Rodents as intermediate hosts of cestode parasites of mammalian carnivores and birds of prey in Poland, with the first data on the life-cycle of *Mesocestoides melesi*

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**Abstract**

**Background:** Rodents constitute an important part of the diet of many carnivore species. This predator-prey food chain is exploited by helminth parasites, such as cestodes, whose larval stages develop in rodents and then mature to the adult stage in predators. The main aim of our study was to use molecular techniques for identification of cestode species recovered from both intermediate and definitive hosts, with a particular focus on the genus *Mesocestoides*.

**Methods:** Larval cestodes were obtained during our long-term studies on rodent helminth communities in the Mazury Lake District in the north-east Poland in 2000–2018. Cestode larvae/cysts were collected from body cavities or internal organs (e.g. liver) during autopsies. Adult tapeworms were derived from nine red foxes, three Eurasian badgers and one Eurasian lynx. PCR amplification, sequencing and phylogenetic analyses were conducted employing three genetic markers: 18S rDNA, mitochondrial (mt) 12S rDNA and the mt cytochrome c oxidase subunit 1 (cox1) gene fragment.

**Results:** Altogether 19 *Mesocestoides* samples were analyzed, including 13 adult tapeworms from definitive hosts and six larval samples from 4 bank voles and 2 yellow-necked mice. Phylogenetic analyses revealed three well-supported trees of similar topology. In each case the *Mesocestoides* samples formed two separate clades. All isolates from foxes, the lynx isolate and two isolates from rodents grouped with *Mesocestoides litteratus*. Four isolates from rodents and all three isolates from Eurasian badgers were resolved in a separate clade, most similar to North American *M. vogae* (syn. *M. corti*). Examination of fixed, stained adult specimens from Eurasian badgers revealed consistency with the morphology of *Mesocestoides melesi*. Therefore, this clade is likely to represent *M. melesi*, a species first described in 1985 from the Eurasian badger *Meles meles*. Molecular analysis allowed also the identification of *Taenia crassiceps*, *Hydatigera kamiyai* and *Cladotaenia globifera* among larvae derived from rodents.
Conclusions: Molecular and phylogenetic analyses support the recognition of *M. melesi* as a valid species. Our data represent the first record of the larvae of this species in rodents. This is the first report on the occurrence of *H. kamiyai* in rodents from Poland.

Keywords: *Mesocestoides*, *Hydatigera*, *Taenia crassiceps*, Rodents, Fox, Badger, Lynx

**Background**

Rodents constitute an important part of the diet of many carnivorous species. This predator-prey food chain is exploited by helminth parasites, such as cestodes, whose larval stages develop in rodents and then mature to the adult stage in predators (both carnivorous mammals and birds of prey). The role of rodents as obligatory intermediate or paratenic hosts of tapeworms exploiting this route of transmission (families Mesocestoididae, Taeniidae and Paruterinidae) is therefore indispensable in enabling the completion of their life-cycles.

In our previous studies on parasite communities of rodents from north-east Poland, we investigated the larval cestodes present in different body cavities and in the liver [1–5]. The larval stages of several cestode species were recognized in bank voles (*Myodes glareolus*) by morphological features, including *Mesocestoides* spp., *Cladotaenia globifera*, *Taenia martis*, *Taenia mustelae* and *Hydatigera taeniaeformis* (syn. *Taenia taeniaeformis*). However, in recent years molecular studies have revealed that some of these species actually comprise complexes that include cryptic species which could not be distinguished earlier by conventional morphological examination. Hence re-description of these species has been necessary and driven primarily by their genetic signatures, i.e. *H. taeniaeformis* parasitizing voles has been re-described as *Hydatigera kamiyai* and *T. mustelae* as *Versteria mustelae* [6, 7]. To the best of our knowledge, no such molecular studies, reporting the presence of newly raised species, have been carried out to date on cestode isolates from rodents in Poland.

