

Molecular systematics and Holarctic phylogeography of cestodes of the genus *Anoplocephaloides* Baer, 1923 s. s. (Cyclophyllidea, Anoplocephalidae) in lemmings (*Lemmus*, *Synaptomys*)

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The present molecular systematic and phylogeographic analysis is based on sequences of cytochrome c oxidase subunit 1 (*cox1*) (mtDNA) and 28S ribosomal DNA and includes 59 isolates of cestodes of the genus *Anoplocephaloides* Baer, 1923 s. s. (Cyclophyllidea, Anoplocephalidae) from arvicoline rodents (lemmings and voles) in the Holarctic region. The emphasis is on *Anoplocephaloides lemmi* (Rausch 1952) parasitizing *Lemmus trimucronatus* and *Lemmus sibiricus* in the northern parts of North America and Arctic coast of Siberia, and *Anoplocephaloides kontrimavichusi* (Rausch 1976) parasitizing *Synaptomys borealis* in Alaska and British Columbia. The *cox1* data, 28S data and their concatenated data all suggest that *A. lemmi* and *A. kontrimavichusi* are both non-monophyletic, each consisting of two separate, well-defined clades, that is independent species. As an example, the sister group of the clade 1 of *A. lemmi*, evidently representing the ‘type clade’ of this species, is the clade 1 of *A. kontrimavichusi*. For *A. kontrimavichusi*, it is not known which one is the type clade. There is also fairly strong evidence for the non-monophyly of *Anoplocephaloides dentata* (Galli-Valerio, 1905)-like species, although an earlier phylogeny suggested that this multi-species assemblage may be monophyletic. The results suggest a deep phylogenetic codivergence of *Lemmus* spp. and *A. lemmi*, primarily separating the two largely allopatric host and parasite species at the Kolyma River in east Siberia. There are also two allopatric sublineages within each main clade/species of *A. lemmi* and *Lemmus*, but the present distributions of the sublineages within the eastern *L. trimucronatus* and clade 1 of *A. lemmi* are not concordant. This discrepancy may be most parsimoniously explained by an extensive westward distributional shift of the easternmost parasite subclade. The results further suggest that the clade 1 of *A. kontrimavichusi* has diverged through a host shift from the precursor of *L. trimucronatus* to *S. borealis*.

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Introduction

The true lemmings (*Lemmus* spp.) and bog lemmings (*Synaptomys* spp.) comprise (with the wood lemming *Myopus schisticolor*) the monophyletic tribe Lemmini, which appears to be one of the basal clades within arvicoline rodents (Cricetidae: Arvicolinae) (see Buzan *et al.* 2008; Robovský *et al.* 2008). The true lemmings, including ca. 5 species (Jarrell & Fredga 1993; Wilson & Reeder 2005), have a wide distribution in northern parts of the Holarctic region, whereas the bog lemmings represent an endemic North American genus of two extant species. Of the latter, the northern bog lemming (*Synaptomys borealis*) has a continent-wide northern distribution from Alaska to Labrador, whereas the southern bog lemming (*Synaptomys cooperi*) has a more restricted (but still extensive), largely non-overlapping range in eastern and central USA and south-eastern part of Canada.

Based on a series of molecular studies (Fedorov *et al.* 1999, 2003; Fedorov & Stenseth 2001), the wide-scale phylogeographic structure of *Lemmus* spp. in northern parts of the Holarctic region is well established (Fig. 1). On the Holarctic scale, the true lemmings comprise four allopatric phylogenetic groups, the main division being located at the Kolyma River in north-eastern Siberia. According to Fedorov *et al.* (1999, 2003), this division, which separates the western *Lemmus sibiricus* and eastern *Lemmus trimucronatus*, has been created by vicariance due to the periodical presence of the Bering Strait between the landmasses of Eurasia and North America, with a subsequent westward shift of the division. The secondary divisions on the two continents (at Lena and McKenzie Rivers) are suggested to be formed by other types of glacial vicariance effects, such as fragmentation and isolation of populations due to glacial barriers. All divisions are evidently significantly older than the latest glacial cycle. The phylogeography of *Synaptomys* spp. has not been studied.

The genera *Lemmus* and *Synaptomys* have, respectively, one and two host-specific cestodes of the genus *Anoplocephaloides* Baer, 1923 s. s. (see Haukisalmi *et al.* 2009); no *Anoplocephaloides* species have evidently been reported from the Eurasian *M. schisticolor*. *Anoplocephaloides lemmi* (Rausch 1952) is a parasite of *Lemmus* spp. in North America and Eurasia. The known distribution of *A. lemmi* spans from north-western Russia in the west (host *L. sibiricus*) to Melville Peninsula (Nunavut, Canada) in the east (host *L. trimucronatus*) (see Rausch 1952; Tenora *et al.* 1990; Yushkov 1995; Haukisalmi & Henttonen 2001). It has also been reported from *Lemmus amurensis* from southern Sakha Republic (former Yakutia) (Gubanov & Fedorov 1970; Revin 1983), but is evidently absent in the westernmost species *Lemmus lemmus* in Fennoscandia (Haukisalmi &

Henttonen 2001; Laakkonen *et al.* 2001). The absence of *A. lemmi* and a few other host-specific parasites of *Lemmus* (*Heligmosomoides* Hall, 1916, *Arostrilepis* Mas-Coma & Tenora, 1997) in Fennoscandia may be due to the strong population bottleneck in *L. lemmus* during the last glacial maximum (Fedorov & Stenseth 2001). *Synaptomys borealis* and *S. cooperi* are parasitized with *Anoplocephaloides kontrimavichusi* Rausch 1976 and *A. bulmeri* Haukisalmi & Eckertlin 2009, respectively.

Haukisalmi *et al.* (2009) have presented a molecular phylogeny for *Anoplocephaloides* s. s. in voles (*Microtus* spp. and *Chionomys nivalis*) and lemmings, including *A. lemmi* and *A. kontrimavichusi*. This analysis confirmed the monophyly of *Anoplocephaloides* s. s. and *A. kontrimavichusi*, but the monophyly of *A. lemmi* remained undefined. However, the *Anoplocephaloides* species of lemmings were represented by a few individuals only, because the main emphasis of the analysis was on the *Anoplocephaloides dentata* (Galli-Valerio, 1905)-like species from voles.

Because of the distinct, allopatric phylogenetic structure of *Lemmus* spp., the true lemmings and their helminths provide an excellent framework for studying host–parasite cophylogeny and cophylogeography on a wide Holarctic scale. Among the helminths of true lemmings, only the cestodes *A. lemmi* (Anoplocephalidae) and *Arostrilepis* spp. (Hymenolepididae) are, however, adequately common and widespread for a Holarctic scale analysis of cophylogeography (Haukisalmi & Henttonen 2001). This study deals with *A. lemmi*; corresponding analyses of *Arostrilepis* spp. in lemmings are being performed by K. Galbreath, A. Makarikov, E. P. Hoberg and others within the framework of the Beringian Coevolution Project (BCP) (Hoberg *et al.* 2003, 2012; Cook *et al.* 2005; Makarikov *et al.* 2013).

Because the isolation of *Lemmus* lineages has extended over several glacial cycles, it can be expected that the host-specific parasites of *Lemmus* would have experienced a corresponding (concomitant) divergence. Such codivergence was indeed found in the cestode *Rauschoides arctica* (Rausch 1952), syn. *Paranoplocephala arctica* (Anoplocephalidae), and its hosts (collared lemmings *Dicrostonyx* spp.) in northern parts of the Holarctic region (Wickström *et al.* 2003). However, the phylogeography of some other anoplocephaline cestodes of arvicoline rodents shows no correspondence to the main phylogeographic structure of their hosts (Haukisalmi *et al.* 2004; Wickström 2004), possibly because of their flexible host selection, which enables them, through ecological fitting, to cross various phylogeographic divisions of the primary hosts (e.g. Brooks & McLennan 2002; Hoberg & Brooks 2008, 2010; Agosta *et al.* 2010). As an example, the phylogeography of a common host generalist cestode of voles [*Eurotaenia gracilis* (Tenora & Murai,

1980)] does not match the shared main phylogeographic split of their hosts in Fennoscandia, whereas two related, more host-specific cestodes [*Anoplocephaloides* cf. *dentata* (Galli-Valerio, 1905) and *Microicola blanchardi* (Moniez, 1891)] show relatively good correspondence to the host split (Wickström 2004).

