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Sarcocystis rileyi (Apicomplexa) in *Anas platyrhynchos* in Europe with a potential for spread

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ABSTRACT

Four specimens of mallard (*Anas platyrhynchos*) shot by local hunters (December 2020 to January 2021 along the eastern coastline of the island of Bornholm in the Baltic Sea) were diagnosed with a heavy load of sarcocysts in the musculature. Morphometric and molecular diagnosis based on rDNA (18 S, ITS1, 28 S) of parasites recovered from two of the birds revealed the causative pathogen to be *Sarcocystis rileyi*. We further present novel sequences for the entire 5.8 S and ITS2 for this species. Elongate cysts (mean length 5.25 (SD 0.6) mm, width 1.37 (SD 0.2) mm) were recorded in all parts of the striated skeletal musculature of the birds. The main part (72%) of the 2585 cysts in one female mallard was located in the outer superficial pectoral musculature, with 11% in the inner pectoral musculature. Minor but significant parts were found in the dorsal, ventral abdominal, neck and head, legs, hand and arm (wing) musculature. No cysts were found in the smooth musculature. Each cyst contained a median of 3.2 mio bradyzoites indicating that more than 8 billion bradyzoites are available for infection of one or more predators/scavengers ingesting the bird. Bradyzoites (median length 13.5 μm (range 12.1–14.5) and median width 2.66 μm (range 2.1–3.3)) were highly resistant to proteinase treatment, which secures the passage through the stomach of the predator to its intestine where wall penetration takes place. One of the birds was ringed (tagged) in Sweden Island Øland in the Baltic Sea two years before being shot. This is documenting immigration of mallards from northern locations. The parasite species was originally described in North America in 1893 and was commonly reported in this region during the 20th century but not in Europe. Recent cases from Norway, Finland, Lithuania, Poland, UK and Hungary suggest that the species may be spreading geographically. Experienced duck hunters with a 40 years record of hunting on the island reported that this type of infection unprecedented. The final host is reported to be canines (fox, raccoon dog), skunk and mustelids, including ermines and American mink. Presence of these hosts in Europe may allow establishment of the life cycle and further colonization of the local duck populations which calls for implementation of a survey program in Europe.

1. Introduction

Sarcocysts are apicomplexan parasites using a two-host life-cycle. They are intracellular protozoans and infect the musculature forming cysts in muscle cells of mammals, birds and reptiles (acting as intermediate hosts). Numerous bradyzoites are produced within the sarcocyst and when a predator (the final host) ingests the infected animal the bradyzoites invade the intestinal wall, conduct sexual propagation and form oocysts leaving the predator along with feces. Oocysts are then infective to the intermediate hosts. Numerous species occur in farm animals such as cattle, sheep, goat, pig, horse (Deplazes et al., 2013) and poultry (Pan et al., 2020). The sarcocysts are difficult to identify to species level macroscopically but molecular tools (sequencing of ribosomal DNA and mtDNA) have proved invaluable for diagnosing. Using these techniques a series of new species have been described and investigations of wild bird populations indicate that a relatively high host specificity exists in several cases. Recent studies described infections with *S. cornixi* in hooded crow (*Corvus cornix*) (Kutkiene et al., 2009), *S. turdusi* in blackbird *Turdus merula* (Kutkiene et al., 2012), *S. columbae, S. halieti, S. lari* and *S. wobeseri* in herring gull (*L. argentatus*) (Prakas et al., 2020b) and *S. kutkenienae* from common raven (*Corvus corax*) (Prakas et al., 2020a) while the sarcocysts in song thrushes await further identification (Cardells-Peris et al., 2020). Older North American studies showed that ducks, including mallard *Anas platyrhynchos,* host a

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Table 1
Primers and primer combinations used for PCR. T_a indicate the annealing temperature use during the PCR.

