Molecular and morphological characterization of *Echinococcus* in cervids from North America

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SUMMARY

Many issues concerning the taxonomy of *Echinococcus* have been resolved in recent years with the application of molecular tools. However, the status of *Echinococcus* maintained in transmission cycles involving cervid intermediate hosts remains to be determined. The recent characterization of the parasite from cervids in Finland has highlighted the paucity of data available, particularly that from North America. In this study, we have characterized a large number of *Echinococcus* isolates from cervids from Western Canada on the basis of morphology and molecular genetic techniques. Our results support earlier studies suggesting that *Echinococcus* of cervid origin is phenotypically and genetically distinct to *Echinococcus* maintained in domestic host assemblages, and also confirms that *Echinococcus* of cervid origin does not constitute a genetically homogeneous group. However, our data do not support the existence of 2 distinct genotypes (strains/subspecies) with separate geographical distributions. Our data appear to support the existence of only 1 species in cervids, but additional isolates from cervids and wolves in other endemic regions should be characterized before a final decision is made on the taxonomic status of *Echinococcus* in cervids.

Key words: *Echinococcus*, *Echinococcus granulosus*, cervids, Canada, molecular characterization, strains/genotypes/subspecies, mitochondrial (COI, NDI and ATP6), ITS-1, G8, G10.

INTRODUCTION

The recent application of molecular tools has helped to resolve many of the taxonomic issues concerning the status of species and strains in the genus *Echinococcus*, and the current situation has been extensively reviewed (Thompson and McManus, 2001, 2002; McManus and Thompson, 2003).

The present understanding of the status of *Echinococcus* species is a series of largely host-adapted species that are maintained in distinct cycles of transmission characterized by the principal intermediate hosts involved (Thompson, 2001; Thompson and McManus, 2002). The most widely distributed species is *E. granulosus* which exists as a series of genetically distinct strains/genotypes, some of which are likely to warrant species status in the future, particularly those in pigs, camels, and cervids (Harandi *et al.* 2002; Thompson and McManus, 2002; Lavikainen *et al.* 2003; Obwaller *et al.* 2004). Until recently, very few isolates of *Echinococcus* of cervid origin had been characterized genetically which is unfortunate in view of the considerable epidemiological and phenotypic features which serve to separate the cervid form of *E. granulosus* from other strains, as well as other species in the genus. Cycles of transmission in which cervids are the intermediate hosts for *E. granulosus* are considered the most important wild-animal cycles for maintaining the parasite. The form of *E. granulosus* in cervids was proposed to be ancestral to *E. granulosus* in domestic ungulates (Rausch, 1986) although this hypothesis has not been supported by phylogenetic analysis of morphological or molecular data (Lymbery, 1995).

Echinococcus granulosus in cervids is primarily perpetuated by a predator-prey relationship involving wolves and large deer, principally moose (*Alces alces*), elk [wapiti] (*Cervus elephus*) and reindeer

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[caribou] (*Rangifer tarandus*), in northern North America and Eurasia (Rausch, 1967*a*, *b*, 1986; Pybus, 1990). Recent research supports the influence such infection has on enhancing moose predation by wolves and the importance such cycles may have in maintaining wolf populations (Joly and Messier, 2004). However, domestic cycles involving dogs and domesticated reindeer operate in parts of Canada, Alaska, Siberia, Finland, Norway and Sweden (Rausch, 1986).

Echinococcus granulosus of cervid origin differs in many respects from other forms of *E. granulosus* (Thompson and Lymbery, 1988). It does not readily infect sheep and other domestic ungulates, exhibits characteristic differences in the type of infection produced in laboratory mice (Webster and Cameron, 1961; Sweatman and Williams, 1963; Safronov and Isakov, 1982, 1984) and develops rapidly in dogs (Mankhaeva and Shumilov, 1982). In contrast with domestic species and strains of *Echinococcus*, many years of clinical experience attest to the cervid form's relatively benign clinical course in the majority of human cases with only rare serious complications (Cameron, 1960; Cameron and Webster, 1961; Wilson, Diddams and Rausch, 1968; Schantz *et al*. 1995; Castrodale *et al*. 2002). It also differs serologically and genetically from domestic forms of the parasite (Cameron, 1960; Bowles, Blair and McManus, 1994). However, the situation may be more complicated with the recent demonstration of 2 genetically distinct forms of *Echinococcus* in cervids (Lavikainen *et al*. 2003).

