

Coconut (*Cocos nucifera* L.) is a member of the palm family (Arecaceae) and the sole recognized species in the genus *Cocos* (Persley, 1992). The general consensus has been that coconut originated in the Indo-Pacific region, around either Melanesia, Malesia or land masses bordering the Indian

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Ocean, and has recorded histories spanning three millennia in Asia Minor, at least two millennia in East Africa and about five centuries in West Africa and the Americas (Clement *et al.*, 2013; Gunn *et al.*, 2011; Schuiling & Harries, 1994). Pan-tropical in its present-day distribution, this species is often cultivated in environments where few alternative crops can thrive, providing a sustainable source of food and shelter, as well as an important source of rural income to small farmers (Persley, 1992). Unfortunately, lethal diseases caused by phytoplasmas pose serious threats to the sustainability of coconut cultivation worldwide.

Abbreviations: CSPW, Cape St Paul wilt; LYD, lethal yellowing-type disease; SNP, single nucleotide polymorphism.

Phytoplasmas are pleomorphic, nutritionally fastidious bacteria for which routine cultivation in cell-free media has not been established. They belong to the class Mollicutes (Gasparich, 2010) and are the presumed aetiological agents of yellows-type diseases affecting at least 1000 plant species worldwide (McCoy et al., 1989; Seemüller et al., 1998). In nature, these bacteria alternately passage between plant and insect hosts, in which they propagate and persist, and depend upon plant-to-plant transmission by phloemfeeding insect vectors of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids (Weintraub & Beanland, 2006). Plant diseases associated with phytoplasma infection are often characterized by symptoms indicative of disturbances in the balance of plant growth regulators (Aguilar et al., 2009; Martinez et al., 2000). From various molecular studies, it is well established that phytoplasmas possess small, A+T-rich genomes (Kollar & Seemüller, 1989; Tran-Nguyen & Gibb, 2007) ranging from 530 to 1350 kb in size (Neimark & Kirkpatrick, 1993; Marcone et al., 1999) and a gene complement indicative of only a limited number of metabolic pathways (Andersen et al., 2013; Bai et al., 2006; Kube et al., 2008; Oshima et al., 2004; Tran Nguyen et al., 2008). These features may be attributable in part to phage-associated genome reductions during an ongoing evolutionary adaptation of phytoplasmas to their plant and insect hosts (Davis et al., 2005; Jomantiene et al., 2007; Wei et al., 2008).

Based on their identification and classification by RFLP analysis of 16S rRNA gene sequences amplified by PCR (Lee *et al.*, 1998), phytoplasmas constitute a genetically diverse taxon of trans-kingdom parasites consisting of at least 28 groups and numerous subgroups (Wei *et al.*, 2007). Furthermore, groups defined by RFLP typing are largely supported by phylogenetic analysis of 16S rRNA gene sequences, upon which the taxonomy of phytoplasmas is currently based (IRPCM, 2004). Thirty-six candidate species of '*Candidatus* Phytoplasma' have so far been formally described (Davis *et al.*, 2013; Nejat *et al.*, 2013; Quaglino *et al.*, 2013), using *Candidatus* concepts for naming incompletely described micro-organisms (IRPCM, 2004; Murray & Stackebrandt, 1995).

In Africa, reliable reports of coconut diseases, later attributed to phytoplasmas, date back to the early 1900s (Eden-Green, 1997). In West Africa, these diseases are known by various local or regional names. They include Awka wilt disease in Nigeria (Ekpo & Ojomo, 1990), Cape St Paul wilt (CSPW) disease in Ghana (Ofori & Nkansah-Poku, 1997) and Côte d'Ivoire (Konan Konan et al., 2013), Kaïncopé in Togo (Dabek et al., 1976) and Kribi disease in Cameroun (Dollet et al., 1977). In East Africa, a lethal disease of coconut is present along the coastal belt of Tanzania and in Kenya (Schuiling & Mpunami, 1992), while a similar disease is active in Mozambique (Bonnot et al., 2010). Collectively, these diseases share a succession of symptoms that closely resemble lethal yellowing, a name first used by Nutman & Roberts (1955) to denote a fatal phytoplasma-associated disease of coconut in Jamaica (Plavsic-Banjac et al., 1972)

