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Metagenomic Description of the Feline Oral Microbiome

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Abstract

The domestic cat oral microbiome constitutes an important microecosystem for both feline and public health, but integrated data on bacteria and viruses at the national level are few. The present work shows that the feline oral microbiome is essentially a separate ecosystem guided above all by geography and climate, with husbandry adding

more subtle control. An OAPGC has been built—a national list of 443 prokaryotic of the 969 high-quality viral genomes studied, the bacteriome seems to be largely occupied by Pseudomonadota, Bacillota, or Bacteroidota, with>34%of species as uncultivated lineages. The virome is mostly Caudoviricetes -based, and almost half its viruses are unclassified, an indication of great novelty in this context. Functionally, the majority of temperate phages appear to change frequently between lytic and lysogenic cycles,a tight regulation of bacterial homeostasis matched to environmental fluctuations. Of particular interest is the fact that monkeypox virus failed to appear in any of the 1，454 tested samples—cats are not now reservoirs or vectors. Taken together, these results reveal great geographic variation, massive microbial“dark matter,”and vital viral control mechanisms. They set out a nation-wide baseline for companion animal oral microbiomes and thus help to evaluate feline pathogenesis, anticipate spillover into humans, and implement“One Health”-based approaches.

KEYWORDS: Metagenomics; Oral microbiome of felines; Virome; Geographical variations; Differences due to rearing; Monkeypox virus

1. Introduction

With the global pet industry expanding rapidly, China possessed 72.89 million urban pet cats in 2024, as well as an estimated stray population of 53 million. From the standpoint of "One Health", such a rapidly growing population elevates zoonotic risks and thus demands urgent attention to the feline oral microbiome as a public health issue [1]. Feline behaviors—biting or licking—facilitate the cross-species transmission of oral microbiota. Among the major zoonotic pathogens—namely Pasteurella multocida and Bartonella henselae—some commonly occur in the feline oral cavity and may endanger human health [2], [3]. Several taxa, such as members of the Bacteroides melaninogenicus group including Porphyromonas gingivalis and P. gulae, contribute to serious feline periodontal disorders and show cross-species transmission potential [4]. There is growing evidence that disruption of the oral microbiota (dysbiosis) is intimately coupled with host viral states, such as FIV [5]. Yet, systematic data concerning the environmental or geographic factors that shape such pathogen networks remains insufficient [1], [6].

China's distinct climatic regions and diverse feline housing conditions—whether characterized by indoor confinement or free-roaming lifestyles—have not yet been thoroughly studied regarding their effects on the oral microecosystem; this omission constitutes a critical gap in existing surveillance infrastructures [7]. Studies on the human oral microbiome have successfully linked geographic variations and lifestyles not only to microbial composition, but ultimately to overall systemic health [8], [9]. Feline oral microbiome research remains relatively limited; indeed, most present studies are

restricted in scope to small-scale, descriptive surveys [10], [11], Moreover, a heavy reliance on 16S rRNA amplicon data fails to achieve full taxonomic clarity or functional depth, and is completely blind to the host virome [2], [12]. Therefore, it is necessary to apply shotgun metagenomics to assess how macro-geographic and micro-environmental factors act synergistically to influence health, and to overcome the limitations of culture-based methodologies [13].

Addressing existing research gaps, the present work utilized a large-scale nationwide cohort to obtain 1,454 oropharyngeal swabs from 22 provinces, representing seven major geographic regions and three types of management settings (catteries, veterinary clinics, and rescue centers for strays). High-throughput shotgun metagenomic sequencing was applied to construct the first national Oral Animal/Pet Genome Collection (OAPGC), which presents an important expansion—relative to prior studies—of the known taxonomic diversity of uncultured core bacteria and novel viruses [14]. The present work provides, for the first time, empirical support for the hypothesis that macro-geographic factors and micro-scale husbandry practices jointly modulate microbial variation in the feline mouth [15]. Cross-kingdom associations between microbes and viruses are closely tied, in a synchronized manner, to environmental fluctuations. As far as emerging hazards are concerned, targeted screening revealed no Mpox virus cases, thus setting up a firm negative baseline among the studied animals.

Crucially, the multi-dimensional baseline data obtained in this work may be regarded as an invaluable resource both to public health and to precision veterinary

medicine. With regard to zoonotic surveillance, it allows the exact identification of pathogen-rich areas—whether geographically defined or linked to management intensity [16]. From an epidemiological standpoint, it clearly elucidates the colonization, movement, and evolutionary trajectories of feline-derived pathogens in various environments. In addition, the dynamic fluctuations of the oral microbiome may be profitably interpreted as a predictive "sentinel" for the early identification of zoonoses or cat-related disease outbreaks [17]. Consequently, this work provides a solid baseline for the downstream analysis of the companion animal microbiome [18]. The OAPGC reference catalog furnishes a necessary foundation for future studies on microbial functions, therapeutics, and probiotics, as well as for health protection under the "One Health" concept [13], [19], [20].

2. Methods

2.1 Collection of Metagenomic Samples from Feline Oral Swabs

From September 1，2022，up to October 30，2024，1，454 feline oropharyngeal swab specimens were obtained in a planned fashion from 22 provinces of China, with three separate types of rearing conditions being considered: catteries (n = 509) , veterinary hospitals (n = 449) , and rescue centers for strays (n = 496) .The entire national sampling effort was spread broadly over seven major geographic areas,the numbers of samples taken from Northeast China amount to 135， from North China to 223， from East China to 285， from Central China to 273， from South China to 160， from Northwest China to 138， and from Southwest China to 240.With regard to each region’s contribution to catteries, veterinary hospitals, or stray rescue groups, a very

fine division has been preserved—so as to achieve wide multi - environmental coverage.

For every one of the specified geographic areas, a stratified pooling method was applied with regard to the actual rearing environments, and this led finally to 63 composite sample pools suited for later metagenomic analysis. Geographically, the macroecological scope of this collection successfully crossed the fundamental Qinling–Huaihe climatic dividing line, thereby capturing a diverse bioclimatic continuum that spanned subarctic, temperate, and subtropical zones characterized by pronounced temperature and humidity gradients **(Fig. 1)**. Such a national, environment - stratified sampling plan enables one to set up a very complete and logically consistent baseline, which in turn makes it possible to study systematically the geographic patterns as well as the localized macroenvironments of rearing in relation to the domestic cat's oral microbiome.

2.2 Genome Acquisition of Feline-Derived Isolates

For April 2025, genomic data concerning prokaryotic and fungal isolates obtained from cats were systematically taken from the NCBI Genome database. Those entries with metadata of BioSample search terms " uncult," " MAG," or " bin " were not included in the analysis, while candidate genomes—whose host or source was explicitly stated (in any of the forms " cat," " feline," " Felis catus," " gut," " feces ", or " intestinal ")—were selected for retention. Manual checks were then applied to each of these candidates in order to confirm definitively their feline provenance, with close attention to BioSample details, to GOLD records, and to supporting literature.

Strict quality control rules were applied in sequence to the verified datasets—each one being handled by its own domain. With regard to prokaryotic genomes, completeness and contamination were analyzed using CheckM2 (v1.0.1) , and possible chimerism was studied with GUNC (v1.0.5);the genome was kept only if it had a completeness of 50% or more, a contamination value under 5%, and a clade separation score less than 0. 45.For fungal genomes, quality evaluation was conducted using BUSCO (v5.4.2) operated in genome mode relative to the fungi_odb10 database, with thresholds of completeness (at least 50%) and contamination (under 5%) used in the selection process.

2.3 Preprocessing and Assembly

Of the 1,454 cat oral swab samples taken, all were pooled into 63 groups for simultaneous preprocessing and assembly before further analysis. The full workflow is described as follows: Initially, quality control and adapter trimming were performed with fastp (v0.20.1). For short reads (≤ 75 bp), the settings -u 30 -q 20 -l 30 -y --trim_poly_g were applied, whereas for long reads, the parameter -l 60 was implemented to filter out low-quality sequences. Then, low-complexity sequences were deleted using the bbduk program of BBTools (v39.00) under the conditions entropy=0.6, entropywindow=50, entropyk=5. Next, clean reads were aligned against the cat genome (GCF_000181335.3) and the phiX174 genome (NC_001422.1) by means of Bowtie2 (v2.4.1), with host or control matches being excluded. Finally, clean reads taken from each sample were assembled separately by means of MEGAHIT (v1.2.9) with parameters --k-list 21,41,61,81,101,121. The resulting assembly gave rise to more than

7.9 million contigs of at least 2 kb in size, or about 68.4 Gb in total, with 35.3% of them being longer than 5 kb. Such data are well suited—both in quantity and quality—for further genome analysis and functional studies

2.4 Prokaryotic Genome Analysis

Regarding metagenome-assembled genome (MAG) reconstruction, only contigs with a length ≥ 2,000 bp were considered. Clean reads were aligned to each contig using Bowtie2, and the sequencing depth for each contig was determined with jgi_summarize_bam_contig_depths. Single-sample binning was executed using four separate algorithms: MetaBAT2 (v2.15), VAMB (v3.0), SemiBin2 (v2.0), and MetaBinner (v1.3). Bins were then combined and dereplicated via DAS Tool (v1.1.2) to obtain high-confidence MAGs. Following reconstruction, MAG completeness and contamination were evaluated via CheckM2 according to MIMAG specifications, adopting thresholds of 50% for completeness and < 5% for contamination. Chimerism was assessed simultaneously using GUNC, and only genomes with a taxonomic separation score below 0.45 were retained. A two-step dereplication procedure was then applied to the remaining high-quality MAGs using dRep (v3.4.0), clustering at 99% average nucleotide identity (ANI) for strains and at 95% ANI for species (defining species-level genome bins, or SGBs). Within each cluster, the genome with maximum completeness and minimum contamination was selected as the representative, defining 443 final SGBs. Their taxonomic affiliations were determined by GTDB-Tk (v2.5.2).