Moreover, some helminths, including *R. arctica*, show distinct lineages in regions where the hosts do not show phylogeographic structure, suggesting the presence of historical, cryptic host divergence (Hoberg 1995; Wickström *et al.* 2003; Nieberding *et al.* 2004; Galbreath & Hoberg 2012).

Besides analysing the cophylogeography of *A. lemmi* and its hosts (*Lemmus* spp.), this study also examines the phylogenetic relationships and molecular systematics of all available species of *Anoplocephaloides* s. s. from lemmings and voles, that is *A. lemmi*, *A. kontrimavichusi*, *A. bulmeri* and several congeneric species from voles of the genera *Microtus* and *Chionomys* (i.e. *Anoplocephaloides dentata* s. l.).

Material and methods

The phylogenetic analysis is based on 27, 18 and 1 isolate (s) of *A. lemmi* (from *Lemmus sibiricus* and *L. trimucronatus*), *A. kontrimavichusi* (from *Synaptomys borealis*) and *A. bulmeri* (from *Synaptomys cooperi*), respectively (Table 1). In addition, the analysis includes previously published sequences of 13 isolates of *A. dentata*-like cestodes from *Microtus* and *Chionomys* voles, representing a minimum of three independent species of *Anoplocephaloides* s. s. (see Haukisalmi *et al.* 2009).

The collection sites of *A. lemmi* and *A. kontrimavichusi* have been depicted in Figs 1 and 2, respectively.

The material of *A. lemmi* from *L. sibiricus* and *L. trimucronatus* originates from four main sources: (i) from various localities along the Siberian Arctic coast, Russia, collected by V. B. Fedorov and K. Fredga during the 'Swedish-Russian Tundra Ecology Expedition' in the summer of 1994 (Fredga *et al.* 1999; see also Haukisalmi & Henttonen 2001), (ii) from Victoria Island/Kent Peninsula region, Nunavut, Canada, collected in early July 1996 by C. J. Krebs and A. Kenney, (iii) from the Canadian Arctic archipelago, collected during the 'Tundra Northwest 1999' expedition by V. B. Fedorov, K. Fredga, C. J. Krebs and A. Angerbjörn (Molau *et al.* 1999) and (iv) from various localities in Alaska, north-western British Columbia, northern Yukon (Canada) and north-eastern Russia (Chukotka), collected by participants of the BCP during 2001–2002 (Hoberg *et al.* 2003; Cook *et al.* 2005).

The material of *A. kontrimavichusi* from *S. borealis* originates from various localities in Alaska and north-western British Columbia, collected by the BCP. The single specimen of *A. bulmeri* used in the present analysis was collected from

S. cooperi in Virginia (USA) by W. Bulmer, R. P. Eckerlin and A. L. Gardner (see Haukisalmi & Eckerlin 2009).

Tissue samples fixed and preserved in 70–96% ethanol, and held at ultralow temperatures (−80 °C), were extracted using E.Z.N.A.TM Tissue Kit (OMEGA Bio-Tek Inc., Norcross, GA, USA). For the amplification of DNA, standard 50 µl PCR was performed using hot start to avoid non-specific amplification of DNA. Successfully amplified DNA was purified using E.Z.N.A.TM Cycle Pure Kit (OMEGA Bio-Tek). Purified PCR products were directly sequenced using dye terminators and visualized with an ABI 3730xl DNA analyser at Macrogen Inc. (Seoul, Korea).

All isolates of *A. lemmi* and *A. kontrimavichusi* were successfully amplified and sequenced for a partial (ca. 640 bp) mitochondrial cytochrome oxidase subunit I (*cox1*) gene, using the primers COX-F 5'-GAT GTT TTC TTT ACA TTT ATC TGG TG-3' and COX-R 5'-GCC ACC ACA AAT CAA GTA TC-3' of Haukisalmi *et al.* (2004) and amplification procedures described in Wickström *et al.* (2003, 2005) and Haukisalmi *et al.* (2004). *Anoplocephaloides bulmeri* could not be successfully amplified for *cox1*.

In addition, eighteen isolates of *A. lemmi*, 10 isolates of *A. kontrimavichusi* and one isolate of *A. bulmeri* were successfully amplified and sequenced for partial 28S ribosomal DNA (domains 1–3) using three alternative pairs of primers: (i) LSU5 (forward, 5' TAGGTCGACCCGCTGAA YTTYAGCA 3') of Littlewood *et al.* (2000), except that one 'A' was replaced with 'Y' (in bold), and 1200R (reverse, 5' GCATAGTTCACCATCTTTTCGG 3') of Lockyer *et al.* (2003) (ca. 1400 bp), (ii) XZ-1 (forward, 5' ACCCGC TGAATTTAAGCATAT 3') of Waeschenbach *et al.* (2007), which differs from the original XZ-1 of Auwera *et al.* (1994) by having one 'Y' replaced with 'T' (in bold), and 1500R (reverse, 5' GCTATCCTGAGGGAACTTCG 3') of Littlewood *et al.* (2008) (ca. 1660 bp) and (iii) U178 (forward, 5' GCACCCGCTGAAYTTAAG 3') and L1642 (reverse, 5' CCAGCGCCATCCATTTTCA 3') (ca. 1500 bp), both from Lockyer *et al.* (2003). The cycling conditions for 28S primers followed those of Lockyer *et al.* (2003), Waeschenbach *et al.* (2007) and Littlewood *et al.* (2008).

Sequences were assembled and edited in Geneious version 5.3.6. (<http://www.geneious.com>, Kearse *et al.* 2012) and aligned with ClustalW (Thompson *et al.* 1997). The length of the edited alignment for *cox1*, 28S and their concatenated data was 499, 1343 and 1788 bp, respectively.

Phylogenetic relationships were reconstructed using the Bayesian approach (Huelsenbeck *et al.* 2001) implemented in the program MrBayes v. 3.5 (Ronquist & Huelsenbeck 2003). For each data set, MrBayes was run for 5 million generations, sampled every 1000 generations, and 500 000 generations were discarded as 'burn-in'. Node support was expressed as posterior probabilities, ≥95% being considered

Table 1 Tapeworm isolates used in the phylogenetic analysis. New sequences in bold. Coordinates, collectors, accession numbers and other details of host specimens having a field code starting with 'AF', 'IF' or 'NK' are available in the Arctos Database (<http://arctos.database.museum/>)

