

COMMENTARY TO HABILITATION THESIS¹

Name of the habilitation thesis: Gastrointestinal Parasites at the Human–Wildlife Interface: Diversity, Transmission and Consequences for Host Health

Name and surname of the applicant: Mgr. Barbora Pafčo, Ph.D.

Gastrointestinal parasites are integral components of natural ecosystems and form long-standing associations with their hosts. They include organisms that persist with little apparent effect on host health, as well as important pathogens capable of causing severe disease. Individual hosts frequently harbour multiple parasite taxa simultaneously, forming complex communities whose composition reflects the interplay between parasite biology, host evolutionary history, ecology and environmental exposure. Understanding these communities therefore requires moving beyond the simple detection of infection towards identifying which parasites are present, which hosts they infect, what determines their distribution, and under which circumstances infection has consequences for host health.

These questions become particularly important at the human–wildlife interface, where humans, wildlife and domestic animals increasingly share landscapes and resources. For many gastrointestinal parasites, transmission does not require direct contact between hosts, as infective stages may persist in soil, vegetation, food or water. Non-human primates provide a particularly informative model for investigating these processes because of their close evolutionary relationship with humans, differences in ecology and behaviour, and varying degrees of contact with human populations. At the same time, many primate species are endangered, making parasite transmission relevant not only to public health but also to wildlife conservation.

My research has focused on gastrointestinal parasites, particularly strongylid nematodes and genus *Strongyloides*, their genetic diversity, host specificity, transmission ecology and potential health consequences. A central question throughout this work has been how parasite diversity and host associations can be reliably characterised, especially when conventional diagnostic methods provide insufficient taxonomic resolution. This has gradually led to broader questions concerning parasite circulation among host species, the effects of ecological and anthropogenic changes on parasite communities, and the circumstances under which infections that are apparently well tolerated may become associated with disease.

The presented habilitation thesis brings together 26 scientific publications addressing these questions in different host species and ecological settings. The research is organised into four interconnected thematic areas: parasite diversity and the development and application of molecular approaches; host specificity, parasite sharing and zoonotic potential; ecological and anthropogenic drivers of parasite communities; and the health consequences of gastrointestinal infections in wild primates. Particular attention is given to African great apes and human populations living in or near their habitats, although the work also includes other primates, domestic animals and parasite systems outside Africa.

An important methodological component of my research has been the combination of classical parasitological approaches with molecular identification and high-throughput sequencing. Microscopy, coproscopic examinations and larval cultivation remain essential for detecting parasite stages and assessing infection intensity, but frequently provide insufficient resolution

¹ The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

for distinguishing closely related species or mixed infections. The application of targeted molecular markers and metabarcoding, particularly ITS-2 sequencing of strongylid nematodes and 18S rRNA and mitochondrial markers for *Strongyloides*, has made it possible to characterise parasite communities and genetic lineages that would otherwise remain unresolved. These approaches have been complemented by phylogenetic analyses, statistical evaluation of parasite communities, ecological and spatial data, and, where available, information on host health. More recently, our research has also begun moving towards genome-wide approaches to address questions that cannot be adequately resolved using individual genetic markers.

The first part of the thesis demonstrates how increasing molecular resolution has changed our understanding of gastrointestinal parasite diversity. Our studies revealed complex strongylid communities in African great apes and humans, including considerable genetic variation within genera *Oesophagostomum* and *Necator*. Comparisons with primates from South-East and East Asia showed markedly different community structures and patterns of genetic diversity. We also applied metabarcoding beyond wildlife systems to investigate strongylid communities and their responses to anthelmintic treatment in cattle. At the same time, these studies highlighted important methodological limitations, particularly the influence of marker selection, amplification biases and incomplete reference databases. Increased molecular resolution does not automatically provide reliable species identification, and molecular findings must therefore be interpreted in relation to parasite taxonomy and biology.

