



Pollination Ecology of Selected *Argostemma* spp. (Rubiaceae)

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ชื่อวิทยานิพนธ์	นิเวศวิทยาการถ่ายละอองเกสรของพืชบางชนิดในสกุล <i>Argostemma</i> spp. (Rubiaceae)
ผู้เขียน	นายรัฐจักร ชูเข้ม
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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการแพร่กระจายและความชุกชุมของ *Argostemma* spp. ในเชิงความสัมพันธ์กับพฤติกรรมการหาอาหารของสัตว์ที่ช่วยให้เกิดการถ่ายละอองเกสรของพืชชนิดนี้ การศึกษาครั้งนี้เลือก *Argostemma* 7 ชนิด จากพื้นที่การศึกษา 4 แห่งในภาคใต้ของประเทศไทย โดยใช้ สวิง กล้องส่องทางไกล และกล้องวิดีโอในการเก็บตัวอย่าง สังเกตและบันทึกพฤติกรรมการหาอาหารของ แมลงที่น่าจะเป็นตัวช่วยถ่ายละอองเกสร ผล-การศึกษาพบแมลงในกลุ่มผึ้ง 2 วงศ์ 4 สกุล และ 9 ชนิด และกลุ่มแมลงวัน 1 ชนิด ผึ้งถูกจัดเป็นแมลงที่มีบทบาทในการผสมเกสร เนื่องจากมีประสิทธิภาพในการสัมผัสอับละอองเกสรและยอดเกสรตัวเมีย อีกทั้งตรวจพบละอองเกสรของ *Argostemma* spp. บนตัวผึ้งเหล่านี้แมลงวันถูกจัดเป็นเพียงผู้มาเยือน เพราะตรวจไม่พบละอองเกสรบนตัวแมลงวันชนิดนี้ แมลงที่ช่วยให้เกิดการถ่ายละอองเกสรถูกจำแนกออกเป็น 2 กลุ่ม ตามพฤติกรรมการหาอาหาร โดยการปลดปล่อยละอองเกสรจากอับละอองเกสร นั่นคือ ผึ้งที่ใช้วิธีการสั่นปีกในการปลดปล่อยละอองเกสร ได้แก่ *Elaphropoda* sp. และผึ้งที่ไม่ใช้วิธีการสั่นปีก ได้แก่ *Nomia* ชนิดที่ 1-5, *Lasioglossum* sp., *Trigona laeviceps* และ *Trigona atripes* โดยผึ้งเหล่านี้จะใช้ปากกัดอับละอองเกสรและใช้ขาในการเก็บละอองเกสรแทน การถ่ายละอองเกสรของ *Argostemma* spp. ส่วนใหญ่อาศัยผึ้งที่ใช้ปากและขา แม้ว่าวิธีการนี้จะมีประสิทธิภาพต่ำกว่าผึ้งที่ใช้การสั่นปีกก็ตาม ทั้งนี้อาจเป็นไปได้ว่าการถ่ายละอองเกสรโดยวิธีนี้จะเป็นตัวกำหนดรูปแบบการแพร่กระจายและความชุกชุมของพืชชนิดนี้ เนื่องจากผึ้งที่ใช้ปากและขามีระยะทางในการหาอาหารที่ใกล้ จึงไม่มีประสิทธิภาพในการแลกเปลี่ยนละอองเกสรและขึ้นระหว่างประชากร ซึ่งพฤติกรรมนี้สามารถอธิบายได้ว่าทำไมประชากรของ *Argostemma* spp. ในบริเวณที่ศึกษาจึงพบเป็นจำนวนน้อย และมีรูปแบบการแพร่กระจายเป็นกระจุกหรืออยู่โดดเดี่ยว

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ABSTRACT

The aim of this research is to investigate the distribution and abundance of *Argostemma* spp. in relation to the foraging behaviour of their pollinators. Seven species of *Argostemma* at four study sites in the southern part of Thailand were chosen. The hand net, a binocular and videotaping method were used to collect, observe and record the foraging behaviour of potential pollinators. Insect visitors belong to 2 families 4 genera and 9 species in the Order Hymenoptera (bees) and 1 species in the Order Diptera (flies). The bees were classified as pollinators since they effectively contacted anthers and stigma and carried pollen of selected *Argostemma* spp. on their bodies. Flies were classified as visitors because they did not carry any pollen on their bodies. The pollinators were divided into two groups according to their foraging strategies in extracting pollen from the anthers, *i.e.*, buzz pollinator and non-buzz pollinator. The buzz pollinator is *Elaphropoda* sp. and the non-buzz pollinators comprise *Nomia* spp. (5 unidentified species), *Lasioglossum* sp., *Trigona laeviceps* and *Trigona atripes*. The non-buzz bees used their mouthparts and legs to collect pollen from the poricidal anthers. The *Argostemma* spp. were observed to be predominantly pollinated by non-buzz bees though this is less effective in comparison with the buzz pollinator. It is suggested that the pollination of *Argostemma* by the non-buzz bees probably determines the distribution patterns and abundance of this plant. Non-buzz bees have shorter foraging distance. This causes an ineffective pollen and gene flow among populations. In view of this behaviour, it may explain why the populations of selected *Argostemma* spp. at all study sites appear in small number and the distribution pattern of the populations of selected *Argostemma* spp. is clumped or isolated.

CONTENTS

	Page
Contents	vi
List of tables	viii
List of figures	ix
List of tables in the appendices	xi
List of figures in the appendices	xii
Chapter 1. Introduction	1
Reasons and justification	1
Literature review	1
Central question	5
Questions	5
Hypothesis	6
Theoretical framework	6
Objectives	9
Significance of the research	9
Chapter 2. Materials and methods	10
Study species and research sites	10
Climate	15
Duration of field work	15
Experimental designs	16
Chapter 3. Results	18
Diversity of pollinators and visitors of <i>Argostemma</i> spp.	18
Insect descriptions	26
Foraging behaviours	36

CONTENTS (continued)

	Page
Chapter 4. Discussion and conclusion	43
Pollinators and the flowers of <i>Argostemma</i> spp.	43
Pollination effectiveness through the insect's foraging behaviour	47
The contribution of the pollinators to the distribution and abundance of selected <i>Argostemma</i> spp.	49
Conclusions	51
Perspectives and recommendations	52
References	53
Appendices	60
Appendix a	61
Appendix b	64
Vitae	69

LIST OF TABLES

Table		Page
1.	Flower visitors recorded on the flowers of <i>Argostemma</i> spp.	19
2.	Floral morphology and pollinators of <i>Argostemma</i> spp.	21

LIST OF FIGURES

Figure	Page
1. Entire distribution of <i>Argostemma</i> in Thailand and surrounding areas	6
2. The solanoid floral morphology of selected <i>Argostemma</i> spp.	7
3. The foraging behaviour of bees promotes out-crossing of <i>Delphinium</i>	8
4. Conceptual framework of this research	8
5. <i>Argostemma</i> in its natural habitat at the study areas	13
6. Map of Southern Thailand showing the location of the study species in the study sites	14
7. Frequency of visits of all pollinators of <i>Argostemma</i> spp. at all study sites	20
8. Pollen grains on the body of <i>Nomia</i> sp. 1 and <i>Nomia</i> sp. 2	22
9. Pollen grains on the body of <i>Nomia</i> sp. 3, <i>Nomia</i> sp. 4, <i>Nomia</i> sp. 5 and <i>Lasioglossum</i> sp.	23
10. Pollen grains on the body of <i>Elaphropoda</i> sp. and <i>Trigona atripes</i>	24
11. Pollen grains on the body of <i>Trigona laeviceps</i>	25
12. The pinned specimens of <i>Nomia</i> spp. (5 unidentified species) and <i>Lasioglossum</i> sp	34
13. The pinned specimens of <i>Elaphropoda</i> sp., <i>Trigona laeviceps</i> and <i>Trigona atripes</i>	35
14. Diagram showing manner of pollinators approaching <i>Argostemma</i> flowers by large, medium and small bees and chance of pollen grains deposit after their visits	37
15. <i>Elaphropoda</i> sp. is buzzing the flowers of <i>A. diversifolium</i> and <i>A. lobulatum</i> var. <i>variable</i>	38
16. Non-buzzing bees are collecting pollen from the star-shaped flowers	39
17. Non-buzzing bees are collecting pollen from the bell-shaped flowers	41
18. Mean time spent of all pollinator species on a flower of <i>Argostemma</i> spp.	42

LIST OF FIGURES (Continued)

Figure		Page
19.	The foraging distance of bees	45
20.	The colour perception of bees	46

LIST OF TABLES IN THE APPENDICES

Table		Page
1.	Temperature and humidity during the observation periods at all studied areas in 2007	65
2.	Temperature and humidity during the observation periods at all studied areas in 2008	67
3.	The time spent of all visitors on a flower of <i>Argostemma</i> spp.	68

LIST OF FIGURES IN THE APPENDICES

Figure		Page
1.	The pollen grains of identified <i>Argostemma</i> in the study areas	62

CHAPTER 1

INTRODUCTION

REASONS AND JUSTIFICATION

Sridith and Puff (1999, 2000) studied the taxonomic status and the geographical distribution of *Argostemma* and noted that the floral morphology of *Argostemma* was very diverse and the distribution of *Argostemma* populations was clumped with isolated and sympatric species (Figure 1). However, the information on its taxonomic status and geographical distribution does not suffice to explain the abundance and distribution of this plant species unless its interactions with pollinators are better understood. The pollination, floral morphology and the distribution of plants are closely interrelated. It is expected that the information on pollinators and their foraging behaviour can help us understand the pollination ecology and reflect the diversity of floral morphology and distribution. Therefore, the focus of this research is to make a detailed study of the interaction between pollinators and their host plants. It is anticipated that the findings can shed light on the mode of reproduction and distribution of the selected *Argostemma* spp. and these will lead to a better understanding of the survival of this plant.

LITERATURE REVIEW

Pollination ecology is the study of the sexuality of seed plants. It is concerned with pollination vectors, attractants, flower morphology and the environment. Research in this field has significant bearing on evolution, systematics, conservation and plant propagation (Catling and Catling, 1991). Hitherto, the pollination ecology of many plants has been explored. However, only a very small proportion of plant species in South-east Asia have been studied. Moreover, the decrease of pollinators has become a global pollination crisis (Allen-Wardell *et al.*, 1998; Kearns *et al.*, 1998) and the International Pollinators Initiative (São Paulo

Declaration on Pollinators) (Eardley *et al.*, 2006) points to a lack of baseline ecological data for plant-pollinator relationships for conservation management. *Argostemma* Wall. (Rubiaceae) is a large group of small perennial herbs which comprise approximately 100 species and mainly distribute in South-east Asia. There are uncommon species and grow naturally in the undisturbed areas with high humidity. The baseline information of *Argostemma* as to its taxonomic status, the abundance and its geographical distribution were documented by Sridith and Puff (1999, 2000, 2001). Thirty one species are recorded in Thailand of which 18 species can be found in the peninsula. Seven species show a high degree of endemism. Some species are rare and are at risk of extinction. The floral morphology of *Argostemma* spp. is very diverse but this does not differ in the pollination syndrome. Pollination syndromes are characteristics of flowers, *e.g.*, size, colour, odour etc. that have evolved in response to natural selection imposed by different pollen vectors, *e.g.*, abiotic (wind or water) or biotic (birds, bees, flies). Flowers of *Argostemma* are bisexual, odourless and nectarless. Flowers are small (*ca* 5-15(25) mm. diameter) with bell- or star- shaped flower. Several to many (occasional few) flowers are arranged in an umbel inflorescence (Figure 3). Flowers curve downward. The corolla lobes are erect with recurved tips, and form a cup around the central column in bell-shaped flowers but the corolla lobes are erect and do not form a cup in star-shaped flowers. The nectarless flowers of *Argostemma* consist of 4 or 5 small, white petals and 5 short anther pores which vary from being apical (poricidal dehiscent anthers) to extending almost the entire length of the anthers (longitudinal dehiscent anthers). The anthers gather at the center of the flower and mostly fused into cone and opened at one end, and the pollen is inside - smooth-grained and firmly attached. Symmetry of the anthers varies with the zygomorphic or actinomorphic anthers which are also diverse in anther's colour. The style is straight and directed forwards so that the stigma is below the anthers. The stigma is minute (less than 0.2 mm. wide).

Harmonious co-operation between members of different species has been an interesting and intriguing biological riddle ever since Charles Darwin's time. Plants lack mobility and cannot crossbreed by itself. As such, the transfer of pollen from one plant to the ovaries of another is made possible with the help of its pollinators. The reward for this trouble is a drink of nectar and/or a good meal of

pollen. Pollination is a critical stage in the reproductive process of plants. In the case of the genus *Argostemma* Wall. (Rubiaceae), its pollen matures and releases in a poricidal/longitudinal dehiscence in cone-like structure of anthers. It therefore requires an external agent to shake its pollen out. As a result, pollination is a critical stage for the survival of this plant. Pollinators play a crucial role in pollination ecology of *Argostemma* because the exchange of genes among individuals in the population depends on the foraging habits of its pollinators. In order for the reproduction of *Argostemma* to be effective, the pollination mechanisms are generally adapted to the habits of its pollinators.

Pollinators play a crucial role in the out-crossing and sexual reproduction of many plants and contribute to the maintenance of biological diversity and natural ecosystems. However, pollinators differ in both a pollinator's quantity (visitation rates) and quality (effectiveness). Pollinator effectiveness is considered from the probability of contacting stigmas and anthers (Lindsey, 1984; Sugden, 1986; Armbruster, 1988) and visit duration, with visit duration being positively related to both pollinator efficiency and effectiveness (Fishbein and Venable, 1996; Ivey *et al.*, 2003). In tropical forests, mammals, birds and insects are important pollinators. In flowering plants, however, insects have been found to play a major role in the transfer of genes via pollen from the reproductive males to the stigma of the females. Insects usually visit flowers to drink nectar, but some insects such as bees require pollen in their diet. For most bees, pollen is a principal protein source. It is collected and carried to the nest as food for larvae and is also eaten by adults, especially females producing eggs. Buchmann (1983) found that 20,000 species in 72 families (only 6-8%) of flowering plants have anthers with poricidal dehiscence, *i.e.*, opening by apical pores. Many of these species are presumed to be buzz-pollinated by bees (Van der Pijl, 1954; Buchmann, 1983). Pollen removal requires bees that land on the flowers and vibrate their indirect flight muscles at a high frequency, which causes pollen to stream out of the anthers. In reference to the sound made by the bee vibrations, this pollination system is called buzz pollination. These buzz-pollinated flowers share a number of floral traits. They are often opening bowl-shaped or white reflexed petals, small or average-sized and often lack floral nectar (Buchmann, 1983). Buzz pollination has attracted extensive interests of pollination biologists in recent years.

Nevertheless, field investigations of the pollination ecology of buzz-pollinated species are few (*e.g.*, Johnson, 1992; Knudsen and Olesen, 1993; Larson and Barrett, 1999a, 1999b; Shelly *et al.*, 2000.; Gross and Kukuk, 2001; Gross *et al.*, 2003.; Coletto-Silva *et al.*, 2004; Duncan *et al.*, 2004; Dupont and Olsen, 2006; Gao *et al.*, 2006; Schwartz-Tzachor *et al.*, 2006) and several questions concerning the response of pollinators to floral traits associated with buzz pollination remain unanswered.

Pollen is an important reward to visiting bees in buzz-pollinated flowers (Vogel, 1978; Bucnmann, 1983; Buchmaan and Cane, 1989). The flowers of *Argostemma* belong to the *Solanum*-type (*sensu* Vogel, 1978) and offers only pollen that contains in the poricidal anthers instead of nectar as a reward for its pollinators. The presumed pollination syndrome as well as the floral morphology of *Argostemma* may restrict the removal of pollen by bees, however an occurrence of buzz pollination has been by far speculative as there is no empirical evidence to support this view. The pollination success by buzzing is different depending on the body size of bees (Drummond, 2003) and the efficient pollination is accomplished by a few species which specialize in sonication or buzz pollination (Javorek *et al.*, 2002; Drummond, 2003). However, it has been proposed that buzz pollination does not appear to be highly specialized because various pollinator species are capable of performing this behaviour (Williams, 1998; Larson and Barrett, 1999), while the general evolution trend is toward specialization (Stebbins, 1970; Faegri and van der Pijl, 1971; Crepet, 1984) that appears to be quite common in the tropics (Armstrong and Irvine, 1989; Gautier-Hion and Maisels, 1994; Ghazoul, 1997; Momose, *et al.*, 1998) and is one of the current debates in pollination ecology (Waser *et al.*, 1996; Gómez and Zamora, 1999; Johnson and Steiner, 2000; Ramirez, 2004). Specialized pollination provides the legitimate pollen because it is pollinated by single functional animals (such as beetles, birds, butterflies, bees) but it is at risk of extinction when its pollinators disappear or the environment changes, and *vice versa*. Generalized pollination chances to lose its pollen because it is pollinated by several functional animals but it is advantageous in ensuring pollination in the event of environmental change (Herrera, 1988), and enables plants to expand their ranges into new habitats (Ghazoul, 1997). Specialization and generalization are concerned because of the decline of pollinators at the hot spot by the increasing forest fragmentation and destruction especially in South-east Asia (Vamosi

et al., 2006). It is notable that plants frequently adopt generalist pollination systems in the relatively unpredictable Mediterranean-type climates (Herrera, 1988; Herrera, 1996; Bosch *et al.*, 1997). Fluctuations in abundances of insect pollinators between years have been noted in other studies (Schemske and Horvitz, 1988; Heard *et al.*, 1990; Eckhart, 1992), and spatial variation was apparent (Herrera, 1988; Eckhart, 1992; Hingston, 2000).

