Molecular systematics and morphometrics of *Anoplocephaloides dentata* (Cestoda, Anoplocephalidae) and related species in voles and lemmings

Voitto Haukisalmi, Lotta M. Hardman, Heikki Henttonen, Juha Laakkonen, Jukka Niemimaa, Michael Hardman & András Gubányi

Submitted: 12 June 2008
Accepted: 25 August 2008
doi:10.1111/j.1463-6409.2008.00363.x

Haukisalmi, V., Hardman, L. M., Henttonen, H., Laakkonen, J., Niemimaa, J., Hardman, M. & Gubányi, A. (2008). Molecular systematics and morphometrics of *Anoplocephaloides dentata* (Cestoda, Anoplocephalidae) and related species in voles and lemmings. — *Zoologica Scripta*, *38*, 199–220.

This study presents extensive molecular phylogenetic and morphometric data for *Anoplocephaloides dentata* (Galli-Valerio, 1905)-like cestodes (Anoplocephalidae) and related species parasitizing arvicoline rodents (voles and lemmings) in the Holarctic region. The molecular phylogeny is based on nucleotide sequences of cytochrome oxidase I (mtDNA) and 28S ribosomal RNA. *Anoplocephaloides dentata*-like cestodes included three main clades, two in western Eurasia and one in the Holarctic region (excluding western Eurasia). Three well-supported sublineages were included in the southern European clade, one of which represents the true *A. dentata* from *Chionomys nivalis* and sympatric *Microtus arvalis* and *Dinaromys bogdanovi*. These clades generally had non-overlapping distributions and showed a preference for certain host species. Multivariate analysis of morphometric data failed to discriminate unambiguously the various *A. dentata*-like lineages recovered in the molecular phylogeny, although two to three of the (sub)lineages were morphologically divergent. The overall evidence suggests, however, that instead of a single host-generalist species there are at least five more or less host-specific species of *A. dentata*-like cestodes. Colonization of new host lineages seems to have been the predominant mode of diversification, suggested by the considerable incongruence between host and parasite phylogenies at multiple taxonomic levels. Based on the results of the molecular survey, a redescription and neotype designation are provided for *A. dentata*.

Corresponding author: *Voitto Haukisalmi*, *Finnish Forest Research Institute*, *Vantaa Research Unit*, *PO Box 18*, *FI-01301 Vantaa*, *Finland. E-mail: voitto.haukisalmi@metla.fi*
Lotta M. Hardman, *Heikki Henttonen*, *Jukka Niemimaa*, *Michael Hardman*, *Finnish Forest Research Institute*, *Vantaa Research Unit*, *PO Box 18*, *FI-01301 Vantaa*, *Finland. E-mails: lotta.hardman@metla.fi*, *heikki.henttonen@metla.fi*, *jukka.niemimaa@metla.fi*, *m.hardman@mac.com*
Juha Laakkonen, *Division of Anatomy*, *Department of Basic Veterinary Sciences*, *Faculty of Veterinary Medicine*, *PO Box 66*, *00014 University of Helsinki*, *Finland. E-mail: juha.laakkonen@helsinki.fi*
András Gubányi, *Department of Zoology*, *Hungarian Natural History Museum*, *Budapest 1088*, *Baross u. 13*, *Hungary. E-mail: gubanyi@mail.zoo.nhmus.hu*

Introduction

The physical environment of intestinal parasites is fairly homogeneous and offers few opportunities for morphological adaptation and specialization. Recent molecular approaches have revealed a structured diversity in various endoparasites and have led to numerous and fairly radical changes in systematic concepts of these groups. For example, anoplocephalid cestodes assumed to represent a single host-generalist species actually consisted of several largely host-specific, more or less cryptic species (Haukisalmi *et al*. 2004; Hu *et al*. 2005; Beveridge *et al*. 2007; Haukisalmi *et al*. 2008). Cryptic anoplocephaline species, once identified through their genetic signatures have been successfully diagnosed according to morphometric characters and using multivariate methods (Haukisalmi *et al*. 2004). In this way, the marriage of molecular heuristics and multivariate morphometrics is providing a more accurate view of parasite diversity within a phylogenetic context.

Cestodes representing the genus *Anoplocephaloides* Baer, 1923 s. str. (Anoplocephalidae) are ubiquitous parasites of voles, lemmings (Cricetidae: Arvicolinae) and pocket gophers (Geomyidae) in Eurasia and North America. Species of *Anoplocephaloides* s. str. are characterized by a short and wide ('wedge-shaped') strobila, a prominent scolex and all have a similar internal morphology (Rausch 1976). In addition, they inhabit the same microhabitat, that is, the terminal ileum, ileo–caecal junction and caecum, in which respect they differ from other *Anoplocephaloides* species and anoplocephalids of rodents that inhabit the small intestine proper; often the duodenum or jejunum (Haukisalmi *et al*. 1998).

Traditionally, all *Anoplocephaloides dentata* (Galli-Valerio, 1905)-like cestodes parasitizing *Microtus* spp. and *Chionomys nivalis* in continental Eurasia, including its junior synonym *Paranoplocephala brevis* Kirshenblat, 1938, were considered a single species (Rausch 1976; Tenora & Murai 1980; Genov & Georgiev 1988). However, molecular phylogenetic analyses suggested that cestodes identifiable as *A. dentata* included multiple phylogenetic lineages each of which may represent an independent species (Wickström *et al*. 2005; Haukisalmi *et al*. 2008). Although those studies did not include the type species *A. infrequens* (Douthitt, 1915) from Nearctic pocket gophers, it is apparently indistinguishable from the other *A. dentata*-like species (Rausch 1976; Genov & Georgiev 1988) and probably belongs to the same clade.

Within *Anoplocephaloides* s. str., the two species inhabiting lemmings, *Anoplocephaloides lemmi* (Rausch, 1952) and *Anoplocephaloides kontrimavichusi* Rausch, 1976 in *Lemmus* spp. and *Synaptomys borealis*, respectively, are morphologically well-differentiated from each other and from all *A. dentata*-like taxa (Rausch 1976). However, the latter assemblage, including cestodes from the snow vole *C. nivalis*, *Microtus* voles and pocket gophers, is believed to be morphologically uniform although a comprehensive study of morphological variation is lacking.

This study presents molecular phylogenetic data for *A. dentata*-like cestodes in the Holarctic and defines the main phylogenetic lineages and their relationships within this apparently cryptic species complex. Morphological differences observed between the recovered lineages (i.e. putative species) are examined using multivariate methods. In order to help clarify the systematics of this group, *A. dentata* is redescribed and a neotype is designated.

Materials and methods

Rodents and cestodes

Most of the rodent and cestode material from Europe, Turkey, Kazakhstan and the Republic of Buryatia (Russian Federation) were collected by H. Henttonen, J. Laakkonen and J. Niemimaa with the help of local collaborators, usually in connection with various projects on Hantaviruses and other rodent-borne zoonoses. Most of the specimens from north-eastern Siberia, Alaska and western Yukon were obtained through the Beringian Coevolution Project (Hoberg *et al*. 2003; Cook *et al*. 2005). For study localities and collectors, see Appendix.

The voles and lemmings were usually dissected for helminths immediately after trapping although frozen hosts or their intestines were used from Finnish and Swedish sources. Cestodes were washed and relaxed in water, fixed flat (without pressure) in 70% ethanol and tissue samples (usually postmature and/or pregravid proglottids) were later taken for DNA-extraction. For morphometrics, cestodes were stained with Mayer's haemalum, Semichon's aceto-carmine or iron aceto-carmine, cleared in eugenol and mounted in Canada balsam. Voucher specimens (whole-mounts) of each putative *A. dentata*-like species and the neotype of *A. dentata* were deposited in the United States National Parasite Collection, Maryland (USNPC), the Museum of Southwestern Biology, Albuquerque, New Mexico (MSB) and the Hungarian Natural History Museum, Budapest (HNHM) (Table 1).

Molecular analysis

Eighty-eight specimens identified as *A. dentata* were sequenced for the partial mitochondrial cytochrome c oxidase I (COI) gene, which has proved to be an excellent heuristic tool in anoplocephaline systematics (Haukisalmi *et al*. 2004; Beveridge *et al*. 2007; Haukisalmi *et al*. 2008). These specimens originated from eight species of *Microtus*, *C. nivalis* and *Myodes rufocanus* from several localities in Eurasia (mainly Europe), Alaska and adjacent regions in north-western North America, and two localities in south-western North America (California and New Mexico) (Fig. 1). In addition, the COI gene was sequenced from five specimens each of *A. lemmi* and *A. kontrimavichusi* to complete the taxonomic sampling of *Anoplocephaloides* s. str. (Wickström *et al*. 2005; Haukisalmi *et al*. 2008). A smaller sample of *A. dentata* ($N = 32$) and 2 + 2 specimens of *A. lemmi* and *A. kontrimavichusi* was sequenced for the 28S ribosomal RNA. Most of these specimens were also sequenced for COI and therefore represent the same host association and distribution (Fig. 1) although we were unsuccessful in sequencing 28S in specimens from California and Turkey.

Seventy-five haplotypes of COI and 17 haplotypes of 28S were recovered and added to existing sequence data sets of related anoplocephaline species including *Microcephaloides* spp. (= *A. variabilis* (Douthitt, 1915) and related species) and *Paranoplocephala* spp., all from rodents, and *Andrya rhopalocephala* (Riehm, 1881) and *Neandrya cuniculi* (Blanchard, 1891) from lagomorphs (Wickström *et al*. 2005; Haukisalmi *et al*. 2008). Following the results of Wickström *et al*. (2005), two species of *Anoplocephala* Blanchard, 1848 from equids and three species of *Moniezia* Blanchard, 1891 from ruminants

Table 1 Specimens of *Anoplocephaloides dentata*-like cestodes used in the morphometric analysis. Locality numbers (in parentheses) refer to Fig. 1. N, number of specimens examined. For collectors, see Appendix.

DNA clade	Host species	Locality (no.)	*N*	Parasite accession numbers
Clade 1a (*N* = 24),				
A. dentata	*C. nivalis*	Monte Bondone, Trento, Italy (3)	8	USNPC 95383, USNPC 95646, MSB Endo 85
	C. nivalis	Bourg-Saint-Maurice, France (2)	6	USNPC 97609
	C. nivalis	Mt. Lafenberg, Austria (4)	1	MSB Endo 88
	C. nivalis	Pirin Mts, Bulgaria (7)	1	MSB Endo 86
	C. nivalis	Sator Mt., Bosnia (6)	2	MSB Endo 87
	D. bogdanovi	Sator Mt., Bosnia (6)	1	—
	M. arvalis	Monte Bondone, Trento, Italy (3)	5	USNPC 97610
Clade 1b (*N* = 6)	*M. guentheri*	Bozdag, Turkey (8)	6	USNPC 97611, MSB Endo 94–96
Clade 1c (*N* = 22)	*M. arvalis*	Taldygorgan, Kazakhstan (30)	10	USNPC 95647, MSB Endo 89–92
	M. arvalis	Novo Granica, Croatia (5)	2	MSB Endo 93
	M. arvalis	Rabaköz and Hansag, Hungary (9)	10	HNHM 11914, 11916, 11932, 11935, 11942, 11947
Clade 2 (*N* = 31)	*M. oeconomus*	Pallasjärvi, Finland (21)	9	USNPC 97612
	M. oeconomus	Kilpisjärvi, Finland (20)	4	USNPC 97616
	M. agrestis	Lammi and Heinola, Finland (25)	5	USNPC 95648, 97613
	M. agrestis	Kilpisjärvi, Finland (20)	9	USNPC 97614
	M. agrestis	Aberdeen, Scotland (11)	4	USNPC 97617
Clade 3 (*N* = 43)	*M. miurus*	GAAR, Alaska (42,43)	10	USNPC 95649, 97618, 97619, 97623, MSB Endo 123–129
	M. miurus	Toolik Lake, Alaska (44)	4	USNPC 97624
	M. miurus	Cape Krusenstern NM, Alaska (40)	2	MSB Endo 119, 120
	M. oeconomus	Toolik Lake, Alaska (44)	4	USNPC 97620
	M. oeconomus	GAAR, Alaska (42,43)	3	USNPC 97625, MSB Endo 130, 131, 133
	M. oeconomus	YUCH, Alaska (47,48)	1	USNPC 97621
	M. oeconomus	Lake Clark NPP, Alaska (51)	2	MSB Endo 117, 118
	M. oeconomus	Kenai Fjords NP, Alaska (52)	1	MSB Endo 121, 122
	M. oeconomus	Yttygran Island, Chukotka, Russia (39)	1	USNPC 97626
	M. oeconomus	Chaunsk Gulf, Chukotka (38)	3	MSB Endo 139–143
	M. oeconomus	Markovo, Vakareva River (37)	2	MSB Endo 151–152
	M. pennsylvanicus	WRST, Alaska (50)	2	USNPC 97627, MSB Endo 137, 138
	M. pennsylvanicus	Tanana River, Alaska (46)	1	USNPC 97628
	M. pennsylvanicus	YUCH, Alaska (47,48)	2	USNPC 97629, MSB Endo 134–136
	M. pennsylvanicus	Stikine River, British Columbia (53)	1	MSB Endo 116
	M. fortis	Nesteriha, Buryatia, Russia (33)	4	USNPC 97630, MSB 115, 153
'Clade' 4 (*N* = 14)	*M. xanthognathus*	Tetlin NWR, Alaska (49)	8	MSB Endo 105–113
	M. xanthognathus	Tanana River, Alaska (46)	6	MSB Endo 97–104

GAAR, Gates of the Arctic National Park and Preserve. YUCH, Yukon-Charley Rivers National Preserve. WRST, Wrangel-St.Elias National Park and Preserve. NM, National Monument. NP, National Park. NPP, National Park and Preserve. NWR, National Wildlife Refuge.

were used as outgroup for COI, and *A. rhopalocephala* and *N. cuniculi* served as outgroup for the 28S analysis.

