A BIOLOGICAL STUDY OF *PACHYCREPOIDEUS VINDEMMIAE* (RONDANI), A HYMENOPTEROUS PARASITOID OF MEDICAL IMPORTANCE

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The biology of a hymenopteran parasitoid, *Pachycrepoideus vindemmiae* Rondani, and its efficiency in the control of flies of medical importance was investigated in the laboratory.

The life cycle study revealed that *Pachycrepoideus vindemmiae,* when developed in house fly puparia, the eggs, the first, second, third, fourth and fifth instar larvae, and prepupa and pupal stages lasted 1-2, 1-2, 2-3, 1-2, 2-3, 3-4, 1-2 and 9-12 days respectively. The average developmental period from egg to adult emergence was 20.35 ± 1.43 days for males and 21.30 ± 1.16 days for females. The characteristics of each developmental stage of the parasitoid were also described in this study.

Adult longevity, when reared with 10% sugar solution, averaged 13.63 ± 4.06 days in males and 18.22 ± 2.93 in females. The mean fecundity was 112.18 ± 25.24 offspring per female. The sex ratio, female per male, of offspring produced by mated females was 1.58:1, while only males were obtained from unmated females. The parasitism rate in *Musca domestica* was 75.12 ± 10.97%.

Regarding the host preference in the laboratory *Musca domestica* was preferred most, followed by *Chrysomya megacephala* and *Parasarcophaga orchidae,* respectively.

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ยุทธนา สามัง : การศึกษาชีววิทยาของ *PACHYCREPOIDEUS VINDEMMIAE* (RONDANI) แตนเบียนของแมลงวันที่มีความสำคัญทางการแพทย์ (A BIOLOGICAL STUDY OF *PACHYCREPOIDEUS VINDEMMIAE* (RONDANI), A HYMENOPTEROUS PARASITOID OF MEDICAL IMPORTANCE). คณะกรรมการควบคุมวิทยานิพนธ์ : สมเกียรติ วงศ์ทางสวัสดิ์, Dr. P.H., จิระศักดิ์ โรจนาเปรมสุข, Dr.P.H., ชำนาญ อภิวัฒนศร, M.T.H., 80 หน้า. ISBN 974-664-787-3

ศึกษาชีววิทยาของ *Pachycrepoideus vindemmiae* Rondani และความสามารถในการควบคุมแมลงวันที่มีความสำคัญทางการแพทย์ โดยทำการศึกษาในห้องปฏิบัติการ

การศึกษาวงจรชีวิตของ *Pachycrepoideus vindemmiae* ที่เจริญเติบโตในระยะดักแด้ของแมลงวัน มีระยะการเจริญเติบโตดังนี้ ระยะไข่ 1-2 วัน, ตัวอ่อนระยะที่ 1 1-2 วัน, ตัวอ่อนระยะที่ 2 2-3 วัน, ตัวอ่อนระยะที่ 3 1-2 วัน, ตัวอ่อนระยะที่ 4 2-3 วัน, ตัวอ่อนระยะที่ 5 3-4 วัน, ระยะ prepupa 1-2 วัน และระยะ pupa 9-12 วัน รวมระยะเวลาในการเจริญเติบโตเฉลี่ยจากไข่เป็นตัวเต็มวัยเพศผู้ 20.35 ± 1.43 วัน และเพศเมีย 21.30 ± 1.16 วัน

ค่าเฉลี่ยอายุของตัวเต็มวัย เมื่อเลี้ยงด้วยน้ำตาลเข้มข้น 10% จะได้เท่ากับ 13.63 ± 4.06 วันในเพศผู้ และ 18.22 ± 2.93 ในเพศเมีย, ค่าเฉลี่ยของความสามารถแพร่พันธุ์เท่ากับ 112.18 ± 25.24 ตัวต่อเพศเมียหนึ่งตัว, อัตราส่วนเพศผู้ : เพศเมีย เมื่อเพศเมียได้รับการผสมพันธุ์เท่ากับ 1.58 : 1 ในขณะที่เพศเมียไม่ได้รับการผสมพันธุ์จะให้เพศผู้ทั้งหมด, parasitism rate ใน *Musca domestica* เท่ากับ 75.12 ± 10.97%

เมื่อทดลองหา Host preference ในห้องปฏิบัติการพบว่า จะมีความชอบ *Musca domestica* มากที่สุด รองลงมาคือ *Chrysomya megacephala* และ *Parasarcophaga orchidae* ตามลำดับ

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ABBREVIATIONS

°C	=	Degrees Celsius
cm	=	Centimetre
oz	=	Ounce
F1	=	First filial generation
Hr (s)	=	Hours
μ	=	Micron
No.	=	Number
et al.	=	And others
mm.	=	Millimetre
etc.	=	Et cetera
i.e.	=	That is, to be precise
W	=	Wattage
km	=	Kilometer
gms	=	Gram
%	=	Percentage

CHAPTER I

INTRODUCTION

Flies are cosmopolitan in distribution, having travelled everywhere man has settled, and unfortunately endeavoring to live in very close association with him. The muscid population is so great that infestations spread into suburban and even urban areas, entering houses where they cause much annoyance, becoming potential disease carriers. They are not only known as carriers of disease but their presence in barns, poultry farms or human communities also gives rise to a serious problem of control. Flies are largely in the families Muscidae (the house fly and its relatives), Calliphoridae (blow flies) and Sarcophagidae (flesh flies). As they pass their larval stages in organic waste materials (poultry manure, garbage, etc.) and excrement, they are involved in the transmission of disease, generally through contamination of food. There are several ways that flies can cause serious problems for man (1). Besides causing annoyance to humans and livestock, house flies transmit various diseases. After feeding and breeding on fecal accumulation or excrement, the fly can freely enter houses and contaminate food and drink. It is a mechanical vector of viruses, bacteria, protozoa and helminths, such as bacillary dysentery, shigellosis, typhoid fever, cholera, anthrax, diarrhea, eye infections, poliomyelitis, certain skin infections, myiasis, etc. (2, 3, 4, 5).

More stringent restrictions on the use of pesticides are emphasized due to the rapid development of resistance in fly populations to those chemicals and their residues in the environment. Insecticides often possess a threat to non-target

organisms, including man, and their application is commonly harzardous. Residues left in food products, plants and animals can accumulate in the human body. Environmental pollution caused by highly residual insecticides has become a matter of genetic, hormonal, integrated or biological method (predators, pathogens and parasitoids) affords new prospects for future fly control, and it is clear that disease-vector control is advancing toward a methodology which will carry fewer and fewer inherent harzards to the environment (6, 7).

Biological control agents are a vital component of integrated pest management strategies to repress populations of synanthropic muscoid flies (8). Biological control which attempts to overcome these flies has been organized throughout the world since the beginning of the 20th century. This kind of control has focussed attention on rearing strains of entomophages which are resistant to natural enemies and diseases. Biological control offers certain advantages that many other methods of insect control do not. It is inherently safe, both in that there is usually little or no danger involved during its application, and that no toxicant contaminates the environment and destroys beneficial animals as well as harmful insects (1).

Hymenopteran parasites seem to be valuable agents in the biological control of flies, especially the house fly. Research on insect parasitoids has increased explosively in the last 15 years (9). Renewed interest in parasitoids as biological control agents has coincided with the adoption of parasitoids as model theoretical and experimental systems (10, 11). That interest has been prompted in part by the deeper understanding of parasitoid behavior and ecology these new approaches bring, and in part by an increasing disenchantment with chemical means of pest control (10).

Parasitoids in the order Diptera are the subject of this review. This order includes an estimated 16,000 described species of parasitoids, or about 20% of the known species with this life-style (9). In Thailand, *Spalangia endius* , *Exoristobia philippinensis* and *Pachycrepoideus vindemmiae* were first reported by Rongsriyam *et al.* (12). They were observed to parasitize the pupal stage of various species of synanthropic flies. *Spalangia endius* was the most widely distributed throughout the study sites on these hosts with 85.3% total recovery, followed by *Spalangia nigroaene* with 44.1%, and *Pachycrepoideus vindemmiae* was the third with 23.5% (1).

As regards biological control, it is emphasized that the successful use as biotic agents requires a fundamental understanding of the interrelationships between them and their potential hosts. As mentioned above, little was known about these parasitoids in Thailand (1, 12, 13, 14), further study is required, and the role of *Pachycrepoideus vindemmiae* in the biological control of flies and its potential should be further evaluated. This study was undertaken to determine the following points that relate as a potential biological agent for fly control.

Research aims

General objective

To conduct a biological study and host preference study of *Pachycrepoideus vindemmiae*.

Specific objective

1. To culture and study the biology of *Pachycrepoideus vindemmiae*, a hymenopterous parasite of fly pupae, including life history, morphology, fecundity, sex-ratio and longevity in the laboratory.

2. To determine host preference of the parasite between 3 important synanthropic flies that are found frequently in Thailand.

Definition of terms

The fecundity of *Pachycrepoideus vindemmiae:* the potential reproductive capacity of *Pachycrepoideus vindemmiae* expressed as the number of viable offspring produced per one female.

Parasitism rate: the number of pupae *Musca domestica,* which are killed by the newly emerged progeny parasitoids.

Longevity of adult *Pachycrepoideus vindemmiae*: the length of time after molting from pupa to the last day of adult male and female *Pachycrepoideus vindemmiae.*

Parasitic : living on or in some other animal or insect in such a way as to derive all nourshment from the tissues of the host

Parasitism : a form of symboisis in which one party lives upon or at the expense of the other, makes no return and destroys it host.

Parasitoids : Insect that parasitize other insects

: Usually parasitic as immatures, free-living as adults

: Only one host is required for complete development

: Population can be maintained at low levels

: Survivorship is usually good

Superparasitism : occurs when a host is attacked by more larvae of some species than can reach maturity in the one host.

Research consensus

The host pupae develop to adult flies and the death of host pupae from other causes (*i.e.* death from microsporidium) assumed as not infected by parasitoid.

CHAPTER II

LITERATURE REVIEW

HYMENOPTERAN PARASITOID AS THE BIO-AGENT FOR FLY CONTROL

The Hymenoptera is an abundant, ubiquitous, highly specialized, and highly successful order of insects that rivals the Diptera and Lepidoptera in number of species, and probably exceeds both in variety of adaptive radiations (15). Hymenopterous parasites appear to be of real value in the natural control of flies, particulary species of the genus *Spalangia* which have been used in field tests in Pacific islands and Puerto Rico with some success (16). Steve (17) showed that the Pteromalid, *Pachycrepoideus vindemmiae* was the pupal parasite of both *Fannia canicularis* and *Fannia scalaris*. McCoy (18) worked on mass liberation of laboratory reared parasites, *Spalangia muscidarum* for control of *Stomoxys calcitrans* in Lancaster Country, Nebraska. Wylia (19) reported some effects of host age on parasitism by *Nasonia vitripennis*. Sacca (20) made a comparative bionomic study in the genus *Musca*. He referred to a number of entomophagous arthropods of housefly which are able to be used as biological control agents. From these studies and reports the percentage of parasitism were found varied considerably and data on true effectiveness were lacking.

Research in this field was studied intensively by Legner and his colleagues, both in the laboratory and in the field. Their results seemed to show some value in controlling flies by these parasites. They started surveying the hymenopterous parasites of flies in Southern California and other areas in 1962. Bay, Legner and

Medved (21) studied *Hippelates collusor* as a host for four species of parasitic Hymenoptera in Southern California- *Phaenopria occidentalis*, *Spalangia drosophila*, *Eupteromalus nidulans* and *Trichopria* n.sp. The *Spalangia drosophila* was the most efficient species in seeking *Hippelates* pupae in the field. Legner (22) reported eight parasitic hymenoptera in five families attacking the larval and pupal stages of the house fly and six other nuisance Diptera in Southern California. The seven host species were the little house fly, *Fannia canicularis*; *Fannia femoralis*; house fly, *Musca domestica*; false stable fly, *Muscina stabulans*; *Ophyra leucostoma*; black blow fly, *Phormia regina;* and stable fly, *Stomoxys calcitrans*. The parasite species were *Figites* sp. (Figitidae); *Trichopria* n.sp. (Diapriidae); *Stilpnus anthomyidiperda* (Ichneumonidae); *Muscidifurax raptor*, *Nasonia vitripennis* (Pteromalidae); *Spalangia cameroni*, *Spalangia endius*, *Spalangia nigroaenea* (Spalangiidae). At poultry farms in Southern California the parasites were found actively suppressing the dung-inhabiting fly population, and *Muscidifurax raptor* and *Spalangia endius* accounted very high activity in parasitizing *Fannia femoralis* and *Ophyra leucostoma*. *Stilpnus* sp. and *Muscidifurax raptor* can also parasitize the puparia of the little house fly (23). Depner (24) had studied the parasitism of the horn fly in six regions of Alberta differing in climate, soil and vegetation. He discovered eight parasitic hymenoptera which were classified to three families, the Pteromalidae, Ichneumonidae and Cynipidae. The parasites were *Spalangia drosophila*, *Spalangia haematobia*, *Habrocytes* sp., *Mesochorus* sp., *Tritneptis* sp., *Dibrachys* sp., *Phygodeuon* sp., and *Kleidotoma* sp.

