



# Molecular Identification of Fall Armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) in Myanmar

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# Outlines

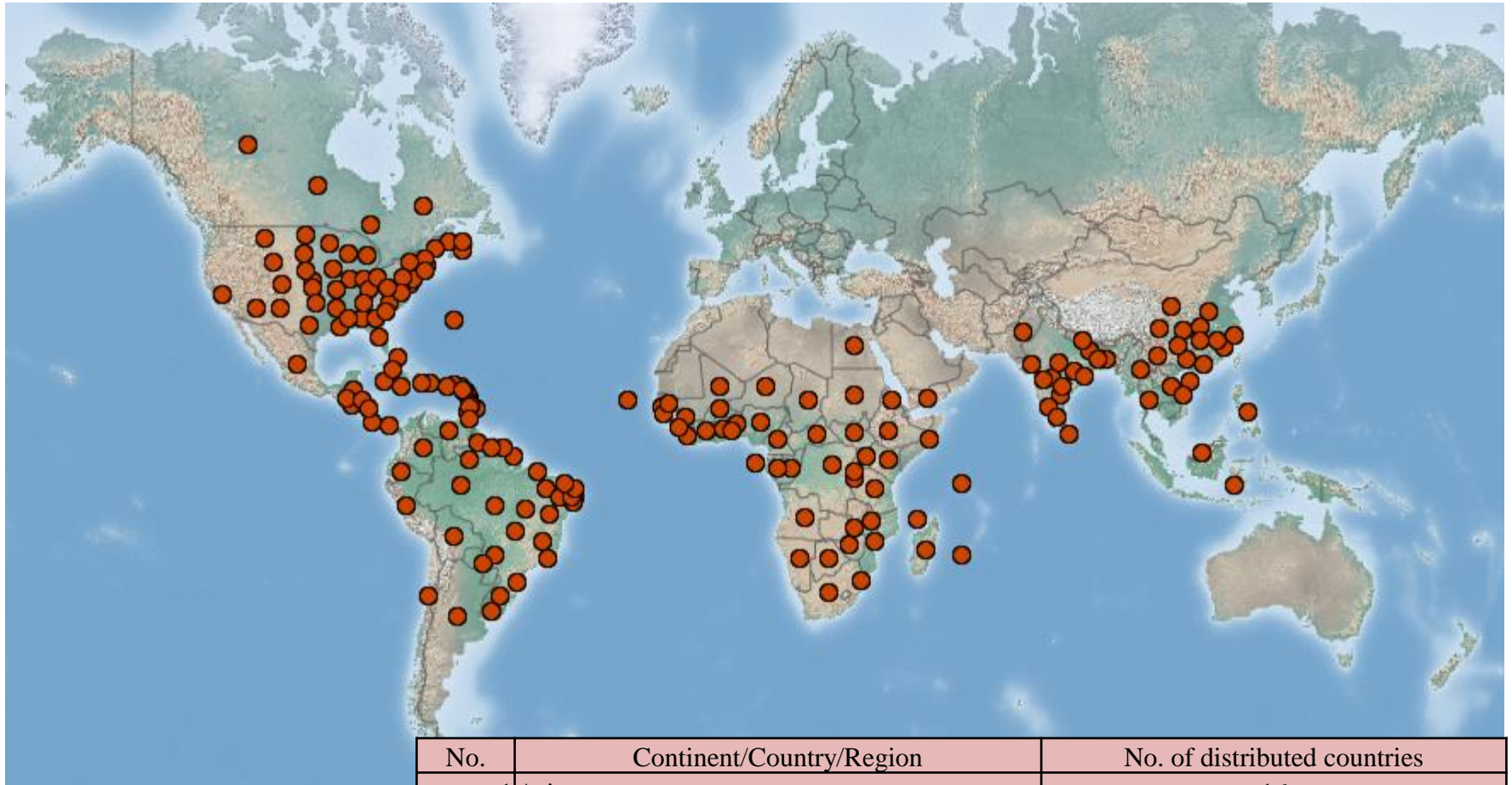
- Introduction
- Objectives
- Materials and Methods
- Statistical Analysis
- Results and Discussions
- Conclusions



# Introduction

- The fall armyworm (*Spodoptera frugiperda* J.E. Smith)
- native to tropical and subtropical regions of the Americas
- **Distribution**
  - central and western African in early 2016,
  - India and Yemen at July 2018,
  - Bangladesh, Sri Lanka and Thailand at December 2018,
  - Myanmar, China, Indonesia, Laos, and Malaysia at June 2019,
  - Vietnam, Egypt, the Republic of Korea and Japan at July 2019.

