



**Full Length Article**

## Detection of a New Strain of Phytoplasma Associated with Lethal Yellowing Disease of Coconut (*Cocos nucifera*) in Côte d'Ivoire

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### Abstract

Coconut palm (*Cocos nucifera* L.) is an important staple food and cash crop worldwide, especially in developing countries. However, in Côte d'Ivoire, coconut cultivation is threatened by the Côte d'Ivoire coconut lethal yellowing disease (CILY), which has led to the destruction of about 400 ha of coconut plantations in Grand-Lahou. Surveys conducted in Aboisso, Bonoua, Grand-Bassam, Grand-Lahou and Jacqueville to assess the progress of CILY infection in coconut plantations exhibiting typical CILY symptoms. Around 100 trunk boring samples were collected from West African Tall and Dwarf hybrid coconut trees with and without symptoms of CILY symptoms. Total DNA was extracted and tested by PCRs using universal and specific primer pairs for the CILY phytoplasma. Disease prevalence was high in all localities regardless of coconut variety grown (> 60%). Phytoplasmas were detected in 45 coconut trunk boring samples corresponding to both symptomatic and symptomless samples in all surveyed areas, 39 of which were detected with the Gh813f/AwkaSR primer pair and 6 with R16mF2n/R16mR2 primer pair. Sequencing of the amplicons and phylogenetic analysis revealed the presence of the 16SrXXII-B subgroup phytoplasma, previously identified in Côte d'Ivoire and Ghana. For the very first time, in Côte d'Ivoire, the 16SrIV phytoplasma group is reported in the Aboisso coconut growing area. © 2022 Friends Science Publishers

**Keywords:** Coconut; Côte d'Ivoire; Phytoplasma; 16SrIV; Yellowing

### Introduction

Coconut palm (*Cocos nucifera* L.) is a perennial staple and cash crop widely cultivated in 90 countries across 12 million hectares by over 11 million farmers (Gurr *et al.* 2016). Global production in 2018 only was around 62 million tons (FAOSTAT 2019). In West Africa, the area under coconut plantations is 128,000 ha, with an annual production of 145.5 million tons of copra (CNRA 2015). Côte d'Ivoire is one of the top 24 copra exporting countries in the world (Allou *et al.* 2012) with an estimated annual production of 55,000 tons. In Côte d'Ivoire, the coconut tree is generally cultivated by smallholder producers living mainly on the Ivorian coast. It thus represents a significant source of employment and income for smallholder coconut men and women farmers (Mahyao *et al.* 2016).

The world coconut cultivation is threatened by the lethal yellowing disease (LYD), also found in several other palm species, but responsible for the most devastating losses in coconut in West African and the Caribbean. In Nigeria, LYD known as Awka wilt, has devastated 98% of West African Tall (WAT) coconut trees (Odewale *et al.*

2010) and in Ghana, the Cape St. Paul Wilt (CSPW), has destroyed about one million coconut trees in the last 30 years (Nipah *et al.* 2007). In Côte d'Ivoire, the Côte d'Ivoire coconut lethal yellowing disease (CILY) was first reported in Grand-Lahou (Konan *et al.* 2013) where nearly 400 ha of coconut plantations were destroyed for over a decade, resulting in a loss of about 12,000 tons of copra/year (Arocha-Rosete *et al.* 2017).

A phytoplasma (class Mollicutes) was associated with CILY (Konan *et al.* 2013). The phytoplasma was identified as a member of the 16SrXXII group, subgroup B, the same phytoplasma associated with CSPW in Ghana (Arocha-Rosete *et al.* 2014). In the Grand-Lahou area, a new phytoplasma of subgroup 16SrXXII-C was detected in Ivorian coconut, oil palm (*Elaeis guineensis* Jacq.) and roaster (*Borassus aethiopicum* Mart.) showing LYD-like symptoms (Kra *et al.* 2017). Recently, LYD-like symptoms have been observed in coconut plantations across the Ivorian southern coast other than Grand-Lahou. The present work assesses for the presence of phytoplasmas that may be affecting five new coconut growing areas in the southern coast of Côte d'Ivoire.

