Molecular characterisation of ampeloviruses associated with mealybug wilt of pineapple disease in Ghana

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Abstract

**Background:** Mealybug wilt of pineapple (MWP) is the most destructive viral disease of pineapple worldwide. The disease is caused by pineapple mealybug wilt-associated virus (PMWaV), a member of the family *Closteroviridae* and the genus *Ampelovirus*, and transmitted by mealybugs.

**Methods:** In order to understand the association between closteroviruses and MWP in Ghana, 24 pineapple plant samples showing typical symptoms of MWP were collected during a survey of the Central Region in 2019. Three quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assays with PMWaV species specific primers were performed to amplify the heatshock protein 70 gene (HSP70) of PMWaV-1,-2 and -3 in the pineapple samples. Purified qRT-PCR products of thirteen isolates which consist of PMWaV-1 (7 isolates), PMWaV-2 (4 isolates) and PMWaV-3 (2 isolates) were sequenced in both directions. Sequence and phylogenetic analyses were then conducted.

**Results:** Three different species of *Ampelovirus* namely PMWaV-1, PMWaV-2, and PMWaV-3, were detected from the plant samples, with abundance of mixed infections. Sixteen out of the 24 samples (66.7%) were infected with at least one of the three species of *Ampelovirus* identified. PMWaV-2 had the highest infection rate of 62.5% across the districts; this was followed by PMWaV-1 and PMWaV-3 with infection rates of 33.3% and 8.3% respectively. Purified qRT-PCR products of thirteen isolates which consist of PMWaV-1 (7 isolates), PMWaV-2 (4 isolates) and PMWaV-3 (2 isolates) were sequenced in both directions. Sequence comparison using BlastN showed that all the seven sequences of the Ghanaian PMWaV-1 isolates (GenBank Accession Nos. MN427634 - MN427639 and MN399973) shared 95.2% to 99.7% nucleotide identity with each other and 95.2-100% with sequences of isolates previously published in GenBank. The four sequences of the Ghanaian PMWaV-2 isolates (GenBank Accession Nos. MN427642 - MN427645) shared
nucleotide identity of 98.9–100% with each other and 98.2–100% nucleotide identity with sequences of isolates previously published in GenBank. Also, the two sequences of the Ghanaian PMWaV-3 isolates (GenBank Accession Nos. MN427640 and MN427641) shared 98.3% nucleotide identity to each other and 97.5–99.3% nucleotide identity with sequences of isolates previously published in GenBank. Phylogenetic analyses of the nucleotide sequences of HSP70 gene of the 13 Ghanaian isolates and 24 sequences previously published in GenBank, clustered the PMWaV-1, PMWaV-2 and PMWaV-3 isolates into three distinct genetic groups with > 95% bootstrap support.

**Conclusion:** The present study shows for the first time the occurrence of PMWaV-1, PMWaV-2 and PMWaV-3 in Ghana pineapple fields as well as in Africa.

**Background**

Pineapple (*Ananas comosus* L. Merrill), a *Bromeliaceae*, is the third most important fresh fruit crop after citrus and banana worldwide [1]. Brazil, Philippines, and Thailand are the leading producers worldwide whilst in Africa, Côte d’Ivoire, Nigeria, Ghana and Kenya are the main pineapple producing countries [2].

In Ghana, the pineapple sector is the most developed horticultural sector [3,4] cultivated mainly in the areas of Central, Greater Accra, Eastern and Volta regions of Ghana, in small and medium scale. Pineapple production is a source of income for thousands of people ranging from farmers to market women and small-holder farmers. The crop provides raw material to feed industries, leading to establishment of cottage industries. Pineapple is a non-traditional export crop in Ghana and hence a source of foreign exchange. It contributed more than USD 283,000,000 in foreign exchange to the economy of Ghana between 1990 and 2013 [5].

Mealybug wilt of pineapple (MWP) is a destructive viral disease that affects pineapple production in many growing regions worldwide including Ghana [6,7]. In Ghana, fruit yield
loss due to MWP attack has been estimated at about US$ 248.00 per hectare [7].
Elsewhere in Hawaii, MWP has been reported to cause reduction in fruit yield by 30-55%,
depending on the age of the plant at the onset of the disease [8].
Mealybug wilt of pineapple is caused by pineapple mealybug wilt-associated virus
(PMWaV), a member of the genus *Ampelovirus* and family *Closteroviridae*. Pineapple
mealybug wilt-associated virus-1 (PMWaV-1), PMWaV-2, PMWaV-3, PMWaV-4, and PMWaV-5
are the five distinct species identified in Hawaii, Australia and Cuba from diseased
pineapple fields [9,10,11]. These viruses are transmitted by two species of mealybugs
namely the gray pineapple mealybug (*Dysmicoccus neobrevipes*, (Beardsley), and the pink
pineapple mealybug (*Dysmicoccus brevipes* (Cockerell) [12], and also by man through
inadvertently planting of infected planting materials (suckers, slips or crowns). These
mealybugs have a symbiotic association with the ants. The ants help the mealybugs in the
foundation of mealybug settlements and consume the honeydew created by the
mealybugs and can suppressively affect the mealybugs' natural enemies [13-16].
Mealybug wilt of pineapple disease symptoms are displayed by serious tip dieback,
downward curling, reddening, and wilting of the leaves which can prompt a complete
breakdown of the plant [17,18] (Figure 1).
Even though PMWaVs have been associated with MWP disease worldwide, there is no
record of such an association in Ghana. Knowledge of ampelovirus species associated with
MWP in Ghana is very important in devising strategies to manage the disease. Dey et al.
[6] reported that PMWaV-2 species alone without the others could make the wilt symptom
of pineapple to develop. Also, in Hawaii, MWP symptoms are strongly associated with
infection by PMWaV-2 [8]. On the other hand, in Australia, the disease is strongly
associated with infections by PMWaV-3 alone or co-infection by PMWaV-1 and -3 [11].
The aim of this study was to identify and characterize ampeloviruses associated with MWP
disease that affects pineapple production in Ghana.

