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Article

Diversity of the aerobic cloacal microbiota of syntopic lizard species (Reptilia: Sauria) from a low-mountain area in Western Bulgaria

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Abstract
Compared to other reptile groups in Europe, lizards have generally been neglected and understudied in terms of microbiota research. In this study, we aimed to isolate, identify and characterize the aerobic cloacal microflora of wild-dwelling lizard hosts. We examined a total of 86 individuals from five species belonging to three families: the European green lizard (*Lacerta viridis*), the common wall lizard (*Podarcis muralis*), the meadow lizard (*Darevskia praticola*) (*Lacertidae*), the European snake-eyed skink (*Ablepharus kitaibelii*) (*Scincidae*) and the European slow worm (*Anguis fragilis*) (*Anguidae*) which co-occur in a low-mountain region in Western Bulgaria. In general, a similar composition of the resident microbial communities in the cloaca was found, accompanied by variation in the relative abundance of some bacterial taxa between the lizard species. A variety of Gram-negative and Gram-positive bacteria was isolated from the cloacal samples. Some of these bacteria are also known as opportunistic pathogens, both for hosts and humans. The bacterial species *Hafnia alvei*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and representatives of *Enterobacter* spp., *Citrobacter* spp. and *Enterococcus* spp. were among the most prevalent.

Key words aerobic cloacal microflora, free-living reptiles, Lacertidae, Scincidae, Anguidae, *Enterobacteriaceae*, *Enterococcaceae*.

Introduction

Microbiome acquisition in reptiles occurs in a variety of ways: parental transmission to offspring, social and sexual interactions with conspecifics, recruitment of microbes through contact with soil/substrate or food (during feeding) (White *et al.* 2011; Singh *et al.* 2014; Kohl *et al.* 2017; Carranco *et al.* 2022; Bunker *et al.* 2022a). Cloacal microbiomes significantly differ from microbiomes in other compartments of the gastrointestinal tract and tend to have lower alpha diversity (Colston *et al.* 2015; Bunker *et al.* 2022b; Forbes *et al.* 2023; Hernandes *et al.* 2023). Aerobic conditions in cloaca, along with external factors such as water and soil, could have an impact on the microbiota composition, leading to a dominance of Proteobacteria (Forbes *et al.* 2023). The role of the cloacal microbiota in host well-being is still poorly understood, as are the interactions between members of the microbial community. A great number of bacteria residing in reptile cloaca are part of the normal microbiota without causing disorders, but in some cases may act as opportunistic pathogens especially in malnourished, poorly maintained or immunosuppressed individuals living under stressful conditions (Romero *et al.* 2015; Divers 2022). Alterations of habitat and behavior can constitute the greatest stress factor predisposing to bacterial infections (Ferreira Junior *et al.* 2009). Since most studies have mainly been conducted on captive animals (Jho *et al.* 2011; Piasecki *et al.* 2014; Romero *et al.* 2015; Marin *et al.* 2021), there is ambiguity in general about the peculiarities of the cloacal microbiota and health status of wild populations. A number of opportunistic microorganisms could be transmitted to other animals and humans as well, and pose a potential health risk (Avsever & Tunaligil 2016; Ebani 2017; Marenzoni *et al.* 2022). For example, *Salmonella* surveys are most often emphasized since reptiles are known to be asymptomatic carriers of various serovars (Schröter *et al.* 2004; Krautwald-Junghanns *et al.* 2013; Pees *et al.* 2013; Whiley *et al.* 2017). Potential pathogens considered to be one of the factors leading to decline in populations of some reptile species worldwide are also understudied (Rose *et al.* 2017; McKnight *et al.* 2020; Galosi *et al*. 2021). Complex research is needed within the One Health concept to demonstrate the microbiota diversity and its importance for the health status of animals, humans and environment. Therefore, large-scale screening in wild reptile populations is needed to fill the existing gap of knowledge.

Regarding the European herpetofauna, investigations on the cloacal microbiota as well as its related pathogenic potential have been carried out in various reptile species: tortoises (Barbour *et al.* 2007; Laroucau *et al.* 2020; Casalino *et al.* 2021; Marenzoni *et al.* 2022), pond turtles (Hacioglu *et al.* 2012; Nowakiewicz *et al.* 2015), snakes (Schmidt *et al.* 2014; Zając *et al.* 2016; Lukač *et al.* 2017; Pawlak *et al.* 2020), etc. Although lizards are among the dominant reptile species in terms of numbers in European ecosystems and their important components (Vacheva 2021), microbiological studies on these vertebrates are few (White *et al.* 2011; Schmidt *et al.* 2014; Dudek *et al.* 2016). Herewith, we aimed to screen the aerobic cloacal microbiota of five syntopic lizard species from three families occurring in Bulgaria. The European green lizard (*Lacerta viridis* Laurenti, 1768), the common wall lizard (*Podarcis muralis* Laurenti, 1768), the meadow lizard (*Darevskia praticola* Eversmann, 1834) (Lacertidae); the European snake-eyed skink (*Ablepharus kitaibelii* Bibron & Bory de Saint-Vincent, 1833) (Scincidae) and the European slow worm (*Anguis fragilis* Linnaeus, 1758) (Anguidae) co-inhabit a low-mountain area in the western part of the country. They exhibit different preferences in terms of microhabitat selection, spatial and trophic niche width (Vacheva 2021). The question arises whether the environment as an external source plays the main formative role for the cloacal microbiota of syntopic lizard species or it is host specific.

Materials and methods

Study area and sample collection

The study area was located in western Bulgaria, along the valley of the Dalbochitsa River, Ihtimanska Sredna Gora Mountain, northeast of the village of Gabrovitsa (N42.2602º, E23.9208º), 430–580 m above sea level. Fieldwork was carried out in May and June 2022. A total of 86 individuals were captured as follows: *D. praticola* n=26; *A. kitaibelii* n=26; *P. muralis* n=17; *L. viridis* n=15; , *A. fragilis* n=2. Lizards were caught by hand. Collection of cloacal samples was performed with sterile cotton swabs inserted carefully into the cloaca pre-wiped with alcohol 70% and with a gentle rotating motion. Cotton swabs were placed immediately in Amies transport medium (Biolab, Hungary) and stored at 4°C for 48 h until further processing in laboratory.

Biometric data (snout-vent length - SVL and weight) were taken on each specimen caught, together with age group and sex determination. After sampling, lizards were released at their place of capture (the location of each individual was recorded with a GPS device). Handling of animals was performed according to the necessary regulations and ethics requirements (Permit № 861/13.01.2021 from the Ministry of Environment and Water, Republic of Bulgaria).

Isolation and Identification of Microorganisms

Cotton swabs were transferred to tubes with 5 ml Nutrient Broth (HI Media, India) to enrich the cultures and incubated at 37°C for 24 h – 48 h depending on bacterial growth. An initial screening of the mixed cultures was performed by plating on petri dishes with different selective and differentiation media: HiCrome UTI Agar (HI Media, India), Cetrimide agar (Merck, Germany), TCBS agar (Biolab, Hungary), Brilliant green agar (after pre-enrichment in Rappaport-Vassiliadis broth) (Biolab, Hungary), *Aeromonas* isolation medium (HI Media, India), KF *Streptococcus* agar (Biolab, Hungary), Mannitol salt agar (Biolab, Hungary), and incubation for another 24 h. Isolated pure cultures obtained from single colonies were identified morphologically, microscopically (Gram staining) and biochemically. Tests for catalase, glucose fermentation (OF test), cytochrome oxidase detection (OXI strip test), tryptophanase (INDOL test), acetoin production (Voges-Proskauer reaction) were performed too. Identification kit MICROLATEST® ID: ENTERO 24N Test (Erba Lachema, Czech Republic) and specialized software ErbaExpert Identification Program (www.erbalachema.com) were used for identification and interpretation of the results about Gram-negative bacteria. Automatic BD PhoenixTM M50 system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was applied for a full biochemical characterization of Gram-negative and Gram-positive isolates by laboratory procedure, as described by the manufacturer. The obtained data were analyzed by EpiCentre™software (V7.45A/V6.71A).