## Materials and Methods

### Study area

Five sites located at the southern coastal region of Côte d'Ivoire were the study areas from west to east: Grand-Lahou, Jacquerville, Grand-Bassam, Bonoua and Aboisso (Fig. 1). Four coconut plantations were surveyed in each site for a total of 20 coconut plantations assessed.

### Plant material

A total 100 trunk boring samples were collected from coconut trees of two different varieties: 65 from the WAT variety and 35 from the Dwarf variety (yellow dwarf and green dwarf). Trunk boring samples corresponded to both symptomless and CILY symptoms-bearing coconut trees.

### Description of symptoms

Outbreaks of the disease including infected coconut trees were also observed and described in the surveyed sites. Symptoms associated with the lethal yellowing disease observed on the organs of infected coconut trees were described according to the description scale of Konan *et al.* (2013).

### Assessment of the CILY prevalence in coconut plantations

Thirty coconut trees per variety from 20 coconut plantations were randomly selected in each surveyed coconut plantation to assess the prevalence of the disease.

- The prevalence of the disease was determined as the ratio of the number of plants showing symptoms of CILY from the total number of plants surveyed. It was calculated according to the following formula (1) from Ackah *et al.* (2008):

$$P_1(\%) = \frac{1}{n} \sum_{i=0}^n \left( \frac{NP_i}{NP_t} \right) \times 100 \quad (1)$$

Where  $P_1$ : prevalence of the disease according to the site,  $n$ : total number of coconut plantations surveyed in the site,  $NP_i$ : number of infected plants and  $NP_t$ : total number of plants surveyed.

The prevalence of the disease in relation to the different coconut varieties grown was assessed as before and calculated according to the following formula (2) of Ackah *et al.* (2008):

$$P_2(\%) = \frac{1}{n} \sum_{i=0}^n \left( \frac{NP_{iv}}{NP_{tv}} \right) \times 100 \quad (2)$$

Where  $P_2$ : prevalence of disease by variety,  $n$ : total number of coconut plantations surveyed in the site,  $NP_{iv}$ : number of symptomatic plants per variety, and  $NP_{tv}$ : total number of inspected plants of the variety in each site.

### Sample collection

The collection of trunk boring samples from symptomatic and asymptomatic coconut trees was carried out between September 2017 and January 2018 in five sites along the southern Ivorian coast.

A total of 20 coconut plantations (4 plantations per site) were randomly selected and surveyed. Trunk boring samples were collected from WAT and Dwarf coconut varieties, using the method of Harrison *et al.* (2013). For this purpose, symptomatic and symptomless coconut trees of WAT and Dwarf varieties were bored using an electric drill (DS 14DVF3, Hitachi Koki Tokyo, Japan) fitted with a 5 mm diameter tendril sterilized in 70% ethanol, at about 1 m above the ground and about 5 cm deep. One hundred trunk boring samples were collected separately in sterile plastic bags and transported to the laboratory in a cooler with ice packs for further analysis (Table 1).

### Molecular identification of the causal agent

**DNA extraction:** Total DNA was extracted from 0.3 g of trunk boring of each 100-coconut trunk boring samples using the method described by Doyle and Doyle (1990). The DNA extracts were eluted in 25  $\mu$ L TE and stored at  $-20^\circ\text{C}$ .

**PCR analysis:** The amplification of the 16S rRNA phytoplasma gene associated with CILY was performed by nested PCR on all DNA extracts as follows:

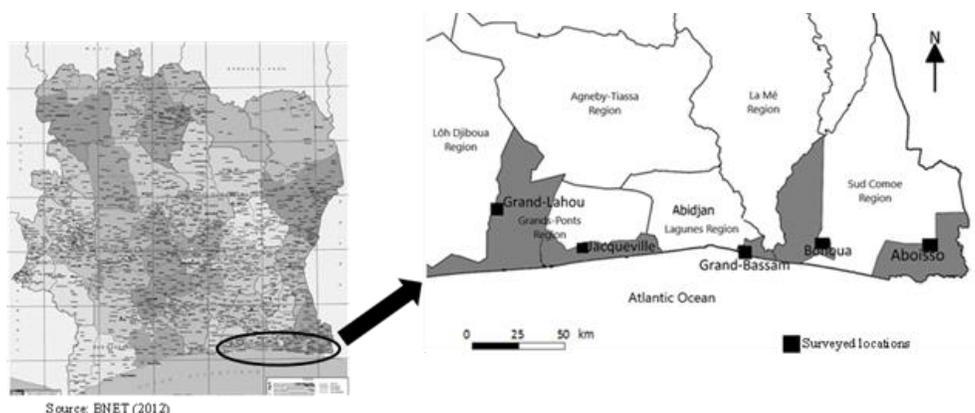
Direct PCR with the phytoplasma universal primer pair P1/P7 (Deng and Hiruki 1991; Schneider 1995) in a reaction volume of 12.5  $\mu$ L; containing 6.25  $\mu$ L of GoTaq G2 Green buffer (Promega, USA), 1.25  $\mu$ L of each primer, 1.75  $\mu$ L of sterile deionized water (Promega, USA) and 2  $\mu$ L of DNA. The PCR program consisted of an initial 3 min denaturation cycle at  $94^\circ\text{C}$ , followed by 35 cycles with denaturation at  $94^\circ\text{C}$  for 40 s, annealing at  $56^\circ\text{C}$  for 40 s, and extension at  $72^\circ\text{C}$  for 1 min 40, followed by a final 10 min extension cycle at  $72^\circ\text{C}$ . For the nested reaction, 2  $\mu$ L of the first-round PCR product was used as a template in a final volume of 25  $\mu$ L using the specific primer pair Gh813f/AwkaSR (Tymon *et al.* 1998). The reaction mixture contained 12.5  $\mu$ L of GoTaq G2 Green buffer (Promega, USA), 2.5  $\mu$ L of each primer and 5.5  $\mu$ L of sterile deionized water (Promega, USA).

The PCR program for the nested PCR reaction included an initial 3 min denaturation cycle at  $94^\circ\text{C}$ , followed by 35 cycles involving denaturation at  $94^\circ\text{C}$  for 40 s, annealing at  $53^\circ\text{C}$  for 40 s and extension at  $72^\circ\text{C}$  for 1 min 40, followed by a final 10 min extension cycle at  $72^\circ\text{C}$ . Direct PCR using the phytoplasma universal primer pair R16mF2/R16mR1 (Lee *et al.* 1993) was performed in a reaction volume of 12.5  $\mu$ L containing 6.25  $\mu$ L GoTaq G2 Green buffer (Promega, USA), 1.25  $\mu$ L of each primer and 1.75  $\mu$ L of sterile deionized water (Promega, USA) and 2  $\mu$ L of DNA. Five  $\mu$ L of the 1:30 diluted R16mF2/R16mR1

**Table 1:** Samples number collected by coconut production localities

Surveyed localities	Number of samples				Total
	WAT variety		Dwarf variety		
	Symptomatic	Symptomless	Symptomatic	Symptomless	
Aboisso	5	6	4	5	20
Bonoua	10	4	5	1	20
Grand-Bassam	19	1	Absent	Absent	20
Grand-Lahou	10	0*	10	0*	20
Jacqueville	17	3	Absent	Absent	20
Total	61	14	19	6	100

\*This type of sample was not collected during sampling



**Fig. 1:** Sampling sites for trunk boring samples of coconut palm (*Cocos nucifera* L.) in the south-eastern coastal area of Côte d'Ivoire

PCR product was used as a template in a nested PCR using the primer pair R16mF2n/R16mR2 (Gundersen and Lee 1996) in a total volume of 50  $\mu$ L with 25  $\mu$ L of GoTaq G2 Green buffer (Promega, USA), 5  $\mu$ L of each primer and 10  $\mu$ L of sterile deionized water (Promega, USA). The PCR reaction was performed.