Preliminary observation found that the distribution pattern of the populations of selected *Argostemma* spp. are clumped with isolated and/or sympatric populations. Grant (1994) and Macior (1983) proposed that ethological as well as mechanical isolation could occur when two or more plant species have different flower structures or grow in sympatric field to reduce or prevent interspecific pollinators. In sympatric species of *Argostemma*, the floral morphology is very similar and flowering is at the same duration. Could it share pollinators? If yes, what is the mechanism to avoid the competition for their pollinators? Therefore, the study of pollination ecology of *Argostemma* in its natural habitats will be of ecological significance to an understanding of the principles underlying the abundance and distribution of these plant species. It is speculated that *Argostemma* may be pollinated by bees and buzz pollination is therefore a possibility to release its pollen from fused or tubular anthers. However, field investigations of the reproductive ecology of buzz-pollinated species are few and several questions relating to the response of pollinators and floral traits associated with buzz pollination remain unanswered. Moreover, the role in buzz pollination of *Argostemma* remains unclear and requires further investigation to support this hypothesis.

CENTRAL QUESTION

How do pollinators contribute to the distribution and abundance of selected *Argostemma* spp.?

QUESTIONS

1. What are the pollinators of selected *Argostemma* spp.?
2. How do pollinators transfer pollen from anthers to the other individuals of the same species?

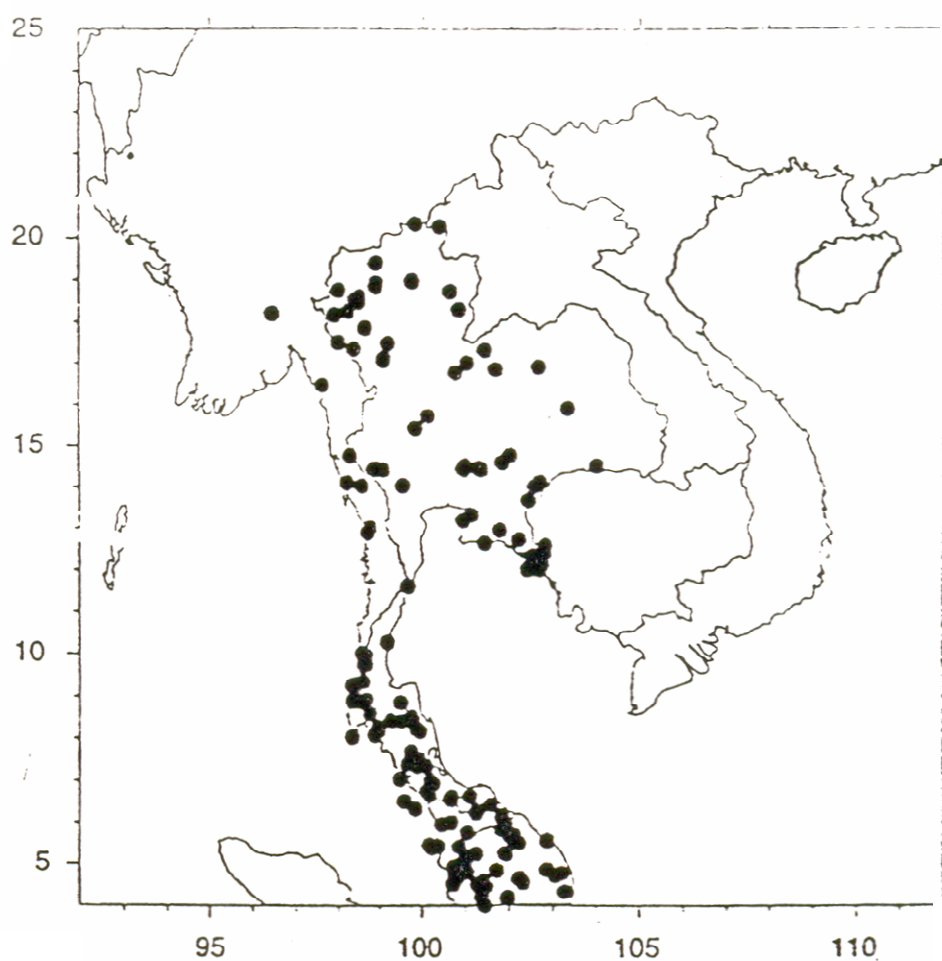


Figure 1. Entire distribution (often scattered or clumped) of *Argostemma* in Thailand and surrounding areas, Source: Sridith and Puff (2000).

HYPOTHESIS

Pollen of selected *Argostemma* spp. is shaken out of its anthers by buzz pollination following which the pollen will be transferred to another of the same species. This behaviour is performed by specialist insects.

THEORETICAL FRAMEWORK

The term “co-evolution” is often used to describe adaptations in one species in relation to adaptations in another where each partner gains a net profit. Price and Waser (1979) suggested that for sexually reproducing plants the healthiest offspring will result from mating between individuals that are not too similar in genetic make up, but not too dissimilar. The plants will benefit from outcrossing and that outcrossing in nature is achieved by the movements of pollinators such as bees. Further, pollen transfer will be most efficient if the bee flies from one plant to another of the same species. In the case of *Argostemma* spp., it is constrained by a lack of scent and nectar which could help attract potential pollinators. Pollination is however made possible only by the *Argostemma*'s pollination syndrome; its floral morphological features and a pollen reward (Figure 2). Small insects are speculated to be the *Argostemma*'s pollinator on grounds of their morphological compatibility with that of *Argostemma*'s flowers. It is further hypothesized that the pollinator should be a specialist insect which is capable of vibrating its wings rapidly in order to release pollen from the porical/longitudinal dehiscence in cone-like structure of anthers. The pollen grains are a source of diet for the insects. This pollination mechanism is called buzz pollination. Pollination syndrome is a speculation on grounds of coevolution. Therefore, pollination syndrome and coevolution are interwoven and help naturalist investigate novel plants to predict what kind of animals pollinate their flowers.

Optimal foraging theory has been widely used by behavioural ecologists to describe foraging behaviour of organisms. They behave in such a way as to find, capture and consume food containing the most calories while expending the least amount of time or energy possible in doing so. (Krebs and Davies, 1993). It influences the foraging patterns of pollinators and the pollen dispersal of flowering plants. In the

case of *Argostemma* spp., the pollen is the only reward for pollinators. The release of pollen is constrained by a small opening anther end and a small amount of pollen which is not enough for a consumer's energy requirement. The pollinators are speculated to find more pollen from other flowers/plants in a single foraging route. It is further hypothesized that the foraging behaviour of the pollinators should promote the out-crossing. A similar figure was found in *Delphinium*, the flowers at the top are males that contain the less nectar and the flowers at the bottom are females that contain the most nectar. When the bees forage, they start at the bottom flowers and move vertically upwards. This is good for the plant because it promotes out-crossing (Figure 3).

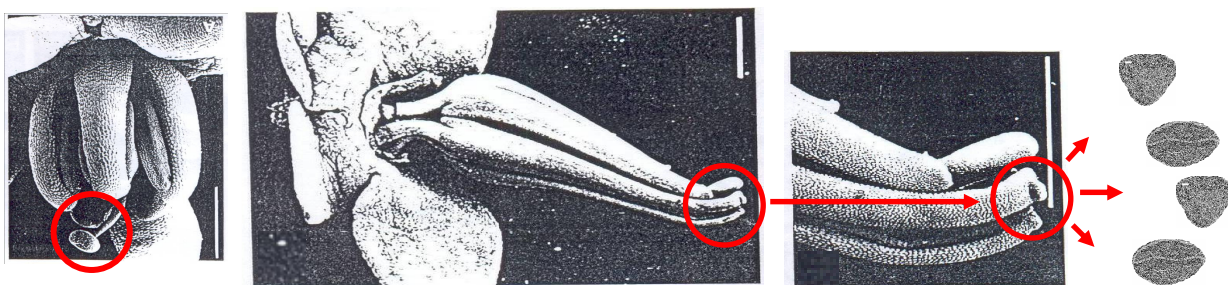


Figure 2. The solanoid floral morphology of selected *Argostemma* spp. The pollen is contained in the poricidal anthers and the stigma protrudes below the anthers. Source: Sridith and Puff (2001) and Sridith (2007).

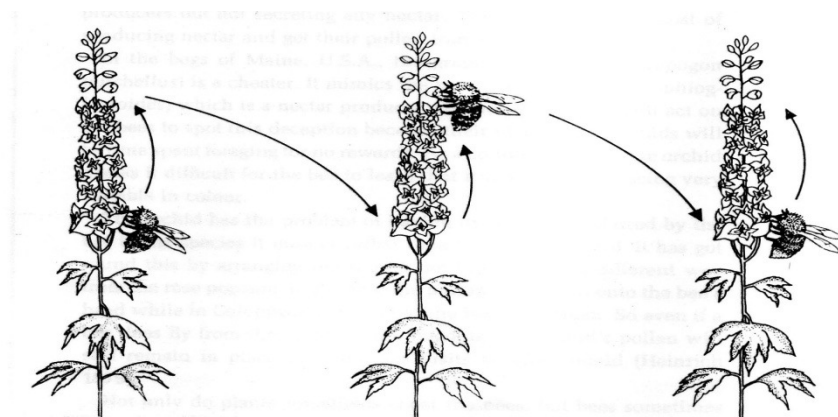


Figure 3. The foraging behaviour of bees promotes out-crossing of *Delphinium*. Source: Krebs and Davies (1993).

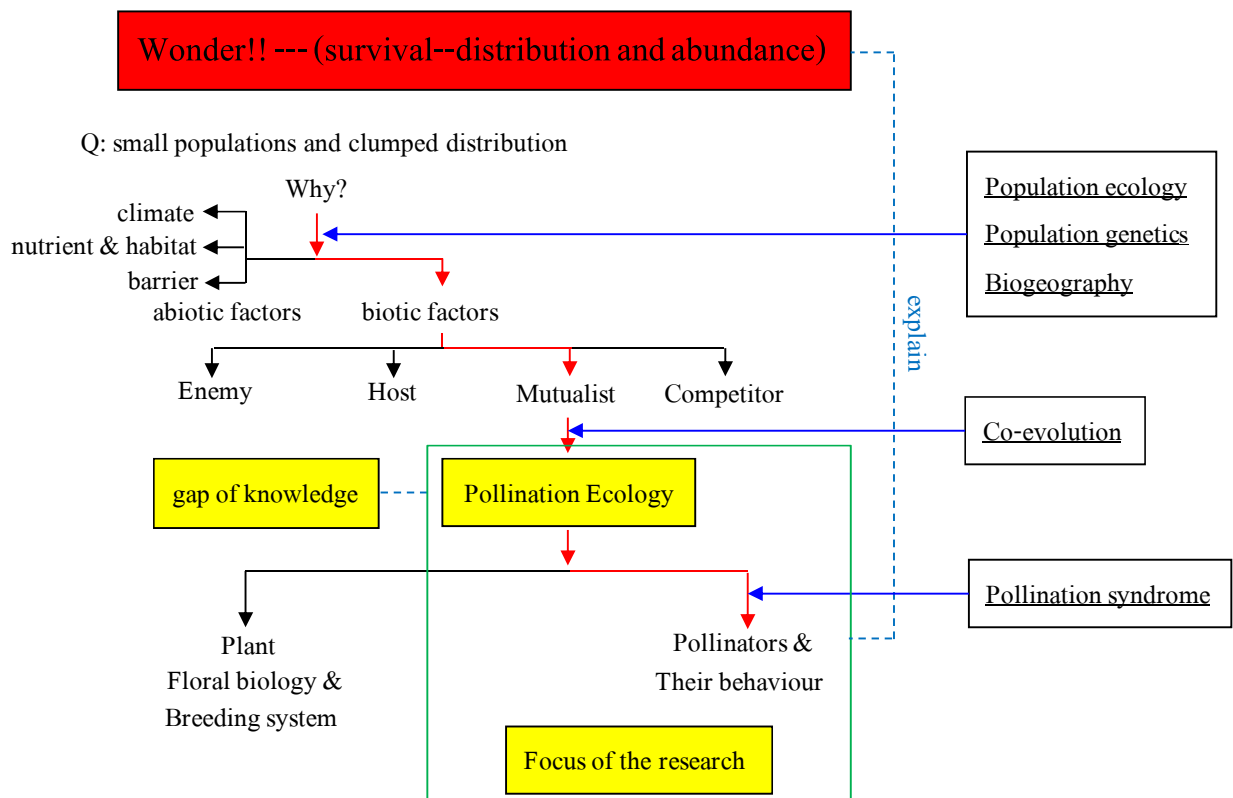


Figure 4. Conceptual framework of this research.

OBJECTIVES

1. To identify and describe the foraging behaviour of the pollinators of selected *Argostemma* species.
2. To explain the distribution and abundance of selected *Argostemma* species.

SIGNIFICANCE OF THE RESEARCH

1. To better understand the successful reproductive strategies of selected *Argostemma* spp. by means of pollination in isolated and sympatric species.
2. To gain knowledge in pollination ecology of selected *Argostemma* species in Peninsular Thailand.

CHAPTER 2

MATERIALS AND METHODS

STUDY SPECIES AND RESEARCH SITES

Seven species of *Argostemma* at four study sites were chosen for this study. They were identified by Associate Professor Kitichate Sridith, Department of Biology, Prince of Songkla University, Hat Yai Campus (personal communication). The species were selected for three reasons. First, these congeners represent isolated and sympatric species. Second, the groups include species with the bell- and star-shaped flowers, poricidal and cone-like anthers (-the longitudinal dehiscent anthers which is fused into the cone-like structure and has a pore at the apical end), colour of anthers ranges from pale yellow, yellow to orange and pale blue, symmetry of anthers comprises actinomorphic (erect anthers), slightly zygomorphic (slightly curved anthers) and zygomorphic (curved anthers). Third, they were easily accessible in terms of distance making a few-day trip for field observation possible. They consist of *A. leave* subsp. *setosum*, *A. propinguum*, *A. diversifolium*, *A. lobulatum* var. *variabile*, *A. condensum*, *A. pictum* and *A. puffii* (Figure 5):

A. leave Benn. subsp. *setosum* (Geddes) Sridith has the star-shaped flowers (pentamerous) and true-anther cones (anthers fused into cone-like structure) with longitudinal dehiscence. The anthers are yellow and zygomorphic symmetry (Figure 5E). There are 1-4 flowers/plant and flowering occurs from January through December. The large populations of *A. leave* subsp. *setosum* grow on the forest ground.

A. propinguum Ridl. has the star-shaped flowers (pentamerous) and true-anther cones (anthers fused into cone-like structure) with longitudinal dehiscence. The anthers are yellow and actinomorphic symmetry (Figure 5F). There are 4-16 flowers/plant and flowering occurs from February to August. A small population of *A. propinguum* grows on the forest ground.

A. diversifolium Ridl. has the star-shaped flowers (pentamerous) and pseudo-anther cones (anthers are not fused, although in a tube-like arrangement) with poricidal dehiscence (Figure 5A). The anthers are pale blue and slightly zygomorphic symmetry. There are 4-14 flowers/plant and flowering occurs from July to August. The small clumped populations of *A. diversifolium* grow on the rocks along the stream.

A. lobulatum Craib var. *variabile* Sridith has the bell-shaped flowers (pentamerous) with pseudo-anther cones (anthers are not fused, although in a tube-like arrangement) with longitudinal dehiscence. The anthers are pale yellow and actinomorphic symmetry (Figure 5B). There are 2-9 flowers/plant and flowering occurs from July to August. A small population of *A. lobulatum* var. *variabile* grows on the rocks near the stream.

A. condensum Craib has the star-shaped flowers (pentamerous) and true-anther cones (anthers fused into cone-like structure) with longitudinal dehiscence. The anthers are yellow and slightly zygomorphic symmetry (Figure 5C). There is 1 (rarely 2-3) flower/plant and flowering occurs on March. A small population of *A. condensum* grows on the rocks near the stream.

A. pictum Wall. has the star-shaped flowers (pentamerous) with pseudo-anther cones (anthers are not fused, although in a tube-like arrangement) with longitudinal dehiscence. The anthers are yellow and actinomorphic symmetry (Figure 5D). There are 6-20 flowers/plant and flowering occurs from June to August. The small clumped populations of *A. pictum* grow on the forest ground along the stream.

A. puffii Sridith has the bell-shaped flowers (pentamerous) with pseudo-anther cones (anthers are not fused, although in a tube-like arrangement) with longitudinal dehiscence. The anthers are yellow and actinomorphic symmetry (Figure 5G). There are 8-12 flowers/plant and flowering occurs on August. The small clumped populations of *A. puffii* grow on the limestone. *A. puffii* are rare and are at risk of extinction.

The following species pairs: *A. leave* subsp. *setosum* and *A. propinguum*, *A. diversifolium* and *A. lobulatum* var. *variabile*, and *A. condensum* and *A. pictum* are sympatric species. The flowering periods of all sympatric species are overlapping. Only *A. puffii* is isolated species. Because the populations of the sympatric species are

rare and their flowering periods occur at approximately the same time, thereby the investigation could not be replicated here.

