



Phylogenetic relationships within Dicrocoeliidae (Platyhelminthes: Digenea) from birds from the Czech Republic using partial 28S rDNA sequences

Jitka Aldhoun¹ · Rasha Elmahy^{1,2} · D.T.J. Littlewood^{1,2}

Received: 18 May 2018 / Accepted: 20 August 2018 / Published online: 5 September 2018
© The Author(s) 2018

Abstract

Partial (D1–D3) 28S rRNA gene sequences from 16 isolates of digenean parasites of the family Dicrocoeliidae recovered from 16 bird species from the Czech Republic were used for phylogenetic reconstruction. Comparison with sequences available from GenBank suggests that the genus *Brachylecithum* is paraphyletic, requiring further validation and possible systematic revision. Although partial 28S rDNA is relatively conserved, analyses suggest that the following taxa are synonymous: *Lutztrema attenuatum* = *L. monenteron* = *L. microstomum*, *Brachylecithum lobatum* = *B. glareoli*. *Zonorchis petiolatus* is reassigned back to the genus *Lyperosomum* with *L. collurionis* as a junior synonym. The study revealed how complicated the systematics of the family Dicrocoeliidae is currently. The morphology of the group is variable, and the current distinguishing characters at species and even generic level are not sufficiently distinctive; it is difficult to identify the specimens correctly and identification of GenBank isolates is not reliable. Extensive sampling of isolates for both molecular and morphological studies is necessary to resolve the relationships within the family.

Keywords Dicrocoeliidae · *Lutztrema* · *Lyperosomum* · *Brachylecithum* · Birds · 28S rDNA

Introduction

The digenean family Dicrocoeliidae Looss, 1899 is a large family that includes more than 400 species with considerable variability in size, shape, and position of internal organs. Dicrocoeliidae include parasites of livestock, as well as wild-life, and their complex life cycles are facilitated by trophic transmission. They develop exclusively in terrestrial environments and have complex life cycles that involve mostly two intermediate hosts: snails and arthropods (Pojmańska 2008). The three subfamilies of the family and the genera are distinguished based on the relative position of the reproductive

organs, ventral sucker, and caeca, as well as on the structure of the vitellarium, and on the length of the caeca (Pojmańska 2008). Species identification is often based on morphometric data (e.g., Hildebrand et al. 2007); however, specimens of the same species vary greatly in dimensions, shape, and positions of the inner organs, and shape and dimensions of the body depending on the age of the specimen, its location in the host, intensity of infection, and on the fixation method (Sitko 1994; Sitko 1995; Sitko et al. 2000). Many species were described based on incomplete and/or single specimens, and the range of within-species morphological variability is therefore unknown. As a result, the boundaries between species and even genera are somewhat blurred, and some species have been assigned to several different genera (see e.g. Denton and Krüssinger 1974; Panin 1984; Sitko 1994; Sitko 1995; Sitko et al. 2000; Sitko 2013). Consequently, the validity and status of many taxa are doubtful. The issue is further aggravated by insufficient knowledge of life cycles and scant molecular data that are known only for a fraction of all the taxa described to date, namely some species of *Dicrocoelium* Dujardin, 1845 (e.g., Maurelli et al. 2007), *Eurytrema* Looss, 1907 (e.g. Cai et al. 2012), *Paraconcinnum* Vassiliades et Richard, 1970 (Ribas et al. 2012), *Brachylecithum* Shtrom, 1940 (Kinsella

Handling Editor: Julia Walochnik

✉ Jitka Aldhoun
jita@nhm.ac.uk

¹ Parasites & Vectors, Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

² Zoology Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

and Tkach 2009; Hildebrand et al. 2016), *Lyperosomum* Looss, 1899, and *Lutztrema* Travassos, 1941 (Hildebrand et al. 2015)).

The highly variable D1-D3 domains of 28S rDNA were shown to be suitable to identify species and determine phylogenetic relationships within Platyhelminthes (e.g., Shylla et al. 2013; Razo-Mendivil et al. 2014). Since the partial (D1-D3) 28S rRNA gene has been the molecular marker of choice in various studies, numerous sequences are available for comparative analysis within GenBank; also, universal primers to amplify the region across various diverse taxa within the Platyhelminthes are available. Therefore, we chose this region and conducted phylogenetic analysis of partial (D1-D3) 28S rRNA gene sequences of 16 isolates of worms parasitizing birds collected in the Czech Republic, including sequences already deposited in GenBank. Our specimens were assigned to eight species of the family Dicrocoeliidae, including three species whose DNA sequences were previously unknown.