Region	Primer	Purpose	Primer sequence (5′→3′)	T_a	Product length	Reference
18 S	SarAF	PCR & Seq.	ctggttgatcctgccagtag	51 °C	960 bp	Kutkienė et al. (2010)
	SarBR	PCR & Seq.	ggcaaatgctttcgcagtag			
	SarCF	PCR & Seq.	tttaactgtcagaggtgaaattctt	51 °C	895 bp	Kutkienė et al. (2010)
	SarDR	PCR & Seq.	gcaggttcacctacggaaa			
ITS1	SU1F	PCR & Seq.	gattgagtgttccggtgaattatt	53 °C	1197 bp	(Gjerde 2014)
	5.8SR2	PCR & Seq.	aaggtgccatttgcgttcagaa			
ITS1-5.8 S-ITS2-28 S	BD1	PCR	gtcgtaacaaggtttccgta	53 °C	3024 bp	Galazo et al. (2002)
	KL-P1R	PCR & Seq.	cccaagtttgacgaacgatt			Kutkienė et al. (2010)
	PTN#390 R	Seq.	gatccgtgtttcaagacggg			This Study
28 S	KL-P1F	PCR & Seq.	tacccgctgaacttaagcat	53 °C	1568 bp	Kutkienė et al. (2010)
	KL-P2R	PCR & Seq.	tgctactaccaccaagatctgc			
	KL-P2F	Seq.	aaccgacccgtcttgaaac			Kutkienė et al. (2010)

Sarcosystis species described as *S. rileyi* (Stiles, 1893). It has been regarded as common in North American birds, both in the nineteenth and twentieth century (Dubey et al. 2003), but European reports on sarcocysts in mallards have been rare. The sarcocysts are conspicuous and easily detectable and as mallard is a popular game bird it suggests absence or extreme rarity of sarcocysts in European mallards in the 20th century. However, in 2002 a Norwegian eider duck was found infected with sarcocysts (Gjerde, 2014) and in 2003 sarcocysts were reported in 1 out of 148 investigated mallards in Poland collected in 1999 and 2000 (Kalisinska et al., 2003). The identification of sarcocysts in mallards to the species *S. rileyi* was later established for ducks in Finland and Lithuania (Prakas et al., 2014), UK (Cromie and Ellis, 2019; Muir et al. 2019) and in Hungary (Szekeres et al., 2019). The present study reports four cases of sarcocystosis caused by *S. rileyi* in mallards from the island of Bornholm in the Baltic Sea, a central location between Scandinavian and European countries and placed on the route of migrating birds between south and north. We present molecular data, distribution of sarcocysts in the muscle compartments, morphometric data of sarcocysts and bradyzoites, and histological examination of the infection site. We discuss the origin, possible introduction and probability of further spreading of the species in Europe.

2. Materials and methods

2.1. Birds

Two local and experienced hunters on the island of Bornholm in the Baltic Sea reported presence of numerous white rice grain like particles in the pectoral musculature of 4 mallards shot during December 2020 and January 2021. The total number of ducks examined was 93. The findings were characterized as unprecedented by the hunters. Two of the birds were discarded but two others (one male, one female) were recovered by the authors and brought to the laboratory for identification. One of the birds (a male) was previously ringed (Sweden, Stockholm, Riksmuseum (SVS), 90 B.15128) showing that it had been tagged on August 28, 2018 on the Swedish island Øland (at the age of 2) (Andfänget, Norrvik, Ottenby, Öland, Sverige, coordinates: 56°13′16.16″N 16°26′32.87″E). This bird was shot on the south coast of the Island of Bornholm on January 9, 2021, a location 163 km and 215 °SV from the tagging locality. The other bird (female) was shot on the east coast of Bornholm January 11, 2021.

2.2. Necropsy

Both birds were necropsied and the body musculature examined for presence of sarcocysts. Subsamples were taken and preserved in ethanol (96%) for molecular analysis. The female bird was skinned and the different compartments of the musculature were separated and the number of sarcocysts enumerated.

2.3. Morphometric and numerical measurements

A total of 50 cysts were measured using a Vernier gauge (0.1 mm accuracy) for length and width. A suspension of bradyzoites was prepared by homogenization of the individual sarcocyst in 10 ml water. The length and width of 50 bradyzoites in ten individual sarcocysts were measured microscopically (magnification ×400–1000) (accuracy 1 μm) (Leica DMLB, Leica Microsystems, Germany). From each sarcocyst suspension five subsamples (volume 1 μl) were taken and the number of bradyzoites enumerated whereafter the total number of bradyzoites per sarcocyst was calculated by multiplying with the dilution factor. A total of 50 samples were counted (5 from each of ten individual cyst suspensions).

2.4. Whole mount

Isolated sarcocysts were liberated from host material, rinsed in water and fixed in 4% neutral formalin for 24 h. They were then cleared in lactic acid and embedded on microscope slides in Aquatex (Merck, Darmstadt, Germany).

