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Animal Models for Exploring the Microbiome

# Wildlife-microbiome interactions and disease: exploring opportunities for disease mitigation across ecological scales

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Captive wildlife are a unique set of animals, whose diverse host-microbe symbioses are underexplored. Compared to their wild counterparts they are particularly susceptible to a variety of diseases, many of which have explicit or purported links to the microbiome. In this perspective, we will examine how the microbiome influences gastrointestinal disorders, metabolic dysregulation, reproduction, and disease susceptibility in captive wildlife. Investigation of wildlife, and specifically captive wildlife, affords a unique opportunity to gain understanding of the broad diversity of the associated microbiota and learn from nature's molecular and microbial responses to disease. Studies like these could lead to the discovery of new interventions, ranging from dietary changes to the use of microbes or their

natural products as treatment. Intervention strategies can lead to the discovery of medically relevant small molecules and the development of a novel platform for N-of-1 targeted medical investigations.

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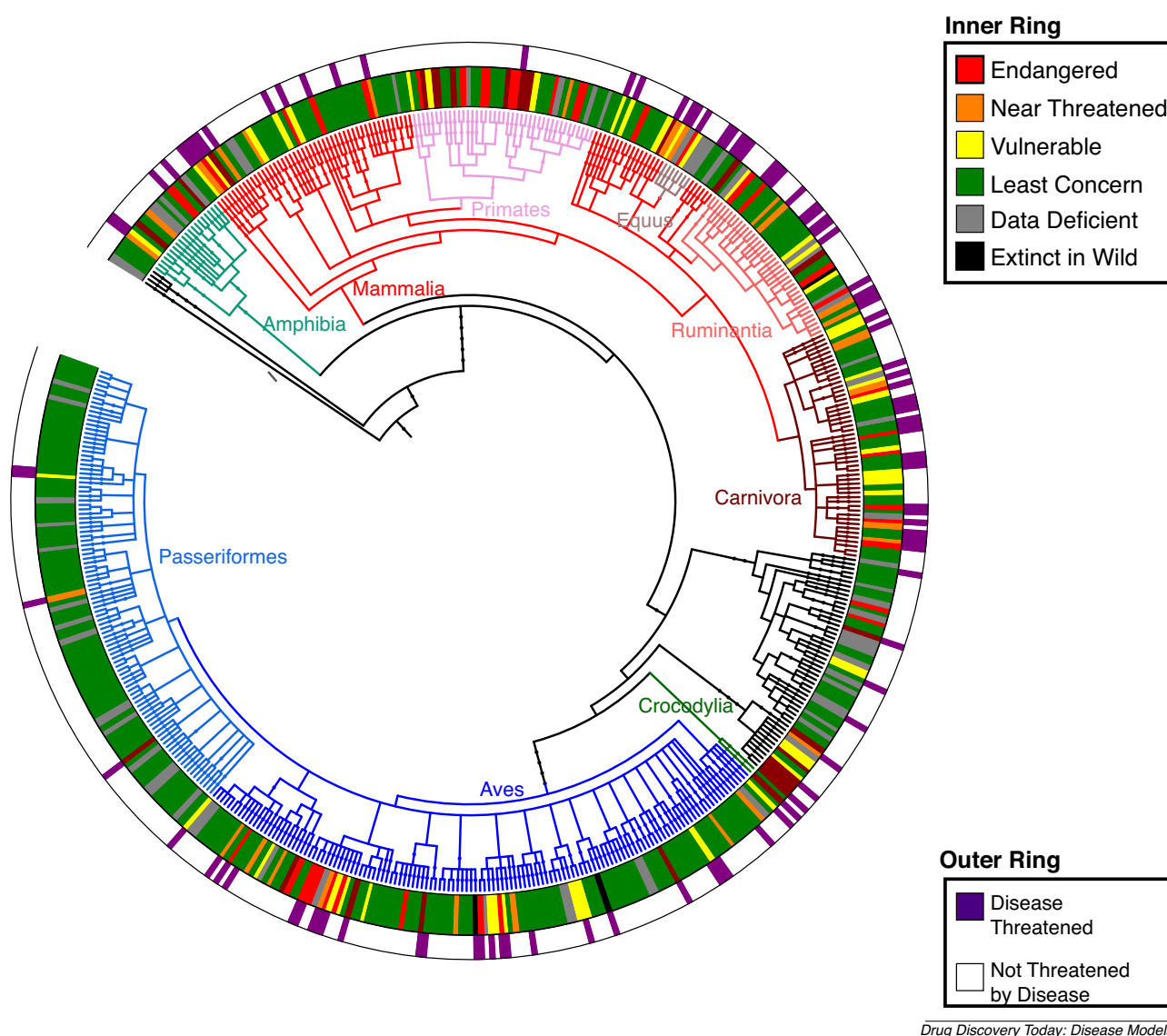
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## Introduction