Materials and methods

Adult specimens of dicrocoeliid worms were collected and identified during long-term helminthological studies of birds in Zahlinice (Central Moravia, Czech Republic—see Table 1). The adults were identified under the microscope based on expertise of the observer, Dr. Jilji Sitko (Komenský Museum in Prerov, Czech Republic), and then preserved in 96% ethanol for molecular studies. Genomic DNA was isolated from single specimens using a QIAamp DNA Mini Kit (Qiagen) according to manufacturer's instructions. Polymerase chain reactions were performed with PuReTaq Ready-To-Go PCR Beads (GE Healthcare), with 5–50 ng of template DNA and 10 μ M of each primer in a reaction volume of 25 μ l. Partial 28S rRNA gene (D1-D3) regions were amplified, using primers LSU5 and 1500R (Waeschenbach et al. 2007). The reaction profile included an initial denaturation step at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 2 min with a final extension step at 72 °C for 7 min. PCR products were purified from the PCR mixture or from agarose gel with QIAquick PCR Purification Kit or QIAquick Gel Extraction Kit (both Qiagen), respectively. Purified PCR products were sequenced directly using the PCR primers and additional sequencing primers as listed in Waeschenbach et al. 2007. The products were sequenced on an Applied Biosystems 377 automated sequencer. The sequences were deposited in GenBank under the accession numbers MG560850–MG560865.

Phylogenetic analysis for the partial 28S rRNA gene was performed using the newly obtained sequences, and sequences downloaded from GenBank (Tables 1 and 2). *Polylekithum* Arnold, 1934 (Trematoda: Allocreadiidae;

accession numbers EF032697 and EF032698) was used as an outgroup to root the phylogeny. Sequences were aligned, and the alignments manually refined in AliView (Larsson 2014). The entire alignment was used, as no ambiguously aligned regions were identified. Maximum likelihood analysis was done in the program Phyml (Guindon and Gascuel 2003) using the GTR+I+ Γ model, as chosen by jModelTest (Posada 2008), with parameters estimated by the software. Nodal support was estimated by bootstrap resampling (1000 replicates). The difference between sequences was expressed as base substitutions per site, i.e., number of substitutions divided by the total number of positions used for the alignment (= 1078). Thus, 0.001 base substitution per site corresponds approximately to one nucleotide difference between the sequences.

Results and discussion

Phylogenetic analysis of partial 28S rRNA gene fragments (1078 positions) included 16 newly obtained sequences and 24 sequences downloaded from GenBank. Bootstrap support within the tree created using maximum likelihood (Fig. 1) was low (< 50) for some branches, suggesting that the relationships between these taxa remain unresolved. The phylogenetic tree reflects the complicated and confusing situation of the systematics of the family.

Lutztrema and *Brachylecithum* are morphologically very similar genera. Their main distinguishing character is the morphology of the intestine: while *Brachylecithum* has two intestinal branches that run from below the pharynx, *Lutztrema* has only one intestinal branch (Sitko and Koubkova 1999). However, since the intestine is often concealed by the uterine loops filled with eggs, and secretory canals, located between the oral and ventral sucker of adults, can be mistaken for the intestine, incorrect identifications are readily made, e.g., specimens originally identified as *Brachylecithum mosquensis* (Skrjabin and Isaitschikoff, 1927) by Kinsella and Tkach (2009) were later redetermined as *Lutztrema monenteron* (Price and McIntosh, 1935) (Hildebrand et al. 2015). Without a special staining method—a combination of boraxcarmine and astra blue staining, which stains the intestine blue, among red gonads (Sitko and Koubkova 1999)—it might be impossible to observe the intestine and reliably determine the genus. In our tree, samples of *Lutztrema* and *Brachylecithum* form a clade together (bootstrap support 61). Within this clade, all *Lutztrema* samples form a monophyletic clade (bootstrap support 100), whereas *Brachylecithum* is paraphyletic. Relationships within *Lutztrema* clade are unresolved; however, we can identify two groups: (1) isolates identified as *L. attenuatum* (Dujardin, 1845) from common blackbird, *Turdus merula* L., and common starling, *Sturnus vulgaris* L., together with *L. microstomum* Denton and Byrd, 1951 and *L. monenteron*; and (2) two samples from Eurasian blackcap,

