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First molecular characterization and morphological aspects of *Sarcocystis fusiformis* infecting water buffalo *Bubalus bubalis* in Egypt

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Abstract

Fresh muscle samples from water buffalo (*Bubalus bubalis*) aged 2–15, from Giza Province, Egypt; were examined for *Sarcocystis* infection. Macroscopic ovoid sarcocysts embedded in the muscle tissues of the examined buffaloes were detected; they measured 152–230 (210 ± 7) µm in length and 37–119 (95 ± 3) µm in width. The esophagus was the most infected organ followed by the diaphragm, and tongue, while the heart muscles were the least infected. The cyst cavity was compartmentalized by septa derived from the ground substance located under the primary cyst wall. Using transmission electron microscopy, the primary cyst wall bordered sarcocysts were determined to be 0.08–0.22 µm in thickness, raised from the parasitophorous vacuolar membrane, and surrounded by a secondary cyst wall of host origin. The primary cyst wall had irregular wall folds with numerous cauliflower-like projections of variable sizes and shapes accompanied by knob-like electron-dense elevations. 18S rRNA gene expression studies confirmed that the present parasite isolates belonged to the genus *Sarcocystis*. The sequence data showed significant identities (>90%) with archived gene sequences from many Eimeriidae organisms, and a dendogram showing the phylogenetic relationship was constructed. The most closely related species was *Sarcocystis fusiformis* KR186117, with an identity percentage of 98%. The recovered sequences were deposited in the GenBank under the accession number MG572125. The present study, to our knowledge, is the first collective ultrastructural and molecular study that confirmed the taxonomy of sarcocysts isolated from water buffaloes in Egypt as *Sarcocystis fusiformis*.

Keywords

Sarcocystis fusiformis, *Bubalus bubalis*, light and transmission electron microscopy, molecular study

Introduction

Sarcocystosis is a zoonotic and protozoan disease, often prevalent in domesticated animals such as buffaloes, cattle, and pigs. *Sarcocystis* is an intracellular, cyst-forming coccidian parasite under the phylum Apicomplexa and family Sarcocystidae, with two obligate hosts: a definitive carnivorous host and an intermediate omnivorous or herbivorous host (Fayer *et al*. 2015; Cerqueira-Cezar *et al*. 2017). Complications from the pathogen in domestic cattle's may lead to severe fatalities such as abortion, reduced milk yield, neurological signs, and weight loss. Infection with macroscopic sarcocystis leads to a low market profile for the meat along with condemnation of the infected hosts (El-Morsey *et al*. 2015; Calero-Bernal *et al*. 2016; Chen *et al*. 2017; Verma *et al.* 2017). The main sources of sarcocysts infection in humans are meat and meat-derivatives, through ingestion of well-developed tissue cysts containing bradyzoites (Abu-Elwafa *et al*. 2015; Daptardar *et al*. 2016; Gjerde *et al*. 2016). The Indian water buffalo (*Bubalus bubalis*) acts as a natural reservoir that harbors the four named species of *Sarcocystis*: *S. fusiformis*, *S. buffalonis*, *S. levinei*, and *S. sinensis* (Gjerde 2016 a, b; Gjerde *et al*. 2017). Infection of Egyptian buffaloes by two *Sarcocystis* species (*S. fusiformis* and *S. levinei*) was revealed in a study by El-Dakhly *et al*. (2011). A similar study was carried out in Sohag, Egypt by Khalifa *et al*. (2008), and they reported the detection of three *Sarcocystis* sp. and a macroscopic fusiform shaped *S. fusiformis* (Scioscia *et al*. 2017).

Hence, the present study aimed to describe the morphological characterization of sarcocysts isolated from different or-

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gans of slaughtered *Bubalus bubalis* by light and transmission electron microscopy. Additionally, molecular characterization of the recovered 18S rRNA sequences was performed out and compared with sequences for the same genus previously deposited in the GenBank in order to determine the exact taxonomic position within Sarcocystidae.

Materials and Methods

Sample collection and light microscopy

A total number of 35 adult male buffaloes *Bubalus bubalis* (Mammalia: Bovidae) freshly slaughtered at El-Bassatine Abattoir, Egypt; were surveyed for the presence of *Sarcocystis* macrocysts during 2015. These sentence should be changed into: " Tissue samples from the slaughter house were preserved in ice bags according to Huong (1997) and transferred to the Laboratory of Parasitology, Faculty of Science, Cairo University, Egypt for later examination. Specimens were stored under refrigeration prior to examination. Macroscopically visible sarcocysts were excised from the esophagus, heart and diaphragm muscles, while small pieces of infected muscles containing visible sarcocysts (1–3 mm long and about 0.2 mm wide) were also collected. The excised sarcocysts and muscle tissue were preserved in 10% buffered formalin for light microscopy, 3% buffered glutaraldehyde for transmission electron microscopy and 70% ethanol for molecular studies. For the histopathological examination, buffered formalin solution 10% (pH 7.3) was used to immobilize small (3 mm) pieces of infected muscle samples for at least 12 hours. Phosphate buffer was used to wash the samples for 10 min two to three times; specimens were dehydrated in a gradual ethanol series (50%, 70%, 80%, 90%, 96% and 100%), cleared in butanol and were finally embedded in paraplast at 62°C. Sections of 5–7 µm thickness were prepared using the rotary microtome. After removing the wax, sections were hydrated in a descending series of ethanol and finally stained with Haematoxylin and Eosin. The stained sections were examined by a photomicroscope (Zeiss Research) and were photographed by the attached digital camera (Canon).

Transmission electron microscopy

Very small pieces from the highly infected muscles were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for at least 24h. Samples were washed four to five times in the same buffer, for 10–15 min each, and were treated with 2% (w/v) osmium tetroxide, rewashed in the buffer, and rinsed in 1% (w/v) uranyl acetate–phosphotungstic acid in ethanol (Mollenhauer 1964). Finally, samples were dehydrated in an ascending series of ethanol, treated with propylene oxide and embedded in araldite mixture (SERVA). Semi-thin and ultrathin sections were cut on a Reichert Ultracut with glass knives. Ultrathin sections were examined with a Jeol EM.

Molecular analyses

From the ethanol preserved samples, genomic DNA was extracted from sarcocysts with a QIAmp DNA Mini Kit (Qiagen, Germany) according to manufacturer's instructions, and were stored at −20°C. PCR reactions were carried out on the isolated sarcocysts using the common primers (S18S F: 5′GGCTGCATGTCTAAGTATAAG3′, S18S R: 5′GCCTCT AAGTGTTAAGGTTC3′) according to Yan *et al*. (2013), to target the 18S rRNA gene in a final reaction, volume of 50 µL containing 1 µL of DNA template (100 ng), 0.5 µL (50 pmol) of each primer, 2 µL of dNTPs (Advanced Bioenzymes, UK), 1 µL DNA polymerase (2 U) (Advanced Bioenzymes, UK), 5 µL of 10× buffer (500 mM KCl, 100 mM Tris HCl pH 9.0, 1.5 mM $MgCl_2$), and 40 µL of dist. H_2O. Initial denaturation occurred at 94°C for 4 min, with 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 90 sec, and then a final extension at 72°C for 1 min. The amplification reactions were carried out in a PCR Thermocycler (PTC 100, MJR Research, USA). Two microliters of bromophenol blue were added to the aliquots of PCR products and the corresponding amplicons were electrophoresed on 2.5% agarose gel, stained with ethidium bromide, visualized using a UV transilluminator, and photographed.

Sequencing and phylogeny

PCR product was purified with a purification kit (Roche Diagnostics, Germany), and sequencing templates were prepared using a plasmid preparation kit (Machery-Nagel) and a BIO Dye Terminator v 3.1 Ready Sequencing Kit (Applied Biosystems) and 310 Automated DNA Sequencer (Applied Biosystems, USA) using universal forward and reverse primers. To identify related sequences, a BLAST search was carried out on the NCBI database. Data of DNA sequences were aligned using CLUSTAL-X multiple sequence alignment (Thompson *et al*. 1997) and compared with previously recorded data from Genbank to analyze the intra-specific differences. The alignment was corrected manually using the alignment editor on the software BIOEDIT 4.8.9 (Hall 1999). A phylogenetic tree was constructed using MEGA version 4 (Tamura *et al*. 2007). *Neospora caninum* BD225295 was employed as an out-group.

