A taxonomic revision of the Taeniidae Ludwig, 1886 based on molecular phylogenies

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Academic dissertation

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Contents

Abstract

Taeniid cestodes (family Taeniidae) require a predator-prey relationship between two mammalian hosts to complete their life cycles. The adult taeniid tapeworm occurs in the gastrointestinal tract of a predatory definitive host, and the cystic larva (metacestode) develops in tissues or body cavities of an herbivorous or omnivorous intermediate host. The most pathogenic zoonotic cestodes of humans belong to this family. The metacestode stages of taeniids are causative agents of severe diseases in humans and domestic animals.

Due to the major medical, veterinary and economic importance, taeniids have been a subject of taxonomic, ecological and epidemiological studies. Intensive scrutiny has resulted in contrasting conclusions about the taxonomic diversity within the family. Two genera, *Echinococcus* and *Taenia*, are currently recognized and placed in monotypic subfamilies Echinococcinae and Taeniinae, respectively. Identification of taeniid tapeworms and their taxonomic classification have traditionally been based on morphological criteria. Development of molecular genetic techniques has provided more accurate tools for identification. Especially within *Echinococcus*, molecular studies have revealed so-called cryptic taxa, which are indistinguishable by traditional methods. These previous molecular studies have focused mainly on zoonotic taeniid species and cosmopolitan species in domestic animals.

The knowledge of taxonomy and evolutionary history of taeniids is essential for better understanding of the epidemiology and transmission of these parasites. The aims of the present thesis were to elucidate evolutionary relationships of taeniids, to explore the diversity within the family and to evaluate the taeniid taxonomy on the basis of phylogenetic relationships. Special emphasis was on northern species that have often been neglected in prior molecular genetic studies.

The parasite material was collected from definitive and intermediate host animals in different parts of the Holarctic region, mainly in Eurasia, during the course of various research projects and field expeditions, as well as in routine meat inspection. In addition, a collection of taeniid DNA specimens from various sources worldwide was used in a final large analysis of taeniid phylogeny. Previously published sequence data were also utilized in the analyses. Short mitochondrial DNA (mtDNA) sequences were used as primary markers for molecular genetic characterization of taeniid taxa and for reconstruction of preliminary phylogenies. Longer mtDNA regions or complete genes were used to improve resolution of phylogenies. Finally, phylogenetic trees were constructed using sequence data of mitochondrial genomes and nuclear genes. Divergence between taeniid species and intraspecific variation was evaluated comparing pairwise nucleotide differences. Sequence data were analyzed applying different phylogenetic approaches including distance, parsimony, likelihood and Bayesian methods.

Based on the molecular analyses, cryptic or previously unknown species or intraspecific entities were detected, and the specific status of some taeniid taxa was confirmed:
(1) A new genotypic group, Fennoscandian cervid strain or genotype G10, of *Echinococcus granulosus* sensu lato was discovered in Finnish and Swedish cervids.

It has now been placed within the recently recognized species *E. canadensis* as a genotypic group.
(2) *Taenia polyacantha* and *T. taeniaeformis* were found to be cryptic complexes, both comprising a pair of hidden species.
(3) The phylogenetic position of *T. krabbei* as sister to *T. multiceps* showed that it is a distinct species rather than a subspecies of *T. ovis* as has been proposed previously.
(4) A new species of *Taenia* in a unique bear-moose cycle was found in Finland, and its geographical distribution across the Holarctic was demonstrated. It was shown to be a sister species of the medically significant species *T. solium*. The new species has later been described morphologically and named as *T. arctos.*
(5) An unknown, putatively new, species of *Taenia* was found in lynx in Finland. It was phylogenetically closely related to *T. hydatigena*, *T. kotlani* and *T. regis.* It differed morphologically from the other *Taenia* spp. recorded in felids from the Holarctic region. This species is currently unnamed.

Molecular phylogenetic analyses demonstrated that *T. mustelae* and a clade formed by *T. krepkogorski*, *T. taeniaeformis* and *T. parva* (referred to as clade II) are only distantly related to other members of *Taenia.* Paraphyly of *Taenia* was strongly suggested. In most phylogenetic trees, *T. mustelae* was more closely related to *Echinococcus* than to other *Taenia* spp. In addition, clade II was sister to all other taeniids in a phylogenetic tree inferred from nuclear protein-coding genes.

Based on the phylogenetic relationships, a generic level taxonomic revision was justified. A new genus, *Versteria*, was created for *T. mustelae* and an old genus, *Hydatigera*, was resurrected for clade II. *Versteria*, which includes parasites of mustelids and rodents, is characterized by morphological miniaturization, especially concerning rostellar hooks. The most characteristic features of *Hydatigera* are large rostellar hooks, a strobilocercus type metacestode and a felid/viverrid-rodent life cycle. The remaining species of *Taenia* were included in *Taenia* sensu stricto, which is monophyletic but a highly diversified assemblage. *Echinococcus* is a compact monophyletic group, in which close genetic relationships imply recent speciation.

The present thesis clarifies the taxonomy of the Taeniidae and creates a framework for further phylogenetic studies, possible additional revisions and comparative research.

List of publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I–VIII):

I **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.

II **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.

III **Lavikainen, A.**, Haukisalmi, V., Lehtinen, M. J., Henttonen, H., Oksanen, A. and Meri, S., 2008. A phylogeny of members of the family Taeniidae based on the mitochondrial *cox1* and *nad1* gene data. *Parasitology* 135: 1457–1467.

IV **Lavikainen, A.**, Haukisalmi, V., Lehtinen, M. J., Laaksonen, S., Holmström, S., Isomursu, M., Oksanen, A. and Meri, S., 2010. Mitochondrial DNA data reveal cryptic species within *Taenia krabbei*. *Parasitol. Int.* 59: 290–293.

V **Lavikainen, A.**, Laaksonen, S., Beckmen, K., Oksanen, A., Isomursu, M. and Meri, S., 2011. Molecular identification of *Taenia* spp. in wolves (*Canis lupus*), brown bears (*Ursus arctos*) and cervids from North Europe and Alaska. *Parasitol. Int.* 60: 289–295.

VI **Lavikainen, A.**, Haukisalmi, V., Deksne, G., Holmala, K., Lejeune, M., Isomursu, M., Jokelainen, P., Näreaho, A., Laakkonen, J., Hoberg, E. and Sukura, A., 2013. Molecular identification of *Taenia* spp. in the Eurasian lynx (*Lynx lynx*) from Finland. *Parasitology* 140: 653–662.

VII Nakao, M., **Lavikainen, A.**, Iwaki, T., Haukisalmi, V., Konyaev, S., Oku, Y., Okamoto, M. and Ito, A., 2013. Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): Proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. *Int. J. Parasitol.* 43: 427–437.

VIII Nakao*, M., **Lavikainen*, A.**, Yanagida, T. and Ito, A., 2013. Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). *Int. J. Parasitol.* 43: 1017–1029.
*Authors contributed equally to this work.

Abbreviations

atp6	ATP synthase subunit 6
bp	Base pair(s)
cox1	Cytochrome *c* oxidase subunit I
DNA	Deoxyribonucleic acid
G1–G10	10 genotypes of *E. granulosus* s.l.
G1	A genotype of *E. granulosus* s.l., corresponds to the sheep strain
G2	A genotype of *E. granulosus* s.l., corresponds to the Tasmanian sheep strain
G3	A genotype of *E. granulosus* s.l., corresponds to the buffalo strain
G4	A genotype of *E. granulosus* s.l., corresponds to the horse strain
G5	A genotype of *E. granulosus* s.l., corresponds to the cattle strain
G6	A genotype of *E. granulosus* s.l., corresponds to the camel strain
G7	A genotype of *E. granulosus* s.l., corresponds to the pig strain
G8	A genotype of *E. granulosus* s.l., corresponds to the cervid strain
G9	A genotype of *E. granulosus* s.l., corresponds to an unnamed Polish strain
G10	A genotype of *E. granulosus* s.l., corresponds to the Fennoscandian cervid strain
gen. nov.	Genus novum, new genus
ITS-1	First internal transcribed spacer of ribosomal DNA
JTT	Jones-Taylor-Thornton (a model of amino acid change, Jones et al. 1992)
KP2	Kimura 2-parameter (a model of nucleotide change, Kimura, 1980)
ME	Minimum evolution
ML	Maximum likelihood
MP	Maximum parsimony
mtDNA	Mitochondrial DNA
nad1	NADH dehydrogenase subunit 1
nad3	NADH dehydrogenase subunit 3
nDNA	Nuclear DNA
NJ	Neighbor-joining
PCR	Polymerase chain reaction
pepck	Phosphoenolpyruvate carboxykinase
pold	DNA polymerase delta
QP	Quartet puzzling
rDNA	Ribosomal DNA
s.l.	Sensu lato; in the broad sense
sp.	Species
sp. nov.	Species novum, new species
spp.	Species (plural)
s.s.	Sensu stricto; in the strict sense

1 Introduction

Cestodes of the family Taeniidae Ludwig, 1886 (Eucestoda: Cyclophyllidea van Beneden in Braun, 1900) are parasites of terrestrial mammals, characteristically occurring as adult tapeworms in predatory definitive hosts, and developing as larval (metacestode) stages in their prey. The most pathogenic zoonotic cestodes of humans belong to taeniids. As causative agents of serious diseases in humans and production losses in domestic livestock, taeniids are of major public health and economic importance (Abuladze, 1964; Eckert et al. 2001; Hoberg, 2002; Murrell, 2005).

The control of zoonoses requires a solid knowledge of the taxonomy and life cycles of the causative agents. Due to their global significance, taeniids have been the focus of intensive taxonomic, ecological and epidemiological studies. A variety of contrasting views of the taxonomic diversity within the family has been presented (e.g. Abuladze, 1964; Verster, 1965, 1969; Rausch, 1967, 1985, 1994b; Wardle et al. 1974). Currently, the validity of two genera, *Echinococcus* Rudolphi, 1801 and *Taenia* Linnaeus, 1758, is widely recognized (Rausch, 1994b). The taeniid taxonomy and identification have been based traditionally on morphological criteria, often completed with some ecological aspects, such as host specificity. Advances in molecular genetic techniques and bioinformatics have provided accurate and effective tools for identification and classification of organisms and for reconstruction of their evolutionary relationships from higher taxa to species and populations (Avise, 2000, 2004; Kunz, 2002). However, prior to the studies comprising this thesis, molecular phylogenies of taeniids narrowly focused either on the genus *Echinococcus* or *Taenia*, and mainly included common zoonotic species and cosmopolitan species in domestic animals (e.g. Bowles et al. 1995; Gasser et al. 1999). Taeniids in wildlife, especially in northern regions, received little attention.

In this thesis, several species of lesser-known taeniids from the Holarctic region are genetically characterized and their phylogenetic relationships are examined. Finally, a taxonomic revision of the genera within the Taeniidae is presented and lists of valid species are updated on the basis of molecular phylogenies. In addition to the present revision, the studies of this thesis serve as a springboard for further revisions at the specific and possibly generic levels, and have already provided a molecular basis to the description of one new species, *Taenia arctos* Haukisalmi, Lavikainen, Laaksonen & Meri, 2011.

The present thesis work promotes the organizing of the diversity within the Taeniidae and elucidates evolution of this significant group of cestodes. Both of these objectives are essential for better understanding of the epidemiology and transmission of these parasites. Furthermore, DNA sequence data published during the course of this long-term project are of diagnostic value making the identification of taeniid species more straightforward and accurate.

2 Review of the literature

2.1 Mammalian hosts of taeniid cestodes

Taeniids are unique among cestodes because they need two obligate mammalian hosts to complete their life cycles (Rausch, 1994b). Each species has a characteristic cycle maintained by a specific predator-prey association between carnivorous (or omnivorous) definitive (final) hosts and herbivorous (or omnivorous) intermediate hosts (Abuladze, 1964; Hoberg et al. 2000; Loos-Frank, 2000).

Carnivorans, mainly canids and to a lesser extent felids, serve as definitive hosts for *Echinococcus* (Thompson, 1995). In addition, adult stages of *Echinococcus* have been reported in hyenas (Nelson and Rausch, 1963; Hüttner et al. 2009). The repertoire of definitive hosts for *Taenia* is more diverse. The majority of *Taenia* spp. use canids or felids as definitive hosts (Abuladze, 1964; Loos-Frank, 2000). Furthermore, several *Taenia* spp. parasitize mustelids or hyaenids, whereas viverrids and procyonids are preferred only by a single species each (Loos-Frank, 2000; Rausch, 2003). The only non-carnivoran definitive hosts of *Taenia* are humans with three host specific species of their own, namely *Taenia solium* Linnaeus, 1758, *Taenia saginata* Goeze, 1782 and *Taenia asiatica* Eom & Rim, 1993. These species are referred to as the human-*Taenia* (Hoberg, 2002).

Most taeniids use ruminants (particularly bovids) or various rodents as principal intermediate hosts (Loos-Frank, 2000; Eckert et al. 2001). A few species use lagomorphs (Loos-Frank, 2000; Xiao et al. 2005). Camelid and equid intermediate hosts are rare (Loos-Frank, 2000; Eckert et al. 2001). Suids are also rare intermediate hosts, but of special medical importance since they are associated with transmission of *T. solium* and *T. asiatica* (Hoberg et al., 2000; Murrell, 2005). Primates are generally considered incidental intermediate hosts of taeniids, except for *Taenia saigoni* Le-Van-Hoa, 1964, whose metacestode stage has been reported only in a macaque species *Macaca fascicularis* (syn. *Macacus cynomolgus*) (Loos-Frank, 2000; Eckert et al. 2001).

Apart from typical hosts involved in the life cycles, taeniid species are often able to infect, as adults or metacestodes, various alternative hosts (Loos-Frank, 2000; Eckert et al. 2001). Metacestodes of *Echinococcus* mainly have low host specificity, actually lower than those of larval *Taenia*, but adult stages are rather host specific (Cameron, 1956; Thompson, 1995). Similar difference in host specificity between stages seems to exist among *Taenia* spp. because the range of their intermediate hosts is often more diverse than that of their definitive hosts (Verster, 1969; Loos-Frank, 2000). Therefore, intermediate host specificity should not be the sole criterion for the diagnosis of a species, and subspecific ranking could be preferable for forms showing different intermediate host preferences (Verster, 1969).

Many of the reported atypical hosts of taeniids may represent ‘dead ends’, i.e. aberrant or accidental hosts, which do not interact in the transmission cycle, or in which the parasite cannot develop or reproduce properly. On the other hand, susceptible atypical hosts can provide niches for colonization enabling adaptation to new life cycles and host switching, which are key processes in speciation and

diversification of parasites (Hoberg, 2000; Emelianov, 2007). A classical example of a recent successful adaptation to a new life cycle comes from Australia, where an introduced taeniid species, *Echinococcus granulosus* (Batsch, 1786), originally a parasite of dogs and sheep, occurs in a sylvatic cycle involving mainly dingoes (*Canis lupus dingo*) as definitive hosts and indigenous macropodid marsupials as intermediate hosts (Jenkins and Macpherson, 2003). A recent experimental study has shown that metacestodes of *E. granulosus* actually develop faster in macropodids than they do in sheep (Barnes et al. 2011).

2.2 General life history and reproduction modes

2.2.1 Adult stage

The adult (strobilar) stage of taeniids occurs in the small intestine of the definitive host. Usually the infection, referred to as taeniosis/taeniasis or echinococcosis, is harmless to the definitive host and causes only mild local inflammation in the intestine, although various symptoms and rarely also complications have been reported (Abuladze, 1964; Thompson, 1995; García et al., 2003). Adult taeniid tapeworms vary considerably in length, ranging from less than two millimeters of the smallest species of *Echinococcus* up to more than ten meters of the largest species of *Taenia* (Abuladze, 1964; Xiao et al. 2005). In general, the adult taeniids have similar structure, although *Taenia* spp. have a ribbon-like strobila consisting of many proglottids, and *Echinococcus* spp. are tiny with not more than seven proglottids (Fig. 1) (Rausch, 1994b). The anterior end of the worm is the scolex, a globular holdfast organ, which has always four suckers and usually a cone-like rostellum armed with a crown of chitinized hooks (Fig. 1.3) (Wardle and McLeod, 1952; Rausch, 1994b). The scolex anchors the worm in the intestinal mucosa. Behind the scolex, there is an unsegmented neck region, where proglottisation, i.e. generation of proglottids, takes place (Wardle and McLeod, 1952; Abuladze, 1964). The proglottid production is a method of asexual multiplication of the adult stage (Whitfield and Evans, 1983). It increases the egg production by continuous proliferation of sexually reproducing units. New proglottids displace the old ones, which will finally be filled with eggs and shed from the posterior terminus of the worm (Smyth, 1994).

