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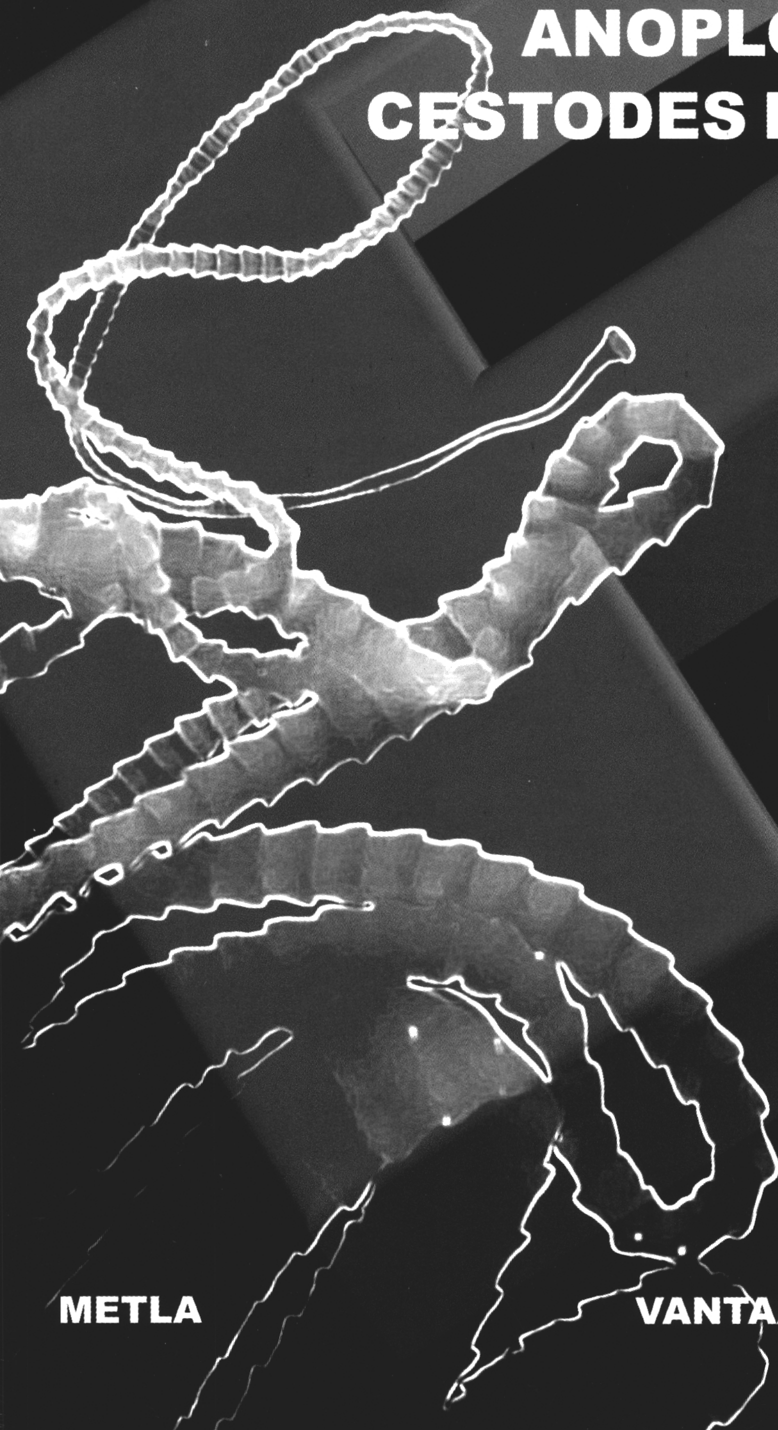
PHYLOGENY, PHYLETIC COEVOLUTION & PHYLOGEOGRAPHY OF ANOPLOCEPHALINE CESTODES IN MAMMALS



LOTTA M. WICKSTRÖM

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Phylogeny, phyletic coevolution and phylogeography of anoplocephaline cestodes in mammals

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Finnish Forest Research Institute
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Academic dissertation

To be presented, with the permission of the Faculty of Biosciences of the University of Helsinki, for public criticism in the Walter auditorium of the EE-building (Agnes Sjöbergin k. 2, Helsinki) on May 27th 2004 at noon.

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It depends on the sailor
how great the swell is going to be.
It's every sailor's dream
to drown in such a flood,
and you're the greatest of sailors.

To experience
'*As it is, we always misunderstand ourselves, and rarely understand others. Experience is of no ethical value. It is merely the name men give to their mistakes.*'
Oscar Wilde, 1890

Preface

This work has been carried out at the Vantaa Research Centre of the Finnish Forest Research Institute (METLA). I am grateful to Dr. Heikki Pajuoja, Director of the Vantaa Research Centre, and Professor emeritus Timo Kurkela, for providing excellent working facilities and an equal opportunity position for a wannabe parasitologist amongst the forest pathologists. Wood decaying fungi will be an active part of my forest walks forever. The pathologists have profoundly changed my view of trees as simply trees (they're not, they're actually universes hosting all kind of good and evil).

I want to thank my three supervisors, Docent Voitto Haukisalmi, Professor Jarkko Hantula and Professor Heikki Henttonen for their always-smiling attitude towards me. Whether it was pity or approval, you never knew. Combining tapeworm morphology with fungal genetics and mammalian ecology is not entirely straightforward. Working with experts in their own separate fields and additionally having these fields combined in the head of a former cell biologist, you're apt for a certain amount of conflict. Saila Varis, my right, left and middle hand, the true description of you is an angel sent from above, continuing as such even when not paid for. I have relied on many people, which evidently is the nature of the type of research I do, but I thrive on it when things go smoothly. Vote, I wish I had your patience and your touch for detail, Jarkko, I wish I was as ever cheerful and full of ideas, Heikki, I wish I saw the big picture and knew how to write shorter and less complicated sentences!

Although genetics and parasite/host biology were well tutored, the field of phylogeny was a great no-mans-land to all of us. I wish to express my gratitude towards the Centre for Evolutionary Biology, Uppsala University, for providing excellent courses and teaching. In particular, Johan Nylander (Uppsala) and Lacey Knowles (Museum of Zoology, University of Michigan), I wish I could have saved you from some of my stupidity; – Thank heavens for e-mail. Our Reverend Bayes has turned in his grave and I've been kneeling and blushing in front of Swofford, Kumar, Nei and the Maddisons, but we have grown towards a greater mutual understanding.

Another source of constant support has been the Department of Parasitic Worms, the Natural History Museum, London. Timothy Littlewood and Suzanne Williams, where would my trees of life be without you?! Tim, you've been a pain in the neck, but you have taught me prioritizing, persistence, language, and the profound meaning of the word disengage. With the LSU primers I finally got to grips with reality, thanks for sending me the first batch. Suzanne, even though I have not been your responsibility either, you have spent extortionate amounts of time on me. You're so sweet! Maarit Jaarola from Lund University and Vadim Fedorov, at the present based at the University of Alaska – fountains of phylogeographical knowledge. Thanks Maarit for pinning through it. I hate that so many people that contributed so much into this are not within hugging distance!

Meetings and workshops have enriched my life, shaken my assessments of right and wrong and opened up my blue eyes for all the greys that fit in-between. Time is not always relevant, plant a thought in my head, and it will quickly grow out of proportions and the need to test the idea becomes unbearable. Thanks to Ian Beveridge, Boyko Georgiev, Joe Cook and all the

others for help and fruitful discussions, and Robin Gasser for running some preliminary tests for me in his lab. I wish to thank Sonia Whitlow for many wonderfully fun shared meetings, sleeping quarters, parties and leisure trips. Thanks Sonia.

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Last but not least, I want to thank my patient family and friends. My mother for her contribution to the cover and the pictures in this thesis, my father and brother for immense help with a lot of small and bigger things, Anders for taking care of the household and Tia for not sighing too loud and for sleeping a lot. – My grandparents, Annika, Pi and friends for still acknowledging my existence. Work around the clock won't leave much for love and caring, but I changed that now.

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Fagerstad, April 2004

List of original articles

This thesis is based on the following articles, which are referred to in the text by the Roman numerals **I-VI**. Articles II-V are reprinted with kind permission of Blackwell Publishing Ltd.

I Wickström LM, Haukisalmi V, Varis S, Hantula J, Henttonen H. (2004) A molecular phylogeny of anoplocephaline cestodes in rodents and lagomorphs. **manuscript**

II Haukisalmi V, Wickström LM, Hantula J, Henttonen H (2001) Taxonomy, genetic differentiation and Holarctic biogeography of *Paranoplocephala* spp. (Cestoda: Anoplocephalidae) in collared lemmings (*Dicrostonyx*; Arvicolinae). *Biological Journal of the Linnean Society* **74**, 171-196.

III Wickström LM, Hantula J, Haukisalmi V, Henttonen H (2001) Genetic and morphometric variation in the Holarctic helminth parasite *Andrya arctica* (Cestoda, Anoplocephalidae) in relation to the divergence of its lemming hosts (*Dicrostonyx* spp.). *Zoological Journal of the Linnean Society* **131**, 443-457.

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

V Haukisalmi V, Wickström LM, Henttonen H, Hantula J, Gubanyi A (2003) Molecular and morphological evidence for multiple species within *Paranoplocephala omphalodes* (Cestoda, Anoplocephalidae) in *Microtus*-voles (Arvicolinae). *Zoologica Scripta* **33**, 277-290.

VI Wickström LM, Haukisalmi V, Varis S, Hantula J, Jaarola M, Henttonen H. (2004) The phylogeography of vole cestodes over a host contact zone in Fennoscandia. **manuscript**

The author's contribution

The author is responsible for all the molecular work and all the phylogenetic and population genetic analyses. Even when not the senior author, she has essentially contributed to the text, also for the non-molecular part of the work. The co-author V. Haukisalmi is responsible for the species descriptions and the morphometric analyses in papers II, III and V.

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1 Introduction

Phylogeography, as a formal discipline, is just over 15 years old (Avise *et al.* 1987) although the field's gestation began in the mid 1970s with the introduction of mitochondrial DNA analyses to population genetics, and the profound shift towards a genealogical perspective at the intraspecific level (now formalized as coalescent theory) that these methods prompted (Avise 1998). Phylogeography integrates molecular genetics, population genetics, phylogenetics, demography, ethology and historical biogeography. Emphasis is put on historical aspects of the contemporary distribution of gene genealogies.

There has been a wealth of studies concentrating on host-parasite coevolution, avian models forming one textbook example (Clayton & Moore 1997); although perhaps the most thoroughly studied system involves lice and their mammalian/avian hosts (Hafner & Page 1995). Also the number of phylogeographical studies has increased exponentially in recent years. Historical distribution and colonization patterns have been inferred for many of the rodent hosts involved in this work. However, few studies to date have strongly focused on the phylogeographic aspect of host-parasite coevolution as has been the aim of this thesis.

1.1 The main hosts: voles and lemmings

Arvicoline rodents (voles and lemmings) are numerically and functionally the dominant mammalian herbivores in the northern parts of the Holarctic region. The subfamily Arvicolinae (Muridae) consists of 26 genera and ca. 140 species, the most diverse genus being *Microtus* with ca. 60 recognized species. According to the molecular phylogenetic study of Conroy & Cook (1999) the evolutionary history of arvicoline rodents is characterized by two pulses of speciation, i.e. episodes of intensive radiation in a historically short period of time. Recent phylogeographic analyses have shown that lemmings and voles in North Europe, Siberia and North America represent divergent intraspecific evolutionary lineages, and that the historical fragmentation of populations and subsequent differentiation in glacial refugia is the underlying reason for the observed genetic structure (Brunhoff *et al.* 2003; Fedorov & Stenseth 2002; Jaarola *et al.* 1999).

The phylogenetic relationship between the main host group (rodents) and its proposed closest sister group (lagomorphs) has been controversial, but the most recent overall molecular evidence shows unequivocally that these mammalian orders are sister taxa and form a monophyletic group known as "Glires" (Murphy *et al.* 2001a; Murphy *et al.* 2001b).

1.1.1 Historical background: impact of Pleistocene glaciations on arvicoline rodents

Glacial and interglacial periods during the Pleistocene have strongly affected the evolution of biotas and fauna in the northern regions both in Eurasia and North America (Pielou 1991). The last Weichselian (=Wisconsin) glaciation covered simultaneously large areas, particularly in northern Europe and North America; however, similar glaciations have occurred repeatedly during the Pleistocene. Isolation of populations due to glacial barriers, or forest advances in the interstadials for cold adapted species, has often led to genetic differentiation, sometimes to closely related (sibling) species. Recolonization of fauna during the last postglacial period has also left its imprints in the genetic structure and diversity

of vertebrates in northern regions (Barnes *et al.* 2002; Hewitt 2000; Hewitt 1999). Furthermore, postglacial movements have brought isolated faunas into contact, thus creating contact and hybrid zones that still persist.

Beringia

Beringia, which spans northeast Siberia, Alaska and northwest Canada, provides an excellent natural laboratory for examining the impact of Pleistocene glaciations and their genetic consequences for northern organisms. Both as a glacial refugium and a route of colonization, the region has played dual roles in structuring the biogeography and genetic diversity of Holarctic species (Guthrie & Matthews 1971; Sher 1999). During the ice ages, Beringia was bounded by complex glacial systems that fragmented and condensed populations, isolating some organisms from conspecifics outside the refugium. Moreover, lowered sea levels during glacial periods exposed the continental shelf between Asia and North America, permitting an exchange of biota between continents. More species moved from Asia into North America than viceversa (Rausch 1994). Not all species took advantage of the land bridge, and some were apparently prevented from crossing by ecological limitations or competitive exclusion (Guthrie 2001; Hoffman 1994). Testable hypotheses have been proposed relating the impact of geologic events in this region to the diversification of mammals (Hoffman 1981; Sher 1999) and other fauna (Hoberg *et al.* 2003; Rausch 1994) and flora (Abbott & Brochmann 2003).

The glacial events have also separated components of the Siberian and North American faunas on a whole, leading to intraspecific divergence and speciation. A recent series of papers by V. Fedorov, K. Fredga and others gives a detailed picture of the evolutionary history of two Holarctic genera of lemmings, *Dicrostonyx* (collared lemmings) and *Lemmus* (true lemmings) (Fedorov *et al.* 1999a; Fedorov *et al.* 1999b; Fedorov 1999; Fedorov & Goropashnaya 1999; Fedorov *et al.* 2003; Fedorov & Stenseth 2002; Fredga *et al.* 1999). Also the historical biogeography of red-backed voles (Cook *et al.* 2004) and tundra voles (Brunhoff 2003) seems to be intimately tied to and affected by the history of northern paleoenvironments.

Fennoscandia

When the ice shield covering Fennoscandia started to melt ca. 12 000 years ago, two main recolonization routes became available: one from the south through present Denmark and southern Sweden, and another from the east through present southeastern Finland. Some of the species that managed to use both colonization routes, including the field vole *Microtus agrestis*, bank vole *Clethrionomys glareolus*, common shrew *Sorex araneus* and brown bear *Ursus arctos*, now have a contact/hybrid zone in central Sweden, where the two main lineages meet (Jaarola *et al.* 1999). The contact zones of various species are largely overlapping, but not identical. The southern route from Denmark to Sweden was intermittently open during the development of the future Baltic Sea, which is reflected in the present genetic structure of bank and field vole populations in southern and central Sweden. A third potential migration route along the northern coast of Russia and Norway could have existed during late glacial times when the sea level was much lower than at present (Björck 1995). There is some evidence of genetic distinctiveness of populations of small rodents along the northern coast of Norway (Brunhoff *et al.* 2003). It is evident that at least two different genetic lineages of field and bank voles have immigrated into Finland from southeast and east, respectively, but the exact distributions of these lineages are still unkno-

wn and, furthermore, they seem to differ between species (Jaarola & Tegelström 1995; Tegelström 1987).

1.2 The parasites: anoplocephaline cestodes

Cestodes of the subfamily Anoplocephalinae (Fig. 1) are known from all continents (except Antarctica), suggesting that this taxon appeared prior to the break-up of Gondwana 130-140 million years ago. The anoplocephaline radiation has been most intense in rodents and lagomorphs in the Holarctic region, but considerable diversity has also been found in Australia, particularly in marsupials (Beveridge 1994). Anoplocephaline cestodes use soil arthropods such as oribatid mites and collembolans as their intermediate hosts (Gleason & Buckner 1979, Fig. 2). The role of these fairly sedentary intermediate hosts in dispersal and speciation of the parasites has not been studied for anoplocephalines parasitizing arvicoline rodents.

Anoplocephaline cestodes of rodents include two recognized genera, *Paranoplocephala* and *Anoplocephaloides*. The more species-rich genus, *Paranoplocephala*, occurs exclusively in rodents, particularly in voles and lemmings. Earlier, also *Andrya* was included (Tenora *et al.* 1986a; Tenora *et al.* 1986b), but species in voles and lemmings have now been transferred to *Paranoplocephala* (Haukisalmi *et al.* 2002) and *Andrya* is presently only found from leporide hosts. Prior to our research, the true diversity of *Paranoplocephala* spp. was very poorly understood, since most of the species were, and partly remain, inadequately described. We now know that there are at least 50 species of *Paranoplocephala* in rodents, ca. 40 of these in arvicolines (Haukisalmi *et al.* 2002). The latter figure includes 15 species described or recognized by our research group (Haukisalmi & Henttonen 2000; Haukisalmi & Henttonen 2001; Haukisalmi & Henttonen 2003; Haukisalmi *et al.* 2002). Based on the number of host species and the number of parasite species in well-examined hosts, it has been predicted that there are at least 120 species of *Paranoplocephala* in arvicoline rodents and possibly up to 200 species of anoplocephaline cestodes in all rodents and lagomorphs (V. Haukisalmi, pers. comm.).

Fig. 1 *Paranoplocephala macrocephala* (Cestoda: Anoplocephalidae) found in a meadow vole in Alaska.

The endoparasite fauna of some of the arvicoline species, particularly in Europe and Alaska, has been intensively studied, but for most of the species the parasitological data are still scarce or absent. However, we do know that the most diverse component of the helminth fauna of arvicoline rodents consists of cestodes of the subfamily Anoplocephalinae (family Anoplocephalidae). Anoplocephaline cestodes are taxonomically a challenging group, since they lack hooks and are also devoid of other good diagnostic characters (Beveridge 1994). Molecular markers are therefore necessary tools in the taxonomy of anoplocephaline cestodes.

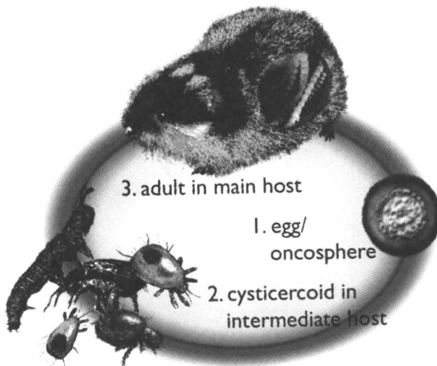

Fig. 2 Anoplocephaline cestodes have a typical cyclophyllidean life cycle, including soil arthropods such as oribatid mites and collembolans as intermediate hosts (2). Soil arthropods are infected when they consume rodent faeces containing gravid proglottids or eggs. The oncosphere (1) penetrates the intestinal wall of the arthropod and enters the hemocoel where it develops into the cysticercoid stage (2). When the infected arthropod is ingested by a rodent definitive host (3), the cysticercoid is liberated in the small intestine and develops into an adult cestode.

1.3 Phyletic coevolution

The phyletic coevolution between parasites and their hosts has been acknowledged as a major scientific problem for at least 150 years, going back to Darwin (see Hoberg *et al.* 1997). The earliest hypotheses emphasized strict cospeciation between parasites and their hosts, to the degree that parasites were used for determining phylogenetic relationships among hosts. This approach was indeed successful, demonstrating, e.g., that humans share related parasites with great apes, our closest living relatives. However, the idea of obligatory cospeciation was later shown to be too simplistic and flawed by circular reasoning, and other determinants of parasite speciation, particularly the historical shift of parasites from one host lineage to another, were also recognized.

Research on the (co)evolutionary history of parasites has been revolutionized by the availability of high-resolution molecular markers. There now exist a number of interspecific phylogenetic studies on a wide array of parasites. Still, surprisingly few analyses on intraspecific phylogeny (phylogeography) and phyletic coevolution between parasites and their hosts are available. The latter aspect has been most thoroughly analysed for lice and their mammalian and avian hosts, the lice-pocket gopher system now forming a textbook example of host-parasite coevolution (e.g. Hafner & Page 1995). However, most mammalian endoparasites differ drastically from lice in host-specificity and life-history features, and the generality of the coevolutionary patterns detected so far remains unsettled.

2 Aims of the study

In this thesis morphometric, modern molecular and analytical approaches were combined to elucidate the morphological and genetic affinities and evolutionary history of intestinal flatworms (Cestoda: Anoplocephalidae) of arvicoline rodents (voles and lemmings) in the Holarctic region. In addition to an attempt at resolving the phylogeny in this group, species complexes within the genera *Paranoplocephala* and *Anoplocephaloides* were explored. Some of the host species (lemmings, voles) have been subjected to detailed phylogeographic analyses in the Holarctic region, particularly in Beringia and Fennoscandia. Patterns of genetic diversity at various levels of evolutionary divergence were contrasted with the knowledge of geological history and phylogeography of the host. As parasites are soft-

bodied animals and no molecular clock therefore can be calibrated from a fossil record, phyletic coevolution between parasites and hosts were scrutinized from a speculative point of view and different patterns discussed and compared.

By assessing patterns of contemporary genetic diversity and combining and comparing the results hereof with morphological analyses performed by my colleague Voitto Haukisalmi, I have aimed to elucidate the phylogenetic and phylogeographic affinities of members of the anoplocephaline subfamily. The first paper in this thesis focuses on phylogeny and taxonomy of anoplocephaline genera from arvicoline hosts. –Does uterine morphology reflect the evolutionary relationships in this group, and are parasite genera in voles and lemmings mono- or paraphyletic? Paper II concentrates on species in collared lemmings, asking what is the true diversity and biogeography of *Paranoplocephala* spp. in collared lemmings. Papers III and IV comprise the phylogeography of a Holarcic species complex, and explore the possibilities of using parasites as additional markers when attempting to reconstruct the colonization history of the hosts. –Does the phylogeography of *Paranoplocephala arctica* and *P. alternata* coincide with the phylogeography of the hosts in the Holarctic? Paper V aims to reveal the true species diversity of a Holarctic host generalist species based on combined molecular and morphological evidence. –How many species/clades of *P. omphalodes* can be genetically and morphologically distinguished? 'Cryptic diversity' is one of the main themes encountered throughout this thesis work. The last paper focuses on phylogeography in Fennoscandia, and in particular, I asked whether a host contact zone generated by post-glacial (re)colonisation, had left its genetic imprints also in three parasite species present in these vole hosts.

3 Materials and methods

3.1 Samples

For this thesis, more than 2700 tapeworm samples from arvicoline rodents have been collected, morphologically determined and preserved for both genetic and morphometric analysis. About 60% of the catalogued specimens belong to the subfamily Anoplocephalinae, and of these over 600 individuals have been subjected to detailed morphological and/or genetic analysis. Total genomic DNA was used for all genetic applications.

3.2 Molecular markers – an overview

As the anoplocephaline cestodes in voles and lemmings have not been subjected to genetic analyses before the current study, the testing of a wide variety of universal markers and markers successfully applied for other cestode or nematode parasites, was followed by rigorous work modifying and designing specific markers that detected variation across the desired species range. A general description of the methods used in this study is presented here. Detailed laboratory procedures and analytical methods are specified in the original papers.

The projects included in this thesis have a great variance as to geographical spread of the data and species level organisation. As the temporal depth of interest varied between projects, almost the whole known genre of molecular markers commonly used in population genetics/phylogenetics have been applied, ranging from micro- and minisatellite based approaches to mitochondrial and nuclear DNA sequences. Microsatellites have been used as a basis for the design of Sequence Tagged Si-

tes (STS) (III), an application that allows the usage of sequences in variable regions of the genome as loci that can be analysed for allelic composition through a variety of electrophoretic approaches. Micro- and minisatellites were successfully applied for distinguishing between species (II, III). However, for in depth population genetics and phylogenetics, mitochondrial cytochrome c oxidase subunit I (COI)(I, IV, V, VI) and nuclear ribosomal sequences (internal transcribed spacer 1; ITS1 in I, II, IV, V and domains D1-D3 of 28S rDNA in I) were employed for improved resolution and accuracy. The evolution of mitochondrial (mt) DNA in hermaphroditic cestodes is rather similar to that in asexually reproducing organisms, as the effective population size of mtDNA equals that of individuals. Hence, for example the three-times rule of thumb (Palumbi *et al.* 2001) that predicts nuclear coalescence from mitochondrial data is not applicable as the predictions are based on sequences from organisms with separate sexes that show a differing ratio between mitochondrial and nuclear evolution than what could be expected for hermaphrodites.

3.3 Phylogenetic analyses – an overview

To the extent that a gene tree is an accurate reflection of a species tree, where the null hypothesis may be that we expect strict cophyly between the two, gene trees can be used for making inferences about a species past. Given the high variance of the coalescent process, confidence limits are expected to be very broad and phylogeographic interpretation needs to be made with much caution. Evolutionary processes other than genetic drift leave different signatures in the structure of evolutionary trees because of how they affect the coalescence time of alleles (Page & Holmes 1998). For example, positive selection (single locus) or a population bottleneck (all loci) produce genealogies with short depth in time, compared to genetic drift, and with very little branching structure near the root. Insufficient time for alleles to reach high frequencies and produce a structured population shows up as polytomies. Polytomies may be the result of homoplasy due to saturation, i.e. 'soft' rather than 'hard' (Maddison & Maddison 2000), or a paucity of synapomorphies along short internodal branches due to rapid pulses of speciation. In a gene tree based approach we can consider a diverse array of processes, ranging from population subdivision, range expansion, geographically structured migration to isolation by distance, but we do not tend to consider the phylogeographic inference in a statistical framework. The gene tree based approach mostly used in this thesis (as opposed to a population genetic approach where a statistical framework is used for parameter estimation) cannot generate support for alternative conclusions and is further compromised by an absence of exact methods for considering probable errors in the inference. Today, there is an obvious trend towards formalizing tests of phylogeographic scenarios. However, depending on the particular methodological approach, there are fundamental differences among these historical inferences. Largely qualitative, post-hoc explanations are mostly applied in my thesis, combined with tests of significant structure.

In addition to the analysis of multiple unlinked marker loci, different methods of phylogenetic inference were employed. Phylogenetic methods can be classified by the method used to build the tree and by the type of data used. A tree building method should have five desirable properties: efficiency, power, consistency, robustness and falsifiability. All current methods emphasize one or more of these criteria at the expense of the remainder (Page & Holmes 1998). In this study neigh-

bour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis of phylogeny have been the methods used. NJ, operating on distance matrices, has been used for its computational speed and uniqueness of result whereas the others, as discrete statistical methods, operate directly on the sequences. MP chooses the tree that requires the fewest evolutionary changes whereas ML chooses the tree that is most likely to have produced the observed data under the model used. As ML is computationally difficult, especially for confidence intervals, Bayesian inference of phylogeny was implemented as it generates numerical solutions to difficult, multi-dimensional problems and is superior in speed. Also, as the full heuristic tree search option in ML seldom can be used for big data sets due to computational restrictions, Metropolis Coupled Markov Chain Monte Carlo simulations implemented in the Bayesian approach offer an exhaustive coverage of the parameter space. The pitfalls in Bayesian inference are, like for ML, the goodness of fit of a model to the observed data and not to get caught in a local optimum, i.e. in Bayesian analysis, getting the chains to converge on a stationary distribution that is not the real optimum but a local optimum. The most common approach to check for convergence of chains is to compare independent runs starting from different points in parameter space. To what extent posterior distribution is influenced by the prior can be evaluated by applying different priors. Different models of nucleotide substitution will generate differing posterior distributions from the same data. Likelihood ratio tests or Akaike's information criterion (*AIC,* Akaike 1974) can be used to examine the goodness of fit of a model to the observed data. Sophisticated models were usually suggested for the data presented herein. The choice of model has been much debated. Gaut & Lewis (1995) and Yang (1997) have shown that a sophisticated model does not necessarily give the correct topology with a higher probability than a simple model. The results of their studies counterbalanced by Bruno & Halpern (1999). However, when the number of sequences is large, a simple model usually gives better results than a complex model as long as the sequence length is relatively short (Nei & Kumar 2000). Recent studies also suggest that substantially more data should be used in phylogeny reconstruction than has to date been fashionable (Rokas *et al.* 2003).

4 Results and discussion

4.1 Phylogeny of the Anoplocephalinae (I, II)

Hoberg and colleagues (1999) have shown that extensive homoplasy of uterine structures is evident in cyclophyllidean cestodes at the family and subfamily level (including Anoplocephalidae). The results of paper I show that a corresponding situation could prevail also at the generic level within Anoplocephalinae. Uterine morphology within *Paranoplocephala* and *Anoplocephaloides* was found to be very variable. The division into three main categories applied by Beveridge and others (e.g. Beveridge 1994) was considered inappropriate, as intermediate forms could not clearly be assigned to one category or the other. The evolution of the arvicoline hosts is characterized by pulses of speciation early in the history of the group detected as 'hard polytomies' (Conroy & Cook 1999; Conroy & Cook 2000). Bootstrap support and high posterior probabilities for nodes above and below polytomies in my data indicate diversification over a short period of time for the parasites, rather than saturation effects. The po-

lytomy observed in uncoupled markers for the anoplocephaline cestodes from rodent hosts (I) suggests phyletic coevolution as the predominant mode of speciation on family level. Host shifts between genera appear to be frequent (*Dicrostonyx, Lemmus, Microtus, Clethrionomys, Chionomys*) and may account for subsequent parasite speciation (I, II, Hoberg 1995).

The results of paper II revealed the diversity of *Paranoplocephala* in collared lemmings. The number of host-specific cestode species matched the pattern of species divergence of collared lemmings in the Palearctic vs. the Nearctic, further corroborating the close relationships between rodent hosts and their parasites from a phylogeographical perspective. The lack of unambiguous descriptive characters, a general problem in anoplocephaline taxonomy, is also apparent for *Anoplocephaloides* and *Paranoplocephala* (I-V). The close relationships between species/genera (I, II) and in some cases substantial intraspecific divergence (III, IV, VI) combined with rapid radiation generate sequence data sets that produce considerable challenge to any tree building method.

4.2 Species complexes within *Paranoplocephala* and *Anoplocephaloides*

4.2.1 Paranoplocephala arctica/alternata (III , IV)

P. alternata and *P. arctica* are host specific cestodes of collared lemmings (*Dicrostonyx*). *P. alternata* has a Holarctic distribution whereas *P. arctica* is Nearctic. Whether the two morphospecies should be regarded as separate species or conspecific is still ambiguous (I). Of the sequence-based phylogenies, 28S rDNA separate the morphospecies, ITS1 recognises *P. alternata* as monophyletic whereas mtDNA (COI) recognises both species as paraphyletic (I). The mtDNA phylogeny (IV) divides the species complex into one main Nearctic and one main Palearctic phylogroup, corresponding to the main phylogeographical division of the host. This main division was not, however, observed in the study based on STS and minisatellite markers (III). This may have been due to lower resolution of STS (or lack of deeper level phylogenetic signal), lower mutation rates, the lack of information on phylogenetic relationships and a high level of homoplasy among alleles (minisatellites). Although phylogeographical congruence was observed between host and parasite in the Palearctic (III, IV), over Bering Strait (IV) and to some extent within the Nearctic (IV), parasite phylogeny and host relationships do not show a complete match. The occurrence of the morphospecies *P. arctica* and several separate mtDNA lineages within eastern Beringia, suggests eastern Beringia as a possible refugial area with a long uninterrupted demographic history. A separate parasite lineage present on only the northernmost Canadian Arctic islands could be interpreted as support for refugial theories proposed for the hosts, involving glacial survival also in northern Arctic Canada (Fedorov & Goropashnaya 1999; Fedorov & Stenseth 2002). Although eastern Beringia might have been most likely to serve as a centre for parasite diversification, it was probably not the only glacial refugium in the Nearctic. Additionally, the parasite phylogeny suggests a history of colonization and secondary patterns of dispersal from Beringia into the Canadian Arctic, an event not proposed by the host phylogenies alone.

Assuming the phylogenetic patterns observed for the parasites are temporally congruent with those of the hosts, the phylogeography of the *P. arctica/alternata* species complex imply strong codivergence with only

occasionally decoupled host and parasite phylogenies (III, IV). However, the geographical distribution of these lineages does not always converge, and cryptic parasite lineages without host lineage counterparts have been detected (IV).

4.2.2 Paranoplocephala omphalodes(V)

In addition to *P. arctica/alternata*, a second species complex was detected within *Paranoplocephala* when analysing the traditionally recognised host generalist *P. omphalodes*, a Holarctic parasite of *Microtus* voles. The increasing use of molecular markers as a taxonomical tool (Blouin 2002; McManus & Bowles 1996) has revealed a considerable degree of 'hidden', or cryptic, diversity in various groups of parasites (e.g. Anderson *et al.* 1998; Nadler 1990). Sequence data from COI (V) and 28S rDNA (I) as well as morphometrics (V) successfully distinguished four separate clades probably representing three distinct, largely host specific species (V). The nominal *P. omphalodes* is shown to be a parasite of *Microtus arvalis* (host species of the holotype), *Microtus agrestis* and *Clethrionomys glareolus* in Europe. *Microtus oeconomus* harbours two host-specific, allopatric and possibly conspecific clades, one with a Holarctic and another with an eastern Beringian (Alaskan) distribution. The eastern Beringian endemic *M. miurus* is also parasitized by a host-specific, morphologically divergent species of *Paranoplocephala*. In addition to these, the species complex included *Paranoplocephala kalelai* from *Clethrionomys rufocanus* (and occasionally from sympatric *C. glareolus*) and *P. macrocephala* from *Microtus* spp. The COI phylogeny positioned the true *P. omphalodes* outside the assemblage (V), whereas 28S rDNA showed less resolution and included this species in the assemblage (I). The combined data set of COI and 28S rDNA placed the nominal *P. omphalodes* as closest sister group to the complex (I).

Four of the six recognised cestode clades belonging to this complex occur in eastern Beringia, supporting the role of Beringia as a centre of diversification. Two clades are probably endemic to this region. Additionally, the Holarctic *P.* cf. *omphalodes* clade includes an eastern Beringian subclade and also *P. macrocephala* show supported structure in Alaska. Assuming that *P. kalelai* has diverged as a consequence of a host shift, the divergence within the species complex (excluding the European true *P. omphalodes*) seems to parallel the evolutionary history of *Microtus*, i.e. the studied species of *Paranoplocephala* spp. in North America have probably diverged since representatives of *Microtus* crossed the Bering Strait (cf. Rauch 1994; Conroy & Cook 2000). The presence of two parasite clades (clade II and III, paper V) in *M. oeconomus* was surprising, as the host *M. oeconomus* is relatively undifferentiated in Alaska (Brunhoff *et al.* 2003). Neither does the structure within the Holarctic parasite clade II reflect the main *M. oeconomus* host split at the Urals. As for the *P. alternata/arctica* species complex, there is deep phyletic coevolution with the hosts, but deviant patterns are also acknowledged.

4.2.3 Other discovered species complexes of anoplocephaline cestodes

The Holarctic *Anoplocephaloides dentata* formed three supported clades in the phylogenies that probably represent separate species (I). The nominal *A. dentata* is a parasite of *Chionomys* and, based on preliminary studies, probably also of sympatric *Microtus* voles in central Europe. Of the two northern clades, one is restricted to northern Europe (individuals 3 and 4, paper I) whereas the other clade (individuals 5, 6 and 7 in paper I) is Holarctic. Neither of these proposed northern species expresses strict host specificity wit-

hin *Microtus*. Morphologically, individuals 6 and 7 from USA occurring in *Microtus* voles were assigned to the Nearctic species *A. troeschi*. *A. troeschi*, however, has been described from *Microtus pennsylvanicus* (Rausch & Schiller 1946). As we have no samples of *A. troeschi* from *M. pennsylvanicus*, and as all Nearctic specimens of *A. troeschi* from *Microtus* hosts mix in the gene trees with non-European Palearctic *A.*cf. *dentata* from *Microtus* hosts, we have assigned all the individuals to the non-European (Holarctic) *A.* cf. *dentata*. This species complex will be subjected to multivariate morphometrics and detailed molecular analyses based on larger samples from the Holarctic region. The species complex seems, at this point, to consist of geographically restricted clades with less host specificity than observed in the *P. omphalodes* species complex.

The Holarctic *A. variabilis* was discovered to be paraphyletic in the 28S rDNA phylogeny (I). *A. variabilis* from Northern Europe formed a species complex with *A. tenoramuraiae* from the Alpine region, whereas *A. variabilis* from the east Beringian endemic *Microtus miurus* (Rausch 1994) formed its own Nearctic lineage. *A. variabilis* is also expected to include multiple (host-specific) species when subjected to a more detailed study. The present analysis (I) and ongoing research clearly demonstrates the importance of broad-scale, synoptic field collections for understanding the true diversity and evolutionary history of northern host-parasite assemblages.

4.3 A host contact zone affecting parasite dispersal in Fennoscandia (VI)

The extent of phylogeographical congruence between hosts and intestinal parasites, even when accounting for historical associations, can be affected by several factors in parasite biology. For example, facultative life cycles through intermediate hosts, inbreeding and small population sizes in comparison to hosts create different underlying assumptions for coalescent processes in the parasites. The phylogeographical patterns of three parasite species with differing abundance and host specificity were compared over a host contact zone in northern central Sweden (Jaarola & Tegelström 1995; Jaarola & Tegelström 1996; Tegelström 1987). As for the main hosts, two main mtDNA lineages were also recorded for all parasite species. Two of the parasite species, *Anoplocpehaloides* cf. *dentata* (Northern European) and *Paranoplocephala blanchardi*, showed phylogeographical patterns that coincided either roughly or strictly with the phylogeographies of their main hosts over the contact zone. The pattern observed for the common host generalist *P. gracilis* did not. Differences observed in phylogeogrphical structure for the parasites correlated with their host specificity and abundance, corroborating the role of host choice and commonness as determinants of parasite dispersal and gene flow. A plausible interpretation of the data is that host contact zones more easily delimit rare host-specific parasites than common generalists.

Assuming the (re)colonization history of parasite lineages is synchronous with that of their hosts, we would expect the phylogeographical patterns of parasites in Fennoscandia to mirror those of their hosts. The east/west pattern that we have observed for the parasites (VI) could be interpreted as synchrony of host-parasite assemblages prior to the colonization of Fennoscandia and later mixing over the host contact zone. This model seems highly likely for *P. blanchardi*, and is also plausible for *A.* cf. *dentata*. An alternative model would involve the presence of mtDNA lineages in both the Eastern and the Western

host lineages during the colonization of Fennoscandia, a scenario likely for *P. gracilis*. However, *P. gracilis* can also be found from other, accidental, host species that do not display geographical subdivision in north-central Sweden. A parasite's ability to cross the main host contact zone and infect main hosts of the opposite evolutionary lineage would create geographical mixing of assumed historically separated lineages for the parasite.

The phylogeographical patterns observed for the parasite species would imply that a host contact zone does act as a limiting factor for dispersal and gene flow for the parasites. The phylogeography of at least two of the three parasite species could serve as additional evidence for the bidirectional colonization of Fennoscandia, if considering the main mtDNA lineages as separate evolutionary units, i.e. synchronic and sympatric host-parasite assemblages prior to the recolonization of Fennoscandia. In addition to *A.* cf. *dentata* (VI), also *P. kalelai* (V) express two separate lineages in northern Fennoscandia. The lineages are supported in COI (V and I) and 28S rDNA (I) sequence data.

5 Conclusions

The evolution of anoplocephaline cestodes of arvicoline rodents seem to be intimately tied to host evolution, and characterized by the same 'pulses of speciation' that are argued to be the reason for unresolved relationships within Arvicolinae. Phyletic coevolution between host and parasite was found to be the predominant mode of speciation at the family and subfamily level. Classification, based primarily on uterine structure, has been the most recently proposed systematic scheme for Anoplocephalinae cestodes. The variability among uterine structures and topologies recovered from the gene trees do not support a classification scheme based on uterine development. The importance of within species sampling for phylogeny reconstruction has been proved essential for anoplocephaline cestodes. As Anoplocephalinae include inadequately known genera devoid of good descriptive morphological characters (Beveridge 1994), a lack of rigorous within species sampling might overlook issues of considerable importance to phylogeny, phylogeographical history and speciation.

The application of molecular markers has revealed pronounced undetected/cryptic diversity, and higher degrees of host-specificity than suggested by traditional taxonomic methods. The *Paranoplocephala omphalodes* and the *A. dentata* species complexes show the drastically changed ideas of species diversity and host-specificity in anoplocephaline cestodes of voles. Species diversity and phylogeographical structure seems to be most pronounced in the Beringian region, emphasising the role of this ice age refugium as a centre of diversification for anoplocephaline parasites. However, phylogenies recovered for the *P. alternata/arctica* species complex do not exclude the possibility of glacial refugia on the northernmost Canadian Arctic Islands, an additional theory proposed for the hosts. In Fennoscandia, the presence of supported Eastern and Western parasite clades in hosts of opposite evolutionary lineages also points towards deep phyletic coevolution. The geographical distribution of the lineages correlates with parasite host specificity and commonness. Several species of anoplocephaline cestodes parasitizing voles and lemmings have been thoroughly studied in this thesis work, but the role of the intermediate hosts in dispersal and speciation has not been evaluated.

Rapid inter- and intraspecific radiation in arvicoline rodents during the Pleistocene environmental changes have provided diverse research opportunities for the study of host-

parasite coevolution in the Holarctic (I-VI). Congruence between host and parasite phylogeographies has been found in many cases. However, almost equally often, discrepancies are observed in the patterns and a lack of congruence recognized in roughly equally many cases. No formal cospeciation analyses have been performed, and considering the unresolved phylogenies for hosts and parasites and the amount of unresolved species issues within Anoplocephalinae, a formal cospeciation analysis actually cannot be performed at present. However, there are situations where the parasites seem to have diversified in the absence of host divergence, and other occasions of host shifts and possible extinctions. Every association is, nonetheless, unique, and general predictions vis-à-vis patterns of host-parasite coevolution could not be made. The consensus of this thesis work would be the recognition of every host-parasite system as a unique assemblage. No general patterns can be applied when dealing with different systems, apparently not even within the same parasite and host genera.

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A molecular phylogeny of anoplocephaline cestodes in rodents and lagomorphs

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Abstract

A molecular phylogeny is presented for the subfamily Anoplocephalinae (including representatives of all genera in the Holarctic region) based on sequence data from mitochondrial cytochrome c oxidase I (COI), the nuclear 28S rRNA gene and the internal transcribed spacer region I of rRNA (ITS1). Emphasis was put on 28 species parasitizing voles and lemmings, and the phylogeny showed considerable disagreement with earlier hypotheses derived from morphological data. In particular, the monophyly of rodent parasite genera with differing uterine development contradicted the view of uterine development being a primary determinant of deeper phylogenetic splits. The relationships between the anoplocephaline genera, *Anoplocephaloides* and *Paranoplocephala* parasitizing rodents, remained unresolved. *Andrya* from leporids was recognised as the closest sister group to the arvicoline-hosted genera (also including *Diandrya* from marmots). The molecular phylogeny provides a framework for the adaptive radiation of anoplocephalines, showing deep cospeciation on the familial level, but probably including frequent shifts between closely related host genera. Consistent support for nodes above and below unresolved polytomies indicates a rapid radiation involving nearly simultaneous diversification of many lineages, a scenario also proposed for the arvicoline hosts.

Keywords: Cestoda, coevolution, Holarctic region, intestinal parasite, molecular evolution, phylogeny

Introduction

The anoplocephaline cestodes (order Cyclophyllidea, family Anoplocephalidae) represent a diverse group of parasites infecting both terrestrial mammals and birds. Based on the number of genera present in these hosts, the most important radiation of the anoplocephalines has been in the rodents and lagomorphs (Beveridge 1994). Also, in a broader phylogenetic context, terrestrial mammals are recognised as the basal hosts for cyclophyllidean diversification (Hoberg *et al.* 1999).

Phylogenetic schemes for anoplocephalid cestodes have been proposed by Baer (1927), Spasskij (1951), Tenora (1976) and Beveridge (1994), but none of these have relied on formal methods of phylogeny construction. Spasskij (1951) incorporated the patterns of uterine development in the systematical arrangement of Anoplocephalidae (sensu Spasskij 1951) by distinguishing the subfamily Anoplocephalinae with a tubular early uterus and the subfamily Monieziinae with a reticulated early uterus. This arrangement was later adopted by Tenora (1976), but not by Yamaguti (1959), Schmidt (1986) and Beveridge (1994), because of ontogenic changes in uterine morphology (Yamaguti, 1959) or because of uncertainties concerning the uterine development in a number of anoplocephalid genera (Schmidt 1986; Beveridge 1994). The most substantiated phylogenetic hypothesis for Anoplocephalinae is that of Beveridge (1994), who based his view on selected morphological features, uterine development being the primary determinant of the deeper phy-

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logenetic splits. Beveridge (1994) suggested that species with partly and completely reticulated uterus form a single lineage, partly reticulated uterus being ancestral to completely reticulated uterus. Taxa with tubular early uteri were also suggested to form a monophyletic group, but their relationship with the group having reticulated uteri was not defined.

Beveridge (1994) also emphasised the importance of the duplication of genitalia, first proposed by Baer (1955), as a mechanism of divergence in Anoplocephalinae. According to Beveridge (1994), the genera in the pairs *Schizorchis-Mosgovoyia* and *Andrya-Diandrya* are separated from their sister taxa primarily by the number of genitalia per segment (single-double).

Most of the species included in this study parasitize voles and lemmings of the genera *Microtus, Clethrionomys, Chionomys, Lemmus, Dicrostonyx* and *Synaptomys* (Arvicolinae, Rodentia). The phylogeny of these genera constitutes a classical example of rapid adaptive radiation resulting in about 65 extant species distributed throughout the Palearctic and the Nearctic regions (Musser & Carleton 1993; Nowak 1999). The majority of extant *Microtus* species do not appear in the fossil record until middle Pleistocene about 0.7-0.5 million years ago (Chaline *et al.* 1999; Rabeder 1986; Richmond 1996), and it has even been suggested that some species trace their origin to the last glaciation (e.g. Brunet-Lecomte & Chaline 1990; Chaline & Graf 1988). The phylogenetic relationships within *Microtus* and its closest relatives are uncertain and difficulties remain both in delimiting species and defining subgenera. Unresolved relationships suggest a rapid and potentially simultaneous radiation of a widespread ancestor early in the history of the genus (Jaarola *et al.* 2004).

Here we present the first molecular phylogenetic hypothesis for species of anoplocephaline cestodes parasitizing rodents and lagomorphs in the Holarctic region, based on data from two nuclear (28S ribosomal RNA gene, 28S rDNA and internal transcribed spacer I, ITS1) and one mitochondrial (cytochrome c oxidase I, COI) sequence. As Jaarola *et al.* (2004) and Jaarola & Searle (2003) demonstrated that speciation still is an ongoing process in *Microtus,* and as our earlier studies have revealed cryptic species complexes within *Paranoplocephala* (Haukisalmi *et al.* 2004; Wickström *et al.* 2001; Wickström *et al.* 2003; Wickström *et al.* unpublished), we have, when available, included several specimens of the same species in the data. The resulting phylogeny is used for inferring the systematics and evolutionary history of anoplocephaline cestodes in the Holarctic region, particularly with respect to the hypotheses of Beveridge. Patterns of phyletic co-evolution between the parasites and the hosts are also discussed.

Material and methods

Samples

The analysed material consisted of most of the known species from the subfamily *Anoplocephalinae* (genera *Anoplocephaloides* and *Paranoplocephala*) in Holarctic arvicoline rodents of the genera *Microtus, Clethrionomys, Dicrostonyx, Lemmus, Synaptomys* and *Chionomys*. The synopsis of the main morphological characters has been given by Haukisalmi *et al.* (2002). Parasite species are represented by at least two individuals in the COI and 28S rDNA phylogenies with the exception of four species for which only one individual was obtained. To evaluate the relationships between the studied genera, we additionally included representatives of four other anoplocephaline genera *(Andrya, Mosgovoyia, Cittotaenia, Schizorchis)* of lagomorphs and two genera (*Anoplocephala, Moniezia*) of ruminants (Table 1). One species, *Monoecocestus americanus*, from the North American porcupine (a hystricomorph rodent) was also included. The inclusion of two species of true *Andrya* from leporids, *A. cuniculi* and *A. rophalocephala,* provided an opportunity to evaluate the generic status of *Andrya*-like species in arvicoline rodents, a long-standing taxonomical problem. Representatives of *Ctenotaenia* and *Diandrya,* two genera parasitizing marmots (*Sciuridae*), were included to achieve a complete representation of the northern hemisphere genera within *Anoplocephalinae* sensu Beveridge (1994). According to Beveridge (1994), *Ctenotaenia* is proposed to be a sister group to *Anoplocephaloides* and *Diandrya* a sister group to *Andrya*. The outgroup comprised species from two other *Cyclophyllidean* families, *Hymenolepididae* and *Taeniidae.* Representatives of the other three subfamilies of the *Anoplocephalidae* were not used as outgroups as morphological evidence indicates the four subfamilies could represent a polyphyletic assamblage of taxa (Beveridge, 1994). Portions of the mitochondrial COI gene and of 28S rDNA were amplified and sequenced from all species listed in Table 1. ITS1 was cloned and sequenced from a subset of the species listed in Table 1. Species of *Paranoplocephala* from arvicoline rodents were selected so that each host genus *(Microtus, Clethrionomys, Dicrostonyx)* was repre-

sented by species with differing uterine morphology (Fig. 1).