For the extraction, amplification and sequencing of COI and 28S see Wickström *et al.* (2003, 2005) and Haukisalmi *et al.* (2004). Sequences were assembled and edited using Align IR^{TM} Sequence Assembly and Alignment Software (LI-COR Inc., Lincoln, NE) and aligned in ClustalW (Thompson *et al.* 1994) with default gap penalties. GenBank numbers for the COI and 28S sequences are given in the Appendix.

Phylogenetic relationships were reconstructed using the Bayesian approach (Huelsenbeck *et al.* 2001) implemented in the program MrBayes v.3.1 (Ronquist & Huelsenbeck 2003). Modeltest (Posada & Crandall 1998) supported the application of the GTR + I + Γ model to first, second and third codon positions of COI separately. GTR + I + Γ was also applied to the 28S data analysed as a single partition. All parameters were unlinked and 3 million (COI) and 4 million generations (28S) were sampled every 1000th and 50% majority-rule consensus trees were computed of the postburnin samples. Of both COI and 28S two independent runs were compared to confirm that the likelihood plateau represented a real rather than local optimum particular to

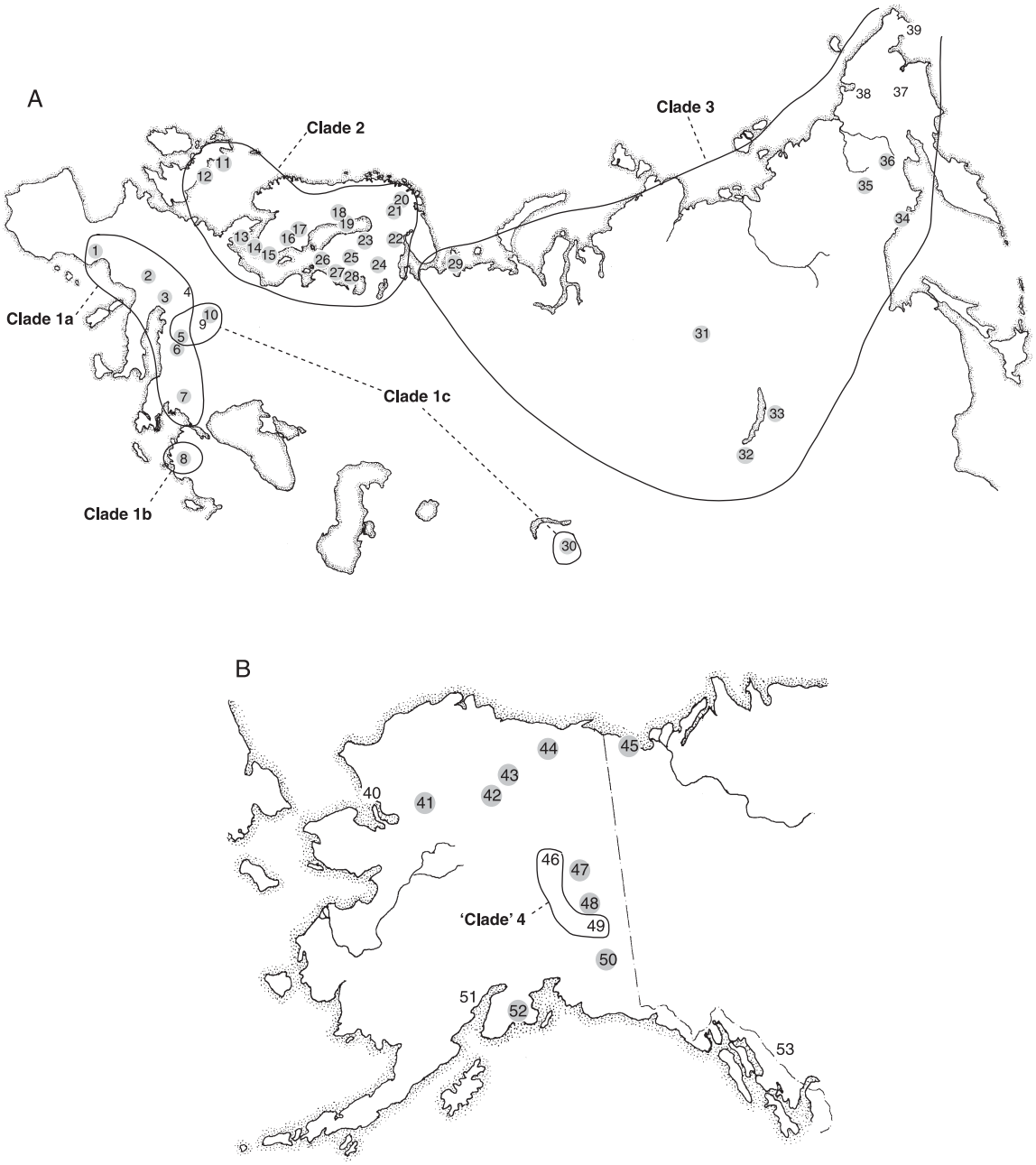

Fig. 1 A, B. Geographic origin of cestodes and distribution of *Anoplocephaloides dentata*-like clades in Eurasia (A) and north-western North America (B). See Table 1 and Appendix for locality names. Specimens used in the molecular phylogenetic analysis are from localities indicated by shaded circles.

one of the two runs. Support for nodes were expressed as posterior probabilities.

Morphometrics

Anoplocephaloides dentata-like cestodes do not have a proper neck (unsegmented region), but there is always a more or less distinct constriction immediately posterior to scolex. The length of the scolex was measured from the anterior tip of the scolex to the level of the constriction. The various internal organs were measured and counted only from specimens in which they were clearly visible; the testes, in particular, were often poorly visible. Because of the shortness of the body and rapid development of proglottids, internal organs were measured and counted from a single proglottid from each

strobila that we considered to represent the same stage. This proglottid is called here the last mature one because the vitellarium and ovary reach their maximum size and subsequently shrink and show clear signs of disintegration in the next. In the examined proglottid, the uterus was a narrow tube in the anterior part of the proglottid but expanded considerably and became filled with eggs in the subsequent proglottid. In addition, the internal seminal vesicle was usually small and empty in the examined proglottid, starting to expand and fill with sperm in the next proglottid.

Replicate measurements were recorded for the diameter of suckers (usually four measurements per individual) and egg length (5–10 measurements per individual). In these cases, the median value of the replicate measurements was used in statistical analyses. The eggs were measured from the terminal, fully gravid proglottid only. Standardization of egg measurements is crucial because the fully developed, thick-walled eggs in the terminal proglottid are consistently *smaller* than the thin-walled ones in the preceding gravid proglottids.

Because the cirrus sac and seminal receptacle continue to increase markedly in size after the last mature proglottid, only their maximum dimensions in each strobila were recorded. The seminal receptacle was measured only if it had reached the typical 'capsular' form, that is, an elongate, expanded structure with distinct walls filling most of the space between the transverse osmoregulatory canals (Fig. 8). Alternatively, the seminal receptacle appeared as a thinner, proximally tapering and less voluminous sac with poorly visible walls even when filled with sperm.

The asymmetrical position of the vitellarium ('index of asymmetry') was quantified as the ratio of the poral distance (measured from the midpoint of the vitellarium to the poral margin of the proglottid) to the corresponding proglottid width. Because it has been previously suggested that the distribution of testes with respect to ovary and vitellarium may provide a key feature for distinguishing between various *A. dentata*-like species (Rausch 1976), we compared the extent of median testes among the six clades separately (Table 3). Fewer ($N = 106$) and partly different specimens were used in this comparison than in the main morphometric analysis.

Statistical analysis

The morphometric analysis was performed on 140 fully gravid specimens of *A. dentata*-like cestodes. All statistical analyses were performed with SPSS® for Windows® vs. 16. Variables that could be obtained from most specimens were used in the morphometric analysis (Table 2). Although the relative measurements are expressed as ratios in Table 2 and in the redescription of *A. dentata*, the statistical testing of the corresponding relationships was based on standardized residuals, obtained from a linear regression of an absolute dimension on the corresponding body, scolex or proglottid width. Residuals, which represent morphometric measures controlled for the varying size of the body, scolex or proglottid are better suited for statistical testing than ratios.

Because many of the morphometric variables were strongly correlated with each other, we performed a principal component analyses (PCA) for a selected set (Table 1) of absolute and relative measurements (residuals). In order to test the power of the selected morphometric variables to correctly classify the observed DNA clades of *A. dentata*, we also performed a set of discriminant analyses. Because of the correlation among variables, an additional discriminant analysis was performed for the six (uncorrelated) principal components produced by the PCA. The canonical discriminant functions were calculated using the variables that best separated the study groups selected by a forward stepwise procedure and using minimization of Wilk's lambda as selection criterion. 'Jack-knifing' was used to evaluate the success of the discriminant functions to correctly classify specimens, that is, each specimen was classified by the discriminant functions derived from all other specimens.

Results

Phylogeny

Aligned COI sequences of *Anoplocephaloides* s. str. (hereafter called the ingroup) contained no indels and had an average length of 605 bp (median = 617 bp, minimum = 514 bp, maximum = 625 bp). The ingroup contained 164 parsimony-informative characters of which 35 were found among first, 8 among second and 168 among third codon position. The 28S ingroup sequences had an average length of 1390 (median = 1390 bp, minimum 1357 bp, maximum = 1407 bp). Indels in the 28S data set were easily aligned and the total alignment of 1452 bp contained 20 parsimony-informative characters among the ingroup (Table 4).

The Bayesian analyses converged independently according to PSRF (potential scale reduction factor) convergence diagnostics and the marginal likelihoods of the two runs were overlapping (COI: –ln8229, 8230 arithmetic means, –ln8306, 8325 harmonic means; 28S: –ln4176, 4177 arithmetic means, –ln4219, 4218 harmonic means). The majority-rule consensus phylograms were based on trees from both runs (4598 post burnin trees sampled for COI, 6538 post burnin for 28S).

In the COI phylogram (Fig. 2), the *A. dentata*-like cestodes appeared as three main, highly supported (posterior $P \geq 0.98$) monophyletic groups (clades 1–3, excluding haplotype C21). Clades 1 and 2 appeared to form a derived clade with a European/western Eurasian distribution, whereas clade 3 had a wide Holarctic distribution but being absent in western Eurasia (Fig. 1). Within clade 1, there were three supported crown clades (subclades 1a, 1b and 1c), which had largely non-overlapping distributions and were primarily associated with different host species.

Table 2 Absolute and relative measurements for the six clades of *Anoplocephaloides dentata*-like cestodes. All measurements in mm, except the egg length (in μm). N, number of specimens examined. *n*, number of measurements.