For subsequent community composition analyses, a custom Kraken2 and Bracken database was built from the 443 prokaryotic SGBs available in the OAPGC. Clean reads

were assigned to taxa using Kraken2 (v2.1.3) with a confidence threshold of 0.1, and species-specific relative abundances were estimated by Bracken (v2.8). Simultaneously, protein-coding genes were predicted from the genomic sequences using Prodigal (v2.6.3) in meta-prokaryotic mode. The predicted open reading frames were annotated for function using DIAMOND (v2.0.13) in BLASTP mode. Alignments with both the KEGG and CAZy databases required a sequence identity ≥ 30% and a minimum coverage of 50% for both the query and subject lengths.

2.5 Viral Genome Analysis

With regard to viral sequence identification, metagenomic contigs of length above 5，000 bp were first chosen and analyzed as a group by means of an integrated pipeline involving many bioinformatic tools. To keep host - related contamination to a minimum, CheckV (v0.7.0) was applied; those sequences having over 10 host - assigned genes or showing a host - to - virus gene ratio higher than 5 were removed systematically, the specific extraction of proviral regions comes next. At the same time, viral identification was applied to the metagenomic contigs using geNomad (v1.11.1) but not its prophage detection part. Contaminant sequences were treated by means of taxonomic classification, and temporary files were deleted or modified, so as to arrive at a clean, high - quality working set of possible viral sequences.

Verification of all downstream candidates and final curation were handled by means of the parallel application of DeepVirFinder (v1.0) and VIBRANT (v1.2.1) .For DeepVirFinder filtering it was required that both a score and a p - value meet strict limits—score > 0.90 and p < 0.01.As for VIBRANT, the standard parameters were used,

with the proviso that any sequence having 5%or more bacterial universal single - copy orthologs (USCOs) would be deleted from the set. Only those sequences with CheckV completeness above 50%were taken as reliable, and such items were joined with verified data to form the completed OAPGC - v viral genome dataset.

3. Results

This paper reports on a national cohort formed of 1，454 oral swab samples taken from domestic cats. Great differences—of a quantitative nature—were found to exist between the macro - geographic areas and the micro - rearing situations surveyed. From a geographical standpoint, seven large regions were covered by the cohort, with East China (n = 285) providing the largest number of samples and South China (n = 160) the smallest such count.A breakdown of the regions under consideration was given as such: East China (n = 285) ， Central China (n = 273) ， Southwest China (n = 240) ， North China (n = 223) ， South China (n = 160) ， Northwest China (n = 138) ， and Northeast China (n = 135) .Such a distribution is tentatively interpreted as an indicator of differences—between regions—in domestic cat numbers, in pet culture, and in logistical access at the time of sampling.

On the micro - environmental level, the present study applied stratification to each macro - region with regard to three specific types of sites: catteries (n = 509) ， veterinary hospitals (n = 449) ， and stray cat rescue units (n = 496) .Among these, the cattery - related samples occurred mainly in East China (n = 111) or North China (n = 96) .The veterinary hospital group was heavily concentrated Among the regions studied, Southwest China (n = 119) and North China (n = 75) had relatively few stray shelter samples, whereas East China (n = 116) and Central China (n = 100) were very heavily represented. Pronounced regional differences are apparent with respect to the relative frequency of the various environmental types; in particular, Southwest China yielded the maximum share of clinical cases, whereas stray cat numbers were more

densely concentrated in East and Central China. Such spatial heterogeneity is plausibly connected to differences between regions concerning veterinary care availability, maturity of stray animal welfare arrangements, traditional husbandry methods, and levels of urbanization. That prompts a basic question about the ecology of feline oral microbes: the extent to which macro-geographic baselines govern community structure versus the dominance of micro-environmental pressures, as well as the underlying causal factors. The landscape of such quantitative facts is an essential prerequisite for separating environmental influences from one another and for determining the biogeography of microbial communities. (in Fig. 1)

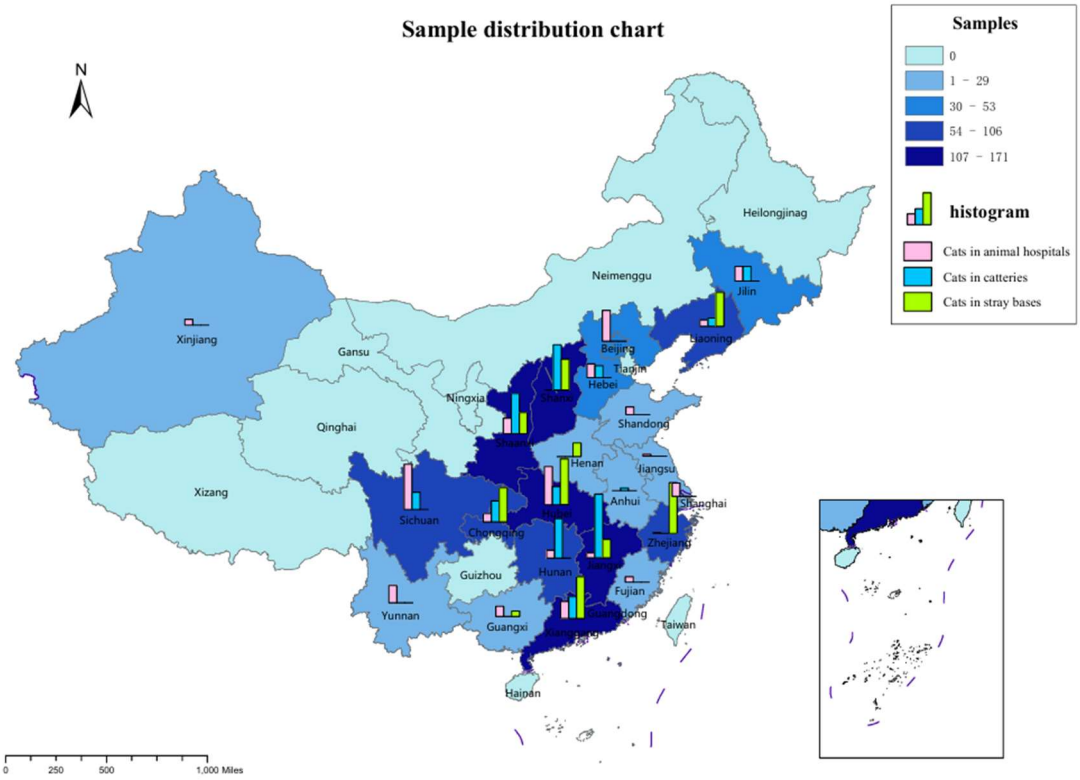

Figure 1: Geographic distribution and source types of feline oral samples in China.

A systematic treatment of the alpha diversity of oral bacteria and viruses found in felines is given in Figure 2, with macro - geographical trends as well as core

microbiome properties clearly laid out. Very different effects seem to emerge—along geographic gradients—from a comparison of evenness with species richness. As far as community evenness (measured by means of the Shannon index) is concerned ,no appreciable variation seems to be present—either for bacterial (Panel A) or for viral (Panel C) communities—in the seven principal geographical zones listed: Northeast (DB) ， East China (HD) ， Central China (HZ) ， North China (HB) ， South China (HN) ， Northwest (XB) ， or Southwest (XN).This suggests, quite clearly, that macro - geographical placement has little effect upon the structure and species distribution evenness for the feline oral microecology. By contrast with this general statement, there were definite regional differences—of a rather large magnitude—in species richness (as measured by the actual count of SGBs) ， with consistent trends for bacteria (Panel B) and viruses (Panel D) .Peak richness is to be found in the Northeast (DB) ， whereas the Southwest (XN) has the minimum values.