Microbiomes have now been characterized from a phylogenetically, geographically, and environmentally diverse set of organisms (Fig. 1; [1–3]) including a broad phylogenetic range of non-human vertebrate species (mammals, amphibians, birds, fish, and reptiles) [4,5] and several sample types [6,7]. Most studies have focused on fecal or skin sampling, though some have characterized other regions such as the mouth or along the gastrointestinal tract [3,5,8–10]. Vertebrates have evolved to use their diverse microbial communities to improve their fitness in a variety of strategies [11]. Microbiota are integral to the survival, maturity and normal functioning of their hosts, playing key roles in their development [12–14], immune function [15], and nutrient acquisition [16].

In light of global declines in biodiversity, the loss of each animal species is profound, but greater still is the loss of their unique microbiota. Each microbiota has the functional potential to act as drugs in the context of live bio therapeutic agents or to synthesize novel biomolecules that could be exploited as new small-molecule drugs [17–19]. Yet, to our knowledge, <1% of threatened vertebrate species' microbiomes have been explored (Fig. 1) ([3]; IUCN 2019).

Captive wildlife represent a unique population of animals for investigating the relationship between the microbiome and health and disease. Understanding the host–microbe relationship of these species may be especially relevant to human health since an increase in urban populations has led to a significant number of individuals who have increasingly



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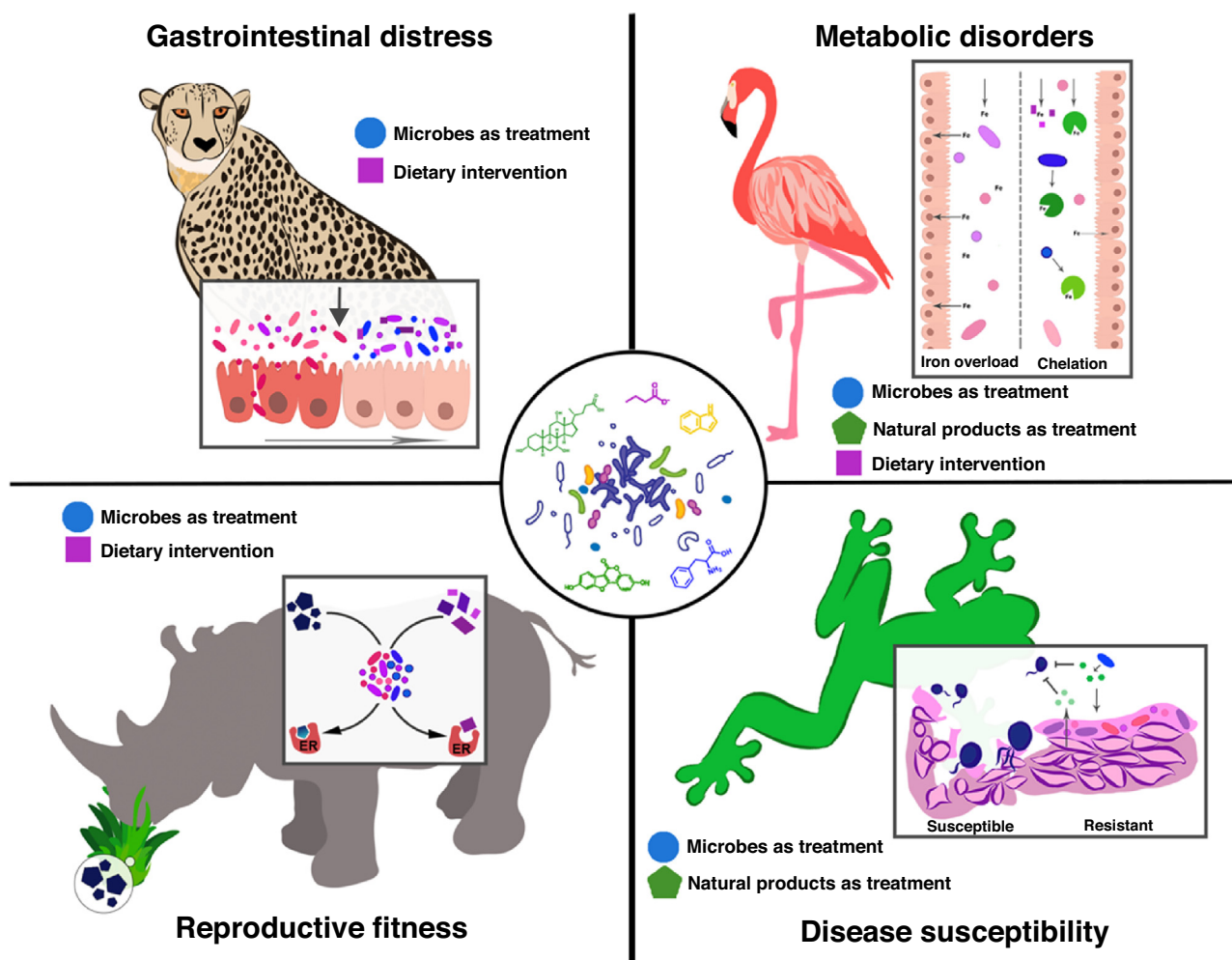
**Fig. 1.** Circular phylogenetic tree of the 527 vertebrate animal species present in the Templeton Project microbiome example dataset [3,29], with major clades color-coded and labeled. This does not reflect sample number — some species have many samples present, some only have one. This is <1% of all IUCN species. The tree was generated using the interactive Tree of Life (iTOL) [141]. The inner ring contains the 2019 IUCN status of the species represented here. The outer ring contains the 2019 IUCN threatened by disease status for the species represented here (19% of taxa).