Cestode species Host species	Host field code	Isolate no.	Country, state/region	Locality (number)	Collectors	cox1 code	GenBank Acc. No.	28S code	GenBank Acc. No.
<i>Anoplocephaloides lemmi</i>									
<i>Lemmus sibiricus</i>	S 51	19	Russia	Yamal Peninsula (1)	V. B. Fedorov, K. Fredga	C1	KJ950149	S1	KJ950124
<i>L. sibiricus</i>	S 243	108	Russia	Taimyr Peninsula (2)	Fedorov, Fredga	C2	AY568197	S2	AY569733
<i>L. sibiricus</i>	S 451	5	Russia	Indigirka Delta (3)	Fedorov, Fredga	C3	KJ950150	—	—
<i>L. sibiricus</i>	S 504	109	Russia	Kolyma River West (4)	Fedorov, Fredga	C4	EU744308	S4	KJ950125
<i>L. sibiricus</i>	S 504	111	Russia	Kolyma River West (4)	Fedorov, Fredga	C5	EU744307	S5	KJ950126
<i>Lemmus trimucronatus</i>	S 492	32	Russia	Kolyma River East (5)	Fedorov, Fredga	C6	AY568198	S6	AY569734
<i>L. trimucronatus</i>	S 488	104	Russia	Kolyma River East (5)	Fedorov, Fredga	C7	KJ950151	S7	KJ950127
<i>L. trimucronatus</i>	S 489	105	Russia	Kolyma River East (5)	Fedorov, Fredga	C8	KJ950152	S8	KJ950128
<i>L. trimucronatus</i>	IF 7769	P74	Russia, Chukotka	Getlyangen Lagoon (6)	BCP	C9	KJ950153	—	—
<i>L. trimucronatus</i>	IF 7796	P76	Russia, Chukotka	Getlyangen Lagoon (6)	BCP	C10	KJ950154	S10	KJ950129
<i>L. trimucronatus</i>	AF 48229	J91	USA, Alaska	Noatak NP (7)	BCP	C11	KJ950155	S11	KJ950130
<i>L. trimucronatus</i>	AF 61679	O12	USA, Alaska	GAAR, Koyukuk River (8)	BCP	C12	KJ950156	S12	KJ950131
<i>L. trimucronatus</i>	AF 61246	P45	USA, Alaska	GAAR, Koyukuk River (8)	BCP	C13	KJ950157	—	—
<i>L. trimucronatus</i>	AF 58976	O76	USA, Alaska	GAAR, Walker Lake (8)	BCP	C14	KJ950158	S14	KJ950132
<i>L. trimucronatus</i>	AF 59924	P55	USA, Alaska	GAAR, Agiak Lake (8)	BCP	C15	KJ950159	S15	KJ950133
<i>L. trimucronatus</i>	AF 63694	P53	USA, Alaska	Mt. Fairplay (9)	BCP	C16	KJ950160	S16	KJ950134
<i>L. trimucronatus</i>	C 101	C13	Canada	North Yukon (10)	BCP	C17	KJ950161	S17	KJ950135
<i>L. trimucronatus</i>	C 103	A5	Canada	North Yukon (10)	BCP	C18	AY568199	—	—
<i>L. trimucronatus</i>	C 100	A61	Canada	North Yukon (10)	BCP	C19	KJ950162	—	—
<i>L. trimucronatus</i>	C 170	996	Canada	Cape Bathurst (11)	BCP	C20	KJ950163	S20	KJ950136
<i>L. trimucronatus</i>	C 170	997	Canada	Cape Bathurst (11)	BCP	C21	KJ950164	—	—
<i>L. trimucronatus</i>	CN 91	B60	Canada	Banks Island (12)	C. J. Krebs, A. Kenney	C22	KJ950165	—	—
<i>L. trimucronatus</i>	CN 305	387	Canada, Nunavut	Walker Bay (13)	Krebs, Kenney	C23	KJ950166	S23	KJ950137
<i>L. trimucronatus</i>	CN 215	438	Canada, Nunavut	Hope Bay (13)	Krebs, Kenney	C24	KJ950167	S24	KJ950138
<i>L. trimucronatus</i>	CN 197	784	Canada, Nunavut	Hope Bay (13)	Krebs, Kenney	C25	KJ950168	—	—
<i>L. trimucronatus</i>	CN 273	789	Canada, Nunavut	Byron Bay (13)	Krebs, Kenney	C26	KJ950169	S26	KJ950139
<i>L. trimucronatus</i>	CN 166	795	Canada, Nunavut	Hurd Island (13)	Krebs, Kenney	C27	KJ950170	—	—
<i>A. kontrimavichusi</i>									
<i>Synaptomys borealis</i>	AF 42746	G71	USA, Alaska	Fairbanks (14)	H. Henttonen <i>et al.</i>	C28	AY568195	S28	AY569731
<i>S. borealis</i>	AF 49369	J51	USA, Alaska	YUCH, Mt. Kathryn (15)	BCP	C29	AY568196	S29	AY569732
<i>S. borealis</i>	AF 45711	J86	USA, Alaska	Tetlin NWR, Wellesley Lake (16)	BCP	C30	EU744305	—	—
<i>S. borealis</i>	AF 63902	N25	USA, Alaska	Mt. Fairplay (17)	BCP	C31	EU744304	—	—
<i>S. borealis</i>	AF 63121	N70	USA, Alaska	WRST, Chokosna Lake (18)	BCP	C32	EU744306	—	—
<i>S. borealis</i>	AF 53505	Y12	USA, Alaska	WRST, Pocket Creek (19)	BCP	C33	KJ950171	S33	KJ950140
<i>S. borealis</i>	AF 61354	O47	USA, Alaska	GAAR, Koyukuk River (20)	BCP	C34	KJ950172	—	—
<i>S. borealis</i>	AF 61654	X86	USA, Alaska	GAAR, Koyukuk River (20)	BCP	C35	KJ950173	S35	KJ950141
<i>S. borealis</i>	AF 61663	X87	USA, Alaska	GAAR, Koyukuk River (20)	BCP	C36	KJ950174	S36	KJ950142
<i>S. borealis</i>	AF 61607	X88	USA, Alaska	GAAR, Koyukuk River (20)	BCP	C37	KJ950175	S37	KJ950143
<i>S. borealis</i>	AF 58570	O73	USA, Alaska	GAAR, Takahula Lake (21)	BCP	C38	KJ950176	S38	KJ950144
<i>S. borealis</i>	AF 58619	X91	USA, Alaska	GAAR, Takahula Lake (21)	BCP	C39	KJ950177	S39	KJ950145
<i>S. borealis</i>	AF 58684	X92	USA, Alaska	GAAR, Takahula Lake (21)	BCP	C40	KJ950178	—	—
<i>S. borealis</i>	AF 58878	O87	USA, Alaska	GAAR, Walker Lake (22)	BCP	C41	KJ950179	S41	KJ950146
<i>S. borealis</i>	AF 59096	X85	USA, Alaska	GAAR, Walker Lake (22)	BCP	C42	KJ950180	—	—
<i>S. borealis</i>	IF 12853	Y1	USA, Alaska	Kenai NWR, Watson Lake (23)	BCP	C43	KJ950181	—	—
<i>S. borealis</i>	IF 6902	T51	Canada, B. C.	Stikine River (24)	BCP	C44	KJ950182	—	—
<i>S. borealis</i>	IF 10167	X98	Canada, B. C.	Unnamed (25)	BCP	C45	KJ950183	S45	KJ950147

