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A synopsis of records of myxozoan parasites (Cnidaria: Myxozoa) from shrews, with additional data on *Soricimyxum fegati* from common shrew *Sorex araneus* in Hungary and pygmy shrew *Sorex minutus* in Slovakia

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Abstract: Myxozoans (Cnidaria: Myxozoa) are almost exclusively endoparasites of aquatic vertebrates and invertebrates, with the notable exception being two species of *Soricimyxum* Prunescu, Prunescu, Pucek et Lom, 2007 described from terrestrial shrews (Soricidae) in central Europe. Myxospores of the two parasites are morphologically indistinguishable, but have SSU rDNA sequences that differ by about 4%. Herein, we report additional molecular and histology data from *Soricimyxum fegati* Prunescu, Prunescu, Pucek et Lom, 2007 from common shrew (*Sorex araneus* Linnaeus) from Hungary, and add a new geographic record for *S. fegati* in pygmy shrew (*Sorex minutus* Linnaeus) from Slovakia. A limited survey of shrews from the northern United States, *Blarina brevicauda* Say and *Sorex* sp. from New York, and *Sorex* spp. from Oregon, did not discover any infections, which is in stark contrast to the relatively high infection rates (up to 66%) in European shrew populations. We also provide a summary and discussion of literature records of species of *Soricimyxum* and a host survey. Given the lack of distinguishing morphological or morphometric characters between *Soricimyxum* spp., and the overlap in vertebrate hosts and geographic ranges, unambiguous identification of these closely related shrew parasites can presently only be achieved through sequence comparison of one or more variable SSU rDNA regions.

Keywords: Eurasian shrew, Eurasian pygmy shrew, myxozoan infection, bile ducts, liver, mammal hosts, Central Europe

Myxozoans are cosmopolitan parasites of aquatic vertebrates, primarily fishes, with more than 2 200 described species (Okamura et al. 2015). In the last ten years, two myxozoan species have been described from terrestrial mammals, specifically, three species of shrews in central Europe (Table 1). *Soricimyxum fegati* Prunescu, Prunescu, Pucek et Lom, 2007 and *Soricimyxum minuti* Székely, Cech, Atkinson, Molnár, Egyed et Gubányi, 2015 were described from bile ducts of *Sorex araneus* Linnaeus and *Sorex minutus* Linnaeus, respectively. Shrews infected with the same parasite species have been identified from localities more than 1 000 km apart, at high infection prevalence up to 66%, in mixed populations of *Sorex* spp. (Table 1, Fig. 1).

Dyková et al. (2007) redescribed *S. fegati* and showed by a study of the type material that the spore dimensions reported in the original description by Prunescu et al. (2007) are erroneously small. Dyková et al. (2007, 2011) also described the same myxozoan from three species of shrews in the southern Czech Republic and show genetic variation in the parasite SSU rDNA sequences (up to 4%; Table 2). A subsequent finding by Székely et al. (2015) of a genetically but not morphologically distinct myxozoan, *S. minuti*, in pygmy shrews, demonstrated that DNA sequencing is necessary to distinguish between the two parasites.

In the present study, we sequenced additional shrew myxozoans from our earlier collections (Székely et al. 2015). We also surveyed additional shrews from localities in northwestern Hungary as part of the National Biodiversity Monitoring Program (Table 1). We extended the geographic range of samples to include shrews from southern Slovakia, which died during the Slovakian small mammal monitoring program in 2012. These samples were provided by Michal Ambros (Slovakian State Nature Conservation Centre, Nitra). To test the hypothesis that shrew myxozoans are common across the northern hemisphere, we also examined small mammals from East and West Coast localities in the United States (Table 1). Trap mortalities from shrew surveys in New York State were provided by Rick Ostfeld and Kelly Oggenfuss (Cary Institute of Ecosystem Studies). We undertook opportunistic sampling of natural mortalities in shrews and voles from Oregon, USA; some

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Table 1. Review of small mammals surveyed for myxozoan infections in central Europe and USA, showing collection locality and prevalence of infections. Type myxozoan records are in bold.

