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ABSTRACT

Comprehensive collections approaching millions of sequenced genomes have become central information sources in the life sciences. However, the rapid growth of these collections makes it effectively impossible to search these data using tools such as BLAST and its successors. Here, we present a technique called phylogenetic compression, which uses evolutionary history to guide compression and efficiently search large collections of microbial genomes using existing algorithms and data structures. We show that, when applied to modern diverse collections approaching millions of genomes, lossless phylogenetic compression improves the compression ratios of assemblies, de Bruijn graphs, and *k*-mer indexes by one to two orders of magnitude. Additionally, we develop a pipeline for a BLAST-like search over these phylogeny-compressed reference data, and demonstrate it can align genes, plasmids, or entire sequencing experiments against all sequenced bacteria until 2019 on ordinary desktop computers within a few hours. Phylogenetic compression has broad applications in computational biology and may provide a fundamental design principle for future genomics infrastructure.

INTRODUCTION

The comprehensive collections of genomes have become an invaluable resource for research across life sciences. However, their exponential growth, exceeding improvements in computation, makes their storage, distribution, and analysis increasingly difficult [1]. As a consequence, traditional search approaches, such as the Basic Local Alignment Search Tool (BLAST) [2] and its successors, are becoming less effective with the available reference data, which poses a major challenge for organizations such as the National Center for Biotechnology Information (NCBI) or European Bioinformatics Institute (EBI) in maintaining the searchability of their repositories.

The key to achieving search scalability are compressive approaches that aim to store and analyze genomes directly in the compressed domain [3,4]. Genomic data have low fractal dimension and entropy [5], which guarantees the existence of efficient search algorithms [5]. However, despite the progress in compression-related areas of computer science [4–14], it remains a practical challenge to compute parsimonious compressed representations of the exponentially growing public genome collections, particularly in light of their heavily biased sampling.

Microbial collections are particularly difficult to compress due to the huge number and the exceptional levels of genetic diversity, which reflect the billions of years of evolution across the domain. Even though substantial efforts have been made to construct comprehensive collections of all sequenced microbial genomes, such as the 661k assembly collection [15] (661k pre-2019 bacteria) and the BIGSIdata de Bruijn graph collection [16] (448k de Bruijn graphs of all pre-2016 bacterial and viral raw sequence), the resulting data archives and indexes range from hundreds of gigabytes (661k) to tens of terabytes (BIGSIdata). This scale exceeds the bandwidth, storage, and data processing capacities of most users, making local computation on these data functionally impossible.

We reasoned that the redundancies among microbial genomes are efficiently predictable, as they reflect the underlying evolutionary and sampling processes. While genomes in nature can accumulate substantial diversity through vertical and horizontal mutational processes, this process is functionally sparse, and at the same time subjected to selective pressures and drift that limit their overall entropy. This is further limited by selective biases due to culture and research or clinical interests, resulting in sequencing efforts being predominantly focused on narrow subparts of the tree of life, associated with model organisms and human pathogens [15]. Importantly, such subtrees have been shown to be efficiently compressible when considered in isolation, as low-diversity groups of oversampled phylogenetically related genomes, such as isolates of the same species under epidemiological surveillance [17,18]. This suggests that the compression of comprehensive collections could be informed by their evolutionary

history. This would reduce the complex problem of general genome compression to a much more tractable problem of local compression of phylogenetically ordered genomes, identified for instance through phylogenetic trees.

Phylogenetic trees are effective at estimating the similarity and compressibility of microbial genomes and their data representations. The closer two genomes are within a phylogeny, the closer they are likely to be in terms of mathematical similarity measures, such as the edit distance or *k*-mer distances [19], and thus also more compressible. Importantly, this principle holds not only for genome assemblies, but also for their similarity-preserving representations, such as de Bruijn graphs or *k*-mer indexes [20]. Phylogenetic trees could be embedded into computational schemes in order to assort similar data together, as a preprocessing step for boosting local compressibility of data. The well-known Burrows-Wheeler Transform [21] has a similar purpose in a different context. Other related ideas have previously been used for scaling up metagenomic classification using taxonomic trees [22–25].

At present, the public version of BLAST is frequently used to identify the species of a given sequence by comparing it to exemplars, but it is impossible to align against *all* sequenced bacteria. Despite the increasing number of bacterial assemblies available in the NCBI repositories, the searchable fraction of bacteria is exponentially decreasing over time (**Fig. 1a**). This limits the ability of the research community to study bacteria in the context of their known diversity, as the gene content of different strains can vary substantially, and important hits can be missed due to the database being unrepresentative.

Here, we present a solution to the problem of searching vast libraries of microbial genomes: *phylogenetic compression*, a technique for an evolutionary-guided compression of arbitrarily sized microbial genome collections. We show that the underlying evolutionary structure of microbes can be efficiently approximated and used as a guide for existing compression and indexing tools. Phylogenetic compression can then be applied to collections of assemblies, de Bruijn graphs, and *k*-mer indexes, and can be run in parallel for efficient processing. The resulting compression yields benefits ranging from a quick download, through a reduction of Internet bandwidth and storage costs, to efficient search on personal computers. We show this by implementing BLAST-like search to all sequenced pre-2019 bacterial isolates, which allow us to align genes, plasmids, and sequencing reads on an ordinary laptop or desktop within a few hours, a task that was completely infeasible with previous techniques. Phylogenetic compression has wide applications in computational biology and may provide a fundamental design principle for future genomics infrastructure.