The PCR program for both primer pairs (R16mF2n/R16mR2) included an initial 2 min denaturation cycle at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 3 min, followed by a final 10 min extension cycle at 72°C. Total DNA from a coconut trunk boring sample collected in Grand-Lahou, confirmed as positive to the CILY phytoplasma (16SrXXII-B) was used as a positive control. All amplifications were performed in a thermal cycler (T100<sup>M</sup> Thermal Cycler, BIORAD, Singapore). After PCR amplification, 10  $\mu$ L of each nested PCR product was separated in a 1.5% agarose gel in 1X TAE buffer (400 Mm Tris-Acetate, 10 Mm EDTA; Promega, USA) stained with 4  $\mu$ L ethidium bromide, for 30 min at 80 volts. The gel was visualised using a UV trans illuminator (EBOX VX5, Vilber Lourmat<sup>TM</sup>, France).

**Sequencing of amplified products:** The Gh813f/AwkaSR and R16mF2n/ R16mR2 amplicons were purified and sequenced (Eurofins, France). The 16S rDNA sequences were assembled using Genious Prime v. 2019.1.3 software. Sequences were compared to those of reference phytoplasmas using BLAST (Altschul *et al.* 1990) in the GenBank (NCBI (<http://www.ncbi.nlm.nih.gov>) to identify

phytoplasma strains present in trunk boring samples from infected coconut trees under study.

**Phylogenetic analysis:** The 16S rRNA gene sequences obtained from PCR products with Gh813f/AwkaSR and R16mF2n/R16mR2 primers and those from reference phytoplasmas retrieved from GenBank were aligned with Clustal X software v. 2.0 (Larkin *et al.* 2007). A phylogenetic tree was generated by the method of Tamura *et al.* (2004) using MEGA X software (Kumar *et al.* 2018).

### Statistical analysis

Prevalence disease data was analyzed by site and by varieties using the RStudio software v. 1.4.1106. The Kruskal-Wallis ANOVA test (MacFarland and Yates 2016a) was used to compare the average of the prevalence of CILY in coconut plantations according to the surveyed sites. In those cases where a significant difference in average of disease prevalence was found at the 5% threshold, multiple comparison of mean ranks was used to obtain homogeneous groups. The Wilcoxon Mann-Whitney test (MacFarland and Yates 2016b) was used to compare the average prevalence of the disease for the different coconut varieties grown.

### Results

#### Diversity of symptoms associated with CILY

CILY outbreaks were randomly distributed in all coconut



**Fig. 2:** CILY symptoms in coconut plantations surveyed in south-eastern Côte d'Ivoire. **a)** Deformed nuts with reduced size after falling down; **b)** Necrosis of a nut; **c)** Necrosis of inflorescences; **d)** Yellowing of young palms; **e)** Yellowing of old palms; **f)** Coconut trees with “telephone pole”

plantations. Typical CILY symptoms were observed on both WAT and Dwarf coconut varieties in coconut plantations, in all surveyed sites. During the surveys carried out in the coconut plantations of the five sites, infected coconut trees showed different types of symptoms: a premature coconut drop and deformation and reduction in size of nuts (Fig. 2a) and nut necrosis (Fig. 2b) referred to stage 1; inflorescence necrosis (Fig. 2c) and the early yellowing of young palms (Fig. 2d) referred to stage 2; the yellowing of old palms before the senescence (Fig. 2e) referred to stage 3 and a complete defoliation leaving the tree topped as a telephone pole referred to stage 4 (Fig. 2f).

#### Prevalence of CILY by site and variety

The prevalence of CILY ranged from 62.75 to 100% depending on the site surveyed.

Indeed, the prevalence of the disease was 62.75% in Bonoua, 81.64%, in Aboisso, 94.74%, in Jacqueline, 98.67% in Grand-Bassam and in Grand-Lahou, 100%. Despite this prevalence varying from one site to another, no significant difference was observed ( $H = 6.4216$ ;  $P > 0.01$ ).

The prevalence of CILY was 95.31% for the WAT variety and 88.89% for the Dwarf variety. No difference was observed in the prevalence of the disease between coconut varieties ( $W = 9$ ;  $P > 0.01$ ).

