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Identification of *Liriomyza* spp. (Diptera: Agromyzidae) on yardlong bean and cucumber in Songkhla province : I. Feeding tunnel patterns, external morphology and male distipallus morphology

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Taxonomic study of agromyzid leafminer *Liriomyza* spp. (Diptera: Agromyzidae) damaging on leaves of yardlong bean, and cucumber in Songkhla Province was conducted. Six hundred and fifty one leafminer larvae in their feeding tunnels were collected and reared in laboratory. Total of 186 adult male and 209 adult female leafminers were emerged. Species identification using feeding tunnel patterns of leafminer larvae and external morphology; colour of the ground, where inner and outer vertical bristles were situated, proportion of the last to the penultimate section of vein M_{3+4} of front wing, colour and patch size on mesopleura and number and location of setae on mesopleura, of the adult flies had considerable variation which cause difficulty and uncertainty. Using male distipallus morphology could separate the leafminer into at least 2 species, *L. sativae and L.trifolii*, but distortion during preparation also caused variation difficut to determine in some samples. DNA polymorphism from the remainder of each flies was impossible to study due to its tiny size.

Key words: *Liriomyza* spp., *Liriomyza trifolii, Liriomyza sativae,* agromyzid leafminer, identification

Introduction

More than 300 species of agromyzid leafminer *Liriomyza* spp. (Diptera:Agromyzidae) were found all over the world (Parrella, 1987 and OEPP/EPPO, 2005). *Liriomyza* larvae attack at least 65 plant species in families Cucurbitaceae, Leguminosae, Fabaceae, Solanaceae and Brassicaceae (CAB International, 2001). However only 23 agromyzid species are eonomic pests and only 3 species, *Liriomyza trifolii* Burgess *L. sativae* Blanchard and

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L.huidobrensis Blanchard, were reported outbreak on vegetables and ornamental plants around the world (Spencer, 1973). Certain external and internal morphological characters used to seperate the *Liriomyza* species were the relatived length of sections along particular wing veins; the presence, position and size of certain setae; the color of the cuticle where particular head setae arise and the form of male genitalia (Spencer, 1973; Zhang *et al*., 2001; Shiao, 2004 and OEPP/EPPO, 2005). Larval feeding patterns were also used in *Liriomyza* identification (Spencer, 1973; OEPP/EPPO, 2005). However, the range of variation of these characters often overlap especially in polyphagous pest species such as *L.sativae, L. huidobrensis, L. trifolii, L. brassicae, L. bryoniae, L. congesta* and *L. strigata* and this obstructed the *Liriomyza* species identification (Collins, 1996; Shiao, 2004; OEPP/EPPO, 2005; Central Science Library, 2007 and Feng *et al*., 2007). The present study was performed to identify the agromyzid leafminer *Liriomyza* spp. attacked yardlong bean *Vigna sesquipedalis* (L.) and cucumber *Cucumis sativus* L. in Songkhla province, southern Thailand using male genitalia and PCR-RAPD techniques.

Materials and methods

Field sampling of leafminer

From July to October 2006 yardlong bean and cucumber leaves damaged by leafminer were collected from seven yardlong bean fields in Bangklam, Rattapum and Had Yai districs and seven cucumber fields in Kuanneang, Bangklam, Rattapum and Had Yai districs. In each field 30 damaged leaves were picked as simple random sampling. Each leaf sample was wraped at base with cotton soaked with water to keep the leaf fresh and the leaf then kept in an insect rearing plastic box of 19×25×10 cm., one leaf/box. Adult leafminers emerged from each leaf were fed with 15% honey solution and bean or cucumber leaf in according to their original food plant. After 2 days, the fullgrown leafminer flies with complety developed color and setae were killed and kept in 95% ethyl alcohol for morphological examination.

Identification of Leafminer

1. Identification to species using mine patterns

1.1. After the adult flies were totally emerged, the mine pattern on damaged leaf was photographed and mine pattern was redrawn on paper. All detail data of each leaf such as sampling place and date, host plant was recorded.

1.2. The redrawn leaf patterns from 1.1 were compared with those in Spencer (1973) and OEPP/EPPO (2005) for species identification.