Results

In the present study, preliminary surveillance of the herds at the slaughter house indicated that there were a few animals with noticeable weight loss and imbalance with no tendency to move, and when they did they were mostly unstable. Post-slaughter, the infected animals showed enlargement and color change of the heart. In total, 18 out of 35 (51.4%) of the examined buffaloes were found to be harboring

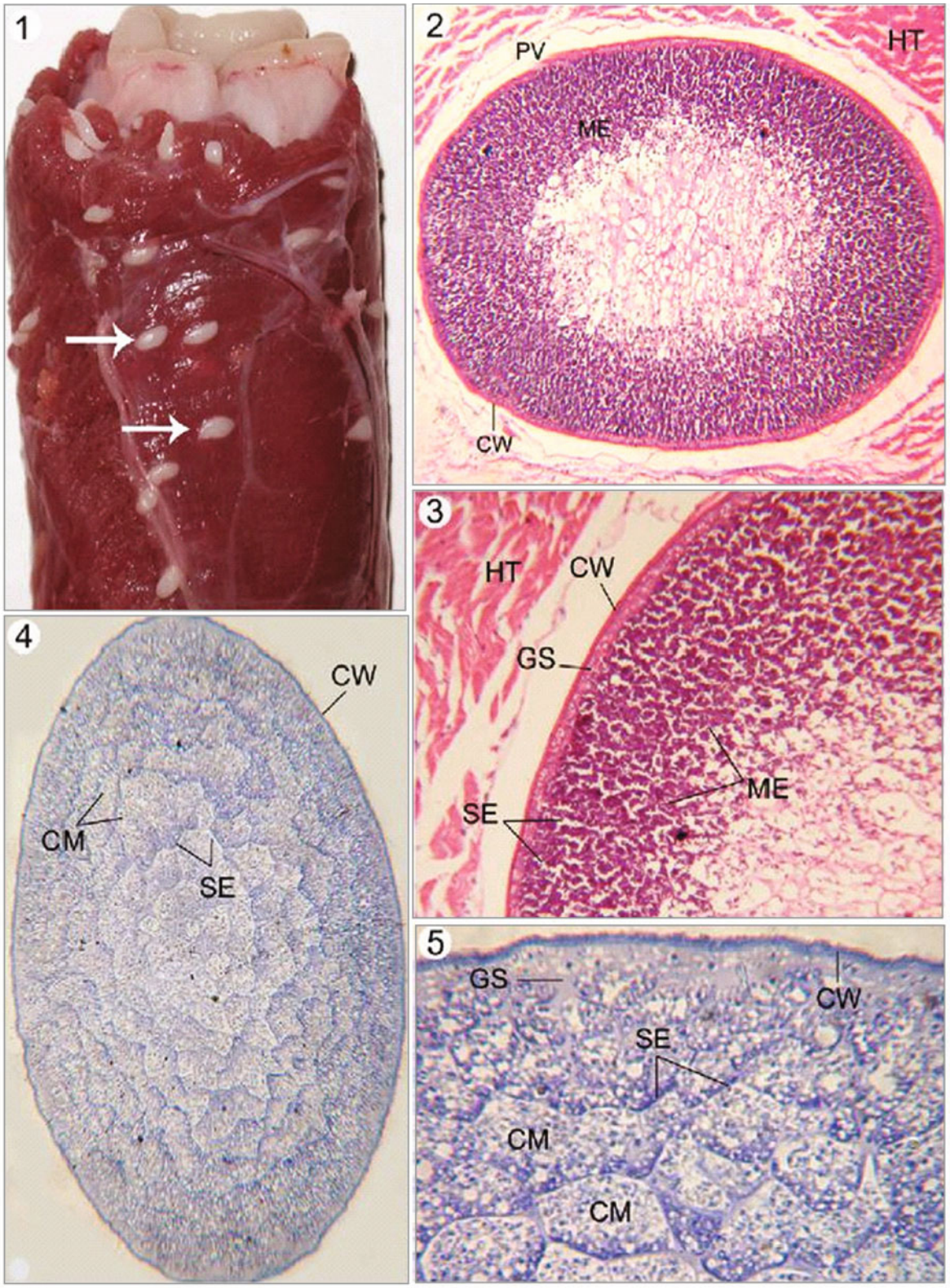
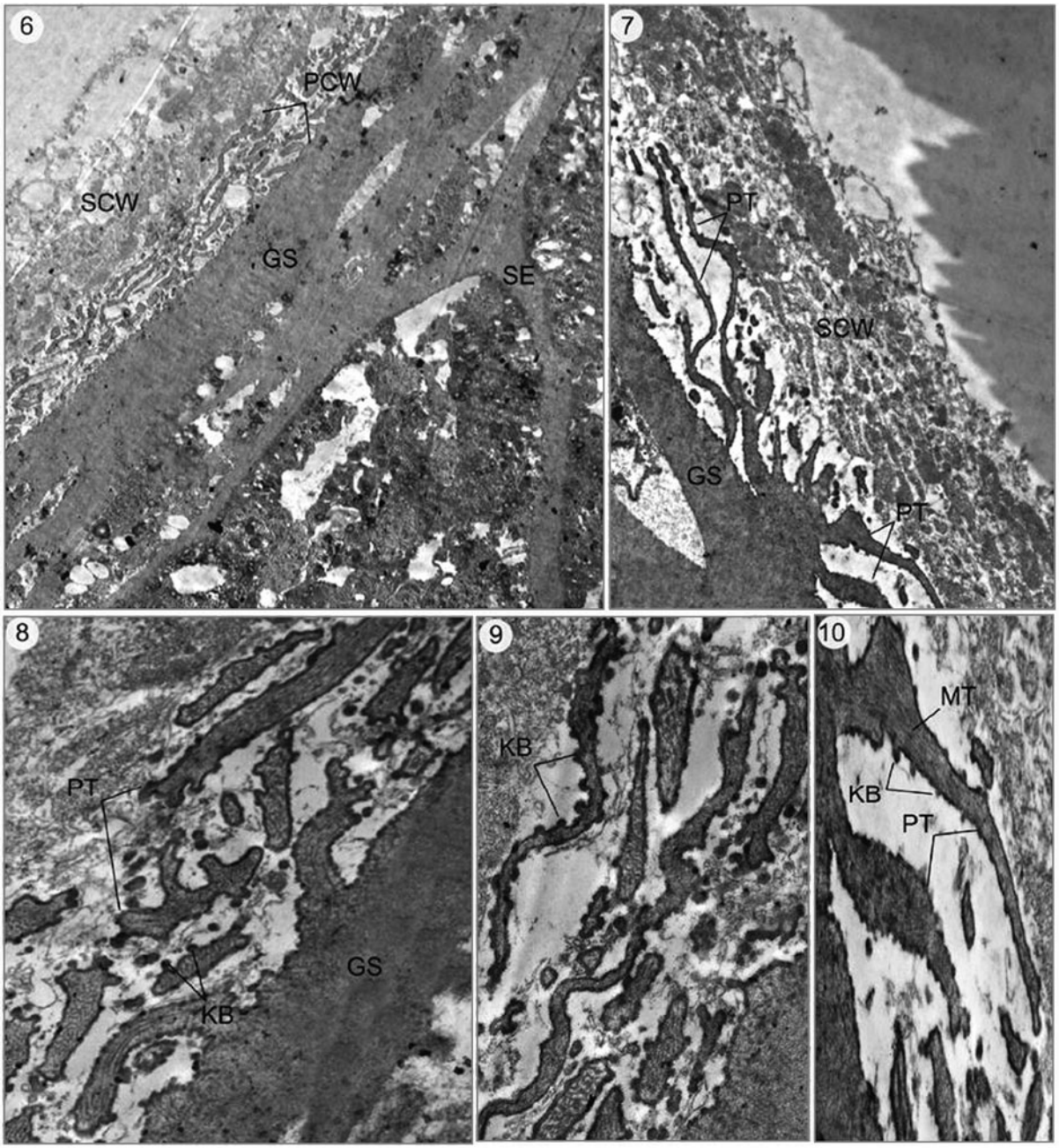


Fig. 1. 1 – Photograph showing a section of buffalo esophagus heavily infected with sarcocysts (arrows) of *Sarcocystis fusiformis*. The infection appeared as macroscopic and whitish sarcocysts are embedded in the host muscles. **2-5 –** Photomicrographs showing the following: 2,3 – Histological sections through host esophagus muscles infected with a parasite sarcocyst within parasitophorous vacuoles (PV) and stained with hematoxylin and eosin (H&E). The cyst is bordered by a cyst wall (CW) underlined by a layer of ground substance (GS) extended into the interior of the cyst as septa (SE) dividing it into compartments enclosing the parasite merozoites (ME) (140×, 560×). **4-5 –** Semi-thin sections through a sarcocyst stained with toluidine blue showing cyst compartments (CM) separated from each other by septa (SE) (240×, 400×)