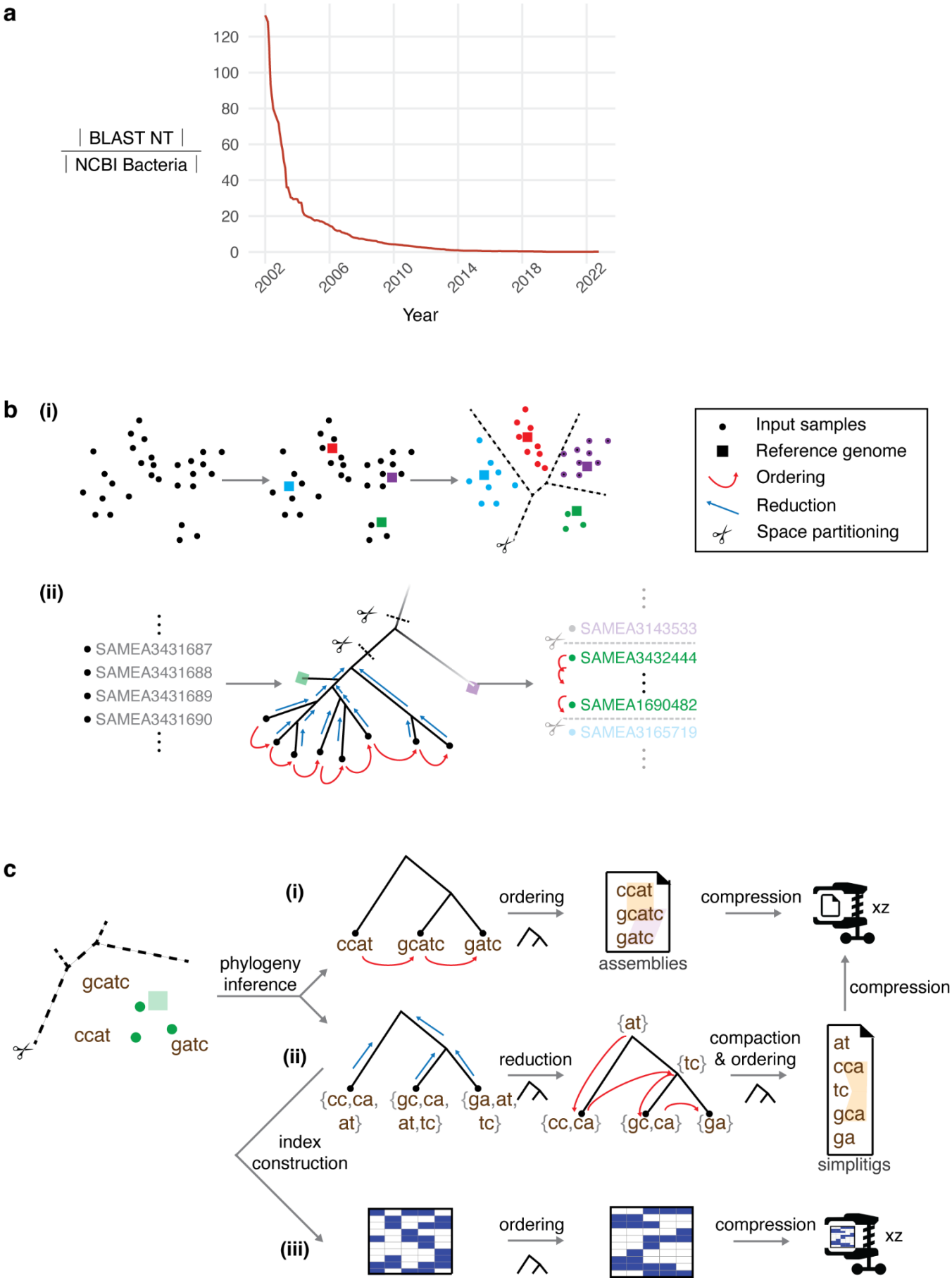

Fig. 1: Overview of phylogenetic compression and its applications to different data types. **a)** Exponential decrease of data searchability over the past two decades – the size of the BLAST NT database divided by the size of the NCBI Bacterial Assembly database, as a function of time (Methods). **b)** The first three stages of phylogenetic compression before the application of a low-level compressor. **(i)** Partitioning genome collection into size- and diversity-balanced batches using metagenomic classification. **(ii)** Reversible reordering of input data according to their phylogeny, applied per batch. **c)** Examples of specific protocols of phylogenetic compression for individual data types, applied per batch. **(i)** For assemblies, data are sorted left-to-right according to the phylogeny and then compressed using a low-level compressor such as XZ or MBGC [17]. **(ii)** For de Bruijn graphs, *k*-mers are propagated bottom-up along the phylogeny, the newly obtained *k*-mer sets compacted into simplitigs, and compressed using XZ. **(iii)** For BIGSI *k*-mer indexes, Bloom filters (in columns) are ordered left-to-right according to the phylogeny and then compressed using XZ.

RESULTS

We developed a technique called phylogenetic compression for evolutionarily informed compression and search of microbial collections (**Fig. 1**). Phylogenetic compression combines four ingredients (**Fig. 1b**): 1) *clustering* of samples into phylogenetically related groups, followed by 2) inference of a *compressive phylogeny* that acts as a template for 3) *data reordering*, prior to an 4) application of a calibrated *low-level compressor/indexer* (Methods). This general scheme can be instantiated to individual protocols for various data types as we show in **Fig. 1c**; for instance, a set of bacterial assemblies can be phylogenetically compressed by XZ (the Lempel-Ziv Markov-Chain Algorithm [7], implemented in XZ utils, https://tukaani.org/xz/) by a left-to-right enumeration of the assemblies, with respect to the topology of their compressive phylogeny obtained through sketching [26].

We implemented phylogenetic compression for assemblies, de Bruijn graphs, and *k*-mer indexes in a framework called Microbes on a Flash Drive (MOF, http://karel-brinda.github.io/mof). We build upon the empirical observation that microbial genomes in public repositories usually form clusters corresponding to individual species [27], which we identify for individual genomes via standard metagenomic classification [28] (**Fig. 1b**, Methods). As some of the resulting clusters may be too large or too small, and thus unbalancing downstream parallelization, we further redistribute the clustered genomes into size- and diversity-balanced batches (Methods, **Supplementary Fig. 1**). This batching

enables compression and search in a constant time (using one node per batch on a cluster) or linear time (using a single machine) (Methods). For every batch, a compressive phylogeny is computed using Mashtree [26] and used for data reordering (Methods). Finally, the obtained reordered data are compressed per batch using particularly optimized XZ, and possibly further re-compressed or indexed using some general or specialized low-level tool, such as MBGC [17] or COBS [29] (Methods).

We calibrated and evaluated MOF using five microbial collections, selected as representatives of compression-related tradeoffs between characteristics including data quality, genetic diversity, genome size, and collection size (Methods, **Supplementary Table 1**). We quantified the distribution of their underlying phylogenetic signal (Methods, **Supplementary Table 2, Supplementary Fig. 2**), used them to calibrate the individual steps of the phylogenetic compression workflow (Methods, **Supplementary Fig. 3, Supplementary Fig. 4, Supplementary Fig. 5**), and evaluated the resulting performance, tradeoffs, and extremal characteristics (Methods, **Supplementary Table 3, Supplementary Fig. 6**). For instance, we found that, as one extreme, 591k SARS-CoV-2 genomes can be phylogenetically compressed using XZ to only 18.1 bytes/genome (Methods, **Supplementary Table 3**, **Supplementary Fig. 4,6**), resulting in a file size of 10.7 Mb (13× more compressed than GZip).

We found that phylogenetic compression improved the compression of genome assembly collections that comprise hundreds of thousands of isolates of over 1,000 species, by more than one order of magnitude compared to the state-of-the-art (**Fig. 2a**, **Supplementary Table 3**). As specialized compressors of high efficiency such as MBGC [17] are not applicable to highly diverse collections, the compression protocols deployed in practice for extremely large and diverse collections are still based on the standard GZip. One example is provided by the 661k datasets, containing all bacteria pre-2019 from ENA (n=661,405), which occupies 805 GB on a public FTP [15]. Here, MOF recompressed the collection to 29.0 GB (impr. 27.8×; 43.8 KB/genome, 0.0898 bits/bp, 5.23 bits/distinct *k*-mer) using XZ as the low-level tool, and even more to 20.7 GB (impr. 38.9×; 31.3 KB/genome, 0.0642 bits/bp, 3.74 bits/distinct *k*-mer) when combined with MBGC [17] that also accounts for reverse complements (**Fig. 2a**, **Supplementary Table 3**, Methods). Additionally, we found that the lexicographically ordered ENA datasets, as being partially phylogenetically ordered, can be used as a first-order approximation of phylogenetic compression, with compression performance, degraded only by a factor of 4.17 compared to the full phylogenetic compression (**Supplementary Table 3**, Methods). Phylogenetic compression proceeded through several hundred batches of at most 4k genomes per batch (**Supplementary Fig. 1**). The resulting compressed files are provided for download from Zenodo (**Supplementary Table 4**).

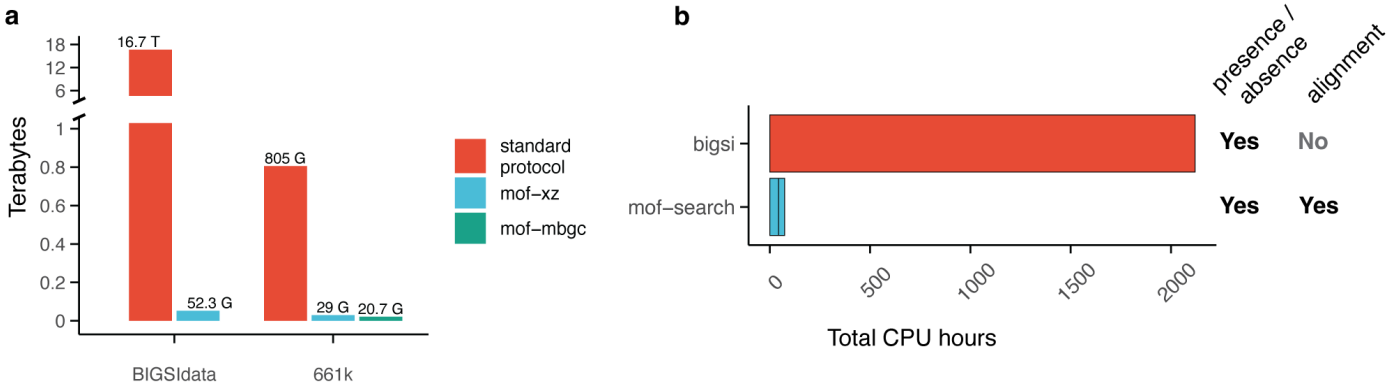

Fig. 2: Results of phylogenetic compression. a) Compression of the two comprehensive genome collections: BIGSI (425k de Bruijn graphs, the standard compression proceeds by McCortex binary files) and 661k (661k bacterial assemblies, the standard protocol is based on GZip). **b)** Comparison of the MOF vs. BIGSI methods on search of all plasmids from the EBI database. For MOF-Search, the split of the times of matching and alignment is denoted by a vertical bar.

