Scaled MinHash Containment enables alignment-free distance estimation across the tree of life

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Abstract

Background Sequence similarity estimation is critical for genome analyses ranging from taxonomic classification to phylogenetic reconstruction. Current practices are still insufficient for environmental samples. Even best-practices samples can only assign a small fraction of metagenomic sequencing to known species... Given the scale of sequencing data now available, there is a great need for tools that can accurately estimate pairwise genome relatedness in an assembly-free and alignment-free manner.

Results Here, we introduce an alignment-free k-mer based method for quickly and accurately estimating pairwise sequence similarity, including Average Nucleotide Identity (ANI) and Average Amino Acid Identity (AAI). Because this method is assembly-independent and sequence-agnostic, it can be applied to both DNA and protein sequence data from across the tree of life. We demonstrate the utility of this technique with two primary examples: phylogenetic reconstruction of the genome-based prokaryotic taxonomy (GTDB) and fast and accurate microbial taxonomic classification. To demonstrate utility in other domains, we demonstrate proof-of-concept analysis on two published eukaryotic and viral datasets.

Conclusions Containment-based pairwise distance estimation via Scaled Minhash enables accurate assembly-free and alignment-free phylogenomic reconstruction and taxonomic classification across a wide range of evolutionary distances.

Keywords (3-10)

Background

As the scale of genomic sequencing continues to grow, alignment-free methods for estimating sequence similarity have become critical for conducting tasks ranging from taxonomic classification to phylogenetic analysis on large-scale datasets [1,2]. The majority of alignment-free methods rely upon exact matching of k-mers: subsequences of length k, that can be counted and compared across datasets, with or without use of subsampling methods such as MinHash. As k-mer based methods rely on exact sequence matches, they can suffer from limited sensitivity when comparing highly polymorphic sequences or classifying organisms from groups that are not well represented in reference databases.

Current best practices methods can still only categorize a fraction of the metagenomic and metatranscriptomic data, especially for understudied and/or diverse habitats (xx% recovery for soil, xx% recovery ocean metagenomes, etc). Even well-studied environments such as human gut can produce significant uncharacterized metagenome content. "For example, a reference-based approach failed to map 35% of reads in the iHMP study on inflammatory bowel disease (Supp. Data. of (Franzosa et al., 2019)), omitting them from any further analysis. These reads may belong to unknown microbes, phage or viruses, plasmids, or accessory elements of known microbes, all of which can play a role in disease.[from RO1]". This phenomenon is not restricted to metagenome samples. Alignment-based estimates can fail at larger evolutionary distances and even rRNA amplicon surveys may underestimate bacterial diversity [3].

To increase sensitivity of alignment-free methods, modified k-mer approaches have been introduced, including spaced seeds /split k-mers, which accommodate polymorphic sites in highly similar genomes (CITE). For larger evolutionary distances, protein-based comparisons have long been the gold-standard approach for taxonomic and functional annotation, as protein sequence is more conserved than the underlying DNA sequence [4,5]. As microbial and viral genomes are gene-dense, [MinHash-based] alignment-free comparisons of translated protein sequence have been shown to increase sensitivity for taxonomic classification and genome discovery [6,7]. Here, we demonstrate the utility of protein k-mer comparisons for phylogenomic reconstruction and taxonomic classification at larger evolutionary distances and across both gene-rich and [gene-sparse] sequences. We use Scaled Minhash subsampling to facilitate conducting these comparisons at scale [8].

Scaled Minhash is a MinHash variant for selecting and hashing a set of representative k-mers from a sequence dataset [8]. Unlike traditional MinHash, Scaled MinHash sketches scale with the size of the dataset, meaning each sketch is comprised of the chosen proportion of k-mers in the input dataset, rather than a chosen number of k-mers. Downsampling sequencing datasets in this way enables estimation of containment, which has been shown to permit more accurate estimation of genomic distance, particularly for genomes of very different lengths [9,10]. Streaming containment estimates have been shown to facilitate genome discovery and correlate with Mash Distance, a proxy for Average Nucleotide Identity (ANI) [7,11].

