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RESEARCH

MALDI-TOF Mass Spectrometry Characterization of Culturable Microbiota Associated with the Skin of Amphibians from the Southern Andes Mountains of Ecuador

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Abstract
Ecuador is recognized for having a high diversity of anuran species, which are distributed mainly south of the Andes mountains. However, due to their geographic location and accessibility, there are few studies related to the culturable microbiota of these amphibians in this region. The objective of this study was to explore the bacterial and fungal biodiversity present on the skin of wild anuran species in the southern Andes of Ecuador and to observe whether geographical barriers in the region could increase the variability of the culturable microbiota through MALDI-TOF mass spectrometry. This analysis revealed the presence of 29 bacterial taxa and 9 fungal taxa, consisting mainly of: *Pseudomonas chlororaphis* (28%), *Acinetobacter iwoffii* (14%), *Pseudomonas fluorescens* (14%), and *Hortaea werneckii* (26.4%), *Fusarium solani* (20.5%), S*yncephalastrum* spp. (20.5%), respectively. Diversity varied across the five sampling locations, with geographic location proving to be a significant driver of diversity. Some of the most abundant bacterial and fungal genera have important associations with skin diseases in wildlife and humans. This work represents a glimpse into the complex biodiversity of bacteria and fungi that inhabit the skin substrate, and further studies will be needed to better understand bacterial and fungal biodiversity with potential implications for establishing conservation strategies, along with the development of necessary animal protection measures.

Keywords Anura · Andes · MALDI-TOF mass spectrometry · Fungi · Bacteria

Introduction

Ecuador is one of the countries with the greatest diversity of amphibians, with approximately 653 described species [1, 2], of which 13% are critically endangered, 23% are endangered, and 20% are vulnerable [3]. Most of these species inhabit aquatic and terrestrial environments, which determine their physiological processes [4], making them susceptible to environmental changes that alter their habitat. It is worth noting that 40% of species are in decline in recent years [5]. The increase in anthropogenic activities resulting in pollution [6, 7] and the expansion of the agricultural frontier [8] have had observable effects on amphibian populations, which are severely threatened and in decline [9–11].

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The microbiota, which represents the set of microorganisms that inhabit both the surface and the interior of organisms, can modulate host health by affecting their development, behavior, metabolism, and inflammatory responses [11]. In amphibians, bacterial communities present on the skin could offer protection against infection by synthesizing antifungal metabolites, acting as an integral part of the animal's immune system [12]. Some bacteria on the skin of amphibians are capable of inhibiting the growth of pathogens in vitro [13, 14], and supplementing amphibian microbiomes with inhibitory bacteria can increase survival in laboratory assays [15, 16]. Furthermore, the composition of bacterial communities in frogs and the persistence of the host population are often correlated [17, 18]. In addition to the knowledge about bacterial microbiomes, there are few studies on fungal microbiomes [19]. Relatively few studies have examined the fungal microbiomes of vertebrate wildlife [20–23]. Furthermore, while these studies provide valuable starting points, they have often had limitations as they were carried out in captivity [21, 24], which disrupts the microbiomes [25, 26]. Previous studies have described that fungi inhabiting the skin of some amphibians are capable of producing antimicrobial compounds such as penicillin [27], although little is known about their effects on amphibian health and how they interact with host immune defenses. It should be mentioned that few studies have been conducted on cutaneous fungal communities in amphibians and the same time has been observed the potential of bacteria for probiotic applications [28, 29].

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been applied in the field of microbiology for many years. However, with the development of new technologies and method optimization, new rapid and accurate approaches have been developed to improve the accuracy of targeted identification. MALDI-TOF spectroscopy is not limited to identifying strains grown on solid media, or in vitro, but can also directly identify them from blood culture samples, cerebrospinal fluid, urine, and skin samples [30–34]. Identification by MALDI-TOF spectroscopy has been used to identify Gram-negative and Gram-positive bacteria, aerobes, anaerobes, mycobacteria, nocardia, yeasts, filamentous fungi, and viruses [35–42]. MALDI-TOF MS is a reliable, simple, and readily available technology [43, 44].

Currently, there is a need to understand the diversity of cultivable microbial communities that contribute to host disease resistance, as well as those of pathogenic communities. The objective of this study was to characterize the composition of the bacterial and fungal skin microbiota in anuran species residing in the south of the Ecuador using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and to determine whether geographic location influenced the species diversity observed on anuran skin.

Materials and Methods

Ethics Statement

This study was conducted in strict accordance with the guidelines for the use of live amphibians and reptiles in field research developed by the American Society of Ichthyologists and Herpetologists, the League of Herpetologists, and the Society for the Study of Amphibians and Reptiles. Specific collecting permits for this study were obtained under authorization from the Ministry of Environment, Water, and Ecological Transition of Ecuador (MAATE), number MAAE-ARSFC-2021–1564. The samples did not include endangered or protected animal or plant species.

Sampling Locations

The study was conducted in eleven locations located from south to north in the foothills of the Andes Mountains during the months of September and November 2021. These locations include: (A) Zamora-Chinchipe Province; (1) the "provincial boundary" sector located in the southeastern part and classified as montane cloud forest, with species from the Areaceae, Poaceae, and Orchidaceae families; (2) the "Padmi sector," characterized as a lowland evergreen forest, located in the central-eastern part. It has shrub species such as Sapium and *Grias peruviana*, secondary forests with *Dictyoloma peruviana*, and also includes sections of tropical rainforest and premontane forest; (3) the "Piuntza sector," which exhibits premontane forest vegetation characterized by high tree and shrub diversity. Volcanic soil conditions, high humidity, and moderate altitude favor endemic communities and ecological transitions between tropical forests and montane ecosystems. (B) Loja Province; (1) "Cerro Pucará Park," adjacent to the northwestern part of Podocarpus National Park. Its ecosystem is described as a montane cloud forest, located between 1,500 and 2,900 m a.s.l.; (2) "Abra del Zamora sector," located on the northern periphery, between Podocarpus National Park and Pucará Park, classified as a montane cloud forest ecosystem. (C) Cañar Province; (1) "Laguna de Guabizhum sector," located in the Soldados parish, belonging to the Déleg canton, has a montane cloud forest, with the presence of Cyperaceae species such as *Barnadesia parviflora*, *Juglans neotropica*, and *Myrcianthes* spp. (D) Azuay Province; (1) "Guangarcucho sector," with vegetation formation characterized by the appearance of humid montane scrub, includes relatively humid valleys between 2,000 and 3,000 m.a.s.l. in the inter-Andean alley,

where native vegetation has been devastated and replaced by agricultural crops and forests of *Eucalyptus globulus*, *Salix humboldtiana* and *Acacia farnesiana*; (2) "Lazareto sector," located in the urban area, which includes the banks of the Milchichig River, here the introduced forests (non-native plants) contain species of the genera *Junglas neotropica*, *Baccharis latifolia* and *Spartium junceum*; (3) "Chanlud sector," classified as a shrubby páramo, composed mainly of grasslands and shrubs, in an altitudinal range of 2600 to 3600 m a.s.l., characterized by the presence of the genus Calamagrostis and shrub species of the genera Baccharis, Gynoxys, and Brachyotum; (4) "Quimsacocha sector," with an altitude of 3100 m a.s.l., is characterized by having characteristics of a shrubby páramo with a significant presence of *Cortaderia nitida*. (E) Morona Santiago Province; (1) "Wapú sector," has an ecosystem classified as an evergreen piedmont forest with an altitude of 800 and 1300 m a.s.l. It has a tree composition native to the area where the canopy can reach 30 m in height (Fig. 1).

Sample Collection

Sampling was carried out on amphibian species observed at the sampling sites, in water bodies, and in the surrounding vegetation. Specimens were located using the "visual encounter" method and subsequently morphologically characterized according to [45, 46]. Specimens were handled using nitrile gloves, and a skin swab was subsequently performed in situ by skin rubbing according to [47] (Supplementary Fig. 1). Two skin swabs were taken from each specimen. One swab was subsequently stored in a Falcon™ tube containing 2 ml of Brain Heart culture medium (Merck®) and the second with TGhL medium with Tryptone