Tapeworms of the genus *Mesocestoides* (Cyclophyllidea, Mesocestoididae) have been reported to parasitize a range of wild and domestic carnivores and even birds of prey as definitive hosts [8–10]. The systematics of *Mesocestoides* spp. is still not fully resolved [11, 12] and the unarmed scolex and pleomorphic metacestodes/larvae (tetrathyridia) found in rodents and other intermediate hosts (insectivore mammals, birds, reptiles, etc.), do not provide sufficient characteristic features to enable unambiguous differentiation between species. To date, 4–7 *Mesocestoides* species have been reported from Europe [13–17]. The two most commonly reported species are *M. litteratus* found in red foxes (originally described as from a ‘fox’), rodents, grey wolves, dogs and cats among others; and *M. lineatus* that has been reported from domestic/wild cats (originally described from wild cats, *Felis sylvestris*) and dogs, jackals and other carnivores [18]. In Poland, only one molecular study has been completed on *Mesocestoides* larvae from rodent hosts, and this identified *M. litteratus* in striped field mice *Apodemus agrarius* and *M. glareolus* from the Wroclaw area (western Poland) [19]. Red foxes (*Vulpes vulpes*) are considered to be the principal hosts of adult *Mesocestoides* spp. in Poland [20]. In recent years we have carried out extensive studies on different parasites of the red fox from different regions of Poland and we have confirmed the high overall prevalence of *Mesocestoides* in foxes, with a prevalence of 88% in all of the sampled populations [21], as in Karamon et al. [20].

The main aim of our current study was to use molecular techniques for identification of, and comparison between, cestode species recovered from both intermediate and definitive hosts: sylvatic rodents, red foxes and other definitive hosts, with a particular focus on *Mesocestoides* spp.

**Methods**

Larval cestodes were obtained during our long-term studies on rodent helminths in the Mazury Lake District in north-east Poland in 2000–2018 [1–5]. In addition, one *Mesocestoides* sample was obtained from a yellow-necked mouse (*Apodemus flavicollis*) from the Białowieża Forest region, north-east Poland. Altogether, ten infected rodents were examined, including five bank voles *M. glareolus*, two yellow-necked mice *A. agrarius* (Table 1). Cestode larvae from body cavities, identified preliminarily as *Mesocestoides* spp., were obtained from seven rodents, including one sample identified later by molecular typing as an undeveloped *Hydatigera* larva. In one sample, cysts found in the body cavity were morphologically identified as *T. crassiceps*. Two larval samples were derived from rodent livers: one mature strobilocercus of *Hydatigera* sp. and numerous *C. globifera* larvae. The host species for each specimen are recorded in Table 1.

Adult *Mesocestoides* tapeworms were selected from eight red foxes (*V. vulpes*) originating from three administrative regions of Poland: the Mazowieckie, Łódzkie and Kujawsko-Pomorskie Voivodeships (Table 1). One adult...
the Mazury Lake District, north-east Poland, respectively, were also included (Table 1).

### Morphological examination of Mesocestoides spp.

Larval Mesocestoides from rodents and adult Mesocestoides from badgers were flattened and fixed in AFA solution (100 ml 40% formaldehyde, 250 ml 95% ethanol, 100 ml glycerine, 50 ml glacial acetic acid, 500 ml distilled water) and stained using borax carmine, dehydrated in an ethanol series and mounted in Canada balsam for microscopic examination. Slides were examined and selected photographs were taken using a NIKON Eclipse E-600 microscope with differential interference contrast, equipped with the NIS Elements Br 3.1 software (Nikon Instruments Co., Tokyo, Japan) for image processing and recording. Photographs were taken using a NIKON DX-1200 digital camera connected to the microscope.

### DNA extraction and amplification

Genomic DNA was extracted from specimens fixed in ethanol (about 20 mg of tissue) using the DNAeasy Blood & Tissue kit (Qiagen, Hilden, Germany) and stored at a temperature of –20 °C.

Molecular typing of tapeworms was performed by amplification and sequencing of three markers: (i) a fragment of 18S rDNA was amplified using the primers P60 for c.1100 bp of 18S rDNA was amplified using the primers Worm A (5′-GCG AAT GGC TCA TTA AAT AG-3′) and 1270R (5′-CGG TCA ATT CCT TTA AGT TT-3′) [23]; (ii) a fragment of c.350 bp of mitochondrial (mt) 12S rDNA was amplified using the primers P60 for 5′-TTA AGA TAT ATG TGG TAC AGG ATT AGA