A. leave subsp. *setosum*, *A. propinguum*, *A. condensum*, and *A. puffi* are endemic to peninsular Thailand.

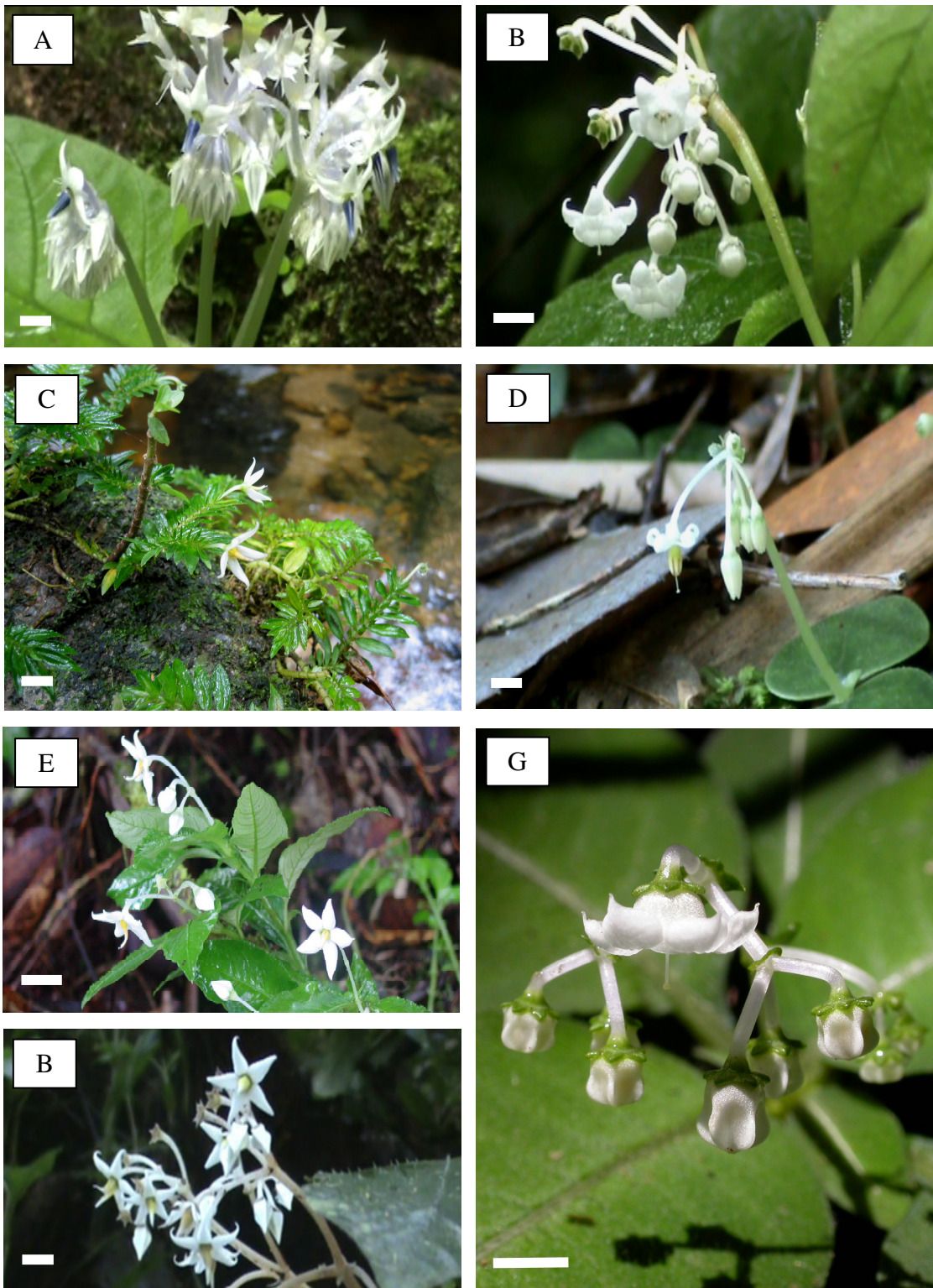
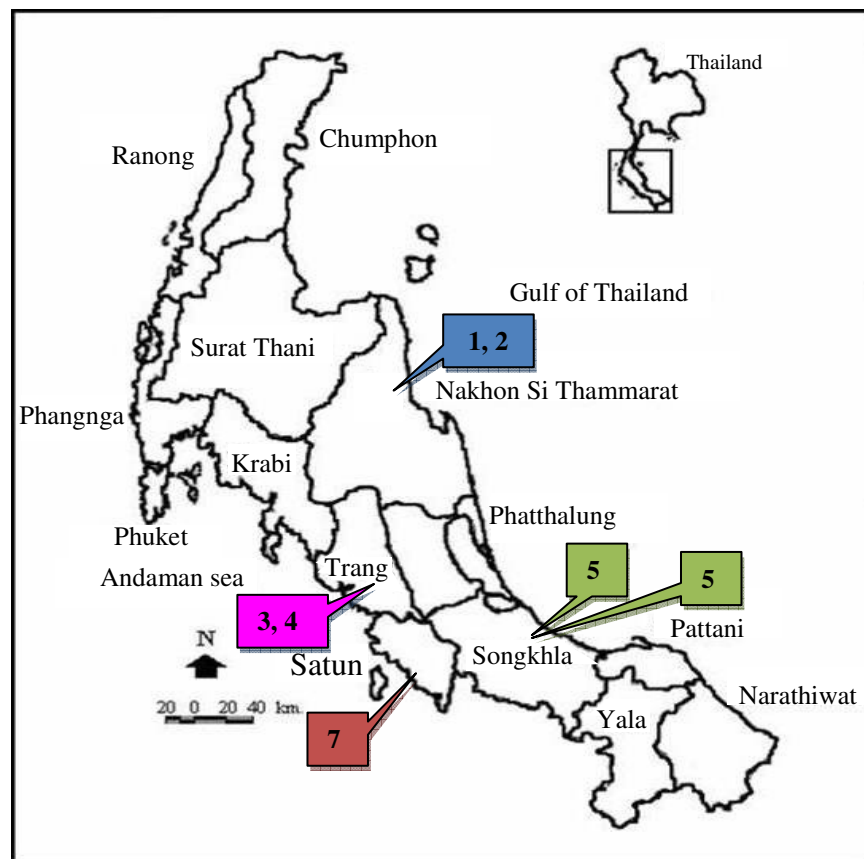


Figure 5. *Argostemma* in its natural habitat at the study areas. A. *A. diversifolium*; B. *A. lobulatum* var. *variabile*; C. *A. condensum*; D. *A. pictum*; E. *A. leave* subsp. *setosum*; F. *A. propinguum*; G. *A. puffii*. Scale bars = 5 mm.

As for the research sites (Figure 6), the first area is Khao Ram-Rom located in Ronphiboon District, Nakhon Si Thammarat Province. There are two species of *Argostemma*: *A. leave* subsp. *setosum* and *A. propinguum*. The second area is Khao Chong located in Na Yong District, Trang Province. There are two species of *Argostemma*: *A. diversifolium* and *A. lobulatum* var. *variabile*. The third area is Ton Nga Chang Waterfall. It is situated in the Ton Nga Chang Wildlife Sanctuary, Songkhla Province. There are two species of *Argostemma*; *A. pictum* occurs on the 5th and 7th tier and *A. condensum* occurs on the 7th tier of Ton Nga Chang Waterfall. The last area is Khao Toh-Ngai located in La-ngu District, Satun Province. There is one species of *Argostemma*. It is *A. puffii*.



- | | |
|---|-------------------------|
| 1 = <i>A. leave</i> subsp. <i>setosum</i> | 5 = <i>A. condensum</i> |
| 2 = <i>A. propinguum</i> | 6 = <i>A. pictum</i> |
| 3 = <i>A. diversifolium</i> | 7 = <i>A. puffii</i> |
| 4 = <i>A. lobulatum</i> var. <i>variabile</i> | |

Figure 6. Map of Southern Thailand showing the location of the study species in the study sites.

CLIMATE

All observations were made on the clear and shiny days, and occasionally on the small rainy days. Temperature (°C) and relative humidity (%) at all study sites were simultaneously measured using thermometer and hydrometer during observation periods. The climatic conditions at all study sites between 2007 and 2008 were very similar. In 2007: the mean temperature was 25.9 ± 0.3 (SE) °C, reaching a minimum of 20.2 °C and a maximum of 32.8 °C and the mean relative humidity was 74.9 ± 1.1 (SE)%, reaching a minimum of 60.0% and a mean maximum of 98.5% at all study sites. In 2008: the mean temperature was 26 ± 0.6 (SE) °C, reaching a minimum of 20.3 °C and a maximum of 32.5 °C and the mean relative humidity was 76.8 ± 2.1 (SE)%, reaching a minimum of 60.7% and a maximum of 97.8% at all study sites.

DURATION OF FIELD WORK

Field work was conducted during the flowering seasons between 2007 and 2008. The study of *A. leave* subsp. *setosum* was carried out from 13-19 July 2007 and 19-25 July 2008 and *A. propinguum* was carried out from 12-18 May 2007 and 21-27 April 2008 at Khao Ram-Rom, Nakhon Si Thammarat Province. The study of *A. diversifolium* was carried out from 20-26 August 2007 and 5-11 September 2008 and *A. lobulatum* var. *variabile* was carried out from 15-21 September 2007 and 22-28 September 2008 at Khao Chong, Trang Province. The study of *A. condensum* was carried out from 22-28 May and *A. pictum* was carried out from 28 July to 5 August 2007 at Ton Nga Chang Waterfall, Songkhla Province. The study of *A. puffii* was carried out from 22-28 September 2007 at Khao Toh Ngai, Satun Province. *A. condensum*, *A. pictum* and *A. puffii* was not studied repeatedly in 2008 because they grow in the conservation areas where permission to use the area is extremely difficult (again).

EXPERIMENTAL DESIGNS

Flower visitors and pollinators

Insect visitors were directly observed by a binocular and the videotaping method (see Dafni, 1992) at all field sites during flowering periods of all study species. Initially, observations on flower visitors were made both during the daytime and nighttime. Due to the apparent lack of floral visitors during the night, later observations were restricted to the period between onset of flowering, which started at about 0500 and the end of flower visits at around 1800. Representative individuals of all insect visitor species were captured immediately by hand net after their foraging behaviour were recorded. After that, they were spread out, fixed their legs and killed in a separate 'killing jar' by adding a few drops of ethyl acetate. They were preserved for subsequent identification, description of their morphology and collection of pollen in the laboratory. All insect visitors were identified by the authors with the assistance of Associate Professor Surakrai Permkam, Department of Pest Management, Prince of Sonkla University, Hat Yai Campus and Associate Professor Savitree Malaipan, Department of Entomology, Kasetsart University, Bangkok Campus (personal communication). The insects were identified by using the key of CSIRO (1991), Saiboon (1996) and Michener (2007). The orders, superfamilies, families, subfamilies, genera and species are given wherever possible. Voucher specimens of all insects were deposited in the collection of Princess Chakri Sirindhorn Natural History Museum, Faculty of Science, Prince of Sonkla University, Hat Yai Campus.

Insect visitors were categorized as pollinator when they effectively contacted anthers and/or stigma during flowering visits, carried pollen of selected *Argostemma* spp. on their body and caused fruit set. Pollen loads of the flower visitors were proved to be the pollen of selected *Argostemma* spp. by comparison with the pollen grains of identified *Argostemma* in the studied areas. Fruit set was investigated after three weeks on a visit.

Foraging behaviour

The foraging behaviour, *i.e.*, visitation, activity, movement of representative individuals of all insect species were observed and described for answering how they transfer pollen from poricidal anthers to the other individuals of the same species. The following characters were recorded: (1) the visitation of each pollinator species (frequency, time of starting and ending and peak of flowering visits), (2) their activities (movement, pollen collecting strategy and time spent on flowers) and (3) their movements between flowers, inflorescences, other plants of the same species and the other species in their foraging trips.

CHAPTER 3

RESULTS

Diversity of pollinators and visitors of *Argostemma* spp.

Nine species of pollinators belonging to 2 families and 4 genera within the Hymenoptera (bees) were found, *i.e.*, *Nomia* spp. (5 unidentified species), *Lasioglossum* sp., *Elaphropoda* sp., *Trigona laeviceps* and *Trigona atripes* (Table 1-2 and Figure 7). It is to be noted here that there were two to five insect species visiting the flowers of each *Argostemma* species. However, when the frequency of the visits was taken into account, two categories could be divided, *i.e.*, the ones that have 40-60% of total visits - major visitors; the other group which has less frequency of the visits - minor visitors (Table 1). The species of the pollinators vary from site to site (Table 1 and Figure 7). One species of pollinators, *i.e.*, *Nomia* sp. 1 is a common pollinator of the genus *Argostemma* in all selected study sites (it was found as a pollinator of *Argostemma* in almost every site except for Khao Toh-Ngai, Satun Province where only one more/less endemic species, *i.e.*, *A. puffii* occurred).

In addition, one visitor was recognized, *i.e.*, *Microdon* sp. It was found at Khao Ram-Rom, Nakhon Si Thammarat Province and Khao Chong, Trang Province (Table 1).

All the carried-pollen grain types of *Nomia* sp. 1 (Figure 8A-E), *Nomia* sp. 2 (Figure 8F), *Nomia* sp. 3 (Figure 9A), *Nomia* sp. 4 (Figure 9B), *Nomia* sp. 5 (Figure 9C, D), *Lasioglossum* sp. (Figure 9E, F), *Elaphropoda* sp. (Figure 10A-D), *Trigona atripes* (Figure 10E, F) and *Trigona laeviceps* (Figure 11A-D) had been compared with the pollen grain of identified *Argostemma* in the studied areas (see Appendix a). Each pollinator had carried not only *Argostemma* pollen grains, but other types of pollen grains from unknown plant species as well.

Table 1. Flower visitors, number of individuals and frequency of total visits (in parenthesis) recorded on the flowers of *Argosstemma* spp. The mean of body length was given in millimeter. Orders, families, subfamilies, genera and species are given as possible. (BL = body length, NO = number observed, NC = number collected)

Visitors	BL	Khao Ram-Rom				Khao Chong				Ton Nga Chang Waterfall				Khao Toh-Ngai	
		Nakhon Si Thammarat Province				Trang Province				Songkhla Province				Satun Province	
		<i>A. leave</i> subsp. <i>setosum</i>		<i>A. propinguum</i>		<i>A. diversifolium</i>		<i>A. lobulatum</i> var. <i>variabile</i>		<i>A. condensum</i>		<i>A. pictum</i>		<i>A. puffii</i>	
		NO	NC	NO	NC	NO	NC	NO	NC	NO	NC	NO	NC	NO	NC
Hymenoptera															
Halictidae															
Nomiinae															
<i>Nomia</i> sp. 1	8.7	27(56%)	6	18(64%)	5	5(12%)	2	13(25%)	1	6(16%)	2	4(6%)	1	-	-
<i>Nomia</i> sp. 2	7.9	-	-	-	-	-	-	-	1	5(13%)	1	4(6%)	1	-	-
<i>Nomia</i> sp. 3	12.9	-	-	-	-	-	-	-	-	-	-	-	-	6(20%)	2
<i>Nomia</i> sp. 4	8.1	-	-	-	-	-	-	-	-	-	-	-	-	15(50%)	8
<i>Nomia</i> sp. 5	11.1	-	-	-	-	12(28%)	6	14(26%)	-	-	-	-	-	-	-
Halictinae															
Halictini															
<i>Lasioglossum</i> sp.	6.7	-	-	-	-	-	-	-	1	6(16%)	3	4(6%)	1	9(30%)	1
Apidae															
Apinae															
Anthophorini															
<i>Elaphropoda</i> sp.	15.7	18(38%)	6	8(29%)	2	3(6%)	1	2(4%)	-	-	-	-	-	-	-
Meliponini															
<i>Trigona laeviceps</i> Smith.	3.6	-	-	-	-	17(40%)	10	21(40%)	24	16(42%)	9	45(66%)	24	-	-
<i>Trigona atripes</i> Smith.	5.6	-	-	-	-	-	-	-	5	5(13%)	2	11(16%)	5	-	-
Diptera															
Syrphidae															
<i>Microdon</i> sp.	6.7	3(6%)	2	2(7%)	0	6(14%)	2	3(5%)	-	-	-	-	-	-	-
Total		48		28		43		53		38		68		30	

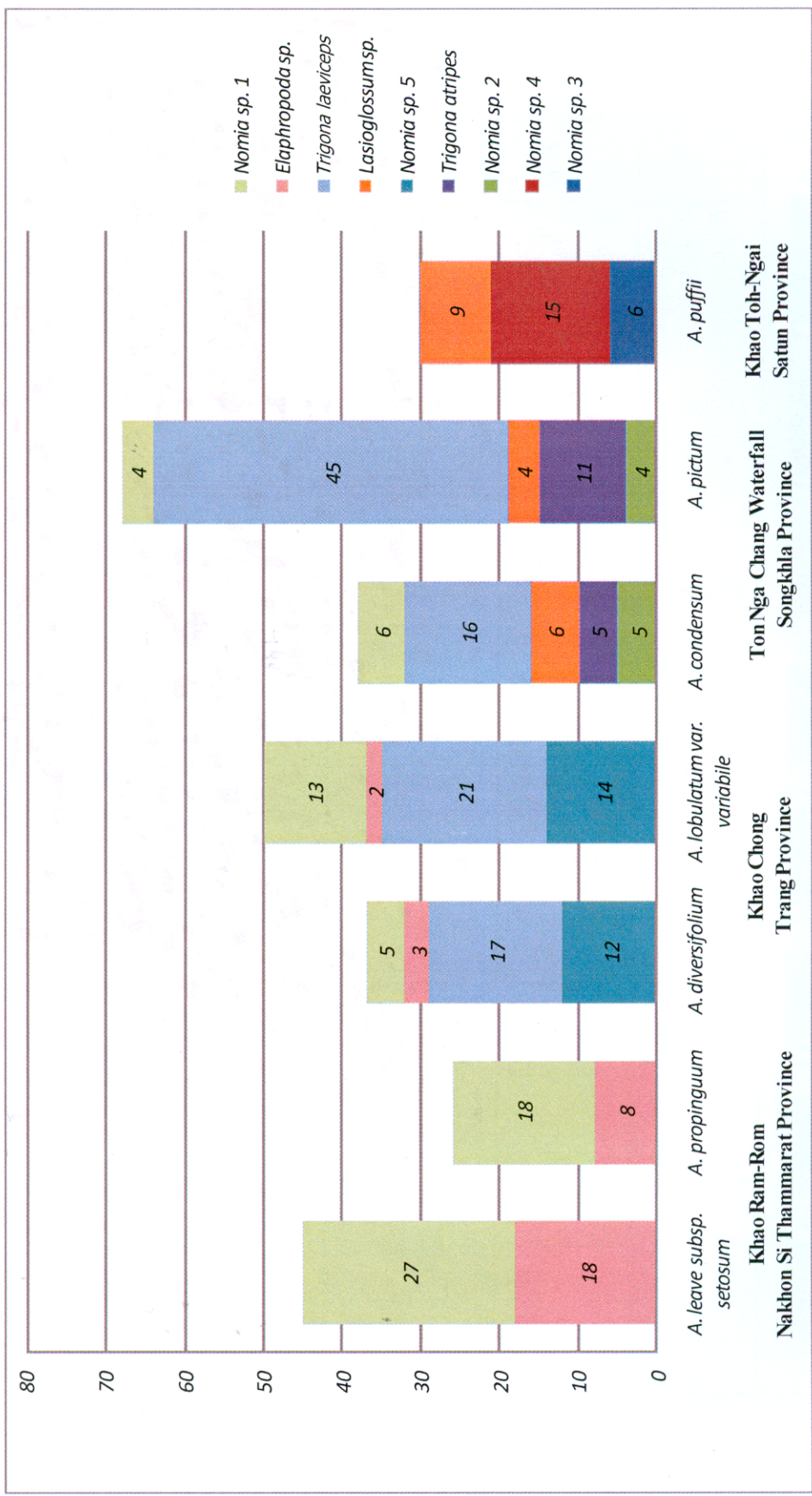




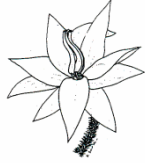

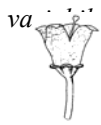









Figure 7. Frequency of visits of all pollinators of *Argostemma* spp. at all study sites.