Genetic analysis of cervid material from North America has been limited to material from 2 moose; one from Minnesota and one from Alaska, which based on mitochondrial gene sequences and internal transcribed spacer 1 (ITS-1) characterization in the case of the Minnesota isolate, were shown to be similar to but distinct from previously reported genotypes, and was referred to as the G8 genotype (Bowles *et al*. 1994, 1995; McManus *et al*. 2002). More recently, 5 isolates (4 reindeer and 1 moose) from north-east Finland were characterized by Lavikainen *et al*. (2003) using the same loci and were shown to be genetically different to the cervid genotype (G8). They denoted this new genotype as G10 and suggested that this novel genotype was representative of the indigenous Fennoscandian form. Clearly there is a need to characterize more isolates of *Echinococcus* of cervid origin from North America. The recent emergence of hydatid disease in farmed elk in Alberta, Western Canada (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex8833? open document) provided most of the material for the present study in which we have characterized a large number of *Echinococcus* isolates from cervids on the basis of morphology and molecular genetic techniques.

MATERIALS AND METHODS

Parasite isolates

Isolates of larval *E. granulosus* were obtained from the lungs of 16 farmed elk, 1 wild elk and 2 wild moose (Table 1). The majority of the elk sampled formed part of an ongoing survey of hydatid disease being undertaken by Alberta Agriculture of farmed elk in Alberta, SW Canada. However, cysts were recovered from 1 wild elk from Manitoba. The 2 hydatid infected lungs from moose were provided by Dr M Pybus of Alberta Agriculture from 2 animals, one from Alberta and one from the State of Washington, USA, that had been removed at post-mortem previously and the lungs kept frozen at −20 °C. Protoscoleces and laminated layer with adhering germinal layer from each cyst were preserved in 10% formalin for morphology and 90% ethanol for molecular characterization. Adult worms of *E. granulosus* were recovered from 1 wolf that died in Banff National Park, Alberta, and worms were recovered directly from the mucosal surface or following examination of gut scrapings. The worms were in poor condition and had lost their hooks but several hundred specimens were preserved in 10% formalin for morphology and 90% ethanol for molecular characterization.

Morphology

Individual protoscoleces were mounted in polyvinyl lactophenol (R. A. Lamb) with sufficient cover-slip pressure to cause the hooks to lie flat. The hook components measured were as reported by Hobbs, Lymbery and Thompson (1990). Measurements of the total length and blade length were made on 3 large and 3 small hooks per rostellum from each of 10 protoscoleces for each isolate. Measurements were made using an Olympus BX50 microscope with a 100× objective and an Optimas image analyser.

Intact whole adult worms were placed in a Petri dish in 70% ethanol and photographed using an Olympus C-3040 digital camera through the eyepiece of a Wild M3 stereomicroscope at 16× magnification using the C3040 ADU coupling attachment. Measurements were made using ImageJ (NIH). Three measurements were made on each worm: total length, length of the last segment, and length of the penultimate segment.

DNA extraction from parasite material

DNA was extracted from 100 μl of packed, washed protoscoleces by initially adding extraction buffer and then performing 5× freeze-thaw processes. A standard method of SDS and proteinase K treatment was applied (Maniatis, Fritsch and

Table 1. Hosts, geographical origins and sequence Accession numbers for the ITS-1, COI, ATP6 and NDI of the analysed *Echinococcus granulosus* isolates from Canada and the USA, and *Echinococcus* species and strains used as reference material

