Торайғыров университетінің
ҒЫЛЫМИ ЖУРНАЛЫ

НАУЧНЫЙ ЖУРНАЛ
Торайгыров университета

ТОРАЙҒЫРОВ УНИВЕРСИТЕТІНІҢ ХАБАРШЫСЫ

Химия-биологиялық сериясы

1997 жылдан бастап шығады

TORAIGHYROV UNIVERSITY

ВЕСТНИК ТОРАЙГЫРОВ УНИВЕРСИТЕТА

Химико-биологическая серия

Издается с 1997 года

ISSN 2710-3544

№ 3 (2023)

Павлодар

НАУЧНЫЙ ЖУРНАЛ
Торайгыров университета

Химико-биологическая серия
выходит 4 раза в год

СВИДЕТЕЛЬСТВО
о постановке на переучет периодического печатного издания,
информационного агентства и сетевого издания
№ KZ84VPY00029266

выдано
Министерством информации и коммуникаций Республики Казахстан

Тематическая направленность
публикация материалов в области химии, биологии, экологии,
сельскохозяйственных наук, медицины

Подписной индекс – 76134

https://doi.org/10.48081/TIDJ1047

Бас редакторы – главный редактор
Ержанов Н. Т.
д.б.н., профессор

Заместитель главного редактора — Ахметов К. К., *д.б.н., профессор*
Ответственный секретарь — Камкин В. А., *к.б.н., доцент*

Редакция алқасы – Редакционная коллегия

Яковлев Р.В., *д.б.н., профессор (Россия);*
Титов С. В., доктор *PhD;*
Касанова А. Ж., доктор *PhD;*
Шокубаева З. Ж. *(технический редактор).*

За достоверность материалов и рекламы ответственность несут авторы и рекламодатели
Редакция оставляет за собой право на отклонение материалов
При использовании материалов журнала ссылка на «Вестник Торайгыров университета» обязательна

© Торайгыров университет

СЕКЦИЯ «СЕЛЬСКОЕ ХОЗЯЙСТВО»

SRSTI 34.15.05

https://doi.org/10.48081/OYSC1016

Altantsesteg Zul, *Undarmaa Davaasambuu
School of Agroecology,
Mongolian University of Life Sciences,
Mongolia, Ulaanbaatar
*e-mail: undarmaa@muls.edu.mn

MOLECULAR IDENTIFICATION OF THRIPS SPECIES, WHICH INFESTS THE GREENHOUSE VEGETABLES INCLUDING EGGPLANT IN THE CENTRAL AGRARIAN REGION OF MONGOLIA

In Mongolia, the first record of onion thrips was informed in vegetable fields by D. Tsedev (Tsendsuren et al., 1979), since that has no detailed research materials relating to the identification of thrips and the Thysanoptera group observed in different crop fields.

In 2017–2019, we have taken the thrips samples from the following areas, which spread on eggplant, cucumber, tomato, paprika, and beans grown in the greenhouses of the Agropark research and training center, the greenhouses of the Bornuur soum in the Tuv province and the Mandal soum of Selenge province and onion field accordingly. The mitochondrial COI gene of selected insect samples has been used for species identification. For the nucleotide sequence of the mitochondrial COI area of the insects, we amplified with universal LCO1490 and HCO2198 primers. We have illustrated the COI gene fragments of thrips samples.

For building a phylogenetic tree was constructed using the Neighbor-Joining method. A bootstrap test with 1000 replications was carried out. The evolutionary distances were calculated using Tamura's three-parameter method. A phylogenetic tree was built for 14 sequences with a total length of 681bp nucleotides.

When conducting phylogenetic analysis using the MEGA X program, it was displayed the phylogenetic origin of our samples in all inferred trees is the same as the Chinese species; Thrips tabaci (China – Zhejiang), which registered with MN036455 accession number in the gene bank of

NCBI. According to these results, the sampled thrips were collected from various vegetables including eggplants in Mongolia identified as Thrips tabaci and we have registered it in the GenBank of NCBI under OP288232 accession number.