sequestered themselves in cities and in sealed, air-conditioned buildings. These types of changes in human habitats have with each generation decreased the exposure to a diverse selection of environmental microbes with which we have naturally co-evolved over millions of years [20]. Many studies have focused on the differences between wild and captive microbiomes [3] noting a higher incidence of disease of unknown etiology in captive animals [21]. Captive animals have extensively manipulated diets and live in built environments. Captivity also leads to increased contact with humans and other animals which they would normally not be exposed to in the wild and exposes them to far fewer natural substrates (microbial as well as inert) and members of their own species. Similar to humans in cities, all of these factors can impact the diversity of microbes that they encounter.

Animals interact with their environment extensively via their skin, oral and gut mucosa, and microbial composition of these compartments are influenced by captivity. For example, the

reduced complexity of the environmental microbiome has potential health implications in captive Komodo dragons (*Varanus komodoensis*) [22]. The habitat and environmental stressors encountered in captivity are undoubtedly unique, yet are similar to that of the human built environment. Much like humans colonize their living spaces with their microbes [23], animals such as the Komodo dragon also influence their captive environment, and their microbiome is in turn also shaped by changes in environmental exposures [22], possibly contributing to observed differences in wild and captive microbiota.

A wide array of diseases and disorders affect wildlife, with varying degrees of evidence linking these conditions to the microbiome. However, as recent studies in humans and laboratory animal models have suggested, the microbiome is involved in a far ranging set of illnesses, both local and systemic [24]. Finding that the microbiome plays a similar role in disease across the animal kingdom would therefore not be surprising. This review will highlight known or



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**Fig. 2.** Schematic displaying highlighted examples of known or possible interactions between microbiota and host systems, particularly those associated with gastrointestinal disease, metabolic disorders, reproductive fitness, and disease susceptibility in wildlife, and the possible interventions that can be discovered or applied to the host system.

possible interactions between microbiota and host systems, particularly those associated with gastrointestinal disease, metabolic disorders, reproduction, and disease susceptibility in wildlife (Fig. 2). It will then explore the novel insights that can be gained by studying disease and disease treatment in wildlife, as well as highlight emerging techniques or treatments from human or laboratory work that can be used to benefit the ongoing efforts to combat these ailments.