#### Diversity of phytoplasmas associated with CILY in surveyed sites

Nested PCR using the Gh813f/AwkaSR primer pair was able to amplify DNA fragments to the expected size of 800 bp (Fig. 3) in 39 out of a total of 100 coconut trunk boring samples analysed. Thus, out of a total of 20 samples tested per site: 15 samples were positive in Grand-Bassam; 5 samples in Jacqueline were positive; in Bonoua, 11

samples; 6 samples in Grand-Lahou and 2 samples in Aboisso were positive. Nested PCR using the primer pair R16mF2/R16mR2 allowed the amplification of DNA fragments to the size of 1.25 kb in 6 samples of coconut trunk boring (Fig. 4). Thus, out of a total of 20 samples tested per site, 3 positive samples were obtained in Aboisso and 3 others in Bonoua. No positive samples were obtained from Grand-Bassam, Jacqueline or Grand-Lahou. Thus, different strains of phytoplasmas were detected in the samples of coconut trunk boring tested in the coconut plantations of the five surveyed sites. Samples that tested positive for phytoplasma were from both symptomatic and symptomless coconut trees.

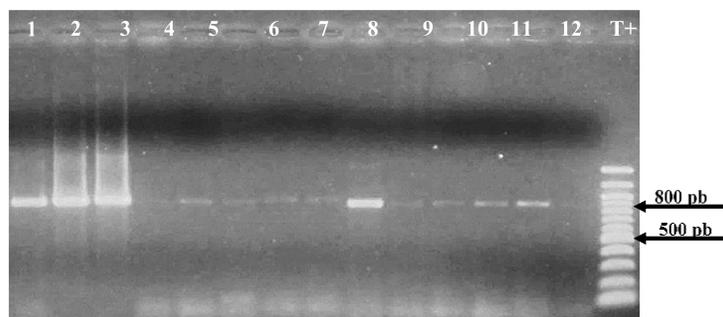
#### Diversity of phytoplasma strains associated with CILY in relation to coconut varieties

Nested PCR using the primer pair Gh813f/AwkaSR allowed the amplification of DNA in 37 coconut trunk boring belonging to the West African Tall variety, from which 33 were from symptomatic coconut trees and 4 from symptomless coconut trees. However, only 2 trunk boring samples from diseased coconut trees of the Dwarf variety tested positive.

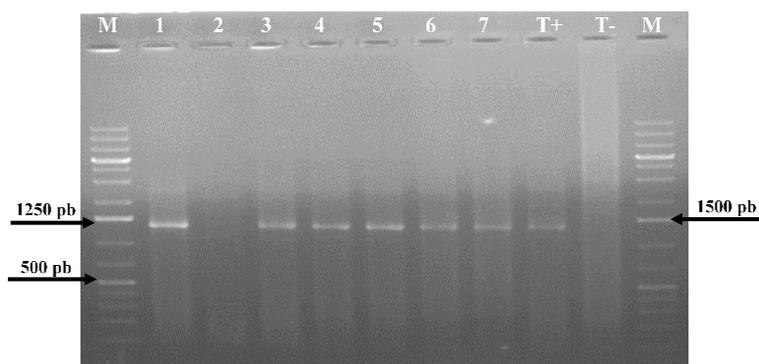
Nested PCR using the primer pair R16mF2/R16mR2 allowed the amplification of DNA in 4 trunk boring from coconut trees belonging to the West African Tall variety, 3 of which were symptomatic and 1 symptomless and from 2 trunk boring samples from symptomatic coconut trees of the Dwarf variety.

#### Sequence analysis and identification of phytoplasmas associated with CILY

BLAST results revealed that the 16S rRNA gene sequence of the CILY phytoplasma strain obtained with the Gh813f/Awka SR primers from Grand-Lahou (MN540266)



**Fig. 3:** 1.5% electrophoresis gel of nested PCR products with the Gh813f/AwkaSR primer pair  
M = 100 bp size marker; 1-12: trunk boring samples of tested coconut trees T+: positive control (trunk boring from an infected coconut tree); T-: negative control (water)  
lanes 1-6: symptomatic coconut trees of the WAT variety; lanes 11-12: symptomatic coconut trees of the Dwarf variety