2.5. Histology

Sections of the pectoral musculature, containing sarcocysts, from the male mallard were fixed in 4% neutral formalin (Hounisen Laboratorieudstyr, Skanderborg, Denmark) for 24 h, transferred to 70% ethanol and dehydrated in graded ethanol series until two shifts of Shondon Xylene Substitute (Fisher Scientific, Denmark) whereafter the specimen was embedded in paraffin. Sections of 4 μm were prepared and stained by haematoxylin and embedded in DePeX mounting medium (Sigma-Aldrich, Denmark). Sections were studied in a Leica DMLB light microscope and micrograph pictures recovered by a Leica CD300 (Leica Microsystems, Germany).

2.6. Molecular analysis

Six individually isolated sarcocysts (three from each of the two birds) were subjected to lysis, DNA-purification, PCR with specific primers (Table 1), product purification, sequencing and BLAST analysis. Genomic DNA from each individual sarcocyst was extracted by use of a QIAGEN® DNeasy Blood & Tissue Kits according to the manufacturer's recommendation. PCR was performed in 60 μl PCR mix which contain 2 μl sample 6 μl forward primer (10 mM), 6 μl reverse primer (10 mM), 6 μl 10x PCR-buffer, 6 μl dNTP (4 × 10 mM), 1.8 μl $MgCl_2$ (50 mM), 0.25 Polymerase (BIOTAQ DNA Polymerase, Saveen & Werner ApS, Denmark) and H_2O. The PCR outline was pre-denaturation at 95 °C for 5 min; amplification by 40 cycles starting with denaturation at 95 °C for 30 s, followed by annealing at an assay specific temperature (Table 1) for 30 s, and ending with elongation at 72 °C for 2 min and finally a post-elongation step at 72 °C for 7 min. The elongation time for the PCR using BD1 vs KL-P1R was 3 min. For the lysis of the bradyzoites 20 μl of

Fig. 1. *Sarcocystis rileyi* sarcocysts in mallard musculature. A. Pectoral musculature, B. Dorsal musculature, C. Hand and arm musculature, D. Leg musculature.

Table 2
Sarcocyst distribution. The number and location of sarcocysts of *S. rileyi* in the different muscular compartments of a female mallard *A. platyrhynchos*.

Musculature compartment	Right side	Left side	Right and left combined	
	No.	No.	No.	% of all
Pectoral musculature superficial	969	893	1862	72.03
Pectoral musculature interior	174	116	290	11.22
Dorsal musculature	6	13	19	0.74
Ventral abdominal musculature	11	13	24	0.93
Leg musculature	38	31	69	2.67
Arm musculature	115	131	246	9.52
Neck musculature	16	10	26	1.01
Head musculature	22	27	49	1.90
Total	1351	1234	2585	100

the Proteinase K solution (>600 mAU/mL) was used in 200 µl lysis reactions, whereby the concentration of Proteinase K was >60 mAU/mL. The lysis had to be prolonged, according to the manufacturer's manual, to 24 h because bradyzoites were found intact (microscopic examination) after 5 h incubation. PCR products purified using illustra™ GFX™ PCR DNA and Gel Band Purification Kit (cat.no. 28-9034-71,VWR, Denmark) were sequenced at Macrogen Europe B.V., Netherlands. All six purified products were subjected to individual sequencing and subsequent analyses. Thus, six independent alignments of sequences were obtained and analyzed individually.

