

***Paraconcinnum leirsi* n.sp. (Trematoda: Dicrocoeliidae) from rodents in Tanzania and its phylogenetic position within the dicrocoeliids**

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The trematode *Paraconcinnum leirsi* n.sp. (Dicrocoeliidae) is described from two rodent species, the African gerbil, *Gerbilliscus vicinus*, and the spiny mouse, *Acomys spinosissimus*, from Tanzania. It differs from the description of *P. hylomisci* found in the Stella wood mouse, *Hylomyscus stella*, in the Democratic Republic of Congo. Molecular studies were performed by sequencing the near complete 18S rDNA gene of the fluke to assess its phylogenetic position within the Dicrocoeliidae. The resulting estimate of evolutionary divergence between the fluke and other dicrocoeliids was $1.60 \pm 0.22\%$ base differences per site. The phylogenetic analysis shows that the fluke is a new species within Dicrocoeliidae falling in a cluster with the genera *Corrigia*, *Lyperosomum*, *Concinnum* and *Eurytrema* although phylogenetic relationships among these genera are not well resolved. This is the first dicrocoeliid reported from rodents in eastern Africa.

Key words: *Acomys*, Dicrocoeliidae, *Gerbilliscus*, *Paraconcinnum*, 18S rDNA gene.

INTRODUCTION

The family Dicrocoeliidae Loos, 1899 comprises over 400 species. It is one of few digenean trematode families that complete their life cycles in the terrestrial environment, usually *via* a snail and an arthropod as first and second intermediate hosts respectively (Pojmaška 2008). These trematodes are mainly found in the bile ducts and gall bladders of birds and placental mammals and more rarely in reptiles and marsupials (Pojmaška 2008).

The helminth parasites of African rodents are poorly known and have only been investigated in a fragmentary way across the continent. The genus *Paraconcinnum* Vassiliadès & Richard, 1970 (family Dicrocoeliidae) is represented by *P. hylomisci* Vassiliadès & Richard, 1970, and found in the murid *Hylomyscus stella* (Thomas, 1911) from the Democratic Republic of Congo and later reported by Quentin (1989) in Central African Republic. The literature review of helminths of African mammals (from 1800 to 1967) by Canaris & Gardner (2003) does not include dicrocoeliids of rodents. Yet, to date *Lyperosomum africanum* Baer, 1957 in *Lophuromys sikapusi* (Temminck, 1853) and *Malacomys longipes edwardsi* (Milne-Edwards,

1877); *Dicrocoelium ivoriense* Baer, 1971 in *Praomys tullbergi* (Thomas, 1894); *Hemixenotrema hunkeleri* Baer, 1971 in *Praomys tullbergi* and *Hybomys trivirgatus* (Temminck, 1853) and *Euparadistomum lophuromidis* Baer, 1971 from *Lophuromys sikapusi*, all reported from the Ivory Coast by Baer (1957, 1971) from the family Dicrocoeliidae have been described in African rodents.

In Tanzania, studies of helminths of rodents (excluding dicrocoeliids) are limited to those of the Gambian pouched rat, *Cricetomys gambianus* Waterhouse, 1840 (Khalil 1973; Gibbons *et al.* 1990) despite the fact that 110 rodent species are found in Tanzania (Senzota *et al.* 2012). A study of helminths of rodents by Ouge *et al.* (1997) in central Kenya reported no dicrocoeliids in any of the studied rodents (*Mastomys natalensis* Smith, 1834 ($n = 14$); *Lemniscomys striatus* (Linnaeus, 1758) ($n = 7$); *Arvicanthis niloticus* (Geoffrey, 1803) ($n = 6$); *Mus minutoides* (Smith, 1834) ($n = 1$), and *Gerbilliscus robustus* (Cretzschmar, 1826) ($n = 1$)).

In order to gain insight into the diversity and composition of the helminth community in rodents, we carried out a study in the Morogoro region. A trematode belonging to the genus *Paraconcinnum* Vassiliadès & Richard, 1970 was recovered and showed morphological and metrical characteristics

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that pointed to a new species. Alongside the morphological and metrical classification we performed a molecular study using the 18S rDNA gene which placed this new species within the Dicrocoeliidae.

MATERIALS & METHODS

Field work

Field sampling took place in mid-December 2009. The study area was located in Maguha in the north of the Morogoro Region, Tanzania (S6°17.187', E37°11.890'). In total, eight grids of 1 ha were set in fallow lands with 100 Sherman traps each. Dissections were performed in a field laboratory to recover fresh material for optimal fixation of the digeneans. The four sampled rodent species were the spiny mouse, *Acomys spinosissimus* Peters, 1852 ($n = 4$), the red rock rat, *Aethomys chrysophilus* (De Winton, 1897) ($n = 2$), the multimammate mouse, *Mastomys natalensis* ($n = 11$) and the African gerbil, *Gerbilliscus vicinus* (Peters, 1878) ($n = 11$). The field identification (based on external but not cranial measurements) of the rodent species was later confirmed by sequencing the cytochrome b gene using L7 and H6 primers (Montgelard *et al.* 2002).

