Identification of a new species of digenean *Notocotylus malhamensis* n. sp. (Digenea: Notocotylidae) from the bank vole (*Myodes glareolus*) and the field vole (*Microtus agrestis*)

K. BOYCE[1], G. HIDE[1], P. S. CRAIG[1], P. D. HARRIS[3], C. REYNOLDS[1], A. PICKLES[2] *and* M. T. ROGAN[1]*

[1] *Centre for Parasitology and Disease Research, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK*
[2] *Field Studies Council at Malham Tarn Field Centre, North Yorkshire BD24 9PU*
[3] *National Centre for Biosystematics, Natural History Museum, University of Oslo, PO Box 1172, N-0318, Oslo, Norway*

(*Received 21 March 2012; revised 2 May 2012; accepted 2 May 2012; first published online 19 July 2012*)

SUMMARY

Notocotylus malhamensis n. sp. is described from the caecum of the bank vole (*Myodes glareolus*) and the field vole (*Microtus agrestis*) from Malham Tarn Nature Reserve in North Yorkshire, UK. In total, 581 specimens were collected from rodents trapped at a wetland site (Tarn Fen) between July 2010 and October 2011 with a prevalence of 66·7% and mean intensity of 94·6 in the bank vole and 50% prevalence and a mean intensity of 4·3 in the field vole. This species appears to be most closely related to other previously described *Notocotylus* species infecting rodents in Europe but differs principally by the metraterm to cirrus sac ratio (1:1·5–1:1·2) in combination with a densely spinulated cirrus, simple caeca and a greater number of ventral glands in the lateral rows (14–17). The use of molecular differentiation was of limited use in this study due to a paucity of relevant information in the DNA sequence databases. However, the complete ITS1-5.8S rDNA-ITS2 and partial 28S gene sequences have been generated to provide a definitive tool for identification of this species in future studies. As far as we know this is the first report of a notocotylid infection in *M. glareolus* in the UK.

Key words: Notocotylidae, *Notocotylus malhamensis*, bank vole, *Myodes glareolus*, field vole, *Microtus agrestis*, DNA.

INTRODUCTION

Wild murid populations worldwide are commonly examined for their helminth fauna. The most commonly reported gastrointestinal digeneans found in the UK wood mouse *Apodemus sylvaticus* tend to be *Corrigia vitta* and *Brachylaemus recurvum* (Elton *et al*. 1931; Behnke *et al*. 1999; Abu-Madi *et al*. 2000). In 2007, however, Rogan *et al*. (2007) reported a rare UK occurrence of the intestinal digenean *Plagiorchis muris* from the small intestine of *A. sylvaticus* at the Malham Tarn Nature Reserve and which had been previously recorded over a 13-year period. Reports of digeneans from UK vole populations appear to be limited; however, here we report the occurrence of another species of the genus *Notocotylus* Diesing, 1839, from the caecum of 2 vole populations at Malham Tarn. Previously, *Notocotylus noyeri* has been recorded from the water-vole *Arvicola amphibius* (syn. *A terrestris*) and the short-tailed field vole *Microtus hirtus* (syn. *M. agrestis*) in Cambridgeshire, UK (Baylis, 1928*b*, 1939). A further 6 *Notocotylus* spp. recorded in the UK have all involved waterfowl as definitive hosts. The current report identifies a new *Notocotylus* species that can be morphologically differentiated from all previously reported notocotylids.

The genus *Notocotylus* Diesing, 1839 is cosmopolitan, parasitizing waterfowl, and small mammals with an affinity for water. The genus has a complicated taxonomic history and misidentification has been commonplace leading to synonymization and much confusion. Morphological differences between species are small, and there is ongoing dispute over reliable diagnostic morphological criteria (Nath and Pande, 1963). As a result many newly described *Notocotylus* species have been suppressed as synonyms and the genus has been continuously revised (Lal, 1935*a*; Harwood, 1939; Dubois, 1951; Stunkard, 1966; Simon-Vicente *et al*. 1985*a*). Even the number of valid species within the genus *Notocotylus* can be problematic, a fact that has been reflected in the variable number of species included in differential keys (Dubois, 1951; Skrjabin, 1953; Yamaguti, 1958). Following an extensive study into the morphology of *Notocotylus* species infecting rodents in Europe, Simon-Vicente *et al*. (1985*a*) identified the existence of 4 stable intraspecific morphological features that are of differential systematic value. Using these criteria, in addition to those previously established by Lal (1935*a*) and

* Corresponding author: Centre for Parasitology and Disease, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK. Tel: 0044 161 295 4083. Fax: 0044 161 295 5015. E-mail: m.t.rogan@salford.ac.uk

doi:10.1017/S0031182012000911

Dubois (1951), the digeneans from bank voles and field voles at Malham Tarn have been designated as a new species in the genus *Notocotylus*.

MATERIALS AND METHODS

The study was carried out at Malham Tarn Nature Reserve located in North Yorkshire, Northwest England at an altitude of 375 m above sea level. Malham Tarn is a 'site of special scientific interest' (SSSI) and is the only upland marl lake in Britain (Rogan *et al*. 2007). This area has previously been investigated for a range of host parasite systems (Allan *et al*. 1999; Hughes *et al*. 2006, 2008; Rogan *et al*. 2007; Behnke *et al*. 2009; Thomasson *et al*. 2011).

Rodents were trapped under permit from the National Trust, using Longworth small mammal traps for 4 days each season between January 2010 and October 2011. The trapping point of each infected rodent was recorded using a Garmin GPS 60 set to the co-ordinate framework WGS84. Four sites were examined throughout the sampling period including 3 woodland sites (Tarn Wood 54°06′03.3″N, 002°09′44.9″W, Spiggot Hill 54°05′72.9″N, 002°10′ 43.1″W; Ha Mire Plantation 54°05′64.5″N, 002°09′ 53.7″W) and 1 wetland site, (Tarn Fen 54°06′00.0″N, 002°10′43.4″W). In total, 126 wood mice (*Apodemus sylvaticus*) and 63 voles (54 *Myodes glareolus* and 9 *Microtus agrestis*) were trapped from all 4 sampling sites. All rodents trapped were examined for a range of helminth parasites using a consistent detailed post-mortem examination. In the collection of *M. glareolus* and *M. agrestis*, *Capillaria* spp., *Hymenolepis diminuta* and *Heligmosomoides glareoli* were also found in addition to *Notocotylus malhamensis*. Helminth species richness varied from 0 to 3 in individual animals.

Rodents were euthanized and examined according to Rogan *et al*. (2007) and morphologically identified to species level using the criteria of Sargent and Morris (2003). Morphological identification of vole species was verified using molecular analysis. A 1 cm^2 section of thigh muscle was aseptically removed from each of the vole species and was placed into an Eppendorf tube containing 400 μl of lysis buffer (100 mM NaCl, 25 mM ethylene diamine tetraacetic acid, 0·5% sodium dodecyl sulphate, 20 mM Tris, pH 8·0). DNA extraction was performed according to Thomasson *et al*. (2011).