Host species	Myxozoan	Nos. infected/ Nos. sampled (prevalence)	Collection year	Collection locality	Reference
Sorex araneus Linnaeus	***Soricimyxum fegati*** Prunescu, Prunescu, Pucek et Lom, 2007	19/46 (41%)	2001–2005	Poland, Bialowieza primeval forest	Prunescu et al. 2007
Sorex araneus	*S. fegati*	10/24 (42%)	2007	Czech Republic, Southern Bohemia	Dyková et al. 2007
Sorex minutus Linnaeus	*S. fegati*[a]	2/29 (7%)	2007		
Neomys fodiens (Pennant)	-	0/1	2007		
Sorex araneus	*S. fegati*	51/98 (52%)	2007–2009	Czech Republic, Southern Bohemia	Dyková et al. 2011
Sorex minutus	*S. fegati*[a]	14/70 (20%)	2007–2009		
Crocidura suaveolens Pallas	*S. fegati*[a]	1/10 (10%)	2007–2009		
Sorex araneus	*S. fegati*	8/21 (38%)	2007–2010	Hungary, Lipót	Székely et al. 2015
Sorex minutus	***Soricimyxum minuti*** Székely, Cech, Atkinson, Molnár, Egyed et Gubányi, 2015	1/3 (33%)	2009		
Sorex araneus	*S. fegati*	2/3 (67%)	2011	Hungary, Egervár	Present study
Sorex araneus	*S. fegati*	1/8 (13%)	2010	Hungary, Barbacsi Lake	
Sorex minutus	*S. fegati*	1/3 (33%)	2012	Slovakia, Gbelce	
Sorex minutus	-	0/2	2010	Hungary, Barbacsi Lake	
Sorex minutus	-	0/1	2011	Hungary, Lipót	
Neomys sp.	-	0/5	2011	Hungary, Barbacsi Lake	
Blarina brevicauda Say	-	0/16	2008–2010	USA, Hudson River Valley, NY	Present study[b]
Sorex sp.	-	0/23	2010		
Microtus canacaudis (Miller)	-	0/6	2011–2012	USA, Corvallis area, Oregon	
Sorex sp.	-	0/3	2011–2012		

[a] PCR not specific for *S. fegati*, thus these infections could be either *S. fegati* or *S. minuti* (or a putative close relative); [b] some of these USA data were reported as a personal communication in Hallett et al. (2015).

Fig. 1. Map showing localities in central Europe where infected shrews have been found. Locations from the present study are indicated in green.

of these data were included as a personal communication in Hallett et al. (2015), but are reported in full here for the first time.

Shrews were necropsied as reported previously (Székely et al. 2015). Briefly, we examined bile, gall bladder and liver as fresh squash preparations for the presence of myxozoan developmental stages and spores; animals from North America had been frozen prior to necropsy. Myxospores were photographed, measured and described per the guidelines of Lom and Arthur (1989). Liver tissue that included both mature spores and developmental stages was subdivided for histology and DNA extraction.

Survey results are shown in Tables 1 and 2. No infections were found in either the small number of *Neomys* sp. examined from Hungary, or in any of the North American shrews or voles. Infected species of *Sorex* were found in both northwestern Hungary and Slovakia. From a subset of visually positive shrews, parasite SSU rDNA was amplified and sequenced using PCR primers and cycling conditions reported previously (Székely et al. 2015). We designed one new *Sorimyxum*-specific primer SOR1050R (CCTCTCGCGCACAAATACC) to improve amplification of the 5' end of the target in semi-nested PCRs. Forward and reverse sequence segments were aligned by eye in

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Table 2. Records of myxozoans from shrews with spore morphology and DNA sequence comparisons. Type myxozoan records are in bold.

Myxozoan	Host (sample code)	H	S	Sampled material	Spore length*	Spore width*	Spore thick-ness*	Polar capsule diameter	Polar filament turns	GenBank accession number (length)	Notes	Reference
Soricimyxum fegati Prunescu, Prunescu, Pucek et Lom, 2007	*Sorex araneus* Linnaeus	yes	no	fixed	6.3–7.5, 7.0	4.3–6.0, 5.4	3.5	1.3–2.1, 1.7	1.5–1.7	-	Measurements in original description	Prunescu et al. 2007
				fixed	11.0–12.0	7.7–8.3	-	-	-	-	Type material re-measured by Dyková et al. 2007	
S. fegati	*Sorex araneus*	yes	yes	fresh	11.7–12.9, 12.3	8.8–10.0, 9.4	-	3.5	2(–3)[a]	EU232760 (1584 nt)	3 shrews sequenced; 99.2–99.3% similar to each other	Dyková et al. 2007
				fixed	11.0–12.0	7.7–8.3	-	-	-			
S. fegati[b]	*Sorex araneus* *Sorex minutus* Linnaeus *Crocidura suaveolens* Pallas	yes	yes	fixed	"Spores ... did not differ morphologically."					not published	Multiple shrews sequenced over a 170 nt variable region; 96.0% similar (163/170 nt) to *S. fegati* EU232760	Dyková et al. 2011
S. fegati	*Sorex araneus* (SA1)	yes	yes	fresh	-	-	-	-	-	KU248477 (1665 nt)	This sample sectionned and sequenced as part of present study; 99.8% similar (1580/1584 nt) to *S. fegati* EU232760	Székely et al. 2015
Soricimyxum minuti Székely, Cech, Atkinson, Molnár, Egyed et Gubányi, 2015	*Sorex minutus* (SM1)	no	yes	fresh	12.3–13.3, 12.6 ± 0.3	8.4–9.6, 9.2 ± 0.5	7.4–8.4, 8.0 ± 0.4	4.0–4.5, 4.3 ± 0.2	2–3	KR673725 (1629 nt)	*S. minuti* type sequence: 96% similar (1357/1413 nt) to *S. fegati* type EU232760	
S. fegati	*Sorex araneus* (SAA, SAR2)	no	yes	fresh	-	-	-	-	-	not published	SAA: 1139 nt; 99.9% similar (977/978) to EU232760; SAR2: 1593 nt; 99.8% similar (1298/1302) to EU232760	Present study
S. fegati	*Sorex minutus* (SM3/4)	no	yes	fresh	12.2–13.4, 12.6 ± 0.5	8.4–10.2, 9.1 ± 0.6	7.7–8.4, 8.1 ± 0.3	3.6–4.8, 4.1 ± 0.4	2–3	KU248478 (2025 nt)	99.8% similar (1580/1584 nt) to *S. fegati* EU232760	

