academicJournals

Vol. 14(52), pp. 3359-3367, 30 December, 2015
DOI: 10.5897/AJB2015.15002
Article Number: B42095056975
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

African fan palm (*Borassus aethiopum)* and oil palm (*Elaeis guineensis*) are alternate hosts of coconut lethal yellowing phytoplasma in Mozambique

J. Bila[1,2]*, N. Högberg[1], A. Mondjana[2] and B. Samils[1]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[2]Departamento de Protecção Vegetal, Faculdade de Agronomia e Engenharia Florestal, Universidade Eduardo Mondlane, Maputo, Mozambique.

Received 21 September, 2015; Accepted 19 November, 2015

In this study, potential alternate hosts of the phytoplasma causing coconut lethal yellowing disease (CLYD) in Mozambique were investigated based on 16S rRNA and *secA* genes. The results reveal that the naturalized palm species, *Elaeis guineensis* and *Borassus aethiopum* are alternate hosts of CLYD phytoplasma in Mozambique. Based on the *i*PhyClassifier online software, the phytoplasma detected in *B. aethiopum* belongs to the 16Sr group XXII-A, which include '*Candidatus* Phytoplasma palmicola' and '*Candidatus* Phytoplasma cocosnigeriae'. This is the first report associating '*Candidatus* Phytoplasma palmicola' with wild naturalized palm species in the world.

Key words: Alternate hosts, *Borassus aethiopum*, '*Candidatus* Phytoplasma palmicola', *Elaeis guineensis,* Mozambique, palm lethal phytoplasma phylogeny.

INTRODUCTION

The coconut palm (*Cocos nucifera*) is a major cash crop widely grown in Mozambique, contributing to the livelihood, income and food security of millions of the inhabitants (Bila et al., 2014). Outbreaks of coconut lethal yellowing disease (CLYD) is now threatening the coconut industry and the livelihood of over three million people in Mozambique. The current disease outbreak has already killed about eight million coconut trees and destroyed coconut associated businesses. Currently, the most sustainable CLYD management strategy is removal and burning of symptomatic coconut trees. Removed palms are replaced by hitherto resistant varieties. However, identifying resistance to the palm phytoplasma in Africa has proven challenging. The varieties that were shown to be resistant to lethal yellowing (LY) in the Caribbean region did not show the same degree of resistance in Africa (Eziashi and Omamor, 2010). The Mozambique giant green tall variety is still considered to be tolerant, since when compared to other varieties, it survives relatively longer to infection with CLYD, and therefore this variety is widely used to replace dead coconut palms in Mozambique. As an obligate parasite, the phytoplasma

*Corresponding author. E-mail: jbilay@gmail.com

Table 1. List of plant species sampled.

Species name		Number of samples
Scientific	**Common (English/Portuguese/local)**	
Hyphaene coriacea	Lala palm/ “Munala”	1
Phoenix reclinata	Senegal date palm or Wild date palm	3
Borassus aethiopum	African fan palm/ “Coqueiro Bravo”	9
Elaeis guineensis	African oil palm/ “Coconorte(eiro)”	28
Pinus sp.	Pine/ “Pinheiro Comum”	14
Total		55

needs another host to survive when the primary host, the coconut palm, is unavailable due to death from the disease or other factors. In the Caribbean, lethal yellowing-type phytoplasmas were detected in grass species associated with coconut farms, such as *Emelia forsbegii* and *Synedrella nodiflora* (Brown et al., 2008). Moreover, transmission of coconut phytoplasma between different host species was observed in Malaysia, where the causal agent of coconut LY-type diseases was also observed in Bermuda grass (*Cynodon dactylon*) and oil palm (*Elaeis guineensis)* (Nejat and Vadamalai, 2010). These findings highlight the need for identification of possible alternate host plant species in Mozambique. The Mozambican and West African phytoplasmas were recently formally described as ‘*Candidatus* Phytoplasma palmicola’ and related strains (Harrison et al., 2014). Bila et al*.* (2014) observed the existence of three different types of phytoplasmas in Mozambique coconut palms: ‘*Ca.* P. palmicola’, another one which was closely related to the East African (Tanzania) type and a third ‘*Ca*. *P. pini*’ related species. Given that several phytoplasma groups are present in Mozambique, different insect vectors and alternate hosts may be associated with their occurrence. Thus, it is pivotal to elucidate the origin and epidemiology of the Mozambican phytoplasmas. In this study, the hypothesis that there are other palmae and *Pinus* sp*.* species which are alternate hosts for phytoplasma species causing CLYD in Mozambique were tested.