Table 1 Samples sequenced in the present study. The first column lists the original species identification based on morphological examination of the parasites; the last column lists the species identification according to the results of the phylogenetic analysis of partial 28 rDNA

Species identification—this study	Host	Host common name	Sample/ GenBank accession number	Identification in GenBank
<i>Brachydistomum ventricosum</i>	<i>Erithacus rubecula</i>	European robin	JS431/MG560855	<i>Brachydistomum ventricosum</i>
<i>Brachylecithum kakea</i>	<i>Acrocephalus arundinaceus</i>	Great reed warbler	JA11/MG560851	<i>Brachylecithum kakea</i>
<i>B. kakea</i>	<i>Acrocephalus scirpaceus</i>	Eurasian reed warbler	JS442/MG560856	<i>B. kakea</i>
<i>B. kakea</i>	<i>Emberiza schoeniclus</i>	Common reed bunting	JS443/MG560857	<i>B. lobatum</i>
<i>B. strigis</i>	<i>Otus scops</i>	Eurasian scops owl	JA16/MG560852	<i>B. strigis</i>
<i>Lutztrema attenuatum</i>	<i>Turdus merula</i>	Common blackbird	JA58/MG560853	<i>Lutztrema attenuatum</i>
<i>Lu. attenuatum</i>	<i>Sturnus vulgaris</i>	Common starling	JS491/MG560858	<i>Lu. attenuatum</i>
<i>Lu. attenuatum</i>	<i>Sylvia atricapilla</i>	Eurasian blackcap	JS492/MG560859	<i>Lutztrema</i> sp.
<i>Lyperosomum collurionis</i>	<i>S. atricapilla</i>	Eurasian blackcap	JA66/MG560854	<i>Lyperosomum collurionis</i>
<i>Ly. collurionis</i>	<i>S. atricapilla</i>	Eurasian blackcap	JS502/MG560860	<i>Ly. collurionis</i>
<i>Stromitrema koshewnikowi</i>	<i>Hirundo rustica</i>	Barn swallow	JA100/MG560850	<i>Stromitrema koshewnikowi</i>
<i>Zonorchis clathratus</i>	<i>Apus apus</i>	Common swift	JS613/MG560862	<i>Zonorchis clathratus</i>
<i>Z. petiolatus</i>	<i>Garrulus glandarius</i>	Eurasian jay	JS507/MG560861	<i>Ly. collurionis</i>
<i>Z. petiolatus</i>	<i>Prunella modularis</i>	Dunnock	JS615/MG560863	<i>Ly. collurionis</i>
<i>Z. petiolatus</i>	<i>T. merula</i>	Common blackbird	JS616/MG560864	<i>Lyperosomum</i> sp.
<i>Z. petiolatus</i>	<i>T. philomelos</i>	Song thrush	JS619/MG560865	<i>Lyperosomum</i> sp.

Sylvia atricapilla (L.), and *Lutztrema* sp. from great reed warbler, *Acrocephalus arundinaceus* (L.). Sitko et al. (2000) conducted a review of the variability of *L. attenuatum* based on morphological examination and statistical evaluation of morphometric data; they concluded that *L. microstomum* and *L. monenteron* are synonyms of *L. attenuatum*. This opinion is supported by our results because the difference between *L. attenuatum* samples from *Sturnus* and *Turdus* (0.002 base substitution per site or 2 nucleotide differences) corresponds to that between them, and *L. microstomum* and *L. monenteron* (0.002–0.003). On the other hand, the difference between *L. attenuatum* from *Turdus* and *Sturnus*, and “*L. attenuatum*” sample from *S. atricapilla* is considerably higher at 0.006–0.007. A GenBank isolate identified as *Brachylecithum* sp. (accession number KU563711, host *S. atricapilla*) groups with the latter sample and *Lutztrema* sp., with the difference within this group being 0.001–0.003 base substitution per site. We conclude that the *Brachylecithum* isolate was most likely misidentified, and all three isolates probably belong to the same species of *Lutztrema*. More molecular data is needed to

decide whether that is *L. attenuatum* or another species of the genus.

