mtDNA "Nomenclutter" and its Consequences on the Interpretation of Genetic Data

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Abstract

Population-based studies of mitochondrial genetic diversity often require the classification of mitochondrial DNA (mtDNA) haplotypes into more than 2000 described haplogroups, and further grouping those into hierarchically higher haplogroups. Such secondary haplogroup groupings (e.g. “macro-haplogroups”) vary across studies, as they depend on the sample quality, technical factors of haplogroup calling, the aims of the study, and the researchers' understanding of the mtDNA haplogroup nomenclature. Retention of historical nomenclature coupled with a growing number of newly described mtDNA lineages results in increasingly complex and inconsistent nomenclature that does not reflect phylogeny well. This “clutter” leaves room for grouping errors and inconsistencies between scientific publications, especially when the haplogroup names are used as a proxy for secondary groupings, and represents a source for scientific misinterpretation.

Here we explore the effects of phylogenetically insensitive secondary mtDNA haplogroup groupings, and the lack of standardized secondary haplogroup groupings on downstream analyses and interpretation of genetic data. We demonstrate that frequency-based analyses produce inconsistent results when different secondary mtDNA groupings are applied, and thus allow for vastly different interpretations of the same genetic data. The lack of guidelines and recommendations on how to choose appropriate secondary haplogroup groupings presents an issue for the interpretation of results, as well as their comparison and reproducibility across studies.

To reduce biases introduced by arbitrarily defined secondary nomenclature-based groupings, we suggest the implementation of phylogenetically meaningful algorithm-based groupings to define a standardized set of “macro-haplogroups”, “meso-haplogroups”, and “micro-haplogroups”. Such phylogenetically informative levels of haplogroup groupings can be easily implemented into haplogroup callers such as *HaploGrep3*. This would foster reproducibility across studies, provide a grouping standard for population-based studies, and reduce errors associated with haplogroup nomenclatures in future studies.

Background

Mitochondrial DNA (mtDNA) is a circular non-recombining ~16.5 kbp long extra-nuclear DNA. Exhibiting a tenfold higher mutation rate compared to nuclear DNA, with even higher mutation rates in the hypervariable segments (HVSI & HVSII), mtDNA shows great variation and diversity across individuals and can be used to study their evolutionary history. As mtDNA is inherited maternally, it is very informative about maternal population history and genetic structure, and it represents one of the commonly studied genetic markers [1, 2].

Early studies of mtDNA diversity across the globe set the stage for the **mtDNA nomenclature** commonly used nowadays (for an overview see [3]). In 1992-1993, three milestone publications [4–6] on the mtDNA of Native Americans and indigenous peoples of Siberia not only proposed a genetic link between these two populations but also established a first nomenclature for mtDNA [3]. Additionally, they introduced the term ***haplogroup***, which stands for "haplotype group", defined by the presence of specific mutations characteristic of a given branch of mtDNA phylogeny [3]. Due to these pioneering studies that introduced the nomenclature, the haplogroups A, B, C, and D were assigned to mtDNA types found in Native Americans and Siberians [6]. Later studies built upon this nomenclature, which turned out to be a suboptimal beginning of nomenclature given that humans originated in Africa and that the Americas were among the last regions humans reached. Over time, by researching diverse populations across the globe and accumulating mtDNA sequences, numerous "region-specific" haplogroups were identified [3]. Consistent with the original alphabetical nomenclature, newly discovered mtDNA haplogroups were assigned new letters. By the time the phylogenetically deepest splitting Sub-Saharan populations were studied, the haplogroups were designated "L" followed by a number [7] (Figure 1). Consequently, the current mtDNA nomenclature mostly reflects the history of research, and not necessarily the nested phylogenetic structure of the mtDNA tree.

To accommodate the ever-growing amounts of mtDNA sequences in the mitochondrial tree, a systematic nomenclature was established (Text Box 1; for a detailed explanation see *"Cladistic notation for mitochondrial clusters"* in [8]). This nomenclature assumes that the first character of the haplogroup name is a capital letter, followed by a number and a lowercase letter in an alternating manner (e.g. C1d1), allowing easy naming for every new sub-branch. Consequently, the number of characters in a haplogroup name appears to reflect a phylogenetic level (which is not necessarily the case). Unfortunately, this naming convention became increasingly inconsistent with the discovery of new lineages and improvements in phylogeny.

Text Box 1 - Cladistic notation for mitochondrial clusters

Richards et al. 1998 outlined several principles for a cladistic notation for mitochondrial clusters:

1) Single capital letters denote principal clusters.

E.g. A, B, C, D...

2) Nesting of clusters is permitted.

E.g. C belongs to M, and M belongs to L3.

3) Subclusters of single-capital-letter-coded clusters can have non-negative integer suffixes that follow hierarchical notation with alternating small letters and numbers.
E.g. C1d1 is a subcluster of C1d which is a subcluster of C1 which belongs to C.

4) Clade can be referred to using the names of its prominent subclades.
E.g. CZ refers to the smallest monophyletic clade containing C and Z.

5) Paraphyletic clusters can be temporarily labeled using an exponent suffix *.
E.g. If U6b1, U6b2, and U6b3 are known then U6b includes all U6b sequences not in already mentioned subclusters.*

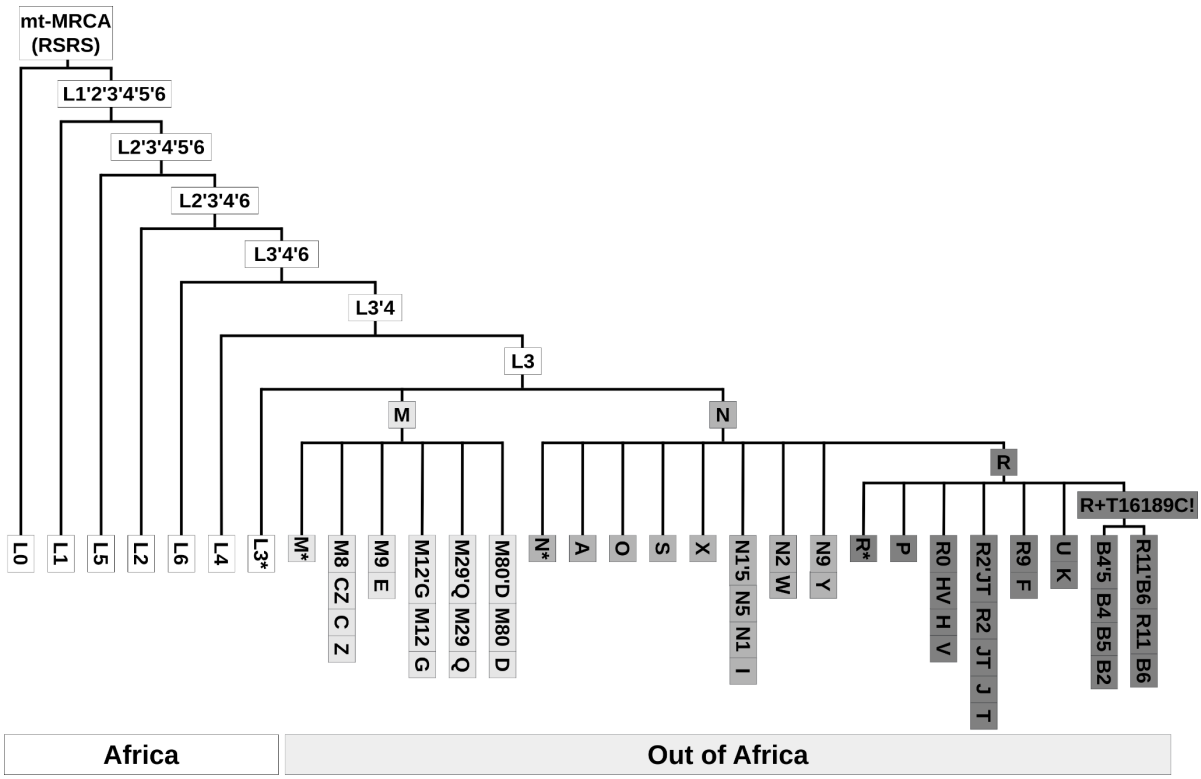

Figure 1. Schematic illustration of mtDNA phylogeny from *PhyloTree build 17* (after van Oven 2015). The figure showcases the complexity in nomenclature as can be seen for example in how single capital letter haplogroups M, N, R, and A are nestled within two-character-long haplogroup L3.

The most comprehensive and commonly used resource for the organization and maintenance of the human mtDNA phylogeny is provided by **PhyloTree** [9, 10]. Between 2009 and 2016, PhyloTree periodically incorporated the ever-growing amount of mtDNA sequences into their phylogenetic tree, with the disclaimer that more branches are yet to be defined due to the biased sampling of populations.

For a given sample, **haplogroup calling** can be performed manually by checking if the haplogroup-specific mutations from *PhyloTree* are present or absent in the sample, or more commonly automatically using methods such as *HaploGrep3* [11]. ***HaploGrep3*** is a tool that assigns individual samples to the best-matching haplogroup according to the presence or absence of haplogroup-defining mutations from *PhyloTree*. The most recent *HaploGrep3* comes with several older *PhyloTree* versions, along with recent additions from forensics [12] and the ability to integrate external custom-made phylogenetic trees, which opens up the possibility for decentralized community-based updates.

Haplogroup callers such as *HaploGrep3* often output too many unique haplogroups to be useful for downstream population-based haplogroup frequency analyses. For example, *PhyloTree17-ForensicUpdate1.2* includes a total of 6,380 haplogroups. This is why scientists often rely on custom secondary **Nomenclature-Based Groupings (NBG)** of haplogroups, in which they calculate the frequency of so-called **"macro-haplogroups"** or groups of related haplogroups (for example by summing frequencies of haplogroups B2a1a and B2a3 into macro-haplogroup B or even B2a). In contrast to the relatively well-defined concept of a **"haplogroup"**, the terms **"macro-haplogroup"** and **"sub-haplogroup"** are not clearly defined. Those two terms are instead often used to describe a relative relationship to the focal haplogroup, rather than representing clearly defined categories consistent across studies (e.g. one can say that B4a1 is a sub-haplogroup of haplogroup B4a, which belongs to macro-haplogroup B).

