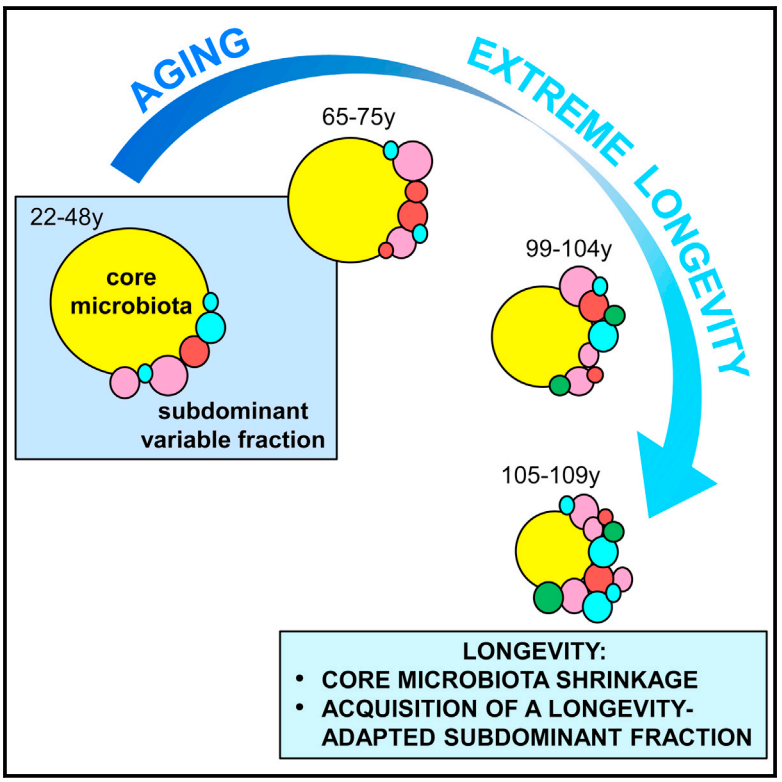
Report

Current Biology

Gut Microbiota and Extreme Longevity

Graphical Abstract



Highlights

- A core microbiota accompanies human life, decreasing in abundance along with aging
- In longevity, the age-related enrichment of subdominant taxa is boosted
- The microbiota of longevous hosts accommodates allochthonous bacteria
- "Longevity adaptation" seems to involve enrichment in health-associated gut bacteria

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In Brief

Biagi et al. reconstructed the longest available human microbiota trajectory by analyzing persons >105 years old, compared to adults, elderly, and centenarians. In longevity, the age-related increase of subdominant species is boosted, accommodating, along with pro-inflammatory species, also health-associated taxa that might support extreme aging.

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Gut Microbiota and Extreme Longevity

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SUMMARY

The study of the extreme limits of human lifespan may allow a better understanding of how human beings can escape, delay, or survive the most frequent age-related causes of morbidity, a peculiarity shown by long-living individuals. Longevity is a complex trait in which genetics, environment, and stochasticity concur to determine the chance to reach 100 or more years of age [1]. Because of its impact on human metabolism and immunology, the gut microbiome has been proposed as a possible determinant of healthy aging [2, 3]. Indeed, the preservation of host-microbes homeostasis can counteract inflammaging [4], intestinal permeability [5], and decline in bone and cognitive health [6, 7]. Aiming at deepening our knowledge on the relationship between the gut microbiota and a long-living host, we provide for the first time the phylogenetic microbiota analysis of semi-supercentenarians, i.e., 105–109 years old, in comparison to adults, elderly, and centenarians, thus reconstructing the longest available human microbiota trajectory along aging. We highlighted the presence of a core microbiota of highly occurring, symbiotic bacterial taxa (mostly belonging to the dominant *Ruminococcaceae*, *Lachnospiraceae*, and *Bacteroidaceae* families), with a cumulative abundance decreasing along with age. Aging is characterized by an increasing abundance of subdominant species, as well as a rearrangement in their co-occurrence network. These features are maintained in longevity and extreme longevity, but peculiarities emerged, especially in semi-supercentenarians, describing changes that, even accommodating opportunistic and allochthonous bacteria, might possibly support health maintenance during aging, such as an enrichment and/or higher prevalence of health-associated groups (e.g., *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*).

