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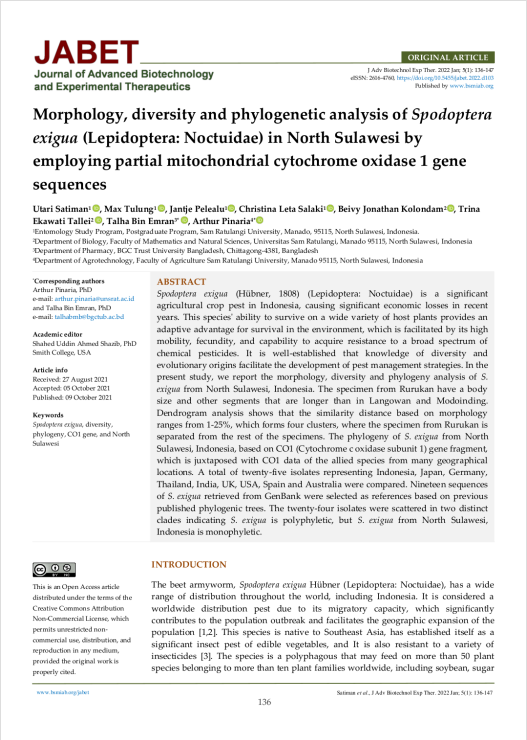

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Morphology, diversity and phylogenetic analysis of *Spodoptera exigua* (Lepidoptera: Noctuidae) in North Sulawesi by employing partial mitochondrial cytochrome oxidase 1 gene sequences

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ABSTRACT

Spodoptera exigua (Hübner, 1808) (Lepidoptera: Noctuidae) is a significant agricultural crop pest in Indonesia, causing significant economic losses in recent years. This species' ability to survive on a wide variety of host plants provides an adaptive advantage for survival in the environment, which is facilitated by its high mobility, fecundity, and capability to acquire resistance to a broad spectrum of chemical pesticides. It is well-established that knowledge of diversity and evolutionary origins facilitate the development of pest management strategies. In the present study, we report the morphology, diversity and phylogeny analysis of *S. exigua* from North Sulawesi, Indonesia. The specimen from Rurukan have a body size and other segments that are longer than in Langowan and Modoinding. Dendrogram analysis shows that the similarity distance based on morphology ranges from 1-25%, which forms four clusters, where the specimen from Rurukan is separated from the rest of the specimens. The phylogeny of *S. exigua* from North Sulawesi, Indonesia, based on CO1 (Cytochrome c oxidase subunit 1) gene fragment, which is juxtaposed with CO1 data of the allied species from many geographical locations. A total of twenty-five isolates representing Indonesia, Japan, Germany, Thailand, India, UK, USA, Spain and Australia were compared. Nineteen sequences of *S. exigua* retrieved from GenBank were selected as references based on previous published phylogenic trees. The twenty-four isolates were scattered in two distinct clades indicating *S. exigua* is polyphyletic, but *S. exigua* from North Sulawesi, Indonesia is monophyletic.

INTRODUCTION

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), has a wide range of distribution throughout the world, including Indonesia. It is considered a worldwide distribution pest due to its migratory capacity, which significantly contributes to the population outbreak and facilitates the geographic expansion of the population [1,2]. This species is native to Southeast Asia, has established itself as a significant insect pest of edible vegetables, and It is also resistant to a variety of insecticides [3]. The species is a polyphagous that may feed on more than 50 plant species belonging to more than ten plant families worldwide, including soybean, sugar

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INTRODUCTION

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beet, cabbage, cauliflower, brussels sprout, tomato, maize, cotton, lettuce, peanut, alfalfa, shallot, pastures crops, and various wild hosts [4].

In Indonesia, the pest was first reported attacking shallot plants in Java in December 1930 in large numbers. Currently, the pest has spread to virtually all parts of Indonesia, including Java, Sumatra, Sulawesi, Kalimantan, Bali, and East Nusa Tenggara. This insect has the capacity to spread rapidly on shallot plants in both the highlands and lowlands and is unaffected by the seasons throughout the year [5]. Based on the level of damage caused by this pest, it generally occurs in onion plants. Hence, it is called an important pest on onion plant [6,7]. Also, the insect is an obstacle to increasing crop yields of scallion due to a decrease in yield quantity and quality of 57-100% [8].

