

RESEARCH

Open Access



The uropygial gland of the European hoopoe as a symbiotic organ

Manuel Martín-Vivaldi^{1,9*}, Ángela Martínez-García², Juan M. Peralta-Sánchez³, Michael Schaub⁴, Raphaël Arlettaz⁵, Antonio M. Martín-Platero^{6,9}, Ester Martínez-Renau⁷, María Dolores Barón⁸, Magdalena Ruiz-Rodríguez^{1,9}, Estefanía López-Hernández^{1,9}, Manuel Martínez-Bueno^{6,9}, Eva Valdivia^{6,9} and Juan J. Soler^{8,9*}

Abstract

Background Animals rely on symbiotic bacteria living within/on their tissues for multiple functions, while simultaneously needing to protect themselves via immune functions or other defenses from potentially pathogenic microorganisms that could invade those tissues. As a result, interactions with complex assemblages of bacteria have driven the evolution of host strategies to control established symbioses. One such strategy involves the development of organs specialized in maintaining associations with beneficial members of the microbial community — so-called symbiotic organs. These organs are characterized by compartmentalizing spaces where favorable conditions for the beneficial bacteria are promoted, while preventing colonization of other tissues. Although several model systems of symbiotic organs have been studied in animals, none have been recognized in non-aquatic vertebrates except for the intestinal crypts of mammals. Here, we propose that bird's uropygial glands may be specialized symbiotic organs.

Results We tested this hypothesis using the uropygial gland of the hoopoe (*Upupa epops*) as a model, which hosts a complex bacterial community that includes antimicrobial-producing symbionts. First, we examined whether the uropygial gland supports a specific symbiotic community, by comparing the microbiome composition of the uropygial secretions and eggshell surfaces in two European hoopoe populations. Additionally, using histological staining and fluorescence in situ hybridization, we looked for structural specializations for compartmentalization and bacterial targeting in the glands of nesting hoopoes in comparison to non-breeding individuals lacking the symbiosis. Results show that, in comparison with bacterial communities of the eggshells, those of the uropygial gland were more conserved between both populations. Moreover, uropygial glands of nesting hoopoes were strictly compartmentalized by a special tissue, properties that are absent in the non-breeding individuals lacking the symbiosis. Finally, bacteria were organized within the organ, suggesting the existence of special physical niches to promote specialized mutualistic symbionts.

Conclusions All evidence supports that the hoopoe uropygial gland is a specialized *symbiotic organ* for bacterial cultivation, paving the way for new insights into vertebrates' exocrine glands' role in microbial symbiosis.

Clinical trial number Not applicable.

*Correspondence:

Manuel Martín-Vivaldi

mmv@ugr.es

Juan J. Soler

jsoler@eeza.csic.es

Full list of author information is available at the end of the article



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Keywords ARISA, Bacterial community, Coevolution, Geographic variation, Illumina, Mutualistic symbiosis, Uropygial secretion, Symbiotic organs

Background

Bacterial symbiosis is currently recognized as one of the main interactions driving evolution of plants and animals, including vertebrates [1]. External and internal epithelial tissues of healthy animals harbor a diversity of microbial assemblages that are consistent across species [2] and populations [3, 4], while also showing individual specificity [5, 6]. The microbiome expands host functionality [7] with stability [8], but also presents adaptability [9, 10], thus facilitating plastic responses to environmental conditions.

The importance of symbiotic bacteria for their animal hosts [11] ranges from providing nutrients [12, 13], to enhancing pathogen resistance by promoting immune response [14], or producing defensive substances [15–21]. Symbionts also play central roles in thermal tolerance [22], sensorial capacity [23], social behavior [24, 25], or mental health [26, 27]. Host traits that favor the acquisition of adequate or optimal symbionts for these functions would increase host fitness [28, 29] and, thus, evolve as adaptations in host populations [30–32]. In this sense, host traits assuring vertical transmission of beneficial microbes have traditionally been recognized as adaptations that ensure the acquisition of the optimal symbionts [33–37]. Highly specific mutualisms can also evolve in the absence of vertical transmission. For example *Drosophila* fruit flies acquire symbionts from the environment to their simple gut microbiomes [38], and the *Euprymna scolopes* squid, recruits *Vibrio fischeri* fluorescent inhabitants from the environment to its luminescent organ [39, 40]. Indeed, it is progressively accepted that, from the hundreds of bacterial strains that come into contact with animals, with apparent stochastic transit, hosts are able to select, maintain and promote particular species or communities ([41], Box 1). Such selection occurs in sites specialized for colonization and growth of those symbionts [42–44]. Favoring *immigration*, *compartmentalization*, *monitoring* and *targeting* (Box 1), hosts can exert *partner choice* (Box 1) and control the symbiont community established [32]. Among host characteristics favoring acquisition of the “chosen” symbionts, the compartmentalization of body regions allows cultivating bacteria in particular locations where the hosts control the chemical and physical environment (thus allowing specific *monitoring* and *targeting*, Box 1). Such compartmentalization strategies would involve the physical delimitation of spaces to promote the establishment and growth of specific symbionts. This can be achieved by facilitating or penalizing bacteria depending on their associated benefits [45]. Examples of hosts

conferring these strategies include insect bacteriocytes housing endosymbionts [46–48], or the root nodules of legumes. In the latter, the plant monitors the level of N₂ fixation by the single bacterial strain responsible for each nodule and decides nutrient rewards or abortion accordingly [49]. A variety of animal cavities also function as physical compartments where mutualistic microbiomes of various levels of complexity are housed. This is the case of luminescent squid crypts [40], the pouches/crypts in the wall of the animals’ gut [50–52] or the cuticle of some ant species [53, 54]. These *physical niches* for symbionts might also be more fluid [45] compartments in time and space, as in the foregut of fruit flies [55]. We are at the beginning of understanding how animals establish mutualistic associations with bacteria, and discovering new special host compartments that provide appropriate niches for bacteria [56].

Some of the special locations where animals cultivate mutualistic bacteria result from evolutionary modifications of cavities in which the secretion of exocrine glands (i.e., glands delivering secretions directly into body cavities or onto external surfaces [58]) accumulates [57, 59]. In the case of squids, the origin of the luminescent organ is related to the ink bladder and probably the accessory nidamental gland [60, 61]. The nidamental gland is also a cavity specialized in hosting symbiotic bacteria that protect the host’s eggs [62], with many similarities to the luminescent organ in the way of obtaining symbionts [63]. In fungus growing ants (subfamily *Myrmicinae*, tribe *Attini*), elaborate cuticular crypts of different depths and shapes for cultivation of antifungal-producing bacteria, are associated with exocrine glands opened in the cuticle [53]. These structures have been highly modified across the ants’ evolutionary history and such modifications are absent in other ant genera that do not cultivate fungus [53]. Exocrine glands in the integument are common places used by invertebrates and aquatic vertebrates [59] to compartmentalize symbionts, letting selective colonization and nurturing by evolving as *symbiotic organs* (Box 1 [57]). Among terrestrial vertebrates, only gut associated compartments are considered symbiotic organs (reviewed in [57]). However, symbiotic bacterial communities are commonly detected in their exocrine glands [64], which may, thus, have evolved as organs specialized in cultivating specific mutualistic bacteria. A prominent example of the exocrine glands supporting symbiosis with bacteria is the uropygial gland of birds.

The uropygial gland (also called preen gland) is the only exocrine gland with external opening in birds [65]. The uropygial secretion serves to waterproof and keep

Box 1 Glossary of terms used in the context of control of host-symbionts associations

Term	Meaning	Source
Partner choice	Active behavioral response in one species that favors cooperation in the other. This is achieved either: (i) interacting more with cooperators, (ii) interacting randomly but rejecting non-cooperators, (iii) interacting randomly but supplying less aid to non-cooperators.	Foster & Wenseleers 2006 [41]
Favoring immigration	Control exerted by host to influence which microbial strains and species it encounters (in the environment) or are acquired by progeny (vertical transmission).	Foster et al. 2017 [32]
Compartmentalization	Physical and chemical barriers (such as epithelia and associated mucus layers) in/on hosts that limit accessibility of microbes to particular tissues or cavities, getting germ-free body locations and separating microbes in space.	Foster et al. 2017 [32]; Chomicki et al. 2020 [45]
Monitoring	Host detects the location and identity/effects of microbes within its body, either with adaptive immunity/ toll-like receptors of innate immunity, or by <i>measuring</i> the net income of beneficial resources obtained from symbionts.	Foster et al. 2017 [32]
Targeting	Host promotion of the right symbionts by providing specific resources they need (positive selection) or secreting specific antimicrobials against undesired invaders (negative selection).	Foster et al. 2017 [32]
Symbiotic organ	Host structures that house populations of beneficial microbes providing a core set of mutualism services to hosts. Microbial communities are filtered by symbiotic organs, mediating which genotypes interact with hosts, gain host resources, and are subsequently passed on to new hosts (see Box 2).	Fronk and Sachs 2022 [57]
Physical niche	A host-constructed niche provides specific sites for adhesion, protected space that limits loss of bacteria, and a specifically tailored nutritional and immune environment that selects the proper strains from the available microbiota.	Ludington 2024 [56]
Complementarity	The microbial partners express metabolic pathways that are lacking in, but imply fitness benefits for the host.	Fronk and Sachs 2022 [57]

integrity of the plumage [66] and may function in scenarios of social communication, parasitism, and predation that may be partially mediated by mutualistic bacterial symbionts (reviewed in [64, 67]). As an exocrine gland, the uropygial gland may be an ideal place for the compartmentalization of beneficial bacteria [68]. Evidence of a diverse range of bird lineages hosting symbiotic bacteria within their uropygial gland is increasing [69–79]. Moreover, in several of those species, injecting antibiotics into the glands influenced the secretion composition and abundance of beneficial substances [69, 70, 72, 80–82]. This shows that symbiotic bacteria in the gland *complement* (Box 1) metabolic pathways of avian hosts. Thus, although the uropygial gland is not included in the list of organs that allow animals to form close association with mutualistic bacteria [45, 57], evidence from well-studied species suggests that it may indeed be a symbiotic organ in some avian groups.

There is strong evidence suggesting that the uropygial gland of Upupiformes is specialized in cultivating beneficial bacteria. The uropygial glands of the European hoopoe (*Upupa epops*) and the green woodhoopoe (*Phoeniculus purpureus*) are special in several aspects related to symbiosis with bacteria. First, their dark and odorous secretions include known chemicals from bacterial metabolites [81, 82]. Moreover, in the case of the European hoopoe, dark and odorous secretions only occur in reproducing females and nestlings (hereafter, both named as nesting individuals). These nesting individuals, in comparison with males and non-reproducing females, have bigger glands that harbor abundant and

complex bacterial communities [82–84]. The existence within the same species of individuals/phases with and without the symbiosis, makes the hoopoe an ideal system to study adaptations caused by the interaction with the symbiotic bacteria. In the case of breeding females, the secretion is used to cover eggshells, which is facilitated by a special eggshell, full of shallow crypts, that help retaining it [85–87] (Fig. 1a, c). Interestingly, experimental antibiotic injection in the glands not only affects the chemical composition and color of the secretion, but also causes a gland-size reduction [84]. This suggests that symbionts mediate the seasonal changes in the gland morphology of the females of this species (i.e., there is a “phenotypically plastic” structure of the organ, Box 2 [57]). The uropygial gland and secretion of green woodhoopoes also contain functional bacterial communities but, in this case, neither the symbiotic association nor gland morphology change between sexes nor throughout the year [72, 81]. It is of interest that these interspecific differences in temporal associations with bacteria and uropygial gland morphology are related to specificity of some of their symbionts. In the case of the European hoopoe, high density of several generalist species (mainly *Enterococcus faecalis* and *Enterococcus faecium*) appeared in the secretion of nesting individuals [18, 73, 88, 89], while in the secretion of green woodhoopoe a new species (*Enterococcus phoeniculicola*) was described [72]. Notably, these two distantly related Upupiform species (included in two different families: Upupidae and Phoeniculidae, [90–92]) living in three different continents (Europe, Asia and Africa) have been found

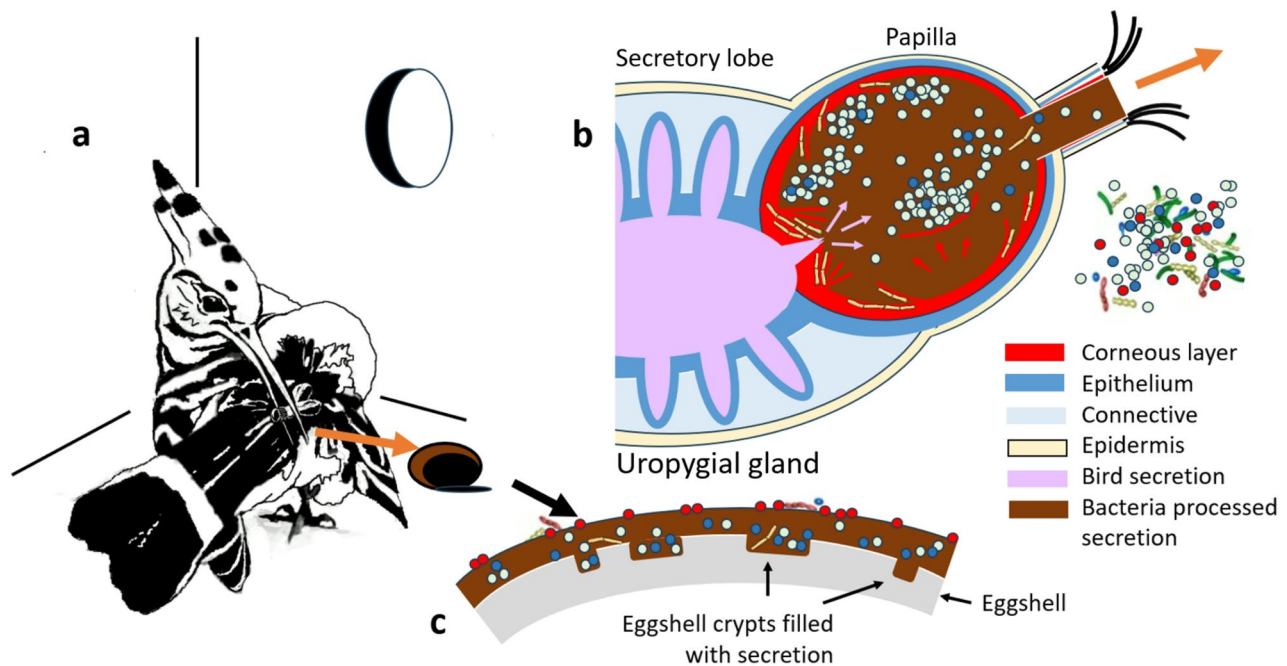


Fig. 1 Structure and function of the hoopoe uropygial gland. **(a)** Nesting female hoopoe using the uropygial secretion to cover eggshell surface within the nest cavity. **(b)** Schematic representation of the section of the uropygial gland of a nesting hoopoe (breeding female or nestling). The papilla is full of dark secretion because of the metabolic activity of symbiotic bacteria living within it. Bacteria do not enter hoopoe tissues nor the cavities of the secretory lobe, since a thick keratin corneous layer compartmentalizes them within the papilla lumen. Symbionts living within the papilla are a selection of those available in the surrounding environment, and use the secretion and probably substances provided by desquamating keratin layers, whose inter-lamina spaces are inhabited only by long bacilli (probably some actinobacteria). **(c)** The surface of eggshells is covered in dark secretion with uropygial symbionts, but incorporate many other bacteria from the environment