RESULTS AND DISCUSSION

Twenty-four semi-supercentenarians (105+; group S), i.e., 105–109 years old (18 females and 6 males; mean age 106.2), were enrolled for this study in Emilia Romagna and surrounding area, Italy. Fifteen young adults (group Y; eight females and seven males; aged 22–48 years; average age 30.5) were enrolled in the same geographic area. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy) as EM/26/2014/U (with reference to 22/2007/U/Tess). Feces were collected, and total bacterial DNA was extracted from all samples (see the Supplemental Experimental Procedures).

To complete a human aging trajectory, we included extracted fecal DNA, stored at −80°C, from 15 centenarians (group C; 14 females and 1 male; aged 99–104 years; mean age 100.4) and 15 younger elderly (group E; seven females and eight males; aged 65–75 years; mean age 72.5; see also Table S2) enrolled in the same geographic area (Emilia Romagna, Italy), obtained by Biagi et al. [4], in the present study.

For detailed information on physical and cognitive status of the subjects enrolled and a summary of the reported dietary habits, see the Supplemental Experimental Procedures and Tables S1 and S2; in brief, young adults were healthy and medication-free, whereas the physical and cognitive health status of 105+ (as well as that of the centenarians enrolled in the previous study) [4], assessed by ADL (activities of daily living) scale [8] and standardized mini-mental state examination test (SMMSE) [9], mirrored that of the majority of Italian centenarians, as previously characterized by Franceschi et al. [10].

The fecal microbiota of the 69 samples was characterized by Illumina sequencing of the V3–V4 region of the bacterial 16S rRNA gene (see the Supplemental Experimental Procedures; sequences are available at the following MG-Rast link: http://metagenomics.anl.gov/linkin.cgi?project=17761). A total of 1,246,682 high-quality reads were obtained with a mean of 18,068 reads per subject. Reads were clustered in 11,587 operational taxonomic units at 97% of identity.

The four age groups showed a good separation on a principal coordinates analysis (PCoA) based on the unweighted UniFrac distance (Figure 1); indeed, corrected p values obtained by permutation test were <0.05 for all possible comparisons with the exception of groups C versus S. PCo1 separated young subjects