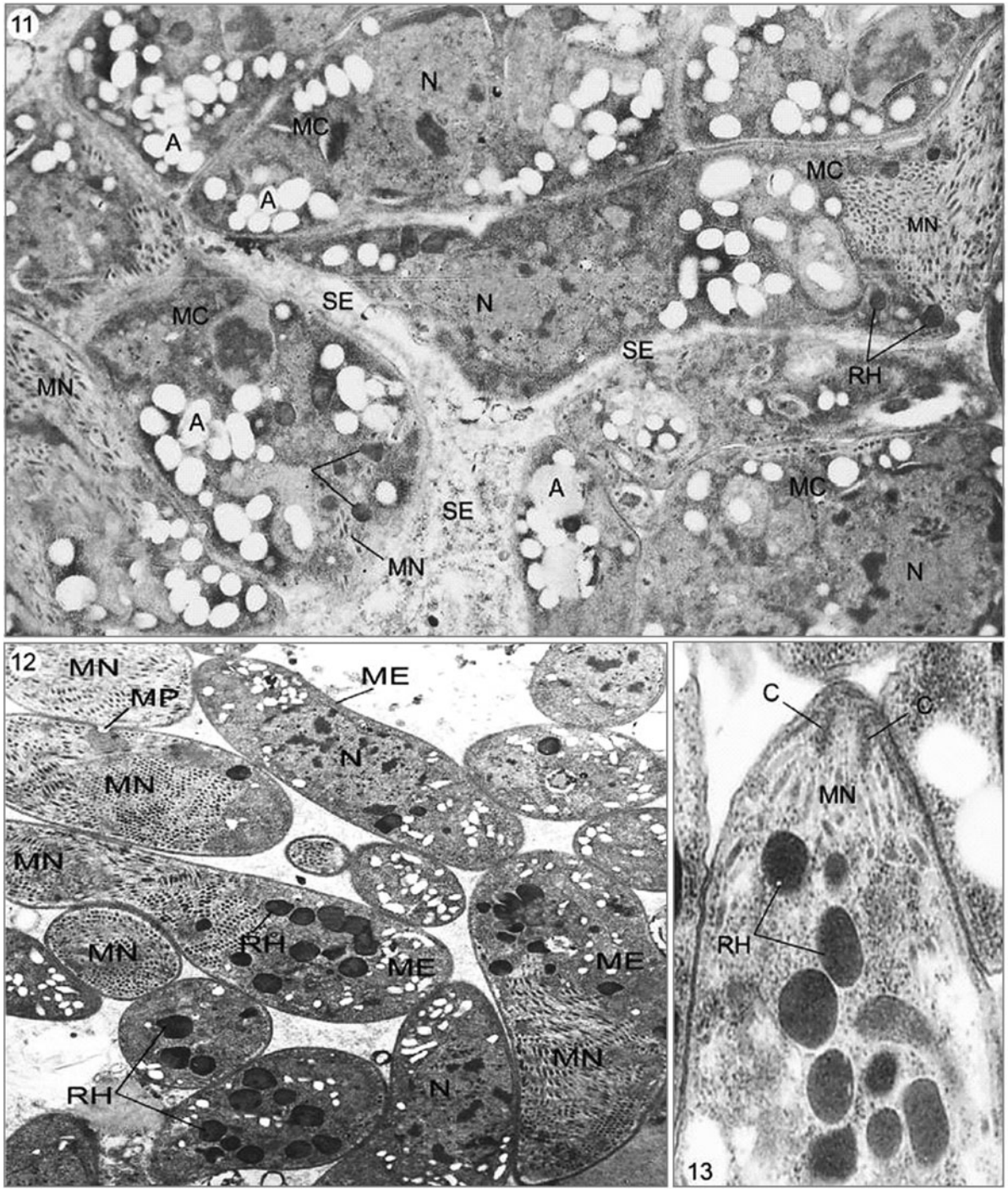
whitish cysts. The infection was observed to be macroscopic cysts embedded in the muscle tissues (Fig. 1). They measured between 33.5–63.5 µm (mean 46.3 ± 2 µm) in width, and 66.3–182 µm (mean 75 ± 4 µm) in length. The esophagus was the most infected organ followed by the diaphragm, tongue, and muscles, while the heart was the least infected organ.

Light microscopy

Ovoid sarcocysts were observed in freshly isolated muscle samples with dimensions of 152–230 µm (mean 210 ± 7 µm) in length and 37–119 µm (mean 95 ± 3 µm) in width. Histological sections (Figs. 2, 3) through the muscle tissue and its sarcocysts showed that the cyst cavity was divided into many



Figs 6–**10.** Transmission electron micrographs of ultrathin sections through the parasite sarcocyst showing the ultrastructural characteristics of the recorded sarcocysts. **6 –** The primary cyst wall (PCW) underlined by a layer of thick ground substance (GS) extended into the interior of the cyst as septa (SE) dividing it into compartments. External to the primary cyst wall is a secondary cyst wall (SW) of host origin (13,000×). **7-9 –** The primary cyst wall has cauliflower-like protrusions (PT) of variable size and shape with knob (KB) like structures surrounding the outer layer of each protrusion. Fig. 7 (16,000×), 8,9 (20,000×). **10 –** High magnification of the primary cyst wall showing a protrusion with its internal microtubules (MT) (22,000×)



Figs 11–**13.** Transmission electron micrographs showing the following: **11 –** Part of the interior of a sarcocyst just underneath the primary cyst wall. The cyst is divided into compartments separated by septa (SE) filled with mother cells or metrocytes (MC). Metrocytes retained most of the apical complex structural characteristics such as micronemes (MN), rhoptries (RH), amylopectin (A), and nucleus (N), 11,400×. **12 –** Different cross-section levels through cyst merozoites (ME). These stages exhibit the apical complex structural elements of motile infective stages as micronemes (MN), rhoptries (RH), micropore (MP) and conoid (C), 17,000×. **13 –** High magnification of the apical complex region of a merozoite showing its elements; the anterior polar ring (APR) is the terminal part of the merozoite, the mechanical penetrating organ represented by two conoids (C), and the secretory organelles are represented by micronemes (MN) and rhoptries (RH), 22,000×

chamber-like compartments separated from each other by septa derived from the ground substance, which was located under the primary cyst wall. In the examined semi-thin sections through sarcocysts, the cyst compartments were enclosed and surrounded by the primary cyst wall underneath a ground substance. Two types of parasitic stages were observed within

the cyst; metrocytes that acquired a faint stain with toluidine blue located directly under the ground substance and cyst merozoites that filled most of the cyst‘s interior area, which was darkly stained with toluidine blue (Figs. 4, 5).

Transmission electron microscopy

In the present study, examination of ultrathin sections through the isolated macroscopic sarcocysts showed that all of the cysts examined bordered by a clearly primary cyst wall (Figs. 6–10) of 0.08–0.22 µm thickness had developed from the parasitophorous vacuolar membrane. An easily observable secondary cyst wall also presided. A crucial identification factor for species identification such as the structure of the primary cyst wall had revealed that this wall, irregular folds with numerous cauliflower-like projections or protrusions of variable sizes and shapes. Additionally, knob-like electron-dense elevations accompanied by projections were observed (Figs. 8–10). Within the projections, many fibrillar elements were observed (Fig. 10). Numerous vesicles, vacuoles, and mito-

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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         5         15         25         35         45         55
S. fusiformis MG572125  -TGGAAATTC TATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. buffalonis           CTGGAAATTC AATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. fusiformis           CTGGAAATTC TATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. cafferi              CTGGCGATGC ACAGTCT-GC CCGGGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAAAT
S. hirsuta              CTGGAAATTC AATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. gigantea             CTGGAAATTG TATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. alceslatrans         CTGGAAATTT TATTTCTAGT GATTGGAATG ATGGGAATTT AAACCCCTTT CAGAGTAACA
S. scandinavica         CTGGAGATTA TATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. tarandi              CTGGAAATTT TATTTCTAGT GATTGGAATG ATGGGAATTY AAACCCCTTT CAGAGTAACA
S. hominis              CTGGAAATTC AATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. tenella              CTGGAAATTT TATTTCTAGT GATTGGAATG ATGGGAATTT AAACCCCTTT CAGAGTAACA
S. moulei               CTGATGGTCG TAGCGATACT GACGTGCAAA TCGTTCGTCA AACTTGGGTA TAGGGGAAAG
S. hirsuta              CTGGAAATTC AATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA
S. masoni               CTGGAAATTT TATTTCTAGT GATTGGAATG ATGGGAATCC AAACCCCTTT CAGAGTAACA

                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        65         75         85         95        105        115
S. fusiformis MG572125  ATTGGAGGGC AAGTCTGGCG CCAGCAGCCG CGGTAATCCC AGCTCCAATA GCGTATATTA
S. buffalonis           ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. fusiformis           ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. cafferi              GCTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGTACTTAAC AGCTCCAATA GCGTATATTA
S. hirsuta              ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. gigantea             ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. alceslatrans         ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. scandinavica         ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. tarandi              ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. hominis              ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. tenella              ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. moulei               ACTAATCGAA CCATCTAGTG CTGGTTTCTT CCGAAGTTTC TCTCAGGATA GCTGGAGTTT
S. hirsuta              ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA
S. masoni               ATTGGAGGGC AAGTCTGGTG CCAGCAGCCG CGGTAATTCC AGCTCCAATA GCGTATATTA