Table 1 Continued

Cestode species Host species	Host field code	Isolate no.	Country, state/region	Locality (number)	Collectors	cox1 code	GenBank Acc. No.	28S code	GenBank Acc. No.
<i>A. bulmeri</i> <i>Synaptomys cooperi</i>	—	Y22	USA, Virginia	Highland County	W. Bulmer, R. P. Eckerling, A. L. Gardner	—	—	S46	KJ950148
<i>A. dentata</i> s. l.									
<i>Chionomys nivalis</i>	I 83+84	E38	Italy	Trentino	H. Henttonen et al.	C47	AY568190	S47	AY569725
<i>C. nivalis</i>	I 103	E52	Italy	Trentino	Henttonen et al.	C48	EU664404	S48	EU664384
<i>Microtus guentheri</i>	T 274	R41	Turkey	Gundalan	Henttonen et al.	C49	EF688329	S49	—
<i>Microtus arvalis</i>	31220503	R31	Croatia	Novo Granica	Henttonen et al.	C50	EU664432	S50	EU664396
<i>M. arvalis</i>	—	Q48	Slovakia	Raros	A. Gubányi	C51	EU664427	S51	EU664392
<i>Microtus agrestis</i>	F 3	240	Finland	Iitti	I. K. Hanski, V. Haukisalmi	C52	AY423811	S52	EU664376
<i>M. agrestis</i>	S 121-92	D72	Sweden	Ödeshög	M. Jaarola, Haukisalmi	C53	EU664403	S53	EU664382
<i>Microtus oeconomus</i>	AF 41837	G47	USA, Alaska	Toolik Lake	Henttonen et al.	C54	EU664406	S54	EU664385
<i>M. oeconomus</i>	AF 63550	P25	USA, Alaska	WRST, Twin Lakes	BCP	C55	EU664425	S55	EU664391
<i>M. oeconomus</i>	C 124	A2	Canada	North Yukon	BCP	C56	AY568193	S56	AY569730
<i>Microtus pennsylvanicus</i>	AF 59122	O96	USA, Alaska	GAAR, Walker Lake	BCP	C57	EU664424	S57	EU664390
<i>Microtus mogollonensis</i>	NK 136 591	X29	USA, New Mexico		J. A. Cook et al.	C58	EU664438	S58	EU664399
<i>Microtus</i> sp.	H 184	R36	Russia, Irkutsk Oblast	Tunguska River	A. Lavikainen	—	—	S59	EU664397
Outgroup species									
<i>Andrya rhopalocephala</i>	—	I41	Hungary	Hódmezővásárhely	Gubányi		AY189958		AY569724
<i>Neandrya cuniculi</i>	—	C38	Spain, Canary Islands	Tenerife	C. Feliu		AY189957		AY569723
<i>Diandrya composita</i>	AF 49540	L25	USA, Alaska	YUCH, Crescent Creek	BCP		AY181550		AY569739

NP, National Preserve; GAAR, Gates of the Arctic National Park and Preserve; YUCH, Yukon-Charley Rivers National Preserve; NWR, National Wildlife Refuge; WRST, Wrangell-St. Elias National Park and Preserve.

significant. The substitution models used, based on the Akaike information criterion in jModelTest (Posada 2008), were GTR + I + Γ for *cox1* data and concatenated data and GTR + Γ for 28S data.

Because of the absence of indels, frame shifts and premature stop codons, the *cox1* sequences probably did not include any *numt* pseudogenes (Triant & DeWoody 2007).

Three other anoplocephalid cestodes, that is *Andrya rhopalocephala* (Riehm, 1881), *Neandrya cuniculi* (Blanchard, 1891) and *Diandrya composita* (Darrach, 1930), were used as out-group in phylogenetic analyses. Of these, *D. composita* belongs to the monophyletic 'arvicoline clade' of anoplocephalid cestodes together with *Anoplocephaloides* spp. s. s., *Microcephaloides* spp. and *Paranoplocephala* spp. s. l., and *A. rhopalocephala* and *N. cuniculi* comprise [with *Ctenotaenia marmotae* (Frölich, 1802)] the sister group of the 'arvicoline clade' (Wickström et al. 2005; Haukisalmi et al. 2010).

The GenBank accession numbers have been listed in Table 1. Of these, ten *cox1* sequences and four 28S sequences of *A. lemmyi* and *A. kontrimavichusi* have been previously used in the phylogenetic analysis of Haukisalmi et al. (2009).

The specific identification of *A. lemmyi* and *A. kontrimavichusi* and their morphological differentiation from

A. dentata-like species are based on Rausch (1976) and Haukisalmi et al. (2009). The deposited whole-mount voucher specimens of *A. lemmyi*, *A. kontrimavichusi* and *A. bulmeri* have been listed in Table S1. The voucher specimens do not usually represent the same individual cestodes used for extracting DNA.

The voucher specimens were fixed in 70% ethanol (most specimens) or 4% formaldehyde, stained with Mayer's hemalum, Semichon's acetocarmine or alum carmine, and mounted in Canada balsam.

Results

The *cox1* data, 28S data and their concatenated data (Figs 3–5) all suggest that *A. lemmyi* and *A. kontrimavichusi* are both non-monophyletic, each consisting of two independent, usually well-defined clades. There is also fairly strong evidence for the non-monophyly of *A. dentata*-like species, although the earlier 28S phylogeny (Haukisalmi et al. 2009) suggested that this assemblage may be monophyletic.

Phylogeny based on *cox1* data

In the *cox1* data (Fig. 3), *A. lemmyi* consists of two main clades, an eastern one parasitizing primarily *L. trimucronatus*

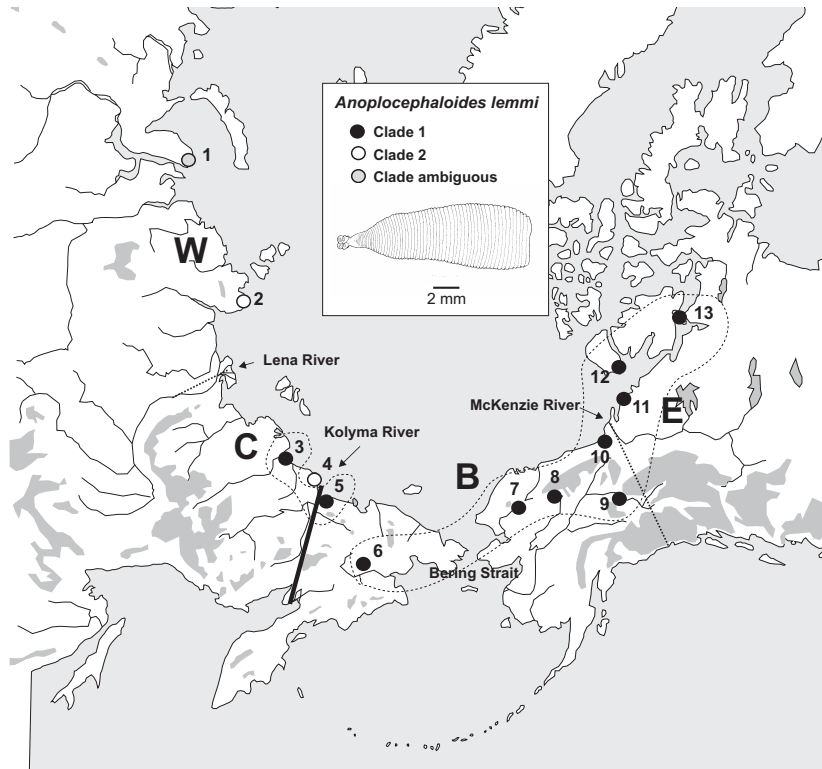


Fig. 1 Sampling localities of *Anoplocephaloides lemmi* in *Lemmus* spp. in Siberia and North America. 1, Yamal Peninsula; 2, Taimyr Peninsula; 3, Indigirka River; 4, Kolyma River West; 5, Kolyma River East; 6, Chukotka; 7, Noatak National Preserve (Alaska); 8, Gates of the Arctic National Park and Preserve (Alaska); 9, Mt. Fairplay (Alaska); 10, North Yukon; 11, Cape Bathurst; 12, Banks Island; 13, Victoria Island/Kent Peninsula region. Locality symbols are coded according to the main phylogenetic group of *A. lemmi* [closed circles, eastern clade (1); open circles, western clade (2); grey circle (loc. 1), clade designation ambiguous]. The two sublineages of the eastern clade of *A. lemmi* (1a and 1b) enclosed by hatched lines. The main phylogenetic division of *Lemmus* spp. (*L. sibiricus* vs. *L. trimucronatus*) at the Kolyma River indicated by a straight solid line, and secondary divisions of these species in Siberia and North America indicated by straight hatched lines. Capital letters stand for the four sublineages of *Lemmus* spp. (W, western; C, central; B, Beringian; and E, eastern), based on Fedorov *et al.* (2003).

in North America and north-eastern Siberia east of the Kolyma River (clade 1) and a western one parasitizing *L. sibiricus* in central and eastern Siberia west of the Kolyma River (clade 2). This pattern is, however, partly disrupted by two samples: the westernmost isolate (C1) of *A. lemmi* from the Yamal Peninsula (loc. 1) is not clearly associated with any of the clades, and the isolate (C3) from the Indigirka River Delta (loc. 3) belongs to the eastern clade of *A. lemmi*, although it is parasitizing the western host species *L. sibiricus* (Fig. 1, Table 1).