We then studied the compression of de Bruijn graphs, which are a popular genome representation directly applicable to raw read data [16,30], and found that phylogenetic compression can improve state-of-the-art approaches by one to two orders of magnitude (**Fig. 2a**, **Supplementary Table 3,** Methods). As de Bruijn graphs lack practical methods for joint compression, single graphs are usually distributed individually [31]. For instance, the graphs of the BIGSIdata collection [16], comprising all viral and bacterial genomes from pre-2016 ENA (n=447,833), are provided in an online repository in the McCortex binary format [32] and occupy in total >16.7 TB (Methods). Here, we managed to retrieve n=425,160 graphs from the Internet (94.5% of the original count) (Methods) and losslessly recompressed them using the MOF methodology, with a bottom-up propagation of the *k*-mer content, to 52.3 GB (impr. 319×; 123. KB/genome, 0.166 bits/simplitig bp [33], 10.2 bits/distinct *k*-mer) (**Fig. 2a**, **Supplementary Table 3**, Methods). As recent advances in de Bruijn graph indexing [20] may lead to more efficient storage protocols in the future, we also compared MOF to MetaGraph [30], an optimized tool for indexing on high-performance servers with a large amount of memory. Here, we found that MOF still provided an improvement of nearly one order of magnitude (Methods).

Phylogenetic compression can be applied to any data structure as long as it is based on a similarity-preserving genome representation. We demonstrate this using the Bitsliced Genomic Signature Index (BIGSI) [16] (**Fig. 1c(iii)**), a *k*-mer indexing method using an array of Bloom filters, which is widely used

for large-scale genotyping and presence/absence queries of genomic elements [15,16]. Using the same data, batches, and orders as inferred previously, we phylogenetically compressed the BIGSI indexes of the 661k collection, computed using a modified version of COBS [29] (**Supplementary Table 5**, Methods). Phylogenetic compression provided an 8.51× overall improvement compared to the original index (from 937 GB to 110 GB), making it finally applicable on ordinary computers. Removing the low-quality genomes from the precomputed batches decreased the uncompressed index size by 4.9% (removing 3.7% of genomes, **Supplementary Fig. 7**), but the resulting phylogenetic compression improved to 12.3× (72.8 GB) (**Supplementary Table 5**).

We found that the most divergent genomes occupied 9.4× higher proportion of the database after compression, both for assemblies and COBS *k*-mer indexes (**Supplementary Fig. 8**). On the other hand, the top ten species (accounting for 80% of the genomic content) occupied less than half of the compressed database after compression. The remarkable similarity of the post-compression species ratios between assemblies and *k*-mer indexes suggests that compressibility is governed by the same rules, regardless of the specific data representation used, with divergent genomes as a major driver of the final size.

To demonstrate the utility of phylogenetic compression in practice, we implemented BLAST-like search across all pre-2019 bacteria for standard desktop and laptop computers (MOF-search, http://github.com/karel-brinda/mof-search). For a given batch of queries, MOF-search first filters reference genomes using phylogenetically compressed COBS *k*-mer indexes [29], and then computes alignment using Minimap 2 [34] while iterating over phylogenetically compressed genome assemblies (Methods). The tool choice was arbitrary, and other programs could readily be used instead. Despite the size of the original database, this resulted in total download and storage requirements of only 102 GB (195 KB/genome, 0.329 bits/bp, 23.0 bits/distinct *k*-mer) and memory requirements starting from 12 GB (user-specified) (**Supplementary Table 7**); therefore, the pipeline is deployable on all modern laptop and desktop computers.

We first evaluated MOF-search with 661k-HQ using three different types of queries - resistance genes (the ARG-ANNOT database of resistance genes [35], n=1,856), plasmids (EBI plasmid database, n=2,826), and a nanopore sequencing experiment (n=158,583 reads), and found consistent performance with results available within several hours (**Supplementary Table 2**). To benchmark against other tools, we were unable to find any tool capable of aligning queries to 661k-HQ in a comparable setup (excluding solutions based on an extensive parallelization on a compute cluster). We therefore used the EBI plasmid dataset to compare MOF-Search to BIGSI with its original database of 448k genomes (which is essentially a subset of the 661k-HQ) [16]. We found that MOF-search was over an order of magnitude faster

(**Fig. 2b, Supplementary Table 6**); the search required 74.1 CPU hours and provided an improvement in performance of a factor of 28.6× compared to the same BIGSI benchmark with its smaller database [16] (1.43× less genomes compared to 661k-HQ) (**Fig. 2b, Supplementary Table 6**), while providing the full alignments rather than presence/absence only (**Fig. 2b)**. This is to our knowledge the first time when alignment on this scale has been performed.

DISCUSSION

It is hard to overstate the impact on bioinformatics of BLAST [2], which has allowed biologists across the world to simply and rapidly compare their sequence of interest with essentially all known genomes – to the extent that the tool name has become a verb. The web version provided by NCBI/EBI is so standard that it is easy not to think how representative or complete its database is. However, twenty-three years on, sequencing data is far outstripping BLAST's ability to keep up, and in fact the publicly BLAST-able fraction of all sequenced microbes is shrinking exponentially (**Fig. 1a**). Much work has gone into approximate solutions [20], but full alignment to the complete corpus of bacterial genomes has remained completely impossible. We have addressed this problem and made significant progress, via phylogenetic compression, a highly efficient general technique using evolutionary history of microbes to improve existing algorithms and data structures. Performance of compression and search improves by one to two orders of magnitude. More concretely, BLAST-like search of all microbes moves from the impossible to the possible, not just for NCBI/EBI, but for anyone on their laptop. There are wide-ranging benefits, ranging from an easy and rapid download of large and diverse genome collections, through reductions in bandwidth, transmission/storage costs and computational time.

As with all compression, our capability to reduce data is fundamentally limited by the underlying information entropy. For genome collections, this is not just introduced by the underlying signal, but also tightly connected with the sequencing process and our ability to reconstruct the genomes from sequencing reads. The underlying *k*-mer histograms (**Supplementary Fig. 7**) suggest that any methods for compression or search will have to address noise in the form of contamination, missing regions, and technological artifacts, with legacy data being a major issue for both storage and analysis. Future methods may choose to incorporate stricter filtering, and as our experiments demonstrated, this will help not only to reduce the data volume, but also improve the quality of the search output. We note that this problem may be mitigated by novel computational approaches such as taxonomic filters [36] or sweep deconvolution [37].

Many elements of our approach have been used previously in other contexts. Reversible reordering for improving compression is in the core of the Burrows-Wheeler Transform [21] and its associated indexes [38,39], and it has also been used for read compression [40]. Tree hierarchies have been applied in metagenomics for lossy [22,23,41] and lossless [24] reference data compression. Finally, a divide-and-conquer methodology has been used for accelerating inference of species trees [42].