Study of the *S. exigua* in Indonesia has been conducted on population [6], pest management [9], life cycle [10], invasion and attack level [11], control using insecticides [9,12], and natural enemies [13]. The phylogeny of the Indonesian *S. exigua*, on the other hand, has not yet been reported in any publications. The knowledge of whether this insect was introduced into Indonesia and/or evolved locally implicates to practical pest management. Therefore, in the present study, we investigated the phylogeny of Indonesian *S. exigua* from North Sulawesi, Indonesia, and compared it with data on the same species in several geographical locations in the world. Evolutionary inferences were made by constructing gene genealogies from partial DNA sequences of the mitochondrial gene cytochrome c oxidase subunit 1 (COI).

MATERIALS AND METHODS

Sample collection

The specimen of *S. exigua* were collected during night time (07.00 – 08.00 p.m.) from different hosts, namely shallots, corn and peanuts in three different regions in North Sulawesi, Indonesia, including Tomohon City, Minahasa Regency and South Minahasa Regency (Figure 1). Adult insects (imago) were collected and stored in glass bottles, covered in gauze, and transported to the laboratory for morphological observation and molecular identification of the specimens.

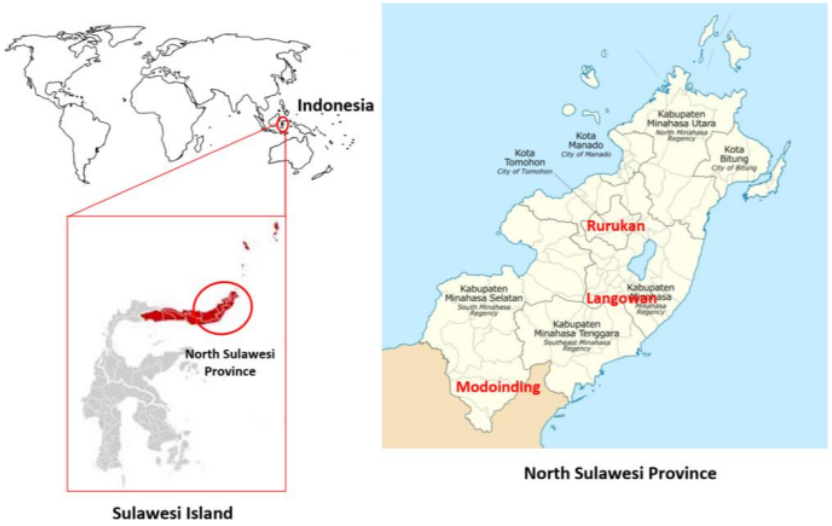

Figure 1. Sampling location.

Multivariate morphometric analysis

Observations of morphological characteristics were conducted using the approach described by Balvin et al. [14]. The qualitative parameters observed included eye color, wing color, wing pattern, body color, and body hair intensity. Quantitative parameters which are morphometric characters included head length (HL), thorax length (THL), abdomen length (ADL), antenna length (ANL), eye diameter (ED), wing length (WL), wingspan (WS), femur length (FL) and tibia length (TL). Observations of these morphological characteristics were carried out with a HIROX KH-8700 Digital Microscope (Hirox, Europe). Multivariate clustering analysis for morphometric characters was performed using SPSS IBM 20.

DNA extraction

Total DNA was extracted from fresh specimens using the innuPrep DNA Micro Kit (Analytik Jena) according to the manufacturer's instructions. To achieve higher concentration of DNA yield, a slight modification was done according to Kolondam [15] by increasing the time for incubation in lysis solution (and proteinase K) to one hour. The purified DNA samples were preserved in the freezer (-20°C).