Species	Genotype	Origin	Sample name	Host	COI	NDI	ATP6
E. granulosus	G10	Canada, Alberta	Elk1	Elk	DQ144012	DQ144029	DQ143992
E. granulosus	G10	Canada, Alberta	Elk2	Elk	—	DQ144030	—
E. granulosus	G10	Canada, Alberta	Elk3	Elk	DQ144013	DQ144031	—
E. granulosus	G8	Canada, Alberta	Elk4	Elk	—	DQ144032	DQ143993
E. granulosus	G10	Canada, Alberta	Elk5	Elk	DQ144014	DQ144033	DQ143994
E. granulosus	G10	Canada, Alberta	Elk6	Elk	DQ144008	DQ144027	DQ143995
E. granulosus	G10	Canada, Alberta	Elk7	Elk	DQ144011	DQ144028	—
E. granulosus	G10	Canada, Alberta	Elk8	Elk	DQ144009	DQ144023	DQ143996
E. granulosus	G10	Canada, Alberta	Elk9	Elk	DQ144006	—	DQ143997
E. granulosus	G10	Canada, Alberta	Elk10	Elk	DQ144007	DQ144024	—
E. granulosus	G10	Canada, Alberta	Elk11	Elk	DQ144022	DQ144041	DQ143998
E. granulosus	G10	Canada, Alberta	Elk12	Elk	—	DQ144025	DQ143999
E. granulosus	G10	Canada, Alberta	Elk13	Elk	DQ144010	DQ144026	DQ144000
E. granulosus	G10	Canada, Alberta	Elk14	Elk	DQ144018	DQ144038	—
E. granulosus	G8	Canada, Alberta	Elk15	Elk	DQ144021	DQ144037	DQ144001
E. granulosus	G8	Canada, Alberta	Elk16	Elk	DQ144019	DQ144040	DQ144002
E. granulosus	G10	Canada, Manitoba	Elk17	Elk	DQ144020	DQ144039	—
E. granulosus	G10	Canada, Alberta	Wolf1	Wolf	DQ144017	DQ144036	DQ144003
E. granulosus	G10	Canada, Alberta	Moose1	Moose	DQ144015	DQ144034	DQ144004
E. granulosus	G10	USA, Washington	Moose2	Moose	DQ144016	DQ144035	DQ144005
E. granulosus	G1	Many countries	G1	Sheep	AF297617	AJ237632	AF297617
E. granulosus	G2	Tasmania	G2	Sheep	M84662	AJ237633	—
E. granulosus	G3	India	G3	Buffalo	M84663	AJ237634	—
E. granulosus	G4	Europe	G4	Horse	M84664	AJ237635	AF346403
E. granulosus	G5	Europe, India	G5	Cattle	M84665	AJ237636	—
E. granulosus	G6	Sudan, Somalia	G6	Camel	M84666	AJ237637	AY056613
E. granulosus	G7	Poland	G7	Pig	M84667	AJ237638	AY056614
E. granulosus	G8	USA	G8	Moose	—	AJ237643	AY056615
E. granulosus	G10	Finland	G10	Reindeer and Moose	AF525457	AF525297	—
E. multilocularis		China, Alaska	Em-M1	Human	M84668	AJ237639	
E. multilocularis		Germany	Em-M2	Rodent	M84669	AJ237640	AB018440
E. oligarthus		Panama	Eo	Rodent*	M84671	AJ237642	AY056611
E. vogeli		South America	Ev	Rodent*	M84660	AJ237641	AY056612
T. solium			T. solium		AB086256	AB086256	AB086256
T. solium			T. solium				

— Sequence not obtained.
* Laboratory strain.

Sambrook, 1982) followed by a glass-milk method (Qiagen, Hilden, Germany) developed by Morgan *et al*. 1995.

DNA amplification and sequencing

DNA was purified and PCRs were performed as previously described, ITS1 (Bowles and McManus, 1993*a*), cytochrome *c* oxidase I (COI) (Bowles, Blair and McManus, 1992), NADH dehydrogenase I (NDI) (Bowles and McManus 1993*b*). The adenosine triphosphate 6 (ATP6) fragment (Le *et al*. 2002; Xiao *et al*. 2005) was amplified using the primers ATP6-F 5′-GCATCAATTTGAAGAGTTGGG-GATAAC-3′ and ATP6-R 5′-CCAAATAATCTA-TCAACTACACAACAC-3′. The PCR (50 μl) was performed in 200 μM of each dGTP, dATP, dCTP, dTTP, 0·2 μM of each primer, 2U Tth plus (Fisher-Biotech, Western Australia) buffer was added following the manufacturer's instructions, 1 μl of DNA template was added. Thermocycler conditions were as follows: initial denaturation step of 98 °C for 30 sec; 35 cycles of 96 °C for 30 sec, 55 °C for 30 sec and 72 °C for 1 min; followed by a final extension step at 72 °C for 7 min and a final hold at 15 °C. Amplicons from all loci were visualized using ethidium bromide in 1% agarose gels after electrophoresis for 30 min at 90 volts.