## *Spodoptera frugiperda* (fall armyworm) Distribution Map



No.	Continent/Country/Region	No. of distributed countries
1	Asia	16
2	Africa	47
3	North America	4
4	Central America and Caribbean	28
5	South America	13
6	Europe	Eradicated in Germany
	Total no. countries	108

# Introduction

## Economic impact

- ❑ The infestation of FAW caused the losses of crop yield 11.57% when 26.4 and 55.9% infestation (Baudron et al., 2019),
- ❑ 58% yield losses when leaf, silk and tassel damage level between 25 and 50% (Chimweta et al., 2019), and
- ❑ 15-73% yield losses when 55-100% plants were infested during the mid to late-whorl stage of maize development (Hruska and Gould, 1997).

# Introduction

- Fall armyworm (FAW) has two subpopulations such as “rice-strain” and “corn-strain”, these two strains were morphologically indistinguishable
- molecular methods are valuable tool
  - ❑ to support national capability in rapid and reliable pest diagnosis and identification
  - ❑ to control and monitoring FAW armyworm infestation,
  - ❑ to understand the migration patterns, genetic diversity of the existing populations, and potential development of new populations

# Introduction

## *FAW armyworm infestation in Myanmar*

- Yee et al. (2019) conducted surveys for fall armyworm on maize in Myingyan township (Mandalay region) on December 2018,
- Hinthada and Zalun township (Ayeyarwady region) on January 2019,
- After rearing at Plant Protection Division, Yangon, and it was positively confirmed as fall armyworm *Spodoptera frugiperda*, based on morphological characteristics of larvae. It is the first detection report for fall armyworm infestation in Myanmar.

# Objectives

- i. to investigate incidence of fall armyworm (FAW) in the target areas,
- ii. to examine the genetic differentiation between FAW and non-FAW at molecular level, and
- iii. to elucidate the genetic variation among and within population of FAW larvae samples.



# Materials and Methods

- Sampling and Collection Sites
- DNA Extraction and Genotyping
- Statistical Analysis



# DNA Extraction and Genotyping



Table 1. Geographical location of fall armyworm collected in Myanmar

No.	Location		Coordinates	Altitude (Above Sea Level) (ft)
	State/Region	Township		
1	Ayeyarwady	Hinthada	17° 15' and 17° 50'N, & 95° 10' and 95° 35'E	44.30
2		Maubin	16° 30' and 16° 57'N, & 95° 24' and 95° 52'E	13.62
3		Zalun	17° 35' and 17° 40'N, & 95° 15' and 95° 45'E	36.17
4	Yangon	Hlegu	16° 59' and 17° 19'N, & 96° 13' and 96° 25'E	45.50
5		Taikyi	17° 10' and 17° 48'N, & 95° 40' and 96° 8'E	100.00
6		Mingaladon	17° 02' and 17° 04' N, & 96° 08' and 96° 15'E	100.00
7	Sagaing	Kanbalu	20° 54' and 23° 43'N, & 95° 19' and 95° 59'E	465.00
8		Kani	22° 49' and 22° 15'N, & 94° 15' and 94° 45'E	265.00
9		Salingyi	21° 49' and 22° 9'N, & 94° 53' and 95° 8'E	1,057.00
10		Yinmabin	20° 59' and 22° 19'N, & 94° 36' and 95° 15'E	400.00
11	Nay Pyi Taw	Zaryarthiri	19° 24' and 20° 18'N, & 95° 40' and 96° 46'E	320.00
12	Kayah	Loikaw	19° 14' and 20° 59'N, & 97° 07' and 97° 42'E	2,950.00