	Clade 1a	Clade 1b	Clade 1c	Clade 2	Clade 3	'Clade' 4
	A. dentata					
Morphometric variables	*N* = 24	*N* = 6	*N* = 22	*N* = 31	*N* = 43	*N* = 14
Proglottids, number (PN)†	26–52 (39.1), *n* = 22	22–35 (28.6), *n* = 5	33–53 (42.9), *n* = 20	29–62 (47.1), *n* = 31	32–54 (43.4), *n* = 43	31–59 (43.1), *n* = 9
Body, length (BL)†	5.5–12.9 (9.5), *n* = 22	6.2–8.5 (7.3), *n* = 5	6.0–12.2 (8.9), *n* = 20	6.7–16.1 (11.6), *n* = 31	7.8–14.5 (11.0), *n* = 43	5.2–12.0 (7.5), *n* = 9
Body, maximum width (BW)†	2.3–4.8 (9.5), *n* = 22	3.8–4.5 (4.0), *n* = 6	2.4–4.1 (3.1), *n* = 21	2.3–4.7 (3.5), *n* = 31	2.5–5.0 (3.6), *n* = 43	1.7–3.4 (2.5), *n* = 10
Scolex, width (SCW)†	0.9–1.5 (1.1), *n* = 24	1.1–1.4 (1.3), *n* = 6	0.7–1.1 (0.9), *n* = 22	0.9–1.4 (1.1), *n* = 31	0.9–1.3 (1.1), *n* = 43	0.8–1.0 (0.9), *n* = 12
Scolex, length (SCL)†	0.52–1.10 (0.83), *n* = 23	0.70–0.85 (0.78), *n* = 6	0.42–0.76 (0.56) *n* = 20	0.58–0.95 (0.71), *n* = 25	0.46–1.0 (0.71), *n* = 33	0.45–0.55 (0.52), *n* = 10
Suckers, diameter (SU)†	0.33–0.45 (0.39), *n* = 24	0.43–0.47 (0.45), *n* = 6	0.32–0.42 (0.37), *n* = 22	0.34–0.47 (0.40), *n* = 31	0.35–0.47 (0.40), *n* = 43	0.32–0.37 (0.34), *n* = 11
Neck, minimum width (NW)†	0.4–1.1 (0.85), *n* = 24	0.9–1.3 (1.15), *n* = 6	0.4–0.9 (0.65), *n* = 22	0.5–1.2 (0.81), *n* = 31	0.5–1.0 (0.81), *n* = 42	0.4–0.6 (0.52), *n* = 11
Testes, number (TN)	27–63 (42.4), *n* = 11	38–67 (53.0), *n* = 5	31–46 (41.0), *n* = 7	37–56 (47.6), *n* = 16	28–60 (42.8), *n* = 20	29–53 (38.3), *n* = 9
Testicular field, width (TW)	0.53–1.20 (0.92), *n* = 11	0.92–1.32 (1.15), *n* = 5	0.60–0.98 (0.85), *n* = 7	0.6–1.4 (0.84), *n* = 16	0.42–1.43 (0.82), *n* = 21	0.27-0-70 (0.44), *n* = 9
Cirrus sac, maximum length (CS)†	0.30–0.48 (0.38), *n* = 24	0.33–0.39 (0.37), *n* = 6	0.20–0.39 (0.28), *n* = 22	0.23–0.38 (0.30), *n* = 31	0.25–0.48 (0.38), *n* = 43	0.25–0.30 (0.27), *n* = 14
Vitellarium, width (VI)	0.20–0.41 (0.33), *n* = 11	0.28–0.34 (0.31), *n* = 5	0.22–0.32 (0.27), *n* = 7	0.23–0.34 (0.26), *n* = 16	0.21–0.40 (0.30), *n* = 23	0.22–0.35 (0.28), *n* = 9
Ovary, width (OV)	0.53–0.89 (0.73), *n* = 11	0.78–0.95 (0.84), *n* = 5	0.55–0.80 (0.72), *n* = 7	0.52–0.94 (0.72), *n* = 16	0.54–0.91 (0.75), *n* = 23	0.45–0.83 (0.64), *n* = 8
Seminal receptacle, maximum length (SR)	0.43–0.61 (0.55), *n* = 5	0.64–0.70, *n* = 3	0.47–0.68 (0.59), *n* = 6	0.45–0.70 (0.57), *n* = 14	0.27–0.88 (0.52), *n* = 14	0.23–0.38 (0.29), *n* = 9
Egg, length†	38–50 (43.5), *n* = 24	38–42 (0.393), *n* = 6	38–48 (43.4), *n* = 22	37–49 (43.1), *n* = 31	30–59 (43.3), *n* = 43	27–37 (31.4), *n* = 14
BL/BW†	1.7–3.0 (2.45), *n* = 22	1.6–1.9 (1.81), *n* = 5	2.4–4.0 (2.91), *n* = 20	2.5–4.7 (3.27), *n* = 31	2.16–4.23 (3.07), *n* = 43	2.5–4.3 (3.16), *n* = 9
SU/SCW†	0.27–0.45 (0.37), *n* = 24	0.32–0.42 (0.35), *n* = 6	0.35–0.47 (0.40), *n* = 22	0.28–0.46 (0.38), *n* = 31	0.31–0.43 (0.38), *n* = 43	0.36–0.44 (0.40), *n* = 11
SCL/SCW†	0.55–0.94 (0.76), *n* = 23	0.53–0.72 (0.60), *n* = 6	0.51–0.71 (0.60), *n* = 20	0.50–0.83 (0.67), *n* = 31	0.44–0.87 (0.67), *n* = 33	0.52–0.66 (0.60), *n* = 10
NW/SCW†	0.45–0.97 (0.79), *n* = 24	0.77–0.99 (0.89), *n* = 6	0.45–0.89 (0.69), *n* = 22	0.54–0.96 (0.76), *n* = 31	0.46–0.94 (0.75), *n* = 42	0.48–0.72 (0.60), *n* = 11
CS/BW†	0.08–0.12 (0.099), *n* = 23	0.09–0.10 (0.091), *n* = 6	0.08–0.12 (0.090), *n* = 21	0.07–0.12 (0.085), *n* = 31	0.09–0.13 (0.105), *n* = 43	0.08–0.15 (0.116), *n* = 10
Index of asymmetry*†	0.29–0.47 (0.36), *n* = 23	0.34–0.40 (0.37), *n* = 6	0.32–0.41 (0.35), *n* = 22	0.30–0.43 (0.38), *n* = 31	0.32–0.44 (0.36), *n* = 42	0.35–0.44 (0.39), *n* = 14

*Poral distance of vitellarium/proglottid width (see Materials and methods). †Variables used in the multivariate morphometric analyses (i.e. those available for most of the specimens).

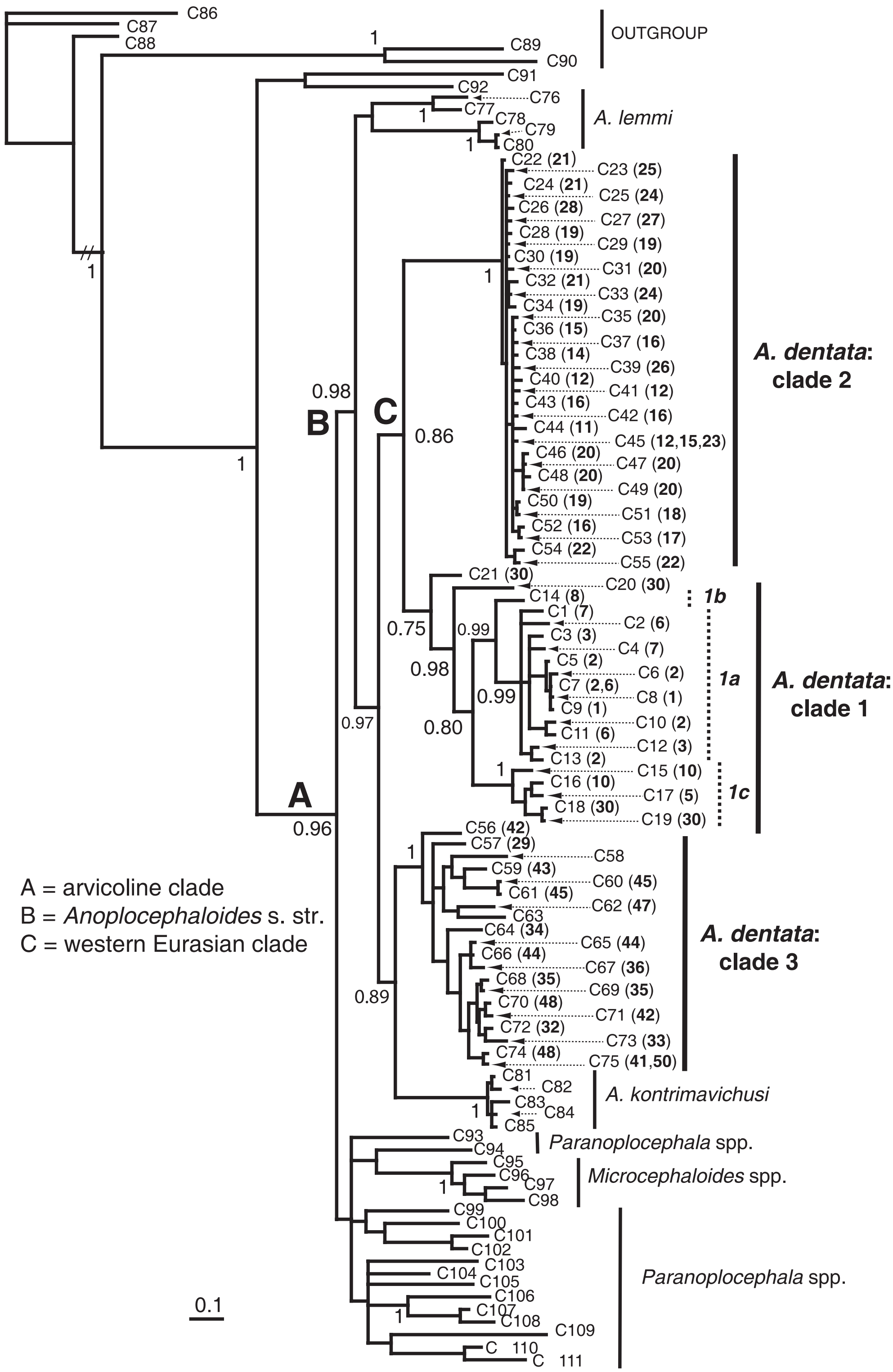

Fig. 2 Bayesian reconstruction of partial cytochrome oxidase I (mtDNA) sequences of *Anoplocephaloides* spp. and related species from rodents. *Moniezia* spp. from ruminants and *Anoplocephala* spp. from equids were used as outgroup. Labels starting with 'C' show the haplotypes; see Appendix. Locality numbers in parentheses (in bold) after the haplotype label; see Figure 1. Values at nodes show the posterior probabilities. Scale bar indicates inferred number of changes per site.

Table 3 Extent of median testes in the six DNA clades of *Anoplocephaloides dentata*-like cestodes. Each specimen is represented by a single, typical character state determined from last mature proglottid.

Clade/species	Overlapping ovary	In contact with vitellarium	Overlapping vitellarium
Clade 1a, *A. dentata* (*n* = 16)	6 (37.5%)	5 (31%)	5 (31%)
Clade 1b (*n* = 6)	2	2	2
Clade 1c (*n* = 7)	3	4	—
Clade 2 (*n* = 38)	15 (39%)	18 (47%)	5 (13%)
Clade 3 (*n* = 30)	4 (13%)	17 (57%)	9 (30%)
'Clade' 4 (*n* = 9)	9	—	—

Subclade 1a, representing the true *A. dentata*, was found primarily from *C. nivalis* in four different mountain ranges in southern Europe (Alps in France and Italy, Pyrenees in Spain, Pirin Mountains in Bulgaria and Dinaric Mountains in Bosnia) but also from *M. arvalis* and *Dinaromys bogdanovi* living in close association with *C. nivalis* at high altitudes (Figs 1 and 3). Subclade 1b, sister to subclade 1a, included a single specimen from *Microtus guentheri* from south-western Turkey. Subclade 1c, sister to subclades 1a + 1b, was found only from *M. arvalis* in Central Europe (Hungary, Slovakia, Croatia) and south-western Asia (Kazakhstan).

Clade 2, possibly the basal lineage within the western Eurasian group, had a more northern distribution than clades 1a–1c, being confined to Fennoscandia and the British Isles (Scotland) and occurring primarily in *Microtus agrestis* but also in *Microtus oeconomus* in northern Fennoscandia. Compared with the other clades, clade 2 was genetically uniform and highly diverged from the other lineages.

Clade 3 had a very wide Holarctic distribution in *Microtus* spp. (mainly *M. oeconomus*), spanning from the Kanin peninsula (north-western Russia) in the west to Buryatia (South-Central Russia), and further east to Alaska, western Yukon and extending to California and New Mexico. Notice that *M. oeconomus* does not occur in south-western North America; the hosts for clade 3 in these regions were *Microtus longicaudus* and *Microtus mexicanus*. Clade 3 included several supported subclades, which had either an Alaskan, Nearctic, Transberingian or wide Holarctic distribution (Fig. 3).

The 28S phylogram (Fig. 4) revealed four supported clades, corresponding to the main clades and subclades of the COI phylogram. Monophyly of the *A. dentata*-like cestodes (clades 1–3) and the western Eurasian clades (1–2) was also highly supported, although the internal topology of the latter differed from that in the COI-phylogram, probably due to the few informative characters in the 28S data. Notice that no specimens from Turkey (clade 1b in COI) were included in the 28S data.

The monophyly of *Anoplocephaloides* s. str. (including all *A. dentata*-like clades, *A. lemmi* and *A. kontrimavichusi*) with respect to other anoplocephaline cestodes was strongly supported by COI and 28S. Based on the 28S phylogram, species from lemmings (*A. lemmi* and *A. kontrimavichusi*) have a basal position within *Anoplocephaloides s*. str.

Table 4 Twenty parsimony-informative sites recovered from the 28S fragment of 17 haplotypes of *Anoplocephaloides dentata*. The site numbering corresponds to GenBank sequence AY569730. A = adenine, C = cytosine, G = guanine, T = thymine, N = A, C, G or T, K = G or T, R = A or G, S = C or G and Y = C or T. Empty cells denote alignment gaps.