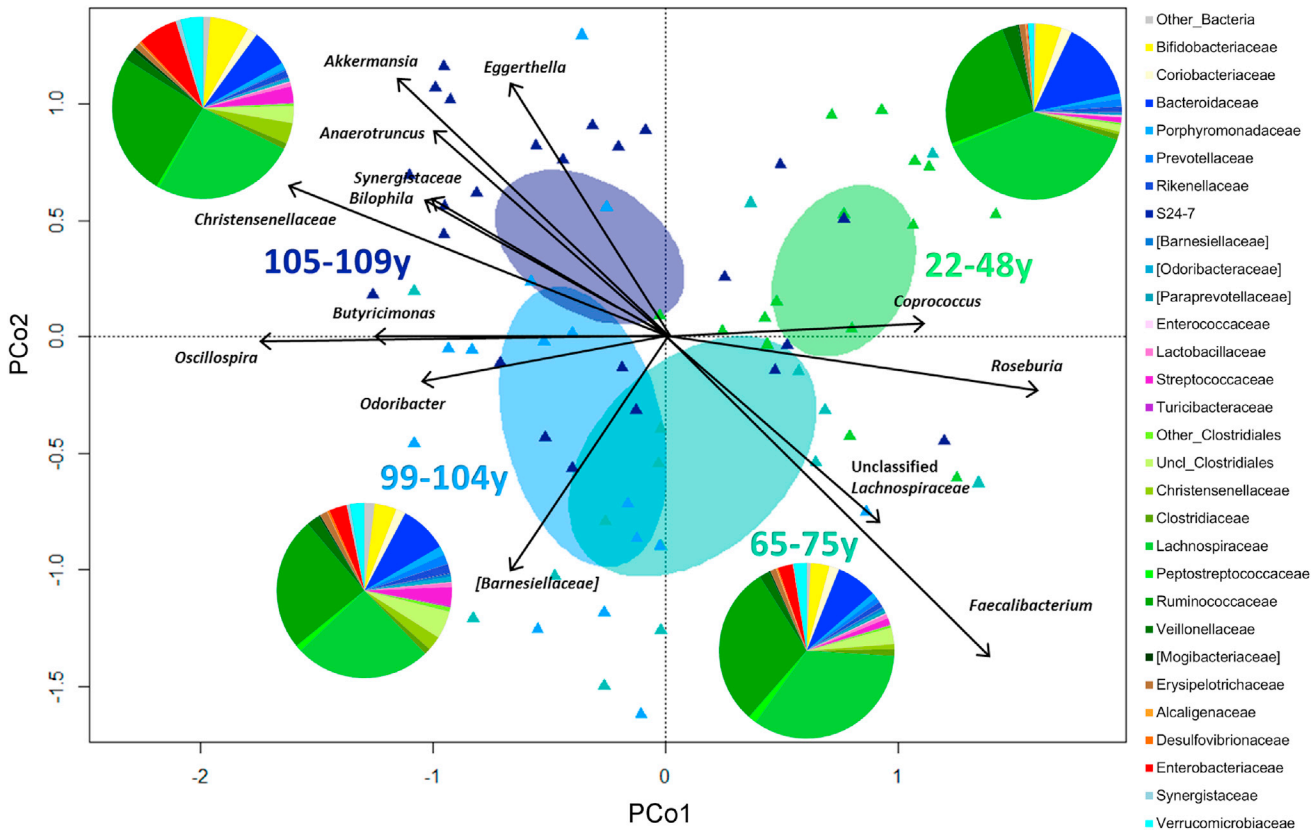

Figure 1. Gut Microbiota Variations across Different Age Groups

PCoA based on unweighted UniFrac distances of the fecal microbiota of the enrolled young adults (green), elderly (turquoise), centenarians (light blue), and semi-supercentenarians (dark blue). SEM-based ellipse around the centroid is plotted. Samples are identified by filled triangles. The first and second principal components (PCo1 and PCo2) are shown, explaining 6.6% and 4.0% of the variance in the dataset, respectively. For each group of subjects, a pie chart based on the average relative abundance at family level is shown; colors for each family are reported in the legend. The biplot of the average bacterial coordinates weighted by the corresponding bacterial abundance per sample was superimposed on the PCoA plot to identify the bacterial genera or families contributing to the ordination space (black arrows). Only the bacterial groups showing a highly significant correlation with the sample separation ($p < 0.005$) were considered. See also Figure S1 and Table S1.

(Y) from elderly (E) and long-living individuals (groups C and S; Pearson's $r = -0.61$; $p < 0.001$). As noticeable in the pie charts in Figure 1, the fecal microbiota in all age groups was dominated by just three families: *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, but their cumulative relative abundance decreased along with aging (77.8% ± 8.5% in Y; 71.1% ± 12.3% in E; 58.7% ± 11.8% in C; 57.7% ± 15.0% in S), highlighting an age-dependent increasing contribution of subdominant families. Seventy-year-old people (group E) showed similarities with young adults, such as the cumulative abundance of *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, but started to show also some of the age-associated features observed in centenarians, as demonstrated by the partial overlapping of the samples of the two groups in the PCoA. Centenarians and 105+ showed very similar relative abundance of *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, as well as overlapping coordinate values on PCo1 (average PCo1 coordinates −0.36 and −0.33 for group C and S, respectively), but they significantly separated on PCo2 (average PCo2 coordinates −0.38 and 0.38 for groups C and S, respectively; pseudo-F-ratio permutational test; $p < 0.05$), hinting that differences were present between the microbiota structures of these two groups even if the age gap was very small, i.e., 6 years only in average.

