BIODIVERSITAS
Volume 25, Number 5, May 2024
Pages: 2104-2114

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250528

Genetic structure and current distribution of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* (Diptera: Agromyzidae) on vegetable crops in Bali, Indonesia

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Manuscript received: 13 March 2024. Revision accepted: 18 May 2024.

Abstract. *Utama IWEK, Supartha IW, Yuliadhi KA, Sudiarta IP, Yudha IKW, Saleh S, Wahyuni S, Wiradana PA. 2024. Genetic structure and current distribution of* Liriomyza huidobrensis, L. sativae *and* L. trifolii *(Diptera: Agromyzidae) on vegetable crops in Bali, Indonesia. Biodiversitas 25: 2104-2114. Liriomyza* spp. (Diptera: Agromizidae) is a polyphagous pest that attacks various types of vegetable and ornamental plants throughout the world. The most damaging species are *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* (Blanchard), and *Liriomyza trifolii* (Burgess). There have been no reports regarding mapping of population distribution and population genetics of *Liriomyza* in the Bali region using the Cytochrome C Oxidase Subunit I (COI) approach. This research aims to map the population and genetic structure of *L. huidobrensis*, *L. sativae* and *L. trifolii* on vegetable plants in Bali. This research uses a purposive sampling method which represents vegetable plantings in each district and city in Bali Province. Identification of the *Liriomyza* spp. gene structure using the COI approach using LCO and HCO primers. The results of the research found that the spatial distribution of invasive leafminer fly species, namely *L. sativae* and *L. trifolii*, was evenly distributed throughout the city districts in Bali. The distribution pattern of *L. trifolii* is uniform with an S2/X value <1, while *L. huidobrensis* is found in the highland areas with a clustered distribution pattern with an S2/. The genetic distance between the three *Liriomyza* species found in vegetable plants in Bali is much longer. This means that the three *Liriomyza* species show very high levels of genetic differentiation in mitochondrial and nuclear genes, and differentiation between species in nuclear genes. This genetic variation can influence the ability of insects to quickly adapt to new environments and contribute to population dynamics and dispersal capacity.

Keywords: COI, genetic diversity, invasive pest, *Liriomyza*

INTRODUCTION

Liriomyza (Diptera: Agromyzidae) is an important quarantine pest because it is capable of affecting the decline of important agricultural commodities in various parts of the world, including Indonesia (Spencer and Steyskal 1986). However, this pest continues to be introduced and reintroduced in several regions of the world (Nakamura et al. 2013; Blacket et al. 2015). International trade in ornamental plants and vegetables facilitates their spread across countries, as their eggs and larvae are not always visible on their hosts (Nakamura et al. 2013; Lonsdale et al. 2023). The number of species in the Genus *Liriomyza* is known to be up to 400 species, which allows them to spread to various regions throughout the world. Most of the Genus *Liriomyza* are leafminers with phytophagous larvae that feed on living plant tissue and attack 140 host plant families throughout the world (Liang et al. 2023). Since the 1970s, *Liriomyza* species have also been found in Florida (United States) (Weintraub et al. 2017). According to Lonsdale et al. (2023), this pest began to spread outside the American continent in 1976, such as to the country of Kenya (Africa) when that country pioneered chrysanthemum flower plantations whose seeds were imported from Florida. Its spread then reached the European continent, such as in England, the Netherlands, Germany and Denmark when these countries imported chrysanthemums from Kenya in 1989.

The spread of *Liriomyza* to the Asian continent (Japan, the Philippines and Korea) began in 1990 (Chang et al. 2020). The *Liriomyza* species in Indonesia was first reported by Rauf (1995), attacking potato plantations in Tugu Selatan Village, Cisarua District, Bogor in mid-1994. In 1998, this pest had spread to various highland vegetable production centers in Java, Sumatra, Bali and Lombok (Rauf 1995; Supartha et al. 2005). In the same year, vegetable crops in South Sulawesi were also reported to have been attacked (Hikmawati et al. 2013). The type of *Liriomyza* that first entered Indonesia was *L. huidobrensis*, followed by *L. sativae*.

Three species of *Liriomyza* were initially reported to be economically damaging in America, namely *Liriomyza*

huidobrensis (Blanchard), *Liriomyza sativae* (Blanchard), and *Liriomyza trifolii* (Burgess) (Sher et al. 2000). These three species have a wide host range and include various ornamental and vegetable crops such as beans (*Vigna angularis*), eggplant (*Solanum melongena*), tomatoes (*Solanum lycopersicum*), pumpkins (*Cucurbita moschata*), chrysanthemums (*Chrysanthemum morifolium*), garbera (*Gerbera jamesonii*), gypsophila (*Gypsophila paniculata*), and marigolds (*Tagetes erecta*) (Liang et al. 2023; Yadav et al. 2024). Variations in pest attacks are greatly influenced by the diversity and abundance of host plants in the field (Muiruri et al. 2019; Supartha et al. 2023). The diversity and abundance of host plants provide a variety of suitable hosts, thus influencing their distribution in the field (Salazar-Mendoza et al. 2021). Previous studies from Wahyuni et al. (2017) also reported strong indications of differences in the adaptability of each *Liriomyza* species to host plants in the field. The *L. sativae* species was initially only found in the lowlands on leguminous plants but has now spread to various high-altitude areas on various types of host plants in the field. Likewise, *L. trifolii*, which is the last species to enter Indonesia, has adapted to various types of host plants at various altitudes, especially in Bali (Supartha et al. 2023). Due to their small size, rapid interspecific competition, invasion rate, and adaptability, insects are sensitive to geographic isolation, hosts, and phenological niches leading to species differentiation (Blacket et al. 2015; Tang et al. 2016; Finch et al. 2021).

To determine the population genetic structure and migration patterns of these insects, several research have analyzed different populations in China using ITS1 and microsatellites (Tang et al. 2016). In previous research from Chen et al. (2019), mitochondrial rDNA-ITS2 and Cytochrome C Oxidase Subunit I (COI) sequences were used to analyze population differentiation in several populations of the invasive species *Liriomyza*. The study of population genetic structure, identification of genetic relationships, construction of genetic maps, and gene mapping is useful in exploring and revealing information about population genetics, molecular systematics, and population ecology (Tang et al. 2016; Xuan et al. 2023).