2. Identification to species using adult flies morphology

Adult flies (from 1.1) were preserved in 95% ethyl alcohol and examined under stereomicroscope. The fly morphology especially the following characters were observed and photographed.

2.1. Colour of the ground where inner and outer vertical bristles were situated (Fig. 1).

2.2. Proportion of the last (a) to the penultimate (b) section of vein M_{3+4} of front wing (Fig. 2).

2.3. Colour, patch size and colour, number and location of setae on mesopleura (Fig. 3).

The flies were identified to species using these characters (from 2.1 to 2.3) in according to Spencer (1973).

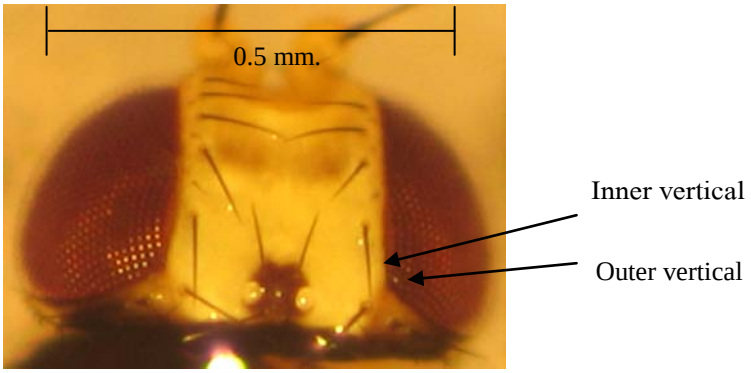


Fig. 1. Head of *Liriomyza* sp. with inner and outer vertical Bristles (40X).

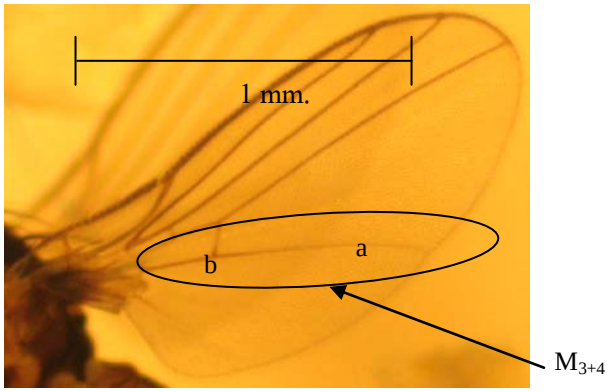


Fig. 2. M_{3+4} vein on front wing of *Liriomyza* sp. (40X).

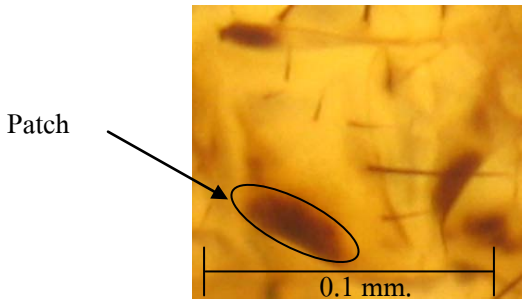


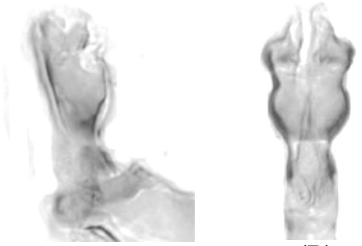
Fig. 3. Mesopleuron of *Liriomyza* sp. (40X).

3. Identification to species using the male distiphallus

3.1. Adult male leafminers (From 2.) in beaker contained 3 ml. of 10% potassium hydroxide were warm over alcohol lamp for 10 minutes to reduce the colour and hardening of their exoskeleton.

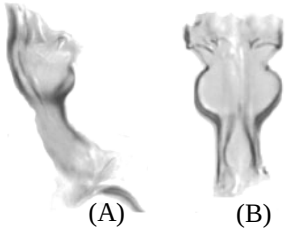
3.2. Under compound, the fly sample which exoskeleton became soft and clear was dissected. The fly phallus (aedeagus) was removed, examined and compared with those in Spencer (1973), Collins, (1996) and OEPP/EPPO (2005) for species identification (Fig. 4 and 5). The distiphallus sample was photographed before it was mounted in slide with Hoyer's solution.

