

Molecular identification of *Sarcocystis funereus* sp. nov. (Apicomplexa, Sarcocystidae) in offspring of Tengmalm's owls *Aegolius funereus* (Aves, Strigidae)

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Research Article

Keywords: transmission of coccidia, vertebrates, voles, ICZN, LSIDs, nomenclatural acts

Posted Date: August 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-755552/v1>

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2 ***Sarcocystidae*) in offspring of Tengmalm's owls *Aegolius funereus* (Aves,**
3 ***Strigidae*)**

4

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15

16 **Abstract**

17 The spectrum of species of *Sarcocystis* in birds and the role of the latter in the transmission of
18 coccidia are still incomplete for many avian species including Tengmalm's owl *Aegolius funereus*
19 (Linnaeus, 1758). Therefore, this study is focused on the morphological and molecular description
20 of a new *Sarcocystis* species found in the intestine of the Tengmalm's owl and its possible role as
21 definitive host. Ten fledgling owls from the Kauhava region of west-central Finland were
22 parasitized by numerous sporocysts and oocysts in the intestinal mucosa scrapings (prevalence
23 100%). Sporulated oocysts and sporocysts measured 16.34–16.96 × 11.47–12.09 μm and
24 11.85–13.52 × 7.77–9.25 μm, respectively. Skeletal and heart muscles were negative for sarcocysts.
25 *Sarcocystis funereus* sp. nov. is closely related to *Sarcocystis strixi* from the barred owl (*Strix varia*
26 Barton, 1799) from the USA and *Sarcocystis* sp. isolate 5 from the European shrew (*Sorex araneus*

27 Linnaeus, 1758) from the Czech Republic. This is the first and most comprehensive record on
28 *Sarcocystis* from owls obtained in Finland, thus highlighting the importance of molecular data in
29 the species identification.

30

31 **Introduction**

32 Cyst-forming coccidia parasites of the genus *Sarcocystis* Lankester, 1882 can infect a wide variety
33 of vertebrates, including birds, which could act as definitive and intermediate hosts in the life cycle
34 of these parasites. However, the spectrum of species of *Sarcocystis* in birds and the role of the latter
35 in the transmission of coccidia are still incomplete for many avian species including Tengmalm's
36 owl *Aegolius funereus* (Linnaeus, 1758). This species is a small nocturnal cavity-nesting owl living
37 in coniferous forests in the boreal zone and in alpine forests further south in the Holarctic region^{1,2}.

38 It feeds mainly on small mammals, among which voles of the genera *Myodes* Pallas, 1779 (= *Clethrionomys*
39 *Tilesius*, 1850) and *Microtus* Schrank, 1798 are their main preys, while shrews of
40 the genus *Sorex* and small forest birds are their most important alternative prey items^{3,4,5}.

41 To date, relatively few studies have been conducted with the *Sarcocystis* species in *A. funereus* in
42 wild; in fact, only Wiesner⁶, in a scientific meeting, reported sporocysts of *Sarcocystis* sp. in the
43 Tengmalm's owl, which were experimentally developed in the bank vole *Myodes glareolus* (= *Clethrionomys*
44 *glareolus*) Schreber, 1780. Whereas Zuo et al.⁷ and Zuo and Yang⁸ were
45 unsuccessful in experimentally infecting *A. funereus* with *Sarcocystis sinensis* Zuo, Zhang et Yie,
46 1990 from China.

47

48 During radio-telemetry research of Tengmalm's owls in Finland, where decreasing densities of
49 main prey (voles) occurred, some fledglings were found dead and subsequently parasitologically
50 examined. Since this owl species has practically no records of species of *Sarcocystis* and the role of
51 owl in the life cycle of parasite is partially known, this study is focused on the morphological and

52 molecular description of a new *Sarcocystis* species found in the intestine of the Tengmalm's owl
53 and its possible role as definitive host.

54

55 **Results**

56 All intestinal samples of the 11 owls examined under light microscopy were positive to oocysts and
57 sporocysts of *Sarcocystis*, whereas samples of muscular tissues were negative.

58

59 Nomenclatural acts

60 This published work and the nomenclatural acts it contains have been registered in ZooBank, the
61 online registration system for the ICZN. The ZooBank Life Science Identifiers (LSIDs) can be
62 resolved and the associated information viewed through any standard web browser by appending
63 the LSID to the prefix "http://zoobank.org/". The LSID for this publication is:.....