Previous *Liriomyza* research was still limited to morphometric identification, host plant types, and parasitoid associations in controlling this pest naturally in its habitat (Shiao et al. 2004; Foba et al. 2015; Wahyuni et al. 2017). Previous research has not yet reached the stage of exploring the involvement of ecological factors, host plants, and climate as important factors in the adaptation process of *Liriomyza* which is polyphagous (Wang et al. 2021) and has the capacity for wide spatial distribution in the Bali region. This research aims to map the population of the invasive pest *Liriomyza* spp. and its genetic structure both intra and inter-species of the three *Liriomyza* species. The findings of this study will hopefully contribute to improving our knowledge of changes in gene structure caused by geographic isolation and population growth of *L. trifolii, L. sativae*, and *L. huidobrensis*, particularly in the Bali Province region of Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted on a field and laboratory scale. Field studies were carried out in all vegetable growing areas covering 8 districts and 1 city in Bali Province, Indonesia (Table 1). Integrated Pest Management Laboratory (IPMLab), Faculty of Agriculture, Universitas Udayana, Bali, Indonesia, to carry out laboratory scale investigations into the maintenance of flies on different host plants.

Research procedure

Determination of sampling location and sample handling

Sampling was carried out in all districts/cities in Bali Province (Figure 1).

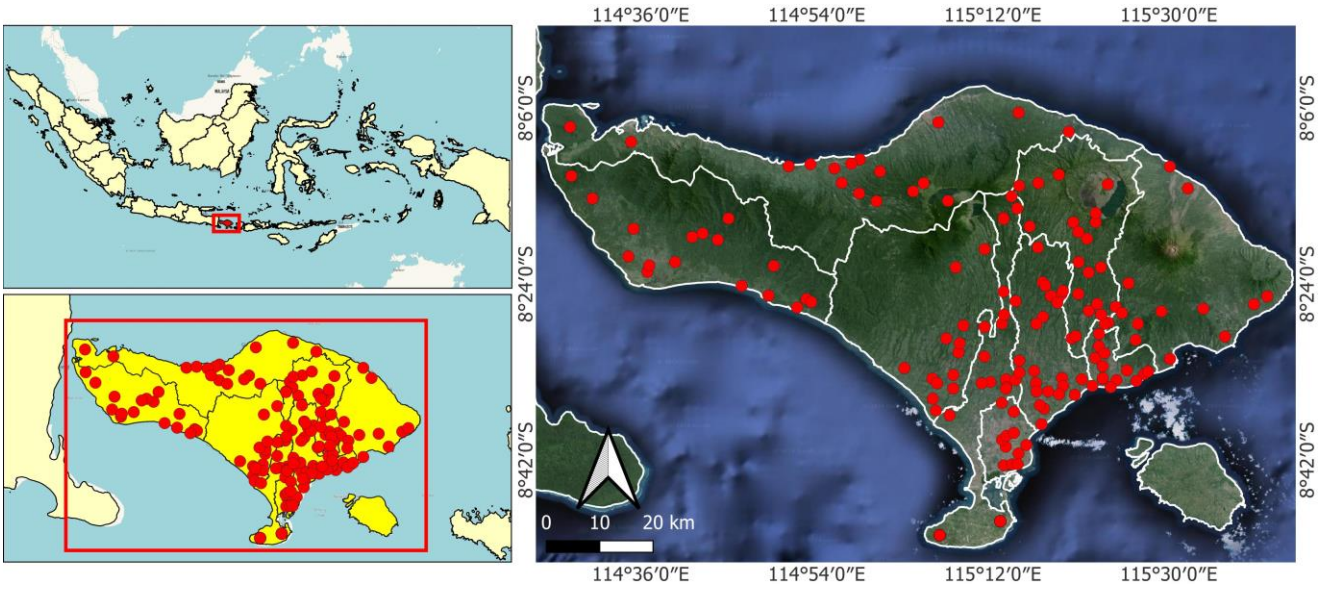

Figure 1. Sampling site in Bali Province, Indonesia

Table 1. Sampling site in Bali Province, Indonesia

District, Sub-District	Village	Coordinate point	Alt. (m asl.)
Badung			
Abian Semal	Sibang Gede	5'75'67'677"S 311'13'7,456E	109
	Sibang Gede	8'75'67'677"S 311'13'7,456E	109
	Sedang	5'60'76'321"S 347'18'8,423E	100
	Gerana	8°28'30" S 115°12'30" E	256
	Anggungan	8°33'33" S 115°11'51" E	98
	Abiansemal	8'75'67'227"S 222'13'6,245E	214
Petang	Pangsan	5'75'67'877"S 311'12'7,959E	357
	Plage	5'75'67'877"S 311'12'7,979E	824
	plage	5'75'67'877"S 311'12'7,979E	827
	petang	5'75'67'2327"S 123'9'6,2451E	745
	Beloksidan	5'75'67'87"S 311'12'7,79E	890
	Catur	8'31'88S 115'26'29E	925
	Sulahan	8'31'87S 115'24'37E	347
Mengwi	Munggu	4'54'67'86,S 112'13'8,111E	37
	Sembung	8°28'56" S 115°11'8" E	242
	Mengwi	5'75'67'877"S 311'12'852E	89
	Baha	8'75'587'87"S 311'12'7,79E	320
	Cempage	3'71'45'227"S 123'25'9,234E	39
	Sembung	8°28'56" S 115°11'8" E	242
	Anggungan	8°33'33" S 115°11'51" E	98
Bangli			
Bangli	Cempage	8'71'45'227"S 123'25'9,234E	39
		8'31'74S 115'21'30E	301
	Pengotan	8'31'83S 115'25'32E	450
	Bambang	8'31'83S 115'27'22E	341
	Bunutin	8'31'76S 115'21'29E	310
	Kayubihi	8'31'87S 115'17'34E	357
Kintamani	Buahan	8'31'87S 115'21'25E	1023
	Kedisan	8'31'87S 115'21'25E	987
	Catur	8°15'19" S 115°18'46" E	1004
	Sekardadi	8°19'49" S 115°20'43" E	974
	Bayung gede	8'31'87S 115'27'29E	987
	Songan	8°12'47" S 115°18'47" E	1330
	Songan A	8'31'90S 115'24'29E	1200
	Belantih	8'31'87S 115'27'29E	1349
Tembuku	Tembuku	8'31'87S 115'22'29E	350
		8'31'87S 115'22'29E	289
	Jehem	8'31'87S 115'23'30E	278
	Yangapi	8'31'85S 115'21'27E	342
Susut	Apuan	8'31'43S 115'21'25E	350
	Demulih	8'31'87S 115'21'25E	459
		8°30'56" S 115°20'43" E	182
	Susut	8'31'87S 115'21'27E	578
Buleleng			
Sukasada	Panca Sari	8'34'75.86"S 144'15'18,107E	1245
		8'23'45,87"S 144'05'18,97E	1245
	Asah Gobleg	8'53'75,102"S 144'05'89,105E	1346
Gerokgak	Celukan Bawang	2'80'89,239"S 142'34'12,108E	14
	Pejarakan	8'25'47,56"S 144'10'15,71E	12
Seririt	Patemon	8'23'45,78"S 144'05'10,78E	125
	Ringdikit	8°13'26" S 114°56'34" E	129
	Kalianget	8°11'28" S 114°57'37" E	10
	Bestala	8°14'52" S 114°56'46" E	265
	Gunuing Sari	8°15'25" S 114°59'34" E	345
	Kalisade	8'25'47,81"S 144'10'18,80E	87
Banjar	Cempaga	8'31'74S 115'21'30E	331
	Gobleg	8°15'41" S 115°4'6" E	939
	Banjar	8'45'101,98"S 144'32'45,94E	68
Denpasar			
Denpasar Utara	Peguyangan Kaja	8'37'47S 115'12'07E	90
Denpasar Selatan	Sidekarya	8'14'19S 115'05'02E	19