The second part focuses on host specificity and parasite circulation among host species. Our studies in the Dzanga-Sangha Protected Areas in the Central African Republic demonstrated that strongylid communities are shaped not only by host evolutionary relationships but also by ecological opportunities for transmission. In particular, humans spending substantial time in the forest shared more similarities in their strongylid communities with gorillas than did people living predominantly in villages. Molecular analyses also revealed the occurrence of closely related parasite lineages across different host species, including *Necator* and *Strongyloides*. Studies involving humans, non-human primates, dogs and other mammals further demonstrated the importance of considering parasite transmission within multihost systems rather than as isolated relationships between two host species. Nevertheless, the identification of identical or closely related parasite sequences in different hosts does not necessarily demonstrate transmission, and important questions remain concerning its direction, frequency and epidemiological significance.

The third part examines how ecological and anthropogenic factors influence gastrointestinal parasite communities. Our research showed that habitat disturbance may be associated with a reduction in gastrointestinal symbiont diversity, but also that the effects of human activities are not uniform across parasite taxa or host populations. Studies of African primates demonstrated differences in parasite occurrence and community composition associated with habitat conditions, host ecology and the degree of contact with humans. In mountain gorillas, we investigated spatial variation in helminth infections and the potential relationships between environmental conditions, physiological stress and parasite dynamics. Together, these findings indicate that parasite communities should not be viewed as static characteristics of their hosts, but as dynamic outcomes of interactions among hosts, parasites and their environments.

The final part addresses a question that has become increasingly important in my research: when and why does an established gastrointestinal infection become associated with disease? This question is particularly relevant for mountain gorillas, where severe gastrointestinal health problems have been observed in association with strongylid infections. Our studies in the Virunga Massif identified changes in strongylid communities associated with clinical disease and investigated the possible role of environmental stressors. Analyses of historical samples provided an opportunity to examine how parasite communities and host–parasite relationships

have changed over time. Research in Bwindi Impenetrable National Park revealed a different pattern of strongylid-associated health problems, further illustrating that similar parasite groups may have different consequences in closely related host populations. These findings are especially interesting when compared with western lowland gorillas, in which some of the same parasite genera occur commonly without comparable clinical problems. However, the mechanisms underlying these differences remain unresolved, and associations between parasite infection and disease should not automatically be interpreted as evidence of causation.

Taken together, the publications presented in this thesis demonstrate the importance of integrating parasite identification with host ecology, environmental conditions and health-related information. They also highlight several questions that remain central to my ongoing research. We still have limited knowledge of which hosts maintain the transmission of many gastrointestinal parasites, how frequently parasite lineages move between host species, and why infections that appear well tolerated in some populations are associated with severe disease in others. Addressing these questions will require further integration of molecular and genome-wide approaches with ecological studies and information on host responses. This is particularly important for understanding the emergence of pathogenic parasite populations and for developing more effective approaches to disease management in endangered wildlife.

The broader conclusion of this habilitation is that gastrointestinal parasites need to be studied as part of dynamic systems involving multiple hosts and their shared environments. A One Health approach provides a useful framework for bringing these components together, but its successful application depends on understanding the biology of the parasites themselves. By combining increasingly detailed molecular identification with ecological and health-related information, we can move beyond describing parasite diversity towards a better understanding of the processes that determine parasite transmission and its consequences.

The publications included in this habilitation thesis represent research carried out in collaboration with colleagues and students from different institutions and countries. My contribution has varied among individual studies and has included the development of research questions and study design, field sampling, parasitological and molecular laboratory work, data analysis and interpretation, supervision of students and research activities, and preparation of manuscripts. In several publications, particularly those arising from research projects that I initiated and supervised, I was responsible for the overall scientific direction and coordination of the work. In other studies, I contributed specific methodological expertise, laboratory analyses or interpretation of parasitological findings within larger collaborative projects. The following section therefore specifies my individual contribution to each of the 26 publications in terms of both its scientific content and the activities undertaken.

[1]² Vlčková K., Kreisinger J., Pafčo B., Čížková D., Tagg N., Hehl A. B., Modrý D. (2018). Diversity of *Entamoeba* spp. in African great apes and humans: an insight from Illumina MiSeq high-throughput sequencing. *International Journal for Parasitology*, 48(7): 519–530.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
40	15	20	20

² Bibliographic record of a published scientific result, which is part of the habilitation thesis.

