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Specific Identification Method based on PCR for *Drosophila melanogaster*

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Abstract. *D. melanogaster* is one of the most harmful citrus fruit flies having a large number of host plants. The molecular diagnostic method has been created for identification the *D. melanogaster* from another non-quarantine species *Drosophila* spp. The proposed method for differentiation is to use the mitochondrial DNA cytochrome oxidase I gene region 709-bp. We amplified samples of DNA with primers Droso-S391 and Droso-A381 by *D. melanogaster*, *D. suzukii*, and *D. Simulans* collections in the laboratory samples from many countries and contrasted with sequences of other GenBank *Drosophila* taxa. The findings of a polymerase chain reaction (PCR) based on DNA sequence polymorphisms showed that these primers accurately identify the area of the gene as well as the unique primers of *Drosophila melanogaster*.

Keywords: Identification, diagnosis, *Drosophila melanogaster*, PCR, plant quarantine

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Introduction

The *Drosophilidae* family consists of more than 4200 species worldwide, of which more than 2000 are *Drosophila* species [1, 2]. *Drosophila* species are well known for their extensive use in genetic studies of *Drosophila melanogaster* and as common vinegar flies associated with over-ripe and rotting fruit. Many exotic species of fruit fly pose a serious threat to the union of Asia, Australia, the United States, Europe and Russia. Most fruit flies are able to infest and cause significant damage to a wide range of commercial and native fruits and vegetables, although the degree of infestation and damage varies between species. Fruit flies are important too [3—5]. On the positive side, they may act as agents for biological pest control, and the species *Drosophila melanogaster*, as a major research model organism, unlocks genetic and even certain disease-related secrets in humans. On the negative side, fruit flies can be a major agricultural threat, with the potential to destroy up to 100 percent of some crops. For the latter reason, countries without fruit fly infestations may enforce heavy quarantine restrictions or even bans in fruit imported from countries where fruit fly is endemic. Human responsibility in caring for nature extends to showing the utmost concern about introducing foreign species into new areas. History is full of cases of invasive species (*Drosophila melanogaster*, *Drosophila suzukii*, Mediterranean fruit fly, sea lamprey, ctenophore *Mniopsis leidyi*, gypsy moth, etc.) that wreak havoc in the habitats into which they were introduced, either intentionally or accidentally [6, 7]. *Drosophila* species are well-known pests in restaurants, grocery stores, and fruit and home markets. *Drosophila* spp. are also identified as a nuisance pest during winemaking and fruit fermentation. *D. melanogaster* identified as a major risk and poses a major challenge to the production of fruit. *D. melanogaster* is a major quarantine pest for growers, gardeners and researchers in Russia's quarantine centers and other farmers around the world. Since there are many environments where opportunistic invasive species can flourish, there is a need to quarantine transported goods and items capable of carrying dormant or active pest species phases [8—12]. The quarantine strategies include regional and international import and export prohibitions, removal of potentially contaminated goods and decontamination in the form of fumigation, steam cleaning and chemical irradiation [13, 14]. Such pathogens and *Drosophila melanogaster* are a major concern and risk for the fruit industry as it is no longer possible to remove or contain them. To reduce the economic impact on fruit production in the Russian Federation, the implementation of targeted integrated pest management (IPM) is crucial. Besides, farmers need to quickly decide if the larvae in harvested berries are *Drosophila melanogaster* [15]. There is no way to distinguish *D. melanogaster* at the moment. The main objective of this research is to classify the molecular species *Drosophila melanogaster* [5, 16—20]. Our goal was to create an alternative identification technique for *Drosophila melanogaster* to improve morphological identification and allow for rapid identification of immature phases without the expense of sequencing DNA. The polymerase chain reaction was created to identify insects as a reliable and cost-effective method.

Materials and Methods

DNA extraction, amplification and sequencing. DNA was extracted from the material under study (insect and larvae) which was performed by treating the specimens with Proteinase K followed by removal of proteins with no extraction with organic solvents

and using DNA Ekstran-2 kit, set № NG-511—100 (“Synthol”, Russian Federation) as per manufacturer’s instructions. Since drosophilids are very small size, physical disruption of tissue was performed by finely chopping with sterile scissors. This is a rapid method of DNA extraction and provides a time advantage, especially for urgent diagnostic needs. DNA extracts were quantified on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA).

