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Review

Emerging Opportunistic *Colpodella* Species: Nutrient Uptake and Approaches to Diagnose Infections

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Abstract: *Colpodella* species are free-living opportunistic pathogens that cause human and animal infections and use ticks and flies as vectors. Trophozoite and cyst stages of *Colpodella* species can be distinguished from stages of the prey *Parabodo caudatus* using Sam-Yellowe's trichrome staining. *Colpodella* species obtain nutrients by attaching to their prey, aspirating the prey's cytoplasmic contents into a posterior food vacuole and encysting. It is unclear whether both trophozoite and cyst stages are present during infection. Molecular techniques identified *Colpodella* species in all reported infections. However, no morphological information was reported to aid life cycle stage identification in hosts. This review discusses the increased incidence of *Colpodella* infection in animals and in vectors and the need to identify stages used for transmission and pathogenesis. The potential for zoonotic infections through tick and fly bites is a public health concern. We previously used Sam-Yellowe's trichrome staining to identify life cycle stages of *Colpodella* sp. In order to gain a better understanding of transmission and pathogenesis, identifying *Colpodella* life cycle stages in infected tissue and in vectors will provide important insights regarding nutrient uptake in hosts by determining whether attachment of trophozoites to host cells occurs, and identifying trophozoites and cysts in infected hosts.

Keywords: apicomplexa; *Colpodella* species; *Colpodella* sp. ATCC 50594; *Colpodella* infection, Sam-Yellowe's trichrome staining; endocytosis; myzocytosis; phagotropy; trogocytosis

1. Introduction

Colpodella species are free-living predatory protists classified as Myzozoans, a diverse group of organisms that includes dinoflagellates, apicomplexans and predatory biflagellated free-living protists. They possess an apical complex consisting of secretory organelles like the rhoptries and micronemes along with microtubular structures like the conoid and polar rings [1,2]. A pseudoconoid (open conoid) contained within the rostrum is used for attachment and predation among the free-living protists, in a process known as myzocytosis where cytoplasmic contents of the prey are aspirated into the predator. In *Plasmodium* species, *Toxoplasma gondii, Cryptosporidium* sp. and among the gregarines, the apical complex organelles also participate in nutrient uptake along with invasion [2–4]. The feeding of gregarines, *Cryptosporidium* sp., *Colpodella tetrahymenae* and *Colpodella gonderi* is characterized as extracellular parasitism [3,5–7]. Feeding on biflagellated bodonid species and algae using the process of myzocytosis leads to the formation of a cyst in some *Colpodella* species. Non-cyst forming species such as *C. unguis* and *C. edax* have been described [8,9]. Free-living *Colpodella* species have been shown to cause opportunistic infections in humans and in animals [10–15]. *Colpodella* sp. have also been identified in ticks and flies [11,16] prompting the concern for potential zoonotic infections from tick and fly bites. A babesiosis-like relapsing fever, with red blood cell infection was reported in a 57 year old woman in Yunnan Province, China [10]. Polymerase chain reaction targeting the 18S rRNA gene and sequence analysis showed that the identified *Colpodella* sp. had 89 % homology with *Colpodella tetrahymenae* [10]. A second human case of *Colpodella* infection was reported in a 55 year old woman in Heilongjiang Province, in Northeast China [11]. Neurological symptoms developed following a tick bite. Four hundred and seventy-four *Ixodes persulcatus* ticks were examined for *Colpodella* sp. from the woodlands surrounding her residence. Two ticks were positive

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for *Colpodella* [10,11]. In both human cases, identification of transmission and pathogenic stages of *Colpodella* were not performed (NP), reported or confirmed by light microscopy (Table 1). *Colpodella gonderi* was identified in a human case of urinary tract infection, although the cause of infection was uncertain [17]. Giemsa staining for light microscopy was performed with the identification of trophozoite stages in urine. However, transmission or pathogenic stages were not described. It was also unclear if *Colpodella gonderi* was the etiological agent for the infection [17].