DNA extraction, PCR amplification and sequencing

A 641bp long fragment was amplified from COI. Amplification from total genomic DNA and sequencing methods for COI are described in Haukisalmi *et al.* (2004). We are confident that our sequences represent the true partial mitochondrial cytochrome oxidase I as there were no anomalies of the type commonly associated with pseudogenes (Bensasson *et al.* 2001; Zhang & Hewitt 1996) and the translated protein sequences obtained matched previously published data for other cestode species (complete mitochondrial genomes of *Hymenolepis diminuta*, GenBank acc. no. NC_002767; *Echinococcus multilocularis*, GenBank acc.no. NC_000928). About 800 bp of domains D1-D3 of 28S rDNA was amplified in a single reaction as in Lockyer *et al.* (2003) and directly sequenced with labelled PCR primers from both directions as in Haukisalmi *et al.* (2004). For methodological notes on amplifying, cloning and sequencing of ITS1, see Haukisalmi *et al.* (2001). Outgroup sequences were retrieved from GenBank. *E. multilocularis* NC_000928 *H. diminuta* NC_002767, AF314223 and *H. nana* AB033412 were used in the COI alignment, *H. microstoma* AF286918, *H. diminuta* AY157181 and *Wardoides nyrocae* AF286919 in the 28S rDNA alignment and *H. diminuta* AF461125, *Rodentolepis microstoma* AY221167 and *E. granulosus* AJ245930, AJ237773 tested as outgroup for ITS1.

Phylogenetic analyses

Sequences were assembled and edited using Align IR™ Sequence Assembly and Alignment Software (LI-COR Inc., Nebraska, USA) and aligned in ClustalW (Thompson *et al.* 1994) with default gap penalties. Further minor adjustments to improve alignments were made by eye. Regions where the alignment was ambiguous were excluded from the analyses. Sites at which an insertion only affected one single taxon were also excluded, because they were phylogenetically uninformative. Data on nucleotide substitutions and amino acid replacement were determined using MacClade version 4 (Maddison & Maddison 2000) and Kimura-2 parameter total distances between haplotypes counted in MEGA v.2.1 (Kumar *et al.* 2001).

The phylogenetic relationships among taxa were reconstructed using the Bayesian approach (Huelsenbeck *et al.* 2001) implemented in the program MrBayes v.3.0B4 (Ronquist & Huelsenbeck 2003) as well as neighbor-joining (NJ) and maximum parsimony (MP) algorithms implemented in PAUP* v. 4.0b10 (Swofford 2002). A consensus tree was constructed from combined nucleotide sequences for COI+28S rDNA and COI+28S rDNA+ITS1, as well as each individual data set. The substitution model used for the combined data sets corresponded to the general time-reversible model with gamma-distributed rate variation among sites approximated with five categories (α shape estimated). This model, GTR+Γ, was the best model found for ITS1 and 28S rDNA sequences using MrModeltest v.1.1b (a variation of ModelTest by Posada & Crandall 1998). The best model suggested for the COI sequence additionally allowed for invariant sites (GTR+Γ+I). The GTR+Γ+I model was used for the COI partition in the combined data set. In the Bayesian analysis, base frequencies were estimated, four chains were used (default temperature) and the starting tree was random. The analysis was run for 11 million generations with a sample frequency of 100. The first 10 000 trees were discarded, so that the final consensus was based on 100 000 trees. Support for nodes were expressed as posterior probabilities (calculated by MrBayes) and also as bootstrap support (1000 replicates). The latter employed the NJ algorithm with maximum likelihood (ML) distances, using the substitution model found by ModelTest (GTR+Γ in combined data sets) and parameters estimated from the MrBayes tree. Three independent runs (shortest run one million generations) were compared to confirm that the likelihood plateau represented a real optimum and not a local optimum, which might have varied between runs. For comparison, analyses were also performed using NJ (using ML and Kimura 2-parameter distances) and MP algorithms in PAUP*. The NJ algorithm implemented with Kimura 2-parameter distances was applied as complicated substitution models have shown inconsistent results for various anoplocephaline taxa (Wickström *et al.* 2003). The parsimony analyses were carried out heuristically with 1000 random additions, TBR swapping and MulTrees option in effect. Bootstrap analyses were conducted for 1000 rearrangements (with 10 random additions). The results of the MP analyses are reported only if different from the NJ analyses with Kimura 2-parameter distances.

Results

Interspecific total distances for COI varied between 2% and 30% within Anoplocephalinae. For species

Table 1. Holarctic anoplocephaline parasite genera/species in alphabetical order. Type of early uterus divided into two main types, reticulated (R) and tubular (T). The arvicoline host genera *Microtus, Chionomys, Synaptomys, Lemmus, Dicrostonyx, Clethrionomys* abbreviated to first letter/s, eg. *M, C, S, L, D, Cl* respectively. The other host genera are spelled out. Parasite specimens are numbered and GenBank accession numbers of cytochrome *c* oxidase I mtDNA (COI), 28S rRNA and ITS1 rRNA sequences are given in the mentioned order (M= missing).

Species	Uterus-type	Host species	Host common name	No	Location	Country	Accession No (COI, 28S, ITS) M = missing
Andrya cuniculi,	R	*Oryctolagus cuniculus*	European rabbit		Tenerife	Spain	AY189957, AY569723, AF314409
A. rhopalocephala	R	*Lepus europaeus*	Brown hare		Hodmezövasarhely	Hungary	AY189958, AY569724, M
Anoplocephala magna	T	*Equus burchellii*	Bruchell's zebra		Verribee, Victoria	Australia	AY568206, AY586610, M
A. perfoliata	T	*Equus caballus*	Horse		Verribee, Victoria	Australia	AY568189, AY569769, M
Anoplocephaloides dentata	T	*C. nivalis*	Snow vole	1	Trentino	Italy	AY568190, AY569725, M
	T	*C. nivalis*	Snow vole	2	Bourg-Saint-Maurice	France	AY568191, AY569726, M
A.cf. dentata I	T	*M. oeconomus*	Root vole	3	Pallasjärvi	Finland	AY423809, AY569727, AYxxxxxx
	T	*M. agrestis*	Field vole	4	Aberdeen, Scotland	UK	AY423834, AY569728, M
A.cf. dentata II	T	*M. oeconomus*	Root vole	5	Kolyma River, Siberia	Russia	AY568192, AY569729, M
	T	*M. oeconomus*	Root (tundra) vole	6	Northern Yukon	Canada	AY568193, AY569730 AYxxxxxx
	T	*M. oeconomus*	Root (tundra) vole	7	Gates of the Arctic NP, Alaska	USA	AY568194, M, M
A. kontrimavichusi	T	*S. borealis*	Northern bog lemming	1	Fairbanks, Alaska	USA	AY568195, AY569731, AYxxxxxx
	T	*S. borealis*	Northern bog lemming	2	Yukon-Charlie NP, Alaska	USA	AY568196, AY569732, M
A. lemmi	T	*L. sibiricus*	Siberian lemming	1	Tajmyr, Siberia	Russia	AY568197, AY569733, AYxxxxxx
	T	*L. trimucronatus*	Brown lemming	2	Kolyma River, Siberia	Russia	AY568198, AY569734, M
		L. trimucronatus	Brown lemming	3	Yukon-Charlie NP, Alaska	USA	AY568199, M, M
A. mamillana	T	*Equus caballus*	Horse			Germany	M, AY569770, M
A. tenoramuraiae	T	*C. nivalis*	Snow vole	1	Trentino	Italy	AY568207, AY569735, AYxxxxxx
		C. nivalis	Snow vole	2	Bourg-Saint-Maurice	France	AY568208, AY569736, M
A. variabilis	T	*M. agrestis*	Field vole	1	Pallasjärvi	Finland	AY568209, AY569737, AYxxxxxx
	T	*M. miurus*	Singing vole	2	Toolik Lake, Alaska	USA	AY686611, AY586607, M
Cittotaenia denticulata	R	*Oryctolagus cuniculus*	European rabbit		North Yorkshire, England	UK	AY568211, AY569771, M
Ctenotaenia marmotae	T	*Marmota marmota*	Alpine marmot		French Alps	France	AY568187, M, M
Diandrya composita	R	*Marmota caligata*	Hoary marmot	1	Yukon-Charlie NP, Alaska	USA	AY181550, AY569739, M
	R	*Marmota broweri*	Alaska marmot	2	Gates of the Arctic NP, Alaska	USA	AY568212, AY569740, M
	R	*Marmota caligata*	Hoary marmot	3	Yukon-Charlie NP, Alaska	USA	AY181551, AY569741, AYxxxxxx
Moniezia sp.	R	*Rangifer tarandus*	Reindeer		Paistunturi	Finland	M, M, AYxxxxxx
Monoecocestus americanus	R	*Erethizon dorsatum*	North American porcupine		Yukon-Charlie NP, Alaska	USA	AY568184, AY569772, AYxxxxxx
Mosgovoyia pectinata	T	*Lepus timidus*	Mountain hare		Häme	Finland	M, M, AYxxxxxx

Paranoplocephala alternata	R	*D. groenlandicus*	Collared lemming	1	Cape Crusenstern, Alaska	USA	AY181502, AY569742, AY299551
	R	*D. torquatus*	Collared lemming	2	Kolyma River, Siberia	Russia	AY181431, AY569743, AYxxxxxx
P. arctica	R	*D. groenlandicus*	Collared lemming	1	Wrangel Island, Siberia	Russia	AY181505, AY569744, AF314412
	R	*D. groenlandicus*	Collared lemming	2	Alaska	USA	AY181507, AY569745, AYxxxxxx
P. blanchardi	R	*M. agrestis*	Field vole	1	Heinävesi-Enonkoski	Finland	AY189955, AY569746, AYxxxxxx
	R	*M. agrestis*	Field vole	2	Stilleryd	Sweden	AY189956, AY569747, M
P. etholeni	R	*M. pennsylvanicus*	Meadow vole	1	Fairbanks, Alaska	USA	AY568186, AY569773, M
	R	*M. pennsylvanicus*	Meadow vole	2	Fairbanks, Alaska	USA	AY568214, AY569774, AYxxxxxx
P. fellmani	R	*L. lemmus*	Norvegian lemming	1	Finse	Norway	AY568200, AY569748, AYxxxxxx
	R	*L. lemmus*	Norvegian lemming	2	Finse	Norway	AY586612, AY569749, M
P. gracilis	R	*M. agrestis*	Field vole	1	Hattusaari, Pielinen	Finland	AY395633, AY569750, AYxxxxxx
	R	*M. agrestis*	Field vole	2	Kielder Forest, Scotland	UK	AY568215, AY569751, M
P. kalelai	R	*Cl. rufocanus*	Grey sided vole	1	Kilpisjärvi	Finland	AY181512, AY569752, AYxxxxxx
	R	*Cl. glareolus*	Bank vole	2	Narvik	Norway	AY181513, AY569753, M
	R	*Cl. glareolus*	Bank vole	3	Narvik	Norway	AY189959, M, M
P. krebsi	R	*D. groenlandicus*	Collared lemming	1	Wrangel Island	Russia	AY568201, AY569754, M
	R	*D. groenlandicus*	Collared lemming	2	Victoria Island, Nunavut	Canada	AY568216, AY569755, AF314416
P. longivaginata	R	*Cl. rufocanus*	Grey sided vole	1	Kolyma River, Siberia	Russia	AY568202, AY569756, AYxxxxxx
	R	*Cl. rufocanus*	Grey sided vole	2	Kolyma River, Siberia	Russia	AY568203, M, M
P. macrocephala	R	*M. pennsylvanicus*	Meadow vole	1	Fairbanks, Alaska	USA	M, AY569757, AYxxxxxx
	R	*M. pennsylvanicus*	Meadow vole	2	Yukon-Charlie NP, Alaska	USA	AY181517, AY569758, M
	R	*M. pennsylvanicus*	Meadow vole	3	Yukon-Charlie NP, Alaska	USA	AY181518, AY586608, M
P. nordenskioeldi	R	*D. groenlandicus*	Collared lemming		Victoria Island, Nunavut	Canada	AY568204, AY569759 AF314411
P. oeconomi	R	*M. oeconomus*	Root (tundra) vole	1	Barbacs	Hungary	AY568217, AY569760, M
	R	*M. oeconomus*	Root (tundra) vole	2	Barbacs	Hungary	AY568205, AY569761, M
P. omphalodes	R	*M. agrestis*	Field vole	1	Espoo	Finland	AY181525, AY569762, M
	R	*M. arvalis*	Common vole	2	Dévaványa	Hungary	AY181536, AY569763, M
P.cf. omphalodes I	R	*M. oeconomus*	Root (tundra) vole	1	Pallasjärvi	Finland	AY181520, M, AYxxxxxx
	R	*M.* sp.		2	Wrangel-StElias NP, Alaska	USA	AY181543, AY586609, M
P.cf. omphalodes II	R	*M. oeconomus*	Root (tundra) vole		Gates of the Arctic NP, Alaska	USA	AY181547, M, M
P.cf. omphalodes III	R	*M. miurus*	Singing vole	1	Gates of the Arctic NP, Alaska	USA	AY189952, AY569764, M
	R	*M. miurus*	Singing vole	2	Noatak NP, Alaska	USA	AY181541, AY569765, M
P. primordialis	R	*M. oeconomus*	Root (tundra) vole		Yukon	Canada	AY568218, AY569766, AYxxxxxx
P. serrata	R	*D. torquatus*	Collared lemming	1	Yamal, Siberia	Russia	AY568220, AY569767, M
	R	*D. groenlandicus*	Collared lemming	2	Byron Bay, Nunavut	Canada	AY568219, AY569768, AF314414
P. spI	R	*C. nivalis*	Snow vole		Bourg-Saint-Maurice	France	AY568188, M, M
Schizorchis caballeroi	T	*Ochotona collaris*	Collared pika		Yukon-Charlie NP, Alaska	USA	M, AY569775, AYxxxxxx

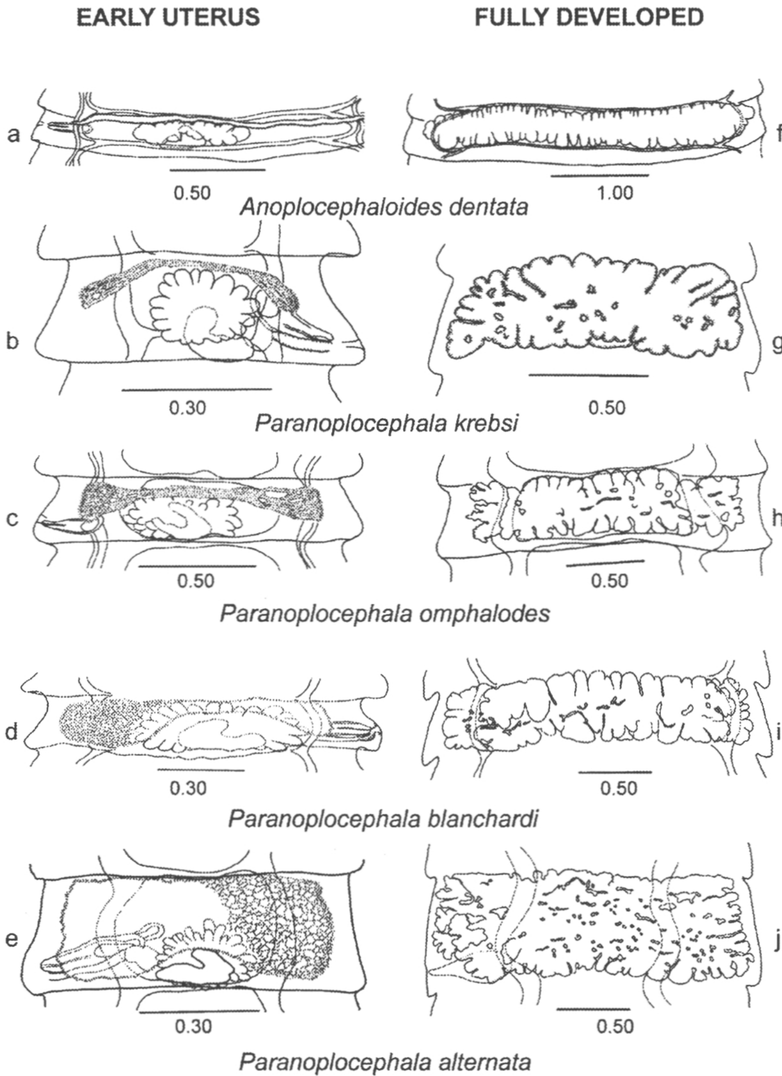

Fig. 1 The early uterus (a-e) and fully developed uterus (f-j) of five species of anoplocephaline cestodes depicting the variability of uterine structures between the two extremes, tubular (a, f) vs. completely reticulated (e, j).

with more than one individual sampled, the corresponding intraspecific distances ranged up to 16% (*Anoplocephaloides lemmi*). ITS1 interspecific total distances varied between 0.3% and 18%, 28S rDNA distances between 0.2% and 1.4%. Corresponding intraspecific distances for 28S rDNA ranged up to 0.8% (*A. dentata*, individuals no. 6 and 7, Table 1). Out of 174 third positions in COI 19 were invariant.

GenBank accession numbers for COI, 28S rDNA and ITS1 sequences are given in Table 1. Some of the taxa did not amplify/amplified poorly with COI and/or 28S rDNA PCR primers and were therefore left out from the combined COI+28S rDNA data set. Out of a total of 1791bp of aligned COI and 28S rDNA sequences from 55 taxa, 571 sites were variable, of which 378 were informative (198 informative sites out of

522bp of COI and 180 informative sites in 28S rDNA). A total of 114bp were excluded from the analysis, all from 28S rDNA. The 2381bp long aligned data set of all three sequences consisted of 25 taxa and displayed 527 variable sites of which 296 were informative. Alignments for the ribosomal sequences have been deposited with EBI/EMBL, and are available by anonymous FTP from FTP.EBI.AC.UK in directory /pub/databases/embl/align or via SRS at http://srs.ebi.ac.uk, under accession numbers ALIGN_xxxxxx and ALIGN_xxxxxx.

The phylogenetic methods used (Bayesian, NJ and MP) displayed trees with very similar topologies (Figs. 2-5). Individual analyses of each sequence showed poor resolution amongst *Paranoplocephala* and *Anoplocephaloides* parasitizing arvicoline rodents (Fig. 2a-b). *Anoplocephaloides mamillana* from horse did not group together with *Anoplocephaloides* from arvicolines. The 'arvicoline' clade additionally included the genera *Diandrya* and *Andrya* in all phylogenies and also *Ctenotaenia* that was present only in the COI phylogeny. Individuals morphologically assigned to the same species within *Paranoplocephala* and *Anoplocephaloides* formed monophyletic groups in all cases except for the earlier studied *P. omphalodes* species complex (Haukisalmi *et al.* 2004), the *P. arctica -P. alternata* species complex (Wickström *et al.* 2001; Wickström *et al.* 2003) and *A. variabilis*. One individual of *A. lemmi* from Tajmyr in Siberia did not group with the other two from Siberia and Alaska respectively in the COI and 28S rDNA trees (Fig. 2a, Table1). Only one individual was included in the ITS1 data set. Additionaly, a third species complex was discovered, splitting the morphological species *A. dentata* into two monophyletic groups. A secondary division within one of the *A. dentata* groups additionally separated individuals from *Chionomys* and *Microtus* hosts. As no conflicting branches with posterior probabilities greater than 95% were found (apart from the *P. omphalodes* species complex where the conflict could have been caused by the lack of *P.* cf. *omphalodes II* in the 28S rDNA data set), the sequences were combined in all further analyses since this has been shown to improve the estimation of phylogeny (Cunningham 1997; Yoder *et al.* 2001). The genera *Cittotaenia* and *Monoecocestus* were specified as outgroups for the combined data sets based on their position in the individual data sets. Taenids and hymenolepidids were not included in the combined data sets as different individuals were used as outgroups in the individually analysed sequence data and as they are probably too distant to function well as outgroups.

All three independent runs of the Bayesian analysis of COI+28S rDNA and COI+28S rDNA+ITS1 respectively converged on the same optimum, and the long runs of 11 million generations showed no increase in log-likelihood scores, suggesting that the trees found under a particular model were stable. The combined data of COI and 28S rDNA is presented in Figs. 3 and 4. The nodes with high posterior probabilities (Fig. 4c) that were not present in NJ and MP topologies (Fig. 3) were not supported by bootstrap analysis. The relationship between bootstrap support and posterior probability appears to be influenced by branch length as all short branches with high posterior probability had only low bootstrap support, suggesting that support for these nodes depends upon only a few sites and are therefore not reliable.

All analyses of COI+28S rDNA positioned *Andrya* from leporids as the closest sister group to the 'arvicoline' clade. Although the intermediate structure in the bayesian topology proved ambiguous, the same well-supported terminal clades were present as in the NJ topology. The *P. omphalodes* species complex was supported in the COI+28S rDNA data set (Figs. 3 and 4, orange bar) and *Paranoplocephala oeconomi* was assigned to the *A. variabilis, A. tenoramuraiae, P. krebsi* species group (Figs. 3 and 4c, yellow bar). Apart from *A. variabilis* and *A. tenoramuraiae*, all species with tubular early uterus parasitizing arvicolines formed a monophyletic group (Figs. 3 and 4, red bar). All other relationships within the 'arvicoline' clade remained ambiguous, and the almost star-like phylogeny of the 'arvicoline' clade is illustrated in Fig. 4b. To test whether outgroup rooting affected the topology/support for branching within the 'arvicoline' clade, an analysis was run with *Andrya rophalocephala* and *A. cuniculi* as outgroups (Fig. 4c). Exclusion of the more distantly related genera did not improve the resolution between rodent parasites.

The phylogenies based on all three sequences showed similar topologies in NJ, MP and Bayesian reconstructions. The clades supported in the Bayesian phylogeny (Fig. 5) by both posterior probabilities and NJ bootrstraps based on ML distances were the same as in the COI+28S rDNA data set.

Phylogeny summary

The most basal clade/clades included a combination of *Cittotaenia denticulata, Monoecocestus americanus, Anoplocephala magna, A. perfoliata, Moniezia* sp. and *Schizorchis caballeroi*. These genera are all

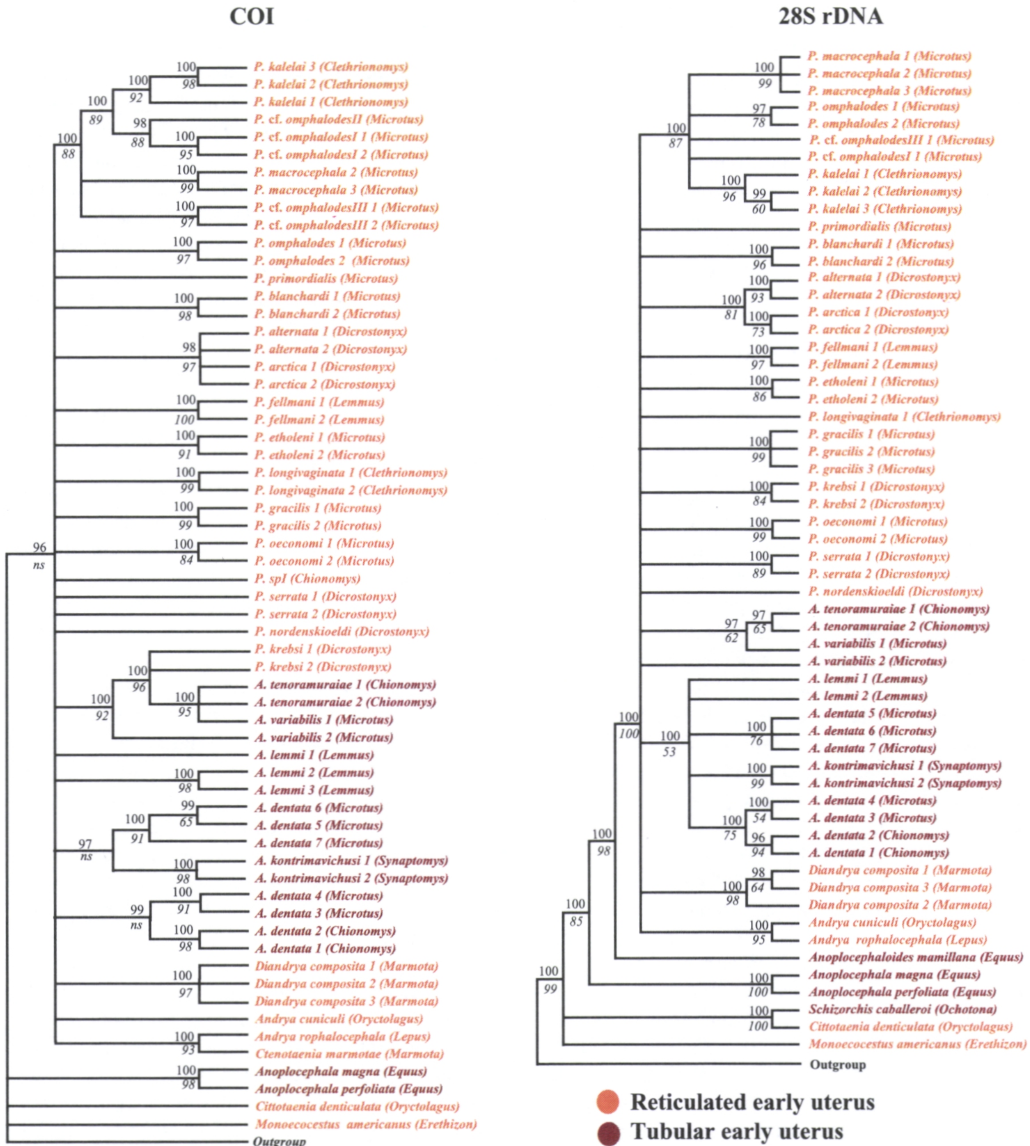

Fig. 2a

Fig. 2a-b Molecular phylogenies of Anoplocephalinae produced by Bayesian analysis of individual gene sequence data from mitochondrial COI (522bp of which 204 informative), ITS1 (408bp of which 227 informative) and 28S rDNA (1158bp, 223 informative). Taxa belonging to the families Hymenolepididae and Taeniidae were used as outgroup for COI, hymenolepidids only for 28S, taeniids only for ITS1. Branches with posterior probabilities <95% have been collapsed. Bootstrap values (>50%, based on NJ with ML distances) are shown in italics below branches. Other branches have less than 50% bootstrap support (ns). Species are colour coded according to uterine morphology; red=tubular, orange=reticulated.

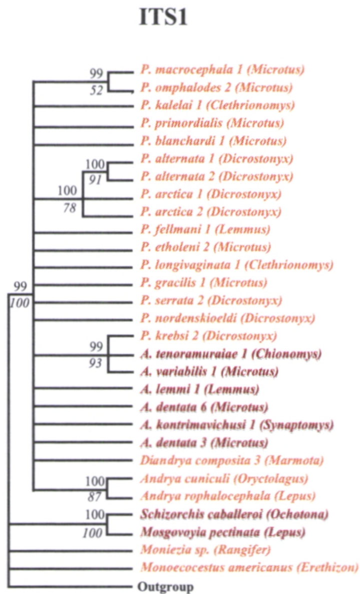

Fig. 2b

found from non-arvicoline hosts. *Anoplocephaloides mamillana* from horse did not group together with *Anoplocephaloides* from arvicoline hosts. All species from arvicoline rodents, including both *Anoplocephaloides* spp. and *Paranoplocephala* spp., formed a monophyletic group together with *Diandrya* and *Ctenotaenia* from marmots. In the combined data sets, *Andrya* spp. from leporids was recognised as the closest sister group to the 'arvicoline' clade. *Diandrya* never formed a monophyletic group with *Andrya* spp. in contrast to previous hypotheses (Beveridge 1994; Rausch 1980).

Although the internal stucture of the 'arvicoline' clade remained mostly unresolved, the combined data set revealed three supported species groups within the 'arvicoline' clade. One of these groups (red bar in Figs. 3-5) included all species of arvicoline *Anoplocephaloides* except *A. variabilis* and *A. tenoramuraiae*. The second group (orange bar in Figs. 3-5) comprised the earlier recognised *Paranoplocephala omphalodes* species complex (Haukisalmi *et al.* 2004) always including *P. omphalodes, P. macrocephala* and *P. kalelai*. A third, well supported group included *P. krebsi, A. variabilis* and *A. tenoramuraiae* (yellow bar in Figs. 3-5). The monophyly of the genera *Anoplocephaloides* and *Paranoplocephala* was not supported by any of the present analyses.

The species *P. arctica* and *P. alternata* should either be regarded as very closely related sister species with huge internal variation or be considered conspecific, whereas *A. dentata*, *A. variabilis* and *A. lemmi* in all reconstructions proved to be non-monophyletic.

Classification of uterine morphology into partly and completely reticulated uterine types in addition to tubular uterus as in Beveridge (1994), did not seem appropriate, as uteri classified by Beveridge as 'completely reticulated' are morphologically heterogeneous if subjected to a detailed comparative analysis (Haukisalmi & Henttonen 2001; Haukisalmi *et al.* 2001). The fully developed uterus of *Moniezia,* for example, retains its complex reticulate structures that are lost or simplified in the fully developed uteri of other anoplocephaline species with completely reticulated early uterus. The anoplocephaline species we have studied display a gradient of differing uterine types (Fig. 1) rather than a clearcut division into three categories. We have therefore chosen to discriminate only between tubular and reticulated uterine types.

As the sequence-based phylogenies did not reveal unambiguous supported structure amongst arvicoline genera, we could not make a detailed comparison between our results and the morphological classification (tubular vs. reticulated uterus, see Figs. 2-5). However, two of the supported species groups proved unambiguous as regards the type of early uterus, and only one supported species group included species with different types of early uterus (*P. krebsi* vs. *A. variabilis* and *A. tenoramuraiae*). Amongst other genera, on the one hand, grouping in accordance to uterus type was prevailing. Only the genus pair *Schizorchis-Cittotaenia* in the 28S rDNA phylogeny had different types of early uteri. On the other hand, species with reticulated uterus in arvicolines were more closely related to other species in arvicolines with tubular uterus than to species with reticulated uterus in other host genera. Thus, the anoplocephalines in voles and lemmings tended to form a monophyletic group in which both uterine types were represented.

Discussion

Our primary goal was to generate a first, relatively large, molecular data set to test taxonomic hypotheses within Anoplocephalinae. The emphasis was placed on species from arvicoline rodent hosts in the Holarctic region, and almost all known species were included in at least one of the three sequence data sets. The com-

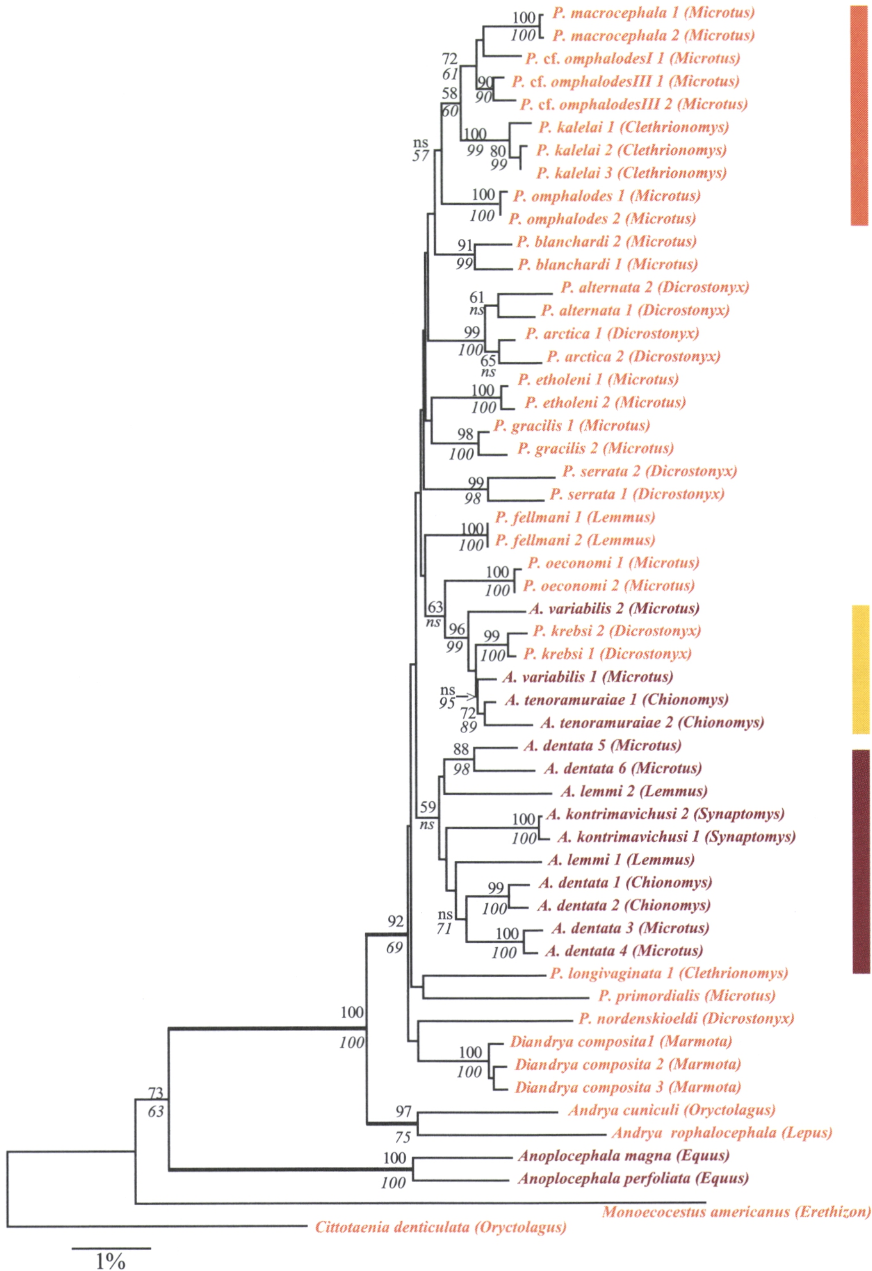

Fig. 3 Molecular phylogeny of anoplocephaline mitochondrial COI and 28S rDNA combined sequences produced by the NJ algorithm implemented in PAUP* with Kimura 2-parameter distances. Bootstrap percentages from 10 000 iterations are shown above branches. Bootstrap support generated from MP analysis (1000 iterations) is shown in italics below branches. Species are colour coded according to uterine morphology; red=tubular, orange=reticulated. The coloured bars represent recognised clades.

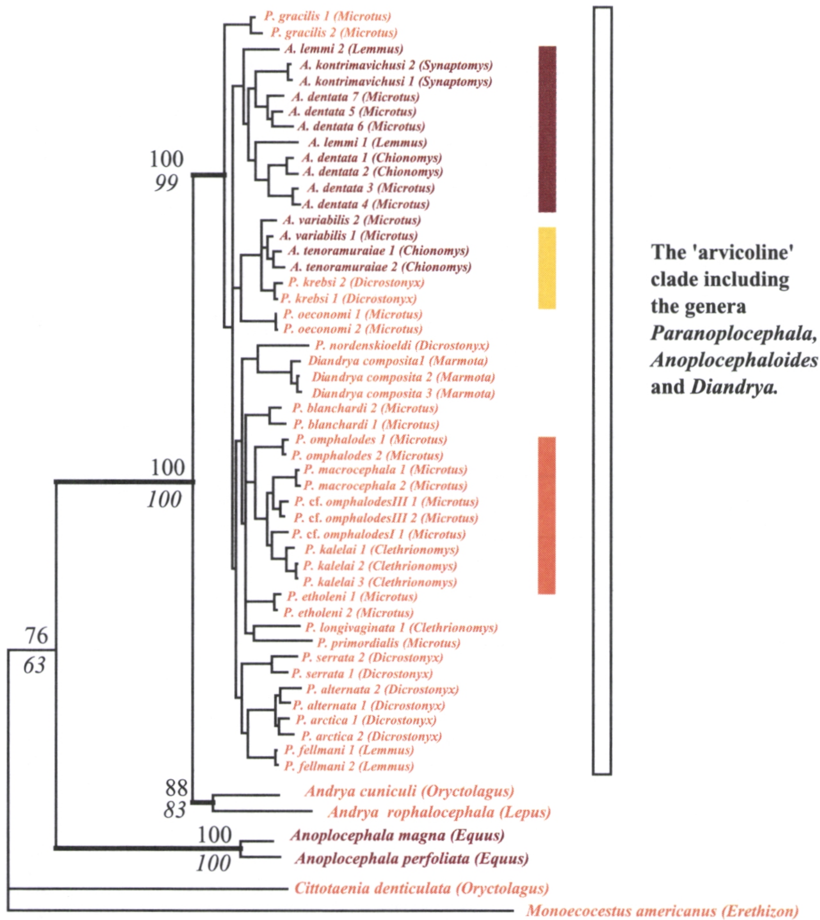

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Fig. 4a

Fig. 4 a) Molecular phylogeny of anoplocephaline mitochondrial COI and 28S rDNA combined sequences produced by Bayesian analysis implemented in MrBayes with GTR+Γ+I (COI partition) and GTR+Γ (28S rDNA partition) distances. Posterior probabilities (>70%) are shown above branches; bootstrap values (>50%, based on NJ with ML distances) are shown in italics below branches; other branches have <70% (Bayesian) or <50% (bootstrap) support (ns). Species are colour coded according to uterine morphology; red=tubular, orange= reticulated. **b,c**) the 'arvicoline' clade analysed with *Andrya rophalocephala* and *A. cuniculi* as outgroups. Fig. 4b is a schematic picture of Fig. 4c, depicting the star-like phylogeny. The coloured bars/circles represent supported clades.

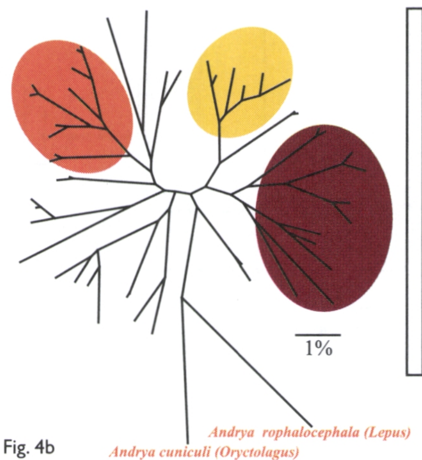

Fig. 4b

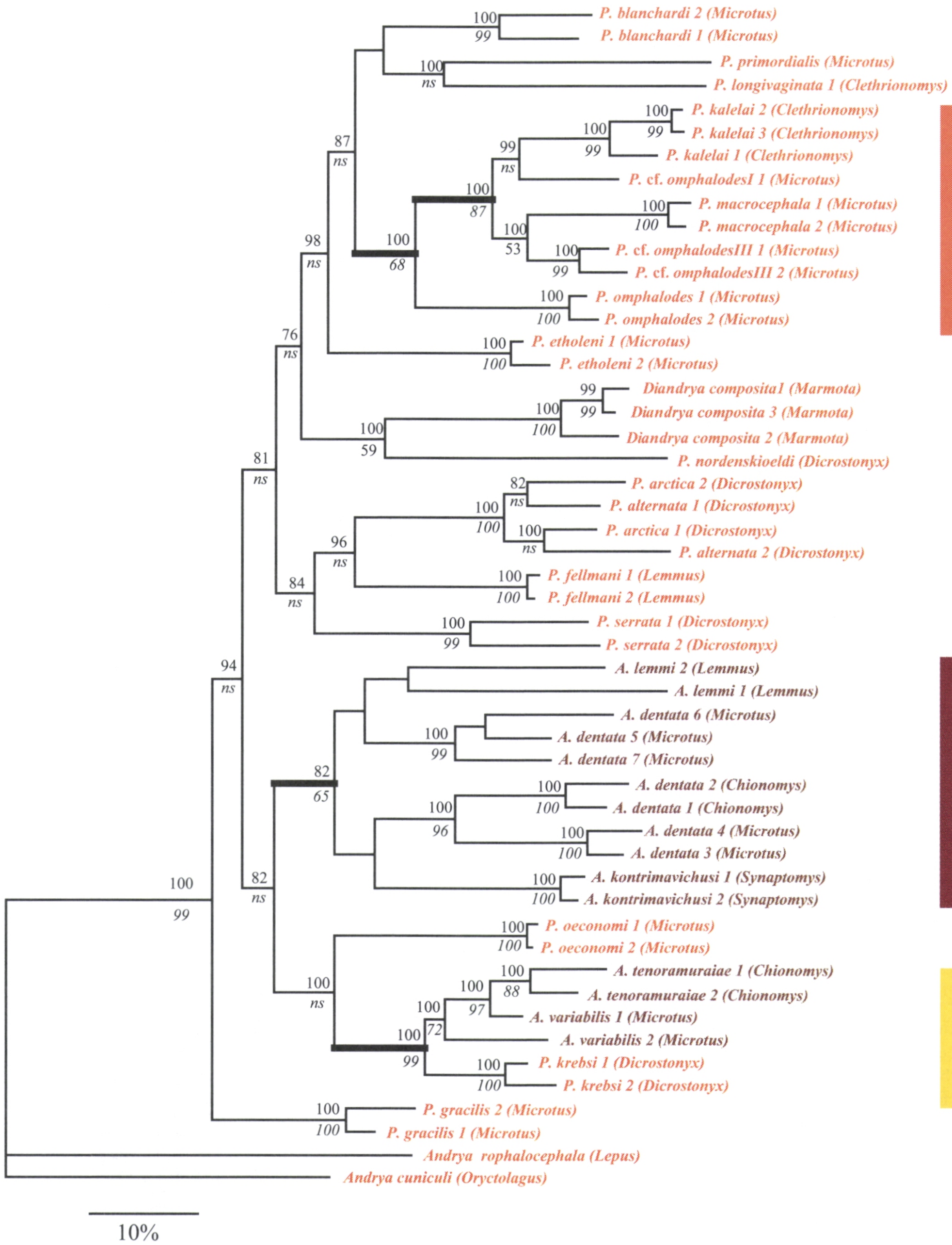

Fig. 4c

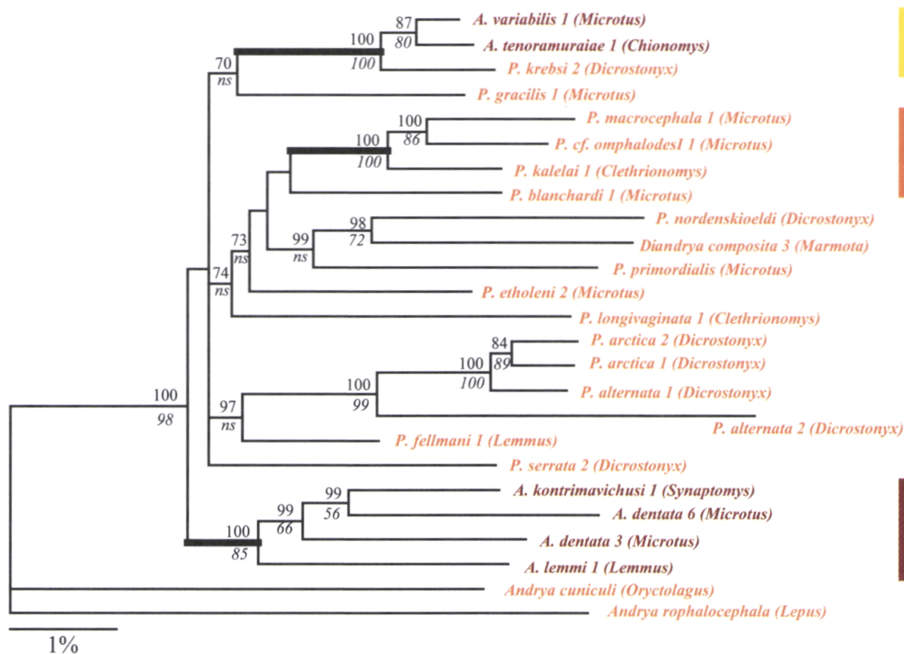

Fig. 5 Molecular phylogenies of anoplocephaline mitochondrial COI, 28S and ITS1 rDNA combined sequence produced by Bayesian analysis implemented in MrBayes with GTR+Γ+I (COI partition) and GTR +Γ (28S rDNA and ITS1 partition) distances. *Andrya rophalocephala* and *A. cuniculi* were used as outgroups. Posterior probabilities (>70%) are shown above branches; bootstrap values (>50%, based on NJ with ML distances) are shown in italics below branches; other branches have <70% (Bayesian) or <50% (bootstrap) support (ns). Species are colour coded according to uterine morphology; red=tubular, orange=reticulated. The coloured bars represent supported clades.

bined data set only marginally improved the resolution between species with arvicoline rodent hosts. Characteristic poor or incomplete resolution within the 'arvicoline' clade in molecular analyses suggests that the anoplocephaline parasites in the arvicoline hosts would have differentiated in a short period of time. For Anoplocephalinae, difficulties remain both in delimiting species and defining genera. A more complete species coverage, including species from yet other arvicoline rodent host genera with to date unknown parasite faunas, combined with considerable additional sequence data may be needed to provide unambiguous resolution. As old evolutionary lineages/deep phylogenetic splits have been encountered within morphological species (i.e. species complexes encountered in this study), vast geographical sampling is also required. Rapid radiation seems to have been the primary mode of speciation in this group.

Uterine morphology versus phylogeny

The subfamily Anoplocephalinae is separated from the other subfamilies of Anoplocephalidae (Linstowiinae, Thysanosomatinae and Inermicapsiferinae) by a saccate uterus that persists in gravid segments (Beveridge 1994). Developing uteri of anoplocephalinae cestodes exhibit structures of varying complexity, all of which usually disappear in fully gravid segments. Spasskij (1951), Rausch (1976) and Beveridge (1994) have distinguished three main types of uterine development within Anoplocephalinae. However, in the genus *Paranoplocephala sensu lato* there are species with intermediate uterine types that cannot clearly be defined as belonging to any of Beveridge's categories; in particular, the division into partly reticulate and completely reticulate type seems to be fairly artificial (i.e. Fig. 1). We therefore employed a more clear-cut division into only two categories, reticulated and tu-

bular early uteri.

The division of Anoplocephalidae into two subfamilies with different uterine morphology (Anoplocephalinae with tubular uterus, Monieziinae with a reticular uterus, Spasskij 1951) is clearly inappropriate as neither of the characters corresponds to monophyletic groups of species (Beveridge 1994). The phylogenetic hypothesis for Anoplocephalinae based primarily on uterine morphology (Beveridge 1994) suggested that partly and completely reticulated uteri would form a single lineage, partly reticulated being the ancestral form. Beveridge (1994) also suggested that anoplocephaline taxa with tubular early uterus would form a monophyletic group, but the relationship with species with a reticulated early uterus was not defined. *Paranoplocephala* and *Anoplocephaloides* from voles and lemmings, and *Diandrya* from marmots formed a monophyletic group in our data set, with *Andrya* from leporids as the closest relative in most of the phylogenetic trees. If we assume *Andrya* as ancestral to the 'arvicoline' clade, then reticulated uterus would be the ancestral uterine type for this group. If reticulated uterus is recognised as the ancestral form for anoplocephalines in arvicolines, then there should also have been one or two conversions to the tubular uterus of *Anoplocephaloides* spp. in arvicolines.

Taxa with tubular uterus in our data set never formed a monophyletic group. Not even the species in arvicoline hosts were monophyletic. At least in the present species assemblage tubular uterus cannot be seen as the ancestral type for anoplocephalines in arvicolines. What had been the ancestral uterine type for the whole anoplocephaline subfamily is still ambiguous. Beveridge (1994) suggested that *Anoplocephala* with tubular uterus is the basal genus in Anoplocephalinae, but the present analysis does not support this, either. The presence of species pairs with different uterus types that appear as sister taxa could mean that speciation in aforementioned cestodes may frequently involve changes in the uterine structure.

Moniezia with double genitals and *Monoecocestus* with single genitals, hypothesised as being sister taxa (Beveridge 1994), also appeared as sister taxa in the ITS1 phylogeny. The genera *Schizorchis* (single genitals) and *Mosgovoyia* (double genitals) were monophyletic in the ITS1 phylogeny whereas the species in the pair *Andrya* (single genitals) - *Diandrya* (double genitals) did not form a monophyletic group in any of the phylogenies. However, the importance of duplication of genitalia in Anoplocephalinae can only be assessed with a more complete set of species also from other host genera than arvicolines.

When scrutinizing the systematic arrangement of the genera for which more than one species was included in the present analysis, we suggest that *Paranoplocephala* could be either mono- or paraphyletic, *Andrya* and *Anoplocephala* is probably monophyletic while *Anoplocephaloides* probably is not.

Phyletic coevolution of anoplocephaline cestodes in arvicoline hosts

Assuming that our molecular phylogenies reflect the true approximation of the evolution of anoplocephaline species, how does parasite evolution fit the phylogenetic hypotheses proposed for the hosts? The most recent, strongly supported, phylogeny of living placental mammals (Murphy *et al.* 2001), places Lagomorpha ancestral to Rodentia. This is also true for the parasite tree within the subfamily Anoplocephalinae. *Andrya, Mosgovoyia, Ctenotaenia, Cittotaenia* and *Schizorchis* from lagomorphs are all basal with respect to *Paranoplocephala* and *Anoplocephaloides* in arvicoline rodents. However, the genera from lagomorphs do not form a monophyletic group, and one rodent parasite species, *Monoecocestus americanus* from porcupine, turned out to be amongst the most basal taxa in this data set. *Anoplocephala* and *Anoplocephaloides* species from ruminants were more closely related to the arvicoline ingroup than several of the species from lagomorphs. The position of parasite genera from marmots (Sciuridae) within the arvicoline parasite clade suggests that host shifts have taken place in the history of the anoplocephalines.