PCR products were purified using Qiagen spin columns (Qiagen, Hilden, Germany) and sequenced using an ABI prism^{TM} Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Sequences were analysed using SeqEd v1.0.3. (Applied Biosystems). Additional *Echinococcus* DNA sequences were obtained from

GenBank. For the ITS, the sequencing primers BD1, 4S2 and 4S (Bowles and McManus, 1993*a*) were used.

Phylogenetic analyses

Previously published sequences of *Echinococcus* isolates were used as the reference material and *Taenia solium* was used as an outgroup (Table 1). Nucleotide sequences were aligned using Clustal W (Thompson, Higgins and Gibson, 1994). Phylogenetic analysis was performed using TREECON (Van de Peer and De Wachter, 1993). Distance-based analyses were conducted using Tajima and Nei distance estimates and trees were constructed using the Neighbour Joining algorithm. Bootstrap analyses were conducted using 1000 replicates.

RESULTS

Morphology

Figure 1 is a scatterplot of blade length and total length of (A) large rostellar hooks, and (B) small rostellar hooks, measured in micrometres. The mean lengths for individual isolates in moose and elk are from this study. The means of individual isolates from 14 Australian mainland sheep, 2 UK horses, and 1 Egyptian camel are from unpublished data of the Hobbs *et al*. (1990) study. The overall mean of 7 horse isolates is from the data of Kumaratilake, Thompson and Eckert (1986); of 7 cattle isolates is from Thompson, Kumaratilake and Eckert (1984); and of 21 camel isolates is from Eckert *et al*. (1989). Overall means of 29 camel strain isolates, and 78 sheep strain isolates from Iran are derived from both published and unpublished data from the study of Harandi *et al*. (2002). As can be seen, the cervid isolates from elk (2 G8 and 2 G10) and moose (G10) group together for both large and small hook length, and are quite distinct from isolates of sheep origin.

The poor quality of adult worms recovered from the wolf only made it possible to obtain some data on strobilar dimensions of 32 worms that remained intact. The mean total length was 3·47 mm (S.D. 0·75) but the value of this measurement is limited without knowledge of the age of the worms. However, of more value are data on proglottid length as a proportion of total length. The mean length of the terminal proglottid was 1·58 (S.D. 0·33), and its proportion to total worm length 0·46 (S.D. 0·04).

Mitochondrial phylogenetic analysis

The neighbour-joining trees based on the alignment of the COI, NDI and ATP6 partial sequences are presented in Fig. 2. Sequences were not attainable for Elk 2, 4 and 12 at the COI locus, Elk 9 at the NDI locus and Elk 2, 3, 7, 10, 14 and 17 at the ATP6 locus.

The phylogenetic analysis at the 3 mitochondrial genes demonstrate that the cervid samples form 2 clusters; one cluster grouping with the G8 and the other grouping with the G10. These 2 clusters are genetically more distinct than G2 and G3, supporting their recognition as different genotypes.

In the G8 cluster are isolates from Elk 4, Elk 15 and Elk 16, forming a distinct group with 94–100% bootstrap support separating them from the other cluster which includes G10. The Elk 4 sequence for the COI was not attainable and the G8 sequence not included due to ambiguities in the sequence. At the NDI, Elk 4 differed from G8 at 1bp and Elk 15 and 16 at a different base. Elk 15 and 16 differ from G8 at the ATP6 by 2bp.

In the G10 cluster are Elk 1-3, Elk 5-14, wolf 1 and Moose 1 and 2. Sequences for all the isolates at the NDI were identical to each other and 1bp different to G10. The moose samples align 100% with the G10 at the COI, NDI sequences for these samples were not attainable. All other isolates differ from the G10 at the COI by only 1 or 2bp. At the ATP6, moose 1 and 2 differ from all other samples in this cluster by 2bp and Elk 11 by 1bp.

