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Distribution and diversity of emergent Banana bunchy top virus infecting banana and plantain in Cameroon, Central Africa

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Abstract

Invasions of arthropod vectors and viruses are the main factors associated with emerging plant viral diseases. The presence of the *Banana bunchy top virus* (BBTV, genus *Babuvirus*), an aphid-transmitted virus responsible for the banana bunchy top disease (BBTD), was first confirmed in 2008 in the South region of Cameroon. This study reports on surveys over 14 years to determine the status of BBTV spread and virus diversity in Cameroon. A total of 544 fields extending through 81 districts in 7 regions were surveyed in 5 phases: (1) 2009–10, (2) 2012, (3) 2013–14, (4) 2016–17, and (5) 2022. BBTV was detected in 36 sites, all located in the Ambam district in the South region, with an incidence in the virus-affected fields ranging from 5% to 40%, with an average incidence of 14.8%. The findings indicate BBTV expansion from the location of first detection in 2008 to about 4–25 km in all directions, with the virus spread range of about 700 km^2, as of the last survey in 2022. Phylogenetic analysis using complete nucleotide sequences of the BBTV-R and BBTV-S gene aligned Cameroon isolates with the sub-Saharan Africa subgroup of the Pacific-Indian Oceans (PIO) group of BBTV isolates, suggesting a likely virus invasion from neighbouring

central African countries where BBTV presence had been reported in the 1980s. Knowledge of BBTV distribution provided through the detection and delimiting surveys has contributed to the efficient targeting of interventions to limit the expansion of an emerging virus threat to banana production in Cameroon.

KEYWORDS
Babuvirus, BBTD, *Musa*, *Pentalonia nigronervosa*, surveillance, virus incidence

1 | INTRODUCTION

Banana and plantain (*Musa* spp., hereinafter referred to as bananas) are important food crops in SSA, where over 100 million people depend on their livelihood (Kilimo, 2014). Cameroon ranks second in banana production in Africa, with total production estimated at 6.1 million tonnes (MT) from 384,289 ha in 2021 (FAO, 2022). Banana cultivation in Cameroon is predominantly a perennial mixed cropping system, with large swaths of plantations in the fields as mixed or mono-crop, along the field boundaries, in the backyards in urban and rural areas, and unmanaged fields along the roadside and forests as wild plants. The bulk of production by smallholder farmers is in the humid forest of the southern half of the country and its Western Highlands (Fonsah & Chidebelu, 2011). Cooking bananas dominates the production, and almost 90% of it is used for domestic consumption and a small proportion to export to neighbouring countries. Mono-crop dessert banana production is concentrated in over 3500 ha of highly intensive agriculture by multinational companies in the Littoral and South-West regions, produced almost exclusively for fruit export to international markets. Traditionally banana farmers use suckers (side shoots) harvested from the existing plantations for establishing new plantations and sometimes acquire from other farmers for free or bartered (Nkengla-Asi et al., 2021). Overall productivity of banana is very low in Cameroon. Many biotic and abiotic factors are responsible for the lower yields.

Banana disease concerns were heightened with the discovery of banana bunchy top virus (BBTV) in 2008 in southern Cameroon (Oben et al., 2009). BBTV, the causal agent of banana bunchy top disease (BBTD), is listed among the top 10 plant viruses in terms of economic impact worldwide (Rybicki, 2015) and is a regulated quarantine pest classified as one of the World's 100 Worst Invasive Alien Species because of the difficulties in containing the virus spread and the threat it poses to banana biodiversity (Lowe et al., 2000). BBTV causes chlorotic hooks on leaf lamina, dark green streaks on leaf petioles, marginal leaf chlorosis, narrow and erect leaves, and a bunchy appearance of the plant due to a drastic reduction of leaf lamina and petiole size. The diseased plant ceases to produce fruits, leading to 100% production loss within one or two seasons after infection (Cook et al., 2012; Hooks et al., 2008). The BBTV genome comprises six circular single-stranded DNA components, each about 1.1 kb long, encoding a single open reading frame (Banerjee et al., 2014). Based on the genomic diversity, BBTV isolates were grouped into Pacific-Indian Oceans (PIO) group comprising the isolates from Africa, Australia, Hawaii, and South Asia; and the South-East Asian (SEA) group comprising the isolates from South-East Asia (Banerjee et al., 2014). The virus spreads through vegetative propagation of banana and the natural transmission from plant to plant is facilitated by the banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), which is found on *Musa* species worldwide. The aphid vector which persistently transmits the virus (Anhalt & Almeida, 2008) is implicated mostly in short-distance virus spread, while the long-distance spread was attributed to the human movement of infected planting materials (Kumar et al., 2011; Nkengla-Asi et al., 2021).

BBTV is considered native to South-East Asia and was first reported from the African continent in Egypt in 1901 (Kumar et al., 2015). At present, the virus has been confirmed in 18 African countries, where it is causing substantial losses in banana and plantain production and trade (Kumar et al., 2015; Shimwela et al., 2022). The patterns of banana cropland and cultural practices, especially the tradition of using suckers sourced from the existing plantations for establishing new plantations, greatly heighten the risk of further invasion and saturation of BBTV (Nkengla-Asi et al., 2021; Xing et al., 2020). Understanding the extent of BBTV spread is crucial to implementing strategies to prevent the expansion of BBTV in Cameroon and elsewhere where the disease has invaded. In this paper, we report the results and analysis of systematic surveys conducted between 2009 and 2022 in all the banana production regions to track the spread of BBTV and delimit its distribution in the banana-growing area of Cameroon, supported by analysis of the molecular diversity of BBTV isolates collected throughout its occurrence in the country. Broadly, the study is used to draw lessons on factors that affect BBTV spread and persistence and to advocate for integrated measures to control the further invasion of BBTV.