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**Table 1** Origin (host species, region and site) and results of genotyping for larval and adult cestodes involved in the study

| Host group   | Host ID | Host species | Region, site, year | Developmental stage, localization | Cestode species (morphological) | GenBank ID | Cestode species (molecular) | GenBank ID | GenBank ID |
|--------------|---------|--------------|--------------------|-----------------------------------|----------------------------------|------------|----------------------------|------------|------------|
| Rodents      | 0005    | *M. glareolus* | Masuria, U, 2018   | Larvae, PC                        | *Mesocestoides* sp. *M. melesi*  | MN512706   | MN505192 M514024          | MN514024   |            |
|             | 0029    | *M. glareolus* | Masuria, U, 2018   | Larvae, PC                        | *Mesocestoides* sp. *M. melesi*  | MN512707   | MN505193 M514025          | MN514025   |            |
|             | 0066    | *M. glareolus* | Masuria, U, 2018   | Larvae, PC                        | *Mesocestoides* sp. *M. melesi*  | MN401347   | MN505194 M514026          | MN514026   |            |
|             | 0130    | *M. glareolus* | Masuria, T, 2018   | Larvae, PC, Liv                   | *Mesocestoides* sp. *M. litteratus* | MN401340   | MN505195 M514027          | MN514027   |            |
|             | 00M3    | A. flavicollis | Masuria, U, 2001   | Larvae, PC                        | *Mesocestoides* sp. *M. melesi*  | MN401345   | MN505196 M514028          | MN514028   |            |
|             | 0177    | A. agrarius   | Masuria, T, 2018   | Larvae, Liv                        | *C. globifera* *C. globifera*     | nd         | MN505197 M514029          |            |            |
|             | 0D45    | M. arvalis    | Masuria, U, 2000   | Larvae, Liv                        | *H. taeniaeformis* *H. kamiyai*   | nd         | MN505198 M514030          |            |            |
|             | 0D53    | M. arvalis    | Masuria, U, 2000   | Larvae, PC                        | *T. crassiceps* *T. crassiceps*   | nd         | MN514031 M514032          |            |            |
|             | D172    | *M. glareolus* | Masuria, U, 2000   | Larvae, PC                        | *Mesocestoides* sp. *H. kamiyai*  | nd         | MN505199 M514033          |            |            |
|             | 0A54    | A. flavicollis | Podlaskie, B, 2016 | Larvae, PC                        | *Mesocestoides* sp. *M. melesi*   | MN401344   | MN505199 M514033          |            |            |
| Canids       | 0079    | V. vulpes     | Mazovia, L, 2017   | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN401342   | MN505200 M514034          |            |            |
|             | 0125    | V. vulpes     | Kujawska-Pomorskie, | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN512708   | MN505201 M514035          |            |            |
|             | 0138    | V. vulpes     | Kujawska-Pomorskie, | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN512709   | nd                       | M514036    |            |
|             | 0143    | V. vulpes     | Kujawska-Pomorskie, | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN401343   | M505202 M514037          |            |            |
|             | 0145    | V. vulpes     | Kujawska-Pomorskie, | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN512710   | nd                       | M514038    |            |
|             | 0146    | V. vulpes     | Kujawska-Pomorskie, | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN512711   | M505203 M514039          |            |            |
|             | 0321    | V. vulpes     | Łódzkie, Wo, 2018  | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN401341   | M505204 M514040          |            |            |
|             | 0322    | V. vulpes     | Łódzkie, M, 2018   | Adult, SI                         | *M. litteratus* *M. litteratus*   | MN512712   | M505205 M514041          |            |            |
|             | 0280    | V. vulpes     | Mazovia, W, 2018   | Adult, SI                         | *T. crassiceps* *T. crassiceps*   | MN512713   | M505206 M514042          |            |            |
| Mustelids    | 0366    | M. meles      | Masuria, Je, 2018  | Adult, SI                         | *Mesocestoides* sp. *M. melesi*   | MN512714   | M505207 nd              |            |            |
|             | 0367    | M. meles      | Masuria, Je, 2018  | Adult, SI                         | *Mesocestoides* sp. *M. melesi*   | MN401346   | M505208 M514043          |            |            |
|             | 0368    | M. meles      | Masuria, Je, 2018  | Adult, SI                         | *Mesocestoides* sp. *M. melesi*   | nd         | M505209 M514044          |            |            |
| Felids       | 0151    | L. lynx       | Podlaskie, L, 2013  | Adult, SI                         | *Mesocestoides* sp. *M. melesi*   | nd         | M505210 nd              |            |            |

Abbreviations: U, Unwitałt; T, Tałty; W, Warsaw; J, Jagodne; K, Kłóbka; Wo, Wolbórz, My, Myslenice, Je, Jedwabno, B, Białowieża, L, Lubaczów; PC, peritoneal cavity; Liv, liver; SI, small intestine; nd, not done
TAC CC-3′) and P375 rev (5′-AAC CGA GGG TGA CGG GCG GTG TGT ACC-3′) [24]; (iii) a fragment of c.400 bp of the cytochrome c oxidase subunit 1 (cox1) was amplified using the primers JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT T-3′) and JB45 (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′) [25]. The PCR reactions were performed in a volume of 20 μl, including 1× PCR Dream Taq Green buffer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1U Dream Taq polymerase (Thermo Fisher Scientific), 0.33 mM dNTPs, 1 μM of each primer and 2 μl of the extracted DNA sample. Negative controls were performed with nuclease-free distilled water, in the absence of template DNA.

