

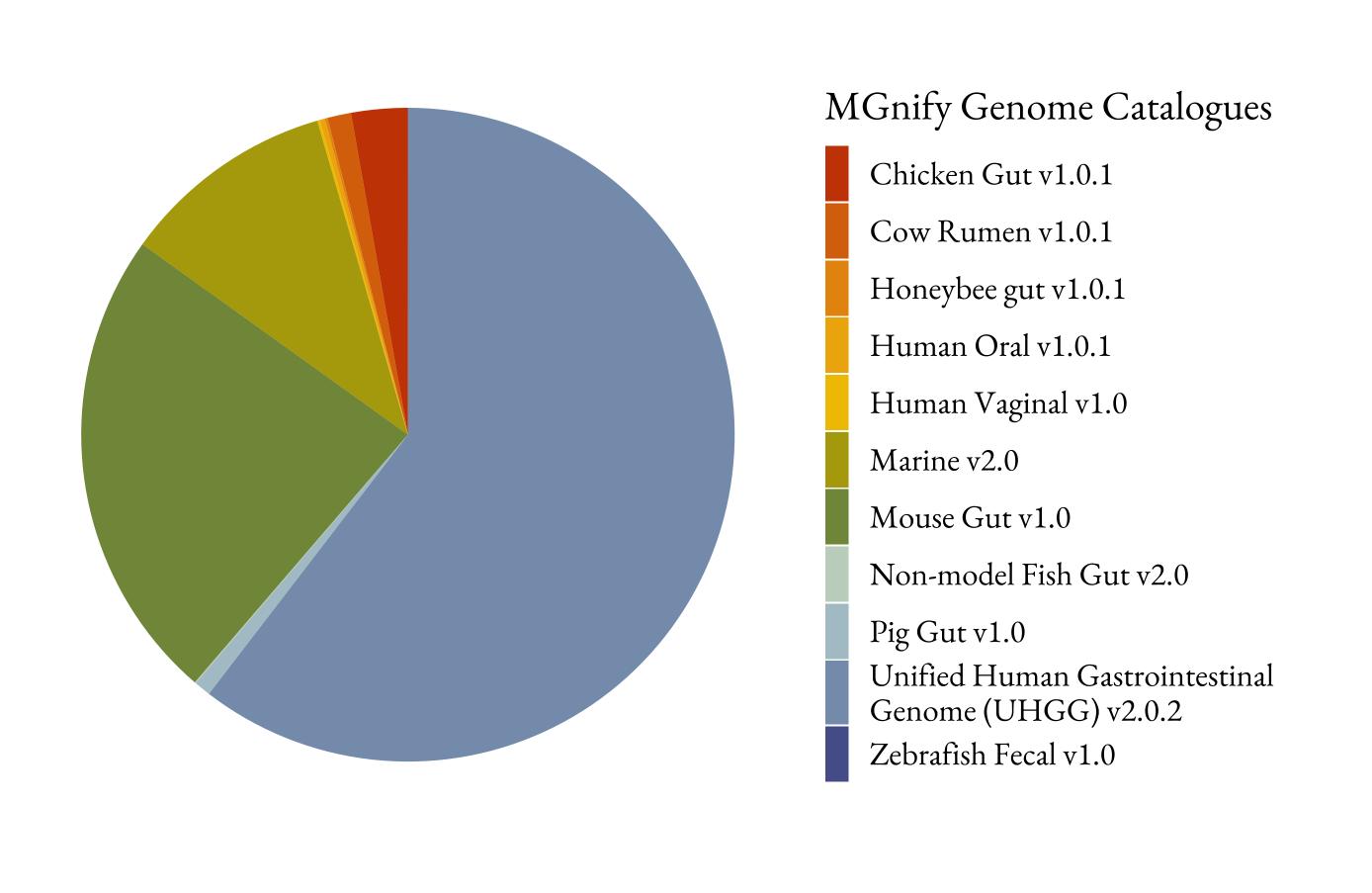
# ESTIMATING COMMUNITY COMPOSITION OF UNDERSTUDIED MICROBIOMES THROUGH NOVEL SPECIES GENOME ASSEMBLIES

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### UNDERSTUDIED MICROBIOMES ABOUND

Most microbiome studies focus on humans, especially the gut.
Microbes living elsewhere are underrepresented in genomic databases. Community composition estimation is confined to known taxa, undermining the breadth of microbiome research. To better study microbiomes, unknown species must be accounted for.