2 | MATERIALS AND METHODS

2.1 | Surveys and sample collection

Field surveys were conducted in seven of the ten administrative regions of Cameroon in five periods from 2009 to 2022: (1) April 2009 to July 2010 in the Center, Littoral, South, South-West and West regions; (2) March to July 2012 in the Center, South and South-West regions; (3) December 2013 to January 2014 in the Center, East, South, South-West and West regions; (4) July 2016 to January 2017 in South, East and Adamawa regions. In October 2022, the survey was limited to assessing the recent spread status in the South region

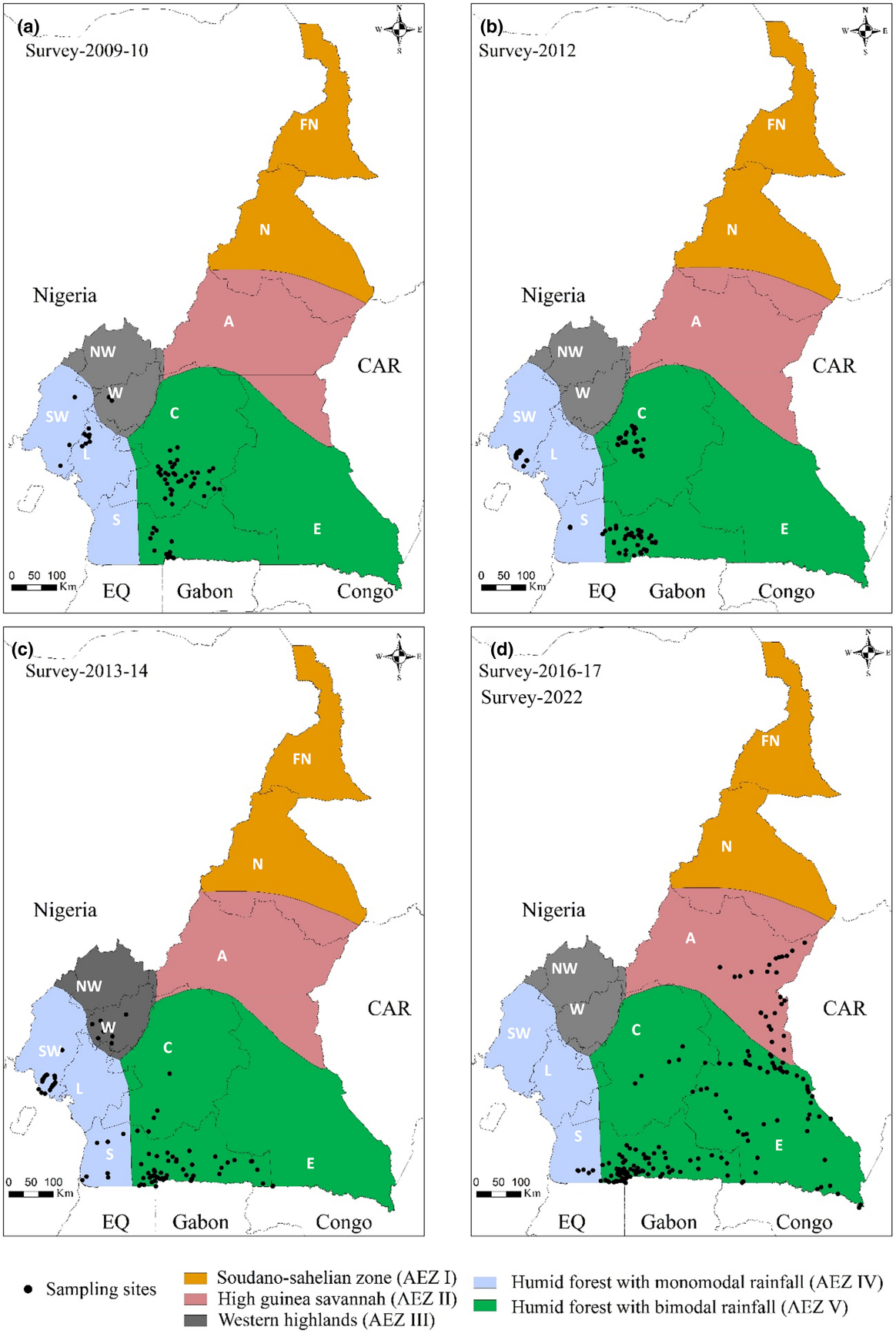

FIGURE 1 Indication of locations surveyed during 2009–10 (a), 2012 (b), 2013–14 (c) and 2016–17 and 2022 (d) in the geographic maps of Cameroon. The five agroecological zones (AEZ) and 10 regions (C, Center; E, East; FN, Far-North; L, Littoral; N, North; NW, North-West; S, South; SW, South-West; W, West) are indicated on the maps. Detailed maps of each survey were presented in Figures S1–S5.

where the BBTV is localized, as other regions were found to be free of BBTV (Figure 1) (see Table S1 for details). Together, the five surveys covered a total of 544 fields located in 81 districts in 7 regions known to produce bananas in Cameroon (Figures S1–S5).

Banana fields were inspected at 20–30 km intervals along motorable roads, except in the BBTV-affected South region, where inspected fields were selected at 2–5 km intervals. In the 2022 survey, fields for BBTV assessment were selected at about 5 km from the most external BBTV-infected location as per the 2017 distribution status to determine the extent of spread and continued at 5 km intervals for delimiting the virus spread in the South region. The geographic coordinates and altitude of each site were obtained using a GPS recorder (eTrex 30, GARMIN International Inc., USA). In each field, 20 banana mats selected in 2 diagonal transects were visually inspected for the common BBTD symptoms used in field visual diagnostics. BBTD symptom severity was scored on a 1–3 rating scale, where 1 = asymptomatic plants, 2 = plants with chlorotic streaks on petioles or leaf lamina, marginal leaf chlorosis with no apparent bunching symptoms, and 3 = narrow and erect leaves, plants with typical bunchy top symptoms (Kumar et al., 2011). Leaf samples were collected from five randomly selected plants while ensuring collection of at least one symptomatic plant in BBTD-affected fields for BBTV detection by polymerase chain reaction (PCR). Leaf samples (about 50 cm^2 from each sampled plant) were wrapped in aluminium foil and transferred on icepacks for transport to the laboratory, where they were stored at −20°C for later PCR analysis to ascertain the infection status of each field.