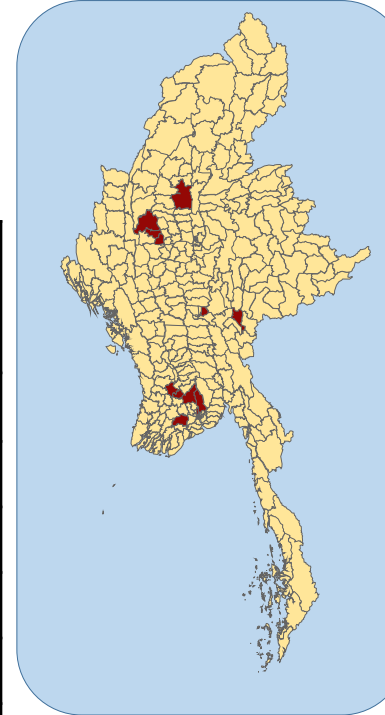


Figure 1. Collection site of fall armyworm, *Spodoptera frugiperda*.

From January to April 2019, larvae were collected from five state/regions (Ayeyarwady, Yangon, Sagaing, Nay Pyi Taw, and Kayah) of Myanmar.



# Sampling and Collection Sites





Hinthada (Shwe Hin Thar Kyune)



Hinthada (Kyaung Say Kyune)



Hlegu



Taikiyi



Yinmabin



Kani

Figure 2. The fall armyworm larvae samples.

Table 2. Occurrence of fall armyworm in the investigated areas and number of larvae samples used in the study.

No.	Township	State/Region	Sub-township/ village	No. of Larvae Sample	Total (State/Region)
1	Hinthada	Ayeyarwady	-Shwe Hin Thar Kyune -Kyaung Say Kyune	4 8	20
2	Maubin	Ayeyarwady		5	
3	Zalun	Ayeyarwady		3	
4	Hlegu	Yangon		FAW-3 Non-FAW -3	13
5	Taikyi	Yangon	Aphauk	5	
6	Mingaladon	Yangon		2	
7	Kanbalu	Sagaing	Kyune Hla	2	12
8	Kani	Yangon	Tha Yet Pin	5	
9	Salingyi	Yangon	Kan village	2	
10	Yinmabin	Yangon		3	
11	Zaryarthiri	Nay Pyi Taw		6	6
12	Loikaw	Kayah		5	5
	Total			56	

# Statistical Analysis

- Molecular variance (AMOVA) and principal coordinate analysis (PCoA) were conducted using GenAlEx version 6.5 (Peakall and Smouse, 2012).
- In order to support identification of larvae samples, cluster analysis was conducted using NTSYS Pc v 2.1 (Rolf, 2000), based Nei and Li genetic distance (Nei and Li, 1979).

# Results and Discussions

- Genotyping the fall armyworm
- Cluster analysis of larvae from Ayeyarwady, Yangon and Sagaing regions