One of the central scientific problems raised by this uncoupled pattern concerns the fact that species richness varies strongly with geography while the basic evenness of the community—across all taxa—remains undistorted. Moreover, it is not yet fully resolved which environmental factors (if any) operate jointly to explain the simultaneous rise or fall of species numbers across kingdoms, and whether such profiles derive mainly from macro - climatic elements (such as average temperature and moisture) ， from host genetics, or from other unexplored ecological influences.The present analytical data suggest that the phyla Pseudomonadota, Bacillota, and Bacteroidota form a predominant part of the oral microbiota of domestic cats. Of the

bacterial species now known or suspected to exist there, nearly half remain uncultivated. The uncharacterized taxa—though not yet fully described—appear to cluster mostly within the three major phyla examined, with their phylogenetic distribution showing some distinct regularity.At the same time, among the culturable species considered, only 13%are known to have originated specifically from felines, while 36%derive from alternative host backgrounds. Hence, the physiological roles performed—or at least attempted—by this large, uncultured core microbiota (which dominates the feline oral cavity) are not yet completely clarified, and the exact relations it sustains with host health or with the " One Health " microecology still leave room for debate. Moreover, it is uncertain whether the extreme phylogenetic concentration seen in these uncultured taxa represents truly singular, conserved ecological behavior within this narrow oral setting or not—a matter of great scientific interest. (in Fig. 2)

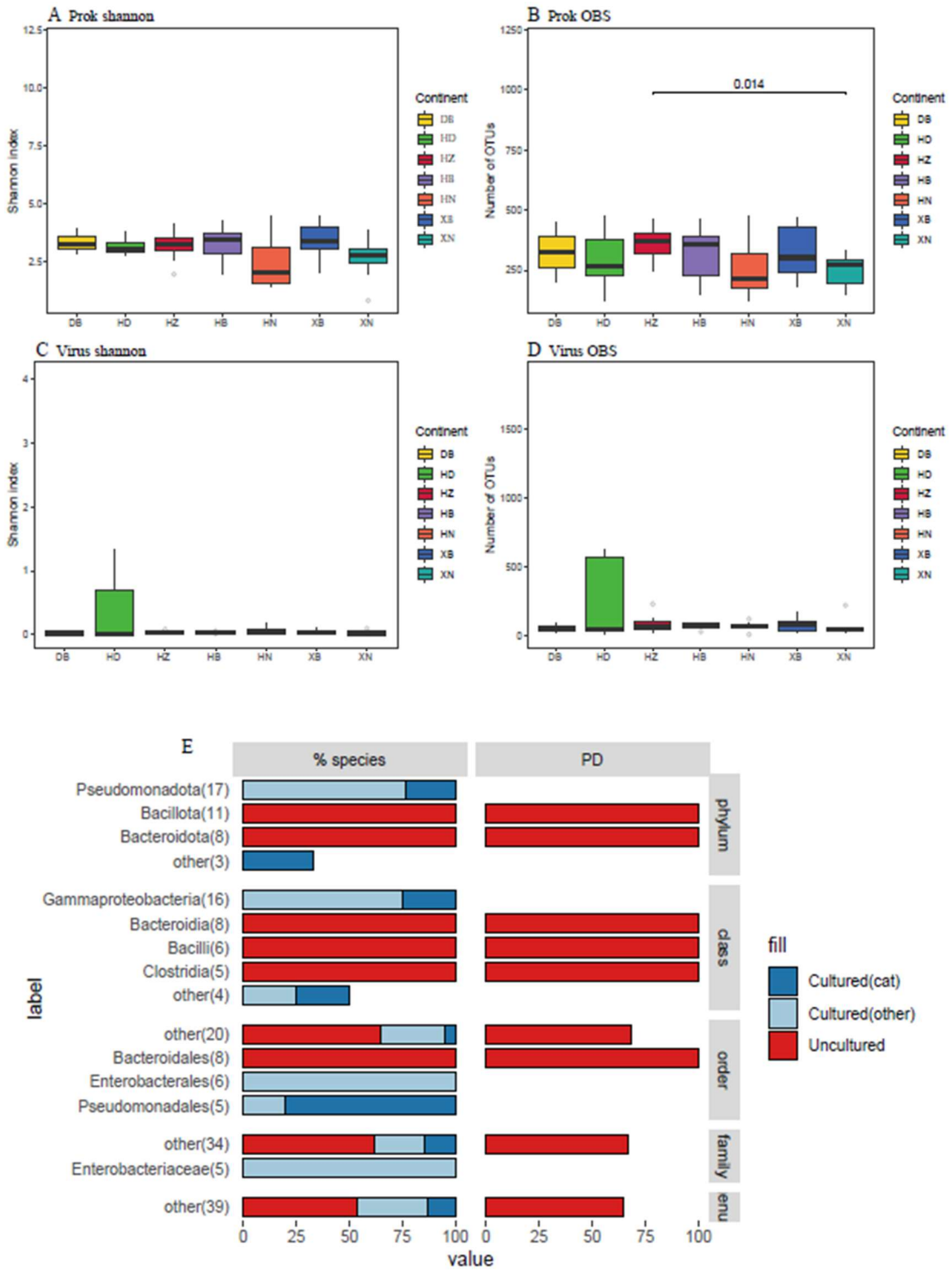

Figure 2: Alpha diversity and community studies of bacteria and viruses in the oral cavity of cats. A: Bacterial Shannon index: An indicator of evenness and of diversity among bacteria; it is higher when the structure of the community is stable or

its species are uniformly spread. There was no significant variation—across the seven areas considered (DB: Northeast, HD: East China, HZ: Central China, HB: North China, HN: South China, XB: Northwest, XN: Southwest). B: Bacterial species richness: Counts the number of observed species - level genome bins (SGBs)—this is a measure of bacterial species richness; Higher values correspond to greater bacterial species richness. Pronounced regional differences were evident: bacterial richness was highest in the Northeast and lowest in the Southwest. C: Viral Shannon index: No significant differences in viral evenness were detected across the seven regions, indicating a minimal impact of geography on viral community stability. D: Viral species richness: Significant regional variation was observed, with viral richness peaking in the Northeast (DB) and reaching its lowest in the Southwest (XN), consistent with the bacterial pattern. E: Feline oral bacterial SGBs were dominated by the phyla Pseudomonadota, Bacillota, and Bacteroidota. Over half (51%) comprised uncultured species, with only 13% culturable from feline hosts and 36% from other hosts. Uncultured species were predominantly concentrated within these three dominant phyla, exhibiting significant phylogenetic enrichment.

The geographic differentiation patterns of oral bacterial and viral communities in domestic cats are systematically characterized in Figure 3 via-diversity and taxonomic composition analyses. The microbial divergence shown in each area has been traced largely to geographic isolation (as perβ- diversity analysis) .Clustering of bacterial types is quite distinct between geographic zones **(Fig. 3A)**, with separate clusters being formed by the Northeast (DB) and Southwest (XN) regions, whereas a relative

aggregation was maintained by samples from alternative regions. The viral communities seem to show still sharper geographic limits and more definite structural differences between regions **(Fig. 3C)**. From this, it follows that the ecological basis of the heightened geographic specificity of the oral virome relative to its bacterial counterpart is still not completely understood, and the environmental pressures common to both that cause such synchronized, cross-kingdom differentiation need more study. Very marked regional enrichment trends appeared at the level of taxonomic composition for both viruses and bacteria. Regarding the virome, a uniform dominance was apparent throughout all regions, led by Siphoviridae (with a relative abundance of 30%–50%), followed by Myoviridae, which were especially abundant (10%–20%) in the XN, XB, and HD zones but rare in DB and HN.

Importantly, more than half of all viral sequences remained unclassified, leaving the issue unresolved as to whether the regional changes observed in major phage groups derive from a biogeographical arrangement of host bacteria or are directly determined by localized environmental pressures. At the same time, Porphyromonas gingivalis and Pasteurella multocida proved to be the principal commensal species present, yet the community under study had substantial colonization by opportunistic pathogens whose regional prevalence—often extreme—was notable (for example, Pseudomonas aeruginosa and Klebsiella pneumoniae in DB, Streptococcus canis or Enterococcus faecium in HD, and Acinetobacter baumannii and Bordetella pertussis in HN). The environmental causes of these localized spikes—possibly tied to the climate patterns of a given region, to animal-rearing methods, or to the local use of veterinary antibiotics—

still await clarification, as do the exact taxonomic relations, ecological roles, and "One Health" threats presented by this considerable quantity of uncultured bacteria and unidentified or unclassified viruses. (in Fig. 3)