All PCR reactions were carried out in identical cycling conditions: primary denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min, and elongation at 72 °C for 1 min, followed by a final elongation step at 72 °C for 7 min and a hold step at 4 °C.

PCR products were subjected to electrophoresis on a 1.5% agarose gel, stained with Midori Green stain (Nippon Genetics, GmbH). PCR products were directly sequenced in both directions by Genomed S.A. (Warsaw, Poland) with the primers used for DNA amplification. Sequences were aligned and visually inspected using Clustal W in MEGA v.7.0 [25]. Consensus sequences were compared with sequences deposited in the GenBank database.

Phylogenetic analyses were conducted separately for each molecular marker (Table 2). Sequences were aligned using E-INS-i algorithm implemented in Mafft version 7.271 [26, 27]. Maximum likelihood trees were obtained in RAxML version 8.2.4 [28] assuming a GTR + G model for the nucleotide substitution process. The topology and branch lengths were optimized starting the analysis 200 times with distinct randomized maximum parsimony trees. Branch support values were obtained during 1000 rapid bootstrap replicates. Bayesian phylogenetic inference was conducted in MrBayes parallel version 3.2.6 [29] with selection of the model of nucleotide substitution (for 12S rDNA: GTR + G; for 18S rDNA: K80 + G; for cox1: GTR + G) by using the BIC implemented in Partition Finder2 [30, 31]. The Bayesian analysis was run for 10 million generations with two independent runs sampled every 1000 generations. The results were combined after discarding 25% of trees considered as ‘burn-in’ phase. The remaining 30,000 trees were summarized as a 50% majority rule consensus tree. Convergence of independent runs and the effective sample size of sampled parameters were inspected in Tracer version 1.6.

Results

Molecular identification of *Mesocestoides* spp.

All eight adult *Mesocestoides* specimens from red foxes and one adult *Mesocestoides* from the Eurasian lynx were identified as *M. litteratus* based on 98–100% identity of the three markers with *M. litteratus* sequences deposited in GenBank (Additional file 1: Tables S1–S3). All three applied genetic markers were successful in amplifying *Mesocestoides* spp. DNA from foxes; however, only 12S rDNA could be amplified from the lynx sample. All the sequences obtained in the present study grouped with sequences of *M. litteratus* from carnivores from a range of European countries (Figs. 1, 2, 3).

Among six larval *Mesocestoides* isolates from rodents, only two (one from a bank vole from Masuria and one from a yellow-necked mouse from Białowieża) were identified as *M. litteratus*, based on 98–100% identity of the three markers used for analysis with *M. litteratus* sequences deposited in GenBank (Additional file 1: Tables S1–S3). A group of four sequences could not be identified due to the lack of identical sequences of 18S rDNA, mt 12S rDNA and cox1 in the GenBank database. These sequences, one derived from *A. flavicollis* and three from *M. glareolus*, both from the Mazury Lake District, displayed the highest similarity (97.4–99.4%) to *M. vogae* (syn. *M. corti*) based on 18S rDNA (Additional file 1: Table S2). Based on mt 12S rDNA and cox1 sequences, percent similarity was markedly lower (90.2–90.5% in 12S rDNA and 88–89% in cox1; Additional file 1: Tables S1, S3), suggesting the presence of a distinct species.

In phylogenetic analyses, these four isolates grouped separately (Figs. 1, 2, 3), distant from *M. litteratus*, *M. lineatus* or *M. canislagopodis*, but displaying closer similarity with North American *M. vogae* (syn. *M. corti*) (Figs. 1, 3). Maximum likelihood and Bayesian trees had very similar topology and therefore we show only ML trees with posterior probability for corresponding bipartitions (Figs. 1, 2, 3).