Materials And Methods

Collection of pineapple leaf samples
Twenty-four pineapple leaf samples showing symptoms of MWP (tip dieback, descending curling, reddening, and wilting of the leaves which can prompt a complete breakdown of the plant) were collected from three districts in the Central Region of Ghana, during field survey in 2019. The districts were Abura-Asebu-Kwamankese (AAK), Ekumfi, and Komenda-Edina-Eguafo-Abirem (KEEA), leading pineapple growing centres in Ghana. The location, climate and vegetation types of the three districts are presented in Table 1.

RNA extraction
Viral RNAs were extracted from leaf tissues of each sample using Quick-RNA™ Plant Miniprep Kit (ZymoResearch Corp.) according to the manufacturer’s instructions.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)
Luna Universal One-Step qRT-PCR Kit (BioLabs Inc.) was used for the qRT-PCR amplification of headstock protein 70 (HSP70) gene of PMWaVs, according to the manufacturer’s instructions. Briefly, an initial reaction volume of 12.6 µL containing 10 µL of 2× Luna Universal One-Step Reaction Mix, 1 µL of 20x Luna WarmStart RT Enzyme Mix, 0.8 µL of 10 µM reverse primer, 0.8 µL of 10 µM forward primer, was prepared and placed in qPCR tubes. Total RNA template (< 1 µg) was added to the mixture in the qPCR tubes and nuclease-free water was added to make up a final reaction volume of 20 µL. The qPCR tubes were then spun in a centrifuge for 1 min at 2,500 rpm to remove the bubbles. The tubes were then incubated in a pre-warmed thermocycler (Applied Biosystems StepOnePlus) according to the programme reaction conditions indicated in Table 1, and SYBR scan mode setting on the real-time instrument (thermocycler). The primer sequences
are shown in Table 2.

**Gel electrophoresis**

The amplification products were assessed by electrophoresis in 1.5% agarose gel in TBE buffer (89 mM Tris-borate and 2 mM EDTA, pH 8.3) and stained with ethidium bromide using a 2 kb ladder. The gel was then visualized in UV light in a gel documentation system and the gel photograph was then taken for further analysis.

**Cleaning and sequencing of PMWaV-1, PMWaV-2 and PMWaV-3**

Purified qRT-PCR products of thirteen isolates which consist of PMWaV-1, PMWaV-2 and PMWaV-3 were sequenced according to Sether *et al.* [20] and Gambley *et al.* [11] in order to assess variation within a virus isolate and to ensure consistent and reliable sequence data. The DNA bands were purified and sequenced in both directions using the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1 (NimaGen BV, The Netherlands). Sequence data were edited and assembled using BioEdit version 7.0.5 [21]. The quality of each nucleotide in the sequence was examined in order to detect and evaluate changes in nucleotides and for each amplicon construct consensus sequences. Both primer and non-coding sequences from the alignments were also removed. Additional published sequences obtained from GenBank were verified and added to the data sets (Table 3). After editing, the final sequences analysed were the partial HSP70 gene of 420 nt of PMWaV-1, 591 nt of PMWaV-2 and 486 nt of PMWaV-3. The deduced amino acid sequences analysed included 140 aa for PMWaV-1, 197 aa for PMWaV-2 and 162 aa for PMWaV-3. The sequences obtained in this work were analysed together with those retrieved from the GenBank (Table 3).

Multiple sequence alignments were made using the ClustalW programme implemented in MEGA version 7.0 [22]. Alignments were also manually altered to guarantee right reading
frames. The analyses included a total of thirteen isolates of PMWaV-1, PMWaV-2 and PMWaV-5 nucleotide sequences of HSP70 genes from pineapple fields in Ghana, and 24 corresponding sequences of isolates previously published in GenBank.

**Sequence comparisons and phylogenetic analyses**

For HSP70 homologous genes of PMWaV-1, PMWaV-2 and PMWaV-3, the nucleotide and the deduced amino acid sequence identities were determined using BioEdit v7.0.5 [21] and BlastN. For HSP70 homologous genes between PMWaV-1, PMWaV-2 and PMWaV-3 sequence alignments, MODELTEST [23] implemented in MEGA version 7 program [22] was conducted to select the most suitable nucleotide substitution model using the Akaike Information Criterion, the Bayesian Information Criterion [24] and the hierarchical probability ratio test. The best fit nucleotide substitution model was then used for phylogenetic analyses using the maximum likelihood method with 1000 replicates of bootstrapping using the MEGA 7 software [22]. The neighbour-joining method also implemented in MEGA 7 was used for comparison.

**Genetic diversity**

The following genetic diversity indices for all samples of the HSP70 homologous gene each of the PMWaV-1, PMWaV-2 and PMWaV-3 were determined using the DnaSP V.5.0 programme [25]: haplotype diversity (h), nucleotide diversity (π), number of segregating sites (S) and total number of mutations (Eta).

**Determination of genetic distance and selection pressure**

For each of PMWaV-1, PMWaV-2 and PMWaV-3 sequence dataset, the overall genetic distance (the number of base substitutions per site from averaging across all sequence pairs in a population) within HSP70 homologous nucleotide sequence data sets were estimated using the Maximum likelihood model [26]. Bootstrap method (1000 replicates)
was used to obtain standard error estimates. The analyses were conducted in MEGA 7. The HyPhy package Maximum Likelihood analysis of the natural codon-by-codon selection technique [27] implemented in MEGA 7 [22] was used to predict the number of synonymous substitutions inferred per synonymous site (dS) and the number of non-synonymous substitutions per non-synonymous site (dN). These estimates were produced using the joint Maximum Likelihood reconstructions of ancestral states under the defaults Muse-Gaut model [28] and General Time Reversible model [29]. The dN-dS test statistic was used to detect codons that were under positive selection.

An overabundance of non-synonymous substitutions shows a positive value for the test statistics. In this case, Kosakovsky and Frost [30] and Suzuki and Gojobori [31] methods were used to calculate the probability of rejecting the null hypothesis of neutral evolution (P-value). Values of P less than 0.05 are considered significant at a 5% level. The overall ratio dN/dS was also calculated from the mean values of dN and dS to compare the selection pressures acting on the HSP 70 gene of each PMWaV-1, PMWaV-2 and PMWaV-3 species. The gene is under positive (or diversifying) selection when the dN/dS ratio is > 1, negative (or purifying) selection when the dN/dS ratio < 1, and neutral selection when dN/dS ratio = 1.