                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       125        135        145        155        165        175
S. fusiformis MG572125  AAGTTGTTGC AGTTAA-AAG CTCGTAGTTG -TATCTGCTG GAAGCAATCA GTCCGCCCTT
S. buffalonis           AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT
S. fusiformis           AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT
S. cafferi              AAGTTGCCTT AAGTACAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT
S. hirsuta              AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT
S. gigantea             AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT
S. alceslatrans         AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATGTCTGCTG GAAGCAATCA GTCCGCCCTA
S. scandinavica         AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTA
S. tarandi              AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTA
S. hominis              AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTA
S. tenella              AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATGTCTGCTG GAAGCAATCA GTC-GCCCTA
S. moulei               GATTAGTTTT ATCAGGAAAG CGAATGATTC -TTTCTCGGG GGCGCAATGC CCTCGACCTA
S. hirsuta              AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCTCTT
S. masoni               AAGTTGTTGC AGTTAAAAAG CTCGTAGTTG ATATCTGCTG GAAGCAATCA GTCCGCCCTT

                    ....|....| ....|....| ....|....| ....|
                       185        195        205        215
S. fusiformis MG572125  TTAGTGAGGG TGTGTACTTG ATGAATTCTG GCATG
S. buffalonis           TTAGTGAAGG TGTGTACTTG ATGAATTCTG GCATG
S. fusiformis           TTAGTGAGGG TGTGTACTTG ATGAATTCTG GCATG
S. cafferi              TTA-TGAGGG TGTGTACTTG ATGAATTCTG GCACC
S. hirsuta              CTAATGAGGG TGTGTACTTG ATGAATTCTG GCATG
S. gigantea             TTCGTAAGGG TGTGCACTTG ATGAATTCTG GCATG
S. alceslatrans         TTTGT-AGGG TGTGCACTTG ATGAATTCTG GCATC
S. scandinavica         TTATC-AGGG TGTGCACCTG ATGAATTCTG GCATG
S. tarandi              TTT-T-AGGG TGTGCACTTG ATGAATTCTG GCATC
S. hominis              TTT-T-AGGG TGTGCACTTG ATGAATTCTG GCATC
S. tenella              TTTGT-AGGG TGTGCACTTG ATGAAT-CTG GCATT
S. moulei               TTCTCAAATT AAATGGGTCA ATGGTTACTT AAGTG
S. hirsuta              TTAATGAGGG TGTGTACTTG ATGAATTCTG GCATG
S. masoni               ATTATAAGGG TGTGCACTTG ATGAATTCTG GCATG
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Fig. 14. Multiple sequence alignment between 18S rRNA gene sequences of the query *Sarcocystis fusiformis* (MG572125) and some species with high percentages of sequence identity using the CLUSTAL-X multiple sequence alignment program

Table I. Sarcocystis species used for the phylogenetic analysis of the sarcocysts recovered in the present study

Species	Accession number	Intermediate host	Host	Final	Sarcocyst size	Identity	Site of infection	Morphology	Reference
S. fusiformis	KR186117	Cattle, Water buffalo	Cat	China	Macrocysts	98%	Oesophagus, tongue, diaphragm and throat muscle	Thin cyst wall (1–3 µm in thickness) projecting highly branched villar protrusions.	Gjerde *et al*. 2016
S. buffalonis	KU247912	Water buffalo	Cat	Sweden	Macrocysts	97%	Oesophagus, tongue, diaphragm and throat muscles	Thick cyst wall (4–6 µm in thickness) projecting highly branched villar protrusions	Gjerde *et al*. 2015
S. hirsuta	AF176940	Cattle	Cat	New Zealand	Microcysts	97%	Heart, diaphragm and oesophagus	The membrane possessed villar projections which were folded to form two to four conical projections.	Yang *et al*. 2001
S. gigantea	KC209733	Sheep	Cat	Australia	Macrocysts	96%	Tongue, oesophagus and flank.	cauliflower like protrusions	Gjerde 2013
S. masoni	KU527113	Camelids	Dog	Argentina and Peru	Microcysts	96%	Neck and lumbar region	The wall (2·5–3·5 µm thick) was striated with conical to cylindrical villar protrusions	More *et al*. 2016
S. scandinavica	EU282020	Moose	Dog Coyote	Norway	Macrocysts	95%	Heart, diaphragm and oesophagus	Spindle shaped cysts, 1.0 × 0.1 mm, with up to 10 µm long, thin, finger-like surface protrusions	Stina *et al*. 2008
S. hominis	AF176945	Cattle	Human	China	Macrocysts	95%	Heart, diaphragm and oesophagus	Thick-walled cyst (5.9 µm), cyst size 787 × 112 µm, villar protrusions were cylindrical, oriented nearly perpendicular to the sarcocyst surface	Yang *et al*. 2001
S. alceslatrans	EU282033	Moose	Dog Coyote	Canada	Microcysts	94%	Heart, diaphragm and oesophagus	Several mm long, thread-like cysts, their surfaces with about 10.0 µm long, thin, flexible, and hair-like protrusions	Stina *et al*. 2008
S. tarandi	EF056018	Red deer	Felines	Northeastern Norway.	Macrocysts	94%	Heart and diaphragm	0.5–2 mm slender spindle shaped cysts with thin, straight, finger-like surface protrusions	Dahlgren and Gjerde 2007
S. tenella	L24383	Sheep	Dog Coyote	Sweden	Macrocysts	93%	Tongue, diaphragm, masseter, limb and intercostals	The cyst wall with villiar-like protrusions about 3.5 µm long	Ellis *et al*. 1995
S. cafferi	KJ778010	African buffaloes	Cat	South Africa	Macrocysts	91%	Heart, skeletal muscle, and tongue	The cyst wall is up to 3.6 µm thick with an outermost parasitophorous vacuolar membrane lined with an electron dense layer, 25 nm thick	Dubey *et al*. 2014

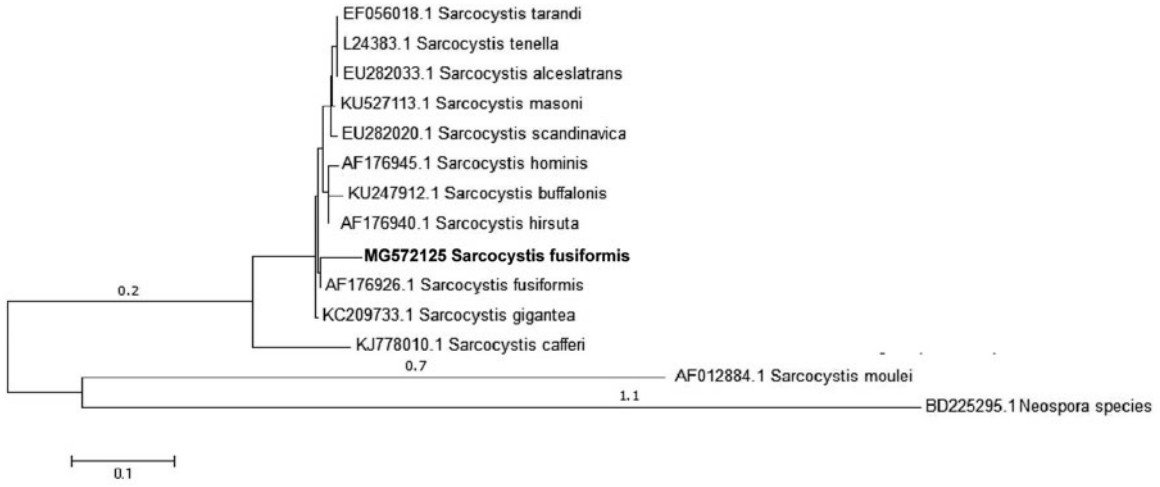

Fig. 15. A dendogram showing the phylogenetic relationship based on 18S rRNA gene sequences. The evolutionary history was inferred using the neighbor-joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method. The analysis involved 14 nucleotide sequences. There were a total of 140 positions in the final dataset. Evolutionary analyses were conducted in MEGA 4. *Neospora caninum* BD225295 was employed as an out-group