The cytochrome oxidase subunit I gene was amplified from vole DNA by PCR using the forward primer RonM ($^{5'}$GGMGCMCCMGATATRGCA-TTCCC$^{3'}$) and reverse primer NancyM ($^{5'}$CCTG-GGAGRATAAGAATATAWACTTC$^{3'}$) according to Pfunder *et al*. (2004). Each 25 μl reaction contained 2·5 μl of 10X DreamTaq buffer including 2 mM $MgCl_2$ (Fermentas, Life Sciences), 0·025 μmol deoxynucleotide triphosphate (dNTPs; 100 mM, Bioline), 1 μM forward primer, 1 μM reverse primer, 2·5 U DreamTaq DNA polymerase and 1 μl gDNA template (50 μg/μl). All PCR reactions were performed using a Robocycler 96 PCR machine (Stratagene, CA, USA) and visualized on a 1% (w/v) Tris-acetate-EDTA (TAE) agarose gel stained with gel red using a G: Box gel imaging system (Syngene, UK). The cycling profile consisted of an initial denaturation of 1 cycle at 95 °C for 15 min, 45 cycles of denaturation at 95 °C for 40 sec, annealing at 50 °C for 40 sec and elongation at 72 °C for 1 min, and 1 final cycle at 72 °C for 7 min. The target bands were excised from the gel using a UV transilluminator and purified using a PCR purification kit (Geneflow) according to the manufacturer's instructions. Samples were commercially sequenced in both directions (Source Bioscience, Nottingham, UK).

Notocotylus was not detected from any of the *A. sylvaticus* despite careful observation. All specimens of *N. malhamensis* from the caecum of the bank voles (*Myodes glareolus*) and the field voles (*Microtus agrestis*) were relaxed in distilled water for morphological examination. Some digeneans were fixed in 5% formal saline and flattened under light cover-slip pressure for morphological analysis. All measurements were taken using a Leica DM500 microscope and eyepiece graticule. Eggs were recovered from the feces of infected rodents and examined under phosphate-buffered saline (PBS) to measure the length of the egg filaments. Twenty specimens were stained in borax carmine and mounted in Canada balsam according to Gurr (1963) with alteration of the staining time to 3 h. The ventral glands and cirrus composition were examined on unstained unflattened specimens. Drawings were made from photographs taken with a Leica ICC50 digital camera attached to a Leica DM500 microscope. The difference in morpho-anatomic measurements between the two specimens of *N. malhamensis* recovered from *M. glareolus* and *M. agrestis* were statistically analysed using a one-way parametric ANOVA with equal replicates.

The remainder of the flukes were fixed in 70% ethanol suitable for molecular analysis. DNA was extracted from individual worms using a phenol: chloroform method modified from Thomasson *et al*. (2011) by halving the amount of reagent at each stage of the protocol. The Internal transcribed spacer (ITS), including the ITS1, 5.8S, ITS2 and flanking regions of the 3′ end of the 18S and 5′ end of the 28S were amplified using the forward universal primer BR ($^{5'}$GTAGGTGAACCTGCGGA$^{3'}$) and reverse digenean specific primer dig11 ($^{5'}$GTGATATGCT-TAAGTTCAGC$^{3'}$) according to Tkach *et al*. (2000*a*). The partial 28S rDNA gene region was amplified using the forward digenean specific primer dig12 ($^{5'}$AAGCATATCACTAAGCGG$^{3'}$)

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

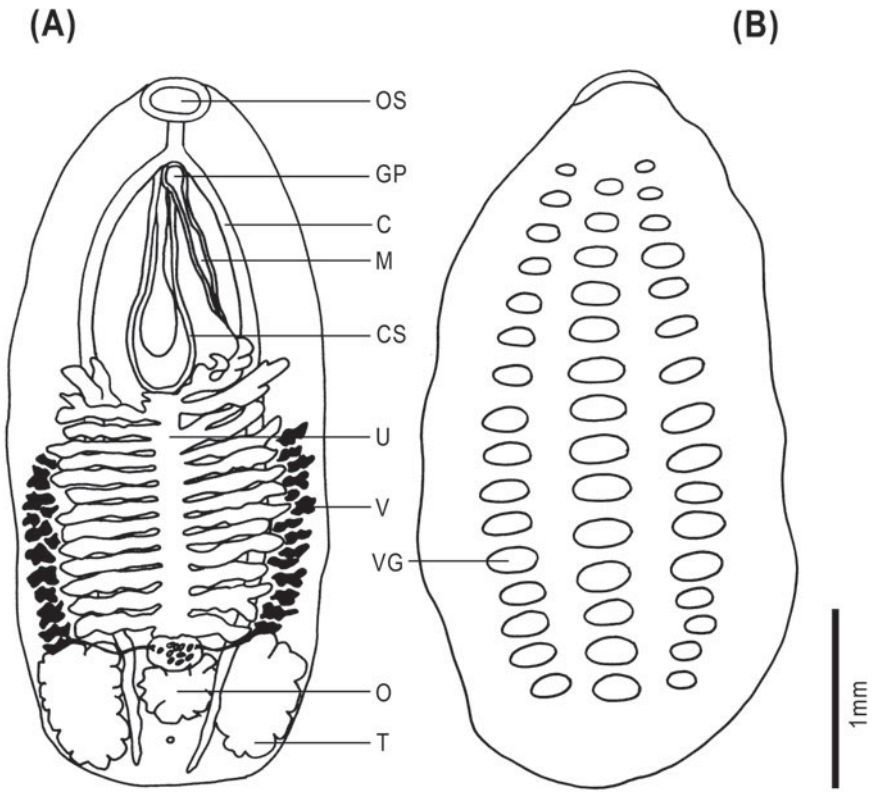

Fig. 1. *Notocotylus malhamensis* n. sp. from *Myodes glareolus*. (A) Dorsal surface. (B) Ventral surface. OS, oral sucker; GP, genital pore; C, caecum; M, metraterm; CS, cirrus sac; U, uterus; V, vitelline glands; VG, ventral glands; O, ovary; T, testis.

and the reverse universal primer Lo ($^{5'}$GCTATCCTGAGRGAAACTTCG$^{3'}$) according to Tkach *et al*. (2000*b*).

Each 50 μl PCR reaction contained 5 μl of 10X DreamTaq buffer including 2 mM $MgCl_2$ (Fermentas, Life Sciences), 0·05 μmol dNTPS (100 mM, Bioline), 2·5 μM forward primer, 2·5 μM reverse primer, 5 U DreamTaq DNA polymerase and 2 μl of gDNA template (50 μg/μl). The amplification profile consisted of 1 cycle at 94 °C for 10 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 54 °C and 1 min at 72 °C and 1 final cycle at 72 °C for 10 min. Excision of the target bands, PCR purification and DNA sequencing was performed as previously described. This same procedure was conducted on 3 independent occasions. In total 9 sequences for each gene were aligned.

RESULTS

Confirmation of vole species

Identification of the 2 vole species was confirmed by molecular barcoding of Cytochrome Oxidase 1 as well as by overall morphology. In both cases, animals identified by morphology as *M. glareolus* and *M. agrestis* were confirmed by 99% sequence homology with NCBI reference sequences (*M. glareolus* AY332679 and *M. agrestis* AY332684). Sequences from Malham rodents have been deposited into GenBank under Accession numbers: (*M. glareolus* JQ794805 and *M. agrestis* JQ794806).