**Neutrality test**

Tajima D and Fu and Li's D and F statistics were used to test the hypothesis that PMWaV diversity trends are consistent with the neutral molecular evolution theory [32-34]. The neutral theory of molecular evolution says that the great majority of molecular-level evolutionary modifications are caused by selectively neutral mutants shifting randomly [32]. The importance of each test statistics was estimated by 10,000 permutations.

**Results**

**Detection of the viral species responsible for MWP**
Three different species of *Ampelovirus* namely PMWaV-1, PMWaV-2, and PMWaV-3 were detected by qRT-PCR from the plant samples during the study (Table 4). Sixteen out of the 24 samples (66.7%) were infected with at least one of the three species of *Ampelovirus* identified. PMWaV-2 had the highest infection rate of 62.5% across the districts, indicating that it is the most prevalent virus species in the region; this was followed by PMWaV-1 and PMWaV-3 with infection rates of 33.3% and 8.3% respectively (Table 4; Figure 1). All the three viral species were detected from pineapple samples from AAK district, whilst only two (PMWaV-1 and PMWaV-2), were detected from Ekumfi and KEEA districts, implying that PMWaV-3 was found only in AAK district.

**Performance of the PMWaVs primers on samples across the growing area**

Fig. 2 shows qRT-PCR amplification of the ampeloviruses with PMWaVs primers of cDNA fragment size that varies from 495 – 610 bp from all the 24 diseased leaf samples (lanes 1-12 and 13- 24) but no band for negative control (NTC).

The amplicon of PMWaV-1 was obtained from pineapple samples using 225 / 226 primer pair of band size 590 bp. The primer pair detected the virus from two out of eight samples from KEEA, and six out of eight samples from AAK, whilst none of the eight samples from Ekumfi was infected by PMWaV-1.

With PMWaV-2 amplicon obtained from pineapple samples using 223 / 224 primer pair of band size 610 bp, the primers detected the virus from two out of eight samples from Ekumfi, four out of eight samples from KEEA, whilst all the eight samples from AAK were infected by PMWaV-2.

PMWaV-3 was detected from only two out of eight samples from AAK using 263/264 primer pair with band size 495 bp. The virus, however, was not detected from any of the samples
from KEEA and Ekumfi districts.

**Mixed viral infections of pineapple samples by pineapple mealybug wilt associated viruses (PMWaVs)**

Mixed viral infections by two or all three viruses identified (PMWaV-1, PMWaV-2, PMWaV-3), were detected in the pineapple samples from two out of the three districts (Table 5). Mixed infections were detected in 6 out of 8 pineapple samples in AAK where they were co-infected by PMWaV-1 and either PMWaV-2 or PMWaV-3 in double infections (4 samples) or co-infected by all three virus species, i.e triple infections (2 samples). Mixed infection was detected on only one sample from KEEA where it was co-infected by PMWaV-1 and PMWaV-2. Mixed infection however was not detected from any of the samples from Ekumfi District.

**Sequence analysis**

Heat shock protein 70 (HSP70) gene was sequenced to confirm identities and estimate the genetic variability among PMWaV-1, PMWaV-2 and PMWaV-3 isolates. Sequence comparison showed that all the seven sequences of the Ghanaian PMWaV-1 isolates (GenBank accession numbers MN427634 - MN427639 and MN399973) shared 95.2 to 99.7% nucleotide identity with each other and 95.2-100% with sequences of isolates previously published in GenBank (Accession numbers KT322152, KT322148, EF620774, HE583225 JX645771, HQ129930, MH704740, AF414119, KJ872494, EU79113, KC800714 and HG940514). The two sequences of the Ghanaian PMWaV-3 isolates (GenBank accession numbers MN427640 and MN427641) shared 98.3% nucleotide identity to each other and 97.5- 99.3% nucleotide identity with sequences of isolates previously published in GenBank (Accession numbers NC043406, MH704742, GU563497, JX508638 and FJ209047). Also, the four Ghanaian PMWaV-2 isolates (GenBank Accession numbers
MN427642 - MN427645) shared 98.9 to 100% nucleotide identity with each other and 98.2 - 100% nucleotide identity with sequences of isolates previously published in GenBank (Accession numbers EU769115, KT322167, FN825676, MH704741, HE583226, NC043105, EU016675 and JX645772). The deduced amino acid sequences of the Ghanaian isolates also ranged from 86.5 to 99.2% for the PMWaV-1, 97.1 to 100% for PMWaV-2 and 95.3% for the PMWaV-3 (Table 6), indicating narrow variability (close identities) within each viral species. Deduced amino acid sequences of the Ghanaian isolates shared identities of 86.5 - 100% for PMWaV-1, 95.5 - 100% for PMWaV-2, and 93.3 - 98.0% for PMWaV-3 with isolates previously published in (Table 6).

3.5 Phylogenetic analyses

The maximum likelihood tree for the partial HSP70 gene nucleotide sequence dataset revealed that the 37 sequences of PMWaV-1, PMWaV-2 and PMWaV-3 isolates from symptomatic pineapple samples formed three main genetic groups corresponding to three clades supported by bootstrap values of greater than 95%. The seven Ghanaian PMWaV-1 isolates (Accession numbers: MN427634 - MN427639 and MN399973) clustered on clade 1 together with twelve isolates previously published in GenBank with 99% bootstrap support. The two PMWaV-3 isolates from Ghana (Accession numbers MN427640 and MN427641) clustered on clade 2 with four corresponding PMWaV-3 isolates previously published in GenBank with 96% bootstrap support. With 99% bootstrap support the four Ghanaian PMWaV-2 isolates (Accession numbers: MN427642 - MN427645) clustered on clade 3 with the corresponding PMWaV-2 isolates previously published in GenBank.

The maximum likelihood phylogenetic tree for the HSP70 ORF amino acid sequences had similar topology as that of nucleotide sequence (three genetic groups or clades) with bootstrap support of greater than 88% (Figure 4). Group 1 containing seven PMWaV-1
isolates from Ghana (Accession numbers MN427634 - MN427639 and MN399973) clustered with PMWAV-1 isolates previously published in GenBank with 89% bootstrap support. Two Ghanaian PMWaV-3 isolates (Accession numbers MN427640 and MN427641) clustered on clade 2 with four corresponding PMWaV-3 isolates previously published in GenBank with bootstrap support of 91%. Group 3 consists of four isolates of PMWaV-2 obtained from pineapple fields in Ghana (Accession numbers MN427642 - MN427645) clustering on clade 3 with PMWaV-2 isolates from GenBank (Figure 4).

