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Loop-mediated isothermal amplification of economically important *Ceratitis* species (Diptera: Tephritidae)

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Ceratitis is an economically important genus of fruit flies that originated in Africa, has a wide host range, and causes serious economic losses due to its invasive damage. As a result, it is critical to identify them accurately and quickly in the world. Loop-mediated isothermal amplification (LAMP), as one of the representatives of isothermal amplification technology, has been widely used in the rapid nucleic acid detection of human pathogens and has shown its advantages in the identification of insect agricultural pests. In this study, using the mitochondrial *cox1* and *cob* genes as target genes, the rapid molecular identification of the *Ceratitis* FARQ complex, *C. cosyra,* and *C. capitata* was realized based on LAMP. The experimental conditions optimization results showed that F3/B3:FIP/BIP = 1:8 was the optimal primer concentration ratio and 63 °C was the optimal reaction temperature. The sensitivity of the primers obtained in this study can reach up to 0.01 ng/μl DNA. A loop-mediated isothermal amplification identification technology system was established based on rapid, rough DNA extraction and visual detection of *Ceratitis* economically important fruit flies. The positive reaction system changed from pink to khaki by visual detection. The identification flow can be completed within 1 hour, including sample processing, DNA extraction, and LAMP visual detection.

Key words Ceratitis, loop-mediated isothermal amplification, identification

Introduction

Tephritid fruit flies (Diptera: Tephritidae) are important agricultural and quarantine pests. There are about 500 genera and 4,500 species worldwide. They include about 250 species of economic importance, which are classified into 6 main genera, namely *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis*, and *Zeugodacus*. (Li et al. 2013a, b). Fruit flies have a wide number of hosts, mostly infesting the fruit-bearing organs. For those found in fruits and vegetables, the female adults lay eggs under the skin of the hosts, and the hatched larvae feed inside them (Ismay 1992), which seriously impedes agricultural production and international trade. Because of this, they have received extensive phytosanitary attention worldwide (Christenson and Foote 1960, Liang et al. 2008, Li et al. 2013). Therefore, in order to reduce their impact via early and accurate detection, the development of rapid and accurate identification technologies is crucial.

Ceratitis, one of the 6 economically important fruit fly genera, includes as major pests *C. capitata* (Wiedemann, 1824), *C. cosyra* (Walker, 1849), and the *Ceratitis* FARQ complex (*C. fasciventris* Bezzi, 1920, *C. anonae* Graham, 1908, *C. rosa* Karsch, 1887, *C. quilicii* De Meyer, Mwatawala & Virgilio, 2016). The *Ceratitis* species are of Afrotropical origin (De Meyer 2001) and have a significant effect on the commercial production and export of fruits. *Ceratitis capitata*, the Mediterranean fruit fly, is the most notorious species in this genus (White et al. 2000). It has been introduced in different regions and is now widely distributed in the world. The other species are restricted to Africa. *Ceratitis cosyra*, also known as the mango fruit fly, has a serious impact on the mango industry, mainly in sub-Saharan Africa (Virgilio et al. 2017). The *Ceratitis* FARQ complex consists of 4 morphologically closely related species: *C. fasciventris*, *C. anonae*, *C. rosa*, and *C. quilicii* (Barr and McPheron 2006, Virgilio et al. 2013, De Meyer et al. 2016). *Ceratitis rosa* and *C. quilicii* are the most important agricultural pests in this complex, and can damage 90 different crops in 25 families (De Meyer et al. 2002, Copeland et al.2006). Because of the economic importance

of these *Ceratitis* fruit flies, their accurate and rapid identification is very important for early quarantine and pest management.

In recent decades, molecular biology techniques have been continuously explored for the identification of fruit flies. These techniques include AFLP (amplified fragment length polymorphism) (Kakouli-Duarte et al., 2001), RFLP (restriction fragment length polymorphism) (Chua et al. 2010, Mezghani Khemakhem et al. 2013), RAPD (random amplified polymorphic DNA) (Sonvico et al. 1996, Zhang et al. 2004), DNA barcoding techniques (Zhang et al. 2019, Li et al. 2022), species-specific PCR and qPCR techniques (Jiang et al. 2016, Koohkanzade et al. 2018, Zheng et al. 2019), etc. These tools are generally cumbersome, require rather expensive equipment and setup, and are generally time consuming. This requires technical personnel who have been trained and skilled in basic molecular experimental operating skills and is not suitable for quarantine testing at port sites. So, it is urgent to develop more fast, accurate, and convenient identification procedures.