2.2 | BBTV detection using PCR

Leaf samples collected during the surveys were analysed using PCR as detailed by Kumar et al. (2011). Total genomic DNA was extracted following the CTAB protocol and used for the BBTV detection using a primer pair [BBTV-1: 5′GCGTGAAACGCACAAAAGGCC3′; and BBTV-2: 5′GCATACGTTGTCAAACCTTCTCCTC3′] corresponding to the 240 base pair (bp) BBTV DNA-R segment. Primers for host plant DNA Brep repetitive genes in *Musa* spp. [BrepF: 5′GATTTTGTAGATTTTGGACACCG3′; and BrepR: 5′GAATAACAAATATGCTCCAATACCC3′] which result in 401 bp products were used as internal control. The PCR profile involved an initial denaturation at 94°C for 5 min, then 35 cycles of 94°C for 45 s, 50°C for 30 s, 72°C for 33 s; and a final extension at 72°C for 5 min and 4°C for the end. PCR products were run on 1.5% agarose gel stained using EZ-Vision (VWR International, Radnor, PA, USA), and DNA was visualized under UV trans illuminator.

2.3 | Sequencing of the BBTV-R and BBTV-S gene and phylogenetic analysis

Four virus-positive samples from surveys were selected for direct sequencing of PCR products by the Sanger dideoxy method at Iowa State University, DNA Sequencing Facility (Ames, Iowa, USA) (Table S2). The complete 1075 bp segment of BBTV-S (encoding for coat protein gene) was amplified using the primer pair BBTV-CP1 [5′-CCCGGGAGAATACTTCACTGGGC-3′] and BBTV-CP2 [5′-CCCGGGCTTCACCTTGCACACCA-3′], and the complete 1111 bp segment of BBTV-R (encoding for the master replication initiation protein) was amplified using the primer pair MREP-F [5′-GAATTCAAGAATGGAATAATTC-3′] and MREP-R [5′-GAATTCCTCTAATAACCC-3′] (Amin et al., 2008). The sequences generated were edited and assembled in BioEdit v7.2.5 and used for construction of a phylogenetic tree with BBTV-R and BBTV-S gene sequences retrieved from the NCBI GenBank (Table S2).

DNA sequences representing the full DNA-S (47) and DNA-R (40) segments were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore) (Table S2) and aligned using MAFFT server (https://mafft.cbrc.jp/alignment/server/) using the auto strategy. In addition to this, full genome of DNA-S and R segment of the abaca bunchy top virus (ABTV), downloaded from the NCBI GenBank, were included in the analysis as an outgroup. The analysis was such that the 5′ UTR (untranslated region), Open Reading Frame (ORF) and the 3′ UTR of BBTV DNA-S and DNA-R components were analysed separately for nucleotide sequence diversity. Based on the varying conservation among the nucleotide sequences, sequence diversity analysis was conducted using full genomic component and solely with the ORF coding for the coat protein (CP) and master replicase initiation protein of DNA-S and DNA-R, respectively. Subsequently, the multiple sequences of only ORF regions of DNA-S and -R were prepared. The corresponding model for nucleotide substitution (S and R segment = TN + F + R2 and TPM2 + F + R2) and amino acid sequences (S and R = JTTDCMut + I and JTTDCMut + G4) were inferred using the ModelFinder (Kalyaanamoorthy et al., 2017). Subsequently, the phylogenetic trees were reconstructed using the maximum likelihood (ML) estimate in the IQ-tree version 2.0 (Trifinopoulos et al., 2016) and branch support was determined using the 1000 ultrafast bootstrap (Hoang et al., 2018). The reconstructed tree was viewed and edited in the Figtree software version 1.4.4. The evolutionary divergence was estimated within and between the identified phylogenetic groups from the reconstructed phylogenetic tree using maximum composite likelihood of 1000 bootstrap replicates (Tamura et al., 2004).

2.4 | Data analysis

BBTD prevalence was estimated as the total number of fields with at least one BBTD-symptomatic plant over the total number of fields inspected in affected regions. BBTD incidence within each field was calculated as the percentage of the number of symptomatic mats over total number of inspected mats. BBTV incidence was calculated as the percentage of BBTV-positive leaf samples over total number of leaf samples. BBTD distribution was mapped with ArcGIS Desktop version 10.7 (ESRI, 2019). Banana production and harvested area were estimated using data from spatial production allocation model (SPAM) developed by the International Food Policy

Research Institute (IFPRI, 2019) for generating spatially disaggregated crop-specific production data. The SPAM spatial model uses a cross-entropy approach to make plausible estimates of crop distribution within disaggregated units by allocating crop production by district, region, and country to a raster grid at a spatial resolution of 5 min of arc represented as a 10 × 10 km pixel.

3 | RESULTS

3.1 | Incidence and distribution of BBTV

Out of the 544 fields surveyed in 7 Cameroon regions, plants with typical BBTD symptoms (Figure 2) were detected in 36 (6.6%) fields in the South region and none in Adamawa, Center, Littoral, South-West and West regions (Figure 3; Table S1).

PCR-based diagnostic amplified BBTV-specific 240 bp amplicon in samples collected from all the symptomatic fields (Figure S6). Overall, 63 (1.5%) of 4200 samples collected from 544 fields tested positive for BBTV. All the positive samples represented plants showing typical BBTD symptoms (Figure 2) from the 36 symptomatic fields in the South region. None of the 4137 asymptomatic plant samples from 508 asymptomatic fields located in the Adamawa, Center, Littoral, South, South-West and West regions tested positive for BBTV (Table 1).

In the South region, BBTV was detected in 36 of 300 (12%) fields between 2009 and 2022 with the following distribution by year of survey: 2 of 15 (13.3%) fields in 2009–10 (Figure S1), 3 of 46 (6.5%) fields in 2012 (Figure S2), 5 of 57 (8.8%) fields in 2013–14 (Figure S3), 21 of 158 (13.3%) fields in 2016–17 (Figure S4) and 5 of 24 (20.8%) fields in 2022 (Figure S5) (Table 1). All the fields with BBTD infection were detected in 20 communities in Ambam district. BBTD incidence in the 20 communities ranged between 5% and 40% with an overall mean incidence of 14.8%. Mean symptom severity on infected plants was consistently high with 2.8 (out of a maximum score of 3), implying that almost all the infected mats had shoots with typical bunchy top symptoms. Out of 255 samples tested from 36 BBTD-affected fields, 63 (24.7%) samples tested positive to BBTV (Table 2). All the BBTV-positive plants were symptomatic, while none of the asymptomatic plants sampled from the symptomatic fields tested positive to BBTV.