Standardized genomic measures of relatedness such as ANI and its protein counterpart, Average Amino Acid Identity (AAI) have shown lasting utility for genome relatedness and phylogenomic analysis. Traditional ANI and AAI describe the sequence similarity of all orthologous genes, either in nucleotide or protein space, respectively. Both been shown to be robust measure of overall pairwise genome relatedness even for highly incomplete datasets, such as those comprised of only ~4% of the genome or 100 genes [12,13]. ANI has emerged as the most widely-accepted method for estimating pairwise similarity of microbial genomes and delimiting species boundaries [14]. Recent research appears to confirm 95% ANI species threshold for prokaryotic species, although there is some debate as to the universality of this threshold [15,16,17]. AAI thresholds have been proposed for higher taxonomic ranks, <45%, 45-65% and 65-95% for family, genus, and species [13,18]. While traditional alignment-based estimation of ANI and AAI are computationally intensive, sketching-based estimates and sketching-facilitated estimates have permitted ANI calculations at the scale of whole-databases [1,7,15].

[Pierce-Ward et al., 2021 (tbd technical paper)] showed that Scaled MinHash containment estimates can be used to approximate both ANI (nucleotide k-mers) and Average Amino Acid Identity (AAI; protein k-mers), while accounting for the non-independence of mutated k-mers [19]. Furthermore, Scaled MinHash containment estimates work well for genome pairs of varying lengths and for compositional analysis of metagenome samples. Taken together, these properties enable robust assembly and alignment-free pairwise relatedness estimation that can be used on sequences separated by a wide range of evolutionary distances. Here, we demonstrate that the utility of Scaled MinHash protein containment, both used directly and a an approximation of ANI and AAI, for taxonomic classification and phylogenomic reconstruction for species across the tree of life.

Notes

- AAI::phylogeny https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1236649/

Results

GTDB "Evolutionary Paths" Dataset

The Genome Taxonomy Database (GTDB) provides a genome-based taxonomy for bacterial and archaeal genomes [20]. To assess the utility of Scaled MinHash techniques across evolutionary distance, we generated a series of "evolutionary paths" from the set of 31k representative GTDB genomes. For each genus with at least two species clusters, one representative genome was randomly selected as a path "anchor" genome. To build the path, one additional genome was selected from the representative genomes matching the anchor's taxonomy at each higher taxonomic rank. Each path thus consisted of seven genomes: an anchor genome, a genome matching anchor taxonomy down to the genus level, one matching anchor taxonomy to the family level, one matching to the order level, and so on. This creates a gradient of similarity, where comparisons to the anchor genome range from genus-level to superkingdom-level. Path selection using the representative genomes in GTDB release 95 resulted in 2957 paths comprised of 6690 unique genomes. These paths include genome comparisons across 33 phyla (29 Bacteria, 4 Archaea), covering roughly a quarter of the 129 phyla (111 Bacteria, 18 Archaea) in GTDB release 95. While paths are limited to taxonomies with at least two GTDB representative genomes for each taxonomic rank, these paths provide a rich resource for comparisons at increasing evolutionary distances.

k-mer containment searches enable similarity detection at increased evolutionary distances (nucl AND protein)

(DNA vs Protein) - *(just containment, no ANI/AAI) - include dayhoff or just protein?*

K-mer analysis methods enable similarity detection as low as a single shared k-mer between divergent genomes. As a result, exact matching long nucleotide k-mers can be used for taxonomic classification between closely related genomes, including at the strain, species, and genus level (k-mer lengths 51, 31, and 21, respectively). At larger evolutionary distances, accumulated nucleotide divergence limits the utility of exact nucleotide k-mer matching.

As protein sequences are more conserved than their coding nucleotide sequences, exact matching of protein k-mers can detect sequence similarity in spite of synonymous nucleotide substitutions that may have accumulated across the two sequences.