**Fig. 4:** 1.5% electrophoresis gel of nested PCR products with primer pair R16mF2n/R16mR2  
M = 1 kb size marker; 1-7: trunk boring samples of tested coconut trunks; T+: positive control (trunk boring from an infected coconut tree); T-: negative control (water)  
lanes 1-4: symptomatic coconut trees of the variety WAT; lane 5: symptomless coconut trees of WAT variety; lanes 6-7: symptomatic coconut trees of Dwarf variety

shared a sequence identity of more than 99% with those strains identified in Grand-Lahou (KU216460, KU216457, KY969444, KY969445, KY969456, KY969461) as well as with the CSPWD phytoplasma strain from Ghana (KU216222.1), which belong to the group 16SrXXII-B ‘*Candidatus Phytoplasma palmicola*’. The partial sequence of the CILY phytoplasma strain obtained with the R16mF2n/R16mR2 primers from the Aboisso site (MN545965), showed a 99% of sequence identity with that of the Mexican phytoplasma strain (KX982667.1), group 16SrIV, ‘*Candidatus Phytoplasma palmae*’. The strain obtained with the R16mF2n/R16mR2 primers from the Bonoua site has not been identified.

### Phylogenetic analysis

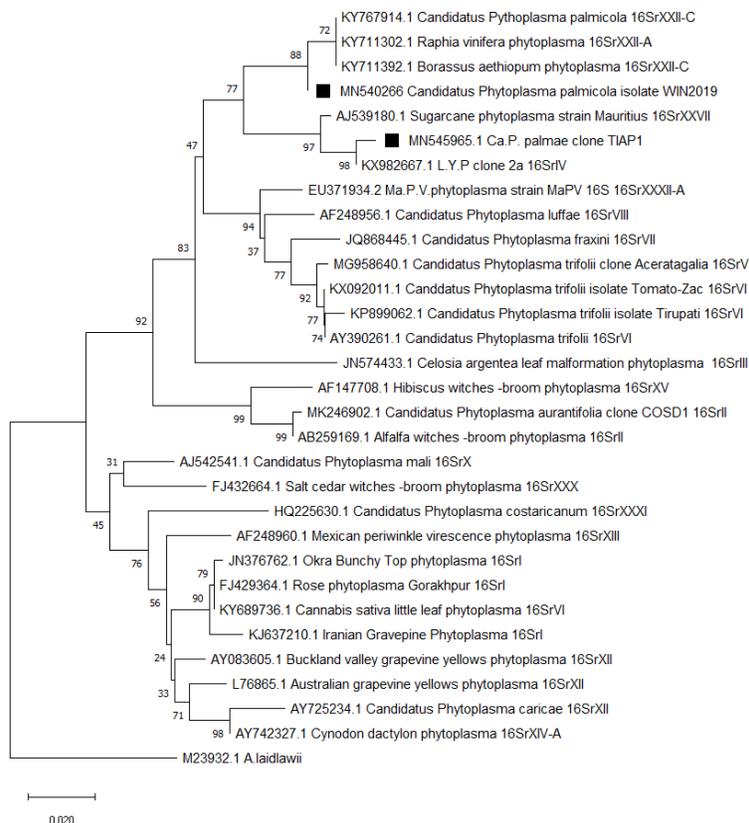
Phylogenetic analysis of the partial 16Sr RNA sequences confirmed the sequence analysis results. The CILY phytoplasma TIAP1 from Aboisso (MN545965) is closely related to the clade that includes the phytoplasma strain associated with LYD coconut palms in Mexico (KX982667) 16SrIV group, ‘*Ca. P. palmae*’. The CILY phytoplasma isolate WIN2019 from Grand-Lahou (MN540266) clusters with the 16SrXXII-B group as the strains already identified previously in coconut trees, palms and raffia plants in Côte d'Ivoire (Fig. 5).

### Mapping the distribution of phytoplasma strains associated with CILY in south-eastern Côte d'Ivoire

Mapping of the phytoplasmas associated with CILY in the five different coconut production sites of the study area (Fig. 6), shows different distribution patterns for each strain identified. ‘*Ca. P. palmicola*’ (16SrXXII-B) appears widely distributed in each site of the study area; however, ‘*Ca. P. palmae*’ (16SrIV) is restricted to the Aboisso site. There is, therefore, a diversity of phytoplasma strains in Aboisso unlike Grand-Lahou, Jacquerville and Grand-Bassam where only one phytoplasma strain has been identified. In Bonoua, the diversity of phytoplasma has not been proved.