64

65 Oocysts/sporocysts were described as follows:

66 Family Sarcocystidae Poche, 1913

67 *Sarcocystis funereus* sp. nov. (Fig. 1)

68 Description: thin-walled sporulated oocysts (Fig. 1a), 16.34–16.96 × 11.47–12.09 (n= 5) and
69 sporocysts (Fig. 1b) were 11.85–13.52 × 7.77–9.25 (n= 35).

70

71 Taxonomy summary

72 *Definitive host*: Tengmalm's owl *Aegolius funereus* Linnaeus, 1758 (Strigiformes: Strigidae).

73

74 *Intermediate host*: Unknown.

75

76 *Distribution*: Kauhava region, west-central Finland (approx. 63° N, 23° E).

77

78 *Site of infection:* Small intestine.

79

80 *Deposited material:* Symbiotype (oocysts/sporocysts in 2.5% potassium dichromate), and genomic
81 DNA in Eppendorf tube were stored at SVI Prague. GenBank sequences MW349706 (*18S* rRNA
82 gene), MW349707 (*28S* rRNA gene), MW373964 (ITS1 region), MW489293 (*cox1* gene).

83

84 *Etymology:* The specific epithet is derived from the species name of its definitive host, i.e.,
85 *funereus*.

86

87 Genetic sequences of 20 *Sarcocystis* isolates (11 birds) were obtained for the *18S* rRNA, *28S* rRNA,
88 ITS1 and *cox1* loci, of which those of six owls were clearly identified as *S. funereus* sp. nov., while
89 those from four and one owls were excluded due to their low quality/short fragments of sequences
90 or failed in amplifying, respectively. All obtained *18S* rRNA sequences were identical; therefore,
91 only one of 1773 bp was submitted to GenBank (MW349706). It shared a 99.69% similarity with
92 an *Sarcocystis* sp. isolate 5 (as named in GenBank) (1594 bp) (AF513487) from the European
93 shrew (*Sorex araneus* Linnaeus, 1758) from the Czech Republic, 99.61% with *S. strixi* Verma, von
94 Dohlen, Mowery, Scott, Cerqueira-Cézar, Rosenthal, Dubey et Lindsay, 2017 (MF162315) in the
95 barred owl (*Strix varia* Barton, 1799) from USA, and 99.55% with *S. corvusi* Prakas, Kutkiené,
96 Butkauskas, Sruoga et Žalakevičius, 2013 (JN256117) in the jackdaw (*Corvus monedula* [Linnaeus,
97 1758]) from Lithuania, *S. halioti* Gjerde, Vikøren et Hammes, 2018 (MH130211, MF946587) in the
98 great cormorant (*Phalacrocorax carbo* [Linnaeus, 1758]) from Lithuania, and the white-tailed sea
99 eagle (*Haliaeetus albicilla* [Linnaeus, 1758]) from Norway. The representative *28S* rRNA sequence
100 (MW349707) was 1509 bp and shared 97.59% genetic similarity with *S. strixi* (MF162316) and *S.*
101 *lari* Prakas, Kutkiené, Butkauskas, Sruoga et Žalakevičius, 2014 (MF946611) in the white-tailed sea
102 eagle from Norway, whereas it shared 97.49% similarity with *S. lutrae* Gjerde et Josefsen, 2015
103 (KM657771) in the Eurasian otter (*Lutra lutra* [Linnaeus, 1758]) from Norway. Analyses of the

104 *coxI* gene sequences (MW489293, 1060 bp) showed a high similarity (99.52%) with *S. strixi*
105 (MF162317), 99.43% with *S. lutrae* (KM657808) and 99.42% with *S. lari* (MF596283, MF946584)
106 from the great black-backed gull (*Larus marinus* [Linnaeus, 1758]) from Lithuania and the white-
107 tailed sea eagle from Norway. Representative nucleotide ITS1 region sequence was 1294 bp
108 (MW373964), with no significant match to other *Sarcocystis* species from GenBank, except 89.57%
109 similar (44% query cover) to *S. halioti* (MF946596) and 89.52% similar (35% query cover) to *S.*
110 *lutrae* (MG372109) in *L. lutra* from the Czech Republic. Single cases of double peaks were noted at
111 28S rRNA at nucleotide positions 666, 667 (TT/CC), while some ITS1 sequences differed only by
112 one single nucleotide polymorphism (T/C) at nucleotide position 466.