H – histology; S – DNA sequenced; * range (above), mean ± standard deviation; in micrometres; [a] images in Dyková et al. 2007 show 2–3 polar filament turns; [b] PCR testing in Dyková et al. 2011 would not have distinguished *S. fegati* from *S. minuti* (which was not known at that time).

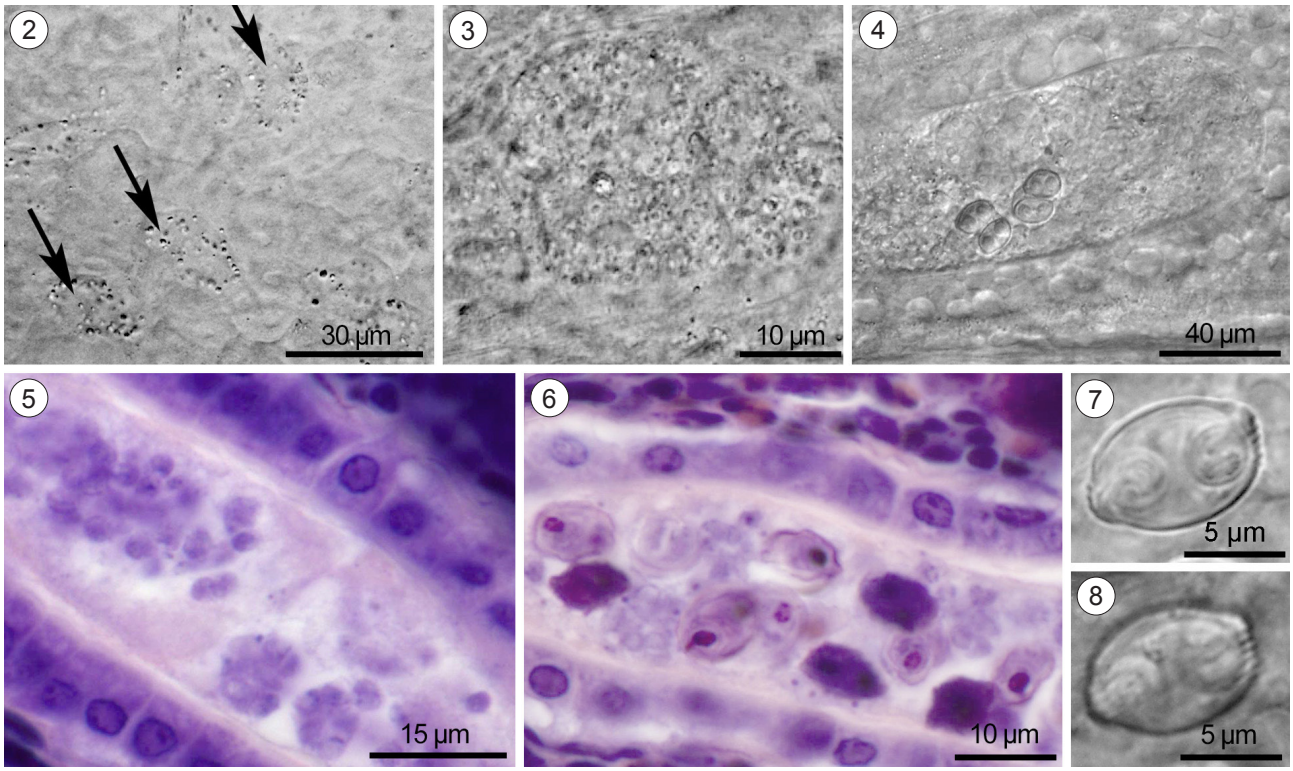

Fig. 2–8. Spores of *Soricimyxum fegati* Prunescu, Prunescu, Pucek et Lom, 2007 from the bile duct of the common shrew *Sorex araneus* Linnaeus from Hungary. **Figs. 2**, **3**, **5**. Early developmental stages (arrowed) in bile duct showing granular appearance and multiple nuclei. **Figs. 4**, **6**. Sporogonic stages with developing plasmodium and mature myxospores from fresh (Fig. 4) and fixed material (Fig. 6 H&E). **Figs. 7**, **8**. Fresh, mature myxospore in sutural view in two different focal planes. Figs. 2–4. Phase contrast. Figs. 5, 6. Bright field. Figs. 7, 8. Nomarski interference contrast.

