



First Detection and Molecular Characterization of *Colpodella* in Goats, Foxes, and Birds

Kyriacos A. Hasapis¹ · Iris Charalambidou² · Catherine O'Dowd Phanis¹ · Stefanie Kazamia¹ · Nicolaos Kassinis³ · Chad Schou¹ · Panagiotis Karanis^{1,4,5}

Received: 23 October 2024 / Accepted: 25 November 2024
© The Author(s) 2024

Abstract

Purpose This study aimed to evaluate the prevalence of *Colpodella* sp. in domestic and wild animals in Cyprus. To the authors' knowledge, this is the first study to report the detection of *Colpodella* sp. in foxes (Cyprus red fox *Vulpes vulpes indutus*), wild birds (Eurasian coot *Fulica atra*, duck *Anas* spp.) and goats (*Capra hircus*) worldwide.

Methods A total of 180 faecal samples (29 foxes, 48 Eurasian coot, 20 Eurasian teal *Anas crecca*, 7 duck, 44 goats – 10 from a farm and 34 free-living individuals – and 32 sheep *Ovis aries*) were analyzed for the parasite by nested PCR and sequencing.

Results Four samples were positive (4/180 = 2.2%), including one goat from a farm (1/10 = 10%), one fox (1/29 = 3.4%), one Eurasian coot (1/48 = 2.1%) and one duck (1/7 = 14.3%).

Conclusion The results of this study support the evidence that *Colpodella* sp. can infect mammals and birds, as well as livestock and wildlife, which could act as zoonotic reservoirs of the parasite and potentially pose a risk to human and animal health.

Keywords *Colpodella* sp. · Cyprus red fox · Eurasian coot · *Anas* sp. · Goats · Cyprus

Introduction

Colpodella are protists that mainly feed on other protists and algae. They are closely related to pathogenic Apicomplexa such as *Cryptosporidium*, *Plasmodium*, and *Toxoplasma gondii*. *Colpodella* sp. are considered to be free-living organisms with a pathogenic capability [1–5]. Recent studies have reported *Colpodella* sp. infections in humans and other

mammals. In two cases from China, *Colpodella* sp. caused severe disease in humans. In the first case, *Colpodella* sp. (isolate HEP) infected the red blood cells of an immunocompromised individual causing anaemia [6]. In the second case, the DNA of *Colpodella* sp. (isolate HLJ) was detected in the cerebrospinal fluid of a patient, causing a neurological disorder [7]. *Colpodella* sp. was also incidentally discovered in the urine of a 70-year-old female patient in Romania who was admitted to the hospital for breathing difficulties [8]. Additionally, *Colpodella* infection caused the death of a South China tiger (*Panthera tigris amoyensis*) in a zoo [9]. Reports of *Colpodella* sp. infections in other mammals are limited. *Colpodella* sp. has been isolated from the blood of two horses (*Equus ferus caballus*) (2/400 = 0.5%) in China [10] and one cow (*Bos taurus*) (1/232 = 0.4%) in Zambia [11], faecal samples of captive Felidae (7/56 = 12.5%) at a zoo in China [12] and ear fragments of Racoons (*Procyon lotor*) (3/170 = 1.8%) in Poland [13]. Moreover, two studies indicated that ticks transferred *Colpodella* sp. to the hosts [7, 9]. Another study found a high incidence of *Colpodella* sp. in ticks collected from goat flocks in China [14].