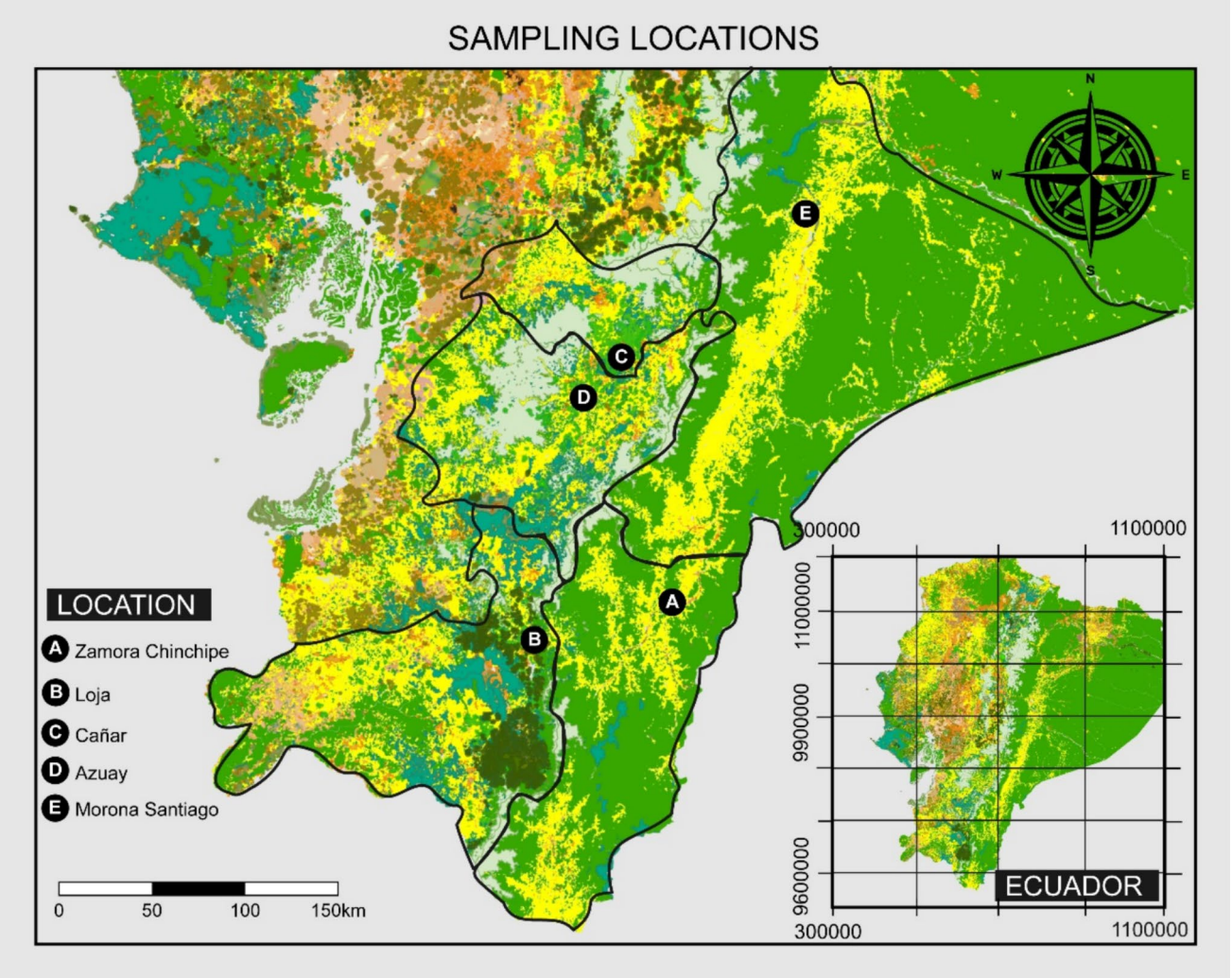

Fig. 1 The left side shows the location of the sampling sites south of the Andes mountain range in the areas corresponding to the provinces of **(A)** Zamora Chinchipe, **(B)** Loja, **(C)** Cañar **(D)** Azuay, and **(E)** Morona Santiago. At the bottom, a map of the Republic of Ecuador is shown

(SIGMA®), Hydrolyzed Gelatin (TM MEDIA®), and Lactose (SIGMA®). The samples were kept in a cooler with dry ice for 24 to 48 h and transferred to the Microbial Ecology and Active Ingredients Laboratory of the Center for Research, Innovation and Technology Transfer (CIITT) of the Catholic University of Cuenca for processing.

Isolation, Culture Conditions, and Activation

Bacterial Isolation and Culture

Bacterial cultures were prepared using a solid medium (95.5% blood agar) dispensed into 9 cm diameter Petri dishes. The medium was sterilized via autoclave (121 °C, 15 psi, 15 min) and allowed to cool under a laminar flow cabinet. Samples, previously stored at 4 °C, were aseptically inoculated onto the agar surface using a sterile inoculation loop, followed by streaking to isolate individual colonies. Plates were incubated at 37 °C for 16 h under anaerobic conditions. After incubation, colony growth was assessed, and a representative colony from each sample was selected for downstream analysis.

Fungal Isolation and Culture

Fungal cultures were prepared with 2% potato dextrose agar (PDA), sterilized via autoclave (121 °C, 15 psi, 15 min), and poured into 9-cm Petri dishes. Mycelial plugs (5 mm diameter) were excised from stock cultures using a sterile punch and transferred to the PDA plates. Inoculated plates were incubated at 25–28 °C under a 12/12 h light–dark photoperiod for 5 days. Mycelial growth was monitored daily, with expansion patterns and morphological features recorded to confirm viability and purity.

MALDI-TOF MS

Bacteria

All samples were analyzed using an Axima™ Confidence MALDI-TOF MS spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan) in positive linear mode (m/z = 2000–20,000). A small number of colonies from each pure culture were transferred to a FlexiMass™ destination well using a disposable loop, covered with 0.5 µl of 2,5-dihydroxybenzoic acid (DHB; 10 mg/ml in acetonitrile/0.1% trifluoroacetic acid 1:1) matrix solution, and air-dehydrated for 1–2 min at 24–27 °C. The reference strain *Escherichia coli* K12 (genotype GM48) was used as a calibration standard and as a reference for quality control. Sample information such as medium and culture conditions were imported into Shimadzu Biotech Launchpad™ software, v.2.8 (Shimadzu-Biotech Corp., Kyoto, Japan). Protein mass profiles were obtained with linear positive mode detection at a laser frequency of 50 Hz and within a mass range of 2000–20,000 Da. The acceleration voltage was 20 kV and the extraction delay time was 200 ns. A minimum of 20 laser shots per sample were used to generate each ion spectrum. For each bacterial sample, 50 protein mass fingerprints were averaged and processed. The spectra were analyzed using SARAMIS™ (Spectral Archive and Microbial Identification System, AnagnosTec GmbH, Potzdam, Germany) with a reliability of 99% [48].

Fungi

Samples were taken from storage at 4 °C, and a portion of mycelium was extracted. Each sample was then homogenized using glass beads in 1 mL of 70% ethanol. Protein extraction was carried out by suspending the mycelium in 300 µL of ultrapure water, then mixed with 900 µL of 70% ethanol. The mycelium was then centrifuged at 13,000 × g for 2 min. The supernatant was discarded and resuspended in 50 µL of 70% formic acid and 50 µL of acetonitrile. The supernatant was then centrifuged, and the supernatant was collected. 1 µL of the protein extract was then spotted onto a MALDI plate by applying it to a stainless steel plate. It was mixed with 1 µL of matrix solution (α-cyano-4-hydroxycinnamic acid [HCCA] in acetonitrile/water/trifluoroacetic acid [50:47.5:2.5]) and dried at room temperature. Data acquisition was carried out using a MALDI-TOF MS spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan) in reflectron mode, with a mass range of 2,000–20,000 Da, and was realized 240 laser shots were performed for each sample (40 shots in 6 positions). The obtained spectra were compared with the Bruker MBT Filamentous Fungi Library 2.0 database. The spectra were analyzed using correlation algorithms (logarithmic score: ≥ 2.0 indicating reliable identification) and with a fidelity of 99%. How positive control was *Candida albicans* ATCC 10231 (spectrum of reference in databases). And as a negative control, a matrix without sample was used to rule out contamination.

Statistical Analysis

All statistical analyses were performed in R Studio (v. 4.4.3). Taxonomic relative abundances were preprocessed by calculating the mean abundance of each taxon across all sampling locations. Alpha diversity metrics (observed species, Shannon index, and Simpson index) were computed using the phyloseq R package (v. 1.50.0). Differences in alpha diversity between groups were evaluated using the non-parametric Kruskal–Wallis test, with post hoc Dunn's test for pairwise comparisons (adjusted via the Benjamini–Hochberg method, $p < 0.05$). Beta diversity was assessed using Bray–Curtis dissimilarity matrices. Non-metric multidimensional scaling

(NMDS) was applied to visualize community dissimilarities, and statistical significance of observed differences was tested with PERMANOVA (999 permutations) using the vegan R package (v. 2.6.10). To further resolve community patterns, a heatmap was generated with the pheatmap package (v. 1.0.12), clustering samples based on Bray–Curtis distances. Intersection patterns of taxa across sampling locations were visualized using an UpSet plot (UpSetR package, v. 1.4.0), chosen over traditional Venn diagrams due to its enhanced readability for complex datasets.

Results

Characterization of Anurans

The collection sites yielded a total of 20 different species of amphibians, distributed across the five sampling locations (Zamora Chinchipe, Loja, Cañar, Azuay, and Morona Santiago). The genera characterized were *Pristimantis* (16 records), *Gastrotheca* (5 records), *Hyloxalus* (3 records), *Ctenophryne* (3 records), *Lithobates* (3 records), *Adenomera*

Table 1 Georeferenced distribution of anuran samples in five southern Ecuadorian provinces (Zamora Chinchipe, Loja, Cañar, Azuay, and Morona Santiago). The columns show: province, study area, anuran species observed, and geographic coordinates (latitude and longitude). The records cover 11 ecologically heterogeneous high Andean areas. The table structure allows for analysis of the spatial association between taxa and geographic variables, highlighting the presence of endemic and exotic species

Location[a]	Sector[b]	Anuran spp.[c]	Coordinates[d]	
			Latitude	Longitude
Zamora Chinchipe	Límite provincial	*Pristimantis andinognomus*	−3,992,947	−79,145,022
		Pristimantis vidua	−3,992,947	−79,145,022
	Criadero Piuntza	*Lithobates catesbeianus*	−3,870,451	−78,881,814
	Padmi	*Adenomera hylaedactyla*	−3,737,577	−78,619,146
		Pristimantis diadematus	−3,737,577	−78,619,146
		Pristimantis conspicillatus	−3,737,577	−78,619,146
Loja	Parque cerro Pucará	*Lithobates catesbeianus*	−4,012724	−79,195,073
		Lithobates catesbeianus	−4,012724	−79,195,073
	Abra del Zamora	*Pristimantis versicolor*	−3,985,234	−79,145,307
		Pristimantis balionotus	−3,985,234	−79,145,307
		Pristimantis samaniegoi	−3,985,234	−79,145,307
		Pristimantis colodactylus	−3,985,234	−79,145,307
		Pristimantis matildae	−3,985,234	−79,145,307
Cañar	Laguna de Guabizhun	*Hyloxalus vertebralis*	−2,803,048	−78,936,164
		Gastrotheca cuencana	−2,803,048	−78,936,164
Azuay	Guangarcucho	*Gastrotheca cuencana*	−2,843,274	−78,885,605
		Gastrotheca cuencana	−2,843,274	−78,885,605
		Ctenophryne aequatorialis	−2,843,274	−78,885,605
		Ctenophryne aequatorialis	−2,843,274	−78,885,605
		Ctenophryne aequatorialis	−2,843,274	−78,885,605
	Lazareto	*Gastrotheca cuencana*	−2,882,491	−79,008925
		Hyloxalus vertebralis	−2,882,491	−79,008925
		Hyloxalus vertebralis	−2,882,491	−79,008925
	Chanlud	*Pristimantis erythros*	−2,895,228	−78,957,036
		Pristimantis erythros	−2,895,228	−78,957,036
		Pristimantis lutzae	−2,895,228	−78,957,036
		Pristimantis lutzae	−2,895,228	−78,957,036
	Quimsacocha	*Gastrotheca pseustes*	−2,682,961	−79,033192
Morona Santiago	Wapú	*Pristimantis conspicillatus*	−2,682,961	−79,033192
		Pristimantis conspicillatus	−2,682,961	−79,033192
		Chiasmocleis bassleri	−2,682,961	−79,033192
		Scinax cruentommus	−3,034273	−79,222,145
		Pristimantis conspicillatus	−2,843,274	−78,885,605
		Dendropsophus bifurcus	−2,843,274	−78,885,605

[a]Ecuador Province. [b]Sector belonging to each study location. [c]Recorded anuran species. [d] Reference geographic coordinate

(1 record), *Chiasmocleis* (1 record), *Scinax* (1 record), and *Dendropsophus* (1 record) (Table 1).