Table 2. Floral morphology and pollinators of *Argostemma* spp. Scale bars = 5mm.

Flowers reproduced from Sridith and Puff (1999).

Study sites	Substrate	Plants	Anther	Pollinators	Pictures
Khao Ram-Rom Nakhon Si Thammarat Province	soil	<i>A. leave</i> subsp. 	- true anther cones - longitudinal dehiscence	A, B	
		<i>A. l...um</i> 	- true anther cones - longitudinal dehiscence	A, B	
Khao Chong Trang	moist rocks along stream	<i>A. diversifolium</i> 	- pseudo cones - poricidal dehiscence	A, B, C, D,	
		<i>A. lobulatum</i> var. 	- pseudo cones - longitudinal dehiscence	A, B, C, D,	
Ton Nga Chang Waterfall Songkhla	moist rocks along stream	<i>A. condensum</i> 	- true anther cones - longitudinal dehiscence	A, C, E, F, G	
		<i>A. nictum</i> 	- pseudo cones - longitudinal dehiscence	A, C, E, F, G	
Khao Toh-Ngai Satun	lime stone	<i>A. nuffii</i> 	- pseudo cones - longitudinal dehiscence	G, H, I	

I = *Nomia* sp.3

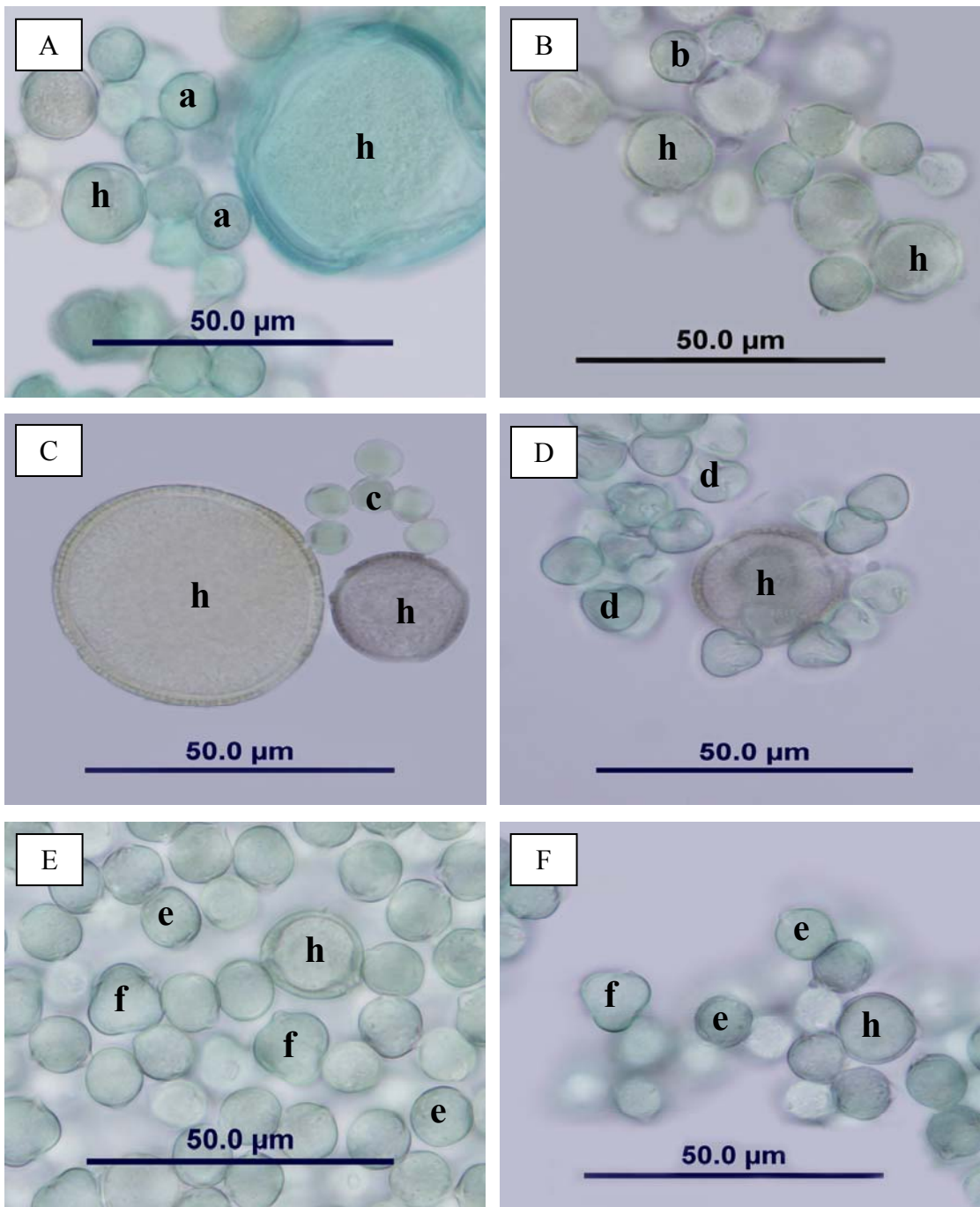


Figure 8. Pollen grains on the body of (A-E) *Nomia* sp. 1 and (F) *Nomia* sp. 2 (magn. 100×) (a = *A. leave* subsp. *setosum*, b = *A. propinguum*, c = *A. diversifolium*, d = *A. lobulatum* var. *variabile*, e = *A. condensum*, f = *A. pictumi*, h = unknown plant species).

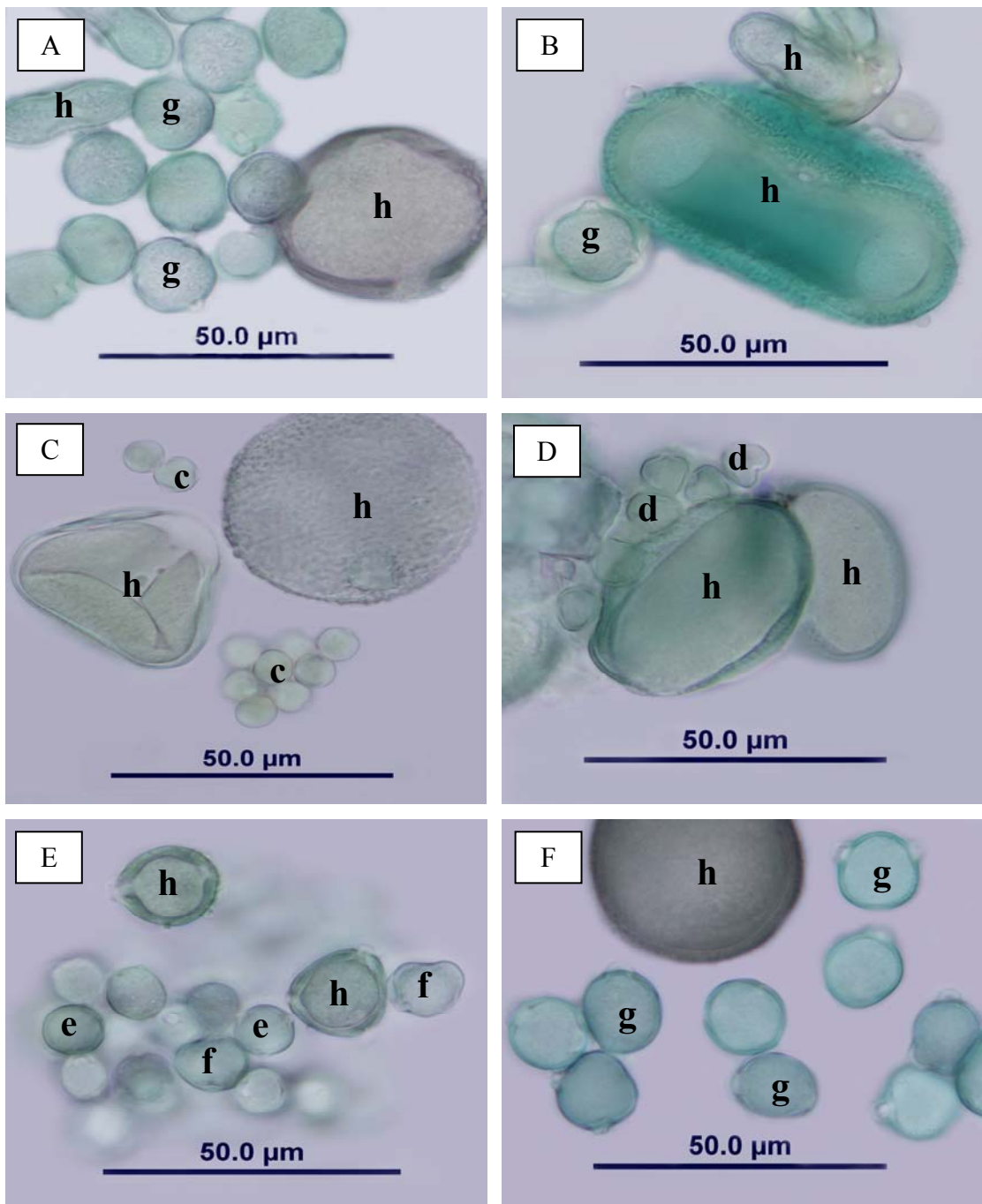


Figure 9. Pollen grain on the body of (A) *Nomia* sp. 3, (B) *Nomia* sp. 4, (C-D) *Nomia* sp. 5, and (E-F) *Lasioglossum* sp. (magn. 100×) (c = *A. diversifolium*, d = *A. lobulatum* var. *variabile*, e = *A. condensum*, f = *A. pictum*, g = *A. puffii*, h = unknown plant species).

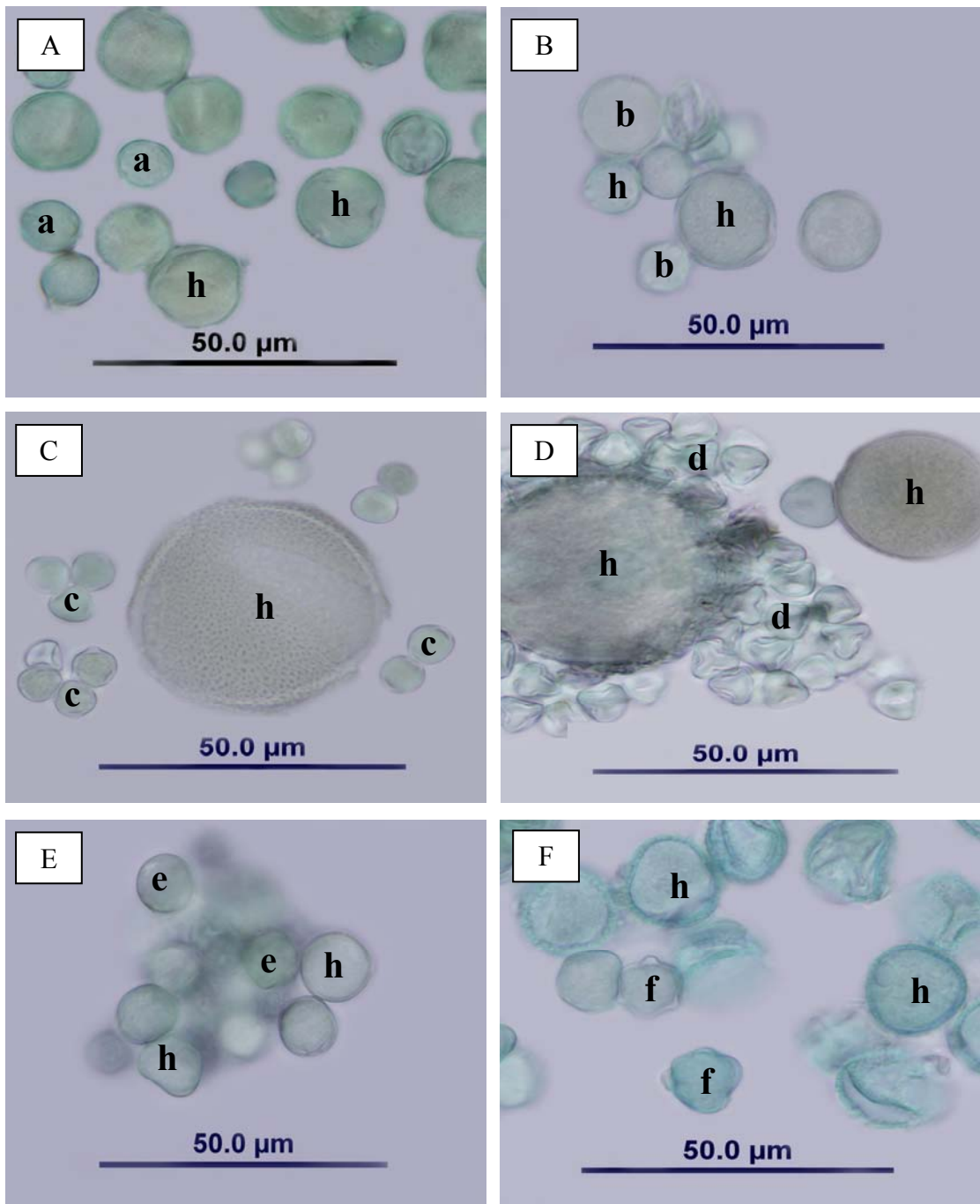


Figure 10. Pollen grains on the body of (A-D) *Elaphropoda* sp., and (E-F) *Trigona atripes* (magn. 100×) (a = *A. leave* subsp. *setosum*, b = *A. propinguum*, c = *A. diversifolium*, d = *A. lobulatum* var. *variabile*, e = *A. condensum*, f = *A. pictum*, h = unknown plant species).

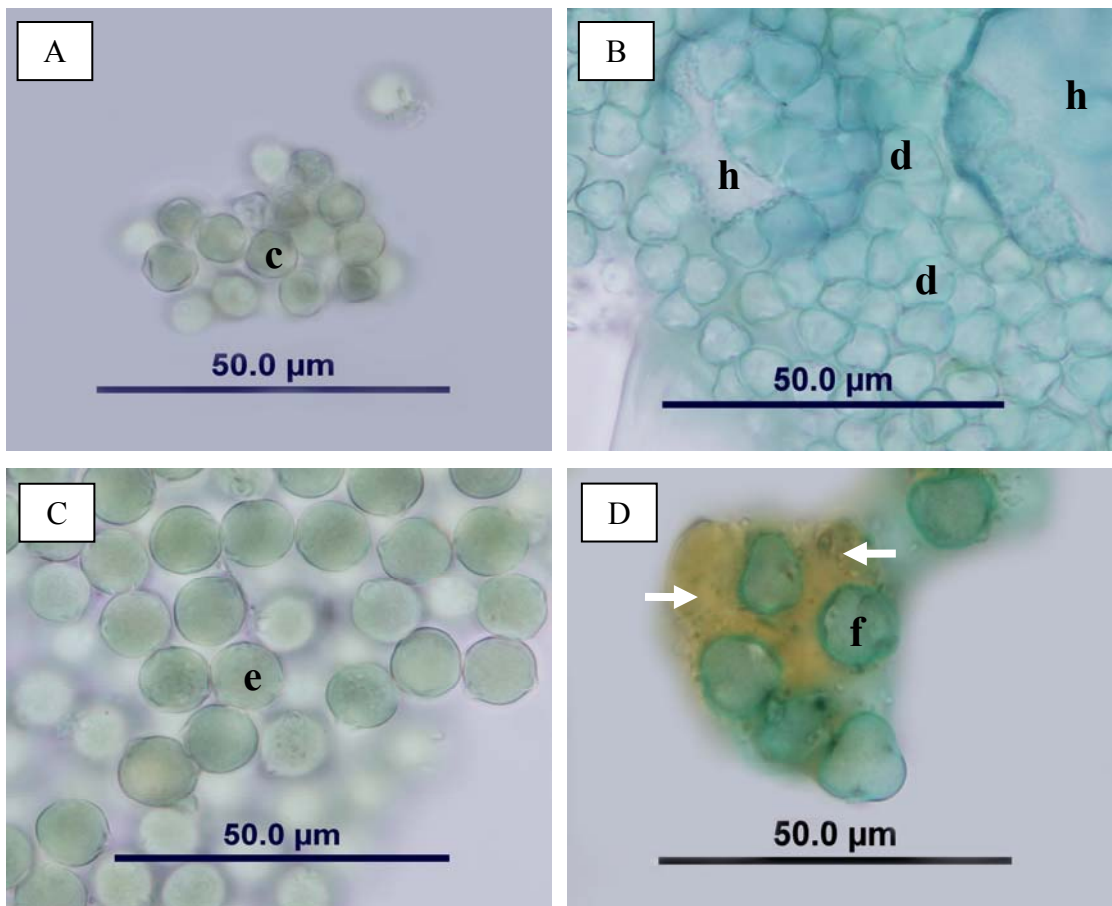


Figure 11. Pollen grains on the body of (A-D) *Trigona laeviceps* (magn. 100×) (c = *A. diversifolium*, d = *A. lobulatum* var. *variabile*, e = *A. condensum*, f = *A. pictum*, h = unknown plant species). Note some pollen grains which were glued with cement-like material (indicated by arrows).

