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'*Candidatus Phytoplasma palmicola*', a novel taxon associated with a lethal yellowing-type disease (LYD) of coconut (*Cocos nucifera* L.) in Mozambique

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1 **‘Candidatus Phytoplasma palmicola’, a novel taxon associated with a lethal yellowing-type**
2 **disease (LYD) of coconut (Cocos nucifera L.) in Mozambique**

3
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22 Running title: ‘Candidatus Phytoplasma palmicola’

23
24 GenBank[/EMBL/DDBJ] accession numbers for the sequences determined in this study are:
25 EU549768, KF364359, KF387570, KF41928, KF751386, KF751388

1 _____
2 In this study, the taxonomic position and group classification of the phytoplasma associated with
3 a lethal yellowing-type disease (LYD) of coconut (*Cocos nucifera* L.) in Mozambique were
4 addressed. Pairwise sequence similarity values based on alignment of near full-length 16SrRNA
5 genes (1530 bp) revealed that the Mozambique coconut phytoplasma (LYDM) shared 100%
6 identity with a comparable sequence derived from a phytoplasma strain (LDN), responsible for
7 Awka wilt disease of coconut in Nigeria, and shared 99-99.6% identity with 16S rRNA
8 sequences from strains associated with Cape St. Paul wilt (CSPW) disease of coconut in Ghana
9 and Côte d'Ivoire. Similarity scores further determined the 16S rRNA gene of LYDM
10 phytoplasma to share <97.5% sequence identity with all prior descriptions of 'Ca. Phytoplasma'
11 species. Presence of unique regions in the 16S rRNA distinguished LYDM phytoplasma from all
12 currently described 'Candidatus Phytoplasma' species, justifying its recognition as reference
13 strain of a novel taxon, 'Candidatus Phytoplasma palmicola'. Restriction fragment length
14 polymorphism (RFLP) profiles of the F2n/R2 portion (1251 bp) of the 16S rRNA gene and
15 pattern similarity coefficient values delineated coconut LYDM phytoplasma strains from
16 Mozambique as new members of established group 16SrXXII, subgroup A (16SrXXII-A).
17 Similarity coefficients of 0.97 were obtained for comparisons between subgroup 16SrXXII-A
18 strains and CSPW phytoplasmas from Ghana and Côte d'Ivoire. On this basis, CSPW
19 phytoplasma strains were designated as members of a new subgroup, 16SrXXII-B.

20 _____
21 Coconut (*Cocos nucifera* L.) is a member of the palm family (Arecaceae) and sole recognized
22 species in the genus *Cocos* (Persley, 1992). The general consensus has been that coconut
23 originated in the Indo-Pacific region either around Melanesia, Malesia or land masses bordering
24 the Indian Ocean, and has recorded histories spanning 3 millennia in Asia Minor, at least two
25 millennia in East Africa, and about five centuries in West Africa and the Americas (Clement et
26 al., 2013; Ohler, 1984; Schuiling and Harries, 1994). Pan-tropical in present day distribution,
27 this species is often cultivated in environments where few alternative crops can thrive, providing
28 a sustainable source of food and shelter, as well as an important source of rural income to small
29 farmers (Persley, 1992). Unfortunately, lethal diseases caused by phytoplasmas pose serious
30 threats to sustainability of coconut cultivation worldwide.