Statistical analysis

Statistical analysis was performed using the programme PAST 4.07 (Hammer *et al.* 2001). Differences between lizard species according to number of bacterial species isolated from each lizard were examined using a diversity permutation test at a 0.05 significance level.

Results

From cloacal samples (n=86) a total number of 357 isolates were obtained. The identified isolates belonged to 18 genera and 28 species. The full list of Gram-negative and Gram-positive bacteria identified is represented in Table 1, along with the frequency of occurrence in the overall dataset.

Table 1. Bacterial species isolated from cloacal microbiota of lizards.

Family	Bacterial species	A total number of individuals-carriers/Percent
Gram-negative bacteria		
Hafniaceae	*Hafnia alvei*	34 (39.5%)
Enterobacteriaceae	*Enterobacter amnigenus* biovar 2	30 (34.9%)
	Enterobacter cloacae	11 (12.8%)
	Enterobacter nimipressuralis	2 (2.3%)
	Citrobacter braakii	28 (32.5%)
	Citrobacter youngae	16 (18.6%)
	Citrobacter freundii	10 (11.6%)
	Citrobacter werkmanii	1 (1.2%)
	Rahnella aquatilis	22 (25.6%)
	Klebsiella oxytoca	18 (20.9%)

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Table 1

	Budvicia sp.	17 (19.8%)
	Buttiauxella sp.	16 (18.6%)
	Escherichia vulneris	10 (11.6%)
	Escherichia coli	4 (4.6%)
	Serratia plymuthica	9 (10.5%)
	Pragia fontium	8 (9.3%)
	Raoultella terrigena	1 (1.2%)
Pseudomonadaceae	*Pseudomonas aeruginosa*	24 (27.9%)
Vibrionaceae	*Vibrio metschnikovii*	13 (15.1%)
Morganellaceae	*Providencia heimbachae*	4 (4.6%)
Gram-positive bacteria		
Enterococcaceae	*Enterococcus casseliflavus/gallinarum*	24 (27.9%)
	Enterococcus faecalis	16 (18.6%)
	Enterococcus faecium	14 (16.3%)
	Enterococcus avium	6 (6.9%)
Bacillaceae	*Bacillus pumilis*	12 (13.9%)
	Bacillus cereus	3 (3.4%)
Staphylococcaceae	*Staphylococcus saprophyticus*	3 (3.4%)
Micrococcaceae	*Kocuria varians*	1 (1.2%)

In terms of cloacal microbiota diversity, no statistically significant difference was observed between lizard species (permutation $p > 0.05$ in all tested combinations; *A. fragilis* was not included). Only 8 bacterial species were identified in *A. fragilis,* probably due to the small sample size. A total number of bacterial species found in the lacertid and scincid species was as follows: 21 in *L. viridis* and *D. praticola,* 20 in *P. muralis* and 19 in *A. kitaibelii*. Differences were observed between lizard species regarding the prevalence of widespread (found in more than 5 individuals) and rare (found in only one individual) bacterial species (Fig. 1). Most bacterial species were found to be present in 2 to 5 individuals. Thirty three percent (5/21) of the bacterial species, identified in *L. viridis* were rare, detected in only 1 individual. In *P. muralis* and *A. kitaibelii* they were 20% (4 bacterial species), while in *D. praticola* only one bacterial species was present exclusively in 1 individual (*B. cereus*). In contrast, in *L. viridis* – only one bacterial species (*K. oxytoca*) was found in more than 5 individuals, while in *D. praticola* and *P. muralis* - 40% of bacterial species (8/20) were widespread.

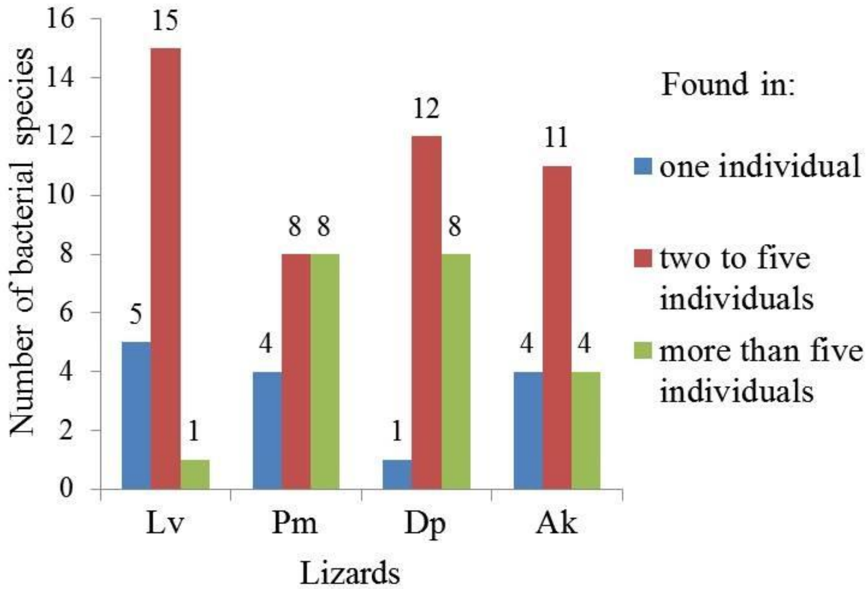

Figure 1. Dispersion mode of widespread and underrepresented bacterial species in: *L. viridis* (Lv); *P. muralis* (Pm); *D. praticola* (Dp) and *A. kitaibelii* (Ak).

Up to 10 bacterial species we succeeded to isolate from each individual. The median number was highest in *P. muralis* – 5 or more bacterial species in the samples were proven in 64.7% (11/17) of the individuals. The mean number of bacterial species per individual, by age and by sex is given in Table 2. No significant difference related to age or sex was found.

Table 2. Number of bacterial species isolated from cloacal microbiota of each lizard species.

Lizard species	Total number of bacterial species	Mean number of bacterial species				
		Per individual (±SD*)	by age		by sex	
			adult	subadult	males	females
L. viridis	21	3.6±2.03	3.0	3.16	- **	- **
P. muralis	20	5.52±2.49	5.5	5.6	5.0	6.5
D. praticola	21	4.54±2.45	4.63	4.57	6.67	4.5
A. kitaibelii	19	3.25±1.66	3.25	3.25	3.13	3.17

* SD (standard deviation)
** In the case of *L. viridis*, most of the specimens caught were subadults

The composition of the cloacal microbiota of each lizard species is represented in Fig. 2. *Anguis fragilis* specimens contained the following bacterial species, which turned out to be among the most common in cloacal swabs: *H. alvei*, *E. amnigenus* biovar 2, *C. braakii*, *C. youngae*, *K. oxytoca*, *Budvicia sp.*, *R. aquatilis* and *P. aeruginosa*. We observed a similar composition of the cloacal microflora, but different relative abundance of the identified bacteria across lizard species (Fig.3). Ubiquitous in all lizards were 42.8% (12/28) of bacterial species, including *H. alvei*, *E. amnigenus*, *Citrobacter* spp., *K. oxytoca*, *P. aeruginosa*, *Enterococcocus* spp. and *B. pumilis*. Distribution of 25% (7/28) of bacterial species was limited to two lizard species. *Podarcis muralis* and *D. praticola* shared 3 bacterial species: *Buttiauxella sp.*, *E. cloacae* and *B. cereus*. *Providencia heimbachae* was detected in *D. praticola* and *L. viridis*, *E. avium* – in *P. muralis* and *A. kitaibelii*, *V. metschnikovii* – in *D. praticola* and *A. kitaibelii*, and *E. coli* – in *L. viridis* and *P. muralis*. Uniquely found only in one lizard species were 17.8% (5/28) of bacteria namely: *C. werkmanii*, *R. terrigena* and *K. varians* in *L. viridis*, *E. nimipressuralis* – in *P. muralis* and *S. saprophyticus* - in *A. kitaibelii*.