MATERIALS AND METHODS

Sampling methodology for pines and palm

The detection of lethal yellowing symptoms among the potential alternate hosts may prove to be difficult since the symptoms occurring in these plants may be different from typical lethal yellowing symptoms and sometimes are similar to those caused by other biotic or abiotic factors (Perera et al., 2012). Furthermore, some plant species are tolerant to phytoplasmas and therefore show mild or no symptoms. Based upon these criteria, plants which showed symptoms or were propagated in the vicinity with diseased coconut palms were selected for this study. A total of 41 palm and 14 pine samples were collected from an equal number of trees (Table 1). The survey was conducted in the Mozambican Nicoadala and Maganja da Costa districts of the Zambezia province, during April 2014. For *Borassus aethiopum* and *Elaeis guineensis* palm tree species, sampling were performed by boring into the trunk using a 10 cm long drill bit that was 10 mm in diameter, while *Hyphaene coriacea* and *Phoenix reclinata* bush like palm species were sampled by cutting the spear leaves (youngest leaves). Finally, for pine trees, samples were collected from needles and stems, by cutting a piece of stem after removing the outer bark, targeting the phloem region. All sampling tools were sterilized in sodium hypochlorite and alcohol before each sample collection. At the sampling site, the collected trunk tissues were dried in tubes containing silica gel and maintained at room temperature until DNA extraction.

DNA extraction

From each tissue sample, 200 mg was ground using a bead beater (Precellys 24 Lysis and Homogenization; Bertin Technologies), and the nucleic acids were extracted using the CTAB extraction procedure described by Harrison and Oropeza (2008), with minor modifications (Bila et al., 2014). Prior to PCR, the extracted DNA was purified using JETquick DNA clean up Spin kit, and quantified on a NanoDrop spectrophotometer (Thermo Fisher Scientific).

PCR analysis

The phytoplasma DNA was amplified from total DNA extracts using direct PCR, followed by nested PCR. For the 16S rRNA genes, the phytoplasma-specific universal primer pairs P1/P7 (Schneider et al., 1995) were used in a first round PCR, in 50 µL reactions containing 25 µL diluted DNA template, 0.2 mM dNTP mix, 0.025 U μL^{-1} DreamTaq, Green DNA polymerase with the supplied reaction buffer (Fermentas), 0.2 µM of each primer and $MgCl_2$ were added to a final concentration of 2.75 mM. All amplifications were performed in a 2720 Thermal Cycler (Applied Biosystems) according to the conditions described by Lee et al. (1993). For nested PCR, the first round of PCR product was diluted 1000-fold and a section amplified by PCR employing three different primer sets specific to sections of the 16S rRNA gene, namely R16F2n/R16R2 (Lee et al., 1993; Gundersen and Lee, 1996), G813/Awka SR (Tymon, 1995) and LY16Sf/LY16Sr (Harrison et al., 2002). The PCR conditions for the primer pair R16F2n/R16R2 were 94°C for 90 s followed by 35 cycles of 94°C for 30 s, 60°C for 50 s and 72°C for 90 s, and a final extension step of 72°C for 10 min. The PCR conditions for the primer pair G813/Awka SR was the same as used for R16F2n/R16R2, except for the annealing temperature being 57°C instead of 60°C. Finally, for LY16Sf/LY16Sr the following PCR conditions was used; 94°C for 150 s followed by 35 cycles of 94°C for 30 s, 60°C for 50 s and 72°C for 80s, and a final extension step of 72°C for 10 min (Harrison et al., 2002). For amplification of the *secA* gene, a semi-

Table 2. Screening of potential CLYD alternate hosts based on the PCR assay.

