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Characteristics of tongue images and tongue coating bacteria in patients with colorectal cancer

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Abstract

Background Tongue diagnosis can assess the physiological functions and pathological conditions of colorectal cancer (CRC).

Objective To investigate the characteristics of tongue images and the bacteria composition of tongue coating in patients with CRC.

Methods A total of 59 CRC patients were enrolled and underwent tongue image analysis using a diagnostic instrument. Third-generation sequencing technique was used to determine tongue coating bacteria. The relationships among tongue images, tongue coating bacteria and clinical information (age, gender, and location) were further analyzed. Statistical analyses were performed to investigate the potential associations between tongue coating microbiota and two key variables (tongue images and age).

Results CRC patients typically present with four distinct tongue images, including yellow greasy coating, petechiae, white greasy coating, and red tongue with little coating. Notably, the microbiota composition of tongue coatings demonstrates a statistically significant correlation with both tongue diagnostic features and patient age compared to other clinical parameters. *Alloprevotella rava* may be primary contributors to the formation of yellow greasy coating, while *Prevotella intermedia* appears to be associated with the development of white greasy coating. Furthermore, *Streptococcus parasanguinis* might emerged as a potential biomarker for CRC patients under 62 years of age, whereas *Prevotella* sp. 000163055 appears to be a predominant species in the tongue coating microbiota of CRC patients over 62 years.

Conclusion Overall, this study emphasizes the variations in the community structure and diversity of tongue coating bacteria in CRC patients through different tongue images. The findings provide novel insights that could enhance

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the predictive capacity of tongue diagnosis for clinical progression monitoring and contribute to the development of early intervention strategies in CRC management.

Importance

Colorectal cancer (CRC) is a prevalent malignant tumor of the digestive system, characterized by high morbidity and mortality rates. In recent years, increasing attention has been directed towards the investigation of tongue imaging in CRC patients. As one of the four fundamental diagnostic methods in Traditional Chinese Medicine (TCM), tongue diagnosis has emerged as a significant auxiliary tool for identifying various diseases. To enhance the sensitivity and specificity of tongue imaging in the early screening and widespread application of CRC, this study aims to establish a connection between tongue images and CRC at a microscopic level. By doing so, it seeks to provide a novel perspective for the early intervention strategies in CRC management and to facilitate the integration of tongue diagnosis into modern clinical practice.

Highlights

- Patients with CRC might exhibit four distinct tongue images, including yellow greasy coating, petechiae, white greasy coating, and red tongue with little coating.
- *Alloprevotella rava* may serve as primary contributors to the formation of yellow greasy coating, whereas *Prevotella intermedia* was potentially associated with the development of white greasy coating.
- *Streptococcus parasanguinis* emerged as a potential biomarker distinguishing CRC patients aged 62 years, whereas *Prevotella* sp. 000163055 appeared to dominate the tongue coating microbiota in CRC patients aged 62 years and above.

Keywords CRC, Tongue images, Tongue coating, Bacteria, Clinical information

Introduction

Traditional Chinese medicine (TCM) is a comprehensive and time-honored medical system that has been developed over thousands of years, focusing on the study of human physiology, pathology, as well as disease diagnosis and prevention [1, 2]. TCM practitioners diagnose a patient's imbalance primarily by 4 traditional methods of diagnosis (including observation, listening, interrogation, and pulse-taking) [3]. As an integral part of the healthcare system, TCM plays a significant role in daily medical treatment and disease prevention across several Asian countries, particularly in China, South Korea, and Japan [4]. Among various diagnostic approaches, tongue diagnosis stands out as a crucial non-invasive technique in TCM, where doctors assess disease symptoms by observing tongue coating color, tongue color, and texture [5]. This traditional diagnostic method has been widely recognized as an effective auxiliary approach for assessing both physiological and pathological changes in the human body, thereby providing valuable insights into an individual's health status [6, 7].

Colorectal cancer (CRC) represents a major global health challenge, ranking as the third most commonly diagnosed malignancy worldwide with over 1.8 million new cases annually, and the second leading cause of cancer-related mortality, responsible for more than 860,000 deaths each year [8]. While multiple factors contribute to CRC development, environmental influences play a predominant role. Among these environmental factors, the involvement of gut microbiota in cancer biology has gained increasing scientific attention [9]. A growing body of research has established significant associations between gut microbial composition and CRC pathogenesis [10–14]. The oral cavity, serving as the primary entry point to the digestive system, shares substantial metabolic regulatory similarities with gut microbiota [15]. Emerging evidence suggested that certain oral-derived microorganisms could translocate to the gastrointestinal tract by overcoming physiological barriers, including epithelial integrity and antimicrobial defenses, under pathological conditions. This ectopic colonization has been mechanistically linked to the pathogenesis of chronic inflammatory disorders, particularly inflammatory bowel disease (IBD) and CRC, through microbiota-mediated immune modulation and epithelial barrier dysfunction [16–18]. This research is supported by Cui et al. [19] who identified distinct alterations in tongue microbiota of gastritis patients through metagenomic sequencing, revealing potential diagnostic biomarkers. Similarly, Lu et al. [20] demonstrated unique microbial profiles in tongue coatings of pancreatic head cancer patients, with *Porphyromonas*, *Haemophilus*, *Fusobacterium*, and *Leptotrichia* emerging as potential discriminative biomarkers between cancer patients and healthy individuals.

The oral microbiome, characterized by its remarkable stability in contrast to the gut microbiome, has emerged as a promising focal point for research into tongue coating. The incorporation of tongue coating microecology into tongue diagnosis research not only offers robust biological evidence for this traditional diagnostic method but also significantly enhances its scientific credibility and clinical applicability. For instance, Chen et al. [21]

identified correlations among tongue images, microbiome composition, and cardiovascular function, suggesting a novel approach to blood pressure management through the modulation of tongue coating bacteria in specific populations. Similarly, Liu et al. [22] observed significant alterations in tongue coating characteristics and microbial profiles across different populations during the progression of CRC, reflecting disease evolution. Our prior research further revealed distinct differences in the microbial community structure of tongue coating between CRC patients and healthy individuals, suggesting that tongue coating microbiota could serve as a potential biomarker for CRC detection [23]. Consequently, we propose that a detailed analysis of tongue coating bacteria can provide critical insights into the relationship between tongue images and clinical data in CRC, paving the way for innovative diagnostic and therapeutic strategies.

However, traditional tongue diagnosis primarily relies on physicians' subjective visual assessments and lacks standardized quantitative measures, often resulting in diagnostic outcomes that are vulnerable to external influences and individual biases [24]. Digital image processing technology, known for its ability to rapidly classify images and efficiently extract features [25], has been widely utilized in various fields, including facial recognition [26], fingerprint identification [27], and satellite cloud image analysis [28]. In this study, the Shanghai Daosheng Tongue Image Acquisition System (DS01-A) was utilized for tongue image analysis and processing, alongside microbial detection using 16 S rRNA sequencing technology. The study compared the characteristics of tongue coating bacteria and tongue images among patients with different clinical manifestations of CRC, and explored the relationships among tongue coating bacteria, tongue images, and clinical characteristics of CRC.