Colpodella sp. have been identified in cattle, ticks infesting cattle, raccoons, horses, in fecal samples from zoo felines, domestic (pet) cats and dogs and in ticks attached to goats [18–22] (Table 1). A routine screening of whole blood samples from 400 horses, identified *Colpodella* species in two samples along with *Babesia caballi* in two samples and *Theileria equi* in 132 samples [18]. These observations suggest that ticks may be potential vectors for transmission and that *Colpodella* species may cause opportunistic infection as a novel tick-borne zoonotic pathogen of public health importance [18]. *Colpodella* species have been identified in a tick causing infection in a human host and in ticks infesting animals [11,19,22]. Tabanid flies and *Stomoxys indicus* were shown to be positive for *Colpodella* species on horse farms in Thailand [16]. Three different *Colpodella* species were identified in raccoons with the suggestion that raccoons may serve as "dispersal vectors" for *Colpodella* sp. [20]. *Colpodella* 18S rDNA was identified from the blood of a South China Tiger that died of infection from a tick bite [12]. DNA sequence had 90.1 % sequence identity to *Colpodella* species strain HEP [10,12] and 90.4 % similarity to *Colpodella* sp. strain Heilonjiang (HLJ) [11,12] (Table 1). Chiu et al. [12], reported symptoms of severe jaundice, and enlarged organs in the babesiosis-like infection in the South China Tiger. Out of 402 adult ticks examined from the tiger enclosure and grasses around the enclosure, 22 were positive for *Colpodella* species [12]. Two *Colpodella* species with sequence homology to *Colpodella* sp. (ATCC 50594) were identified in horse blood [18]. None of the animal studies reported life cycle stage identification through staining for light microscopy, differential interference contrast (DIC) microscopy or by electron microscopy. The morphology of the *Colpodella* species identified is unknown. Life cycle stages involved in transmission and pathogenesis are unknown and the mechanism of infection, including the types of nutrients taken up from the host during infection have not been described. It is unclear how *Colpodella* sp. survive in mammalian and arthropod hosts.

Among myzozoans, predators aspirate large particulate material from the prey's cytoplasm during myzocytosis. This suggests that nutrient uptake occurs using the pseudoconoid and serves as an early mode of attachment to prey and is similar to nutrient uptake in the basal apicomplexan lineages such as in the archigregarines and in the free-living myzozoans [3]. The position and function of the food vacuole among apicomplexans like *Selenidium pendula* Giard, 1884 which feeds by myzocytosis may influence the development of the life cycle stages following nutrient uptake. A flask-shaped organelle found to contain digested pieces of host cell organelles and debris is thought to be the food vacuole with nutrient uptake resulting from phagocytosis in *S. pendula* Giard, 1884 [23]. The presence of a posterior food vacuole in *Colpodella* sp. (ATCC 50594), differs from the food vacuoles located in the anterior end of the trophozoite in *S. pendula* Giard, 1884 [23] and located near rhoptries in the anterior end. Trophozoites of *Colpodella* sp. (ATCC 50594) initiate myzocytosis by binding with a myzocytic aperture posterior to the apical tip, and can feed intermittently on multiple prey. *Colpodella* species can attach to prey, commence feeding and then detach from the prey, to seek new prey [24]. In previous studies we showed that the process of myzocytosis in *Colpodella* sp. (ATCC 50594) occurs sequentially, beginning with attachment to the prey *P. caudatus*, engulfment of the plasma membrane of the prey, destruction of the prey's plasma membrane and aspiration of the prey's cytoplasmic contents into a posterior food vacuole [25]. In addition to myzocytosis for nutrient uptake, *Colpodella* sp. (ATCC 50594) trophozoites can also carry out endocytosis in culture [25]. Following myzocytosis, and nutrient acquisition, *Colpodella* sp. (ATCC 50594) encysts. Nutrients transported to the posterior food vacuole aid cyst development and maturation. This is followed by mitosis and cytokinesis to produce juvenile trophozoites. Different methods are used by pathogenic protists to obtain nutrients within their hosts. *Cryptosporidium* sp. use a feeder organelle that is formed at the parasite-host cell interface for obtaining nutrients from the host cell [26]. Free living

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opportunistic amoeba such as *Naegeria fowleri* that infect the nervous system of the host through the olfactory nerve, use food cups (amoebastomes) which are cytoplasmic extensions of the amoeba surface, to digest brain tissue [27]. The biflagellate trophozoite stage of *N. fowleri* initiates infection in human and animal infections. Target cells in the brain are destroyed by "nibbling" and ingestion of the host tissue in a process known as trogocytosis. The process of adhesion and attachment have been identified by microcopy with Nf-actin identified by immunofluorescence [28,29]. Both cyst and trophozoite stages of *N. fowleri* were identified in infected host specimens using light microscopy [30]. Investigations aimed at providing accurate diagnosis of amoebic keratitis to distinguish *Acanthamoeba* from non-*Acanthamoeba* amoebic keratitis and the presence of mixed infections show the importance of using a combination of methods to identify and accurately diagnose infection. The use of culture, microscopy and PCR is emphasized for identifying parasite stages [31]. Cytolytic effects of *Acanthamoeba castellanii* in vitro have been identified by light microscopy [32] and phagocytosis of erythrocytes, leukocytes and bacteria by *Trichomonas vaginalis* in vitro demonstrates how *T. vaginalis* obtains nutrient during host infection [33].

