A phylogeny of members of the family Taeniidae based on the mitochondrial *cox1* and *nad1* gene data

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SUMMARY

The cestode family Taeniidae consists of 2 genera, *Taenia* and *Echinococcus*, which both have been the focus of intensive taxonomic and epidemiological studies because of their zoonotic importance. However, a comprehensive molecular phylogeny of this family has yet to be reconstructed. In this study, 54 isolates representing 9 *Taenia* species were characterized using DNA sequences in the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes. Phylogenetic relationships within the family Taeniidae were inferred by combining *cox1* and *nad1* sequence data of the present and previous studies. In the phylogenetic analysis, the genus *Echinococcus* was shown to be monophyletic, but *Taenia* proved to be paraphyletic due to the position of *T. mustelae* as a probable sister taxon of *Echinococcus*. This indicates that *T. mustelae* should form a genus of its own. *Taenia ovis krabbei* was placed distant from *T. ovis ovis*, as a sister taxon of *T. multiceps*, supporting its recognition as a distinct species, *T. krabbei*. High intraspecific sequence variation within both *T. polyacantha* and *T. taeniaeformis* suggests the existence of cryptic sister species.

Key words: phylogeny, Taeniidae, *Taenia*, *Echinococcus*.

INTRODUCTION

Taeniid tapeworms (Eucestoda: Cyclophyllidea: Taeniidae) are parasites of mammals, with carnivores as definitive and mostly herbivores as intermediate hosts. The family Taeniidae consists of 2 genera, *Taenia* and *Echinococcus*, which both have a global socioeconomic impact by causing morbidity in humans and domestic livestock (Eckert *et al*. 2001; Hoberg, 2002). Because of their medical and veterinary significance, taeniids have been the focus of intensive epidemiological, ecological and taxonomic studies.

Traditionally, the specific identification of taeniids has been based on morphological criteria, usually taking into account also ecological and biological aspects like host specificity (e.g. Abuladze, 1964). The development of molecular genetic techniques has provided improved tools for the identification of taeniid species and for investigating relationships among them. In particular, mitochondrial DNA sequencing has been successfully used for the identification and genetic characterization of these parasites (e.g. Bowles *et al*. 1992; Bowles and McManus, 1994). To date, whole mitochondrial genomes of 8 *Echinococcus* spp. and 3 *Taenia* spp. have been published (Le *et al*. 2000, 2002; Nakao *et al*. 2002, 2003, 2007; Jeon *et al*. 2005).

Sequence analyses have assisted in the revision of the taxonomy of the genus *Echinococcus* (Le *et al*. 2002; Nakao *et al*. 2007; Hüttner *et al*. 2008). Recently reconstructed, robust molecular phylogenies supported strongly the validation of 9 *Echinococcus* spp. (Nakao *et al*. 2007; Hüttner *et al*. 2008). Several molecular phylogenies of *Taenia* have also been published (e.g. Okamoto *et al*. 1995*a*; de Queiroz and Alkire, 1998; von Nickisch-Rosenegk *et al*. 1999), but unfortunately they all suffer from an insufficient number of species to represent the diversity within this genus. The most comprehensive hypotheses regarding the phylogenetic relationships within *Taenia* were presented by Hoberg *et al*. (2000, 2001) and Hoberg (2006) on the basis of morphological characteristics. Currently, the genus *Taenia* contains 42 valid species (Hoberg, 2006), but most of them are still genetically uncharacterized. Moreover, to date, there has been no comprehensive phylogenetic analysis of members of the family Taeniidae using relatively large sample sizes for both genera in the same analysis. In the current study, we

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Table 1. Hosts and geographical origins of the *Taenia* specimens, and GenBank accession numbers for the partial *cox1* and *nad1* sequences

Sample code	Species	Host	Origin; collected by	Accession numbers	
				cox1	*nad1*
TcSv1	*Taenia crassiceps*	*Vulpes lagopus*	Svalbard, Norway; E. Fuglei	EU544546	EU544599
TcSv2	*T. crassiceps*	*Microtus levis*	Svalbard, Norway; H. Stein	EU544547	EU544600
TcYa	*T. crassiceps*	*Microtus gregalis*	Yamal Peninsula, Russia; V. Fedorov and K. Fredga[3]	EU544548	EU544601
TcBu	*T. crassiceps*	*Microtus fortis*	Kamensk, Buryatia, Russia; H. Henttonen *et al.*	EU544549	EU544602
TcAl	*T. crassiceps*	*Microtus pennsylvanicus*	Fairbanks, Alaska, USA; H. Henttonen *et al.*	EU544550	EU544603
ThFi1	*T. hydatigena*	*Ovis aries*	Suomussalmi, Finland	EU544551	EU544604
ThFi2[1]	*T. hydatigena*	*Rangifer tarandus*	Kuusamo, Finland	EU544552	EU544605
TmaDe1[1]	*T. martis*	*Myodes glareolus*	Flöjstrup, Denmark; H. Henttonen and J. Niemimaa	EU544553	EU544606
TmaDe2	*T. martis*	*Myodes glareolus*	Hörret, Denmark; H. Henttonen and J. Niemimaa	EU544554	EU544607
TmaCr	*T. martis*	*Myodes glareolus*	Migalovci, Croatia; H. Henttonen and J. Niemimaa	EU544555	EU544608
TmaTu	*T. martis*	*Apodemus sylvaticus*	Ayder, Turkey; H. Henttonen *et al.*	EU544556	EU544609
TmaBu	*T. martis*	*Myodes rufocanus*	Utochkina Pad', Buryatia, Russia; H. Henttonen *et al.*	EU544557	EU544610
TmaChi	*T. martis*	*Myodes rufocanus*	Fenglin, Heilongjiang Province, China; E. Kallio	EU544558	EU544611
TmuFi1	*T. mustelae*	*Myodes glareolus*	Hankasalmi, Finland; H. Henttonen *et al.*	EU544559	EU544612
TmuFi2	*T. mustelae*	*Myodes glareolus*	Savonlinna, Finland; H. Henttonen *et al.*	EU544560	EU544613
TmuFi3-4	*T. mustelae*	*Myodes glareolus*	Laihia, Finland; E. Kallio	EU544561-2	EU544614-5
TmuFi5-6	*T. mustelae*	*Myodes glareolus*	Ähtäri, Finland; E. Kallio	EU544563-4	EU544616-7
TmuFi7	*T. mustelae*	*Myodes glareolus*	Pallasjärvi, Finland; H. Henttonen	EU544565	EU544618
TmuFi8	*T. mustelae*	*Myodes rufocanus*	Pallasjärvi, Finland; H. Henttonen	EU544566	EU544619
TmuFi9[1]	*T. mustelae*	*Myodes rutilus*	Pallasjärvi, Finland; H. Henttonen	EU544567	EU544620
TmuIr1-2	*T. mustelae*	*Myodes rutilus*	Lower Tunguska River, Central Siberia, Russia; A. Lavikainen	EU544568-9	EU544621-2
TmuEv1	*T. mustelae*	*Myodes rufocanus*	Lower Tunguska River, Central Siberia, Russia; A. Lavikainen	EU544570	EU544623
TmuEv2[1,2]	*T. mustelae*	*Myopus schisticolor*	Lower Tunguska River, Central Siberia, Russia; A. Lavikainen	EU544571	EU544624
TkSv1[1,2]-8	*T. ovis krabbei*	*Vulpes lagopus*	Svalbard, Norway; E. Fuglei	EU544572-9	EU544625-32
TpaSp[1]	*T. parva*	*Apodemus sylvaticus*	Galicia, Spain; H. Henttonen *et al.*	EU544580	EU544633
TpoTu	*T. polyacantha*	*Microtus guentheri*	Gundalan, Turkey; H. Henttonen *et al.*	EU544581	EU544634
TpoSc	*T. polyacantha*	*Myodes glareolus*	Kielder Forest, Scotland, UK; H. Henttonen and X. Lambin	EU544582	EU544635
TpoDe	*T. polyacantha*	*Myodes glareolus*	Hörret, Denmark; H. Henttonen and J. Niemimaa	EU544583	EU544636
TpoFi1	*T. polyacantha*	*Myodes glareolus*	Lappeenranta, Finland; H. Henttonen *et al.*	EU544584	EU544637
TpoFi2	*T. polyacantha*	*Myodes glareolus*	Pallasjärvi, Finland; H. Henttonen	EU544585	EU544638
TpoFi3[1,2]	*T. polyacantha*	*Microtus oeconomus*	Pallasjärvi, Finland; H. Henttonen	EU544586	EU544639
TpoFi4-6	*T. polyacantha*	*Vulpes vulpes*	Kuusamo, Finland; S. Laaksonen	EU544587-9	EU544640-1
TpoSv1[1,2]-4	*T. polyacantha*	*Vulpes lagopus*	Svalbard, Norway; E. Fuglei	EU544590-3	EU544642-5
TpoGr	*T. polyacantha*	*Dicrostonyx groenlandicus*	Constable Point, Greenland, Denmark; V. Fedorov, H. P. Gelter and G. H. Jarrell	EU544594	EU544646