Table 1 Characteristics of study participants

Clinical information		**CRC (*n* = 59)**
Gender	Male	37
	Female	22
Age		62.82 ± 14.14
Coated Tongue	Yellow and greasy fur	19
	Petechia	11
	White and greasy tongue coating	15
	Red tongue with little moss	14
Stage	I	9
	II	12
	III	17
	IV	21
Location	Rectum	25
	Colon	34

Methods

Inclusion and exclusion criteria of samples

A total of 59 CRC patients were enrolled in the Oncology Department of Huzhou Central Hospital from 2021 to 2022, including 34 colon cancer patients and 25 rectal cancer patients. The CRC patients enrolled in this study were carefully selected to ensure homogeneity across key clinical parameters. The inclusion criteria comprised individuals of the yellow race with pathologically confirmed CRC, all hailing from the same geographical region (Huzhou, Zhejiang Province) and sharing comparable dietary habits and nutritional patterns. The exclusion criteria were as follows: ① Combined with other malignant tumors; ② Serious heart and lung disease; ③ Patients who had taken antibiotics, immunosuppressants or bacteria regulation drugs within 1 month before admission; ④ Other intestinal diseases, such as ulcerative colitis and Crohn's disease; ⑤ Patients with mental illness and communication disorders; ⑥Patients with dental caries, periodontitis, and oral mucosal lesions (confirmed through comprehensive dental examinations). All participants were instructed to refrain from eating, drinking, and performing oral hygiene procedures for a minimum of 2 h prior to the examination.

All CRC patients were pathologically diagnosed and clinically staged in accordance with the American Joint Committee on Cancer (AJCC) cancer staging guidelines. All subjects signed informed consent in accordance with the guidelines approved by the Ethics Committee of Huzhou Central Hospital. The characteristics of the study participants are shown in Table 1. Standardized tongue image acquisition and coating sample collection were performed during preoperative clinical assessment.

Tongue images acquisition

The participants' tongues were photographed before breakfast, and they were instructed to gargle with water before the imaging process. The tongue coating images of all participants were captured and analyzed using the DS01-A Tongue Surface Information Identification and Acquisition System (Dow, Shanghai, China). The analysis of tongue coating encompassed various parameters, including the assessment of presence, thickness, color, moisture, greasiness, indentation, tingling, crevices, ecchymosis, and other relevant characteristics.

Tongue coating sample collection

Once again, all participants underwent tongue coating sampling before breakfast, with instructions to gargle with water prior to sample collection. A sterile spoon was used to scrape the front and middle regions of the tongue, and saline was added to the test tube. This process was repeated three times, followed by centrifugation (2000 rpm) for 5 min. The resulting sediment was stored in an ultra-low-temperature (-80 ℃) refrigerator for subsequent use.

Collection and processing of clinical information

The data were sourced from Huzhou Central Hospital. In light of the incomplete data for certain aspects of patient examinations, 59 CRC samples were included in the microbiome analysis based on the available data. Clinical information for the selected samples encompassed demographic details such as age and gender, as well as pertinent medical information including tongue images, cancer stage, and cancer site.

Acquisition of microbial genomes

DNA extraction kit (a E.Z.N.A.® Tongue Coat DNA kit) was used to extract DNA from tongue coat samples (Omega Bio-Tek, Norcross, GA, USA). Specific primers with barcodes were synthesized. The V1-V9 region of the bacteria 16S ribosomal RNA gene were amplified by PCR (95°C for 2 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s and a final extension at 72°C for 5 min) using primers 27F 5'-AGRGTTYGATYMTGGCTCAG-3' and 1492R 5'-RGYTACCTTGTTACGACTT-3'. Amplicons were purified using a magnetic bead-based DNA cleanup kit (Agencourt AMPure XP, Beckman Coulter, Brea, CA, USA) to remove primer dimers and other artifacts.

16 S rDNA full-length sequencing technology was used to analyze the DNA sequence encoding 16 S RNA of bacterial ribosomes [29]. All amplicon sequencing was performed by Shanghai Lingen Biotechnology Co. Ltd (Shanghai, China). PacBio raw reads were processed using the SMRT Link Analysis software version 9.0 to obtain demultiplexed circular consensus sequence (CCS) reads. Raw reads were processed through SMRT Portal. Sequences were further filtered by removing barcode, primer sequences, chirmas and sequences, if they contained 10 consecutive identical bases. OTUs were clustered with 98.65% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16 S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU132)16 S rRNA database using confidence threshold of 70% [30, 31].

Data analysis

Rarefaction analysis revealed diversity index, including Chao, ACE and Shannon diversity index. Beta diversity analysis was performed using UniFrac to compare the results of the principal component analysis (PCA) using the community ecology package and R-forge (Vegan 2.0 package was used to generate a PCA figure). R (pheatmap package) and Cytoscape (http://www.cytoscape.org) were used to visualize relationships via correlated heat maps and network maps, respectively. Statistical analysis was performed by SPSS software (V: 16.0; SPSS Inc., Chicago, IL) w. Data were expressed as mean ± standard deviation (SD). T-test was used to compare the means $p < 0.05$, and the difference was statistically significant.

Results

Relationship between tongue images and clinical information in patients with CRC

The study was conducted according to the process shown in Fig. S1. Tongue images and tongue coatings of 59 participants were collected and analyzed. Given that tongue coating thickness is influenced by factors such as age, gender, BMI, tumor site, and neoplasm staging, these variables were statistically analyzed for all participants. The histogram analysis of color ratios of tongue revealed that the tongue images of CRC patients predominantly exhibited yellow and red hues, with no statistically significant differences observed among the groups (Fig. S2). A comprehensive analysis of tongue images using a tongue diagnosis instrument identified four distinct types in CRC patients: yellow greasy coating, petechiae, white greasy coating, and red tongue with little coating (Fig. 1A). The tongue image collection report indicated that CRC patients frequently exhibited thicker and greasier tongue coatings, often accompanied by tooth marks. Furthermore, correlation analysis between different tongue images and clinical information suggested that petechiae had a relatively stronger correlation with clinical data (Fig. 1B). Specifically, petechiae showed a positive correlation with gender ($r = 0.26$, $p < 0.05$) and a negative correlation with disease stage (r=-0.25, $p < 0.05$). Thus, there might be potential relationships between tongue images and CRC.