Are the reports of *Colpodella* sp. in human, animal and arthropod hosts, true infections, infestations or contamination of specimens from the soil and aquatic environments? If these are indeed true opportunistic infections leading to pathogenesis, then the morphological identity of *Colpodella* sp. life cycle stages is urgently needed to aid better characterization and prioritization of which species and strains of *Colpodella* to emphasize in investigations. *Colpodella* sp. can be cultured in vitro in diprotist cultures using Hay medium, allowing for investigations in vitro [24]. Giemsa staining is routinely used to stain specimens containing parasitic protists. However, differentiation of life cycle stages may be challenging depending on the life cycle stage that needs to be identified. We developed Sam-Yellowe's trichrome staining protocols to identify life cycle stages of *Colpodella* sp. (ATCC 50594) [34]. The staining protocol was used to identify previously undocumented life cycle stages of *Colpodella* sp. (ATCC 50594) and facilitated interpretations of transmission electron micrographs [24,35]. Staining specimens obtained from *Colpodella* infected hosts and from the arthropod vectors for light microscopy will provide a better understanding of *Colpodella*-host cell interactions, identify life cycle stages present in the hosts and determine stages involved in transmission and pathogenesis. We showed in previous studies that unattached *Colpodella* sp. (ATCC 50594) trophozoites can endocytose nanoparticles of 40 and 100 nm from culture suggesting that in addition to myzocytosis, the predator can acquire nutrients by endocytosis [25]. Whether endocytosis is sufficient to form the food vacuole and lead to encystation is unknown and requires further investigation. The process of endocytosis may be used by *Colpodella* species for nutrient uptake during host infection. Alternately, *Colpodella* sp. may carry out contact-dependent interaction with host cells leading to cell and tissue destruction or invade human cells as described [10]. Understanding the biology of *Colpodella* species is crucial to identifying transmission stages initiating infection in human and animal hosts, and identifying stages of *Colpodella* associated with pathogenesis. Morphological identification of life cycle stages by staining and light microscopy is required to identify the distribution of the protist within infected host tissues. Markers identifying transmission and pathogenic stages of *Colpodella* sp. in the life cycle are unknown. In *P. falciparum* infected erythrocytes, uptake of the host cytosol into the food vacuole has been described using fluorescent dextran which was identified in vesicles inside the intracellular parasite [36]. Proteins having roles in endocytosis such as Kelch 13, AP-2μ and Eps-15 were identified in *P. falciparum*, as markers of endocytosis [36–38]. Additionally, the protein VPS45 identified in *P. falciparum* is involved in host cell cytosol uptake (HCCU) [36]. Inactivation of the genes encoding these proteins resulted in decreased hemoglobin uptake [36–39]. In future experiments, it will be important to identify markers for endocytosis, myzocytosis, encystation and excystation in *Colpodella* species. In particular, Kelch 13 a protein associated with endocytosis has been identified in all apicomplexans and myzozoans examined [40] and its presence and role in endocytosis will provide key insights into the similarities of endocytosis across the apicomplexa, including in *Colpodella* species infecting human and animal hosts. Apicomplexans utilize apical phagotrophy, phagocytosis, osmotrophy, pinocytosis and endocytosis for nutrient uptake, with the cytostome and micropore implicated for endocytosis [3,26].