In the light of technological development, the benefits of phylogenetic compression will grow in time. Only a fraction of the world's microbial diversity has been sequenced, but as more is sequenced, the tree of life will not change, thus the relative advantage of phylogenetic compression will improve. We foresee its use from mobile devices to large distributed cloud environments, and anticipate promising applications in global epidemiological surveillance [43] and rapid diagnostics [44]. Overall, phylogenetic compression of data structures has broad applications across computational biology and provides a fundamental design principle for future genomics infrastructure.

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METHODS

Analysis of the decrease in bacteria BLAST searchability

BLAST NT size estimation. The estimates of the size of the BLAST NT database (n=27) for the time period between 2002-01-01 and 2022-11-01 were inferred from five types of online resources. First, most recent values were recorded manually from the file size reported on the official NCBI website https://ftp.ncbi.nih.gov/blast/db/FASTA/ (n=11, between 2020-04-05 and 2022-11-01); second, additional values were obtained from the snapshots of this website and its other NCBI mirrors on http://web.archive.org (n=7, between 2012-10-11 and 2022-06-06); third, the archived versions of the NT database at selected time points were found in online repositories (n=3, between 2017-10-26 and 2021-01-15); fourth, the size of the database was also captured in a software documentation (n=1, 2013-12-03); and fifth, the number of base pairs was also provided in scientific literature (n=5, between 2002-01-01 and 2010-01-01) (**Supplementary Table 6**). To convert the size of the NT database between the number of nucleotides and the size of the FASTA file after compressing using GZip; the compression ratio was estimated using the NT version from 2022-06-20 to be approximately 2.04 bits per bp.

NCBI Assembly DB size estimation. The number of bacteria in the NCBI Assembly database and their compressed size were estimated from the GenBank assembly summary file (https://ftp.ncbi.nlm.nih.gov/genomes/genbank/bacteria/assembly_summary.txt, downloaded on 2022-11-02, n=1,280,758 records), and subsequently sorted according to the 'seq_rel_date' field. The resulting file was used for calculating the number of published assemblies till a given date, further aggregated per month. The total size of the assemblies was estimated from the average size of genome assembly in the 661k collection, which is 3.90 Mbp, and the corresponding GZip size was estimated as previously. We note that updates in the assembly_summary.txt file could affect the old statistics, such as the removal of old contaminated records, but a manual inspection and comparison during a several-months-long period revealed that these changes have only a negligible impact on the resulting statistics.

Comparison. The BLAST scalability plot (**Fig 1a**) depicts the estimated size of the BLAST NT database (in the .fasta.gz format) divided by the estimated size of the bacteria in the NCBI Assembly Database [45] (https://www.ncbi.nlm.nih.gov/assembly/) (also in the .fasta.gz format) at the same time, as a function of time from 2002 to 2022. Both types of values, i.e., the sizes of the NT database and the bacteria in the NCBI Assembly database, were interpolated in the logarithmic scale by piecewise linear functions. The resulting interpolations were used for enumerating the estimated proportion of the sizes of NT and the bacteria in the NCBI Assembly database, by calculating their values at regular intervals (for each month).

Although the provided calculation may involve little inaccuracies (for instance, the average bacterial genome size or GZip compression ratio might differ for the NCBI Assembly database), this would have only negligible impact on the overall exponential decrease of data searchability. The resulting approximations were used for plotting **Fig 1a**.

Conceptual overview of phylogenetic compression

General overview. Phylogenetic compression is a general approach for compressing arbitrarily-sized genome collections and indexes and to search them. While the existing compression techniques excel in local compression, they struggle with widely distributed redundancies. As genomic data result from a superposition of evolutionary and sampling processes, genomic collections feature a tree-like geometrical structure reflecting vertical descent and partially confounded less frequent horizontal transfer. Reordering according to the tree topology co-localizes correlated information within the input data, and thus increases the local compressibility of data – consecutive genomes in phylogenetic orders will often be highly similar. To organize input genomes into phylogenetic trees in a scalable manner, phylogenetic compression combines four conceptual steps.

Step 1: Clustering/batching (Fig. 1b(i)). The goal of this step is to separate genomes into batches of phylogenetically related genomes of limited size and diversity that can be easily compressed and searched together. In downstream compression, indexing, and analyses, individual batches are processed individually, in separation, and the guarantees on the maximum batch size and diversity enable us to establish upper bounds on the maximum time and space necessary for processing a single batch. The clustering and batching is achieved via metagenomic classification [28]; it is known from the literature that microbial genomes in public repositories form distinct clusters, usually (but not always) corresponding to individual species [27] and metagenomic classification assigns genomes to individual clusters, defined by the genomes used in the corresponding reference database (e.g., RefSeq). Clusters of divergent genomes that are too small are put into a separate joint pseudo-cluster called dustbin. As some of the obtained (pseudo)-clusters can be too big (such as the clusters corresponding to oversampled species; e.g., *S. enterica*), they are further divided into smaller batches in a way that provides guarantees on downstream computational resources.

Step 2: Inference of a compressive phylogeny (Fig. 1b(ii)). The second step, already performed per individual batches of a limited size and diversity independently, consists in inferring a so-called *compressive phylogeny* that sufficiently approximates the true phylogenetic signal for compression purposes. While phylogenies computed using an accurate inference method such as BAPS [46] are

preferable, in most practical scenarios these are not available and would be too costly to compute or require particular adjustments for different species. In such cases, a rapidly estimated phylogeny, for instance using MashTree [26], is sufficient.

Step 3: Data reduction/reordering (Fig. 1b(ii)). The role of the computed compressive phylogeny is to act as a template for the reduction and re-ordering of input data according to their evolutionary history. This can have multiple different forms, based on the specific application and type of compression (e.g., lossy vs. lossless), and it can involve two directions. Either the collection/batch is only reordered left-to-right according to the topology of the compressive phylogeny, or the genomic data are propagated bottom-up along the phylogeny (i.e., shared genomic content is propagated up, and thus reduced, before the left-to-right enumeration is performed).

Step 4: Compression or indexing using a calibrated low-level tool (Fig. 1c). Once the data are reordered (and possibly reduced) using the compressive phylogenies, the last step is the final compression or indexing using a low-level tool that can exploit local redundancies in the data. At this stage, all the data are highly locally compressible thanks to both the phylogeny-based clustering and phylogeny-based reordering. Many general and specialized genome compressors are available and can be used at this step; however, it is important to ensure that the parameters of the underlying algorithms correspond to the characteristics of genome data; for instance, the window/dictionary of a Lempel-Ziv-based compressor needs to be sufficiently large to span multiple genomes and to store a sufficient amount of phrases (**Supplementary Fig. 3a**). General compressors usually need to be particularly tested and calibrated, whereas specialized compressors for genomes are usually calibrated by default. Furthermore, general compressors may require additional data re-formatting; for instance, for efficient multi-genome compression using general compressors, it is important to ensure that FASTA files have one sequence per one line (**Supplementary Fig. 3b**).

The Microbes on Flash Drive (MOF) workflow for phylogenetic compression

MOF implements several protocols of phylogenetic compression for compression of assemblies, de Bruijn graphs, and for search genome; more information and links can be found on the associated website (http://karel-brinda.github.io/mof).

Clustering/batching. As individual genome collections encountered in practice can have very different properties and associated data available, for the use with MOF the clustering and batching steps are expected to be performed by the user. The recommended procedure is to identify species using standard

metagenomic approaches, such as those implemented in the Kraken software suite [47] (e.g., Kraken 2 [48] and Bracken [49] applied on the original read sets) and divided into smaller batches analogically to the examples in **Supplementary Figure 1**. The protocol can be further customized based on the specific performance of algorithms downstream, e.g., by increasing/decreasing batch size or adjusting parameters for building dustbin batches. The clustering/batching step is not necessary if the number of genomes is sufficiently small (the order of thousands).

