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TITLE: Biological Aspect of Eucalyptus Gall Wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) and Its Parasitoids in *Eucalyptus camaldulensis* Dehnh. Plantations, Tha Muang and Phanom Thuan Districts, Kanchanaburi Province

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THESIS

BIOLOGICAL ASPECT OF EUCALYPTUS GALL WASP, *Leptocybe invasa* Fisher & La Salle (HYMENOPTERA: EULOPHIDAE) AND ITS PARASITOIDS IN *Eucalyptus camaldulensis* Dehnh. PLANTATIONS, THA MUANG AND PHANOM THUAN DISTRICTS, KANCHANABURI PROVINCE

BENJAKHUN SANGTONGPRAOW

A Thesis Submitted in Partial Fulfillment of
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Benjakhun Sangtongpraow 2011: Biological Aspect of Eucalyptus Gall Wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) and Its Parasitoids in *Eucalyptus camaldulensis* Dehnh. Plantations, Tha Muang and Phanom Thuan Districts, Kanchanaburi Province. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Kosol Charernsom, M.S. 228 pages.

The objectives of this research were to study the biological aspect of *Leptocybe invasa* and its parasitoids and to assess their population dynamics in *Eucalyptus camaldulensis* plantations, Kanchanaburi province. The experiments were undertaken in laboratory, greenhouses and plantations.

It was found both female and male *L. invasa*. Their morphologies were described. By feeding with 6 different diets, honey solution could prolong the largest mean longevity of female (7.67 days) and male (5.67 days). Different diets had significant effects on the means of their longevities at P=0.05. The average potential fecundity of female of all sizes and ages was 158.70 eggs per a female. There was significant positive relationship between female sizes and egg loads (R^2=0.772). The mean realized fecundity of a female was 61.53 progenies per a female. *L. invasa* is a pro–ovigenic species. The female oviposited eggs in vascular bundles in young tissues of *E. camaldulensis*. Eggs development stimulated gall development. The mean development time from eggs to adult was 45.96 days. This research found two local parasitoids of *L. invasa*; namely *Aprostocetus* sp. (abbreviated to Asp) and *Megastigmus* sp. (abbreviated to Msp). Their morphologies of both female and male were described. By feeding with six different diets, honey solution could prolong largest mean longevity with 18.67 and 13.33 days for female and male Asp; and 9.83 and 7.83 days for female and male Msp. Different diets had significant effects on the means of their longevities at P=0.05. The average potential fecundity of female Asp, of all size and ages, was 6.31 eggs per a female; and that of female Msp was 2.98 eggs per a female. There was significantly positive relationship between female sizes and egg loads (R^2=0.250 for Asp and R^2=0.156 for Msp). The mean realized fecundity of a female Asp was 51.10 progenies per female while that of a female Msp was 13.20 progenies per female. Asp and Msp are synovigenic species. Asp was a solitary endoparasitoid and Msp was a solitary ectoparasitoid. Mean development time of Asp was 12.92 days while that of Msp was 17.00 days. The populations of *L. invasa*, Asp and Msp were monthly fluctuated. The population of each species showed the peak on May and declined later in rainy period. The insect similarity, index of insect diversity and evenness index were also determined. The findings suggest that *Aprostocetus* sp. is more suitable than *Megastigmus* sp. to be used as biocontrol agent of *L. invasa.* However, the potential combination of these parasitoids to control *L. invasa* is recommended.

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Benjakhun Sangtongpraow

April, 2011

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INTRODUCTION

Eucalyptus camaldulensis trees are widely planted in state lands, private lands and farmlands of Thailand. This fast growing tree species provides wood materials, products and other indirect benefits for domestic, commercial and industrial uses.

The area of *E. camaldulensis* plantations in 19 provinces of the northeastern Thailand was 4,229,247 rais (6.25 rais=1 hectare), represented 4.03% of the total area of the northeast, and the most planting areas were found in Nakhon Ratchasima followed by Surin and Udon Thani provinces (Forest Research Center, 2007). So the total area of *E. camaldulensis* plantations in Thailand is more than 4 million rais.

Most *E. camaldulensis* plantations use some selected clones and also used monoculture system in vast areas. It was believed this tree species produced high wood yield and was more tolerant to plant pests than other tree species. Unfortunately, many years ago, *E. camaldulensis* plantations in some provinces were damaged by the leaf–gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae).

This leaf–gall wasp is an exotic and invasive species. The attack of *L. invasa* in Thailand was first observed in Chachoengsao province in 2004 on young coppice shoots of *E. camaldulensis* in a plantation. The damages gradually become notable and spread to the other parts of the country. Today the injuries by *L. invasa* are also found in Lao and Vietnam (Thu *et al*., 2009). In Mediterranean region, *L. invasa* was

discovered in Israel in 2000, subsequently spreaded to Africa in 2002 and to India and Southeast Asia in 2004 (IFGTB, n.d.). Recently, *L. invasa* attack was also found in Florida and Brazil (Kim *et al*., 2008).

Leptocybe invasa is the devastating pest of eucalypt. It damages newly young leaves and young twigs of young coppice shoots of *E. camaldulensis* in the plantations. This wasp also attacks small plants from cuttings in the uncontrolled nurseries (Kim *et al*., 2008). *Eucalyptus camaldulensis* harmed by *L. invasa* forms numerous galls on petioles and midribs of young leaves and on young twigs. Their attacks result in the deformity of leaf shape, the reduction of mature leaf size, the loss of plant vigor, the abnormality of plant growth, the reduction of wood yields and the delay of growers' incomes (Mendel *et al*., 2004).

In order to control *L. invasa*'s attack, Thai researchers in some private sectors carry out *E. camaldulensis* improvement to produce new clones with high wood yields and which tolerate to *L. invasa*'s attacks. Some clones could tolerate to *L. invasa* in the first few years. They become susceptible later because this gall wasps adapt themselves and can produce offsprings which are able to damage those clones.

The researchers in Israel carry out biological control to overcome *L. invasa* attack. They believe that *L. invasa* is native in Australia and then invaded to Israel. The damage in Australia is little because *L. invasa* is controlled by local parasitoid species. The local parasitoids were used to control *L. invasa* in Israel and Turkey (Protasov *et al*., 2008). Two parasitoid species were imported from Australia to Israel to be used in control this gall wasp. The parasitoids showed well establishment in Israel (Kim *et al*., 2008). However, they reported that the success of parasitoids to control *L. invasa* needed more investigations.

The biocontrol of *L. invasa* should be undertaken in Thailand in parallel with *E. camaldulensis* improvement. However, it is essential to investigate the local parasitoids and to determine the biological parameters of *L. invasa* and those local parasitoids. The biological knowledge on *L. invasa* in Israel and Turkey are not

appropriate to Thailand because of the differences in region and environment. Moreover, the knowledge about *L. invasa* in Thailand is extremely limited.

Therefore, this research determines the biological aspect of *L. invasa* and its parasitoids in *E. camaldulensis* plantations. The research was carried out in Kanchanaburi province because this province is one which *E. camaldulensis* trees are widely planted.

The data, obtained from this research, will provide the biological parameters and other knowledge about *L. invasa* and local parasitoids in Thailand. They will be used to compare with those in other countries. The data on their biological parameters will be beneficial in the selection of suitably local parasitoids to control *L. invasa*. Finally, successfully biological control of *L. invasa* will be a supplement to *E. camaldulensis* improvement. This is called integrated *L. invasa* management.

OBJECTIVES

1. To study the biological aspect of eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle in *Eucalyptus camaldulensis* Dehnh. plantations, Tha Muang and Phanom Thuan districts, Kanchanaburi province.

2. To determine the biological aspect of the parasitoids of *L. invasa* in these two districts.

3. To assess the population dynamics of *L. invasa* and the parasitoids in these two districts and to compare their population dynamics between Tha Muang district and Phanom Thuan district.

The scope of the biological aspect in this research covered the followings: morphology, longevity, fecundity, reproductive organs, and development of *L. invasa* and the parasitoids.

LITERATURE REVIEW

The literature review relating to this research title covered the following topics.

1. *Eucalyptus camaldulensis* Dehnh.
2. *Leptocybe invasa* Fisher & La Salle
3. Parasitoids of *Leptocybe invasa* Fisher & La Salle
4. Biological control
5. Study areas

***Eucalyptus camaldulensis* Dehnh.**

Family: Myrtaceae
Trade name: Red gum, river red gum

The genus *Eucalyptus* was described and named in 1788 by French botanist I' Héritier. The flowers of the various *Eucalyptus* species are protected by operculum. By this characteristic, the generic name was created from the Greek words "eu" (well) and "kalyptos" (covered) (Orwa *et al*., 2009).

Eucalyptus camaldulensis is one species in the genus *Eucalyptus* which covers more than 500 species (Chippendale, 1988). The genus *Eucalyptus* is almost entirely Australian with only two species not found there: *E. urophylla* in Timore and some adjacent islands of Indonesia and *E. deglupta* in Papua New Guinea, Irian Jaya and the Moluccas of Indonesia, and Mindanao in the southern Philippines (George, 1981; Eldridge *et al*., 1997) (Figure 1). Some taxonomists proposed that the genus *Eucalyptus* is able to be divided into 8 subgenera (Eldridge *et al.*, 1997).

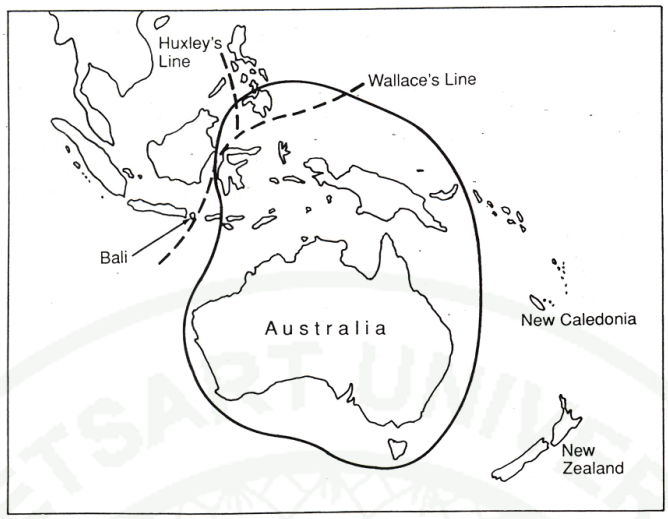

Figure 1 The limit of natural distribution of the genus *Eucalyptus*.

Source: Eldridge *et al*. (1997)

Vernacular Name

Vernacular names (common name, local names) of *E. camaldulensis* are extremely different among countries as shown below (Orwa *et al*., 2009).

Thai	(yukhalip)
Arabic	(ban, kafur)
Australian	(gum tree)
Burmese	(pyilon–chantha)
English	(long beak eucalyptus, murray red gum, red gum, river gum, river red gum, red river gum)
French	(eucalyptus rouge, eucalyptus)
German	(roter eucalyptus, Rotgummibaum)
Indonesian	(ekaliptus)
Italian	(eucalipto rostrato)
Spanish	(eucalipto, eucalipto rojo)
Vietnamese	(bajch dafn usc)

Botanic Description of Mature Tree

General botanic description of mature *E. camaldulensis* was described by Orwa *et al*. (2009) as follows:

General feature: medium to large tree, commonly grows to 20 m tall, possibly reaching 35 m; a clear bole of 20 m in plantation. *Bark*: rather smooth, white, grey, brown; bark shedding in strips or irregular flakes; rough bark occupies the 1st 1–2 m of the trunk.

Leaves: simple, drooping; alternate arrangement; upper surface dark–green, lower surface grey–green; narrow lanceolate leaf shape, tapering, often curved; leaf size of big tree 1.5–3 cm wide and 18–24 cm long; mature leaf size of young coppice shoots 4–5 cm wide and 11–13 cm long; margin entire; acute to acuminate apex; obtuse base. *Inflorescence*: white, axillary, 7–11 flowered; bisexual; stamen with anther and long filament like the rose apple; operculum hemispherical or conical, 4– 6 x 3–6 mm, obtuse. *Fruit*: very small capsules at the end of thin stalks, hemispherical or cup like, 5–8 mm wide, valves 4, containing minute seeds. Some morphological characteristics of *E. camaldulensis* are shown in Figure 2.

Time of flowering in natural stands depends on the geography of a given location. Pollination is mostly by insects. Seeds ripen about ≥3 months later. This species does not develop resting buds and grows whenever environmental conditions are favorable.

Chaisalee (2000) studied the anatomy of mature leaf of *E. camaldulensis* and found this species has single epidermal layer on both adaxial and abaxial leaf surfaces. The epidermis is heavily cutinized on both surfaces.

The mesophyll comprises of palisade and spongy parenchyma. The palisade parenchyma exists on both surfaces of the leaf and is densely arranged in two layers along the upper and lower epidermis. The spongy parenchyma is loosely arranged

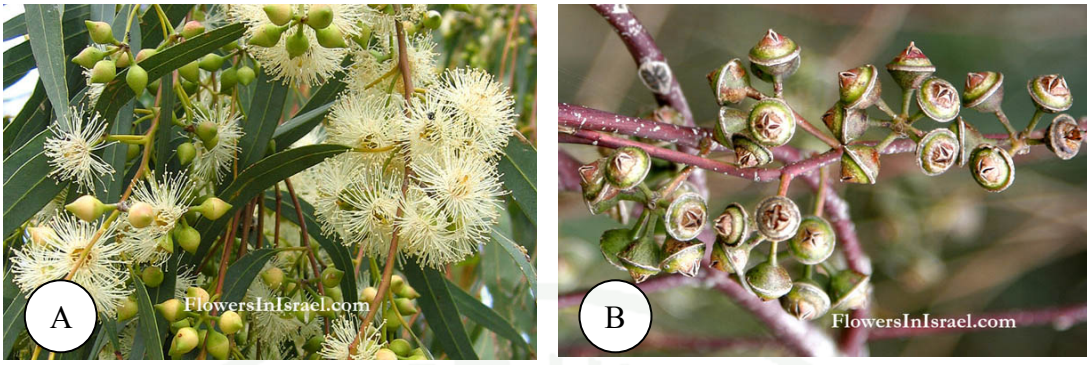

Figure 2 *Eucalyptus camaldulensis*: (A) flowers; (B) fruits.

Sources: Modzelevich, (n.d.)

in the middle of the blade. Oil glands appear in the mesophyll layer scattering in the layers of dense palisade tissues. The vascular bundles exist in pairs oriented with xylem towards the center of the organ. The smaller bundles embed in the mesophyll layer.

Eucalyptus camaldulensis leaf is amphistomatous, with numerous stomata on both surface of the leaf. Stomata are distributed over the whole leaf.

Natural Distribution

According to Orwa *et al*. (2009), the natural distribution of *E. camaldulensis* covers most of Australia's mainland. Under natural conditions, this species occurs typically along the banks of inland rivers and on periodic flood plains. Very occasionally in southern Australia, it extends to hills or ranges usually in open forest and woodland.

In Australia, it grows under a wide range of climatic conditions from temperate to hot and from humid to arid zones. The length of the dry season may vary from 0 to 8 months, and the rainfall distribution from a winter maximum in southern

regions to a monsoon type with summer rain in northern areas. The limits of the natural distribution of *E. camaldulensis* are as follows:

1. It grows well at altitude 0–1,500 m, mean annual temperature 3–22 to 21–40 ºC, and mean annual rain fall 250–2,500 mm.

2. It grows best on deep, silty or loamy soils with accessible water table, tolerates to water logging and periodic flooding. It is one of the species found to be most tolerant to acid soils.

At present, the native *E. camaldulensis* of Australia is introduced to other countries for various purposes. The countries where this species has been planted are Thailand, Albania, Argentina, Bangladesh, Brunei, Cambodia, Eritrea, Ethiopia, Greece, India, Indonesia, Israel, Italy, Kenya, Laos, Malaysia, Multa, Morocco, Myanmar, Namibia, Nepal, Nigeria, Pakistan, Philippines, Spain, Sudan, Tanzania, Turkey, Uganda, and United Kingdom. It is the exotic species of these countries. It can grow in most of the tropical and temperate regions between latitudes 45 ºS and 40 ºN (Pryor, 1976; Eldridge *et al.*, 1997).

The success of *E. camaldulensis* as an exotic species is attributed to its superiority to other tree species in the production of wood on infertile and dry sites, the tolerance to drought and high temperature, the rapid growth in soil with available water and the tolerance to periodic water logging and soil salinity (Eldridge *et al.,* 1997).

Benefits

Eucalyptus camaldulensis provides both direct benefits (products) and indirect benefits (services). The data from Pousajja (1996) and Orwa *et al.* (2009) were integrated and summarized as follows.

1. Direct benefits (products)

1.1 Timber: The wood of this tree species is suitable for many constructional works (for example; pile, pole), furniture and house construction. Pile and pole are the big market of *E. camaldulensis* in Thailand. Farmers earn a high income from their eucalypt wood production. For furniture and housing from wood of *E. camaldulensis*, the small sized wood commonly breaks or twists. At a certain log size with special techniques, the eucalypt wood is used for furniture, house and log cabin construction.

It was a great honour for the Royal Forest Department to have opportunity to construct a Eucalyptus House at the Phupink Royal Palace, Chiang Mai province for Her Majesty in early 1992. The eucalypt house was also mainly furnished with eucalypt furniture. The Ratchaburi RFD Research Station built a eucalypt log cabin for visitors in 1988 and furnished it with eucalypt furniture, and it remains in good condition.

1.2 Pulp and paper: The fiber is used as raw material for pulp and paper industry. It is also planted for making wood chip in hardboard, fibreboard and particleboard productions. The pulp and paper industry has an important role for economic development of the country and for generating more job opportunities for the labor force. There are many eucalypt pulp and paper mills and eucalypt wood chip factories in Thailand.

1.3 Firewood and charcoal: The firewood is suitable for industrial use in brick kilns. In some countries it is not preferred for domestic use because it burns too fast. In many areas it is used for charcoal production. Thailand and many other developing countries in Asia and the Pacific region are facing the problem of energy and fuel shortage, especially at household level. The household cooking energy consumption in rural areas is still in the form of traditional energy, mainly fuelwood and charcoal. Thus the annual demand for fuelwood and charcoal is enormous. *E. camaldulensis* wood from man–made plantations can support this demand.

1.4 Dye: The parts of tree provide a gum or tannin that can be used for silk dying and cotton yarn production.

1.5 Eucalyptus oil: Some tropical provenances of *E. camaldulensis* are rich in 1, 8–cineole leaf oil and are commercial sources of medicinal eucalyptus oils. The oils are used as an inhalant for relief of colds.

1.6 Honey: *Eucalyptus camaldulensis*, insect pollinated, is a source of honey in some countries. It produces yields of nectar in good seasons. The honey color is light gold with a distinctive flavor and has been marketed in a straight line. The tree is a source for building up bee populations in some areas.

1.7 Edible mushroom: The root system of *E. camaldulensis* is associated with mycorrhiza. The mycorrhiza encourages the growth rate of eucalypts and it can produce edible mushrooms in rainy season. Eucalypt plantations which are able to produce edible mushrooms will be the source of extra income of the farmers.

2. Indirect benefits (services)

2.1 Shade, shelter and windbreak: *Eucalyptus camaldulensis* is widely planted for shade and shelter. Some countries in desert or arid region, such as Sudan, it is planted to protect crops from blowing sand.

2.2 Intercropping: With its light crown, *E. camaldulensis* is well suited for growing in aerable fields. Intercropping this tree species with agricultural crops and planting this trees with appropriate spacing, give satisfactory yields of trees and crops.

2.3 Increasing green area: The green area helps reduce greenhouse gases, greenhouse effect and global warming.

2.4 Enhancement of ecosystem: *Eucalyptus camaldulensis* is a producer in the ecosystem of an area. It supports many levels of consumers, such as wild animals. In ecological point of views, this makes the food chain of living things in that area going well.

Eucalyptus Plantation Establishment

The exotic *Eucalyptus* species were firstly planted in Thailand at Doi Suthep, Chiang Mai province in 1950. From 1964 the species trials of *Eucalyptus* species were carried out in different parts of the countries. It is found from the studies that only four species of this genus show fast growth rate and maximum wood yield in Thailand. Those are *E. camaldulensis*, *E. citriodora*, *E. deglupta*, and *E. tereticornis* (Chokepatana, 2003).

Among these four species, *E. camaldulensis* was best in growth rate and wood yield. *E. camaldulensis* is also more superior when compared to other fast growing tree species which are native to Thailand (Chokepatana, 2003).

Eucalyptus camaldulensis grows best in optimum environment. However it grow well in semi–arid areas with amount of rainfall less than 650 mm/yr, even in the site of periodic flooding and in moderately saline soil. It is one of the species found to be most tolerant to acid soil. However it shows less tolerance to calcareous soil rich in $CaCO_3$. In this soil condition, it shows chlorosis symptom on leaves (FAO, 1981).

1. Necessity of plantation establishment

Long–term deforestation together with population growth in Thailand led to insufficiency of timber and wood materials in the country. In order to solve the national demands of the materials, more import of timbers/woods and more economic tree species planting had been carried out. The establishment of *E. camaldulensis* plantations has been continuously promoted by the government and the private sectors to supply wood materials for domestic and industrial uses.

2. *Eucalyptus camaldulensis* planting

Formerly, seedlings of *E. camaldulensis* obtained from seed germination were used in plantings, even in plantations, with various spacing. However it was not good because tree height, growth rate and yield were uneven.

At present the cuttings (or rooted cuttings or propagules) from a single individual of a selected hybrid are used in producing planting materials and, then, they will be planted in the plantations. The planting materials produced vegetatively by this procedure are named “clone”. They bring about even tree height, even growth rate and steadily maximum woodyield.

The clone means any group of plants derived from a single individual by vegetative propagation. All members of a clone have the same genotype and consequently tend to be uniform (Ford–Robertson, 1971).

Eucalyptus clones are better than seedlings (from seed germination) in the following performance: survival rate, diameter growth, height growth and weight of woody parts. However Aramsri (1999) studied clonal test by using 3 clones of 5-year-old *E. camaldulensis*, T5, P7 and O2, planted in 3 different sites, and reported that one clone showed best performance in one site. Site had effect on clone performance. T5 was best in every site while the others were best in some sites.

3. Planting management

The space of planting varies from planting at home to close space in commercial plantations. It depends on the objectives and the end products required (Orwa *et al.*, 2009).

The planting space in commercial plantations in Thailand is 2 x 3 m; 2 m between tree and 3 m between row. Application of NPK fertilizer to each tree at the beginning of planting is common to assist establishment and early growth. Poor

competition ability with weeds necessitates frequent weeding up to 3 times a year until the canopies close. Tree growth may exceed 3 m per year for good clone on favorable site.

Generally trees will be cut by using coppicing system at 3–5 years of planting for commercial utilization, such as piles, poles, posts, fuelwood, pulpwood, etc. Each stool produces many new coppice shoots (Figure 3). If the trees are not cut and let them grow, it will produce the other products, such as sawn timber. Coppicing means cutting tree close to the ground level for producing new coppice shoots. The second cutting of new coppice shoots will be done again when they are 3–5 years old. The cutting like this can be done six times or more on the same stool. This is a good income of the farmer.

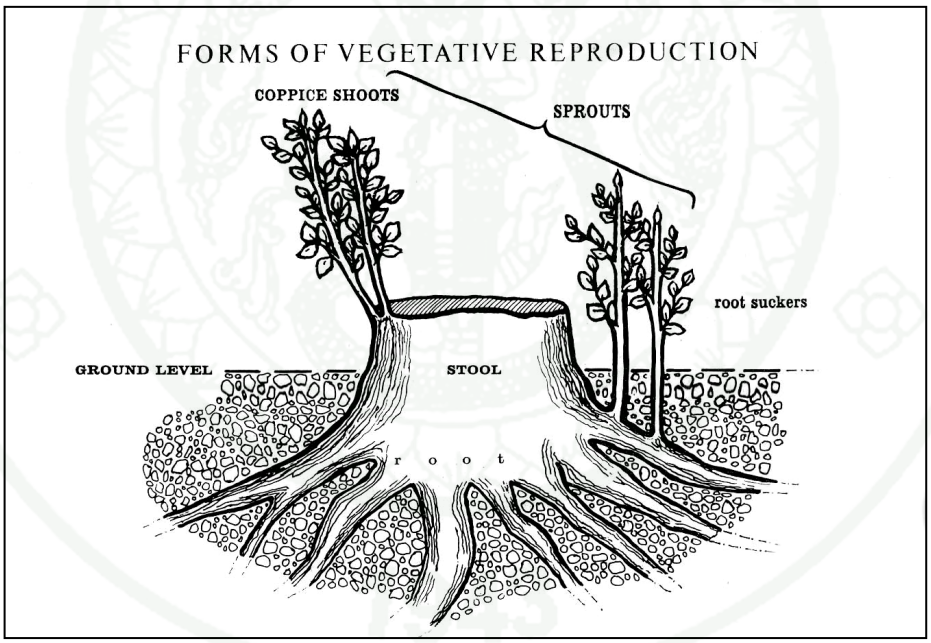

Figure 3 Vegetative reproduction of *Eucalyptus* species.

Source: Ford–Robertson (1971)

Like any other fast growing plants, the eucalypts require large amounts of water to sustain their rapid growth. The studies of water use indicated that eucalypts

were comparable with other agricultural crops and more frugal than many slow growing native trees in the number of ton of water required per ton of dry matter harvested (Tiwari and Mathur, 1983; Eldridge *et al*., 1997).

4. Disease and insect pests

In the common nursery, small *E. camaldulensis* is susceptible to some fungi causing leaf diseases and damping–off. Some insect species are troublesome to young tree planting outdoor. Both physical and chemical measures are used in some areas to control them. Young trees weakened by drought in some areas can be badly infected by moth larvae and other insects (Orwa *et al.*, 2009).

At present, the invasive leaf–gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), is the devasting insect pest of some clones of *E. camaldulensis* in many provinces of Thailand. The attack of this insect was first observed in Chachoengsao province in 2004 and spreaded to other parts of the country.

Leptocybe invasa Fisher & La Salle

Taxonomic placing:

Class Insecta
Subclass Pterygota
Infraclass Neoptera
Division Exopterygota
Order Hymenoptera
Suborder Apocrita
Superfamily Chalcidoidea
Family Eulophidae
Subfamily Tetrastichinae

Common name: Eucalyptus gall wasp

From Mendel *et al.* (2004), *L. invasa* is a new genus and a new species of Family Eulophidae. From etymology, Lepto was derived from Greek *Leptos* meaning fine, weak and thin, and cybe from *kybe* meaning head. Thus *Leptocybe* signified the weak area on the head around the ocellar triangle.

Approximately 100 species of Chalcidoidea are known to be associated with eucalypts galls (Noyes, 2003). The most common group of Australian gall inducer on eucalypts is the family Eulophidae (La Salle, 2005).

Gall wasps recorded in *Eucalyptus* comprise of 5 leaf gall wasp species: *Epichrysocharis burwelli* Schauff (Eulophidae); *Leptocybe invasa* Fisher & La Salle (Eulophidae); *Ophelimus eucalypti* (Gahan) (Eulophidae); *Ophelimus maskelli* (Ashmead) (Eulophidae) and *Nambouria xanthops* Berry & Withers (Pteromalidae). Seed gall wasps of *Eucalyptus* are in the family Eulophidae. They include 3 species: *Leprosa milga* Kim & La Salle; *Moona spermophaga* Kim & La Salle and *Quadrastichodella nova* Girault (Berry and Withers, 2000; Withers *et al*., 2000; Mendel *et al.*, 2004; Kim *et al*., 2005; Protasov *et al*., 2007; Kim and La Salle, 2008).

Galls are defined as any deviation in the normal plant growth produced by a specific reaction to the presence and the activity of a foreign organism (Bloch, 1965). Dreger–Jauffret and Shorthouse (1992) defined gall as the modified plant tissues in order to supply the causal agent with both suitable shelter and nutritious food.

Morphological Characteristics of Adult

Mendel *et al*. (2004) reported that only the female *Leptocybe invasa* was found in Israel. The morphology of the adult female is described below.

Length: tiny, 1.10–1.40 mm; head and body brown with slight to distinct blue to green metallic shine. *Head*: weak, with distinct groove and weakened area around

ocellar triangle; antenna with 4 anelli, 3 funicullar segments, 3 club segment. *Thorax*: pronotum short; midlobe of mesoscutum without median line, with short adnotaular setae at lateral margin; scutellum with submedian and sublateral lines; propodeum without median lines or lateral carinae or plicae, but with a raised lobe of the callus that partially overhangs the outer of the spiracle; spiracular depression open to anterior margin of propodeum. *Wings*: submarginal vein generally with 3–4 dorsal setae; postmarginal vein short, less than 0.25 length of stigmal vein; basal cell without setae; basal vein usually with 1 seta. *Gaster*: short, ovate; hypopygium extending just over half the length of the gaster.

Doğanlar (2005) reported that it was found both female and male *L. invasa* in Turkey. Gupta and Poorani (2009) found both female and male of *L. invasa* in India. They reported that the female was similar to that in Israel but they describe briefly on the morphology of male *L. invasa*. The male is similar to female in general appearance and coloration. *Head*: antennal formula 11343 with scape having a narrow and elongate sensory organ on lateral margin, annelli 3 segments, funicle 4 segments with long lateral–terminal bristle and less stout than in female, club 3 segments. *Gaster*: somewhat tubular.

Distribution

Leptocybe invasa, a gall–inducing invasive wasp, is assumed to have originated from Australia and spreads to many countries. From Australia, *L. invasa* spreaded to the Middle East in 2000, to Mediterranean countries and northeast Africa in 2002 (Mendel *et al*., 2004), to India and Southeast Asia in 2004 (CABI, 2007). Recently *L. invasa* was also found in Brazil and Florida (Kim *et al.*, 2008). World distribution of *L. invasa* was shown in Figure 4.

In Thailand, *L. invasa* was first observed in 2004 on approximately 6-month-old coppice shoots of *E. camaldulensis* in a plantation in Chachoengsao province. The damages by this gall wasp gradually become notable later and spread to the other parts of the country.

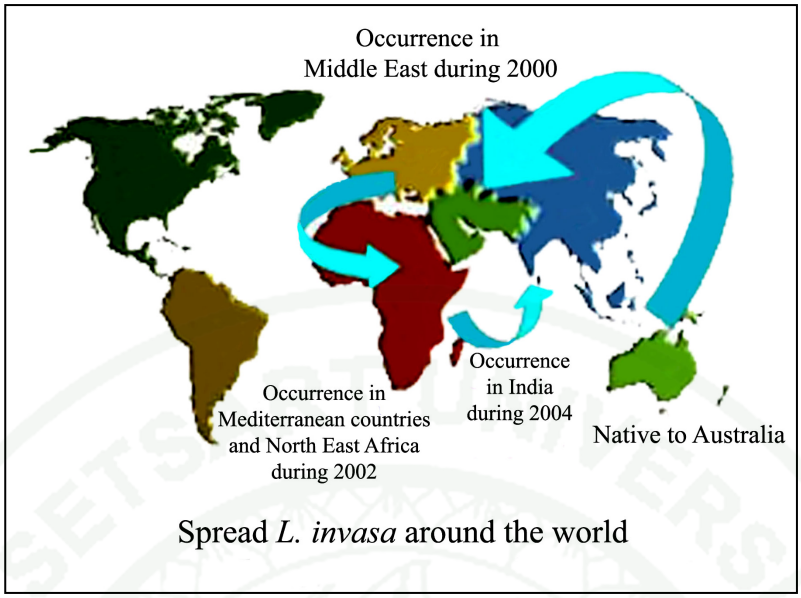

Figure 4 World distribution of *Leptocybe invasa*.

Source: IFGTB (n.d.)

Damages to *Eucalyptus camaldulensis*

Leptocybe invasa causes damage to planting materials and small *E. camaldulensis* in uncontrolled nurseries and in plantations. Newly developed shoots of saplings and young coppices in the plantations are also susceptible to *L. invasa*. This gall wasp damages newly young leaves and young twigs of *E. camaldulensis* (Mendel *et al*., 2004). They form numerous galls on petioles and midribs of young leaves and on young twigs. Their attacks result in the deformity of leaf shape. In India, it quickens abscission of leaves and perhaps drying up of shoots (IFGTB, n.d.). They cause the reduction of mature leaf size, the loss of plant vigor, the abnormality of plant growth, the reduction of wood yields and of growers' incomes.

Longevity

Generally, the longevity of *L. invasa* refers to the period from adult to its expiration. The life–span of an individual insect can be divided into two phases: the development period from hatching of egg to adult eclosion and the period of adult life which usually refers to as longevity (Jervis, 2005).

Mendel *et al.* (2004) studied the longevity of female *L. invasa* in Israel by using six food treatments. They found that wasps which were supplied with honey solution could prolong the largest mean longevity (6.50±0.20 days). The wasp longevity fed by this diet was significantly longer than wasps supplied with other diets.

Fecundity

1. Potential fecundity and realized fecundity

The fecundity refers to an animal's reproductive output. It may express as the total number of eggs produced or be laid over a specified period. Fecundity can be divided into potential fecundity and realized fecundity (Jervis, 2005).

The potential fecundity of a species is usually taken to be the maximum number of eggs that can potentially be laid by an adult female. In a strictly pro–ovigenic species, the number of all mature eggs at emergence counted by ovary dissection is the potential fecundity (Jervis, 2005). Mendel *et al*. (2004) reported that *L. invasa* is the pro–ovigenic species. In synovigenic species, the potential fecundity is the number of mature eggs plus the number of immature eggs at emergence (Jervis, 2005).

The realized fecundity is defined as the total number of eggs actually laid over the life–time of an adult female when excess hosts are provided in the laboratory (Jervis, 2005). It is possible to obtain realized fecundity without actually counting

eggs. Takagi (1985) and Benzemer and Mills (2003) counted the number of adult offsprings produced and took account of the intervening mortality processes, so deriving the estimated number of eggs originally deposited.

2. Reproductive mode

The reproductive modes of insect (Hymenoptera) are divided into arrhenotoky, thelytoky and deuterotoky (Jervis, 2005).

Arrhenotoky refers to male progenies developed parthenogenetically from unfertilized eggs (haploid) and female progenies developed from fertilized eggs (diploid). As a result, sons are haploid received genetic material from their mother only, and are therefore 100% related to their mother and unrelated to their father. On the other hand, daughters are diploid received one haploid copy of their genome from each of their parents and are therefore 50% related to their mother and 50% to their father (Godfray, 1994).

Unmated females can lay only haploid eggs and so produce progenies consisting solely of males. Mated females store sperm in a spermatheca and can control the sex of their offsprings when ovipositing, by selectively releasing sperm to an egg as the latter pass down to common oviduct (Godfray, 1994).

Thelytoky denotes the entirely parthenogenetical reproduction. There are no males and unfertilized eggs give rise to diploid females, resulting in 100% of daughters related to their mother (Jervis, 2005).

Deuterotoky refers to female production with rare males. It differs from arrhenotoky in that both sexes of the offsprings develop from unfertilized eggs. The male offsprings are non–functional (Jervis, 2005).

The female *L. invasa* in Israel produce offsprings by a reproductive mode called thelytoky which refers to the entirely parthenogenetical reproduction (Jervis, 2005).

Luck *et al*. (1993) pointed out that the distinction between thelytoky and deuterotoky is ambiguous. The reason is that some parasitoid wasp species originally designate as thelytokous but when the maternal females have been exposed to high temperature, small number of male offsprings can be produced. It becomes deuterotoky. There is evidence that in some cases are not only capable of mating but also able to pass on their genes to progeny, which produce thelytokously.

Development

Development of female and male *L. invasa* covers from egg stage to adult stage. Mendel *et al.* (2004) reported that development time from oviposition to wasp emergence of *L. invasa* was 132.6±8.1 days. Development of *L. invasa* associated with the development of leaf gall.

Leaf gall development comprised of five stages as follows (Mendel *et al*., 2004):

Stage 1 began 1–2 week after oviposition, with the first symptoms of cork tissues appearing at the egg insertion spots. At the end of the stage, the galls were easily recognized by their spherical appearance. Eggs of the wasp were hatched.

Stage 2 was characterised by development of the typical bump shape and the galls reached their maximum size (2.7±0.5 mm width). Mainly small larvae of the wasp were found.

Stage 3 was characterised by the fading of the green color on the gall surface that tended to change to pink while retaining its typical gloss. The wasps were mainly mature larvae and prepupae.

Stage 4 was characterised by the loss of the glossiness of gall surface and the color changed to light or dark red. The wasps were mainly pupae and preadult.

Stage 5 was characterised by the appearance of emergence holes of the adults and the gall color changed to light brown.

Variation in leaf structures, chemical and nutritional properties might associate with young leaves or mature leaves and proceeded to the susceptibility to galling (Ohmart and Edwards, 1991). The rapid spread and the fast population growth were probably the results of *L. invasa*'s thelytokous reproduction (all unfertilized eggs give rise to diploid female), multivotinous development and perhaps the absence of its principle–natural enemies (Mendel *et al.,* 2004).

Galls are defined as any deviation in the normal pattern of plant growth produced by a specific reaction to the presence and activity of a foreign organism (Bloch, 1965). Raman *et al*. (2003) define gall as a complex biological expression that principally involves two participants: an arthropod that induces the gall and the plant that responds by developing a gall.

The ability of arthropod to induce a gall does not occur in all orders of Arthropoda. Within plant–feeding groups, gall–inducing capability exists in Acarina, Thysanoptera, Hemiptera, Diptera and Hymenoptera. The relatively few gall inducers exist among the Coleoptera and Lepidoptera (Raman *et al*., 2003).

During the gall development or cecidogenesis, the parasitic arthropod inhibits the normal growth processes of the host plant, but induces new morphogenetic and physiological responses. Such newly induced responses are so specific that the gall inducer is able to obtain appropriate nutrition and confined environment (Raman *et al*., 2003).

The leaf is the most susceptible plant organ for gall development. Fewer galls occur on shoot axes, roots, vegetative buds and floral buds (Dreger–Jauffret and

Shorthouse, 1992). A chemical stimulus from the arthropod accelerates the velocity of cell division at the potential gall site on the host plant (Harper *et al*., 2004).

Gall development comprises of four basic stages; initiation, growth and differentiation, maturation and dehiscence (Rohfritsch, 1992). He also described the detail of each stage of gall development as follows.

1) Gall initiation stage was characterized by the isolation and withdrawal of cells from normal growth of host tissues. These were done by adult female at oviposition. The ovipositor poured some fluids on plant cells around egg to modify cell walls or to liquidize cell contents. This process gave rise to egg chamber. During gall development, normal growth processes was inhibit but induced new morphogenetic and physiological responses that were useful to egg and larva.

2) Gall growth and differentiation stage was observed at growth of gall by stimulating of young larva. It vastly accelerated cell division (hyperplasy) and cell enlargement (hypertrophy) by the feeding of young larva.

During feeding activities, the young larva poured salivary fluids on surrounding plant cells to modify cell wall or to liquidize the cell contents. The cells reacted to this stimulation by cell differentiation and proliferation. These gave rise to the occurrence of nutritive tissue along the inner surface of gall chamber. The young larva poured saliva to digest nutritive tissue to white fluid and filled in gall chamber. These also produced new vascular tissues arising among the mass of growing cells and joined the normal vascular tissues of the attached organ.

3) Gall maturation stage occured while the insect was in mature larval stage which consumed the largest amount of food. When it reached to pupa stage, it stopped feeding and did not pour saliva to digest nutritive tissue. The cells around the layer of nutritive tissue differentiated to sclerenchyma sheath to protect the pupa.

4) Gall dehiscence or gall opening stage: This stage was a period of major physiological and chemical changes in the gall tissue. For example, the flow of sap and water to the gall stopped and the gall was no–longer a nutrient sink. Although it was known that ripening of the gall tissues allowed the escape of some insects. The factors that trigger ripening are not known.

The development of sclerenchyma sheath around the pupa of *L. invasa* inside leaf gall of *E. camaldulensis* derives from the following processes. The larva dilivers saliva to digest nutritive tissue surrounded larval chamber. Later, the pupa which develops from the larva stops feeding. No more saliva to digest nutritive tissue, so the cells around larval chamber develop and form crust like. This is called sclerenchyma sheath to protect the pupa inside leaf gall.

Management

To manage *L. invasa* problems, the following interim control measures in India were suggested (IFGTB, n.d.).

1. Periodical monitoring the infestation in nurseries and plantations.

2. Destroying the severely affected plants in nursery by burning; pruning unwanted coppice shoots and burning them to reduce breeding site for gall insects.

3. Restriction of the production, supply and planting of susceptible clones.

4. Pruning the coppice shoots in harvested plots as soon as possible when the visible symptoms of infestations were noticed.

5. Application of systematic pesticides.

Searching of parasitoids and natural enemies to control *L. invasa* is initiated in many countries. Israel and Turkey use these parasitoids to control *L. invasa*;

Megastigmus sp. (Torymidae), *Selitrichodes* sp. (Eulophidae) and *Quadrastichus* sp. (Eulophidae) (Kim *et al.*, 2008; and Protasov *et al.*, 2008).

Parasitoids of *Leptocybe invasa* Fisher & La Salle

The native *L. invasa* does not damage eucalypts in Australia. This suggests that there are natural enemies of *L. invasa* which play significant role in reducing the population of this wasp species to below the observational threshold in Australia.

Two parasitoid species of Tetrastichinae (Hymenoptera: Eulophidae) in Australia, *Quadrastichus mendeli* Kim & La Salle and *Selitrichodes kryceri* Kim & La Salle, were introduced to Israel to be used in classical biological control program (Kim *et al.*, 2008). Two species of local parasitoids, *Megastigmus* species (Hymenoptera: Torymidae) are used to control *L. invasa* in Israel and Turkey (Protasov *et al*., 2008).

Morphological Characteristics of Adult Parasitoids

The morphological characteristics of three parasitoid species; *Quadrastichus mendeli* Kim & La Salle and *Selitrichodes kryceri* Kim & La Salle in Australia and *Megastigmus* sp. in Israel and Turkey are described below.

1. *Quadrastichus mendeli* Kim & La Salle

Kim *et al.* (2008) reported this parasitoid was introduced from Australia to Israel. Only female is found in Australia. *Quadrastichus mendeli* is uniparental.

Length: 1.15–1.35 mm; body mainly yellow with dark brown marking. *Head*: frons with a median area dorsally, bordered laterally by sutures which extend from frontal suture halfway to level of toruli; vertexal suture very weak and extending from lateral ocellus to eyes; antenna with three funicular segments and one large anellus. *Thorax*: mid lobe of mesoscutum with distinct median line and 3–4

adnotaular setae; scutellum wider than long; submedian lines and sublateral lines present; two pairs of setae on scutellum. *Wings*: submarginal vein with 1 seta; parastigma and stigmal vein without a hyaline break; post marginal vein rudimentary. *Gaster*: longer than the head plus mesosoma.

2. *Selitrichodes kryceri* Kim & La Salle

This parasitoid is found both female and male in Australia. *Selitrichodes kryceri* is biparental. (Kim *et al*., 2008)

Length of adult female: 1.35–1.75 mm; body almost completely yellow or mainly yellow with dark brown marking. *Head*: frontal suture transverse medially with lateral sides turning ventrally, placed ventral to medium ocellus, separated from median ocellus; antenna with 3 funicular segments and 3 anelli. *Thorax*: pronotum very short; midlobe of mesoscutum with very weak median line and with one row of 7–9 adnotaular setae on each side. *Wings*: submarginal vein usually with 2 dorsal setae; parastigma with a hyaline break and without hyaline break on stigmal vein. *Gaster*: slightly longer than head plus mesosoma.

Length of adult male: 1.00–1.15 mm; body color patterns similar to female, but with more black marking on vertex, midlobe of mesoscutum, scutellum, propodeum and gaster. *Head*: antenna with 3 anelli and only 3 funicular segments.

3. *Megastigmus* sp.

Protasov *et al.* (2008) reported that two local *Megastigmus* species are found in Israel and Turkey. They are parasitoids of *L. invasa*. Both species are biparental. The morphological differences between the two species were reported.

Megastigmus sp.-I (Israel): distance between the tip of mesonotum and the rear end of the scutellum is shorter than the width of the mesonotum. The female

forewing has stigma almost twice as long as broad. The male forewing has almost circular stigma. The male gaster is only slightly longer than its breadth.

Megastigmus sp.-T (Turkey): distance between the tip of mesonotum and the rear end of the scutellum exceeds the width of the mesonotum. The female forewing has stigma almost three times as long as broad. The male forewing has stigma 1.6x as long as broad. The male gaster length is more than twice its breadth.

Doğanlan and Hassan (2010) reported that it is found five species of *Megastigmus* which are the parasitoids of *L. invasa*; namely *Megastigmus judikingae*, *M. leptocybus*, *M. thailandiensis*, *M. thitipornae* and *M. zvimendeli*. Two species of *Megastigmus*; *M. thailandiensis* and *M. thitipornae* are new species of Thailand.

Longevity and Fecundity of Parasitoids

Kim *et al*. (2008) reported that adults of *Quadrastichus mendeli*, *Selitrichodes kryceri* and *Megastigmus* sp.-I had largest longevities when fed with honey solution at 25 ºC. Protasov *et al*. (2008) studied on the longevities of female and male *Megastigmus* sp.-I found in Israel by feeding with 8 different diets. They found that the longevities of female and male *Megastigmus* sp.-I fed with honey solution, honey solution+young leaves and honey solution+galled leaves of *E. camaldulensis* were longer than those fed with other diets.

The report on the longevity, particularly the fecundity and reproductive organs of parasitoids of *L. invasa,* are extremely limited.

Development of Parasitoids

Quadrastichus mendeli and *Selitrichodes kryceri* are solitary ectoparasitoids. The entire development from egg to emergence was completed in 30 days under 28 º C. They developed on both young and mature larvae of *L. invasa* (Kim *et al.*, 2008).

Megastigmus sp.–I is also a solitary ectoparasitoid on larvae and pupae of *L. invasa*. The development time took 41–43 days under 23–31 º C. In Adana province of Turkey, *Megastigmus* sp.–I could produce at least 3 generations during the period from May to November each year. However its effectiveness on *L. invasa* control was very low (Protosov *et al*., 2008).

Population Dynamics of Parasitoids

The reports on population dynamics of *L. invasa* and parasitoids are very extremely limited. The population dynamic of *Megastigmus* species in *E. camaldulensis* plantations in Israel and Turkey was reported by Protasov *et al.* (2008) in restricted areas. From sampling, *Megastigmus* sp.–I could be detected in small number in July, reached it peak emergence in August and disappeared in October. There is no report on population dynamics of *Q. mendeli* and *S. kryceri.*

Biological Control

Definition

Biological control represents the action of living natural enemies in suppressing the abundance or activity of pests. The natural enemies include predators (that must consume many prey individuals to complete their development), pathogens (bacteria, fungi and viruses), parasites (soil–inhabiting entomopathogenic nematodes), antagonists (competitors) and parasitoids. The parasitoids are the most important group in the context of biological control of insect pests (Mills and Wajnberg, 2008).

From Debach and Rosen (1991), the biological control is a part of the broader overall phenomenon of natural control. Natural control may be defined as the regulation of populations within certain more or less regular upper and lower limits over period of the time by anyone or any combination of natural factors. Such factors have sometimes been classed into two groups: biotic (living) and abiotic (non–living).

The most important factors in natural control are 1) natural enemies (predators, pathogens and parasites), 2) weather and other physical factors, 3) food (quantity and quality), 4) interspecific competition (other than natural enemies), 5) intraspecific competition, and 6) spatial or territorial requirements.

Biological control in an ecological sense can be defined as the regulation by natural enemies of another organism's population density at a lower average than would otherwise occur.

Biological control may be variously defined as an applied field of endeavor or as a natural phenomenon. In the applied sense, it may be defined simply as the utilization of natural enemies to reduce the damage caused by noxious organisms to tolerable levels. On the other hand, from a more scientific standpoint, this term may be used to denote one of the major ecological forces of nature, and the regulation of the number of plant and animal by natural enemies.

Uses of Parasitoids in Biological Control

There are three broad categories how parasitoids can be used in biological control; importation, augmentation and conservation (Mills, 2000; Daane *et al*., 2002; Mills and Wajnberg, 2008).

Importation (or classical biological control) makes use of host–specific parasitoids imported from the region of origin of invasive pests and has received that greatest amount of attention.

Augmentation (or augmentative biological control) involves the periodic release of insectary–produced parasitoids. It can be effective when parasitoids of invasive or indigenous pests are unable to persist year round or to increase in numbers quickly enough to suppress pest damage. Augmentation has been used most effectively in protected or semi–protected environments such as glasshouses and cattle or poultry houses, with rather less success under open field conditions.

Augmentation can be approached through inoculation or inundation. Inoculation of small numbers of parasitoids can be used to improve colonization at critical periods for season–long pest suppression. Alternatively, inundation of large number of parasitoids can be used for immediate suppression.

Conservation biological control focuses on the enhancement of both introduced and indigenous parasitoid populations through provisioning of limiting resources or alteration of crop production practices. Parasitoids are limited by the availability of essential resources such as nectar and are excluded from crops by use of incompatible pesticides.

Flint and Dreistadt (1998) expanded the importance of augmentation. Augmentation supplements the numbers of naturally occurring natural enemies with releases of laboratory–reared or field–collected natural enemies. Two approaches to augmentation are inoculative release and inundative release.

Inoculative releases are used to build up populations of a natural enemy earlier in the season than usual or to establish a natural enemy in orchards or field where it is not present, often because populations were destroyed by pesticide applications, unfavorable weather or cultural activities. Once releases are made, the natural enemy is expected to reproduce and increase its populations on its own.

In inundative releases (or periodic release programs), the introduced natural enemies are not expected to reproduce and increase in numbers, biological control is achieved through the activities of the released individuals. Additional releases may be required throughout the season if pest populations approach damaging levels. A wide variety of predators and parasitoids have been used in inundative releases.

From Debach and Rosen (1991), natural enemies can be utilized in three major ways: 1) importation of exotic species and their establishment in a new habitat (classical biological control); 2) augmentation of established species through direct

manipulation of their populations, as by insectary mass production and periodic colonization; and 3) their conservation through manipulation of the environment.

When it is successful, the utilization of natural enemies is an inexpensive and non–hazardous means of reducing pest populations and maintaining them below economic injury levels.

In biological control balance, natural enemies are referred to as parasitoids, predators or pathogens. The first two are termed entomophagous, the latter "entomopathogenic". Both parasitoids and predators are animals that feed on other animals. The parasitoid completes its development on a single host, whereas a predator consumes several (or many) prey individuals during its development.

The modern concept of biological pest control has been developed and in practice is normally taken to mean the use of living natural enemies to control pest species. This can be accomplished either through 1) importation of exotic enemies against either exotic or native pests (classical biological control) or 2) augmentation and conservation of natural enemies that are already in place or are readily available. Most authors define augmentation as actions that increase the populations of natural enemies and conservation as actions that preserve or protect natural enemies (Ehler, 1998).

Populations of all living organisms are, to some degree, reduced by the natural actions of their predators, parasitoids, antagonists and diseases. This process has been referred to as "natural control" but when pests are controlled, this is often called biological control (sometimes shortened to biocontrol) and the agents that exert the control are frequently called natural enemies (Hajek, 2004).

Study Areas

Kanchanaburi province which locates in western Thailand, covers approximately an area of 19,483 km² (Wikipedia, 2009). This province is about 129 km northwest of Bangkok. It comprises of 13 districts (Figure 5). Kanchanaburi is a province which *E. camaldulensis* is widely planted.

The field studies were carried out in *E. camaldulensis* plantations at Ban Sra Kloy, Rang Sali subdistrict, Tha Muang district, and the Siam Forestry Company Limited, Rang Wai subdistrict, Phanom Thuan district, in Kanchanaburi province (Figure 5). The study site in Tha Muang district located at latitude 13º 49′ 01.06″N and 99º 30′ 03.65″E and that in Phanom Thuan district located at 14º 15′ 13.86″N and 99º 44′ 30.72″E (Figure 6). The topography of both sites encompasses flat plain.

Tha Muang district: It locates in the southeast of Kanchanaburi province and covers an area of 610.97 km² (Wikipedia, 2009). The topography encompasses flat plain. The main soil feature is arranged in Yang Talat series. The properties of this soil series are as follows; low soil fertility, coarse–loamy soil texture, sandy loam soil, good soil infiltration and drainage, deep soil, low percentage of organic matter, soil pH 5.5–6.5, low cation–exchanged capacity, low available phosphorus and available potassium (Department of Agricultural Extension, 2010).

Phanom Thuan district: It locates in the southeast of Kanchanaburi province and covers an area of 535.78 km² (Wikipedia, 2009). The topography encompasses flat plain, highland and scattered limestone hills. The main soil feature is arranged in Yang Talat series. The soil properties in this district are similar to those in Tha Muang district (Department of Agricultural Extension, 2010).

Figure 5 Location of the study areas (red circle) in Tha Muang and Phanom Thuan districts, Kanchanaburi province.

Source: Wikipedia (2009)

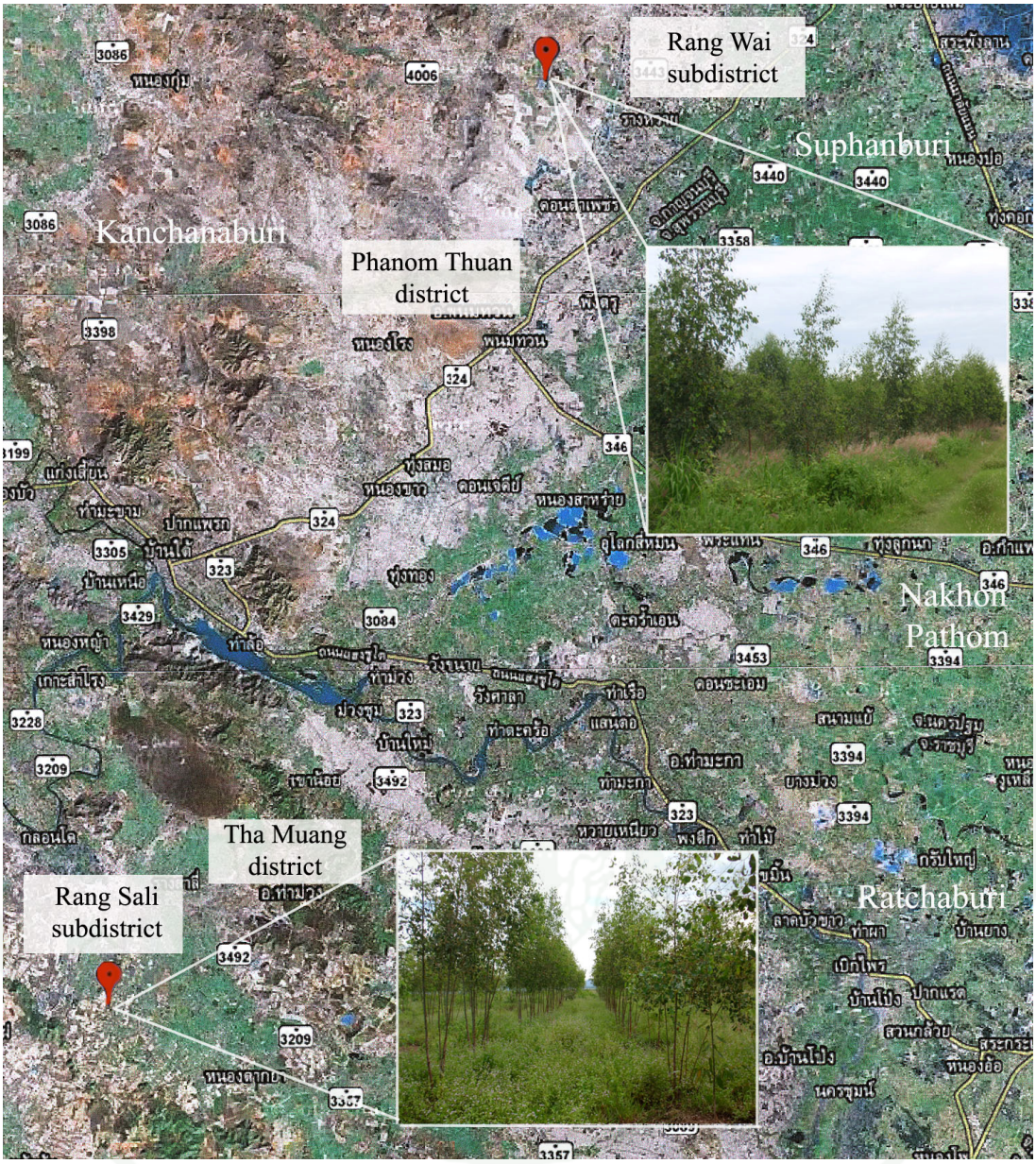

Figure 6 Locations of the study areas in Tha Muang district and in Phanom Thuan district, Kanchanaburi province.

Source: Anonymous (2010); *Eucalyptus camaldulensis* plantations were added by the author.

