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Characterization and molecular identification of the lumpy skin disease virus in cattle in the Mekong Delta of Vietnam

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ABSTRACT

Background: Lumpy skin disease (LSD) is caused by a virus belonging to the genus *Capripoxvirus*, exhibiting clinical symptoms ranging from mild signs to the development of nodules. LSD emerged in Asia and Southeast Asia, including Vietnam, in October 2020 and has since spread throughout the region, resulting in productivity and economic losses.

Aim: This study aimed to investigate the virus-causing papular dermatitis in cattle from the Mekong Delta region of Vietnam by analyzing its GPCR gene and assessing its evolutionary relationship with sequences in the GenBank database.

Methods: Blood samples (n = 180) were collected from cattle farms in Ben Tre, Tien Giang, and Tra Vinh provinces. PCR targeting the P32 antigen gene was utilized to detect LSDV presence, and GPCR gene amplification was performed to assess genetic variability.

Results: LSDV was detected in 8.33% (15/180) of the samples using PCR targeting the P32 antigen gene. Each sample that tested positive for LSDV demonstrated complete amplification of the GPCR gene. Sequence alignments and phylogenetic analyses of the GPCR gene revealed that Mekong Delta LSDV isolates shared genetic similarities and possessed a 12-nucleotide insertion comparable to strains from China in 2019 and Northern Vietnam in 2020.

Conclusion: This study provides preliminary insights into the molecular characteristics of LSDV in cattle from the Mekong Delta region of Vietnam. The observed genetic relatedness to other LSDV sequences from Asia and Southeast Asia underscores the importance of regional surveillance and control measures. These findings contribute to the development of effective strategies for LSDV control and prevention.

Keywords: GPCR, Lumpy skin disease virus, Characterization, Mekong Delta, Vietnam.

Introduction

Lumpy skin disease (LSD) is classified by the World Organisation for Animal Health (OIE) as a transboundary disease due to its rapid spread and the significant financial losses it incurs (Tuppurainen and Oura, 2012). LSD, caused by the Neethling strain of the *Capripoxvirus* from the Poxviridae family, was first identified in Zambia in 1929. It is prevalent across much of Africa, certain regions of the Middle East, and Turkey. According to data from 2020, the LSD epidemic has affected over 20 countries and territories, including Bangladesh, Bhutan, China, Taiwan, Djibouti, Hong Kong, India, Iraq, Mozambique, Myanmar, Namibia, Nepal, Russia, Saudi Arabia, Sri Lanka, Switzerland, Syria, Thailand, and Turkey (WAHIS, 2022).

According to the online animal disease information system (WAHIS), LSD was first identified in Lang Son province of Vietnam in October 2020. Since then, LSD cases have been reported in 55 out of 63 provinces and cities across the country, spanning 457 districts and 4,280 communes. This has resulted in a total of 200,831 infected buffaloes and cows and the culling of 28,600 animals. Specifically, the provinces of Tien Giang, Long An, Tra Vinh, Vinh Long, and Ben Tre have reported 31 affected districts and 188 communes, with 4,558 infected cows and 803 culled cows (DAH, 2021). LSD is characterized by round, slightly elevated papules approximately 2–7 cm in diameter, which typically appear on the neck, legs, tail, and back shortly after the onset of fever (**Şevik** and Doğan, 2017). The disease leads to a significant decrease in milk

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production (ranging from 10% to 85%) due to elevated body temperature and the development of secondary mastitis. Additional consequences of the disease include irreversible skin damage, reduced growth rates in cattle, temporary or permanent sterility, miscarriage, and potential fatality (Şevik *et al.,* 2016).

Several specimens commonly used for viral genome detection include nodules, ulcers, secretions, semen, and blood from infected cattle (Bedeković *et al.,* 2018; Wolf *et al.,* 2021). PCR techniques focusing on P32 and GPCR genes are commonly used for identifying and analyzing LSDV (Seerintra *et al.,* 2022; Koirala *et al.,* 2022). Capripoxviruses can be genetically distinguished by the GPCR gene, found on the double-stranded DNA (dsDNA) of the LSDV genome and playing a role in immunomodulation in the host (Le Goff *et al.,* 2009). Comparing the GPCR gene sequences of LSDV from wild field isolates and vaccine strains, it was shown that the wild type of LSDV genomes had a particular 12-bp deletion that was not present in vaccine strains (El-Tholoth and El-Kenawy, 2016; Lu *et al.,* 2019). This discovery emphasizes the significance of the GPCR gene as a potential target for the creation of a "DIVA" (differentiation of infected from vaccinated animals) technique, which may accurately identify LSDV infections in herds that have been immunized with similar vaccinations (Agianniotaki *et al.,* 2017; Bedeković *et al.,* 2018). Although prior studies have examined the frequency of LSDV in Vietnam (Tran *et al.,* 2021), there is less data available regarding the molecular attributes of the present LSDV strains found in the Mekong Delta region of Vietnam. Comprehending these molecular characteristics is crucial for the field of molecular epidemiology and the use of vaccines. Consequently, the purpose of this research was to examine the GPCR gene-based molecular features of LSDV strains in the blood of recently infected Vietnamese cows. Additionally, we looked at the phylogenetic link between the isolated Vietnamese LSDV strains and the sequences published in the database.

Materials and Methods

Specimen collection and DNA extraction

This study was conducted in three of Vietnam's Mekong Delta provinces: Ben Tre, Tra Vinh, and Tien Giang (Fig. 1). Blood samples were collected from 180 cows suspected of being affected by LSD during the outbreak between January and December 2023. These samples were obtained from cows displaying LSD-like clinical signs and had not been vaccinated against LSDV previously. Approximately 1–3 ml of blood was collected and deposited in sterile tubes containing whole blood (EDTA tubes). The samples were collected aseptically following the guidelines outlined by the TCVN 8400:2010 (TCVN, 2010), TCCS 04:2020/TY–DT (MARD, 2020), and OIE (2017). Detailed information regarding suspected LSD-affected cattle, including age, gender, breed, and clinical signs, was also recorded. Each clinical sample was labeled with a unique sample ID upon collection. The samples were transported in a cooler box with ice packs to the Faculty of Veterinary Laboratory at Can Tho University and stored at −80°C for further molecular analysis.

DNA extraction and PCR assay

The whole viral DNA was isolated from the acquired whole blood sample using the TopPURE® Serum Viral Extraction Kit (ABT, Vietnam) by the manufacturer's instructions. The genomic DNA was extracted using the kit and then kept at a temperature of −20°C until it was needed for subsequent experiments. Next, the DNA extracts from LSDV-infected samples were used for conventional PCR amplification with a 192 bp region in the P32 (LSDV074) gene using pairs of primers: forward primer, 5′-TTTCCTGATTTTTCTTACTAT-3′, and reverse primer, 5′-AAATTATATACGTAAATAAC-3′, according to the PCR procedures, as described in a previous study (Ireland and Binepal, 1998).

GPCR gene amplification

All positive samples were processed to amplify the G-protein-coupled-chemokine-like receptor (GPCR) (LSDV011) gene using PCR according to primer forward 5′-GATGAGTATTGATAGATAC CTAGCTGTAGTT-3′, and reverse primer, 5′-TGAGACAATCCAAACCACCAT-3′, were used for further DNA sequencing as previously described (Le Goff *et al.,* 2009). The PCR amplification reactions were performed in a 25 µl volume containing 12 µl GoTaq® DNA Polymerase (Promega, USA), 3 µl template DNA, 1 µl of 10 µM each primer (forward and reverse), and 8 µl ultrapure water. The PCR was performed with the following thermal conditions: an initial denaturation step at 96°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The PCR amplification results were analyzed using electrophoresis on a 1.5% agarose gel with ethidium bromide (1 µg/ml) in 1X Tris-acetate-EDTA (TAE) buffer. The visualization and imaging of the products were done using UV transillumination using a BIO-Rad instrument from the USA.

Nucleotide sequencing and analysis

The amplicons of GPCR (1,158 bp) were purified from the PCR products using the TopPURE® PCR/Gel ADN purification kit (ABT, Vietnam). The products were sequenced using both forward and reverse strands at a commercial sequencing company (NamKhoa-Biotech, Vietnam). The Sanger sequencing technique, which used the ABI Prism BigDyeTM Terminator v1.1 cycle sequencing kit, was carried out with the assistance of an ABI PRISM 3,500 × l Genetic Analyzer that was utilized. The resulting LSDV GPCR sequences were repeatedly aligned using Geneious Prime® version 2024.0.2 and BioEdit® version 7.1.9, with final adjustments made manually, then evaluated for similarity to GenBank

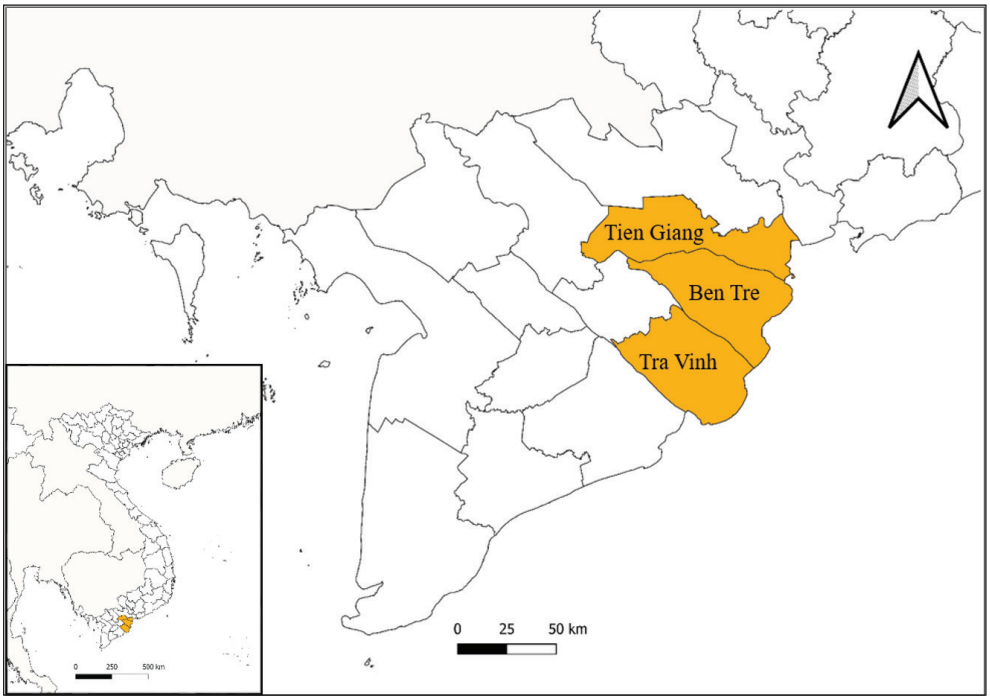

Fig. 1. The study area map shows three provinces in the Mekong Delta of Vietnam where the blood samples were collected.