Polymerase chain reaction

Table 1

Qualitative time PCR assay for the identification of *Drosophila melanogaster*: Reaction composition and temperature cycling conditions

Target genes	Primer	Primer sequence (5'-3')	
<i>Drosophila melanogaster</i>	Droso-S391	AAATAACAATACAGGACTCATATcc	Sint et al. 2014
	Droso-A381	gTAATACGCTTACATACATaAAGGTATA	Sint et al. 2014

A denotes the forward and S the reverse primer. Lower-case letters in the group primer sequences indicate modifications of the original primers [21]

Primer Droso-S391 (5-AAATAACAATACAGGACTCATATcc –3') as a forward and Droso-A381 (5-gTAATACGCTTACATACATaAAGGTATA –3') as a reverse were used. In order to make PCR mix we used 0.5 µl (10 p mol) µl of each primer, 5 µl of screen-mix (HS-5x), 17 µl H₂O and 1 µl DNA (table 2). The total volume should be 25 µl. After that put tubes in PCR machine, in a Veriti™ thermocycler (Applied Biosystems, USA). The reaction mixture was as follows: ready-to-use PCR mixture Screen Mix-HS (Evrogen, Russia). PCR conditions: denaturation at 95 °C for 90 sec. followed by 40 cycles, including 15 sec. at 90 °C; primer annealing for 30 sec. at 63 °C; elongation for 30 sec. at 72 °C; final elongation for 5 min at 72 °C. The *Drosophila spp.* primers, Droso-S391 and Droso-A381, are targeting several *Drosophila* species and generate an amplicon of 220-bp length. PCR conditions were identical for both primer pairs: each 25 µL reaction included 2 µl of DNA extract (10 pmol), 5x PCR Master Mix, Screen-mix (HS-5x), 0.5 µM each primer, 17 µl water.

PCR-products purification. Added a 1:1 volume of Binding Buffer to completed PCR mixture (e.g. for every 100 µL of the reaction mixture, add 100 µL of Binding Buffer). Mixed thoroughly. Transferred up the solution to the GeneJET purification column. Centrifuged for 30—60 s. discarded the flow-through. Added 700 µL of Wash Buffer to the GeneJET purification column. Centrifuged for 30—60 s. discarded the flow-through and place the purification column back into the collection tube. Then centrifuged the empty GeneJET purification column for an additional 1 min after that transferred the GeneJET purification column to a clean 1.5 mL micro centrifuged tube. Added 50 µL of Elution Buffer to the center of the GeneJET purification column membrane and centrifuged for 1 min.

Sequencing. Sequencing was done by the generally accepted protocol with the use of Genetic Analyzer AB-3500 (Applied Biosystems, USA). Primary comparison for the results of the sequence with the GeneBank genetic sequence database was performed by the NCBI BLAST web site (<http://www.ncbi.nlm.nih.gov/BLAST>). BioEdit v.7.0.5.3, sequence alignment editor was used for sequence checking, alignment, and editing.

Sanger Dideoxy directly sequenced PCR products in both directions. Sequences of forward and reverse DNA strands were then edited and aligned manually using SEAVIEW

software. MEGA6 and SEAVIEW were used for sequence analysis. Blast searches at NCBI were performed for species identification. For multiple sequence alignment, complete COI gene sequences of some dipteran insects were collected from NCBI and their list is shown in Table 2. Sequences were aligned using CLUSTAL W. For phylogenetic analysis, MEGA6 and SEAVIEW were used. A neighbor-joining tree was constructed using MEGA6 to observe phylogenetic relationships of this fly with another dipteran genus. For protein coding nucleotide sequences, genetic code was selected for “invertebrate mitochondrial” and a number of bootstrap replicons was set to 100.