Most of Legner and his colleagues' work had been done with house flies in Southern California. They obtained three important genera which seemed to be the

most successful parasitoids in the laboratory. They were *Muscidifurax raptor*, *Nasonia vitripennis* and many species of *Spalangia*. However, from field observation, even though *Nasonia vitripennis* could parasitize many species of Diptera, the females lacked the ability to penetrate into the ground to find a suitable host. This disadvantage prevented its utilization as an effective control agent. For *Spalangia* spp., which is the most active genus in finding a host, *Spalangia endius* tends to be the most effective one. The sustained release of this microhymenopteran pupal parasite at a commercial poultry installation in North Florida completely suppressed a population of house flies within 35 days (25). Morgan (25) concluded that *Spalangia endius* was as effective as the recommended insecticidal treatments in the control of house flies. The costs of producing and releasing the parasites were relatively low and slightly less than those for pesticides. This approach is highly specific compared with pesticides and prevents interference with the ecological system. Morgan and Patterson (26) reported that *Muscidifurax raptor* was the most prevalent house fly parasite collected in Florida with *Spalangia nigroaenea* and *Pachycrepoideus vindemmiae* also being quite common. In South Carolina, Ables and Shepard (27) reported that *Spalangia nigroaenea* was the most abundant and *Muscidifurax raptor* one of the least abundant house fly parasites collected. After an additional year of monitoring, however, Ables and Shepard (28) concluded that *Muscidifurax raptor* was one of the 3 predominant house fly parasites in South Carolina along with *Spalangia nigroaenea* and *Spalangia endius*. Pickens, Miller and Centala (29) studied to determine the biology, population dynamics, and host-finding efficiency of *Pachycrepoideus vindemmiae* in box stalls and a poultry houses. Weidhaas *et al.*, (30) have constructed a model that permits us to

study the interactions of populations of house flies (*Musca domestica*) and *Spalangia endius* and how the density of the host could change when the population is exposed to assumed numbers of parasites under a variety of conditions. Rutz and Axtell (31) conducted a comprehensive survey of the occurrence, relative abundance and seasonal abundance of indigenous house fly parasites in 3 geographic regions of North Carolina. Eight parasite species, *Muscidifurax raptor*, *Spalangia cameroni*, *Spalangia endius*, *Spalangia nigra*, *Spalangia nigroaenea*, *Spalangia* n.sp. near *drosophila*, *Pachycrepoideus vindemmiae*, and *Nasonia vitripennis* were found. In the same year there were reports that *Muscidifurax raptor*, *Spalangia* undescribed sp. near *drosophila*, *Spalangia cameroni*, *Pachycrepoideus vindemmiae and Nasonia vitripennis* invaded manure at new caged-layer poultry houses near Raleigh, North Carolina within 8 weeks after the chickens were placed in the houses. During the 2-year study, *Muscidifurax raptor*, *Pachycrepoideus vindemmiae* and *Spalangia cameroni* ranked 1st, 2nd and 3rd, respectively, in relative abundance of all parasites collected. *Mucidifurax raptor* and *Pachycrepoideus vindemmiae* were abundant from June through November (32). Rueda and Axtell (33) carried out a comparative survey of hymenopterous parasites of the house fly, *Musca domestica*, pupae in different livestock and poultry production systems, and reported that *Muscidifurax raptor*, *Pachycrepoideus vindemmiae* and *Spalangia cameroni* were the most prevalent parasites in both the confined systems and the pastures, accounting for 95 to 98% of all parasites recovered. In 1986, there were preliminary studies of *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) by Panicker on the biology and biocontrol efficiency for controlling houseflies, *Musca domestica* (34). Gerald (35) reported on

the status of the parasite populations during the winter months in Florida. The predominant parasite observed attacking muscoid flies (75% for stable flies and 58% for house flies) was *Spalangia cameroni*. Mann, Stinner and Axtell (36) studied the effects of host-parasitoid densities and host distribution for four species of Pteromalidae (Hymenoptera), *Muscidifurax raptor*, *Muscidifurax zaraptor*, *Spalangia cameroni* and *Spalangia endius* to house fly (*Musca domestica*) pupae. In peninsular Malaysia there was a survey of microhymenoptera. Nine species of parasitoids were found parasitizing the pupae of fifth flies breeding in refuse and poultry farms. *Spalangia* were most common, consisting of *Spalangia endius*, *Spalangia cameroni*, *Spalangia gemina*, *Spalangia nigroaenea*, and two undescribed species. Other parasitoids collected were *Pachycrepoideus vindemmiae*, *Dirhinus himalayanus*, and an unidentified hymenoptera (37). Smith and Rutz (38) study measured the incidence and seasonal occurrence of parasitoids attacking the house fly, *Musca domestica* and the stable fly, *Stomoxys calcitrans* pupae at dairy farms in central New York. The mean relative abundance of each species was 59% for *Muscidifurax*, 14% for *Urolepis rufipes*, 11% for *Phygadeuon fumator*, 10% for *Spalangia cameroni*, 3% for *Spalangia nigroaenea* and 2% for *Trichomalopsis dubius*. Rare species included *Pachycrepoideus vindemmiae*, *Nasonia vitripennis*, *Dibrachys carus*, *Spalangia nigra*, and *Macroneura vesicularis*. Geden *et al.*, (39) studied the to susceptibility of house flies, *Musca domestica* and five pupal parasitoids : *Urolepis rufipes*, *Muscidifurax raptor*, *Nasonia vitripennis*, *Pachycrepoideus vindemmiae* and *Spalangia cameroni* to Abamectin and seven commercial insecticides (Atroban, Ciodrin, Rabon, Ectrin, Cygon, Vapona and Pyrenone). Assays of five commercial insecticides applied as

residual sprays at label rates to plywood indicated the most toxic insecticides overall for pteromalid parasitoids of house flies, *Musca domestica* were Atroban, Ciodrin, Robon, Ectrin and Cygon. Abamectin was more toxic to all of the parasitoids except *Nasonia vitripennis* and *Spalangia cameroni* than to newly colonized house flies. Space sprays with Vapona killed all of the parasitoids and susceptible flies. Space sprays with Pyrenone killed >86% of all insects.

Research on insect parasitoids has increased. In 1997, Rueda *et al.*, (40) conducted surveys of different poultry and livestock facilities, refuse dumps, and garbage dumpsters in South Korea. Five species of hymenopterous parasitoids: *Spalangia nigroaenea, Spalangia nigra, Muscidifurax raptor, Pachycrepoideus vindemmiae* and *Nasonia vitripennis* were found parasitizing pupae of house flies, *Musca domestica.* Four hymenopterous parasitoids: *Spalangia nigroaenea, Spalangia nigra, Muscidifurax raptor* and *Pachycrepoideus vindemmiae* were recovered from the pupae of stable flies, *Stomoxys calcitrans* and blowflies, *Chrysomya megacephala.* Only 2 parasitic species : *Spalangia nigroaenea* and *Pachycrepoideus vindemmiae* were recovered from the pupae of blowflies, *Phaenicia sericata. Pachycrepoideus vindemmiae* was the most abundant parasitic species in swine barns. The effects of age and burial of house flies (Diptera: Muscidae) pupae on parasitism by *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) indicated the greater effectiveness of the former than the latter and was most pronounced for buried hosts. Among unburied hosts, the effectiveness of *Spalangia cameroni* at killing hosts was most pronounced in young hosts and more effective than *Muscidifurax raptor. Spalangia cameroni* produced offspring regardless of host age whereas. *Muscidifurax*

raptor produced fewer offspring from young hosts than from old hosts (41). Jones (42) reported geographical and temporal variation in Pteromalid (Hymenoptera : Pteromalideae) parasitism of the stable fly, *Stomoxys calcitrans* and house fly, *Musca domestica*, puparia were collected at cattle feedlots in south central and north-western Illinois from May through August 1991-1993.

HOUSEFLIES (4)

The well-known "common house fly", *Musca domestica* is a complex species occuring in all parts of the world in various forms that are synanthropic and mainly endophilic. The connexion between the house fly and man is manifold. It breeds in accumulation of waste, manure, etc., it feeds on his food and waste, it uses his buildings for shelter and frequents the skin of man and his animals. By these habits, it becomes of public health importance, as an annoyance and as a potential carrier of disease. There are many synanthropic flies of public health importance. However, *Musca domestica* is the most common and wide-spread species.

Life cycle

There are four distinct stages in the life of a fly: egg, larva or maggot, pupa and adult (Fig. 1). Depending on the temperature, it takes from 6 to 42 days for the egg to develop into the adult fly. The length of life is usually 2-3 weeks but in cooler conditions it may be as long as three months.

Eggs are usually laid in masses on organic material such as manure and garbage. Hatching occurs within a few hours. The young larvae burrow into the

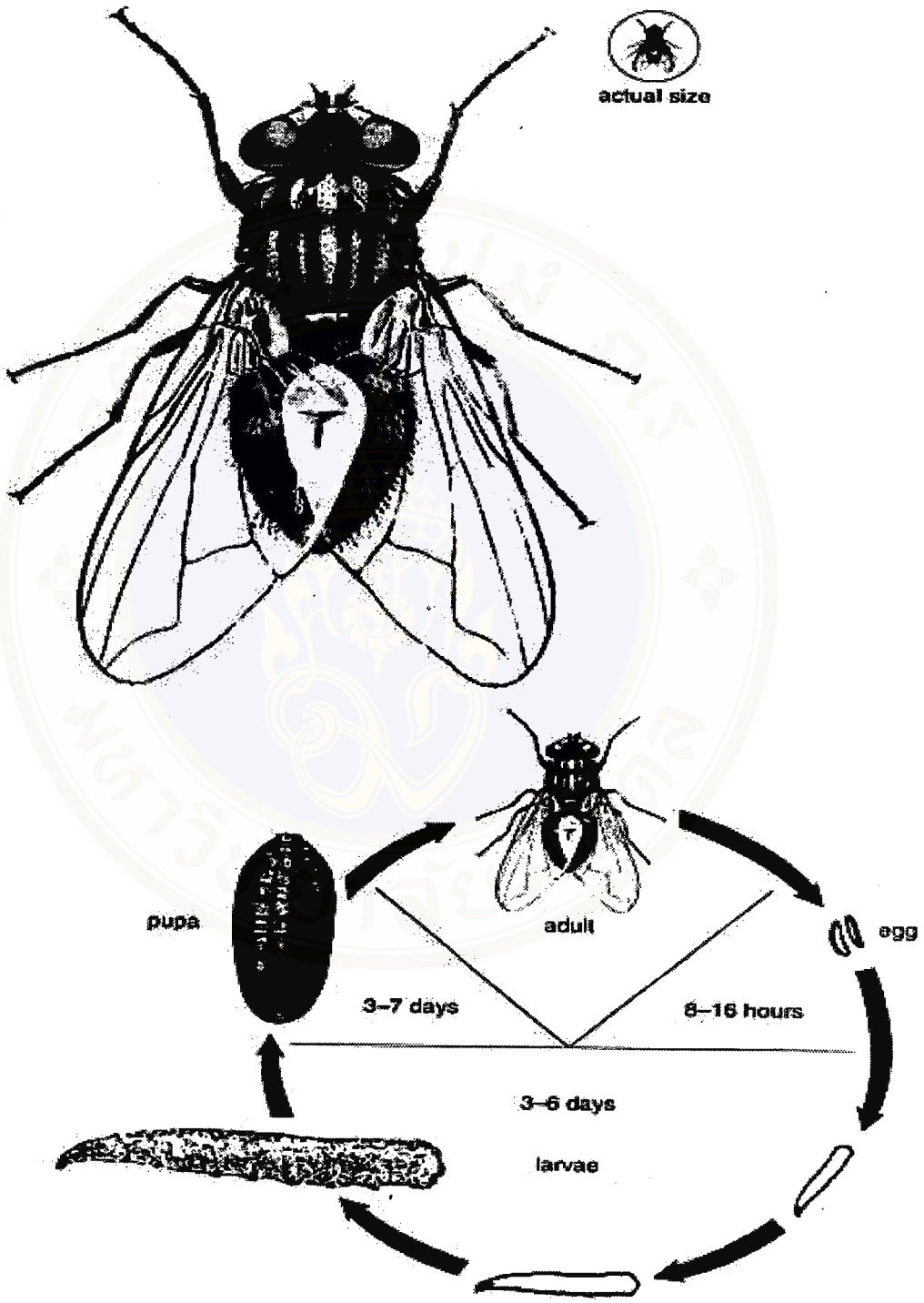


Fig 1 The life cycle of the fly.

breeding material; they must obtain oxygen from the atmosphere and can, therefore, survive only where sufficient fresh air is available. When the b reeding medium is very wet they can live on its surface only, whereas in drier materials they may penetrate to a depth of several centimetres.

The larvae of most species are slender, white, legless maggots that develop rapidly, passing through three instars. The time required for development varies from a minimum of three days to several weeks, depending on the species as well as the temperature, and the type and quantity of food available. After the feeding stage is completed, the larvae migrate to a drier place and burrow into the soil or hide under objects offering protection. They form a capsule-like case, the puparium, within which the transformation from larva to adult takes place. This usually takes 2-10 days, at the end of which the fly pushes open the top of the case and works its way out and up to the surface. Soon after emergence the fly spreads its wings and the body dries and hardens. The adult fly is grey, 6-9 mm long and has four dark stripes running lengthwise on the back. A few days elapse before the adult is capable of reproduction. Under natural conditions, an adult female rarely lays eggs more than five times, and seldom lays more than 120-130 eggs on each occasion.

Food

Both male and female flies feed on all kinds of human food, garbage and excreta, including sweat, and on animal dung. Under natural conditions, flies seek a wide variety of food substances. Because of the structure of their mouthparts, food must be either in the liquid state or readily soluble in the salivary gland secretions or in the crop. Liquid food is sucked up and solid food is wetted with saliva, to be dissolved

before ingestion. Water is an essential part of a fly's diet and flies do not ordinarily live more than 48 hours without access to it. Other common sources of food are milk, sugar, syrup, blood, meat broth and many other materials found in human settlements. The flies evidently need to feed at least two or three times a day.

Breeding sites

Female flies deposit their eggs on decayed, fermenting or rotting organic material of either animal or vegetable origin. Unlike blowflies and fleshflies, houseflies rarely breed in meat or carrion.

Dung

Heaps of accumulated animal feces are among the most important breeding sites for houseflies. The suitability of dung for breeding depends on its moisture (not too wet), texture (not too solid) and freshness (normally within a week after deposition).

Garbage and waste from food processing

Garbage provides the main medium for breeding. It includes waste associated with the preparation, cooking and serving of food at home and in public places, and with the handling, storage and sale of food, including fruit and vegetables in markets.