GenBank Number	81	170	264	667	679	680	683	685	811	823	839	966	1008	1010	1023	1316	1317	1369	1386	1391
EU664389	c	g	c	a	t	c	—	—	c	t	g	a	c	t	a	t	a	c	g	c
AY569727	c	g	t	a	t	c	—	—	c	t	g	a	c	t	a	t	a	c	g	c
EU664392	c	g	c	a	t	a	—	—	c	c	g	a	c	c	g	a	a	c	g	c
EU664396	c	g	c	a	t	a	—	—	c	c	g	a	c	c	g	a	a	c	g	c
EU664384	t	g	c	a	t	c	—	—	c	c	g	a	c	t	g	a	a	c	g	c
AY569726	t	g	N	a	t	c	—	—	c	c	g	a	c	t	g	a	a	c	g	c
EU664386	t	t	Y	a	t	c	—	—	c	c	g	a	c	t	g	a	a	c	g	c
AY569728	c	t	K	a	t	c	—	—	c	t	g	a	c	t	a	t	a	c	g	c
EU664388	c	t	Y	a	t	c	—	—	c	t	g	a	c	t	a	t	a	c	g	c
EU664375	c	a	N	a	t	c	—	—	c	t	g	a	c	t	a	t	a	c	g	c
EU664385	c	K	c	t	c	c	t	g	t	c	a	g	t	t	g	a	t	g	a	t
EU664398	c	g	R	t	c	c	t	t	t	c	a	g	t	t	g	a	t	g	a	t
AY569729	c	g	t	t	c	c	c	c	t	c	a	g	t	t	g	a	t	g	a	c
EU664399	c	g	t	t	c	c	c	c	t	c	a	g	t	t	g	a	t	g	a	c
EU664397	c	S	c	t	c	c	t	c	t	c	a	g	t	t	g	a	t	g	a	t
AY569730	c	g	c	t	c	c	c	c	t	c	a	g	t	t	g	a	t	g	a	c
EU664391	c	g	c	t	c	c	t	c	t	c	a	g	t	t	g	a	t	g	a	t

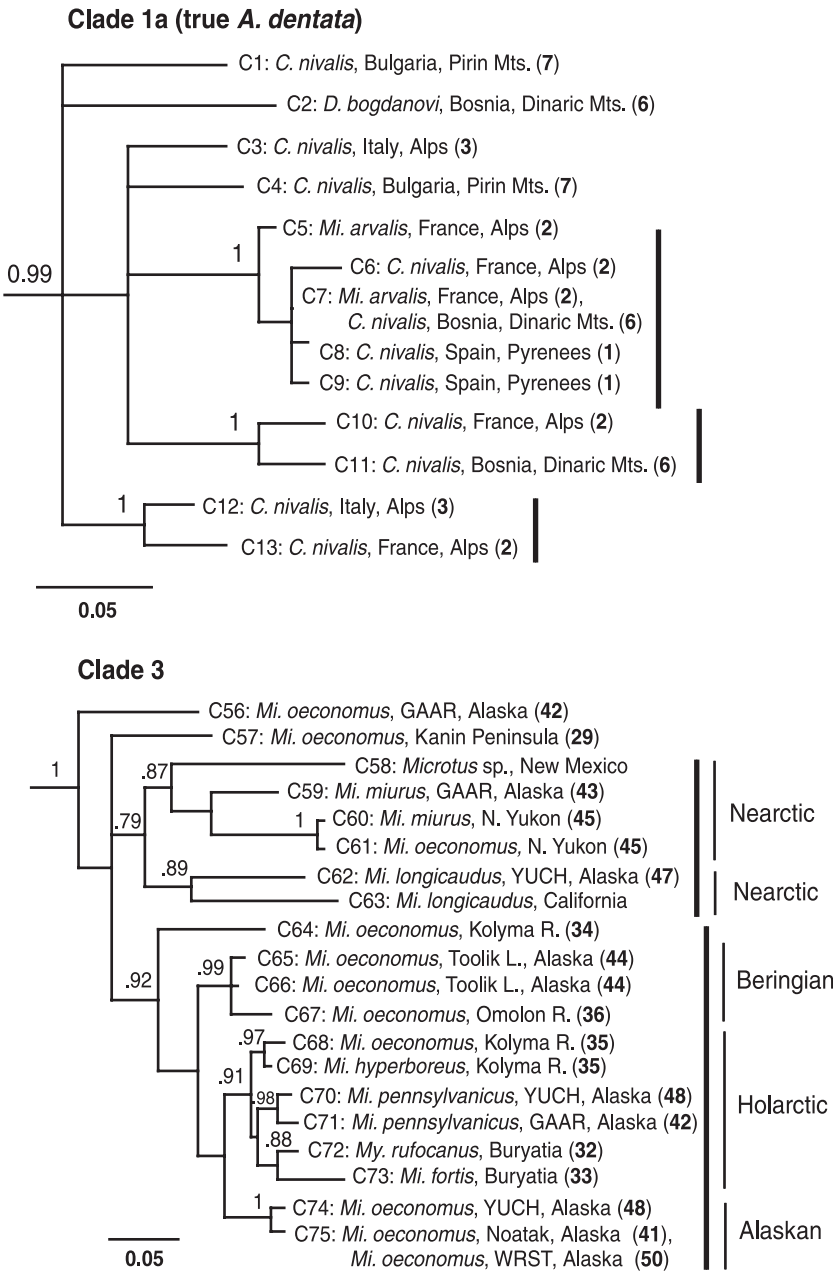

Fig. 3 Bayesian reconstruction of partial cytochrome oxidase I (mtDNA) sequences of *Anoplocephaloides dentata*-like clades 1a and 3. Labels show the haplotype, host, region and locality number (in bold within parentheses; see Fig. 1). Values at nodes show the posterior probabilities. Scale bar indicates inferred number of changes per site.

Morphometrics

Based on the phylogenetic analysis (sub)clades 1a, 1b, 1c, 2 and 3 were used as putative species in the following morphometric analysis. Specimens from *Microtus xanthognathus* from Alaska could not be successfully sequenced for COI or 28S, but because of their morphological distinctiveness were included as a class in the morphometric analysis.

The PCA extracted six principal components (PC's) each having an Eigenvalue > 1 and collectively accounting for 84% of the total variance. Four of the PC's differed significantly between the six clades (one-way ANOVA, Fig. 5). Of these, PC1 (31% of the variance) reflected primarily the variation in the width of the body (BW), scolex (SW) and neck (NW), and in the absolute length of the cirrus sac (CS).

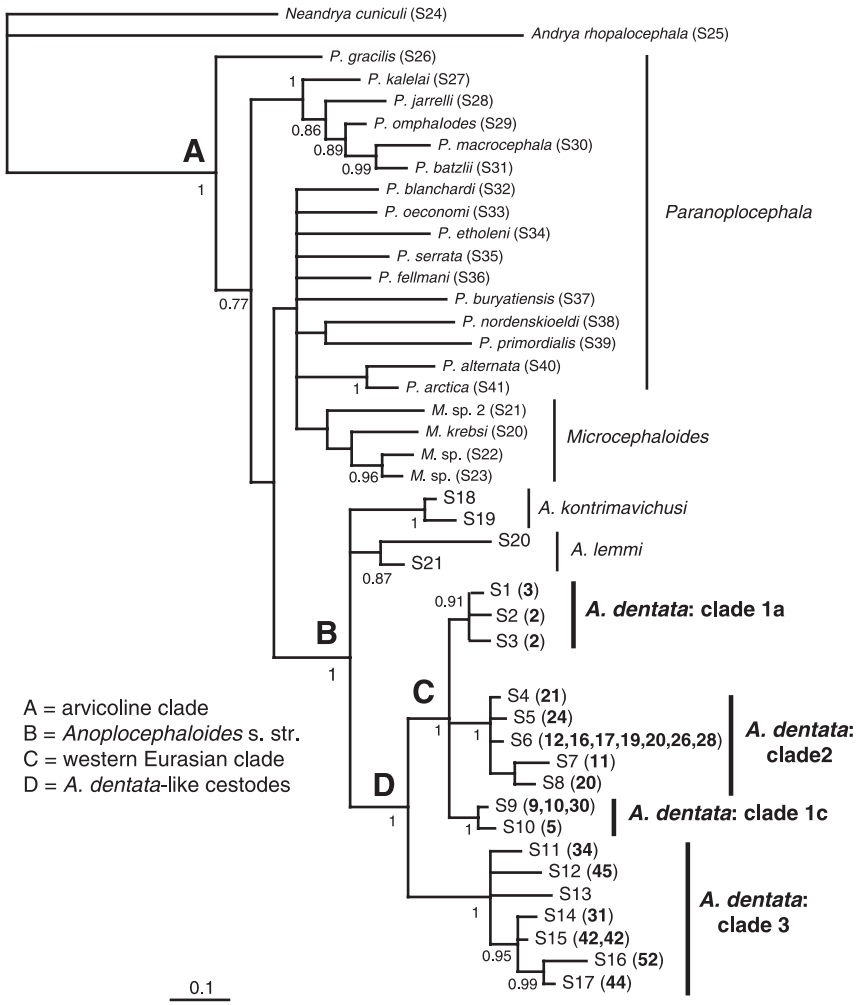

Fig. 4 Bayesian reconstruction of 28S ribosomal RNA sequences of *Anoplocephaloides* spp. and related species from rodents. *Andrya rhopalocephala* and *Neandrya cuniculi* from lagomorphs were used as outgroup. Labels starting with 'S' show the haplotypes; see Appendix. Locality numbers in parentheses (in bold) after the haplotype label; see Fig. 1. Values at nodes show the posterior probabilities. Scale bar indicates inferred number of changes per site.

PC2 (19%) correlated most strongly with the number of proglottids (PN) and the length (BL) and length/width ratio (BL/BW) of the body. PC3 (11%) measured largely the absolute (SU) and relative (SU/SW) size of the suckers. PC6 (7%) showed highest correlation with the index of asymmetry (i.e. transverse position of the vitellarium).

Statistically significant pair-wise differences for each PC are shown in Fig. 5. The PC's, particularly PC2 and PC3, suggested that clade 1b is different from the other clades, and that PC1 separates clades 1c and 4 from the other clades but not from each other. Thus, various external measurements, body shape and maximum length of the cirrus sac are most useful in the separation of various *A. dentata*-like taxa. However, it should be noticed that, with the exception of PC1, the PC's accounted for a relatively small proportion of the total variation (7–19%), and have therefore weak explanatory power when used independently in the morphometric separation of the clades.

The canonical discriminant functions were calculated using the length of the scolex, relative length of the body and cirrus sac, minimum width of the neck and egg length, as selected by the step-wise procedure. The overall classification success of the discriminant analysis was low (63%), meaning that the available morphometric measures do not adequately discriminate the six (sub)clades. The classification success of clades 1b (83%) and 4 (86%) was somewhat higher than that of the other clades (51–71%). Because the sample sizes of the clades 1b and 4 were low, discriminant analysis was also

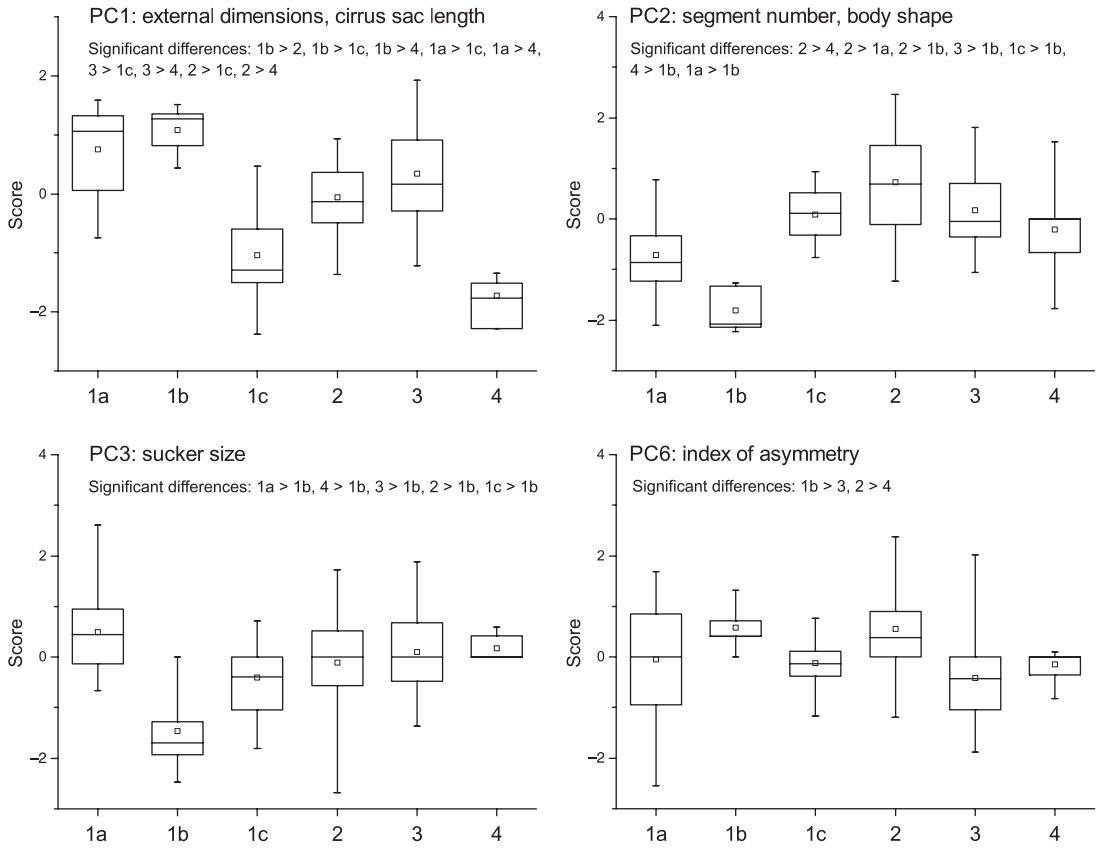

Fig. 5 Principal components (PC's) showing statistically significant (one-way ANOVA, $P < 0.05$) differences between the DNA clades of *Anoplocephaloides dentata*-like cestodes. The graphs show the range ('whiskers'), lower and upper quartiles (box), median (transverse line within the box) and mean (small square within the box). Significant pairwise differences inserted in figures.

performed for the four clades with largest samples (1a, 1c, 2 and 3). This did not have a significant effect on the overall classification success (66%). In addition, because the original measurements were frequently correlated with each other, discriminant analysis was performed for all six clades using the six (uncorrelated) principal components. Again, no significant change was seen in the overall classification success (64%) although clade 1b was perfectly (100%) distinguished from the other clades.

