

# Eggplant solanum melongena has been cultivated biology essay

[Science](#), [Biology](#)



## Abstract

Eggplant (*Solanum melongena*) is an economically important vegetable crop in many countries in South and Southeast Asia. Eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis* Guenée, has been reported as the most detrimental pest of eggplant in South and Southeast Asia. Phylogeographical patterns of contemporary genetic variation in *L. orbonalis* is essential for proposing successful integrated pest management (IPM) strategy for this insect pest. In this study, population genetic diversity and structure, and geographic distributions of this species were examined using the partial mitochondrial cytochrome c oxidase subunit I (COI) DNA sequences. Examination of eight populations of *L. orbonalis* from six South and Southeast Asian countries revealed 25 haplotypes. No significant correlation between genetic diversity and geographic distance was detected among populations from distinct geographical locations. Relatively low levels of haplotype diversity ( $h = 0.200$ ) and nucleotide diversity ( $\pi = 0.00034$ ) were observed in the Philippines population, suggesting a recent colonization. Gene flow analysis showed that there had been no significant gene flow among local populations in different countries, and that the Vietnam population is highly differentiated from all the other populations ( $F_{ST} = 0.87278-0.96837$ ) and may be ascribed to a new subspecies or race of *L. orbonalis*. Moreover, India was revealed to be the source of genetic variation in *L. orbonalis* populations. Our study showed that *L. orbonalis* formed subpopulations for each local region, and the corresponding IPM technology should be developed at the country scale.

## Introduction

Eggplant (*Solanum melongena*) has been cultivated for the last 4000 years, and is a common and popular vegetable crop grown in tropical and subtropical zones (Owusu, 1980). The place of origin of the eggplant is probably India, with the secondary center of diversity in China. It is commonly cultivated in southern Asia, Malay Peninsula, Indonesia, tropical Africa, and South America (Srinivasan, 2009). This crop is currently cultivated on more than 1.81 million ha worldwide with a production of 46.69 million t; of which, Asia accounts for 1.69 million ha with a production of 44.16 million t (FAO, 2011). Eggplant is naturally low in calories, but contains dietary fiber, folate, potassium and phosphorus (Chen & Li, 1996). The nutrients that it contributes to the diets of the poor are especially important during times when other vegetables are in short supply (Srinivasan, 2009). Eggplant is practically the only vegetable that is available at an affordable price for the rural and urban poor (Alam et al., 2003). Among the plethora of arthropod pests that damage eggplant in Asia and Africa, eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée (Lepidoptera: Pyralidae) has been considered as the most destructive pest, especially in South and Southeast Asia (Chen et al., 2001). *L. orbonalis* larvae are mostly monophagous, feeding mainly on eggplant. During this developmental stage, they cause severe damage to their host plants. The caterpillars bore into the nearest tender shoot, flower or fruit within one hour after hatching. Soon after boring into the plant, they plug the entrance hole with excreta and remain concealed inside that particular plant part. Other vegetable crops belonging to the family Solanaceae, including black

nightshade (*Solanum nigrum*), Turkey berry (*S. torvum*), potato (*S. tuberosum*), Indian nightshade (*S. aculeatissimum*), and tomato (*Solanum lycopersicum*) are also reported to be hosts of *L. orbonalis* (AVRDC, 1990). Larval feeding inside shoots results in progressive wilting of the young shoot, a characteristic symptom called "dead-heart." Larvae feeding inside the fruit prevents the fruit from being marketable and leads to economic yield loss (Alam & Britain, 2006). *L. orbonalis* has been reported to reduce yield in eggplant by as much as 90% in India (Kalloo, 1988) and comparable losses were also documented in Bangladesh (Ali et al., 1980). The pest management tactics targeting *L. orbonalis* are limited only to spraying of chemical insecticides, giving rise to the over-application and abuse of pesticides in order to increase the proportion of marketable eggplant fruits. For example, farmers in certain areas of the Philippines spray chemical insecticides up to 56 times for a seasonal crop; the total quantity of pesticide used per ha of eggplant was about 41 liters (Gapud & Canapi, 1994; Orden et al., 1994). Such insecticide use not only pollutes the environment but is also detrimental to human health, not to mention the increased cost of production that makes the vegetable more expensive for the poor consumers. Integrated pest management (IPM) practices are crucial for effective and sustainable control of *L. orbonalis* (Srinivasan, 2008). Development and application of IPM requires a thorough understanding of the phylogeography of the target pest species as the population structure and dynamics usually varies from region to region. Although *L. orbonalis* is an agriculturally and economically important insect pest, there has been no previous study investigating the molecular population structure and the

