International Journal for Parasitology 46 (2016) 361–374

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Reappraisal of *Hydatigera taeniaeformis* (Batsch, 1786) (Cestoda: Taeniidae) sensu lato with description of *Hydatigera kamiyai* n. sp. ☆

Antti Lavikainen [a,*,1], Takashi Iwaki [b,1], Voitto Haukisalmi [c], Sergey V. Konyaev [d], Maurizio Casiraghi [e], Nikolai E. Dokuchaev [f], Andrea Galimberti [e], Ali Halajian [g], Heikki Henttonen [h], Madoka Ichikawa-Seki [i], Tadashi Itagaki [i], Anton V. Krivopalov [d], Seppo Meri [a], Serge Morand [j], Anu Näreaho [k], Gert E. Olsson [l], Alexis Ribas [m,n], Yitagele Terefe [o], Minoru Nakao [p]

[a] *Department of Bacteriology and Immunology/Immunobiology Research Program, Faculty of Medicine, P.O. Box 21, FI-00014 University of Helsinki, Finland*
[b] *Meguro Parasitological Museum, Shimomeguro, Meguro-ku, Tokyo 153-0064, Japan*
[c] *Finnish Museum of Natural History Luomus, P.O. Box 17, FI-00014 University of Helsinki, Finland*
[d] *Institute Systematics and Ecology of Animals, Siberian Branch Russian Academy of Sciences, Novosibirsk 630091, Russia*
[e] *University of Milano-Bicocca, ZooPlantLab, Department of Biotechnology and Biosciences, Piazza della Scienza 2, 20126 Milano, Italy*
[f] *Institute of Biological Problems of the North, Far East Branch, Russian Academy of Sciences, Magadan 685000, Russia*
[g] *Department of Biodiversity (Zoology), University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa*
[h] *Natural Resources Institute Finland, Vantaa Unit, P.O. Box 18, FI-01301 Vantaa, Finland*
[i] *Laboratory of Veterinary Parasitology, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan*
[j] *CNRS-CIRAD, Centre d'Infectiologie Christophe Mérieux du Laos, P.O. Box 3888, Vientiane, Lao Democratic People's Republic*
[k] *Department of Veterinary Biosciences, Faculty of Veterinary Medicine, P.O. Box 66, FI-00014 University of Helsinki, Finland*
[l] *Department of Wildlife, Fish, and Environmental Studies, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[m] *Biodiversity Research Group, Faculty of Science, Udon Thani Rajabhat University, 41000 Udon Thani, Thailand*
[n] *Laboratory of Parasitology, Faculty of Pharmacy, University of Barcelona, Avda Diagonal s/n, 08028 Barcelona, Spain*
[o] *College of Veterinary Medicine, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia*
[p] *Department of Parasitology, Asahikawa Medical University, Asahikawa, Hokkaido 078-8510, Japan*

ARTICLE INFO

Article history:
Received 21 October 2015
Received in revised form 15 January 2016
Accepted 29 January 2016
Available online 5 March 2016

Keywords:
Hydatigera kamiyai n. sp.
Hydatigera taeniaeformis
Cryptic species
Phylogeny
Taeniidae

ABSTRACT

The common cat tapeworm *Hydatigera taeniaeformis* is a complex of three morphologically cryptic entities, which can be differentiated genetically. To clarify the biogeography and the host spectrum of the cryptic lineages, 150 specimens of *H. taeniaeformis* in various definitive and intermediate hosts from Eurasia, Africa and Australia were identified with DNA barcoding using partial mitochondrial cytochrome *c* oxidase subunit 1 gene sequences and compared with previously published data. Additional phylogenetic analyses of selected isolates were performed using nuclear DNA and mitochondrial genome sequences. Based on molecular data and morphological analysis, *Hydatigera kamiyai* n. sp. Iwaki is proposed for a cryptic lineage, which is predominantly northern Eurasian and uses mainly arvicoline rodents (voles) and mice of the genus *Apodemus* as intermediate hosts. *Hydatigera taeniaeformis* sensu stricto (s.s.) is restricted to murine rodents (rats and mice) as intermediate hosts. It probably originates from Asia but has spread worldwide. Despite remarkable genetic divergence between *H. taeniaeformis* s.s. and *H. kamiyai*, interspecific morphological differences are evident only in dimensions of rostellar hooks. The third cryptic lineage is closely related to *H. kamiyai*, but its taxonomic status remains unresolved due to limited morphological, molecular, biogeographical and ecological data. This *Hydatigera* sp. is confined to the Mediterranean and its intermediate hosts are unknown. Further studies are needed to classify *Hydatigera* sp. either as a distinct species or a variant of *H. kamiyai*. According to previously published limited data, all three entities occur in the Americas, probably due to human-mediated introductions.

1. Introduction

Cestodes of the genus *Hydatigera* Lamarck, 1816 (Cyclophyllidea: Taeniidae) occur, as adult tapeworms, in the small intestine of felid or viverrid definitive hosts, and develop as metacestode stages in tissues or body cavities of rodent intermediate hosts.

☆ Nucleotide sequence data reported in this paper are available in DDBJ/EMBL/GenBank databases under the accession numbers KT693044–KT693095 and LC008533–LC008539.

* Corresponding author. Tel.: +358 294126889.
E-mail address: antti.lavikainen@helsinki.fi (A. Lavikainen).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijpara.2016.01.009
0020-7519/

The taxonomic status of *Hydatigera* has been scrutinised by a number of workers. Based on morphological observations, some authors (e.g. Wardle and McLeod, 1952; Yamaguti, 1959; Abuladze, 1964) have recognised *Hydatigera* as valid, whereas the majority has treated this genus as a junior synonym of *Taenia* Linnaeus, 1758 (e.g. Esch and Self, 1965; Verster, 1969; Rausch, 1994; Hoberg et al., 2000; Loos-Frank, 2000). Nuclear and mitochondrial DNA (mtDNA) sequence evidence, however, strongly supports the distinctiveness of *Hydatigera*, and thus the resurrection of the genus was proposed in a recent revision of the Taeniidae (Nakao et al., 2013a). Currently, the genus consists of only three valid species, *Hydatigera taeniaeformis* (Batsch, 1786), *Hydatigera krepkogorski* Schulz and Landa, 1934, and *Hydatigera parva* (Baer, 1924) (see Nakao et al., 2013a). Among taeniids, species of *Hydatigera* are characterised by large rostellar hooks and a special larval form, the strobilocercus, which is a metacestode with a prominent segmented strobila.

Hydatigera taeniaeformis, the type species of its genus, is the most common and widespread tapeworm of domestic cats and various wild felids (Abuladze, 1964). As a metacestode, it typically parasitises mice and rats (Murinae). This species is admittedly one of the best-known taeniids; it was used, in addition to *Taenia solium* Linneaus, 1758, as a model of the taeniid life cycle, when the link between the adult and larval forms was first solved during the second half of the 19th century (reviewed in Abuladze, 1964). Although *H. taeniaeformis* was considered a single species in many handbooks and revisions (e.g. Yamaguti, 1959; Abuladze, 1964; Verster, 1969), it might represent a cryptic species complex. Intraspecific differences in the host specificity, particularly in infectivity for mice and rats, have been demonstrated experimentally by various authors (e.g. Brandt and Sewell, 1981; Conchedda and Ferretti, 1983). In the 1990s, a laboratory-reared isolate (referred to as "ACR"), originating from the grey-sided vole (*Clethrionomys rufocanus bedfordiae*, at present *Myodes rufocanus*) in Hokkaido, Japan, was shown to differ from Belgian and Asian isolates of murine origins in several criteria, including infectivity, development, morphology and biochemistry (Nonaka et al., 1994; Iwaki et al., 1994; Azuma et al., 1995; Okamoto et al., 1995b). Furthermore, remarkable DNA sequence differences were detected in a region of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene (Okamoto et al., 1995a). It was suggested that the isolate ACR might represent a distinct strain or even a separate, new species (Iwaki et al., 1994; Okamoto et al., 1995a).

Subsequent molecular studies have confirmed the presence of two divergent entities within *H. taeniaeformis* that could, according to the genetic variability, be recognised as separate species (Lavikainen et al., 2008; Galimberti et al., 2012a; Jia et al., 2012; Nakao et al., 2013a). Analysed isolates of one of these cryptic entities have originated mainly in Asia, whereas another group (corresponding to the ACR isolate in Hokkaido) seems to be predominantly European (Jia et al., 2012). In a previous study (Nakao et al., 2013a), the former was designated as sp. A and the latter as sp. B. It was suggested that sp. A can be treated as *H. taeniaeformis* sensu stricto (s.s.) because it can infect mice and rats from which *Cysticercus fasciolaris* Rudolphi, 1808, a historical synonym for *H. taeniaeformis*, has been found. Recently, Galimberti et al. (2012a) identified a third *cox1* lineage of *H. taeniaeformis* in European wildcats (*Felis silvestris silvestris*), domestic cats and their hybrids from Italy, and suggested that it might represent a third cryptic species. In the present article, these previously identified three molecular lineages or clades are referred to as A, B and C, respectively.

Despite the broad consensus on the presence of cryptic species within *H. taeniaeformis* (Okamoto et al., 1995a; Lavikainen et al., 2008; Galimberti et al., 2012a; Jia et al., 2012; Nakao et al., 2013a), there has been no attempt to validate their taxonomic status. The number of published *H. taeniaeformis* isolates has remained too low to reliably determine the geographic distributions of the cryptic lineages. In addition, the intermediate host associations suggested by previous infection experiments (Nonaka et al., 1994; Azuma et al., 1995) are only indicative due to a limited number of isolates analysed and lack of data on natural infections. In this study, many isolates of *H. taeniaeformis* in various intermediate and definitive hosts from different continents (including Eurasia, Africa and Australia) were analysed to elucidate the geographical distribution and natural hosts of the three lineages. The taxonomy of *H. taeniaeformis* sensu lato (s.l.) is clarified by defining molecular clade A as *H. taeniaeformis* s.s. and by describing molecular clade B as a new species. However, we remain undecided about the specific status of clade C from the Mediterranean due to a lack of data on its morphology and intermediate hosts.

2. Materials and methods

2.1. Parasite specimens

A total of 158 specimens of *H. taeniaeformis* s.l. were collected from various definitive and intermediate hosts in Eurasia, Africa and Australia (Table 1; for details, see Supplementary Table S1). Thirty-eight specimens were adult tapeworms from 15 host individuals representing three felid species, and the remaining 120 specimens were strobilocerci from 115 host individuals representing 25 rodent species. Domestic cats were killed in traffic accidents, died of illness or were euthanised by veterinarians for humane reasons (independent from the present work). Wild felids were killed in traffic accidents or found dead due to other causes. Rodents were mainly trapped in the course of rodent research projects, with the exception of the specimens of muskrats (*Ondatra zibethicus*) in Finland, which were zoo animals, and brown rats (*Rattus norvegicus*) in South Africa, which were laboratory animals accidentally infected with contaminated food. Parasite specimens were preserved in 70–95% ethanol at −20 °C.

The materials were obtained according to the laws of the countries in which they were collected. Approval notices for trapping and investigation of rodents were given by the Ministry of Health Council of Medical Sciences, National Ethics Committee for Health Research, Lao People's Democratic Republic (No. 51/NECHR); by the Ethical Committee of Mahidol University, Bangkok, Thailand, (No. 0517.1116/661); by the Environmental Protection Agency, Sweden (No. NV-02939–11); and by the Board of Agriculture, Sweden (ethical permit No. Dnr_A78-08). In the other countries, licenses were not required for snap trapping of unprotected rodent species, or to collect specimens from naturally or accidentally died felids during necropsies performed by veterinary authorities or researchers. The necropsies of Finnish cats were performed with the permission of the cat owners, and the sampling was included to the necropsy referral documents. Zoo and laboratory animals were not infected experimentally and they died of natural causes, and therefore the sampling during necropsy did not require animal ethics permissions.