To identify the bacterial genera or families with the most significant contribution (permutational correlation test; $p < 0.005$) to the sample ordination, we superimposed the genus/family abundance on the PCoA plot, identifying spatial correlations between samples and bacterial groups (Figure 1). The bacterial genera or families plotted in Figure 1 showed an interesting age-related ascending or descending trajectory (Figure 2). In particular, the abundance of *Coprococcus*, *Roseburia*, and *Faecalibacterium*, belonging to the *Lachnospiraceae* and *Ruminococcaceae* families, was negatively associated with age. The trend observed for *Coprococcus* and *Faecalibacterium* was already reported in Chinese centenarians [11], suggesting that they can be part of the aging process itself, regardless of lifestyle and dietary habits. On the contrary, *Oscillospira* was positively correlated with age, as well as two subdominant members of the Bacteroidales order (*Odoribacter* and *Butyricimonas*). Interestingly, a group of subdominant genera and families (*Eggerthella*, *Akkermansia*, *Anaerotruncus*, *Synergistaceae*, *Bilophila*, and *Christensenellaceae*) described a steeper, increasing trajectory along with aging

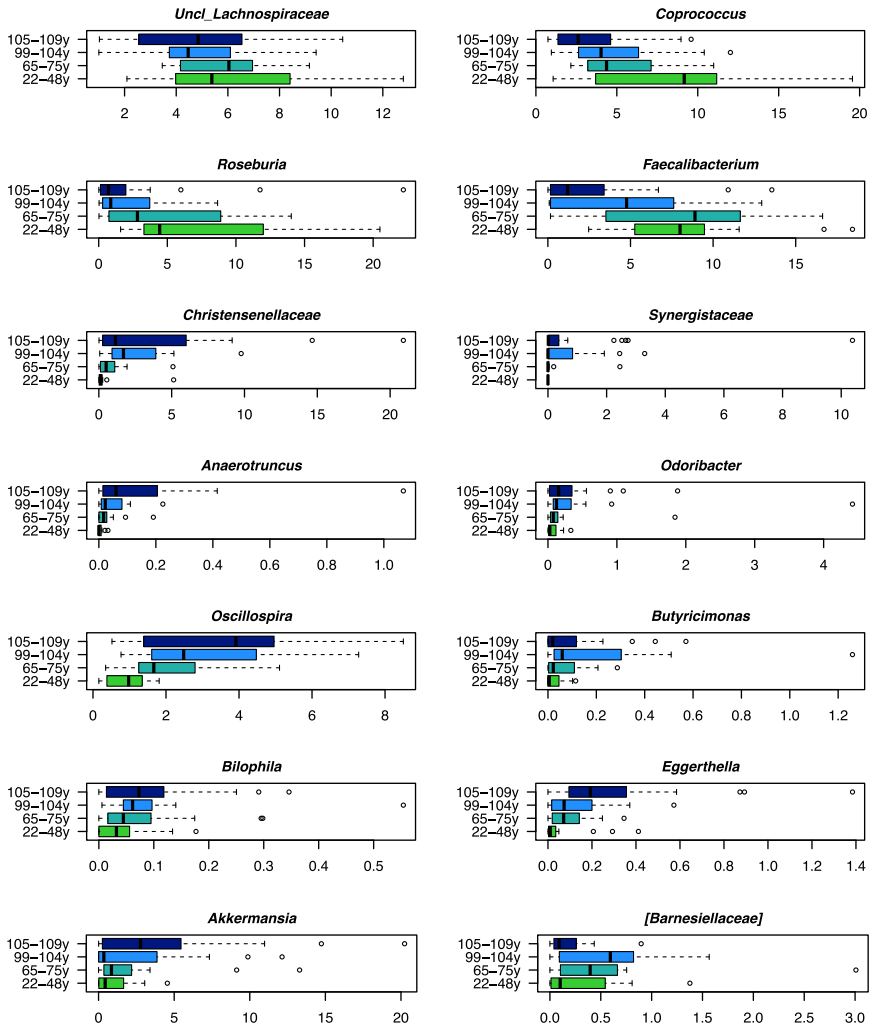

Figure 2. Aging-Related Trajectories of Selected Gut Microbiota Genera and Families

Box-and-whisker plot of the relative abundance distributions of the bacterial genera and families identified in the biplot in Figure 1. The distribution for each age group is shown (young adults, green; elderly, turquoise; centenarians, light blue; semi-supercentenarians, dark blue). Bacterial abundance is given as percentage of total sequences obtained for each sample. The central box of each dataset represents the distance between the 25th and the 75th percentiles. The median between them is marked with a black line. Whiskers identify the 10th and the 90th percentiles.

with a more prominent increase in the 105+ group, being responsible for the separation of the S group in the top-left portion of the plot (Figures 1 and 2).

In order to explore the evolution of the gut microbiota network along with human aging, we performed an analysis of co-occurrence, meaning the frequency of concomitant detection of two bacterial groups. Co-occurrence associations between genera were obtained as detailed in the Supplemental Experimental Procedures, and genera were clustered into four co-occurrence groups (COGs) ($p < 0.01$; permutational multivariate