113

114 The phylogenetic trees showed different topologies and relationships between the new species with
115 its congeners according to the availability of sequences. Phylogenetic trees based on 18S rRNA, 28S
116 rRNA and *coxI* genes showed a clade formed by the new species and *S. strixi*, as well as
117 *Sarcocystis* sp. 5 (in the case of 18S rRNA) (Fig. 2a, b, d), while tree of ITS1 region showed *S.*
118 *funereus* sp. nov. in a single clade since the ITS1 sequence of *S. strixi* was not available, although
119 the new species formed a group with other *Sarcocystis* spp. (Fig. 2c).

120

121 **Discussion**

122 The first published finding and description of the oocysts/sporocysts of *Sarcocystis* sp. in the
123 Tengmalm's owl was made by Wiesner⁶ in Europe. Other *Sarcocystis* using owls as definitive hosts
124 are: *S. espinosai* (Espinosa, Sterner, Blixt et Cawthorn, 1988) Odening, 1997 in the northern saw-
125 whet owl *Aegolius acadicus* (Gmelin, 1788) from the USA⁹; *S. dispersa* Černá et Sénaud, 1977 in
126 the long-eared owl *Asio otus* (Linnaeus, 1758), barn owl *Tyto alba* (Scopoli, 1769), masked owl *T.*
127 *novaehollandiae* (Stephens, 1826) and *Ninox novaeseelandiae* (Gmelin, 1788) in the Czech Republic
128 and Australia^{10,11,12,13,14}; *S. rauschorum* Cawthorn, Gajadhar et Brooks, 1984 in the snowy owl
129 *Bubo scandiacus* (reported as *Nyctea scandiaca*) (Linnaeus, 1758) from Canada¹⁴; *S. scotti* Levine

130 et Tadros, 1980 (this species is considered invalid¹⁵) and *S. sebeki* (Tadros et Laarman, 1976)
131 Levine, 1978 both in the tawny owl *Strix aluco* Linnaeus, 1758 from Europe^{16,17}; and *S. strixi* in the
132 barred owl *Strix varia* from USA¹⁸. Hoberg et al.¹⁹ reported a coccidian (resembling *Frenkelia* or
133 *Sarcocystis*) in the northern spotted owl *Strix occidentalis caurina* Xantus de Vesey, 1860 from the
134 USA, although the proper identity of the parasite was undetermined. There also are reports of owls
135 acting as intermediate hosts for *S. falcatula* Stiles, 1983 in the great-horned owl *Bubo virginianus*
136 (Gmelin, 1788) from the USA²⁰ and *S. otus* Krone, Rudolphi et Jakob, 2000 (invalid species
137 according to ¹⁵) in *As. otus* from Germany²¹. Most of these were solely morphologically studied,
138 while only sporocysts of *S. dispersa* in *Tyto alba* (18S rRNA)²² and *S. strixi* (18S rRNA, 28S rRNA
139 and *cox1*)¹⁸ were morphological and molecularly characterized.

140

141 The size of fully sporulated sporocysts of *Sarcocystis espinosai*, *S. rauschorum*, *S. sebeki* and *S.*
142 *strixi* are within the range for those of the new species (9.5–14.0 × 8.0–12.0 μm, 9.6–14.0 ×
143 7.0–10.0 μm and 11.2–13.7 × 8.8–10.9 μm vs 11.85–13.52 × 7.77–9.25 μm), and partially with the
144 sporocysts and oocysts of *Sarcocystis* sp. in *S. o. caurina*, *S. dispersa* and *S. sebeki* (11.0–12.0 ×
145 5.0–6.0 μm, 11–14 × 8–11 μm and 10.0 × 14.0 μm vs 11.85–13.52 × 7.77–9.25 μm; 12.4–15.5 ×
146 9.3–12.4 and 17–20 × 10–13 μm vs 16.34–16.96 × 11.47–12.09). Since the morphological
147 parameters of oocysts/sporocysts are insufficient to distinguish species, the comparison of these
148 *Sarcocystis* with the new species is unreliable. The sporocysts and oocysts of *Sarcocystis* sp. of
149 Wiesner⁶ were not described, so it is impossible to say if it belongs to *S. funereus* sp. nov., although
150 they could be conspecific.