Conroy & Cook (1999) verified the monophyly for the Arvicolinae and suggested that the lack of resolution among the arvicoline genera is due to pulses of speciation. The evolutionary history of the genus *Microtus* has been studied in detail (Conroy & Cook 2000; Jaarola *et al.* 2004), and is characterised by bursts of rapid diversification. A first radiation of *Microtus* can be traced back to 2 million years ago. However, the majority of extant *Microtus* species do not appear in the fossil record until Middle Pleistocene about 0.7-0.5 million years ago (Jaarola *et al.* 2004). A pulse of diversification early in the history of the anoplocephaline genera prasitizing rodents and lagomorphs is a very likely explanation for the observed polytomy in our data. Since the species are closely related, (and not all third positions in COI were variable), the polytomy observed cannot be ascribed to saturation. Instead, consistent support for nodes above and below unresolved polytomies indicate a rapid radiation involving

nearly simultaneous diversification of many lineages (cf. Jaarola *et al.* 2004; Conroy & Cook 1999; Lessa & Cook 1998). For *Paranoplocephala* and *Anoplocephaloides* in arvicoline rodents, co-speciation seems to be basal, and apart from host switches between closely related genera, seems to characterise the phyletic coevolution of anoplocephaline cestodes and their hosts.

Acknowledgements

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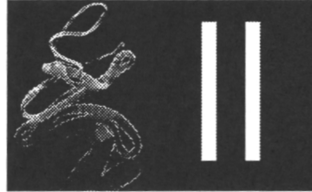

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Taxonomy, genetic differentiation and Holarctic biogeography of *Paranoplocephala* spp. (Cestoda: Anoplocephalidae) in collared lemmings (*Dicrostonyx*; Arvicolinae)

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The present study reviews the taxonomy of anoplocephalid cestodes of collared lemmings (*Dicrostonyx* spp.) and describes the patterns of cestode biogeography in the Holarctic region. The morphological differentiation of cestode species is augmented with a genetic differentiation based on three independent markers. We show that collared lemmings are parasitized by five host-specific species of *Paranoplocephala*, three of which are described here as new: *P. arctica* (Rausch, 1952), ***P. alternata* sp. nov.**, *P. serrata* Haukisalmi & Henttonen, 2000, ***P. nordenskioeldi* sp. nov.** and ***P. krebsi* sp. nov.** The redescription of *P. arctica* shows that the original description of this species is composite. *Paranoplocephala alternata*, *P. serrata* and *P. nordenskioeldi* are shown to have a Holarctic distribution, whereas *P. arctica* and *P. krebsi* are restricted to the Nearctic region, including Wrangel Island. It is suggested that the Holarctic species colonized North America concomitantly with their hosts and that the appearance of the Nearctic species is connected with the subsequent divergence of collared lemmings in North America. Geographical distribution and sequence data for the first transcribed spacer (ITS1) of nuclear rDNA show that *P. alternata* and *P. arctica* are sister taxa and that the latter species probably diverged from *P. alternata* in eastern Beringia. Other phylogenetic relationships among cestode species remained largely unsettled.

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ADDITIONAL KEY WORDS: tapeworms – Arctic – helminths – zoogeography – Beringia – fingerprinting – ITS1 – sequencing – phylogeny.

INTRODUCTION

Collared lemmings (*Dicrostonyx* Gloger) are the northernmost genus of arvicoline rodents with a nearly circumpolar distribution. The most recent taxonomy (Jarrell & Fredga, 1993; Fredga *et al.*, 1999) acknowledges four species, three of which (*D. groenlandicus* (Traill), *D. richardsoni* Merriam and *D. hudsonius* (Pallas)) are Nearctic and one (*D. torquatus* (Pallas)) is Palearctic (Fig. 1). A number of studies (Rausch & Rausch, 1972; van Wynsberghe & Engstrom, 1992; Engstrom *et al.*, 1993; Fedorov & Goropashnaya, 1999) suggest that vicariant events generated by the climatic oscillations during the Pleistocene have promoted the intra- and interspecific divergence of collared lemmings, supporting the hypothesis of multiple glacial refugia for Arctic mammals, first outlined by Macpherson (1965).

There is accumulating evidence that the historical fragmentation of host populations in the Arctic may also have promoted the divergence of their parasites. Small, isolated parasite populations are prone to genetic drift leading to the appearance of divergent, allopatric parasite lineages. Consequently, the fragmentation of parasite populations may have favoured the evolution of cryptic species flocks in Arctic vertebrates (Hoberg *et al.*, 1999). There is also evidence suggesting that the probability of host shifts and subsequent parasite speciation may be high in small, refugial host populations (Hoberg, 1995).

Paranoplocephala Lühe, 1910 (Cestoda: Anoplocephalidae) are ubiquitous cestode parasites of

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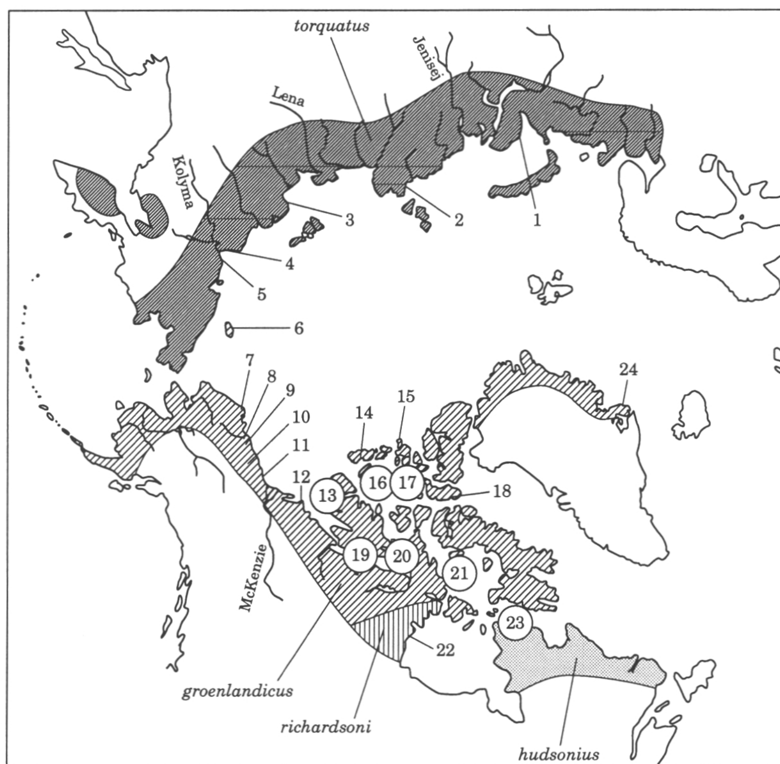

Figure 1. Geographical distribution of *Dicrostonyx* spp. according to Jarrell & Fredga (1993) and Fredga *et al.* (1999) and the localities for lemming and helminth samples. 1, Yamal Peninsula; 2, Taymyr Peninsula; 3, North-east of Yana Delta; 4, Western Kolyma Delta; 5, Eastern Kolyma Delta; 6, Wrangel Island; 7, Point Barrow; 8, Colville River; 9, Kuparuk; 10, Lake Schrader; 11, Beaufort Lagoon; 12, Cape Bathurst; 13, Banks Island; 14, Prince Patrick Island; 15, Ellef Ringnes Island; 16, Melville Island; 17, Bathurst Island; 18, Devon Island; 19, Southern Victoria Island, Kent Peninsula and adjacent mainland; 20, King William Island; 21, Melville Peninsula; 22, Rankin Inlet; 23, Cap de Nouvelle—France, Ungava Peninsula; 24, Constable Point, Greenland.

northern arvicoline rodents (Rausch, 1976; Haukisalmi, Henttonen & Batzli, 1995). Two species of host-specific cestodes are known from collared lemmings: *P. arctica* (Rausch, 1952) and *P. serrata* Haukisalmi & Henttonen, 2000. Despite the vast range and deep phylogenetic splits of the host genus, both cestode species have a wide, Holarctic distribution (Haukisalmi & Henttonen, 2000). However, morphological and genetical data show that the population of *P. arctica* on Wrangel Island may be specifically distinct from the Siberian and North American mainland populations (Wickström *et al.*, 2001).

The aim of the present study is to review the taxonomy of anoplocephalid cestodes of collared lemmings and to describe the patterns of cestode biogeography with respect to the known evolutionary history of the hosts in the Holarctic region. Because intraspecific morphological variation in the Holarctic *Paranoplocephala* may be considerable (e.g. Haukisalmi & Henttonen, 2000) and because cryptic species are known to exist in anoplocephalid cestodes (Baverstock, Adams & Beveridge, 1985), the morphological differentiation was augmented by a genetical analysis based on three independent markers.

MATERIAL AND METHODS

LEMMINGS

A total of 240 collared lemmings from the Holarctic region were examined by us for helminths. The

Palearctic lemming material was collected during the Swedish–Russian Tundra Ecology Expedition in the summer of 1994 along the Arctic coast of Siberia, including the New Siberian Islands and Wrangel Island ($N=78$) (Fredga *et al.*, 1999). The lemmings from Siberia were collected by V. Fedorov and K. Fredga from several localities, ranging from Pechora Bay in the west to the eastern Kolyma Delta and Wrangel Island in the east (Fig. 1). The collared lemmings from Siberia represent *D. torquatus*, except for those from Wrangel Island that belong to the Nearctic *D. groenlandicus* (Fedorov & Goropashnaya, 1999).

The Nearctic lemming material originates from four sources: (1) the lemmings collected during the Tundra Northwest 1999 expedition into the Canadian Arctic archipelago ($N=85$) by V. Fedorov, K. Fredga, C. J. Krebs and A. Angerbjörn. Most of these specimens represent *D. groenlandicus*; only one individual of *D. hudsonius* from Ungava Peninsula was caught and examined for helminths. (2) A material of *D. groenlandicus* ($N=62$) from the southern Victoria Island/Kent Peninsula region in Nunavut, central Arctic Canada, collected by C. J. Krebs and A. Kenney during the summer of 1996. (3) Ten alcohol-preserved intestines of *D. groenlandicus* from northern Alaska (Kuparuk and Colville River Delta), collected by D. J. Helmericks in July–August 1997, and deposited in the University of Alaska Museum, Fairbanks (UAM 502317–18, 502334–37, 502354, 502356, 502469 and 502470). (4) Five alcohol-preserved intestines of *D. groenlandicus* from Constable Point, eastern Greenland, collected by V. Fedorov, H. P. Gelter and G. H. Jarrell, in late July 1995. Three of these specimens were deposited in the University of Alaska Museum (UAM 33909, 33910 and 33915).

All lemmings or their intestines, except for those from North Alaska and Greenland, were frozen for later examination of helminths. Specimens from Alaska and Greenland were preserved intact in 70% alcohol and their intestines were later removed for a helminthological investigation.

CESTODES

Dissection of collared lemmings yielded *c.* 475 specimens of *Paranoplocephala*, of which *c.* 200 specimens were stained and mounted for taxonomical scrutiny. All specimens of *Paranoplocephala alternata* sp. nov. were not mounted because the strobila of this species is dorsoventrally thin and transparent, and all the major organs can easily be seen even in unstained and unmounted specimens. In addition, we had an opportunity to examine 15 mounted specimens of *Paranoplocephala* from the personal collection of Robert L. Rausch (RLR), originating from Point Barrow (2 specimens), Beaufort lagoon (2) and Lake Schrader (1) in North Alaska, Prince Patrick Island (2), Devon Island (2), Victoria Island (1) and Bathurst Island (1) in the Canadian Arctic Archipelago, and Rankin Inlet (4) in the west coast of Hudson Bay, Canada. Of these, two specimens from Point barrow (type locality of *P. arctica*; RLR 4) and two specimens from Rankin Inlet (*P. alternata*; RLR 42999) were included in the morphometric analysis.

The cestodes found were washed and relaxed in tap water for 1–2 h and fixed in 70% alcohol (most specimens) or 10% formalin, stained with Mayer's haemalum (most specimens) or Semichon's acetic carmine, cleared in eugenol and mounted in Canada balsam.

For genetic studies, cestode DNA was extracted from 0.5–2 mm^3 tissue samples as described previously by Vainio, Korhonen & Hantula (1998). The protocol included cell disruption (using quartz sand), four phenol–chloroform (1:1) extractions, one chloroform–isoamyl alcohol (24:1) extraction, precipitation with polyethylene glycol (PEG) and drying. The DNA was resuspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

MORPHOLOGICAL DIFFERENTIATION

The morphometric analysis follows the procedure used in our previous taxonomical studies (e.g. Haukisalmi & Henttonen, 2000). The length and maximum width of body and the number of segments were recorded from fully gravid specimens only. The median of maximum diameter of four suckers was used as a representative measure of sucker size for each individual. The length/width ratio of segments were usually measured from three segments for each developmental stage (immature/premature, mature, postmature/pregravid and gravid segments). The first mature segment was defined as the one in which the internal seminal vesicle was first seen clearly differentiated; the last mature segment was the one in which the vitellarium was last seen compact (no visible disintegration). If possible, various reproductive organs were measured and counted from at least three mature segments from each individual. Testes were counted by drawing them on paper.

The index of asymmetry of vitellarium and associate organs was measured as the distance from the midpoint of vitellarium to the poral margin of the segment/width of segment at the level of vitellarium. We also report the vagina/cirrus sac ratio (length of vagina/length of cirrus sac), and the relative size of some of the reproductive organs in mature segments. The maximum length of cirrus sac and seminal receptacle were measured from postmature segments. Egg dimensions are usually based on five measurements from each fully gravid strobila. If possible, egg morphology was examined from fresh (unfixed) specimens.

The pattern of the alternation of genital openings for *P. alternata* was determined by recording the number of segments in each unilateral set; the median number of such segments was used as an index of the frequency of genital pore alternation in each strobila (low value of index indicates frequent alternation). All measurements are in millimetres if not otherwise stated.

The type specimens of the new species have been deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland.

GENETICAL DIFFERENTIATION

The preliminary morphological analyses revealed that 3–4 species of *Paranoplocephala* exist in collared lemmings (excluding *P. arctica* and *P. alternata*). However, because of the pronounced morphological variability within species, varying age and condition and the relatively small number of available specimens especially for the rare taxa, the taxonomical status and geographical distribution of some of the forms could not be assessed definitively. We therefore applied genetical differentiaton based on micro- and minisatellite fingerprinting on 13 specimens of *Paranoplocephala* with a 'paranoplocephaloid' early uterus (see Discussion). *Paranoplocephala arctica* and *P. alternata* were excluded from this analysis because of their fundamentally different uterus development, and therefore, independent taxonomical status.

Most of the taxonomical uncertainties concerned the cestodes now assigned to *P. nordenskioeldi* sp. nov. Specifically, the available material did not allow us to determine definitely whether the specimens from Siberia and North America are conspecific and whether this taxon is specifically distinct from *P. serrata*, particularly in North America where the testis distribution of *P. serrata* resembles that of *P. nordenskioeldi* (see Haukisalmi & Henttonen, 2000 for morphological variation of *P. serrata*). Moreover, *P. nordenskioeldi* sp. nov. is morphologically related to the Palearctic host-generalist *Paranoplocephala gracilis* Tenora & Murai, 1980. *Paranoplocephala gracilis* and *P. serrata* were therefore included in the genetical differentiation based on fingerprinting technique. For the specimens now assigned to *P. krebsi*, the genetical analysis aimed to determine whether the few, morphologically slightly differentiated specimens from Wrangel Island and Ungava Peninsula should be classified within this species.

An additional genetical differentiation using the sequencing of the first internal transcribed spacer (ITS1) of the nuclear rDNA was applied on seven specimens of *Paranoplocephala*, including *P. arctica* and *P. alternata*. The sequencing of the ITS1 region has been used successfully for taxonomical differentiation in other organisms, including helminths (Morgan & Blair, 1995) and parasitic protozoans (Hnida & Duszynski, 1999). This analysis also enables phylogenetic inference among cestode taxa. Based on the unpublished phylogenetic studies of Wickström *et al.* on anoplocephalid cestodes of rodents and leporids, *Andrya cuniculi* Blanchard, 1891 from the European rabbit *Oryctolagus cunilus* (Linnaeus) was used as an outgroup.

MICRO- AND MINISATELLITE FINGERPRINTING

Two primers were used for fingerprinting: a RAMS (random amplified microsatellite; Hantula, Dusabenyagasani & Hamelin, 1996) primer GT= YHY(GT)$_7$G, where H=A/C/T and Y=A/C/G, and a M13 minisatellite primer (GAG GGT GGC GGT TCT) (Stenlid, Karlsson & Högberg, 1994). The GT and M13 fingerprints were amplified in 50-µl reaction volume essentially as described in Vainio & Hantula, 1999. Annealing temperatures and times were 58°C and 45 sec for the GT primer and 48°C and 60 sec for the M13 primer, respectively. The final extensions at 72°C were 7 and 10 min for GT and M13 primers, respectively.

Reaction products were separated by electrophoresis on agarose gels containing 1.0% agarose (FMC BioProducts, Rockland, ME, USA) and 1.0% SynerGel (Diversified Biotech, Boston, MA, USA). Electrophoreses were run in TAE-buffer (40 mM Tris-Acetate pH 8.0, 1 mM EDTA) and reaction products were detected by ethidium bromide staining. Gel interpretations were made from photographic prints. Only clear and distinct bands were considered. The approximate lengths of the amplification products were estimated by comparing them to a 100 bp DNA ladder (Gibco BRL, Gaithersburg, MD, USA).

ANALYSIS OF FINGERPRINT DATA

The fragments scored from the fingerprint profiles were treated as binary characters (presence/absence), and relationships between isolates were assessed by distance-based methods. A band-sharing similarity index s was calculated for each pair of isolates as the number of shared fragments divided by the total number of fragments in the two isolates (Lynch, 1990); the corresponding divergence measure is $d=1-s$. Each unique fingerprint profile was treated as a separate unit, even if some units may belong to the same species. The distances (counted as both separate and combined distances for M13 and GT fingerprints) were illustrated by neighbour-joining (NJ) (Saitou & Nei, 1987) and UPGMA clustering using MEGA software (Kumar, Tamura & Nei, 1993).

SEQUENCING OF THE RDNA ITS1 REGION

The ITS1 region of ribosomal DNA was cloned and sequenced from all species included in the fingerprint

study and also from three additional species (GeneBank accession numbers AF314409–AF314416). The reaction conditions for the PCR amplification of ITS sequences were the same as for the micro- and minisatellite primers described above. The ITS primers (Ferris, Ferris & Faghihi, 1993) take advantage of the conserved regions of 18S and 28S to amplify the ITS1, 5.8S and ITS2 regions in between (White *et al.*, 1990). In PCR amplifications the samples were denatured by 10 min incubation at 95°C after which 31 cycles of amplification were carried out (1 min denaturation at 95°C, 1 min annealing at 45°C, 1 min primer extension at 72°C). The reaction was terminated with a 7 min extension at 72°C. The product (*c.* 1400 bp) from this amplification was monitored by electrophoresis in agarose gels as described above, purified and ligated into pCR 2.1 vector using the TOPO/TA Cloning Kit (Invitrogen, Carlsbad, CA, U.S.A.). The resulting recombinant plasmids were transformed and isolated as in Wickström *et al.*, 2001. The orientation of the insert in the vector was determined by PCR reactions with a primer designed into the 5.8S rDNA (5′-CAA GAT GTC GAT GTT CAA AG-3′) and either the universal M13F or M13R primer (M13F = −20 forward and M13R = reverse sequencing primer for vector pCR 2.1, Invitrogen). The reaction conditions were the same as mentioned above, except that the annealing temperature was 54°C. The ITS1 region (601–627 bp) of the cloned inserts was sequenced by A.L.F. DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). Either universal M13F or universal M13R, 5.8S and two intermediate primers (forward 5′-GCA AGG CAT AAG AGG TTT GG-3′ and reverse 5′-CCA AAC CTC TTA TGC CTT GC-3′) were used. Sequencing was performed with Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, England). The nucleotide positions 378–397 in the ITS sequence of *P. serrata* (GeneBank accession number AF314414) corresponds to the annealing site of the internal primers.

ANALYSIS OF SEQUENCE DATA

The boundaries separating the 18S and 5.8S ribosomal gene sequences from the ITS1 sequence were determined approximately by comparisons with the rDNA sequences of an other cestode, *Echinococcus granulosus* (Batsch, 1786) (Taeniidae) (GeneBank acc. no. AJ245930 and AJ237773). Sequences were aligned by eye and a multiple alignment of the ITS1 sequences was computed using CLUSTAL X (default gap and weighting values) (Thompson *et al.*, 1997). Optimization of the multiple alignment was also performed by eye and all regions containing insertions/deletions or unambiguous alignment were omitted from the phylogenetic analysis. Therefore only nucleotides corresponding to sites 51–60, 66–96, 102–169, 177–299, 309–328, 367–435, 440–556 and 560–702 of *P. serrata* ITS sequence (GeneBank accession number AF314414) were included in the data set. The dendrograms were constructed by maximum parsimony (MP), maximum likelihood (ML) and neighbour-joining (NJ) methods in PAUP* beta version 4.0b4a for Macintosh (Swofford, 1998). NJ and ML dendrograms were constructed using the Tamura–Nei model of substitution (Tamura & Nei, 1993) with default options as well as with models and frequencies obtained with Modeltest3 (Posada & Crandall, 1998). Indications of support for various clades were obtained by bootstrapping ($N = 500$) applying the heuristic option of tree searching. Trees were drawn from PAUP* tree-files using the program TreeView (Page, 1996).

RESULTS

GENETICAL DIFFERENTIATION

The NJ-dendrogram based on genetic distances of the fingerprint data shows that the specimens with a 'paranoplocephaloid' early uterus form four distinct clusters, two of which (*P. serrata* and *P. gracilis*) represent well-defined species used here for comparison with other taxa (Fig. 2). The remaining specimens form two clusters corresponding to *P. krebsi* sp. nov. and *P. nordenskioeldi* sp. nov. Thus, despite its morphological affinity with *P. gracilis* and North American *P. serrata*, *P. nordenskioeldi* is a separate species with a Holarctic distribution. The distance data also show that *P. krebsi* is a Nearctic species that evidently occurs throughout the North American range of *Dicrostonyx*, including Wrangel Island and Ungava Peninsula. The UPGMA-dendrogram (not shown) gave an identical clustering of cestode specimens.

The independent status and geographical distribution of these three species of *Paranoplocephala* is fully supported by the the sequence data for the ITS1 region of nuclear rDNA (Fig. 3). The sequence data also show that the two species with a completely reticulated early uterus, *P. alternata* and *P. arctica*, form a strongly supported clade with respect to other cestode species of collared lemmings. However, the supraspecific branching order of the tree is poorly supported by the bootstrap analysis, and the phylogenetic relationships among the observed clades (species) remain largely unsettled. The main phylogenetic patterns were not affected by the clustering algorithm used (maximum parsimony, neighbor-joining or maximum likelihood).

PARANOPLOCEPHALA ARCTICA (RAUSCH, 1952)
(Figs 4, 5; Table 2)

Paranoplocephala arctica was found only in *D. groenlandicus* from Wrangel Island and north-western Alaska (Table 1). The redescription is based on the

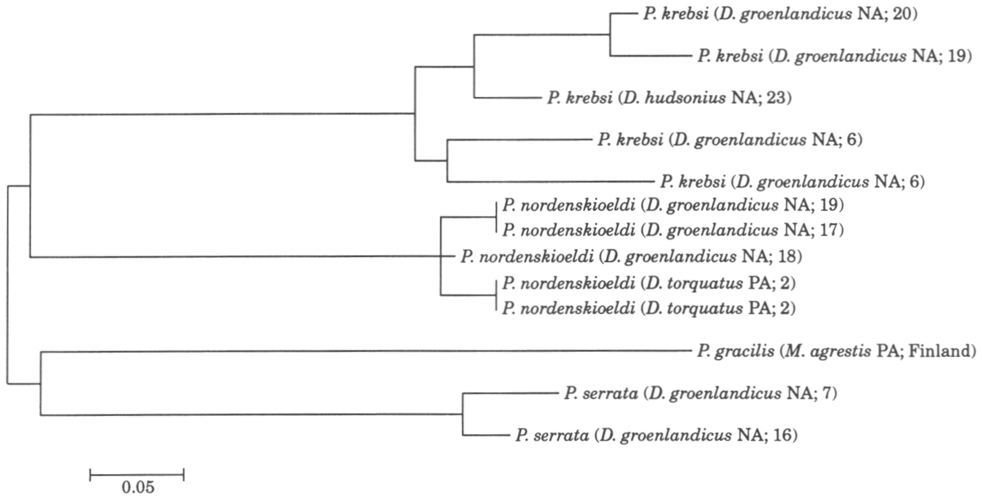

Figure 2. Dendrogram for genetic distances of anoplocephaline cestodes of collared lemmings (host species in parentheses) based on combined mini- and microsatellite data using neighbour-joining (NJ) tree reconstruction. NA = Nearctic, PA = Palearctic; numbers in parentheses refer to the sample localities in Figure 1.

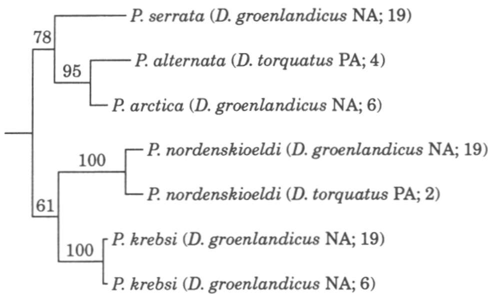

Figure 3. Dendrogram for sequence differences in the ITS1 region of anoplocephaline cestodes of collared lemmings (host species in parentheses) based on parsimony analysis with PAUP*. *Andrya cuniculi* was used as an outgroup (not shown). Values indicate bootstrap support from 250 replicates if 50% or higher. Gaps treated as missing data. NA = Nearctic, PA = Palearctic; numbers in parentheses refer to the sample localities in Figure 1.

holotype (USNPC 37356) from Point Barrow, North Alaska, and two other specimens from the type locality (personal collection of R. L. Rausch).

Redescription . Strobila relatively short and thin. Maximum width attained in gravid segments. Total number of segments 213–242; number of mature segments variable (12–45). Scolex small, globular. Suckers ovoid, directed antero-laterally. Length of neck *c.* 0.7; of uniform width. Segments craspedote. Length/width ratio of segments high, decreasing slightly from premature (0.44–1.07, mean 0.70) to mature (0.46–0.74, 0.63) and postmature (0.52–0.73, 0.62) segments, then increasing in pregravid and gravid segments (0.60–0.94, 0.77). Genital pores opening in posterior half of segment margin. Genital pores unilateral, either sinistral or dextral.

Ventral longitudinal osmoregulatory canals thin (0.020–0.040), connected by transverse canals. Dorsal osmoregulatory canals not observed. Genital ducts passing dorsally across longitudinal osmoregulatory canals.

Testes situated antiporally and anteriorly to ovary. Several testes antiporal to antiporal v.l.o.c. (= ventral longitudinal osmoregulatory canal). Poral testes usually in contact with poral v.l.o.c. and may overlap it, but never lie poral to this canal in anterior segment. Occasionally 1–2 isolated testes seen posterior to cirrus sac, either overlapping poral v.l.o.c. or extending across it porally. Few testes may overlap anterior and/or antiporal margin of ovary. Diameter of testes 0.04–0.06.

Cirrus sac large; its length and width increase further in postmature segments. Proximal cirrus sac extends invariably across poral v.l.o.c. In mature segments, length of cirrus sac slightly less than 1/3 of segment width. Muscle layers of proximal cirrus sac fairly well developed; maximum thickness in postmature segments 0.015. Ductus cirri armed with short spines. Internal seminal vesicle less than 1/3 of length of cirrus sac in mature segments; maximum absolute

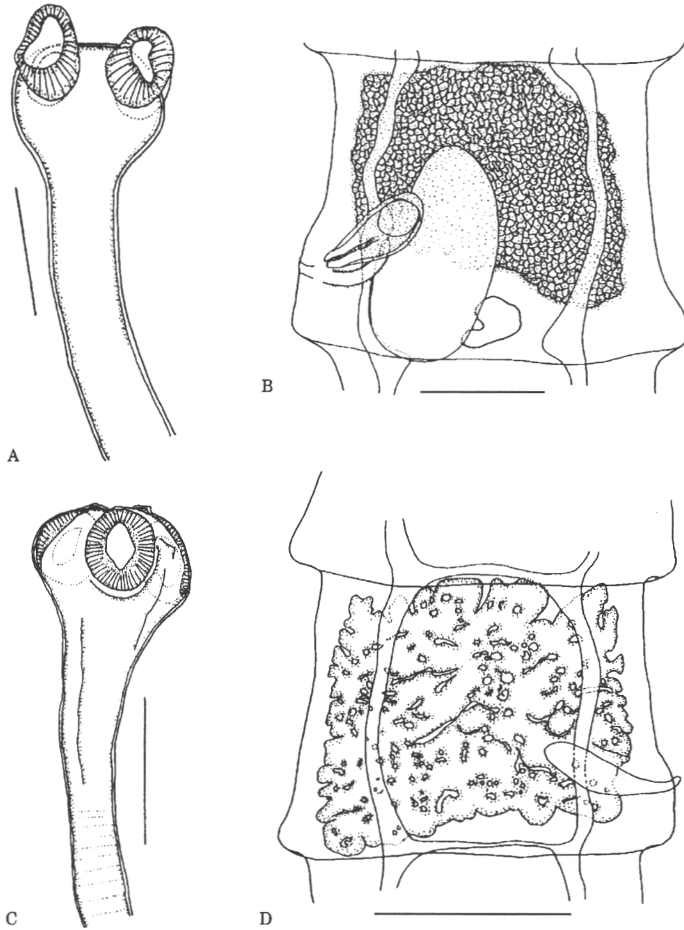

Figure 4. *Paranoplocephala arctica* (Rausch, 1952). A, scolex. Holotype from Point Barrow, Alaska, host *Dicrostonyx groenlandicus* (scale bar=0.20 mm). B, uterus in late mature segment. Holotype (scale bar=0.20 mm). C, scolex. Wrangel Island, host *D. groenlandicus* (scale bar=0.30 mm). D, uterus in pregravid segment. Holotype (scale bar=0.50 mm).

and relative size attained in postmature segments (more than $\frac{1}{2}$ of cirrus sac length). External seminal vesicle elongate, its surface covered with thin, loose cell layer. External seminal vesicle elongates further in postmature segments and attains pedunculated form.

Vagina tube-like organ of uniform width; situated posteriorly to cirrus sac. Vagina slightly shorter than cirrus sac. Proximal vagina curved anteriad and distinctly set off from seminal receptacle. No hairs or other lining observed on internal surface of vagina; external surface covered with thin cell layer. Seminal receptacle large, ovoid. Seminal receptacle increases markedly in size in late mature segments, its length maximally almost half of segment width. Vitellarium relatively large, asymmetrically bilobed, may overlap slightly seminal receptacle. Mehlis' gland ovoid or spherical. Position of vitellarium and ovary median. Ovary large, lobed; usually overlaps longitudinal ventral osmoregulatory canals bilaterally. Width of ovary *c.* $\frac{1}{2}$ of segment width.

In late mature segments, uterus seen as fine reticulum that covers most of medulla, excluding posteriormost part of segment, and extends markedly across ventral longitudinal osmoregulatory canals bilaterally. In pregravid segments, uterus with anterior, posterior and lateral sacculations, and numerous

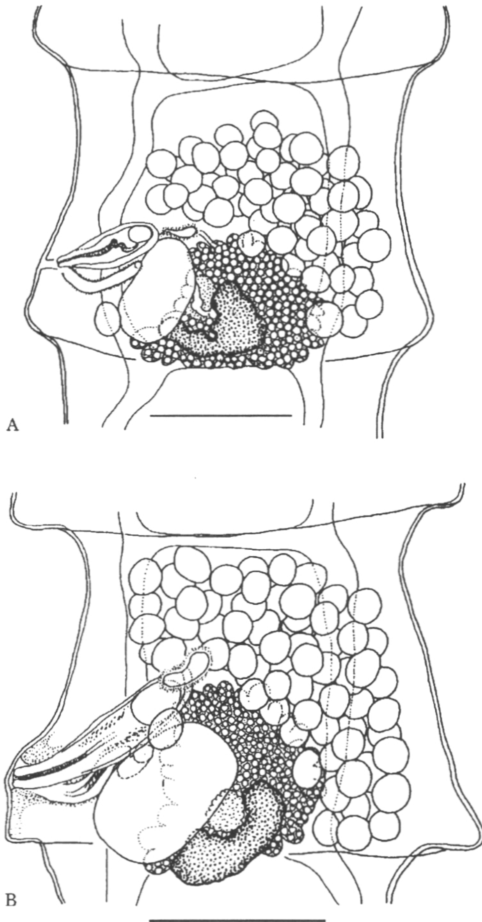

Figure 5. *Paranoplocephala arctica* (Rausch, 1952): mature segments. A, holotype from Point Barrow, Alaska, host *Dicrostonyx groenlandicus* (scale bar = 0.20 mm). B, Wrangel Island, host *D. groenlandicus* (scale bar = 0.30 mm).

internal trabecula. Eggs large, spherical in surface view; distinctly ovoid in side view. Pyriform apparatus present.

Specimens from Wrangel Island and north-eastern Alaska. The three main characters that link the specimens from Wrangel Island with those from the type locality of *P. arctica* are unilateral genital pores, large eggs and testes distribution (no testes extending porally across poral v.l.o.c. in anterior segment) (Figs 4, 5); these characters also serve to distinguish *P. arctica* from *P. alternata* (below). The three additional specimens from north-eastern Alaska (Kuparuk and Colville River) were deteriorated, but they could also be identified as *P. arctica* according to the features listed above. The specimens from Wrangel Island differed from the Alaskan specimens by larger body width, larger scolex and larger dimensions of some organs (Table 2). However, the ovary of Alaskan specimens was larger than the ovary of *P. arctica* on Wrangel Island. Such metric differences obviously have less taxonomical value than the set of correlated (qualitative) characters listed above, especially because the sample sizes were small. We conclude that despite the observed metric differences, both populations represent *P. arctica*.

A representative slide of *P. arctica* from Wrangel Island has been deposited in the United States National Parasite Collection (USNPC 88813).

Remarks. Rausch (1952) described *P. arctica* from *D. groenlandicus* from Point Barrow, North Alaska. In addition to the type host and type locality, *P. arctica* was recorded from the Prince Patrick Island (host *D. groenlandicus*), from Churchill, Manitoba (host *D. richardsoni*), and from *Lemmus trimucronatus* (Richardson), *Clethrionomys rutilus* (Pallas) and *Microtus miurus* Osgood in Alaska (Rausch, 1952). Since the original descripton, *P. arctica* has been found as an accidental parasite of *M. miurus* in Alaska (Haukisalmi *et al.*, 1995), and as a common and widespread parasite of *Dicrostonyx* spp. in the Holarctic (Haukisalmi & Henttonen, 2000; Wickström *et al.*, 2001). However, our re-examination of the two specimens from *M. miurus* showed that they represent an unknown species of *Paranoplocephala*, and the species previously identified as *P. arctica* in collared lemmings outside the northern Alaska is here shown to represent a separate, new species (*P. alternata*).

There are several reasons to assume that the original description *P. arctica* is composite, and may include three different species. First, the reported host spectrum is unusually wide for any species of anoplocephaline cestode in rodents. We have examined cestode material from *L. trimucronatus*, *C. rutilus* and *Microtus* spp. from Alaska without finding specimens corresponding to the holotype of *P. arctica*; other species of *Paranoplocephala* do, however, occur in these arvicoline rodents in Alaska (Haukisalmi *et al.*, 1995; Haukisalmi & Henttonen, 2000). Rausch (1952) also suspected that the Fennoscandian *Lemmus lemmus* (Linnaeus) might be parasitized by *P. arctica*, but we have recently shown that the cestode in the Norwegian lemming is a distinct species specific to the genus *Lemmus* Link (Haukisalmi & Henttonen, 2001).

According to Rausch, 1952, the genital pores of *P. arctica* alternate irregularly, but we found that they are in fact strictly unilateral in the holotype as well

Table 1. Prevalence (%) and geographical distribution of cestodes of collared lemmings in the present material. The numbers refer to the localities (Fig. 1) in which the species was found. Notice that Wrangel Island (locality 6) has been included in the Nearctic. The Palearctic and some of the Nearctic records for *Paranoplocephala serrata* are from Haukisalmi & Henttonen, 2000

	Palearctic ($N=68$)		Nearctic ($N=172$)	
	%	Localities 1–5	%	Localities 6–24
Paranoplocephala arctica	—	—	5	6–9
***P. alternata* sp. nov.**	28	1–5	43	10–22, 24
P. serrata	11	1, 3, 4	29	6, 8, 9, 11–13, 15–19, 22
***P. nordenskioeldi* sp. nov.**	5	2, 4	2	17–19
***P. krebsi* sp. nov.**	—	—	10	6, 13, 18–21, 23, 24

as in the other two specimens from the type locality. This discrepancy suggests that the original description of *P. arctica* also includes specimens of *P. alternata*, a species that has frequently (and irregularly) alternating genital pores (below). The wide variability of egg dimensions originally reported for *P. arctica* supports this conclusion; *P. alternata* has significantly smaller eggs than *P. arctica* (Table 3). We found that the specimens from Prince Patrick Island, identified as *P. arctica* by Rausch (1952), represent *P. alternata*.

Finally, Rausch (1952) states in the description of *P. arctica* that "Testes in some cases not seen farther poral than poral edge of ovary", a characteristic that does not occur in *P. arctica* (or *P. alternata*) in the type material. We therefore believe that the original description might also refer to *Paranoplocephala serrata*, a common anoplocephaline cestode of collared lemmings in the Palearctic and Nearctic regions, including Alaska. *Paranoplocephala serrata* is most clearly distinguished from *P. arctica* and *P. alternata* by different distribution of testes and development of uterus (Haukisalmi & Henttonen, 2000).

PARANOPLOCEPHALA ALTERNATA SP. NOV.

(Figs 6, 7; Table 3)

Holotype. USNPC 89644, from collared lemming, *Dicrostonyx torquatus*, collected from western Kolyma Delta (69° N, 162° E), Russia, on 10 July 1994; an entire gravid specimen.

Paratypes. (i) USNPC 88811, from *Dicrostonyx torquatus*, collected from western Yamal Peninsula (72° N, 68° E), Russia, on 18 June 1994; an entire gravid specimen, one slide. (ii) USNPC 88812, from *Dicrostonyx torquatus*, collected from western Kolyma Delta, Russia, on 10 July 1994; an entire gravid specimen. (iii) USNPC 88814, from *Dicrostonyx groenlandicus*, collected from Byron Bay, Southern Victoria Island (68°45′N, 109°04′W), Nunavut, Canada, on 8 July 1996; an entire gravid specimen.

Location in the host. Small intestine (jejunum).

Etymology. Derived from its frequently alternating genital pores.

Prevalence and geographical distribution. P. alternata is the most common (28–43%) cestode of collared lemmings throughout the Holarctic region, with the exception of eastern Beringia. It was found in *D. torquatus* from Siberia, *D. groenlandicus* from northeastern Alaska, Canadian Arctic Archipelago and Greenland, and *D. richardsoni* from the western coast of Hudson Bay (Table 1; Fig. 1).

Description. The description is based on *c.* 70 mounted specimens from Siberia and Arctic Canada, of which 31 gravid/pregravid individuals were subjected to a detailed morphometric analysis: 8 from Yamal Peninsula, 1 from Taymyr Peninsula, 8 from western Kolyma Delta, 12 from southern Victoria Island/Kent Peninsula region (Nunavut, Canada) and 2 from Rankin Inlet, western coast of Hudson Bay. 28 of these were used in a morphometric analysis of *P. alternata* by Wickström *et al.* (2001), then included in *Andrya arctica*.

Strobila long, ribbon-like; segments dorso-ventrally thin, transparent. Maximum width attained in pregravid or gravid segments. Scolex small, not distinct from neck. Suckers spherical or ovoid, directed laterally or antero-laterally, often partly covered by lids. Length of neck variable; of uniform width or tapering posteriad; minimum width attained *c.* 0.5 from scolex. Segments craspedote, but velum short. Total number of segments 187–229 (mean 244.5, $N=19$); number of mature segments 14–42 (mean 28.6, $N=30$). Length/width ratio of segments variable (on average 0.30–0.50); decreasing from premature to mature and postmature segments, then increasing in pregravid and gravid segments. Genital pores opening in posterior half of segment margin. Genital pores always frequently alternating; on average 2.7 segments in each

Table 2. Measurements and other morphological features of *Paranoplocephala arctica* based on the original description (Rausch, 1952), material from the type locality (Point Barrow) including the holotype ($N=3$), and specimens from Wrangel Island ($N=4$). All metric data are in millimeters, except for the egg dimensions which are in microns

	Rausch, 1952	Present study: Point Barrow, Alaska			Present study: Wrangel Island	
Characters	Range	Mean	Range	No. measurements	Range	No. measurements
Genital pore alternation	Irregularly alternating	Unilateral			Unilateral	
Body length	50–145	90.7	84–98	3	90	1
Maximum width	1–2.4	1.48	1.28–1.58	3	1.80–2.96	4
Width of mature segments	—	0.66	0.52–0.80	11	0.61–0.95	3
Scolex, diameter	0.216–0.560	0.233	0.22–0.25	2	0.31–0.33	2
Suckers, diameter	0.080–0.110	0.130	—	1	0.160–0.165	2
Testes, total number	40–50	51.8	45–60	11	49–80	3
Testes, no. antiporal to a.v.l.o.c.[a]	Several	9.8	8–12	11	9–16	3
Testes, no. poral to p.v.l.o.c.[b]	0	0.5	0–2	11	0–1	3
Cirrus sac, length	0.194–0.352	0.182	0.140–0.245	11	0.22–0.34	3
Cirrus sac, width	0.057–0.136	0.071	0.055–0.090	11	0.065–0.085	3
Cirrus sac, maximum length[c]	—	0.270	0.26–0.28	3	0.25–0.30	2
Internal seminal vesicle, length	—	0.049	0.035–0.075	11	0.050–0.095	3
External seminal vesicle, length	—	0.066	0.035–0.120	11	0.075–0.110	3
Ovary, width	—	0.288	0.26–0.32	6	0.20–0.23	3
Vitellarium, width	—	0.180	0.110–0.260	11	0.200–0.225	3
Index of asymmetry	—	0.50	0.45–0.56	11	0.48–0.51	3
Vagina, length	—	0.159	0.13–0.20	10	0.17–0.23	2
Vagina, maximum width	—	0.021	0.018–0.025	10	0.030–0.040	3
Vagina/cirrus sac ratio	—	0.907	0.82–1.00	10	0.77–0.85	2
Seminal receptacle, length	—	0.204	0.10–0.35	11	0.17–0.26	3
Seminal receptacle, width	—	0.138	0.070–0.280	11	0.135–0.180	3
Seminal receptacle, max. length	—	0.353	0.35–0.36	3	0.45–0.57	3
Egg, length	40–72	67.0	60–76	15	61–73	20
Egg, width	26–65	61.1	55–67	15	58–70	20

[a] a.v.l.o.c., antiporal ventral longitudinal osmoregulatory canal.
[b] p.v.l.o.c., poral ventral longitudinal osmoregulatory canal.
[c] Postmature segments.

unilateral set or 57–92 changes in fully gravid strobilae.

Ventral longitudinal osmoregulatory canals (v.l.o.c.) 0.035–0.100, connected by transverse canals. Dorsal longitudinal osmoregulatory canals *c.* 0.01, lateral to ventral osmoregulatory canals or overlapping them dorsally. Genital ducts passing dorsally across longitudinal osmoregulatory canals.

Testes situated in antiporal and anterior parts of segment. Several testes antiporally to antiporal v.l.o.c; few testes always extend porally across poral v.l.o.c. Testes do not usually overlap ovary. Diameter of testes 0.05–0.09; testes in lateral fields usually larger than those in median field.

Cirrus sac prominent, usually extending across poral v.l.o.c. Length of cirrus sac slightly less than 1/3 of

Table 3. Measurements of ***Paranoplocephala alternata* sp. nov.** in the type locality (western Kolyma Delta) and in the combined material from Siberia (including western Kolyma Delta), Arctic Canada and western coast of Hudson Bay. All metric data are in millimeters, except for the egg dimensions which are in microns

	Western Kolyma Delta ($N=8$)			Whole material ($N=31$)		
	Mean	Range	No. measurements	Mean	Range	No. measurements
Body length	112.9	90–136	7	102.0	74–147	22
Maximum width	2.36	2.05–2.84	8	2.35	1.73–2.96	25
Segments/unilateral set	2.42	1.82–2.74	7	2.66	2.42–2.94	28
Length/width ratio Premature segments	0.41	0.22–0.81	24	0.40	0.17–0.81	85
Mature segments	0.38	0.22–0.62	24	0.37	0.14–0.75	87
Postmature segments	0.35	0.25–0.51	24	0.30	0.16–0.51	87
Gravid segments	0.53	0.35–0.84	18	0.45	0.24–0.84	70
Scolex, diameter	0.339	0.30–0.38	8	0.307	0.24–0.50	29
Suckers, diameter	0.166	0.155–0.180	8	0.163	0.140–0.230	28
Neck, length	0.51	0.30–0.65	8	0.62	0.10–1.10	26
Neck, minimum width	0.150	0.11–0.21	8	0.14	0.08–0.24	26
Testes, total number	82.3	70–101	23	73.7	45–101	82
Testes, no. antiporal to a.v.l.o.c.[a]	14.9	9–26	20	16.3	2–29	72
Testes, no. poral to p.v.l.o.c.[b]	6.2	2–10	20	4.8	0–10	69
Cirrus sac, length	0.325	0.24–0.43	23	0.289	0.22–0.43	83
Cirrus sac, width	0.112	0.085–0.154	23	0.096	0.069–0.154	83
Cirrus sac, maximum length[c]	0.468	0.40–0.50	8	0.477	0.40–0.62	31
Internal seminal vesicle, length	0.128	0.080–0.175	23	0.108	0.060–0.200	81
External seminal vesicle, length	0.120	0.085–0.159	22	0.105	0.055–0.170	81
Ovary, width	0.407	0.32–0.55	23	0.377	0.23–0.57	78
Vitellarium, width	0.227	0.18–0.29	23	0.196	0.13–0.33	80
Index of asymmetry	0.52	0.46–0.60	22	0.51	0.43–0.64	78
Vagina, length	0.238	0.20–0.27	13	0.224	0.17–0.28	59
Vagina, maximum width	0.029	0.023–0.037	15	0.033	0.023–0.045	61
Vagina/cirrus sac ratio	0.76	0.65–0.92	13	0.79	0.65–1.00	56
Seminal receptacle, length	0.303	0.13–0.51	23	0.250	0.09–0.51	74
Seminal receptacle, width	0.168	0.090–0.292	23	0.160	0.070–0.292	74
Seminal receptacle, mx. length[c]	0.66	0.40–0.80	7	0.59	0.40–0.90	29
Egg, length	46.7	43–51	35	49.1	40–61	145
Egg, width	45.1	41–48	35	47.4	40–60	145

[a] a.v.l.o.c., antiporal ventral longitudinal osmoregulatory canal.
[b] p.v.l.o.c., poral ventral longitudinal osmoregulatory canal.
[c] Postmature segments.