Polymerase chain reaction (PCR)

The PCR of samples were carried out using MyTaq HS Red Mix (Bioline) PCR kit. Every 40 µl reaction contained 20 µl of PCR premix, 15 pmol of each primer used, and 1 µl of DNA sample. Autoclaved MilliQ water was used to complete the volume to 40 µl. The primers employed in this research were based on Folmer et al. [16] as follows: LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') as forward primer and HC02198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') as reverse primer. The Thermocycler (TPersonal, Biometra) setting was 95°C (3 minutes) for initial denaturation and continued with 35 cycles of 95°C (20 seconds) of denaturation, 50°C (30 seconds) of primer annealing, and 72°C (20 seconds) of DNA elongation. DNA separation of PCR products were done in agarose gel electrophoresis (0.8%) contained ethidium bromide. The PCR products were visualized using UV light to detect the 710 bp amplified band in PCR reaction.

DNA sequencing

The Sequencing were conducted by BigDye™ Terminator v3.1 cycle sequencing kit chemistry in ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems) by First Base C.O. (Malaysia). All of the samples were sequenced bi-directionally with both primers used in the PCR reaction. Chromatograms were assembled using MUSCLE algorithm [17], and edited under Geneious v5.6 [18] platform. The 658 bp long fragment generated were used for data analysis.

Molecular identification of specimens

Sequences of local *S. exigua* were deposited in GenBank. The GenBank accession numbers of Indonesian *S. exigua* as well accompanied data are shown in Table 1. Identification was accomplished through the use of the BLAST identity search feature supplied by the same platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 1. Sources and accession of *S. exigua* (No. 1-24;), and *S. litura* (No. 25) used in this study.

No.	GenBank accession	Host	Region	Country of Origin
1	HQ950505.1	N/A	Junction of Shaw River, W. Australia	Australia
2	HQ950504.1	N/A	Derby, W. Australia	Australia
3	HQ950506.1	N/A	Calperum, S. Australia	Australia
4	GU707393.1	N/A	Jenkofen, Bavaria	Germany
5	JF415658.1	N/A	Oberschleissheim, Bavaria	Germany
6	HM914242.1	N/A	Rinnkendlstei, Bavaria	Germany
7	JQ064572.1	N/A	N/A	India
8	AB733673.1	N/A	Kinpou-cho, Kagoshima	Japan
9	AB733674.1	N/A	Kinpou-cho, Kagoshima	Japan
10	AB733675.1	N/A	Kinpou-cho, Kagoshima	Japan
11	FN908024.1	N/A	N/A	Spain
12	FN908004.1	N/A	N/A	Thailand
13	FN907974.1	N/A	N/A	UK
14	FN907975.1	N/A	N/A	UK
15	FN907973.1	N/A	N/A	UK
16	HM756079.1	N/A	N/A	USA
17	HM756080.1	N/A	N/A	USA
18	HM756077.1	N/A	N/A	USA
19	MZ323866.1	Scallion	Rurukan	Indonesia
20	MZ323861.1	Scallion	Modoinding	Indonesia
21	MZ323862.1	Scallion	Tompaso	Indonesia
22	MZ323865.1	Corn	Pineleng	Indonesia
23	MZ323864.1	Peanut	Langowan	Indonesia
24	MZ323863.1	Onion	Tompaso	Indonesia
25	HQ950413.1 (*S. litura*)	N/A	Keep River NP, Northern Territory	Australia

Phylogenetic analysis

The sequences of Indonesian *S. exigua* were aligned with reference sequences obtained from GenBank using the multiple alignment program CLUSTALW (v.1.83) plug-in integrated in Geneious v.5.3.6. The alignment was edited manually using Geneious v.5.3.6 and all polymorphisms were confirmed by re-examining the electropherograms. The evolutionary history was inferred by the Maximum Likelihood (ML) method based on the Kimura 2-parameter (K2P) model [19]. Evolutionary analyses were conducted in MEGA v10.0.4 [20].