Inference of a compressive phylogeny. The user can either provide a custom tree, tailored for the specific collection/batch, such as a tree computed by RHierBAPS [46], or leave MOF-Compress to compute a compressive phylogeny by Mashtree [26], which is based on estimating *k*-mer-set Jaccard index using locality sensitive hashing using MinHash sketches [50] and estimating mutation rate under a simple evolutionary model [51] using the so-called Mash distance [50]; the obtained distances are then used for estimating the likely phylogeny using the Neighbor-Joining algorithm [52,53] as implemented in QuickTree [54].

MOF-Compress (http://github.com/karel-brinda/mof-compress)**.** This is a central package of MOF that performs phylogenetic compression of a single batch and calculates the associated statistics. It implements the following three protocols: 1) phylogenetic compression of assemblies based on a left-to-right reordering, 2) phylogenetic compression of de Bruijn graphs represented by simplitigs [33] based on the left-to-right reordering, and 3) phylogenetic compression of de Bruijn graphs using bottom-up *k*-mer propagation using ProPhyle [25,55]. The *k*-mer propagation proceeds recursively inside the compressive phylogeny in a bottom-up fashion – at every internal node, *k*-mer sets of the child nodes are loaded, their intersection computed, stored at the node, the intersection subtracted from the child nodes, and all three *k*-mer sets saved in the form of simplitigs. This progressively reduces the *k*-mer content within the phylogeny in a lossless fashion. More details on this technique can be found in ref [55]. In all three protocols, the output is a TAR file with ordered text files with sequences – for assemblies in the one-line FASTA format and for simplitigs in a text file with eol-separated simplitigs. The TAR file is subsequently compressed using XZ with the parameters 'xz -9 -T1' (see calibration). MOF-Compress also computes extensive statistics for all three protocols, including the size of the corresponding *k*-mer multiset, *k*-mer set, number of sequences, their cumulative length, and the resulting compressed sizes (see section Statistics). The output .tar.xz file from MOF-Compress can be used for additional recompression or indexing in the same order by other low-level tools.

MOF-Compress statistics. MOF-Compress computes a multitude of statistics characterizing the compressibility using the three implemented protocols, and these are further used for computing global statistics such as phylogeny-explained redundancy. For each of the three protocols, the following

559 statistics are calculated: set (the size of the *k*-mer set of all sequences), multiset (size of the *k*-mer
560 multiset of all sequences), sum_ns (number of sequences), and sum_cl (total sequence length), recs
561 (number of records), and xz_size (size after compression using XZ). The sizes of *k*-mer sets and multi-
562 sets are obtained from *k*-mer histograms computed by JellyFish 2 [56]. Based on these numbers the various
563 compression-related statistics used in this paper are computed, such as bits per distinct *k*-mer or
564 kilobytes per genome.
565
566 **Phylogeny-explained redundancy.** Comparing the sizes of *k*-mer sets and multisets before and after
567 reduction using *k*-mer propagation along phylogenies allows further quantification of the proportion of
568 the *k*-mer signal that is explained by a given compressive phylogeny. The so-called *removed k-mer*
569 *redundancy* quantifies the proportion of *k*-mer occurrences that were removed by *k*-mer propagation out
570 of those that could be removed if the phylogeny perfectly explained the distribution of *k*-mers (i.e., every
571 *k*-mer occurring only once after propagation), and the corresponding formula is
572
573 removed_redundancy = (|multiset_preprop|-|multiset_postprop|)/(|multiset_preprop|-|set|)
574
575 **MOF-COBS-Build.** MOF-COBS-build (https://github.com/leoisl/mof-cobs-build) is a pipeline that can
576 be appended to MOF-Compress for constructing phylogenetically compressed ClaBS COBS indexes
577 (Classical Bit-sliced index) for creating XZ-compressed COBS indexes from batches of phylogenetically-
578 ordered samples. ClaBS is a mode of COBS that is conceptually analogous to the original BIGSI data
579 structure [16], using Bloom filters of the same size, which is a key property that guarantees that Bloom
580 filters of phylogenetically close datasets are mutually compressible (different sizes of Bloom filters would
581 shift bits corresponding to the same *k*-mers to different positions). MOF-COBS-build is built as a
582 Snakemake [57] workflow reading two directories: the first describes the sample batches as well as their
583 ordering (in detail, this directory contains a list of text files with each such file listing the samples in the
584 batch and their ordering, with one sample name per line). The second is a directory with the assemblies
585 themselves. The workflow comprises four main steps: 1) creating groups of assemblies in the order
586 specified by the input (in details this is done by creating a directory with symbolic links to the original
587 assemblies, with these links having artificial names sorted by their phylogenetically order, forcing COBS
588 to process them in such order); 2) building COBS classic indexes by “cobs classic-construct -T 8 {batch}
589 {output}.cobs_classic”; 3) compressing the COBS classic indexes with “xz -9 -T1 -e -k -c --
590 lzma2=preset=9,dict=1500MiB,nice=250”; 4) combining all compressed indexes into a single TAR file
591 that can be further used for distribution.
592
593

Overview of the five test microbial collections

GISP. The collection consists of 1,102 draft assemblies constructed from clinical isolates of *N. gonorrhoeae* collected in the US from 2000 to 2013 by the Centers for Disease Control and Prevention within the Gonococcal Isolate Surveillance Project [58]; the isolates were previously sequenced using Illumina HiSeq and assembled using Velvet [59]. The collection presents a model of high-quality genomic data from a low-diversity species sequenced and assembled using identical protocols.

NCTC3k. The collection consists of 1,065 draft and complete assemblies constructed from strains from the National Collection of Type Cultures (NCTC) collection, analyzed by Public Health England, the Wellcome Trust Sanger Institute, and Pacific Biosciences within the NCTC 3000 project; the isolates were sequenced using the PacBio Single Molecule, Real-Time (SMRT) DNA Sequencing technology, assembled using automated pipelines, and are provided online through the https://www.sanger.ac.uk/resources/downloads/bacteria/nctc/ website. The collection presents a model of nearly complete high-quality genomes of diverse species.

SC2. The collection is a snapshot of the GISAID database [60] from 2021-05-18 of 590,779 SARS-CoV-2 isolates (complete assemblies) with a known phylogeny and available complete genomic sequences, collected and sequenced from 2020 to 2021 by various laboratories, and provided online through https://gisaid.org/ and analyzed using the sarscov2phylo software (https://github.com/roblanf/sarscov2phylo/, ref [61]). The collection presents a model of a large number of genomes of varying quality from epidemiological surveillance of a single species collected across the globe.

BIGSIdata. The BIGSIdata collection is a snapshot of bacterial and viral isolates present in the European Nucleotide Archive (ENA) on December 2016 as published in ref [16], consisting of 425,160 cleaned de Bruijn graphs (k=31) that we managed to download from the associated FTP website (http://ftp.ebi.ac.uk/pub/software/bigsi/nat_biotech_2018, out of the original 447,833 that were mentioned in ref [16]); the isolates had originally been collected and sequenced using various laboratories, deposited to some repository that is synchronized with ENA (i.e., ENA, NCBI SRA, or DDBJ Sequence Read Archive), downloaded and transformed into cleaned de Bruijn graphs using McCortex [32,62] by the European Bioinformatics Institute (EBI) and provided on the FTP website together with metadata on Figshare [63]. The collection presents a model of a large number of microbial isolates collected and sequenced across the globe using various sequencing technologies that are represented in a searchable representation other than genome assembly.

661k. This collection is an assembled snapshot [15] (draft assemblies) of all 661,405 Illumina-sequenced bacterial isolates present in the ENA on 2018-11-26; the isolates had originally been collected and sequenced using various laboratories, deposited to some repository that is synchronized with ENA (i.e., ENA, NCBI SRA, or DDBJ Sequence Read Archive), downloaded and assembled using a single unified pipeline (https://github.com/iqbal-lab-org/assemble-all-ena) based on Shovill (https://github.com/tseemann/shovill) by the European Bioinformatics Institute (EBI), and provided on FigShare [64] (metadata) on FTP (https://ftp.ebi.ac.uk/pub/databases/ENA2018-bacteria-661k/, assemblies). The collection presents a model of a large number of assembled microbial isolates collected and sequenced across the globe using a single sequencing technology, i.e., the state-of-the-art of the short read-assembly era.

