stereo microscope Leica M250 C to determine the type of gall and its size. All collected insect galls were deposited at IPB University, Bogor and Museum Zoologicum Bogoriense, Indonesian Institute of Sciences as the voucher specimens.



**Figure 1** The location for gall eucalypt collection in Soe reforestation garden

### 1.1.1. Insect identification

Collected insects were preserved in tubes containing 70% EtOH. Insects were identified based on morphological characteristics performed under the Olympus SZ51 stereo microscope as well as on the Leica M205C microscope along with the Leica DFC450 digital camera and the LAS V.4.4.0 (Build: 454) application that is connected to a PC computer. Identification of morphology based on Mendel literature [5]. The identification will also be done using molecular technique based on the DNA sequence of CO-I.

### 1.2. Our Contribution

This paper presents the first report of the *L. invasa* insect as a cause of gall on eucalyptus plants in East Nusa Tenggara Province, Indonesia, based on morphological characters and has been molecularly confirmed. This finding also implies that special precautions are needed for the eucalyptus forest industry on other islands such as Java, Sumatra, and Kalimantan where these insects still do not exist.

### 1.3. Paper Structure

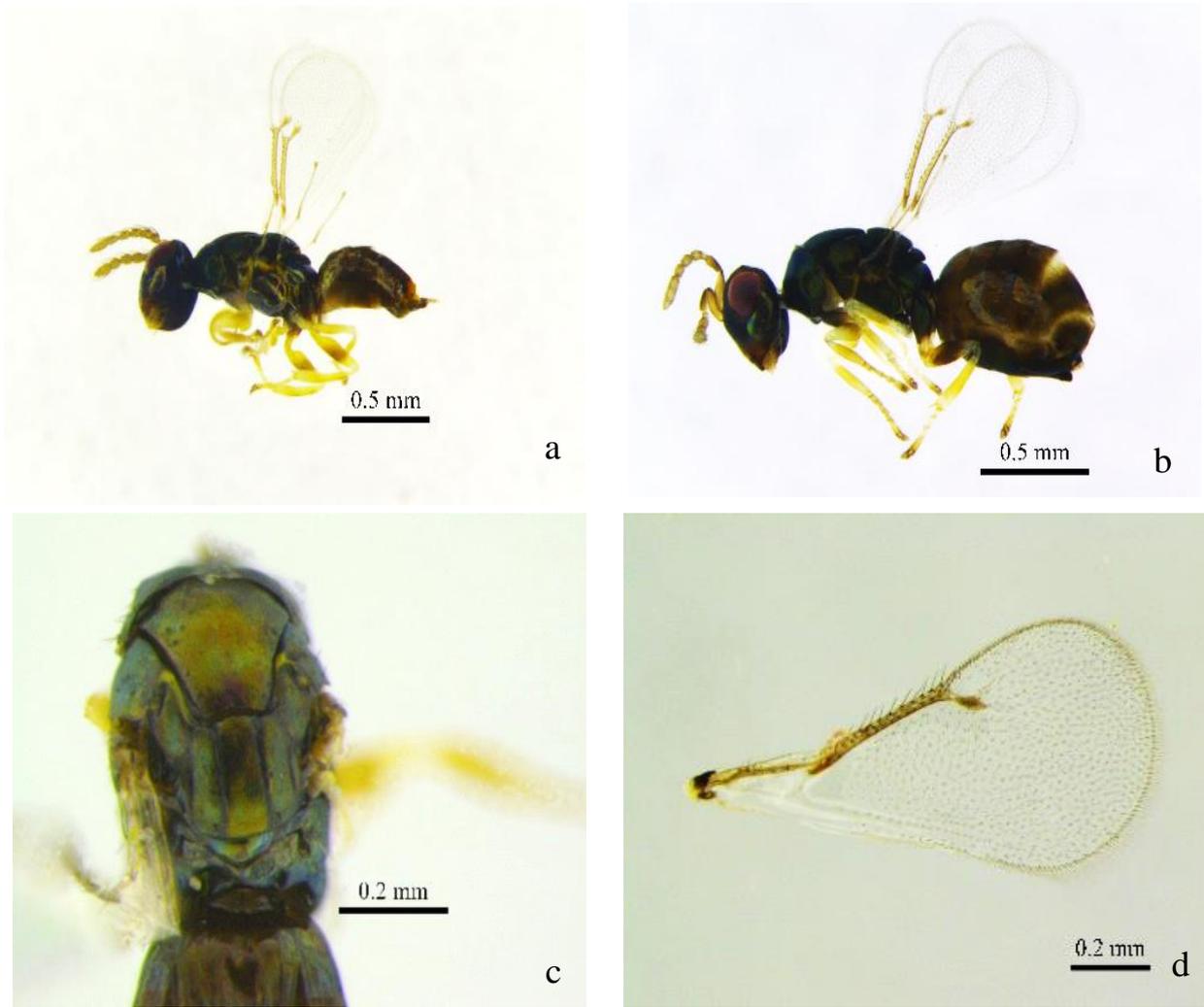
The rest of the paper is organized as follows. Section 2 introduces the preliminaries used in this paper, which includes collecting symptoms of the eucalyptus plant, describing the gall symptoms and shape of the gall. The symptomatic leaves and branches are taken and stored in plastic containers so that the observed insects come out. Identifying morphologically based on related literature and comparing it with Mendel *et al.* [2], Doganlar [8] and confirm it with molecular identification. Finally, Section 3 concludes the paper and presents direction for future research