Box 2 Key features of symbiotic organs (modified from [57])

Feature	Function	Origin
Infection specificity	Symbiotic organs filter microbial partners.	a) Mechanical structures that spatially and/or temporally restrict access. b) Chemical attractants that help recruit symbionts. c) Antibiotic selectors that remove non-symbiotic strains.
Structure favoring compartmentalization	Structures that reduce symbiont interference with other host processes while creating microenvironments that optimize the services provided by symbionts.	The structure might be <i>canalized</i> (unaffected by symbiont colonization), or <i>phenotypically plastic</i> (promoted by symbiont colonization).
Ability to maintain beneficial partnerships	a) Within organ promotion of beneficial symbionts. b) Mediate transmission of best cooperators.	a) Host investment that generates nutritional support to beneficial symbionts or sanctioning of strains that disrupt the symbiotic organ or its function. b) Host physically transfers beneficial partners from symbiotic organs.

to harbor similar symbionts within their glands (species of the Genus *Enterococcus*, hereafter, enterococci) when studied with aerobic culturing methods [72, 89, 93]. For the European hoopoe, molecular analyses of the microbial communities indicate that a variety of taxa, other than enterococci, dominate the uropygial bacterial community [83, 94, 95] which is very different from those of other sympatric bird species [79]. Although the bacterial community of woodhoopoe glands has not been characterized with molecular methods, the similarity in the bacterial metabolites found in the secretions of the two Upupiform groups [81, 82] suggests that their symbiotic microbiota may share constituents, as would be expected

for symbiotic microbiotas of specialized organs (“infection specificity”, Box 2, [57]).

In this study, we tested if the uropygial gland of the European hoopoe is a specialized organ for the selective cultivation of a particular microbiome and, thus, it meets the criteria assigned to typical symbiotic organs (Box 2 [57]). Briefly, we first analyze the specificity of host-bacterial association by comparing two populations breeding in two spatially independent countries (Switzerland and Spain) and with different migratory strategies [96, 97]. If the gland is acting to select a particular kind of symbiont, we expect to find congruence in the communities of uropygial secretions of both European hoopoe populations.

Moreover, given that female hoopoes cover eggshells with uropygial secretion [85–87], but the eggshell community is more exposed to environmental influence [98], we expect to find higher inter-population variation and more diverse microbiomes on eggshells than for glands. Second, we study the anatomy and morphology of the uropygial gland of the European hoopoe. We expect to find morphological differences in glands of nesting and non-nesting hoopoes that point at the existence of compartmentalization favoring bacterial cultivation (Fig. 1b). Third, we study microscopically the organization of bacterial morphs within the gland of nesting hoopoes. We expect that different types of bacteria are associated with distinct within-gland spaces (Fig. 1b), implying selective compartmentalization/specificity of symbionts related to gland structure.

Methods

Study species

The European hoopoe (*Upupa epops*) inhabits open woods or open areas such as steppes, grasslands, pastures, semi-deserts, or field crops with scattered trees, walls or buildings providing holes for nesting, and fields with patches of bare ground for feeding [99–101]. Females lay one, two or rarely three clutches of 5–10 eggs over the breeding season, between February and July [102]. Often, they use the same nest hole through the years and between clutches in the same year [102, 103]. Incubation, performed only by females, lasts 16–17 days and starts with the first, second or third egg. Eggs laid after the start of incubation will hatch asynchronously at 24 h or even greater intervals [104–106], which causes a marked size hierarchy within the brood, frequently resulting in the death or cannibalism of last hatched nestlings [107–109]. The female stays within the nest, brooding chicks, at least for eight days after the start of hatching, and, for her whole stay since start of laying, all food for her and nestlings is provided by the male, which rarely enter the nest cavity [110].

Study area

The study was carried out in two distant wild populations in Spain and Switzerland sampled during the 2010 and 2014 breeding seasons, respectively. The Spanish population is located in the Hoya de Guadix (37°24′08″N, 3°04′04″W), southern Spain, where hoopoes breed in nest-boxes placed in trees or buildings in crops, forests and gullies [102]. This population is a mixture of sedentary and migratory individuals spending the winter in Africa [96, 97]. The Swiss population is located on the plain of the Rhône, in Valais, southwestern Switzerland (46°16′10″N, 7°7′29″E). This area is a semi-open to open landscape with 700 nest-boxes installed mostly in agricultural sheds among fruit tree plantations and vineyards

[111]. This population is integrated by long distance Palaearctic-African migrant individuals, which spend the non-breeding season in the Sahelian belt south of Sahara [96, 97, 112]. They typically return to the breeding grounds in March-May, start breeding in April, and the last nestlings usually fledge in August [103, 113].

General methods

Nest-boxes were visited twice per week from mid-February to the end of July in Spain and once per week from mid-April to the end of July in Switzerland, to record laying date, clutch size and hatching date. All individuals were banded with metal numbered rings for individual recognition. Incubating females were caught 14 days after laying the first egg within the nest-box by hand, quickly sampled, and released again within the nest-box to minimize disturbance. Nestlings were sampled 20 days (± 1 day) after hatching of the first egg. All sampling was carried out with sterilized material and reagents, whether prepared at the laboratory or from the commercial supplier. For each capture, we wore new latex gloves cleaned with 70% ethanol for the whole process to reduce the risk of bacterial contamination. Before collecting uropygial secretion samples, we gently washed the circlet of feathers and skin surrounding the uropygial gland with a cotton swab dipped in ethanol to prevent contamination with external bacteria. Then, the secretion was extracted by inserting a sterile micropipette tip (1–10 ml Finpipette micropipette) into the opening while gently pressing the papilla (Fig. 1b), and transferred to a sterile microfuge tube. Bacterial samples from eggshells were collected by rubbing the entire surface of all eggs in the clutch with a sterile swab slightly wet with sterile phosphate buffer (PBS, Na_2HPO_4 , 0.1 M and NaH_2PO_4 0.1 M, pH 7.4) and individually stored in a sterile microfuge tube with 1.2 ml of the buffer solution [114]. Samples were kept cool (i.e., 3–4 °C) in the field until being stored at -20 °C in the lab for further molecular analyses.

Bacterial diversity and taxonomic identification

Bacterial genomic DNA was extracted from uropygial secretions (10–20 μl per sample) using FavorPrep™ Blood Genomic DNA Extraction Kit (Favorgen Biotech). For eggshells, before DNA extraction, samples were sonicated in an ultrasonic bath (50/60 Hz) for 90 s to free cells from the cotton swab. Afterwards vials were centrifuged for 5 min at maximum speed. The pellets were treated with a lysozyme solution (10 mg/ml, 37 °C for 30 min) and extracted with the Chelex method [115]. The extracted DNA was eluted in sterile distilled water and stored at -20 °C until processing for microbiota analyses.

The bacterial communities were studied by two different approaches: 16S rRNA amplicon sequencing in the Illumina MiSeq platform [116] and ARISA (automated

rRNA Intergenic Spacer Analysis [117]). We used ARISA in previous experimental studies exploring the dynamics of the bacterial community of the hoopoe secretion [87, 95, 118, 119], followed by studies based on 16S rRNA amplicon sequencing exploring the relationships of this community with nest odor and ectoparasitism risk [120]. Here, we used both ARISA and 16S rRNA amplicon sequencing of the v6-v8 region of 16S rRNA genes for microbiota characterization (see details of these protocols in Additional file 1). We used all available samples ($N=342$) for ARISA, and a subset of them ($N=84$) for 16S rRNA amplicon sequencing. The use of both methods allowed us to check for consistency of results between approaches in some analyses, and to study the most probable correspondence of ARISA peaks with taxa identified by 16S rRNA amplicon sequencing (Additional file 3). That exercise was used to discuss our results considering information about the hoopoe microbiota previously acquired with ARISA.

For ARISA analyses, we processed a total of 342 samples from 32 breeding attempts in Spain and 39 in Switzerland. Of these samples, 60 were eggshells (30 Spain, 30 Switzerland), 69 were female secretions (30 Spain, 39 Switzerland) and the remaining 213 were nestlings' secretions (68 Spain, between 1 and 3 nestlings/brood; 145 Switzerland, between 1 and 7 nestlings/brood). For 16S rRNA amplicon sequencing, we used a subsample ($N=84$) including 7 broods from Spain (2 nestlings per brood, 8 female secretions, eggshell samples from 6 clutches) and 15 broods from Switzerland (2 nestlings per brood, 14 female secretions, eggshell samples from 12 clutches). For both 16S rRNA amplicon sequencing and ARISA analyses, negative controls were run together with samples and in no case did they produce amplification. Detailed methods on sequence library processing of samples are included in Additional file 1.

The nomenclature used for the taxonomy assigned to 16S rRNA amplicon sequencing ASVs follows the database silva-138-99-tax, although some names have changed [121]. For example, we maintain *Firmicutes* for the actual phylum *Bacillota* or *Proteobacteria* for the actual phylum *Pseudomonadota*.

Uropygial gland morphology and anatomy

Fresh uropygial glands of hoopoes found killed by predators in the field in the Spanish population (nine individuals), or found dead accidentally in our captive breeding population (eight individuals, [118]) were dissected and mounted for the study of internal anatomy and morphology. Glands were immediately fixed in 4% paraformaldehyde, washed in sterile distilled water, dehydrated in successive 50%, 80%, and 96% ethanol baths, rinsed in distilled water and stored in PBS at 4 °C until use.

Dehydrated glands were embedded in Embed 812 resin. Semi-thin sections of the samples were stained with hematoxylin-eosin and observed in an Olympus BX51 microscope and an Olympus DP72 camera (Olympus, Shinjuku, Tokyo, Japan) for histological study, or used for Fluorescence In Situ Hybridization (FISH) with fluorescently labelled probes for bacteria (see below).

Glands from 17 individuals were used for the microscopic study: four breeding females, four breeding males, three non-breeding females, three non-breeding males and three nestlings with functional glands (i.e., older than 8 days).

Fluorescence in situ hybridization (FISH)

Semifine sections of the uropygial gland of a breeding hoopoe female were used to study the location of bacteria within the gland using FISH techniques. Samples were hybridized with Eub338 [122] labeled with cyanine 3 (Cy3) as a universal probe for bacterial RNA (Thermo Scientific, Ulm, Germany). As positive control for the presence of bacterial DNA, we incubated slides with the DNA intercalating agent Hoechst (Sigma-Aldrich, St. Louis, MO, USA). For a detailed explanation of the hybridization protocol followed, see Additional file 1. Using an Olympus BX51 fluorescence microscope and an Olympus DP72 camera, we obtained pictures at different magnifications of the gland around the papilla and secretory lobes. Pictures were taken through the 4',6'-diamidino-2-phenylindole (DAPI, blue), fluorescein-5-isothiocyanate (FITC, green-yellow), and tetramethylrhodamine-isothiocyanate (TRITC, red) filters, and superimposed with Photoshop.2024 when needed for a better visualization of the location within the gland.

Statistical analyses

QIIME2 v2021.11 (Quantitative Insights In Microbial Ecology [123]), was used to generate alpha-diversity estimates for samples (number of ASVs, Berger-Harper dominance and Faith phylogenetic distance [124]), and beta-diversity matrices of distances among samples (weighted and unweighted UniFrac distances [125]). We have used UniFrac distances of rarefied samples (Additional file 1) as an analytical approach to compare composition of communities [126, 127]. Comparisons among types of samples were performed with Primer 7.0.13 (PRIMER-e) and Statistica TIBCO 2023.

Comparison of alpha-diversity indices was performed using General Linear Mixed Models (GLMM), with sample type (nestling secretion, female secretion and eggshells) and population (Spain, Switzerland) as fixed factors, and nest identity (nested within population) as random factor. A factorial design including the interaction between sample type and population explored whether population affected differences among sample

types, and when non-significant, the interaction was excluded from the final model.

The beta-diversity of bacterial communities was compared among sample types and populations (Spanish and Swiss) using the same design described above, by means of PERMANOVA with 9999 permutations [128]. Distance matrices were calculated using unweighted and weighted UniFrac distances generated with QIIME2 for 16S rRNA amplicon sequencing at the ASV level, and Jaccard distances for ARISA ITSs.

Classical multidimensional scaling analysis (MDS after bootstrap analyses with 50 repeats per group) was used to graphically visualize bacterial community composition of the samples in multidimensional space. This technique represents the communities on a plot with canonical axes, where the proximity between communities shows their underlying similarity [129].

To explore whether the bacterial communities of the two populations (Spain and Switzerland) are congruent, we analyzed the relationship between prevalence in Switzerland (dependent variable) and Spain (continuous explanatory variable) for ASVs in Generalized Linear Models (GLZ) with a Poisson distribution. Sample type and its interaction with prevalence in Spain were included as fixed factors. To elucidate the predicted greater dissimilarity between populations for eggshells than for secretions, the models were repeated including only the two types of secretion samples.

All samples were used to generate the abundance-occupancy plot of ASVs expected under a neutral model for secretions and eggshells [130]. This allowed us to evaluate the position of ASVs out of the confidence interval of the neutral model, suggesting positive or negative selection [131]. Additionally, we determined the members of the core microbiome, i.e., consistent ASVs of our data set that can be hypothesized to reflect underlying functional relationships with the host [131, 132]. For this approach we calculated the consistency index for each taxon found in secretions using the available script for spatial study designs provided by Shade and Stopnisek (2019) [131]. As cut-off criterion we used the last taxon (ranked by the consistency index) increasing more than 2% the beta diversity of the core [131].

Results

Influence of sample type and population on microbiome composition

The 16S rRNA amplicon sequencing of the 85 hoopoe samples produced 1,922,850 reads (mean(SD)=22,621.76(7,226.21)) classified into 677 ASVs. After rarefaction to the minimum sampling depth (3,864 reads, see Additional file 1), the number of ASVs was reduced to 661 for the 84 samples retained. Taxonomy assignment detected 12 bacterial phyla, with a much higher relative abundance

of *Firmicutes* (78.4% of reads) followed by *Bacteroidota* (12.2%), *Actinobacteriota* (6.7%), *Proteobacteria* (1.3%), and *Campylobacterota* (1.3%). The remaining seven phyla except *Fusobacteriota* were exclusively found on eggshells (classes written in black in Fig. 2). The Phyla *Proteobacteria* and *Actinobacteriota* were significantly more frequent in eggshells samples (Fig. 2, Kruskal-Wallis tests on relative abundance, $H(2, N=84)=49.1, p<0.001$; $H(2, N=84)=23.2, p<0.001$, respectively), while Ph. *Firmicutes* reached higher values in secretions (Fig. 2, Kruskal-Wallis test, $H(2, N=84)=11.97, p<0.005$), without significant differences between nestlings and female secretions (Additional file 2, Table S1). Within the Ph. *Firmicutes*, the relative abundance of Cl. *Bacilli* was much higher in eggshells, whereas Cl. *Clostridia* was dominant in secretion samples (Fig. 2).