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Brugerolle [41] described the ultrastructure of *Colpodella vorax*, showing aspiration of the prey's organelles though a channel formed after attachment and the resultant encystation following feeding. Similarly, *C. tetrahymenae*, ectoparasitic to the ciliate *Tetrahymena* aff. *pyriformis*, encysts following myzocytosis. However, an enlargement of the food vacuole and a precyst stage was not described [6]. The use of staining protocols such as Giemsa and Sam-Yellowe's trichrome staining which can be performed in less than 10 minutes will provide important insights regarding morphological similarities and differences in each of the infections described and in the ticks and flies shown to harbor *Colpodella* species. Two new species of *Colpodella*; *Colpodella* sp. *struthionis* and *Colpodella* sp. *yiyuansis* were named by Qi et al. [22]. However, morphological characteristics of the cells were not described, infectivity of the life cycle stages are unknown and the mode of survival and nutrient uptake within the infected hosts are unknown. Endocytosis has not been described in other *Colpodella* species besides *Colpodella* sp. (ATCC 50594). Therefore, it is unclear if similar mechanisms are used, particularly in species that feed on ciliates and algae. The biology of *Colpodella* species is still unclear and although investigations of the model *Colpodella* sp. (ATCC 50594) is beginning to provide insights into life cycle stage transitions, the diprotist culture conditions in bacterized media pose a challenge to studies focused on *Colpodella* sp. (ATCC 50594). Effects of infection on host tissue in human and animal cases will need to be investigated to gain insights into the mechanisms of pathogenesis in the host and the differences in host tissue specificity and tropism of life cycle stages once inside the host. These novel opportunistic infections by *Colpodella* species are considered underreported emerging zoonotic pathogens requiring urgent attention [18,42]. The identification of *Colpodella* species in wide ranging animal hosts such as cattle and wild life [43], horses [18], and raccoons [20] and in the ticks infesting the animals such as in the ticks infecting camels [44] and goats [22], poses a public health threat for humans in close contact with the animals. So far, *Colpodella* species have been identified in the ticks, *Ixodes persulcatus*, *Rhipicephalus microplus*, *Dermacentor*, *Haemaphysalis longicornis* and *Hyalomma dromedarii* [11,14,15,19,22,44]. These ticks are found infesting animals that are constantly in close contact with humans as work animals, agricultural animals, pets, and recreational animals associated with tourists [11,14,15,19,22,44]. These infections, mechanisms of transmission and pathogenicity, and the biology of *Colpodella* species merit further investigation.

The following questions will need to be answered to provide clarity to the mechanisms of transmission and pathogenesis of *Colpodella* sp. infections. 1) What are the life cycle stages of *Colpodella* species causing infection and pathogenesis? 2) Are *Colpodella* trophozoites able to attach to and feed on host cells such as erythrocytes, leukocytes or epithelial cells? 3) Can *Colpodella* trophozoites invade host cells? 4) Myzocytosis in culture and in the environment occurs when the *Colplodella* sp. trophozoite engulfs the plasma membrane of the prey, dissolves the membrane and aspirates the cytoplasmic contents of the prey. What is the nutrient source for *Colpodella* sp. in the human and animal hosts? 5) Where in the ticks and flies are *Colpodella* sp. life cycle stages located? 6) What is the nutrient source for *Colpodella* in the tick and fly vectors? 7) Are ticks and flies mechanical or biological vectors? 8) How are nutrients trafficked in *Colpodella* sp. within the host? 9) Is a food vacuole formed and are cyst stages formed within the host? 10) Do *Colpodella* sp. coinfections occur with other apicomplexans such as *Plasmodium* species or *Toxoplasma gondii*? The use of culture and microscopy along with molecular methods is necessary for identification of the life cycle stages transmitting infection and causing pathogenesis [31]. Giemsa staining has been useful in identifying trophozoites of *Colpodella* sp. and its prey *Parabodo caudatus*, particularly being able to differentiate the kinetoplast and nucleus of the prey. Sam-Yellowe's trichrome staining performed in less than ten minutes, can identify and differentiate precyst and cyst stages of the predator and prey and help with identification of the stage of maturity of both trophozoites and cysts if present in tissue specimens. Additional investigations will be needed to identify markers of transmission and pathogenesis. It will be important to know if recently identified species are different from previously described species. Morphological identification of life cycle stages of *Colpodella* species obtained from infected hosts and from vectors, stained for light microscopy will provide much needed information regarding the morphology of transmission stages and the distribution of life cycle stages present in the host. Diagnosis using molecular techniques while very useful should be aided by staining for light

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microscopy, further evaluation of the ultrastructure of the identified *Colpodella* species and culturing the cells to allow for further cell biological and molecular investigations required to aid clinical investigations and diagnosis.

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Table 1. *Colpodella* species infecting humans, animals, ticks and flies.