RESULTS

Morphometric analysis of *S. exigua* from North Sulawesi

The morphometric study employed specimens from three localities in North Sulawesi, namely Tomohon, Minahasa, and South Minahasa. Visual examinations using a magnifying equipment revealed similarities in the morphology of moths from three districts in Tomohon City (Rurukan), Minahasa Regency (Langowan), and South Minahasa Regency (Modoinding). The color of the forewings was grayish brown, while the hind wings were white and slightly brownish, black eyes, antennae like threads and at rest the position of the wings like a precarious arrangement on the abdomen. However, as seen in Table 2, the average morphometry of all specimens varied.

Table 2. Average morphometry (mm) of specimens from Rurukan, Langowan, and Modoinding.

Morphological Character	Rurukan		Langowan		Modoinding	
	Morphometry	SD	Morphometry	SD	Morphometry	SD
Head length	1.561	0.364	0.349	0.019	0.353	0.037
Thoracic Length	3.588	0.021	0.850	0.021	0.869	0.033
Abdomen Length	5.384	0.467	1.218	0.213	1.490	0.112
Antenna Length	6.166	0.364	1.928	0.082	1.878	0.018
Eye Diameter	0.957	0.034	0.290	0.228	0.217	0.003
Front Wing Length	8.991	0.762	2.303	0.107	2.339	0.051
Front Wingspan	4.656	1.119	1.783	0.031	1.817	0.005
Femur Length	2.558	0.240	0.482	0.051	0.508	0.065
Tibia Length	1.837	0.205	0.456	0.118	0.406	0.042

Morphological characteristics that can be used in general as a marker or identification of this moth species are the wings. The forewings are rather narrow, while the hind wings are broad, and are mostly covered by feathers or scales, have irregular stripes and black spots on the sides. The outer edge of the wing is dark, with dark irregular stripes with yellow-orange spots. Some have silver spots on the forewings, quadruped cubital hind wings, and long and slender filamentous antennae in males and females, which varies in the color of the thorax and back. The color of the abdomen and the intensity of the hair on the organs, especially the legs, the appendages on the genitalia in the form of a collection of feathers lighter in color than the abdomen.

Cluster analysis of *S. exigua* from North Sulawesi

Cluster analysis is a statistical clustering method used to analyze large amounts of data divided into clustered segments [21]. Clustering has been widely used since the 1990s aimed at grouping objects based on the similarity of characteristics between these objects. In this study, cluster analysis was carried out to determine the similarity of the morphological characteristics of *S. exigua* in different habitats, namely Rurukan, Langowan, and Modoinding. Each of these areas was represented by three samples with nine main morphological characters used, namely head length (HL), thorax length (THL), abdomen length (ADL), antenna length (ANL), eye diameter (ED), wing length (WL), wingspan (WS), femur length (FL) and tibia length (TL). The morphological character variables have distances which are arranged in Table 3.

Table 3. Distance between variables of morphological character of *S. exigua* from Rurukan, Langowan and Modoinding.

Case	Matric File Input								
	1. T1	2. T2	3.T3	4. L1	5. L2	6. L3	7. M1	8. M2	9. M3
HL	,000	1653020,8	8998207, 7	155008886,6	15136263,1	150337281,6	148815037,3	149135421,2	148006665,0
THL	1653020,8	,000	14802539,	134509677,6	125343410,9	130266824,6	128793586,5	128964773,0	127930573,2
AL	8998207,7	14802539,	,000	227488174,0	215465545,3	221965027,4	220026797,9	220297076,4	218884500,3
ANL	155008886,5	134509677,6	227488174,0	,000	1058630,89	80106,610	172828,434	210705,630	224155,630
ED	145136263,1	125343410,9	215465545,3	1058630,890	,000	978829,960	678772,492	654762,740	636671,740
WL	150337281,6	130266824,6	221965027,4	80106,610	978829,960	,000	140795,304	150579,200	146488,800
WS	148815037,3	128793586,5	220026797,8	172828,434	678772,492	140795,304	,000	50672,784	52132,884
FL	149135421,2	128964773,0	220297076,5	210705,630	654762,740	150579,200	50672,784	,000	3757,000
TL	148006665,0	127930573,2	218884500,3	224155,630	636671,740	146488,800	52132,884	3757,000	,000

The distance matrix table shows the distances between variables in nine morphological characters of *S. exigua* in North Sulawesi represented by three onion-producing centers, namely Tomohon (Rurukan), Minahasa (Langowan) and South Minahasa (Modoinding). The smaller the Euclidean distance, the more similar the two morphometric character variables were analyzed together from the three habitats of the *S. exigua* moth. The more similar characters will form a cluster (Table 4).