Loop-mediated isothermal amplification (LAMP) is an in vitro isothermal DNA amplification technology (Notomi 2000) . LAMP can be implemented at constant temperature (generally 60–65 °C for 30–60 min) and relies on the use of 4–6 specific primers, which generally allow amplifying 6 regions of a target gene. Amplification is promoted by the DNA polymerase Bst (i.e., from *Bacillus stearothermophilus*) and produces large amounts of DNA (Nagamine et al. 2002). Usually, results for LAMP identification are visualized via agarose gel electrophoresis. Hydroxynaphthol blue (HNB) is commonly used as a metal ion indicator for LAMP visual detection, after heating for less than 1 h, and following color change of the amplification mixture (Huang and Weng 2015). In recent years, scientists have used LAMP to rapidly identify tephritid flies such as *Dacus ciliatus* Loew 1862, *Zeugodacus scutellatus* (Hendel, 1912), *Bactrocera tryoni* (Froggatt 1897), among others (Sabahi et al. 2018, Blacket et al. 2020, Kitano and Takakura 2020). The first LAMP identification of Tephritidae was achieved in 2009, who succeeded in distinguishing *C. capitata* from 3 other genera of fruit flies within 45 min (Huang et al. 2009).

In our study, we focused on economically significant species including *C. capitata*, *C. cosyra*, and the *Ceratitis* FARQ complex. Based on the mitochondrial *cox1* gene and referencing 986 mitochondrial genome sequences of 58 *Ceratitis* species on NCBI, as well as the sequences obtained from our previous sequencing (Zhang et al. 2019), we designed and screened species-specific LAMP primers. Based on these primers, we have established a rapid and efficient visual identification system for *Ceratitis* fruit flies.

Materials and Methods

Sample Collection and DNA Extraction

All *Ceratitis* specimens used in this study were obtained from collections in the Royal Museum of Central Africa (Tervuren, Belgium). Each sample was reidentified by examining traditional taxonomic characteristics through an Olympus CX31 optical microscope, following the descriptions of Wu (2009) and Virgilio et al. (2014). Genomic DNA was subsequently extracted from individual samples using a TIANamp Micro DNA Kit (Tiangen, Beijing, China) and assessed for quality using a Quawell UV-Vis Spectrophotometer Q5000 (USA). Detailed information for each sample can be found in Table 1.

Table 1. Samples information of fruit flies for LAMP technique research

No.	Species	Geographic origin
1	*C. fasciventris*	Nyarubanga, Burundi (4.219 °S, 29.993 °E)
2	*C. anonae*	Bikok, Cameroon (3.642 °N, 11.532 °E)
3	*C. rosa*	Morogoro, Tanzania (6.955 °S, 37.535 °E)
4	*C. quilicii*	Mlangali, Tanzania (7.043 °S, 37.574 °E)
5		Ruiru, Kenya (1.148 °S, 36.960 °E)
6		Mpumalanga, South Africa (23.988 °S, 31.555 °E)
7	*C. rubivora*	Mlangali, Tanzania (7.043 °S, 37.574 °E)
8	*C. cosyra*	Mpumalanga, South Africa (23.988 °S, 31.555 °E)
9		Cuamba, Mozambique (14.816°S, 36.535°E)
10		Nasarawa, Nigeria (9.082 °N, 8.675 °E)
11		Ruiru, Kenya (1.148 °S, 36.960 °E)
12	*C. capitata*	Mpumalanga, South Africa (23.988 °S, 31.555 °E)
13		Ruiru, Kenya (1.148 °S, 36.960 °E)
14		Kabare, Congo (2.23 °S, 28.83 °E)
15	*C. quinaria*	Singa, Sudan (13.145 °N, 33.927 °E)

Primer Design

Due to their rapid mutation rate and short coalescence time, mitochondrial genomes have been established as powerful genetic markers for studying phylogenetic relationships and species diagnosis (Harrison, 1989). Therefore, in our primer design, we specifically targeted the 13 protein-coding genes within the mitochondrial genome to develop *Ceratitis*-specific LAMP primers. We obtained 986 mitochondrial genome sequences of 58 *Ceratitis* species from both GenBank (https://www.ncbi.nlm.nih.gov/) and the Barcode of Life Data System (https://www.boldsystems.org/). The primers were chosen by considering 2 factors: sufficient representation of various species and a homologous sequence database. This ensured that the primers are conserved within each species and specific between different species, thus increasing the accuracy of the designed primer sets. Since *cox1* sequences were the most abundant among publicly available sequences, we prioritized designing LAMP primers for the *cox1* gene. To reduce the risk of suboptimal primer design for *cox1*, we screened alternative/complementary sites in the remaining 12 mitochondrial genes and noncoding regions.