31

1 Phytoplasmas are unculturable, pleomorphic bacteria that belong to the class Mollicutes
2 (Bertaccini, 2007; Gasparich, 2010; Hogenhout et al., 2008). They are the presumed aetiological
3 agents of yellows-type diseases affecting at least 1000 plant species worldwide (McCoy et al.,
4 1989; Lee et al., 2000; Seemüller et al., 1998). In nature, these bacteria alternate passage
5 between plant and insect hosts in which they propagate and persist and depend upon plant to
6 plant transmission by phloem-feeding insect vectors of the order Hemiptera, primarily,
7 leafhoppers, planthoppers and psyllids (D'Arcy & Nault, 1982; Weintraub & Beanland, 2006).
8 Plant diseases associated with phytoplasma infection are often characterized by symptoms
9 indicative of disturbances in the balance of plant growth regulators (Aguilar et al., 2009;
10 Martinez et al., 2000). From various molecular studies, it is well established that phytoplasmas
11 possess small A+T rich genomes (Kollar & Seemüller, 1989; Tran-Nguyen & Gibb, 2007)
12 ranging from 530-1,350 kb in size (Neimark & Kirkpatrick, 1993; Marcone et al., 1999), and a
13 gene complement indicative of only a limited number of metabolic pathways (Andersen et al.,
14 2013; Bai et al., 2006; Kube et al., 2008; Oshima et al., 2004; Tran Nguyen et al., 2008). These
15 features may be attributable in part to phage-associated genome reductions during an ongoing
16 evolutionary adaptation of phytoplasmas to their plant and insect hosts (Davis et al., 2005;
17 Jomantiene et al., 2007; Wei et al., 2008).

18
19 Based on their identification and classification by restriction fragment length polymorphism
20 (RFLP) analysis of 16S ribosomal RNA gene sequences amplified by polymerase chain reaction
21 (PCR) assay (Lee et al., 1998; 2000), phytoplasmas constitute a genetically diverse taxon of
22 trans-kingdom parasites consisting of at least 28 groups and numerous subgroups (Wei et al.,
23 2007). Furthermore, groups defined by RFLP typing are largely supported by phylogenetic
24 analysis of 16S rDNA sequences upon which the taxonomy of phytoplasmas is currently based
25 (IRPCM, 2004). Thirty-six 'Candidatus Phytoplasma' species descriptions have thus far been
26 formally described (Davis et al., 2013; Nejat et al., 2013; Quaglino et al., 2013), using
27 'Candidatus' concepts for naming incompletely described microorganisms (IRPCM, 2004;
28 Murray & Stackebrandt, 1995).

29
30 In Africa, reliable reports of coconut diseases, later attributed to phytoplasmas (Dabek et al.,
31 1976; Dollet et al., 1977; Dollet & Giannotti, 1976; Nienhaus & Steiner; 1976), date back to the

1 early 1900s (Eden-Green, 1997). In West Africa, these diseases are known by various local or
2 regional names. They include Awka wilt disease in Nigeria (Ekpo and Ojomo, 1990; Osagie &
3 Asemota, 1997), Cape St. Paul wilt (CSPW) disease in Ghana (Ofori & Nkansah-Poku, 1997;
4 Danyo, 2011; Sangaré et al., 1992) and Côte d'Ivoire (Konan Konan et al., 2013), Kaïncopé in
5 Togo (Meiffren, 1951; Agounké, 1997) and kribi disease in Cameroun (Dollet et al., 1977). In
6 East Africa, a lethal disease of coconut is present along the coastal belt of Tanzania and in Kenya
7 (Schuiling & Mpunami, 1992a), while a similar disease is active in Mozambique (Bonnot et al.,
8 2010). Collectively, these diseases all share a succession of symptoms closely resembling lethal
9 yellowing, a name first used by Nutman & Roberts (1955) to denote a fatal phytoplasma-
10 associated disease of coconut in Jamaica (Beakbane, et al., 1972; Heinze et al., 1972; Plavsic-
11 Banjac et al., 1972) that has since spread to other parts of the Caribbean basin (Ntushelo et al.,
12 2013). Seemingly identical aetiologies and shared symptoms initially supported the view of a
13 common origin for these coconut lethal yellowing-type diseases (LYD) (Ogle & Harries, 2005;
14 Ollagnier & Weststeijn, 1962). However, differences in epidemiology (Danyo, 2011; Nkansah-
15 Poku et al., 2009; Philippe et al., 2009; Schuiling et al., 1992b) and coconut ecotype
16 susceptibility (Dery et al., 2008; Schuiling et al., 1992c) indicated that coconut-associated
17 phytoplasmas in Africa were probably distinct from those affecting palms in the Caribbean basin
18 (Eden-Green, 1997; Eziashi & Omamar, 2010).