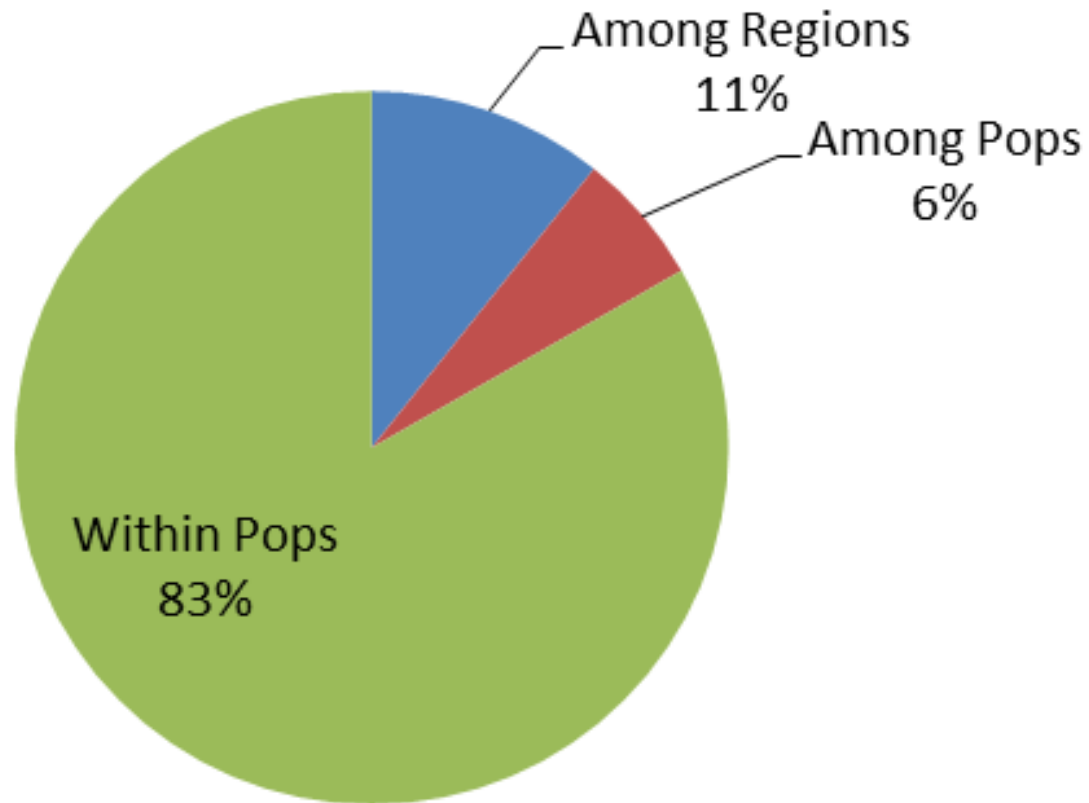
# Genotyping the fall armyworm

Table 4. Markers used in genotyping of all larvae samples.

No.	Marker Name	No. of alleles	PIC	Expected Size (bp)
1	Stv_Spf00918	3	0.21	115-125
2	Stv_Spf00343	3	0.15	100-120
3	Stv_Spf00544	2	0.07	120-130
4	Stv_Spf01856	2	0.27	115-120
5	Stv_Spf01587	3	0.40	95-110
6	282F-850R	3	0.15	400-500
	average	2.67	0.21	

Figure 3. Analysis of molecular variance (AMOVA) for fall armyworm in twelve investigated areas.