3. Results

3.1. Infection data

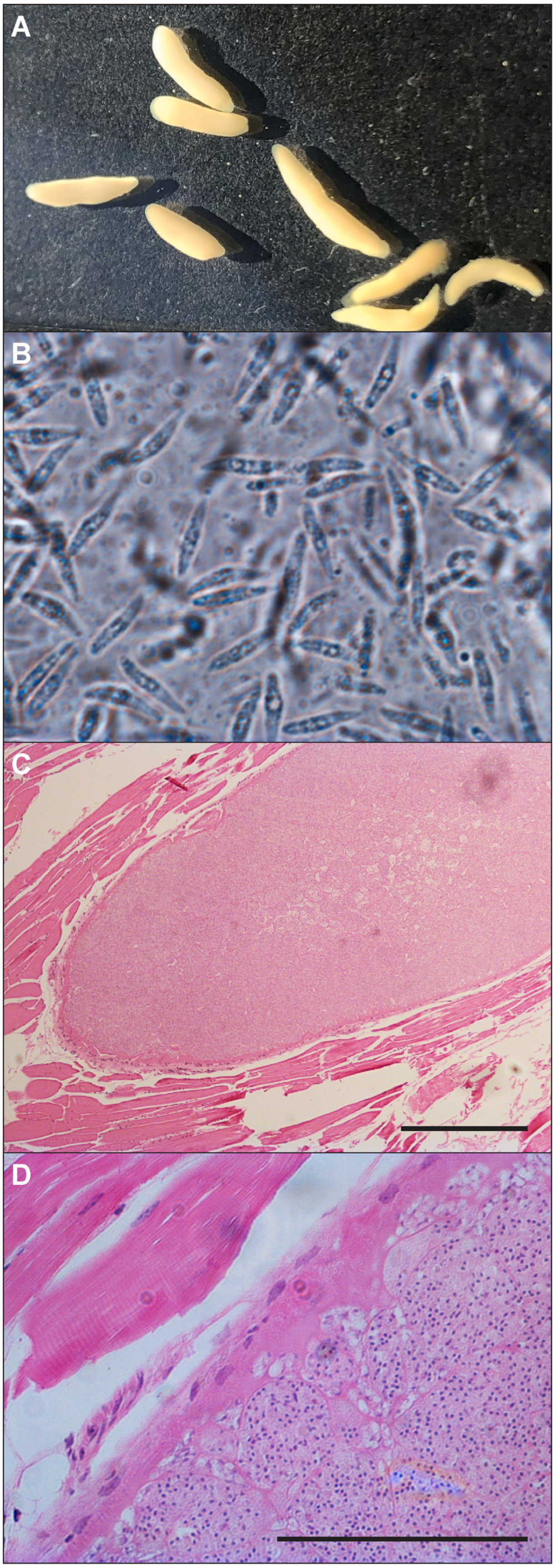
The two mallards, of which at least one of the birds had immigrated from Sweden, as documented by the ring data, were found heavily infected with cysts of *Sarcocystis rileyi* throughout their body musculature (Fig. 1). A total of 2585 sarcocysts were recovered from a single mallard. The main part of the cysts and the bradyzoites within them were located in the superficial pectoral muscles (accounting for 72% of all cysts) but also the back, ventral abdomen, neck, head, leg and wing (arm/hand) muscles were infected (Table 2).

3.2. Morphometric data

The sarcocysts were rice grain shaped (Fig. 2A) and contained numerous bradyzoites, which we released by homogenization in water (Fig. 2B). The histology showed the thin cyst wall surrounding an ordered system of bradyzoites (Fig. 2C and D). The sarcocysts were surrounded by a single cyst wall and in some cases, but not all, encapsulated by a collagenous amorphous layer in the striated muscle tissue. Some sections revealed accumulation of host cells (Fig. 2 C and D). The bradyzoites in the intact sarcocyst were densely packed and the nuclei heavily stained (Fig. 2D). In addition, the morphometric data of sarcocysts and bradyzoites (Table 3) complied with the species descriptions provided by Dubey et al. (2003). The mean size of the cysts was 5.25 (SD 0.6) mm in length and the width 1.37 (SD 0.20) mm. The elongated bradyzoites with a subterminally located nucleus had a median length of 13.5 (range 12.1–14.5) µm and a median width of 2.6 (range 2.1–3.3) µm. Each of the cysts contained a median number of 3.2 mio bradyzoites (range 1.9–5.0 mio) and with a total of 2585 cysts in one single host, the bird represents an infective potential of more than 8 billion bradyzoites.