19
20 The involvement of mutually distinct phytoplasmas with coconut diseases in West Africa
21 (Nigeria and Ghana) versus East Africa (Kenya and Tanzania) was clearly demonstrated by
22 specific amplification of 16S rRNA genes in PCR assays employing primer pairs based upon
23 unique sequences in rRNA operons of the respective pathogens and augmented by RFLP
24 analysis of PCR products (Tymon et al., 1997; 1998; Wei et al., 2007). In addition, PCRs
25 incorporating primer pair G813/AKSR, designed to amplify DNA of West African coconut
26 phytoplasmas (Tymon et al., 1997), were also later found to amplify rDNA sequences from
27 coconuts with LYD symptoms in Mozambique, East Africa (Mpunami et al., 1999). However,
28 not all diseased palms in Mozambique yielded phytoplasma positive results when PCRs were
29 primed by G813/AKSR, an outcome that upon further study was attributed to variability in the
30 region of the 16-23S intergenic spacer region (ISR) sequence corresponding to primer AKSR
31 (Dollet et al., 2009). Phylogenetic analysis of near full-length 16S rRNA gene sequences

1 revealed that coconut phytoplasmas from West Africa (Nigeria and Ghana) and East Africa
2 (Tanzania) formed two distinct subclades designated as (xii) and (xiv), respectively (Tymon et
3 al., 1998); both subclades were separate from subclade (vii), which encompassed coconut
4 phytoplasmas from the Caribbean region (Gundersen et al., 1994). Based on these findings, it
5 was informally proposed that these subclades represented three separate ‘Candidatus
6 Phytoplasma’ species (IRPCM, 2004). Further support for this proposal has since been obtained
7 from phylogenetic analysis of *rrn* operon sequences consisting of the 16-23S ISR and 5’-end of
8 the 23S rRNA gene, and by similar analysis of *secA* gene sequences, both of which also
9 differentiated African and Caribbean coconut phytoplasmas into three distinct clusters or groups
10 (Hodgetts et al., 2008), but formal descriptions of these taxa remain to be published.

11
12 Coconut palms with symptoms indicative of LYD were reported near Quelimane in Zambesia
13 Province, Mozambique during 2007 (Dollet et al., 2009) and on 2013 were observed for the first
14 time, in the Grand Lahou district on the southern coastal belt of Côte d’Ivoire (Konan Konan et
15 al., 2013). Although the coconut-infecting phytoplasmas detected in both countries were judged
16 most similar to strains previously associated with Awka wilt disease in Nigeria and CSPW
17 disease in Ghana (Dollet et al., 2011; Konan Konan et al., 2013), their precise taxonomic
18 positions and group affiliations were not determined. In this communication, we report results
19 from nucleotide and phylogenetic analysis of 16S rRNA gene sequences, and propose that
20 phytoplasma strains associated with coconut LYD in Mozambique, Nigeria, Ghana and Côte
21 d’Ivoire, represent a distinct species level lineage and novel taxon, ‘Candidatus Phytoplasma
22 *palmicola*’.

23

24 **Mozambique coconut phytoplasma (LYDM) represents a novel taxon**

25 To characterize phytoplasmas associated with coconut LYD in Mozambique and Côte d’Ivoire,
26 DNA fragments consisting of a portion of the *rrn* operon (1.8 kb) were amplified by PCR assay
27 employing phytoplasma universal primer pair P1 (Deng & Hiruki, 1991) and P7 (Smart et al.,
28 1996) and CTAB extractions of total DNAs (Harrison et al., 2013) from inflorescence or stem
29 tissues as template. Resulting PCR products, each comprising a near full-length 16S rRNA gene,
30 16-23S rRNA intergenic spacer and partial 23S rRNA gene, were purified separately on spin
31 columns using a Wizard PCR Preps purification system (Promega, Madison, WI) and sequenced