that has since spread to other parts of the Caribbean basin (Ntushelo *et al.*, 2013). Seemingly identical aetiologies and shared symptoms initially supported the view of a common origin for these coconut lethal yellowing-type diseases (LYD) (Ogle & Harries, 2005). However, differences in epidemiology (Danyo, 2011; Schuiling *et al.*, 1992a) and coconut ecotype susceptibility (Dery *et al.*, 2008; Schuiling *et al.*, 1992b) indicated that coconut-associated phytoplasmas in Africa were probably distinct from those affecting palms in the Caribbean basin (Eziashi & Omamor, 2010).

The involvement of mutually distinct phytoplasmas with coconut diseases in West Africa (Nigeria and Ghana) and East Africa (Kenya and Tanzania) was clearly demonstrated by specific amplification of 16S rRNA genes in PCR assays employing primer pairs based upon unique sequences in the rRNA operons of the respective pathogens and augmented by RFLP analysis of PCR products (Tymon et al., 1997, 1998; Wei et al., 2007). In addition, PCRs incorporating primer pair G813/AKSR, designed to amplify DNA of West African coconut phytoplasmas only (Tymon et al., 1997), were also later found to amplify rRNA gene sequences from coconuts with LYD symptoms in Mozambique, in East Africa (Mpunami et al., 1999). However, not all diseased palms in Mozambique yielded positive results when PCRs were primed by G813/AKSR, an outcome that, upon further study, was attributed to variability in the region of the 16S-23S rRNA intergenic spacer region (ISR) sequence corresponding to primer AKSR (Dollet et al., 2009). Phylogenetic analysis of nearly full-length 16S rRNA gene sequences revealed that coconut phytoplasmas from West Africa (Nigeria and Ghana) and East Africa (Tanzania) formed two distinct subclades designated (xii) and (xiv), respectively (Tymon et al., 1998); both subclades were separate from subclade (vii), which encompassed coconut phytoplasmas from the Caribbean region (Gundersen et al., 1994). Based on these findings, it was informally proposed that these subclades represented three separate candidate species of 'Candidatus Phytoplasma' (IRPCM, 2004). Further support for this proposal has since been obtained from phylogenetic analysis of rrn operon sequences consisting of the 16S-23S rRNA ISR and the 5' end of the 23S rRNA gene, and by similar analysis of secA gene sequences, both of which also differentiated African and Caribbean coconut phytoplasmas into three distinct clusters or groups (Hodgetts et al., 2008), but formal descriptions of these taxa remain to be published.

Coconut palms with symptoms indicative of LYD were reported near Quelimane in Zambesia Province, Mozambique, during 2007 (Dollet *et al.*, 2009) and, in 2013, were observed for the first time in the Grand Lahou district on the southern coastal belt of Côte d'Ivoire (Konan Konan *et al.*, 2013). Although the coconut-infecting phytoplasmas detected in both countries were judged most similar to strains previously associated with Awka wilt disease in Nigeria and CSPW disease in Ghana (Dollet *et al.*, 2011; Konan *et al.*, 2013), their precise taxonomic

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#### Table 1. Reference strains of candidate species of 'Candidatus Phytoplasma' and their 16S rRNA gene RFLP group/subgroup classifications

16Sr RFLP group/subgroup classification status of phytoplasmas is based on Lee et al. (1998, 2000, 2006, 2011), Wei et al. (2007), Davis et al. (2013) and Nejat et al. (2013). ND, Not determined.