2.2.2 Fertilization

As most of the cestodes, the taeniids are hermaphrodites. Sexual reproduction of taeniids may occur by both self- and cross-fertilization. The mode of fertilization has a fundamental influence on the genetic variation and speciation (Smyth and Smyth, 1964; Rausch, 1985; Lymbery, 1992), and has therefore been scrutinized, especially within the medically important genus *Echinococcus*. It is still, however, arguable whether selfing or outcrossing predominates in *Echinococcus* (Haag et al. 2011), and very little is known about the fecundation of other taeniids.

Smyth and Smyth (1964) suggested that selfing is the main method of fertilization in *Echinococcus.* Together with extensive asexual proliferation of metacestodes, it could promote expression of mutants, their rapid selection and establishment in new hosts. This has become a widely cited explanation for the extensive variation within

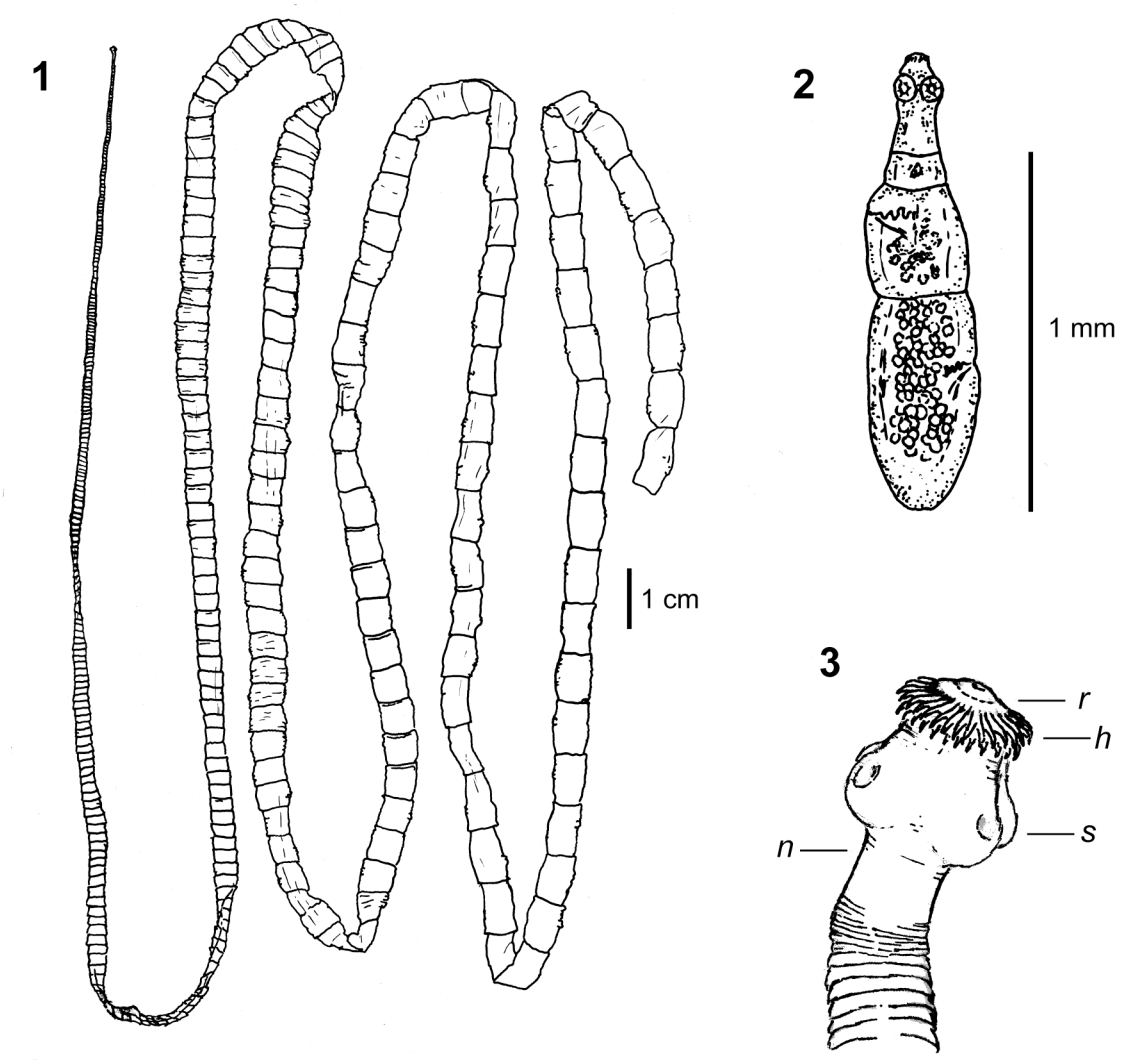

Figure 1. General structure of adult taeniid tapeworms. (1) The strobilar stage of a species of *Taenia* from the spotted hyena (*Crocuta crocuta*); (2) the strobila of *Echinococcus shiquicus*; (3) the scolex of *Taenia laticollis*; *h*, rostellar hooks; *n*, neck; *r*, rostellum; *s*, suckers.

Echinococcus, and has provided a model for the process of speciation (Smyth and Smyth, 1964; Haag et al. 2011). In theory, self-fertilizing hermaphrodites, such as parasitic cestodes, may speciate instantaneously and sympatrically without geographic variation (Mayr, 1949). However, obligatory or habitual self-fertilization increases homozygosity and makes formation of favourable gene combinations difficult thereby inflicting a loss of evolutionary plasticity (Dobzhansky, 1959). Rausch (1985) suggested that taeniids mainly reproduce by cross-fertilization, which may maintain adaptability and mediate gene flow leading to intraspecific phenotypic uniformity in natural populations. In that case, speciation would mainly be allopatric. Great degree of intraspecific variation within some species of *Taenia* and *Echinococcus* would be due to selection, and would associate with synathropy and recent adaptation to different domesticated hosts (Rausch, 1985).

The evidence, which has been presented on the fertilization method in taeniids, is contrasting. Adults of some species of *Taenia* usually occur alone in the small intestine of the final host having no other choice than to self-fertilize (Pawlowski, 2002; de Meeûs, 2003; Yamane et al. 2012), whereas some other taeniid species aggregate in large numbers within specific areas of the host intestine ensuring close contacts of strobilae and cross-fertilization (Rausch, 1985). Self-insemination between two proglottids of the same strobila may take place in *Taenia*, but is unlikely in *Echinococcus* because of the small size of the worms and absence of two mature proglottids in a single strobila (Kumaratilake et al. 1986). Self-insemination by a single proglottid has been observed by light microscopy in *Echinococcus* spp. (Leuckart, 1863; Smyth and Smyth, 1969; Kumaratilake et al. 1986), while cross-insemination has never been directly documented.

Several genetic studies have provided evidence for predominance of selfing (such as high degree of homozygosity at several loci and linkage disequilibrium) in *E. granulosus*, *Echinococcus multilocularis* Leuckart, 1863, *T. asiatica* and *T. saginata*, but have also reported the presence of heterozygotes indicating that low levels of outcrossing occurs as well (e.g. Lymbery et al. 1990, 1997; Haag et al. 1998, 1999; Nakao et al. 2003; Badaraco et al. 2008; Okamoto et al. 2010). Recent contrasting results, however, suggest a major role for outcrossing at least in *E. granulosus* (Haag et al. 2011). Furthermore, in *Echinococcus*, population genetic effects typical to selfing can be explained by cross-fertilization between clonal descendants of a single proliferative metacestode (Smyth, 1969; Moore, 1981; Lymbery et al. 1997). It seems that the breeding system of *Echinococcus* (or generally that of the taeniids) could be defined as a balance between cross- and self-fertilization, which keeps the parasite evolving in a patchy and heterogeneous environment by maintaining adaptability and enabling rapid response to host selection (Haag et al. 1998, 1999, 2011).

2.2.3 Oncosphere and metacestode stage

Taeniid eggs are practically uniform in morphology differing from other cyclophyllidean eggs especially by their characteristic thick, striated embryophore (Abuladze, 1964; Smyth, 1969; Fairweather and Threadgold, 1981; Rausch, 1994b). Eggs or egg-filled gravid proglottids are passed to environment with faeces of the final host, and ingested by the intermediate host, for example, with contaminated plant material or water (Abuladze, 1964; Lawson and Gemmel, 1983). The oncosphere, so-called hexacanth (‘six-hooked’) embryo, hatches in the small intestine of the intermediate host and actively penetrates the intestinal wall (Wardle and McLeod, 1952; Smyth, 1969; Thompson, 1995). It is carried by blood or lymphatic circulation to the specific predilection site, where development of the metacestode takes place (Heath, 1971; Garrido et al. 2007).

Taeniid metacestodes can parasitize various internal organs and tissues or develop free in body cavities (Abuladze, 1964; Loos-Frank, 2000). Organ involvement reflects the predator-prey association aiming at ingestion by the correct final host. An extreme example is the metacestode of *Taenia olngojinei* Dinnik & Sachs, 1969, which occurs in the sacral epidural space of Alcelaphinae antelopes ensuring access only for the bone-cracking hyaenids (Dinnik and Sachs, 1969; Jones and Pybus, 2001). In addition

to typical species-specific locations, taeniid metacestodes can be found in various other organs (Abuladze, 1964), especially in aberrant hosts.

The basic form of the taeniid metacestode is the cysticercus, a bladder-like cestode larva with an invaginated scolex (Chervy, 2002). A variety of terms have been utilized to describe modifications of cysticerci (Chervy, 2002). The following types are generally recognized (Fig. 2): (1) cysticercus; (2) coenurus, a polycephalic cysticercus; (3) strobilocercus, a strobilate metacestode with segmentation, a well-developed scolex and terminal bladder; (4) fimbriocercus, an elongated solid-bodied larva; (5) echinococcus, a cystic or multicystic structure enveloped by a laminated layer and producing protoscoleces in brood capsules (includes cystic, alveolar and polycystic forms) (Rausch 1994b; Hoberg 2000; Chervy 2002; D'Allesandro and Rausch 2008). In addition, the term 'hydatid' is widely used for the last (5) type (Freeman, 1973; Pawlowski, 1997). The metacestode structure has been used as a primary criterion in establishing certain taeniid genera, but its taxonomic significance has been questioned (Rausch, 1959; Hoberg, 2000).

Taeniid infections in intermediate hosts caused by larval stages, especially in humans and domestic animals, are called after the respective metacestode type (e.g. cysticercosis, coenurosis and echinococcosis/hydatidosis) (Pawlowski, 1997; Hoberg, 2002). Infections often remain asymptomatic, but can also cause various organ dysfunctions or even death (Abuladze, 1964; Eckert *et al*. 2001). The medical, veterinary and economic importance of taeniids is almost completely confined to infections with the metacestode stage (Eckert *et al*. 2001; Hoberg, 2002).

2.2.4 Asexual multiplication of the metacestode

Asexual reproduction of the metacestode stage is rare among cestodes in general (Whitfield and Evans, 1983; Mackiewicz, 1988). It appears in less than 1% of the cestode species representing only six (Whitfield and Evans, 1983; Mackiewicz, 1988) of over 70 families recognized in a summary of cestode taxonomy (Georgiev, 2003). Most of these species belong to cyclophyllideans, and within those predominantly to taeniids (Whitfield and Evans, 1983). Among taeniids, asexual multiplication is common appearing in all species of *Echinococcus* and in one fourth of the species of *Taenia* (Rausch 1994b; Loos-Frank, 2000).

Asexually proliferating forms have been observed in all types of taeniid metacestodes, and proliferation can occur in several ways (Šlais, 1973; Hoberg et al. 2000). Briefly, these include: multiplication of scoleces from infolds or external buds of the bladder wall or from internal buds of brood capsules; proliferation of vesicles by exogenous or endogenous budding or by transverse fission; and metastatic formation of secondary metacestodes (Rausch, 1954, 1959; Freeman, 1962; Opuni, 1970; Šlais, 1973; Eckert et al. 1983). Reproductive potential is greatest in *Echinococcus*, especially in *E. multilocularis*, which possesses a nearly unlimited capacity of proliferation using most of the aforementioned mechanisms (Rausch, 1954; Eckert et al. 1983). The diversity of proliferative forms and their presence in phylogenetically unrelated species suggest multiple and independent origins of asexual reproduction in taeniids (Moore and Brooks, 1987; Hoberg et al. 2000; Trouvé et al. 2003).

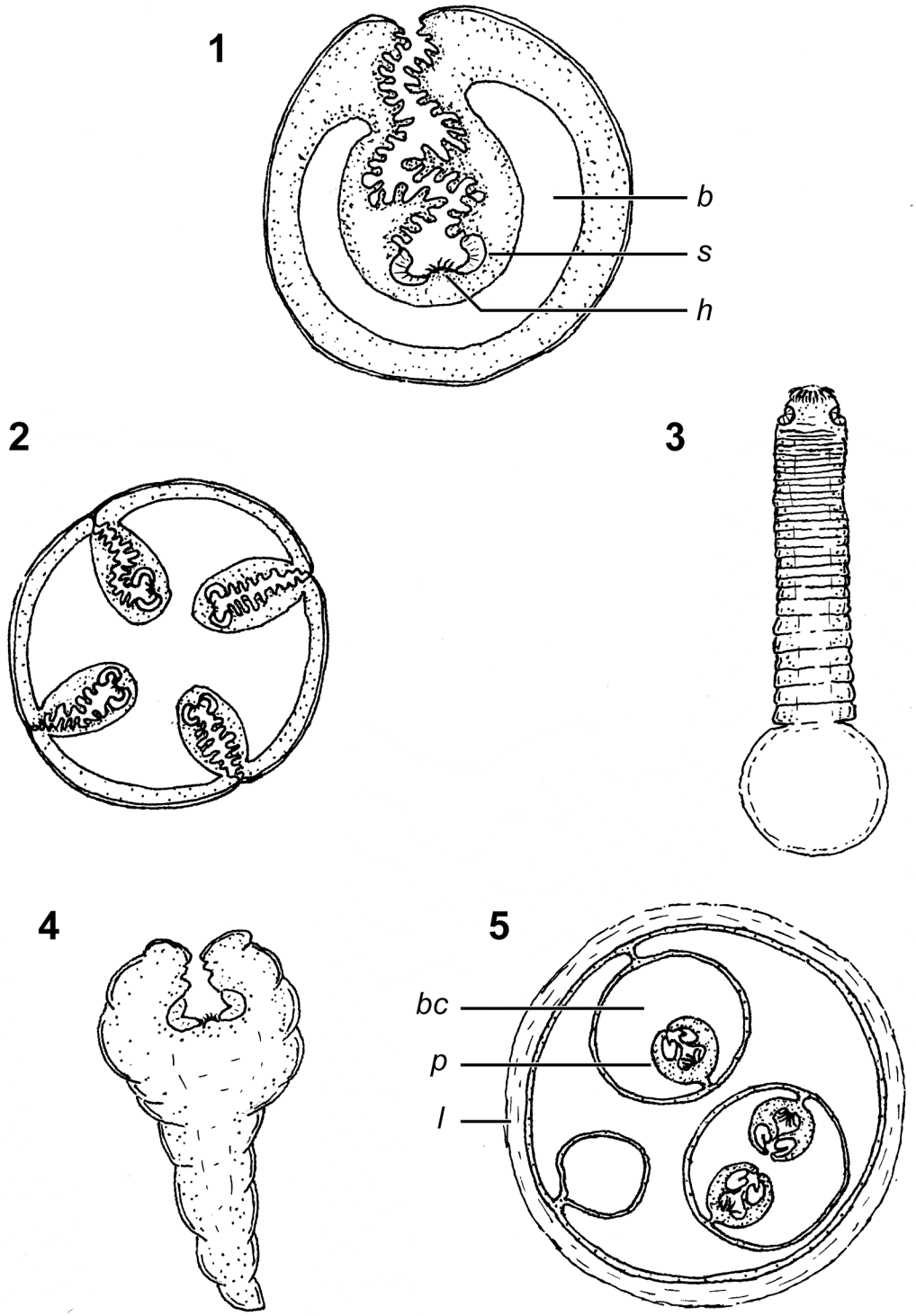

Figure 2. Types of taeniid metacestodes. (1) Cysticercus; *b*, bladder; *h*, rostellar hooks; *s*, sucker; (2) coenurus; (3) strobilocercus; (4) fimbriocercus; (5) echinococcus; *bc*; brood capsule; *l*, laminated layer; *p*, protoscolex. The drawings have been modified from Šlais (1973), Thompson (1995) and Chervy (2002).