### Disease and the microbiome in captive species

Captive animals are particularly susceptible to various ailments, ranging from gastrointestinal distress, and metabolic disease, to reduced reproductive fitness, and skin infection. We will highlight the potential role of the microbiome in diseases observed in captive animals and articulate how these studies have improved our understanding of the host-microbe relationship and how these species could be studied as models of disease.

#### Gastrointestinal distress

Maintaining wildlife in captivity presents many challenges. One major challenge is providing diets that mimic those found in the wild. As diet is known to be a major driver of gastrointestinal microbiota [1,25], *ex situ* diets play a significant role in the differences observed between *in situ* and *ex situ* gut microbiota of many captive animals [3,9,26–29]. In most of these studies, captive wildlife had reduced microbial diversity compared to their wild counterparts, most likely driven by the reduction in diversity of diet items. As reduced microbial diversity has been associated with disease states in other species [30], it is probable that these differences may also be a contributing factor for captivity-associated gastrointestinal disease that plagues many species [31,32]; reviewed by Refs. [9,33–35].

Carnivore diets are challenging to replicate in captivity because the natural prey animals are not readily available. Domestic whole prey animals, or, more frequently, homogenized meat-only products are substituted for their diet and strongly influence gastrointestinal health [32]. For example, captive cheetahs suffer from higher rates of veno-occlusive disease and gastrointestinal distress than wild cheetah [36]. One possible cause could be the *ex situ* diet composed largely of meat-only products supplemented with plant fiber (cf. with those fed whole prey items, which contain stomach and intestinal contents, organs, and other components typically consumed by a predator) that lack indigestible animal components such as bone [32]. Compositional differences in diet may lead to higher levels of putrefaction metabolites, influencing gut health [37,38]. A microbial link is likely, as nutrient uptake is impacted and possibly mediated by the gut microbiome. In addition, *Helicobacter* spp., which is linked to gastritis in humans, were found in both wild and captive cheetahs, despite the increased prevalence of gastritis in captive cheetahs [39]. Cheetahs may therefore provide an interesting natural disease

model for analysis of host factors important in the development of *Helicobacter*-induced gastritis.

Giant pandas (*Ailuropoda melanoleuca*) are unique—taxonomically classified as carnivores, but subsist on a completely herbivorous diet, primarily of bamboo [40,41], and also suffer from gastrointestinal disease under captive management [42,43]. Both wild and captive giant pandas have seasonal shifts in bamboo part preference [41,44] with corresponding changes in gut microbiota [45,46]. However, following this transition, only captive pandas experience gastrointestinal distress, resulting in the production of mucous stools [35,47]. Williams et al., proposed that the reduction in fiber during the dietary shift from culm to leaves induces an alteration in gut microbiota (increased abundance of *Escherichia-Shigella* spp., *Clostridium* spp., and Pasteurellaceae), leading to mucosal injury [34]. The excretion of mucus then serves to reset the system, removing mucosal layers and their associated microbiota. Similar findings have been observed in rhesus macaques (*Macaca mulatta*), a species that suffers greatly from idiopathic chronic diarrhea (ICD) [48] linked to altered microbial fucose utilization and mucin degradation in captivity [49]. Since these types of distress only occur in captivity, it is unclear what characteristic of captive diets is driving gastrointestinal distress.

In general, herbivores can be challenging to manage in captive settings due to balancing the proper diet for a healthy gut microbiome, particularly folivores [33]. Specifically, several colobine species appear to have a higher predisposition to gastrointestinal distress in captivity, including the doucs (*Pygathrix* spp.) and red colobus (*Procolobus* spp.). Amato et al. examined the link between diet, gut microbiota, and disease outcome in captive colobus, and found that microbiota were different between gastrointestinally healthy and unhealthy monkeys [9]. One major difference between the two groups was the decreased relative abundance in unhealthy individuals of *Akkermansia* spp., a taxa with strain specific anti-inflammatory function in humans [50]. Clayton et al. found that captive doucs did not maintain a “wild” microbiota in captivity, with keystone species being replaced with increased levels of human-associated *Prevotella* and *Bacteroides* [26]. Both taxa are efficient at fermenting a diverse range of plant and host-derived carbohydrates [51]. For folivorous primates, an imbalance in carbohydrate complexity can lead to gastritis and malnutrition [52]. Additional studies are necessary to determine whether the loss of wild keystone microbial species is driving gastrointestinal distress observed in captivity and may provide the framework to examine how dietary differences may contribute to changes in the microbiome and lower bowel disease across vertebrates.