Interestingly, all three *Mesocestoides* sequences derived from adult worms from Eurasian badgers were very similar (Additional file 1: Tables S1–S3) to these four isolates from rodents. In all phylogenetic trees, the four sequences from rodents and all available sequences from badgers formed one phylogenetic group, distant from *M. litteratus*, other species and a range of recently identified *Mesocestoides* genotypes from Italy and Tunisia [32, 33]. This group of sequences displayed the highest similarity to *M. vogae* (syn. *M. corti*) based on 18S rDNA and cox1 markers (Figs. 1, 3). Some minor diversity (1–3
SNPs) among this group of sequences was also observed (Figs. 1, 2, 3; Additional file 1: Tables S1–S3). There were also some differences between different M. litteratus sequences/isolates (Figs. 2, 3; 125 and cox1).

Morphological examination of Mesocestoides spp.

The larvae of putative M. melesi were half the size of M. litteratus larvae (Additional file 2: Figure S1) and additional morphological evaluation of slides with stained adult tapeworms from Eurasian badgers (Additional file 3: Figure S2) revealed no obvious differences between the present cestodes and those described as M. melesi. Although mean sucker length and width of the adult tapeworms from badgers were slightly larger than the means reported by Yanchev and Petrov [34] (Additional file 4: Table S4), they were well within the range described for M. melesi. Interestingly, the dimensions of the larval suckers of M. melesi identified in this study were half the size of the reported dimensions of suckers in adult worms. Fixed, stained preparations of these worms were compared also with other Mesocestoides spp. in the collection of the Natural History Museum, London (R. A. Bray and P. Olson, personal communication) and it was concluded that M. melesi could not be eliminated as the identity of these worms and with the additional genetic evidence provided in this paper, it was concluded that they were most likely to be M. melesi. A slide with adult tapeworms has been deposited in the Natural History Museum, London, UK, under the accession number NHMUK 2019.9.23.1.

Molecular identification of other larval and adult cestodes

Two isolates were identified as Taenia crassiceps based on 18S rDNA (GTR + G model). Numbers along branches are bootstrap support (BS) and posterior probability (PP) values if corresponding bipartition was found in Bayesian 50% majority rule consensus tree. Only values of BS higher than 75% and PP higher than 0.95 are shown. The scale-bar indicates the expected number of nucleotide substitutions per site.
Again, for these larval isolates from bank voles and sequences from the GenBank database (NC037071). 

rDNA sequences, respectively, were amplified successfully. We were able to obtain cox1 and 12S rDNA sequences for C. globifera larvae from A. agrarius. However, we found no match with any available sequences deposited in GenBank for both markers, so the sequences were deposited as C. globifera based on morphological identification (number and dimensions of larval hooks).

two isolates grouped with other T. crassiceps in one clade of the phylogenetic tree based on cox1 sequences (Fig. 3). The two Hydatigera larvae were identified as H. kamiyai based on 100% similarity of our cox1 sequences with sequences from the GenBank database (NC037071). Again, for these larval isolates from bank voles and common voles sampled in 2000, only cox1 and cox1 and 12S rDNA sequences, respectively, were amplified successfully.
Fig. 3  Maximum likelihood tree for Mesocestoides and relatives based on cox1 gene fragment (GTR + G model). Numbers along branches are bootstrap support (BS) and posterior probability (PP) values if corresponding bipartition was found in Bayesian 50% majority rule consensus tree. Only values of BS higher than 75% and PP higher than 0.95 are shown. The scale-bar indicates the expected number of nucleotide substitutions per site.
Table 2: Characteristics of the nucleotide datasets used in phylogenetic analyses

|                  | 12S rDNA | 18S rDNA | cox1 |
|------------------|----------|----------|------|
| Number of sequences | 51       | 33       | 63   |
| Sequence length variation (bp) | 222–337 | 593–1133 | 366–388 |
| Number of aligned positions | Total    | 350      | 1026 | 388 |
|                    | Constant | 193      | 1010 | 235 |
|                    | Autapomorphic | 28    | 86   | 9   |
|                    | Parsimony informative | 129  | 110  | 144 |
|                    | Containing gaps | 171  | 707  | 29  |
| Percentage of gaps/missing data | 10.35 | 11.42 | 0.93 |