	Research Study Title	Year of Publication	Location	Host Species	Tick/Flies Species	Staining For Light Microscopy	Identification Method	DNA Sequence Homology with *Colpodella* sp.	DIC/Electron Microscopy
1	*Colpodella* spp.–like Parasite Infection in Woman, China [10]	2012	Kunming City, Yunnan Province, China	Human	N/A	Giemsa Stain	Polymerase Chain Reaction	*Colpodella tetrahymenae* (89% similarity)	*NP*
2	Molecular detection of pathogens in ticks infesting cattle in Nampula province, Mozambique [19]	2017	Nampula province, Mozambique	Cattle	*Rhipicephalus microplus*	NP	Polymerase Chain Reaction	*Colpodella* sp. (89% and 86% similarity)	NP
3	Potential novel tick-borne *Colpodella* species parasite infection in patient with neurological symptoms [11]	2018	Heilongjiang Province, China	Human	*Ixodes persulcatus*	NP	Polymerase Chain Reaction	*Colpodella* sp. (89%-90% similarity)	NP
4	Raccoons foster the spread of freshwater and terrestrial microorganisms—Mammals as a source of microbial eDNA [20]	2020	Warta Mouth National Park, Western Poland	Racoon Dog	N/A	NP	Polymerase Chain Reaction	*Colpodella* sp. (99.13% similarity)	NP
5	Investigation of the piroplasm diversity circulating in wildlife and cattle of the greater Kafue ecosystem, Zambia [43]	2020	The Greater Kafue Ecosystem, Zambia	Cattle	N/A	NP	Polymerase Chain Reaction	*Colpodella* sp. (79.6% similarity to human cases) *Colpodella* sp. (100% similarity to racoon dog case)	NP
6	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 [17]	2021	Cluj-Napoca, Romania	Human	N/A	Giemsa Stain	Morphological Criteria though Staining	N/A	NP
7	Cross-genera amplification and identification of *Colpodella* sp. with *Cryptosporidiu*m primers in fecal samples of zoo felids from northeast China [21]	2021	Harbin Zoo, China	Fecal Matter	N/A	NP	Polymerase Chain Reaction	*Colpodella* sp. (97% similarity with *Cryptosporidium* sp.)	NP
8	*Colpodella* sp. (Phylum Apicomplexa) Identified in Horses Shed Light on Its Potential Transmission and Zoonotic Pathogenicity [18]	2022	Ordos City, Inner Mongolia, located in northern China	Horses	N/A	NP	Polymerase Chain Reaction	*Colpodella* sp. (99.18% and 98.73% similarity with *Colpodella* sp. ATCC 50594)	NP
9	A multipronged next-generation sequencing metabarcoding approach unearths hyperdiverse and abundant dog pathogen communities in Cambodia [14]	2022	Cambodia	Dogs	N/A	NP	Next-generation sequencing (NGS)-based metabarcoding protocol	*Coldpodella* sp. (95% similarity with Horse Infection #MW261750.1)	NP
10	Molecular identification of *Colpodella* sp. of South China tiger *Panthera tigris* amoyensis (Hilzheimer) in the Meihua Mountains, Fujian, China [12]	2022	Meihua Mountains, Fujian, China	Tiger	Unidentified Tick	NP	Polymerase Chain Reaction	*Colpodella* sp. (91.1% similarity to *Colpodella* sp. strain human erythrocyte parasite (HEP, MH208621) and 90.4% similar to the *Colpodella* sp. strain Heilongjiang (HLJ, KT364261).	NP

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11	Preliminary study on prevalence of hemoprotozoan parasites harbored by *Stomoxys* (Diptera: Muscidae) and tabanid flies (Diptera: Tabanidae) in horse farms in Nakhon Si Thammarat province, Southern Thailand [16]	2023	Nakhon Si Thammarat province, Southern Thailand	Horse	*Stomoxys indicus*	NP	Polymerase Chain Reaction	*Colpodella tetrahymenae (89.46% similarity)*	NP
12	Molecular epidemiological investigation of piroplasms carried by pet cats and dogs in an animal hospital in Guiyang, China [15]	2023	Guiyang, China	Cats and Dogs	N/A	NP	Polymerase Chain Reaction	*Colpodella* sp. (84.71% simialirty to *Colpdoella* sp. ATCC 50594)	NP
13	Potential novel Colpodella spp. (phylum Apicomplexa) and high prevalence of *Colpodella* spp. in goat-attached *Haemaphysalis longicornis* ticks in Shandong province, China [22]	2024	Shandong province, China	Goats and Dogs	*Haemaphysali longicornis*	NP	Polymerase Chain Reaction	*Colpdoella sp. in Dog Tick 38 (98.26% similarity with 2018 Human Infection.* *Colpodella sp. struthionis* in Goat Tick 168 (93.66% similarity with *Cryptosporidium struthionis)* *Colpodella* sp. *yiyuansis* in Goat Tick 161 (92.98% similarity with *Colpodella tetrahymenae*	NP
14	Discovery of *Colpodella* spp. in ticks (*Hyalomma dromedarii*) infesting camels in southern Egypt [44]	2024	Egypt	Camels	*Hyalomma dromedarii*	NP	Polymerase Chain Reaction	*Colpodella* sp. in *H. dromedarii* ticks 98.4 % similarity with *Colpodella angusta*	NP
15	Eosinophilic pericardial effusion and pericarditis in a cat [13]	2023	North Carolina, United State	Female spayed domestic shorthair cat.	N/A	Wright Giemsa Stain	Polymerase Chain Reaction and Staining	*Colpdoella* sp. (90% similarity)	NP