Asexual multiplication of taeniid metacestodes is associated with a relatively small size and short lifespan of the adult stage (Moore, 1981). Proliferation at the larval stage could thus compensate for the decrease in fecundity of adults (Moore, 1981). An asexually reproducing taeniid species may act as a colonial organism, which collectively produces many more eggs than a solitary large-sized worm but may also require a large infrapopulation for survival in a final host (Moore, 1981; Mackiewicz, 1988). From an evolutionary point of view, asexual proliferation may have a fundamental role in speciation since multiplication of successful variants in new intermediate hosts promotes host switching (Smyth and Smyth, 1964).

2.3 Taxonomy of the Taeniidae

Taeniids, in particular *Taenia* spp., are among the oldest known helminths, with written records and the history of the term 'Taenia' reaching into antiquity (Wardle and McLeod, 1952). During the Linnaean times, various tapeworms were placed in the genus *Taenia*, a 'ragbag of miscellaneous forms' as defined by Wardle and McLeod (1952). The picture was further complicated by classifying adults and larvae of single species into different genera prior to discovering the life history of taeniids (Abuladze, 1964). The old-fashioned Latin names for the metacestode types date back to that period. In this section, the major conflicts and the most widely accepted consensus on the taeniid taxonomy in the last century are briefly outlined.

2.3.1 Higher taxonomic levels

The Cyclophyllidea, established in the beginning of the 20th century, is the taxonomically most diverse order of cestodes currently including 18–19 families, about 380 genera and 3,100 species (Hoberg et al. 1999; Georgiev, 2003). This order thus represents more than half of the cestode biodiversity. Adult cyclophyllideans are parasites in tetrapods, mostly in birds and mammals, and larval stages occur in invertebrates and/or vertebrates (Georgiev, 2003).

The family Taeniidae was invented to replace similar earlier terms for a diverse group of tapeworms (Wardle and McLeod, 1952; Abuladze, 1964). By the end of the 19th century, the Taeniidae was a very large taxonomic group of approximately 2,000 species, but since then it has been reduced in size by synonymization and the transfer of genera and species to other taxa (Wardle and McLeod, 1952; Wardle et al. 1974). Especially Russian researchers have widely adopted division of cyclophyllideans to suborders (e.g. Spasskii, 1951; Abuladze, 1964), where the Taeniidae is classified as the sole family in the suborder Taeniata Skrjabin & Shul'ts, 1937. In addition, two taeniid subfamilies have been generally recognized: Taeniinae Stiles, 1896 for *Taenia* (other genera with a ribbon-like strobila also included by some authors) and Echinococcinae Abuladze, 1960 for *Echinococcus* (e.g. Abuladze, 1964; Rausch, 1994b). Ordinal level revisions, such as removal of the taeniids with their supposed relatives from the Cyclophyllidea to a distinct family (Freeman, 1973) or promotion of the taeniids to ordinal status (Wardle et al. 1974), have been poorly justified and have thus not received general acceptance.

2.3.2 Taxonomy of taeniid genera

During the 20th century, the main disagreements between different concepts for taeniid taxonomy concerned the number of genera in the family (examples provided in Table 1), the number of valid species within genera and the criteria used to specify these taxa (e.g. Abuladze, 1964; Verster, 1969).

Wardle and McLeod (1952) recognized seven valid genera within the Taeniidae (*Taenia*; *Anoplotaenia* Beddard, 1911; *Cladotaenia* Cohn, 1901; *Echinococcus*; *Hydatigera* Lamarck, 1816; *Multiceps* Goeze, 1782; *Taeniarhynchus* Weinland, 1858), and listed four genera (*Dasyurotaenia* Beddard, 1912; *Fossor* Honess, 1937 [homonymous; later replaced with *Monordotaenia* Little, 1967]; *Insinuarotaenia* Spasskii, 1948; *Paracladotaenia* Yamaguti, 1935), whose taxonomic status or affinities with the Taeniidae were regarded unclear or doubtful. A couple of similar opinions with multiple valid genera have been published (reviewed in Rausch, 1985). Among these, the broadest and most cited concept was presented by Abuladze (1964) (Table 1), who included in the Taeniidae all the aforementioned 11 genera plus two additional genera, *Alveococcus* Abuladze, 1960 and *Tetratirotaenia* Abuladze, 1964. Bessonov et al. (1994) defended this taxonomy with a minimal modification (*Dasyurotaenia* was ignored).

Rausch (1959) stated that the establishment of taeniid genera based on metacestode characteristics is untenable due to the pleomorphism of the larval structure. Thus *Alveococcus*, previously created for *E. multilocularis* with alveolar hydatid metacestode, was subsumed as a synonym within *Echinococcus* (Rausch and Nelson, 1963). Verster (1969) concluded that neither the metacestode structure nor a single adult character can be a sole criterion for the creation of a genus. *Hydatigera*, *Multiceps* and *Tetratirotaenia* that were characterized primarily by the metacestode structure, as well as *Monordotaenia* and *Taeniarhynchus*, both based only on characteristics of rostellar armature, were relegated to synonymy with *Taenia* by Verster (1969). Subsequently, *Fimbriotaenia* Kornyushin & Sharpilo, 1986 was established on the basis of the metacestode morphology, but it was similarly synonymized with *Taenia* (Rausch, 1994b).

Anoplotaenia, *Dasyurotaenia*, *Cladotaenia* and *Paracladotaenia* were excluded from the Taeniidae on zoogeographical, morphological and ontogenetic grounds by Rausch (1985, 1994b). *Cladotaenia* and *Paracladotaenia*, both occurring in raptors as adult tapeworms, are currently treated as synonyms with each other and placed in the family Paruterinidae Fuhrmann, 1907 (Rausch, 1985, 1994b; Chervy, 2002). *Anoplotaenia* and *Dasyurotaenia* are parasites of carnivorous marsupials in Australia, and their similarity with taeniids may be due to convergent evolution (Beveridge, 1984; Rausch, 1985). *Anoplotaenia* is currently an unplaced genus, although its affinities with the Linstowiidae Fuhrmann, 1907 and Dilepididae Fuhrmann, 1907 have been noted (Beveridge, 1984; Schmidt, 1986; Chervy, 2002). The tentative placement of *Dasyurotaenia* within the family Davaineidae Braun, 1900 seems to be artificial (Schmidt, 1986; Jones in Caira et al. 2012). The systematic status of *Insinuarotaenia* has also remained uncertain. Because of the incomplete description, this genus cannot be assigned to any family (Rausch, 1994b).

Table 1. Genera assigned to the family Taeniidae by Abuladze (1964) and Rausch (1994b).

Genus	Abuladze (1964)	Rausch (1994b)
Taenia Linnaeus, 1758	+	+
Echinococcus Rudolphi, 1801	+	+
Alveococcus Abuladze, 1960	+	+ (syn. of *Echinococcus*)
Fimbriotaenia Kornyushin & Sharpilo, 1986		+ (syn. of *Taenia*)
Fossor Honess, 1937	+	+ (syn. of *Taenia*)
Hydatigera Lamarck, 1816	+	+ (syn. of *Taenia*)
Monordotaenia Little, 1967		+ (syn. of *Taenia*)
Multiceps Goeze, 1782	+	+ (syn. of *Taenia*)
Taeniarhynchus Weinland, 1858	+	+ (syn. of *Taenia*)
Tetratirotaenia Abuladze, 1964	+	+ (syn. of *Taenia*)
Anoplotaenia Beddard, 1911	+	
Cladotaenia Cohn, 1901	+	
Dasyurotaenia Beddard, 1912	+	
Insinuarotaenia Spasskii, 1948	+	?
Paracladotaenia Yamaguti, 1935	+	

+ = placed in the Taeniidae; ? = cannot be assigned to family; syn. = a junior synonym

As the result of the revisions by Verster (1969) and Rausch (1994b), the number of genera within the Taeniidae was reduced to two. In conclusion, the following taxonomy of the taeniids has been widely accepted:

Order Cyclophyllidea van Beneden in Braun, 1900

Suborder Taeniata Skrjabin & Shul'ts, 1937

Family Taeniidae Ludwig, 1886

Subfamily Taeniinae Stiles, 1896
Genus *Taenia* Linnaeus, 1758 (Type genus of the family)
Type species *Taenia solium* Linnaeus, 1758

Subfamily Echinococcinae Abuladze, 1960
Genus *Echinococcus* Rudolphi, 1801
Type species *Echinococcus granulosus* (Batsch, 1786)

2.3.3 Taxonomy of taeniid species and ranking of intraspecific entities

Extensive scrutiny received by taeniids resulted in a massive accumulation of synonyms. Wardle and McLeod (1952) and Abuladze (1964) listed 25 old generic synonyms of *Taenia*, and as many as 89 full or partial synonyms of *T. solium*, which is the species of the greatest medical and veterinary interest. The early taxonomic disarray in *Echinococcus* was quite similar. A list of old synonyms contains nine generic synonyms of *Echinococcus* and 83 synonyms of the most significant species *E. granulosus* (Abuladze, 1964). Most of the historical synonyms of *E. granulosus*

seem to be just Latin names coined for hydatids from different organs or hosts, and their assignment to any species is unclear.

The major problems in taeniid taxonomy have been incomplete original descriptions, lack of distinctive morphological characters or poor knowledge of their taxonomic value, as well as undefined limits of taxonomically significant variation and inadequate knowledge of the influence of the host species or the age of the infestation to such variation (Rausch 1953, 1967; Verster 1965, 1969). Several characters are dependent on the method of fixation (such as measurements of strobila, scolex and suckers) or cannot be reliably examined from improperly relaxed specimens (e.g. morphology of the genitalia) (Verster, 1969). Rostellar hooks, which are widely used in species identification and are relatively simple to measure, are easily lost from adult specimens, rather variable in shape and probably dependent on the age of the infestation and the host species (Verster, 1965, 1969; Rausch 1963, 1985). Although taxonomically valuable characters have been listed and adequate methodology described by some authors (e.g. Rausch 1953; Verster, 1965, 1969; Loos-Frank, 2000), it is almost impossible to construct simple keys for quick identification because of the similarity of a number of features (Loos-Frank, 2000).

Verster (1969) thoroughly revised the genus *Taenia* by examining 70 recognized species. The validity of 31 species (one of which was new) and three subspecies was confirmed, while the remaining species were considered synonymous, species inquirendae or invalid taxa. In the same study, standards for diagnostically significant characters were set (Verster, 1969). The revision was subsequently updated and new species were added, leading to the recognition of 42 valid species and three subspecies by the early years of the present century (Loos-Frank, 2000; Rausch, 2003; Hoberg, 2006). In addition, the presence of two species within *Taenia taeniaeformis* (Batsch, 1786) was suggested by various criteria, e.g. host specificity, isoenzyme profiles and mitochondrial DNA (mtDNA) sequences, but these cryptic entities did not receive taxonomic status (Iwaki et al. 1994; Okamoto et al. 1995a, 1995b).

During the last century, taxonomic studies on *Echinococcus* focused on evaluation of the validity of 16 recognized species and 13 subspecies (Kumaratilake and Thompson, 1982). Finally, only four species were regarded as valid, and the rest were relegated to synonymy (mostly with *E. granulosus*) because they could not be differentiated by morphological criteria (reviewed in Kumaratilake and Thompson, 1982). Most subspecies of *E. granulosus* were invalidated since they were not shown to be isolated geographically or ecologically from the nominotypical subspecies (Rausch, 1967; Schantz et al. 1976). An informal term ‘strain’ was adopted to categorize the intraspecific phenotypic variants of *E. granulosus* s.l. (‘sensu lato’, referring hereafter to extended definition of this species including strains) (Smyth and Smyth, 1964; Rausch, 1967; Kumaratilake and Thompson, 1982). The strains were mainly named after their typical intermediate hosts (Thompson and Lymbery, 1988).

Molecular studies later characterized nine mitochondrial genotypes (G1–G9) that closely followed the pattern of phenotypic strain variation in *E. granulosus* s.l. (Bowles et al. 1992, 1994; Bowles and McManus, 1993a; Scott et al. 1997). Taking into account evident genetic and phenotypic differences, and previous descriptions, the status of valid species was proposed for *Echinococcus ortleppi* López-Neyra & Soler Planas, 1943 and for the former subspecies *Echinococcus granulosus equinus*

Williams & Sweatman, 1963 corresponding to the cattle (G5) and horse (G4) strains of *E. granulosus* s.l., respectively (Thompson and McManus, 2002). The sheep strain with closely related strains (G1–G3) formed *E. granulosus* s.s. ('sensu stricto') but the taxonomic status of the group including the camel (G6), pig (G7) and cervid (G8) strains remained unclear.

2.4 Phylogenetic hypotheses

2.4.1 Phylogenetics and molecular markers in taeniid systematics

Phylogenetic systematics, bringing shared-derived traits and common ancestry in classification (e.g. Hennig, 1966), was introduced to systematic practice in the 1950s and has since then become an almost universally accepted approach to assessment of relationships of organisms and resolution of taxonomic problems (Avise, 2004). The recent development of phylogenetic algorithms and DNA sequencing has further revolutionized the taxonomic classification and reconstruction of evolutionary histories (Avise, 2004; Felsenstein, 2004). Phylogenetic methodology has been applied to the systematics of cestodes at different taxonomic ranks since the late 1970s, first based on comparative morphology (reviewed in Brooks and McLennan, 1993) and later increasingly on DNA sequence data, as was initiated for taeniids by Bowles et al. (1995) and Okamoto et al. (1995a).

Molecular markers are of great utility in diagnosing closely related taxa, especially the so-called cryptic species, which are indistinguishable by traditional morphological methods (Hebert et al. 2003; Avise, 2004). In particular, mtDNA has been an attractive marker of molecular biodiversity because it is typically nonrecombining, maternally inherited (clonal), rapidly evolving and intronless, and appears in multiple copies in every cell (Avise, 2000, 2004). It has become a popular tool for phylogenetic studies and DNA barcoding diagnostics leading to the accumulation of comparative information in databases (Avise, 2004; Savolainen et al. 2005). mtDNA has been applied extensively in studies of taeniids since Bowles et al. (1992) first used a region of the cytochrome *c* oxidase subunit I (*cox1*) gene for characterizing isolates of *Echinococcus*. Soon thereafter, phylogenetic analyses of mtDNA sequences confirmed cryptic species complexes within taeniids (Bowles et al. 1995; Okamoto et al. 1995a).