chondria of the host cell were observed surrounding these projections. In longitudinal sections, these protrusions measured 0.49–0.83 µm (mean 0.67 ± 0.02 µm). Under the primary cyst wall, a relatively thick homogenous ground substance was observed with 0.074–0.53 µm (mean 0.29 µm) thickness. The ground substance (Fig. 11) extended to the inside of the cyst as septa divided the cavity into compartments containing the parasite merozoites and metrocytes. The globular metrocytes occupied the peripheral portion of the cyst directly under the ground substance, while the banana-shaped merozoites were concentrated in the central region. Metrocytes were structurally similar to merozoites, but did not possess a conoid and had less osmiophilic cytoplasm. They were oval to globular in shape with a relatively large nucleus, and were 6.5–8.4 µm (mean 7.1 ± 3 µm) long and 9.1–10.4 µm (mean 9.7 ± 3 µm) wide. Metrocytic lipid droplets were removed. Also, large amylopectin granules were observed in the cytoplasm, measuring 0.44–0.63 µm (mean 0.57 ± 0.02 µm) in length. Merozoites of the isolated sarcocysts (Fig. 12) showed the same ultrastructural patterns as the apicomplexan merozoites of Eimeriidae organisms. These stages were banana shaped, with a blunt posterior and pointed anterior end. They were 13.2–16.4 µm (mean 13.9 ± 4 µm) long and 2.9–6.4 µm (mean 5.3 ± 2 µm) wide. The outer boundary of each merozoite consisted of two layers; an outermost plasmalemma and an inner membranous layer together formed the pellicle. Ultrastructurally, the apical complex (Fig. 13) of the sarcocyst merozoites consisted of the following: the pellicle was the outer covering of each motile infective stage (merozoite), formed of three layers, the outer most plasmalemma, the intermediate electron-pale space, and an inner membranous double layer complex. A conoid was observed at the anterior polar ring; this was a hollow cone-like structure consisting of spirally arranged fibrillar structures with one or two conoidal rings. Rhoptries were located in the anterior region; they are paired club-shaped and dense structures with a gland like appearance. Micronemes, unlike the implication of their name, are structures shaped like rice grains and were numerous filling the whole anterior region.

Molecular analyses

Construction of DNA sequences using forward and reverse primers resulted in a fragment containing 215 consensus nucleotides. Sequence data from the SSU rRNA gene of the present sarcocysts showed significant identity with archived SSU rRNA sequences from many Eimeriidae organisms (Figs. 14,15; Table I). The percentage of identity between them and the species under investigation was recorded. *S. fusiformis* KR186117 had the highest degree of sequence identity (98%) to those of the present species, followed by *S. buffalonis* KU247912.1 (97%), *S. hirsuta* AF176940.1 (97%), *S. gigantea* KC209733.1 (96%), *S. masoni* KU527113.1 (96%), *S. scandinavica* EU282032.1 (95%), *S. hominis* AF176945.1 (95%), *S. tarandi* EF056018.1 (94%), *S. alceslatrans* EU282033.1 (94%), and *S. tenella* L24383.1 (93%), while the lowest degree of sequence identity was observed for *S. cafferi* KJ778010 (91%). The recovered sequences were deposited in the GenBank under the accession number MG572125.

Discussion

A wide range of mammals harbor *Sarcocystis*, and it is uncommon in ectothermic vertebrates (El-Morsey *et al*. 2015, Dubey *et al*. 2017). The coccidian nature of *Sarcocystis* was

elucidated by (Fayer 1972, Rommel *et al*. 1972, Jäkel 1995, Oryan *et al*. 1996, Moré *et al*. 2016). El-Bassatine Abattoir showed a high infection profile in slaughtered buffaloes, leading to the indication that these domestic animals are at a high risk of infection because of their surreal inter-relationships ±with each other. The indication that this parasite is highly contagious agrees forth with the studies performed by Collier *et al*. (1998), which observed that it was involved in zoonotic transmission, sporocystic sheddings, and concomitant resistance of oocysts in an external micro-environment. Similar results were previously reported from studies on domestic mammals, and were as high as 100% in sheep (Ford 1987), 93% in donkeys (El-Ganayni 2003), 90% in cattle (Fukuyo *et al*. 2002), 73.1% in lions in Argentina (Moré *et al*. 2011) but, Khalifa *et al.* (2008) reported 84% in cattle and 28% in buffaloes in Sohag, Egypt. In goats, a percentage of 77% (Al-Hoot *et al*. 2005) and 79.4% (Morsy *et al*. 2011) were recorded. Horses were found to be 93% infected in Mongolia (Fukuyo *et al*. 2002), while, Sakran *et al*. (2013) stated that it was 25%. In camels, a percentage of 64% of animals were recorded to be infected (Abdel-Ghaffar *et al*. 2009, Al-Quraishi *et al*. 2005). In pigs, infection percentages of both 68% (Lukesová *et al*. 1986), and 34.7% (Grikienienė 1994) have been recorded, while in cats, the percentage found varied from 17.6% (Lukesová *et al*. 1986), to 10% (Gillis *et al*. 2003), and 0.03% (Barutzki and Schaper 2011). In birds, 36.5% (Černá 1984), 62% (Olias *et al*. 2011), 8% in common moorhen (*Gallinula chloropus*) (El-Morsey *et al*. 2013), and 25% in jackdaw (*Corvus monedula*) (Praka *et al*. 2013) were reported, and buffaloes in Assiut Province were found to be 25.5% infected (Metwally *et al*. 2014). Sarcocysts vary in morphology depending on species and age (JyothiSree *et al*. 2017). Common shapes are filamentous, elongated, or globular (Dubey *et al*. 1989). At the same time, authors have reported *Sarcocystis* with different forms according to its species: they were stumpy, as in *S. zamani* (Mehlhorn *et al*. 1976); spindle-shaped, as in *S. idahoensis* (Bledsoe 1980); streak-like, as in *S. crotali* (Entzeroth *et al*. 1985); slender, as in *S. clethrionomyelaphis* (Matuschka 1986); spherical, as in *S. hoarensis* (Matuschka *et al*. 1987); ovoid, as in *S. moulei* (Abdel-Ghaffar *et al*. 1989); filiform, as in *S. dubeyella* (Stolte *et al*. 1998); ellipitical, as in *S. felis* in cats (Elsheikha *et al*. 2006); or thick thread-like, as in *Sarcocystis corvusi* in jackdaw (Prakas *et al*. 2013). As mentioned earlier, the morphological characteristics of the primary cyst wall play a pivotal role in the genus identification of *Sarcocystis* (Mehlhorn and Heydorn 1978, Dubey *et al*. 2006). According to the primary cyst wall, mature sarcocysts are basically differentiated into thick-walled, if the outer portions of their walls have prominent long projections, and thin walled, if these protrusions are short or disappear (Dar *et al*. 2017). Henceforth, the comparative parameter of species differences should be restricted to mature cysts (Bashtar *et al*. 1988, Abdel-Ghaffar *et al*. 1990, 2009, Modrý *et al*. 2000, Mehlhorn 2008, Latif *et al*. 2015) because young cysts are somewhat similar. In the present study, a unipolar membrane limited coccidian parasitophorous vacuole led to the rise of recovered macroscopic sarcocysts (Scholtyseck *et al*. 1974, Mehlhorn and Heydorn 1978, Abdel-Ghaffar *et al*. 1989, Abdel-Ghaffar and Al-Johany 2002, Al-Quraishi *et al*. 2005, Gjerde and Hilali 2016). Table I shows a comparison between the present *S. fusiformis* and some species of the same genus from different host species. The general developmental steps for the formation of the cyst wall from the parasitophorous vacuolar membrane were observed in the cysts of the *Sarcocystis* sp. examined. The origin of this cyst wall is controversial. Some authors concluded that it was of host origin, others suggested that it was of parasitic origin, while the third opinion claimed that this wall might be derived from both sources (Mehlhorn and Heydorn 1978). After the formation of the parasitophorous vacuole wall and cyst formation began, the underlying layer of osmiophilic material strengthened and the complex structure formed become the primary cyst wall (Mehlhorn 2008, Dubey *et al*. 2016). This wall varies widely among different stages of development of the parasite; thus, it was postulated that the fine structure of the sarcocyst wall could be used with care as a criterion to identify different species of *Sarcocystis* (Scholtyseck *et al*. 1974, Modrý *et al*. 2000, Dubey *et al*. 2006). In some *Sarcocystis* spp., this wall was thick and folded many times to form irregular wall folds, with numerous cauliflower projections and knob-like electron-dense elevations. Similar results were obtained from the study of buffaloes by Abdel-Ghaffar *et al*. (1989). In addition to this primary wall and its complex structures, a layer of fibril material of host origin enclosing the parasitized host cell may also appear; this is known as a secondary cyst wall, representing a significant response from the host towards the parasite in accordance with previous studies (Mehlhorn and Scholtyseck 1973, Abdel-Ghaffar *et al*. 1989, Dubey *et al*. 2014, Ahmadi *et al*. 2015). Under the primary cyst wall a relatively thin homogenous layer known as the ground substance extended outward through the middle portion of the protrusions, leaving a clear zone from each side and inward through the cyst as septa; this is one of the main features characterizing sarcocysts of the genus *Sarcocystis* (Matuschka and Bannert 1989, Dubey *et al*. 2017, Hu *et al*. 2017). The fine structural characteristics of merozoites were similar to those described for many other *Sarcocystis* species: a cyst enclosed by a complex membranous structure composed of two layers, interrupted by anterior polar rings and a micropore. In the anterior part of the currently recorded cyst there was a hallmark organelle, polar rings, conoid, micronemes, and rhoptries, resembling those reported previously (Abdel-Ghaffar and Al-Johany 2002, Dubey *et al*. 2017). In the posterior merozoite there was a globular nucleus, mitochondria and apicoplast, reserve food materials presented in two forms (lipid and amylopectin granules) were founded scattered randomly, in accordance with (Abdel-Ghaffar and Al-Johany 2002, Abdel-Ghaffar *et al*. 2009). Metrocytes or mother cells represent one of the main components of the cyst, which is the main stage of asexual reproduction or multiplication inside the cyst (Mehlhorn and Heydorn 1978, Dubey *et al*. 2017). They possessed all the api-