#### Metabolic disorders

With the loss of *in situ* keystone species and an *ex situ* diet, host-microbe interactions may lead to increased metabolic disorders



in captive wildlife. Metabolic disturbances have been identified in an array of wildlife, such as reduced insulin sensitivity and systemic inflammation [53], hyperglycemia and fatty liver [54], acquired lysosomal storage disease [55], and wasting syndrome in marmosets (*Callithrix jacchus*) [56]. Notably, iron storage disease (ISD) has been observed across many different taxa, including, but not limited to, avian species such as mynah birds, birds of paradise, toucans and flamingos [57], captive Egyptian fruit bats (*Rousettus aegyptiacus*) [58,59], and black rhinoceros (*Diceros bicornis*) [60], with a higher incidence of disease reported in captive species [61].

#### Ties between gut microbiota and iron storage disease

Organisms, from microbes to humans, have evolved complex regulatory systems to ensure adequate uptake of iron, while preventing excessive accumulation, which can lead to toxicity due to its propensity to promote oxidation of cellular components [62,63]. The majority of iron found in the body is contained in hemoglobin in red blood cells and recycled by the liver, while intracellular stores are bound to the labile iron chelator, ferritin [64]. ISD refers to the accumulation of iron, predominantly in the liver, as hemosiderin, a less accessible iron-storage complex found in hepatocytes, or higher circulating iron levels [58,65]. However, ISD is often only detected as a potential factor in illness and disease upon post mortem necropsy, where 75–90% of susceptible species have hemosiderin accumulation [66].

The current understanding of disease associated with excess iron accumulation and the broad susceptibility of this disease in captive species points towards a possible cause unique to captivity, including diet. Hemosiderin is found exclusively in captive black rhinos in comparison with their wild counterparts and increases with time in captivity [67]. The cause of ISD in one of the most commonly reported species, mynah bird (*Gracula religiosa*), has been linked to maintenance of iron uptake from the gastrointestinal tract, despite excess hepatic iron accumulation [68], making it a viable option as a translational model to investigate the role of the microbiome in acquired ISD.

A genetic propensity to accumulate iron or an inability to control intestinal absorption may be a primary cause in some species. An evaluation of ISD in frugivorous species and the common vampire bat (*Desmodus rotundus*), an obligate sanguivore, showed differences in the regulation of iron absorption [59]. These differences were linked to differential expression of the iron regulatory hormone, hepcidin, with a much higher tolerance in the common vampire bat, likely due to the fundamental difference in diet [69–71]. Secondary causes, such as stress, liver disease, systemic inflammatory processes or local inflammation in the gastrointestinal tract [discussed above], could lead to an immune response and dysregulation of iron homeostasis in the liver, or increased host sequestration of iron. Interactions between gut micro-

biota and iron have been well documented in the literature [72,73], as well as a role for dietary transition metals to modulate host–microbe interactions [74]. Further investigation is needed to gain an understanding of how the interplay between gut microbiota and metals impacts organismal health and disease, and how this relationship could be chemically or microbiologically altered to treat disease.

Metabolic diseases also interact, leading to negative host outcomes. Using equids as a model for rhinoceros, a potential link between insulin resistance and iron storage was observed [75]. Insulin resistance has also been linked to low fertility [76], and this may therefore be the case in other wildlife species. As captive breeding programs are paramount for species survival, additional knowledge about primary effects that influence fertility is critical.

#### Microbiome and reproductive success in captive wildlife

Conservation breeding programs are an integral part of the management of *ex situ* wildlife. Of the approximately 9000 threatened vertebrate species, nearly 10% have captive breeding recommendations currently (IUCN 2019). Captive breeding continues to be challenging for many wildlife species, as few vertebrates display consistent reproduction and survivorship [77]. To overcome these issues, assisted reproduction techniques have been developed to enhance captive breeding of wildlife via artificial insemination and *in vitro* fertilization, often paired with exogenous hormone administration [78–81]. While these techniques have been somewhat successful in some species, a better understanding of what stimuli are required to improve natural breeding is needed.