The sequences of all available *Ceratitis* homologous genes were multiple sequence aligned in MEGA 7 (Kumar et al. 2016). The intraspecific conserved and specific loci of the target fruit fly species were manually screened, as marked in Geneious 10.1.3 (Kearse et al. 2012). Primer Explore V4 (PrimerExplore V4 2003) (http://primerexplorer.jp/elamp4.0.0/index.html) was used for the design of 6 LAMP primer pair. (F1c/B1c = 20–28 bp, F2/B2 = 18–25 bp, F3/B3 = 20–28 bp; Tm: F1c/B1c = 59–61 °C, F2/B2 = 54–56 °C, F3/B3 = 54–56 °C; GC rate = 40–50%) (Zhao and Zou 2007). Alternative primers to F2/B2 for the amplification of the 3' end of gene were also obtained inPRIMER EXPLORE (settings: |5'dG|>4, |3'dG|>4, |dimer dG|< 2) and tested. The primers were synthesized by Tsingke Biological Technology (Beijing, China).

Primer Specificity Test

The specificity of LAMP primers was tested by 2% agarose gel electrophoresis. As a positive control the genomic DNA of a single *Ceratitis* fruit fly from each geographic region was used, and negative controls were DNA samples from other *Ceratitis* species and ddH_2O. Primers were considered as diagnostic only when generating positive reactions for all samples from a species, and for none of the other species. The total volume of LAMP amplification is 25.0 μL, including Warm Start LAMP 2 × Master Mix E1700S (New England Biolabs, MA, US). 12.5 μL, 0.5 μL each of 10 μM F3 and B3 primers, 4.0 μL 10 μM each of FIP (Forward Inner Primer) and BIP (Backward Inner Primer), template DNA 1.0 μL, ddH_2O 2.5 μL. After incubation at 63 °C for 40 min, the reaction was terminated

at 85 °C for 3 min. After the amplification, 5 µL products were added into agarose gel electrophoresis channels for 30 min at 220 V. Then, examine the results under UV light using Gel Logic 212 PRO (Carestream Kodak, USA) to determine whether the primers were specific or not. The experiments were at least repeated 3 times.

Optimization of LAMP Reaction Conditions

Referred to the LAMP primer ratio and amplification temperatures (Li et al. 2020), and used the WarmStart LAMP Kit (DNA and RNA) E1700S (New England Biolabs Inc., MA, USA) to optimize the *Ceratitis* LAMP reaction systems. The optimal concentration ratio was screened from the primer concentration ratio (F3/B3:/BIP = 1:4, 1:6, 1:8, 1:10), and the optimal reaction temperature was screened from 4 reaction temperature conditions, including 61, 63, 65, and 67 °C for 40 min; the reaction ended at 85 °C for 3 min. The detection method was the same as in the primer specificity test. The experiments were at least repeated 3 times. Results are shown in Supplementary File (Supplementary Fig. S1).

Primer Sensitivity Test

After selecting LAMP-specific primers and determining the optimal primer concentration ratio and reaction temperature, we tested the sensitivity of LAMP primers by changing the concentration of template DNA through gradient dilution. We used ddH_2O as a negative control to detect whether the LAMP reaction could effectively amplify template DNA at concentrations of 10.0, 1.0, 0.1, and 0.01 ng/µl. The detection method was similar to that used in the primer specificity test. The experiments were at least repeated 3 times. Results are presented in Supplementary File (Supplementary Fig. S2).

LAMP Visual Detection

In the LAMP visual detection test, the total reaction volume is 40.0 µl, including WarmStart Colorimetric LAMP 2 × Master Mix M1800S (New England Biolabs, MA, US) 25.0 µl, 0.5 µl each of 10 µM F3 and B3 primers, 4.0 µl each of FIP and BIP primers, template DNA 2.0 µl, ddH_2O 4 µl. The mixture was incubated at 63 °C for 40 min, and after the amplification, we observed the color change to judge whether the visual detection could be effectively realized. The experiments were at least repeated 3 times.