General Composition of the Anuran Species, Bacteria, and Fungi Assemblage

An overall species diversity was observed within the five study areas (Zamora, Chinchipe, Loja, Cañar, Azuay, and Morona Santiago), where *Pristimantis conspicillatus* (11.8%) and *Gastrotheca cuencana* (11.8%) were the most abundant species, followed by *Hyloxalus vertebralis* (8.8%), *Ctenophryne aequatorialis* (8.8%), and *Lithobates catesbeianus* (8.8%), which were in higher proportions compared to other identified species. Likewise, species of the genus *Pristimantis*, such as *P. erythros* (5.8%), *P. lutzae* (5.8%), *P. matildae* (2.9%), and *P. colodactylus* (2.9%), are well represented in the sample, demonstrating the high richness of this genus in the study locations (Fig. 2A). Microbial associations were identified in *Gastrotheca cuencana* (Azuay) with the presence of *Pseudomonas antarctica* and *Pseudomonas fluorescens*. In contrast, *Hyloxalus vertebralis* (Azuay) showed the presence of *Acinetobacter iwoffii* and *Staphylococcus xylosus*. In *Lithobates catesbeianus* (Loja), a dominance of *Lactobacillus curvatus* and *Chryseobacterium joostei* was observed. In *Pristimantis conspicillatus* (Morona Santiago) the species was observed *Serratia marcescens*. Furthermore, in *Pristimantis diadematus* (Zamora), co-occurrence of *Bacillus pumilus* and *Pseudomonas brassicacearum* was recorded. However, in *Pristimantis samaniegio* (Loja) and *Pristimantis vidua* (Zamora Chinchipe) no bacterial species were detected (Fig. 3).

The total relative abundance of bacteria in anurans was mainly composed of species of the genera *Pseudomonas*, *Bacillus*, *Pantoea*, *Corynebacterium* and *Acinetobacter.* Within these, *Pseudomonas chlororaphis* was present (28%), followed by *Acinetobacter iwoffii* and *Pseudomonas fluorescens* (14%). The species *Pseudomonas Antarctica* was present (12%), followed by *Pseudomonas orientalis*, *Pantoea agglomerans* and *Pseudarthrobacter oxydans* in an equal percentage of 10%. While *Pseudomonas jensenii* and *Rahnella aquatilis* was (8%). The species with the lowest percentage were *Pseudomonas kilonensis* (6%), *Pseudomonas thivervalensis*, *Bacillus pumilus* and *Kluyvera ascorbata* (6%). We must highlight that the bacterial species with intermediate prevalence were *Bacillus infantis*, *Pseudomonas azotoformans*, *Staphylococcus xylosus*, *Pseudomonas rhodesiae*, *Pseudomonas taetrolens*, *Lactobacillus curvatus* and *Aeromonas bestiarum* in similar values of (4%). The least prevalent species were *Corynebacterium striatum*, *Chryseobacterium joostei*, *Pseudomonas japonica*, *Serratia marcescens*, *Comamonas testosteroni*, *Proteus mirabilis*, *Pseudomonas brassicacearum*, *Pseudomonas extremerientalis*, and *Providencia rettgeri* in (2%) (Fig. 2B).

On the other hand, the relative abundance of total fungal species in anurans showed a higher prevalence of *Hortaea werneckii* (26.4%), recognized for its ability to colonize the skin of amphibians and that could be involved in cutaneous infection processes, followed by *Fusarium solani* (20.5%), *Syncephalastrum* spp. (20.5%), *Fusarium concentricum* (8.8%). Species such as *Fusarium oxysporum*, *Fusarium proliferatum* and *Aspergillus niger* (5.8%) showed a notable abundance. Finally, the species that showed less abundance were represented by *Fusarium* spp. (2.9%) and *Bipolaris* spp. (2.9%) (Fig. 2C).

Relative Composition of the Bacterial Population

The total number of detections was 92, distributed among 29 bacterial species on the skin of anurans spp. at the sampling sites was 29, distributed in the provinces of Zamora, Chinchipe, Loja, Cañar, Azuay, and Morona Santiago (Supplementary Fig. 2).

Subkingdom Negibacteria

This subkingdom comprised a total of 79.31% of all observed species, including *Acinetobacter iwoffii* (3.45%), *Chryseobacterium joostei* (3.45%), *Pseudomonas antarctica* (3.45%), *Pseudomonas azotoformans* (3.45%), *Pseudomonas chlororaphis* (3.45%), *Pseudomonas fluorescens* (3.45%), *Pseudomonas japonica* (3.45%), among others.

Subkingdom Posibacteria

A total of 6 species were observed (20.69%). They were represented by: *Bacillus infantis* (3.45%), *Corynebacterium striatum* (3.45%), *Staphylococcus xylosus* (3.45%), *Bacillus pumilus* (3.45%), *Pseudarthrobacter oxydans* (3.45%), and *Lactobacillus curvatus* (3.45%).

Phylum

Proteobacteria

This phylum was the dominant one in the samples from the studied locations, exhibiting 22 species and representing 75.86% of the bacterial composition. Species such as *Acinetobacter iwoffii* (3.45%), *Pseudomonas Antarctica* (3.45%), *Pseudomonas azotoformans* (3.45%), *Pseudomonas chlororaphis* (3.45%), *Pseudomonas fluorescens* (3.45%), *Pseudomonas japonica* (3.45%), *Pseudomonas jensenii* (3.45%), *Pseudomonas kilonensis* (3.45%), and *Pseudomonas orientalis* (3.45%) stood out here (Fig. 3).

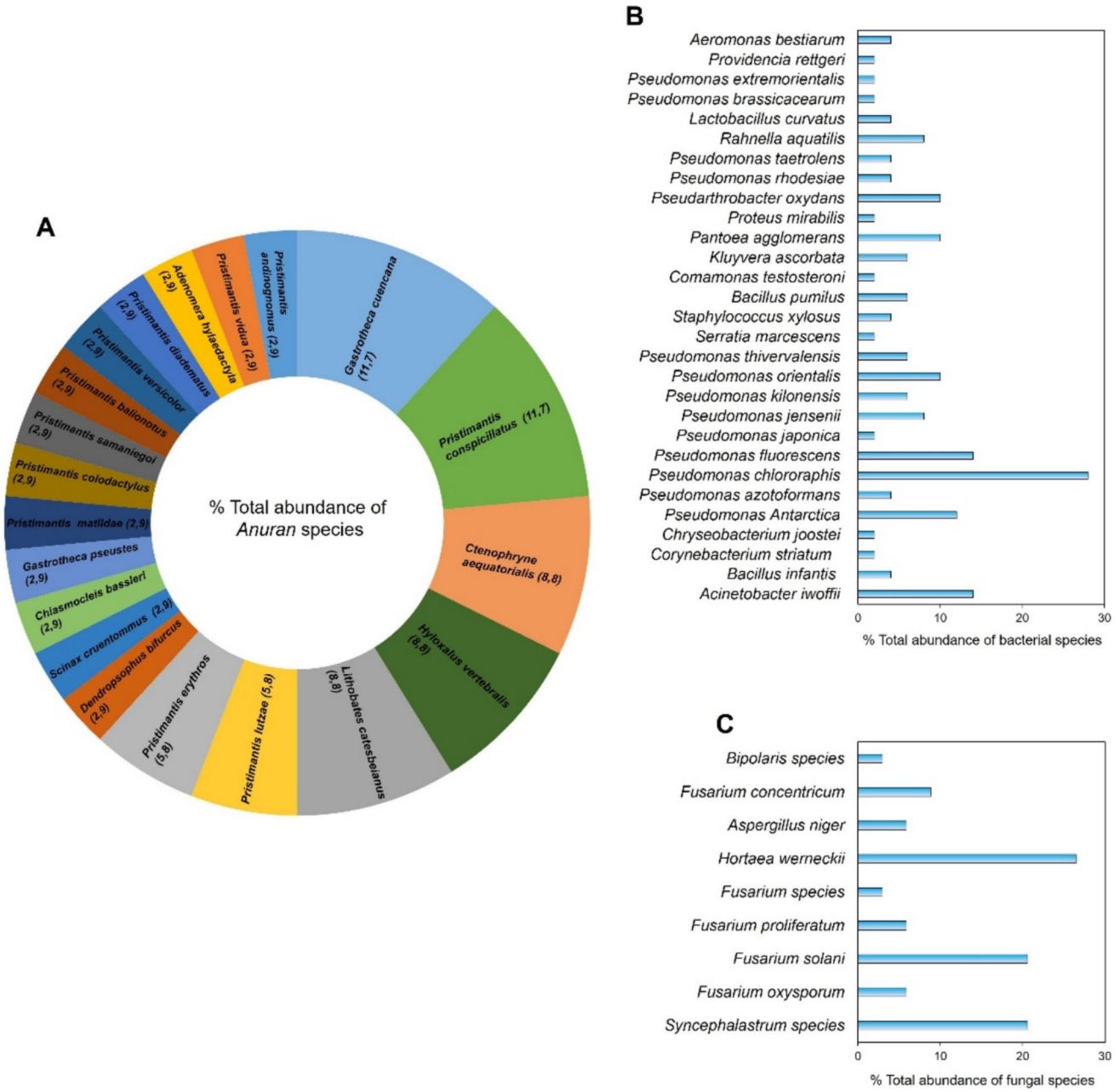

Fig. 2 Total abundance of the species observed in the provinces of Zamora Chinchipe, Loja, Cañar, Azuay, and Morona Santiago. **(A)** Ring diagram illustrating the relative percentage abundance of sampled anuran species, with color codes and percentages next to each taxonomic name. **(B)** Horizontal bar graph showing the percentage proportion of isolated bacterial species, sorted by decreasing abundance, with the x-axis indicating the total percentage of bacterial abundance. C) Horizontal bar graph presenting the percentage abundance of culturable fungal species, with the x-axis representing the total percentage of fungal abundance. All axes include comparable numerical scales

Firmicutes

These covered of a total of 4 species that together represented 13.79% of the samples and consisted of *Bacillus infantis* (3.45%), *Staphylococcus xylosus* (3.45%), *Bacillus pumilus* (3.45%), and *Lactobacillus curvatus* (3.45%) (Fig. 4).