Species name		Total number of samples	PCR positive results for different primer pairs (number of samples)					
			16Sr				*secA*	
Scientific	**Common**		**P1P7**	**R16F2n/ R16R2**	**G813/ AwaK Sr**	**LY16F/ LY16R**	**secA For1/ SecA rev3**	**secA For2/ SecA rev 3**
Hyphaene coriacea	Lala palm	1	1 (100%)	1 (100%)	-	-	-	-
Phoenix reclinata	Senegal date palm	3	1 (33.3%)	1 (33.1%)	-	-	-	-
Borassus aethiopum	African fan palm	9	66.7% (6)	55.6% (5)	11.1% (1)	11.1% (1)	66.7% (6)	66.7% (6)
Elaeis guineensis	African oil palm	28	32% (23)	53.4% (15)	4% (1)	-	46.4% (13)	50% (14)
Pinus sp.	Pine	14	50% (7)	100% (14)	-	-	14.3% (2)	50% (7)
Total		55						

nested PCR assay was used as described by Hodgetts et al. (2008) using a 50°C rather than 53°C annealing temperature. Primer pair secAfor1/secArev3 (Hodgetts and Dickinson, 2010) was used in the first round. For semi-nested PCR, the first round PCR product was diluted 1000-fold and a section used as a DNA template in the second round PCR employing primer pair secAfor2/secArev3 (Hodgetts and Dickinson, 2010). A control reaction using water as a template was performed in all experiments to confirm that there was no contamination of the PCR reactions. A 5 µL aliquot of each final PCR reaction was analyzed on 1% agarose gel containing Nancy-520 (Sigma). The DNA was visualized by UV trans-illumination and photographed.

Purification and sequencing of PCR products

The PCR positive products were purified using spin columns (Cycle-Pure Spin PCR purification kit). The purified products were quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and dried overnight in an incubator at 37°C. ABI sequencing was performed on both strands of the PCR products using the PCR primers.

Sequence analysis

Sequence editing and assembly of forward and reverse sequences were performed using the SEQMAN PRO software (DNASTAR Lasergene 12 core suite). When sequences were retrieved from two different 16S rRNA primer sets from the same sample, they were assembled into one contig (Table S1). The 16S rRNA gene sequences obtained in this study were compared with those of known phytoplasmas using BLAST searches (Altschul et al., 1990) from the National Centre for Biotechnology Information (NCBI). The sequences were aligned using CLUSTALW (Larkin et al., 2007). Phylogenetic analyses of the phytoplasma sequences were performed with MEGA v. 6.06 (Tamura et al., 2013) using the neighbor joining (NJ) and maximum likelihood (ML) methods evaluated with 1000 bootstrap replicates. The *Bacillus subtilis* 16S rRNA (AB042061) sequence was used as an out-group to root the trees (Hodgetts et al., 2008). Sequences of the 16S rRNA genes of other phytoplasmas were obtained from GenBank and used as reference sequences for phylogenetic analyses. The phytoplasma groups were assigned using the *i*PhyClassifier (Zhao et al., 2009) online interactive software tool.

RESULTS

Out of 55 plants sampled, the positive PCR ratio for 16S rRNA genes primers ranged from 4 (1 out of 28 *E. guineensis* palms) to 100% on both pine and *H. coriacea* palm samples, while for *secA* genes primers, the positive PCR ratio ranged from 0% for both *H. coriacea* and *P. reclinata* palms to 67% on *B. aethiopum* palms. A higher ratio of PCR positive samples with both 16S rRNA and *secA* genes primers was achieved on *B. aethiopum* and *E. guineensis* palm tree samples collected using stem boring method (Table 2 and Table S1). The three phytoplasma sequences used in this study were retrieved from samples MZ- Eg19 and MZ- Ba20 detected by G813/Awka SR and LY16Sf/LY16Sr primer pairs (Tables 2 and S1). None of the *SecA* PCR positive samples yielded a phytoplasma sequence. Thus, other PCR-positive samples (Tables 2 and S1) from both 16S rRNA and *secA* genes were determined to belong to other Gram positive bacteria than phytoplasmas after sequencing (data not shown). For instance, *Bacillus pumilis* sequences were retrieved from the samples MZ-Eg19 and MZ-Ba20 *secA* genes PCR product, while phytoplasma sequences were retrieved from the 16S rRNA PCR product of the same samples.