biogeographical history of this species. Phylogeographical patterns of contemporary genetic variation in organisms have shown to be remarkably useful in revealing their migration histories and demographic records (Avisé, 2000; Hewitt, 2000). Nevertheless, lack of *L. orbonalis* population diversity data, including DNA sequences, molecular markers or information on the population structure, hinders the development of IPM for managing this pest. In the current study, our aim was to examine the partial mitochondrial cytochrome c oxidase subunit I (COI) DNA sequences to better understand phylogeographical relationships among the *L. orbonalis* populations predominantly occurring in major eggplant growing regions of South and Southeast Asia and to aid our knowledge of factors shaping the distribution of this species. This research addressed whether there is geographical variation in *L. orbonalis* in South and Southeast Asia and unmasked their genetic structure.

## **Materials and methods**

**Sample collection** A total of 74 *Leucinodes orbonalis* larvae were collected on eggplants from India, Thailand, Lao PDR, Vietnam, Taiwan, and the Philippines. The exact locations, their geographical coordinates and the time of collection are given in Table 1. The gathered samples were stored in 95% ethanol and shipped to AVRDC headquarters in Taiwan. Genomic DNA isolation, polymerase chain reaction (PCR), DNA sequencing After removing the larvae from the 95% ethanol, they were washed with double distilled water. The head was decapitated and used for the DNA extraction using the Easy DNA High-speed Extraction Tissue Kit (Saturn Biotech, Taiwan) following

the manufacturer's instruction. The extracted DNA was stored at  $-20^{\circ}\text{C}$  for subsequent PCR. Oligonucleotide primers EBCOI-F 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' and EBCOI-R 5'-GGTCAACAAATCATAAAGATATTGG-3' were used for PCR amplification of the mitochondrial COI DNA barcode region. PCR reactions were performed in 25  $\mu\text{l}$  reaction volumes that contained 250–750 ng of template DNA, 2.5 pmol of each primer, 1X PCR buffer, 0.04 unit/ $\mu\text{l}$  of Super-Therm Gold DNA Polymerase (Bertec Enterprise, Taipei, Taiwan), 0.2 mM dNTP, and 2.5 mM  $\text{MgCl}_2$ . Thermal cycling was carried out on a MJ Research PTC-200 Thermal Cycler (Watertown, MA, USA), and performed using a  $95^{\circ}\text{C}$  denaturation step for 10 min, followed by 35 cycles of  $95^{\circ}\text{C}$  for 30 s,  $55^{\circ}\text{C}$  for 45 s,  $72^{\circ}\text{C}$  for 1 min and a final extension of  $72^{\circ}\text{C}$  for 8 min. A 1  $\mu\text{l}$  aliquot of PCR product was separated on a 1.5% agarose gel in 1X TBE at 100 V for 1 h and 20 min followed by ethidium bromide staining, and data was acquired on an AlphaDigiDoc gel documentation system (Bio-Rad, Hercules, CA, USA). The remaining PCR product was sent to Genomics BioSci & Tech (Taipei, Taiwan) for purification and sequencing. Molecular divergence and population genetic analysesThe obtained raw sequence data were edited and assembled into contigs with ContigExpress of the Vector NTI Advance® program (Invitrogen, Carlsbad, CA). The number of variable nucleotide sites, number of haplotypes, nucleotide diversity, and haplotype diversity were calculated for investigating COI sequence diversity using the software package DnaSP 5.10 (Librado & Rozas, 2009). Tajima's D and Fu's FS values were used to study population demographic history and evolutionary neutrality of *L. orbonalis*, implemented by DnaSP 5.10 considering only the number of