The median extent of the testes was usually largely overlapping between the six clades, with the exception of the 'clade' 4, in which the testes were never in contact with or overlapping the vitellarium (Table 3). In clade 1c, the testes did not overlap the vitellarium, but the sample size was small. A corresponding interspecific difference was observed for the absolute width of the testicular field (TW), which was significantly lower in clade 4 than in the other clades (Table 2). It should be noted that the position of testes with respect to the *antiporal* longitudinal osmoregulatory canals is constant among the *A. dentata*-like cestodes, with testes typically in contact or overlapping these canals but not extending beyond them (Figs 6 and 8).

Overall, various morphometric measures were rather unsuccessful in separating *A. dentata*-like clades/species and we therefore refrain from describing any of them as new species. However, we present a redescription for *A. dentata*, which can be used in future taxonomic analyses of this species complex. The morphology of the other supposed *A. dentata*-like species is depicted in Figs 7 and 8.

Family ANOPLOCEPHALIDAE
Genus *Anoplocephaloides* Baer, 1923
Anoplocephaloides dentata (Galli-Valerio, 1905)
Syns. *Anoplocephala dentata* (Galli-Valerio, 1905), *Paranoplocephala dentata* (Galli-Valerio, 1905) Spasskii, 1951.

Material examined. 24 specimens from *C. nivalis*, *M. arvalis* and *D. bogdanovi* from five high-altitude localities in southern Europe, including eight specimens from the type host (*C. nivalis*) in the type region (Italian Alps) (Table 1).

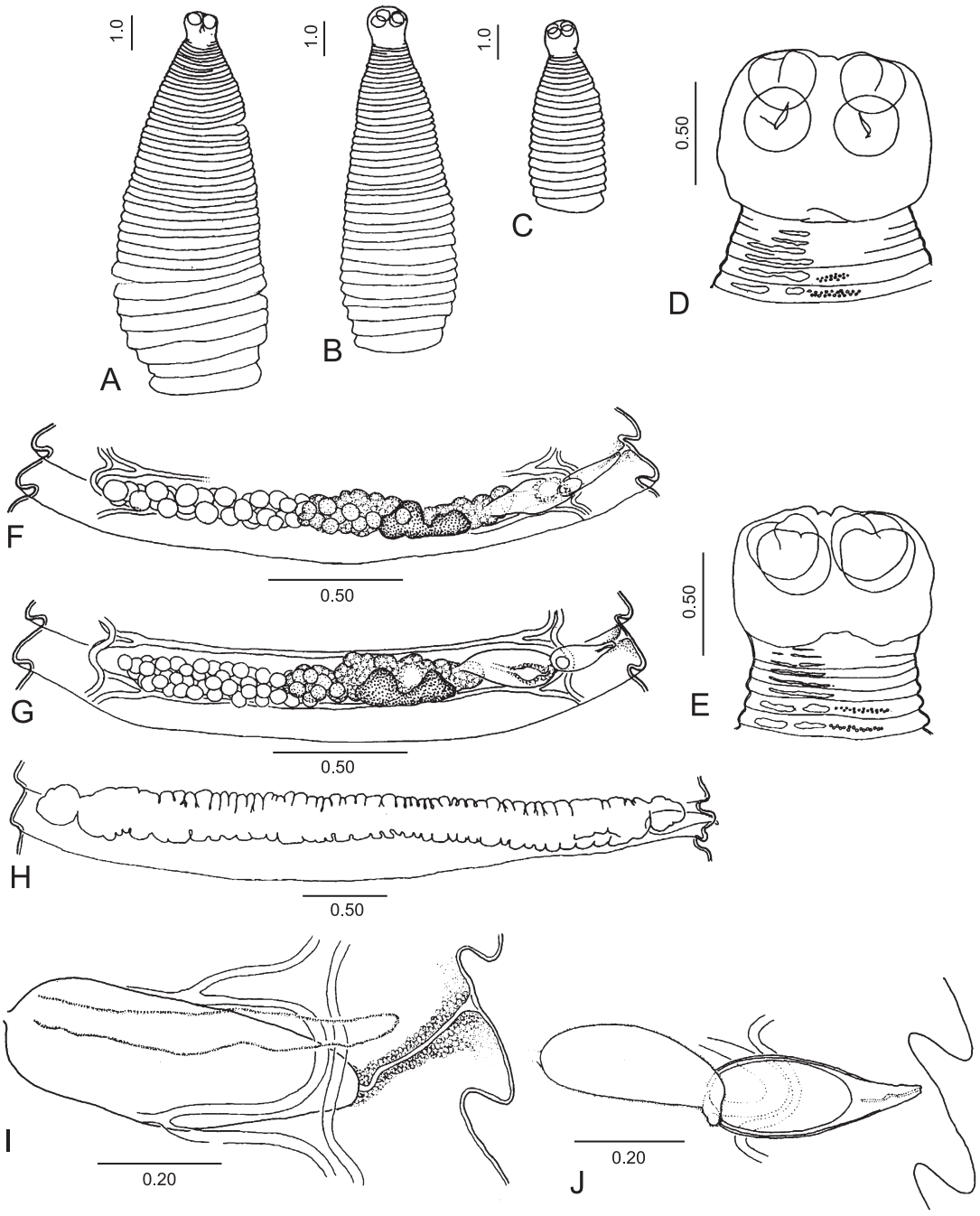

Fig. 6 A–J. Morphology of clade 1a or the true *Anoplocephaloides dentata* from the type host *Chionomys nivalis*. —A–C. Strobila (Italy). —D. Scolex and neck region (France). —E. Scolex and neck region (Italy). —F. Last mature proglottid (Italy). —G. Last mature proglottid (France). —H. Pregravid proglottid (Italy). —I. Early uterus and female genital ducts (Italy). —J. Male genital ducts (Italy). Scale-bars in mm.

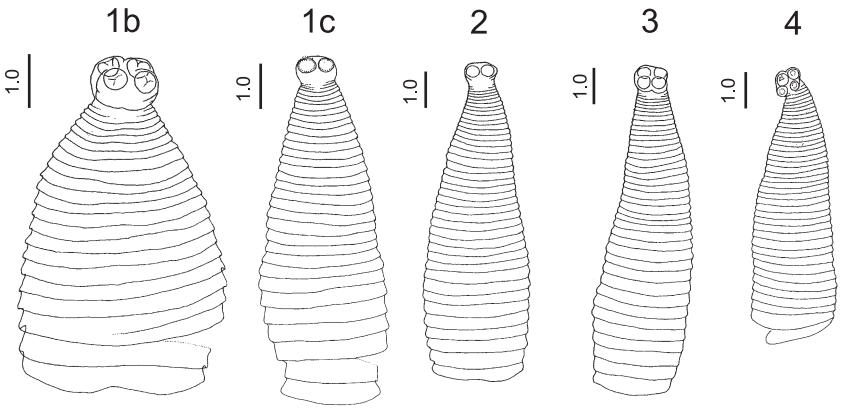

Fig. 7 Examples of strobilae of other *Anoplocephaloides dentata*-like cestodes (clades 1b-4). 1b, from *Microtus guentheri*, Turkey. 1c, from *Microtus arvalis*, Kazakhstan. 2, from *Microtus agrestis*, Finland. 3, from *Microtus miurus*, Alaska, USA. 4, from *Microtus xanthognathus*, Alaska, USA. Scale-bars in mm.

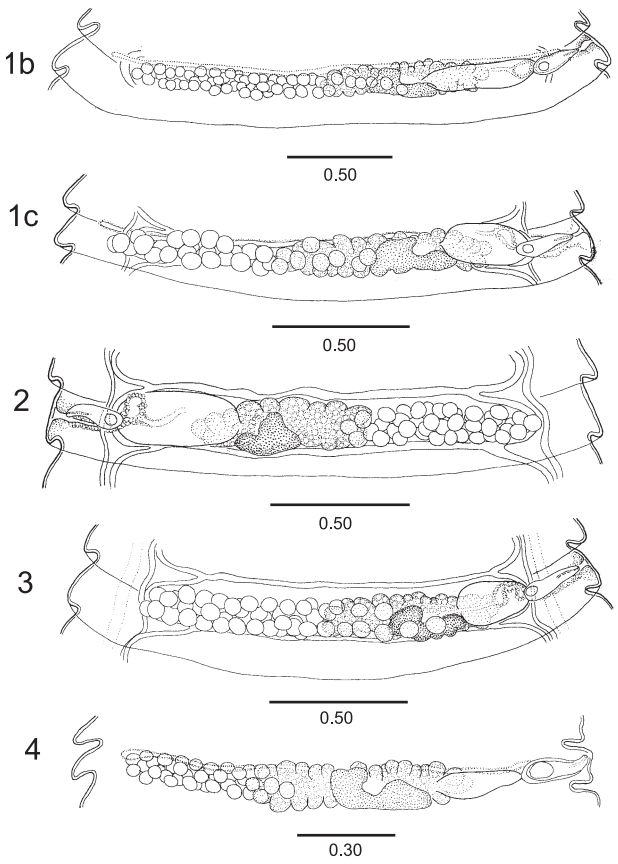

Fig. 8 Examples of mature proglottids of other *Anoplocephaloides dentata*-like cestodes (clades 1b-4). 1b, from *Microtus guentheri*, Turkey. 1c, from *Microtus arvalis*, Kazakhstan. 2, from *Microtus oeconomus*, Finland. 3, from *Microtus miurus*, Alaska, USA. 4, from *Microtus xanthognathus*, Alaska, USA. Scale-bars in mm.

Redescription (Fig. 6). The mean and sample size (*n*, number of measurements) follow in parentheses after the range. All measurements are in millimeters.

Body 5.5–12.9 (9.5, *n* = 22) long, with 26–52 (39.1, *n* = 22) proglottids. Maximum width 2.3–4.8 (3.9, *n* = 23), attained in early gravid proglottids. Velum long, covering *c.* 1/3 of subsequent proglottid; curved posteriorly in surface view. All proglottids much wider than long, length : width ratio 0.07–0.11 (0.087, *n* = 11) in last mature proglottid; last gravid proglottids longer and thinner than preceding ones. Scolex 0.85–1.50 (1.08, *n* = 24) wide and 0.52–1.10 (0.83, *n* = 23) long, usually with expanded posterior part, giving scolex more or less rectangular shape. Posterior scolex often demarcated from neck region by distinct transverse ridge. Suckers embedded within scolex, 0.33–0.46 (0.393, *n* = 24) in diameter, directed anteriorly or slightly antero-laterally. Minimum width of neck region 0.44–1.07 (0.85, *n* = 24) or 45–97% (79%, *n* = 24) of scolex width.

Developing genital organs visible in first proglottids. Genital pores unilateral, opening slightly anterior to middle of proglottid margin in last mature proglottids. Genital ducts passing dorsally across longitudinal osmoregulatory canals and nerve cord. Ventral longitudinal osmoregulatory canals 0.015–0.028 (0.022, *n* = 5) wide in last mature proglottid, strongly arched, connected by transverse canals *c.* 0.02 wide; junction between longitudinal and transverse canals deeply 'y'-shaped. Dorsal longitudinal osmoregulatory canals 0.02–0.03 (*n* = 3) wide in last mature proglottid, occasionally wider than ventral canals of the same proglottid, positioned lateral to ventral canals or partly overlapping them.

Cirrus sac elongate, its proximal part typically extending across longitudinal osmoregulatory canals. Muscle layers of cirrus sac initially very thin, widening slightly in postmature

and pregravid proglottids. Maximum length and width of cirrus sac 0.30–0.48 (0.379, *n* = 24) and 0.12–0.17 (0.141, *n* = 5), respectively, attained in pregravid or early gravid proglottids. Ductus cirri straight, provided with minute spines. Internal seminal vesicle initially spherical, starting to fill and expand in first postmature proglottid, reaching length of 0.10–0.30 (0.18, *n* = 15) in pregravid proglottids, covering 32–79% (50%, *n* = 15) of cirrus sac length. External seminal vesicle initially irregularly shaped sac covered by thick cell layer, expanding and elongating considerably in postmature proglottids when filled with sperm, reaching length of 0.16–0.34 (0.26, *n* = 10). Expanded external seminal vesicle with thin distal loop and without visible cell covering. Testes 27–63 (42.4, *n* = 11) in number, forming compact transverse band from antiporal osmoregulatory canals to midline of proglottid or slightly beyond (up to 10 testes may lie in poral field). Testes either in contact with antiporal canals, often overlapping them, or being separated by distinct gap. Median testes always overlapping ovary, often contacting or overlapping vitellarium. Testes ovoid, 0.05–0.10 (0.070, *n* = 20) long in last mature proglottid, attaining length of 0.08–0.12 (0.098, *n* = 14) in postmature proglottids. Testes shifted medially in postmature proglottids, partly replacing female glands.