Cryptic speciation not only complicates identification of cestodes but also, particularly together with the self-fertilizing mode of reproduction, obscures the definition of species. The widely accepted biological species concept defines species as interbreeding and reproductively isolated entities (Mayr, 1949). These criteria might not be applicable in self-fertilizers, not at least for obligate ones. Using *Echinococcus* as an example, Lymbery (1992) proposed application of a phylogenetic species concept for mainly self-fertilizing parasites. This concept would delimit species on the basis of monophyly and genetic distinctness. The latter, referred to as 'genetic yardstick' (i.e. genetic differentiation in relation to that between conventional species; Lymbery, 1992), is close to a more recent ideology of DNA barcoding taxonomy (Savolainen et al. 2005). The general use of barcoding approach in taxonomy remains contentious since the determination of universal distance-based

thresholds for delineation of species boundaries is problematic (Hebert et al. 2004; Savolainen et al. 2005; Galtier et al. 2009).

2.4.2 Phylogeny of the Cyclophyllidea

The Cyclophyllidea, as currently defined (Georgiev, 2003), constitutes a nearly monophyletic assemblage (Hoberg et al. 1997, 1999; Justine, 1998; Mariaux, 1998; Olson et al. 2001; Waeschenbach et al. 2007, 2012). Uncertainty remains in the position of the family Mesocestoididae Perrier, 1897, which is placed within the Cyclophyllidea in phylogenetic studies based on comparative morphology and ontogeny, but outside the order in analyses of nuclear ribosomal DNA (rDNA) and mtDNA (Hoberg et al. 1997, 2001b; Mariaux, 1998; Olson et al. 2001; Waeschenbach et al. 2007, 2012). Although some authors (e.g. Wardle and McLeod, 1952; Rausch, 1994a; Miquel et al. 1999) have pointed out distinctive characters of the Mesocestoididae, its promotion to ordinal rank has been considered premature. This is due to incompleteness of molecular data and instability of molecular phylogenies, whose topology in this part was not supported by a total evidence analysis (Hoberg et al. 2001b; Olson et al. 2001; Waeschenbach et al. 2012).

Cyclophyllideans have been placed as a highly derived group among cestodes by early evolutionary hypotheses (reviewed in Hoberg et al. 1997) as well as by advanced phylogenetic studies (Hoberg et al. 1997, 2001b; Justine, 1998; Mariaux, 1998; Olson et al. 2001; Waeschenbach et al. 2007, 2012). In these recent phylogenetic analyses, the Cyclophyllidea, Mesocestoididae, Nippotaeniidea Yamaguti, 1939 and Tetrabothriidea Baer, 1954 form a well-supported monophyletic clade, referred to as ‘higher’ or ‘most derived’ acetabulates (Olson et al. 2001; Waeschenbach et al. 2007), but interrelationships within this group remain uncertain. As adults, the higher acetabulates are parasites of tetrapods except for nippotaeniideans that parasitize freshwater teleost fishes.

2.4.3 Phylogenetic placement and relationships of the Taeniidae

The early attempts to define relationships for taxa within the Cyclophyllidea were mainly based on larval morphology and development (Hoberg et al. 1999). Spasskii (1951) speculated about the evolution of the taeniid life cycle, particularly how a putative primitive cycle including an invertebrate intermediate host changed to the present taeniid cycle involving only vertebrates. He concluded that the taeniids possessing this ‘peculiar biology’ diverged early from other cyclophyllidean lineages, perhaps earlier than the mesocestoidids. Jarecka (1975) defined, based on larval morphology and ontogeny, two lineages in the cyclophyllidean evolution, one leading to the hymenolepidids using mainly birds as definitive hosts and having cysticercoid metacestodes in invertebrate intermediate hosts, and the other resulting in the taeniids with mammalian hosts and cysticercus metacestodes. The taeniids were considered evolutionarily highest among the cyclophyllideans, but phylogenetic relationships within the order were not outlined (Jarecka, 1975).

Freeman (1973) presented the first explicit hypothesis for relationships among the cyclophyllideans based on morphologic and ontogenetic characters of the larval

stages. The Cyclophyllidea was polyphyletic and divided into two main 'stems' determined by a presence or absence of a single structure, the cercomer, which is a tail-like appendage at the posterior end of a larval cestode. The taeniids were placed apically in the 'taeniid stem', whereas the hymenolepidids were the most derived group in the other main stem (Freeman, 1973). Three taxa, currently treated as the families Amabiliidae Braun, 1900, Dilepididae and Linstowiidae, formed the sister group of the Taeniidae. The main developmental characters used by Freeman (1973), however, appear to be phylogenetically uninformative or strongly influenced by homoplasy (Hoberg et al. 1999).

A preliminary cladistic analysis including only four cyclophyllidean families, and utilizing limited data from comparative morphology, suggested a basal position for taeniids and placed the anoplocephalids, hymenolepidids and mesocestoidids in a polytomy next to the taeniids (Brooks and McLennan, 1993). The family Taeniidae was found to be monophyletic and closely related to the genus *Dasyurotaenia* by a comprehensive phylogenetic analysis of the families within the Cyclophyllidea based on a large character matrix from comparative morphology and ontogeny (Hoberg et al. 1999). A clade formed by the Metadilepididae Spasskii, 1959 and Paruterinidae, both taxa using non-aquatic birds as definitive hosts, was the sister group of the Taeniidae + *Dasyurotaenia* (Hoberg et al. 1999). The tree differed significantly in topology from the dendrogram presented by Freeman (1973). Also, the previously proposed basal divergence of the taeniids (Spasskii, 1951; Brooks and McLennan, 1993) was not supported either.

Only two molecular phylogenetic studies on family level relationships within the Cyclophyllidea have been published. One was based on mitochondrial 12S rDNA (von Nickisch-Rosenegk et al. 1999a), and the other on nuclear 18S rDNA (Foronda et al. 2004). A limited number of taxa was used in both analyses, the first including seven cyclophyllidean families and the latter only five. Metadilepidids or *Dasyurotaenia* were not represented in either study. In the phylogeny by Foronda et al. (2004), a member of the Paruterinidae was placed distant to taeniids, and relationships of the Taeniidae with the other families remained uncertain. The phylogeny by von Nickisch-Rosenegk et al. (1999a), in which paruterinids were not included, implied a sister group relationship between the taeniids and dipylidiids (represented by the canine tapeworm *Dipylidium caninum* [Linnaeus, 1758]) as well as a possible close relationship between taeniids and mesocestoidids. The considerable differences between the molecular phylogenies and the comprehensive morphology-based phylogeny by Hoberg et al. (1999) can simply be explained by the incomplete sampling of taxa and by the use of distant outgroups in molecular studies.

2.4.4 Phylogenetic relationships among the taeniid genera

Freeman (1973) envisioned paraphyly of *Taenia* by placing *Echinococcus* among members of *Taenia* in his hypothesis of the cyclophyllidean evolution. Evaluation of the relationships within the Taeniidae was based only on the proliferation and type of the metacestode. In the dendrogram, *Echinococcus* was the most derived group among taeniids with proliferating metacestodes. A sister group relationship for *Echinococcus* and *Taenia* possessing the 'multigerminocysticercus' type of metacestode (i.e. *Taenia crassiceps* [Zeder, 1800] with exogenously proliferating

cysticerci) was suggested. This is, however, an apparent typing error since the similarity of *Echinococcus* and species of *Taenia* with the 'multicephalocysticercus' (i.e. coenurus, larvae of *Taenia multiceps* Leske, 1780 and *Taenia mustelae* Gmelin, 1790 as examples) was emphasized elsewhere in the text (e.g. p. 523 in Freeman, 1973).

Notwithstanding the limited phylogenetic basis, Freeman's (1973) evolutionary tree was the first of the few hypotheses of interrelationships within the taeniid family dealing with both of its genera. Subsequent phylogenetic studies, both morphological and molecular, having similar approach and using relevant outgroups outside of the taeniids, suggested that *Echinococcus* and *Taenia* are monophyletic entities (Moore and Brooks, 1987; Okamoto et al. 1995a; de Quieroz and Alkire, 1998; von Nickisch-Rosenegk et al. 1999b). The majority of the studies on taeniid phylogeny, however, have separately focused on either *Echinococcus* or *Taenia*, using the other one as an outgroup (e.g. Bowles et al. 1995; Hoberg et al. 2000).

2.4.5 Phylogenetic studies of *Taenia* spp. until the early 21st century

Perhaps the first ideas about the evolutionary history of species of *Taenia* concerned the origin of human-*Taenia* spp. and were based on life cycle associations (e.g. Baer, 1940; Cameron, 1956). Baer (1940) speculated that the ancestor of *T. solium* could have been a parasite of large carnivorans, such as extinct felids, and humans acquired the infection by hunting the same prey, i.e. wild pigs. Then the life cycle was possibly maintained by cannibalism in human populations until domestic dogs and later pigs inherited the role of the intermediate host. In contrast, the origin of *T. saginata* in humans would have been more recent and linked to the domestication of cattle (Baer, 1940). Cameron (1956) suggested that *T. solium* originated from a parasite using dogs as the final host, and colonization of humans by this tapeworm would thus be associated with the domestication of dogs.

Verster (1969) divided the genus *Taenia* into two major groups on the basis of the spatial arrangement of genital ducts in the adults: in the group I the terminal genital ducts pass between the longitudinal osmoregulatory canals, and in the group II they are ventral to the canals. The group I, linked to *T. solium* as a typical species, was larger and included species in canids, felids, hyaenids and humans. The smaller group II included *T. taeniaeformis* (the type) and *Taenia selousi* Mettrick, 1962 of cats and, in addition, species parasitizing mustelids and viverrids, which were considered to be relatively older taxa (Verster, 1969). However, any justification for this view on the evolutionary age was not presented.

A phenetic study based on distance comparisons for morphometric characters of hooks identified distinct groups among 18 species of *Taenia*, and this was proposed to indicate that more than one genus are involved (Gubányi, 1995). However, the approach of the study was strictly numerical taxonomic and a phylogenetic context was lacking. The first actual cladistic analysis of the relationships of taeniids included 13 species of *Taenia* and applied morphological characters of the strobilar stage (Moore and Brooks, 1987). The resultant cladograms supported the monophyly of *Taenia*, and *T. mustelae* was the most basal species. The hypothesis was in general

agreement with Verster's (1969) groups by placing species of the group II relatively basally except for *T. taeniaeformis*, which was located apically.

A nearly comprehensive cladistic analysis of relationships among *Taenia* based on a large set of morphological characters of both adult and metacestode stages was published by Hoberg et al. (2000), and slightly modified and expanded by adding more taxa by Hoberg et al. (2001a) and Hoberg (2006). Only ten species with incomplete data were excluded. These thorough analyses did not provide support for recognition of the previously proposed diversity of genera or higher taxa within *Taenia* (see e.g. Abuladze, 1964). The most basal position was occupied by *T. mustelae*, and Verster's (1969) grouping was only partly supported by the relatively basal placement of members of the group II including *T. taeniaeformis*. However, several species of the group I were represented among basal species. Coevolution with respect to definitive hosts and *Taenia* appeared to have been limited, and extensive host switching among phylogenetically unrelated predators was postulated. Two independent origins for the human-*Taenia* (one for *T. solium* and another for *T. saginata* + *T. asiatica*) were indicated, and human-*Taenia* spp. appeared to be closely related to species occurring at present in large African carnivorans. The results suggested a more dominant role for coevolution with intermediate hosts. Almost all basal species have rodent intermediate hosts but more derived ones are mainly linked to artiodactyls. Thus the colonization of artiodactyls appeared to have been a single event during the evolution of *Taenia.*

Pioneering molecular phylogenies for species of *Taenia* were inferred from short regions of mtDNA (366–471 bp) or nuclear rDNA (199 bp) (Okamoto et al. 1995a; de Queiroz and Alkire, 1998; Gasser et al. 1999; von Nickisch-Rosenegk et al. 1999b). In addition, these studies included only a limited number (7–11) of species. Taxon sampling in von Nickisch-Rosenegk et al. (1999b) was most diverse including species using canids, felids, humans, mustelids or viverrids as definitive hosts. Other studies mainly included cosmopolitan species occurring as adults in humans, dogs and cats. Later Zhang et al. (2007) extended a little the prior studies by adding two species from African wildlife. Basal placement of *T. mustelae* was demonstrated (Okamoto et al. 1995a; de Queiroz and Alkire, 1998; von Nickisch-Rosenegk et al. 1999b), but *T. taeniaeformis* was located ambiguously either in the apical (Okamoto et al. 1995a; de Queiroz and Alkire, 1998) or basal part of the phylogenetic trees (von Nickisch-Rosenegk et al. 1999b; Zhang et al. 2007). Two distinct origins of human-*Taenia* were evident (de Queiroz and Alkire, 1998; Gasser et al. 1999; von Nickisch-Rosenegk et al. 1999b; Zhang et al. 2007). The phylogenies did not clearly support extensive coevolution between *Taenia* spp. and their hosts, although species using artiodactyls as intermediate hosts formed a monophyletic clade in several trees (Gasser et al. 1999; von Nickisch-Rosenegk et al. 1999b; Zhang et al. 2007). Overall, agreement with morphology-based phylogenies (Hoberg et al. 2000, 2001a; Hoberg, 2006) was limited. Incongruence can mainly be explained by homoplasy in most morphological characters assessed in cladistic analyses as well as by incomplete sampling of taxa in molecular phylogenetic studies.

2.4.6 Phylogenetic studies of *Echinococcus* spp. until the early 21st century

There are very few early hypotheses about evolutionary relationships within *Echinococcus.* Referring to the original life cycles and the domestication history of ungulates, Rausch (1986) considered the cervid strain (or the northern biotype) of *E. granulosus* s.l. to be ancestral to the strains in synanthropic hosts, but did not evaluate other relationships within the genus. Due to the paucity of clear distinctive characters, morphology-based studies of relationships within *Echinococcus* are almost lacking. A phenetic grouping based on a set of morphological characters of four species of *Echinococcus* and three strains of *E. granulosus* s.l. suggested that *Echinococcus oligarthra* (Diesing, 1863) and *Echinococcus vogeli* Rausch and Bernstein, 1972 were intermediate between *E. granulosus* s.l. and *E. multilocularis*, and the cattle strain of *E. granulosus* s.l. was quite distant from the horse and sheep strains (Kumaratilake in Thompson and Lymbery, 1988). In a unique cladistic analysis by Lymbery (1992), relationships for *E. multilocularis* and six strains of *E. granulosus* s.l. were examined using a limited number of morphological characters. The topology of the phylogenetic tree was not particularly robust, and several strains appeared in polytomy. Nevertheless, paraphyly of *E. granulosus* s.l. was suggested. The sheep strains were separate from other strains, and the horse and cattle strains were placed as sister taxa. The cervid strain was not included.

The first phylogenetic analysis of *Echinococcus* based on mtDNA data (*cox1* and NADH dehydrogenase subunit 1, *nad1*) supported paraphyly of *E. granulosus* s.l. (Bowles et al. 1995). The Neotropical species *E. oligarthra* and *E. vogeli* were basal, and *E. multilocularis* was placed among strains of *E. granulosus* s.l. The camel (G6), pig (G7) and cervid (G8) strains formed a monophyletic clade, which was sister to the cattle strain (G5). The cervid strain was represented by isolates from a single moose in North America (Bowles et al. 1994), and its position remained somewhat uncertain due to the ambiguous *cox1* sequence (Bowles et al. 1995).

Further studies by Le et al. (2002) and McManus et al. (2002) using these and a few additional mitochondrial genes did not improve the robustness of the phylogeny. In contrast to Bowles et al. (1995), *E. multilocularis* was found to be the basal taxon. The cattle strain (G5), which is a critical taxon considering the status of the G5–G8 cluster, was not included in the analyses by Le et al. (2002) and McManus et al. (2002). The sequences of G8 were unambiguous, but the number of analyzed isolates remained low and their origin was restricted to North America.

A nuclear DNA (nDNA) region, the first internal transcribed spacer (ITS-1) of rDNA, was also analyzed in the dawn of the molecular phylogenetic era of the *Echinococcus* research to resolve phylogenetic relationships within the genus (Bowles et al. 1995; Kędra et al. 1999; van Herwerden et al. 2000). The paraphyly of *E. granulosus* s.l. was supported. However, the interpretation of the results was difficult due to the high variability of this fragment, and its use in molecular diagnostics of *Echinococcus* was questioned (Kędra et al. 1999). This revealed the need for utilizing other regions of nDNA in molecular phylogenetic studies.