In the BBTV-affected district of Ambam, banana-harvested areas vary between 300 and 1000 hectares (Figures 3 and 4). During the 2009–10 survey, BBTV was detected only in the localities of Abang Minko'o and Kou'ou-si, which are 2 and 4 km, respectively, from the Gabon border. In 2012, in addition to the previous localities where the virus had persisted, BBTV was detected in 2 new localities about 15 km eastward from the initial focus. In 2013–14, BBTV was also detected in 4 new localities inside Cameroon at about 5 km northward and 20 km eastward from the initial areas. During the survey in 2016–17, BBTV was detected in previously non-infected localities at about 30 km from the initial infected area in the north-west direction (Figure 4). In 2022, BBTV was detected for the first time in 5 locations, north-eastward, at Ebozi – 22.1 km from the initial point (2.1 km from a previously infected field) and Nkemeyen – 29.8 km from the initial point (9.4 km from a previously infected field). North-westward, BBTV was detected in Akoulouzok at 21.2 km from the initial point (5.5 km from a previously infected field).

Westward towards Equatorial Guinea road, BBTV was detected at Zaminkane – 32.1 km from the initial point (3.9 km from a previously infected field) and Yama – 28.3 km from the initial point (5.1 km from a previously infected field). The near-approximate Euclidean distances between the BBTV-affected sites detected in 2008 (Oben et al., 2009) and sites discovered in all five surveys of this study were about 22 km east, 25 km west, 19 km north and 3 km south (to the edge of the international border with Gabon) with the diseased sites spread approximately in an area covering 700 sq. km (Figure 4).

FIGURE 2 Severe bunchy top symptoms in the local plantain cultivars (white circles) in various banana farmers' fields in the Ambam district in Cameroon.

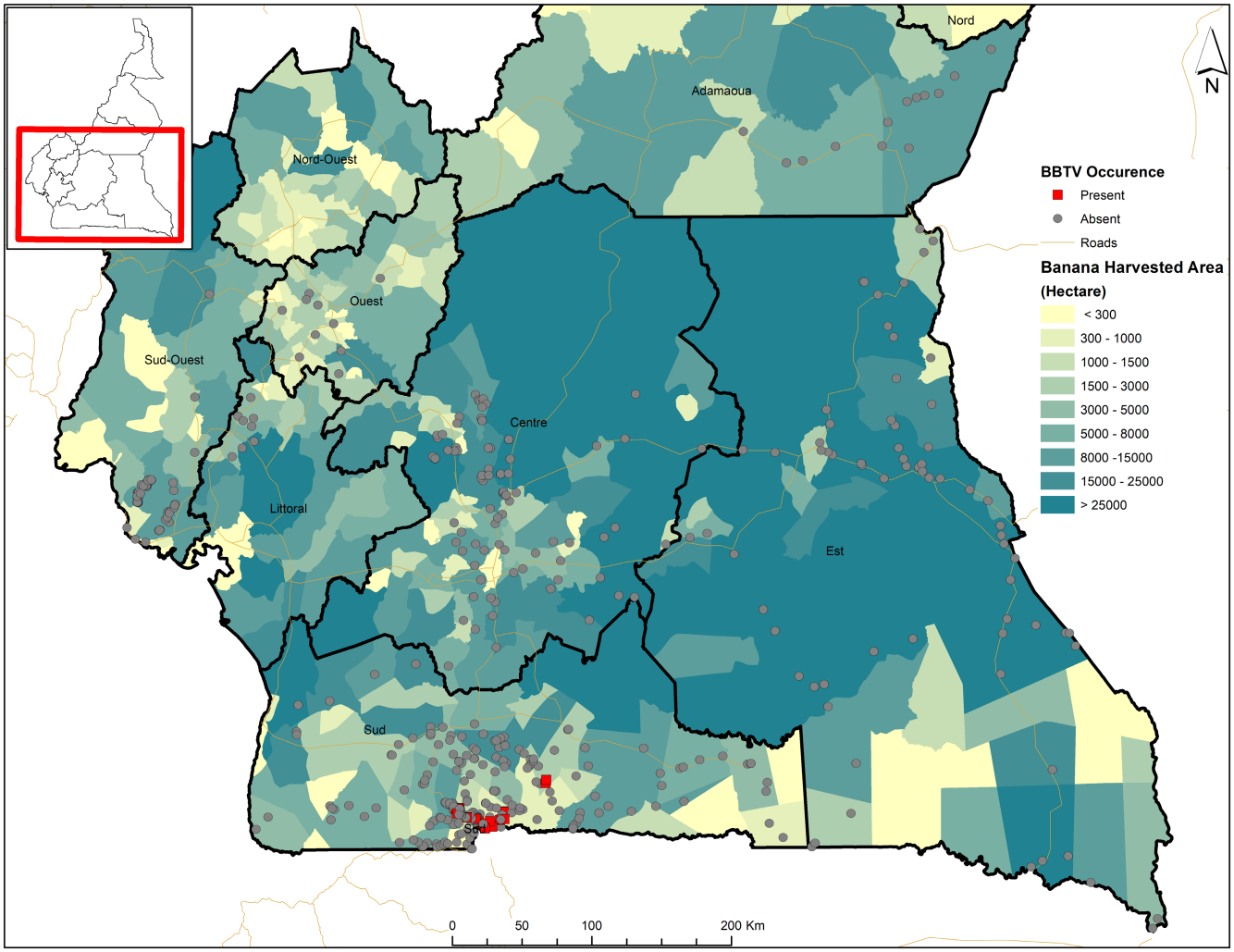

FIGURE 3 Geographic distribution of survey sites and BBTV-positive locations in Cameroon during 2009–10, 2012, 2013–14, 2016–17 and 2022 surveys. The banana production area indicated in the map is based on the SPAM model (IFPRI, 2019).

Although this represents a large area, the number of infected fields within this area was few and widely distributed.

The sources of planting materials for all but one surveyed field were suckers sourced from own fields or neighbours within the community. Banana plants with a tissue culture origin were found in only one farm in the south-west region in the 2009–10 survey; the field belonged to a commercial company producing dessert bananas (Cavendish-AAA) (Table 1). Local plantain cultivars (AAB) were observed in 539 of 544 inspected fields (99.1%), while dessert banana cultivars were found in 146 (26.8%) fields. Cooking bananas (AAB) and synthetic hybrids (tetraploid bananas) were found in 12 (2.2%) and 5 (0.9%) fields, respectively (Table 1). Inspected fields had an average age of 3.9 ± 0.85 years.