segregating sites with the significance level evaluated using the coalescent algorithm with 1, 000 replicates. Pairwise  $F_{ST}$  values used to appraise the genetic structure among populations were obtained with 1, 000 permutations and a significance level of 0. 05 using the Kimura 2-parameter (K2P) model (Kimura, 1980). The geographical structure among populations was examined using a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) with 1, 000 permutations and the K2P model, in which populations were grouped according to their localities. The tests described above were both conducted by Arlequin 3. 5 (Excoffier & Lischer, 2010). A Mantel test (Mantel, 1967) was performed with the web tool IBD 3. 23 (Jensen et al., 2005) to examine the correlations between genetic differentiation and geographic distance. The distances between populations were measured using the shortest straight distance from one population to another based on their longitudes and latitudes. Phylogenetic and network analyses Nucleic acid sequences of COI were aligned using ClustalX2 (Thompson et al., 1997). The alignment result was analyzed with the neighbor-joining method using a K2P distance matrix with MEGA5 (Tamura et al., 2011). The clustering probabilities of each resulting phylogenetic tree node were statistically tested by bootstrap method of 1, 000 replicates. Genealogical relationships among *L. orbonalis* COI sequences were examined by establishing a median-joining haplotype network with the software Network 4. 6 (Fluxus Technology Ltd, UK) using an epsilon value of 10 and maximum parsimony post-processing that removed superfluous nodes and links (Polzin & Daneshmand, 2003).

## Results

COI variation The length of the amplified partial COI gene from 74 individuals of *L. orbonalis* was approximately 700 bp. The sequence data obtained in this study were submitted to the NCBI GenBank, and the accession numbers ranged from JN580318 to JN580392. Analysis of the average nucleotide compositions in the *L. orbonalis* COI gene fragments showed a high A+T content (71.3%) and a low content of G+C (28.7%), which is consistent with the AT-rich nature of this gene previously reported in other insects (Simons et al., 1994). A total of 43 polymorphic sites were identified from the COI sequences, 33 of which were parsimony-informative and 10 of which were singleton. No insertions or deletions were found in the sequences. The total nucleotide diversity of samples from all *L. orbonalis* populations was 0.01183, varying from 0.00034 (PH) to 0.00932 (KA) (Table 2). A total of 25 haplotypes were identified within *L. orbonalis*, most of which were not shared among different countries. The number of haplotypes observed in each population ranged from six in Jharkhand (India) and Vietnam to two in the Philippines. The values of haplotype diversities were from 0.200 (PH) to 0.952 (VI), with the total haplotype diversity 0.85820 (Table 2). The lowest haplotype diversity and nucleotide diversity were both occurred in the Philippines. In contrast, samples from Karnataka (India) were found to have the highest nucleotide diversity and the second highest haplotype diversity of 0.844. Tajima's D test showed positive values, except in Jharkhand (India), Vietnam, and the Phillipines populations, and all of these values were non-significant. Similarly, apart from population JH, VI and PH, positive values were observed from Fu's FS test without significance. Population genetic



diversity The  $F_{ST}$  values produced from all population pairwise comparisons ranged from  $-0.08984$  to  $0.96837$  (Table 3). Negative  $F_{ST}$  values are likely an artifact of the statistical software and can be interpreted to mean that population structure is too small to be detectable. The Vietnam population was highly significantly differentiated from all the other populations based on pairwise  $F_{ST}$  values ( $0.87278-0.96837$ ;  $P < 0.01$ ). The lowest  $F_{ST}$  value was calculated between the TH and LA populations in the Indo-China Peninsula. Low  $F_{ST}$  values were also observed between populations from Tamil Nadu, Karnataka, and Jharkhand in India ( $-0.04673$  to  $0.05151$ ). AMOVA analysis was performed with the populations grouped by geographical distributions, i. e., the samples from the same country as a single group (Table 4). The results showed a significant level of genetic structure among countries ( $P < 0.05$ ) with the main contribution to total genetic variation (67.15%). The lack of distinct geographic structure of subregions in India was further supported by AMOVA with no significant difference among populations within countries. The Mantel test examining whether the pairwise  $F_{ST}$  matrix correlates with the pairwise geographic distance matrix detected no significant correlation among populations ( $r = -0.0410$ ;  $P = 0.6010$ ; Fig. 1). Phylogenetic pattern The intraspecific phylogenetic relationships based on the COI sequences of *L. orbonalis* are shown in Fig. 2. According to the phylogenetic tree, the *L. orbonalis* populations from selected countries in South and Southeast Asia were categorized into four groups. Group I included all Philippine samples, most Lao PDR, Thailand, and Jharkhand (India) samples, and part of the Taiwan samples. Group II was mainly comprised of Taiwan and Thailand samples,