### SPECIES LACKING A REFERENCE GENOME

Identified through *de novo* (co)assembly of samples into metagenome-assembled genomes (MAGs);

Added to reference databases to better estimate sample composition; A study-specific effort is necessary for understudied microbiomes.

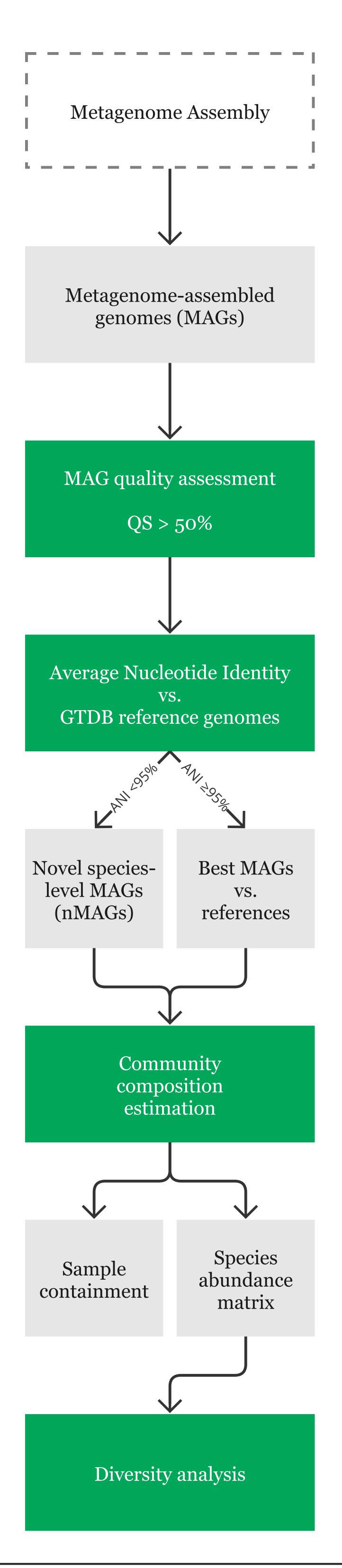
### **OBJECTIVES**

## BETTER ESTIMATE COMMUNITY COMPOSITION BY IDENTIFYING NOVEL SPECIES-LEVEL MAGS

- 1. Identify novel species from a set of MAGs;
- Evaluate the effect of nMAGs addition to genome database on increasing the containment therein of the samples from which the nMAGs were assembled;
- 3. Explore the effects of better community composition estimation on a simple comparative diversity analysis.

#### **METHODS**

- 1. MAG quality score (QS; Completeness  $-5 \times$  Contamination) > 0.50
- 2. Novel species-level MAGs (nMAGs): Average Nucleotide Identity (ANI) < 95 % with every known representative genome (GTDB r214)
- 3. MAGs with >= 95 % ANI are substituted if better QS than reference
- 4. Estimate sample containment\* and community composition with default and improved reference database (Sourmash gather)
- Explore effects of improvement on sample diversity (Shannon, rarefaction)
- \* Jaccard containment : abundance-weighted intersection of sample and reference database k-mers.



### NOVEL AND BETTER MAGS IDENTIFIED FROM TWO BIOMES

	Human saliva 26 samples (13 persons)	Boreal mosses 131 samples (4 species)
Species representative MAGs	130	153
Good quality MAGs (QS > 0.50)	110	107
nMAGs (ANI < 95%)	12	102
non-novel species (ANI ≥ 95%)	98	5
of which QS > reference QS	20	1