Notocotylus malhamensis n. sp. (Figs 1–4)

Type-locality: Tarn Fen wetlands (54°06′00.0″N, 002°10′43.4″W, WGS84 framework), Malham Tarn Nature Reserve, North Yorkshire, UK.
Type-hosts: *Myodes glareolus, Microtus agrestis*.
Site in host: Caecum.
Prevalence and intensity: 568 specimens were collected from *M. glareolus* (overall prevalence 66·7%, 6/9) and 13 from *M. agrestis* (overall prevalence 50%, 3/6) from the Tarn Fen area. Infected *M. glareolus* had infection intensities ranging from 1 to 294 flukes (median = 48) and *M. agrestis* had intensities ranging from 1 to 6 flukes (median = 6).
Type specimens: 45 specimens have been deposited at the Department of Zoology, Natural History Museum, Cromwell Road, London, UK (holotype NHMUK 2012.3.14.1, and paratypes NHMUK 2012.3.14.2-30 (10 borax carmine-stained specimens and 35 specimens stored in 70% molecular grade ethanol). A further 10 borax carmine-stained specimens and 120 specimens stored in 70% molecular grade ethanol are held at The School of Environment

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

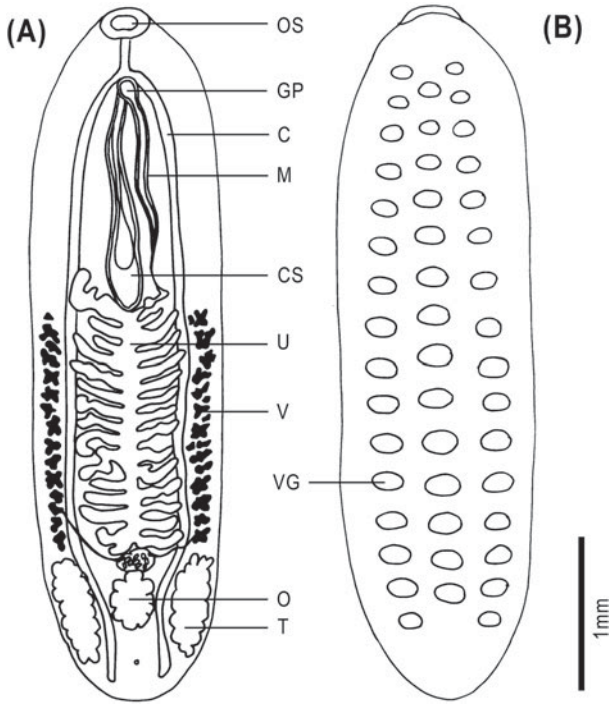

Fig. 2. *Notocotylus malhamensis* n. sp. from *Microtus agrestis*. (A) Dorsal surface. (B) Ventral surface. OS, oral sucker; GP, genital pore; C, caecum; M, metraterm; CS, cirrus sac; U, uterus; V, vitelline glands; VG, ventral glands; O, ovary; T, testes.

and Life Science, The University of Salford, Salford Crescent, Manchester, UK.

Morphological description

Figure 1 illustrates the ventral and dorsal morphology of *N. malhamensis* recovered from *M. glareolus*. Measurements were based on 10 specimens. In live specimens the fluke is dorso-ventrally flattened with lateral margins that fold dorsally to provide a curvature to the body and create a ventral concavity. Tegument is unspined. In flattened specimens the anterior end of the body attenuates and is bluntly pointed in comparison to the posterior end, which is generally rotund. The body length ranges from 2·47 to 4·86 mm (mean 4·04 mm) and the maximum body width (midway across the uterus) from 1·17 to 1·53 mm (mean 1·39 mm). The ventral surface possesses 3 rows of protrusible glands, 42–47 in total; lateral rows consisting of 14–17 glands (most commonly 16) and a median row of 14–15 glands (most commonly 15). The first median gland is positioned half an interval behind the first lateral glands.

The oral sucker ranges from 170 to 288 μm in length (mean 234 μm) by 180 to 300 μm (mean 258 μm) in width. The oesophagus measures 150–170 μm (mean 164 μm) in length and bifurcates in front of the genital pore extending into very long blindly ending caeca. The caeca extend posteriorly

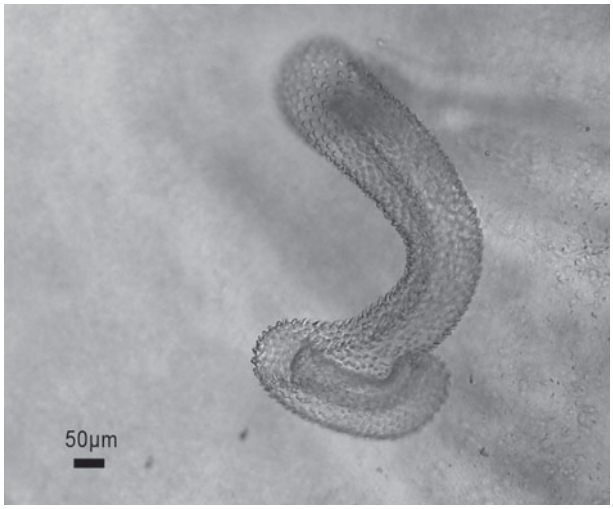

Fig. 3. Spinulated cirrus of *Notocotylus malhamensis*.

underlying the uterine loops and curving in-between the laterally positioned testes and a single centrally located ovary. The caeca appear red in live specimens.

The laterally positioned testes measure 650 and 800 μm in length are lobed in form and extracaecal. The claviform cirrus sac extends from 840 to 1380 μm (mean 1170 μm) in length with the proximal extremity positioned at 40–45% the length from the anterior. The length of the cirrus was difficult to measure. The cirrus is coiled in relaxed specimens when everted (Fig. 3). The width of the cirrus measured from 84 to 86 μm in diameter at the distal end, and is densely spinulated over its entire surface.

The genital pore lies behind the intestinal bifurcation. The uterus is transversely coiled tightly between the base of the cirrus sac and the anterior border of the vitelline reservoir. There are 12–14 uterine loops that overflow the caecal field and 3–4 uterine coils lie ahead of the vitelline glands. The anterior border of the vitelline glands is positioned at 48–50% from the anterior of the body length. Two lateral groups of 14–18 follicles extend to the posterior border of the uterus. All principal uterine loops are posterior to the base of the cirrus sac. Secondary uterine loops can be observed on the lateral side of the cirrus sac, which adjoin the metraterm. The metraterm is long and rectilineal in form and measures from 1:1·5 to 1:1·2 the length of the cirrus sac. Metratermic glands can be observed along the full length of the metraterm. The egg measures 20 μm × 10 μm and bears 2 polar filaments, one on either side (Fig. 4). The filaments range in length from 60 μm to 180 μm and are often unequal in length.

Variation in specimens recovered from Microtus agrestis

The following description of a variant type is based on 9 specimens. An illustration of the morphology

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

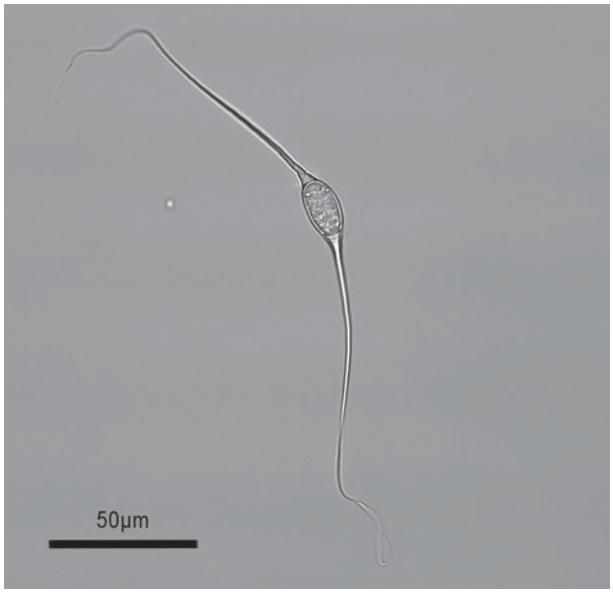

Fig. 4. Egg recovered from the feces of an infected bank vole indicating the extended egg filaments.

can be seen in Fig. 2. The body appears identical in form to those recovered from *M. glareolus* but is more elongated with a body length ranging from 3·73 to 5·05 mm (mean 4·52 mm) and a maximum body width (midway across the uterus) from 1·10 to 1·47 mm (mean 1·36 mm). This elongation of the worms appears to create a more bluntly pointed posterior. Three protrusible rows of ventral glands are present conforming to the pattern of the glands observed in the specimens recovered from *M. glareolus*, differing only by the last lateral glands extending slightly further towards the posterior and bypassing the last median gland.