along with six samples of all the three states from India, while the remnant 17 samples from Tamil Nadu, Karnataka and Jharkhand states in India together formed three subgroups of group III. The Vietnam population had been exclusively clustered under group IV and differed from all the other populations. The network analyses generated three distantly connected haplotype networks that were generally consistent with the constructed phylogenetic tree (Fig. 3). Six nucleotide substitutions separated network A from network B, whereas there were over 18 base changes between haplotypes in network A and C. Of the 25 revealed haplotypes, 17 were singletons (4–10, 12, 15, 17–19 and 21–25), four were shared between individuals within the same population (13, 14, 16 and 20), and the other four haplotypes were dispersed among distinct populations (1–3 and 11). Haplotype 3 was the most common haplotype in the present study, containing 24 individuals across five populations. Haplotype 2 and 11 were present solely within Indian populations. All haplotypes from Vietnam (20–25) formed a unique network C. Moreover, six potential median vectors were constructed within the network with maximum parsimony.

## Discussion

Genetic structureThe haplotype diversity and nucleotide diversity indices provide information about the haplotype uniqueness and population structure in *L. orbonalis* (Nei, 1987; Nei & Li, 1979). Among the eight populations examined, PH revealed both low haplotype and nucleotide diversities ( $h = 0.2$ ;  $\pi = 0.00034$ ), which suggests a recent colonization or founder effect and supports previous research that *L. orbonalis* was

established in the Philippines relatively recently (Navasero, 1983). The low nucleotide diversity ( $\pi < 0.005$ ) and high haplotype diversity ( $h > 0.5$ ) observed in JH and VI may represent the expansion of populations after population bottlenecks or founder events, after which nucleotide mutations were rapidly accumulated (Avice, 2000). This finding was further supported by Tajima's D and Fu's FS tests with negative values in PH, JH and VI (Table 2), showing these populations experienced demographic expansion events (Fu, 1997; Tajima, 1989); however, such population growth could have been confined to separate local regions and thereby statistically non-significant numbers were observed (Liao et al., 2010). The other five populations, TN, KA, TH, LA and TW, with both high haplotype and nucleotide diversities could be the result of a large stable population with long evolutionary history (Grant & Bowen, 1998). Of the 25 haplotypes identified, only haplotype 1 and 3 were shared among countries, suggesting independent histories of dispersal and gene flow (Conn et al., 1993; Conn et al., 1998; Fairley et al., 2000). AMOVA analysis revealed that 67.15% of the genetic variation was contributed by differences among countries, indicating that each country formed a unique genetic group (Table 4). Additionally, there was no significant correlation between the genetic distances and geographic distances based on the Mantel test (Fig. 1), suggesting that the isolation by distance (IBD) model cannot explain the differences among populations. However, it should not be excluded that, because the adult *L. orbonalis* are comparatively small and only fly for short distances, IBD may be inapplicable to such low-vagility species (Darling et al., 2004; Miller, 1997; Olson et al., 2009; Peterson & Denno, 1998). Other factors may be also responsible for

shaping the current population genetic structure of *L. orbonalis*. Overall, the evidence indicates that *L. orbonalis* adapted to its local environment rapidly and diversified to different populations independently, so pest control aimed at *L. orbonalis* may have to be developed at the country scale, rather than continental or super-regional scale. Phylogeographical history

The phylogenetic construction, including the COI nucleotide sequences of two larvae of *Dichocrocis* spp. that morphologically resemble *L. orbonalis* as the outgroup, was carried out in the present study (Fig. 2). All the samples from Vietnam formed a monophyletic clade (group IV), which supported the finding that the VI population shares little or no genetic diversity with other populations based on pairwise  $F_{ST}$  values. The majority of Indian samples (82%) are paraphyletic and were placed in the basal groups (III. 1, III. 2 and III. 3), raising the likelihood that most extant populations descend from ancestral populations in India. This assumption is further strengthened by the fact that the Indian populations all had high gene haplotype diversity, and is congruent with the idea that India is the most probable region of origin for *L. orbonalis* (Waterhouse, 1998). It is noteworthy that six Indian samples formed a monophyletic group II with samples from Thailand, Lao PDR and Taiwan, whereas five Tamil Nadu samples were discretely distributed in group I, suggesting that *L. orbonalis* may have spread from India to Southeast Asia via more than one migration route. The haplotype network divided the populations of *L. orbonalis* into three clades that were basically congruent with the topology of the phylogenetic tree, indicating distribution of genetic variation due to geographical separation. The star-like pattern of the Indian populations in network A may infer the potential