The higher bacterial diversity on eggshells was also evident at the ASV level. Eggshell samples presented significantly higher alpha-diversity than secretion samples for number of ASVs and Faith phylogenetic diversity, which did not differ between nestling and female secretions (Fig. 3, Additional file 2: Table S2). The Berger-Parker dominance index was significantly higher for nestling secretions than for eggshells, with an intermediate mean value in female secretions (Fig. 3, Additional file 2: Table S2). The number of ARISA ITSs per sample showed a different pattern, with significantly higher values for Spanish than Swiss secretions and the opposite for eggshells (Additional file 2: Fig. S1).

Using beta-diversity estimates to study community composition, we found greater distances within populations between eggshells and secretions than between nestling and female secretions, both with unweighted UniFrac distances for 16S rRNA amplicon sequencing ASVs and with ARISA ITS identities (Fig. 4; Table 1, Additional file 2: Table S3 for ARISA). This pattern was less clear using weighted UniFrac distances for Spain, without any significant pairwise comparison (Table 1). The differences between populations were greater for eggshells than for both secretion types when using unweighted UniFrac distances (Fig. 4a, see average distances in Table 1). A similar trend but much less pronounced was obtained for weighted UniFrac distances and similarity in ARISA ITS composition (Fig. 4b-c; Table 1 and Additional file 2: Table S3 for ARISA). The beta dispersion around centroids were not significantly different between populations for any sample-type x beta diversity index, except for Jaccard distances in nestling secretions (Permdisp results in Table 1 and Additional file 2: Table S3). However, in most cases, the comparisons of beta dispersions were significant for nestling and female secretions within countries, coinciding with non-significantly different or less distant communities

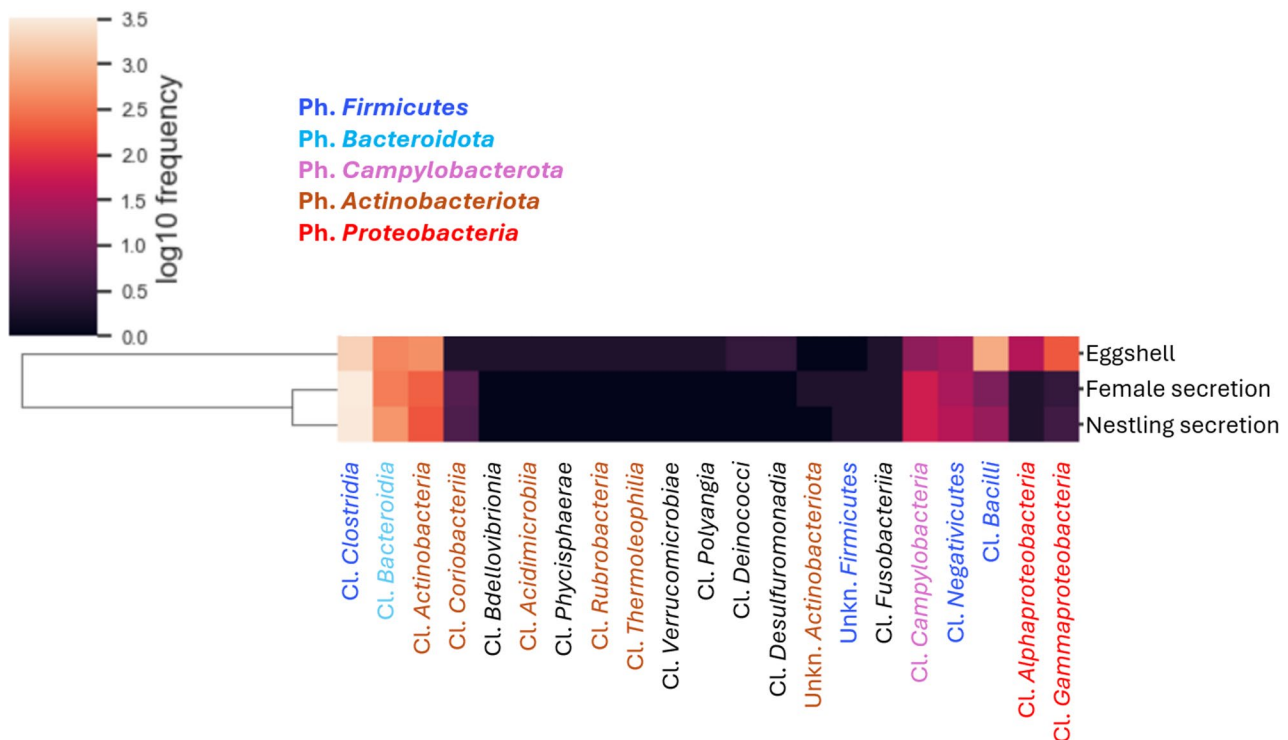


Fig. 2 Heatmap showing differences in the relative abundances of bacteria at the Class level among three sample types obtained from nesting hoopoes. Nestling and female uropygial gland secretions clustered together and showed similar profiles. Eggshells were differentially enriched in both *Alpha*- and *Gammaproteobacteria*, as well as *Bacilli*. Bacterial classes label colors refer to their taxonomic classification into the phyla listed in the figure (the seven classes written in black are from seven different minoritarian phyla exclusive of eggshells (except *Fusobacteriia*), whose names are not included in the legend)

of secretions when compared with Permanovas (Table 1 and Additional file 2: Table S3).

Prevalence of bacterial ASVs associated positively between Spanish and Swiss populations (Fig. 5), while the strength of the association depended on the considered type of sample (GLZ, interaction term, 16S rRNA amplicon sequencing ASVs: Wald = 1757.194, $p < 0.001$, Fig. 5). The significant interaction with type of sample for ASVs was mainly due to differences between eggshells and both types of uropygial secretions, since it became non-significant when eggshells were excluded (Wald = 1.325, $p = 0.250$, Fig. 5). Given the great similarity in the profiles of female and nestling uropygial secretions within populations (Figs. 2, 3, 4 and 5; Table 1), we have considered them together for subsequent analyses.

The taxonomic affiliation of ASVs of the core microbiomes showed that a few from four phyla dominated all communities (Fig. 6, only analyzed for 16S rRNA amplicon sequencing, the correspondence of ARISA ITSs with identified ASVs is explored in Additional file 3). The same ASVs with the highest prevalence in secretions: one *Porphyromonas* (Ph. *Bacteroidota*), one *Varibaculum* (Ph. *Actinobacteriota*), one *Campylobacter* (Ph. *Campylobacterota*), and nine AVSs from phylum *Firmicutes* including one *Negativicoccus*, one *Lachnospiraceae*, two

Fastidiosipila and five *Peptostreptococcales* (one *Anaerococcus*, two *Peptoniphilus*, one *Murdochella* and other unidentified), also occupied the highest rank positions in the core microbiome of eggshells (Fig. 6). However, the remaining part of the core was very different between secretions and eggshells. The eggshell community was enriched with numerous ASVs of *Bacilli* (Ph. *Firmicutes*), *Proteobacteria*, *Bacteroidota* and groups of the Phylum *Actinobacteriota* not present in the core of secretions. On the other hand, the composition of the core microbiome of secretions was more consistent in its whole range, with a great proportion of potentially redundant ASVs. In many cases, the core ASVs of secretions were variants of the same genus or closely related genera, mainly within *Firmicutes*. For instance, 13 ASVs were *Fastidiosipila* variants (Ph. *Firmicutes*, O. *Clostridia*), five were *Murdochella* (Ph. *Firmicutes*, O. *Peptostreptococcales*), the genus *Peptoniphilus* with the most prevalent (found in 100% of secretions) and abundant ASV, presented 4 variants (also O. *Peptostreptococcales*), and there were 15 additional *Peptostreptococcales* ASVs (two *Helcococcus* and 13 unidentified, Fig. 6a). In many cases, variants of the same genus were negatively correlated or even incompatible (results not shown) suggesting they are alternative symbionts filling the same niche. Two ITSs known

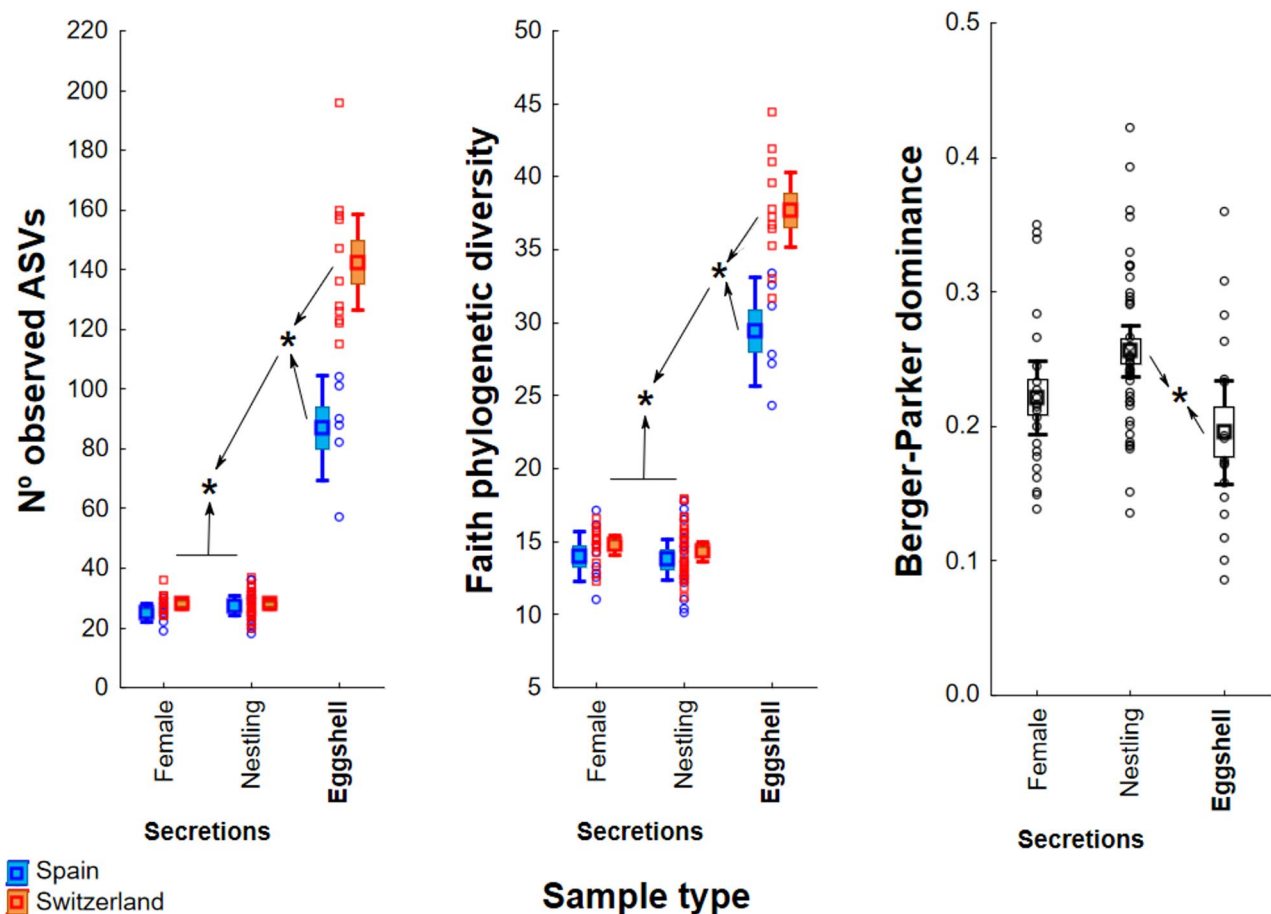


Fig. 3 Differences among the three sample types and between populations in three alpha-diversity estimates (boxplots show Mean $\pm$ 95% CI and SE; points are raw data). For N° of ASVs and Faith phylogenetic diversity there was a significant interaction between population and sample type, while population had no effect on Berger-Parker dominance (Additional file 2: Table S2). Asterisks mark significant differences in post-hoc Tukey tests between groups connected by arrows ($p < 0.05$)

to depend on vertical transmission from female glands to nestlings corresponded to genera of the order *Peptostreptococcales* (*Helcococcus* and *Peptoniphilus*) included in the core of secretions (Additional file 3: Table S2). Prevalence of most of the 59 ASVs of the core of secretions (32 out of the 59) was higher than expected by their relative abundances under a neutral model (Fig. 6a). When the core microbiomes were studied separately by population, a high proportion (76.8%) of the total 82 ASVs included in secretions were shared, while only 25% of 179 core ASVs from eggshells were shared between the Spanish and Swiss populations (Chi-Square test: $\chi^2 = 153.37$, $p < 0.0001$).

Specializations of uropygial gland in nesting individuals

The external morphology and the internal anatomy and histology of nesting individuals (nestlings and breeding females) greatly differed from those of males and non-nesting females (Fig. 7). Regarding external morphology, the uropygial gland of nesting individuals shows a more

voluminous papilla than that of non-nesting hoopoes (cf. Figure 7a, i). Because the stored secretion is dark in nesting individuals, the color can be seen through the skin, resulting in darker papilla compared to the secretory elements (lobules) (Fig. 7a-b). This is not true for non-nesting adults (Fig. 7f-g, i) whose secretions are white (Fig. 7i).