Two commonly occurring NBGs are **Single Character grouping (SC),** where grouping is based on the first haplogroup letter, and **Single Character and L with one digit grouping (SCL),** in which grouping is the same as SC except that haplogroups starting with "L" are grouped into more groups based on the first letter and the first digit (see Text Box 2 - mtDNA groupings). SCL makes more sense than SC as it better represents the haplogroup diversity within Africa. Besides those two NBGs, other modifications of secondary haplogroup groupings can be found in the literature. The newly released *Haplogrep3* introduces a slight modification to SCL by including HV as an additional category, resulting in 33 top-level haplogroups/clusters. In addition, custom groupings tailored to fit the study's research question are also common in the literature (e.g. focusing on a particular haplogroup in more detail and lumping all the others in a single category). Consequently, the choice of NBG will influence the granularity (i.e. the phylogenetic level) of haplogroups and this can further influence the downstream analysis and interpretation of the data.

Text Box 2 - mtDNA groupings

Throughout this paper, we consider three different types of *mtDNA groupings*:

1) Haplogroup calling - i.e. mtDNA haplogroup classification. A primary assignment of haplogroup names to haplotypes based on a set of mutations present in a given sequence. This step is commonly performed by haplogroup callers such as *HaploGrep3* [11], *HaploCart* [13], and *HaploGrouper* [14].

2) Nomenclature-Based Groupings (NBG) - i.e. creation of broader secondary mtDNA groupings based on traditional nomenclature, whose granularity level can vary from study to study (e.g. grouping samples assigned to haplogroups B4a1a1, B4a2b1a, B4a3, B4a4, B4a5 into B4a, or B4, or even B). Two commonly occurring NBGs are:

i) Single Character grouping (SC) - i.e. grouping based on the first haplogroup letter (e.g. grouping haplogroups C1b13c1 and C4a1a3a1 into C); or

ii) Single Character and L with one digit grouping (SCL) - i.e. grouping based on the first haplogroup letter except for those starting with "L", for which the first letter and the first digit are used (e.g. grouping haplogroups L0d2c1a1 and L0k1a1c into L0).

3) Algorithm-Based Groupings (ABG) - i.e. computational grouping of mtDNA based on the sequence independent of traditional haplogroup nomenclature. Ideally, such groups of mtDNA should be in accordance with sequence similarities and/or mtDNA phylogeny. ABGs usually assign an arbitrary name or a number to a group of related mtDNA sequences. Such groupings can be obtained from mtDNA phylogenetic trees (e.g. using *TreeCluster* [15]), or directly from MSA in FASTA format (e.g. using *rhierBAPS* [16]).

The complexity of mitochondrial nomenclature and custom-made phylogenetic levels for secondary haplogroup groupings may lead to misunderstandings and inconsistencies between scientific publications. This is especially true when the lengths of haplogroup names are used as a proxy for their phylogenetic level and informativeness to create secondary groupings.

As mentioned above, the *PhyloTree17-ForensicUpdate1.2* has 6380 haplogroups, but nearly one-quarter of them have names that do not follow strict cladistic nomenclature, i.e. haplogroup names start with capital letters and are followed with alternating numbers and small letters (Table S1). For example, 1.8% of haplogroup names include two consecutive uppercase letters denoting nested haplogroups (e.g. haplogroups J and T are within JT; see Figure 1), 2.8% contain two consecutive lowercase letters (e.g. H3ag1), nearly 19% have one or more of the symbols *()@+" denoting mutated positions and/or lack of them (e.g. D5a2a1+@16172). Furthermore, many haplogroups were named by merging the names of two sub-branches, separated with an apostrophe (e.g. haplogroups M9a and M9b are nested within M9a'b). Besides the name itself, the phylogenetic relationship between haplogroups presents another obstacle. For example, haplogroups D1, D2, and D3 are nested within D4 instead of a more logical assumption of them being at the same or similar phylogenetic level. All these historically accumulated naming conventions make modern mtDNA nomenclature complex [3], unintuitive to navigate, and difficult to work with in bioinformatics pipelines, which is especially true for researchers new to the field.

In addition, the richness of defined haplogroup names depends on the studied populations, which results in systematic biases. European individuals and populations are often overrepresented in genetic databases [17]. Consequently, an mtDNA sample is more likely to be assigned to a haplogroup with a high resolution if they resemble well-studied and described populations, like Europeans. Moreover, *PhyloTree* has not been updated since 2016, which opens up the question about the future maintenance and standardization of mtDNA haplogroup nomenclature.

Many studies assume that **SC** groups are “macro-haplogroups” (e.g. assuming that both haplogroup L1 and L0d belong to "macro-haplogroup L") (e.g. [18–21]). Such practice creates confusion as there is no haplogroup L, and since all non-L haplogroups are nested within L3. In reality, the hierarchically highest "L" haplogroup nomenclature designations all have two characters (L0 - L7) [10, 22]. Phylogenetically speaking, all haplogroups that contain "L" at the beginning of their name refer to the most recent common ancestor (MRCA) and should be grouped under mt-MRCA (that splits into L0 and L’1’2’3’4’5’6). What authors actually assume with SC grouping is a non-monophyletic grouping of haplogroups, whose names start with the letter L. Subsequently, this grouping leads to a disproportionate loss of phylogenetic resolution for the deepest lineages of the mtDNA phylogeny and thus has the strongest effect on African populations that harbor the highest frequencies and diversity of such haplogroups [23]. The effect on African populations is less severe when **SCL** is employed as it includes at least two characters for haplogroups whose name starts with the letter "L" (e.g. [24–26]. Even though slightly better than SCL, the 33 top-level haplogroups/clusters provided by *Haplogrep3* still do not account completely for the nested phylogenetic structure of haplogroups. For example, frequencies of M, N, and R are calculated separately as if R is not nested within N [21, 27]. Furthermore, there are small inconsistencies between *Haplogrep3* clusters depending on their phylogenies. For example, *Haplogrep3* “Cluster R” is defined differently, depending on whether PhyloTree 17 - Forensic Update 1.2 (https://haplogrep.i-med.ac.at/phylogenies/phylotree-fu-rcrs@1.2/clusters/R; accessed on 30.09.2023) or PhyloTree 17.1 (https://haplogrep.i-med.ac.at/phylogenies/phylotree-rsrs@17.1/clusters/R; accessed on 30.09.2023) is used. In the first case “Cluster R” includes 198 haplogroups (including HV*, JT, and all that start with the letter R), while in the second case, it includes only the 39 haplogroups that start with R0 and all other haplogroups whose name starts with “R” are included in “Cluster N”.

In summary, complex nomenclature and lack of standardized or recommended grouping levels for haplogroups may introduce biases into downstream analyses and consequent interpretations of data. A better alternative to traditional nomenclature-based haplogroup groupings could be an **Algorithm-Based Grouping (ABG)** utilizing sequence similarities or phylogenetic trees. Such grouping could circumvent complex nomenclature and reduce human bias in choosing secondary haplogroup groupings.

Here we aim to explore how the categorization of human mitochondrial diversity into secondary NBGs can affect downstream haplogroup frequency-based analyses and our interpretations of the underlying genetic data. To do so, common NBGs are compared with ABGs produced by *rhierBAPS* and *TreeCluster*. Lastly, we propose that defining a standardized set of “macro-haplogroups”, “meso-haplogroups”, and “micro-haplogroups” in a phylogenetically meaningful way would be beneficial to the fields of population genetics, forensics, and medical genetics.

Materials and methods

Data and data preparation

We retrieved full mitochondrial sequences from 660 individuals free of relatives belonging to seven African Ancestry populations (ACB - African Caribbean in Barbados; ASW - African Ancestry in SW USA; MSL - Mende in Sierra Leone; GWD - Gambian in Western Division - Mandinka; ESN - Esan in Nigeria; YRI - Yoruba in Ibadan, Nigeria; LWK - Luhya in Webuye, Kenya) from the Phase 3 release of the 1KGP (1000 Genomes Project) [28]. The revised Cambridge Reference Sequence (rCRS) [29] was added as a reference for multiple sequence alignment. In addition, we added the Reconstructed Sapiens Reference Sequence (RSRS) [30] for rooting the phylogenetic tree. Positions known to interfere with multiple-sequence alignment were removed or altered manually using *Unipro UGENE v42.0* [31]. The spacer in the rCRS at position 3107 was changed from an "N" to an indel. The same was done for the spacers in the RSRS [30] at positions 523-524. The positions in the poly-c region 303–315 and 16183–16194 were removed from all sequences.

Multiple sequence alignment (MSA)

MSA was performed using *MAFFT* [32]. Following good practice recommendations, manual post-alignment base correction in the region around the original 3107 rCRS spacer was performed using *Unipro UGENE*, ensuring that all indels align in the same pattern.

Phylogeny construction and visualization

mtDNA phylogenetic trees were constructed by Maximum Likelihood (ML) with *IQ-TREE* [33]. The *ModelFinder Plus* setting was used to determine the best-fitting model by calculating the Bayesian Information Criterion (BIC) and choosing the model which minimizes the BIC. Phylogenetic trees were rooted using the RSRS [30]. To obtain a schematic reduced phylogenetic tree (Figure 3) results from secondary nomenclature-based groupings (NBG) and algorithm-based groupings (ABG) were combined to identify a minimal set of representative samples that capture all unique combinations of different groupings across individuals. The phylogenetic tree was visualized using the R package *ggtree* [34].

mtDNA groupings

Haplogroup calling was performed using *Haplogrep3 (v.3.2.1)* command line tool [11], with FASTA as the input file, and the *PhyloTree17 - Forensic Update (rCRS Human mtDNA) Version 1.2 (phylotree-fu-rcrs@1.2) as the tree*. Based on the output of *HaploGrep3* we created two commonly occurring **secondary NBGs: SC** and **SCL** in R [35]. To obtain mitochondrial groupings based on mtDNA sequence similarity independent of traditional nomenclature, we performed two **ABGs:** *rhierBAPS and TreeCluster.*

The rhierBAPS groupings (rhb) were performed directly on MSA FASTA using the R package *rhierBAPS* [16]. The *rhierBAPS* analysis was run with

keep.singletons=TRUE, max.depth=10, and the default value for *n.pop*. For visualization purposes, we focussed on the first 3 levels of the *rhierBAPS* output.