1 directly on automated equipment at the University of Florida's Core Genomics Service
2 laboratory to obtain at least 3X coverage per base position. Sequences obtained from three
3 diseased palms in both Mozambique and Côte d'Ivoire were archived in the GenBank nucleotide
4 database as accessions EU549768, KF751386, KF751388 and KF364359, KF387570,
5 KF419286, respectively. The portion of the *rrn* operon corresponding to the 16S rRNA gene
6 (positions 1..1530) in each accession contains a sequence $_{266}5'$ -
7 CAAGACGATGATGTGTAGCTGGACT-3' $_{290}$ matching the signature sequence (5'-
8 CAAGAYBATKATGKTAGCYGGDCT-3') that defines the taxa in the provisional genus
9 'Candidatus Phytoplasma' (IRPCM, 2004).

10

11 To determine their taxonomic position, near full-length (1530 bp) 16S rRNA gene sequences
12 derived from Mozambique and Côte d'Ivoire phytoplasma strains, from other West African
13 coconut phytoplasmas, from reference strains of all formally described 'Ca Phytoplasma'
14 species, and from strains representative of additional phytoplasma groups, were retrieved from
15 the GenBank nucleotide database. A global alignment of sequences was constructed using
16 CLUSTAL W in the MEGALIGN program (DNASTAR, Madison, WI) and queried using the
17 sequence distances option to obtain % identity values. The results confirmed that all three
18 LYDM phytoplasma sequences from Mozambique were mutually identical as were CSPW
19 phytoplasma sequences obtained from Côte d'Ivoire in this study. In addition, LYDM
20 phytoplasma sequences shared 100% identity with that of Awka wilt phytoplasma strain LDN
21 from Nigeria (GenBank accession Y14175), for which a provisional name of 'Candidatus
22 Phytoplasma cocosnigeriae' was previously proposed (IRPCM, 2004). By comparison, LYDM
23 sequences shared 99.6% identity with those of CSPW-associated phytoplasmas from Ghana
24 (accessions Y13912, JQ868442) and Côte d'Ivoire (accessions KC999037, KF364359,
25 KF387570, KF419286), but less than 97.5% identity with all previously described 'Ca.
26 Phytoplasma' species reference strains.

27

28 Differences in sequence identity (%) values between near full length 16S rRNA gene sequences
29 (1530 bp) of LYDM phytoplasmas and those of Côte d'Ivoire strains were attributable to a total
30 of six single nucleotide polymorphisms (SNPs) in base positions as follows: A/G₂₅₂, T/C₂₆₂,
31 T/C₆₁₉, T/C₉₈₃, T/G₁₀₀₆ and A/G₁₀₁₉ in this study. These same six SNPs were identified when

1 LYDM sequences were aligned with a comparable length (1530 bp) 16S rRNA gene sequence of
2 CSWP strain LDG (Y13912) and with lesser length 16S rRNA gene sequences of strains
3 CSPWB (JQ868442) from Ghana and CILY (KC999037) from Côte d'Ivoire that are 1508 bp
4 and 1251 bp in length, respectively. Strain LDG (Y13912) also possessed a putative SNP,
5 namely G/T₆₄₆, that was absent from Mozambique strains and from all other West African
6 coconut phytoplasmas. The putative SNP G/T₃ was unique to the CSPWB (JQ868442) sequence
7 while the following seven SNPs with their respective positions, G/A₆, A/G₇, C/T₈, A/G₁₆, C/T₄₉,
8 A/G₂₉₅ A/T₁₂₂₆ within the sequence derived from strain CILY (KC999037), were absent from all
9 other West African coconut strains and from the Mozambique strains.

10

11 The 'Ca Phytoplasma' species most closely related to LYDM phytoplasmas was 'Candidatus
12 Phytoplasma castaneae' whose 16S rRNA gene sequence shared 94% identity with the former
13 LYDM query sequences. Guidelines for the recognition of a novel 'Ca. Phytoplasma' species
14 (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004) state
15 that a strain can be described as a novel 'Ca. Phytoplasma' species if its 16S rRNA gene
16 sequence has <97.5% similarity to that of any previously described 'Ca. Phytoplasma species
17 (IRPCM, 2004). Our data illustrate that LYDM phytoplasmas meet the criterion for recognition
18 as a novel 'Ca. Phytoplasma' species for which we propose the name 'Candidatus Phytoplasma
19 palmicola'.