Insect descriptions

Nomia sp. 1

(Figure 12A)

Material Examined: PSUZC-EN-HY.10.1, SUZC-EN-HY.10.2; 2 females, BL 8.8, 8.6 mm, Nakhon Si Thammarat Province, Khao Ram-Rom, 17 May 2007, 15 June 2008, PSUZC-EN-HY.10.3; 1 female, BL 8.7 mm, Trang Province, Khao Chong, 23 August 2007.

Description: *Body length:* 8.7 mm (range 8.6-8.8 mm, n = 3).

Coloration: head, antennae, legs, mesonotum and abdomen black, the basal segments 1st and basal half 2nd of abdominal tergites pale red, the segments 2nd-4th of apical abdominal tergites with transverse opaque white narrowed bands; most part of mandible black or blackish brown. **Pubescence:** head, face, the side of thorax, postscutellum, abdomen and legs covered with short pale pubescence, most dense on the side of median segment. **Structure/Integument:** head and mesonotum finely and densely punctured; clypeus slightly convex and raised, transverse anteriorly with a medial longitudinal carina; scutellum prominent, finely and densely punctured; the space at the base of median segment concave and coarsely longitudinally rugose; abdomen oval, shining, finely and densely punctured; scopae on hind legs from coxa to basitarsus, ventral abdomen and lateral thorax; wing hyaline and iridescent, the vein and tegulae darkish brown.

***Nomia* sp. 2**

(Figure 12B)

Material Examined: PSUZC-EN-HY.10.4, PSUZC-EN-HY.10.4; 2 females, BL 8.0, 7.8 mm, Trang Province, Khao Chong, 15 September 2007, 6 September 2008.

Description: *Body length:* 7.9 mm (range 7.8-8.0 mm, n = 2). *Coloration:* head, antennae, mesonotum and mandible blackish brown; legs and abdomen pale brown, the apical abdominal tergites without coloured bands. *Pubescence:* head, face, the side of thorax, the apex of abdomen and legs covered with short pale pubescence. *Structure/Integument:* head, mesonotum, postscutellum, and median segment finely and closely punctured; clypeus slightly convex and raised, transverse anteriorly with a medial longitudinal carina; scutellum prominent, finely and closely punctured; median segment densely punctured, the space at the base concave and coarsely longitudinally rugose; abdomen oval, shining, finely and closely punctured except the depressed marginal zones of abdominal tergites largely impunctated; scopae on hind legs from coxa to tibia and ventral abdomen; wing hyaline and iridescent, the vein brown.

***Nomia* sp. 3**

(Figure 12C)

Material Examined: PSUZC-EN-HY.10.5; 1 female, BL 12.9 mm, Satun Province, Khao Toh-Ngai, 23 September 2007.

Description: *Body length:* 12.9 mm (n = 1). *Coloration:* head, antennae, mesonotum, mandible and legs blackish brown; abdomen black, the segments 1st-4th of apical abdominal tergites with transverse bright green bands; *Pubescence:* head, the side of thorax, postscutellum and legs covered with minute pale pubescence, most dense on the side of face, cheeks and the side of median segment; abdomen covered with short black pubescence. *Structure/Integument:* head and mesonotum finely and densely punctured; clypeus convex with a median sharp

vertical carina, coarsely punctured; mesonotum broad, slightly convex with longitudinally-impressed parallel lines on the disc; scutellum prominent, finely and closely punctured; base of median segment longitudinally rugose; abdomen broadly oval, shining, very finely and densely punctured; scopae on hind legs from femur to tibia and ventral abdomen; wing hyaline and iridescent, the vein brown.

Note: The abdomen of *Nomia* sp. 3 looks like *Nomia* sp. 1. The base 4 segments of apical abdominal tergites of *Nomia* sp. 3 with transverse bright green bands. In *Nomia* sp. 1, the first segment without the coloured band and the segments 2-4 of apical abdominal tergites with transverse opaque white narrowed bands.

***Nomia* sp. 4**

(Figure 12D)

Material Examined: PSUZC-EN-HY.10.6, PSUZC-EN-HY.10.7, PSUZC-EN-HY.10.8, PSUZC-EN-HY.10.9; 4 females, BL 8.1, 8.2, 7.9, 8.0 mm, Satun Province, Khao Toh-Ngai, 24, 26 September 2007.

Description: *Body length:* 8.1 mm (range 7.9-8.2 mm, n = 4). *Coloration:* head, mesonotum, mandible and abdomen black; the apical abdominal tergites without coloured bands, the depressed marginal zones of abdominal tergites pale brown; antennae blackish brown; legs brown. *Pubescence:* head, the side of thorax, abdomen and legs covered with short pale pubescence, most dense on the side of face and cheeks; postscutellum covered with a dense pad of pale pubescence. *Structure/Integument:* head and mesonotum finely and densely punctured; clypeus transverse anteriorly; scutellum prominent, finely and closely punctured; median segment with a narrow depress transverse band at the base; abdomen oval, shining, finely and densely punctured except the depressed marginal zones of abdominal tergites largely impunctated; scopae on hind legs from coxa to tibia and ventral abdomen; wing hyaline and iridescent, the vein brown.

***Nomia* sp. 5**

(Figure 12E)

Material Examined: PSUZC-EN-HY.10.10, PSUZC-EN-HY.10.11; 2 females, BL 11.2, 11.0 mm, Trang Province, Khao Chong, 8 September 2007, 15 September 2008.

Description: *Body length:* 11.1 mm (range 11.0-11.2 mm, n = 4). *Coloration:* head, antennae, mandible, mesonotum, postscutellum and legs black; abdomen red brown, the apical abdominal tergites without coloured bands. *Pubescence:* head, face, the side of thorax, abdomen and legs covered with short pale pubescence. *Structure/Integument:* head and mesonotum finely and densely punctured; clypeus slightly convex and raised, transverse anteriorly with a medial longitudinal carina; mesonotum broad; scutellum prominent, finely and densely punctured; median segment short, vertical posteriorly, with a median vertical carina, the space at the base slightly concave; abdomen broadly oval, shining, finely and densely punctured except the depressed marginal zones of abdominal tergites largely impunctated; scopae on hind legs from femur to tibia and ventral abdomen; wing hyaline and iridescent, the vein and tegulae darkish brown.

Note: *Nomia* sp. 5 looks like *Nomia* sp. 3 but *Nomia* sp. 5 is bigger than *Nomia* sp. 3.

***Lasioglossum* sp.**

(Figure 12F)

Material Examined: PSUZC-EN-HY.10.12; 1 female, BL 6.5, mm Songkhla Province, Ton Nga Chang Waterfall, 2 August 2007, PSUZC-EN-HY.10.13; 1 female, BL 6.9 mm, Trang Province, Khao Chong, 8 September 2008,

Description: *Body Length* 6.7 mm (range 6.5-6.9 mm, n = 2). *Coloration:* head, antennae, mandible, mesonotum, abdomen and legs black; *Pubescence:* clypeus, the back of head, pronotum, the side of thorax, median segment and legs covered with minute pale pubescence; postscutellum covered with a pad of thin pale pubescence. *Structure/Integument:* head and mesonotum finely and densely punctured; mesonotum broad; scutellum small, finely and densely punctured; a broad depress transverse band at the base of median segment concave and coarsely longitudinally rugose; abdomen oval, smooth and shining, impunctated; scopae on hind legs from femur to tibia and ventral abdomen; wing hyaline and iridescent, the vein and tegulae darkish brown.

***Elaphropoda* sp.**

(Figure 13A)

Material Examined: PSUZC-EN-HY.10.14, PSUZC-EN-HY.10.15; 2 females, BL 15.7, 15.4 mm, Nakhon Si Thammarat, Khao Ram-Rom, 19 July 2007, 19 Jun 2008, PSUZC-EN-HY.10.16, PSUZC-EN-HY.10.17; 2 females, BL 16.0, 15.7 mm, Trang Province, Khao Chong, 8, 10 September 2008.

Description: *Body Length:* 15.7 mm (range 15.4-16.0 mm, n = 4). *Coloration:* head, antennae, mesonotum and abdomen blackish brown; the segments 1st-2nd of abdominal tergites red brown; the base of mandible and labrum pale yellow; clypeus darkly yellow with two divergent abbreviated black line at base; legs brown. *Pubescence:* head and thorax densely, clypeus, face below the antennae, postscutellum and legs covered with short pale pubescence; each side of the median segment covered with a tuft of white pubescence; the base of abdominal segments

covered with broadly and densely pale pubescence. **Structure/Integument:** head, almost thorax and scutellum finely punctured underneath the short pale pubescence; clypeus strongly convex and raised, finely and densely punctured; abdomen oval, shining, impunctated; scopae on outer tibia and basitarsus of hind legs, ventral abdomen and lateral thorax; wing hyaline, the vein and tegulae darkish brown.

***Trigona laeviceps* Smith, 1857**

(Figure 13B)

Trigona laeviceps (Bingham 1897: 563; Saiboon 1996: 34-37, Figure 10-13)

Material Examined: PSUZC-EN-HY.10.18, PSUZC-EN-HY.10.19; 2 females, BL 3.5, 3.5 mm, Songkhla Province, Ton Nga Chang Waterfall, 30 July 2007, 3 August 2007, PSUZC-EN-HY.10.20, PSUZC-EN-HY.10.21; 2 females, BL 3.7, 3.5 mm, Trang Province, Khao Chong, 21 September 2007, 8 September 2008.

Description: **Body Length:** 3.6 mm (range 3.5-3.7 mm, n = 4). **Coloration:** head, mandible, thorax, abdomen and legs black; antennae, clypeus and labrum darkish brown; the base of the clypeus black. **Pubescence:** head, face, clypeus, thorax, scutellum, abdomen and legs covered with minute pale brown pubescence; labrum covered with dense brown pubescence; each side of the median segments covered with a tuft of white pubescence; the apical abdominal tergites covered with transverse pale brown pubescence. **Structure/Integument:** head, thorax and abdomen smooth, shining, impunctated; mesonotum and scutellum densely punctuated; the vertex of the medium segment very slightly polished; the space at the base of median segment concave and coarsely longitudinally; clypeus slightly convex, impunctuated; abdomen oval; corbiculae on femur of hind legs; wing subhyaline and iridescent.

***Trigona atripes* Smith, 1857**

(Figure 13C)

Trigona atripes (Bingham 1897: 985; Saiboon 1996: 49-52, Figure 26-29)

Material Examined: PSUZC-EN-HY.10.22, PSUZC-EN-HY.10.23, PSUZC-EN-HY.10.24; 3 females, BL 5.7, 5.4, 5.6 mm, Songkhla Province, Ton Nga Chang Waterfall, 1, 4 August 2007.

Description: *Body Length:* 5.6 mm (range 5.4-5.7 mm, n = 3). *Coloration:* head, antennae, mandible, thorax, abdomen and legs red brown; labrum and mandible pale yellow; the apex of the mandible darkish black. *Pubescence:* head, face, clypeus, labrum, thorax, abdomen and legs covered with minute brown pubescence; postscutellum covered with a pad of thin pale pubescence; each side of the median segment covered with a tuft of white pubescence. *Structure/Integument:* head finely punctate; mesonotum with a few coarse punctures; thorax, median segment and abdomen smooth and shining; scutellum prominent, finely and densely punctate; the space at the base of median segment concave and coarsely longitudinally rugose; clypeus slightly convex; abdomen oval; corbiculae on femur of hind legs; wing reddish brown, milky-white beyond the stigma.

***Microdon* sp.**

(Figure 13D)

Material Examined: PSUZC-EN-DI.10.1, PSUZC-EN-DI.10.2; 2 females, BL 6.7, 6.9 mm, Nakhon Si Thammarat, Khao Ram-Rom, 17, 18 May 2007. PSUZC-EN-DI.10.3, PSUZC-EN-DI.10.4; 2 females, BL 6.5, 6.8 mm, Songkhla Province, Ton Nga Chang Waterfall, 29 July 2007.

Description: *Body Length:* 6.7 mm (range 6.5-6.9 mm, n = 3). *Coloration:* head, thorax and legs brown; antennae black; arista brown; abdomen black, with yellow marking. *Pubescence:* vertex and front of head covered with blackish brown pubescence; Thorax covered with brown pubescence. *Structure/Integument:* head slightly broader than thorax; vertex only slightly raised above level of eyes; head, thorax and abdomen smooth and shining, superficially resembling bees; antennae short, the last segment bear a strong hair (seta); scutellum with hind corners produced into two blunt teeth; proboscis long and slender; abdomen broadly oval, bent downward; wing hyaline, the vein brown, spurious vein occurs between the radius and the media.

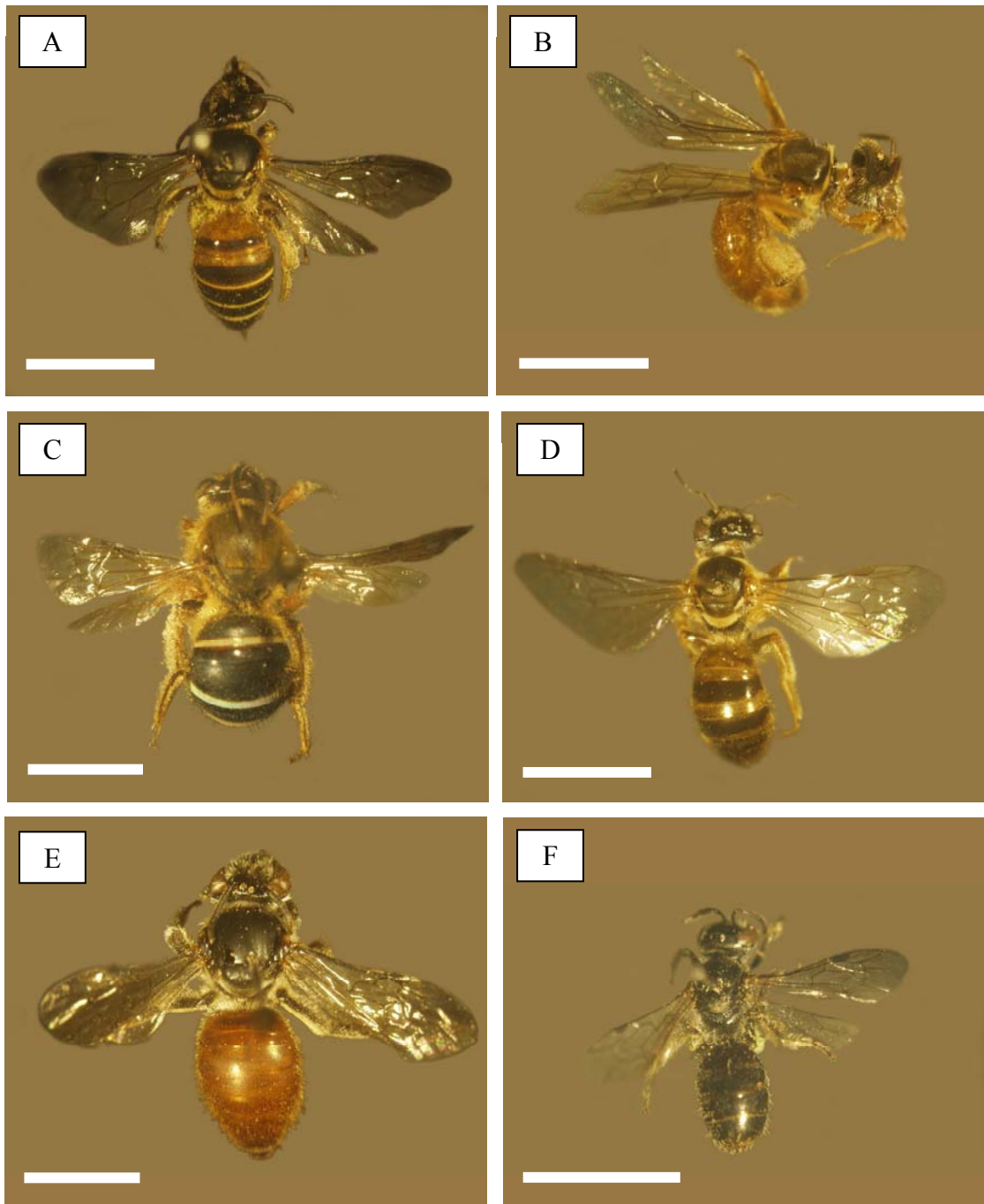


Figure 12. The pinned specimens of (A) *Nomia* sp. 1 (PSUZC-EN-HY.10.1), (B) *Nomia* sp. 2 (PSUZC-EN-HY.10.4), (C) *Nomia* sp. 3 (PSUZC-EN-HY.10.5), (D) *Nomia* sp. 4 (PSUZC-EN-HY.10.6), (E) *Nomia* sp. 5 (PSUZC-EN-HY.10.10), and (F) *Lasioglossum* sp. (PSUZC-EN-HY.10.12). Scale bars = 5 mm.



Figure 13. The pinned specimens of (A) *Elaphropoda* sp. (PSUZC-EN-HY.10.14), (B) *Trigona laeviceps* (PSUZC-EN-HY.10.18), (C) *Trigona atripes* (PSUZC-EN-HY.10.22), and (D) *Microdon* sp. (PSUZC-EN-DI.10.1). Scale bars = 5 mm.