We found interesting locations of symbiotic bacteria within the glands' internal structure of nesting individuals (see female glands in Fig. 7 and Additional file 2: Fig. S2, nestling glands only in Additional file 2: Fig. S2). First, within the papilla, bacteria concentrate close to the internal walls or around corneous lamina disaggregated from it (see green arrows in Fig. 7c-d and specific fluorescent stained bacteria in Fig. 8a). Second, the internal wall of the papilla is separated from its lumen by a continuous keratin multi-stratified layer covering the epithelium, which is engrossed and folded around the connection between the papilla and the ducts coming from secretory lobes (yellow arrows in Fig. 7c). The magnification of that

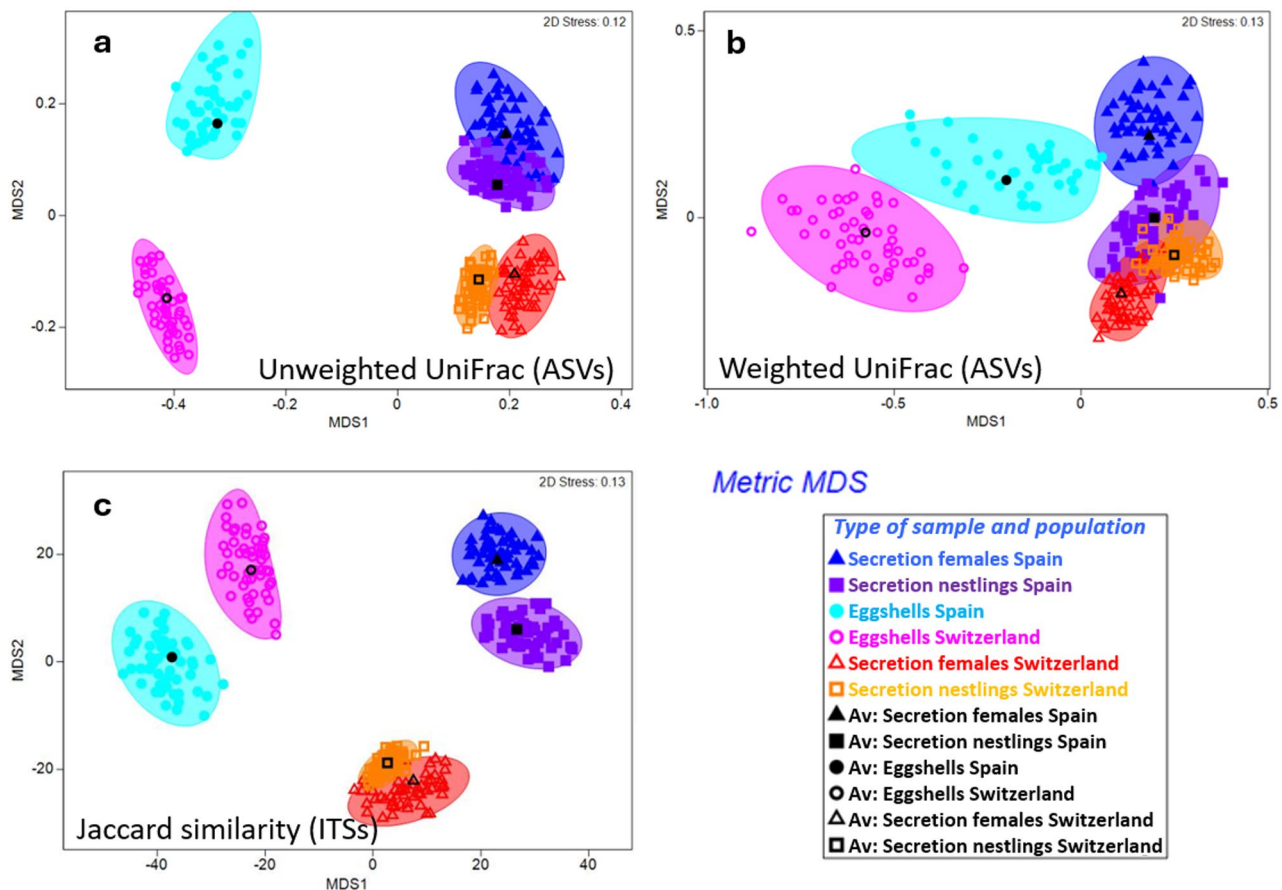


Fig. 4 Differences in the composition of the hoopoe bacterial communities present in the two types of uropygial secretions (females and nestlings) and eggshells, for the Spanish and Swiss populations. Graphs show the average positions of communities in a metric multidimensional space, after bootstrap analyses with 50 repeats per group. **(a)** Comparison based on unweighted UniFrac distances at ASV level, **(b)** Comparison based on weighted UniFrac distances at ASV level **(c)** Comparison based on the 63 most prevalent ARISA ITSs based on Jaccard similarity. Black symbols mark the average positions obtained in the bootstrap analyses and shaded areas the 95% confidence surface of such means. See Permanova Models for comparisons in Additional file 2: Table S3

layer shows dense concentrations of bacteria, especially in the spaces left by disaggregating laminae (Fig. 7c-e). Such corneous layers are not present neither in males (seven individuals examined) nor in non-breeding female glands (three individuals examined), where the basal and proliferative layers of the epithelium appear in direct contact with the lumen of the papilla (blue arrows in Fig. 7h and j). Finally, an apparent additional difference between the images of the glands of the two types of individuals is the aspect of the material filling the secretory tubules, lobe spaces and papilla. Preparations of non-breeding adults normally show empty papillae and lobe spaces (asterisks in Fig. 7h and j), as well as abundant oil droplets inside cells in the intermedium-transitium layers of the epithelium of secretory tubules (Additional file 2: Fig. S3c, S3f). On the contrary, glands of nesting individuals stay filled with material after fixation, both in the papilla lumen and within the lobe spaces (Fig. 7c, Additional file 2: Fig. S2a, c). Moreover, the cells within the secretory

tissues of tubules lack oil droplets in both breeding females and nestlings (Additional file 2: Fig. S2d-e).

Compartmentalization and sorted organization of bacteria

The observation of fluorescently labeled bacteria confirmed compartmentalization and some level of organization of different types of symbionts within the papilla of the gland (Fig. 8). The corneous layer compartmentalizes the symbionts strictly in the papilla, with dense concentrations on the lumen side of the epithelium, but no bacteria in the lobes or live host tissues behind the corneum (Fig. 8a-b). Moreover, while open spaces are full of cocci (Fig. 8b), the engrossed areas of corneous material around the base of the papilla appear as loose laminae leaving narrow spaces occupied only by chains of long bacilli (Fig. 8c-d).

Table 1 Results of Permanova analyses comparing the composition of the hoopoe bacterial communities present in the two types of uropygial secretions (females and nestlings) and eggshells, for the Spanish and Swiss populations

Permanova											
a) Unweighted UniFrac					b) Weighted UniFrac						
Whole model					Whole model						
Factor		Nested in	df	PseudoF	p	Factor		Nested in	df	PseudoF	p
1) Population	Fixed		1	2.15	0.0001	1) Population	Fixed		1	2.33	0.02
2) Sample type	Fixed		2	18.38	0.0001	2) Sample type	Fixed		2	9.07	0.0001
3) Nest	Random	Populat.	22	4.68	0.0001	3) Nest	Random	Populat.	22	3.57	0.0001
1 × 2	Fixed		2	2.44	0.0001	1 × 2	Fixed		2	2.32	0.02
2 × 3	Random		34	1.71	0.0001	2 × 3	Random		34	1.55	0.03
Res.			22			Res.			22		
Pair-Wise tests (1 × 2)					Pair-Wise tests (1 × 2)						
Factor	Within		Distance	t	p	Factor	Within		Distance	t	p
Population	Eggshells		0.59	1.69	0.0003	Population	Eggshells		0.88	1.39	0.098
Population	Nestlings' secretions		0.51	1.12	0.141	Population	Nestlings' secretions		0.70	0.89	0.572
Population	Females' secretions		0.47	1.57	0.002	Population	Females' secretions		0.67	1.97	0.002
Factor	Within Spain		Distance	t	p	Factor	Within Spain		Distance	t	p
Sample type	Eggshells-Fem. Secret.		0.75	2.88	*0.02	Sample type	Eggshells-Fem. Secret.		0.73	1.14	0.320
Sample type	Eggshells-Nestl. Secret.		0.78	2.59	0.02	Sample type	Eggshells-Nestl. Secret.		0.77	1.51	0.159
Sample type	Fem.-Nestl. Secretions		0.47	1.29	*0.174	Sample type	Fem.-Nestl. Secretions		0.64	1.64	0.109
Factor	Within Switzerland		Distance	t	p	Factor	Within Switzerland		Distance	t	p
Sample type	Eggshells-Fem. Secret.		0.76	4.54	*0.0001	Sample type	Eggshells-Fem. Secret.		1.06	3.43	*0.001
Sample type	Eggshells-Nestl. Secret.		0.76	4.67	0.0001	Sample type	Eggshells-Nestl. Secret.		0.99	3.97	0.0001
Sample type	Fem.-Nestl. Secretions		0.47	1.29	*0.01	Sample type	Fem.-Nestl. Secretions		0.66	2.05	*0.02

The matrix of distances among samples used is based on: (a) Unweighted UniFrac and (b) Weighted UniFrac distances, calculated with the profiles of ASVs obtained with 16S rRNA amplicon sequencing rarified to 3864 sequences per sample. The whole model as well as the pair-wise tests comparing the same type of sample between populations (factor 1 × 2 within type of sample) are presented. Asterisks on pair-wise *p* values, mark when the comparison of beta dispersions using a Permdisp analysis resulted significant (*p* < 0.05)

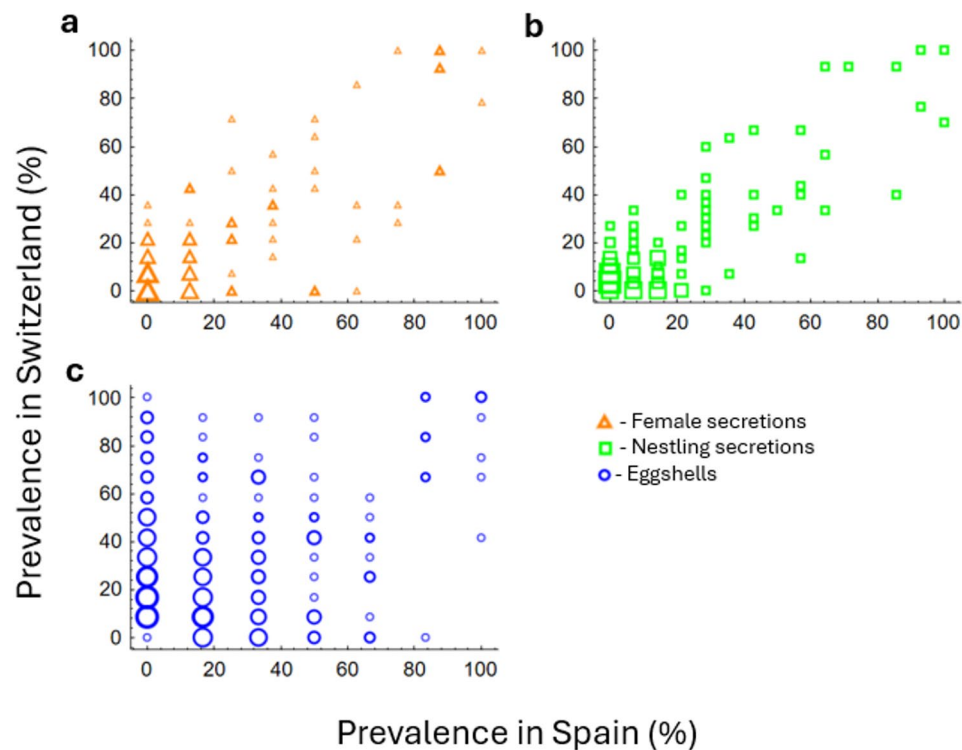


Fig. 5 Relationships between the prevalences in Spain and Switzerland for 16S rRNA amplicon sequencing ASVs in each of three sample types: (a) female secretions, (b) nestling secretions and (c) eggshells. Number of ASVs per point is represented on a log scale

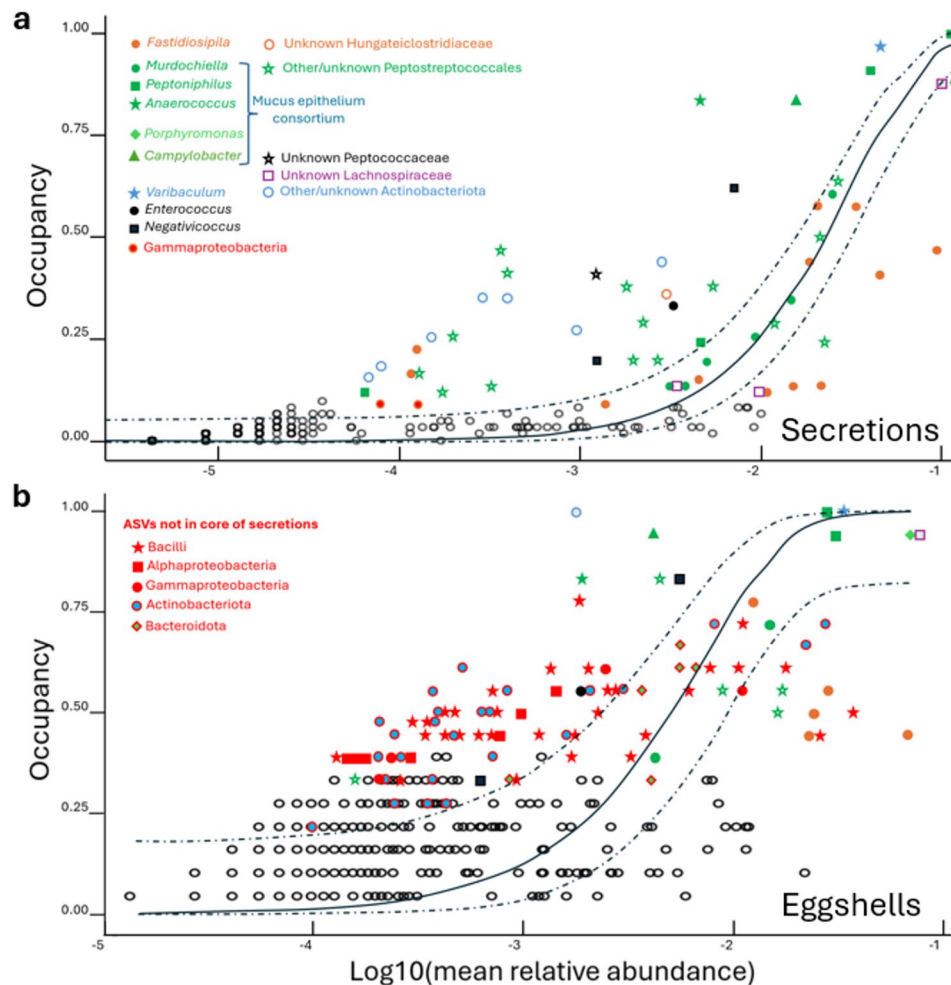


Fig. 6 Contribution of the different ASVs found in hoopoe uropygial secretions to the similarity in the composition of bacterial communities among samples. **(a)** Fit of the neutral model to the relationship between abundance and occupancy for the ASVs found in uropygial secretions of both Spanish and Swiss hoopoes. The solid line represents the expected relationship of the neutral model, and dashed lines are 95% CI around the neutral model. Points represent different ASVs, the 59 included in the core are determined taxonomically as indicated by color and shape in the legend. Those out of the CI interval are more (above) or less (below) prevalent than expected by their abundance under a neutral model. **(b)** Fit of the neutral model for eggshells in both populations. ASVs not present in the core microbiome of secretions are marked with red symbols and their taxonomic affiliation indicated in the legend. The ASVs shared with secretions maintain the same symbols from subfigure a

Discussion

We have found that, in comparison with the bacterial community of the eggshell surface, the microbiota hosted within the uropygial gland of nesting hoopoes is very similar across populations. Moreover, most of its core components are more prevalent than expected by neutral processes, possibly implying some kind of filtering or promotion explaining its maintained composition. Indeed, the microscopic study of those glands showed that the bacteria are strictly compartmentalized within the papilla and concentrated on locations where the nesting hoopoes provide their special secretion and engrossed keratin layers, suggesting the possibility of host control. Moreover, morphologically distinct bacteria are associated with disaggregating keratin laminae. Such structures and organization are not present in the glands

of males and non-breeding females, whose secretions differ from those of nesting individuals in bacteriological, physical and chemical properties [82–84]. Previous studies demonstrated that these symbiotic bacteria benefit their nesting hoopoe hosts [18, 73, 82, 85, 88, 93, 120, 133], suggesting that the glands of nesting hoopoes have evolved as specialized organs for growing a particular beneficial microbiome.