Microbial characteristics of tongue coating in patients with CRC

The microbial communities present in tongue coating samples were analyzed using metagenomic sequencing technology. The analysis revealed a predominance of *Streptococcus* and *Neisseria* at the genus level. Specifically, *Streptococcus*, *Neisseria*, *Veillonella* and *Prevotella* were the most abundant genera, while *Neisseria subflava_A*, *Streptococcus oralis_S*, *Haemophilus haemolyticus*, and *Streptococcus salivarius* were the top four species identified (Fig. 2A-B). Further analysis explored the correlation between tongue coating microbiota and clinical parameters. At the genus level (Fig. 2C), *Streptococcus* exhibited a positive correlation with age ($r = 0.37$, $p < 0.05$), whereas *Proteiniphilum* (r=-0.39, $p < 0.05$) and *Prevotellamassilia* (r=-0.36, $p < 0.05$) showed negative correlations with age. Additionally, *Gemella* was positively correlated with gender ($r = 0.40$, $p < 0.05$). Conversely, *Alloprevotella* (r=-0.32,

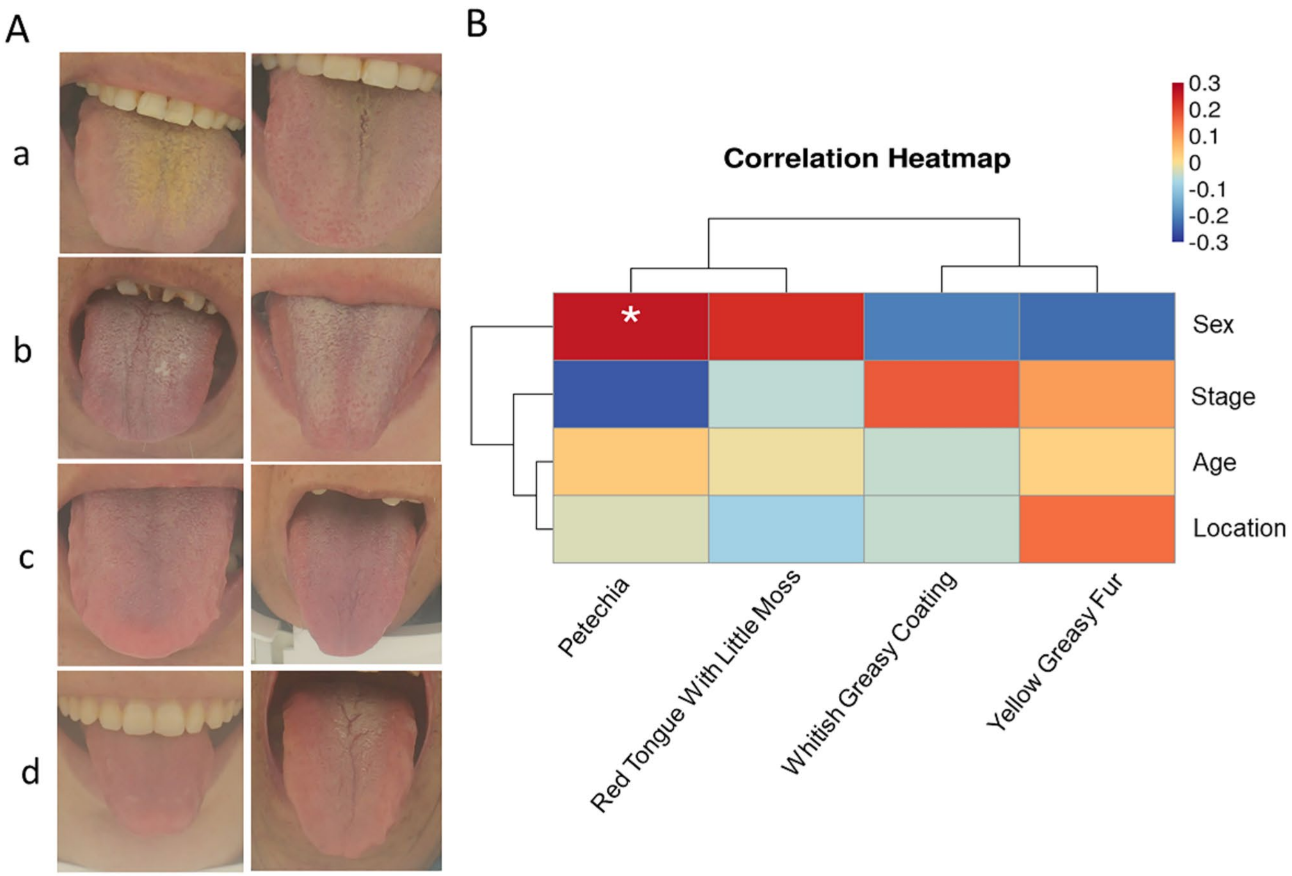

Fig. 1 Relationship between tongue images and clinical information in patients with CRC. **A**: four tongue images of CRC patients. **a**. yellow greasy coating, **b**. white greasy coating, **c**. red tongue with little coating, **d**. petechiae. **B**: Heat map of tongue images correlation with gender, stage, age and location

$p < 0.05$), *Lachnoanaerobaculum* (r=-0.27, $p < 0.05$), *Actinomyces* (r=-0.26, $p < 0.05$) were negatively correlated with tongue images. At the species level (Fig. 2D), *Streptococcus parasanguinis_B* showed a significant positive correlation with age ($r = 0.38$, $p < 0.05$), while *Prevotella* sp. 000163055 exhibited a significant negative correlation with age (r=-0.41, $p < 0.05$). Furthermore, *Rothia mucilaginosa_B* and *Rothia* sp. 001808955 showed distinct correlations with tongue images, demonstrating their highest relative abundance in red tongue with little coating (Fig. S3). This study highlights the intricate relationships between tongue microbiota composition and clinical factors, providing insights into potential microbial biomarkers associated with age and tongue characteristics.

Microbial characteristics of tongue coating in CRC patients with different tongue images

Participants were stratified by age, gender, disease stage, disease location, and tongue imaging characteristics. At the species level, we analyzed the relative abundance of 30 bacteria across these groups (Fig. S4). Figure 3A demonstrated the species-level bacterial distribution across different tongue image categories, revealing *Neisseria subflava_A* as the most abundant species across four tongue image types. We further examined variations in alpha diversity of tongue coating microbiota across different tongue image types. Utilizing eight distinct metrics, we comprehensively evaluated alpha diversity patterns. As shown in Fig. 3B, all diversity indices consistently demonstrated no significant variation in microbial alpha diversity across the four age groups. Subsequent beta diversity analysis, illustrated in Fig. 3C, revealed considerable overlap in microbial community structures among the four tongue coating types, as indicated by confidence ellipses. These findings were statistically validated through permutational multivariate analysis of variance (PERMANOVA), confirming the absence of significant intergroup differences (Table S1, $p = 0.07$).