### Discussion

The CILY survey in the south-eastern part of the Ivorian coast revealed the presence of a variety of CILY symptoms on coconut trees. These symptoms included premature fall and deformation of the nuts, necrosis of the inflorescences, yellowing of the palm leaves, desiccation of the palms and topping of the coconut trees. Based on the fact that symptoms were on the coconut trees during the survey with a random distribution across all the coconut plantations and the confirmation of the detection of phytoplasmas in all symptomatic trees, it suggests that symptoms are mainly

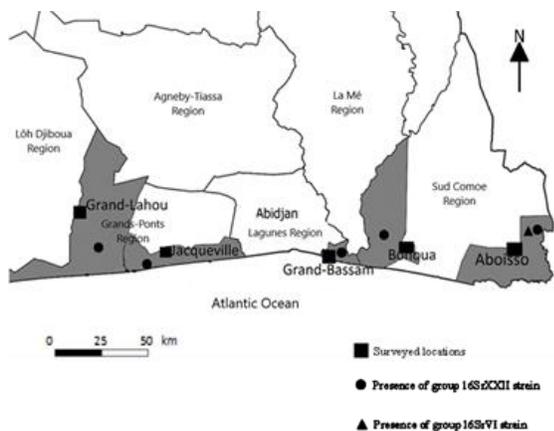


**Fig. 5:** Phylogenetic tree based on the Gh813f/AwkaSR and R16mF2n/R16mR2 sequences of the Lethal Yellowing Disease phytoplasma and the 16Sr RNA gene of phytoplasma reference sequences constructed by the Neighbour-joining method with MEGA X. The species *Acholeplasma laidlawii* was chosen to root the tree. Bootstrap values from 1000 replicates are shown on the branches. GenBank accession numbers for each sequence are given before the name of the phytoplasma. ■ - Strains obtained in this study

associated with an infection caused by phytoplasmas. CILY symptoms have already been observed and described in Grand-Lahou in Côte d'Ivoire (Arocha-Rosete *et al.* 2015), which are mostly similar to CSPWD symptoms in Ghana (Danyo 2011). Indeed, when the phytoplasma infects the

coconut tree, it localises in the phloem of the tree and causes a functional disorder (Lee *et al.* 2000). The consequences of this functional disorder are the closure of stomata leading to a decrease in photosynthetic activity and the subsequent appearance of yellowing symptoms in young and mature palms. In addition, the presence of phytoplasmas in coconut palms degrades the content of elaborated sap, leading to necrosis of the phloem and inflorescences (Lee *et al.* 2000). The phytoplasma also causes infected coconut palms and all aerial organs to fall off, leaving the tree without any crown resembling a telephone pole (Brown *et al.* 2007).

The assessment of CILY revealed a high prevalence of the disease in all the coconut plantations surveyed. This high prevalence could be explained by the susceptibility of two coconut varieties to phytoplasmas. Thus, the spread of the disease may not be related to the site surveyed, nor to the coconut variety grown. Both, the West African Tall (WAT) variety widely grown in the plantations and the Dwarf (Malaysian yellow dwarf) variety are known by their high susceptibility to CSPWD in Ghana (Nipah *et al.* 2007; Dery and Philippe 2008). According to Kumari *et al.* (2019), the diversity of symptoms and the high prevalence of symptoms associated with yellowing disease may be due to the presence of different strains of phytoplasmas infecting the coconut.