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References

1. Bargieri, D.; Lagal, V.; Andenmatten, N.; Tardieux, I.; Meissner, M.; Ménard R. Host cell invasion by apicomplexan parasites: the junction conundrum. PLoS Pathog. 2014 Sep 18;10(9):e1004273.
2. Gubbels, M-J.; Duraisingh, M.T. Evolution of apicomplexan secretory organelles. Int J Parasitol., 2012, 42:1071-1081.
3. Valigurová, A.; Florent, I. Nutrient Acquisition and Attachment Strategies in Basal Lineages: A Tough Nut to Crack in the Evolutionary Puzzle of Apicomplexa. Microorganisms. 2021, 9: 1430.
4. Desai, S. A. Why do malaria parasites increase host erythrocyte permeability? Trends Parasitol., 2014, 30:151-159.
5. Olmo, J.L.; Esteban, G.F.; Finlay, B.J. New records of the ectoparasitic flagellate *Colpodella gonderi* on non-*Colpoda* ciliates. J Int Microbiol, 2011, 14:207-211.
6. Cavalier-Smith T; Chao, E.E. Protalveolate phylogeny and systematics and the origins of Sporozoa and dinoflagellates (phylum Myzozoa nom. Nov.). Eur. J. Protistol. 2004, 40: 185-212.
7. Simdyanov, T.G.; Paskerova ,G.G.; Valigurová, A.; Diakin, A.; Kováčiková, M.; Schrével, J.; Guillou, L.; Dobrovolskij, A.A.; Aleoshin, V.V. First Ultrastructural and Molecular Phylogenetic Evidence from the Blastogregarines, an Early Branching Lineage of Plesiomorphic Apicomplexa. Protist, 2018, 169:697-726.
8. Mylnikov, A.P.; Mylnikova, Z.M. Feeding spectra and pseudoconoid structure in predatory alveolate flagellates. Inland Water Biol. 2008, 1:210-216.
9. Mylnikov, A.P. Ultrastructure and phylogeny of colpodellids (Colpodellida, Alveolata). Biol Bulletin, 2009, 36:582-590.
10. Yuan, C.L.; Keeling, P.J.; Krause, P.J. et al. *Colpodella* spp.–like Parasite Infection in Woman, China. Emerg Infect Dis. 2012, 18:125-127.
11. Jiang, J-F.; Jiang, R-R.; Chang, Q-C.; Zheng, Y-C.; Jiang, B-G.; Sun, Y.; Jia, N.; Wei, R.; Bo, H-B.; Huo, Q-B.; Wang, H.; von Fricken, M. E.; Cao, W-C. Potential novel tick-borne *Colpodella* species parasite infection in patient with neurological symptoms. PLOS Negl. Trop. Dis., 2018, 12(8):e0006546
12. Chiu, H. C.; Sun, X.; Bao, Y.; Fu, W.; Lin, K.; Chen, T.; Zheng, C.; Li, S.; Chen, W.; Huang, C. Molecular identification of *Colpodella* sp. of South China tiger *Panthera tigris amoyensis* (Hilzheimer) in the Meihua Mountains, Fujian, China. Folia Parasitol (Praha). 2022, 69; 2022.019.
13. Wheatley, M.A.; Shamoun, J.; Maggi, R.; Breitschwerdt, E.B.; Sommer, S.L.; Cullen, J.M.; Stowe, D.M. Eosinophilic pericardial effusion and pericarditis in a cat. JFMS Open Rep., 2023 9:20551169231213498
14. Huggins, L. G.; Colella, V.; Koehler, A. V.; Schunack, B.; Traub, R. J. A multipronged next-generation sequencing metabarcoding approach unearths hyperdiverse and abundant dog pathogen communities in Cambodia. *Transbound Emerg Dis*. **2022**, 69:1933-1950.
15. Wu, S. Meng, J.; Yu, F.; Zhou, C.; Yang, B.; Chen, X.; Yang, G.; Sun, Y.; Cao, W.; Jiang, J.; Wu, J.; Zhan, L. Molecular epidemiological investigation of piroplasms carried by pet cats and dogs in an animal hospital in Guiyang, China. *Front Microbiol*. **2023**,14:1266583.
16. Phetkarl, T.; Fungwithaya, P.; Udompornprasith, S.; Amendt, J. Sontigun, N. Preliminary study on prevalence of hemoprotozoan parasites harbored by *Stomoxys* (Diptera: Muscidae) and tabanid flies (Diptera: Tabanidae) in horse farms in Nakhon Si Thammarat province, Southern Thailand. *Vet World*. **2023**, 16:2128-2134.
17. Neculicioiu, V. S.; Colosi, I. A.; Toc, D. A.; Lesan, A.; Costache, C. 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 the literature. *Biology* (Basel) **2021** Jun; 10 (6):476.
18. Xu, M.; Hu, Y.; Qiu, H.; Wang, J.; Jiang, J. *Colpodella* sp. (Phylum Apicomplexa) Identified in Horses Shed Light on Its Potential Transmission and Zoonotic Pathogenicity. Front Microbiol. 2022, 13:857752.
19. Matsimbe, A, M.; Magaia, V.; Sanchez, G. S.;Neves, L.; Noormahomed, E.; Antunes, S.; Domingos, A. Molecular detection of pathogens in ticks infesting cattle in Nampula province, Mozambique. *Exp. Appl. Acarol*. **2017**, 73, 91-102.
20. Solarz, W.; Najberek, K; Wilk-Wozniak, E.; Biedrzycka, A. Raccoons foster the spread of freshwater and terrestrial microorganisms-mammals as source of microbial eDNA. *Divers. Distrib*. **2020**, 26 453-459.
21. Hussein, S.; Li, X.; Bukharr, S. M.; Zhou, M.; Ahmad, S.; Amhad, S.; Javid, A.; Guan, C.; Hussain, A.; Ali, W.; Khalid, N,;Ahmad, U, Tian, L.; Hou, Z. Cross-genera amplification and identification of *Colpodella* sp.