The eastern clade (1) of *A. lemmi* includes two supported subclades (1a and 1b), their division being positioned between the Kolyma River (loc. 5) and central Chukotka (loc. 6) (Figs 1 and 3). However, there was no supported phylogeographic structure within the widespread subclade 1b of *A. lemmi* either at the Bering Strait or in North America.

The sister group for the eastern clade (1) of *A. lemmi* is formed by the clade 1 of *A. kontrimavichusi*, whereas the

western clade (2) of *A. lemmi* has an unresolved basal position (Fig. 3). *Anoplocephaloides kontrimavichusi* clade 2 is sister to the Holarctic clade 1 of *A. dentata*-like cestodes. There is no supported phylogeographic structure within either of the *A. kontrimavichusi* clades (Fig. 3).

Of the two independent clades of *A. kontrimavichusi*, clade 2 is evidently more common and seems to have a more extensive distribution than the clade 1 (the latter was absent in south-eastern Alaska), although both extend from northern Alaska [Gates of the Arctic National Park and Preserve (GAAR)] to north-western British Columbia (Fig. 2). The distribution of the two clades is at least partly overlapping, because they were collected from the same study site in GAAR and from two nearby sites in British Columbia.

Phylogeny based on 28S data and concatenated data

In the 28S data (Fig. 4), the clade 1 of *A. lemmi* is confirmed to be sister to the clade 1 of *A. kontrimavichusi*, but

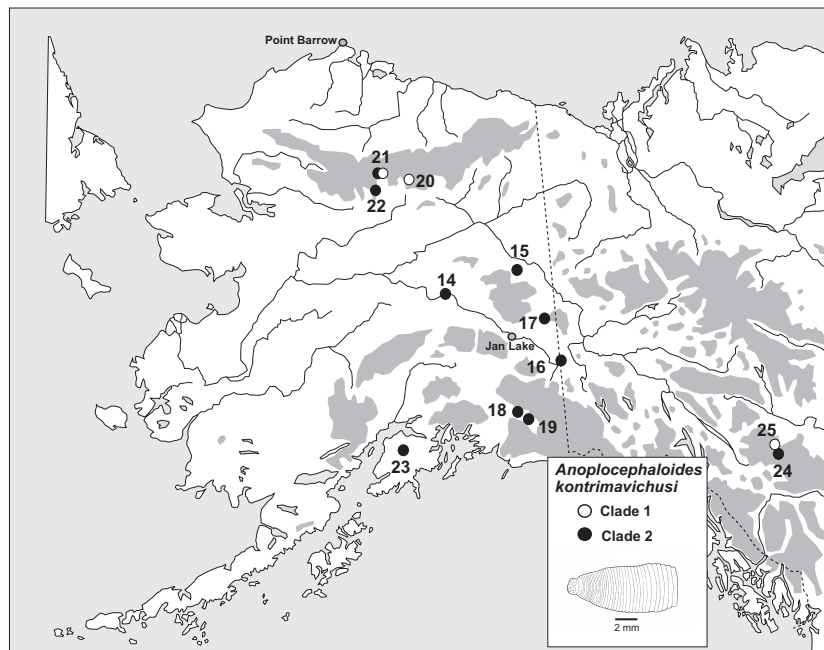


Fig. 2 Sampling localities of *Anoplocephaloides kontrimavichusi* in *Synaptomys borealis* in Alaska and British Columbia. 14, Fairbanks; 15, Mt. Kathryn, Yukon-Charley Rivers National Preserve; 16, Wellesley Lake, Tetlin National Wildlife Refuge; 17, Mt. Fairplay; 18, Chokosna Lake, Wrangell-St. Elias National Park and Preserve (WRST); 19, Pocket Creek, WRST; 20, Koyukuk River, Gates of the Arctic National Park and Preserve (GAAR); 21, Takahula Lake, GAAR; 22, Walker Lake, GAAR; 23, Watson Lake, Kenai National Wildlife Refuge; 24, Stikine River, British Columbia; 25, Unnamed locality, British Columbia. Locality symbols are coded according to the main phylogenetic group of *A. kontrimavichusi* (open circles, clade 1; closed circles, clade 2). The map also shows the type localities of *Anoplocephaloides lemmi* (Point Barrow) and *A. kontrimavichusi* (Jan Lake).

they now form a more inclusive clade with specimens representing the clade 2 of *A. lemmi*. The isolate of *A. lemmi* from Yamal (S1) is now firmly positioned within the clade 1 of *A. lemmi*, whereas in *cox1* data (C1), it has an unresolved basal position. The phylogenetic position of a specimen of *A. lemmi* from Chukotka (S10, C10) is also slightly different in the two sequence data sets.

The association between the clade 2 of *A. kontrimavichusi* and the clade 1 of *A. dentata*-like cestodes is also suggested by the 28S data, albeit with a lower support than in the *cox1* data (Fig. 4). This clade also includes *A. bulmeri* as a separate, divergent branch.

The isolate from the Yamal Peninsula (C1+S1) was excluded from the final concatenated analysis (*cox1* + 28S), because it showed a very deviant phylogenetic position in the *cox1* and 28S data and a very long branch in the preliminary concatenated tree. The final concatenated phylogeny (Fig. 5) reveals a topology that follows that of the 28S data, although some of the nodes are less strongly supported in the former. As an additional feature, concatenated tree shows a fairly well-supported (93% posterior probability) dichotomy within the western clade 2 of *A. lemmi* (2a and 2b), located between Taymyr Peninsula (loc. 2) and western Kolyma River (loc. 4) (Fig. 1).

Discussion

Taxonomy of *Anoplocephaloides lemmi* and *A. kontrimavichusi*

Anoplocephaloides lemmi was described from *L. trimucronatus* from Point Barrow at the northern coast of Alaska (type

locality), Nunivak Island in the Bering Sea, and Melville Peninsula and Chesterfield Inlet in Nunavut, Canada (Rausch 1952). Considering the geographical distribution of the main clades of *A. lemmi*, the Holarctic clade 1 (the only clade present in North America) evidently represents the true *A. lemmi*. According to the present data, the distribution of the true *A. lemmi* spans into the north-eastern Siberia, at least to the Indigirka River (parasitizing *L. sibiricus* in the latter area).

Because of the strongly supported non-monophyly of *A. lemmi*, the western clade 2 of *A. lemmi* should be treated an independent, new species. However, the distribution of this species remains ambiguous because of the low number of isolates from western Siberia and the unsettled phylogenetic position of the single isolate from the Yamal Peninsula (C1, S1). Thus, Taymyr Peninsula and western Kolyma River are the only localities where the presence of the putative new species can presently be confirmed. However, the two main lineages seem to overlap at least in the region between the western Kolyma River and Indigirka River.