sequences using the BLAST program offered by NCBI (https://www.ncbi.nlm.nih.gov/). The Maximum Likelihood method in MEGA X software (Kumar *et al.,* 2018) was employed for phylogenetic analysis to generate the phylogenetic tree for phylogenetic study. The confidence of the branching patterns of the trees was estimated using bootstrap analysis with 1,000 replications. The trees were visualized and annotated using the interactive tree of life (iTOL) (Letunic and Bork, 2024). The multiple sequence alignments of the partial GPCR genes were visualized using BioEdit® version 7.1.9. The GPCR sequences of the Mekong Delta Vietnam were submitted to the GenBank database and are available under accession numbers PP645759-PP645773.

Ethical approval

This study was carried out on naturally infected animals. The samples used for this study were diagnostic, and no experimental procedures were carried out in any animal. Written informed consent was obtained from the owners for the participation of their animals in this study.

Results

LSD outbreak investigation

One hundred and eighty cattle suspected of being infected with LSDV from three provinces in the Mekong Delta of Vietnam were sampled: Tien Giang, Ben Tre, and Tra Vinh (Fig. 1). All sampled animals had not been previously vaccinated against LSDV. Cattle that were suspected of being infected with LSD had a wide range of clinical symptoms, such as depression, lack of appetite, fever, nasal and ocular discharges, enlarged superficial lymph nodes, circumscribed skin nodules on different regions of the body, and a fall in body condition score (Fig. 2). LSDV was isolated from fifteen cows and confirmed by PCR. Among these cows, eleven were calves between one and six months old. The remaining cows included two lactating cows and one dry cow (Table 1).

Phylogenetic analysis

LSDV was detected in fifteen out of 180 blood samples using PCR based on the P32 antigen gene. All fifteen positive samples exhibited clinical signs of LSD. In addition, partial GPCR sequences were amplified in approximately all positive samples. After quality checking and editing, the GPCR sequences of the Vietnam LSDV in beef cattle were deposited in GenBank under the accession numbers (Table 1). All fifteen LSDV samples from the Mekong Delta showed close genetic relatedness to LSDV strains from North Vietnam (accession number OK258127), Thailand (accession number ON184045), China (accession number MN598006, MN508357, and MN864147), and Taiwan (accession number MZ934385), with similarities ranging from 99.7% to 100%. Additionally, they exhibited similarities to LSDV strains from Russia (accession number MH029290 and MK358808), Myanmar (accession number OM674470), Bangladesh (accession number MT448701), and Egypt (accession

Fig. 2. Clinical signs of lumpy skin disease in cattle were observed in this study. The dairy calf with nodular skin (A)–(D) was infected with the lumpy skin disease virus and presented numerous circumscribed skin nodules throughout the body.

number FJ869377, KP071936, and MG970343), ranging from 98.0% to 99.3% (Table S1).

Phylogenetic relationships among fifteen LSDV isolates from the Mekong Delta in Vietnam and all 26 LSDV GPCR genes from the GenBank database were analyzed. Additionally, five vaccine strains, three goatpox virus strains, and three sheeppox virus strains were included in the phylogeny (Fig. 3). The LSDV isolates from the Mekong Delta in Vietnam were discovered in the same group as other LSDV isolates from various countries, including North Vietnam (OK258127), China (MN598006), Russia (MH029290), Thailand (ON184045), and Taiwan (MZ934385) (Fig. 3). In addition, the study of the GPCR gene revealed an insertion of 12 base pairs (nucleotides 94 to 105) in the GPCR gene of cattle from the provinces of Tien Giang, Ben Tre, and Tra Vinh in the Mekong Delta region of Vietnam (Fig. 4; Fig. S1).

The absence of specific amino acid residues (L32, S33, T34, and I35) and the presence of particular mutations (I28F and M318T) are identified as unique signatures among isolates from the Mekong Delta region and strains originating from North Vietnam, China, Thailand, Taiwan, and Russia. In comparison with the reference sequence MK302091.Eastern Africa/1971 (Fig. 5; Fig. S2).

Discussion

The current study examined and verified instances of LSD in the Mekong Delta region of Vietnam using PCR. The LSDVs obtained from the observed outbreaks were also analyzed utilizing GPCR gene sequencing. This research represents the first known instance of molecular characterization and phylogenetic analysis of the LSDV observed in this location in 2023.

LSD is considered one of the most economically significant viral infections in cattle due to its detrimental effects on hides, milk production, mastitis, fertility, weight loss, and mortality. While the morbidity rate of LSD might exceed 100%, the fatality rate is below 10% (OIE, 2021). The initial occurrence of LSD in cattle was documented in Lang Son province, Vietnam, towards the conclusion of October 2020 (Tran *et al.,* 2021). Following this, the LSDV subsequently disseminated to many additional regions within Vietnam. A prompt preventative strategy was implemented, utilizing the emergency vaccination Lumpyvac® (Vetal, Adiyaman, Turkey) sourced from Turkey. Furthermore, it was advised that farm owners in both infected and non-infested zones/provinces should also adopt biosecurity measures. Despite the implementation of a vaccination regimen for cattle and buffalo, inadequate vaccine coverage has resulted in ongoing LSD outbreaks that

Table 1. The summary of signalments, collected location, and clinical symptoms of 15 LSD-positive cattle.

Accession numbers	Isolate	Date of investigation	Age (months)	Sex	Clinical symptoms
PP645759	CTU/LSDV/TraVinh-TV01.2023	Jan 30, 2023	2	Male	Pyrexia, nodular skin lesions on the neck and shoulder
PP645760	CTU/LSDV/TraVinh-TV02.2023	Jan 30, 2023	48	Female	Pyrexia, nodular skin lesions
PP645761	CTU/LSDV/TraVinh-TV03.2023	Apr 02, 2023	3	Male	Pyrexia, nodular skin lesions, generalized enlarged lymph nodes
PP645762	CTU/LSDV/TraVinh-TV04.2023	May 01, 2023	2	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645763	CTU/LSDV/TraVinh-TV05.2023	May 12, 2023	3	Female	Pyrexia, nodular skin lesions
PP645764	CTU/LSDV/BenTre-BT01.2023	May 12, 2023	5	Male	Pyrexia, nodular skin lesions on the neck and shoulder
PP645765	CTU/LSDV/BenTre-BT02.2023	Dec 11, 2023	4	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645766	CTU/LSDV/BenTre-BT03.2023	Jun 02, 2023	56	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645767	CTU/LSDV/BenTre-BT04.2023	Aug 12, 2023	3	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645768	CTU/LSDV/BenTre-BT05.2023	Jan 30, 2023	2	Male	Pyrexia, nodular skin lesions on the neck and shoulder
PP645769	CTU/LSDV/TienGiang-TG01.2023	Apr 02, 2023	1	Male	Pyrexia, nodular skin lesions, generalized enlarged lymph nodes
PP645770	CTU/LSDV/TienGiang-TG02.2023	Apr 02, 2022	36	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645771	CTU/LSDV/TienGiang-TG03.2023	May 02, 2023	3	Male	Pyrexia, nodular skin lesions on the neck and shoulder
PP645772	CTU/LSDV/TienGiang-TG04.2023	Dec 01, 2023	4	Female	Pyrexia, nodular skin lesions on the neck and shoulder
PP645773	CTU/LSDV/TienGiang-TG05.2023	Jun 22, 2023	2	Female	Pyrexia, nodular skin lesions, generalized enlarged lymph nodes

continue to inflict significant economic damage on the cattle industry. The current investigation used PCR targeting the P32 gene to identify the presence of LSDV in the blood of infected cattle. Using blood samples for early virus detection before cows show signs of lumps on the skin is a proactive approach to managing LSD in cattle, allowing for timely intervention and potentially reducing the spread of the disease (Tuppurainen *et al.,* 2005; Aerts *et al.,* 2021). The positive samples were then amplified, explicitly targeting the GPCR gene to detect and analyze LSDV outbreaks in some provinces in the Mekong Delta area of Vietnam. The presence of viral DNA was identified in 15 out of 180 samples (Table 1). All positive samples were obtained from cattle that had clinical symptoms of LSD and had not received prior vaccination against LSDV. Primarily, cows become infected with LSD when they are aged between one and six months. The positive rate of samples collected from suspected animals varied due to the occasional detection of LSDV viral DNA in blood during a short period of viremia from 4–11 days after infection (Aerts *et al.,* 2021). There was no correlation between the duration of the viremia phase and the severity of clinical symptoms (Tuppurainen *et al.,* 2005).

The G protein-coupled chemokine receptor (GPCR) gene emerges as a potential candidate for elucidating the evolutionary connection between members of the *Capripoxvirus* genus and LSDV. This study, utilizing wild field and vaccine strains, employed primers from previous research to amplify LSDV, explicitly targeting the GPCR sequences (Le Goff *et al.,* 2009). Multiple sequence alignments of the fifteen targeted LSDV genes within the GPCR revealed complete identity among

Table S1. Nucleotide sequence identity distances for the GPCR gene of local LSDV isolates compared to reference LSDV, sheep pox virus, and goat pox virus published in GenBank.