✉ Panagiotis Karanis
karanis.p@unic.ac.cy

¹ Department of Basic and Clinical Sciences, University of Nicosia Medical School, Nicosi 24005, Cyprus

² Department of Life Sciences, School of Life and Health Sciences, University of Nicosia, Nicosia, Cyprus

³ Game and Fauna Service, Nicosia, Cyprus

⁴ Medical Faculty and University Hospital, The University of Cologne, Cologne 50923, Germany

⁵ Department of Basic and Clinical Sciences, University of Nicosia Medical School, 21 Ilia Papakyriakou, 2414 Engomi, P.O. Box 24005, Nicosi CY-1700, Cyprus

In several studies, *Colpodella* sp. was accidentally detected following PCR amplification with primers specifically designed for *Cryptosporidium* sp. or for other parasites, targeting a part of the 18S rRNA gene [10, 12, 14–17]. Similarly, in the present study *Colpodella* sp. was accidentally detected by PCR and sequencing using specific primers designed for the detection of *Cryptosporidium* sp [18–20]. The present study aimed to evaluate the prevalence of *Colpodella* sp. in 180 animal faecal samples by nested PCR and sequencing.

The relatively wide distribution of the genus *Colpodella* sp. and the possibility of infection are a potential public health concern. Here, we provide evidence that this pathogenic protist can infect a great variety of animal hosts, therefore drawing attention to *Colpodella* sp. as an under-reported protist infection in humans, livestock, and wildlife.

Materials and Methods

Sample Collection

One hundred eighty animal faecal samples from livestock and wildlife were collected in Cyprus between January 2021 and April 2023 (Table 1). Specifically, 32 samples were collected from sheep (*Ovis aries*), 10 from goats (*Capra hircus*) at a farm in Famagusta District and 34 from free-ranging goats at Paphos State Forest (Paphos District). At Oroklini Lake (Larnaca District), 29 faecal samples of the Cyprus red fox (*Vulpes vulpes indutus*) were collected. All avian faecal samples were collected from Phassouri reedbeds at Akrotiri wetlands (Limassol District), including 48 Eurasian coot (*Fulica atra*), 20 Eurasian teal (*Anas crecca*)

Table 1 *Colpodella* positives from the analysis of faecal samples of livestock and wildlife in different areas in Cyprus

Area	Host Species name/ Common name	% positive for <i>Colpodella</i> spp. (No. positive/ No. sampled)
Farm, Famagusta District	<i>Ovis aries</i> Sheep	0 (0/32)
	<i>Capra hircus</i> Goat	10 (1/10)
	<i>Capra hircus</i> Goat (Free-ranging)	0 (0/34)
Paphos State Forest, Paphos District	<i>Vulpes vulpes indutus</i> Cyprus Red fox	3.4 (1/29)
Phassouri Reedbeds, Akrotiri Wetlands, Limassol District	<i>Fulica atra</i> Eurasian Coot	2.1 (1/48)
Limassol District	<i>Anas crecca</i> Eurasian Teal	0 (0/20)
	<i>Anas</i> spp. Duck	14.3 (1/7)
Total		2.2 (4/180)

and 7 duck sp. (*Anas* sp.). All faecal samples were collected from the ground while fresh and placed in sterile 50 ml tubes with animal species/group records, date, location and identification numbers. The samples were transferred to the laboratory on the same day and stored at -20°C until DNA extraction within three weeks after collection. A map containing the collection sites for every animal faecal sample is shown in Fig. 1.

DNA Extraction and PCR

According to the manufacturer's instructions, the total genomic DNA was extracted from the faecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). A nested Polymerase Chain Reaction (PCR) targeting a 600 bp fragment of the 18S rRNA gene of *Colpodella* sp. was performed. The primers used for the first amplification were SHP1 (forward) 5'-ACC TAT CAG CTT TAG ACG GTA GGG TAT-3' and SHP2 (reverse) 5'-TTC TCA TAA GGT GCT GAA GGA GTA AGG-3'. The primers used for the second amplification were SHP3 (forward) 5'-ACA GGG AGG TAG TGA CAA GAA ATA ACA-3' and SSU-R3 (reverse) 5'-AAG GAG TAA GGA ACA ACC TCC A-3'. The conditions used in both amplifications were 94°C for 3 min, 35 cycles of 94°C for 45 s, 56°C for 45 s and 72°C for 60 s, followed by a final extension of 72°C for 7 min. For every reaction, a positive and a negative control were used [18].

