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Seasonal and altitudinal variation of gut microbiota in Alpine Accentor (*Prunella collaris*) in the Western Carpathians

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Abstract. This study investigated seasonal and altitudinal variation in the gut microbiota of the Alpine Accentor (*Prunella collaris*) in the Western Carpathians during 2022–2025. Fecal samples were collected from alpine breeding sites (1600–2600 m a.s.l.) and lowland overwintering sites in southern Slovakia (400–500 m a.s.l.). Samples were cultured on non-selective and selective media (CIN, ENDO, XLD, and blood agar) and evaluated using the ENTEROtest 24N kit. The most prevalent bacterial genera were *Serratia* spp. (27.14%), *Enterobacter* spp. (20.71%), Gram-positive bacteria (10.71%), *Yersinia* spp. (7.14%), and *Pantoea* spp. (4.29%). A significant seasonal variation was observed, with the highest bacterial occurrence in autumn. No statistically significant relationship was found between altitude and bacterial presence. These findings highlight the influence of environmental seasonality on gut microbiota composition in Alpine Accentor.

Key words: Prunella collaris, gut microbiota, *Enterobacter*iaceae, alpine environment, seasonality, Western Carpathians

Introduction

Gut microbiota play a crucial role in avian health, influencing digestion, immunity, and adaptation to environmental stressors (Grond *et al.* 2018; Baldelli *et al.* 2021). Members of the family *Enterobacter*iaceae are common in avian intestines and are known to interact with the host immune system and participate in metabolic processes (Janda and Abbott 2021). The Alpine Accentor (*Prunella collaris*), a high-altitude species, inhabits harsh alpine environments characterized by temperature extremes, high UV exposure, and limited food availability (Carey and Morton 1976). Seasonal and elevational changes in diet and habitat may therefore influence intestinal microbial communities and their ecological functions (Zilber-Rosenberg and Rosenberg 2008).

Previous studies have documented seasonal shifts in gut microbiota among mountain and migratory birds (Grond *et al.* 2018; Liukkonen *et al.* 2024), suggesting that microbial composition reflects ecological adaptation. However, information on high-altitude passerines of the Western Carpathians remains limited. This study aims to assess the effects of season and altitude on gut microbiota composition in *P. collaris* populations, hypothesizing that (1) bacterial communities vary seasonally, and (2) altitude influences the diversity of gut bacteria.

Material and Methods

Study area and sampling

Fecal samples of *P. collaris* were collected from the High Tatras (Western Carpathians) and overwintering sites at Šomoška and Jasenov castle ruins in southern Slovakia (Fig. 1). Samples were collected at altitudes of 1600–2600 m a.s.l. (breeding season) and 400–500 m a.s.l. (winter). A total of 140 samples were obtained during 2022–2025, using sterile tubes, and stored at 4 ˚C until they were processes in the laboratory, within 24 hours.

Laboratory analysis

Samples were inoculated in a liquid nutrient medium and incubated at 22–24 ˚C for 48 hours. After bacterial growth, cultures were transferred to selective media (CIN, ENDO, XLD, and blood agar) and incubated at 37 ˚C for 24–30 hours. Isolated colonies were identified using ENTEROtest 24N, Indole, OXI, VP, and OF tests.

Statistical analysis

Data were analyzed using Statistica 10 (StatSoft Inc., Tulsa, USA). Normality was tested by the Shapiro-Wilk test. Due to non-normal data distribution, non-parametric tests were used. The Kruskal-Wallis test examined altitude effects on bacterial abundance, while the Pearson's Chi-square test compared bacterial occurrence across seasons.