As mentioned above, *Brachylecithum* is a paraphyletic taxon based on the tree. GenBank isolates of *Brachylecithum lobatum* (Railliet, 1900); *B. glareoli* Hildebrand, Okulewicz and Popiołek, 2007, *B. strigis* (Yamaguti, 1939); and *B. capilliformis* Oshmarin, 1952 form a clade in sister position to the *Lutztrema* clade, with all isolates except *B. capilliformis* being identical. The results of molecular analysis of partial 28S rDNA and partial mitochondrial cytochrome c oxidase subunit I gene (cox1) conducted by Hildebrand et al. (2016) were very similar and led the authors to the conclusion that *B. strigis* is a synonym of *B. lobatum*; however, they decided to keep *B. glareoli* as a separate species based on differences in morphometric data. Our molecular results indicate *B. glareoli* should be considered a synonym of *B. lobatum*, especially as various authors have shown that dimensions can vary to a large extent between populations of the same species from different hosts (e.g., Kostadinova 1996, Sitko and Okulewicz 2002, Sitko et al. 2000). Our isolate from common

Table 2 Dicrocoeliid trematode sequences downloaded from GenBank, with reported hosts, used in this study

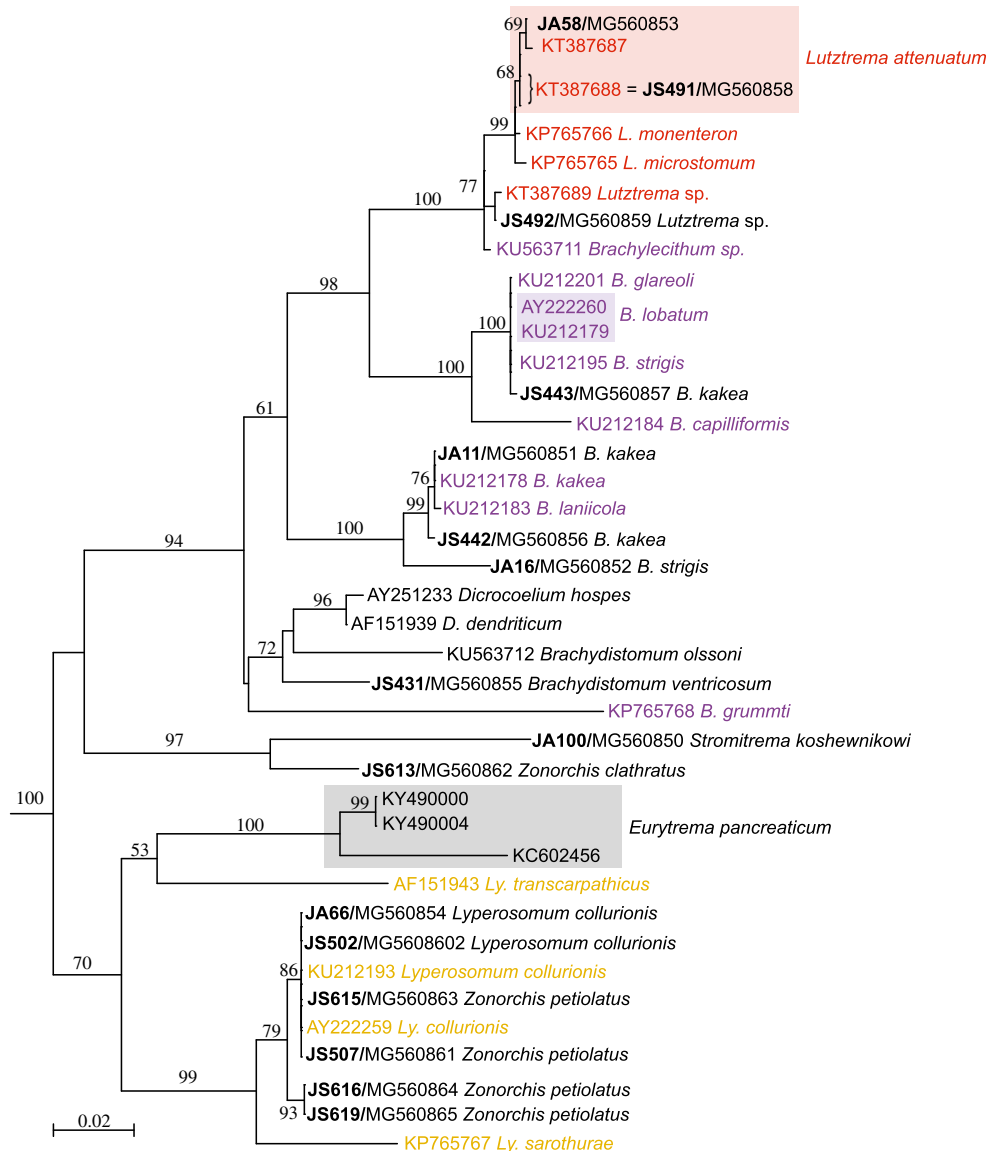
Species	Host	Host common name	GenBank accession number
<i>Brachydistomum olssoni</i>	<i>Apus apus</i>	Common swift	KU563712
<i>Brachylecithum</i> sp.	<i>Sylvia atricapilla</i>	Eurasian blackcap	KU563711
<i>B. capilliformis</i>	<i>Locustella fluviatilis</i>	River warbler	KU212184
<i>B. glareoli</i>	<i>Myodes glareolus</i>	Bank vole	KU212201
<i>B. grummti</i>	<i>Attila cinnamomeus</i>	Cinnamon attila	KP765768
<i>B. kakea</i>	<i>Acrocephalus arundinaceus</i>	Great reed warbler	KU212178
<i>B. laniicola</i>	<i>Lanius collurio</i>	Red-backed shrike	KU212183
<i>B. lobatum</i>	<i>Accipiter nisus</i>	Eurasian sparrowhawk	KU212179
<i>B. lobatum</i>	<i>Corvus corone</i>	Carrion crow	AY222260
<i>B. strigis</i>	<i>Otus scops</i>	Eurasian scops owl	KU212195
<i>Dicrocoelium dendriticum</i>	<i>Marmota bobak</i>	Bobak marmot	AF151939
