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SHORT COMMUNICATION

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Molecular epidemiology of peste des petits ruminants virus in Nigeria: An update

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Abstract

Peste des petits ruminants (PPR) is a highly contagious viral disease that mainly affects goats and sheep in Asia, Africa and the Middle East. The PPR virus (PPRV) can be classified into four genetically distinct lineages (I, II, III and IV). All have been historically present in Africa, except the Asian lineage IV that has been spreading across the globe and across Africa in recent decades. Previous studies have identified the presence of lineage IV in Nigeria since 2010. In the present study, samples were taken from 429 small ruminants with PPR symptoms across Nigeria in 2017–2020 to provide an update on the distribution and genetic diversity of PPRV in the country. Sequences from a portion of the PPRV nucleoprotein (N) gene were obtained from 91 samples, 90 belonging to lineage IV and one to lineage II. Phylogenetic analysis identified at least four lineage IV sub-clusters in Nigeria, grouping samples across multiple regions. Our results suggest extensive endemic circulation of a wide range of PPRV strains across Nigeria and across borders with neighbouring countries, underlining the difficulty involved in controlling the disease in the region.

KEYWORDS
distribution, genetic diversity, Morbillivirus, Nigeria, Phylogeny, transboundary disease

Peste des petits ruminants (PPR) is a devastating disease infecting mainly goats and sheep in Africa, Asia, and the Middle East. The disease is caused by the peste des petits ruminants virus (PPRV) of the family *Paramyxoviridae* and genus *Morbillivirus* (species name: *Small ruminant morbillivirus*) (Amarasinghe et al., 2017). PPR-related morbidity in a flock can reach 100%, and mortality is estimated at 90% (Baron et al., 2016). Due to the severe impact of the disease on the economy and food security, PPR is now the target of a global eradication programme launched by International Epizootics Organization (OIE) and the Food and Agriculture Organization of the United Nations (FAO) (OIE & FAO, 2015). The PPRV genome is composed of single-stranded negative-sense RNA (between 15,948 and 15,954 nucleotides in length) that encodes six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (H) and the RNA-dependent viral polymerase (L), as well as two non-structural proteins, C and V (Parida et al., 2015). PPRV is classified into four genetically distinct lineages (lineage I to lineage IV) based on molecular characterization using a partial sequence of the N gene (Dundon et al., 2020; Kwiatek et al., 2007). Lineage I is only present in West Africa, and recent results suggest only limited circulation in past decades (Souley et al., 2019; Tounkara et al., 2018). This lineage has now been mostly replaced by lineage II throughout West Africa (Adombi et al., 2017; Tounkara et al., 2019). Lineage III is restricted to East Africa and some parts of the Middle East, whereas Lineage IV, which was first identified in India in 1987, has spread rapidly into the Middle

East and on the African continent (Banyard et al., 2010; Dundon et al., 2020; Kwiatek et al., 2011). This lineage is currently rapidly moving into West Africa, notably through extensive and poorly controlled transboundary animal movements (Souley et al., 2019; Tounkara et al., 2018; Tounkara et al., 2019).

Among West African countries, Nigeria has over the years played a preponderant role in the production of small ruminants and is a hub of exchanges with frontier countries, in particular for the import of live small ruminants from Niger, Chad and Cameroon. The trade in and movement of animals is accompanied by the spread of animal diseases, including peste des petits ruminants (Apolloni et al., 2019). The results obtained in previous studies suggest that PPR is an endemic disease in Nigeria (Luka et al., 2011; Mantip et al., 2016; Woma et al., 2016). Molecular characterization of PPRV isolated in Nigeria suggests only lineage II was present until 2007–2009 (Luka et al., 2011), followed by circulation of both lineages II and IV in 2010–2013 (Mantip et al., 2016; Woma et al., 2016). These authors identified two sub-clusters of lineage II, but one of them (II-NigB) grouped with vaccine sequences, and until proven otherwise, should thus be assumed to be the result of laboratory contamination (Dundon et al., 2020). On the other hand, PPRV lineage IV isolates were separated into two sub-clusters (IV-NigA and IV-NigB), and these results were further confirmed with samples collected in Niger in 2011–2017 (Souley et al., 2019). The present study provides an update on the distribution and genetic diversity of PPRV in Nigeria.