The *TreeCluster* groupings (tc) were performed on the mtDNA consensus trees outputted by the *IQ-TREE* using the command line program *TreeCluster* [15] with eight different threshold values (*-t*) ranging from 0.001 to 0.008, and method (*-m*) set to its default: *max_clade*. The Max Clade method of *TreeCluster* clusters the leaves of the provided phylogenetic tree ensuring that the maximum pairwise distance between leaves in the cluster is at most equal to the specified threshold. For visualization purposes, we focussed on the output of *TreeCluster* with threshold values 0.003-0.006.

Frequency-based analysis

Frequency bar plots

Frequency bar plots based on different mitochondrial groupings were created in R using packages *tidyverse* [36] and *ggpubr* [37].

Correspondence analysis (CA)

CA was performed using the R package *FactoMineR* [38] and visualized using the R package *factoextra* [39].

Pairwise distance-based analyses

Pairwise distances between individuals from the same mitochondrial group were calculated in R using the packages *ape* [40] and *tidyverse* [36].

Multidimensional scaling (MDS)

MDS analysis was performed on the mtDNA pairwise distance matrix using the function *cmdscale()* from the R package *stats* [35] *and visualized using ggplot2* [37].

Geographic map

The geographic map with populations was created using R packages *rworldmap* [41] *and tidyverse* [36].

Literature meta-analysis

For a systematic overview of NBGs used in the literature, we assembled a list of research articles published in years 2020-2023 through Nature Publishing Group that contained the search term: *"africa* haplogrep* human* mtdna* haplogroup* OR africa* haplogrep* human* mitochondria* haplogroup*"* (Table S2). Each research article we categorized based on the NBG employed.

Results and Discussion

Comparison of NBG with ABG

To illustrate the differences between NBGs and ABGs we chose seven African Ancestry populations (AFR) from the 1000 Genomes Project (1KGP) Phase 3 (Figure 2A). Those populations are suitable as they harbor a high mtDNA diversity (Figure 2B, Figure S1, Figure S2) and many haplogroups, whose names are starting with the letter L that are often lumped together into “L macro-haplogroup” (assuming SC grouping). The majority of “non-L haplogroups” (A, B, C, D, H, J) in those populations are found in low frequency only in two populations from Americas (ACB - African Caribbean and ASW - African Ancestry SW) known to be admixed with non-African populations.

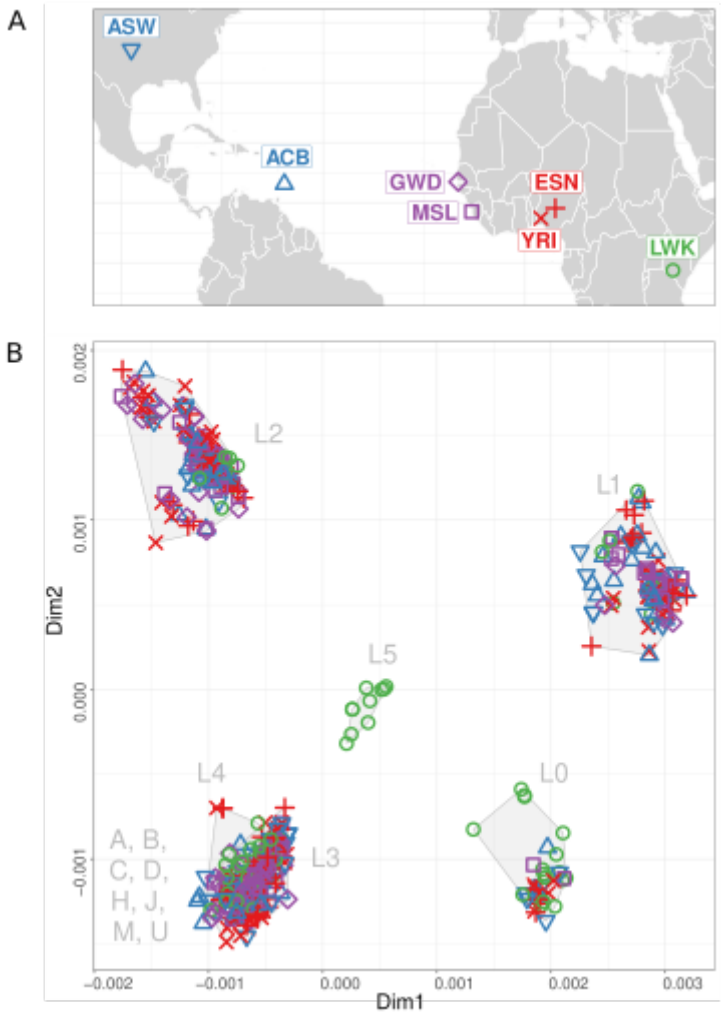

Figure 2. African Ancestry populations from the 1KGP Phase 3 and mtDNA pairwise-distance-based MDS plots. A) Map of sampling locations. ACB - African Caribbean in Barbados; ASW - African Ancestry in SW USA; MSL - Mende in Sierra Leone; GWD - Gambian in Western Division - Mandinka; ESN - Esan in Nigeria; YRI - Yoruba in Ibadan, Nigeria; LWK - Luhya in Webuye, Kenya. **B)** Two-dimensional MDS plots based on mtDNA pairwise distances colored according to populations. Population symbols correspond to those in panel A. SCL groupings are indicated with gray shadings and labels. Note that L3, L4, and “non-L” haplogroups are overlapping on this two-dimensional MDS plot.

The NBGs (SC and SCL) reflect phylogenetic structure poorly (Figure 3; for the full tree colored according to SCL see Figure S1). This is especially true for SC, which lumps the majority of mtDNA diversity into a single haplogroup and gives disproportionately more resolution to lineages commonly found outside of Africa. A noticeable improvement is seen with SCL, which gives slightly more resolution to haplogroups beginning with the letter L. On the other hand, *rhierBAPS* defines groups of sequences at several phylogenetic levels of resolution based on sequence similarity. Even though it provides more resolution for the phylogenetically deeper splitting haplogroups (i.e. haplogroups whose name starts with the letter L), *rhierBAPS* still produces phylogenetically inaccurate groups (e.g. phylogenetically related L2 haplogroups are not grouped together in *rhb_01*; a monophyletic group composed of L3b and L3d is nested within another group that would be monophyletic if it would include them in *rhb_02*).

The best accordance with phylogeny is seen for *TreeCluster* groupings. This is expected as *TreeCluster* relies on a phylogenetic tree when making groupings, and the granularity of the groups can be adjusted with the threshold. In our case, the threshold of 0.006 (*tc_0.006*) resulted in the creation of groups equivalent to SCL groups L0, L1, L2, and L5. However, all other SCL groups are contained in the same group in *tc_0.006*.

As expected, decreasing the threshold values for the maximum pairwise distance between leaves in the cluster (*tc_0.005, tc_0.004, tc_0.003*) resulted in the creation of new groups predominantly in haplogroups whose name starts with the letter L (i.e. phylogenetically deeper splitting haplogroups). For example, only at *tc_0.003*, we can see the differentiation between non-L haplogroups, which are grouped into only 3 groups (first comprising haplogroup A, second comprising haplogroups B, J, U, and H, and third comprising haplogroups C, D, and M), while there are 26 groups whose haplogroup names start with letter L (which are all grouped into single L macro-haplogroup when SC is applied). Even though phylogenetically more meaningful and informative for African populations, such groupings would not be optimal for research focusing on non-African admixture in African Americans and African Caribbeans. However, this limitation could be resolved by further decreasing the threshold values, which would result in phylogenetically finer and more homogeneous groupings and thus distinction between non-L haplogroups. The choice of the exact threshold values would thus depend on the study and the question in mind.