cal complex structural characteristics of the merozoite stage. Their nuclei were relatively large and have peripherally arranged chromatin materials. In the present study, metrocytes had a shape variation from oval to globular with a proportionately large nucleus, and their measurements are similar to those of sarcocysts reported by Abdel-Ghaffar *et al*. (1978).

Lately, the 18S rRNA sequences have been widely used as appropriate targets for the accurate hierarchical identification and phylogenetic analyses of *Sarcocystis* spp. (Ellis *et al*. 1995; Holmdahl *et al*. 1999; Li *et al*. 2002; Dahlgren and Gjerde 2007; Stina *et al*. 2008; Gjerde 2013). The perpetuated regions of 18S rRNA gene play a pivotal role in primer designing, which can be used to amplify identical genes in related species. Improving of phylogenetic analysis techniques have led to easier identification of the mosaic structure of the parasite (Olsen and Woese 1993; Gjerde *et al*. 2015). In addition, within the *Sarcocystis* species, the gene sequence has variable genotypic behaviors (Holmdahl *et al*. 1999; Hamidinejat *et al*. 2015). Holmdahl *et al*. (1994) had reported a rather high identity value of 18S rRNA genes within species; hence, molecular analysis and sequencing may stand apart from species identification. Phylogenetic analysis showed that the present species is *Sarcocystis fusiformis* according to both morphological and molecular studies. Additionally, the sequence alignment, as well as the cladogram, revealed a high nucleotide homology (98%) of the test organism to that of *Sarcocystis fusiformis* KR186117, in addition to the ultrastructural similarities in the primary cyst wall between the two species; this confirmed that the sarcocyst under study is for *Sarcocystis fusiformis*. In addition, large sequence identities (>95%) were observed for some of the aligned species, such as *S. buffalonis* KU247912 (97%), *S. hirsuta* AF176940 (97%), *S. gigan*tea KC209733 (96%), and S. masoni KU527113 (96%) but by comparing the ultrastructural characteristics between these species and the recorded sarcocysts in the current study, it was revealed that there is no relation between them in the morphology of the primary cyst wall. The findings of Holmdahl *et al*. (1994) indicated that most of the *Sarcocystis* species, with the exception for *S. buffalonis* and S. *hirsute* (13 positional differences), showed polymorphism in more than 100 bp positions. Correspondingly, the *S. fusiformis* recorded by Holmdahl *et al*. (1999) and Yang *et al*. (2001) from China and Sweden, respectively, had a sequential difference that 3% different to each other. Hence, inspection of more *Sarcocystis* from other geographically isolated areas and sequencing at other different genetic loci (for example, 18S rRNA and ITS-1) may reveal the prevalence of further ameliorations within similar species. Another approach to nucleotide polymorphism within the same *Sarcocystis* species is that, water buffaloes in different geographical regions may act as a reservoir for other species too. Oryan *et al*. (1996) and Hu *et al*. (2016) reported that the 18S rRNA gene comprises multivariate genes which may lead to diversity among multiple copies of this specific gene that was amplified from different merozoites within the sarcocysts.

Compliance with Ethical Standards

Parasite collection from the examined host was carried out according to the regulatory laws and ethical considerations regarding experimental ethics of animal use and collecting permits.

Conflict of interest

The authors have indicated that they have no conflict of interest regarding the content of this article.

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References

Abdel-Ghaffar F., Al-Johany A.M. 2002. A light and electron microscope study of *Sarcocystis mitrani* (sp. nov.) infecting the skink *Scincus mitranus* in the central region of Saudi Arabia. *Parasitology Research*, 88, 102–106

Abdel-Ghaffar F., Bashtar A.R., Ashour M.B., Sakran T.H. 1990. Life cycle of *Sarcocystis gongyli* Trinci 1911 in the skink *Chalcides ocellatus* and the snake *Spalerosophis diadema*. *Parasitology Research*, 76, 444–450

Abdel-Ghaffar F., Heydorn A.O., Mehlhorn H. 1989. The fine structure of cysts of *Sarcocystis moulei* from goats. *Parasitology Research*, 75, 416–418

Abdel-Ghaffar F., Hilali M., Scholtyseek E. 1978. Ultrastructure study of *Sarcocystis fusiformis* (Railliet 1897) infecting the Indian water buffalo (*Bubalus bubalis*) of Egypt. *Tropical Medicine and Parasitology*, 29, 289–294

Abdel-Ghaffar F., Mehlhorn H., Bashtar A.R., Al-Rasheid K., Sakran T.H., Al-Fayoumi H. 2009. Life cycle of *Sarcocystis camelicanis* infecting the camel (*Camelus dromedarius*) and the dog (*Canis familiaris*), light and electron microscopic study. *Parasitology Research*, 106, 189–195

Abu-Elwafa S., Al-Araby M., Abbas I. 2015. *Sarcocystis fusiformis* infecting water buffaloes (*Bubalus bubalis*) in Dakahlia province, Egypt. *International Journal of Advanced Research*, 3, n116–120

Ahmadi M.M., Hajimohammadi B., Eslami G., Oryan A., Yasini Ardakani S.A., Zohourtabar A., Zare S. 2015. First identification of *Sarcocystis hominis* in Iranian traditional hamburger. *Journal of Parasitic Diseases*, 39, 770–772

Al-Hoot A.S., Al-Qureishy S.A., Al-Rashid K., Bashtar A.R. 2005. Microscopic study on *Sarcocystis moulei* from sheep and goats in Saudi Arabia. *Journal of the Egyptian Society of Parasitology*, 35, 295–312

Al-Quraishi S.A., Bashtar A.R., Al-Rasheid K.A.S., Abdel-Ghaffar F. 2005. The prevalence and ultrastructure of *Sarcocystis* species infecting camels (*Camelus dromedaries*) Slaughtered in Riyadh city, Saudi Arabia. *Saudi Journal of Biological Sciences*, 11,135–142

Barutzki D., Schaper R. 2011. Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. *Parasitology Research*, 109, 545–560

Bashtar A.R., Abdel-Ghaffar F., El-Sayed M.A. 1988. Electron microscopic study on *Sarcocystis capracanis* infecting goats at Aswan area. *Egyptian Journal of Histology*, 11, 211–219

Bledsoe B. 1980. *Sarcocystis idahoensis* sp. n. in deer mice *Peromyscus maniculatus* (wagner) and gopher snakes *Pituophis melanoleucus* (Daudin). *The Journal of Protozoology*, 27, 93–102

Calero-Bernal R., Cerqueira-Cézar C.K., Verma S.K., Mowery J., Carmena D., Beckmen K., Dubey J.P. 2016. *Sarcocystis arctica* (Apicomplexa: Sarcocystidae): ultrastructural description and its new host record, the Alaskan wolf (*Canis lupus*). *Parasitology Research*, 115, 2893–2897

Černá Ž. 1984. The role of birds as definitive hosts and intermediate hosts of heteroxenous coccidians. *The Journal of Protozoology*, 31, 579–581

Cerqueira-Cezar C.K., Thompson P.C., Verma S.K., Mowery J., Calero-Bernal R., Antunes Murata F.H., *et al.* 2017. Morphological and molecular characterization of *Sarcocystis arctica*-like sarcocysts from the Arctic fox (*Vulpes lagopus*) from Alaska, USA. *Parasitology Research*, 116, 1871–1878

Chen X., Wen T., Hu J., Liu T., Esch G.W., Liang Y., *et al.* 2017. *Sarcocystis dehongensis* n. sp. (Apicomplexa: Sarcocystidae) from water buffalo (*Bubalus bubalis*) in China. *Parasitology Research*, 116, 2145–2150