Table 1. (*Cont.*)

Sample code	Species	Host	Origin; collected by	Accession numbers	
				cox1	*nad1*
TpoCa	*T. polyacantha*	*Lemmus trimucronatus*	Cape Bathurst, Canada; V. Fedorov, K. Fredga, C. J. Krebs and A. Angerbjörn[4]	EU544595	EU544647
TtaTu[1,2]	*T. taeniaeformis*	*Apodemus sylvaticus*	Elmabag, Turkey; H. Henttonen *et al.*	EU544596	EU544648
TtaKa[1,2]	*T. taeniaeformis*	*Apodemus sylvaticus*	Taldykorgan, Kazakhstan; H. Henttonen *et al.*	EU544597	EU544649
TtaFi	*T. taeniaeformis*	*Felis catus*	Porvoo, Finland; A. Lavikainen	EU861478	EU861479
TtwChu[1]	*T. twitchelli*	*Gulo gulo*	Getlyangen Lagoon, Chukotka, Russia; BCP[5]	EU544598	EU544650

[1] The *cox1* and *nad1* sequences of the isolate were included into the phylogenetic analysis.
[2] Heterogeneity of amplified DNA examined by cloning.
[3] Swedish-Russian Tundra Ecology Expedition (1994).
[4] Tundra Northwest 1999 Expedition.
[5] Beringian Coevolution Project.

genetically characterized specimens of 9 *Taenia* spp. by sequencing 2 mitochondrial DNA regions, and investigated variation within the species. Furthermore, we inferred phylogenetic relationships within the family Taeniidae by combining sequence data from the present and previously published studies.

MATERIALS AND METHODS

Parasite specimens and DNA extraction

Fifty-four specimens of *Taenia* (larval and strobilate stages), representing 9 different taxa (*T. crassiceps*, $n=5$; *T. hydatigena*, 2; *T. martis*, 6; *T. mustelae*, 13; *T. ovis krabbei*, 8; *T. parva*, 1; *T. polyacantha*, 15; *T. taeniaeformis*, 3; *T. twitchelli*, 1), were collected from various intermediate and definitive hosts from different geographical regions (Table 1 and Fig. 1). Specimens of *T. hydatigena* were collected during routine meat inspection in slaughterhouses. The rest of the specimens were collected during several research projects (Table 1). The species were identified primarily based on the number, size and shape of the rostellar hooks, according to Verster (1969) and Loos-Frank (2000). All of the samples were fixed and stored in ethanol. Genomic DNA was extracted using the DNeasy™ Tissue Kit (Qiagen) and stored at −20 °C.

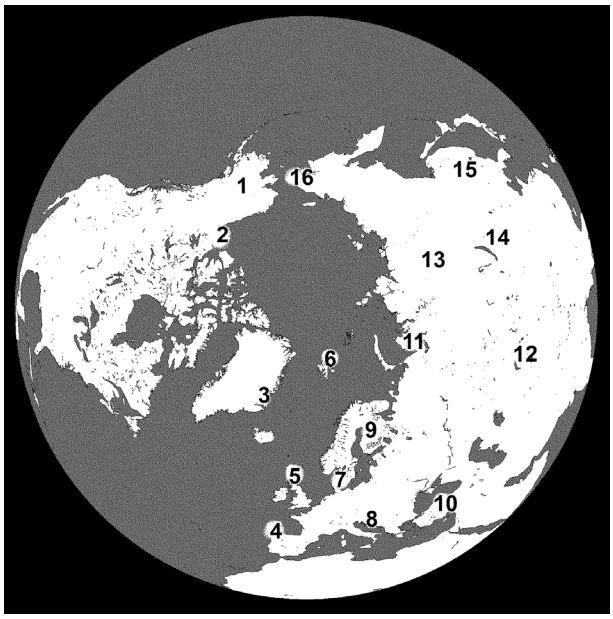

Fig. 1. Sampling locations of *Taenia* spp. in this study. (1) Alaska; (2) northern Canada; (3) Greenland; (4) Spain; (5) Scotland; (6) Svalbard; (7) Denmark; (8) Croatia; (9) Finland; (10) Turkey; (11) Yamal Peninsula; (12) Kazakhstan; (13) Central Siberia; (14) Buryatia; (15) northeastern China; (16) Chukotka. For the detailed locations of the single specimens, see Table 1.