Sampling was conducted between 2017 and 2020 in 18 states (Figure 1). Field sampling was carried out in accordance with local legislation and with the approval of National Veterinary Research Institute (NVRI) Animal Ethics Committee (ref: AEC/02/87/20). In 2017 and 2018, goats and sheep suspected of acute PPR infection were identified in one major market per state based on clinical symptoms that included fever over 40°C, weight loss, ocular, nasal, or ocular-nasal discharge and diarrhoea. The suspected animals were purchased from their owners, euthanized humanely and subjected to an autopsy to collect tissue samples (lungs and mesenteric lymph nodes). Additionally, ocular swab samples were collected in 2020 in multiple sites in Plateau and Bauchi states as part of a research project focusing on these regions (Table 1). The samples were transported to the PPR laboratory of the NVRI, Vom, Nigeria, and stored at −70°C until being shipped to CIRAD, Montpellier, France, for further analysis in a Biosafety level-3 laboratory facility.

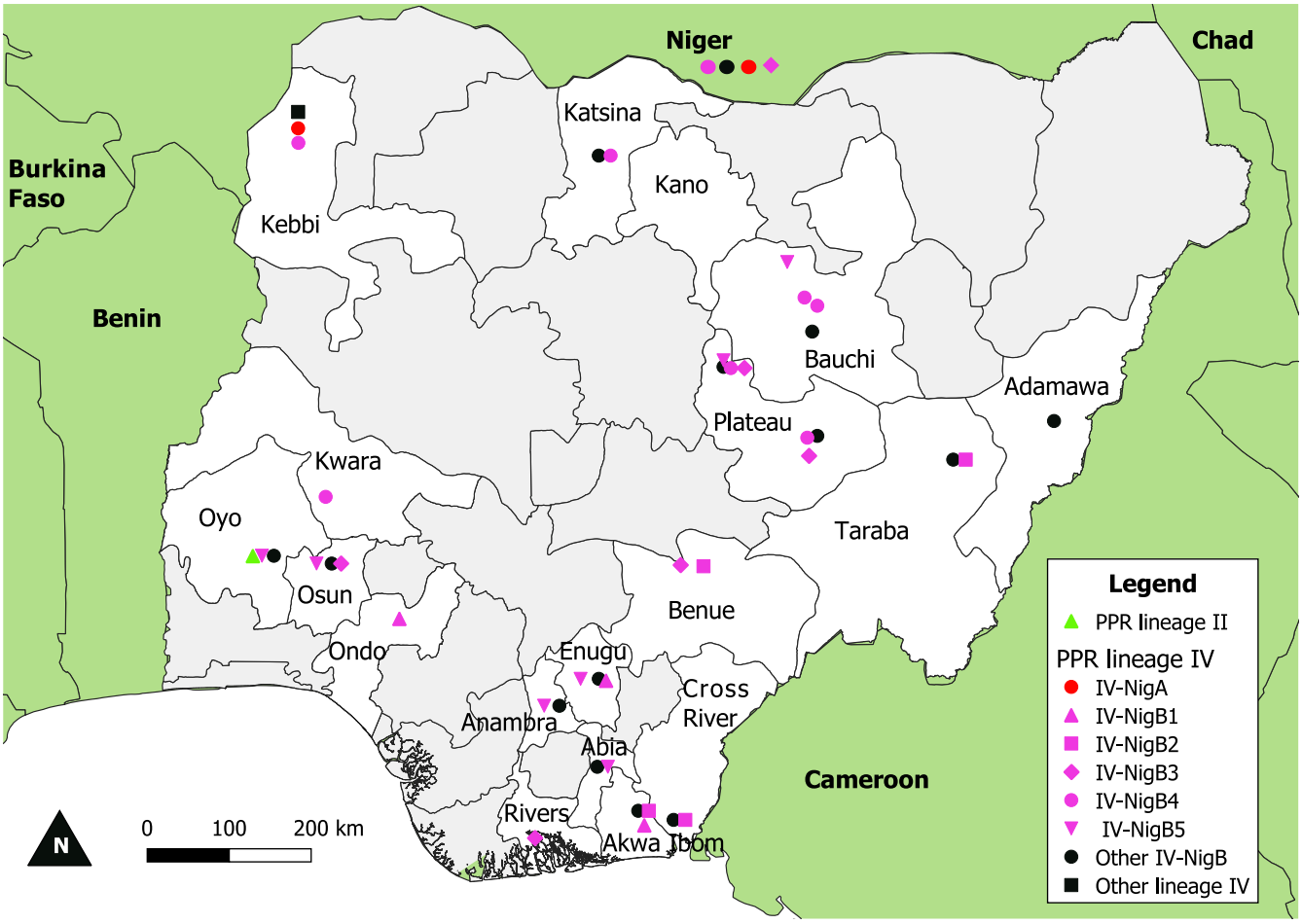

FIGURE 1 Distribution of PPRV lineages and genetic clusters identified in Nigeria in 2017–2020. Samples collected in Nigeria in the present study are indicated by icons based on phylogenetic clustering (see Figure 2). Icons are also shown in Niger if sequences previously obtained in Niger clustered with sequences from Nigeria

TABLE 1 List of PPRV-positive samples and partial N gene sequences obtained in the present study

Location	Species	Sample	No	Year	Accession number
North Central Zone					
Plateau state	Goats	Lg, Ln	8/20	2017–2018	MT193239, MT193240 MT193241, MT193242