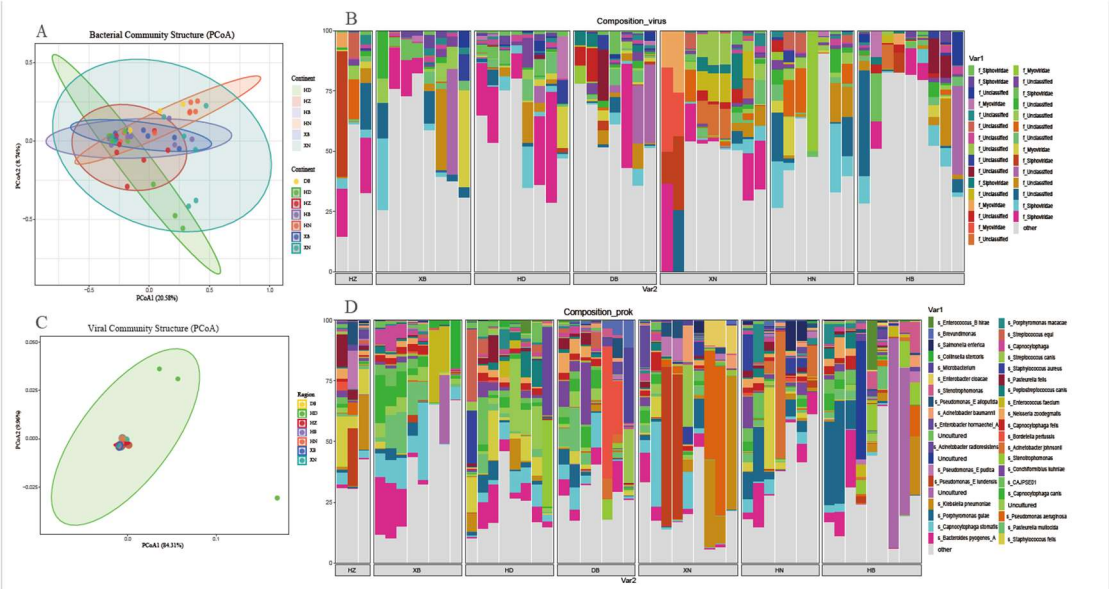

Figure 3: Geographical differentiation of oral bacterial and of viral communities among domestic cats. A: Bacterial PCoA and β-diversity: In terms of bacterial β-diversity, distinct geographical groupings were observed, namely separate clusters for the Northeast and Southwest, and a single grouping for all other areas. B: Stacked bar plot of viral family composition: Among the viruses found in the oral cavity of cats, the majority belong to the families Siphoviridae or Myoviridae, with many unclassified ones making up over half of the community. There are distinct regional differences in the distribution of viral families, especially regarding Myoviridae in the Southwest and Northwest areas. Siphoviridae consistently represents the main group throughout all regions (30%–50%), remaining by far the most common phage group among the feline oral viruses. The Myoviridae are the second most common family there and are

relatively abundant (between 10% and 20%) in the Southwest, Northwest, and East China, but somewhat less frequent in the Northeast or South China. C: Viral PCoA and β-diversity: From a standpoint of β-diversity, geographic location appears to be an important factor in determining phage community structure. Viral communities have very definite regional boundaries—their geographical specificity is much greater than that of bacterial communities. D: Stacked bar plot of bacterial species composition: Shows the relative frequencies of bacterial species-level genome bins (SGBs) in seven areas (DB: Northeast; HD: East China; HZ: Central China; HB: North China; HN: South China; XB: Northwest; XN: Southwest). The bacterial community of the feline oral cavity seems to be largely occupied—without obvious pathology—by certain dominant commensals (Porphyromonas gingivalis, Pasteurella multocida, Staphylococcus spp.) as well as by opportunistic pathogens (Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii). Region-specific taxa having unusually high abundance are listed here: Northeast (DB): many cases of P. aeruginosa or K. pneumoniae; East China (HD): more frequent appearances of Streptococcus canis or Enterococcus faecium; South China (HN): greater numbers of A. baumannii and Bordetella pertussis.

The current work reports on 969 complete viral genomes obtained simultaneously with the help of the VIBRANT and DeepVirFinder algorithms (**Fig. 4A**). Within specific lineages, co-detection by the two algorithms is established for 32 cases, and 25 further genomes are uniquely identified by one or the other method. With such a dual-algorithm cross-validation setup, the risks of erroneous positive or negative findings

associated with single-pipeline methods proved to be effectively mitigated, which is helpful in raising the baseline probability of viral sequence identification. From that standpoint, a very strong prevalence of lysogeny rather than of lytic behavior was found for the feline oral cavity (**Fig. 4 B**). Most of the phages present in this niche appear to be temperate, with their genomes preferentially integrated into host bacteria rather than undergoing direct host lysis. A relative stability of the resident microbial community appears to be ensured—on a general level—by this lysogeny-centered strategy. With regard to specific environmental pressures (host immunity or local nutrient competition among microbes), the factors that encourage or preserve a predominantly temperate phage mode of life are not yet completely known, nor are the details of how lysogenic alignment affects, in turn, the eventual behavior and functional state of the bacterial community in question.

Among other things, the class Caudoviricetes has been singled out by a random forest model-oriented study of feature importance as an essential taxonomic factor in viral community differentiation (**Fig. 4C**). The top contribution values pertain to certain newly defined clusters such as Caudoviricetes_13_59 or Caudoviricetes_31_1 (and also to Caudoviricetes_5_18), while high levels of importance are simultaneously shown by a substantial part of the unclassified (unplaced) viral sequences. With an eye to these data, one may now consider the host range, genome structure, and exact ecological function of each of the high-contribution Caudoviricetes lineages as topics ripe for study. Also worth investigation is the biological complexity—often great but not yet cataloged—of these powerful yet unclassified viruses, with regard to their structural

roles in the feline oral microbiome (in particular, their exact impact). (in Fig. 4)

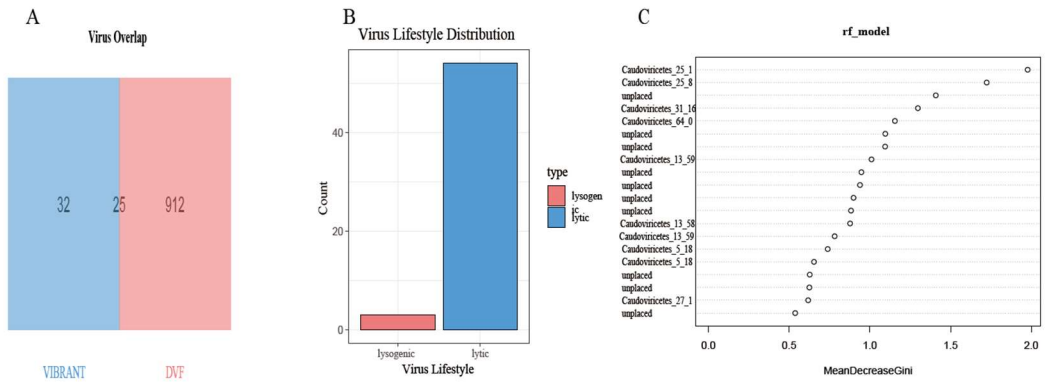

Figure 4: Identification methods, lifestyle characteristics, and their bearing upon community structure in the domestic cat oral virome. A: Joint identification using VIBRANT and DeepVirFinder: A total of 969 viral genomes were found, of which 32 were simultaneously recognized by both tools and 25 by only one of them. B: Lifestyle analysis: Among domestic cats, oral phage lifestyles are mostly lysogenic, with lytic forms being of secondary importance. C: Random Forest (RF) assessment of feature importance: Caudoviricetes (the tailed phages) is shown to be a central taxon for viral community organization, and Caudoviricetes_13_59, Caudoviricetes_31_1, and Caudoviricetes_5_18 hold the top three importance scores.

The effects—differential or not—of the rearing environment (grouped under the three DLM categories) and of geographic region upon the abundance of feline oral bacterial and viral communities are shown by means of the volcano plot in Figure 5. With regard to the rearing environment (stray versus pet cats), there is clear evidence of more marked viral enrichment among stray cats at the virome level (Panel A). Among the most highly upregulated taxa, one finds them to be mostly unclassified viruses (e.g.,

v001372 and v014896), as well as one definite viral lineage of the family Siphoviridae (v007223). At the same time, the major bacterial enrichment observed in stray cats is mirrored at the bacteriome level (Panel B). Among the top upregulated bacterial taxa is an uncultured species called SGB0234, a non-feline culturable commensal (Barnesiella intestinihominis).Among the microbes studied are two species of bacteria—SGB0321 and a feline-associated unnamed one (SGB0411). On the other hand, many feline culturable bacteria show a definite downregulation, namely Campylobacter rectus (SGB0775), Corynebacterium aurimucosum (SGB0649), or Acinetobacter pittii (SGB0128). The shaping of microbial communities by means of intricate environmental or dietary factors is strongly demonstrated by the marked enrichment—within this particular context—of unclassified viruses and of uncultured/non-feline bacteria, along with the downregulation of a few host-adapted culturable taxa. On the basis of such observations, it is still uncertain what role—if any—the current abundance of unclassified viruses and of uncultured/non-feline bacteria might play in stray cat health (in relation to immunity or disease). Moreover, whether the present highly specific community configuration mainly results from diet, antibiotic use differences, or contact with environmental microbes is a matter of unresolved importance.