Organic manure

Fields that are heavily manured with organic matter such as dung, excrement, garbage and fish-meal may provide suitable breeding places for flies.

Sewage

Houseflies also breed in sewage sludge and solid organic waste in open drains, cesspools (underground pools for household sewage) and cesspits.

Accumulated plant materials

Piles of decaying grass clippings, compost heaps and other accumulations of rotting vegetable matter serve as good breeding places for flies.

BLOW FLIES

Blow flies are generally large metallic green or blue flies. The larvae are scavengers on dead animals and fish and are also found in garbage and fecal meterial. The success of this group of flies in the human environment is probably due to the facultative feeding habits of the larvae stages and the strong flying ability of the adults. The pest status of blow flies is based in part on their presence indoors. The adults can be annoying because of their large size and buzzing sound when they travel back and forth in a room and knock into windows and light fixtures and people. The adults also contaminate food with bacteria and other disease organisms because of their habit of visiting garbage sites and excrement.

Most blow fly species are strong fliers and can travel long distances on warm, sunny days. For example, the black blow fly *Phormia regina* can disperse as far as 46 km from a point of origin. This flight ability and the broad range of larval feeding habits permits many blow fly species to move easily between agricultural and human environments. The blue bottle fly *Calliphora vomitaria* may be found in natural forested regions with the larvae feeding on decaying organic matter; however, pest populations can also occur in urban areas, where larvae feed on garbage and decaying organic matter, and adults actively invade buildings. In Southeast Asia the calliphorid *Chrysomya megacephala* occurs in large populations at urban garbage dumps. It moves from these sites to the residential areas nearby.

Biology

The larval feeding habits of blow flies are not restrictive and they can utilize a range of organic meterial. The female blow flies usually lay their eggs on exposed meat ('blowing') or dead animals, but when these substrates are not available they will deposit eggs on decaying plant material or animal excrement. Dog feces form an important breeding site in urban and suburban habitats. The egg may be laid in wounds of animals and humans, and when the eggs hatch and the larvae begin feeding in the flesh the condition is known as myiasis.

In the urban environment, blow flies commonly breed in the carcasses of dead birds or rodents (including squirrels) in attics or wall voids indoors. When full grown, blow fly larvae usually leave the carcass and crawl to a drier location to pupate. Sometimes larvae are found crawling from fireplaces or dropping from

ceilings because of dead animals in these locations. If larvae go unnoticed, within a few weeks there may be a sudden infestation of blow fly adults indoors.

FLESH FLIES

These are mostly rather large flies, grey in color with longitudinal black stripes on the dorsal surface of the thorax and a somewhat shimmering and variable diced or chequered pattern on the dorsal surface of the abdomen. In life, the feet are noticeably large but when the flies are dried the part of the feet (pulvilli) that give this appearance shrivel up somewhat. Some species are brownish in color or brownish-yellow or even golden, but the longitudinal black stripes, one median and one on each side of the centre one, are always present.

The larvae are found naturally in decaying organic matter of a great variety of kinds. Sometimes they infest wounds, sores and the natural cavities of the body. The adults are larviparous, and consequently a relatively small number of maggots may be expected in any given infestation. Foul emanations from wounds and sores may attract the gravid female flies to deposit larvae in them.

The maggots resemble those of the Blue Bottles except that, in the second and third stages, which are the ones likely to come to notice, the posterior spiracles are sunk in a cleft in the truncated posterior part of the body. The slits in these spiracles are nearly vertical. Species of *Sarcophaga* are found throughout the world.

PARASITOID INSECTS

Only the larvae of holometabolous insects, especially Diptera and Hymenoptera, are parasitoids. Their unique attributes combine certain features of true parasites and predators. Parasitoids differ from predators in the following features:

1. only one host is required
2. the host is larger than the parasitoid
3. parasitoids are frequently host-specific, attacking one or several related host species
4. a lower density of host population will sustain a parasitoid population
5. the victim is usually searched for and selected by the diurnally active, adult female.

A species of parasitoid may be specific not only in the choice of host species, but also in the choice of the life stages of the host to be attacked. Eggs or young, or both, are most frequenetly attacked, but pupae and sometimes adult hosts are also eaten.

Parasitoids may feed externally as ectoparasitoids or internally as endoparasitoids. Exposed hosts are usually attacked by endoparasitoids, whereas hosts in protected situations such as leaf mines, galls, or nests, are attacked by either endoparasitoids or ectoparasitoids. When the larva of a species characteristically develops in the ratio of one to a host, the species is termed a *solitary parasitoid.* When several larvae of the same species normally develop in a single host, the species is called a *gregarious parasitoid.* If the host is a phytophagous insect, the parasitoid is a

primary parasitoid. Parasitoids that attack other parasitoids are called *hyperparasitoids* or secondary parasitoids. *Multiple parasitoidism* occurs when two or more species of primary parasitoids attack one host individual. *Superparasitoidism* occurs when a host is attacked by more larvae of some species than can reach maturity in the one host.

Adult parasitoids are free-living, and most of them are winged except for female velvet ants (Mutillidae) and certain other scolioid wasps. Energy for their activity and egg production is derived from food stored by the larva or by feeding as an adult. In the latter case, nectar and honeydew are frequently consumed. Some parasitoid wasps feed on the body fluids that are released from the host's body when it is punctured by the parasitoid's ovipositor. This is called *host feeding*. In some instances, the host is inside a cocoon or for some other reason can be reached only by the parasitoid's ovipositor. A tube of coagulated host's hemolymph, or possibly of a secretion produced by the parasitoid, forms around the ovipositor. When the ovipositor is withdrawn, the parasitoid is able to suck the host's hemolymph as it wells up in the tube.

The adult parasitoid searches first for the habitat in which the appropriate host exists. Primary parasitoids are often attracted mainly by the plants that shelter favored insect hosts. In this behavior the parasitoids respond like phytophagous insects to shapes, colors, and odors of plants, yet they do not feed on plant tissues. Once near the host, female Hymenoptera usually search for suitable individuals on which to oviposit, using their tactile and olfactory senses. Their movements may be essentially random or somewhat systematic in response to stimuli detected at a distance. Female

Diptera lack the well-developed antennae of wasps and the needlelike ovipositor for precisely inserting eggs in hosts. Consequently, the flies commonly depend on first instar larvae to actually locate hosts after the eggs are laid in the proper habitat.

RELATIVE EFFECTIVENESS

Whereas insect predators immediately kill or disable their prey, pests attacked by parasitoids die more slowly. Some hosts are paralyzed, while others may continue to feed or even lay eggs before succumbing to the attack. Parasitoids, however, often complete their life cycle much more quickly and increase their numbers much faster than many predators. Parasitoids can be the dominant and most effective natural enemies of some pest insects, but their presence may not be obvious. It is often necessary, to determine the extent of parasitism, to dissect or rear samples of pest insects to see if any adult parasitoids emerge.

Parasitoids can be parasitized by other parasitoids. This phenomenon, known as hyperparasitism, is a natural occurrence, can be common, and may reduce the effectiveness of some beneficial species. Little can be done to manage hyperparasitism.

PESTICIDE SUSCEPTIBILITY

Parasitoids are often more susceptible to chemical insecticides than predators. Adult parasitoids are usually more susceptible than their hosts. Immature parasitoids, especially if protected within the egg of their host or in their own cocoon,

may tolerate pesticides better than adults, but immature parasitoids will usually die if their host is killed.

PACHYCREPOIDEUS VINDEMMIAE (RONDANI) AS BIOLOGICAL AGENT FOR FLY CONTROL

Since chemical control measures involve the drawbacks of resistance and residues, a great variety of alternatives has been considered. Above all, there is hope that "biological control" may help in solving the problem. So over 40 years a number of novel biological control methods has been tried out. For biological control of fly populations, several species of small parasitoid wasps are specific to fly pupae, and these parasitoids should be an important part of integrated fly control. Under relatively stable conditions of fly breeding habitats, and in a suitable climate, some predators and parasitoids may effectively regulate the population and possibly limit it to an acceptable level. The effect of other predators is less known, but the parasitoid wasps may at times kill a high percentage of pupae of house flies and other species. Recent emphasis was placed on these parasitoids as it was recognized that many species were influential in fly population regulation, especially during the pupal stage.

Among the parasitoids that have been introduced for fly control, *Pachycrepoideus vindemmiae* showed promising results. The classification of this small parasitoid is :-

Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera

Suborder	Apocrita
Super family	Chalcidoidea
Family	Pteromalidae
Subfamily	Sphegigasterinae
Genus	*Pachycrepoideus*
Species	*vindemmiae*

The Pteromalid, *Pachycrepoideus vindemmiae* (Rondani) (=*dubius* Ashmead), was reported by Girault and Sanders (43) as parasitizing several species of Diptera. Further observations on this parasite were discontinued upon discovery of Crandell's (44) excellent biological and morphological account of this species. It is easily reared and attacks a variety of hosts (45,17), and might, therefore, be a promising candidate for a control program against eusynanthropic, endophilous house flies, *Musca domestica*, symbovine stable flies, *Stomoxys calcitrans*, and eusynanthropic, exophilus *Fannia* spp; *Chrysomya megacephala.*

From the previous studies of *Pachycrepoideus vindemmiae,* much is known about the field survey (1, 12, 26, 31, 33, 37, 38, 40, 42). Only a few research, which had been conducted by Crandell (44), Pickens (29), and Panicker (34), was concerned with biology.

CHAPTER III

MATERIALS AND METHODS

Study design

The study is an experimental study.

Materials

1. Fly Hosts

1.1 House fly : *Musca domestica* (Diptera: Muscidae)

1.2 Blow fly : *Chrysomya megacephala* (Diptera: Calliphoridae)

1.3 Flesh fly : *Parasarcophaga orchidae* (Diptera: Sarcophagidae)

The fly hosts were collected from the Faculty of Tropical Medicine, Mahidol University, and were colonized in the laboratory of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

2. Parasitic Hymenoptera

Pachycrepoideus vindemmiae. (Hymenoptera: Pteromalidae)

The parasitoids were available from the laboratory of the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

3. Cages and plastic jar for rearing flies and parasitoids.

Adults and larvae of the house fly and parasitoids employed in this study were maintained in two insectaria at a temperature of 27±1°C, and 80% relative humidity. Each room was illuminated daily for 8 hours from 40 w cool white fluorescent tubes. The adult flies were reared in a cage measuring 30 x 30 x 30 cm. Access to the cage was made through an 20 cm. circular opening with 30 cm. sleeve attached, and was securely knotted when out of use. The larvae were reared in a transparent plastic jar 8 inches in diameter by 8 inches in height.

The adult parasitoids were reared in a transparent plastic jar which had same size as using the fly larvae.

METHODS

1. Rearing methods

1.1 Mass rearing of flies

1.1.1 House fly: *Musca domestica* (Diptera: Muscidae)

Adult flies were fed with 10% sugar solution soaked in cotton wool in a 4 oz. ice-cream cup and were given constant access to cubes of sugar. Six to eight days after emergence, the mixture of rice bran and fish powder and water (1:1:1) was mixed well and put in an ice-cream cup then inserted into the house fly cage for oviposition. About two days after continuous feeding, the female stock oviposited the eggs, which hatched within 6 hours. About 500 newly hatched larvae were transferred to a transparent plastic jar containing about 20 oz. of the same medium, then covered with sawdust, 2-5 inches in depth. The larvae took about 4-5 days for development. The late third stage larvae crept up to pupate in the sawdust layer. When the larvae became

pupae, some of them were collected for the experiment and the rest were separated from the sawdust by sieving and then transferred to a cup. The cup was placed in the cage for the adults to emerge within 4-5 days.

1.1.2 Blow fly: *Chrysomya megacephala.* (Diptera: Calliphoridae)

The same procedure was used as in *Musca domestica,* but slices of fresh beef were used for oviposition instead of the mixture of bran and fish powder.

1.1.3 Flesh fly: *Parasarcophaga orchidae* (Diptera: Sarcophagidae)

The female of this species is larviparous. It lays first instar larvae on the breeding medium or slices of beef. The following steps for rearing this species were essentially the same as for *Chrysomya megacephala.*

1.2 Mass rearing of parasitoids

Parasitoid insect: *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae)

Adults were reared in a plastic jar with 10% sugar solution soaked in a pad of cotton wool in a small plastic container to supply carbohydrate food for male and female parasitoids. Fly pupae 6-24 hours old were supplied to parasitoids every day as hosts for oviposition, as food for development of immature parasites, and as the source of protein food for adult parasites (46). The parasitized pupae, after having been exposed to parasites for 24 hours, were separated from the ovipositing females and kept in a small plastic container before a new batch of pupae was put in the colonized cage. The parasitized puparia were kept until the new progeny of parasitoids emerged for further study.

2. Experimental methods

2.1 Biological study of the parasitoids

2.1.1 Life cycle and development

Laboratory experiments were conducted at 25-27°C and 60-80% relative humidity. The parasites were reared on 12-48 hours' old puparia of the housefly (*Musca domestica*) in screened plastic containers, supplied with 10% glucose solution soaked in a pad of cotton wool. Ovipositing females were separated from hosts at the 24-hour exposure period. Daily dissections of 20 host puparia were made to determine parasite development. All immature stages of parasites were observed in normal saline solution after removal from the host under a stereomicroscope. The adult male and female parasitoids were observed under the stereomicroscope both alive (anesthetized by ether) and dead (mounted in Hoyer's medium).

2.1.2 Longevity, fecundity, sex ratio and parasitism rate

Forty-four pairs of newly emerged adult parasites from the stock of each sex were used. Each pair of insects was kept separately in a glass vial. An excess amount of young fly pupae (about 15-20 pupae per vial) were supplied daily to the parasitoids until the ovipositing females died. The sugar pad was changed every day until all of the insects died. The longevity of each insect was recorded. The daily removed parasitized puparia were labelled and kept individual in a glass vial until the adult parasitoids (F_1 progeny) emerged. The number and sexes were checked.

In order to determine the sex of the offsprings of the unmated females another group of 20 newly emerged female parasitoids from the stock was used. Each female parasitoid was kept separately in a glass vial. The procedure followed as the same as above.