## 2. BACKGROUND

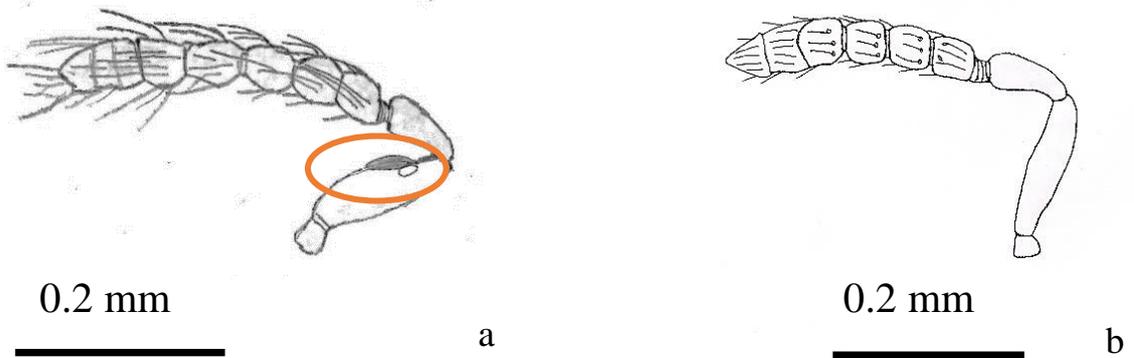
*Leptocybe invasa* is a novel forest pest responsible for significant economic loss in *Eucalyptus* spp. plantations worldwide. *L. invasa* was not identified when it was first recorded in Turkey [9] and Italy in 2001 as *Aprostocetus* sp. [10]. Simultaneously, severe damage to eucalyptus plantations in Israel and the economic losses that resulted from this pest led to the study of the unknown eulophid wasp-causing gall on eucalypts [2].

Wasp specimens (Figure 2) were identified in insect biosystematics laboratory, Faculty of Agriculture, IPB University with the scientific name *L. invasa*, by comparing the descriptions given by Mendel *et al.* [2]. The length of female wasp ranged from 1.1-1.44 mm with a body and head size larger than the males (Figure 2a, 2b). Body length of male ranged from 0.78-1.23 mm; mesosoma brown with metallic shine (blue or dark green); metasoma brown with a slight metallic hint on the dorsal portion. Prothorax is short; well-developed mesothorax; scutellum divided into three separated zones by sublateral lines (Figure 2c). Wings hyaline with yellow veins. Hyaline wings completely covered with setae and slight wing venation. Post marginal vein is shorter than stigmal vein. Submarginal vein generally with 3–4 dorsal setae. Scutellum with submedian and sublateral lines (Figure 2c). Original and detailed description is available in Doganlar [8].