#### Interplay between microbiota and host fertility

Few studies have explored interactions between wildlife microbiota and fertility, and most are descriptive, correlating changes in microbiota and reproductive cycle and fertility [82,83]. Gut microbiota are known to play fundamental roles in regulating host endocrine function, specifically through hormone modification and transformations [84–86]. Similar functions have also been observed with exogenous compounds, like endocrine disrupting chemicals (EDC) [87–89]. EDCs come in many forms, from natural compounds like phytoestrogens to compounds of industrial origins like persistent organic pollutants, pesticides, and plasticizers [90]. In captivity and the growing anthropogenic landscape, interactions between EDC and wildlife are of concern [91]. Therefore, further investigations of interactions between gut microbiota and endogenous/exogenous hormones are critical for reproductive success in animal species.

#### Microbiome and endocrine dysfunction: a case study in rhinoceros

Recent work by Williams et al., explored the role of gut microbiota in rhinoceros fertility [92]. This relationship

appears important to southern white rhinoceros (*Ceratotherium simum simum*, SWR), as phytoestrogen-associated endocrine disruption has been strongly linked to the reduced fertility of this species [93]. Rhinoceros world-wide face great pressure, as increased poaching has led to estimates of *in situ* extinction in the next two decades [94], and captive populations are no longer self-sustaining [95].

Linking gut microbiota function to fertility is challenging. A multi-disciplinary integration of 16S rRNA sequencing, targeted mass spectrometry, *in vitro* estrogen receptor assays, and fertility measurements, revealed that rhinos that excreted fecal samples with the highest overall estrogenicity had the highest fertility [92]. This high activation of estrogen receptors was attributed to equol, a microbially-derived daidzein metabolite [92], which was previously associated with infertility [96]. Antwis et al. speculates that rare taxa are associated with fertility in captive black rhinoceros [82], and taxa associated with phytoestrogen transformation, the *Coriobacteriaceae* and the *Eubacterium* [97], were both found in low abundance in SWR [92]. The involvement of novel microbiota and novel metabolites is possible, as SWR microbiota were poorly classified and a targeted mass spectrometry approach was used. Therefore, future directions in studies should be aimed to identify specific microbiota involved in these processes, as novel applications can be applied to enhance captive breeding.

#### Microbiome and chemical communication among vertebrates

Chemical communication between conspecifics is widespread in the animal kingdom, with several taxa known to transmit key information regarding, sex, age, individual and reproductive status [98]. In reproduction specifically, species that have structured social groups can use chemical communication in addition to behavioral cues to alert potential mates regarding their reproductive status [99]. This is more difficult for solitary species, like giant pandas, which rely heavily on chemical communication to do so [95].

Microbiota associated with skin and external glands may play an important role in the generation of these molecules for chemical communication. In spotted hyenas (*Crocuta crocuta*), scent pouches contain fermentative microbiota (*Propionibacterium* and Firmicutes members) that produce volatile compounds of likely microbial origin used for chemical signaling [100]. Similar findings have also been observed in Malagasy lemurs (Lemuroidea) by Greene et al., further supporting the bacterial fermentation hypothesis in chemical communication [101]. The previous studies have not directly linked associated microbiota to the production of communication molecules, but work by Brunetti et al. did [102]. Microorganisms isolated from the skin of Burmeister's tree frog (*Boana prasina*), produced several compounds within the volatile profile associated to sex differences. One isolate in particular, a *Pseudomonas* spp., produced several methoxy-

pyrazines, a chemical class of volatile compounds [102] previously described as pheromones in insects [103–105] that may play a similar role in amphibians. These examples highlight the potential role of host-microbiota interplay driving reproductive behaviors via microbially-mediated chemical communication.

#### Host-microbe interactions at the skin surface

As the first barrier of interaction with the environment [106,107], characterizing the skin microbiome of wildlife is a fundamental step to understanding environmental adaptation of animals and captivity and defining differences between healthy and diseased states [5]. Additionally, for many taxa, symbiotic relationships between host, environment, and microbiota are mediated by small molecules, some beneficial [17,108], and some associated to human disease [109]. Therefore, defining the mechanism of these symbioses, either through the microbes themselves or the microbial natural products they produce is a fundamental component of skin microbiome studies and should be considered in determining the role of microbiomes in animal health.