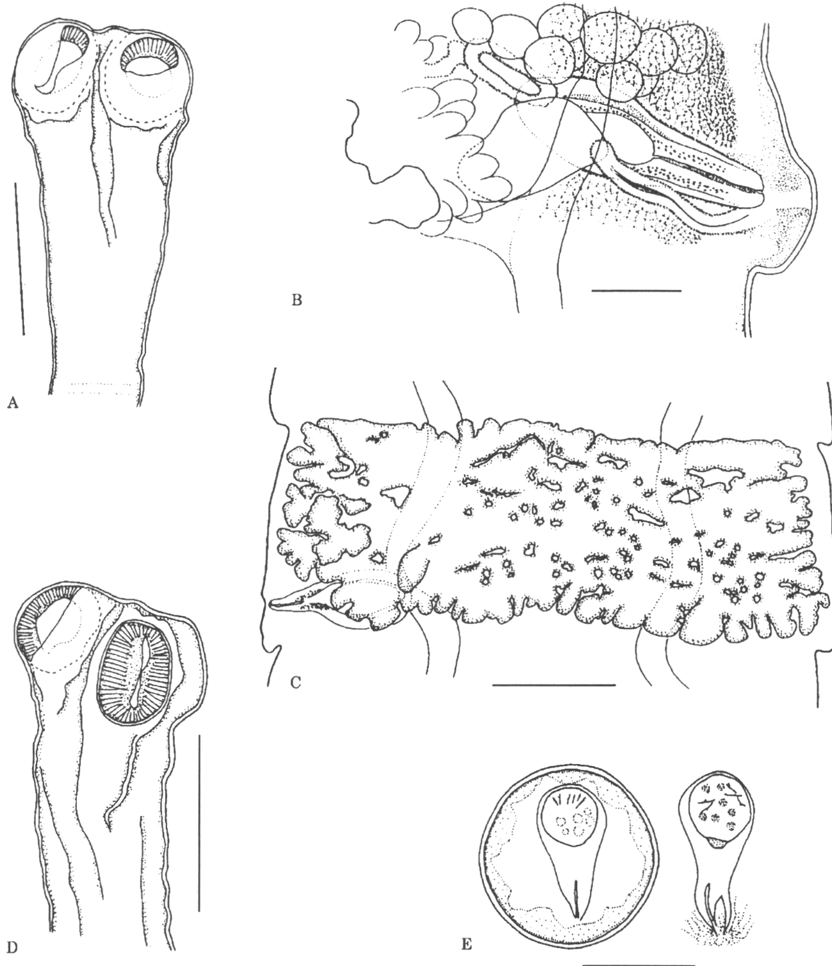

Figure 6. *Paranoplocephala alternata* sp. nov. A, scolex. Western Kolyma Delta, host *Dicrostonyx torquatus* (scale bar = 0.30 mm). B, genital ducts and a part of early uterus in mature segment. Western Kolyma Delta, host *D. torquatus* (scale bar = 0.10 mm). C, uterus in pregravid segment. Western Kolyma Delta, host *D. torquatus* (scale bar = 0.50 mm). D, scolex. Byron Bay, host *D. groenlandicus* (scale bar = 0.30 mm). E, egg and oncosphere with pyriform apparatus. Western Kolyma Delta, host *D. torquatus* (scale bar = 0.030 mm).

segment width in mature segments; absolute and relative length increase in postmature segments. Muscle layers of proximal cirrus very thick, 0.035–0.075 in postmature segments. Cirrus sac covered with thick layer of intensely stained, rounded cells. Ductus cirri armed with short spines. Length of internal seminal vesicle *c.* 40% of length of cirrus sac in mature segments, up to 70% in postmature segments. External seminal vesicle elongate, increases in length and attains pedunculated form in postmature segments. Surface of external seminal vesicle covered with thick layer of intensely stained cells.

Vagina tube-like organ of uniform width; distal part situated posteriorly and proximal part ventrally or postero-ventrally to cirrus sac. Vagina slightly shorter than cirrus sac (*c.* 4/5), clearly set off from seminal receptacle. Expanding seminal receptacle distorts vagina in late mature segments. Internal surface of proximal vagina lined with fine hairs; external surface covered with dense layer of small intensely stained

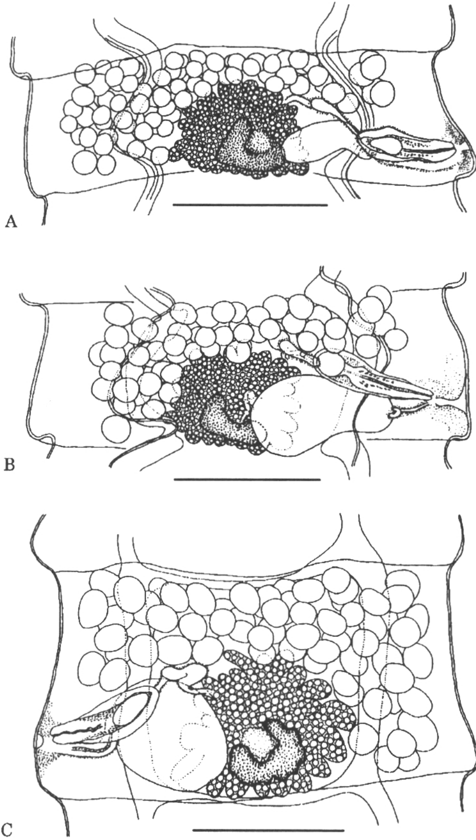

Figure 7. *Paranoplocephala alternata* sp. nov.: mature segments. A, Western Kolyma Delta, host *Dicrostonyx torquatus* (scale bar = 0.50 mm). B, Rankin Inlet, host *D. richardsoni* (scale bar = 0.30 mm). C, Byron Bay, host *D. groenlandicus* (scale bar = 0.30 mm).

cells. Seminal receptacle very large, especially in postmature segments; ovoid or irregularly shaped. Position of ovary median, vitellarium median or slightly antiporal. Vitellarium asymmetrically bilobed, may overlap slightly seminal receptacle. Mehlis' gland ovoid or spherical. Ovary distinctly lobed; width of ovary *c.* 1/3 of segment width.

Uterus first seen in late premature segments as dorso-ventrally thin, complex reticulum formed by fine threads, situated ventrally to other organs and covering whole medulla. In mature segments, reticulum becomes denser and thickens dorso-ventrally; lateral margins more densely reticulated than median uterus. With further development, irregular marginal sacculations and internal compartments with visible lumen appear within uterus. Fully developed uterus (pregravid segments) with numerous internal trabecula and fenestrations without lumen. Marginal sacculations disappear, but internal trabecula remain in fully gravid segments. Eggs spherical in surface view, ovoid in side view. Pyriform apparatus with two horns, one of which may be branched; tip of horns armed with bunch of fine hairs.

Remarks. Paranoplocephala alternata and *P. arctica* are the only species in collared lemmings having a completely reticulated early uterus, which suggests a close relationship between these taxa. However, *P. alternata* differs markedly from *P. arctica* on morphological grounds. The new species has frequently alternating genital pores (unilateral in *P. arctica*), different distribution of testes and significantly smaller eggs than *P. arctic*. There are also several other morphometric differences, but these cannot be given too much weight because of the small sample size for *P. arctica* (Tables 2 and 3).

Because of their allopatric distribution, these taxa could be treated either as subspecies of a single Holarctic species or as separate, full species. We have chosen the latter alternative, because there is a distinct morphological gap between the two taxa (no intermediate forms have been found) and because the existing morphological differences, especially the alternation of genital pores and distribution of testes, do not show significant intraspecific variation in other anoplocephaline species.

In addition to *P. alternata*, there are three other species of *Paranoplocephala* with alternating genital pores and testes distributed in the antiporal, median and poral fields: *P. sciuri* (Rausch, 1947), *P. aquatica* Genov, Vasileva & Georgiev, 1996 and *P. genovi* Gubányi, Tenora & Murai, 1998. Of these, *P. aquatica* is known to have a completely reticulated early uterus (Genov, Vasileva & Georgiev, 1996), but the uterine development of *P. sciuri* and *P. genovi* has not been described in detail. *Paranoplocephala alternata* can be easily distinguished from *P. sciuri and P. aquatica* both on morphological and morphometric grounds. For example, both species differ from *P. alternata* by having less frequently alternating genital pores, different type of seminal receptacle, larger scolex, shorter cirrus sac and shorter vagina relative to the length of the cirrus sac (Table 4). *Paranoplocephala genovi* is closely related to *P. aquatica*, and both species differ in the same respects from *P. alternata*. All three species compared with *P. alternata* also differ from the new species by occurring in different host species in more southern regions.

Wickström *et al.* (2001) compared the morphometrics of *P. alternata* (then included in *Andrya arctica*) among three localities in the Holarctic region and showed

Table 4. Main morphological and morphometric features of ***Paranoplocephala alternata*** **sp. nov.** and two related species, *Paranoplocephala sciuri* (Rausch, 1947) and *Paranoplocephala aquatica* Genov, Vasileva & Georgiev, 1996. Measurements are mean values and range. All metric data are in millimeters, except for the egg dimensions which are in microns

Parasite species Host species Distribution Source	***P. alternata*** **sp. nov.** *Dicrostonyx* spp. Holarctic Present study		*P. sciuri* *Glaucomys sabrinus* Nearctic Rausch, 1947; Genov *et al.*, 1996		*P. aquatica* *Arvicola terrestris* Palearctic Genov *et al.*, 1996	
Genital pore alternation	frequently alternating		infrequently alternating		infrequently alternating	
Position of genital pores	posterior half of segment margin		posterior half of segment margin		middle of segment margin	
Position of femal glands	median		median		poral	
Shape of seminal receptacle	ovoid		elongate		elongate	
Body length	102.0	74–147	—	170	—	178
Maximum width	2.35	1.73–2.96	—	2	2.30	1.86–2.65
Scolex, diameter	0.307	0.24–0.50	0.380	—	0.475	0.407–0.619
Suckers, diameter	0.163	0.140–0.230	0.151	0.148–0.152	0.187	0.152–0.237
Testes, total number	73.7	45–101	100	93–109	92	76–110
Cirrus sac, length	0.289	0.22–0.43	0.179	0.170–0.187	0.184	0.157–0.201
Vagina/cirrus sac ratio	0.79	0.65–1.00	0.46	0.43–0.50	0.33	0.30–0.38
Seminal receptacle, length	0.250	0.09–0.51	0.407	0.34–0.49	0.262	0.215–0.339
Egg, length	49.1	40–61	—	52–56	39	38–41

that the populations in western Arctic Siberia (Yamal Peninsula, host *D. torquatus*) and Arctic Canada (Victoria Island/Kent Peninsula, host *D. groenlandicus*) were morphometrically indistinguishable, both differing significantly from the population in eastern Siberia (western Kolyma Delta, host *D. torquatus*). A corresponding pattern was observed in three genetical markers. However, there are no differences in taxonomically important characters (e.g. distribution of testes, alternation of genital pores) or in any qualitative feature between specimens in different regions, which suggests that all studied populations are conspecific and that *P. alternata* has a Holarctic distribution.

The present study also shows that the cestodes from *D. richardsoni*, originating from the western coast of Hudson Bay, represent *P. alternata*. The two specimens examined had a characteristic distribution of testes (Fig. 7B), alternation of genital pores and egg size (0.048–0.055). On the other hand, the diameter of scolex (0.42–0.50) and the total number of testes (51–63) were significantly different in the specimens from *D. richardsoni* compared with *P. alternata* from other host species and regions.

PARANOPLOCEPHALA NORDENSKIOELDI SP. NOV.

(Figs 8, 9; Table 5)

Holotype. USNPC 90540, from *Dicrostonyx torquatus*, collected from western Kolyma Delta (69° N, 162° E), Russia, on 10 July 1994; an entire gravid specimen.

Paratypes. (i) USNPC 90541, from *Dicrostonyx torquatus*, collected from north-western Taymyr Peninsula (77° N, 105° E), Russia, on 28 June 1994; gravid specimen without scolex. (ii) USNPC 90542, from *Dicrostonyx groenlandicus*, collected from southern Bathurst Island (75°04′N, 98°34′W), Nunavut, Canada, on 13 July 1999; gravid specimen without scolex.

Location in the host. Small intestine (duodenum).

Etymology. The new species is named after Adolf Erik Nordenskiöld (1832–1901), a Finnish-born explorer and scientist who was the first to navigate the North-East passage. A large number of biological samples was collected during Nordenskiöld's expeditions.

Prevalence and geographical distribution. P. nordenskioeldi is a rare (2–5%), but wide-spread parasite

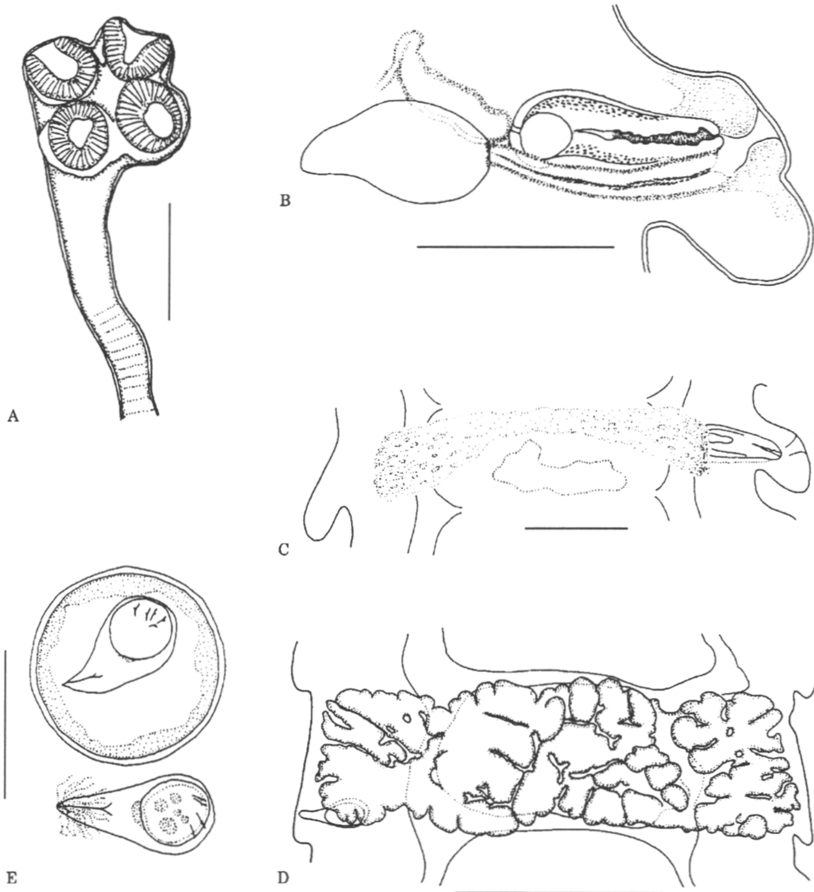

Figure 8. *Paranoplocephala nordenskioeldi* sp. nov. A, scolex. Devon Island, host *Dicrostonyx groenlandicus* (scale bar=0.40 mm). B, genital ducts. Taymyr Peninsula, host *D. torquatus* (scale bar=0.20 mm). C, early uterus in mature segment. Taymyr Peninsula, host *D. torquatus* (scale bar=0.30 mm). D, uterus in pregravid segment. Western Kolyma Delta, host *D. torquatus* (scale bar=1.0 mm). E, egg and oncosphere with pyriform apparatus. Bathurst Island, host *D. groenlandicus* (scale bar=0.040 mm).

of collared lemmings; it has a Holarctic distribution in *D. torquatus* and *D. groenlandicus* (Table 1; Fig. 1).

Description. The description is based on eight gravid specimens: six from *D. groenlandicus* from Arctic Canada and two from *D. torquatus* from central Siberia (Taymyr Peninsula and western Kolyma delta).

Strobila relatively long; maximum width attained in pregravid or gravid segments. Scolex small, distinctly set off from thin neck. Suckers protruding, large compared with size of scolex. Total number of segments c. 400 ($N=1$), number of mature segments 30–50 (mean 43.3, $N=5$). Segments craspedote with distinct velum. All segments wider than long; maximum length/width ratio always in gravid segments.

Genital pores opening in middle of segment margin or slightly more posteriad. Genital atrium deep, directed antero-laterally. Genital pores unilateral or infrequently alternating with 1–11 changes per strobila.

Ventral longitudinal osmoregulatory canals (v.l.o.c.) variable in width (0.02–0.12), connected by thin transverse canals. Dorsal osmoregulatory canals not observed, but probably present. Genital ducts passing dorsally across longitudinal osmoregulatory canals.

Testes 29–52 in number, situated antiporally and anteriorly to ovary, dorsoventrally in 1–3 layers. Few

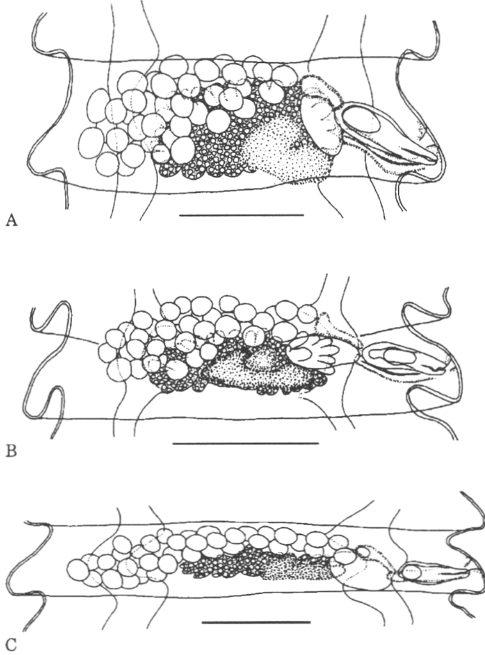

Figure 9. *Paranoplocephala nordenskioeldi* sp. nov.: mature segments. A, Bathurst Island, host *Dicrostonyx groenlandicus* (scale bar = 0.30 mm). B, Taymyr Peninsula, host *D. torquatus* (scale bar = 0.50 mm). C, Western Kolyma Delta, host *D. torquatus* (scale bar = 0.30 mm).

testes lie antiporally to antiporal v.l.o.c. Porally testes reach poral v.l.o.c. and occasionally overlap it. Testes overlap antiporal and anterior parts of ovary. Diameter of testes 0.05–0.08; testes in lateral field usually larger than those in median field. Length of cirrus sac 14–37% of segment width. Position of cirrus sac relative to v.l.o.c. variable (not reaching, overlapping or extending across v.l.o.c.). Muscle layers of proximal cirrus sac of variable thickness (0.013–0.031). Cirrus sac covered with loose layer of rounded cells. Ductus cirri armed with short spines. Length of internal seminal vesicle *c.* 1/3 of cirrus sac length in mature segments. External seminal vesicle straight or slightly looped, *c.* half of cirrus sac length in mature segments. External surface of external seminal vesicle lined with dense layer of rounded, intensely stained cells.

Vagina situated ventrally, postero-ventrally or posteriorly to cirrus sac. Vagina usually slightly shorter than cirrus sac, but occasionally exceeds length of cirrus sac. Vagina distinctly set off from seminal receptacle. Vagina of uniform width or distal vagina slightly wider than proximal part. Fine hairs usually observed on internal surface of vagina. External surface of vagina covered with dense layer of small, intensely stained cells. Seminal receptacle elongate or subspherical in shape. Vitellarium asymmetrically bilobed. Width of ovary 35–55% of segment width, fills most of space between v.l.o.c.s. Position of vitellarium usually slightly poral or median, occasionally slightly antiporal; ovary median.

Uterus appears ventrally as transverse band in anteriormost part of early mature segments, extending across ventral osmoregulatory canals bilaterally. Lateral parts of uterus slightly wider than median part, small fenestrations without lumen present in lateral extremities. Margins of early uterus irregular. In early postmature segments, median part of uterus spreads posteriad overlapping testes and develops visible lumen. Fully developed uterus (pregravid segments) with large anterior, posterior and lateral sacculations and internal trabecula. Fenestrations and reticulations absent. All sacculations and diverticula disappear in fully gravid segments, but internal trabecula persist. Eggs spherical or slightly ovoid in surface view, slightly flattened in side view. Pyriform apparatus with two slender horns; additional horn may be present. Tip of horns armed with bunch of fine hairs.

Remarks. We compare *P. nordenskioeldi* with species having testes distributed both antiporally and anteriorly to ovary and having a 'paranoplocephaloid' early uterus with slightly fenestrated lateral 'wings' (Table 6).

Paranoplocephala macrocephala (Douthitt, 1915) from *Geomys bursarius* (Shaw) and *P. kalelai* (Tenora, Haukisalmi & Henttonen, 1985) from *Clethrionomys rufocanus* (Sundevall) both differ from *P. nordenskioeldi* by their larger scolex, fewer testes antiporal to antiporal v.l.o.c., shorter vagina and smaller eggs. A few other morphometric differences are also evident in both species. In addition, the morphology of genital ducts of *P. macrocephala* and *P. kalelai* differs fundamentally from that of *P. nordenskioeldi* (cf. Douthitt, 1915; Tenora, Haukisalmi & Henttonen, 1985a).

Paranoplocephala gracilis (hosts *Microtus* spp. and *Clethrionomys* spp.) is morphometrically fairly similar to *P. nordenskioeldi*, the main differences being the smaller cirrus sac, larger seminal receptacle and smaller eggs in *P. gracilis*. In addition, a few testes usually extend across poral v.l.o.c. in *P. gracilis*, which has not been observed in the new species. *Paranoplocephala janickii* Tenora, Murai & Vaucher, 1985 from *Microtus arvalis* (Pallas) is a slightly smaller (average length 50 mm) cestode than *P. nordenskioeldi*, and it also differs from the new species by a shorter cirrus sac, absolutely and relatively shorter vagina and smaller eggs. The independent taxonomical status of *P. gracilis*

Table 5. Measurements of ***Paranoplocephala krebsi*** **sp. nov.** and ***P. nordenskioeldi*** **sp. nov.** All metric data are in millimeters, except for the egg dimensions which are in microns

	P. krebsi sp. nov. ($N=12$)			*P. nordenskioeldi* sp. nov. ($N=8$)		
	Range	Mean	*N*	Range	Mean	*N*
Body length	36–85	60.4	8	70–145	103.0	4
Maximum width	1.40–3.35	2.56	11	1.40–2.87	2.04	7
Length/width ratio						
Premature segments	0.07–0.51	0.19	16	0.16–0.51	0.32	12
Mature segments	0.08–0.35	0.19	29	0.15–0.43	0.25	21
Postmature segments	0.09–0.40	0.20	29	0.13–0.47	0.25	21
Gravid segments	0.13–0.81	0.32	28	0.24–0.92	0.50	20
Scolex, diameter	0.30–0.45	0.35	9	0.37–0.61	0.46	5
Suckers, diameter	0.130–0.193	0.159	9	0.175–0.250	0.207	5
Neck, length	0.26–0.45	0.34	7	0.27–0.45	0.36	3
Neck, minimum width	0.13–0.32	0.22	8	0.10–0.15	0.13	3
Testes, total number	27–47	37.8	32	29–52	39.7	18
Testes, no. antiporal to a.v.l.o.c.[a]	3–16	9.0	29	0–8	4.1	18
Cirrus sac						
Length	0.17–0.26	0.209	26	0.19–0.31	0.243	23
Width	0.050–0.096	0.072	26	0.060–0.100	0.079	23
Maximum length[b]	0.20–0.29	0.225	11	0.23–0.31	0.266	8
Internal seminal vesicle, length	0.050–0.130	0.083	24	0.050–0.110	0.075	19
External seminal vesicle, length	0.070–0.160	0.111	25	0.075–0.170	0.118	11
Ovary, width	0.30–0.67	0.474	30	0.300–0.630	0.416	19
Vitellarium, width	0.14–0.28	0.209	32	0.14–0.38	0.234	23
Index of asymmetry	0.373–0.539	0.452	32	0.38–0.59	0.477	23
Vagina, length	0.12–0.20	0.162	21	0.16–0.27	0.198	22
Vagina, maximum width	0.019–0.051	0.034	21	0.030–0.050	0.043	21
Vagina/cirrus sac ratio	0.62–0.95	0.83	20	0.68–1.05	0.82	22
Seminal receptacle						
Length	0.10–0.28	0.177	23	0.14–0.25	0.190	22
Width	0.037–0.160	0.089	23	0.080–0.150	0.111	22
Maximum length[b]	0.26–0.60	0.417	11	0.25–0.55	0.348	8
Egg, length	39–55	46.3	55	46–56	50.9	35
Egg, width (larger)	36–51	43.8	55	42–54	48.3	35
Egg, width (smaller)	30–37	33.4	30	33–39	36.5	10

[a] a.v.l.o.c., antiporal ventral longitudinal osmoregulatory canal.
[b] Postmature segments.

and *P. nordenskioeldi* was supported by the genetical markers (above).

Of the other related species, *P. longivaginata* Chechulin & Gulyaev, 1998 from *Clethrionomys rutilus* has a distinctly longer vagina than any other species of *Paranoplocephala*. *Paranoplocephala nevoi* Fair, Schmidt & Wertheim, 1990 from *Nannospalax ehrenbergi* (Nehring) is a short-bodied species that has a short cirrus-sac (0.12–0.16). *Paranoplocephala neotomae* (Voge, 1946) from *Neotoma fuscipes* Baird is

Table 6. Comparison of the main morphometric features of ***Paranoplocephala nordenskioeldi* sp. nov.** with those of *P. macrocephala* (Douthitt, 1915), *P. kalelai* (Tenora, Haukisalmi & Henttonen, 1985), *P. gracilis* Tenora & Murai, 1980 and *P. janickii* Tenora, Murai & Vaucher, 1985. The measurements of the four last species that differ significantly from those of *P. nordenskioeldi* have been indicated in bold. All metric data are in millimeters, except for the egg length which is in microns

Parasite species	***P. nordenskioeldi* sp. nov.**	*P. macrocephala*	*P. kalelai*	*P. gracilis*	*P. janickii*
Host species	*Dicrostonyx* spp.	*Geomys bursarius*	*Clethrionomys rufocanus*	*Microtus* spp., *Clethrionomys* spp.	*Microtus arvalis*
Distribution	Nearctic	Nearctic	Palearctic	Palearctic	Palearctic
Source	Present study	Douthitt, 1915 Haukisalmi, unpubl.	Tenora *et al.*, 1985	Tenora *et al.*, 1985	Tenora *et al.*, 1985
Genital pore alternation	unilateral/infrequently alternating	infrequently alternating	**frequently alternating**	unilateral/infrequently alternating	unilateral
Body length	70–145	100–200	66–191	60–120	40–100
Maximum width	1.4–2.9	1.5–1.9	1.1–2.3	1.5–3.0	1.6–2.5
Scolex, diameter	0.37–0.61	**0.60–0.95**	**0.53–1.16**	0.37–0.60	0.32–0.45
Suckers, diameter	0.18–0.25	**0.30–0.41**	0.20–0.40	0.18–0.24	0.17–0.22
Testes, total number	29–52	32–57	22–35	40–55	**50–60**
Antiporal to a.v.l.o.c.[a]	0–8	**0**	**0**	few	few
Cirrus sac, length (mat.)	0.19–0.31	**0.11–0.18**	0.14–0.24	**0.18–0.20**	**0.17–0.22**
Maximum (postmat.)	0.23–0.31	**0.21**	—	0.25–0.28	0.25
Vagina, length	0.16–0.27	**0.09–0.13**	**0.10–0.18**	0.20–0.30	**0.09–0.16**
Vagina/cirrus sac ratio	0.68–1.05	0.71–1.00	<1	*c.* 1	*c.* **0.5**
Seminal receptacle, length (mat.)	0.14–0.25	0.11–0.29	0.14–0.23	**0.22–0.50**	0.20–0.36
Maximum (postmat.)	0.25–0.55	0.32–0.45	—	—	—
Egg, length	46–56	**30–40**	**27–43**	**37–48**	**30–40**

[a] a.v.l.o.c., antiporal ventral longitudinal osmoregulatory canal.

a poorly described species that differs from *P. nordenskioeldi* by the distribution (no testes antiporal to antiporal v.l.o.c.) and higher number of testes (60–74).

In addition to *P. nordenskioeldi*, there are two other species of *Paranoplocephala* in collared lemmings with a 'paranoplocephaloid' early uterus, i.e. *P. serrata* and *P. krebsi* sp. nov. (below). They both differ from *P. nordenskioeldi* by the distribution of testes (only in the antiporal part of the segment). However, in North America the testes of *P. serrata* occasionally reach the poral v.l.o.c. (but not in Siberia) (Haukisalmi & Henttonen, 2000), a pattern that also characterizes *P. nordenskioeldi*. Additional differences between *P. serrata* and *P. nordenskioeldi* include slightly shorter and thinner body, shorter cirrus sac (postmature segments), smaller number of antiporal testes and smaller eggs in the latter species. The other taxonomically important features, e.g. uterine development and the structure of cirrus sac and vagina, are similar in the two taxa.

PARANOPLOCEPHALA KREBSI SP. NOV.

(Figs 10, 11; Table 5)

Holotype. USNPC 90537, from *Dicrostonyx groenlandicus*, collected from Byron Bay (68°45′N, 109°04′W), Southern Victoria Island, Nunavut, Canada, on 6 July 1996; an entire gravid specimen.

Paratypes. (i) USNPC 90538, from *Dicrostonyx groenlandicus*, collected from Hope Bay (68°06′N, 106°43′W), on the southern coast of the bay separating Kent Peninsula from the mainland, Nunavut, Canada, on 4 July 1996; an entire gravid specimen. (ii) USNPC 90539, from *Dicrostonyx hudsonius*, collected from Cap de Nouvelle-France (62°20′N, 73°40′W), Ungava Peninsula (Quebec), Canada, collected on 2 July 1999; an entire gravid specimen.

Location in the host. Small intestine (duodenum).

Etymology. The new species is named after Charles J. Krebs in recognition of his long-term ecological research on northern mammals. He also provided (with A. Kenney) the lemmings from southern Victoria Island/Kent Peninsula region, the type locality of *Paranoplocephala krebsi*.

Prevalence and geographical distribution. P. krebsi was found to be a relatively rare (10%) and sporadic parasite of collared lemmings in the Nearctic. However, it has a geographically wide distribution, ranging from Wrangel Island in the west (host *D. groenlandicus*) to Ungava Peninsula in the east (host *D. hudsonius*). It was also found from Greenland and other High Arctic localities (host *D. groenlandicus*) (Table 1; Fig. 1).

Description. The description is based on 12 gravid specimens from the Nearctic: 11 from *D. groenlandicus* and one from *D. hudsonicus* (Ungava Peninsula).

Strobila relatively short and wide; maximum width usually attained in gravid segments. Scolex small, not distinctly set off from neck. Suckers spherical in surface view, directed laterally. Suckers large compared with size of scolex. Neck short and thick, with distinct longitudinal grooves and ridges. Segments craspedote with pronounced velum. All segments markedly wider than long; maximum length/width ratio always in gravid segments. Total number of segments 192–233 ($N=2$); number of mature segments 18–31 (mean 24.1, $N=9$).

Genital pores opening in middle of segment margin or slightly more posteriad. Genital atrium deep, directed laterally. Genital pores usually unilateral, either sinistral (6/12) or dextral (4/12). Two specimens with alternating genital pores; in these position of genital pores changed infrequently (one and three changes per strobila).

Ventral longitudinal osmoregulatory canals (v.l.o.c.) 0.025–0.070 in width, connected by thin transverse canals. Dorsal osmoregulatory canals present, but usually seen poorly. Genital ducts passing dorsally across longitudinal osmoregulatory canals.

Testes 27–47 in number, situated entirely in antiporal half of segment in 1–3 dorsoventral layers. Several testes lie antiporally to antiporal v.l.o.c. Antiporal testes overlap ovary considerably, reaching level of antiporal margin of vitellarium. Diameter of testes 0.05–0.07. Cirrus sac short relative to width of segment (12–25%). Cirrus sac overlaps poral v.l.o.c., but rarely extends across it. Muscle layers of proximal cirrus sac fairly well developed; maximum thickness (0.015–0.025) attained in postmature segments. Cirrus sac covered with loose cell layer. Ductus cirri armed with short spines. Length of internal seminal vesicle variable; *c.* 28% of cirrus sac length in mature segments. External seminal vesicle straight or slightly looped, relatively long, *c.* half of cirrus sac length in mature segments. External surface of this vesicle lined with dense layer of small, intensely stained cells.

Vagina situated postero-ventrally to cirrus sac. Vagina always slightly shorter than cirrus sac; distinctly set off from seminal receptacle. Vagina of uniform width or distal vagina slightly wider than proximal part. No hairs or other lining observed on internal surface of vagina. External surface of vagina covered with dense layer of small, intensely stained cells. Seminal receptacle usually elongate, ovoid or irregularly shaped in some specimens. Seminal receptacle increases markedly in size in postmature segments. Vitellarium asymmetrically bilobed. Ovary large, filling most of space between v.l.o.c.s; width of ovary 31–51%

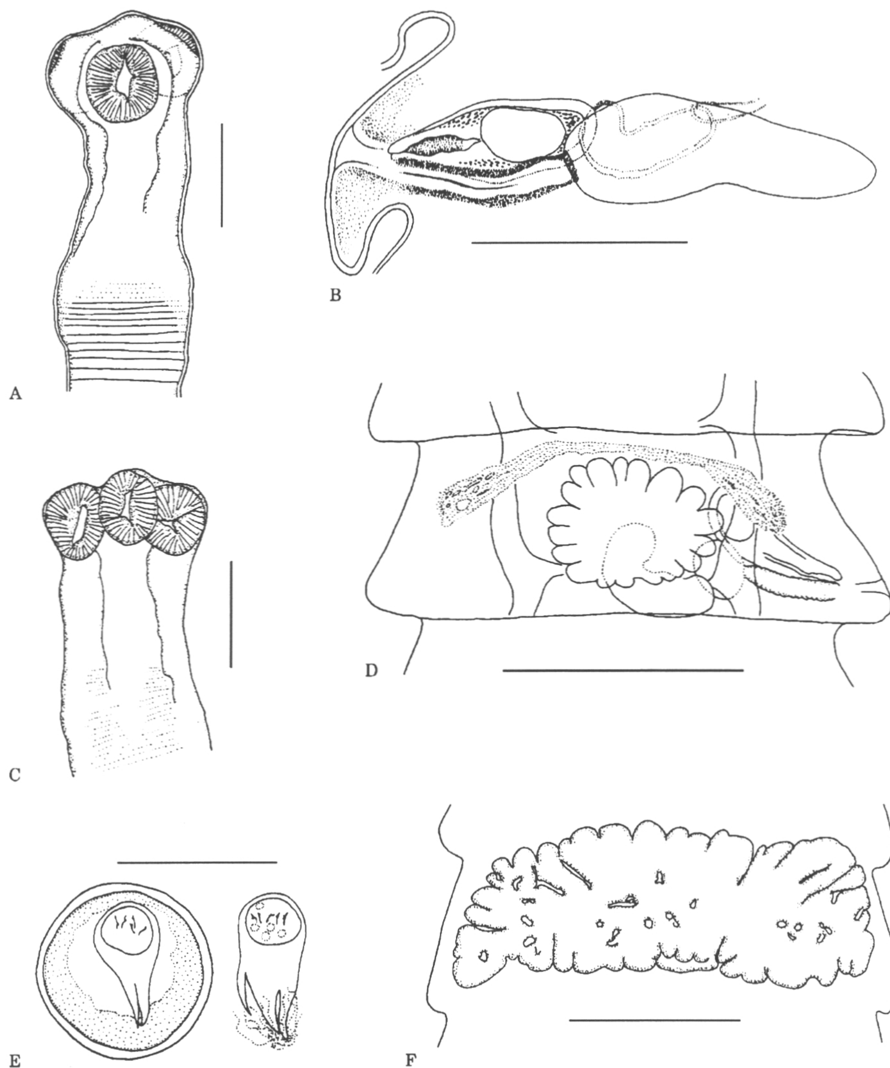

Figure 10. ***Paranoplocephala krebsi*** **sp. nov.** A, scolex. Byron Bay, host *Dicrostonyx groenlandicus* (scale bar = 0.20 mm). B, genital ducts. Hope Bay, host *D. groenlandicus* (scale bar = 0.20 mm). C, scolex. Wrangel Island, host *D. groenlandicus* (scale bar = 0.20 mm). D, early uterus in mature segment. Banks Island, host *D. groenlandicus* (scale bar = 0.30 mm). E, egg and oncosphere with pyriform apparatus. Ungava Peninsula, host *D. hudsonius* (scale bar = 0.040 mm). F, uterus in pregravid segment. Banks Island, host *D. groenlandicus* (scale bar = 0.50 mm).

of segment width. Position of female glands usually slightly poral, occasionally median.

Uterus appears ventrally as thin, transverse cord in anteriormost part of early mature segments, extending across ventral osmoregulatory canals bilaterally. In earliest discernible stage lateral parts of uterus slightly wider than median part with distinct, elongate fenestrations without lumen. Margins of early uterus regular and well-defined. With further development, compartments with lumen appear simultaneously in lateral 'wings' and median part of uterus, and uterus spreads rapidly posteriad pushing testes backwards,

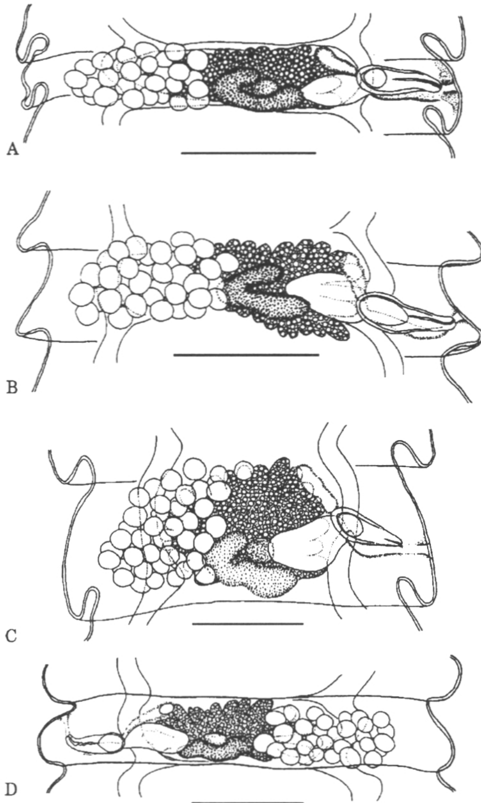

Figure 11. ***Paranoplocephala krebsi*** **sp. nov.**: mature segments. A, Byron Bay, host *Dicrostonyx groenlandicus* (scale bar = 0.30 mm). B, Ungava Peninsula, host *D. hudsonius* (scale bar = 0.30 mm). C, Wrangel Island, host *D. groenlandicus* (scale bar = 0.30 mm). D, Greenland, host *D. groenlandicus* (scale bar = 0.30 mm).

partly overlapping them. Fully developed uterus (pregravid segments) with large anterior and posterior sacculations, smaller lateral diverticula and internal structures (trabecula), but no fenestrations or reticular structures. All sacculations and diverticula disappear in fully gravid segments, but internal trabecula persist. Eggs spherical or slightly ovoid in surface view, slightly flattened in side view. Pyriform apparatus with two slender horns; tip of horns armed with bunch of fine hairs.

Remarks. We compare *P. krebsi* with three related species characterized by unilateral or infrequently alternating genital pores and antiporal distribution of testes: *P. serrata*, *P. blanchardi* (Moniez, 1891) sensu Tenora, Murai & Vaucher, 1985b and *P. bairdi* (Schad, 1954). Each of these species can be distinguished from *P. krebsi* by several morphometric features (Table 7). *Paranoplocephala serrata*, a species of collared lemmings, has a distinctly longer strobila, larger cirrus sac and larger eggs than *P. krebsi. Paranoplocephala blanchardi resembles P. krebsi* in having short and relatively wide strobila, but differs from the new species by the number and distribution of testes, larger maximum size of cirrus sac, absolutely and relatively shorter vagina and smaller eggs. Moreover, *P. blanchardi* is only known from *Microtus* spp. in Europe and western Russia. *Paranoplocephala bairdi*, a parasite of the heather vole, *Phenacomys ungava*, has a longer and more slender body and smaller suckers than *P. krebsi*, and also a slightly different distribution of testes.

The structure of early uterus provides the main qualitative difference between *P. krebsi* and the three related species. The early uterus of the new species is very thin and the lateral wings are less pronounced than in the other species (see Haukisalmi & Henttonen, 2000 for uterus development of *P. serrata* and *P. bairdi*). The structure of early uterus of *P. blanchardi* has not been described in detail, but our unpublished observations show that it is a broad, densely reticulated band that covers almost the entire medulla; in this respect it resembles *P. arctica* and *P. alternata*.

There are three other species of *Paranoplocephala* and *Andrya* with unilateral genital pores and antiporal distribution of testes, i.e. *P. mascomai* Murai, Tenora & Rocamora, 1980, *P. maseri* Tenora, Gubányi & Murai, 1999 and *P. apodemi* Iwaki, Tenora, Abe, Oku & Kamiya, 1994, but they all can easily be separated from *P. krebsi* by the dimensions of the strobila and scolex and other morphometric features, by the development of uterus, and by the host and geographical distribution (Murai, Tenora & Rocamora, 1980; Iwaki *et al.*, 1994; Tenora, Gubányi & Murai, 1999; Tenora *et al.*, 1999).

DISCUSSION

TAXONOMY AND PHYLOGENY

The generic assignment in the *Andrya/Paranoplocephala*-complex is based on a single morphological feature, the structure of the early uterus (Rausch, 1976). The early uterus in *P. omphalodes* (Hermann, 1783), the type species of the genus *Paranoplocephala*, is restricted to the anterior part of segment with reticulated lateral 'wings' (Rausch, 1976). Among cestodes of collared lemmings, this pattern occurs in *P. serrata* (Haukisalmi & Henttonen, 2000), *P. krebsi* and *P. nordenskioeldi*. The early uterus of *P. arctica* and *P. alternata* is a fine, complete reticulum that covers most of the segment, a pattern that seemingly corresponds to that in *A. rhopalocephala* (Riehm, 1881), the type species of the genus *Andrya*

Table 7. Main morphometric features of ***Paranoplocephala krebsi* sp. nov.**, *P. serrata* Haukisalmi & Henttonen, 2000, *P. blanchardi* (Moniez, 1891) and *P. bairdi* (Schad, 1954). The measurements of the last three species that differ significantly from those of *P. krebsi* have been indicated in bold. All metric data are in millimeters, except for the egg length which is in microns

Parasite species	***P. krebsi* sp. nov.**	*P. serrata*	*P. blanchardi*	*P. bairdi*
Host species	*Dicrostonyx* spp.	*Dicrostonyx* spp.	*Microtus* spp.	*Phenacomys ungava*
Distribution	Nearctic	Holarctic	Palearctic	Nearctic
Source	Present study	Haukisalmi & Henttonen, 2000	Tenora *et al.*, 1985	Schad, 1954; Haukisalmi & Henttonen, 2000
Body length	36–85	**95–186**	40–85	**200**
Maximum width	1.4–3.4	2.0–3.4	2.8 (2.5–4.1)[a]	**2**
Scolex, diameter	0.30–0.45	0.28–0.47	0.42–0.52	0.30–0.34
Suckers, diameter	0.13–0.19	0.16–0.20	0.15–0.18	**0.12–0.13**
Testes, total number	27–47	36–60	44–50 **(52–62)**	31–51
Antiporal to a.v.l.o.c.[b]	3–16	5–24	— **(0–3)**	**1–5**
Cirrus sac, length (mat.)	0.17–0.26	**0.20–0.38**	0.18–0.22	0.15–0.25
Maximum (postmat.)	0.20–0.29	**0.28–0.43**	**0.25 (0.33)**	0.25
Vagina, length	0.12–0.20	0.13–0.32	**0.10–0.14**	0.16–0.19
Vagina/cirrus sac ratio	0.62–0.95	0.61–0.95	*c.* **0.5 (<0.5)**	0.77–0.97
Seminal receptacle, length (mat.)	0.10–0.28	0.15–0.32	0.22–0.60	0.10–0.23
Maximum (postmat.)	0.26–0.60	0.21–0.54	— (0.64)	0.35
Egg, length	39–55	**53–73**	**33–40**	40

[a] Measurements for *P. blanchardi* from Fennoscandia (in parentheses) shown only if the measurement differs markedly from that given by Tenora *et al.*, 1985b. Source for Fennoscandian *P. blanchardi*: Haukisalmi & Henttonen, unpubl,; $N=9$).

[b] a.v.l.o.c., antiporal ventral longitudinal osmoregulatory canal.

Railliet, 1893. However, there are fundamental differences in the uterine morphology between the true *Andrya* in leporids (*A. rhopalocephala* and *A. cuniculi*) and the species with a reticulated, *Andrya*-like uterus in arvicoline rodents (Haukisalmi *et al.*, unpublished; see also Genov *et al.*, 1996).

Recent studies, including the present one, have revealed considerable interspecific diversity in the uterine development of *Andrya/Paranoplocephala* (Genov *et al.*, 1996; Haukisalmi & Henttonen, 2000a), suggesting that the phylogenetic relationships and generic taxonomy of this group should be revised using a larger number of morphological characters. Beveridge (1994) included the structure of external seminal vesicle in the diagnosis of the genus *Andrya*, but Genov *et al.* (1996) showed that the presence or absence of 'prostatic cells' on the external seminal receptacle does not clarify the generic concept in these genera. Based on the above facts, we have assigned all species occurring in arvicoline rodents to *Paranoplocephala* irrespective of their uterine development. This classification scheme, that is supported by the unpublished phylogenetic study of Wickström *et al.*, has earlier been applied by Tenora, Murai & Vaucher (1986) and Genov *et al.* (1996).

The present genetical data show that the two species of *Paranoplocephala* with a completely reticulated early uterus (*P. arctica* and *P. alternata*) form a monophyletic group with respect to other species of collared lemmings. This suggests that all species sharing this characteristic (Genov *et al.*, 1996) should be assigned under the same (new) genus. However, the limited coverage of species and poor resolution of the present phylogenetic analysis does not allow to propose new classification schemes for anoplocephalid cestodes of rodents. The possible reasons for the lack of resolution of the phylogenetic tree are varied, but it may reflect rapid divergence of cestode taxa or 'pulses of speciation', a phenomenon that has characterized the evolutionary history of arvicoline hosts of anoplocephaline cestodes (Conroy & Cook, 1999; Martin *et al.*, 2000).

Morphological and genetical evidence shows that there are five species of anoplocephalid cestodes in collared lemmings, all assigned here to the genus *Paranoplocephala*. Cestodes representing other genera of Anoplocephalidae (*Anoplocephaloides* Baer, 1923) or other families (Hymenolepididae, Catenotaeniidae) were not found. The five species of *Paranoplocephala* are host-specific parasites of collared lemmings, since they have not been found from sympatric *Microtus*-voles or true lemmings (*Lemmus* spp.) studied by us in Siberia, Alaska and northern Yukon (Haukisalmi *et al.*, 1995; Haukisalmi & Henttonen, 2001 & unpublished). Other cestodes reported from collared lemmings are *Paranoplocephala omphalodes*, *Anoplocephaloides dentata* (Galli-Valerio, 1905) (Yushkov, 1995), *A. variabilis* (Douthitt, 1915); Schad, 1954) and *Hymenolepis horrida s.l.* (von Linstow, 1901) (Luzhkov, 1964; Gubanov & Fedorov, 1970). However, the taxonomical status of these species may not be properly evaluated, because no descriptions were provided.

Of these species, *H. horrida s.l.* (Hymenolepididae) is a host-generalist in various species of rodents, and *P. omphalodes* and *A. dentata* are typically parasites of *Microtus*-voles. These three taxa must be considered as accidental parasites of collared lemmings. However, the cestodes from *D. hudsonius* from the Ungava Peninsula, identified as *A. variabilis* by Schad (1954), may in fact represent *P. krebsi*, a species that was found by us from *D. hudsonius*. *Anoplocephaloides variabilis* is presently thought to be a host-specific parasite of pocket gophers (*Geomys bursarius*) (Rausch, 1976), and it is unlikely that it would also parasitize collared lemmings. These two species are similar in gross morphology, but they can be distinguished by the structure of the early uterus (tube-like in *A. variabilis*). Thus, *P. krebsi* seems to be the only species of cestode found from collared lemmings of the Ungava Peninsula.

BIOGEOGRAPHY AND EVOLUTIONARY HISTORY

The main phylogenetic split within *Dicrostonyx* is at the Bering strait, separating the western (Siberian) *D. torquatus* and the eastern (North American) clade consisting of *D. groenlandicus*, *D. hudsonius* and *D. richardsoni* (Fedorov, Fredga & Jarrell, 1999). It should be noticed that the collared lemmings on Wrangel Island, which lies north-west of the Bering strait, belong to the Nearctic *D. groenlandicus*. Recent genetical evidence suggests that the time of divergence between the Palearctic and Nearctic taxa of *Dicrostonyx* is *c.* 1 Myr BP. There also exists more shallow genetic splits within *D. torquatus* in Siberia and within *D. groenlandicus* in the Nearctic; these sublineages have probably been caused by Pleistocene glacial barriers *c.* 0.5 Myr BP (Fedorov & Goropashnaya, 1999). The data of Fedorov & Goropashnaya (1999) also show that the Nearctic representatives of *Dicrostonyx* originate from a single invasion from the Palearctic, and the present taxa have all diverged since that colonization event. The Siberian *D. torquatus* probably originates from a single ice age refugium in eastern Siberia (Fedorov *et al.*, 1999).

Three of the cestode species, *P. alternata*, *P. serrata* and *P. nordenskioeldi*, were found to have a Holarctic distribution, whereas the other two, *P. arctica* and *P. krebsi*, were only recorded from the Nearctic. A similar pattern exists in nematodes of collared lemmings: two species (*Heligmosomoides hudsoni* (Cameron, 1937) and *Syphacia arctica* Tiner & Rausch, 1950) have a

wide Holarctic distribution, whereas an undescribed species of *Heligmosomoides* is evidently restricted to the Nearctic, including Wrangel Island (Rausch & Rausch, 1972; Haukisalmi & Henttonen, unpublished). Thus, the number of host-specific helminth species of collared lemmings is higher in the Nearctic than in the Palearctic, which corresponds to the species diversity of the hosts on the two continents. This could mean that the factors that have been responsible for the divergence of the hosts in the Nearctic have also contributed to the divergence of their helminths. The preponderance of Holarctic helminth taxa in Arctic rodents is supported by a recent biogeographical analysis on helminths of true lemmings (*Lemmus*) (Haukisalmi & Henttonen, 2001).

Although we do not have intraspecific phylogenetic data for helminths, it is plausible to assume that the Holarctic helminth species represent an ancient faunal element that was present in the precursor of all the extant species of collared lemmings and have colonized the Nearctic concomitantly with their host. Such an evolutionary history has been earlier suggested by Rausch & Rausch (1972) for a Holarctic nematode of collared lemmings, *Heligmosomoides hudsoni* (see also Rausch, 1994). Accordingly, the Nearctic helminth species probably appeared after the North American continent was colonized by collared lemmings.