DNA amplification and sequencing

Two mitochondrial DNA regions, including parts of the cytochrome *c* oxidase subunit 1 gene (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes, were amplified using previously published primers (Bowles *et al.* 1992; Bowles and McManus, 1993). PCR and sequencing were carried out as described previously (Lavikainen *et al.* 2006). The sizes of the amplification products were assessed by electrophoresis in 1·5% (w/v) Tris-borate/EDTA agarose gels and ethidium bromide staining. Before sequencing, the amplicons were purified enzymatically with ExoSAP-IT® (USB, Cleveland, Ohio) treatment or excised from the agarose gels and purified using Qiaquick® Gel Extraction Kit (Qiagen). Both strands of DNA were sequenced with the same primers as used for the primary PCR. Because of

difficulties in sequencing *nad1* of *T. parva* in the reverse direction, 2 internal reverse sequencing primers, Tpa1 (5′-ACGGAGTACGATTAGTT-TCACAGA-3′) and Tpa2 (5′-CCATTAAACAA-GCCTCAAACCT-3′), were designed.

Investigation of pseudogene contamination

Because nuclear mitochondrial pseudogenes have been detected previously in *Echinococcus* (Obwaller *et al*. 2004), we examined possible heterogeneity within PCR products from single isolates, which can indicate pseudogene 'contamination' (see Zhang and Hewitt, 1996). For this purpose, amplicons from selected isolates of *T. mustelae*, *T. ovis krabbei*, *T. polyacantha* and *T. taeniaeformis* (shown in Table 1) were excised from the agarose gels, purified (Qiaquick® Gel Extraction Kit, Qiagen) and then cloned using the TOPO TA Cloning® Kit (Invitrogen). Plasmid DNA was purified using QIAprep® Spin Miniprep Kit (Qiagen). The presence of plasmid inserts was confirmed by restriction analysis (*Eco*RI, New England BioLabs). Four clones from each amplicon were sequenced as described previously (Lavikainen *et al*. 2006) using the plasmid-specific sequencing primers M13 forward and M13 reverse.

The cloned sequences were aligned with the directly sequenced PCR products by the Megalign™ module of the DNASTAR Lasergene® software. The theoretical *Taq* polymerase error rate (p) was calculated using the formula $p = 2f/n$, where f is the observed error frequency and n is the number of the cycles (Eckert and Kunkel, 1991). This error rate was then compared with previously published *Taq* error rates (see e.g., Eckert and Kunkel, 1991). The cloned sequences were also examined for frameshift mutations and internal stop codons, which are anomalies of the type commonly associated with pseudogenes (Zhang and Hewitt, 1996).

Calculation of intraspecies variation

To evaluate intraspecific variation, the directly sequenced amplicons were aligned species by species using Megalign. Alignments were modified manually by removing all sites with ambiguous nucleotides. The levels of sequence difference (D), based on pairwise comparisons, were calculated according to Chilton *et al*. (1997) using the formula $D = 1 - (M/L)$, where M is the number of alignment positions at which the 2 sequences have a base in common, and L is the total number of positions over which the sequences are compared. The sequences were also compared with previously published sequences of the same species, if such data of either of the gene regions were available. The level of sequence difference was expressed as percentage ($100\% \times D$).

Phylogenetic analysis

Eleven *Taenia* isolates of this study representing 8 species (directly sequenced amplicons, Table 1), and previously published sequences of 10 *Taenia* spp. and 9 *Echinococcus* spp./genotypes were included into the phylogenetic analysis, with *Hymenolepis diminuta* as an outgroup (Bowles *et al*. 1992; Bowles and McManus, 1993, 1994; Gasser *et al*. 1999; Le *et al*. 2000, 2002; von Nickisch-Rosenegk *et al*. 2001; Nakao *et al*. 2002, 2003; Lavikainen *et al*. 2003; Jeon *et al*. 2005; Xiao *et al*. 2005; Zhang *et al*. 2007; Hüttner *et al*. 2008). The genotype G10 of *E. granulosus sensu lato* was chosen to represent a cluster of closely related genotypes G6-G10. These genotypes have been proposed to form a single species based on mitochondrial DNA sequence data (see e.g., Nakao *et al*. 2007; Moks *et al*. 2008; Hüttner *et al*. 2008). Sequences of 2 isolates from *T. mustelae*, *T. polyacantha* and *T. taeniaeformis* were selected because of the finding of the intraspecific variation. The partial sequences of *cox1* and *nad1* genes were first aligned using ClustalW and further manually adjusted (Thompson *et al*. 1994). Gaps and all codons with ambiguous sites were deleted from the alignments that were then concatenated. The final alignment contained 810 nucleotides.

An evolutionary model for the alignment was selected with Akaike Information Criterion implemented in the Modeltest v.3.06 program (Posada and Crandall, 1998). Modeltest selected the model TVM + I + G to best describe the evolutionary information in the alignment. Transversional model (TVM) rate matrix parameters were a = 1·7351, b = 19·1170, c = 1·5266, d = 10·2675, e = 19·1170, and f = 1·0. The proportion of invariable sites (I) was estimated to be 0·3022 and the alpha parameter of gamma distribution (G) 0·5788. Phylogenies were constructed with PAUP* 4.0b10 (Sinauer Associates Inc., Sunderland, MA, USA) using minimum evolution (ME), maximum parsimony (MP) and maximum likelihood (ML) criteria. Heuristic Tree Bisection Reconnection algorithm was repeated 100 times with a random addition of sequences to construct an ML tree, which was assessed with 100 replicates of bootstrapping. For the ME approach, the neighbor-joining algorithm was used to construct a phylogenetic tree. Statistical support for the nodes in the ME and MP trees was assessed with 1000 replicates of bootstrapping.

RESULTS

Assessing the quality of amplicons

Single amplicons of the expected sizes for both *cox1* and *nad1* were detected on agarose gels for each examined *Taenia* isolate, except for a *T. polyacantha* isolate (TpoFi5) whose *nad1* gene fragment was

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not amplified; *cox1* and *nad1* sequences of 396 bp and 488 bp, respectively, were obtained for each species, with the exception of *T. parva*, which had an *nad1* sequence of 491 bp with ambiguous nucleotides between positions 441 and 446. All nucleotide sequences have been deposited in the GenBank database under the Accession numbers EU544546-EU544650, EU861478 and EU861479 (see Table 1).

To detect potential pseudogenes, sequence heterogeneity within amplicons from selected isolates (Table 1) was examined by cloning. In total, 16 *cox1* clones (6336 bp) and 24 *nad1* clones (11 712 bp) were sequenced. The number of *cox1* clones was smaller, as the amplicons from the *T. mustelae* isolate TmuEv2 and from the *T. polyacantha* isolate TpoFi3 could not be cloned. When sequences of the clones were compared with directly sequenced PCR products, 60% of the *cox1* clones and 50% of the *nad1* clones exhibited no nucleotide differences. The obtained differences were single nucleotide substitutions, typically 1–2 per clone (the maximum was 4 nucleotide differences in a *nad1* clone representing *T. mustelae*). Neither indels nor internal stop codons were detected. For both genes, the substitution frequency was $1{\cdot}4 \times 10^{-3}$. The error rate *per* nucleotide *per* cycle was estimated as 8×10^{-5}, which is in accordance with the published *Taq* error rates which range from $<1 \times 10^{-5}$ to 2×10^{-4} (Eckert and Kunkel, 1991).