#### Skin infections

Few studies have explored the role of skin-microbiota broadly across captive vertebrates, with most studies centered around amphibians and chytridiomycosis, a disease responsible for the greatest recorded loss of biodiversity [110]. The skin microbiome may also play a role in Tasmanian devils (*Sarcophilus harrisii*). Devils face the threat of extinction, most notably from their susceptibility to a transmissible cancer, devil facial tumor disease (DFTD; [111]). To better understand the potential role of skin microbiota and DFTD, Cheng et al., compared wild and captive devils microbiomes and found increased levels of *Mycobacterium* levels in captive devils [112]. This microorganism is responsible for skin infections commonly observed under captive conditions highlighting the negative impact of captivity on the health status of animals [112]. Although differences in the microbiome from wild and captive animals were found, further studies are necessary to actually demonstrate if the alterations in the microbiome from captive animals will have any impact on their survival in the wild [112].

#### Protective role of skin symbiotic microorganisms

Several species of amphibians are especially susceptible to fungal pathogens, such as chytridiomycosis, an infectious and lethal disease caused by two fungal species, *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal) [113,114]. Currently, only few antifungal compounds produced by symbiotic bacteria from amphibians skin have been reported [115], and these appear to be species-specific, and in some cases within certain populations of a species [114]. This specificity may be driven by the unique composition of the

skin microbiome across species, leading to varying infection and disease susceptibility [116].

Using microbiota to combat these infections shows promise. For several frog species, the treatment of *Janthinobacterium lividum*, a bacterium isolated from amphibian skin, has shown anti-Bd properties through the production of antifungal metabolites, such as violacein [117]. Other treatments, such as the use of probiotic bacteria against chytridiomycosis has revealed a skin defense peptide from the endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) [118]. This probiotic treatment triggers activation of host antimicrobial peptide (AMP) as a defense mechanism. Therefore, skin symbiotic microbiota have indeed been shown to have a protective role for amphibians against chytridiomycosis, not just by their chemical arsenal, but also inducing an immune response in the host. Work by Kearns et al., demonstrated the use of a fungal probiotic, *Penicillium expansum*, may also be useful, as it did not elicit a host stress or immune response, suggesting that symbiotic fungi may provide greater host protection from Bd, compared to bacterial species [116].

Like amphibians, bats are susceptible to fungal diseases, especially white-nose syndrome (WNS) caused by *Pseudogymnoascus destructans* (Pd) [119]. Recent work by Ange-Stark et al. found that the impact of WNS on the skin microbiome was species-specific, with lower bacterial diversity found in *Myotis lucifugus* where the Pd was present, but had no significant effect on *Eptesicus fuscus* or *Perimyotis subflavus* [120]. Interestingly, *M. lucifugus* and *P. subflavus* are both heavily susceptible to Pd in comparison to lower susceptibility in *E. fuscus*. Like the amphibian examples above, probiotic treatment with bacteria isolated from the bat have been implemented with some success. Several *Pseudomonas* isolates inhibited Pd *in vitro* [121], and when trialed as a probiotic treatment reduced WNS mortality was observed [122]. These examples highlight how symbiotic microbiota can be used to mitigate disease in wildlife.

## Drug discovery and interventions

The study of host–microbe interactions with relationship to disease in wildlife can result in the discovery of potential new medical interventions that can be validated under laboratory conditions. These interventions may range from using microbes as a treatment themselves, to small molecule, peptide, or enzymatic drug discovery, and the development of a novel platform for N-of-1 investigations. In turn, recent developments in treatment and screening garnered from model systems approaches and human clinical trials can be repurposed to benefit disease mitigation in wild and captive animals.

### Microbes as treatment

Animal microbiome studies have led to putative mechanisms behind their protective role for maintaining health, from the

production of microbial compounds to induction of immune responses, in other words, the involvement of natural products in the maintenance of health. As discussed in this review, several diseases have been observed in captive animals and been associated with the captive environment and changes in diet.