2.2. DNA amplification and sequencing

A total of 150 specimens were identified genetically (Table 1). Extraction of genomic DNA from strobilocerci and adult proglottids, enzymatic amplifications and sequencing of PCR products were carried out as described previously (Lavikainen et al., 2008; Nakao et al., 2013a; Terefe et al., 2014). The universal primers JB3 and JB4.5 (Bowles and McManus, 1994) were used for the amplification of a partial sequence (396 nucleotide sites) of *cox1*.

Table 1
Genetically identified specimens of *Hydatigera taeniaeformis* sensu lato in this study.

Clade	Country	Stage	Hosts	*n*	*cox1* haplotypes
A	South Africa	Larva	*Rattus norvegicus*[a]	4	A25
	Ethiopia	Larva	*Rattus rattus*[a]	7	A19, A24
	Spain	Larva	*Mus domesticus*[a]	2	A12
	Russia (Far East)	Larva	*Apodemus agrarius*[a], *Rattus norvegicus*[a]	4	A13, A14, A15, A16
		Adult	*Prionailurus bengalensis euptilurus*	7	A13, A22, A23
	Japan	Adult	*Felis silvestris catus*	4	A13
	Cambodia	Larva	*Bandicota savilei*[a], *Berylmys berdmorei*[a], *Maxomys surifer*[a], *Niviventer fulvescens*[a], *Rattus argentiventer*[a], *Rattus exulans*[a], *Rattus norvegicus*[a], *Rattus tanezumi*[a]	29	A1, A2, A3, A4, A5, A8, A26, A27, A28
	Laos	Larva	*Bandicota indica*[a], *Berylmys berdmorei*[a], *Leopoldamys edwardsi*[a], *Rattus andamanensis*[a], *Rattus exulans*[a], *Rattus tanezumi*[a]	11	A1, A2, A6, A32, A34, A35, A36
	Thailand	Larva	*Bandicota indica*[a], *Berylmys berdmorei*[a], *Mus cervicolor*[a], *Rattus exulans*[a], *Rattus tanezumi*[a]	7	A2, A6, A7, A8, A9, A10, A33
	Vietnam	Larva	*Bandicota indica*[a], *Rattus andamanensis*[a], *Rattus argentiventer*[a], *Rattus norvegicus*[a], *Rattus tanezumi*[a]	22	A1, A2, A3, A8, A29, A30, A31
	Australia	Adult	*Felis silvestris catus*	1	A11
B	Norway	Larva	*Microtus agrestis*[b]	1	B5
	Sweden	Larva	*Arvicola amphibius*[b]	5	B3, B6, B7, B11
	Finland	Larva	*Microtus agrestis*[b], *Ondatra zibethicus*[b]	5	B3, B4
		Adult	*Felis silvestris catus*, *Lynx lynx*	19	B3, B4, B6, B7, B8, B9, B10, B12, B13
	Latvia	Larva	*Apodemus flavicollis*[a]	2	B1
	Bosnia	Larva	*Apodemus flavicollis*[a]	1	B2
	Russia (Europe)	Larva	*Apodemus uralensis*[a], *Microtus*[b] sp.	7	B2, B3, B17, B18, B19
	Russia (western Siberia)	Larva	*Alticola strelzowi*[b], *Microtus agrestis*[b], *Microtus oeconomus*[b], *Mus musculus*[a], *Myodes rufocanus*[b], *Myodes rutilus*[b]	11	B3, B7, B19, B20, B21, B22
C	France	Adult	*Felis silvestris catus*	1	C1

cox1, mitochondrial cytochrome *c* oxidase subunit 1 gene.
[a] Murinae (mice and rats).
[b] Arvicolinae (voles).

The usefulness of the primers in DNA barcoding of taeniids (including *Hydatigera*) has been demonstrated repeatedly (e.g. Lavikainen et al., 2008; Galimberti et al., 2012a), and large number of previously published sequences are available for this region.

Isolate HCFr in the present material and a previously published isolate 01364 from Galimberti et al. (2012a) were selected for further sequencing as representatives of the recently discovered (Galimberti et al., 2012a) molecular clade C of *H. taeniaeformis* s.l. For details of these isolates, see Supplementary Table S1. Nuclear DNA sequences (18S ribosomal DNA (rDNA), phosphoenolpyruvate carboxykinase (*pepck*) and DNA polymerase delta (*pold*)) of these isolates were determined as previously described (Nakao et al., 2013a; Terefe et al., 2014). Furthermore, isolate HCFr was subjected to sequencing of the mitochondrial genome (mitogenome) as reported previously (Nakao et al., 2003, 2013a).

2.3. DNA barcoding and phylogenetic analysis

Haplotypes of *cox1* were compared with previously published sequence data of *H. taeniaeformis* s.l. (Table 2) and classified into molecular clades A, B and C. The analyses were performed in MEGA 6 (Tamura et al., 2013). A total of 81 sequences, including present and previously published haplotypes and a *cox1* fragment of *H. krepkogorski* (derived from mitogenomic data, DDBJ/EMBL/GenBank accession AB731762), were aligned using ClustalW (Chenna et al., 2003). The final alignment contained 380 nucleotide sites. Pairwise nucleotide sequence divergences were calculated using the Kimura 2-parameter (K2P) model (Kimura, 1980) with a gamma setting of 0.5. A neighbour-joining (NJ) phenetic tree, based on the comprehensive dataset with *H. krepkogorski* as the outgroup, was constructed using the K2P distances, and assessed with 10,000 bootstrap replicates. Phylogenetic relationships within the main clades were analysed separately for clade A (haplotype B20 as an outgroup) and closely related clades B + C (haplotype A1 as an outgroup). The best fitting nucleotide substitution model GTR+G+I (Tavaré, 1986) was selected for the datasets with Akaike information criterion, and phylogenies were reconstructed with the maximum likelihood (ML) method. Initial trees were built with the NJ algorithm, and the nearest-neighbour-interchange method was used to search for the best trees. Robustness of the trees was tested with 500 bootstrap replicates. The phylogenetic analyses were repeated at least twice to verify the consistency of the results.

Representatives of *H. taeniaeformis* s.l. and *H. krepkogorski* were included in the phylogenetic analysis of nuclear sequences (seven operational taxonomic units (OTUs)) and mitogenomic sequences (four OTUs). The sequences were aligned using MAFFT (Katoh and Standley, 2013), and all of the alignment gaps were removed. The data set of nuclear 18S rDNA comprised 2,472 nucleotide sites, while the data set of nuclear protein-coding genes (*pepck* and *pold*) consisted of 3,141 sites including introns. The intronic regions could be well aligned due to close genetic relationships among the OTUs. The data set of mitogenomes was made up of 10,065 sites from all protein-coding genes. The substitution models were selected using MEGA 6. The model HKY+G+I (Hasegawa et al., 1985) was applied to the data sets of 18S rDNA and nuclear protein-coding genes. The model GTR+G+I was used to the set of mitogenomes. Phylogenies were reconstructed with the ML method implemented in the program PhylML 3.0 (Guindon et al., 2010) and with the Bayesian method in MrBayes 3.2.1 (Ronquist et al., 2012) using the aforementioned substitution models. In the ML analysis, a parsimony tree was used as a starting tree, and the robustness of the phylogeny was tested by bootstrapping with 500 replicates. In the Bayesian analysis, the Markov chain Monte Carlo analysis was run for 1 million generations and sampled every 100 generations to estimate the posterior probabilities of phylogenetic trees. The run produced 10,000 trees, of which the initial 1,000 trees were treated as burn-in. The ML and Bayesian analyses were conducted at least twice for each data set to verify the consistency of the results. All of the resultant phylogenetic trees were rooted with the same outgroup species (*H. krepkogorski*).

Table 2
Previously published isolates of *Hydatigera taeniaeformis* sensu lato, whose partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene sequences were used in this study.

Clade	Country	Stage	Hosts	*n*	Accession nos[c]/isolate codes	Reference	*cox1* haplotypes
A	Japan	Larva	*Apodemus argenteus*[a], *Rattus norvegicus*[a]	6	TtSRN, TtMar, TtTom, TtKaRN, TtKaAA, TtNop	Okamoto et al. (1995a)	A13, A17
	Malaysia	Larva	*Rattus norvegicus*[a]	1	TtKRN	Okamoto et al. (1995a)	A18
	Belgium	Larva	*Mus musculus*[a]	1	TtBMM	Okamoto et al. (1995a)	A19
	China	Larva	*Mus musculus*[a]	1	TtChi	Okamoto et al. (1995a)	A20
	Kazakhstan	Larva	*Apodemus sylvaticus*[a]	1	EU544597/TtaKa	Lavikainen et al. (2008)	A21
	China	Adult	*Felis silvestris catus*	1	FJ597547	Liu et al., 2011	A1
B	Japan	Larva	*Myodes rufocanus*[b]	1	TtACR[d]	Okamoto et al. (1995a)	B1
	Finland	Adult	*Felis catus*	1	EU861478 / TtaFi[e]	Lavikainen et al. (2008)	B3
	Turkey	Larva	*Apodemus sylvaticus*[a]	1	EU544596 / TtaTu	Lavikainen et al. (2008)	B14
	Italy	Adult	*Felis silvestris silvestris*	1	FN547850	Galimberti et al. (2012a)	B15
	Germany	Adult	*Felis silvestris catus*	1	JQ663994/Tt-GER	Jia et al. (2012)	B16
C	Italy	Adult	*Felis silvestris catus*, *Felis s. silvestris*, *Felis s. catus* × *silvestris*	61	FN547823-49, FN547851-84	Galimberti et al. (2012a)	C2–C22

[a] Murinae (mice and rats).
[b] Arvicolinae (voles).
[c] Six-digit numbers are the accession numbers of *cox1* sequences in DDBJ/EMBL/GenBank databases.
[d] Specimens derived from this isolate are used as paratypes of *Hydatigera kamiyai* n. sp. (Meguro Parasitological Museum (MPM) Collection No. 20885).
[e] Used as holotype of *H. kamiyai* n. sp. (MPM Coll. No. 20884).

2.4. Morphological observations

Representatives of clade B, including specimens from the present and previous studies (TtaFi in Lavikainen et al., 2008, and ACR in Iwaki et al., 1994), were used in the morphological examination and description of the new species. The material consists of 12 adult tapeworms obtained from five domestic cats (natural infections) and a Eurasian lynx (*Lynx lynx*; natural infection) from Finland, and a domestic cat from Japan (experimental infection; Iwaki et al., 1994). In addition, 12 strobilocerci from rodents from Finland, Russia and Sweden were examined.

For comparative purposes, morphological data of *H. taeniaeformis* s.s. (clade A) were obtained from 16 adult specimens from a domestic cat from Japan and seven Amur leopard cats (*Prionailurus bengalensis euptilurus*) from the Russian Far East, as well as from five strobilocerci from rodents from southeastern Asia.

Altogether (including clades A and B) 17 specimens were used for observing the size of the strobila or metacestode, 16 for proglottid morphology, 34 for hook lengths, and nine for hook morphometrics. For detailed origins of the specimens used in the morphological examination, see Supplementary Table S1. The new specimens subjected to morphological examination were identified genetically except for eight specimens (one specimen of the new species and seven of *H. taeniaeformis* s.s.) which, however, were from the same host individuals as sequenced specimens.

After relaxation in tap water, adult cestodes were fixed flat (without pressure) and preserved in 70% ethanol. Fragments of cestodes were stained with alum carmine, cleared in xylene or eugenol and mounted in Canada balsam. Cysticerci were fixed and preserved in 70% or 95% ethanol. The hook crowns extracted from cysticerci were mounted in glycerin or chloral-gum medium for study. Only well-aligned rostellar hooks were used for the morphometric analysis.