LAMP Detection System Based on Rapid DNA Extraction

To expedite testing, we referred to a rapid DNA extraction method (Kitano and Takakura 2020)). First, a portion of thoracic muscle tissue from a single fruit fly specimen was taken and placed in a 0.2-ml centrifuge tube. Then, 30 µl of TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA pH 8.0) was added, and the sample was thoroughly ground. After grinding, the centrifuge tube was placed in a water bath at 95 °C for 3 minutes for rapid extraction of DNA, which was present in the supernatant. We took 2.0 µl of the supernatant from the above reaction as a DNA template for LAMP visual detection. The LAMP reaction system was the same as that in the LAMP visual detection. The experiments were at least repeated 3 times.

Results

Specific Primer Design and Screening Results

LAMP-specific primers of the *Ceratitis* FARQ complex were designed and screened on the *cob* gene, and the specific primers of *C. cosyra* and *C. capitata* were designed and screened on the *cox1* gene (Fig. 1). Primer sequences and lengths are provided in Table 2.

Specificity Test

The specificities of the 3 groups of primers in Table 2 were detected, by taking the *Ceratitis* FARQ complex, *C. cosyra*, and *C. capitata* as positive controls, and 7 other different species and geographical populations of *Ceratitis* fruit flies listed in Table 1 as negative controls. The results of agarose gel electrophoresis showed evident trapezoidal strips, *Ceratitis*-specific LAMP primers sets showed strong specificity to the target samples (Fig. 2).

Optimization of the LAMP Reaction

The *Ceratitis* FARQ complex LAMP-specific primer pair FARQ-F3/FARQ-B3/FARQ-FIP/FARQ-BIP designed and screened in this study was used to match the *C. fasciventris*, *C. anonae*, *C. rosa*, and *C. quilicii* samples at the respective primer concentrations. The LAMP amplification reaction was carried out under 4 conditions of ratio F3/B3: FIP/BIP = 1:4, 1:6, 1:8, and 1:10, and the results of the products detected by agarose gel electrophoresis indicated that the samples of *C. fasciventris*, *C. anonae*, *C. rosa*, and *C. quilicii* can be successfully amplified under the conditions of the 4 primer concentration ratios. And under the condition that the concentration ratio of F3/B3:FIP/BIP is 1:8, and the electrophoresis band is the clearest and brightest, that means the amplification efficiency is the highest. The other 2 fruit flies, *C. capitata* and *C. cosyra*, also showed the same result (Supplementary Fig. S1).

Furthermore, the effects of various reaction temperatures (61, 63, 65, and 67 °C) were also examined. The results of agarose gel electrophoresis showed that samples of *C. fasciventris*, *C. anonae*, *C. rosa*, and *C. quilicii* could be successfully amplified at 3 reaction temperatures: 61, 63, and 65 °C. And at the reaction temperatures of 61 and 63°C, the amplification efficiency is the highest. This is basically the same result as the screening of the optimal reaction temperature for *C. cosyra* and *C. capitata* (Supplementary Fig. S2). Therefore, the conditions of ratio F3/B3: FIP/BIP = 1:8 and a reaction temperature of 63 °C were used in the experiments.

Sensitivity Test

The samples of *C. fasciventris*, *C. anonae*, *C. rosa*, and *C. quilicii* were serially diluted to 10.0, 1.0, 0.1, and 0.01 ng/µl, using the *Ceratitis* FARQ complex LAMP-specific primer pairs of FARQ-F3/FARQ-B3/FARQ-FIP/FARQ-BIP to carry out LAMP amplification reactions, respectively. The results of agarose gel electrophoresis showed that the 4 fruit fly samples could be successfully amplified at the 4 concentrations, showing a typical ladder-shaped band, but the amplification efficiency was lower at the concentration of 0.01 ng/µl; the bands are dim and unclear. Therefore, for the sensitivity of the LAMP primer for the *Ceratitis* FARQ complex, when the concentration of template DNA reaches 0.1 ng/µl, a bright and clear ladder-like band can be produced. As for *C. capitata* and *C. cosyra*, their primers' highest sensitivity can reach up to 0.1 and 0.01 ng/µl (Supplementary Fig. S3). These results imply that the 3 groups of LAMP primers could be used for *Ceratitis* with rather low DNA concentrations.