Intraspecific variation

Within-species variation in each *cox1* and *nad1* was examined by pair-wise comparison. Nucleotide differences between sequences can be interpreted reliably, because the errors introduced by *Taq* polymerase are not detectable by direct sequencing. A single nucleotide difference between sequences corresponded to differences of 0·3% in *cox1* and 0·2% in *nad1*.

Low intraspecific sequence variation was detected within *T. crassiceps*, *T. martis* and *T. ovis krabbei*, and between the 2 *T. hydatigena* isolates (Table 2). Sequences of *T. crassiceps*, *T. hydatigena* and *T. o. krabbei* were also compared with the previously published sequences for these species. The Alaskan *T. crassiceps* isolate (TcAl) used on this study had identical sequences with that in the complete mitochondrial genome of *T. crassiceps* (see Le *et al.* 2000). The previous *T. hydatigena* isolates differed in sequence from isolates studied herein by 0·3–1·0% in *cox1* and 0·2–1·9% in *nad1* (Okamoto *et al.* 1995*a*; Gasser *et al.* 1999; Nakao *et al.* 2000; Zhang *et al.* 2007). Kedra *et al.* (2001) reported up to 5·5% variation for *nad1* among *T. hydatigena* isolates, but these sequences were not compared in the present study, because of their multiple ambiguous nucleotides and shorter lengths, which most likely exaggerated the variation. The differences between the *T. o. krabbei* and previously published *T. ovis* (presumably *T. ovis ovis*) sequences (Bowles and McManus, 1994; Gasser *et al.* 1999) were as high as 13·4% in *cox1* and 16·4–16·8% in *nad1*.

Table 2. Intraspecific nucleotide sequence variations (%) in *cox1* and *nad1*

Species (no. of specimens)	Variation in *cox1*	Variation in *nad1*
Taenia crassiceps (5)	0·0–0·8%	0·0–1·6%
T. hydatigena (2)	0·8%	0·6%
T. martis (6)	0·0–0·3%	0·0–0·6%
T. ovis krabbei (8)	0·0%	0·0–0·2%
T. mustelae (13)	0·0–3·4%	0·0–5·4%
T. polyacantha (15)	0·0–6·8%	0·0–9·2%
T. taeniaeformis (3)	1·3–9·8%	0·8–11·3%

On the basis of the nucleotide sequence differences (Table 2), the *T. mustelae* isolates were divided into 2 groups. This grouping was concordant with the geographical origins (Finland and Siberia) of the specimens. Sequence variations within the geographical groups were 0·0–0·8% in *cox1* and 0·0–1·4% in *nad1*, whereas between the groups they were 2·3–3·4% and 4·5–5·4%, respectively. The Siberian isolates differed from a previously published *cox1* sequence of *T. mustelae* from Japan by 0·5–0·8% (Okamoto *et al.* 1995*a*), whilst differences between the Finnish and Japanese *T. mustelae cox1* sequences were 3·1–3·4%.

The *T. polyacantha* isolates were also divided into 2 groups (Table 2). The geographically southernmost group contained specimens from Finland, Denmark, Scotland and Turkey, and the northern group from Svalbard, Canada and Greenland. Between the southern and northern groups, the sequence differences were 5·8–6·8% in *cox1* and 9·0–9·2% in *nad1*. The sequence variations within the southern group were 0·0–0·3% in *cox1* and 0·0–0·4% in *nad1*, and within the northern group 0·0–2·8% and 0·0–3·3%, respectively. Within the northern group, the Greenlandic isolate was the most divergent (differing by 2·3–2·8% in *cox1* and 2·9–3·3% in *nad1*), whereas the isolates from Svalbard and Canada were closely related to each other exhibiting differences only up to 0·8% in *cox1* and 1·0% in *nad1*.

Within the 3 *T. taeniaeformis* isolates, remarkable sequence differences were detected (Table 2). The *cox1* sequence of the Kazakhstan isolate (TtaKa) resembled the majority of the previously published *cox1* sequences of *T. taeniaeformis*, whereas the Turkish (TtaTu) and Finnish (TtaFi) isolates were close to a divergent isolate (TtACR) from Japan (Table 3; Okamoto *et al.* 1995*a*).

Table 3. Pairwise comparison of nucleotide sequence differences (%) in *cox1* (362 bp) between the present and previously published *Taenia taeniaeformis* specimens

(In parenthesis, geographical origins of the previously published specimens.)

Samples	Tt1[1] (Australia)	TtBMM[2] (Belgium)	TtKRN[2] (Malaysia)	TtChi[2] (China)	TtSRN[2] (Japan)	TtNop[2] (Japan)	TtACR[2] (Japan)
TtaTu[3]	9·9	9·1	9·1	9·4	8·8	9·1	1·4
TtaFi[3]	10·2	8·8	9·4	9·1	9·1	9·4	1·1
TtaKa[3]	1·9	1·7	1·1	1·9	0·8	3·3	9·7

[1] From Gasser *et al*. (1999).
[2] From Okamoto *et al*. (1995*a*).
[3] Specimens of this study. For details, see Table 1.

Phylogenetic relationships

An heuristic search for the best maximum likelihood (ML) phylogeny resulted in 2 trees that had very similar likelihoods and topologies. The differences in the tree topologies were all in a monophyletic group formed by the genus *Echinococcus*. We addressed the *Echinococcus* group separately using ML criterion with branch and bound algorithm. The resultant topology was identical with that of the *Echinococcus* group in one of the 2 trees found in the heuristic search. This topology was also better supported by the previously published data on the phylogeny of *Echinococcus* (Bowles *et al*. 1995; Nakao *et al*. 2007) and, therefore, only the tree with this topology was considered for further analyses.

Minimum evolution (ME) and maximum parsimony (MP) trees had minor differences to the ML tree presented in Fig. 2. The slight differences were detected within the monophyletic *Echinococcus* group and positions of *T. solium*, *T. pisiformis* and *T. serialis*. In all trees, *T. taeniaeformis* isolates clustered with *T. parva*, and *T. crassiceps* with *T. martis* and *T. twitchelli* (in well-supported clades). In addition, the position of *T. regis* and *T. hydatigena* as sister species was also strongly supported. The bootstrap supports for the node grouping *T. mustelae* with *Echinococcus* were 67% in MP, and 76% in ME trees. Furthermore, the sister species relationship between *T. multiceps* and *T. ovis krabbei* was supported with 91% and 69% (bootstrap proportions) in the MP and ME trees, respectively.