Table 2

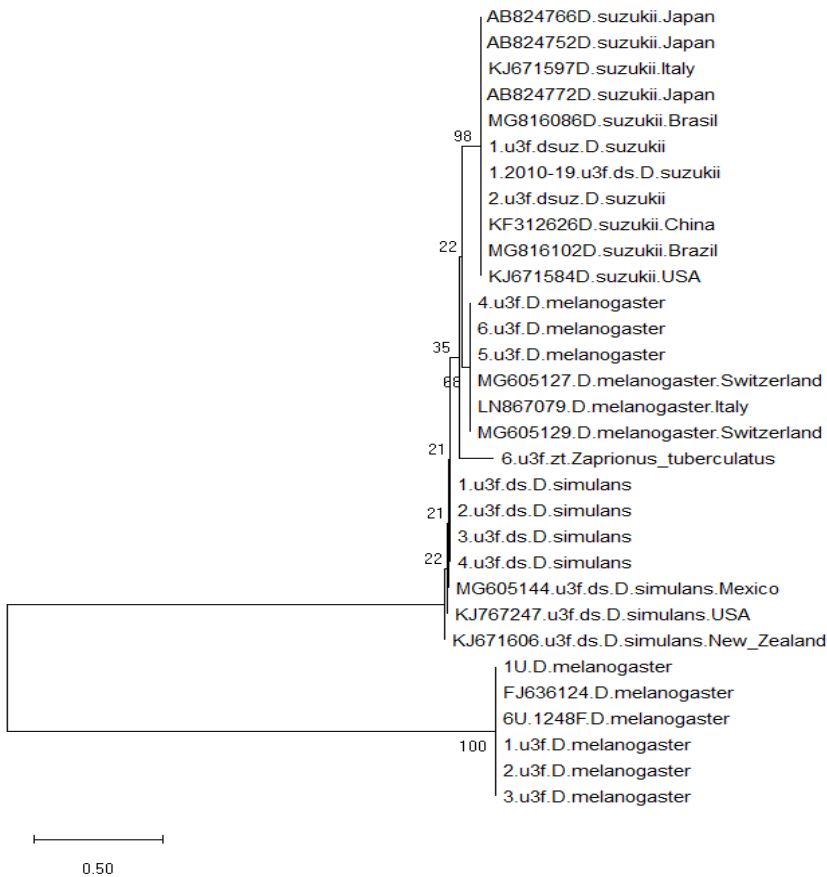
A list of sequences for the present study

No	Species and Accession number	Country	Data from laboratory of Russian quarantine	Data from GenBank
1	1U.D. <i>Melanogaster</i>	Turkey	✓	
2	6U.1248F.D. <i>melanogaster</i>	Egypt	✓	
3	1.u3f.D. <i>melanogaster</i>	Turkey	✓	
4	2. u3f.D. <i>melanogaster</i>	Egypt	✓	
5	3. u3f.D. <i>melanogaster</i>	Turkey	✓	
6	4. u3f.D. <i>melanogaster</i>	Turkey	✓	
7	5. u3f.D. <i>melanogaster</i>	Turkey	✓	
8	6. u3f.D. <i>melanogaster</i>	Mexico	✓	
9	MG605127.D. <i>melanogaster</i>	Switzerland		✓
10	LN867079.D. <i>melanogaster</i>	Italy		✓
11	MG605129.D. <i>melanogaster</i>	Switzerland		✓
12	FJ636124.D. <i>melanogaster</i>	Switzerland		✓
13	1.u3f.ds.D. <i>Simulans</i>	Turkey	✓	
14	2.u3f.ds.D. <i>Simulans</i>	Iran	✓	
15	3.u3f.ds.D. <i>Simulans</i>	Turkey	✓	
16	4.u3f.ds.D. <i>Simulans</i>	Mexico	✓	
17	MG605144.u3f.ds.D. <i>Simulans</i>	Mexico		✓
18	KJ767247.u3f.ds.D. <i>Simulans</i>	USA		✓
19	KJ671606.u3f.ds.D. <i>Simulans</i>	New Zealand		✓
20	1.u3f.dsuz.D. <i>Suzukii</i>	Canada	✓	
21	2.u3f.dsuz.D. <i>Suzukii</i>	Turkey	✓	
22	1.2010–19.u3f.ds.D. <i>Suzukii</i>	Mexico	✓	
23	AB824772D. <i>Suzukii</i>	Japan		✓
24	KJ671597D. <i>Suzukii</i>	Italy		✓
25	AB824766D. <i>Suzukii</i>	Japan		✓
26	AB824752D. <i>Suzukii</i>	Japan		✓
27	KF312626D. <i>Suzukii</i>	China		✓
28	MG816102D. <i>Suzukii</i>	Brazil		✓
29	MG816086D. <i>Suzukii</i>	Brazil		✓
30	KJ671584D. <i>Suzukii</i>	USA		✓
31	6.u3f.zt. <i>Zaprionus Tuberculatus</i>	Mexico	✓	