The two phytoplasma sequences were deposited in GenBank under the accession numbers KP938847 and KP938848. Sample MZ-Ba20 was collected from African fan palm (*B. aethiopum)* in the district of Nicoadala showing

Figure 1. Palm species associated with CLYD phytoplasma in this study. (a) African fan palm (*Borassus aethiopum)* showing the symptoms of a skirt shaped brown discoloration (necrosis) of the old leaves; (b) African oil palm (*Elaeis guineensis*) exhibiting skirt shaped brown discoloration of the older leaves and (c) collapse of the necrotic crown.

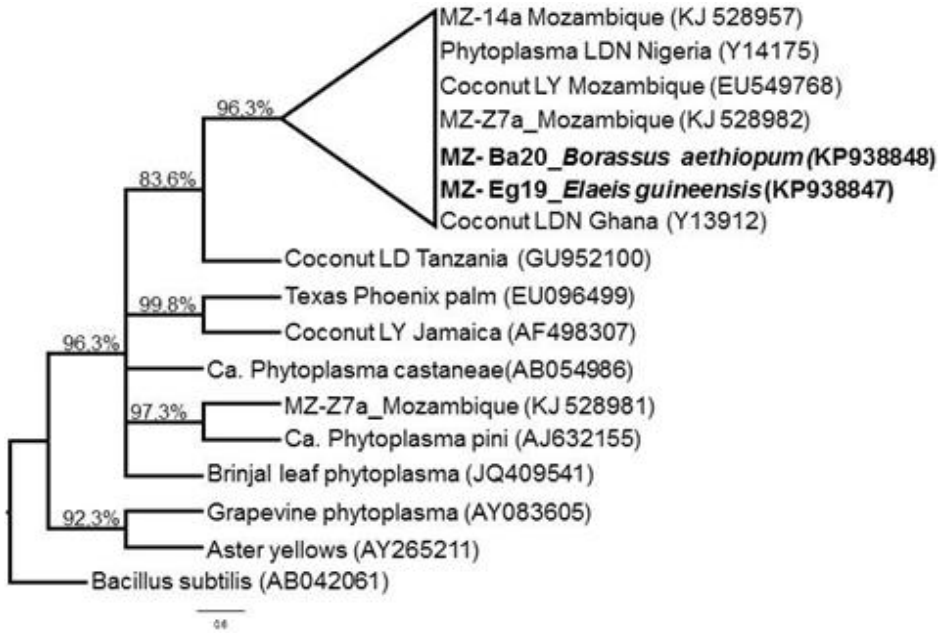

Figure 2. Dendrogram constructed with the maximum likelihood method showing the phylogenetic relationships among the wild naturalized palm phytoplasma samples from Mozambique compared with representatives from other 16Sr groups. The test samples included in the analysis are indicated by MZ-Ba20 and MZ-Eg19. The GenBank accession numbers are shown in parentheses. Bootstrap values greater than 80% based upon 1000 replicates are shown.

the symptoms of a skirt shaped brown discoloration (necrosis) of the old leaves, and sample MZ- Eg19 was collected from African oil palm (*E. guineensis*) in the same district of Nicoadala exhibiting brown discoloration (necrosis) of the mature and spear leaves (Figure 1). The retrieved sequences were used in a phylogenetic analysis together with other phytoplasma sequence, where the 16S rDNA NJ and ML trees had similar topologies (data not shown), and the ML tree is shown in Figure 2.