<i>D. hospes</i>	Not stated		AY251233
<i>Eurytrema pancreaticum</i>	<i>Bos indicus</i>	Zebu	KC602456
<i>E. pancreaticum</i>	Sheep		KY490000
<i>E. pancreaticum</i>	sheep		KY490004
<i>Lutztrema</i> sp.	<i>Acrocephalus arundinaceus</i>	Great reed warbler	KT387689
<i>L. attenuatum</i>	<i>Turdus merula</i>	Common blackbird	KT387688
<i>L. attenuatum</i>	<i>T. merula</i>	Common blackbird	KT387687
<i>L. microstomum</i>	<i>Cyanocitta cristata</i>	Blue jay	KP765765
<i>L. monenteron</i>	<i>Turdus migratorius</i>	American robin	KP765766
<i>Lyperosomum collurionis</i>	<i>Sylvia atricapilla</i>	Eurasian blackcap	KU212193
<i>L. collurionis</i>	<i>S. atricapilla</i>	Eurasian blackcap	AY222259
<i>L. sarothurae</i>	<i>Sarothrura pulchra</i>	White-spotted flufftail	KP765767
<i>L. transcarpathicus</i>	<i>Sorex minutus</i>	Eurasian pygmy shrew	AF151943

reed bunting, *Emberiza schoeniclus* (L.), identified as *B. kakea* (Bhalerao, 1926) was most likely misidentified and belongs to *B. lobatum* as it is nearly identical in gene sequence to the other *B. lobatum* isolates (difference 0.001). Another clade, in a basal position to the *B. lobatum*/*B. capillaris* and *Lutztrema* spp. clade, includes GenBank samples identified as *B. kakea* and *B. laniicola* (Layman, 1926), and our isolates identified as *B. kakea* and *B. strigis*. All isolates except for the latter belong to the same species (difference 0.001). The GenBank isolate of *B. laniicola* could have been misidentified, possibly belonging instead to *B. kakea*, or the two species are synonymous. However, as we did not see the specimens used for these published DNA studies we cannot refute or support either possibility. Our isolate of *B. strigis* is not identical to that taken from GenBank; instead, the former is a distinct species in sister position to the *B. kakea*/*B. laniicola* clade, whereas the latter falls within the *B. lobatum* clade and is identical to *B. lobatum* isolates. It seems likely that this isolate was in fact *B. lobatum*