Strain or taxon	GenBank accession no.	16S RFLP group classification	16S rRNA gene sequence		Reference
			Length (bp)	Identity with ' <i>Ca</i> . Phytoplasma palmicola' (%)	
Formally described members of 'Ca. Phytoplasma'					
<i>Ca.</i> Phytoplasma palmicola' strain LYDM 178 <sup>R</sup>	KF751387	16SrXXII-A	1530	(100)	This study
<i>Ca.</i> Phytoplasma asteris'	M30790	16Sr1-B	1530	89.3	Lee et al. (2004a)
'Ca. Phytoplasma aurantifolia'	U15442	16SrII-B	1513	90.7	Zreik et al. (1995)
<i>Ca.</i> Phytoplasma australasia'	Y10097	16SrII-D	1521	90.8	White <i>et al.</i> (1998)
<i>Ca.</i> Phytoplasma pruni'	JQ044393	16SrIII-A	1519	92.9	Davis et al. (2013)
<i>Ca.</i> Phytoplasma ulmi'	AY197655	16SrV-B	1487	92.2	Lee et al. (2004b)
'Ca. Phytoplasma ziziphi'	AB052876	16SrV-B	1529	92.7	Jung et al. (2003a)
<i>Ca.</i> Phytoplasma rubi	AY197648	16SrV-D	1529	93.0	Malembic-Maher et al. (2011)
<i>Ca.</i> Phytoplasma balanitae'	AB689678	ND	1528	92.9	Win et al. (2013)
<i>Ca.</i> Phytoplasma trifolii'	AY390261	16SrVI-A	1531	93.4	Hiruki &Wang (2004)
'Ca. Phytoplasma sudamericanum'	GU292081	16SrVI-I	1530	93.1	Davis et al. (2012)
<i>Ca.</i> Phytoplasma fraxini'	AF092209	16SrVII-A	1496	93.5	Griffiths et al. (1999)
<i>Ca.</i> Phytoplasma phoenicium'	AF515636	16SrIX-D	1502	90.7	Verdin et al. (2003)
<i>Ca.</i> Phytoplasma mali'	AJ542541	16SrX-A	1514	90.8	Seemüller & Schneider (2004)
<i>Ca.</i> Phytoplasma pyri'	AJ542543	16SrX-C	1514	90.8	Seemüller & Schneider (2004)
<i>'Ca.</i> Phytoplasma spartii'	X92869	16SrX-D	1515	90.5	Marcone et al. (2004a)
<i>Ca.</i> Phytoplasma prunorum'	AJ542544	16SrX-F	1514	90.7	Seemüller & Schneider (2004)
<i>'Ca.</i> Phytoplasma oryzae'	AB052873	16SrXI-A	1523	93.1	Jung et al. (2003b)
<i>'Ca.</i> Phytoplasma solani'	AF248959	16SrXII-A	1524	88.4	Quaglino et al. (2013)
<i>Ca.</i> Phytoplasma australiense'	L76865	16SrXII-B	1377	89.2	Davis et al. (1997)
<i>Ca.</i> 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)
<i>'Ca.</i> Phytoplasma brasiliense'	AF147708	16SrXV-A	1528	90.2	Montano et al. (2001)
<i>Ca.</i> Phytoplasma graminis'	AY725228	16SrXVI-A	1521	84.8	Arocha et al. (2005)
<i>Ca.</i> Phytoplasma caricae'	AY725234	16SrXVII-A	1516	84.5	Arocha et al. (2005)
<i>Ca.</i> Phytoplasma americanum'	DQ174122	16SrXVIII-A	1503	88.2	Lee et al. (2006)
<i>Ca.</i> Phytoplasma castaneae'	AB054986	16SrXIX-A	1522	94.0	Jung et al. (2002)
<i>Ca.</i> Phytoplasma rhamni'	X76431	16SrXX-A	1473	90.3	Marcone et al. (2004a)
<i>Ca.</i> 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)
<i>'Ca.</i> 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)

#### Table 1. cont.

Strain or taxon	GenBank accession no.	16S RFLP group	16S rRNA gene sequence		Reference
			Length (bp)	Identity with ' <i>Ca</i> . Phytoplasma palmicola' (%)	
<i>'Ca.</i> 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 candidate species of 'Ca. Phytoplasma'					
and other incidentally cited strains					
<i>'Ca.</i> 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)
<i>'Ca.</i> Phytoplasma vitis'	AF176319	16SrVIII-A	1529	93.2	IRPCM (2004)
<i>'Ca.</i> 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)