3.2 | BBTV diversity

The 1075 bp BBTV-S gene that encodes for coat protein (S) from four virus-positive isolates representing Abang Minko'o location in 2010 and 2014, and Mengama in 2016 and 2017 (NCBI Acc.# MT553857, MT553856, ON109391 and MT553855, respectively) were sequenced along with a 1111 bp BBTV-R gene that encodes master replication initiation protein from Mengama (NCBI Acc.# ON109390). The BBTV-S translated into one open reading frame (ORF) of 175 amino acids that showed 99% homologies with BBTV-S segments of PIO group of isolates. The mean pairwise nucleotide (nt) homology between the 3 BBTV-S sequences was 99.7%. The mean nt homology between the isolates collected from Abang Minko'o was 99.7% at nt level and 100% at the amino acid (aa) level, whereas the mean nt and aa homology were 99.6% and 98.8%, respectively, between the isolates from Abang Minko'o and Mengama. A similar level of nt homologies (99%) was observed between the two BBTV-S segments sequenced (NCBI Acc.# JF755978 and JF755979) from samples collected in 2008 survey. The BBTV-R translated into ORF of 301 amino acids which showed 99%–100% identity with homologous sequences of PIO group of isolates. The phylogenetic analysis clustered the four BBTV-S isolates sequenced in this study to PIO cluster of BBTV isolates (Figure 5).

TABLE 1 Types of planting materials and bananas cultivated during surveys conducted between 2009 and 2022 in Cameroon.

				Types of planting materials			Banana types (genomic groups)			
							Natural hybrids		Synthetic hybrids	
Region	Survey year	Total fields surveyed	Total fields with BBTV infection	Suckers from old fields	Tissue culture	Plantains (AAB)	Dessert bananas (AAA)	Cooking bananas (AAB)	Hybrid bananas	Hybrid plantains
Adamawa	2016–17	13	0	13	0	9	0	11	0	0
Center	2009–10	34	0	34	0	34	23	0	1	1
	2012	43	0	43	0	43	18	0	0	0
	2013–14	4	0	4	0	4	1	0	0	0
	2016–17	7	0	7	0	7	0	0	0	0
East	2013–14	2	0	2	0	2	1	0	0	0
	2016–17	70	0	70	0	70	2	0	0	0
Littoral	2009–10	9	0	9	0	9	1	0	0	0
South	2009–10	15	2	15	0	15	1	0	0	0
	2012	46	3	46	0	46	9	0	0	0
	2013–14	57	5	57	0	57	17	1	0	0
	2016–17	158	21	158	0	158	31	0	1	2
	2022	24	5	24	0	24	16	0	0	0
South-West	2009–10	2	0	1	1	1	2	0	0	0
	2012	30	0	30	0	30	9	0	0	0
	2013–14	20	0	20	0	20	6	0	0	0
West	2009–10	2	0	2	0	2	1	0	0	0
	2013–14	8	0	8	0	8	8	0	0	0
Total		544	36	543	1	539	146	12	2	3

TABLE 2 Summary of BBTV-positive fields in the South region.

Survey period	Number of fields surveyed	Number of BBTV-positive fields	BBTD prevalence (%)	Mean BBTD incidence (%)	Mean symptom severity[a]	Samples tested (symptomatic/asymptomatic (total))	Total positive	Virus incidence (%)
2009–10	15	2	13.3	25	3	7/13 (20)	7	35
2012	46	3	6.5	16.6	2.7	8/22 (30)	8	26.7
2013–14	57	5	8.8	12	2.8	11/39 (50)	11	22
2016–17	158	21	13.3	9.5	2.7	27/78 (105)	27	25.7
2022	24	5	20.8	11	2.7	10/230 (240)	10	4.2
Total	300	36	12.5	14.8	2.8	63/382 (445)	63	22.7

Abbreviations: BBTD, banana bunchy top disease; BBTV, banana bunchy top virus.

[a]Severity assessed on 1–3 scale, where 1 = no symptoms and 3 = severe bunchy top symptoms.

The nt diversity in SSA ranged from 0% to 3%. Low levels of diversity between BBTV isolates in SSA and that of PIO were observed in several BBTV isolates sequenced in SSA (Kumar et al., 2011; Shimwela et al., 2022; Ximba et al., 2022). Based on the nucleotide sequence analysis, the 3′ and 5′ sequences flanking the CP encoding gene segment had a higher sequence diversity (6.8% and 3.9%, respectively) compared to the CP coding portion (2.9%) (Table 3). The phylogenetic tree constructed for the CP gene had a similar organization to those described by Kumar et al. (2011) with well-supported distinct clades (PIO = Pacific and Indian Ocean group; BS = 98% and the South-East Asian group; BS = 99%). The CP sequences of BBTV isolates from this study clustered into subgroup PIO-1B, representing the sub-Sahara subgroup (BS = 98%).

A similar nucleotide diversity conservation pattern was observed in the nucleotide sequence BBTV-R component. However, the phylogenetic tree generated from the BBTV-R gene had a slightly different organization from that obtained from the coat protein, with the presence of PIO-1C, composed of isolates from Pakistan (BS = 47%). The isolates from this study clustered with BBTV isolates from Central Africa region (PIO-1B) (Figure 6).

4 | DISCUSSION

Agriculture and other managed and natural ecosystems are constantly threatened by biological invasions that often cause substantial damage to their productivity and biodiversity with ever-increasing costs to producers, consumers and the environment (Mormul et al., 2022). Numerous cases of invasive virus species in agricultural systems have been documented, and many have turned from epidemics to pandemics that threaten agricultural production and food security, with over 300 billion US$ in overall crop and market losses (Jones, 2020; Kumar et al., 2019). This study assessed the extent of BBTV spread in Cameroon through frequent surveys conducted since the first confirmation of the virus in the country from 2009 to 2022, covering all the banana-producing regions in Cameroon.