The male antennae are covered with longer hairs compared to the female (Figure 3a, 3b). The male antennae with yellow scape become darker dorsally in the apex and ventral plaque. Pedicellus is yellow, with a darker dorsal basal portion, and a club yellow funicle. The scape is longer than the pedicellum with broadened middle section. Flagellum with six segments separated by four annelli, the basal segments constitute a funiculum and three apical segments form the antennal mass (Figure 3b).



**Figure 2** Morphology of *L. invasa*: (a) male, (b) female, (c) mesosoma dorsal, (d) forewing



**Figure 3** Morphology *L. invasa*: (a) antenna male, (b) antenna female

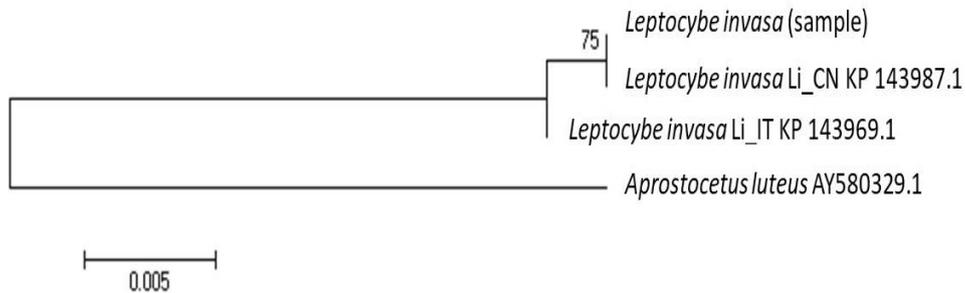
*Leptocybe invasa* is considered one of the most dangerous gall-inducer in eucalyptus. The level of damage to *E. alba* caused by *L. invasa* can reach 64%. The formation of gall caused by *L. invasa* yield several varieties. Gall in *E. alba* occurs early in the development of veins, leaves, twigs, branches and stems (Figure 4). The variety in gall shapes are possible through the interaction with other insects in the site of infection. This is in line with a report made by Cornell [11] stating that the diversity in gall shapes correspond to a higher variety of interaction between insects.

In Soe reforestation area, Timor Tengah Selatan District, East Nusa Tenggara Province, the number of gall

caused by *L. invasa* in *E. alba* is 1-84 gall with an average per leaf of 3.17 gall. The gall is oval shaped at the top and bottom of the leaf surface. While on the stems and twigs, the gall formed by this insect is malformed with a length of 0.5-2.2 cm. The identification based on molecular character utilized 28S DNA sequences with primers D2 3549 (5'-AGT CGT GTT GCT TGA TAG TGC AG-3') and D2 4068 (5'-TTG GTC CGT GTT TCA AGA CGG G-3') showing 100% homology with the *L. invasa* DNA sequences deposited in GenBank (accession # KP143987.1). It is the sister group of *L. invasa* originating from China (Figure 5).



**Figure 4** *Eucalyptus alba* leaf. Galls induced by *L. invasa*: (a) vein leaves, (b) stem, (c) twig



**Figure 5** Phylogeny of *Leptocybe invasa* using software Mega 6 with Neighborhood joining tree, Bootstrap 1000x

### 3. CONCLUSION

*Leptocybe invasa* was first reported on *Eucalyptus alba* in the Timor Tengah Selatan District, East Nusa Tenggara Province, Indonesia identified based on its morphological and molecular characteristics. *L. invasa* is considered one of the most dangerous gall inducers on eucalyptus and it is reported to have high occurrence in Timor Tengah Selatan District, East Nusa Tenggara Province. This preliminary finding is an early warning for eucalyptus forest industry in Indonesia.

### ACKNOWLEDGMENT

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