misidentified as *B. strigis*, and the two species are not synonymous. *Brachylecithum grummti* Odening, 1964 isolate from GenBank is entirely outside the other *Brachylecithum* isolates, in sister position to a clade containing *Brachydistomum* Travassos, 1944 and *Dicrocoelium*. However, since the boundaries between dicrocoeliid genera are rather fluid, with *Brachydistomum* and *Brachylecithum* morphologically similar (to the extent that Panin (1984) considered the former a synonym of the latter, and Sitko and Oculewicz (2002) transferred *Brachylecithum mosquensis* to *Brachydistomum*), there is a possibility that the isolate in fact belongs to another genus.

Zonorchis petiolatus (Railliet, 1900) isolates form a clade with isolates determined as *Lyperosomum collurionis* (Skrjabin and Isaitschikoff, 1927). All *L. collurionis* isolates and isolates determined as *Z. petiolatus* from dunnoek, *Prunella modularis* (L.) and from Eurasian jay, *Garrulus glandarius* (L.), are identical; we conclude that they all belong to the same species. The other two isolates determined as *Z. petiolatus* (from common blackbird, *Turdus merula*, and

Fig. 1 Phylogenetic tree constructed by maximum likelihood based on partial 28S rDNA sequences of microcoeliid samples from this study and downloaded from GenBank. Bootstrap values of 50 or lower are not plotted.



song thrush, *T. philomelos* Brehm, 1831, respectively) are identical and they differ from the *L. collurionis* isolates very slightly (difference 0.003), which suggests that they might belong to a different species of the same genus or even to *L. collurionis* as well. An isolate from common swift, *Apus apus* (L.), determined as *Z. clathratus* (Deslongschamps, 1824) is in sister position to an isolate of *Stromitrema koshewnikowi* (Skrjabin et Massino, 1924) and belongs to a different genus than the *Z. petiolatus* isolates. It is worth mentioning that both *Z. petiolatus* and *Z. clathratus* were repeatedly assigned to the genus *Lyperosomum* in the past (e.g., Denton and Krissinger 1974), which again shows how close different genera of the Dicrocoeliidae are in terms of morphology. Based on our results, *Z. petiolatus* should be reassigned back to the genus *Lyperosomum*, with *L. collurionis* becoming a junior synonym.

There is considerable difference between GenBank isolates of *Eurytrema pancreaticum* (Janson, 1889) from sheep and that from zebu, *Bos indicus* L., (base substitution difference per site is 0.03), which suggests that they are in fact two different species.

Our study generated 16 new partial 28S rDNA sequences of microcoeliids from birds and thus considerably increased the number of isolates whose sequences are deposited in GenBank. However, it also laid bare the issues that complicate the studies of the family and highlight the limitations of both morphology and choice of gene in revising this family adequately. Morphology, predominantly focused on dimensions and shape of the body and inner organs, is very variable between specimens of the same species, and the distinguishing characters are not sufficiently distinctive. Some species and genera described as separate taxa are in fact synonymous.

Therefore, it is hard to identify the specimens correctly, and some of the isolates deposited in GenBank have clearly been misidentified. The systematics of the family is in need of revision, and an extensive sampling for both molecular, using a combination of nuclear and mitochondrial markers, and morphological studies with associated voucher specimens acquired is necessary to achieve this important goal.