The surveys demonstrated the spread and confinement of BBTV to only the Ambam district in the South region. BBTD-symptomatic plants were encountered in 36 locations in 300 locations surveyed between 2008 and 2022. The expansion pattern suggests that BBTD spread has occurred largely from infected fields to neighbouring fields and their vicinity, indicating that the aphid vector plays an important role in local spread. Five BBTD-infected fields were detected about 15 km eastward in 2013–14, and 21 BBTD-infected fields about 30 km north-westward in 2016–17 from the early infection sites in the Ambam district. In 2022, BBTV was newly detected in five locations in Ambam district situated at 2–10 km from old limit north-eastward towards Equatorial Guinea road. This relatively long-distance spread of BBTV was most likely due to the movement of infected suckers. Therefore, this study implicates both human-mediated transmission and plant-to-plant (or field-to-field) by the banana aphid vector.

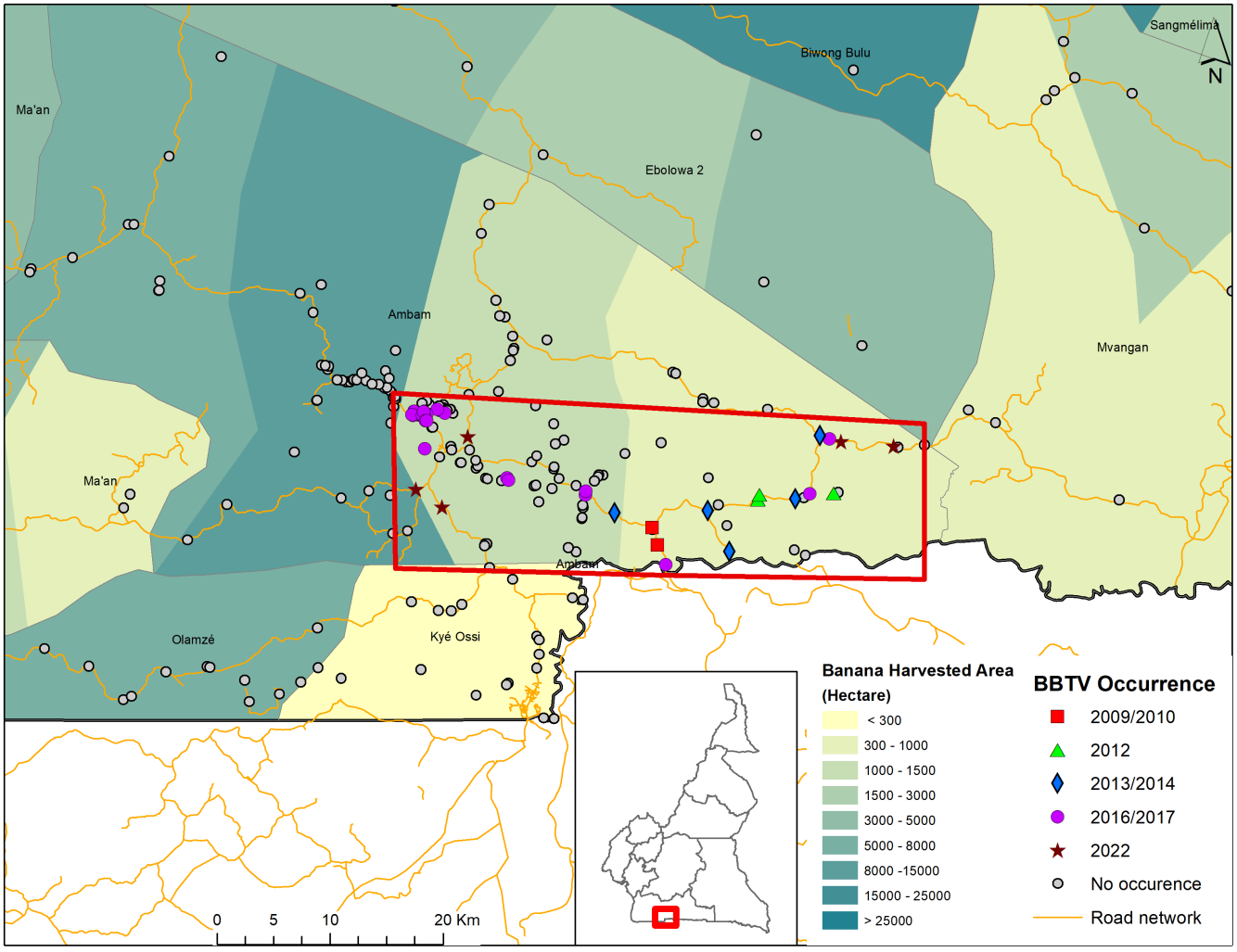

FIGURE 4 Geographic distribution of survey sites and BBTV-positive fields detected during the four surveys conducted in 2009–10, 2012, 2013–14, 2016–17 and 2022 in Ambam district in South region of Cameroon. The banana production area indicated in the map is based on the SPAM model (IFPRI, 2019). The BBTV-affected region is marked with a polygon box.

Farmers have limited knowledge of the disease symptoms and the risk of virus spread along with banana planting materials; therefore, they do not prioritize healthy planting materials for establishing new fields (Abiola et al., 2020; Nkengla-Asi et al., 2021). This appears to be one of the main factors for sustaining the disease in the South region as farmers abandon banana fields after 2–3 years and establish new fields with suckers from existing plantations. The abandoned fields continue to be a source of the virus and become difficult to reach as they turn into bush fallow with overgrown weeds. Contiguous banana plantations, susceptible *Musa* cultivars grown in the region and the ecology that supports high aphid activity, combined with subsistence-type farms with very little or no farm management, are the likely reasons for the spread and persistence of the disease in the South region.

The 2008 survey by Oben et al. (2009) reported the occurrence of BBTD-symptomatic plants in three locations in the South region, along with sporadic occurrence of asymptomatic, virus-positive plants in seven locations in South-West and Littoral regions, three locations in the West and one location in the Central regions. In contrast, the present study did not detect any BBTD-symptomatic and BBTV-positive plants in the Center, Littoral, South-West and West regions. We presume the symptomatic plants reported in some of these locations by Oben et al. (2009) could have been the primary infected sources spread along with the movement of infected suckers from the South region, which were removed by rouging (i.e. removal and destruction of infected) following the advice on good agronomic practices by the 2008 survey staff. The lack of spread of BBTV to other regions could also be due to the direction of planting materials flow. The planting material for most production regions is sourced and transported from the major banana production zones of the BBTV-free Center, Littoral and South-West regions to the South region and not the reverse direction. The direction of movement of suckers from no-BBTV zones could have prevented the northward spread of the virus.