Exact matching of k-mers has long been deemed a shortcoming for k-mer based analyses, limiting similarity detection power across larger evolutionary distances. However, protein k-mers (and k-mers leveraging reduced protein alphabets)

Using nucleotide k-mersThis property allows for low-level homology detection at the n

Protein sequences are more conserved than their underlying DNA sequences. Whole-proteome MinHash sketches are more similar than whole-genome DNA sketches, enabling us to find protein-level similarity across divergent genomes.

For , e.g. Pseudomonas, XX% of k-mers are shared within the chosen/published genomes within species. For all published genomes within the genus, a median of xx% of k-mers are shared between genomes of one species and genomes of the a different species in the same genus.

rankinfo ... at ksize of 10... -xx% of DNA k-mers are shared within-species -yy% of protein k-mers are shared within-species - zz% of DNA k-mers are shared within-genus ... etc == median or mean containment at rank? containent = % of a genome's k-mers that are shared – do using ALL of gtdb, BUT, start with just a single set of genomes.. e.g. Pseudomonas? == similar to "shared k-mers" paper [21]
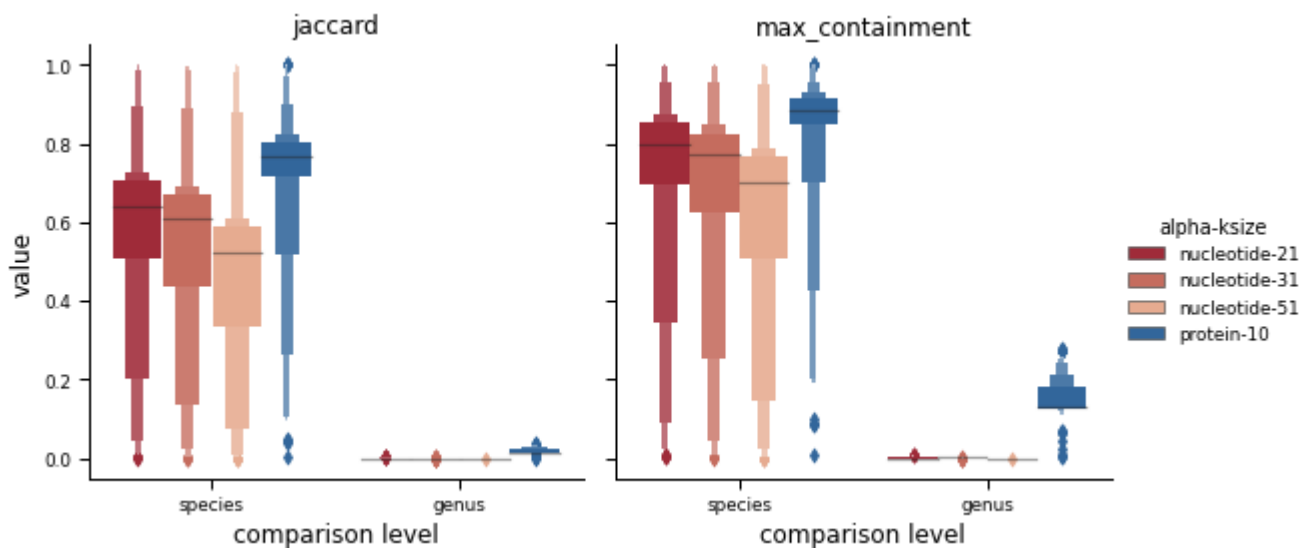
Figure 1: **Protein k-mer containment facilitates genus-level comparisons** 10k pseudomonas genome sequences, median containment at each alphabet

k-size selection for optimal comparisons / distance estimation

- num shared k-mers at different ksizes
- e.g. k=7 much more common – share far more k-mers. I assumed this would hurt, rather than help classification. Check!
- do rankinfo on each database??