Phylogenetic analysis of the 16S rDNA sequences

showed that the phytoplasmas detected in *B. aethiopum* (MZ-Ba20) and *E. guineensis* (MZ-Eg19) belong to the same clade as the '*Ca*. P. palmicola' which cause CLYD in Mozambique, Nigerian phytoplasma sequence (Y14175.1) and coconut LY in Ghana (Y13912) (Figure 2). Based on the *i*PhyClassifier online software tool, the phytoplasma sequence from sample *B. aethiopum* (MZ-Ba20) is classified as '*Ca.* P. cocosnigeriae' reference strain or closely related. Furthermore the virtual RFLP pattern derived from the same program (*i*PhyClassifier) assigned the phytoplasma sequence to 16Sr group XXII, subgroup A (GenBank accession: Y14175). Unfortunately, phytoplasma sequence retrieved from sample MZ-Eg19 was not long enough to be supported by *i*PhyClassifier online software tool. The phytoplasma sequence from sample MZ-Eg19 (*E. guineensis)* clustered together with MZ-Ba20 in the phylogenetic analysis with 96.3% similarity. Thus, the results show that *E. guineensis* and *B. aethiopum* palm species are alternate hosts of CLYD phytoplasma (Figure 2).

DISCUSSION

Identification of alternate hosts is essential to develop sustainable control strategies against the spread of CLYD. The results reveal that *B. aethiopum* and *E. guineensis* palm species are alternate hosts of CLYD in Mozambique. Other than *C. nucifera,* this is the first report associating '*Ca.* P. palmicola' with wild naturalized palm species in the world. A Bermuda grass white leaf phytoplasma related strain has been detected in date palm in North Sudan (Cronje et al*.,* 2000). Palm species other than *C. nucifera* have been found harbouring lethal yellowing phytoplasma in other parts of the world. *E. guineensis* has previously been reported as susceptible to oil palm stunting disease in India, caused by '*Ca.* P. asteris'-related strain (Mehdi et al*.,* 2012) and to Malaysian coconut yellow decline in Malaysia (Nejat et al*.*, 2009). Members of the subgroup 16SrIV of the LY phytoplasmas infecting coconut, have also been found causing LY-like symptoms in silver date (*Phoenix sylvestris*), edible date (*Phoenix dactylifera*), queen palms (*Syagrus romanozoffiana*), Mexican fan palms (*Washingtonia robusta*) sabal palms (*Sabal palmetto*), Bismarck palm (*Bismarckia nobilis*), royal palm (*Roystonea regia*) and foxtail palm (*Wodyetia bifurcata*) (Myrie et al*.,* 2014; Harrison and Oropeza*,* 2008).

Based on PCR, RFLP and sequence analysis, a low detection rate was also reported by Brown et al*.* (2008) when searching for potential alternate hosts of coconut LY phytoplasma among weeds in Jamaica. They found a detection rate of 9/51 in *Emilia fosbergiii* and 4/36 in *Synedrella nodiflora*. Moreover, Myrie et al*.* (2014), screening for potential alternate hosts of coconut LY phytoplasma on other palm species, found only phytoplasma in one out of nine palm species, which increased to six out of nine by using real-time PCR, however no phytoplasma sequence retrieval attempt was reported. Detection of '*Ca*. P. palmicola' type in naturalized palm species suggests that this phytoplasma may switch from the wild naturalized palm host to coconut or vice versa, and might be transmitted between plant species by an unknown insect vector species.