A first condition that the uropygial gland of hoopoes should fulfill to be considered as a symbiotic organ is that, independently of the environmental conditions, it should harbor consistent bacterial communities among populations (specificity, Box 2 [57]). Our results support that prediction. First, and although samples from the two populations were taken in different years, the communities of secretions were more similar between populations

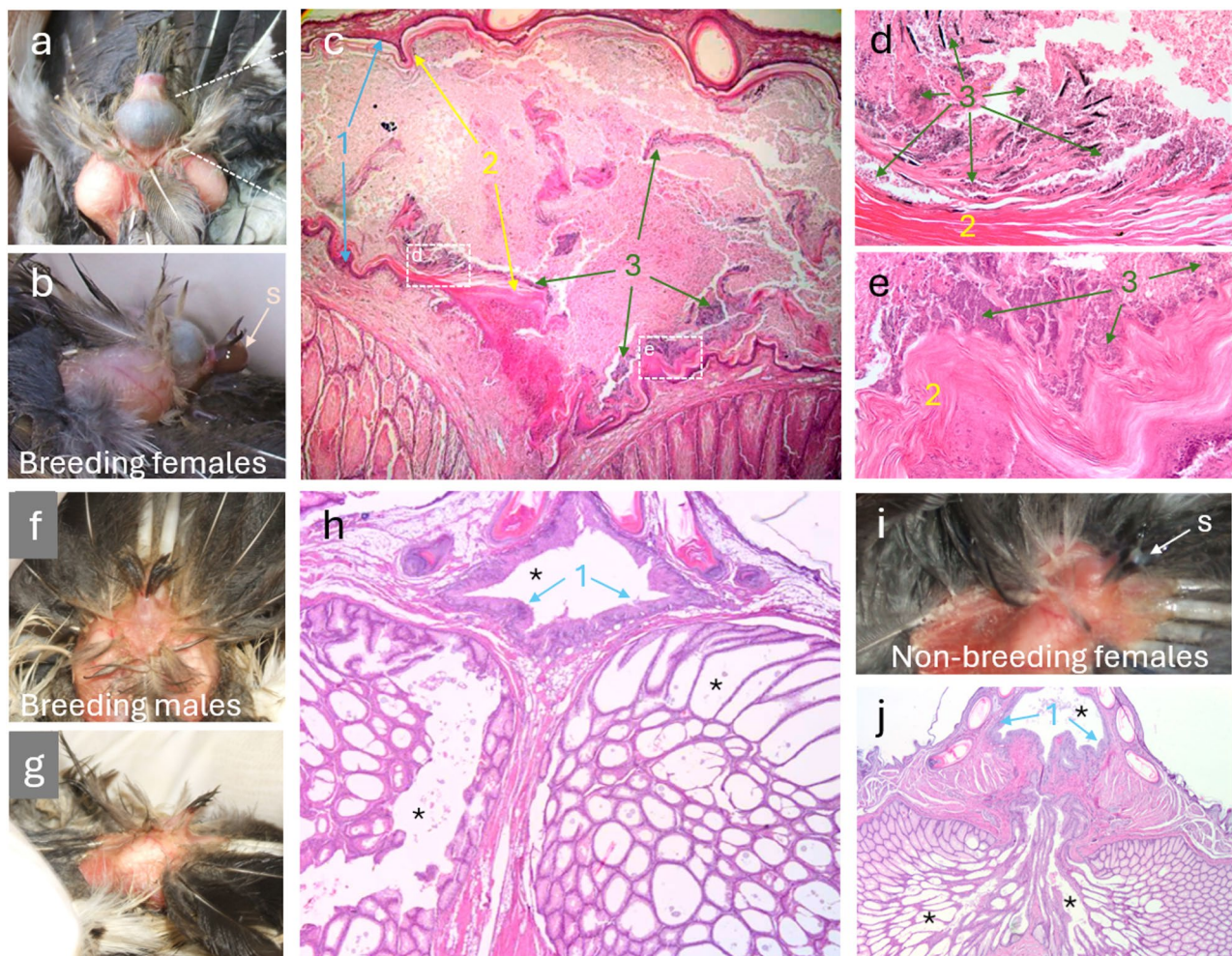


Fig. 7 Morphology and anatomy of the uropygial gland of hoopoe breeding females (a–e), breeding males (f–h) and non-breeding females (i–j). Images c, d, e, h and j are hematoxylin-eosin-stained sections of glands, showing the internal anatomy of the papilla and its connection with the secretory lobes (the approximate spatial correspondence is indicated with dashed lines in image a). Images d and e show details of the internal wall of the papilla at the sites indicated in image c. Numbers mark: **1** basal and proliferative layers of the epithelium of the papilla wall, **2** corneous layers of the papilla wall, **3** masses of bacteria (thin grains violet-stained). The **S** in b and i marks a drop of secretion at the tip of the papilla. Asterisks mark empty spaces at the papilla and secretory lobes. Images c, h and j 40x, images d and e 400x

than those of eggshells. Second, the prevalence of bacterial ASVs in different populations were more consistent when considering uropygial secretions than eggshells bacterial communities. Moreover, alpha diversity was significantly higher in eggshell than in secretion microbiomes, and the prevalence of ASVs of the core microbiome was higher than expected by neutral processes. Taken together, these results suggest facilitation of the acquisition and growth of the particular community found in the uropygial gland of hoopoes.

The specificity of the core microbiome was even clearer when looking at the taxonomy of the detected bacterial ASVs of the uropygial secretion of nesting hoopoes in Spain and Switzerland. Bacterial ASVs that differed between both populations were mostly variants of the same genera. The taxonomic spectrum of this core

microbiome is therefore very narrow. Moreover, seven ASVs were almost omnipresent in secretions from both populations, suggesting they are key constituents of this microbiome. We found that these seven ASVs were also present in the very different core microbiome of eggshells, suggesting their arrival after female application of secretions to eggs [85, 87, 95]. Five of them are typical components of the “mucosal consortium” (name given in [134]) characteristic of some regions of the mammalian intestine. Another 15 variants in the core were also unknown *Peptostreptococcales-Tissierellales* and therefore closely related to members of the consortium. These genera, and other main components of the core microbiome of glands (*Lachnospiraceae*, *Negativicoccus*, *Varibaculum*, *Fastidiosipila*, *Enterococcus*) are common constituents of the gut microbiome of healthy mammals

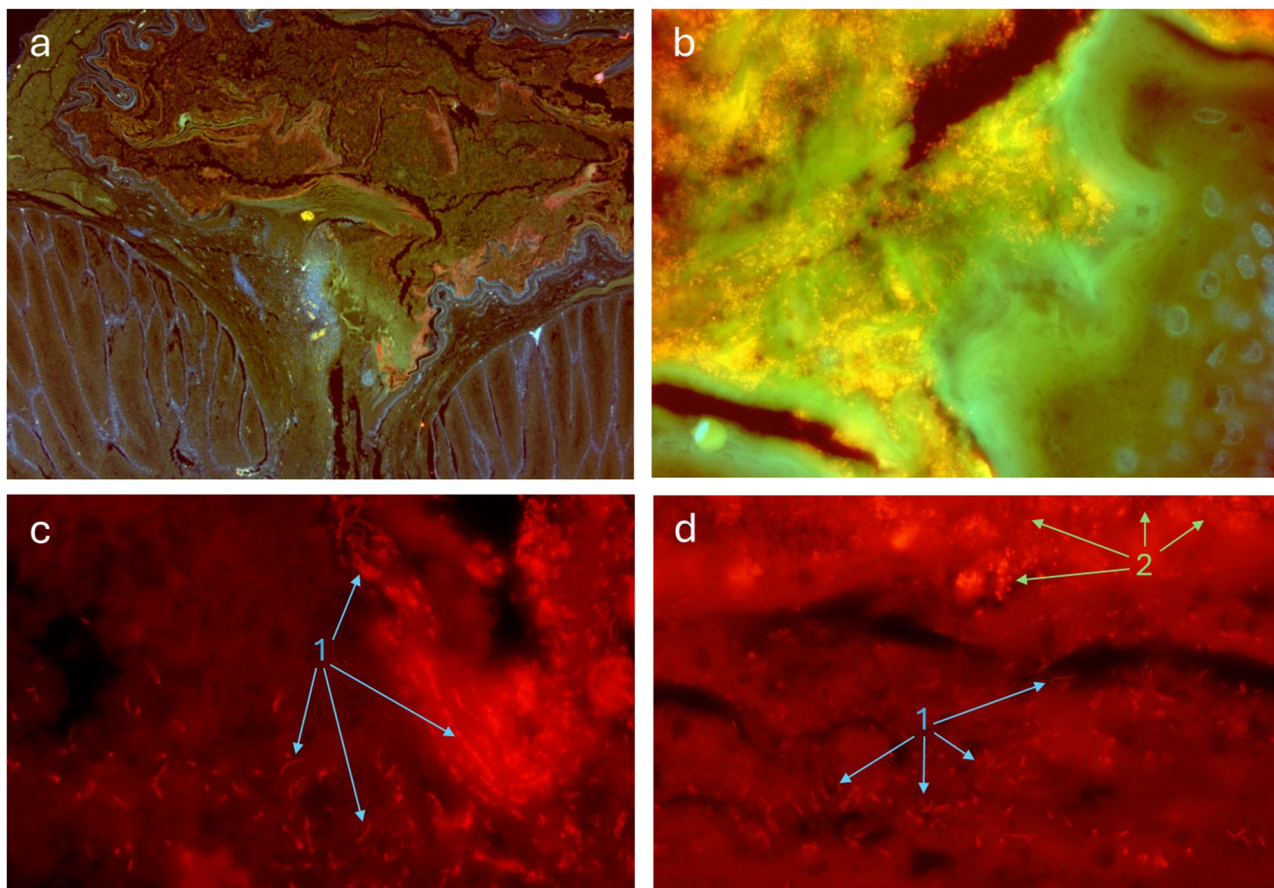


Fig. 8 Fluorescence microscopical images of uropygial glands of hoopoe breeding females showing the localization of symbiotic bacteria within the papilla. Bacteria are fluorescently labelled in red with Cy3-EUB338 universal bacterial probe. In blue, nucleic acids of the bird cells nuclei stained with Hoescht. Images **a** and **b** are superpositions of the red, blue and green filters pictures so that the labelled bacteria look pale orange. **(a)** General view of the same section of the papilla presented in Fig. 6c. The whole content of the papilla is full of fluorescently marked bacteria, but the highest densities are located close to the papilla walls and detached corneous layers. **(b)** Detail of dense masses of bacteria filling all spaces among detached corneous layers in the papilla lumen. The bacteria do not surpass the thick integer keratinized surface of this layer. **(c-d)** High magnification of the interface between the papilla lumen and the thick corneous layer of the papilla wall. Bacteria of different morphologies are located within the detaching layers (chains of long bacilli indicated with **1**) and in the secretion filling the open areas (masses of cocci, indicated with **2**). Image **a** 40x, image **b** 600x, image **c** and **d** 1000x

and/or birds, many of them with known key functions in regulation of immune function or complementing metabolic routes [134–142]. If the same functions are demonstrated for the species/variants living in hoopoe uropygial glands, this would imply that these glands are recruiting a consortium of commensal gut bacteria into an organ not involved in food processing, but in bird-secretion processing instead.

A second property of symbiotic organs is the existence of specialized structures compartmentalizing bacteria (Box 2 [57]). The hoopoe is the only bird species in which, the conduits transporting the secretion from the two lobes of the uropygial gland to the opening form a common, wide cavity in the papilla, not divided by longitudinal septa and opening in only one orifice [65, 143]. Even the specialized papillae present in passerines, with valves preventing the backflow of secretion to the lobes, is divided by a septum and open in two orifices [143].

Inside that cavity is where nesting hoopoes store a dense, dark, stinky secretion [144] full of bacteria [84]. In the present study, we have shown that the stratum corneum of the epithelium lining the interior of the papilla in nesting hoopoes is constituted by a thick layer of long cornified keratin laminae similar to the structure of bird skin in unfeathered and exposed areas [145]. There is a striking difference with the papilla of males and non-breeding females, in which the corneous layer lacks such laminae. Instead, corneocyte apoptosis apparently happens in loose fragmented cell debris, similar to the non-cornified squamous epithelium typical of the internal ducts or the cavities of the papilla of other birds, such as passerines or pigeons [65]. These particularities of the papilla of nesting hoopoes may be an adaptation to compartmentalize that cavity, allowing its use as a reactor for cultivating bacteria while reducing the risk of invasion of hoopoe tissues. Indeed, corneous layers of epithelia are known

barriers protecting against bacteria penetrating tissues at animal surfaces [146], as mucus layers do in internal organs such as the digestive tract [147–149].

In accordance with the existence of a compartmentalized cavity for bacterial cultivation, the FISH images showed that the papilla is full of bacteria, but they do not enter the lobes or penetrate the papilla wall. Moreover, bacteria appeared in association with disaggregated corneous laminae, which may serve as a source of nutrients for enhancing the growth of beneficial symbionts (third property of symbiotic organs, Box 2 [57]). It is known that the stratum corneum of the avian skin allows the transport of substances to the surface. This occurs by means of lipids of multigranular bodies in droplets freed in the intercellular spaces [150], which, for example, are used by pitohuis to channel defensive toxic alkaloids to the skin surface [151]. This functionality of the corneous layers could serve to provide a variety of substances to the epithelium surface [152] and drive the composition of the microbiome, as shown for the animal skin [146, 153]. Magnification of the corneous layers of the papilla in nesting hoopoes showed that only a particular type of long bacilli occupied spaces between those laminae. This suggests specialization in the use of nutrients of such location, either from the droplets present among laminae or from the very keratin of laminae (Fig. 1b).