151

152 On the other hand, of those species molecularly characterized, as *S. dispersa* (18S rRNA) and *S.*
153 *strixi* (18S rRNA, 28S rRNA and *cox1*), the first formed a different clade than that of *S. funereus* sp.
154 nov., while the second grouped together with the new species in the three genes. Apparently, *S.*
155 *strixi* and *Sarcocystis* sp. 5 are closely related (sister) to *S. funereus* sp. nov., but with genetic

156 differences to be still considered as separated species. The ITS1 region is more sensitive to the
157 genetic differences among *Sarcocystis* species (see^{23,24,25}), while the *18S* rRNA gene is now
158 considered of limited taxonomic help¹⁵. Unfortunately, ITS1 region was not used previously in *S.*
159 *dispersa* nor *S. strixi*, thus making their comparison with the new species limited. This is the first
160 time that the ITS1 region was sequenced for a *Sarcocystis* from owls as definitive hosts and clearly
161 revealed the differences among species.

162

163 The intermediate host of *S. funereus* sp. nov. is unknown, but apparently rodents (different species
164 of mice and voles inhabiting the study area) (see^{4,26}) play that important role. Experimentally,
165 Wiesner⁶ observed that the bank vole *M. glareolus* acts as intermediate host and it could also be the
166 potential host for the new species, while the northern saw-whet owl *A. acadicus*, a congeneric owl
167 species from the USA, used the deer mice (*Peromyscus maniculatus*) (see⁹). According to Mikkola¹,
168 König and Weick², there are more than 47 mammalian and 66 bird species used as preys by owls in
169 Europe, which could act as intermediate hosts for *S. funereus* sp. nov. The most common small
170 mammals used as prey by the Tengmalm's owl are the bank vole, field vole (*Microtus agrestis*
171 [Linnaeus, 1761]), sibling vole (*Microtus levis* [syn. *M. rossiaemeridionalis*] Miller, 1908) and
172 harvest mouse (*Micromys minutus* [Pallas, 1771]); less commonly are shrews (genus *Sorex*)^{4,26}. In
173 the study area, the main preys of fledglings and adult Tengmalm's owls are bank voles, field voles
174 and sibling voles, whose abundances regularly fluctuate in high-amplitude (100–200-fold) three-
175 year cycle^{27,28,29,30}. Accordingly, the abundances of individual vole species vary strongly. The
176 overall prey abundance could be 0.2–13.1 and 0.6–28.2 vole individuals per 100 trap-nights as
177 revealed by regular long-term snap-trapping in the study area during spring and autumn,
178 respectively, thus differing up to 65-fold times between different years/phases of the vole
179 cycle^{4,29,31}.

180

181 It has been mentioned that species of *Sarcocystis* are more specific to their intermediate than
182 definitive hosts, especially those using rodents as intermediate hosts (see¹⁵). In the case of *M.*
183 *glareolus*, it has been found as intermediate host of several types and unnamed species of
184 *Sarcocystis* from the Czech Republic³², Baltic region³³ and Lithuania³⁴, as well as of *S.*
185 *clethrionomyelaphis* Matuschka, 1986 in Germany, which uses canids, mustelids, snakes or birds of
186 prey as definitive hosts (see^{35,36}). One of the forms from Lithuania showed similar features (dense
187 hair-like projections on cyst wall) than that of *Sarcocystis* sp. described by Wiesner⁶ (see³⁴), thus
188 corroborating the role of bank vole in the life cycle of the parasite. In the present case, even though
189 the intermediate host is unknown, the molecular analysis indeed determined that the developmental
190 stages in the definitive host belong to a new species, as herein stated.

191

192 The cause of death of Tengmalm's owls was undetermined, but the occurrence of *Sarcocystis* in
193 these birds should be monitored, since other taxa of this genus (*S. falcatula*, *Sarcocystis* sp. isolate
194 from chicken) have been reported as causing encephalitis in free-ranging great owls (*B. virginianus*)
195 and meningoencephalitis in chickens, respectively (see^{20,37}).

