

TITLE: IDENTIFICATION OF *Mycoplasma* spp. IN CAPTIVE SNAKES BY CULTURE, PCR AND DNA SEQUENCING.

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ABSTRACT

Among the agents associated with respiratory signs in reptiles, mycoplasmas are considered one of the main causes. *Mycoplasma agassizii* and *M. testudineum* may cause nasal and ocular discharge, palpebral edema and rhinitis in chelonians and crocodylians. There are few reports associating the presence of clinical signs with mycoplasmosis in snakes or other reptiles, to such an extent that mycoplasmas' pathogenic potential has been questioned in animals from Squamata Order. The aim of this study was to identify *Mycoplasma* spp. by culture, PCR and DNA sequencing in snakes belonging to the Riozoo S/A, located at Rio de Janeiro, Brazil. Tracheal swabs from 26 captive snakes were collected and immersed into microtubes containing modified Frey's medium. For the isolation of *Mycoplasma* spp., serial dilutions were made up to 10⁵, being the dilutions 10³ and 10⁵ submitted to growth in modified solid Frey's medium. Dilution broths and plates were incubated at 37°C under microaerophilya for 21 days. The Dienes and digitonin tests were used to confirm the positive isolates. An aliquot of medium from each tube containing immersed swab was used for PCR. *Mycoplasma* DNA was extracted using the Wizard® Genomic DNA Purification kit. Specific primers were used for the detection of conventional *Mycoplasma* spp. Samples presenting positive results by PCR were submitted to purification and sent to Sequencing Platform subunit RPT01A of Oswaldo Cruz Institute. Positive samples were obtained for *Mycoplasma* spp. in 11.5% (3/26) of samples cultured and 65.4% (17/26) by PCR. The sequences obtained presented similarity of 95% to 97% with strains of *M. agassizii* and 95% to 96% with strains of *M. testudineum*. It was possible to detect *Mycoplasma* spp. in snakes in captivity at Rio de Janeiro Zoo by culture and PCR. The mycoplasmas found presented genetic similarity with *M. agassizii* and *M. testudineum*, pathogenic species found in chelonians. More studies on the prevalence and agent-host relation regarding mycoplasmas in Squamata Order are necessary.

Keywords: *Mycoplasma*, snakes, Squamata Order

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