DISCUSSION

The current analysis represents the most comprehensive molecular phylogeny to date for the family Taeniidae. Because the parasite material of the present study was limited and somewhat biased with an over-presentation of some isolates over others, the specification and phylogeography could not be viewed in detail. Despite these limitations, however, some fundamental conclusions could be drawn. The main new findings of this study were the paraphyly of the genus *Taenia* and of the species *T. ovis*, and the high intraspecific variation within *T. polyacantha*. In addition, the previously discovered high genetic variation within *T. taeniaeformis* (see Okamoto *et al*. 1995*a*) was confirmed. These results suggest that a taxonomic revision of *Taenia* at the specific and possibly generic levels is warranted. Partial sequences of the *cox1* and *nad1* genes have been commonly used for genetic characterization of taeniids. Therefore, a relatively large amount of sequence data is available for phylogeny reconstruction. In addition, mitochondrial DNA has proved to be a useful molecular marker in evolutionary biology, in spite of some limitations associated with its use in phylogenetic studies (see e.g., Harrison 1989; Zhang and Hewitt, 1996). One of the problems is the occurrence of mitochondrial-like sequences in the nuclear genome, so-called pseudogenes, which have been discovered in a wide range of taxa, including *Echinococcus* (Obwaller *et al*. 2004). Nuclear mitochondrial pseudogenes can confound phylogenetic studies, particularly if they occur in high copy numbers and universal primers are used. In this study, no evidence of pseudogene contamination was found.

Contrasting phylogenies

In the present analysis, the genus *Echinococcus* was found to be compact and monophyletic. The topology of the *Echinococcus* clade is consistent with the previous studies (e.g. Nakao *et al*. 2007; Hüttner *et al*. 2008) placing *E. oligarthrus* and *E. vogeli* basally, and distinguishing 3 pairs of sister taxa: *E. granulosus sensu stricto* – *E. felidis*, *E. ortleppi* (the genotype G5 of *E. granulosus sensu lato*) –*Echinococcus* sp. (the genotype cluster G6-10 represented by G10 in the current analysis), and *E. multilocularis* – *E. shiquicus*. Molecular phylogenies for *Taenia* presented by Gasser *et al*. (1999) and Zhang *et al*. (2007) are in essence similar to the current analysis, although these prior studies, based on the same gene regions, are more limited in the numbers of species investigated.

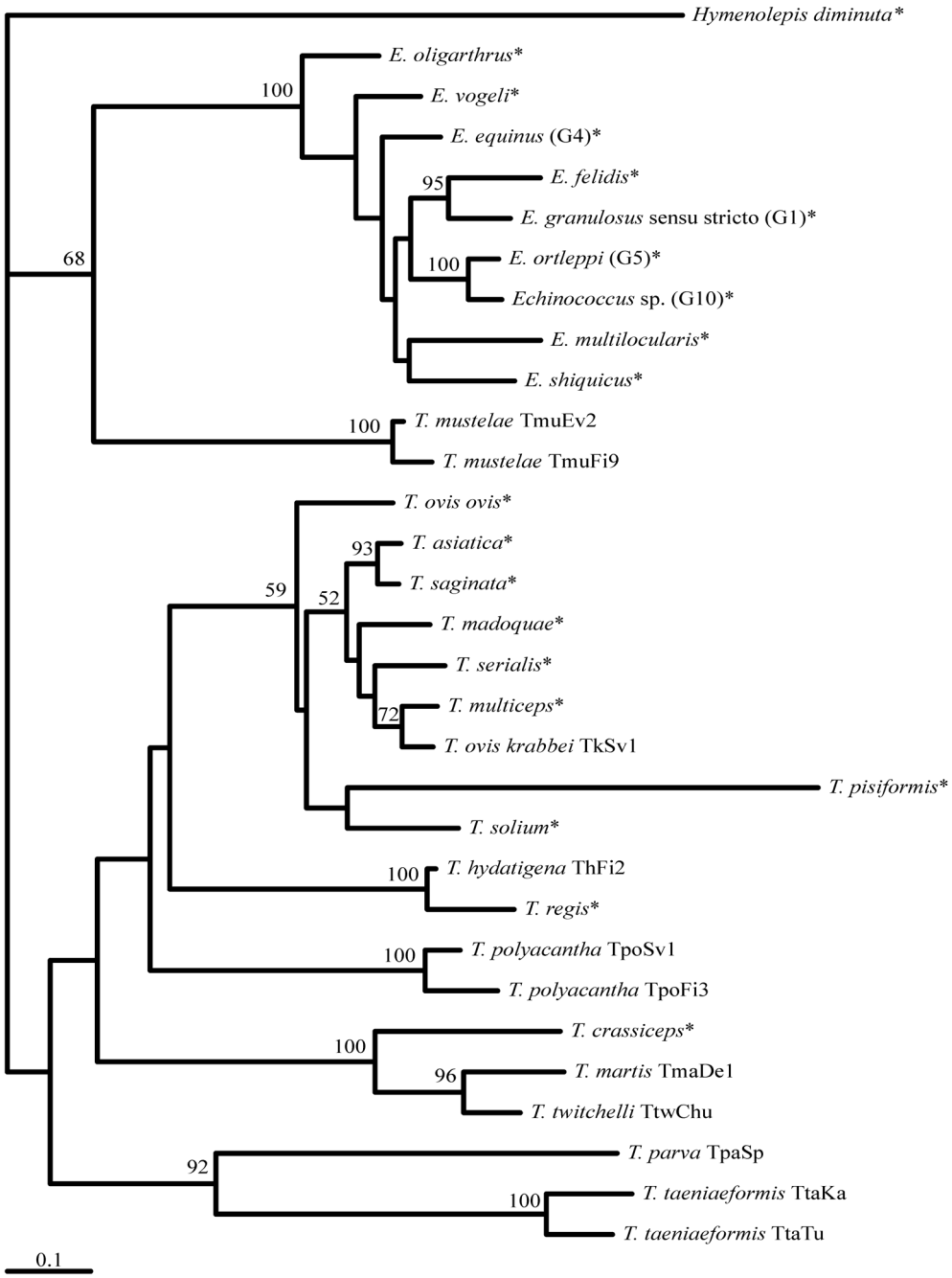

Fig. 2. Maximum likelihood tree inferred from the concatenated sequences of *cox1* and *nad1* genes of Taeniidae and *Hymenolepis diminuta* (outgroup). Bootstrap values >50% are shown. The scale bar is proportional to 0·1 substitutions per site. Abbreviations: *E* – *Echinococcus*; *T* – *Taenia*; G1, G4, G5, G10 – genotypes of *E. granulosus sensu lato*; TmuEv2, TmuFi9, TkSv1, ThFi2, TpoSv1, TpoFi3, TtwChu, TpaSp, TtaKa, TtaTu – isolates of this study (for details, see Table 1). Marked with an asterisks (*) are the selected reference sequences from previous studies (Bowles *et al*. 1992; Bowles and McManus, 1993, 1994; Gasser *et al*. 1999; Le *et al*. 2000, 2002; von Nickisch-Rosenegk *et al*. 2001; Nakao *et al*. 2002, 2003; Lavikainen *et al*. 2003; Jeon *et al*. 2005; Xiao *et al*. 2005; Zhang *et al*. 2007; Hüttner *et al*. 2008).