ANOVA; see also Figure S1) according to the co-occurrence pattern. Co-occurrence network plots for all samples together (Figure 3A) and for the four age groups (Figures 3B–3E) were obtained, using the prevalence of bacterial genera in the microbiota of all samples or each age group (i.e., the percentage of subjects in each group in which a genus was present) as factor proportional to the dimension of the spots. Plotted genera showed relative abundance >0.5% in at least 30% of subjects in the considered group. The four COGs were named *Bacteroides* (yellow), *Roseburia* (pink), *Lachnospira* (red), and *Dialister* (cyan) COG. The *Bacteroides* COG defined a sort of core microbiome including highly co-occurring genera, almost always present, with high prevalence, in all age groups. These genera together represented the majority of the intestinal ecosystem in terms of relative abundance, accounting for 68.6% in average in all samples, but with a coverage decreasing along with age: 75.3% in group Y; 70.9% in group E; 65.7% in group C; and 64.9% in group S (see also Figure S1). Genera in this COG did not show sensible variations in prevalence across age groups, with the exception of *Faecalibacterium*, for which a marked decrease in prevalence was found (100% in Y and E, 93% in C, and 79% in S), and *Bifidobacterium* that showed a decreasing trend in E and C (80% and 87%, respectively) compared to Y (93%) to go up again in S (92%). On the contrary, aging involved a reassembly of the other three COGs, which emerged as ancillary, mutually exclusive groups of genera poorly co-occurring among themselves. The *Dialister* COG was the most variable in terms of present genera and co-occurrence network in the different age groups: in group Y, only three genera were present; in group E, more genera appeared; whereas in group C, some of those disappeared, leaving space for unclassified members of the *Mogibacteriaceae* family. Semi-supercentenarians' co-occurrence and prevalence network shared some features with the centenarians' one (presence of unclassified *Christensenellaceae* in the *Roseburia* COG; presence of unclassified *Enterobacteriaceae* in the *Lachnospira* COG) but showed also some peculiarities, such as the high prevalence of *Akkermansia* in the *Roseburia* COG and *Phascolarctobacterium* in the *Lachnospira* COG and the mono-genus arrangement of the *Dialister* COG, in which only the unclassified *Mogibacteriaceae* are present with a very high prevalence (92%).