No.	Strain CePV	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	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	CTU/LSDV/TraVinh-TV01.2023																																																				
2	CTU/LSDV/TraVinh-TV02.2023	100																																																			
3	CTU/LSDV/TraVinh-TV03.2023	99.74	99.74																																																		
4	CTU/LSDV/TraVinh-TV04.2023	99.74	99.74	100																																																
5	CTU/LSDV/TraVinh-TV05.2023	99.74	99.74	100	100																																															
6	CTU/LSDV/BenTre-BT01.2023	99.39	99.39	99.65	99.65	99.65																																														
7	CTU/LSDV/BenTre-BT02.2023	99.48	99.48	99.74	99.74	99.74	99.91																																													
8	CTU/LSDV/BenTre-BT03.2023	99.74	99.74	100	100	100	99.65	99.74																																												
9	CTU/LSDV/BenTre-BT04.2023	99.74	99.74	100	100	100	99.65	99.74	100																																										
10	CTU/LSDV/BenTre-BT05.2023	100	100	99.74	99.74	99.74	99.39	99.48	99.74	99.74																																									
11	CTU/LSDV/TienGiang-TG01.2023	100	100	99.74	99.74	99.74	99.39	99.48	99.74	99.74	100																																								
12	CTU/LSDV/TienGiang-TG02.2023	99.74	99.74	100	100	100	99.65	99.74	100	100	99.74	99.74																																								
13	CTU/LSDV/TienGiang-TG03.2023	99.74	99.74	100	100	100	99.65	99.74	100	100	99.74	99.74	100																																							
14	CTU/LSDV/TienGiang-TG04.2023	99.39	99.39	99.65	99.65	99.65	100	99.91	99.65	99.65	99.39	99.39	100	100																																						
15	CTU/LSDV/TienGiang-TG05.2023	99.48	99.48	99.74	99.74	99.74	99.91	100	99.74	99.74	99.48	99.48	100	100	99.91																																					
16	OK258127.LSDV_VietNam-LangSon/2020	99.74	99.74	100	100	100	99.65	99.74	100	100	99.74	99.74	100	100	99.65	99.74																																				
17	MT448701.LSDV_Bangladesh/2019	99.21	99.21	99.13	99.13	99.13	98.78	98.86	99.13	99.13	99.21	99.21	99.13	99.13	98.78	98.86	99.13																																				
18	ON184045.LSDV_ThaiLand/2021	99.64	99.64	99.91	99.91	99.91	99.55	99.64	99.91	99.91	99.64	99.64	99.91	99.91	99.55	99.64	99.91	99.01																																				
19	OM674470.LSDV_Myanmar/2020	99.21	99.21	99.13	99.13	99.13	98.78	98.86	99.13	99.13	99.21	99.21	99.13	99.13	98.78	98.86	99.13	100	99.01																																				
20	MZ934385.LSDV_Taiwan/2020	99.74	99.74	100	100	100	99.65	99.74	100	100	99.74	99.74	100	100	99.65	99.74	100	99.13	99.91	99.13																																				
21	MN598006.LSDV_China/2019	99.65	99.65	99.91	99.91	99.91	99.56	99.65	99.91	99.91	99.65	99.65	99.91	99.91	99.56	99.65	99.91	99.04	99.82	99.04	99.91																																				
22	MN608357.LSDV_China/2019	99.91	99.91	99.83	99.83	99.83	99.48	99.56	99.83	99.83	99.91	99.91	99.83	99.83	99.48	99.56	99.83	99.3	99.73	99.3	99.83	99.74																																				
23	MN864147.LSDV_China/2019	99.81	99.81	100	100	100	99.81	99.9	100	100	99.81	99.81	100	100	99.81	99.9	100	99.23	100	99.23	100	99.9	99.9																																			
24	MK358808.LSDV_Russia/2018	99.04	99.04	99.30	99.30	99.30	98.95	99.04	99.30	99.30	99.04	99.04	99.30	99.30	98.95	99.04	99.30	98.43	99.19	98.43	99.3	99.21	99.13	99.23																																		
25	MH029290.LSDV_Russia/2017	98.69	98.69	98.95	98.95	98.95	98.60	98.69	98.95	98.95	98.69	98.69	98.95	98.95	98.60	98.69	98.95	98.08	98.83	98.08	98.95	98.87	98.78	98.85	99.65																																		
26	MK765544.LSDV_Kazakhstan/2016	99.61	99.61	99.61	99.61	99.61	99.36	99.49	99.61	99.61	99.61	99.61	99.61	99.61	99.36	99.49	99.61	100	99.61	100	99.61	99.49	99.74	99.61	98.84	98.33																																		
27	MW452646.LSDV_India/2019	99.21	99.21	99.13	99.13	99.13	98.78	98.86	99.13	99.13	99.21	99.21	99.13	99.13	98.78	98.86	99.13	100	99.01	100	99.13	99.04	99.3	99.23	98.43	98.08	100																																		
28	FJ869376.LSDV_South_Africa/1954	98.60	98.60	98.86	98.86	98.86	98.52	98.60	98.86	98.86	98.60	98.60	98.86	98.86	98.52	98.60	98.87	97.99	98.74	97.99	98.87	98.78	98.69	98.76	99.56	99.74	98.2	97.99																																		
29	MK302091.LSDV_Eastern_Africa/1971	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97																																		
30	FJ869377.LSDV_Egypt/1989	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100																																		
31	KP071936.LSDV_Egypt/2015	98.59	98.59	98.32	98.32	98.32	97.97	98.06	98.32	98.32	98.59	98.59	98.32	98.32	97.97	98.06	98.32	99.21	98.18	99.21	98.32	98.24	98.5	98.64	97.62	97.27	99.23	99.21	97.18	99.21	99.21																																		
32	MG970343.LSDV_Egypt/2016	98.68	98.68	98.59	98.59	98.59	98.23	98.32	98.59	98.59	98.68	98.68	98.59	98.59	98.23	98.32	98.59	99.47	98.45	99.47	98.59	98.5	98.77	98.94	97.88	97.53	99.61	99.47	97.44	99.47	99.47	99.74																																		
33	KR024779.LSDV_Turkey/2015	99.23	99.23	99.33	99.33	99.33	99.14	99.23	99.33	99.33	99.23	99.23	99.33	99.33	99.14	99.23	99.33	100	99.23	100	99.33	99.23	99.33	99.4	98.56	98.17	100	100	98.08	100	100	99.33	99.62																																		
34	KP663691.LSDV_Ethiopia/2011	99.03	99.03	98.94	98.94	98.94	98.59	98.68	98.94	98.94	99.03	99.03	98.94	98.94	98.59	98.68	98.94	99.82	98.91	99.82	98.94	98.85	99.12	99.13	98.24	97.88	99.87	99.82	97.8	99.82	99.82	99.03	99.29	99.9																																		
35	MK302073.LSDV_Ethiopia/2011	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100	100	99.21	99.47	100	99.82																																		
36	MW748479.LSDV_Bostwana/2019	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100	100	99.21	99.47	100	99.82	100																																	
37	MW344046.LSDV_Syria_2020	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100	100	99.21	99.47	100	99.82	100	100																																
38	MT015606.LSDV_Iran/2015	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100	100	99.21	99.47	100	99.82	100	100	100																															
39	KJ818281.LSDV_Kenyan/2014	99.21	99.21	99.13	99.13	99.13	98.78	98.86	99.13	99.13	99.21	99.21	99.13	99.13	98.78	98.86	99.13	100	99.01	100	99.13	99.04	99.3	99.23	98.43	98.08	100	100	97.99	100	100	99.21	99.47	100	99.82	100	100	100	100																														
40	FJ869369.LSDV_Sudan/2006	99.12	99.12	99.03	99.03	99.03	98.68	98.76	99.03	99.03	99.12	99.12	99.03	99.03	98.68	98.76	99.03	99.91	98.91	99.91	99.03	98.94	99.21	99.23	98.32	97.97	100	99.91	97.88	99.91	99.91	99.12	99.38	99.9	99.74	99.91	99.91	99.91	99.91	99.91																																
41	MW115948.LSDV_Namibia/2019	99.21	99.21	99.12	99.12	99.12	98.78	98.85	99.12	99.12	99.21	99.21	99.12	99.12	98.78	98.85	99.12	100	99.00	100	99.12	99.03	99.29	99.23	98.41	98.06	100	100	97.97	100	100	99.21	99.47	100	99.82	100	100	100	100	99.91																															
42	KX764643.LSDV_SIS-Lumpyvax_vaccine	47.08	47.08	47.21	47.21	47.21	47.12	47.21	47.21	47.21	47.08	47.08	47.21	47.21	47.12	47.21	47.17	47.17	47.22	47.17	47.17	47.26	47.17	46.94	47.06	46.81	48.03	47.17	46.90	47.17	47.17	47.35	47.44	47.45	47.26	47.17	47.17	47.17	47.17	47.17	47.08	47.17																															
43	KX764644.LSDV_Neethling-Herbivac_vaccine	47.08	47.08	47.21	47.21	47.21	47.12	47.21	47.21	47.21	47.08	47.08	47.21	47.21	47.12	47.21	47.17	47.17	47.22	47.17	47.17	47.26	47.17	46.94	47.06	46.81	48.03	47.17	46.90	47.17	47.17	47.35	47.44	47.45	47.26	47.17	47.17	47.17	47.17	47.17	47.08	47.17	100																															
44	KX764645.LSDV_Neethling-LSD_vaccine-OBP	47.08	47.08	47.21	47.21	47.21	47.12	47.21	47.21	47.21	47.08	47.08	47.21	47.21	47.12	47.21	47.17	47.17	47.22	47.17	47.17	47.26	47.17	46.94	47.06	46.81	48.03	47.17	46.90	47.17	47.17	47.35	47.44	47.45	47.26	47.17	47.17	47.17	47.17	47.17	47.08	47.17	100	100																															
45	AF409138.LSDV_Neethling_vaccine_LW_1959	47.08	47.08	47.21	47.21	47.21	47.12	47.21	47.21	47.21	47.08	47.08	47.21	47.21	47.12	47.21	47.17	47.17	47.22	47.17	47.17	47.26	47.17	46.94	47.06	46.81	48.03	47.17	46.90	47.17	47.17	47.35	47.44	47.45	47.26	47.17	47.17	47.17	47.17	47.17	47.08	47.17	100	100	100																															
46	MT130592.LSDV_Neethling-RIBSP_vaccine	47.23	47.23	47.36	47.36	47.36	47.27	47.36	47.36	47.36	47.23	47.23	47.36	47.36	47.27	47.36	47.32	47.32	47.28	47.32	47.32	47.41	47.32	46.94	47.23	46.96	47.77	47.32	47.05	47.32	47.32	47.50	47.59	47.62	47.41	47.32	47.32	47.32	47.32	47.23	47.32	98.06	98.06	98.06	98.06																															
47	MN072622.GTPV_Turkey	46.81	46.81	47.03	47.03	47.03	46.94	47.03	47.03	47.03	46.81	46.81	47.03	47.03	46.94	47.03	46.99	46.90	47.22	46.90	46.99	47.08	46.90	46.64	46.90	46.63	47.51	46.90	46.72	46.90	46.90	47.08	47.17	47.45	46.99	46.90	46.90	46.90	46.90	46.90	46.81	46.90	96.16	96.16	96.16	96.16	96.21																															
48	MN072621.GTPV_Vietnam	46.71	46.71	46.94	46.94	46.94	46.84	46.94	46.94	46.94	46.71	46.71	46.94	46.94	46.84	46.94	46.89	46.80	47.12	46.80	46.89	46.98	46.80	46.54	46.80	46.53	47.38	46.80	46.62	46.80	46.80	47.08	47.07	47.45	46.89	46.80	46.80	46.80	46.80	46.80	46.71	46.80	96.36	96.36	96.36	96.36	96.5	99.64																															
49	MN072624.GPTV_Sudan	46.81	46.81	47.03	47.03	47.03	46.94	47.03	47.03	47.03	46.81	46.81	47.03	47.03	46.94	47.03	46.99	46.90	47.12	46.90	46.99	47.08	46.90	46.64	46.90	46.63	47.77	46.90	46.72	46.90	46.90	47.08	47.17	47.35	46.99	46.90	46.90	46.90	46.90	46.90	46.81	46.90	95.81	95.81	95.81	95.81	96.21	98.25	98.13																															
50	MN072629.SPPV_Pendik	46.80	46.80	47.03	47.03	47.03	46.94	47.03	47.03	47.03	46.80	46.80	47.03	47.03	46.94	47.03	46.98	46.89	47.03	46.89	46.98	47.07	46.89	46.64	46.71	46.44	47.24	46.89	46.53	46.89	46.89	47.07	47.17	47.25	46.98	46.89	46.89	46.89	46.89	46.89	46.80	46.89	96.00	96.00	96.00	96.00	96.41	95.64	95.64	95.64																															
51	MW167071.SPPV_Egypt	46.80	46.80	47.03	47.03	47.03	46.94	47.03	47.03	47.03	46.80	46.80	47.03	47.03	46.94	47.03	46.98	46.89	47.03	46.89	46.98	47.07	46.89	46.64	46.71	46.44	47.24	46.89	46.53	46.89	46.89	47.07	47.17	47.25	46.98	46.89	46.89	46.89	46.89	46.89	46.80	46.89	96.00	96.00	96.00	96.00	96.41	95.64	95.64	95.64	100																															
52	MN072628.SPPV_Nigeria	46.71	46.71	46.94	46.94	46.94	46.84	46.94	46.94	46.94	46.71	46.71	46.94	46.94	46.84	46.94	46.89	46.80	46.93	46.80	46.89	46.98	46.80	46.54	46.62	46.34	47.11	46.80	46.44	46.80	46.80	46.98	47.07	47.16	46.89	46.80	46.80	46.80	46.80	46.80	46.71	46.80	95.91	95.91	95.91	95.91	96.32	95.56	95.56	95.56	99.91	99.91	