Fungal infections threaten a wide range of wild animals, including frogs and bats [123,124], as described previously. Investigation of microbial metabolites have led to discovery of antifungal natural products such as violacein, a deep violet pigment produced by *Chromobacterium violaceum* and *Janthinobacterium lividum*, [117,125,126]. This natural product triggers activation of host antimicrobial peptide (AMP) as a defense mechanism against Bd. Not only can microbes be used as a medication, but also as the source of a compound administered as a treatment, such as iron chelators of microbial origin (siderophores: of marine, terrestrial, and synthetic origin [127,128]) used to treat ISD. General principles regarding host–microbe interactions are lacking for most wildlife species, requiring an expansion of our understanding of the protective role of microbiota, not just by their potential to exclude pathogens or their chemical arsenal, but also their role in modulating host immune responses.

Microbes can also be used to treat other diseases, specifically through intestinal transfaunation, such as rumen fluid transfer or fecal microbiota transfer (FMT). In several species, vertical transmission of microbes is essential, particularly dietary specialists like koalas (*Phascolarctos cinereus*, [129]) and vampire bats (*Desmodus rotundus*, [130]) to transfer microbiota required to degrade their specialized diets. In koalas, mothers feed a coprophagic pap that contains high concentrations of enterobacteria, microbes known for their ability to degrade eucalypts' tannin-protein-complex [129]. This is a clear example of how maintaining natural microbiota is essential for host fitness, as koalas rely on their resident gut microbiota to combat plant herbivory defenses [129]. Toxic plant secondary metabolites are diverse among eucalypt species and vary across the landscape, leading to differing exposure across koala populations [131], with corresponding changes in gut microbiota necessary for toxin degradation [132]. These eucalypt-specific microbiota demonstrate the importance of functionally appropriate microbiota, not only for specialist herbivores, but also more broadly for wildlife. How might time in captivity lead to the loss of critical microbiota, subsequently driving the diseases discussed above?

### Zoo animals as translational models

Captive wildlife can potentially be models for individualized precision medicine. Individual animals are of high value in multiple ways and receive intensive individual veterinary care similar to human hospitals. Access to consistent veterinary care also enables the collection of detailed health

information and a controlled environment, in contrast to human subjects, unless they are sequestered for the duration of the study in question. By studying individual animals, or congregating information from multiple sites, a better understanding of variation in disease progression can be gained. For example, wild mice have been used to improve understanding of normal immunologic processes since they are exposed to more dynamic environmental factors than their laboratory counterparts [133]. Furthermore, N-of-1 studies employing personalized medical interventions in captive wildlife may be more informative for understanding naturally occurring disease, as they have not been genetically manipulated to present certain disease symptoms. While sample size may be low, showing success in a potentially more outbred population that interacts with the external environment and diet perturbations may give a better indication if interventions are more likely to translate to real world results.

#### Application of emerging methodology

An array of recently developed methods applied for human microbiome studies can also be applied to investigate animal microbiome and explore potential treatments for diseases threatening captive wildlife. Multi-omics approaches have been applied to understand human diseases [134–136] as the goal is to obtain and integrate the maximum of information from biological samples. Techniques such as untargeted metabolomics (discovery of markers and molecules [137]), metagenomics [138], and functional analyses, like activity-based labeling [139,140], can provide the necessary information that may lead to the development of new treatments for captive wildlife, as well as to survey and study wild populations. These techniques can be applied to many different disease models to enhance the discovery of novel compounds, microbes, and the unique functions they play that are fundamental across systems. With the increasing advance of technology and sensitivity of these techniques, the research efforts can begin to focus on *in situ* detection of these natural products to provide a deeper understanding of their function in the studied environment.

Determining the impact of the transition from a wild to a captive environment could help to answer questions of whether changes in microbial community structure impact disease susceptibility, and the role that nutrition or other environmental aspects of the built environment play in host microbe interactions and host health status.

Understanding the connections between the microbiome and host in captive wildlife affords the opportunity to view the disease state as a type of model system. In this way we can understand how different diseases manifest across a range of biological diversity, as well as identify potential targets for drug therapy, either molecular or microbial. Medical interventions are closely regulated in captive animals, whose symptoms and medical treatment is similar to humans, but

the ability to collect additional health information may serve as a model for N-of-1 studies, to better understand individualized targeted medical approaches.

#### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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