According to these observations, extreme longevity seems to involve an invasion of the gut ecosystem by micro-organisms typical from other niches, such as *Mogibacteriaceae* and *Synergistaceae*, known to be abundant in the periodontal environment [12–14]. However, extremely long-living people seem to experience a parallel increase in several health-associated taxa. In particular, the family *Christensenellaceae*, which increased in

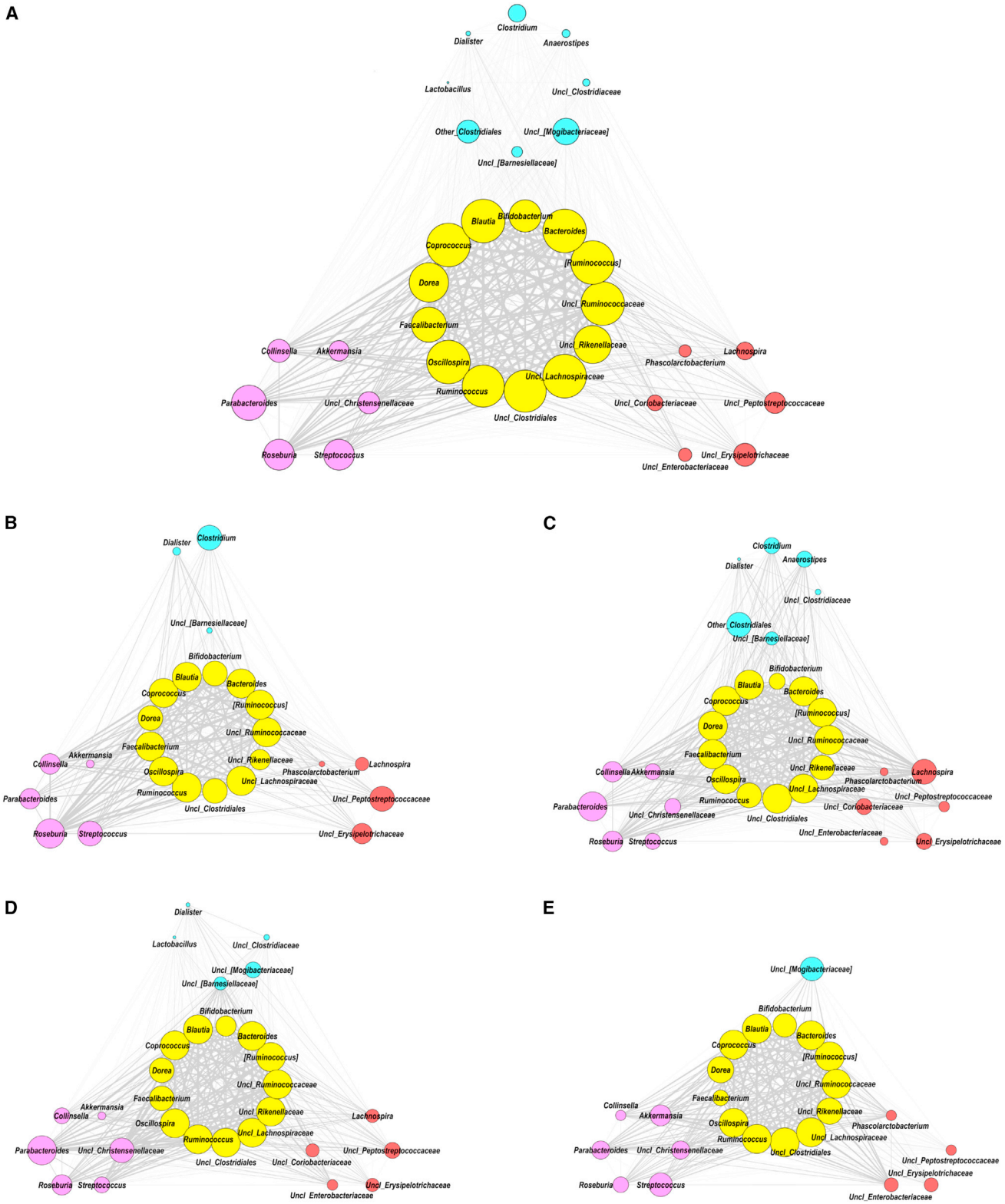

Figure 3. Co-occurrence Network and Prevalence of Genera among Age Groups

Network plots describing co-occurrence and prevalence of bacterial genera in the gut microbiota of all samples (A), young adults (B), elderly (C), centenarians (D), and semi-supercentenarians (E). Bacterial genera with at least 0.5% of relative abundance in at least 30% of the samples in each group were plotted, with the

(legend continued on next page)

terms of both relative abundance and prevalence in centenarians and 105+, is a recently reported health-associated bacterial taxon that has been inversely correlated to BMI [15] and positively associated to improved renal function [16]. Together with *Akkermansia* and *Bifidobacterium*, well-known health-associated genera whose abundance and/or prevalence interestingly increased in semi-supercentenarians, known to promote immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis [17, 18], *Christensenellaceae* might represent a signature of the ecosystem of extremely longevous people. Moreover, the family *Christensenellaceae* has recently emerged as the gut microbiota component whose abundance is the most significantly influenced by host genetics [15], suggesting an interesting possible link to the heritable component of human longevity [19].

In conclusion, we presented the longest available trajectory of the human gut microbiota along aging, with a focus on longevity and extreme longevity, represented by a group of 105+, a demographically very selected group of subjects, as the ratio between centenarians and 105+ is 21.7 (one 105+ every 21 100+ subjects). Confirming the known features of an aging microbiota, we highlighted the presence of a core microbiota of highly occurring, symbiotic bacterial groups, which remains approximately constant during aging but varies in the cumulative relative abundance of its members. The aging-associated microbiota is characterized by an increasing contribution of subdominant species, as well as