The oral sucker measures 210–300 μm (mean 286 μm) in width and 170–230 μm (mean 213 μm) in length. The oesophagus is more elongated ranging from 220 to 290 μm (mean 260 μm) in length. The lobed testes measure 700–900 μm (mean 800 μm) in length. The cirrus sac is more elongated extending from 1050 to 1900 μm (mean 1610 μm). The majority of uterine loops lie intracaecal with only a few loops slightly overlapping the caecal field and there appears to be more space separating the uterine loops. Three to four uterine coils lie ahead of the vitelline glands although in one specimen the vitelline glands extended as far as the anterior border of the uterine coils. The metraterm is more elongated with a range of 770 to 1460 μm (mean 1165 μm); however, the metraterm to cirrus sac ratio remained stable measuring 1:1·5 to 1:1·2 the length of the cirrus sac. Despite size variation seen in *N. malhamensis* between the two host species, these measurements were not found to be significantly different using a one-way ANOVA with equal replicates ($P=0{\cdot}5233$, D.F. = 1, $F=0{\cdot}41$). DNA sequencing of the ITS region furthermore indicates a 100% sequence homology between *N. malhamensis* recovered from both *M. glareolus* and *M. agrestis*.

Differential comparison of N. malhamensis *with other* Notocotylus *spp.*

Following an extensive literature search, 68 *Notocotylus* species names were identified worldwide. Of these, 63 taxa appear to be valid. Cribb (1991) listed 41 species in his comparison with *Notocotylus johnstoni*. Kinsella and Tkach (2005) added a further 8 species. From these 49 species, both *Notocotylus gippyensis* and *N. tadornae* (see Bisset, 1977), which possess a single row of ventral glands, have been re-located into *Uniserialis* Beverley-Burton, 1958 (Barton and Blair, 2005). Two further species have been described since 2005 *N. biomphalaria* (Flores and Brugni, 2005) and *N. loeiensis* (Chaisiri *et al.* 2011), and 15 names not mentioned, including *N. linearis* (Rudolphi, 1819), *N. triserialis* Diesing, 1839, *N. urbanensis* Cort, 1914, *N. chionis* (Baylis, 1928*a*), *N. intestinalis* (Tubangui, 1932), *N. babai* (Bhalerao, 1935), *N. lucknowensis* (Lal, 1935*a*), *N. anatis* and *N. orientalis* (Ku, 1937), *N. dafilae* and *N. porzanae* (Harwood, 1939), *N. stagnicolae* (Herber, 1942), *N. solitaria* (Singh, 1954), *N. wetlugensis* (Shaldybin, 1965), and *N. lianhuensis* (Qiongzhang, 1988), include species that are more controversially valid.

Cribb (1991) regarded *N. anatis, N. babai, N. lucknowensis* and *N. solitaria* as synonyms of *N. imbricatus* Looss, 1893, and while *N. triserialis* and *N. attenuatus* may be synonyms, the relative precedence and validity of the two forms remains disputed (Dubois, 1951; Beverley-Burton, 1961, 1972; Pike, 1969). In the current context, the most important controversial taxon is *N. wetlugensis*, described from voles in European Russia (Shaldybin, 1965). Frequently considered a synonym of *N. noyeri*, the taxon was re-established by Tenora *et al.* (1983), a decision supported by Simone-Vicente *et al.* (1985*a*).

Notocotylus malhamensis n. sp. could be differentiated from all of these species using morphological characteristics, including the arrangement and composition of the ventral glands, the positioning of the genital pore, the structure of the caecum, the metraterm to cirrus sac ratio, cirrus surface composition and the number and positioning of the uterine coils.

The group of species that include *N. gibbus* Mehlis, 1846 (Beverley-Burton, 1961), *N. pacifera* (Noble, 1933), *N. porzanae* (Harwood, 1939), and *N. regis* (Harwood, 1939), have short oval bodies and only 4–5 ventral glands in the central row that are flat and non-protrusible. This group of species is easily distinguishable from *Notocotylus sensu stricto*.

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

Table 1. The number of ventral glands found in each of the three rows of previously described *Notocotylus* species that can be differentiated from *N. malhamensis* on this basis

		Ventral gland composition		
Species	Reference	Lateral	Median	Lateral
N. malhamensis	**Current study**	**14–17**	**14–15**	**14–17**
N. ephemera	Nitzsch, 1817 (Kanev *et al*. 1994)	9–12	11–14	9–12
N. orientalis	Ku (1937)	23	23	23
N. lopezneyrai	Dubois and Vigueras (1953)	11–12	13	11–12
N. skrjabini	Ablasov, 1953 (Bisset, 1977)	5	5	5
N. ratti	Ye *et al*. (1956)	10–11	5–6	10–11
N. duboisianus	Odening (1964)	10–13	9–11	10–13
N. kiangsuensis	I-Ping (1965)	10	5	10
N. breviserialis	Stunkard (1967)	4	5	4
N. barmarensis	Gupta (1970)	11	4–5	11
N. panjnadensis	Bhutta and Khan (1975)	12	12	12
N. anseri	Gupta and Gupta (1976)	23	20	23
N. schmidti	Brooks and Heard (1977)	4	3	4
N. kanpurensis	Gupta and Gupta (1977)	23	19	23
N. casarcai	Gupta and Jehan (1977)	6–10	6–10	6–10
N. gallinulae	El-Naffer and Khalifa (1983)	10–13	9–11	10–13
N. paithanensis	Deshmukh (1985)	11	5	11
N. lianhuaensis	Qiongzhang (1988)	4–6	6–7	4–6
N. polylecithus	Qiongzhang (1992)	27–28	24–25	27–28
N. biomphalariae	Flores and Brugni (2005)	11	4	11

N. malhamensis could be differentiated from a further 19 *Notocotylus* species by the arrangement and composition of the ventral glands (Table 1).

N. malhamensis has a post-bifurcal genital pore and can therefore be differentiated from *N. naviformis* (Tubangui, 1932), *N. vinodae* (Gupta and Singh, 1983), *N. johnstoni* (Cribb, 1991), *N. fosteri* Kinsella and Tkach (2005), and *N. loeiensis* (Chaisiri *et al*. 2011), all of which possess a prebifurcal genital pore. *N. tachyeretis* (Duthoit, 1931), *N. lucknowensis* (Lal, 1935*a*), *N. mamii* (Hsu, 1954), *N. nathipandei* (Odening, 1964), and *N. poecilorhynchai* (Gupta and Jehan, 1977), all have a genital pore that is located ventral to the intestinal bifurcation. Additionally, *N. tachyeretis* possesses from 21 to 26 uterine coils and a cirrus sac length from 2500–2800 μm, *N. lucknowensis* has a metraterm to cirrus sac ratio of 1:3, *N. mamii* has a metraterm that is slightly longer than the cirrus sac and *N. nathipandei* possesses more than 20 uterine coils, 7 of which are positioned ahead of the vitelline glands.

N. malhamensis has a spinulated cirrus and can additionally be distinguished from *N. triserialis* Diesing, 1839 (Pike, 1969), *N. filamentis* (Barker, 1915), and *N. solitaria* (Singh, 1954), which have a papillated cirrus, and *N. noyeri* (Joyeux, 1922), *N. intestinalis* (Tubangui, 1932), and *N. gonzalezi* Simon-Vicente *et al*. 1985*a*, all of which possess a cirrus smooth in composition.