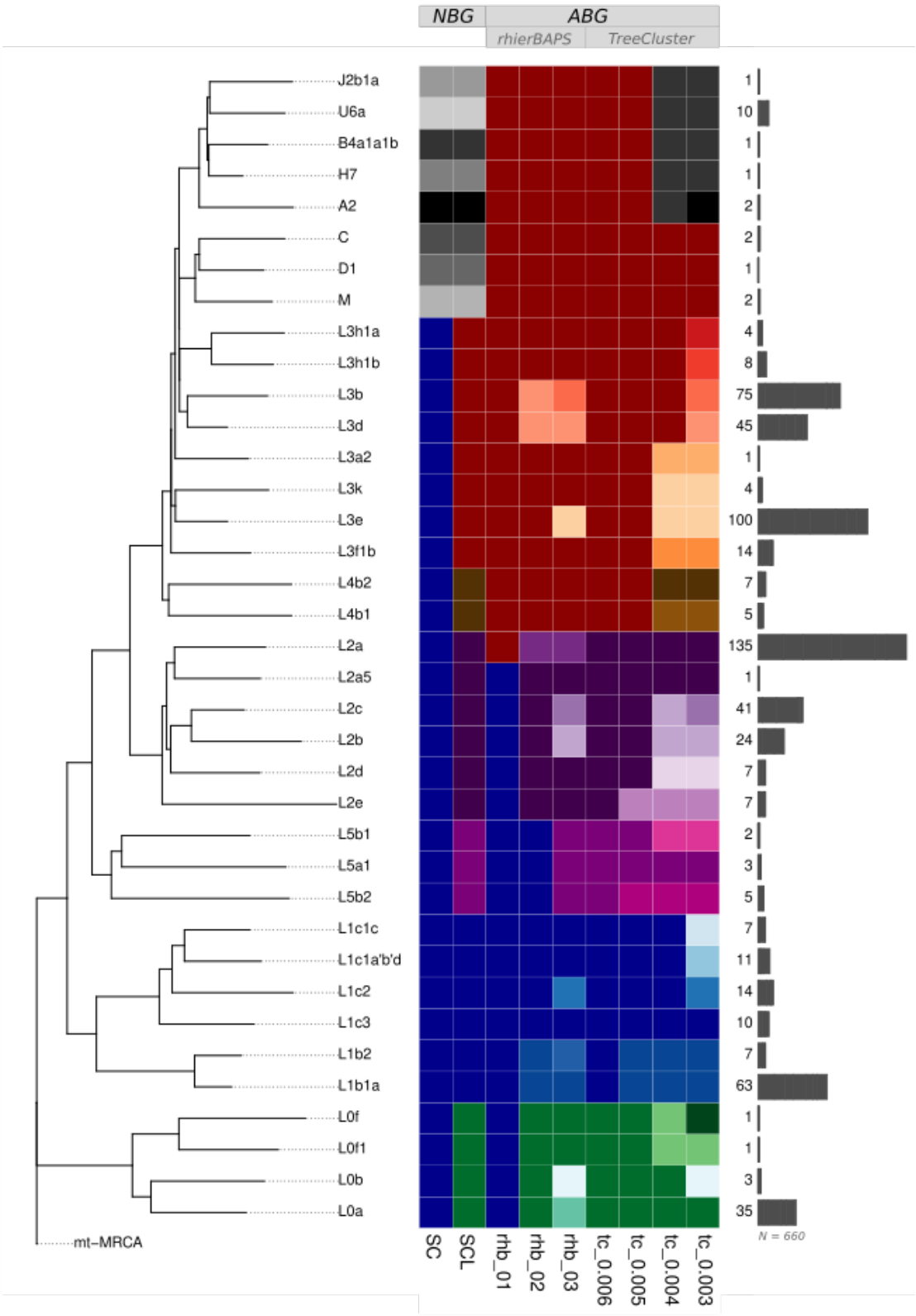

Figure 3: Comparison between nomenclature-based (NBG) and algorithm-based (ABG) groupings. The reduced phylogeny (left panel) is based on the minimal number of samples needed to capture the diversity based on combinations of all grouping designations. The total number of samples (N=660) belonging to each grouping is indicated with bar plots and numbers (right panel). Colors serve as an aid for an easier visual distinction of groups (vertically) and as an illustration of correspondence between grouping approaches (horizontally). SC - single-character grouping; SCL - single character and L with one digit grouping; rhb - *rhierBAPS* (levels: 1, 2, and 3); tc - *TreeCluster* (thresholds: 0.006, 0.005, 0.004, and 0.003). RSRS (mt-MRCA) was added for rooting the tree.

Mitochondrial grouping choices affect the interpretation of results

Different approaches to mitochondrial groupings are expected to have a direct effect on haplogroup frequency-based downstream analyses (e.g. haplogroup frequency plots such as pie charts or bar plots, and correspondence analysis). Here we demonstrate the effect of NBGs and ABGs on the outcome of such analysis. The SC approach distinguishes 9 groups from which the "L haplogroup" accounts for the majority of the data (Figure 4A). Besides being uninformative for African populations, SC could lead to a biologically misleading conclusion (e.g. that a given African population exhibits lower macro-haplogroup diversity when compared to non-African populations (see Table S2 for more examples).

By implementing the SCL approach, the number of groups increases to 14 (Figure 4B), which starts to reveal the diversity of haplogroups in the sample. Comparisons between populations based on SCL allow more meaningful differentiation between African populations (e.g. L5 occurs only in LWK, while the majority of non-L haplogroups occur in two populations from the Americas). The comparison of frequency bar plots between SC and SCL is a striking example illustrating how NBGs could lead to drastically different interpretations of the same genetic data: The first level of *rhierBAPS* identified only two groups, the second level seven groups, and the third level 16 groups (Figure 4C-E). These different levels of granularity, however, might invite different conclusions. Based on *rhb_01,* there are hardly any differences between populations. Based on *rhb_02,* one could say that GWD shows the lowest haplogroup diversity, while all the other populations show comparable haplogroup diversity. Based on *rhb_03* YRI and GWD have the lowest haplogroup diversities and LWK clearly has the highest haplogroup diversity.

Decreasing the threshold value in *TreeCluster* groupings leads to a drastic increase in the number of defined groups (e.g. threshold of 0.006 produces 5 groups, while the threshold of 0.003 produces 29 groups). Even though an increased number of groups allows more precise inferences in phylogenetically more recent times (e.g. detection of admixture from non-African sources in African American and African Caribbean populations) it hinders visualization and interpretation of the data.

To assess how different the sequences belonging to the same secondary haplogroup groupings are, we calculated the pairwise distances between individuals belonging to the same group (Figure 5C-D, Figure S2). When examining the pairwise distances for NBGs, it is noticeable that the SC grouping "L" encompasses a large range of pairwise distances (Figure 5A, Figure S2A). This diversity is separated into 6 groups (L0-L5) when SCL is applied, which results in generally reduced mean pairwise distances within groups (Figure 5B, Figure S2B). However, the SCL groups still exhibit considerably high levels of pairwise differences.
The ABGs allow us to define groups at different granularity levels by adjusting parameters and thresholds. The increased granularity from level 1 to level 3 in *rhierBAPS* groupings is reflected in lower within-group pairwise distances in higher levels (Figure 5C-E, Figure S2C-E). Still, level 3 (*rhb_03*) produces groups with very high variation in average and maximal within-group pairwise distances (e.g. groups 36, 31, and 1 show high (max values >0.004), while groups 34 and 41 show small within-group pairwise distances (max values <0.001)). On the other hand, *TreeCluster* allows strict control of the maximal within-group pairwise distances, and it uses information from a phylogenetic tree, which ultimately creates phylogenetically meaningful groups of comparable within-group pairwise distances, but at the expense of the increased number of groups (Figure 5F-I, Figure S2F-I).

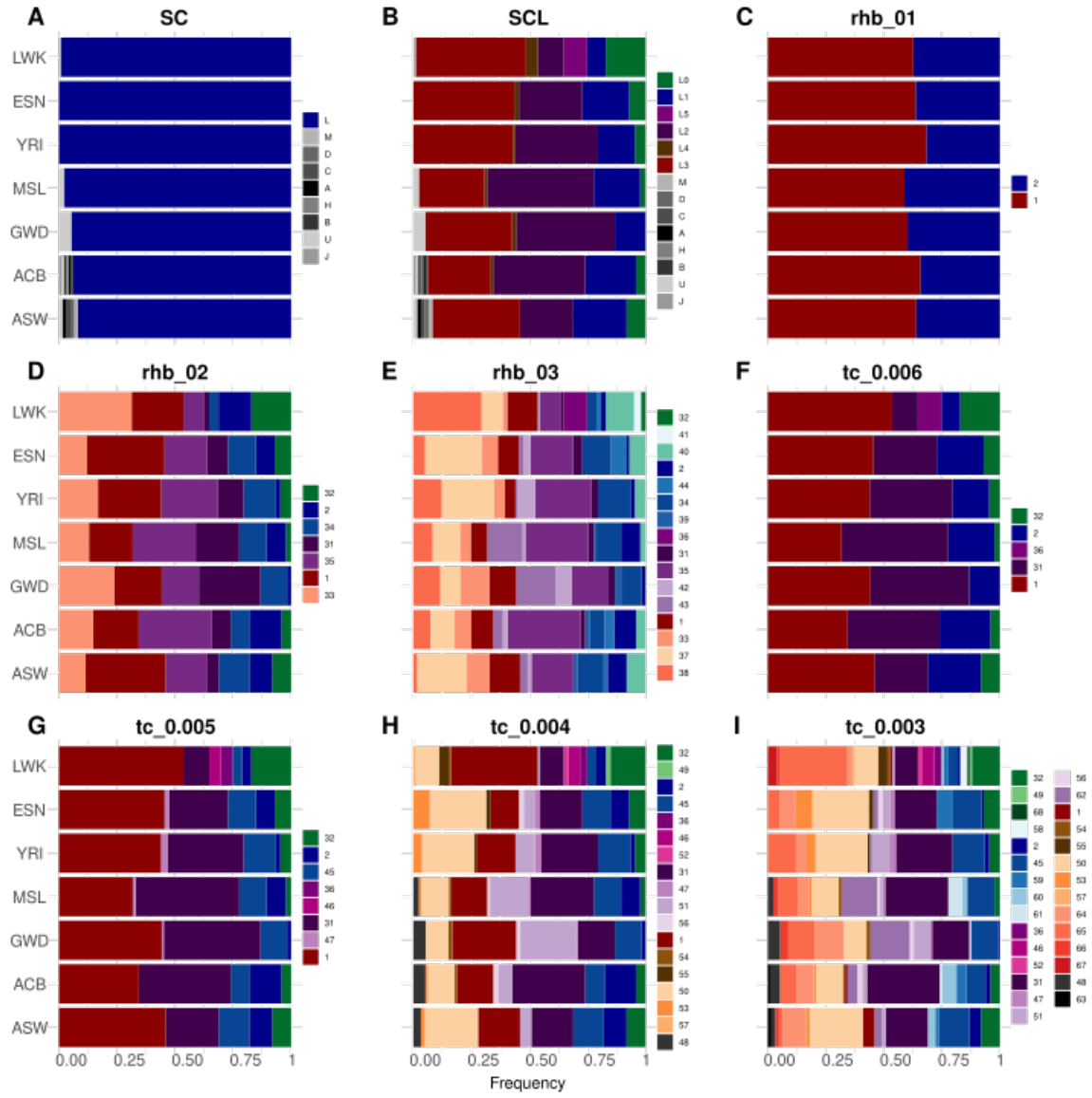

Figure 4. Frequency bar plots for African Ancestry populations from 1KGP based on mtDNA groups produced by NBGs: A) SC, and B) SCL; and ABGs: C-E) *rhierBAPS*, and F-I) *TreeCluster*. Note remarkable differences between SC and SCL as well as an increase in the number of haplogroups with increasing threshold values for ABGs. ACB - African Caribbean in Barbados; ASW - African Ancestry in SW USA; MSL - Mende in Sierra Leone; GWD - Gambian in Western Division - Mandinka; ESN - Esan in Nigeria; YRI - Yoruba in Ibadan, Nigeria; LWK - Luhya in Webuye, Kenya.