There is no weather station of Meteorological Department in Tha Muang and Phanom Thuan districts. The climatic data at the weather station in Muang district which is the nearest station to the study areas are shown in Table 1. The total rainfall was 1,081.3 mm a year. The average rainfull was maximum on September (205.6 mm) and was little between January to March and November to December. The annual average temperature was 28.6 ºC, with maximum on April and minimum on December. The annual mean of relative humidity was 69.41% ranging from 63–78%.

Table 1 Monthly distribution of climatic data at the station in Muang district, Kanchanaburi province, average for 10 years (1999–2008).

Month	Average rain (mm)	Average rainy day	Air temperature (ºC)			Mean RH (%)
			Mean Max	Mean Min	Average	
January	5.0	1.2	32.9	20.9	26.7	64
February	37.2	2.2	34.6	22.3	28.3	64
March	50.1	4.3	36.2	24.6	30.2	63
April	124.2	7.6	37.1	25.9	31.3	65
May	163.6	15.3	34.4	25.5	29.7	73
June	98.4	14.3	34.3	25.5	29.7	72
July	93.3	15.9	33.7	25.2	29.2	72
August	74.8	15.0	33.6	25.1	29.1	72
September	205.6	17.9	33.4	24.7	28.7	75
October	181.8	13.9	32.0	24.1	27.9	78
November	41.9	4.4	31.5	22.4	26.9	71
December	5.4	1.6	31.0	20.2	25.6	64
Total	1,081.3					
Average		9.46	33.72	23.86	28.60	69.41

Source: Climatological Group (2010).

MATERIALS AND METHODS

Materials

1. Stereoscope and light microscope
2. Digital camera
3. Computer
4. Statistical programs
5. Geographic Position System (GPS map 60 CSx)
6. Ventilated greenhouses
7. Petri dishes
8. Glass vials (6 cm long x 3.5 cm diam. and 7 cm long x 2 cm diam.)
9. Plastic boxes (different sizes)
10. Forceps
11. Dissecting equipments
12. Saline solution (7.5 g NaCl/1,000 cc of water)
13. Gall wasp diets
14. Small cotton balls
15. Sweep nets
16. Shears and long handle–shears
17. Plastic bags (different sizes, with ring rubbers)
18. Foam boxes (different sizes)
19. Marking pens
20. Ethyl alcohol 70%
21. Ethyl acetate
22. Rotary microtome
23. Slides and cover slips
24. Canada balsam

Methods

Selection of *Eucalyptus camaldulensis*

The CT 76 clone of *E. camaldulensis* was used in this study. The author selected one plot of CT 76 clone (about 1 rai in area) in the plantations of Ban Sra Kloy, Rang Sali subdistrict, Tha Muang district, and one plot of CT 76 clone in the plantations of Siam Forestry Co. Ltd., Rang Wai subdistrict, Phanom Thuan district, Kanchanaburi province. Generally, the environmental conditions of two studied sites were unsimilar.

There were two reasons to select the CT 76 clone of *E. camaldulensis* for this study. Firstly, this clone formerly gave acceptably good results in growth performance, but at present it is susceptible to *L. invasa* attack. Prior to CT 76 clone, CT 394 clone was popular but it was later devastated by this gall wasp. Thus the new CT 76 clone is produced and replaced the CT 394 clone. This phenomena suggest that the exotic and invasive *L. invasa* is able to adapt and to produce new progenies which can attack the new clone. Secondly, the biological knowledge of *L. invasa* and its parasitoids obtained from CT 76 clone will be benefit to the biocontrol of *L. invasa* which may attack the new clones produced in the future.

Ten trees of *E. camaldulensis* (CT 76) were selected by simple random sampling in the plot of each study area. From preliminary survey, the trees in the areas were formerly cut and let them produced new coppice shoots (about 4 coppice shoots/tree by average). The height of coppice shoots at the beginning of this study (April, 2009) were about 2 m in Tha Muang and 3–4 m in Phanom Thuan. Tree spacing is 2 x 3 m. The gall–leaf samples and sweeping were carried on the ten sampled trees.

Study on Biological Aspect of *L. invasa*

1. Preparation of stock culture of host plants and gall wasps

A) Small plants from cuttings of non–infected CT 76 were prepared, planted in the pots and let them grow well in ventilated greenhouses which covered with fine mesh. These small plants were the stock of host plants in the experiments of this study.

B) The leaves with mature galls of host plants (CT 76) were collected from the sampled plots in the study areas. These were the preparation of gall wasp prior to studies. The mature galls of the leaves sampled from the plantation in each district, were removed from the leaves, put them in plastic boxes with small moist cotton balls inside and kept the boxes at room temperature. The wasps emerged from the galls were used in the experiments.

The emerging wasps were determined their morphologies and were separated into *L. invasa* and parasitoids. Their morphological characteristics and genders were described under stereoscope and the photos were taken with digital camera.

2. Assessment of *L. invasa* longevity

From the preparation in 1B), the emerging *L. invasa* from leaf galls (< 6 hrs–old) were sampled. Only large wasps with similar size and great vigor were selected and used in the longevity assessment. The emerging *L. invasa* were also separated into female and male wasps.

The longevity of each sex was determined in different six diets as follows: no-diet, pure water, pure water plus fresh flowers of *E. camaldulensis*, honey solution (honey:water = 1:1), honey solution plus fresh flowers of *E. camaldulensis* and fresh

flowers of *E. camaldulensis*. The diets were refreshed daily until all wasps were dead. Pure water and honey solution were dropped on small cotton balls to feed the wasps.

Each treatment comprised of nine replications and ten wasps per replication. The experiments were conducted in the small glass vials, and each was covered with fine mesh to provide ventilation. The experiments were carried out in laboratory at room temperature. The numbers of dead and living *L. invasa* were counted daily. *Leptocybe invasa* longevity and survival patterns were determined in each diet. The wasp longevity was analyzed by F–test, using statistical program.

3. Determination of *L. invasa* fecundity

3.1 Potential fecundity

From the preparation in 1B), the newly emerging *L. invasa* from leaf galls (< 2hrs–old) were separated into three sizes; large, medium and small sizes. The wasps of each size were kept in small glass vials covered with fine meshes. They were fed with honey solution, dropping on small cotton balls in the vials. The wasps of each size used five vials and ten wasps/vial. The females in each size were killed at 2 hrs, 12 hrs, 1 day, 2 days and 3 days–old by freezing.

Their ovaries were dissected in saline solution under a stereoscope. The number of mature eggs in each ovary were counted. The length of hind tibiae of females of all sizes and ages were measured. The relationship between female sizes and the number of mature eggs were determined by regression analysis, using statistical program.

3.2 Realized fecundity

From plant preparation in 1A), sixty shoots of plants were sampled. Each shoot was enveloped with fine mesh. A single female, <6 hrs post–emergence and fed them with honey solution prior to use, was introduced into each shoot which

was enveloped with fine mesh. The female was left inside for 24 hrs. The next day, that female was transferred to the new shoot and left for 24 hrs. It was conducted like this until that female died. During these tests, the female was fed with honey solution on the small cotton ball hanging on the shoot in a ventilated greenhouse covered with fine mesh.

After shoots were exposed to female gall wasps, the fine meshes were removed from the shoots. The infested plants were kept in ventilated greenhouse covered with fine mesh to prevent the entering of external *L. invasa*. The plants were left in the greenhouse until the mature galls occurred on the leaves.

Before the emergence of adult offsprings from mature galls, the infested shoots were enveloped with fine mesh again for a period. The number of emerging wasps was counted. The realized fecundity was determined by counting the total number of emerging adult offsprings over her life–time of a female. The sex ratio of emerging wasps was determined.

4. Determination of *L. invasa* reproductive organs

Two day–old female adults and male adults of *L. invasa* were killed by freezing. They were dissected in saline solution under stereoscope to determine their reproductive organs. The female genitalia was mounted on slide with Canada balsum and covered wirh glass cover slip. The fresh reproductive organ and the female genitalia were photographed by digital camera attached to the stereoscope.

The reproductive organ and the male genitalia were performed in the same manner as described in female.

5. Study on the development *L. invasa*

The development of *L. invasa* covered from egg stage to adult emergence stage. Oviposition behaviors were observed closely and carefully and the photographs were taken by digital camera. The positions of egg insertion inside plant tissues were investigated. The location and development of eggs in plant tissues at 3, 15, 20 and 30 days–old were determined by using paraffin embedding technique and by sectioning with rotary microtome as described by Johansen (1960). The anatomy of egg stage to prepupal stage in plant tissues were studies and described under light microscope. The photographs were taken with digital camera attached to the microscope.

From plant preparation in 1A), ten plants were randomly selected to be used in the study of development time of *L. invasa*. Each shoot of plants was enveloped with fine mesh. Each shoot was exposed to five females *L. invasa* and left them inside the fine mesh for 24 hrs. The egg stage started after female wasps were introduced to shoot. The females were <6 hrs post–emergence and feed them with honey solution prior to use. The study was carried out in ventilated greenhouse. After exposure, the fine mesh was removed from shoot. Five leaves were collected at 3 days interval. The galls were removed from leaves and were dissected every three days until the emerging wasp was completed. The fresh larvae and pupae were studied and photographed. The development time of *L. invasa* was determined. The sizes of galls and the size of galled leaves were measured, the relation between gall and leaf developments and the immature development of *L. invasa* were carried out.

Determination of Biological Aspect of Parasitoids of *L. invasa*

1. Morphological study of parasitoids

From the preparation in 1B), the parasitoids emerging from the galls were identified and their morphological characteristics and genders were described under the stereoscope and were photographed by digital camera. The morphological differences between female and male parasitoids were also described.

2. Assessment of parasitoid longevity

From the preparation in 1B), the longevity of female and male of each parasitoid species emerging from the galls was assessed their longevities and survival patterns in a similar manner of *L. invasa* longevity study. The parasitoid longevity was analyzed by F–test, using statistical program.

3. Determination of parasitoid fecundity

3.1 Potential fecundity

From the preparation in 1B), the newly emerging parasitoids from leaf galls were determined their potential fecundity in the similar manner of *L. invasa* potential fecundity study.

3.2 Realized fecundity

From plant preparation in 1A), the determination of appropriate gall stage for parasitism of the parasitoid was carried out as follows:

Two hundred shoots of plants were sampled. Each shoot was enveloped with fine mesh. Each shoot was exposed to *L. invasa* female (<6 hrs–old) for 24 hrs and five females per shoot. *L. invasa* females produced a range of gall stages as follows: a) egg stage, b) young larval stage, c) mature larval stage and d) pupal stage. Five shoots were used in each stage. When the development of *L. invasa* reached to each stage, one pair of parasitoids (female and male; 1 day–old) which were fed by honey solution prior to uses, were introduced to each infested shoot for 24 hrs. After exposure, the fine mesh was removed from shoot. Before the emergence of *L. invasa* and parasitoids, the gall leaves of each shoot were enveloped with fine mesh. The number of emerging wasps was determined. The experiment was carried out in ventilated greenhouse.

From plant preparation in 1A), the determination of realized fecundity of parasitoids was carried out as follows:

Two hundred shoots of plants were sampled. Each shoot was enveloped with fine mesh. Each shoot was exposed to *L. invasa* females (<6 hrs–old) for 24 hrs and five females per shoot. After exposure to *L. invasa*, the leaf galls would develop into various stages. When the gall stage reached to the appropriate gall stage for parasitism, one pair of parasitoids (female and male; 1 day–old) which were fed on honey solution prior to use, was introduced to each infested shoot for 24 hrs. On the next day, the pair of parasitoids was transferred to the new infested shoot and left them for 24 hrs. It was conducted daily like this until the parasitoids died. During these studies, the parasitoids were fed on honey solution on the small cotton ball hanging on the shoots. The studies were conducted in a ventilated greenhouse covered with fine mesh. Before the emergence of *L. invasa* and parasitoids, gall leaves of each shoot were enveloped with fine mesh. The numbers of emerging *L. invasa* and parasitoids were counted. The realized fecundities of the parasitoids were determined. Their sex ratio were considered.

4. Determination of parasitoid reproductive organs

This studies were derived in a similar manner as the determination of *L. invasa* reproductive organs.

5. Study on development of immature parasitoids

The development of parasitoids covered from egg stage to adult emergence stage. The egg stage of parasitoid was initiated after female parasitoid was introduced to infested shoot (caused by *L. invasa*). Oviposition behaviors of parasitoids were observed closely and carefully and the photographs were taken by digital camera. Preference of parasitoids to parasitize in different developmental stages of *L. invasa* was determined.

From plant preparation in 1A), ten plants were randomly selected to be used in the study of development time of parasitoids. Each shoot of plants was enveloped with fine mesh. Each shoot was exposed to five female *L. invasa* and left them inside the fine mesh for 24 hrs. The females *L. invasa* used in this study were < 6 hrs post–emergence and fed them on honey solution prior to use. After exposure to *L. invasa*, the leaf galls would develop in various stages. Two pairs of parasitoids (24 hrs–old), fed on honey solution prior to use, were introduced to each infested shoot with appropriate gall stage for 24 hrs. This study was conducted in ventilated greenhouse covered with fine mesh.

After exposure, the fine mesh was removed from shoot. Five leaves were collected at 2 days interval. The galls were removed from leaves and were dissected every two days until the emergence of parasitoids were completed. The fresh larvae and pupae of parasitoids were studied and photographed. The development time of each parasitoid species was determined.

Assessment of the Population Dynamics of *L. invasa* and Parasitoids in Two District

The assessment of their population dynamics was carried out in Tha Muang district and Phanom Thuan district. One plot was selected in each district. Tree spacing in each plot was 2 x 3 m. Ten trees by simple random sampling in each plot were used in the assessment of their population dynamics. A tree had four coppice shoots by average.

Two methods were used in the assessment of their population dynamics; leaf–gall sampling and sweeping around canopy of coppice shoots of *Eucalyptus camaldulensis* trees. Each method was carried out monthly for one year.

1. *Eucalyptus* eaf–gall sampling:

Each tree comprised of many coppice shoots. Four coppice shoots of different directions (north, south, east and west) were selected. Twenty–five leaves with mature galls were sampled from each coppice shoot, or 100 leaves per tree. Ten sampled trees were used. The sampled leaves of each tree were immediately stored in the plastic bag, one tree in one bag, all plastic bags were kept in foam box containing ice pieces and brought back to the laboratory.

The galls were removed from the sampled leaves, left them in plastic boxes containing small moist cottons and all plastic boxes were maintained in the laboratory at room temperature. The number of individuals of emerging *L. invasa* and parasitoids were counted daily and their genders were determined. Monthly population dynamics of each species in a year were determined in each district and compared between the districts.

2. Sweeping around canopy of coppice shoots of *Eucalyptus* trees:

Ten trees were sampled. The insects around canopy of coppice shoots of each tree were swept by sweep net. The insects were stored immediately in plastic bags, one tree in one bag, killed them by ethyl acetate, left them in foam box containing ice pieces and brought them back to the laboratory. The insects were preserved in 70% ethyl alcohol. The number of individuals of *L. invasa* and parasitoids were counted and their genders were determined. Monthly population dynamics of each species in a year were assessed in each district and compared between the districts.

The other insect species were identified and their roles were determined. The vegetative species of ground covers in the plantations were also noted. The climatic data in the study areas were collected.

2.1 The similarity between insect species in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts was determined by using similarity index (Kimmins, 1987) as shown below:

$$\text{Similarity index (\%)} = \frac{2ab}{a+b} \text{ x } 100$$

When a = Number of insect species in area A

b = Number of insect species in area B

ab = Number of insect species found in both area A and B

The maximum value of similarity index is 100 percent. If the value of similarity index is closed to 100%, the similarity between insect species in area A and area B is maximum.

2.2 The index of insect diversity was calculated by using Shannon–Weiner's Index of Diversity (Kreb, 1987). Shanon–Wiener function was shown as follows:

$$H' = - \sum_{i=1}^{S} (p_i \log_2 p_i)$$

When H' = Index of insect diversity

S = Total number of insect species in the study area

p_i = Ratio of the number of individuals of an insect species to the number of individuals of all insect species

H′=0 when it is found only one insect species in the study area and H′ is maximum when the number of individuals of each insect species is equal.

2.3 The evenness index of insects was calculated by this formula (Krebs, 1987):

$$E = H' / \ln S$$

When E = Evenness index

H′ = Index of insect diversity

S = Number of insect species in the study area

The more E value is closed to 1.0, the more evenness of insects is found in the study area.

Places and Duration of the Study

This research was carried out in *E. camaldulensis* plantations in Tha Muang district, *E. camaldulensis* plantations of Siam Forestry Company in Phanom Thuan district of Kanchanaburi province, ventilated greenhouse of SCG Paper Public Company Limited in Ban Pong district, Ratchaburi province, and in the laboratory of the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok.

The study was undertaken from March 2009 to April 2011.

RESULTS AND DISCUSSION

The findings from this research were reported in sequence of the objectives.

Biological Aspect of Eucalyptus Gall Wasp, *Leptocybe invasa* Fisher & La Salle

1. Morphology of the adult *L. invasa* (Hymenoptera: Eulophidae)

It was discovered that eucalyptus gall wasp found in Kanchanaburi province was *L. invasa*. This was determined by relying on the diagnosis of *L. invasa* described by Mendel *et al,* (2004).

Wasp: female and male small, blue to green metallic shine. *Head*: weak, with distinct groove and weakened area around ocellar triangle, malar sulcus distinctly curved. *Mesoscutum*: without median line, and with 2–3 small adnotaular setae. *Postmarginal vein*: short, less than 0.25 stigmal vein length. *Propodeum*: with a raised lobe of the callus that partially overhangs the outer rim of the spiracle, spiracular depression opens to anterior margin of propodeum; two longest cercal setae subequal in length and straight or only slightly curved.

This research also found both female and male *L. invasa* in *E. camaldulensis* plantations, in Tha Muang and Phanom Thuan districts, Kanchanaburi province. Their general and comprehensive morphologies were described below.

1.1 Female *L. invasa*

General feature: small size, length from head to abdomen tip 1.30 mm by average, ranging from 1.10 to 1.60 mm; head and body slightly blue color and to green metallic shine; antenna light brown; wings hyaline (glass–like colored) or very faintly, with light brown veins; fore coxa legs and tarsi yellow, middle and hind tibia coxa base blue to green metallic shine, last tarsal segment brown

The comprehensive morphology of the female *L. invasa* in the study areas was described below (Figure 7).

Head: generally strongly collapsing, particularly along weakened areas associated with a deep sulcus around the ocellar triangle; antennal torulus insertion halfway between clypeal margin and median ocellus , and above level of ventral eye margin; scrobal cavity with median line; malar sulcus distinctly curved; gena relatively large and rounded behind malar sulcus; eye moderately protruded; clypeus weakly bilobed.

Antenna: with 4 anelli, 3 funicular segments, 3 club segments; scape not reaching upper margin of vertex; pedicel long, over half the length of the scape; funicular segments all roughly quadrate.

Mesosoma: pronotum short; midlobe of mesoscutum without median line, with 2–3 weak short adnotaular setae at lateral margin; scutellum with submedian and sublateral lines; propodeum without median lines or lateral carinae; propodeum with a raised lobe of the callus that partially overhangs the outer rim of the spiracle; spiracular depression open to anterior margin of propodeum; propodeal callus with 2 setae.

Forewing: submarginal vein generally with 3–4 dorsal setae; postmarginal vein absent; basal cell without setae; basal vein usually with 1 setae; speculum small; cubital line of setae not extending all the way to basal vein, leaving the speculum open behind.

Gaster: lightly shorter than the head plus mesosoma, ovate; hypopygium extending just over half the length of the gaster; two longest cercal setae subequal in length and straight or only slightly curved; ovipositor sheaths short, not reaching apex of abdomen.

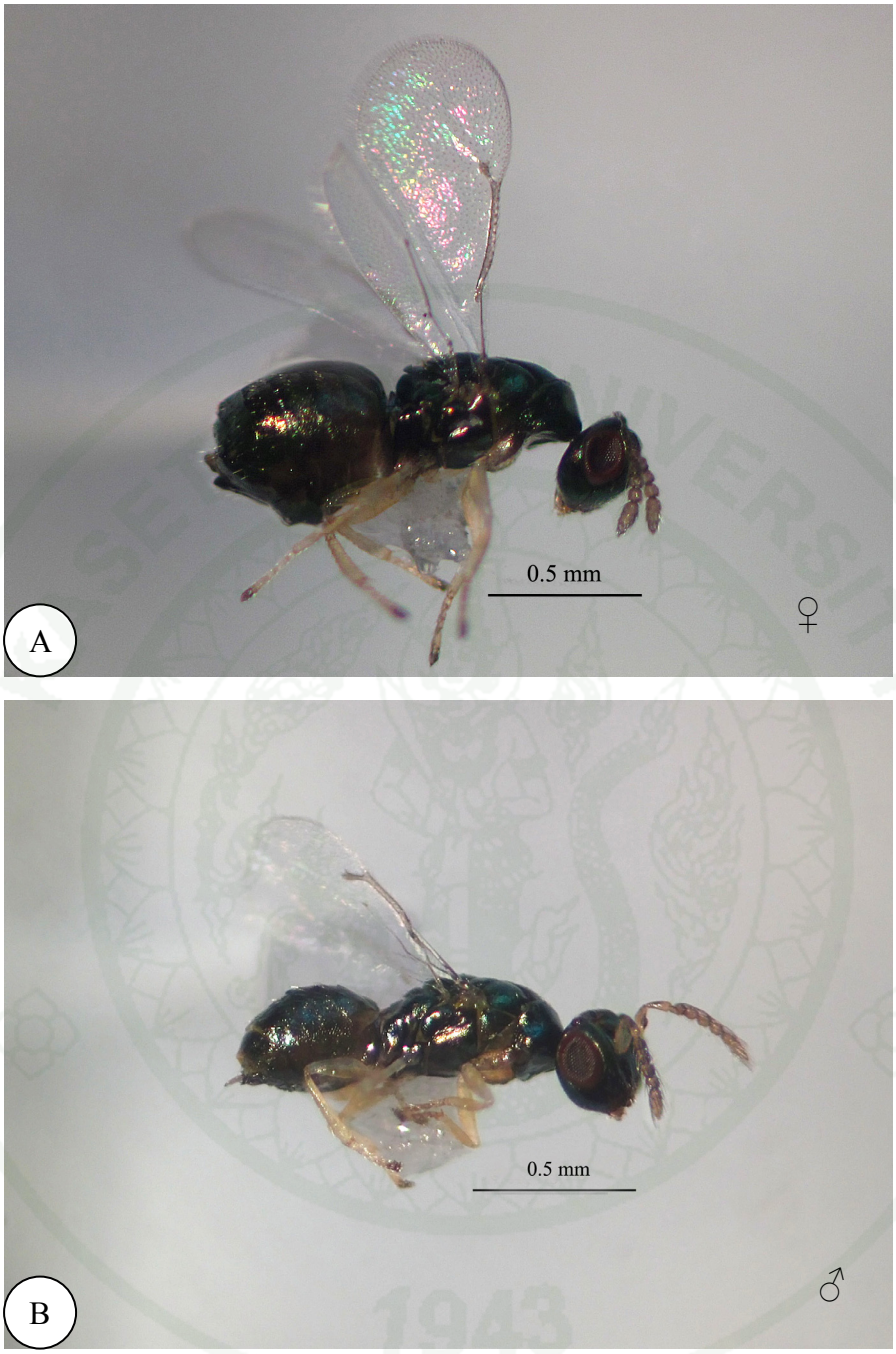

Figure 7 *Leptocybe invasa* found in *Eucalyptus camaldulensis* plantations, in Tha Muang and Phanom Thuan districts, Kanchanaburi province: (A) female; and (B) male.

1.2 Male *L. invasa*

General feature: small size, length from head to abdomen tip 1.16 mm by average, ranging from 0.80 to 1.40 mm; head and mesosoma brown with distinct blue to green metallic shine metasoma brown with slightly metallic finge dorsally; color of antenna light brown; wingd hyaline, veins light brown; legs pale yellow, mid and hind coxae metallic shine, and last tarsal segment brown.

The comprehensive morphology of the male *L. invasa* in the study areas was described below (Figure 7).

Head: strongly collapsing, particularly along weakened area around the ocellar triangle; antennal torulus inserted about halfway between clypeal margin and median ocellus, and above level of ventral eye margin; scrobal cavity with median line; malar sulcus distinctly curved; gena relatively large and rounded behind malar sulcus; clypeus weakly bilobed.

Antenna: with 4 anelli, 4 funicular segments, 3 club segments; funicle and clava with compact subbasal with the whorls of long setae; scape not reaching upper margin of vertex; pedicel long, over half the length of the scape; funicular segments all roughly quadrate.

Mesosoma: pronotum short; midlobe of mesoscutum without median line, with 2–3 weak, short adnotaular setae at lateral margin; scutellum with submedian and sublateral lines; propodeum without median lines or lateral carinae, propodeum with a raised lobe of the callus that partially overhangs the outer rim of the spiracle; spiracular depression open to anterior margin of propodeum; propodeal callus with 2 setae.

Forewing: submarginal vein with 3–4 dorsal setae; post marginal vein rudimentary; basal without setae; basal vein with 4 setae; cubital line of setae extending all the way to basal vein, closing speculum.

Gaster: shorter than the head plus mesosoma, slightly flatted.

The female *L. invasa* differed from the male in the characteristics that shown in Table 2 and Figure 8.

Table 2 Different characteristics between female and male *Leptocybe invasa*.

Characteristic	*L. invasa*	
	Female	Male
Funicle and clava of antenna with the whorls of long setae	No	Yes
Seta numbers of basal vein of forewing	1	4
Cubital line of setae of forewing	Not extending to basal vein, speculum opened	Extending to basal vein, speculum closed
Gaster shape	Ovate	Slightly flatted
Size	Larger	Smaller

The morphological description of female and male *L. invasa* was unpublished in Thailand. From the diagnosis and full description in this study showed that female *L. invasa* in Thailand was similar to that in Israel as described by Mendel *et al.* (2004), and to that in Turkey and India as described by Doğanlar (2005) and Gupta and Poorani (2009), respectively. The male *L. invasa* in Thailand was also similar to that in Turkey and India as described by Doğanlar (2005) and Gupta and Poorani (2009). The female size in Israel and Turkey was between 1.10–1.40 mm. The male size in Turkey was between 0.80–1.20 mm. The sizes of female and male *L. invasa* in Thailand were between 1.10–1.60 mm and 0.80–1.40 mm, respectively.

The findings of both female and male *L. invasa* in this research were characterized the first time and were the new record of Thailand. Sex differentiation of *L. invasa* was determined by relying on their mating behaviors and morphologies.

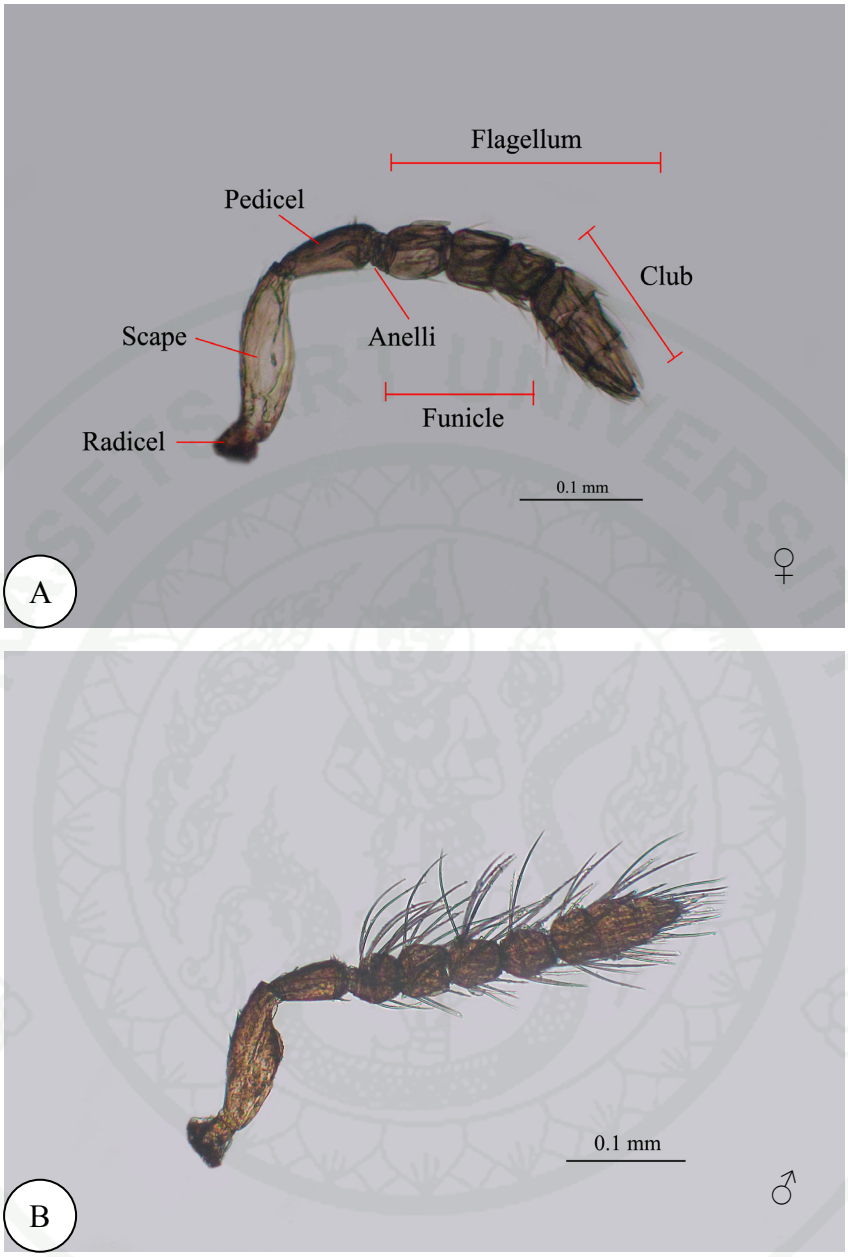

Figure 8 Some morphological distinctions between female and male *Leptocybe invasa*: (A–B) female and male antennae; (C–D) female and male forewings.

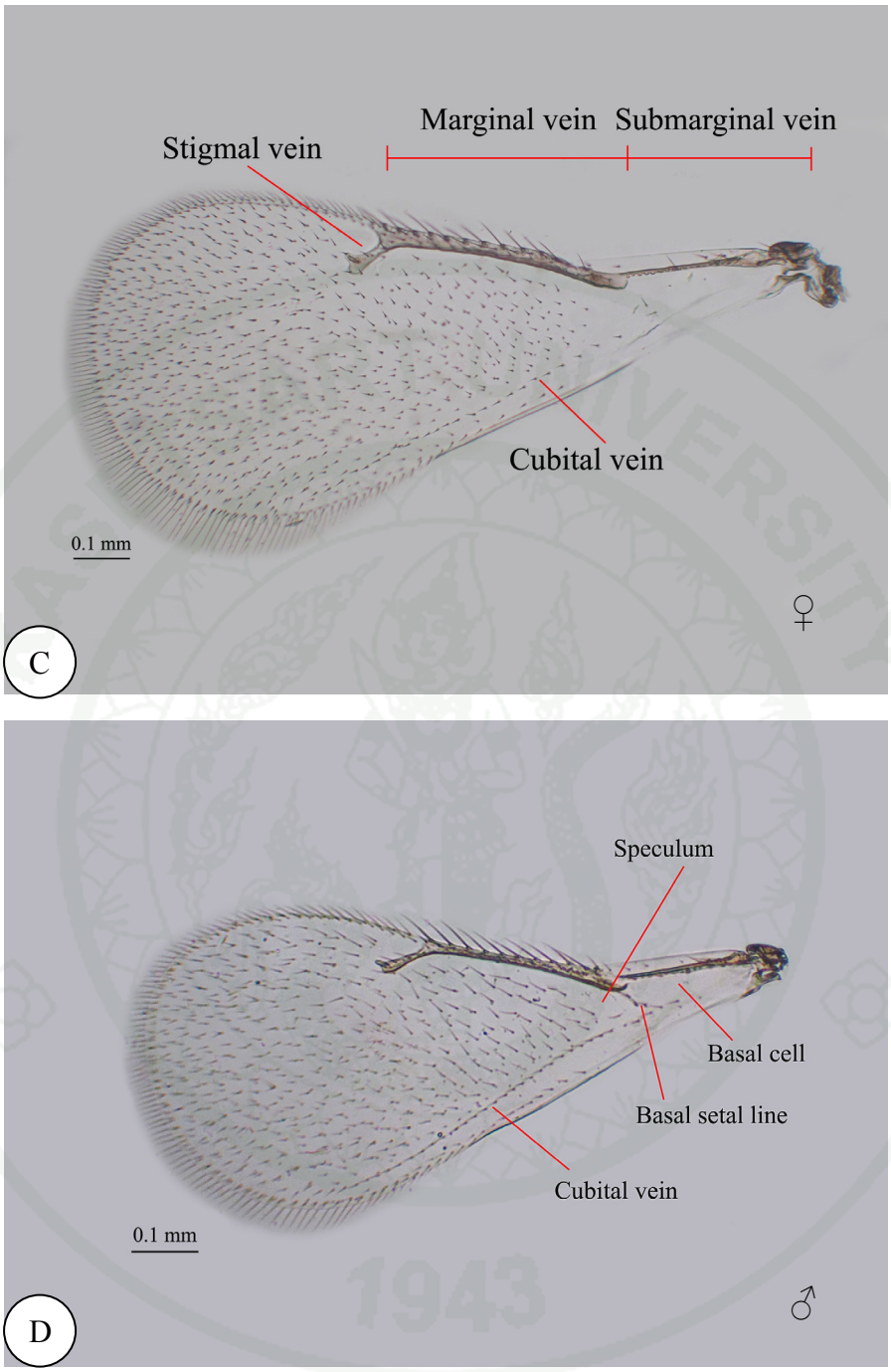

Figure 8 (Continued)

2. Longevity of *L. invasa*

2.1 Mean longevity of adult female and male *L. invasa*

The longevity of *L. invasa* refers to the period from adult, emerging from leaf gall of *E. camaldulensis*, to its expiration. Longevity study in the laboratory can provide information on the extension of *L.invasa* longevity and the application of this gall wasp as host to increase parasitoid production in the controlled conditions. Finally, the mass production of the parasitoid from the laboratory will be introduced to control *L. invasa* in *E.camaldulensis* plantations.

In the laboratory, it was found that the mean longevity of female and that of male *L. invasa* were rather short. The application of 6 different diets on the longevity study of *L. invasa* showed that honey solution and honey solution+flowers could prolong the large mean longevities by 7.67 and 6.11 days for female and by 5.67 and 5.22 days for male, respectively (Table 3).

To feed with no–diet, water, water+flowers, and flowers of *E. camaldulensis* disclosed that the mean longevities of females were only 1.33, 1.56, 1.89, and 2.00 days respectively, and those of males were 1.00, 1.11, 1.56, and 1.22 days, respectively (Table 3).

These findings suggest that mean longevity of *L. invasa* in the plantations is rather short. However, to feed them with honey solution and honey solution+flowers could extend their longevities. It was noticed that feeding with *E. camaldulensis* flowers could not extend *L. invasa* longevity. The possible explanations were that 1) the flowers of this species produced nectar in strong sunshine days, and 2) the nectar was not much, and 3) the nectar was produced at the base of flowers and the stamens were the barrier to reach the nectar. Thus, the nectar of *E. camaldulensis* is not much beneficial to extend the longevity of *L. invasa*.

Table 3 Mean longevities (days) of *Leptocybe invasa* fed with different diets.

Diet	Female	Male
No–diet	1.33±0.17 a	1.00±0.00 a
Water	1.56±0.18 a	1.11±0.11 a
Water+flowers	1.89±0.26 a	1.56±0.18 a
Honey solution	7.67±0.93 b	5.67±0.41 b
Honey solution+flowers	6.11±0.79 b	5.22±0.47 b
Flowers	2.00±0.40 a	1.22±0.15 a

Female: F=27.434; df=5; P–value=0.000
Male: F=64.463; df=5; P–value=0.000
Means followed by the same letter within each column were not significantly different (Tukey's HSD, P=0.05).

The statistical analysis of the means of female longevity and the means of male longevity by F–test indicated that both P–values=0.000 were lower than P=0.05, thus different diets had significant effects on the means of longevity of female and male *L. invasa*

It was noticeable that feeding with honey solution and honey solution +flowers of *E. camaldulensis* were not statistical different, Thus the prolongation of longevity of female and male *L. invasa* by feeding with honey solution alone were more practical and more convenient than feeding with honey solution+flowers.

To compare the findings from this research with those carried out in the other countries, it was found that only longevity of female *L. invasa* in Israel was reported. Thus, this was the first time that the longevity of male *L. invasa* was reported and this finding was new. These longevities of female and male *L. invasa* also were the new record of Thailand.

The reasons to use such six different diets in this study were that those diets were inconsistent with the natural diets found in *E. camaldulensis* plantations. Moreover, honey/or nectar of the flowers was the common diet of most insects (Hymenoptera).

To determine the longevity of female *L. invasa* obtained from this research with that carried out in Israel, it was found that there were difference in some findings. Mendel *et al.* (2004) studied on the longevity of female *L. invasa* in Israel by using 6 dietary supplements: no–diet, water, honey solution, honey solution plus young leaves, flowers, and young leaves of *E.camaldulensis*. He reported that honey solution prolonged the largest mean longevity of the female *L. invasa* (6.50 days). The means of female longevity fed with no–diet, water, flowers and young leaves of *E. camaldulensis* were shorter than that fed with honey solution. Different dietary supplements had significant effects on the means of female longevity.

The honey solution could prolong more mean longevity of female *L. invasa* in Thailand (7.67 days) than in Israel (6.50 days). The difference in the means of female longevity was possibly explained in terms of body sizes as follows: a) the body size had a positive correlation with longevity (Jervis, 2005), and b) the size of female *L. invasa* in Thailand (1.10–1.60 mm in length) was larger than that in Israel (1.10–1.40 mm in length). The larger size of *L. invasa* might consume more diet and might be stronger, then these led to the longer longevity.

2.2 Survival patterns of adult female and male *L. invasa*

Survival pattern denotes the percent of survivors in each day within the range of longevity when *L. invasa* is fed with a diet.

The results showed that the survival patterns of female and male were able to divided into two groups of survivors. The longest survivals were among the females that were fed with honey solution and honey solution+flowers, ranging from

1 to 13 days and 1 to 11 days, respectively. Estimated 50% female survival periods were 5 and 4 days respectively (Figure 9).

The short survivals were among the females that were supplied with no–diet, water, water+flowers, and flowers, ranging from 1 to 4 days. Estimated 50% female survival periods were approximate 2 days (Figure 9).

For the male *L. invasa*, the longest survivals were among the males that were fed with honey solution and honey solution+flowers, ranging from 1 to 8 days. Estimated 50% male survival periods were 4 and 3 days respectively. The short survivals were among the males that were supplied with no–diet, water, water+ flowers, and flowers, ranging from 1 to 3 days. Estimated 50% male survival periods were ~1 day (Figure 10).

The findings from the survival patterns also suggested that honey solution and honey solution+flowers of *E. camaldulensis* were the best diets to prolong survival periods of female and male *L. invasa*. However, feeding with honey solution alone was more practical and more convenient than feeding with honey solution+ flowers.

To compare the survival patterns of female *L. invasa* in laboratory of this study with that in Israel, Mendel *et al*. (2004) reported that the longevity of the females fed with honey solution ranged from 1 to 8 days. The distinction between the survival patterns of the female *L. invasa* in this research and that in Israel might concerned with the differences of body sizes. The body size had a positive correlation with longevity (Jervis, 2005). The size of female *L. invasa* in Thailand was larger than that in Israel.

Mendel *et al*. (2004) also reported that feeding the female *L. invasa* with young foliages of *E. camaldulensis* could prolong the mean longevity only 3 days. Thus the future study should be carried out whether there were the alternate

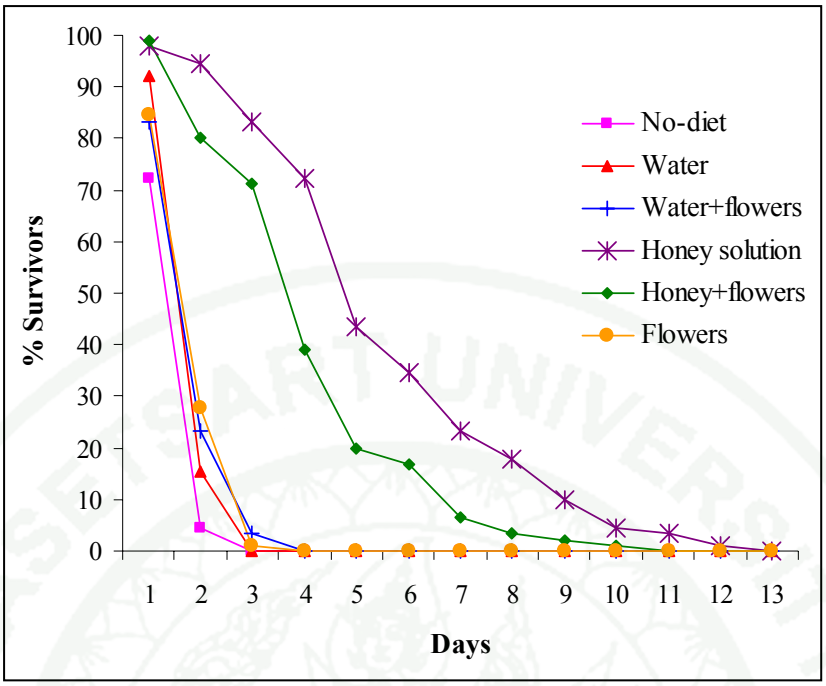

Figure 9 Survival patterns of female *Leptocybe invasa* fed with different diets.

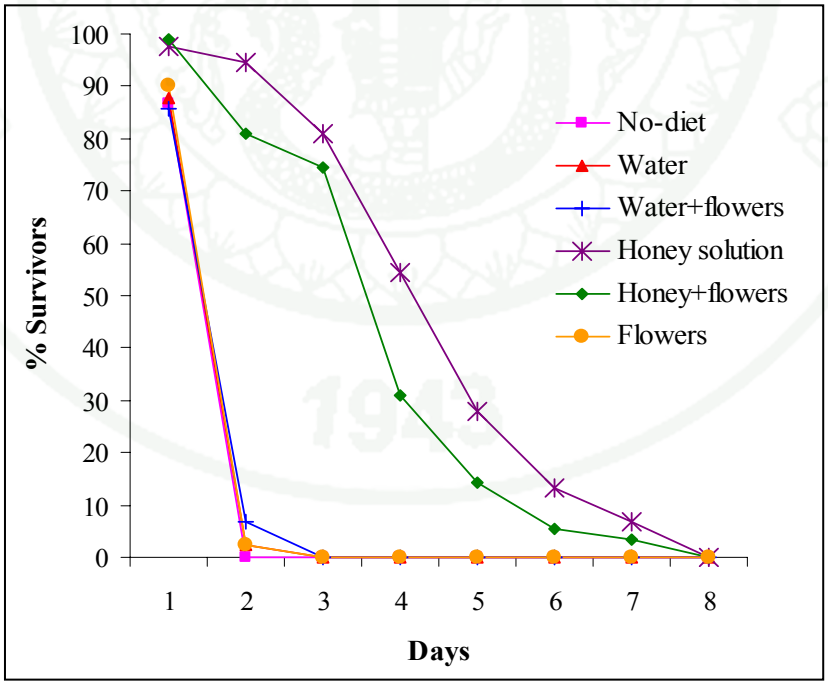

Figure 10 Survival patterns of male *Leptocybe invasa* fed with different diets.

foods of *L. invasa* in *E. camaldulensis* plantations. These were interested because natural diets in the plantations seasonally changed in quantities and qualities, such as flowers, young leaves of *E. camaldulensis* and so on. Moreover, the natural diets probably were not only one factor that caused the widespread and population expansion of *L. invasa* in *E. camaldulensis* plantations, but also the other factors might involved.

3. Fecundity of *L. invasa*

3.1 Potential fecundity of *L. invasa*

The potential fecundity denotes the maximum number of mature eggs in ovary (or egg load) that can potentially be laid by an adult female. It could be counted directly in the ovary of adult female by dissection.

In the laboratory, all eggs in ovaries of newly emerging female *L. invasa* from leaf gall were already mature. The potential fecundity in a pair of ovaries of a female *L. invasa* was shown in Figure 11. The research found that the average potential fecundity of the females, of all sizes and ages, was 158.70±4.62 eggs per female, ranging from 39 to 298 eggs per female.

The details of the potential fecundity of adult female *L. invasa* were investigated for the first time and the findings from this research were new and were documented. The egg load of *L. invasa* was rather high and it might be influenced by temperature and humidity of the study areas. This was possible because Heidari (1989) reported that high temperature and low humidity trended to increase potential fecundity of coccinellid predators.

The future study should be carried on the effects of high temperature and low humidity on the increasing potential fecundity of *L. invasa*. The results can be used to predict the degree of damages caused by *L. invasa* in the future.

Figure 11 Mature eggs in a pair of ovaries of a female *Leptocybe invasa*.

To use hind tibia length as a substitute for female size, the average of all sizes of females *L. invasa* was 0.28 mm, ranging from 0.20–0.35 mm. It was able to divide the female sizes into 3 classes as follows: small size (0.20–0.24 mm), medium size (0.25–0.29 mm), and large size (0.30 mm or more). The percentages of small size:medium size:large size of the female *L. invasa* were 17.55:48.58:33.86, respectively (Table 4). The results revealed that the female size of *L. invasa* in Tha Muang and Phanom Thuan districts were medium size (48.58 %) and large size (33.86 %).

The results also showed that increasing of female sizes trended to increase potential fecundities or to produce more egg loads. The average potential fecundity of a) the small size of female was 90.64±3.32 eggs/female, ranging from 39–140 eggs/female, b) the medium size of female was 160.58±3.82 eggs/female, ranging from 112–213 eggs/female, and c) the large size of female was 209.03±4.41 eggs/female, ranging from 141–298 eggs/female (Table 5).

The effects of female sizes of *L. invasa* all ages on mean egg loads were determined by ANOVA regression analysis. The results showed that sizes had significant effects on mean egg loads at P=0.05 (F=532.257; P–value=0.000). There was significantly positive relationship between sizes and egg loads of female *L. invasa* ($y=1578.834x - 283.230$; $R^2=0.772$; n=159). The larger sizes of female tended to produce more eggs than the smaller size (Figure 12). The $R^2=0.772$ indicated that the sizes influenced on egg loads at about 77.20%. The rest might be other variables which could involve and had influenced on egg loads.

This study used hind tibia length as a substitute for female size of *L. invasa*. Usually, width or length of some body parts such as head, thorax, or hind tibia are used to represent the body size (Jervis, 2005). Unfortunately, the insects in Family Eulophidae change their width and length from normal heads to crumpling and distorted head after death. This caused difficulty in head measurement. The length of hind tibia was unchanged and it related to body size. Thus, the hind tibia length was reasonable to be used as representative of body size.

Table 4 The average of female sizes and percentage in each size class of female *Leptocybe invasa.*

Size class (mm)	Female size average (mm)	Percentage
Small (0.20–0.24)	0.239 ± 0.002 [1/]	17.55
Medium (0.25–0.29)	0.279 ± 0.001	48.58
Large (0.30 or more)	0.311 ± 0.002	33.86

[1/] Mean±S.E.

Table 5 Relationship between size class and potential fecundity of female *Leptocybe invasa*.

Size class	Potential fecundity (eggs/ female)
Small	90.64±3.32 [1/] (39–140)
Medium	160.58±3.82 (112–213)
Large	209.03±4.41 (141–298)

[1/] Mean±S.E., with range in the bracket.

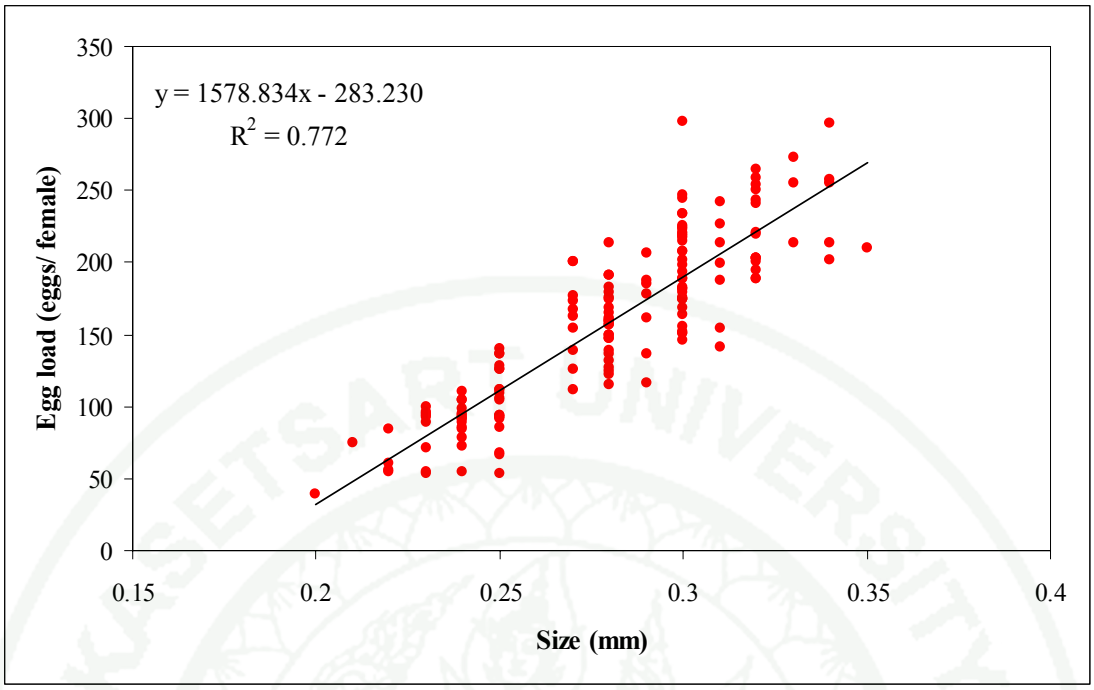

Figure 12 Relationship between size and egg load of female *Leptocybe invasa*.

The percentages of small size, medium size, and large size of *L. invasa* were 17.55, 48.58 and 33.86 respectively, indicating that most female *L. invasa* in the study areas were medium to large sizes. These might be resulted from some factors which were reported by some researchers. Jervis (2005) explained that the size might be influenced by a) larval feeding history, i.e. host species during development, host size, quality of host diet, and clutch size; and b) temperature during larval development. Zheng *et al*. (1993) reported that the size of lacewing *Chrysopera carnea* was influenced by larval food consumption. Ernsting and Huyer (1984) found that the increasing of temperature during larval development stimulated the adult size of two species of carabid beetle, *Notiohilus rufipes* and *N. biguttatus*.

Increasing sizes of female *L. invasa* trended to produce more egg loads and there was a significantly positive correlation between female size and egg load. The influence of female sizes on egg loads found in this study was in line with Jervis (2005), and Mozaddedul and Copland (2002) who studied on the potential

fecundity of parasitoid of long–tailed mealybug, and Mills and Kuhmann (2000) who determined the potential fecundity of *Trichogramma* parasitoids.

The effects of female ages and sizes on mean egg loads were shown in Figure 13. In each age group, mean egg loads increased with increasing sizes; but in each size group, mean egg loads in different ages were slightly different.

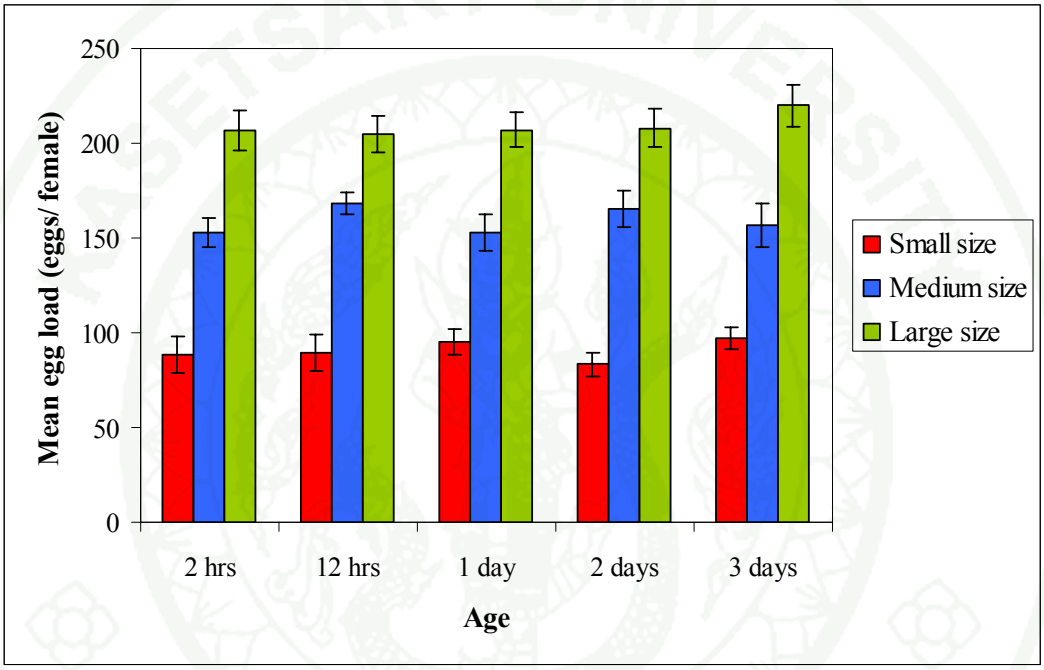

Figure 13 Mean egg loads in different ages and sizes of female *Leptocybe invasa*. The vertical bars indicate standard errors.

By two–way analysis of variance, ages had no effect on mean egg loads at P=0.05 (F=0.281, df =4, P–value=0.890). Sizes had significant effect on mean egg loads at P=0.05 (F=211.973, df =2, P–value=0.000). Age x size had no effect on mean egg loads at P=0.05 (F=0.416, df =8, P–value=0.910). So the effect of female sizes on egg loads did not vary with ages (Table 6 and Table 7).

Table 6 Mean egg loads in different ages and sizes of female *Leptocybe invasa*.

Age	Size	n	Mean	S.E.
2 hrs	Small	8	88.375	10.799
	Medium	7	153.000	11.545
	Large	11	206.727	9.209
12 hrs	Small	10	89.000	9.659
	Medium	13	165.462	8.471
	Large	14	204.568	8.163
1 day	Small	10	95.200	9.659
	Medium	8	152.875	10.799
	Large	15	207.133	7.886
2 days	Small	11	83.273	9.209
	Medium	8	165.500	10.799
	Large	14	208.143	8.163
3 days	Small	11	97.000	9.209
	Medium	7	156.571	11.545
	Large	12	219.833	8.817

Table 7 Effects of ages and sizes on mean egg loads of female *Leptocybe invasa* by two–way ANOVA.

Source	df	SS	MS	F	P-value
Age	4	1,048.102	262.025	0.281	0.890
Size	2	395,513.057	197,756.528	211.973	0.000
Age x Size	8	3,105.867	388.233	0.416	0.910
Error	144	134,342.273	932.932		
Total	159	4,529,495.000			

The two–way analysis of variance which showed that different ages of female *L. invasa* had no significant effect on mean egg loads, was consistent with Bernardo *et al*. (2005). He studied on the potential fecundity of *Thripobius semiluteus* Bouček (Hymenoptera: Eulophidae) and reported that the mean egg loads among females of different ages did not significantly differ.

3.2 Realized fecundity of *L. invasa*

Generally, realized fecundity refers to the total number of eggs actually laid over the life–time of an adult female *L. invasa*. Practically, realized fecundity is determined by counting the number of eggs laid in host per day until a female died, and then calculates the total number of eggs laid in host over the life–time of a female.

Takagi (1985) and Hardy *et al.* (1992) determined the realized fecundity of parasitoid wasps without counting the number of eggs. They counted the number of adult offsprings produced and combined with dead eggs, so deriving the estimated number of eggs originally deposited. Their researches were carried out in the parasitoid wasps which their realized fecundity measurements were less complex and less difficult than *L. invasa.*

In the case of *L. invasa*, the adult female laid her eggs in tissues of *E. camaldulensis*, so the eggs laid in each day and the dead eggs in tissues were difficult to detect. Thus this research determined the realized fecundity of *L. invasa* by counting the total number of emerging progenies (emerging adult offsprings) over the life–time of a female and expressed in terms of progenies per a female.

The reasons to determine the realized fecundity by counting the total number of emerging progenies over the life–time of a female and expresses in terms of progenies per a female were as follows: a) the eggs of a female were laid in *Eucalyptus* tissues, so the eggs were difficult to detect; b) counting total eggs laid in plant tissues were laborious because it must be carried out by plant microtechniques;

and c) a number of eggs might be dead at ovipositing time, and then could not observe them.