The metraterm to cirrus sac ratio of 1:1·5–1:1·2 observed in *N. malhamensis* can furthermore be used for differentiation from the following described species. The ratios have been indicated in parentheses: *N. linearis* (Rudolphi, 1819) (1:3), *N. aegyptiacus* Odhner, 1905 (<1:2) (Dubois, 1951), *N. urbanensis* Cort, 1914 (1:2) (Harrah, 1922), *N. magniovatus* Yamaguti, 1934 (1:4–1:2), *N. minutus* (Stunkard, 1960) (1:2), *N. atlanticus* (Stunkard, 1966) (<1:2), *N. neyrai* Gonzalez Castro, 1945 (<1:3·3) (Simon-Vicente *et al*. 1985*a*) and *N. zduni* Chiaberachvili and Djavelidze 1968 (1:2) (Vassilev and Kanev, 1984). The eggs of *N. zduni* furthermore possess a long filament at one end and a tuft of short and thin filaments at the opposite end (Vassilev and Kanev, 1984). All eggs observed for *N. malhamensis* possess one long filament at each end of the egg (Fig. 4).

Furthermore, the length of the cirrus of *N. marinus* (Ginetsinskaya and Naumov, 1958), equals twice the length of the uterine coils and the longest length recorded for *N. indicus* (Lal, 1935*b*) was much less than the smallest record for *N. malhamensis*. The cirrus composition of *N. indicus* is furthermore long, thin and feeble with no mention of spines on the surface. In addition, *N. urbanensis* possesses 10 uterine coils that lie ahead of the vitelline glands.

The species which *N. malhamensis* most closely resembles, in terms of possessing a long and rectilineal metraterm in combination with spinulation of the cirrus, is *N. wetlugensis* (Shaldybin, 1965). This species, however, also possesses caecal diverticulations, which are absent in *N. malhamensis*.

The number and arrangement of the uterine coils can also be used for differentiation. *N. malhamensis* possesses 12–14 uterine coils that overflow the caecal field. The uterine coils of *N. attenuatus*

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

(Rudolphi, 1809) are strictly confined to the caecal field. This species was furthermore ruled out using molecular analysis. The uterine coils of *N. imbricatus* Looss, 1893 are also confined to the caecal field and this species has a metraterm that is only 1:1·8 the length of the cirrus sac (Beverley-Burton, 1961). The following species can furthermore be ruled out on having a greater number of uterine coils, as indicated in parenthesis: *N. chionis* (Baylis, 1928*a*) (16–26), *N. parviovatus* (Yamaguti, 1934) (>21), *N. ralli* (Baylis, 1936) (37–34) and *N. anatis* (Ku, 1937) (22–27). Additionally, *N. babai* (Bhalerao, 1935) has 10–11 uterine loops that lie ahead of the vitelline glands and a metraterm to cirrus sac ratio of 1:4–1:2, *N. micropalmae* (Harwood, 1939), has 9 coils and *N. dafilae* (Harwood, 1939), has 6–8 coils ahead of the vitelline glands. In addition, *N. stagnicolae* (Herber, 1942), can be ruled out by the anterior half of the body being covered with spines, the caeca possessing dilatations and indentations and although not mentioned in the text, the figure indicates 18–19 uterine coils that are all confined to the caecal field.

Molecular characterization

Amplification of the internal transcribed spacer (ITS) including the 3′ end of the 18S, ITS1, 5.8S, ITS2 and the 5′ end of the 28S generated a sequence of 1236 bp (GenBank Accession number: JQ766940). Sequences obtained from specimens recovered from the caecum of *M. glareolus* and *M. agrestis* were 100% identical.

Amplification of the 28S gene generated a partial sequence of 1260 bp. The 28S sequence of *N. malhamensis* was compared against 3 available DNA sequences that were available from the NCBI database: *N. attenuatus* (AF184259) collected from an athyid duck in southern Ukraine (Tkach *et al.* 2001), *Notocotylus* sp. UK-PO-2003 (AY222219), based on sporocyst material from *Lymnaea* in the UK, and *Notocotylus* BH-2008 (EU712725) based on larval material from *Physa* from Nebraska, USA (Hanelt, 2009). The sequence for *N. malhamensis* appeared most closely related to *Notocotylus* BH-2008 sharing 99% sequence homology, followed by 98% with *Notocotylus* sp. UK-PO-2003 and only 96% with *N. attenuatus*. The 28S sequence of *N. malhamensis* has been deposited into GenBank under Accession number: JQ766939.

DISCUSSION

Members of the genus *Notocotylus* predominantly infect waterfowl; however, a limited number of species infect rodents. From the 63 described species only 10 have previously been recorded as infecting rodents naturally of which 4 have been recorded within Europe: *N. noyeri* Joyeux, 1922, *N. neyrai* Gonzalez Castro, 1945, *N. wetlugensis* Shaldybin, 1965, and *N. gonzalezi* Simon-Vicente *et al.* 1985 (Tenora *et al.* 1983; Simon-Vicente *et al.* 1985*a*). *Notocotylus malhamensis* n. sp appears to be most closely related to these other *Notocotylus* species from European rodents, sharing similarities in the number and arrangement of ventral glands, the number and positioning of the uterine coils and the positioning of the genital pore. As shown by Simon-Vicente *et al.* (1985*a*), the metraterm to cirrus sac ratio in combination with cirrus composition can be used as a basis for differentiating these species. Those criteria in combination with the structure of the caecum can furthermore be used to distinguish *N. malhamensis* from the other European forms. The presence of a long and rectilineal metraterm, dense spinulation of the cirrus and simple caeca are a combination seen in *N. malhamensis* that has not been observed in any of the other European forms.

Notocotylus malhamensis from the field vole *Microtus agrestis* were morphologically comparable to the specimens from *Myodes glareolus* when using the above key taxonomic features. The *M. agrestis* specimens were, however, more elongated with a greater range in length, smaller width and elongation of internal structures, although these differences were not statistically significant. A difference in the width and spatial separation of the uterine loops was also observed. In the specimens from *M. glareolus* the uterine loops consistently extend beyond the caecal field, whereas in specimens from *M. agrestis* the uterine loops appear to only slightly extend beyond the caeca. The specimens from *M. agrestis* may represent juvenile adults that had not fully developed, as Kinsella (1971) demonstrated that in *Quinqueserialis quinqueserialis,* a species closely related to *Notocotylus,* at 12 days post-infection the uterine coils were confined within the caecal field, while by day 15 they had begun to overlap the gut caeca.

The detailed life cycles of most *Notocotylus* species remain unknown. Typically, a brackish or freshwater lymnaeid or hydrobiid becomes infected by eating eggs released into water with feces of the definitive host (Murrils *et al.* 1988). Following development in the snail, actively swimming eyed cercariae are released which quickly encyst on nearby solid objects such as aquatic vegetation (Simon-Vicente *et al.* 1985*b*) or even the snail shell. The definitive host becomes infected by ingestion of the encysted metacercariae together with vegetation during foraging.

Notocotylus malhamensis was recovered from *M. glareolus* and *M. agrestis* captured from the wetland site (Tarn Fen) only. No infection was observed in either host captured from any of the 3 woodland sites (0/48), suggesting that flooding of the wetland area is potentially important in terms of transmission of *N. malhamensis* to the vole populations. Typically,

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

voles may be restricted to feeding at the water edge and as such will be susceptible to infection by metacercariae present only around the water margin (Webber *et al*. 1987). During periods of flooding, invertebrate species inhabiting the water column are likely to be conveyed into the typical foraging range of the vole communities along with floating aquatic vegetation and detritus. As the water recedes, both snails and plants infected with metacercariae are likely to be left on the ground. Aquatic plants have been observed littering this area on several occasions, a factor that could contribute to exposure of voles to infective metacercariae while foraging.