According to the *i*PhyClassifier software tool, the phytoplasma sequence from *B. aethiopum* (MZ-Ba20) species was classified as '*Ca. P. cocosnigeriae*' 16Sr group XXII, subgroup A, which is the same subgroup as '*Ca*. P. palmicola', the main causal agent of CLYD in Mozambique. It is concluded that the source of infection in our alternate host samples is likely to be phytoplasma infections from cultivated *C. nucifera* since there is no sequence divergence from '*Ca*. P. palmicola'. This pattern is different from the situation in the USA where phytoplasma that infect palm species other than coconut, belongs to group 16SrIV, the same group as lethal yellowing phytoplasma of coconut (16SrIV-A) but different subgroups (16SrIV-B, 16SrIV-C, 16SrIV-D and 16SrIV-F) (Harrison and Oropeza, 2008; Hodgetts and Dickinson, 2010). Interestingly, closely related 16SrIV phytoplasmas found in other palm species in USA, such as date palm (Harrison and Oropeza, 2008) and in coconut palm in Jamaica, were detected in weed members of the family Asteraceae such as *E. fosbergii* and *S. nodiflora,* collected in the vicinity of coconut palms in Jamaica (Brown et al*.,* 2008). In Malaysia, the pattern is similar to Mozambique since the phytoplasma infecting coconut, Bermuda grass (*Cynodon dactylon*), and oil palm (*E. guineensis)* belongs to the same 16SrXIV phytoplasma group (Nejat et al., 2009). The same authors also reported another novel phytoplasma species infecting coconuts, oil palms and Madagascar periwinkle (*Catharanthus roseus*).

Phytoplasma was not detected in the other tested plants species in the present study. Even though Bila et al. (2015) suspected *Pinus* sp. as potential alternate host for CLYD phytoplasma, our results did not confirm that. The lack of consistency between the number of PCR products and phytoplasma sequences recovered is likely to be associated with primer specificity. Thus, from a substantial number of PCR positive samples, bacterial rather than phytoplasma sequences were retrieved. Investigations of diseases affecting Phoenix palms as well as other plant species have reported amplification of rDNA products from non-target Gram-positive bacteria by PCR assays employing phytoplasma universal rRNA gene primer pair P1/P7 followed by either R16F2n/R16R2 or LY16Sf/LY16Sr (Harrison and Oropeza, 2008; Myrie et al., 2014). Furthermore, Harrison et al. (2002) reported detection of nontarget *Bacillus megaterium* related rDNA sequences of similar size with expected phytoplasmas from trunk phloem of *Phoenix canariensis* symptomatic palms using P1/P7-primed PCR. Since the other tested plant species were

also PCR positive, the possibility of harboring coconut phytoplasma or related strains cannot be excluded. Moreover, only a few plants from a single growing season were screened from the many plant species available. The results of this work underscore the need for sequencing before making conclusions. This work also demonstrates the need to search for more reliable specific primers for detecting the '*Ca*. P. palmicola' and related strains. Better detection ratio with both 16S rRNA and s*ecA* genes primers was achieved on palm samples collected using stem boring compared to spear leaves methods. This finding could suggest that the viability of phytoplasmas on the spear leaves, which were kept dried under silica gel, may be limited, and instead a cool box with ice should be used. Mpunami et al*.* (1999) suggest keeping the sample at 4°C and processed within three days. Likewise, Nejat et al. (2009) kept the sample in clean plastic bags stored on ice for transport back to the laboratory. There is also a possibility that the phytoplasmas titre on spear leaves was lower than in the stem, since phytoplasma DNA levels vary according to season, plant organs and plant species throughout the disease progress stage (Oropeza et al*.*, 2011; Nejat and Vadamalai, 2010).

The presence of '*Ca*. P. palmicola' in alternate palm species needs to be taken into account in future management strategies against this plant disease. Not only *C. nucifera* but also other palm species must be monitored for the presence of phytoplasma infections. Recently, Mozambique has been implementing a costly and large scale CLYD management strategy which comprised removal of symptomatic coconut trees and replanting with the giant green tall coconut variety. However, alternate hosts were not considered in this effort. In future, a more comprehensive sampling of taxa and with larger sample sizes for each taxon may reveal more alternate hosts among, for example grasses. Other challenges within the field of CLYD epidemiology include the identification of insect vectors and screening for the disease resistant varieties against CLYD.

Conflict of interests

The authors have not declare any conflict of interest.