2.2 Host preference

Host preference among 3 species of fly, which are medically important in Thailand, was determined. They are :-

- *Musca domestica*
- *Chrysomya megacephala*
- *Parasarcophaga orchidae*

Determinations were done in 2 types :-

2.2.1 Single exposed type

Using a single female exposed to 15 fly puparia from each species of flys; altogether 45 puparia were used.

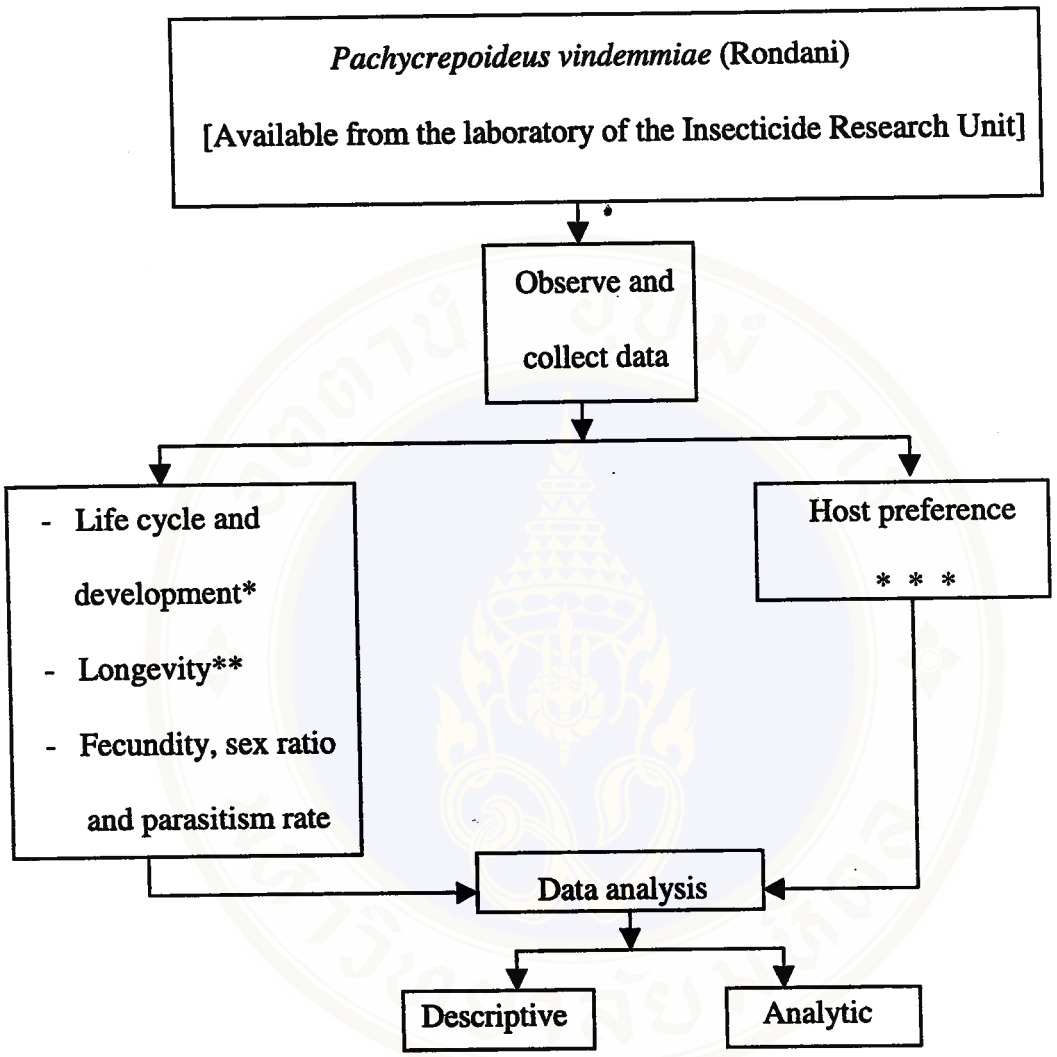
2.2.2 Group exposed type

Using a group of 5 females exposed to 50 fly puparia from each species of fly; altogether 150 puparia were used.

The number of puparia used in this experiment was chosen from the preliminary tests. The complex of various numbers of each species of fly puparia were exposed to *Pachycrepoideus vindemmiae* females. If the number of puparia was too low (less than 10 puparia/female/fly species) most of the puparia were parasitized. In case of these being too many puparia (over 20 puparia/female/fly species), a large number of them was wasted. After the preliminary experiments, it was found that for a single exposure to female *Pachycrepoideus vindemmiae,* 15 puparia of each host species provided the best result, whilst 50 puparia of each species would be best for group exposure.

Each experiment used 3 day old mated females and 12-48 hr. old puparia. The parasitized females were exposed to the host puparia for 24 hr; 10 replications were made in both experiments. After removal of the parasitized female, the host puparia were kept until the unparasitized flies emerged from the puparia and the new progeny parasites emerged. The percentage of parasitization in each host species was checked and the difference between different host species and between single exposed types and group types were compared.

Research framework



* Life cycle and development

1. Egg size
2. Morphology
3. Duration

** Longevity

1. Male
2. Female

.*** Host preference

1. Single exposed type
2. Group exposed type

CHAPTER IV

RESULTS

1. Biological study

1.1 Life history and description

1.1.1 The egg

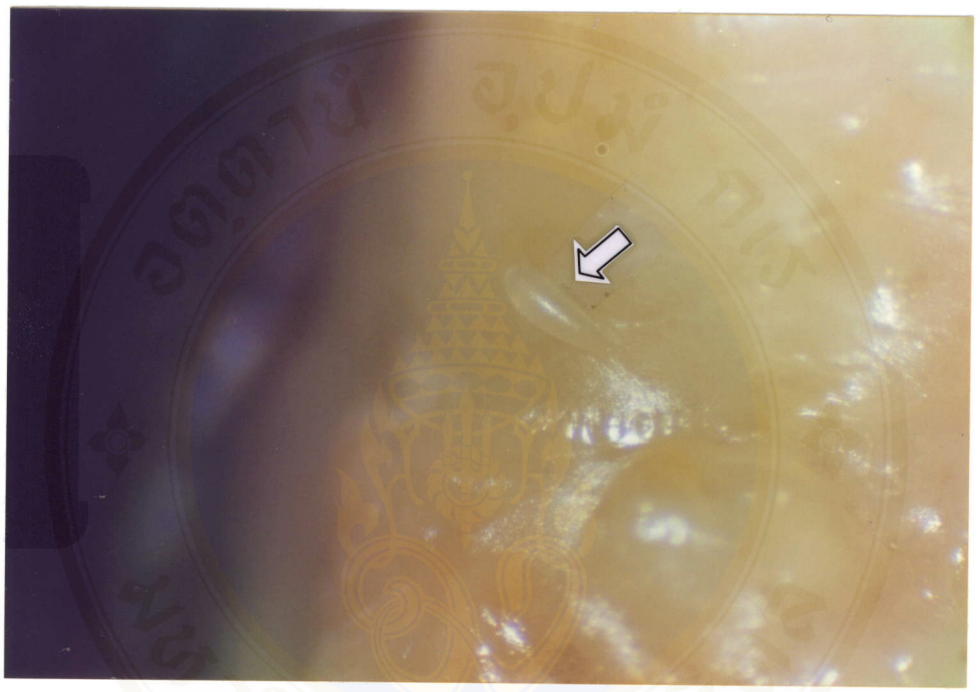
The egg of *Pachycrepoideus vindemmiae* has a smooth chorion. It is opaque white or creamy white in color. It has an elongate oval shape without stalk. The average size of the egg is 371μ in length and 146μ in width (Table 1). Its anterior end is narrower than the posterior. The average duration of the egg stage is 2 days (Figure 2).

1.1.2 The larval stage

The first instar larva

The newly emerged larva was of minute size, the larva always being slightly less than the egg in length. It has a thin and transparent cuticle, except for an elongate, median, opaque zone which owes its opaqueness to the presence of food particles in the alimentary tract. In addition to the cephalic segment, thirteen segments of the body, composed of a prominent head, the prothoracic or first segment being slightly the widest and the remaining segments narrowing posteriorly. Laterally, each thoracic segment was provided with a pair of minute hair-like papilae.

The head is more or less semicircular in outline and is superficially inserted in the prothorax (Figure 3).



A

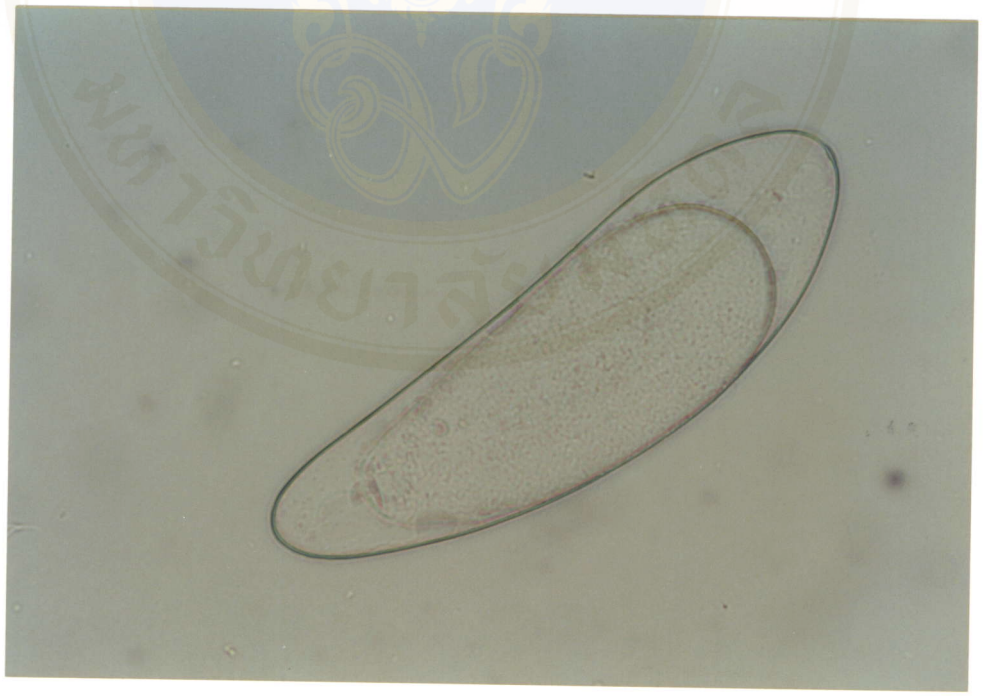
Figure 2. Egg of *Pachycrepoideus vindemmiae*

A. Parasitoid egg on the fly pupa when removed from the pupal case.(10 x 6.3)

B. The elongate oval shape of the *Pachycrepoideus vindemmiae* egg. (10 x 6.3)

C. The embryonated egg. (40 x)

B



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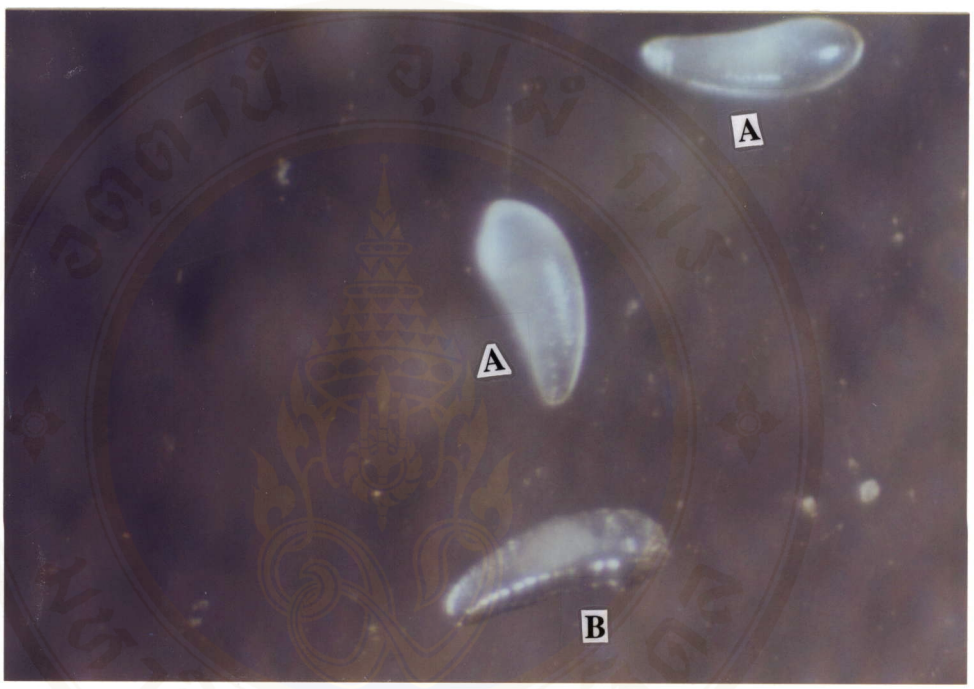


Figure 3. The eggs (A) and the first instar larva (B) of *Pachycrepoideus vindemmiae* (10 x 6.3)

The second instar larva

In this stage the parasite was much bigger than the first instar. It was about 540μ x 240μ (length x width). It has 14 segments, including the cephalic segment. Nine additional pairs of spiracles, one on each of the body segments, two to ten inclusive; those on segments two, four, five and six being larger. The sac-like gut had a dark brown color and increased in size to occupy a major part of the body cavity (Figure 4).

The third instar larva

The form is broadly spindle-shaped, the cephalic end being somewhat blunt. The dorsal is moderately arched. The arrangement of the setae and spines on the body segments is similar to that of the second instar.

The mature third-instar larva averages 680μ in length and 320μ at the greatest width (Figure 4, 5).

The fourth instar larva

In most respects, the fourth-instar larva is merely a larger edition of the third-instar larva. The color, however, is a dilute milky white.

The mature fourth-instar larva averages 1,060μ in length and 500μ at the greatest width (Figure 5).