If phyletic coevolution has been the predominant pattern in the evolutionary history of collared lemmings and their Holarctic cestodes, we would expect the morphological and/or genetical divergence of parasites to parallel the divergence of their hosts. The recent analysis by Wickström *et al.* (2001) shows, however, that the genetical and morphological variation among Holarctic cestode populations may show complex patterns that do not necessarily match with the corresponding patterns in the hosts. Wickström *et al.* (2001) showed that the main genetic and morphometric split within *P. alternata* (then included within *Andrya arctica*) is not at the Bering strait, as in the hosts, but between the Siberian populations. Wickström *et al.* (2001) suggested that there has been no severe parasite population bottlenecks in the Nearctic, which would explain the genetical and morphometric similarity of the Nearctic and Palearctic populations as whole and the high degree of within-population genetical variation in *P. alternata* in the Nearctic. In contrast, in the Palearctic the observed genetic/morphometric entities of parasites corresponded to the main chromosome races and phylogenetic groupings of the hosts (*D. torquatus*).

The study of Wickström *et al.* (2001) also suggested that the population of *Andrya arctica* on Wrangel Island is genetically so divergent that it may be separate from the other studied populations in the Nearctic and Palearctic. The independent status of *P. arctica* in eastern Beringia has been confirmed in the present study. The existing facts suggest that *P. arctica* has probably diverged from *P. alternata*. First, the morphological and genetical data (ITS1 sequences) show that *P. arctica* and *P. alternata* are sister taxa. Second, the ranges of these species are allopatric, and the range of *P. arctica* is restricted within the range of the western (Beringian) clade of *D. groenlandicus*, which is the most recent phylogenetic entity among Nearctic *Dicrostonyx* (Fedorov & Goropashnaya, 1999). Thus, the divergence of these species has probably occurred fairly recently in eastern Beringia during the period(s) of isolation between the two clades of *D. groenlandicus*. That the ranges of the two cestode species have remained non-overlapping suggests an existence of a parasite contact zone in the north-eastern Alaska between Kuparuk and Lake Schrader, although no clear geographical barriers exist in that part of the coastal plain of North Alaska (cf. Hafner *et al.*, 1998).

The other strictly Nearctic cestode species, *P. krebsi*, has a wide distribution in Arctic North America, suggesting that its evolutionary history differs from that of *P. arctica*. The geographical and host distribution of *P. krebsi* indicates that it appeared very early in the Nearctic *Dicrostonyx*, probably before the precursor of the Nearctic collared lemmings had diverged into the three extant species. Phylogenetic data (Wickström *et al.*, unpublished) suggest that the evolutionary history of *P. krebsi* may include a shift from other arvicoline rodents.

The fact that all three Nearctic helminth species of collared lemmings occur on Wrangel Island and north-western Alaska may indicate that (eastern) Beringia has played a central role in the helminth evolution. Thus, among the several ice-age refugia suggested by Macpherson (1965), only the Bering region seems to have beeen involved in the divergence of helminths of collared lemmings. Distribution patterns suggesting a Beringian origin have also been indicated for the fleas of collared lemmings and other arctic mammals (Holland, 1956), and for a nematode of the lake trout (Black, 1983).

Overall, the evolutionary history of cestodes of collared lemmings shows a mixture of various patterns, including broad coadaption with hosts, i.e. no specific divergence in parasites despite deep phylogenetic splits in the hosts, congruent divergence and speciation with hosts, and a probable case of host-shift. In addition, the previous biogeographical analysis on helminths of true lemmings (*Lemmus*) suggested that the divergence and speciation of a refugial host population may be accompanied with the loss of helminth species (Haukisalmi & Henttonen, 2001). In these respects, the evolutionary history of cestodes and their lemming hosts resembles the complex history of other Arctic

host–parasite assemblages (Hoberg, 1992, 1995; Hoberg *et al.*, 1999; Rausch, 1994).

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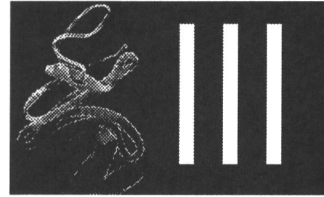
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Genetic and morphometric variation in the Holarctic helminth parasite *Andrya arctica* (Cestoda, Anoplocephalidae) in relation to the divergence of its lemming hosts (*Dicrostonyx* spp.)

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Andrya arctica is a cestode parasite of the family Anoplocephalidae (Cyclophyllidea), parasitizing lemmings of the genus *Dicrostonyx* throughout the Holarctic region. The population structure of this intestinal parasite was studied from eight different regions, six of which represented different genetic entities of lemming hosts. Molecular sequence tagged site markers and minisatellite fingerprints as well as morphology and morphometrics were used to reveal the population structure of *A. arctica* in the Holarctic region. The results suggest that the evolutionary history of this cestode species has included different processes acting on different geographical regions. On the Siberian mainland (host *D. torquatus*), the division of the parasites into different genetic entities agreed perfectly with the chromosomal races of the lemming hosts that points towards a shared evolutionary history between the host and the parasite ('cospeciation'). The main phylogenetic split of *Dicrostonyx* between Eurasia and North America was not, however, observed in *A. arctica*. This suggests that in the Nearctic (host *D. groenlandicus*) the parasite has remained relatively unmodified because of the large cohesive populations ('coadaptation'). The uniqueness of the Greenland population, and possibly also that of the Wrangel Island, can be explained by peripheral isolation, refugial effects or founder effects. © 2001 The Linnean Society of London

ADDITIONAL KEYWORDS: Arctic–host parasite relationships – population history – DGGE – morphology – Pleistocene glaciations.

INTRODUCTION

Fluctuations of glacial and interglacial periods during the Pleistocene have promoted speciation and intraspecific divergence in the northern fauna. Isolation due to glacial barriers has led to genetic differentiation and subsequent postglacial distribution shifts have created contact zones that still persist (Hewitt, 1999).

Collared lemmings, *Dicrostonyx* Gloger, are a Holarctic genus of arvicoline rodents inhabiting the Arctic tundra; they are absent only from Fennoscandia (Jarrell & Fredga, 1993). Collared lemmings are morphologically very similar throughout the Holarctic region. Their taxonomy has not been resolved until quite recently, when chromosome surveys (Gileva, 1983; Jarrell & Fredga, 1993; Fredga *et al.*, 1995a,b) and phylogeographic patterns of mtDNA genealogies (Fedorov, Fredga & Jarrell, 1999a) revealed the divergence of this genus in the Eurasian Arctic. According to Fedorov *et al.* (1999a), the main phylogeographic split in *Dicrostonyx* is between the Palearctic and the Nearctic. Collared lemmings inhabiting the Palearctic mainland are divided into four chromosomal races and five phylogeographic clades; the two groupings are largely congruent. Wrangel Island is considered to be colonized by lemmings of Nearctic origin (Fedorov *et al.*, 1999a). All Siberian populations (Fig. 1) are now assigned to *D. torquatus* (Pallas) and those on the Wrangel Island and western Nearctic to *D. groenlandicus* (Traill) (Jarrell & Fredga, 1993). The collared lemmings of the Nearctic (Fig. 1) are currently considered as three species, *D.*

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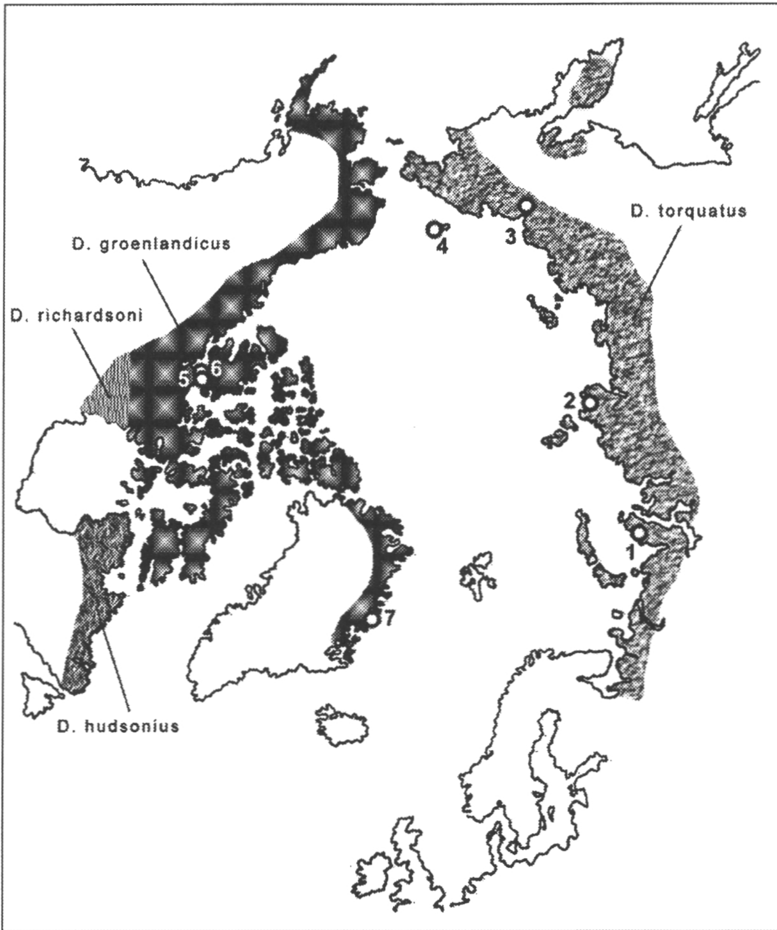

Figure 1. The distribution range of collared lemmings *Dicrostonyx* in the Holarctic. Sampling locations for this study: 1. Northern Yamal Peninsula. 2. Northern Taimyr Peninsula. 3. Kolyma River Delta. 4. Wrangel Island. 5. Byron Bay on Victoria Island. 6. Three sampling locations in the Kent Peninsula region (Walker Bay, Hope Bay and Breakwater). 7. Eastern Greenland.

groenlandicus, *D. richardsoni* Merriam and *D. hudsonius* (Pallas) (Jarrell & Fredga, 1993).

Although the genus *Dicrostonyx* is widely distributed and many aspects of its ecology and evolution have been studied (Stenseth & Ims, 1993; Fedorov *et al.*, 1999a) the existing knowledge of anoplocephalid cestodes and other helminth parasites of collared lemmings is scanty. Rausch (1952) described the species *Andrya arctica* from Alaska and Arctic Canada (host *D. groenlandicus*), and Schad (1954) reported another anoplocephalid species from Quebec and Labrador (host *D. hudsonius*). The only extensive study from the Palearctic is that of Juškov (1995), who identified two anoplocephalid cestodes of uncertain identity from northwestern Russia (host *D. torquatus*) (c.f. Haukisalmi & Henttonen, 2000).

Our ongoing studies have shown that collared lemmings have at least four species of anoplocephalid cestodes, two of which (*Andrya arctica* and *Paranoplocephala serrata* Haukisalmi & Henttonen) have a Holarctic distribution (Haukisalmi & Henttonen, 2000), and the other two (*Paranoplocephala* spp.) are restricted to the Nearctic (Haukisalmi, Wickström, Hantula & Henttonen, unpublished). All these species appear to be specific to collared lemmings, because none has been found in sympatric true lemmings

(*Lemmus* spp.) or voles of the genus *Microtus* Schrank from either the Palearctic or the Nearctic. The most common helminth species of collared lemmings is *Andrya arctica* (see Haukisalmi & Henttonen, 2000).

In concordance with refugial hypotheses based on cyclical glacials and interglacials (Hewitt, 1996; Rausch, 1994) and a model for cospeciation, we would predict the cestodes of collared lemmings to show divergence that coincides with the different genetic entities of the hosts. The aim of this study was to find out whether different genetic and morphological entities occur in *Andrya arctica* in the Holarctic region, and whether the groupings, if observed, follow the chromosomal races and phylogenetic groupings of the hosts as revealed for the Eurasian Arctic by Fedorov *et al.* (1999a) and Fedorov & Goropashanya (1999). We applied sequence tagged sites (STS) and a minisatellite fingerprints to examine genetic variation in *A. arctica*. The genetic patterns were compared with intraspecific differences in morphology and morphometrics of *A. arctica*.

MATERIAL AND METHODS

LEMMINGS

The Palearctic material of collared lemmings (Fig. 1) (*Dicrostonyx torquatus*) from the Arctic coast of Siberia and Wrangel Island ($n = 81$) was collected by V. Fedorov and K. Fredga during the 'Swedish–Russian Tundra Ecology Expedition' during the summer of 1994 (Fredga, Fedorov & Jarrell, 1999). Collared lemmings were obtained from four localities, ranging from Yamal Peninsula in the west to the Wrangel Island in the east (Fig. 1). The main Nearctic material of collared lemmings (*Dicrostonyx groenlandicus*) originates from southern Victoria Island and Kent Peninsula region in Nunavut (formerly Northwest Territories), Central Arctic Canada ($n = 62$) (Fig. 1). Lemmings from Arctic Canada were trapped by C. J. Krebs and A. Kenney during the summer of 1996. Lemming intestines from Siberia and Arctic Canada were frozen after trapping for later examination of helminths.

A small number ($n = 3$) of intestines of collared lemmings (*D. groenlandicus*) was also obtained from Greenland; these lemmings were collected by V. Fedorov, H. P. Gelter and G. H. Jarrell on 23 July 1995, preserved entire in 70% alcohol and deposited at the University of Alaska Museum Mammal Collection (UAM NO 33909). Later, the intestines were removed from the bodies and examined for helminths.

CESTODES

Andrya arctica was found from all localities studied, its prevalence varying from 17% (Taimyr Peninsula, $n = 30$) to 61% (Yamal, $n = 18$). Of 39 adult worms, DNA was successfully extracted from 30 specimens (Table 1) originating from (Fig. 1) western Yamal Peninsula (73 °N, 70 °E), Taimyr Peninsula (77 °N, 105 °E), western Kolyma River Delta (69 °N, 162 °E), Wrangel Island (72 °N, 180 °E), Greenland (Ostgronland, Hurry Fjord, Hareelv, 70°42′N, 22°40′W) and four localities in Nunavut, Canada (Byron Bay 68°45′N, 109°04′W, Hope Bay 68°06′N, 106°43′W, Breakwater Island 67°55′N, 108°30′W and Walker Bay 67°N, 106°W). Two of the specimens from Yamal originated from the same lemming host, as did two from Kolyma, Greenland, Walker Bay, Hope Bay and Byron Bay. The pairs from Yamal, Kolyma, Greenland and Walker Bay had identical genetic markers, while the pairs from Hope Bay and Byron Bay possessed different markers.

After dissection, the cestodes recovered from frozen intestines were relaxed in tap water for 1–2 hours and fixed in 70% alcohol (most specimens) or 10% formalin. The specimens used in morphological analysis were stained with Mayer's haemalum or Semichon's acetic carmine, cleared in eugenol and mounted in Canada balsam. The tissue samples for genetic analysis were obtained from specimens preserved in 70% alcohol or from specimens frozen in extraction buffer (50 mM Tris/HCl, pH 7.2; 50 mM EDTA; 3% SDS; 1% beta-mercaptoethanol) prior to DNA extraction.

The material for morphometric analysis included 28 specimens of *A. arctica* from three locations: Yamal Peninsula ($n = 8$), western Kolyma Delta ($n = 8$) and Nunavut, Canada ($n = 12$). In morphological analyses, we used specimens with gravid or pregravid segments only (pregravid segments are those immediately anterior to fully gravid segments). The specimens from other locations (Taimyr, eastern Kolyma, Wrangel Island and Greenland) were too few for proper morphometric analysis. However, we describe the main morphological features of specimens from Wrangel Island ($n = 5$), because they deviated partly from the other sampling locations studied. The following representative specimens of *A. arctica* have been deposited in the US National Parasite Collection (Beltsville, Maryland): USNPC 88811 (Yamal), USNPC 88812 (Western Kolyma), USNPC 88813 (Wrangel Island) and USNPC 88814 (Victoria Island).

To determine the geographical and host distribution of *Andrya arctica*, we examined preliminarily the holotype (USNPC 37356) from Point Barrow, North Alaska (host *D. groenlandicus*) and several specimens from the personal collection of Robert L. Rausch (hosts *D. groenlandicus* and *D. richardsoni*). The detailed taxonomy of these specimens will be presented in a later publication (Haukisalmi, Wickström, Hantula & Henttonen, submitted).

MORPHOMETRIC ANALYSES

From each cestode specimen, we recorded the body length and maximum body width (pregravid segments)

Table 1. Distribution of alleles (markers STS-A and STS-B) and M13 markers (900 to 185) among *Andrya arctica* specimens. Homozygotes for STS-A or STS-B are depicted with only one number and alleles with the same migration rate were scored as equal. Groups with abbreviations starting with P and N are from the Palearctic and Nearctic, respectively. The grouping of the Palearctic individuals (P1–P3) is made according to the chromosomal races of the host

		STS		M13 markers (900–185)																			
Sampling location and country	Group	A	B	900	860	850	650	600	550	530	470	450	400	380	340	300	285	280	270	260	250	190	185
Yamal Peninsula, western central Siberia, Russia	P1	2	1/2	1*	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	2/5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	2/5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Taimyr Peninsula, central Siberia, Russia	P1	2/5	1	0	1	0	1	1	1	0	0	0	0	0	1	0	1	0	0	1	1	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	5	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	5	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	6	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Ostgronland, Hurry Fjord, Hareelv, Greenland	N1	3/5	3	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0
Ostgronland, Hurry Fjord, Hareelv, Greenland	N1	3/5	3	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	0	1
Walker Bay, Kent Peninsula, Nunavut, Canada	N2	4	3	0	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	1	0	1	0
Walker Bay, Kent Peninsula, Nunavut, Canada	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	1/2	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	1/2	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Breakwater Island, Nunavut, Canada	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	5	3	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	1/2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0

* 1 and 0 indicate the presence and absence of the marker, respectively.

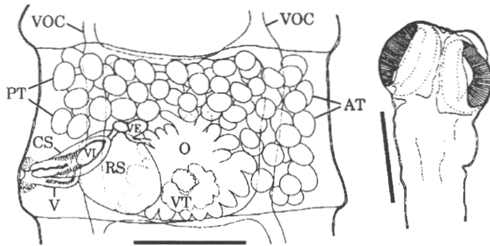

Figure 2. Mature segment and scolex of *Andrya arctica* from Victoria Island, Nunavut (host *Dicrostonyx groenlandicus*). Abbreviations: VOC, ventral osmoregulatory canal; PT, poral testes; AT, antiporal testes; CS, cirrus sac; VI, vesicula seminalis interna; VE, vesicula seminalis externa; V, vagina; RS, seminal receptacle; O, ovary; VT, vitellarium. Scale bars: 0.30 mm (segment) and 0.20 mm (scolex).

and the diameter of the scolex and suckers (if present). The width of segment, number of testes and the length and width of various reproductive organs were measured from three mature segments (Fig. 2), and the maximum length of cirrus sac and seminal receptacle were recorded from postmature segments (postmature segments are those immediately posterior to fully mature segments). The first mature segment was defined as the one in which the internal seminal vesicle was first seen clearly differentiated; the last mature segment was the one in which the vitellarium was last seen compact (no visible disintegration).

Egg size is based on the measurement of the maximum diameter of five eggs from fully gravid segments. When multiple measurements were made from a single strobila, we used the median of these values in statistical analyses. We also determined the pattern of the alternation of genital openings by counting the number of sets with unilateral segments, i.e. those having the genital pore on the same side of the segment.

We used both univariate and multivariate statistical methods to study the morphometric differences among the specimens of *A. arctica* from three sampling locations. As a first step, we performed linear regression analysis between the width of mature segments and each of the other variables. If necessary, logarithmic transformation was performed on the variables to obtain a better fit to the normal distribution. Several tests indicated a significant positive association between the two variables (Table 2). Therefore, we calculated standardized residuals from the significant linear regressions between the body width and other variables, which were then used in subsequent statistical analyses as size-adjusted morphometric measures.

The following step was to calculate rank correlation (Spearman) between various variables using either the absolute values or those adjusted for the effect of body width (residuals). Most of the variables showed significant positive correlation at least with one of the other variables, usually with several of them. We therefore performed a principal component analysis (PCA), based on a correlation matrix, for 15 variables, including the length and width of various reproductive organs and eggs (Table 3). The PCA creates new, uncorrelated variables from a set of intercorrelated variables; the new variables (principal components) are interpreted and used in subsequent statistical comparisons. The other variables were not included in the PCA either because of too many missing values (scolex, suckers and body length) or because of a lack of significant correlation with other variables (maximum length of cirrus sac and seminal receptacle). The meristic (countable) variables (genital pore alternation and number of testes) were also excluded from the PCA. The few missing values were replaced with the mean from the particular location. Variables excluded from the PCA were subjected to univariate comparisons (Kruskall-Wallis non-parametric ANOVA) using either the original values or residuals (Table 2).

DNA EXTRACTION

DNA was extracted from 0.5–2 mm^3 tissue samples as described previously (Vainio, Korhonen & Hantula, 1998). The protocol included cell disruption (using quartz sand), four phenol-chloroform (1:1) extractions, one chloroform:isoamyl alcohol (24:1) extraction, precipitation with polyethylene glycol (PEG) and drying. The DNA was resuspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

PCR AMPLIFICATION USING RANDOM AMPLIFIED MICROSATELLITE PRIMERS (RAMS)

The PCR reactions were carried out in reaction conditions recommended by the manufacturer of the Dynazyme II DNA-polymerase (Finnzymes Ltd., Finland), except that the concentration of the RAMS (Hantula, Dusabenyagasani & Hamelin, 1996) primers was 2 µM. In PCR amplifications the samples were denatured by 10 min incubation at 95°C after which 37 cycles of amplification were carried out (30 s denaturation at 95°C, 45 s annealing at a temperature depending on the primer, 2 min primer extension at 72°C). The annealing temperature was 58°C for both GT ($YHY(GT)_7G$, where H = A/C/T and Y = A/C/G) and TCC primer ($YDD(TCC)_5$, where Y = A/C/G and D = A/G/T). The reaction was terminated with a 7 min extension at 72°C. Reaction products were resolved by electrophoresis on agarose gels containing 1.0% agarose (FMC BioProducts) and 1.0% SynerGel (Diversified Biotech). The lengths of the amplification products were estimated by comparing them to a 100 bp

Table 2. Variation of metric and meristic characteristics of *Andrya arctica* in three sampling locations. The last column shows the statistical difference in the mean values between locations. Results for pairwise tests in parentheses; statistically similar groups combined by '+'

	Yamal Peninsula (Y)				Western Kolyma (K)				Nunavut (N)				
Variable	*N*	Mean	SD	Range	*N*	Mean	SD	Range	*N*	Mean	SD	Range	Kruskall–Wallis test
Body length	3	106.0	37.3	74–123	7	112.9	16.0	90–136	9	94.0	16.0	74–123	NS
Scolex, diameter[1]	6	0.275	0.010	0.26–0.29	8	0.339	0.024	0.30–0.38	10	0.280	0.028	0.24–0.32	P=0.003 (Y+N,K)
Suckers, diameter[1]	5	0.154	0.006	0.150–0.165	8	0.166	0.008	0.155–0.180	10	0.159	0.012	0.140–0.180	NS
Testes number													
Total[1]	7	73.3	8.38	65–89	8	82.4	4.43	75–88	12	70.1	7.40	59–83	P=0.005 (Y+N,Y+K)
Antiporal	7	19.6	3.26	15–23	7	14.1	1.68	12–17	11	15.9	5.17	8–23	P=0.030 (Y+N,K+N)
Poral	7	6.0	1.82	3–8	7	6.6	1.27	4–8	11	3.7	2.53	0–8	P=0.036 (Y+K+N)
Mature segment, width	7	0.89	0.07	0.82–1.01	8	1.14	0.19	0.92–1.43	12	1.03	0.21	0.82–1.49	P=0.015 (Y+N,K+N)
Cirrus sac, max. length	7	0.483	0.035	0.43–0.53	8	0.034	0.034	0.40–0.50	12	0.449	0.034	0.40–0.50	NS
Seminal receptacle, max. length	7	0.519	0.080	0.40–0.61	8	0.657	0.120	0.40–0.80	12	0.610	0.127	0.45–0.90	NS
Unilateral segments[2]	11	2.94	0.71	2.3–4.8	7	2.42	0.30	1.82–2.74	10	2.62	0.17	2.42–2.90	NS

[1] Statistical test based on residuals from the regression between the width of mature segment and the particular measurement.
[2] Number of segments in a set of unilateral consecutive segments.

Table 3. Variables used in the principal component analysis and their loadings with the two main principal components (PC). Highest loadings in bold

Variables	Principal components PC1	PC2
Ovary, width[1]	0.095	**−0.385**
Ovary, length	**0.288**	−0.086
Vitellarium, width[1]	0.231	−0.280
Vitellarium, length	**0.260**	0.056
Cirrus sac, width	**0.303**	−0.094
Cirrus sac, length[1]	**0.302**	0.126
Internal seminal vesicle, width	**0.266**	−0.300
Internal seminal vesicle, length[1]	**0.274**	−0.099
External seminal vesicle, width	**0.305**	−0.008
External seminal vesicle, length[1]	**0.312**	0.089
Vagina, length	0.213	0.237
Seminal receptacle, width	**0.294**	0.025
Seminal receptacle[1], length	**0.304**	−0.105
Egg, width	−0.150	**−0.531**
Egg, length[1]	−0.146	**−0.531**

[1] Because of the significant correlation between the width of mature segments and the particular variable, size-adjusted values (residuals) were used in the analysis. The original values were used for the other variables.

DNA ladder (Gibco BRL). Electrophoreses were run in TAE-buffer (40 mM Tris-Acetate pH 8.0, 1 mM EDTA) and reaction products were detected by ethidium bromide staining.

CLONING OF RAMS FRAGMENTS FOR SEQUENCE TAGGED SITES (STS)

Two RAMS amplification products obtained with two different primers (*A. arctica* 580 bp TCC fragment and *A. arctica* 990 bp GT fragment) were chosen as suitable for development of specific STS primers. Amplification products obtained using GT and TCC primers were ligated into pCR/2.1 vector using the TOPO/TA Cloning Kit (Invitrogen). The resulting recombinant plasmids were used to transform *Escherichia coli* DH5α as recommended by the manufacturer. Recombinant colonies were identified by colour selection after overnight growth at 37°C on Luria-Bertani agar plates (Sambrook, Fritsch & Maniatis, 1989) containing 150 μg/ml of ampicillin and 27 μg/ml of both X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside, Promega) and IPTG (isopropylthio-β-D-galactoside). For screening of the inserts, the plasmids were isolated according to Birnboim & Doly (1979). The sought-after fragments were selected by PCR amplifications using TCC or GT primers according to the protocol described above. Prior to sequencing the plasmids were isolated using QIAGEN Spin Plasmid Kit (QIAGEN GmbH, Germany). Selected cloned inserts were sequenced by A.L.F. DNA sequencer (Pharmacia Biotech, Uppsala, Sweden) using M13 reverse and forward primers and Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, England). The primers designed based on these sequences (not shown) were as follows: for *A. arctica* 580 bp TCC-fragment forward primer 5′ TTC CTC TCA GCT TGG CTA CC 3′ and reverse primer 5′ TTC GCA GTT AAG TCA GCA TAG C 3′ (Sequence Tagged Site A = STS-A) and for *A. arctica* 990 bp GT-fragment, forward primer 5′ TTA CCT TCT CGG TTG GTC TCA 3′ and reverse primer 5′ AAT GGC CTA ACT TCA CCG C 3′ (Sequence Tagged Site B = STS-B). For efficient separation of the amplification products of the STSs in DGGE, a 41 bp long GC-clamp was added to STS-A-F and STS-B-R. Thus the primers used were: 5′ CGC CCG CGG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCT TCC TCT CAG CTT GGC TAC C 3′ (GC-clamped STS-A-F) , 5′ TTC GCA GTT AAG TCA GCA TAG C 3′ (STS-A-R), 5′ 5′ TTA CCT TCT CGG TTG GTC TCA 3′ (STS-B-F) and 5′ CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCA ATG GCC TAA CTT CAC CGC 3′ (GC-clamped STS-B-R).

STS ANALYSIS

Optimization of PCR conditions for the specific markers resulted in the following protocol: denaturation by 10 min incubation at 95°C after which 37 cycles of amplification were carried out (30 s denaturation at 95°C, 1 min annealing at 49°C for specific marker STS-A and 48°C for specific marker STS-B, 1 min primer extension at 72°C). The reaction was terminated with a 7 min extension at 72°C. The concentration of the primers was 1 μM. Amplification products were checked by electrophoresis on agarose gels as described above. The denaturing gradient gel electrophoresis was run according to the instructions of the manufacturer of the D-GENE denaturing gradient gel system (BioRad) using 7.5% (w/v) acrylamide/bisacrylamide (37.5:1) gels with a denaturant gradient of 25–40% for STS-A and 35–50% for STS-B. The 100% denaturing solution contained 40% deionized formamide and 7 M urea. Also these electrophoreses were run in TAE-buffer and reaction products were detected by ethidium bromide staining.

HIERARCHICAL ANALYSIS OF GENETIC DIVERSITY

The analysis of molecular variance was carried out using AMOVA software (Excoffier, Smouse & Quattro, 1992). Haplotypes for both STS-A and STS-B polymorphic loci were used, and every specimen listed in Table 1 was included in the analyses. The material

was divided into two main regions; the Palearctic and the Nearctic. Within the Palearctic the material was divided into three groups according to different chromosome races of the hosts (Fedorov *et al.*, 1999a); Group P1: Yamal and Taimyr, Group P2: Kolyma and Group P3: Wrangel Island. The Nearctic region was divided as follows; Group N1: Greenland, Group N2: Walker Bay, Hope Bay and Breakwater Island Nunavut, Canada, and Group N3: Victoria Island Nunavut, Canada (see Table 1 for groups). The wide strait separating Victoria Island from the mainland and the smaller islands justified the division of the Nunavut area into two groups. As this strait is approx. 30 km at its narrowest part, we addressed the possibility of a lack of recent gene flow between the two Nunavut populations (corresponding to our groups N2 and N3) in Canada.

In addition to the AMOVA analysis, also the coefficient of gene differentiation (G_{st}) was calculated according to Nei (1973). The G_{st} listed in the results table was calculated as an average of both markers studied.

MINISATELLITE SURVEY

DNA was amplified using the M13 core sequence as primer (Stenlid, Karlsson & Högberg, 1994). The reaction conditions for the PCR were the same as for the RAMS-primers described above. The cycling parameters were: 37 cycles of denaturing, 95°C 30 s, annealing, 48°C 1 min, extension, 72°C 2 min, final extension, 72°C 10 min. The amplified products were treated as the RAMS products and gel interpretations were made from photographic prints. Only clear and distinct bands were considered.

The analysis of genetic diversity of M13-RFLP fragments was carried out using AMOVA software and also Nei's coefficient of gene differentiation was calculated as an average of all markers (putative loci) studied. In these analyses the grouping was done as in the analyses of STS data described above.

RESULTS

MORPHOLOGY

According to Rausch (1952), the combination of morphological features that distinguish *Andrya arctica* from related species are irregularly alternating genital pores, very large cirrus sac and seminal receptacle, distribution of testes (antiporally and anteriorly to ovary), large eggs and completely reticulate early uterus. The last characteristic also serves as the main feature for the generic assignment of *A. arctica*.

With the exception of the material from Wrangel Island, all the specimens studied by us agree completely with the description of Rausch (1952), and have therefore been identified as *A. arctica* (Figs 2 and 3). Haukisalmi & Henttonen (2000) provided detailed morphological differences between *A. arctica* and *Paranoplocephala serrata*, the other anoplocephalid cestode described from collared lemmings.

The specimens from Wrangel Island were unique in two respects. Genital openings were strictly unilateral and no testes were situated on the poral side of the poral ventral osmoregulatory canal in any specimen (in other locations the mean number of poral testes ranged between 3.7–6.6) (Fig. 3, Table 2). However, the uterine development shows that the specimens from Wrangel Island also belong to *Andrya*, and they are therefore closely related with the more widespread taxon. The two specimens from Greenland did not differ morphologically from the rest of the specimens studied.

We also examined several more detailed morphological features, though none differed between the locations studied by us, including the Wrangel Island specimens. These were the structure of the cirrus sac (strongly developed musculature) and vagina (tube-like organ of uniform width), covering of the external seminal vesicle (dense layer of small 'prostatic' cells), distribution of testes (not overlapping ovary) and the position of vitellarium and ovary (median). The role of these characteristics in the taxonomy of *Andrya* and *Paranoplocephala* has been discussed by Genov, Vasileva & Georgiev (1996).

MORPHOMETRICS

Five of the ten univariate morphometric comparisons showed a statistically significant difference among the three locations in Table 2. According to the pairwise comparisons, the specimens from Yamal and Nunavut were indistinguishable in all five cases, the specimens from Kolyma and Nunavut in three cases, and those from Yamal and Kolyma in one case only. Univariate morphometric tests thus link better the Palearctic and Nearctic populations than the two Palearctic ones.

We considered two vectors (principal components) of the principal component analysis that had the highest eigenvalues (both >1). The first principal component (PC1) was responsible for 51.6% of the total variance and showed high loadings with most of the variables included in the analysis, excluding the egg dimensions (Table 3). The PC1 can thus be interpreted as a measure of general organ size in *A. arctica*. The PC2 explained 15.3% of the variance, and showed highest loadings with the egg dimensions and width of ovary. Both PC1 and PC2 were significantly different among the three locations (Table 3). Pairwise tests showed that PC1 was similar in Yamal and Nunavut, and both of them showed significantly lower values than the specimens from Kolyma (Fig. 4). PC2 showed two homogeneous groupings: Yamal + Kolyma and Kolyma + Nunavut.

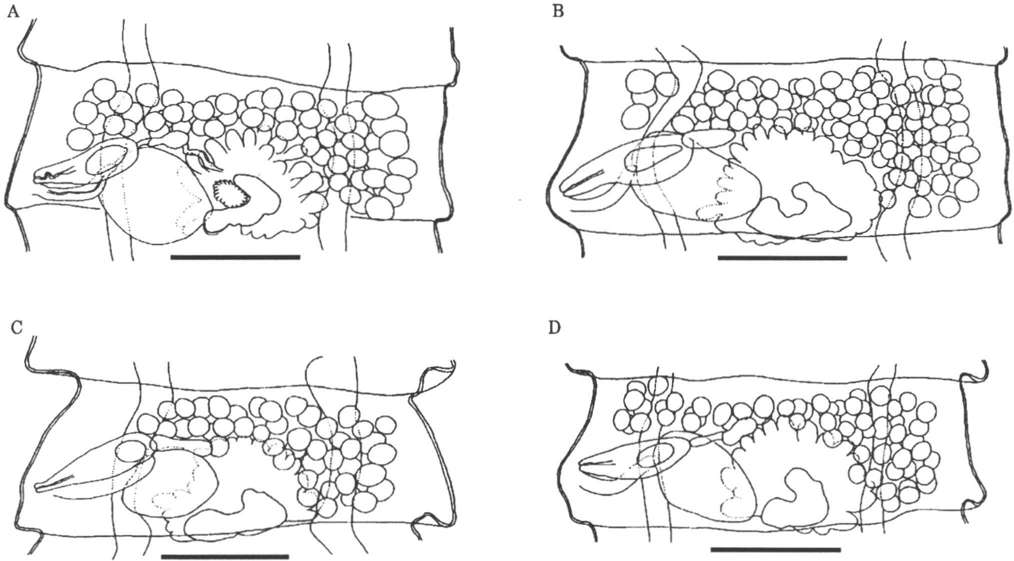

Figure 3. Representative mature segments of *Andrya arctica* from different locations. A, Yamal Peninsula (host *Dicrostonyx torquatus*); B, Western Kolyma Delta (host *Dicrostonyx torquatus*); C, Wrangel Island (host *Dicrostonyx groenlandicus*); D, Kent Peninsula region, Nunavut (host *Dicrostonyx groenlandicus*). Scale-bars: 0.30 mm.

RAMS-PATTERNS

The patterns of amplification products using GT primer showed substantial variation within *A. arctica* (not shown). With TCC primer, only two clear and reproducible bands were observed (not shown). The 580 bp TCC fragment and the 900 bp GT fragment were selected for development of specific STS markers due to the possible intraspecies length polymorphism observed in these fragments. The amplification product sizes expected with the designed primers were about 320 bp and 410 bp, respectively.

STS ANALYSIS OF ALLELIC VARIATION

For *A. arctica*, six alleles at the locus STS-A and five alleles at the locus STS-B were detected within the Holarctic region (Table 1). For the locus STS-A, the most common allele was STS-A2, which occurred in 18 of the specimens scored, ten of them being heterozygous. The allele STS-A5 was found in 13 specimens, five of which were heterozygous. Alleles four and six at locus STS-A occurred only once in the material. The most common allele for locus STS-B was STS-B3. Twenty specimens carrying this allele were scored, and seven of these were heterozygotes. All specimens in the survey carrying alleles STS-B2 or STS-B4 in locus STS-B were heterozygotes with B1 and B3, respectively. Twelve of the 30 specimens analysed in total were heterozygous for STS-A, 13 of 30 for STS-B and seven specimens of 30 were heterozygous for both STS-A and STS-B.

GEOGRAPHICAL DISTRIBUTION OF ALLELES

In the Palearctic, the differences in allelic composition between locations were more pronounced for STS-B, as all groups (Table 1) had varying allelic makeup in this locus (except for the one specimen from Taimyr that could not be distinguished from the Yamal specimens). The Yamal and Taimyr specimens could be identified by the presence of the alleles STS-B1, B2 and A2. These did not occur in any other Palearctic group. From the Kolyma River delta, only one allele for each locus was observed, namely STS-B3 and STS-A5. STS-B3 separated this group from the other Siberian groups. The specimens from Wrangel Island displayed two locality specific alleles, STS-B5 and STS-A6. These did not occur in any other studied specimen throughout the whole Holarctic region (Table 1).

As might be suspected from the distances between the survey sites, the Canadian groups were more alike than the Siberian groups. Considering locus STS-A, only Walker Bay (allele A4) and Victoria Island (allele A5) could be separated from other Canadian groups (alleles A1 and A2 occurred in all Canadian groups).

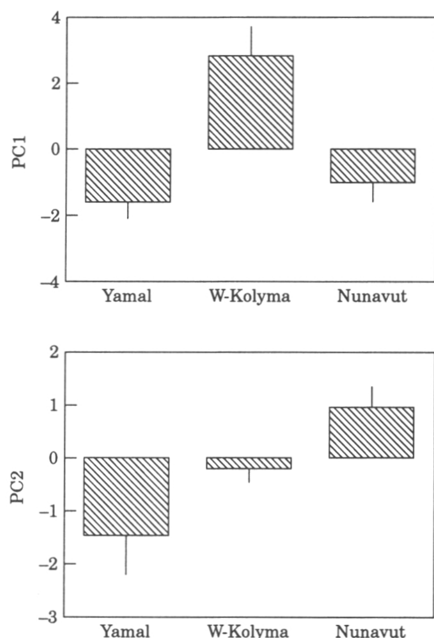

Figure 4. Mean values and standard errors of the mean for the two main principal components (PC) based on the dimensions of the reproductive organs and eggs of *Andrya arctica*. See text for statistical significances of differences between the three locations.

The majority of the Canadian specimens were homozygous for allele STS-B3 or STS-B3/B4 heterozygotes. In addition, the specimens from Greenland were STS-B3 homozygotes. Allele STS-A3, however, distinguished Greenland from all other localities. Comparing the Palearctic and the Nearctic, no major differences were seen in the allelic arrays, besides the absence of allele STS-B4 from the Palearctic and Greenland.

Hierarchical analysis of genetic diversity (Table 4; groups explained in materials and methods and depicted in Table 1) indicated that 12% of the genetic diversity was due to variation between the Palearctic and the Nearctic (Analysis 1, Table 4). However, this figure was not statistically significant ($P=0.16$). In the same test, the variance among all Holarctic groups was 46% ($P<0.0001$), and within groups 42% ($P<0.0001$). When analysing the Palearctic and Nearctic groups separately, the variance among groups in the Palearctic (Analysis 3, Table 4) was 73% ($P<0.001$) and within groups 27% ($P<0.0001$). The corresponding figures for the Nearctic (Analysis 4, Table 4) were 33% ($P<0.0001$) between groups N1, N2 and N3, and 67% ($P<0.0001$) within groups. When excluding Greenland (N1) from the Nearctic (Analysis 5, Table 4), the variance among Canadian groups (N2 and N3) became even smaller (18%, $P=0.05$) which was expected as the geographical range of the sampling locations was restricted to approx. 125 km. Analysis 2 in Table 4 was done as a comparison to the level of genetic differentiation among all groups studied (Table 5). In this analysis the variance among all groups (when not divided into Palearctic and Nearctic regions) was 56% ($P<0.0001$) and within groups 44% ($P<0.0001$).

The level of genetic differentiation (Table 5) calculated according to Nei, resulted in high levels of differentiation among the Palearctic groups ($G_{st}=0.53$). When all groups studied were included the differentiation was equally high ($G_{st}=0.53$). The genetic differentiation among the Nearctic groups showed a lower level of differentiation ($G_{st}=0.26$) and when excluding Greenland (N1) and considering only the Canadian groups (N2 and N3), an even lower level of genetic differentiation was reached ($G_{st}=0.09$). For STS-A and STS-B the same general patterns were obtained both from AMOVA and G_{st} analyses.

VARIATION REVEALED BY AMPLIFICATION OF MINISATELLITE DNA

As the sample sizes of groups studied here are very small, we carried out a complementary analysis for the results obtained with STS markers. This test was done using M13 fingerprints, which are based on minisatellites. DNA was successfully amplified from all specimens in Table 1 using the M13 core sequence as primer. Eight to ten amplified fragments were scored from all other locations except from Greenland where only five and six clear bands were amplified. A distinct pattern of bands was recorded from Yamal, Taimyr, Kolyma and Wrangel, whereas the Canadian specimens showed variation even within the locations. The pattern for the M13 minisatellite primer basically shows the same result as the STS analysis suggesting that despite the small sample sizes the overall picture emerging is reliable. Differences are mainly recorded between groups in the Palearctic, whereas in the Nearctic the diversity is mainly recorded within groups. Of 18 scored fragments, 17 were shared by Palearctic and Nearctic groups and only one fragment (~530 bp), that could be found from the Nearctic specimens, was totally missing from the Palearctic (Fig. 5, Table 1).

Analysis of genetic diversity (Table 4) with AMOVA (Excoffier *et al.*, 1992) indicated that approximately 69% ($P<0.0001$) of the total genetic diversity (Analysis 1) was due to variation among all Holarctic groups (groups explained in Table 1), while the variance within groups was *c.* 18% ($P<0.0001$). The variance among the Palearctic and Nearctic regions was *c.* 13%, but

Table 4. Analysis of molecular variance (AMOVA) based on STS and M13 markers. Five analyses were carried out. Analysis 1 was carried out in three hierarchical levels (between regions N vs. P, within regions both among parasite groups and within parasite groups). The other analyses were carried out in only two hierarchical levels. Among vs. within all groups N+P (analysis 2), among vs. within Palearctic (P) groups (analysis 3), among vs. within Nearctic (N) groups (analysis 4) and among vs. within Canadian groups (analysis 5). For further details on grouping, see Table 1

	STS (microsatellite markers)				M13 (minisatellite markers)			
	Variance component	φ-statistics	Prop. of variance component	*P*-value*	Variance component	φ-statistics	Prop. of variance component	*P*-value
1. Among regions (N vs. P)	0.36	0.12	12%	0.1538	0.35	0.13	13%	0.2283
Among groups within regions (N+P)	1.35	0.52	46%	<0.0001	1.80	0.79	69%	<0.0001
Within groups (N+P)	1.24	0.58	42%	<0.0001	0.48	0.82	18%	<0.0001
2. Among groups (N+P)	1.57	0.56	56%	<0.0001	2.02	0.81	81%	<0.0001
Within groups (N+P)	1.24		44%		0.48		19%	
3. Among groups (P)	2.20	0.73	73%	<0.0001	2.72	1.00	100%	<0.0001
Within groups (P)	0.83		27%		0.00		0%	
4. Among groups (N)	0.74	0.33	33%	<0.0001	1.44	0.74	74%	<0.0001
Within groups (N)	1.49		67%		0.50		26%	
5. Among groups (Canadian)	0.34	0.17	18%	0.0467	0.07	0.13	13%	0.0887
Within groups (Canadian)	1.60		82%		0.44		87%	

* *P* = probability of obtaining equal or larger value determined by 9999 randomization of the treatments.

Table 5. Genetic differentiation (G_{st}) among groups counted as mean values for all markers studied in *Andrya arctica*. The grouping is equal to the grouping used in Table 4 (excluding the analysis in three hierarchical levels)

	Genetic differentiation G_{st}	
	STS	M13
Among all groups (6*)	0.53	0.74
Among all Palearctic groups (3)	0.53	0.38
Among all Nearctic groups (3)	0.26	0.47
Among Canadian groups only (2)	0.09	0.02

* Number of groups.

the differences were not statistically significant ($P=0.23$). As no variance was recorded within groups in the Palearctic (Analysis 3), the variance among groups was 100% ($P<0.0001$) in this region. The variance among all the Nearctic groups (Analysis 4) was 74% ($P<0.0001$), and when excluding Greenland (Analysis 5) the variance fell to 13%, and was not significant any more ($P=0.09$).

Considering the levels of genetic differentiation (Table 5) as a mean value of all markers included (putative loci), the highest level of genetic differentiation was reached among all groups studied ($G_{st}=0.74$). Because of the many shared fragments (Table 1) in the Palearctic groups, the G_{st} remained at 0.38 compared to a G_{st} of 0.53 for the STS-analysis. The specimens from Greenland with lots of markers differing from the Canadian specimens resulted in a marked raising of the genetic differentiation ($G_{st}=0.47$) compared with the G_{st} for only the Canadian specimens ($G_{st}=0.02$).

DISCUSSION

PHYLOGEOGRAPHY OF COLLARED LEMMINGS

It has been suggested that populations living in areas glaciated during the Pleistocene tend to be genetically less variable than populations in nonglaciated areas (Sage & Wolff, 1996; Hewitt, 1996). For the High Arctic genus *Dicrostonyx*, the matter seems to be somewhat different as the populations of collared lemmings were depressed during the warm periods of the interglacials. Palaeontological data as well as the present distribution of *Dicrostonyx* show that this northernmost genus of rodents evolved in tundra landscapes and was always restricted to cold and dry environment (Fedorov *et al.*, 1999a). Forest expansion during warm climatic events disrupted the range of collared lemmings (Sher, 1991) and the genetic variation observed in the phylogeographical groups might have resulted from divergence *in situ* following bottleneck events

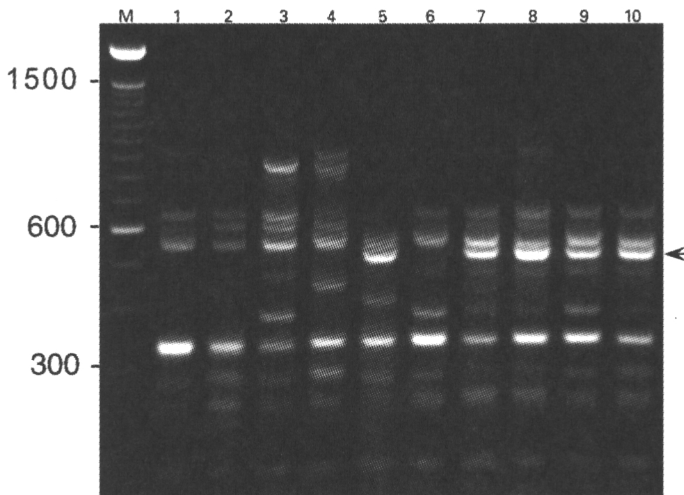

Figure 5. Banding patterns obtained with the M13 minisatellite primer. 1 – Yamal, 2 – Taimyr, 3 – Kolyma, 4 – Wrangel Island, 5 – Greenland, 6 and 7 – Walker Bay, 8 – Hope Bay, 9 – Byron Bay, 10 – Breakwater Island, M – fragment size marker (100 bp DNA ladder). The arrow indicates the ~530 bp fragment present only in Nearctic specimens.

during climatic warming of the Holocene. This hypothesis is also supported by the higher genetic diversity in the lemming populations from the area east of the Kolyma River and from Wrangel Island compared with western Siberian lemming populations. Eastern Kolyma was not affected by forest expansion during the Holocene (Khotinskiy, 1984) and Wrangel Island provided a relict of Pleistocene dry tundra in the Holocene (Vartanyan, Garutt & Sher, 1993). The variation in *D. groenlandicus* is more pronounced on Wrangel Island than in other locations probably due to the lack of bottlenecks and extinctions.

The main phylogenetic division in *Dicrostonyx* lies between the Eurasian *D. torquatus* and the North American group of species and is probably the result of intermittent inundation of the Bering Strait during the interglacials (Fedorov *et al.*, 1999a). However, Wrangel Island is considered to be colonized by lemmings of Nearctic origin (Fedorov *et al.*, 1999a). In North America the following vicariant separation in three refugial areas probably generated the extant species diversity (Fedorov *et al.*, 1999b). The amount of divergence among the three North American species suggests that the vicariant events predated the latest glaciation (Weichsel; 10–115 kyr; Andersen & Borns, 1997). *D. groenlandicus* evolved in ice free areas to the north of the main ice sheet, whereas the *D. hudsonius* and *D. richardsoni* likely derived from the southeastern and southwestern periglacial areas, respectively (Chaline, 1987; Engstrom *et al.*, 1993; Eger, 1995).