In addition, the driving role of geographic areas for community organization was studied systematically. Regarding the virome (**Fig. 5C**), geographic features appear to exert substantial control over the distribution of viruses, and individual taxa (such as v010814, v000339, or v007309) are strongly associated with limited local territories. With regard to the bacteriome (**Fig. 5D**), the effect of geography on community

differentiation is shown to have been especially marked. Among the taxa greatly enriched in particular regions, one finds mainly non-feline culturable bacteria such as Pantoea sp. (SGB0840), Leclercia adecarboxylata (SGB0783), or Cronobacter dublinensis (SGB0625). On the other hand, the localized lack of certain species is mostly borne out by uncultured bacteria (SGB0793, SGB0870) as well as the non-feline culturable Streptococcus equi (SGB0779). In general, a distinct local overabundance of selected viruses or bacteria—specifically of non-feline culturable types—combined with a relative lack of other classes, seems to result from geographic causes. Hence, the precise environmental provenance of these highly abundant, non-feline culturable bacteria enriched in specific regions is still not fully resolved, and it is uncertain whether such features derive from native soil/water matrices or from alternate animal sources, or whether they form a definite regional microbial "fingerprint." In addition, the exact roles played—individually and collectively—by climate trends, by altitude, and by localized fauna in selecting and guiding this extremely region-specific oral biogeography are well worth further study.(in Fig. 5)

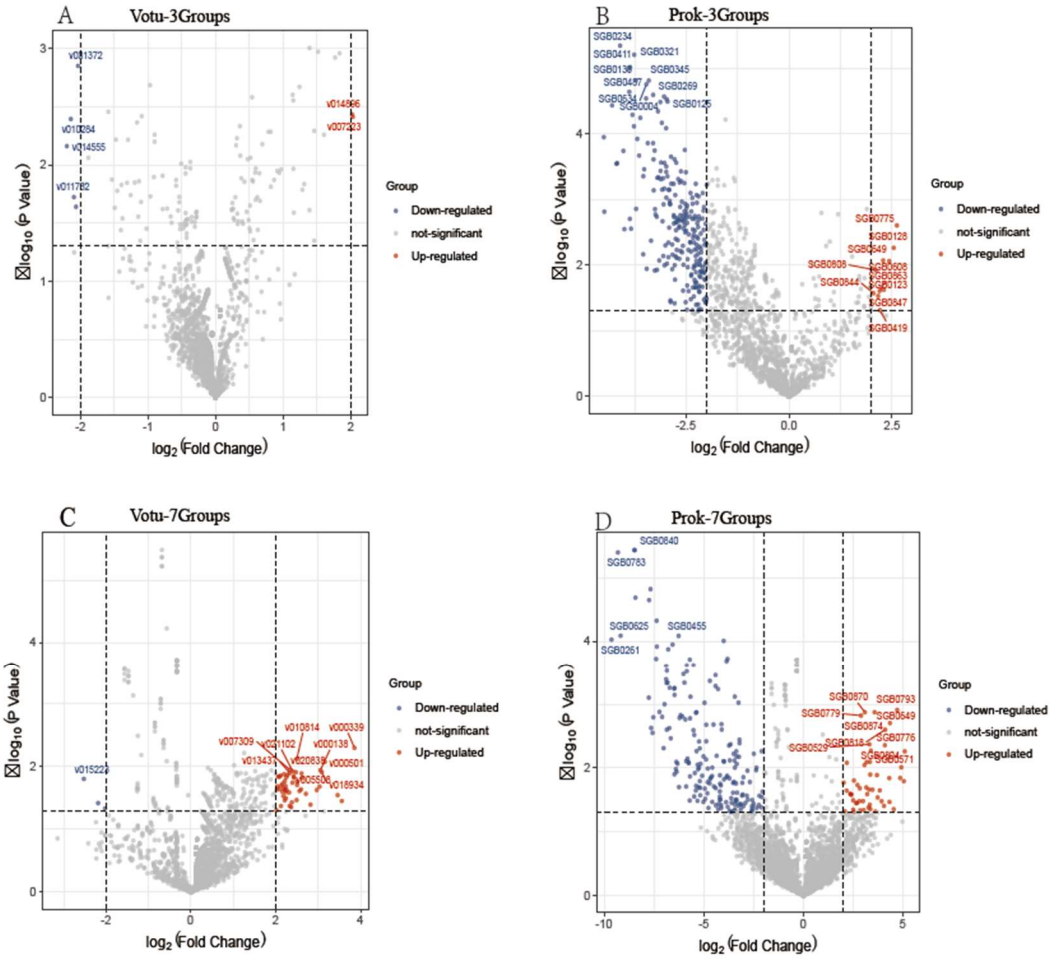

Figure 5: Volcano plots of differentially abundant bacteria and viruses. (A/B) Comparisons among three DLM groups; (C/D) Comparisons across seven geographic regions. A: DLM-based volcano plot of viral abundance: Among the stray cats, viral enrichment appears especially strong, with nearly all the unclassified viral sequences being of Siphoviridae type or similar (with very few exceptions). Of these, v001372 (Unclassified), v014896 (Unclassified), and v007223 (Siphoviridae) are by far the most highly upregulated taxa. B: DLM bacterial differential abundance volcano plot: Bacterial enrichment is especially strong in the stray cat group. Among SGBs, SGB0234 (Uncultured), SGB0321 (culturable non-feline-origin commensal

Barnesiella intestinihominis), and SGB0411 (culturable feline-origin unnamed bacterium) have the maximum upregulation; SGB0775 (culturable feline-origin Campylobacter rectus), SGB0649 (culturable feline-origin Corynebacterium amycolatum), and SGB0128 (culturable feline-origin Acinetobacter pittii) are associated with the most extreme downregulation. C: Volcano plot of viral differential abundance for seven areas: Geographic influence is very marked in regard to viral communities—v010814, v000339, and v007309 occur predominantly or strongly in certain localities. D: Volcano plot of bacterial differential abundance for seven regions: Geography seems to cause more marked bacterial community divergence, with SGB0840 (culturable non-feline-origin Pantoea sp. 002920175), SGB0783 (culturable non-feline-origin Leclercia adecarboxylata), and SGB0625 (culturable non-feline-origin Cronobacter dublinensis) being strongly upregulated in one or more areas; SGB0793 (Uncultured), SGB0870 (Uncultured), and SGB0779 (culturable non-feline-origin Streptococcus equinus) show the major downregulation.

Among the bacteria of the feline oral microbiome, there appears to be a dominant role played by a small subset of core organisms—a fact illustrated by the co-occurrence network shown in Figure 6. A distinct core-periphery type of network is formed here, with bacterial SGB0405 (degree ≈ 128) and SGB0534 (degree ≈ 125) acting as principal core hubs. With regard to these primary hubs, nearly all the network nodes are connected, with the named taxa being keystone species whose presence is fundamental to structural stability. At the same time, substantial regulatory roles—modular or otherwise—are played by SGB0759 and SGB0665 (secondary core nodes). By contrast,

the 136 peripheral viral nodes tend to form a radial cluster about each of these core bacteria, with virtually all of them being unclassified viruses in appearance.

The fact that the feline oral virome is largely made up of bacteriophages is well confirmed by the present structural arrangement, with the survival or functioning of such viruses—whether active or dormant—being closely tied to their bacterial hosts. Of particular importance is the maintenance of direct interaction with each of the four core bacteria by ten viral nodes (e.g., v013271, v000275, and v014519), which are thereby positioned as vital hubs of cross-kingdom connectivity. The exact biological role of each such "bridge" virus is still an unresolved ecological problem, with uncertainty as to whether they denote general-host-range phages (able to attack many principal bacterial species) or rather more complex ecological relations—possibly involving auxiliary interbacterial interactions. These material interactions are indicated here by the links. To determine the exact manner in which several core bacterial populations are at once shaped by the given bridge viruses is to embark on an investigation that critically depends upon a thorough description of such viruses, with an eye toward eventual clarification of the downstream control of oral microecological stability and of its functions. (in Fig. 6).