Results

A total of 233 isolates were obtained. *Serratia* spp. and *Enterobacter* spp. dominated the samples. The high-

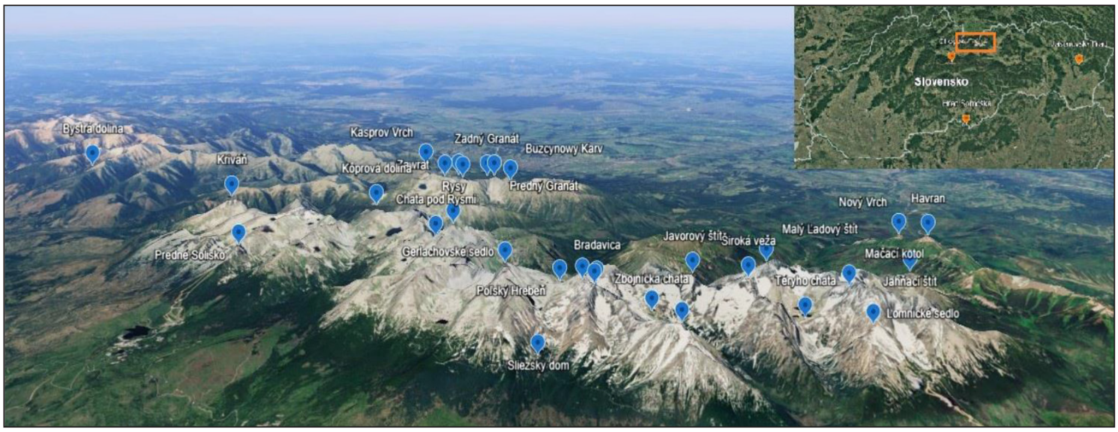

Fig. 1. Sampling sites (Source: Google Earth).

est sample positivity occurred in autumn (82 positive samples), followed by summer (26 positive samples), winter (18 positive samples), and spring (16 positive samples). The Chi-square test ($p = 0.00003$) indicated significant seasonal variation in bacterial occurrence (Table 1, Fig. 2), whereas altitude showed no significant influence (Kruskal–Wallis $H = 5.99$; $p = 0.74$).

Genus	Season	N	χ^2	P value
Enterobacter	Spring	1	48.1665	0.00003
	Summer	7		
	Autumn	11		
	Winter	9		
Pantoea	Spring	0		
	Summer	1		
	Autumn	2		
	Winter	3		
Serratia	Spring	0		
	Summer	3		
	Autumn	30		
	Winter	5		
Yersinia	Spring	2		
	Summer	3		
	Autumn	5		
	Winter	0		
G+	Spring	7		
	Summer	2		
	Autumn	6		
	Winter	0		

Table 1. Seasonal prevalence of bacterial genera detected in fecal samples of *Prunella collaris* (N = 140). Significant seasonal differences were confirmed by Pearson's Chi-square test ($p = 0.00003$).

Enterobacter spp. were present year-round, whereas *Serratia* spp. were observed most frequently in autumn. *Yersinia* spp. occurred primarily in summer and autumn. Gram-positive bacteria were absent in winter. These findings indicate clear seasonal trends in microbial prevalence linked to temperature and food availability (Table 2). No statistically significant relationship between altitude and bacterial abundance was detected (Table 3).

Discussion

The present study provides new insights into the seasonal and altitudinal variability of gut microbiota in the Alpine Accentor (*Prunella collaris*). The data revealed that bacterial community composition is strongly affected by season, while altitude had no

Positivity	Season	N	χ^2	P value
Positive	Spring	16	1.4156	0.7019
	Summer	26		
	Autumn	82		
	Winter	18		
Negative	Spring	8		
	Summer	17		
	Autumn	58		
	Winter	8		

Table 2. Comparison of positive and negative fecal samples of *Prunella collaris* by season (N = 233). No significant seasonal difference was detected ($p = 0.70$).

Variables	N	Statistical method (p value)
Bacterial genera vs. Altitude	140	K-W H test = 5.99 (0.74)

Table 3. Relationship between bacterial genera abundance and altitude (Kruskal–Wallis $H = 5.99$; $p = 0.74$). No significant correlation was found (N = number of samples).