The secretion produced by hoopoe tissues before reaching the papilla (i.e., in secretory lobes of the gland) was different in chemical nature between nesting and non-nesting hoopoes. This was shown by the dissolution of the content in cavities and ducts with non-polar solvents (i.e., ethanol used for fixing glands, e.g., [154]), only in non-nesting individuals. This suggests that, in the secretions produced by nesting individuals, the lipidic fraction is not so important as in non-breeding ones, as well as in most bird species [65]. In accordance with this possibility, only in these non-nesting individuals, the cells in the intermediate layers of secretory tubules of the gland showed lipid-bearing spheres filling the cytoplasm (as it is common in most birds, e.g., [154, 155]). These results indicate that the kind of substances produced in the secretory lobes of hoopoe uropygial glands change substantially between the non-nesting and the nesting phase. Among other mechanisms, hosts regulate the dynamics of bacterial assemblages by secretions providing particular nutrients [44], or substances that enhance (or prevent) recruiting particular symbionts [49]. The shift in secreted substances detected in hoopoes may allow them to control the community hosted (Fig. 1b).

Symbiotic communities can be the result of an appropriate selection of symbionts by a combination of selective feeding of beneficial colonizers and vertical transmission [156]. Behavioral traits of hoopoes favoring vertical transmission of beneficial bacteria [87] or from

nest materials from previous reproduction [157] could also explain the detected specificity of the bacterial community of the uropygial secretion of nesting hoopoes. Evidence of vertical transmission comes from correlational and experimental studies [118, 119, 158] in which fingerprinting techniques allowed to conclude that ITSs of sizes 346 and 466 were those most likely transferred from mothers to offspring [118]. Here, our results suggest that these ITSs correspond with the genera *Peptoniphilus* and *Helcococcus*. Further, two ITSs identified here as *Peptoniphilus* (sizes 346 and 566) were negatively related to the abundance of enterococci within the gland [118], and enterococci are responsible for the production of potent bacteriocins within hoopoe glands with beneficial effects for the host [18, 93, 118]. Bacteriocins of enterococci are key for their ability to colonize symbiotic communities [159], and direct competence among bacterial strains via antimicrobials has a central role in the configuration of stable microbiomes [160–163]. Therefore, vertically transmitted *Peptoniphilus* and *Helcococcus* may be host-controlled symbionts conditioning [164] the establishment of particular enterococci and/or other symbionts with high competitive ability within the glands of hoopoes, either directly or indirectly. The hoopoe uropygial gland may thus be an organ specialized in providing a space facilitating competence among antimicrobial producers, with safety for the host (outside the digestive tract), to get the best producers of these antimicrobial metabolites for use on body and egg surfaces (Fig. 1c).

The variety of types of organization of uropygial glands in birds is huge, including the location of cavities storing the secretion (lobes or papilla), connection between lobes and papilla, and types of lining of the ducts and cavities (with or without layers of corneous laminae) [65, 143, 154]. Evidence suggesting presence of bacterial communities in the uropygial gland of other bird species is accumulating, and similar host-control mechanisms mediated by gland structure and function, as those suggested for hoopoes, may be widespread in the avian phylogeny.

Conclusions

In conclusion, the hoopoe uropygial gland is an organ specialized in culturing symbiotic bacteria, at least by compartmentalizing and nurturing a specific microbial assemblage in the papilla, to obtain its special bacteria-processed secretion that the bird uses in several ecological contexts. The patterns described here, supporting that the uropygial gland of European hoopoes is a symbiotic organ, might also appear in other exocrine glands of terrestrial vertebrates known to harbor bacteria (e.g., odor glands of mammals [24, 165–169]). Future studies should consider them as a type of *symbiotic organ* in animals. The uropygial gland of the hoopoe is a key model for the study of symbiotic organs selecting for defensive bacteria.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42523-026-00543-y>.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

We thank Natalia Juárez García Pelayo and Anna Sandor for the help in field work with hoopes; Olga Corona Forero and Sonia M. Rodríguez-Ruano for the help in laboratory work; Manuel Soler for help in the establishment and Laura Arco for caring of the captive population; Ana Santos, Ana Belén García Martín and Juan de Dios Bueno for help in gland-processing for microscopical study, Javier Martín-Vivaldi for help in image processing. Gustavo Tomás and three anonymous referees read a previous version of the MS providing comments that improved it in several aspects. The research group also benefits from facilities, including accommodation, provided by the City Hall of Guadix, where a small lab to quickly process the samples was installed.

Author contributions

M. M-V., J. J. S., M. M-B. and E. V. conceived the idea and designed the study of the Spanish population. M. S. and R. A. designed the study of the Swiss population. A. M-G., M. M-V., J. J. S., J. M. P-S. and M. R-R. carried out fieldwork in Spain and A. M-G., helped by M. S. and R. A., in Switzerland. A. M-G., E. L-H, M. M-B. and A. M. M-P. performed the lab work for study of bacterial communities. M. M-V., J. M. P-S., M. R-R. and E. V. were responsible for FISH procedures and performed the microscopical study of glands. M. M-V. and A. M-G. proceeded with bioinformatics with help from M. M-B., A. M. M-P, E. M-R. and M. D. B. Finally, M. M-V. performed statistical analyses and wrote the first draft with great help from J. J. S., E. M-R. and M. D. B. All authors read and approved the final version of the manuscript.

Funding

This work was supported by Spanish Ministerio de Economía y Competitividad, European funds (FEDER) (CGL2013-48193-C3-1-P, CGL2013-48193-C3-2-P), by Ministerio de Ciencia e Innovación /Agencia Estatal de Investigación/<https://doi.org/10.13039/501100011033> with the project's numbers PID2020-117429GB-C21, PID2020-117429GB-C22, PID2024-159017NB-C31 and PID2024-159017NB-C32. A.M-G. had a predoctoral grant from the Junta de Andalucía (P09-RNM-4557).

Data availability

The datasets generated and/or analysed during the current study are available in the [NCBI] repository, [PRJNA1364298 [ID 1364298 - BioProject - NCBI]](<https://www.ncbi.nlm.nih.gov/bioproject/1364298>) and [Digibug] repository [<http://hdl.handle.net/10481/108046>].

Declarations

Ethical approval

This research complies with all applicable guidelines for the care of animals. Necessary permits were obtained from the relevant authorities in each country: Spain (approved by Consejería de Medio Ambiente de la Junta de Andalucía, reference SGYB/FOA/AFR), Switzerland (research permit from the Swiss Confederation and Valais-Vaud animal welfare authorities, reference VS 02/14).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Departamento de Zoología, Universidad de Granada, Granada 18071, Spain

²IES Padre Manjón, Granada 18003, Spain

³Departamento de Zoología, Universidad de Sevilla, Sevilla 41012, Spain

⁴Swiss Ornithological Institute, Sempach 6204, Switzerland

⁵Division of Conservation Biology, Institute of Ecology and Evolution, University of Bern, Bern 3012, Switzerland

⁶Departamento de Microbiología, Universidad de Granada, Granada 18071, Spain

⁷Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

⁸Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (CSIC), Almería 04120, Spain

⁹Coevolución: Cucos, Hospedadores y Bacterias Simbiontes, Unidad Asociada (CSIC), Universidad de Granada, Granada 18071, Spain

Received: 5 December 2025 / Accepted: 2 March 2026

Published online: 30 April 2026

References

- McFall-Ngai M. Symbiosis takes a front and center role in biology. *PLoS Biol.* 2024;22(4):e3002571.
- Mallott EK, Amato KR. Host specificity of the gut microbiome. *Nat Rev Microbiol.* 2021;19(10):639–53.
- Rudman SM, Greenblum S, Hughes RC, Rajpurohit S, Kiratli O, Lowder DB, Lemmon SG, Petrov DA, Chaston JM, Schmidt P. Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proc Natl Acad Sci.* 2019;116(40):20025–32.
- Govender P, Ghai M. Population-specific differences in the human microbiome: Factors defining the diversity. *Gene.* 2025;933:148923.
- Sato Y, Yamagishi J, Yamashita R, Shinozaki N, Ye B, Yamada T, Yamamoto M, Nagasaki M, Tsuboi A. Inter-individual differences in the oral bacteriome are greater than intra-day fluctuations in individuals. *PLoS ONE.* 2015;10(6):e0131607.
- Cafaro MJ, Poulsen M, Little AEF, Price SL, Gerardo NM, Wong B, Stuart AE, Larget B, Abbot P, Currie CR. Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. *Proc Royal Soc B-Biological Sci.* 2011;278(1713):1814–22.
- Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, Brady A, Creasy HH, McCracken C, Giglio MG, McDonald D, Franzosa EA, Knight R, White O, Huttenhower C. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature.* 2017;550(7674):61–6.
- Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science.* 2015;350(6261):663–6.
- Barreto HC, Gordo I. Intra-host evolution of the gut microbiota. *Nat Rev Microbiol.* 2023;21(9):590–603.
- Dapa T, Wong DP, Vasquez KS, Xavier KB, Huang KC, Good BH. Within-host evolution of the gut microbiome. *Curr Opin Microbiol.* 2023;71:102258.
- McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Neelson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ. Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci USA.* 2013;110(9):3229.
- Douglas AE, Minto LB, Wilkinson TL. Quantifying nutrient production by the microbial symbionts in an aphid. *J Exp Biol.* 2001;204:349–58.
- Douglas AE. Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. *Ann Rev Entomol.* 1998;43:17–37.
- McLaren MR, Callahan BJ. Pathogen resistance may be the principal evolutionary advantage provided by the microbiome. *Philos Trans R Soc Lond B Biol Sci.* 2020;375(1808):20190592.
- Currie CR. Ants, agriculture, and antibiotics. A quadripartite symbiosis. In: Seckbach J. (Ed.) *Symbiosis: Mechanisms and model systems.* 2002, Kluwer academic: Dordrecht. 687–99.
- Kroiss J, Kaltenpoth M, Schneider B, Schwinger MG, Hertweck C, Maddula RK, Strohm E, Svatos A. Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. *Nat Chem Biol.* 2010;6(4):261–3.
- Shao Y, Chen B, Sun C, Ishida K, Hertweck C, Boland W. Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. *Cell Chem Biology.* 2017;24(1):66–75.
- Martín-Platero AM, Valdivia E, Ruiz-Rodríguez M, Soler JJ, Martín-Vivaldi M, Maqueda M, Martínez-Bueno M. Characterization of antimicrobial substances