3 Aims of the study

The taxonomy of the Taeniidae has been a subject of endless debate and several revisions. Molecular phylogenetic studies have indicated the presence of cryptic species complexes within the family. Approximately one third of the taeniid taxa occur mainly in the northern Boreal and Arctic regions but they have often been neglected in earlier molecular genetic studies.

This thesis work attempted to investigate the above-mentioned unexplored and disputable areas of the taeniid systematics. The general aims were to elucidate evolutionary relationships of taeniids, to explore the diversity within the family and to evaluate the taeniid taxonomy on the basis of phylogenetic relationships. The special emphasis of the present work was on the northern species.

The specific aims were:

- to characterize genetically the *Echinococcus* strain occurring in cervids in Finland and Sweden, and to assess the phylogenetic relationships of this strain within the genus *Echinococcus*, especially defining its position in relation to other strains of *E. granulosus* s.l.;

- to characterize genetically specimens of several species of *Taenia* especially focusing on northern species in wildlife;

- to screen for communities of *Taenia* in large/medium-sized carnivorans by mtDNA barcoding;

- to examine unknown species of *Taenia* by mtDNA markers to find out their hosts, life cycles and geographical distribution;

- to analyze molecular phylogenetic relationships within the family Taeniidae;

- to update the taxonomy of *Echinococcus* spp. and *Taenia* spp. by applying the phylogenetic species concept;

- to revise the genus *Taenia* based on molecular phylogenies.

4 Materials and methods

4.1 Parasite specimens and taxon sampling

4.1.1 *Echinococcus* isolates from cervids and phylogeny of *Echinococcus*

To characterize genetically the *Echinococcus* strain occurring in cervids in Finland, four isolates from semidomestic reindeer (*Rangifer tarandus*) and one from a moose (or Eurasian elk, *Alces alces*) from northeastern Finland were compared with species and strains of *Echinococcus* (I). The phylogenetic analysis included, in addition to sequences of new specimens, previously published sequence data of four species of *Echinococcus* and eight strains (G1–G8) of *E. granulosus* s.l.

Genetic variation among *Echinococcus* isolates of cervid origin was further studied by identifying molecularly 24 isolates from reindeer and nine from moose (II). One reindeer isolate was from northern Sweden while the rest of the specimens originated from northern and eastern Finland. All hydatids from reindeer were found at routine meat inspection, and the moose isolates were collected from lungs provided by hunters. Phylogenetic analysis focused especially on the relationships between the G5–G8 genotypes and the strain occurring in Finnish and Swedish cervids. New DNA sequence data from a cattle strain (G5) isolate in Netherlands were included, as well as previously published data from five strains of *E. granulosus* s.l., *E. multilocularis* and *T. crassiceps* (outgroup).

Finally, the phylogenetic relationships within *Echinococcus* were studied utilizing mitochondrial genomes (mitogenomes) (VIII). By that time, mitogenomes of most species and strains of *Echinococcus* had already been published (Le et al. 2002; Nakao et al. 2002, 2007), and the mitogenome-based phylogeny was now revised by adding two missing taxa, i.e. the strain originally found from Finnish cervids and the African species *Echinococcus felidis* Ortlepp, 1937 (‘lion strain’ of *E. granulosus* s.l.). The comprehensive analysis comprised 12 taxonomic units including all currently recognized species of *Echinococcus* and the most important genotypes. *Taenia mustelae* was used as an outgroup.

4.1.2 *Taenia* samples from mammals in the Holarctic

To analyze molecular phylogenetic relationships within the family Taeniidae (III), *Taenia* specimens were collected from definitive and intermediate hosts in different parts of the Holarctic region, mainly in Eurasia, during the course of several research projects and field expeditions. Altogether 54 specimens representing nine species were examined. The species were determined primarily based on the number and morphology of the rostellar hooks. In the phylogenetic analysis, 30 taxonomic units, comprising 26 taeniid species and 4 subspecies or other subspecific entities, were included. Choice of a relatively distant cyclophyllidean species *Hymenolepis diminuta* (Rudolphi, 1819) for outgroup was due to the availability of its mtDNA sequences.

4.1.3 Muscle cysticerci from cervids and adult stages from carnivorans

In article IV, muscle cysticerci from two moose, representing an unknown species of *Taenia*, were characterized morphologically and molecularly. The animals were shot in eastern and northeastern Finland during legal hunting. The numbers and morphology of the rostellar hooks of the cysticerci were compared with morphological data of selected *Taenia* spp. In the phylogenetic analysis, the unknown species was compared to published sequence data of *Taenia* with the same sampling of taxa as in article III. Two relevant outgroups, *E. oligarthra* and *T. mustelae*, were selected based on the observations of intrafamilial phylogenetic relationships (III).

To find out the definitive host of the enigmatic *Taenia* sp. discovered in moose, a screening of adult stages of *Taenia* in brown bears (*Ursus arctos*) and wolves (*Canis lupus*) was carried out by mtDNA barcoding (V). A total of 104 bear intestines from Finland were examined. All obtained *Taenia* tapeworms, i.e. four individuals from two bears, were identified molecularly. In addition, nearly 300 tapeworms from 35 Finnish and Swedish wolves were identified. Furthermore, to find out causative agents of muscle cysticercosis in cervids in other parts of the Holarctic, eight specimens from moose (*Alces americanus*) and three specimens from Grant's caribou (*Rangifer tarandus granti*) from Alaska, and three specimens from Svalbard reindeer (*Rangifer tarandus platyrhynchus*) were analyzed. The wildlife specimens were collected mainly in collaboration with local hunters. Haplotypes of all discovered species were phylogenetically compared with most closely related species of *Taenia.*

Taeniid diversity in carnivorans in Finland was further investigated by identifying 135 tapeworm specimens from 72 hunter-harvested lynx (*Lynx lynx*) using mtDNA sequences (VI). The trigger for this survey was observation of a large-sized unknown species of *Taenia* in lynx. Rostellar hooks and other available morphological characters of rather decomposed specimens were examined. A specimen of *Taenia omissa* Lühe, 1910 from a cougar (*Puma concolor*) from Canada, and specimens of *Taenia laticollis* Rudolphi, 1819 and *T. taeniaeformis* from lynx were used for molecular and morphological comparisons. The molecular phylogenetic analyses comprised 11–22 species of *Taenia* and several subspecific entities or haplotypes depending on analyzed sequence regions and the availability of published sequences. Outgroups were the same as in article IV.

4.1.4 DNA specimens for the multilocus phylogenetic analysis of the Taeniidae

Finally, persevering international research collaboration culminated with a multilocus phylogenetic analysis of the Taeniidae (VII) that utilized a large set of taeniid DNA specimens from various sources, including essential specimens from study III. The parasite material consisted of 38 specimens representing 25 taeniid species and several intraspecific variants. *Dipylidium caninum* was used as an outgroup because of availability of a DNA specimen for this species and its close placement to the Taeniidae in a previous family level molecular phylogeny by von Nickisch-Rosenegk et al. (1999a).

4.2 Molecular markers

4.2.1 mtDNA markers

Extensive use of mtDNA sequences in the present work was not only due to the benefits of mtDNA as a molecular marker, but also an opportunistic decision. These commonly used DNA regions were chosen in order to maximize the availability of previously published comparative sequences.

Partial DNA sequences from the mitochondrial *cox1* and *nad1* genes, 396 bp and 488 bp long, respectively, were used as primary markers for molecular genetic characterization of taeniid species, reconstruction of preliminary phylogenies and barcoding identification of specimens. Previously published primers were used for the enzymatic amplifications (Bowles et al. 1992; Bowles and McManus, 1993a). These mtDNA regions were analyzed because they have proved to be appropriate for identification of species and strains of *Echinococcus* and species of *Taenia*, and they are generally accepted for these purposes (Bowles et al. 1992; Bowles and McManus, 1993a; Okamoto et al. 1995a; Gasser et al. 1999; McManus, 2002).

Longer mtDNA regions or complete genes were used attempting to improve the resolution of phylogenies. In study II, mtDNA sequences were analyzed to resolve the phylogeny of the strains/genotypes of *E. granulosus* s.l. Phylogenetic analysis was based on concatenated sequences of complete ATP synthase subunit 6 (*atp6*) and NADH dehydrogenase subunit 3 (*nad3*) genes, and fragments of *cox1* (another region than above) and *nad1* (the same region as above); the alignment consisted of over 1,500 nucleotide sites in total. These mtDNA regions were selected because they were available for the G6–G8 genotypes. New primers were designed to amplify *atp6*, *nad3* and the region of *cox1*. In studies VI and VII the complete sequences of *nad1* (ca 900 bp) and *cox1* (ca 1,600 bp), respectively, were used to resolve phylogenetic relationships of *Taenia* isolates. Primers published by Hüttner et al. (2008) were used to amplify *nad1* (VI) and new primers were designed for *cox1* (VII).

In the final parts of this thesis (VII, VIII), phylogenies were inferred from full mitogenome datasets. Either all genes (VIII) or 12 protein-coding genes (VII) were analyzed. The number of analyzed nucleotide sites was thus multifold compared to the earlier studies of this thesis, the final alignments consisting of over 13,000 and 6,700 sites, respectively (in the latter case, third positions were deleted to reduce the influence of synonymous substitutions). The mitogenomes were amplified using noncontiguous sequence islands (Nakao et al. 2003). Briefly, at first primers designed from conserved areas were used to amplify regions of mtDNA, from which new primers were designed for amplification of the remaining regions. Sequencing was performed by primer walking.

4.2.2 Nuclear markers

Despite several advantages of mtDNA as a genetic marker, it is not without complication. The main problem is that maternally inherited mtDNA does not necessarily reflect the organismal evolutionary history as a whole (Avise, 1991; Ballard and Whitlock, 2004). Further, mitogenome is linked and behaves as a single

locus, whereupon different mitochondrial genes do not give statistically independent information about the species level phylogeny (Ballard and Whitlock, 2004). Instead of employing mtDNA data alone for inferring phylogenies, a broader use of multiple nuclear markers has been recommended (e.g. Ballard and Whitlock, 2004; Ballard and Rand, 2005). Analysis of nuclear data sets is necessary especially when major taxonomic revisions are proposed, as was done in article VII.

In the first work of the present thesis (I), the complete DNA sequence of the nuclear ITS-1 region, ca 1,000 bp, was used for strain characterization of *Echinococcus* isolates of cervid origin. Previously published primers were used for the enzymatic amplifications (Bowles and McManus, 1993b). The PCR products were cloned in plasmid vectors before sequencing. The ITS-1 region was analyzed since this region of nDNA has been used in addition to mtDNA sequences for strain identification and phylogenetic analyses of *Echinococcus* (Bowles and McManus 1993b; Bowles et al. 1995; Kędra et al. 1999; van Herwerden et al. 2000). The aligned and refined data set consisted of ca 650 nucleotide sites.

In study VII, nuclear DNA sequences of 18S rDNA and two protein-coding genes, phosphoenolpyruvate carboxykinase (*pepck*) and DNA polymerase delta (*pold*), were used to reconstruct phylogeny of the Taeniidae. Previously published primers were used in enzymatic amplifications (Littlewood and Olson, 2001; Knapp et al. 2011). Since 18S rDNA occurs in multiple copies, high fidelity PCR and cloning in plasmid vectors were used to obtain sequences. *Pepck* and *pold* were selected as nuclear markers because of their single-copy presence in many organisms including *E. multilocularis* (Knapp et al. 2011). To obtain *pepck* and *pold*, the PCR products were first directly sequenced by primer walking but if double peaks were detected in the sequencing reaction, cloning in a plasmid vector was performed and then inserts were sequenced to confirm allelic polymorphism. The alignment of 18S rDNA contained ca 1,800 nucleotide sites while exon alignments of concatenated protein-coding genes consisted of ca 2,800 sites.

4.3 Phylogenetic methods and data analysis

The degree of divergence between taeniid species and intraspecific variation was evaluated by comparing simple proportions of pairwise nucleotide differences (or identities) of aligned sequences (I–VI). In study VII, sequence characteristics were examined with MEGA5 (Tamura et al. 2011) by calculating pairwise divergence values of nucleotide sequences and their means within each genus under the Kimura 2-parameter model (KP2) (Kimura, 1980) and pairwise divergence of deduced amino acid sequences under the JTT model (Jones et al. 1992).

Phylogenetic analyses were based on DNA sequences and, in study VII, also on the amino acid sequence data of the mitochondrial protein-coding genes. The sequences were aligned using programs of the Clustal series (Thompson et al. 1994; Chenna et al. 2003) (I–VI), T-Coffee (Notredame et al. 2000; Taly et al. 2011) (VII, VIII) and CodonAlign 2.0 (Hall, 2004) (VII). To assess the effect of the tree construction method to tree topologies, different phylogenetic approaches were used including distance, parsimony, likelihood and Bayesian methods.

Minimum-evolution (ME) (Rzhetsky and Nei, 1992) and a simplified version, neighbor-joining (NJ) (Saitou and Nei, 1987), were the methods operating on distance matrices (I–VI). The other methods employed in the present thesis use sequence data sets directly. Maximum parsimony (MP) chooses the tree that requires the least evolutionary change to explain observed data (Fitch, 1971). Maximum likelihood (ML) method chooses the most likely tree, which has the highest probability of producing the observed data under the evolutionary model used (Felsenstein, 1981). Quartet puzzling (QP) method reconstructs ML trees for quartets of taxa, combines these into intermediate trees and finally builds a consensus tree (Strimmer and von Haeseler, 1996). Bayesian inference of phylogeny uses Bayes' theorem to combine the prior probability of a phylogeny with the likelihood of data to produce not a single optimal tree but a posterior probability distribution on trees (Huelsenbeck et al. 2001). The selection of phylogenetic methods was dependent on the aim of each study and qualities of sequence data sets; e.g. the simple and rapid NJ algorithm implemented with KP2 distances was the main method when short mtDNA sequences were utilized primarily in distinguishing different taxa and in sorting haplotypes (V, VI), whereas only ML and Bayesian inference with sophisticated substitution models were used in multilocus analyses of nuclear genes and mitogenomes (VII).

Phylogenetic analyses were conducted in computer programs TREE-PUZZLE (Schmidt et al. 2002) (QP; I), MEGA2.1 (Kumar et al. 2001) (MP and NJ; I), PAUP* 4.0b10 (Swofford, 2002) (NJ, ME, MP and ML; II–VI), PhyML 3.0 (Guindon et al. 2010) (ML; VII, VIII) and MrBayes 3.2.1 (Ronquist et al. 2012) (Bayesian approach; VII). Versions of MODELTEST (Posada and Crandall, 1998) or the program MEGA5 were used to select substitution models for ML and Bayesian analyses. Support for nodes was assessed by bootstrapping (NJ, ME, MP and ML), by puzzling steps (providing reliability values in QP) or by estimating posterior probabilities using Markov chain Monte Carlo analysis (Bayesian tree). In study VII, an approximate likelihood ratio test (aLRT) (Guindon et al., 2010) was conducted to evaluate tree topologies.

5 Results and Discussion

In this thesis, the molecular phylogenetic basis for taxonomic revisions at the specific and generic levels within the Taeniidae is presented. The majority of the studies (I–VI) used mainly short mtDNA regions in characterizing taeniid species and in reconstructing their evolutionary relationships. Phylogenies presented in those studies should be considered as preliminary. Mitogenomic and nDNA analyses of the final studies (VII, VIII) produced more strongly supported phylogenetic hypotheses. As a result of recent research (Nakao et al. 2007; Knapp et al. 2011; Nakao et al. 2013; VIII), relationships within *Echinococcus* are now almost completely resolved. Due to the lack of several taxa, the analysis of relationships within the Taeniidae was not comprehensive. Thus the molecular taxonomy presented in article VII plays an initiating role in creating a framework for further phylogenetic studies and additional revisions of the family.