### Percentages of Molecular Variance



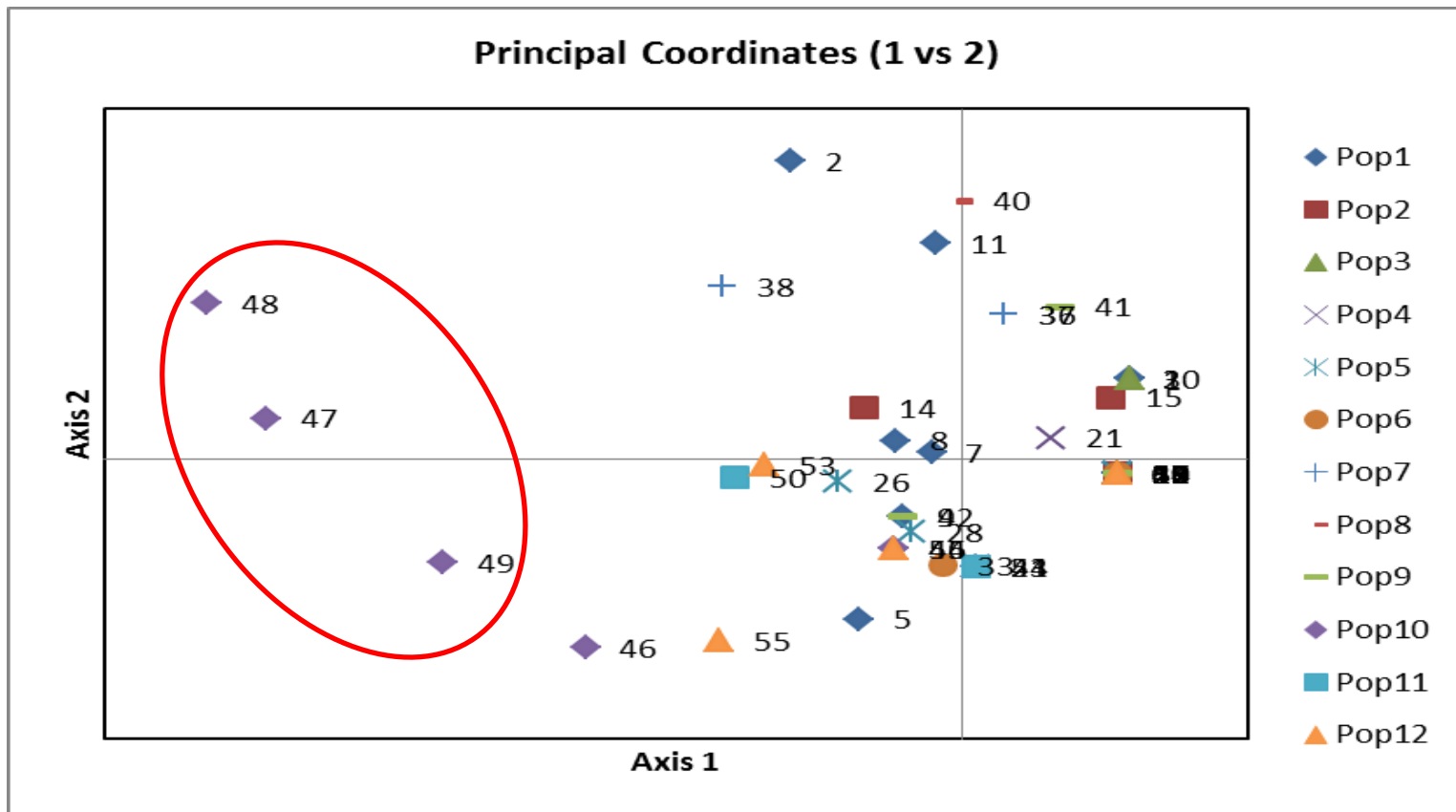


Figure 4. Principal coordinate analysis of (56) larvae at twelve populations (pop 1 to 12: Hinthada, Maubin, Zalun, Loikaw, Yezin, Kanbalu, Kani, Salingyi, Yinmabin, Hlegu, Mingaladon and Taikyi, respectively) level generated from six molecular markers.

The first two principal components (PC1 and PC2), account for 24.98% and 15.37% of total variance.

# Cluster analysis of larvae from Ayeyarwady, Yangon and Sagaing regions

Table 6. Markers used in genotyping of larvae samples from Ayeyarwady, Yangon and Sagaing regions.

No.	Marker Name	No. of alleles	PIC	Expected Size (bp)
1	Stv_spf 00918	3	0.27	115-120
2	Stv_spf 00343	2	0.10	100-120
3	Stv_spf 00544	2	0.05	120-130
4	Stv_spf 01856	2	0.34	115-120
5	Stv_spf 01587	3	0.36	95-110
6	282F-850R	3	0.20	400-500
7	Stv_spf 01685	2	0.38	140-145
8	Spf 05	2	0.40	200-300
9	Sf_COIII	2	0.09	710-720
10	891F-1472	2	0.17	600-630
	average	2.3	0.24	

Interestingly, marker *Spf05* showed heterozygous alleles at 12 fall armyworm larvae (5, 2, 2, 1 and 2 larvae samples from Hinthada, Maubin, Zalun, Kani and Taiky Township, respectively).