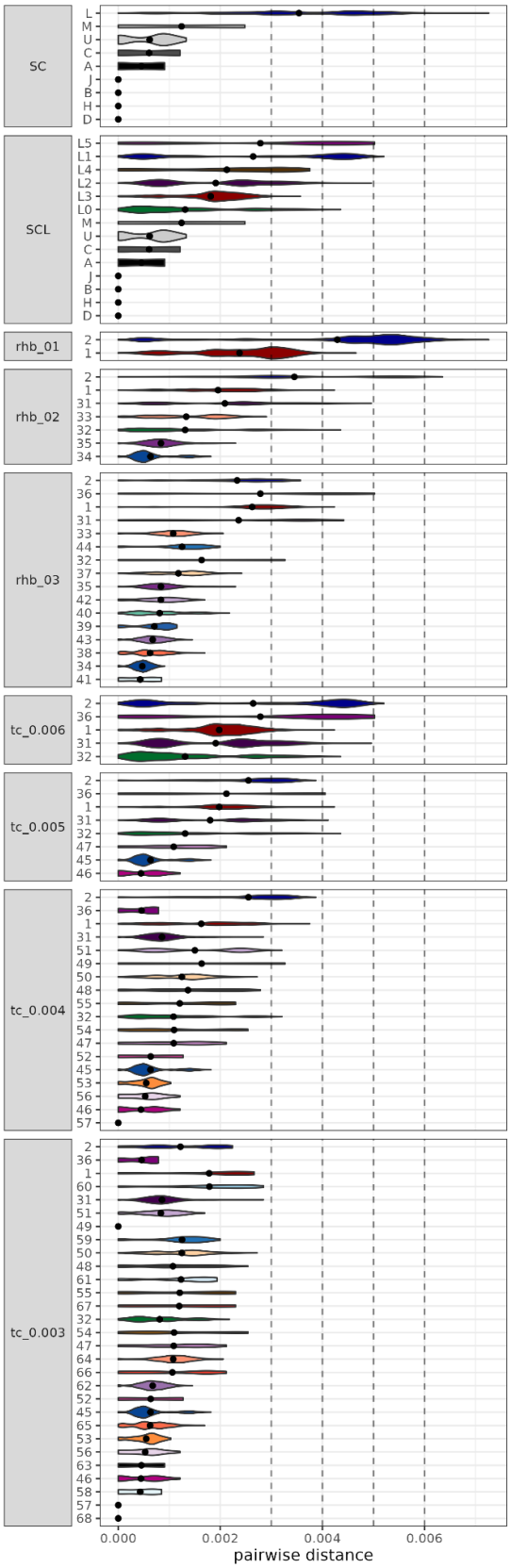

Figure 5. Violin plots of the mtDNA pairwise distances between individuals belonging to the same cluster produced by NBGs (SC and SCL), and ABGs (rhierBAPS and TreeCluster). Dashed lines indicate the threshold values used in TreeCluster runs. Note remarkable differences between SC and SCL, as well as an increase in the number of groupings with increasing rhierBAPS levels or decreasing threshold values for TreeCluster.

445 *Correspondence analysis*

446 Correspondence Analysis (CA) is a multivariate statistical technique useful for
447 visualizing the relative relationships between and within two groups of variables. In
448 population genetic studies, CA is commonly used to explore the relationships among
449 the frequencies of haplogroups and populations. Thus, CA is often used to estimate
450 "similarity" or "difference" between a given set of populations based on their
451 haplogroup frequencies. This, of course, heavily depends on the secondary
452 haplogroup groupings (i.e., a phylogenetic level at which haplogroup calls are
453 made). To investigate how different secondary haplogroup groupings affect CA, and
454 ultimately the conclusions drawn from it, we compared eight different CA plots based
455 on the NBGs (SC and SCL), and ABGs (*rhierBAPS* levels 2 and 3, and *TreeCluster*
456 groupings with thresholds 0.006, 0.005, 0.004, and 0.003).

457 The SC and SCL CA plots exhibit striking differences in the clustering of populations
458 on the first two dimensions. For the CA based on SC groupings (Figure 6A), the first
459 two dimensions separate populations from Americas ASW and ACB from all the
460 other populations that are almost indistinguishable from each other. The separation
461 of populations from Americas is driven by the presence of non-African haplogroups
462 (with the exception of haplogroup U).

463 The CA based on the SCL groupings (Figure 6B) still preserves the separation of
464 populations from Americas ACB and ASW on the second dimension, while the first
465 dimension separates East African LWK. This separation on the first dimension is
466 primarily driven by haplogroup L5, which is unique to LWK in the dataset. The
467 distinction between “L haplogroups” in SCL grouping thus adds valuable information
468 for population differentiation within Africa.

469

470 Visually striking differences between SC and SCL CA could have a profound effect
471 on the interpretation of the very same underlying genetic data. When interpreting
472 the SC grouping, one can create a narrative, according to which the ACB and ASW
473 populations are genetically more different from the continental African populations
474 due to their non-African admixture. On the other hand, the SCL grouping could
475 evoke an interpretation that the LWK population is genetically distinct from all of the
476 other populations due to being East African and that the ACB and ASW populations
477 outside of continental Africa are genetically distinct due to the presence of non-
478 African haplogroups, yet still are genetically more similar to the West-African
479 populations (ESN, GWD, MSL, YRI) than to the East-African populations (LWK).
480 This could be explained by the trans-Atlantic slave trade, which mostly affected
481 West-African populations [42]. While interpretations based on SC and SCL can both
482 be inspired and supported (to some extent) by population history and thus both seem
483 plausible, the question remains, which one of them is "better" or less biased for
484 population genetics research?

485 Interpretations of CA based on ABGs pose a similar issue to NBGs. Even though
486 East African LWK is always separated on the first axis, the separation of populations
487 on the second axis is not always comparable across different ABGs. For example,
488 *rhierBAPS* Levels 2 (*rhb_02*) and 3 (*rhb_03*) show different clustering of populations,
489 especially for GWD, which appears much more distant from MSI in *rhb_02* than in
490 *rhb_03* (Figures 5C-D). The separation of populations from Americas is not obvious
491 based on the first two dimensions in *TreeCluster* groupings except for *tc_0.005*.

Based on comparisons between NBGs and ABGs we can conclude that the SC and SCL groupings put more emphasis on the non-L haplogroups due to the biases in haplogroup nomenclature, while the ABGs give more resolution to the phylogenetically diverse haplogroups whose names start with letter “L”.

However, these conclusions may be dependent on the dataset and haplogroup composition, as populations analyzed here predominantly consist of "L haplogroups".

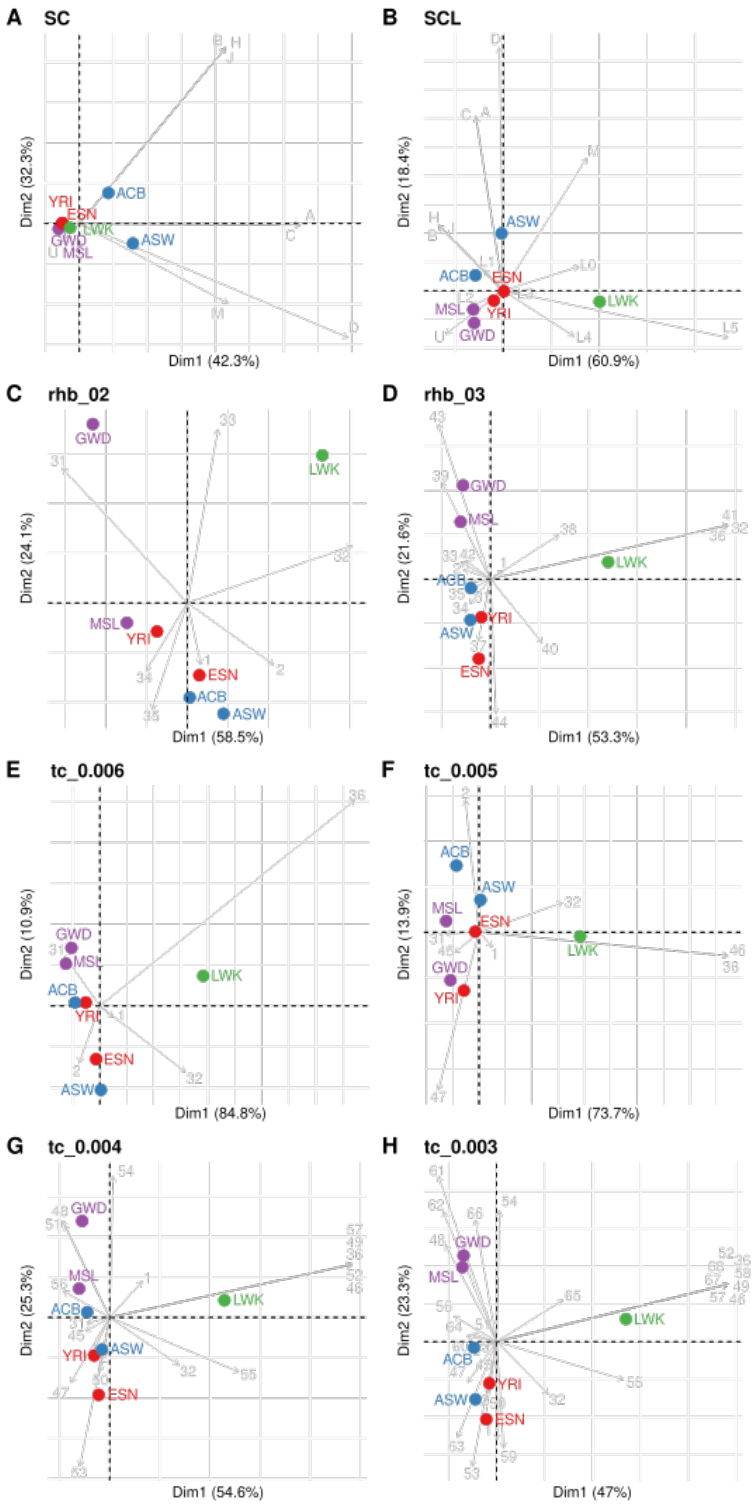

Figure 6: Correspondence analysis (CA) plot of haplogroup frequencies of African Ancestry populations from 1KGP based on groupings produced by A) SC, B) SCL, C) *rhierBAPS* level 2, D) *rhierBAPS* level 3, E) *TreeCluster* with threshold 0.006, F) *TreeCluster* with threshold 0.005, G) *TreeCluster* with threshold 0.004, H) *TreeCluster* with threshold 0.003. Arrows indicate the strength and direction of associations between mtDNA clusters and populations based on the first two dimensions (x- and y-axis). Colors indicate geographic regions: green - East Africa (LWK), blue - Americas (ACB and ASW), red - eastern West Africa (YRI and ESN), purple - western West Africa (GWD and MSL). ACB - African Caribbean in Barbados; ASW - African Ancestry in SW USA; MSL - Mende in Sierra Leone; GWD - Gambian in Western Division - Mandinka; ESN - Esan in Nigeria; YRI - Yoruba in Ibadan, Nigeria; LWK - Luhya in Webuye, Kenya.