- produced by *Enterococcus faecalis* MRR 10–3, isolated from the uropygial gland of the hoopoe *Upupa epops*. *Appl Environ Microbiol.* 2006;72:4245–9.
19. Brunetti AE, Lyra ML, Bauermeister A, Bunk B, Boedeker C, Mücken M, Neto FC, Mendonça JN, Caraballo-Rodríguez AM, Melo WGP, Pupo MT, Haddad CFB, Cabrera GM, Overmann J, Lopes NP. Host macrocyclic acylcarnitines mediate symbiotic interactions between frogs and their skin microbiome. *iScience.* 2023;26(11).
 20. Flórez LV, Biedermann PHW, Engl T, Kaltenpoth M. Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. *Nat Prod Rep.* 2015;32(7):904–36.
 21. Flórez LV, Scherlach K, Gaube P, Ross C, Sitte E, Hermes C, Rodrigues A, Hertweck C, Kaltenpoth M. Antibiotic-producing symbionts dynamically transition between plant pathogenicity and insect-defensive mutualism. *Nat Commun.* 2017;8(1):15172.
 22. Wang Z, Wu Y, Li X, Ji X, Liu W. The gut microbiota facilitate their host tolerance to extreme temperatures. *BMC Microbiol.* 2024;24(1):131.
 23. O'Donnell MP, Fox BW, Chao PH, Schroeder FC, Sengupta P. A neurotransmitter produced by gut bacteria modulates host sensory behaviour. *Nature.* 2020;583(7816):415–20.
 24. Theis KR, Venkataraman A, Dycus JA, Koonter KD, Schmitt-Matzen EN, Wagner AP, Holekamp KE, Schmidt TM. Symbiotic bacteria appear to mediate hyena social odors. *Proc Natl Acad Sci.* 2013;110(49):19832.
 25. Zhu L, Editorial. Animal social behaviour and gut microbiome. *Front Microbiol.* 2023;14:1210717.
 26. Dinan K, Dinan T. Antibiotics and mental health: The good, the bad and the ugly. *J Intern Med.* 2022;292(6):858–69.
 27. Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. Microbiota and the social brain. *Science.* 2019;366(6465).
 28. Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, Ludington WB. Microbiome interactions shape host fitness. *Proc Natl Acad Sci USA.* 2018;115(51):E11951–60.
 29. Matthews MK, Wilcox H, Hughes R, Veloz M, Hammer A, Banks B, Walters A, Schneider KJ, Sexton CE, Chaston JM. Genetic influences of the microbiota on the life span of *Drosophila melanogaster*. *Appl Environ Microbiol.* 2020;86(10).
 30. Wilde J, Slack E, Foster KR. Host control of the microbiome: Mechanisms, evolution, and disease. *Science.* 2024;385(6706):eadi3338.
 31. Sharp C, Foster KR. Host control and the evolution of cooperation in host microbiomes. *Nat Commun.* 2022;13(1):3567.
 32. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum. The evolution of the host microbiome as an ecosystem on a leash. *Nature.* 2017;548(7665):43–51.
 33. Douglas AE, Werren JH. Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio.* 2016;7(2):e02099–15.
 34. Douglas AE. Host benefit and the evolution of specialization in symbiosis. *Heredity.* 1998;81:599–603.
 35. Yamamura N. Vertical transmission and evolution of mutualism from parasitism. *Theor Popul Biol.* 1993;44(1):95–109.
 36. Fisher RM, Henry LM, Cornwallis CK, Kiers ET, West SA. The evolution of host-symbiont dependence. *Nat Commun.* 2017;8(1):15973.
 37. Leftwich PT, Edgington MP, Chapman T. Transmission efficiency drives host-microbe associations. *Proc Royal Soc B: Biol Sci.* 2020;287(1934):20200820.
 38. Pais IS, Valente RS, Sporniak M, Teixeira L. *Drosophila melanogaster* establishes a species-specific mutualistic interaction with stable gut-colonizing bacteria. *PLoS Biol.* 2018;16(7):e2005710.
 39. Nyholm SV, McFall-Ngai MJ. The winnowing: Establishing the squid-Vibrio symbiosis. *Nat Rev Microbiol.* 2004;2(8):632–42.
 40. Nyholm SV, Stabb EV, Ruby EG, McFall-Ngai MJ. Establishment of an animal-bacterial association: Recruiting symbiotic vibrios from the environment. *Proc Natl Acad Sci USA.* 2000;97(18):10231–5.
 41. Foster KR, Wenseleers T. A general model for the evolution of mutualisms. *J Evol Biol.* 2006;19(4):1283–93.
 42. Maritan E, Quagliariello A, Frago E, Patarnello T, Martino ME. The role of animal hosts in shaping gut microbiome variation. *Philos Trans R Soc Lond B Biol Sci.* 2024;379(1901):20230071.
 43. Nishino T, Moriyama M, Mukai H, Tanahashi M, Hosokawa T, Chang H-Y, Tachikawa S, Nikoh N, Koga R, Kuo C-H, Fukatsu T. Defensive fungal symbiosis on insect hindlegs. *Science.* 2025;390(6770):279–83.
 44. Schluter J, Foster KR. The evolution of mutualism in gut microbiota via host epithelial selection. *PLoS Biol.* 2012;10(11):e1001424.
 45. Chomiccki G, Werner GDA, West SA, Kiers ET. Compartmentalization drives the evolution of symbiotic cooperation. *Philos Trans R Soc Lond B Biol Sci.* 2020;375(1808):20190602.
 46. Douglas AE. Mycetocyte symbiosis in insects. *Biol Rev.* 1989;64(4):409–34.
 47. Moran NA, Telang A. Bacteriocyte-associated symbionts of insects. *Bioscience.* 1998;48(4):295–304.
 48. Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci USA.* 2010;107(2):769–74.
 49. Montoya AP, Wendlandt CE, Benedict AB, Roberts M, Piovio-Scott J, Griffiths JS, Porter SS. Hosts winnow symbionts with multiple layers of absolute and conditional discrimination mechanisms. *Proc Biol Sci.* 2023;290(1990):20222153.
 50. Salem H, Florez L, Gerardo N, Kaltenpoth M. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proc Roy Soc B.* 2015;282(1804).
 51. Salem H, Bauer E, Kirsch R, Berasategui A, Cripps M, Weiss B, Koga R, Fukumori K, Vogel H, Fukatsu T, Kaltenpoth M. Drastic genome reduction in an herbivore's pectinolytic symbiont. *Cell.* 2017;171(7):1520–e15311513.
 52. Itoh H, Jiang S, Takeshita K, Ohbayashi T, Ohnishi N, Meng X-Y, Mitani Y, Kikuchi Y. Host-symbiont specificity determined by microbe-microbe competition in an insect gut. *Proc Natl Acad Sci USA.* 2019. 116(45): 22673–22682.
 53. Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J. Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science.* 2006;311:81–3.
 54. Li H, Sosa-Calvo J, Horn HA, Pupo MT, Clardy J, Rabeling C, Schultz TR, Currie CR. Convergent evolution of complex structures for ant-bacterial defensive symbiosis in fungus-farming ants. *Proc Natl Acad Sci USA.* 2018;115(42):10720–5.
 55. Dodge R, Jones EW, Zhu H, Obadia B, Martinez DJ, Wang C, Aranda-Díaz A, Aumiller K, Liu Z, Voltolini M, Brodie EL, Huang KC, Carlson JM, Sivak DA, Spradling AC, Ludington WB. A symbiotic physical niche in *Drosophila melanogaster* regulates stable association of a multi-species gut microbiota. *Nat Commun.* 2023;14(1):1557.
 56. Ludington WB. The importance of host physical niches for the stability of gut microbiome composition. *Phil Trans Royal Soc B: Biol Sci.* 2024;379(1901):20230066.
 57. Fronk DC, Sachs JL. Symbiotic organs: the nexus of host-microbe evolution. *Trends Ecol Evol.* 2022;37(7):599–610.
 58. Khan S, Fitch S, Knox S, Arora R. Exocrine gland structure-function relationships. *Development.* 2022;149(1):dev197657.
 59. Davis MP, Sparks JS, Smith WL. Repeated and widespread evolution of bioluminescence in marine fishes. *PLoS ONE.* 2016;11(6):e0155154.
 60. Montgomery MK, McFall-Ngai M. Embryonic development of the light organ of the sepiolid squid *Euprymna scolopes* Berry. *Biol Bull.* 1993;184(3):296–308.
 61. Belcaid M, Casaburi G, McNulty SJ, Schmidbaur H, Suria AM, Moriano-Gutierrez S, Pankey MS, Oakley TH, Kremer N, Koch EJ, Collins AJ, Nguyen H, Lek S, Goncharenko-Foster I, Minx P, Sodergren E, Weinstock G, Rokhsar DS, McFall-Ngai M, Simakov O, Foster JS, Nyholm SV. Symbiotic organs shaped by distinct modes of genome evolution in cephalopods. *Proc Natl Acad Sci.* 2019;116(8): 3030–3035.
 62. Collins AJ, LaBarre BA, Won BSW, Shah MV, Heng S, Choudhury MH, Haydar SA, Santiago J, Nyholm SV. Diversity and partitioning of bacterial populations within the accessory nidamental gland of the squid *Euprymna scolopes*. *Appl Environ Microbiol.* 2012;78(12):4200–8.
 63. Kerwin AH, McNulty SJ, Nyholm SV. Development of the accessory nidamental gland and associated bacterial community in the Hawaiian bobtail squid, *Euprymna scolopes*. *Biol Bull.* 2021;240(3):205–18.
 64. Mazorra-Alonso M, Tomás G, Soler JJ. Microbially mediated chemical ecology of animals: A review of its role in conspecific communication, parasitism and predation. *Biology (Basel).* 2021;10(4):274.
 65. Jacob J, Ziswiler V. The uropygial gland. *Avian Biology.* London: Academic; 1982. pp. 199–324.
 66. Møller AP, Laursen K. Function of the uropygial gland in eiders (*Somateria mollissima*). *Avian Res.* 2019;10(1):24.
 67. Praveenkumar D, Vinothkumar A, Saravanan G, Selvakumar M, Vijayakumar AS, Kolanchinathan P, Kamalakkannan S, Achiraman S. Symbiotic microbes play a role more important than preen gland in avian pheromone production—A review. *Avian Biol Res.* 2023;16(1):32–41.
 68. Soler JJ, Martín-Vivaldi M, Peralta-Sánchez JM, Ruiz-Rodríguez M. Antibiotic-producing bacteria as a possible defence of birds against pathogenic microorganisms. *Open Ornithol J.* 2010;3:93–100.
 69. Whittaker DJ, Slowinski SP, Greenberg JM, Alian O, Winters AD, Ahmad MM, Burrell MJE, Soini HA, Novotny MV, Ketterson ED, Theis KR. Experimental evidence that symbiotic bacteria produce chemical cues in a songbird. *J Exp Biol.* 2019;222(20):jeb202978.

70. Seibel E, Um S, Bodawatta KH, Komor AJ, Decker T, Fricke J, Murphy R, Maiah G, Iova B, Maus H, Schirmeister T, Jönsson KA, Poulsen M, Beemelmanns C. Bacteria from the *Amycolatopsis* genus associated with a toxic bird secrete protective secondary metabolites. *Nat Commun*. 2024;15(1):8524.
71. Bodawatta KH, Schierbech SK, Petersen NR, Sam K, Bos N, Jönsson KA, Poulsen M. Great tit (*Parus major*) uropygial gland microbiomes and their potential defensive roles. *Front Microbiol*. 2020;11:1735.
72. Law-Brown J, Meyers PR. *Enterococcus phoeniculicola* sp nov., a novel member of the enterococci isolated from the uropygial gland of the red-billed wood-hoopoe, *Phoeniculus purpureus*. *Int J Syst Evol Microbiol*. 2003;53:683–5.
73. Soler JJ, Martín-Vivaldi M, Ruiz-Rodríguez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M, Peralta-Sánchez JM, Méndez M. Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropygial gland. *Funct Ecol*. 2008;22(5):864–71.
74. Braun MS, Wang E, Zimmermann S, Wagner H, Wink M. *Kocuria tytonis* sp. nov., isolated from the uropygial gland of an American barn owl (*Tyto furcata*). *Int J Syst Evol Microbiol*. 2019;69(2):447–51.
75. Braun M, Wang E, Zimmermann S, Wink M. *Corynebacterium heidelbergense* sp. nov., isolated from the preen glands of Egyptian geese (*Alopochen aegyptiacus*). *Syst Appl Microbiol*. 2018;41:564–9.
76. Braun MS, Wang E, Zimmermann S, Boutin S, Wink M. *Kocuria uropygioeca* sp. nov. and *Kocuria uropygialis* sp. nov., isolated from the preen glands of great spotted woodpeckers (*Dendrocopos major*). *Syst Appl Microbiol*. 2018;41(1):38–43.
77. Braun MS, Zimmermann S, Danner M, Rashid Ho, Wink M. *Corynebacterium uropygiale* sp. nov., isolated from the preen gland of turkeys (*Meleagris gallopavo*). *Syst Appl Microbiol*. 2016;39(2):88–92.
78. Barón MD, Stanback M, Martínez-Renau E, José Soler J, Martín-Vivaldi M. Characterizing bacterial communities of wild birds: insights from three southern African hornbill species. *J Avian Biol*. 2025(3): e03347.
79. Martínez-Renau E. Pathogen risks of nesting wild birds drives their skin and uropygial microbiome. In: *Microbiota asociada a la glándula uropigial de aves: diversidad y funciones en escenarios de parasitismo y comunicación social*. Granada: Universidad de Granada. 2024. <https://hdl.handle.net/10481/89880>
80. Law-Brown, J. Chemical defence in the red-billed woodhoopoe, *Phoeniculus purpureus*. 2001. Cape Town, University of Cape Town.
81. Burger BV, Reiter B, Borzyk O, Plessis D. Avian exocrine secretions. I. Chemical characterization of the volatile fraction of the uropygial secretion of the green woodhoopoe, *Phoeniculus purpureus*. *J Chem Ecol*. 2004;30(8):1603–11.
82. Martín-Vivaldi M, Peña A, Peralta-Sánchez JM, Sánchez L, Ananou S, Ruiz-Rodríguez M, Soler JJ. Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proc Biol Sci*. 2010;277(1678):123–30.
83. Rodríguez-Ruano SM, Martín-Vivaldi M, Peralta-Sánchez JM, García-Martín AB, Martínez-García A, Soler JJ, Valdivia E, Martínez-Bueno M. Seasonal and sexual differences in the microbiota of the hoopoe uropygial secretion. *Genes*. 2018;9(8):407.
84. Martín-Vivaldi M, Ruiz-Rodríguez M, Soler JJ, Peralta-Sánchez JM, Méndez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M. Seasonal, sexual and developmental differences in hoopoe preen gland morphology and secretions. Evidence for a role of bacteria. *J Avian Biol*. 2009;40:191–205.
85. Martín-Vivaldi M, Soler JJ, Peralta-Sánchez JM, Arco L, Martín-Platero AM, Martínez-Bueno M, Ruiz-Rodríguez M, Valdivia E. Special structures of hoopoe eggshells enhance the adhesion of symbiont-carrying uropygial secretion that increase hatching success. *J Anim Ecol*. 2014;83(6):1289–301.
86. Soler JJ, Martín-Vivaldi M, Peralta-Sánchez JM, Arco L, Juárez-García-Pelayo, N. Hoopoes color their eggs with antimicrobial uropygial secretions. *Naturwissenschaften*. 2014;101(9):697–705.
87. Soler JJ, Martínez-García A, Rodríguez-Ruano S, Martínez-Bueno M, Martín-Platero AM, Peralta-Sánchez JM, Martín-Vivaldi M. Nestedness of hoopoes' bacterial communities: symbionts from the uropygial gland to the eggshell. *Biol J Linn Soc*. 2016;118:763–73.
88. Ruiz-Rodríguez M, Martínez-Bueno M, Martín-Vivaldi M, Valdivia E, Soler JJ. Bacteriocins with a broader antimicrobial spectrum prevail in enterococcal symbionts isolated from the hoopoe's uropygial gland. *FEMS Microbiol Ecol*. 2013;85(3):495–502.
89. Ruiz-Rodríguez M, Valdivia E, Martín-Vivaldi M, Martín-Platero AM, Martínez-Bueno M, Méndez M, Peralta-Sánchez JM, Soler JJ. Antimicrobial activity and genetic profile of enterococci isolated from hoopoes uropygial gland. *PLoS ONE*. 2012;7(e41843):41841–6.
90. Mayr G, Manegold A, Johansson US. Monophyletic groups within 'higher land birds'- comparison of morphological and molecular data. *J Zoological Syst Evolutionary Res*. 2003;41(4):233–48.
91. Mayr G. New specimens of the Eocene *Messelirrisoridae* (Aves: *Bucerotes*), with comments on the preservation of uropygial gland waxes in fossil birds from Messel and the phylogenetic affinities of *Bucerotes*. *Palaeontologische Zeitschrift*. 2006;80(4):390–405.
92. Feduccia A. The bony stapes in the *Upupidae* and *Phoeniculidae*: evidence for common ancestry. *Wilson Bull*. 1975;87(3):416–7.
93. Soler JJ, Barón MD, Martínez-Renau E, Zhang L, Liang W, Martín-Vivaldi M. Nesting hoopoes cultivate in their uropygial gland the microbial symbionts with the highest antimicrobial capacity. *Sci Rep*. 2024;14(1):30797.
94. Rodríguez-Ruano S, Martín-Vivaldi M, Martín-Platero AM, López-López JP, Peralta-Sánchez JM, Ruiz-Rodríguez M, Soler JJ, Valdivia E, Martínez-Bueno M. The hoopoe's uropygial gland hosts a bacterial community influenced by the living conditions of the bird. *PLoS ONE*. 2015;10(10):e0139734.
95. Martínez-García A, Soler JJ, Rodríguez-Ruano SM, Martínez-Bueno M, Martín-Platero AM, Juárez-García-Pelayo, N., and Martín-Vivaldi, M. Preening as a vehicle for key bacteria in hoopoes. *Microb Ecol*. 2015;70(4):1024–33.
96. van Wijk RE, Schaub M, Hahn S, Juárez-García-Pelayo N, Schäfer B, Viktora L, Martín-Vivaldi M, Zischewski M, Bauer S. Diverse migration strategies in hoopoes (*Upupa epops*) lead to weak spatial but strong temporal connectivity. *Naturwissenschaften*. 2018;105(7–8):42.
97. Reichlin TS, Hobson KA, Van Wilgenburg SL, Schaub M, Wassenaar LI, Martín-Vivaldi M, Arlettaz R, Jenni L. Conservation through connectivity: can isotopic gradients in Africa reveal winter quarters of a migratory bird? *Oecologia*. 2013;171(2):591–600.
98. Martínez-García A, Martín-Vivaldi M, Rodríguez-Ruano S, Peralta-Sánchez JM, Valdivia E, Soler, Juan J. Nest bacterial environment affects microbiome of hoopoe eggshells, but not that of the uropygial secretion. *PLoS ONE*. 2016;11(7):e0158158.
99. Rehsteiner U. Abundance and habitat requirements of the hoopoe *Upupa epops* in Extremadura (Spain). *Der Ornithologische Beobachter*. 1996;93:277–87.
100. Barro L, Couzi L, Bretagnolle V, Nezan J, Vetillard F. Multi-scale habitat selection and foraging ecology of the Eurasian hoopoe (*Upupa epops*) in pine plantations. *Biodivers Conserv*. 2008;17(5):1073–87.
101. Schaub M, Martinez N, Tagmann-Ioset A, Weisshaupt N, Maurer ML, Reichlin TS, Abadi F, Zbinden N, Jenni L, Arlettaz R. Patches of bare ground as a staple commodity for declining ground-foraging insectivorous farmland birds. *PLoS ONE*. 2010;5(10):e13115–13111.
102. Martín-Vivaldi M, Palomino JJ, Soler M, Soler JJ. Determinants of reproductive success in the hoopoe (*Upupa epops*), a hole-nesting non-passerine bird with asynchronous hatching. *Bird Study*. 1999;46:205–16.
103. Hoffmann J, Postma E, Schaub M. Factors influencing double brooding in Eurasian hoopoes *Upupa epops*. *Ibis*. 2015;157(1):17–30.
104. Bussmann J. Zur Brutbiologie des Wiedehopfes. *Ornithol Beob*. 1950;47:141–51.
105. Gupta RC, Ahmad I. On the clutch size, egg laying schedule, hatching patterns and stay of nestlings of Indian hoopoe. *Geobios*. 1993;20:148–50.
106. Cramp S. The birds of the western palearctic. Oxford: Oxford University Press; 1985.
107. Ferrer-Pereira P, Martínez-Renau E, Martín-Vivaldi M, Soler JJ. Food supply and provisioning behavior of parents: Are small hoopoe nestlings condemned to die? *Behav Ecol*. 2023;34(6):992–1001.
108. Barón MD, Martín-Vivaldi M, Martínez-Renau E, Soler JJ. Extra nestlings that are condemned to die increase reproductive success in hoopoes. *Am Nat*. 2024;203(4):503–12.
109. Soler JJ, Martín-Vivaldi M, Nuhlicková S, Ruiz-Castellano C, Mazorra-Alonso M, Martínez-Renau E, Eckenfellner M, Svetlik J, Hoi H. Avian sibling cannibalism: Hoopoe mothers regularly use their last hatched nestlings to feed older siblings. *Zoological Res*. 2022;43(2):265–74.
110. Arco L, Martín-Vivaldi M, Peralta-Sánchez JM, García-Pelayo NJ, Soler M. Provisioning challenge: self-consumption versus nestling provisioning, an experimental study. *Anim Behav*. 2022;190:153–65.
111. Arlettaz R, Schaub M, Fournier J, Reichlin TS, Sierro A, Watson JEM, Braunisch V. From publications to public actions: When conservation biologists bridge the gap between research and implementation. *Bioscience*. 2010;60:835–42.
112. Bächler E, Hahn S, Schaub M, Arlettaz R, Jenni L, Fox JW, Afanasyev V, Liechti F. Year-round tracking of small trans-saharan migrants using light-level geolocators. *Plos One*. 2010. 5(3): e9566.