3.3. Sequence data

PCR studies were conducted individually for six isolated sarcocysts. Performing five PCR resulted in overlapping products (GenBank accession nos. MZ468637 to MZ468641). These products may be combined into a single sequence 5349 bp long comprising the partial 18 S, the



(caption on next column)

Fig. 2. Sarcocyst and bradyzoite morphology. A. Isolated *S. rileyi* sarcocysts (mean length 5.25 mm) mounted in Aquatex, B. Wet smear of liberated bradyzoites (mean length 13.48 μm) from a sarcocyst of *S. rileyi*,
C. Histological
section of a sarcocyst in the musculature of the mallard (scale bar 100 μm), D. Histological section of a *S. rileyi* sarcocyst in pectoral musculature of male mallard showing densely packed bradyzoites with strongly stained nuclei. Haematoxylin staining. DePeX mounting, (scale bar 100 μm).

complete ITS1, the complete 5.8 S, the complete ITS2 and partial 28 S. All six analyzed sarcocysts displayed the same sequences. Fig. 3 presents an alignment of the obtained sequences of the five PCRs together with 100% similar sequences at GenBank (See Table 1 for obtained length of PCR products, annotated using the internet resource Rfam). The molecular diagnosis (Table 4) gave a precise designation with a high ITS sequence similarity to parasites recovered in the USA, Norway & UK (Table 4) supported by a high 18 S similarity to parasites recovered in the Norway, UK and Lithuania and by a high 28 S similarity to parasites recovered in Norway, Lithuania and USA. Only 60 ITS2 sequences of the family Sarcocystidae (designated *Sarcocystis* sp. with accession numbers MH590230 & MH590233) (Lee, 2019) representing seven species were available at GenBank. Despite the high quality of sequence data from the present work (PCR product using BD1 vs KL-P1R), the ITS2 of *S. rileyi* had no obvious similarity to any ITS sequence at GenBank. Using the least stringent BLAST option ("Somewhat similar sequences"), the only hit at Genbank was a 73 bp long fragment with no annotation from the *Rhinatrema bivittatum* (two-lined caecilian) chromosome 12 (LR584398) with 78% similarity towards the obtained ITS2. The 5′end and the 3′end of this sequence of the PCR product overlapped the GenBank *S. rileyi* sequences KJ396584 and HM185743 with 1070 bp and 1004 bp, respectively. The ITS2 of *S. rileyi* is 285 bp and 321 bp longer than the 2 reported *Sarcocystis* sp. sequences. The resulting 5349 bp long sequence of *S. rileyi* has been uploaded to GenBank (accession number MZ151434) which represent the first report of the complete ITS2 of *S. rileyi*.

4. Discussion

The two mallards obtained from local recreational hunters on the island of Bornholm in the central Baltic Sea were found heavily infected with cysts of *Sarcocystis rileyi* throughout their body musculature. The main part of the cysts were located in the superficial pectoral muscles but also the dorsal, leg and arm muscles were highly infected and thereby infective to a predator or scavenger. The molecular diagnosis gave a precise designation with a high ITS1 sequence similarity to the species records in the USA, Norway, UK, Finland and Lithuania (Gjerde, 2014; Muir et al. 2019; Prakas et al.,2020). By combining various primer sets and analyzing overlaps we were able to present novel sequences for rDNA segments 5.8 S and ITS2 for *S. rileyi*. In addition, the morphometric data complied with the species descriptions provided by Dubey et al. (2003). At least one of the infected birds had immigrated from Sweden, as documented by the ring data, and it shows that the distribution area of a certain avian parasite species can be expanded easily with bird migration. In this context it should be considered that mallard migration and distribution entails a potential for fast and wide dispersal of parasites easily covering the whole Baltic sea (van Toor et al., 2018). During the 20th century reports on occurrence of sarcocysts in European mallards were limited whereas North America appeared as the main area of distribution of sarcocyst infected birds. Sarcocysts were observed in North American birds by Riley in 1869 and *S. rileyi* was then described by Stiles in 1893, whereafter numerous studies through the 20th century documented the species in American ducks (Dubey et al., 2003). The occurrence of a sarcocyst infected mallard in Poland 1999) to 2000 (finding one infected bird out of 148 examined) (Kalisinska et al., 2003) and one infected eider duck in Northern Norway in 2002 (Gjerde, 2014) were considered unusual findings. The parasite was later recorded from Finland and Lithuania (18 and 12 birds, respectively, found infected in

Table 3
Enumeration of bradyzoites per sarcocyst and bradyzoite size (length and width) measurements. A total of 50 bradyzoites were size-measured. The number of bradyzoites was counted in ten sarcocysts.