Collier L., Balows A., Sussman M. 1998. *Sarcocystis* Isospora and Cyclospora. In: Gransden W.R, editor. Topley and Welson's:Microbiology and Microbial Infections. 9th ed. Vol. 5. New York: Oxford University Press Inc, pp. 319–326

Dahlgren S., Gjerde B. 2007. Genetic characterization of six *Sarcocystis* species from reindeer (*Rangifer tarand us tarand us*) in Norway based on the small subunit rRNA gene. *Veterinary Parasitology*, 146, 204–213

Daptardar M., Singh B.B., Aulakh R.S., Gill J.P 2016. Prevalence and first molecular identification of *Sarcocystis* species in cattle and water buffaloes in India. *Acta Parasitologica*, 61, 523–528

Dar K.H., Bhat M.M., Shafi M. 2017. Prevalence and organwise distribution of sarcocystiosis in buffaloes of Mohali, Punjab. *Journal of Parasitic Diseases*, 41, 318–321

Dubey J.P., Lane E.P., Wilpe E., Suleman E., Reininghaus B., Verma S.K., *et al.* 2014. *Sarcocystis cafferi* n. sp. (Protozoa: Apicomplexa) from the African Buffalo (*Syncerus caffer*). *Journal of Parasitology*, 100, 817–827

Dubey J.P., Moré G., van Wilpe E., Calero-Bernal R., Verma S.K., Schares G. 2016. *Sarcocystis rommeli*, n. sp. (Apicomplexa: Sarcocystidae) from Cattle (Bostaurus) and its Differentiation from *Sarcocystis hominis. Journal of Eukaryotic Microbiology*, 63, 62–68

Dubey J.P., Rosenthal B.M., Morales J.A., Alfaro A. 2006. Morphologic and genetic characterization of *Sarcocystis*sp. from the African grey parrot, *Psittacus erithacus*, from Costa Rica. *Acta Parasitologica*, 51, 161–168

Dubey J.P., Speer C.A., Fayer R. 1989. Sarcocystosis of animals and man, 1st ed., CRC Press, Boca Raton

Dubey J.P., Naji N., Mowery J., Verma S., Calero-Bernal R. 2017. Identification of macroscopic sarcocysts of *sarcocystis cameli* from one- humped camel (*camelus dromedarius*) in Iraq. *Journal of Parasitology*, 103, 168–169

El-Dakhly K.M., El-Nesr K.A., El-Nahass S., Hirata A., Sakai H., Yanai T. 2011. Prevalence and distribution patterns of *Sarcocystis* spp. In buffaloes in Beni-Suef, Egypt. *Tropical Animal Health and Production*, 43, 1549–1554

El-Ganayni S.E. 2003. Ultrastructure of *Sarcocystis fayeri* infecting donkeys (*Equusasinus*) in El-Menia Governorate, Egypt. *Journal of the Egyptian-German Society of Zoology*, 42, 33–46

Ellis T.J., Luton K., Baverstock P.R., Whitworth G., Tenter A.M., Johnson A.M. 1995. Phylogenetic relationships between *Toxoplasma* and *Sarcocystis* deduced from a comparison of 18S rDNA sequences. *Parasitology*, 110, 521–528

El-Morsey A., El-Seify M., Desouky A.R., Abdel-Aziz M.M., El-Dakhly K.M., Kasem S., *et al.* 2015. Morphologic and molecular characteristics of *Sarcocystis atraii* n. sp. (Apicomplexa: Sarcocystidae) infecting the common coot (*Fulica atra*) from Egypt. *Acta Parasitologica*, 60, 691–9

El-Morsey A., El-Seify M., Desouky A.R., Abdel-Aziz M.M., Sakai H., Yanai T. 2013. Morphologic identification of a new *Sarcocystis* sp. in the common moorhen (*Gallinula chloropus*) (Aves: Gruiformes: Rallidae) from Brolos Lake, Egypt. *Parasitology Research*, 113, 391–397

Elsheikha H.M., Kennedy F.A., Murphy A.J., Soliman M., Mansfieled L.S. 2006. Sarcocystosis of *Sarcocystis felis* in cats. *Journal of the Egyptian Society of Parasitology*, 36, 1071–1079

Entzeroth R., Chobotar B., Scholtyseck E., Nemesri L. 1985. Light and electron microscope study of *Sarcocystis* sp. from the fallow deer (*Cervivs lama*). *Parasitology Research*, 71, 33–39

Fayer R. 1972. Gametogeny of *Sarcocystis* in cell culture. *Science*, 175, 65–67

Fayer R., Esposito D.H., Dubeya J.P. 2015. Human infections with *Sarcocystis* species. *Clinical Microbiology Reviews*, 28, 295–311

Ford G.E. 1987. Hosts of two canid genera, the red fox and the dog, as alternate vectors in the transmission of *Sarcocystis tenella* from sheep. *Veterinary Parasitology*, 26, 13–20

Fukuyo M., Battsetseg G., Byambaa B. 2002. Prevalence of *Sarcocystis* infection in horses in Mangolia. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 33, 718–719

Gillis K.D., Mackay R.J., Yowell C.A., Levy J.K., Greiner E.C., Dame J.B., *et al.* 2003. Naturally occurring *sarcocystis* infection in domestic cats (*Felis catus*). *International Journal of Parasitology*, 33, 877–883

Gjerde B. 2013. Phylogenetic relationships among ***Sarcocystis*** species in cervids, cattle and sheep inferred from the mitochondrial cytochrome ***c*** oxidase subunit I gene. *International Journal of Parasitology*, 43, 579–591

Gjerde B. 2016a. Molecular characterisation of *Sarcocystis bovifelis*, *Sarcocystis bovini* n. sp., *Sarcocystis hirsuta* and *Sarcocystis cruzi* from cattle (*Bos taurus*) and *Sarcocystis sinensis* from water buffaloes (*Bubalus bubalis*). *Parasitology Research* 115, 1473–1492

Gjerde B. 2016b. The resurrection of a species: *Sarcocystis bovifelis* Heydorn *et al*., 1975 is distinct from the current *Sarcocystis hirsuta* in cattle and morphologically indistinguishable from *Sarcocystis sinensis* in water buffaloes. *Parasitology Research*, 115, 1–21

Gjerde B., Giacomelli S., Bianchi A., Bertoletti I., Mondani H., Gibelli L.R. 2017. Morphological and molecular characterization of four *Sarcocystis* spp., including *Sarcocystis linearis* n. sp., from roe deer (*Capreolus capreolus*) in Italy. *Parasitology Research*, 116, 1317–1338

Gjerde B., Hilali M. 2016. Domestic cats (*Feliscatus*) are definitive hosts for Sarcocystis sinensis from water buffaloes (*Bubalus bubalis*). *The Journal of Veterinary Medical Science*, 78, 1217–1221

Gjerde B., Hilali M., Abbas I.E. 2016. Molecular differentiation of Sarcocystis buffalonis and *Sarcocystis levinei* in water buffaloes (*Bubalus bubalis*) from *Sarcocystis hirsuta* and *Sarcocystis cruzi* in cattle (*Bos taurus*). *Parasitology Research*, 115, 2459–2471

Gjerde B., Hilali M., Mawgood S.A. 2015. Molecular characterization of three regions of the nuclear ribosomal DNA unit and the mitochondrial *cox1* gene of *Sarcocystis fusiformis* from water buffaloes (*Bubalus bubalis*) in Egypt. *Parasitology Research*, 114, 3401–3413

Grikienienė J. 1994. *Sarcocystis* in cattle and swine: significance for man and man's role in its spreading. Proceedings of the sci-

entific conference Animal husbandary and ecology, Kaunas, Lithuania, pp. 76–80

Hall T.A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95−98

Hamidinejat H., Razi Jalali M.H., Gharibi D., Molayan P.H. 2015. Detection of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffaloes (*Bubalus bubalis*) in Iran by PCR-RFLP. *Journal of Parasitic Diseases*, 39, 658–62

Holmdahl O.J., Mattsson M.J.G., Uggla A., Johansson K.E. 1994. The phylogeny of Neos- poracaninum and *Toxoplasma gondii* based on ribosomal RNA sequences. *FEMS Microbiology Letters*, 119, 187–192

Holmdahl O.J., Morrison D.A., Ellis J.T., Huong L.T. 1999. Evolution of ruminant *Sarcocystis* (Sporozoa) parasites based on small subunit rDNA sequences. *Molecular Phylogenetics and Evolution*, 11, 27–37

Hu J.J., Huang S., Wen T., Esch G.W., Liang Y., Li H.L. 2017 Morphology, Molecular Characteristics, and Demonstration of a Definitive Host for *Sarcocystis rommeli* from Cattle (Bostaurus) in China. *Journal of Parasitology*, 103, 471–476