In ventilated greenhouse, all eggs in ovaries of newly emerging female *L. invasa* were mature. The research found that the female *L. invasa* oviposited on the first day after her emergence and lasted on the sixth day. The mean progeny/ female was maximum on the first day with 30.47±7.41 progenies/female and declined in the following days and lasted on the sixth day (Table 8 and Figure 14).

Table 8 Relation between oviposition day and mean progeny/female of *Leptocybe invasa*.

Oviposition day [1/]	Mean progeny/ female
1	30.47±7.41 [2/]
2	19.07±3.86
3	7.27±2.35
4	3.20±1.15
5	1.33±0.73
6	0.20±0.20
7	-

[1/] Day after emergence of a female

[2/] Mean±S.E.

It was found that the mean of realized fecundity of a female from the first day to the sixth day was 61.53±8.94 progenies per a female, ranging from 18 to 130 progenies/female. After the sixth day, all females were dead. The progenies emerged in the laboratory of this study comprised of more females than males.

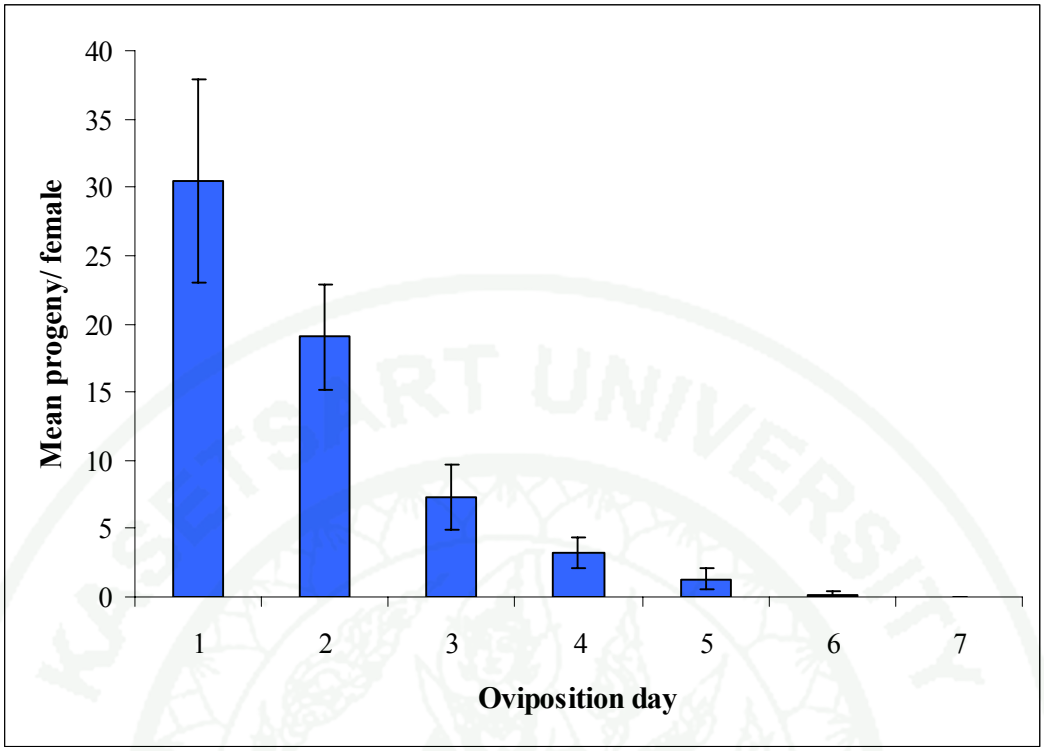

Figure 14 Mean progeny/female as related to oviposition day of *Leptocybe invasa*. The vertical bars indicate standard errors.

The mean progeny/female which was maximum on the first day, was probably due to the fact that *L. invasa* had rather short longevity or short life–time. So the female had to produce a large number of progenies as fast as possible. This report on realized fecundity of *L. invasa* from this research was new and was documented.

The results from this realized fecundity indicated that *L. invasa* is a pro–ovigenic species, because a) all eggs in ovaries of newly emerging female *L. invasa* are mature (as determined by size and shape of eggs), and b) the adult females oviposite on the first day after emergence, and the mean progeny/female is maximum on the first day or on early adult–life.

It was in line with Mendel *et al*. (2004) who reported that the dissection of newly emerging *L. invasa* showed strict pro–ovigenesis. Flanders (1950) classified parasitoid wasps that had all or nearly all of their eggs matured prior to the

start of oviposition as pro–ovigenic, and those that continued to mature eggs throughout their reproductive life as syn–ovigenic. Flander (1950) also reported that pro–ovigenic wasps actually had shorter life than syn–ovigenic ones. The life–span or the longevity was negatively correlated with the proportion of oocytes mature upon emergence.

The mean realized fecundity of female *L. invasa* from the first day to the sixth day was 61.53±8.94 progenies per a female, ranging from 18 to 130 progenies/female. On the seventh day, all females were dead. The mean of the remaining eggs in ovaries at their death was 131.40±11.94 eggs/female.

It was observed that the mean number of female progenies:male progenies was 40.80:20.73 or 1.97:1. Thus the sex ratio of female:male progenies of *L. invasa* ~ 2:1. From the observation of mating behavior, the male offsprings did not mate with female offsprings because the male offsprings were non–functional. The unmated female offsprings could oviposit and the eggs could develop into both female and male progenies. The finding from this study indicated that the reproductive mode of *L. invasa* is deuterotoky. This mode was observed both in ventilated greenhouse and in outdoor.

Jervis (2005) divided reproductive modes of wasps into 3 groups; arrhenotoky, thelytoky, and deuterotoky.

Arrhenotoky refers to male progenies develop parthenogenetically from unfertilized eggs and female progenies develop from fertilized eggs.

Thelytoky denotes the entirely parthenogenetical reproduction. There is no male and unfertilized eggs give rise to diploid females, resulting in 100% of daughters related to their mother.

Deuterotoky means the female production with more female offsprings and rare male offsprings. The male offsprings are non–functional. Both sexes of offsprings developed from unfertilized eggs.

Luck *et al.* (1993) pointed out that some parasitoid wasp species originally designated as thelytoky. During the maternal female development, they had been exposed to high temperature and small number of male offsprings could be produced, and it finally became deuterotoky. The male offsprings were initially considered to be non–functional.

The findings from this research were beneficial as follows:

a) The female *L. invasa* had rather high potential fecundity (158.70±4.62 eggs/female) but low realized fecundity (61.53±8.94 progenies/female). The sex ratio between female and male offsprings was 2:1. These data can be used as a guide line for a measure of *L. invasa* control.

b) The female *L. invasa* is a pro–ovigenic species. This can be used to compare with parasitoids about their weak points and strenuous points.

c) The reproductive mode of female *L. invasa* is deuterotoky. Jervis (2005) described this mode that the maternal female (diploid) produced more female offsprings (diploid) but the rare male offsprings (haploid). Both offsprings developed entirely from unfertilized eggs. With this reproductive mode, the female offsprings possibly had slightly variation in genetic background. Then the occurrence of new genes in female offsprings which was capable to increase more damage to *E. camaldulensis*, would be less. Moreover, the male offsprings were non–functional.

4. Reproductive organs of *L. invasa*

Generally the external morphology is used to identify insect species. In some cases, only the external morphology is not adequate to separate two insect

species which are similar in shape. Thus the internal morphology, such as the reproductive organs and genitalia will be beneficial to identify the distinction between them.

4.1 Reproductive organs of females *L. invasa*

The reproductive organs of female differ greatly from male. This research found that the reproductive organs of female *L. invasa* consisted of a pair of ovaries and short lateral oviducts, a short common oviduct, a pair of accessory glands, a spermatheca, an ovipositor (first and second valvulae) and a pair of ovipositor sheaths, first valvifer and second valvifer (Figure 15A and B).

The ovaries of female *L. invasa* contained numerous mature eggs and filled in the abdomen cavity above the gut. The immature eggs had none. The accessory glands located with the anterior end or the common oviduct. The spermatheca was very small and noticeably pigmented yellow. The ovipositor was not longer than the tip of abdomen. The length of ovipositor was 0.35–0.40 mm. The tip of ovipositor sheaths had some sensory hairs.

4.2 Reproductive organs of male *L. invasa*

The reproductive organs of male *L. invasa* comprised of a pair of testes, vasa deferentia and seminal vasicles, an ejaculatory duct, a pair of accessory glands, digitae and parameres, and an aedeagus (Figure 15C and D).

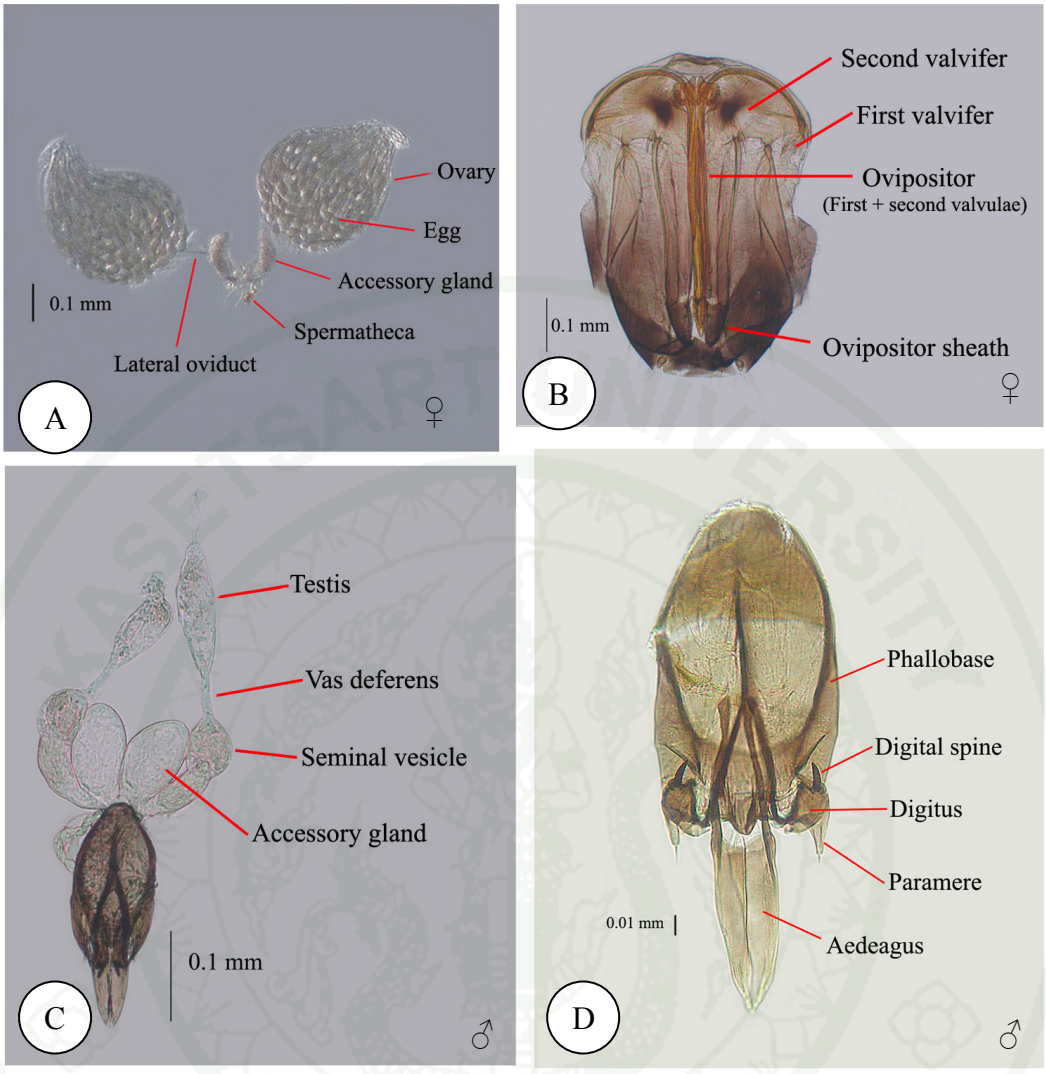

Figure 15 Reproductive organs of *Leptocybe invasa*: (A–B) female; and (C–D) male.

The testes were slightly oval to elongate. The accessory glands were well developed and oval shaped. The tip of digitus had a large digital spin. The paramere was short and had one seta on the tip. The aedeagus was cylindrical.

This research reported for the first time on the reproductive organs of the female and male *L. invasa* and these findings were documented. However, the structure of reproductive organs, which were found in this study, were close to those of some species in Hymenoptera as described by some researchers, such as Sanger and King (1971) and Jervis (2005).

5. Development of *L. invasa*

Development of female and male *L. invasa* in this study covered from egg stage to young larva, mature larva to prepupa, pupa, and adult stage (emergence stage or adult emerging from *E. camalulensis* tissues).

5.1 Oviposition behavior and egg development of *L. invasa*

It was found from this research that adult female *L. invasa* oviposited in newly developed leaves and in young twigs since the first day of her emergence. When the female found them, she explored to search the most appropriate position for her egg laying by: a) using antennae knocking, mostly on the surface of leaf midrib, petiole and young twig, and b) testing the tissues at several points by inserting a strong ovipositor in them before the real ovipositing. After finding the suitable position, the female *L. invasa* inserted eggs in those tissues (Figure 16 and Figure 17).

The female *L. invasa* inserted eggs in the midribs at the lower surface of lamina, in the petioles and in the tissues of young twigs. The attack took place approximately within 1 week after bud break. Generally, the eggs were laid at a distance ~ 0.3–0.5 mm from each other and in line on midrib and petiole. It was noticed in the study areas that nearly one hundred percent of newly developed leaves were attacked by this gall wasp.

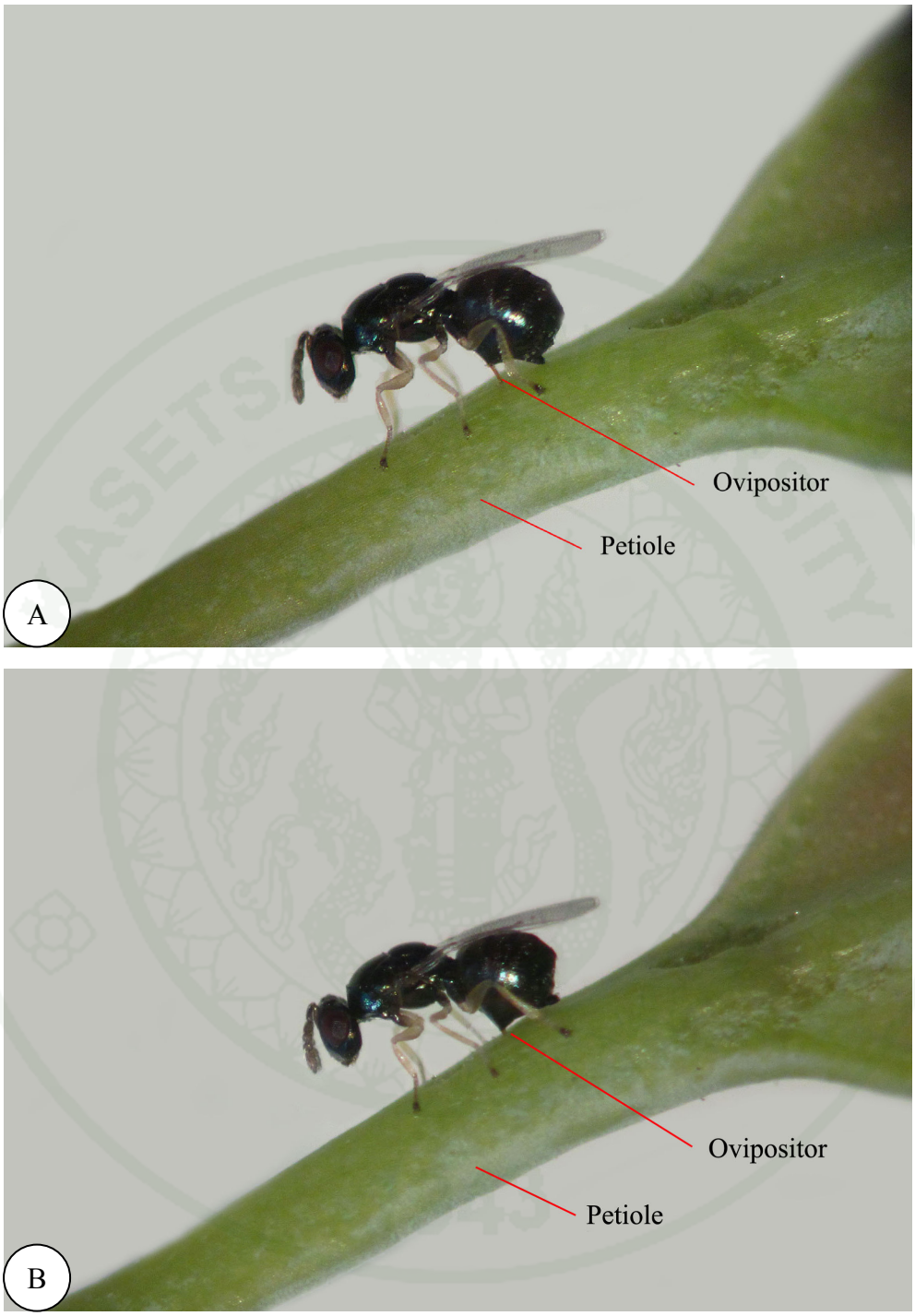

Figure 16 Oviposition behavior of female *Leptocybe invasa* on the petiole of the newly developed leaf of *Eucalyptus camaldulensis*: (A) testing with ovipositor to search the suitable position for egg laying; and (B) ovipositing in tissues of the petiole.

Figure 17 Oviposition behavior of female *Leptocybe invasa* in young tissues of *Eucalyptus camaldulensis*: (A) midrib; (B) petiole; and (C) twig.

The adult female oviposited since the first day of her emergence because her longevity was short. The female *L. invasa* oviposited in midrib and in petiole because these organs were probably able to support egg and gall developments. Her eggs were mostly inserted in midribs of the lower surface of lamina because these positions were close to the phloem and/or might decrease the effects of strong sunlight.

Besides *E. camaldulensis*, Mendel *et al.* (2004) found that nine species were found to be suitable hosts of *L. invasa* in Israel. Those were *E. botryoides*, *E. bridgesiana*, *E. globulus*, *E.gunii*, *E. grandis*, *E. robusta*, *E. saligna*, *E. tereticonis* and *E. viminalis*. Outdoors in the summer or on the strong sunshine days, the females were observed on young growth during the morning and in late afternoon. Indoors, the females were ready to attack newly developed leaves and young twigs at any time during the day.

About a minute after oviposition in the tissues, white and transparent liquid, probably gum, secreted from the wounds caused by the ovipositor and blocked the opening of the injury. The liquid might protect the wounds at the egg insertion points. Few days later, symptom of cork tissues appeared at the egg insertion spots. Then the cork scars caused by oviposition increased in sizes (Figure 18). This symptom was called cork tissue and cork scar by Mendel *et al.* (2004).

This research found that the female *L. invasa* oviposited eggs closely to/ or in vascular bundles of midrib and petiole of newly developed leaves, and of young twigs (Figure 19).

Such position of oviposition followed by gall inducing caused retardation in leaf growth and deformation and in shoot growth of *E. camaldulensis*. Egg overloading in some months gave rise to the severe reduction of leaf and shoot growth. The larvae of *L. invasa* in the galls took away mineral salts and nutrients from young plants. These might kill juvenile shoots or young plants. Moreover, egg overloading in some months in combination with other stress factors in the plantations

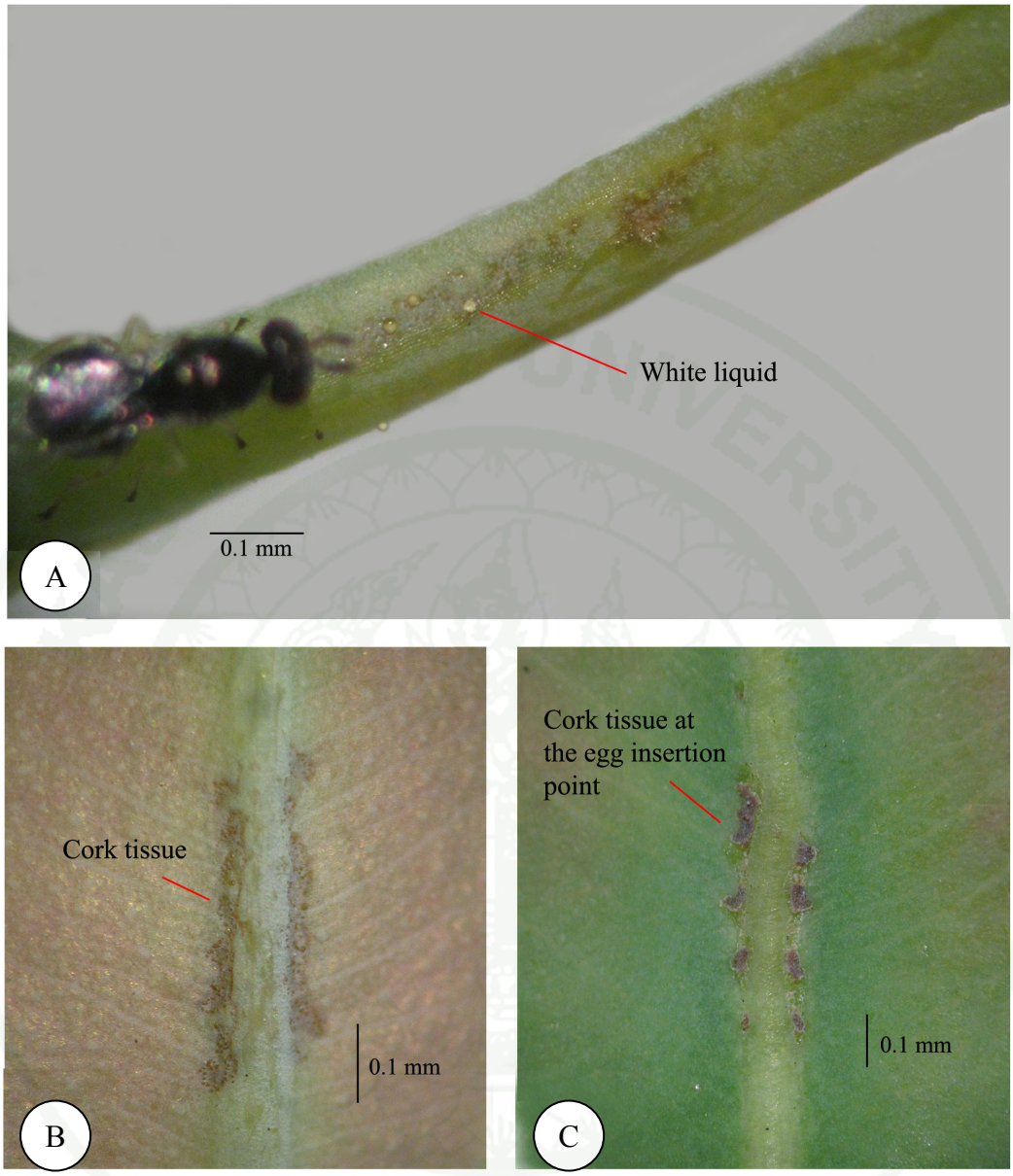

Figure 18 Wounds at the egg insertion points cause by ovipositors of *Leptocybe invasa*: (A) white liquid at the wound caused by ovipositor; and (B, C) cork tissues at the points of egg insertion.

could seriously damage or kill young *E. camaldulensis* or young coppice shoots which were less vigor.

Generally, from the outer layer to inner one of young midrib and petiole, the tissues comprise of epidermis, ground tissue (comprises of collenchyma and parenchyma) and vascular bundle (xylem and phloem). Xylem is oriented towards the center of the organ while the phloem is oriented to the lower midrib. Collenchyma is the supporting tissue. The tissues of young twig were composed of epidermis, cortex, vascular bundle and pith. Leaf tissues consisted of upper (adaxial) epidermis with cuticle (waterproof covering), palisade mesophyll (tightly packed layer of photosynthetic tissues), spongy mesophyll (loosely packed layer of photosynthetic tissues), veins (xylem, phloem, strengthening sclerenchyma), and lower (abaxial) epidermis.

Development of *L. invasa* eggs inside vascular bundles also stimulated the development of gall. Later the gall formed typical bump–shape. Heavy galling prevented further development and growth of infested tree.

The development from eggs to larvae of *L. invasa* in the vascular bundles of midribs of young *E. camaldulensis* leaves were shown in Figure 19 to Figure 21. It began with the insertion of egg in vascular bundle and was followed by the occurrence of chamber around the egg. When the egg developed to larva, the gall chamber and the nutritive tissue were observed around the larva. The growth of larva might cause the disruption and deformation of vascular tissue. The development of eggs and growth of larvae were accompanied by the formation and development of galls.

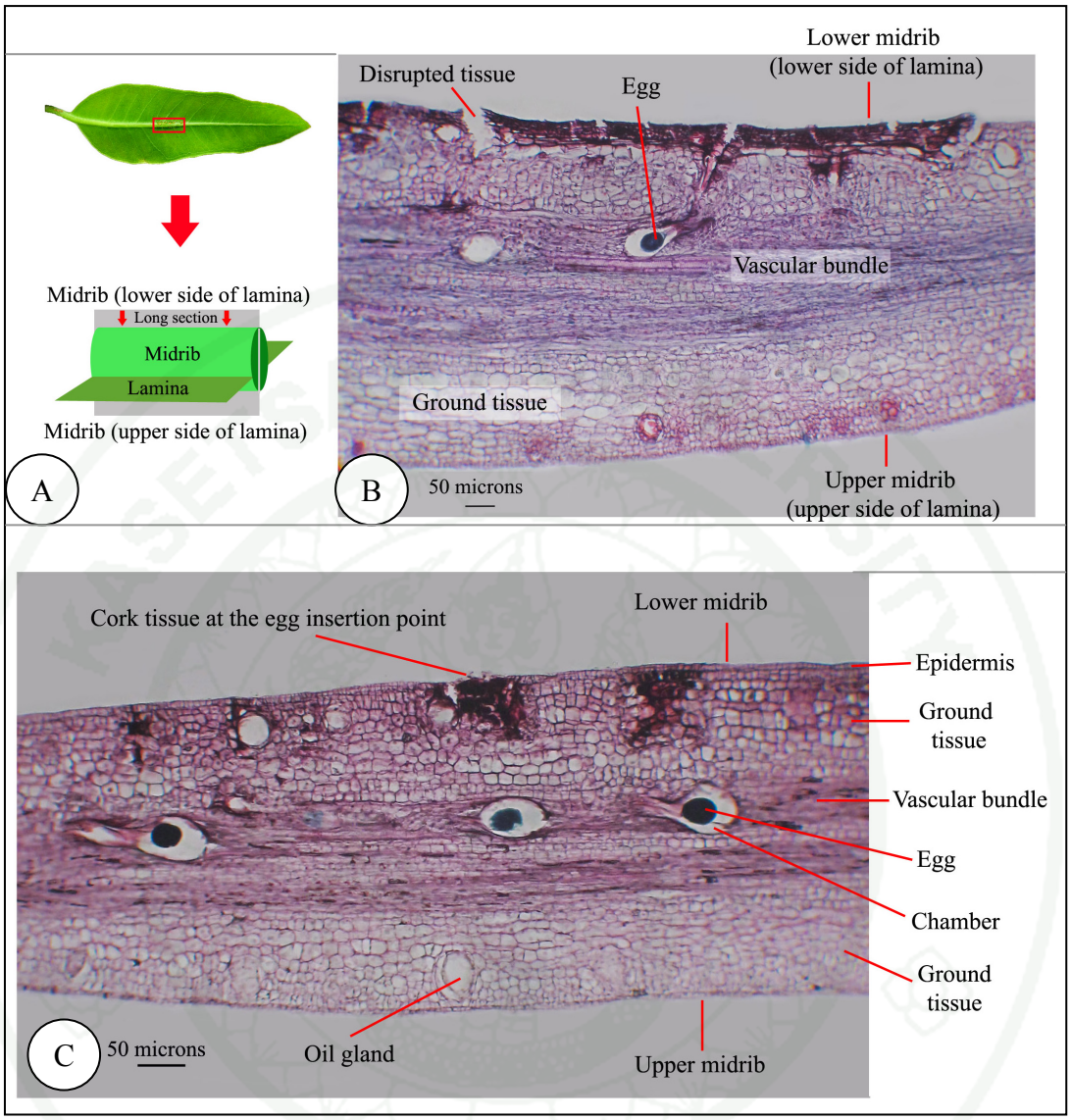

Figure 19 Longitudinal section of young midribs of *Eucalyptus camaldulensis*: (A) direction of longitudinal section; (B–C) 3–day old eggs after eggs insertion of *Leptocybe invasa* in vascular bundles.

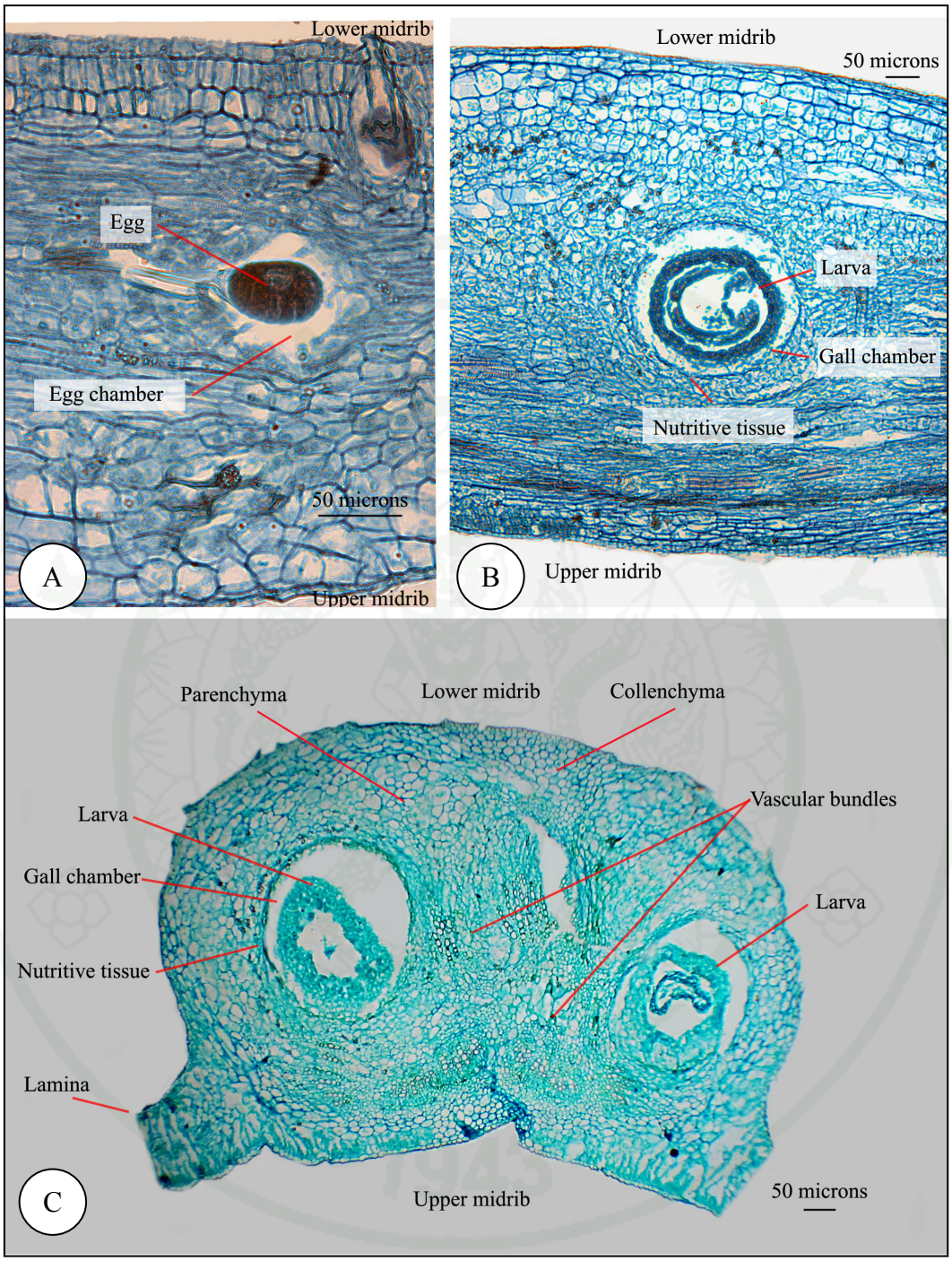

Figure 20 Eggs and larvae in vascular bundles of young midribs of *Eucalyptus camaldulensis*: (A) longitudinal section showing 3–day old egg in gall chamber; (B) longitudinal section illustrating 15–day old larva in gall chamber; and (C) transverse section indicating 15–day old larvae in a pair of vascular bundles.

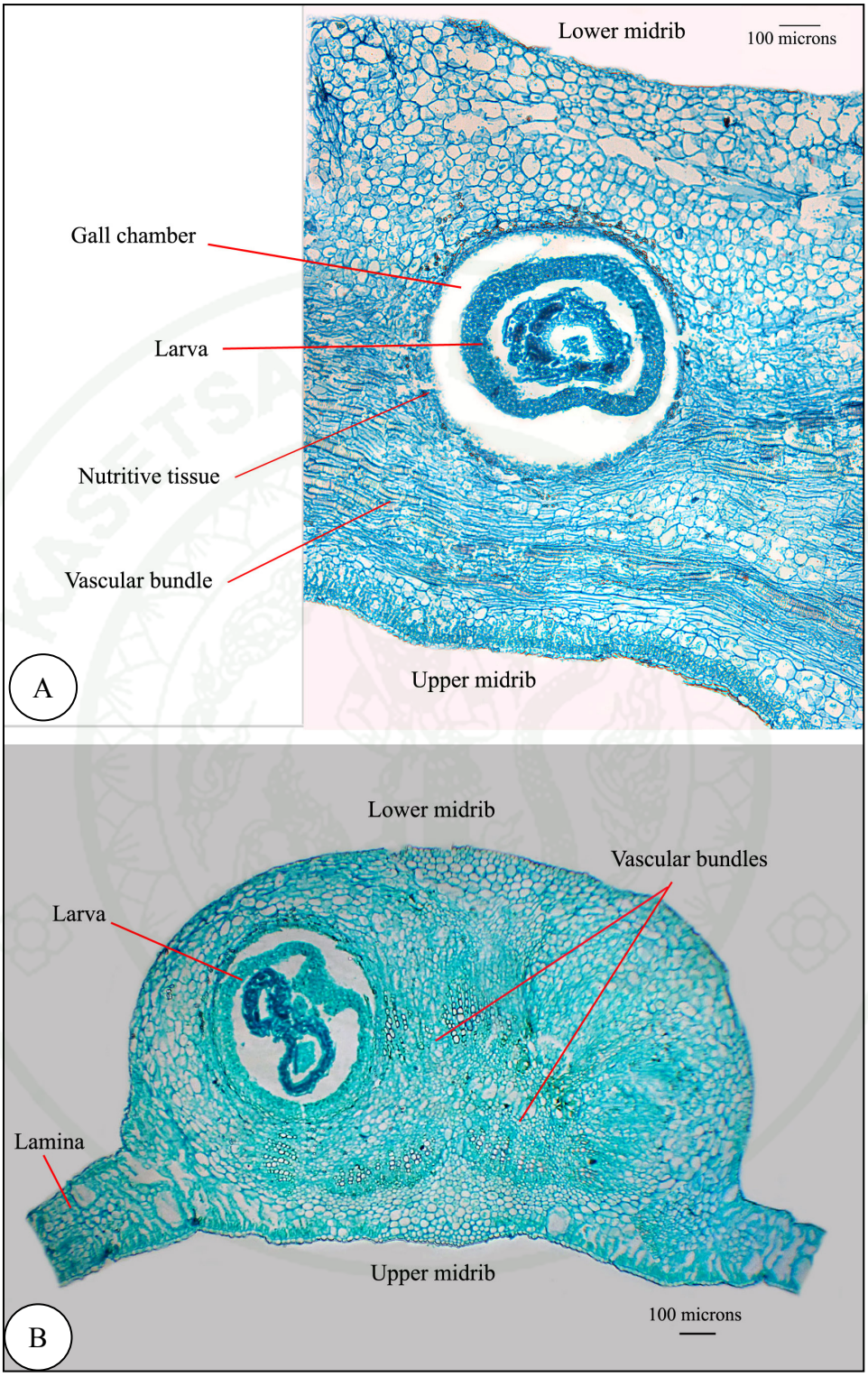

Figure 21 Larvae in vascular bundles of young midribs of *Eucalyptus camaldulensis*: (A) longitudinal section showing 30–day old larva; and (B) transverse section illustrating 30–day old larva.

It was informed by some specialists in *E. camaldulensis* that young leaves of CT 76 clone has single epidermal layer with thick cuticle. There is no answer why this clone is susceptible to *L. invasa* but tolerate to pathogens.

Rohfritsch (1992) reported on the gall development that there were 4 basic stages of gall development as follows; initiation stage, growth and differentiation stage, maturation stage, and dehiscence stage. The description of each stage was shown as follows.

1) Gall initiation stage: It was characterized by the isolation and withdrawal of cells from normal growth of host tissues. These were done by female adult at oviposition. The ovipositor poured some fluids on plant cells around egg to modify cell walls or to liquidize cell contents. This process gave rise to egg chamber. During gall development, normal growth processes was inhibit but induced new morphogenetic and physiological responses that were useful to egg and larva.

2) Gall growth and differentiation stage: It was observed at growth of gall by stimulating of young larva. It vastly accelerated cell division (hyperplasy) and cell enlargement (hypertrophy) by the feeding of young larva.

During feeding activities, the young larva poured salivary fluids on surrounding plant cells to modify cell wall or to liquidize the cell contents. The cells reacted to this stimulation by cell differentiation and proliferation. These gave rise to the occurrence of nutritive tissue along the inner surface of gall chamber. The young larva poured saliva to digest nutritive tissue to white fluid and filled in gall chamber. These also produced new vascular tissues arising among the mass of growing cells and joined the normal vascular tissues of the attached organ.

3) Gall maturation stage: It occurred while the insect was in mature larval stage which consumed the largest amount of food. When it reached to pupa stage, it stopped feeding and did not pour saliva to digest nutritive tissue. The cells

around the layer of nutritive tissue differentiated to sclerenchyma sheath to protect the pupa.

4) Gall dehiscence or gall opening stage: This stage was not found in *L. invasa*. The adult *L. invasa* made exit hole to emerge the gall by itself.

Bronner (1992) defined nutritive tissue as the tissue found between gall chamber and cortex or lignified sheath. Nutritive cells had high concentrations of starch, soluble sugars, lipids and proteins.

Raman *et al*. (2003) reported that the vast increasing of gall development by rapid cell division and cell enlargement in stage 2, was mainly due to IAA (indole–3–acetic acid). IAA biosynthesis at gall sites could be phenolic compounds from larva saliva. Larva feeding accelerated the velocity of cell division and cell enlargement and nutritive tissue differentiation of host plant.

Hori (1992) pointed out that IAA and amino acids in larva saliva were the important factors in gall formation. Miles (1999) found that larval feeding gave rise to the appearance of auxin and a vigorous uptake of oxygen in gall tissues.

Raman *et al*. (2003) concluded that feeding activities of larva induced two key responses from the host plant: a) accelerating the velocity of cell division and cell enlargement and b) induction the differentiation of nutritive tissue.

5.2 Development time from eggs to adult emergence of *L. invasa*

To follow Mendel *et al*. (2004), the development of *L. invasa* in this study was divided into 5 stages as follows: stage 1 (egg), stage 2 (young larva), stage 3 (mature larva to prepupa), stage 4 (pupa) and stage 5 (adult).

In ventilated greenhouse, it was found that the mean development time of *L. invasa* from eggs to adults was 45.96±0.52 days, ranging from 35 to 73

days. The egg stage, young larval stage, mature larval to prepupal stage, and pupal stage took 10.94±0.25 days, 18.07±1.16 days, 30.13±1.05 days and 37.70±1.07 days, respectively. The development time was minimum in egg stage and maximum in larval stage and in pupal stage. The detail of *L. invasa* development time was shown on Figure 22.

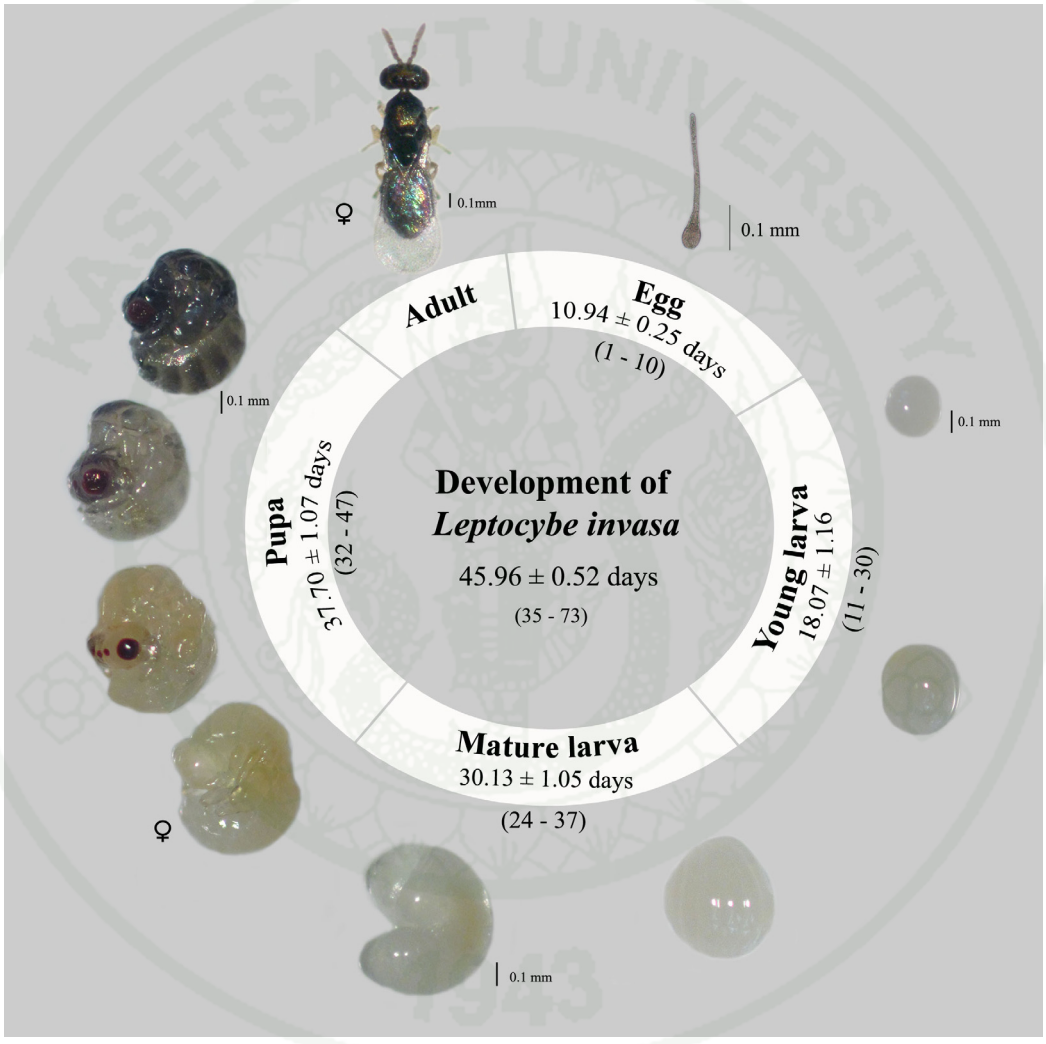

Figure 22 Development time at each stage of *Leptocybe invasa* in young *Eucalyptus camaldulensis* leaf tissues.

The external morphology of the immature found at each stage was described below and shown in Figure 23A–D.

Stage 1 (egg): The egg of *L. invasa* comprised of an oval body and a long narrow anterior stalk. The size of individual egg ranged from 0.30–0.33 mm in length. The egg was grayish white in color and the entire surface was smooth, lacking ornamentation.

Stage 2 (young larva) and stage 3 (mature larva to prepupa): The young larva and mature larva were rounded shape but differed in size and segmentation. The mature larva was bigger in size and had distinct segments. The rounded shapes of young larva and mature larva were rather smooth, and lacked of tubercles or spines. The larva was grayish white in color and opaque. The thoracic legs and abdominal prolegs were absents. The young larva had no segment but the mature larva had it. Head capsule was not developed. The mandibles were strongly sclerotised. Soon after the mature larva began to expel its meconium (the midgut waste materials) at posterior end, it turned to prepupa. The prepupa was characterized by C–shape with thoracic segment elongated. The number of instars was not determined in this study.

Stage 4 (pupa): *L. invasa*, like other Hymenoptera, produced exarate pupa with clearly visible mouthparts, antennae and legs. The C–shape of pupae were not protected by any special cocoon. The newly formed pupa was whitish with no pigmentation. A few days later, eyes of pupa became reddish. In the next days, the pupal cuticle was sclerotized from a light gray to black. No morphological difference was observed between the different stages of pupal development.

Stage 5 (adult emergence): The adult *L. invasa* perforated from vascular bundle to epidermis to make emergence hole from the gall. When the adult emerged from the gall, the tissues filled up the exit hole and gall cavity. It was observed that adult males of *L. invasa* emerged from the gall before adult females.

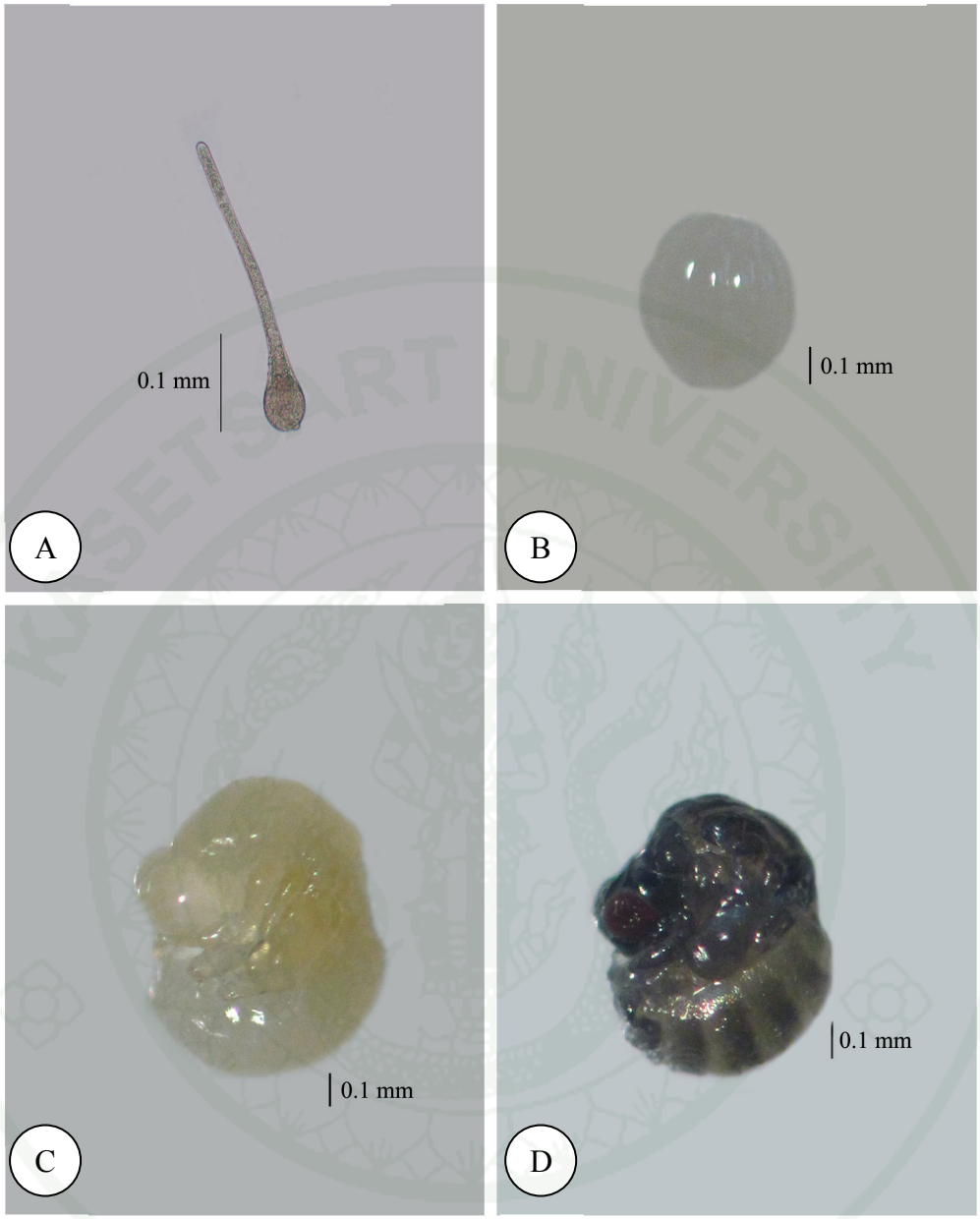

Figure 23 Morphology of immature *Leptocybe invasa* in *Eucalyptus camaldulensis* leaf tissues: (A) egg; (B) young larva; (C) mature larva to prepupa; and (D) late pupa.

The findings from this research showed that the mean development time of *L. invasa* from eggs to adults in ventilated greenhouse was 45.96±0.52 days. Mendel *et al*. (2004) reported that the mean development time of *L. invasa* in room temperature was 132.60 days. The difference in development time probably resulted from the difference in temperature and environmental conditions of the study. Generally the development time of insects was accelerated in the warmer air. As this result, global warming may accelerate the development of *L. invasa* which may have more negative effects on *E. camaldulensis* plantations.

At the adult emergence, the adult male *L. invasa* emerged from the gall before adult female. This could be explained that the longevity of this gall wasp was rather short. Thus the males probably had to wait and be ready to mate with females which came out later from the exit hole at the galls. However, the male offsprings were non–functional. So this occurrence needs more studies in the future.

5.3 Relation between gall and leaf developments of *E. camaldulensis* and immature development of *L. invasa*

The immature development of *L. invasa* occurs in parallel with gall and leaf developments. The study on the development of eggs to adult in leaf galls is laborious and consumes time and expenditure. Thus, the research on the relation between gall and leaf developments of *E. camaldulensis* and immature development of *L. invasa* is more practical. If we measured the gall size or leaf area, we can estimate the developmental stage of the immature *L. invasa* in plant tissue.

There was a relation between immature development and gall development. The results from this study were described below.

Stage 1 (egg): This corresponded to the gall initiation stage. The midrib and petiole occurred the numerous wounds from oviposition and followed by white and transparent liquid secreted from the wounds and blocked the opening of the injuries. Few days later, symptoms of cork tissues appeared at the egg insertion spots.

Then the cork scars developed and increased in width and length. The gall formation at stage 1 was not distinct.

Stage 2 (young larva): It corresponded to the gall growth and differentiation stage. The cork scars on midrib and petiole expanded and finally disappeared. The typical bump shape of galls developed. The gall surface was smooth and glossy green, covered with wax in some parts. At this stage, the growth of galls was very rapid.

Stage 3 (mature larva to prepupa): This corresponded to the gall maturation stage. The larvae and the galls still developed very fast. The gall surface was not smooth and the gall color tended to change to pale pink while retaining its gloss. The galls reached the maximum size about the end of stage 3 (mature larva).

The gall sizes and shapes were related to the number of egg loadings in leaf tissues. If egg over loadings occurred closely and in line along the midrib and petiole, each gall fused together and formed a largely long – shape gall.

Stage 4 (pupa): It remained in the gall maturation stage. The larvae developed to pupae. The gall sizes were maximum and remained unchanged. The galls lossed glossiness and the color was pink, or red pink. The pupa in gall could be observed as dark spot when the gall was peered closely to the sunshine.

Stage 5 (adult, emergence): This remained in the gall maturation stage. The pupae developed to adults. The emergence holes of the wasps were noticed on gall surface. The adults used mandibles to make the exit holes. Plant tissues filled up the exit holes and gall cavities after wasp emergence. The gall color changed to pale pink or light brown.

This study suggested that changing in gall development and gall morphology could estimate the developmental stages of immature *L. invasa*. The gall color was probably used as an indicator of immature development of *L. invasa*.

There was a relation between gall width and leaf area of *E. camaldulensis* and immature development of *L. invasa*. The results were shown on Figure 24 and were described below:

a) during the initiation of gall development and the newly developed leaf, the immature development was in stage 1 (egg);

b) during the rapid growth of gall width and galled leaf area, the immature development was in stage 2 (young larva) to the beginning of stage 3 (mature larva to prepupa);

c) during the slow growth of gall width and galled leaf–area, the immature development was in stage 3 (mature larva to prepupa) to beginning of stage 4 (pupa);

d) reaching the maximum size of gall width and galled leaf–area, the immature development was in stage 4 (pupa). The maximum size of gall width (0.30 cm) and galled leaf area (18.34 cm^2) were found on the thirty–third day.

e) appearance of exit holes at gall surface, the immature development was in stage 5 (adult stage or emergence stage).

The results from this study suggested that measurement of gall width and galled leaf area could estimate the developmental stage of immature *L. invasa* in leaf gall.

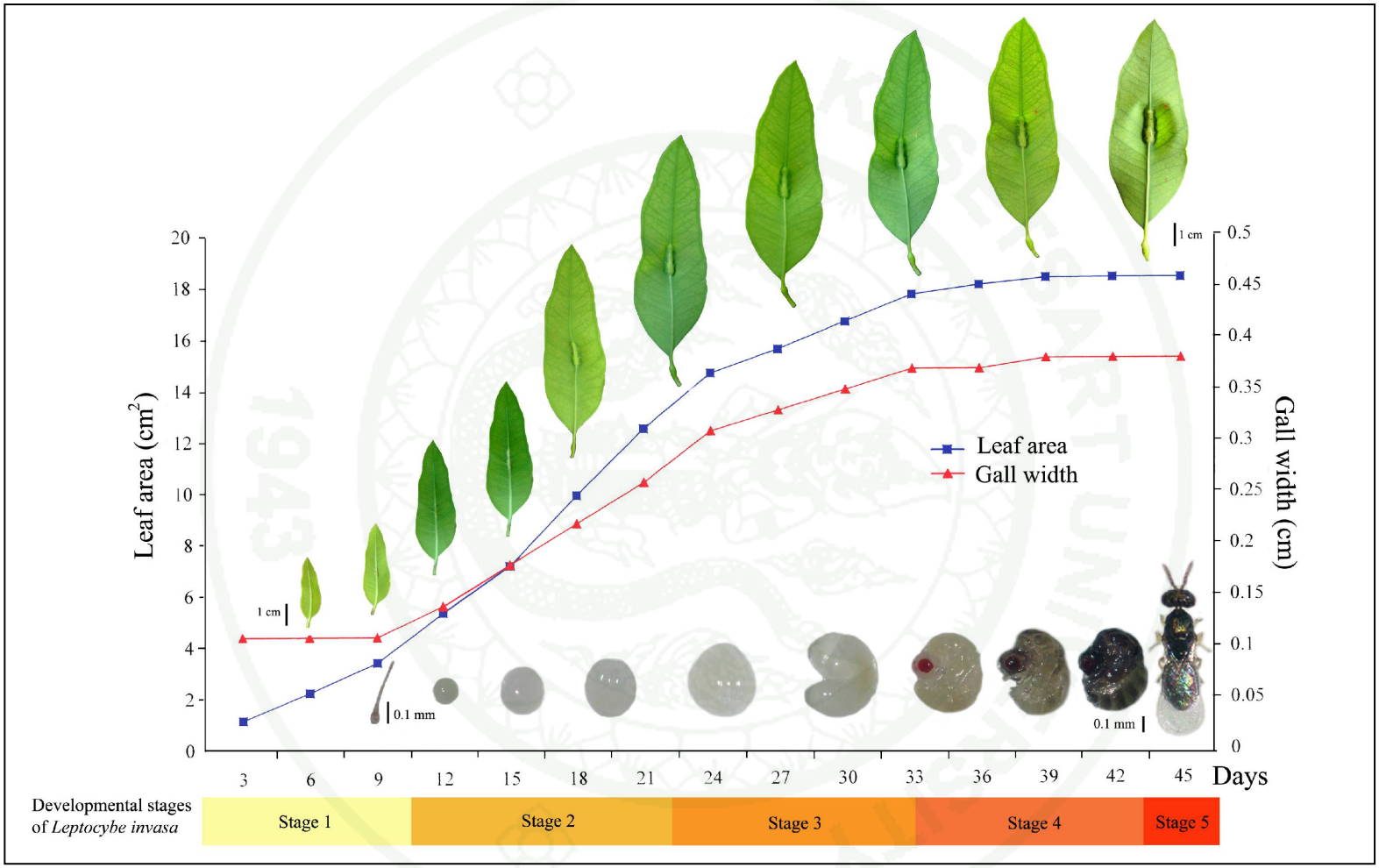

Figure 24 Relation between gall width and leaf area of *Eucalyptus camaldulensis*

In this study, the increasing of gall widths was used to relate with the stages of immature development of *L. invasa* because the females inserted eggs along the midrib or petiole and the fusion of each gall into a long–developed gall occurred in later period. Thus the gall lengths were not appropriate to relate with the stages of immature development but it should be used to relate with the stages of immature development plus the number of immatures. By this method and couple with adequate leaf samples could be used to relate between gall size and immature development.