196

197 In the last decades, the integrative taxonomy by using morphological features and molecular
198 analysis has uncovered the huge diversity of species in various groups of organisms, including
199 protists¹⁵. Additionally, it particularly improved the recognition of the specificity of *Sarcocystis* in
200 their intermediate and definitive hosts around the world. Apparently, *S. funereus* sp. nov. is specific
201 to *A. funereus*, which acted as natural definitive host of this parasite, thus representing the first host
202 record in *A. funereus* and the ninth owl species with a *Sarcocystis* species. Interestingly, the
203 Tengmalm's owl was experimentally infected with *S. sinensis*, but sporocysts and oocysts were no
204 found after some days of infection (see⁸); this could indicate the host specificity of the new species.
205 However, birds of prey might be infected by more than one *Sarcocystis* species, as *S. halieti* and *S.*

206 *lari* in the white-tailed sea eagle (*H. albicilla*) (see³⁸); therefore, more Tengmalm's owls, other owl
207 species and birds of prey should be examined to determine the presence of other species or forms.

208

209 Tengmalm's owls are nomadic and the natal dispersal movements of those juveniles hatched in
210 Finland could extend more than 1000 km^{39,40}, while adult females show long distance breeding
211 dispersal up to > 600 km in Finland^{4,39} and adult males are usually resident after their first breeding
212 attempt⁴. They can also move over long distances and are widely distributed in North and Central
213 Europe including the Italian Alps and Pyrenees in North Spain¹. Therefore, it is highly probable that
214 Tengmalm's owls could spread *S. funereus* sp. nov. out of Finland to various other locations within
215 their distribution range. For instance, during a long-term study of Tengmalm's owl in the Czech
216 Republic (years 2010–12 and 2015) a prevalence of 40% was found for a *Sarcocystis* sp. in 10
217 fledglings^{41,42}. Thus, these parasites seem to be present in that country, although the species
218 identification should be confirmed to determine the real distribution of *S. funereus* sp. nov.
219 However, Svobodová⁴³ examined two Tengmalm's owls in the Czech Republic, which were
220 negative to the presence of oocysts/sporocysts of *Sarcocystis*.

221

222 If we considered that family Strigidae comprises 223 species of owls reported around the world,
223 more studies are needed to elucidate the parasite fauna and involvement of these birds in the life
224 cycles of parasites. Thus, new findings will help in increasing the knowledge about this interesting
225 group of predators, as well as their role as predators of rodents, which also act as intermediate hosts
226 of several *Sarcocystis*.

227

228 This work provided the first and the most comprehensive record on *Sarcocystis* from owls in
229 Finland, thus highlighting the importance of molecular data in the species identification. It also
230 contributes to the better understanding of species diversity and current taxonomic status of the new
231 species within the genus *Sarcocystis*. Further works including examinations of owl populations and

232 particularly their preys in Finland, the Czech Republic and worldwide are required to elucidate the
233 life cycle of the parasite.

234

235 **Materials and methods**

236 The carcasses of 11 specimens (29–47 days old from hatching, 98–136 g in weight) from 7
237 different nests (10 died due to starvation and infection, 1 per marten *Martes martes* Linnaeus, 1758
238 predation) (MK and EK unpublished data) were examined in this study. They were collected in the
239 Kauhava region of west-central Finland (63°N, 23°E) during a radio-tracking study of Tengmalm's
240 owl fledglings during the post-fledging dependence period in 2019. The study area is located
241 30–120 m above sea level and mostly covered by forest (for detailed description of the study area
242 see^{4,31,44}). Aerial distances (n= 21) between involved nest boxes from which the fledglings
243 originated and later subjected to necropsy were 19.2 ± 9.3 km on average (range 3.8–38.7 km).
244 Necropsies were carried out in the State Veterinary Institute (SVI) Prague, Czech Republic, where
245 the intestinal content and muscular samples (breast, legs, heart) of thawed birds were
246 parasitologically examined in wet mouth using water or glycerine for orientation purposes. After
247 parasite finding, intestinal mucosa scrapings were used for flotation-centrifugation coprological
248 method under light microscopy for the final evaluation and presence of oocysts/sporocysts using a
249 Leica DMLB optical microscope with a Leica DFC420 digital camera (Leica Microsystems,
250 Wetzlar, Germany), and isolation to Eppendorf tubes for DNA extraction. All measurements are
251 given in micrometres, unless otherwise mentioned.