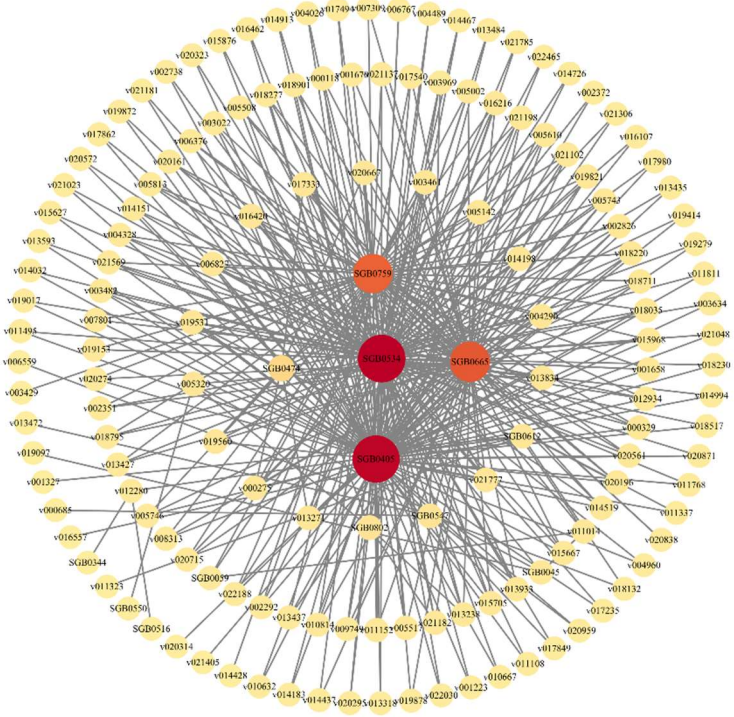

Figure 6: Cross-kingdom bacterial-viral co-occurrence network in the feline oral cavity.

Significant variations—of the kind seen in the feline oral microbiota (top 50 taxa)—in relation to various housing situations are indicated by the analytical results. When analyzing the bacterial community as a whole, differences between groups become quite marked: several potential pathogens (for example, Streptococcus equi or Campylobacter jejuni) appear to be enriched in cats kept in the stray cat shelter (Group L), while data from Group D suggest a relatively large abundance of gut-related or uncultured bacteria, such as Blautia and Faecalibacterium. By contrast, an overrepresentation of ordinary commensals—for example, Ligilactobacillus animalis or Sellimonas intestinalis—is characteristic of the oral microbiota of cats examined at the veterinary hospital (Group D), and seems to indicate a configuration of the community closer to a healthy homeostatic condition.

While presenting a particular case of homogenization—namely one involving minimal intra-group microbial variation and maximal homogeneity—which is characteristic of the cattery (Group M) population compared with other groups, pronounced compositional contrasts similar to those of the bacterial microecology seem simultaneously to define the virome of these animals. With regard to viral diversity, stray cats (Group L) are not merely more varied in this respect but also host many unclassified viral taxa, whose oral phage life-histories show a marked bias toward lysis. By contrast, cats kept at animal hospitals (Group D) sustain a far more regular oral virome, mainly of lysogenic origin and largely belonging to the families Siphoviridae or Myoviridae. By now it is evident that the co-enrichment of lytic phages and possible pathogens (such as Campylobacter jejuni) in the oral cavity of stray cats may be interpreted either as a controlled, phage-guided adjustment of pathogen numbers (i.e., an ecological balance of sorts) or as an extreme microbial disturbance with substantial likelihood of clinical sequelae—a matter still unresolved. The identification of cross-kingdom interactions in particular ecological settings is considered central to any thorough evaluation of animal health and to planning of useful therapeutic or preventive measures. (in Fig. 7).

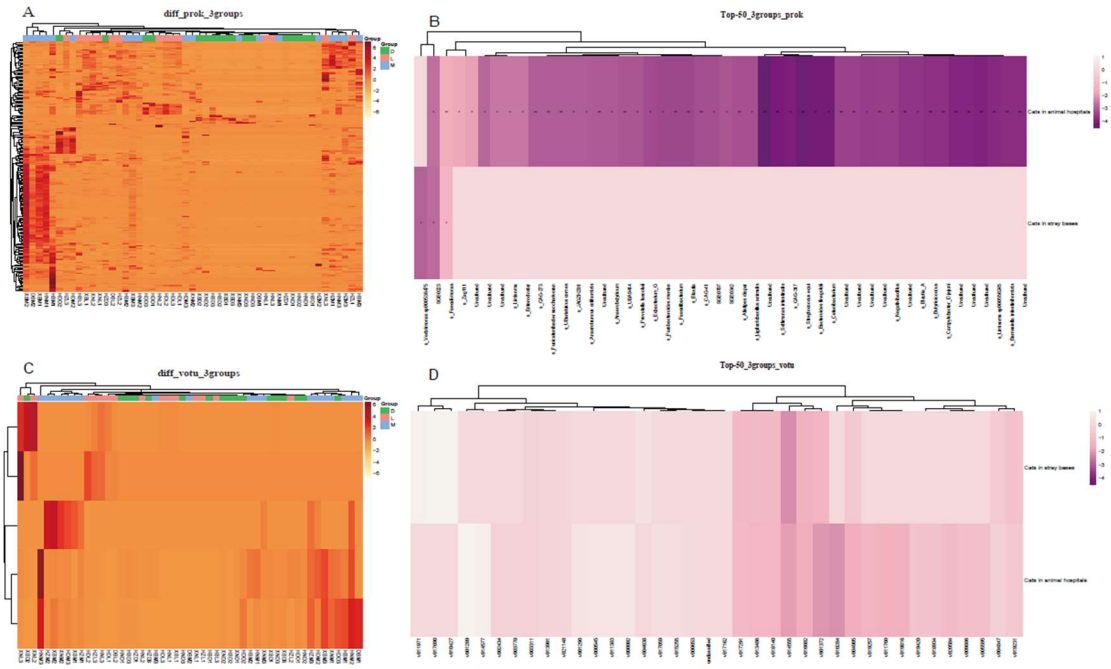

Figure 7: Top 50 distinct microorganisms that are differentially abundant across groups.(A/B) Heatmap with a note on the statistical significance of bacterial abundance. A: DLM three-component bacterial differential heatmap: There is definite evidence of variation in bacterial communities. Stray cats tend to be colonized by possible pathogens (such as Streptococcus or Campylobacter), whereas cats from animal hospitals have more commensals; meanwhile, the cattery group is associated with the least microbiota divergence. B: DLM three-component bacterial top-50 heatmap: Among stray cats, certain commensals or opportunistic pathogens—such as Streptococcus equi, Campylobacter jejuni, Blautia, and Faecalibacterium—were found to be more common, suggesting higher pathogen carriage. For cats treated in animal hospitals, the overrepresentation of the commensal taxa Ligilactobacillus animalis, Sellimonas intestinalis, and Barnesiella spp. denotes a healthier state of microbiota—one dominated by commensals. There is also considerable evidence of culturable

bacteria (in higher relative abundances) for some of these stray cats. C: DLM three-component viral differential heatmap: Viruses of all sorts appear to be tightly associated with bacterial patterns. Stray cats show enrichment for lytic phage species, while animal hospital cats were associated mainly with lysogenic phages. Among the cattery groups, there was very weak viral divergence, with unique bacterial-phage relationships for each environment. D: DLM three-component viral top-50 heatmap: Stray cats had a much greater variety of viruses and of unclassified taxa. By contrast, the animal hospital group was overwhelmingly occupied by known long-tailed or contractile-tailed phages, which suggest a highly uniform viral population pattern.

3.1 Compositional Analysis of the Feline Oral Microbiome

3.1.1 Metagenomic Assembly and Quality Control of the Feline Oral Microbiome

To fully characterize the feline oral microbiota, 1,454 oral swab samples were combined into 63 composite samples for deep metagenomic analysis. De novo assembly followed by binning of all contigs longer than 2,000 bp produced initially 998 metagenome-assembled genomes (MAGs). Using a multilayered quality control system—predicated on CheckM2 criteria (completeness >70% and contamination <5%) with chimeric sequences removed via GUNC—this collection was successfully narrowed to 184 high-quality MAGs. Each of these genomes was integrated with 2,325 bacterial isolates of cat origin retrieved from the NCBI RefSeq database. Species-level dereplication of this expanded group yielded a non-redundant dataset of 443 species-level genome bins (SGBs). The initial integrated set—without further restriction—exhibited extraordinary genomic variety (size range: 0.20–22.30 Mbp; GC values:

19.00%–74.00%), but the target genomes proved well-bounded (280 kbp–6.5 Mbp). With an average completeness of 58.44% and a very low contamination rate (mean 4.16%), this catalog offers a firm basis for future taxonomic or functional studies.

3.2 Taxonomic Diversity and Profiling of Feline Oral Prokaryotes and Viruses

A taxonomic survey of the 443 SGBs by means of GTDB-Tk suggests a very diverse prokaryotic community with 14 classes, 269 orders, 188 families, and 167 genera, predominantly made up of the three phyla Pseudomonadota, Bacillota, and Bacteroidota (together accounting for >75% of all abundances). Of special interest is the fact that 34.1% of the identified prokaryotic species had not been isolated or described before, which points to an immense reservoir of "microbial dark matter" as it occurs in the feline oral cavity. A parallel viromic analysis of contigs longer than 5 kbp yielded 8,962 tentative viral sequences, of which CheckV quality assessment later indicated 969 to be high-quality or complete genomes (12.73%). Among the viruses found in this oral setting there was a marked dominance of the class Caudoviricetes (over 90%), with Siphoviridae and Myoviridae being the most frequent families. Importantly, 48.7% of all detected viral types were unclassified, which illustrates the great taxonomic novelty of the feline oral virome.