Thorough morphological phylogenies by Hoberg *et al*. (2000) and Hoberg (2006) are consistent with the present study, particularly in the relatively basal placement of *T. parva*, *T. taeniaeformis*, *T. twitchelli*, *T. martis* and *T. crassiceps*, and in the relationships between them, whereas congruence regarding distal branches of the trees is more limited. In contrast to the current study, *T. taeniaeformis* is placed in the crown of the trees constructed by Okamoto *et al*. (1995*a*) and de Queiroz and Alkire (1998), based on *cox1*, and a combination of *cox1* and a region of nuclear ribosomal DNA, respectively. This is the major difference also between our results and a morphological phylogeny by Moore and Brooks

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(1987). A phylogenetic tree by von Nickisch-Rosenegk *et al*. (1999), based on mitochondrial 12S rDNA data, is quite different from the current phylogeny, except for some similarity in the basal species.

Revising taeniid taxonomy at the generic level

The genus *Taenia* is apparently much more diverse than *Echinococcus*. Some authors have even recognized a number of distinct genera within *Taenia*, based primarily on the morphology of the metacestode (e.g. Abuladze, 1964; Bessonov *et al*. 1994). In agreement with several previous studies (Verster, 1969; de Queiroz and Alkire, 1998; Loos-Frank, 2000; Hoberg *et al*. 2000), our results do not support the recognition of the genera *Taeniarhynchus*, *Multiceps* or *Fimbriotaenia*. *Taeniarhynchus* consists of *T. saginata* (and *T. asiatica*). From the species of this study, *T. multiceps*, *T. serialis* and *T. twitchelli* are included in *Multiceps* (see e.g. Abuladze, 1964), and on the other hand, *T. martis*, *T. mustelae* and *T. twitchelli* into *Fimbriotaenia* (Korniushin and Sharpilo, 1986). According to the present results, these putative genera either are polyphyletic, or their recognition would make *Taenia* paraphyletic. Due to the partly low resolution of the current phylogeny, the status of *Hydatigera* (including *T. taeniaeformis* and *T. parva*) and *Tetratirotaenia* (*T. polyacantha*) remains unsolved. Furthermore, other related genera, such as *Fossor*, cannot be commented on here because of insufficient material.

Several previously proposed taeniid genera were ranked to synonymy with *Taenia* in a landmark taxonomic revision published by Verster (1969). In the same article, *Taenia* was divided into 2 groups on the basis of the relative positions of the genital and osmoregulatory ducts. One of these groups was postulated to be older and parasitize mainly mustelids and viverrids. *Taenia parva*, *T. taeniaeformis*, *T. twitchelli* and *T. martis*, which were included into this group, are basal also in the present phylogeny. However, the placement of *T. crassiceps* among the 'older' species differs from Verster's grouping. Furthermore, one member of this group, *T. mustelae*, is placed outside the genus *Taenia* in the current analysis.

Taenia mustelae is located in the echinococcal branch of the present phylogenetic tree. This is not surprising, because in all available phylogenies of *Taenia*, both morphological and genetic, in which *T. mustelae* has been included, it has been placed as the basal species (Moore and Brooks, 1987; Okamoto *et al*. 1995*a*; de Queiroz and Alkire 1998; Nickisch-Rosenegk *et al*. 1999; Hoberg *et al*. 2000). However, the current placement of *T. mustelae* renders *Taenia* paraphyletic, and consequently raises a need of a generic level revision of Taeniidae. Because of obvious morphological differences of the metacestode and adult parasite, *T. mustelae* cannot be included into *Echinococcus*, but judging by the phylogenetic position, it could form a genus of its own. *Taenia mustelae* differs from most of the other *Taenia* spp. by its numerous and very small rostellar hooks (Loos-Frank, 2000), which, together with additional characteristics, might be used in the generic distinction of *T. mustelae*.

A common feature of *T. mustelae* and *Echinococcus* is the ability for asexual reproduction in the metacestode stage. The larvae of *Echinococcus* are proliferative, whereas 2 larval forms, uniscolex cysticercus and multiscolex coenurus, occur in *T. mustelae*. Coenuri of *T. mustelae* have been reported in various intermediate hosts from North America (Locker, 1955; Freeman, 1956). Future studies may determine whether these different larval forms are associated with genetically distinct lineages. Asexual reproduction occurs also among some other species of *Taenia* (Loos-Frank, 2000). Most of these species were located basally in the present phylogeny, and thus, the potential of scoleces to multiply in the intermediate host seems to be a very basic feature of the family Taeniidae.

Rehabilitating T. krabbei *to an independent species*

Taenia krabbei, described by Moniez in 1879, was lowered in rank to a subspecies of *T. ovis* by Verster (1969). These 2 species were considered to be biologically distinct but morphologically nearly indistinguishable. Recently, the subspecific ranking of *T. o. krabbei* has been defended (Loos-Frank, 2000; Hoberg, 2006), but also evidence supporting its specific status has been presented (Priemer *et al*. 2002). In the current analysis, *T. o. krabbei* proved to be rather distant from *T. ovis*, and was placed as a sister taxon of *T. multiceps*. Judging by the host (sheep) and geographical origin (New Zealand), the former *T. ovis* sequences were from the subspecies *ovis* (see Gasser *et al*. 1999). Thus, the present results strongly support the recognition of *T. krabbei* as a valid species. *Taenia krabbei* has not been implicated in human infections, whereas *T. multiceps* is known to be the causative agent of coenurosis in humans (Hoberg, 2002). The close relationship between these species raises the question as to whether *T. krabbei* could also have zoonotic potential.

Cryptic species within T. taeniaeformis *and* T. polyacantha

Pairs of closely related sister taxa seem to occur commonly among taeniids. Some of these sisters were originally regarded as distinct species, for example, *T. hydatigena* versus *T. regis*, whereas the status of the others is controversial, for example, *E. ortleppi* versus the genotype group G6-G10 of

E. granulosus sensu lato (Lavikainen *et al*. 2006; Moks *et al*. 2008; Hüttner *et al*. 2008). Within the current *T. polyacantha* specimens, and within *T. taeniaeformis* isolates of this and previous studies, a relatively high level of intraspecific sequence variation was detected, in accordance to that demonstrated previously between different species (e.g. Gasser *et al*. 1999; Zhang *et al*. 2007).