with *Cryptosporidium* primers in fecal samples of zoo felids from northeast China. *Braz J Biol*. **2021** Sep 6; 83:e247181.doi.10.1590/1519-6984.247181 eCollection 2021.

22. Qi, Y.; Wang, J.; Lu, N.; Qi, X.; Yang, C.; Liu, B.; Lu, Y.; Gu, Y.; Tan, W.; Zhu, C.; Ai, L.; Rao, J.; Mao, Y.; Yi, H.; Li, Y.; Yue, M. 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*. **2024**, 15:102328.
23. Schrével, J.; Valigurová, A.; Prensier, G.; Chambouvet, A.; Florent, I.; Guillou, L. Ultrastructure of *Selenidium pendula*, the Type Species of Archigregarines, and Phylogenetic Relations to Other Marine Apicomplexa. Protist, 2016, 167:339-368..
24. Sam-Yellowe, T.Y.; Fujioka, H.; Peterson, J.W. Ultrastructure of Myzocytosis and Cyst Formation, and the Role of Actin in Tubular Tether Formation in *Colpodella* sp. (ATCC 50594). Pathogens, 2022, 11:455.
25. Sam-Yellowe, T.Y.; Asraf, M.M.; Peterson, J.W.; Fujioka, H. Fluorescent Nanoparticle Uptake by Myzocytosis and Endocytosis in *Colpodella* sp. ATCC 50594. *Microorganisms* 2023, *11*, 1945.
26. Piro, F.; Focaia, R.; Dou, Z.; Masci, S.; Smith, D.; Di Cristina, M. An Uninvited Seat at the Dinner Table: How Apicomplexan Parasites Scavenge Nutrients from the Host. Microorganisms. 2021 Dec 15;9(12):2592.
27. Marciano-Cabral, F. M.; Fulford, D. E. Cytopathology of pathogenic and nonpathogenic *Naegleria* species for cultured rat neuroblastoma cells. Appl Environ Microbiol. 1986, 51:1133-7
28. Sohn, H. J.; Kim,J. H.; Shin, M. H.; Song, K. J.; Shin, H. J. The Nf-actin gene is an important factor for food-cup formation and cytotoxicity of pathogenic Naegleria fowleri. Parasitol Res. 2010, 106:917-24.
29. Sohn, H. J.; Song, K. J.; Kang, H.; Ham, A. J.; Lee, J. H.; Chwae, Y. J.; Kim, K.; Park, S.; Kim, J. H.; Shin, H. J. Cellular characterization of actin gene concerned with contact-dependent mechanisms in Naegleria fowleri. Parasite Immunol. 2019, 41(8):e12631.
30. Chomba, M.; Mucheleng'anga, L.; Fwoloshi, S.; Ngulube, J.; Mutengo, M. M. A case report: primary amebic meningoencephalitis in a young Zambian adult. BMC Infect Dis. 2017, 17:532.
31. Moran, S.; Mooney, R.; Henriquez, F. L. Diagnostic considerations for Non-Acanthamoeba amoebic keratitis and clinical outcomes. Pathogens. 2022, 11, 219.
32. Gonzalez-Robles, A.; Castanon, G.; Cristobal-Ramos, A. R.; Lazaroo-Haller, A.; Omana-Molina, M.; Bonilla, P.; Martinez-Palomo, A.Acanthamoeba castellanii: Structural basis of the cytopathic mechanisms. Exp Parasitol. 2006, 114:133-140.