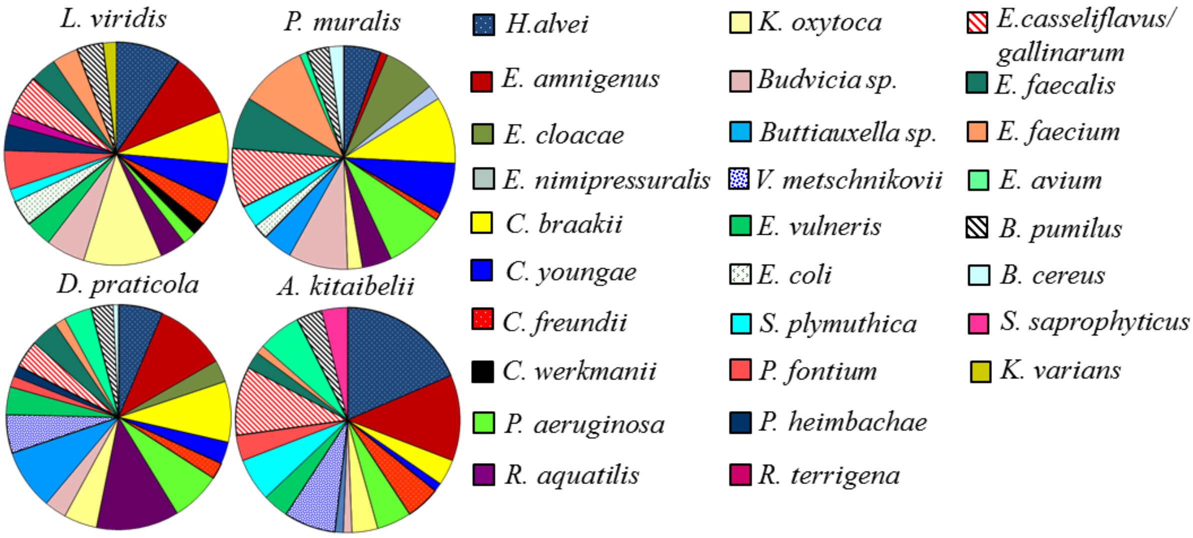

Figure 2. Composition of the cloacal microbiota of each lizard species.

Certain bacterial species predominated in a particular lizard species, occurring in more than 40-50% of the individuals, but being poorly present in the other species (Fig. 3). Almost 58% of the *A. kitaibelii* individuals were carriers of *H. alvei*, while in the rest it did not exceed an average of 30%. *Buttiauxella sp.* and *R. aquatilis* were spread in 42.3% and 57.7% of *D. praticola* specimens, respectively, but both reached only 23.5% in other species. *Budvicia sp.*, *E. cloacae*, *E. faecium* and *E. faecalis* were encountered in 41 - 53% of *P. muralis* specimens, while in the rest they vary between 3 and 20%.

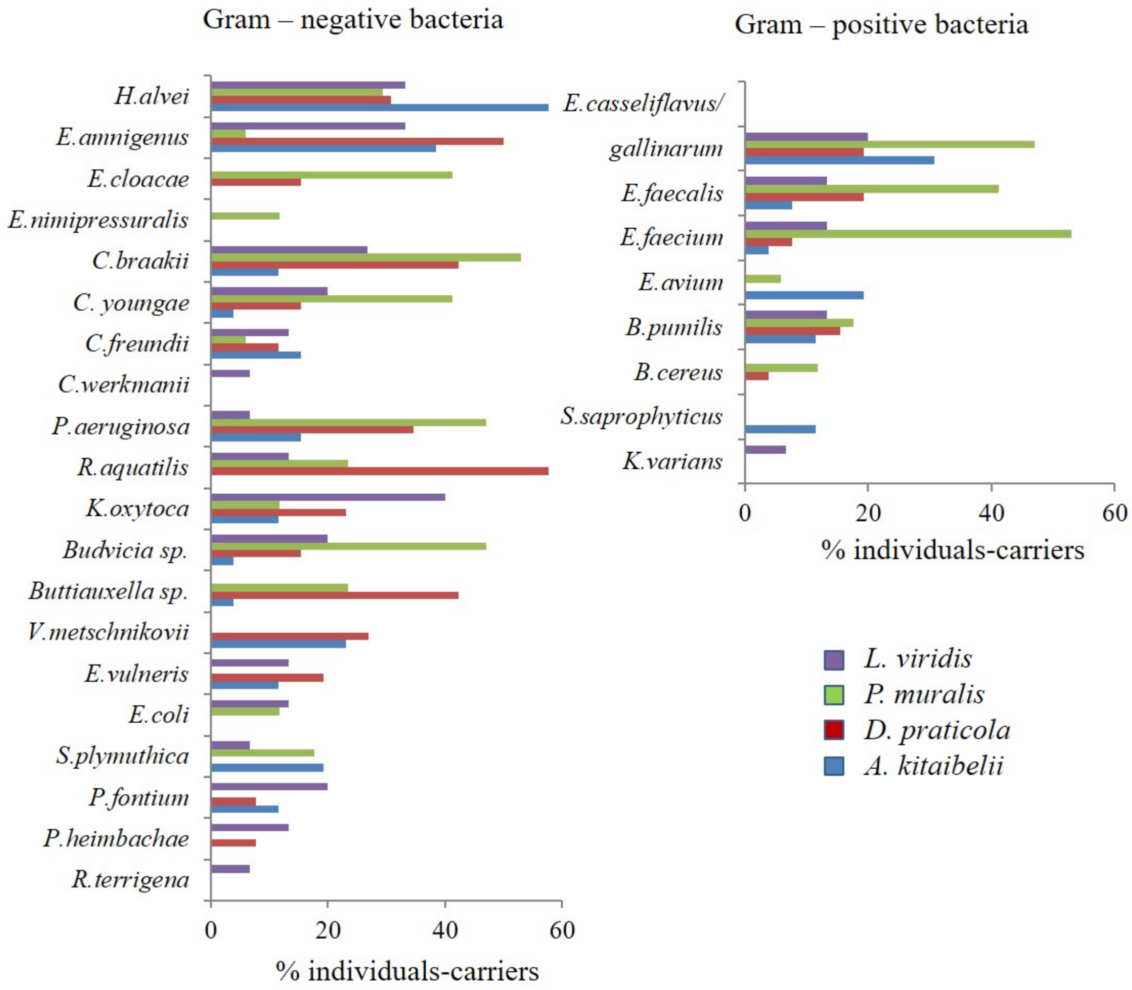

Figure 3. Relative abundance of the isolated bacterial species across the lizard population.