		Lg, Ln	3/4	2020	MW600929
Langtang	Goats	Oc, Lg, Ln	6/7 *(3)*	2020	MW600925–MW600927
	Sheep	Oc, Lg, Ln	0/3	2020	-
Wase	Sheep	Oc, Lg, Ln	0/10	2020	-
Mikang	Goats	Oc, Lg, Ln	0/3	2020	-
	Sheep	Oc, Lg, Ln	3/7 *(2)*	2020	MW600928
Benue state	Goats	Lg, Ln	2/20	2018	MT193243
Kwara state	Goats	Lg, Ln	3/17	2017–2018	MW600924
	Sheep	Lg, Ln	0/3	2017–2018	-
North-East Zone					
Bauchi state	Goats	Lg, Ln	4/15	2017–2018	MT193241, MT193244
		Lg, Ln	1/5	2020	MW600933
	Sheep	Lg, Ln	1/5	2017–2018	MT193241
		Lg, Ln	2/10 *(1)*	2020	MW600932
Ningi	Goats	Oc, Lg, Ln	4/5	2020	MW600930
	Sheep	Oc, Lg, Ln	2/5	2020	MW600930
Ganjuwa	Goats	Oc, Lg, Ln	2/3 *(1)*	2020	MW600931
	Sheep	Oc, Lg, Ln	3/7 *(1)*	2020	MW600931
Adamawa state	Goats	Lg, Ln	0/12	2017–2018	-
	Sheep	Lg, Ln	1/8	2017–2018	MT193245
Taraba state	Goats	Lg, Ln	2/14	2017–2018	MT193243, MT193246
	Sheep	Lg, Ln	0/6	2017–2018	-
North-West Zone					
Kano state	Goats	Lg, Ln	0/14	2017–2018	
	Sheep	Lg, Ln	0/6	2017–2018	
Katsina state	Goats	Lg, Ln	3/13	2017–2018	MT193241, MW600924
	Sheep	Lg, Ln	1/7	2017–2018	MT193237
Kebbi state	Goats	Lg, Ln	5/6	2018	MT193234, MT193247, MT193248, MW600924
	Sheep	Lg, Ln	7/14	2018	MT193234, MW600924
South-East Zone					
Enugu state	Goats	Lg, Ln	2/11	2018	MT193236, MT193249
	Sheep	Lg, Ln	1/9	2018	MT193241
Abia state	Goats	Lg, Ln	3/13	2018	MT193238, MT193241
	Sheep	Lg, Ln	4/7	2018	MT193241, MT193250
Anambra state	Goats	Lg, Ln	6/13	2018	MT193241, MT193251
	Sheep	Lg, Ln	3/7	2018	MT193241, MT193246
South-West Zone					
Oyo state	Goats	Lg, Ln	5/20	2018	MT193241, MT193246, MT193252
Ondo state	Goats	Lg, Ln	1/20	2018	MT193249
Osun state	Goats	Lg, Ln	3/20	2018	MT193239, MT193241