3.3. Slide mounted distiphallus with the fly information was then kept in oven at 35°C until the media became harden. The slide was sealed and kept in a slide box.



(A) (B)

Fig. 4. Male distipallus of *Liriomyza sativae* Blanchard400)x), (A) lateral) B (dorsal
From: Collins, 1996



(A) (B)

Fig. 5. Male distipallus of *Liriomyza trifolii* Burgess400) x)**,** (A) lateral) B (dorsal
From: Collins, 1996

Results and discussion

Field sampling of leafminer

Although yardlong bean leaf was smaller than cucumber leaf but total numbers of mine on each leaf were about the same (1.49 mines/ yardlong bean leaf and 1.61 mines/ cucumber leaf). However, there were 174 adult flies (81 males and 93females, Male: Female = 1.00: 1.15) and 73 *Phedrotoma* sp. (leafminer's parasitoid) emerged from 30× 7yardlong bean leaves (collected from seven yardlong bean fields in Bangklam, Rattapum and Had Yai districts) while there were 221 adult flies (105 males and 116 females, Male: Female = 1.00: 1.10) and 53 *Phedrotoma* sp.emerged from 30 × 7 cucumber leaves (collected from seven cucumber fields in Kuanneang, Bangklam, Rattapum and Had Yai districts).

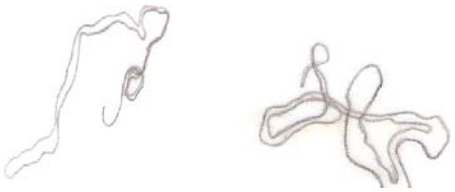
Identification of Leafminer

1. Identification to species using mine patterns

Patterns of leaf mine on yardlong bean and cucumber leaf samples were compared with those in Spencer (1973) and were separated into 2 groups, the loose; irregular scrolled mine and the convoluted mine (Figs. 6 and 7). Both forms of mines were found either on yardlong bean or cucumber leaf and within each form there was no other difference which abled to indicate species of the leaf miner. Jiao *et al.* (1998) also reported that mine patterns could not use to identify species of miner

(A) (B)

Fig. 6. Examples of *Liriomyza* spp. mine on yardlong bean leaves.
(A) Loose, irregular scrolled mine (B) Convoluted mine



(A) (B)

Fig. 7. Examples of *Liriomyza* spp. mine on cucumber leaves.
(A) Loose, irregular scrolled mine (B) Convoluted mine

Patterns of leaf mine on leaf samples were compared with those in Spencer (1973) and found that we was not able to identify the leaf miner flies from mine patterns only. Collins (1996) and Jiao *et al*. (1998) also mentioned that for polyphagous agromyzid species, mine configuration is affected by the host plant species, by the physical and physiological condition of leaf and by the number of larvae mining this same leaf.

2. Identification to species using adult flies morphology

2.1 Colours of the ground where inner and outer vertical bristles were situated.

The inner vertical bristles of the leaf miner fly samples were always found on yellow areas while the outer vertical bristles were situated on areas where the colors varied from pale yellow to dark brown difficult to distinguish (Fig. 8) and were not able to identify the fly species using only this character.

2.2 Proportion of the last (a) to the penultimate (b) section of vein M_{3+4} of front wing

The proportions of the last (a) to the penultimate (b) section of vein M_{3+4} of fly samples front wings were varied from 1.95 to 4.2 times. These proportions alone were not accurated enough to identify the insect specimens. However, after the identification using male distiphallus (which some of them were also unobvious for species identification). The flies were separated into 2 species, *Liriomyza sativae* and *L. trifolii.* The proportion of (a) and (b) were found varied from 2.13 to 4.20 times in *L. sativae* and 1.95 to 4.20 times in *L. trifolii*.

