

**Title: NOVEL NOSTOCACEAN CYANOBACTERIA ISOLATED FROM ATLANTIC FOREST (SÃO PAULO, BRAZIL)**

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**Abstract:**

The Atlantic Forest represents the second most extensive tropical forest in Brazil and harbors an underexplored microbial diversity. A key process for the sustainability of tropical forests is the biological nitrogen fixation (BNF) performed by specific microbial groups of Bacteria and Archaea. It is known that one of the main inputs of nitrogen in Atlantic Forest is through BNF, which occurs in several surfaces of terrestrial environments. In this study, nitrogen fixing cyanobacteria from the order Nostocales were isolated and cultivated from macroscopic and microscopic biofilms on trunks, rocks, leaves and waterfalls in Parque Estadual da Serra do Mar, São Paulo state. These strains have been maintained in the Laboratory of Cell and Molecular Biology collection (CENA/USP), Piracicaba, São Paulo State, Brazil. Morphological identification of these strains was conducted using an optical light microscopy and specific literature. The 16S rRNA genes were sequenced and phylogenetic inferences were performed comparing these sequences with related ones retrieved from GenBank. The 16S/23S intergenic spacer (ITS) of the strains was also characterized. The seven novel isolated strains were primarily identified as *Nostoc* morphotypes, due to isopolar and non-branched trichomes and the presence of differentiated cells to BNF (heterocytes), besides a complex life cycle and reproduction by hormogonia. However, the 16S rRNA gene sequences of these strains shared less than 98% with the sequences available on GenBank and grouped into a new and exclusive clade in the phylogenetic tree, distantly related to the typical *Nostoc* clade, containing the type species of this genus (*Nostoc commune*). The novel strains were also phylogenetically distant from others morphologically related genera as *Halotia*, *Mojavia* and *Desmonostoc*. The 16S/23S ITS sequences of the novel strains presented 584 nucleotides length, Ala and Ile anticodon tRNAs and presenting highly similar D1-D1', Box B, V2 and V3 conserved regions. These results indicate that genetic diversity of these novel diazotrophic cyanobacteria strains overlaps the morphological variations and, considering the polyphasic approach (morphology evaluation, environment of origin and phylogeny), traditionally used for Cyanobacteria taxonomy, these strains could represent a novel generic entity.

**Key words:** *Nostoc*, Phylogeny, Polyphasic approach, 16S rRNA, 16S/23S ITS

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