The main difference between the COI and the NDI trees is the location of G6 and G7 genotypes. The location of G6 and G7 is the same for ATP6 and COI, but different for NDI.

ITS1 phylogenetic analysis

The phylogenetic tree obtained for 18 *Echinococcus* species/isolates sequenced in the present study and by other authors at the ITS1 locus showed a very different topology with all the cervid isolates typed clustering with 3 G10 variants, and with the single G8 isolate very distinct (data not shown). As the cervid samples were not cloned and the ITS1s region is known to be problematic for phylogenetic analysis of *E. granulosus* (Kedra *et al*. 1999; Lavkainen *et al*. 2003) these data are not useful in ascertaining relationships. Many copies of the ITS would need to be cloned and sequenced in order to detect all possible variants.

DISCUSSION

Our results support earlier studies suggesting that *Echinococcus* of cervid origin is phenotypically and genetically distinct from *Echinococcus* maintained in domestic host assemblages (Cameron, 1960; Webster and Cameron, 1961; Sweatman and Williams, 1963; Wilson *et al*. 1968; Bowles *et al*. 1994; Castrodale *et al*. 2002). Our results also confirm those of Lavikainen *et al*. (2003) that *E. granulosus* of cervid origin does not constitute a genetically homogeneous group. However, the present study has raised doubts of there being 2 distinct genotypes (strains/subspecies) with separate geographical distributions.