LSDVs from three provinces in the Mekong Delta of Vietnam. Analysis of these sequences unveiled a 12-base pair nucleotide insertion between positions 94 and 105 (CTCAGTACAATT), leading to the alteration of four amino acids (LSTI) (aa 33–35) and a nucleotide change at position C1050T (aa M318T), thereby modifying the GPCR gene in LSDV in cattle. This characteristic was also observed in LSDV isolates from North Vietnam (OK258127), Thailand (ON184045), China (MN598006), Taiwan (MZ934385), Russia (MT129673), and Kenya (KJ818281) (Lu *et al.,* 2019; Trinh *et al.,* 2022; Singhla *et al.,* 2022). Phylogenetic analysis indicated that LSDV strains found in 15 samples from three provinces in the Mekong Delta of Vietnam exhibited similarity to LSDV strains previously recovered from North Vietnam, China, Russia, and Thailand, suggesting a common progenitor. However, GPCR sequences obtained from strains in Eastern Africa (MK302081), Iran (MT015606), Egypt (MG970343), Botswana (MW748479), and Syria (MW818281) showed a distinct loss of 12 base pairs; these findings provide evidence that LSDV strains originating from Africa exhibit more significant genetic variation compared to Eurasian strains (Sameea Yousefi *et al.,* 2018; Sprygin *et al.,* 2020; Chibssa *et al.,* 2021; Badhy *et al.,* 2021). Strains originating from North Vietnam, China, Thailand, and Russia were classified as a distinct cluster due to their status as newly generated recombinant strains; these strains exhibited sequences similar to the Neethling vaccination strains, as previously described (Dao *et al.,* 2020; Aleksandr *et al.,* 2020; Ma *et al.,* 2022; Zan *et al.,* 2022). However, conducting a comprehensive genome sequence analysis is necessary to elucidate the discrepancy in the partial sequence alignment among the 15 strains and to track any genetic mutations in the LSDV.

The molecular analysis of the gene sequences provided evidence that LSDV field strains were involved in the outbreak. LSDV has been documented in Southeast Asian and Asian countries, including Thailand, Laos, Cambodia, Myanmar, Malaysia, China, and Russia (Arjkumpa *et al.,* 2021; Roche *et al.,* 2021; Azeem *et al.,* 2022). The disease may spread across borders by arthropod vectors, including stable flies, horse flies, and mosquitoes, facilitating the transmission of the virus from neighboring nations (Tuppurainen *et al.,* 2021). A

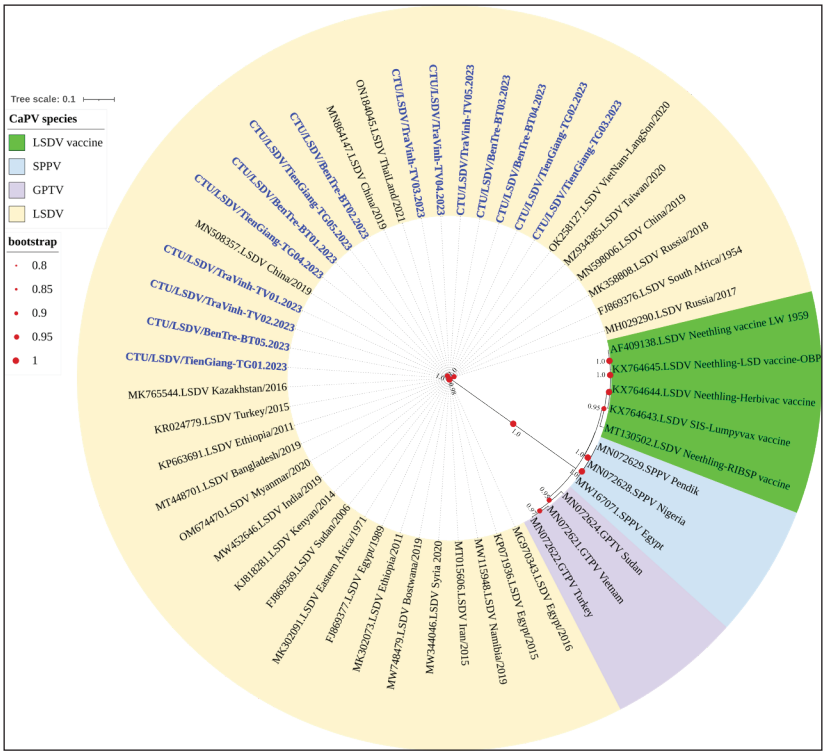

Fig. 3. Phylogenetic tree demonstrating the relationship between GPCR gene sequences obtained from the Mekong Delta in Vietnam (shown in blue and bold) and other Capripoxvirus GPCR gene sequences acquired from GenBank. LSDV-lumpy skin disease virus; GTPV-goat pox virus; SPPV-sheep pox virus.

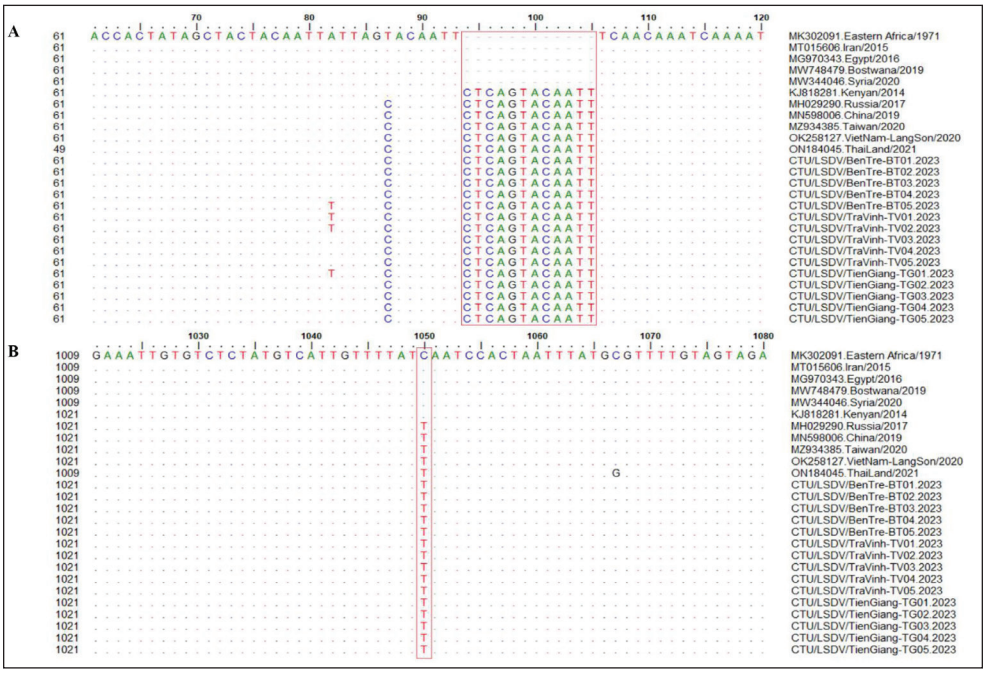

Fig. 4. Multiple sequence alignments of the partial nucleotide sequences of the GPCR gene. The isolates from the Mekong Delta in Vietnam were aligned with representative LSDV sequences retrieved from GenBank. (A) Insertion of 12 nucleotides from positions 94 to 105 is evident in the sequences of the newly isolated LSDV when compared with strain MK302091. (B) The C to T change is highlighted in the red box. The dots indicate identical nucleotides in the alignment.

