Proceedings of
The 3rd National Meeting on Biodiversity Management in Thailand
การประชุมวิชาการการบริหารจัดการความหลากหลายทางชีวภาพแห่งชาติ ครั้งที่ 3 (2016) : 157–162

CO-10

Survey of diurnal parasitic wasps family Braconidae (Hymenoptera: Ichneumonoidea) at Samaesan Islands, Chonburi Province

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Abstract : Braconid wasps are classified in the superfamily Ichneumonoidea, order Hymenoptera, same as sawflies, bees, ants and other wasps. Braconidae are highly diverse, in both life history and morphological characters. They are parasitoids of other insects, the parasitoid larva consume the host's tissues and finally killed its host and emerged as adult parasitoid. They mostly attack the insect hosts in the early stage of development, especially larvae of Coleoptera, Diptera and Lepidoptera. At present, there are at least 17,000 species known worldwide within 1,000 genera, however the scientists estimated that there are at least 60,000 braconid species around the world. Diurnal braconid wasps play an important role in the terrestrial ecosystems by controlling population of other insects, and can be used as natural enemies to control population of insect pests in biological control programme. However, the knowledge of braconid wasp species diversity in Thailand is very limited. Therefore, this research aims to study the taxonomy of braconid wasps at Khao Mah Cho, Samaesan and Chuang Islands, Chonburi Province as part of the Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG). This area has been poorly studied for insect diversity in general, including braconid wasps, and may even harbor new species of braconid wasps. Diurnal parasitic wasps were collected using 3 different methods: Malaise traps, yellow pan traps and an aerial net. Two Malaise traps, 20 yellow pan traps were placed at each study site. Specimens were collected every 2 months and the Malaise traps were maintained every month. The specimens had been collected 6 times started from November 2014, January, March, May, July to September 2015 and brought back to the Integrative Ecology laboratory for taxonomic study. Samaesan Island has the highest number of braconids same as the study of nocturnal braconid wasps because this area has relative larger size compare to the other 2 study sites and has more diverse microhabitats.

Keywords : Parasitic wasps, Braconidae, Taxonomy, New taxon, Samaesan Islands

Introduction

Parasitic wasps are classified in the order Hymenoptera same as bees, ants and other wasps, and are considered to be the highest evolved order of the insects (Quicke, 1997; 2014). Female parasitic wasps lay eggs on or inside their hosts, then the eggs hatched and became larvae that consume the host's tissues and finally kill their hosts when they develop into adults. Adult parasitic wasps are free living, after mating the female will search for potential insect hosts to lay eggs.

Braconid wasps are classified in the Superfamily Ichneumonoidea which are highly diverse in both morphological characters and life histories. Even though they play an important role in both ecosystem and economic, however, there are not enough studies on them, especially their diversity and biology. Braconidae can be used as natural enemies to control population of agricultural insect pests in biological control programmes and many are successful compared to other groups of natural enemies (Shaw and Huddleston, 1991). The unique characters of these wasps are as follow: fore wings vein without vein 2m-cu; 1Rs+M in the fore wings mostly

presented (Figure 1A); hind wings with vein 1r-m (Figure 1B). Second and third metasomal tergites combined (Goulet and Huber, 1993).

Superfamily Ichneumonoidea comprises of 2 large families, Ichneumonidae and Braconidae. Nowadays, more than 17,532 braconid wasps in 1,000 genera can be found across the world. However, the scientists estimated that there are approximately 100,000 species of Ichneumonidae and 60,000 species of Braconidae have not yet been discovered (Ghahari *et al*., 2006). Nevertheless, there is very few information on diversity, taxonomy, evolution and relationships of parasitic wasps and their hosts around the world. In Thailand, there is very few data on parasitic wasp diversity and very limited dichotomous keys, therefore, it is difficult to identify exact species of the Braconidae in Thailand (Santos *et al*., 2010). Butcher *et al*., 2012 discovered 176 new species of braconid wasps in only one genus, *Aleiodes* spp., in Thailand. There are more reports on the new species braconid wasps found in Thailand such as *Ischnobracon albitarsus, Confusocentrus panturat, Yelicones samaesanensis* (Butcher and Quicke, 2010; 2011a; 2011b; 2011c; Butcher, 2014). These previous studies confirmed that Thailand has highly diverse braconid wasps and most of them have not yet been discovered and known to science.