		8'14'16S 115'05'02E	18
		8°28"12 S 115°26'57" E	8
		8°28"12 S 115°26'57" E	6
	Pegok	8°28'27" S 115°23'19" E	27
		8'14'17S 115'05'02E	12
	Suwung	8°42'24" S 115°14'34" E	2
	Panjer	8'14'17S 115'05'02E	14
	Renon	8'36'35S 115'13'05E	10
	Penatih	8'37'49S 115'13'07E	80
	Sanur Kaje	8'36'38S 115'14'05E	9
Denpasar Barat	Dauh Puri	8°40'32" S 115°12'59" E	12
	Dauh Puri Kelod	8°40'32" S 115°12'59" E	18
Denpasar Timur	Sumerta	8°40'17" S 115°15'9" E	12
Gianyar			
Sukawati	Batuan	8'23'19'S 115'22'23E	97
	Ketewel	8'25'58'S 115'22'23E	25
		8°36'57" S 115°17'51" E	27
	Guwamg	8'23'45'S 115'22'19E	29
	Batuan Kaler	8'22'45'S 115'22'23E	98
	Sukawati	8'23'21'S 115'22'23E	97
	Batuan	8'23'19'S 115'22'23E	95
	Sakah	8'23'42'S 115'22'18E	92
	Singepadu Kaler	8'21'32'S 115'22'23E	101
Blahbatuh	Bona	8'23'54'S 115'22'23E	96
	Keramas	8'23'55'S 115'22'24E	81
		8°34'41" S 115°19'25" E	18
	Bon Biu	8°11'28" S 114°57'37" E	10
	Pering	8'23'55'S 115'22'23E	90
	Medahan	8'23'55'S 115'22'23E	87
Payangan	Kerta	8'20'50'S 115'22'23E	552
	Melinggih	8'20'53'S 115'22'23E	342
Tegalalang	Tegalalang	8°28'5" S 115°16'39" E	371
	Bayad	8'32'24'S 115'22'24E	359
	Sapat	8'32'24'S 115'22'30E	375
	Kendran	8'20'48'S 115'22'23E	321
	Taro Kelod	8'30'22'S 115'21'28E	531
Tampak Siring	Tampak Siring	8'32'42'S 115'22'23E	342
	Basangambu	8'32'34'S 115'22'27E	355
Ubud	Singekerta	8°31'41" S 115°14'37" E	178
	Sayan	8'22'42'S 115'19'20E	107
Gianyar	Temesi	8'18'52'S 115'22'23E	78
Jembrana			
Mendoyo	Madewi	8'10'S 114'40'E	47
	Lelateng	8'16'S 114'28'E	32
	Poh Santen	8'16S 114'41'E	112
	Gumrih	8'15'S 114'31'E	79
	Yeh Embang Kauh	8'18'S 114'27'E	90
	Dangin Tudak Aya	8'15'S 114'38'E	79
	Pergung	8'14S 114'35'E	65
		8'17'S 114'40'E	79
	Penyaringan	8'17S 114'31'E	70
		8'18'S 114'30'E	45
	Rambut Siwi	8'10'S 114'35'E	35
		8'9'S 114'40'E	4
Pekutatat	Pekutatan	8'10'S 114'23'E	69
		8'16'S 114'40'E	23
	Pangyangan	8'15'S 114'33'E	12
		8'10'S 114'27'E	12
	Gumrih	8'10'S 114'37'E	28
		8'15'S 114'31'E	78
Negara	Negara	8'18'S 114'40'E	98
	Baluk	8'18'S 114'39'E	23
	Lelateng	8'16'S 114'28'E	32
		8'10'S 114'39'E	7
Karangasem			
Manggis	Bugbug	8°29'16" S 115°35'52" E	58
		8'225S 115'439'E	34
	Manggis	8'229'S 115'451'E	5

Sidemen	Talibeng	8°29'25" S 115°26'20" E	225
		8'220'S 115'461'E	345
	Sidemen	8'229'S 115'472'E	380
Rendang	Menanga	8'312'S 115'471'E	1015
	Nongan	8°26'28" S 114°24'51" E	515
Kubu	Kubu	8'234S 115'461'E	25
	Sukadana	8'229S 115'461'E	20
Selat	Selat Duda	8'312'S 115'451'E	450
		8'234'S 115'461'E	670
Karangasem	Seraya Barat	8'227S 115'451'E	350
	Seraya	8'227S 115'452'E	346
	Bugbug	8°29'16" S 115°35'52" E	58
Bebandem	Bebandem	8'326'S 115'451'E	157
		8'326'S 115'451'E	159
Klungkung			
Banjarangkan	Desa Aan	8'18'20,S 115'09'03,E	45
		8°30'13" S 115°22'39" E	267
	Desa Getakan	8'18'21,S 115'09'03,E	54
	Takmung	8'14'18,S 115'04'02,E	29
		8°33'17" S 115°23'15" E	14
	Desa Nyanglan	8'18'21,S 115'09'034,E	102
	Tihingan	8'45'22,S 115'09'04,E	157
	Tegal Besar	8'14'14,S 115'05'02,E	9
	Nyanglan	8°28'8" S 115°23'31" E	374
	Timuhun	8°28'27" S 115°23'19" E	363
Klungkung	Takmung	8°33'41" S 115°23'13" E	15
	Klotok	8'14'17,S 115'05'02,E	9
	Manduang	8'29'47,S 115'15'07,E	98
	Gelgel	8'38'53,S 115'12'07,E	45
		8°33'49" S 115°24'42" E	11
Dawan	Dawan	8'14'18,S 115'08'02,E	38
	Gunaksa	8'14'18,S 115'07'02,E	27
		8°33'8" S 115°25'48" E	41
	Pesinggahan	8'14'20,S 115'05'02,E	10
	Kusamba	8'14'12,S 115'05'02,E	5
		8'14'57,S 115'15'09,E	7
Tabanan			
Baturiti	Bedugul	8'75'67'87"S 321'13'9,1021E	1123
	Baturiti	8'75'67'987"S 321'13'9,1032E	1068
Tabanan	Subamia	8'75'54'23"S 321'14'6,124E	83
	Tunjuk	8°28'56" S 115°9'8" E	267
	Bakisan	8°31'10" S 115°8'15" E	164
	Gubug	8°33'6" S 115°6'15" E	69
	Tajen	8°26'44" S 115°8'52" E	291
	Gubug	8°29'16" S 115°35'52" E	58
	Buahan	8'31'90S 115'25'28E	179
Kediri	Pejaten	8'75'54'37"S 321'13'6,141E	90
	Beraban	8'75'67'9965"S 321'13'9,982E	60
		8'75'67'777"S 321'13'9,432E	45
	Pandak	8'34'75,86"S 144'15'18,107E	76
	Bebalang	8'31'87S 115'17'34E	475
	Kediri	8'75'54'27"S 321'13'6,131E	85
Selemadeg Timur	Tanggun Titi	8°32'37" S 115°2'24" E	22
		8'45'56'89"S 102'9'5,90E	90