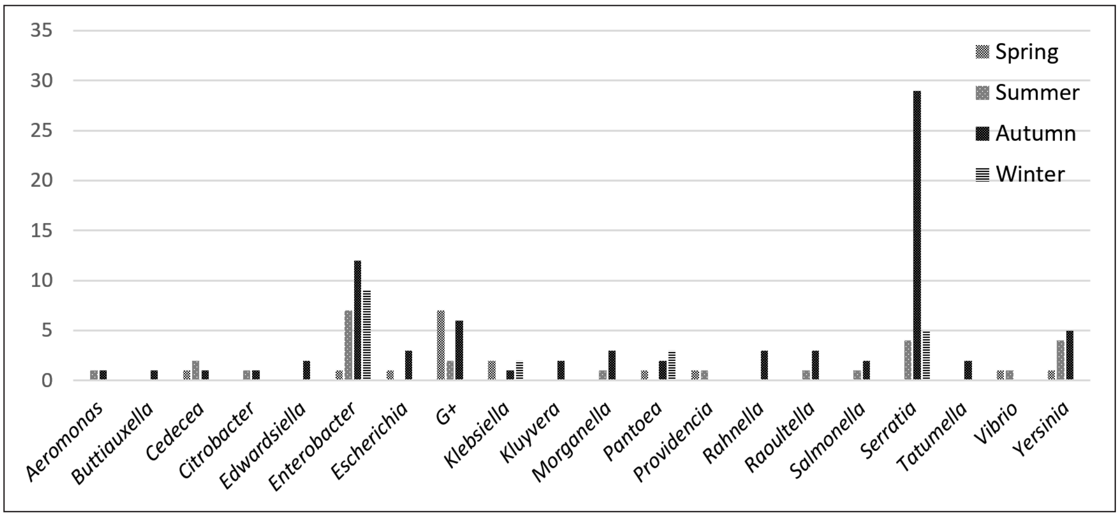

Fig. 2. Percentage of positive fecal samples for each bacterial genus of *Prunella collaris* across seasons (spring, summer, autumn, winter). The highest positivity was recorded in autumn.

significant effect. This finding aligns with previous studies on alpine and migratory birds, which also demonstrated that diet and environmental factors associated with seasonality play a dominant role in shaping intestinal microbiota (Grond *et al.* 2018; Liukkonen *et al.* 2024).

Seasonal dynamics

The significant seasonal differences observed in this study (p = 0.00003) suggest that microbial community structure in *P. collaris* is highly responsive to seasonal shifts in food sources and environmental conditions. During autumn, the highest bacterial positivity, and the dominance of *Serratia* spp. were recorded. The genus *Serratia* is known to thrive in moist environments and has been associated with decaying plant material and seed surfaces (Grimont and Grimont 2006; Kulkova *et al.* 2024). As *P. collaris* shifts its diet towards seeds and plant material in autumn, this dietary change promotes *Serratia* proliferation in the gut. Similar seasonal enrichment of *Enterobacter*iaceae has been reported in other alpine birds, reflecting changes in nutrient intake and microbial exposure (Tang *et al.* 2023).

Conversely, *Enterobacter* spp. were consistently detected throughout all seasons, indicating that they represent a stable and functionally important component of the gut microbiota. Members of this genus play essential roles in nitrogen cycling and energy metabolism and have been observed in feces of other granivorous passerines (Glünder 1981). Their persistence may also be linked to their ability to tolerate temperature fluctuations and utilize diverse substrates.

Yersinia spp. were observed during summer and autumn. The presence of *Yersinia enterocolitica* and related species in wild birds has been widely documented (Niskanen *et al.* 2003; Novotný *et al.* 2007), suggesting that Alpine Accentors could serve as potential reservoirs for these bacteria. The absence of *Yersinia* in winter may be due to temperature constraints or changes in the feeding ecology of the species. Gram-positive bacteria exhibited moderate seasonal variability, occurring mostly in spring and autumn. Their prevalence may be related to the availability of specific carbohydrate substrates or host immune modulation during these transitional periods (Baldelli *et al.* 2021).