[2] Pafčo B., Kreisinger J., Čížková D., Pšenková-Profousová I., Shutt-Phillips K., Todd A., Fuh T., Petrželková K. J., Modrý D. (2019). Genetic diversity of primate strongylid nematodes: Do sympatric nonhuman primates and humans share their strongylid worms?. *Molecular Ecology*, 28(21): 4786–4797.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
80	20	70	50

[3] Barelli C., Pafčo B., Manica M., Rovero F., Rosà R., Modrý D., Haufe H.C. (2020). Loss of protozoan and metazoan intestinal symbiont biodiversity in wild primates living in unprotected forests. *Scientific Reports*, 10: 10917.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
35	25	35	10

[4] Bradbury R.S., Pafčo B., Nosková E., Hasegawa H. (2021). *Strongyloides* genotyping: a review of methods and application in public health and population genetics. *International Journal for Parasitology*, 51: 1153–1166.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	45	40	45

[5] Shutt-Phillips K., Pafčo B., Heistermann M., Kasim A., Petrželková K. J., Profousová-Pšenková I., Modrý D., Todd A., Fuh T., Dicky J.-F., Bopalanzognako J.-B., Setchell J. M. (2021). Fecal glucocorticoids and gastrointestinal parasite infections in wild western lowland gorillas (*Gorilla gorilla gorilla*) involved in ecotourism. *General and Comparative Endocrinology*, 312: 113859.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
65	10	45	30

[6] Petrželková K. J., Uwamahoro C., Pafčo B., Červená B., Samaš P., Mudakikwa A., Muvunyi R., Uwingeli P., Gilardi K., Nziza J., Noheri J. B., Eckardt W., Ndagijimana F., Ssebide B., Okwirokello R., Nizeyimana F., Kambale Syaluha E., Nzayisenga G., Flores Girón L., Bahizi M., Ntwari A. E., Lukusa J.-P., Tumushime J. C., Mangura D., Mapilanga J., Kalonji A., Aruho R., Stryková A., Tehlarová Z., Cameira R., Lowenstine L., Šlapeta J., Romportl D., Ferrari N., Cranfield M., Modrý D. (2021). Heterogeneity in patterns of helminth infections across populations of mountain gorillas (*Gorilla beringei beringei*). *Scientific Reports*, 11: 10869.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	10	25	25

[7] Mason B., Piel A.K., Modrý D., Petrželková K.J., Stewart F.A., Pafčo B. (2022). Association of human disturbance and gastrointestinal parasite infection of yellow baboons in western Tanzania. *PLOS ONE*. 17: e0262481.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
40	50	45	40

- [8]** Mason B., Petrželková K. J., Kreisinger J., Bohm T., Cervena B., Fairet E., Fuh T., Gomez A., Knauf S., Maloueki U., Modry D., Shirley M. H., Tagg N., Wangue N., Pafco B. (2022). Gastrointestinal symbiont diversity in wild gorilla: A comparison of bacterial and strongylid communities across multiple localities. *Molecular Ecology*, 31(15): 4127–4145.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	80	40	75

- [9]** Petrželková K. J., Samaš P., Romportl D., Uwamahoro C., Červená B., Pafčo B., Prokopová T., Cameira R., Granjon A. C., Shapiro A., Bahizi M., Nziza J., Noheri J. B., Syaluha E. K., Eckardt W., Ndagijimana F., Šlapeta J., Modrý D., Gilardi K., Muvunyi R., Uwingeli P., Mudakikwa A., Mapilanga J., Kalonji A., Hickey J. R., Cranfield M. (2022). Ecological drivers of helminth infection patterns in the Virunga Massif mountain gorilla population. *International Journal for Parasitology: Parasites and Wildlife*, 17: 174–184.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	10	20	20

