

# Opening a next-generation black box: ecological trends for hundreds of species-like taxa uncovered within a single bacterial >99% 16S rRNA operational taxonomic unit

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## Abstract

Current knowledge on environmental distribution and taxon richness of free-living bacteria is mainly based on cultivation-independent investigations employing 16S rRNA gene sequencing methods. Yet, 16S rRNA genes are evolutionarily rather conserved, resulting in limited taxonomic and ecological resolutions provided by this marker. We used a faster evolving protein-encoding marker to reveal ecological patterns hidden within a single OTU defined by >99% 16S rRNA sequence similarity. The studied taxon, subcluster PnecC of the genus *Polynucleobacter*, represents a ubiquitous group of planktonic freshwater bacteria with cosmopolitan distribution, which is very frequently detected by diversity surveys of freshwater systems. Based on genome taxonomy and a large set of genome sequences, a sequence similarity threshold for delineation of species-like taxa could be established. In total, 600 species-like taxa were detected in 99 freshwater habitats scattered across three regions representing a latitudinal range of 3400 km (42°N to 71°N) and a pH gradient of 4.2 to 8.6. Besides the unexpectedly high richness, the increased taxonomic resolution revealed structuring of *Polynucleobacter* communities by a couple of macroecological trends, which was previously only demonstrated for phylogenetically much broader groups of bacteria. A unexpected pattern was the almost complete compositional separation of *Polynucleobacter* communities of Ca<sup>2+</sup>-rich and Ca<sup>2+</sup>-poor habitats, which strongly resembled the vicariance of plant species on silicate and limestone soils. The presented new cultivation-independent approach opened a window to an incredible, previously unseen diversity, and enables investigations aiming on deeper understanding of how environmental conditions shape bacterial communities and drive evolution of free-living bacteria.

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