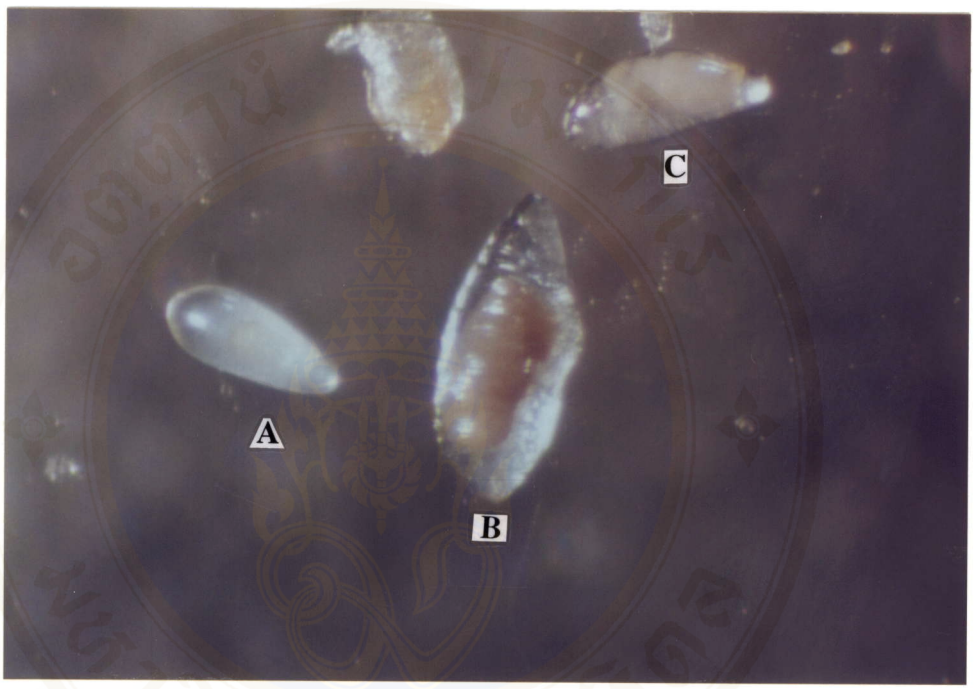


Figure 4. The egg (A), the third instar larvae (B) and the second instar larvae (C) of *Pachycrepoideus vindemmiae* (10 x 6.3)

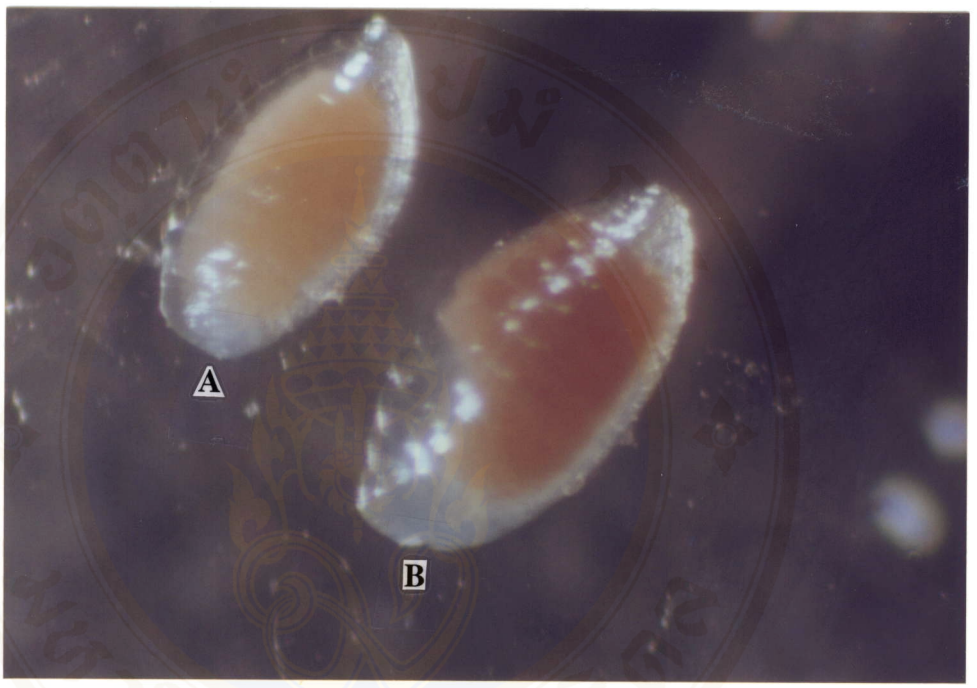


Figure 5. The third instar larvae (A) and the fourth instar larvae (B) of *Pachycrepoideus vindemmiae.* (10 x 4.5)

The fifth instar larva

It is grayish white in color, except for a darkish elongate, median, dorsal area occupied by the heart. The mature larva is elongate, cylindrical, and tapers gradually in width toward either end, the cephalic end being somewhat more blunt than the apical. The body is decidedly arched dorsally and consists of thirteen segments, the apical segment being dorso-ventrally bilobed.

The mature fifth-instar larva averages 1,900μ in length and 800μ in width (Figure 6).

The prepupa

The appearance of this stage was the intermediate form of the late fifth instar larva and the pupal stage. Externally the prepupa stage differs from the fifth instar larva through its evenly constricted body, especially the thoracic appendages. The size of the prepupa was smaller than the fifth instar larva because of the striations. It is ivory white and differentiated into two distinct regions, a relatively narrow anterior region and a wider, ovoid, posterior region which corresponds to the abdomen (Figure 7).

The pupa stage

It is of a typical naked chalcid type. A curious feature, however, is the presence of a cushion, composed of numerous moderately long bristles, on the vertex of the head. Melanization would occur about three days after pupation. It occurred gradually day by day beginning with the eyes, appendages, petiole, dorsal surface, ventral

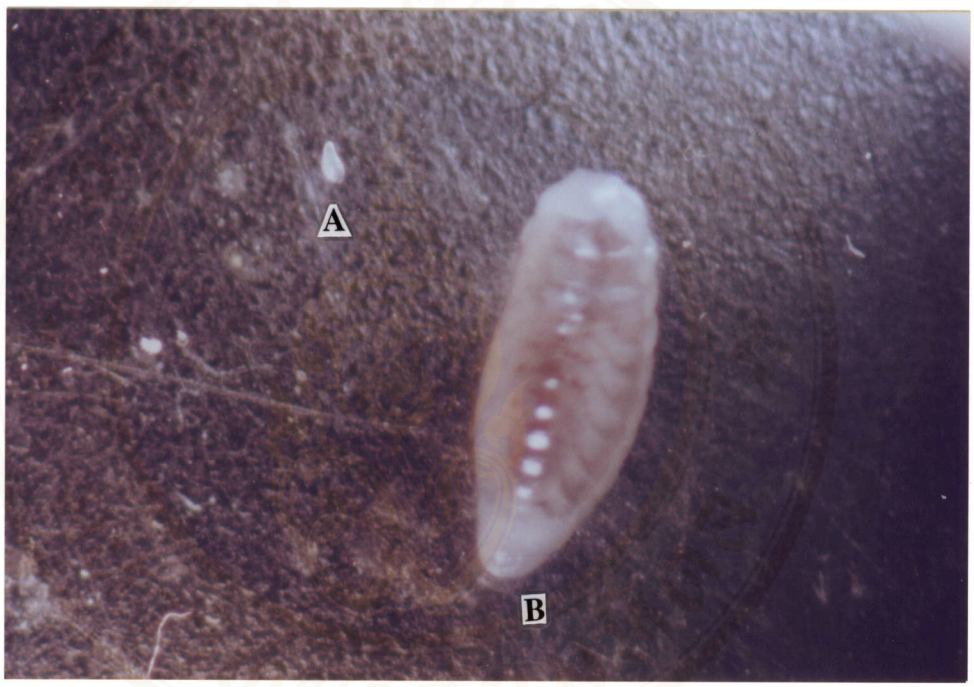


Figure 6. Size difference between the egg (A) and the fifth instar larvae (B) of *Pachycrepoideus vindemmiae.* (10 x 3.2)

surface, the thorax, head and abdomen. The old pupa had a dark metallic color which was the color of an adult. The size that was of the adult, about 2 mm (Figure 8).

The adult stage

Pachycrepoideus vindemmiae is a member in the superfamily Chalcidoidea order Hymenoptera. It has a black metallic color with a body length of about 2 mm. The wing venation is much reduced. The male and female are easily differentiated by the shape of the abdomen. For the female the abdomen is smooth and shining, polished black, distinctly petiolate; segments two and three subequal, long, the second longer, together occupying about half the surface, caudal margins of the second and third segments concavely curved in the dorsal aspect, convexly curved in the lateral aspect, bilobed and incised at the meson in the ventral aspect; segment four variably shorter than segment three but longer than segment five; remaining segments subequal, segment five about half the length of segment three, segment seven acute and apparently apical. The last abdominal sternite is divided longitudinally, the ovipositor issuing from anterior to tip of abdomen and provided with a pair of narrow exserted sheaths as long as the ovipositor (Figure 9).

For the male the abdomen is more distinctly petiolate; obovate, widest at the third segment; segments two and three subequal, long, the second longer, together occupying about half the surface; the caudal margin of segment two concavely curved in the dorsal aspect; the fourth, fifth, sixth, and seventh segments subequal, segment four about half the length of segment two, the other segments successively slightly shorter; segment eight acute and apical; abdomen moderately concave dorsal, decidedly convex ventral (Figure 10).

Figure 7. The prepupal stage of *Pachycrepoideus vindemmiae.* (10 x 4.3)

A

B

Figure 8. The pupal stage of *Pachycrepoideus vindemmiae.*

A. The newly-formed pupa (dorsal view). (10 x 4.0)

B. The newly-formed pupa (ventral view). (10 x 4.0)

C

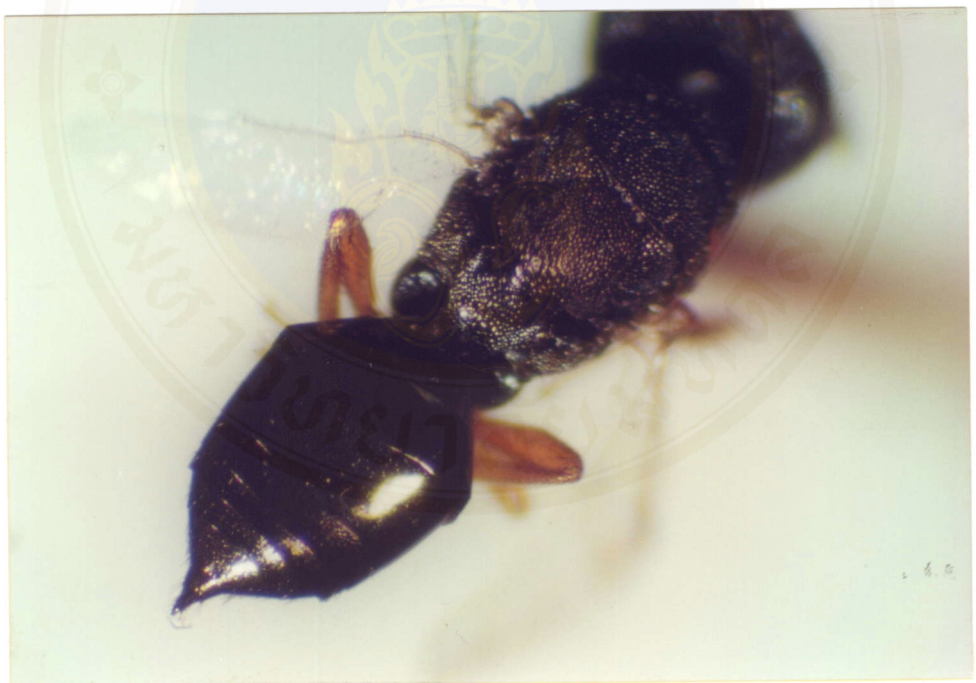
D

Figure 8. The pupal stage of *Pachycrepoideus vindemmiae.* (continue)