TABLE 1 (Continued)

Location	Species	Sample	No	Year	Accession number
South-south Zone					
Rivers state	Goats	Lg, Ln	1/20	2018	MT193235
Cross-rivers state	Goats	Lg, Ln	2/20	2018	MT193243, MT193254
Akwa-Ibom State	Goats	Lg, Ln	4/20	2018	MT193249, MT193255, MT193256
Total			99/429		33

Note: Location refers to the state where the samples were collected. Unless stated otherwise, samples were collected in the main market of the state capital. No, number of positive samples relative to the total number samples tested; if a partial PPR gene sequence was obtained only from a subset of positive samples, the number obtained is given in italics in brackets. Multiple accession numbers are given for one location when multiple non-identical sequences were obtained from PPRV-positive samples. Accession numbers are repeated when the same sequence was obtained in several locations. Total accession number is the number of non-identical sequences obtained.

Abbreviations: Accession number, GenBank accession number; Lg, lung; Ln, lymph node; Oc, ocular swab.

RNA was extracted from homogenized lung and lymph node tissue samples and swabs resuspended in 1 ml of Minimum Essential Medium (MEM). The extraction was carried out with an extraction robot KingFisher ™ and ID Kit Gene ™ Mag Universal Extraction (IDvet), according to the manufacturer's instructions. An RT-PCR was performed using the qScript XLT One-Step RT-PCR Kit (Quantabio, VWR) to amplify a 351 base pair (bp) segment of the PPRV N gene with the NP3/NP4 primer pair modified from Couacy-Hymman et al., (2002). (Forward NP3: 5′-GTC-TCG-GAA-ATC-GCC-TCA-CAG-ACT-3′ and Reverse NP4: 5′-CCT-CCT-CCT-GGT-CCT-CCA-GAA-TCT-3′) at a final concentration of 0.6 µM. PCR was set up under the following programme: 50°C for 30 min; 95°C for 15 min and 40 amplification cycles (10 s at 95°C, 30 s at 60°C and 30 s at 72°C) and a final extension step at 72°C for 5 min. The PCR products were resolved on 1.5% agarose gel to reveal the expected band size.

Ninety-nine out of 429 animals sampled (23%) were identified as PPRV positive by RT-PCR (Table 1). At least one sample was found to be PPRV positive in 17 states: Plateau, Benue, Kwara, Bauchi, Adamawa, Taraba, Katsina, Kebbi, Enugu, Abia, Anambra, Oyo, Ondo, Osun, Rivers, Cross Rivers and Akwa-Ibom. No positive samples among the twenty (20) samples tested were found in Kano state. Positive PCR products were cleaned up and sequenced in both forward and reverse directions by Genewiz. A partial N gene sequence was obtained from 91 out of 99 positive samples. The sequences were submitted to GenBank (Table 1). Forward and reverse DNA sequences were assembled using BioEdit and trimmed to remove poor quality portions of the sequences (final size = 255 bp). Corrected sequences were aligned using MEGA 7 with a data set of PPRV N gene sequences available in GenBank, representative of the four genetic lineages, excluding sequences suspected to be the result of contamination. Phylogenetic trees were constructed using the maximum likelihood method as implemented in MEGA 6, with node supports evaluated by bootstrap analyses with 1,000 replicates.