Foraging behaviours

Two groups of pollinators could be recognized according to their behaviours, *i.e.*

- buzz pollinators, there is one species of the bee which is a buzz pollinator, *i.e.*, *Elaphropoda* sp.

Considering this “buzzing” behaviour, this might be explained when the large *Elaphropoda* sp. (15-16 mm) typically darted and hovered zigzag around the flowers before landings on the top (Figure 14A), after that, it grabbed onto the flower with its mouthparts and legs, then rapidly arched its abdomen beneath the anthers and immediately vibrated its wings very rapidly (Figure 15). These series of activities were performed in a short period (1-2 seconds) on a given flower (Figure 18).

Pollen grains were shaken out of its poricidal anthers or from the apical pore of the anthers cone (- the longitudinal dehiscent anthers which is fused into the cone-like structure and has a pore at the apical end) in some *Argostemma* spp. (such as *A. leave* subsp. *setosum* etc.) and attached on its body. The stigma was frequently contacted with its ventral thorax and abdomen where the pollen scattered. A short loud buzz sound was heard when it was buzzing. In general, *Elaphropoda* sp. was able to seek for the pollen grain in a long distance over the clumped populations of *Argostemma* spp. It sometimes stopped on other plant species and groomed off pollen grains from its body and appendages and then transferred backward to the scopae (pollen-transporting brushes) for transport.

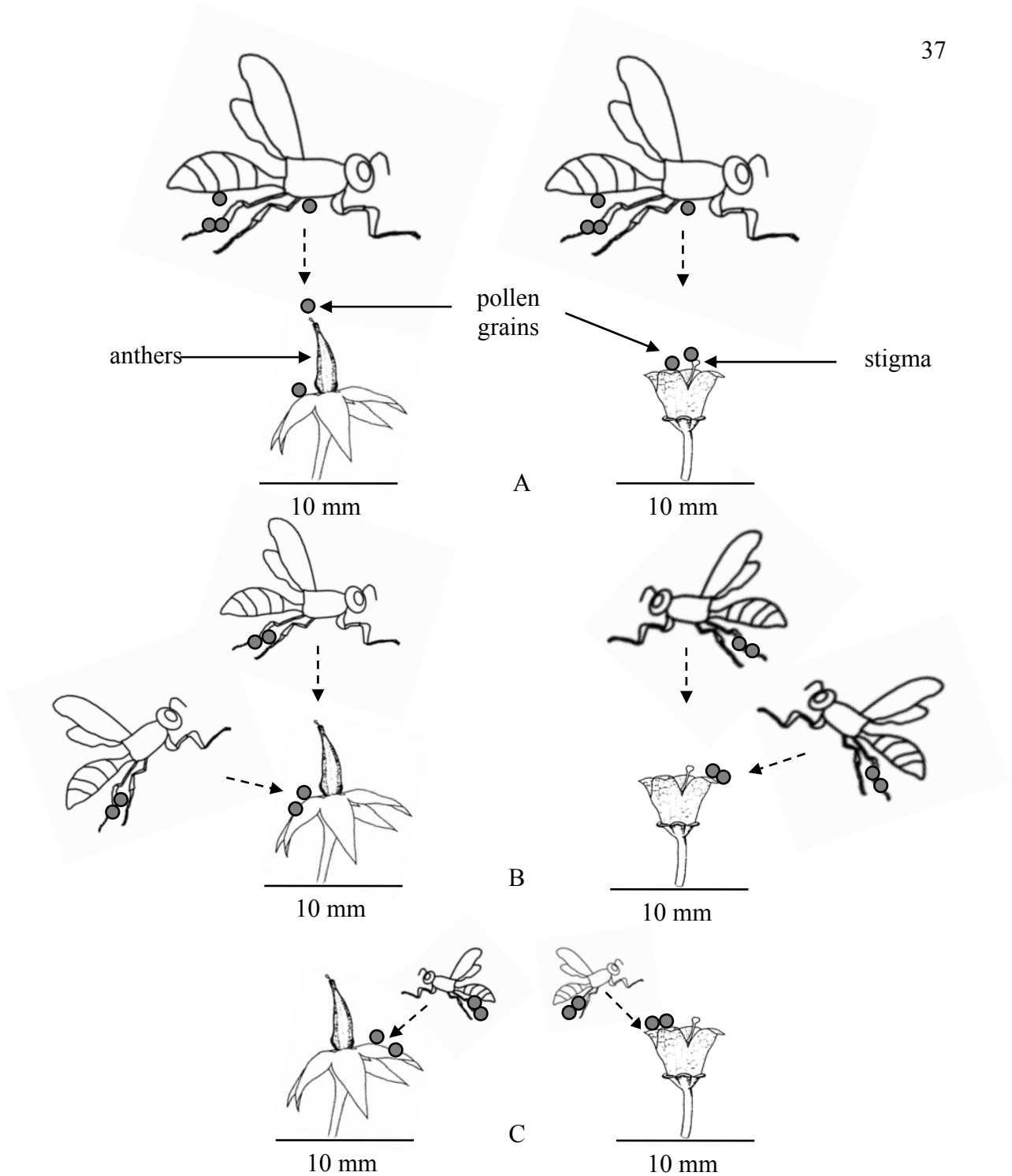


Figure 14. Diagram showing manner of pollinators approaching *Argostemma* flowers (star- and bell-shaped flower) by large, medium and small bees and chance of pollen grains deposit after their visits. A. top-approaching to the flower by large bees which promote pollen transfer to the stigma. B. top- and side-approaching to the flower by medium bees. C. side-approaching to the flower by small bees which pollen carried on their bodies may not contact the stigma. Flowers reproduced from Sridith and Puff (1999) and insects reproduced from Tangmitcharoen (2006).

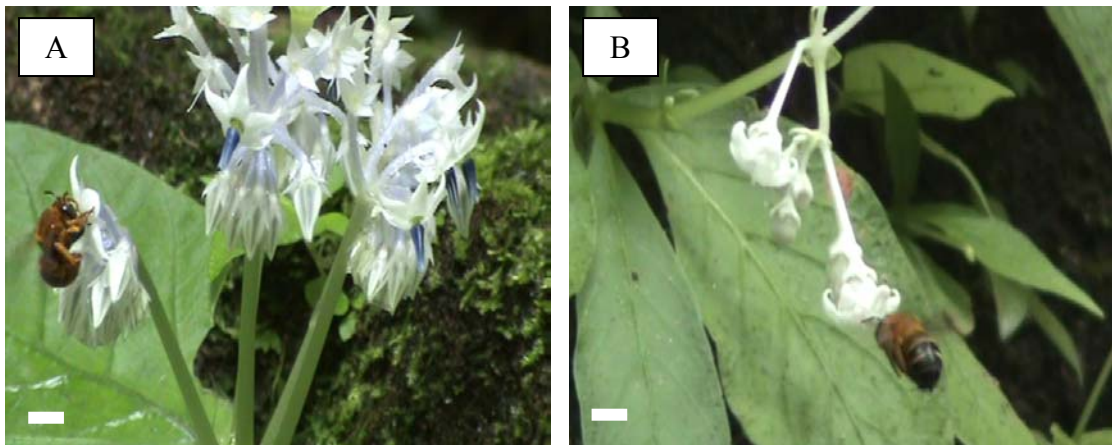


Figure 15. *Elaphropoda* sp. is buzzing the flowers of (A) *A. diversifolium* and (B) *A. lobulatum* var. *variable*. Scale bars = 5 mm.

- non-buzzing pollinators, *i.e.*, *Nomia* spp. (5 unidentified species), *Lasioglossum* sp., *Trigona laeviceps* and *Trigona atripes*

For the “non-buzzing” behaviour, there are two slightly differences in flowers approachings according to their body sizes.

1. The medium *Nomia* spp. (5 unidentified species) (8-13 mm), like the buzzing pollinator (*Elaphropoda* sp.), it darted and hovered zigzag around the flowers before landing either both on the flower top or sides (side lobes of the petal(s) (Figure 14B).

2. The small stingless bees (≤ 7 mm), *i.e.*, *Lasioglossum* sp., *Trigona larviceps* and *Trigona atripes* would fly slowly and then approach flowers from the side (Figure 14C).

After flower approaching, the pollinators would then seek for the pollen grains, however, it was slightly different according to the morphology of *Argostemma* flowers (sensu Sridith, 2007), *i.e.*, star-shaped vs. bell-shaped flowers

- For the star-shaped flowers, after landing on the top or the side of the flowers, the pollinators would climb up from the petals towards the anthers. After climbing back and forth towards the length of the anthers, they bit the anthers from the base to the apex by their mouthparts. Finally they stopped at the apex of the anthers and arched their abdomen beneath the anthers, repeatedly bit at the top of the anthers and collected pollen grain by their forelegs (Figure 16).

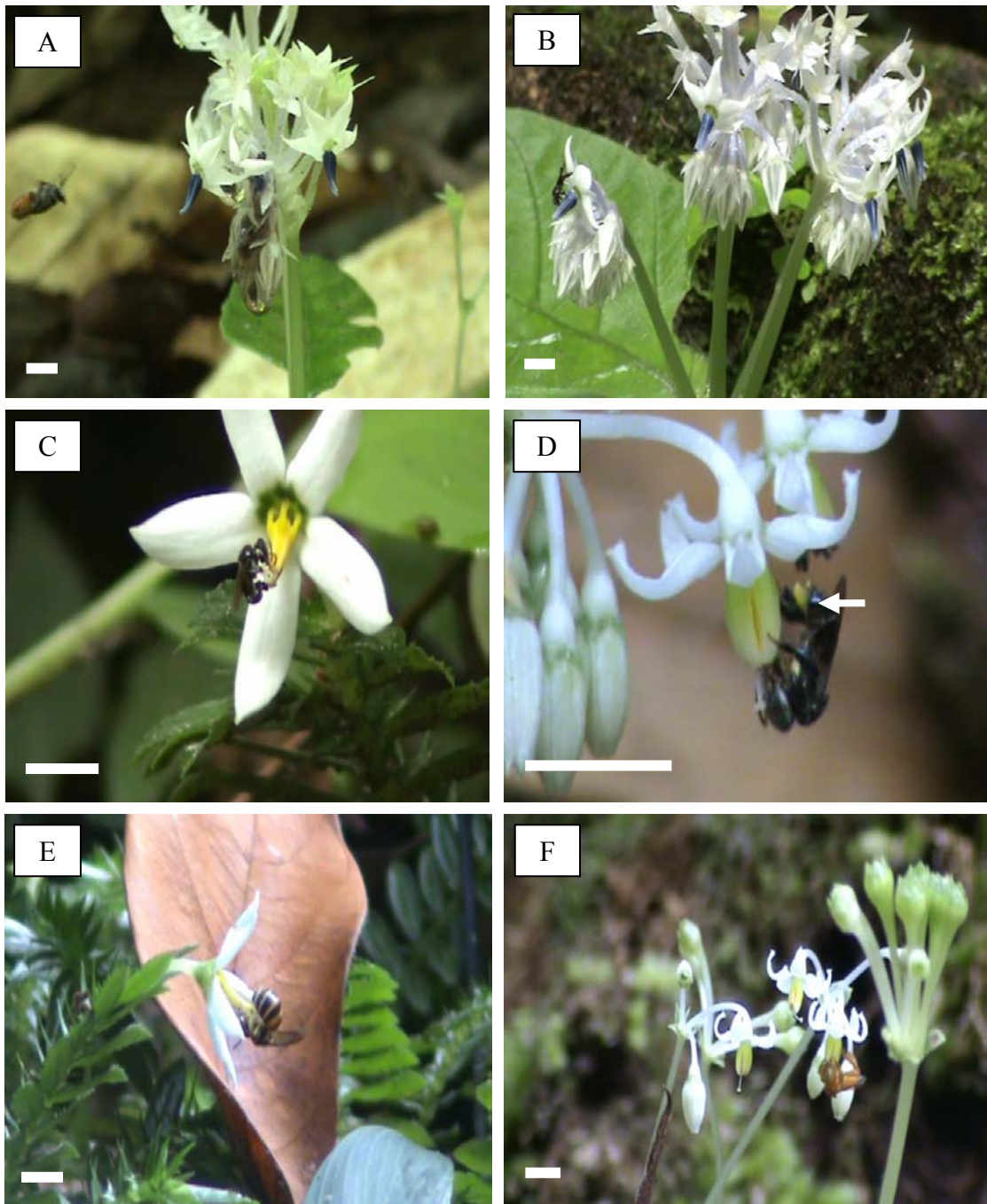


Figure 16. Non-buzzing bees are collecting pollen from the star-shaped flowers. A. *Nomia* sp. 5; B, C and D. *Trigona laeviceps*; E. *Nomia* sp. 1; F. *Trigona atripes*. Note pollen was gathered inside the corbiculae of hind legs (indicated by arrow). Scale bars = 5 mm.

- For the bell-shaped flowers, after landing on top or side of the flowers, the pollinators stopped at the apex of the corolla tube and hid their head into the base of the corolla tube where the anthers/anthers-cone situated. They bit the anthers from the base to the apex by their mouthparts. Then, like the formers, they repeatedly bit at the top of the anthers and collected pollen by their forelegs as well (Figure 17).

Pollen grains were transferred from the fore- to the mid- and then to the hind legs, where they were gathered inside the scopae or corbiculae (pollen basket) for transporting. The non-buzzing pollinators would spend longer period on the flowers than the buzzing pollinators (Figure 18). They sometimes contacted the stigma with their antennae, legs and thorax while collecting pollen from the anthers. Generally, the non-buzzing bees almost sought for pollen grain in a short distance.

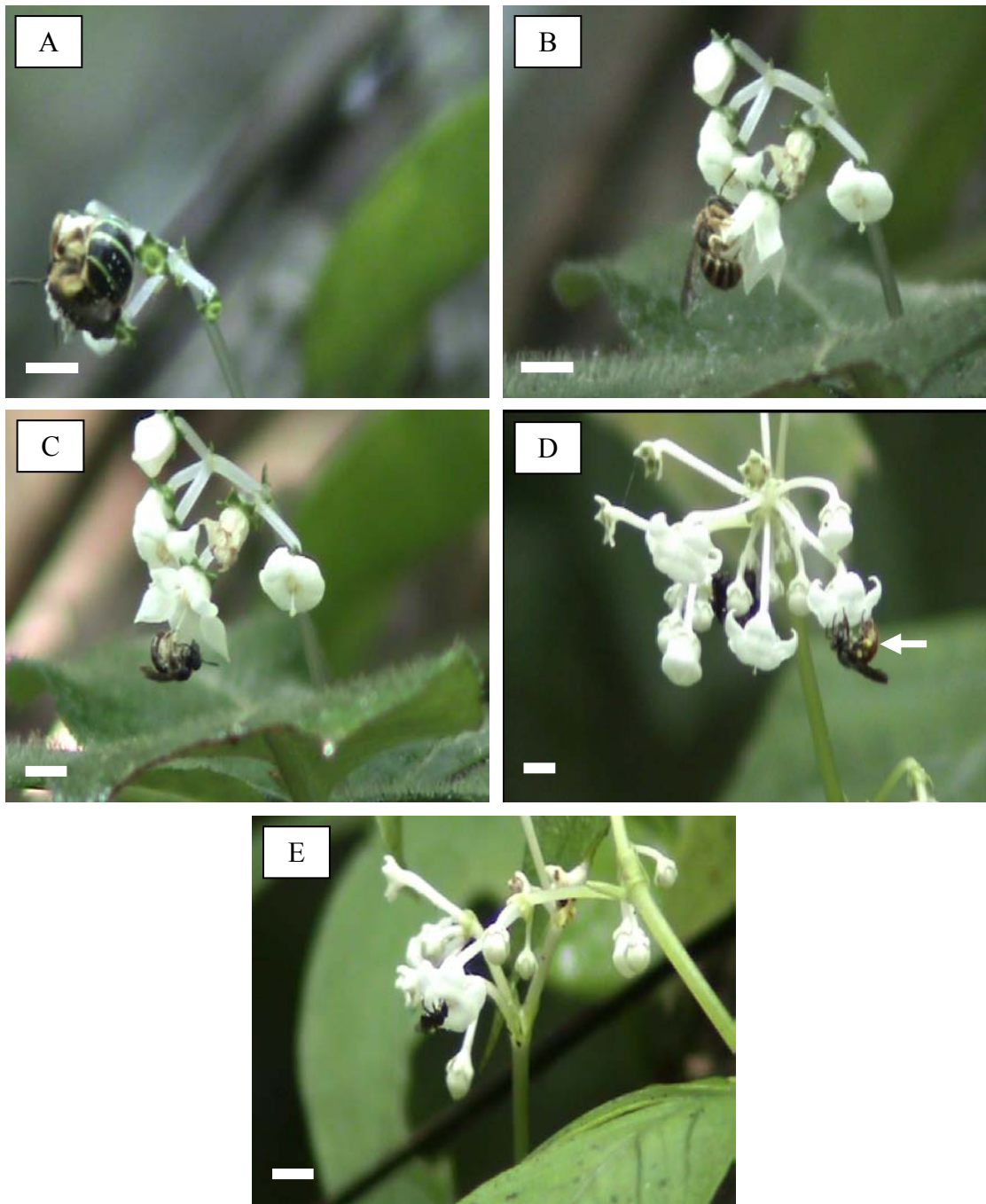


Figure 17. Non-buzzing bees are collecting pollen from the bell-shaped flowers. A. *Nomia* sp. 3; B. *Nomia* sp. 4; C. *Lasioglossum* sp.; D. *Nomia* sp. 5; E. *Trigona laeviceps*. Note pollen which was gathered inside the scopae of hind legs (indicated by arrow). Scale bars = 5 mm.