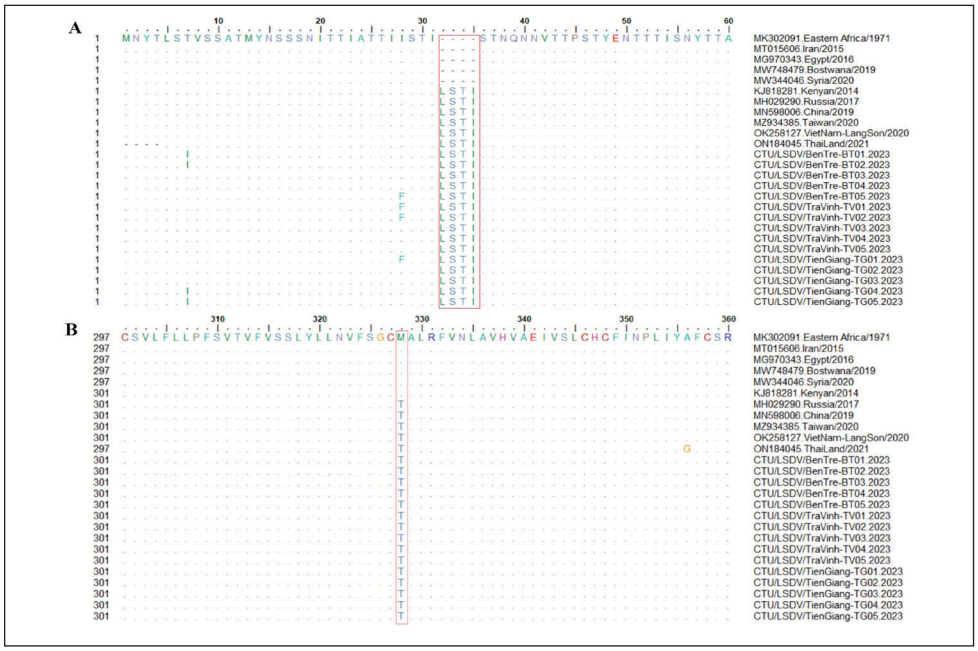

Fig. 5. Multiple sequence alignments of the partial amino acids sequences of the GPCR gene. The isolates from the Mekong Delta in Vietnam were aligned with representative LSDV sequences retrieved from GenBank. (A) Insertion of 4 amino acids from positions 32 to 35 is evident in the sequences of the newly isolated LSDV when compared with strain MK302091. (B) The M (Methionine) to T (Threonine) change is highlighted in the red box. The dots indicate identical amino acids in the alignment.

genetic comparison of LSDV strains from three provinces in the Mekong Delta of Vietnam showed that the 2023 strains were 100% identical to the strains collected in North Vietnam in 2020, suggesting that a single strain of the virus caused the outbreaks in Vietnam. Hence, to decrease losses to the farming community, LSD control methods must include immunization, biosecurity, vector management, and cow movement regulation. In addition, to effectively control and prevent the spread of LSDV, it is necessary to implement surveillance measures in various provinces to assess the prevalence of circulating LSDV strains. This will help identify the predominant LSDV candidates and facilitate the development of a suitable vaccine for the control of LSDV.

Conclusion

This study utilizes GPCR gene sequencing to provide information on the molecular characteristics and phylogenetic analysis of LSDV circulating in the Mekong Delta of Vietnam. According to the findings, the LSDV strains obtained from this location have genetic resemblances to the strains discovered in China in 2019 and North Vietnam in 2020. This research has revealed a substantial amount of evidence that supports the extensive distribution of LSDVs throughout Asian nations and Southeast Asian countries. This data will be advantageous for researchers seeking a deeper understanding of LSDV molecular epidemiology. They will assist the government of Vietnam in enhancing its efforts to develop effective control measures against local LSDV outbreaks in the Mekong Delta and throughout the country. Furthermore, the results of this study are crucial for bolstering the national vaccination campaign against the LSD virus and for advancing the strategies for creating a potent vaccine against the LSD virus in Vietnam.

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Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

This research was conducted with the contributions of all the authors. The authors all participated in the study design, analyzing the results, interpreting the results, and preparing the manuscript. All authors read and approved the final manuscript.

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Data availability

The following supporting information can be downloaded at: https://s.net.vn/KciJ.

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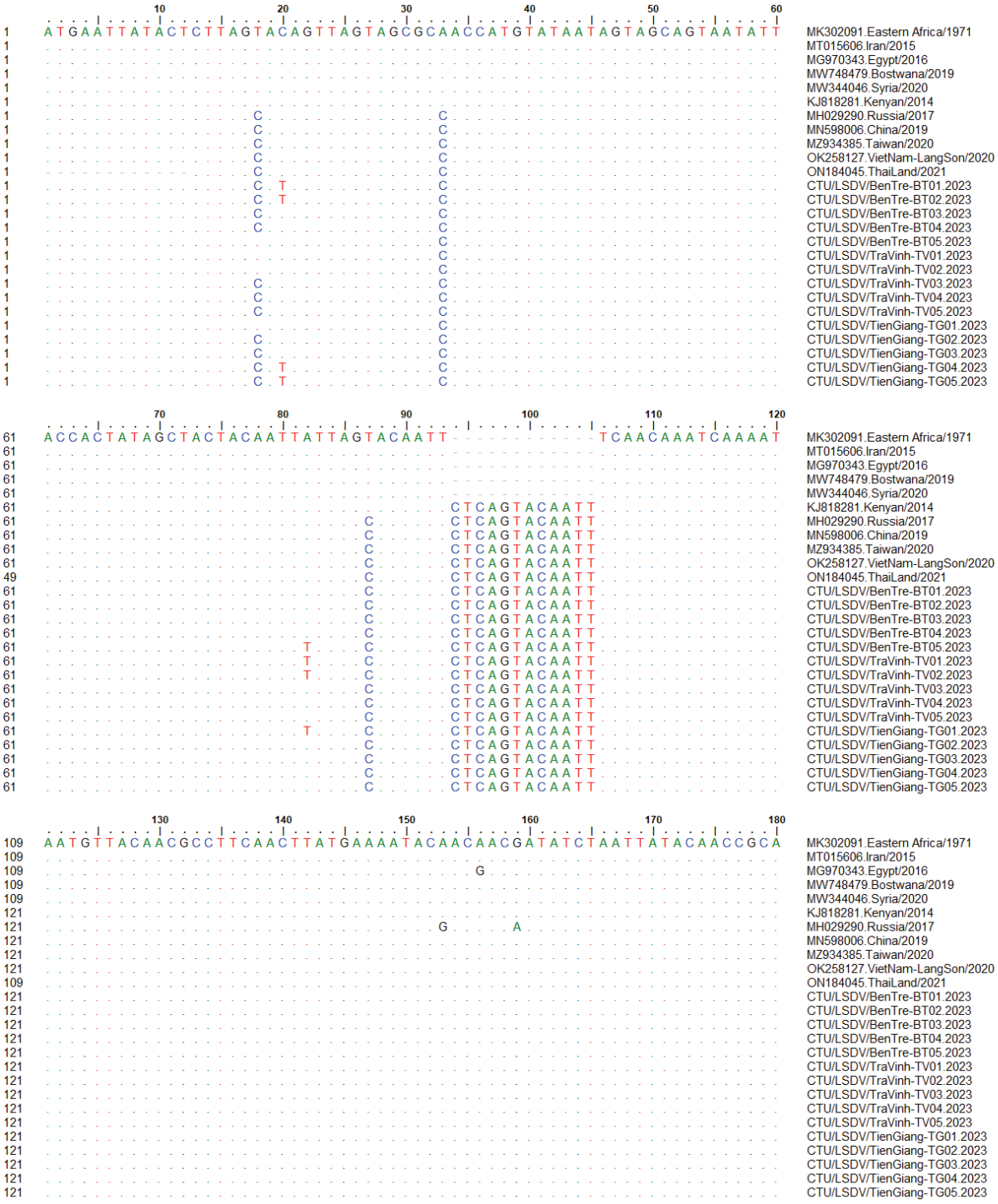
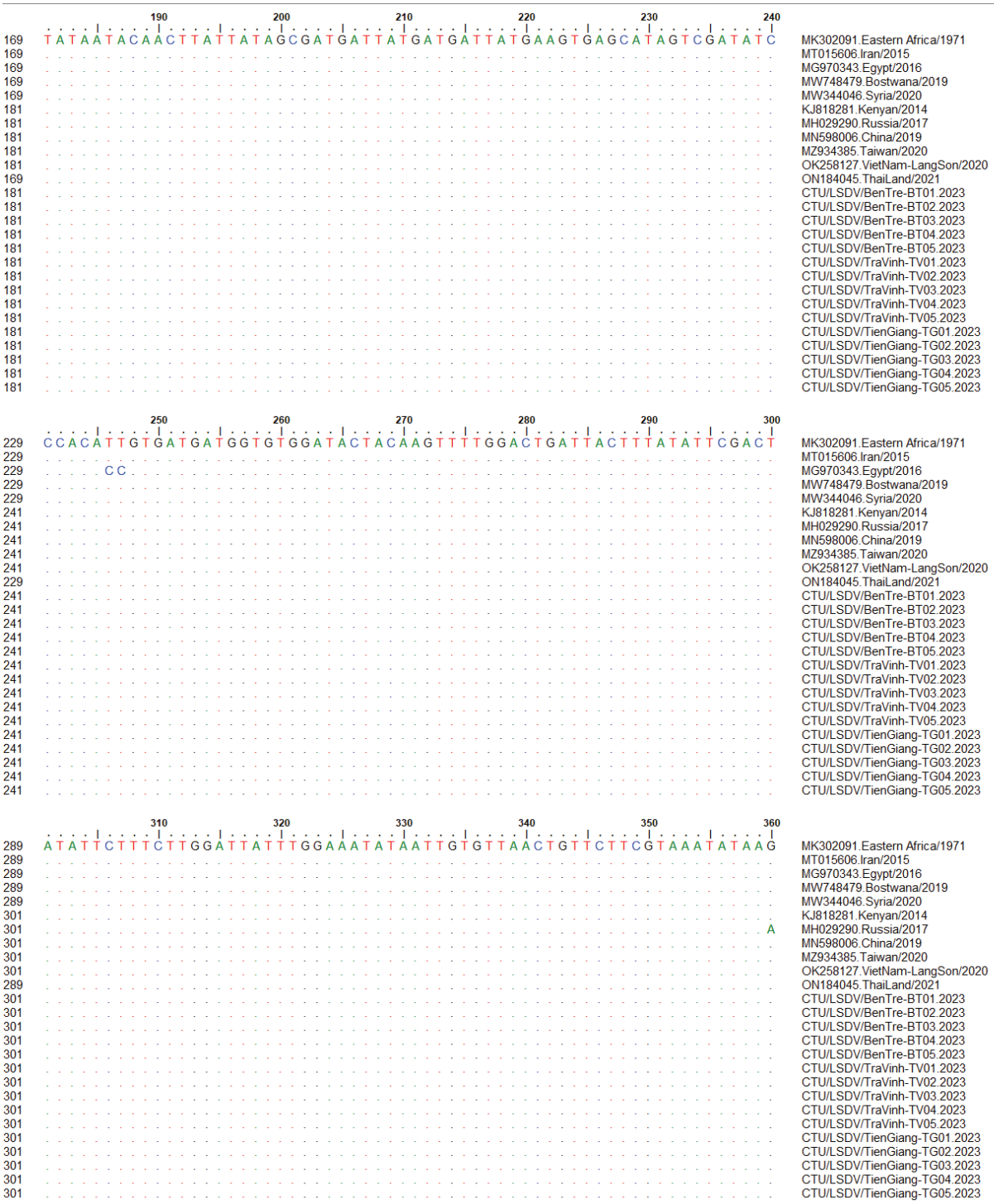