Actinobacteria

This phylum presented intermediate abundance among the total species with a (6.90%). *Corynebacterium striatum* (3.45%) and *Pseudarthrobacter oxydans* (3.45%) (Fig. 4).

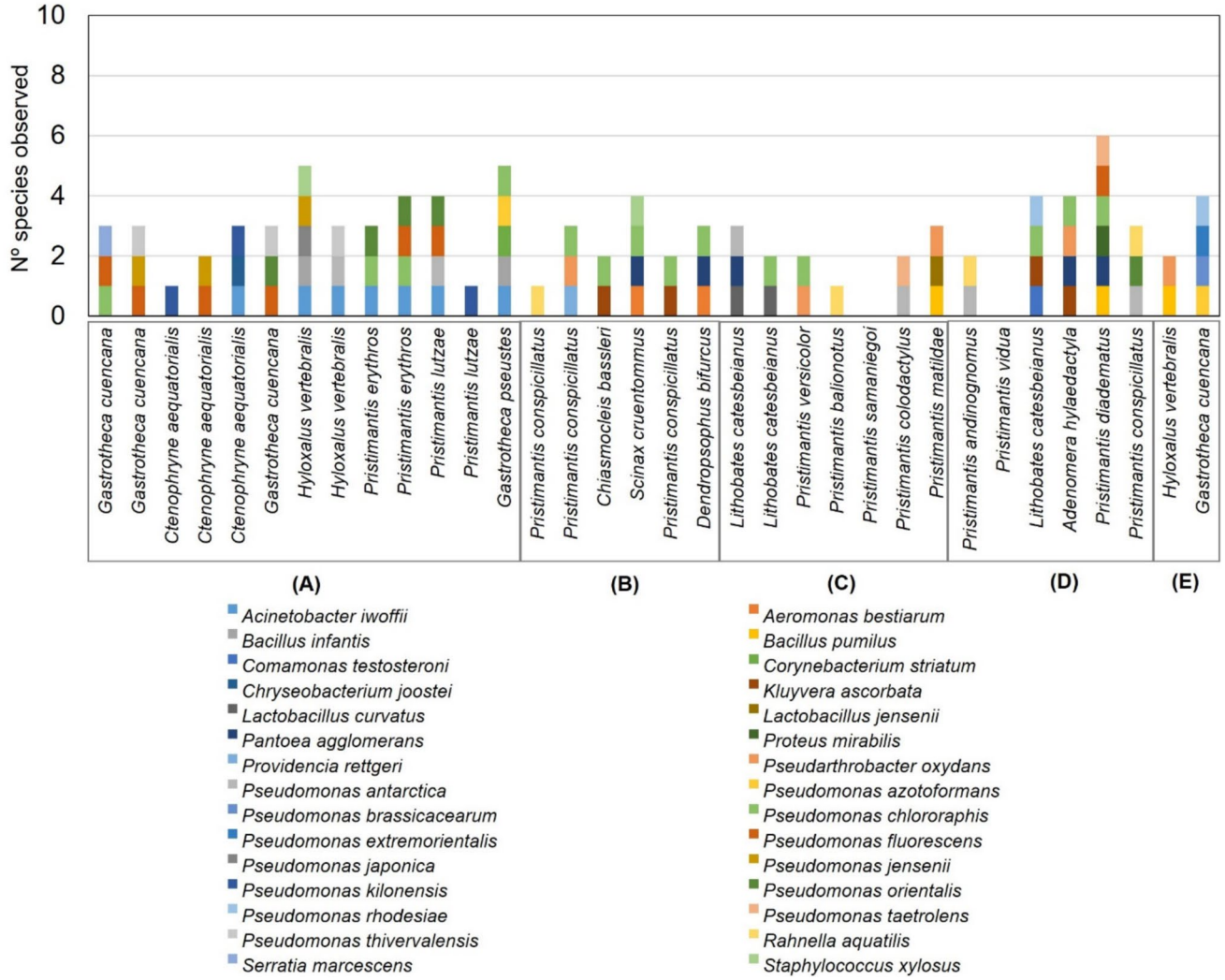

Fig. 3 General abundance at the species level recorded on the skin of anuran spp. in the locations of **(A)** Azuay, **(B)** Morona Santiago, **(C)** Loja, **(D)** Zamora Chinchipe and **(E)** Cañar (Ecuador)

Bacteroidetes

This phylum showed the lowest abundance of species, with a total representation of (3.45%), with *Chryseobacterium joostei* (Fig. 4).

Composition of the Fungal Assemblage

A total of 41 fungal detections were obtained on the skin of anuran spp., distributed across 9 species, in the provinces of Zamora Chinchipe, Loja, Cañar, Azuay, and Morona Santiago (Supplementary Fig. 3).

Subkingdom Dikarya

Ascomycota

Within Ascomycota, Sordariomycetes was the dominant class at the sampling locations, with an average abundance of 44.12%, followed by Dothideomycetes (29.41%), and Eurotiomycetes with an (5.88%).

The predominant genus was Fusarium (44.12%), represented by *Fusarium solani* (20.59%), *Fusarium concentricum* (8.82%), *Fusarium oxysporum* (5.88%), *Fusarium proliferatum* (5.88%), and *Fusarium* spp. (2.94%). The

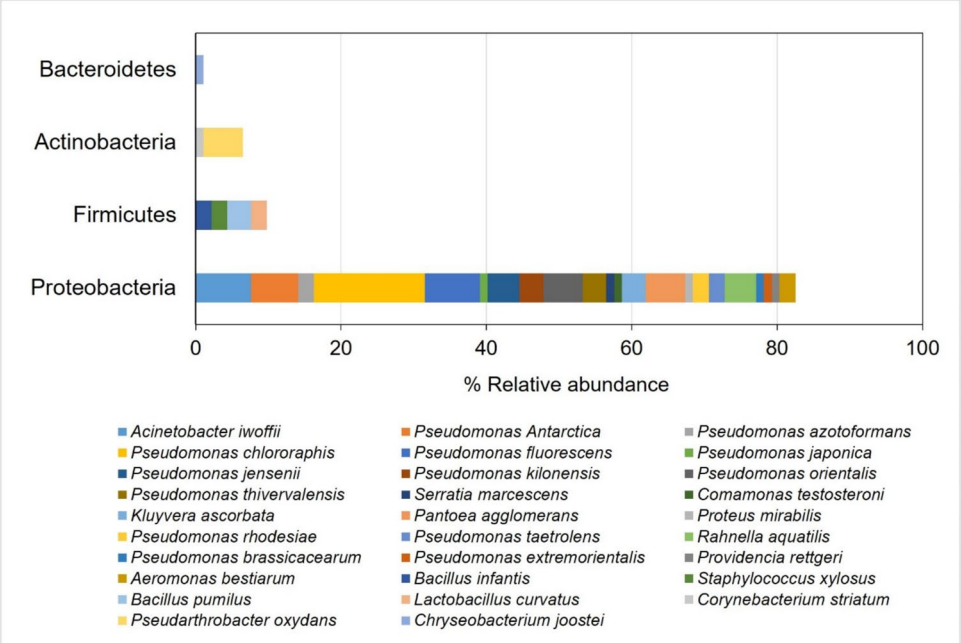

Fig. 4 Relative abundances of bacteria at the phylum level, recorded on the skin of anuran spp. in the locations of Azuay, Morona Santiago, Loja, Cañar and Zamora Chinchipe (Ecuador)

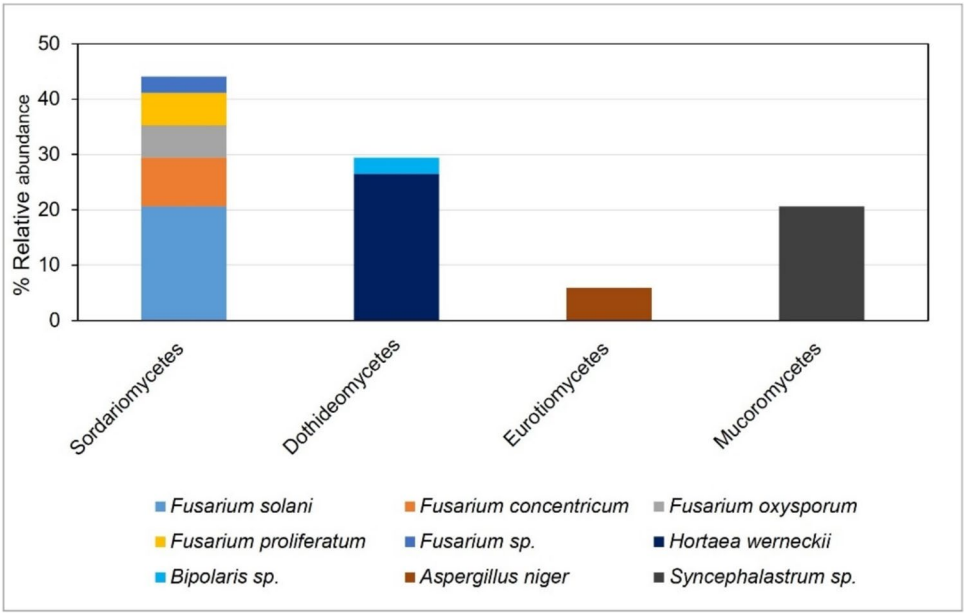

Fig. 5 Relative abundances at the class level, recorded on the skin of anuran species in the locations of Azuay, Morona Santiago, Loja, Cañar and Zamora Chinchipe (Ecuador)

genus *Hortaea* showed average values of 26.47%. The genus *Aspergillus* was represented with (5.88%). Finally, the genus *Bipolaris* was the least predominant with (2.94%) (Fig. 5).