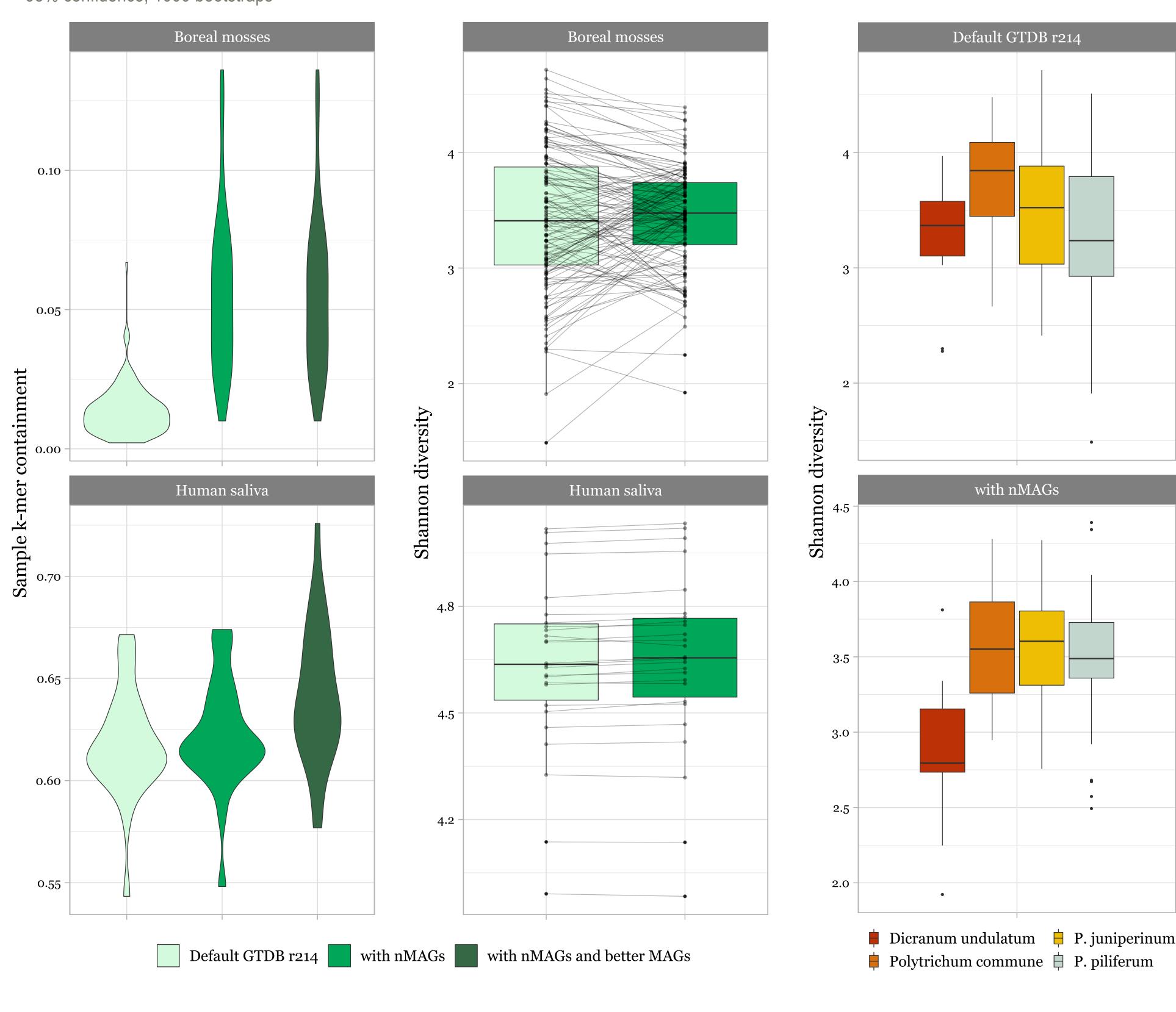
### NOVEL AND BETTER MAGS IMPROVE SAMPLE CONTAINMENT

nMAGs increase sample containment only in Moss dataset; better MAGs increase only in saliva.

**Human saliva:** 1.04-fold mean increase (Cohen's paired d = 1.03)

**Boreal mosses:** 4.84-fold mean increase (2.1 to 9.1-fold for 90% samples\*; Cohen's paired *d* = 1.72)

\* 95% confidence, 1000 bootstraps



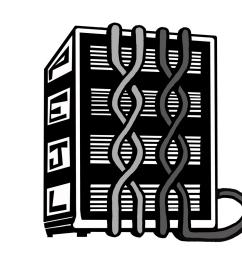
### NOVEL MAGS DIFFERENTIALLY AFFECT DIVERSITY ESTIMATIONS

Mean diversity doesn't change, but variance halves (from 0.37 to 0.20 when adding nMAGs)

Sample-wise diversities shift without pattern (mean change 2.6 % ± 17.7 %)

Variance explained by linear regression on host species triples ( $R^2$ : 0.04, p = 0.04 to  $R^2$ : 0.18, p < 10-5)

Including nMAGs reduces noise and could provides a better estimate of diversity.

















3. BE consistent in your presentation of the various references.