Table 4. Formation of the morphological cluster of S. exigua from North Sulawesi.

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	8	9	3757,000	0	0	2
2	7	8	51402,834	0	1	4
3	4	6	80106,610	0	0	4
4	4	7	174258,833	3	2	5
5	4	5	801533,564	4	0	8
6	1	2	1653020,840	0	0	7
7	1	3	11900373,760	6	0	8
8	1	4	166465306,785	7	5	0

Following cluster analysis, groups are determined based on the degree of similarity. The dendrogram analysis of *S. exigua* insect samples from Rurukan, Langowan, and Modoinding using SPSS IBM ver. 21.0 resulted in a similarity index as shown in Figure 2.

The dendrogram study of insects from Rurukan, Langowan, and Modoinding revealed that the distance of similarity based on morphology was between 1 and 25%, forming four groups. The first cluster, consisting of insects from Langowan (L1, L2, L3) and Modoinding (M1, M2, M3), demonstrated morphological resemblance by creating a single group with a similarity level of 1%. The second cluster, insects from Rurukan (T1 and T2), also demonstrated morphological similarity at a 1% similarity index, resulting in the formation of a new group distinct from the first cluster. At a 2% similarity index, the third grouping, insects from Rurukan (T1 and T3), demonstrated morphological resemblance. Meanwhile, the fourth cluster demonstrates that the third cluster, *S. exigua* from Rurukan (T1 and T3), forms a single group with the first cluster, at a 25 percent similarity index.

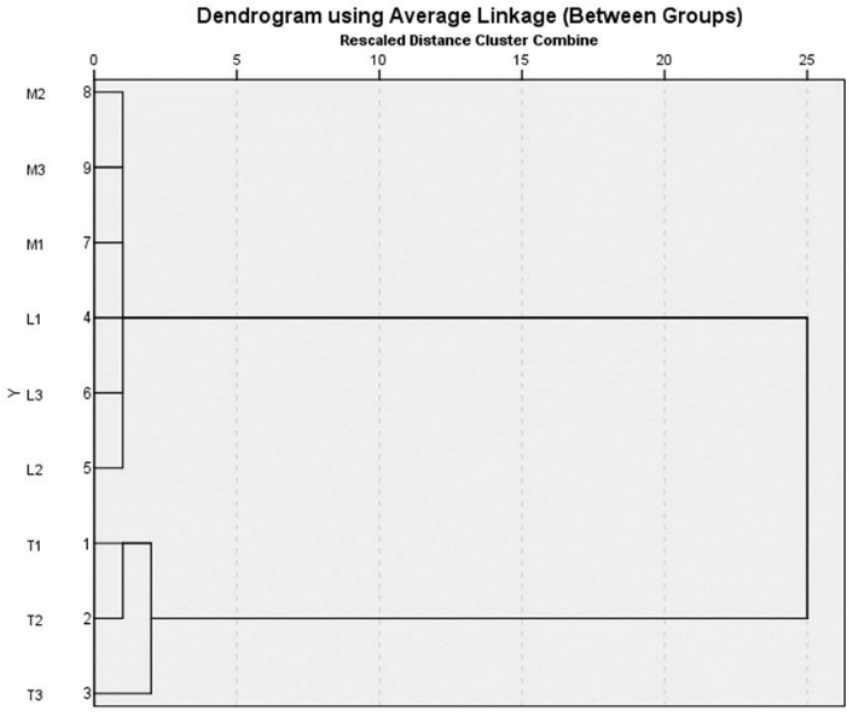

Figure 2. Dendrogram of morphological character grouping of *S. exigua* sampled from Rurukan, Langowan, and Modoinding.