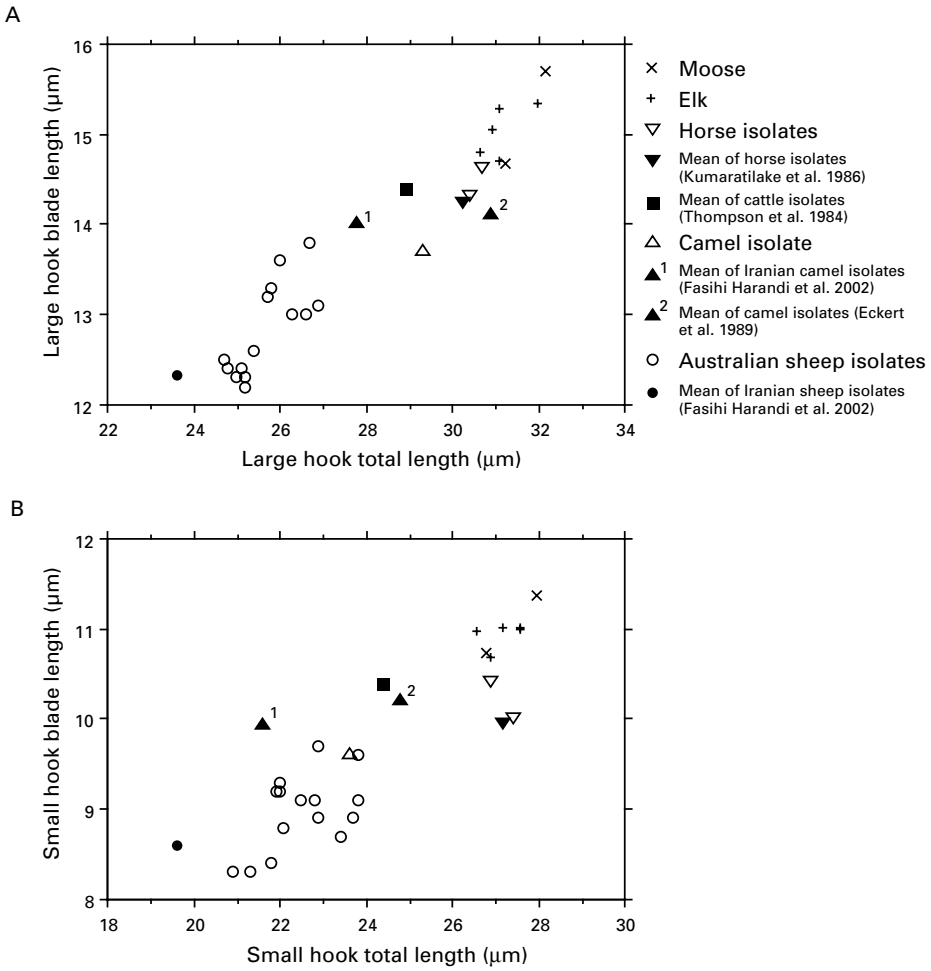

Fig. 1. Scatterplot of blade length and total length of (A) large rostellar hooks, and (B) small rostellar hooks, measured in micrometres. Mean lengths for individual isolates in moose and elk are from this study. Means of individual isolates from 14 Australian mainland sheep, 2 UK horses, and 1 Egyptian camel are from unpublished data of the Hobbs *et al*. (1990) study. The overall mean of 7 horse isolates is from data of Kumaratilake *et al*. (1986); of 7 cattle isolates is from Thompson *et al*. (1984); and of 21 camel isolates is from Eckert *et al*. (1989). Overall means of 29 camel strain isolates, and 78 sheep strain isolates from Iran are derived from both published and unpublished data from Harandi *et al*. (2002).

Originally, 2 subspecies of *E. granulosus* were described for the parasite in cervids. *E. granulosus canadensis* was proposed by Webster and Cameron (1961) who considered their description to be representative for material of indigenous cervid origin in North America. However, on the basis of detailed comparative studies involving experimental infections and morphological analysis of material of moose and reindeer origin, Sweatman and Williams (1963) proposed an additional subspecies, *E. g. borealis*, on the basis of morphological differences with *E. g. canadensis*, and further proposed that since the description by Cameron and Webster (1961) was based on material of reindeer origin that were likely to have been recently introduced into Canada, *E. g. canadensis* should be applied to the introduced form, and *E. g. borealis* to the indigenous form found in moose and elk. The indigenous reindeer (*Rangifer tarandus*) was never domesticated by aboriginal peoples in North America, and it was not until the early 20th century that domestic herds were introduced in north western Canada from Lapland in Norway (Sweatman and Williams, 1963; Rausch 1967*b*; Bergerud and Mercer, 1989; Long, 2003). Herd dogs accompanied the introduced reindeer and it was suggested that they probably brought hydatid infection with them (Sweatman and Williams, 1963).

The detailed studies undertaken by Sweatman and Williams (1963) demonstrated that *E. g. canadensis* and *E. g. borealis* shared a number of morphological characteristics, particularly those associated with larval and adult rostellar hooks, that were quite distinct from those of *E. granulosus* of sheep origin. With the adult worms, although the reproductive anatomy and strobilar dimensions of the terminal segment of *E. g. canadensis* were quite different to those of adult *E. granulosus* of sheep-dog origin, *E. g. borealis* was somewhat intermediate in its adult morphology. A subsequent study of adult