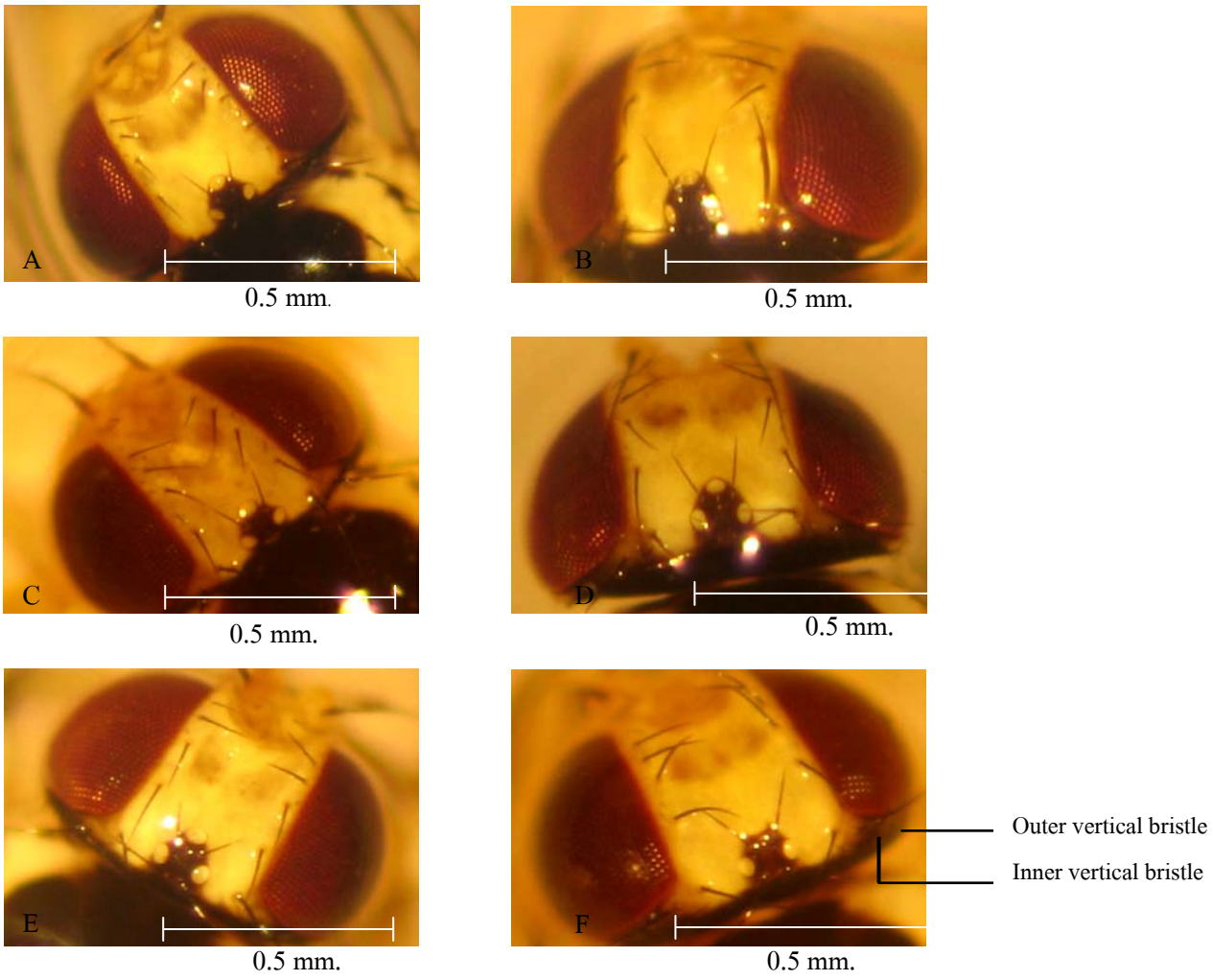


Fig. 8. Examples of heads of *Liriomyza* spp. showed variation of colours on areas where the outer vertical bristles located (40X).

The overlaped proportions (Fig. 9) made impossible to separate the 2 species, *L. sativae* and *L. trifolii*, the same as whats Collins (2004) had pointed out that although species identification of *Liriomyza* spp. could be done using proportion of the last (a) to the penultimate (b) section of vein M_{3+4} of front wing, colour of the ground where inner and outer vertical bristles were situated but the overlapping of these characters in polyphagous species made it become complicated. *L. sativae* and *L. trifolii* have been known to be polyphagous (Spencer, 1973 and CABI, 2001). It is assumed that there may be 2 species of *Liriomyza*, *L. sativae* and *L. trifolii*, in fly samples.