In the following sections, the main results of this thesis are presented and contrasted with previous research and with the most recent studies in this field. First, the undetected diversity of species revealed by molecular markers will be analyzed. Secondly, phylogenetic relationships of the main taeniid clades and the new generic level revision will be explored.

5.1 Hidden diversity in taeniids

5.1.1 Fennoscandian cervid strain G10 and its phylogenetic position within *Echinococcus canadensis* (I, II, VIII)

The first publication of this thesis (I) focused on the molecular genetic characterization of *Echinococcus* isolates in cervids from Finland. The specimens possessed unique *cox1* and *nad1* sequences that resembled closely those of the genotypes G6–G8 of *E. granulosus* s.l. Also, sequence variants of ITS-1 suggested a close relationship with the genotypes G6–G8. The difference between the new specimens and the closest genotypes was at similar level as between some of the established genotypes/strains of *E. granulosus* s.l. This novel variant of *E. granulosus* s.l. was therefore denoted as a distinct genotype, G10, and named as the Fennoscandian cervid strain according to the intermediate hosts and the biogeographical region of the discovery (I). This informal naming followed the same principle as in the case of the Tasmanian sheep strain (G2) (Thompson and Lymbery, 1988; Thompson, 1995).

We supposed at first that the distinct genotypes in cervids from different continents, G8 and G10, are probably related to the previously described subspecies *Echinococcus granulosus borealis* Sweatman & Williams, 1963 and *Echinococcus granulosus canadensis* Webster & Cameron, 1961 of North-American and Fennoscandian origins, respectively (I). Only G10 was found in further screening of *Echinococcus* isolates in cervids from Finland and Sweden suggesting that it could be the sole genotype in the North-European wildlife (II). Subsequent studies have proven that these conclusions, and the distinct strain status of G10, were fallacious. As shown

by several later reports, both G8 and G10 occur in North America and Eurasia (e.g. Thompson et al. 2006; Moks et al. 2008; Konyaev et al. 2013). Sympatric occurrence of these genotypes indicates that they cannot be considered to represent distinct subspecies. In addition, although they represent different genotypes or mitochondrial lineages, they do not fulfill criteria of distinct strains as 'characters of actual or potential significance to the epidemiology and control of hydatid disease' (Thompson and Lymbery, 1988) cannot be demonstrated. Nevertheless, our studies showed that *Echinococcus* in cervids does not constitute a genetically homogeneous group.

The phylogenetic analysis of mtDNA sequences showed that a clade formed by the genotypes G6–G8 and G10 was sister to the cattle strain G5 (II). Within the clade, the cervid strain G8 was basal, and G10 was sister to G6 + G7. The bootstrap supports for the nodes were relatively low, but the robust mitogenomic analysis (VIII) confirmed later this topology. The genotypes G6–G8 and G10 have also clustered in phylogenies based on nDNA, although topologies have been slightly variable (Saarma et al. 2009; Knapp et al. 2011). The dubious genotype G9 is nearly identical to G7, and it was not included in the comparisons due to the incomplete sequence data. In article II we concluded that the genotypes G6–G10 belong to a single species, which should be separated from *E. granulosus* s.s. Thompson (1995) elevated a former subspecific name to a specific rank proposing '*E. canadensis*' as a possible taxonomic designation for the cervid strain. Nakao et al. (2007) proposed, based on mitogenome data from G6–G8, the name *E. canadensis* for the G6–G10 cluster. This nomination was advocated in article VIII. However, it should be pointed out that morphological support for this taxonomic decision is lacking. Furthermore, the use of the genotypic codes G6–G8 and G10 is still recommended due to genetic and ecological complexity of this taxon.

Konyaev et al. (2013) further elucidated the genetic diversity of *E. canadensis* in cervids by identifying the camel strain G6 in a reindeer in Yakutia. Three genotypes, G6, G8 and G10 were found in wolves or cervids in Yakutia suggesting that they occur in the same life cycle. Phylogenies presented in articles II and VIII suggest that the form of *E. canadensis* in the wolf-cervid life cycle is ancestral to the genotypes G6 and G7 in domestic cycles. This hypothesis was earlier outlined in a broader sense by Rausch (1986). One explanation for the genotypic diversity of *E. canadensis* in Yakutia could be that this region is the cradle of the species. On the other hand, the presence of the above-mentioned genotypes could just reflect historical contacts between nomadic peoples of Siberia and Mongolia, i.e. camel and reindeer nomads and hunters, and anthropogenic movements of host animals.

5.1.2 Cryptic species within *Taenia taeniaeformis* and *Taenia polyacantha* (III, VII)

Remarkable differences in the partial sequences of the *cox1* and *nad1* genes indicated the presence of two distinct lineages in *T. taeniaeformis* (III). These lineages were also demonstrated in the phylogenetic analyses of mitogenomes and nDNA (VII). Branch lengths in the mitogenomic trees and pairwise divergence values of the complete sequences of *cox1* gene showed that the difference was at the specific level. The hidden taxa were tentatively named as sp. A and sp. B. The results were concordant with earlier studies, which have suggested that cryptic species are

included in *T. taeniaeformis* (Iwaki et al. 1994; Okamoto et al. 1995a, 1995b). Similar conclusions were drawn by Jia et al. (2012) based on mitogenomic data. Galimberti et al. (2012) revealed still more crypsis in *T. taeniaeformis* by discovering a third lineage (herein sp. C), sister to sp. B., in the Apennine Peninsula. According to our large unpublished data (Lavikainen et al. unpubl.), geographical distributions of these hidden taxa are different but partly overlapping: sp. A is mainly Asian and spread worldwide probably with introduced cats, sp. B occurs in Europe, northern Eurasia and Japan, while sp. C is restricted to the Mediterranean region.

Similar sequence differences in *cox1* and *nad1* were detected among isolates of *Taenia polyacantha* Leuckart, 1856 (III), which is a taeniid parasite in a canid-rodent life cycle. Based on the sequence data, the specimens were divided into southern and northern groups. The former included specimens from Europe and the latter from the Arctic region. These groups probably are related to two recognized subspecies of *T. polyacantha*, i.e. southern or boreal *T. p. polyacantha* and Arctic *Taenia polyacantha arctica* Rausch & Fay, 1988. However, morphological identification of our specimens to subspecies, based on hook numbers and measurements, was partly inconsistent, suggesting that the hook characteristics vary more or in a different manner than previously known. Elevation of *T. p. arctica* to a specific rank would require re-evaluation of the diagnostic criteria and additional molecular analysis of specimens identified by morphology.

5.1.3 Repromotion of *Taenia krabbei* to a specific rank and discovery of a new *Taenia* sp. in bear-moose cycle (III–V)

In phylogenetic analyses (III, IV, VI, VII), the nominotypical subspecies of *Taenia ovis* (Cobbold, 1869), occurring in a pastoral dog-sheep life cycle worldwide, was placed relatively basally in one of the main clades of *Taenia* (clade Ia in VII). The subspecies *Taenia ovis krabbei* (Moniez, 1879) was located in the same clade but surprisingly as a sister taxon of *T. multiceps*, distant to *T. o. ovis* (III). *Taenia krabbei*, which uses cervids as intermediate hosts, was originally described as a distinct species but, in the absence of other distinctive criteria than the host specificity, it was relegated to a subspecific rank under *T. ovis* by Verster (1969). The phylogenetic placement indisputably indicates that the recognition of *T. krabbei* as a valid species is justified.

The analyzed specimens of *T. krabbei* in study III were from Arctic foxes (*Vulpes lagopus*) from Svalbard. The placement of *T. krabbei* as sister to zoonotic *T. multiceps* inspired us to examine additional specimens. Muscle cysticerci from two Finnish moose were sequenced, and amazingly they showed a sister species relationship with *T. solium* (IV). A survey of taeniids in carnivorans proved that the definitive host of this parasite is the brown bear (V). The prevalence of the parasite in bears was 2%, and other tapeworms were not found. Strobilate stages of this parasite were not found in wolves (n=35, from which 293 tapeworms were identified), nor in a later survey (VI) in lynx (n=72, 135 tapeworms identified), although other taeniids using cervid intermediate hosts were detected. The Holarctic distribution of this previously unknown species was shown by identifying its cysticercal stages in Alaskan moose (V). The unknown species in the bear-moose life cycle was finally

described as *T. arctos* sp. nov. by Haukisalmi et al. (2011) based on specimens of studies IV and V.

Taenia arctos is the only taeniid species, which uses bears as principal definitive hosts. In addition, it is of special evolutionary significance because of the close relationship to *T. solium.* Raundrup et al. (2012) reported, based on molecular diagnosis, an infection with sterile cysticerci of *T. arctos* (misnaming causative agent as '*T. o. krabbei*') in a muskox (*Ovibos moschatus*) from an area in Greenland, where polar bears (*Ursus maritimus*) apparently do not occur. The only predatory mammals there are Arctic foxes and humans. Given a probable sporadic human activity in tundra, the Arctic fox may be a candidate for a definitive host of *T. arctos* – a topic which requires further investigation. The sister species relationship between *T. arctos* and *T. solium* was recently supported by phylogenetic analyses of mitogenomic and nDNA data (Yitagele et al. 2014). Phylogenies suggested that *T. arctos* and *T. solium* are related to two species of *Taenia* in the spotted hyena (*Crocuta crocuta*). The results do not fully confound a previously proposed hypothesis of the African origin of human-*Taenia* (Hoberg et al. 2001a). Nevertheless, they raise the question whether the host switch from carnivorans to hominins could have occurred in the Palearctic region during the evolution of *T. solium*.

Concerning *T. krabbei*, our findings in Finland, Sweden, Svalbard and Alaska confirmed the Holarctic distribution of this parasite (III, V). *Taenia cervi* Christiansen, 1931, described originally from the roe deer (*Capreolus capreolus*) in Denmark, was considered to be synonymous with *T. o. krabbei* by Verster (1969). A recent molecular analysis of cysticerci in a Danish roe deer confirmed conspecificity of *T. cervi* and *T. krabbei* (Al-Sabi et al. 2013).

5.1.4 Unknown species of *Taenia* in lynx, a member of the felid-*Taenia* clade (VI)

A survey of taeniids in lynx from Finland (VI) revealed low species diversity. The presence of only two species was demonstrated by mtDNA sequencing and comparative morphology. *Taenia laticollis* was found to be common. The second species, found only in four lynx out of 72, was a large-sized tapeworm with unique mtDNA profiles. In addition, it differed from the other species of *Taenia* in felids from the Holarctic region based on the morphology of the rostellar hooks. This putative new *Taenia* sp. was phylogenetically related to *Taenia hydatigena* Pallas, 1766, a common species occurring in canid-ruminant life cycles worldwide, and *Taenia regis* Baer, 1923 in African large felids (*Panthera* spp.). Originally the life cycle of *Taenia* sp. in lynx remained unknown but recently cysticerci were found in cervids (Lavikainen et al. unpubl.).

Phylogenetic placement of the unknown *Taenia* sp. is shown in Fig. 3. Sequences of two species, which were not included in study VI, have now been added. The unknown species is located in a clade consisting of several species with felid definitive hosts (hereafter called as 'felid-*Taenia* clade'). Basally diverged species parasitize lynx or cougar, which are phylogenetically related cats (Johnson et al. 2006). Next are species parasitizing *Panthera* spp. The species of veterinary importance, *T. hydatigena*, has evolved as a result of a host switch from felids to

canids. This tree is based on a single short mtDNA region, and thus further sequencing is required to confirm the topology of the felid-*Taenia* clade.

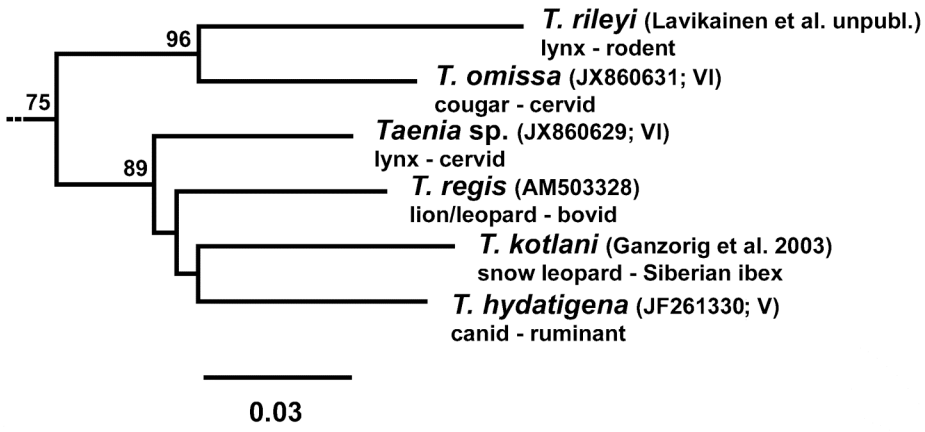

Figure 3. Felid-*Taenia* clade; a detail from a NJ tree inferred with KP2 distances from the *cox1* sequence data set of 22 species of *Taenia* with PAUP* 4.0b10. GenBank accession numbers or references for sequences are shown in parentheses. Life cycles of each species are presented. Bootstrap values (>50%) are shown above branches. The scale bar is proportional to the substitutions per site.

5.2 Generic level revision of the Taeniidae

5.2.1 General overview on molecular phylogenies of the Taeniidae (III, IV, VI–VIII)

In the present thesis, partial and complete mtDNA genes, as well as whole mitogenomes and nDNA regions, were used to reconstruct phylogenetic relationships within the Taeniidae. The results clearly demonstrated that *Echinococcus* is a compact monophyletic group, whereas *Taenia* is a highly diversified assemblage. This was also shown in a recent phylogenetic study (Knapp et al. 2011) based on nuclear protein-coding genes and the same parasite material as used in studies III and VII.

Phylogeny of Echinococcus

The phylogenies of *Echinococcus* inferred from concatenated *cox1* and *nad1* sequences (III) and mitogenomes (Nakao et al. 2007; complete version in article VIII) were essentially similar. Neotropical species were basal, the *E. granulosus* s.l. complex was paraphyletic and identical pairs of sister species were present. Two first points were already visible in the first phylogenetic tree by Bowles et al. (1995) based on *cox1* and *nad1* data. Details of the topologies differed and bootstrap values in the phylogenies based on short sequences (Bowles et al. 1995; III) were very low. The

topology of the nDNA phylogeny by Knapp et al. (2011) was mostly similar to that of the robust mitogenomic phylogeny (compared in article VIII). Another nDNA phylogeny (Saarma et al. 2009) differed by placing *E. multilocularis* as the most basal taxon.

Phylogeny of Taenia

In the phylogenies based on short mtDNA sequences (III, IV, VI), mitogenomes (VII) and nDNA (VII), *Taenia* was divided into similar main clades. The essentially same branching order of the main clades, which was supported by low bootstrap values in the phylogenies inferred from concatenated *cox1* and *nad1* sequences (III, IV), appeared in the more robust mitogenomic analysis (VII) (outlined in Fig. 4). The nDNA trees were very similar to the mitogenome trees especially concerning robust internal nodes within the main clades, but some disagreement was seen particularly in deeper nodes and in the basal topology.