LAMP Visual Detection

Furthermore, visual detection was developed to make it easier to observe LAMP outcomes in realistic applications. After amplifying the corresponding positive samples with specific LAMP primers, the color of the 25 µl LAMP reaction system single-tube changed from pink to khaki, while the negative control remained pink (Supplementary Fig. S4). The results showed that the 3 groups of specific primers all achieved visual detection, thus making the practical application of LAMP possible.

Fig. 1. Alignment of sequences of *Ceratitis*-specific LAMP primer sets. The LAMP primers are shown as directions (5′–3′), wherein primer F1c and primer F2 constitute the upstream inner primer FIP, and primer B1c and primer B2 constitute the downstream inner primer BIP. A) The position of LAMP primers of Ceratitis FARQ complex in cob gene. B) The position of LAMP primers of *C. cosyra* in cox1 gene. C) The position of LAMP primers of *C. capitata* complex in cox1 gene.

Table 2. Three *Ceratitis* economically important fruit flies LAMP-specific primers

Species	Primers	Sequences (5′–3′)	Length
Ceratitis FARQ complex	FARQ-F3	GGATTATTCCTTGCTATACATTAC	24 bp
	FARQ-B3	GTTGGGGTGAATAAATAAGATC	22 bp
	FARQ-FIP	TCATCCATAATTTACATCTCGACAACAGCAGATATTAATTTAGCTTTC	48 bp
	FARQ-BIP	ATTACGAACTATACACGCTAACGCATAATACATTCCACGTCCTAC	45 bp
C. cosyra	Ccos-F3	GGGTTTGGAATAATTTCCCATA	22 bp
	Ccos-B3	AGTGTAGCAAGTCAACTGAA	20 bp
	Ccos-FIP	CCAAGTAAACCGATTGCTAATATAGCACAAGAATCAGGGAAAAAGGAGA	49 bp
	Ccos-BIP	TTGTTTGAGCCCACCATATATTTACAGGCAATAATTATTGTTGCTGATGT	50 bp
C. capitata	Ccap-F3	AGGAGCTGTAAATTTTATCACA	22 bp
	Ccap-B3	GTTGGTATAAAATAGGGTCTCC	22 bp
	Ccap-FIP	TGCAGTAAGAACTACTGCTCAAACAACAGTAATTAATATACGATCTACCG	50 bp
	Ccap-BIP	ACCAGTTTTAGCAGGAGCTATTACCTCCAGCTGGGTCAAAGA	42 bp

LAMP Detection Based on Rapid DNA Extraction

After immersing the muscle tissue of *Ceratitis* samples in TE buffer and treating them at 95 °C for 3 min, the supernatant was used as a DNA template. All geographic population samples were subjected to LAMP reactions using the specific primers, and the amplification results can be directly observed with the eyes. The color of the positive control obviously changed from pink to khaki, while the color of the negative control remained pink (Fig. 3).

Discussion

In this study, we achieved accurate and rapid identification of *C. capitata*, *C. cosyra*, and the *Ceratitis* FARQ complex based on LAMP. We validated the outcomes of an optimized reaction system with primer ratios of 1:8 for the inner and outer layers and 63 °C for the reaction temperature. By combining DNA rapid extraction and visualization detection, the identification of the target species can be completed within 1 h, providing a new solution for port and field quarantine.