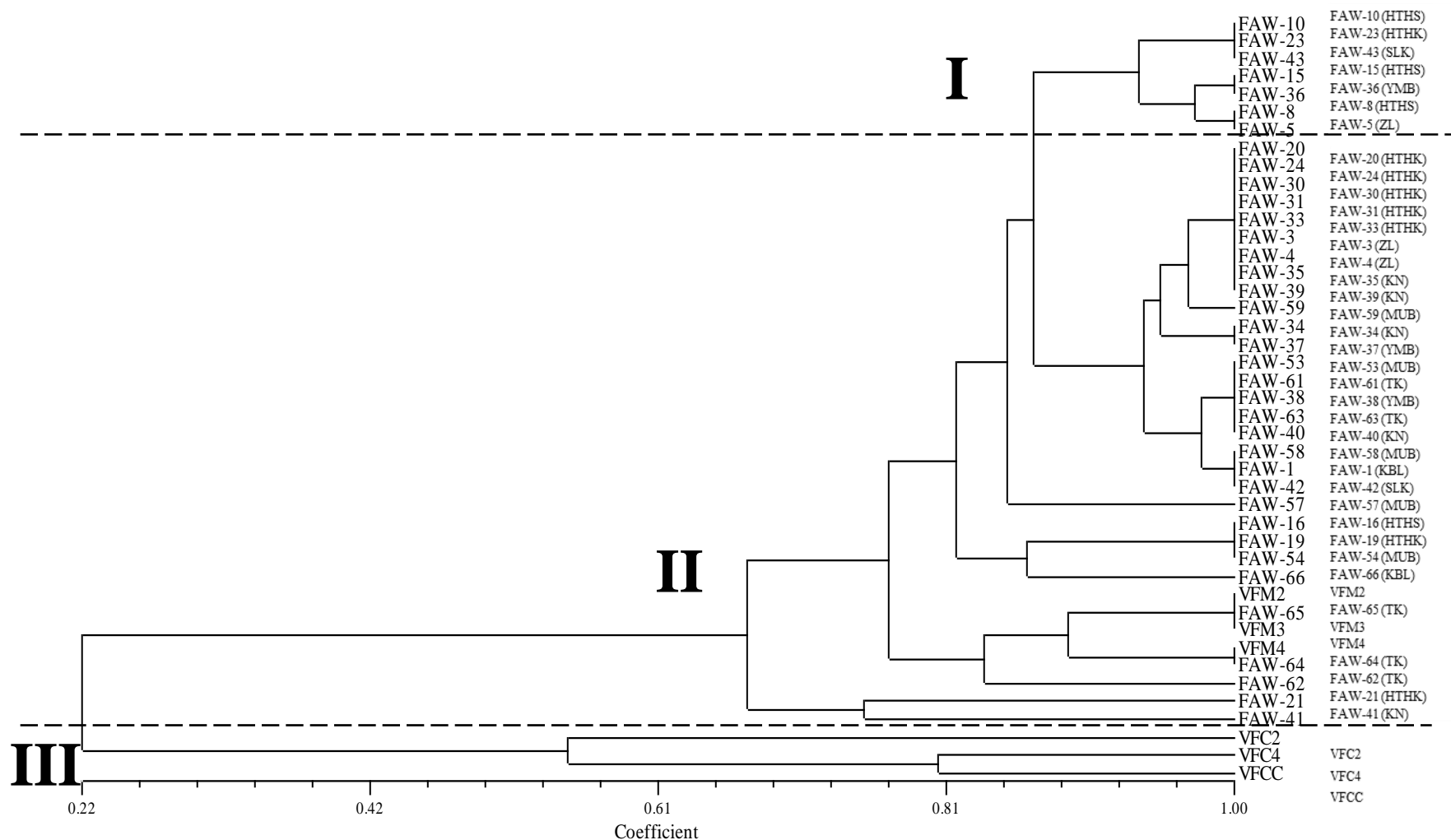


Figure 5. Dendrogram of fall and non-fall armyworm from Ayeyarwady, Yangon and Sagaing regions, using Nei and Li genetic distance (1979). (HTHS means Hinthada (Shwe Hin Thar Kyune), HTHK – Hinthada (Kyaung Say Kyune), MUB-Maubin, ZL- Zalun, VF-Hlegu, TK- Taikyi, KBL-Kanbalu (Kyune Hla) , KN- Kani (Tha Yet Pin), SLK- Salingyi (Kan village), and YMB- Yinmabin Township, respectively).

# Conclusions

- This study suggests that the fall armyworm incidence at twelve townships have a considerable variation at population level even molecular identification is started immediately after the fall armyworm invasion to Myanmar.
- The six molecular markers which used in this study were sufficiently discriminate FAW and non-FAW and also useful for the genetic studies of *S. frugiperda*.
- Since the FAW populations from Ayeyarwady, Yangon and Sagaing were clustered independently from their collection sites, it might be due to migration of the populations in the early infestation period to Myanmar.
- In order to capture the genetic variation and population structure of the fall armyworm more precisely, additional molecular and adequate individual per population should be studied.

# Acknowledgement

- Regional Directors from five state/region
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- Extension staff

THANK YOU.