Discussion

In the present study, three genetic markers were used for identification of cestode species recovered from both intermediate (rodents) and definitive hosts (red fox, Eurasian lynx and Eurasian badger) with a particular focus on *Mesocestoides* spp. We demonstrated that *M. litteratus* is a dominant species, occurring in red foxes in Poland and also in the Eurasian lynx from Podkarpackie, south-east Poland and in rodents. However, four isolates from rodents from the Mazury Lake District and all three isolates from Eurasian badgers from the same region created a separate clade, distant from all known species or genotypes available in the GenBank database, but most similar to North American *M. vogae* (syn. *M. corti*) or recently described *M. canislagopodis* [16]. Although genetic divergence for 18S rDNA between our unique isolates and these *Mesocestoides* spp. was only about 1–3%, much higher divergence was noted for the mitochondrial markers, 9–10% for 12S rDNA and 11–12% for cox1, which is enough to consider that these isolates must be a distinct tapeworm species with a novel genetic signature [6, 11, 35, 36]. On balance, taking into account both our morphological observations on adult worms and the genetic analysis, the samples in this clade are most likely to represent *M. melesi*. Our larval and adult cestodes of putative *M. melesi* revealed no obvious differences with the description of *M. melesi*, a species that was first described in 1985 from the Eurasian badger *M. meles* [34]. This first robust description of *M. melesi* was based on a significant number of tapeworms from 42 Eurasian badgers from Bulgaria and detailed several morphological features enabling differentiation of these worms as a new species distinct from *M. lineatus* and *M. erschovi*. The authors did not suggest any intermediate hosts for the new species at that time.

Moreover, although our four *M. melesi* samples from rodents displayed the highest genetic similarity to *M. vogae* (syn. *M. corti*), it is unlikely that they could represent a variant of *M. vogae*. Phylogenetic analyses clearly separated our sequences from *M. vogae*. Besides, *M. corti* was described in the USA by Hoeppli [37] based on about 100 tapeworms (adults, 8 cm long) recovered from the intestines of *Mus musculus* in Colorado in 1909 and recorded in the collection of Professor W.W. Cort. Later, others found only tetrathyridia in mice and rodents and small adults in cats, dogs and skunks [38, 39]. The original description by Hoeppli [37] was eventually questioned [38], especially as the original description was based solely on one archival field sample and rodents are now known not to serve as definitive hosts of *Mesocestoides* spp. These serious concerns led to the description of a new species by Etges [39], *M. vogae*, based on metacestodes from the body cavities and livers of fence lizards (*Sceloporus occidentalis biseriatus*) from California [40]. This description was approved and *M. corti* was synonymized with *M. vogae*. However, no data on definitive hosts was presented in the description of this new species. Then in 2004, Padgett and Boyce [8] provided detailed molecular data on the definitive hosts of *M. vogae*, including coyotes (*Canis latrans*) and domestic dogs, and proposed rodents (deer mice *Peromyscus maniculatus*) as intermediate hosts of this cestode. This biological data support differentiation of *M. vogae* (syn. *M. corti*) from *M. melesi*, with its life-cycle based on Eurasian badgers and European rodents (*Myodes* spp., *Apodemus* spp.).

To the best of our knowledge, our study is one of the first presenting the molecular characteristics of tapeworms derived from both intermediate and definitive hosts. Our analyses have demonstrated clearly that larval and adult *Mesocestoides* derived from rodents and Eurasian badgers, respectively, are closely related and genetically very similar, distant from other *Mesocestoides* species/genotypes, representing a badger-specific species. Thus, taking into account the previous description of *Mesocestoides* from Eurasian badgers as a new species by Yanchev and Petrov [34], we provide evidence for recognition of *M. melesi* as a valid species.

Our study supports the dominant occurrence of *M. litteratus* in rodents and carnivores from central Europe, in accordance with previous studies [14, 15, 41]. This species appears to be a generalist, occurring in a wide range of carnivores (but not in Eurasian badgers); in our study it was found in red foxes from different regions of Poland and in a Eurasian lynx from south-east Poland (Podkarpackie Voivodeship). In a recent molecular study of tapeworms, only this *Mesocestoides* species was found in dogs and cats in south-east Poland [42]. A few years ago, tetrathyridia of *M. litteratus* were identified molecularly in *M. glareolus* and *A. agrarius* from the Wroclaw area, south-west Poland [19]. Both rodent species, in which we identified *M. litteratus* larvae, *M. glareolus* and *A. flavicollis*, are known
intermediate hosts of this species. Interestingly, phylogenetic analyses of *M. litteratus* mitochondrial sequences obtained in this study from carnivores and rodents revealed some degree of diversity, suggesting the existence of several genotypes within the species.

The molecular characteristics of tapeworms derived from both intermediate and final hosts allowed us to conclude that the same genotype of *T. crassiceps* was present in rodents (*M. arvalis*) and red foxes, the definitive hosts of this species.