Based on the severity of the symptoms in the virus-affected fields and farmers' knowledge, BBTV was predicted to have occurred in the South region since 2006–07. The localities where BBTV was first detected, Kou'ou-si and Abang Minko'o in Ambam district border Gabon,

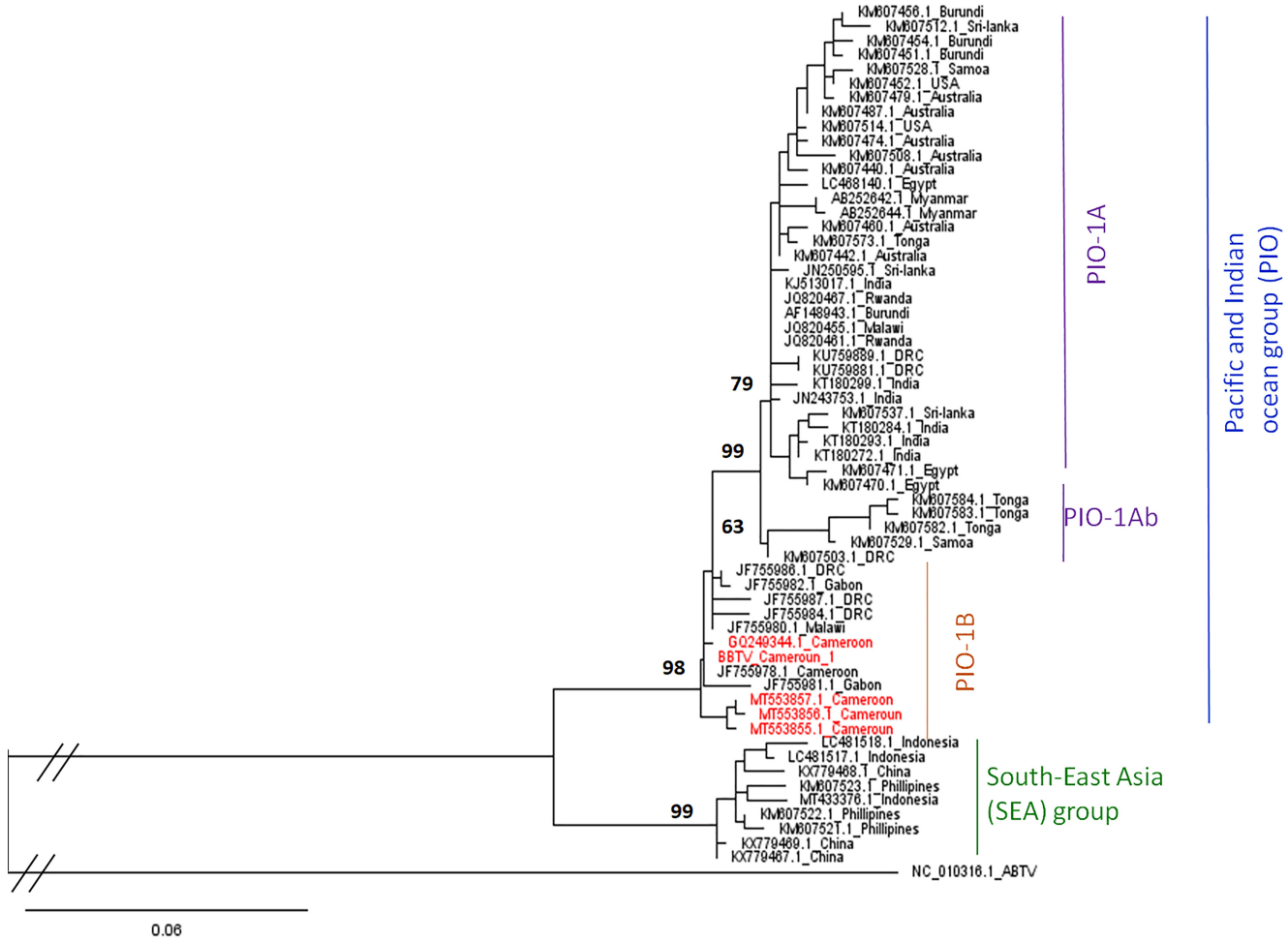

FIGURE 5 Diversity of banana bunchy top virus (BBTV) as inferred by maximum likelihood at 1000 bootstrap replicates inferred using the open reading frame coding the coat protein (CP) gene of the DNA-S. The corresponding bootstrap supporting the clades is presented beside each branch. Nucleotide sequences generated in this study are indicated in red font.

TABLE 3 Nucleotide diversity for the different regions of the BBTV DNA components S and R.

	BBTV-S		BBTV-R	
Gene portion	π	η	π	η
3′ UTR	0.0680	0.0826	0.0876	0.0122
5′ UTR	0.0394	0.0742	0.0187	0.0446
Coding region	0.0296	0.0418	0.0317	0.0430

Note: π = nucleotide diversity; η = per site diversity; BBTV-S and DNA-R = DNA segments encoding for structural coat protein and replication initiation proteins, respectively.

where BBTD symptomatic plants had been reported in the 1980s (Foure & Manser, 1982). Abang Minko'o is a cosmopolitan town inhabited and frequented by people from various areas of Cameroon, Gabon and Equatorial Guinea owing to its location as a trade hub between the three countries (Nkengla-Asi et al., 2019). The economic and intercultural exchanges have likely contributed to the BBTV spread through the inadvertent exchange of infected planting material from Gabon.

The phylogenetic analysis of the BBTV-S and R genes presented in this study suggests a close relationship between Cameroon and Gabon isolates, and it confirms the Central African origin of the Cameroon BBTV isolate. High nucleotide homologies between the BBTV-S isolates obtained in this study and two BBTV-S isolates sequenced earlier (Kumar et al., 2011; Oben et al., 2009) support the hypothesis of a single introduction of BBTV into Cameroon and its subsequent spread in the country, which was similar to the situation observed in South Africa (Ximba et al., 2022).