Basic characteristics of the test collections, including the size of the original files, the number of samples, as well as the number of species, and the number of distinct *k*-mers are provided in **Supplementary Table 1**.

Acquisition of the test collections

BIGSIdata. The files corresponding to individual samples of the collection [16] were downloaded from the associated FTP (http://ftp.ebi.ac.uk/pub/software/bigsi/nat_biotech_2018/), including cleaned de Bruijn graphs, and taxonomic information as inferred using metagenomic classification using Kraken [22] and abundance reports computed using Bracken [49]. The download was done in groups corresponding to individual EBI prefixes (e.g., DRR000) using RSync by

```
rsync -avP --min-size=1 --exclude '*stats*' --exclude '*uncleaned*' --exclude '*bloom*' --exclude
'*log* "rsync://ftp.ebi.ac.uk/pub/software/bigsi/nat_biotech_2018/ctx/${prefix}"
```

The prefixes were further organized into batches by 100, which resulted in 15 batches in total. The batches were processed sequentially, and the individual contained groups were downloaded on a research computing cluster in parallel using Slurm, with jobs deployed using Snakemake [57] (between 2020-08-01 and 2020-09-15). From the downloaded McCortex files, unitigs were extracted using McCortex ("mccortex31 unitigs -m 3G -") and stored locally, after which the McCortex files were deleted. Only graphs with unitigs of length at least 2 kbp, with less then 15 M *k*-mers (to remove contaminated datasets), and without any file system errors were used in the subsequent processing. This resulted in n=425,161 de Bruijn graphs (out of the original 463,331 files) that were used in the subsequent analyses.

661k. All assemblies were retrieved in March 2022 from the official FTP repository provided in ref[15], by running

rsync -avp rsync://ftp.ebi.ac.uk/pub/databases/ENA2018-bacteria-661k/Assemblies/{pref}

for individual prefixes ranging from 000 to 661. This resulted in n=661,405 .fa.gz files, occupying in total 805,255,364,491 bytes (i.e., 805 GB).

GISP. The GISP collection was obtained from the https://github.com/c2-d2/rase-db-ngonorrhoeae-gisp, published in ref [44]; the original data were originally analyzed in ref [58] and the resulting data later provided for download also on Zenodo [65]. The GISP assemblies (n=1,102) were obtained from the "isolates/contigs" subdirectory of Github repository, and the associated phylogenetic tree, computed using BAPS [66] (Bayesian Analysis of Population Structure) after correction for recombination using Gubbins[67], downloaded from the "tree/" subdirectory of the same repository.

SC2. The following SARS-Cov-2 data were downloaded from the GISAID website (https://www.gisaid.org/, as of 2021-05-18): an assembly file (sequences_fasta_2021_05_18.tar.xz, n=1,593,858) and a Sarscov2phylo phylogeny[68] data file (gisaid-hcov-19-phylogeny-2021-05-11.zip, n=590,952). Both datasets were converted to the same set of identifiers, and isolates with missing data discarded. This resulted in 590,779 genomes accompanied with their corresponding phylogenetic tree (**Tab. 2**; the SC2 collection). Out of the downloaded 1,593,858 sequences that were available in May 2021, we first extracted those with known phylogenetic position within the global Sarscov2phylo phylogeny [68]; this resulted in 590,779 genomes accompanied with their corresponding phylogenetic tree (**Tab. 2**; the SC2 collection) (as of May 2021; n=590,779 sequences with phylogenetic information out of the total of 1,593,858; Methods)

NCTC3k. The assemblies were downloaded in the GFF format using FTP from ftp://ftp.sanger.ac.uk/pub/project/pathogens/NCTC3000 by

wget -m -np -nH --cut-dirs 3 --retr-symlinks ftp://ftp.sanger.ac.uk/pub/project/pathogens/NCTC3000 .

converted them to the FASTA format by any2fasta (https://github.com/tseemann/any2fasta, v0.4.2) parallelized by GNU Parallel [69], and finally uploaded to Zenodo (http://doi.org/10.5281/zenodo.4838517). Species were counted based on the data in the main Sanger/Public Health England assembly table for NCTC3000 as provided online (https://www.sanger.ac.uk/resources/downloads/bacteria/nctc/, retrieved on 2022-09-14). The HTML table was first manually exported to the XLSX, and then used for constructing a translation table from

NCTC accession numbers to the corresponding species. Finally, the accessions of the assemblies contained in our collection were extracted from file names, translated to species, and the species counted. Overall, this resulted in 1,065 assemblies of 259 species.

Calibration and evaluation of phylogenetic compression using the test collections

For calibration, two collections from epidemiological surveillance (SC2 and GISP) were used to model similar genomes, and one additional high-diversity isolate collection (NCTC3k) was used to model divergent genomes (**Supplementary Tab. 1**). These three collections were used for calibrating and comparing individual low-level compressors, as well as for evaluating the compressibility of the datasets (**Supplementary Fig. 3–5**).

Calibration of the XZ compressor (Supplementary Fig. 3). The comparison was performed using the GISP collection and the GZip, BZ2, and XZ low-level compressors with range of their presets, and in the case of XZ with only 1 thread. For the right panel, line length was progressively modified using seqtk seq (the "-l" parameter), the collection recompressed, and all the final results compared in function of line length.

Comparison of scaling modes (Supplementary Fig. 4). The SC2 collection was provided in the left-to-right order according to the topology of the phylogeny and compressed, with genomes being progressively subsampled. The compression methods in this experiment included XZ ("xz -9 -T1"), BZip2 ("bzip2 --best"), GZip ("gzip -9"), and Re-Pair [70,71] (implementation from https://github.com/rwanwork/Re-Pair, "repair -v -i", version "Oct 26 2021"). As Re-Pair was only little scalable and suffered from various technical issues, the integrity of the output files was always verified via their decompression. The comparison for the NCTC3k collection was done analogically via MOF-Compress with individual subsampling, and additional re-recompression using GZip and BZip2 with the same parameters as previously. In the case of the SC2 collection, sequence names were not included in the benchmark to their long names given the short genomes.

Order comparison (Supplementary Fig. 5). For SC2, the isolates with phylogenetic information (n=590,779) were used for the compression analysis using three orderings: the original ordering (corresponding to the lexicographical ordering by sequence names), the left-to-right ordering of the phylogeny, and a randomized order. In all cases, a custom Python script using BioPython [72] was used to order the FASTA file and remove sequence names, and its output was compressed by the XZ compressor

using 1 thread and maximum compression ('xz -T1 -9'), and the sizes of the resulting files measured using wc ('wc -c'). The comparisons for GISP and NCTC3k was performed analogically.

Summary of the findings. The most popular method, GZip, always performed poorly for bacteria, but provided a moderate scaling for viruses. Stronger compressors such XZ achieved steep compression curves for high-diversity collections, with compression ratio improving by one order per one order increase of #genomes, for both viruses and bacteria. On the other hand, NCTC3k was little compressible even with the best approaches (<1 order of magnitude of compression after a 3 orders-of-magnitude increase of #genomes), indicative of that divergent genomes is the fundamental compression bottleneck within comprehensive collections. Finally, we compared the best experimental grammar-based compressor (Re-Pair [70,71]) to XZ, and found they achieved similar asymptotics, suggesting the potential of grammar compression for phylogenetic compression. As for the orders, we found that phylogeny reordering always boosted compression (reduction to 38%–67% compared to the random order), for both low- and high-diversity collections. We also found that trees computed using rapid heuristics (MashTree [26]) performed nearly as well as an accurate Bayesian approach (Bayesian Analysis of Population Structure [46]). Overall, based on the observed tradeoffs, we selected "xz -9 -T1" as the compression procedure for MOF-Search and Mashtree as a sufficiently accurate method for generating compressive phylogenies.