because kmer size matters –> conversion to AAI is useful!? conversion to AAI does two things: accounts for k-mer length, …
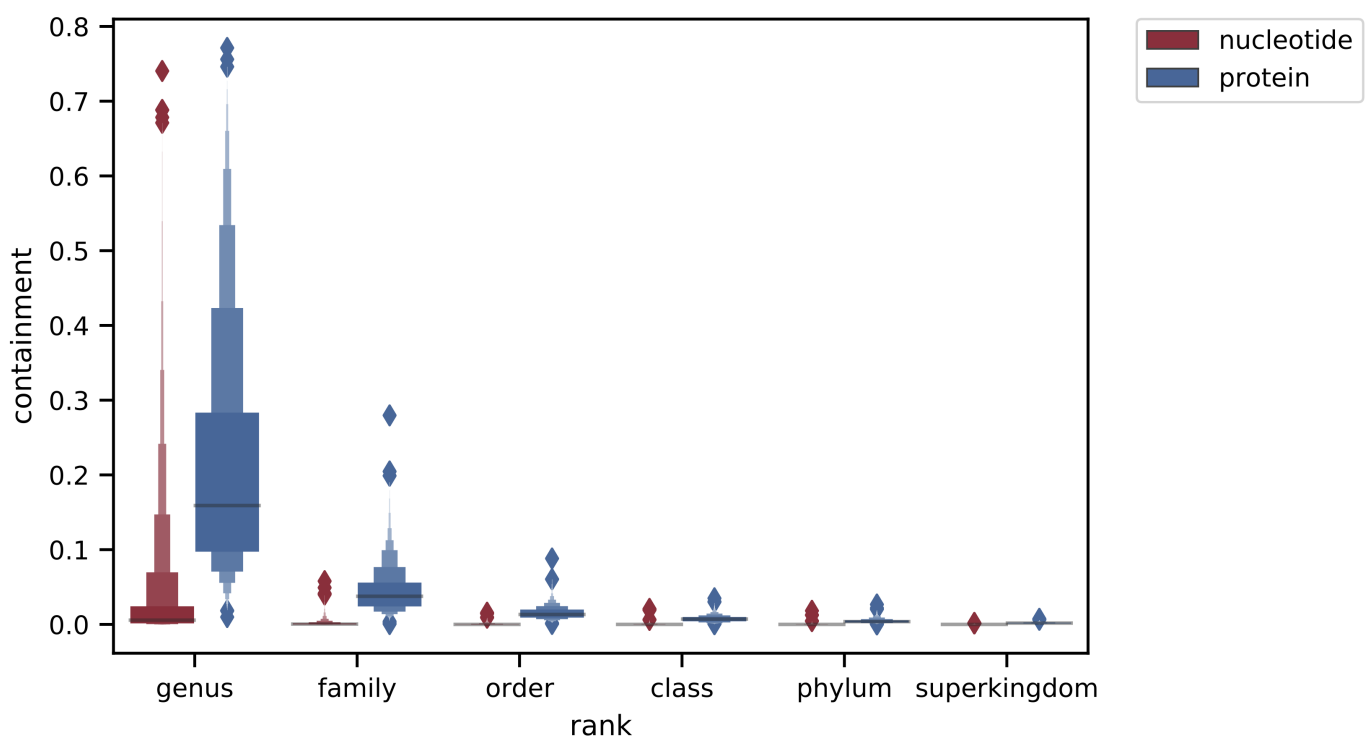
Figure 2: **Protein k-mers facilitate comparisons across species** This currently uses the evolutionary paths dataset. Perhaps better to demonstrate with a different test set – say, just the species, genus family level, using something