Morphological analysis

Trematodes were isolated from pancreatic ducts and a portion was preserved in 70% ethanol for molecular studies and the remainder were fixed in Bouin's solution for morphological studies. The latter were dehydrated through a series of graded ethanols, cleared with xylene and mounted in Canada balsam directly or after staining in Semichon acetocarmine. Measurements of the holotype and eight paratypes were taken using a microscope-mounted camera. Illustrations were drawn with the aid of a camera lucida (Figs 1a & 2).

Molecular analysis

Total DNA was extracted from a single worm using the DNeasy tissue kit (Qiagen) and eluted in 50 μ l of AE buffer. Since the number of available sequences from the Dicrocoeliidae is limited in public databases, we also extracted DNA from a specimen of *Corrigia vitta* (Dujardin, 1845) previously isolated from the rodent *Apodemus* sp. from Montseny Natural Park (Spain). Polymerase chain reaction (PCR) amplification was performed in 25 μ l volume containing 0.2 μ M of each primer, 0.2 mM of each dNTP, 2.5 mM MgCl₂, 1X DreamTaq buffer, 0.6 unit of DreamTaq DNA Poly-

merase (Fermentas) and 3 μ l of DNA template. Thermal cycling profile was as follows: an initial denaturing step at 94°C for 3 min, followed by 40 cycles at 94°C for 30 s, 54°C for 30 s and 72°C for 2 min and ending with an extension step of 72°C for 10 min. Near-complete 18S rDNA sequences (~1800 bp) were amplified using primers Worm-A and Worm-B (Littlewood & Olson, 2001). PCR products were visualized on a 1.4% agarose gel, and were purified and sequenced by VIB Genetic Service Facility (University of Antwerp, Belgium) using the same primers that generated the PCR products together with two internal primers: 1270R and 930F (Littlewood & Olson, 2001). The two new partial 18S rDNA sequences have been deposited in GenBank (AN: JN831598 and JN831599).

Sequences were aligned using Clustal W (Thompson *et al.* 1994) together with all Dicrocoeliidae 18S rDNA available in GenBank: *Concinnum ten* (Yamaguchi, 1939) (AB521801), *Brachylecithum lobatum* (Railliet, 1900) (AY222144), *Lyperosomum collurionis* (Skrjabin & Issatschikov, 1927) (AY222143), *Dicrocoelium chinensis* (Sudarikov and Ryjikov, 1951) Tang & Tang, 1978 (syn: *Dicrocoelium orientalis* (Sudarikov and Ryjikov, 1951) and *Dicrocoelium suppereri* (Sudarikov and Ryjikov, 1951) Hinaidy, 1983) (EF547131), *D. dendriticum* (Rudolphi, 1819) (Y11236), *Eurytrema coelmaticum* (Giard & Billet, 1892) (DQ401035) and *E. pancreaticum* (Janson, 1889) (DQ401034). *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 (Encyclometridae; AY222142) and *Orchipedum tracheicola* Braun, 1901 (Orchipedidae; AJ287551) were used as outgroups. The final alignments included 1769 nucleotides. Distances were computed using the p-distance method in MEGA 5.05 (Tamura *et al.* 2011). jModelTest 0.1.1 (Posada 2008; Guindon & Gascuel 2003) was used to evaluate the fit of 88 nested models of nucleotide substitution to the data using the Akaike Information Criterion (AIC). The AIC indicated that the model best fitting the data is the general time-reversible model with a proportion of invariables sites (GTR+I). Phylogenetic reconstruction was performed by Maximum Likelihood (ML) with the PhyML online web server (Guindon *et al.* 2005). Branch supports were evaluated by a non-parametric bootstrap analysis (1000 replicates) and an approximate likelihood-ratio test (aLRT) relying on a non-parametric Shimodaira-Hasegawa (SH)-like procedure (Guindon *et al.* 2010).