From both HSP70 nucleotide (Figure 3) and deduced amino acid (Figure 4) phylogenetic trees, it is clear that PMWaV-2 isolates have long evolutionary relationship with both PMWaV-1 and PMWaV-3. It is also evident from Figures 3 and 4 that PMWaV-1 and PMWaV-3 are more closely related i.e. they have close evolutionary relationship.

Genetic diversity within HSP70 gene of PMWaV-1, PMWaV-2 and PMWaV-3 isolates

Analysis of genetic diversity within the HSP70 genes of PMWaV-1, PMWaV-2 and PMWaV-3 isolates showed that the genes were variable with high number of mutations, high number of polymorphic sites and very high haplotype diversity but low nucleotide diversity (Table 7).

Analyses of genetic distance and the natural selection within HSP70 gene of the PMWaV-1, PMWaV-2 and PMWaV-3 isolates

The overall mean genetic distances within the nucleotide sequence datasets for HSP70 gene for PMWaV-1, PMWaV-2 and PMWaV-3 were determined using Maximum Likelihood model [32]. The mean genetic distance within the isolates of PMWaV-1, PMWaV-2 and PMWaV-3 were 0.018±0.002, 0.007±0.002 0.020±0.004 respectively (Table 8).

Using the Maximum Likelihood method via the HyPhy package [35] 17 detected codon
positions in the HSP70 gene of PMWaV-1, 7 for PMWaV-2 and 4 codon positions in the HSP70 gene for PMWaV-3, were under significant positive selection ($P < 0.05$) (Table 8). This provided evidence of heterogenous selection pressures among codon sites in HSP70 genes for PMWaV-1, PMWaV-2 and PMWaV-3 datasets. There was also comparison for the overall selection intensity in the HSP70 genes. The results showed that the selection intensity (mean pairwise $d_N/d_S$) for this gene was 0.2587 for PMWaV-1, 0.2696 for PMWaV-2 and 0.1545 for PMWaV-3) (Table 8). Thus, overall, the values of the $d_N/d_S$ were low, i.e. $d_N/d_S < 1$, implying that the HSP70 gene of PMWaV-1, PMWaV-2 and PMWaV-3 was under negative selection.

**Neutrality tests**

The results for the various neutrality tests are summarised in Table 9. Tajima’s D test and Fu and Li’s $F^*$ test for PMWav-1 were significant in terms of neutrality deviation ($P < 0.05$), but the rest of the tests (Fu and Li’s $D^*$ and Fu and Li’s $F^*$ tests) did not detect significant neutrality deviation ($P > 0.05$) for the PMWaV-1, PMWaV-2, and PMWaV-3 populations.

**Discussion**

Over the years, symptoms alone have not been effective in the detection of the plant viral disease [36]. The detection of PMWaVs by molecular means has however been shown to be reliable and efficient [11, 20]. To identify ampeloviruses associated with MWP disease, viral RNA was detected by qRT-PCR using species specific primers developed by Sether et al. [20] and Melzer et al. [37] and subsequently confirmed by phylogenetic analyses of nucleotide and deduced amino acid sequences of HSP70 gene of the PMWaV isolates. The qRT-PCR assays successfully detected three ampeloviruses namely PMWaV-1- PMWaV-2 and PMWaV-3 from MWP-symptomatic pineapple samples collected from the three districts, which are leading pineapple producing centres in Ghana. This finding is
consistent with report of Dey et al. [6] which states that PMWaV is a currently recognized as a complex of viruses belonging to three recognized species, designated as Pineapple mealybug-wilt associated virus 1 (PMWaV-1), PMWaV-2, PMWaV-3, and the putative PMWaV-4 and PMWaV-5. This is the first time these three ampelovirus species have been identified in one country in Africa. Similarly, all the three virus species have been identified from Hawaii [6], Australia [11], Taiwan [38], Mexico, China, Cuba etc. These countries are major pineapple countries in the world, suggesting that these viruses are prevalent in all major pineapple growing countries worldwide as reported by Sether et al. [20] and Dey et al. [6]. The presence of these three ampelovirus species in Ghana, has serious consequence in the epidemiology of MWP disease in the country, hence threatening the pineapple industry. It has been reported by Dey et al. [6] that PMWaV-2 species alone without the others could make the wilt symptom of pineapple to develop. Also, whilst in Hawaii, MWP symptoms are strongly associated with infection by PMWaV-2 alone [17] in Australia, the disease is strongly associated with infections by PMWaV-3 alone or co-infection by PMWaV-1 and PMWaV-3 [11]. The study also detected multiple viral infections of pineapple samples from AAK and KEEA districts by these three ampeloviruses (PMWaV-1, PMWaV-2 and PMWaV-3). This has serious consequences in the epidemiology and management of MWP in these districts especially at AAK where double and triple infections were detected from six out of eight pineapple samples tested. Mixed infections can result in recombination of viral species leading to the generation of variants showing novel genetic features, which may cause severe damage to crops [39]. It was observed that AAK district had higher levels of PMWaV infection than KEEA and Ekumfi districts. This finding could be attributed to the presence of large numbers of viruliferous mealybugs feeding on the pineapple plants in the AAK districts as reported by
Dey et al. [6]. The least PMWaVs infection of pineapple samples from Ekumfi could also be as a result of the small number of samples tested or exposure to non-viruliferous mealybugs under field conditions or farmers practising proper agronomic practices such as good sanitation and proper pest management which reduce the abundance of the viruliferous mealybugs on their farm as reported by Sether and Hu [17].