**Fig. 1.** Phylogenetic tree derived from analysis of 16S rRNA gene sequences. Maximum-parsimony analysis was performed using the close neighbour interchange (CNI) algorithm with search level 3 in MEGA4. The initial tree for the CNI search was obtained with the random addition of sequences (10 replicates). The reliability of the analysis was subjected to a bootstrap test with 1000 replicates. Percentages (>60 %) of replicate trees in which the associated taxa clustered together in the bootstrap

analysis are shown next to branches. The taxa used in the phylogenetic tree reconstruction included the '*Candidatus* Phytoplasma palmicola' reference strain (in bold), '*Candidatus* Phytoplasma palmicola'-related strains (labelled with superscript RS), reference strains of 36 previously described candidate species of '*Candidatus* Phytoplasma' (superscript R), provisional candidate species of '*Candidatus* Phytoplasma' (superscript P) and other incidental strains (superscript S). *Acholeplasma palmae* J233<sup>T</sup> served as an outgroup during phylogenetic tree reconstruction. Bar, 20 nucleotide substitutions.

positions and group affiliations were not determined. In this communication, we report results from nucleotide and phylogenetic analysis of 16S rRNA gene sequences, and propose that phytoplasma strains associated with coconut LYD in Mozambique, Nigeria, Ghana and Côte d'Ivoire represent a distinct species-level lineage and novel taxon, *'Candidatus* Phytoplasma palmicola'.

# Mozambique coconut phytoplasma (LYDM) represents a novel taxon

To characterize phytoplasmas associated with coconut LYD in Mozambique and Côte d'Ivoire, DNA fragments consisting of a portion of the rrn operon (1.8 kb) were amplified by a PCR employing phytoplasma universal primer pair P1 (Deng & Hiruki, 1991) and P7 (Smart et al., 1996) and CTAB extractions of total DNAs (Harrison et al., 2013) from inflorescence or stem tissues as template. Resulting PCR products, each comprising a nearly fulllength 16S rRNA gene, 16S-23S rRNA ISR and partial 23S rRNA gene, were purified separately on spin columns using the Wizard PCR Preps purification system (Promega) and sequenced directly on automated equipment at the University of Florida's Core Genomics Service laboratory to obtain at least  $3 \times$  coverage per base position. Sequences were obtained from three diseased palms in both Mozambique and Côte d'Ivoire. The portion of the rrn operon corresponding to the 16S rRNA gene (positions 1-1530) in each accession contains a sequence 5'-266 CAAGACGATGATGTGTAGC-TGGACT<sup>290</sup>-3' that matches the signature sequence (5'-C-AAGAYBATKATGTKTAGCYGGDCT-3') that defines taxa in the provisional genus 'Candidatus Phytoplasma' (IRPCM, 2004).

To determine their taxonomic position, nearly fulllength (1530 bp) 16S rRNA gene sequences derived from Mozambique and Côte d'Ivoire phytoplasma strains, from other West African coconut phytoplasmas, from reference strains of all formally described candidate species of 'Candidatus Phytoplasma' and from strains representative of additional phytoplasma groups were retrieved from the GenBank nucleotide database. A global alignment of sequences was constructed using CLUSTAL W in the MEGALIGN program (DNASTAR) and queried using the sequence distances option to obtain percentage identity values. The results confirmed that all three LYDM phytoplasma sequences from Mozambique were mutually identical, as were the CSPW phytoplasma sequences obtained from Côte d'Ivoire in this study. In addition, the LYDM phytoplasma sequences shared 100% identity with that of Awka wilt phytoplasma strain LDN from

Nigeria (GenBank accession no. Y14175), for which the provisional name '*Candidatus* Phytoplasma cocosnigeriae' was proposed previously (IRPCM, 2004). By comparison, LYDM sequences shared 99.0–99.6% identity with those of CSPW-associated phytoplasmas from Ghana (GenBank accession numbers Y13912 and JQ868442) and Côte d'Ivoire (KC999037, KF364359, KF387570 and KF419286), but less than 97.5% identity with reference strains of all previously described candidate species of '*Candidatus* Phytoplasma' (Table 1).