The differences of the top 20 microbial species across patient subgroups stratified by disease stage (I-IV), anatomical tumor location (colon or rectum), and gender (male or female) were shown in FIG S5. Among these, *Parvimonas micra*, *Prevotella loescheii* and *Alloprevotella rava* exhibited the most pronounced variations across tongue image categories. *Alloprevotella rava* showed

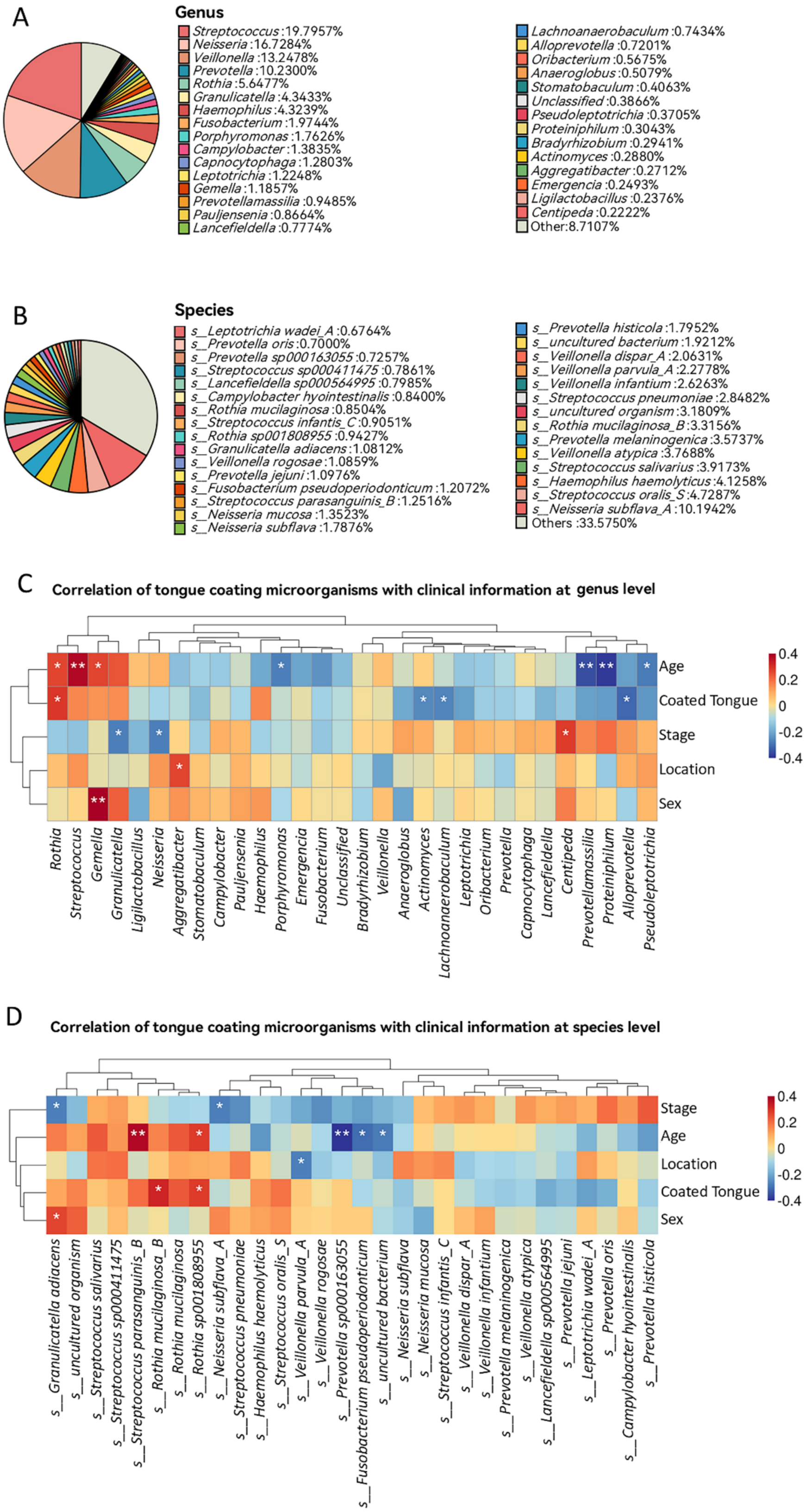

Fig. 2 Microbial characteristics of tongue coating in patients with CRC. **A**: Pie chart of proportion of tongue coating bacteria at genus level. **B**: Pie chart of proportion of tongue coating bacteria at species level. **C**: Correlation heat map of tongue coating bacteria with age, gender, location, stage and tongue coating at generic level. * signifies $p<0.05$, ** signifies $p<0.01$. **D**: Correlation heat map of tongue coating bacteria with age, gender, location, stage and tongue coating at species level. * signifies $p<0.05$, ** signifies $p<0.01$