Scoleces, rostellar hooks and proglottids were drawn using a camera lucida. Observations of mature and gravid proglottids were done for five consecutive proglottids of each strobila. Measurements were taken from these drawings or photographs using a personal computer with ImageJ software (U.S. National Institutes of Health, available at http://rsbweb.nih.gov/ij/), or using a Nikon microscope digital camera system (DS-Fi1 and DS-L2). The number of testes was counted from the drawings. For comparison of the numbers and lengths of rostellar hooks, one to five large and small hooks were measured from each worm. Detailed morphometric analysis of the rostellar hooks was based on adult worms, and included eight different measurements (total length, total width, basal length, apical length, guard length, guard width, blade curvature and handle width) (Gubányi, 1995; Haukisalmi et al., 2011). The total length was recorded from five small and large hooks from each crown (except for four large hooks from a single worm), but the other variables were recorded from three representative large and small hooks from each crown. The differences in the measurements between two species were statistically analysed by a Student's *t*-test. To analyse the ability of hook measurements to separate the species A and B, increasing stepwise discriminant function analysis was performed using R version 3.2.3 in the package MASS version 7.3-45.

Type and voucher specimens of the new species, and vouchers of *H. taeniaeformis* s.s., have been deposited in the Meguro Parasitological Museum (MPM), Tokyo, Japan. Two vouchers of the new species from lynx and four from muskrats have been deposited in the Finnish Museum of Natural History, Helsinki, Finland. The collection numbers of the museum specimens are shown in Supplementary Table S1.

3. Results

Original data of this study including nucleotide sequence alignments, numerical morphological data and a drawing (atypical proglottid from a lynx) are available at Mendeley Data (http://dx.doi.org/10.17632/f34pw8mf4y.1).

3.1. DNA barcoding and phylogenetic relationships

A total of 52 *cox1* haplotypes (sequence types), of which 48 were new, were identified among the 150 specimens of *H. taeniaeformis* s.l. Taking into account previously published data, the complete *cox1* data set included 80 haplotypes. Three main clades A, B and C, corresponding to the previously identified genetic entities of *H. taeniaeformis* s.l. (Okamoto et al., 1995a; Galimberti et al., 2012a), were detected (see the phenogram in Supplementary Fig. S1). Bootstrap supports for the clades were: A, 99%; B, 52%; and C, 99% (B + C, 97%). Clade A consisted of 36

Table 3
Variation in the partial sequence of the mitochondrial cytochrome *c* oxidase subunit 1 gene within and between the clades of *Hydatigera taeniaeformis* sensu lato (s.l.), and between *H. taeniaeformis* s.l. and *Hydatigera krepkogorski*.

Comparisons	Pairwise divergences
Clade A	0.003–0.051
Clade B	0.003–0.035
Clade C	0.003–0.014
Clade A vs. clade B	0.091–0.133
Clade A vs. clade C	0.106–0.150
Clade B vs. clade C	0.047–0.079
Clade A vs. *H. krepkogorski*	0.109–0.133
Clade B vs. *H. krepkogorski*	0.094–0.114
Clade C vs. *H. krepkogorski*	0.129–0.147

haplotypes (designated as A1–A36), whereas clades B and C included 22 haplotypes each (B1–B22 and C1–C22, respectively).

Genetic divergence was estimated by comparing K2P distances of the partial *cox1* sequences within and between the three clades (Table 3). Pairwise divergence values were low (<1.4%) within clade C, but higher within clades A and B, reaching up to 5.1% and 3.5%, respectively. Within a single definitive host individual, distances among the haplotypes of clade B reached 2.2%. In the phylogenetic analysis, clade A was divided into two subclades with bootstrap supports of 61% and 84% (ML tree in Supplementary Fig. S2A). Pairwise divergence values within these subclades (0.3–3.5%) overlapped with values between them (1.6–5.1%). Clade A diverged from the other clades with distance values comparable with the distances between *H. taeniaeformis* s.l. and *H. krepkogorski*, i.e. the distances ranged at the interspecific level as was also observed in previous studies (e.g. Lavikainen et al., 2008; Galimberti et al., 2012a; Nakao et al., 2013a). Between clades B and C, divergence values were lower, but equal to or slightly higher than values between the subclades of clade A. Phylogenetic relationships of the haplotypes within the clades remained mostly uncertain due to low bootstrap values (for most nodes <50%; Supplementary Fig. S2).

To investigate the evolutionary relationships of three clades of *H. taeniaeformis* s.l., rooted phylograms were inferred using ML and Bayesian methods from nuclear and mitogenomic data sets. As shown in Fig. 1, the analyses of nuclear 18S rDNA clearly showed that clade A is distant from clades B and C. The data sets of nuclear protein-coding genes (*pepck* and *pold*) including introns and all mitochondrial protein-coding genes, also demonstrated clade A to be distinct from clades B and C. In all phylogenies examined, ML and Bayesian statistics yielded identical topologies and robust support for the three clades. Clades B and C formed a monophyletic entity, which was sister to clade A.

3.2. Phylogeography and hosts

The majority of the new and previously published isolates of clade A originated in eastern or southeastern parts of Asia, where the highest diversity of haplotypes was also demonstrated (Fig. 2). However, this lineage has a wide geographic distribution as shown by findings in central Asia, Europe, Africa and Australia. One of the two subclades within clade A included haplotypes only from Asia (southeastern Asia, Japan and the Russian Far East), whereas another contained both Asiatic and non-Asiatic haplotypes, suggesting a common origin for the non-Asiatic haplotypes (see Supplementary Fig. S2A).

Clade B is distributed across Europe to western Siberia (Fig. 2). In addition, there is an isolated focus in Hokkaido, Japan, represented by the ACR isolate (haplotype B1 in Fig. 2) in Okamoto et al. (1995a). No clear geographic structure could be inferred from the haplotype tree (Supplementary Fig. S2B). Finnish haplotypes, which were most numerous in the present analysis, were placed throughout clade B. On the other hand, such distant localities as Latvia and Japan share a common haplotype (B1). Clade C has the most restricted distribution area. In the present study, a member of clade C was identified for the first time outside Italy in Mosset, France, which might suggest a wider distribution of this clade in the Mediterranean region.

Previously published records and the present host data are summarised in Tables 1 and 2. Strobilocerci of clade A were found in murines (Muridae), especially in various rats (tribe Rattini), but also in mice of the genera *Apodemus* and *Mus*. In southeastern Asia,

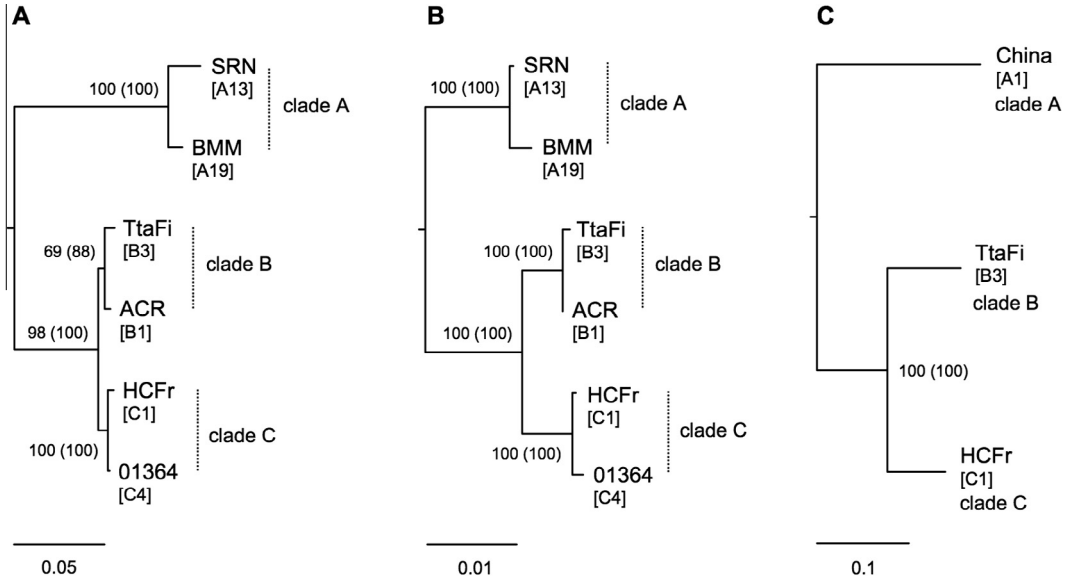

Fig. 1. Phylogenetic trees of the *Hydatigera taeniaeformis* species complex based on sequences of nuclear and mitochondrial DNA. An outgroup taxon, *Hydatigera krepkogorski*, was omitted from the trees. Scale bars represent the estimated number of substitutions per site. Values of nodes are bootstrap percentages of maximum likelihood analysis. Posterior probability percentages of Bayesian analysis are shown in parentheses. Representative isolates (for details see Tables 1 and 2 and Supplementary Table S1) of the mitochondrial DNA lineages A, B and C were used for the analyses; the respective mitochondrial cytochrome *c* oxidase subunit 1 haplotype of each isolate is shown in brackets. (A) Phylogram from 18S ribosomal DNA. (B) Phylogram from concatenated sequences of nuclear protein-coding genes (DNA polymerase delta and phosphoenolpyruvate carboxykinase) including introns. (C) Phylogram from concatenated sequences of all protein-coding genes of mitogenomes.

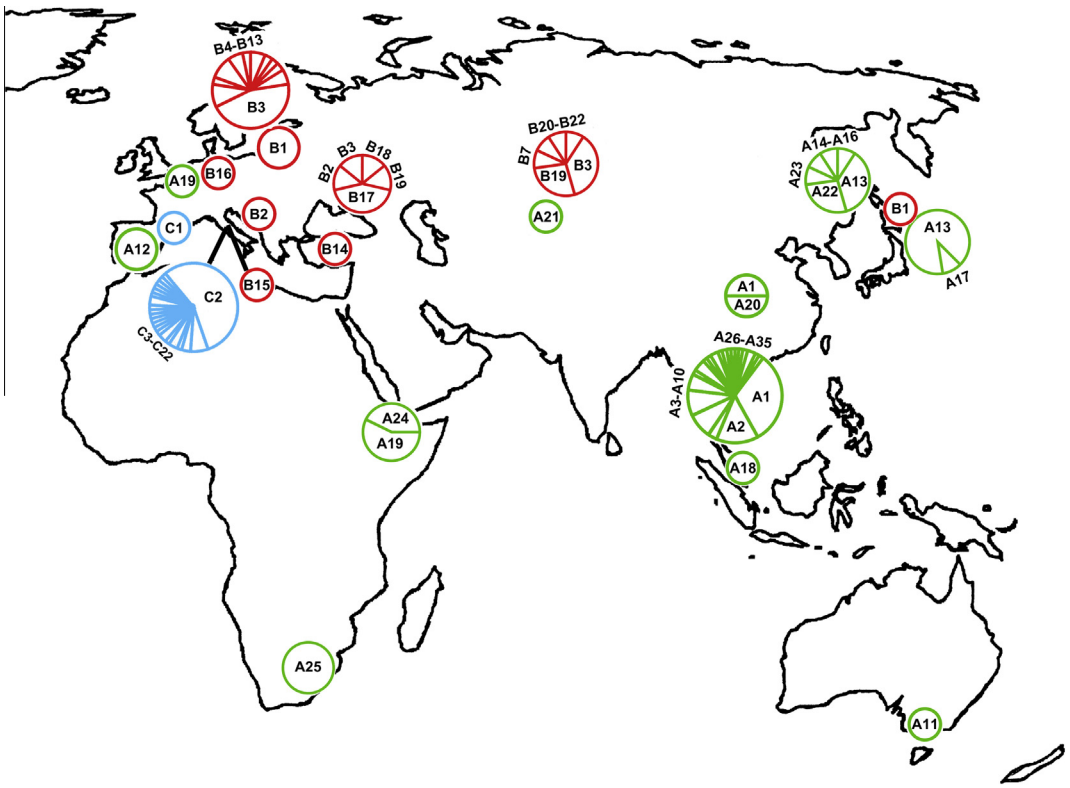

Fig. 2. Geographical distribution of mitochondrial cytochrome *c* oxidase subunit 1 haplotypes of the *Hydatigera taeniaeformis* species complex based on previously published (references in Table 2) and new data. The circle areas are proportional to the sample sizes in each region. Haplotypes are grouped (A1–A36 (green), B1–B22 (red), C1–C22 (blue)) according to the three molecular lineages A, B and C.

clade A was recorded in rodents in different habitats including forest, agricultural areas and human settlements. Clade B occurred mainly in arvicolines (Cricetidae) but also in *Apodemus*, and was found once in *Mus* in the present material. Intermediate hosts of clade C remain unknown due to a lack of larval specimens. At the adult stage, all lineages are found in domestic cats. In addition, we identified clade A in the Amur leopard cat and clade B in the Eurasian lynx. Clade C has been reported in the European wildcat (Galimberti et al., 2012a).