Results and Discussion

Drosophila melanogaster is a small, common fly found near unripe and rotten fruit. It has been in use for over a century to study genetics and behavior [1, 22–27]. Thomas Hunt Morgan was the preeminent biologist studying *Drosophila* early in the 1900s. He was the first to discover sex linkage and genetic recombination, which placed the small fly at the forefront of genetic research. Due to its small size, ease of culture and short generation time, geneticists have been using *Drosophila* ever since. Fruit flies are easily obtained from the wild and many biological science companies carry a variety of different mutations [8–29]. In addition, these companies sell any equipment needed to culture

the flies. Costs are relatively low and most equipment can be used year after year. There is a variety of laboratory exercises one could purchase, although the necessity to do so is questionable. Compared to GenBank entries using BLAST, the sequences produced from PCR products obtained by testing field-collected predators were used to verify target DNA amplification. All matches for the *Drosophila* spp [30]. Primers consisted of *Drosophila* sequences with a similarity of 98...100 %. As mentioned, correct identification of them is almost unlikely unless they are brought up to adults. This can be a high-risk operation, as many facilities around the world may not have the quarantine protection needed to rear pests like *D. melanogaster* and egg rearing failure may be very high. However, the long-term portion of identification rearing may be challenging when fresh produce worth hundreds of thousands to millions of dollars is at stake. In such cases, the solution may be given by molecular recognition techniques [7, 31, 32]. For several decades, polymerase chain reaction (PCR)-based approaches have been used to classify pests and diseases worldwide. Results showed that these primers accurately identify the area of the gene as well as the particular region of *D. melanogaster*. When samples are too poorly maintained for adequate morphological identification or when only immature specimens are available, the suggested PCR molecular diagnosis can be used as a quick and efficient identification method. For this economically important invasive species, a different identification strategy may allow for more precise monitoring and detection and may prevent misidentification (fig.).



Conclusion

In conclusion, with our selection of DNA, we optimized the PCR process with Russian chemistry. Created here is suitable for regular use by diagnostic and research organizations to promote exports and imports, as well as globally reducing and monitoring the spread of this pest by border security organizations. This assay provides a quick, accurate and precise alternative methods to the identification *D. melanogaster*. Because PCR machines are accessible in a 96-well or other configuration, this technique is suitable for high-performance applications that are often needed during large-scale studies of infestation during an incursion. This assay has been fully optimized for instant jobs in the Russian Federation. We recommend pre-deployment testing in places outside Moscow to guarantee that no false positive is detected, although this would be extremely unlikely.