In this study the PCR primers used for the amplification of the *Colpodella* sp. sequences were originally designed and used for the detection of *Cryptosporidium* sp [18–20]. In the agarose gel electrophoresis analysis, the PCR bands of both *Cryptosporidium* sp. and *Colpodella* sp. have approximately the same size (around 600 bp) and they are equally strong, so the only way to distinguish them is through the sequencing of the purified PCR products. Mixed infection with both parasites in the same animal can be excluded because of the apparent discrimination peaks of the chromatograms of all the sequenced results.

Sequencing and Phylogenetic Analysis

The bands of the PCR products were gel-extracted from the agarose gel and were purified using the Blirt ExtractMe DNA Kit (Blirt, Gdansk, Poland). The final purified products were sent for sequencing to Macrogen Ltd Europe, Amsterdam (using the SHP3 forward primer of the nested PCR reaction). To identify the species, the sequences obtained from Macrogen were subjected to Nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov/Blast>) at NCBI GenBank. Furthermore, the sequences were deposited in the NCBI GenBank under the accession numbers OR380968-OR380971. A

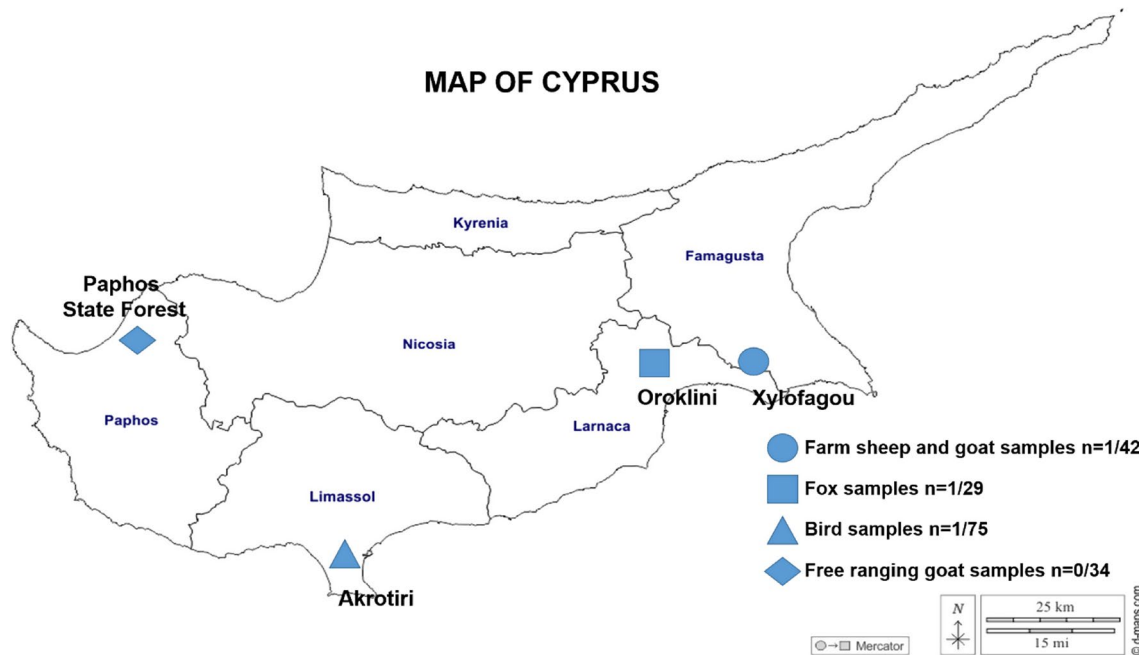


Fig. 1 Map of Cyprus indicating the collection sites of the animal faecal samples that were analysed in this study and also the number of positive samples for *Colpodella* sp. and the number of total samples collected per area

phylogenetic tree including all the sequences isolated in this study and several reference sequences from the GenBank was constructed using the Neighbor-Joining method by the MEGA 11 software (Bootstrap 1000 replicates). Evolutionary distances were calculated using the Tamura-3 parameter model (<https://www.megasoftware.net/>).