3.3. Species description

Hydatigera kamiyai n. sp. is proposed for clade B based on molecular distinctiveness and morphological characteristics presented herein, while clade A is assigned to *H. taeniaeformis* s.s., and clade C remains a putative unnamed species of *Hydatigera*.

3.3.1. *Hydatigera kamiyai* n. sp. Iwaki

ZooBank reference LSID: urn:lsid:zoobank.org:pub:A9232AD0-05E5-4446-BC2D-5E3FBDAA1D38

Type-host: Felis silvestris catus, domestic cat.
Other definitive host: Lynx lynx, Eurasian lynx.
Type-locality: Porvoo, Finland (holotype), Hokkaido, Japan (paratype).
Site: Small intestine (anterior jejunum).
Type-material: Holotype MPM Coll. No. 20884 (3 slides), paratypes MPM Coll. No. 20885 (3 worms, 6 slides).
Voucher material: MPM Coll. No. 21115–21118 (Meguro Parasitological Museum, Japan; 6 specimens, 10 slides, 6 vials) from domestic cats from Espoo, Loviisa, Helsinki and Orimattila, Finland. MZH 123007 and MZH 123008 (Finnish Museum of Natural History, Finland; 2 specimens, 2 slides, fragments in ethanol) from lynx from Jyväskylä, Finland.
Etymology: Named for Masao Kamiya, a professor emeritus at the Hokkaido University, who initially supervised a series of comparative studies on *H. taeniaeformis* s.l. including clade B (Nonaka et al., 1994; Iwaki et al., 1994; Azuma et al., 1995; Okamoto et al., 1995a,b).
Intermediate hosts: Alticola strelzowi, Apodemus flavicollis, Apodemus sylvaticus, Apodemus uralensis, Arvicola amphibius, Microtus agrestis, Microtus oeconomus, Mus musculus, M. rufocanus, Myodes rutilus, O. zibethicus.
Site of metacestode: liver.
Voucher material of metacestode: MPM Coll. No. 21119 and 21176-21178 from *A. amphibius* from Uddevalla, Sweden; MPM Coll. No. 21120 from *M. agrestis* from Vantaa, Finland; MPM Coll. No. 21121 from *Microtus* sp. from Oboyan, Russia; and MPM Coll. No. 21122 from *A. uralensis* from Oboyan, Russia (7 specimens, 4 slides, 7 vials). MZH 127092 and MZH 127093 from *O. zibethicus* from Ähtäri, Finland (5 specimens in ethanol).

3.3.1.1. Description of the adult stage (Fig. 3, Tables 4–6). Based on 6 pregravid and gravid specimens (means in parentheses).

In holotype (pregravid whole-mount specimen), length 24.2 cm, maximum width 5.9 mm at mature proglottids, number of proglottids 239. In 3 paratypes (gravid specimens), length 21.2–24.0 cm, maximum width 6.3–6.8 mm, number of proglottids 171–182.

Scolex 1.77–2.17 (1.96) mm wide. Diameter of rostellum 731–910 (824) µm. Rostellar hooks in two rows. Number of hooks 30–40 (33) with equal number in each row. Large hooks 396–456 (426) µm long and small hooks 213–275 (253) µm long (based on 5 well-aligned hooks). Other hook dimensions in Table 5.

Table 4
Morphological comparison between *Hydatigera kamiyai* n. sp. and *Hydatigera taeniaeformis* sensu stricto (s.s.) in this study.

Species	*H. kamiyai* n. sp.		*H. taeniaeformis* s.s.	
	Adult[a]	Larva[a]	Adult[a]	Larva[a]
Total length (cm)	21.2–24.2		19.5[b]	
Maximum width (mm)	5.9–6.8		5.1–6.3	
Number of proglottids	171–239		222[b]	
Width of scolex (mm)	1.77–2.17 (1.96)		1.19–1.44 (1.30)	
Diameter of rostellum	731–910 (824)		703–779 (736)	
Number of hooks	30–40 (33)	28–36 (32)	36–42 (38)	36–44 (40)
Length of large hooks	396–456 (426)	421–461 (441)	393–467 (429)	379–432 (412)
Length of small hooks	213–275 (253)	242–283 (263)	249–281 (266)	245–274 (259)
Length × width of sucker	396–510 (445) × 333–463 (399)		288–321 (300) × 228–268 (248)	
Length × width of mature proglottids (mm)	1.26–2.47 (1.69) × 5.53–6.22 (5.86)		0.70–1.27 (1.02) × 3.32–6.60 (4.86)	
Number of testes	367–529 (424)		384–627 (486)	
Length × width of cirrus sac in mature proglottid	374–627 (475) × 76–119 (97)		340–551 (427) × 58–83 (70)	
Length × width of ovary	376–752 (560) × 1349–2034 (1728)		253–475 (352) × 844–1866 (1311)	
Length × width of vitellarium	149–302 (235) × 1119–1675 (1356)		91–221 (149) × 627–1565 (1126)	
Number of uterine branches (unilateral)	6–11 (8)		5–12 (9)	
Dimensions of eggs	27–34 (30) × 25–32 (28)		26–33 (28) × 23–33 (27)	

[a] All measurements are in micrometres unless otherwise indicated, given as a range with the mean in parenthesis.
[b] The total length and the number of proglottids were available only from a single specimen from an Amur leopard cat from Russia. For other parameters, multiple measurements were made.

Table 5
Various hook dimensions of adult worms of *Hydatigera kamiyai* and *Hydatigera taeniaeformis* sensu stricto (s.s.).

Species	*H. kamiyai*			*H. taeniaeformis* s.s.			*P*
	n	Mean	Range	*n*	Mean	Range	
Large hooks							
Total length (TL)	50	426	396–456	59	425	393–467	NS
Total width (TW)	12	162	150–171	15	181	170–194	<0.001
Basal length (BL)	12	265	249–277	15	286	256–314	<0.001
Apical length (AL)	12	192	179–210	15	202	193–209	0.006
Guard length (GL)	12	75	71–78	15	83	72–95	<0.001
Guard width (GW)	12	62	58–66	15	68	59–85	0.008
Blade curvature (BC)	12	37	32–43	15	41	35–49	0.013
Handle width (HW)	12	48	42–55	15	64	53–78	<0.001
Small hooks							
Total length (TL)	50	253	213–275	60	266	249–281	<0.001
Total width (TW)	12	114	110–118	15	123	111–137	<0.001
Basal length (BL)	12	126	111–155	15	150	145–159	<0.001
Apical length (AL)	12	141	131–148	15	154	146–166	<0.001
Guard length (GL)	12	55	50–62	15	55	48–60	NS
Guard width (GW)	12	44	35–57	15	50	40–62	0.014
Blade curvature (BC)	12	27	20–34	15	38	32–44	<0.001
Handle width (HW)	12	31	25–35	15	34	29–40	0.024

For explanation of the measurements, see Fig. 5. All measurements are in micrometres. Statistical significance (*P*) refers to the differences between the mean values of these two species (present study) based on independent sample *t*-tests. NS, not significant ($P > 0.05$); *n*, number of measurements.

Suckers 396–510 (445) μm long, 332–463 (399) μm wide. Unsegmented neck region absent.

Proglottids craspedote. Mature proglottids 1.26–2.47 (1.69) mm long, 5.53–6.22 (5.86) mm wide (measurements based on 5 proglottids). Genital pores irregularly alternating, positioned at middle of lateral margin in mature proglottids. Ventral longitudinal osmoregulatory canal on each side of proglottids. Transverse connecting canals narrower. Dorsal canal narrower than other canals, positioned slightly median to ventral canal. Terminal genital ducts pass longitudinal osmoregulatory canals ventrally.

Testes 316–529 (426) in number, positioned mainly in one dorso-ventral layer. Testicular fields confluent anteriorly, but testes relatively few in antero-median region; testes usually not confluent posterior to vitellarium. In one specimen from lynx, testes clearly confluent posterior to vitellarium. Cirrus sac elongate, 374–627 (475) μm long, 76–119 (97) μm wide, usually overlapping or extending across longitudinal ventral osmoregulatory canal. Vas deferens forming 1–2 loops inside cirrus-sac; proximal part of vas deferens long, prominently convoluted.

Ovary bilobed, 376–752 (560) μm long and 1349–2034 (1728) μm wide, antiporal lobe slightly larger than poral lobe. Vitellarium 149–302 (235) μm long and 1119–1675 (1356) μm wide, not as wide as ovary. Vagina runs posterior to cirrus-sac and vas deferens, slightly widened and undulating or sometimes looped distally, covered externally by thin cell layer, lined with delicate hair-like structures throughout its length, distinct vaginal sphincter present. Uterus in pregravid and gravid proglottids with 6–11 primary branches on either side in paratype specimens. Eggs (in paratypes) spherical or subspherical, 27–34 μm long, 25–32 μm wide, thickness of striated outer egg shell 3–4 μm in whole-mounts.

3.3.1.2. Description of metacestodes. Measurements based on 8 ethanol-fixed specimens (7 for hooks).

Strobilocercus metacestode with evaginated scolex, well-defined pseudosegmented strobila and small terminal bladder. Total length 16–124 mm in small rodents, but up to 450 mm in muskrats. Bladders usually 3–10 × 3–6 mm, but lacking in some larvae. Rostellar hooks in two rows. Number of hooks 28–36 (32)

Table 6
Morphological comparison among adult worms of *Hydatigera* spp.