Vagina 0.17–0.22 (*n* = 3) long, thick-walled tube of fairly uniform width, usually slightly curved. Internally vagina lined with delicate hairs. Seminal receptacle typically an elongate sac with poorly visible walls, even when filled with sperm (73% of specimens, *n* = 15). Alternatively (27% of specimens), sac expands markedly forming 'capsular' shape with distinct walls and filling entire space between transverse osmoregulatory canals. Maximum length and width of 'capsular' seminal receptacle in postmature/pregravid proglottids 0.43–0.61 (0.55, *n* = 5) and 0.17–0.22, respectively. Ovary coarsely lobed, transversely elongated, 0.53–0.89 (0.75, *n* = 11) wide in last mature proglottid, positioned mostly in poral field. Median margin of ovary always extending across midline of proglottid (transverse distance between median margin of ovary and midline of proglottid 0.03–0.23, mean = 0.12, *n* = 11); poral margin of ovary does not reach longitudinal canals. Vitellarium asymmetrically bilobed, 0.20–0.41 (0.33, *n* = 11) wide and 0.10–0.17 (0.14, *n* = 11) long in last mature proglottid, positioned porally with respect to midline of proglottid and ovary (index of asymmetry 0.29–0.47, 0.36, *n* = 23). Uterus appears early as transverse cord in anterior part of premature proglottids. In last mature proglottids, uterus seen as narrow tube with well-defined lumen, positioned ventral to testes and extending ventrally and bilaterally across longitudinal osmoregulatory canals; in mid-region early uterus lies within ovarian lobes. In first postmature proglottids, uterus rapidly expands posteriorly and develops anterior and posterior sacculations; number of anterior sacculations 40–55 and posterior ones 35–46. Eggs 0.038–0.050 (0.0435, *n* = 24) long, thick-walled, spherical in surface view, ovoid or slightly citriform in side view, often with thickened poles. Main trunk of pyriform apparatus not divided, but ends bearing two delicate horns crossing each other, one of which may be noticeably thicker than the other.

Remarks. *Anoplocephaloides dentata* was described as *Anoplocephala dentata* Galli-Valerio, 1905 from the snow vole (*C. nivalis*) near Valtellina in the Italian Alps (Galli-Valerio 1905). Therefore, clade 1a that is primarily associated with *C. nivalis* in southern Europe, including the Italian Alps, evidently represents the true *A. dentata*. Because the whereabouts of the original material (one specimen without a scolex) are unknown, we propose that a specimen from *C. nivalis* from Monte Bondone, northern Italy (MSB Endo 85), be designated as the neotype of *A. dentata*. Two specimens from the same host species and locality have been deposited in the USNPC (numbers 95383 and 95646). For other voucher specimens, see Table 1. It should be noted that the earlier morphometric characterizations and redescriptions of *A. dentata* (see Rausch 1976; Tenora & Murai 1980; Genov & Georgiev 1988) have been based partly or entirely on materials that are probably not conspecific.

Discussion

Phylogeny

As suggested earlier (Wickström *et al*. 2005), the *A. dentata*-like cestodes, *A. lemmi* and *A. kontrimavichusi* (i.e. *Anoplocephaloides* s. str.) are part of a large 'arvicoline clade' of cestodes including *Paranoplocephala* spp. and *Microcephaloides* spp. from arvicoline rodents (voles and lemmings) and *Diandrya composita* Darrah, 1930 from marmots (Figs 2 and 4). The monophyly of *Anoplocephaloides* s. str. was strongly supported by the COI and 28S data, confirming the observations of Wickström *et al*. (2005) and Haukisalmi *et al*. (2008). The unique body shape and other morphological features support the notion of *Anoplocephaloides* s. str. as a monophyletic taxon.

Phylogenetic relationships among lineages of the 'arvicoline clade' are partly unresolved and it is not yet possible to define unambiguously the sister group of *Anoplocephaloides* s. str. However, the available phylogenies reject a sister group relationship between *Anoplocephaloides* s. str. and *Microcephaloides*, both of which have a tubular early uterus with similar subsequent development (the early uterus of *Paranoplocephala* Lühe, 1910 and *Diandrya* Darrah, 1930 is more or less reticulated). It is clear that uterine development, earlier supposed to be a key feature in the classification of anoplocephaline cestodes, is a poor index of phylogenetic affinity (Wickström *et al*. 2005). Because the sister group of the 'arvicoline clade' is formed by two species with a reticulated early uterus (*A. rhopalocephala* and *N. cuniculi*) (see Wickström *et al*. 2005),

Anoplocephaloides s. str. and *Microcephaloides* may represent independent reversals to the ancestral character state of Anoplocephalinae. In addition, the intestinal position of *Anoplocephaloides* s. str. (posterior ileum, ileo-caecal junction or caecum) is evidently derived with respect to that of *Paranoplocephala* spp., *Microcephaloides* spp. and other anoplocephaline cestodes (small intestine proper).

The 28S data (Fig. 4) are the first to provide support for the monophyly of *A. dentata*-like cestodes (from voles) with respect to *A. lemmi* and *A. kontrimavichusi* (from lemmings). However, COI suggested a conflicting though poorly supported affinity between the Holarctic *A. dentata* (clade 3) and *A. kontrimavichusi*. An earlier phylogenetic analysis of Wickström *et al*. (2005) based on a few specimens and utilizing a combination of COI, 28S and internal transcribed spacer 1, yielded a high (0.99) posterior probability for the latter clade. However, in the data set of Wickström *et al*. (2005), COI provided the greatest number of informative sites and convincing phylogenetic results. Thus, given the conflicting evidence, the monophyly of *A. dentata*-like cestodes and their relationships with other clades of *Anoplocephaloides* s. str. requires additional testing and independent markers.

The position of *A. lemmi* and *A. kontrimavichusi* as basal lineages within *Anoplocephaloides* s. str. suggests that the early diversification of this clade occurred in lemmings (*Lemmus* and *Synaptomys*). Because lemmings (tribe Lemmini) are phylogenetically basal to voles (tribe Arvicolini) (Galewski *et al*. 2006), the early diversification in *Anoplocephaloides* s. str. may have coincided with diversification of the hosts. However, it should be noted that *A. lemmi* and *A. kontrimavichusi* were never recovered as sister species although their hosts (*Lemmus* and *Synaptomys*, respectively) form a well-supported clade (Conroy & Cook 1999) and in general there is little evidence for cophylogeny of hosts and *Anoplocephaloides* s. str.

Because clades 1–3 are predominantly parasites of *Microtus* spp., the true *A. dentata* (clade 1a), which is primarily associated with *C. nivalis*, has probably diverged through a host shift from *Microtus* to *C. nivalis* in south-western Eurasia. This divergence has probably been reinforced by the ecological separation of the montane *C. nivalis* from the lowland *M. guentheri*, the host of clade 1b. It is probable that all *A. dentata*-like cestodes of other rodents co-occurring with *C. nivalis* at high altitudes represent the true *A. dentata*. For example, Genov & Georgiev (1988), who described *A. dentata* from high-altitude rodent populations in Bulgaria, could not find any major morphometric differences between specimens from *M. subterraneus* and those from the sympatric *C. nivalis*.

Since the COI lineages within the true *A. dentata* (clade 1a) are not confined to any single mountain region (Fig. 3), lineage diversity within this species is not due to *in situ* divergence in their fragmented extant host population. It is probable that each of the mountain regions has been recolonized from multiple glacial refugia, and a corresponding recolonization pattern is predicted to have occurred also in *C. nivalis*. Unfortunately, phylogeographical studies of the snow vole that might test this scenario are unavailable.

The high divergence and limited genetic variation of the northern European clade 2, compared with other clades, may have been caused by bottle-neck events in a glacial refugium and/or founder effects during the postglacial colonization of northern Europe. The primary host of clade 2, the field-vole *M. agrestis*, has colonized northern Europe from the south-west through present-day Denmark and southern Sweden, and from the east through present-day Finland. Today, these lineages meet at a narrow contact zone in central Sweden (slightly south of Umeå) (Jaarola & Tegelström 1996). It has been proposed that the two northern lineages of *M. agrestis* originate from different glacial refugia, possibly situated in Central Europe and south-western Asia, respectively (Jaarola & Searle 2002). However, the shallow phylogenetic structure and lack of lineage diversity in clade 2 suggest that it has not codiversified with or tracked the phylogeography of its primary host. Rather, clade 2 probably originates from a single glacial refugium from where it has colonized northern Europe and crossing the northern phylogeographical border within *M. agrestis*. Because specimens of clade 2 from Scotland are genetically similar to those from Fennoscandia (Fig. 2), it can be postulated that clade 2 survived the glaciations in a central European refugium rather than a south-western Asian one. However, in the absence of sequences outside northern Europe and Scotland, the glacial and postglacial history of clade 2 remains speculative.

Clade 3 was shown to have an extremely wide Holarctic distribution with multiple divergent lineages. In Eurasia, clade 3 seems to be mainly associated with *M. oeconomus*, a Holarctic species that has colonized north-western North America rather recently. In North America, clade 3 occurs in several *Microtus* species, including the south-western *M. mexicanus*. The presence of multiple well-supported subclades within clade 3 may be due to periodical blockage of gene flow in its principal host (*M. oeconomus*) within Beringia (Brunhoff *et al*. 2003; Galbreath & Cook 2004). However, the major phylogeographical split in *M. oeconomus* between Beringian and Asian clades, located at the Kolyma/Omolon Rivers in eastern Russia (Galbreath & Cook 2004), is not reflected in the geographical distribution of the various sublineages within the Holarctic clade (3) of *A. dentata*.

Although the geographical distribution of various (sub)clades of *A. dentata*-like cestodes is determined mainly by the distribution of their primary hosts, a notable exception is seen in northern Europe. The Holarctic clade 3 is replaced there by the northern European clade 2, which parasitizes both

M. agrestis and *M. oeconomus*. There is a major phylogeographical split within *M. oeconomus* at the Ural mountains (Brunhoff *et al*. 2003), but this split is probably not responsible for the absence of clade 3 in northern Fennoscandia since the latter species has been found much further west than the Ural mountains in the Kanin Peninsula. Thus, it appears that factors unrelated to the evolutionary history of the hosts, such as competitive exclusion among parasites, has been partly responsible for the present distribution of these clades.

The intra- and interspecific phylogenetic patterns within *Anoplocephaloides* s. str. suggest extensive colonization of new host lineages with limited codivergence between hosts and parasites. This is in line with the earlier phylogenetic studies of anoplocephalids (Hu *et al*. 2005; Wickström *et al*. 2005; Beveridge *et al*. 2007; Haukisalmi *et al*. 2008). The main reason for the absence of host–parasite cophylogeny may be the potentially flexible host selection of *A. dentata*-like cestodes. Though each cestode species is primarily associated with a single host species in a given region, they regularly parasitize 'alien' host species, at least in certain parts of their range. For example, clade 3 has been found several times in *Myodes rufocanus* (syn. *Clethrionomys rufocanus*) in Siberia (Tunguska River region and Buryatia; unpublished observations). On the island of Hokkaido (Japan), this association seems to have led to the divergence of *A. dentatoides* Sato, Kamiya, Tenora & Kamiya, 1993, a specific parasite of *My. rufocanus* (Sato *et al*. 1993) (there are no extant *Microtus* species in Hokkaido). The largely non-overlapping distributions of cestodes suggest that their speciation events have been promoted by local or regional allopatry of hosts.

Two other monophyletic assemblages of anoplocepahaline cestodes (*Paranoplocephala* s. str. and *Microcephaloides* spp.) parasitizing arvicoline rodents in the Holarctic have previously been studied with molecular phylogenetic techniques (Haukisalmi *et al*. 2004; Haukisalmi *et al*. 2008). These two assemblages and *Anoplocephaloides* s. str. are all primarily parasites of *Microtus* voles, have similar overall species diversity (5–6 species) and relatively high host-specificity but there is little correspondence between their patterns of host use, diversification and geographical distribution. However, in all three assemblages host colonization seems to have been the primary determinant of diversification and speciation and the observed clades/species have largely non-overlapping geographical distributions that suggest an allopatric model of divergence. In each assemblage there are at least two species that have probably speciated through a shift to another host genus. Interestingly, all three have colonized Nearctic pocket gophers (*Geomys* and *Thomomys*), suggesting a shared colonization episode. However, the other supposed colonizations are independent, including shifts in different regions to different host genera.