Fig. S1. Multiple sequence alignments of the partial nucleotide sequences of the GPCR gene.



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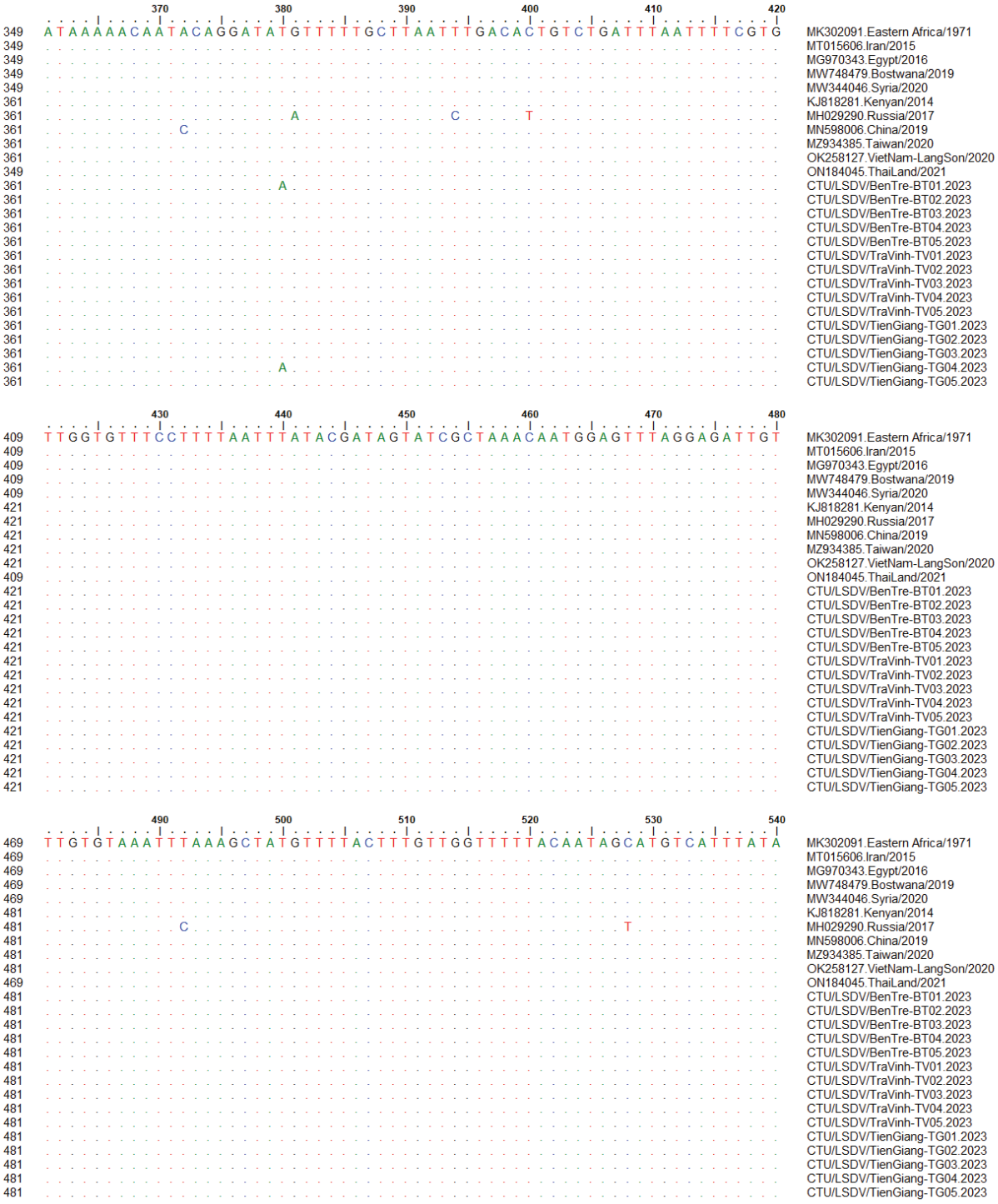

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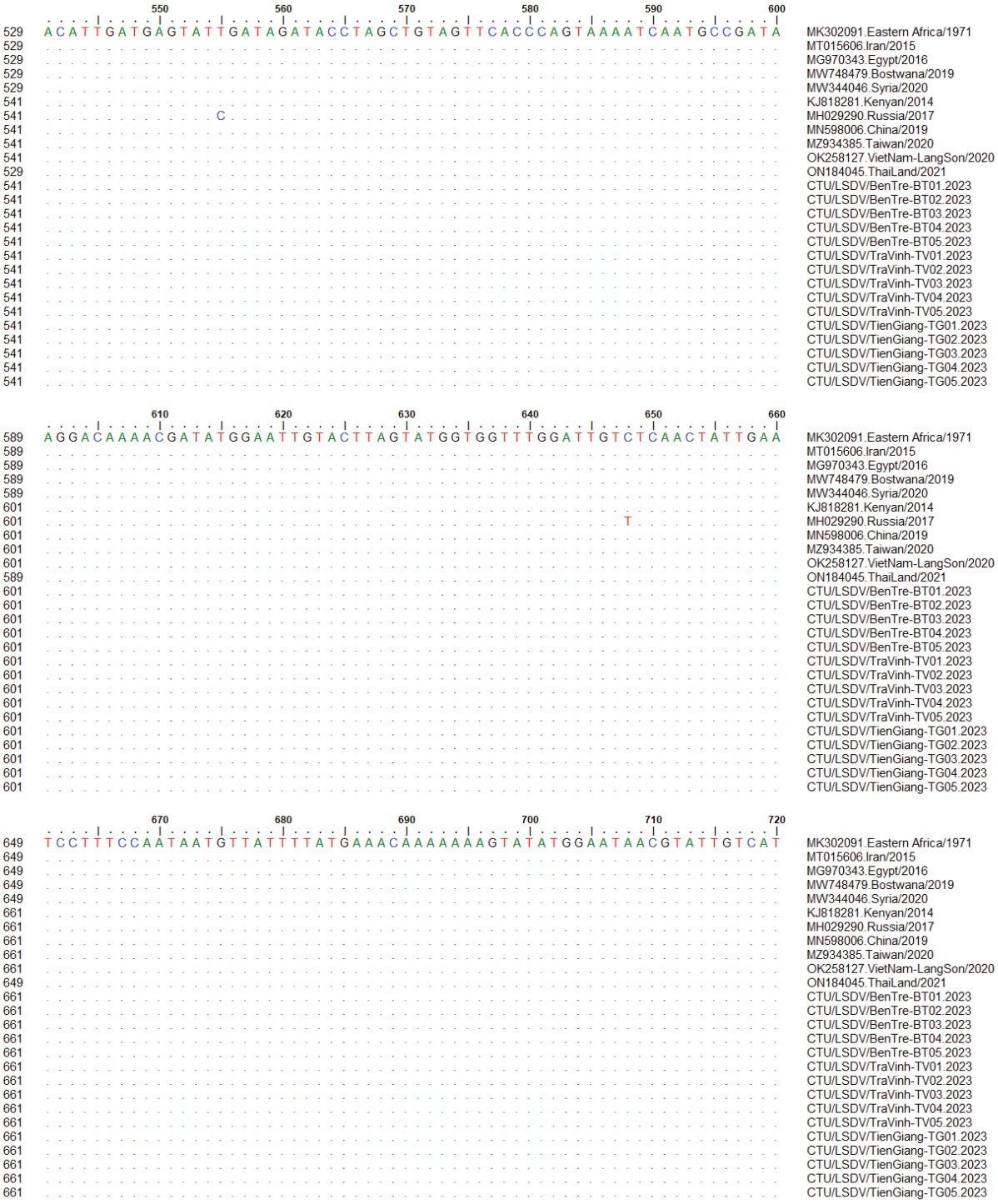