Keywords. Eggplant damage, thrips species identification, COI gene, phylogenetic origin, PCR.

Introduction

The onion thrips – Thrips tabaci Lindeman (Thysanoptera: Thripidae) is polyphagous and a cosmopolitan pest and infests the plant leaves in protected and open field crops such as onion, leek, eggplant, cucumber, paprika, tomato, and various ornamentals [1].

About 15 genera are considered members of this genus group, with 300 species in the genus Thrips. Of the other 14 genera, eight each include a single species, and the remaining six comprise a total of 67 species [2] (Mound, Laurence A. et al).

In Mongolia, the first record of onion thrips was found on the greenhouse vegetables by D. Tsedev et al in Zuunkharaa province (A. Tsendsuren et al., 1979) [3], since has been no detailed research material relating to the identification of thrips, which are observed in different crop fields. According to Loredo Varela, the list of 391 different crops belonging to families like Asteraceae, Fabaceae, Brassicaceae, Poaceae, and Solanaceae, were infested by onion thrips. Interestingly, he found the thrips generation related to tobacco plants from Europe and the middle east [4].

Among thrips species, the ones recognized as pests that can feed both on leaves and in flowers, damaging their host plants by puncturing and sucking their cells, like *Thrips palmi, T. tabaci and Frankliniella ssp,* which are remarkably similar in appearance and size too. Therefore, thrips are considered cryptic insects to discriminate [1].

Materials and methods

Insect Sampling

With a goal to determine the species of thrips that occur mainly in the vegetable field, we collected samples of thrips from the greenhouses of the Agropark research and training center, the greenhouses of the Bornuur soum the Tuv province, and the Mandal soum of Selenge province accordingly.

The samples for identification were collected from June to September 2017–2019 from vegetable fields in Mongolia. The thrips were taken out from leaves to plastic containers with a brush, and then preserved in 99 % ethanol and

stored at – 20 ^{0}C. The specimens used in this study were labeled and kept at the Mongolian University of Life Sciences.

DNA extraction, amplification, and sequencing

Due to the limitation of morphological classification, the first time we have done molecular identification of thrips according to the following procedures.

The Molecular Analysis for thrips identification was done following steps:

→To extract the total genome DNA of the insect.

→To amplify by polymerase chain reaction (PCR) using universal primers of animal DNA barcode.

→To identify the nucleotide sequence of the insect mitochondrial locus (COI).

→Identify a species using a nucleotide sequence of thrips DNA compared to according to NCBI genebank library.

Total genomic DNA was extracted using a Tissue genomic DNA Mini KIT GT (Geneaid) according to the given protocol and amplified by PCR with universal primers LCO1490, and HCO2198.

The sequence of universal primers of animal DNA barcode:

**LCO1490: 5`-** GGTCAACAAATCATAAAGATATTGG-3`
(Folmer et al. 1994) [5]

**HCO2198: 5`-** TAAACTTCAGGGTGACCAAAAAATCA-3`
(Folmer et al. 1994)

Table 1 – PCR condition; total 28 cycles

Initial denaturation	94 °C	5 min
Denaturation	94 °C	30 sec
Annealing	53 °C	30 sec
Extension	72 °C	1 min
Final extension	72 °C	8 min
Keep	4 °C	

Phylogenetic Analysis

The phylogenetic tree was constructed using the Neighbor-Joining (Saitou N. and Nei M. (1987) [6] method. A bootstrap test with 1000 replications was performed [7] (Felsenstein J. (1985)]. Evolutionary distances were calculated using the Tamura 3-parameter method (Tamura K. (1992) [8]. A phylogenetic tree was constructed for 14 sequences with a total length of 633 nucleotides. When

the phylogenetic analysis was performed in the program MEGA X (Kumar S. et al, 2018) [9].