252

253 Genomic DNA was extracted by glass bead disruption from 22 isolates (two from each owl) of
254 oocysts/sporocysts using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)
255 according to the manufacturer's recommendations. DNA was stored at -20 °C until further use.
256 Polymerase chain reaction (PCR) and nested-PCR were carried out by using primers for *18S* rRNA
257 (ERIB1/A2R, A1F/S2r, A2F/Primer BSarc and Fext/Rext, Fint/Rint, respectively)^{38,45,46,47,48},

258 28S rRNA (KL_P1R/KL_P1F)⁴⁹, ITS1 region (ITS-F/ITS-R)⁴⁹ and *cox1* genes (SF1/SR10)^{50,51}; all
259 these primers worked properly. The PCR mixture (20 µl of reaction mixture and 5 µl of DNA
260 extract) comprising of GoTaq® G2 Green Master Mix (Promega, Madison, Wisconsin, USA), 0.4
261 µM of each primer, DNA template and nuclease-free water to obtain a final volume of 25 µl. PCR
262 conditions consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for
263 30 s, 52–60 °C for 30 s, 72 °C for 1 min, and then a final extension step at 72 °C for 10 min. The
264 amplification products were resolved on 1.5% agarose gels and visualized by ethidium bromide
265 staining. The PCR products were purified using the ExoSAP-IT™ Express PCR Product Cleanup
266 Reagent kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Purified PCR
267 products were directly sequenced in both forward and reverse directions using the same primers as
268 for PCR through the commercial company Eurofins Genomics (Ebersberg, Germany).

269 Representative nucleotide sequences of *18S* rRNA, *28S* rRNA, ITS1 and *cox1* loci of *S. funereus* sp.
270 nov. have been deposited in GenBank under the accession numbers MW349706, MW349707,
271 MW373964, MW489293, respectively. Sequences from both forward and reverse strands were
272 assembled and manually edited using FinchTV software (Geospiza Inc., Seattle, Washington)
273 followed by BLAST (Basic Local Alignment Search Tool) program at the NCBI (National Center
274 for Biotechnology Information) server, searches were conducted on obtained sequences for
275 genus/species identification. Sequence chromatograms obtained in this study were aligned using
276 MAFFT software version 7⁵².

277

278 Phylogenetic trees for all datasets were generated from nucleotide sequences of the selected
279 *Sarcocystis* species using the MEGA X⁵³ and reconstructed by using the Neighbor-Joining (NJ) and
280 Maximum Likelihood (ML) methods. A NJ phylogenetic tree for *18S* rRNA gene (dataset with 25
281 nucleotide sequences with a total of 1644 aligned nucleotide positions) was computed according to
282 the Tamura-Nei model with a gamma distribution (TN93+G). Other phylogenetic trees were
283 generated using ML analyses based on the Kimura 2-parameter model with a gamma distribution

284 rate and a proportion of invariant sites (K2+G+I) for 28S rRNA gene (25 sequences with 1442
285 positions); for *cox1* gene (18 sequences with 1013 positions) the Hasegawa-Kishino-Yano model
286 with a gamma distribution (HKY+G) was used, while the Tamura-Nei model with a gamma
287 distribution rate and a proportion of invariant sites (TN93+G+I) was used for ITS1 region (24
288 sequences with 1426 positions). All four phylogenetic trees were rooted using *Toxoplasma gondii*
289 sequence. Consensus trees were obtained after bootstrap analysis with 1000 replications.

290

291 **Ethics approval**

292 Not applicable.

293

294 **Data availability**

295 The sequences generated in the present study were submitted to the GenBank database under the
296 accession numbers MW349706, MW349707, MW373964, MW489293.

297

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426

427 **Acknowledgements**

428 The authors thank to all colleagues from Laboratory of Parasitology (SVI Prague), especially E.
429 Dubská. Thank are also to T. Bušina and K. Hongisto for their great help during the field work in
430 Kauhava study area, as well as O. Doskočil for making the graphical abstract. This research was
431 performed within the Centre for Infectious Animal Diseases. Succumbed fledglings of Tengmalm's
432 owls were collected, preserved and transported to State Veterinary Institute Prague, Czech Republic
433 under the permission obtained from the Centre for Economic Development, Transport and the
434 Environment in Southwest Finland (permit number VARELY/5933/2019 to Erkki Korpimäki).

435

436 **Funding**

437 Open access funding was provided by the Faculty of Agrobiolgy, Food and Natural Resources,
438 Czech University of Life Sciences Prague. The research project of owls in the Kauhava region was
439 financially supported by the grant provided by the Regional Fund of the South Ostrobothnia of the
440 Finnish Cultural Foundation.