Results

Four of the 180 faecal samples ($4/180=2.2\%$) were positive for *Colpodella* sp. These included one goat from a farm ($1/10=10\%$), one fox ($1/29=3.4\%$), one Eurasian coot ($1/48=2.1\%$) and one duck ($1/7=14.3\%$) (Table 1).

The validity and elucidation of our results were confirmed after phylogenetic analysis using the Mega11 software. All four *Colpodella* sp. sequences isolated in this study formed a well-defined cluster with every other *Colpodella* sp. sequence (isolated from humans and animals) available in GenBank, including isolates HEP (accession number MH208621) and HLJ (accession number KT364261), as shown in Fig. 2. Interestingly, all four sequences were similar even though they were isolated from different animal species. Also, they all had very high similarity with the *Colpodella* sp. sheep reference sequences from Nigeria, forming a sub-cluster (Fig. 2).

In contrast, the *Cryptosporidium* sp. sequences that were previously isolated by our group [19, 20] formed a distant

different cluster despite the fact that they were isolated using the same PCR primers (Fig. 2).

Discussion

Our findings support previous studies showing that *Colpodella* sp. species are associated with vertebrates. So far, *Colpodella* sp. has been identified in mammalian hosts, including humans [6, 7, 10] felids in China [9, 12] cattle in Zambia [11] and Nigeria (unpublished results, nucleotide sequences found in the GenBank), and racoons in Poland [13]. Additionally, it has been identified in ticks of goats in China [14]. In this study, we have identified goats and foxes as two additional mammalian hosts for *Colpodella* sp., and the positive samples from the bird droppings show for the first time that birds can also host these parasites. This data reinforces the evidence that livestock and wildlife can serve as *Colpodella* sp. reservoirs. More investigations are needed to determine whether a tick vector is involved in the transmission of these avian and mammalian *Colpodella* sp. infections. In addition, the public health risk needs to be evaluated since this protist infection has been under-reported in the literature. Researchers have emphasized the pathogenic importance of *Colpodella* sp. after two case reports of human diseases in China [6, 7], one case report in Romania [8], and the death of a South China tiger in a zoo [9]. Therefore, assessing the zoonotic potential of *Colpodella* sp. infection is crucial for avoiding misdiagnosis in