Mendel *et al*. (2004) divided gall development on *E. camaldulensis* leaves into five stages by using gall shape and gall color. He carried out his research in Israel and reported that the development of gall shape and gall color related to the stage of immature development. The findings in Israel resembled the results from this study.

6. Damage to *E. camaldulensis* by *L. invasa*

The results from this research revealed that *E. camaldulensis* (CT 76 clone) in the plantations of two study areas was damaged by *L. invasa* throughout the year. This clone becomes susceptible to this gall wasp.

Leptocybe invasa induced galls mostly at midribs and petioles of newly developed leaves (~1 cm in length) and young twigs. The female wasps oviposited by inserting eggs in those young tissues. They oviposited eggs closely to/or in vascular bundles of midribs, petioles and young twigs. Afterwards, the gall development occurred at them (Figure 25A and B).

The oviposition closely to/ or in vascular bundles together with immature and gall development at midribs and petioles of newly developed leaves caused damage to *E. camaldulensis* by retardation in growth and deformation of leaves and shoots. The immature *L. invasa* in the galls took away mineral salts and nutrients from plants.

Eucalyptus camaldulensis produced new leaves throughout the year. The degrees of damage concerned with the environment. During the adequate rain period, *E. camaldulensis* produced much new leaves. The heavy galling on young leaves and twigs was observed distinctly. During the dry period, the trees produced less new leaves, the gall inducing at young leaves and twigs were decline. This suggested that there was a seasonal fluctuation of food supplied for *L. invasa* in the study areas which brought to the fluctuation of *L. invasa* attack.

In the period of optimum environment, over egg loadings caused heavy galling and gave rise to the severe reduction of leaf and shoot growth of *E. camaldulensis* (Figure 25C, D and E). The larvae in the galls took away mineral salts and nutrients from young plants.

When the new young leaves and twigs of young coppice shoots were attacked by *L. invasa*, they tried to produce new leaves but they were attacked by this gall wasp again. Repeated attack like this caused severe reduction in growth and deformation of leaves and shoots of young coppices. Such attacks reduced the vigor and could serious damage or kill young coppices. In some months, over egg loadings in combination with other stress factors in the plantations, such as drought, could seriously damage young *E. camaldulensis* which was less vigor (Figure 25F, G and H).

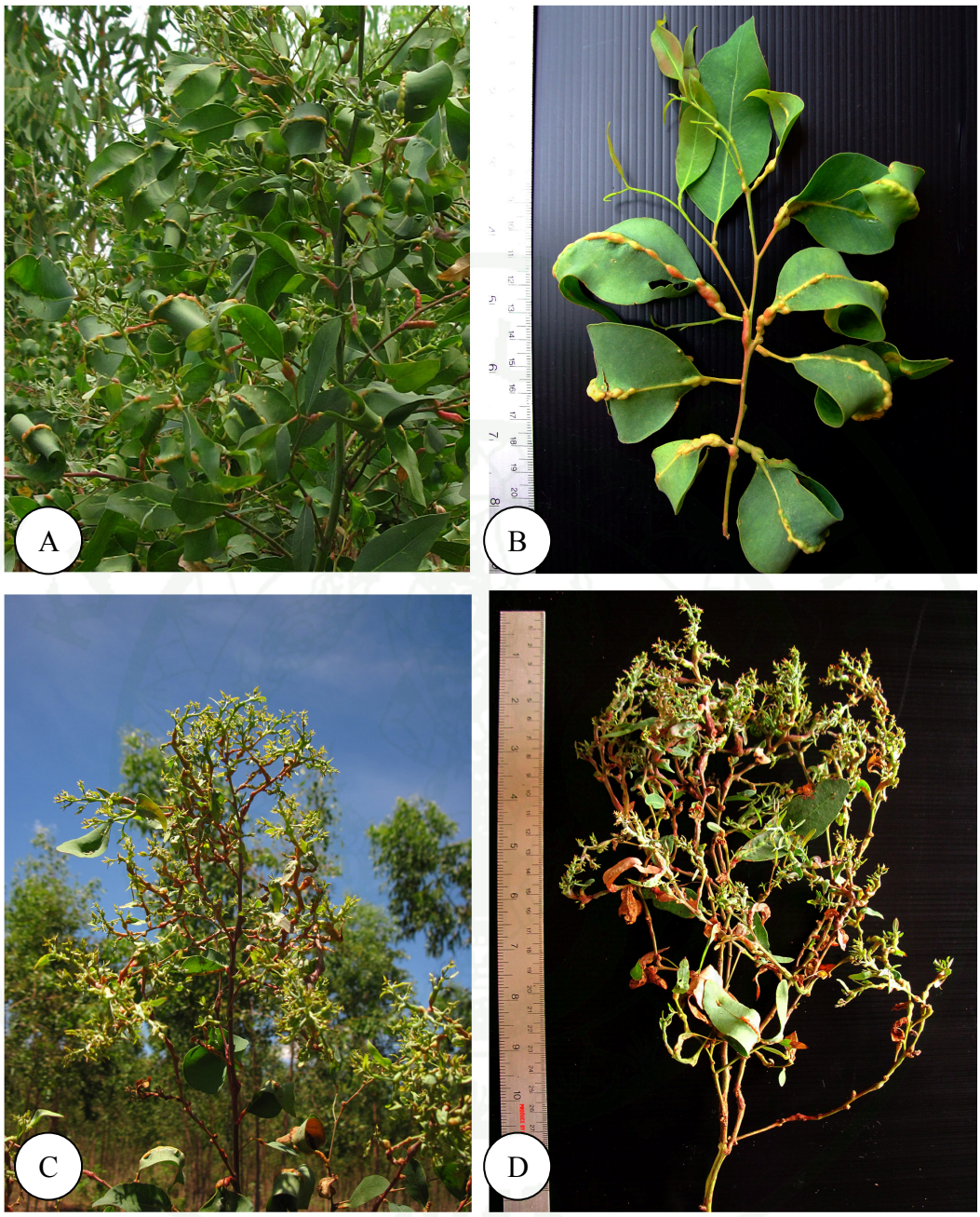

Figure 25 Damage to young coppice shoots of *Eucalyptus camaldulensis* by *Leptocybe invasa* in Tha Muang district: (A–B) general leaf gall induced by *L. invasa*; (C–E) heavy galling; and (F–H) over egg loadings in combination with some stress factors.

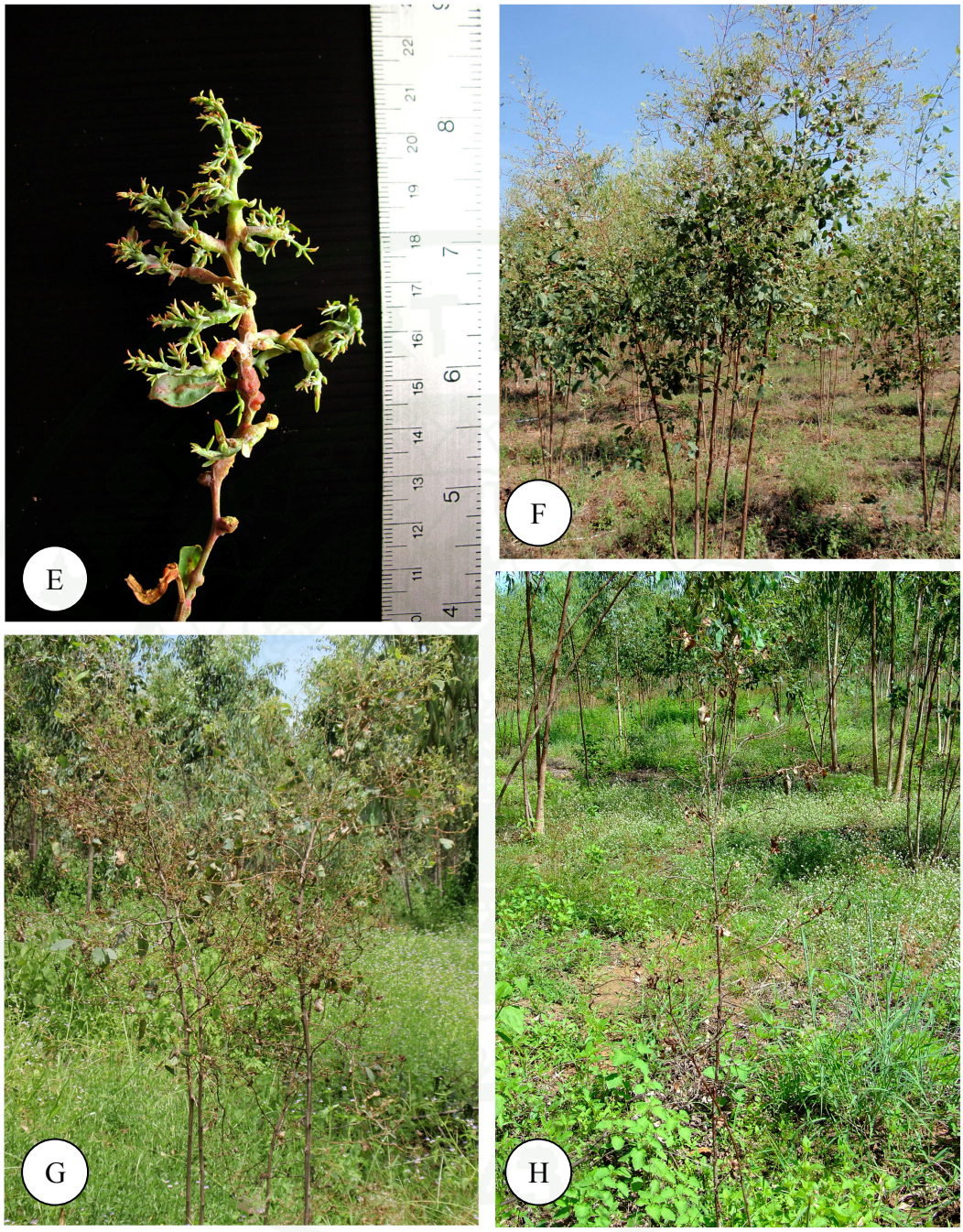

Figure 25 (Continued)

The average number of coppice shoots of *E. camaldulensis* in Tha Muang district was 3.89 coppice shoots/tree. Some coppice shoots grew very rapidly but some grew slowly. The coppice shoots which had opportunities or conditions to grow rapidly until their height reaching about 6 meters or over, were able to tolerate to *L. invasa* attack (Figure 26A and B). At this height, their young leaves and mature leaves were physiological mature. The slow growth of some coppice shoots remained being attacked by *L. invasa*.

Ohmart and Edwards (1991) reported in some tree species that variation in details of leaf structure and chemical and nutritional properties might associate with young leaves or mature leaves and proceeded to the susceptibility of galling.

The mature leaves of *E. camaldulensis* at about 6 meters height are narrowly lanceolate in shape, tapering and often curved. The upper surface is dark–green and the lower surface is gray–green. The leaf blade is leathery and hard (Figure 26C). The young and mature leaves at this height might change their internal structures, physiological ages and chemical properties when compared to young and mature leaves of small coppice shoots. Such changing of leaves might cause improper diets for *L. invasa*. Besides changing in leaf properties, the height of coppice shoots at 6 meters and over would expose to strong sunshine and wind. This also gave rise to the unfavorable habitats for *L. invasa*.

The findings from this study suggested that *E. camaldulensis* (CT 76 clone) was susceptible to *L. invasa*. The degrees of damage were varied and might concern with the following factors; egg loading of the female, height of coppice shoot, physiological age of leaves, chemical properties of leaves, environmental conditions of the site, and population dynamics of *L. invasa*.

In Tha Muang and Phanom Thuan districts, *E. camaldulensis* (CT 76 clone) in the plantations was seriously damage by *L. invasa*. Apart from this gall wasp attack, it was found that other insects could harm *E. camaldulensis* in some seasons. However the degrees of damage by those insects were lesser than those by *L. invasa*.

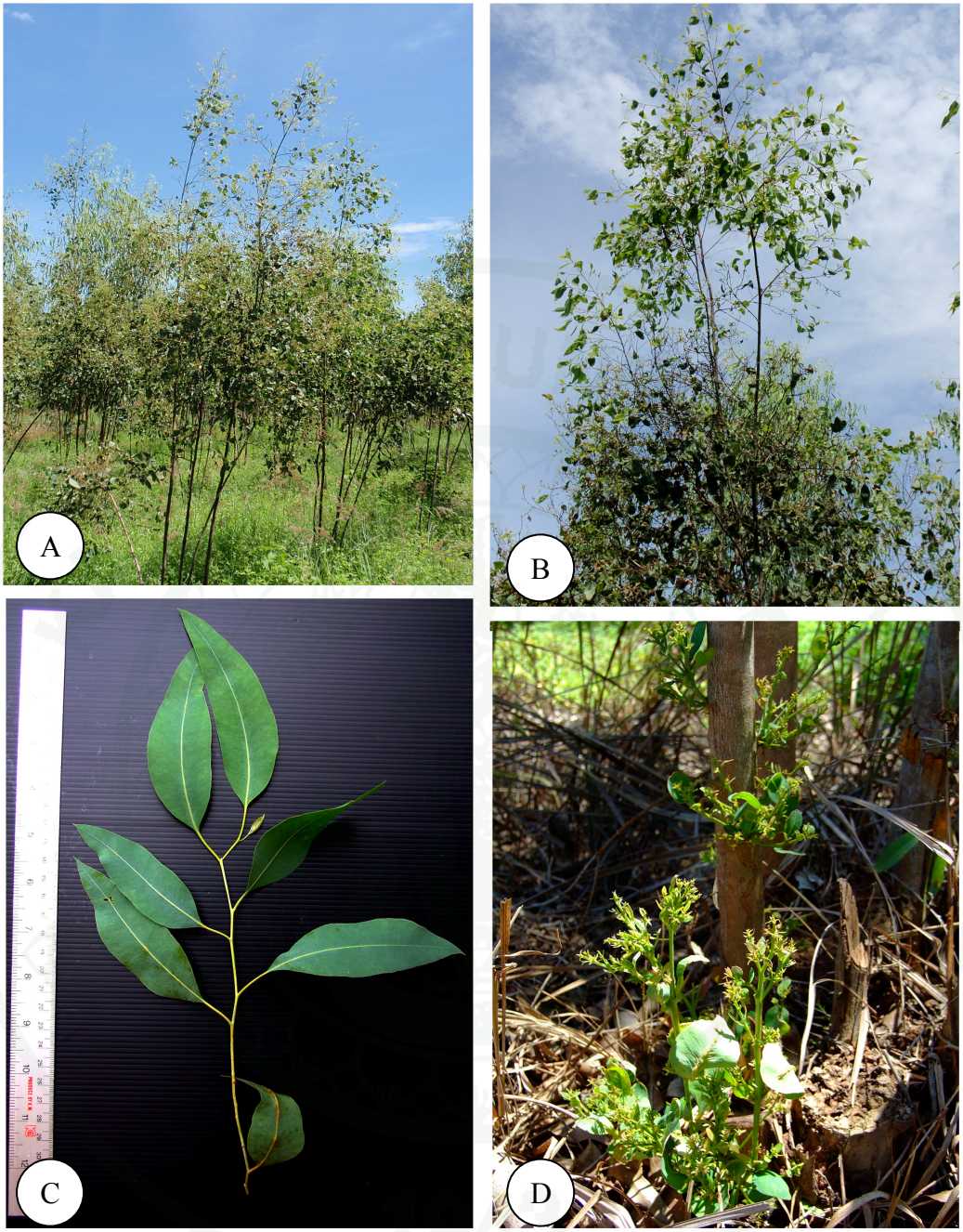

Figure 26 Coppice shoots at height about 6 meters and over of *Eucalyptus camaldulensis* in Tha Muang district: (A–B) tolerant coppice shoots at 6–7 m height; (C) mature leaves; and (D) susceptibility of juvenile shoots of over 6 m height coppices.

The insects found to damage the coppice shoots of *E. camaldulensis* in the study areas were as follows (Figure 27 and Figure 28):

- Leaf roller in Order Lepidoptera; between November and January.

- Leaf roller (*Strepsicrates semicanella* (Walker)) Family Tortricidae; between November and January.

- Leaf eating caterpillar (*Pataeta carbo* (Guenee)) Family Noctuidae; between November and January.

- Leaf eating caterpillar (*Ophiusa disjungens* (Walker)) Family Noctuidae; between November and January.

- Root eating beetle (*Lepidiota stigma* Fabricius) Family Scarabaeidae; or root eating by underground white grub; between May and June.

- Leaf eating weevil (*Sepiomus* sp.) Family Curculionidae; throughout the year.

- Leaf eating weevil (*Hypomeces squamosus* (Fabricius)) Family Curculionidae; throughout the year.

- Psyllid (sap sucker) Family Psyllidae; throughout the year.

- Planthopper (sap sucker) (*Ricania marginalis* Walker) Family Ricaniidae; throughout the year.

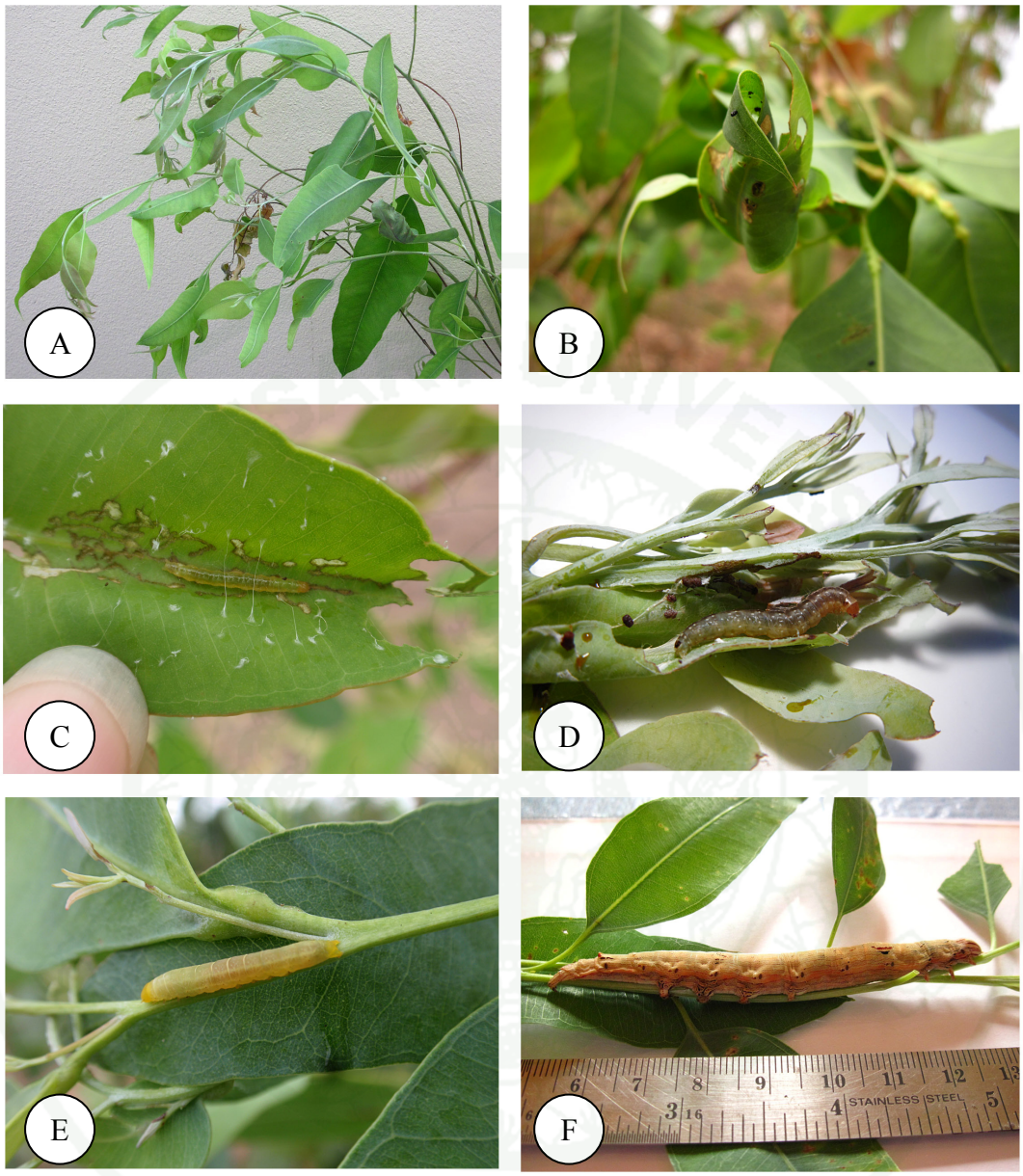

Figure 27 Damages to *Eucalyptus camaldulensis* coppice shoots by other insects: (A–B) leaf roller; (C–D) *Strepsicates semicarnella*; (E) *Pataeta carbo*; and (F) *Ophiusa disjungens*.

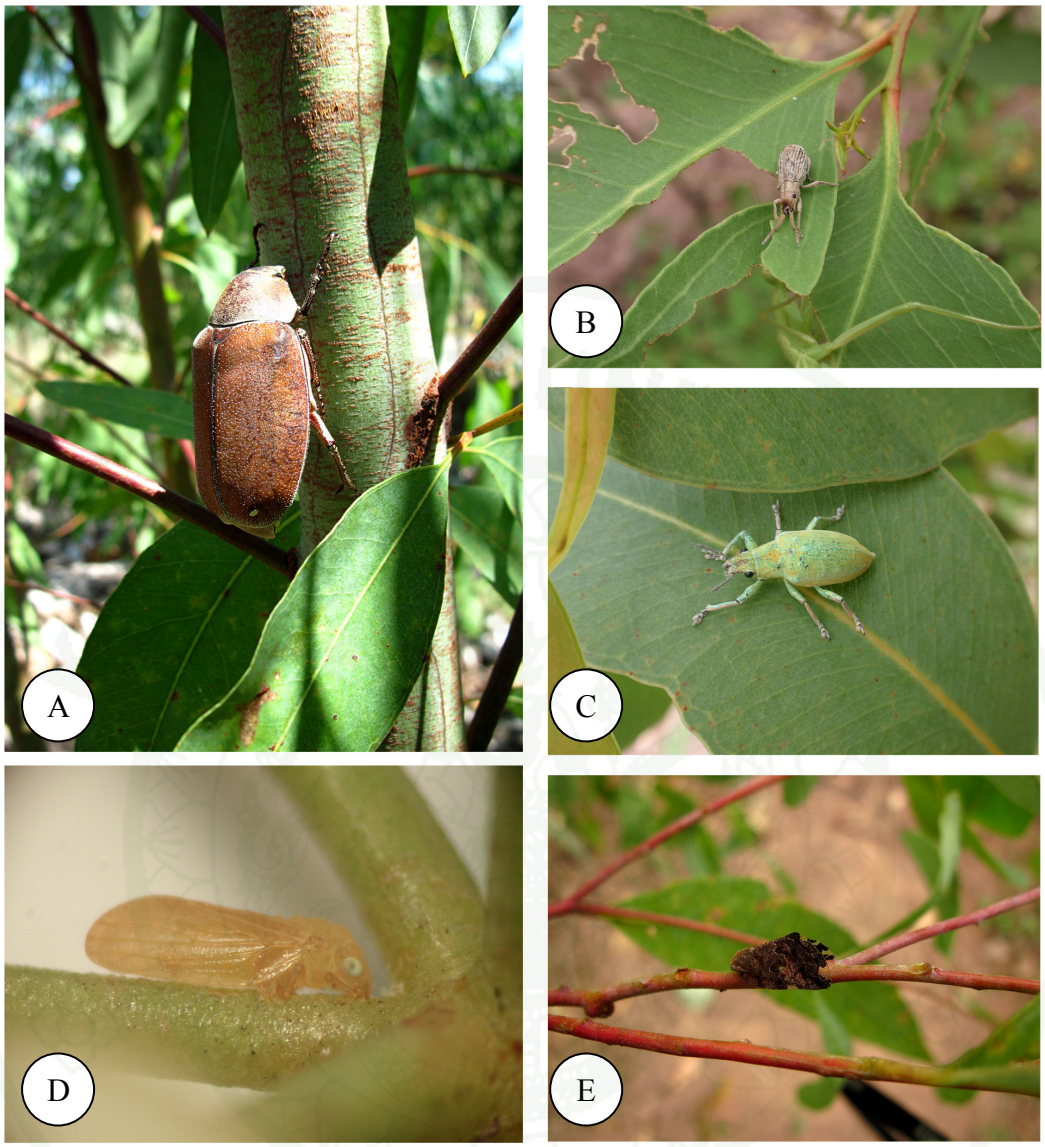

Figure 28 Damages to *Eucalyptus camaldulensis* coppice shoots by other insects: (A) *Lepidiota stigma*; (B) *Sepiomus* sp.; (C) *Hypomeces squamosus*; (D) psyllid (sap sucker); and (E) *Ricania marginalis*.

Biological Aspect of Parasitoids of *Leptocybe invasa* Fisher & La Salle

This research found two species of parasitoids in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts, Kanchanaburi province. They were *Aprostocetus* sp. and *Megastigmus* sp. which are the parasitoids of *L. invasa*. These two parasitoids, *Aprostocetus* sp. and *Megastigmus* sp. were found both female and male.

The genera *Aprostocetus* and *Megastigmus* were described by Dr. John La Salle in Australia (personal communication) but the identification of the species did not finish.

1. Morphology of adult *Aprostocetus* sp.and *Megastigmus* sp.

Aprostocetus sp. (Hymenoptera) is belonged to the family Eulophidae and *Megastigmus* sp. (Hymenoptera) to the family Torymidae.

1.1 Morphology of adult *Aprostocetus* sp.

Diagnosis of the genus *Aprostocetus* found in the Tha Muang and Phanom Thuan districts, was determined by relying on Hesami *et al.* (2010), who described as follows:

Female: *Antennal funicle*: all segments longer than broad. *Mesoscutum*: with or without median line, with one or two rows of adnotaular setae. *Scutellum*: normally with 2 pairs of setae, submedian lines usually distinct.

Male: *Antennal funicle*: with 4 segments; funicle and club with whorled long dark setae; scape with ventral plague.

The comprehensive morphology of female *Aprostocetus* sp. in Tha Muang and Phanom Thuan districts was described and shown in Figure 29.

General feature: small size, the length from head to abdomen tip (not included ovipositor) 1.21±0.02 mm, ranging from 1.05–1.30 mm; body, head and antenna orange brown; wings hyaline and vein light brown; legs yellow and the last tarsal segment brown; gaster orange brown with pale transverse stripes on gastral tergites; Ovipositor sheaths dark brown.

Head: in front view, head broader than height; face with frontal line as usual T-like; frons smooth; scrobes with median; antennal torulus located at middle of face, distinctly above the lower margin of eye; malar sulcus moderately curved; gena only slightly swollen; subtorular grooves present; clypeal margin strongly bidentate.

Antenna: with 3 anelli, 3 funicular segments, 3 club segments; scape slender, 2 times longer than pedicel, apex scape not exceeding median ocellus; pedicel slightly longer than the first funicular segment; all funicular segments longer than width.

Mesosoma: pronotum short; midlobe of mesoscutum with weak median line, with 6 pairs of adnotaular setae at lateral margin; notaulus quite deep; mesoscutum longer than scutellum, distinctly convex; scutellum with distinct submedian and sublateral lines, anterior pairs of setae located slightly posterior to middle, posterior pairs of setae located near posterior margin; frenum very narrow; propodeum without median carina; outer rim of propodeal spiracle partially covered by a raised lobe of callus; spiracular depression open to anterior margin of propodeum; propodeal callus with 2 setae.

Forewing: submarginal vein with 3–4 setae; postmarginal vein rudimentary; cubital line of setae extending all the way to basal vein, closing speculum; basal cell without setae; basal vein with 3 setae.

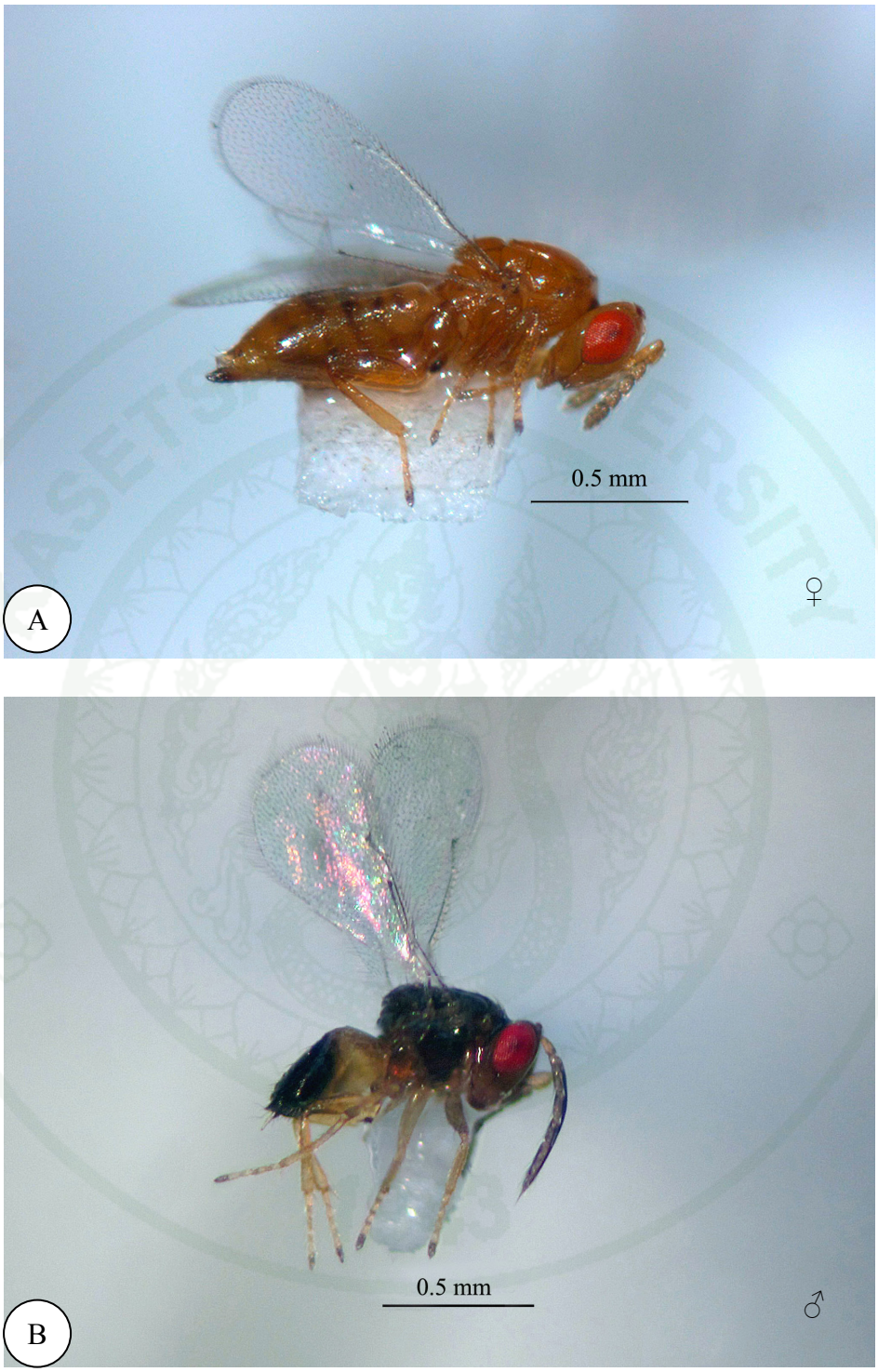

Figure 29 *Aprostocetus* sp. found in *Eucalyptus camaldulensis* plantations in Tha Muang and Phanom Thuan districts, Kanchanaburi province: (A) female; and (B) male.

Gaster: longer than the head plus mesosoma; hypopygium reaching the level of 4th gastral tergite; cercus with 3 setae, the longest one slightly curved; ovipositor sheaths slightly protruding, very short in dorsal view.

The comprehensive morphology of male *Aprostocetus* sp. in Tha Muang and Phanom Thuan districts was described and shown in Figure 29.

General feature: small size, the length from head to abdomen tip 1.01±0.03 mm, ranging from 0.80–1.20 mm; head brown; thorax and gaster dark brown with a large white to yellow area at the base of gaster; scape and pedicel yellow; flagellum brown; wings hyaline, vein light brown; legs yellow and the last tarsal segment brown.

Head: similar to female.

Antenna: with 3 anelli, 4 funicular segments, 3 club segments; scape 2 times longer than pedicel; the first funicular segment shorter than pedicel and other funicular segments; whorled setae of funicular and club very long, those on the first funicular segment reaching mid–length of the fourth one.

Mesosoma: pronotum short; mid–lobe of mesoscutum with weak median line; 4 pairs of adnotaular setae at the lateral margin; notauli quite deep; mesoscutum longer than scutellum, distinctly convex; scutellum with distinct submedian and sublateral lines, anterior pairs of setae located slightly posterior to middle, posterior pairs of setae located near posterior margin; frenum very narrow; propodeum without median carina; outer rim of propodeal spiracle partially covered by a raised lobe of callus; spiracular depression open to anterior margin of propodeum; propodeal callus with 2 setae.

Forewing: submarginal vein with 2–3 setae; postmarginal vein rudimentary; cubital line of setae extending to basal vein, closing speculum; basal cell without setae; basal vein with 3 setae.

Gaster: as long as the head plus mesosoma.

The female *Aprostocetus* sp. differed from the male in the characteristics that shown in Table 9 and Figure 30.

Table 9 Different characteristics between female and male *Aprostocetus* sp.

Characteristic	*Aprostocetus* sp.	
	Female	Male
Numbers of funicular segment of antenna	3	4
Whorled long seta of funicular and club	No	Yes
Numbers of adnotaular seta	6	4
Body color	Orange brown	Dark brown with white area at the base of gaster
Size	Larger	Smaller

Kulkarni *et al*. (2010) reported that *Aprostocetus gala* and *Aprostocetus* sp. were the parasitoids of *L. invasa* in India. They found both female and male, but they did not described the morphology of those two parasitoids in their report.

Moreover, there were no reports on the morphology of *Aprostocetus* spp. which associated with *L. invasa*. Thus the finding and description of *Aprostocetus* sp., both female and male, in these study areas were the new record of Thailand.

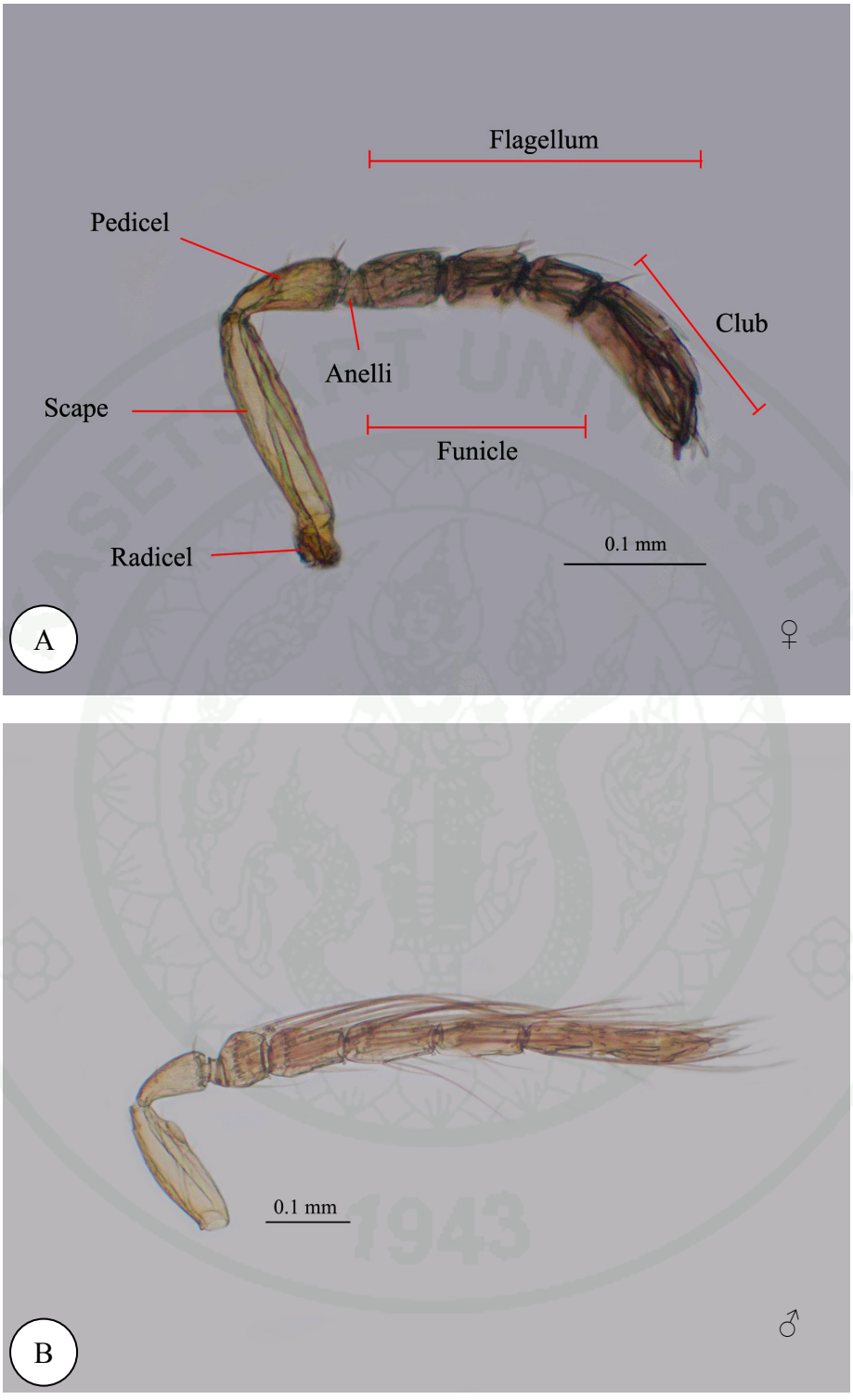

Figure 30 Some morphological distinctions between female and male *Aprostocetus* sp.: (A–B) female and male antennae; (C–D) similar forewings of female and male.

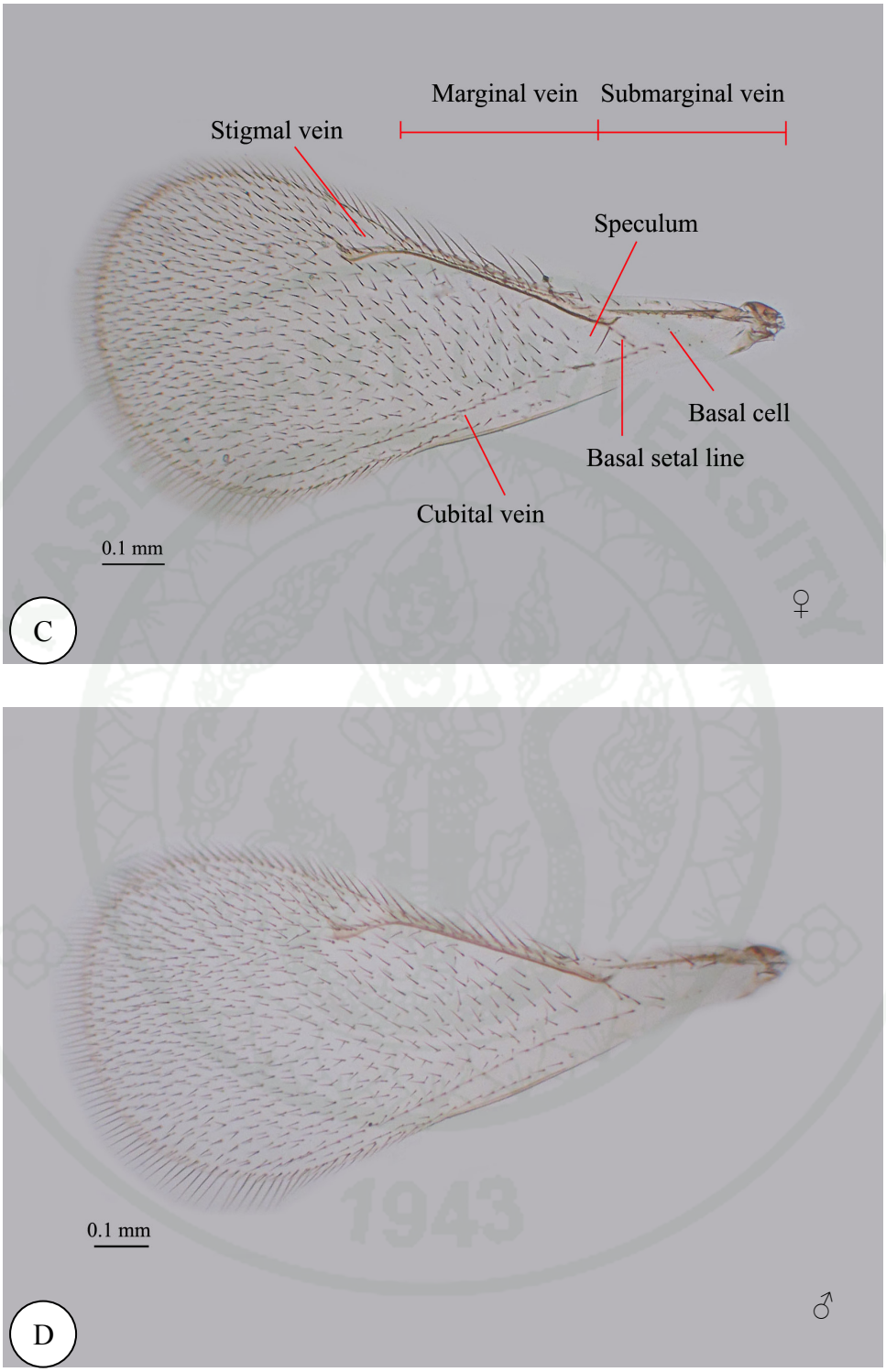

Figure 30 (Continued)

1.2 Morphology of adult *Megastigmus* sp.

Diagnosis of the genus *Megastigmus*, found in Tha Muang and Phanom Thuan districts, was determined by relying on Doğanlan and Hassan (2010) who described as follows:

Body: without any metallic color; lower clypeal margin with deep median incision, hence appearing 2–toothed. *Antenna*: scape in female not exceeding median ocellus, in male antenna normal, similar to that of female. *Thorax*: either rather shiny or, if sculptured, then at least on scutum with transverse striae, thorax pilosity usually conspicuous, and midlobe of mesonotum with one pairs of setae longitudinally along notauli; scutellum only rarely engraved–reticulate, usually with frenal cross–line. *Forewing*: rarely darkened at stigma, then vaguely delimited.

The comprehensive morphology of female *Megastigmus* sp. in Tha Muang and Phanom Thuan districts was described and shown in Figure 31.

General feature: small size, the length from head to abdomen tip (not included ovipositor) 1.33±0.02 mm, ranging from 1.10–1.80 mm in length; average length of ovipositor 0.79±0.05 mm, ranging from 0.60–1.00 mm; head and body brownish yellow except vertex around ocelli; antenna brown with scape and pedicel yellowish beneath; wings hyaline, veins and stigma brown; legs yellow; ovipositor sheaths black.

Head: in front view, head broader than height; vertex weakly strigate; ocellar area, frons and lower surface strigate–reticulate; head covered with sparse black setae; antennal scrobes deep and reaching median ocellus; antennal torulus inserted above level of ventral margin of eyes; malar sulcus distinct.

Antenna: slightly clavate with 1 anellus, 7 funicular segments, 3 club segments; scape cylindrical reaching level of vertex, with 2 times longer than pedicel; anellus slightly transverse to quadrate; flagellomere shorter than pedicel.

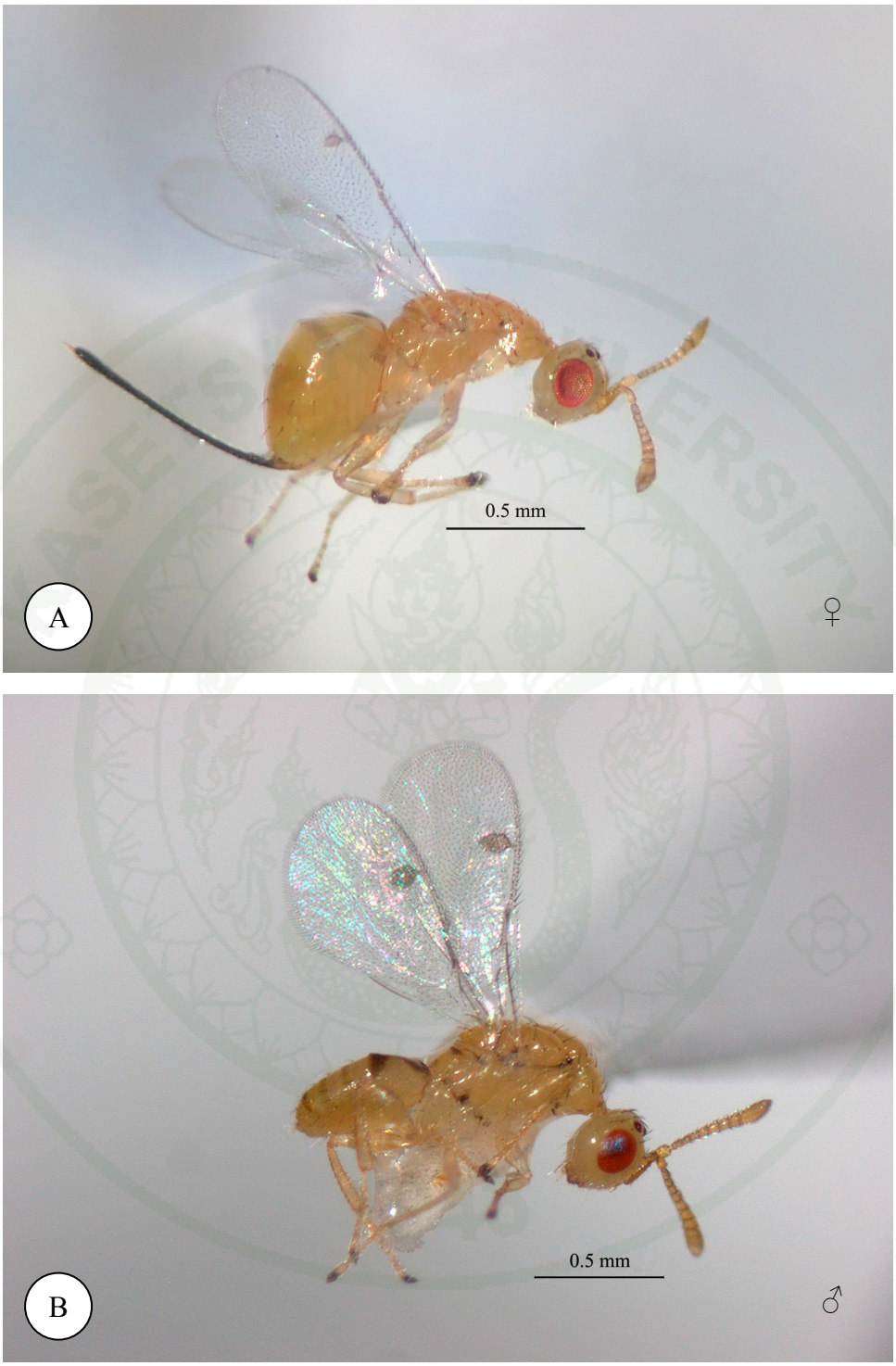

Figure 31 *Megastigmus* sp. found in *Eucalyptus camaldulensis* plantations in Tha muang and Phanom Thuan districts, Kanchanaburi province: (A) female; and (B) male.

Mesosoma: pronotum broad and large, strigate–reticulate, with 4 rows setae; mesoscutum slightly longer than pronotum; midlobe of mesoscutum without median line, with 4 setae at anterior margin and 2 pairs of adnotaular setae at lateral margin, scapulae reticulate; lateral lobe of mesoscutum with 4 setae; scutellum as long as broad, sculptured–like mesoscutum, without submedian lines, with 3 setae on each lateral side; frenum not indicated; propodeum with strongly raised reticulation and median carina present; propodeal callus with 7 setae; prepectus broad and triangular.

Forewing: submarginal vein with 6 setae; marginal vein slightly shorter than postmarginal vein; stigmal vein a little longer than half length of marginal vein; stigma conspicuous darkened, with uncus distinct; stigmal lobe narrow–oval shape; basal vein distinct; speculum rather large and closed below; cubital line complete.

Gaster: slightly longer than thorax; ovipositor sheaths weakly clavate, longer than abdomen, with setae along the length.

The comprehensive morphology of male *Megastigmus* sp. in Tha Muang and Phanom Thuan districts was described and shown in Figure 31.

General feature: small size, the length from head to abdomen tip 1.37±0.05 mm, ranging from 0.90–1.70 mm in length; head and thorax yellow except gaster; antenna pale yellow; notaulus, sublateral line and lateral of axilla dark brown; lateral panel of metanotum with dark brown strips; propodeum with dark brown markings (T–like shape); wings hyaline, veins and stigma brown; legs pale yellow, last tarsal segment brown; gaster with T1 to T5 more extensively dark brown strips.

Head: in front view, head broader than height; vertex weakly strigate; ocellar area, frons and lower face strigate–reticulate; head covered with sparse black setae; antennal scrobes deep and reaching median ocellus; antenna torulus inserted above level of ventral margin of eye; malar sulcus distinct.

Antenna: slightly clavate, with 1 anellus, 7 funicular segments, 3 club segments; scape reaching level of vertex, with 2 times longer than pedicel; pedicel rather rounded; anellus slightly transverse to quadrate; flagellomere shorter than pedicel.

Mesosoma: pronotum broad and large, strigate–reticulate, with 4 rows of setae; mesoscutum longer than pronotum; midlobe of mesoscutum without median line, with 4 long setae at anterior margin and 4 pairs of adnotaular setae at lateral margin, scapulae reticulate; lateral lobe of mesoscutum with 4 long setae; scutellum as long as broad, sculpture–like mesoscutum, without submedian lines, with 3 setae on each lateral side; frenum not indicated; propodeum with strongly raised reticulation, median carina absent; propodeal callus with 7 setae; prepectus broad and triangular.

Forewing: submarginal vein with 6 setae; marginal vein slightly shorter than postmarginal vein; stigmal vein longer than half length of marginal vein; stigma conspicuous darkened, with uncus distinct; stigmal lobe oval; basal vein distinct; speculum rather large, closed below; cubital line complete.

Gaster: shorter than head plus mesosoma.

The female *Megastigmus* sp. differed from the male in the characteristics that shown in Table 10 and Figure 32.

Table 10 Different characteristics between female and male *Megastigmus* sp.

Characteristic	*Megastigmus* sp.	
	Female	Male
Shape of stigma on forewing	Narrow oval	Oval
Numbers of adnotaular seta at midlobe of mesoscutum	Two pairs	Four pairs
Ovipositor	Very long	Absent
Body color	Brownish yellow	Yellow

Doğanlan and Hassan (2010) reported that it was found five species of *Megastigmus* which were parasitoids of *Leptocybe invasa*; namely *Megastigmus judikingae*, *M. leptocybus*, *M. thailandiensis*, *M. thitipornae* and *M. zvimendeli*. Two species of *Megastigmus*, *Megastigmus thailandiensis* and *M. thitipornae* are the new species of Thailand.

However, the detailed comparisons on morphology between *Megastigmus* sp. found from this research in Tha Muang and Phanom Thuan district, Kanchanaburi province, and *M. thailandiensis* and *M. thitipornae*, showed that there were differences in some characters:

a) female *Megastigmus* sp. in this research was close to female *M. thailandiensis* and *M. thitipornae*, but the shapes of antenna and stigma of forewing of this *Megastigmus* sp. differed from those of *M. thailandiensis*.

For this *Megastigmus* sp., the shape of antenna was quadrangular and short, and the shape of stigma of forewing was oval and narrow. For *M. thailandiensis*, the shape of antenna was elongate and long, and the shape of stigma of forewing was oval and broad.

b) the color markings on body of the *Magestigmus* sp. found in this research differed from that of *M. thitipornae*.

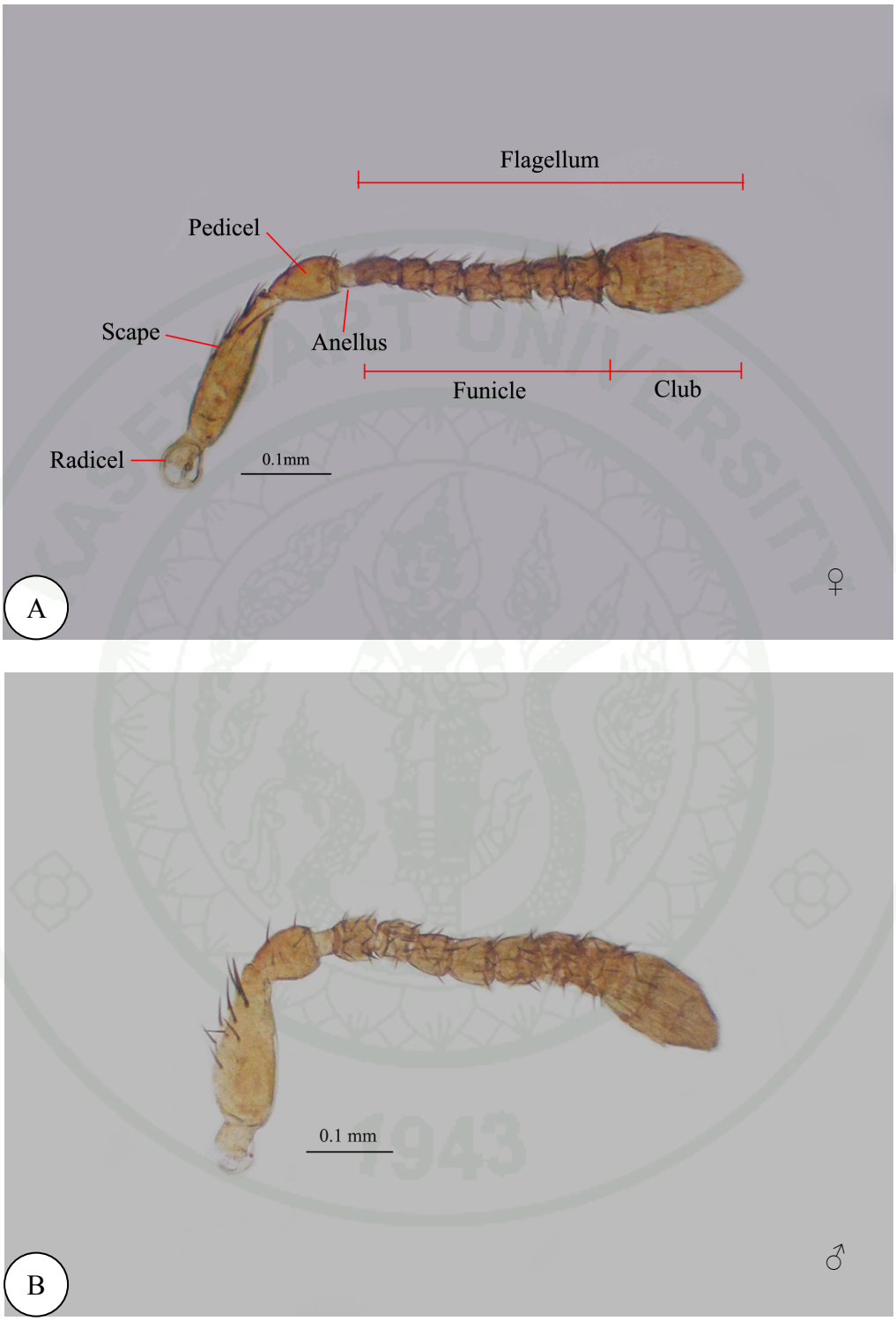

Figure 32 Some morphological distinctions between female and male *Megastigmus* sp.: (A–B) similar antennae of female and male; (C–D) female and male forewings.