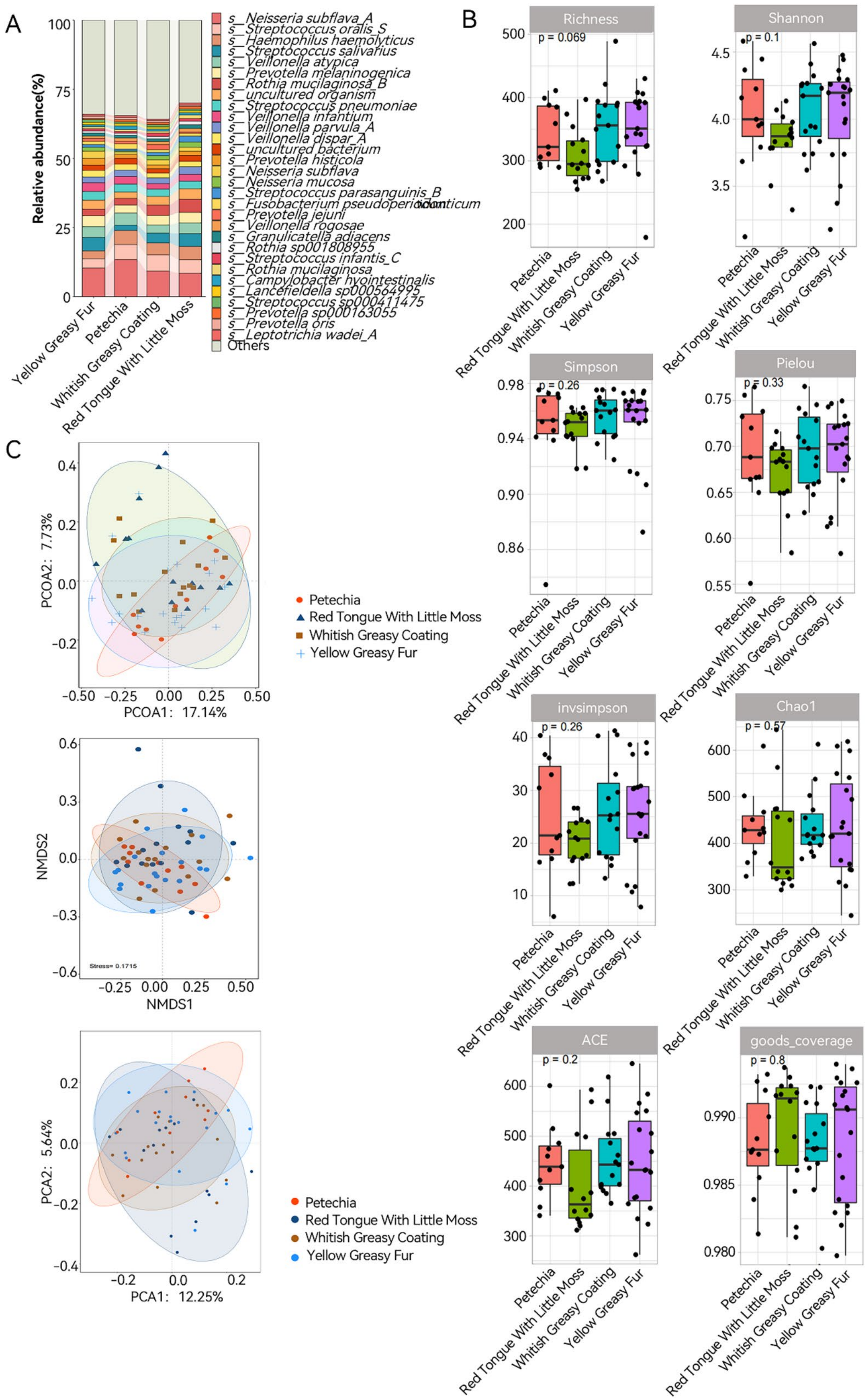

Fig. 3 Microbial characteristics of tongue coating in CRC patients with different tongue images. **A**: Histogram of the relative abundance of tongue coating bacteria in four kinds of tongue images. **B**: Alpha diversity of tongue coating bacteria in four tongue images. **C**: Beta diversity of tongue coating bacteria in four tongue images. Points with different colors or shapes represent different groups of samples. The closer the two sample points are, the more similar the species composition of the two samples

particular prominence in yellow greasy coating, while *Parvimonas micra* and *Prevotella loescheii* were more prevalent in whitish greasy coating (Fig. 4A). Chord diagram analysis revealed strong associations between *Alloprevotella rava* and yellow greasy coating, while *Prevotella intermedia* showed stronger correlations with whitish greasy coating (Fig. 4B). The specific numerical data are provided in Supplementary File 1. To explore potential microbial interactions within tongue image categories, we performed extensive correlation analyses of microbial taxa. *Neisseria cinerea* and *Neisseria flavescens_A* demonstrated significant associations with petechiae. Our correlation network analysis identified four bacterial strains (*Lactobacillus helsingborgensis*, *Acinetobacter sp012371325*, *Pseudoclavibacter chungangensis* and *Staphylococcus hyicus*) showing strong positive intercorrelations (Fig. S6A, $r = 0.99$, $p < 0.05$). Conversely, Fig. S7 showed *Rothia mucilaginosa_A* exhibited negative correlations with seven bacterial species, including *Treponema* sp. 000775995 (r=-0.51, $p < 0.05$), *Fusobacterium polymorphum* (FIG. S6A, r=-0.49, $p < 0.05$), *Filifactor alocis* (r=-0.42, $p < 0.05$), *Catonella morbi* (r=-0.41, $p < 0.05$), *Alloprevotella rava* (r=-0.40, $p < 0.05$), *Porphyromonas endodontalis* (r=-0.40, $p < 0.05$), and *Prevotella intermedia* (r=-0.39, $p < 0.05$).

Microbial characteristics of tongue coating in CRC patients of different ages

CRC patients were stratified into two age groups based on the mean age of 62 years. Given the pronounced correlation between age and tongue coating microbiota, this study specifically investigated the characteristics of tongue coating bacteria in CRC patients across these age groups. Figure 5A illustrated the relative abundance of the top 30 bacteria in each age group. The analysis revealed distinct microbial profiles: in patients over 62 years, the predominant species were *Neisseria subflava_A*, *Haemophilus haemolyticus*, and *Streptococcus oralis_S*, whereas in patients under 62 years, the dominant species were *Neisseria subflava_A*, *Streptococcus salivarius*, and *Streptococcus oralis_S*. To evaluate age-related variations in the alpha diversity of tongue coating bacteria, eight different metrics were employed. As depicted in Fig. 5B, significant differences in alpha diversity between the two age groups were only observed using the Richness index, with other metrics showing no significant variation. Figure 5C presented considerable overlap in microbial community structures between the two age groups as well. These findings were statistically validated using PERMANOVA, which confirmed the lack of significant intergroup differences (Table S2, $p = 0.352$).

Further analysis focused on the top 20 age-associated bacterial species. Differential analysis highlighted *Prevotella copri_A*, *Agathobacter faecis*, and *Streptococcus cristatus* as showing the most significant age-related differences (Fig. 6A). Chord diagrams indicated that *S. parasanguinis_B* was more prevalent in patients under 62 years, while *Prevotella* sp. 000163055 was more associated with patients over 62 years (Fig. 6B). The specific numerical data are provided in Supplementary File 2. The correlation heatmap (FIG. S6B) demonstrated strong positive correlations among *S. parasanguinis_B*, *S. parasanguinis_C*, and *S. parasanguinis_D*, with the strongest correlation observed between *S. parasanguinis_B* and *S. parasanguinis_D* ($r = 0.95$, $p < 0.05$). Additionally, *S. parasanguinis* exhibited significant negative correlations with *Fusobacterium pseudoperiodonticum* (r=-0.65, $p < 0.05$) and *Prevotella* sp. 000163055 (r=-0.54, $p < 0.05$).

Discussion

Tongue diagnosis, a distinctive approach in TCM for evaluating health status through the analysis of tongue images, represents a fundamental component of TCM diagnostic practices. With the rapid evolution of computer information technology, advancements in artificial intelligence have attracted considerable attention. Particularly, image recognition technology has become a crucial element in the collection and interpretation of tongue image data. Studies have revealed that changes in tongue coating characteristics are closely associated with variations in the composition of tongue microbiota [32]. Tongue images were associated with the development and progression of various diseases [33–35]. In this research, we utilized the Shanghai Daosheng Tongue Image Acquisition System (DS01-A) to examine and process tongue images from CRC patients, combined with 16 S rRNA sequencing technology to identify microbial communities. This comprehensive methodology allowed us to explore the potential relationships between tongue image features and the clinical profiles of CRC patients.