Phylogeny analysis of *S. exigua* using the CO1 gene

This CO1 gene has been frequently utilized in insect research to assess inter- and intraspecific genetic variation [22]. It is also used to supplement standard morphological-based species identification for more precise findings [23,24]. Figure 3 depicts a molecular phylogenetic study using the neighbor joining method and the K2P model. The tree reveals that specimens form two clades, while specimens from North Sulawesi are clustered in one clade. Clade I include *S. exigua* from Japan (3 specimens), Germany (3 specimens), Thailand (1 specimen), Indonesia (6 specimens), India (1 specimen), UK (3 specimens), USA (3 specimens), and one specimen from Spain, with strong bootstrap support (100 %). Clade II is made up of only three specimens of the Australian *S. exigua* with bootstrap support of 100 %.

Table 5 summarizes the genetic distances between the specimens examined. *S. exigua* (HQ950504) from Australia and *S. litura* (HQ950413) from the Northern Territory of Australia have a genetic distance of 0.075. The farthest genetic distance between *S. exigua* was between specimens HQ950504 (Australia) and JF415658 (Germany), which was 0.052. Meanwhile, the genetic distance between *S. litura* (HQ950413) and *S. exigua* varies between 0.071 and 0.073.

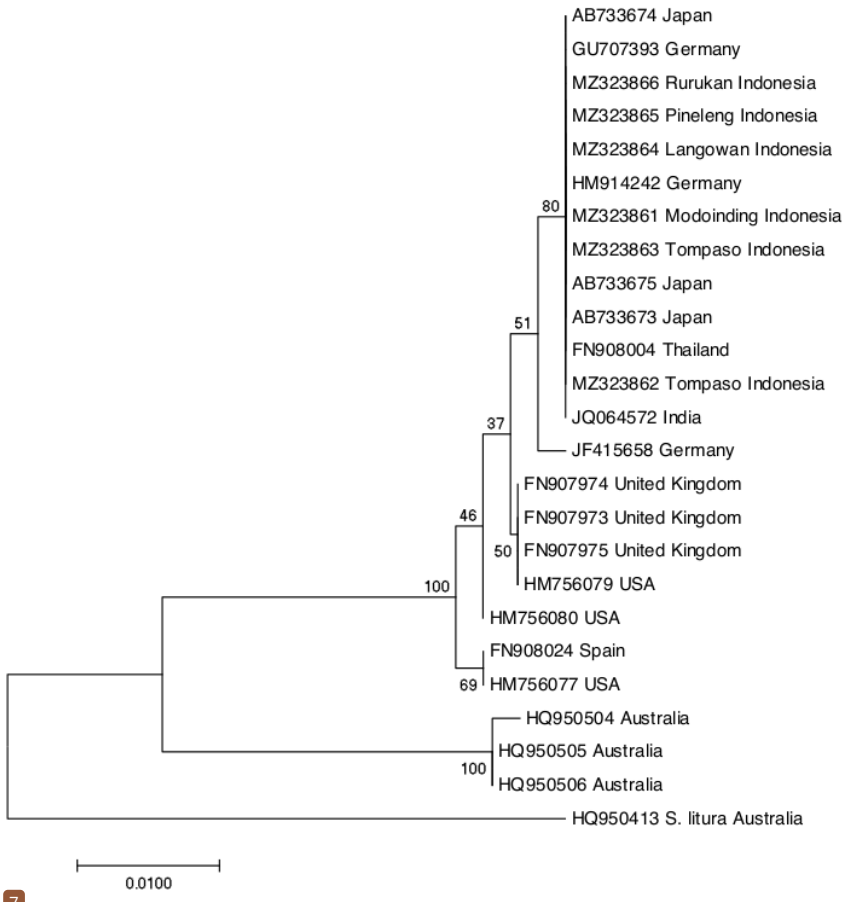

Figure 3. Molecular phylogenetic analysis inferred by using Maximum Likelihood method based on Kimura 2-parameter (19). The percentage of trees with the relevant taxa clustered together is presented alongside the branches.