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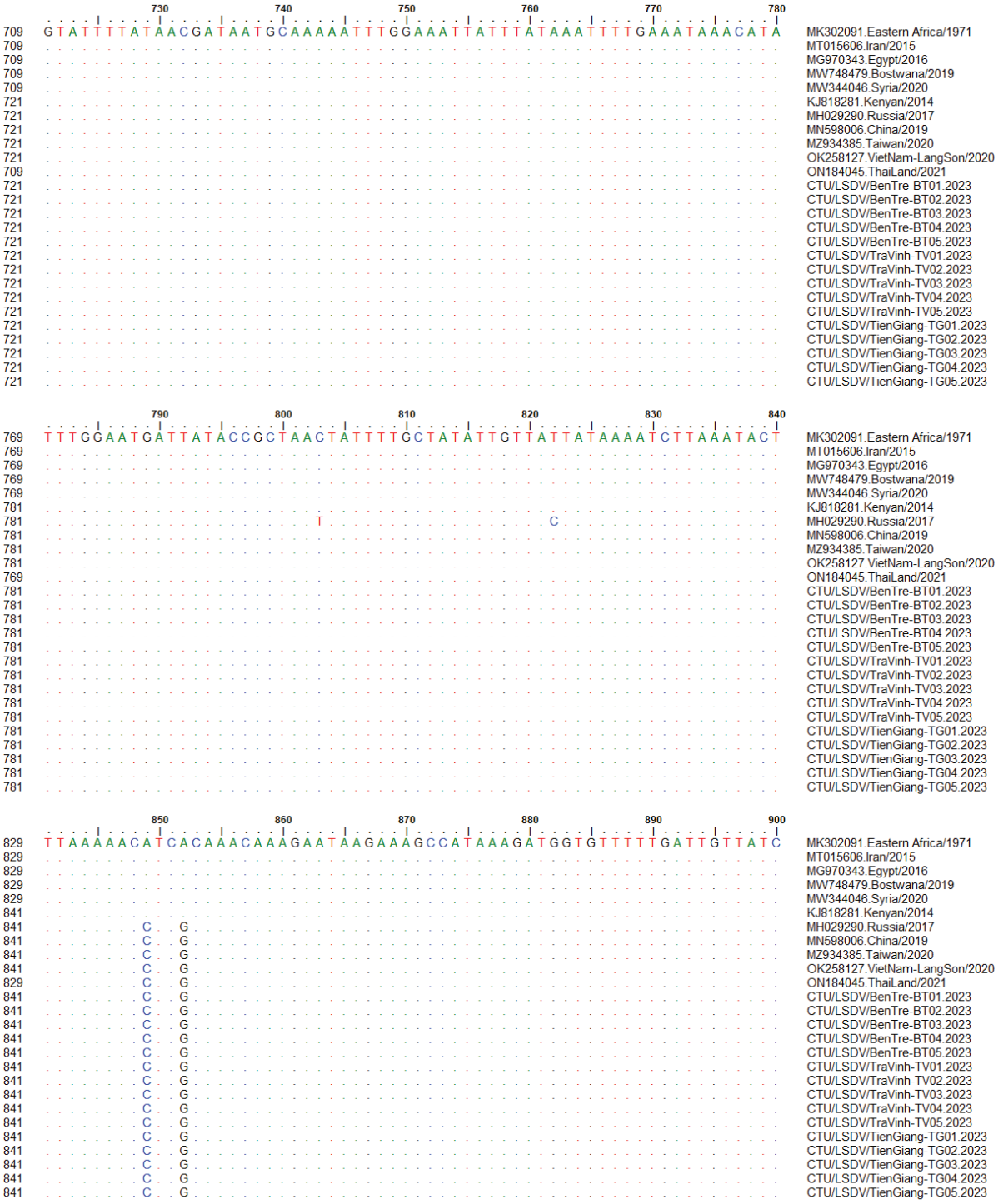

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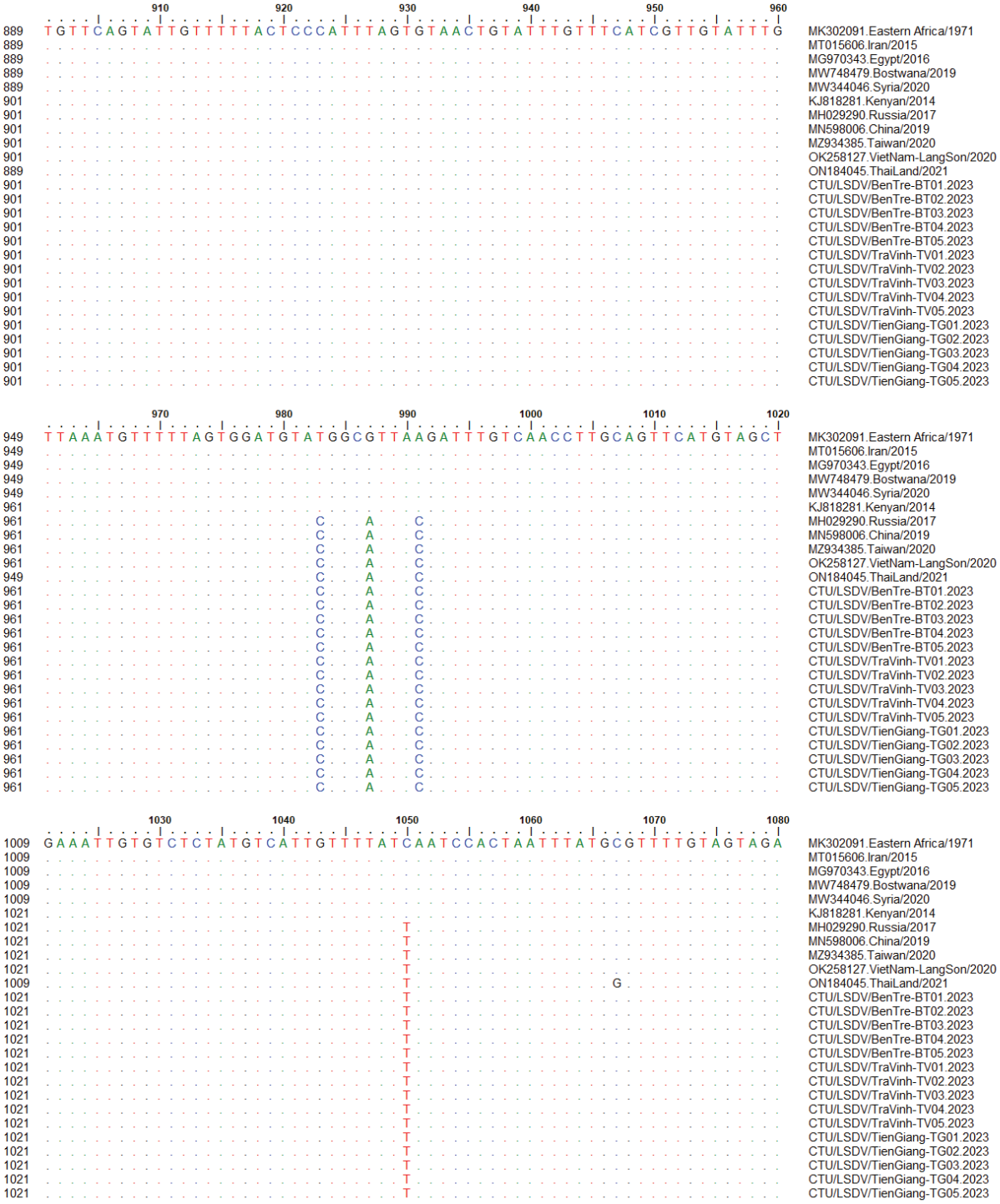