like Pseudomonas that has a lot of published genomes. Also show jaccard to emphasize how it gets progressively worse when you start comparing genomes that are different sizes? Or separate figure for this...?
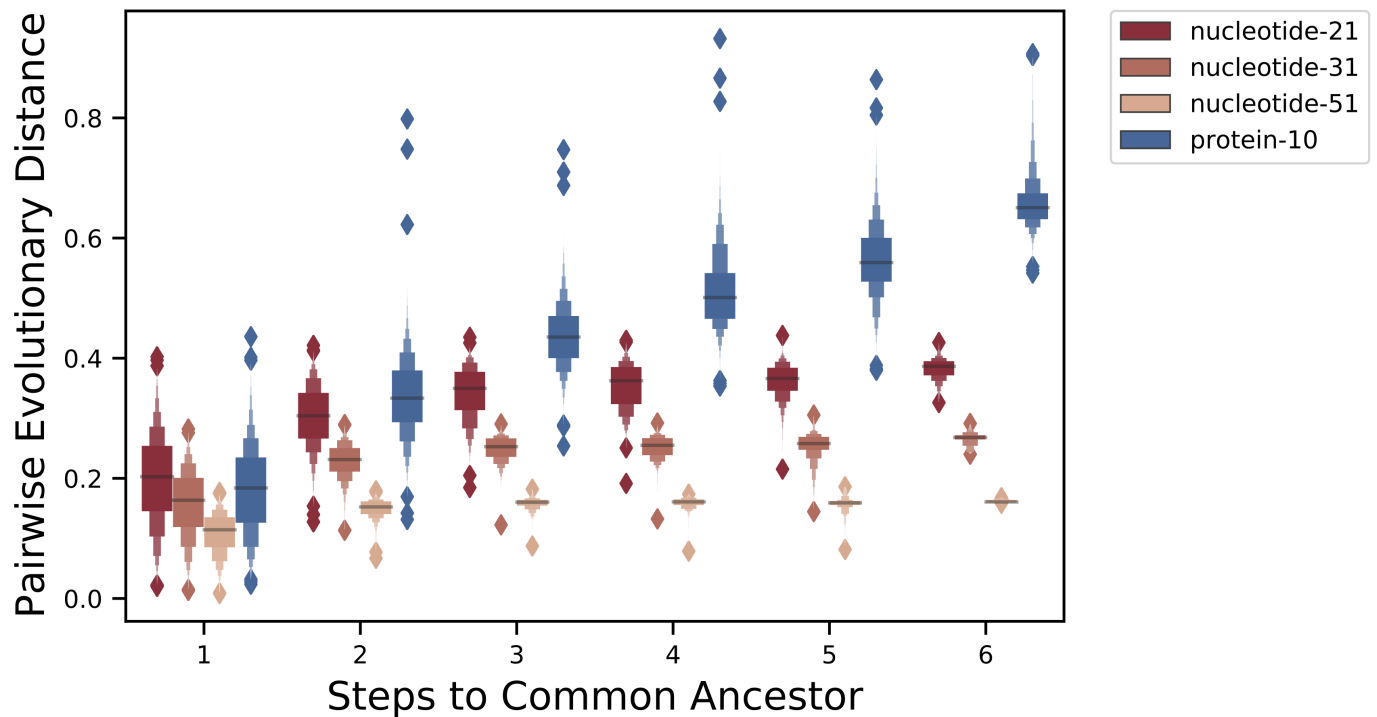
Figure 3: **Containment-based ANI, AAI estimates, evolpaths**

```
** core vs accessory distances **
ANI/AAI == really getting at _core_
```