3.3 Geographical Factors

Analysis of alpha diversity indicates a distinct relationship—sometimes conflicting—between community richness and evenness in each of the seven areas **(Fig. 2A–D)**. With regard to evenness (as determined by the Shannon index), neither bacteria

nor viruses show any marked variation in their regional distributions ($P > 0.05$), so the basic structure and rank-abundance of the oral core microbiota seem to be tightly constrained, suggesting that the host's intrinsic physiological homeostasis is in control of this phenomenon. By contrast, species richness (as measured by the actual count of SGBs) was not uniform geographically ($P < 0.05$), reaching an unexpectedly high value in Northeast China (DB) and its minimum in Southwest China (XN). Such a pattern implies that although the host's inner environment provides a steady ecological setting, macro-geographic gradients as well as regional environmental filters appear to act separately in determining whether transient or marginal microbes will reach and colonize the feline oral cavity. From an ecological standpoint it is clear that Pseudomonadota, Bacillota, and Bacteroidota are the main bacterial phyla of the oral cavity, and that Gammaproteobacteria, Bacteroidia, and Bacilli predominate among their classes. Among the findings noted is the fact that uncultured taxa amount to more than 34%, which implies a considerable unexplored "microbial dark matter" in the feline oral microbiome **(Fig. 2E)**. In addition, there is a definite positive relationship—of statistical significance—between bacterial and viral alpha diversity ($r = 0.62$, $P < 0.001$), suggesting joint control of both types of communities by geography and by climatic conditions **(Fig. 2B)**.

β-diversity analysis gives additional support to the hypothesis that geographic variables are by far the main drivers of variation in oral microbial communities (and not merely the living environment). The PERMANOVA data confirm that geographic region (Region) is responsible for a much larger share of the variance of bacterial

structure ($R^2 = 0.081$, $P < 0.01$) than is the living environment (Group: $R^2 = 0.032$, $P < 0.05$). With regard to the viral community structure, geographic region accounted for more of the variance ($R^2 = 0.067$, $P < 0.01$) than did the living environment ($R^2 = 0.028$, $P < 0.05$). Principal coordinates analysis (PCoA) showed strict clustering of samples according to geographic origin. This PCoA ordination yielded no exceptions in terms of geographical clustering among the samples. The first two principal coordinates explained 20.58% and 8.74% of the total variation for the bacterial community, while the same axes accounted for 84.31% and 9.96% of variation in the viral community. By contrast, the clustering related to living environment is very weak, and this fact supports the conclusion that geography governs oral microbial community structure **(Fig. 3C/D)**.

3.4 Feeding Factors

Differential abundance analysis has been used to assess the joint influence of geography and environment upon the oral microbiome of cats, with volcano plots helping to define group-specific changes in taxonomy **(Fig. 5)**. Studies of particular regions identify certain lineages as being enriched geographically, as well-illustrated by the localized prevalence of Pantoea sp. (SGB0840) and Leclercia adecarboxylata (SGB0783) **(Fig. 5C/D)**. At the level of the microenvironment, specific husbandry conditions seem to strongly influence microbiota composition—they lead to selective enrichment of certain functional taxa **(Fig. 5A/B)**. Among stray cats there is an appreciable prevalence of genera adapted to environmental stress (such as Pseudomonas or Acinetobacter), which likely represent responses to complex or variable ecological pressures. By contrast, cats kept in catteries or under veterinary care

exhibit a marked surge of opportunistic agents (including Staphylococcus and Klebsiella), a phenomenon closely linked to high-density living and to medical treatments such as antibiotic use.

With regard to the environmental dependencies mentioned above, a random forest classifier was built using oral viromic data to assess host housing conditions. It yielded a list of 20 top-ranked viral biomarkers useful for environmental discrimination, achieving strong predictive capacity—with an Area Under the Curve (AUC) of 0.88 **(Fig. 4B)**. Such robust classification performance is strong evidence of the oral virome's extreme sensitivity to anthropogenic or lifestyle factors. From this perspective, the feline oral virome appears well-suited to act as a high-resolution "environmental signature," one that facilitates fast and accurate identification of cats under varied care regimens.

3.5 Bacteria-Virus Interactions

With regard to α-diversity, the evenness of oral communities (as assessed by the Shannon index) was found to be uniform for all the cohorts examined here, but species richness varied greatly over space—both bacteria and viruses being most numerous in Northeast China and least so in Southwest China **(Fig. 2)**. Principal coordinates analysis (PCoA) with Bray-Curtis distances yielded clear clustering of samples by their geographic origin, clearly demonstrating that macro-geographic factors are the chief determinants of community architecture. Geographic variation explains 20.58% (PCoA1) and 8.74% (PCoA2) of the total variance in the bacterial communities **(Fig. 3A)**, or 84.31% (PCoA1) and 9.96% (PCoA2) in the viral case **(Fig. 3C)**. A

biogeographic survey of the seven main Chinese regions yielded very distinct regional distribution patterns **(Fig. 3B/D)**.Of all the viruses considered, the relative abundance of Siphoviridae is strongly increased in the Northeast (42.15%) and North (38.72%) when compared with the Southwest (25.33%), and for Myoviridae there is a downward trend—from the Southwest (10.28%) to the South (7.56%) or Northeast (5.92%); in particular, unclassified viral families occur very frequently in the Southwest (64.39%) and in the South (58.71%).Among prokaryotes, the MaAsLin2 analysis yielded 428 species that were geographically associated (FDR-adjusted $P < 0.05$; Supplementary Table 11), or more precisely 29 core species with an average abundance ≥0.5%. Of these regional core microbiota, Porphyromonas turned up frequently in the Northeast yet was absent or rare in the Southwest, and Fusobacterium was strongly overrepresented in the North, with Pseudomonas being especially enriched in the South. Spatial separation is not the only factor involved in structuring the microbiota of the feline oral cavity—bacterial and viral communities are closely linked and change in very similar ways, suggesting firm trans-kingdom interactions **(Fig. 3–4)**. With regard to many areas and to various management conditions, viral operational taxonomic units (vOTUs) and species-level genome bins (SGBs) were found to co-aggregate frequently, to share differential abundance, and to vary transcriptionally together. Such tight coordination was especially notable—across a range of experimental conditions—between the major bacterial phyla (Pseudomonadota, Bacillota, and Bacteroidota) and the main viral families (Myoviridae and Siphoviridae), which seem to act in concert with regard to the three levels of community structure (composition, enrichment, and

regulation). All of this implies quite strongly that phages contribute powerfully—both infectively and regulatively—to the shaping of their host bacteria's ecology, and to the balance of the feline oral microbiome.

3.6 Environmental Modulation of Phage Lifestyles and Targeted Surveillance of Mpox Virus

With regard to taxonomic composition, the feline oral virome presents specialized replicative lifestyles that are sensitive to environmental change. Of all the viruses present in the oral virome, approximately 68.3% were temperate phages and only 31.7% were virulent lytic phages. Importantly, the relative prevalence of each type of viral replication varied strongly among host habitats: the proportion of lytic phages among stray cats (38.5%) was substantially higher than among cats kept in catteries (27.9%) or brought to veterinary clinics (28.7%). Such a divergence may be taken as evidence of a marked, environment-related change in phage–host relations, possibly tied to differences in host condition and in the local bacterial density—across the various types of care or management described **(Fig. 4B)**.

At the same time—given emerging biosecurity concerns—a focused screening protocol was applied to the available data with regard to the Mpox virus. With the aid of an exacting computational workflow (multi-stage filtering, highly sensitive alignment, and rigorous validation of control cases), no homologous sequences of the Mpox virus were found among all 1,454 samples analyzed in this study. Such results confirm that the oral cavities of Chinese domestic cats do not currently serve as a

principal source or transmission conduit for the virus. Therefore, the present investigation yields a reliable negative baseline, providing significant value both to health planning and to the epidemiological study of companion animals.

4. Discussion

Of the 1,454 domestic cat oral swab samples taken nationwide, a spatial pattern emerged—one that was tied to regional socioeconomic conditions and to local pet-care practices (for example, stray shelter enrichment in East/Central China and a high density of veterinary hospitals in the Southwest)—but this imbalance provided a convenient natural experiment for evaluating whether macro-geography or the micro-environment dominates in shaping the feline oral microbiome. The results of PERMANOVA and PCoA both point to macro-geographical factors as the main determinants of variation between communities, with 8.1% of bacteria and 6.7% of viruses accounted for by them; these figures are substantially higher than the 3.2% or 2.8% derived from rearing environment data. Mechanistically, such variance appears to be controlled largely by widespread environmental filtering along climate zones divided by the Qinling–Huaihe boundary; in this case, hydrothermal gradients act directly on host mucosal physiochemistry (e.g., with regard to saliva or to osmotic conditions) and indirectly limit the availability of the exogenous "total species pool" from soil, air, or water. Therefore, although micro-rearing conditions encourage local fine-tuning—of diet and of behavior—the feline oral microecology is ultimately governed by an interaction of macro- and micro-level controls (dominance and regulation) that, at the molecular level, facilitate the cross-regional handling of infectious threats to companion animals.