Altitudinal influence

Although this study found no statistically significant relationship between altitude and bacterial occurrence (p = 0.74), previous research has demonstrated that high-altitude environments can influence gut microbial diversity through selective pressures such as hypoxia, temperature, and UV radiation (Simonson *et al.* 2010; Beall 2011). Liu *et al.* (2022) observed that while microbial diversity decreases at higher altitudes, functional diversity may increase, reflecting adaptive specialization to harsh conditions. The absence of altitudinal effects in this study could result from the narrow elevational gradient, or the high mobility of *P. collaris*, which may homogenize microbial communities across sites.

Ecological and adaptive implications

The observed seasonal variation in microbiota composition supports the hypothesis that gut microbial communities are dynamic and adapt to environmental changes. This flexibility contributes to physiological resilience in high-altitude birds, aiding digestion efficiency, immune regulation, and energy metabolism under fluctuating conditions (Zilber-Rosenberg and Rosenberg 2008; Zhang *et al.* 2016). The high autumnal bacterial load may reflect preparatory physiological adaptations for overwintering, where increased microbial diversity enhances metabolic efficiency.

The predominance of *Serratia* and *Enterobacter* spp. further suggests that the gut microbiota of *P. collaris* may play an important ecological role in nutrient cycling within alpine habitats, as these bacteria are known for their roles in organic matter decomposition and heavy metal tolerance (Korzeniowska and Krąż 2020; El-Beltagi *et al.* 2024). This ecological link between microbial activity and habitat quality warrants further exploration.

Future perspectives

Future research should employ molecular approaches such as 16S rRNA sequencing or metagenomic profiling to identify microbial taxa more precisely, and to assess their functional roles. Expanding sampling across multiple years and mountain ranges could reveal long-term patterns of microbiome adaptation. Additionally, experimental studies on dietary manipulation or environmental stress could help clarify causal relationships between ecological variables and microbial structure.

Conclusion

Seasonal variation strongly affects gut microbiota in the Alpine Accentor, with the highest bacterial prevalence observed in autumn. No significant effect of altitude was found. The dominance of *Serratia* and *Enterobacter* spp. highlights their ecological role in alpine passerine microbiomes. Broader spatial and temporal sampling, combined with molecular analyses, is recommended to understand the adaptive significance of gut microbiota in alpine birds.

References

Baldelli, V., Scaldaferri, F., Putignani, L. and Del Chierico, F. 2021: The role of *Enterobacter*iaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms*, **9**: 697. https://doi.org/10.3390/microorganisms9040697

Beall, C.M. 2011: Genetic changes in Tibet. *High. Alt. Med. Biol.*, **12**: 101–102. https://doi.org/10.1089/ham.2011.1007

Carey, C. and Morton, M.L. 1976: Aspects of circulatory physiology of montane and lowland birds. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **54**: 61–74. https://doi.org/10.1016/S0300-9629(76)80073-4

El-Beltagi, H.S., Halema, A.A., Almutairi, Z.M., Almutairi, H.H., Elarabi, N.I., Abdelhadi, A.A., Henawy, A.R. and Abdelhaleem, H.A. 2024: Draft genome analysis for *Enterobacter kobei*, a promising lead bioremediation bacterium. *Front. Bioeng. Biotechnol.*, **11**: 1335854. https://doi.org/10.3389/fbioe.2023.1335854

Glünder, G. 1981: Occurrence of *Enterobacter*iaceae in feces of granivorous passeriform birds. *Avian Dis.*, **25**: 195–198.