A total of 33 different sequences were obtained (Table 1, Genbank accession numbers: MT193234-MT193256; MW600924-MW600933). Phylogenetic analyses showed that the sequence of 32 PPR-positive samples belonged to lineage IV. A single sequence from the state of Oyo belonged to lineage II (Figure 2). In previous publications, PPRV lineage II strains were also only identified in this Western part of Nigeria in 2010–2013 (Mantip et al., 2016; Woma et al., 2016). The lineage II sequence provided poor node support for clustering (< 50%) with sequences from Senegal and Mauritania, well separated from other lineage II sequences (II-NigA) obtained in Nigeria in 2010–2013. Phylogenetic analysis revealed the presence of multiple well-supported sub-clusters within lineage IV in Nigeria (Figure 2). The IV-NigA sub-lineage was identified with good support (83%), while support for IV-NigB was low (51%). Only two sequences from Kebbi belonged to sub-lineage IV-NigA. They were closely related to samples from Niger (Figure 2). Five clusters were observed within Group IV-NigB, each including 3–7 sequences from multiple states, here called IV-NigB1 to IV-NigB5 (Figures 1 and 2). Clade IV-NigB1 included sequences from Ondo, Akwa-Ibom and Enugu. Clade IV-NigB2 included sequences from Akwa-Ibom, Benue, Taraba and Cross-River. Clade IV-NigB3 included strains from Rivers, Plateau, Benue and Osun with lower support (61%). Clade IV-NigB4 included sequences from Plateau, Bauchi, Kwara, Katsina and Kebbi (62% support). Identical sequences were collected in seven different states and are identified as IV-B5 in our tree. Sequences from Niger were found in sub-clusters IV-B3 and IV-B4. Many additional sequences (15/33) obtained here belonged to IV-Nig-B but could not be grouped in a specific clade. Notably, one sequence from the southern state of Cross River clustered with a sequence from Niger. One sequence from Kebbi did not belong to either IV-NigA or IV-NigB (Figures 1 and 2).

The results obtained here provide further support for extensive circulation of PPRV across Nigeria, and between Nigeria and neighbouring countries. Notably, clear evidence for movement of PPR between Nigeria and Niger was found, confirming the results obtained in a previous study (Souley et al., 2019). Sequences from different genetic clusters were found at the same time in the same market, implying co-circulation of different strains of PPR in the markets. These results support the role of markets in grouping of animals from different origins in the transmission and spread of PPR. The large majority of sequences obtained during this sampling effort that belong to lineage IV (IV-NigB) suggest differences in the circulation dynamics of lineage II and IV in Nigeria (possibly associated