- [10]** Ilík V., Kreisinger J., Modrý D., Schwarz E. M., Tagg N., Mbohli D., Nkombou I.C., Petrželková K. J., Pafčo, B (2023). High diversity and sharing of strongylid nematodes in humans and great apes co-habiting an unprotected area in Cameroon. *PLoS Neglected Tropical Diseases*, 17: e0011499.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	80	40	75

- [11]** Nosková E., Modrý D., Baláž V., Červená B., Jirků-Pomajbíková K., Zechmeisterová K., Leowski C., Petrželková K. J., Pšenková I., Vodička R., Kessler S. E., Ngoubangoye B., Setchell J. M., Pafčo B. (2023). Identification of potentially zoonotic parasites in captive orangutans and semi-captive mandrills: Phylogeny and morphological comparison. *American Journal of Primatology*, 85(4): e23475.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	80	40	75

- [12]** Mason B., Cervena B., Frias L., Goossens B., Hasegawa H., Keuk K., Langgeng A., Majewski K., Matsumoto T., Matsuura K., Mendonça R., Okamoto M., Peter S., Petrzelkova K. J., Sipangkui S., Xu Z., Pafco B., MacIntosh A. J. J. (2024). Novel insight into the genetic diversity of strongylid nematodes infecting South-East and East Asian primates. *Parasitology*, 151(5): 514–522.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
35	60	40	40

[13] Pafčo B., Nosková E., Ilík V., Anettová L., Červená B., Kreisinger J., Pšenková I., Václavek P., Vyhlídalová T., Ježková J., Malát K., Mihalca A. D., Modrý, D (2024). First insight into strongylid nematode diversity and anthelmintic treatment effectiveness in beef cattle in the Czech Republic explored by HTS metagenomics. *Veterinary Parasitology: Regional Studies and Reports*, 47: 100961.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
75	30	80	60

[14] Nosková E., Svobodová V., Hyská V., Cerezo-Echevarria A., Kurucová T., Ilík V., Modrý D., Pafčo, B (2024). High-throughput sequencing of *Strongyloides stercoralis* - a fatal disseminated infection in a dog. *Parasitology*, 151: 587–593.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	80	35	40

[15] Ilík V., Schwarz E. M., Nosková E., Pafčo B. (2024). Hookworm genomics: dusk or dawn. *Trends in Parasitology*, 40: 452–465.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	75	30	45

[16] Nosková E., Sambucci K. M., Petrželková K. J., Červená B., Modrý D., Pafčo, B (2024). *Strongyloides* in non-human primates: significance for public health control. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 379: 20230006.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	75	45	80

[17] Červená B., Prokopová T., Cameira R. M., Pafčo B., Samaš P., Romportl D., Uwamahoro C., Noheri J. B., Ntwari A. E., Bahizi M., Nzayisenga G., Nziza J., Gilardi K., Eckardt W., Ndagijimana F., Mudakikwa A., Muvunyi R., Uwingeli P., Cranfield M., Šlapeta J., Petrželková K. J., Modrý D. (2024). Anoplocephalid tapeworms in mountain gorillas (*Gorilla beringei beringei*) inhabiting the Volcanoes National Park, Rwanda. *Parasitology*, 151(2): 135–150.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	15	10	25

- [18]** Zhao H., Haidamak J., Nosková E., Ilík V., Pafčo B., Ford R., Masiria G., Maure T., Kotale N., Pomat W., Gordon C., Navarro S., Horwood P. F., Constantinoiu C., Greenhill A. R., Bradbury R.S (2025). Insights into Infant Strongyloidiasis, Papua New Guinea. *Emerging Infectious Diseases*, 31: 1793–1801.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	20	15	10

- [19]** Uwamahoro C., Holubová N., Jirků M., Sak B., Květoňová D., Samaš P., Červená B., Romportl D., Eckardt W., Ndagijimana F., Muvunyi R., Uwingeli P., Mapilanga J., Kalonji A., Aruho R., Cranfield M., Gilardi K., Nziza J., Ssebide B., Kambale Syaluha E., Pafčo B., Jirků K., Kváč M., Petrželková K. J. (2025). *Cryptosporidium*, Microsporidia and *Giardia* infections in endangered mountain gorillas inhabiting Virunga Massif. *Journal of Vertebrate Biology*, 74(25035):25035.1–15.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
15	5	10	10