Mucoromycotina

Mucoromycotina was the least predominant class at the sampling locations (20.59%)and was represented by *Syncephalastrum* spp. (Fig. 5, Supplementary Fig. 4).

Alpha Diversity Patterns Across Localities

Bacteria

Bacterial alpha diversity, quantified using the Shannon, Simpson, and Chao1 indices (Fig. 6), demonstrated marked variability across localities. Azuay exhibited the highest overall diversity (Shannon = 2.36 ± 0.08; Simpson = 0.88 ± 0.01) and richness (Chao1 = 17.7 ± 3.3), closely followed by Zamora (Shannon = 2.20 ± 0.17; Simpson = 0.87 ± 0.02; Chao1 = 15.4 ± 3.1). Loja showed intermediate diversity

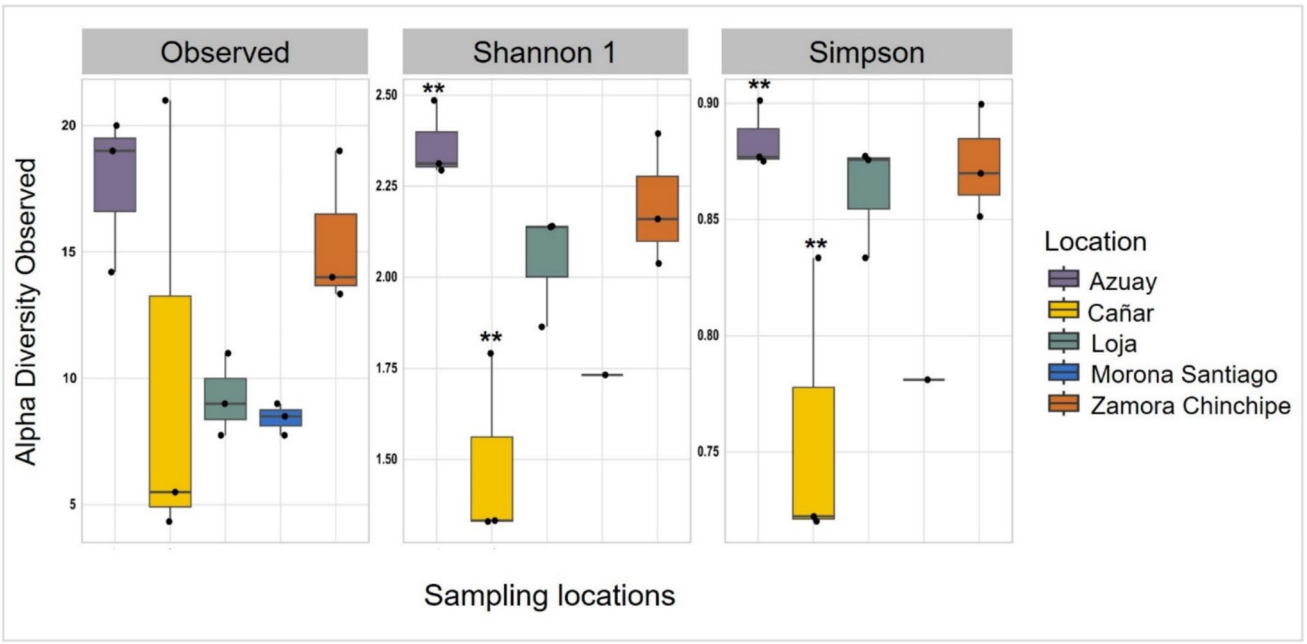

Fig. 6 Alpha diversity metrics (Observed, Shannon, and Simpson) assessed in five provinces south of the Andes in Ecuador (Azuay, Cañar, Loja, Morona Santiago, and Zamora Chinchipe). Richness (Observed), heterogeneity (Shannon), and dominance (Simpson) indices are shown, spatially correlated with sampling locations. Data arrangement allows for comparisons of microbial biodiversity patterns across high Andean zones. (**) indicates significant differences between locations according to the Kroskal-Wallis test for each index. Observed variability values ($P = < 0.05$)

(Shannon = 2.05 ± 0.15; Simpson = 0.86 ± 0.02) but lower richness (Chao1 = 9.3 ± 1.6). In contrast, Morona displayed reduced diversity (Shannon = 1.66 ± 0.07; Simpson = 0.77 ± 0.02) and the lowest richness (Chao1 = 7.4 ± 1.3). Notably, Cañar presented stark contrasts: one sample showed high richness (Chao1 = 21) with moderate diversity (Shannon = 1.79; Simpson = 0.83), while others were species-poor (Chao1 = 4.3 ± 0.6; Shannon = 1.33 ± 0.002; Simpson = 0.72 ± 0.01). Kruskal–Wallis tests confirmed significant differences across localities for Shannon and Simpson indices ($p < 0.05$), with post-hoc Benjamini–Hochberg adjustments highlighting Cañar's differences from Azuay.

Fungi

Fungal alpha diversity, revealed pronounced contrasts among localities (Fig. 7). Loja exhibited the highest richness (Chao1 = 7.4 ± 3.0) and diversity (Shannon = 1.62 ± 0.10; Simpson = 0.79 ± 0.02), followed by Azuay (Chao1 = 6.9 ± 2.3; Shannon = 1.55 ± 0.17; Simpson = 0.76 ± 0.03). Zamora displayed moderate diversity (Shannon = 1.34 ± 0.32; Simpson = 0.70 ± 0.11) but lower richness (Chao1 = 4.8 ± 1.8). In contrast, Morona showed reduced diversity (Shannon = 1.02 ± 0.05; Simpson = 0.61 ± 0.03) and minimal richness (Chao1 = 3 ± 0). Notably, Cañar was statistically distinct ($p < 0.05$, Kruskal–Wallis with Benjamini–Hochberg adjustment), with no detectable fungal diversity (Shannon = 0; Simpson = 0) and extremely low richness (Chao1 = 1 ± 0), indicating a near-absence of viable fungal communities. Post hoc analyses confirmed Cañar's divergence from all other localities, which exhibited overlapping but variable profiles.

Beta Diversity and Community Structure

Bacteria

Pairwise PERMANOVA analysis, based on Bray–Curtis dissimilarity, revealed distinct patterns of bacterial community differentiation between localities. While adjusted p-values (p-adjusted = 1) did not reach statistical significance after Benjamini–Hochberg correction, effect size metrics (R^2) and F-statistics highlighted biologically meaningful trends. The NMDS ordination plot, based on Bray–Curtis dissimilarity, corroborated pairwise PERMANOVA results, revealing distinct spatial clustering of bacterial communities (Fig. 8).

Zamora, Loja, and Morona formed well-separated clusters, with 95% confidence ellipses showing minimal overlap, underscoring their unique taxonomic assemblages. This segregation aligns with their divergent alpha diversity profiles and suggests strong environmental filtering or niche specialization. The hierarchical clustering patterns observed in the Bray–Curtis-derived heatmap (Fig. 9) further validated the spatial structuring of bacterial communities.

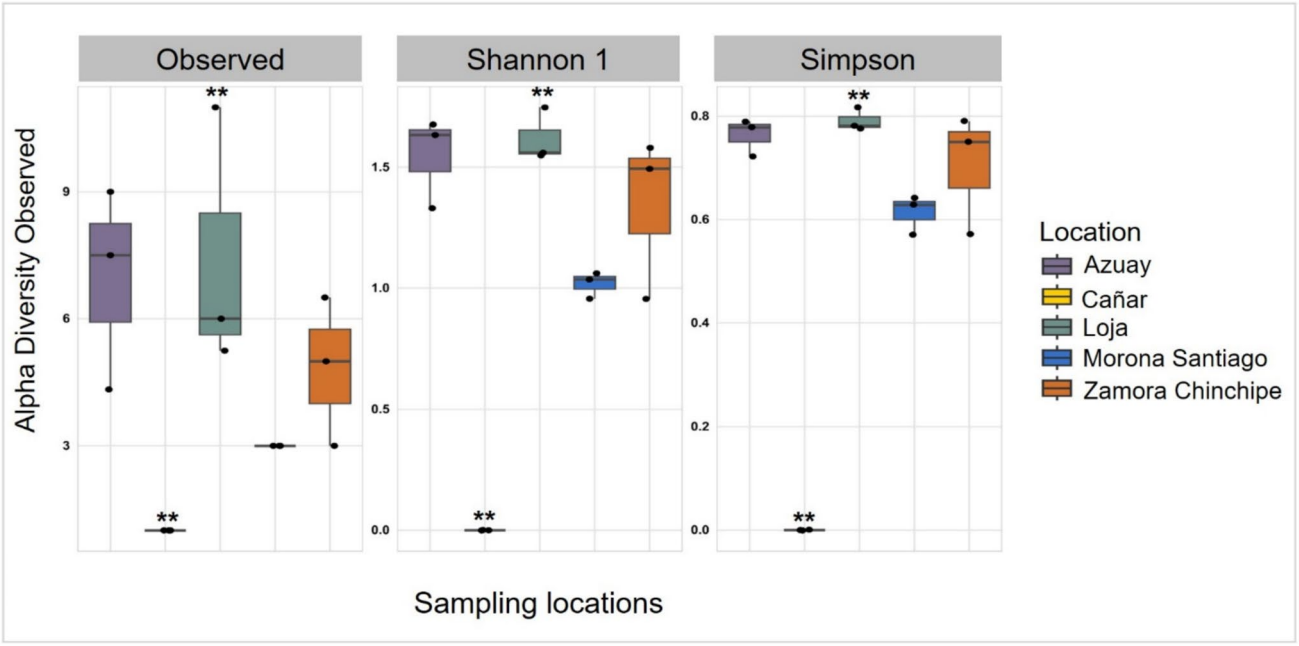

Fig. 7 Estimated alpha diversity in fungi for the sampling locations (Azuay, Cañar, Loja, Morona Santiago and Zamora Chinchipe). Richness (Observed), heterogeneity (Shannon), and dominance (Simpson) indices are shown, spatially correlated with sampling locations. Data arrangement allows for comparisons of microbial biodiversity patterns across high Andean zones. (**) show significant differences between the three groups according to the Kruskal–Wallis test for each index. Observed variability values ($P = < 0.05$)