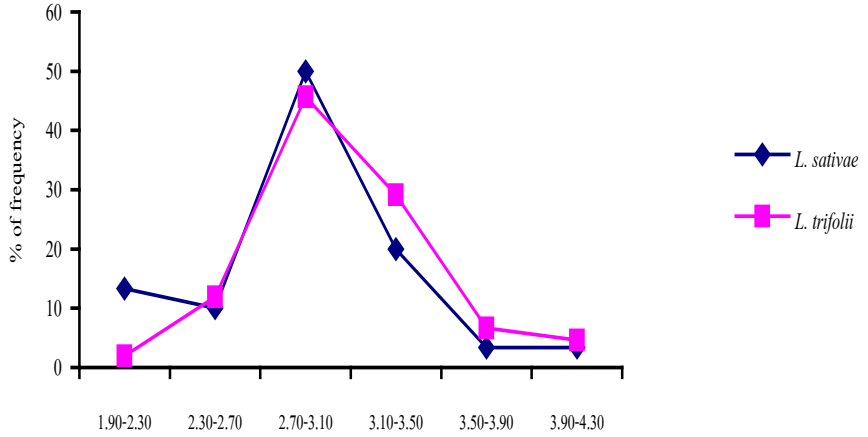


Fig. 9. Proportion of the last (a) to the penultimate (b) section of vein M_{3+4} of males *Liriomyza sativae* Blanchard and *Liriomyza trifolii* Burgess front wings.

2.3 Colour, patch size and colour, seta number and location on mesopleura

There was a small patch at the front lower margin of the mesopleura (anepisternum) as seen in Fig. 10. The patch was about 1/8 of the whole area varying in shape and colour (from light brown to black). There were 4-9 setae with one macroseta on the upper hind margin. All of these characters were compared among each of the fly samples, it was difficult to separate the difference and identify to species.

3. Identification to species using the male distiphallus

Male distiphallus photomicrographs of 186 male leafminer flies, two species of *Liriomyza*, *L. sativae* and *L. trifoli* were identified, although some of them had unclear figure which may cause by sample preparation. *L. sativae* male distiphallus laterally had no strongly curved basal and one distal or apical bulb, posteriory slight constrict between apical and basal parts of the bulp (Fig. 11) while *L. trifoli* male distiphallus laterally had strongly curved basal and one distal or apical bulb, posteriory marked constrict between apical and basal parts of the bulp (Fig. 12).