Data analysis

All sequences obtained in this study were compared with those on GenBank and BOLD using «BLAST» (http://www.ncbi.nlm.nih.gov/blast/) and species identification. For nucleotide sequence alignments, Geneblast, and Neighbor-Joining (NJ) clustering analysis, and for the phylogenetic analysis, program MEGA X was used respectively.

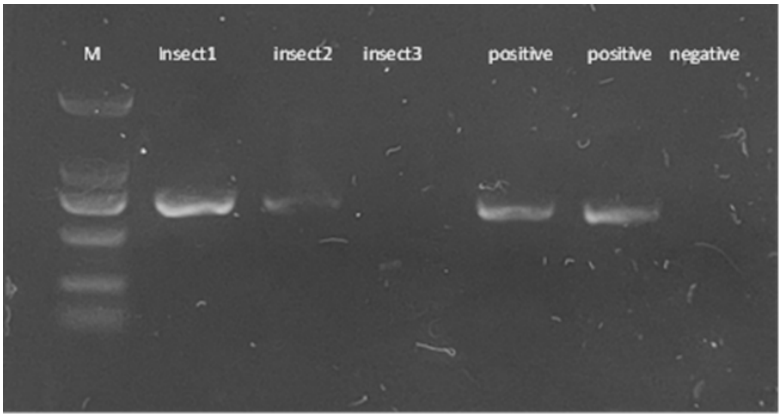
Results

In 2017–2019, we have taken thrips samples from the following areas, which are written in Table 1. The thrips have infested the eggplant, cucumber, tomato, paprika, and bean plants.

Table 2 – Thrips species that were used to test the specificity of the probe-based Tissue genomic DNA Mini Kit GT100 (Geneaid) assay

Species	Location	Crop	Results of assay
Thrips sample 1	Ulaanbaatar, Agropark station	Eggplant, cucumber, bean, tomato	+
Thrips sample 2	Tuv province, Bornuur soum	Cucumber, eggplant, paprika	+
Thrips sample 3	Selenge, Mandal soum	Cucumber, tomato, eggplant, paprika	NA

**remarks: «+» for the corresponding thrips samples and those that did not amplify were presented «NA».*



Picture 1 – The PCR product of insect samples

The PCR product was viewed as running the electrophoresis through 1.2 % agarose gel and harvested PCR product ~700 bp in size as estimated as illustrated in picture 1.

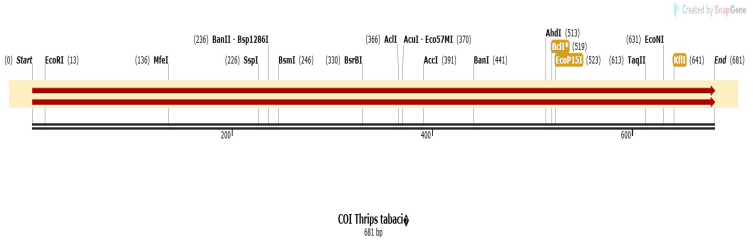
This one was eliminated from a further step in the sample (insect 3) that did not harvest PCR product. After lyophilizing, the amplified products were sequenced at Macrogen Co., Ltd (s. Korea).

The result of the nucleotide sequence

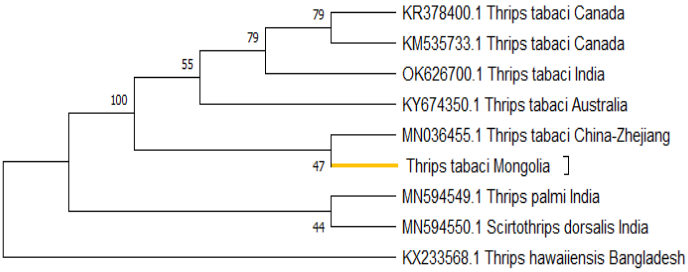
681 bp fragment of thrips COI gene locus, was amplified by PCR, and it was determined below shown.