In previous studies, a very wide range in the numbers and measurements of rostellar hooks of *T. taeniaeformis* has been reported, which can indicate that more than one species are included (Loos-Frank, 2000). In addition, it has been suggested that a divergent *T. taeniaeformis* isolate, found from the grey-sided vole (*Myodes rufocanis bedfordiae*, former *Clethrionomys r. b.*) from Japan, could be regarded as a distinct species (Iwaki *et al*. 1994; Okamoto *et al*. 1995*a*). This isolate differed from all other *T. taeniaeformis* isolates in various criteria, including morphology, infectivity, protein composition of metacestodes, isoenzyme profiles, DNA fingerprints and mitochondrial *cox1* sequences (Iwaki *et al*. 1994; Azuma *et al*. 1995; Okamoto *et al*. 1995*a*, *b*). It has been proposed that this isolate has adapted to voles as intermediate hosts, and that it is either European in origin, or alternatively indigenous to Hokkaido Island, where it was found (Iwaki *et al*. 1994; Okamoto *et al*. 1995*b*). Two of the *T. taeniaeformis* specimens, a metacestode from a wood mouse (*Apodemus sylvaticus*) from Turkey and a strobilate stage from a domestic cat from Finland, used herein, were genetically close to this isolate. The *T. taeniaeformis* specimen from Kazakhstan resembled closely the other former isolates. A genetic difference between these 2 lineages suggests that they are distinct species.

Two subspecies of *T. polyacantha* have been described based on differences in the numbers and sizes of rostellar hooks: *T. p. polyacantha*, distributed in Eurasia south of the tundra zone, and *T. p. arctica*, present throughout the holarctic tundra (Rausch and Fay, 1988). The present results support this division in that *T. polyacantha* specimens from the Arctic (Svalbard, Greenland and northern Canada) and western Eurasia were genetically clearly distinct. However, the morphological determination of the subspecies was partly inconsistent (data not shown). According to the numbers of the rostellar hooks of the larval stages, the specimens from Greenland and Canada belonged to *T. p. arctica*, but the Danish specimen was also *T. p. arctica* or intermediate, whereas the rest fell into *T. p. polyacantha*. Unfortunately, we did not have metacestodes from Svalbard, and the hook numbers cannot be counted reliably from adults because they fall off easily. The hook lengths of the specimens from Svalbard, however, matched those of *T. p. arctica*. Consequently, it seems that 2 genetically distinct allopatric species/subspecies (West-Eurasian and Arctic) occur, but their morphological characteristics vary more or in a different manner than has been previously described. Furthermore, the degree of genetic difference between these taxa supports their recognition as distinct species.

The current molecular phylogeny for Taeniidae is not complete. More than half of the recognized species of *Taenia* still remains to be genetically characterized. In addition, larger DNA fragments, preferably complete genes, should be sequenced to improve the resolution of analyses. Hence, further studies are required to resolve the phylogenetic relationships and taxonomy within this important cestode family.

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REFERENCES

Abuladze, K. I. (1964). *Essentials of Cestodology. Vol IV. Taeniata of Animals and Man and Diseases Caused by them* (ed. Skrjabin, K. I.). Nauka, Moskow. English translation (1970), Israel Program of Scientific Translations, Jerusalem.

Azuma, H., Okamoto, M., Oku, Y. and Kamiya, M. (1995). Intraspecific variation of *Taenia taeniaeformis* as determined by various criteria. *Parasitology Research* **81**, 103–108.

Bessonov, A. S., Movsessian, S. O. and Abuladze, K. I. (1994). On the classification and validity of superspecies taxons of the cestodes of the suborder Taeniata Skrjabin et Schulz, 1937. *Helminthologia* **31**, 67–71.

Bowles, J., Blair, D. and McManus, D. P. (1992). Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–174.

Bowles, J., Blair, D. and McManus, D. P. (1995). A molecular phylogeny of the genus *Echinococcus*. *Parasitology* **110**, 317–328.

Bowles, J. and McManus, D. P. (1993). NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *International Journal for Parasitology* **23**, 969–972.

Bowles, J. and McManus, D. P. (1994). Genetic characterization of Asian *Taenia*, a newly described taeniid cestode of humans. *American Journal of Tropical Medicine and Hygiene* **50**, 33–44.

Chilton, N. B., Gasser, R. B. and Beveridge, I. (1997). Phylogenetic relationships of Australian strongyloid nematodes inferred from ribosomal DNA sequence data. *International Journal for Parasitology* **27**, 1481–1494.

Eckert, J., Gemmell, M. A., Meslin, F.-X. and Pawlowski, Z. S. (eds) (2001). *WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern*. World Organization for Animal Health and World Health Organization, Paris, France.

Eckert, K. A. and Kunkel, T. A. (1991). DNA polymerase fidelity and the polymerase chain reaction. *PCR Methods and Applications* **1**, 17–24.

Freeman, R. S. (1956). Life history studies on *Taenia mustelae* Gmelin, 1790 and the taxonomy of certain taenioid cestodes from Mustelidae. *Canadian Journal of Zoology* **34**, 219–242.

Gasser, R. B., Zhu, X. and McManus, D. P. (1999). NADH dehydrogenase subunit 1 and cytochrome *c* oxidase subunit I sequences compared for members of the genus *Taenia* (Cestoda). *International Journal for Parasitology* **29**, 1965–1970.

Harrison, R. G. (1989). Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution* **4**, 6–11.

Hoberg, E. P. (2002). *Taenia* tapeworms: their biology, evolution and socioeconomic significance. *Microbes and Infection* **4**, 859–866.

Hoberg, E. P. (2006). Phylogeny of *Taenia*: Species definitions and origins of human parasites. *Parasitology International* **55**, S23–S30. doi: 10.1016/jparint.2005.11.049.

Hoberg, E. P., Alkire, N. L., de Queiroz, A. and Jones, A. (2001). Out of Africa: orgins of the *Taenia* tapeworms in humans. *Proceedings of the Royal Society of London, B* **268**, 781–787. doi: 10.1098/rspb.2000.1579.

Hoberg, E. P., Jones, A., Rausch, R. L., Eom, K. S. and Gardner, S. L. (2000). A phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). *The Journal of Parasitology* **86**, 89–98.

Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J. D. F., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T. and Ito, A. (2008). Genetic characterization and phylogenetic position of *Echinococcus felidis* Ortlepp, 1937 (Cestoda: Taeniidae) from the African lion. *International Journal for Parasitology* **38**, 861–868. doi: 10.1016/j.ijpara.2007.10.013.

Iwaki, T., Nonaka, N., Okamoto, M., Oku, Y. and Kamiya, M. (1994). Developmental and morphological characteristics of *Taenia taeniaeformis* (Batsch, 1786) in *Clethrionomys rufocanus bedfordiae* and *Rattus norvegicus* from different geographical locations. *The Journal of Parasitology* **80**, 461–467.