Thus, there have been frequent colonizations among cestodes of arvicoline rodents inhabiting the same region and/or biome, but the present distributions suggest that final divergences were attained in allopatry without secondary mixing of populations. The evidence for cospeciation is weak. There is also incongruity between phylogenies of *Paranoplocephala* s. str. and *Microtus* voles (Haukisalmi *et al*. 2004; but see Wickström *et al*. 2003). This may in part be due to the ability of these cestodes to parasitize other (usually congeneric) host species, leading to the breakage of strict host–parasite phylogenetic correspondence. As expected for species that are specialized to a certain host-species/lineage, a closer correspondence between the phylogeographical divisions of the host and the parasite have been observed (Wickström *et al*. 2003; Nieberding *et al*. 2004; Nieberding *et al*. 2005).

How many species?

Based on the present phylogenetic analysis, there are 4–5 monophyletic groups of *A. dentata*-like cestodes in Eurasia and western North America that together form a clade. Therefore, *A. dentata*-like cestodes can be regarded either as a single species with significant genetic substructuring or as 2–5 independent species. The apparent allopatry of the main clades supports the idea of a single variable species. Although the morphometric differences among *A. dentata*-like cestodes are generally low, there are a few morphologically deviant clades that probably deserve specific status. In particular, clade 1b is clearly different from all other clades and clades 1c and 4 also differ from the other clades (Figs 7 and 8) but not from each other (notice that there were no genetic data for 'clade' 4). If the specific status of clades 1b and 1c is accepted, it is logical to assume a corresponding status also for the other *A. dentata*-like clades and subclades. Additional support for the existence of multiple biologically distinct species comes from their largely non-overlapping host preferences, particularly in western Eurasia. The present data thus support the existence of five allopatric and more or less host-specific species of *A. dentata*-like cestodes in Eurasia, one of which extends into North America.

Besides *A. dentata*, there are four nominal species of *A. dentata*-like cestodes, two in Eurasia (*Paranoplocephala brevis* and *A. dentatoides*) and two in North America [*A. infrequens* and *A. troeschi* (Rausch, 1946)]. In his authoritative review, Rausch (1976) considered *A. dentata*, *A. infrequens* and *A. troeschi* valid species but listed *P. brevis* as a junior synonym of *A. dentata*. However, the comparative material of *A. dentata* used by Rausch (1976) originated mostly from *M. oeconomus* in north-eastern Siberia, therefore probably representing clade 3. This means that none of the other nominal species have been compared with the true *A. dentata*. Also, the present analysis shows that morphological criteria are generally too variable and overlapping to reliably separate *A. dentata*-like

species. Based on host preference, *A. dentatoides*, *A. infrequens* and *A. troeschi* are not conspecific with *A. dentata* although we have yet to distinguish them from the latter species or from each other on morphological grounds. The host and geographical distribution of *A. infrequens* and *A. troeschi* are unknown, and one or both of them may be conspecific with clade 3 which extends far into south-western North America. *Paranoplocephala brevis* is considered here *species inquirenda* because the original description lacks many important details and no type specimen exists (Kirshenblat 1938). 'Clade' 4 of *A. dentata* (lacking sequence data) appears to be morphologically differentiated from the related species and a host-specific parasite of the Nearctic *M. xanthognathus*.

Because *A. dentata*-like cestodes exhibit relatively high host-specificity and because some of them have restricted geographical distributions, future studies based on combined molecular and morphometric evidence are expected to reveal additional diversity within this complex. For example, the comprehensive host-parasite database maintained by the Natural History Museum in London (Gibson *et al.* 2005) shows that at least 33 species of rodents have been reported as hosts for *A. dentata* or *Paranoplocephala brevis* in Eurasia. Likewise, the compendium of Ryzhikov *et al.* (1978) reports that *A. dentata* parasitizes 22 species of rodents in the territory of the former Soviet Union alone. Thus, it is too early to estimate the total species diversity of *A. dentata*-like cestodes. The use of molecular methods in future taxonomic analyses of the *A. dentata* complex is highly recommended.

Acknowledgements

Our studies on molecular systematics of anoplocephalid cestodes have been funded primarily by the Research Council for Biosciences and Environment, Finnish Academy (grants 40813 and 50474 to H. H. and a postdoctoral fellowship 108372 to L. M. H.). Most of the cestodes from north-eastern Siberia and Alaska were collected in connection with the Beringian Coevolution Project (BCP), funded by the US National Park Service and National Science Foundation (DEB 0196095 and 0415668), and planned and coordinated by Joseph A. Cook and Eric P. Hoberg. Authors thank all members of various BCP crews for their invaluable efforts under harsh field conditions. Anna-Paola Rizzoli and other members of the ECODIS project helped in the collection of voles and their helminths from Italy (Centro di Ecologia Alpina). Authors also acknowledge the crucial role of all other collectors and local collaborators (see Appendix) in making the present wide-scale study possible. E. P. Hoberg, Patricia Pilitt (USNPC), J. A. Cook and Anson Koehler (MSB) have kindly helped in the examination and deposition of museum specimens. The advice and materials given generously by Robert L. Rausch and E. P. Hoberg during various stages of the study are highly appreciated.

References

Beveridge, I., Shamsi, S., Hu, M., Chilton, N. B. & Gasser, R. B. (2007). Genetic variation in the mitochondrial cytochrome c oxidase subunit 1 within Progamotaenia festiva (Cestoda: Anoplocephalidae) from macropodid marsupials. *Parasitology*, *134*, 1465–1476.

Brunhoff, C., Galbreath, K. E., Fedorov, V. B., Cook, J. A. & Jaarola, M. (2003). Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. *Molecular Ecology*, *12*, 957–968.

Conroy, C. J. & Cook, J. A. (1999). MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. *Journal of Mammologyalian Evolution*, *6*, 221–245.

Cook, J. A., Hoberg, E. P., Koehler, A., Henttonen, H., Wickström, L., Haukisalmi, V., Galbreath, K., Chernyavski, F., Dokuchaev, N., Lahzuhtkin, A., MacDonald, S. O., Hope, A., Waltari, E., Runck, A., Veitch, A., Popko, R., Jenkins, E., Kutz, S. & Eckerlin, R. (2005). Beringia: Intercontinental exchange and diversification of high latitude mammals and their parasites during the Pliocene and Quaternary. *Mammal Study*, *30*, S33–S44.

Galbreath, K. E. & Cook, J. A. (2004). Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. *Molecular Ecology*, *13*, 135–148.

Galewski, T., Tilak, M., Sanchez, S., Chevret, P., Paradis, E. & Douzery, E. J. P. (2006). The evolutionary radiation of Arvicolinae rodents (voles and lemmings): relative contribution of nuclear and mitochondrial DNA phylogenies. *BMC Evolutionary Biology*, *6*, 80.

Galli-Valerio, B. (1905). Einige Parasiten von *Arvicola nivalis*. *Zoologisher Anzeiger*, *28*, 519–522.

Genov, T. & Georgiev, B. B. (1988). Review of the species of the genus *Anoplocephaloides* Baer, 1923 emend Rausch, 1976 (Cestoda: Anoplocephalidae) parasitizing rodents in Bulgaria, with an analysis of the taxonomic structure of the genus. *Parasitologica Hungarica*, *21*, 31–52.

Gibson, D. I., Bray, R. A. & Harris, E. A. (2005). *Host-Parasite Database of the Natural History Museum, London*. Available at http://www.nhm.ac.uk/research-curation/projects/host-parasites/.

Haukisalmi, V., Hardman, L. M., Hardman, M., Rausch, R. L. & Henttonen, H. (2008). Molecular systematics of the Holarctic *Anoplocephaloides variabilis* (Douthitt, 1915) complex, with the proposal of *Microcephaloides* n. g. (Cestoda: Anoplocephalidae). *Systematic Parasitology*, *70*, 15–26.

Haukisalmi, V., Heino, M. & Kaitala, V. (1998). Body size variation in tapeworms (Cestoda): adaptation to intestinal gradients? *Oikos*, *83*, 152–160.

Haukisalmi, V., Wickström, L. M., Henttonen, H., Hantula, J. & Gubányi, A. (2004). Molecular and morphological evidence for multiple species within *Paranoplocephala omphalodes* (Cestoda, Anoplocephalidae) in *Microtus* voles (Arvicolinae). *Zoologica Scripta*, *33*, 277–290.

Hoberg, E. P., Kutz, S. J., Galbreath, K. E. & Cook, J. A. (2003). Arctic biodiversity: from discovery to faunal baselines — revealing the history of a dynamic ecosystem. *Journal of Parasitology*, *89* (Suppl.), S84–S95.

Hu, M., Gasser, R. B., Chilton, N. B. & Beveridge, I. (2005). Genetic variation in the mitochondrial cytochrome *c* oxidase subunit 1 within three species of *Progamotaenia* (Cestoda: Anoplocephalidae) from macropodid marsupials. *Parasitology*, *130*, 117–129.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R. & Bollback, J. P. (2001).

Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, *294*, 2310–2314.

Jaarola, M. & Searle, J. B. (2002). Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. *Molecular Ecology*, *11*, 2613–2621.

Jaarola, M. & Tegelström, H. (1996). Mitochondrial DNA variation in the field vole (*Microtus agrestis*): regional population structure and colonization history. *Evolution*, *50*, 2073–2085.

Kirshenblat, Y. D. (1938). Zakonomernosti dinamiky parazitofauni myschevidnyh gryzunov. *Izdanie Lenindgradskogo Gosudarstvenogo Universiteta*, 92 pp.

Nieberding, C., Libois, R., Doady, C. J., Morand, S. & Michaux, J. R. (2005). Phylogeography of a nematode (*Heligmosomoides polygyrus*) in the western Palearctic region: persistence of northern cryptic populations during ice ages? *Molecular Ecology*, *14*, 765–779.

Nieberding, C., Morand, S., Libois, R. & Michaux, J. R. (2004). A parasite reveals cryptic phylogeographic history of its host. *Proceedings of the Royal Society of London Series B Biology Sciences*, *271*, 2559–2568.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818.

Rausch, R. L. (1976). The genera *Paranoplocephala* Lühe, 1910 and *Anoplocephaloides* Baer, 1923 (Cestoda: Anoplocephalidae), with particular reference to species in rodents. *Annales de Parasitologie Humaine et Comparée*, *51*, 513–562.

Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Ryzhikov, K. M., Gvozdev, E. V., Tokobaev, M. M., Shaldybin, L. S., Macaberidze, G. V., Merkusheva, I. V., Nadtochii, E. V., Hohlova, I. G. & Sharpilo, L. D. (1978). *Opredelitel' gelmintov gryzunov fauny SSSR. Cestody i trematod*. Moskva: Nauka.

Tenora, F. & Murai, É. (1980). The genera *Anoplocephaloides and Paranoplocephala* (Cestoda) parasites of Rodentia in Europe. *Acta Zoologica Academiae Scientiarum Hungarica*, *26*, 263–284.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680.

Wickström, L. M., Haukisalmi, V., Varis, S., Hantula, J., Fedorov, V. B. & Henttonen, H. (2003). Phylogeography of the circumpolar *Paranoplocephala arctica* species complex (Cestoda: Anoplocephalidae) parasitizing collared lemmings (*Dicrostonyx* spp.). *Molecular Ecology*, *12*, 3359–3371.

Wickström, L. M., Haukisalmi, V., Varis, S., Hantula, J. & Henttonen, H. (2005). Molecular phylogeny and systematics of anoplocephaline cestodes in rodents and lagomorphs. *Systematic Parasitology*, *62*, 83–99.

Appendix

Cytochrome oxidase I (COI) and rRNA 28S haplotypes used in the phylogenetic analysis. GenBank numbers in parentheses after the haplotype labels.