ACKNOWLEDGEMENTS

This work was funded by the Swedish International Development Agency (SIDA). The authors are also thankful to Madal Company and the small-scale coconut producers from Zambezia province of Mozambique for access to samples from their coconut farms. Finally the authors are grateful to Dr. William J. Baker from Royal Botanic Gardens, Kew, UK for the provided palm species identifications support.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. Mol. Biol. 215:403-410.

Bila J, Mondjana A, Berit S, Högberg N (2014). High diversity, expanding populations and purifying selection in phytoplasmas causing coconut lethal yellowing in Mozambique. Plant Pathol. 64:597-604.

Bila J, Mondjana A, Berit S, Högberg N (2015). Potential novel 'Candidatus Phytoplasma pini'-related strain associated with coconut lethal yellowing in Mozambique. Phytopathogenic Mollicutes 5:S59-S60.

Brown S, Been BO, McLaughlin WA (2008). First report of the presence of the lethal yellowing group (16Sr IV) of phytoplasmas in the weeds Emelia fosbergii and Synedrella nodiflora in Jamaica. Plant Pathol. 57:770.

Cronje P, Dabek AJ, Jones P, Tymon AM (2000). First report of a phytoplasma associated with a disease of date palms in North Africa. Plant Pathol. 49:801.

Eziashi E, Omamor I (2010). Lethal yellowing disease of the coconut palms (Cocos nucifera l.): An overview of the crises. Afr. J. Biotechnol. 9: 9122-9127.

Gundersen D, Lee I-M (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assay using two universal primer pairs. Phytopathol. Mediterr. 35:144-151.

Harrison NA, Davis RE, Oropeza C, Helmick EE, Narvaez M, Eden-Green S, Dollet M, Dickinson M (2014). 'Candidatus Phytoplasma palmicola', associated with a lethal yellowing-type disease of coconut (*Cocos nucifera* L.) in Mozambique. Int. J. Syst. Evol. Microbiol. 64:1890-1899.

Harrison NA, Oropeza C (2008). Coconut lethal yellowing. In: Harrison NA, Rao GP, Marcone C, eds. Characterization, Diagnosis and Management of Phytoplasmas. Studium Press LLC, Houston, TX, USA.

Harrison NA, Womack M, Carpio ML (2002). Detection and characterization of a lethal yellowing (16SrIV) group phytoplasma in Canary Island date palms affected by lethal decline in Texas. Plant Dis. 86:676-681.

Hodgetts J, Boonham N, Mumford R, Harrison N, Dickinson M (2008). Phytoplasma phylogenetics based on analysis of secA and 23S rRNA gene sequences for improved resolution of candidate species of 'Candidatus Phytoplasma'. Int. J. Syst. Evol. Microbiol. 58:1826-1837.

Hodgetts J, Dickinson M (2010). Phytoplasma phylogeny and detection based on genes other than 16S rRNA. In: Weintraub PG, Jones P, eds. Phytoplasmas: Genomes, Plant Hosts and Vectors. CAB International, Wallingford, UK.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). CLUSTALW and CLUSTALX version 2.0. Bioinformatics 23:2947-2948.

Lee LM, Hammond W, Davis RE, Gundersen DE (1993). Universal amplification and analysis of pathogen 16s rDNA for classification and identification of mycoplasma like organisms. Phytopathology 83:834-842.

Mehdi A, Baranwal VK, Kochu Babu M, Praveena D (2012). Sequence Analysis of 16S rRNA and secA Genes Confirms the Association of 16SrI-B Subgroup Phytoplasma with Oil Palm (Elaeis guineensis Jacq.) Stunting Disease in India. J. Phytopathol. 160:6-12.

Mpunami A, Tymon A, Jones P, Dickinson MJ (1999). Genetic diversity in the coconut lethal yellowing disease phytoplasmas of East Africa. Plant Pathol. 48:109-114.

Myrie WA, Harrison NA, Douglas L, Helmick E, Gore-Francis J, Oropeza C, McLaughlin WA (2014). First report of lethal yellowing disease associated with subgroup 16SrIV-A phytoplasmas in Antigua, West Indies. New Dis. Rep. 29:12.