a rearrangement in their co-occurrence network. These features are maintained in longevity and extreme longevity, but peculiarities emerged, especially in semi-supercentenarians. The microbial ecosystem found in extremely old people, even accommodating opportunistic and allochthonous bacteria, is enriched in health-associated *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*. It is not possible to know whether these health-associated features were already present at a younger age in these exceptional individuals, and/or they are somewhat related to the past lifestyle, due to the cross-sectional nature of the study; indeed, only longitudinal studies, which would be very difficult to apply to the field of human longevity, could explain whether these gut bacteria are always lost during aging and reacquired by the subjects who get to live longer or whether they are maintained across aging and longevity only by long-living subjects. However, it is tempting to hypothesize that these particular bacterial taxa might be involved in the establishment of a new homeostasis with the aging host, thus contributing to reach the extreme limits of human life.

ACCESSION NUMBERS

The accession number for the sequences reported in this paper is MG-Rast: 17761.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.04.016.

AUTHOR CONTRIBUTIONS

Conceptualization, C.F., P.B., and M. Candela; Investigation, E.B., C.C., and S.Q.; Formal Analysis, E.B., S.R., and M. Severgnini; Writing – Original Draft, E.B., S.R., and M. Candela; Writing – Review & Editing, E.B., P.B., and S.T.; Resources and Data Curation, R.O. and M. Scurti; Funding Acquisition, C.F., D.M., M. Capri, and P.B. All authors discussed the results and commented on the manuscript.

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exception of the network involving all samples (A) for which the genera present in at least one of the other networks were plotted. Co-occurrence groups (COGs) are named after the included genera with the highest relative abundance and are color coded as follows: *Bacteroides* COG (yellow); *Roseburia* COG (pink); *Lachnospira* COG (red); and *Dialister* COG (cyan). Circle size represented the prevalence, i.e., the percentage of subjects in each group in which a genus was present at 0.1% of relative abundance. The thickness of connection between nodes represented the co-occurrence. See also Figure S1.

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