Solutions to reduce biases in mitochondrial groupings

The complexity of traditional nomenclature and the biases it introduces in downstream analyses and interpretation of genetic data could be at least partially accounted for in several ways. Each requires different efforts for implementation and the likelihood of acceptance by the broader scientific community.

A radical solution to allow easier handling of mitochondrial haplogroups would be the development of a completely **new nomenclature**. Even though several minor conflicts in mtDNA nomenclature for specific sets of haplogroups were already successfully resolved and incorporated into *PhyloTree* [10, 43–45], drastic changes in the mitochondrial nomenclature would come with issues and resistance in the scientific community, due to the widespread usage and well-established nature of the current nomenclature that ensures continuity with the previous mtDNA research [30]. A possible new nomenclature system would require well-documented resources to aid with translation from an old to a new system, ensuring that previous research using old nomenclature could be related to future research and new nomenclature. Lastly, such a drastic change in nomenclature would have to be centralized, approved, and accepted by the global scientific community. Such centralization would also entail regular updating of the database and nomenclature to incorporate new mtDNA sequences, improving the accuracy and quality of haplogroup calling.

Alternatively, **algorithm-based groupings (ABGs)** could be useful in minimizing biases introduced by scientists when making secondary haplogroup groupings. For example, tools that group sequences based on sequence similarity [46, 47], or tools that make groups based on phylogenetic trees [15] can be very useful. However, ABGs can be labor-intensive, complicated to install and run, and might require additional data. Even though ABGs may introduce more reliable and reproducible groupings that are in better accordance with phylogeny, they are still not completely unbiased and still depend on arbitrarily chosen cutoffs.

The most optimal solution, in our opinion, would be the **implementation of informed grouping recommendations**. Similar recommendations were made for the Y chromosome, when the major changes in nomenclature were made [48]. The consortium had to make somewhat arbitrary decisions on which branches of the Y chromosome tree would represent the "main" branches. Nevertheless, even the arbitrary choice helped in establishing a centralized reference. In 2014, a reduced Y chromosome reference phylogeny and new nomenclature were suggested based on well-defined mutations in branches, presenting a common standard and intuitive overview for its usage [49]. Regarding mtDNA, *PhyloTree* provides a simplified grouping scheme (Figure 1A) for accessing the subtrees [10]. This reduced scheme, however, was not intentionally made to serve as a reference phylogeny, but only as a navigational aid for *PhyloTree's* webpage. Interestingly, the newest *HaploGrep3* provides a first step towards standardization of secondary haplogroup groupings by clustering all haplogroups into 33 top-level haplogroups/clusters for simplified overview and navigation [11]. However, those clusters are still at different phylogenetic levels and many of them are nested within each other.

557 A reasonable solution to the current problems of phylogenetically insensitive
558 nomenclature could be an implementation of informed grouping recommendations
559 (i.e. strictly defining **"macro-haplogroups"**, **"meso-haplogroups"**, and **"micro-**
560 **haplogroups"**) as well as the implementation of a new algorithm-guided
561 nomenclature concept in the output of haplogroup callers such as *HaploGrep3*. As
562 a new nomenclature (supplementary to traditional nomenclature), we propose
563 implementing a concept similar to the "*SNP-address"* [50]. This nomenclature,
564 originally developed for pathogens, can capture the relationship between isolates to
565 the single nucleotide resolution. The *"SNP address"* concept relies on the
566 hierarchical single linkage clustering of sequences based on the pairwise SNP
567 distances at seven descending thresholds of SNP distances (250, 100, 50, 25, 10,
568 5, 0). This clustering produces for each haplotype a unique name made of seven
569 numbers separated by dots. Each number in the name indicates the cluster
570 membership at each of the seven predefined SNP distance thresholds. The *"SNP*
571 *address"* thus provides a nomenclature that is phylogenetically meaningful and
572 ensures that two isolates with identical sequences would have the same SNP
573 address. For example, SNP addresses 1.1.1.1.1.1.1 and 1.1.1.1.1.1.2 indicate that
574 two isolates belong to the same clusters based on 250, 100, 50, 25, 10, and 5 SNP
575 distance thresholds but that they differ from each other by at least one SNP. A similar
576 concept can be developed for mtDNA (e.g. *"**mtHg-address**"*) where several
577 phylogenetic levels of haplogroups could be defined based on the phylogenetic tree
578 and pairwise distances between haplotypes. For example, "*mtHg-address"* could be
579 defined using *TreeCluster* with several different threshold values. Each threshold
580 value would ensure that the maximum pairwise distance between leaves of the
581 provided phylogenetic tree is at most equal to the specified threshold (e.g. seven
582 threshold values ranging from 0.006 to 0). Some of those levels, carefully chosen to
583 be informative about mtDNA biogeography, could also be used as guides for
584 defining **macro-, meso-, and micro-haplogroups** using the traditional
585 nomenclature (see example in Table 1). The creation of *"mtHg-addresses"* and
586 micro-, meso-, and macro-haplogroups could be performed on the single mtDNA
587 tree including all known mtDNA diversity, and then implemented in *HaploGrep3*.
588
589

SampleID	Haplogroup	Micro-haplogroup	Meso-haplogroup	Macro-haplogroup	mtHg-address
NA19454	L0f1	L0f1	L0f	L0a'b'f'g	2.2.1.1.1.1.1
NA19904	L0a2a2a	L0a2	L0a	L0a'b'f'g	2.1.1.1.1.1.1
NA19463	L4b2a2b	L4b2	L4	L3'4	1.2.1.1.1.1.1
HG02445	B4a1a1b	R	N	L3'4	1.1.2.2.1.1.1
HG00106	J2b1a	R	N	L3'4	1.1.2.1.1.1.1
HG02511	A2n*2	A	N	L3'4	1.1.1.1.1.1.1

590
591 **Table 1. Hypothetical illustration of how *HaploGrep3* output could be updated to include**
592 **information about predefined and standardized micro-, meso-, and macro-haplogroups, as**
593 **well as the *mtHg-address* unique to each haplotype.** Note that the categories presented here
594 are only for illustration purposes, and thus they are not proposals for actual standardization.

The changes we proposed here would overcome problems related to traditional mtDNA nomenclature, reduce biases introduced by arbitrarily defined secondary nomenclature-based groupings, foster reproducibility across studies, and importantly, could be implemented into haplogroup callers such as *HaploGrep3*. Such changes would be of great importance and relevance for population genetics, forensics, and medical genetics.

Key words

Mitochondria, mtDNA, mtDNA nomenclature, haplogroup, classification, grouping, PhyloTree, HaploGrep.

Declarations

Data Availability

All scripts and output data are available on GitHub: https://github.com/bajicv/mtDNA_nomenclutter
Zenodo: https://doi.org/10.5281/zenodo.10156923

All mentioned data, software, tools, and packages are publicly available and listed below:

Data	Source
1KGP mtDNA	http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/supporting/MT/chrMT_sequences_2534.20160505.fasta.gz
Related individuals	http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/20140625_related_individuals.txt
rCRS	http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/supporting/MT/rCRS.fasta.gz
RSRS	https://www.cell.com/cms/10.1016/j.ajhg.2012.03.002/attachment/34f040af-c97e-4bfa-854b-d2238be91d2e/mmc2.zip

Software/tool/package	Source
UGENE (v42.0)	http://ugene.net
MAFFT (v7.310)	http://mafft.cbrc.jp/alignment/software /
IQ-TREE (v2.0.3 Linux)	http://www.iqtree.org
HaploGrep3 (v3.2.1)	https://haplogrep.i-med.ac.at/
TreeCluster	https://github.com/niemasd/TreeCluster
R (v4.2.0)	https://www.cran.r-project.org/bin/macosx/
R Studio (v1.1.383)	https://www.rstudio.com/products/rstudio/download/
rhierBAPS	https://github.com/gtonkinhill/rhierbaps
ape	https://www.cran.r-project.org/web/packages/ape/index.html
seqinr	https://www.cran.r-project.org/web/packages/seqinr/index.html
phytools	https://www.cran.r-project.org/web/packages/phytools/index.html
ggtree	https://www.bioconductor.org/packages/release/bioc/html/ggtree.html
tidyverse	https://www.tidyverse.org
FactoMineR	https://www.cran.r-project.org/web/packages/FactoMineR/index.html
factoextra	https://www.cran.r-project.org/web/packages/factoextra/index.html
ggpubr	https://cran.r-project.org/web/packages/ggpubr/index.html
cowplot	https://cran.r-project.org/web/packages/cowplot/index.html
rworldmap	https://cran.r-project.org/web/packages/rworldmap/index.html

Authors' contributions

Vladimir Bajić and Vanessa Hava Schulmann shared first authorship.