Genetic variability of PMWaV-1, PMWaV-2 and PMWaV-3 populations infecting pineapple crops in the Central Region of Ghana was analysed using the sequences encoding HSP70 homologous genes of the viral genome. The results revealed that the PMWaV-1, PMWaV-2 and PMWaV-3 isolates clustered into three different genetic groups (evolutionary divergent lineages) corresponding to three clades supported by bootstrap values more than 98%, irrespective of the geographical origin. Thus all the Ghanaian PMWaV-1, PMWaV-3 and PMWaV-2 isolated clustered together with This affirms the reports of Dey et al. [6] and Gambley et al. [11] which describe PMWaV-1, PMWaV-2 and PMWaV-3 isolates as three distinct closterovirus species. It was however interesting to note that the HSP70 ORF amino acid phylogenetic tree revealed that PMWaV-1 isolates which clustered at clade 1 had two significant sub-clusters supported by 92% bootstrap support with our field isolate with accession number MN427634 clustering with isolate from Genbank with accession number HG940514 (Figure 4). On the other hand, PPMWaV-2 and PMWaV-3 isolates did not form sub-clusters. This suggests that HSP70 gene of PMWaV-1 is more diverse than that of PPMWaV-2 and PMWaV-3. This is supported by the relatively higher nucleotide and haplotype diversities recorded for PMWaV-1 than that of PMWaV-2 and PMWaV-3 (Table 7). This could be due to the greater number of mutations and recombination in the gene of PMWaV-1 compared to that PMWaV-2 and PMWaV-3, which is consistent with the finding of Melzer et al. [18]. According to report by Roossinck, [40] mutation and recombination are the initial sources of variation in populations. RNA viruses use all
known genetic variation processes to guarantee their survival, mutation and recombination are the main cause of errors that occur during replication of RNA viruses resulting in a high degree of variability [41].

In spite of the high number of mutations and the consequent high number of haplotypes recorded for the PMWaV-1, 2 and 3 HSP70 genes, the genetic diversity was low (0.0172 ± 0.0032 for the PMWaV-1, 0.0199 ± 0.0024 for PMWaV-3 and 0.0074 ± 0.0016 for PMWaV-2), suggesting genetic homogeneity. This is in line with Sacristan and García-Arenal [42] and Elena et al. [41] which indicated that populations of plant viruses are not extremely variable despite high genetic variation potential and high mutation rates are not necessarily adaptive as a portion of the mutations are deleterious. It has also been reported that analysed populations of plant viruses are genetically stable, and this is so regardless of the many haplotypes that may occur in the population [41]. This assertion is further supported by the finding from our study on the selection pressure where HSP70 gene for all the three ampelovirus species (PMWaV-1, PMWaV-2 and PMWaV-3) were found to be under negative selection (Table 8).

The high rate of mutation in RNA viruses could not be due to an evolutionary strategy but to the need for replication of their chemically unstable RNA genome [40]. However, high mutation rates for RNA viruses have been revealed to represent an evolutionary strategy [42].

An indication of population substructuring was the important neutrality deviation observed from the neutrality trials. All the tests for neutrality showed negative values (see Table 24), indicating that all PMWaV-1, PMWaV-2 and PMWaV-3 populations were in active evolution.

Conclusions

Three different ampeloviruses namely PMWaV-1, PMWaV-2 and PMWaV-3, were detected
from the plant samples following qRT-PCR assays with PMWaVs species specific primers and sequence and confirmed phylogenetic analyses of nucleotide and amino acid sequences of HSP70 gene. The HSP70 gene of the PMWaV-1, PMWaV-2 and PMWaV-3 had low nucleotide diversity and was under negative selection. Mixed viral infections by 2 or all three viral species were detected in the pineapple samples from two out of the three districts in the Central region. This is the first report of PMWaV-1, PMWaV-2 and PMWaV-3 in Ghanaian pineapple and in Africa.

Declarations

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Authors’ contributions

EAB conceived and designed the manuscript; JN performed the experiment and wrote the draft; GVP and ATA performed analyses. All authors read and approved the manuscript

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Availability of data and materials

The HSP70 nucleotide sequences of the Ghanaian PMWaV-1, -2, and -3 isolates used in the study have been deposited in GenBank under the accession numbers as follows:

MN427634-MN427639 and MN399973 (PMWaV-3 isolates); MN427642-MN427645 (PMWAV-2 isolates) and MN427640and MN427641 (PMWaV-3 isolates)

Ethics approval and consent to participate

Not applicable

Consent for publication
Not applicable

**Competing interests**

The authors declare that they have no competing interests

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**Abbreviations**

HSP70 heatshock protein 70 gene; PMWaV pineapple mealybug wilt associated wilt; qRT-PCR quantitative reverse transcription-polymerase chain reaction; MWP mealybug wilt of pineapple

**References**

1. Usman IS, Abdulmalik MM, Sani LA, Muhammad AN. Development of an efficient protocol for micropropagation of pineapple (Ananas comosus L. var. smooth cayenne). Afr J Agric Res. 2013 May 16;8(18):2053-6.

2. Agriculture in Ghana Facts and Figures. Statistics. Accra, Ghana: Research and Information Directorate (SRID) of Ministry of Food and Agriculture. 2006.pp 36.

3. Kleemann L, Abdulai A. Organic certification, agro-ecological practices and return on investment: Farm level evidence from Ghana. Kiel Working Paper; 2012.

4. Kuwornu JK, Mustapha S. Global GAP standard compliance and smallholder pineapple farmers' access to export markets: implications for incomes. J Econ Behav Stu. 2013 Feb 1;5(2):69.

5. Ghana Export Promotion Authority, Developing Regional Export Trade in Ghana, (2014).
6. Dey KK, Green JC, Melzer M, Borth W, Hu JS. Mealybug Wilt of Pineapple and Associated Viruses. Horticulturae. 2018 Dec;4(4):52.

7. Sarpong TM, Asare-Bediako E, Acheampong L. Perception of Mealybug Wilt Effect and Management among Pineapple Farmers in Ghana. J Agr Ext. 2017;21(2):1-6.

8. Sether DM, Hu JS. Yield impact and spread of Pineapple mealybug wilt associated virus-2 and mealybug wilt of pineapple in Hawaii. Plant Dis. 2002 Aug;86(8):867-74.

9. Sether DM, Karasev AV, Okumura C, Arakawa C, Zee F, Kislan MM, Busto JL, Hu JS. Differentiation, distribution, and elimination of two different pineapple mealybug wilt-associated viruses found in pineapple. Plant Dis. 2001 Aug;85(8):856-64.

10. Sether DM, Melzer MJ, Busto J, Zee F, Hu JS. Diversity and mealybug transmissibility of ampeloviruses in pineapple. Plant Dis. 2005 May;89(5):450-6.

11. Gambley CF, Steele V, Geering AD, Thomas JE. The genetic diversity of ampeloviruses in Australian pineapples and their association with mealybug wilt disease. Aust J Plant Pathol. 2008 Mar 1;37(2):95-105.