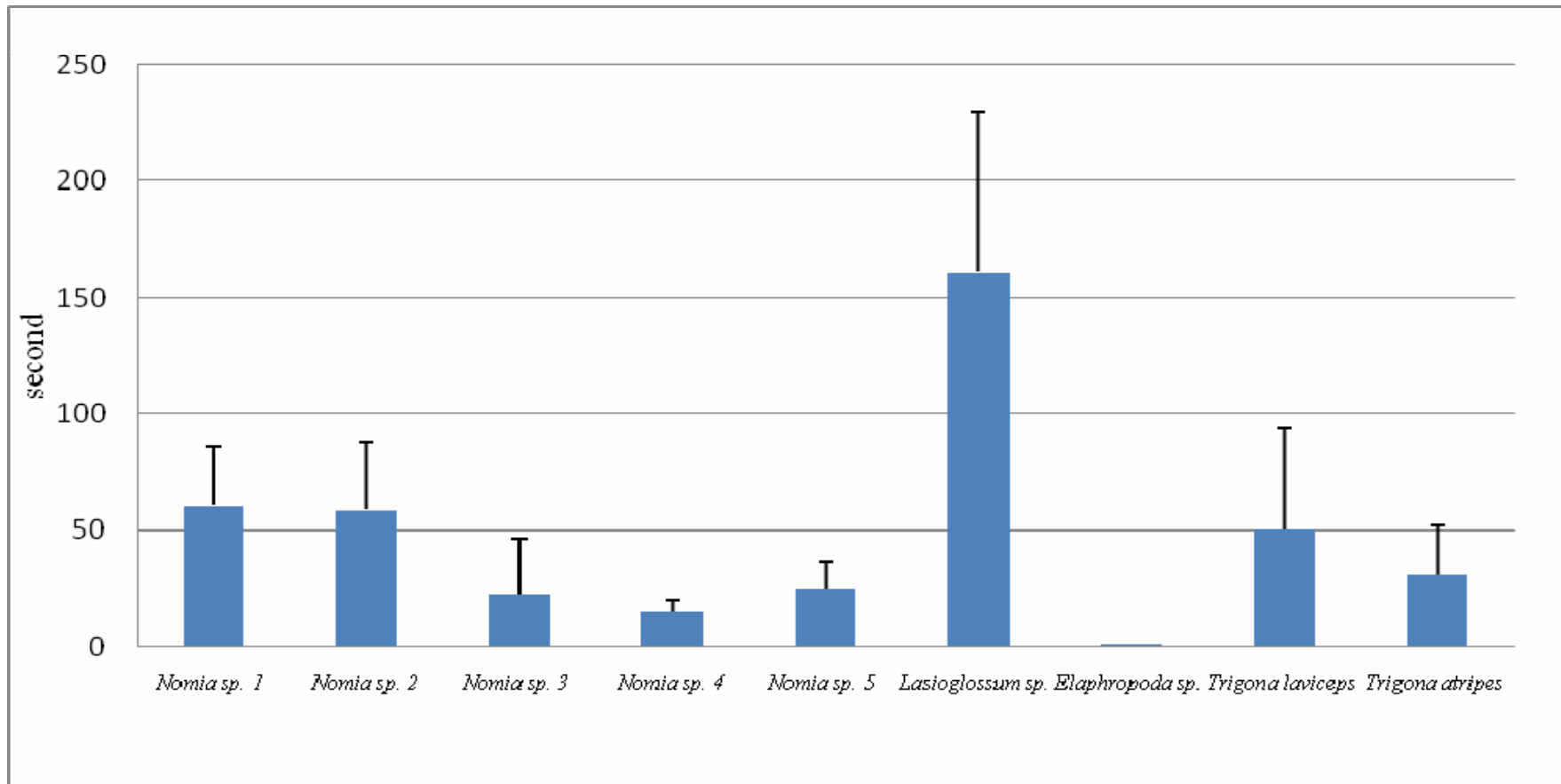


Figure 18. Mean time spent of all pollinator species on a flower of *Argostemma* spp.

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Pollinators and the flowers of *Argostemma* spp.

4.1.1 Identification of pollinators

All bees were identified to the genus level using the keys of Bingham (1897), Saiboon (1996), and Michener (2007) based on the external morphological characteristics. The identification to the species level will be made possible by an analysis of the male genitalia. The present results showed that only female bees visited the flowers. Moreover, from my personal observation, the surrounding areas where *Argostemma* spp. grow appear to have no plants that can provide nectar to the male bees. It is commonly found that female bees act as primary pollinators by taking pollen while the male bees of nearly all species take nectar from flowers instead (Bell *et al.*, 2006). *Argostemma* offers only pollen as a reward for their pollinators. This finding is consistent with Michener (2007) who reported that female bees collected pollen as the principal protein source for their own food and especially for feeding their larvae. This may be a plausible explanation why the male bees were not observed in this study.

4.1.2 Compatibility of pollinators with the floral morphology of *Argostemma* spp.

Some insects and their entomophilous flowers have evolved to form a mutualistic relationship. It has been also generally held that mutualism is a result of co-evolution. Plant-insect interaction has been recognized as a good example of co-evolutionary process. Flowers of selected *Argostemma* spp. were hypothesized to be pollinated by small insects. The present experimental results support this hypothesis. Bees are found to be the pollinators of the selected *Argostemma* spp. Although their

floral morphology was very diverse in terms of signal designs, *i.e.*, shape, colour, they are however dependent on only nine species of bees for pollen transfer.

Flowers of selected *Argostemma* spp., both star- and bell shaped are small in size (ca 5-15(25) mm in diameter). Their anther colours range from pale yellow (*A. lobulatum* var. *variabile* and *A. pictum*), yellow (*A. condensum*, *A. propinguum* and *A. puffii*) to orange (*A. leave* subsp. *setosum*) and pale blue (*A. diversifolium*) (Sridith and Puff, 2001). The bees are small in size (3.6-15.7 mm) and are equal in size to the flowers (Table 2 and Figure 14). A similar size coupled with their compatible shapes help facilitate a pollination mechanism. As a consequence, each visit resulted in an attachment of a certain amount of pollen to the bee's body and then caused fruit set. All anther colours are in range of the bees colour vision (Figure 20B). The photoreceptor of bees can perceive the colour range from UV to yellow (wavelength 325-560 nm) (Chittka, 1996) (Figure 20A). The anthers of selected *Argostemma* spp. might be easily noticed by the bees because their anther colours contrast with the white petal background (the white is perceived by the bees as the UV colour). However, some *Argostemma* species have white anther colour. Greenleaf and her colleagues (2007) found that the foraging range of bees positively correlates with the body size (Figure 19A). The body size (the distance between the wing bases, IT span) of bees can be used to predict the foraging distance. Thereby the large *Elaphropoda* sp. might forage in longer distance than the smaller individuals (Figure 19B).

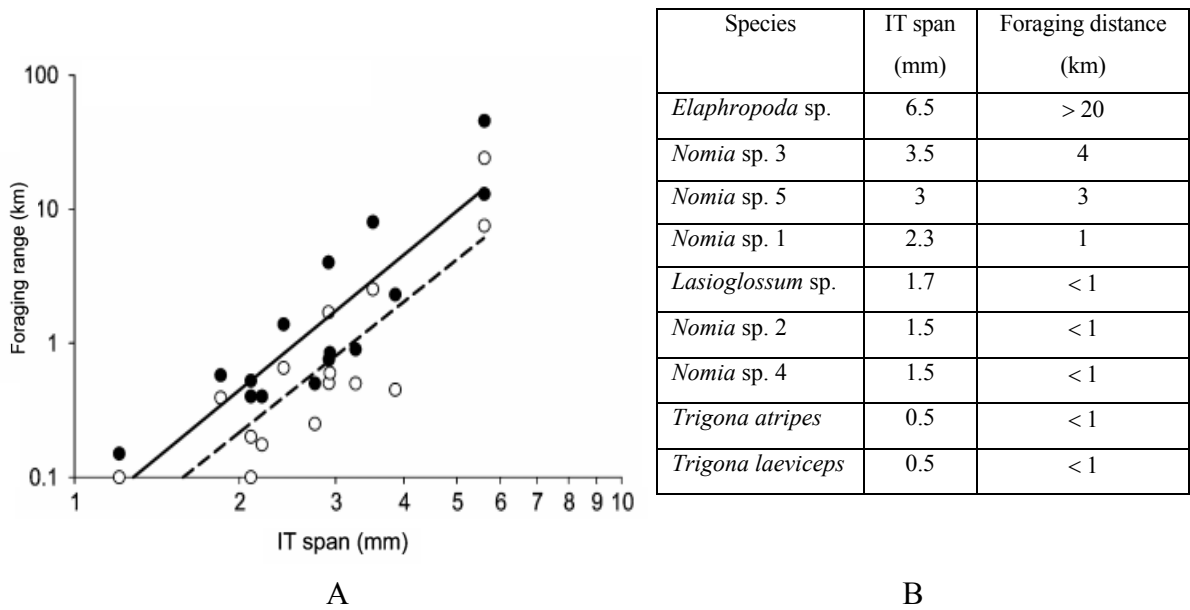


Figure 19. The foraging distance of bees. A. the relationship between bee foraging distance and body size (the distance between the wing bases, IT span). B. the approximate foraging distance of the pollinators. Source: Greenleaf (2007).

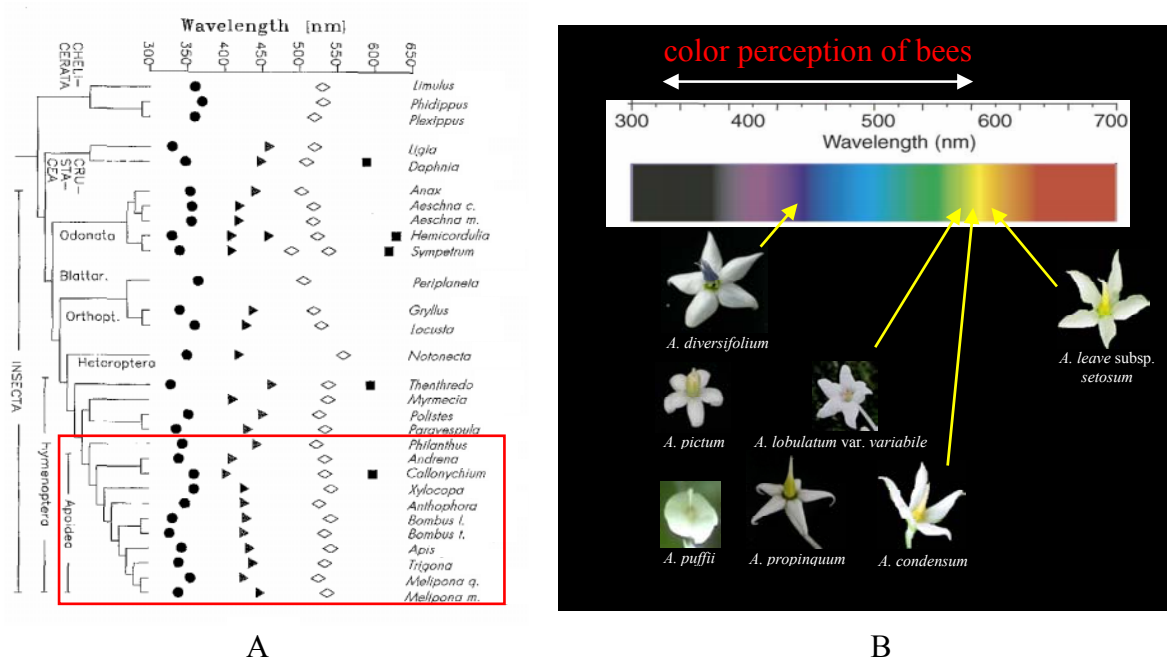


Figure 20. The colour perception of bees. A. photoreceptor of bees comprises with UV, blue and green. B. The anther colours of flowers of selected *Argostemma* spp. and the bees colour visual perception. Source: Chittka (1996).

Most plants use odour, nectar to attract their pollinators. Nevertheless *Argostemma* offers only pollen as a reward to their pollinators. Pollen grains of *Argostemma* are kept in the poricidal anthers with its opening at one end. As a result, it is difficult to harvest the pollen by visiting pollinators. For this reason, only a small numbers of bees were observed to collect pollen from the flowers of *Argostemma* spp. (Table 1 and Figure 7). Small numbers of pollinating bees seem to be a common phenomenon of plants with poricidal anthers (Johnson, 1992; Larson and Barrett, 1999; Coletto-Silva *et al.*, 2004; Kawai and Kudo, 2009). All the bees have a structural adaptation by the modification of hair structure, the scopae or corbiculae for pollen gathering, manipulation and transport. The peak visitation of the pollinators and the anthesis of the flowers took place together in the morning, thus it might be assumed that the amount of pollen was highest in the morning, leading to high visitations at that time period.

The results from this study showed that different *Argostemma* spp. growing at particular study sites have different sets of pollinators. In terms of a plant and its pollinator relationship, this observation may be indicative of a trend in species specificity (Table 1, 2 and Figure 7). The distance between populations of *Argostemma* spp. might be too far for the bees to move from one location to the others. For sympatric species, they always have the same pollinators because their flowers are not different in the pollination syndrome so the pollinators can use the same foraging behavior protocol for harvesting pollen. Sharing of pollen-collecting pollinators among co-occurring poricidal plants has been observed several times (*e.g.*, Macior, 1971; Buchmann *et al.*, 1977; Renner, 1986). Nevertheless, they seemingly avoid the competition for their pollinators by flowering in different periods. One species flowers earlier than another species, *i.e.*, the flowering of *A. diversifolium* occurs before *A. lobulatum* var. *variabile*.

Based on the criteria set forth in Chapter 2, the pollinators of selected *Argostemma* spp. were a functional group of insects (only bees). In view of the co-evolutionary principle, *Argostemma* spp. and their pollinators have adapted their morphology, physiology, and behaviours to affect mutual benefits to both. This co-adaptation supports the pollination syndrome concept, the theoretical framework, which holds that particular classes of pollinators are specifically associated with

particular floral traits, *i.e.*, size, shape, colour and reward (Van der Pijl, 1934; Fenster *et al.*, 2004).

4.2 Pollination effectiveness through the insect's foraging behaviour

Pollen grains of *Argostemma* are kept in the poricidal anthers with its opening at one end, so they were hypothesized to be shaken out of its anthers by buzz pollination. The present experimental results do not support this hypothesis. The pollen grains of selected *Argostemma* spp. are mainly transferred by non-buzzing bees using their mouthparts and legs. Only *Elapthropoda* sp. was observed to perform buzz pollination. Contrary to the reports by Buchmann (1983), Vogel (1978) and other authors who studied poricidal plants, they all proposed that reproduction of the studied species was made possible by buzz pollination. This study has shed new light on the pollination of the poricidal plant in its natural habitat in South-east Asia that has not been previously reported while other studies were mostly conducted in the temperate region or in different parts of the tropical zone. We hope the work presented here will stimulate others to continue the important studies on other poricidal plants of South-east Asia. Why do *Argostemma* spp. lack of buzz pollinators of *Argostemma* spp. is whether no buzzing bees in the study areas or they could not entice them. If there are quite a few buzzing bees, then

With regard to the pollination effectiveness, it refers to a plant's reproductive success as a result of one visit of one pollinator (Dafni, 1992). Different groups of pollinators have different behaviour and with different degree of effectiveness (Schemske and Horvitz, 1984). The present experimental results found that the pollination of selected *Argostemma* spp. is made by two groups of bees, buzzing and non-buzzing behaviour.

4.2.1 buzzing behaviour

It was reported that during buzzing pollen had been expelled thoroughly onto both thorax and abdominal tergites (Johnson, 1992; Larson and Barrett, 1999) which were subsequently dislodged from the insect's scopae or corbiculae onto the recipient flowers (Michener, 2007). Thereby buzzing behaviour effects high pollination efficiency in poricidal plants (Larson and Barrett, 1999; Kawai and Kudo, 2009). This behavior is accomplished by a few bees that specialized in buzz pollination or sonication (Javorek *et al.*, 2002; Drummond, 2003). Increasing body size was reported to foster pollination effectiveness (Drummond, 2003). The present finding is in line with these authors because only the large *Elaphropoda* sp. was observed to perform buzz pollination (Figure 14) following which the pollen grains were scattered over its thorax and abdominal tergites. Thereby *Elaphropoda* is believed to be the most effective pollinator than other non-buzzing bees leading high pollination effectiveness. As the pollen grains of *Argostemma* are noticeably small, it is probable that the grains will be expelled more easily from the poricidal anthers during the vibration of the bees. It was reasoned by some authors (Erickson, 1975; Thorp, 1979; Corbert *et al.*, 1982; Erickson and Buchmann, 1983) that the presence of electrostatic force at the moment of pollination by vibration could enhance the attachment of the pollen to the body of the bees which would ensure the success of the transfer of the pollen to the stigma of the recipient.

4.2.2 non-buzzing behaviour

Other pollinators cannot use the strategy of buzz pollination. Buchmann (1983) reported that it was very difficult to remove from poricidal anthers without high-frequency vibration. This structure obviously poses problem for non-buzzing bees to harvest the pollen. As a consequence, the non-buzzing bees have to recourse to other strategy in order to successfully collect the pollen. The present study found that although all non-buzzing bees could not perform the buzzing behaviour, they could collect pollen by biting the anthers and as consequence damaged the anthers. By so doing, they are regarded as merely a thief of buzz-pollinated plants

(Johnson, 1992; Larson and Barrett, 1999; Coletto-Silva *et al.*, 2004). However, it was observed that they frequently contacted the stigma while collecting the pollen. The pollen grains were gathered inside the scopae or cubicalae that has been proposed by Michener (2007), it is probably less likely to pollinate the next flowers. Thus they are regarded as the less effective pollinators leading to low pollination effectiveness.