Prior to sampling, the last bout of flooding took place on 13 October 2011 at which point the water level at Tarn Fen rose to 200 mm above normal levels (Hodgson, *personal communication*). *Microtus agrestis* were trapped on the 28 October 2011. If flooding is an important criterion for infection to occur then it might be speculated that the estimated age of the *N*. *malhamensis* samples recovered from *M*. *agrestis* would be less than 15 days old, indicating that the uterine coils may not yet be fully developed.

The elongate appearance and increased range of internal measurements of *N*. *malhamensis* from *M*. *agrestis* may, however, represent their ability to grow to a much larger size in this host than in *M*. *glareolus*. Variation in morpho-anatomic measurements of digeneans in different host species has been previously reported to be host-dependent (Kinsella, 1971). This intraspecific variation observed in morpho-anatomic measurements between the two host species at Tarn Fen reinforces the fact that no reliance can be placed upon the size of the worm or its internal organs for species differentiation and that key taxonomic features which remain constant across a species should instead be used (Lal, 1935*a*).

A maximum of 6 *N*. *malhamensis* were recovered from any individual *M*. *agrestis* infection, while up to 294 worms were recovered from the caecum of a single *M*. *glareolus*. The gross anatomy of the caecum differs between the two rodent species, being only 13 cm in *M*. *glareolus* compared to 24 cm in *M*. *agrestis* (see Lange and Staaland, 1970). This difference alone may contribute to 'crowding' during heavy infections (Read, 1951). *N*. *malhamensis* may therefore appear more stunted in *M*. *glareolus* due to adaptation in response to limitations in physical space and may undergo morpho-anatomic variation in size according to host species.

The origin of *N*. *malhamensis* at Malham Tarn could be avian. Malham Tarn boasts a diverse population of birds of which as many as 200 species have been recorded (Sutton and Shorrock, 2008). Other species of *Notocotylus* such as *N*. *imbricatus* Looss, 1893 (Cribb, 1991) and *N*. *ephemera* Nitzsch 1817 (Gibson *et al*. 2005) have been recorded from both bird and mammalian hosts. Ablasov and Iksanov (1958) identified *N*. *noyeri* Joyeux, 1922, from the caeca of a piscivorous bird and commented that this species, that is frequently recovered from the caecum of murids, might actually be a parasite of birds (Simon-Vicente *et al*. 1985*a*). Further research into the life cycle of *N*. *malhamensis* at Tarn Fen is required. Five species of freshwater snail (*Lymnaea peregra, Lymnaea truncatula, Anisus leucostoma, Psidium* sp. and *Potamopygrus antipodarum*) have been repeatedly sampled from Tarn Fen throughout the study period, but to date no snails infected with *N*. *malhamensis* have been identified, although it is likely that one of these snail species is the intermediate host at Tarn Fen.

Confirmation of a new species can be simplified by combining the use of modern molecular tools with the classical approach of species identification. Currently, DNA sequences for the genus *Notocotylus* are poorly represented with only 5 sequences (3 for 28S rDNA, 2 for 18S rDNA) available in NCBI. Furthermore, 2 of the 3 28S sequences can only be matched with *Notocotylus* at the genus level, as they were derived from incompletely identified larval material. This genus fully illustrates the value of molecular differentiation in conjunction with classical identification for genera where differences in morphology are minute and many life cycles are incompletely understood. Reports that include the DNA sequence of a common gene in addition to a full morphological description will be beneficial for future workers, in particular if we are to record new parasite occurrences.

ACKNOWLEDGEMENTS

This project was funded by the University of Salford Ph.D. Studentship scheme. We would like to thank The National Trust for granting the licence which enabled us to carry out this work and all staff at Malham Tarn FSC field centre, in particular the current warden Mike Cawthorn and Kate Martin for support and assistance during the sampling period. We would also like to thank Professor Richard Birtles and Professor Jerzy Behnke for advice provided during species identification and Dr Belgees Boufana and Mr Tony Bodell for technical advice. We would furthermore like to thank David Hodgson for providing important information and finally Jaroslav Bajnok and Michelle Dasic for assistance during the summer sampling and the many undergraduate and post-graduate students who contributed to the fieldwork during the autumn sampling period.

REFERENCES

Ablasov, N. A. and Iksanov, K. I. (1958). Trematode fauna of piscivorous birds of Kirgizia. Rab. Gelm. 80-let. Skrjabin. Izd. AN SSSR, Moskva, 15–22 (in Russian).

Abu-Madi, M. A., Behnke, J. M., Lewis, J. W. and Gilbert, F. S. (2000). Seasonal and site specific variation in the component community structure of intestinal helminths in *Apodemus sylvaticus* from three contrasting habitats in south-east England. *Journal of Helminthology* **74**, 7–15.

Allan, J. C., Craig, P. S., Sherington, J., Rogan, M. T., Storey, D. M., Heath, S. and Iball, K. (1999). Helminth parasites of the wild rabbit *Oryctolagus cuniculus* near Malham Tarn, Yorkshire, UK. *Journal of Helminthology* **73**, 289–294.

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press

Barker, F. D. (1915). Parasites of the American Muskrat (*Fiber zibethicus*). *Journal of Parasitology* **1**, 184–197.

Barton, D. P. and Blair, D. (2005). Family Notocotylidae Lühe, 1909. In *Keys to the Trematoda, Vol. 2* (ed. Jones, A., Bray, R. A. and Gibson, D. I.), pp. 383–396. Cabi Publishing: Wallingford, Oxon, London, UK.

Baylis, H. A. (1928*a*). A new species of *Notocotylus* (Trematoda), with some remarks on the genus. *The Annals and Magazine of Natural History, 10th Series* **2**, 582–585.

Baylis, H. A. (1928*b*). Records of some parasitic Worms from British Vertebrates. *The Annals and Magazine of Natural History, 10th Series* **3**, 329–343.

Baylis, H. A. (1936). A new species of *Notocotylus* (Trematoda), from the water rail. *Annals and Magazine of Natural History, 10th Series* **17**, 474–477.

Baylis, H. A. (1939). Further records of Parasitic Worms from British Vertebrates. *The Annals and Magazine of Natural History, 11th Series* **23**, 473–798.

Behnke, J. M., Eira, C., Rogan, M., Gilbert, F. S., Torres, J., Miquel, J. and Lewis, J. W. (2009). Helminth species richness in wild wood mice, *Apodemus sylvaticus*, is enhanced by the presence of the intestinal nematode *Heligmosomoides polygyrus*. *Parasitology* **136**, 793–804.

Behnke, J. M., Lewis, J. W., Mohd Zain, S. N. and Gilbert, F. S. (1999). Helminth infections in *Apodemus sylvaticus* in Southern England: interactive effects of host age, sex and year on the prevalence and abundance of infections. *Journal of Helminthology* **73**, 31–44.

Beverley-Burton, M. (1961). Studies on the trematoda of British freshwater birds. *Proceedings of the Zoological Society of London* **137**, 13–39.

Beverley-Burton, M. (1972). Helminths from wild Anatids in Great Britain. *Journal of Helminthology* **46**, 345–355.

Bhalerao, G. D. (1935). On two new monostomes (Trematoda) from avian hosts in British India. *Indian Journal of Veterinary Science and Animal Husbandry* **5**, 49–63.

Bhutta, M. S. and Khan, D. (1975). *Digenetic trematodes of vertebrates from Pakistan*. Bulletin of the Department of Zoology, University of the Panjab (new series), article 8: Lahore, Pakistan.

Bisset, S. (1977). *Notocotylus tadornae* n.sp. and *Notocotylus gippyensis* (Beverley-Burton, 1958) (Trematoda: Notocotylidae) from waterfowl in New Zealand: morphology, life history and systematic relations. *Journal of Helminthology* **51**, 365–372.

Brooks, D. R. and Heard, R. W., III (1977). Parasites of the Clapper Rail, *Rallus longirostris* Boddaert. III. Description of *Notocotylus schmidti* sp. n. *Proceedings of the Helminthological Society of Washington* **44**, 63–65.