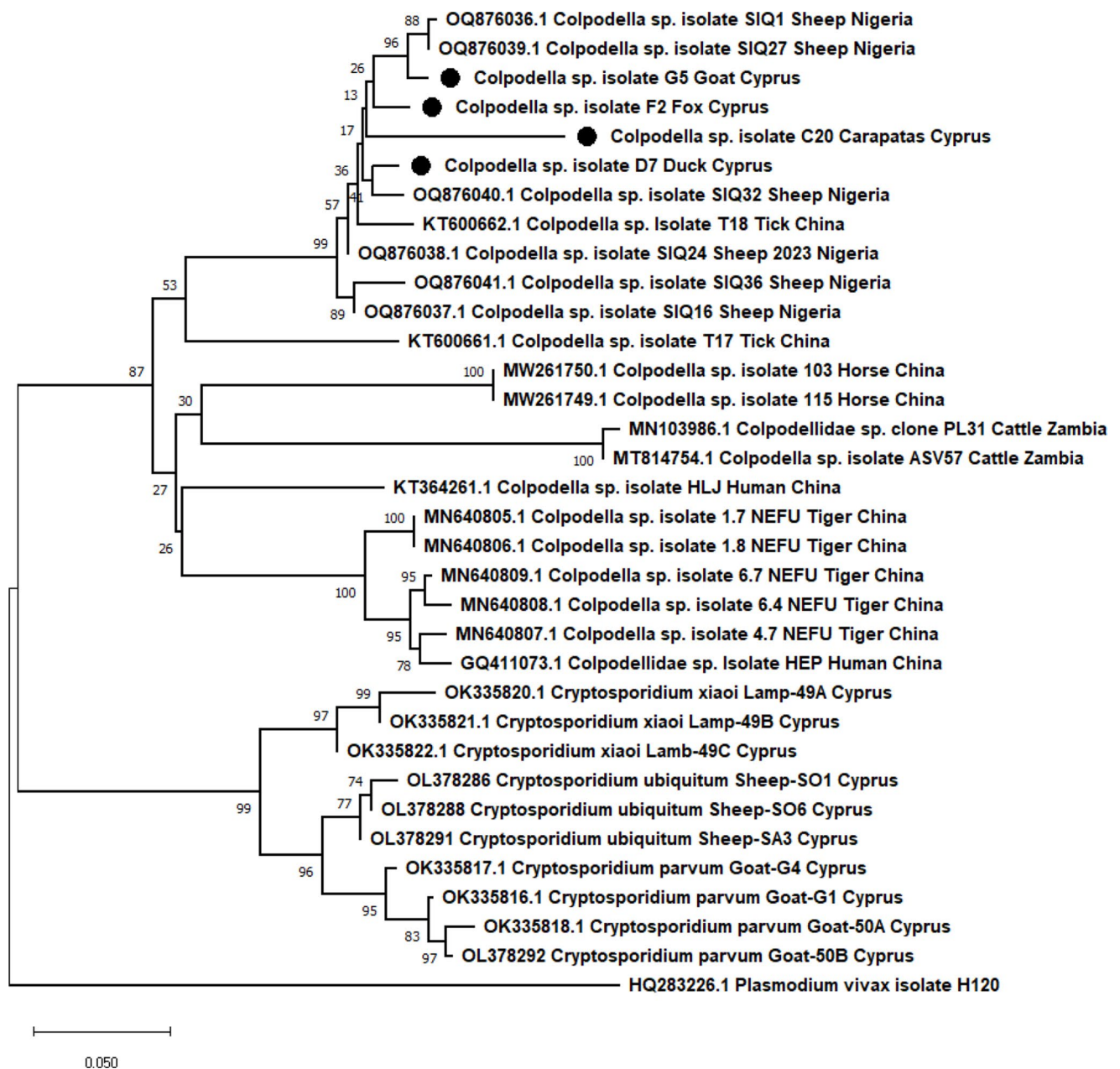


Fig. 2 Phylogenetic analysis of *Colpodella* subtypes based on the 600 bp sequence of the 18 S rRNA gene, using the Neighbor-Joining method with Bootstrap 1000 replicates. Evolutionary distances were calculated using the Tamura-3 parameter model. The percentage of bootstrap samplings is indicated by the numbers above the branches. The phylogenetic tree was constructed using the MEGA 11.0 software. The four *Colpodella* species isolated in this study are marked with a

solid circle. Some *Cryptosporidium* species isolated in Cyprus using the same PCR primers as with *Colpodella* sp. (see Hasapis et al. 2023, Schou et al. 2022) are also added in the phylogenetic tree. Finally, *Plasmodium vivax* (HQ283226) 18 S rRNA gene sequence was used as the outgroup. All the other sequences are reference sequences deposited in Genbank

humans and animals [11]. The state of disease due to the *Colpodella* sp. infection has most likely been underestimated, and many case reports have not been documented since there is no established detection protocol for this pathogenic protist.

Conclusion

This is the first *Colpodella* sp. isolation and molecular characterization report in goats, foxes and birds worldwide. The results of this study contribute to the growing body of evidence suggesting that *Colpodella* sp. may not be a free-living organism and can be pathogenic to its hosts. Therefore,

we assume that this organism may be an under-reported potential pathogenic protozoon. Subsequently, there is a need to develop monitoring systems for this protist, and further investigation is required to evaluate the risk of zoonotic transmission in humans and animals.