COI Thrips tabaci:

ATAAAGATAT TGGAATTCTT TACTTCATTT TTGGATTTTG GTCAGGAATG ATAGGGCTTT CTTTAAGAAT AATTATTCGA TTAAATTTAC GAACATCAAT AAAACTATTC ATTAGAAACG ATCAATTTTA CAATTCAATT GTTACAGCTC ACGCTTTTGT AATAATTTTT TTTACAGTTA TACCTATTAT AATTGGTGGA TTTGGAAACT GATTGGTTCC TTTAATATTA GGAGCCCCTG ACATAGCATT CCCTCGATTA AATAATATAA GATTCTGACT TTTACCCCCT TCTCTGGGAT TATTAATTAT AGGACTTTAT AAAGAAGGAG CGGGAACGGG ATGAACAGTA TATCCACCTT TATCAACGTT TTATCATTCA GGACCTTCAG TAGACTTAAC AATTTTTTCT TTACACCTTG CAGGGATTTC TTCAATTTTA GGTGCCTTAA ATTTTATTAC TACAATTATT AATCTTAAAG CAAAAAACCT TTCAGCAGAA AAAATTAGAC TATTTGTCTG ATCAGTTATT TTAACAGCCA TTCTTCTTCT TTTATCTTTA CCAGTGTTAG CGGGAGCTAT CACAATACTT TTAACTGACC GAAACTTAAA TACCTCTTTT TTTGACCCTA GAGGAGGAGG GGACCCTGTT TTATATCAAC ACCTTTTTTG ATTTTTTGGT C



Picture 2 – Alignment of COI of Thrips tabaci



Picture 3 – The phylogenetic relationship of thrips species

According to phylogenetic analysis, it was displayed the phylogenetic origin of our samples in all inferred trees is the same as the Chinese species. Thrips tabaci (China – Zhejiang), which registered with MN036455 accession number in the gene bank of NCBI. According to these results, the sampled thrips were collected on the above-mentioned vegetables in Mongolia identified as Thrips tabaci. Afterward, we registered the Thrips tabaci in the GenBank of NCBI with OP288232 accession number as a presence in Mongolia.

Discussion

The insects of the *Thripidae* family are not well studied in Mongolia, as increasing the type of crops grown in the greenhouses, the infestation rate of thrips is becoming a reason for yield loss. Therefore, we aimed to identify the thrips, which are infesting the vegetables in Mongolia using modern molecular techniques.

To avoid misidentifications based on morphology, and to recognize cryptic diversity amongst thrips species, an accurate and effective molecular approach is required.

Molecular methods for identifying thrips species represent a valuable alternative for situations in which correct identification using classical morphological methods is difficult, time-consuming, or virtually impossible (Mehle, N et al,2012) [10].

For discrimination of cryptic insect species, the most accurate molecular approach is DNA barcoding with mt DNA as mentioned in the literature. The insect mitochondrial DNA is a short stretch of insect DNA to detect a species.

Mitochondrial DNA (mtDNA) is a widely used molecular marker. It is easy to use and has favorable biological properties, such as near-neutrality, lack of recombination, and a clock-like evolutionary rate. Mitochondrial DNA has several over than nuclear DNA for species identification purposes, including a lack of

sequence ambiguities from heterozygous genotypes, and a faster rate of mutation (Rasmussen and Morrissey, 2008) [11].

The literature by Yan Lan Xie et al. 2022 demonstrates that COI barcoding can reliably and efficiently identify *Panchaetothripinae* based on a broad-scale sampling. Thirty-two of 40 morphospecies were successfully identified by COI barcoding [12].

DNA barcoding is generating a wealth of computable data that in many ways are much easier to work with than classical taxonomic descriptions, but many of the sequences are not identified to species level (Roderic D. M.) [13].