Our comprehensive analysis of 59 CRC patients identified four predominant tongue image characteristics: yellow greasy coating, petechiae, white greasy coating, and red tongue with minimal coating. To elucidate the potential relationship between tongue coating microbiota and these clinical manifestations, we conducted an extensive microbial analysis of tongue coating samples from the enrolled CRC cohort. The results demonstrated that *Streptococcus* and *Neisseria* were the most abundant microorganisms in the tongue coating of CRC patients, consistent with the findings reported by Liu et al. [22]. *Neisseria subflava*, typically a commensal bacterium colonizing the human respiratory tract as part of the normal flora, can transform into an opportunistic pathogen under specific conditions, potentially leading to various health complications [36]. Beyond its pathogenic potential, this bacterium is also recognized as a component of symbiotic microbial communities frequently observed on

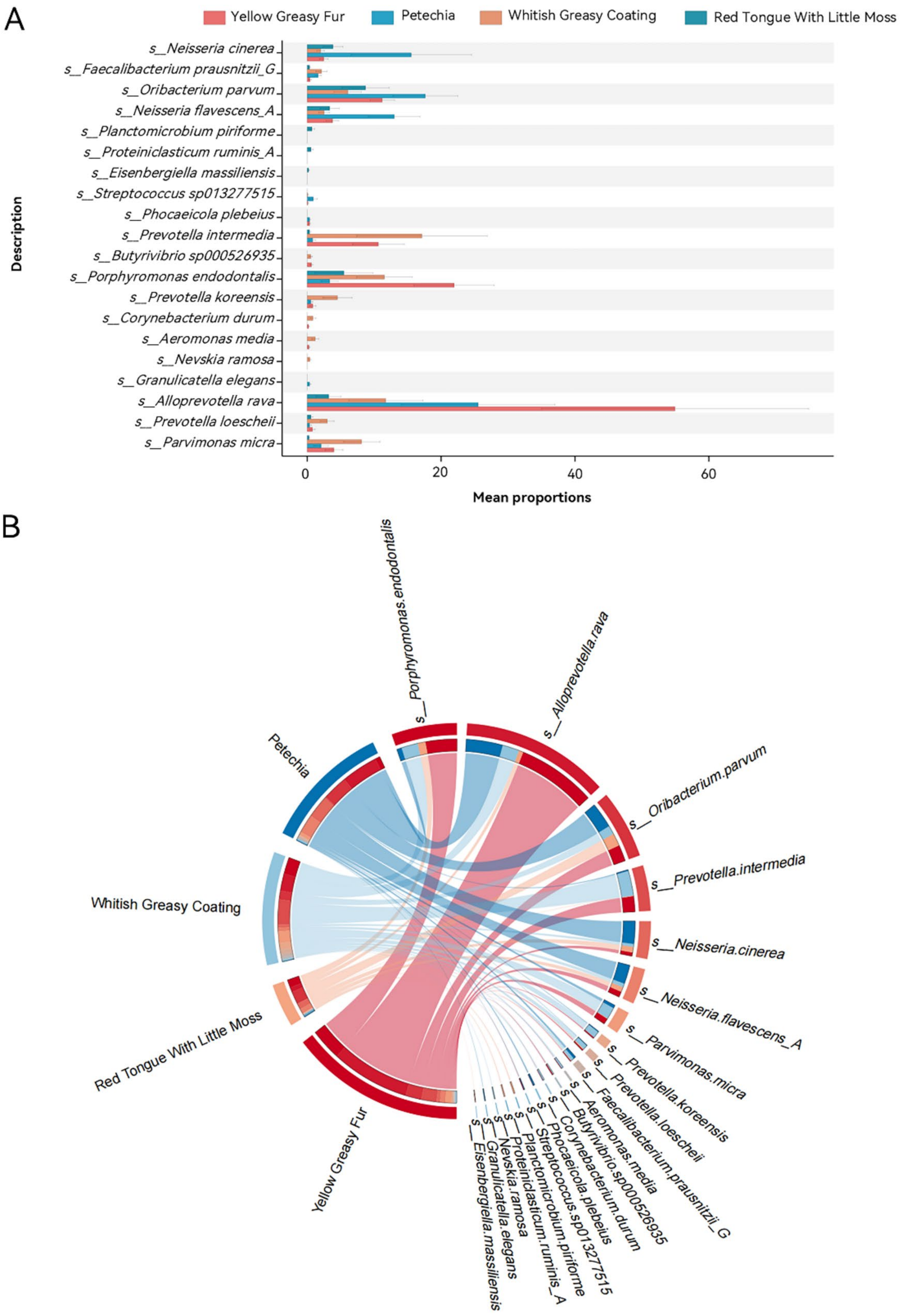

Fig. 4 The difference and correlation between tongue coating bacteria and tongue images. **A**: Difference analysis of tongue coating bacteria in four tongue images. **B**: Chords of tongue coating bacteria in four tongue images. The distinct colors correspond to different tongue images and different bacteria, while the strength of the relationships is indicated by the varying thickness of the connecting lines