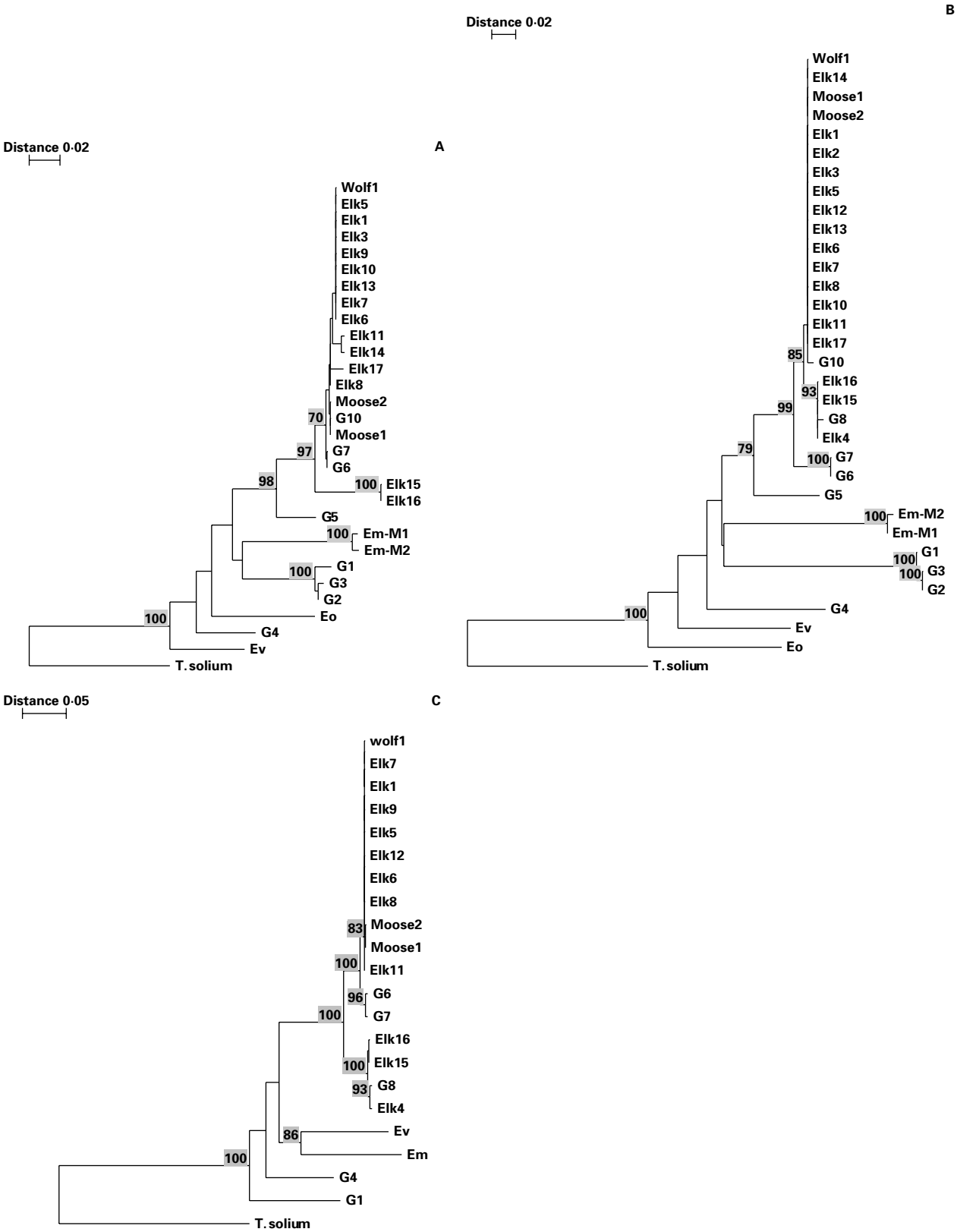

Fig. 2. Phylogenetic trees obtained for *Echinococcus* species/isolates sequenced in the present study and by other authors at the COI locus (A), NDI locus (B) and ATP6 locus (C). The method of Tajima and Nei (1984) was applied and calculating distances and tree topology was inferred by Neighbour joining. The TREECON program was used for analysis. Numbers at the nodes indicate percentage bootstrap support obtained in 1000 replications. *Taenia solium* was used as an outgroup to root the tree.

Echinococcus recovered from a naturally infected wolf in Canada found that the adult worms possessed similar characteristics to those reported by Sweatman and Williams (1963) for *E. g. canadensis* (Kumaratilake, 1982).

Rausch (1967*a*) did not support the designation of 2 subspecies for *E. granulosus* in cervids. He considered the introduction of a distinctive organism into Canada as doubtful and further pointed out that an introduced subspecies of *E. granulosus* could not retain its genetic identity in sympatry without an identifiable segregating mechanism.

Lavikainen *et al.* (2003) examined 5 isolates of *E. granulosus* from 4 reindeer and 1 moose from north-east Finland at the mitochondrial COI and NDI, and ribosomal ITS-1 loci and demonstrated genetic differences between their isolates and previously published data obtained from 1 infected moose from Minnesota, USA that formed the basis for denoting the cervid G8 genotype (Bowles *et al.* 1994. 1995). Although Lavikainen *et al.* (2003) demonstrated some molecular similarity with the G8 genotype in mitochondrial NDI and some ITS-1 sequence variants, they also found clear differences in these sequences, particularly in the COI sequence and some of the ITS-1 clones. Consequently, they denoted the Finnish isolates of *E. granulosus* as a distinct genotypic grouping, G10, and referred to it as the Fennoscandian cervid strain, suggesting it to be indigenous to this geographical region thus supporting the earlier suggestion of Sweatman and Williams (1963) for *E. g. canadensis*. It is certainly possible that *E. granulosus* could have been introduced into Canada in domestic reindeer and/or accompanying herd-dogs from Scandinavia in the early part of the last century. There were 2 such introductions of reindeer into Newfoundland and the Northwest Territories in 1908 and 1932 respectively (Sweatman, 1964; Bergerud and Mercer, 1989; Long, 2003). Indeed, the latter introduction may have been the source of material from which Cameron and Webster (1961) undertook their studies and which led to the description of *E. g. canadensis*. However, whether these introductions were the original source of the '*canadensis*/Scandinavian' form in Canada leading to its subsequent maintenance together with a closely related indigenous form is not known.