The purposive sampling method was used to collect plant leaves that showed symptoms of leafminer fly attacks (symptoms of attack are characterized by white spots and white grooves on the surface of the leaves), 50-100 leaves per location based on the presence of the host plant. Leaf samples were collected at each location and sealed in sterile 1 liter plastic bags, then labeled and transported to the laboratory. The leaf samples were then split depending on the development of the larval attack and cultivated in clear plastic containers with dimensions according to research by Yuliadhi et al. (2021), namely 10 cm high and 8 cm wide, the container was covered with gauze. The imago of *Liriomyza* spp. maintained in a controlled manner on a laboratory scale until they develop into adults.

Inventory and morphological identification of Liriomyza *spp.*

Leaf miner flies and parasitoids that developed during the rearing period were measured and documented based on their morphology before being collected separately in bottles containing 80% ethanol for further identification. Following that, each collection bottle was labeled with the location, host plant, and sampling date (Yuliadhi et al. 2021). The leaf-miner fly was identified based on morphological characteristics described by Liang et al. (2023).

Molecular identification of *Liriomyza* spp.

DNA extraction

DNA extraction was performed using the procedure described by Hamid et al. (2018) and Zhong et al. (2020). In summary, adults of *Liriomyza* spp. those found in the study area were preserved in 70% alcohol and stored in a -20°C freezer until the material was needed for isolation. Samples were isolated, collected, and dried for 30 minutes on paper towels. The larvae are then soaked in hot water at 85°C for 30 minutes until they are slightly yellowish in color. Next, the adults were excised and placed in a 1.5 mL tube. 5 µL of proteinase K was added and crushed. The crushed material was dissolved in 300 µL of TNES buffer containing 1 M Tris HCl (pH 7.5), 5 M NaCl, 0.5 M EDTA, ddH2O, and 20% SDS), homogenized and incubated at 60°C for 3 hours. After the incubation period was complete, 85 µL of 5 M NaCl was added and centrifuged for 10 minutes at 14000 rpm. The supernatant was collected in large quantities (up to 400 µL) and placed in a fresh tube with isopropanol, up to 60% of the volume of the supernatant taken. After that, put it in the freezer for 20 minutes. Centrifuge again at 14000 rpm for 5 minutes. The supernatant was removed, and 500 µL of cold 70% alcohol was added before centrifuging for 15 minutes at 14000 rpm. The supernatant was discarded once again and dried for 24 h at room temperature. After drying, 20 µL TE buffer was added (1st Base, Malaysia). Before use, the DNA suspension was stored at -20°C.

DNA amplification and sequencing

The Cytochrome C Oxidase Subunit I (COI) region was amplified using the forward primer LCO (5'-ATT CAA CCA ATC ATA AAG ATAT-3') and the reverse primer HCO (5'-TAA ACT TCT GGA TGT CCA AAAA-3'). Herlinda et al. (2022) following the amplification process. Briefly, each PCR included 5 µL PCR buffer pH 8.3 (10 Mm Tris-HCl, pH 8.3; 1.5 Mm MgCl2; and 50 Mm KCl; 0.01% NP-40), 35 mL distilled water, 200 mM dNTP, 1-unit Taq Polymerase, 0.3 M primer, and 1-4 µL DNA template. PCR is carried out in stages, namely one cycle for 1 minute at 94°C, five cycles for 1 minute at 94°C, 1.5

minutes at 45°C, 1.5 minutes at 72°C, 35 cycles for 1 minute at 94°C, 1.5 minutes at 50°C, and 1 minute at 72°C, and a final cycle of 5 minutes at 72°C. The PCR reaction was then electrophoresed on % agarose gel using 1 µL Ethidium Bromide (EtBr: 10 mg/mL/20 mL agarose) for 70 min at 55 V. A UV transilluminator was used to visualize the information (UVP, USA). The sequencing data was examined at PT. Genetika Science Indonesia.

Data analysis

Data analysis using the Bioedit Version 7.0.5.3 program, the basesequences of the two samples were evaluated in sequenceto determine the difference in the base composition of the protein. The analytical findings were then sent to the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sample similarities and potential identities. To determine genetic distance, the MEGA program version 6.06 for Windows was utilized. The COI gene phylogenetic analysis was then performed using the Maximum Likelihood technique with 1000 bootstrap replicates (Tamura - Nei model). The NCBI program (https://www.ncbi.nlm.nih.gov/) provided the reference strain utilized in this investigation.

RESULTS AND DISCUSSION

Genetic structure of *Liriomyza huidobrensis, Liriomyza sativae* and *Liriomyza trifolii in* Bali

The presence of a clear and thick DNA band as a result of the electrophoresis process can be evidence of successful amplification. Then the PCR results were sequenced to see the nucleotide structure and aligned with the *Liriomyza* sequence taken from GenBank to see the genetic similarity of each *Liriomyza* found in Field (Figure 2).

The genetic identity of a species shows the unique characteristics of each species. Genes that come from ancestors are still passed down. The greater the value of the genetic distance (p-distance) between a population or individual, the more isolated they are from each other (Shi and Mou 2016; Uffelmann et al. 2021). Genetic distance indicates the possible influence of geographic isolation on a population (Guo et al. 2023; Song et al. 2023).