geographical origin of *L. orbonalis* populations, which coincides with the observation from the phylogenetic tree. The separation of network A and B matches the grouping structure of the phylogram (group I plus III and group II, respectively), but the mechanism is unclear. It is probable that physical barriers separated the original populations followed by secondary contact between diverging populations, yet the genetic differences were preserved. In network C composed of Vietnam populations, five out of six haplotypes were not shared between populations. The pattern might be explained by specific habitat requirements or mating preferences that segregated populations (Collin & Fumagalli, 2011; Rice, 1987; Via, 1999). These specific behavioral patterns may thus keep groups within Vietnam from interbreeding, and also keep Vietnam populations from breeding with populations in other countries (Zhang et al., 2010). Phylogeographical implications Phylogeographical patterns are widely recognized as a tool for investigating the origin location and the dispersal direction of a species (Zhang et al., 2010). In this study, India was identified as the source of genetic variation in *L. orbonalis* populations. Insect pest populations are likely to emerge and adapt to the environments where host plants were cultivated. Hence, as it is certain that eggplant has originated in India and spread to other countries in Southeast Asia (Chen, 1997; Doganlar et al., 2002; Vavilov, 1935; Wang et al., 2008), *L. orbonalis* may have similar evolutionary history: arising in India and spread to contiguous regions such as Thailand, Lao PDR, and Taiwan, forming local populations. Although no significant correlation between genetic differentiation and geographic distances was detected, given the poor dispersal abilities of *L. orbonalis* and

taking into account that its primary host plant, eggplant, is an economically important and very popular vegetable crop in South and Southeast Asia, it is plausible that anthropogenic activities (e. g., unintended introduction through agricultural or other means of transportation) play a critical role in shaping the population structure of this species. Additional research focusing on *L. orbonalis* populations in China and the African continent is required to validate this hypothesis. The VI population is unique in differing enormously in genetic structure and phylogenetic relationship from all the other *L. orbonalis* examined. It is well known that subspecies must be sharply genetically differentiated and that  $F_{ST}$  must be at least 0.25–0.30 for subspecies, or races, to be recognized (Srinivasan, 2008). In the current study, pairwise  $F_{ST}$  values between VI and other populations are greater than 0.87 and demonstrate a highly significant difference. As insect samples from Vietnam are morphologically identical to *L. orbonalis*, we are of the opinion that the VI population might be a new subspecies or race. This discovery needs additional evidence from taxonomic and cross-mating for support from future studies. Distinguished from other sampled locations, the insular nature of the Philippines should prevent crops on the islands from frequent and rapid insect pest introgression. Our results suggest that the PH population represents a recent colonization event and we deduce that the potential factor responsible for to the introduction of the *L. orbonalis* population could be direct dispersal via human transportation (Alvarez et al., 2007; Castoe et al., 2007; Li et al., 2011). This anthropogenic effect on dispersal might explain the phylogeographical pattern of the PH population. For example, in 1977–1987, U. S. Customs ports of entry had reported 1291

cases of *L. orbonalis* interception, most of which are on eggplants in passengers' luggage. (Whittle & Ferguson, 1987), and such issues should be recognized, prevented, and treated in appropriate IPM packages. The importance of insect pest diversity has been commonly recognized but no effort has been made to establish the evolutionary basis for *L. orbonalis*. Gaining insight into *L. orbonalis* phylogeography, including the gene flow patterns, the geographical origin and distribution of populations, and the extant genetic structure is essential for proposing successful IPM for this pest. It should be noted that this study has examined only small-sized samples, and selected populations may not be representative of the true distribution of *L. orbonalis* in South and Southeast Asia. In addition to mitochondrial COI gene data, nuclear DNA markers such as single nucleotide polymorphisms (SNPs) or simple sequence repeats (SSR) may provide more comprehensive information and should be examined in order to obtain a deeper understanding of phylogeographical patterns of *L. orbonalis*.

## **Acknowledgements**

The authors are grateful to Fu-Cheng Su, Mei-Ying Lin, Yun-Che Hsu, Yuan Liang, Li-Hua Yang, Chun-Chu Huang, Ruey-Chuan Hu, and Malini Periasamy who worked on this project, and for providing the materials necessary for the study. We especially thank Kun-Chan Tsai for his valuable comments and suggestions on several population genetic analyses. This study received financial support from the Federal Ministry for Economic Cooperation and Development, Germany through an internship to Mr. Daniel Ponnath.

## Tables

Table 1. Time and location with latitude and longitude of sample collection.