Table 5. Estimation of evolutionary divergence amongst *S. exigua* and *S. litura* specimens based on K2P method. The number of base substitutions per site from between sequences are shown in the lower diagonal; the number of base differences per site from between sequences are shown on the upper diagonal.

	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	HQ950413 *S. litura* Australia		0,069	0,069	0,071	0,069	0,069	0,069	0,069	0,069	0,069	0,067	0,069	0,069	0,069	0,069	0,069	0,069	0,069	0,069	0,069	0,069	0,069	0,067	0,069	0,067
2	HQ950505 Australia	0,073		0,000	0,002	0,048	0,044	0,044	0,044	0,044	0,042	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,042
3	HQ950506 Australia	0,073	0,000		0,002	0,048	0,044	0,044	0,044	0,044	0,042	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,044	0,042
4	HQ950504 Australia	0,075	0,002	0,002		0,050	0,046	0,046	0,046	0,046	0,044	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,046	0,044
5	JF415658 Germany	0,073	0,050	0,050	0,052		0,004	0,004	0,004	0,004	0,008	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,006	0,008
6	MZ323862 Tompaso, Indonesia	0,073	0,046	0,046	0,048	0,004		0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
7	FN907974 United Kingdom	0,073	0,046	0,046	0,048	0,004	0,004		0,000	0,000	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,000	0,002	0,004
8	FN907973 United Kingdom	0,073	0,046	0,046	0,048	0,004	0,004	0,000		0,000	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,000	0,002	0,004
9	FN907975 United Kingdom	0,073	0,046	0,046	0,048	0,004	0,004	0,000	0,000		0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,000	0,002	0,004
10	FN908024 Spain	0,071	0,044	0,044	0,046	0,008	0,008	0,004	0,004	0,004		0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,004	0,002	0,000
11	FN908004 Thailand	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
12	AB733673 Japan	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
13	AB733675 Japan	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
14	MZ323863 Tompaso, Indonesia	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
15	MZ323861 Modoinding Indonesia	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
16	HM914242 Germany	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
17	MZ323864 Langowan, Indonesia	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000	0,004	0,006	0,008
18	MZ323865 Pineleng, Indonesia	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,004	0,006	0,008
19	MZ323866 Tomohon, Indonesia	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,004	0,006	0,008
20	AB733674 Japan	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,000	0,004	0,006	0,008
21	GU707393 Germany	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,000	0,004	0,006	0,008
22	JQ064572 India	0,073	0,046	0,046	0,048	0,004	0,000	0,004	0,004	0,004	0,008	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000		0,004	0,006	0,008
23	HM756079 USA	0,073	0,046	0,046	0,048	0,004	0,004	0,000	0,000	0,000	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,004		0,002	0,004
24	HM756080 USA	0,073	0,046	0,046	0,048	0,006	0,006	0,002	0,002	0,002	0,002	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,002		0,002
25	HM756077 USA	0,071	0,044	0,044	0,046	0,008	0,008	0,004	0,004	0,004	0,000	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,004	0,002	

DISCUSSION

Despite extensive use of agrochemicals, insect pests continue to pose a significant threat to agricultural output and yields. For this reason, correct identification of the species is critical in the establishment of integrated pest control programs (IPM). Kinship relationship may be used to quantify the degree of similarity between species or populations [25]. This is in contrast to the diversity coefficient, which is used to quantify the degree of variation across species or populations on a set of characteristics [26]. It may be deduced from this connection that the further the kinship relationship, the greater the amount of variety and the lesser the level of uniformity, and vice versa.

In comparison to other animal species, insects have the greatest phenotypic plasticity within the animal group [27]. As a result, even within a single species, numerous morphological differences were discovered in insects. Environmental factors such as habitat and ecosystem conditions at the sampling location greatly impact the factors that determine the size difference across several populations [28]. Morphological variety in insects occurs as a result of environmental variables such as food and climatic availability, geographic position, and the existence of natural enemies, as well as the process of natural selection [29]. Morphological diversity occurs mostly in the shape and size of bodily organs in insects [30].