Phylogenetic compression of the BIGSIdata collection of de Bruijn graphs

Clustering and batching. For every sample, the output of Kraken [22] and Bracken [49] were extracted from the downloaded data. Clusters were then defined based on the most prevalent species in a sample, as identified in the corresponding Bracken report and batching proceeded as depicted in **Supplementary Fig. 1**. The genomes of the 1,443 identified species (clusters) were redistributed into 568 regular batches and 6 dustbin batches, resulting in a total of 574 batches.

Phylogenetic compression. Phylogenetic compression first proceeded through a workflow that later resulted in MOF-Compress For individual batches, compressive phylogenies were computed using Mashtree with the default parameters. The resulting trees were then used with ProPhyle and unitig files to propagate *k*-mers along the compressive phylogenies and to compute simplitigs using ProphAsm [33]. After the resulting files were ordered and compressed by XZ ("xz -v -z -9 -T8 --stdout"), the resulting files (occupying 74.4 GB) were deposited on https://doi.org/10.5281/zenodo.4086456 and https://doi.org/10.5281/zenodo.4087330. Furthermore, an analogical version of the propagated simplitig files, but without sequence headers and with compression using a single thread only, was later

771 created using the MOF compress pipeline and resulted in files occupying in total 52.3 GB that were
772 subsequently deposited on https://doi.org/10.5281/zenodo.5555253.

773 **Phylogenetic decompression.** To decompress the files obtained through phylogenetic compression
774 based on *k*-mer propagation back to the original de Bruijn graphs, the original graphs need to be
775 reconstructed by collecting all *k*-mers along root-to-leaf paths, which we implemented a program called
776 MOF-Client (https://github.com/karel-brinda/mof-client). The program downloads individual data files
777 from Zenodo from the accessions above and decompresses them using the following procedure. For it
778 decompresses the XZ file of a given batch, splits it according to files corresponding to individual nodes of
779 the compressive phylogeny, recompressed individual nodes using GZip parallelized using GNU
780 Parallel [69], and for all leaves (genomes) it collects the corresponding *k*-mer sets from by merging all GZip
781 files along the corresponding root-to-leaf paths using the Unix cat command. The correctness of the
782 resulting files was confirmed using JellyFish [56].

783 **Comparison to the original compression.** As the samples in our BIGSIdata collection do not fully
784 correspond to the selected data used in the original publication [16], we calculated the original size of the
785 published McCortex files of our graphs based on the FTP listoff files as provided within individual
786 subdirectories of http://ftp.ebi.ac.uk/pub/software/bigsi/nat_biotech_2018/ (as of 2021-08-27). These
787 were downloaded per individual prefix directories recursively using wget by

788 *wget -nv -e robots=off -np -r -A .html*
789 *"http://ftp.ebi.ac.uk/pub/software/bigsi/nat_biotech_2018/ctx/${prefix}/",*

790 The corresponding parallelized Snakemake pipeline was run on a desktop computer. This resulted in a
791 table containing 484,463 files, out of which 162,645 had a bz2 suffix. The individual file records were
792 compared with the list of accessions of files that were previously retrieved and sorted in our BIGSIdata
793 collection, and the volume of the source graphs on FTP calculated to be 16.7 TB.

794 **Comparison to Metagraph** [30]. The size of the phylogenetically compression BIGSIdata collection was
795 compared to the size of an analogical Metagraph index from the original paper [30] based on the statistics
796 in Table 1 and Supplementary Table 1 (SRA-Microbe collection): n=446,506 indexed datasets, 39.5 G
797 canonical *k*-mers (with the same *k*-mer size *k*=31), and the size of the annotated de Bruijn graph being
798 291 GB (graph 30 GB + annotations 261 GB). This index was constructed from the same data as in the
799 original BIGSI paper [16], but using a slightly different computational methodology. In consequence, the
800 index of Metagraph contained a by 4% lower number of distinct *k*-mers compared to BIGSIdata as
801 constructed in this paper, indicative of either lower diversity of the samples included or of their
802 additional cleaning. To compare the two compression approaches (MOF with bottom-up *k*-mer
803 propagation and XZ as a low-level tool vs. SRA-Microbe compressed using Metagraph), both applied to
804 the similar but different input data, we used the number of bits per distinct *k*-mer as the statistics to

compare, which was found to be 10.2 and 58.9, respectively, Therefore, the MOF compression was more efficient by an estimated factor of 5.78, but this number might be underestimated due to a different noise level. We note that phylogenetic compression could be directly embedded into Metagraph in the future, which may help to reduce the size of its index substantially.

Phylogenetic compression of the 661k assembly collection

Batches. Clusters were identified based on the species identified using Kraken 2 [48] + Bracken [49], as provided in the supplementary materials in ref [15] (the File1_full_krakenbracken.txt file, the V2 column), and further split into batches as displayed in **Supplementary Fig. 1**.

Phylogenetic compression using MOF-Compress. The individual batches of the collection were compressed using the MOF-Compress pipeline, compressive phylogenies computed using MashTree [26], left-to-right reordering of the assemblies, left-to-right re-ordering of simplitigs of the de Bruijn graphs, bottom-up *k*-mer propagation and simplitig computation by ProPhyle, and storing simplitigs and assemblies as text and FASTA file, respectively, followed by a compression by 'xz -9 -T1'. The resulting files were deposited on https://doi.org/10.5281/zenodo.4602622.

Calculations of the statistics. All the statistics used in the plots and tables were calculated based on the numbers obtained from MOF-Compress. Additionally, the total number of *k*-mers was calculated using JellyFish [56] (v2.2.10) by

> *jellyfish count --mer-len 31 --size 200G --threads 32 --output kmer_counting.jf --out-counter-len=1 --canonical*

which resulted in 44,349,827,744 distinct *k*-mers (28,706,296,898 unique *k*-mers) for the 661k collection and in 35,524,194,027 distinct *k*-mers (22,904,412,202 unique *k*-mers) for the 661k-HQ collection. We note that the files uploaded to https://doi.org/10.5281/zenodo.4602622 are higher by approximately 0.2 GB (approx. 0.7% of the total size) compared to the value **Supplementary Table 3** as the Zenodo submission was done with an older version of the pipeline with slightly different trees.

Recompression using MBGC. Individual phylogenetically compressed batches from the previous step were converted to single FASTA files by '*tar -xOvf {input.xz}*' and then compressed using MBGC v1.2.1 with 8 threads and the maximum compression level (3) by '*mbgc -i {input.fa} -c 3 -t 8 {output.mbgc}*'. This resulted in files occupying in total 20,726,725,129 bytes, which were then uploaded to Zenodo (https://doi.org/10.5281/zenodo.6347064).

Compression in the lexicographic order. As data in ENA and other similar repositories have identifiers assigned in the order in which they are uploaded, individual uploads typically proceed by uploading entire projects, and these typically involve phylogenetically very close genomes; for instance, genomes from a study investigating a hospital outbreak often occupy a range of accessions. As such, lexicographically sorted genomes from ENA can be considered as a first approximation of phylogenetic compression. To compare the compressibility of the 661k collection in the ENA accession order to the full phylogenetic compression, we streamed the genomes from the main collection file provided on http://ftp.ebi.ac.uk/pub/databases/ENA2018-bacteria-661k/661_assemblies.tar, decompressed them on-the-fly, converted them to the one-line FASTA format, and compressed using XZ with 32 threads, all by

pv 661_assemblies.tar | tar -xOf - | gunzip -c | seqtk seq | xz -9 -T32 -v

The computation, performed on a dedicated server, required 23.5 h of wall clock time and 757 CPU hours, and the resulting file had 120,701,329,280 bytes. We note that compression using a single thread was not possible in this case due to the size of the file; nevertheless, as the individual blocks used for XZ parallelization were guaranteed to be a multiple of the dictionary size (that is 68.7 MB with the '-9' preset), ensuring their sufficient size for the comparison to be correct.