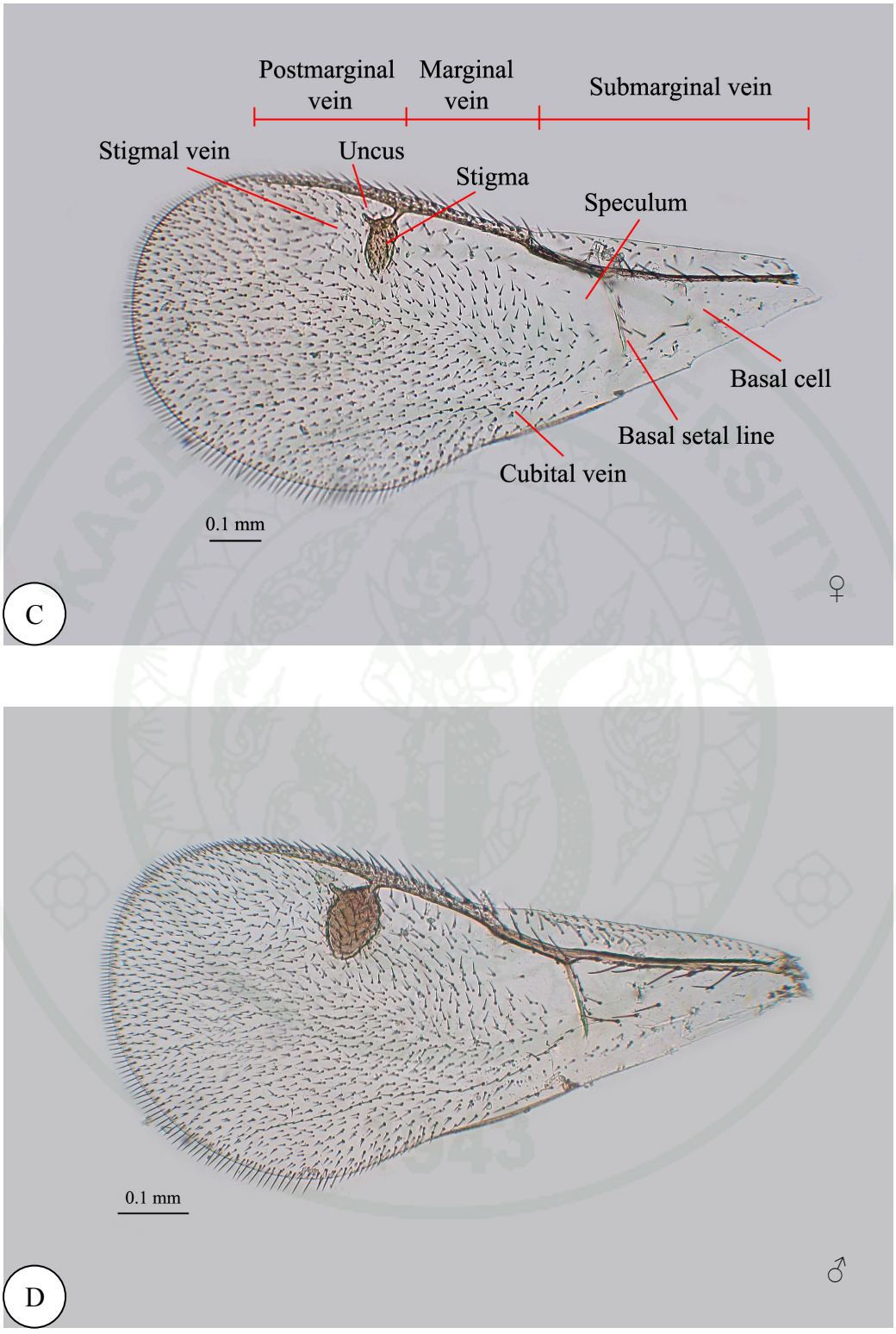

Figure 32 (Continued)

For *Megastigmus* sp., the body had no color marking. But for *M. thitipornae*, the brown markings were found on anterior base and sides of axillae, metasoma (except dorsellum), median area sutures of thorax, and on base of metasoma.

This findings suggested that *Megastigmus* sp. found in Tha Muang and Phanom Thuan districts was rather different from *M. thailandiensis* and *M. thitipornae* which are the new species of Thailand.

2. Longevity of *Aprostocetus* sp. and *Megastigmus* sp.

2.1 Longevity of *Aprostocetus* sp.

2.1.1 Mean longevity of adult female and male *Aprostocetus* sp.

The longevity of *Aprostocetus* sp. refers to the period from adult to its expiration. Longevity of parasitoid is one of three key parameters for basic requirements of the successfully biological control (Grabenweger *et al*., 2009). Three basic knowledges for the successful biocontrol are the data of longevity, fecundity and development of the parasitoid. Longevity study of parasitoid will provide information how to extend its longevity for mass rearing in the laboratory. Finally, the mass production of parasitoid from the laboratory will be introduced to control *L. invasa* in *E. camaldulensis* plantations.

In the laboratory, it was found from this research that the mean longevity of female and male *Aprostocetus* sp. were long. The mean longevity of female was longer than that of male. The mean longevity of each sex could be divided into two groups. The application of 6 different diets on the longevity study of *Aprostocetus* sp. showed that honey solution and honey solution+*E. camaldulensis* flowers could prolong the large mean longevities; 18.67 and 19.33 days for females, and 13.33 and 15.50 days for male, respectively (Table 11).

To feed with no–diet, water, water+flower, and flowers of *E. camaldulensis* disclosed that the mean longevities of females were 1.00, 1.17, 1.67 and 1.33 days, and those of males were 1.00, 1.50, 1.83 and 1.17 days, respectively (Table 11).

Table 11 Mean longevities (days) of *Aprostocetus* sp. fed with different diets.

Diet	Female	Male
No–diet	1.00±0.00 a	1.00±0.00 a
Water	1.17±0.17 a	1.50±0.22 a
Water+flowers	1.67±0.21 a	1.83±1.40 a
Honey solution	18.67±1.93 b	13.33±1.75 b
Honey solution+flowers	19.33±2.03 b	15.50±1.48 b
Flowers	1.33±0.21 a	1.17±0.17 a

Mean±S.E.

Female: F=63.270; df =5; P–value=0.000

Male: F=50.386; df =5; P–value=0.000

Means followed by the same letter within each column were not significantly different (Tukey's HSD, P=0.05)

The statistical analysis of the means of female longevity and the means of male longevity by F–test indicated that both P–values=0.000 were lower than P=0.05, thus different diets had significant effects on the means of longevity of both female and male *Aprostocetus* sp.

To feed with honey solution and honey solution+flowers of *E. camaldulensis* were not statistically different, so the prolongation of longevity of female and male *Aprostocetus* sp. by feeding with honey solution alone were more practical and more convenient than feeding with honey solution+flowers.

There was no report on longevity of female and male *Aprostocetus* sp. fed with different diets in the laboratory. Thus the findings from this research were the first time and documented. These also were the new record of Thailand.

To compare the mean longevities of female and male *Aprostocetus* sp. with that of female and male *L. invasa* in the laboratory of this research, the results showed that the mean longevities of *Aprostocetus* sp. fed with honey solution were 18.67 days for females and 13.33 days for males (Table 11) but the mean longevities of *L. invasa* fed with honey solution were 7.67 days for females and 5.67 days for males.

The mean longevities of female and male *Aprostocetus* sp. which supplied with honey solution prolonged twice as much as *L. invasa*. The more prolonged longevities of *Aprostocetus* sp. suggested that this parasitoid could be potentially used in *L. invasa* control.

However, the results from this study were carried out in normal laboratory conditions. The future study should be focused on the optimum temperature for mass rearing of *Aprostocetus* sp.

2.1.2 Survival patterns of adult female and male *Aprostocetus* sp.

Survival pattern denoted the percent of survivors in each day within the range of longevity when fed with a diet.

The results showed that the survival patterns of female and male *Aprostocetus* sp. were able to divided into two groups of survivors. The longest survivals were among the females that were fed with honey solution and honey solution+flowers, ranging from 1 to 26 days and 1 to 25 days. Estimated 50% female survival period were 12 and 9 days, respectively (Figure 33).

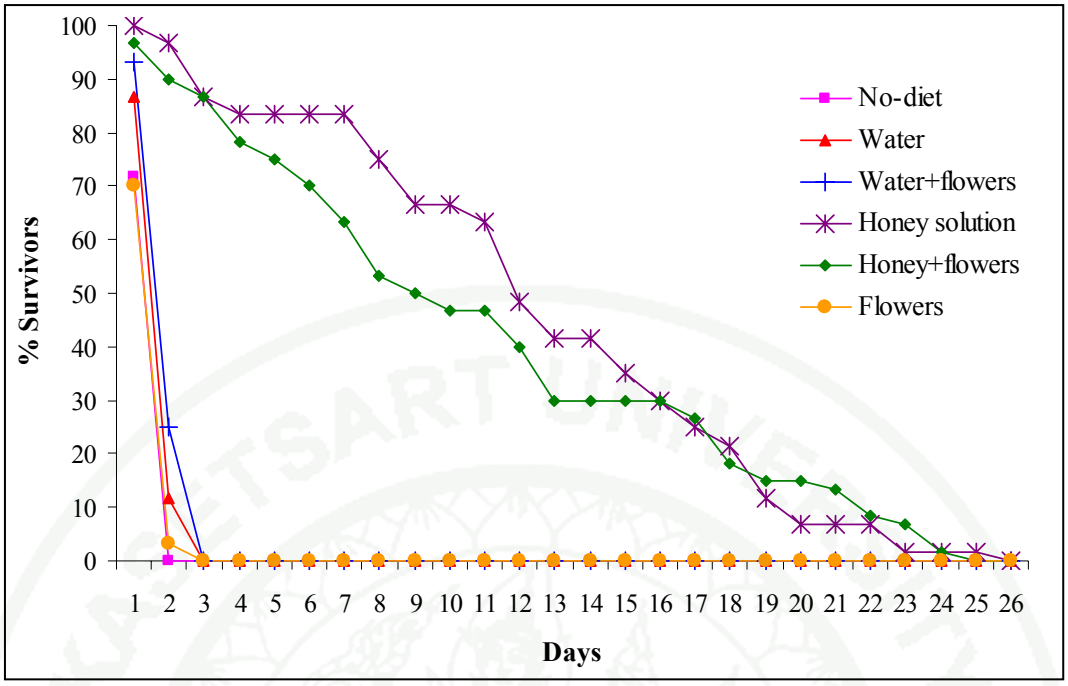

Figure 33 Survival patterns of female *Aprostocetus* sp. fed with different diets.

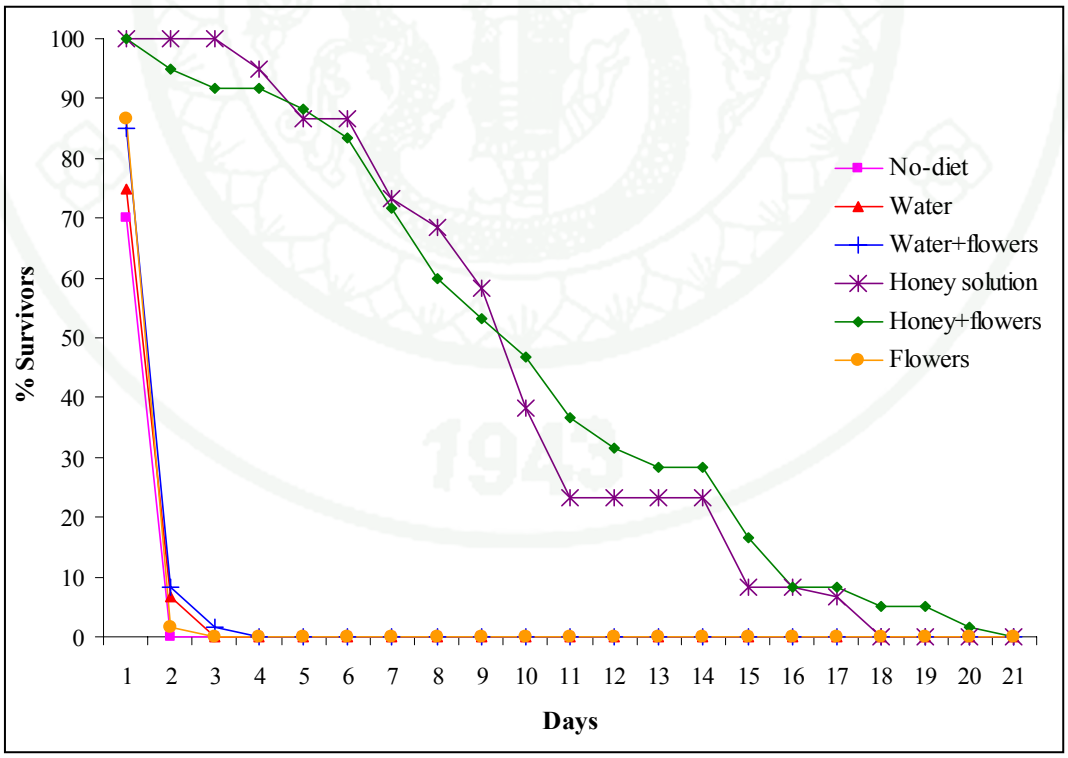

Figure 34 Survival patterns of male *Aprostocetus* sp. fed with different diets.

The short survivals were among the females that were supplied with no–diet, water, water+flowers, and flowers, ranging from 1 to 3 days. Estimated 50% female survival period was approximate 2 days (Figure 33).

For the male *Aprostocetus* sp., the longest survivals were among the males that were fed with honey solution and honey solution+flowers, ranging from 1 to 18 days and 1 to 21 days respectively. Estimated 50% male survival period was 9 days. To fed with other diets, the longevity of male *Aprostocetus* sp. ranged from 1 to 3 days and estimated 50% male survival period was only 1 days (Figure 34).

To compare survival patterns of female and male *Aprostocetus* sp. with those of female and male *L. invasa* supplied with honey solution, the results showed that estimated 50% survival periods of *Aprostocetus* sp. were 12 days for females and 9 days for males; while those of *L. invasa* were only 5 days for females and 4 days for males.

The finding from this study suggested that estimated 50% survival periods of female and male *Aprostocetus* sp. were twice longer than those of female and male *L. invasa*. These findings suggested that *Aprostocetus* sp. could be potentially used in *L. invasa* control.

2.2 Longevity of *Megastigmus* sp.

2.2.1 Mean longevity of adult female and male *Megastigmus* sp.

In the laboratory, the results showed that the mean longevity of female and male *Megastigmus* sp. were moderate. The mean longevity of female was longer than that of male. The mean longevity of each sex could be divided into two groups. The application of 6 different diets on the longevity study of *Megastigmus* sp. showed that honey solution and honey solution+flowers could

prolong the large mean longevity; 9.83 and 9.17 days for females, and 7.83 and 8.00 days for males (Table 12).

To feed with no–diet, water, water+flowers, and flowers alone disclosed that the mean longevities of females were 1.00, 1.17, 1.33 and 1.00 days and those of males were 1.00, 1.00, 1.17 and 1.00 days, respectively (Table 12).

Table 12 Mean longevities (days) of *Megastigmus* sp. fed with different diets.

Diet	Female	Male
No–diet	1.00±0.00 a	1.00±0.00 a
Water	1.17±0.17 a	1.00±0.00 a
Water+flowers	1.33±0.21 a	1.17±0.17 a
Honey solution	9.83±0.60 b	7.83±0.48 b
Honey solution+flowers	9.17±0.48 b	8.00±1.58 b
Flowers	1.00±0.00 a	1.00±0.00 a

Mean±S.E.
Female: F=170.294; df =5; P–value=0.000
Male: F=128.491; df =5; P–value=0.000
Means followed by the same letter within each column were not significantly different (Tukey's HSD, P=0.05)

The statistical analysis of the means of female longevity and the means of male longevity by F–test indicated that both P–values=0.000 were lower than P=0.05, thus different diets had significant effects on the means of longevity of both female and male *Megastigmus* sp.

To feed with honey solution and honey solution+flowers of *E. camaldulensis* were not statistically different, so the prolongation of longevity of female and male *Megastigmus* sp. by feeding with honey solution alone were more practical and more convenient than feeding with honey solution+flowers.

Protasov *et al*. (2008) studied on the longevities of female and male *Megastigmus* sp. which found in Israel by feeding with 8 different diets: no–diet, water, honey solution, honey solution+young leaves, honey solution+galled leaves, young leaves, galled leaves, and flowers. They reported that the mean longevities of female and male *Megastigmus* sp.-I fed with honey solution, honey solution+young leaves, and honey solution+galled leaves, were longer than those fed with no–diet, water, young leaves, galled leaves, and flowers of *E. camaldulensis* alone. They stressed that flowers of *E. camaldulensis* could not extend the mean longevities of female and male *Megastigmus* sp.

The findings from this research that honey solution could prolong the largest mean longevity of female and male *Megastigmus* sp. were in line with those found by Protasov *et al*. (2008).

To compare the mean longevities of female and male *Megastigmus* sp. with those of female and male *L. invasa* in this research, it was found that the mean longevities of *Megastigmus* sp. fed with honey solution were 9.83 days for females and 7.83 days for males (Table 11) but the mean longevities of *L. invasa* fed with honey solution were 7.67 days for females and 5.67 days for males.

The mean longevities of female and male *Megastigmus* sp., which supplied with honey solution, did not differ much from those of female and male *L. invasa*. These findings suggested that *Megastigmus* sp. might not be a potential parasitoid to control *L. invasa*.

2.2.2 Survival patterns of adult female and male *Megastigmus* sp.

It was found that the females *Megastigmus* sp. were able to divided into two groups of survivors. The longest survivals were among the females that were fed with honey solution and honey solution+flowers, ranging from 1 to 13 days and 1 to 12 days. Estimated 50% female survival period were 8 and 6 days respectively (Figure 35).

The short survivals were among the females that were supplied with no–diet, water, water+flowers, and flowers, ranging from 1 to 3 days. Estimated 50% female survival period was only 1 days (Figure 35).

For the male *Megastigmus* sp., the longest survivals were among the males that were fed with honey solution and honey solution+flowers, ranging from 1 to 10 days and 1 to 11 days respectively. Estimated 50% male survival period was 4 days. To fed with other diets, the longevity of male *Megastigmus* sp. ranged from 1 to 3 days and estimated 50% male survival period was only 1 days (Figure 36).

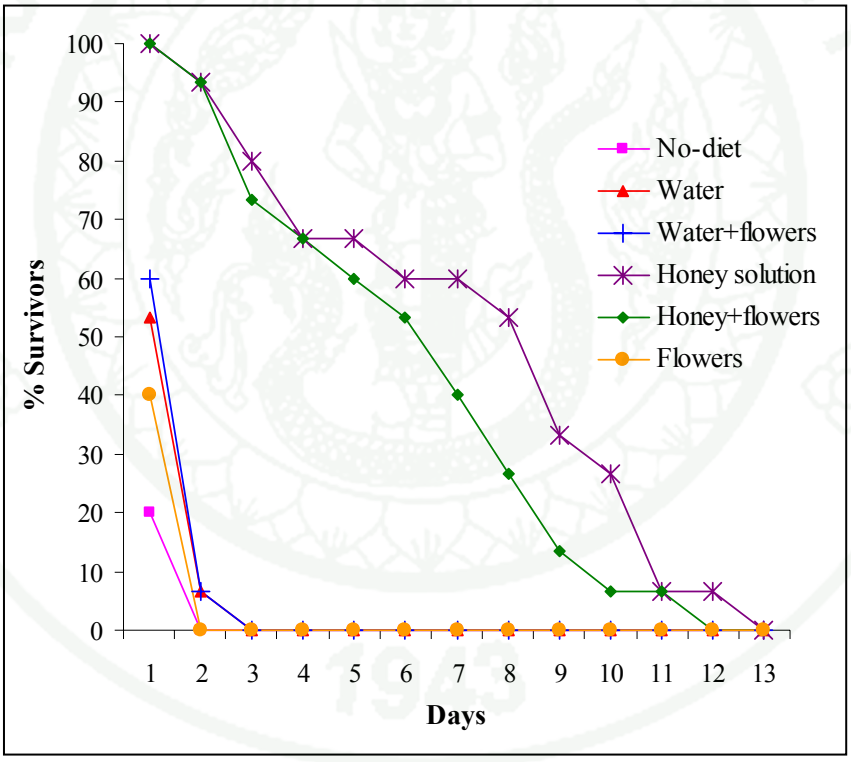

Figure 35 Survival patterns of female *Megastigmus* sp. fed with different diets.

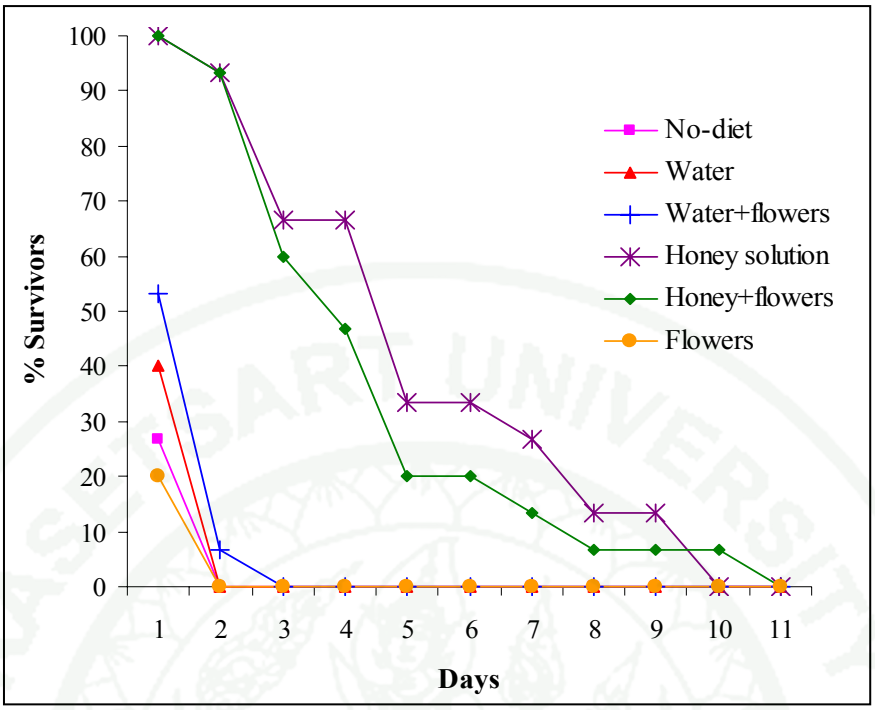

Figure 36 Survival patterns of male *Megastigmus* sp. fed with different diets.

To compare survival patterns of female and male *Megastigmus* sp. with those of female and male *L. invasa* supplied with honey solution, the results showed that estimated 50% survival periods of *Megastigmus* sp. were 8 days for females and 4 days for males; while those of *L. invasa* were only 5 days for females and 4 days for males.

The findings from this study suggested that estimated 50% survival periods of female and male *Megastigmus* sp.did not greatly differ from those of female and male *L. invasa*. Thus, only *Megastigmus* sp. could not be potentially used in *L. invasa* control.

In principles, the parasitoids (*Aprostocetus* sp. and *Megastigmus* sp.) should be live–longer than the host (*L. invasa*). The longer a male parasitoid could live, the more female parasitoids he could inseminate, and therefore the more eggs he could fertilize. On the other hands, the longer a female parasitoid

could live, the more eggs she would lay. So the population of parasitoid would increase faster than that of the host (Jervis, 2005; Grabenweger *et al*., 2009).

3. Fecundity of *Aprostocetus* sp. and *Megastigmus* sp.

3.1 Fecundity of *Aprostocetus* sp.

3.1.1 Potential fecundity of *Aprostocetus* sp.

The potential fecundity refers the maximum number of eggs in ovary that can potentially be laid by an adult female *Aprostocetus* sp. It can be counted directly in the ovary of adult female by dissection.

In the laboratory, all eggs in ovaries of newly emerging female *Aprostocetus* sp. were immature. The potential fecundity in a pair of ovaries of a female was shown in Figure 37. The results showed that dissection of the ovaries of 12 hrs–old female *Aprostocetus* sp. could not observed the mature eggs. Some eggs in ovaries were mature when the females were 1 day–old after emergence. The average potential fecundity of the females, of all sizes and ages, was 6.31±0.23 eggs per female, ranging from 2 to 11 eggs per female.

The potential fecundity of female *Aprostocetus* sp. was investigated for the first time and the findings from this research were new and were documented. To compare *Aprostocetus* sp. with *L. invasa*, it was found that the average potential fecundity of the female *Aprostocetus* sp. (6.31 eggs per female, ranging from 2 to 11 eggs per female) was much lower than that of the female *L. invasa* (158.70 eggs per female, ranging from 39 to 298 eggs per female).

To use hind tibia length as a substitute for female size, it was found that the average of all female sizes of *Aprostocetus* sp. was 0.26±0.003 mm, ranging from 0.20 to 0.30 mm.

Figure 37 Eggs in a pair of ovaries of female *Aprostocetus* sp. after emergence: (A) immature eggs of 12 hrs–old; and (B) mature eggs of 1 day–old.

The effects of female sizes of *Aprostocetus* sp. of all ages on mean egg loads was determined by ANOVA regression analysis. The results showed that sizes had significant effects on mean egg loads at P=0.05 (F=29.345, P–value= 0.000). There was significantly positive relationship between sizes and egg loads of female *Aprostocetus* sp. (Y=43.589x – 5.133; R^2=0.250; n=90). The larger sizes of female tended to produced more eggs than the smaller size (Figure 38). The R^2=0.252 indicated that the sizes influenced on egg loads at about 25.00%. The other variables probably involved and had influence on egg loads.

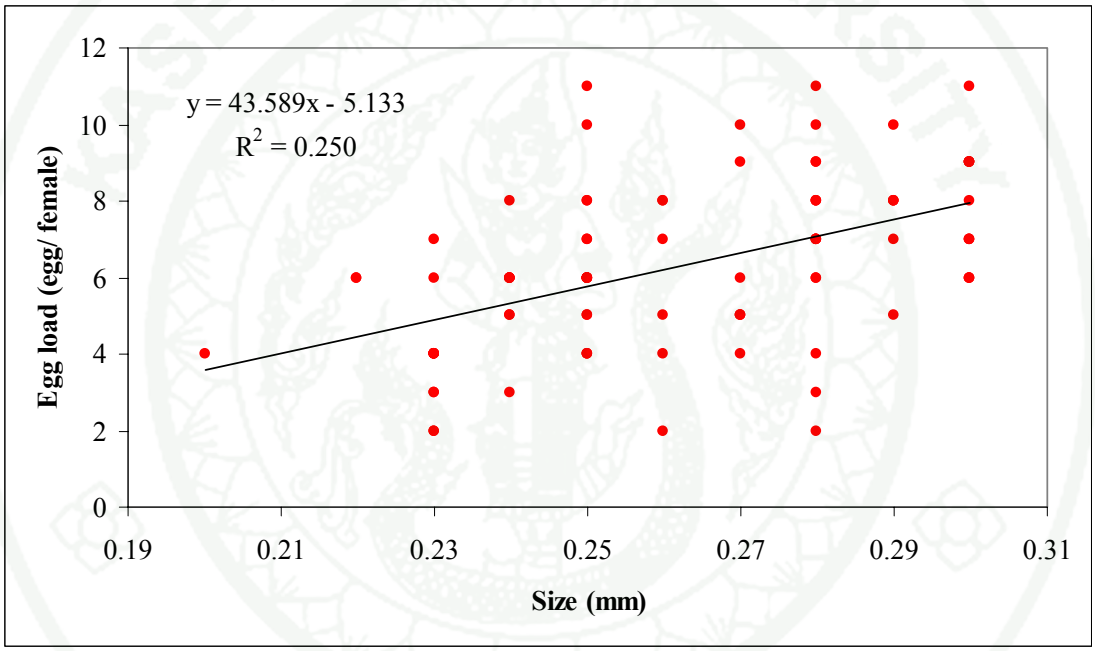

Figure 38 Relationship between size and egg load of female *Aprostocetus* sp.

It was noticed that R^2 or coefficient of determination of this equation was low. This probably arised from the reasons that a) *Aprostocetus* sp. was a synovigenic species, or b) the sample size (n=90) was probably not large enough for this species to be tested in this experiment. However, this was a constraint because the population of *Aprostocetus* sp. in *E. camaldulensis* plantations in Tha Muang and Phanom Thaun districts was naturally small.

The effects of female ages (days after eclosion) on egg loads of *Aprostocetus* sp. were shown in Figure 39. By one–way analysis of variance, the result showed that mean egg loads in different ages were different, but not significantly at P=0.05 (F=1.390; df =5; P–value=0.236).

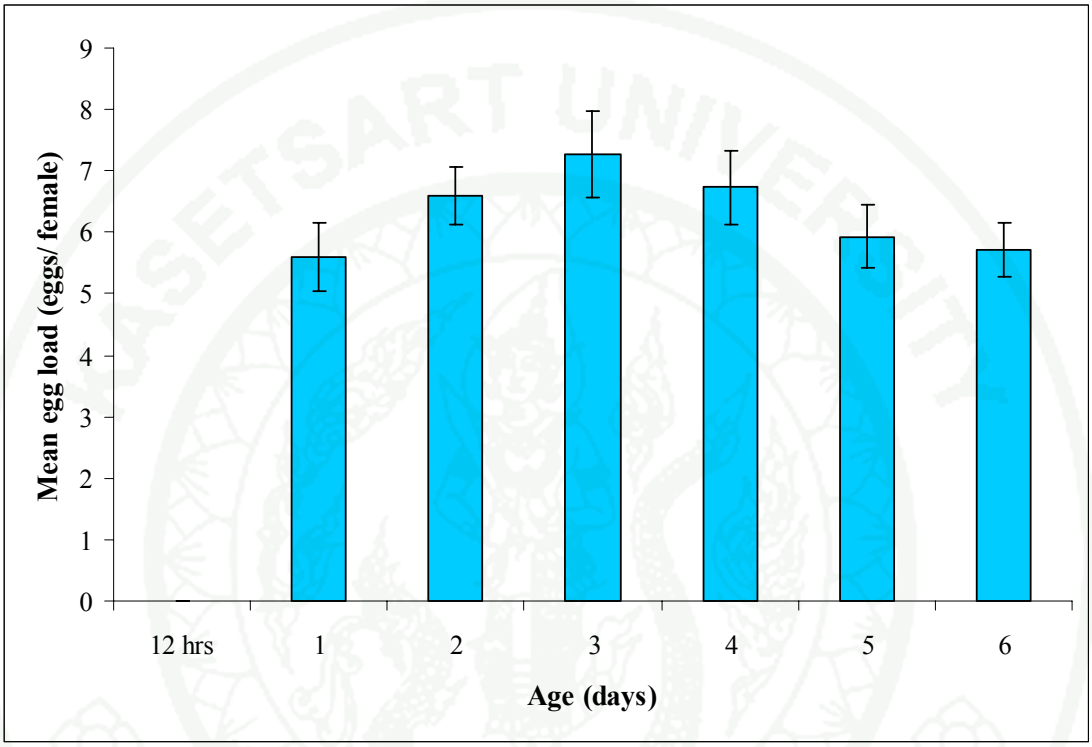

Figure 39 Mean egg loads in different ages of female *Aprostocetus* sp. The vertical bars indicate standard errors.

Different ages of *Aprostocetus* sp. had no significant effect on egg loads. This was consistent with Bernado *et al.* (2005) who studied on the potential fecundity of *Thripobius semiluteus* Bouček (Hymenoptera: Eulophidae).

3.1.2 Realized fecundity of *Aprostocetus* sp.

Realized fecundity refers to the total number of eggs actually laid over the life–time of a female *Aprostocetus* sp. Practically, realized fecundity is determined by counting the real number of eggs laid on host per day until a female

died, and then calculates the total number of eggs laid on host over the life–time of a female.

The eggs of *L. invasa* were in leaf gall of *E. camaldulensis*, so the eggs of *Aprostocetus* sp. laid on host in each day were difficult to detect. This research determined the realized fecundity of *Aprostocetus* sp. by counting the total number of emerging progenies (adult offsprings) over the life–time of a female and expressed in terms of progenies per a female.

In ventilated greenhouse, all eggs in ovaries of newly emerging female *Aprostocetus* sp. were immature. Some eggs in ovaries were mature when the females were 1 day–old after emergence, but continued to mature in the next days. The results showed that the female *Aprostocetus* sp. started to oviposit on the first day after emergence and lasted on the eighteenth day. The mean progeny/female gradually increased and was maximum on the ninth day with 9.60±1.24 progenies per female, and then gradually declined in the later days (Figure 40).

It was found that the mean of realized fecundity of a female from the first day to the eighteenth day was 51.10±3.28 progenies per a female, ranging from 38 to 65 progenies per female.

To compare *Aprostocetus* sp. with *L. invasa*, it was found as follows. For the female *Aprostocetus* sp., a) they oviposited on the first day after emergence and lasted on the eighteenth day, b) the mean progeny/female was maximum on the ninth day with 9.60±1.24 progenies per female, and c) the mean of realized fecundity of a female from the first day to the eighteenth day was 51.10±3.28 progenies per a female, ranging from 38 to 65 progenies per female. For the female *L. invasa*, a) they oviposited on the first day after emergence and lasted on the sixth day only, b) the mean progeny/female was maximum on the first day with 30.47±7.41 progenies per female, and c) the mean of realized fecundity of a female from the first day to the sixth day was 61.53±8.94 progenies per a female, ranging from 18 to 130

progenies/female. These comparisons suggested that *L. invasa* was advantageous in the first period but *Aprostocetus* sp. was not much disadvantageous in the later period.

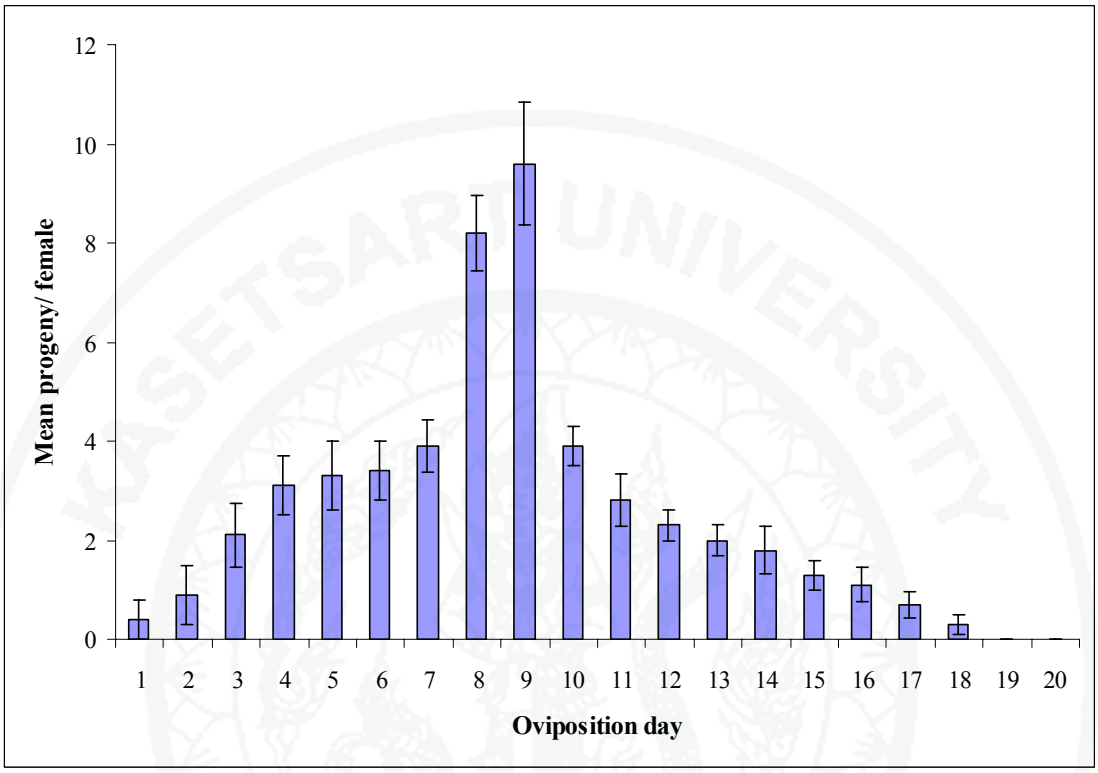

Figure 40 Mean progeny/ female as related to oviposition day of *Aprostocetus* sp. The vertical bars indicate standard errors.

The results from this research indicated that *Aprostocetus* sp. was a synovigenic species, because a) all eggs in ovaries of newly emerging adult *Aprostocetus* sp. were immature but continued to mature in the next days, b) the female adults oviposited on the first day after emergence, and the mean progeny/ female increased and was maximum on middle adult–life (on the ninth day of this study), and c) the mean realized fecundity was higher than the mean potential fecundity.

There was no report on the realized fecundity and synovigenic sp. of *Aprostocetus* sp., thus the results obtained from this research were new and were documented. It was also the new record of Thailand.

The data on synovigenic species were reported by many authors. Jervis *et al*. (2001) denoted synovigenic species as the species at emerging time had small number of mature eggs but the large number of immature eggs could gradually mature within its life–time. The potential fecundity of newly emerged female was significantly less than the measured realized fecundity.

Gordh *et al*. (1999) reported that synovigenic species generally synchronize oogenesis with oviposition and the oviposition period of an adult female was relatively long. Synovigenic species were more effective in biological control because they were lived–longer and could reproduce at lower density of the host population. They also reported that temperature and humidity influenced on oviposition and efficiency of parasitoids. Low temperature could reduced the oviposition capacity.

Adult nutrition might have important effects on life–time and reproductive success of female parasitoids (Jervis *et al*., 1996). Under natural conditions, synovigenic parasitoid species might exploit both host food (such as haemolymph) and non–host food (such as honeydrew and nectar). The females of many synovigenic parasitoids not only parasitized hosts but also fed on them. Host–feeding supplied the females with materials for continued egg production and for somatic maintenance (Jervis and Kidd, 1986).

Waage *et al*. (1985) reported that most parasitic Hymenoptera that did not feed on host, could naturally produce mature eggs with a source of carbohydrate such as honey.

Many adult parasitoids required nutrients in the form of nectar, honey, and pollen. Carbohydrate–rich nectar provided energy. Pollen might provide nutrients for egg production in some synovigenic species (Jervis *et al*., 1996).

Gordh *et al.* (1999) reported that host feeding was a phenomenon in which female parasitoids wounded hosts with the ovipositor or

mandibles and consumed haemolymph at the wound. Host feeding prolonged the life of females and supplied protein needed for oogenesis.

In the case of parasitoid of gall insect (such as *Aprostocetus* sp.), the larvae of gall insect (such as *L. invasa*) developed in the plant tissues, so the adult parasitoid could not feed on haemolymph of the larvae living inside the galls. Thus, the parasitoid might alternatively feed on flower's nectar.

It was noticed that, at the emergence time in ventilated greenhouse of this research, only the male progenies of *Aprostocetus* sp. were observed. So the sex ratio of the progenies of this parasitoid was called male–biased sex ratio.

In prolonged culture of parasitoids which was carried out by Etzel and legner (1999), they reported that sex ratio change in parasitoids were complexity. Fuester *et al*. (2003) reported that there were many factors contributing to male–bias sex ratio; such as failure of females to mate, refusal of older virgin female to mate, parental age at time of mating, excessive mating, host size or stage, maternal crowding, sex ratio of parents, clutch size, local mate competition, microhabitat, and storage of parents at low temperature.

Fuester *et al*. (2003) also reported that many species of parasitic Hymenoptera mated at temperature which might be somewhat lower than the optimum temperature for oviposition and development. Finney and Fisher (1964) reported that some species of parasitoid required a period of inactivity after copulation to facilitate fertilization, which did not occur until the sperm reached the spermathecal duct and entered the receptacle. Thus, if the female were exposed to hosts too soon after mating, the sex ratio in their progenies might be male–biased (Fuester *et al*. 2003). Waage *et al*. (1985) reported that female parasitoids tended to lay male eggs in small hosts and in already parasitized hosts.

It was noticed from this research that the small *E. camaldulensis* when planted in the pots, and left them outside the greenhouse, many

females and males of *Aprostocetus* sp. were observed on their shoots. In the outdoor conditions, not only both of females and males were found but also the population of females was more than that of males.

Thus, the future studies on sex ratio of *Aprostocetus* sp. should be carried out and should focus on the factors affecting on sex ratio of this parasitoid. The progenies at emerging time should compose of females and males. This would be beneficial in control of *L. invasa*.

The reproductive mode of *Aprostocetus* sp. was probable arrhenotoky. This mode was observed both in ventilate greenhouse and in outdoor. The arrhenotoky referred to male progenies developing parthenogenetically from unfertilized eggs, and female progenies developed from fertilized eggs (Jervis, 2005).

3.2 Fecundity of *Megastigmus* sp.

3.2.1 Potential fecundity of *Megastigmus* sp.

In the laboratory, all eggs in ovaries of newly emerging female *Megastigmus* sp. were immature. Some eggs in ovaries were mature when the female were 2 days–old after emergence. It was found from this study that the average potential fecundity of the female *Megastigmus* sp., of all sizes and ages, was 2.98±0.11 eggs per female, ranging from 1–5 eggs per female.

The potential fecundity of female *Megastigmus* sp. was also investigated for the first time and the findings from this research were new and were documented. Bouaziz and Roques (2006), who studied the potential fecundity of *Megastigmus wachtli* Seither (seed eater of cypress; *Cupressus sempervirens* L.), found that the eggs in ovaries were mature when the females were 12–days old after emergence. The average potential fecundity of the female *M. wachtli* was 7.80±6.40 eggs per female.

To compare *Megastigmus* sp. with *L. invasa*, it was found that the average potential fecundity of female *Megastigmus* sp. (2.98 eggs per female, ranging from 1 to 5 eggs per female) was enormously lower than that of the female *L. invasa* (158.70 eggs per female, ranging from 39 to 298 eggs per female).

To use hind tibia length as a substitute for female size, the results showed that the average of all sizes of female *Megastigmus* sp. was 0.31±0.002 mm, ranging from 0.25 to 0.36 mm.

The effects of female sizes of *Megastigmus* sp. of all ages on mean egg loads was determined by ANOVA regression analysis. It was found that sizes had significant effects on mean egg loads at P=0.05 (F=13.569; P–value = 0.000). There was significantly positive relationship between sizes and egg loads of female *Megastigmus* sp. ($y=15.704x - 1.784$; $R^2=0.156$; n=75). The larger sizes of female tended to produce more eggs than the smaller sizes (Figure 41). The $R^2=0.156$ indicated that the sizes influenced on egg loads at about 15.60 %. The other variables probably involved and had influences on egg loads.

It was noticed that R^2 or coefficient of determination of this equation was low. This probably arised from the reasons that a) *Megastigmus* sp. was a synovigenic species, or b) the sample size (n=75) was not probably large enough for this species to be tested in this experiment. However, this was a constraint because the population of *Megastigmus* sp. in *E. camaldulensis* plantations in Tha Muang and Phanom Thaun districts was naturally small.

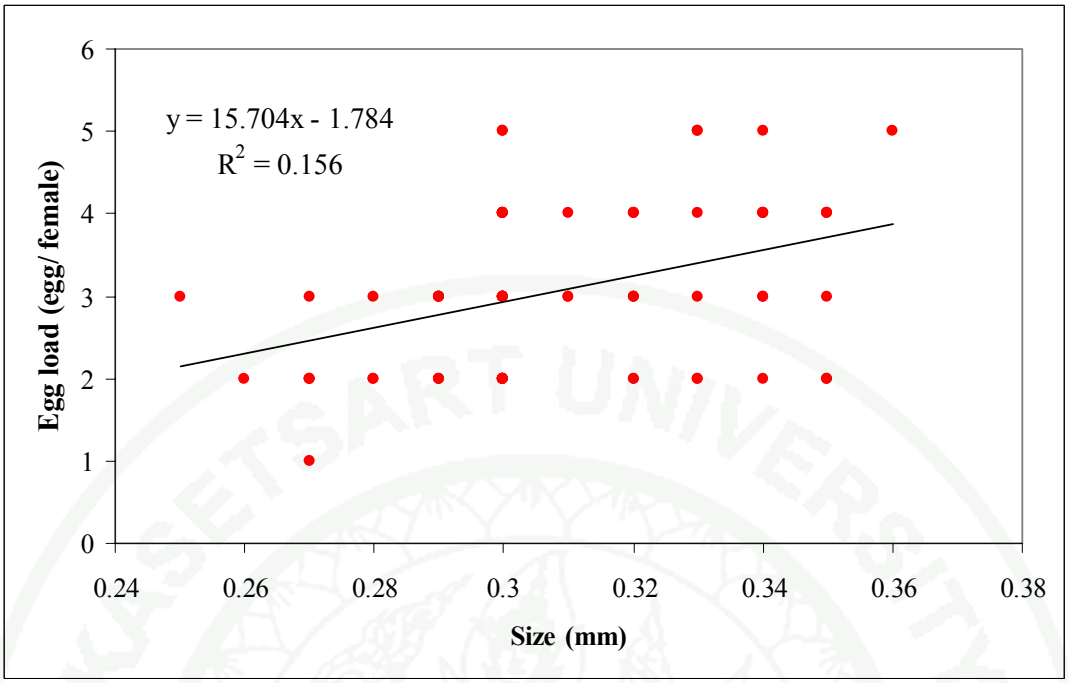

Figure 41 Relationship between size and egg load of female *Megastigmus* sp.

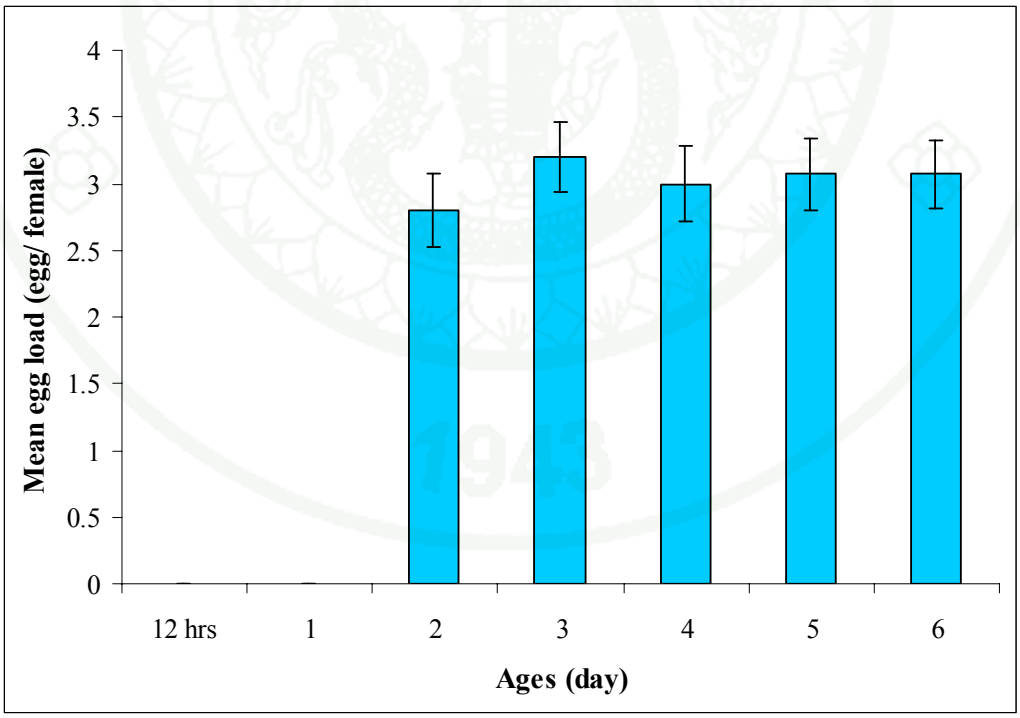

Figure 42 Mean egg loads in different ages of female *Megastigmus* sp. The vertical bars indicate standard errors.

The effects of female ages (days after eclosion) on egg loads of *Megastigmus* sp. was shown in Figure 42. By one–way analysis of variance, the results showed that mean egg loads in different ages were different, but not significantly at P=0.05 (F=0.308; df =4; P–value=0.872).

3.2.2 Realized fecundity of *Megastigmus* sp.

In ventilated greenhouse, all eggs in ovaries of newly emerging female *Megastigmus* sp. were immature. Some eggs in ovaries were mature when the females were 2 days–old after emergence, but the rest continued to mature in the next days. The results showed that the female *Megastigmus* sp. started to oviposit when the females were 2 days–old and lasted on the tenth day. The mean progeny/ female of *Megastigmus* sp. gradually increased and was maximum on the fourth day with 2.87±0.55 progenies per female and then gradually declined in the later days (Figure 43).

It was also found that the mean of realized fecundity of a female from the second day to the tenth day was 13.20±1.95 progenies per a female, ranging from 5 to 30 progenies per female.

To compare *Megastigmus* sp. with *L. invasa*, it was found as follows. For the female *Megastigmus* sp., a) they oviposited on the second day after emergence and lasted on the tenth day, b) the mean progeny/female was maximum on the fourth day with 2.87±0.55 progenies per female, and c) the mean of realized fecundity of a female from the second day to the tenth day was 13.20±1.95 progenies per a female, ranging from 5 to 30 progenies per female. For the female *L. invasa*, a) they oviposited on the first day after emergence and lasted on the sixth day only, b) the mean progeny/female was maximum on the first day with 30.47±7.41 progenies per female, and c) the mean of realized fecundity of a female from the first day to the sixth day was 61.53±8.94 progenies per a female, ranging from 18 to 130 progenies per female. These comparisons suggested that *L. invasa* was more advantageous than *Megastigmus* sp. and it was not easy to use this parasitoid to control *L. invasa*.

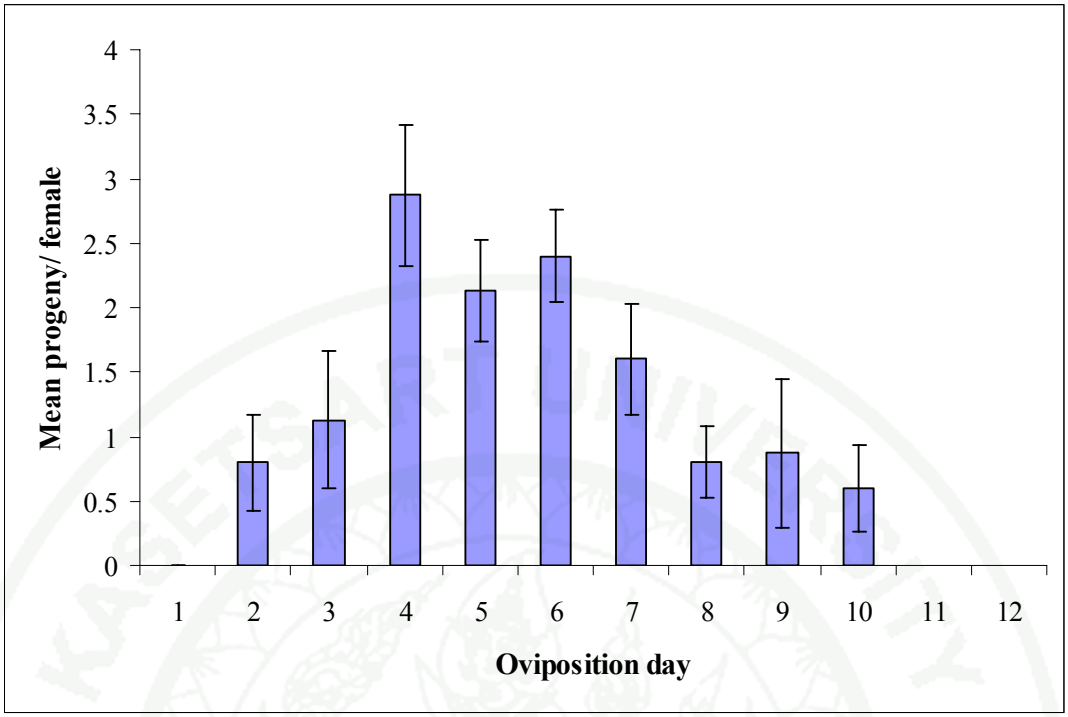

Figure 43 Mean progeny/ female as related to oviposition day of *Megastigmus* sp. The vertical bars indicate standard errors.

The result from this research indicated that *Megastigmus* sp. was a synovigenic species, because a) all eggs in ovaries of newly emergence of adult *Megastigmus* sp. were immature and continued to mature in the later days, b) the female adults oviposited on the second day after emergence and the mean progeny/ female increased and was maximum on nearly middle adult–life (on the fourth day of this study), and c) the mean realized fecundity was higher than the mean potential fecundity.

There was no report on the realized fecundity of *Megastigmus* sp., thus the results obtained from this research were new and were documented.

It was found from this study that, at the emergence time in ventilated greenhouse, the average number of female progenies:male progenies= 4.33:8.87, or about 1:2. So the sex ratio of *Megastigmus* sp. progenies was called male–biased sex ratio.

The reproductive mode of *Megastigmus* sp. in the study areas was probable arrhenotoky. This mode was observed both in ventilated greenhouse and in outdoor. The arrhenotoky referred to male progenies developing parthenogenetically from unfertilized eggs, and female progenies developed from fertilized eggs (Jervis, 2005).

4. Reproductive organs of *Aprostocetus* sp. and *Megastigmus* sp.

4.1 Reproductive organs of *Aprostocetus* sp.

4.1.1 Reproductive organs of the female *Aprostocetus* sp.

The reproductive organs of the female *Aprostocetus* sp. differed from the male one. The research found that the reproductive organs of female *Aprostocetus* sp. comprised mainly of a pair of ovaries and short lateral oviducts, a common oviduct, a pair of accessory glands, a spermatheca, an ovipositor (first and second valvulae), and a pair of ovipositor sheaths, first valvifer and second valvifers (Figure 44A and B).

Each ovary composed of 2–6 ovarioles and located in the abdomen above the gut. The lateral oviducts and the common oviduct were short. The accessory glands were ovate and located with the anterior end of the common oviduct. The spermatheca was noticeably pigmented yellow.

The female genitalia of *Aprostocetus* sp. was elongated. The ovipositor sheaths were longer than the tip of abdomen. The length of ovipositor was 0.40–0.50 mm. The posterior margin of ovipositor sheaths, along the ovipositor, had a row of setae. The tip of ovipositor sheaths has long sensory hairs.

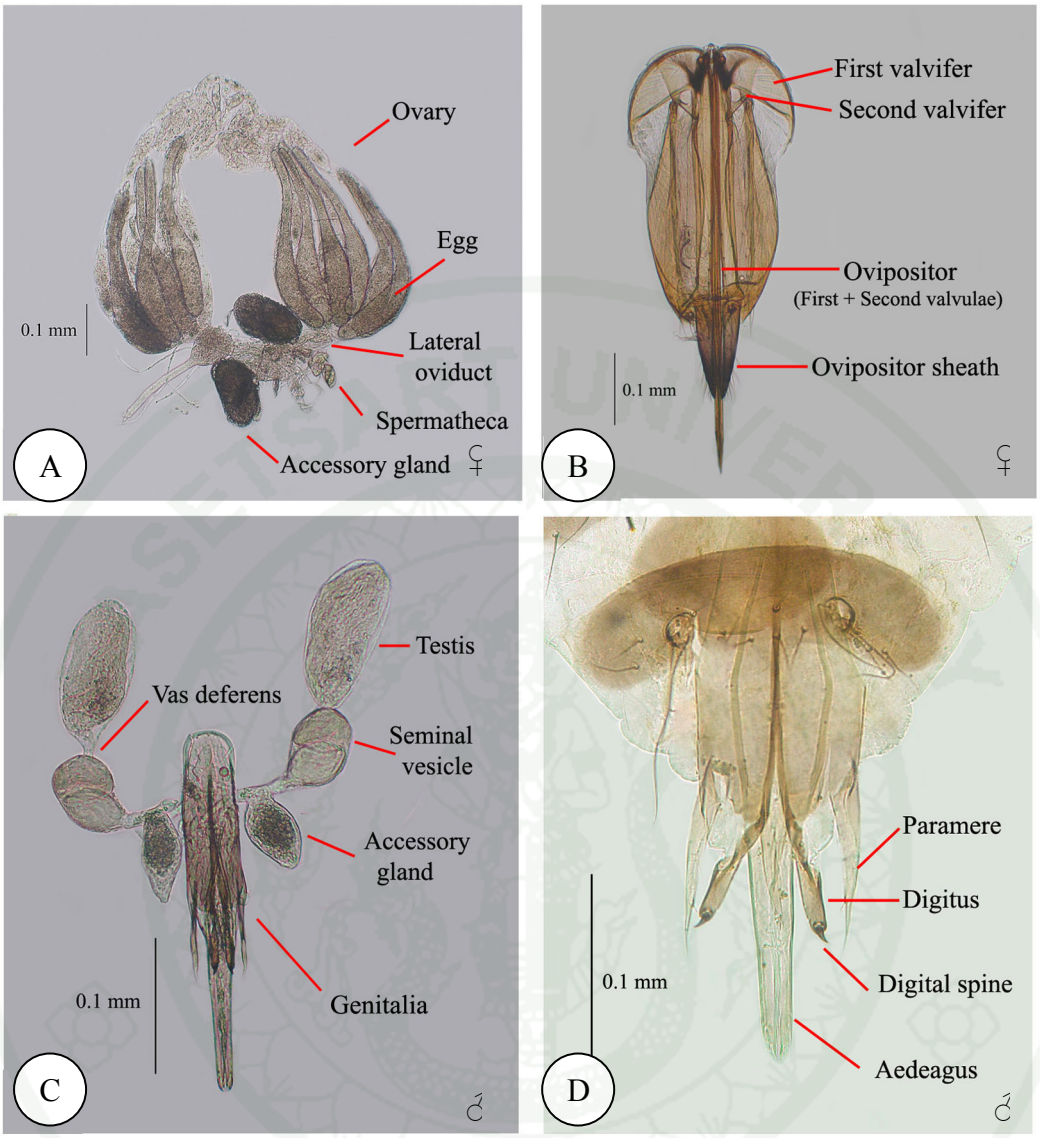

Figure 44 Reproductive organs of *Aprostocetus* sp.: (A–B) female; and (C–D) male.