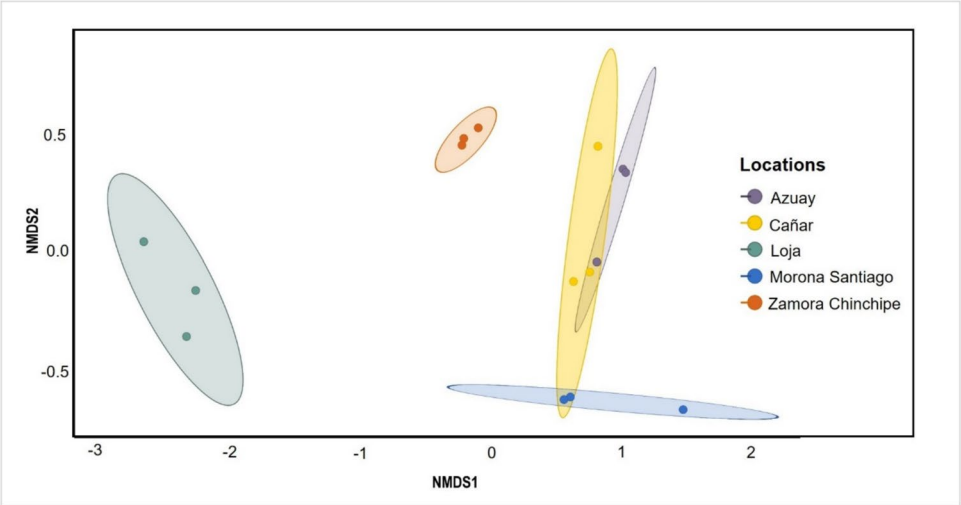

Fig. 8 Principal coordinate analysis (PCoA) based on Bray–Curtis distances of bacterial communities on the skin of anuran spp. divided according to the sampling location (Azuay, Cañar, Loja, Morona Santiago and Zamora Chinchipe)

An UpSet plot analysis showed the distribution of bacterial species found on anuran skin at the study locations (Morona Santiago, Azuay, Cañar, Loja, and Zamora Chinchipe). It revealed the presence of eight bacterial species shared by all locations. This finding suggests the existence of widely distributed taxa, potentially adapted to a diverse range of environmental conditions. Certain locations shared certain bacterial species, and in several cases, taxa existed that were unique to specific combinations from two locations. Furthermore, each location harbors potentially unique bacteria, demonstrating a possible degree of microbial endemism, taking into account the scope of the technique used. This differentiation could be associated with variation in the composition of the amphibian community (Supplementary Fig. 5).

Fungi

Pairwise PERMANOVA analysis, based "n" Bray–Curtis dissimilarity, revealed biologically meaningful

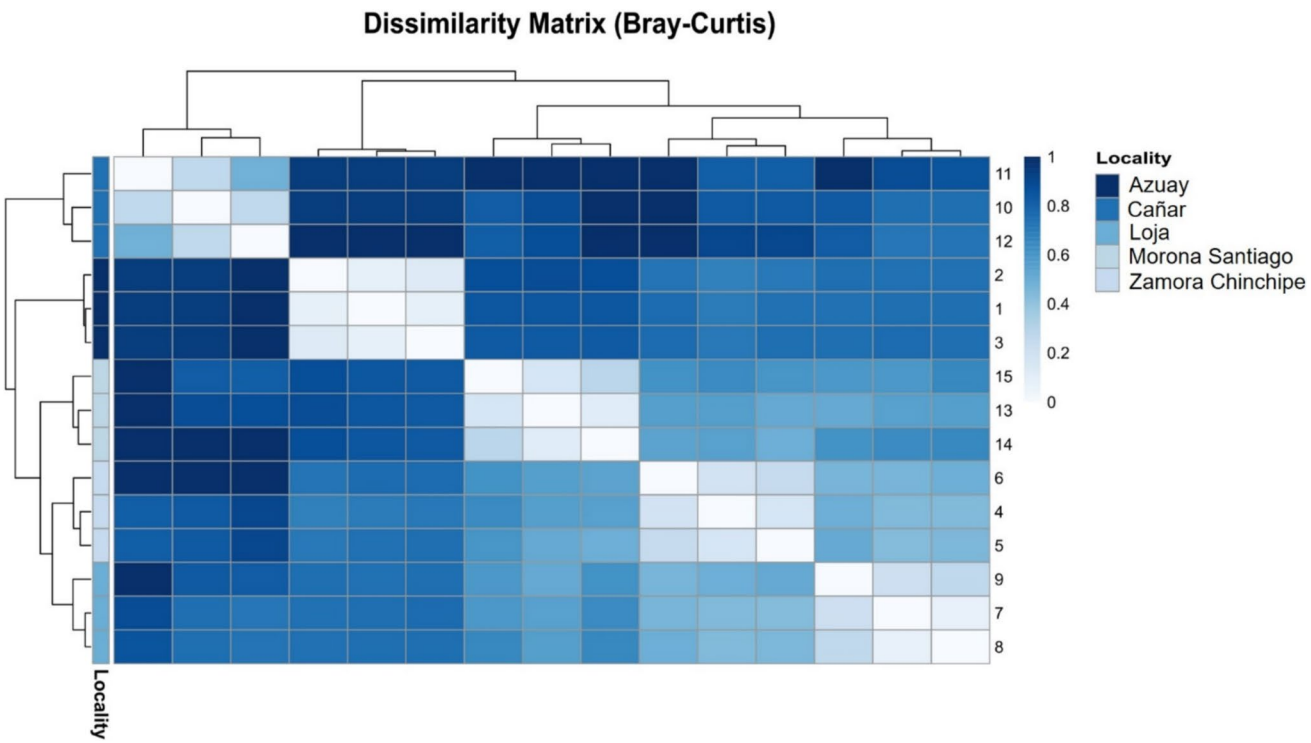

Fig. 9 Comparative heatmap of the bacterial community in the localities of Azuay, Cañar, Loja, Morona Santiago, and Zamora Chinchipe. Color intensities (light, low dissimilarity—dark, high dissimilarity) reflect variations in the relative abundance of taxa, highlighting clusters through dendrograms

differentiation in fungal community composition across localities, despite the lack of statistical significance after Benjamini–Hochberg correction (all adjusted p-values = 1). Several comparisons showed high R^2 values, and large F-statistics, indicating strong effect sizes and potential ecological relevance. The NMDS ordination plot (based on Bray Curtis dissimilarity; Fig. 10) supported these trends, revealing partial spatial separation of fungal communities.

Although some confidence ellipses overlapped, notable clustering was observed, particularly among the Zamora, Loja, and Morona samples, suggesting underlying ecological or host-driven factors shaping fungal assemblages. Complementarily, the UpSet plot (Supplementary Fig. 6). highlighted the distribution of fungal taxa across localities. Three fungal taxa were shared among Morona, Zamora, Azuay and Loja, suggesting the presence of a core mycobiome possibly

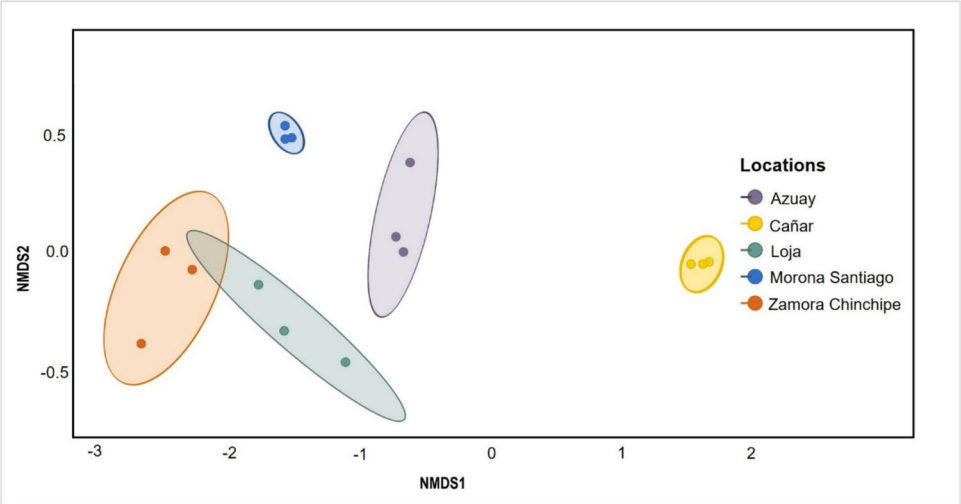

Fig. 10 Principal coordinate analysis (PCoA) based on Bray–Curtis distances of the fungal communities on the skin of anuran spp. divided according to sampling location (Azuay, Cañar, Loja, Morona Santiago and Zamora Chinchipe)

adapted to a broad range of environmental or host-related conditions. However, numerous taxa were exclusive to specific site combinations, and each locality also harbored unique taxa, indicating a high degree of microbial endemism and potential ecological specialization. The heatmap based on Jaccard dissimilarity (Fig. 11) further reinforced the observed spatial structure. Hierarchical clustering grouped localities according to similarities in fungal composition, reflecting consistent biogeographic patterns. Together, these findings underscore a non-random distribution of skin-associated fungi in anurans across the Andean and Amazonian transition zone.

Discussion

This study provides an overview of the diversity of bacterial and fungal communities on the skin of anuran spp., where each habitat and geographic location can serve as a selective filter determining local microbial diversity, a phenomenon known as the Baas-Becking principle [49]. Previous studies have shown that the skin microbiota profile is influenced by the phylogenetic identity of the host amphibian [16]. Our understanding of the composition and role of the microbiota associated with plants and animals, including humans, is increasing due to the application of technologies such as targeted metagenomics and MALDI-TOF MS. Microbial communities living on animal skin are of great interest, as they are continuously exposed to the influence of the external environment. However, most of these studies have largely focused on the human skin microbiome. [50]. Among wild animals, amphibians, due to their absence of fur or feathers, provide an excellent model system to study skin-associated microbial communities, which are thought to mediate disease susceptibility by providing the first line of defense against pathogens [51–53]. Knowledge about host-associated microbial communities can assist with conservation actions for endangered species, as well as play a role as a bioindicator of a pathogen-free population [54–56]. Therefore, the aim of this study was to analyze the diversity of bacteria and fungi on the skin of anuran spp. in wild habitats using MALDI-TOF MS mass spectrometry in five locations: Azuay, Cañar, Loja, Morona Santiago and Zamora Chinchipe in the Republic of Ecuador.