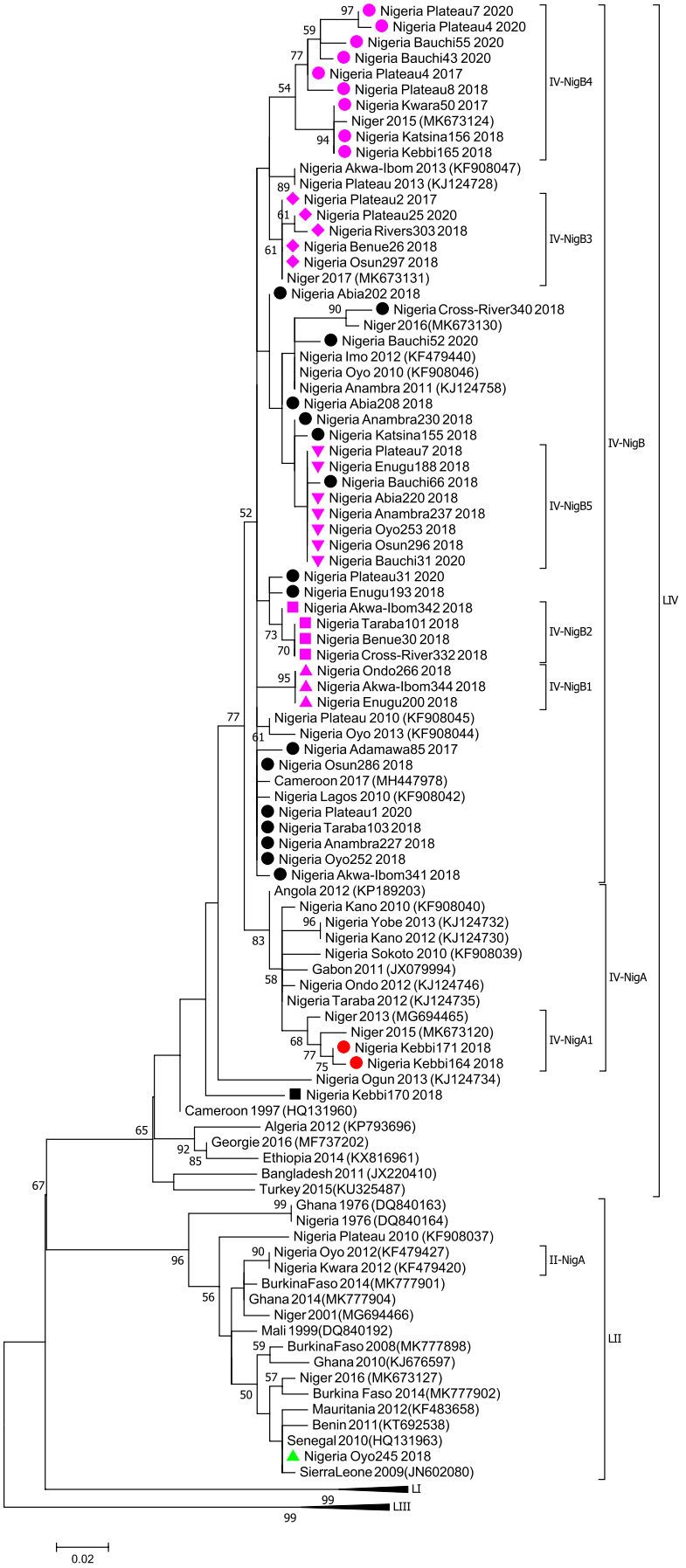

FIGURE 2 Peste des petits ruminants partial N gene phylogenetic analysis. Phylogenetic tree constructed using a maximum likelihood inference method showing the relationship based on N gene sequences of peste des petits ruminants virus (PPRV) derived from samples collected in Nigeria and that are publicly available. Samples collected in this study are indicated by icons based on phylogenetic clustering. The numbers at the nodes are bootstrap values obtained from 1,000 replicates, shown if >50%

with hosts and seasons), or strong overall dominance of lineage IV in Nigeria. Further sampling in different types of markets and ruminant populations at multiple time points would help unravel this issue. The identification of many new sequences of lineages II and IV with no clear links with sequences isolated previously in Nigeria and other West African countries also suggests that our understanding of the distribution and genetic diversity of PPRV in West Africa are still limited.

To summarize, this study provides an update on the diversity of PPRV strains circulating in Nigeria. Lineages II and IV continued to circulate in Nigeria between 2017 and 2020. This study, combined with previous studies on the molecular epidemiology of PPRV in West Africa, suggests the presence of a wide range of PPRV strains circulating across the region with limited control. Analyses based on larger sampling campaigns encompassing the entire region, using complete genomes of PPRV, and associated with detailed data on animal movement and epidemiological data, are necessary to better understand the phylogenetic relationships within PPRV and the transmission dynamics of the virus in Nigeria and in West Africa in general. This information is vital to develop appropriate and effective surveillance and control strategies against PPR.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

Approval was received to carry out this study from the NVRI Animal Ethics Committee with the Ref. No. AEC/02/87/20.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are freely available in GenBank at https://www.ncbi.nlm.nih.gov/ (under the following accession numbers: MT193234-MT193256; MW600924-MW600933).

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