# Evidence of an environmental reservoir for emergent *Mycobacterium colombiense*

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## Abstract

*Mycobacterium colombiense*, which belongs to the *M. avium* complex, is reported to have been isolated from cases of disseminated infection in both immunocompromised and immunocompetent patients. During the isolation of protists from water samples in French Guyana, we co-isolated a flagellated green alga (*Polytoma* sp.) and a mycobacterium identified as *M. colombiense*.


**Keywords:** Environmental reservoir, human infection, *Mycobacterium colombiense*, *Polytoma*sp., RpoB
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Non-tuberculous mycobacteria (NTMs) are widely distributed in the environment. NTMs are thought to be amoeba-resistant bacteria having the ability to survive within free-living amoeba trophozoites and being able to resist the cyst-forming process [1]. Several species can be isolated from water, especially from cooling towers, by using co-culture with amoebae [2]. In the past 16 years, our laboratory has developed an amoeba co-culture strategy to isolate intra-amoebal microorganisms, especially giant viruses [3]. For this purpose, in August 2013 100 µL of water were collected from Canal de Laussat at Cayenne, French Guyana (04°56.063N, 052°20.094W) and inoculated into a 25-$cm^2$ culture flask containing 10 mL of starvation buffer [4] incubated at 25°C. To avoid any bacterial contamination, we added an antibiotic mixture (vancomycin 10 µg/mL, ciprofloxacin 20 µg/mL, and imipenem 10 µg/mL). The growth of protists was monitored and observed under an inverted microscope every 48 h for 1 week. After 3 days of culture, a flagellate could be observed in this sample. To obtain this flagellate in axenic culture, we managed to eliminate the contaminating flora by using an antibiotic mixture as previously described. For the preliminary characterization, using DAPI (4′,6-diamidino-2-phenylindole) staining to search for giant viruses, we were astonished to observe bacteria-like bodies associated with this protist and in the medium. To perform identifications, we used the standard protocol with Hotstar® polymerase and specific primers targeting RNA polymerase subunit β (RpoB) against *Mycobacterium* spp. (Myco-Forward: GGCAAGGTCACCCCGAAGGG and Myco-Reverse: AGCGGCTGCTGGGTGATCATC). We sequenced the PCR amplicons using the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Based on microscopic observation and rRNA 18S gene amplification and sequencing, the protist was identified as a species of *Polytoma*, a colourless saprozoic green alga, with 100% identity with the reference strain *Polytoma uvella* strain SAG 62-2c (NCBI number U22941.1). Then the Ziehl–Nielssen stain enabled us to observe acid-fast bacilli close to *Polytoma* and in the supernatant. RpoB gene amplification and sequencing (NCBI number KU351672) performed on the protist and on DNA extracted from colonies obtained after 3 weeks of incubation on plates of Columbia agar + 5% sheep blood allowed us to identify this mycobacterium as *M. colombiense* (Fig. 1) with a similarity of 99% to reference strain CIP108962 (NCBI GQ153308.1). A pure culture of *Polytoma* sp. was obtained by treating the co-culture with a mix of clarithromycin and rifampicin (5 µg/mL and 200 µg/mL respectively) for 10 weeks. The mycobacterial strain has been deposited in the CSUR culture collection with number CSURP297. *M. colombiense* is a recently described mycobacterium belonging to the *Mycobacterium avium* complex (MAC), first described in blood and sputum from Columbian HIV patients [5]. Since then it has also been isolated from cases of disseminated cutaneous infections and lymph nodes in both immunocompromised and immunocompetent patients [6–8]. It has also been demonstrated that mycobacteria belonging to the MAC can survive within trophozoites and cysts of the free-living amoeba *Acanthamoeba polyphaga* [9]. Currently, the reservoir of *Mycobacterium colombiense* remains unclear. Recently, draft genomes were obtained from the soil of a swine farm in Japan [10]. The results reported herein show that the reservoir of *M. colombiense* also includes an aquatic environment in the proximity of the population of Cayenne city, French Guyana.



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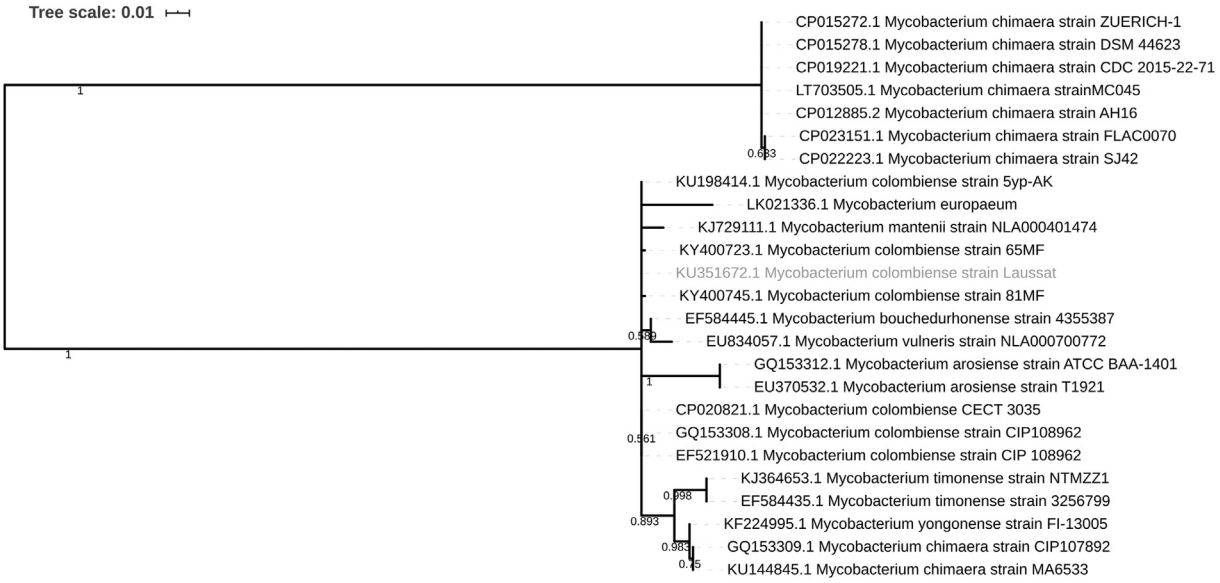


**FIG. 1.** Phylogenetic tree analysis of *Mycobacterium colombiense* and relationship with some non-tuberculous mycobacteria (NTM) based on the *RpoB* gene of *Mycobacterium colombiense*. Sequences were obtained from genbank (NCBI). Alignment was performed using MEGA6.0 software with Muscle tool. The phylogenetic tree was designed by maximum likelihood method on 776 positions with the Jukes–Cantor model and with 1000 bootstrap replications. Bootstrap proportion values are indicated at the node. Visualization was done by iTOl online with collapsed branches when values are <0.5.

## Transparency declaration

The authors declare no conflicts of interest. This study was supported by IHU Méditerranée Infection, Marseille, France and by the French Government under the 'Investissements d'avenir' (Investments for the Future) programme managed by the Agence Nationale de la Recherche (ANR, fr: National Agency for Research) (reference: Méditerranée Infection 10-IAHU-03). This work was also supported by Region Provence-Alpes-Cote d'Azur and European funding FEDER PRIMI.

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