References

1. Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, et al. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*. 2015; 88(3):469–494. doi: 10.1007/s10340-015-0681-z
2. Kanzawa T. Studies on *Drosophila suzukii* mats. Kofu: Yamanashi Agricultural Experimental Station; 1939.
3. Burrack HJ, Fernandez GE, Spivey T, Kraus DA. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore. *Pest Management Science*. 2013; 69(10):1173–1180. doi: 10.1002/ps.3489
4. Parchami-Araghi M, Pont AC, Gilasian E, Basavand F, Mousavi H. First Palaearctic record of the genus *Pygophora* Schiner, 1868 (Diptera: Muscidae) from Iranian Baluchestan. *Zoology in the Middle East*. 2017; 63(3):280–282. doi: 10.1080/09397140.2017.1331589
5. Naserzadeh Y, Mahmoudi N, Pakina E, Wase M, Anne I, Heydari M, et al. Parameters Affecting the Biosynthesis of Gold Nanoparticles Using the Aquatic Extract of *Scrophularia striata* and their Antibacterial Properties. *Journal of Nanoanalysis*. 2019; 6(2):105–114. doi: 10.22034/JNA.2019.667091
6. Mans DR, Sairras S, Ganga D, Kartopawiro J. Exploring the global animal biodiversity in the search for new drugs–insects. *J Transl Sci*. 2016; 3(1):371–386. doi: 10.15761/JTS.1000164
7. Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, et al. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*. 2011; 2(1): G1–G7. doi: 10.1603/IPM10010
8. Nikolouli K, Colinet H, Renault D, Enriquez T, Mouton L, Gibert P, et al. Sterile insect technique and *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. *Journal of pest science*. 2018; 91(2):489–503. doi: 10.1007/s10340-017-0944-y
9. Rota-Stabelli O, Ometto L, Tait G, Ghirrotto S, Kaur R, Drago F, et al. Distinct genotypes and phenotypes in European and American strains of *Drosophila suzukii*: implications for biology and management of an invasive organism. *Journal of Pest Science*. 2020; 93(1):77–89. doi: 10.1007/s10340-019-01172-y
10. Diepenbrock LM, Burrack HJ. Variation of within crop microhabitat use by *Drosophila suzukii* (Diptera: Drosophilidae) in blackberry. *Journal of Applied Entomology*. 2016; 141(1–2):1–7. doi: 10.1111/jen.12335
11. Poyet M, Eslin P, Héraude M, Le Roux V, Prévost G, Gibert P, et al. Invasive host for invasive pest: when the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. *Agricultural and forest entomology*. 2014; 16(3):251–259. doi: 10.1111/afe.12052
12. Correa SC, Wille CL, Hoffer H, Boff MI, Franco CR. Oviposition preference and biology of fruit flies (Diptera: Tephritidae) on grape vine genotypes. *Revista Caatinga*. 2018; 31(4):850–859. doi: 10.1590/1983-21252018v31n407rc
13. Hauser M. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest management science*. 2011; 67(11):1352–1357. doi: 10.1002/ps.2265
14. Landolt PJ, Adams T, Rogg H. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *Journal of Applied Entomology*. 2012; 136(1-2):148–154. doi: 10.1111/j.1439-0418.2011.01646.x