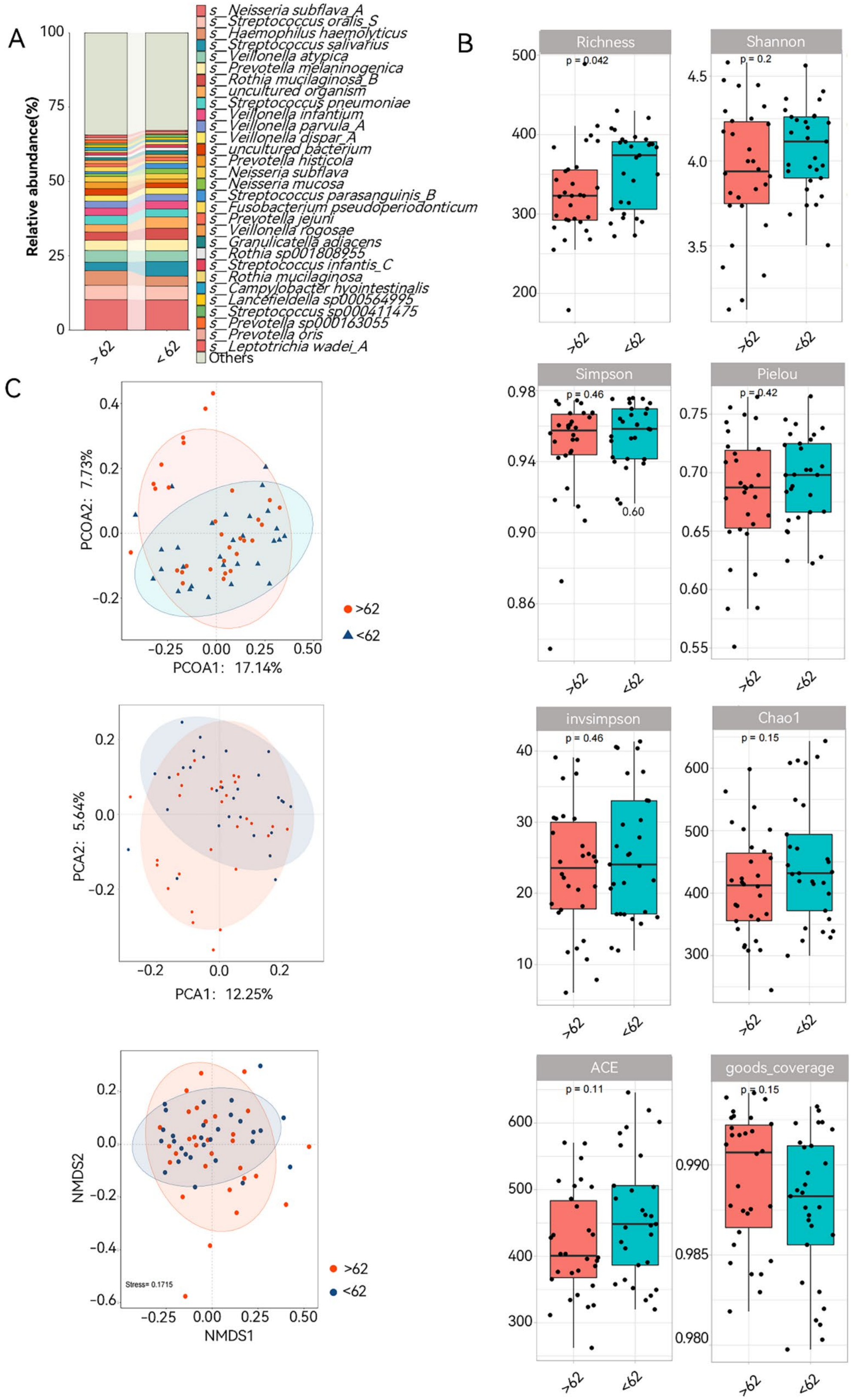

Fig. 5 Microbial characteristics of tongue coating in CRC patients of different ages. **A**: Histogram of the relative abundance of tongue coating bacteria in different age groups. **B**: Alpha diversity of tongue coating bacteria in different age groups. **C**: Beta diversity of tongue coating bacteria in different age groups. Points with different colors or shapes represent different groups of samples. The closer the two sample points are, the more similar the species composition of the two samples

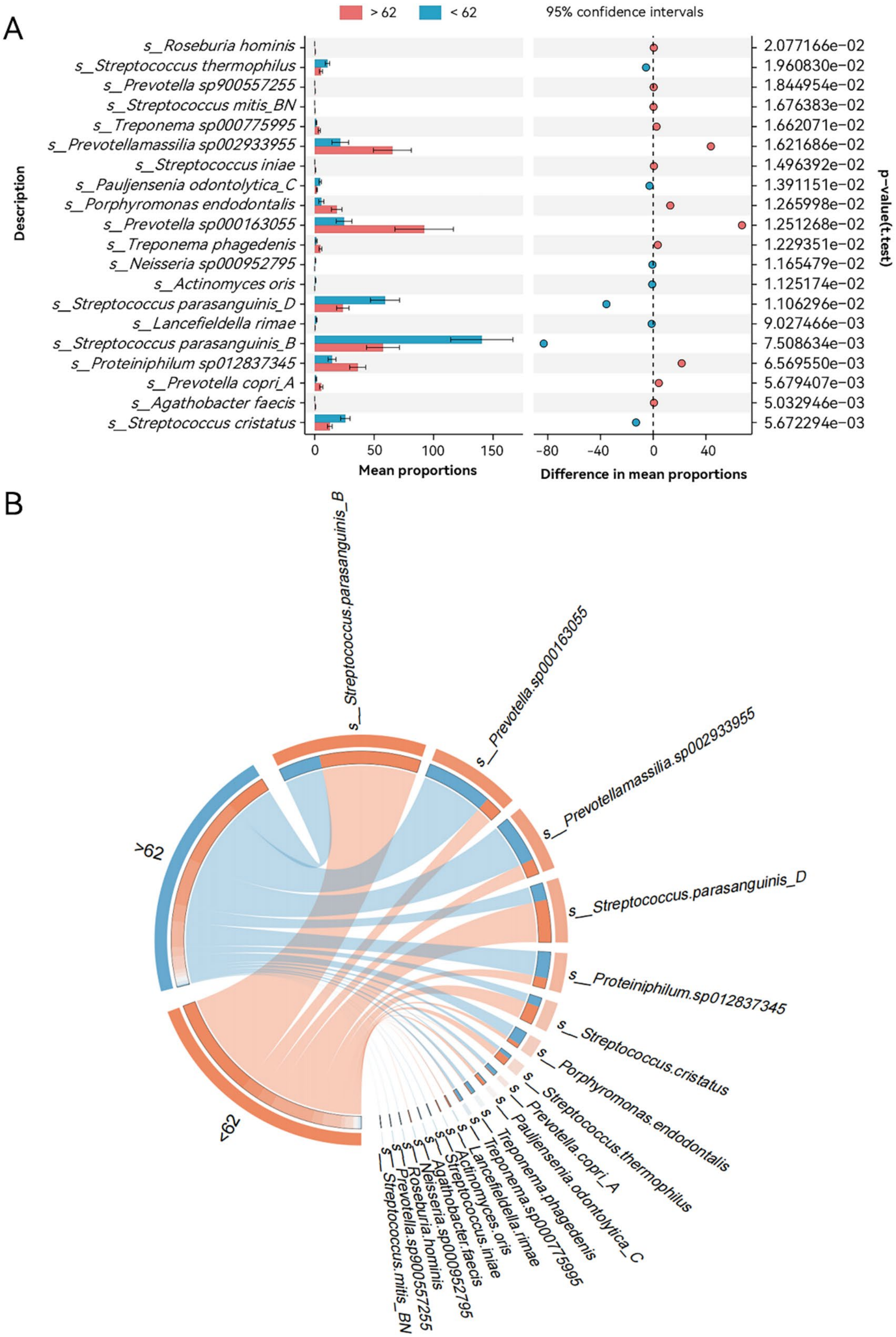

Fig. 6 The difference and correlation between tongue coating bacteria and age. **A**: Difference analysis of tongue coating bacteria in different age groups. **B**: Chords of tongue coating bacteria in different age groups. The distinct colors correspond to different tongue images and different bacteria, while the strength of the relationships is indicated by the varying thickness of the connecting lines

the tongues of elementary school children, where it participates in one of two competing symbiotic groups [37]. Although not a core constituent of the oral microbiome, *Neisseria subflava* may significantly influence microbiome dynamics in CRC patients, despite not being the primary driver of tongue microbiome alterations.