Scaled Minhash distance estimation is robust to completeness

(unlike standard minhash
https://drep.readthedocs.io/en/latest/choosing_parameters.html#importance-of-genome-completeness)

Protein containment searches enable Sensitive/fast/accurate taxonomic classification

(just containment, no ANI/AAI)

to do, classification: 0. fix thumper (refactor branch) –> working + tests 1. implement "leave one xx clade out classification check" –> instead of just ignoring exact matches, ignore any matches in same species/genus/family 2. prelim figure for tara classification vs GTDB-Tk vs BAT - classification of incomplete genomes - for "contaminated" genomes .. can we randomly add contigs from diff species, see the impact? Like classification still works until xx% contaminated with something present in our database? 3. ksize diffs for classification? k=7 vs k=10 vs k=11? - time, sensitivity, specificity 4. virus development! (could be separate paper)

While more protein k-mers are shared across genomes within the same genus (and different species), min-set-cov + LCA allows us to find/report the most similar genome.

benchmarking :: Leave one out classification

leave one clade out version? see CAT/BAT paper)

[protein vs dna]
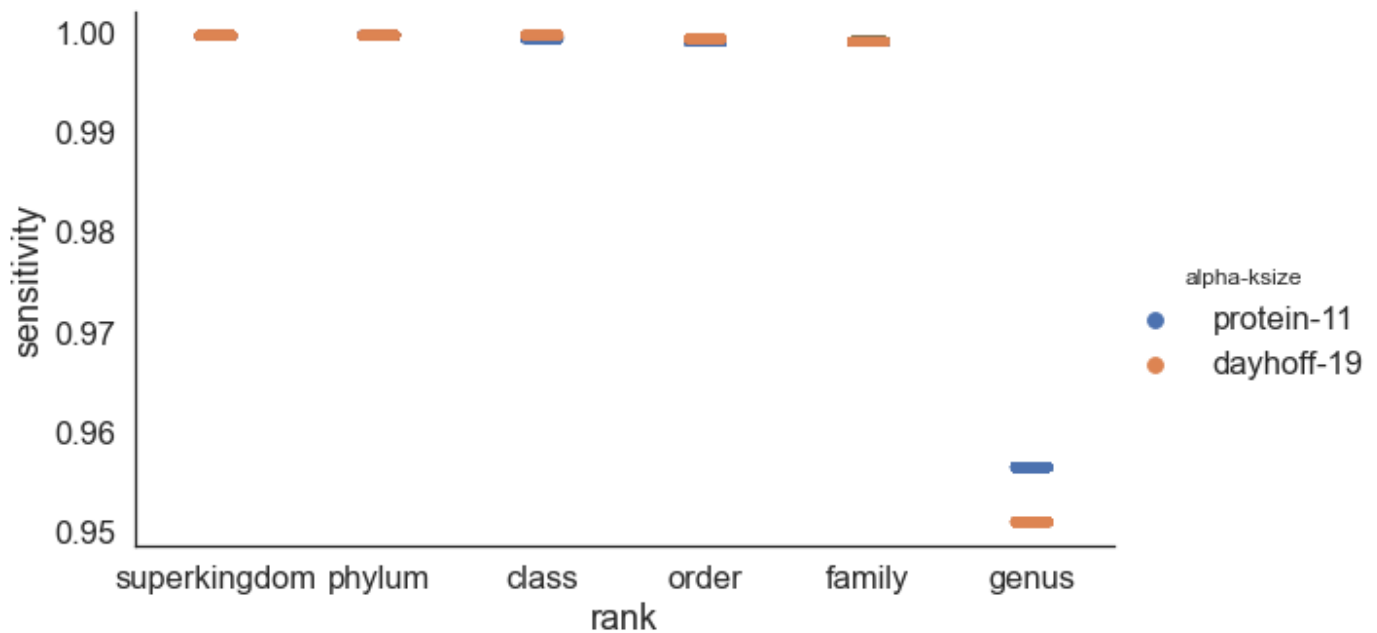
Figure 4: Protein classification sensitivity

include 6-frame translation works well for database search (sensitivity/specificity of Prodigal-translated vs 6-frame translated)

CAT/BAT paper [22] ("cat" = contig annotation, "bat" = bin annotation)

main point: more k-mers are shared = more k-mers available for matching

# Classification of incomplete and contaminated genomes

virus classification

euk classification?? Too much.

Containment-AAI enable alignment-free phylogenomic reconstruction

evolpaths analysis

Containment searches enable similiarity estimation, especially between genomes of different lengths.