The main clades of the Taeniidae are presented in Fig. 4. Clade Ia consists of species using canid, ursid or human definitive hosts and ruminant, suid or lagomorph intermediate hosts. Clade Ib includes *T. crassiceps*, *Taenia martis* (Zeder, 1803) and *Taenia twitchelli* Schwartz, 1924. The first of these occurs in a canid-rodent life cycle and the latter two in mustelid-rodent cycles. Uncertainty remains on the topology due to differences between phylogenies based on nDNA and mitogenomic data. The placement of *T. polyacantha* in an independent clade is also uncertain because this taxon was not included in study VII. The branch length and unstable position of *Taenia pisiformis* (Bloch, 1780) within clade Ia (III, IV) probably indicate the presence of a long-branch attraction artefact. Adding closely related taxa to analyses would clarify the relationships of *T. pisiformis.*

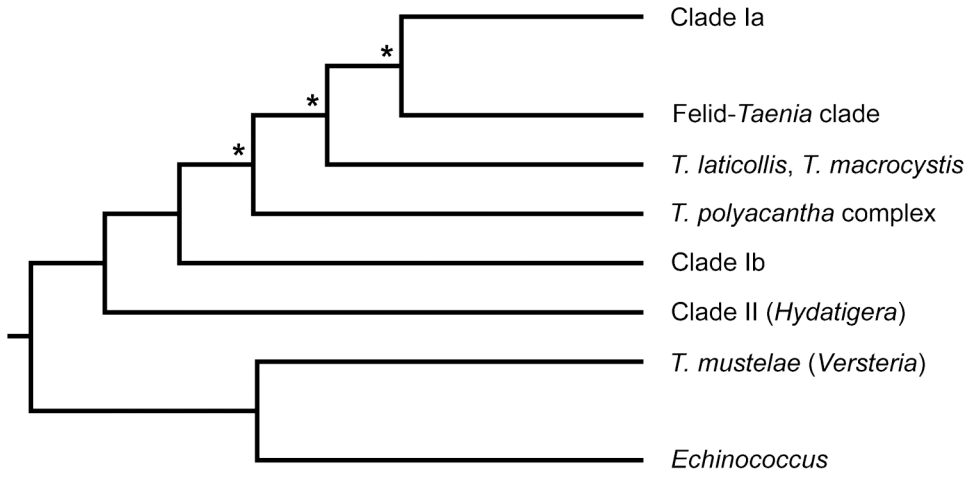

Figure 4. Hypothesis for a branching pattern of the main taeniid clades. The cladogram is based on mtDNA phylogenies in articles III, IV, VI and VII. Nodes with low support are marked with asterisks. For definitions of the clade names, see text and article VII.

The most significant discovery of this thesis is the paraphyly of *Taenia.* The paraphyly is primarily caused by the position of *T. mustelae* as sister to *Echinococcus* (III, VII). In addition, the phylogeny based on nuclear protein-coding genes placed a clade that includes *Taenia krepkogorski* (Schultz & Landa, 1934), *Taenia parva* Baer, 1924 and *T. taeniaeformis* (clade II) as sister to all other taeniids, which renders *Taenia* paraphyletic. This topology was also observed by Knapp et al. (2011). In the mitogenomic phylogeny, clade II was located most basally within *Taenia.* Analysis of the 18S rDNA data set suggested different topology, in which *Taenia* was monophyletic. However, this topology was not robust. Unrooted trees based on nuclear protein-coding gene and mitogenomic data sets demonstrated that the clades II and *T. mustelae* + *Echinococcus* are distantly related to other members of the Taeniidae.

A comparison between our results and previously published molecular phylogenies of *Taenia* is difficult because fewer taxa were included in the early analyses. The basic topology of the phylogenies in this thesis is similar to that of the trees in Gasser et al. (1999) and Zhang et al. (2007) based on mtDNA and rDNA data. A phylogeny based on 12S rDNA by von Nickisch-Rosenegk et al. (1999) differed by placing *T. taeniaeformis* and *T. martis*, and *T. parva* and *T. mustelae*, as sisters. The position of *T. taeniaeformis* among the other members of *Taenia* is the major difference in the phylogeny by Okamoto et al. (1995) based on the short *cox1* fragment. Similar placement of *T. taeniaeformis* was observed in study VI when this region was used to construct a NJ tree. The partial *cox1* sequence (350–400 bp) can be used alone in distinguishing different taeniid species and in some cases in visualizing relationships of closely related taxa (e.g. Fig. 3), as shown by previous studies, this thesis and recent barcoding analyses (e.g. Galimberti et al. 2012), but in general it is not sufficient as a sole marker of phylogeny.

The internal topology of clade Ib is supported by morphology-based phylogenies (Hoberg et al. 2000, 2001a; Hoberg, 2006). This is the major agreement between molecular and morphological phylogenies; otherwise results are only roughly congruent. The grouping of species as basal or apical is similar. Extensive coevolution between taeniid parasites and their definitive hosts is generally not supported by either of the approaches, but two different origins of human-*Taenia* spp. are suggested by both. The phylogenies of this thesis did not clearly demonstrate that colonization of artiodactyl intermediate hosts would have been a single event, as morphological phylogenies (e.g. Hoberg et al. 2000) suggested, since it is possible that clade Ia and the felid-*Taenia* clade have evolved independently. Homoplastic morphological characters used in cladistic analyses may explain most mismatches between the phylogenies.

5.2.2 Taxonomic interpretations

***New taeniid genus,* Versteria**

Based on the strongly supported sister taxon relationship of *T. mustelae* and *Echinococcus*, clearly resulting in paraphyly of *Taenia*, a generic level revision was warranted. Thus, creation of *Versteria* gen. nov. for *T. mustelae* (as the type species) was proposed in article VIII. *Taenia brachyacantha* Baer & Fain, 1951, which is

morphologically very similar, was included in the new genus, although its sequence data have not been published. Morphologically, the new genus can be differentiated from *Taenia* especially based on the short rostellar hooks and small scolex, rostellum and suckers. These characters may represent synapomorphies with *Echinococcus.* Species of *Versteria* use mustelids as definitive hosts and rodents as intermediate hosts.

Recently, a fatal case of disseminated infection caused by an unknown species of *Versteria* was reported in a captive orangutan (*Pongo pygmaeus*) in USA (Goldberg et al. 2014). Our unpublished molecular data suggest that this species probably represents a Nearctic form of *Versteria* using at least muskrats (*Ondatra zibethicus*) as intermediate hosts (Lavikainen et al. unpubl.). Proliferative metacestodes of *Versteria* have often been reported in North America (e.g. Freeman, 1956). Interestingly, multiplying metacestodes, obviously belonging to *Versteria*, have been described only once in Eurasia (see Kirschenblatt in Abuladze, 1964, pp. 232–233). A tendency to asexual multiplication may be a characteristic feature of the Nearctic species differentiating it from Eurasian (*Versteria mustelae* [Gmelin, 1790]) and African (*Versteria brachyacantha* [Baer & Fain, 1951]) species. Infection in an orangutan raises concerns about the zoonotic potential of this parasite (Goldberg et al. 2014).

Resurrection of Hydatigera

Molecular phylogenies clearly indicated that clade II is distantly related to other members of *Taenia* (VII). It was separated by a long branch from other taeniids. Furthermore, it was sister to all other taeniids in a phylogeny based on nuclear protein-coding genes suggesting paraphyly of *Taenia*. In the mitogenomic and 18S rDNA analyses, clade II was sister to *Taenia* (excluding *Versteria*). These results justified the recognition of clade II as a distinct genus. Members of clade II have earlier been placed in the genus *Hydatigera* (see e.g. Abuladze, 1964). Therefore, this generic name was resurrected (VII).

The type species of the genus is *Hydatigera taeniaeformis* (Batsch, 1786). The key characteristics of the genus are large rostellar hooks and strobilocercus as a metacestode. In addition, terminal genital ducts pass the longitudinal osmoregulatory canals ventrally indicating the membership in Verster's group II. Definitive hosts are felids or viverrids, and intermediate hosts are rodents. Concerning these characteristics, there is considerable overlap with some other taeniid species. Among those, *T. selousi* fulfills other criteria but has somewhat smaller rostellar hooks. Considering this single character as non-critical, *T. selousi* could be placed within *Hydatigera*, but molecular data are not available. Another candidate, *Taenia rileyi* Loewen, 1929, possessing strobilocercus larva (or hemistrobilocysticercus as proposed by Rausch, 1981), seems to belong to the felid-*Taenia* clade instead of *Hydatigera* according to our preliminary molecular data (Fig. 3, Lavikainen et al. unpubl.).

Taenia *sensu stricto and* Echinococcus

The remaining members of *Taenia* were classified within *Taenia* sensu stricto in article VII. Species, which were represented in the phylogenetic analyses, formed a monophyletic but genetically diversified group. Deep branching and diverse host associations suggest that this group is evolutionarily older than other taeniid genera. Some of the clades may require taxonomic re-evaluation in the future, perhaps subgeneric or even generic ranking. *Taenia martis*, the type species of *Fimbriotaenia*, is located in clade Ib (VII). *Taenia polyacantha*, the type species of *Tetratirotaenia*, is possibly occupying a relatively basal clade of its own (III). Although these clades are related to other members of *Taenia* s.s., there remains some uncertainty about their taxonomic status due to their basal divergence and branch lengths. However, recognition of *Fimbriotaenia* or *Tetratirotaenia* as valid genera based on the phylogenies presented in this thesis would be clearly premature and would raise needs for further untimely splitting of *Taenia*.

Advances in systematics of *Echinococcus* from the past to the present are thoroughly reviewed in article VIII. Monophyly of *Echinococcus* is well established, and species composition within this genus is almost clarified. Close genetic relationships among the species of *Echinococcus* imply that the genus is a young, rapidly speciated group.

Invalid or uncertain taxa

The phylogenetic analyses of this thesis did not support recognition of the genera *Alveococcus*, *Multiceps* or *Taeniarhynchus*. Their recognition would make either *Echinococcus* or *Taenia* paraphyletic since type species are located within well-supported clades – *E. multilocularis* (*Alveococcus*) within *Echinococcus*, and *T. multiceps* (*Multiceps*) and *T. saginata* (*Taeniarhynchus*) within clade Ia. Moreover, *Multiceps* sensu Abuladze (1964) would be a polyphyletic taxon. *Fimbriotaenia* and *Tetratirotaenia* can also be considered invalid taxa as mentioned above. The validity of *Monordotaenia* cannot be evaluated since the type species, currently known as *Taenia taxidiensis* Skinker, 1935, or other species that might be placed in this genus, were not included.

Subfamilies Taeniinae and Echinococcinae were not supported. The Taeniinae, as definied by Rausch (1994b), would be paraphyletic. Cladistic relationships presented in article VII indicate that the subfamilial classification of taeniid tapeworms should be invalidated. Alternatively, the Taeniinae needs to be refined and new subfamilies created for *Hydatigera* and *Versteria*. Such a revision would, nevertheless, confuse more than clarify the taxonomic classification of taeniid tapewoms.

6 Concluding remarks

The studies comprising this thesis cover a long time period. The first article (I) was published in 2003. During the past eleven years the use of molecular methods in the identification of taeniids has considerably increased. Active research has expanded our knowledge of the taeniid diversity, molecular epidemiology and evolutionary history. The studies of the present thesis contributed to this development by characterizing molecularly several taeniid taxa and by presenting hypotheses for their phylogenetic relationships.

6.1 Summary

Based on molecular analyses, cryptic or previously unknown species or intraspecific variants were detected, and the specific status of some taeniid taxa was confirmed.
(1) A new genotypic group, the Fennoscandian cervid strain or genotype G10, of *E. granulosus* s.l. was characterized in cervids in Finland and Sweden. Its position within a recently recognized species *E. canadensis* was confirmed by mitogenomic data. Based on later findings, its status as a distinct strain should be rejected, but the use of the genotypic code G10 is still recommended for ecological and epidemiological considerations.
(2) Two cryptic complexes of closely related species were detected. *Taenia polyacantha* was shown to comprise two species, which are probably related to the subspecies *T. p. polyacantha* and *T. p. arctica* with southern and northern distributions, respectively. The presence of two cryptic species within *T. taeniaeformis* was confirmed.
(3) In phylogenetic analyses, *T. ovis krabbei* was placed as sister to *T. multiceps*, distant to *T. o. ovis*. This result clearly supports recognition of *T. krabbei* as a distinct species.
(4) An unknown species of *Taenia* was discovered in moose and brown bears. A Holarctic distribution of this parasite was demonstrated. Phylogenetic analysis suggested a sister species relationship between this species and *T. solium*. The new species has later been morphologically described and named as *T. arctos*.
(5) Another unknown species of *Taenia* was discovered in lynx from Finland. Based on the morphology of rostellar hooks, it clearly differed from the other *Taenia* spp. recorded in felids from the Holarctic region. This species is phylogenetically closely related to *T. hydatigena*, *T. kotlani* and *T. regis.*

The most significant result of the phylogenetic analyses was that *T. mustelae* and a clade formed by *T. krepkogorski*, *T. taeniaeformis* and *T. parva* (‘clade II’) are only distantly related to other *Taenia* spp. *Taenia mustelae* was placed as sister to *Echinococcus* in most phylogenies, and clade II was sister to other taeniids in a phylogenetic tree inferred from nuclear protein-coding genes, both of these topologies indicating paraphyly of *Taenia*. In conclusion, a generic level revision was justified. A new genus *Versteria* was created for *T. mustelae* and an old genus *Hydatigera* was resurrected for clade II. In addition, *T. brachyacantha* was included in *Versteria* due to morphological similarities.

The remaining *Taenia* s.s. (excluding *Hydatigera* and *Versteria*) is a monophyletic but diversified assemblage. *Echinococcus* is a monophyletic group, close genetic relationships within this genus implying young evolutionary age and recent rapid speciation and global radiation.

6.2 Future prospects

The phylogeny of taeniid tapeworms is not yet conclusively resolved. Open questions remain especially concerning the basal topology within *Taenia* s.s. Additional taxa would clarify relationships of the main clades of *Taenia* s.s. and confirm their taxonomic status.

One problem in resolving taeniid phylogeny is that an optimal outgroup has not been determined. In our studies, rather distant taxa were used. Only a couple of incomplete family level molecular studies on cyclophyllidean phylogeny (von Nickisch-Rosenegk et al. 1999a; Foronda et al. 2004) have been published. The phylogeny of cyclophyllidean families would be a reasonable subject for further research. A morphological phylogeny suggested that *Dasyurotaenia* is closely related to the Taeniidae (Hoberg et al. 1999). However, members of *Dasyurotaenia* are parasites of protected carnivorous marsupials, and specimens are thus very difficult to obtain. Other putatively closely related taxa, such as metadilepidids and paruterinids, could be tested as outgroups.

Short mtDNA sequences are useful in the identification of taeniids. However, complete genes or mitogenomes are preferable in phylogenetic analyses. Hardman and Hardman (2006) evaluated the phylogenetic performance of platyhelminth mitochondrial protein-coding genes and their fragments of different lengths. They noted that accurate phylogeny could be obtained from several hundred bp but nodal supports remained low. Our observations were similar. Hardman and Hardman (2006) recovered all expected nodes with > 90% bootstrap support when 4,000 bp were sampled. They recommended the use of NADH dehydrogenase subunit 2 due to its superior perfomance and *cox1* due to its wide use in barcoding studies. A molecular phylogeny of *Echinococcus* inferred from a set of four mitochondrial genes (ca 5,200 bp) (Hüttner et al. 2008) differs very little from mitogenomic trees in topology, and most nodes are strongly supported. In addition to mitochondrial genes, the use of nuclear sequences is recommended in phylogenetic studies. Particularly, they are essential when reproductive isolation and possible hybridization of taxa are being evaluated.

When the first paper of this project was published, only a couple of taeniid mitogenomes were available in the GenBank database. Now nuclear genomes for *E. granulosus* s.s., *E. multilocularis* and *T. solium* have been published affording large material for evolutionary studies (Tsai et al. 2013). The past 15 years have been the golden age of taeniid mitochondrial genomes but perhaps a new era of nuclear genomes has now begun. One interesting topic would be a comparison between genomes of *Echinococcus* and *Versteria.* In spite of these predictions, one should not underappreciate the value of mitochondrial genes or small sets of nuclear genes in future research. They will still remain useful and practical tools for inferring phylogenies.