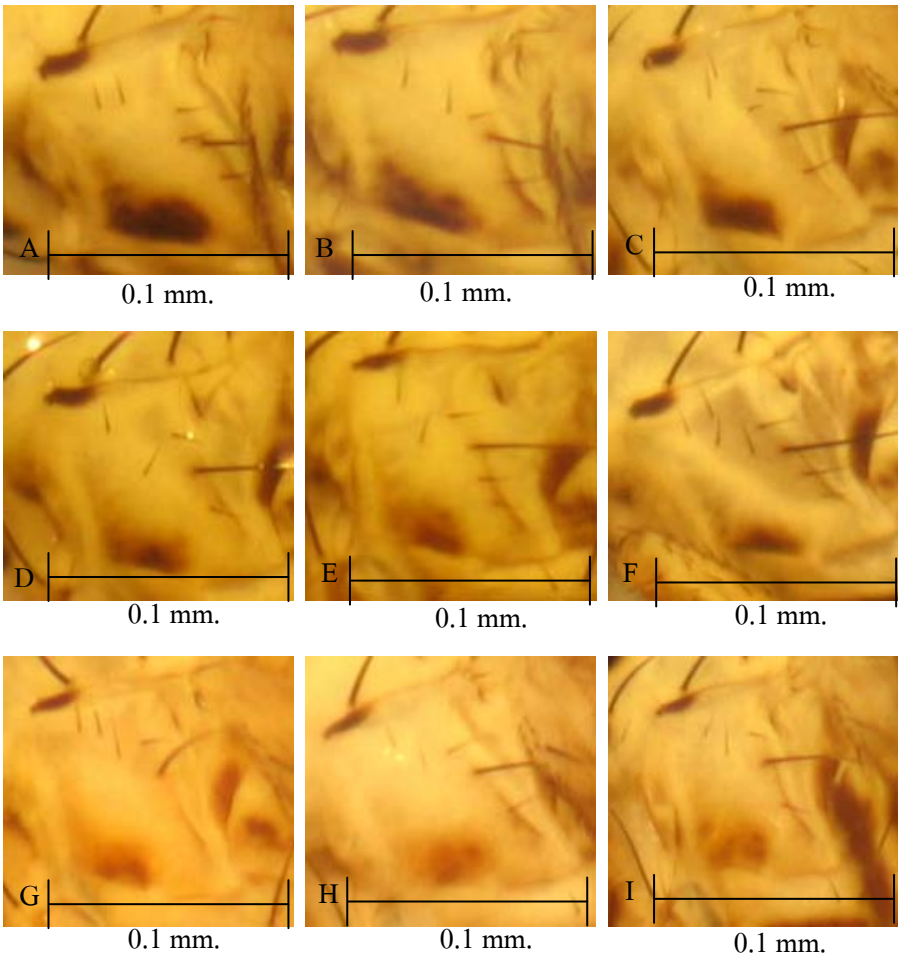


Fig. 10. Examples of patch size-colour and seta number-location on mesopleurons of collected *Liriomyza* spp. (40X).

Although some of the male distiphallus photomicrographs were not distincted, 15 males agromyzid leafminer from yardlong bean leaves collected in Songkhla were identified as *L. sativae* and the less 66 males were *L. trifolii* (Table 1) while from cucumber leaves, 15 males were *L. sativae* and the less 156 males were *L. trifolii* (Table 2).

After morphological examination for species identification had been done, the DNA sequence through PCR-RAPD technique of an insect bodypart remainder was tried it was not able to do because an insect body size was too small.

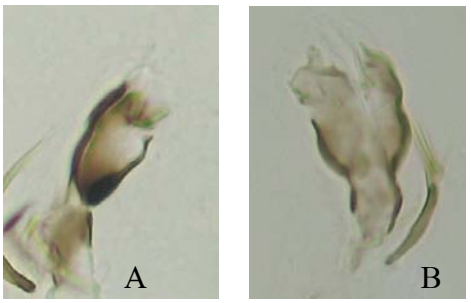


Fig.11. Male distiphallus of *Liriomyza sativae* Blanchard (200X).
A) lateral, B) posterior

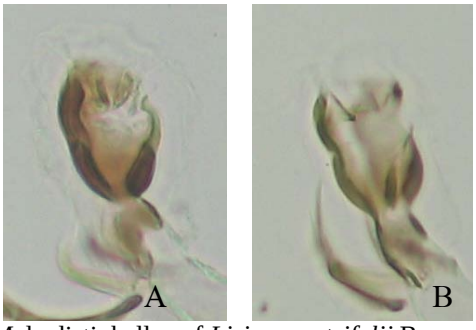


Fig. 12. Male distiphallus of *Liriomyza trifolii* Burgess (200X).
A) lateral, B) posterior

Table 1. Identification of male leafminer *Liriomyza* spp., collected from yardlong bean leaves in Songkhla Province, using male distiphallus.

Locations	Number	
	L. sativae	***L. trifolii***
1. M.4 Ban Han, Bang Klam district	3	16
2. M.14Thachamuang, Rattapum district	2	11
3. M. 9.Klong Hair, Hat Yai	5	12
4. M. 3.Khor Hong , Hat Yai	1	6
5. M. 8.Tha Kam, Hat Yai	2	7
6. M.7 Tung Tum Sao, Hat Yai	0	7
7. M.5 Tung Yai, Hat Yai	2	7
Total	15	66

Table 2. Identification of male leafminer *Liriomyza* spp., collected from cucumber leaves in Songkhla Province, using male distiphallus.

Locations	Number	
	L. sativae	***L. trifolii***
1. M 6.Bangreang, Kuanneang district	3	14
2. M 4.Ban Han, Bang Klam district	1	11
3. M 5.Thachamuang, Rattapum district	1	6
4. M 7.Thachamuang, Rattapum district	3	17
5. M 9.Klong Hair, Hat Yai	1	14
6. M 3.Khor Hong , Songkhla	3	14
7. M 7.Tung Tum Sao, Hat Yai	3	14
Total	30	156

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