4.1.2 Reproductive organs of the male *Aprostocetus* sp.

The male reproductive organs of *Aprostocetus* sp. consisted mainly of a pair of testes, vasa deferentia and seminal vesicles, an ejaculatory duct, a pair of accessory glands, digitae and parameres and an aedeagus (Figure 44C and D).

The testes were elliptic. The accessory glands were oval shaped with acute apex and smaller than testes. The male genitalia of *Aprostocetus* sp.

was cylindrically elongated. The digitae and the parameres were elongated. Each of digitae had a large digital spine on the tip. Each of parameres had one seta on the tip and one seta at the lateral side. The aedeagus was very long.

This research reported for the first time on the reproductive organs of the female and male *Aprostocetus* sp. and these findings were documented. However, the structure of reproductive organs of *Aprostocetus* sp., which were found in this study, were close to those of many species in Hymenoptera.

4.2 Reproductive organs of *Megastigmus* sp.

4.2.1 Reproductive organs of the female *Megastigmus* sp.

The reproductive organs of the female *Megastigmus* sp. comprised mainly of a pair of ovaries and lateral oviducts, a common oviduct, a pair of accessory glands, a spermatheca, an ovipositor (first and second valvulae), and a pair of ovipositor sheaths, first valvifer and second valvifers (Figure 45A and B).

Each ovary composed of 1–3 ovarioles. The accessory glands were slightly rounded. The female genitalia of *Megastigmus* sp. was long curved. The ovipositor and the ovipositor sheaths were longer than the tip of abdomen. The ovipositor sheaths were darked brown and were mostly covered with long sensory hairs.

4.2.2 Reproductive organs of the male *Megastigmus* sp.

The male reproductive organs of *Megastigmus* sp. comprised mainly of a pair of testes, vasa deferentia and seminal vesicles, an ejaculatory duct, a pair of accessory glands, digitae and parameres and an aedeagus (Figure 45C and D).

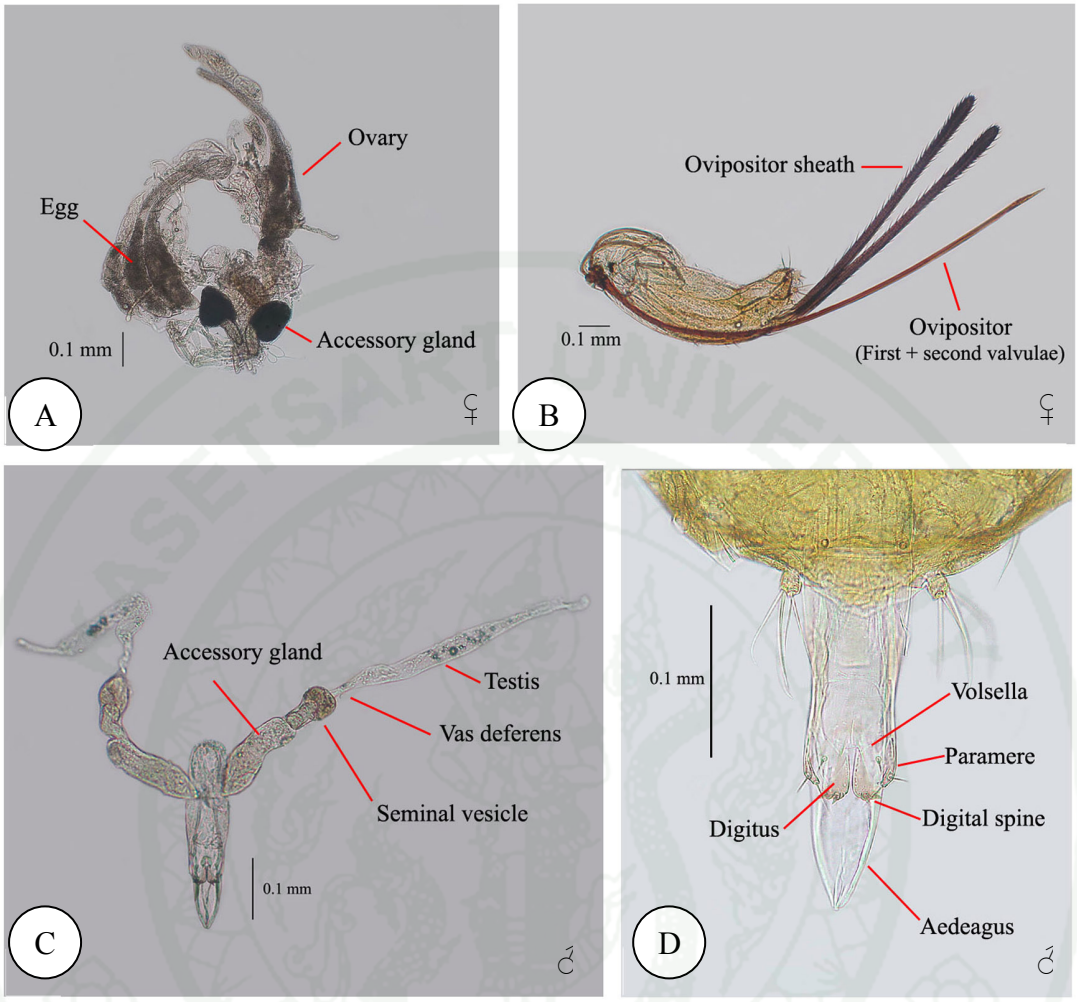

Figure 45 Reproductive organs of *Megastigmus* sp.: (A–B) female; and (C–D) male.

The testes were very long and narrow. The accessory glands were oval shaped. The male genitalia was oval shaped with long tip. Each of digitae was dark brown and had a digital spine on the tip. Each of parameres was brown and had one seta on the tip and one seta at the lateral side. The aedeagus was short.

The reproductive organs of the female and male *Megastigmus* sp. were reported for the first time in this research. The structure of reproductive organs of this species were also close to those of many species in Hymenoptera.

5. Development of *Aprostocetus* sp. and *Megastigmus* sp.

5.1 Development of *Aprostocetus* sp.

Development of *Aprostocetus* sp. in this study covered from egg stage to young larva, mature larva, larva to prepupa, pupa, and adult stage (or emergence stage).

5.1.1 Oviposition behavior and egg development of *Aprostocetus* sp.

Generally, the parasitoid was specific to the host. The results showed that adult female *Aprostocetus* sp. oviposited in mature larva and in pupa of *L. invasa* since the female was 1 day–old after her emergence. The mature larva and the pupa of *L. invasa* were in stage 3 and stage 4 of this gall wasp development. The preferences of *Aprostocetus* sp. to parasitize in mature larva and in pupa of *L. invasa* were shown in Table 13. Adult female *Aprostocetus* sp. preferred to parasitize more in mature larva than in pupa of host.

Table 13 Preference of *Aprostocetus* sp. to parasitize in different developments of *Leptocybe invasa*.

Development of *L. invasa*	Number of individuals	
	Emerging *L. invasa*	Emerging *Aprostocetus* sp.
Stage 1 (egg)	168	0
Stage 2 (young larva)	220	0
Stage 3 (mature larva to prepupa)	156	31
Stage 4 (pupa)	158	8

When the female *Aprostocetus* sp. found leaf galls which had the immatures of *L. invasa* living inside, she explored to search the most appropriate position for her egg laying by a) using antennae knocking at the surface of the gall, and b) inserting an ovipositor in the gall before the real ovipositing. After finding the suitable position, the female *Aprostocetus* sp. laid a single egg in the mature larva or in the pupa of *L. invasa* living inside the leaf gall (Figure 46). The single egg of female *Aprostocetus* sp. developed as a solitary endoparasitoid.

If the egg of *Aprostocetus* sp. developed inside the mature larva of *L. invasa*, the mature larva of the host fractured and its haemolymph came out and stored in leaf–gall chamber. The larva of *Aprostocetus* sp. lived and developed to the last stage within this haemolymph in the gall chamber. Finally the mature larva of *L. invasa* became a tiny remains or no remains left. If the egg of *Aprostocetus* sp. developed inside the pupa of *L. invasa*, the larva of parasitoid lived and developed there until the last stage. Finally the pupa collapsed and became the small remains at the outer surface of the larva of *Aprostocetus* sp. (Figure 47).

There were several researchers reported about endoparasitoid. Gordh *et al.* (1999) reported that endoparasitoid developed inside the body of host. Solitary parasitism was a condition in which a parasitoid larva completed development in one host. Tscharntke *et al*. (1991) reported that *Aprostocetus calamarious*, *A. gratus*, *A. longiscapus*, *A. orithyia*, and *A. phragmiticola* were endoparasitoid of *Giraudiella inclusa* (Diptera: Cecidomyiidae), gall midge of common reed grass. Sampson *et al.* (2006) reported that *Aprostocetus* sp. was solitary endoparasitoid of gall midge on blueberries.

Bouček (1988) reported that many species in the Genus *Aprostocetus* developed in plant tissue, mostly being associated with galls caused by other insects, mainly Cecidomyiidae. They seemed to be primary parasites attacking eggs or various larval instars of their hosts. Many species were specialized for a certain gall or host.

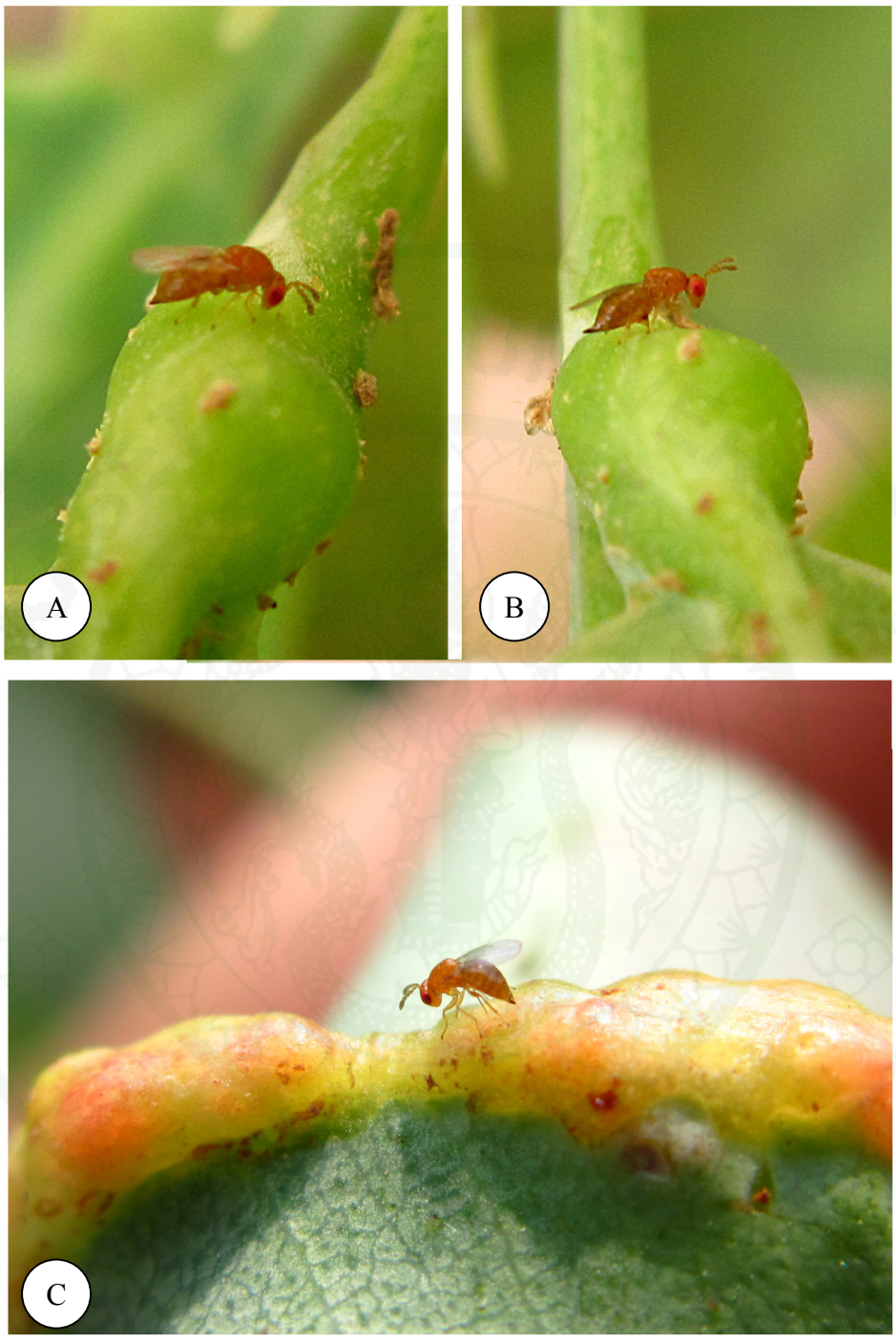

Figure 46 Oviposition behavior of female *Aprostocetus* sp.: (A) searching the larva or the pupa of *Leptocybe invasa* living inside the leaf gall of *Eucalyptus camaldulensis*; and (B–C) ovipositing.

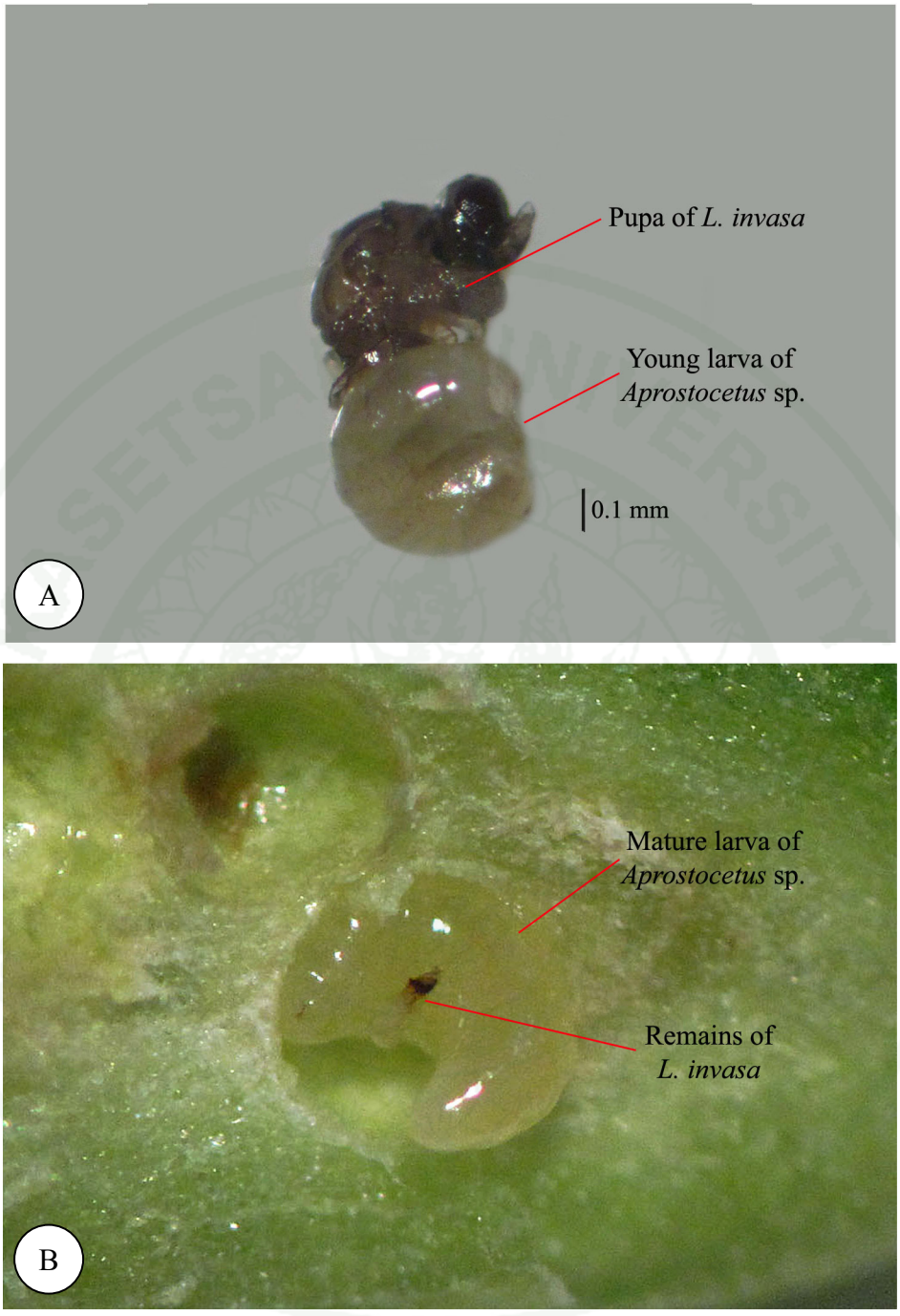

Figure 47 The larva of *Aprostocetus* sp.: (A) young larva of *Aprostocetus* sp. developed inside the abdomen of the pupa of *Leptocybe invasa*; (B) small remains of *L. invasa* on the outer surface of mature larva of *Aprostocetus* sp.

5.1.2 Development time from egg to adult emergence of *Aprostocetus* sp.

The development of *Aprostocetus* sp. in this study was divided into 5 staged as follows: stage1 (egg), stage2 (young larva), stage3 (mature larva to prepupa), stage4 (pupa), and stage5 (adult).

In ventilated greenhouse, the results showed that the mean development time of *Aprostocetus* sp. from oviposition to adult emergence was 12.92 ±0.29 days, ranging from 10 to 19 days. The egg stage, young larval stage, mature larval to prepupal stage, and pupal stage took 1.33±0.13 days, 2.85±0.17 days, 5.18± 0.34 days, and 7.08±0.36 days, respectively. The detail of *Aprostocetus* sp. development time was shown in Figure 48. The development time was minimum in egg stage and maximum in larval stage and in pupal stage.

The external morphology of the immature *Aprostocetus* sp. found at each stage was described below and shown in Figure 49 A–F.

Stage1 (egg): The egg of *Aprostocetus* sp., seen as stalked egg, comprised of an elongate–oval body (sausage like), and a narrow anterior stalk with as long as the body length. The size of individual egg ranged from 0.20–0.30 mm in length. The egg was greyish white in color and the entire surface was smooth, lacking of ornamentation.

Stage2 (young larva) and stage3 (mature larva to prepupa): The number of instars of *Aprostocetus* sp. larva was not determined. The young larva and mature larva were sac–like grubs or hymenopteriform as classified by Gauld and Botton (1996), with a weakly head capsule, and without legs. All of mouthparts were extremely reduced except the mandibles. The head typically beared a pair of mandibles with strongly sclerotized.

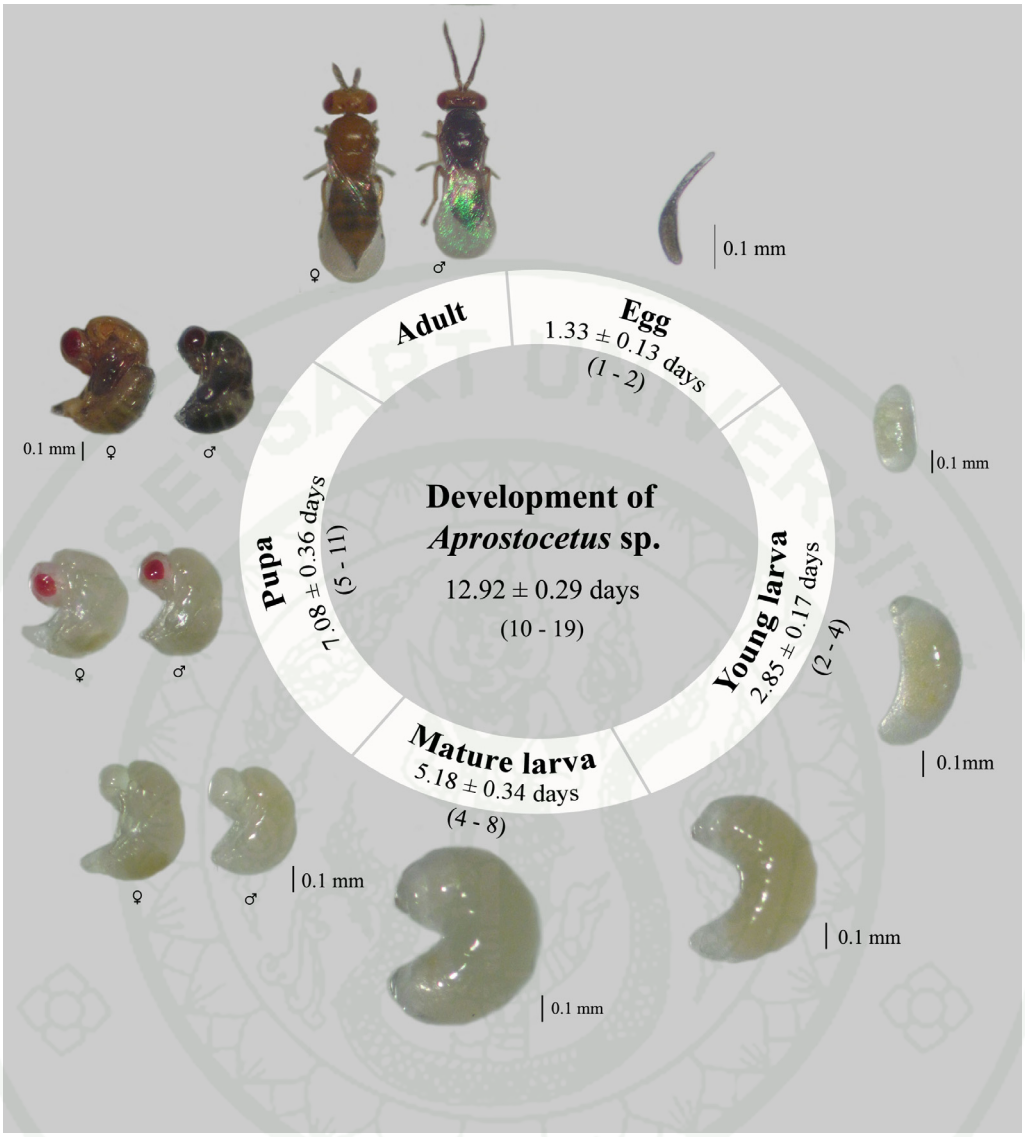

Figure 48 Development time at each stage of *Aprostocetus* sp. as endoparasitoid of *Leptocybe invasa*.

The larva was greyish white and translucent skin that was rather smooth. The young larva differed from the mature larva in size, curve of body, and segmentation. The mature larva was bigger in size, had curved body (C-shape), and had distinct segment. Soon after the mature larva began to expel its meconium at posterior end, it turned to the prepupa.

Stage4 (pupa): *Aprostocetus* sp., like other Hymenoptera, produced exarate pupa with clearly visible mouthparts, antennae, and legs. The C–shaped pupa was not protected by any special cocoon.

The newly formed female and male pupa (or early pupa) were whitish with no pigmentation. A few days later, eyes of pupa became reddish. In the next days (or late pupa), the pupal cuticle was sclerotized from a light–grey to orange–brown in female pupa and to dark brown in male pupa. Morphological difference was not observed between the different stages of pupal development. The late pupa of female was separated from the late pupa of male by the appearance of the ovipositor of female pupa.

Stage5 (adult emergence): the adult *Aprostocetus* sp. perforated from leaf galls of *E. camaldulensis* by using mandibles. It was observed that adult males of *Aprostocetus* sp. emerged from the galls before adult females.

There was no report on the oviposition behavior, egg development, development time, and morphology and development of immature *Aprostocetus* sp. which associated with Eucalyptus galls. Thus, these findings were new and were also the new record of Thailand. Tschudi–Rein and Dorn (2001) studied in other Eulophidae and reported that endoparasitic species tended to have three larval instars and ectoparasitoids tended to have five larval instars.

To compare the mean development time from eggs to adult emergence of two species, the result showed that the mean development time of *Aprostocetus* sp. was 12.92±0.29 days, while that of *L. invasa* was 45.96±0.52 days. This biological parameter suggested that *Aprostocetus* sp. would be advantageous to be used in the control of *L. invasa*.

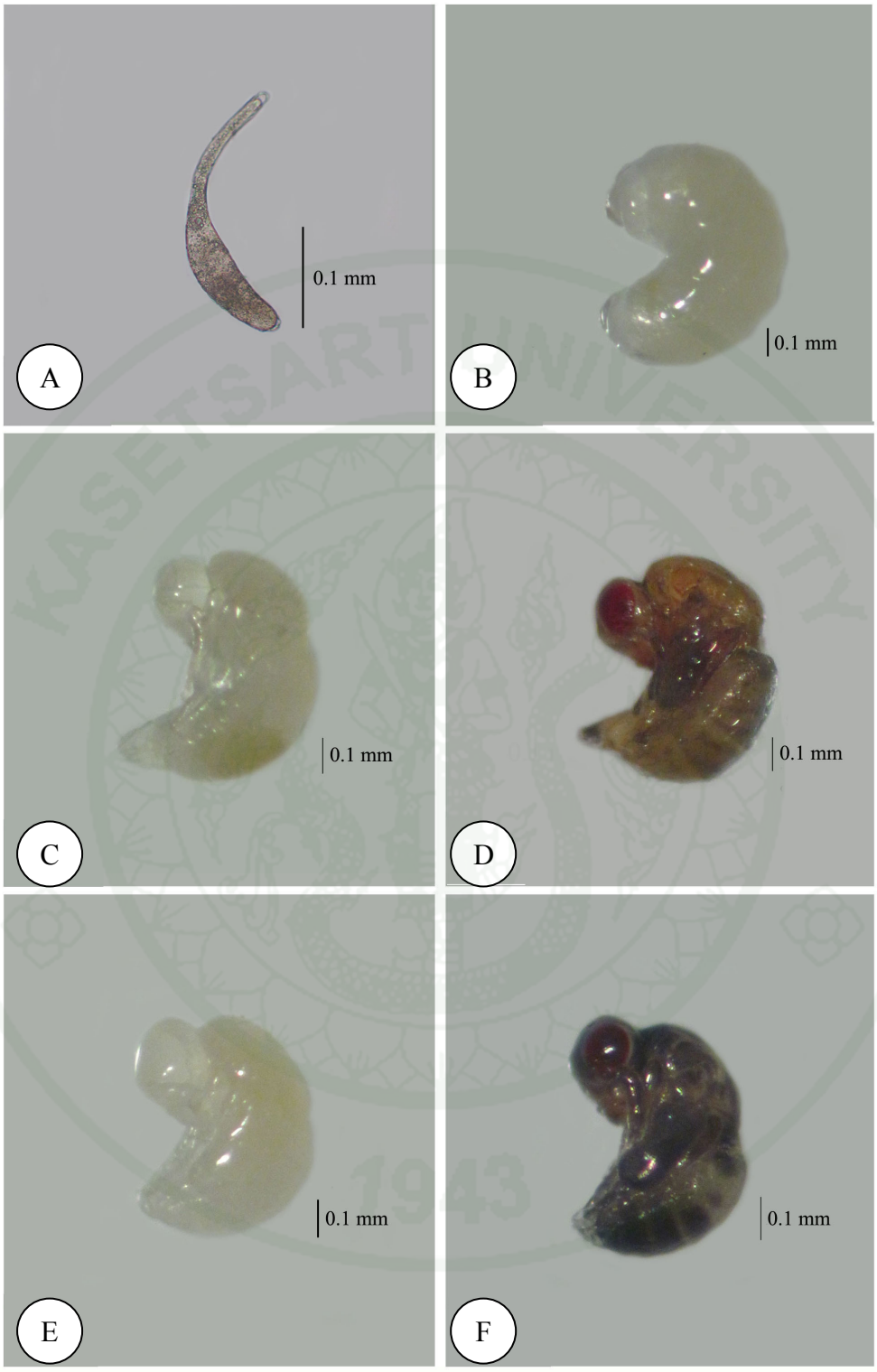

Figure 49 Morphology of immature *Aprostocetus* sp.: (A) egg; (B) mature larva; (C) early pupa of female; (D) late pupa of female; (E) early pupa of male; and (F) late pupa of male.

5.2 Development of *Megastigmus* sp.

Development of *Megastigmus* sp. in this study also covered from egg stage to young larva, mature larva to prepupa, pupa, and adult stage (or emergence stage).

5.2.1 Oviposition behavior and egg development of *Megastigmus* sp.

The parasitoid was usually specific to the host. It was found from this research that adult female *Megastigmus* sp. oviposited on mature larva and on pupa of *L. invasa* since the female was 2 days–old after her emergence. The mature larva and the pupa of *L. invasa* were in stage 3 and stage 4 of this gall wasp development. The preferences of *Megastigmus* sp. to parasitize on mature larva and on pupa of *L. invasa* were shown in Table 14. Adult female *Megastigmus* sp. preferred to parasitize more on pupa than on mature larva of host.

When the female *Megastigmus* sp. found the leaf galls which had the immatures of *L. invasa* living inside, she explored to search the most appropriate position for her egg laying in the same manner of *Aprostocetus* sp. (Figure 50). After finding the suitable position, the female *Megastigmus* sp. laid a single egg on the mature larva or on the pupa of *L. invasa* living the leaf gall. The single egg of female *Megastigmus* sp. developed as a solitary ectoparasitoid. Ectoparasitoid developed outside the body of host.

The mature larva or the pupa parasitized by *Megastigmus* sp. gradually became softly, changed the body color to dark brown, and dried out. Finally, only the remains of integument of *L. invasa* was left inside leaf–gall chamber (Figure 51). The larva of *Megastigmus* sp. developed to the last stage in the gall chamber and emerging from the gall.

Table 14 Preference of *Megastigmus* sp. to parasitize in different developments of *Leptocybe invasa*.

Development of *L. invasa*	Number of individuals	
	Emerging *L. invasa*	Emerging *Megastigmus* sp.
Stage 1 (egg)	193	0
Stage 2 (young larva)	278	0
Stage 3 (mature larva to prepupa)	217	3
Stage 4 (pupa)	152	5

The findings from this research, that *Megastigmus* sp. was a solitary ectoparasitoid on the larva or on the pupa of *L. invasa*, was in line with Protasov *et al*. (2008). They studied the other species of *Megastigmus* and reported the same results.

Bouček (1988) wrote in his book that most species of *Megastigmus* in the Holarctic region were phytophagous in seeds of conifers, but some were parasitic in galls of cynipids. In southern Asia, the hosts were more varied and some species developed even in figs. In Australia some species were claimed to be phytophagous.

Figure 50 Behavior of female *Megastigmus* sp. in searching the larva or the pupa of *Leptocybe invasa* living inside the leaf gall of *Eucalyptus camaldulensis* before ovipositing.

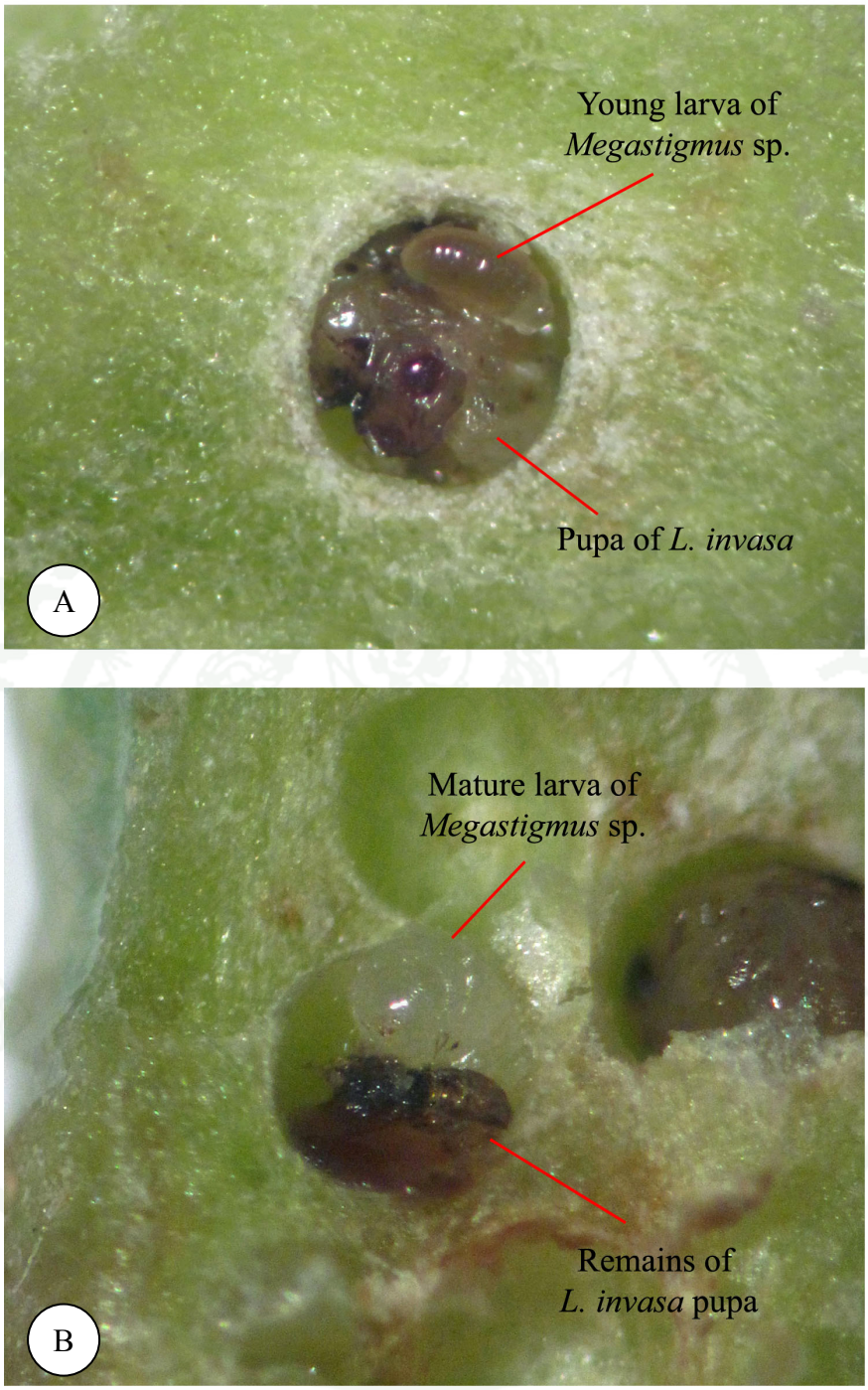

Figure 51 The larva of *Megastigmus* sp.: (A) young larva of *Megastigmus* sp. developed on the pupa of *Leptocybe invasa*; (B) mature larva of *Megastigmus* sp. on the remains of *L. invasa* pupa.

5.2.2 Development time from egg to adult emergence of *Megastigmus* sp.

The development of *Megastigmus* sp. in this study was divided into 5 staged as follows: stage1 (egg), stage2 (young larva), stage3 (mature larva to prepupa), stage4 (pupa), and stage5 (adult).

In ventilated greenhouse, it was found that the mean development time of *Megastigmus* sp. from oviposition to adult emergence was 17.00 ±0.44 days, ranging from 12 to 32 days. The egg stage, young larval stage, mature larval to prepupal stage, and pupal stage took 1.87±0.17 days, 5.00±0.58 days, 9.00± 0.55 days, and 12.38±0.38 days, respectively. The detail of *Megastigmus* sp. development time was shown in Figure52. The development time was minimum in egg stage and maximum in larval stage and in pupal stage.

The external morphology of the immature *Megastigmus* sp. found at each stage was described below and shown in Figure 53A–F.

Stage1 (egg): The egg of *Megastigmus* sp. seen as stalked egg, comprised of an elongate–oval body (sausage like), a short spur–like posterior stalk, and a long narrow anterior stalk. The size of individual egg ranged from 0.50–0.55 mm in length. The egg was greyish white in color and the entire surface was smooth, lacking of ornamentation.

Stage2 (young larva) and stage3 (mature larva to prepupa): The number of instars of *Megastigmus* sp. larva was not determined. The young larva and mature larva were hymenopteriform, with a head capsule, and without legs. All of mouthparts were reduced except the mandibles. The head typically beared a pair of mandibles with strongly sclerotized. The well–developed setae were presented on the head.

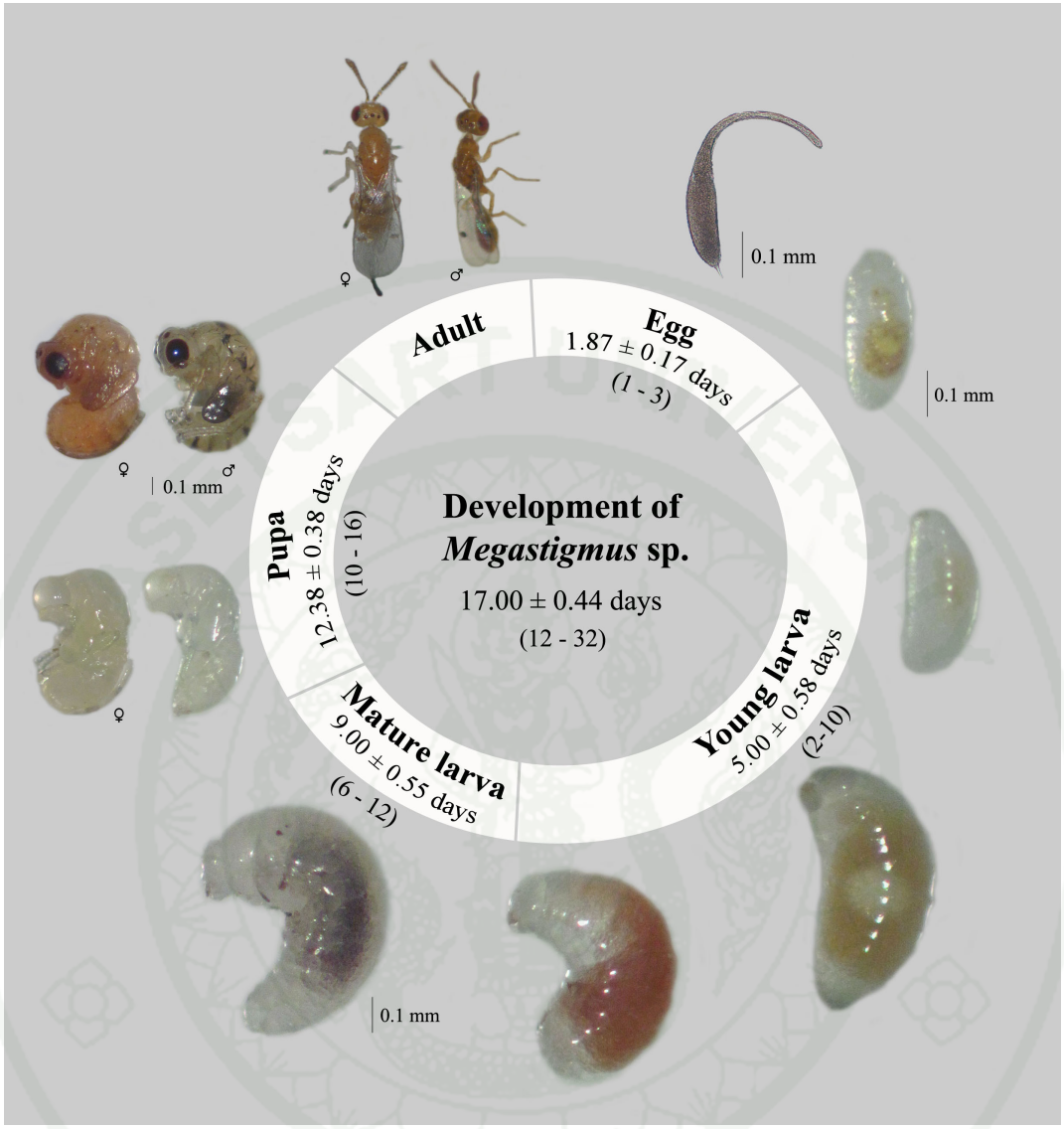

Figure 52 Development time at each stage of *Megastigmus* sp. as ectoparasitoid of *Leptocybe invasa*.

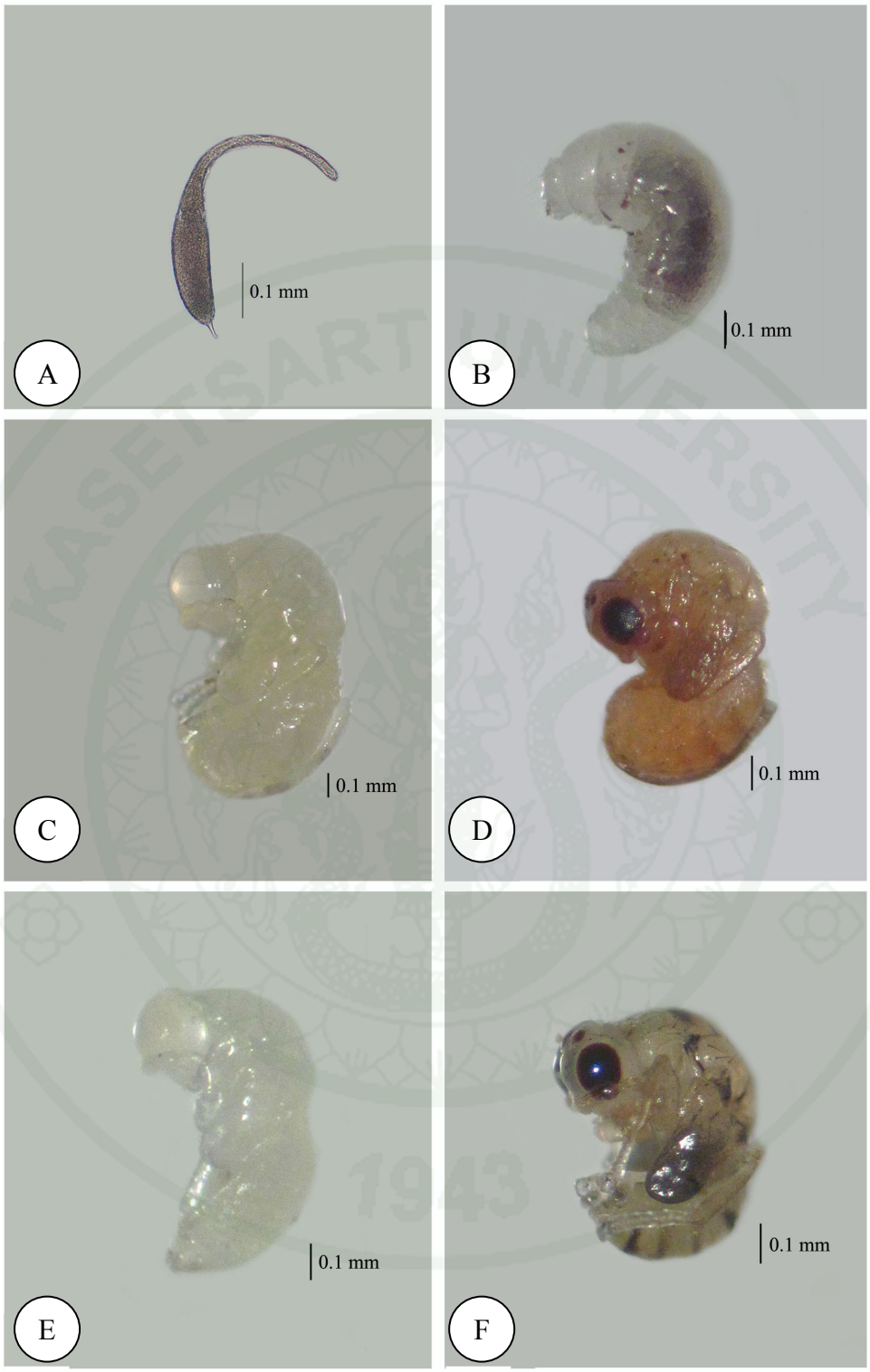

Figure 53 Morphology of immature *Megastigmus* sp.: (A) egg; (B) mature larva; (C) early pupa of female; (D) late pupa of female; (E) early pupa of male; and (F) late pupa of male.

The larva was greyish–white and translucent skin that was rather smooth. The young larva differed from the mature larva in size, curve of body, and long setae on head. The mature larva was bigger in size, had curved body (C–shape), and had long setae on head. Soon after the mature larva began to expel its meconium at posterior end, it turned to the prepupa.

Stage4 (pupa): The pupa of *Megastigmus* sp. was exarate pupa, with clearly visible mouthparts, antennae, and legs. The C–shaped pupa was not protected by any special cocoon.

The newly formed female and male pupa (or early pupa) were whitish with no pigmentation. A few days later, eyes of pupa became reddish. In the next days (or late pupa), the pupal cuticle was sclerotized from a light–grey to orange–brown in female pupa and to yellow with dark markers in male pupa. Morphological difference was not observed between the different stages of pupal development. The late pupa of female was separated from the late pupa of male by the appearance of the ovipositor of female pupa.

Stage5 (adult emergence): the adult *Megastigmus* sp. perforated from leaf galls of *E. camaldulensis* by using mandibles. It was observed that adult males of *Megastigmus* sp. emerged from the galls before adult females.

The development time of *Megastigmus* sp.–I (found in Israel) was studied by Protasov *et al*. (2008). They reported that the development time of *Megastigmus* sp.–I from eggs to adult emergence took 41–43 days in greenhouse under a temperature regime of 23–31 ºC. Their findings were different from the results of this research. It probably arose from the different species of *Megastigmus* or the difference in experimental conditions.

There was a report on larval instars of a species of *Megastigmus*. Milliron (1949) reported that *Megastigmus nigrovariegatus* had five larval instars. The results of oviposition behavior, egg development, and development

time of *Megastigmus* sp. associated with Eucalyptus galls in Tha Muang and Phanom thuan district, Kanchanaburi province, were new and were also the new record of Thailand. Moreover, there was no report on morphology and development of immature *Megastigmus* sp. which associated with Eucalyptus galls.

To compare the mean development time from eggs to adult emergence of two species, the result showed that the mean development time of *Megastigmus* sp. was 17.00±0.44 days, while that of *L. invasa* was 45.96±0.52 days. Based on this biological parameter, it suggested that *Megastigmus* sp. would be advantageous to be used in the control of *L. invasa*.

6. Comparison between biological aspects of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp.

The biological aspects of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp., were compared and summarized in Table 15. The findings in the table suggested the advantages and disadvantages of *L. invasa* and the parasitoids.

However, if based on those biological parameters, *Aprostocetus* sp. had more potentials than *Megastigmus* sp. to be used in the management or in the control of *L. invasa* in *E. camaldulensis* plantations.

Table 15 Summary of the biological aspects of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp.

Biological aspect	*Leptocybe invasa*	*Aprostocetus* sp.	*Megastigmus* sp.
Mean longevity of adult (days) [1/]			
Female	7.67	18.67	9.83
Male	5.67	13.33	7.83
Estimated 50% survival period (days) [1/]			
Female	5	12	8
Male	4	9	4
Fecundity of adult			
1) Mean potential fecundity of female (eggs/female) [1/]	158.70	6.31	2.98
2) Mean realized fecundity of female (progenies/female) [2/]	61.53	51.10	13.20
3) Life – history strategy	Pro-ovigenic sp.	Synovigenic sp.	Synovigenic sp.
4) Sex ratio (female progeny: male progeny) [2/]	2 : 1	Male - biased	Male - biased
5) Reproductive mode	Deuterotoky	Arrhenotoky	Arrhenotoky
Development [2/]			
1) Egg development	In leaf tissue	In mature larva and pupa (Endoparasitoid)	On mature larva and pupa (Ectoparasitoid)
2) Mean development time from egg to adult (days)	45.96	12.92	17.00

[1/] In the laboratory.

[2/] In ventilated greenhouse.

Population Dynamics of *L. invasa* and the Parasitoids in Two Districts

The research on population dynamics of *L. invasa* and the parasitoids in Tha Muang and Phanom Thuan districts, Kanchanaburi province, were carried out in *Eucalyptus* plantations from May 2009 to April 2010, and were assessed by two methods; namely *Eucalyptus* leaf–gall sampling and sweeping around canopy of coppice shoots of *Eucalyptus* trees.

1. Population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. by *Eucalyptus* leaf–gall sampling

1.1 Tha Muang district

The results showed that the populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp., in *E. camaldulensis* plantations in Tha Muang district, fluctuated from month to month.

By *Eucalyptus* leaf–gall sampling, the maximum populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. occurred on May and March. After May, the populations of 3 species declined and varied monthly from June to February. *Aprostocetus* sp. and *Megastigmus* sp. were rare from August to February. The populations of *L. invasa* were larger than the populations of parasitoids in every month. The details were shown in Table 16 and Figure 54.

It was noticed that the maximum population of *L. invasa* on May was 973 individuals/1,000 leaves (with 967 females and 6 males), and on March was 1,058 individuals/1,000 leaves (with 1,040 females and 18 males). The maximum population of *Aprostocetus* sp. on May was 80 individuals/1,000 leaves (with 46 females and 34 males) and on March was 18 individuals/1,000 leaves (with 14 females and 4 males). The maximum population of *Megastigmus* sp. on May was 201 individuals /1,000 leaves (with 86 females and 115 males), and on March was 136 individuals/1,000 leaves (with 59 females and 77 males) (Table 16).

Table 16 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by *Eucalyptus* leaf–gall sampling in Tha Muang district, From May 2009 to April 2010.

Months	Number of individuals/1,000 leaves								
	Leptocybe invasa			*Aprostocetus* sp.			*Megastigmus* sp.		
	Total	Female	Male	Total	Female	Male	Total	Female	Male
May-09	973	967	6	80	46	34	201	86	115
Jun-09	250	249	1	2	2	0	131	40	91
Jul-09	417	416	1	13	8	5	19	10	9
Aug-09	202	200	2	4	4	0	2	1	1
Sep-09	461	459	2	9	8	1	0	0	0
Oct-09	145	142	3	3	3	0	2	0	2
Nov-09	214	209	5	3	1	2	0	0	0
Dec-09	104	103	1	5	5	0	0	0	0
Jan-10	241	232	9	7	5	2	1	1	0
Feb-10	409	396	13	18	11	7	7	3	4
Mar-10	1,058	1,040	18	18	14	4	136	59	77
Apr-10	383	383	0	1	1	0	89	47	42
Total [1/]	4,857	4,796	61	163	108	55	588	247	341
%	100	98.74	1.25	100	66.25	33.74	100	42.00	57.99

[1/] Total number of individuals/12,000 leaves/year.

In the aspect of total population throughout the year, the results showed that a) the small populations of 3 species were found from June to February, b) the total population of *L. invasa* was 4,857 individuals/12,000 leaves/year (with 4,796 females and 61 males), *Aprostocetus* sp. was 163 individuals/12,000 leaves/year (with 108 females and 55 males), and *Megastigmus* sp. was 588 individuals/12,000 leaves/year (with 247 females and 341 males), c) the females of *L. invasa* and *Aprostocetus* sp. covered a large proportion of males, and d) the fluctuation of *L. invasa* had tendency to be consistent with parasitoids (Table 16 and Figure 54).

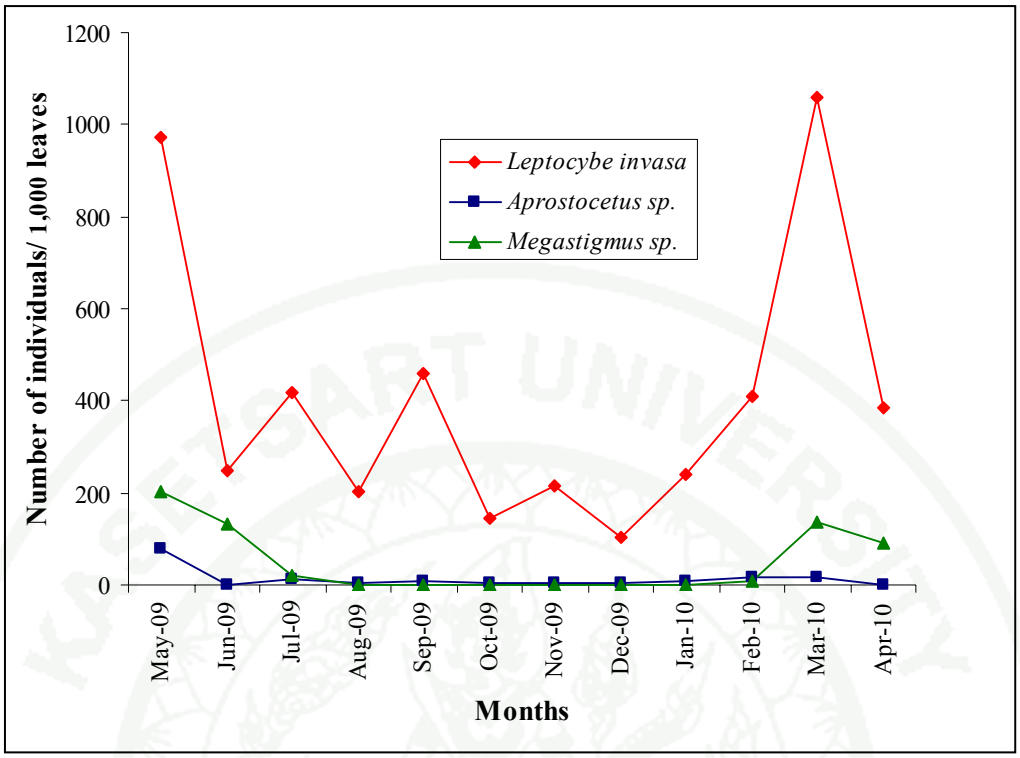

Figure 54 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by *Eucalyptus* leaf–gall sampling in Tha Muang district, from May 2009 to April 2010.

1.2 Phanom Thaun district

It was found that the populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp., in *E. camaldulensis* plantations in Phanom Thaun district, also fluctuated from month to month.

By *Eucalyptus* leaf–gall sampling, the maximum population of *L. invasa* occurred on May and, then, decreased variously from June to April. The population of *Aprostocetus* sp. was rare from May to April. The maximum population of *Megastigmus* sp. was found only on June and became rarely from July to April. The populations of *L. invasa* were larger than the populations of parasitoids in every month. The details were shown in Table 17 and Figure 55.

Table 17 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by *Eucalyptus* leaf–gall sampling in Phanom Thaun district, From May 2009 to April 2010.

Months	Number of individuals/1,000 leaves								
	Leptocybe invasa			*Aprostocetus* sp.			*Megastigmus* sp.		
	Total	Female	Male	Total	Female	Male	Total	Female	Male
May-09	2,841	2,809	32	36	18	18	189	88	101
Jun-09	567	566	1	26	14	12	406	171	235
Jul-09	219	218	1	7	6	1	14	8	6
Aug-09	130	130	0	2	2	0	2	1	1
Sep-09	355	345	10	3	2	1	0	0	0
Oct-09	181	178	3	1	1	0	8	1	7
Nov-09	297	293	4	6	2	4	0	0	0
Dec-09	89	82	7	0	0	0	0	0	0
Jan-10	107	105	2	1	1	0	0	0	0
Feb-10	297	290	7	2	2	0	0	0	0
Mar-10	519	517	2	0	0	0	3	0	3
Apr-10	209	209	0	1	0	1	6	2	4
Total [1/]	5,811	5,742	69	85	48	37	628	271	357
%	100	98.81	1.18	100	56.47	43.52	100	43.15	56.84

[1/] Total number of individuals/12,000 leaves/year.