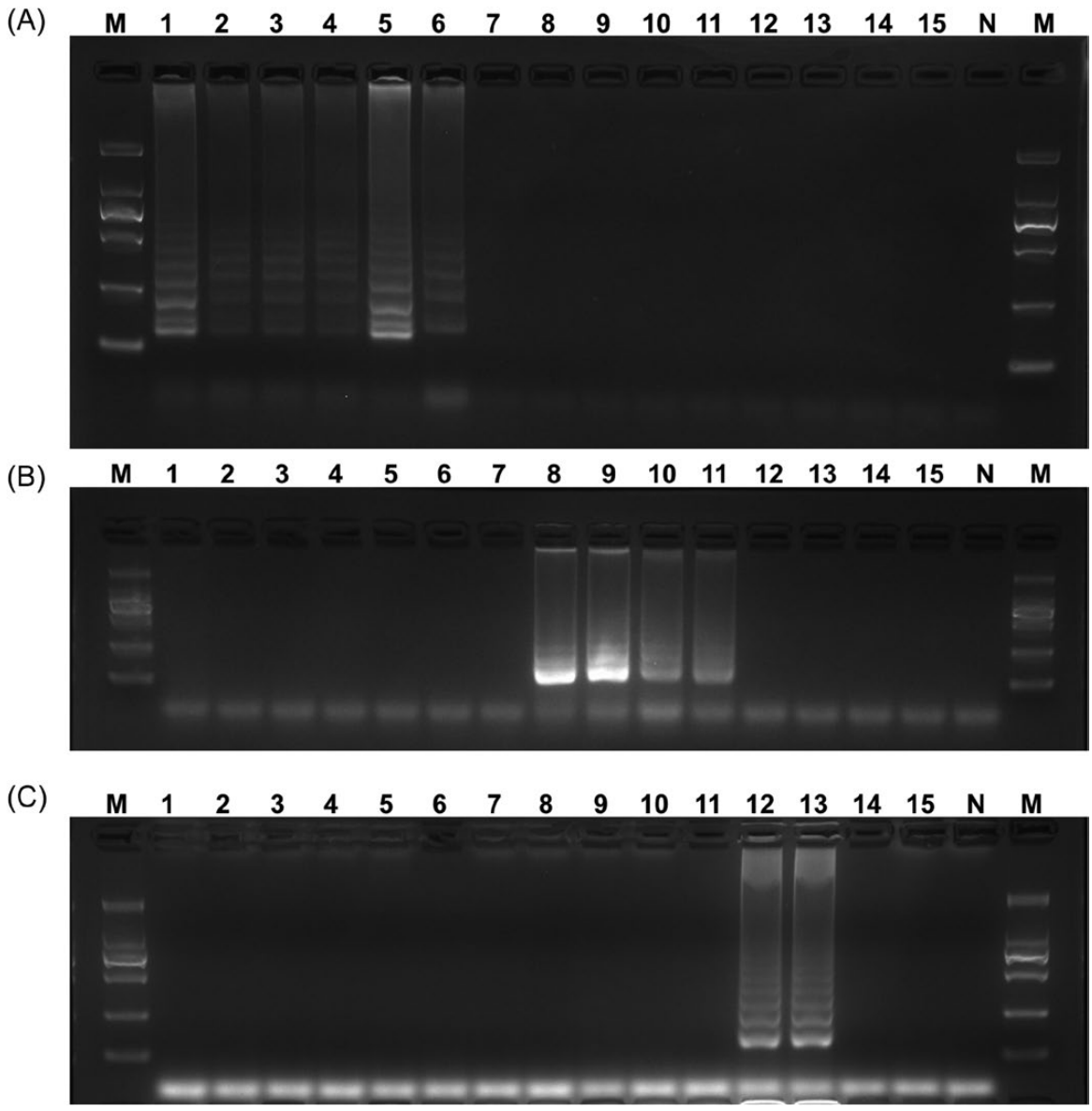

Fig. 2. *Ceratitis* LAMP primers sets specificity verification by agarose gel electrophoresis. Lane M. D2000 Marker, Lane 1. *C. fasciventris* Burundi, Lane 2. *C. anonae* Cameroon, Lane 3. *C. rosa* Tanzania, Lane 4. *C. quilicii* Tanzania, Lane 5. *C. quilicii* Kenya, Lane 6. *C. quilicii* South Africa, Lane 7. *C. rubivora* Tanzania, Lane 8. *C. cosyra* South Africa, Lane 9. *C. cosyra* Mozambique, Lane 10. *C. cosyra* Nigeria, Lane 11. *C. cosyra* Kenya, Lane 12. *C. capitata* South Africa, Lane 13. *C. capitata* Kenya, Lane 14. *C. quinaria* Sudan, Lane 15. *C. quinaria* Sudan, Lane N. negative control. A) *Ceratitis* FARQ complex. B) *Ceratitis cosyra.* C) *Ceratitis capitata.*

In the current quarantine interception process, larvae and pupae of the fruit fly are primarily encountered, which contribute to their long-distance dispersal (Li et al., 2013). However, in our study, we used adult specimens of the fruit fly as the source for DNA extraction, which is consistent with many other studies (Zhong et al. 2019, Kitano and Takakura 2020). This is because the different developmental stages of the insect do not result in genetic sequence variations. The only difference lies in the size of the insect body, which affects the quantity of DNA template obtained, and this is closely related to the sensitivity of the detection technique. In our study, the detection system we established has a sensitivity of up to 0.01 ng/μl, which is sufficient for detecting larvae and insect remnants. Furthermore, it is important to note that the 4 species within the *Ceratitis* FARQ complex can only be distinguished based on subtle morphological differences in adults. It is nearly impossible to differentiate them during the larval stage (De Meyer et al. 2016). Therefore, to ensure the accuracy of the species DNA templates used, we did not conduct tests on larvae.