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Formal Analysis	Vladimir Bajić, Vanessa Hava Schulmann
Funding Acquisition	Katja Nowick
Investigation	Vanessa Hava Schulmann, Vladimir Bajić
Methodology	Vladimir Bajić
Project Administration	Vladimir Bajić
Resources	Vladimir Bajić, Katja Nowick
Software	Vladimir Bajić, Vanessa Hava Schulmann
Supervision	Vladimir Bajić, Katja Nowick
Validation	Vladimir Bajić, Vanessa Hava Schulmann
Visualization	Vladimir Bajić
Writing – Original Draft Preparation	Vanessa Hava Schulmann, Vladimir Bajić
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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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Abbreviations

NBG	-	Nomenclature-Based Groupings
SC	-	Single Character grouping
SCL	-	Single Character and L with one digit grouping
ABG	-	Algorithm-Based Groupings
mtDNA	-	Mitochondrial DNA
rCRS	-	The revised Cambridge Reference Sequence
RSRS	-	The Reconstructed Sapiens Reference Sequence
MDS	-	Multidimensional scaling
CA	-	Correspondence analysis
rhb_01	-	Results of *rhierBAPS* level 1
rhb_02	-	Results of *rhierBAPS* level 2
rhb_03	-	Results of *rhierBAPS* level 3
tc_0.006	-	Results of *TreeCluster* with the threshold value of 0.006
tc_0.005	-	Results of *TreeCluster* with the threshold value of 0.005
tc_0.004	-	Results of *TreeCluster* with the threshold value of 0.004
tc_0.003	-	Results of *TreeCluster* with the threshold value of 0.003

Population abbreviations

ACB	-	African Caribbean in Barbados
ASW	-	African Ancestry in SW USA
MSL	-	Mende in Sierra Leone
GWD	-	Gambian in Western Division - Mandinka
ESN	-	Esan in Nigeria
YRI	-	Yoruba in Ibadan, Nigeria
LWK	-	Luhya in Webuye, Kenya

Supplementary Material

Supplementary Tables

[Symbol]	No.	%	Example	Comment
[-]	**1**	0,02%	mt-MRCA	
[.]	**1**	0,02%	R0a+60.1T	Insertions are indicated by the position number preceding the insertion followed by a dot (.), the relative insert position, and the inserted base(s) (from PhyloTree.org - mtDNA tree Build 17).
["]	**2**	0,03%	M4"67+16311	(as M4"67 in tree.xml)
[( ] and [ )]	**31**	0,49%	M7b1a1+(16192)	(always occurs together with +) Mutations between brackets () are recurrent/unstable within the respective clade, or are yet uncertain based on current data (from PhyloTree.org - mtDNA tree Build 17).
[@]	**34**	0,53%	U5a1+@16192	
[']	**109**	1,71%	U2c'd	
[A-Z][A-Z]	**116**	1,82%	CZ, HV, JT, R2'JT	(excluding mt-MRCA) A clade can be referred to using the names of its prominent subclades. E.g. CZ refers to the smallest monophyletic clade including Y and Z (Richards et al. 1998).
[a-z][a-z]	**179**	2,81%	A2aa	(excluding mt-MRCA)
[+]	**305**	4,78%	U2+152	
[*]	**945**	14,81%	U6b*	Haplogroup followed by * denotes all sequences outside of its already named clades. E.g. if U6b1, U6b2, and U6b3 are named then: U6b* = U6b - (U6b1 ∪ U6b2 ∪U6b3). Such derived cluster notations are only preliminary and may change in the future (Richards et al. 1998).
at least one of ["*()@+"]	**1199**	18,79%	V+@72, U5b1+16189+@16192	
at least one of ["*-()@+"]	**1306**	20,47%	X2+225+@153, U6a+16189+(103), U3a'c	(including mt-MRCA)
at least one exception	**1559**	24,44%	A2ae*2, HV+16311*, X2m'n	
without exception	**4821**	75,56%	X2e2a1	

Table S1. The number of haplogroup names from *phylotree-fu-rcrs-1.2* (total of 6380 haplogroups) that do not follow strict cladistic nomenclature with haplogroup names starting with capital letters followed with alternating numbers and small letters. Note that the "cladistic notation for mitochondrial clusters" established by Richards et al. 1998 (see Test Box 1) permits composed names to refer to monophyletic clades based on subclades (e.g. CZ), and usage of * that designates all sequences that do not belong to any of the already named subclades (green rows in the table).

Table S2. Overview of Nomenclature-Based Groupings (NBGs) used in the research articles published in years 2020-2023 through Nature Publishing Group (https://www.nature.com/search/advanced) with the search term: "africa* haplogrep* human* mtdna* haplogroup* OR africa* haplogrep* human* mitochondria* haplogroup*". Each research article was categorized based on the NBG employed.

Supplementary Figures

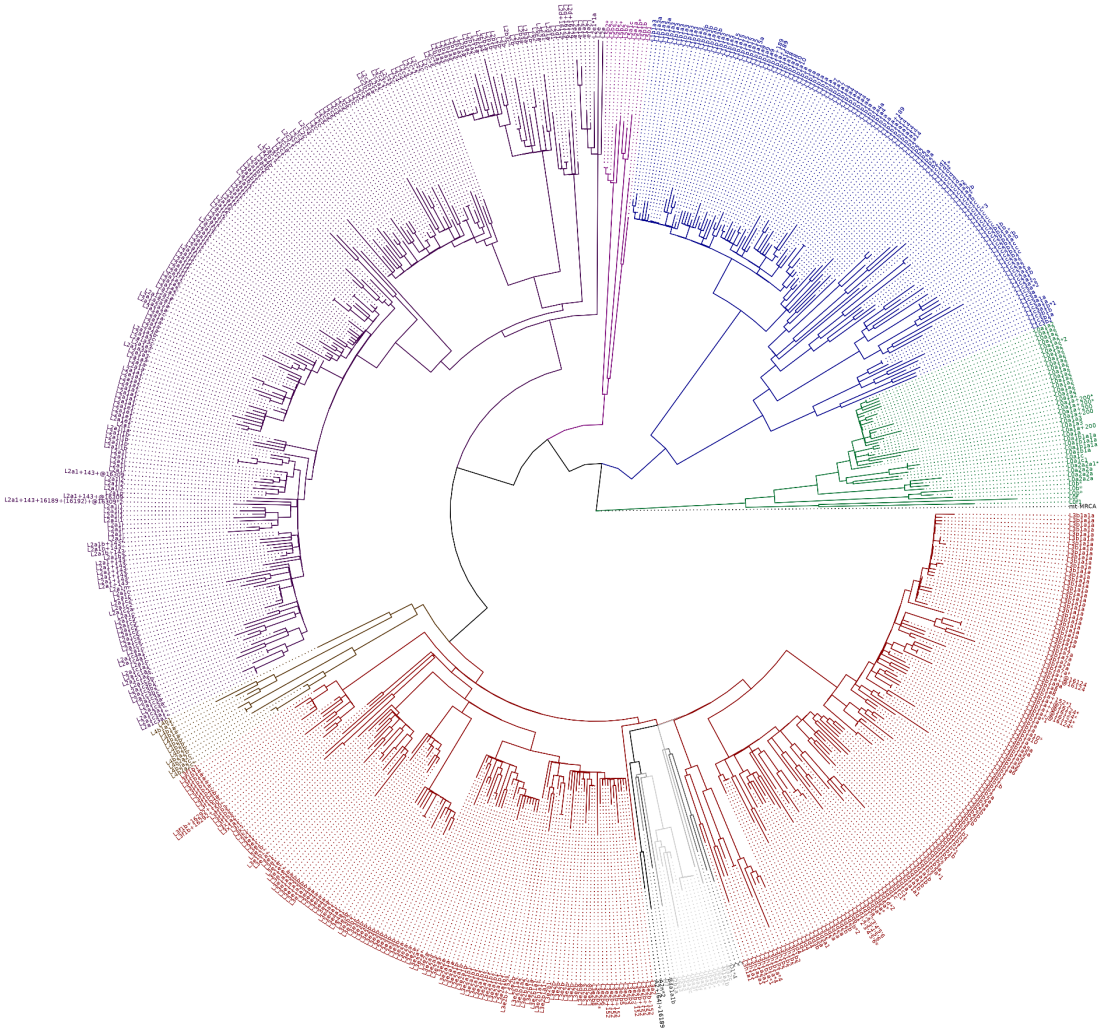

Figure S1. The mtDNA ML consensus trees of 660 samples belonging to seven African Ancestry populations (ACB, ASW, MSL, GWD, ESN, YRI, LWK) from the Phase 3 release of the 1KGP. Colors indicate SCL membership (single character and L with one-digit grouping). RSRS (mt-MRCA) was added for rooting the tree. The labels indicate haplogroup reported by *HaploGrep3*.