Discussion

The cloaca, in particular, is inhabited by its own set of microbes being both the terminal section of the intestine through which urea and feces pass, and part of the reproductive system. Therefore, it may be influenced by mating behavior and sexually transmitted microbes, as well as by inoculation during defecation with microbes from the upper gut (Bunker *et al*. 2022b). Most likely, the cloacal microbiota largely comes from the aerobic environment rather than from the digestive or reproductive tract. The composition and diversity of the cloacal microbiota can be modulated by external factors such as water, air and soil (Forbes *et al.* 2013), and by several host traits, such as taxonomic affiliation, sex, size and social interactions as well (Bunker *et al*. 2022b). Both ambient influences and host relatedness have an effect on the cloacal microbiome, whereby the host's internal environment may define the microbial pool available to colonize a host (Bunker & Weiss, 2022). Not enough is known about interspecific relationships in the cloacal microbial community. For instance, some taxa of the dominant family *Enterobacteriaceae* have been suggested to have antimicrobial properties, which can reduce diversity if they are abundant (Bunker *et al*. 2022a), as well as antifungal activity to protect eggs from fungal infection during incubation (Bunker *et al.* 2022b). Overall, we found a similar bacterial composition in all lizard species, but different proportion of certain bacteria species. Although *L. viridis, P. muralis, D. praticola* and *A. kitaibelii* differ to some extent in their microhabitat selection preference, their spatial niches largely overlap (Vacheva 2021). This implies similar environmental pressure. The abundance of some bacteria in a particular lizard species and low presence in another suggests a more likely host-relatedness than microhabitat influence. Host taxonomy, rather than habitat, is considered a determinant of the cloacal microbiota of colubrid snakes as well (Dallas *et al.* 2021). Unlike lacertids and skink, *A. fragilis* leads a more secretive and semifossorial life (Vacheva 2021). Surprisingly, we found that the cloacal microbiota of the slow worm was characterized by poor diversity, comprising only the most common types of bacteria identified in the entire set of cloacal swabs.

Consistent with studies in other lizard species (Singh *et al.* 2013; Bunker & Weiss 2022c), we reaffirmed the prevalance of *Enterobacteriaceae* and *Enterococcaceae* in the cloacal microbiota. Bacterial species identified tend to be part of the normal cloacal microbiota of the lizards, but are also known to be opportunistic pathogens of reptiles and humans (Divers 2022; Ebani 2017). However, infections caused by them are rare and mostly affect immunocompromised hosts. The overwhelming majority of bacterial isolates belonged to genera *Hafnia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Pseudomonas* and *Enterococcus.*

Multiple findings of *H. alvei* were observed in all lizard species, but most prevalent in cloacal microflora of *A. kitaibelii*. It was found in remarkably high relative abundance in the gut microbiota of *A. kitaibelii* as well (Lazarkevich *et al.* 2024a). *Hafnia alvei* participates in the intestinal microbiota of humans and various animals, including snakes and skinks (Padilla *et al.* 2015). As opportunistic pathogen, *H. alvei* may be associated with septicaemia, endocarditis, meningitis, pneumonia and other disorders in immunocompromised patients, and it has also been reported in outbreaks of disease in a variety of animals such as poultry, ruminants, snails, fish and bees (Padilla *et al.* 2015).

We found widespread distribution of members of the genus *Citrobacter*, detected in 45.3% of individuals, with *C. braakii* being the most common. The highest prevalence of *Citrobacter* spp. was observed in *P. muralis*, where 64.7% of the individuals were carriers of at least one species, followed by *D. praticola* and *L. viridis* (53.5%), and *A. kitaibelii* (23.1%). *Citrobacter* spp. have been frequently reported in various reptiles (Silvestre *et al.* 2003; Singh *et al*. 2013; Romero *et al.* 2015; Nowakiewicz *et al.* 2015; Pawlak *et al*. 2020). The leading *Citrobacter* species linked to human infections were *C. braakii*, *C. freundii* and *C. koseri* (Wang & Chang 2016). Especially *C. freundii* causes nosocomial infections and is increasingly becoming multi-drug resistant (MDR) (Liu *et al.* 2017). In a number of cases, *C. freundii* has been recognized as a causative agent of fatal infections in various animals – sheep (Liu *et al.* 2018), whales (Fernandes *et al.* 2011), birds (Godoy & Matushima 2010), giant salamanders (*Andrias davidianus*) (Gao *et al.* 2012), turtles (Hossain *et al.* 2017; Inurria *et al.* 2024), and was also involved in the etiology of Septicemic Cutaneous Ulcerative Disease (SCUD) in reptiles (Divers 2022).

Of interest were findings of certain bacterial species. *Pragia fontium*, along with *Budvicia* spp. are closely related atypical enterobacterial species belonging to a relatively small group of H_2S-producers (Snopková *et al.* 2017). *Budvicia* was originally isolated from freshwater, but several isolates have been described from the intestinal microflora of insects, millipedes and salmonids, while *Pragia* appears to occupy environmental niches with no association with hosts although few strains came from human clinical material (Snopková *et al.* 2017) and gecko eggs (Singh *et al.* 2014). *Rahnella aquatilis* is an important pathogen of several aquatic organisms and widely distributed in the freshwater, soil, fish and human clinical samples (Liang *et al.* 2020). We identified *V. metschnikovii* in cloacal microbiota of *D. praticola* and *A. kitaibelii.* This microorganism was involved in outbreaks in fish, farm animals, poultry and wild birds (Zheng *et al.* 2021; Xiao *et al*. 2022) and is considered to be one of twelve pathogenic *Vibrio* species associated with diseases in humans (Ayala & Ogbunugafor 2022), although clinical cases are sporadic (Magalhães *et al.* 1996, Linde *et al.* 2004, Konechnyi *et al.* 2019). Vibrios comprise Gram-negative, facultatively anaerobic bacteria naturally occuring in freshwater, marine and estuarine ecosystems, with a preference for warm, brackish waters, capable of colonizing fish and marine invertebrates or associated with plankton and algae (Baker-Austin *et al*. 2018, Ebani 2023). Sea turtles have been repeatedly documented as carriers of *Vibrio* spp. (Hacıoğlu *et al.* 2012; Ebani 2023) and freshwater turtles (*E. orbicularis, M. rivulata*) have been reported as well (Hacıoğlu *et al*. 2012). Also, numerous studies point to migratory and sedentary birds, including those not strictly associated with aquatic environments, as hosts for a spectrum of potentially pathogenic *Vibrio* spp. (Páll *et al.* 2021, Zheng *et al.* 2021; Ayala. & Ogbunugafor 2022). *Vibrio* spp. have been proven in fecal samples from an invasive lizard species of *Anolis* in the Canary Islands (Abreu-Acosta *et al*. 2023).

However, in the available literature, evidence for the presence of *Vibrio* spp. in the microbiota of terrestrial reptiles is scarce.

In *D. praticola* and *L. viridis*, *P. heimbachae* was detected. *Providencia* - closely related to genera *Proteus* and *Morganella* – has been isolated from multiple animal hosts, including flies, birds, cats, dogs, cattle, sheep, guinea pigs, penguins, and is resident in the oral flora of reptiles such as pythons, vipers and boas (Charbek 2019). Some species have been reported to cause enteritis in chickens and cows, as well as meningitis/septicemia in crocodiles (Charbek 2019). In one individual of *L. viridis*, *R. terrigena* was identified. *Roultella* spp. not commonly found in reptile microbiota (Singh *et al.* 2013, Artavia-Leon *et al.* 2018), but are considered rare enteropathogens with a high mortality rate from this infection and a multidrug resistance profile (Lekhniuk *et al*. 2021).