Species Source	*H. kamiyai*[a] This study	*H. taeniaeformis* sensu lato (s.l.)[a] Abuladze (1964)	*H. taeniaeformis* s.l.[a] Verster (1969)	*H. taeniaeformis* s.l.[a] Loos-Frank (2000); after authors except Verster (1969)	*H. krepkogorski*[a] Abuladze (1964)
Total length (cm)	21.2–24.2	15–60			6–11
Maximum width (mm)	5.9–6.8	5–6			
Number of proglottids	171–239				80–110
Width of scolex (mm)	1.77–2.17		1.00–1.18		0.83–1.07
Diameter of rostellum	731–910		546–918		706–785
Number of hooks	30–40	26–52	34–36	24–52	64–76
Length of large hooks	396–456	380–420	370–402 (384)	300–530	265–345
Length of small hooks	213–275	250–270	210–261 (241)	187–293 /⩽360	182–204
Dimensions of sucker	396–510 × 333–463	460–470	291–491		314–345
Length × width of mature proglottids (mm)	1.26–2.47 × 5.53–6.22				2.28–2.52 × 1.75–2.10
Number of testes	367–529	Numerous	450–500	370–670	
Length × width of cirrus sac in mature proglottid	374–627 × 76–119		301–412 × 64–82	⩽659 × 155	
Length × width of ovary	376–752 × 1349–2034				
Length × width of vitellarium	149–302 × 1119–1675				
Vaginal sphincter present	+	+	+	+	?
Number of uterine branches (unilateral)	6–11		5–9		8–10
Dimensions of eggs	27–34 × 25–32	31–37		31–36	31–34 × 24–27

Species Source	*H. krepkogorski*[a] Bray (1972)	*H. krepkogorski*[a] Loos-Frank (2000); after authors except Bray (1972)	*H. parva*[a] Abuladze (1964)	*H. parva*[a] Verster (1969)	*H. parva*[a] Loos-Frank (2000); after authors except Verster (1969)
Total length (cm)	1.2–3.1		⩽5.5		
Maximum width (mm)	3		3.2		
No. of proglottids	⩽99		80		
Width of scolex (mm)	0.56–0.84		1	0.68–1.00	
Diameter of rostellum	400–560			546–655	
No. of hooks	68–76	⩽60	44	38–48	30 ⩽
Length of large hooks	300–330	265–354	344–376	302–370	267–424
Length of small hooks	200–222	⩽182	228–248	192–238	⩽266
Dimensions of sucker	230 (rounded)/ 232–280 × 125–212		200	165–237	
Length × width of mature proglottid (mm)	0.4 × 2.7				
No. of testes	360–400		500	500–600	
Length × width of cirrus sac in mature proglottid	310–430 × 70–90		440 × 80	297–470 × 69–110	
Length and width of ovary	500 wide				
Length and width of vitellarium					
Vaginal sphincter present	–		?	–	–
No. of uterine branches (unilateral)	4–5		7–12	7–12	
Dimensions of eggs	30–33 × 23–25	30–34	23–27	25–29	

[a] All measurements are in micrometres unless otherwise indicated.

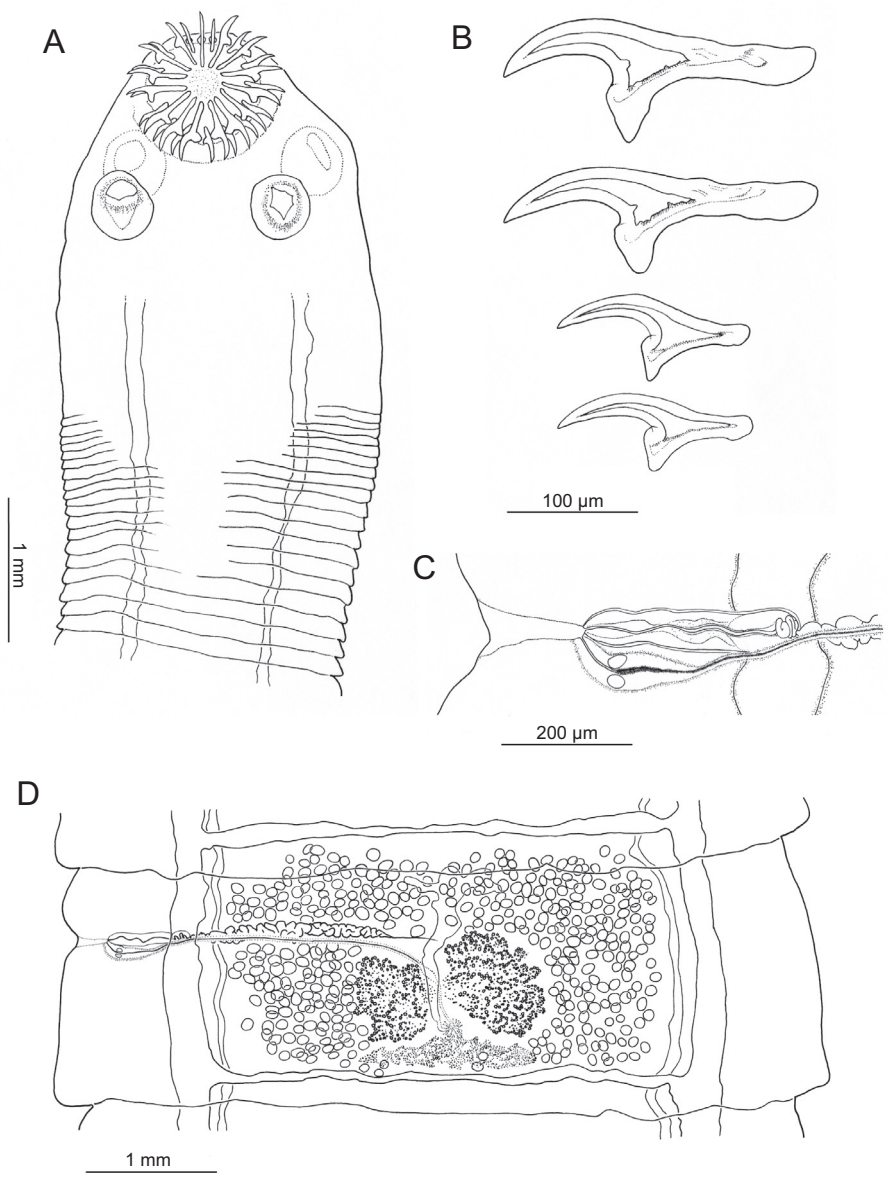

Fig. 3. *Hydatigera kamiyai* n. sp. (holotype) (A) scolex; (B) rostellar hooks; (C) terminal genital ducts in a mature proglottid; and (D) mature proglottid.

with equal number in each row. Large hooks 421–461 (441) µm long, small hooks 242–283 (263) µm long (based on each 5 well-aligned hooks).

3.3.2. Remarks

Hydatigera kamiyai n. sp. is compared with three congeners, all of which have a strobilocercus-type metacestode (Tables 4 and 6). *Hydatigera* sp. (clade C) cannot be compared due to lack of material.

Most of the morphological characters of *H. kamiyai* are similar and overlap with those of *H. taeniaeformis* s.s. from specimens of this study and *H. taeniaeformis* s.l. by previous authors (Figs. 4 and 5; Tables 4–6). According to the morphometric analysis of the rostellar hooks, significant differences were found in seven characters of large and small hooks by independent-samples *t*-test. The stepwise discriminant analysis with all eight variables gave the following final functions (the abbreviations shown in Table 5):

$$\text{Large hooks} : Z = -0.35TW = 60.02$$

$$\text{Small hooks} : Z = -3.05BC - 2.67BL - 2.76HW + 1.60TL - 0.97TW + 271.58$$

Based on the resulting discriminant scores for large and small hooks, the individuals were assigned to the correct species with the probability of 93% and 100%, respectively. This shows that the measurements of rostellar hooks can be used reliably for the separation of *H. kamiyai* and *H. taeniaeformis* s.s. However, the range of dimensions of these characteristics is overlapping. According to Loos-Frank (2000) the number of hooks, length of large and small hooks, and number of testes, are the most important characters for identification of *Taenia* spp. These characters cannot, however, differentiate *H. kamiyai* and *H. taeniaeformis* s.s.

Iwaki et al. (1994) reported differences in the length of the small hooks, number and distribution of testes, and length of the cirrus sac between isolate ACR derived from voles (corresponding to *H. kamiyai* in this study) and the other two isolates from rats (representing *H. taeniaeformis* s.s.). In contrast, no significant difference in these characters was found in the present study. This could be because Iwaki et al. (1994) observed only a small local

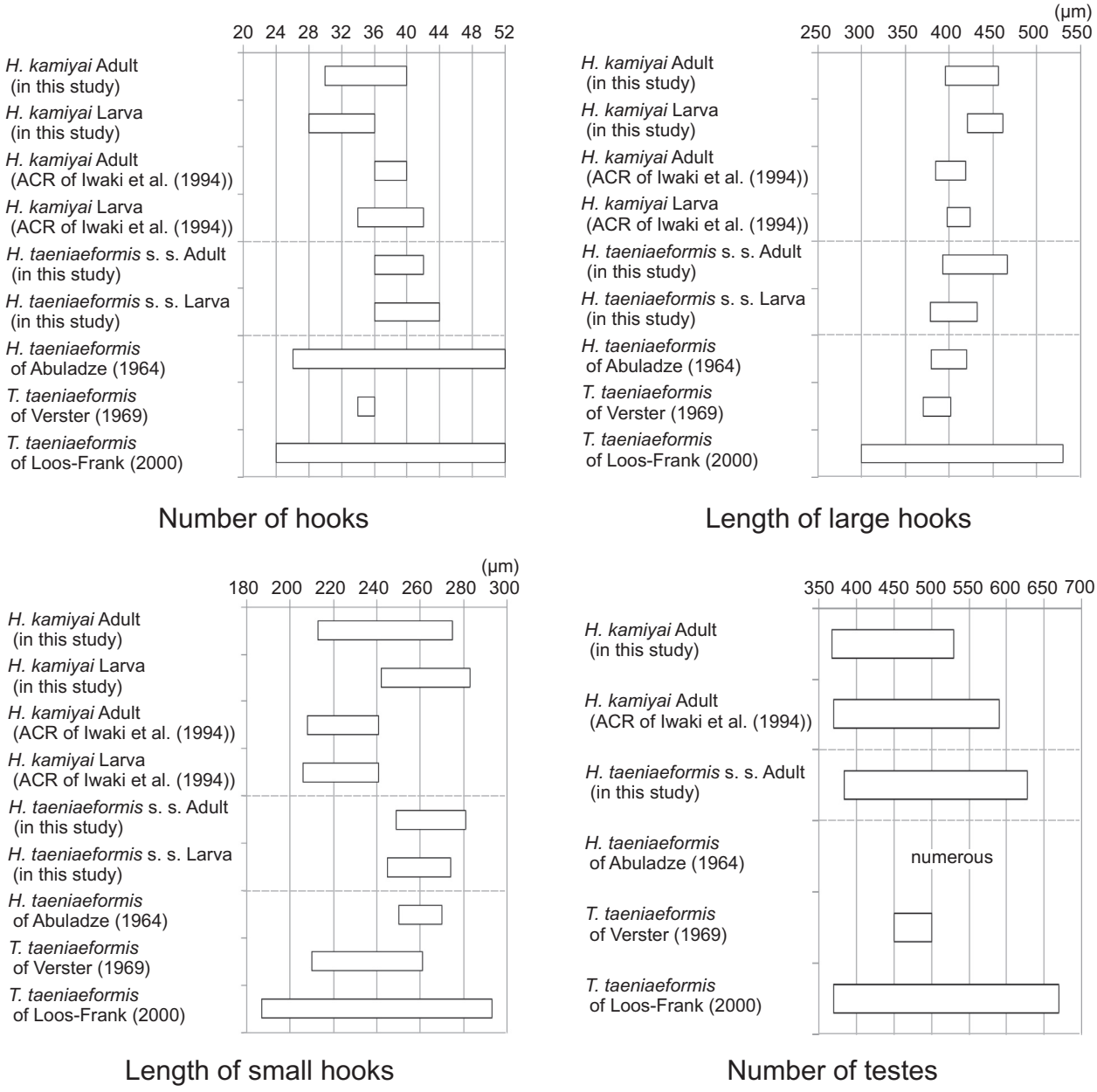

Fig. 4. Comparison of the number and length of the rostellar hooks, and the number of testes between *Hydatigera kamiyai* and *Hydatigera taeniaeformis* sensu stricto (s.s).

population of *H. kamiyai*. In addition, methodological inconsistency can partly explain the conflicting results. In particular, the uterine branches of isolate ACR were possibly counted bilaterally leading to a double (15–23) result observed by Iwaki et al. (1994).

Regarding the distribution of testes of *H. taeniaeformis* s.l., previous authors (Abuladze, 1964; Verster, 1969; Loos-Frank, 2000) concluded that testes "do not reach the vitellarium" or "are not confluent posterior to it". Most of the specimens of *H. kamiyai* are consistent with this observation. However, in one specimen of *H. kamiyai* from a lynx, the testes were clearly confluent posterior to vitellarium in all proglottids examined. This atypical specimen may represent an extreme of the intraspecific variation in this characteristic.