Grimont, F. and Grimont, P.A. 2006: The genus *Serratia*. *Prokaryotes*, **6**: 44. https://doi.org/10.1146/annurev.mi.32.100178.001253

Grond, K., Sandercock, B.K., Jumpponen, A. and Zeglin, L.H. 2018: The avian gut microbiota: community, physiology, and function in wild birds. *J. Avian Biol.*, **49**: 01788. https://doi.org/10.1111/jav.01788

Janda, J.M. and Abbott, S.L. 2021: The changing face of the family *Enterobacter*iaceae (Order: "*Enterobacter*ales"): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. *Clin. Microbiol. Rev.*, **34**: 1128. https://doi.org/10.1128/CMR.00174-20

Korzeniowska, J. and Krąż, P. 2020: Heavy Metals Content in the Soils of the Tatra National Park Near Lake Morskie Oko and Kasprowy Wierch - A Case Study (Tatra Mts, Central Europe). *Minerals*, **10**: 1120. https://doi.org/10.3390/min10121120

Kulkova, I., Wróbel, B. and Dobrzyński, J. 2024: *Serratia* spp. as plant growth-promoting bacteria alleviating salinity, drought, and nutrient imbalance stresses. *Front. Microbiol.*, **15**: 1342331. https://doi.org/10.3389/fmicb.2024.1342331

Liu, G., Li, C., Liu, Y., Zheng, C., Ning, Y., Yang, H., Suo, L., Qi, X., Li, H., Wen, W., Wang, Y., Qi, H., Cai, P. and Gong, M. 2022: Highland adaptation of birds on the Qinghai Tibet Plateau via gut microbiota. *Appl. Microbiol. Biotechnol.*, **106**: 6701–6711. https://doi.org/10.1007/s00253-022-12171-y

Liukkonen, M., Muriel, J., Martínez-Padilla, J., Nord, A., Pakanen, V.M., Rosivall,B., Tilgar, V., van Oers, K., Grond, K. and Ruuskanen, S. 2024: Seasonal and environmental factors contribute to the variation in the gut microbiome: A large-scale study of a small bird. *J. Anim. Ecol.*, **93**: 1475–1492. https://doi.org/10.1111/1365-2656.14153

Niskanen, T., Waldenström, J., Fredriksson-Ahomaa, M., Olsen, B. and Korkeala, H. 2003: virF-positive *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* found in migratory birds in Sweden. *Appl. Environ. Microbiol.*, **69**: 4670–4675. https://doi.org/10.1128/AEM.69.8.4670-4675.2003

Novotný, M., Fečková, M., Janiga, M., Lukáň, M., Novotná, M. and Kovalčíková, Z. 2007: High incidence of *Yersinia enterocolitica* (*Enterobacter*iaceae) in Alpine Accentors *Prunella collaris* of the Tatra Mountains. *Acta Ornithol.*, **42**: 137–143. https://doi.org/10.3161/000164507783516872

Simonson, T.S., Yang, Y., Huff, C.D., Yun, H., Qin, G., Witherspoon, D.J., Bai, Z., Lorenzo, F.R., Xing, J., Jorde, L.B., Prchal, J.T. and Ge, R. 2010: Genetic evidence for high altitude adaptation in Tibet. *Science*, **329**: 72–75. https://doi.org/10.1126/science.1189406

Tang, K., Tao, L., Wang, Y., Wang, Q., Fu, C., Chen, B. and Fu, Y. 2023: Temporal variations in the gut microbiota of the globally endangered Sichuan partridge (*Arborophila rufipectus*): Implications for adaptation to seasonal dietary change and conservation. *Appl. Environ. Microbiol.*, **89**: e00747-23. https://doi.org/10.1128/aem.00747-23

Zhang, Q., Gou, W., Wang, X., Zhang, Y., Ma, J., Zhang, H. and Zhang, H. 2016: Genome resequencing identifies unique adaptations of Tibetan chickens to hypoxia and high-dose ultraviolet radiation in high-altitude environments. *Genome Biol. Evol.*, **8**: 765–776. https://doi.org/10.1093/gbe/evw032

Zilber-Rosenberg, I. and Rosenberg, E. 2008: Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.*, **32**: 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

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