12. Sether DM, Ullman DE, Hu JS. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (Dysmicoccus spp.). Phytopathology. 1998 Nov;88(11):1224-30.

13. Jahn GC. The ecological significance of the big-headed ant in mealybug wilt disease of pineapple (Doctoral dissertation).

14. Petty GJ, Tustin H. Ant (Pheidole megacephala F.)-mealybug (Dysmicoccus brevipes Ckll.) relationships in pineapples in South Africa. InI International Pineapple Symposium 334 1992 Nov 2 (pp. 387-396).

15. Rohrbach KG, Phillips DJ. Postharvest diseases of pineapple. InSymposium on Tropical Fruit in International Trade 269 1989 Jun 4 (pp. 503-508).

16. Jahn GC, Beardsley JW, González-Hernández H. A review of the association of ants
with mealybug wilt disease of pineapple. 2003

17. Sether DM, Hu JS. Closterovirus infection and mealybug exposure are necessary for the development of mealybug wilt of pineapple disease. Phytopathology. 2002 Sep;92(9):928-35.

18. Melzer MJ, Karasev AV, Sether DM, Hu JS. Nucleotide sequence, genome organization and phylogenetic analysis of pineapple mealybug wilt-associated virus-2. J Gen Virol. 2001 Jan 1;82(1):1-7.

19. Ministry of Food and Agriculture (MoFA) Agriculture in Ghana: Facts and Figures for 2010. Statistics, Research and Information. Directorate (SRID), MoFA, Accra. Ghana, (2011).

20. Sether DM, Melzer MJ, Borth WB, Hu JS. Genome organization and phylogenetic relationship of Pineapple mealybug wilt associated virus-3 with family Closteroviridae members. Virus genes. 2009 Jun 1;38(3):414-20.

21. Hall T. BioEdit v7. 0.5. Biological sequence alignment editor. Department of Microbiology, North Carolina State University. (Online) Available at: http://www.mbio.ncsu.edu/BioEdit/Bioedit.html [accessed 2 March 2006]. 2005.

22. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016 Mar 22;33(7):1870-4.

23. Posada D, Crandall KA. Modeltest: testing the model of DNA substitution. Bioinformatics (Oxford, England). 1998 Jan 1;14(9):817-8.

24. Fraley C, Raftery AE. Model-based clustering, discriminant analysis, and density estimation. J AM Stat Assoc. 2002 Jun 1;97(458):611-31.

25. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009 Jun 1;25(11):1451-2.

26. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular
evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011 Oct 1;28(10):2731-9.

27. Pond SL, Muse SV. HyPhy: hypothesis testing using phylogenies. InStatistical methods in molecular evolution 2005 (pp. 125-181). Springer, New York, NY.

28. Muse SV, Gaut BS. A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. Mol Biol Evol. 1994 Sep 1;11(5):715-24.

29. Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford university press; 2000 Jul 27.

30. Kosakovsky Pond SL, Frost SD. Not so different after all: a comparison of methods for detecting amino acid sites under selection. Mol Biol Evol. 2005 Feb 9;22(5):1208-22.

31. Suzuki Y, Gojobori T. A method for detecting positive selection at single amino acid sites. Mol Biol Evol. 1999 Oct 1;16(10):1315-28.

32. Kimura M. DNA and the neutral theory. Philosophical Transactions of the Royal Society of London. B, Biological Sciences. 1986 Jan 29;312(1154):343-54.

33. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989 Nov 1;123(3):585-95.

34. Fu YX, Li WH. Statistical tests of neutrality of mutations. Genetics. 1993 Mar 1;133(3):693-709.

35. Pond SL, Frost SD. Datamonkey: rapid detection of selective pressure on individual sites of codon alignments. Bioinformatics. 2005 Feb 15;21(10):2531-3.

36. Agrios GN. Plant pathology. Academic press,; 2005.

37. Melzer MJ, Sether DM, Karasev AV, Borth W, Hu JS. Complete nucleotide sequence and genome organization of pineapple mealybug wilt-associated virus-1. Arch Virol. 2008 Apr 1;153(4):707-14.
38. Shen BN, Zheng YX, Chen WH, Chang TY, Ku HM, Jan FJ. Occurrence and molecular characterization of three Pineapple Mealybug Wilt-Associated Viruses in pineapple in Taiwan. Plant Dis. 2009 Feb;93(2):196-.

39. Syller J. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol Plant Pathol. 2012 Feb;13(2):204-16.

40. Roossinck MJ. Mechanisms of plant virus evolution. Annu Rev Phytopath. 1997 Sep;35(1):191-209.

41. Elena SF, Fraile A, García-Arenal F. Evolution and emergence of plant viruses. In Advances in Virus Research 2014 Jan 1 (Vol. 88, pp. 161-191). Academic Press.

42. García-Arenal F, Fraile A, Malpica JM. Variability and genetic structure of plant virus populations. Annu Rev Phytopath. 2001 Sep;39(1):157-86

Tables

Table 1 Location, Climate and Vegetation types of the three districts collected from Ghana meteorological service during the study

| District | Altitude | Longitude/Latitude | Relative humidity (%) | Temperature (°C) | Rainfall (mm) | *Vegetation type |
|----------|----------|--------------------|-----------------------|-----------------|--------------|-----------------|
| KEEA     | 31.1     | 01° 15’ W 05° 06’ N | 86                    | 26-35           | 9201         | Coastal savannah |
| AAK      | 31.1     | 01° 20’ W 05° 05’ N | 86                    | 22-30           | 1940.2       | Deciduous forest |
| Ekumfi   | 15.2     | 00° 37’ W          | 81                    | 24-28           | 631.2        | Coastal savannah |

(Source: *MoFA, [19]; Ghana Meteorological Service, 2018)

Table 1: Reaction conditions used for qRT-PCR amplification of heatshock protein 70 gene of PMWaVs
| Cycle step       | Temperature (°C) | Time       | Cycles |
|-----------------|-----------------|------------|--------|
| Reverse
transcription | 55              | 10 minutes | 1      |
| Initial
denaturation  | 95              | 1 minutes  | 1      |
| Denaturation     | 95              | 10 seconds | 40-45  |
| Extension        | 60              | 30 seconds |        |
| Melt curve       | 60-95           | Various    | 1      |