An examination of α-diversity indicated that macro-geographic isolation significantly modulates species richness but does not alter community evenness (as

measured by Pielou's evenness index), thereby suggesting an essential evolutionary compromise between host homeostasis and the environment [21]. Robust intrinsic physiological systems—like routine salivary flushing or immunoglobulin production—appear to maintain a remarkably constant national architecture of community evenness [22], whereas species richness reflects an open window onto the external environment, varying with local climate patterns and husbandry practices. Importantly, while bacterial and viral α-diversity are tightly coupled ($r = 0.62$, $P < 0.001$), they do not share the same degree of geographic fluidity, with viral communities exhibiting far sharper β-diversity boundaries. This cross-kingdom asymmetry is mechanistically rooted in the strict "lock-and-key" or "Red Queen" coevolutionary dynamics among bacteriophages. Geographic isolation constrains host population interactions, thereby accelerating the local drift of bacterial genomes and driving highly specific sequence recombinations in associated phages. Consequently, environmental filtering defines the geographical range of host bacteria, and each localized virus–bacterium alignment cumulatively amplifies the spatial signal, ultimately producing a fine-scale geographic fingerprint for the feline oral virome.

The attempt to construct an oral microbial genome catalog (OAPGC) has uncovered a major amount of "microbial dark matter," namely 51% of known bacteria for which no culturable representatives appear in global databases. Such non-random clustering—within the three principal phyla (Pseudomonadota, Bacillota, or Bacteroidota)—suggests that the relevant taxa are part of the resident core rather than of a temporary passenger population; indeed, their lengthy coevolution with host-

specific oral pressures seems to have caused the loss of autonomous metabolic pathways, making traditional monoculture difficult to apply because of a necessary metabolic syntrophy [23], [24]. With the "One Health" perspective in mind, the uncultured core taxa are both an asset and a potential danger: they contribute strongly to normal oral niche dynamics and so help to prevent the establishment of foreign (pathogenic) organisms; however, they must also be regarded as undercharacterized sources of new virulence elements and of latent antimicrobial resistance genes (ARGs)—both of which may cause zoonotic disease if transmitted across species when the host is immunocompromised. At the same time, an improved-resolution map has revealed a definite regional pattern of highly prevalent opportunistic pathogens, connected to climate and to forms of animal husbandry: specifically, the long, cold, and damp winters of Northeast China were favorable for biofilm-producing environmental pathogens (Pseudomonas aeruginosa or Klebsiella pneumoniae), whereas East and South China—because of their dense pet trades and their clinical use of antibiotics—strongly selected for multidrug-resistant variants (Enterococcus faecium and Acinetobacter baumannii) [25], [26]. Ultimately, such localized microbial fingerprints confirm that the cat's oral cavity is an excellent "biochemical sentinel"—one capable of revealing local environmental facts and human influences—and thus furnishes important molecular clues for precision veterinary care and for AMR-directed containment measures [27].

With the aid of high-throughput shotgun metagenomics and an integrated identification method, this work has analyzed thousands of well-defined viral genomes, uncovering a very large reserve of viral "dark matter" largely composed of unclassified

Caudoviricetes [23], [28]. Random forest analysis points to certain order- or family-level taxa as principal factors in determining community structure [29]; The relevant phages are marked by specific genomic properties—such as specialized tail fibers or transcriptional modules—rendering them capable of acting as "apex regulators" which control core bacterial populations over spatial gradients. To chart these dynamics, a cross-kingdom co-occurrence network suggests an unusual "core-periphery" configuration, with many as yet unclassified peripheral viruses arranged radially about a small set of hub bacteria (e.g., SGB0405 and SGB0534), which seem to show strong host dependency. Among the ten "bridge" viruses, all four main core bacteria were simultaneously engaged, and this fact suggests two ecological possibilities: either these are very rare, broadly applicable (polyvalent) phages making use of surface receptors—common to many mucosal microbes—within the biofilm, or they derive from cross-kingdom relations shaped by metabolism, with a specific lysis of one hub sufficient to destabilize the others (nutritionally or structurally) [30]. In light of such bridging phenomena, the classical "one bacterium, one phage" rule is called into question, and new molecular candidates emerge for polyvalent phage-guided therapies in domesticated animals [30].

A characterization of phage life-history strategies indicates that most bacteriophages found in the healthy feline oral cavity use an evolutionarily conserved, lysogeny-dominant approach. That lifestyle seems well adapted—evolutionarily—to a mucosal environment of long-term stability but of intense competition ("high-density, low-turnover"); under such conditions, temperate phages avoid extinction by means of

integration with the host genome, thereby resisting salivary washout and host immune clearance [31], [32], Under these conditions, temperate phages evade extinction via genome integration, thereby buffering the viral population against salivary washout and host immune clearance, while simultaneously conferring superinfection immunity and metabolic augmentation to the resident microbiota [33], [34]. Nevertheless, such a conservative balance is greatly modified in stray cat communities; exposure to contaminated items or to wild prey introduces new environmental or gastrointestinal-derived bacteria, which tend to promote the co-enrichment of lytic phages and opportunistically acting pathogens, such as Campylobacter jejuni [35]. Rather than being evidence of irreversible dysbiosis, this phenomenon is best interpreted as an adaptive feedback mechanism compatible with the "Kill the Winner" hypothesis, in which limited pathogen proliferation leads to more frequent phage-host collisions, thus initiating lytic cycles and enabling lytic phages to serve as "ecological safety valves" that rein in pathogens at subclinical levels [36]. Crucially, if malnutrition or co-infections (such as FIV) compromise host immunity, then massive endotoxin and cellular debris release—byproducts of this extreme lytic state—may cause an acute exacerbation of feline chronic gingivostomatitis (FCGS) or raise zoonotic spillover probabilities [37], [38], These findings highlight the imperial necessity for microecological surveillance of stray animals, calling for an integrated "One Health" approach to mitigate public health risks [39], [40].

5. Conclusion

With a view to nationwide metagenomic data, this study systematically determines

the underlying mechanisms—geographical and environmental—by which macro-scale patterns and local-scale factors affect the oral bacterial and viral communities of cats in China. Geographical variation is strongly reflected in the oral microbiota, and its diversity appears tightly bound to China's climatic zones. Among the dominant bacterial phyla are Pseudomonadota, Bacillota, and Bacteroidota. Within the virome, most elements belong to Caudoviricetes, and many unassigned novel taxa—so far unnamed—play an appreciable part in oral microecosystem regulation via bacteria–virus interactions. Environmental variation is a key driver causing the differentiation of microbial guilds: stray cats seem to be dominated by resilient organisms and show a greater prevalence of clinically significant opportunistic pathogens, while catteries or veterinary hospitals present a more stable commensal profile. Viral biomarkers selected by means of random forest analysis yield useful distinctions between various living environments. Targeted screening for monkeypox virus yielded exclusively negative results, which strongly suggests that domestic cats' oral cavities in China are not currently important reservoirs or transmitters of this pathogen. Taken together, these data provide a national baseline for monitoring companion animal health and for early warnings of zoonoses—as well as for One Health-inspired prevention—and so help to guide future microbiome-related studies.

6. Abbreviations

D : cats in animal hospitals; L : cats in stray shelters; M : cats in catteries;

Caudoviricetes_13_59 : "Caudoviricetes_13_59" denotes a phage assigned to the 59th novel family within the 13th unclassified novel order of the class Caudoviricetes.

7. Data availability

The raw metagenomic sequencing data generated in this study have been deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB104049. The complete Oral Animal/Pet Genome Collection (OAPGC), including non-redundant prokaryotic, fungal, and viral genome sequences, associated annotation files, and the custom Kraken2 database, has been permanently and publicly archived in the Zenodo repository under the accession number 17695899 (https://doi.org/10.5281/zenodo.17695899). Source data for the main figures are provided with this paper.

8. Authors' contributions

Q.L. and Z.L. conceived this study.J.Z.,L.C., designed and performed this research. Q.Y. and Z.P. wrote this manuscript. W.Y. and D.Z. improved the language. All authors reviewed this manuscript.

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10. Ethics approval and consent to participate

All procedures involving the newly sequenced feline oral samples in this study were reviewed and approved by the Animal Ethics Committee of Wuhan University of Technology (Approval No. 2024-034). All methods were performed in accordance with the relevant guidelines and regulations established by the committee. The publicly available metagenomic datasets analyzed in this study were sourced from the NCBI Sequence Read Archive (SRA) and EMBL-EBI ENA databases. Their use in this secondary analysis is consistent with the terms of access and intended use specified by the original submitters. Informed consent was obtained from all cat owners prior to the collection of new samples.

11. Consent for publication

Not applicable.

12. Competing interests

The authors declare that they have no competing interests.

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