

1 First report of strains related to the phytoplasma associated with Tanzanian Lethal

2 Decline on *Cocos nucifera* on the western coast of Madagascar

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25 Madagascar is a high diversity hotspot in the world, and palms are highly represented with
26 nearly 200 endemic species (Rakotoarinivo *et al.*, 2014). Coconut tree (*Cocos nucifera*)
27 could have been introduced in Madagascar by Austronesians around AD 400 or 700
28 (Beaujard, 2011). Sporadic coconut trees showing very severe wilt were observed in 2016
29 in three localities of the western and northern coast of the island: Katsepy (Sample MG16-
30 001), Antsohyhi (MG16-004 and MG16-005) and Ambaritsatrana (MG16-010). Symptoms
31 correspond on a severe ascendant wilt of the leaves, associated with necrosis of the
32 inflorescences and absence of nuts and death of all trees was confirmed eventually. We
33 investigated the implication of phytoplasma because of the apparent similarity in the
34 symptomatology with Coconut Lethal Yellowing Disease and Coconut Lethal Decline
35 occurring in East Africa (Mpunami *et al.*, 1999), and because the western coast of
36 Madagascar faces the Mozambican channel only 400 km apart from areas along the East
37 African coast where those two diseases occur.

38 Symptomatic (n=4) and asymptomatic (n=6) coconut trees were sampled by stem drilling.
39 DNA was extracted from sawdust samples using a modified CTAB protocol (Mpunami *et*
40 *al.*, 1999). A direct polymerase chain reaction (PCR) targeting the 16S rRNA gene plus
41 Internal transcribed spacer with the P1-1T (AAGAGTTTGATCCTGGCTCAGGAT)/P7
42 primers (Schneider *et al.*, 1998) amplified a product of about 1.8 kb for MG16-001 and
43 MG16-005 samples only, while the four DNA extracts from symptomatic trees showed a
44 1.2 kb amplicon by nested PCR using R16F2n/R16R2 primer pairs in the second round
45 (Lee *et al.*, 1998). Amplification of the *secA* gene using the primer pair
46 *secAFor1/secARev3* (Hodgetts *et al.*, 2008) was performed in a single round and gave a

product of 850 bp exclusively for the symptomatic tree DNAs. All amplicons were double strand sequenced (Genewiz, UK). Corresponding high quality sequences were deposited in GenBank and submitted to Blastn on NCBI.

The partial 16S rRNA gene sequences (accessions MN264629 to MN264632) obtained using R16F2n/R16R2 primers presented the highest similarity (from 99.47 to 99.56%) to the reference sequence for the phytoplasma associated with the Tanzanian Lethal Decline (GenBank accession X80117). This genetic proximity of the Malagasy strains was confirmed by the partial *secA* gene sequences (accessions MN267853 to MN267856) presenting the highest similarity (from 89.92 to 90.70%) to the Tanzanian Lethal Decline phytoplasma *secA* gene partial sequence (Genbank accession KJ462071).

Full-length 16S rRNA gene sequences of MG16-001 and MG16-005 strains (accessions MN388765 and MN388766) were submitted to iPhyClassifier virtual RFLP tool (Zhao *et al.*, 2009). The iPhyClassifier tool confirmed that Malagasy strains are related to the reference strain X80117 but belong to a different 16Sr subgroup (similarity coefficient from 0.90 to 0.93, Dev. 1). Both Malagassy strains and LDT phytoplasma should be assigned to a new 16Sr group since X80117 is itself erroneously assigned to 16SrIV group while the closest reference sequence AF509322, 16SrXXIV-A, shared only a similarity of 0.83 (Dev. 1).

Occurrence of a phytoplasma associated with a lethal yellowing type syndrome in Madagascar could represent a dangerous threat to coconut crops that play an important socio-economic role in the coastal areas, but also to the many endemic palm species already on high extinction risk.

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Figure S1. Symptoms of severe wilt observed on coconut tree in Antsohyhi, Madagascar (sample MG16-004).

1828x1219mm (72 x 72 DPI)



Figure S2. Symptoms of wilt observed on inflorescences of a coconut tree in Antsohyhi, Madagascar (sample MG16-004).

1828x1219mm (72 x 72 DPI)