Acknowledgements This project was supported by the Disease Initiative of the Natural History Museum in London. We are indebted to Dr. Jilji Sitko from the Moravian Ornithological Station of the Komensky Museum in Prerov, the Czech Republic, for his help in sourcing, supplying, and identifying parasite material.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Cai Z, Zhang Y, Ye X (2012) Phylogenetic relationships of the genus *Eurytrema* from domestic and wild animal based on 18S rRNA sequences. *Parasitol Res* 111:1637–1644
- Denton JF, Krissinger WA (1974) The occurrence and morphology of *Brachylecithum transversum* (Travassos, 1917) comb.n., in the eastern kingbird, *Tyrannus tyrannus* (L.), from Georgia. *Proc Helm Soc Wash* 41:191–194
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704
- Hildebrand J, Okulewicz J, Popiolek M (2007) A new dicrocoeliid from the bank vole *Clethrionomys glareolus* (Rodentia: Microtidae) from Poland. *J Parasitol* 93:151–154
- Hildebrand J, Pulis EE, Tkach VV (2015) Redescription and phylogenetic relationships of the rare *Lyperosomum sarothurae* Baer, 1959 (Digenea: Dicrocoeliidae). *Acta Parasitol* 60:371–377
- Hildebrand J, Sitko J, Zalesny G, Jezewski W, Laskowski Z (2016) Molecular characteristics of representatives of the genus *Brachylecithum* Shtrom, 1940 (Digenea, Dicrocoeliidae) with comments on life cycle and host specificity. *Parasitol Res* 115:1417–1425
- Kinsella JM, Tkach VV (2009) Molecular identification of an avian dicrocoeliid, *Brachylecithum mosquensis*, from a Vagrant Shrew, *Sorex vagrans*, in Montana, U.S.A. *Comp Parasitol* 76:287–289
- Kostadinova A (1996) Morphological variability of *Brachylecithum microtesticulatum* (Digenea: Dicrocoeliidae) in the Black Sea region. *Folia Parasitol* 43:47–51
- Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30:3276–3278
- Maurelli MP, Rinaldi L, Capuano F, Perugini AG, Veneziano V, Cringoli G (2007) Characterization of the 28S and the second internal transcribed spacer of ribosomal DNA of *Dicrocoelium dendriticum* and *Dicrocoelium hospes*. *Parasitol Res* 101:1251–1255
- Panin V (1984) Dicrocoeliid trematodes of the world fauna. *Acad Sci Kazakh.SSR Inst Zool, Alma-Ata*
- Pojmańska T (2008) Family Dicrocoeliidae Looss, 1899. In: Bray RA, Gibson DL, Jones A (eds) *Keys to the Trematoda*. CABI Publishing and The Natural History Museum, London, pp 233–260
- Posada D (2008) jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256
- Razo-Mendivil U, Perez-Ponce de Leon G, Rubio-Godoy M (2014) Testing the systematic position and relationships of *Paracreptotrema heterandriae* within the Allocreadiidae through partial 28S rRNA gene sequences. *J Parasitol* 100:537–541
- Ribas A, Makundi RH, Göüy de Bellocq J (2012) *Paraconcinnum leirsi* n.sp. (Trematoda: Dicrocoeliidae) from rodents in Tanzania and its phylogenetic position within the dicrocoeliids. *Afr Zool* 47:326–331
- Shylla JA, Ghatani S, Tandon V (2013) Utility of divergent domains of 28S ribosomal RNA in species discrimination of paramphistomes (Trematoda: Digenea: Paramphistomoidea). *Parasitol Res* 112:4239–4253
- Sitko J (1994) Revision of the genus *Brachydistomum* Travassos, 1944 (Digenea: Dicrocoeliidae). *Helminthologia* 31:57–65
- Sitko J (1995) Variability and systematic status of *Zonorchis clathratum* (Trematoda: Dicrocoeliidae), a parasite of swifts and swallows. *Folia Parasitol* 42:193–198
- Sitko J (2013) Redescription of *Skrjabinus skrjabini* and validity reassessment of selected species of *Skrjabinus* (Digenea, Dicrocoeliidae). *Helminthologia* 50:281–286
- Sitko J, Koubkova B (1999) A simple differentiation of two genera *Brachylecithum* and *Lutztrema* (Trematoda: Dicrocoeliidae) based on Borax carmine and Astra blue staining method. *Helminthologia* 36:119–121
- Sitko J, Okulewicz J, Noga L (2000) Variability and systematic status of *Lutztrema attenuatum* (Dujardin, 1845) comb. N. (Trematoda: Dicrocoeliidae) parasitizing passeriform birds. *Helminthologia* 37:97–111
- Sitko J, Okulewicz J (2002) Redescription and systematic status of *Brachydistomum ventricosum* (Rudolphi, 1809) comb. N. (Trematoda: Dicrocoeliidae) parasitizing passeriform birds. *Helminthologia* 39:103–110
- Waeschenbach A, Webster BL, Bray RA, Littlewood DT (2007) Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. *Mol Phylogenet Evol* 45:311–325