Table 2 Primers used for qRT-PCR detection of *Ampelovirus* species

| Primer name | Primer sequence (5’ – 3’) | Viral species | Reference          |
|-------------|---------------------------|---------------|--------------------|
| 225         | ACA GGA AGG ACA ACA CTC AC| PMWaV-1       | Sether et al., 2001|
| 226         | CGC ACA AAC TTC AAG CAA TC|               |                    |
| 224         | CAT ACG AAC TAG ACT CAT ACG| PMWaV-2       | Sether and Hu, 2002|
| 223         | CCA TCC ACC AAT TTT ACT AC|               |                    |
| 264         | ATT GAT GGA TGT GTA TCG   | PMWaV-3       | Sether et al., 2001|
| 263         | AGT TCA CTG TAG ATT TCG GA|               |                    |

Table 3 Pineapple mealybugs wilt associated viruses isolates from GenBank

| Name of isolates | GenBank accession     | Country  | Genomic region | Authors                   |
|------------------|-----------------------|----------|----------------|---------------------------|
| KT322148         | KT322148.1            | Thailand | Partial genome | Srikumphung and Chiensor |
| HQ129930         | HQ129930.1            | Cuba     | Partial genome | Hernandez and Ramos (2012)|
| KT322152         | KT322152.1            | Thailand | Partial genome | Srikumphung and Chiensor |
| EU769113         | EU769113.1            | Taiwan   | Partial genome | Shen et al. (2009)       |
| HG940514         | HG940514.1            | Thailand | Partial genome | Koohapitagtam (2014)      |
| MH704740         | MH704740.1            | USA      | whole genome   | Green et al. (2018)       |
| Accession   | Accession Description | Country   | Length of Genome | Contributors                  |
|-------------|-----------------------|-----------|------------------|-------------------------------|
| HE583225    | HE583225.1            | Thailand  | Partial genome   | Koohapitagtam and Hongpr.     |
| EF620774    | EF620774.1            | Thailand  | Partial genome   | Chiemson and Maneech.         |
| KC800714    | KC800714.1            | Mexico    | Partial genome   | Ochoa-Martinez et al. (2013)  |
| KJ872494    | KJ872494.1            | China     | Whole genome     | Yu et al. (2014)              |
| AF414119    | AF414119.3            | USA       | Whole genome     | Melzer et al. (2009)          |
| JX645771    | JX645771.1            | Cuba      | Partial genome   | Hernandez-Rodriguez (2012)    |
| PMWaV-2     | KT322167.1            | Thailand  | Partial genome   | Srikumphung and Chiemson.     |
| FN825676    | FN825676.1            | Cuba      | Partial genome   | Hernandez et al. (2010)       |
| EU769115    | EU769115.1            | Taiwan    | Partial genome   | Shen et al. (2009)            |
| MH704741    | MH704741.1            | USA       | Whole genome     | Green et al. (2018)           |
| HE583226    | HE583226.1            | Thailand  | Partial genome   | Koohapitagtam and Hongpr.     |
| NC043105    | NC_043105.1           | USA       | Partial genome   | Melzer et al. (2000)          |
| EU016675    | EU016675.1            | Thailand  | Partial genome   | Chiemson et al. (2007)        |
| JX645772    | JX645772.1            | Cuba      | Partial genome   | Hernandez-Rodriguez (2012)    |

| Accession   | Accession Description | Country   | Length of Genome | Contributors                  |
|-------------|-----------------------|-----------|------------------|-------------------------------|
| PMWaV-3     | GU563497.1            | Cuba      | Partial genome   | Hernandez et al. (2010)       |
| MH704742    | MH704742.1            | USA       | Whole genome     | Green et al. (2018)           |
| NC_043406   | NC_043406.1           | USA       | Whole genome     | Sether et al. (2009)          |
| JX508636    | JX508636.1            | Cuba      | Partial genome   | Hernandez-Rodriguez (2012)    |

Table 4 Ampelovirus species detected from 24 diseased pineapple plant samples from three districts in the Central Region
## District Sample number

| District | Sample number |
|----------|---------------|
| AAK      | 17            |
| AAK      | 18            |
| AAK      | 19            |
| AAK      | 20            |
| AAK      | 21            |
| AAK      | 22            |
| AAK      | 23            |
| AAK      | 24            |
| Ekumfi   | 1             |
| Ekumfi   | 2             |
| Ekumfi   | 3             |
| Ekumfi   | 4             |
| Ekumfi   | 5             |
| Ekumfi   | 6             |
| Ekumfi   | 7             |
| Ekumfi   | 8             |
| KEEA     | 9             |
| KEEA     | 10            |
| KEEA     | 11            |
| KEEA     | 12            |
| KEEA     | 13            |
| KEEA     | 14            |
| KEEA     | 15            |
| KEEA     | -             |

## Virus species

|              | PMWaV-1 (Ct) | PMWaV-2 (Ct) | PMWaV-3 (Ct) |
|--------------|--------------|--------------|--------------|
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | +            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | +            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| AAK          | +            | +            | -            |
| Ekumfi       | -            | +            | -            |
| Ekumfi       | -            | +            | -            |
| Ekumfi       | -            | +            | -            |
| Ekumfi       | -            | +            | -            |
| Ekumfi       | -            | +            | -            |
| Ekumfi       | -            | +            | -            |
| KEEA         | -            | +            | -            |
| KEEA         | -            | +            | -            |
| KEEA         | -            | +            | -            |
| KEEA         | -            | +            | -            |
| KEEA         | -            | +            | -            |
| KEEA         | -            | +            | -            |

Present (+) and Absent (-); KEEA: Komenda-Edina-Eguafo-Abirem; AAK: Abura-Asebu-Kwamankese

### Table 5 Mixed infections of pineapple samples by pineapple mealybug wilt associated viruses

| District | Double | Triple | Total (%) |
|----------|--------|--------|-----------|
| AAK      | 4      | 2      | 6 (75)    |
| Ekumfi   | 0      | 0      | 0 (0)     |
| KEEA     | 1      | 0      | 1 (12.5)  |