20

21 The global alignment of 16S rRNA gene sequences was assessed further to identify signature
22 sequences unique to LYDM phytoplasmas. Comparisons of the aligned sequences revealed at
23 least four unique regions. They include: ₁₆₉5'-AATGAGGCATCTCGTTAT-3'₁₈₆, ₁₉₆5'-
24 ACCTTCGCAAGAAGGT-3'₂₁₁, ₄₄₈5'-CGCTAGTGGAAAACTAGT-3'₄₆₆ and ₁₂₃₇5'-
25 CAGCTGAAACGTGAGTTGTTAGCT-3'₁₂₆₀ which differ from three to nine, from four to
26 nine, from five to nine and from three to 10 base positions, respectively, from the corresponding
27 regions in the 16S rRNA genes of all previously described 'Ca. Phytoplasma' species.

28

29 **New phytoplasma subgroup 16SrXXII-B**

30 To augment description of LYDM phytoplasmas and to further clarify their interrelationships
31 with strains affecting coconut palms in West Africa, the F2n/R2 portion of the 16S rRNA gene

1 (Lee et al., 1998) was subjected to virtual restriction site analysis employing 17 recommended
2 restriction endonucleases (Zhao et al., 2009b). Resulting RFLP patterns were visualized and
3 compared on virtual 3% gels using pDRAW32 (AcaClone software, <http://www.acaclone.com>).
4 Differences in fragment profiles between strains were evident for digestions with HaeIII only.
5 This endonuclease cuts the F2n/R2 sequence of LYDM and Awka wilt (LDN) phytoplasmas at
6 positions 121 and 187, producing fragments of 66, 121, and 1064 bp in size, respectively. By
7 comparison, the endonuclease cuts F2n/R2 sequences of all Ghana and Côte d'Ivoire strains just
8 once at position 187 producing fragments of 187 and 1064 bp, respectively. The base difference
9 at SNP T/C₂₆₂ eliminates a restriction site for the latter strains that all three LYDM strains and
10 Awka wilt phytoplasmas possess. The F2n/R2 sequences were also queried using the
11 iPhyClassifier program (Zhao et al., 2009b). A similarity coefficient value of 1.0 was obtained in
12 all pairwise comparison of LYDM strains with Awka wilt strain LDN from Nigeria. The latter
13 strain was previously identified and classified as sole member of RFLP group 16SrXXII,
14 subgroup A (i.e. 16SrXXII-A) (Wei et al., 2007). These values affirmed LYDM phytoplasmas as
15 new members of this established subgroup. However, virtual RFLP analysis of sequences
16 derived from strains LDG (Y14175) and CSPWB (JQ868442) from Ghana, as well as CILY
17 (KF387570) and additional Côte d'Ivoire strains (KF364359, KF387570, KF419286) from this
18 study, each generated a similarity coefficient value of 0.97 in all pairwise comparisons with
19 LYDM or LDN phytoplasmas. Thus, coconut phytoplasmas resident in Ghana and Côte d'Ivoire
20 were judged most similar to those of subgroup 16SrXXII-A; a value of 0.97 indicates that the
21 latter strains warrant classification as a new subgroup within group 16SrXXII. Therefore, we
22 assign LDG, CSPB and CILY and additional Côte d'Ivoire strains to subgroup 16SrXXII-B and
23 members of this subgroup as 'Ca. *Phytoplasma palmicola*'-related strains.

24

25 **Description of 'Candidatus *Phytoplasma palmicola*'**

26 'Candidatus *Phytoplasma palmicola*' (palmi- combining form [L, fr. palma palm] -cola n combining
27 form -s [NL, fr. L] : inhabitant, referring to the palm-inhabiting phytoplasma).