From the sequencing results obtained, it was then continued with Pairwise distance calculation analysis to see the genetic relationship between *L. huidobrensis* taken from Genbank and *L. huidobrensis* specimens in Bali (Table 2). *L. huidobrensis* found in Bali shows an identity value of 1.00 to the *L. huidobrensis* sequence found in Zambia (KX373670.1), Sri Lanka (KX373669.1). Zimbabwe (KU244272.1), India_LHP1 (MW467884.1), India_LHP3 (MW467886.1), India_LHP2 (MW467885.1), South_Korea_LHS2-4 (KC136094.1), South_Korea_LHS2-2 (KC136092.1). Then, an identity value of 0.99 was shown for the *L. huidobrensis* sequences South_Korea_LHS2-4 (KC136094.1), South_Korea_LHS2-2 (KC136092.1). Meanwhile, the lowest identity values were shown in the sequences of *L. huidobrensis* Philippines (DQ150841.1) with a value of 0.46 and *L. huidobrensis* Japan (AB721350.1) with a value of 0.45.

Table 2. The genetic similarity of the *Liriomyza huidobrensis* in this study (Bali, Indonesia) and other country

Sequences	1	2	3	4	5	6	7	8	9	10
*L.huidobrensis*__Bali	ID									
*L.huidobrensis*_Zambia_KX373670.1	1.00	ID								
*L.huidobrensis*_Srilanka_KX373669.1	1.00	1.00	ID							
*L.huidobrensis*_Zimbabwe_KU244272.1	1.00	1.00	1.00	ID						
*L.huidobrensis*_India_LHP1_MW467884.1	1.00	1.00	1.00	1.00	ID					
*L.huidobrensis*_India_LHP3_MW467886.1	1.00	1.00	1.00	1.00	1.00	ID				
*L.huidobrensis*_India_LHP2_MW467885.1	1.00	1.00	1.00	1.00	1.00	1.00	ID			
*L.huidobrensis*_South_Korea_LHS2-1_KC136091.1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	ID		
*L.huidobrensis*_South_Korea_LHS2-3_KC136093.1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	ID	
*L.huidobrensis*_South_Korea_LHS2-2_KC136092.1	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	ID

Table 3. The genetic similarity of the *Liriomyza sativae* in this study (Bali, Indonesia) and other country

Sequences	1	2	3	4	5	6	7	8	9	10
*L.sativae*_Bali	ID									
*L.sativae*_India_MN525177.1	0.99	ID								
*L.sativae*_India_MN525176.1	0.99	1.00	ID							
*L.sativae*_Bangladesh_KF962579.1	0.99	0.99	0.99	ID						
*L.sativae*_Thailand_OM327488.1	0.99	1.00	1.00	1.00	ID					
*L.sativae*_China_KX373677.1	0.99	1.00	1.00	0.99	1.00	ID				
*L.sativae*_Sri_Lanka_KU244270.1	0.99	1.00	1.00	0.99	1.00	1.00	ID			
*L.sativae*_Pakistan_KY831315.1	0.98	1.00	1.00	0.99	0.99	1.00	1.00	ID		
*L.sativae*_French_Polynesia_KX053848.1	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	ID	
*L.sativae*_Hong_Kong_ON368812.1	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	ID

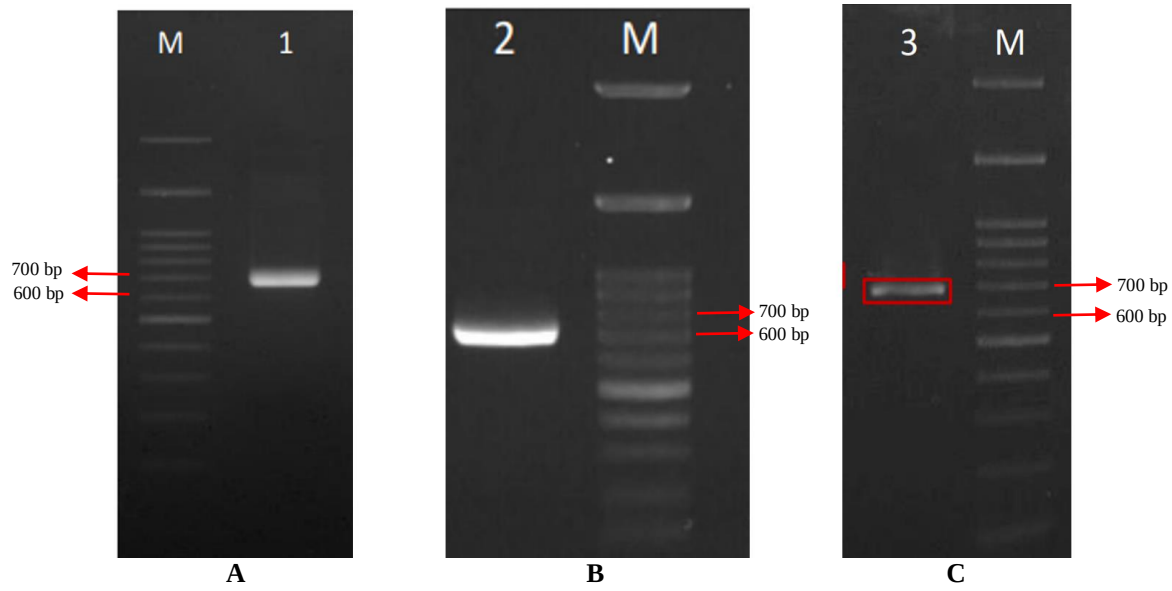

Figure 2. Results of DNA amplification by PCR method using COI mitochondrial primer pair. Sequence of: A. *Liriomyza sativae*; B. *Liriomyza huidobrensis*; C. *Liriomyza trifolii*. M: Marker

The *pairwise distance calculation* method was also carried out on *L. sativae* sequences found in Bali compared with *L. sativae* sequences taken from GenBank. The genetic similarity results of *L. sativae* in Bali showed a value of 0.99 for the *L. sativae* sequences from India (MN525177.1), India (MN525176.1), Bangladesh (KF962579.1), Thailand (OM327488.1), China (KX373677). 1), Sri Lanka (KU244270.1), French Polynesia (KX053848.1), Hong Kong (ON368812.1). Meanwhile, the Pakistan sequence (KY831315.1) shows a similarity value of 0.98. (Table 3).

The results of the pairwise distance calculation analysis on *L. trifolii* sequences in Bali compared with *L. trifolii* sequences taken from GenBank show a value of 1.00 for the *L. trifolii* sequences New_Zealand (KX373675.1), Thailand (OM327504.1), Mexico (MK111692. 1), Mexico (MK111677.1). Against *L. trifolii* Canada (MH169712.1) with a value of 0.96, South_Korea (KC136098.1) with a value of 0.88 and the Philippines (DQ150853.1) and China (DQ874615.1) with a value of 0.46 (Table 4).