For *D. torquatus*, the phylogenetic grouping is due to historical population fragmentation and allopatric bottleneck events that divided the now continuously distributed species into five clades of haplotypes specific to different geographical regions within the Palearctic. The results of Fedorov *et al.* (1999a) suggest that during one of the last interglacials the distribution range of *D. torquatus* in Eurasia was contracted to a single refugium, probably situated to the east of the Kolyma river. The main phylogenetic split of *Dicrostonyx* is at the Bering Strait and corresponds to the main chromosome division between the Beringian and Eurasian groups of karyotypes. This suggests that despite the intermittent Bering land bridge, Palearctic and Nearctic forms have been separated since the mid Pleistocene *c.* 1 Myr ago (Fedorov & Goropashnaya, 1999).

GENETIC AND MORPHOMETRIC DIVERGENCE IN *ANDRYA ARCTICA*

The present results unequivocally show that the intestinal parasite *A. arctica* does not follow the major Palearctic–Nearctic phylogenetic split recorded for its host, *Dicrostonyx* (Fedorov *et al.*, 1999a). Morphometrics and all genetic comparisons show that the largest differences between *A. arctica* populations are found within the Palearctic region and not between the Palearctic and the Nearctic. In the Palearctic, three different genetic entities of *A. arctica* were found (Yamal, Taimyr and Kolyma) and two (Yamal, Kolyma) according to morphometrics. These regions correspond to the main chromosome races and phylogenetic groupings of the hosts (*D. torquatus*).

In principle, the lack of pronounced differences between the Palearctic and the Nearctic could be explained by long-distance dispersal of *A. arctica* by means of infected intermediate hosts (the intermediate host for anoplocephalid cestodes are soil arthropods, particularly oribatid mites; Gleason & Buckner, 1979; Denegri, 1993). However, this leaves open the question about differences in local divergence and degree of genetic variation in the Palearctic and Nearctic, respectively. Instead, the following explanation based on different evolutionary histories of *A. arctica* on the two continents would fit the observed patterns.

Originally a single genetically variable *A. arctica* population would have occupied the whole Holarctic area. Later the Palearctic and the Nearctic populations were separated from each other, and two equally variable parasite populations existed. This is supported by the fact that most of the genetic markers are observed both in the Nearctic and the Palearctic. After the separation, the Palearctic and the Nearctic host and parasite populations would have had different evolutionary histories. In the Palearctic, the populations would have gone through bottlenecks, which would have contributed to the low amount of within-population variation. This could have been due to contractions into several small refugia (random genetic drift acting within them) or by contraction to a single refugium and later dispersal events (resulting in the founder effect). This history in the Palearctic corresponds to the chromosome races and phylogenetic groupings of the hosts (*D. torquatus*) and suggests that the current groupings of the parasite may have the same origin as those of the host.

In the Nearctic, no severe population bottlenecks would have occurred, which would explain the high degree of within-population variation in *A. arctica*. The Nearctic thus contrasts with the history of the Palearctic in that there was no major spatial partitioning of the host and parasite populations within *D. groenlandicus*.

However, our sample from the Nearctic is restricted as all the parasite specimens came from a single host species (*D. groenlandicus*) and most of them from a single region. Samples from the northern and western Alaska, in particular, would be needed to assess the evolutionary history of *A. arctica* in the Nearctic. Interestingly, the holotype of *A. arctica*, which originates from North Alaska, was found to be morphologically

similar to the specimens from the Wrangel Island (unilateral genital pores, no testes poral to the poral ventral osmoregulatory canal). This could mean that there exists a Beringian form of *A. arctica* that is restricted to the western clade of *D. groenlandicus*, occurring in Alaska west of McKenzie River and on the Wrangel Island (Fedorov & Goropashnaya, 1999). The *A. arctica* populations on the Wrangel Island and in northern Alaska are morphologically and genetically so different from the Canadian and Siberian populations that these two taxa should be assigned to different species (Haukisalmi, Wickström, Hantula & Henttonen, submitted).

In the absence of phylogenetic hypothesis for different populations within *A. arctica* we can not determine plausibly whether the possible Beringian form of *A. arctica* has been derived from its wide-spread sister taxa by peripheral isolation or whether it is the ancestral form among the studied cestode populations. However, due to the restricted distribution of the 'Beringian form', we prefer the former possibility; this taxon would thus have originated in an isolated host population in eastern Beringia, possibly during the late Pleistocene (cf. Fedorov & Goropashnaya, 1999). We assume that the more widespread form of *A. arctica* was present in the precursor of all extant taxa of *Dicrostonyx*, and dispersed with its host to the Nearctic; this is supported by the presence of a morphologically identical form of *A. arctica* in *D. richardsoni* on the western coast of Hudson Bay (Rankin Inlet; Haukisalmi, Hoberg, Rausch & Beveridge, unpubl.). On the other hand, *A. arctica*-like cestodes have not yet been reported from the most divergent Nearctic representitive of *Dicrostonyx*, *D. hudsonius* (Schad, 1954).

Assuming that the host and parasite colonized Greenland postglacially from the ice-free areas of the Canadian Arctic islands (Fedorov & Goropashnaya, 1999), the genetic divergence of the Greenland population of *A. arctica* (host *D. groenlandicus*) can be explained by the founder effect (migration of a small number of individuals). It is also possible that collared lemmings 'overwintered' in the coastal part of North Greenland (Macpherson, 1965; Eger, 1995); in this case the parasite divergence would reflect genetic drift in a small refugial lemming population.

The genetical and morphological divergence of *A. arctica* suggests that the evolutionary history of this cestode species has included different processes acting on different geographical regions. In the Palearctic, the divergence of *A. arctica* has paralleled that of the hosts; this mechanism is analogous to the 'cospeciation' of host and parasite populations, although no taxonomical boundaries are recognized in Arctic Siberia. On the other hand, the similarity of most of the Palearctic and Nearctic populations is compatible with the concept of 'coadaption'; i.e. the parasite has remained relatively unmodified through a series of host speciation/divergence events because of large cohesive populations (cf. Brooks & McLennan, 1993). The uniqueness of the Greenland parasite population, and possibly also that of the Wrangel Island, can be explained by peripheral isolation, refugial effects or founder effects.

The present results contradict the pattern emerging from the studies on comparative biogeography of arctic marine homeotherms and their cestodes (Hoberg, 1992, 1995), a system in which the colonization of new host species by parasites in glacial refugia has been the principal mode of parasite speciation. However, the present study and those of Hoberg both show that the evolutionary history of host-parasite associations with broad geographical ranges are likely to be complex, and are seldom structured strictly by cospeciation processes.

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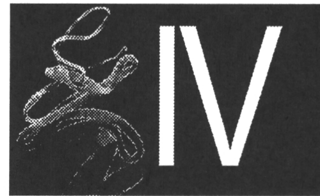
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Phylogeography of the circumpolar *Paranoplocephala arctica* species complex (Cestoda: Anoplocephalidae) parasitizing collared lemmings (*Dicrostonyx* spp.)

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Abstract

The *Paranoplocephla arctica* complex (Cyclophyllidea, Anoplocephalidae), host-specific cestodes of collared lemmings *Dicrostonyx*, include two morphospecies *P. arctica* and *P. alternata*, whose taxonomical status now must be considered ambiguous. The genetic population structure and phylogeography of the *P. arctica* complex was studied from 83 individuals sampled throughout the Holarctic distribution range using 600 bp of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). The mitochondrial DNA (mtDNA) phylogeny divides the species complex into one main Nearctic and one main Palaearctic phylogroup, corresponding to the main phylogenetic division of the hosts. In the Palearctic phylogroup, the parasite clades correspond to the host clades although the parasites from Wrangel Island form an exception as the host on this island, *D. groenlandicus*, belongs to the Nearctic phylogroup. In the Nearctic, northern refugia beyond the ice limit of the Pleistocene glaciations are proposed for the hosts. All reconstructions of parasite phylogeny show a genetically differentiated population structure that in the Canadian Arctic lacks strict congruence between phylogeny and geography. The parasite phylogeny does not show complete congruence with host relationships, suggesting a history of colonization and secondary patterns of dispersal from Beringia into the Canadian Arctic, an event not proposed by the host phylogenies alone.

Keywords: Cestoda, co-evolution, COI, Holarctic region, intestinal parasite, phylogeography

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Introduction

Collared lemmings (*Dicrostonyx*) are an almost circumpolar genus of arvicoline rodents inhabiting the Arctic tundra, being absent only from Fennoscandia. Three of the four recognized species have a Nearctic distribution (*D. groenlandicus*, *D. richardsoni* and *D. hudsonius*) and one (*D. torquatus*) inhabits the Palearctic (Jarrell & Fredga 1993; Fredga *et al.* 1999). A number of studies (Engstrom *et al.* 1993; Rausch & Rausch 1972; van Wynsberghe & Engstrom 1992; Fedorov *et al.* 1999) suggest that vicariant events generated by the climatic oscillations during the Pleistocene have promoted intra- and interspecific divergence of collared lemmings, supporting the hypothesis of multiple glacial refugia for Arctic mammals, first outlined by Macpherson (1965). The mitochondrial DNA (mtDNA) phylogeography of *D. groenlandicus* in the North American Arctic and *D. torquatus* on the Siberian mainland has been extensively studied and several mtDNA lineages and phylogeographical groups have been detected (Ehrich *et al.* 2000; Fedorov & Goropashnaya 1999; Fedorov *et al.* 1999; Fedorov & Stenseth 2002). The main phylogenetic split between *D. torquatus* and the Nearctic group of species at Bering Strait is ≈ 1 Myr old. Secondary divisions can be seen among *D. torquatus* populations (200 000 years) and between Alaskan and Canadian Arctic *D. groenlandicus* populations (100 000 years) (Fig. 1). In the Canadian Arctic an even younger population division (60 000 years) probably reflects postglacial colonization from multiple

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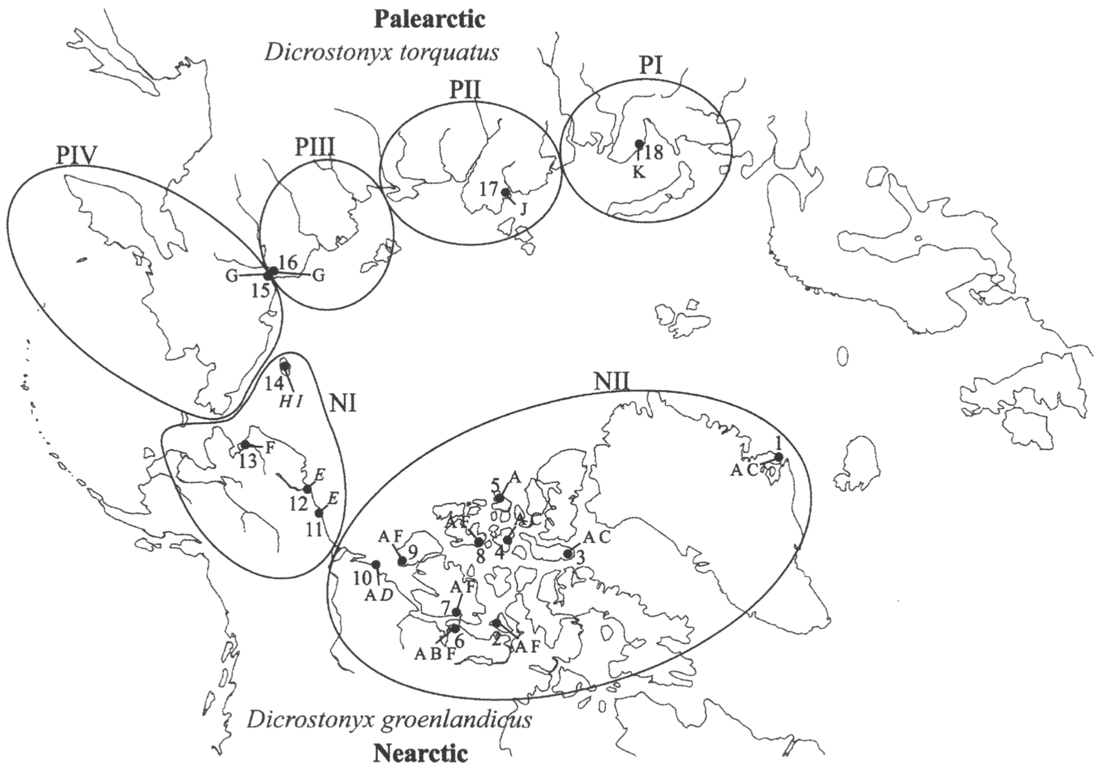

Fig. 1 Map showing the approximate host mtDNA phylogroups (PI-PIV for *Dicrostonyx torquatus*; NI and NII for *D. groenlandicus*). Groups adapted from Fedorov *et al.* (1999) and Fedorov & Goropashnaya (1999). For sampling localities of parasites (1–18) see Table 2. Lettering at sampling localities refers to the phylogenetic lineages (subclades A to K in Figs 2–4).

Table 1 Distribution of the five species of *Paranoplocephala* parasites in the Nearctic lemming host *Dicrostonyx groenlandicus* and the Palearctic host *D. torquatus*

	P. arctica	*P. alternata*	*P. krebsi*	*P. nordenskioeldi*	*P. serrata*
D. groenlandicus	X	X	X	X	X
D. torquatus		X		X	X

glacial refugia after the last glaciation (Fedorov & Stenseth 2002). If refugia within the glaciated region played an important role in (re)colonization, genetic differentiation should be pronounced, thus implying a 'persistence scenario', in contrast to colonization from nonglaciated regions further away with rapid expansion that would generate little genetic differentiation in (re)colonized areas (cf. Hewitt 1996).

Paranoplocephala spp. (Cestoda: Anoplocephalidae) are tapeworms parasitizing arvicoline rodents (voles and lemmings) in the Holarctic (Rausch 1976; Haukisalmi *et al.* 2002). Collared lemmings are parasitized by five host-specific species of *Paranoplocephala* (Table 1) (Haukisalmi *et al.* 2001). Of these, three species have a Holarctic distribution (*P. alternata*, *P. serrata* and *P. nordenskioeldi*) and two occur only in the Nearctic and on Wrangel Island (*P. arctica* and *P. krebsi*). This study covers *P. alternata* and *P. arctica* (Table 1) and when referring to both proposed species we use *P. arctica* species complex as the term of reference. An initial population genetic study of *P. arctica* and *P. alternata* (Wickström *et al.* 2001; both species then included in *Andrya arctica*) using sequence tagged sites (STS) and minisatellite data revealed strict co-divergence with the host *D. torquatus* on the Siberian mainland. The main phylogenetic split of *Dicrostonyx* between Eurasia and the North American Arctic was not, however, observed in the *P. arctica* species complex. In the previous study no samples from the western Nearctic host subclade (west of Mackenzie River, Alaska) were obtained, and we were not, therefore, able to extend that work (Wickström *et al.* 2001)

to include the phylogeography of the parasites in the Nearctic. This survey includes the whole distribution range for *D. groenlandicus* in the North American Arctic, in addition to the Palearctic material already screened using minisatellites and microsatellite-based (STS) markers by Wickström *et al.* (2001).

As mtDNA sequences are widely used for intraspecific phylogeny assessment in arvicoline rodents (voles and lemmings) (Avise 2000; Fedorov & Stenseth 2002; Jaarola & Searle 2002; Haynes *et al.* 2003), we used partial cytochrome *c* oxidase subunit I (COI), a mitochondrial gene, to infer parasite phylogeny. The *P. arctica* species complex was chosen for this study as *P. alternata* is the most widespread and locally most abundant taxon of the host-specific cestodes of *Dicrostonyx* spp., and as population genetic analyses on this particular species complex had already been initiated.

Host–parasite co-evolution is a richly complex interaction of phylogenetic history, temporal association and ecological factors that must all be implemented in the development of causal explanations (Hoberg 1997). In our study system, there are five proposed species of *Paranoplocephala* in the four acknowledged *Dicrostonyx* lemming hosts. *D. groenlandicus* harbours all five parasite species, whereas all except *P. krebsi* and *P. arctica* can be found from *D. torquatus* (Table 1). Overall, this suggests a complex history of co-speciation, co-adaptation and colonization that can only be resolved in the context of comprehensive phylogenetic information yet to be gathered, and which is beyond the scope of this study. Here we focus on comparison between mtDNA phylogeography of the *P. arctica* species complex and the two well-studied host species, the Eurasian *D. torquatus* and the North American *D. groenlandicus* (Fedorov & Goropashnaya 1999; Fedorov *et al.* 1999; Ehrich *et al.* 2000; Fedorov & Stenseth 2002).

Co-speciation analysis attempts to assess the degree of congruence/incongruence between host and parasite phylogenies and the history of the association. The occurrence of the same parasite species in at least two host species could represent either host speciation in the absence of parasite speciation (a form of co-adaption) or host switching. It is rather unusual to find host and parasite associations that match perfectly; rather, there is typically a mixture of congruence and incongruence (Page 1993). Because of its stochastic nature, false congruence is not likely to be common, but a more serious problem is false incongruence. False incongruence can be caused in two main ways: (i) extinctions, 'missing the boat' or sampling errors, i.e. three different types of parasite lineage sorting; and (ii) redundant parasite distribution (*sensu*, Page 1993). Redundant distribution of the parasites could be mediated by intermediate host migration and/or diverging mating behaviour of hermaphroditic parasites in relation to their hosts. Differences between host and parasite in substitution rates and population effective sizes are other factors generating incongruence. As spermatozoa of tapeworms lack mitochondria (Justine 1991, 2001), mtDNA evolution in cestodes is rather similar to that in asexually reproducing organisms and the population size of mtDNA equals that of individuals. Consequently, clonality is facilitated and haplotypes may persist for long periods.

As vicariant separation into different glacial refugia has been suggested for the hosts, we would expect pronounced genetic differentiation for the parasites but not strict congruence of phylogeny and geography as a consequence of recurrent range shifts of different host and parasite phylogenetic lineages.

The aim of this study is to examine the co-evolutionary history of *P. arctica* and *P. alternata* and their hosts, and assess whether parasite phylogeny can serve as a model for tracing host evolution in Arctic species.

Materials and methods

Eighty-three individuals (seven morphotyped as *Paranoplocephala arctica*, the rest as *P. alternata*) from 21 localities across the Holarctic region were screened in this study (Table 2, Fig. 1). Tissue samples for genetic analyses were obtained from specimens preserved in 70% ethanol or from specimens frozen in extraction buffer (50 mM Tris–HCl, pH 7.2; 50 mM EDTA; 3% SDS; 1% β-mercaptoethanol) at −20 °C. Total genomic DNA was extracted from 0.5–2 mm^3 tissue samples as described previously (Vainio *et al.* 1998).

A 641-bp fragment was amplified from COI using a hot-start polymerase chain reaction (PCR) and a reaction volume of 50 μL. DNA amplification and sequencing methods for COI are described in Haukisalmi *et al.* (2003). We are confident that our sequences represent the true partial COI as there were no anomalies of the type commonly associated with pseudogenes (Zhang & Hewitt 1996) and the translated protein sequences obtained matched previously published data for other cestode species (complete mitochondrial genomes of *Hymenolepis diminuta*, GenBank Accession no. NC_002767; *Echinococcus multilocularis*, GenBank Accession no. NC_000928) and previously cloned partial COI sequences of other anoplocephalid cestode species amplified using a different primer pair (LM Wickström *et al.* unpublished data). In addition, ≈ 650 bp of internal transcribed spacer (ITS)1 and ≈ 300 bp of 12*S* ribosomal DNA (rDNA) were screened in geographically distant specimens, but because of low variation in these partial sequences throughout the Holarctic region, they could not be used to infer phylogenies on a population level. However, ITS1 was used to assess the taxonomical relationship of *P. arctica* and *P. alternata*. For methodological notes on amplifying and sequencing of 12*S* rDNA see von Nickisch-Rosenegk *et al.* (1999) and for ITS1 see Haukisalmi *et al.* (2001).

Table 2 Sampling regions and localities, number of individuals screened in each locality (n), haplotype designations and GenBank Accession nos (AY181426–AY181509) for *Paranoplocephala arctica* (haplotype designated in bold) and *P. alternata* partial COI sequences

Region	Locality code in figs	Locality	n	Haplotypes	GenBank Accession numbers, AY181xxx. Haplotype designation in parenthesis, if several
Greenland	1	Constable Point	2	19, 35	454 (19), 453 (35)
Central Canadian Arctic	2	King William Island	5	16, 30, 32	465, 466, 467 (16), 476 (30), 477 (32)
Northern Canadian Arctic	3	Devon Island	5	16, 20, 37	488 (16), 489 (20), 482, 492, 495 (37)
	4	Bathurst Island	13	16, 23, 36, 38, 39, 42	463, 469, 470, 484, 481, 496, 498 (16), 464, 480 (23), 483 (36), 500 (38), 499 (39), 487 (42)
	5	Ellef Ringnes Island	1	16	462
Kent Peninsula region	6	Breakwater	1	28	444
		Hope Bay	9	16, 18, 22, 24, 25, 26, 27	448, 449 (16), 434 (18), 438 (22), 439 (24), 447, 450 (25), 435 (26), 437 (27)
		Hurd	1	25	452
		Walker Bay	2	40, 25	432 (40), 433 (25)
Victoria Island	7	Byron Bay	6	16, 24, 33	441, 442, 443, 446 (16), 451 (24), 440 (33)
Western Canadian Arctic	8	Melville Island	8	16, 31, 33, 41	472, 473, 490, 491, 497 (16), 471 (31), 475 (33), 474 (41)
	9	Banks Island	7	16, 17, 30, 33, 34	485, 486 (16), 501 (17), 460, 461 (30), 494 (33), 493 (34)
Western Canadian mainland	10	Cape Bathurst	4	16, 21, **15**	468, 479 (16), 478 (21), 503 (15)
Northern Alaska	11	Prudhoe Bay	1	**13**	507
	12	Colville River Delta	2	**14**	506, 508
Western Alaska	13	Cape Krusenstern	1	29	502
Eastern Siberia	14	Wrangel Island	3	**10**, **11**, **12**	504 (10), 505 (11), 509 (12)
	15	Eastern Kolyma	2	6, 8	429 (6), 426 (8)
	16	Western Kolyma	3	7, 9	430 (7), 431, 436 (9)
Central Siberia	17	Tajmyr	1	5	459
Western Siberia	18	Yamal	6	1, 2, 3, 4	457 (1), 428, 458, 455 (2), 427 (3), 456 (4)

Data on nucleotide substitutions and amino acid replacement were determined using MACCLADE Version 4 (Maddison & Maddison 2000). Nucleotide and haplotype diversity estimates were calculated according to Nei (1987). Nucleotide diversities and their bootstrap standard errors were counted as Kimura 2-parameter distances in MEGA 2.1 (Kumar *et al.* 2001). Phylogenetic analyses of haplotypes were performed using maximum parsimony (MP) and maximum likelihood (ML) algorithms implemented in PAUP* beta Version 4.0b10 (Swofford 2002) and the neighbour-joining (NJ) method (Saitou & Nei 1987) in the MEGA 2.1 package. The parsimony analyses were carried out heuristically with 1000 random additions, TBR swapping and MulTrees option in effect. Bootstrap analyses were conducted for 1000 rearrangements (with 10 random additions). The computer program MODELTEST Version 3.06 (Posada & Crandall 1998) was used to identify the most appropriate substitution model for our data. The selected models were HKY85 + I + G (using the hierarchical likelihood ratio test, hLRT, with outgroups included) and GTR + I + G (without outgroups by hLRT and constantly by the Akaike Information Criterion, *AIC*). GTR + I + G was implemented with unequal base frequencies, a gamma distributed shape parameter ($\alpha = 1.0236/0.8750$) and the proportion of invariable sites ($I = 0.6046/0.6733$) with/without outgroups. These models and several simpler substitution models were used in the ML analyses and assessed with 100 bootstrap replicates. The NJ algorithm in MEGA 2.1 was implemented with Kimura 2-parameter distances, as more complicated models did not give better results, and assessed with 10 000 bootstrap replicates. The Bayesian method of phylogeny (MB) was tested using MRBAYES 3.0B4 (Ronquist & Huelsenbeck 2003). Two independent Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were completed, both started with random trees for each of four simultaneous chains and run for 10^7 generations with a burn-in of 10% of the sampled trees (trees were sampled every 50 generations). Models GTR + I + G and HKY + I + G were implemented separately for the whole data with and without third codon position uncoupled for the shape parameter estimate (α-value). Trees were viewed using TREEVIEW (Page 1996). Three species of anoplocephaline cestodes, *Andrya rhopalocephala* (GenBank Accession no. AY189958, host; European hare *Lepus europaeus*), *Andrya cuniculi* (AY189957, host; European rabbit *Oryctolagus cuniculus*) and *Diandrya composita* (AY181550, host; hoary marmot *Marmota caligata*) were used as outgroups in the phylogenies. Owing to uncertain phylogenetic relationships within *Paranoplocephala* spp. (LM Wickström *et al.* unpublished COI, and ITS1 data), the sister taxon for *P. alternata* and *P. arctica* remains unknown. Haukisalmi *et al.* (2001) indicate a putative sister species relationship with *P. serrata*, but also indicate that these relationships are poorly supported. *Andrya*, *Diandrya* and *Paranoplocephala* are morphologically closely related to each other (Rausch 1976, 1980), and the unpublished molecular phylogenetic data of Wickström *et al.* suggest that *Andrya* and *Diandrya* may form the sister group for *Paranoplocephala sensu lato*. Because of uncertainty regarding the correct outgroup species, the initial phylogenetic analysis was conducted without outgroups. Unrooted analyses may avoid certain difficulties associated with outgroup sequences (Stanhope *et al.* 1993; Swofford *et al.* 1996; Burk *et al.* 1999; Eizirik *et al.* 2001) and recent studies have highlighted the importance of unrooted analysis in recovering monophyletic groups (Lin *et al.* 2002; Scally *et al.* 2002).

Results

Genetic diversity

Eighty-three variable sites were found (total number of mutations was 93, no gaps) in COI, corresponding to 14% of the sequence length. Ten of the sites showed more than one type of substitution. The majority of polymorphic sites were at the third position (82%) followed by the first (17%) and the second (1%). As expected, most nucleotide substitutions were transitions (78%) and silent (93%). We found 42 haplotypes among the 83 individuals sequenced for the 605 bp partial COI (Table 2). Twelve different haplotypes were recorded among the 15 individuals from the Palearctic (Wrangel Island included), corresponding to a haplotype diversity of 0.80. Among the 68 individuals from the Nearctic, we recorded 30 different haplotypes, giving a haplotype diversity of 0.44. The nucleotide diversity in the Palearctic ($2.66 \pm 0.45\%$) was also higher than in the Nearctic ($1.58 \pm 0.29\%$), despite the Nearctic individuals being sampled over an equally vast geographical region. The total haplotype variation (among all individuals) was 0.51, and total nucleotide diversity $2.5 \pm 0.35\%$. The total raw DNA divergence (Dxy) was $4.55 \pm 0.51\%$ between the Palearctic and the Nearctic and the net divergence (Da) was $2.43 \pm 0.53\%$. On the Siberian mainland (and on Wrangel Island), haplotypes were not shared among regions. The most common haplotype (no. 16, Table 2) was found in the Nearctic and shared between 25 Nearctic individuals from 9 geographical regions. Haplotypes from Alaska were not found elsewhere, also in the Canadian Arctic mostly region-specific haplotypes were found. The diverging haplotype pattern within the Canadian Arctic is a result of a few haplotypes common to geographically distant regions, with the most common one (haplotype 16) occurring in nearly all Canadian localities screened in this study. The differences in haplotype structure and diversity in the Palearctic and Nearctic are concordant with the results of the more limited survey of Wickström *et al.* (2001)

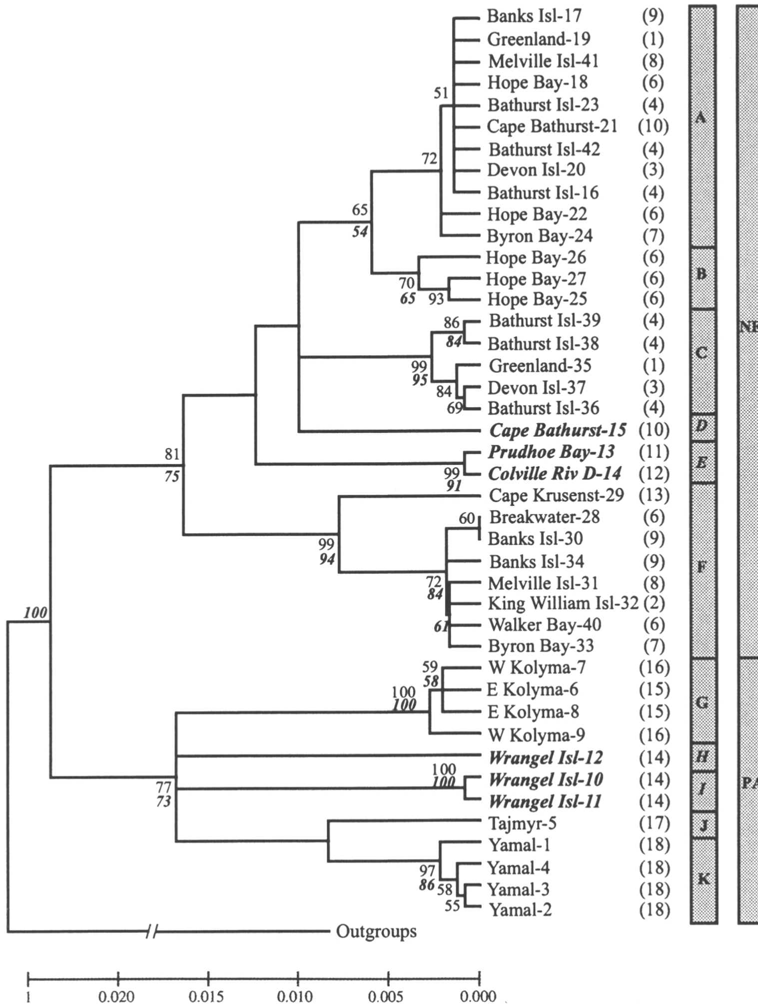

Fig. 2 Neighbour-joining (NJ) tree of the *Paranoplocephala arctica* species complex mtDNA haplotypes (for haplotype designations 1–41, see Table 2). Sampling localities in parentheses refer to Fig. 1. The haplotypes that refer to the morphospecies *P. arctica* are given in bold italics. PA = Palearctic clade, NE = Nearctic clade. Lettering A to K designate the recognized NJ and MP subclades. Subclades representing the morphospecies *P. arctica* in italics. Bootstrap percentages from 10 000 iterations are shown at nodes, maximum parsimony (MP) bootstrap values (1000 iterations) are shown in bold italics.

considering molecular variance in STS and minisatellite data between *Paranoplocephala arctica* populations from the Holarctic region.

Phylogeography of the P. arctica *species complex*

The haplotype trees obtained using NJ methods (Fig. 2) were minimally influenced by the substitution model or outgroup, although the highest bootstrap values for the rooted trees were found with all three outgroup taxa included. The NJ method revealed one main Nearctic (NA) and one main Palearctic (PA) clade. (Bootstrap support for these clades was 99% in unrooted trees.) The Palearctic subclades (G, J, K) on the Siberian mainland were identified previously using STS and minisatellite data (Wickström *et al.* 2001), but the COI sequence data also revealed two subclades (H and I) on Wrangel Island (morphospecies *P. arctica*), and grouped the Wrangel Island subclades within the Palearctic clade. Within the Nearctic clade the five main branches stemmed from a polytomy when ignoring poorly supported structure (i.e. bootstrap support < 50%). Two branches represented the

morphospecies *P. arctica*, one from Cape Bathurst, Canada, east of Mackenzie River (subclade D), and the other one from Prudhoe Bay and Colville River Delta in northeastern Alaska, west of Mackenzie River (subclade E). The other branches represented the morphospecies *P. alternata*. Of the four *P. alternata* subclades, one is geographically widespread occurring all over the Canadian Arctic (subclade A, Fig. 1) including the haplotypes from the central Canadian Arctic mainland and islands, and from the High Arctic islands (Fig. 1), whereas the other two well-supported clades (C and F) consist of haplotypes from the High Arctic islands (subclade C) and from the western–central Canadian Arctic only (subclade F) (Fig. 1). Subclade F also included the only specimen/haplotype of morphospecies *P. alternata* from western Alaska, being basal to this clade. Subclade B consisted of haplotypes from the Kent Peninsula region only.

Parsimony analysis with PAUP* on the 61 informative sites generated four minimal trees of 319 steps, a consistency index of 0.69, a homoplasy index of 0.31 and a retention index of 0.83 when using all three outgroup species for rooting. (Other outgroup setups resulted in longer trees, lower consistency index and/or more trees.) The division into a Palearctic and a Nearctic clade was supported (bootstrap percentages presented in bold italics in the NJ tree, Fig. 2), and the same subclades were present (A to K) as in the NJ trees. A strict consensus of the four best trees also suggested that subclades D and E (morphospecies *P. arctica* from the Beringian region) are basal to the rest of the Nearctic subclades (both in rooted and unrooted trees), but this topology was not supported by bootstrap analyses. The Palearctic–Nearctic division was strongly supported (100%) in unrooted MP trees (not shown).

The ML algorithm in PAUP* implemented with GTR + I + G and HKY + I + G with values calculated using MODELTEST generated trees with subclades A to K always present and supported, but the internal branching order (or lack thereof) was very much dependent on the model used when outgroups were included (trees not shown). GTR and HKY recognized the Palearctic clade, whereas the Nearctic subclades appeared as a polytomy directly from the root in the consensus trees. With simpler models (for example, F81 + I + G) and with GTR + SS, HKY + SS the consensus trees showed a reversed basal structure; the Nearctic clade supported and the Palearctic subclades derived from the root. In both rooted and unrooted analyses, the Beringian subclades (D and E) were basal to the other Nearctic clades but, as for MP, the topology was very unstable. Unrooted ML topologies also recognized a main Palearctic–Nearctic division.

Bayesian inference of phylogeny implemented with the same models as for ML generated topologies very similar to ML, MP and NJ. The unrooted topology (Fig. 3) very strongly supported monophyly for the Palearctic and the Nearctic clades. In the rooted trees (not shown) the pattern observed for ML was repeated, i.e. the Palearctic clade was recognized, but the Nearctic subclades often appeared as polytomies originating from the root. Using GTR + I + G in MB and also allowing the third codon position to have its own, potentially different, α-value, rooted the tree from within the Palearctic, rendering a similar topology as for GTR + SS or HKY + SS in PAUP*. All phylogenetic reconstruction methods used in this study produced trees of similar topology. The sensitivity of the rooting to the choice of model in MB and ML is discussed below.

The haplotypes representing the morphospecies *P. arctica* (subclades D, E, H and I) did not group together in the COI gene trees, but were split and included within the subclades representing the *P. alternata* morphospecies in all the genealogies. As these results contradicted the proposition of *P. arctica* and *P. alternata* being two separate species, as stated based on morphology in Haukisalmi *et al.* (2001), we also screened 635 bp of the nuclear rDNA ITS1 region (GenBank Accession nos; AY299542–AY299562, AF314412, AF314413). The ITS1 data consisted of a subset of both Palearctic and Nearctic *P. alternata* (18 individuals), and the five *P. arctica* individuals for which an unambiguous sequencing product could be obtained. The sequence revealed 21 variable sites (sites with gaps in ambiguous regions excluded). Of the six parsimony informative sites, five agreed on separating the species complex into a Holarctic group (morphospecies *P. alternata*) and a western Beringian (morphospecies *P. arctica*) group, whereas the sixth site distinguished the Wrangel Island *P. arctica* individuals from all the others. The 300 bp of 12*S* rDNA analysed from an even smaller subset of individuals (from the Nearctic only) generated only three parsimony informative sites that give little phylogenetic information.

Discussion

mtDNA diversity in a Holarctic parasite

Considering the deep phylogenetic division of the host, the vast geographical range sampled and the historical/geographical barriers to dispersal (Fedorov & Goropashnaya 1999; Fedorov *et al.* 1999; Fedorov & Stenseth 2002), we expected a higher degree of sequence variation within the *P. arctica* species complex. A large portion of the Canadian COI haplotypes differed in only a few nucleotide positions from the most common haplotype (no. 16) found throughout the Canadian Arctic. In the Palearctic, nucleotide diversity was 1.7 times higher, which together with the lack of shared haplotypes between regions sampled on a continental scale, implies a longer period of separation and/or regional bottleneck events. The relatively high level of diversity on Wrangel Island,

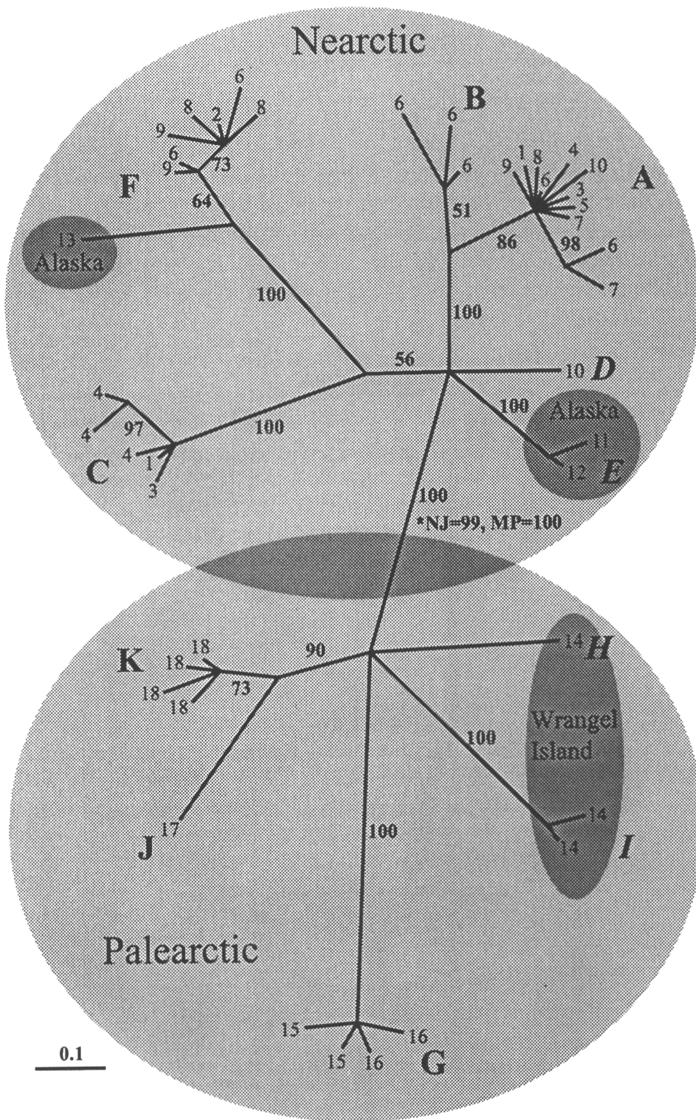

Fig. 3 Unrooted tree of the *Paranoplocephala arctica* species complex mtDNA haplotypes constructed with Bayesian inference of phylogeny. Posterior probabilities presented as percentages. The subclades recovered (A to K) are lettered as in Figs 1 and 2. Locality codes (as in Fig. 1) presented for the individual branches. Subclades D, E, H and I represent the morphospecies *P. arctica*. Bootstrap percentages for the Palearctic–Nearctic division (*) recovered from unrooted trees with other phylogenetic methods are presented after the asterisk (NJ = neighbour-joining, MP = maximum parsimony).

reflected by the two divergent haplotype lineages, may imply a longer *in situ* demographic history on Wrangel compared with the Siberian mainland populations.

The observed pattern is congruent with that of mtDNA (cytochrome *b*) haplotypes recorded for the hosts in the Palearctic vs. the Nearctic. Studies on the genetic population structure of the hosts (Ehrich *et al.* 2000; Fedorov *et al.* 1999) have recognized unusually low mtDNA diversity compared with that generally recorded for rodents (reviews in Plante *et al.* 1989; Hayes & Harrison 1992; Riddle *et al.* 1993; Jaarola & Tegelström 1995; McKnight 1995). Differing scales, duration and regional extent of isolating events associated with alternating stadials and interstadials are the most probable reasons for small effective populations, founder effects and peripheral isolates. Signs of such events may be most evident at species or intraspecies levels (e.g. Hewitt 2000; Galbreath 2002; Hoberg *et al.* 2003). Range contractions suggested for the hosts (induced by Pleistocene–Holocene climatic warming events) have probably resulted in temporarily greatly reduced host population sizes that could be argued to harbour extremely reduced parasite populations.

We have previously shown that *P. arctica* and *P. alternata* are monophyletic with respect to other species of *Paranoplocephala* in collared lemmings (Haukisalmi *et al.* 2001). However, the COI data show that neither the morphospecies *P. arctica* nor *P. alternata* is monophyletic. The conspecificity of the two morphospecies is supported by the generally low degree of genetic divergence within this complex and common alleles in STS and minisatellite data (see Wickström *et al.* 2001).

Differences between the Palearctic and Nearctic in sequence diversity in COI compared with molecular variance based on STS and minisatellite markers in Wickström *et al.* (2001) agree very well, and the sparse information obtained from 12*S* rDNA did not suggest any kind of phylogenetic structure other than that obtained with COI. In contrast to these three data sets, the ITS sequences do not imply separate lineages in Siberia or between Palearctic and Nearctic populations. Instead, five of six parsimony-informative sites separated an eastern Beringian lineage (morphospecies *P. arctica*) from the main Holarctic lineage. However, because of the small number of parsimony informative sites, too much emphasis should not be given to the ITS data. The discussion below is therefore based on COI data only.

Historical patterns over Bering Strait; Palearctic vs. Nearctic

The NJ/MP trees (Fig. 2) and all unrooted topologies (for example, Fig. 3) suggest a main phylogenetic split at Bering Strait for the parasites, concordant with the main split for the hosts (Fedorov & Goropashnaya 1999; Fedorov & Stenseth 2002). The Nearctic (Alaskan and Canadian) haplotypes never intermixed with the Palearctic clade even if they did not always form a single monophyletic group. The ML and MB gene trees, although otherwise very similar to the NJ and MP topologies, rooted (with complicated models) either within the Palearctic or the Nearctic clade. Even though likelihood ratio tests or AIC often are used to examine the goodness of fit of a model to the observed data, Takahashi & Nei (2000) showed, through computer simulations, that when the number of sequences is large a simple model usually gives better results than a complex model as long as the sequences are relatively short. Also, theoretical studies (Gaut & Lewis 1995; Yang 1997) indicated that sophisticated models might not necessarily give the correct topology with a higher probability than a simple model. For our topologies this seems to be true, the conclusion further corroborated by the generally consistent phylogenetic estimate obtained from NJ and MP (and ML/MB with simpler models, i.e. analyses which involved fewer parameter estimates). The outgroup sequences were divergent among themselves and possibly too distant to function well as outgroups. Lack of robustness in the data and/or long branch attraction may also contribute to the instability of the basal nodes in the current topologies.

The origin and/or separation of the Palearctic and Nearctic parasite subclades and the origin of the main Palearctic–Nearctic split may be separated by only a relatively short time interval and the evolutionary traces of these events may, therefore, be obscure. However, in the absence of a calibrated molecular clock for the parasites we are not able to place these events on a reliable time scale. The only time scale available is that for the hosts (Fedorov *et al.* 1999; Fedorov & Stenseth 2002), and as the parasite tree in most aspects resembles the host tree, we assume that separation of the Palearctic clades from the Nearctic clades corresponds temporally (1 Myr) to the major split recorded for the hosts, i.e. indicating deep co-speciation. For further assessment of the stability of the clades, additional loci and/or more extensive sampling, particularly in Beringia, would be required. However, most of the evidence indicates that there is a main division over Bering Strait for the parasites. The mtDNA genealogy of the parasites matches that of the host (Fig. 4) and suggests co-divergence between host and parasite, with Wrangel Island populations as the main exception. The failure to detect a main split over Bering Strait in Wickström *et al.* (2001) may have been due to the lower resolution of the markers (STS) because of lower mutation rates, the lack of information on phylogenetic relationships and high level of homoplasy among alleles (minisatellites).

Parasite phylogeography in the Palearctic region

The greater sequence diversity between the Palearctic subclades and the lack of shared haplotypes within them imply that the parasite populations may have undergone bottleneck events within geographical regions (see Wickström *et al.* 2001). As all the Siberian subclades stem from a polytomy, they may all be approximately the same age, and probably originate from the same vicariant separation by glacial barriers (200 000 years ago) and regional bottlenecks that have generated similar phylogeny among the host populations (Fedorov *et al.* 1999). The COI genealogy and the difference in morphology suggest a differing/longer demographic history for Wrangel Island haplotypes for the parasite. Similarly, a higher level in mtDNA diversity was recorded for the host on Wrangel Island, but also in the Kolyma River region in the eastern Palearctic (Fedorov *et al.* 1999). Kolyma River constitutes the geographical border between the two easternmost Palearctic host phylogroups (Fedorov *et al.* 1999). Such a clear-cut geographical distinction cannot be seen for the parasite haplotypes at the Kolyma River implying incongruence between host and parasite and a possible lack of separate phylogroups for the parasites. However, as anoplocephalid cestodes are found to cross host

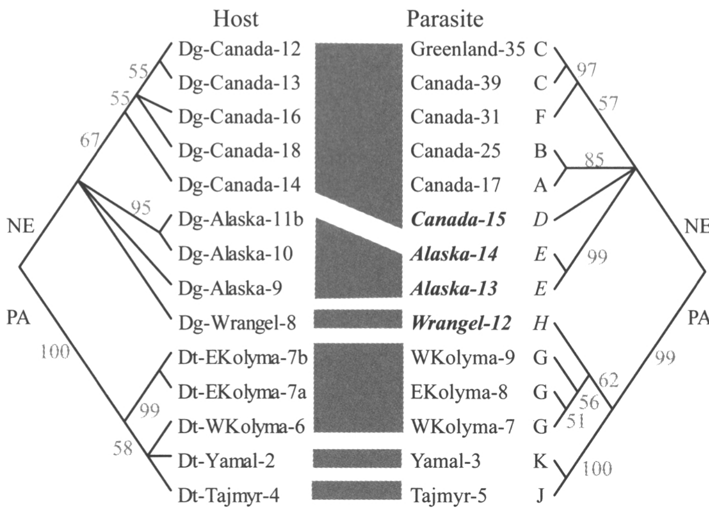

Fig. 4 Comparative neighbour-joining phylogeny of midpoint-rooted host and parasite consensus trees of major mtDNA clades recovered from cytochrome *b* sequences for the hosts (*Dicrostonyx torquatus* = Dt, *D. groenlandicus* = Dg, numbering refers to the sampling localities/individuals in Fedorov & Goropashnaya 1999; Fig. 1) and COI for the parasites (*Paranoplocephala*, numbering refers to COI haplotypes, listed in Table 2, lettering refers to subclades A to K as in Figs 1–3). Subclades representing the morphospecies *P. arctica* are shown in bold italics. NE = Nearctic, PA = Palearctic clades. Bootstrap percentages shown at nodes (10 000 iterations).

contact zones (LM Wickström *et al.* unpublished) and as the Kolyma samples are from the host contact region, we cannot exclude the possibility of the presence of two parasite clades in eastern Siberia as recorded for the host.

Parasite phylogeography in the Nearctic region

The well-resolved Nearctic *P. arctica* and *P. alternata* subclades stem from a polytomy in all obtained topologies when ignoring poorly supported nodes. This topology supports the 'persistence scenario' suggested for the hosts on the basis of phylogeography, palaeoecology and geology (cf. Fedorov & Stenseth 2002) in the Canadian Arctic and opposes a single (or repeated) dispersal from the Beringian region. The main Nearctic split of the hosts into an Alaskan and northern Canadian phylogroup (Ehrich *et al.* 2000; Fedorov & Stenseth 2002) cannot be seen as such for the parasites (Figs 2 and 3). The well-supported subclades (C and A + B) probably originated from refugial populations surviving the last glaciation in the Canadian High Arctic. The division of the parasites into two solely Canadian Arctic subclades suggests at least two separate refugial areas northwest of the ice sheet, as proposed for the hosts (Fedorov & Stenseth 2002). The fact that the two haplotypes from Greenland grouped within different subclades, could imply colonization from two separate refugia situated somewhere in the nonglaciated parts of the Canadian High Arctic Archipelago. Subclade F (including the only specimen representing morphospecies *P. alternata* from Alaska) may represent a dispersal event from the Beringian region and a subsequent eastward spread along the southern Canadian Arctic. This hypothesis is supported by the fact that haplotypes belonging to this subclade cannot be found from the northernmost Arctic islands (i.e. sampling localities 1, 3, 4 and 5 in Fig. 1). Retreat of the Wisconsian glaciation starting at ≈ 13 000 years ago could have let the lemmings and their parasites spread over the whole Canadian Arctic in a relatively short period as the ice withdrew. The widespread Nearctic subclade A could thus be the result of this second/first colonization of deglaciated areas. The uncertainty concerning the exact location of the High Arctic refugia (Ehrich *et al.* 2000; Fedorov & Stenseth 2002) cannot be answered by the parasite phylogenies either, but the occurrence of genetically differentiated parasite clades and the patchy, partly redundant, geographical distribution of parasite haplotypes clearly supports the 'persistence scenario' obtained for the hosts, and also implies strong co-divergence with only occasionally decoupled host and parasite phylogenies (Fig. 4).