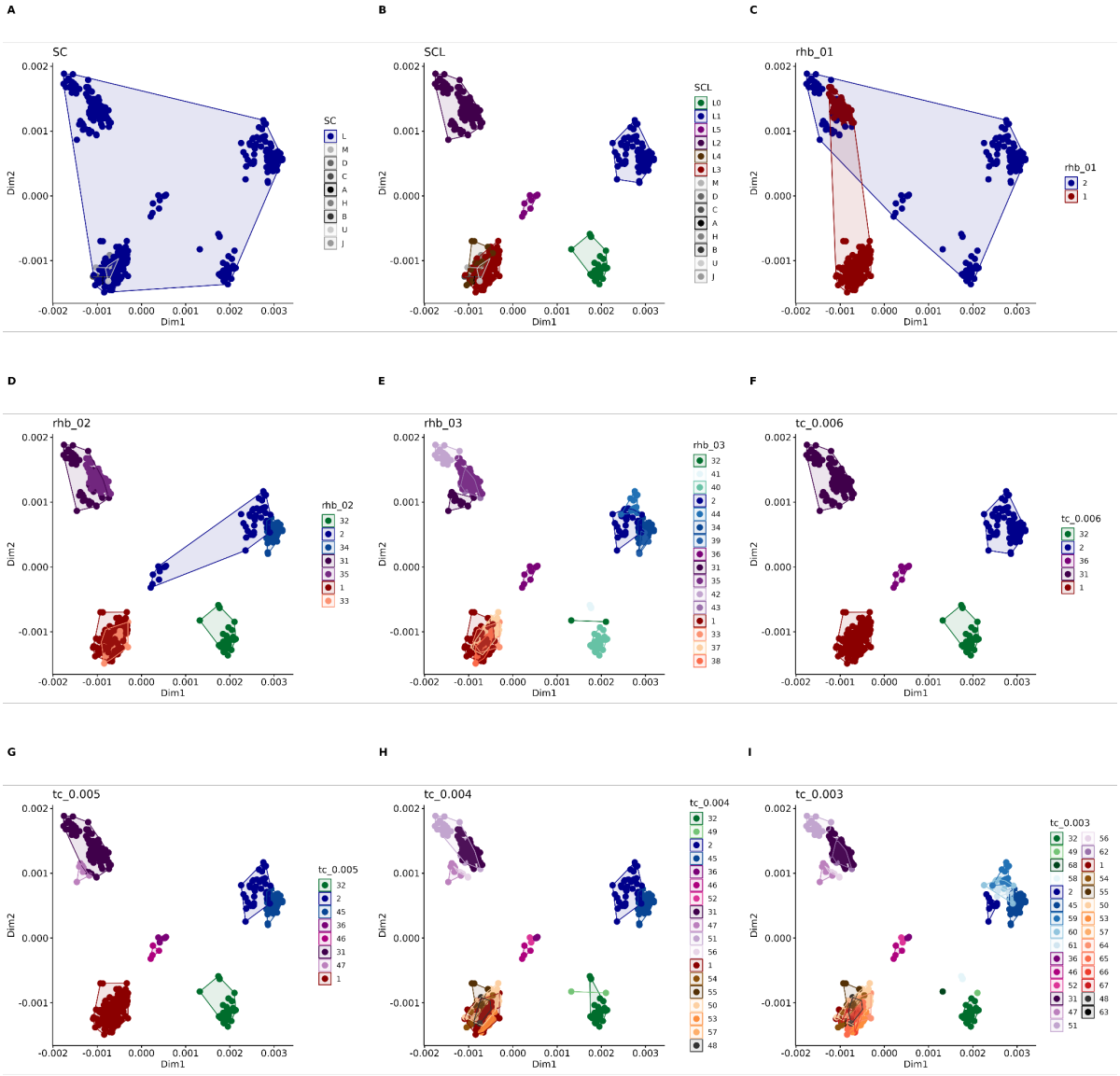

Figure S2. MDS plots based on the mtDNA pairwise distances between individuals colored by NBGs: A) SC, and B) SCL; and ABGs: C-E) *rhierBAPS*, and F-I) *TreeCluster*. Note remarkable differences between SC and SCL as well as an increase in the number of haplogroups with increasing threshold values for ABGs.

References

1. Kivisild T. Maternal ancestry and population history from whole mitochondrial genomes. Investigative Genetics. 2015;6.
2. Underhill PA, Kivisild T. Use of y chromosome and mitochondrial DNA population structure in tracing human migrations. Annual Review of Genetics. 2007;41:539–64.
3. Torroni A, Achilli A, Olivieri A, Semino O. Haplogroups and the history of human evolution through mtDNA. Hum Mitochondrial Genome From Basic Biol to Dis. 2020;:111–29.
4. Torroni A, Schurr TG, Yang C-C, Szathmary EJE, Williams RC, Schanfield MS, et al. Native American Mitochondrial DNA Analysis Indicates That the Amerind and the Nadene Populations Were Founded by Two Independent Migrations. 1992.
5. Torroni A, Sukernik R 1, Schurr TG, Starikovskaya YB, Cabell MF, Crawford MH, et al. mtDNA Variation of Aboriginal Siberians Reveals Distinct Genetic Affinities with Native Americans. 1993.
6. Torroni A, Schurr TG, Cabell MF, Brown MD, Neel J V, Larsen M, et al. Asian Affinities and Continental Radiation of the Four Founding Native American mtDNAs. 1993.
7. Chen Y-S, Torroni A, Excoffier L, Santachiara-Benerecetti AS, Wallace' DC, Wallace DC. Analysis of mtDNA Variation in African Populations Reveals the Most Ancient of All Human Continent-Specific Haplogroups. 1995.
8. RICHARDS MB, MACAULAY VA, BANDELT H-J, SYKES BC. Phylogeography of mitochondrial DNA in western Europe. Ann Hum Genet. 1998;62:241–60.
9. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009;30.
10. van Oven M. PhyloTree Build 17: Growing the human mitochondrial DNA tree. Forensic Sci Int Genet Suppl Ser. 2015;5:e392–4.
11. Schönherr S, Weissensteiner H, Kronenberg F, Forer L. Haplogrep 3 - an interactive haplogroup classification and analysis platform. Nucleic Acids Res. 2023. https://doi.org/10.1093/nar/gkad284.
12. Dür A, Huber N, Parson W. Fine-tuning phylogenetic alignment and haplogrouping of mtdna sequences. Int J Mol Sci. 2021;22.
13. Rubin JD, Vogel NA, Gopalakrishnan S, Sackett PW, Renaud G. HaploCart: Human mtDNA haplogroup classification using a pangenomic reference graph. PLOS Comput Biol. 2023;19:e1011148.
14. Jagadeesan A, Ebenesersdóttir SS, Guðmundsdóttir VB, Thordardottir EL, Moore KHS, Helgason A. HaploGrouper: A generalized approach to haplogroup classification. Bioinformatics. 2021;37:570–2.
15. Balaban M, Moshiri N, Mai U, Jia X, Mirarab S. TreeCluster: Clustering biological sequences using phylogenetic trees. PLoS One. 2019;14.
16. Tonkin-Hill G, Lees JA, Bentley SD, Frost SDW, Corander J. RhierBAPs: An R implementation of the population clustering algorithm hierbaps [version 1; referees: 2 approved]. Wellcome Open Res. 2018;3.
17. Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature. 2016;538:161–4.
18. Yamamoto K, Sakaue S, Matsuda K, Murakami Y, Kamatani Y, Ozono K, et al. Genetic and phenotypic landscape of the mitochondrial genome in the Japanese population. Commun Biol. 2020;3.
19. Swerdlow RH, Hui D, Chalise P, Sharma P, Wang X, Andrews SJ, et al. Exploratory analysis of mtDNA haplogroups in two Alzheimer's longitudinal cohorts. Alzheimer's Dement. 2020;16:1164–72.
20. Wei W, Gomez-Duran A, Hudson G, Chinnery PF. Background sequence characteristics influence the occurrence and severity of disease-causing mtDNA mutations. PLoS Genet. 2017;13.
21. Chen C, Li Y, Tao R, Jin X, Guo Y, Cui W, et al. The genetic structure of Chinese Hui ethnic group revealed by complete mitochondrial genome analyses using massively parallel sequencing. Genes (Basel). 2020;11:1–17.
22. Maier PA, Runfeldt G, Estes RJ, Vilar MG. African mitochondrial haplogroup L7: a 100,000-year-old maternal human lineage discovered through reassessment and new sequencing. Sci Rep. 2022;12.
23. Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, et al. The Genetic Structure and History of Africans and African Americans. 2009.
24. Rishishwar L, Jordan IK. Implications of human evolution and admixture for mitochondrial replacement therapy. BMC Genomics. 2017;18.
25. Røyrvik EC, Burgstaller JP, Johnston IG. mtDNA diversity in human populations highlights the merit of haplotype matching in gene therapies. Mol Hum Reprod. 2016;22:809–17.
26. Pipek OA, Medgyes-Horváth A, Dobos L, Stéger J, Szalai-Gindl J, Visontai D, et al. Worldwide

human mitochondrial haplogroup distribution from urban sewage. Sci Rep. 2019;9.
27. Wang H, Chen M, Chen C, Fang Y, Cui W, Lei F, et al. Genetic Background of Kirgiz Ethnic Group From Northwest China Revealed by Mitochondrial DNA Control Region Sequences on Massively Parallel Sequencing. Front Genet. 2022;13.
28. Auton A, Abecasis GR, Altshuler DM, Durbin RM, Bentley DR, Chakravarti A, et al. A global reference for human genetic variation. Nature. 2015;526:68–74.
29. Richard M. Andrews IKPFCRNLDMT& NH. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet . 1999;23.
30. Behar DM, van Oven M, Rosset S, Metspalu M, Loogväli EL, Silva NM, et al. A “copernican” reassessment of the human mitochondrial DNA tree from its root. Am J Hum Genet. 2012;90:675–84.
31. Okonechnikov K, Golosova O, Fursov M, Varlamov A, Vaskin Y, Efremov I, et al. Unipro UGENE: A unified bioinformatics toolkit. Bioinformatics. 2012;28:1166–7.
32. Katoh K, Misawa K, Kuma K-I, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. 2002.
33. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268–74.
34. Yu G, Smith DK, Zhu H, Guan Y, Lam TTY. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol. 2017;8:28–36.
35. R Core Team. R: A Language and Environment for Statistical Computing. 2021.
36. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. J Open Source Softw. 2019;4:1686.
37. Kassambara A. ggpubr: “ggplot2” Based Publication Ready Plots. 2020.
38. Lê S, Josse J, Rennes A, Husson F. FactoMineR: An R Package for Multivariate Analysis. 2008.
39. Alboukadel Kassambara [aut cre], FM [aut]. Package “factoextra” Type Package Title Extract and Visualize the Results of Multivariate Data Analyses. 2022.
40. Paradis E, Schliep K. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 2019;35:526–8.
41. South A. rworldmap: A New R package for Mapping Global Data. 2011.
42. Micheletti SJ, Bryc K, Ancona Esselmann SG, Freyman WA, Moreno ME, Poznik GD, et al. Genetic Consequences of the Transatlantic Slave Trade in the Americas. Am J Hum Genet. 2020;107:265–77.
43. van Oven M. Revision of the mtDNA tree and corresponding haplogroup nomenclature. Proceedings of the National Academy of Sciences of the United States of America. 2010;107.
44. Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, et al. Updating the East Asian mtDNA phylogeny: A prerequisite for the identification of pathogenic mutations. Hum Mol Genet. 2006;15:2076–86.
45. Kong QP, Bandelt HJ, Zhao M, Zhang YP, Yao YG. Reply to van Oven: Suggestions and caveats for naming mtDNA haplogroup. Proc Natl Acad Sci U S A. 2010;107:E40–1.
46. Wei D, Jiang Q, Wei Y, Wang S. A novel hierarchical clustering algorithm for gene sequences. BMC Bioinformatics. 2012;13.
47. Tonkin-Hill G, Lees JA, Bentley SD, Frost SDW, Corander J. Fast hierarchical Bayesian analysis of population structure. Nucleic Acids Res. 2019;47:5539–49.
48. Hammer M. A nomenclature system for the tree of human Y-Chromosomal binary haplogroups. Genome Res. 2002;12:339–48.
49. van Oven M, van Geystelen A, Kayser M, Decorte R, Larmuseau MH. Seeing the wood for the trees: A minimal reference phylogeny for the human Y chromosome. Hum Mutat. 2014;35:187–91.
50. Dallman T, Ashton P, Schafer U, Jironkin A, Painset A, Shaaban S, et al. SnapperDB: a database solution for routine sequencing analysis of bacterial isolates. Bioinformatics. 2018;34:3028–9.