In our previous studies, cysts containing *strobiocercus* larvae, morphologically identified as *T. taeniaeformis*, were found in the livers of *M. glareolus* [3–5] and *Arvicola terrestris* (Bajer, unpublished) from the same region of Poland. However, following a recent reappraisal of *H. taeniaeformis* and the description of *H. kamiyai* (previously *Taenia taeniaeformis* complex; [6, 7]), here we were able to confirm the occurrence of *H. kamiyai* in voles as intermediate hosts. Moreover, we have now added a third species of *Microtus*, the common vole *M. arvalis*, and the bank vole *Myodes glareolus* to the published list of intermediate hosts for this cestode [6]. To the best of our knowledge, the present study is also the first to report the molecular detection of *H. kamiyai* in Poland, in addition to the recent identification of *H. taeniaeformis* in cats [42].

Conclusions

Molecular and phylogenetic analyses support the recognition of *M. melesi* as a valid species. To the best of our knowledge, our data represent the first record of the larvae of this species in rodents and the first report of the occurrence of *H. kamiyai* in rodents from Poland.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-3961-2.

Additional file 1: Table S1. Similarity (%) between selected 12S rDNA sequences of *Mesocestoides* generated in the present study and sequences of *Mesocestoides* spp. from the GenBank database. Table S2. Similarity (%) between selected 18S rDNA sequences of *Mesocestoides* generated in the present study and sequences of *Mesocestoides* spp. from the GenBank database. Table S3. Similarity (%) between selected cox1 sequences of *Mesocestoides* generated in the present study and sequences of *Mesocestoides* spp. from the GenBank database. Additional file 2: Figure S1. Images of the larvae of *M. melesi*. Larvae from bank vole no. 029: free larva from peritoneal cavity (a–c) and liver cyst (d). Additional file 3: Figure S2. Images of the adult *M. melesi* from the Eurasian badger no. 367. a Scolices with suckers. b Uterine proglottid, cirrus pouch visible. c Gravid proglottids with paruterine organ and cirrus pouch. d Scolices and gravid proglottid. Additional file 4: Table S4. Comparison of the measurements of larval and adult *M. melesi* with *M. litteratus* and data from Yanchev and Petrov [34].
References

1. Bajer A, Behnke JM, Pawelczyk A, Kulik K, Sereda MJ, Siński E. Medium-term temporal stability of the helminth component community structure in bank voles (Clethrionomys glareolus) from the Mazury Lake District region of Poland. Parasitology. 2005;130:213–28.

2. Behnke JM, Barnard CJ, Bajer A, Bray D, Dinmore J, Frake K, et al. Variation in the helminth community structure in bank voles (Clethrionomys glareolus) from three comparable localities in the Mazury Lake District region of Poland. Parasitology. 2001;123:401–14.

3. Behnke JM, Bajer A, Harris PD, Newington L, Pidgeon E, Rowlands G, et al. Temporal and between-site variation in helminth communities of bank voles (Myodes glareolus) from NE Poland. 2. The infracommunity level. Parasitology. 2008;135:995–1018.

4. Girybek M, Bajer A, Bednarńska M, Alsaaraf M, Behnke-Borowczyk C, Harris P, et al. Long-term spatiotemporal stability and dynamic changes in helminth infracommunities of bank voles (Myodes glareolus) in NE Poland. Parasitology. 2015;142:1722–43.

5. Lavikainen A, Iwaki T, Haukisalmi V, Konyaev SV, Casiraghi M, Dokuchaev NE, et al. Reappraisal of Hydatigera taeniaformis (Batsch, 1786) (Cestoda: Taeniidae) sensu lato with description of Hydatigera kantiensis n. sp. Int J Parasitol. 2016;46:36174.

6. Nakao M, Lavikainen A, Iwaki T, Haukisalmi V, Konyaev S, Oku Y, et al. Molecular phylogeny of the genus Taenia (Cestoda: Taeniidae): proposals for the resurrection of Hydatigera Lammarck, 1816 and the creation of a new genus Versteria. Int J Parasitol. 2013;43:427–37.

7. Padgett KA, Boyce WM. Life-history studies on two molecular strains of Mesocestoides (Cestoda: Mesocestoidea): identification of sylvatic hosts and infectivity of immature life stages. J Parasitol. 2004;90:108–13.

8. Padgett KA, Nadler SA, Munson L, Sacks B, Boyce WM. Systematics of Mesocestoides (Cestoda: Mesocestoidea): evaluation of molecular and morphological variation among isolates. J Parasitol. 2005;91:1435–43.