Acknowledgements We thank the staff of the Game and Fauna Service as well as (in alphabetical order) Alexandra Aristou, Katerina Georgiou, Konstantina Sotiriadi and Eleni Tsouma for their assistance collecting the samples. This work was supported in part by personal funding of Chad Schou.

Author Contributions KAH wrote the first draft of the manuscript, provided guidance for the molecular analysis, performed molecular reactions and designed the phylogenetic tree, IC collected samples, supported the first draft, reference searching, sorting and provided guidance and advice for the analysis and references, CODP performed molecular reactions, reference searching, sorting, and edited the manuscript, SK performed molecular reactions, reference searching and sorting, NK collected the samples, CS provided resources, funding support, collected samples, edited the manuscript, PK was responsible whole conceptualization and administration of the project, for drafting the paper and the manuscript revision. All authors read and approved the final manuscript.

Funding Open access funding provided by the Cyprus Libraries Consortium (CLC).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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

References

- Patterson DJ, Simpson AG (1996) Heterotrophic flagellates from coastal marine and hypersaline sediments in Western Australia. *Eur J Protistol* 32(4):423–448. [https://doi.org/10.1016/S0932-4739\(96\)80003-4](https://doi.org/10.1016/S0932-4739(96)80003-4)
- Kuvardina ON, Leander BS, Aleshin VV, Myl'nikov AP, Keeling PJ, Simdyanov TG (2002) The phylogeny of colpodellids (*Alveolata*) using small subunit rRNA gene sequences suggests they are the free-living sister group to apicomplexans. *J Eukaryot Microbiol* 49(6):498–504. <https://doi.org/10.1111/j.1550-7408.2002.tb00235.x>
- Leander BS, Keeling PJ (2003) Morphostasis in alveolate evolution. *Trends Ecol Evol* 18(8):395–402. [https://doi.org/10.1016/S0169-5347\(03\)00152-6](https://doi.org/10.1016/S0169-5347(03)00152-6)
- Yadavalli R, Sam-Yellowe TY (2017) Developmental stages identified in the trophozoite of the free-living Alveolate flagellate *Colpodella* sp. (Apicomplexa). *Int J Microbiol* 20(4):178. <https://doi.org/10.2436/20.1501.01.301>
- Getty TA, Peterson JW, Fujioka H, Walsh AM, Sam-Yellowe TY (2021) *Colpodella* sp. (ATCC 50594) life cycle: myzocytosis and possible links to the origin of intracellular parasitism. *Trop Med Infect Dis* 6(3):127. <https://doi.org/10.3390/tropicalmed6030127>
- Yuan CL, Keeling PJ, Krause PJ, Horak A, Bent S, Rollend L, Hua XG (2012) *Colpodella* spp.-like parasite infection in woman, China. *Emerg Infect Dis* 18(1):125. <https://doi.org/10.3201%2Feid1801.110716>
- Jiang JF, Jiang RR, Chang QC, Zheng YC, Jiang BG, Sun Y, Jia N, Wei R, Liu HB, Huo QB, Wang H, von Fricken ME, Cao WC (2018) Potential novel tick-borne *Colpodella* species parasite infection in patient with neurological symptoms. *PLoS Negl Trop Dis* 12(8):1–3. <https://doi.org/10.1371/journal.pntd.0006546>
- Neculicioiu VS, Colosi IA, Toc DA, Lesan A, Costache C (2021) When a ciliate meets a flagellate: A rare case of *Colpoda* spp. and *Colpodella* spp. isolated from the urine of a human patient. Case report and brief review of literature. *Biol* 10(6):476. <https://doi.org/10.3390/biology10060476>
- Chiu HC, Sun X, Bao Y, Fu W, Lin K, Chen T, Zheng C, Li S, Chen W, Huang C (2022) Molecular identification of *Colpodella* sp. of South China tiger *Panthera tigris amoyensis* (Hilzheimer) in the Meihua Mountains, Fujian, China. *Folia Parasitol* 69:1–9. <https://doi.org/10.14411/fp.2022.019>
- Xu M, Hu Y, Qiu H, Wang J, Jiang J (2022) *Colpodella* sp. (Phylum Apicomplexa) identified in horses shed light on its potential transmission and zoonotic pathogenicity. *Front Microbiol* 13:1–5. <https://doi.org/10.3389/fmicb.2022.857752>
- Squarre D, Nakamura Y, Hayashida K, Kawai N, Chambaro H, Namangala B, Sugimoto C, Yamagishi J (2020) Investigation of the piroplasm diversity circulating in wildlife and cattle of the greater Kafue ecosystem. *Zambia Parasit Vectors* 13(1):1–11. <https://doi.org/10.1186/s13071-020-04475-7>
- Hussain S, Li X, Bukhari SM, Zhou M, Ahmad S, Ahmad S, Javid A, Guan C, Hussain A, Ali W, Khalid N, Ahmad U, Tian L, Hou Z (2021) Cross-genera amplification and identification of *Colpodella* sp. with *Cryptosporidium* primers in fecal samples of zoo felids from northeast China. *Braz J Biol* 83:1–6. <https://doi.org/10.1590/1519-6984.247181>
- Solarz W, Najberek K, Wilk-Woźniak E, Biedrzycka A (2020) Raccoons foster the spread of freshwater and terrestrial microorganisms—mammals as a source of microbial eDNA. *Divers Distrib* 26(4):453–459. <https://doi.org/10.1111/ddi.13027>
- Qi Y, Wang J, Lu N, Qi X, Yang C, Liu B, Lu Y, Gu Y, Tan W, Zhu C, Ai L (2024) Potential novel *Colpodella* spp. (phylum Apicomplexa) and high prevalence of *Colpodella* spp. in goat-attached *Haemaphysalis longicornis* ticks in Shandong province, China. *Ticks Tick-borne Dis* 15(3):102328. <https://doi.org/10.1016/j.ttbdis.2024.102328>
- Liu Y, Xiang J, Gao Y, Wang J, Liu L, Li R, Wang J (2023) Rapid detection of *Cryptosporidium* spp. in diarrheic cattle feces by isothermal recombinase polymerase amplification assays. *Heliyon* 9(10). <https://doi.org/10.1016/j.heliyon.2023.e20794>