15. Nestel D, Nemny-Lavy E, Chang CL. Lipid and protein loads in pupating larvae and emerging adults as affected by the composition of Mediterranean fruit fly (*Ceratitis capitata*) mericid larval diets. *Archives of Insect Biochemistry and Physiology*. 2004; 56(3):97–109. doi: 10.1002/arch.20000
16. Chang CL. Effect of amino acids on larvae and adults of *Ceratitis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America*. 2004; 97(3):529–535. doi: 10.1603/0013–8746(2004)097[0529:EOAOL]2.0.CO;2
17. Rashid MA, Andongma AA, Dong YC, Ren XM, Niu CY. Effect of gut bacteria on fitness of the Chinese citrus fly, *Bactrocera minax* (Diptera: Tephritidae). *Symbiosis*. 2018; 76(1):63–69. doi: 10.1007/s13199-018-0537-4
18. Green L, Battlay P, Fournier-Level A, Good RT, Robin C. Cis- and trans-acting variants contribute to survivorship in a naïve *Drosophila melanogaster* population exposed to ryanoid insecticides. *Proceedings of the National Academy of Sciences*. 2019; 116(21):10424–10429. doi: 10.1073/pnas.1821713116
19. Lachaise D, Cariou ML, David JR, Lemeunier F, Tsacas L, Ashburner M. Historical biogeography of the *Drosophila melanogaster* species subgroup. In: Hecht MK, Wallace B, Prance GT. (eds). *Evolutionary biology*. Boston, MA: Springer; 1988. p.159–225. doi: 10.1007/978–1–4613–0931–4_4
20. Lee CH, Rimesso G, Reynolds DM, Cai J, Baker NE. Whole-genome sequencing and iPLEX MassARRAY genotyping map an EMS-induced mutation affecting cell competition in *Drosophila melanogaster*. *G3: Genes, Genomes, Genetics*. 2016; 6(10):3207–3217. doi: 10.1534/g3.116.029421
21. Lillesaar C, Gaspar P. Serotonergic Neurons in Vertebrate and Invertebrate Model Organisms (Rodents, Zebrafish, *Drosophila melanogaster*, *Aplysia californica*, *Caenorhabditis elegans*). In: Pilowsky PM. (ed.) *Serotonin*. Academic Press; 2019. p.49–80. doi: 10.1016/B978–0–12–800050–2.00003–6
22. Lynch ZR, Schlenke TA, de Roode JC. Evolution of behavioural and cellular defences against parasitoid wasps in the *Drosophila melanogaster* subgroup. *Journal of Evolutionary Biology*. 2016; 29(5):1016–1029. doi: 10.5061/dryad.5t5m4
23. Mahmoudi N, Naserzadeh Y, Pakina EN, Limantceva LA, Nejad DK. Molecular identification of *Ditylenchus destructor* nematode with PCR Species-Specific primers in the Moscow region. *RUDN Journal of Agronomy and Animal Industries*. 2019; 14(4):430–436. doi: 10.22363/2312–797X-2019–14–4–430–436
24. Melcarne C, Ramond E, Dudzic J, Bretscher AJ, Kurucz É, Andó I, et al. Two Nimrod receptors, NimC1 and Eater, synergistically contribute to bacterial phagocytosis in *Drosophila melanogaster*. *The FEBS Journal*. 2019; 286(14):2670–2691. doi.org/10.1111/febs.14857
25. Naserzadeh Y, Kartoolinejad D, Mahmoudi N, Zargar M, Pakina E, Heydari M, et al. Nine strains of *Pseudomonas fluorescens* and *P. putida*: Effects on growth indices, seed and yield production of *Carthamus tinctorius* L. *Research on Crops*. 2018; 19(4):622–632. doi: 10.31830/2348–7542.2018.0001.39
26. Naserzadeh Y, Nafchi AM, Mahmoudi N, Nejad DK, Gadzhikurbanov ASH. Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran. *RUDN Journal of Agronomy and Animal Industries*. 2019; 14(3):209–224. doi: 10.22363/2312–797X-2019–14–3–209–224
27. Yassin A, Debat V, Bastide H, Gidaszewski N, David JR, Pool JE. Recurrent specialization on a toxic fruit in an island *Drosophila* population. *Proceedings of the National Academy of Sciences*. 2016; 113(17):4771–4776. doi: 10.1073/pnas.1522559113
28. Naserzadeh Y, Mahmoudi N, Pakina E. Antipathogenic effects of emulsion and nanoemulsion of cinnamon essential oil against *Rhizopus* rot and grey mold on strawberry fruits. *Foods and Raw Materials*. 2019; 7(1):210–216. doi: 10.21603/2308-4057-2019-1-210-216.

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Идентификация *Drosophila melanogaster* методом полимеразной цепной реакции

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Аннотация. *D. melanogaster* — одна из наиболее вредоносных плодовых мух, повреждающих цитрусовые и многие другие сельскохозяйственные растения. Молекулярный метод диагностики был создан для идентификации *D. melanogaster* от другого некарантинного вида *Drosophila* spp. Предложенный метод дифференциации заключается в использовании фрагмента гена цитохромоксидазы субъединицы 1 митохондриальной ДНК размером 709 п. н. С помощью праймеров Droso-S391 и Droso-A381 были амплифицированы образцы ДНК *D. melanogaster*, *D. suzukii* и *D. Simulans* в лабораторных образцах из разных стран, далее их сравнили с последовательностями других таксонов *Drosophila* из базы GenBank. Результаты проведения полимеразной цепной реакции (ПЦР) на основе полиморфизма нуклеотидных последовательностей показали, что данные праймеры точно идентифицируют участок гена, также как и специфические праймеры *Drosophila melanogaster*.

Ключевые слова: идентификация, диагностика, *Drosophila melanogaster*, полимеразная цепная реакция, ПЦР, карантин растений

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