In this study, the fragment of the mitochondrial cytochrome C oxidase subunit I (COI) gene has been used for the discrimination of thrips species. We have determined 681 bp fragment of thrips COI gene locus, was amplified by PCR, and its nucleotide sequence was determined as shown results above.

According to phylogenetic analysis using the MEGA X program [4], it was displayed the phylogenetic origin of our samples in all inferred trees is the same as the Chinese species; Thrips tabaci (China –Zhejiang), thus the sampled thrips were found in Mongolia identified as Thrips tabaci. The keys for morphological characters of onion thrips have shown comparable results, which we have done before it is not yet published.

The sequences of *T. tabaci*, which were found in Mongolia were not strong by geographical distance. With the results of this study, we submitted DNA barcodes of *T. tabaci,* to register to the international database, that indicated the onion thrips *(T. tabaci)* existed in Mongolia.

Overall, the relationship between intraspecific divergence and geographical distance was not strong. For example, sequences of *Heliothrips haemorrhoidalis* obtained from China, Spain, Australia, and the United Kingdom lacked barcode divergence, but *Caliothrips quadrifasciatus* collected from sites in China (Yan Lan Xie et al. 2022) [12].

A phylogenetic tree is a diagram describing the evolutionary relationships between organisms. Phylogenetic trees are hypotheses, not facts. The branches of a phylogenetic tree indicate the relationships between species or certain groups that have several parts close to each other, that is, how they arose. In addition, it shows how alleles are repeated at a certain position (locus) of the genome and the difference in repeated repeats.

The genetic diversity of the thrips sample collected in Mongolia is similar to *onion thrips* which are registered as Thrips tabaci (China –Zhejiang). In Contrast, the genetic diversity of other thrips species is more distant such as *Thrips palmi* in India.

Conclusion

According to results of DNA barcoding with mt DNA, thrips were found in Mongolia identified as *Thrips tabaci.* DNA barcodes of *T. tabaci*, to register to the international database, indicated that onion thrips *(T. tabaci)* existed in Mongolia, which was not strong by geographical distance from China. We registered the onion thrips *(T. tabaci)* in the GenBank of NCBI with OP288232 accession number.

References

1 **Norman, C. Leppla, Marshall, W. Johnson, Joyce, L. Merritt, Frank, G. Zalom.** Applications and Trends in Commercial Biological Control for Arthropod Pests of Tomato. // In Sustainable Management of Arthropod Pests of Tomato. – 2018. – P. 283–303.

2 **Mound, Laurence, A., Zhaohong, Wang, Élison, F. B. Lima, and Rita, Marullo,** Problems with the Concept of «Pest» among the Diversity of Pestiferous Thrips // Insects. – Vol. 1. – 2022. – P. 61.

3 **Tsendsuren, A, Ulikpan, K**, The Insects of Mongolia. – Ulaanbaatar : Publ industry of Mongolia, 1979. – P. 25.

4 **Loredo Varela, R. C. Fail, J.** Host Plant Association and Distribution of the Onion Thrips, Thrips tabaci Cryptic Species Complex // Insects – Vol. 13. – 2022. – P. 298.

5 **Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. // Mol Mar Biol Biotechnol. – 3(5). – October, 1994. – P. 294–299.

6 **Saitou, N. and Nei, M.** The neighbor-joining method : A new method for reconstructing phylogenetic trees. // Molecular Biology and Evolution. – Vol. 4. – 1987. – pp. 406–425.

7 **Felsenstein, J.** Confidence limits on phylogenies : An approach using the bootstrap // Evolution, 1985. – P. 783–791.

8 **Tamura, K.,** Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases // Molecular biology and Evolution. – Vol. 9. – 1992. – P. 678–687.

9 **Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K.** MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecilar biology and evolution. – Vol. 35. – 2018. – P. 1547–1549.

10 **Mehle, N., Trdan, S.** Traditional and modern methods for the identification of thrips (Thysanoptera) species // Journal of Pest Science. – Vol. 85. – 2012. – P. 179–190.