Hu J.J., Wen T., Chen X.W., Liu T.T., Esch G.W., Huang S. 2016. Prevalence, Morphology, and Molecular Characterization of *Sarcocystis heydorni* Sarcocysts from Cattle (*Bos Taurus*) in China. *Journal of Parasitology*, 102, 545–548

Huong L.T.T., Dubey J.P., Nikkilä T., Uggla A. 1997. *Sarcocystis buffalonis* n. sp. (Protozoa: Sarcocystidae) from the water buffalo (*Bubalus bubalis*) in Vietnam. *Journal of Parasitology*, 83, 471–474

Jäkel T. 1995. Cyclic transmission of *Sarcocystis gerbilliechis* sp. n. by the Arabian saw scaled viber, *Echiscoloratus* to rodents of the sub family *Gerbillinae*. *Journal of Parasitology*, 81, 626–631

JyothiSree C., Venu R., Samatha V., Malakondaiah P., Rayulu V.C. 2017. Prevalence and microscopic studies of *Sarcocystis* infection in naturally infected water buffaloes (Bubalusbubalis) of Andhra Pradesh. *Journal of Parasitic Diseases*, 41, 476–482

Khalifa R.M., El-Nadi N.A., Sayed F.G., Omran E.K. 2008. Comparative morphological studies on three *Sarcocystis* species in Sohag, *Journal of the Egyptian Society of Parasitology*, 38, 599–608

Latif B., Kannan Kutty M., Muslim A., Hussaini J., Omar E., Heo C.C., *et al.* 2015. Light microscopy and molecular identification of *Sarcocystis* spp. in meat producing animals in Selangor, Malaysia. *Tropical Biomedicine*, 32, 444–452

Li Q.Q., Yang Z.Q., Zuo Y.X., Attwood S.W., Chen X.W., Zhang Y.P. 2002. A PCR-based RFLP analysis of *Sarcocystiscruzi* (Protozoa: Sarcocystidae) in Yunnan province, PR China, reveals the water buffalo (*Bubalus bubalis*) as a natural intermediate host. *Journal of Parasitology*, 88, 1259–1261

Lukesová D., Nevole M., Haidová B. 1986. The extent of the incidence of sarcocystosis in cattle and pig farms. *Veterinary Medicine*, 31, 521–530

Matuschka F.R. 1986. *Sarcocystis clethrionomyelaphis* n. sp. from snakes of the genus *Elaphe* and defferent voles of the family Arvicolidae. *Journal of Parasitology*, 72, 226–231

Matuschka F.R., Bannert B. 1989. Recognition of cyclic transmission of *Sarcocystis stehlinii* n. sp. in the Gran Canarian giant lizard. *Journal of Parasitology*, 75, 383–387

Matuschka F.R., Mehlhorn H., Abd-Al-Aal Z. 1987. Replacement of *Besnoitia* Matuschka and Häfner 1984 by *Sarcocystis hoarensis*. *Parasitology Research*, 74, 94–96

Mehlhorn H. 2008. Encylopedic reference of parasitology 3rd ed. Springer, Verlag, Berlin

Mehlhorn H., Hartly W.J., Heydorn A.O. 1976. A comparative ultrastructural study of the cyst wall of 13 *Sarcocystis* species. *Protistologica*, 12, 451–467

Mehlhorn H., Heydorn A.O. 1978. Sarcosporidia (Protozoa, Sporozoa): Life cycle and fine structure. *Advances in Parasitology*, 16, 73–92

Mehlhorn H., Scholtyseck E. 1973. Elektron en mikrokopischeUntersuchungen an Cystenstadien von *Sarcocysti stenella* aus der OesophagusMuskulatur des Schafes. *Parasitology Research*, 41, 291–310

Metwally A.M., Abd-Ellah M.R., Al-Hosary A.A., Omar M.A. 2014. Microscopical and serological studies on Sarcocystis infection with first report of *Sarcocystis* cruzi in buffaloes (*Bubalus bubalis*) in Assiut, Egypt. *Journal of Parasitic Diseases*, 38, 378–82

Modrý D., Koudela B., Slapeta J.R. 2000. *Sarcocystis stenodactylicolubris* n. sp., a new sarcosporidian coccidium with a snake-gecko heteroxenous life cycle. *Parasite*, 7, 201–207

Mollenhauer H.H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technology*, 39, 111–114

Moré G., Abrahamovich P., Jurado S., Bacigalupe D., Marin J.C., Rambeaud M., *et al.* 2011. Prevalence of *Sarcocystis* spp. in Argentinean cattle. *Veterinary Parasitology*, 177, 162–165

Moré G., Regensburger C., Gos L., Pardini L., Verma S., Ctibor J., *et al.* 2016. *Sarcocystis masoni*, n. sp. (Apicomplexa: Sarcocystidae), and redescription of *Sarcocystis aucheniae* from llama (*Lama glama*), guanaco (*Lama guanicoe*) and alpaca (Vicugna pacos). *Parasitology*, 143, 617–626

Morsy K., Saleh A., Al-Ghamdi A., Abdel-Ghaffar F., Al-Rasheid K., Bashtar A.R., *et al.* 2011. Prevalence pattern and biology of *Sarcocystis capracanis* infection in the Egyptian goats: a light and ultrastructural study. *Veterinary Parasitology*, 181, 75–82

Olias P.P., Olias L., Krücken J., Lierz M., Gruber A.D. 2011. High prevalence of *Sarcocystis calchasi* sporocysts in European *Accipiter* hawks. *Veterinary Parasitology*, 175, 230–236

Olsen G.J., Woese C.R. 1993. Ribosomal RNA: A key to phylogeny. *Federation of American Societies for Experimental Biology Journal*, 1, 113–123

Oryan A., Moghaddar N., Gaur S.N.S. 1996. The distribution pattern of *Sarcocystis* sp., their transmission and pathogenesis in sheep in Fars province of Iran. *Veterinary Research Communications*, 20, 243–253

Prakas P., Kutkienė L., Butkauskas D., Sruoga A., Zalakevičius M. 2013. Molecular and morphological investigations of *Sarcocystis corvusi* sp. nov. from the jackdaw (*Corvusmonedula*). *Parasitology Research*, 112, 1163–1167

Rommel M., Heydorn A.O., Gruber V. 1972. Beitragezum Lebenszyklus der Sarkosporidien. I. Die Sporozyste von *S. tenella* in den Fazes der Katze. *Berliner und Münchener tierärztliche Wochenschrift*, 85, 101–105. (In German)

Sakran T., Al-Hroub A., Ahmed A. 2013. Studies on *Sarcocystis* infecting domestic horse. *American Journal of Research Communication*, 1, 39–53

Scholtyseck E., Mehlhorn H., Miiller B.E.G. 1974. Feinstruktur der Cyste und Cystenw and von *Sarcocystis tenella*, *Besnoitia jellisoni*, *Frenkelia* sp. und *Toxoplasma gondii*. *The Journal of Protozoology*, 21, 284–294

Scioscia N.P., Olmos L., Gorosábel A., Bernad L., Pedrana J., Hecker Y.P., *et al.* 2017. Pampas fox (*Lycalopex gymnocercus*) new intermediate host of *Sarcocystis svanai* (Apicomplexa: Sarcocystidae). *Parasitology International*, 66, 214–218

Stina S., Dahlgren S.S., Gjerde B. 2008. Molecular identification and phylogeny of six *Sarcocystis* species in moose, and a morphological description of three new species. *Parasitology Research*, 103, 93–110

Stolte M., Odening K., Quand T.S., Bengis R.G., Bockhardt I. 1998. *Sarcocystis dubeyella* n. sp. and *Sarcocystis phacochoeri* n. sp. (Protozoa: Sarcocystidae) from the warthog (*Phaco-*

choerus aethiopicus) in South Africa. *Journal of Eukaryotic Microbiology*, 45, 101–104

Tamura K., Dudley J., Nei M., Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882

Verma S.K., Lindsay D.S., Mowery J.D., Rosenthal B.M., Dubey J.P. 2017. *Sarcocystis pantherophisi* n. sp., from Eastern Rat Snakes (*Pantherophis alleghaniensis*) as Definitive Hosts and Interferon Gamma Gene Knockout Mice as Experimental Intermediate Hosts. *Journal of Parasitology*, 103, 547–554

Yan W., Qian W., Li X., Wang T., Ding K., Huang T. 2013. Morphological and molecular characterization of *Sarcocystis miescheriana* from pigs in the central region of China. *Parasitology Research*, 112, 975–980

Yang Z.Q., Zuo Y.X., Yao Y.G., Chen X.W., Yang G.C., Zhang Y.P. 2001. Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. *Molecular and Biochemical Parasitology*, 115, 283–288

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