The study sites are located at Khao Ma Cho, Samaesan and Chuang Islands, Sattahip district, Chonburi Province. These sites have many different habitats such as strands, mangroves, cliff- vegetations, dry evergreen forests and secondary growth and have not been thoroughly explore for both flora and fauna, including the parasitic wasps. Recently, nocturnal braconid wasps have been studied in these areas but not for the diurnal ones. Therefore, this study aims to explore the diversity of diurnal Braconidae in this area and create a preliminary database of both nocturnal and diurnal of the braconid wasps in these areas. In addition, this study is part of braconid wasp diversity in Thailand, the results will be added to the Thai braconid database.

Methodology

1. Study sites

The study sites are located at Samaesan subdistrict, Sattahip district, Chonburi Province. Locations of the study sites recorded by Garmin eTrex 30 are as follow: Khao Ma Cho (N 12°36.148' E 100'56.926'), Samaesan Island (N 12°35.159' E 100'56.841') and Chuang Island (N 12°31.361' E 100'57.301') (Figure 2).

a. Khao Ma Cho covers 0.03 km^2 where Thai Island and Sea Natural History Museum is located. The area is connected to the sea, opposite to the Samaesan Island.

b. Samaesan Island is a rather large Island, covering 5 km^2. This Island is a popular tourist attraction, with nature trails, snorkeling and other activities. However, there are some areas on the Island that have not been disturbed by the tourists and human activities.

c. Chuang Island covers 1.2 km^2 under the control of Naval Special Warfare Command, Royal Thai Fleet. Chuang Island situated 7.6 kilometers away from the Samaesan Island and 14 kilometers away from the coast. This Island is closed and tourists are not permitted to visit the Island.

2. Collecting methods

Diurnal parasitic wasps were collected using 3 different methods: Malaise traps, yellow pan traps and an aerial net.

a. Malaise trap is a tent-like trap made from black and white netting. It used primarily for the collecting flying insects, especially flies and parasitic wasps (Figure 3A). The location of each Malaise trap was changed every 2 months and the collecting bottles were replaced every month (Figure 3B).

b. Twenty yellow traps were placed at each study site, with 1 meter away from each other. Each trap was filled with mixture of water and detergent. The specimens were collected daily to avoid rotting. To properly preserve

the specimens, filter the mixed water and detergent solution using plastic sieve before keeping them in 95% ethanol (Figure 4).

c. Aerial net is used to collect flying braconid wasps (Figure 5).

Diurnal braconid wasps were collected every 2 months for 13 months, started from November 2014 to November 2015. Temperature and humidity were recorded by HTC-2 digital Hygro - thermometer.

3. Taxonomic methods

a. Identification and classification

Specimens from the collecting bottles were sorted for only the braconid wasps using Olympus stereo microscope (SZ60 model), then pinned the specimens to make them more convenient for identifying and photographing. Some of them were stored in 100% ethanol for future molecular studies. Each specimen was given voucher number and labeled, and identify the specimens to at least subfamily level using dichotomous keys by Achterberg (1993) as well as other related keys, such as Hymenoptera of the world (Goulet and Huber, 1993) and bibliography of the family Braconidae (Hymenoptera: Ichneumonoidea) (1964-2003) (Ghahari *et al*., 2006). Preliminary database of diurnal braconid wasps recorded from this study was created and compared to the nocturnal braconid wasps database created by Vachirapong Charoennitiwat (unpublished the data). The specimens were deposited at Insect Museum, Museum of Natural History, Chulalongkorn University.

b. Description

In case of finding new braconid wasp species, the morphological characters will be described in details.

c. Dichotomous key

After finishing the field work, every species recorded from this study will be used to construct dichotomous key for the braconids at Samaesan Island and the surroundings areas.