11 **Rosalee, M. T. M. Rasmussen S.** DNA-Based Methods for the Identification of Commercial Fish and Seafood Species // Comper.Reviews,. – Vol. 7. – No. 3. – 2008. – P. 280–295.

12 **Yan Lan Xie, Laurence A Mound, Élison Fabrício Bezerra Lima, Shu Qi He, Hong Rui Zhang, Ya Jin Li.** Molecular Studies of Relationships and Identifications Among Insects of the Subfamily Panchaetothripinae (Thysanoptera, Thripidae) // Journal of Insect Science. – Vol. 22. – No. 5. – 2022.

13 **Roderic, D. M.** DNA barcoding and taxonomy : dark taxa and dark // R. Soc, 2016. – P. 371.

14 **Glover, R. H., Collins, D. W., Walsh, K. and Boonham,** Assessment for loci for DNA barcoding in the Thrips (Thysanoptera) // Mol.Ecol.Resour, 2010. – P. 51–59.

Accepted for publication on 15.09.23.

*Алтанцецег Зул, *Ундармаа Даваасамбуу*
Агроэкология мектебі,
Моңғол өмір туралы ғылымдар университеті,
Моңғолия, Ұлан-Батор қ.
Басып шығаруға 15.09.23 қабылданды.

МОҢҒОЛИЯНЫҢ ОРТАЛЫҚ АГРАРЛЫҚ АЙМАҒЫНДАҒЫ ЖЫЛЫЖАЙ КӨКӨНІСТЕРІНЕ, СОНЫҢ ІШІНДЕ БАКЛАЖАНҒА ӘСЕР ЕТЕТІН ТРИПС ТҮРЛЕРІНІҢ МОЛЕКУЛАЛЫҚ ИДЕНТИФИКАЦИЯСЫ

Моңғолияда көкөніс алқаптарында пияз трипсінің алғашқы тіркелуі туралы Д. Цэдав хабарлады (Tsendsuren et al., 1979), осыдан кейін әртүрлі егістік алқаптарында байқалған трипс пен Thysanoptera отрядын анықтауға қатысты егжей-тегжейлі зерттеу материалдары болған жоқ.

2017–2019 жылдары біз «Агропарк» ғылыми-оқу орталығының жылыжайларында, Тува облысының Борнуур сум және Селенг облысының Мандала сум жылыжайларында және сәйкесінше пияз алқабында өсірілген баклажан, қияр, қызанақ, паприка және бұршақтарға таралған трипс үлгілерін таңдадық. Түрлерді анықтау үшін таңдалған жәндіктер үлгілерінің митохондриялық COI гені пайдаланылды. Жәндіктердің COI митохондриялық аймағының нуклеотидтер тізбегін алу үшін біз әмбебап LCO1490 және HCO2198

праймерлерімен күшейттік. Біз трипс үлгілерінің COI генінің фрагменттерін анықталдық.

Филогенетикалық ағашты салу үшін көрші-біріктіру әдісі қолданылды. Жүктеу сынағы 1000 репликациямен жүргізілді. Эволюциялық қашықтықтар үш параметрлі Тамура әдісімен есептелді. Филогенетикалық ағаш жалпы ұзындығы 681 а.к. болатын 14 тізбек үшін салынған. нуклеотидтер.

MEGA X бағдарламасы арқылы филогенетикалық талдау жүргізу кезінде барлық салынған ағаштардағы үлгілеріміздің филогенетикалық шығу тегі NCBI гендік банкінде MN036455 кіру нөмірімен тіркелген қытайлық Thrips tabaci (Қытай-Чжэцзян) түрімен сәйкес келетіні көрсетілді. Осы нәтижелерге сәйкес, Моңғолиядағы баклажандарды қоса алғанда, әртүрлі көкөністерден жиналған трипс Thrips tabaci ретінде анықталды және біз оларды NCBI ген қорында OP288232 кіру нөмірімен тіркедік.