4.3 The contribution of the pollinators to the distribution and abundance of selected *Argostemma* spp.

It has been proposed that only a small fraction of pollen grains is removed from a single visit because the expulsion is limited by dispensing mechanism of its poricidal anthers (Harder, 1990; Harder and Barclay, 1994; King and Buchmann, 1996; Larson and Barrett, 1999; Kawai and Kudo, 2009). Thereby pollinators should find more pollen from many flowers/plants. The present results showed that all bees were observed to visit several neighboring *Argostemma* in a single foraging route. The foraging movement of all bees indicates a great opportunity for promoting out-crossing and is in line with the optimum foraging theory.

The abundance of plants in a habitat is strongly determined by its reproductive success. The pollination effectiveness would be most successful when it is pollinated by its effective pollinators. Although *Elaphropoda* sp. is the most effective pollinator of the selected *Argostemma* spp., the plants are however predominantly pollinated by non-buzzing bees, the less effective pollinators (Table 1 and Figure 7). The pollination by non-buzzing bees is probably limits the abundance of this plant. This is a reason why the populations of selected *Argostemma* spp. at all study sites appear in small number.

In the best interest of all plants, an individual should disperse all its pollen to the stigma of other individuals of the same species. In all animal-pollinated plants, the dispersal of their pollen depends upon the foraging distance of their pollinators. The information on foraging distance is critical for understanding the role of pollinators in effecting the dispersal of plants. Long foraging distance of *Elaphropoda* sp. is an important in terms of effective gene flow between the populations of selected *Argostemma* spp. However they are predominantly pollinated

by non-buzzing bees, short foraging distance (Figure 18B). This does not give a proper impression of effective pollen and gene flow between populations. Therefore pollination by non-buzzing bees probably limits the distribution of this plant. This is a reason why the distribution pattern of the populations of selected *Argostemma* spp. is clumped. Most *Argostemma* are not substrate specific and occur in the same climatic condition (moist to wet places with high humidity) (Sridith and Puff, 2001). However due to the limitation of gene flow they cannot spread continuously. Moreover, it has been proposed that immigration of gene flow between populations can also lead to no combination of the two gene pools and increases the genetic variation that would have led to full speciation (Enger *et al.*, 2007). The limitation of gene flow by short foraging distance is probably a reason why *Argostemma* have numerous species occurring in a given area.

4.4. Conclusions

1. Nine species of bees and 1 species of fly were observed to visit the flowers of *Argostemma* spp. The bees were categorized as pollinators while the fly was categorized as a visitor.

2. Pollination of selected *Argostemma* spp. was performed by both buzzing and non-buzzing bees. Pollen grains of selected *Argostemma* spp. were mainly transferred by non-buzzing bees using their mouthparts and legs although they are regarded as the less effective pollinators on the bases of smaller body size and shorter foraging distance. Only one species, *Elaphropoda* sp. was found to perform buzzing behaviour to collect pollen from the poricidal anthers. It is believed to be the more effective pollinator in view of its larger body size and longer foraging distance.

3. The distribution pattern and the abundance of selected *Argostemma* spp. might be determined by the foraging behaviour of the non-buzzing bees.

PERSPECTIVES AND RECOMMENDATIONS

1. Identification of bees to species level is badly needed. As such, capturing of male bees is required so that their genitalia can be used in the identification.

2. Studies of pollinators with respect to their distribution, foraging distance, life cycle, and sources of foods etc. should be conducted with a view to understanding the degree of mutual adaptation between pollinators and plants

3. The pollination effectiveness of *Argostemma* spp. should be investigated to understand the reproduction effectiveness of pollinators, e.g., pollen removal, pollen deposition, seed yield as a result of their visits etc. This information will provide an opportunity to assess the population density of these plants.

4. The breeding system of *Argostemma* should be investigated simultaneously in order to understand the role of pollinators in the pollination of this plant.

5. Further studies on the reproductive success of sympatric species are still needed.

6. A decline in number of the endemic *A. leave* subsp. *setosum* at Khao Ram-Rom, Nakhon Si Thammarat Province is noticeable. *Argostemma* spp. might be at risk of extinction soon. To conserve the species, a study on their life system is highly recommended,

7. In order for the interpretation of experimental results to be conclusive, sufficient replication of field data is required.

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Appendices

Appendix a

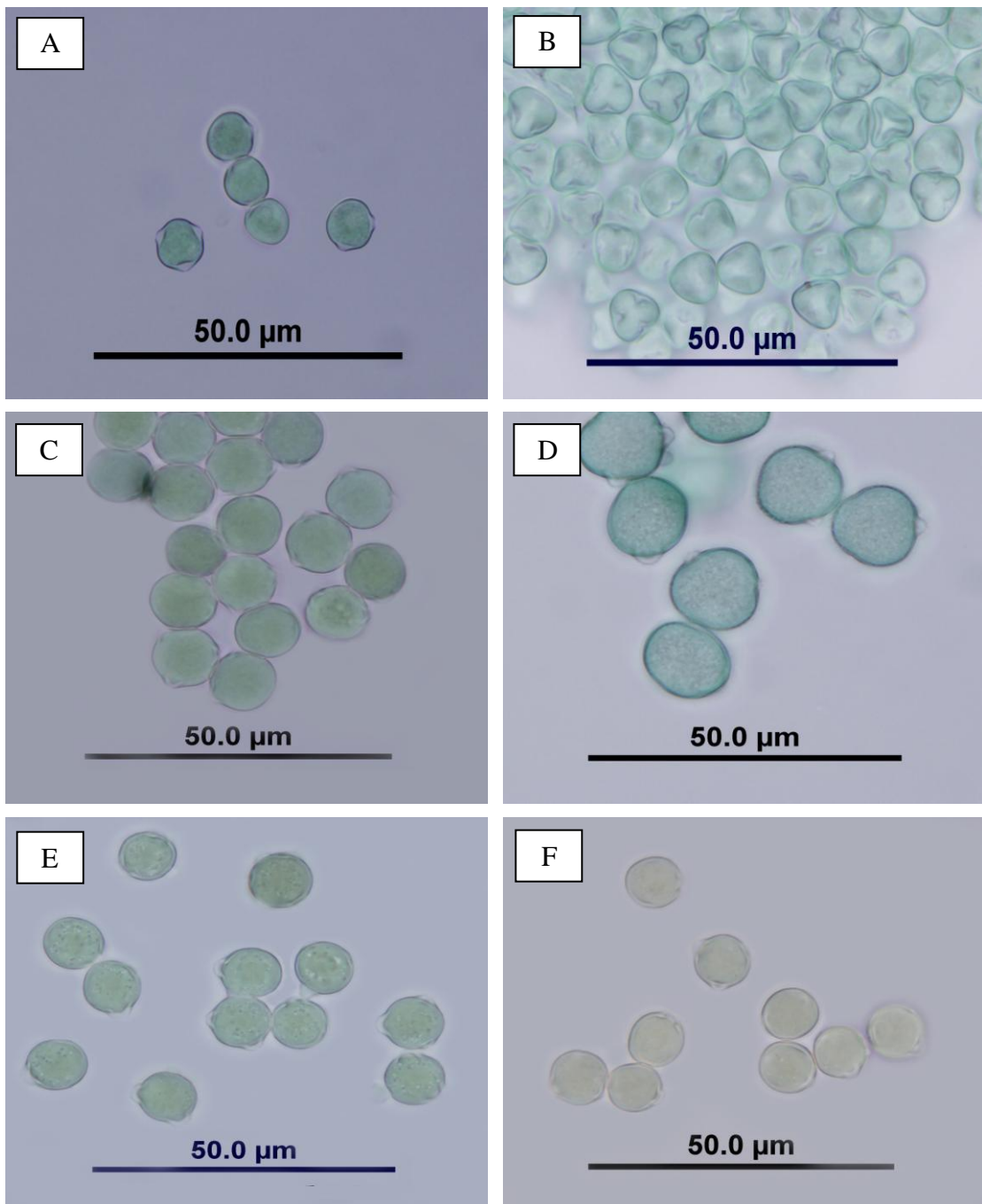


Figure 1. The pollen grain of identified *Argostemma* in the studied areas. A. *A. diversifolium*; B. *A. lobulatum* var. *variabile*; C. *A. condensum*; D. *A. pictum*; E. *A. leave* subsp. *setosum*; F. *A. propinguum* and G. *A. puffii*.

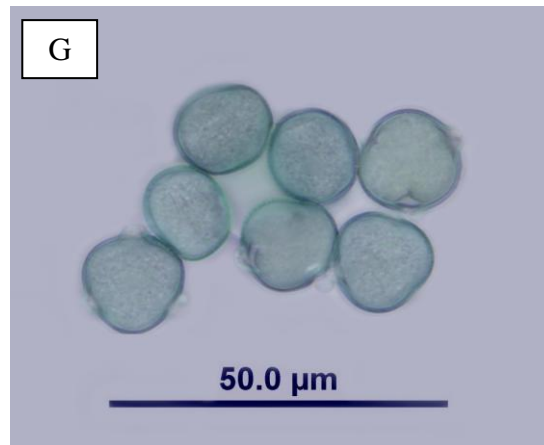


Figure 1. The pollen grain of identified *Argostemma* in the studied areas (continued).

G. *A. puffii*.

Appendix b

Table 1. Temperature and humidity during the observation periods at all studied areas in 2007.

Collection dates	Temperature (°C)	Humidity (%)
Khao Ram-Rom, Nakhon Si Thammarat Province		
12 May 2007	20.3, 30.2, 27.0	95.4, 61.9, 65.2
13 May 2007	21.1, 32.8, 25.2	95.4, 62.7, 69.8
14 May 2007	22.4, 29.0, 27.1	98.5, 64.0, 72.6
15 May 2007	21.3, 30.2, 25.0	94.6, 63.2, 73.0
16 May 2007	22.1, 29.9, 24.0	96.1, 60.9, 64.8
17 May 2007	21.9, 29.8, 26.9	91.5, 65.0, 69.5
18 May 2007	22.0, 31.0, 25.4	96.9, 65.6, 77.1
19 May 2007	21.3, 29.7, 26.5	94.3, 63.1, 74.6
20 May 2007	20.3, 28.9, 24.9	94.4, 62.0, 73.9
13 July 2007	21.7, 31.3, 27.5	97.5, 67.4, 75.0
14 July 2007	22.1, 29.8, 26.3	93.5, 64.2, 73.0
15 July 2007	20.8, 29.2, 27.6	93.1, 65.4, 67.1
16 July 2007	23.3, 30.5, 26.9	90.5, 60.7, 70.1
17 July 2007	21.9, 29.4, 27.0	94.9, 62.8, 70.8
18 July 2007	23.2, 32.8, 25.2	92.4, 62.8, 66.5
19 July 2007	22.5, 30.6, 25.5	90.9, 65.4, 68.7
20 July 2007	21.7, 28.5, 25.8	90.7, 66.5, 69.0
Ton Nga Chang waterfall, Songkhla Province		
28 July 2007	22.5, 31.3, 24.2	94.5, 66.3, 68.0
29 July 2007	20.4, 29.9, 24.3	93.4, 63.5, 67.2
30 July 2007	21.4, 31.3, 25.8	88.8, 66.7, 65.4
31 July 2007	20.2, 30.9, 26.2	92.4, 63.7, 65.5
1 August 2007	22.8, 31.0, 25.3	90.9, 64.9, 62.7
2 August 2007	22.1, 29.9, 26.9	89.9, 62.8, 63.3
3 August 2007	24.0, 30.9, 25.7	94.5, 64.4, 65.5
4 August 2007	22.1, 30.2, 24.8	90.3, 62.7, 63.5
5 August 2007	21.2, 28.9, 23.8	89.7, 66.9, 68.0
Khao Chong, Trang Province		
20 August 2007	21.2, 31.4, 25.5	94.7, 64.6, 70.0
21 August 2007	23.1, 31.9, 26.0	90.9, 62.5, 64.0
22 August 2007	22.6, 29.8, 24.6	95.9, 60.0, 66.6
23 August 2007	21.3, 29.7, 23.8	93.7, 61.4, 69.5
24 August 2007	20.2, 30.5, 26.5	94.8, 64.3, 65.4
25 August 2007	20.4, 30.3, 27.0	98.3, 65.2, 68.7
26 August 2007	21.7, 30.0, 24.4	96.8, 61.0, 68.9
15 September 2007	21.3, 29.8, 25.9	91.4, 62.1, 64.7
16 September 2007	20.2, 29.9, 24.3	89.9, 65.3, 66.0
17 September 2007	22.0, 29.5, 26.5	90.6, 63.0, 65.1
18 September 2007	22.4, 30.4, 26.1	96.0, 68.0, 69.1

Table 1. Temperature and humidity during the observation periods at all studied areas in 2007 (continued).

Collection dates	Temperature (°C)	Humidity (%)
Khao Chong, Trang Province		
19 September 2007	21.6, 30.1, 24.4	89.8, 64.9, 69.3
20 September 2007	21.1, 29.5, 25.3	92.5, 62.8, 64.0
21 September 2007	20.2, 25.9, 23.5	95.7, 63.6, 70.1
Petra National Park, Satun Province		
22 September 2007	21.2, 31.5, 25.6	89.6, 60.4, 70.0
23 September 2007	22.0, 30.9, 24.9	92.5, 61.3, 63.0
24 September 2007	22.1, 32.4, 25.8	93.2, 62.1, 67.3
25 September 2007	21.8, 31.7, 26.0	95.7, 60.7, 64.2
26 September 2007	22.9, 31.9, 25.9	94.5, 64.0, 65.8
27 September 2007	24.0, 30.9, 24.8	92.1, 62.1, 66.5
28 September 2007	22.0, 30.5, 27.8	91.0, 63.5, 65.4

Mean temperature 25.913 SE (0.3111), SD (3.6942) Minimum 20.2, Maximum 32.8, N = 141

Mean relative humidity 74.938 SE (1.1302), SD (13.42) Minimum 60.0, Maximum 98.5, N = 141

Table 2. Temperature and humidity during the observation periods at all studied areas in 2008.

Collection dates	Temperature (°C)	Humidity (%)
Khao Ram-Rom, Nakhon Si Thammarat Province		
15 Jun 2008	21.2, 31.2, 27.0	96.4, 64.7, 66.5
16 Jun 2008	22.1, 32.5, 25.9	96.4, 64.7, 65.9
17 Jun 2008	21.0, 30.0, 27.1	97.8, 66.0, 70.5
18 Jun 2008	22.2, 30.1, 25.0	95.8, 65.1, 73.0
19 Jun 2008	22.0, 29.8, 24.3	96.0, 62.6, 66.7
20 Jun 2008	22.0, 29.8, 25.0	92.5, 66.0, 69.8
21 Jun 2008	22.0, 31.0, 24.2	96.8, 65.4, 75.1
Khao Chong, Trang Province		
5 September 2008	20.3, 29.9, 26.3	95.3, 63.5, 73.3
6 September 2008	20.9, 31.9, 25.0	94.9, 62.6, 72.9
7 September 2008	21.5, 31.9, 25.5	97.0, 67.0, 75.3
8 September 2008	22.4, 29.9, 24.3	93.9, 64.0, 72.0
9 September 2008	21.0, 30.2, 25.6	94.4, 65.5, 67.2
10 September 2008	23.5, 31.4, 25.7	93.2, 60.7, 70.0
11 September 2008	20.9, 30.6, 26.0	94.6, 62.7, 71.1

Mean temperature 25.955 SE (0.5968), SD (3.8679) Minimum 20.3, Maximum 32.5, N = 42

Mean relative humidity 76.781 SE (2.1182), SD (13.728) Minimum 60.7, Maximum 97.8, N = 42

Table 3. The time spent of all visitors (data of the same visitors are in parenthesis) on a flower of *Argostemma* spp. Data is given in second.

Visitors	<i>A. diversifolium</i>	<i>A. lobulatum</i> var. <i>variabile</i>	<i>A. condensum</i>	<i>A. pictum</i>	<i>A. leave</i> subsp. <i>setosum</i>	<i>A. propinguum</i>	<i>A. puffii</i>
Hymenoptera							
Halictidae							
Nomiinae							
<i>Nomia</i> sp. 1	67,49	52,63,47,33	70,29,58	89,49	49,52,64,29,50	120,70,(50,38),55	-
<i>Nomia</i> sp. 2	-	-	25,80, 48,59	104,37	-	-	-
<i>Nomia</i> sp. 3	-	-	-	-	-	-	25,5,6,(68,11,20)
<i>Nomia</i> sp. 4	-	-	-	-	-	-	12,(12,18,8),24,(8,22),17,13,12,16,18
<i>Reepenia</i> sp.	18,25,37,49,30,12,28	(23,10,26,45, 13), (14,20),24	-	-	-	-	-
Halictinae							
Halictini							
<i>Lasioglossum</i> sp.	-	-	205,180,189,190, 243,99	210,87,190	-	8(29%)	-
Apidae							
Apinae							
Anthophorini							
<i>Elaphropoda</i> sp.	1,(1,1)	(1,1),1	-	-	1,2,2,1(1,2),2,1	(1,1),2,2,1	-
Meliponinae							
<i>Trigona laeviceps</i> Smith.	12,12,48,56,39,120, 57,66,91,37,48	34,171,40,18,20,60 50,(20,24),(20,15), 10,(25,54,52, 14)	(15,120),11,200,58 87 60,54 24,90	18,35,21,37,20,28, 44,151,52,17,160, 17,50,85,61,152,58 (16,79),(30,33),16, (110,10),49,7,5 (13,7)	-	-	-
<i>Trigona atripes</i> Smith.	-	13,17,29,7,45,46,9 65,35	-	9,5,9,30,54,34,64,9 10,30,52,33,59	-	-	-
Diptera							
Syrphidae							
<i>Microdon</i> sp	30,12,14,22	58,72,24	-	-	78,50	120	-

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Chucham, R. and Sotthibandhu, S. 2009. The Pollination of buzz-pollinated *Argostemma puffii* (Rubiaceae). Proceedign of the 15th National Graduate Research Conference. Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima Province, Thailand, 14-15 December. CD.