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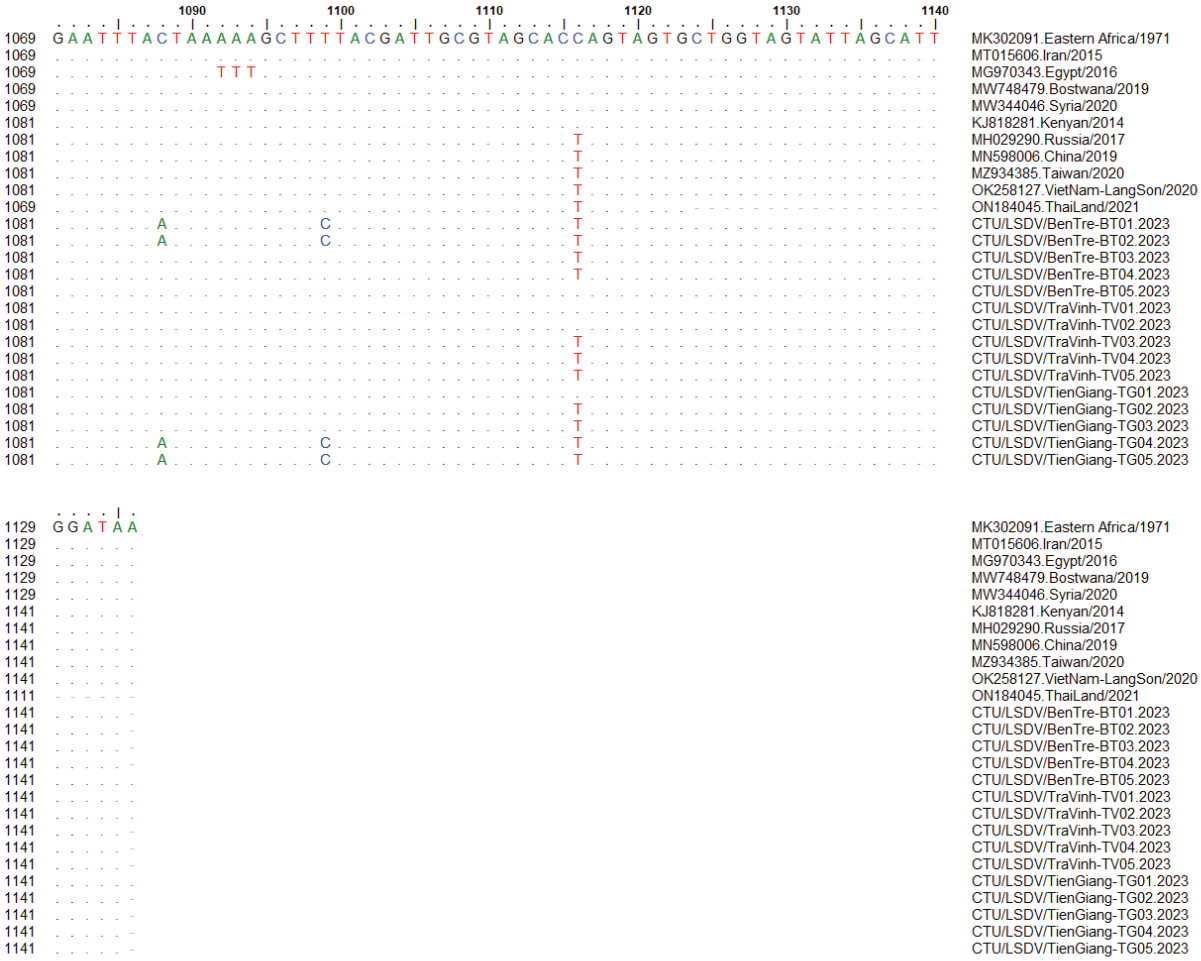

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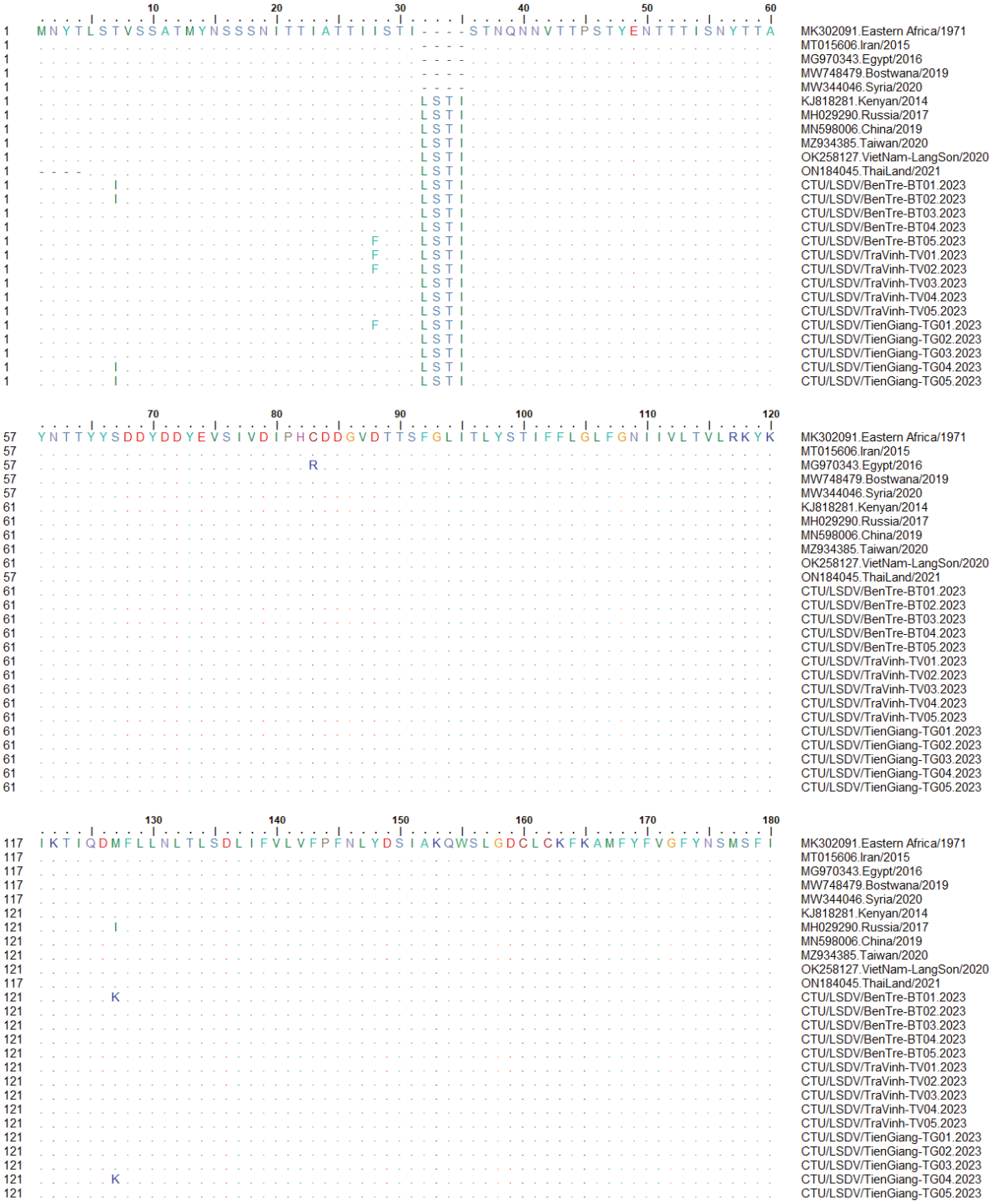

Fig. S2. Multiple sequence alignments of the partial amino acids sequences of the GPCR gene.

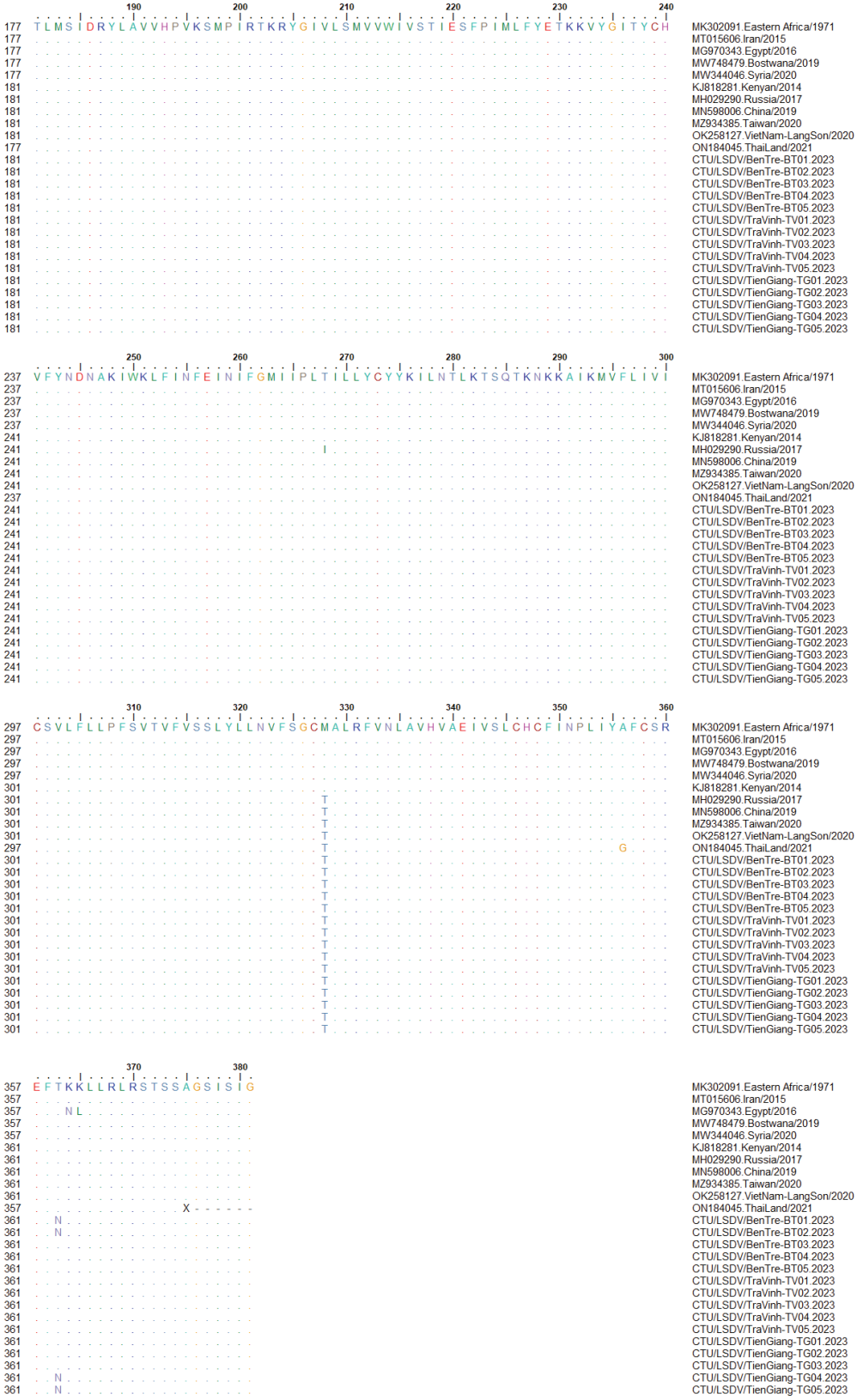

Fig. S2. Continued.