The genus *Enterococcus* was the most prevalent of the Gram-positive bacteria identified, with 51.2% of the individuals harboring one or more enterococcal species. Interestingly, a mass presence was observed in *P. muralis*, where only two cloacal samples were negative. Although largely considered commensals that participate in the gastrointestinal microbiota of a diverse range of taxa, including mammals, birds, reptiles and insects, enterococci are opportunistic and gradually being recognized as pathogenic agents of both human and animals (Rose *et al*. 2017). Members of the *Enterococcus* genus, primarily *E. faecium* and *E. faecalis*, have been reported in clinical manifestations as lethargy, bacteremia, septicemia, necrotizing osteomyelitis, irregular swellings in soft tissues and death in various reptile species (Schröter *et al*. 2005; Innis *et al*. 2014; Rose *et al*. 2017). It is suggested that the putative pathogens implicated in outbreaks in different parts of the world in populations of *Gekko monarchus*, *Anolis carolinensis*, *Cordylus cordylus*, *Lacerta trilineata*, *Lacerta viridis*, *Podarcis muralis*, are actually *Enterococcus* species previously classified within the genus *Streptococcus* (Rose *et al*. 2017).

Two *Bacillus* species were found to be present in the cloacal samples at a relatively high frequency (in 17.4% of individuals). *Bacillus pumilus* and *B. cereus* were detected predominantly in *D. praticola* and *P. muralis* (10 out of 15 total findings). The presence of *B. cereus* was reported in various reptile species (Schmidt *et al*. 2014; Nfor *et al*. 2015; Lukač *et al.* 2017; Sayyadi *et al*. 2019), including *A. fragilis* (Schmidt *et al*. 2014). *Bacillus* spp. are facultatively anaerobic, toxin-producing, endospore-forming Gram-positive bacteria, widely distributed in the environment. Although *B. pumilus* is commonly used as a probiotic in animals and has plant growth-promoting effects, increasing research has revealed that some strains are pathogenic both to humans, causing foodborne illness and cutaneous infections, and plants (Wang *et al.* 2022). Antagonistic action through the production of antimicrobial agents has also been reported in *B. pumilus* (Wang *et al.* 2022). *Bacillus cereus* is associated mainly with food poisoning, but also being increasingly reported as an etiological agent in multitude of serious and potentially fatal infections, anthrax-like progressive pneumonia, devastating central nervous system infections, nosocomial acquired bacteremia, wound infections, septicemia, as well as endophthalmitis, which can lead to vision loss (Bottone 2010). The pathogenicity of *B. cereus*, whether intestinal or nonintestinal, is closely linked to the production of tissue-destructive exoenzymes. The natural environmental reservoir for *B. cereus* consists of decaying organic matter, fresh and marine waters, and the intestinal tract of invertebrates, from which soil and food products may become contaminated, leading to the transient colonization of the animal intestine. Spores germinate when they come into contact with organic matter or within an insect or animal host (Bottone 2010). *Kocuria varians* was isolated from one individual of *L. viridis*. *Kocuria* inhabits human skin and mucus membranes, such as the oral cavity, and is usually considered non-pathogenic, but it can be implicated in bacteremia, skin and soft tissue infections, endophthalmitis, infective endocarditis and peritonitis (Ziogou *et al*. 2023).

In another study we have discussed the pathogenic potential of the isolated strains, assessed on the basis of their antimicrobial resistance, production of extracellular enzymes considered as virulence factors and biofilm-forming capacity (Lazarkevich *et al*. 2024b). The opportunistic nature of the identified bacterial species should be kept in mind, although the studied lizards are assumed to be of minor importance as a threat to public health. However, besides direct human-animal contact, indirect infection is possible through contamination of the environment with feces. The probable role of lizards as reservoirs and spreaders of diseases is of interest because some populations are common in urbanized areas and live in close proximity to humans (Singh *et al.* 2013; Ajayi *et al*. 2015; Sayyadi *et al.* 2019).

Conclusion

Our study sheds light on a still understudied field as a reptile-associated microbiota from free-ranging populations. No significant difference in the bacterial diversity was observed between syntopic lizard species. Cloacal samples were loaded with a similar in composition microbial community, but in different proportion of certain bacterial species. A host-relatedness was more likely than a habitat influence. Also, lizards could be carriers of some opportunistic human pathogens that may pose a potential risk of infection when handling animals.

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References

Abreu-Acosta, N., Pino-Vera, R., Izquierdo-Rodríguez, E., Afonso, O. & Foronda, P. (2023) Zoonotic Bacteria in *Anolis sp*., an Invasive Species Introduced to the Canary Islands (Spain). *Animals*, 13, 414. https://dx.doi.org/10.3390/ani13030414

Ajayi, J., Ogunleye, A., Happi, A. & Okunlade, A. (2015) Bacteria isolated from the oral and cloaca swabs of lizards co-habitating with poultry in some poultry farms in Ibadan, Oyo State, Nigeria. *African Journal of Biomedical Research*, 18 (3), 211–215.

Artavia-León, A., Romero-Guerrero, A., Sancho-Blanco, C., Rojas, N. & Umaña-Castro, R. (2017) Diversity of aerobic bacteria isolated from oral and cloacal cavities from free-living snakes species in Costa Rica rainforest. *International Scholarly Research Notices*, 8934285. https://dx.doi.org/10.1155/2017/8934285

Avsever, M. & Tunaligil, S. (2016) First isolation of enteropathogenic *Escherichia coli* from land turtles (*Testudo graeca ibera*) cultured in Turkey. *Ankara Üniversitesi Veteriner Fakultesi Dergisi*, 63 (4), 407–410. https://dx.doi.org/10.1501/Vetfak_0000002761

Ayala, A. &, Ogbunugafor, B. (2022) When Vibrios take flight: a meta-analysis of pathogenic vibrios species in wild and domestic birds. *In*: Almagro-Moreno, S, Pukatzki, S. (Eds.), *Vibrio spp. Infections*. *Advances in Experimental Medicine and Biology*. Springer, Cham, 295–336. https://dx.doi.org/10.1007/978-3-031-22997-8_15

Baker-Austin, C., Oliver, J., Alam, M., Ali, A., Waldor, M., Qadri, F. & Martinez-Urtaza, J. (2018) *Vibrio* spp. infections. *Nature Reviews Disiease Primers*, 4, 1–19. https://dx.doi.org/10.1038/s41572-018-0005-8

Barbour, E., Chacra, N., Gali-Mouhtaseb, H., Jaber, L., Nehme, P., Shaib, H., Sadek, R. & Usayran N. (2007) Performance, bacterial shedding and microbial drug resistance in two tortoise species. *Veterinary Record*, 161 (2), 62–65. https://dx.doi.org/10.1136/vr.161.2.62

Bottone, E. (2010) *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Reviews*, 23 (2), 382–398. https://dx.doi.org/10.1128/CMR.00073-09

Bunker, M., Arnold, A. & Weiss, S. (2022a). Wild microbiomes of striped plateau lizards vary with reproductive season, sex, and body size. *Scientific Reports*, 12, 20643. https://doi.org/10.1038/s41598-022-24518-6

Bunker, M., Martin, M. & Weiss, S. (2022b). Recovered microbiome of an oviparous lizard differs across gut and reproductive tissues, cloacal swabs, and faeces. *Molecular Ecology Resources*. 22 (5):1693–1705. https://dx.doi.org/10.1111/1755-0998.13573

Bunker, M. & Weiss, S. (2022c). Cloacal microbiomes of sympatric and allopatric *Sceloporus* lizards vary with environment and host relatedness. *PloS One*, 17 (12), e0279288. https://dx.doi.org/10.1371/journal.pone.0279288