The main discrepancy between host and parasite colonization patterns in the Nearctic is the lack of a deep division into an eastern Beringian and a Canadian Arctic phylogroup. There is no evidence of secondary colonization from the Beringian region for the hosts, as may be argued for the parasite. Although screening of more Beringian material is definitely needed, a dispersal event from eastern Beringia for the parasites may shed light on the occurrence of the proposed central Canadian host haplotype clade with a west–east spread (see Fedorov & Stenseth 2002). Despite the observed discrepancies, the refugial hypothesis proposed for the hosts (Fedorov & Stenseth 2002) was not contradicted by the parasite mtDNA phylogeny. Considering these facts, it may be argued that parasites serve as indicators of fine-scaled (temporal and geographical) events that are not (or not as

clearly) apparent in the assessments of the biogeographical history of the hosts. Cryptic host divergence may be enough to drive parasite speciation, whereas the host populations do not speciate. An example of parasite diversification in the absence of host divergence across Bering Strait is the presence of two host-specific, allopatric and possibly conspecific clades of *P. omphalodes* in the root/tundra vole, *Microtus oeconomus*. One parasite clade has a Holarctic and the other an eastern Beringian (Alaskan) distribution (Haukisalmi *et al.* 2003). Because tundra vole populations in Alaska are surprisingly undifferentiated (Brunhoff *et al.* 2003), the scenario proposed would imply that the parasite clades have diverged in the absence of corresponding host divergence. Parasites may therefore provide an additional means of inferring patterns of historical biogeography and phylogeography of the hosts in Arctic regions, as they probably often express a more detailed/conserved picture of the evolutionary history than that of the hosts. It should, however, be noticed that the presence of statistically supported phylogenetic lineages in population data do not necessarily imply that these lineages reflect evolutionary history. It is obvious that more material is needed to get a deeper understanding of the patterns emerging. Meanwhile, we would consider the historical scenarios suggested for the parasite as working hypotheses, not facts.

Beringia, a centre for parasite diversification?

The partial incongruence between the parasite and the host tree reflects the fact that parasites from Wrangel Island are more closely related to parasites from the Palearctic, although the hosts on this Beringian island belong to the Nearctic species *Dicrostonyx groenlandicus*. This incongruence may be the result of one or two host shifts and one extinction within Beringia. There are several studies proposing Beringia as a centre for diversification of cold-adapted organisms (Guthrie & Matthews 1971; Hopkins 1982; Rausch 1994; Sher 1999). In the *P. arctica* phylogeny, there are several factors pointing towards eastern Beringia as a region for parasite diversification, although it has not been the only glacial refugium in the Nearctic. Evidence for Beringian diversification includes the occurrence of the *P. arctica* morphospecies and the COI genealogies that separate several Beringian lineages. That the COI genealogies initially divided the *P. arctica* morphospecies into two lineages (Nearctic vs. Wrangel Island) and the Nearctic *P. arctica* into an Alaskan and a western Canadian subclade, suggests that there has also been a subdivision within eastern Beringia with the nonglaciated part of the Canadian coast to the east of Mackenzie Delta (locality 10 in Fig. 1) as a possible refugial area (cf. Fedorov & Stenseth 2002). The secondary division at Mackenzie River parallels the host (*D. groenlandicus*) subdivision into an eastern Beringian/Canadian Arctic clade, ≈ 100 000 years ago (Fedorov & Stenseth 2002). In the initial study of Fedorov & Goropashnaya (1999), the host *D. groenlandicus* was divided into two supported clades (the Alaskan and the central Canadian), but there were also two haplotypes, one from Wrangel Island and the other from east Alaska, that did not group into either of the supported clades. A secondary division within the Beringian refugium was argued for these haplotypes. The several distinct parasite subclades from Alaska and Wrangel Island support this hypothesis.

As Beringia has been proposed as a refugial area throughout several glacial cycles (Hopkins 1982; Sher 1999), one could argue that the pronounced variation in eastern Beringia is a result of a long uninterrupted demographic history. Subsequent inundations of Bering Strait opening and closing the dispersal corridor between east and west could have generated the phylogeographical patterns observed in Beringia for the parasites and the host. Beringia has evidently been a region of importance for species with a Holarctic distribution range, but other refugial areas have probably also played a prominent role in shaping phylogeographical patterns of collared lemmings and their parasites in the Arctic.

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This study is included as a part of L.M. Wickström's PhD project conducted at the Finnish Forest Research Institute under the supervision of V. Haukisalmi, J. Hantula and H. Henttonen. Our group is dealing with helminths of small rodents on a Holarctic scale with an emphasis on population genetics, morphology and phylogeography. V. Fedorov, at the present working at the University of Alaska, is involved in evolutionary history and population genetics of the hosts (lemmings). S. Varis has performed the sequencing of the material in collaboration with LMW.

V

Molecular and morphological evidence for multiple species within *Paranoplocephala omphalodes* (Cestoda, Anoplocephalidae) in *Microtus* voles (Arvicolinae)

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The present study was designed to test the hypothesis that the anoplocephalid cestode *Paranoplocephala omphalodes* (Hermann, 1783), a Holarctic parasite of *Microtus* voles, is a complex of host-specific species, rather than a single host-generalist species, using uni- and multivariate morphometrics and DNA sequence data from the mitochondrial cytochrome oxidase I gene. The phylogenetic methods applied to the mtDNA sequence data showed consistently that the cestodes morphologically recognizable as *P. omphalodes* include four well-supported monophyletic groups, representing at least three distinct, largely host-specific species. Multivariate morphometrics (discriminant analysis) successfully distinguished the four main mtDNA clades of *P. omphalodes*-like cestodes. The true *P. omphalodes* is shown to be a parasite of *Microtus arvalis*, *M. agrestis* and *Clethrionomys glareolus* in Europe. *Microtus oeconomus* harbours two host-specific, allopatric and possibly conspecific clades, one with a Holarctic and another with an (eastern) Beringian (Alaskan) distribution. The eastern Beringian endemic *M. miurus* is also parasitized with a host-specific, morphologically divergent species of *Paranoplocephala*. The cestode clades recognized in *M. oeconomus* and *M. miurus* represent 2–3 undescribed species. Molecular phylogenetic analyses supported the monophyly of the 'northern clade' of *Paranoplocephala* spp., an assemblage including *P. kalelai* from *Clethrionomys* spp., *P. macrocephala* from *Microtus* spp. and all clades of *P. omphalodes*-like cestodes except those representing the true *P. omphalodes* from Europe. The intra- and interspecific phylogeny within the northern clade is compared tentatively with the known evolutionary history of the hosts.

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Introduction

The large-bodied *Taenia omphalodes* Hermann, 1783 was one of the first cestodes described from rodents. Hermann (1783) based his description on specimens probably recovered from the common vole *Microtus arvalis* ('In unserer kleinen gewöhnlichen Feldmaus, die aber einen etwas dickern Kopf hat ...') from Germany. *Taenia omphalodes* was later transferred to *Anoplocephala* Blanchard, 1848 by von Janicki (1906) and finally to *Paranoplocephala* Lühe, 1910 (Anoplocephalidae) as the type species of this genus (Lühe 1910). The taxonomic history of *Paranoplocephala omphalodes* has been reviewed by Rausch (1976), Tenora & Murai (1980) and Tenora *et al.* (1985b).

Paranoplocephala omphalodes is currently regarded as a Holarctic species (Rausch 1994), its range spanning from western Europe to Alaska. While it has been recorded from a wide range of host species (Tenora & Murai 1980), it is characteristically a parasite of *Microtus* voles. In Central Europe, it is a typical parasite of *M. arvalis* (Pallas) (Tenora & Murai 1980; Gubányi *et al.* 1992), but in northern Europe (Haukisalmi 1986; Haukisalmi *et al.* 1994) and in northern and western Siberia (Chechulin *et al.* 1989; Shahmatova *et al.* 1989; Chechulin 1998), where *M. arvalis* is absent, it is found in the field vole *M. agrestis* (Linnaeus), narrow-headed vole *M. gregalis* (Pallas) and root/tundra vole *M. oeconomus* (Pallas). In Alaska, it has been found in *M. oeconomus* and singing vole

M. miurus Osgood (Rausch 1952, 1976; Haukisalmi *et al.* 1995), including its insular sister species *M. abbreviatus* Miller (Rausch & Rausch 1968).

The main morphological features of *P. omphalodes* have been described by Tenora & Murai (1980) and Tenora *et al.* (1985b) in Europe and Rausch (1952, 1976) in Alaska and eastern Siberia. However, there are no detailed morphometric comparisons between different host species or between different regions. It is therefore possible that *P. omphalodes* includes more than one (host-specific) species, as suggested by Tenora & Murai (1980) and Tenora *et al.* (1986), based on the supposed morphological differences between *P. omphalodes*-like taxa.

The existence of multiple species within *P. omphalodes* would explain its peculiar host preference in northern Europe. In northern Fennoscandia, where the ranges of *M. agrestis* and *M. oeconomus* overlap broadly, the former has never been found to be infected by *P. omphalodes*, although the sympatric *M. oeconomus* is (Haukisalmi 1986). However, *P. omphalodes* is a frequent parasite of *M. agrestis* in central and southern Finland (Haukisalmi *et al.* 1994), where *M. oeconomus* is absent. It is therefore plausible to assume that these host species are parasitized by two host-specific, morphologically poorly differentiated species of *P. omphalodes*-like cestodes in northern Europe. The evidence for multiple species is, however, still circumstantial, reflecting the lack of large-scale surveys covering the potential host species throughout the Holarctic range of *P. omphalodes*-like cestodes. Such material has only recently become available through the combined efforts of our research program and the Beringian Coevolution Project (see Hoberg *et al.* 2003).

The goal of the present study was to test the hypothesis that *P. omphalodes* is a complex of host-specific species, rather than a single host-generalist species, using uni- and multivariate morphometrics and DNA sequence data from the mitochondrial (mt) cytochrome oxidase I (COI) gene. The present analysis also includes two other species of *Paranoplocephala* which parasitize voles, *P. kalelai* (Tenora, Haukisalmi & Henttonen, 1985) and *P. macrocephala* (Douthitt, 1915), both morphologically closely related to *P. omphalodes* (see Haukisalmi & Henttonen 2003). The molecular data are also used for inferring phylogenetic relationships and evolutionary history of various clades of *P. omphalodes* and various species of *Paranoplocephala s.s.*, as defined by Haukisalmi & Henttonen (2003), parasitizing voles in the northern parts of the Holarctic region.

Materials and methods

Cestodes

Thirty-five specimens of *Paranoplocephala* spp. (plus three outgroup specimens) and 44 specimens of *P. omphalodes* were subjected to molecular phylogenetic and morphometric analyses (Tables 1 and 2). Cestodes were saved from several host species and localities in Europe, eastern Siberia and Alaska (Fig. 1). The European rodent and cestode material was collected by H. Henttonen, J. Niemimaa and V. Haukisalmi (from Finland, Norway and Italy) and by A. Gubányi (from Hungary). Henttonen, Niemimaa and J. Laakkonen collected the samples from Toolik Lake and Fairbanks, Alaska. All other specimens from eastern Siberia and Alaska were obtained in connection with the Beringian Coevolution Project (Hoberg *et al.* 2003), organized by the University of Alaska Museum at Fairbanks (project leaders Joseph A. Cook, Eric Hoberg and Sam Telford).

The voles were usually dissected for helminths immediately after trapping, although frozen vole material was used in some

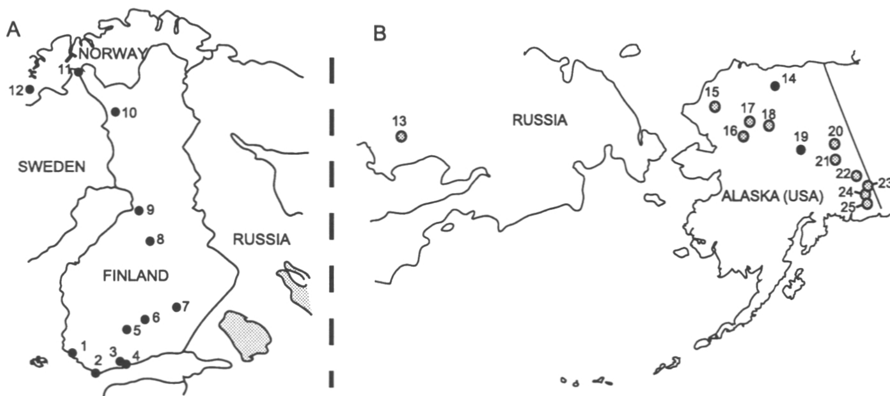

Fig. 1 A, B. Sampling localities in (A) Finland and northern Norway and (B) Alaska and eastern Russia. Shaded symbols indicate the samples collected in connection with the Beringian Coevolution Project (Hoberg *et al.* 2003). Locality numbers refer to Table 1.

Table 1 Specimens of *Paranoplocephala* spp. used in the molecular phylogenetic analysis and their haplotype designations. Locality numbers refer to Fig. 1.

Cestode species Host species	Country, state	Locality (no.)	Coordinates	Haplotypes (*n*)	GenBank acc. no.§
P. omphalodes					
M. agrestis	Finland	Turku (1)	60°30′ N, 22°19′ E	agr-1 (1)	1526
M. agrestis	Finland	Espoo (3)	60°12′ N, 24°40′ E	agr-2 (1)	1525
M. arvalis	Italy	Trento	46°04′ N, 11°08′ E	arv-1 (1)	1527
M. arvalis	Hungary	Dévaványa	47°02′ N, 20°59′ E	arv-2 (2)	1535, 1536
M. miurus	Alaska, USA	Toolik Lake (14)	68°38′ N, 149°36′ W	miu-1 (1)	1528
M. miurus	Alaska, USA	Noatak NP (15)	ca. 68°20′ N, 158°00′ W	miu-2 (2)	1541, 1545
M. miurus	Alaska, USA	GAAR*-1(17)	68°03′ N, 152°34′ W	miu-3 (1)	9952
M. oeconomus	Hungary	Tóköz	47°63′ N, 17°32′ E	oec-1 (1)	1540
M. oeconomus	Finland	Pallasjärvi (10)	68°03′ N, 24°09′ E	oec-2 (1)	1520
M. oeconomus	Finland	Kilpisjärvi (11)	69°03′ N, 20°49′ E	oec-2 (1)	1523
M. oeconomus	Russia	Magadan Obl (13)	62°50′ N, 148°15′ E	oec-3 (1)	1534
M. oeconomus	Alaska, USA	WRST†-1 (23)	62°02′ N, 141°08′ W	oec-4 (1)	1542
M. oeconomus	Alaska, USA	WRST-2 (25)	61°19′ N, 142°31′ W	oec-5 (1)	1546
M. oeconomus	Alaska, USA	Fairbanks (19)	64°51′ N, 147°43′ W	oec-6 (1)	1531
M. oeconomus	Alaska, USA	YUCH‡-1 (21)	64°29′ N, 143°28′ W	oec-7 (1)	9953
M. oeconomus	Alaska, USA	GAAR-2 (18)	67°16′ N, 150°31′ W	oec-8 (1)	1547
M. oeconomus	Alaska, USA	Toolik Lake (14)	68°38′ N, 149°36′ W	oec-9 (2), oec-10 (1)	1529, 1532, 1533
M. pennsylvanicus	Alaska, USA	Fairbanks (19)	64°51′ N, 147°43′ W	oec-6 (1)	1530
M. xanthognathus	Alaska, USA	GAAR-3 (16)	67°03′ N, 154°09′ W	oec-8 (1)	1548
Microtus sp.	Alaska, USA	WRST-3 (24)	62°02′ N, 141°08′ W	Msp-1 (1)	1543
C. glareolus	Hungary	Hanság	47°68′ N, 17°12′ E	gla-1 (1), gla-2 (1)	1537, 9954
P. macrocephala					
M. longicaudus	Alaska, USA	Mt. Fairplay (22)	63°40′ N, 142°13′ W	lon-1 (1)	1549
M. pennsylvanicus	Alaska, USA	Fairbanks (19)	64°51′ N, 147°43′ W	pen-1 (1), pen-2 (1)	1514, 1515
M. pennsylvanicus	Alaska, USA	YUCH-2 (20)	61°10′ N, 143°05′ W	pen-3 (1), pen-4 (1)	1517, 1518
P. kalelai					
C. rufocanus	Finland	Kilpisjärvi (11)	69°03′ N, 20°49′ E	ruf-1 (2)	1511, 1512
C. glareolus	Norway	Narvik (12)	68°28′ N, 17°26′ E	gla-3 (1), gla-4 (1)	1513, 9959

*Gates of the Arctic National Park and Preserve, †Wrangel-St. Elias National Park and Preserve, ‡Yukon-Charley Rivers National Preserve. §Only the last four digits shown, e.g. 1526 = AY181526.

Table 2 Specimens of *P. omphalodes* used in the morphometric analyses. Locality numbers refer to Fig. 1.

mtDNA clade	Host species	Locality (no.)	*n*	Coordinates	Voucher specimens acc. no.
I	*M. agrestis* (Finland)	Turku (1)	1	60°30′ N, 22°19′ E	
		Tvärminne (2)	1	59°50′ N, 23°15′ E	
		Helsinki (4)	1	60°15′ N, 25°03′ E	HNHM 67466, 67467
		Lammi (5)	1	61°05′ N, 22°00′ E	
		Heinola (6)	3	61°15′ N, 26°04′ E	
		Juva (7)	1	60°54′ N, 27°51′ E	
		Maaninka (8)	1	63°09′ N, 27°18′ E	
		Ylikiiminki (9)	1	65°02′ N, 26°04′ E	USNPC 92584
II	*M. oeconomus* (Finland)	Pallasjärvi (10)	5	68°03′ N, 24°09′ E	
		Kilpisjärvi (11)	5	69°03′ N, 20°49′ E	HNHM 67468
III	*M. oeconomus* (Alaska)	Toolik Lake (14)	10	68°38′ N, 149°36′ W	USNPC 92587
IV	*M. miurus* (Alaska)	Toolik Lake (14)	10	68°38′ N, 149°36′ W	USNPC 92588

cases. Cestodes found were fixed flat in 70% ethanol and small tissue samples (*c.* 10 postmature or pregravid proglottides) were later taken for DNA extraction.

Total genomic DNA was extracted from 0.5 to 2 mm^3 tissue samples as described previously (Vainio *et al.* 1998). The protocol included cell disruption (using quartz sand), four phenol chloroform (1 : 1) extractions, one chloroform isoamylalcohol (24 : 1) extraction, precipitation with polyethylene glycol (PEG) and drying. The DNA was resuspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

The cestodes used for morphometric analysis were stained with Mayer's haemalum or Semichon's acetic carmine, cleared in eugenol and mounted in Canada balsam. Representative specimens (whole mounts) of each of the main mtDNA clades of *P. omphalodes* have been deposited in the Hungarian Natural History Museum, Budapest (HNHM) and the United States National Parasite Collection, Maryland, USA (USNPC). Voucher specimens of *P. kalelai* (HWML 16698, 16699) and *P. macrocephala* (HWML 16694–16696) have earlier been deposited in the Harold W. Manter Laboratory of Parasitology, Nebraska State Museum, USA (see Haukisalmi & Henttonen 2003).

Amplification of DNA fragments

About 600 bp of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified with hot start PCR in 50 µL reaction volume essentially as described in Vainio & Hantula (1999). 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 our design amplify a region of 641 bp corresponding to positions 135–160 (COX-F) and 802–812 (COX-R) in *Hymenolepis nana* (von Siebold, 1852) (GenBank no. AB033412). The cycling conditions for COI fragments were 8 min at 95 °C (initial denaturation), then 30 s at 95 °C, (denaturation), 60 s at 53 °C (annealing), 60 s at 72 °C, (primer extension) for 36 cycles, followed by a final extension for 7 min at 72 °C . The products from the amplifications were monitored by electrophoresis in agarose gels and purified as described in Haukisalmi *et al.* (2001).

Sequencing

The partial COI fragments from all specimens were directly sequenced with a NEN Global IR2 DNA sequencer (LI-COR Inc., Nebraska, USA) and deposited in GenBank (accession numbers listed in Table 1). Sequencing was performed with Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, England) for A.L.F. or SequiTherm Excel II DNA sequencing kit (Epicentre Technologies, USA) with fluorescent-labelled COX-F and COX-R primers (MWG Biotech, Germany) for the IR2 sequencer.

Analysis of molecular data

Sequences were edited and aligned using Align IR Sequence Assembly and Alignment Software (LI-COR Inc., Nebraska, USA) and data on nucleotide substitutions and amino acid replacement determined using MacClade version 4 (Maddison & Maddison 2000).

Phylogenetic analyses were performed using maximum parsimony (MP) and maximum likelihood (ML) algorithms implemented in PAUP* 4.0b10 (Swofford 2002), neighbour-joining (NJ; Saitou & Nei 1987) algorithm in MEGA2.1 (Kumar *et al.* 2001) and Bayesian inference using MRBAYES 3.064 (Ronqvist & Huelsenbeck 2003). The parsimony analyses were carried out heuristically with 1000 random additions, TBR swapping and MulTrees option. Bootstrap analyses were conducted for 1000 rearrangements (with 10 random additions). Modeltest v.3.06 (Posada & Crandall 1998) was used to identify the most appropriate substitution model for our data. The one selected was the HKY + G with different transition/transversion ratio and a gamma-distributed shape parameter ($\alpha = 0.1422$). This model and several simpler and more complex substitution models were implemented in the ML analyses and assessed with 250 bootstrap replicates. The NJ algorithm in MEGA was implemented with Kimura-2 parameter distances, and assessed with 10^4 bootstrap replicates. For the Bayesian inference, two independent Markov Chain Monte Carlo (MCMC) runs were completed, both started with random trees for each of the four simultaneous chains and run for 10^8 generations with a burn-in corresponding to 10% of the sampled trees (trees were sampled every 100th generation). Models implemented were HKY + G and HKY + I + G.

We initially used two sequences of *Paranoplocephala blanchardi* (Moniez, 1891) (GenBank nos. AY189955–6), a European species not included within *Paranoplocephala s.s.* by Haukisalmi & Henttonen (2003), as an outgroup. However, this species tended to form a polytomy with the two main clades of the ingroup, and we therefore selected three more distant anoplocephaline cestodes to be used as outgroups: *Andrya rhopalocephala* (Riehm, 1881) from the European hare *Lepus europaeus* Pallas (AY189958), *Andrya cuniculi* (Blanchard, 1891) from the European rabbit *Oryctolagus cuniculus* (L.) (AY189957) and *Diandrya composita* Darrah, 1930 from the hoary marmot *Marmota caligata* (Eschscholtz) (AY181550). *Andrya* Railliet, 1893, *Diandrya* Darrah, 1930 and *Paranoplocephala* are morphologically closely related (Rausch 1976, 1980), and the unpublished molecular phylogenetic data of Wickström *et al.* suggest that *Andrya* and *Diandrya* may form the sister-group for *Paranoplocephala s.l.*

Morphometrics

In total, 40 fully gravid specimens, 10 from each of the main mtDNA clades of *P. omphalodes*, were subjected to uni- and multivariate morphometric analysis (Table 2). The specimens representing the European clade (I) were collected from *M. agrestis* from eight localities in south-central Finland and those representing clade II from *M. oeconomus* from two localities in north-western Finland (Lapland) (Fig. 1). The morphometric material for clades III and IV was obtained from a single locality on the North Slope of Alaska (Toolik Lake) from *M. oeconomus* and *M. miurus*, respectively (Fig. 1).

Fourteen absolute and 10 relative measurements were recorded from each individual (Tables 4, 5). Absolute measurements included the dimensions for body, scolex, suckers

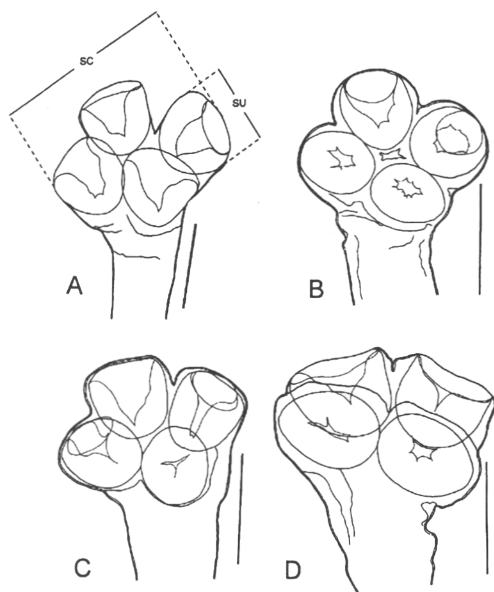

Fig. 2 A–D. Scoleces of *P. omphalodes*-like cestodes representing the four mtDNA clades. —A. Ex. *Microtus agrestis* from Turku, Finland (true *P. omphalodes*; clade I). —B. Ex. *Microtus oeconomus*, Pallasjärvi, Finland (clade II). —C. Ex. *Microtus oeconomus*, Toolik Lake, Alaska (clade III). —D. Ex. *Microtus miurus*, Toolik Lake, Alaska (clade IV). Scale bar: A–D = 0.50 mm.

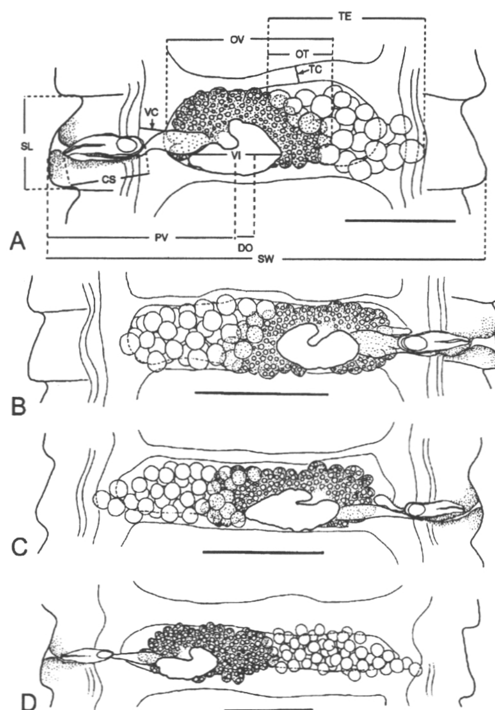

Fig. 3 A–D. Mature proglottides of *P. omphalodes*-like cestodes representing the four mtDNA clades. A–D per Fig. 2. Scale bars: A = 0.30 mm; B–D = 0.50 mm.

and eggs, and dimensions and position of various organs in mature proglottides (Figs 2A, 3A). Relative measurements were calculated as a ratio between an organ dimension and the corresponding width or length of the mature proglottid. Because our earlier analysis on a related species group of *Paranoplocephala* had shown that replicate measurements are not necessary for assessing interspecific morphometric differences (Haukisalmi & Henttonen 2003), organ dimensions were calculated from a single representative, centrally situated, mature proglottid from each individual. However, replicate measurements were recorded for the diameter of suckers (usually four measurements per individual) and length of eggs (5–10 measurements per individual). In these cases, the median value of the replicate measurements was used in the statistical analyses.

The width of the larger of the two (sinistral and dextral) ventral longitudinal osmoregulatory canals, measured at the mid-level of the proglottid, was used as a measure of the size of this organ (VC). The size of the transverse commissures of the canal (TC) was determined as the width of the larger of the two canals anterior and posterior to the mature proglottid being examined.

The number of testes was not used in the morphometric analysis, because they could not always be counted reliably. The poral distance of vitellarium (PV) was measured from the midpoint of the vitellarium to the poral margin of the proglottid, and the distance between the vitellarium and the ovary (DO) was measured as the distance between the midpoints of these glands.

Univariate comparisons of absolute and relative measurements between the main mtDNA clades of *P. omphalodes* were performed by parametric one-way analysis of variance (ANOVA). If this indicated significant differences among the groups of cestodes being examined ($P_{group} < 0.05$), Tukey's HSD was used to evaluate the various pairwise differences. The pairwise differences are expressed as homogeneous groups, i.e. groups that do not differ from each other while being significantly different from all others. Analysis of covariance (ANCOVA), using mature proglottid width or length as a covariate, was also applied to morphometric ratios to test whether the observed differences among groups remained significant when controlled for the possible allometric effect of proglottid width or length.

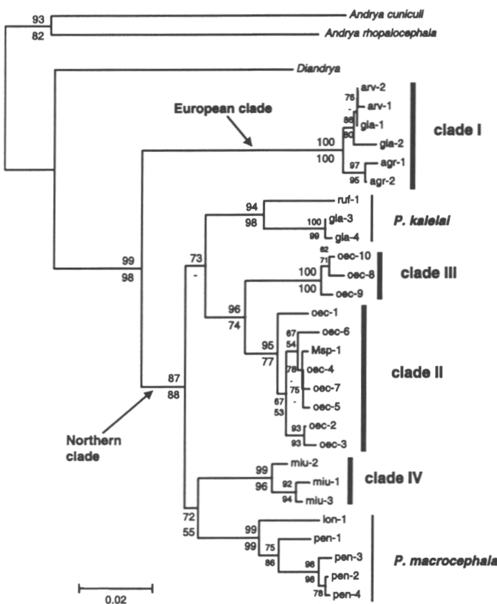

Fig. 4 Neighbour-joining (NJ) tree depicting the phylogenetic relationships among the 28 mtDNA haplotypes of *Paranoplocephala* spp. Upper values show the bootstrap support for the nodes in the NJ tree; lower values show the bootstrap support for the corresponding nodes in the maximum parsimony tree. See Table 1 for haplotype designations.

Discriminant analysis (DA) was used as a multivariate morphometric tool for assessing the general morphological (dis)similarity of the main mtDNA clades of *P. omphalodes*. The canonical discriminant functions (DFs) were calculated using the variables that best separated the study groups. These variables were selected by a forward stepwise procedure, using minimization of Wilks' lambda as a selection criterion. Jack-knifing was used to evaluate the success of the DFs at correctly classifying the specimens being studied, i.e. each specimen was classified by the DFs derived from all specimens other than that specimen. DA was performed on 40 specimens representing the four main clades, using all 24 morphometric variables simultaneously.

Four additional 'test' specimens were included in the classification phase of the DA (Table 7). Two of these represented a known clade of *P. omphalodes*, but were collected from a region and/or host species that was different from those used for calculation of the DFs. The other two, both museum specimens of an unknown clade, were the holotype of *P. microti* (Hansen 1947) (USNPC 36951) (Fig. 8) and a voucher specimen of *P. 'macrocephala'* from *M. pennsylvanicus* (Parasitic Worms Division, Natural History Museum, London, NHM 1970.1.12.40). The mature proglottid of the latter has been illustrated in Haukisalmi & Henttonen (2003: fig. 20). These *P. omphalodes*-like taxa from the North American interior were included to determine their morphometric relationships with the known mtDNA clades of *P. omphalodes*.

To improve fit to the normal distribution, arcsine $\sqrt{x}$-transformation was performed on all ratios. Statistical analyses were performed with SPSS for Windows 10.0.05 (standard version).

Results

Molecular data

We found 28 haplotypes among the 35 specimens of *Paranoplocephala* spp. sequenced for the 574 bp long partial COI. Twenty of these represented *P. omphalodes* (Table 1). In total, 122 variable sites were found (number of mutations 146), corresponding to 21% of the sequence. Twenty-two of these showed more than one type of substitution, with 126 synonymous and 17 replacement changes recorded for the whole data set (without outgroup). In addition, there were three codons in which the number of replacement and synonymous changes could not be determined. However, most nucleotide substitutions were transitions (66%) and silent (86%).

All phylogenetic methods applied to the molecular data showed that cestodes morphologically recognizable as *P. omphalodes* included four well-supported clades (Figs 4, 5), the only exception being the ML tree, where support for clade II was low. These four clades represent 3–4 distinct, largely host-specific species. The existence of four distinct clades with high bootstrap values was also revealed by the analyses using *P. blanchardi*, instead of *Andrya* and *Diandrya*, as an outgroup (not shown, see Material and methods). The two other species of *Paranoplocephala* (*P. kalelai* and *P. macrocephala*) appeared as strongly supported clades in all phylogenetic trees. The results suggest a nonmonophyletic origin for *P. omphalodes*-like cestodes.

Clade I specimens were collected from *M. arvalis*, *M. agrestis* and *Clethrionomys glareolus* (Schreber) from Europe. The haplotypes from *M. agrestis* from northern Europe (Finland) and those from *M. arvalis* and *C. glareolus* from central and southern Europe (Hungary and Italy) were consistently recognized as monophyletic sister-groups.

Clade II occurred primarily in *M. oeconomus* throughout its Holarctic range (Europe, eastern Siberia, central and southeastern Alaska). The haplotypes of *M. oeconomus* from Hungary had a basal position within this clade, while those from the northern Palearctic (Finland and eastern Siberia) appeared consistently as a monophyletic subgroup.

Clade III occurred in *M. oeconomus* and *Microtus xanthognathus* (Leach) (one case) in two localities in northern Alaska.

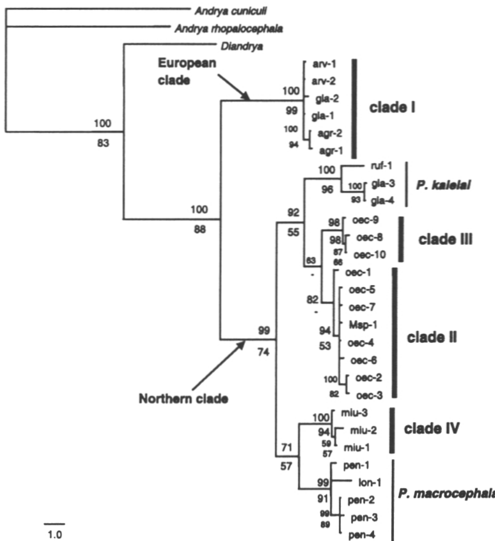

Fig. 5 Bayesian inference tree depicting the phylogenetic relationships among the 28 mtDNA haplotypes of *Paranoplocephala* spp. Upper values show the posterior probabilities for the nodes in the Bayesian tree; lower values show the bootstrap support for the corresponding nodes in the maximum likelihood tree. See Table 1 for haplotype designations.

Clade IV was specific to *M. miurus*, an eastern Beringian (Alaskan) endemic.

All four methods recognized two main clades of *Paranoplocephala* spp., the divergent European clade (I) of *P. omphalodes* and the 'northern clade', consisting of clades II–IV of *P. omphalodes* together with *P. kalelai* and *P. macrocephala*. The bootstrap support for the latter increased further when *P. blanchardi* was used as an outgroup (not shown).

Although the phylogenetic relationships within the large northern clade were not fully resolved, most of the analyses suggested that *P. macrocephala* may be the sister species to clade IV, and that *P. kalelai* and clades II and III form a monophyletic assemblage. However, the topology within the latter subclade varied according to the phylogenetic method used.

The pairwise Kimura 2-parameter distances between *P. kalelai*, *P. macrocephala* and the four clades of *P. omphalodes* varied from 0.042 (clade II vs. clade III) to 0.116 (clade I vs. *P. kalelai*), with a mean value of 0.0823 ($n = 15$) (Table 3).

Morphometrics

According to the one-way ANOVA, 15 (62.5%) of the 24 morphometric variables differed significantly between the four main clades of *P. omphalodes* (Tables 4, 5). Pairwise

Table 3 Pairwise Kimura 2-parameter distances between the four mtDNA clades of *P. omphalodes*, *P. kalelai*, *P. macrocephala* and the outgroups (*Diandrya and Andrya* spp.).

	P. o. II	*P. o.* III	*P. o.* IV	*P. k.*	*P. m.*	Outgroups
P. omphalodes						
clade I	0.106	0.115	0.099	0.116	0.110	0.170
clade II		0.042	0.066	0.063	0.071	0.156
clade III			0.074	0.068	0.086	0.161
clade IV				0.070	0.063	0.144
P. kalelai					0.085	0.151
P. macrocephala						0.161

comparisons showed that in seven instances clade IV was significantly different from the others. The corresponding figures for clades I, II and III were 1, 0 and 0, respectively. Thus, the univariate tests suggest that clade IV is morphologically unique and that the others are relatively poorly differentiated from each other, albeit with 2–5 pairwise differences between them.

Controlling for the effect of the mature proglottid width or length on morphometric ratios by means of ANCOVA changed the outcome of the test for three variables, compared with the corresponding ANOVA (Table 5). Length/width ratio of mature proglottides (PL/PW) and the relative width of the longitudinal osmoregulatory canals (VC/PW) differed significantly between the clades only when PW was used as a covariate. Corrected means based on ANCOVA (not shown) and the corresponding scatter plots (Fig. 6) showed that clade IV has a significantly higher PL/PW ratio and relatively wider canals than the other three, which further emphasizes its uniqueness. ANCOVA and scatter plots also revealed that increasing proglottid width is associated with decreasing PL/PW and VC/PW, reflecting a negative allometric growth pattern. On the other hand, comparison of the ANOVA and ANCOVA showed that a significant difference in the transverse position of the vitellarium (PDV/PW) was largely due to the varying proglottid width of the different clades (Table 5).

The DA produced three DFs that explained *c.* 77, 19 and 4% of the variance, respectively (Table 6). The stepwise procedure selected six variables for the calculation of these (SU, EG, TC, OT/PW, TE/PW, CS/PW). DF1 showed the highest correlations with the absolute and relative width of the transverse canal (TC, TC/PW), the relative width of the ovary (OW/PW) and the relative overlap of testes and ovary (OT/PW). DF2 primarily reflected the variation in size of suckers and eggs, while DF3 was highly correlated with TE/PW. The relationship between DFs 1 and 2, together accounting for 96% of the variance, is shown in Fig. 7. DF1 clearly distinguished clade IV, and partially separated clade II from I and III. DF2 most clearly differentiated clades I, II and III from each other, with IV largely overlapping with the others.

Table 4 Variation of the absolute measurements of the four mtDNA clades of *P. omphalodes*. The statistical significance of differences among the clades (P_{group}) has been assessed by one-way ANOVA. Significance of pairwise differences, expressed as homogeneous groups, was determined by Tukey's HSD. All measurements are in micrometers, except BL, BW and SC (mm). See Materials and methods for explanation of measurements (also Figs 2A, 3A); n = 10 for all samples.

	M. agrestis Finland clade I (A)		*M. oeconomus* Finland clade II (B)		*M. oeconomus* Alaska clade III (C)		*M. miurus* Alaska clade IV (D)		ANOVA	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	P_{group}	Homogeneous Groups
Body, length (BL)	141.1	112–196	183.1	142–235	167.0	141–195	220.0	190–242	< 0.001	A + C, B + C, D
Body, maximum width (BW)	2.80	2.2–4.0	3.23	2.0–4.5	3.01	2.0–4.0	4.54	3.2–5.0	< 0.001	A + B + C, D
Scolex, diameter (SC)	0.976	0.84–1.21	0.775	0.60–0.91	0.853	0.69–1.03	1.030	0.73–1.22	< 0.001	A + C, B + C, A + D
Suckers, diameter (SU)	432	350–500	338	280–378	351	295–390	443	345–490	< 0.001	A + D, B + C
Egg, length (EG)	35.4	32.0–40.0	41.2	38.5–44.0	39.5	36.5–41.5	42.2	38.5–48.5	< 0.001	A, B + C, B + D
Ventral canals, width (VC)	8.9	5.0–15.0	10.7	6.4–14.0	8.0	4.6–11.0	13.0	5.9–18.2	0.004	A + B + C, B + D
Transverse canals, width (TC)	5.4	2.5–8.1	4.6	2.3–6.4	4.7	2.0–7.0	15.1	7.1–22.0	< 0.001	A + B + C, D
Testes, distribution (TE)	54.0	41.1–89.0	49.4	35.0–75.0	54.3	42.7–69.0	64.2	41.7–89.5	0.073	—
Overlap of ovary and testes (OT)	19.2	11.5–36.0	15.7	8.4–26.5	21.6	14.5–30.5	6.1	0.3–10.3	< 0.001	A + B + C, D
Poral distance of vitellarium (PV)	60.6	49.0–80.0	59.6	48.5–69.0	57.4	45.0–78.0	66.1	56.7–83.0	0.215	—
Distance of ovary and vitellarium (DO)	5.22	1.5–13.0	5.41	0.0–11.3	5.02	0.0–12.0	8.01	5.3–12.0	0.175	—
Vitellarium, width (VI)	26.8	20.0–36.0	28.0	20.0–34.8	28.7	23.0–39.0	25.3	19.5–35.0	0.418	—
Ovary, width (OV)	56.5	5.0–94.0	60.8	39.0–81.8	57.8	50.0–71.6	58.2	48.5–79.0	0.895	—
Cirrus sac, length (CS)	23.2	19.5–27.5	21.7	17.0–26.5	23.2	20.0–25.7	23.8	20.0–30.0	0.377	—

Table 5 Variation of the relative measurements (ratios) of the four mtDNA clades of *P. omphalodes*. The ratios are based on the relationships between a linear measurement and the width or length of the corresponding mature proglottid. In addition to one-way ANOVA, analysis of covariance (ANCOVA) was performed on ratios, with parasite clade as a categorical variable and proglottid width (PW) or length (PL) as a covariate. P_{cov} shows the significance of the covariate. For abbreviations, see Table 4; n = 10 for all samples.

	M. agrestis Finland clade I (A)		*M. oeconomus* Finland clade II (B)		*M. oeconomus* Alaska clade III (C)		*M. miurus* Alaska clade IV (D)		ANOVA		ANCOVA	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	P_{group}	Homogeneous groups	P_{group}	P_{cov}
PL/PW	0.148	0.09–0.23	0.139	0.11–0.18	0.168	0.13–0.22	0.170	0.11–0.22	0.136	—	0.001	< 0.001
VC/PW	0.060	0.04–0.11	0.069	0.04–0.09	0.056	0.04–0.08	0.071	0.03–0.12	0.359	—	0.046	0.014
TC/PL	0.248	0.16–0.36	0.218	0.10–0.32	0.198	0.12–0.27	0.487	0.30–0.71	< 0.001	A + B + C, D	< 0.001	0.575
TE/PW	0.354	0.31–0.39	0.309	0.24–0.38	0.379	0.35–0.44	0.342	0.27–0.40	0.001	A + C + D, B + D	< 0.001	0.098
OT/PW	0.124	0.09–0.19	0.098	0.05–0.15	0.152	0.10–0.23	0.033	0.00–0.06	< 0.001	A + B, A + C, D	< 0.001	0.472
PV/PW	0.401	0.35–0.43	0.377	0.35–0.43	0.402	0.35–0.45	0.355	0.34–0.39	0.001	A + B + C, B + D	0.064	0.026
DO/PW	0.033	0.01–0.06	0.034	0.00–0.08	0.034	0.00–0.08	0.043	0.03–0.06	0.508	—	0.803	0.403
VI/PW	0.179	0.15–0.22	0.177	0.15–0.21	0.200	0.17–0.24	0.136	0.11–0.16	< 0.001	A + B + C, D	< 0.001	0.145
OV/PW	0.367	0.28–0.41	0.381	0.29–0.45	0.405	0.35–0.45	0.314	0.25–0.42	0.001	A + B + C, A + D	< 0.001	0.412
CS/PW	0.156	0.11–0.19	0.138	0.11–0.17	0.164	0.14–0.19	0.128	0.11–0.15	< 0.001	A + C, A + B, B + D	0.014	< 0.001

Overall, 85% of the specimens included in the classification phase of the DA were assigned to the correct clade, with a success rate varying between 80% (clades I and III) and 90% (clades II and IV). The high success rates support the clades' morphometric distinctiveness. Two of the 'test' specimens (nos. 1 and 2) were assigned correctly (to clades I and II, respectively). The data points of DFs 1 and 2 for these specimens fell either within or close to the data point range of the correct clade (Fig. 7). Nos. 3 and 4 were both placed close to the data points of clade I, and thus assigned to it. Thus, the multivariate morphometrics suggest that the European clade of *P. omphalodes* may be present in North America although it did not occur in the present molecular data from Alaska.

Discussion

'Hidden' parasite diversity

The increasing use of molecular markers as a taxonomic tool (McManus & Bowles 1996; Blouin 2002) has revealed a considerable degree of 'hidden' diversity in various groups of parasites (e.g. Nadler 1990; Anderson *et al.* 1998). Cryptic speciation, i.e. speciation without obvious morphological divergence, is expected to be common in endoparasites, because host divergence is unlikely to exert strong selection

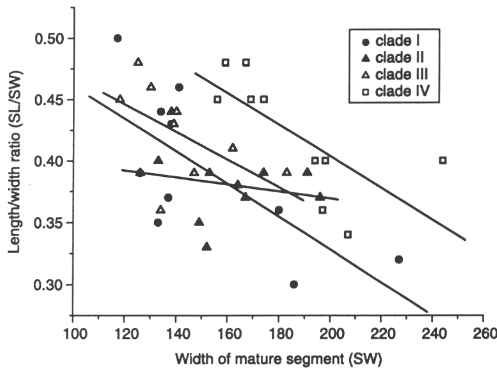

Fig. 6 Relationship between width and length/width ratio of mature proglottides for the four mtDNA clades of *P. omphalodes*-like cestodes. The significant difference between clade IV and the other clades holds even if the clade (II) with a deviating slope is ignored (cf. Table 5).

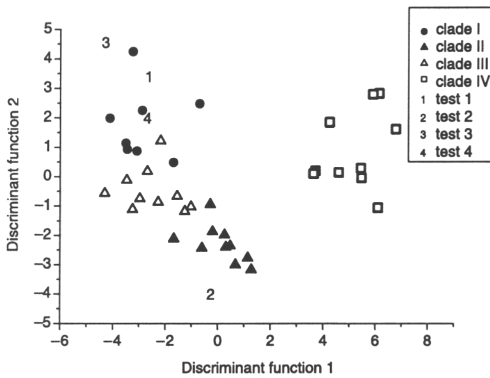

Fig. 7 Relationship between discriminant functions 1 and 2 for the four mtDNA clades of *P. omphalodes*-like cestodes. For the identity of the 'test' specimens, see Table 7.

pressures for morphological adaptation (Rausch 1982; Väinölä *et al.* 1994). Cryptic speciation has been suggested as characterizing the parasites of Arctic vertebrates (Hoberg *et al.* 1999), because long-term climatic oscillations have caused repeated fragmentation and isolation, and subsequent divergence of host populations, including those of arvicoline rodents (i.e. voles and lemmings; Fedorov *et al.* 1999; Fedorov & Goropashnaya 1999; Brunhoff *et al.* 2003).

The cestodes representing the family Anoplocephalidae, characterized by the absence of hooks and general paucity of reliable diagnostic features (Beveridge 1994), seem to include a number of diverse species flocks that have been hidden by their apparent morphological similarity. In a study based on

Table 6 Summary of the discriminant analyses. The upper panel shows the standardized canonical discriminant function (DF) coefficients for the variables that were selected by a stepwise procedure, and the correlation coefficients for the relationships between a variable and a DF. Variables are ordered by absolute size of correlation with a DF, and the highest correlation coefficients for each DF have been indicated in bold. The lower panel shows the Eigenvalues and the percentage of the total variation explained by DFs. For abbreviations, see Tables 4 and 5; n = 10 for all samples.

	Function 1		Function 2		Function 3	
Variable	coeff.	*r*	coeff.	*r*	coeff.	*r*
TC/PL	—	**0.51**	—	0.36	—	0.17
TC*	0.84	**0.46**	0.09	0.39	0.25	0.32
OV/PW	—	**−0.45**	—	−0.32	—	0.09
OT/PW*	−0.53	**−0.45**	−0.38	−0.14	−0.09	0.29
BL	—	0.30	—	0.03	—	0.03
VI/PW	—	−0.29	—	−0.02	—	0.07
VC	—	0.28	—	0.02	—	−0.08
PV/PW	—	−0.27	—	0.07	—	−0.16
OT	—	−0.27	—	−0.14	—	0.20
BW	—	0.25	—	−0.14	—	0.07
VC/PW	—	0.17	—	−0.04	—	−0.04
SU*	0.11	0.15	0.72	**0.65**	−0.37	−0.27
EG*	0.82	0.28	−0.66	**0.65**	−0.37	−0.27
SC	—	−0.04	—	0.33	—	−0.15
DO/PW	—	0.10	—	−0.33	—	0.09
DO	—	0.18	—	−0.31	—	−0.01
OV	—	−0.08	—	−0.15	—	−0.03
TE/PW*	−0.35	−0.09	0.56	0.23	0.80	**0.75**
CS/PW*	−0.44	−0.23	0.35	0.09	0.42	0.37
PL/PW	—	−0.13	—	−0.09	—	0.33
TE	—	0.13	—	0.16	—	0.28
PV	—	0.09	—	0.10	—	−0.22
CS	—	0.04	—	0.19	—	0.21
VI	—	0.02	—	0.04	—	−0.08
Eigenvalue	11.49		2.77		0.86	
% of variance	77.4		18.7		3.9	

*Variable used in the calculation of the DFs.

isozyme electrophoresis, Baverstock *et al.* (1985) showed that the anoplocephaline cestode *Progamotaenia festiva* (Rudolphi, 1819), thought to be a host-generalist parasite of wombats, wallabies and kangaroos, includes at least 12 biological species, most of which are strictly host-specific. Another isozyme study by Ba *et al.* (1993) showed that *Moniezia expansa* (Rudolphi, 1805) and *Moniezia benedini* (Moniez, 1879) (Anoplocephalinae) from African domesticated ruminants actually represent five distinct, largely host-specific entities. Rausch (1976) indicated several potential cases of cryptic speciation in anoplocephalid cestodes (*Paranoplocephala* spp. and *Anoplocephaloides* spp.) of arvicoline rodents.