In terms of visualization detection, previous studies have more often combined calcium yellow-green and HNB with LAMP products to achieve the identification of unknown species by color change visible by the naked eye (Sial et al. 2020, Zeng et al. 2021). In positive reactions, the color changes from brownish-yellowish green to violet-sky blue. However, the difference between these 2 colors is not very obvious, especially when the dye is diluted to 25.0 μl, the color becomes lighter, and the color change is even less obvious (light brown to light yellow-green or lavender to light blue), thus greatly affecting the accuracy and reliability of the results. In this study, the WarmStart Colorimetric LAMP 2 × Master Mix kit from New England Biolabs was used in a visualization assay. Based on

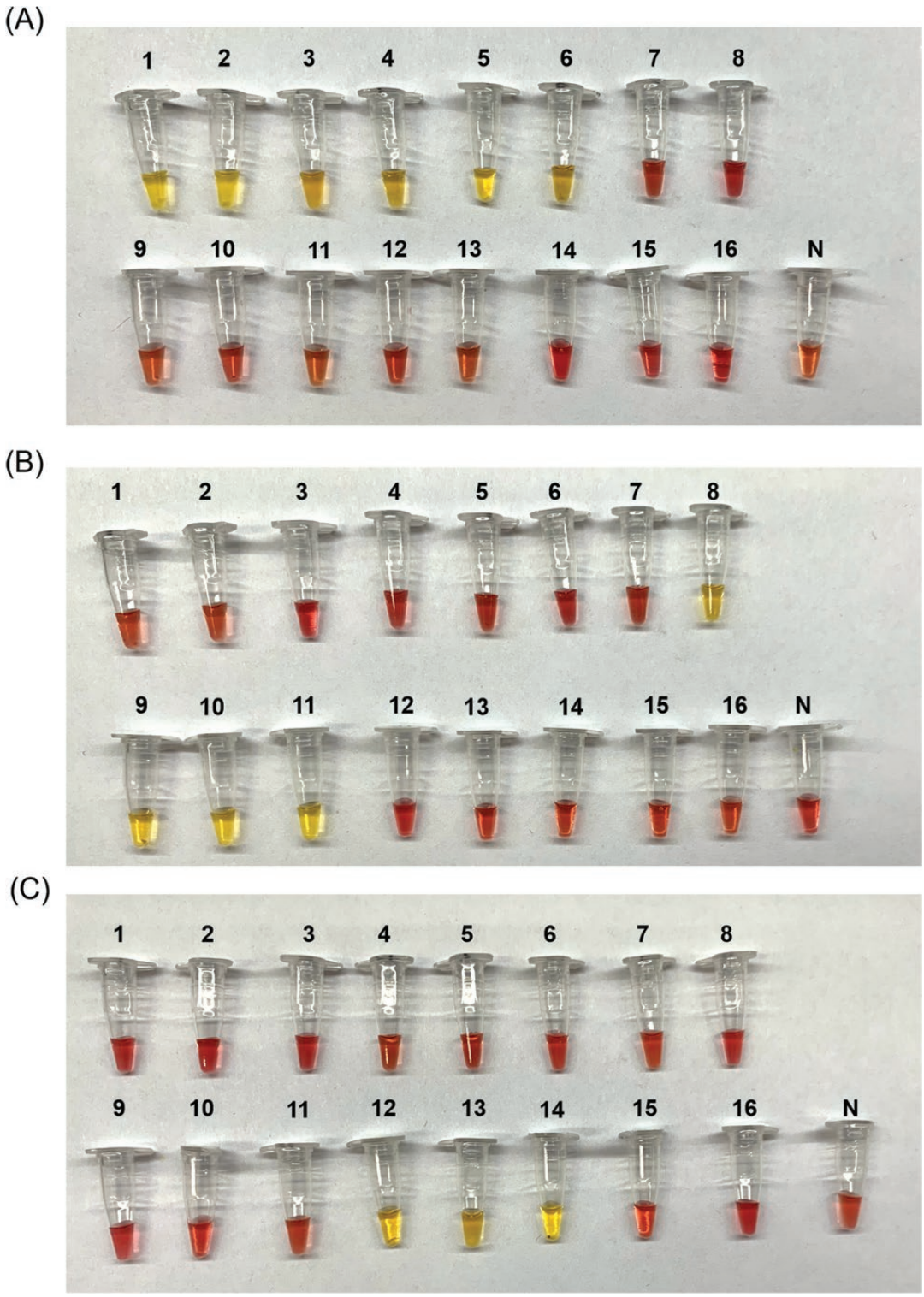

Fig. 3. Visualization of *Ceratitis* LAMP assay based on rapid DNA extraction. PCR tube 1. *C. fasciventris* Burundi, PCR tube 2. *C. anonae* Cameroon, PCR tube 3. *C. rosa* Tanzania, PCR tube 4. *C. quilicii* Tanzania, PCR tube 5. *C. quilicii* Kenya, PCR tube 6. *C. quilicii* South Africa, PCR tube 7. *C. rubivora* Tanzania, PCR tube 8. *C. cosyra* South Africa, PCR tube 9. *C. cosyra* Mozambique, PCR tube 10. *C. cosyra* Nigeria, PCR tube 11. *C. cosyra* Kenya, PCR tube 12. *C. capitata* South Africa, PCR tube 13. *C. capitata* Kenya, PCR tube 14. *C. capitata* Congo, PCR tube 15. *C. quinaria* Sudan, PCR tube 16. *C. quinaria* Sudan, PCR tube N. negative control. A) *Ceratitis* FARQ complex. B) *Ceratitis cosyra.* C) *Ceratitis capitata.*

the change in the PH value of the reaction system, the color changes from pink to earthy yellow and can be easily observed by the naked eye as there is no ambiguous intermediate color.

Although the present study was able to achieve accurate and rapid identification of C. *capitata*, C. *cosyra*, and the *Ceratitis* FARQ complex, effective identification of the 4 species belonging to the FARQ complex was still not possible. Previous studies have shown that the mitochondrial molecular markers *cox1*, *nad6*, and 16S rRNA and the nuclear genomic markers, *period* and ITS1 are also ineffective in recovering the monophyly of the 4 species (Barr and