Chaisiri, K., Morand, S. and Ribas, A. (2011). *Notocotylus loeiensis* N. sp. (Trematoda: Notocotylidae) from *Rattus losea* (Rodentia: Muridae) in Thailand. *Parasite (Paris: France)* **18**, 35–38.

Cribb, T. H. (1991). Notocotylidae (Digenea) from the Australian water rat *Hydromys chrysogaster* Geoffroy, 1804 (Muridae). *Systematic Parasitology* **18**, 227–237.

Deshmukh, A. L. (1985). A new species of *Notocotylus* (Trematoda: Notocotylidae) from a Cattle Egret, *Bubulcus ibis*. *Indian Journal of Parasitology* **9**, 251–252.

Dubois, G. (1951). Etude des trematodes nord-americains de la collection E. L. Schiller et revision de genre *Notocotylus* Diesing, 1839. *Bulletin de la Societe Neuchateloise des Sciences Naturelles* **74**, 42–76.

Dubois, G. and Vigueras, J. P. (1953). *Notocotylus lopezneyrai* n. sp. (Trematoda, Notocotylidae), parasito del intestino des *Rosthramus sociabilis* levis Friedmann (Aves). *Memorias de la Sociedad Cubana Hisoria Natural* **21**, 251–255.

Duthoit, C. M. G. (1931). A new species of the trematode genus *Notocotylus*. *Annals and Magazine of Natural History* **7**, 290–293.

El-Naffar, M. K. and Khalifa, R. (1983). *Notocotylus gallinulae* sp.n. (Trematoda, Notocotylidae), a new digenetic trematode from the Egyptian moorhen *Gallinula Chloropus Chloropus* (L.) *Acta Parasitologica polonica* **28**, 247–252.

Elton, C., Ford, E. B. and Baker, J. R. (1931). The health and parasites of a Wild Mouse population. *Proceedings of the Zoological Society of London* **101**, 657–721.

Flores, V. and Brugni, N. (2005). *Notocotylus biomaphalariae* n. sp (Digenea: Notocotylidae) from *Biomphalaria peregrine* (Gastropoda: Pulmonata) in Patagonia, Argentina. *Systematic Parasitology* **61**, 207–214.

Gibson, D. I., Bray, R. A. and Harris, E. A. (Compilers) (2005). *Host-Parasite Database of the Natural History Museum, London*. Available Internet: www.nhm.ac.uk [Accessed 08/08/2011].

Ginetsinskaya, T. A. and Naumov, D. V. (1958). The helminth fauna of some species of Charadriiformes in the white sea. In *Papers on Helminthology Presented to Academician K. I. Skryabin on his 80th Birthday*. pp. 99–108. Moscow, USSR.

Gupta, P. C. and Gupta, S. P. (1976). On two trematodes of the genus *Notocotylus* Diesing, 1839, from avian hosts of Kanpur. U. P. *Indian Journal of Zootomy* **17**, 1–4.

Gupta, P. C. and Gupta, S. P. (1977). Two new avian trematodes. *Psilotrema nettapusi* n. sp. and *Notocotylus kanpurensis* n. sp. from Kanpur. *Indian Journal of Parasitology* **1**, 133–135.

Gupta, P. C. and Singh, R. B. (1983). On four new species of the genus *Notocotylus* Diesing, 1839 (Digenea: Notocotylidae) from avian hosts of Uttar Pradesh. *Kanpur University Research Journal* **4**, 27–35.

Gupta, P. D. (1970). Fauna of Rajastan, India. Part 8. Trematoda. *Records of the Zoological Survey of India* **62**, 171–190.

Gupta, V. and Jehan, A. (1977). Some trematodes from avian hosts of India. *Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico, Serie Zoologia* **48**, 13–26.

Gurr, G. T. (1963). *Biological Staining Methods*, 7th Edn. George T. Gurr Ltd, London, UK.

Hanelt, B. (2009). Hyperparasitism by *Paragordius varius* (Nematomorpha: Gordiida) Larva of Monostome Redia (Trematoda: Digenea). *Journal of Parasitology* **95**, 242–243.

Harrah, E. C. (1922). *Notocotylus urbanensis*. North American monostomes primarily from freshwater hosts. *Illinois Biological monographs* **7**, 51–53.

Harwood, P. D. (1939). Notes on Tennessee helminths. IV. North American trematodes of the subfamily Notocotylinae. *Journal of the Tennessee Academy of Science* **14**, 421–437.

Herber, E. C. (1942). Life history studies of two trematodes of the sub family Notocotylinae. *Journal of Parasitology* **28**, 179–196.

Hughes, J. M., Thomasson, D. T., Craig, P. S., Georgin, S., Pickles, A. and Hide, G. (2008). *Neospora canimum*: detection in wild rabbits and coinfection with *Toxoplasma gondii* by PCR analysis. *Experimental Parasitology* **120**, 255–260.

Hughes, J. M., Williams, R. H., Morley, E. K., Cook, D. A. N., Terry, R. S., Murphy, R. G., Smith, J. E. and Hide, G. (2006). The prevalence of *Neospora caninum* and co-infection with *Toxoplasma gondii* by PCR analysis in naturally occurring mammal populations. *Parasitology* **132**, 29–36.

Hsu, P. K. (1954). A new species of *Notocotylus* from Canton (Trematoda: Notocotylidae). *Acta Zoologica Sinica* **6**, 117–122.

I-ping, S. (1965). On some digenetic trematodes from small mammals including descriptions of two new species. *Acta Zootaxonomica Sinica* (04). Available internet: http://en.cnki.com.cn/Article_en/CJFDTOTAL-DWFL196504003.htm [Accessed: 31/07/11].

Joyeux, C. H. (1922). Recherches sur les Notocotyles. *Bulletin de la Societe de Pathologie Exotique* **15**, 331–339.

Kanev, I., Vassilev, I., Dimitrov, V. and Radev, V. (1994). Life cycle delimitation and redescription of *Catatropis verrucosa* (Frolich, 1789) Odhner, 1905 (Trematoda, Notocotylidae). *Systematic Parasitology* **29**, 133–148.

Kinsella, J. M. (1971). Growth, development, and intraspecific variation of *Quinqueserialis quinqueserialis* (Trematoda: Notocotylidae) in rodent hosts. *The Journal of Parasitology* **57**, 62–70.

Kinsella, J. M. and Tkach, V. V. (2005). *Notocotylus fosteri sp*. Nov. (Trematoda, Notocotylidae) from the rice rat, *Oryzomys palustris* in Florida. *Acta Parasitologica* **50**, 194–198.

Ku, C. (1937). Two new trematodes of the genus *Notocotylus*, with a key to the species of the genus. *Peking Natural History Bulletin* **12**, 113–122.

Lal, M. B. (1935*a*). A review of the genus *Notocotylus* with description of a new trematode parasite of *Mareca Penelope* from Lucknow. *Proceedings of the Indian Academy of Sciences, Section B* **2**, 457–466.

Lal, M. B. (1935*b*). On the morphology of a new species of monostome of the genus *Notocotylus* Diesing, 1839. *Proceedings of the Indian Academy of Sciences, Section B* **2**, 419–423.

Lange, R. and Staaland, H. (1970). Adaptations of the caecum-colon structure of rodents. *Comparative Biochemistry and Physiology* **35**, 905–919.

Murrills, R. J., Southgate, V. R. and Reader, T. A. J. (1988). Studies on the invasion of *Notocotylus attenuatus* (Notocotylidae: Digenea) into its snail host *Lymnaea peregra: in vivo* observations 1 and 7 days post infection. *International Journal for Parasitology* **18**, 161–166.