All our isolates were from Canada, apart from 1 isolate from a moose in neighbouring Washington State, USA, and the majority conformed to the G10 genotype thus questioning which, if either, of the G8 and G10 genotypes is indigenous to North America. Most of our isolates originated from the southern province of Alberta whereas the postulated introduction of *E. granulosus* in domestic reindeer was into north western Canada. Therefore, for an introduced strain to have become the dominant form being transmitted in Alberta seems unlikely. Although in our study, the majority of G10 isolates were from elk, elk were also found to be infected with the G8 genotype. Similarly, the G10 genotype was not restricted to Canadian elk and was also found in moose and a wolf.

The limited morphological results of the present study support those of Sweatman and Williams (1963) who found marked differences in hook length between protoscoleces of sheep and those of cervid (both *E. g. canadensis* [reindeer] and *e. g. borealis* [moose]) origin. The proportions of the strobila of adult worms from a wolf in this study were also similar to those of *E. g. canadensis* and *E. g. borealis* (Sweatman and Williams, 1963). These authors emphasized the long gravid segment of worms of both reindeer and moose origin compared to worms of sheep origin. The characteristically long terminal proglottid seen by Sweatman and Williams (1963) in their worms of cervid origin is a feature shared by *Echinococcus* of cattle origin (*E. ortleppi*) as well as the camel and pig strains which are closely grouped genetically. A major need of future research is to examine adult worms of cervid origin of known age raised in experimentally infected definitive hosts so that their strobilar morphology and reproductive anatomy can be compared with those of described species and strains of *Echinococcus*.

The major question arising from this study is do we really have 2 evolutionary lineages of *Echinococcus* in cervids, and if there are, how prevalent is the G8 genotype and what is its distribution? Only a few isolates of *E. granulosus* from cervids have so far been characterized from Scandinavia, and future research may show that the G8 genotype is not confined to North America. Whilst the present results do not support the existence of geographical variants of *E. granulosus* in cervids, they do raise the question of the status of the 2 strains/genotypes. To date, only a few isolates of the G8 genotype have been characterized from cervids and additional isolates need to be characterized. However, on the basis of the present results, it does appear that 2 genetically distinct forms occur, with both genotypes occurring in moose and elk. From a taxonomic viewpoint, they cannot be considered to represent subspecies due to their sympatric occurrence, and neither the morphological or genetic data would support recognizing the 2 forms as 2 distinct species. It is possible that the G8 genotype has a limited distribution and differs from the G10 genotype in being more virulent than the more widespread G10 genotype. It was the G8 genotype that was recovered from the recent severe clinical case in Alaska (Castrodale *et al.* 2002; McManus *et al.* 2002). However, such a hypothesis requires the genotypic characterization of *Echinococcus* isolates from many more clinical cases, particularly those from asymptomatic individuals. The data would appear to support the existence of only 1 species, which in terms of priority should be

E. canadensis. However, additional isolates from cervids and wolves in additional endemic regions in North America and Northern Eurasia should be characterized before a final decision is made on the taxonomic status of *Echinococcus* in cervids.

Finally, the phylogenetic analyses undertaken in the present study support the close relationships of the cervid, camel and pig strains which is also complemented by the morphological similarities of their adult, strobilar morphology (Thompson and Lymbery, 1988). Consequently, all 3 strains may belong to a single species (Thompson *et al*. 1995; Thompson and McManus, 2001; Xiao *et al*. 2005).

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