Hydatigera krepkogorski and *H. parva* can be differentiated from the new species by the absence of a vaginal sphincter, a smaller strobila, a lower number of proglottids, a greater number and smaller length of hooks, as well as by the proliferation of metacestodes. The range in the hook number and the length of small hooks of *H. parva* slightly overlap with those of the new species.

4. Discussion

The hidden diversity within *H. taeniaeformis* s.l. was first uncovered based on phenotypic differences (e.g. Nonaka et al., 1994; Iwaki et al., 1994), and, subsequently, it has been supported by increasing molecular evidence (e.g. Okamoto et al., 1995a; Lavikainen et al., 2008). In the present study, a new species *H. kamiyai* is described to organise cryptic complexity of this group. Cryptic complexes are not unusual among taeniids, and actually they occur within every taeniid genus (Thompson and McManus, 2002; Lavikainen et al., 2008; Goldberg et al., 2014).

The discussion of taxonomic problems related to cryptic species has been ongoing for many decades (Dobzhansky, 1959; Nadler and Pérez-Ponce de León, 2011). Taxonomy is still largely based

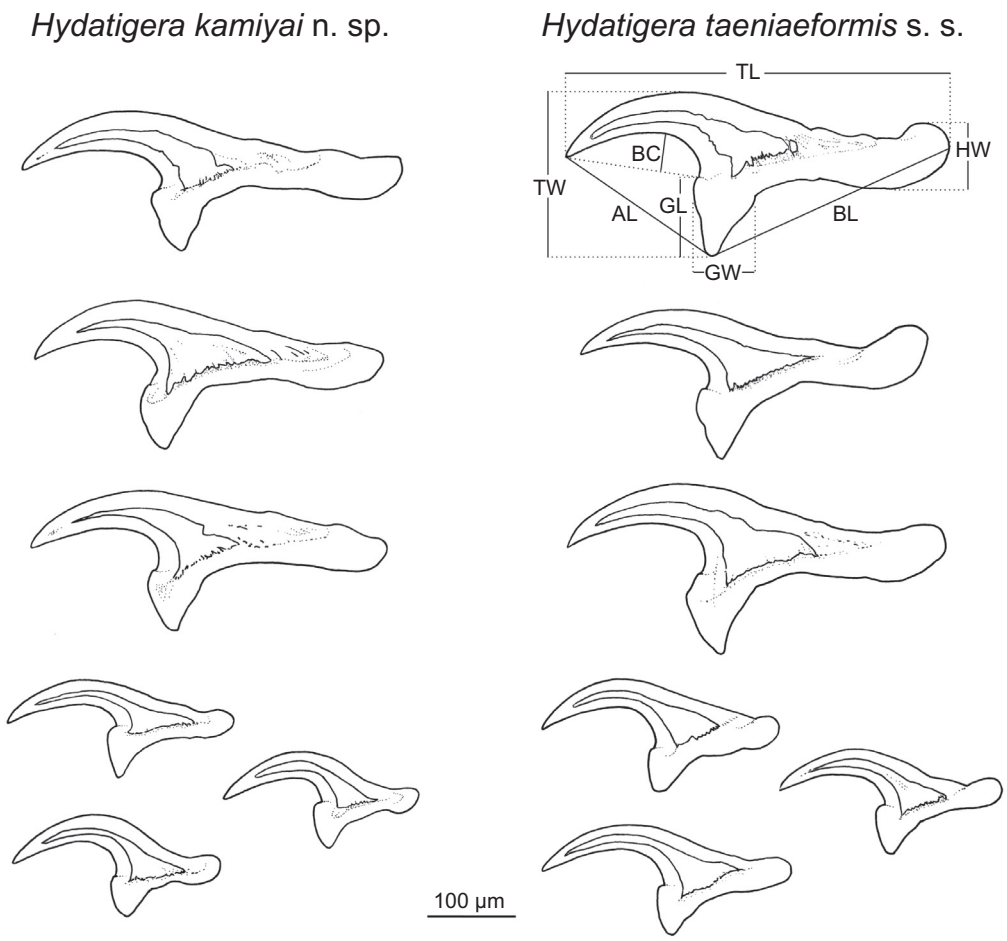

Fig. 5. Examples of large and small rostellar hooks of adults of *Hydatigera kamiyai* and *Hydatigera taeniaeformis* sensu stricto (s.s.). Each hook represents a different rostellar crown and host individual. From top to bottom, hooks are from *H. kamiyai*: TtaFi (holotype), HBJa1 (ACR) (paratype), HBJa2 (ACR) (paratype); *H. taeniaeformis* s.s.: HARu12, HAJa3 and HAJa5. TL, total length; TW, total width; AL, apical length; GL, guard length; BC, blade curvature; GW, guard width; BL, basal length; HW, handle width.

on visible diagnostic criteria. Therefore, it is not straightforward to describe and name species that cannot be differentiated using traditional morphological methods. This is especially true if the hidden species are biologically very close to one another, as in the case of the *H. taeniaeformis* s.l. complex. Although PCR and DNA sequencing have simplified species identification, the use of DNA sequences in taxonomy is not straightforward. A major problem is the lack of common criteria to differentiate interspecific and intraspecific genetic variations. The general use of the DNA barcoding approach in taxonomy remains contentious because the determination of universal distance-based thresholds for delineation of species boundaries, namely the genetic "yardstick approach", differs in each species (Nadler et al., 2000; Hebert et al., 2004; Savolainen et al., 2005; Galtier et al., 2009; Nadler and Pérez-Ponce de León, 2011).

Attempts to set optimal barcoding thresholds for delimitation of taeniid species have been made by applying mean K2P distances of the partial *cox1* sequence (Galimberti et al., 2012a; Zhang et al., 2014). Slightly different threshold values have been presented (3.6% by Galimberti et al., 2012a; 2.0% by Zhang et al., 2014), depending on the calculation method and sequence length. The variation between molecular lineages of the *H. taeniaeformis* species complex clearly exceeds these limits. In addition, phylogenetic analyses of mitogenomes and nuclear protein-coding genes strongly support the presence of three distinct clades. In the present study, however, quite a high level of variation in *cox1* sequences was also revealed within clades A (proposed *H. taeniaeformis* s.s.) and B (*H. kamiyai* n. sp.). Evolutionary relationships among the haplotypes of clade B remain unclear, but clade A seems to include two subclades. There is no evidence for distinct evolutionary trajectories supporting further cryptic species either in clade A or B. Our results suggest that the barcoding thresholds should be considered with caution. When species boundaries are evaluated utilising molecular data, adjunct operational criteria linked to ecology, biogeography and evolution should also be taken into account (Brooks and McLennan, 2002; Galimberti et al., 2012b).

Molecular approaches (including phylogeny reconstruction and DNA barcoding) are fundamental tools needed to discover cryptic diversity, whereas classical taxonomic approaches provide the basis for their evaluation (Hoberg, 2006; Galimberti et al., 2012b). Data on reproductive isolation, allopatric/sympatric occurrence and host specificity are the key information in separating parasite groups into distinct species (Hoberg, 2006). On the whole, sufficient evidence is available to explicitly differentiate and describe clades A and B as distinct species. The taxonomic status of clade C, which is sister to clade B, remains unresolved due to limited biogeographical and ecological data and due to the lack of evidence for the reproductive isolation. Further studies are required to classify clade C (*Hydatigera* sp.) either as a distinct species or a variant of *H. kamiyai.* Morphological attributes are just a part of the evaluation and less important in cryptic species, but an appropriate holotype is essential for the formal description.

Host data on natural infections suggest that there are differences in intermediate host spectra among members of the *H. taeniaeformis* species complex. Intermediate hosts of *H. taeniaeformis* s.s. belong to the subfamily Murinae (Muridae), whereas *H. kamiyai* uses mainly members of the Arvicolinae (Cricetidae) from various voles to muskrats. These observations are supported by negative evidence from the Russian Far East where only *H. taeniaeformis* s.s. was detected in definitive hosts: despite extensive trapping of rodents, strobilocerci have not been found in voles (S. Konyaev, unpublished data).

Findings regarding natural intermediate hosts are mainly in concordance with the results from experimental infections by Iwaki et al. (1994), Nonaka et al. (1994) and Azuma et al. (1995), who did not succeed in infecting grey-sided voles with isolates of *H. taeniaeformis* s.s., or laboratory mice or rats with *H. kamiyai* (isolate ACR). Differences in infectivity and cyst development in mice and rats among isolates of *H. taeniaeformis* s.s. were also reported (Iwaki et al., 1994; Azuma et al., 1995). In addition, Iwaki et al. (1994) noted that development of ACR was incomplete in *Apodemus*. However, according to our observations, fully developed strobilocerci of *H. taeniaeformis* s.s. and *H. kamiyai* are often found in naturally infected *Apodemus* spp., indicating that both species can use field mice as intermediate hosts. We also recorded *H. kamiyai* in a house mouse *M. musculus* from Novosibirsk, western Siberia. Among parasite populations, intraspecific differences in infectivity for different hosts are possible. Also, it should be noted that before the infection experiments in the 1990s, the isolates were maintained in a laboratory from the middle of the 1980s (Azuma et al., 1995; Okamoto et al., 1995b), for multiple generations in the same rodent species from which they originally were isolated (*H. kamiyai* in voles and *H. taeniaeformis* s.s. in rats or mice), thus exposing them to an artificial selection pressure.

A fundamental question related to the recognition of cryptic species is which member of a complex should hold the original name and which one would either require a new name or removal to another previously described species. According to the International Code of Zoological Nomenclature (http://iczn.org/code), the type locality and type host are relevant information for a parasite species. In the description of *H. taeniaeformis*, the geographical origin of specimens was not given (Batsch, 1786). The author, August Batsch, was German and one can speculate that his specimens probably (but not definitely) were from Europe. However, all clades of *H. taeniaeformis* s.l. occur in Europe.

Host specificity has been used to support taxonomic decisions and resurrection of "old" taxa of taeniids (see e.g. Thompson and McManus, 2002; Nakao et al., 2013b). Batsch (1786) described *H. taeniaeformis* based on metacestodes and assigned the species to the genus *Hydatigena* Goeze, 1782 (*Hydatigera* was created later). According to his original description, the intermediate hosts were *Mus norvegicus* (*R. norvegicus*), *Mus rattus* (*Rattus rattus*), *Mus decumanus* (*Mus* sp.), *Mus silvestris* (*Apodemus* sp.) and *Mus amphibius* (*A. amphibius*). The type host was not specified. Thus, both Murinae and Arvicolinae were represented without priority. If the cryptic lineages were strictly host-specific, this would render *H. taeniaeformis* sensu Batsch (1786) a composite of clades A and B. We think, however, that the widely accepted taxon *H. taeniaeformis* should not be invalidated based on this indirect evidence. Intermediate host associations, observed experimentally (Iwaki et al., 1994; Nonaka et al., 1994; Azuma et al., 1995) or based on the present data of natural infections, are hardly absolute and exclusive. We propose that clade A, which is the most widespread and mainly associated with rats and mice as traditionally considered to be characteristic for *H. taeniaeformis*, should retain the original name.

Synonyms of *H. taeniaeformis* have been listed by various authors (Wardle and McLeod, 1952; Abuladze, 1964; Loos-Frank, 2000). None of these can be definitely associated with *H. kamiyai*, and thus cannot be resurrected as a prior name. In some cases, murine intermediate hosts (e.g. *Taenia hepatica* von Linstow, 1872) or Asiatic origin (e.g. *Hydatigera himalayotaenia* Malhotra and Capoor, 1982) may suggest synonymy with *H. taeniaeformis* s.s. However, many of the old names can be considered "nomina nuda" because descriptions are minimal and type specimens have not been assigned.