Nath, D. and Pande, B. P. (1963). On a monostome of Indian Domestic Fowl with remarks on the genus *Notocotylus* Diesing 1839. *Parasitology* **53**, 45–48.

Noble., A. E. (1933). Two new trematodes from the American coot. *Transactions of the American microscopical Society* **52**, 353–360.

Odening, K. (1964). Zur trematoden fauna von *Nettapus C. moromandelianus* in Indien. *Angewandte Parasitologie* **5**, 228–241.

Pfunder, M., Holzgang, O. and Frey, J. E. (2004). Development of microarray-based diagnosis of voles and shrews for use in biodiversity monitoring studies, and evaluation of mitochondrial cytochrome oxidase I vs. cytochrome *b* as genetic markers. *Molecular Ecology* **13**, 1277–1286.

Pike, A. W. (1969). Observations on the life cycles of *Notocotylus triserialis* Diesing, 1839, and *N. imbricatus* (Looss, 1839) sensu Szidat, 1935. *Journal of Helminthology* **43**, 145–165.

Qiongzhang, L. (1988). On the trematode fauna from the domestic geese and ducks in Lianhua County, Jiangxi Province. *Chinese Journal of Animal and Veterinary Sciences*. http://en.cnki.com.cn/articleen/CJFDTOTAL-XMSY198802013.htm

Qiongzhang, L. (1992). A new species and two new records of the genus *Notocotylus* (Trematoda: Notocotylidae). *Acta Veterinaria et Zootechnica Sinica* **23**, 262–266.

Read, C. P. (1951). The crowding effect in tapeworm infections. *The Journal of Parasitology* **37**, 174–178.

Rogan, M. T., Craig, P. S., Hide, G., Heath, S., Pickles, A. and Storey, D. M. (2007). The occurrence of the trematode *Plagiorchis muris* in the wood mouse *Apodemus sylvaticus* in North Yorkshire, UK. *Journal of Helminthology* **81**, 57–62.

Rudolphi, C. A. (1809). *Entozoorum sive vermium intestinalium historia naturalis, vol 2*. Amstelaedami: Sumtibus tabernae librariae et atrium. P328-329. http://www.biodiversitylibrary.org/item/50355#page/334/mode/1up

Rudolphi, C. A. (1819). *Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi*. Berolini: Sumtibusa Rucker. http://www.archive.org/stream/entozoorumsynops00rudo#page/86/mode/2up

Sargent, G. and Morris, P. (2003). *How to find and identify Mammals*. The Mammal Society, London, UK.

Shaldybin, L. S. (1965). A new trematode of *Clethrionomys glareolus*. *Gelmintologia Sbornik* **4**, 93–105 (in Russian).

Simon-Vicente, F., Mas-Coma, S., Lopez-Roman, R., Tenora, F. and Gallego, J. (1985*a*). Review of *Notocotylus* species (Trematoda: Notocotylidae) parasitizing rodents in Europe. *Folia Parasitologica (Praha)* **32**, 21–33.

Simon-Vicente, F., Mas-Coma, S., Lopez-Roman, R., Tenora, F. and Gallego, J. (1985*b*). Biology of *Notocotylus neyrai* Gonzalez-Castro, 1945 (Trematoda). *Folia Parasitologica (Praha)* **32**, 101–111.

Singh, K. S. (1954). Some trematodes collected in India. *Transactions of the American Microscopical Society* **73**, 202–210.

Skrjabin, K. I. (1953). *Trematodes of Animal and Man, Principles of Trematodology Vol*. 8. Publishing House of the USSR Academy of Science, Moscow, USSR.

Stunkard, H. W. (1960). Studies on the morphology and life history of *Notocotylus minutus* n. sp. a digenetic trematode from ducks. *Journal of Parasitology* **46**, 803–809.

Stunkard, H. W. (1966). The morphology and life-history of *Notocotylus atlanticus* n. sp. A digenetic trematode of eider ducks, *Somateria mollissima* and the designation *Notocotylus duboisi* nom. nov for *Notocotylus imbricatus* (Looss, 1839) Szidat, 1935. *The Biological Bulletin* **131**, 501–515.

Stunkard, H. W. (1967). The morphology, life history, and systematic relations of the digenetic trematode, *Uniserialis breviserialis* sp. nov., (Notocotylidae), a parasite of the bursa fabricius of birds. *The Biological Bulletin* **132**, 266–276.

Sutton, R. and Shorrock, B. (2008). *Checklist of the Birds of Malham Tarn*. Malham Tarn Field Centre, UK.

Tenora, F., Henttonen, H. and Haukisalmi, V. (1983). On helminths of rodents in Finland. *Annales Zoologici Fennici* **20**, 37–45.

Thomasson, D., Wright, E. A., Hughes, J. M., Dodd, N. S., Cox, A. P., Boyce, K., Gerwash, O., Abushahma, M., Lun, Z. R., Murphy, R. G., Rogan, M. T. and Hide, G. (2011). Prevalence and co-infection of *Toxoplasma gondii* and *Neospora caninum* in *Apodemus sylvaticus* in an area relatively free of cats. *Parasitology* **138**, 1117–1123.

Tkach, V., Pawlowski, J. and Mariaux, J. (2000*b*). Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. *International Journal for Parasitology* **30**, 83–93.

Tkach, V., Pawlowski, J., Mariaux, J. and Swiderski, Z. (2001). Molecular phylogeny of the suborder Plagiorchiata and its position in the system of Digenea. In *Interrelationships of the Platyhelminthes* (ed. Littlewood, D. T. J. and Bray, R. A.), pp. 186–193. Systematics Association Special Publication, Taylor & Francis, London, UK.

Tkach, V. V., Pawlowski, J. and Sharpilo, V. P. (2000*a*). Molecular and morphological differentiation between species of the *Plagiorchis vespertilionis* group (Digenea, Plagiorchiidae) occurring in European bats, with a re-description of *P. vespertilionis* (Müller, 1780). *Systematic Parasitology* **47**, 9–22.

Tubangui, M. A. (1932). Trematode parasites of Philippine vertebrates. V. Flukes from birds. *Philippine Journal of Science* **47**, 369–404.

Vassilev, I. and Kanev, I. (1984). Morphology and biology of Notocotylus zduni Chiaberachvili and Djavelidze 1966 in Bulgaria. In *Faunae, Taxonomy and Ecology of Helminths on Birds* (ed. Vassilev, I.), pp. 66–74. Publishing House of the Bulgarian Academy of Sciences: Sofia.

Webber, R. A., Rau, M. E. and Lewis, D. J. (1987). The effects of *Plagiorchis noblei* (Trematoda: Plagiorchiidae) metacercariae on the susceptibility of *Aedes aegypti* larvae to predation by guppies (*Poecilia reticulata*) and meadow voles (*Microtus pennsylvanicus*). *Canadian Journal of Zoology* **65**, 2346–2348.

Yamaguti, S. (1934). Studies on the helminth fauna of Japan. Part 3. Avian trematodes II. *Japanese Journal of Zoology* **5**, 543–583.

Yamaguti, S. (1958). *Systema helminthum*, Vol. 1, Parts 1 and 2. *Digenetic Trematodes of Vertebrates*. Interscience Publishers, New York, USA and London, UK.

Ye, Y., Qiu, M., Wen, T., Li, S. and Li, G. (1956). *Shanghai mice, a parasitic new species of trematode Notocotylus ratti sp. n.* (Trematoda) *a description of the form*. Available internet: http://wenku.baidu.com/view/54455ec1d5bbfd0a79567383.html [Accessed: 31/07/11] (in Chinese with additional Russian description).

https://doi.org/10.1017/S0031182012000911 Published online by Cambridge University Press