	Median	Min	Max	75% quantile	95% quantile
Length, μm	13.50	12.10	14.50	12.86	12.90
Width, μm	2.60	2.10	3.30	2.40	2.41
Number/cyst	3.2×10^6	1.9×10^6	5.0×10^6	1.5×10^6	2.0×10^6

the period 2011–2013) (Prakas et al., 2014), UK (90 reports 2015–2017) (Cromie and Ellis, 2019; Muir et al., 2019), Hungary (12 infections reported, identification of *S. rileyi* in two birds) (Szekeres et al., 2019) and from Denmark (Baltic region) (4 infected out of more than 93 ducks examined) (present study). This is the first record of *S. rileyi* in Denmark and the finding, characterized by local hunters as highly unusual and unprecedented, suggests that the species has a potential for spread in Europe. It may be discussed if the occurrence has been underreported in Europe (Szekeres et al., 2019; Muir et al., 2019) but a local hunter from the island of Bornholm, with 40 years experience and always having a habit of removing skin of the duck, claimed that he had never seen such an infection before. Alternatively, the parasite could have been present at very low prevalence in Europe and due to changes of one or more environmental factors (abiotic or biotic) the transmission, and thereby the prevalence in mallards, would rise.

An introduced parasite species with a complicated two-host life cycle may become established in a certain geographic region depending on the presence of suitable hosts. The birds act as intermediate hosts and final host species recorded for *S. rileyi* are skunk (Wicht 1981; Dubey et al., 2003), fox and raccoon dog (Prakas et al., 2015; Moré et al., 2016; Szekeres et al., 2019). Further, it was suggested by Gjerde (2014) that apart from canids, such as polar fox, also American mink (*Mustela vison*) and stout (*Mustela erminea*) could serve as final hosts. European countries are populated by foxes, raccoon dogs, stout and naturalized American mink, whereby the potential for further spread in European mallard populations is present. Even on the island of Bornholm (where fox, raccoon dog and stout are absent) the possibility for establishment is present due to a significant population of American mink. Our histological sections showed that the bradyzoites in the sarcocysts are densely packed but when the cysts are homogenized in water the elongate cells are stretched. The resistance to Proteinase K treatment indicates that the bradyzoites are able to resist the enzymes in the predator's stomach and intestine following ingestion.

No controlled pathogenicity studies in birds infected by *S. rileyi* are available but histopathological studies have shown various (from light to severe) forms of necrosis and inflammation in the muscles of infected birds (Muir et al., 2019). The heavy infections in the entire musculature in the present study suggests that flying ability may be depressed in these birds. Severe disease signs, including muscular malfunctions, may appear in chickens infected with other species of *Sarcocystis* (Munday et al. 1977; Mutalib et al. 1995), a situation known from infections in other animals acting as intermediate hosts (Deplazes et al., 2013). It cannot be excluded that *S. rileyi* could spread further and impact European duck populations. It is therefore recommended that European bird populations become surveyed and sarcocyst infections recorded.

5. Conclusion

Infections with *S. rileyi* in mallard *A. platyrhynchos* have been prevalent in North America but probably absent or very rare in Europe during the 20th century. Since turn of the millennium an increasing

Table 4
Sequences for *Sarcocystis rileyi* obtained from two mallards (6 identical sequences from 6 sarcocysts) shot on the island of Bornholm (Baltic Sea) aligned with GenBank sequences for *S. rileyi* in North America, UK, Norway and Lithuania. PCR primer binding sites were excluded from the sequences before comparison. The ITS2 of *S. rileyi* is exclude from this table because it exhibited no obvious similarity to any ITS sequence at GenBank.

Region	Aligned Length	GenBank acc. No.	Host	Country	Percentage identity
18 S	1784	KJ396583	*Somateria mollissima*	Norway	100
		LT992317	*Anas platyrhynchos*	United Kingdom: Perthshire	100
		HM185742	*Anas platyrhynchos*	Lithuania	100
ITS1	937	LT992314	*Anas platyrhynchos*	United Kingdom: Perthshire	100
		KJ396584	*Somateria mollissima*	Norway	100
		GU188427	*Anas platyrhynchos*	USA: Colorado	99
28 S	1525	GU188426	*Anas platyrhynchos*	USA: Colorado	100
		HM185743	*Anas platyrhynchos*	Lithuania	100
		KJ396585	*Somateria mollissima*	Norway	99