C. The pupa (dorsal view). (10 x 4.1)

D. The pupa (ventral view). (10 x 4.1)

A

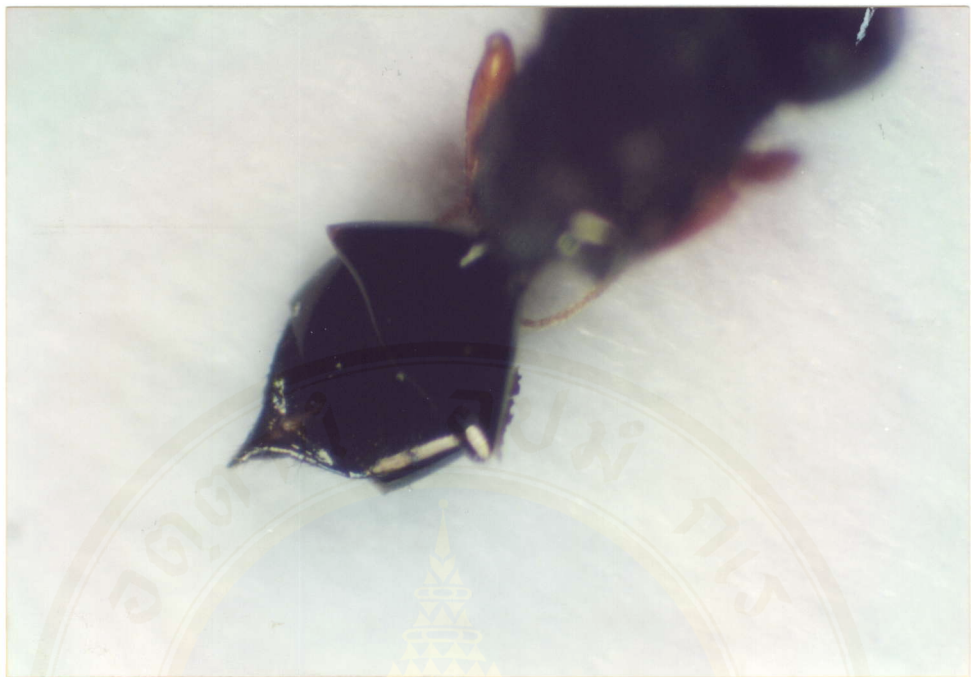


B

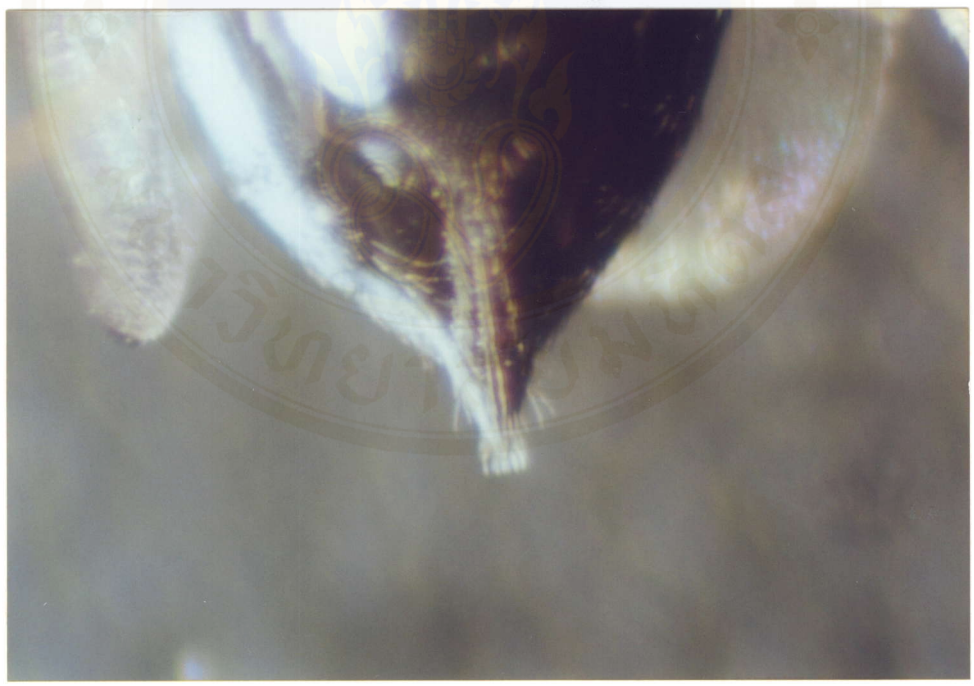
Figure 9. The adult stage female *Pachycrepoideus vindemmiae.*

A. Adult female (side view). (10 x 3.3)

B. Adult female abdomen typical of live specimen (dorsal view). (10 x 6.3)



C

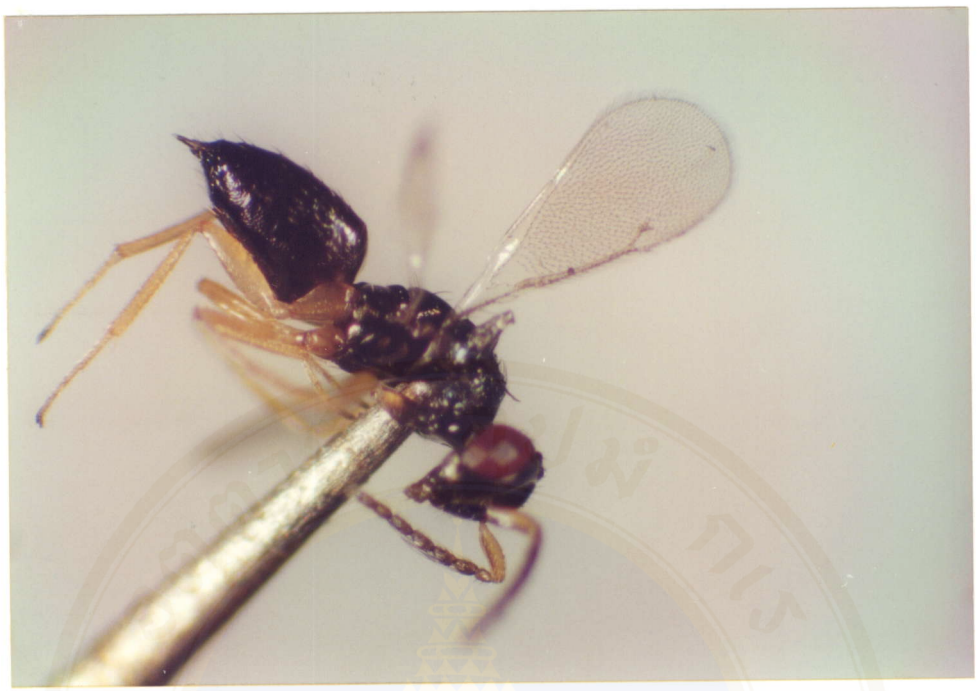


D

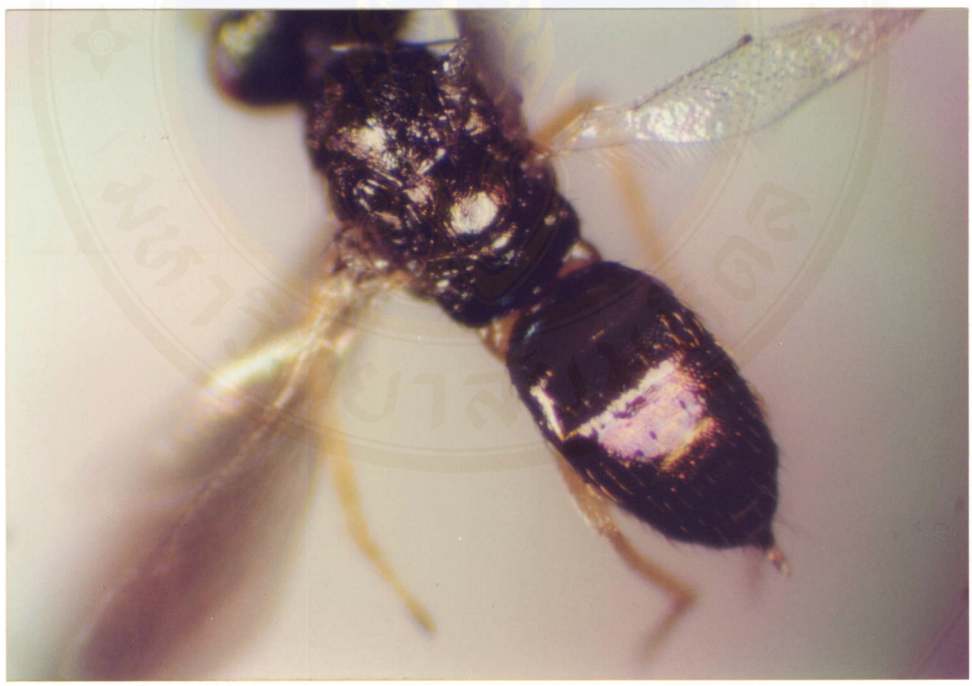
Figure 9. The adult stage female *Pachycrepoideus vindemmiae.* (contiune)

C. Abdomen of dried female (dorsal view). (10 x 6.3)

D. The apical segment acute of the adult female (ventral view). (10 x 6.3)



A

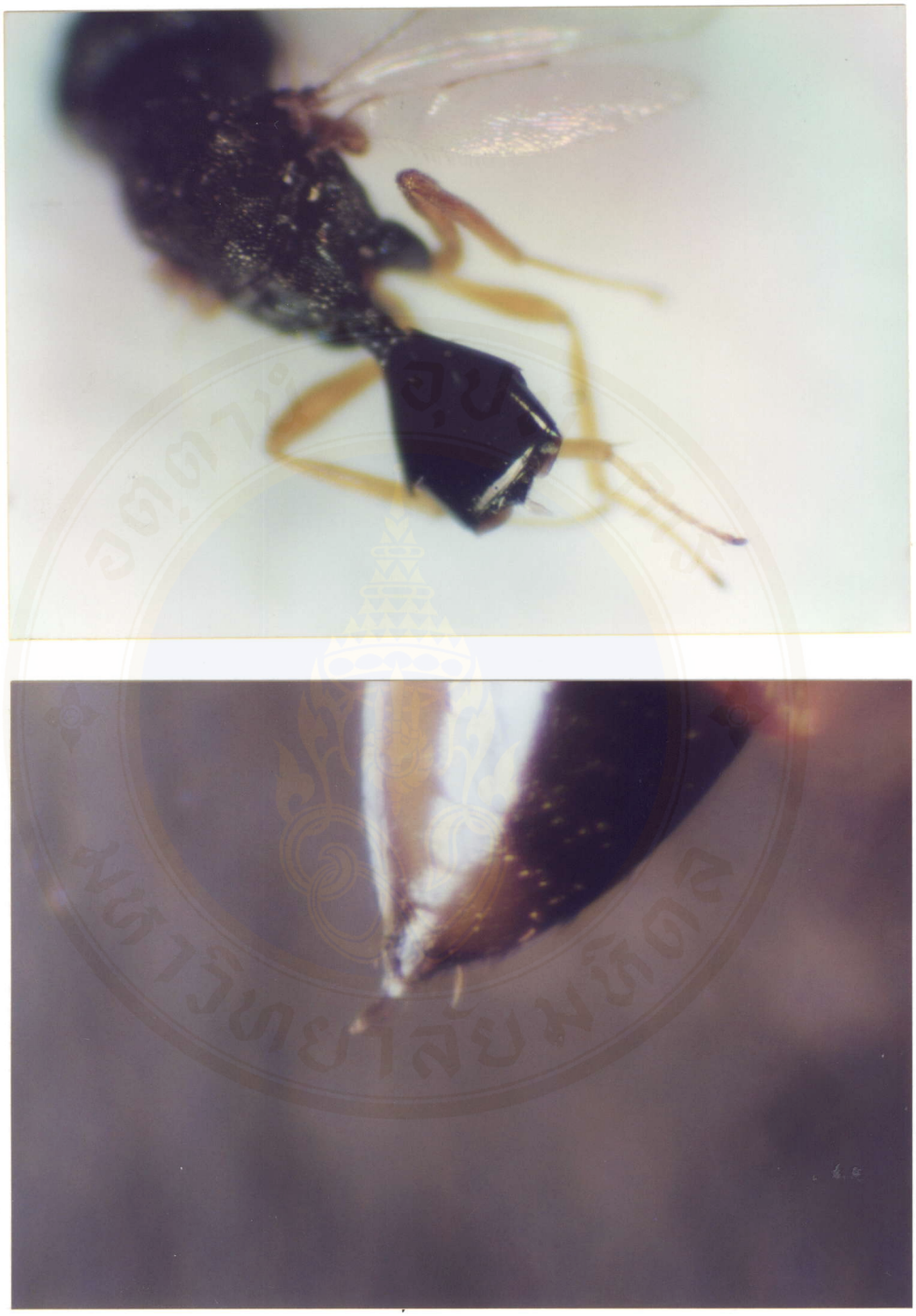


B

Figure 10. The adult stage male *Pachycrepoideus vindemmiae.*

A. Adult male (side view). (10 x 3.8)

B. Adult male abdomen typical of live specimen (dorsal view). (10 x 6.3)



C

D

Figure 10. The adult stage male *Pachycrepoideus vindemmiae.* (continue)

C. Abdomen of dried male (dorsal view). (10 x 6.3)

D. The apical segment acute of the adult male (ventral view). (10 x 6.3)

Figure 11. The difference of apical segment acute between the male (A) and female (B). (10 x 5.3)

1.2 Life cycle and developmental history

Both males and females *Pachycrepoideus vindemmiae* were sexually mature on emergence and mated as soon as they emerged from the host puparium. The female could lay eggs within 24 hours after emergence, whether she had mated or not. If mating were unsuccessful prior to oviposition, her offspring were all males. Successful mating gave rise to male and female offspring at about a 1:1.58 ratio (*i.e.* 1 male to 1.58 females, see Table 2).

Pachycrepoideus vindemmiae females laid their eggs on the external surface of the fly pupa after piercing the host puparium by a specific organ, an ovipositor or sting (Figure 12), which was derived from the modified sclerites and appendages of abdominal segments eight, nine and ten and which lies hidden in a pouch between segment seven and eight when retracted. The egg was laid singly, attached to the surface of the cuticle. The female liked to deposit her eggs on the dorsal surface of the growing fly or in the lateral recess between the head and the thorax. Other sites were also chosen but less frequently. *Pachycrepoideus vindemmiae* was a solitary insect. However, superparasitism was also observed. The highest number of eggs deposited in one pupa was 12 (range from 2-12 eggs per pupa) (Figure 13). In pupae with many parasitoid eggs inside only one or non of them could develop into an adult parasite inside.

Development of the parasitoids took place inside the host puparium. The immature stages fed on the pupal component. When the parasitoid reached the adult stage, it bored out from the host puparium and fed freely on the nectar or sugar solution and the droplet extruded from the pupae after the puncturing of the pupae by the female with has ovipositor. Development in days from egg deposition was:- egg –

first instar larva mean 1.1 ± 4.4, first instar larva – second instar larva mean 1.5 ± 4.71, second instar larva – third instar larva mean 1.6 ± 4.75, third instar larva – fourth instar larva mean 0.85 ± 3.75, fourth instar larva – fifth instar larva mean 2.85 ± 6.96, fifth instar larva – prepupa mean 2.05 ± 5.82, prepupa – pupa mena 0.55 ± 2.03, pupa – adult mean 9.5 ± 9.98. The developmental period from egg to adult averaged from 763 females was 21.30 ± 1.16 days and ranged from 18-25 days. In the male the average was 20.35 ± 1.43 days ranging from 17-25 days in 662 parasite emergences.