Since the original description (Rausch 1952), new observations on the morphology of *A. lemmi*-like cestodes have been published by Rausch (1976), Gubanov & Fedorov (1970) and Tenora *et al.* (1990). Rausch's (1976) data are based on the type material and additional materials from *L. trimucronatus* from eastern Brooks Range and western Alaska, all evidently representing the true *A. lemmi*. Gubanov & Fedorov (1970) provided limited morphological data of *A. lemmi* from *Lemmus obensis* (now a synonym of

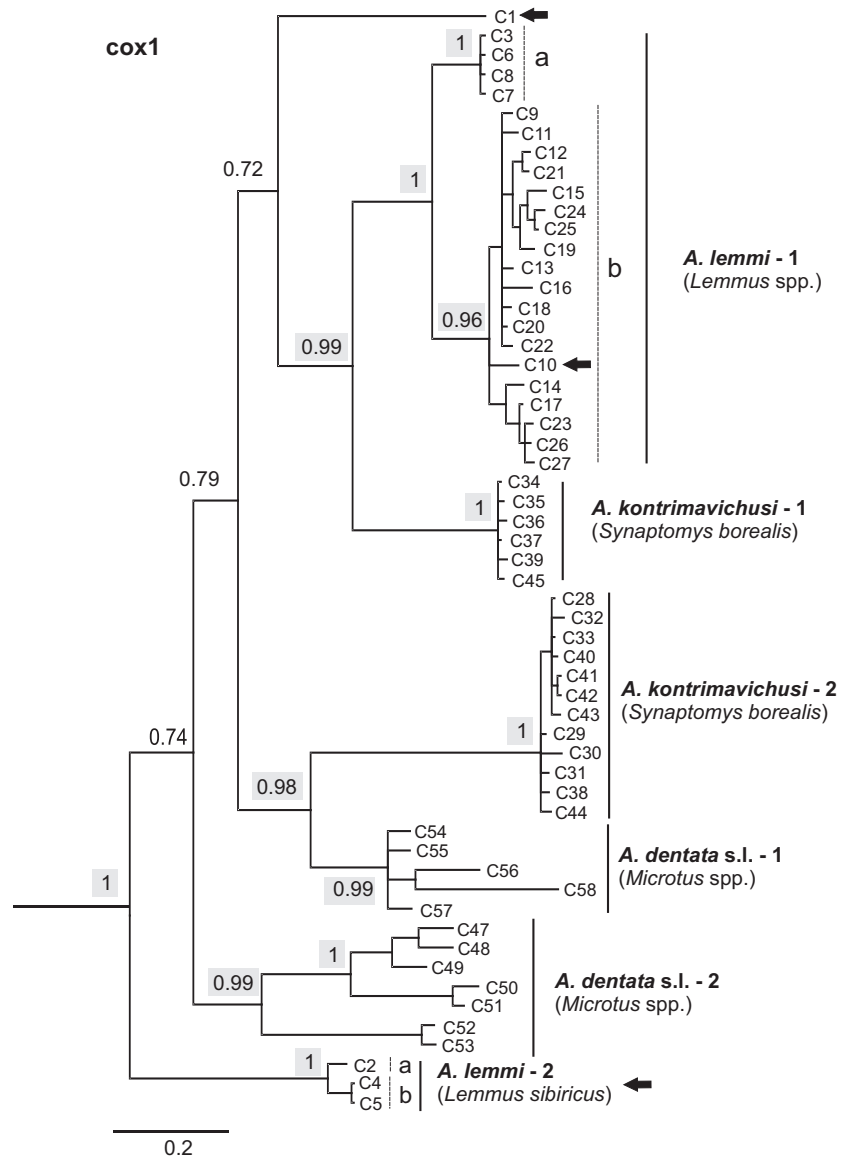


Fig. 3 A Bayesian phylogeny reconstruction of partial cytochrome oxidase I (mtDNA) sequences of *Anoplocephaloides* spp. Arrows indicate isolates and clades that showed different positions in the *cox1* and 28S phylogenies. For sequence codes, see Table 1.

L. sibiricus) from Yakutia (presently Sakha Republic) in eastern Russia. Based on the distribution of the two main lineages of *A. lemmi*, the latter material probably represents the undescribed species.

The observations of Tenora *et al.* (1990) are based on a material collected from *L. sibiricus* from the Taimyr Peninsula, which probably also belongs to the undescribed Siberian species of *A. lemmi*. Tenora *et al.* (1990) compared their material with those of Rausch (1952, 1976) and Gubanov & Fedorov (1970) and concluded that it differs somewhat from all of them. However, Tenora *et al.* (1990) designated their cestodes as *Anoplocephaloides* cf. *lemmi*, not describing them as a new

species. Tenora *et al.* (1990) emphasized the differences in the external morphology of the strobila, the presence/absence of a neck (unsegmented region), the presence of spines on the cirrus and the number of testes. However, the shape of the strobila and prominence of the neck can be strongly affected by the degree of relaxation. Indeed, the holotype of *A. lemmi* appears to be excessively relaxed, which may explain some of the differences mentioned by Tenora *et al.* (1990). Also, the presence of spines on the cirrus is usually not a reliable characteristic, because spines are frequently lost in mounted specimens. The number of testes may prove to be a reliable differentiating feature, but it is usually subject to considerable

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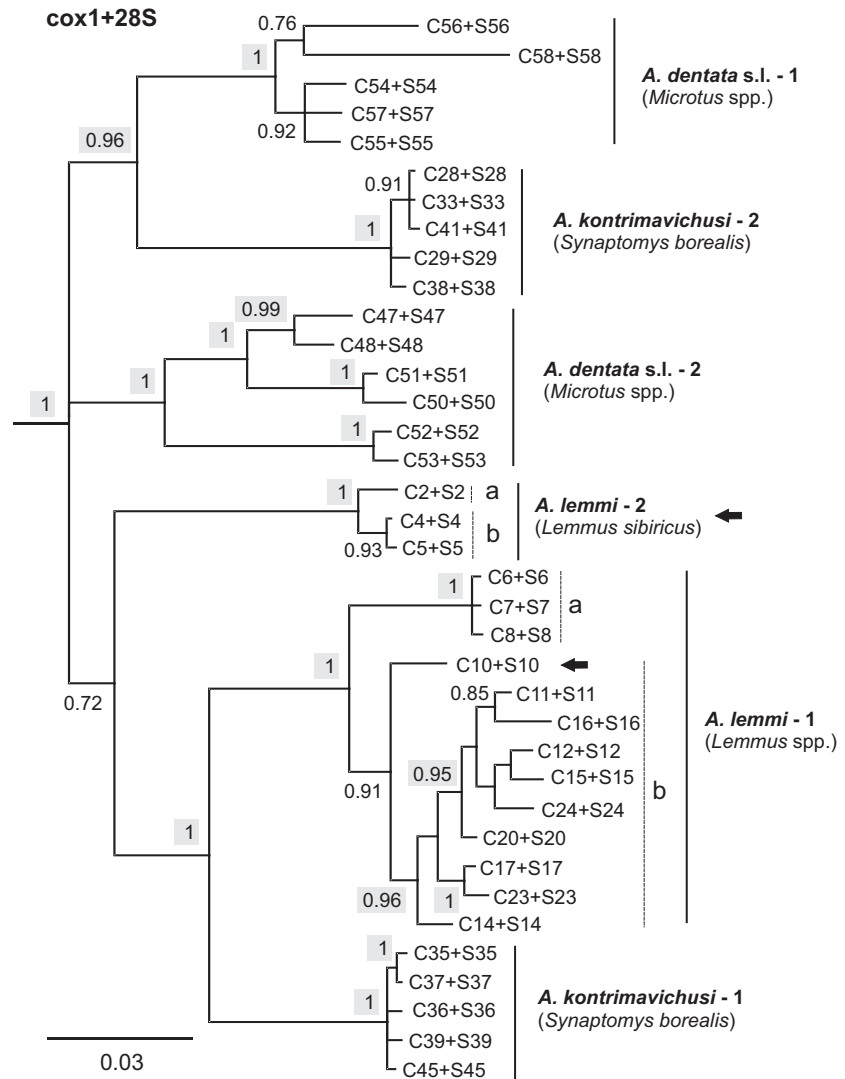


Fig. 5 A Bayesian phylogeny reconstruction of concatenated cytochrome oxidase I (mtDNA) and 28S ribosomal DNA sequences of *Anoplocephaloides* spp. The enigmatic isolate (C1, S1) from the Yamal Peninsula was omitted from the analysis. Arrows indicate isolates and clades that showed different positions in the *cox1* and 28S phylogenies. For sequence codes, see Table 1.

clades of *A. kontrimavichusi* both have an extensive, at least partly overlapping distribution in the north-western North America, and it cannot be determined to which clade the type specimen from Jan Lake belongs.