Max containment normalizes the shared content by the smaller of the two genomes
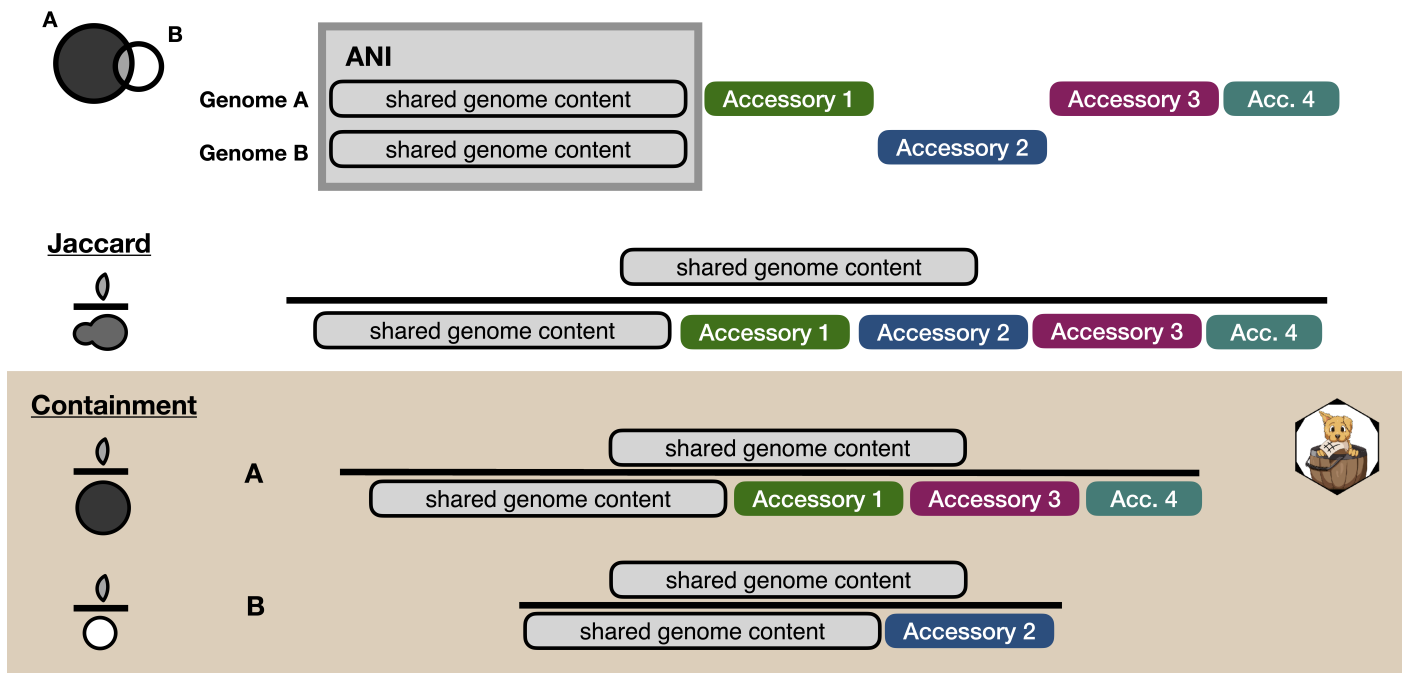
Figure 5: Max Containment to ANI and AAI. Containment calculation is guaranteed to be more similar to traditional calculation of Average Nucleotide Identity and Average Amino Acid Identity, which compared only the sections of genome that align. The shared k-mer content (containment numerator) can be thought of as the alignable sections of the genomes. The denominator of the Jaccard index is the alignable sections + the unalignable sections. The lower bound of the containment denominator will be the exact same as the numerator at 100% containment, where all k-mers are found within the comparison dataset. The upper bound will be the same as the Jaccard denominator, where all k-mers of the comparison dataset are found within the query dataset, and it is the query that contains any additional nonshared k-mers/unalignable sequence.

median AAI across GTDB?

alphabet and k-size selection for optimal distance estimation

- num shared k-mers at different ksizes
- e.g. k=7 much more common – do rankinfo on each database!

Comparison with other alignment-free methods (advantages, disadvantages, etc)

Alignment-based metrics are looking at the specific sequence variation of aligned regions, while k-mer based comparisons are comparing shared k-mers vs distinct k-mers. Since each nucleotide polymorphims generates mutated k-mers with an expected frequency, k-mer containment estimates can be used to accurately estimate both the Average Nucleotide Identity and Average Amino Acid Identity [1,19]

Discussion

Comparison with other alignment-free methods (advantages, disadvantages, etc)

Conclusions

Methods

Availability of data and materials

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Author	Contributions
NTP	Conceptualization; Methodology; Writing - Original Draft; Writing - Review and Editing; Visualization; Supervision; Funding Acquisition
…	…
…	…
…	…
CTB	Conceptualization; Methodology; Writing - Original Draft; Writing - Review and Editing; Visualization; Supervision; Funding Acquisition

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