Additionally, our findings suggested that *Alloprevotella rava* may be primary contributors to the formation of yellow greasy coating, while *Prevotella intermedia* appears to be associated with the development of white greasy coating. However, the physiological and biochemical metabolic pathways of these bacterial species remain largely unexplored, necessitating further investigation. Previous studies have consistently shown a strong association between *Alloprevotella* and gastric cancer [38]. *Prevotella intermedia*, a well-established periodontal pathogen [39], has recently emerged as a potential oncogenic contributor in CRC [40, 41] and promotes the migration and invasion of CRC cells [42]. Its dual presence in both oral and gut ecosystems suggested its potential as a predictive microbial biomarker for CRC development. The oral microbiome is shaped by a dynamic equilibrium of multifactorial determinants. Salivary flow imposes selective pressures on microbial adhesion to oral substrates, while the colonization of newly available substrates is counterbalanced by mechanical disruption of biofilms or shedding of underlying epithelia [43, 44]. This ecological resilience is further governed by host-microbiota crosstalk encompassing immune surveillance and bidirectional modulation of nutrient/solute gradients [45, 46]. Successful microbial persistence in this niche requires continuous adaptation to these evolutionary forces, while host homeostasis necessitates physiological adaptation to maintain symbiotic equilibrium with colonizing microbiota. This contributes to significant variations in tongue coating microbiota profiles among CRC patients. The phenotypic manifestation of tongue coatings, encompassing chromatic variation and structural thickness, may be regulated by microbial ecology through two distinct mechanisms: firstly, through the species-specific pigmentation of oral microbiota, and secondly, via the chromatic metabolites produced through microbial metabolic activities [47, 48]. The stratified deposition patterns of these microbial metabolites account for the characteristic variations observed in tongue coating morphology. Certain tongue coating microbiota may produce carcinogenic metabolites, potentially establishing a direct link between tongue microbiome and CRC. Further identification and characterization of these microbial-derived carcinogens are warranted to validate this association.

The study uncovered a significant and notably strong correlation between age-related variations in tongue coating microbiota composition. Age-stratified analysis demonstrated distinct microbial patterns, with *Streptococcus* showing a positive correlation with advancing age, while *Proteiniphilum* and *Prevotella massilia* exhibited negative correlations. These findings are consistent with previous research by Wang et al. [49], reinforcing the validity of our observations. Of particular interest is the age-dependent distribution of specific bacterial species. *S. parasanguinis* might emerged as a potential biomarker for CRC patients under 62 years of age, whereas *Prevotella* sp. 000163055 predominated in patients over 62 years. The significance of *S. parasanguinis*, an early oral colonizer and symbiotic bacterium, has been previously established in pediatric caries research, where its abundance patterns were associated with deciduous dentition development [37]. Although direct evidence linking *S. parasanguinis* to host age remains elusive, we hypothesize that age-related physiological changes may influence its colonization dynamics and metabolic activity, potentially contributing to CRC pathogenesis. The role of *Prevotella* in oral and gut microbiota warrants special attention. As one of the most abundant genera in the oral microbiome [50], *Prevotella* species have been increasingly detected in the gut microbiota of newly diagnosed CRC patients [51, 52]. This genus' significance extends beyond CRC, as it has been implicated in aging processes [53]. The potential mechanisms may involve age-related gastrointestinal tract changes that compromise barrier function, potentially initiating a cascade of progressive health deterioration. The colonization of *Prevotella intestinalis* induces alterations in microbial metabolism, leading to reduced IL-18 production, which exacerbates intestinal inflammation and may contribute to systemic autoimmunity [54].These findings highlight promising avenues for future research into the intricate relationship between aging and tongue coating microbiota. Such investigations could provide novel insights for developing interventions targeting age-related complications in CRC patients, potentially opening new therapeutic possibilities in microbiome-based medicine.

However, this study has several limitations that warrant consideration. Firstly, the present study primarily focused on investigating the association between tongue coating microbiota and clinical characteristics, including tumor stage and location, in colorectal cancer patients. But this disease-specific parameters could not be conducted in healthy controls. Nevertheless, the inclusion of a normal control group would have enhanced the comprehensiveness and comparative value of our dataset. Secondly, the study represented a novel approach in the field, characterized by its innovative methodology and the significant challenges associated with sample acquisition. To address these limitations and bolster the validity of our conclusions, we recommend that future research initiatives prioritize multi-center collaborations to achieve larger sample sizes and more comprehensive clinical data collection. The implementation of longitudinal studies tracking CRC patients' progression, combined with systematic monitoring of microbial changes in tongue coating, would generate more robust and clinically meaningful data. Moreover, the complex symbiotic relationships between individual tongue coating microbiota and their host organisms present a significant challenge in distinguishing between correlative and causative relationships. The in vitro cultivation of certain tongue coating microbes becomes significantly more challenging when removed from their native human oral environment, compounded by the absence of specialized culture media optimized for oral microbial isolation and cultivation. This complexity highlights the critical need for additional mechanistic studies to precisely elucidate the intricate relationships among tongue coating microbiota, tongue image characteristics, and clinical parameters in CRC patients.

Conclusion

This study employed the TCM technique of tongue diagnosis to explore the microbiological characteristics of tongue images and tongue coating microbiota in patients with CRC. Through a comprehensive analysis, the research characterized the tongue manifestations in CRC patients, elucidated the community structure and diversity of tongue coating bacteria, and delineated the complex relationships among tongue image features, clinical information, and tongue coating microbiota. The tongue image analysis revealed four predominant characteristics in CRC patients: yellow greasy coating, petechiae, white greasy coating, and red tongue with little coating. Notably, the study revealed that *Alloprevotella rava* may play significant roles in the formation of yellow greasy coating formation, while *Prevotella intermedia* was associated with the development of white greasy coating. Additionally, *S. parasanguinis* emerged as a potential biomarker for distinguishing CRC patients aged 62 years, whereas *Prevotella* sp. 000163055 appeared to dominate the tongue coating microbiota in CRC patients aged 62 years and above. These findings underscore a significant correlation between tongue image characteristics, tongue coating microbiota, and clinical parameters in CRC patients. The study provides novel insights into the potential of tongue diagnosis for predicting clinical progression and informing early therapeutic strategies in CRC management, thereby contributing to the broader understanding of the disease's pathophysiology and its diagnostic markers.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions
Duan Jinlong, Liu Jiang and Han Shuwen conceived and drafted the manuscript. Wu Zheng, Wu Yinhang and Chu Jian wrote the paper. Qu Zhanbo, Jean-Marie NIANGA, Louis Wang, and Wang Zefeng analyzed the data. Yang Shujing, Yu Xiaojian and Jin Congjian collected the basic patient information and clinical indicators. Wu Zheng and Huang Jiaqun designed and drew figures. All authors read and approved the paper.

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Data availability
The datasets generated for this study can be accessed from the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA 1116065 (http://www.ncbi.nlm.nih.gov/bioproject/1116065).

Declarations

Ethics approval and consent to participate
This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The clinical protocols, involving the patients and the informed consent form, were approved by the Ethics Committee of Huzhou Central Hospital (No. 20191101-01). All participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations in ethics approval and consent to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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