Based on the analysis of the phylogenetic tree of *L. huidobrensis* specimens using the Neighbor-Joining method with Bootstrap 1000x repetitions, it shows that analysis using COI gene fragments will produce geographic kinship groupings (phylogeography). The phylogenetic tree (Figure 3) shows that the *L. huidobrensis* found in Bali is close to the Zambian *L. huidobrensis* sequence (KX373670.1) as shown by the adjacent branch, followed by the Sri Lankan sequence (KX373669.1). Zimbabwe (KU244272.1), India_LHP1 (MW467884.1), India_LHP3 (MW467886.1), India_LHP2 (MW467885.1), South_Korea_LHS2-4 (KC136094.1), South_Korea_LHS2-2 (KC136092.1) which are located in one branch . For comparison, the sequences of *L. trifolii* and *L. sativae* are presented which function as outgroups.

The description of the phylogenetic tree of *L. sativae* in Bali shows the results of the closest relationship with the sequence of *L. sativae* Bangladesh (KF962579.1) which is drawn with adjacent branch positions. Then proceed with the sequences of *L. sativae* India (MN525177.1), *L. sativae* India (MN525176.1), *L. sativae* Thailand (OM327488.1), *L. sativae* China (KX373677.1), *L. sativae* Sri Lanka (KU244270.1), *L. sativae* French Polynesia (KX053848.1), and *L. sativae* Hong Kong (ON368812.1). Also presented are *L. trifolii* sequences as an out group (Figure 4).

Based on the phylogenetic tree analysis of *L. trifolii* specimens using the Neighbor-Joining method, it is known that the *L. trifolii* specimens in Bali are close to the Thai *L. trifolii* specimens (OM327504.1) as evidenced by one branch. Then it is identical to the Mexican *L. trifolii* sequence (MK111692.1), Mexico (MK111677.1) as depicted by adjacent branches as well as to the *L. trifolii* sequences New_Zealand (KX373675.1), Canada (MH169712.1) and South_Korea (KC136098.1) in another branch. For comparison, the *L. huidobrensis* sequence is also displayed as an out group (Figure 5).

Haplotypes model *Liriomyza trifolii, Liriomyza sativae,* and *Liriomyza huidobrensis* in Bali

Based on the haplotype analysis carried out on the *L. trifolii*, *L. sativae* and *L. huidobrensis* sequences in Bali and compared with the *Liriomyza* sequences taken from Genbank, four haplotype groups were found in the *L. trifolii* sequence, five haplotype groups in the *L. sativae* sequence and one haplotype groups in the *L. huidobrensis* sequence (Figure 6). The *L. trifolii* sequence found in Bali shows genetic similarities to the *L. trifolii* sequence found in Thailand which is indicated by one haplotype group, whereas there is one base difference in the sequences found in New Zealand and Mexico, then there are twenty-three different bases in the sequences found in Bali. *L. trifolii* sequences in Bali were compared with *L. trifolii* sequences that had been found in Canada. In the *L. sativae* Bali

sequence, one different haplotype was found to all *L. sativae* sequences taken from Genbank, with a difference level of four bases to the Bangladesh *L. sativae* sequence, six different bases to the *L. sativae* Thailand sequence, eight different bases to the haplotype group *L. sativae* consists of sequences from India, French Polynesia, Hong Kong, Sri Lanka and China and there are nine different bases to the *L. sativae* sequence from Pakistan.

Table 4. The genetic similarity of the *Liriomyza trifolii* in this study (Bali, Indonesia) and other country

Sequences	1	2	3	4	5	6	7
*Liriomyza_trifolii*_Bali	ID						
*Liriomyza_trifolii*_New_Zealand_KX373675.1	1.00	ID					
*Liriomyza_trifolii*_Thailand_OM327504.1	1.00	1.00	ID				
*Liriomyza_trifolii*_Mexico_MK111692.1	1.00	0.99	1.00	ID			
*Liriomyza_trifolii*_Mexico_MK111677.1	1.00	0.99	1.00	1.00	ID		
*Liriomyza_trifolii*_South_Korea_KC136098.1	0.88	0.88	0.88	0.88	0.88	ID	
*Liriomyza_trifolii*_Canada_MH169712.1	0.96	0.96	0.96	0.96	0.96	0.89	ID