1.3 Longevity

In this work, longevity implied only the duration that the adult insect could live after emergence from the host puparia in the laboratory. The life spans of male and female *Pachycrepoideus vindemmiae* were slightly different. By calculation, the female by average could live longer than the male. In Figure 14, mean longevity of the 44 females was 18.22 ± 2.93 (range from 13-25 days) while that of the 44 males of was 13.63 ± 4.06 (range from 5-20 days).

1.4 Fecundity, parasitism rate and sex ratio

As can be see from Table 2, the female could produce about 112.18 successful eggs (eggs that develop until the young parasitoid emerge from the fly pupae) through her life span (range from 45-164 eggs). Oviposition activities did not appear to be confined to any particular portion of the day, but seemed to be largely a matter of individual activity on the part of the female. High temperature is favorable, some females were observed ovipositing at any daylight hour, while others were resting inactive on the sides of the cage.

Figure 12. Egg laying of a parasitic female on a house fly puparium.

A. The early stage of ovipositing period. (10 x 1.3)

B. The ovipositor is distinctly observed. (10 x 1.8)

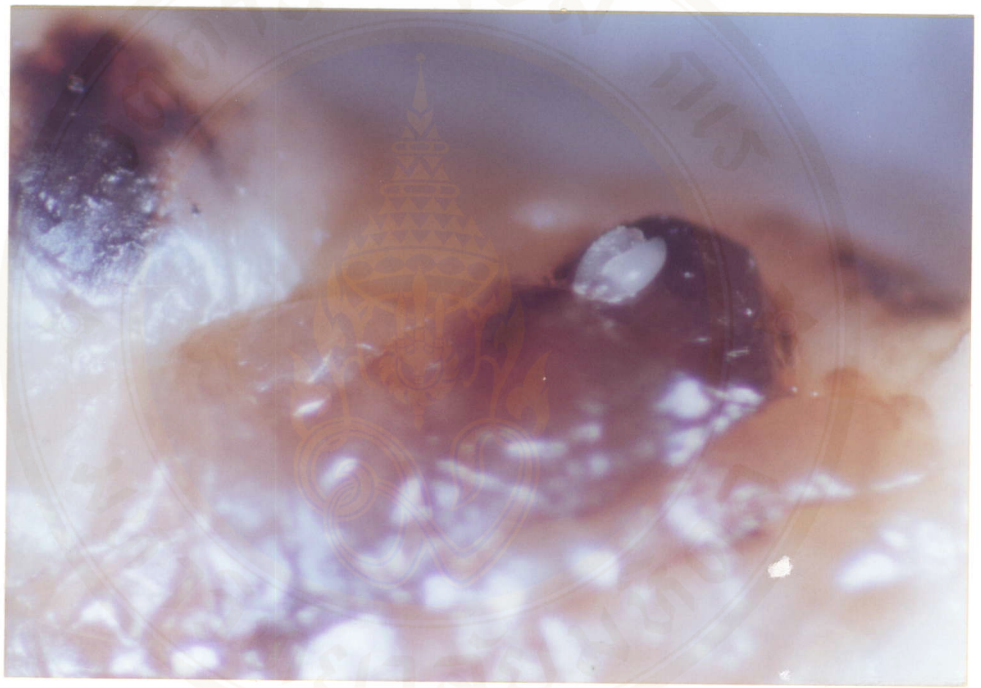


Figure 13. Two parasite eggs were found within a single puparium. (10 x 6.3)

Activity was more pronounced, however, in the morning when the sun was shining in the windows of the laboratory, which faces east, and the light intensity was high. The parasites were usually inactive at night. Artificial light, however, stimulated oviposition, and females may be induced to continue their ovipositional activities at night.

When preparing to oviposit, the female explored the host puparium by rapidly touching or tapping its surface with the extreme tips of her antennae. When a suitable place is discovered, the female assumes a somewhat upright position, brings the tip of the abdomen forward, and places the ovipositor at the desired spot by feeling the surface with the tactile extremities of the ovipositor sheaths. After the ovipositor is oriented, the tip of the abdomen is returned to about its normal position. After the puparium has been punctured, the female may immediately deposit an egg or she may pierce the pupa, ostensibly to feed on the exudation of host body fluid resulting from the wound. During oviposition, the ovipositor is inserted to its full length several times and in various directions, into the pupa skin which lines the interior of the puparium, as if creating a free space for the egg. The egg is deposited externally on the pupa, usually lateral to the position of the parasite, the actual deposition requiring a few seconds. On several occasions, the actual expulsion of the egg from the ovipositor was observed; the ovipositor is immediately withdrawn after egg deposition. The tip of the abdomen may or may not be brought forward to receive it and to prod the oviposition puncture as though sealing it. Oviposition may occur at any point on the puparium, inter- or intrasegmentally. Oviposition began in less than 24 hours after emergence and continued until the reproductive rate gradually reduced in the latter part of her life. From the experiment, 10,585 fly pupae were given to 44 female *Pachycrepoideus*

vindemmiae for ovipositing sites. The 4,155 pupae developed into adult flies and 5,676 pupae were infected and produced young parasitoids. Therefore, the parasitism rate was 75.12±10.97 percent. The mean sex ratio of female and male progeny was 1.58:1.

For the 20 unmated females, the total number of offspring obtained from 4,889 offerred pupae was 1,923. All of these offsprings were males (Table 3).

2. Host preference

Pachycrepoideus vindemmiae is not host species specific. It, however, showed preference for *Musca* sp., when pupae of three fly species were offered at the same time to the parasitized females. When 15 pupae of each fly species to one female was offered, the mean parasitization rates of pupae from 10 experiments were, 20.39%, 5.38% and 1.69% for *Musca domestica, Chrysomya magacephala* and *Parasarcophaga orchidae* respectively (Table 4). When 50 puparia of face fly species were offered to a group of 5 female parasitoids, the mean parasitization rates of pupae in 10 experiments were 40.09%, 21.57% and 14.18% for *Musca domestica, Chrysomya magacephala* and *Parasarcophaga orchidae* (Table 5). These also indicated that the parasite had a preference for the house fly pupa and the remaining two species were less preferred (Tables 4, 5).

Table 1 Egg size (μ) of *Pachycrepoideus vindemmiae*

Egg No.	Length	Width	Egg No.	Length	Width
1	340	130	26	390	150
2	360	140	27	380	160
3	400	140	28	430	170
4	380	160	29	420	170
5	360	140	30	370	150
6	380	140	31	380	140
7	380	140	32	380	150
8	360	140	33	370	150
9	380	150	34	380	140
10	370	140	35	380	160
11	400	140	36	370	140
12	390	140	37	390	160
13	360	130	38	370	150
14	370	140	39	380	150
15	340	130	40	380	150
16	370	140	41	400	140
17	380	150	42	390	160
18	390	160	43	380	150
19	390	140	44	370	140
20	410	160	45	370	150
21	380	140	46	380	140
22	380	150	47	370	140
23	370	140	48	360	150
24	380	150	49	380	140
25	370	150	50	370	140
			Mean	378.6	146.4
			Range	340-430	130-170
			S.D.	16.41	9.42

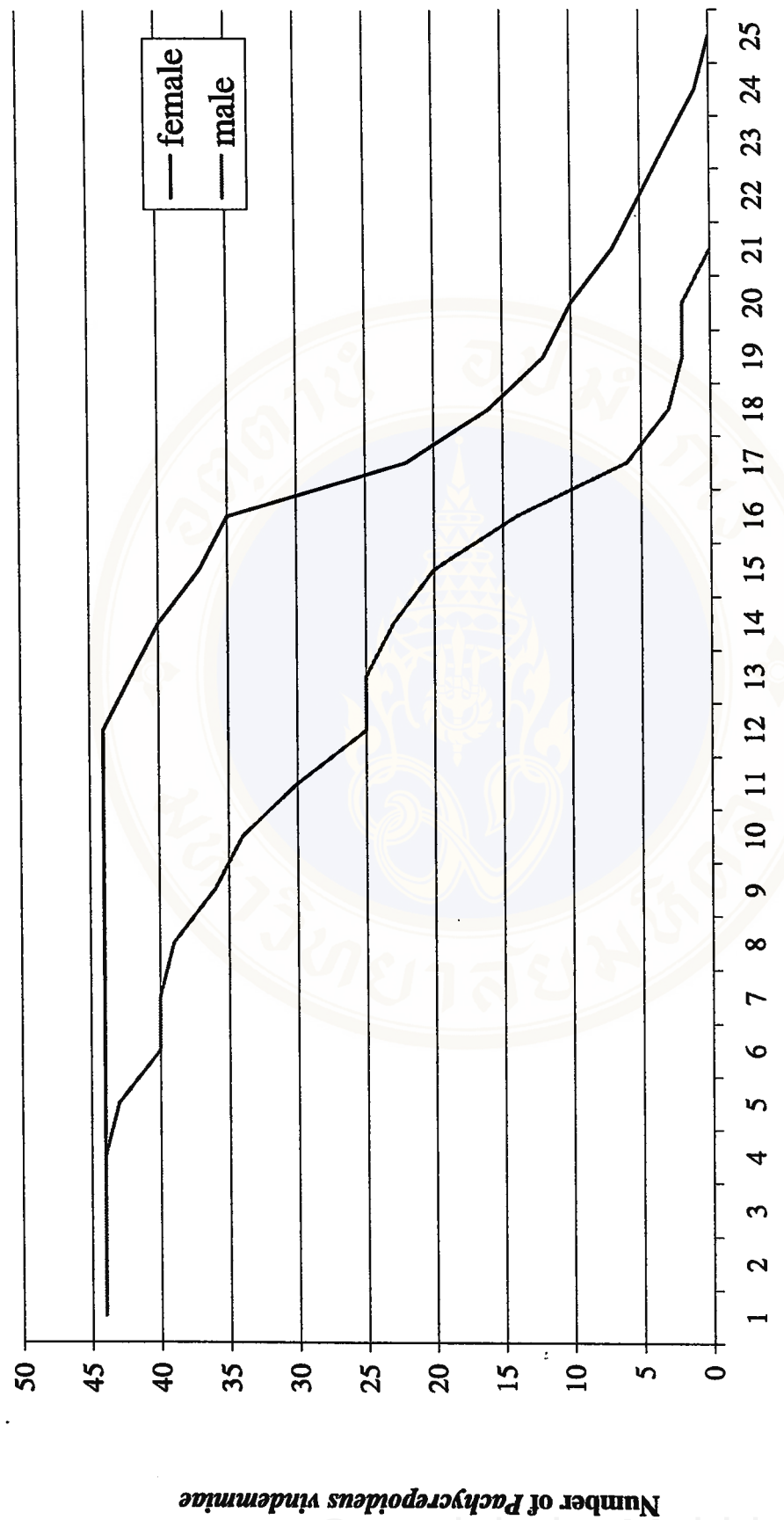


Fig. 2 Longevity of female and male *Pachycrepoideus vindemmiae* under insectary condition (25-27°C, 60-80% RH)

Table 2 **Fecundity, parasitism rate and sex ratio of *Pachycrepoideus vindemmiae.***

Rep. no.	Total no. of fly pupae offered	Average no. of fly pupae offered per day	Total no. of adult progeny per female life span	Percent parasitism	Sex ratio female / male
1	179	12.0	113	79.02	1.25
2	205	13.6	57	90.47	5.50
3	203	11.7	138	83.13	0.71
4	246	17.1	135	85.98	2.42
5	215	14.2	130	80.74	1.40
6	261	17.0	131	76.16	1.43
7	190	15.1	60	47.24	1.67
8	195	13.0	119	83.80	1.45
9	217	14.4	101	74.81	0.80
10	354	15.4	147	70.33	0.10
11	187	17.2	96	75.0	1.04
12	274	18.9	119	72.12	1.20
13	245	15.5	105	78.35	6.20
14	182	16.7	101	92.66	2.50
15	277	17.1	119	78.80	1.22
16	200	17.0	99	77.34	2.53
17	218	14.4	123	78.34	1.55
18	200	10.6	141	85.97	1.72
19	226	15.9	116	87.21	3.25
20	315	16.8	143	80.33	0.42
21	304	17.0	164	82.41	0.45
22	310	14.1	93	63.26	2.20
23	313	19.3	130	67.35	0.13
24	197	13.1	80	82.47	3.01
25	243	14.4	104	59.09	1.52

Table 2 **Fecundity, parasitism rate and sex ratio of *Pachycrepoideus vindemmiae.* (continue)**

Rep. no.	Total no. of fly pupae offered	Average no. of fly pupae offered per day	Total no. of adult progeny per female life span	Percent parasitism	Sex ratio female / male
26	277	18.0	139	90.25	0.86
27	303	14.4	102	76.69	2.00
28	232	17.0	118	84.28	2.18
29	208	13.8	45	47.36	1.34
30	203	10.8	112	78.87	0.85
31	267	17.5	150	83.33	0.05
32	208	14.6	104	64.59	1.25
33	294	15.5	149	84.65	1.40
34	276	17.0	88	70.40	1.00
35	287	14.2	110	52.38	1.67
36	213	13.4	118	69.41	0.85
37	195	13.0	94	68.61	1.23
38	196	16.7	83	57.63	0.39
39	212	15.7	95	81.19	2.05
40	200	12.6	139	83.23	0.44
41	231	17.0	87	59.18	1.05
42	247	17.2	107	78.67	1.33
43	264	17.3	106	74.64	2.67
44	316	14.4	126	67.74	1.35
$\bar{x}$	240.56	15.26	112.18	75.12	1.58
Range	179 – 354	10.6 – 19.3	45 – 164	47.24 – 92.66	0.10 – 6.20
S.D.	45.11	2.10	25.24	10.97	1.20

Table 3 **Fecundity and sex of offspring of unmated female *Pachycrepoideus vindemmiae.***