113. Fournier J, Arlettaz R. Food provision to nestlings in the hoopoe *Upupa epops*: implications for the conservation of a small endangered population in the Swiss Alps. *Ibis*. 2001;143(1):2–10.
114. Peralta-Sánchez JM, Martín-Vivaldi M, Martín-Platero AM, Martínez-Bueno M, Oñate M, Ruiz-Rodríguez M, Soler JJ. Avian life history traits influence eggshell bacterial loads: a comparative analysis. *Ibis*. 2012;154(4):725–37.
115. Martín-Platero AM, Peralta-Sánchez JM, Soler JJ, Martínez-Bueno M. Chelex-based DNA isolation procedure for the identification of microbial communities of eggshell surfaces. *Anal Biochem*. 2010;397(2):253–5.
116. Comeau AM, Li WK, Tremblay J-E, Carmack EC, Lovejoy C. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE*. 2011;6(11):e27492.
117. Fisher MM, Triplett EW. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol*. 1999;65(10):4630–6.
118. Martín-Vivaldi M, Soler JJ, Martínez-García A, Arco L, Juárez-García-Pelayo N, Ruiz-Rodríguez M, Martínez-Bueno M. Acquisition of uropygial gland microbiome by hoopoe nestlings. *Microb Ecol*. 2018;76(1):285–97.
119. Martínez-García A, Martín-Vivaldi M, Ruiz-Rodríguez M, Martínez-Bueno M, Arco L, Rodríguez-Ruano S, Peralta-Sánchez JM, Soler JJ. The microbiome of the uropygial secretion in hoopoes is shaped along the nesting phase. *Microb Ecol*. 2016: 1–10.
120. Mazorra-Alonso M, Peralta-Sánchez JM, Martín-Vivaldi M, Martínez-Bueno M, Gómez RN, Soler JJ. Volatiles of symbiotic bacterial origin explain ectoparasitism and fledging success of hoopoes. *Anim Microbiome*. 2024;6(1):26.
121. Schoch CL, Ciufo S, Domrachev M, Hottot CL, Kannan S, Khovanskaya R, Leipe D, McVeigh R, O'Neill K, Robertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S, Karsch-Mizrachi I. NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database (Oxford)*. 2020;2020.
122. Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol*. 1990;56(6):1919–25.
123. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciorek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Löffelholz E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Priesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ull-Hasan S, van der Hoof JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson AD, Xu ZZ, Zaneveld J, Zhang Y, Zhu Q, Knight R, and Caporaso JG. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*. 2019;37(8): 852–857.
124. Cassol I, Ibañez M, Bustamante JP. Key features and guidelines for the application of microbial alpha diversity metrics. *Sci Rep*. 2025;15(1):622.
125. Lozupone C, Knight R, Unifrac A. A new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*. 2005;71(12):8228–35.
126. Schloss PD. Waste not, want not: revisiting the analysis that called into question the practice of rarefaction. *mSphere*. 2023;0(0):e00355–00323.
127. Schloss PD. Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. *mSphere*. 2024;9(2):e0035423.
128. Anderson, MJ. Permutational multivariate analysis of variance (Permanova). In: Balakrishnan N, Colton T, Everitt B, Piegorsch W, Ruggeri F, Teugels JL, editors. *Wiley StatsRef: Statistics Reference Online*. 2017. p. 1–15.
129. Legendre P, Legendre L. Numerical ecology: developments in environmental modelling. *Developments Environ Modelling*. 1998;20(1):1–853.
130. Burns AR, Stephens WZ, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJM. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J*. 2016;10(3):655–64.
131. Shade A, Stopnisek N. Abundance-occupancy distributions to prioritize plant core microbiome membership. *Curr Opin Microbiol*. 2019;49: 50–8.
132. Neu AT, Allen EE, Roy K. Defining and quantifying the core microbiome: Challenges and prospects. *Proc Natl Acad Sci USA*. 2021;118(51):e2104429118.
133. Ruiz-Rodríguez M, Valdivia E, Soler JJ, Martín-Vivaldi M, Martín-Platero AM, Martínez-Bueno M. Symbiotic bacteria living in the hoopoe's uropygial gland prevent feather degradation. *J Exp Biol*. 2009;212(22):3621–6.
134. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H, Thom SR, Bushman FD, Vinogradov SA, Wu GD. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology*. 2014;147(5):1055–e10631058.
135. Flynn KJ, Ruffin MT, Turgeon IV, D.K., and, Schloss PD. Spatial variation of the native colon microbiota in healthy adults. *Cancer Prev Res*. 2018;11(7):393–402.
136. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2016;14(1):20–32.
137. Lin Q, Lin S, Fan Z, Liu J, Ye D, Guo P. A review of the mechanisms of bacterial colonization of the mammal gut. *Microorganisms*. 2024;12(5):1026.
138. Zhao Q, Maynard CL. Mucus, commensals, and the immune system. *Gut Microbes*. 2022;14(1):2041342.
139. Yao Y, Shang W, Bao L, Peng Z, Wu C. Epithelial-immune cell crosstalk for intestinal barrier homeostasis. *Eur J Immunol*. 2024;54(6):2350631.
140. Detman A, Mielecki D, Pleśniak Ł, Bucha M, Janiga M, Matyasik I, Chojnacka A, Jędrysek M-O, Błaszczak MK, Sikora A. Methane-yielding microbial communities processing lactate-rich substrates: a piece of the anaerobic digestion puzzle. *Biotechnol Biofuels*. 2018;11(1):116.
141. Thie N, Corl A, Turjeman S, Efrat R, Kamath PL, Getz WM, Bowie RCK, Nathan R. Linking migration and microbiota at a major stopover site in a long-distance avian migrant. *Mov Ecol*. 2022;10(1):46.
142. Videvall E, Strandh M, Engelbrecht A, Cloete S, Cornwallis CK. Measuring the gut microbiome in birds: Comparison of faecal and cloacal sampling. *Mol Ecol Resour*. 2018;18(3):424–34.
143. Paris P. Recherches sur la glande uropygienne des oiseaux. *Archives de Zool Expérimentale et Générale*. 1913;53:139–276.
144. Ghidini A. Un organo poco conosciuto nell'Upupa. *Avicula*. 1906;10:80.
145. Menon GK, Maderson PFA, Drewes RC, Baptista LF, Price LF, Elias PM. Ultrastructural organization of avian stratum corneum lipids as the basis for facultative cutaneous waterproofing. *J Morphol*. 1996;227(1):1–13.
146. Corvec S, Dagnelie M-A, Khammari A, Dréno B. Skin barrier and microbiome. In: *Comprehensive gut microbiota*. Glibetic M. Elsevier: Oxford. 2022;435–44.
147. Bergstrom K, Xia L. The barrier and beyond: Roles of intestinal mucus and mucin-type O-glycosylation in resistance and tolerance defense strategies guiding host-microbe symbiosis. *Gut Microbes*. 2022;14(1):2052699.
148. Wu CM, Wheeler KM, Cárcamo-Oyarce G, Aoki K, McShane A, Datta SS, Welch M, Tiemeyer JL, Griffen M, A.L., and, Ribbeck K. Mucin glycans drive oral microbial community composition and function. *NPJ Biofilms Microbiomes*. 2023;9(1):11.
149. Coates M, Lee MJ, Norton D, MacLeod AS. The skin and intestinal microbiota and their specific innate immune systems. *Front Immunol*. 2019;10:2950.
150. Menon GK, Menon J. Avian epidermal lipids: Functional considerations and relationship to feathering. *Am Zool*. 2000;40(4):540–52.
151. Menon GK, Dumbacher JP. A 'toxin mantle' as defensive barrier in a tropical bird: evolutionary exploitation of the basic permeability barrier forming organelles. *Exp Dermatol*. 2014;23(4):288–90.
152. Menon GK, Lee SE, Lee S-H. An overview of epidermal lamellar bodies: Novel roles in biological adaptations and secondary barriers. *J Dermatol Sci*. 2018;92(1):10–7.
153. Chen YE, Fischbach MA, Belkaid Y. Skin microbiota–host interactions. *Nature*. 2018;553:427.
154. Lucas AM, Stettenheim PR. Uropygial Gland. *Avian Anatomy. Integument. Agric. Handbook*. U.S. Dep. Agric. 1972;362.
155. Maiti BR, Ghosh A. Probable role of androgen in the regulation of the uropygial gland. *Gen Comp Endocrinol*. 1972;19(3):527–35.
156. Coyte KZ, Rao C, Rakoff-Nahoum S, Foster KR. Ecological rules for the assembly of microbiome communities. *PLoS Biol*. 2021;19(2):e3001116.
157. Díaz-Lora S, Martín-Vivaldi M, Juárez García-Pelayo N, Azcárate García M, Rodríguez-Ruano SM, Martínez-Bueno M, Soler JJ. Experimental old nest material predicts hoopoe *Upupa epops* eggshell and uropygial gland microbiota. *J Avian Biol*. 2019;50(9):jav.02083.
158. Ruiz-Rodríguez M, Soler JJ, Martín-Vivaldi M, Martín-Platero AM, Méndez M, Peralta-Sánchez JM, Ananou S, Valdivia E, Martínez-Bueno M. Environmental factors shape the community of symbionts in the hoopoe uropygial gland more than genetic factors. *Appl Environ Microbiol*. 2014;80(21):6714–23.

159. Kommineni S, Bretl DJ, Lam V, Chakraborty R, Hayward M, Simpson P, Cao Y, Bousounis P, Kristich CJ, Salzman NH. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature*. 2015;526(7575):719–22.
160. Kern L, Abdeen SK, Kolodziejczyk AA, Elinav E. Commensal inter-bacterial interactions shaping the microbiota. *Curr Opin Microbiol*. 2021;63:158–71.
161. Klein TA, Ahmad S, Whitney JC. Contact-dependent interbacterial antagonism mediated by protein secretion machines. *Trends Microbiol*. 2020;28(5):387–400.
162. Peterson SB, Bertolli SK, Mougous JD. The central role of interbacterial antagonism in bacterial life. *Curr Biol*. 2020;30(19):R1203–14.
163. Heilbronner S, Krismer B, Brötz-Oesterhelt H, Peschel A. The microbiome-shaping roles of bacteriocins. *Nat Rev Microbiol*. 2021;19(11):726–39.
164. Tanger IS, Stefánschitz J, Schwert Y, Roth O. The source of microbial transmission influences niche colonization and microbiome development. *Proc Biol Sci*. 2024;291(2016): 20232036.
165. Rojas CA, Marks SL, Borrás E, Lesea H, McCartney MM, Coil DA, Davis CE, Eisen JA. Characterization of the microbiome and volatile compounds in anal gland secretions from domestic cats (*Felis catus*) using metagenomics and metabolomics. *Sci Rep*. 2023;13(1):19382.
166. Buesching CD, Tinnesand HV, Sin Y, Rosell F, Burke T, Macdonald DW. Coding of group odor in the subcaudal gland secretion of the European badger *Meles meles*: chemical composition and pouch microbiota. *Chemical Signals in Vertebrates 13*, ed. B.A. Schulte, T.E. Goodwin, and M.H. Ferkin, Cham: Springer International Publishing. 2016; 45–62.
167. Yamaguchi MS, Ganz HH, Cho AW, Zaw TH, Jospin G, McCartney MM, Davis CE, Eisen JA, Coil DA. Bacteria isolated from Bengal cat (*Felis catus* × *Prionailurus bengalensis*) anal sac secretions produce volatile compounds potentially associated with animal signaling. *PLoS ONE*. 2019;14(9):e0216846.
168. Sun Y-S, Zhao L, Zheng C-L, Yan X-T, Li Y, Gao X-L, Xue T-F, Zhang Y-M, Li Z-P, Heller R, Feng C-G, Xu C, Wang K, Qiu Q. Convergent musk biosynthesis across host and microbiota in musk deer and muskrat. *Zoological Res*. 2025;46(3):505–17.
169. Leclaire S, Jacob S, Greene LK, Dubay GR, Drea CM. Social odours covary with bacterial community in the anal secretions of wild meerkats. *Sci Rep*. 2017;7(1):3240.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.