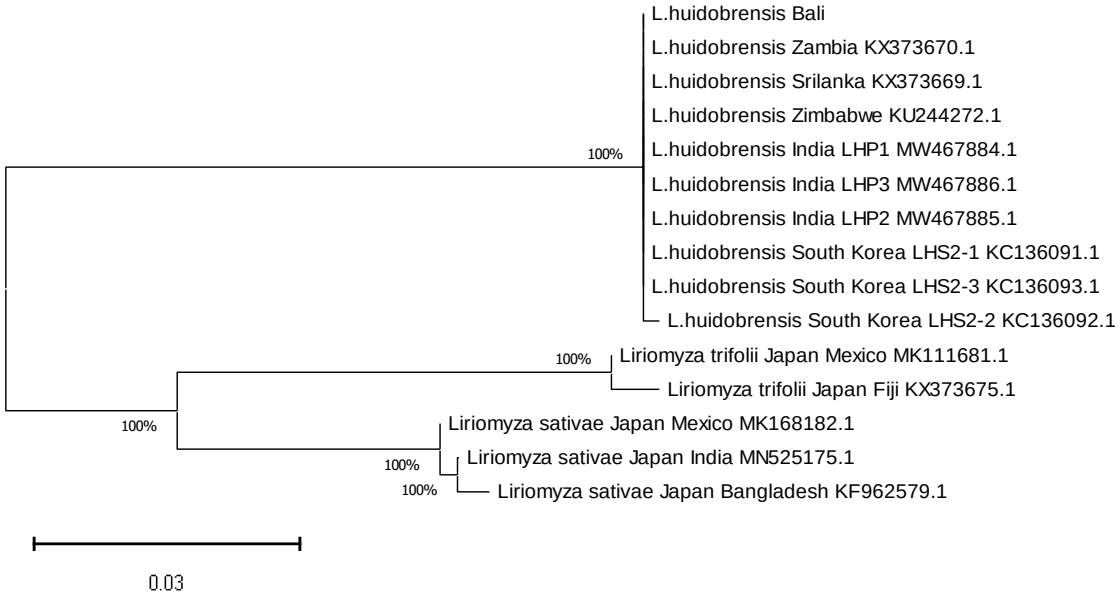

Figure 3. Phylogenetic tree of *Liriomyza huidobrensis*

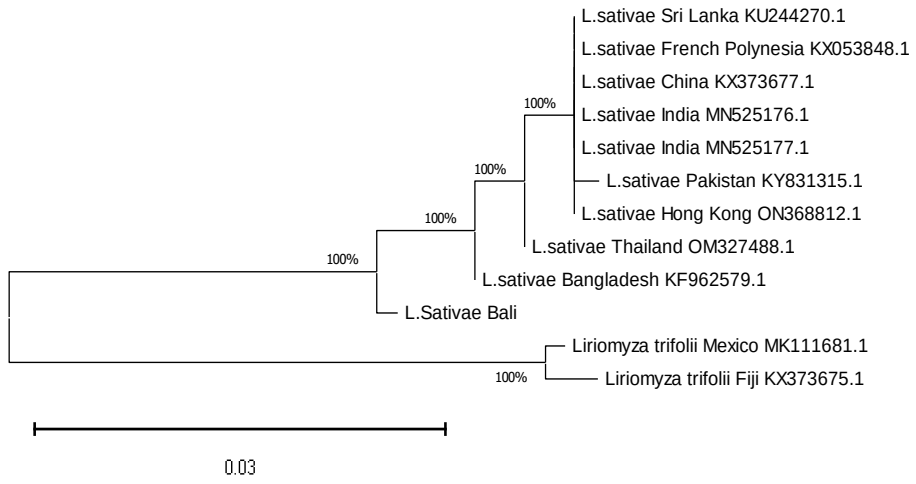

Figure 4. Phylogenetic tree of *Liriomyza sativae*

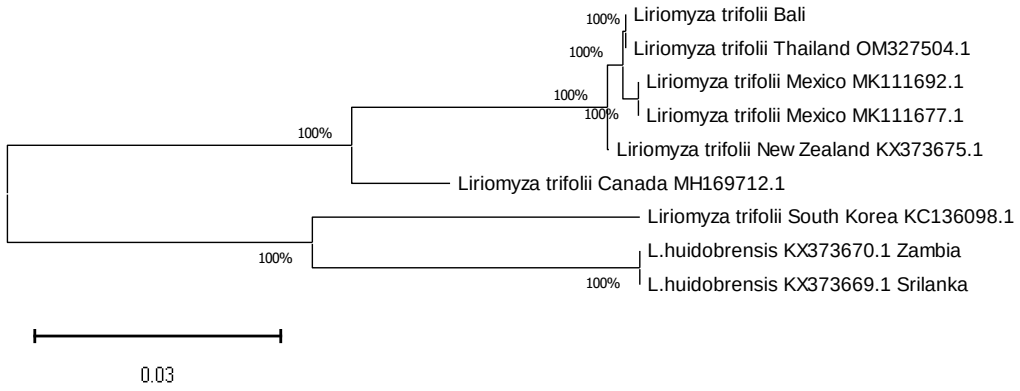

Figure 5. Phylogenetic tree of *Liriomyza trifolii*

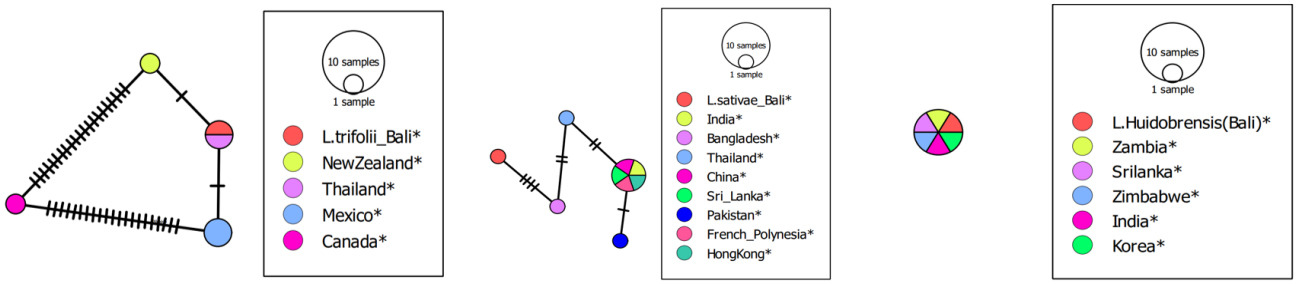

Figure 6. Haplotype model of *Liriomyza trifolii*, *Liriomyza sativae*, and *Liriomyza huidobrensis* on several host plants in Bali Province, Indonesia

Population structure and genetic diversity are important factors influencing the survival and adaptability of invasive species (Wang et al. 2017; Vera-Escalona and Brante 2024). The results of genetic differentiation research carried out through searches using haplotype networks show that there are genetic variations between the species *L. sativae*, *L. trifolii* and *L. huidobrensis* found in vegetable plants. The existence of these variations is strengthened by variations that occur in nucleotide bases caused by mutations. This mutation is the main cause of differences in nucleotide base variations in the COI gene (Matumba et al. 2020; Petit-Marty et al. 2020). Small variations can affect the identity of a species and can even affect the sequence of amino acids that code for proteins (Vihinen 2021).

In this study, analysis of phylogenetic trees, haplotype networks and pairwise structures showed that the degree of differentiation and direction of nuclear and mitochondrial genes were not completely consistent. COI in five *Liriomyza* species showed very conservative characteristics, but the mutation rate was higher, and the results of the phylogenetic tree showed that the haplotypes of *L. trifolii*, *L. sativae* and *L. huidobrensis* could not be distinguished (Chen et al. 2019). The results of the Alignment analysis show that the genetic distance between the three *Liriomyza* species found in vegetable plants in Bali is much longer. This means that the three *Liriomyza* species show high levels of genetic differentiation in mitochondrial and nuclear genes, as well as interspecies differentiation in nuclear genes. Genetic variation can give insects the ability to adapt quickly to new environments and contribute to their dispersal capacity (Webster et al. 2023).

Spatial distribution of populations of invasive species of leafminer flies (Agromyzidae) on vegetable plants in Bali

The results of observations of the spatial distribution of the leafminer fly species *Liriomyza huidobrensis*, *Liriomyza sativae*, and *Liriomyza trifolii* are presented in Figure 7. The distribution of *L. huidobrensis*, *L. sativae*, and *L. trifolii* in Bali province in each district has a varied distribution. The spatial distribution of *L. huidoberensis* is limited to highland areas which include Buleleng, Bangli, Badung, Tabanan and Karangasem Districts with population abundance varying between 1-58 individuals. While the spatial distribution of *L. sativae* has a varied distribution, it is spread evenly throughout all districts/cities, including at different altitudes, namely from the lowlands to the highlands in the province of Bali, with a population abundance ranging from 1-3492 individuals. In contrast to the spatial distribution of the *L. trifolii* species, which is relatively new to the province of Bali, it has spread widely throughout all districts/cities in Bali Province, with a population abundance ranging from 1-778 individuals (Figure 7).