Natural geographic borders of the lineages of *H. taeniaeformis* species complex are difficult to outline due to human-mediated introductions of rodents and domestic cats worldwide. The distribution areas are partly overlapping in Eurasia. At present, the domestic cat is the most important definitive host of the *H. taeniaeformis* s.l. complex and it has had an essential role in spreading these parasites worldwide. Due to the short history of cat domestication and restricted geographical origin of its ancestors (Driscoll et al., 2007), domestic cats obviously were not responsible for the original life cycles of the *H. taeniaeformis* species complex. It is difficult to determine original definitive hosts of each species due to the present dominance of domestic cats in life cycles worldwide. Furthermore, host data from molecularly determined specimens cover only a small part of the wide range of wild felid taxa reported as the hosts of *H. taeniaeformis* s.l. (Abuladze, 1964; Jones and Pybus, 2001). Reports on canids as definitive hosts of *H. taeniaeformis* s.l. (e.g. Abuladze, 1964; Hrčkova et al., 2011) are doubtful. For example, Jia et al. (2012) showed that mtDNA sequences of "*Taenia taeniaeformis*" (*H. taeniaeformis* s.l.) in red foxes (*Vulpes vulpes*) from Slovakia reported by Hrčkova et al. (2011) actually represented other taeniid species.

This study confirmed that *H. taeniaeformis* s.s. is a widespread species occurring in Asia, Europe, Africa and Australia. The high diversity of haplotypes in southeastern Asia and the linkage to the Rattini strongly suggest that the species originates from this region. The placement of non-Asiatic haplotypes in a single subclade and their small genetic variation suggests that the worldwide invasion of *H. taeniaeformis* s.s. was a single, rapid event in the recent past. It was possibly related to the global expansion of black rats (*R. rattus*) reaching Europe during pre-Roman times 4000–2300 years BC (Wilson and Reeder, 2005). Brown rats (*R. norvegicus*) colonised the world much later, perhaps just 300 years ago (Wilson and Reeder, 2005), and such a recent spread of *H. taeniaeformis* s.s. appears unlikely. Given the Near Eastern origin of domestic cats (Driscoll et al., 2007), they or their wild ancestors probably were not involved in the original life cycles of *H. taeniaeformis* s.s. associated with rats in southeastern Asia. Instead, *Prionailurus* or other endemic small felids in southeastern Asia more likely played the role of the definitive host in early life cycles of the parasite. Our finding of *H. taeniaeformis* s.s. in various rats from different habitats in southeastern Asia reveals coexisting sylvatic and synanthropic cycles at the present time, and suggests an easy switch between definitive hosts which enabled the conquest of the world in the past. This is consistent with the hypothesis that the dynamics of host switching and diversification for taeniids is related to colonisation events driven by guild structure (Hoberg, 2006).

Even though *H. kamiyai* was detected in a lynx, it does not appear to be a significant, and hardly an original, host for this species. A survey in Finland showed that lynx are rarely infected with this parasite (Lavikainen et al., 2013), and our specimens originated from a sporadic case. Phylogenetic analysis of the *cox1* haplotypes might suggest another origin for *H. kamiyai.* The isolate HBRu18 (haplotype B20) in flat-headed vole (*A. strelzowi*) from the Altay Mountains was located basally in clade B (Supplementary Fig. S2B). This isolate originates in a montane steppe region with minor human influence. Domestic cats are unlikely to occur in that remote area, but a probable definitive host could be the Pallas' cat (*Otocolobus manul*). If this topology reflects the true history of *H. kamiyai*, the evolution and early radiation of the species could be tracked to grasslands and montane steppe of central Asia.

The geographical distribution of *H. kamiyai* in Eurasia reaches across Europe to western Siberia with a mysterious independent refugium in Hokkaido, Japan. The distribution overlaps with the distribution of *H. taeniaeformis* s.s. in Europe and Hokkaido, and that of lineage C (*Hydatigera* sp.) in the Mediterranean. *Hydatigera* spp. on the main islands of Japan are fully dependent on domestic cats since the only endemic Japanese wild felids, Iriomote and Tsushima leopard cats (*Prionailurus bengalensis iriomotensis* and *P. b. euptilura*, respectively), occur on small islands close to Korea and Taiwan, respectively (Tamada et al., 2008). Lynx had been distributed in Japan from the late Pleistocene to the early Holocene (Hasegawa et al., 2011), but domestic cats were introduced probably during the 6th century (Wastlhuber, 1991). Thus, *H. taeniaeformis* s.s. was undoubtedly introduced with domestic cats. Low diversity of haplotypes of *H. taeniaeformis* s.s. in Japan reflects the recent introduction. Because only *H. taeniaeformis* s.s. occurs in the mainland Far East, the presence of *H. kamiyai* in Hokkaido indicates a more western, perhaps European, contact which led to recent artificial importation of the parasite either with domestic cats or rodents.

An exception to speculations about uncertain original life cycles is *Hydatigera* sp. (clade C), which is clearly associated with European wildcats in Italy, although it also infects domestic cats (Galimberti et al., 2012a). Consistently, we found clade C in a domestic cat in the Pyrenees, southernmost France, in an area where European wildcats have been recorded (Muséum National d'Histoire Naturelle de Paris, France, 2015, http://inpn.mnhn.fr/collTerr/commune/66119/tab/especes?lg=en; A. Ribas, unpublished data). The Italian Peninsula was a refugium in southern Europe during the Pleistocene, and is considered as a hotspot of biodiversity (Colangelo et al., 2012). Consequently, vicariance of host animals in the refugium seems to be responsible for creating clade C. Further studies in other parts of the nowadays fragmented distribution area of European wildcats would confirm the host association and distribution of clade C. For validating the taxonomic status of this cryptic entity, molecular and morphological comparisons should include specimens from both domestic and wild cats, as well as from rodents.

The focus of the present study is on the Old World. We conclude that the clades of *H. taeniaeformis* s.l. could originally be associated with Old World small felids belonging to the most recently derived lineages among the modern felids (Johnson et al., 2006). Further cryptic diversity might be hiding in the New World, because morphologically identified specimens of *H. taeniaeformis* s.l. have been reported in endemic Nearctic and Neotropical felids, closely related to the above-mentioned Old World cat lineages, as well as in endemic rodents (Abuladze, 1964; Jones and Pybus, 2001; Charles et al., 2012; Miño et al., 2012). In addition, extensive human-mediated introductions are obvious.

A few DNA data are available from the western hemisphere. Martínez et al. (2013) identified a strobilocercus from a brown rat from Argentina using 28S rDNA and a short fragment of *cox1*. Although the *cox1* sequence is not available for comparison, their results suggest that the specimen represented *H. taeniaeformis* s.s. The species is probably wide-spread because metacestodes of *H. taeniaeformis* s.l. have been recorded in murines across the New World (e.g. Rodríguez-Vivas et al., 2011; Duque et al., 2012; museum specimens in the Arctos database, e.g. http://arctos.database.museum/guid/HWML:Para:10302). Domestic cats and murines did not occur in the Americas before European colonisation, suggesting human-mediated introductions of *H. taeniaeformis* s.s.

In a very recent study, taeniid egg specimens from seven domestic cats from Alberta and Saskatchewan, Canada, were identified based on a partial sequence of the mitochondrial NADH dehydrogenase subunit 1 gene (Hoopes et al., 2015). Comparison with mitogenomic data reveals that *H. kamiyai* and clade C were present in both provinces, and the latter was more prevalent (5/7 specimens). Absence of *H. kamiyai* in northeastern Eurasia in our material and the very restricted geographical distribution of clade C in Europe suggest that both of them were probably introduced with domestic cats to North America. If our results are biased due to incomplete sampling or discontinuous and artificially disturbed distribution of the current parasite populations, an alternative hypothesis arises: the history of these taxa in the North might be deeper in time, and they may have occurred across the Holarctic region spreading from Eurasia into North America with their arvicoline intermediate hosts. Multiple dispersal events for rodents on intercontinental scales coincided with temperate climates in Beringia (Hoberg et al., 2012). Concurrent occurrence of small felids in more northern latitudes may have allowed the expansion of *Hydatigera* spp. to North America. In this hypothesis, their original wild definitive host in North America before the arrival of domestic cats would be the bobcat (*Lynx rufus*), which is known to be a suitable host for *H. taeniaeformis* s.l. (Jones and Pybus, 2001).

From a taxonomical point of view, Canada seems to be an attractive area for further evaluation of the specific status of clade C due to sympatry with *H. kamiyai*. Furthermore, a dedicated phylogeographical survey could clarify the diversity, geographical distribution and colonisation history of *Hydatigera* spp. in the western hemisphere.

Acknowledgements

Ian Beveridge (University of Melbourne, Australia), Marja Isomursu (Finnish Food Safety Authority Evira, Finland), Antti Oksanen (Evira, Finland), Ivan Seryodkin (Pacific Institute of Geography FEB RAS, Russia) and Egor Vlasov (Kursk State University, Russia) are acknowledged for providing specimens for the present study. This study was supported in part by a Grant-in-Aid for Scientific Research (no. 26460503) from the Japan Society for the Promotion of Science. Computations were partially performed on the supercomputer system of the Research Organization of Information and Systems, National Institute of Genetics, Japan. Sampling in France and Spain was supported by Agency for Management of University and Research Grants, Government of Catalonia (grant 2014 SGR 1241). Sampling in southeastern Asia was supported by the French National Research Agency (grant ANR 07 BDIV 012 "CERoPath" and grant ANR 11 CPEL 002 "BiodivHealthSEA").

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2016.01.009.

References

Abuladze, K.I., 1964. Taeniata of Animals and Man and Diseases Caused by Them. Essentials of Cestodology, vol. IV, Nauka, Moskow (English translation, Israel Program for Scientific Translation, Jerusalem, 1970).

Azuma, H., Okamoto, M., Oku, Y., Kamiya, M., 1995. Intraspecific variation of *Taenia taeniaeformis* as determined by various criteria. Parasitol. Res. 81, 103–108.

Batsch, A.J.G.C., 1786. Naturgeschichte der Bandwurmgattung überhaupt und ihrer Arten insbesondere, nach den neuern Beobachtungen in einem systematischen Auszuge. Johann Jacob Gebauer, Halle.

Bowles, J., McManus, D.P., 1994. Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. Am. J. Trop. Med. Hyg. 50, 33–44.

Brandt, J.R.A., Sewell, M.M.H., 1981. Varying infectivity of *Taenia taeniaeformis* for rats and mice. Vet. Res. Commun. 5, 187–191.

Bray, R.A., 1972. The cestode *Taenia krepkogorski* (Schulz & Landa, 1934) in the Arabian sand cat (*Felis margarita* Loche, 1758) in Bahrain. Bull. Br. Mus. (Nat. Hist.) (Zool. Ser.) 24, 183–194.

Brooks, D.R., McLennan, D.A., 2002. The Nature of Diversity: An Evolutionary Voyage of Discovery. University of Chicago Press, Chicago, Illinois, USA.