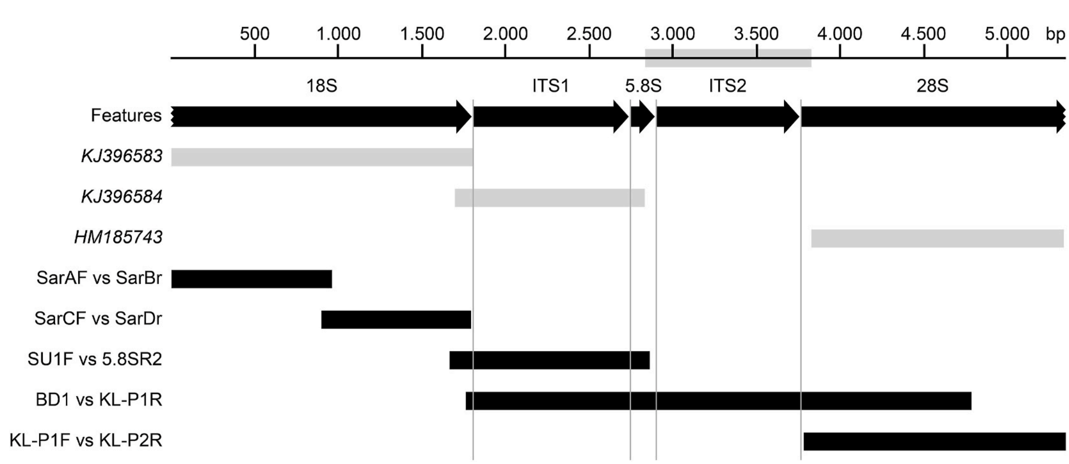


Fig. 3. Alignment of PCR products. The shading on the scale at the top indicates a part of sequences, which resulted in no proper similarity towards any sequence at GenBank. Arrows indicate the features of the combined sequence (MZ151434). The grey bars indicate GenBank sequences with 100% similarity to the obtained sequences. The black bars indicate obtained PCR products, which notations at the left are "Forward primer vs Reverse primer".

number of cases have been reported from Europe and it cannot be excluded that the parasite species is spreading in European countries. The severe infection affecting the entire striated body musculature and with a major sarcocyst occurrence in the flying apparatus (pectoral muscle, wings) suggests a pathogenic effect on the host. Pathogenicity studies should be implemented and surveys for monitoring the infection status in European bird populations should be prioritized.

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Declaration of competing interest

The authors declare that they have no conflicts of interests.

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References

Cardells-Peris, J., Gonzalvez, M., Ortega-Porcel, J., Ybanez, M. R. R. d, Martinez-Herrero, M.C., Garijo-Toledo, M.M., 2020. Parasitofauna survey of song thrushes (*Turdus philomelos*) from the eastern part of Spain. Parasitol. Int. 79, 102176.

Cromie, R., Ellis, M., 2019. Sarcocystis Survey. Sarcocystis Survey Feedback Report – the UK Wildfowl Sarcocystis Survey. www.sarcocystissurvey.org.uk/2015-2018-feedback-report/. accessed April 29, 2021.

Deplazes, P., Eckert, J., Samson-Himmelstjerna, G.v., Zahner, H., 2013. Lehrbuch der Parasitologie für die Tiermedizin. Enke Verlag Stuttgart.

Dubey, J.P., Cawthorn, R.J., Speer, C.A., Wobeser, G.A., 2003. Redescription of the sarcocyst of *Sarcocystis rileyi* (Apicomplexa: Sarcocystidae). J. Eukaryot. Microbiol. 50 (6), 476–482.

Galazzo, D.E., Dayanandan, S., Marcogliese, D.J., McLaughlin, J.D., 2002. Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA sequence data and comparisons with European congeners. Can. J. Zool. 80, 2207–2217. https://doi.org/10.1139/Z02-198.

Kalisinska, E., Betlejewska, K.M., Schmidt, M., Gozdzicka-Jozefiak, A., Tomczyk, G., 2003. Protozoal macrocysts in the skeletal muscle of a mallard duck in Poland: the first recorded case. Acta Parasitol. 48 (1), 1–5.

Kutkienė, L., Prakas, P., Sruoga, A., Butkauskas, D., 2009. Sarcocystis in the birds family Corvidae with description of *Sarcocystis cornixi* sp. nov. from the hooded crow (*Corvus cornix*). Parasitol. Res. 104, 329–336. https://doi.org/10.1007/s00436-008-1196-9.