Rep. no.	Total no. of fly pupae offered	Total no. of adult progeny per female life span	Percent parasitism	Sex of offspring	
				Male	female
1	127	61	79.22	61	-
2	241	90	76.92	90	-
3	292	96	68.08	96	-
4	251	100	67.11	100	-
5	126	45	60	45	-
6	250	115	71.87	115	-
7	174	82	87.23	82	-
8	268	155	88.06	155	-
9	261	55	45.08	55	-
10	136	66	78.57	66	-
11	316	134	79.76	134	-
12	299	132	69.10	132	-
13	237	99	79.83	99	-
14	146	54	66.66	54	-
15	319	127	76.50	127	-
16	315	153	77.27	153	-
17	249	113	70.62	113	-
18	262	106	65.43	106	-
19	300	119	68	119	-
20	320	91	46.90	91	-
$\bar{x}$	244.45	99.65	71.11		
Range	126 – 320	45 – 155	45.08 – 88.06		
S.D.	65.27	31.54	10.95		

Table 4 **The number of parasitized pupae of 3 host species when 15 of each group of host puparia were exposed to individual parasitizing females at the same time, for 24 hours.**

Rep. no.	No. of parasitized pupae on different hosts				No. of unparasitized pupae
	M	C	P	Total	
1	7	2	4	13	32
2	8	2	0	10	35
3	7	2	0	9	36
4	6	3	0	9	36
5	6	1	0	7	38
6	7	3	0	10	35
7	7	4	0	11	34
8	6	1	1	8	37
9	9	1	1	11	34
10	9	0	0	9	36
Total	72	19	6	97	353
Percentage	20.39	5.38	1.69	22	78

M = *Musca domestica*

C = *Chrysomya megacephala*

P = *Parasarcophaga orchidae*

Table 5 **Number of parasitized pupae of 3 host species when 50 of each group of host puparia were exposed to a group of 5 parasitizing females at the same time, for 24 hours.**

Rep. no.	No. of parasitized pupae on different hosts				No.of uparasitized pupae
	M	C	P	Total	
1	36	15	0	51	99
2	39	10	1	50	100
3	33	28	17	78	72
4	32	10	8	50	100
5	35	18	11	64	86
6	44	15	17	76	74
7	39	22	12	73	77
8	34	22	18	74	76
9	23	20	22	65	85
10	27	24	15	66	84
Total	342	184	121	647	853
Percentage	40.09	21.57	14.18	43	57

M = *Musca domestica*

C = *Chrysomya megacephala*

P = *Parasarcophaga orchidae*

CHAPTER V

DISCUSSION

A good biological agent has to be considered for its development period, longevity and fecundity, since an insect with high fecundity and a short life cycle is easy to colonize for mass releasing into the field. *Pachycrepoideus vindemmiae* is one of the parasitic Hymenoptera which tends to have the abouve characteristics. It is the pupal parasite of the house fly. The egg of *Pachycrepoideus vindemmiae* has an elongate oval shape without stalk, the same appearance as the egg of *Dirhinus crythrocerus,* but the size is different (14). The other instars have the same shape as *Dirhinus crythrocerus,* but difference in the numbers of instars *Pachycrepoideus vindemmiae* consists of 5, where 3 is obtained for *Dirhinus crythrocerus.*

The developmental period from egg to adult of other promising fly parasites in the same genus, such as *Spalangia endius,* which has an average life cycle of 18.38 days for males and 19.95 days for females, (13) *Pachycrepoideus vindemmiae* resembles this species, as its life cycle averages 13.63±4.06 days for males ranging from 5-20 days and 18.22±2.93 days for females ranging from 13-25 days. The life cycle of *Muscidifurax raptor* was 20.7 days, *Muscidifurax zaraptor* was 22.8 days and *Spalangia cameroni* was 35.2 days at a temperature of 25°C (36). In another genus of fly parasite, *Exoristobia philippinensis* took about 16 days (47). This parasite is smaller than *Pachycrepoideus vindemmiae* and it is gregarious strain; many offspring can develop on one host but the ability to find the host in the field was poor, thus it can not be a good agent in field control (47) *Pachycrepoideus vindemmiae* is not only of

worldwide distribution, but also has very high searching ability, which is good for release in field control (34).

Apart from the fact that *Pachycrepoideus vindemmiae* females lay eggs throughout their life span, they can parasitize more host pupae at the early age of life and steadily decrease as they get old. The same results were shown in *Exoristobia philippinensis* (47). The females of some species may deposit their entire complement of eggs in a relatively short period of time. This would be a good point of *Pachycrepoideus vindemmiae,* in that she can parasitize the host at any age in her life, and whenever she can find the host.

The average longevity of both sexes of *Pachycrepoideus vindemmiae* was not much different. It was about 17 days on average. However, there was a greater variation in the male than the female. Minimum survival was 5 days and maximum was 20 days in the male, whereas it was 13 days and 25 days in the female. Compared with *Spalangia endius,* which had an average longevity of 21.63 days for females and 11.18 days for males (13) the female of this species had a shorter longevity, and it was vice versa in the male.

The average fecundity of *Spalangia endius* was also greater than *Pachycrepoideus vindemmiae.* It was 375.8 offspring per female for the former and 112 offspring per female for the latter. Sex ratio is also a factor to be considered in a good biological agent. Since the female was the one that destroy fly pupae, therefore, the species which gave a high sex ratio in favor of the female had a greater chance in pest population control. *Pachycrepoideus vindemmiae* gave a mean sex ratio of 1:1.5 in favor of female. It was less than *Spalangia endius* which had a sex ratio of 1:4 in favor of female (13). However, the sex ratio of a given parasitic species is not a fixed

figure. It will vary (i) with the sex ratio of the host; (ii) with successive generations upon the same or a different host generation; (iii) with different hosts, (iv) upon the same host and in the same season, but in different geographical regions; and (v) in successive years when the host population is increasing or declining rapidly (48, 46). Host size and temperature also influence the sex ratio of certain parasitic species. The large host tended to give female progeny, while the small one gave male. Temperature may influence the sex ratio of certain species by adversely affecting sperm viability in the spermathecae of the mated female. If the sperm in the spermathecae died, it would be similar to the unmated female, in which the offspring were all male (49). This was also found in the unmated female *Pachycrepoideus vindemmiae.* The same results were discussed by Linquist (50) in the horn fly parasite *Spalangia. muscidarum stomoxsae,* and by DeBach and Schlinger (49), and Doutt (46) in most parasitic hymenoptera.

Superparasitism is defined as the deposition of a clutch of eggs in a host already parasitized by a member of the same species. Because the progeny of a superparasitizing female are normally at a competitive disadvantage relative to the progeny of the previous parasitoid, natural selection should favor females with the ability to discriminate parasitized from unparasitized hosts. Quantitive observational studies in the field and experimental studies in the laboratory, reaching back nearly 100 years, have documented such host discrimination in a total of 150-200 species of parasitic Hymenoptera, representing nearly all families that oviposit directly on the host (51, 52, 53). These parasitoids use a variety of cues to distinguish between the parasitized and unparasitized host, including externally and internally applied chemical markers, visual or tactile detection of eggs or larvae on the cuticle of the

host, presence of necrotic host tissue, or absence of movement by the host (52). After establishment of the widespread recognition of previously parasitized hosts by parasitic Hymenoptera, researchers have recently focused on the conditions under which superparasitism, or the apparent absence of such recognition, is adaptive (52, 54, 55).

Superparasitism was found in many parasitic specie, even in natural conditions. The female *Muscidifurax raptor* showed a tendency to superparasitize host puparia, even when the host pupae were present in excess (56). The same event occurred in *Spalangia cameroni* (57). In the laboratory, superparasitism is a common occurrence. Dissections of parasitized hosts frequently revealed the presence of two or more parasite eggs. Supernumerary parasitism ordinarily results in either only one of the parasites completing its development and emerging as an adult, or all dying. Therefore, superparasitism caused reduction in efficiency per female due to the wasting of eggs, especially if a solitary specie is involved in which the excess larvae are eliminated in the first instar larva. Superparasitism was also found in *Pachycrepoideus* species. Dissection of 20 pupae found a minimum number of 2 eggs and a maximum of 12 eggs per pupa. It was generally known that superparasitism among parasitic hymenoptera may often result in loss of all the parasites on a given host through the lack of sufficient food to support the development of the parasites or the suppression on larvae which are situated in a less favorable position in the host puparium (58). This inefficiency may be alleviated by maintaining a more optimum host-parasite population ratio. With a solitary specie a high percentage of parasitization is usually accompanied by high superparasitism. However, almost 100 percent parasitism can be obtained with very rare superparasitism with a species that

14. *Parasarcophaga orchidae*

15. *Periplaneta americana*

16. *Blattella germanica*

It can not be successful for release as a biological agent to control all of them. From this study it showed preference for *Musca domestica, Chyrsomya megacephala* and *Parasarcophaga orchidae,* respectively. According to Sucharit *et al.,* (60) *Musca,* especially *Musca domestica,* is the most prevalent fly in Thailand and *Chrysomya, Parasarcophaga* and *Lucilia,* as well as flies in other genera, are less frequent. Therefore, this parasite should be very good in controlling the *Musca domestica* in Thailand.

From the overall results, the use of *Pachycrepoideus vindemmiae* for the control of flies seemed to be an important element in the natural control of the fly population, which includes other natural limiting factors. In nature, biological control by parasites is usually inconspicuous and often unsuspected, but its operation can often be demonstrated by experimental means. With rare exceptions, biological control by parasitoids is notably free from many defects that are commonplace in chemical control. It has no harmful effects on man, cultivated plants or domesticated animals. The method produces no environmental pollution and no undesirable residues in food. There is no adverse effect on wildlife through persistent toxicity passing through food chains. While chemical control is basic to current methods of controlling flies, the development of insecticide resistance and concern over the undesirable side-effects of persistent insecticides encourage research into other means of control, including biological methods. In general, the aim is the development of forms of integrated control that minimize the use of insecticides. The problem in colonizing the

parasitoids is the supply of host pupae in adequate numbers and at the suitable time. Even the generation time of flies and *Pachycrepoideus vindemmiae* are different. The flies take a shorter period than *Pachycrepoideus vindemmiae.* However, insufficient supply of host pupae can occur when both of the flies and the parasitoid start the new generation at the same time. Therefore, to colonize in large numbers and with healthy *Pachycrepoideus vindemmiae* depends most on the host. The big, healthy host pupae will give rise to healthy parasite offspring. In order to get more and healthy pupae for serving as parasitoid hosts the food and other factors affecting larval development should be considered, for example, the quantity of food media adequate for a certain number of larvae, the number of larvae for each container, the heat that liberates from the accumulated food in the container. Too much food media in the container would cause high temperature in the surrounding, especially at the center of the container, which might result in the death of larvae. For the adult fly, the number of flies should be related to the size of the cage. An overcrowded population will also cause death to the adult fly. The colony not tested; however, the microsporidium has been found in almost all colonies of *Muscidifurax raptor* than been found in almost all, usually at high rates. The microsporidium severely reduces fecundity of *Muscidifurax raptor* (61), and fecundity values observed in the study's colony were similar to those of infected wasps. The microsporidium also occurs in natural populations of *Muscidifurax raptor* and in parasitoids from insectaries, but has not been found in *Spalangia cameroni* (61). Although it is possible to reduce or even eliminate infection, whether this will be done in commercial insectaries remains to be seen. However, in general, colonizing of *Pachycrepoideus vindemmiae* is not very difficult. It is a practical method for mass rearing *Pachycrepoideus vindemmiae* for use as a bio-agent.

Improvement of some techniques should be done in order to gain more yield. Nevertheless, these are still many parasitic species, which are the natural enemics of the fly, that have not been studied.

CHAPTER VI

CONCLUSION

Laboratory study of *Pachycrepoideus vindemmiae* which is known to be one of biological agents to control the flies of medical importance was carried out in various aspects in order to gain basic knowledge of this parasitic Hymenoptera.

Biological studies were done in *Musca domestica* puparia together with morphology of immature (parasitic) stages, developmental history, longevity, fecundity, sex ratio and host preferance.

Pachycrepoideus vindemmiae is an oviparous insect laying eggs on the host pupa after piercing the host puparium. The developmental stages included egg, 5 larval instars, prepupa, pupa and adult. The egg measured 371μ in lengh and 146μ in width.

The first instar larva had a thin and transparent cuticle. The head is more or less semicircular in outline and is superficially inserted in the prothorax. The second instar larva was about 540μ x 240μ. It has 14 segments and 9 pairs of spiracles. The third instar larva is broadly spindle-shaped in form. The fourth instar larva is merely a larger edition of the third instar larva. The fifth instar larva is grayish white in color, except for a darkish elongate, median, dorsal area occupied by the heart.

The prepupa is ivory white and differentiated into two distinct regions, a relatively narrow anterior region and a wider, ovoid, posterior region which corresponds to the abdomen. The pupa showed all the adult appendages. The old pupa was dark metallic, which was the same as the adult color.

The full grown adult, which is ready to bore out from the host puparium was about 2 mm. The female fed on sugar solution for carbohydrate and host pupa for getting protein for ovisynthesis. The male fed on sugar solution only.

The development of the parasitoid took place inside the host puparium. It took 1-2 days for egg, 1-2 days for first instar larva, 2-3 days for second instar larva, 1-2 days for third instar larva, 2-3 days for fourth instar larva, 3-4 days for fifth instar larva, 1-2 days for prepupa and 9-11 days for pupa. The total life cycle was 20.35 ± 1.43 days for males and 21.30 ± 1.16 days for females.

The longevity of the parasitoids was 13.63 ± 4.06 days and 18.22 ± 2.93 days for the male and female, respectively. The fecundity was 112.18 ± 25.24 offspring / female. The percentage parasitism was 75.12 ± 10.97. The mean sex ratio of female and male was 1.58:1.

Host preference was also studied in three species of synanthropic flies which were found frequently in Thailand. *Pachycrepoideus vindemmiae* is not host species specific, it readily attacks the first encountered host. However when 3 host species were offerred at the same time it showed the greatest preference for *Musca domestica,*followed by *Chrysomya megacephala* and *Parasarcophaga orchidae,* respectively.

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APPENDIX

APPENDIX A

Hoyer's Medium

Distilled water -	50cc
Gum Arabic crystals -	30gms
Chloral Hydrate -	200gms
Glycerin -	200cc

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