Charles, R.A., Kjos, S., Ellis, A.E., Dubey, J.P., Shock, B.C., Yabsley, M.J., 2012. Parasites and vector-borne pathogens of southern plains woodrats (*Neotoma micropus*) from southern Texas. Parasitol. Res. 110, 1855–1862.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., Thompson, J. D., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31, 3497–3500.
Colangelo, P., Aloise, G., Franchini, P., Annesi, F., Amori, G., 2012. Mitochondrial DNA reveals hidden diversity and an ancestral lineage of the bank vole in the Italian peninsula. J. Zool. 287, 41–52.
Conchedda, M., Ferretti, G., 1983. Vaccination of susceptible hosts with uninfective strains of the same parasite (*Taenia taeniaeformis*, Cestoda) provide protection against an infective strain. J. Parasitol. 69, 1166–1167.
Dobzhansky, T., 1959. Genetics and the Origin of Species, third revised ed. Columbia University Press, New York, USA.
Driscoll, C.A., Menotti-Raymond, M., Roca, A.L., Hupe, K., Johnson, W.E., Geffen, E., Harley, E.H., Delibes, M., Pontier, D., Kitchener, A.C., Yamaguchi, N., O'brien, S.J., Macdonald, D.W., 2007. The Near Eastern origin of cat domestication. Science 317, 519–523.
Duque, B.A., Aranzazu, D., Agudelo-Flórez, P., Londoño, A.F., Quiroz, V.H., Rodas, J.D., 2012. *Rattus norvegicus* como indicador de la circulación de *Capillaria hepatica* y *Taenia taeniaeformis* en la Plaza Minorista de Medellín, Colombia. Biomédica 32, 510–518 (in Spanish).
Esch, G.W., Self, J.T., 1965. A critical study of the taxonomy of *Taenia pisiformis* Bloch, 1780; *Multiceps multiceps* (Leske, 1780); and *Hydatigera taeniaeformis* Batsch, 1786. J. Parasitol. 51, 932–937.
Galimberti, A., Romano, D.F., Genchi, M., Paoloni, D., Vercillo, F., Bizarri, L., Sassera, D., Bandi, C., Genchi, C., Ragni, B., Casiraghi, M., 2012a. Integrative taxonomy at work: DNA barcoding of taeniids harboured by wild and domestic cats. Mol. Ecol. Resour. 12, 403–413.
Galimberti, A., Spada, M., Russo, D., Mucedda, M., Agnelli, P., Crottini, A., Ferri, E., Martinoli, A., Casiraghi, M., 2012b. Integrated operational taxonomic units (IOTUs) in echolocating bats: a bridge between molecular and traditional taxonomy. PLoS One 7, e40122.
Galtier, N., Nabholz, B., Glémin, S., Hurst, G.D.D., 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Mol. Ecol. 18, 4541–4550.
Goldberg, T.L., Gendron-Fitzpatrick, A., Deering, K.M., Wallace, R.S., Clyde, V.L., Lauck, M., Rosen, G.E., Bennett, A.J., Greiner, E.C., O'Connor, D.H., 2014. Fatal metacestode infection in Bornean orangutan caused by unknown *Versteria* species. Emerg. Infect. Dis. 20, 109–113.
Gubányi, A., 1995. Morphometrics of taeniid tapeworms 1. Multivariate analysis of distance measurements of the rostellar hooks. Parasitol. Hung. 28, 21–41.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.
Hasegawa, Y., Kaneko, H., Tachibana, M., Tanaka, G., 2011. A study of the extinct Japanese *Lynx* from the Late Pleistocene to the Early Holocene. Bul. Gunma Mus. Nat. Hist. 15, 43–80 (in Japanese).
Haukisalmi, V., Lavikainen, A., Laaksonen, S., Meri, S., 2011. *Taenia arctos* n. sp. (Cestoda: Cyclophyllidea: Taeniidae) from its definitive (brown bear *Ursus arctos* Linnaeus) and intermediate (moose/elk *Alces* spp.) hosts. Syst. Parasitol. 80, 217–230.
Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., Hallwachs, W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. U.S.A. 101, 14812–14817.
Hoberg, E.P., 2006. Phylogeny of *Taenia*: species definitions and origins of human parasites. Parasitol. Int. 55, S23–S30.
Hoberg, E.P., Jones, A., Rausch, R.L., Eom, K.S., Gardner, S.L., 2000. A phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). J. Parasitol. 86, 89–98.
Hoberg, E.P., Galbreath, K.E., Cook, J.A., Kutz, S.J., Polley, L., 2012. Northern host–parasite assemblages: history and biogeography on the borderlands of episodic climate and environmental transition. Adv. Parasitol. 79, 1–97.
Hoopes, J., Hill, J.E., Polley, L., Fernando, C., Wagner, B., Schurer, J., Jenkins, E., 2015. Enteric parasites of free-roaming, owned, and rural cats in prairie regions of Canada. Can. Vet. J. 56, 495–501.
Hrčkova, G., Miterpáková, M., O'Connor, A., Šnábel, V., Olson, P.D., 2011. Molecular and morphological circumscription of *Mesocestoides* tapeworms from red foxes (*Vulpes vulpes*) in central Europe. Parasitology 138, 638–647.
Iwaki, T., Nonaka, N., Okamoto, M., Oku, Y., Kamiya, M., 1994. Developmental and morphological characteristics of *Taenia taeniaeformis* (Batsch, 1786) in *Clethrionomys rufocanus bedfordiae* and *Rattus norvegicus* from different geographical locations. J. Parasitol. 80, 461–467.
Jia, W., Yan, H., Lou, Z., Ni, X., Dyachenko, V., Li, H., Littlewood, D.T., 2012. Mitochondrial genes and genomes support a cryptic species of tapeworm within *Taenia taeniaeformis*. Acta Trop. 123, 154–163.
Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E., O'Brien, S.J., 2006. The late Miocene radiation of modern Felidae: a genetic assessment. Science 311, 73–77.
Jones, A., Pybus, M.J., 2001. Taeniasis and echinococcosis. In: Samuel, W.M., Pybus, M.J., Kocan, A.A. (Eds.), Parasitic Diseases of Wild Mammals, second ed. Manson Publishing, London, UK, pp. 150–192.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Lavikainen, A., Haukisalmi, V., Lehtinen, M.J., Henttonen, H., Oksanen, A., Meri, S., 2008. A phylogeny of members of the family Taeniidae based on the mitochondrial *cox1* and *nad1* gene data. Parasitology 135, 1457–1467.
Lavikainen, A., Haukisalmi, V., Deksne, G., Holmala, K., Lejeune, M., Isomursu, M., Jokelainen, P., Näreaho, A., Laakkonen, J., Hoberg, E., Sukura, A., 2013. Molecular identification of *Taenia* spp. in the Eurasian lynx (*Lynx lynx*) from Finland. Parasitology 140, 653–662.
Liu, G.H., Lin, R.Q., Li, M.W., Liu, W., Liu, Y., Yuan, Z.G., Song, H.Q., Zhao, G.H., Zhang, K.X., Zhu, X.Q., 2011. The complete mitochondrial genomes of three cestode species of Taenia infecting animals and humans. Mol. Biol. Rep. 38, 2249–2256.
Loos-Frank, B., 2000. An up-date of Verster's (1969) 'Taxonomic revision of the genus *Taenia* Linnaeus' (Cestoda) in table format. Syst. Parasitol. 45, 155–183.
Martínez, M.L., Domínguez, M.G., Morici, G.E., Cavia, R., Montes de Oca, D.P., Lovera, R., Schapiro, J.H., Caracostantogolo, J.L., 2013. Identificación morfológica y molecular de *Cysticercus fasciolaris* aislado de un roedor (*Rattus norvegicus*) de la provincia de Buenos Aires (Argentina). Rev. Argent. Microbiol. 45, 150–153 (in Spanish).
Miño, M.H., Rojas Herrera, E.J., Notarnicola, J., Robles, M.R., Novane, G.T., 2012. Diversity of the helminth community of the Pampean grassland mouse (*Akodon azarae*) on poultry farms in central Argentina. J. Helminthol. 86, 46–53.
Nadler, S.A., Pérez-Ponce de León, G., 2011. Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138, 1688–1709.
Nadler, S.A., Adams, B.J., Lyons, E.T., DeLong, R.L., Melin, S.R., 2000. Molecular and morphometric evidence for separate species of *Uncinaria* (Nematoda: Ancylostomatidae) in California sea lions and northern fur seals: hypothesis testing supplants verification. J. Parasitol. 86, 1099–1106.
Nakao, M., Sako, Y., Ito, A., 2003. The mitochondrial genome of the tapeworm *Taenia solium*: a finding of the abbreviated stop codon U. J. Parasitol. 89, 633–635.
Nakao, M., Lavikainen, A., Iwaki, T., Haukisalmi, V., Konyaev, S., Oku, Y., Okamoto, M., Ito, A., 2013a. 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.
Nakao, M., Lavikainen, A., Yanagida, T., Ito, A., 2013b. Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). Int. J. Parasitol. 43, 1017–1029.
Nonaka, N., Iwaki, T., Okamoto, M., Ooi, H.K., Oku, Y., Ohbayashi, M., Kamiya, M., 1994. Infectivities of four isolates of *Taenia taeniaeformis* to various rodents. J. Vet. Med. Sci. 56, 565–567.
Okamoto, M., Bessho, Y., Kamiya, M., Kurosawa, T., Horii, T., 1995a. Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequence of the cytochrome *c* oxidase subunit I gene. Parasitol. Res. 81, 451–458.
Okamoto, M., Ito, A., Kurosawa, T., Oku, Y., Kamiya, M., Agatsuma, T., 1995b. Intraspecific variation of isoenzymes in *Taenia taeniaeformis*. Int. J. Parasitol. 25, 221–228.
Rausch, R.L., Family Taeniidae Ludwig, 1886, 1994. In: Khalil, L.F., Jones, A., Bray, R. A. (Eds.), Key to the Cestode Parasites of Vertebrates. CAB International, Wallingford, UK, pp. 665–672.
Rodríguez-Vivas, R.I., Panti-May, J.A., Parada-López, J., Hernández-Betancourt, S.F., Ruiz-Piña, H.A., 2011. The occurrence of the larval cestode *Cysticercus fasciolaris* in rodent populations from the Cuxtal ecological reserve, Yucatan, Mexico. J. Helminthol. 85, 458–461.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K., Lane, R., 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. Phil. Trans. R. Soc. B 360, 1805–1811.
Tamada, T., Siriaroonrat, B., Subramaniam, V., Hamachi, M., Lin, L.-K., Oshida, T., Rerkamnuaychoke, W., Masuda, R., 2008. Molecular diversity and phylogeography of the Asian leopard cat, *Felis bengalensis*, inferred form mitochondrial and Y-chromosomal DNA sequences. Zool. Sci. 25, 154–163.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures Math. Life Sci. 17, 57–86.
Terefe, Y., Hailemariam, Z., Menkir, S., Nakao, M., Lavikainen, A., Haukisalmi, V., Iwaki, T., Okamoto, M., Ito, A., 2014. Phylogenetic characterization of *Taenia* tapeworms in spotted hyenas and reconsideration of the "Out of Africa" hypothesis of *Taenia* in humans. Int. J. Parasitol. 44, 533–541.
Thompson, R.C.A., McManus, D.P., 2002. Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol. 18, 452–457.
Verster, A., 1969. A taxonomic revision of the genus *Taenia* Linnaeus, 1758 s. str. Onderstepoort J. Vet. Res. 36, 3–58.
Wardle, R.A., McLeod, J.A., 1952. The Zoology of Tapeworms. University of Minnesota Press, Minneapolis, Minnesota, USA.
Wastlhuber, J., 1991. History of domestic cat and cat breeds. In: Pedersen, N.C. (Ed.), Feline Husbandry. American Veterinary Publications Inc., Goleta, CA, USA, pp. 1–59.
Wilson, D.E., Reeder, D.M. (Eds.), 2005. Mammal Species of the World. A Taxonomic and Geographic Reference, third ed. Johns Hopkins University Press, Baltimore, Maryland, USA, <http://www.press.jhu.edu>, accessed on 31 August 2015.
Yamaguti, S., 1959. Systema Helminthum. Vol. II. The Cestodes of Vertebrates. Interscience Publishers Inc., New York, USA.
Zhang, G., Chen, J., Yang, Y., Liu, N., Jiang, W., Gu, S., Wang, X., Wang, Z., 2014. Utility of DNA barcoding in distinguishing species of the family Taeniidae. J. Parasitol. 100, 542–546.