9. Padgett KA, Crossbie PR, Boyce WM. Mesocestoides. In: Liu D, editor. Molecular detection of human pathogens. Boca Raton: CRC Press, Taylor and Francis Group, 2013. p. 277–85.

10. Kukec-B.BL, Traub NJ, Tkach VV, Shirley TN, Rollins D, Fedynich A. Mesocestoides sp. in wild northern bobwhite (Colinus virginianus) and scaled quail (Callipepla squamata). J Wild Dis. 2018;54:612–6.

11. McAllister CT, Tkach VV, Conn DB. Morphological and molecular characterization of post-larval pre-tetrathyridia of Mesocestoides sp. (Cestoda: Cyclophyllidea) from ground skink, Scincella lateralis (Sauria: Scincidae), from southeastern Oklahoma. J Parasitol. 2018;104:246–53.

12. Chertkova AN, Kosupko GA. Podjedmond Mesocestoidata Skrjabin, 1940. Osnovy cestodologii, 9. Moskva: Nauka; 1978. p. 118–229.

13. Littlewood DTJ, Olson PD. Small subunit rDNA and the platyhelminthes: signal, noise, conflict and compromise. In: Littlewood DTJ, Bray RA, editors. Interrelationships of the platyhelminthes. London: Taylor & Francis, 2001. p. 262–78.

14. Von Nickisch-Rosenegk M, Silva-Gonzalez R, Lucius R. Modification of universal 125 rDNA primers for specific amplification of contaminated Taenia spp. (Cestoda) gDNA enabling phylogenetic studies. Parasitol Res. 1999;85:819–25.

15. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–40.

16. Bowles J, Blair D, McManus DP. A molecular phylogeny of the human schistosomes. Mol Phylogenet Evol. 1995;4:103–9.

17. Katoh K, Misawa K, Kumra T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30:3059–66.

18. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.

19. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3.

20. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Algar D, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–42.

21. Lanfear R, Frandsen PB, Wright AM, Sefc KM, Tavare S, Bapteste E. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Ecol Evol. 2017;72:3–3.

22. Montalbano Di Filippo M, Meoli R, Cavaliero S, Elena C, De Liberato C, Berrilli F. Molecular identification of Mesocestoides sp. metacestodes in a captive gold-handed tamarin (Saguinus midas). Infect Genet Evol. 2016;55:399–405.

23. Varacca A, Sanna D, Casu M, Lahmar S, Dessi G, Pipa AP, et al. Species delimitation based on mtDNA genes suggests the occurrence of new species of Mesocestoides in the Mediterranean region. Parasite Vectors. 2018;11:619.

24. Yanchev YI, Petrov IK. Mesocestoides melesi sp. n. (Cestoda, Mesocestoidea) in Meles meles L. from Bulgaria. Comp Res Anim Vet Sci. 1985;36:247–51.

25. Galimberti A, Romano DF, Genchi M, Paolini D, Vercillo F, Bizzarri L, et al. Integrative taxonomy at work: DNA barcoding of taenids harboured by wild and domestic cats. Mol Ecol Resour. 2012;12:403–13.

26. Zhang L, Hu M, Jones A, Alsopp BA, Beveridge L, Schindler AR, et al. Characterization of Taenia multiceps and Taenia regis from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationship with other selected taenids. Mol Cell Probes. 2007;21:379–85.

27. Hooije RJC. Mesocestoides corti, a new species of cestode from the mouse. J Parasitol. 1925;12:91–6.

28. Beaver PC. Mesocestoides corti: mouse type host, uncharacteristic or questionable? J Parasitol. 1989;75:815.

29. Etges FJ. The proliferative tetrathyridium of Mesocestoides vogae sp. n. (Cestoda). J Helminth Soc Wash. 1991;58:181–5.

30. Specht D, Voige M. Asexual multiplication of Mesocestoides tetrathyridia in laboratory animals. J Parasitol. 1965;51:268–72.

31. Hrckova G, Miterpavkova M, O’Connor A, Snelil V, Olsson PD. Molecular and morphological circumscription of Mesocestoides tapeworms from red foxes (Vulpes vulpes) in central Europe. Parasitology. 2011;138:638–47.

32. Karamon J, Sroka J, Dąbrowska J, Kochanowski M, Siński E. Prevalence of intestinal helminths of red foxes (Vulpes vulpes) in central Europe (Poland): a significant zoonotic threat. Parasit Vectors. 2018;11:436.