28

29 The reference strain LYDM-178^R is associated with succession of symptoms that include fruit
30 abortion, inflorescence necrosis, foliar discoloration and mortality of coconut palm. [(Mollicutes)
31 NC; NA; O, wall-less; NAS (GenBank accession number KF751386), oligonucleotide sequences

1 of unique regions of the 16S rRNA gene are: $_{169}5'$ -AATGAGGCATCTCGTTAT- $3'_{186, 196}5'$ -
2 ACCTTCGCAAGAAGGT- $3'_{211, 448}5'$ -CGCTAGTGGAAAACTAGT- $3'_{466}$, and $_{1237}5'$ -CAG
3 CTGAAACGTGAGTTGTTAGCT- $3'_{1260}$; P (Cocos nucifera, phloem); M]. Harrison et al., this
4 study.

5

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Table 1. ‘Candidatus Phytoplasma’ species reference strains and their 16S rRNA gene RFLP group/subgroup classifications.

Strain name	GenBank accession no.	16S RFLP group classification*	16S rRNA gene sequence length (bp)	Sequence identity (%) with ‘Ca. Phytoplasma palmicola	Reference
Formally described ‘Ca. Phytoplasma’ species					
‘Ca. Phytoplasma palmicola’ strain LYDM 178 ^R	KF751386	16SrXXII-A	1530	100	This study
‘Ca. Phytoplasma asteris’	M30790	16SrI-B	1530	89.3	Lee et al. (2004a)
‘Ca. Phytoplasma aurantifolia’	U15442	16SrII-B	1513	90.7	Zreik et al. (1005)
‘Ca. Phytoplasma australasia’	Y10097	16SrII-D	1521	90.8	White et al. (1998)
‘Ca. Phytoplasma pruni’	JQ044393	16SrIII-A	1519	92.9	Davis et al. (2013)
‘Ca. Phytoplasma ulmi’	AY197655	16SrV-B	1487	92.2	Lee et al. (2004b)
‘Ca. Phytoplasma ziziphi’	AB052876	16SrV-B	1529	92.7	Jung et al. (2003b)
‘Ca. Phytoplasma rubi’	AY197648	16SrV-D	1529	93.0	Malembic-Maher et al. (2011)
‘Ca. Phytoplasma balanitae’	AB689678	ND	1528	92.9	Win et al. (2012)
‘Ca. Phytoplasma trifolii’	AY390261	16SrVI-A	1531	93.4	Hiruki & Wang (2004)
‘Ca. Phytoplasma sudamericanum’	GU292081	16SrVI-I	1530	93.1	Davis et al. (2012)
‘Ca. Phytoplasma fraxini’	AF092209	16SrVII-A	1530	93.5	Griffiths et al. (1999)
‘Ca. Phytoplasma phoenecium’	AF515636	16SrIX-D	1496	90.7	Verdin et al. (2003)
‘Ca. Phytoplasma mali’	AJ542541	16SrX-A	1514	90.8	Seemüller & Schneider (2004)
‘Ca. Phytoplasma pyri’	AJ542543	16SrX-C	1514	90.8	Seemüller & Schneider (2004)
‘Ca. Phytoplasma spartii’	X92869	16SrX-D	1515	90.5	Marcone et al. (2004a)
‘Ca. Phytoplasma prunorum’	AJ542544	16SrX-F	1514	90.7	Seemüller & Schneider (2004)
‘Ca. Phytoplasma oryzae’	AB052873	16SrXI-A	1523	93.1	Jung et al. (2003)
‘Ca. Phytoplasma solani’	AF248959	16SrXII-A	1524	88.4	Quaglino et al. (2013)
‘Ca. Phytoplasma australiense’	L76865	16SrXII-B	1377	89.2	Davis et al. (1997)
‘Ca. Phytoplasma japonicum’	AB010425	16SrXII-D	1521	89.6	Sawayanagi et al. (1999)
‘Ca. Phytoplasma fragariae’	DQ086423	16SrXII-E	1333	88.6	Valiunas et al. (2006)

