

# The stick insect genus *Bacillus* and the role of small mitochondrial highly transcribed RNAs (smithRNAs) in hybridization and speciation.

Iuri Icaro, Diego Carli, Alessandro Formaggioni, Federico Plazzi, Marco Passamonti

Department of Biological, Geological and Environmental Sciences (Bi.Ge.A), University of Bologna, via Selmi, 3, 40126 Bologna (IT)

## Introduction

The stick insect genus *Bacillus* is known for its complex reproductive dynamics, with species such as *B. grandii*, *B. rossius*, and *B. atticus* engaging in both bisexual and parthenogenetic reproduction. These species exhibit a fascinating pattern of hybridization, leading to hybrids like *B. whitei* and *B. lynceorum*. Mitochondrial DNA analysis has revealed asymmetrical hybridization events, consistently showing *B. rossius* as the maternal parent. Recent research has turned its focus to small mitochondrial highly transcribed RNAs (smithRNAs), a class of small non-coding RNAs encoded by the mitochondrial genome that are implicated in regulating nuclear gene expression. This study investigates the distribution and characteristics of smithRNAs across parental and hybrid *Bacillus* species, aiming to understand their role in cyto-nuclear interactions, incompatibilities, and the evolution of isolating mechanisms that drive speciation.



*Bacillus grandii* (Nascetti e Bullini, 1982)



*Bacillus rossius* (Rossi, 1978)



*Bacillus atticus* (Brunner von Wattenwyl, 1882)

## Materials and Methods

### Sample Collection

Samples of *B. grandii*, *B. rossius*, *B. atticus*, and their hybrids (*B. whitei* and *B. lynceorum*) were collected from various locations across their natural habitats. The samples were carefully identified and preserved for further analysis.

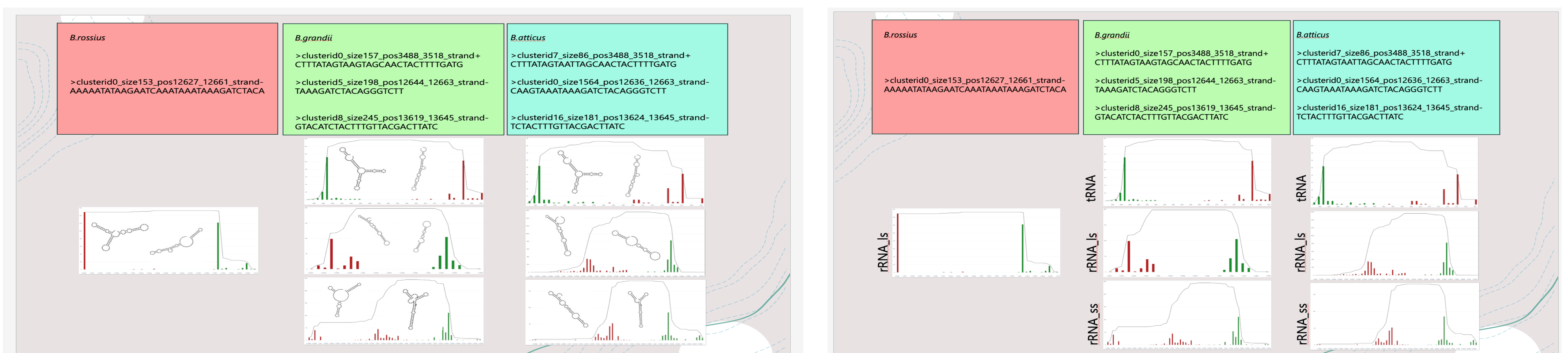
### smithRNA Identification and Quantification

The presence of smithRNAs was determined using Trascriptome sequencing. Libraries for total RNA sequencing were prepared using the TruSeq Small RNA Library Prep Kit (Illumina). Libraries for smallRNA sequencing were prepared using NEBNext small RNA Sequencing. The resulting reads were processed and aligned to the mitochondrial genome of *Bacillus* species.

### Dna extraction, Species Identification and Differentiation

To distinguish between species and hybrids, sequencing of the cytochrome c oxidase subunit I (cox1) gene was performed. Additionally, the following restriction enzymes were used for further differentiation: [ApaI] GTGCAC; [XmnI] GAANNNTTC. These enzymes were chosen for their ability to recognize specific restriction sites, providing clear-cut distinctions between different species based on the patterns of DNA fragments generated.

## Results



### SmithRNA Sequence and Structure Analysis

The second set of images provides detailed sequence and secondary structure analysis of smithRNAs identified in the different *Bacillus* species.

- ***B. rossius***: A single prominent smithRNA sequence identified: AAAAATATAAGAATCAATAATAAAATAAGATCTACA. The secondary structure analysis reveals a simple hairpin loop structure with fewer complex formations.
- ***B. grandii***: Multiple smithRNA sequences identified, including: CTTTATAGTAGACAACACTACTTTTGATG; TAAAGATCTACAGGTTCTT;GTACATCTACTTTGTTAGCACTTATC. The secondary structures are more complex, with multiple loops and stem formations, indicating higher structural diversity.
- ***B. atticus***: Several smithRNA sequences identified, similar to *B. grandii*: CTTTATAGTAGACAACACTACTTTTGATG; CAAGTAAATAAGATCTACAGGTTCTT; TCTACTTTGTTAGCACTTATC. The secondary structures show high complexity, with intricate loop and stem configurations.

### Conclusion from the Results

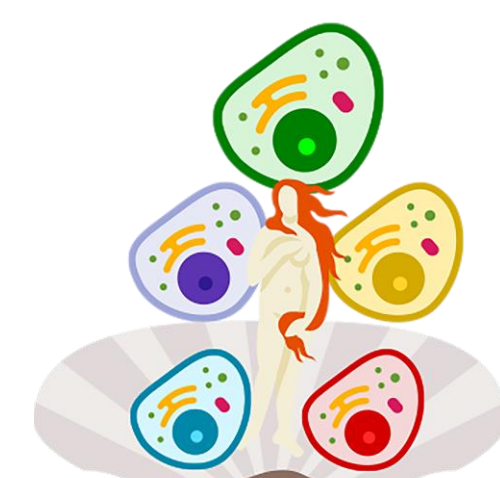
The gel electrophoresis results and the detailed smithRNA sequence and structure analysis provide clear evidence of the differential presence and complexity of smithRNAs across the *Bacillus* species. *B. grandii* and *B. atticus* exhibit higher smithRNA quantities and structural diversity compared to *B. rossius*. These findings support the hypothesis that smithRNAs play a significant role in cyto-nuclear interactions and probably in hybrid viability, potentially driving speciation within the *Bacillus* genus. These probably suggest that smithRNAs contribute to the genetic and reproductive stability of hybrid species. The study is still ongoing to allow for the analysis of hybrid forms of the species and to compare the parental species with the hybrids. This will provide more details on the nucleus-mitochondria communication in *Bacillus* species.



Iuri Icaro

[iuri.icaro2@unibo.it](mailto:iuri.icaro2@unibo.it)

<https://www.unibo.it/sitoweb/iuri.icaro2/>



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