Multiple species within P. omphalodes

The present analysis provides substantial molecular and morphological evidence for the existence of multiple host-specific

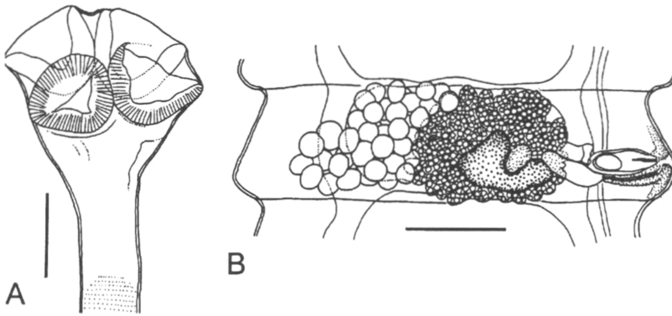

Fig. 8 A, B. Scolex (A) and mature proglottid (B) of *Paranoplocephala microti* (holotype) from *Microtus ochrogaster*. Scale bars: A = 0.50 mm; B = 0.30 mm.

species within *P. omphalodes*, a traditionally recognized host-generalist species of arvicoline rodents. The patterns of host-specificity within the *P. omphalodes* complex contrasts those within the *Arostrilepis horrida* complex (Hymenolepididae) in Holarctic arvicoline rodents, since the species within the latter assemblage appear to be largely partitioned between host genera rather than host species (Hoberg *et al.* 2003).

Because *P. omphalodes* was originally described from the common vole *M. arvalis* from Germany (Hermann 1783), the European clade (I), which includes the central and southern European haplotypes from *M. arvalis*, most likely represents the true *P. omphalodes*. The haplotypes from the bank vole *C. glareolus* were nearly identical to those from the sympatric common voles, indicating an accidental shift from the primary host (common vole) to the secondary one (bank vole) (cf. Gubányi *et al.* 2002). It is obvious that the specimens from the field vole (*M. agrestis*) from northern Europe (Finland), which consistently formed a well-supported subgroup within this clade, should also be assigned to *P. omphalodes*.

Despite its genetic divergence, clade I was morphometrically related to clade III (*M. oeconomus* from Alaska), particularly with respect to the first discriminant function which explained most (77%) of the variance (Fig. 7). However, three morphometric variables (SU, EG, OT/PW) differed significantly between the clades in ANOVA (Tables 4, 5), supporting the morphological distinctiveness of clade I. Small eggs seem to be the most important diagnostic feature of the true *P. omphalodes*, since it differed significantly from all other clades in this respect (Table 4). Clade I also resembled clade IV in having large scolex and suckers, but other evidence confirms their morphometric divergence.

Molecular and morphometric results showed unambiguously that clades II–IV represent 2–3 undescribed species of *Paranoplocephala*. Clade IV from *M. miurus* is easily separated from all other clades, for example, by its larger body (BL, BW), wider transverse commissures of the osmoregulatory canal (TC, TC/PL) and smaller transverse overlap between the testes and ovary (OT, OT/PW) (Fig. 3). Of these variables, the second and third have seldom been quantified for *Paranoplocephala* spp., which may partly explain why the morphologically distinct species from *M. miurus* has remained undetected (e.g. Rausch 1976; Haukisalmi *et al.* 1995).

Clades II and III both appeared to be specific to *M. oeconomus*, although they were occasionally found in other sympatric *Microtus* species. These two clades were morphometrically related to each other (and also to clade I, see above), but three relative measurements (TE/PW, OT/PW, CS/PW) were found to differ significantly between them (Table 5). The significant ANOVA and ANCOVA results support the observation that the distribution of testes in relation to other organs (Fig. 3) is the main qualitative difference between these clades. The testicular field of clade II (Finland) is narrower and therefore overlaps the ovary and antiporal longitudinal osmoregulatory canal to a lesser extent than in clade III (Alaska). In a preliminary taxonomic analysis of *P. omphalodes*-like cestodes, Tenora *et al.* (1986) identified three supposed species in *M. oeconomus*, differing mainly by the distribution of testes in relation to the canal. Although the morphotype in which the testes do not overlap the canal is characteristic of clade II (Fig. 3A), it does occur occasionally in all clades of *P. omphalodes*-like cestodes considered here. Moreover, the degree of overlap shows pronounced variation within local cestode populations and also within individual strobilae, and therefore this feature alone cannot be used to delineate species within *Paranoplocephala s.s.*

Although the relatively low genetic distance between clades II and III supports their conspecificity, these taxa are morphologically and phylogenetically differentiated entities that could equally be regarded as independent species. The conspecificity cannot be determined definitely, because the phylogenetic relationships between these clades and *P. kalelai* remain unresolved.

Evolutionary history

The species-rich genus *Microtus* Schrank, which probably has its origins in Eurasia (Conroy & Cook 2000), colonized

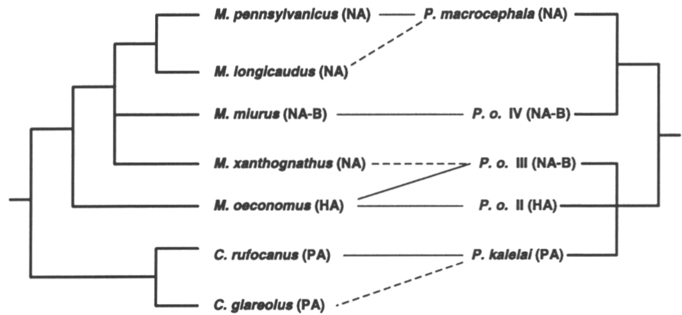

Fig. 9 Phylogenetic relationships of the northern mtDNA clade of *Paranoplocephala* spp. (right) and their hosts (left). The latter are based on Conroy & Cook (2000). Solid lines show the characteristic host–parasite associations; dashed lines show the associations that are assumed to be accidental or atypical. Geographic distributions: PA, Palearctic; NA, Nearctic; NA-B, Nearctic (Beringian); HA, Holarctic.

North America at least 2.1 Mya (Repenning *et al.* 1990). The major Nearctic radiation within this genus took place much later: 0.5 Mya according to the fossil records (Hoffman *et al.* 1985) and 1.3 Mya according to a molecular clock estimate (Conroy & Cook 2000). The North American representatives of *Microtus*, excluding *M. oeconomus*, are possibly monophyletic (Graf 1982; Conroy & Cook 2000).

The classification procedure of the DA suggested that *Microtus* voles in the North American interior harbour cestodes that are morphometrically more closely related to the true European *P. omphalodes* than to any of the *P. omphalodes*-like cestodes from Alaska (Fig. 7). The holotype of *P. microti*, in particular, is very similar to the true *P. omphalodes* and should therefore be treated as a synonym of the latter (cf. Figs 3A, 8B). The type host of *P. microti* is *M. ochrogaster* (Wagner) (Hansen 1947), a species that probably stems from one of the earliest invasions of the *Microtus* lineage into North America (Conroy & Cook 2000). Interestingly, some of the phylogenetic analyses suggest that the western Eurasian *M. agrestis* and *M. arvalis*, the primary hosts for the true *P. omphalodes*, may be the closest relatives of the North American *Microtus* clade (Conroy & Cook 2000). It is therefore plausible to assume that the first *Microtus* species colonizing North America harboured the true *P. omphalodes* or its precursor, which still exists in the most 'primitive' Nearctic representatives of the genus. This hypothesis could be tested by a molecular phylogenetic analysis including (host-specific) *P. omphalodes*-like cestodes from *M. ochrogaster* and other suspected early colonizers (see Hoffman & Koeppl 1985; Conroy & Cook 2000).

The present analysis was not specifically designed to test the monophyly of *Paranoplocephala s.s.* (as defined in Haukisalmi & Henttonen 2003). However, the sequence data do support the monophyly of the northern clade, an assemblage including all other haplotypes except those representing the true *P. omphalodes* from Europe. The following discussion outlines tentatively the host–parasite associations and patterns of phyletic coevolution within the northern clade (Fig. 9).

Table 7 Absolute and relative measurements for the 'test' specimens that were included in the classification phase of the discriminant analysis. For abbreviations, see Tables 4 and 5; $n = 1$ for all samples

	Specimen 1	Specimen 2	Specimen 3	Specimen 4
	P. omphalodes *M. arvalis* Trento, Italy USNPC 92585	*P. omphalodes* *M. oeconomus* Fairbanks, Alaska USNPC 92586	*P. microti* *M. ochrogaster* Nebraska, USA USNPC 36951	*P. 'macrocephala'* *M. pennsylvanicus* Michigan, USA NHM 1970.1.12.40
BL	164	154	157	171
BW	3.40	3.58	2.30	3.33
SC	1.04	0.84	1.16	0.95
SU*	43	31	52	46.5
EG*	35	45	33	35
VC	7.0	9.0	8.2	10.0
TC*	5.0	4.5	2.8	5.5
TE	65.5	75.0	49.5	62.5
OT	11.5	39.5	15.0	27.2
PV	52.5	75.0	49.0	61.0
DO	4.0	10.2	5.5	6.0
VI	29.0	47.0	28.0	29.5
OV	57.5	90.0	47.0	58.0
CS	21.5	23.6	20.0	17.0
PL/PW	0.16	0.18	0.26	0.16
VC/PW	0.046	0.046	0.063	0.065
TC/PW	0.20	0.13	0.08	0.23
TE/PW*	0.43	0.38	0.38	0.41
OT/PW*	0.075	0.203	0.115	0.177
PV/PW	0.34	0.38	0.38	0.40
DO/PW	0.026	0.052	0.042	0.039
VI/PW	0.19	0.24	0.22	0.19
OV/PW	0.38	0.46	0.36	0.38
CS/PW*	0.141	0.121	0.154	0.110

*Variable used in the calculation of the discriminant functions.

Because of the varying resolution of the parasite and host phylogenies (Conroy & Cook 2000), and because of the still incomplete sampling of voles and their cestodes, a formal cospeciation analysis is not presented.

The northern clade consists of two main branches that are associated with hosts originating either from Eurasia or

North America (Fig. 9). Assuming that *P. kalelai* has diverged as a consequence of a host shift (see below), the divergence within the northern clade seems to parallel the evolutionary history of *Microtus*, i.e. the North American subclade of *Paranoplocephala* spp. has probably diverged since representatives of *Microtus* crossed the Bering strait (cf. Rausch 1994; Conroy & Cook 2000). The reconstruction of the evolutionary history of the North American subclade is complicated by the fact that *P. macrocephala*, as presently understood, probably represents a cryptic species flock parasitizing *Geomys* spp. (Geomyidae; type host) and several Nearctic *Microtus* species (Rausch 1976; Haukisalmi & Henttonen 2003; see also Timm 1985).

The recent mtDNA (cyt *b*) sequence analysis of Brunhoff *et al.* (2003) shows that the main phylogeographical splits of *M. oeconomus*, the only Holarctic *Microtus* species, are located in northern Europe (Scandinavia), the Ural mountains and eastern Siberia. The lack of differentiation between the tundra vole populations in eastern Siberia and Alaska was suggested to be due to periodic gene flow across the Bering Strait. The colonization of north-western North America by *M. oeconomus* has obviously been accompanied by a colonization of a host-specific *Paranoplocephala*-species, as suggested by Rausch (1994). If we assume that clades II and III are monophyletic, the cestodes parasitizing *M. oeconomus* may represent a case of intrahost speciation, i.e. parasite divergence in the absence of corresponding host divergence (see Hoberg *et al.* 1997; Paterson & Gray 1997). The divergence of these clades may thus be due to a 'cryptic' isolation event of the hosts, which has been sufficient to drive the divergence of the parasites but not that of the hosts. It can also be expected that the episodes of gene flow between the Beringian parasite populations have been less frequent than those between the corresponding host populations, which would have reinforced parasite isolation and subsequent divergence relative to those of the host. Hoberg (1995) has provided evidence for speciation of cestodes (*Anophryocephalus* spp.) of pinnipeds in the Arctic basin, including Beringia, in the absence of concomitant host speciation. Thus, intrahost speciation may have been a characteristic feature in the evolution of endoparasites in Beringia due to the complex geological and biogeographical history of this region.

Within *Paranoplocephala s.s.*, the western Eurasian *P. kalelai* is the only species that is specific to the voles of the genus *Clethrionomys* Tilesius (Tenora *et al.* 1985a; Haukisalmi & Henttonen 2003). This could mean that the clear incongruence between the host and parasite phylogenies (Fig. 9) is due to a shift from *M. oeconomus* or its precursor to the Palearctic *Clethrionomys rufocanus* (Sundevall), the primary host for *P. kalelai* (*C. glareolus* is known to host *P. kalelai* only in northern Fennoscandia, where it lives in sympatry with *C. rufocanus*; Tenora *et al.* 1985a; Haukisalmi *et al.* 1987). The hypothesis for the origin of *P. kalelai* is supported by the crown position of this species within the clade typically parasitizing *Microtus* spp. *Microtus oeconomus* and *C. rufocanus* currently have largely overlapping ranges in northern Eurasia, a situation that has probably prevailed throughout their evolutionary history, providing necessary opportunities for a host shift. The phylogeny for the northern clade also shows unequivocally that *P. kalelai* and *P. macrocephala*, species that share a characteristic distribution of testes (Haukisalmi & Henttonen 2003), are non-monophyletic, and that this feature has been acquired independently by these species.

The present analysis supports the role of Beringia as a centre of diversification of northern biota (e.g. Guthrie & Matthews 1971; Weider & Hobaek 2000), including parasites (Rausch 1994; Hoberg *et al.* 2003). Four of the six cestode clades recognized by us occur in eastern Beringia; two (clades II and IV) are probably endemic to this region, one Holarctic clade (II) includes an (eastern) Beringian subclade and most of the (sub)clades, including *P. macrocephala*, show supported substructure within Alaska (Figs 4, 5). Moreover, we have earlier shown that the anoplocephalid cestodes of collared lemmings (*Dicrostonyx* Gloger) also include one species of strictly (eastern) Beringian distribution (Haukisalmi *et al.* 2001), and new species of cestodes in voles from Alaska are being detected and described within the framework of the Beringian Coevolution Project (Haukisalmi *et al.* 2002; Hoberg *et al.* 2003).

Hidden diversity revisited

The fact that a relatively simple morphometric analysis successfully distinguished the four main mtDNA clades of *P. omphalodes*-like cestodes indicates that the true species diversity within this assemblage has remained unrecognized because of the lack of multivariate morphometrics and because taxonomic analyses have traditionally been based on a few key characters, such as the distribution of testes. Thus, in the strict sense, the labels of 'cryptic speciation' and 'hidden diversity' do not fit the morphologically divergent species assemblage identified by us. The characters that have been neglected in the taxonomy of *Paranoplocephala* spp., including, for example, the various relative measurements, may prove to be useful in the separation of other 'cryptic' species complexes of anoplocephaline cestodes (cf. Haukisalmi & Henttonen 2003). We predict that two other Holarctic taxa of anoplocephaline cestodes of arvicoline rodents, i.e. *Anoplocephaloides dentata* (Galli-Valerio, 1905)/*A. troeschi* (Rausch, 1946) complex and *Anoplocephaloides variabilis* (Douthitt, 1915) *s.l.* (see Rausch 1976), will be shown to include multiple (host-specific) species when subjected to multivariate morphometric and/or molecular phylogenetic analyses.

Finally, the present analysis clearly demonstrates the importance of broad-scale, synoptic field collections for

understanding the true diversity and evolutionary history of northern host–parasite assemblages (cf. Hoberg *et al.* 2003).

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VI

The phylogeography of vole cestodes over a host contact zone in Fennoscandia

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Abstract

Due to several factors in parasite biology, like facultative life cycles through intermediate hosts, small population size in comparison to hosts, and inbreeding, the extent of phylogeographical congruence of intestinal cestodes and their host may vary. We examined variation in approximately 600 bp of the mitochondrial (mt) cytochrome oxidase I gene in three intestinal parasites (Cestoda, Anoplocephalidae) with differing abundance and host specificity. The phylogeographical patterns of the three parasites were compared with the phylogeographies of their rodent hosts in Fennoscandia. Eastern and Western mtDNA lineages of two hosts, the field vole (*Microtus agrestis)* and the bank vole (*Clethrionomys glareolus*), meet in a contact zone in north-central Sweden. The separate mtDNA lineages found in both species reflect late glacial recolonization from separate refugia. Our results show that the three parasite species are also divided into Eastern and Western main mtDNA lineages. In fact, the mtDNA phylogeographical patterns of the cestode species *Paranoplocephala blanchardi* and *Anoplocephaloides* cf. *dentata* were similar to their host's and coincided to various degrees with the host contact zones. The common host generalist *P. gracilis*, however, exhibited a phylogeographical pattern that did not match that of its main hosts. A plausible interpretation of our data is that host contact zones more easily delimit rare host-specific parasites than common generalists.

Keywords: Anoplocephalid cestodes, voles, mtDNA, phylogeography, coevolution

Introduction

Fennoscandia (Scandinavia, Finland and the northwestern part of Russia) was entirely covered by ice during the last glacial maximum 21 000-17 000 ^{14}C years ago (BP) (Andersen & Borns 1997). The region was recolonized in late glacial and post-glacial times from the east and/or south. Mammals are reviewed in Jaarola *et al.* (1999); snakes Carlsson & Tegelström (2002); fish Koskinen *et al.* (2000), Kontula & Väinöla (2001); plants Malm & Prentice (2002), Nyberg Berglund & Westerbergh (2001) and a virus Hörling *et al.* (1996).

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Several of the species that had bi-directional immigration routes formed intra-specific contact zones in north-central Sweden, thereby creating one of the four main suture zones described in Europe (Jaarola & Tegelström 1995; Taberlet *et al.* 1998). The phylogeography and postglacial colonization history of the field vole (*Microtus agrestis*) and the bank vole (*Clethrionomys glareolus*), the main hosts of the cestode parasites studied here, have been extensively studied in Fennoscandia (Jaarola & Searle 2002; Jaarola & Tegelström 1995; Jaarola & Tegelström 1996; Jaarola *et al.* 1999; Tegelström & Jaarola 1998). For both species, an early bi-directional colonization has been inferred (Jaarola & Tegelström 1995; Jaarola & Tegelström 1996; Tegelström 1987). Thus, Fennoscandia offers opportunities for bi- or unidirectional colonization for rodent parasites depending on the degree of

cospeciation or coadaptation with their hosts.

The patterns of dispersal and recolonization of parasites with complex life cycles, like the anoplocephalid cestodes in our study, may differ in many respects from those of their hosts. Even though the main hosts might act as drivers for patterns of distribution and isolation, dispersal of the parasites is constrained by the availability of facultative intermediate hosts, i.e. small non-volant soil arthropods such as oribatid mites and collembolans (Gleason & Buckner 1979). Also, host specificity and abundance of parasites could have pronounced effects on their population structure and potential for dispersal. Furthermore, inference of population history from mitochondrial genes in cestodes introduces another possible discrepancy in comparison to the host, as the effective population size of mtDNA in hermaphroditic cestodes equals that of individuals.

To compare the phylogeography of hosts and their parasites in Fennoscandia we studied three intestinal cestodes (Anoplocephalidae) of voles. Based on morphology (Beveridge 1994; Rausch 1976; Spasskij 1951), the three species represent separate lineages within the subfamily Anoplocephalinae. However, relationships among lineages are ambiguous (Beveridge 1994). The commonness and host specificity of these parasites vary from a common host generalist to a rare host specialist. The aim of this study was to assess whether phylogeographical patterns found in the hosts can be traced in their parasites as well. If phylogeographical structure is recognised for the parasite, is it topologically and spatially congruent with that of the host? Furthermore, does the host contact zone act as a dispersal barrier for parasites, and is there any correlation with the degree of host specificity and commonness of the parasite?

Material and methods

Species studied and working hypotheses

1) *Paranoplocephala gracilis* is a common host generalist of *Microtus* voles (*M. agrestis, M. arvalis, M. oeconomus*) and *Clethrionomys glareolus* (Haukisalmi *et al.* 1994; Henttonen *et al.* 1998). It is also found, although less numerous, in other voles such as *Arvicola terrestris, C. rutilus* and *C. rufocanus* (Haukisalmi & Henttonen 1993b; Haukisalmi *et al.* 1987; Tenora & Murai 1980; Tenora *et al.* 1985). As *P. gracilis* parasitizes a wide variety of arvicoline species in Fennoscandia, we would expect this cestode to have frequent opportunities for dispersal and gene flow and therefore possibly express nonspecific phylogeographical structure in relation to its main hosts (*M. agrestis* and *C. glareolus*) in Fennoscandia.

2) *Anoplocephaloides* cf. *dentata* belonging to the *A. dentata* species complex is a less common parasite than *P. gracilis* in Fennoscandia. *A. dentata sensu lato* is a cestode species complex with a Holarctic distribution parasitizing *Microtus* and *Chionomys* and only rarely have we found it in *Clethrionomys* voles in Fennoscandia (Haukisalmi & Henttonen 1993a; Haukisalmi *et al.* 1987; Henttonen *et al.* 1998). Initial genetic population studies (mtDNA and ribosomal DNA sequences, Wickström *et al.* in prep.) reveal subdivision into European and Nearctic/Siberian species. The European species was divided into a well-defined northern European and a southern European (Alpine) clade that also could represent separate species. The nominal *A. dentata*, described from Italy (host species *Chionomys nivalis*) (Galli-Valerio 1905) equals the Alpine clade. Samples belonging to the northern European clade, here named *A.* cf. *dentata*, collected from Fennoscandia, Denmark and Scotland, were all from *M. agrestis* and *M. oeconomus*. This leaves us with a *Microtus*-specific parasite that uses a more restricted host range and is less common than *P. gracilis* in Fennoscandia. Therefore, we expect it to have a geographically more distinct genetic structure than *P. gracilis*, but still one that is less pronounced than that of *Paranoplocephala blanchardi* (see below).

3) *Paranoplocephala blanchardi* (sensu Tenora *et al.* 1985) is a rare host specialist of *M. agrestis*, found in low numbers throughout Fennoscandia (Haukisalmi *et al.* 1994). In fact, we have never found it in other vole species in Fennoscandia during our 25 years of rodent parasite research. As rare parasite species are more likely to express genetic differentiation as a consequence of their restricted population sizes and patchy geographical distributions, we would expect this parasite to show a phylogeographical pattern that is congruent with that of its host.

DNA-analyses

This study includes a total of 47 *P. gracilis*, 33 *A.*cf. *dentata* and 24 *P. blanchardi* samples collected from Fennoscandia and a few additional European localities (Table 1, Fig. 1) Approximately 600 bp of the mitochondrial cytochrome oxidase one gene (COI) was sequenced from all individuals as described in (Haukisalmi *et al.* 2004).

Estimates of nucleotide diversity within phylogeographical groups and total and net sequence divergence (Dxy and Da; Nei 1987) between phylogeographical groups and their standard errors were calculated employing Kimura 2-parameter distances (Kimura 1980) in MEGA v. 2.1 (Kumar *et al.* 2001). Phylogenetic analyses were conducted in MEGA using the neighbor-joining (NJ) algorithm with the Kimura 2-parameter model of nucleotide substitution. Bootstrap analyses were performed with 10 000 replicates. The anoplocephaline species *Diandrya composita, Andrya cuniculi* and *Paranoplocephala omphalodes* (GenBank acc. nos AY181550, AY189957, AY189952, AY189953, AY189954) were used for outgroup rooting.

Results

1) The host generalist P. gracilis

We sequenced 641 bp of the COI gene from 47 individuals resulting in 33 different haplotypes Their GenBank accession numbers are given in Table 1. A total of 45 segregating sites were found, and of these 26 were informative.

The NJ reconstructions of phylogeny (Fig. 2) discriminated between two major lineages in Fennoscandia. This main division was well supported in the unrooted haplotype tree (Fig. 2). In trees rooted with an outgroup (not shown) the eastern and western major

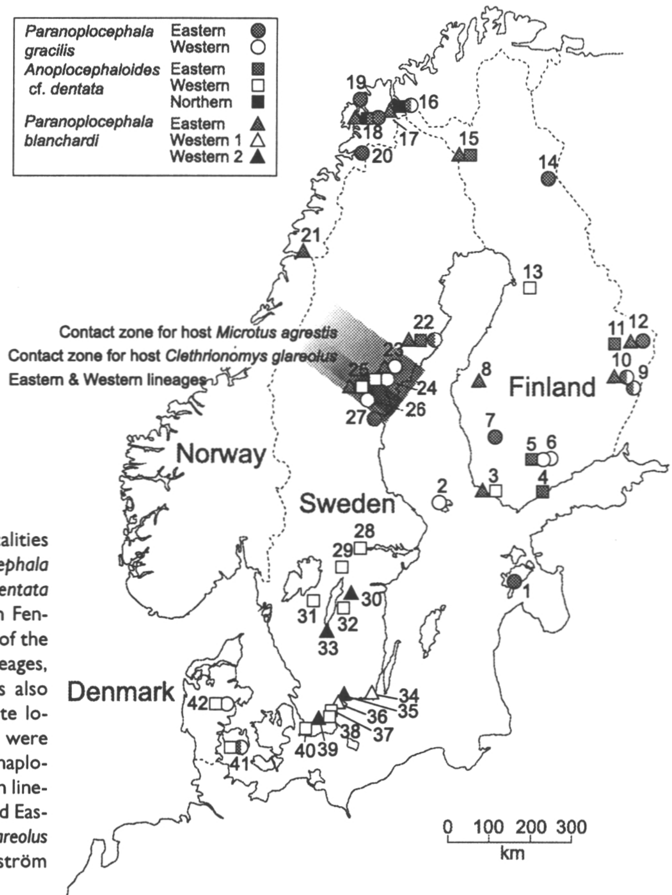

Figure 1. Sample sites (numbers refer to localities in table 1) of the parasites *Paranoplocephala gracilis* (circles), *Anoplocephaloides* cf. *dentata* (squares) and *P. blanchardi* (triangles) in Fennoscandia. The geographic distribution of the major cytochrome oxidase I (COI) lineages, Eastern (grey) and Western (white), is also depicted. Two-coloured symbols denote localities where both COI main lineages were present. Black colour designates COI haplotypes not belonging to either of the main lineages. Host contact zones of Western and Eastern *Microtus agrestis* and *Clethrionomys glareolus* lineages adapted from Jaarola & Tegelström (1995).

Table 1. Sampling localities, hosts (*Microtus arvalis; M.arv, M. agrestis; M.agr, M. oeconomus; M.oec, Clethrionomys glareolus; C.glar, C. rutilus; C.rut, Arvicola terrestris;A.terr*), number of individuals screened in each locality (*n*), parasite haplotype designations (G1-G32 = *Paranoplocephala gracilis*, D1-D29= *Anoplocephaloides* cf. *dentata*, B1-B11= *P. blanchardi*) and GenBank accession nos for the partial cytochrome oxidase I sequences. *M. agrestis* hosts with a Y after the species name have a unique mutation in their Y chromosome (Fredga & Jaarola 1997).

Country	Locality in fig 1	Locality (region or closest city)	Host	*n*	Haplotype, G=*P. gracilis* D=*A.* cf. *dentata*, B=*P. blanchardi*	GenBank accession number
Estonia	1	Saaremaa	*M.arv*	2	G1, G2	AY395648, AY395647
Finland	2	Ahvenanmaa	*M.agr*	1	G29	AY395655
	5	Iitti	*M.agr*	1	G3	AY395636
	6	Päijänne	*M.agr*	3	G4, G5, G6	AY395643, AY395634, AY395642
	7	Parkano	*A.terr*	1	G7	AY395666
	9	Punkaharju	*M.agr*	3	G7, G8, G9	AY395637, AY395650, AY395635
	10	Heinävesi-Enonkoski	*M.agr*	3	G7, G10, G22	AY395639, AY395651, AY395640
	12	Pielinen	*M.agr*	3	G1, G11, G12	AY395641, AY395633, AY395649
	14	Pyhätunturi	*M.agr*	1	G13	AY395683,
	15	Pallasjärvi	*M.agr*	4	G1, G14, G15, G32	AY395653, AY395652, AY395676, AY395682
	16	Kilpisjärvi	*M.agr, C.rut, C.ruf*	5	G1, G13, G16, G17, G18	AY395638, AY395681, AY395674, AY395673, AY395675
Norway	18	Kirkesdalen	*M.agr*	1	G19	AY395684
	19	Vasstrand	*M.oec*	1	G1	AY395654
	20	Narvik	*M.agr*	3	G1, G20, G21	AY395646, AY395645, AY395644
Sweden	22	Umeå	*M.agr*	3	G1, G23, G24	AY395669, AY395671, AY395670
	23	Rötjärn	*M.agr*	3	G18, G25, G26	AY395658, AY395657, AY395659
	24	Kubbe	*M.agr*	2	G23, G27	AY395662, AY395663
	26	Bispfors	*M.agr*	1	G23	AY395660
	27	Njurunda	*M.agr*	1	G28	AY395656
Denmark	41	Fyn Island	*M.agr, C.glar*	2	G1, G31	AY395668, AY395664
	42	Århus, mainland	*C.glar*	2	G18, G30	AY395667, AY395665
Italy	-	Trento	*M.arv*	1	-	AY395672
Finland	3	Turku	*M. agr*	1	D1	AY423822
	4	Espoo	*M. agr*	1	D2	AY423815
	5	Iitti	*M. agr*	1	D3	AY423811
	11	Maaninka	*M. agr*	1	D4	AY423814
	12	Pielinen	*M. agr*	1	D5	AY423810
	13	Muhos	*M. agr*	1	D6	AY423820
	15	Pallasjärvi	*M. oec*	4	D7, D8, D9	AY423807, AY423808, AY423809+AY423812
	16	Kilpisjärvi	*M. agr*	3	D10, D27, D29	AY423813, AY459357, AY459355

Norway	18	Kirkesdalen	*M. agr*	4	D11, D27, D28	AY423836, AY459358+AY459360, AY459359
Sweden	22	Umeå	*M. agr*	4	D12, D13, D14	AY423827+AY423833, AY423826, AY423825
	24	Kubbe	*M. agr*	1	D16	AY423818
	25	Strömsund	*M. agr*	1	D15	AY423828
	28	Örebro	*M. agr*	1	D17	AY423831
	29	Hallsberg	*M. agr*	1	D18	AY423830
	31	Skara	*M. agr*	2	D20, D21	AY423832, AY423819
	32	Ödeshög	*M. agr*	1	D19	AY423829
	37	Åhus	*M. agr*	1	D22	AY423816
	38	Yngsjö	*M. agr*	1	D23	AY423817
	40	Oxie	*M. agr*	1	D24	AY423821
Denmark	41	Fyn Island	*M. agr*	1	D25	AY423824
	42	Århus, mainland	*M. agr*	1	D26	AY423823
Finland	3	Turku	*M.agr*	2	B1	AY423883+ AY423884
	8	Lapua-Kauhava	*M.agr*	1	B2	AY423882
	10	Heinävesi-Enonkoski	*M.agr*	1	B3	AY189955
	12	Pielinen	*M.agr*	1	B4	AY423874
	15	Pallasjärvi	*M.agr*	1	B5	AY423892
Norway	17	Brennfjäll	*M.agr*	1	B2	AY423873
	18	Kirkesdalen	*M.agr*	2	B2	AY604728+ AY604729
	21	Mo i Rana	*M.agr*	1	B2	AY423891
Sweden	22	Umeå	*M.agr*	2	B2	AY423886+AY423888
	23	Rötjärn	*M.agr*	1	B2	AY423877
	25	Strömsund	*M.agr*	1	B2	AY423887
	30	Motala	*M.agr* Y	1	B6	AY423889
	33	Jönköping	*M.agr* Y	2	B6	AY423885+ AY423890
	34	Jämjö	*M.agr*	1	B7	AY423881
	35	Stilleryd	*M.agr* Y	1	B8	AY423875
	36	Gualöv	*M.agr*	2	B9	AY423876+ AY423880
	39	Revinge	*M.agr* Y	2	B8, B10	AY423878, AY423879
Kazakhstan	-	Taldykorgan	*M.arv*	1	B11	AY423895

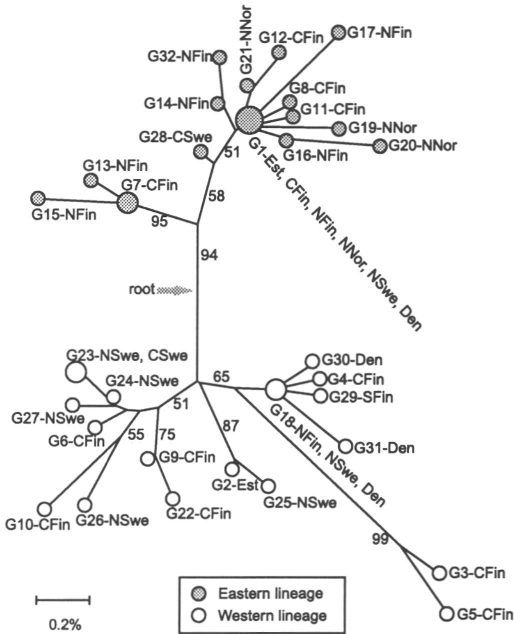

Figure 2. Unrooted neighbour-joining (NJ) tree of *Paranoplocephala gracilis* cytochrome oxidase I (COI) haplotypes (G1-G32). Bootstrap percentages from 10 000 iterations are shown at nodes. Haplotypes belonging to the main Eastern lineage in grey, Western lineage in white. SFin= southern, CFin=central, NFin=northern Finland, Swe= Sweden, Nor=Norway, Den=Denmark, Est=Estonia. The sizes of the haplotypes reflect the number of specimens sharing the same haplotype (for exact *n*, see Table 1).

lineages persisted regardless of outgroup species used but the bootstrap support was lower. The haplotypes of the Eastern *P. gracilis* lineage all grouped north of the host *M. agrestis* and *C. glareolus* contact zones except for the most abundant haplotype G1 (n=8), that was found also from one of the two sampling sites south of the host contact zone (Denmark, Fig. 1). Haplotypes belonging to the Western *P. gracilis* COI lineage were found from both north and south of the host contact zone, i.e. from hosts belonging to both the Eastern and the Western major host lineages.

The estimated nucleotide diversity (Table 2) within the Western COI phylogroup was higher than in the Eastern phylogroup. The divergence between the Western and Eastern lineages was estimated at 0.9 -1.5%. An Italian haplotype (Table 1) not included in Fig. 2 was the most divergent differing by approximately 2%.

2) The Microtus *parasite* Anoplocephaloides *cf.* dentata

Analysis of 544 bp of the COI gene from 33 Fennoscandian individuals of *A.* cf. *dentata* resulted in 29 different haplotypes (GenBank accession nos given in Table 1). A total of 44 segregating sites were found of which 20 were informative.

The NJ phylogenetic reconstruction of *A.* cf. *dentata* COI haplotypes distinguished three Fennoscandian lineages (Fig. 3) of which the Northern one had a restricted distribution (localities 16 and 18, Fig. 1). All individuals belonging to the Eastern COI lineage were found north of the host contact zone, whereas parasite individuals belonging to the Western lineage were found mostly south of or in the host contact zone (with the exception of two haplotypes, D1 and D6, from south-central Finland). The topology was not dependent on outgroup rooting although bootstrap support for the lineages was lower in trees rooted with *Diandrya composita* and *Andrya cuniculi.* The divergence estimates between the Eastern and Western lineages of *A.* cf. *dentata* were similar to those obtained for *P. gracilis* and the nucleotide diversities within the two *A.* cf *dentata* phylogroups were also similar (Table 2).

3) The M. agrestis specialist Paranoplocephala blanchardi

Analysis of 641 bp of COI revealed 41 segregating sites of which 30 were informative. Altogether ten haplotypes represented the 24 Fennoscandian individuals analysed; an eleventh haplotype was found from Kazakhstan (Table 1).

The NJ tree (Fig. 4) revealed a clear and well-supported topology that was not dependent on outgroup rooting. An Eastern lineage was found from Finland, northern Norway and northern Sweden whereas two well-supported Western lineages (W1 and W2) were observed in specimens from southern Sweden (Fig. 1). Even though there was an expected phylogeographical split between W1 and the Eastern lineage at the host contact zone in northern central Sweden, the deepest phylogeographical split in *P. blanchardi* was that between the W2 lineage and the other Fennoscandian haplotypes. Using criteria of Haukisalmi *et al.* (2002), W2 could not be distinguished morphologically from the W1 or the Eastern lineage but it differed 4.8-5.2% from the other lineages in COI (Table 2). A *P. blanchardi* haplotype from Kazakhstan was situated between the W1 and W2 lineages from southern Sweden

Table 2. Diversity estimates for the major cytochrome oxidase I phylogroups in *Paranoplocephala gracilis, Anoplocephaloides* cf. *dentata* and *P. blanchardi*. Numbers along diagonals are nucleotide diversities (in bold). Below the diagonal is the total, raw DNA divergence (Dxy) and above the diagonal is the net divergence (Da). Standard errors based on 10 000 bootstrap replicates are given in parentheses. All estimates are expressed as percentages.

Taxon	Phylogroup	Western (1)	Northern/ Western 2	Eastern
Paranoplocephala gracilis	Western	**0.81** (0.20)		0.91 (0.35)
	Eastern	1.54 (0.39)		**0.46** (0.12)
Anoplocephaloides cf. *dentata*	Western	**0.63** (0.16)	1.00 (0.41)	0.87 (0.37)
	Northern	1.46 (0.44)	**0.31** (0.13)	1.62 (0.53)
	Eastern	1.60 (0.42)	2.20 (0.58)	**0.84** (0.20)
Paranoplocephala blanchardi	Western 1	**0.10** (0.10)	5.07 (0.89)	1.30 (0.40)
	Western 2	5.19 (0.90)	**0.13** (0.10)	4.82 (0.87)
	Eastern	1.53 (0.43)	5.08 (0.91)	**0.36** (0.12)

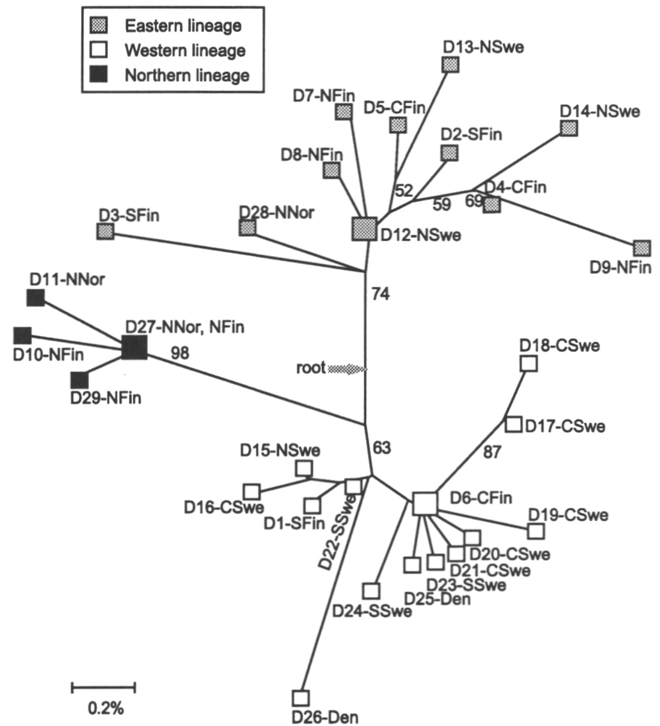

Figure 3. Unrooted neighbour-joining (NJ) tree of *Anoplocephaloides* cf. *dentata* cytochrome oxidase I (COI) haplotypes (D1-D29). Bootstrap percentages from 10 000 iterations are shown at nodes. Haplotypes belonging to the main Eastern lineage in grey, Western lineage in white. SFin=southern, CFin=central, NFin=northern Finland, Swe=Sweden, Nor=Norway, Den=Denmark. The sizes of the haplotypes reflect the number of specimens sharing the same haplotype (for exact *n*, see Table 1).

and differed 5.2 ±0.9% from W2, 3.0±0.6% from W1 and 2.9±0.6% from the Eastern phylogroup. The intralineage nucleotide diversity was low. Of the 12 individuals composing the Eastern phylogroup, only two samples from the southernmost Finnish coast differed in more than one nucleotide position. The W2 lineage differed by 27 mutations from the W1 and the Eastern lineage, whereas the haplotype from Kazakhstan differed by 12 mutations from the W1 and the Easten lineage. The parasite haplotypes in W2 were all found from *M. agrestis* with a rearranged Y chromosome that is unique to southwest Sweden (Fredga & Jaarola

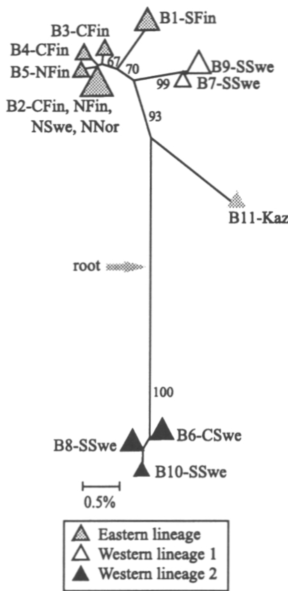

Figure 4. Unrooted neighbour-joining (NJ) tree of *Paranoplocephala blanchardi* cytochrome oxidase I (COI) haplotypes (B1-B11). Bootstrap percentages from 10 000 iterations are shown at nodes. Haplotypes belonging to the main Eastern lineage in grey, Western lineage 1 in white, Western lineage 2 in black. SFin=southern, CFin=central, NFin=northern Finland, Swe=Sweden, Nor=Norway, Kaz=Kazakhstan. The sizes of the haplotypes reflect the number of specimens sharing the same haplotype (for exact *n*, see Table1).

1997). This 'Lund' population is also characterized by a specific mtDNA lineage (Jaarola & Tegelström 1996).

Discussion

All the three parasite species studied exhibited Western and Eastern main COI lineages. Two of the parasite species, *A.* cf. *dentata* and *P. blanchardi*, showed phylogeographical patterns that were roughly congruent with the phylogeographies of their main host, whereas one parasite species, *P. gracilis*, did not. The partitioning of *A.* cf. *dentata* and *P. blanchardi* COI lineages is geographically roughly congruent with the definite mtDNA partitioning of Eastern and Western *M. agrestis* host lineages in northern-central Sweden.

If we assume that the recolonization history of parasite lineages is synchronic with that of their host, we would expect congruence between the phylogeographical patterns of parasites and hosts in Fennoscandia. The east/west pattern that we have observed for the parasites could be interpreted as synchrony of host-parasite assemblages prior to the colonization of Fennoscandia and later mixing over the host contact zone. This model seems highly likely for *P. blanchardi* and can probably also be used to explain the phylogeographical pattern of *A. cf. dentata*. However, the evolutionary histories of the parasite COI lineages cannot be determined without sampling outside Fennoscandia. Therefore, an alternative model would involve the presence of parasite COI lineages in both the Eastern and Western host lineages during the colonization of Fennoscandia. Our results suggest that this may be a likely scenario for *P. gracilis,* and we cannot entirely dismiss this model for *A. cf. dentata.* Uneven historical distributions of the two parasite lineages in the two host populations, differences in effective population sizes, and restricted dispersal over the host contact zone (see below) are other factors that may have affected the parasite phylogeographical patterns.

The nucleotide diversity between Eastern and Western COI phylogroups was similar for the three parasite species studied (0.9-1.6%, considering W1 for *P. blanchardi*). Given COI probably evolves at near equal rate in closely related organisms, the Eastern and Western main lineages of the different parasite species may be of equal age. However, there were differences in the distribution of the Eastern and Western lineages of the tree parasite species that could be related to their abundance and host specificity. Haplotypes of the Western COI lineage of the common host generalist *P. gracilis* were frequently found north of the host contact zone in Sweden and even in Finland. *P. gracilis* is also found from other, accidental host species (Haukisalmi *et al.* 1987), that do not display phylogeographical subdivision in north-central Sweden. A parasites' ability to cross the main host contact zone and infect main hosts of the opposite evolutionary lineage would create geographical mixing of assumed historical lineages for the parasite. A host generalist like *P. gracilis* has more frequent opportunities to disperse over the host contact zone as a function of the greater number of suitable host individuals. Being a common parasite adds to the probability of occurring in a migrating host. This would give a common host generalist a dispersal benefit compared to rare host specialists/generalists and could generate a phylogeographical pattern that differed from that of the main hosts. In cont-

rast, *P. blanchardi* showed a phylogeographical pattern that matched that of its host, *M. agrestis*, both regards to the definite partitioning at the host contact zone as regards to the recognition of a divergent 'Lund' population. This rare host specialist probably lacks frequent opportunities for dispersal and gene flow as a function of patchy distribution and host specificity and therefore has less potential for parasitizing a migrating host. A rare host specific parasite is not necessarily less prone to disperse, but compared to a common generalist, dispersal and gene flow is probably slower. Given a short population history in Fennoscandia, such a time lag could create the differences observed even if the historical associations between host and parasite lineages for all parasite species studied were the same. Stochastic lineage sorting has probably also played a role in shaping these patterns, and especially so in the host specialists (see below).

In view of the recent colonisation history in Fennoscandia, and studies of another paranoplocepaline cestode on a Holarctic scale (Wickström *et al.* 2003), low nucleotide diversities were expected. However, the topologies of the gene trees and the relatively high degrees of net nucleotide diversity do not imply a drastic population expansion in Fennoscandia. Our results are consistent with the fact that the hosts *M. agrestis, M. oeconomus* and *C. glareolus* generally show high levels of genetic diversity in Fennoscandia (Brunhoff et al. 2003; Jaarola et al. 1999; Tegelström 1987). If we assume that the main parasite phylogroups represent historical populations, the greater geographical spread of the Western phylogroups could reflect the complex colonization history of their hosts, probably involving several colonization waves along a land bridge connecting southern Sweden to Denmark during three periods between 11 200- 8 200 BP (Björck 1995a; Björck 1995b; Jaarola & Tegelström 1996).

The Western (W2) lineage of *P. blanchardi* (Fig. 4) parasitizing *M. agrestis* of the 'Lund' population (Fredga & Jaarola 1997; Jaarola & Tegelström 1996) might, as its host, have gone through a severe population bottleneck during the initial stages of recolonization of southern Sweden. Moreover, due to the possibility of selection against hybrid hosts (Jaarola *et al.* 1997), the parasite lineage might have become genetically isolated from neighbouring populations. According to this scenario, the deviant W2 lineage would result from severe inbreeding of a very small population. However, the 5% COI net divergence between the 'Lund' parasite phylogroup and other groups is so great that it is difficult to reconcile with even the combined effects of drastic and prolonged bottlenecks and inbreeding. In comparison, the mtDNA divergence between the *M. agrestis* 'Lund' lineage and other lineages in southern and central Sweden is 0.4-0.9% (Jaarola &Tegelström 1996), whereas the genetic differences in mtDNA between Eastern and Western *M. agrestis* host lineages is 0.5-1.5% (Jaarola & Searle 2002). In comparison, the genetic divergence 0.9-1.6% between east and west in our cestodes is quite similar to that observed in *M. agrestis*, whereas the divergence of the 'Lund' parasite lineage is five to ten times as high as that of the 'Lund' host lineage. Unfortunately, we do not have molecular clock estimate for cestode COI, but in rodent host mtDNA a divergence of 6-10% would approach 1 million years (Jaarola & Searle 2002).

A third, highly divergent lineage was observed in *A.* cf. *dentata* from northern Norway-Finland (localities 18 and 16 in figure 1). Unfortunately, no genetic data for *M. agrestis* is yet available from this specific region. Phylogeographical studies of another northern vole species, *M. oeconomus*, demonstrate a distinct mtDNA sublineage on three north Norwegian islands (just north and south of locality 19 in Fig. 1). *M. oeconomus* belonging to this sublineage have also been found in northwest Russia (Brunhoff 2003; Brunhoff *et al.* 2003). Brunhoff *et al.* (2003) suggested that these island populations represent remnants of a first recolonization wave along the northern coast of Russia and Norway during late glacial times when the sea level was much lower than at present. Interestingly, Haukisalmi *et al.* (2004) showed a separate local lineage (cryptic species) in the *Paranoplocephala omphalodes* group in northern Alaska in the absence of genetic structure in the host *M. oeconomus*.

Phylogeographical patterns observed in the intestinal parasite species studied here would imply that a host contact zone can act as a limiting factor for dispersal and gene flow for the parasites as well. The parasite species addressed showed differences in phylogeographical structure that correlated with host specificity and commonness of the parasite. This corroborates the role of host choice and abundance as determinants of parasite dispersal and gene flow.

It has been shown that intestinal parasites can serve as additional and sensitive tools for inferring the evolutionary history of their hosts (Wickström *et al.* 2003). The phylogeography of two of the three parasite species studied here could serve as further evidence for bidirectional faunal recolonization of Fennoscandia if considering the parasite COI lineages as separa-

te evolutionary units, i.e. synchronic and sympatric host-parasite associations prior to recolonization. Cestodes are prone to cryptic speciation due to their smaller population sizes compared to the host and selfing facilitating clonality. An extreme example of this phenomenon could be the 'Lund' population of *P. blanchardi*. Genetic studies of parasites from central Europe and western Russia could shed light on the postglacial immigration routes for the parasites. We encourage further research on parasite phylogeography to find more detailed patterns that add to the complexity of the colonization models and phylogeographical scenarios proposed for host-parasite associations.

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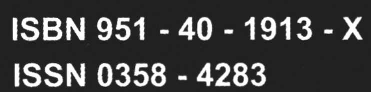
The perfectionist

Precisely,
the true perfectionist
making a full stop
after the last sentence.
Here to, but no longer,
will I spill my thoughts.
So aware
of the limitations
of a full stop.
So lovingly making it,
so contentedly finishing
the signature of his life.
Precisely,
the thoughts of a dreamer
are not mine.
They never end
at a full stop.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