Although the present study does not include information on the morphology of *A. lemmi* and *A. kontrimavichusi*, the two species were easily identifiable and no obvious morphological differences between the main clades of either species were observed. However, because *A. lemmi* and *A. kontrimavichusi* are both non-monophyletic, the morphological similarity of the two main clades must have developed independently at least in one of the morphospecies (probably in *A. kontrimavichusi*).

Evidently host-induced, convergent patterns of morphological evolution further complicate the morphology-based taxonomy of *Anoplocephaloides* s. s. It has been shown earlier that several phylogenetically well-defined *A. dentata*-like

species in voles do not show sufficient morphological differences for identification and description of new species (Haukisalmi et al. 2009). *Anoplocephaloides dentata*-like species may also manifest convergent evolution, because this morphologically rather uniform assemblage was suggested to be non-monophyletic in the present analysis.

To resolve the taxonomical issues in *A. lemmi* and *A. kontrimavichusi*, extensive morphological analyses based on specimens with known phylogenetic status are naturally required. In an optimal case, (multivariate) morphometrics will be able to distinguish the main clades within each species, which could allow determining indirectly the phylogenetic status of the type specimen of *A. kontrimavichusi* and might subsequently allow describing one of the clades as a new species. For *A. lemmi*, new materials from Siberia are critical, especially from the Yamal Peninsula and elsewhere from West Siberia.

Cophylogeography of *Anoplocephaloides lemmi* and *Lemmus* spp.

The reconstruction of the cophylogeography of *Anoplocephaloides lemmi* and *Lemmus* spp. (Fig. 6) is based on the concatenated tree (*cox1*+28S; Fig. 5). Although the deepest node (*A. lemmi* clade 2 vs. *A. lemmi* clade 1 + *A. kontrimavichusi* clade 1) was not strongly supported (72%) in the concatenated tree, this topology is accepted here, because it was highly supported (100%) in the 28S tree. 28S is a more conservative marker than *cox1* and should better identify deep phylogenetic structure in anoplocephalid cestodes than the latter (Wickström et al. 2005).

It is clear that the cophylogeography does not show simple correspondence between the parasites and the hosts, the main discrepancy concerning the inclusion *A. kontrimavichusi* clade 1 within *A. lemmi*. The obvious implication is that *S. borealis* was colonized by the ancestor of the eastern clade (1) of *A. lemmi* parasitizing *L. trimucronatus*, with a subsequent genetic and morphological divergence of the parasite in a new host. Such host colonization seems plausible, as *S. borealis* and *L. trimucronatus* have extensively overlapping distributions in north-western North America. Episodic expansion and colonization at inter- and intracontinental scales have been fundamental processes serving as determinants of faunal assembly across the Holarctic and within North America (Hoberg et al. 2012).

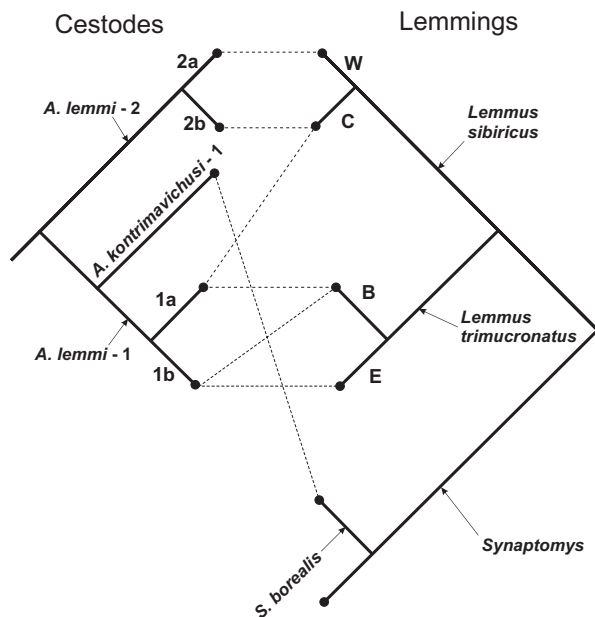


Fig. 6 Schematic phylogenetic relationships and host–parasite associations for the *Anoplocephaloides lemmi* clade and its lemming hosts (*Lemmus* spp. and *Synaptomys borealis*). The phylogenetic relationships of *Lemmus* spp. are based on Fedorov et al. (2003). Capital letters stand for the four sublineages of *Lemmus* spp. (W, western; C, central; B, Beringian; and E, eastern).

However, ignoring the independent colonization event to *S. borealis*, the cophylogeography basically shows two main lineages of *A. lemmi* corresponding to the two main lineages of *Lemmus* (i.e. *L. trimucronatus* and *L. sibiricus*), which suggests a deep codivergence of the host and the parasite. There are also two sublineages in each main clade of *A. lemmi*, which match the sublineages of *L. trimucronatus* and *L. sibiricus*. We assume that all the main phylogenetic splits in the parasite are due to the isolation and divergence of lemmings in different glacial refugia over several glacial cycles (Fedorov et al. 1999, 2003). On the other hand, the current host–parasite associations and geographical distributions of various host and parasite lineages do not show strict correspondence.

Assuming initial codivergence of the hosts and the parasites, the present distribution of *A. lemmi* suggests an extensive westward shift by the clade 1b, crossing the boundary between the two sublineages of *L. trimucronatus* in North America, and further across the Bering Strait, resulting in a wide Holarctic distribution. The East Siberian subclade 1a of *A. lemmi* also seems to have experienced a westward, but a more limited distributional shift across the Kolyma River, which forms the boundary between *L. trimucronatus* and *L. sibiricus*. The absence of the subclade 1a in Alaska can be explained either by the fact that its initial distribution never exceeded north-eastern Siberia or that it was replaced by the easternmost sublineage 1b.

For the eastern clade 1 of *A. lemmi*, other possible but less parsimonious explanations include divergence of parasite clades 1a and 1b within host clade B or within the precursor of B+C, and subsequent colonization of host clade E by parasite subclade 1b (Fig. 7). The latter scenarios also require that 1–2 parasite lineages within host clade E have been lost or have not been found yet. Because the possible colonisations of host clades B and E have not led to genetic divergence in the parasite (subclade 1b), these events are likely to be relatively recent, probably postglacial.