BioEdit (Hall 1999) and ambiguous bases clarified with reference to the corresponding ABI chromatograms. Sequence contigs were compared with reference sequences of species of *Soricimyxum* from NCBI GenBank (Table 2).

Despite the morphological indistinguishability of the two species of *Soricimyxum*, the DNA sequence data were unambiguous – we amplified only *S. fegati* from both *S. araneus* from Hungary and *S. minutus* from Slovakia. Sequence chromatograms did not show any evidence of mixed infections of *S. fegati* and *S. minuti*. The Slovakian infection was sequenced with primers ERIB1-SOR1050R, MYXGEN4F-ERIB10 to generate a near-complete SSU rDNA sequence, which was 99.8% similar with reference sequence of *S. fegati* EU232760. We also sequenced isolates from three infections of *S. araneus* from the northwestern Hungary (including one sample mentioned in Székely et al. 2015), SA1, sequenced with primers ERIB1-SREV3, SFOR3-ERIB10. The parasite in all three of these *S. araneus* samples was 99.8–99.9% similar to the reference sequence of *S. fegati*.

Histological sections and digital images were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, collection numbers HNHM-18386 and HNHM-18387.

The earliest observed parasite stages in the bile ducts were oval or ramified, multinucleate, finely granular plasmodia 30–50 µm × 10–30 µm, which contained early sporogonic stages (Figs. 2, 3, 5). More advanced infections had 4 to 8 maturing spores in bisporic sporoblasts together with early sporogonic stages. (Figs. 4, 6). We did not observe any host reaction in the liver. Mature spores were slightly sigmoidal in sutural view (Figs. 7, 8), ellipsoidal in valvular view. Valves had a relatively thick suture running the length of the spore, and fine, longitudinal, surface ridges, parallel with the suture, distinctly visible in sutural view only (Fig. 8), with the greatest relief at ends of spore and very shallow to non-existent towards the middle of the spore. Two polar capsules, situated at the opposite ends of the spore, near-spherical, equal sized. Spore measurements are shown in Table 2 and are not significantly different from previous measurements of *S. fegati* or *S. minuti*. We could not identify any additional morphological characters to distinguish these two parasites.

Dyková et al. (2011) sequenced three short but relatively variable regions to assess infection status in three species of shrews (Table 1). Whereas the primers they designed distinguish between species of *Soricimyxum* and other myxozoan genera, we can now say that they are not specific enough to distinguish between *S. fegati* and *S. minuti*, which was discovered later. Hence, identification of *S. fegati* in the three shrew species by Dyková et al. (2011) may be ambiguous, particularly given that the variation they report (up to 4% in the 170 nt variable region) is within the range expected of *S. fegati* and *S. minuti*; these sequences are not yet available in GenBank for comparison. In the current study, we have demonstrated conclu-

sively that *S. fegati* can indeed infect both common shrew and pygmy shrew. Given the lack of distinguishing morphological or morphometric characters, and the overlap in vertebrate hosts and geographic range, unambiguous identification of these very closely related shrew parasites can only be achieved through sequencing of one or more regions that vary between these parasites, i.e. regions around SSU rDNA positions 490, 750, 970 and 1 600.

The high prevalence of myxozoan infection in shrews. (typically 10–50%) indicates that this parasite has been extraordinarily successful in adapting from purely aquatic ancestors to a terrestrial mode of existence. We suggest that future work should establish the range of infections in shrew populations outside of central Europe and extend surveys of shrews in North America, with an emphasis on aquatic shrew species: could those hosts have been a bridge for myxozoan parasites out of the aquatic environment? Or are semi-aquatic oligochaetes the 'bridging host'? Life cycle of any of the species of *Soricimyxum* Prunescu, Prunescu, Pucek et Lom, 2007 has not yet been determined, but given that all known myxozoan life cycles involve alternation between vertebrate and invertebrate hosts, it has been suggested that terrestrial oligochaetes may be alternate hosts, with trophic transmission between shrews and earthworms (Dyková et al. 2007). However, different dietary preferences of the known host shrew species suggest multiple invertebrate hosts may be present or that direct transmission may occur (Hallett et al. 2015). Ultimately, determination of life cycles of *Soricimyxum* and transmission routes will provide novel insight into this unique branch of the myxozoan evolutionary tree.

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