Carranco, A., Romo, D., de Lourdes Torres, M., Wilhelm, K., Sommer, S. & Gillingham, M. A. (2022) Egg microbiota is the starting point of hatchling gut microbiota in the endangered yellow-spotted Amazon river turtle. *Molecular Ecology*, 31 (14), 3917–3933. https://dx.doi.org/10.1111/mec.16548

Casalino, G., Bellati, A., Pugliese, N., Camarda, A., Faleo, S., Lombardi, R., Occhiochiuso, G., Circella, E. & D'Onghia, F. (2021) *Salmonella* infection in turtles: A risk for staff involved in wildlife management? *Animals*, 11, 1529. https://dx.doi.org/10.3390/ani11061529

Charbek, E. (2019). *Providencia* Infections. Medscape https://emedicine.medscape.com/article/226541-overview?form=fpf (July 2019)

Colston, T., Noonan, B., Jackson, C. (2015) Phylogenetic analysis of bacterial communities in different regions of the gastrointestinal tract of *Agkistrodon piscivorus*, the Cottonmouth Snake. *PLoS One* 10(6): e0128793. https://dx.doi.org/10.1371/journal.pone.0128793

Dallas, J., Meshaka, W., Zeglin, L. & Warne, R.(2021) Taxonomy, not locality, influences the cloacal microbiota of two nearctic colubrids: a preliminary analysis. *Molecular Biology Reports*, 48, 6435–6442. https://dx.doi.org/10.1007/s11033-021-06645-x

Divers, S. (2022) Bacterial Diseases of Reptiles, *MSD Veterinary manual***.** https://www.msdvetmanual.com/exotic-and-laboratory-animals/reptiles/bacterial-disases-of-reptiles (June 2020)

Dudek, K., Koczura, R., Dudek, M., Sajkowska, Z. & Ekner-Grzyb, A. (2016) Detection of *Salmonella enterica* in a sand lizard (*Lacerta agilis*, Linnaeus, 1758) city population. *Herpetological Journal*, 26, 57–60.

Ebani, V. (2017) Domestic reptiles as source of zoonotic bacteria: A mini review. *Asian Pacific Journal of Tropical Medicine*, 10 (8), 723–728. https://dx.doi.org/10.1016/j.apjtm.2017.07.020

Ebani, V. (2023) Bacterial infections in Sea Turtles. *Veterinary Sciences*, 10, 333. https://dx.doi.org/10.3390/vetsci10050333

Fernández, A., Vela, A., Andrada, M., Herraez, P., Diaz-Delgado, J., Dominguez, L. & Arbelo, M. (2011) *Citrobacter freundii* septicemia in a stranded newborn Cuvier's beaked whale (*Ziphius cavirostris*). *Journal of Wildlife Diseases*, 47 (4), 1043–1046. https://dx.doi.org/10.7589/0090-3558-47.4.1043

Ferreira Junior, R., Siqueira, A. K., Campagner, M., Salerno, T., Soares, T., Lucheis, S., Paes, A. & Barraviera, B. (2009) Comparison of wildlife and captivity rattlesnakes (*Crotalus durissus terrificus*) microbiota. *Pesquisa Veterinária Brasileira*, 29, 999–1003. https://dx.doi.org/10.1590/S0100-736X2009001200008

Forbes, Z., Scro, A., Patel, S., Dourdeville, K., Prescott, R. & Smolowitz, R. (2023) Fecal and cloacal microbiomes of cold-stunned loggerhead *Caretta caretta*, Kemp's ridley *Lepidochelys kempii*, and green sea turtles *Chelonia mydas*. *Endangered Species Research*, 50, 93–105. https://dx.doi.org/10.3354/esr01220

Galosi, L., Attili, A.R., Perrucci, S., Origgi, F.C., Tambella, A.M., Rossi, G., Cuteri, V., Napoleoni, M., Mandolini, M., Perugini, G. & Loehr, V.J. (2021) Health assessment of wild speckled dwarf tortoises, *Chersobius signatus*. *BMC veterinary research*, 17, 1–11. https://dx.doi.org/10.1186/s12917-021-02800-5

Gao, Z., Zeng, L., Meng, Y., Liu, X. & Zhang, B. (2012) Isolation and identification of *Citrobacter freundii* from diseased giant salamander, *Andrias davidianus*. *Acta Microbiologica Sinica*, 52 (2), 169–176.

Godoy, S. & Matushima, E. (2010) A survey of diseases in passeriform birds obtained from illegal wildlife trade in Sao Paulo city, Brazil. *Journal of Avian Medicine and Surgery*, 24 (3), 199–209. https://dx.doi.org/10.1647/2009-029.1

Hammer, Ø., Harper, D. & Ryan P. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4 (1), 1–9. http://palaeo-electronica.org

Hacioglu, N., Dulger, B., Caprazli, T. & Tosunoglu, M. (2012) A study on microflora in oral and cloacal of freshwater turtles (*Emys orbicularis* Linnaeus, 1758 and *Mauremys rivulata* Valenciennes, 1833) from Kavak delta (Canakkale). *Fresenius Environmental Bulletin*, 21 (11b), 3365–3369.

Hernandez, M., Ancona, S., Hereira-Pacheco, S., Díaz de la Vega-Pérez, A. & Navarro-Noya, Y. (2023) Comparative analysis of two nonlethal methods for the study of the gut bacterial communities in wild lizards. *Integrative Zoology*, 18 (6), 1056–1071. https://dx.doi.org/10.1111/1749-4877.12711

Hossain, S., Hakuruge, S., Wimalasena, M., De Zoysa, M. & Heo, G. (2017) Prevalence of *Citrobacter* spp. from pet turtles and their environment. *Journal of Exotic Pet Medicine*, 26 (1), 7–12. https://dx.doi.org/10.1053/j.jepm.2016.10.004

Huang, Z., Yu, K., Lan, R., Morris, J., Xiao, Y., Ye, J., Zhang, L., Luo, L., Gao, H., Bai, X. & Wang, D. (2022) *Vibrio metschnikovii* as an emergent pathogen: analyses of phylogeny and O-antigen and identification of possible virulence characteristics. *Emerging Microbes & Infections*, 12 (2), 2252522. https://dx.doi.org/10.1080/22221751.2023.2252522

Innis, C., Braverman, H., Cavin, J., Ceresia, M., Baden, L., Kuhn, D., Frasca Jr, S., McGowan, J.P., Hirokawa, K., Weber 3[rd], E.S., Stacy, B. & Merigo, C. (2014) Diagnosis and management of *Enterococcus* spp. infections during rehabilitation of cold-stunned Kemp's Ridley turtles (*Lepidochelys kempii*): 50cases (2006-2012). *Journal of the American Veterinary Medical Association*, 245 (3), 315–323. https://dx.doi.org/10.2460/javma.245.3.315

Inurria, A., Suárez-Pérez, A., Calabuig, P. & Orós, J. (2024) *Citrobacter freundii*-associated lesions in stranded loggerhead sea turtles (*Caretta caretta*). *Veterinary Pathology*, 61 (1), 140–144. https://dx.doi.org/10.1177/03009858231183983

Jho, Y., Park, D., Lee, J., Cha, S., & Han, J. (2011) Identification of bacteria from the oral cavity and cloaca of snakes imported from Vietnam. *Laboratory animal research*, 27 (3), 213–217. https://dx.doi.org/10.5625/lar.2011.27.3.213

Kohl, K., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J., Caviedes-Vidal, E. & Bordenstein, S. (2017) Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Molecular ecology*, 26 (4), 1175–1189. https://dx.doi.org/10.1111/mec.13921

Konechnyi, Y., Khorkavyi, Y., Ivanchuk, K., Kobza, I., Sękowska, A., & Korniychuk, O. (2021) *Vibrio metschnikovii*: Current state of knowledge and discussion of recently identified clinical case. *Clinical Case Reports*, 9 (4), 2236–2244.