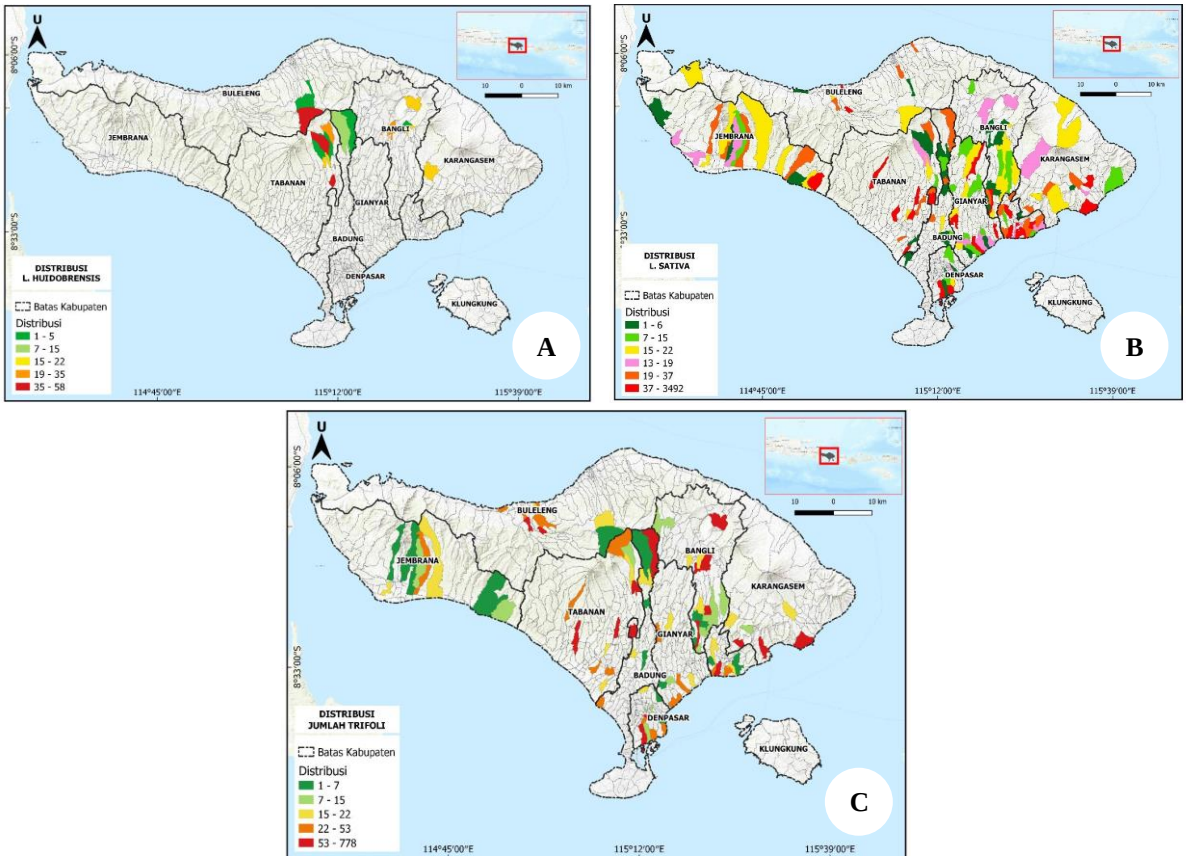

Figure 7. Spatial distribution of leafminer species in Bali, Indonesia. A. *Liriomyza huidobrensis*; B. *Liriomyza sativae*; C. *Liriomyza trifolii*

Table 5. Distribution pattern of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* in Bali, Indonesia

Species	District								
	Badung	**Bangli**	**Buleleng**	**Denpasar**	**Gianyar**	**Jembrana**	**Klungkung**	**Karangasem**	**Tabanan**
L. trifolii	0.5	0.5	0.5	0.05	0.05	0.05	0.05	0.5	0.5
L. huidobrensis	1.8	1.8	1.8	0	0	0	0	1.8	1.8
L. sativae	0.5	0.5	0.5	0.05	0.05	0.05	0.05	0.5	0.5

Distribution pattern of *L. huidobrensis*, *L. sativae* and *L. trifolii* have diverse distribution areas, *L. huidobrensis* is only found in the highlands while *L. sativae* and *L. trifolii* are found at all altitudes in the province of Bali. From the research results, the population distribution patterns of *L. huidobrensis*, *L. sativae* and *L. trifolii* show different distribution patterns, in the districts of Badung, Bangli, Buleleng, Karangasem and Tabanan, the distribution patterns of *L. trifolii* are uniform (uniform) with values S2 /X <1, while the distribution pattern of *L. huidobrensis* has a clustered distribution pattern with a value of S2 /. The distribution pattern of *L. trifolii* and *L. sativae* in the cities of Denpasar, Gianyar, Jembrana and Klungkung has a uniform distribution pattern with S2 /X values <1 (Table 5).

The distribution of *L. huidobrensis*, *L. sativae* and *L. trifolii* is strongly influenced by the presence of host plants. Distribution of *Liriomyza* spp. depending on the availability of host plants that act as sources of nutrients in a particular region (Rodríguez-Castañeda et al. 2017). As a result, populations of *Liriomyza* spp. will grow progressively in geographical areas with a wider range of host plants and adapt to the presence of minimal natural enemies (Xu et al. 2021). The temperature difference factor is one of the most significant environmental elements that influences insect distribution and is related to altitude (Capinha et al. 2014; Supartha et al. 2023). The microclimatic conditions experienced by leafminers in plants are important to consider regarding the risk of invasion by leafminers (Rodríguez-Castañeda et al. 2017; Kirichenko et al. 2018; Dantas et al. 2021).

ACKNOWLEDGEMENTS

The author thanks the Head of IPMLab, Faculty of Agriculture, Universitas Udayana, Bali, Indonesia who has supported this research by helping to facilitate and supervise this project. The authors also thank Sandikayaa and Mahendra Putra for their assistance in the sampling

and data analysis stages. The authors thank Universitas Udayana for funding support under the *Penelitian Hibah Kerjasama Dalam Negeri* (DIKTI) with contract number B/603-10/UN14.4.A/PT.01. 03/2023. The authors also thank the head of IPMLab, Faculty of Agriculture, Universitas Udayana, who has facilitated this research.

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