Nejat N, Sijam K, Abdullah SNA, Vadamalai G, Dickinson M (2009). Phytoplasmas associated with disease of coconut in Malaysia: phylogenetic groups and host plant species. Plant Pathol. 58:1152-1160.

Nejat N, Vadamalai G (2010). Phytoplasmas detection in coconut palms and other tropical crops. Plant Pathol. J. 9(3):110-121

Oropeza C, Cordova I, Chumba A, Narvaez M, Saenz L, Ashburner R & Harrison N (2011). Phytoplasma distribution in coconut palms affected by lethal yellowing disease. Ann. Appl. Biol. 159:109-117.
Perera L, Meegahakumbura MK, Wijesekara HRT, Fernando WBS, M.J. Dickinson MJ (2012). A phytoplasma is associated with the weligama coconut leaf wilt disease in Sri Lanka. Plant Pathol. 94:1
Schneider B, Seemueller E, Smart CD, Kirkpatrick BC (1995). Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG, eds. Molecular and Diagnostic Procedures in Mycoplasmology. Academic Press, San Diego, CA, USA.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30:2725-2729.
Tymon A (1995). Etiology and Control of Lethal Yellowing-Type Diseases of Coconut Palm in Africa. STD III Contract TS3*-92–0055.
Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE (2009). Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). Int. J. Syst. Evol. Microbiol. 59:2582-2593.

Table S1. PCR results for plant samples used in this study.

Species scientific name	Sample ID	PCR results for 16S rRNA and *secA* genes primer pairs					
		16Sr				*SecA*	
		P1P7	R16F2n/ R16R2	G813/ AwaK Sr	LY16F/ LY16R	secA For1/ SecA rev3	secA For2 /SecA rev 3
Borassus aethiopum	MZ-Ba2	+	+			+	+
	MZ-Ba3	+	+			+	+
	MZ-Ba10	+	+				
	MZ-Ba18	+				+	
	MZ-Ba20	+	+	**+**	**+**	+	+
	MZ-Ba21	+	+			+	+
	MZ-Ba25						+
	MZ-Ba44					+	+
	MZ-Ba49						
Elaeis guineensis	MZ- Eg19	+	+	**+**		+	+
	MZ- Eg22					+	
	MZ- Eg23	+	+			+	+
	MZ- Eg24	+	+			+	+
	MZ- Eg27	+	+			+	+
	MZ- Eg28	+	+			+	+
	MZ- Eg32					+	
	MZ- Eg33		+				
	MZ- Eg36					+	+
	MZ- Eg37	+	+			+	+
	MZ- Eg38	+	+			+	+
	MZ- Eg39	+	+			+	+
	MZ- Eg40		+				
	MZ- Eg45	+	+			+	+
	MZ- Eg46		+				+
	MZ- Eg48						
	MZ- Eg50		+				
	MZ- Eg51						
	MZ- Eg53		+				
	MZ- Eg54		+			+	
	MZ- Eg55						
	MZ- Eg56						
	MZ- Eg57						
	MZ- Eg58						+
	MZ- Eg59						+
	MZ- Eg60						+
	MZ- Eg61						
	MZ- Eg62						
Hyphaene coriacea	MZ-Hc15	+	+				
Phoenix reclinata	MZ-Pr14	+	+				
	MZ-Pr26						
	MZ-Pr52						
Pinus sp.	MZ-P4		+				
	MZ-P4.1	+	+				

Table S1. Contd.

	MZ-P5	+	+			+	+
	MZ-P6					+	+
	MZ-P7		+				+
	MZ-P8	+	+				
	MZ-P9		+				+
	MZ-P11	+	+				
	MZ-P13		+				
	MZ-P16		+				+
	MZ-P17		+				+
	MZ-P41		+				
	MZ-P42	+	+				+
	MZ-P42	+	+				
Total	55	69% (38)	65% (36)	0.4% (2)	0.2% (1)	38% (21)	49% (27)