Kutkienė, L., Prakas, P., Sruoga, A., Butkauskas, D., 2010. The mallard duck (*Anas platyrhynchos*) as intermediate host for *Sarcocystis wobeseri* sp. nov. from the barnacle goose (*Branta leucopsis*). Parasitol. Res. 107, 879–888. https://doi.org/10.1007/s00436-010-1945-4.

Kutkienė, L., Prakas, P., Butkauskas, D., Sruoga, A., 2012. Description of *Sarcocystis turdusi* sp. nov. from the common blackbird (*Turdus merula*). Parasitology 139, 1438–1443. https://doi.org/10.1017/S0031182012000819.

Lee, F.C.H., 2019. Finding *Sarcocystis* spp. on the Tioman Island: 28S rRNA gene next-generation sequencing reveals nine new *Sarcocystis* species. J. Water Health 17 (3), 1–12. https://doi.org/10.2166/wh.2019.124.

Moré, G., Maksimov, A., Conraths, F.J., Schares, G., 2016. Molecular identification of Sarcocystis spp. in foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Germany. Vet. Parasitol. 220, 9–14.

Muir, A., Ellis, M., Blake, D.P., Chantrey, J., Strong, E.A., Reeves, J.P., Cromie, R.L., 2019. *Sarcocystis rileyi* in UK free-living wildfowl (Anatidae): surveillance, histopathology and first molecular characterization. Vet. Rec. https://doi.org/10.1136/vetrec-2019-105638.

Munday, B.L., Humphrey, J.D., Kila, V., 1977. Pathology produced by, prevalence of, and probable life-cycle of a species of *Sarcocystis* in the domestic fowl. Avian Dis. 21, 697–703.

Mutalib, A., Keirs, R., Maslin, W., Topper, M., Dubey, J.P., 1995. Sarcocystis-associated encephalitis in chickens. Avian Dis. 39, 436–440.

Pan, J., Ma, C., Huang, Z., Ye, Y., Zeng, H., Deng, S., Hu, J., Tao, J., 2020. Morphological and Molecular Characterization of *Sarcocystis Wenzeli* in Chickens (*Gallus gallus*) in China. Parasites Vectors13, p. 512. https://doi.org/10.1186/s13071-020-04390-x.

Prakas, P., Oksanen, A., Butkauskas, D., Sruoga, A., Kutkiene, L., Svazas, S., Isomursu, M., Liaugaudaite, S., 2014. Identification and intraspecific genetic diversity of *Sarcocystis rileyi* from ducks, *Anas* spp., in Lithuania and Finland. J. Parasitol. 100 (5), 657–661.

Prakas, P., Liaugaudaitė, S., Kutkiene, L., Sruoga, A., Svazas, S., 2015. Molecular identification of *Sarcocystis rileyi* sporocysts in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) in Lithuania. Parasitol. Res. 114, 1671–1676 doi. org10.1007/s00436-015-4348-8.

Prakas, P., Butkauskas, D., Juozaitytė-Ngugu, E., 2020a. Molecular and morphological description of *Sarcocystis kutkienae* sp. nov. from the common raven (*Corvus corax*). Parasitol. Res. 119, 4205–4210. https://doi.org/10.1007/s00436-020-06941-8.

Prakas, P., Butkauskas, D., Juozaitytė-Ngugu, E., 2020b. Molecular identification of four *Sarcocystis* species in the herring gull, *Larus argentatus*, from Lithuania. Parasites Vectors 13, 2. https://doi.org/10.1186/s13071-019-3869-x.

Szekeres, S., Juhhaszi, A., Kondor, M., Takacs, N., Sugar, L., Hornok, S., 2019. *Sarcocystis rileyi* in Hungary: is rice breast disease underreported in the region? Acta Vet. Hung. 67 (3), 401–406 doi: 10.1556/004.2019.040 0236-6290/$ 20.00 ©.

van Toor, M.L., Avril, A., Wu, G., Holan, S.H., Waldenström, J., 2018. As the duck flies—estimating the dispersal of low-pathogenic avian influenza viruses by migrating mallards. Front. Ecol. Evol. 6, 208.

Wicht, R.I., 1981. Transmission of *Sarcocystis rileyi* to the striped skunk (*Mephitis mephitis*). J. Wildl. Dis. 17, 387–388.