The diversity of anurans in Ecuador is recognized as one of the highest in the world due to its complex topography and habitat heterogeneity [57], presenting unique biogeographic patterns that vary significantly between provinces. Analysis of amphibian species composition at Zamora Chinchipe, Loja, Cañar, Azuay, and Morona Santiago highlighted endemism and anthropogenic pressures. The observed data showed a marked heterogeneity in anuran species richness, with increased abundance of genera such as *Pristimantis*, *Gastrotheca*, and *Hyloxalus*, and the presence of invasive species such as *Lithobates catesbeianus*. In Zamora Chinchipe, the presence of species such as *Pristimantis andinognomus* and *Pristimantis vidua*, which are endemic to the montane forests of southern Ecuador [57], suggests a high specialization to humid microhabitats between 1,800–2,500 m. The coexistence of *P. diadematus* and *P. conspicillatus*, both species associated with lower vegetation strata, and *Adenomera hylaedactyla*, which is typical of floodable soils [58], reflected the ecological heterogeneity of the region. However, the detection of *Lithobates catesbeianus*, an

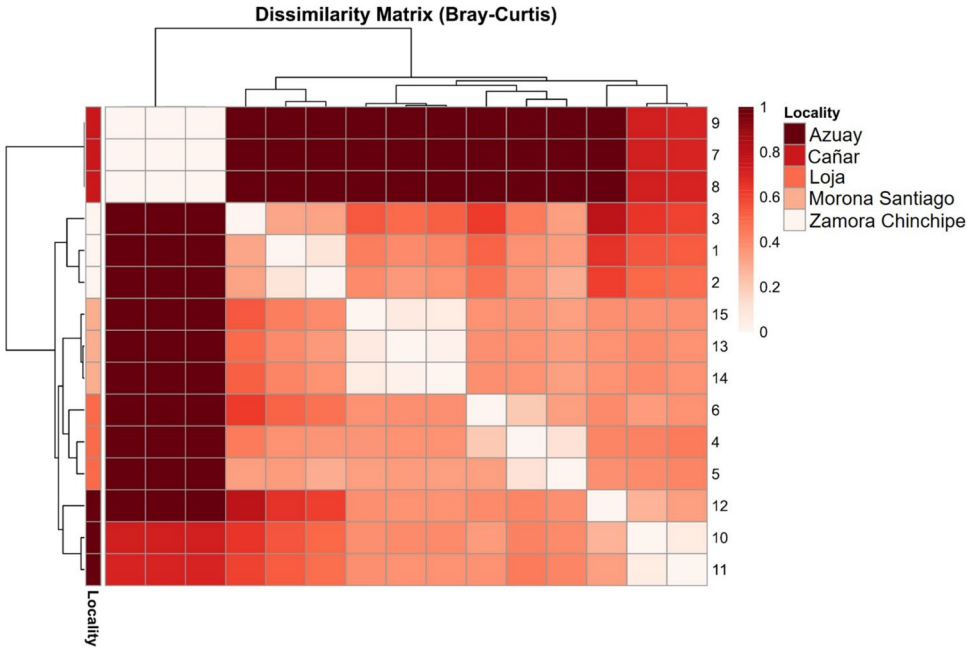

Fig. 11 The comparative Bray–Curtis dissimilarity matrix (Heatmap) illustrates the variation in fungal composition among localities (Azuay, Cañar, Loja, Morona Santiago, and Zamora Chinchipe). The color gradient, from light (low dissimilarity) to dark (high dissimilarity), reflects differences in the abundance and presence of taxa

invasive species [59], in riparian areas indicates possible anthropogenic alterations, since this anuran competes with native species for resources. In the province of Loja, the dominance of the genus *Pristimantis* with the species *versicolor*, *balionotus*, and *samaniegoi* highlights the role of páramos and montane forests as centers of speciation [60]. *Pristimantis matildae*, recently described in the Tapichalaca Reserve [57], evidences the presence of microendemisms critical for conservation. However, the recurrence of *Lithobates catesbeianus* in multiple records suggests a worrying expansion of this species, associated with aquaculture activities [61]. In Cañar and Azuay, the presence of *Gastrotheca cuencana*, an ovoviviparous species endemic to the central Andes [62] and *Hyloxalus vertebralis* associated with ravines in cloud forests [63, 64] reflects adaptations to cold and humid environments (> 3,000 m). The abundance of *Ctenophryne aequatorialis* in Azuay, a cryptically red microhylid, suggests evolutionary strategies to avoid predators in fragmented habitats [64]. The absence *of Lithobates catesbeianus* in these sites could be related to thermal limitations, although physiological studies are required to confirm this. Meanwhile, in Morona Santiago, the coexistence of *Pristimantis conspicillatus* shared with Zamora Chinchipe with *Scinax cruentomma* (typical of the Amazonian lowlands) [64], indicates a transition zone between the Andes and the Amazon. *Chiasmocleis bassleri*, a fossorial microhylid, highlights the importance of non-flooded soils in primary forests [65]. However, the absence of records of high Andean species suggests a biogeographic boundary defined by an altitude < 1,500 masl in this region. When comparing sampling locations, significant differences were observed where Zamora Chinchipe and Loja shared a higher richness of *Pristimantis* (6 and 5 species, respectively), while Azuay stands out for the diversity of *Gastrotheca* and *Ctenophryne*. This reflects altitudinal gradients where Zamora Chinchipe (800–2,500 masl) and Loja (1,500–3,000 masl) host mid-mountain species, while Azuay (> 3,000 masl) showed high Andean taxa. The presence of *Lithobates catesbeianus* in Zamora Chichipe and Loja, but not in Azuay, suggests that its invasion is limited by climatic or anthropogenic factors. Microendemic species, such as *Pristimantis matildae* (Loja) and *Gastrotheca cuencana* (Azuay-Cañar), face critical risks from deforestation and the effects of climate change. For example, 30% of the cloud forests in Azuay have been converted to grasslands, reducing habitats for *Hyloxalus vertebralis* [66].

The environmental microbiota is a critical component of ecosystem health, particularly in amphibians, where skin infections contribute significantly to global population decline. In Azuay, the bacterial community identified on the skin of anuran spp. was characterized by the presence of 15 species distributed in different percentages. The most abundant taxon was *Acinetobacter iwoffii* ($n = 7$), followed by *Pseudomonas fluorescens* ($n = 6$), *Pseudomonas chlororaphis* ($n = 4$), and *Serratia marcescens* ($n = 1$). The high frequency of *A. iwoffii* is relevant, since species of the genus *Acinetobacter* have been associated with skin infections in anuran spp., particularly under conditions of altered natural microbiome [67–69]. In anurans, an imbalance in the cutaneous microbiota could facilitate colonization by this pathogen, leading to dermatitis or systemic infections. *P. fluorescens* has been reported to cause necrotic skin lesions in *Lithobates catesbeianus*, especially in eutrophic environments [70]. Its high prevalence suggests a risk for anurans in altered habitats. However, studies by [71] on *Gastroteca* spp. showed that *P. fluorescens* had inhibitory functions on the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which is linked to many declines in anuran populations. *Serratia marcescens* has been reported to induce deep and extensive ulcers in the tree frog (*Litoria caerulea*) and is considered an important pathogen.

Likewise, the various representatives of the genus *Pseudomonas*, which include *P. azotoformans*, *P. japonica*, *P. jensenii*, *P. kilonensis*, *P. orientalis*, and *P. thivervalensis*, constituted an important part of the microbial community in Azuay. This genus is known for its metabolic versatility and its ability to produce bioactive metabolites. However, in scenarios where there is an imbalance in the microbial community, some species of *Pseudomonas* can act as opportunistic pathogens, generating skin infections, which, in combination with environmental stress, can trigger complex clinical pictures in anurans [72]. In Zamora Chinchipe, anuran populations showed bacterial communities composed of 13 taxa, where *Pseudomonas* was predominant along with other environmental taxa. This location showed the presence of *Pseudomonas chlororaphis* ($n = 3$) and *Pseudomonas antarctica* ($n = 2$), as well as *Pantoea agglomerans* ($n = 2$) and *Rahnella aquatilis* ($n = 2$). The presence of *Pantoea agglomerans* is of particular interest because despite being a common inhabitant of the environment, its bacteria have been implicated in opportunistic infections in animals and humans, and can cause skin irritations [73]. *Pseudomonas chlororaphis* has been reported to secrete phenazines, antifungal compounds that inhibit Bd [74]. However, in Zamora Chinchipe, its high abundance could displace commensal microbiota, increasing susceptibility to secondary infections. Furthermore, the diversity of *Pseudomonas* in Zamora Chinchipe suggests a dynamic bacterial ecosystem where competition between commensal and pathogenic species can determine the health status of anuran skin. The presence of *Pseudomonas orientalis, Pseudomonas rhodesiae* and *Pseudomonas taetrolens* in anuran spp. in smaller proportions reinforces the idea that microbial dysbiosis could be related to infectious outbreaks under conditions of environmental alteration, such as changes in temperature or pollution [75, 76]. On the other hand, Loja showed a community composed