Krautwald-Junghanns, M., Stenkat, J., Szabo, I., Ortlieb, F., Blindow, I., Neul, A., Pees, M. & Schmidt, V. (2013) Characterization of *Salmonella* isolated from captive and free-living snakes in Germany. *Berliner Und Munchener Tierarztliche Wochenschrift*, 126 (5-6), 209–215.

Marenzoni, M., Stefanetti, V., Del Rossi, E., Zicavo, A., Scuota, S., Origgi, F., Deli, F., Corti, C., Marinucci, M. & Olivieri, O. (2022) Detection of Testudinid alphaherpesvirus, *Chlamydia* spp., *Mycoplasma* spp., and *Salmonella* spp. in free-ranging and rescued Italian *Testudo hermanni hermanni*. *Veterinaria Italiana*, 58 (1), 25–34. https://dx.doi.org/10.12834/VetIt.1915.13833.1

Laroucau, K., Ortega, N., Vorimore, F., Aaziz, R., Mitura, A., Szymanska-Czerwinska, M., Cicerol, M., Salinas, J., Sachse, K. & Caro, M. (2020) Detection of a novel *Chlamydia* species in captive spur-thighed tortoises (*Testudo graeca*) in southeastern Spain and proposal of Candidatus *Chlamydia testudinis*. *Systematic and Applied Microbiology*, 43 (2), 126071. https://dx.doi.org/10.1016/j.syapm.2020.126071

Lazarkevich, I., Engibarov, S., Mitova, S., Vacheva, E., Popova, S., Stanchev, N., Eneva, R., Gocheva, Y., Boyadzhieva, I. & Gerginova, M. (2024a) 16S rRNA Gene sequencing-based identification and comparative analysis of the fecal microbiota of five syntopic lizard species from a low-mountain area in western Bulgaria. *Applied Microbiology*, 4, 181–193. https://dx.doi.org/10.3390/applmicrobiol4010013

Lazarkevich, I., Engibarov, S., Mitova, S., Popova, S., Vacheva, E., Stanchev, N., Eneva, R., Gocheva, Y., Lalovska, I., Paunova-Krasteva, Ts., Ilieva, Y. & Najdenski, H. (2024b) Pathogenic potential of opportunistic Gram-negative bacteria, isolated from cloacal microbiota of free-living reptile hosts, originating from Bulgaria. *Life*, 14, 566. https://dx.doi.org/10.3390/life14050566

Lekhniuk, N., Fesenko, U., Pidhirnyi, Y., Sękowska, A., Korniychuk, O. & Konechnyi, Y. (2021) *Raoultella terrigena*: Current state of knowledge, after two recently identified clinical cases in Eastern Europe. *Clinical Case Reports*, 9 (5), e04089. https://dx.doi.org/10.1002/ccr3.4089

Liang, J., Hu, X., Lü, A. & Sun, J. (2020) First report on the characterization of pathogenic *Rahnella aquatilis* KCL-5 from crucian carp: Revealed by genomic and proteomic analyses. *Journal of Fish Diseases*, 43 (8), 889–914. https://dx.doi.org/10.1111/jfd.13200

Linde H, Kobuch R, Jayasinghe S, Reischl U, Lehn N, Kaulfuss S, Beutin L. (2004) *Vibrio metschnikovii*, a rare cause of wound infection. *Journal of Clinical Microbiology*, 42 (10), 4909–4911. https://dx.doi.org/10.1128/JCM.42.10.4909–4911.2004

Liu, L., Lan, R., Liu, L., Wang, Y., Zhang, Y., Wang, Y. & Xu, J. (2017) Antimicrobial Resistance and Cytotoxicity of *Citrobacter* spp. in Maanshan Anhui Province, China. *Frontiers in Microbiology*, 8,1357. https://dx.doi.org/10.3389/fmicb.2017.01357

Liu, H., Zhao, Z., Xue, Y., Ding, K. & Xue, Q. (2018) Fatal cases of *Citrobacter freundii* septicemia and encephalitis in sheep. *Journal of Veterinary Diagnostic Investigation*, 30 (2), 245–248. https://dx.doi.org/10.1177/10406387177310

Lukač, M., Tomić, D., Mandac, Z., Mihoković, S. & Prukner-Radovčić, E. (2017) Oral and cloacal aerobic bacterial and fungal flora of free-living four-lined snakes (*Elaphe quatuorlineata*) from Croatia. *Veterinarski Arhiv*, 87 (3), 351–361.

Magalhães, V., Branco, A., Lima, R. & Magalhães, M. (1996) *Vibrio metschnikovii* among diarrheal patients during cholera epidemic in Recife Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 38 (1), 1–3. https://dx.doi.org/10.1590/s0036-46651996000100001

Marenzoni, M., Stefanetti, V., Del Rossi, E., Zicavo, A., Scuota, S., Origgi, F., Deli, G., Corti, C., Marinucci, M. & Olivieri, O. (2022) Detection of Testudinid alphaherpesvirus, *Chlamydia* spp., *Mycoplasma* spp. and *Salmonella* spp. in free-ranging and rescued Italian *Testudo hermanni hermanni*. *Veterinaria Italiana*, 58 (1), 2534. https://dx.doi.org/10.12834/VetIt.1915.13833.1

Marin, C., Lorenzo-Rebenaque, L., Laso, O., Villora-Gonzalez, J. & Vega, S. (2021) Pet reptiles: a potential source of transmission of multidrug-resistant *Salmonella*. *Frontiers in Veterinary Science*, 7, 613718. https://dx.doi.org/10.3389/fvets.2020.613718

McKnight, D., Zenger, K., Alford, R. & Huerlimann, R. (2020) Microbiome diversity and composition varies across body areas in a freshwater turtle. *Microbiology*, 166 (5), 440–452. https://dx.doi.org/10.1099/mic.0.000904

Nfor, N., Lapin, C. & McLaughlin, R. (2015) Isolation of *Bacillus cereus* group from the fecal material of endangered wood turtles. *Current Microbiology*, 71 (4), 524–527. https://dx.doi.org/10.1007/s00284-015-0875-x

Nowakiewicz, A., Ziółkowska, G., Zięba, P., Dziedzic, B., Gnat, S., Wójcik, M., Dziedzic, R. & Kostruba, A. (2015) Aerobic bacterial microbiota isolated from the cloaca of the European pond turtle (*Emys orbicularis*) in Poland. *Journal of Wildlife Diseases*, 51 (1), 255–259. https://dx.doi.org/10.7589/2013-07-157

Padilla, D., Acosta, F., Ramos-Vivas, J., Grasso, V., Bravo, J., El Aamri, F. & Real, F. (2015) The pathogen *Hafnia alvei* in veterinary medicine: a review. *Journal of Applied Animal Research*, 43 (2), 231–235. https://dx.doi.org/10.1080/09712119.2014.963086

Páll, E., Niculae, M., Brudas, G., Ravilov, R., Sandru, C., Cerbu, C., Olah, D., Zablau, S., Potârniche, A., Spinu, M., Duca, G., Rusu, M., Rzewuska, M. & Vasiu, A. (2021) Assessment and antibiotic resistance profiling in *Vibrio* species isolated from wild birds captured in Danube Delta Biosphere Reserve, Romania. *Antibiotics*, 10 (3), 333. https://dx.doi.org/10.3390/antibiotics10030333

Pawlak, A., Morka, K., Bury, S., Antoniewicz, Z., Wzorek, A., Cieniuch, G., Korzeniowska-Kowal, A., Cichoń, M. & Bugla-Płoskońska, G. (2020) Cloacal Gram-Negative Microbiota in Free-Living Grass Snake *Natrix natrix* from Poland. *Current Microbiology*, 77 (9), 2166–2171. https://dx.doi.org/10.1007/s00284-020-02021-3

Pees, M., Rabsch, W., Plenz, B., Fruth, A., Prager, R., Simon, S., Schmidt, V., Munch, S. & Braun, P. (2013) Evidence for the transmission of *Salmonella* from reptiles to children in Germany, July 2010 to October 2011. *Eurosurveillance*, 18 (46), 20634.