16. de Mendonça PG (2018) Cross-genera PCR amplification of DNA from apicomplexan parasites. *J Arthropod Borne Dis* 12(3):321 PMID: 30584555; PMCID: PMC6297730
 17. Koehler AV, Korhonen PK, Hall RS, Young ND, Wang T, Haydon SR, Gasser RB (2017) Use of a bioinformatic-assisted primer design strategy to establish a new nested PCR-based method for *Cryptosporidium*. *Parasit Vectors* 10:1–8. <https://doi.org/10.1186/s13071-017-2462-4>
 18. Silva SO, Richtzenhain LJ, Barros IN, Gomes AM, Silva AV, Kozerski ND, de Araújo Ceranto JB, Keid LB, Soares RM (2013) A new set of primers directed to 18S rRNA gene for molecular identification of *Cryptosporidium* spp. and their performance in the detection and differentiation of oocysts shed by synanthropic rodents. *Exp Parasitol* 135(3):551–557. <https://doi.org/10.1016/j.exppara.2013.09.003>
 19. Schou C, Hasapis K, Karanis P (2022) Molecular identification of *Cryptosporidium* species from domestic ruminants and wild reptiles in Cyprus. *Parasitol Res* 121(7):2193–2198. <https://doi.org/10.1007/s00436-022-07527-2>
 20. Hasapis KA, Charalambidou I, Tsouma E, Sotiriadi K, Kassinis N, Schou C, Karanis P (2023) First detection of *Cryptosporidium proventriculi* from wild birds in Cyprus. *Parasitol Res* 122:201–205. <https://doi.org/10.1007/s00436-022-07717-y>
- Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.