Кілтті сөздер: баклажанның зақымдануы, трипс түрлерін анықтау, COI гені, филогенетикалық шығу тегі, ПТР.

Алтанцецег Зуль, Ундармаа Даваасамбуу
Школа агроэкологии,
Монгольский университет наук о жизни,
Монголия, г. Улан-Батор.
Принято к изданию 15.09.23.

МОЛЕКУЛЯРНАЯ ИДЕНТИФИКАЦИЯ ВИДОВ ТРИПСОВ, ПОРАЖАЮЩИХ ТЕПЛИЧНЫЕ ОВОЩИ, ВКЛЮЧАЯ БАКЛАЖАНЫ, В ЦЕНТРАЛЬНОМ АГРАРНОМ РЕГИОНЕ МОНГОЛИИ

В Монголии о первой регистрации лукового трипса на овощных полях сообщил Д. Цэдэв (Tsendsuren et al., 1979), после этого подробных материалов исследований, связанных с идентификацией трипсов и отряда Thysanoptera, наблюдаемых на различных полях сельскохозяйственных культур, не было.

В 2017–2019 гг. нами были отобраны образцы трипсов, распространенных на баклажанах, огурцах, томатах, паприке и фасоли, выращенных в теплицах научно-учебного центра «Агропарк», теплицах Борнуурского сум Тувской области и Мандальского сум Селенгской области и на луковом поле соответственно.

Митохондриальный ген COI отобранных образцов насекомых был использован для видовой идентификации. Для получения нуклеотидной последовательности митохондриальной области COI насекомых мы амплифицировали с помощью универсальных праймеров LCO1490 и HCO2198. Мы проиллюстрировали фрагменты гена COI образцов трипсов.

Для построения филогенетического дерева использовали метод Neighbor-Joining. Был проведен бутстреп-тест с 1000 репликациями. Эволюционные расстояния рассчитывали с помощью трехпараметрического метода Тамуры. Филогенетическое дерево было построено для 14 последовательностей общей длиной 681 п.н. нуклеотидов.

При проведении филогенетического анализа с помощью программы MEGA X было показано, что филогенетическое происхождение наших образцов во всех построенных деревьях совпадает с китайским видом Thrips tabaci (Китай-Чжэцзян), который зарегистрирован с номером доступа MN036455 в банке генов NCBI. Согласно этим результатам, трипсы, собранные с различных овощей, включая баклажаны в Монголии, были идентифицированы как Thrips tabaci, и мы зарегистрировали их в Генбанке NCBI под номером доступа OP288232.

Ключевые слова: Повреждение баклажанов, идентификация видов трипсов, ген COI, филогенетическое происхождение, ПЦР.

Теруге 15.09.2023 ж. жіберілді. Басуға 29.09.2023 ж. қол қойылды.
Электронды баспа
2,13 МБ RAM
Шартты баспа табағы 9,90.
Таралымы 300 дана. Бағасы келісім бойынша.
Компьютерде беттеген А. К. Темиргалинова
Корректорлар: А. Р. Омарова, Д. А. Кожас
Тапсырыс № 4185

Сдано в набор 15.09.2023 г. Подписано в печать 29.09.2023 г.
Электронное издание
2,13 МБ RAM
Усл. п. л. 9,90. Тираж 300 экз. Цена договорная.
Компьютерная верстка А. К. Темиргалинова
Корректоры: А. Р. Омарова, Д. А. Кожас
Заказ № 4185

«Toraighyrov University» баспасынан басылып шығарылған
Торайғыров университеті
Павлодар мемлекеттік университеті
140008, Павлодар қ., Ломов к., 64, 137 каб.

«Toraighyrov University» баспасы
Торайғыров университеті
140008, Павлодар қ., Ломов к., 64, 137 каб.
8 (7182) 67-36-69
e-mail: kereku@tou.edu.kz
www.vestnik-cb.tou.edu.kz