### Table 6 Nucleotide (nt) and amino acid (aa) sequence identities of pineapple mealybug
wilt associated virus (PMWaV) isolates from Ghana and selected published isolates retrieved from GenBank

| Isolates         | Sequence identity (%) | Nucleotide          | Amino acid        |
|------------------|-----------------------|---------------------|-------------------|
| PMWaV-1 (HSP70)  |                       |                     |                   |
| Between sequenced isolates | 95.2 - 99.7          | 86.5 - 99.2        |
| Between sequenced isolates and published isolates | 95.2 - 100          | 86.5 - 100        |
| PMWaV-2 (HSP70)  |                       |                     |                   |
| Between sequenced isolates | 98.9 - 100          | 97.1 - 100        |
| Between sequenced isolates and published isolates | 98.2 - 100          | 95.5 - 100        |
| PMWaV-3 (HSP70)  |                       |                     |                   |
| Between sequenced isolates | 98.3               | 95.3               |
| Between sequenced isolates and published isolates | 97.5 - 99.3        | 93.3 - 98.0       |

Table 7 Genetic variability within HSP70 DNA sequences of PMWaV-1, PMWaV-2 and PMWaV-3 isolates

| Dataset     | Number of sequences | Number of polymorphic sites (S) | Number of mutations (Eta) | Nucleotide diversity (π) | Haplotype diversity (h) |
|-------------|---------------------|---------------------------------|---------------------------|--------------------------|-------------------------|
| PMWaV-1     | 19                  | 64                              | 69                        | 0.0172 ± 0.0032          | 1.000 ± 0.0029          |
| PMWaV-2     | 13                  | 23                              | 23                        | 0.0074 ± 0.0016          | 0.9870 ± 0.0035         |
| PMWaV-3     | 6                   | 27                              | 28                        | 0.0199 ± 0.0024          | 1.000 ± 0.0076          |

Table 8 Mean pairwise genetic distance and the selective pressures within HSP70 genes of the PMWaV-1, 2 and 3 isolates
| Specie   | Mean genetic distance | d_N   | d_S   | dN/dS | Total number of codons | Codon positions under positive selection |
|----------|-----------------------|-------|-------|-------|-------------------------|------------------------------------------|
| PMWaV-1  | 0.018±0.002           | 0.0652| 0.2521| 0.2587| 189                     | 17                                       |
| PMWaV-2  | 0.007±0.002           | 0.0199| 0.0738| 0.2696| 196                     | 7                                        |
| PMWaV-3  | 0.020±0.004           | 0.0214| 0.1385| 0.1545| 160                     | 4                                        |

Table 9 Neutrality test for HSP70 gene of PMWaV-1, PMWaV-2 and PMWaV-3

| Species   | Tajima’s D   | P-value | Fu and Li’s D* | P-value | Fu and Li’s F* | P-value |
|-----------|--------------|---------|----------------|---------|----------------|---------|
| PMWaV-1   | -2.07401     | < 0.05a | -2.26722       | > 0.05b | -2.57250       | < 0.05  |
| PMWaV-2   | -1.79390     | <0.05a  | -2.14661       | >0.05b  | -2.34611       | >0.05   |
| PMWaV-3   | -0.87754     | <0.5a   | -0.70049       | >0.05b  | -0.81754       | >0.05   |

^aSignificant at P < 0.05  
^b Not significant (P > 0.05)  

Figures
Figure 1

Infection rate of pineapple mealybug wilt associated virus-1 (PMWaV-1), PMWaV-2 and PMWaV-3 on 24 pineapple samples from three districts in the Central Region of Ghana.
Figure 1

Infection rate of pineapple mealybug wilt associated virus-1 (PMWaV-1), PMWaV-2 and PMWaV-3 on 24 pineapple samples from three districts in the Central Region of Ghana.
The amplicons of pineapple mealybug wilt associated virus-1 (PMWaV-1), PMWaV-2 and PMWaV-3 obtained from MWP symptomatic-pineapple samples using 225/226, 223/224, 263/264 primer pairs respectively. The amplification was done in two parts; Samples 1 to 12 and samples 13 to 24 with band sizes of 500 – 610 bp; Samples 1-8 were obtained from Ekumfi district; Samples 9-16 were obtained from KEEA district and samples 17-24 were obtained from AAK district.
The amplicons of pineapple mealybug wilt associated virus-1 (PMWaV-1), PMWaV-2 and PMWaV-3 obtained from MWP symptomatic-pineapple samples using 225/226, 223/224, 263/264 primer pairs respectively. The amplification was done in two parts; Samples 1 to 12 and samples 13 to 24 with band sizes of 500 – 610 bp; Samples 1-8 were obtained from Ekumfi district; Samples 9-16 were obtained from KEEA district and samples 17-24 were obtained from AAK district.
Figure 3

Maximum-likelihood phylogenetic tree of nucleotide sequences of partial HSP70 genes of PMWaV-1, -2, and -3 isolates (n=37) sampled from the Central Region of Ghana and those from GenBank. Accession numbers of Ghanaian isolates are in green boxes whilst the rest are accession numbers of isolates published in GenBank. The scale bar signifies a genetic distance of 0.10 nucleotide substitutions per site.
Figure 3

Maximum-likelihood phylogenetic tree of nucleotide sequences of partial HSP70 genes of PMWaV-1, -2, and -3 isolates (n=37) sampled from the Central Region of Ghana and those from GenBank. Accession numbers of Ghanaian isolates are in green boxes whilst the rest are accession numbers of isolates published in GenBank. The scale bar signifies a genetic distance of 0.10 nucleotide substitutions per site.
Figure 4

Maximum likelihood phylogenetic tree (abridged) of hsp70 amino acid sequences of PMWaV-1, -2 and -3 isolates (n= 37) sampled in the Central Region of Ghana and corresponding isolates from GenBank. Accession numbers of Ghanaian isolates are in green boxes whilst the rest are accession numbers of isolates from GenBank. The scale bar signifies a genetic distance of 0.20 nucleotide substitutions per site.
Maximum likelihood phylogenetic tree (abridged) of hsp70 amino acid sequences of PMWaV-1, -2 and -3 isolates (n= 37) sampled in the Central Region of Ghana and corresponding isolates from GenBank. Accession numbers of Ghanaian isolates are in green boxes whilst the rest are accession numbers of isolates from GenBank. The scale bar signifies a genetic distance of 0.20 nucleotide substitutions per site.

Figure 4