Results and Discussion

In total, there were 6 collectings trips as follows November 2014, January, March, May, July and September 2015. The total number of specimens are 718. Of which, 86, 474 and 158 specimens were collected from Khao Ma Cho, Samaesan and Chuang Islands, respectively. The specimens belong to 9 subfamilies: Aphidiinae, Alysiinae, Cheloninae, Doryctinae, Euphorinae Macrocentrinae, Microgastrinae, Orgilinae and Pambolinae. 718 of braconid wasps were collected from three sampling methods; Malaise trap, yellow pan trap and aerial net. Malaise traps are best trap to collect the parasitic wasp.The highest number of diurnal braconid wasps collected from this study was Cheloninae (61%) (Figure 6). Cheloninae is one of the largest subfamilies of the Braconidae, all are endoparasitoids of lepidopteran larvae. Characteristics of the Cheloninae are postpectal carina strong and complete, first-third metasomal tergites immovably joined and vein r-m of fore wing present.

This results agreed with the study by Vachirapong *et al.*, 2013 (unpublished data) the highest number of braconid wasps are classified in subfamily Cheloninae (43%), this maybe because these areas have highly abundance of the Cheloninae's hosts which lead to high diversity of the Cheloninae.

Conclusion

In total, 718 specimens belong to 9 subfamilies, 41 morphospecies of Braconidae have been collected from this study. Samaesan Island has the highest number of braconids because it has relative larger size and has more diverse microhabitats.

Acknowledgements

We are grateful to the members of the Integrative Ecology Laboratory for their help with collecting the specimens. Animal Systematics Research Unit, Chulalongkorn University for Cell^D facilities. This work is funded by the Plant Genetic Conservation Project Under the Royal Initiative of Her Royal Highness Princess Maha Chakri

Sirindhorn (RSPG, RSPG-CU), the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Finally, many thanks to the RSPG-Navy for their help and made our fieldwork possible.

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Table 1. Numbers of specimens collected from each study site

Study sites	Number of braconids (individuals)						total
	Nov 2014	Jan 2015	Mar 2015	May 2015	Jul 2015	Sep 2015	
Khao Ma Cho	3	13	31	28	-	11	86
Samaesan island	7	61	54	37	3	312	474
Chuang island	4	6	10	16	-	122	158

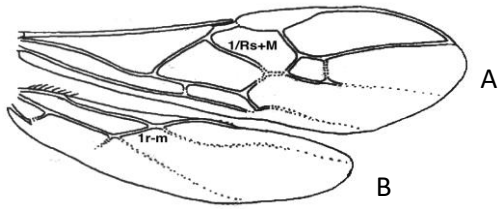

Figure 1. (A) fore wing vein 1/ Rs+M ; (B) hind wings vein 1r-m (Achterberg, 1993)

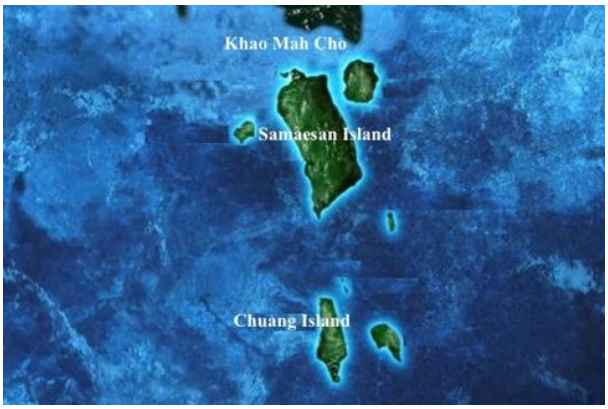

Figure 2. The map shows three study sites: Khao Ma Cho, Samaesan and Chuang Islands

Figure 3A. Malaise trap set at Chuang Island

Figure 3B. Collecting bottle

Figure 4. Yellow pan trap laid on the ground at Samaesan Island

Figure 5. Aerial net

Figure 6. Light microscope photograph of braconid wasp in the subfamily Cheloninae