McPheron 2006, Virgilio et al. 2008, De Meyer et al. 2016). So far, the only molecular marker that can effectively distinguish between the 4 *Ceratitis* FARQ complex species is the set of 16 microsatellite markers (Virgilio et al. 2013), but the phylogenetic relationships of the 4 species within the complex are also unknown. In future research, whole genome resequencing (WGRS) technology will show strong advantages. This technique has been applied to the genetic structure research of fruit fly populations (Zhang et al. 2022). Compared to the limited molecular markers currently used, WGRS can be used to screen among millions of SNPs to effectively distinguish the loci of the 4 species of the *Ceratitis* FARQ complex and design primers based on the screened SNPs to achieve accurate identification of the *Ceratitis* FARQ complex.

In conclusion, we designed 3 sets of LAMP primers to achieve accurate and rapid identification of economically important fruit flies (*C. capitata*, *C. cosyra*, and *Ceratitis* FARQ complex) using LAMP technology. Compared with traditional molecular identification techniques, such as PCR, LAMP technology does not rely on expensive equipment and can take less time to achieve accurate identification of target species and even residues. Combined with the rapid DNA extraction method, our research has improved the level of application, not limited to the port quarantine but also taking into account the field application scenarios, providing a new way of thinking for more agricultural pest workers.

Author Contributions

Yue Zhang (Conceptualization [Equal], Methodology [Equal], Validation [Equal], Writing—original draft [Equal]), Weisong Li (Conceptualization [Equal], Methodology [Equal], Validation [Equal], Writing—original draft [Equal]), Massimiliano Virgilio (Data curation [Equal], Resources [Equal], Supervision [Equal], Writing—review & editing [Equal]), Marc De Meyer (Project administration [Equal], Resources [Equal], Supervision [Equal], Writing—review & editing [Equal]), and Zhihong Li (Conceptualization [Equal], Funding acquisition [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Writing—review & editing [Equal])

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Conflicts of interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material is available at *Journal of Economic Entomology* online.

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