Differences in sequence identity between nearly full-length 16S rRNA gene sequences (1530 bp) of LYDM phytoplasmas and those of Côte d'Ivoire strains in this study were attributable to a total of six single nucleotide polymorphisms (SNPs) in base positions as follows:  $A/G^{252}$ ,  $T/C^{262}$ ,  $T/C^{619}$ ,  $T/C^{983}$ ,  $T/G^{1006}$  and  $A/G^{1019}$ . These same six SNPs were identified when LYDM sequences were aligned with a comparable length (1530 bp) 16S rRNA gene sequence of CSWP strain LDG (GenBank accession no. Y13912) and with shorter 16S rRNA gene sequences of strains CSPWB (JO868442) from Ghana and CILY (KC999037) from Côte d'Ivoire, which are 1508 and 1251 bp long, respectively. Strain LDG (GenBank accession no. Y13912) also possessed a putative SNP, namely G/T<sup>646</sup>, that was absent from the Mozambique strains and from all other West African coconut phytoplasmas. The putative SNP G/T<sup>3</sup> was unique to the CSPWB sequence (GenBank accession no. JQ868442), while the following seven SNPs, G/A<sup>6</sup>, A/G<sup>7</sup>, C/T<sup>8</sup>, A/G<sup>16</sup>,  $C/T^{49}$ ,  $A/G^{295} A/T^{1226}$ , within the sequence derived from strain CILY (KC999037) were absent from all other West African coconut strains and from the Mozambique strains.

The candidate species of '*Candidatus* Phytoplasma' that was most closely related to the LYDM phytoplasmas was '*Candidatus* Phytoplasma castaneae' (GenBank accession no. AB054986), whose 16S rRNA gene sequence shared 94% identity with the LYDM query sequences. Guidelines for the recognition of a novel candidate species of '*Candidatus* Phytoplasma' (IRPCM, 2004) state that a strain can be described as a novel candidate species of '*Candidatus* Phytoplasma' if its 16S rRNA gene sequence has <97.5% similarity to that of any previously described candidate species of '*Candidatus* Phytoplasma' (IRPCM, 2004). Our data illustrate that the LYDM phytoplasmas meet the criterion for recognition as a novel candidate species of '*Candidatus* Phytoplasma', for which we propose the name '*Candidatus* Phytoplasma palmicola'.

The global alignment of 16S rRNA gene sequences was assessed further to identify signature sequences unique

to LYDM phytoplasmas. Comparisons of the aligned sequences revealed at least four unique regions. They include 5'-<sup>169</sup>AATGAGGCATCTCGTTAT<sup>186</sup>-3', 5'-<sup>196</sup>AC-CTTCGCAAGAAGGT<sup>211</sup>-3', 5'-<sup>448</sup>CGCTAGTGGAAAA-ACTAGT<sup>466</sup>-3' and 5'-<sup>1237</sup>CAGCTGAAACGTGAGTTGT-TAGCT<sup>1260</sup>-3', which differ in three to nine, four to nine, five to nine and three to 10 base positions, respectively, across corresponding regions in the 16S rRNA genes of all previously described candidate species of '*Candidatus* Phytoplasma'. Phylogenetic analysis of the 16S rRNA gene sequences using MEGA4 software (Tamura *et al.*, 2007) yielded a phylogenetic tree in which the LYDM phytoplasmas clustered tightly together with strain LDN from Nigeria, forming a distinct subclade together with CSPW phytoplasmas from Ghana and Côte d'Ivoire (Fig. 1).