The relatively slow spread of BBTD in Cameroon contrasts with the devastating effect of BBTD on monocultures of Cavendish cultivars (AAA group dessert banana) in Australia (Allen, 1987) and central Malawi (Kumar et al., 2011). It is well known that crop monocultures of homogeneous genetic backgrounds are more susceptible to severe disease outbreaks than crop mixtures (Garrett & Mundt, 1999). In the BBTD-affected South region of Cameroon, banana cultivation is a variety mixture predominantly of AAB local plantains (Table S1), which are moderately susceptible to BBTD with a variable rate of spread, as shown in two trials organized in different locations in the South of Cameroon (Ngatat

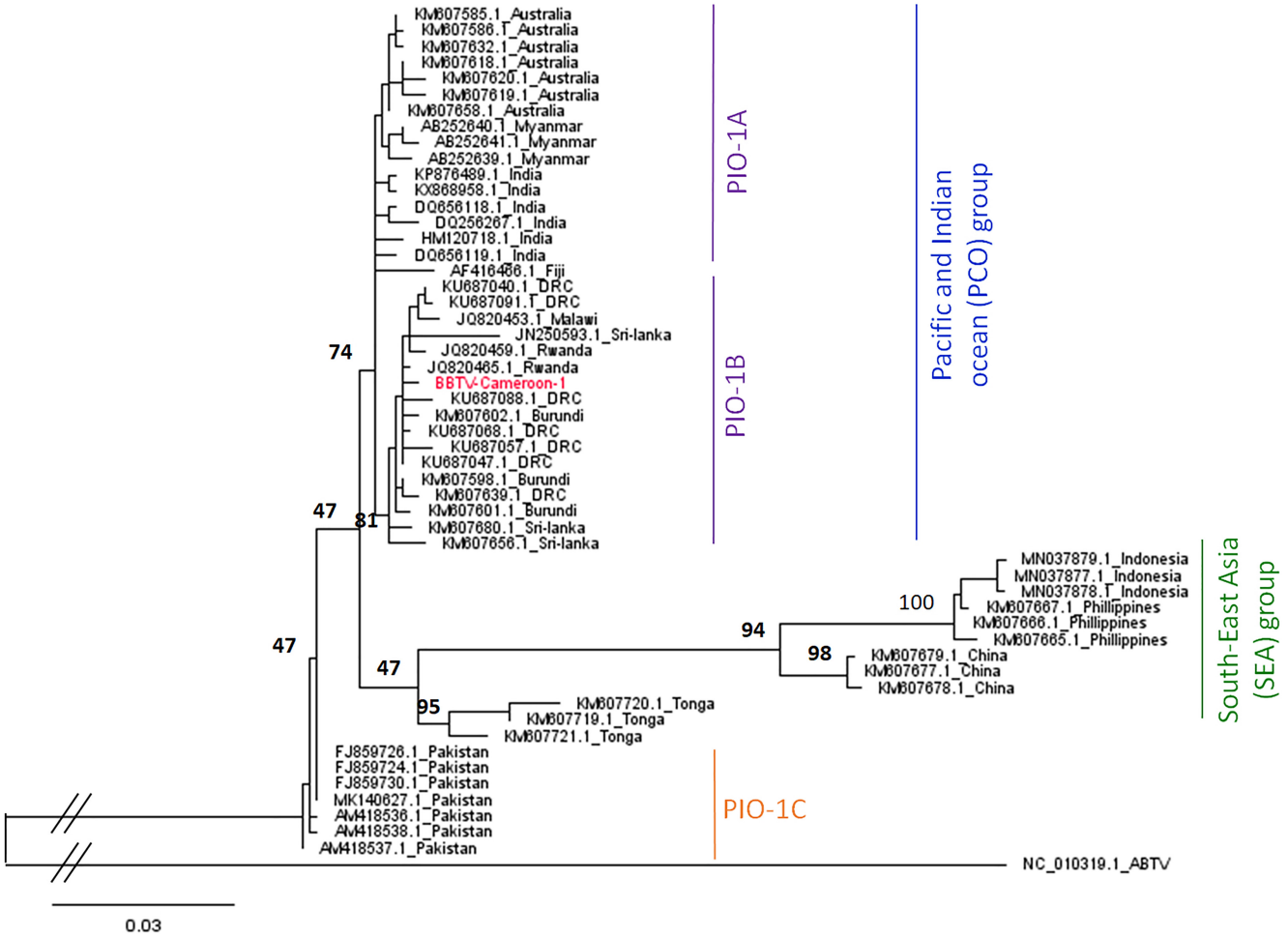

FIGURE 6 Diversity of banana bunchy top virus (BBTV) as inferred by maximum likelihood at 1000 bootstrap replicates using the open reading frame for the master replication initiation protein of the DNA-R component. The corresponding bootstrap supporting the clades is presented beside each branch. Nucleotide sequences generated in this study are indicated in red font.

et al., 2017, 2022). Banana crop land connectivity risk indexing based on network modelling has suggested influence of several factors, including agroecology, cultivars and crop management practices on rate of pathogen spread in a geographic region (Xing et al., 2020).

5 | CONCLUSIONS

This survey helped map geographic areas affected by the virus, identify areas of high risk for virus invasion and design mitigation strategies as part of the ALLIANCE for BBTV control in sub-Saharan Africa (www.bbtvallince.org). These measures include training in the application of virus diagnostics, surveillance and eradication to extension agents; advocacy and awareness to prevent the movement of planting material from the diseased areas; and monitoring surveys to check the virus spread, phytosanitation of infected plants and use of certified clean planting materials for establishing new fields. Several of these measures, which have been and continue to be implemented in the South of Cameroon, are presumed to have helped to limit the further spread of BBTV in the country.

This study demonstrates the usefulness of regular surveys for the containment of an emerging virus disease by mounting intensive efforts to eradicate infected sources to minimize disease spread and even achieve complete eradication. Although the latter is generally considered a very difficult challenge, keeping such a high-intensity goal seems vital for maintaining the pressure to reduce inoculum levels while increasing quarantine monitoring to control the movement of planting material within and between neighbouring countries.

AUTHOR CONTRIBUTIONS

S.N., R.H. and P.L.K.: Conceptualization, investigation, data analysis, visualization, preparation of first draft and final editing; S.N., B.O. A.R.P.D.F, J.A.L., A.F.K., B.N. and K.K.M.F: surveys, analysis, investigation and manuscript drafting; T.A., P.L.K. and S.N.N: GIS mapping; T.A.: SPAM modelling for production area; P.L.K., R.H., K.K.M.F. and G.S.D. Funding acquisition. All authors reviewed and edited the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available in the CKAN repository under the link https://doi.org/10.25502/gamf-4v42/d.

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