**Country**

**City**

**Latitude**

**Longitude**

**Time of collection**

India	Namakkal	11° 29' N	78° 02' E	February 2011	
India	Bangalore	12° 53' N	77° 35' E	May 2011	
India	Ranchi	23° 20' N	85° 18' E	January 2011	
Lao	PDR	Vientiane	17° 57' N	102° 36' E	November 2010
Philippines	Quezon City	14° 38' N	121° 30' E	March 2011	
Taiwan	Tainan	23° 60' N	120° 17' E	July 2011	
Thailand	Nakhon Pathom	13° 53' N	100° 01' E	March 2011	
Vietnam	Hanoi	14° 23' N	109° 20' E	July 2011	

Table 2. List of number of samples studied, number of haplotypes, haplotype diversity (h), nucleotide diversity ( $\pi$ ), Tajima's D and Fu's FS tests for eight *Leucinodes orbonalis* populations from six countries in South and Southeast Asia.



**Region**

**subregion**

**Code**

**No. of**

**samples**

**No. of**

**haplotypes**

**Haplotype**

**diversity (h)**

**Nucleotide**

**diversity ( $\pi$ )**

**Tajima's D**

**Fu's FS**

**India**

Tamil Nadu TN940. 8060. 007020. 332142. 217 Karnataka KA1050. 8440.  
009320. 355141. 925 Jharkhand JH960. 8330. 00497-1. 51869-1. 162

**Thailand**

TH1030. 7780. 007461. 335602. 731

**Lao PDR**

LA950. 8330. 007530. 590190. 987

**Vietnam**

VI760. 9520. 00408-0. 14112-2. 808

**Taiwan**

TW1030. 6000. 006741. 310264. 161

**Philippines**

PH1020. 2000. 00034-1. 11173-0. 339

**Total**

74250. 8580. 01183-0. 70527-3. 980

Table 3. Pairwise FST values (below diagonal) and distance matrix (above diagonal; in the unit of kilometer) comparing eight populations of *Leucinodes orbonalis*.

**Population****TN****KA****JH****TH****LA****VI****TW****PH****TN**

163152623992735340546654715

**KA**

-0.04673141924282738343246514740

**JH**

0.051510.0372118711895271335543916

**TH**

0.178670.109700.11244530100624062316

**LA**

0.14452\*0.088520.05136-0.0898482119532050

**VI**

0.89822\*\*0.87278\*\*0.92398\*\*0.90581\*\*0.89999\*\*15691310

**TW**

0.30032\*0.21266\*0.30745\*-0.029440.025020.90545\*\*1049

**PH**

0.29129\*\*0.22591\*\*0.016760.293740.20158\*0.96837\*\*0.50975\*\* FST values were significant at  $P < 0.05$ ; \*\* highly significant at  $P < 0.01$ . Table 4. Result of AMOVA analysis of eight *Leucinodes orbonalis* populations grouped into six countries based on COI sequence data.

**Source of  
variation**

**df**

**Sum of  
squares**

**Variance**

**components**

**Percentage of**

**variation**

**Fixation**

**indices**

Among countries 5231. 5173. 80977\*67. 15  $\Phi$ CT = 0. 67154 Among

populations within countries 24. 8540. 067731. 19  $\Phi$ SC = 0. 03635 Within

populations 66118. 5171. 79571\*\*31. 65  $\Phi$ ST = 0. 68348 Total 73354. 8885.

67321\* significant at  $P < 0. 05$ ; \*\* highly significant at  $P < 0. 01$ .

## Figure Legends

**Fig. 1.** Comparison of the scatter plot was made between geographic distance (km) and pairwise  $F_{ST}$  values from Table 3.

**Fig. 2.** Neighbor-joining tree inferred the evolutionary relationships of 74 COI mtDNA sequences in *Leucinodes orbonalis* isolated from distinct geographical locations, plus two outgroup *Dichocrocis* spp. The percentages of bootstrap support above 50% are shown next to the branches. Four defined groups were labeled in roman numerals.

**Fig. 3.** The haplotype network of *Leucinodes orbonali* was made using the median-joining method. Each circle represents a single haplotype and the size is proportional to the corresponding haplotype frequency. The length of the connected line between two haplotypes is proportional to the number of nucleotide substitutions. Empty circles indicate unsampled or extinct intermediate haplotypes. Abbreviations of locations can be found in Table 2.