Based on a Holarctic scale phylogeographic analysis of *Lemmus* spp., Fedorov et al. (2003) concluded that the Beringian lemming populations have not acted as a source of postglacial colonization either in the Arctic Eurasia or North America. The Beringian true lemmings (*L. trimucronatus*) formed a well-defined clade (B) occurring on both sides of the Bering Strait without discernible phylogeographic structure. This and the signs of demographic expansion suggested that major distributional changes have occurred relatively recently within clade B of *Lemmus* (Fedorov et al. 2003). Similarly, the evolutionary history of *A. lemmi* does not suggest a major role for Beringia in the postglacial colonization, and in fact, most of Beringia has probably been colonized rather recently by an eastern parasite lineage (1b) originating outside Beringia. The latter

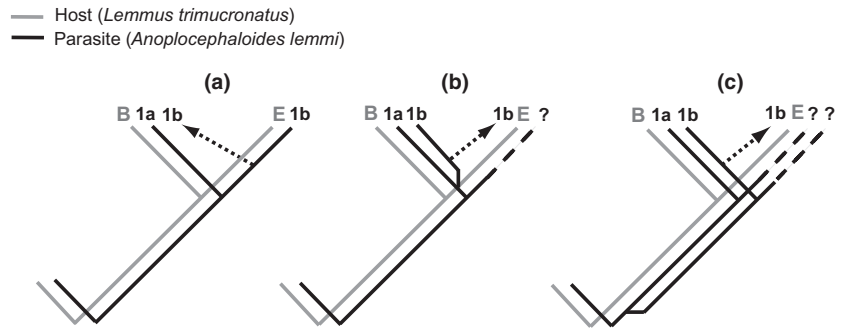


Fig. 7 Three possible reconstructions of the host–parasite cophylogeography in the clade 1 of *A. lemni* in *Lemmus trimucronatus*. For symbols of host and parasite clades, see Fig. 1 and Figs 3–5, respectively. For simplicity, the clade 2 of *A. lemni* and the clade 1 of *A. kontrimavichusi*, and their respective hosts, have been omitted.

parasite lineage has probably ‘overwintered’ south of the main continental ice sheet of North America with its host (Fedorov *et al.* 2003), and its westward range shift has therefore occurred more recently than the supposed westward range expansion of the Beringian *Lemmus* clade B, which is supposed to have ‘overwintered’ in eastern Beringia.

The cophylogeography of another Holarctic, host-specific anoplocephalid tapeworm (*Rauschoides arctica*, syn. *Paranoplocephala arctica*) and its hosts (collared lemmings, *Dicrostonyx* spp.) (see Wickström *et al.* 2003) shows similarities with the *A. lemni*–*Lemmus* system. *Rauschoides arctica* shows deep codivergence with *Dicrostonyx*, the main division of both being located at the Bering Strait (separating the western *Dicrostonyx torquatus* and eastern *D. groenlandicus*). Also, the secondary divisions in Eurasia, at the Lena River, are congruent between the host and the parasite and also correspond to the probable location of the split in *A. lemni* and its host (*L. sibiricus*). However, in North America, the phylogeny of *R. arctica* does not show correspondence to host relationships and lacks strict congruence between phylogeny and geography, particularly in the Canadian Arctic. In addition, the parasite lineage on the Wrangel Island represents the Siberian clade of *R. arctica*, although this island is inhabited by the eastern host species (*D. groenlandicus*). These incongruences were interpreted to indicate extensive and complex postglacial colonization from at least two glacial refugia, one in Beringia (Alaska) and another in north-eastern Canadian Arctic (Wickström *et al.* 2003).

The correspondence between the cophylogeographies of these two host–parasite systems suggests that the deep phylogeographic patterns of host-specific cestodes of Arctic lemmings are generally determined by the divergence and subsequent allopatry of their respective hosts. Both host and parasite systems also suggest existence of two major glacial refugia in North America, of which Beringia is shared between the two systems, and extensive postglacial colonization, which has not been determined by the

expansion and distribution of the host clades only and which differs between the two parasite species. Although it is obvious that the utilization of different eastern glacial refugia (south and north-west of the continental ice, respectively) has shaped the postglacial colonization patterns of the two parasites in North America, it is unclear why we see a westward expansion in *A. lemni*, whereas there is indication of extensive eastward colonization(s) from the Beringian refugium in *R. arctica*. The latter colonisations have been suggested to reflect cryptic host divergence in *Dicrostonyx* (Wickström *et al.* 2003), but a similar explanation does not appear to fit the supposed colonization pattern of *A. lemni*. It is also unclear why we see congruence among a set of hosts and parasites in Eurasia but extensive incongruence in North America, although there are big rivers on both continents that have evidently acted as efficient, shared colonization barriers for lemmings. Eurasia and North America differed with respect to the extent of continental ice during the Pleistocene and probably also during the previous glaciations (glaciations were much more extensive in North America), but it does not seem to explain why the parasites of lemmings have had partly independent evolutionary histories in the latter continent. The degree to which histories for lemmings and this assemblage of anoplocephalines are idiosyncratic can be revealed in a broader context for rodent cestode faunas with respect to shifts in climate and habitat that characterized the Quaternary (e.g. Waltari *et al.* 2007; Hoberg *et al.* 2012).

The tundra vole (*Microtus oeconomus*) is a Holarctic species which shows distinct phylogeographic breaks at the Kolyma/Omolon Rivers, Lena River and Ural Mountains, probably caused by isolation in different glacial refugia (Brunhoff *et al.* 2003; Galbreath & Cook 2004). There are two common Holarctic anoplocephalid cestodes (*Anoplocephaloides* cf. *dentata* and *Paranoplocephala jarrelli*) that show preference for the tundra vole, but often parasitize other congeneric host species and even *Myodes* voles (but not lemmings). Although *A.* cf. *dentata* and *P. jarrelli* show

multiple well-supported subclades, the main phylogeographic splits in *M. oeconomus* are not reflected in the phylogeography of the parasites (Haukisalmi et al. 2004, 2009). Both species also show distinct subclades in Beringia, a region where the host does not show phylogeographic structure. In addition, other *Paranoplocephala* species of voles (with relatively flexible host selection) have not tracked the main, shared phylogeographic split of their hosts in Europe (Wickström 2004). Such host flexibility or a capacity to use otherwise conserved host resources in a broad sloppy fitness space is consistent with a role for ecological fitting in establishing the potential for host switching (e.g. Agosta et al. 2010). Opportunity for host and geographical colonization reflects the interaction between episodic climate change and habitat perturbation over the past 2–3my (Hoberg & Brooks 2008; Hoberg et al. 2012).

Because the examined parasites of lemmings and voles are closely related, the observed differences are probably not due to phylogenetic bias. In addition, there are evidently no differences in mobility of the definitive and intermediate hosts that could account for the observed differences in cophylogeography (intermediate hosts of anoplocephalid cestodes of rodents are usually oribatid mites; e.g. Gleason & Buckner 1979). Our conclusion is that the difference in host specificity is the most probable reason for the contrasting phylogeographic patterns in anoplocephalid cestodes of lemmings and voles. Neither of the lemming cestodes has been found from other sympatric hosts, although *Lemmus* and *Dicrostonyx* are widely overlapping with each other and with northern species of *Microtus* and *Myodes* voles throughout the Holarctic. The parasites with flexible host selection are more likely to cross various intra- and interspecific borders of host populations, whereas strictly host-specific parasites are more likely to track phylogeography of their hosts. Indeed, the best examples of strict cophylogeny and cophylogeography come from host-specific ecto- and endoparasites of rodents (Hafner & Page 1995; Nieberding et al. 2004), but even the former has been misinterpreted and in reality represents a history of episodic host switching (Brooks et al. 2015).

Overall, the present study provides an example of host–parasite codivergence on the Holarctic scale, the main distributional incongruence being explained by a relatively recent range shift in the parasite independently of the host. Comparison with other Holarctic host–parasite systems suggests that host specificity may be one of the main determinants of phylogeographic correspondence or lack thereof. Postglacial colonisations also differ between the cestodes of lemmings, suggesting that stochastic or other undefined factors have also played a role in the recent range shifts of Arctic parasites, contributing to the larger

picture of assembly of faunal mosaics across the Holarctic in space and time.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Whole-mount voucher specimens of *Anoplocephaloides lemmi*, *A. kontrimavichusi* and *A. bulmeri* deposited in the Finnish Museum of Natural History, Helsinki (MZH), United States National Parasite Collection, Maryland (USNPC) and Museum of Southwestern Biology, Albuquerque (MSB).