Piasecki, T., Chrząstek, K. & Wieliczko, A. (2014) *Salmonella* serovar spectrum associated with reptiles in Poland. *Acta Veterinaria Brno*, 83 (4), 287–294. https://dx.doi.org/10.2754/avb201483040287

Romero, S., Čížek, A., Masaříková, M. & Knotek, Z. (2015) Choanal and cloacal aerobic bacterial flora in captive green iguanas: a comparative analysis. *Acta Veterinaria Brno*, 84 (1), 19–24.

https://dx.doi.org/10.2754/avb201584010019
Rose, K., Agius, J., Hall, J., Thompson, P., Eden, J. S., Srivastava, M., Tiernan, B., Jenkins, C. & Phalen, D. (2017) Emergent multisystemic *Enterococcus* infection threatens endangered Christmas Island reptile populations. *PloS One*, 12 (7), e0181240. https://dx.doi.org/10.1371/journal.pone.0181240
Sayyadi, F., Rastegar-Pouyani, N., Azadbakht, M. & Chehri, K. (2019) First comprehensive report of bacteria spp. associated with cloaca of *Laudakia nupta* (Sauria: Agamidae) in Iran using molecular studies. *Laboratory Animal Research*, 35, 3. https://dx.doi.org/10.1186/s42826-019-0001-5
Schmidt, V., Mock, R., Burgkhardt, E., Junghanns, A., Ortlieb, F., Szabo, I., Krautwald-Junghanns, M. (2014) Cloacal aerobic bacterial flora and absence of viruses in free-living slow worms (*Anguis fragilis*), grass snakes (*Natrix natrix*) and European Adders (*Vipera berus*) from Germany. *EcoHealth*, 11 (4), 571–580. https://dx.doi.org/10.1007/s10393-014-0947-6
Schröter**,** M., Roggentin, P., Hofmann, J., Speicher, A., Laufs, R. & Mack, D. (2004) Pet snakes as a reservoir for *Salmonella enterica subsp. diarizonae* (Serogroup IIIb): a prospective study. *Applied and Environmental Microbiology*, 70 (1), 613–615. https://dx.doi.org/10.1128/AEM.70.1.613-615.2004
Schröter, M., Heckers, K., Rüschoff, B., Laufs, R. & Mack, D. (2005) Severe case of osteomyelitis due to *Enterococcus* spp. in a three-year old rhinoceros horned viper, *Bitis nasicornis. Journal of Herpetological Medicine and Surgery*, 15 (5), 3–56. https://dx.doi.org/10.5818/1529-9651.15.3.53
Silvestre, M., Silveira, L., Mateo, J., Urioste, J., Rodríguez Domínguez, M. & Pether, J. (2003) Cloacal microbiology in threatened captive giant lizards from the Canary Islands (genus *Gallotia*). *Revista Espanola de Herpetologia*, 17, 29–37.
Singh, B., Singh, V., Ebibeni, N. & Singh, R. (2013) Antimicrobial and herbal drug resistance in enteric bacteria isolated from faecal droppings of Common House Lizard/Gecko (*Hemidactylus frenatus*). *International Journal of Microbiology*, 2013, 340848. https://dx.doi.org/10.1155/2013/340848
Singh, B., Singh, V., Ebibeni, N. & Singh, R. (2014) Maternal transfer of bacteria to eggs of common house gecko (*Hemidactylus frenatus*). *Journal of Microbiology Research*, 4 (2), 78–85. https://dx.doi.org/10.5923/j.microbiology.20140402.06
Snopková, K., Sedlář, K., Bosák, J., Chaloupková, E., Sedláček, I., Provazník, I., & Šmajs, D. (2017) Free-living enterobacterium *Pragia fontium* 24613: Complete genome sequence and metabolic profiling. *Evolutionary Bioinformatics*, 13, 1176934317700863. https://dx.doi.org/10.1177/1176934317700
Vacheva, E. (2021) Relationships between Representatives of Three Lizard Families—Anguidae, Lacertidae and Scincidae (Reptilia: Squamata: Sauria), in Regard to Their Spatial and Dietary Niches in Western Bulgaria. Ph.D. Thesis, National Museum of Natural History—Bulgarian Academy of Sciences, Sofia, Bulgaria. (In Bulgarian)
Wang, J. & Chang, S. (2016). *Citrobacter* species, Antimicrobe. http://www.antimicrobe.org/b93.asp (April 2024).
Wang, Q., Zhang, L., Zhang, Y., Chen, H., Song, J., Lyu, M., Chen, R. & Zhang, L. (2022) Comparative genomic analyses reveal genetic characteristics and pathogenic factors of *Bacillus pumilus* HM-7. *Frontiers in Microbiology*, 7 (13), 1008648. https://dx.doi.org/10.3389/fmicb.2022.1008648
Whiley, H., Gardner, M. & Ross, K. (2017) A review of *Salmonella* and squamates (lizards, snakes and amphisbians): implications for public health. *Pathogens*, 6 (3), 38. https://dx.doi.org/10.3390/pathogens6030038
White, J., Richard, M., Massot, M. & Meylan, S. (2011) Cloacal bacterial diversity increases with multiple mates: evidence of sexual transmission in female common lizards. *PLoS One*, 6 (7), e22339. https://dx.doi.org/10.1371/journal.pone.0022339
Xiao, Z., Li, X., Xue, M., Zhang, M., Liu, W., Fan, Y., Chen, X., Chu, Z., Gong, F., Zeng, L., & Zhou, Y. (2022) *Vibrio metschnikovii*, a potential pathogen in freshwater-cultured hybrid sturgeon. *Animals*, 12 (9),1101. https://dx.doi.org/10.3390/ani12091101

Zając, M., Wasyl, D., Różycki, M., Bilska-Zając, E., Fafński, Z., Iwaniak, W., Krajewska, M., Hoszowski, A., Fafńska, P. & Szulowski, K. (2016) Free-living snakes as a source and possible vector of *Salmonella* spp. and parasites. *European Journal of Wildlife Research*, 62, 161–166.

Zheng, L., Zhu, L-W., Jing, J., Guan, J., Lu, G., Xie, L., Ji, X., Chu, D., Sun, Y., Chen, P. & Guo, X-J. (2021) Pan-genome analysis of *Vibrio cholerae* and *Vibrio metschnikovii* strains isolated from migratory birds at Dali Nouer Lake in Chifeng, China. *Frontiers in Veterinary Science*, 8, 638820. https://dx.doi.org/10.3389/fvets.2021.638820

Ziogou, A., Giannakodimos, I., Giannakodimos, A., Baliou, S. & Ioannou, P. (2023) *Kocuria* species infections in humans-a narrative review. *Microorganisms*, 11 (9), 2362. https://dx.doi.org/10.3390/microorganisms11092362