A great challenge for future research is to uncover hidden diversity within taeniid tapeworms. The diversity is huge as suggested by our findings: two new species, *Taenia* sp. in lynx and *T. arctos*, were discovered in Europe. The diversity is obviously much higher in southern regions; e.g. Yitagele et al. (2014) found three species of *Taenia*, of which two were previously unknown, in spotted hyenas in Ethiopia with a rather limited sampling of 11 animals. It seems that undetected diversity within taeniids is a continuum from 'true' cryptic taxa, which are practically impossible to differentiate by morphology (e.g. *H. taeniaeformis* complex), to species, which have been neglected due to superficial inspection of specimens (e.g. *Taenia* sp. in lynx and *T. arctos*).

mtDNA barcoding is a highly efficient tool for the identification of specimens and for revealing the presence of cryptic lineages within known taeniid species, as shown by our studies and those of others (e.g. Galimberti et al. 2012). Although DNA methods have simplified identification and cleared a path for modern thinking, the use of molecules in taxonomy is not simple. One problem is the undetermined and variable limits of intraspecific genetic variation. Another problem is hybridization, which blurs the boundaries of species, as recently found between *T. asiatica* and *T. saginata* (Okamoto et al. 2010; Yamane et al. 2012). In addition, taxonomy is still largely based on visible diagnostic criteria. It is not straightforward to describe and name species that cannot be differentiated by traditional methods. This is especially true if the hidden species are ecologically almost identical with their sisters, as in the case of the *H. taeniaeformis* complex. The discussion of taxonomic problems related to cryptic species has been going on for very long. Nevertheless, as stated by Dobzhansky (1959), species are phenomena of nature, which exist regardless of our ability to distinguish them.

But why should relationships or diversity of tapeworms be scrutinized? Some anthropocentric answers can be found in the pages of this thesis. *Versteria*, a negligible group of parasites in mustelids and rodents, is closely related to *Echinococcus*, which is of a major medical significance. *Taenia krabbei*, which harms cervids in northern regions, is sister to global zoonotic *T. multiceps.* A rare parasite of bears, *T. arctos*, is sister to the causative agent of the human neurocysticercosis, *T. solium*, which is regarded as the most important species of human-*Taenia.* Knowledge of evolutionary relationships and taxonomy provides a basis for comparative research, which could clarify, for example, the mechanisms of pathogenicity and host specificity of parasites. On the other hand, as Yitagele et al. (2014) recently showed by analyzing relationships of the human-*Taenia* and *Taenia* spp. in large carnivorans, understanding the evolution of human parasites may provide us with an insight into the history of our own.

6.3 Taxonomic summary with comments on phylogeny and DNA diagnostics

The following taxonomy of taeniids is proposed:

Family Taeniidae Ludwig, 1886

A cyclophyllidean family with four genera and 54 valid species.

Familial diagnosis according to Rausch (1994b), briefly summarized as follows: Strobila large, ribbon-like, with many proglottids, or tiny, with few proglottids. Rostellum well developed with usually two rows of hooks with epiphysis. Single set of genitalia in each segment. Genital pores marginal. Ovary median, bilobed; vitelline gland compact, median, posterior to ovary. Testes numerous. Uterus typically with numerous branches. Eggs with thick striated embryophore. Metacestode a cysticercus or modification thereof. Mammals as definitive and intermediate hosts.

Monophyletic; DNA data, including *cox1* and *nad1* barcodes, currently available for about 60% of the species.

Type genus *Taenia* Linnaeus, 1758 sensu stricto

For generic synonyms, see Rausch (1994b).

Type species *Taenia solium* Linnaeus, 1758; 40 valid species listed in Table 2. For synonyms of specific names, see Wardle and McLeod (1952), Abuladze (1964), Verster (1969), Loos-Frank (2000).

Valid generic diagnosis presented by Rausch (1994b), briefly as follows: Typically medium or large-sized taeniids, some species small. Rostellum typically with two rows of hooks; rarely with one row or absent. Testes abundant. Terminal genital ducts usually pass between longitudinal osmoregulatory canals; in some species genital ducts ventral to osmoregulatory canals. Metacestode a cysticercus, coenurus, fimbriocercus or, in some species, strobilocercus.

Remarks: Basic structure is similar to that of *Hydatigera* and *Versteria.* In addition, some species possess one or more typical characteristics of *Hydatigera*.

DNA data have been published for half of the species. Barcoding based on *cox1* or *nad1* sequences is useful for the identification of species and subspecific entities. The genus appears to be monophyletic but genetically diverse; several subclades with uncertain taxonomic status are present.

Genus *Echinococcus* Rudolphi, 1801

For generic synonyms, see Rausch (1994b).

Type species *Echinococcus granulosus* (Batsch, 1786); nine valid species listed with synonyms in VIII; for other possible synonyms see Abuladze (1964).

Valid generic diagnosis presented by Rausch (1994b), briefly as follows: Tiny strobila up to 12 mm in length, with no more than seven proglottids, very small rostellar hooks in two rows. Gravid uterus with or without lateral branches; fully gravid uterus only in terminal proglottid. Testes relatively few. Metacestode a cystic or multicystic structure producing protoscoleces in brood capsules. Adults typically in canids or felids.

Remarks: Easily distinguishable from other members of the family based on the miniature adult and hydatid metacestode. Measurements of scolex and hooks similar to those of *Versteria.*

DNA data have been published for all species and for several subspecific entities, which are identifiable by barcoding using *cox1* or *nad1*. Various alternative molecular markers and methods have also been developed for identification. In most molecular phylogenies, the placement is basal and as sister to *Versteria*.

Genus *Hydatigera* Lamarck, 1816

Synonym: *Reditaenia* Sambon, 1924.

Type species *Hydatigera taeniaeformis* (Batsch, 1786); three valid species listed in Table 3. For synonyms, see article VII; other possible synonyms listed in Wardle and McLeod (1952), Abuladze (1964), Verster (1969), Loos-Frank (2000).

Generic diagnosis presented in article VII. Briefly, small to medium-sized taeniids; large rostellar hooks in two rows; strobilocercus metacestode in rodent intermediate host. Terminal genital ducts pass longitudinal osmoregulatory canals ventrally. Adults in felids or viverrids.

Remarks: There is considerable overlap in one or more key characteristics with some species of *Taenia* s.s. The most closely resembling species is *Taenia selousi* Mettrick, 1962, which fulfills the other criteria but has somewhat smaller hooks. Old diagnostic criteria of the genus *Hydatigera*, including large rostellum, projecting suckers and absence of neck, cannot be employed before thorough reassessment because they are influenced by the fixation method.

DNA data have been published for all species (Table 3). Barcoding based on *cox1* or *nad1* sequences is useful in the identification of species and cryptic entities within the type species. The placement in molecular phylogenies is basal or nearly basal.

Genus *Versteria* Nakao, Lavikainen, Iwaki, Haukisalmi, Konyaev, Oku, Okamoto & Ito, 2013

Type species *Versteria mustelae* (Gmelin, 1790). Another species *Versteria brachyacantha* (Baer & Fain, 1951). Synonyms and species with doubtful status listed in Table 4.

Generic description presented in article VII. Briefly, taeniids with small-sized strobila and small scolex, rostellum and suckers; rostellum with two rows of very small hooks; testes relatively few. Terminal genital ducts pass longitudinal osmoregulatory canals ventrally. Adults in mustelids. Intermediate hosts rodents; metacestode a cysticercus or coenurus.

Remarks: There is some overlap with *Hydatigera* and *Taenia* s.s. in the number of testes and the length of the strobila.

DNA data have been published for the type species and for a Nearctic species with unclear taxonomic status (Table 4). Barcoding based on *cox1* or *nad1* sequences is able to distinguish species. The genus is placed as sister to *Echinococcus* in most molecular phylogenies.

Table 2. Valid species of *Taenia* sensu stricto and three unknown species.

	Species	Valid taxon according to[1]	Distribution[2]	Typical life-cycle	DNA data[3]
1	*Taenia acinomyxi* Ortlepp, 1938	V	Afrotropical	Felid – ?	–
2	*Taenia arctos* Haukisalmi, Lavikainen, Laaksonen & Meri, 2011	Ha	Holarctic	Ursid – cervid	+
3	*Taenia asiatica* Eom & Rim, 1993	Ho	East Asia, Indochina	Human – suid	+
4	*Taenia crassiceps* (Zeder, 1800)	V	Holarctic	Canid – rodent	+
5	*Taenia crocutae* Mettrick & Beverley-Burton, 1961	V	Afrotropical	Hyaenid – bovid	Y
6	*Taenia dinniki* Jones & Khalil, 1984	L	Afrotropical	Hyaenid – ?	–
7	*Taenia endothoracicus* (Kirschenblatt, 1948)	V	Palearctic	Canid – rodent	–
8	*Taenia gonyamai* Ortlepp, 1938	V	Afrotropical	Felid – bovid	–
9	*Taenia hyaenae* Baer, 1924	V	Afrotropical	Hyaenid – bovid	–
10	*Taenia hydatigena* Pallas, 1766	V	Cosmopolitan	Canid – bovid	+
11	*Taenia ingwei* Ortlepp, 1938	V	Afrotropical	Felid – ?	–
12	*Taenia intermedia* Rudolphi, 1809	Ra	Holarctic	Mustelid – rodent	–
13	*Taenia jaipurensis* Sharma, Bhalya, Seth & Capoor, 1983	L	India	Felid – ?	–
14	*Taenia kotlani* Murái, Gubányi & Sugar, 1993	L	Mongolia	Felid – bovid	+
15	*Taenia krabbei* Moniez, 1879	This study; Ha	Holarctic	Canid – cervid	+
16	*Taenia laticollis* Rudolphi, 1819	V	Holarctic	Felid – lagomorph	+
17	*Taenia macrocystis* (Diesing, 1850)	V	Holarctic	Felid – lagomorph	+
18	*Taenia madoquae* (Pellegrini, 1950)	L	Afrotropical	Canid – bovid	+
19	*Taenia martis* (Zeder, 1803)	V	Palearctic	Mustelid – rodent	+
20	*Taenia multiceps* Leske, 1780	V	Cosmopolitan	Canid – bovid	+
21	*Taenia olngojinei* Dinnik & Sachs, 1969	V	Afrotropical	Hyaenid – bovid	–
22	*Taenia omissa* Lühe, 1910	V	Nearctic, Neotropical	Felid – cervid	+
23	*Taenia ovis* (Cobbold, 1869)	V	Cosmopolitan	Canid – bovid	+
24	*Taenia parenchymatosa* Pushmenkov, 1945	V	Palearctic	Canid – cervid	–
25	*Taenia pencei* Rausch, 2003	Ra	Nearctic	Procyonid – rodent	–

26	*Taenia pisiformis* (Bloch, 1780)	V	Cosmopolitan	Canid – lagomorph	+
27	*Taenia polyacantha polyacantha* Leuckart, 1856	V	Palearctic	Canid – rodent	+ (?)
	T. polyacantha arctica Rausch & Fay, 1988	L	Holarctic	Canid – rodent	+ (?)
28	*Taenia pseudolaticollis* Verster, 1969	V	Nearctic	Felid – ?	–
29	*Taenia regis* Baer, 1923	V	Afrotropical	Felid – bovid	+
30	*Taenia retracta* Linstow, 1904	L	Mongolia	Canid – lagomorph	–
31	*Taenia rileyi* Loewen, 1929	V	Nearctic	Felid – rodent	(+)
32	*Taenia saginata* Goeze, 1782	V	Cosmopolitan	Human – bovid	+
33	*Taenia saigoni* Le-Van-Hoa, 1964	L	Indochina	? – primate	–
34	*Taenia selousi* Mettrick, 1962	V	Afrotropical	Felid – rodent	–
35	*Taenia serialis serialis* (Gervais, 1847)	V	Cosmopolitan	Canid – lagomorph	+
	T. serialis brauni (Setti, 1897)	V	Afrotropical	Canid – rodent	–
36	*Taenia simbae* Dinnik & Sachs, 1972	L	Afrotropical	Felid – bovid	–
37	*Taenia solium* Linnaeus, 1758	V	Cosmopolitan	Human – suid	+
38	*Taenia talicei* Dollfus, 1960	Ro	Neotropical	Canid (experimental) – rodent	–
39	*Taenia taxidiensis* Skinker, 1935	V	Nearctic	Mustelid – rodent	–
40	*Taenia twitchelli* Schwartz, 1924	V	Holarctic	Mustelid – rodent	+
Species yet to be described:					
	Taenia sp. 'AL-2012'[4]		Palearctic	Felid – cervid	+
	Taenia sp. 'MPM<JPN>:20922'[4]		Afrotropical	Hyaenid – bovid	Y
	Taenia sp. 'MZH:127001 MZH:127052'[4]		Afrotropical	Hyaenid – ?	Y

[1] V = Verster, 1969; L = Loos-Frank, 2000; Ra = Rausch, 2003; Ho = Hoberg, 2006; Ro = Rossin et al. 2010; Ha = Haukisalmi et al. 2011.
[2] Presented at level of ecozones; smaller biogeographic region given for species with apparently restricted distribution.
[3] + = DNA data (previously published or new) used in this study; (+) = unpublished data, see Fig. 3; (?) = further morphological examination required for subspecific identification of DNA samples; Y = Yitagele et al. 2014; – = DNA data not available.
[4] Codes of unknown taxa in GenBank database.

Table 3. Valid species of *Hydatigera* and a cryptic complex within *Hydatigera taeniaeformis*.

Species	Distribution	Life-cycle	DNA data[1]
Hydatigera taeniaeformis (Batsch, 1786)			
Species A	Cosmopolitan	Felid – rodent	+
Species B	Palearctic?	Felid – rodent	+
Species C	Italy	Felid – rodent	+
Hydatigera krepkogorski Schulz & Landa, 1934	Southwest and Central Asia	Felid – rodent	+
Hydatigera parva (Baer, 1924)	Mediterranean, Afrotropical	Viverrid – rodent	+

[1] + = DNA data (previously published or new) used in this study.

Table 4. Species of *Versteria* with updated lists of synonyms.

Valid species

Species [synonyms]	Distribution	Life-cycle	DNA data[1]
Versteria mustelae (Gmelin, 1790) [*Taenia mustelae* Gmelin, 1790; *Halysis mustelae* (Gmelin, 1790) Zeder, 1803; *Cysticercus talpae* Rudolphi, 1819; *Taenia brevicollis* Rudolphi, 1819; *Taenia tenuicollis* Rudolphi, 1819; *Cysticercus innominatus hypudaci* Leuckart, 1857; *Taenia tenuicollis armata* Joyeux & Baer, 1934; *Taenia joyeuxiana* Hughes, 1941; *Fimbriotaenia mustelae* (Gmelin, 1790) Kornyushin & Sharpilo, 1986]	Palearctic (Holarctic?)	Mustelid – rodent	+
Versteria brachyacantha (Baer & Fain, 1951) [*Taenia brachyacantha* Baer & Fain, 1951; *Fimbriotaenia brachyacantha* (Baer & Fain, 1951) Kornyushin & Sharpilo, 1986]	Afrotropical	Mustelid – rodent	–

Species of doubtful identity and species yet to be described

Species [synonyms]	Taxonomic status	Distribution	Known hosts	DNA data
Versteria sp. 'TLG-2013'[2]	undescribed	Nearctic	*Ondatra zibethicus*; *Pongo pygmaeus*[3]	G
Versteria parviuncinata (Kirschenblatt, 1939) [*Coenurus parviuncinatus* Kirschenblatt, 1939; *Taenia parviuncinata* (Kirschenblatt, 1939) Kirschenblatt, 1948; *Multiceps parviuncinatus* (Kirschenblatt, 1939) Abuladze, 1964; *Fimbriotaenia parviuncinata* (Kirschenblatt, 1939) Kornyushin & Sharpilo, 1986]	inquirenda	Armenia	*Spermophilus citellus*; *Spalax leucodon*	–
Versteria michiganensis (Cower, 1939) [*Taenia michiganensis* Cower, 1939; *Fimbriotaenia michiganensis* (Cower, 1939) Kornyushin & Sharpilo, 1986]	inquirenda	Michigan, US	*Erethizon dorsata*	–

[1] + = DNA data used in this study; – = DNA data not available; G = Goldberg et al. 2014.
[2] Code of the unknown taxon in GenBank database.
[3] Aberrant host.

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Antti Lavikainen
Espoo, May 2014

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