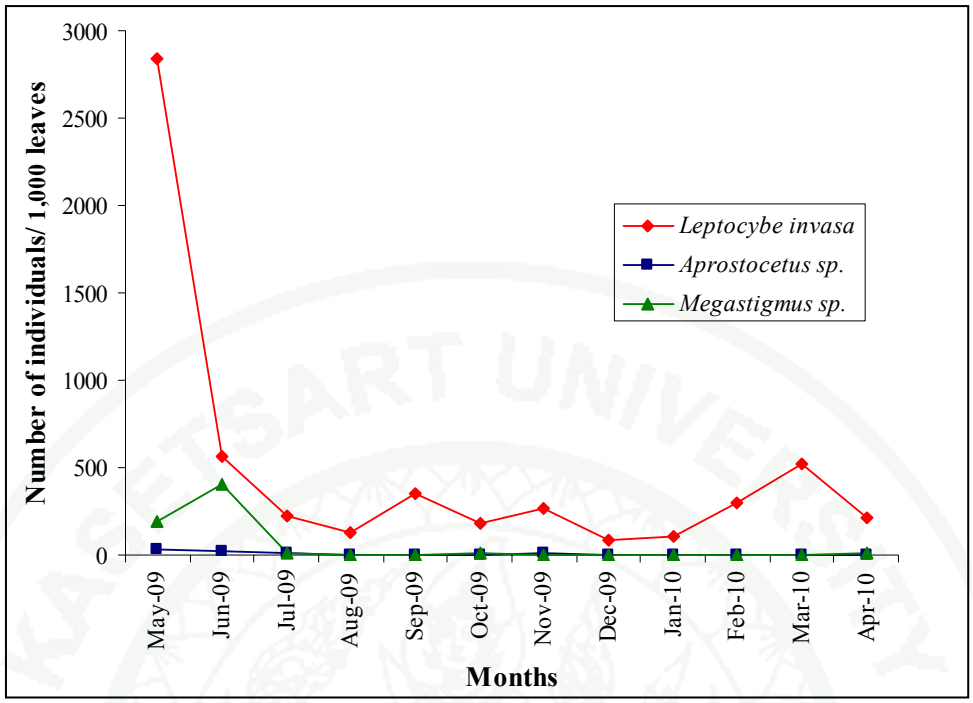

Figure 55 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by *Eucalyptus* leaf–gall sampling in Phanom Thaun district, from May 2009 to April 2010.

It was noticed that the maximum population of *L. invasa* on May was 2,841 individuals/1,000 leaves (with 2,809 females and 32 males). The maximum population of *Aprostocetus* sp. on May was 36 individuals/1,000 leaves (with 18 females and 18 males). The maximum population of *Megastigmus* sp. on June was 406 individuals/1,000 leaves (with 171 females and 235 males) (Table 17).

In the aspect of total population throughout the year, the results showed that a) the small populations of 3 species were found from July to April, b) the total population of *L. invasa* was 5,811 individuals/12,000 leaves/year (with 5,742 females and 69 males), *Aprostocetus* sp. was 85 individuals/12,000 leaves/year (with 48 females and 37 males), and *Megastigmus* sp. was 628 individuals/12,000 leaves/ year (with 271 females and 357 males), c) the females of *L. invasa* and *Aprostocetus* sp. covered a large proportion of males, and d) the fluctuation of *L. invasa* had tendency to be consistent with parasitoids (Table 17 and Figure 55).

2. Population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. by sweeping around canopy of coppice shoots of *Eucalyptus* trees

2.1 Tha Muang district

The results disclosed that the populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp., in *E. camaldulensis* plantations in Tha Muang district, fluctuated monthly.

By sweeping around canopy of coppice shoots of *Eucalyptus* trees, the maximum populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. occurred on May, and then declined and varied monthly from June to April. Only *Megastigmus* sp. was rare from August to April. The details were shown in Table 18 and Figure 56.

It was noticed on May that the maximum population of *L. invasa* was 218 individuals/10 trees (with 125 females and 93 males), while the maximum population of *Aprostocetus* sp. was 327 individuals/10 trees (with 268 females and 59 males) and that of *Megastigmus* sp. was 163 individuals/10 trees (with 91 females and 72 males) (Table 18).

In the aspect of total population throughout the year, the results showed that a) the small populations of 3 species were found from June to April, b) the total population of *L. invasa* was 903 individuals/120 trees/year (with 727 females and 176 males), *Aprostocetus* sp. was 596 individuals/120 trees/year (with 462 females and 134 males), and *Megastigmus* sp. was 203 individuals/120 trees/year (with 122 females and 81 males), c) the females of 3 species covered a large proportion of males, and d) the fluctuation of *L. invasa* had tendency to be consistent with parasitoids (Table 18 and Figure 56).

Table 18 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by sweeping in Tha Muang district, From May 2009 to April 2010.

Months	Number of individuals/10 trees								
	Leptocybe invasa			*Aprostocetus* sp.			*Megastigmus* sp.		
	Total	Female	Male	Total	Female	Male	Total	Female	Male
May-09	218	125	93	327	268	59	163	91	72
Jun-09	65	43	22	25	20	5	30	25	5
Jul-09	89	69	20	32	28	4	8	4	4
Aug-09	73	54	19	15	8	7	1	1	0
Sep-09	131	117	14	41	19	22	1	1	0
Oct-09	72	69	3	7	7	0	0	0	0
Nov-09	13	13	0	0	0	0	0	0	0
Dec-09	51	51	0	27	22	5	0	0	0
Jan-10	9	9	0	9	8	1	0	0	0
Feb-10	68	63	5	53	32	21	0	0	0
Mar-10	102	102	0	60	50	10	9	9	0
Apr-10	12	12	0	0	0	0	0	0	0
Total [1/]	903	727	176	596	462	134	203	122	81
%	100	80.50	19.49	100	77.51	22.48	100	60.09	39.90

[1/] Total number of individuals/120 trees/year.

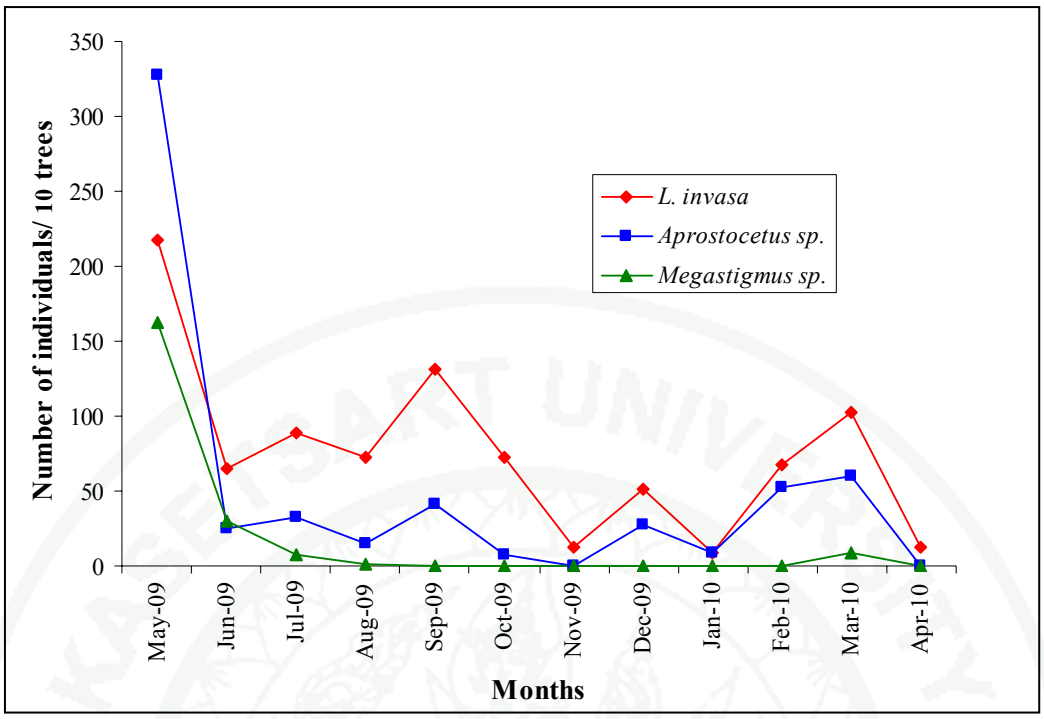

Figure 56 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by sweeping in Tha Muang district, from May 2009 to April 2010.

2.2 Phanom Thaun district

It was found that the populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp., in *E. camaldulensis* plantations in Phanom Thaun district, fluctuated from month to month.

By sweeping around canopy of coppice shoots of *Eucalyptus* trees, the maximum populations of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. occurred on May, and then declined and fluctuated monthly from June to April. Only *Megastigmus* sp. was rare from August to April. The details were shown in Table 19 and Figure 57.

It was noticed on May that the maximum population of *L. invasa* was 218 individuals per 10 trees (with 197 females and 21 males), while the maximum population of *Aprostocetus* sp. was 113 individuals per 10 trees (with 81 females and 32 males) and that of *Megastigmus* sp. was 69 individuals per 10 trees (with 29 females and 40 males) (Table 19).

Table 19 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by sweeping in Phanom Thuan district, From May 2009 to April 2010.

Months	Number of individuals/10 trees								
	Leptocybe invasa			*Aprostocetus* sp.			*Megastigmus* sp.		
	Total	Female	Male	Total	Female	Male	Total	Female	Male
May-09	218	197	21	113	81	32	69	29	40
Jun-09	99	77	22	47	29	18	17	16	1
Jul-09	73	27	46	83	58	25	7	3	4
Aug-09	27	21	6	38	21	17	2	1	1
Sep-09	58	25	33	1	0	1	1	1	0
Oct-09	114	113	1	41	35	6	1	1	0
Nov-09	46	46	0	43	39	4	0	0	0
Dec-09	28	28	0	5	4	1	0	0	0
Jan-10	8	8	0	7	5	2	0	0	0
Feb-10	3	3	0	0	0	0	0	0	0
Mar-10	44	44	0	0	0	0	0	0	0
Apr-10	2	2	0	0	0	0	0	0	0
Total [1/]	720	591	129	378	272	106	97	51	46
%	100	82.08	17.91	100	71.95	28.04	100	52.57	47.42

[1/] Total number of individuals/120 trees/year.

In the aspect of total population throughout the year, the results showed that a) the small populations of 3 species were found from June to April, b) the total population of *L. invasa* was 720 individuals/120 trees/year (with 591 females and 129 males), *Aprostocetus* sp. was 378 individuals/120 trees/year (with 272

females and 106 males), and *Megastigmus* sp. was 97 individuals/120 trees/year (with 51 females and 46 males), c) the females of 3 species covered a large proportion of males, and d) the fluctuation of *L. invasa* had tendency to be consistent with parasitoids (Table 19 and Figure 57).

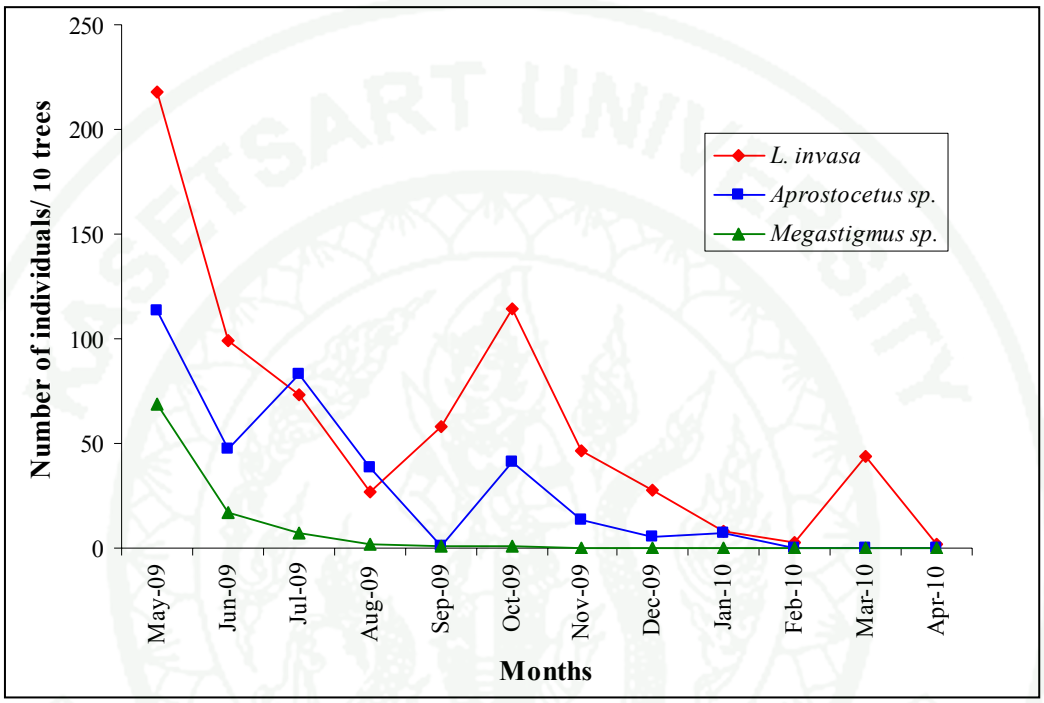

Figure 57 Number of individuals of *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp., collected per month by sweeping in Phanom Thaun district, from May 2009 to April 2010.

3. Comparison between population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp.

The population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. in Tha Muang and Phanom Thaun districts, by *Eucalyptus* leaf–gall sampling and by sweeping around canopy of coppice shoots of *Eucalyptus* tree, were compared and shown in Table 20.

Table 20 Comparison between population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. in Tha Muang and Phanom Thuan districts, by leaf–gall sampling and by sweeping.

Population dynamic	*L. invasa*	*Aprostocetus* sp.	*Megastigmus* sp.
By leaf–gall sampling in Tha Muang:			
1. Peak of population	May, Mar	May, Mar	May, Mar
2. Period of small population	Jun–Feb	Jun–Feb	Jun–Feb
3. Abundance at peak (individuals/1,000 leaves)	973	80	201
4. Total (individuals/12,000 leaves/ yr)	4,857	163	588
	F 98.74%	F 66.25%	F 42.00%
	M 1.25%	M 33.74%	M 57.99%
By leaf–gall sampling in Phanom Thuan:			
1. Peak of population	May	May	June
2. Period of small population	Jun–Apr	Jun–Apr	Jul–Apr
3. Abundance at peak (individuals/1,000 leaves)	2,841	36	189
4. Total (individuals/12,000 leaves/ yr)	5,811	85	628
	F 98.81%	F 56.47%	F 43.15%
	M 1.18%	M 43.52%	M 56.84%
By sweeping in Tha Muang:			
1. Peak of population	May	May	May
2. Period of small population	Jun–Apr	Jun–Apr	Jun–Apr
3. Abundance at peak (individuals/10 trees)	218	327	163
4. Total (individuals/120 trees/yr)	903	596	203
	F 80.50%	F 77.51%	F 60.09%
	M 19.49%	M 22.48%	M 39.90%

Table 20 (Continued)

Population dynamic	*L. invasa*	*Aprostocetus* sp.	*Megastigmus* sp.
By sweeping in Phanom Thuan:			
1. Peak of population	May	May	May
2. Period of small population	Jun–Apr	Jun–Apr	Jun–Apr
3. Abundance at peak (individuals/10 trees)	218	113	69
4. Total (individuals/120 trees/yr)	720	378	97
	F 82.08%	F 71.95%	F 52.57%
	M17.91%	M 28.04%	M 47.42%

Notes: F=Female adult; M=Male adult.

The results summarized in Table 20 showed that:

1. The maximum populations of *L. invasa*, *Aprostocetus* sp, and *Megastigmus* sp. occurred on May.

These probably dued to the large amount of precipitation on May (Figure 58 and Figure 59). After the occurrence of long dry period, the precipitation gradually increased from March until reaching the large amount of precipitation on May. *Eucalyptus camaldulensis* probably produced more new leaves on this month. More food supply on May would lead to the occurrence of maximum population of *L. invasa* and the parasitoids on this month.

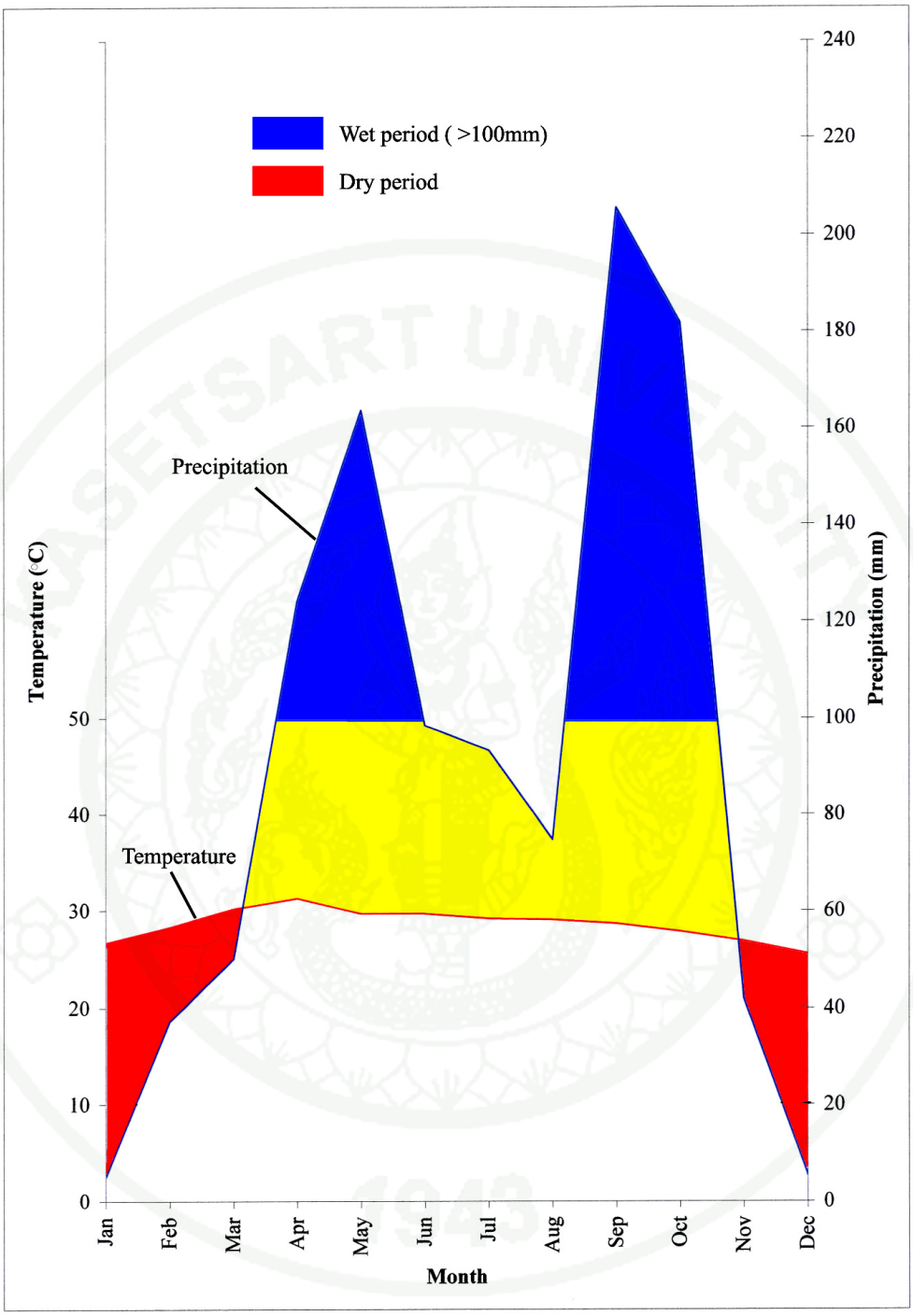

Figure 58 Climate diagram of Muang district, Kanchanaburi province, drawn according to Walter and Breckle (2002), showing distribution of monthly mean temperature and precipitation by 10 years average (1999–2008).

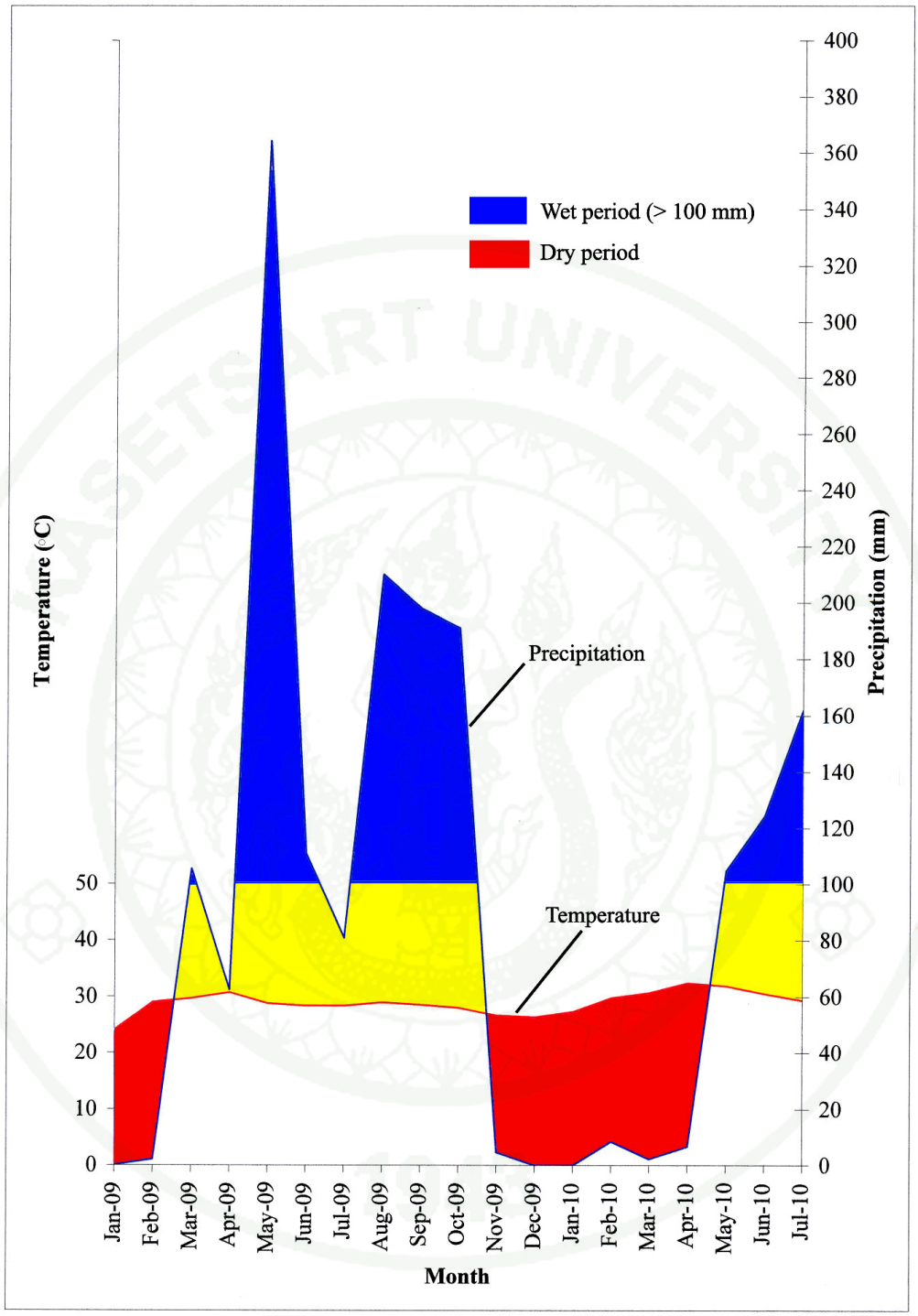

Figure 59 Climate diagram of Muang district, Kanchanaburi province, drawn according to Walter and Breckle (2002), showing distribution of monthly mean temperature and precipitation between January 2009 and July 2010.

2. After May, the precipitation was still rather high but the population of *L. invasa* decreased. This might be resulted from the severe damage of *E. camaldulensis* leaves on May. It perhaps might arise from the large amount of precipitation and strong wind. The average rainy day between May and October was more than other months (Table 1). After November was a long dry period and less new leaf production, thus this period minimized *L. invasa* and parasitoid populations.

The findings suggested whenever there was the adequate precipitation after long dry periods, the enhancement of the population of *L. invasa* and the parasitoids would occur.

There were no weather stations in Tha Muang and Phanom Thuan districts. Thus it was necessary to use climatological data of the weather station in Muang district which was the nearest station to the study areas. The data of precipitation and temperature of that station were accepted by the local people in two districts about the consistency of the data to the real happening in the study sites.

3. The population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. from leaf–gall samplings showed the extremely large number of individuals of adult *L. invasa* and extremely small number of individuals of adult parasitoids. They also showed the wonderingly monthly distributions between *L. invasa* and the parasitoids. Their wondering dynamics were probably explained by the following reasons.

a) These data were obtained from the laboratory conditions which might be their unfamiliar conditions. Leaf–gall were sampled and then only the galls were separated from leaves and were kept in plastic boxes which the humidity were controlled by soaked cottons. The inside humidity was possibly different from the atmospheric humidity in the plantations. Beside this, the other factors inside the leaf–galls might be involved and were beyond the control.

b) The leaf–gall samples were in gall–maturation stage, without emergence holes of the wasps. Naturally, the population of *L. invasa* in leaf–galls was larger than that of the parasitoids. Moreover, the developmental stages of *L. invasa* varied within each point of long gall. Thus, *Aprostocetus* sp. and *Megastigmus* sp. which parasitized on larva or pupa of *L. invasa* inside leaf–galls had also different stages of development.

The leaf–galls which were kept under laboratory conditions might change the physical and physiological properties inside the galls. These changes might affect the development of egg to larval, pupal, and to adult stages of *L. invasa* and those of the parasitoids. If the mature larvae could not develop further to pupa, the immatures of *L. invasa* and those of the parasitoids would die inside the galls. Thus they could not emerge as adults.

Although one portion of *L. invasa* inside the galls could not survive, but the large portion of *L. invasa* population remained. The reasons were that the initial population of *L. invasa* inside the galls was enormously high and higher than those parasitoids, and furthermore, it was probably that *L. invasa* was more tolerant than *Aprostocetus* sp. and *Megastigmus* sp. under warm and humid conditions in the laboratory. *Leptocybe invasa* was the alien species, it perhaps could tolerate to a wide ranges of environmental conditions.

Thus, the above explanations including the relating factors probably were the causes of occurrence of the extremely large number of individuals of *L. invasa* and the extremely small number of individuals of *Aprostocetus* sp. and *Megastigmus* sp. from leaf–gall sampling.

4. The population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. from sweeping around canopy of coppice shoots of *Eucalyptus* trees in the plantations showed a possible proportion between *L. invasa* and the parasitoids. The number of individuals distributed in each month were found in the following order; *L. invasa*>*Aprostocetus* sp.>*Megastigmus* sp. The data from sweeping could be

used as a guideline for the management of *L. invasa* in the plantations. However, it was noticed that:

a) *Leptocybe invasa*, *Aprostocetus* sp., and *Megastigmus* sp. were naturally emerging adults and were in and around coppice shoots for seeking the opportunity for oviposition. From sweeping in each month, the number of individuals of *L. invasa* were larger than *Aprostocetus* sp. and those of this parasitoid were larger than *Megastigmus* sp. The monthly fluctuation of *L. invasa* was in consistent with that of *Aprostocetus* sp. The data suggested that *Megastigmus* sp. did not ecologically succeeded in *E. camaldulensis* plantations because of its low fecundity.

b) The number of individuals of *L. invasa* and the parasitoids in Tha Muang district were larger than those in Phanom Thuan district because Tha Muang had probably more precipitation and had shorter dry period than in Phanom Thuan. The sex ratio of adult *L. invasa* and the parasitoids in the plantations in Tha Muang and Phanom Thuan were females>males.

5. From observation on every month for a year, the number of species of ground cover in the plantations in Tha Muang was richer than that in Phanom Thaun.

The ground cover species provided the habitats and food sources to the large number of insect species. The ground cover species probably provided alternative food sources, such as nectar, to prolong the longevity of *L. invasa*, *Aprostocetus* sp, and *Megastigmus* sp. Waage *et al.* (1985) reported that most parasitic Hymenoptera that did not feed on host, could naturally produce mature eggs with a source of carbohydrate, such as honey, from other sources. Jervis *et al*. (1996) reported that some parasitoid species required nutrient in the form of nectar, honey and pollen to provide their energy and nutrient for egg production in the nature. Jervis and Kid (1996) suggested that synovigenic parasitoids might exploit both host food (haemolymph) and non–host food (nectar, honey drew).

Some distinct species of vegetative ground cover in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts were shown in Appendix Figure 1 and Appendix Figure 2.

4. Similarity and species diversity of insects in Tha Muang and Phanom Thaun districts

From monthly sweeping around canopy of coppice shoots of *E. camaldulensis* trees in the plantations from May 2009–April 2010, it was discovered 208 species in Tha Muang district and 159 species in Phanom Thaun district. Sixty–one species were found in both districts.

To determine the similarity between insect species in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts, the results showed that the similarity between insect species in two study sites was only 33.00 percent.

To calculate the index of insect diversity by using Shannon–Weiner's Index of Diversity, it was found that the index of insect diversity in Tha Muang (H′= 2.300) was higher than that in Phanom Thaun (H′=2.133). Evenness Index in Tha Muang (E=0.402) was also higher than that in Phanom Thaun (E=0.373). These results were shown in Table 21.

Table 21 Shannon–Weiner's Index of Diversity and Evenness Index of insects in *Eucalyptus camaldulensis* plantations of two sites.

Site	Number of species	Number of individuals	H′ Index	Evenness Index (E)
Tha Muang	208	2,874	2.300	0.402
Phanom Thuan	159	1,933	2.133	0.373

E. camaldulensis plantations in Tha Muang district had more number of insect species, more number of individuals, higher H´ Index and Evenness Index, than those in Phanom Thuan. The possible explanations were that a) Tha Muang had more number of species of vegetative ground cover which provided habitats and food sources to insect species, and b) Tha Muang had probably shorter dry period than Phanom Thuan.

Generally, the plantation, such as *E. camaldulensis* plantation, was a monocrop system and was an imbalance ecosystem when compared to natural forest. The enhancement of species diversity of vegetative ground covers would help balance the ecosystem in the plantation at one level. They could raise the diversity of insect species in the plantations, including insect natural enemies. *Leptocybe invasa* is an exotic species and alien species in *E. camaldulensis* plantations. This wasp tolerated in diverse environments. It caused problems to the plantations because of the sufficient food supply and inadequacy of insect natural enemies. Thus, the improvement and management of *E. camaldulensis* and the enhancement of insect natural enemies would probably be the answers of this problem.

5. Other insects and their roles

From monthly sweeping around the canopy of coppice shoots of *E. camaldulensis* trees in the plantations from May 2009–April 2010, it was discovered 208 insect species in Tha Muang. They belong to 77 families and 10 orders: Coleoptera, Collembola, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Psocoptera and Thysanoptera.

It was found 159 insect species in Phanom Thuan. They belong to 58 families and 10 orders: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera, Psocoptera and Thysanoptera.

The order Hymenoptera had the highest number of species (37% in Tha Muang; and 40% in Phanom Thuan) and followed by Diptera and Hemiptera (30%

and 13% respectively in Tha Muang; and 24% and 14% respectively in Phanom Thuan). The details were shown in Figrure 60.

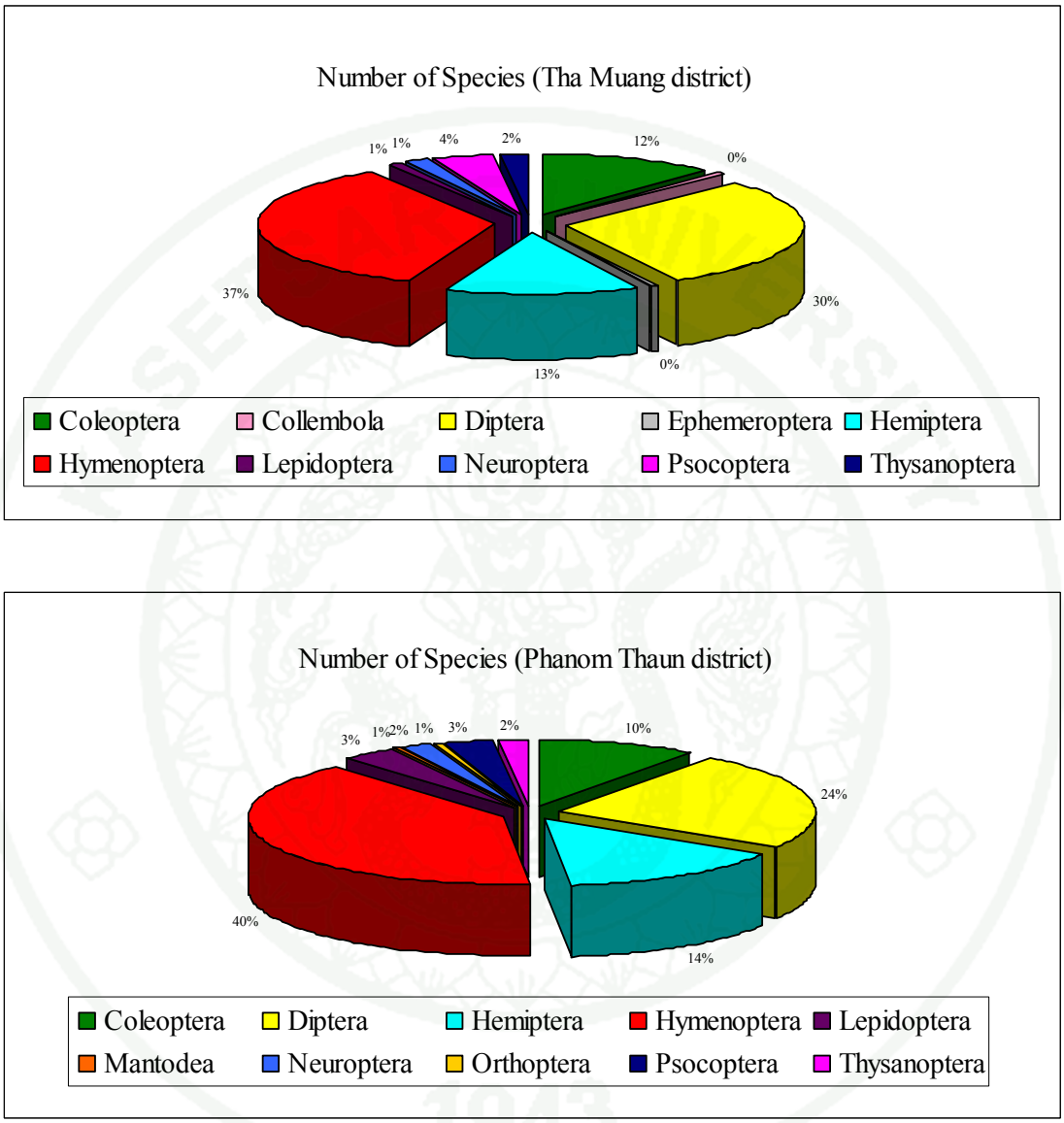

Figure 60 Proportion of insect orders with number of species (%) found in the canopies of *Eucalyptus camaldulensis* plantations: (Top) Tha Muang district; (Bottom) Phanom Thaun district.

The roles of insects in the canopies of *E. camaldulensis* plantations in the research areas, could be divided into 5 groups: a) parasitoid; b) predator; c) phytophaga; d) scavenger; and e) blood sucker.

Parasitoid had the highest number of species (65 species in Tha Muang; and 60 species in Phanom Thuan), and followed by phytophaga and scavenger (63 species and 43 species respectively in Tha Muang; 47 species and 22 species respectively in Phanom Thuan). The details were shown in Table 22 and Figure 61.

Table 22 Number of insect species and their roles in the canopies of *Eucalyptus camaldulensis* plantations in Tha Muang and Phanom Thuan districts.

Insect role	Tha Muang		Phanom Thuan	
	No. of species	%	No. of species	%
Parasitoid [1/]	65	31.25	60	37.73
Predator	19	9.13	13	8.17
Phytophaga [2/]	63	30.28	47	29.55
Scavenger	43	20.67	22	13.83
Blood–sucker	18	8.65	17	10.69
Total	208	100.00	159	100.00

[1/] Included *Aprostocetus* sp. and *Megastigmus* sp.

[2/] Included *Leptocybe invasa*.

Parasitoid is host specific. Thus *L. invasa* was only the host of *Aprostocetus* sp. and *Megastigmus* sp. Most species of blood–sucker were in Family Ceratopogonidae which were blood–sucker of mammal including human. They came from the cattle farms nearby *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts. The distinct insect species easily found in the canopies of *E. camaldulensis* plantations in two research areas are shown in Appendix Figure 3–Appendix Figure 7. Many species of spider which were the predators of *L. invasa* and other insects were shown in Appendix Figure 8.

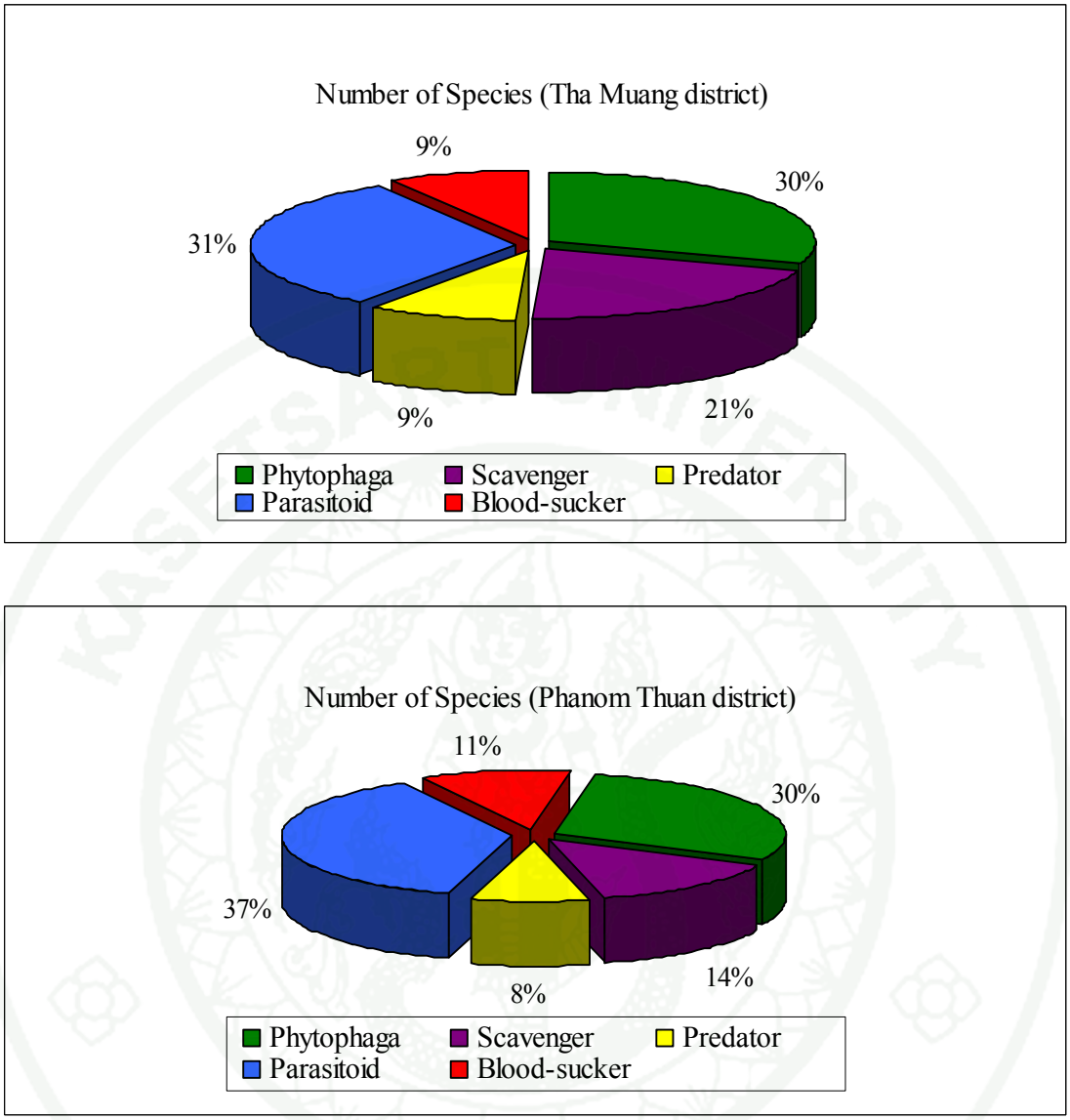

Figure 61 Proportion of insect roles with number of species (%) found in the canopies of *Eucalyptus camaldulensis* plantations: (Top) Tha Muang district; (Bottom) Phanom Thaun district.

The details of other insect species including their roles were shown in Table 23. In Tha Muang, Psyllid 2 and *Trissolcus* sp.1 had highest frequency (92.31%), and follow by Pentatomid 1 (76.92%) and *Corticaria* sp.1 (69.23%). In Phanom Thuan, *Corticaria* sp.1 had highest frequency (92.31%), and followed by Psyllid 1 and Psyllid 2 (84.62%) and *Coniopteryx* sp.1 (76.92%).

Table 23 Other insects and their roles found in the canopies of *Eucalyptus* plantations in Tha Muang and Phanom Thuan districts.

Order	Family	Scientific name	Role	TM	PT
1. Coleoptera	1.1 Anthicidae	Anthicid 1	S	✓	✓
		Anthicid 2	S		✓
	1.2 Aderidae	Aderid 1	S	✓	
		Aderid 2	S	✓	
	1.3 Bruchidae	Bruchid 1	PH	✓	
		Bruchid 2	PH	✓	
		Bruchid 3	PH	✓	✓
		Bruchid 4	PH	✓	
		Bruchid 5	PH		✓
		Bruchidius sp.1	PH	✓	
		Caryedon sp.1	PH	✓	
		Callosobruchus chinensis	PH	✓	
	1.4 Carabidae	Carabid 1	PR	✓	
	1.5 Chrysomelidae	Chrysomelid 1	PH	✓	
		Chrysomelid 2	PH	✓	
	1.6 Coccinellidae	*Cryptolaemus montrouzieri*	PR		✓
		Menochilus sexmaculatus	PR		✓
		Micraspis discolor	PR	✓	
		Stethorus sp.1	PR	✓	✓
		Stethorus sp.2	PR		✓
	1.7 Curculionidae	*Apion* sp.1	PH	✓	
		Curculionid 1	PH	✓	
		Curculionid 2	PH	✓	
		Curculionid 3	PH	✓	
		Curculionid 4	PH		✓
		Curculionid 5	PH		✓
		Hypomeces squamosus	PH		✓
		Sepiomus sp.1	PH	✓	
	1.8 Elateridae	Elaterid 1	PH	✓	
		Elaterid 2	PH	✓	
	1.9 Lathridiidae	*Corticaria* sp.1	S	✓	✓
	1.10 Melyridae	Melyrid 1	PR	✓	
	1.11 Mordellidae	Mordellid 1	PH		✓
		Mordellid 2	PH		✓
		Mordellid 3	PH		✓
	1.12 Phalacridae	Phalacrid 1	PH	✓	
	1.13 Tenebrionidae	Tenebrionid 1	S		✓
2. Collembola	2.1 Entomobryidae	Entomobryid 1	S	✓	
3. Diptera	3.1 Agromyzidae	Agromyzid 1	PH	✓	✓
		Agromyzid 2	PH	✓	✓

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
3. Diptera	3.1 Agromyzidae	Agromyzid 3	PH		✓
		Agromyzid 4	PH		✓
		Agromyzid 5	PH	✓	
		Agromyzid 6	PH	✓	
	3.2 Anthomyiidae	Anthomyiid 1	PH	✓	
		Anthomyiid 2	PH	✓	
	3.3 Asilidae	Asilid 1	PR		✓
	3.4 Calliphoridae	Calliphorid 1	S	✓	
	3.5 Cecidomyiidae	Cecidomyiid 1	PH	✓	
		Cecidomyiid 2	PH	✓	
		Cecidomyiid 3	PH	✓	
		Cecidomyiid 4	PH	✓	
		Cecidomyiid 5	PH	✓	✓
		Cecidomyiid 6	PH		✓
	3.6 Celyphidae	*Celyphus* sp.1	S		✓
	3.7 Ceratopogonidae	Ceratopogonid 1	BS	✓	✓
		Ceratopogonid 2	BS	✓	✓
		Ceratopogonid 3	BS	✓	✓
		Ceratopogonid 4	BS	✓	
		Ceratopogonid 5	BS	✓	
		Ceratopogonid 6	BS	✓	
		Ceratopogonid 7	BS	✓	✓
		Ceratopogonid 8	BS	✓	
		Ceratopogonid 9	BS		✓
		Ceratopogonid 10	BS		✓
		Ceratopogonid 11	BS	✓	✓
		Ceratopogonid 12	BS		✓
		Ceratopogonid 13	BS		✓
		Ceratopogonid 14	BS	✓	✓
		Culicoides sp.1	BS	✓	✓
		Culicoides sp.2	BS	✓	
		Culicoides sp.3	BS	✓	
		Forcipomyia sp.1	BS	✓	✓
		Forcipomyia sp.2	BS	✓	✓
		Forcipomyia sp.3	BS		✓
		Leptoconops sp.1	BS	✓	
		Stilobezzia sp.1	BS	✓	✓
		Stilobezzia sp.2	BS	✓	✓
		Stilobezzia sp.3	BS		✓
	3.8 Chironomidae	Chironomid 1	S	✓	✓
		Chironomid 2	S	✓	
		Chironomid 3	S	✓	

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
3. Diptera	3.8 Chironomidae	Chironomid 4	S	✓	✓
		Chironomid 5	S		✓
		Chironomid 6	S		✓
		Chironomid 7	S		✓
	3.9 Chloropidae	Chloropid 1	S	✓	✓
		Chloropid 2	S	✓	
		Chloropid 3	S		✓
	3.10 Culicidae	Culicid 1	BS	✓	
	3.11 Dolichopodidae	Dolichopodid 1	PR	✓	
		Dolichopus sp.1	PR	✓	
		Dolichopus sp.2	PR	✓	✓
	3.12 Drosophilidae	*Drosophila* sp.1	S	✓	
		Drosophila sp.2	S	✓	
	3.13 Lauxaniidae	Lauxaniid 1	S	✓	
		Lauxaniid 2	S	✓	
	3.14 Lonchaeidae	Lonchaeid 1	S	✓	
		Lonchaeid 2	S	✓	
	3.15 Muscidae	*Musca domestica*	S		✓
	3.16 Phoridae	Phorid 1	S	✓	✓
		Phorid 2	S	✓	
	3.17 Platystomatidae	Platystomatid 1	S	✓	
	3.18 Psychodidae	Psychodid1	S	✓	
	3.19 Scatopsidae	Scatopsid 1	S	✓	
		Scatopsid 2	S	✓	
	3.20 Sciaridae	Sciarid 1	S	✓	✓
		Sciarid 2	S	✓	
		Sciarid 3	S	✓	
	3.21 Simuliidae	Simuliid 1	S	✓	
	3.22 Stratiomyidae	Stratiomyid 1	S	✓	
		Stratiomyid 2	S	✓	
		Stratiomyid 3	S	✓	
	3.23 Tachinidae	Tachinid 1	PA	✓	
	3.24 Tephritidae	Tephritid 1	PH	✓	
	3.25 Tipulidae	Tipulid 1	S	✓	
		Tipulid 2	S		✓
		Tipulid 3	S		✓
4. Ephemeroptera	4.1 Beatidae	Beatid 1	S	✓	
5. Hemiptera	5.1 Aleyrodidae	Aleyrodid 1	PH		✓
	5.2 Aphididae	Aphidid 1	PH	✓	
		Aphidid 2	PH		✓
		Aphidid 3	PH		✓
		Aphidid 4	PH		✓

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
5. Hemiptera	5.3 Cercopidae	Cercopid 1	PH	✓	
		Cercopid 2	PH	✓	
		Cercopid 3	PH		✓
	5.4 Cicadellidae	Cicadellid 1	PH	✓	✓
		Cicadellid 2	PH		✓
		Cicadellid 3	PH	✓	✓
		Cicadellid 4	PH		✓
		Cicadellid 5	PH	✓	✓
		Cicadellid 6	PH	✓	
		Cicadellid 7	PH	✓	
	5.5 Coreidae	Coreid 1	PH	✓	
		Coreid 2	PH	✓	
	5.6 Delphacidae	Delphacid 1	PH	✓	
		Delphacid 2	PH	✓	
		Delphacid 3	PH		✓
	5.7 Flatidae	Flatid 1	PH	✓	✓
	5.8 Geocoridae	Geocorid 1	PH	✓	
	5.9 Hebridae	Hebrid 1	PH		✓
	5.10 Lygaenidae	Lygaenid 1	PH		✓
	5.11 Meenoplidae	Meenoplid 1	PH	✓	
	5.12 Membracidae	*Leptocentrus* sp.1	PH	✓	
	5.13 Miridae	*Cyrtorhinus livinipennis*	PH	✓	✓
		Mirid 1	PH	✓	✓
		Mirid 2	PH	✓	✓
	5.14 Pentatomidae	Pentatomid 1	PH	✓	✓
	5.15 Plataspidae	Plataspid 1	PH	✓	
	5.16 Pseudococcidae	Pseudococcid 1	PH	✓	
		Pseudococcid 2	PH	✓	
	5.17 Psyllidae	Psyllid 1	PH	✓	✓
		Psyllid 2	PH	✓	✓
	5.18 Ricaniidae	*Ricania speculum*	PH	✓	
		Ricaniid 1	PH	✓	
	5.19 Scutelleridae	Scutellerid 1	PH		✓
		Chrysocoris stollii	PH		✓
	5.20 Tingidae	Tingid 1	PH		✓
6. Hymenoptera	6.1 Bethylidae	Bethylid 1	PA	✓	
		Bethylid 2	PA	✓	
	6.2 Braconidae	*Bracon* sp.1	PA	✓	
		Bracon sp.2	PA		✓
		Bracon sp.3	PA		✓
		Braconid 1	PA	✓	
		Braconid 2	PA		✓

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
6. Hymenoptera	6.2 Braconidae	Braconid 3	PA		✓
		Microplitis sp.1	PA		✓
		Phanerotoma sp.1	PA	✓	
		Spathius sp.1	PA	✓	
	6.3 Ceraphronidae	Ceraphronid 1	PA	✓	✓
		Ceraphronid 2	PA	✓	✓
		Ceraphronid 3	PA	✓	
		Ceraphronid 4	PA	✓	
	6.4 Chalcididae	*Brachymeria* sp.1	PA		✓
	6.5 Dryinidae	*Dryinus* sp.1	PA	✓	
	6.6 Elasmidae	*Elasmus* sp.1	PA		✓
		Elasmus sp.2	PA		✓
		Elasmus sp.3	PA	✓	✓
		Elasmus sp.4	PA		✓
	6.7 Encyrtidae	*Anagyrus* sp.1	PA	✓	
		Anagyrus sp.2	PA	✓	
		Anagyrus dactylopii	PA		✓
		Bacalusa sp.1	PA		✓
		Cheilonerus sp.1	PA	✓	
		Cremesina sp.1	PA		✓
		Encyrtid 1	PA	✓	
		Encyrtid 2	PA	✓	
		Encyrtid 3	PA	✓	
		Encyrtid 4	PA	✓	
		Encyrtid 5	PA	✓	
		Encyrtid 6	PA		✓
		Encyrtid 7	PA		✓
		Gyranusoidea tebygi	PA	✓	
		Leptomastidea sp.1	PA		✓
		Metaphycus sp.1	PA	✓	
		Metaphycus sp.2	PA		✓
		Psyllaephagus sp.1	PA	✓	✓
	6.8 Eulophidae	*Asecodes* sp.1	PA		✓
		Aprostocetus sp.1	PA	✓	✓
		Eulophid 1	PA	✓	✓
		Eulophid 2	PA	✓	
		Eulophid 3	PA		✓
		Eulophid 4	PA		✓
		Eulophid 5	PA		✓
		Eulophid 6	PA		✓
		Eulophid 7	PA		✓

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
6. Hymenoptera	6.8 Eulophidae	Eulophid 8	PA	✓	
		Euplectrus sp.1	PA	✓	✓
		Euplectrus sp.2	PA	✓	
		Euplectrus sp.3	PA	✓	✓
		Leptocybe invasa	PH	✓	✓
		Pediobius sp.1	PA	✓	✓
		Pediobius sp.2	PA	✓	✓
		Quadrastichus sp.1	PA	✓	
		Quadrastichus sp.2	PA	✓	
		Quadrastichus sp.3	PA	✓	
		Quadrastichus sp.4	PA	✓	
		Quadrastichus sp.5	PA		✓
		Quadrastichus sp.6	PA		✓
		Quadrastichus sp.7	PA		✓
		Quadrastichus sp.8	PA		✓
	6.9 Eupelmidae	*Anastatus* sp.1	PA	✓	✓
		Eupelmus sp.1	PA		✓
	6.10 Eurytomidae	*Eurytoma* sp.1	PA	✓	✓
	6.11 Figitidae	Figitid 1	PA	✓	
		Figitid 2	PA	✓	✓
		Figitid 3	PA		✓
	6.12 Formicidae	*Anoplolepis gracilipes*	PR		✓
		Camponotus sp.1	PR	✓	
		Camponotus sp.2	PR		✓
		Formicid 1	PR	✓	
		Formicid 2	PR	✓	
		Formicid 3	PR	✓	
		Monomorium destructor	PR	✓	
		Monomorium floricola	PR	✓	
		Paratrechina longicornis	PR	✓	✓
		Paratrechina sp.1	PR	✓	
		Tapinoma melanocephalus	PR	✓	
	6.13 Mymaridae	Mymarid 1	PA	✓	✓
	6.14 Plastygastridae	Plastygastrid 1	PA	✓	
	6.15 Pteromalidae	*Dinarmus* sp.1	PA		✓
		Pteromalid 1	PA	✓	
		Pteromalid 2	PA	✓	
		Pteromalid 3	PA	✓	✓
		Pteromalid 4	PA		✓
		Pteromalid 5	PA		✓
		Pteromalid 6	PA		✓

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
6. Hymenoptera	6.15 Pteromalidae	*Trichomalopsis* sp.1	PA	✓	
	6.16 Scelionidae	*Ceratobaeus* sp.1	PA		✓
		Ceratobaeus sp.2	PA	✓	
		Doddiella sp.1	PA	✓	✓
		Idris sp.1	PA	✓	✓
		Idris sp.2	PA	✓	
		Idris sp.3	PA	✓	
		Idris sp.4	PA		✓
		Odontacolus sp.1	PA		✓
		Scelio sp.1	PA	✓	✓
		Scelio sp.2	PA		✓
		Scelio sp.3	PA		✓
		Scelionid 1	PA	✓	
		Scelionid 2	PA	✓	
		Scelionid 3	PA		✓
		Telenomus sp.1	PA	✓	✓
		Telenomus sp.2	PA	✓	
		Telenomus sp.3	PA	✓	
		Trissolcus sp.1	PA	✓	✓
		Trissolcus sp.2	PA	✓	
	6.17 Signiphoridae	Signiphorid 1	PA	✓	
	6.18 Sphecidae	Sphecid 1	PA	✓	
	6.19 Tiphiidae	Tiphiid 1	PA	✓	
		Tiphiid 2	PA	✓	
	6.20 Torymidae	*Megastigmus* sp.1	PA	✓	✓
		Podagrion sp.1	PA		✓
7. Lepidoptera	7.1 Cosmopterigidae	Cosmopterigid 1	PH		✓
	7.2 Pterophoridae	Pterophorid 1	PH		✓
	7.3 Tortricidae	Tortricid 1	PH	✓	✓
		Tortricid 2	PH	✓	
		Tortricid 3	PH		✓
		Strepsicrates sp.1	PH		✓
8. Mantodea	8.1 Mantidae	Mantid 1	PR		✓
9. Neuroptera	9.1 Chrysopidae	*Chrysopa* sp.1	PR		✓
	9.2 Coniopterygidae	*Coniopteryx* sp.1	PR	✓	✓
		Coniopteryx sp.2	PR	✓	
	9.3 Hemerobiidae	*Micromus* sp.1	PR		✓
		Hemerobiid 1	PR	✓	
10. Orthoptera	10.1 Gryllidae	*Oecanthus* sp.1	PH		✓
11. Psocoptera	11.1 Archipsocidae	Archipsocid 1	S	✓	
	11.2 Caeciliusidae	Caeciliusid 1	S	✓	

Table 23 (Continued)

Order	Family	Scientific name	Role	TM	PT
11. Psocoptera	11.2 Caeciliusidae	Caeciliusid 2	S	✓	
		Caeciliusid 3	S		✓
		Caeciliusid 4	S		✓
	11.3 Trichopsocidae	Trichopsocid 1	S	✓	
		Trichopsocid 2	S	✓	
		Psocop 1	S	✓	
		Psocop 2	S	✓	
		Psocop 3	S	✓	✓
		Psocop 4	S	✓	
		Psocop 5	S		✓
		Psocop 6	S		✓
12. Thysanoptera	12.1 Phlaeothripidae	Phlaeothripid 1	PH	✓	✓
		Phlaeothripid 2	PH	✓	
	12.2 Thripidae	Thripid 1	PH	✓	
		Thripid 2	PH	✓	
		Thripid 3	PH		✓
		Thripid 4	PH		✓

Denoted: PA=Parasitoid; PR=Predator; PH=Phytophaga; S=Scavenger; BS=Blood–sucker; TM=Tha Muang district; PT=Phanom Thuan district

CONCLUSION AND RECOMMENDATIONS

Conclusion

The findings from this research were concluded in sequence of the objectives as follows.