of 9 taxa, with a predominance of bacteria belonging to the genera *Pseudomonas* and *Pseudarthrobacter*, as well as lactobacilli. An equitable distribution of bacterial species was also observed, where *Pseudomonas antarctica*, *Pseudomonas chlororaphis* and *Pseudarthrobacter oxydans*. Additionally, *Lactobacillus curvatus* ($n = 2$) and *Lactobacillus jensenii* (n = 1) were detected. The presence of lactobacilli on the skin of anurans could have a protective effect, since these microorganisms are known to produce lactic acid and bacteriocins, which inhibit the growth of pathogens and modulate the host immune response [77]. However, the coexistence with potential pathogens such as *P. chlororaphis* (a pathogen that causes dermatitis in amphibians) suggests that an imbalance in the bacterial community could trigger skin infections [12]. Likewise, *Pseudarthrobacter oxydans*, another species observed in anurans from this location, has been related to the degradation of contaminants and has shown to have a notable biotechnological potential, which opens the possibility of using it in environmental and remediation applications [78]. In Cañar, the bacterial community observed on the skin of anurans spp. was characterized by a very homogeneous distribution, with 6 taxa present. The taxa identified at this location were *Bacillus pumilus*, *Pseudarthrobacter oxydans*, *Pseudomonas azotoformans*, *Pseudomonas brassicacearum*, *Pseudomonas extremorientalis*, and *Pseudomonas rhodesiae*. The uniformity in the abundance of each observed species could indicate a stable microbial ecosystem that is less exposed to environmental disturbances. However, it should be noted that some species of the genus *Pseudomonas* have been implicated in skin infections in amphibians, especially under stress conditions or in the presence of skin lesions [79]. On the other hand, *Bacillus pumilus* is known for its ability to produce enzymes and antimicrobial compounds, which could contribute to skin defense in anurans [80]. Finally, in Morona Santiago the bacterial community consisted of 8 taxa. The predominant species was *Pseudomonas chlororaphis* ($n = 5$), followed by *Aeromonas bestiarum*, *Kluyvera ascorbata* and *Pantoea agglomerans* ($n = 2$), while *Providencia rettgeri*, *Pseudarthrobacter oxydans*, *Rahnella aquatilis* and *Staphylococcus xylosus* showed lower percentages. Similarly, *Aeromonas bestiarum* is a recognized pathogen in fish and amphibians, responsible for causing septicemia and dermatitis, which could pose a high risk to anuran populations in this region, under adverse environmental conditions. The coexistence of these pathogenic taxa with others that act as commensals raises the need to evaluate the microbial balance in anuran skin, since dysbiosis can facilitate the transition from symbiotic relationships to pathological states.

The presence of bacteria with biotechnological potential, such as *Pseudomonas fluorescens* in Azuay and *Pseudarthrobacter oxydans* in Loja and Cañar, represents an opportunity for the development of bioremediation and biological control strategies. *Pseudomonas fluorescens* has been the subject of numerous studies due to its ability to produce natural antibiotics and toxic compound-degrading enzymes, which could be exploited for the decontamination of environments affected by agrochemicals and other pollutants [81]. Similarly, *Pseudarthrobacter oxydans* has demonstrated potential in the degradation of hydrocarbons and in promoting plant growth in contaminated soils, making it an attractive candidate for biotechnological applications in environmental contexts [82]. On the other hand, some studies have shown that alterations in bacterial composition can facilitate the invasion of external pathogens, such as *Batrachochytrium dendrobatidis*, the etiological agent of chytridiomycosis, which has significantly contributed to the global decline of amphibians [83]. It has also been suggested that variability in bacterial composition can influence the immune response of anurans, affecting their ability to resist secondary infections caused by opportunistic bacteria [84].

Regarding the observed fungal communities, these analyses revealed seven fungal taxa in Azuay: *Aspergillus niger* ($n = 1$), *Bipolaris sp.* ($n = 1$), *Fusarium concentricum* ($n = 2$), *Fusarium solani* ($n = 1$), *Hortaea werneckii* ($n = 3$), *Syncephalastrum* sp. ($n = 1$), and *Fusarium proliferatum* ($n = 1$). The high prevalence of *Hortaea werneckii* is significant, since this fungus, in addition to being involved in the pathogenesis of tinea nigra in humans, can cause skin disorders in amphibians, promoting the appearance of hyperpigmented spots and keratosis [85, 86]. The presence of *Fusarium concentricum*, *Fusarium solani* and *Fusarium concentricum* in Azuay, highlights the possibility of fusariosis, a disease characterized by the formation of erythematous and ulcerated lesions that can affect both the skin and underlying structures in amphibians [87]. The detection of *Bipolaris* spp., a fungus that in humans is associated with photoreaction and subcutaneous mycosis, reinforces the need to consider its role as a potential pathogen in the skin of anurans [88]. Furthermore, the presence of *Aspergillus niger* in Azuay not only indicates its biotechnological potential (given its ability to produce pectins and industrial enzymes), but also alerts to the risk of cutaneous aspergillosis in immunocompromised patients [89]. In Loja, six fungal taxa were reported: *Fusarium oxysporum* ($n = 1$), *Fusarium solani* ($n = 1$), *Fusarium* spp. ($n = 1$), *Hortaea werneckii* ($n = 1$), *Syncephalastrum* spp. ($n = 2$), and *Aspergillus niger* ($n = 1$). In this locality, the highest abundance corresponds to *Syncephalastrum* spp. indicating that this taxon could be playing a predominant role in the cutaneous fungal community. *Syncephalastrum* spp. it is generally considered a saprophytic fungus, its involvement in invasive mycoses in contexts of immunosuppression is cause for alarm, because skin infections can be complicated in environments with high humidity and stress in the host. On the other hand, the presence of *Fusarium oxysporum* and *Fusarium solani*, reinforces the

concern about the possible incidence of fusariosis [87]. The detection of *Aspergillus niger* is relevant, since, in contexts of microenvironmental imbalance, it can act as an opportunistic pathogen, causing cutaneous and systemic aspergillosis in compromised individuals [89]. In the locality of Cañar, the fungal community was summarized in two taxon: *Fusarium concentricum* and *Fusarium proliferatum* with a representation of 50% each. The exclusive presence of *F. concentricum* and *F. proliferatum* is potentially important, since this fungus has been associated with skin infections and can produce mycotoxins that affect skin integrity. Furthermore, studies have shown that certain members of the *Fusarium* complex have a high invasive capacity, which could lead to dermatomycosis in anurans, especially under conditions of environmental stress or previous lesions in the epidermis [90]. In the locality of Zamora Chinchipe, the data indicated the presence of six fungal taxa: *Syncephalastrum* spp. ($n=1$), *Hortaea werneckii* ($n=1$), *Fusarium oxysporum* ($n=1$), *Fusarium solani* ($n=3$), *Fusarium concentricum* ($n=1$), and *Fusarium proliferatum* ($n=1$). The high prevalence of *Fusarium solani* is particularly relevant since this fungus is known to be an etiological agent in cutaneous fungal infections including amphibians, producing keratomycosis and cutaneous fusariosis, conditions that worsen in the presence of environmental stress or skin lesions [87]. Furthermore, *Fusarium proliferatum*, was present in Zamora chinchipe, this is an opportunistic pathogen that produces mycotoxins and can contribute to dermatomycosis, affecting the cutaneous integrity of anurans [88]. The presence of *Hortaea werneckii*, a halophytic fungus, suggests that under specific conditions it could contribute to alterations in the cutaneous barrier of amphibians, generating hyperpigmentation or irritation [85]. Likewise, *Syncephalastrum* spp., although less reported in skin infections, has been documented as a causative agent of mycosis in immunocompromised patients, which opens the possibility that a similar picture may manifest in debilitated anurans [91]. Finally, in Morona Santiago, the fungal community was composed of six taxa: *Fusarium solani* ($n=2$), *Hortaea werneckii* ($n=4$), *Syncephalastrum* spp. ($n=3$), *Aspergillus niger* ($n=1$), *Bipolaris* spp. ($n=2$), and *Fusarium proliferatum*. The predominance of the specie *Hortaea werneckii* in Morona Santiago is of particular interest, since its high abundance will could predispose to the appearance of skin infections in anures [85]. The joint presence of *Fusarium solani* in and *Syncephalastrum* spp. in Morona Santiago suggests a complex fungal ecosystem, in which the interaction between pathogenic and saprotrophic fungi could influence the susceptibility of anurans to diseases such as fusariosis and syncephalasrosis, conditions that have been documented in clinical and experimental studies [87].

The detection of fungal taxa with biotechnological potential, such as *Aspergillus niger* in Azuay and Loja, and the presence of *Fusarium* species in various locations, offer interesting opportunities for the development of industrial and environmental applications. *Aspergillus niger*, for example, is widely recognized for its ability to produce enzymes, organic acids and secondary compounds of industrial relevance, which has allowed its application in the production of pectins, citrates and other biotechnological products [92, 93]. Similarly, some *Fusarium* isolates have shown the ability to degrade toxic compounds and participate in bioremediation processes, which could be used for the treatment of contaminated environments, always considering the duality of these fungi as pathogens and potential agents in biotechnological processes [94, 95].

In summary, the geographic variability of the cutaneous microbiota showed that the composition of bacteria and fungi on the skin of anurans varied significantly among the five analyzed locations, with Azuay and Zamora Chinchipe exhibiting the highest bacterial diversity (Shannon = 2.36 and 2.20, respectively). This suggests that environmental factors, such as altitude and ecosystem type, act as ecological filters, modulating the microbial structure through selection mechanisms and limited dispersal. The functional duality of the dominant taxa: The genera Pseudomonas (28% relative abundance) and *Fusarium* (44.12% in Ascomycota) stood out for their prevalence, showing contrasting roles. While *Pseudomonas fluorescens* and *P. chlororaphis* have probiotic potential against pathogens such as *Batrachochytrium dendrobatidis*, their dominance could indicate dysbiosis under environmental stress. Similarly, *Hortaea werneckii* and *Fusarium solani* pose pathogenic risks, highlighting the complexity of microbiota-host interactions. MALDI-TOF MS identification allowed the characterization of culturable microbes but underestimated overall diversity by missing non-culturable symbionts. The findings underscore the need to integrate metagenomics to explore cryptic communities and assess causal relationships between dysbiosis, emerging pathogens, and population declines.

Therefore, it is essential to continue research to establish the causal relationship between bacterial and fungal composition, the presence of pathogens, and their possible relationship with the development of skin diseases in amphibians. This research, in turn, can guide intervention and risk mitigation strategies in ecosystems affected by human activity and climate change. This work represents a first look at bacterial and fungal diversity in the skin of wild anurans South of the Ecuadorian Andes. Additional studies are needed to better assess this diversity, along with the development of necessary measures for its protection and conservation.

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Author Contribution J.S. and J.G. designed the study. F.S., J.S., J.G., R.F., collected the samples. R.F., A.M., M.C., performed the laboratory cultures and isolation. J.S., J.G., and A.V-T. performed MALDI—TOF MS. A.V-T. performed the bioinformatics analyses of the data. G.V-G. and A.V-T. performed the statistical analysis of the data. AV-T. analyzed and discussed the results and wrote the article. All authors have read and agreed to the published version of the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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