Phylogenetic compression of the 661k/661k-HQ k-mer indexes

Phylogenetic compression of 661k-HQ COBS index. We built a phylogenetically compressed COBS index from the 661-HQ dataset using the MOF-COBS-build pipeline. In short, individual COBS indexes were constructed per individual batches, with low-quality genomes removed, with the variant of the index called ClaBS (Classical Bit-sliced index), analogical to the original BIGSI data structure [16]. In this index, all columns (Bloom filters) have the same size and the genomes were provided in the left-to-right phylogenetic order as illustrated in **Fig. 1c(i)**, after which every index was compressed using XZ, resulting in 72.8 GB (1.06 GB when uncompressed, 14.5× reduction) (**Supplementary Table 5**). See the section about MOF-COBS-build for more information. The resulting indexes were then used in MOF-Search for the initial filtration of reference.

Phylogenetic compression of 661k-HQ COBS index. To evaluate the gain of phylogenetic compression in the specific case of COBS indexes, we performed a series of additional experiments (see their overview in **Supplementary Table 5**). In particular, we also created a phylogenetically compressed index of the entire 661k collection, including the low-quality genomes, resulting in 110. GB

(2.46 TB when uncompressed, 22.5× reduction); here, the compression optically seems to be more efficient, but the only reason is that contaminated genomes too much increase the size of the Bloom filters, adding many additional rows that are predominantly composed of zeros.

Comparisons to baselines. To evaluate the improvement of phylogenetic compression for COBS, we needed also to construct the compacted indexes (the default mode of the COBS program), with adaptive adjustments of Bloom filter sizes through subindexes. This was more challenging as this required to work simultaneously with the entire 661k dataset at the same time. For the entire 661k compact COBS index, we used the official one, available online (http://ftp.ebi.ac.uk/pub/databases/ENA2018-bacteria-661k/661k.cobs_compact, retrieved on 2022-09-08), as published with the original manuscript [15]. The index had originally been constructed by COBS, version 7c030bb, using the "compact-construct" subcommand with default options; i.e., without any batches and with adaptively sized Bloom filters (937 GB). In a similar fashion, we also constructed an analogical compact COBS index for the 661K-HQ collection (893 GB). Both indexes were then compressed on a highly performant server in a combination with XZ parallelization using 32 cores ("xz -9 -T32", resulting in 3.86× and 4.35× reductions, respectively). However, we note such indexes are not suitable for personal computers due to both the space requirements and the necessity to uncompress the index in its entirety at once. The resulting comparison of phylogenetic compression of COBS indexes is provided in **Supplementary Table 5**.

MOF-Search pipeline for BLAST-like search across all pre-2019 bacteria from ENA

Overview of the pipeline. MOF-Search (https://github.com/karel-brinda/mof-search) uses phylogenetically compressed assemblies (661k) and phylogenetically compressed COBS indexes (661-HQ) as described in the corresponding sections. Upon first execution, the pipeline downloads all the input reference files from the Internet (29.2 GB of assemblies and 72.8 GB of COBS indexes, in total 102 GB). The search then consists of two phases – matching of queries against the *k*-mer indexes using COBS [29], and then aligning the identified candidates using Minimap 2 [34]. MOF-Search is developed as a Snakemake [57] pipeline using Bioconda [73], with the standard Snakemake resource management [57] to control the assignments of CPU cores and limiting the RAM usage (up to a user-specified threshold).

Matching. Matching of queries is performed iteratively per individual batches. Individual phylogenetically compressed COBS indexes are decompressed either on-the-fly (faster, but requires additional memory for decompression), or on disk, and then they are queried using a modified version of COBS (see below, v0.2.1 with a pre-specified *k*-mer threshold (the minimum required proportion of matching *k*-mer). The output matches are either or stored on disk entirely, or only a user-defined number

of best hits (in terms of the number of matching *k*-mers) of interest (plus ties). To balance resources, the number of threads used by COBS is adjusted based on the size of individual batches (bigger batches are processed using more threads). Finally, the obtained results are aggregated across batches and for every query only the pre-specified number of best matches (plus ties) is kept.

Alignment. Alignment of queries is performed also iteratively per individual batches. For every batch, a dedicated Python script iterates over the phylogenetically compressed genomes and if at least one of the queries was identified in the previous step as a potential hit for the current genome, its Minimap 2 [34] (v 2.24) index is built on-the-fly and all the relevant queries aligned with user-specified parameters and the output provided to the user.

Modified COBS. To enable the integration of COBS into MOF-Search, a new major version of COBS [29] was created (v2, https://github.com/iqbal-lab-org/cobs), fixing multiple bugs, implementing a support for OS X, integrating more tests, and supporting streamed loading of indexes into memory. The new versions of COBS are provided in the form of Github releases (https://github.com/iqbal-lab-org/cobs/releases), as well as pre-built packages on Bioconda [73].

Evaluating MOF-search

Overview of the benchmarking procedure. The search using MOF-search was evaluated using three datasets, representative of different query scenarios: a database of antibiotic resistance genes, a database of plasmids, and an Oxford nanopore sequencing experiment. In all cases, the search parameters were adjusted to the query type, including the number of top hits, the COBS *k*-mer threshold, and the Minimap preset. The experiments were run on an iMac18,3, Quad-Core Intel CPU i7, 4.2 GHz with 42.9 GB (40 GiB) RAM with 4 physical (8 logical) cores.

Time measurement. The wall clock and CPU time were measured using GNU time, and were calculated as 'real' and 'usr+sys', respectively. The measurements were done for both search phases separately (matching and alignment).

Memory measurement. We have not found any reliable way of measuring peak memory consumption on macOS: GNU time was systematically providing incorrect values for our Snakemake pipeline, with values several times lower compared to the expected ones, as did another method based on the psutil Python library. Therefore, we performed additional measurements using the SLURM job manager on a Linux cluster using jobs allocated with a configuration similar to our iMac computer. We found that for

'max_ram_gb' equal to 30 GB, the peak memory consumption was 26.2 GB. This discrepancy is an expected behavior because the 'max_ram_gb' parameter defines an upper bound for the Snakemake resource management [57], corresponding to the worst-case scenario of parallel job combinations.

Resistance genes – ARGannot. The resistance genes search as performed using the ARG-ANNOT database [35], as distributed within the the SRST2 software toolkit [74] in the file https://github.com/katholt/srst2/blob/master/data/ARGannot_r3.fasta (retrieved on 2022-07-24), consisting of 1,856 genes/alleles. The search parameters were set to require at least 50% matching *k*-mers, 1,000 best hits taken for every gene (although yielding always a much higher number of them due to a high number of equally scoring matches), and the Minimap present for short reads.

Plasmids – the EBI plasmid database. The list of EBI plasmid was downloaded from https://www.ebi.ac.uk/genomes/plasmid.details.txt (retrieved on 2022-04-03), and individual plasmids then downloaded from the EBI ENA using curl and GNU parallel [69] (size characteristics provided in **Supplementary Table 6**). The search parameters were set to at least 40% matching *k*-mers (as in ref [16]), 1,000 best hits taken for every plasmid, and the Minimap present for mapping long highly divergent sequences ('asm20').

Oxford Nanopore reads – ERR9030361. This experiment corresponds to 159k nanopore reads of an isolate of Mycobacterium tuberculosis; the reads were downloaded from SRA NCBI and the search parameters were adjusted for a sensitive identification of the nearest neighbors in the database, with at least 40% matching *k*-mers, 10 best hits taken, and the Minimap present for mapping nanopore reads ('map-ont').

Comparison to BIGSI. As we were unable to reproduce the original plasmid search experiment [16] with BIGSI on our iMac computer (due to the size of files to transfer exceeding 1.43 TB), we resorted to the values provided in the paper [16] . For a fair comparison, we focused our comparison on the the total CPU time (sys+usr) and verified that our parallelization is close from the maximal possible (680% out of 800% possible, based on the values in **Supplementary Table 6**).

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