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460 **Contributions**

461 O.M. conceived and designed the study, M.K. and E.K. conducted field research/collection, O.M.
462 performed laboratory analyses and analyzed data, O.M., D.G.S., wrote the main manuscript. All
463 authors read and approved the final manuscript.

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468 **Ethic declarations**

469 Competing interests

470 The authors declare no competing interests.

471

472 ARRIVE guidelines

473 This survey was carried out in compliance with the ARRIVE guidelines.

474

475 **Figure legends**

476 **Figure 1** *Sarcocystis funereus* sp. nov. from *Aegolius funereus*, light micrographs of oocyst (**a**) and
477 numerous sporocysts (**b**) in intestinal mucosa. Arrow indicates thin oocyst wall

478

479 **Figure 2** Phylogenetic trees of the species of *Sarcocystis* from various hosts based on sequences of

480 *18S* rRNA (**a**), *28S* rRNA (**b**), ITS1 (**c**) and *cox1* (**d**) loci. The numbers on phylogenetic trees

481 represent bootstrap values based on 1000 replications. Genbank accession numbers follow

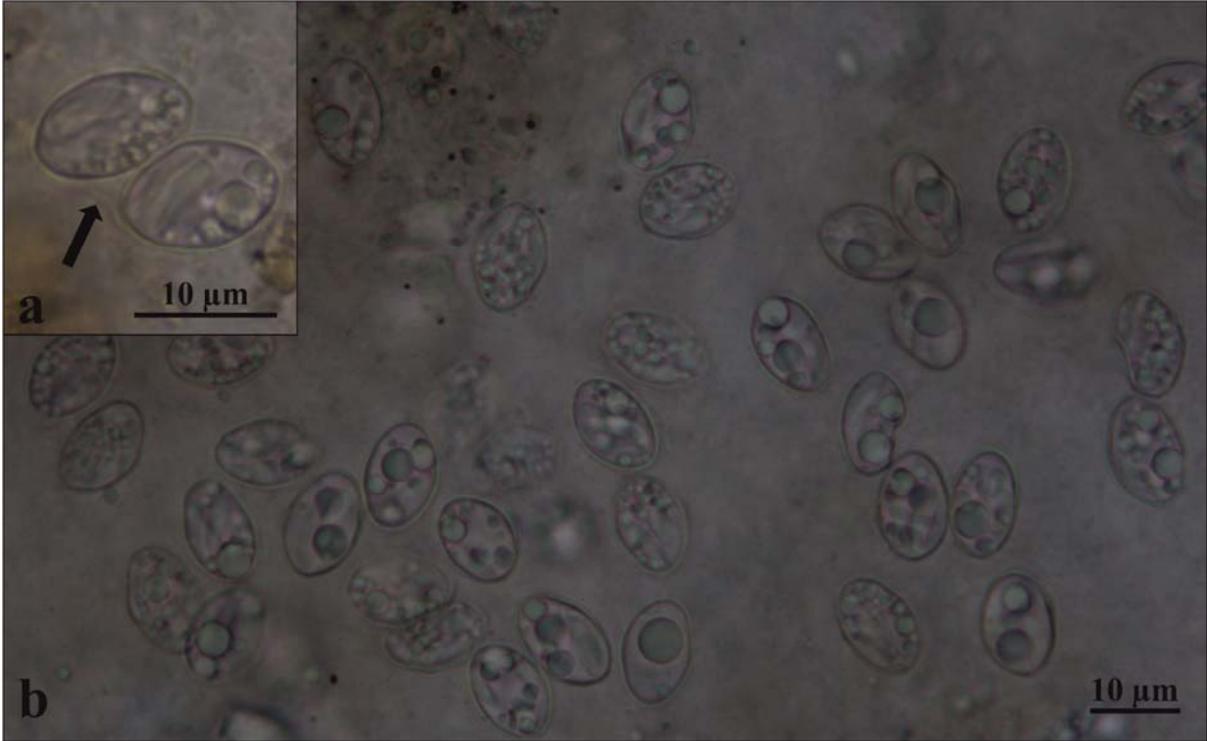
482 *Sarcocystis* taxa

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486 Figure 1



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495 Figure 2