- [20]** Mason B., Ilík V., Červená B., Cranfield M., Nziza J., Ssebide B., Gilardi K., Uwamahoro C., Eckardt W., Ndagijimana F., Muvunyi R., Uwingeli P., Mudakikwa A., Aruho R., Whittier C. A., Kreisinger J., Samaš P., Nosková E., Modrý D., Pafčo B., Petrželková K. J. (2025). Untangling parasite epidemiology of mountain gorillas through historical samples: Strongylid nematodes are friends or foe. *Biological Conservation*, 310: 111319.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
35	60	40	50

- [21]** Šlapeta J., Krücken J., Rojas A., Chambers A., Melville L. A., Martínez-Valladares M., Canton C., Francis E. K., Zahid O., Albuquerque A. C. A., Bartley D. J., Bassetto C. C., Byrne O., Colella V., Costa-Junior L. M., Doyle S. R., Evans M., Ghafar A., Godoy P., Hayashi N., Helal M. A., Huggins L. G., Jabbar A., Jones R. A., Karani B. E., Liron J. P., Maté L., McEvoy A., Mohammedsalih K. M., Mulcahy G., Nielsen M. K., Pafčo B., Peachey L. E., Robleto-Quesada J., von Samson-Himmelstjerna G., de Sousa-Paula L. C., Gilleard J. S. (2026). Ten simple rules for implementing deep amplicon sequencing in parasitology. *International Journal for Parasitology*, 56(6): 104751

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	0	5	10

[22] Upadhayay P., Kváč M., Sambucci K. M., Nosková E., Ilík V., Pafčo B., Holubova N., Červená B., Schoof V. A. M. (2026). Overlap in parasite communities among vervet monkeys, humans, livestock and dogs at Lake Nabugabo, Uganda. *Parasitology*, 153(6): 796–812.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
40	30	30	25

[23] Pafčo B., Kreisinger J., Romportl D., Mason B., Červená B., Uwamahoro C., Eckardt W., Ndagijimana F., Nziza J., Kambale Syaluha E., Ssebide B., Noheri J. B., Bahizi M., Okwirokello R., Nizeyimana F., Lukusa J.-P., Gilardi K., Muvunyi R., Uwingeli P., Mudakikwa A., Mapilanga J., Kalonji A., Aruho R., Hickey J., Šlapeta J., Modrý D., Lowenstine L., Hasegawa H., Cranfield M., Petrželková K. J. (2026). Emerging pathogenic strongylid infections in Virunga Massif mountain gorillas: Role of environmental stressors. *Journal of Applied Ecology*, 63(1): e70258.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
60	65	75	65

[24] Sambucci K. M., Samaš P., Ssebide B., Petrželková K. J., Okello R. O., Nizeyimana F., Bukamba N., Smiley-Evans T., Gilardi K., Pafčo B., Červená B. (2026). Shifts in strongylid communities associated with chronic wasting in mountain gorillas. *International Journal for Parasitology*, 56(7):104848.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
35	60	45	45

[25] Bukamba N., Sambucci K. M., Nosková E., Baláž V., Okello R. O., Muhumuza D. D., Nizeyimana F., Sargison N. D., Kelly R. F., Ssebide B., Evans T. S., Gilardi K., Petrželková K. J., Pafčo B., Červená B. (2026). The potential threat of *Strongyloides* spp. to mountain gorillas and public health in Bwindi Impenetrable National Park, Uganda. *Parasitology Research*, 125(1):52.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	5	10	10

[26] Nosková E., Ilík V., Niatou F. S. S., Dumas L., Fuh T., Dicky J. F., Kurucová T., Baláž V., Petrželková K. J., Pafčo B. (2026). *Strongyloides* Genetic Diversity among Humans, Dogs, and Nonhuman Primates, Central African Republic, 2016-2022. *Emerging Infectious Diseases*, 32(3):349–359.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
40	85	45	85