'Ca. Phytoplasma cynodontis'	AJ550984	16SrXIV-A	1501	93.1	Marcone et al. (2004b)
'Ca. Phytoplasma brasiliense'	AF147708	16SrXV-A	1528	90.2	Montano et al. 2001)
'Ca. Phytoplasma graminis'	AY725228	16SrXVI-A	1521	84.8	Arocha et al. (2005)
'Ca. Phytoplasma caricae'	AY725234	16SrXVII-A	1516	84.5	Arocha et al. (2005)
'Ca. Phytoplasma americanum'	DQ174122	16SrXVIII-A	1503	88.2	Lee et al. (2006)
'Ca. Phytoplasma castaneae'	AB054986	16SrXIX-A	1522	94.0	Jung et al. (2002)
'Ca. Phytoplasma rhamni'	X76431	16SrXX-A	1473	90.3	Marcone et al. (2004a)
'Ca. Phytoplasma pini'	AJ632155	16SrXXI-A	1528	93.4	Schneider et al. (2005)
'Ca. Phytoplasma omanense'	EF666051	16SrXXIX-A	1423	93.1	Al-Saady et al. (2008)
'Ca. Phytoplasma tamaricis'	FJ432664	16SrXXX-A	1516	90.6	Zhao et al. (2009a)
'Ca. Phytoplasma costaricanum'	HQ225630	16SrXXXI-A	1502	89.3	Lee et al. (2011)
'Ca. Phytoplasma malaysianum'	EU371934	16SrXXXII-A	1527	93.3	Nejat et al. (2013)
'Ca. Phytoplasma allocasuarinae'	AY135523	ND	1152	90.1	Marcone et al. (2004a)
'Ca. Phytoplasma lycopersici'	EF199549	ND	1523	87.1	Arocha et al. (2007)
'Ca. Phytoplasma convolvuli'	JN833705	ND	1496	89.1	Martini et al. (2012)
Provisional novel 'Ca. Phytoplasma' species and other incidentally cited strains:					
'Ca. Phytoplasma palmae'	AF498307	16SrIV-A	1524	94.8	IRPCM (2004)
'Ca. Phytoplasma cocostanzaniae'	X80117	16SrIV-C	1524	94.9	IRPCM (2004)
Sabal palm decline phytoplasma	FJ217386	16SrIV-D	1441	94.7	Harrison et al. (2009)
'Ca. Phytoplasma vitis'	AF176319	16SrVIII-A	1529	93.2	IRPCM (2004)
'Ca. Phytoplasma luffae'	AF086621	16SrXII-A	1530	94.1	IRPCM (2004)
Strains of 'Ca. Phytoplasma palmicola'					
LDN phytoplasma, Nigeria	Y14175	16SrXXII-A	1530	100	Tymon et al. (1998)
LYDM-182 phytoplasma, Mozambique	EU549768	16SrXXII-A	1530	100	This study
LYDM-185 phytoplasma, Mozambique	KF751388	16SrXXII-A	1530	100	This study
'Ca. Phytoplasma palmicola'-related strains:					