**Fig. 6:** Mapping of the different phytoplasma strains associated with CILY in the surveyed sites

The presence of phytoplasmas was confirmed in trunk boring samples of both varieties, with and without symptoms. Therefore, the absence of symptoms would not be sufficient to conclude the absence of a phytoplasma infection. The presence of phytoplasmas in symptomless coconut could be related to the incubation time of the phytoplasma which may have been too short for symptoms to develop at the time of the study. LYD symptoms vary according to the palm species and in the case of coconuts, the cultivar or variety involved. The disease develops very fast and infected trees often die within 4 to 6 months after the onset of symptoms (Eziashi and Omamor 2010). Trunk boring samples from coconut trees without any apparent symptoms tested positive for the presence of phytoplasmas. This has been reported in several plant species, including coconut, in Ghana (Nipah *et al.* 2007) and may have important implications for disease development as sources of inoculum for disease spread (Donkersley *et al.* 2019). Some coconut trees yielded negative results for the presence of phytoplasmas despite exhibiting CILY symptoms. This could be explained by the well know random distribution and low concentration of phytoplasmas within the phloem tissues (Firrao *et al.* 2007).

Two phytoplasma strains were identified from the five sites surveyed. '*Ca. P. palmicola*' (16SrXXII-B) was found in all the sites while '*Ca. P. palmae*' (16SrIV) was restricted to the Aboisso site. The 16SrIV group has been also found in Africa, subgroup -C in Tanzania and Kenya, and subgroups -B and -C in Mozambique (Bila *et al.* 2015; Gurr *et al.* 2016) in both palm species and coconut trees. It is not clear how the 16SrIV phytoplasma spread to Côte d'Ivoire, however, results evidence that both strains can co-exist within the same geographical site and co-infect the same host. Aboisso 16SrIV strain is thought to be similar to the Mexican strain but differs from those obtained in Grand-Lahou and Ghana. The group 16SrIV phytoplasma is present in Mexico, Jamaica, Dominican Republic, Honduras, Cuba and other Caribbean countries and it associated with LYD in several species of palms such as coconut causing similar symptoms (Myrie *et al.* 2006). The presence of another phytoplasma associated with lethal yellowing in Côte d'Ivoire could suggest co-infection by two phytoplasmas. Coconut trees in Côte d'Ivoire have been previously shown to host more than one phytoplasma (Arocha-Rosete *et al.* 2014; Kra *et al.* 2017) The 16SrI group and a 16SrXXII-C subgroup phytoplasma were found infecting coconut and other palm species Therefore, results from the present study show that CILY is expanding to other coconut growing areas other than Grand-Lahou, known so far as the primary outbreak focus (Arocha-Rosete *et al.* 2014, 2017). In addition, this is the first report of the identification of the 16SrIV phytoplasma group in West Africa, particularly in Côte d'Ivoire, and confirms that both phytoplasma groups, 16SrXXII-B and 16SrIV can co-infect the same host within the same geographical site. Further

research will be focused on surveying other coconut growing areas, making the full length 16S rRNA gene sequences available to identify subgroups and potential '*Ca. P. species*', using primers targeting non-ribosomal genes to characterize the diversity of CILY phytoplasmas, and determining the epidemiological factors that govern the occurrence of both '*Ca. P. palmicola*' and '*Ca. P. palmae*' strains in Côte d'Ivoire.

## Conclusion

The present study reports for the very first time the presence of CILY in the southern coconut growing areas of Côte d'Ivoire, and the occurrence of two strains associated with the disease, '*Ca. P. palmicola*' (16SrXXII-B) and '*Ca. P. palmae*' (16SrIV) This is the first report of the presence of the 16SrIV phytoplasma in Côte d'Ivoire and in West Africa. Both WAT and Dwarf are susceptible to both phytoplasma strains. '*Ca. P. palmicola*' (16SrXXII-B) is the most widespread strain in the southern plantations of Côte d'Ivoire compared to '*Ca. P. palmae*' (16SrIV). Results suggest that there is a diversity of phytoplasmas occurring in coconut plantations of Côte d'Ivoire that may represent an epidemiological threat for the spread of CILY to coconut trees and other palm species.

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## Author Contributions

BWMO, KDK and HAD; experimental design. BWMO and KDK; data collection with support from MNYT and YFRK. BWMO with YFRK; data analysis and manuscript writeup. All authors read and approved the final manuscript

## Conflicts of Interests

The authors declare that they have no competing interests

## Data Availability

Not applicable

## Ethics approval

Not applicable

## Funding Source

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**Consent for publication**

Not applicable

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