Cestode species Host species	Country, state	Locality (number)	Collectors	Clade	COI haplotypes (GenBank numbers)	28S sequences (GenBank numbers)
Anoplocephaloides dentata						
C. nivalis	Bulgaria	Pirin Mts. (7)	P. Nikolov, T. Genov	1a	C1 (EU664440), C4 (EU664439)	—
C. nivalis	Italy	Trento (3)	H. Henttonen, V. Haukisalmi, J. Niemimaa	1a	C3 (AY568190), C12 (EU664404, EU664405)	S1 (AY569725, EU664384)
C. nivalis	Spain	Catalonia (1)	C. Feliu	1a	C8 (EU664428), C9 (EU664429)	—
C. nivalis	France	Bourg-Saint-Maurice (2)	N. G. Yoccoz	1a	C6 (EU664411), C10 (EU664412), C13 (AY568191)	S2 (AY569726)
C. nivalis	Bosnia	Sator Mnt. (6)	Henttonen, Niemimaa, B. Kryštufek	1a	C11 (EU664444), C7 (EU664415)	—
M. arvalis	France	Bourg-Saint-Maurice (2)	Yoccoz	1a	C5 (EU664413), C7 (EU664414)	S3 (EU664386)
M. arvalis	Italy	Trento (3)	Henttonen, Haukisalmi, Niemimaa	1a	C3 (EU664421)	—
D. bogdanovi	Bosnia	Zelengora (6)	Henttonen, Niemimaa, Kryštufek	1a	C2 (EU664443)	—
M. guentheri	Turkey	Gundalan (8)	Henttonen, Niemimaa, J. Laakkonen, A. Karatas, M. A. Öktem	1b	C14 (EF688329, EU664434, EF688329)	—
M. arvalis	Slovakia	Ráros (10)	A. Gubányi	1c	C15 (EF688330), C16 (EU664427)	S9 (EU664392)
M. arvalis	Hungary	Budakeszi-Telki (9)	Gubányi	1c	—	S9 (EU664387)
M. arvalis	Croatia	Novo Granica (5)	Henttonen, Niemimaa	1c	C17 (EU664432, EU664433)	S10 (EU664396)
M. arvalis	Kazakhstan	Taldygorgan (30)	Henttonen, Niemimaa, Laakkonen	1c	C18 (EU664430), C19 (EU664431)	—
M. arvalis	Kazakhstan	Taldygorgan (30)	Henttonen, Niemimaa, Laakkonen	—	C20 (EU744301), C21 (EU744302)	S9 (EU664393, EU664394, EU664395)
M. oeconomus	Finland	Pallasjärvi (21)	Henttonen, Niemimaa	2	C22 (AY423807), C24 (AY423808), C32 (AY423809), C36 (EU744303)	S4 (AY569727, EU664381)
M. oeconomus	Finland	Taivalkoski (22)	S. Savola	2	C54 (EU664442), C55 (EU664441)	—
M. agrestis	Finland	Kilpisjärvi (20)	Henttonen, Niemimaa	2	C35 (AY459356), C46 (AY423813), C47 (AY459355)	S6 (EU664377, EU664378) S8 (EU664388)
M. agrestis	Finland	Pallasjärvi (21)	Henttonen, Niemimaa	2	C32 (AY423812)	—
M. agrestis	Finland	Luhanka (25)	Henttonen, Niemimaa	2	C23 (EU664400)	—
M. agrestis	Finland	Pielinen (24)	J. Sundell	2	C25 (AY423810)	S5 (EU664375)
M. agrestis	Finland	Iitti (28)	I. K. Hanski	2	C26 (AY423811)	S6 (EU664376)
M. agrestis	Finland	Espoo (27)	Haukisalmi	2	C27 (AY423815)	—
M. agrestis	Finland	Maaninka (24)	R. Väänänen	2	C33 (AY423814)	—
M. agrestis	Finland	Turku (26)	K. Norrdahl	2	C39 (AY423822)	S6 (EU664379)
M. agrestis	Finland	Muhos (23)	Niemimaa	2	C45 (AY423820)	—
M. agrestis	Sweden	Umeå (19)	G. Olsson	2	C28 (AY423826), C29 (AY423827), C30 (AY423833), C34 (AY423825)	S6 (EU664381)
M. agrestis	Sweden	Åhus (15)	M. Jaarola	2	C36 (AY423816)	—

Appendix *Continued.*

Cestode species Host species	Country, state	Locality (number)	Collectors	Clade	COI haplotypes (GenBank numbers)	28S sequences (GenBank numbers)
M. agrestis	Sweden	Skara (16)	Jaarola	2	C37 (AY423819), C42 (AY423832)	—
M. agrestis	Sweden	Oxie (14)	Jaarola	2	C38 (AY423821)	—
M. agrestis	Sweden	Ödeshög (16)	Jaarola	2	C43 (AY423828)	S6 (EU664382)
M. agrestis	Sweden	Kubbe (19)	Jaarola	2	C50 (AY423818)	—
M. agrestis	Sweden	Strömsund (18)	Jaarola	2	C51 (AY423828)	—
M. agrestis	Sweden	Hallsberg (16)	Jaarola	2	C52 (AY423830)	—
M. agrestis	Sweden	Örebro (17)	Jaarola	2	C53 (AY423831)	S6 (EU664383)
M. agrestis	Sweden	Yngsjö (15)	Jaarola	2	C45 (AY423817)	—
M. agrestis	Norway	Kirkesdalen (20)	Henttonen, Niemimaa	2	C31 (AY459359), C48 (AY423836), C49 (AY459357, AY459358, AY459360)	—
M. agrestis	Denmark	Flöjstrup (12)	Henttonen, Niemimaa, H. Leirs	2	C40 (AY423823), C41 (AY423824)	S6 (EU664380)
M. agrestis	Scotland	Aberdeen (11)	Henttonen, Niemimaa, X. Lambin	2	C44 (AY423834)	S7 (AY569728)
M. agrestis	Scotland	Kielder (12)	Henttonen, Niemimaa, Lambin	2	C45 (AY423835)	—
M. oeconomus	Alaska, USA	GAAR (42,43)	BCP	3	C56 (EU664423)	—
M. oeconomus	Alaska, USA	Toolik Lake (44)	Henttonen, Niemimaa, J. Laakkonen	3	C65 (EU664406), C66 (EU664407)	S17 (EU664385)
M. oeconomus	Alaska, USA	YUCH (47,48)	BCP	3	C74 (EU664420)	—
M. oeconomus	Alaska, USA	WRST (50)	BCP	3	C75 (EU664425)	—
M. oeconomus	Alaska, USA	Kenai Fjords National Park (52)	BCP	3	—	S16 (EU664398)
M. oeconomus	Alaska, USA	Noatak National Preserve (41)	BCP	3	C75 (EU664417)	—
M. oeconomus	Russian Fed.	Kanin Peninsula (29)	V. Fedorov, K. Fredga	3	C57 (EU664401)	—
M. oeconomus	Russian Fed.	Ust Omchug (34)	BCP	3	C64 (AY568192)	S11 (AY569729)
M. oeconomus	Russian Fed.	Kegali River (36)	BCP	3	C67 (EU664408)	—
M. oeconomus	Russian Fed.	Elichan Lakes (35)	BCP	3	C68 (EU664409)	—
M. miurus	Alaska, USA	GAAR (42,43)	BCP	3	C59 (EU664422)	—
M. miurus	Canada	North Yukon (45)	BCP	3	C60 (EU664402)	—
M. hyperboreus	Russian Fed.	Elichan Lakes (35)	BCP	3	C69 (EU664410)	—
M. pennsylvanicus	Alaska, USA	YUCH (47,48)	BCP	3	C70 (EU664418)	—
M. pennsylvanicus	Alaska, USA	GAAR (42,43)	BCP	3	C71 (EU664424)	S15 (EU664390, EU664390)
M. oeconomus	Canada	North Yukon (45)	BCP	3	C61 (AY569193)	S12 (AY569730)
M. longicaudus	Alaska, USA	YUCH (47,48)	BCP	3	C62 (EU664419)	—
M. longicaudus	California, USA	Yosemite National Park	C. J. Conroy	3	C63 (EU664437)	—
M. mexicanus	New Mexico, USA		Henttonen, Niemimaa, J. Cook	3	C58 (EU664438)	S13 (EU664399)
M. fortis	Buryatia, Russian Fed.	Nesteriha (33)	Henttonen, Niemimaa, Laakkonen, G. Murueva	3	C73 (EU664436)	—
Microtus sp.	Russian Fed.	Tunguska River (31)	A. Lavikainen	3	—	S14 (EU664397)
My. rufocanus	Buryatia, Russian Fed.	Verhnaya Berezovka (32)	Henttonen, Niemimaa, Laakkonen, Murueva	3	C72 (EU664435)	—

Zoologica Scripta, **38**, 2, March 2009, pp199–220 • © 2008 The Authors. Journal compilation © 2008 The Norwegian Academy of Science and Letters

Appendix *Continued.*

Cestode species Host species	Country, state	Locality (number)	Collectors	Clade	COI haplotypes (GenBank numbers)	28S sequences (GenBank numbers)
Anoplocephaloides lemmi						—
L. trimucronatus	Canada, Yukon		Fedorov, Fredga, C. J. Krebs, A. Angerbjörn		C76 (AY568199)	—
L. trimucronatus	Russian Fed.	E. Kolyma River	BCP		C77 (AY568198)	S20 (A569734)
L. sibiricus	Russian Fed.	Taimyr Peninsula	Fedorov, Fredga		C78 (AY568197)	S21 (AY569733)
L. sibiricus	Russian Fed.	W. Kolyma River	Fedorov, Fredga		C79 (EU744308), C80 (EU744307)	—
Anoplocephaloides konrtimavichusi						—
S. borealis	Alaska, USA	Fairbanks	Henttonen, Niemimaa, Laakkonen		C81 (AY568195)	S18 (AY569731)
S. borealis	Alaska, USA	WRST	BCP		C82 (EU744306)	—
S. borealis	Alaska, USA	Tetlin NWR	BCP		C83 (EU744305)	—
S. borealis	Alaska, USA	YUCH	BCP		C84 (AY568196)	S19 (AY569732)
S. borealis	Alaska, USA	Tanacross	BCP		C85 (EU744304)	—
Microcephaloides krebsi	Canada, Nunavut	Byron Bay	Krebs, A. Kenney		C95 (AY568216)	S20 (AY569755)
Microcephaloides sp.	Finland	Pallasjärvi	Henttonen		C96 (EF688324)	S23 (AY569737)
Microcephaloides sp.	Alaska, USA	Toolik Lake	Henttonen, Niemimaa, Laakkonen		C97 (AY586611)	S21 (AY586607)
Microcephaloides sp.	Alaska, USA	GAAR	BCP		C98 (EF688306)	—
Microcephaloides sp.	Italy	Trento	Henttonen, Haukisalmi, Niemimaa		—	S22 (AY569735)
Paranoplocephala alternata	Russian Fed.	E. Kolyma River	Fedorov, Fredga		C101 (AY181426)	S40 (AY569743)
P. arctica	Alaska, USA	Kuparuk	D. J. Helmericks		C102 (AY181508)	—
P. arctica	Russian Fed.	Wrangel Island	Fedorov, Fredga		—	S41 (AY569744)
P. batzlii	Alaska, USA	GAAR	BCP		—	S31 (AY569764)
P. blanchardi	Norway	Kirkesdalen	Henttonen, Niemimaa		C103 (AY604729)	—
P. blanchardi	Finland	Heinävesi	Niemimaa		—	S32 (AY569746)
P. buryatiensis	Russian Fed.	Buryatia	Henttonen, Niemimaa, Laakkonen, Murueva		C110 (AY568203)	S37 (AY569756)
P. etholeni	Alaska, USA	Fairbanks	Henttonen, Niemimaa, Laakkonen		C104 (AY568214)	S34 (AY569774)
P. fellmani	Norway	Finse	Henttonen, Niemimaa, Laakkonen		C99 (AY568200)	S36 (AY569748)
P. gracilis	Scotland	Aberdeen	Henttonen, Lambin		C93 (AY395680)	S26 (AY569751)
P. jarrelli	Alaska, USA	WRST	BCP		—	S28 (AY586609)
P. kalelai	Norway	Narvik	Henttonen, Niemimaa		C106 (AY181513)	S27 (AY569753)
P. longivaginata	Russian Fed.	Buryatia	Henttonen, Niemimaa, Laakkonen, Murueva		C111 (DQ445261)	—
P. macrocephala	Alaska, USA	Fairbanks	Henttonen, Niemimaa, Laakkonen		C107 (AY181515)	—
P. macrocephala	Alaska, USA	Tanacross	BCP		C108 (AY181549)	—
P. macrocephala	Alaska, USA	YUCH	BCP		—	S30 (AY569758)
P. nordenskioeldi	Canada, Nunavut	Byron Bay	Krebs, Kenney		C109 (AY568204)	S38 (AY569759)

Appendix *Continued.*

Cestode species Host species	Country, state	Locality	Collectors	COI haplotypes (GenBank numbers)	28S sequences (GenBank numbers)
P. oeconomi	Hungary	Barbacs	Gubányi	C94 (AY568205)	S33 (AY569761)
P. omphalodes	Hungary	Dévaványa	Gubányi	—	S29 (AY569763)
P. primordialis	Canada, Yukon	Ivvavik NP	Fedorov, Fredga, Krebs, Angerbjörn	C105 (AY568218)	S39 (AY569766)
P. serrata	Russian Fed.	Yamal Peninnsula	Fedorov, Fredga	C100 (AY568220)	S35 (AY569767)
Outgroup					
Moniezia expansa	—	—	—	C86 (AB099693)	—
Moniezia benedini	—	—	—	C87 (AB099692)	—
Moniezia sp.	Finland	—	—	C88 (AY568213)	—
Anoplocephala magna	Australia	—	—	C89 (AY568206)	—
Anoplocephala perfoliata	Australia	—	—	C90 (AY568189)	—
Neandrya cuniculi	Spain	—	—	C91 (AY189957)	S24 (AY569723)
Andrya rhopalocephala	Hungary	—	—	C92 (AY189958)	S25 (AY569724)

GAAR, Gates of the Arctic National Park and Preserve. YUCH, Yukon-Charley Rivers National Preserve. WRST, Wrangel-St.Elias National Park and Preserve. NWR, National Wildlife Refuge. BCP, Beringian Coevolution Project (numerous collectors).

Zoologica Scripta, **38**, 2, March 2009, pp199–220 • © 2008 The Authors. Journal compilation © 2008 The Norwegian Academy of Science and Letters