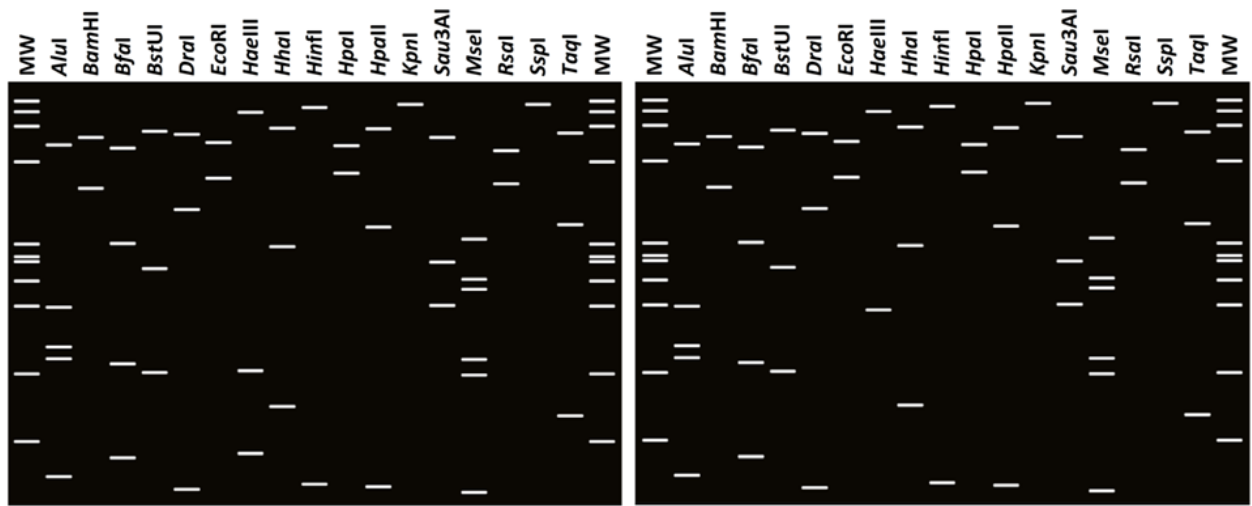
CSPW (LDG) phytoplasma, Ghana	Y13912	16SrXXII-B	1530	99.5	Tymon et al. (1998)
CSPW phytoplasma (DNA31), Côte d'Ivoire	KF387570	16SrXXII-B	1530	99.6	This study
CSPW phytoplasma (DNA19), Côte d'Ivoire	KF364359	16SrXXII-B	1530	99.6	This study
CSPW phytoplasma (DNA43), Côte d'Ivoire	KF419286	16SrXXII-B	1530	99.6	This study
CSPWB phytoplasma, Ghana	JQ868442	16SrXXII-B	1508	99.5	Makarova et al. (2012)
CILY phytoplasma, Côte d'Ivoire	KC999037	16SrXXII-B	1251	99.0	Konan Konan et al. (2013)

ND , Not determined

*16Sr RFLP group/subgroup classification status of the phytoplasma strains are based on Lee et al. (1998, 2000, 2006, 2011), Wei et al., (2007), Davis et al. (2013), Nejat et al. (2013).



1 **Fig. 1.** Phylogenetic tree inferred from analysis of 16S rRNA gene sequences. Maximum-
2 parsimony analysis was performed using the close neighbour interchange (CNI) algorithm in
3 MEGA4. The initial tree for the CNI search was obtained with the random addition of sequences
4 (10 replicates). The reliability of the analysis was subjected to a bootstrap test with 1000
5 replicates. The percentages (>60) of replicate trees in which the associated taxa clustered
6 together in the bootstrap analysis are shown next to the branches. The taxa used in the
7 phylogenetic tree construction included 'Ca. Phytoplasma palmicola' reference strain
8 (underlined), reference strains of 36 previously described 'Ca. Phytoplasma' species, provisional
9 'Ca. Phytoplasma' species (labelled with superscript **P**), and other incidental strains (labeled
10 with superscript **S**). *Acholeplasma palmae* served as an outgroup during the phylogenetic tree
11 construction. Bar, 20 nt substitutions.



1 **Fig. 2.** Virtual RFLP patterns derived from in silico digestions, using pDRAW32 of F2n/R2
2 fragments phytoplasma the 16S rRNA gene with 17 restriction endonuclease enzymes: AluI,
3 BamHI, BfaI, BstUI, DraI, EcoRI, HaeIII, HhaI, HinfI, HpaI, HpaII, KpnI, Sau3AI, MseI, RsaI,
4 SspI, and TaqI. The restriction fragments were resolved by in silico electrophoresis through a 3%
5 agarose gel. Subgroup 16SrXXII-A coconut phytoplasma strain LYDM-178^R (KF751386) (left)
6 and subgroup 16SrXXII-B phytoplasma strain CSPWB-DNA43 (KF419286) (right). MW,
7 Φ X174 DNA–HaeIII digest.