Jeon, H. K., Lee, K. H., Kim, K. H., Hwang, U. W. and Eom, K. S. (2005). Complete sequence and structure of the mitochondrial genome of the human tapeworm, *Taenia asiatica* (Platyhelminthes; Cestoda). *Parasitology* **130**, 717–726. doi: 10.1017/S0031182004007164.

Kedra, A. H., Tkach, V. V., Swiderski, Z. and Pawlowski, Z. (2001). Intraspecific variability among NAHD dehydrogenase subunit 1 sequences of *Taenia hydatigena*. *Parasitology International* **50**, 145–148.

Korniushin, V. V. and Sharpilo, L. D. (1986). Novyi rod teniid (Cestoda, Taeniidae) – parazitov kun'ikh. *Vestnik zoologii* **20**, 10–16.

Lavikainen, A., Lehtinen, M. J., Laaksonen, S., Ågren, E., Oksanen, A. and Meri, S. (2006). Molecular characterization of *Echinococcus* isolates of cervid origin from Finland and Sweden. *Parasitology* **133**, 565–570. doi: 10.1017/S0031182006000667.

Lavikainen, A., Lehtinen, M. J., Meri, T., Hirvelä-Koski, V. and Meri, S. (2003). Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. *Parasitology* **127**, 207–215. doi: 10.1017/S0031182003003780.

Le, T. H., Blair, D., Agatsuma, T., Humair, P. F., Campbell, N. J., Iwagami, M., Littlewood, D. T., Peacock, B., Johnston, D. A., Bartley, J., Rollinson, D., Herniou, E. A., Zarlenga, D. S. and McManus, D. P. (2000). Phylogenies inferred from mitochondrial gene orders – a cautionary tale from the parasitic flatworms. *Molecular Biology and Evolution* **17**, 1123–1125.

Le, T. H., Pearson, M. S., Blair, D., Dai, N., Zhang, L. H. and McManus, D. P. (2002). Complete mitochondrial genomes confirm the distinctiveness of the horse-dog and sheep-dog strains of *Echinococcus granulosus*. *Parasitology* **124**, 97–112. doi: 10.1017/S0031182001008976.

Locker, B. (1955). The identification of *Taenia tenuicollis* Rudolphi, 1819, in North America. *The Journal of Parasitology* **41**, 51–56.

Loos-Frank, B. (2000). An up-date of Verster's (1969) 'Taxonomic revision of the genus *Taenia* Linnaeus' (Cestoda) in table format. *Systematic Parasitology* **45**, 155–183.

Moks, E., Jõgisalu, I., Valdmann, H. and Saarma, U. (2008). First report of *Echinococcus granulosus* G8 in Eurasia and reappraisal of the phylogenetic relationships of 'genotypes' G5-G10. *Parasitology* **135**, 647–654. doi: 10.1017/S0031182008004198.

Moore, J. and Brooks, D. R. (1987). Asexual reproduction in cestodes (Cyclophyllidea: Taeniidae): Ecological and phylogenetic influences. *Evolution* **41**, 882–891.

Nakao, M., McManus, D. P., Schantz, P. M., Craig, P. S. and Ito, A. (2007). A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* **134**, 713–722. doi: 10.1017/S0031182006001934.

Nakao, M., Sako, Y. and Ito, A. (2003). The mitochondrial genome of the tapeworm *Taenia solium*: a finding of the abbreviated stop codon U. *The Journal of Parasitology* **89**, 633–635.

Nakao, M., Sako, Y., Yokoyamo, N., Fukunaga, M. and Ito, A. (2000). Mitochondrial genetic code in cestode. *Molecular and Biochemical Parasitology* **111**, 415–424.

Nakao, M., Yokoyama, N., Sako, Y., Fukunaga, M. and Ito, A. (2002). The complete mitochondrial DNA sequence of the cestode *Echinococcus multilocularis* (Cyclophyllidea: Taeniidae). *Mitochondrion* **1**, 497–509.

von Nickisch-Rosenegk, M., Brown, W. M. and Boore, J. L. (2001). Complete sequence of the mitochondrial genome of the tapeworm *Hymenolepis diminuta*: gene arrangements indicate that Platyhelminths are Eutrochozoans. *Molecular Biology and Evolution* **18**, 721–730.

von Nickisch-Rosenegk, M., Silva-Gonzalez, R. and Lucius, R. (1999). Modification of universal 12S primers for specific amplification of contaminated *Taenia* spp. (Cestoda) gDNA enabling phylogenetic studies. *Parasitology Research* **85**, 819–825.

Obwaller, A., Schneider, R., Walochnik, J., Gollackner, B., Deutz, A., Janitschke, K., Aspöck, H. and Auer, H. (2004). *Echinococcus granulosus* strain differentiation based on sequence heterogeneity in mitochondrial genes of cytochrome *c* oxidase-1 and NADH dehydrogenase-1. *Parasitology* **128**, 569–575. doi: 10.1017/S0031182004004871.

Okamoto, M., Bessho, Y., Kamiya, M., Kurosawa, T. and Horii, T. (1995*a*). Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequence of the cytochrome *c* oxidase subunit I gene. *Parasitology Research* **81**, 415–458.

Okamoto, M., Ito, A., Kurosawa, T., Oku, Y., Kamiya, M. and Agatsuma, T. (1995*b*). Intraspecific variation of isoenzymes in *Taenia taeniaeformis*. *International Journal for Parasitology* **25**, 221–228.

Posanda, D. and Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Priemer, J., Krone, O. and Schuster, R. (2002). *Taenia krabbei* (Cestoda: Cyclophyllidea) in Germany and its delimitation from *T. ovis*. *Zoologischer Anzeiger* **241**, 333–337.

de Queiroz, A. and Alkire, N. L. (1998). The phylogenetic placement of *Taenia* cestodes that parasitize humans. *The Journal of Parasitology* **84**, 379–383.

Rausch, R. L. and Fay, F. H. (1988). Postoncospheral development and cycle of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). *Annales de Parasitologie Humaine et Comparée* **63**, 263–277 and 334–348.

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

Verster, A. (1969). A taxonomic revision of the genus *Taenia* Linnaeus, 1758 s. str. *Onderstepoort Journal of Veterinary Research* **36**, 3–58.

Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P. M., Craig, P. S. and Ito, A. (2005). *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *International Journal for Parasitology* **35**, 693–701. doi: 10.1016/j.ijpara.2005.01.003.

Zhang, D.-X. and Hewitt, G. M. (1996). Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution* **11**, 247–251.

Zhang, L., Hu, M., Jones, A., Alsopp, B. A., Beveridge, I., Schindler, A. R. and Gasser, R. B. (2007). Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. *Molecular and Cellular Probes* **21**, 379–385. doi: 10.1016/j.mcp.2007.05.003.