33. Rendon-Maldonado, J. G.; Espinosa-Cantellano, M.; Gonzalez-Robles, A.; Martinez-Palomo, A. Trichomonas vaginalis: In vitro phagocytosis of Lactobacilli, vaginal epithelial cells, leukocytes and erythrocytes. Exp Parasitol. 1998, 89:241-250.
34. Sam-Yellowe, T.Y.; Addepalli, K.; Yadavalli, R.; Peterson, J.W. New trichrome stains identify cysts of *Colpodella* sp. (Apicomplexa) and *Bodo caudatus*. J Int Microbiol, 2019, 23:303-311.
35. Getty, T.A.; Peterson, J.W.; Fujioka, H.; Walsh, A.M.; Sam-Yellowe, T.Y. *Colpodella* sp. (ATCC 50594) Life Cycle: Myzocytosis and Possible Links to the Origin of Intracellular Parasitism. Trop Med Infect Dis., 2021, 6:127.
36. Jonscher, E.; Flemming, S.; Schmitt, M.; Sabitzki, R.; Reichard, N.; Birnbaum, J.; Bergmann, B.; Höhn, K.; Spielmann, T. PfVPS45 Is Required for Host Cell Cytosol Uptake by Malaria Blood Stage Parasites. Cell Host Microbe, 2019, 25:166-173.
37. Elsworth, B.; Keroack, C.D.; Duraisingh, M.T. Elucidating Host Cell Uptake by Malaria Parasites. Trends Parasitol., 2019, 35:333-335.
38. Edgar, R.C.S.; Counihan, N.A.; McGowan, S.; de Koning-Ward, T.F. Methods Used to Investigate the *Plasmodium falciparum* Digestive Vacuole. Front Cell Infect Microbiol., 2022, 11:829823.
39. Spielmann, T.; Gras, S.; Sabitzki, R.; Meissner, M. Endocytosis in *Plasmodium* and *Toxoplasma* Parasites. Trends Parasitol., 2020, 36:520-532.
40. Koreny, L.; Mercado-Saavedra, B.N.; Klinger, C.M.; Barylyuk, K.; Butterworth, S.; Hirst, J.; Rivera-Cuevas, Y.; Zaccai, N.R.; Holzer, V.J.C.; Klingl, A.; Dacks, J.B.; Carruthers, V.B.; Robinson, M.S.; Gras, S.; Waller, R.F.. Stable endocytic structures navigate the complex pellicle of apicomplexan parasites. Nat Commun., 2023, 14:2167.
41. Brugerolle, G. *Colpodella vorax*: Ultrastructure, predation, life-cycle, mitosis, and phylogenetic relationships. Europ. J. Protistol., 2002, 38:113-125.

 doi:10.20944/preprints202405.1321.v1

42. Chang, Q.; Chen, Z.; von Fricken, M. E.; Liu, Q. Editorial: New infectious agents in arthropod vectors. Front Microbiol. 2022, 13:1105082.
43. Squarre, D.; Nakamura, Y.; Hayashida, K.; Kawai, N.; Chambaro, H.; Namangala, B.; Sugimoto, C.; Yamagishi, J. Investigation of the piroplasm diversity circulating in wildlife and cattle of the greater Kafue ecosystem, Zambia. Parasit Vectors. 2020,13:599.
44. Soliman, A. M.; Mahmoud, H. Y. A. H.; Hifumi, T.; Tanaka, T. Discovery of *Colpodella* spp. in ticks (*Hyalomma domedarii*) infecting camels in southern Egypt. Ticks and Tick-borne Diseases. 2024, 5:102352.