Biological Aspect of Eucalyptus Gall Wasp, *Leptocybe invasa* Fisher & La Salle

1. Morphology of adult *L. invasa* (Hymenoptera: Eulophidae)

L. invasa is a small insect, less than 1.50 mm in length by average from head to abdomen tip. This research found both female and male *L. invasa* in *Eucalyptus camaldulensis* plantations in Tha Muang and Phanom Thuan districts, Kanchanaburi province. The female *L. invasa* differed from the male in antenna, forewing, gaster shape, and size. Their detailed characteristics were described.

2. Longevity of *L. invasa*

Mean longevity of adult female and male *L. invasa*: Their mean longevities were rather short. In the laboratory, feeding with 6 different diets showed that honey solution could prolong largest mean longevity with 7.67 days for female and 5.67 days for male. Different diets had significant effects on the means of longevity of female and male at P=0.05 (female: F=27.434; df =5; P–value=0.000; Male: F=64.463; df =5; P–value=0.000).

Survival patterns of adult female and male *L. invasa*: Feeding with 6 different diets in the laboratory indicated that the longest survival of *L. invasa* was among the females that were fed with honey solution, ranging from 1 to 13 days. Estimated 50% female survival period was 5 days. The longest survival was among the males that were fed with honey solution, ranging from 1 to 8 days. Estimated 50% male survival period was 4 days.

3. Fecundity of *L. invasa*

Potential fecundity of *L. invasa*: In the laboratory, all eggs in ovaries of newly emerging female *L. invasa* were mature. The average potential fecundity of the female *L. invasa*, of all sizes and ages, was 158.70±4.62 eggs per female, ranging from 39 to 298 eggs per female.

To use hind tibia length as a substitute for female size, the average of all sizes of female *L. invasa* was 0.28 mm, ranging from 0.20–0.35 mm. The percentage of small size:medium size:large size were 17.55:48.58:33.86 respectively. These indicated that most female *L. invasa* in *E. camaldulensis* in Tha Muang and Phanom Thuan were medium size (0.25–0.29 mm) and large size (0.30 and over).

Increasing of female size tended to increase potential fecundity or to produce more egg loads. By ANOVA regression analysis, female sizes (1 day–old age) had significant effects on mean egg loads at P=0.05 (F=532.257; P–value= 0.000). There was significantly positive relationship between female sizes and egg loads ($y=1578.834x – 283.230$; $R^2=0.772$; n=159). In contrast, the ages of females had no effect on egg loads at P=0.05 (F=0.281; df =4; P–value=0.890).

Realized fecundity of *L. invasa*: In ventilated greenhouse, all eggs in ovaries of newly emerging female *L. invasa* were mature. The female oviposited on the first day after her emergence and lasted on the sixth day. The mean progeny/ female was maximum on the first day with 30.47±7.41 progenies/female and declined in the following days. The mean of realized fecundity of a female from the first day to the sixth day was 61.53±8.94 progenies per a female.

The results indicated that *L. invasa* was a pro–ovigenic species. The sex ratio of female progenies:male progenies was nearly 2:1. The reproductive mode of *L. invasa* was deuterotoky.

4. Reproductive organs of *L. invasa*

The reproductive organs of female *L. invasa* consisted mainly of a pair of ovaries and lateral oviducts, a common oviduct, a pair of accessory glands, a spermatheca, an ovipositor, and a pair of ovipositor sheaths. The reproductive organs of male *L. invasa* composed mainly of a pair of testes, vasa deferentia and seminal vasicles, an ejaculatory duct, a pair of accessory glands, digitae and parameres, and an aedeagus.

5. Development of *L. invasa*

Oviposition behavior and egg development of *L. invasa*: The adult female *L. invasa* oviposited since the first day of her emergence on petioles and midribs of newly developed leaves, or young twigs of *E. camaldulensis*. The female oviposited eggs closely to/ or in vascular bundle tissues. Development of eggs in vascular bundles stimulated the development of galls. Later, the gall formed typical bump–shaped. Heavy galling prevented further development and growth of infested trees.

Development time from eggs to adult emergence of *L. invasa*: Development of *L. invasa* was divided into 5 stages as follows: egg, young larva, mature larva to prepupa, pupa, and adult. In ventilated greenhouse, mean development time from eggs to adults was 45.96±0.52 days, ranging from 35 to 73 days. The development time was minimum in egg stage and maximum in larval stage and in pupal stage.

There were the relationships between gall and leaf developments of *E. camaldulensis* and immature development of *L. invasa* in leaf gall. Measurement of gall size or leaf area could estimate the developmental stage of immature *L. invasa* inside the leaf gall.

Young leaves of small coppice shoots of *E. camaldulensis* were seriously damaged by *L. invasa*. If any coppice shoots had some opportunities or

some conditions to grow up to six meters height or above, their young leaves and mature leaves at this height became tolerate to the attack of *L. invasa*.

Biological Aspect of Parasitoids of *Leptocybe invasa* Fisher & La Salle

This research found two species of parasitoids in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts, Kanchanaburi province. They were *Aprostocetus* sp. and *Megastigmus* sp. which were the parasitoids of *L. invasa*. These two parasitoids were found both female and male.

1. Morphologies of adult *Aprostocetus* sp. and *Megastigmus* sp.

Morphology of adult *Aprostocetus* sp. (Hymenoptera: Eulophidae): *Aprostocetus* sp. was a small insect, with less than 1.50 mm in length by average from head to abdomen tip. The female differed from the male in antenna, thorax, body color, and size. Their detailed characteristics were described.

Morphology of adult *Megastigmus* sp. (Hymenoptera: Torymidae): *Megastigmus* sp. was a small insect, with less than 1.50 mm in length by average from head to abdomen tip. The female differed from the male in forewing, thorax, body color, and size. Their detailed characteristics were described.

Megastigmus sp. found in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan districts was rather different from *Megastigmus thailandiensis*, and *M. thitipornae* which are the new species of Thailand.

2. Longevity of *Aprostocetus* sp. and *Megastigmus* sp.

2.1 Longevity of *Aprostocetus* sp.

Mean longevity of adult female and male *Aprostocetus* sp.: The mean longevity of female and male *Aprostocetus* sp. were long. In the laboratory, feeding

with 6 different diets showed that honey solution could prolong largest mean longevity with 18.67 days for female and 13.33 days for male. Different diets had significant effects on the means of longevity of female and male at P=0.05 (Female: F=63.270; df =5; P–value=0.000; Male: F=50.386; df =5; P–value=0.000).

Survival patterns of adult female and male *Aprostocetus* sp.: Feeding with 6 different diets in the laboratory indicated that the longest survival of *Aprostocetus* sp. was among the females that were fed with honey solution, ranging from 1 to 26 days. Estimated 50% female survival period was 12 days. The longest survival was among the males that were fed with honey solution, ranging from 1 to 18 days. Estimated 50% male survival period was 9 days.

2.2 Longevity of *Megastigmus* sp.

Mean longevity of adult female and male *Megastigmus* sp.: The mean longevity of female and male *Megastigmus* sp. were moderate. In the laboratory, feeding with 6 different diets indicated that honey solution could prolong largest mean longevity with 9.83 days for female and 7.83 days for male. Different diets had significant effects on the means of longevity of female and male at P=0.05 (Female: F=170.294; df =5; P–value=0.000; Male: F=128.491; df =5; P–value=0.000).

Survival patterns of adult female and male *Megastigmus* sp.: Feeding with 6 different diets in the laboratory indicated that the longest survival of *Megastigmus* sp. was among the females that were fed with honey solution, ranging from 1 to 13 days. Estimated 50% female survival period was 8 days. The longest survival was among the males that were fed with honey solution, ranging from 1 to 10 days. Estimated 50% male survival period was 4 days.

3. Fecundity of *Aprostocetus* sp. and *Megastigmus* sp.

3.1 Fecundity of *Aprostocetus* sp.

Potential fecundity of *Aprostocetus* sp.: In the laboratory, all eggs in ovaries of newly emerging female *Aprostocetus* sp. were immature. Some eggs in ovaries were mature when the females were 1 day–old after emergence. The average potential fecundity of the female *Aprostocetus* sp., of all sizes and ages, was 6.31±0.23 eggs per female, ranging from 2 to 11 eggs per female.

To use hind tibia length as a substitute for female size, the average of all sizes of female *Aprostocetus* sp. was 0.26 mm, ranging from 0.20–0.30 mm.

Increasing of female size tended to increase potential fecundity or to produce more egg loads. By ANOVA regression analysis, female sizes of all ages had significant effects on mean egg loads at P=0.05 (F=29.345; P–value=0.000). There was positive relationship between female sizes and egg loads of female *Aprostocetus* sp. ($y=43.589x - 5.133$; $R^2=0.250$; n=90). In contrast, means of egg loads in different ages (days after exclosion) were different but not significantly at P=0.05 (F=1.390; df =5; P–value=0.236).

Realized fecundity of *Aprostocetus* sp.: In ventilated greenhouse, all eggs in ovaries of newly emerging female *Aprostocetus* sp. were immature. Some eggs in ovaries were mature when the females were 1 day–old after emergence, but continued to mature in the next days.

The female *Aprostocetus* sp. started to oviposit when the female were 1 day–old after her emergence and lasted on the eighteenth day. The mean progeny/female gradually increased and was maximum on the ninth day with 9.60±1.24 progenies per female, and then gradually declined in the later days. The

mean realized fecundity of a female from the first day to the eighteenth day was 51.10±3.28 progenies per female.

The results indicated that *Aprostocetus* sp. was a synovigenic species. The synovigenic species were more effective in biological control because they were lived longer and could reproduce at lower density of the host population. The sex ratio in progenies of *Aprostocetus* sp. was called male–biased sex ratio. The reproductive mode of *Aprostocetus* sp. was arrhenotoky.

3.2 Fecundity of *Megastigmus* sp.

Potential fecundity of *Megastigmus* sp.: In the laboratory, all eggs in ovaries of newly emerging female *Megastigmus* sp. were immature. Some eggs in ovaries were mature when the females were 2 days–old after emergence. The average potential fecundity of the female *Aprostocetus* sp., of all sizes and ages, was 2.98±0.11 eggs per female, ranging from 1 to 5 eggs per female.

To use hind tibia length as a substitute for female size, the average of all sizes of female *Megastigmus* sp. was 0.31 mm, ranging from 0.25 – 0.36 mm.

Increasing of female size tended to increase potential fecundity or to produce more egg loads. By ANOVA regression analysis, female sizes of all ages had significant effects on mean egg loads at P=0.05 (F=13.569; P–value=0.000). There was positive relationship between female sizes and egg loads of female *Megastigmus* sp. ($y=15.704x - 1.784$; $R^2=0.156$; n=75). In contrast, means of egg loads in different ages (days after exclosion) were different but not significantly at P=0.05 (F=0.308; df = 4; P–value=0.872).

Realized fecundity of *Megastigmus* sp.: In ventilated greenhouse, all eggs in ovaries of newly emerging female *Megastigmus* sp. were immature. Some

eggs in ovaries were mature when the females were 2 days–old after emergence, but continued to mature in the next days.

The female *Megastigmus* sp. started to oviposit when the female were 2 days–old after her emergence and lasted on the tenth day. The mean progeny/female gradually increased and was maximum on the fourth day with 2.87±0.55 progenies per female, and then gradually declined in the later days. The mean realized fecundity of a female from the second day to the tenth day was 13.20±1.95 progenies per female.

The results indicated that *Megastigmus* sp. was a synovigenic species. The sex ratio in progenies of *Megastigmus* sp. was called male–biased sex ratio. The reproductive mode of *Megastigmus* sp. was arrhenotoky.

4. Reproductive organs of *Aprostocetus* sp. and *Megastigmus* sp.

The reproductive organs of female and male *Aprostocetus* sp. and those of female and male *Megastigmus* sp. comprised mainly of the organs which were similar to the reproductive organs of female and male *L. invasa*.

5. Development of *Aprostocetus* sp. and *Megastigmus* sp.

5.1 Development of *Aprostocetus* sp.

Oviposition behavior and egg development of *Aprostocetus* sp.: The adult female *Aprostocetus* sp. oviposited in mature larva and in pupa of *L. invasa* since the female was 1 day–old after her emergence. The female inserted ovipositor into the leaf–gall where the immatures of *L. invasa* lived inside and laid a single egg in the mature larva or in the pupa of *L. invasa*.

The single egg of female *Aprostocetus* sp. developed as a solitary endoparasitoid. The mature larva or pupa of *L. invasa* parasitized by

Aprostocetus sp. gradually collapsed. The larva of this parasitoid developed in leaf–gall until reaching adult stage and then it emerged from the leaf–gall.

Development time from egg to adult emergence of *Aprostocetus* sp.: In ventilated greenhouse, the mean development time of *Aprostocetus* sp. from eggs (oviposited in mature larva of *L. invasa* in leaf–gall) to adult emergence (adult *Aprostocetus* sp. emerging from leaf–gall) was 12.92±0.29 days, ranging from 10 to 19 days. The development time was minimum in egg stage and maximum in larval stage and in pupal stage.

5.2 Development of *Megastigmus* sp.

Oviposition behavior and egg development of *Megastigmus* sp.: The adult female *Megastigmus* sp. oviposited on mature larva and on pupa of *L. invasa* since the female was 2 days–old after her emergence. The female inserted ovipositor into the leaf–gall where the immatures of *L. invasa* lived inside and laid a single egg on the mature larva or on the pupa of *L. invasa*.

The single egg of female *Megastigmus* sp. developed as a solitary ectoparasitoid. The mature larva or the pupa of *L. invasa* parasitized by *Megastigmus* sp. gradually collapse and dried out. The larva *Megastigmus* sp. developed in leaf–gall until reaching adult stage and then it emerged from the leaf–gall.

Development time from egg to adult emergence of *Megastigmus* sp.: In ventilated greenhouse, the mean development time of *Megastigmus* sp. from eggs (oviposited on mature larva of *L. invasa* in leaf–gall) to adult emergence (adult *Megastigmus* sp. emerging from leaf–gall) was 17.00±0.44 days, ranging from 12 to 32 days. The development time was minimum in egg stage and maximum in larval stage and in pupal stage.

Population Dynamics of *L. invasa* and the Parasitoids in Two Districts

The population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. were carried out from May 2009 to April 2010, and were assessed by two methods; namely *Eucalyptus* leaf–gall sampling and sweeping around canopy of coppice shoots of *Eucalyptus* trees.

By *Eucalyptus* leaf–gall sampling, the populations of *L. invasa* and two parasitoids in *E. camaldulensis* plantations in Tha Muang and Phanom Thuan had monthly variation. The population peak of 3 species in Tha Muang and Phanom Thuan occurred on May (after long dry period) and tended to decline later in rainy period. The sex ratio among progenies of each species was female>male, except *Megastigmus* sp.

By sweeping around canopy of coppice shoots, the populations of 3 species in *E. camaldulensis* plantations in Tha Muang and Phanom Thaun also fluctuated monthly. The population peak of each species also occurred on May and tended to decline later in the rainy period. The sex ratio among progenies of each species was female>male.

The similarity between insect species in Tha Muang and that in Phanom Thuan was only 33.00%. The index of insect diversity in Tha Muang (H′=2.300) was higher than that in Phanom thuan (H′=2.133). Evenness index in Tha Muang (E=0.402) was also higher than that in Phanom Thuan (E=0.373).

It was found 208 insect species from sweeping around canopy of coppice shoots in Tha Muang and 159 insect species in Phanom Thuan. The order Hymenoptera had the highest number of species. The number of insect species and their roles in *E. camaldulensis* plantations in two districts, were also assessed. Most insect species played their roles as parasitoids (with 31.25% in Tha Muang and 37.73% in Phanom Thuan) and small number of insect species played their roles as predator.

After a long search for the prior reports from many countries, all results obtained from this research could be divided into two groups. The findings which were new and reported for the first time were as followed: the longevity of adult male *L. invasa*; the potential fecundity and the realized fecundity of adult female *L. invasa*; the longevity, potential fecundity, realized fecundity, and the development time of female *Aprostocetus* sp. and *Megastigmus* sp.

The findings which were the new records of Thailand were as followed: the discovery of female and male *L. invasa* with their morphological descriptions; the longevity of female and male, the potential fecundity, and the realized fecundity of female *L. invasa*; the discovery of *Aprostocetus* sp. and *Megastigmus* sp. with their female and male descriptions in Kanchanaburi province; the longevity of female and male; the potential fecundity, realized fecundity, and the development time of female *Aprostocetus* sp. and *Megastigmus* sp.

Recommendations

Recommendations for Practical Uses

The findings from this research provide a wide knowledge of the biological aspect of *L. invasa* and its parasitoids in *E. camaldulensis* plantations (CT 76 clone) in Kanchanaburi province, Thailand. Many findings from this research can be practically or partly used in the integrated management or in the biological control of *L. invasa.*

1. The study on the biological aspects of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. were carried out in the laboratory and in the ventilated greenhouse. They covered the mean longevity of adult female and male, fecundity of adult female, life–history strategy, reproductive mode, and mean development time from eggs to adult. Based on these biological results, they suggest that:

1.1 *Aprostocetus* sp. has more potential to control *L. invasa*, while *Megastigmus* sp. has less potential in this matter. However, the potential combination of these two parasitoids should carry out in the biocontrol of *L. invasa*.

1.2 To feed with honey solution could extend the longevity and fecundity of female and male *Aprostocetus* sp. and *Megastigmus* sp. The data will be useful for mass production of these parasitoids in the laboratory and then they will be introduced to control *L. invasa* in *E. camaldulensis* plantations. These are possible because there are alternative food sources, such as nectar, from ground cover species. Moreover, from sweeping around the canopy of coppice shoot of *E. camaldulensis* in the plantations found the adequate number of individuals of *Aprostocetus* sp. and *Megastigmus* sp.

2. The reproductive mode of *L. invasa* is deuterotoky. This term refers to female production with rare male. The male offsprings are non–functional. Thus, the sterility of *L. invasa* by some procedures may not overcome this problem.

3. The reproductive organs, particularly the genitalia, can be used to classify the species when their external morphologies are very similar. This research showed the descriptions of genitalia of *Aprostocetus* sp. and *Megastigmus* sp.

Two species of *Megastigmus* are found in Thailand; *M. thailandiensis* and *M. thitiporne*, but only their morphologies were described and reported. Thus *Megastigmus* sp., found from this research in *E. camaldulensis* plantations in Kanchanaburi province, may be the other new species if the genitalia is used to compare between this *Megastigmus* sp., *M. thailandiensis*, and *M. thitiporne*.

4. From the assessment of the population dynamics of *L. invasa*, *Aprostocetus* sp., and *Megastigmus* sp. in Tha Muang and Phanom Thuan districts showed that the populations of *L. invasa* and two parasitoids were maximum on May and declined later in the rainy period and dry period. Before May (January to March) was a long dry period. May had an adequate precipitation, so *E. camaldulensis* produced more

newly young leaves which became the abundance of food source and habitat of *L. invasa*. Although *E. camaldulensis* produced newly young leaves throughout the year, the decline of the population in long rainy months (from June to October) followed by long dry period (from November to February) might result from several factors.

Thus, in short–term solution, *E. camaldulensis* cutting for sale should carry out on June or July and let them produce new coppice shoots and newly young leaves in the rainy months. The growth of coppices should be enhanced by some procedures to stimulate the coppice growth reaching up approximately 6 meters in height from July to March. The leaves of the coppice shoots of *E. camaldulensis* at this height develop to mature leaves which are tolerate to *L. invasa* attack. By this management, it is able to reduce the problems from the attacks of *L. invasa* at one level.

5. From monthly sweeping around the canopy of coppice shoots of *E. camaldulensis* trees in the plantations, it was found 208 species of insects in Tha Muang district and 159 species in Phanom Thuan district. The index of insect diversity and Eveness Index in Tha Muang (H′=2.300 and E=0.402) were higher than those in Phanom Thuan (H′=2.133 and E=0.373). The plantations in Tha Muang had more number of species of vegetative ground cover than those in Phanom Thuan. The order Hymenoptera had the highest number of species. The roles of all insect species could be divided into parasitoid, predator, phytophaga, scavenger, and blood sucker.

These findings suggest that it will be beneficial to conserve some ground cover species in the plantations after the coppice shoots of *E. camaldulensis* are tall enough. The ground cover species help the balance of ecosystem and the balance of natural enemies. Many weedy species at ground level provide the habitats and the alternative food sources, such as nectar, for egg production and for the prolongation of adult *Aprostocetus* sp., *Megastigmus* sp. and *L. invasa*. The gall wasp may get less benefits from ground cover species because *L. invasa* has shorter longevity.

6. In order to overcome the attack of *L. invasa* in *E. camaldulensis* plantations, planting with more tolerant clone and the integrated control or the

integrated management are suggested to be carried out. The integrated control covers tree improvements to obtain the new clones which can tolerate to *L. invasa*, the control of *L. invasa* by parasitoids, and the proper management of *E. camaldulensis* plantations. The control of *L. invasa* by *Aprostocetus* sp. and *Megastigmus* sp. will be successful if the data dealing with their biological aspects are carefully utilized.

Recommendations for Future Studies

The findings from this research show that there are some questions waiting for future studies.

1. The egg load or the potential fecundity of female *L. invasa* in the laboratory was rather high. Is the high potential fecundity influenced by high temperature and low humidity? Are there alternative food sources of *L. invasa* in *E. camaldulensis* plantations? The results will be benefit to predict the degree of damage caused by *L. invasa* in the future.

2. The development time from egg to adult *L. invasa* was comparatively long. Generally, the development time of insects is accelerated in the warmer air. Will global warming which accelerate the development time of *L. invasa* have more severely effects on *E. camaldulensis* plantations?

3. The means of longevity of female and male *Aprostocetus* sp. were long and those of *Megastigmus* sp. were short in the laboratory. What is the optimum temperature for mass rearing of *Aprostocetus* sp. and *Megastigmus* sp.?

4. Temperature and humidity may influence on oviposition and efficacy of parasitoids. Do the low/high temperature and humidity reduce the oviposition capacity of *Aprostocetus* sp. and *Megastigmus* sp.?

5. The sex ratio in the progenies of *Aprostocetus* sp. and *Megastigmus* sp. in the ventilated greenhouse was male–biased (male progenies>female progenies). The

sex ratio of progenies at emerging time which will be useful in biocontrol, should comprise of more females than males. What are the factors affecting the sex ratio of *Aprostocetus* sp. and *Megastigmus* sp.?

6. The mass rearing of *Aprostocetus* sp. and *Megastigmus* sp. in the laboratory are essential, and then introduce them to *E. camaldulensis* plantations to control *L. invasa*. What are the optimum environments for mass production of those two parasitoids in the laboratory?

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APPENDIX

Appendix Figure 1 Vegetative ground cover in rainy season, in Tha Muang district:

(A–B) vegetative ground cover in *Eucalyptus camaldulensis* plantations;

(C) *Rhynchelytrum repens* (Willd.) C.E. Hubb., Family Poaceae;

(D) *Cyperus* sp., Family Cyperaceae;

(E) *Gomphena celosioides* Mart., Family Amaranthaceae;

(F) *Richardia brazilliensis* Gomez, Family Rubiaceae;

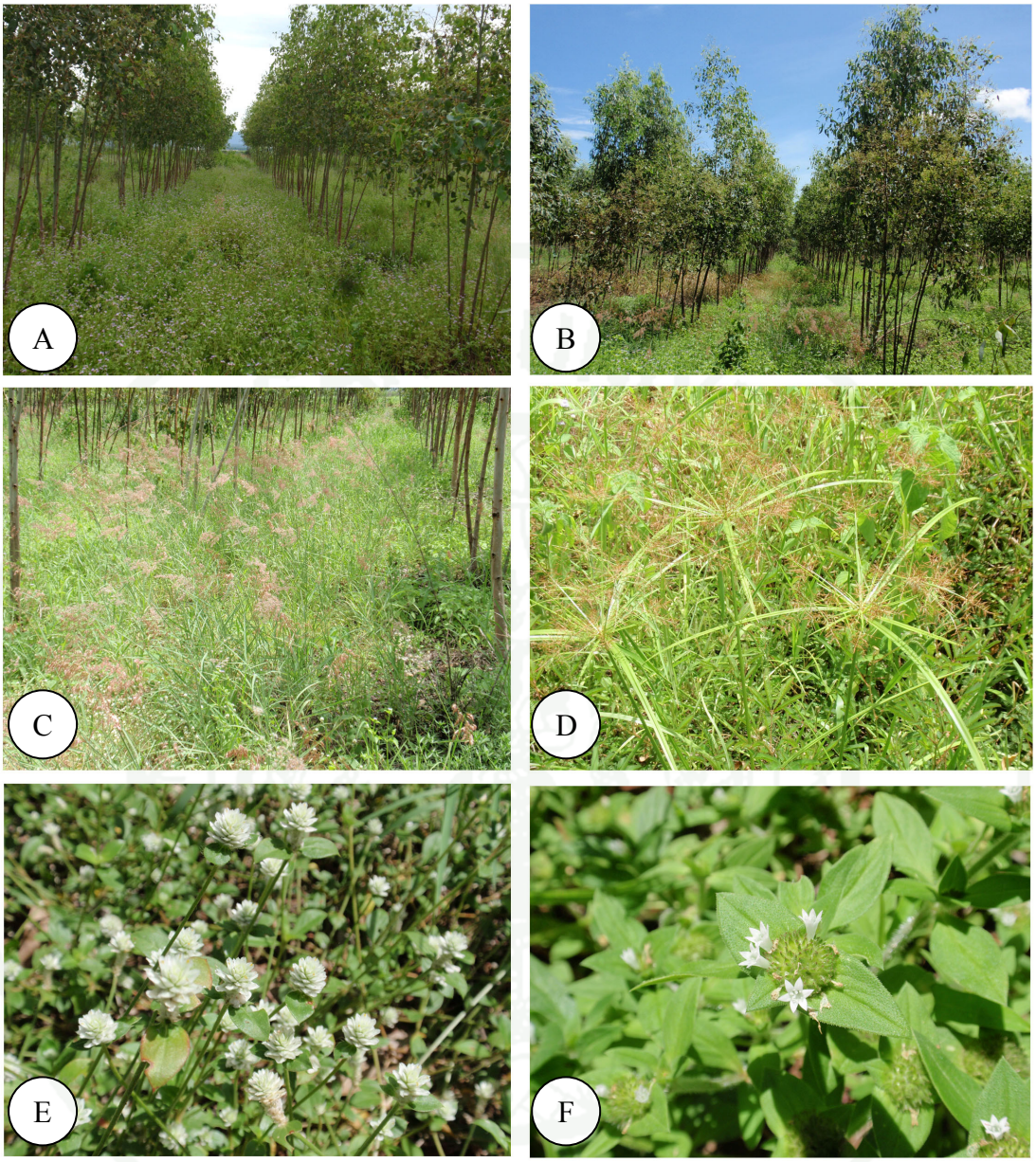
(G) *Ageratum conyzoides* Linn., Family Compositae;

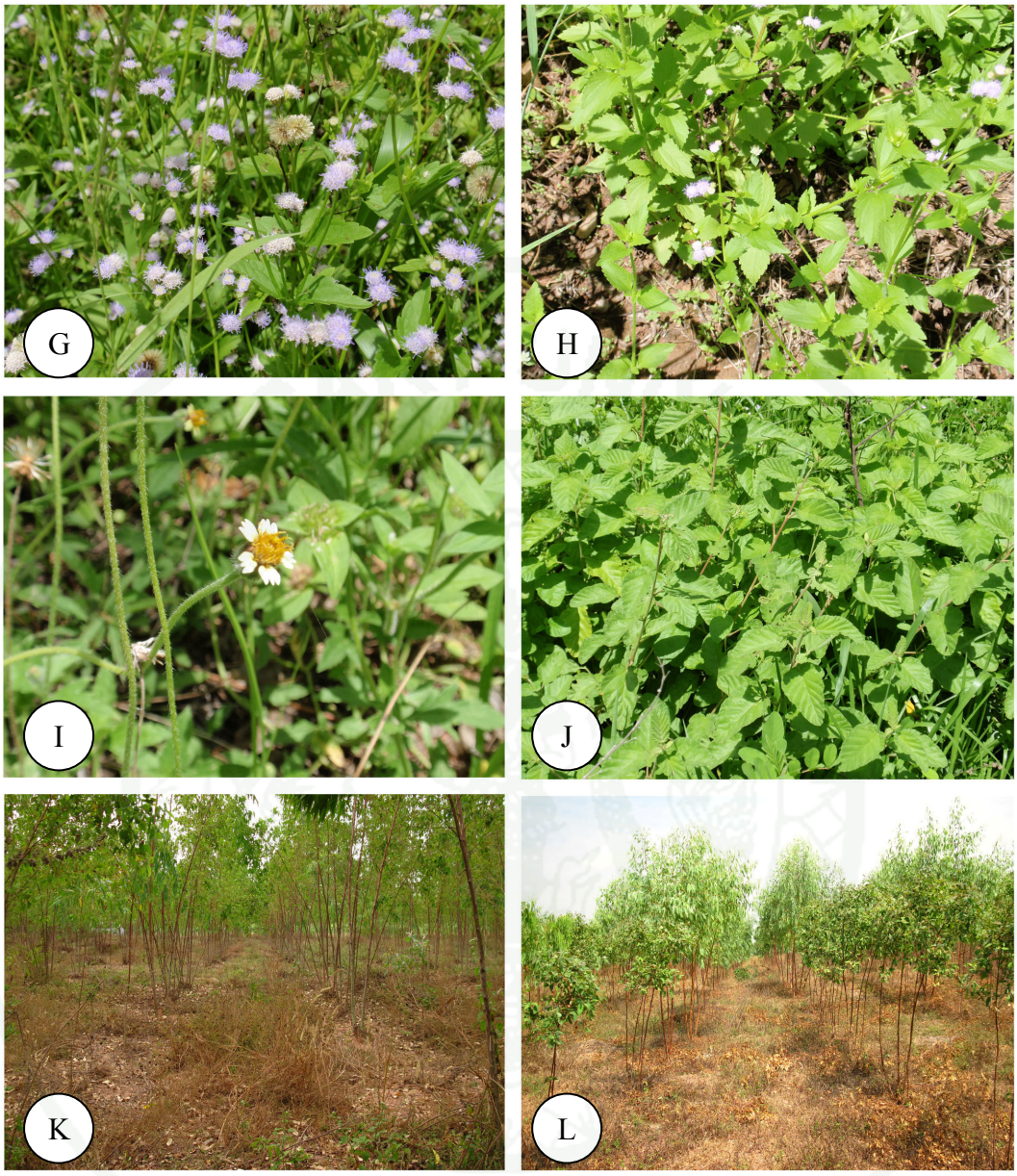
(H) *A. conyzoides*;

(I) *Tridax procumbens* Linn., Family Compositae;

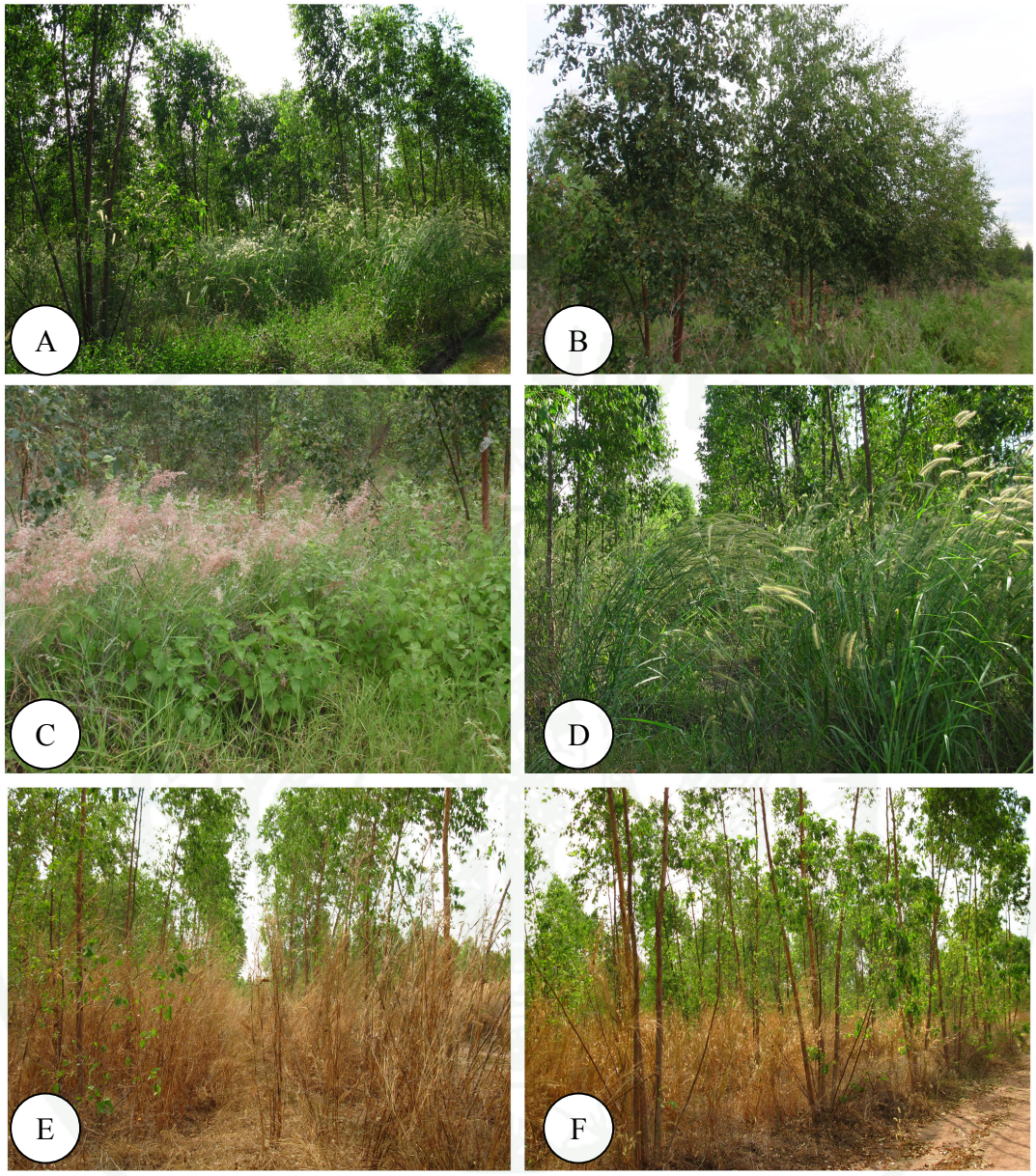
(J) *Hyptis suaveolens* (L.) Poit., Family Lamiaceae; and

(K–L) vegetative ground cover in dry season.

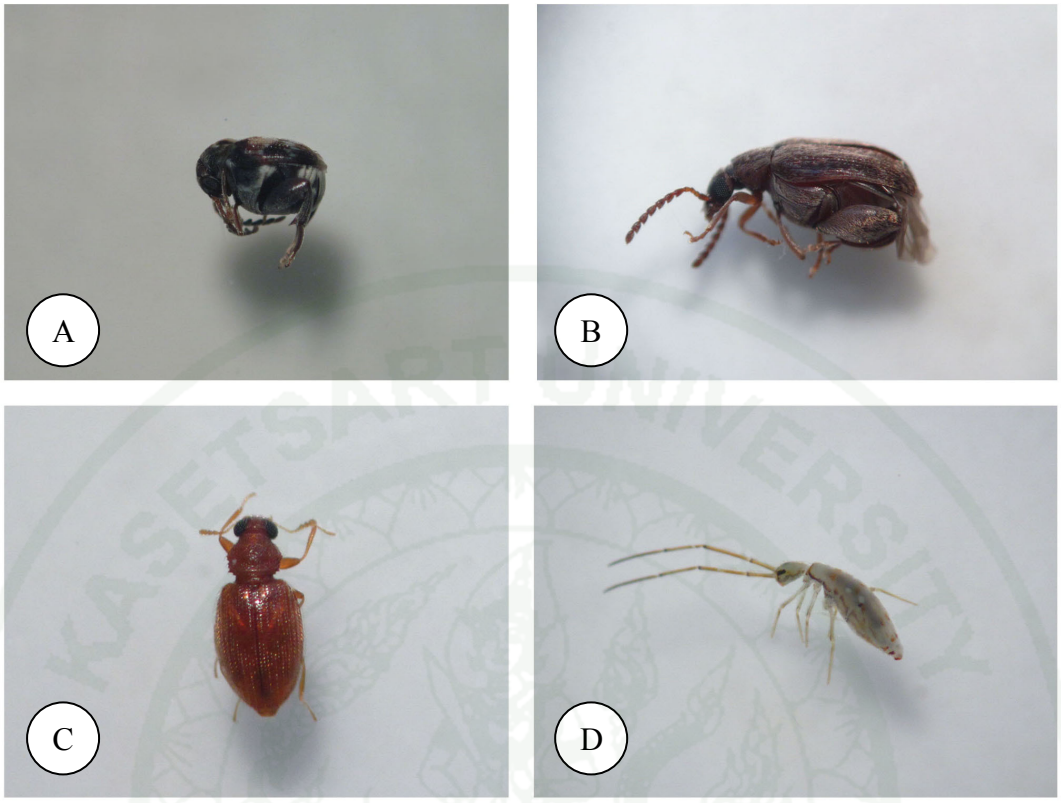




Appendix Figure 1 (Continued)



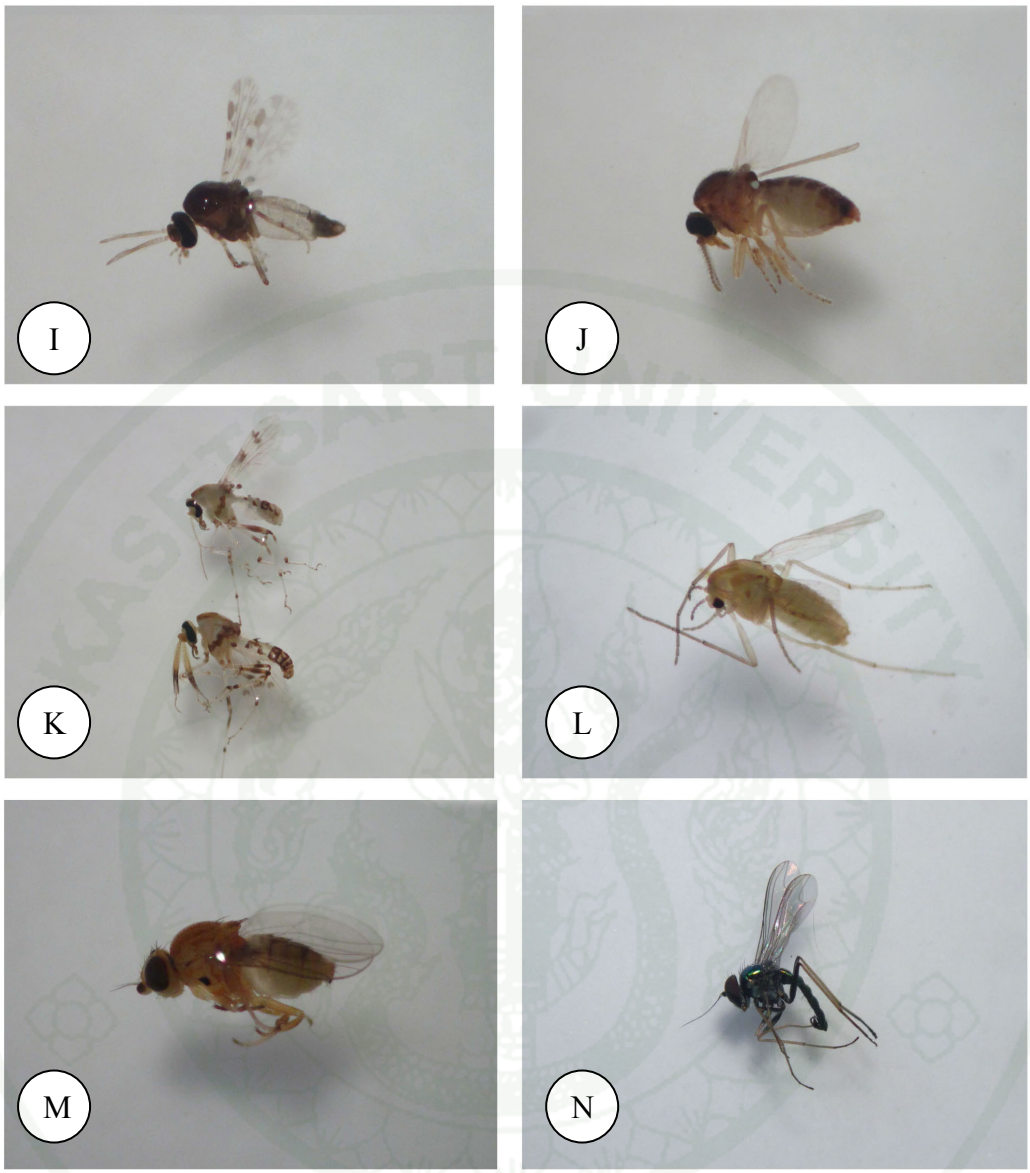
Appendix Figure 2 Vegetative ground cover in rainy season, in Phanom Thuan district: (A–B) vegetative ground cover in *Eucalyptus camaldulensis* plantations; (C) *Rhynchelytrum repens* (Willd.) C.E. Hubb., Family Poaceae; (D) *Pennisetum polystachyon* (L.) Schult., Family Poaceae; and (E–F) vegetative ground cover in dry season.



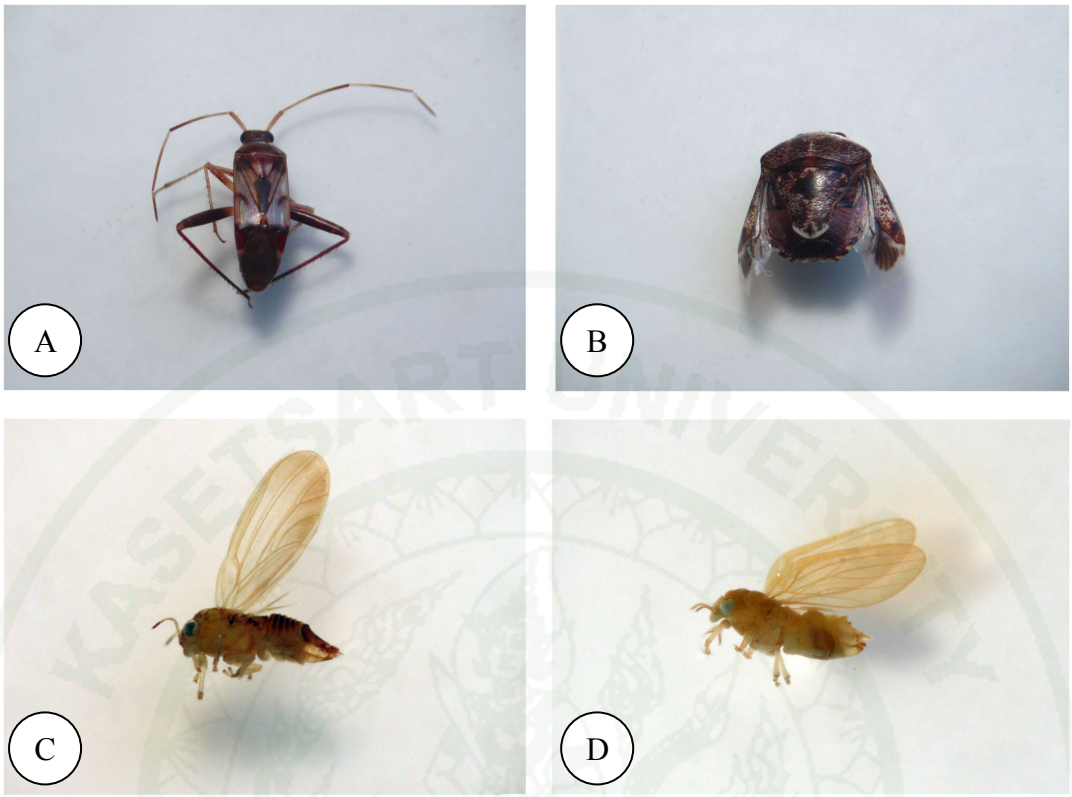
Appendix Figure 3 The distinct insect species in the order Coleoptera and Collembola found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thaun districts: (A) *Callosobruchus chinensis*, Family Bruchidae; (B) *Caryedon* sp.1, Family Bruchidae; (C) *Corticaria* sp.1, Family Lathridiidae; and (D) Entomobryid1, Family Entomobryidae.

Appendix Figure 4 The insect species in the order Diptera easily found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thuan districts:

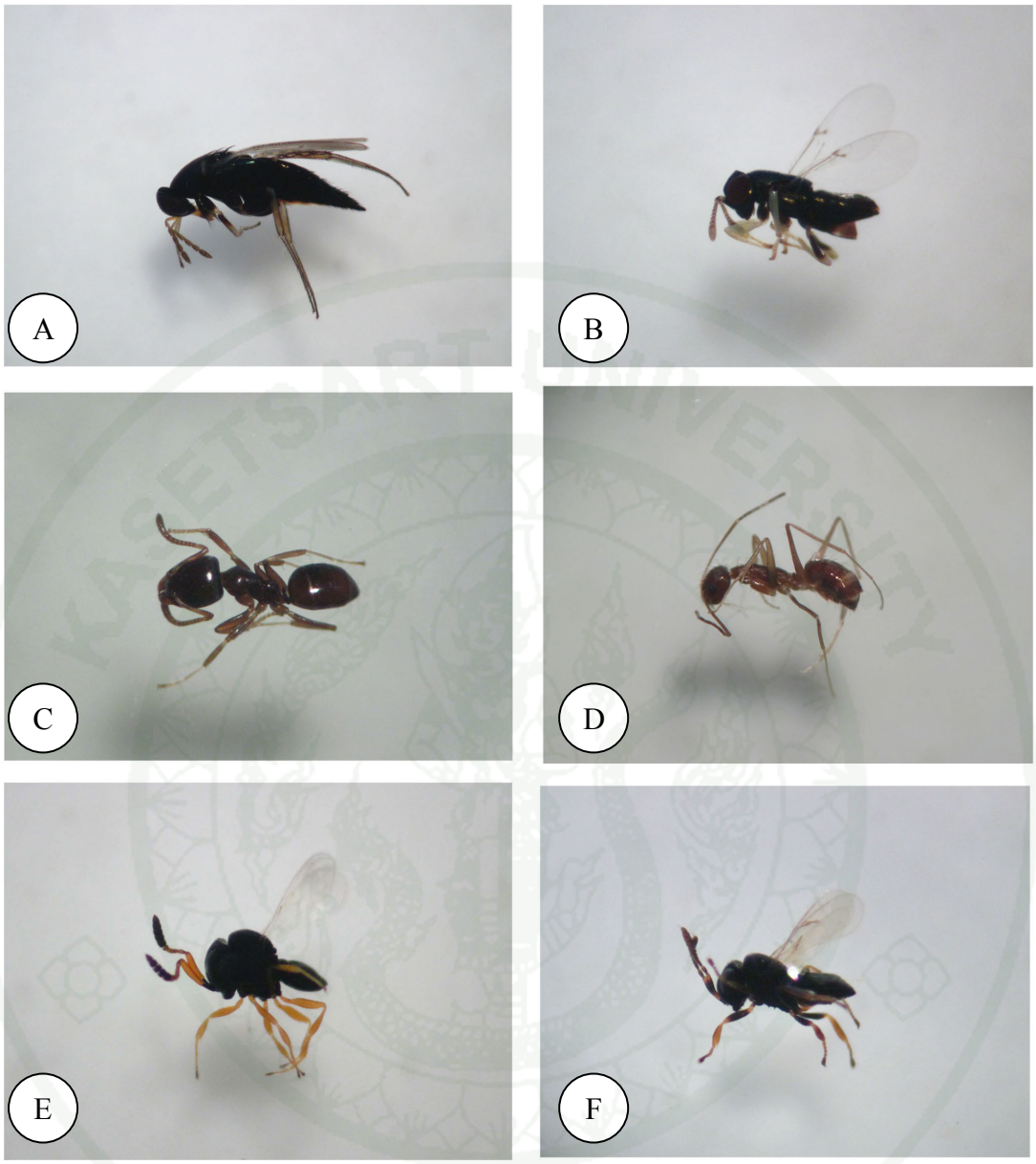
(A) Agromyzid1, Family Agromyzidae;
(B) Cecidomyiid1, Family Cecidomyiidae;
(C) Cecidomyiid5, Family Cecidomyiidae;
(D) Ceratopogonid2, Family Ceratopogonidae;
(E) Ceratopogonid8, Family Ceratopogonidae;
(F) Ceratopogonid11, Family Ceratopogonidae;
(G) *Culicoides* sp.1, Family Ceratopogonidae;
(H) *Culicoides* sp.2, Family Ceratopogonidae;
(I) *Culicoides* sp.3, Family Ceratopogonidae;
(J) *Forcipomyia* sp.1, Family Ceratopogonidae;
(K) *Stilobezzia* sp.1, Family Ceratopogonidae;
(L) Chironomid2, Family Chironomidae;
(M) Chloropid1, Family Chloropidae; and
(N) *Dolichopus* sp.1, Family Dolichopodidae.



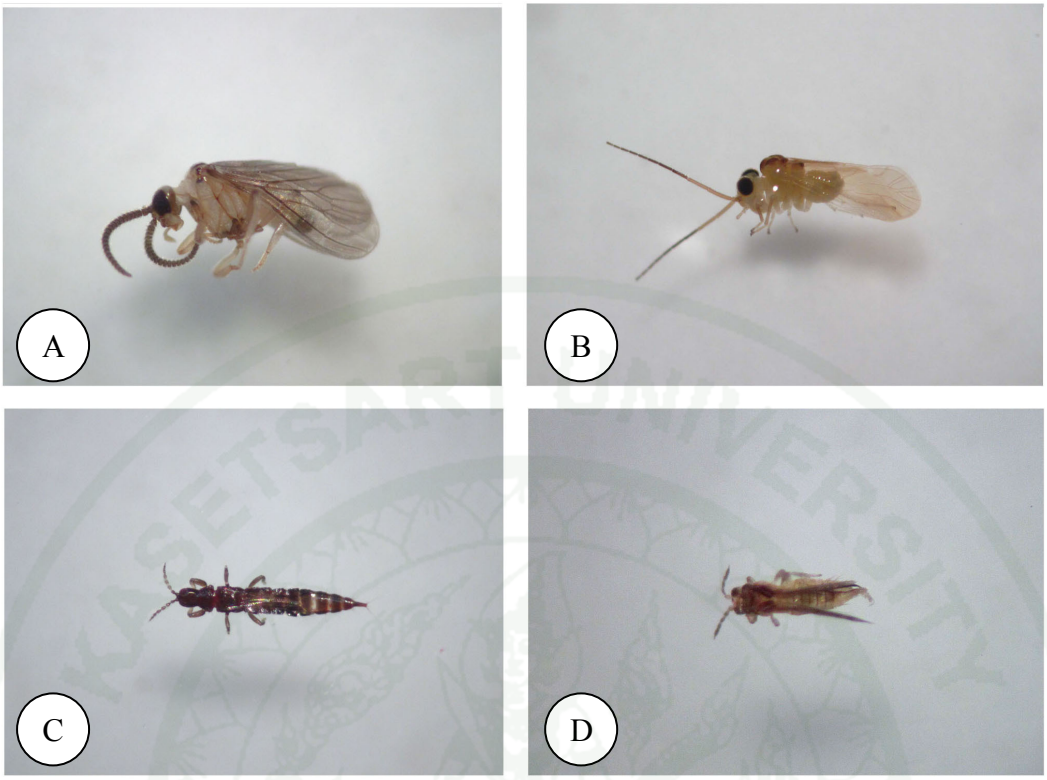
Appendix Figure 4 (Continued)



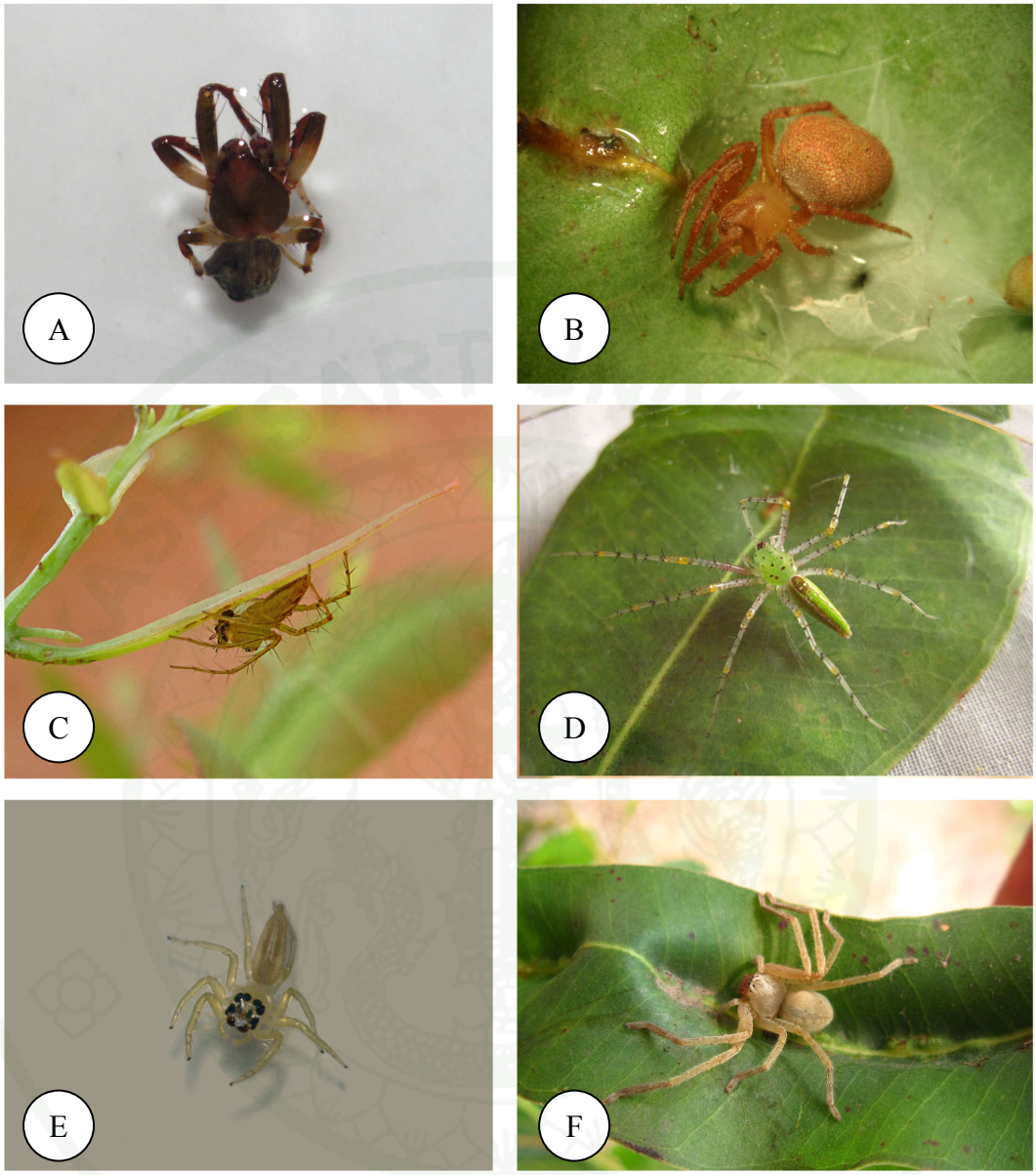
Appendix Figure 5 The distinct insect species in the order Hemiptera found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thuan districts: (A) Mirid1, Family Miridae; (B) Pentatomid1, Family Pentatomidae; (C) Psyllid1, Family Psyllidae; and (D) Psyllid2, Family Psyllidae.



Appendix Figure 6 The insect species in the order Hymenoptera easily found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thuan districts: (A) *Elasmus* sp.3, Family Elasmidae; (B) *Psyllaephagus* sp.1, Family Encyrtidae; (C) *Camponotus* sp.1, Family Formicidae; (D) *Paratrechina longicornis*, Family Formicidae; (E) *Trissolcus* sp.1, Family Scelionidae; and (F) *Trissolcus* sp.2, Family Scelionidae.



Appendix Figure 7 The distinct insect species in the order Neuroptera, Psocoptera, and Thysanoptera found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thuan districts: (A) *Coniopteryx* sp.1, Family Coniopterygidae; (B) Caeciliusid1, Family Caeciliusidae; (C) Phlaeothripid1, Family Phlaeothripidae; and (D) Thripid1, Family Thripidae.



Appendix Figure 8 The spiders easily found in the canopies of *Eucalyptus camaldulensis* plantations, Tha Muang and Phanom Thuan districts: (A) Araneid1, Family Araneidae; (B) Araneid2, Family Araneidae; (C) *Oxyopes* sp.1, Family Oxyopidae, while eating *L. invasa*; (D) *Peucetia* sp.1, Family Oxyopidae; (E) Salticid1, Family Salticidae; and (F) Sparassid1, Family Sparassidae.

CURRICULUM VITAE

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