Another aspect that contributed to the Rurukan specimens having the lowest degree of kinship with the Langowan and Modoinding specimens was the considerably lower intensity of pesticide usage to suppress *S. exigua* on leek plants in the Rurukan region. This was feasible due to the low prevalence of *S. exigua* infestation on leek plants in the Langowan and Modoinding regions. According to farmers in Langowan and Modoinding, leeks were frequently treated with pesticides four to five times during the growing season, but farmers in Rurukan sprayed insecticides just two to three times during the growing season. According to Benítez et al. [29], morphological variation is widespread among insects because it is associated with adaptation to the environment in which they exist. *S. exigua* that resides in the Rurukan was assumed to undergo character changes, both in terms of nature and appearance or phenotype. These are referred to as environmental variants since they are generated only by changes in the environment while the genetic information stays unchanged. Environmental effects might manifest themselves in the form of climate variables and established agricultural practices [31].

While morphometric study revealed morphometry variation across *S. exigua* specimens from North Sulawesi, genetic distance analyses using the K2P method revealed no genetic variation among all of these specimens. This indicates that the environment has an effect on the morphological distinctions between the specimens without creating any intra-specific variation. Thus, *S. exigua* from North Sulawesi exhibited solely morphological plasticity.

Intra-specific genetic variation among specimens in Clade I ranges from 0.000-0.008, whereas among specimens from Australia (Clade II), it ranges from 0.000-0.002. According to Ashfaq et al. [32], intraspecific divergences in 81 butterfly species in North-central Pakistan ranged from 0.0 to 1.6% with a mean of 0.2%. Based on these findings, it may be concluded that the specimens of *S. exigua* examined in this study are most likely no longer *S. exigua*, since intraspecific variation has reached 0.052 (5.2%). As a result, a revisiting of the species *S. exigua* based on a more comprehensive morphometric investigation and multi-barcode regions is highly suggested.

Result of our analyses confirms that *S. exigua* seems well differentiated into two clades and that may correspond to two distinct species. The clade one constitutes a first

putative species cluster whereas the clade two from Australia constitute another one. It is supported by Barcoding of life database (BOLD) that *S. exigua* specimens from Australia are grouped into a distinct barcode cluster (BOLD:AAA6645) that differ from another barcode cluster (BOLD:AAA6644) grouping all remaining *S. exigua* individuals. The result of this study is consistent with Shashank et al. [33] and Dumas et al. [34]. They reported that *S. exigua* is divided into two cluster groups and the Australian population of *S. exigua* is entirely different from other populations. This result requires more study using specific gene in order to determine whether these specimens correspond to a new species or to a case of cryptic species complex. The present study of phylogeny has confirmed that *S. exigua* is polyphyletic, whereas *S. exigua* from North Sulawesi, Indonesia is monophyletic.

CONCLUSIONS

The specimens from Rurukan have a body size and other segments that are longer than in Langowan and Modoinding. The similarity distance based on morphology ranges from 1-25%, which forms four clusters, where the specimen from Rurukan is separated from the rest of the specimens. Morphometric study revealed morphological variation across *S. exigua* specimens from North Sulawesi. However genetic distance analyses using the K2P method revealed no genetic variation among all of these specimens. The phylogeny of *S. exigua* from North Sulawesi, Indonesia, based on the CO1 (Cytochrome c oxidase subunit 1) gene fragment, which is juxtaposed with the CO1 data of the allied species from many geographical locations, is polyphyletic. However, *S. exigua* from North Sulawesi, Indonesia is monophyletic.

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AUTHORS CONTRIBUTION

Conceptualization, U.S., M.T. and A.P.; Methodology, U.S., J.P. and C.L.S.; Formal analysis, T.E.T. and A.P.; Data curation, U.S. and B.J.K.; Writing-original draft preparation, U.S., A.P., B.J.K., T.E.T. and T.B.E.; Writing- review and editing, A.P, T.E.T. and T.B.E.; Visualization, U.S. and T.E.T.; Supervision, M.T., J.P. and T.E.T.; Critical revisions and writing, T.E.T., A.P. and T.B.E. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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