#### Novel phytoplasma subgroup 16SrXXII-B

To augment the description of the LYDM phytoplasmas and to clarify further their interrelationships with strains affecting coconut palms in West Africa, the F2n/R2 portion of the 16S rRNA gene (Lee et al., 1998) was subjected to virtual restriction site analysis employing 17 recommended restriction endonucleases (Zhao et al., 2009b). The resulting RFLP patterns were visualized and compared on virtual 3 % gels using pDRAW32 (AcaClone software; http://www. acaclone.com). Differences in fragment profiles between strains were evident for digestions with HaeIII only (Fig. 2). This endonuclease cuts the F2n/R2 sequence of LYDM and Awka wilt (LDN) phytoplasmas at positions 121 and 187, producing fragments of 66, 121 and 1064 bp, respectively. By comparison, the endonuclease cuts the F2n/R2 sequences of all Ghana and Côte d'Ivoire strains just once, at position 187, producing fragments of 187 and 1064 bp. The base difference at SNP T/C<sup>262</sup> eliminates a restriction site for the latter strains that is possessed by all three LYDM strains and Awka wilt phytoplasmas. The F2n/R2 sequences were also queried using the *i*PhvClassifier program (Zhao et al., 2009b). A similarity coefficient value of 1.0 was obtained in all pairwise comparisons of LYDM strains with Awka wilt strain LDN from Nigeria. The latter strain was previously identified and classified as the sole member of RFLP group 16SrXXII, subgroup A (16SrXXII-A) (Wei et al., 2007). These values affirmed the LYDM phytoplasmas as novel members of this established subgroup. However, virtual RFLP analysis of sequences derived from strains LDG (GenBank accession no. Y14175) and CSPWB (JQ868442) from Ghana, as well as CILY (KF387570) and additional Côte d'Ivoire strains (KF364359, KF387570 and KF419286) from this study, each generated a similarity coefficient value of 0.97 in all pairwise comparisons with LYDM or LDN phytoplasmas. Thus, coconut phytoplasmas resident in Ghana and Côte d'Ivoire were judged most similar to those of subgroup 16SrXXII-A; however, a value of 0.97 indicates that the latter strains warrant classification in a novel subgroup within group 16SrXXII. Therefore, we assign LDG, CSPWB, CILY and additional Côte d'Ivoire strains to subgroup 16SrXXII-B as 'Candidatus Phytoplasma palmicola'-related strains.

# Description of 'Candidatus Phytoplasma palmicola'

'*Candidatus* Phytoplasma palmicola' [pal.mi'co.la. L. fem. n. *palma* palm; L. suff. -*cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *palmicola* palm-dweller].

The reference strain LYDM-178<sup>R</sup> is associated with a succession of symptoms that include fruit abortion,



**Fig. 2.** Virtual RFLP patterns derived from *in silico* digestions, using pDRAW32, of F2n/R2 fragments of the 16S rRNA gene with 17 restriction endonuclease enzymes: *Alul, Bam*HI, *Bfal, Bst*UI, *Dral, Eco*RI, *Hae*III, *Hhal, Hinfl, Hpal, Hpall, Kpnl, Sau*3AI, *Msel, Rsal, Sspl* and *Taql*. The restriction fragments were resolved by *in silico* electrophoresis through a 3% agarose gel. Sequences were from subgroup 16SrXXII-A coconut phytoplasma strain LYDM-178<sup>R</sup> (GenBank accession no. KF751387) (left) and subgroup 16SrXXII-B phytoplasma strain CSPWB-DNA43 (KF419286) (right). MW, *φ*X174 DNA *Hae*III digest.

inflorescence necrosis, foliar discoloration and mortality of coconut palm. [(*Mollicutes*) NC; NA; O, wall-less; NAS (GenBank accession no. KF751387), oligonucleotide sequences of unique regions of the 16S rRNA gene are: 5'-<sup>169</sup>AATGAGGCATCTCGTTAT<sup>186</sup>-3', 5'-<sup>196</sup>ACCTTCG-CAAGAAGGT<sup>211</sup>-3', 5'-<sup>448</sup>CGCTAGTGGAAAAACTAG-T<sup>466</sup>-3' and 5'-<sup>1237</sup>CAGCTGAAACGTGAGTTGTTAGC-T<sup>1260</sup>-3'; P (*Cocos nucifera*, phloem); M]. Harrison *et al.*, this study.

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