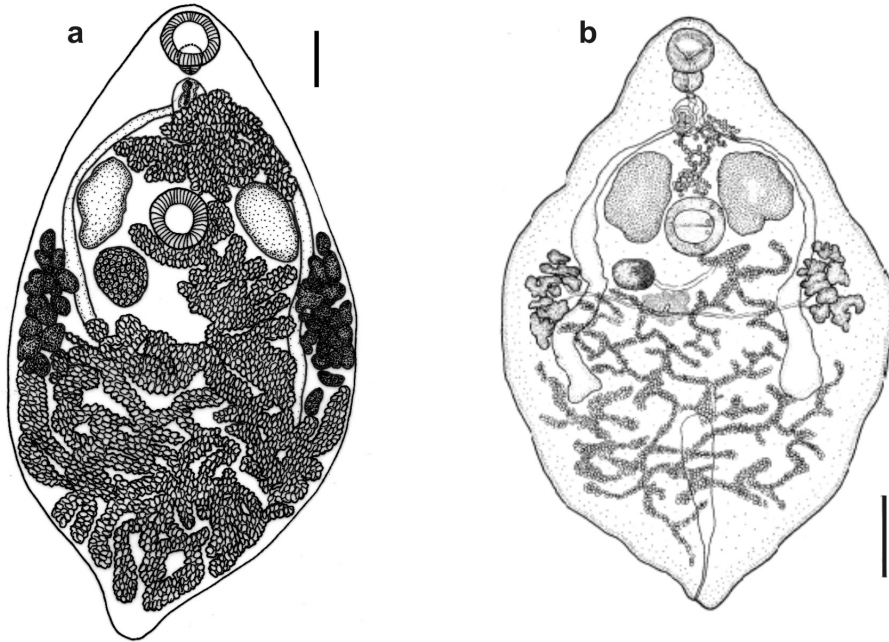


Fig 1. a, *Paraconcinnum leirsi* n.sp. Holotype, ventral view of whole individual. Scale bar = 400 μ m. b, *Paraconcinnum hylomisci* (from the original description of Vassiliadès & Richard (1970)). Scale bar = 500 μ m.

RESULTS & DISCUSSION

Paraconcinnum leirsi n.sp., Fig. 1a & 2

Description (n = 9)

Dicrocoeliidae, with characteristics of genus *Paraconcinnum* according to Pojmaška (2008). Body elongate, lancet-shaped, 4086 μ m (3220–4829 μ m) long, tegument not spined. Body width at level of the acetabulum 1949 μ m (1548–2281 μ m); widest at vitellaria level 2302 μ m (1786–2908 μ m). Anterior and posterior ends bluntly pointed. Oral sucker subterminal; 363 μ m (294–431 μ m) long; 440 μ m (392–484 μ m) wide. Acetabulum closer to anterior extremity than to equator, 436 μ m (394–458 μ m) long; 437 μ m (363–503 μ m) wide. Distance anterior end to beginning ventral sucker 1215 μ m (1024–1473 μ m). Sucker ratio (maximum diameter oral sucker–ventral sucker 0.99 (0.77–1.16 μ m)). Pharynx 182 μ m (158–218 μ m) long; 212 μ m (184–263 μ m) wide. Oesophagus totally covered by cirrus sac. Caeca descending by the internal border of vitellogen glands, extending to the second third of body. Genital pore opening immediately posterior to oral sucker. Testes longer than wide, elliptical, with irregular contour, intracaecal, at level of centre of acetabulum, right testis: 604 μ m (324–899 μ m) long; 348 μ m (200–517 μ m) wide; left

testis: 522 μ m (324–908 μ m) long; 419 μ m (224–704 μ m) wide. Cirrus sac elongate, 295 μ m (236–382 μ m) long; 165 μ m (114–287 μ m) wide. Orientation of cirrus sac variable, from parallel to perpendicular with respect to the body. Ovary oval, 378 μ m (258–472 μ m) long; 338 μ m (263–413 μ m) wide, closer to right testis. Uterus

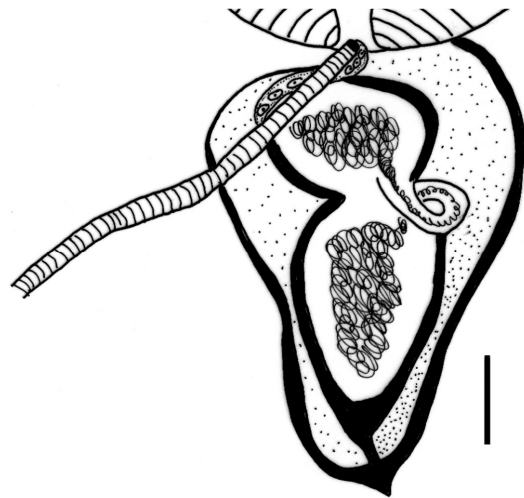


Fig. 2. *Paraconcinnum leirsi* n.sp. Cirrus sac. Scale bar = 50 μ m.

descending to posterior part of body and then ascending occupying intra-testicular space, once past testis occupies anterior part of body to genital pore. Vitellaria begin at level of distal half of testes; completely extra-caecal; right vitelline gland 966 μm (703–1196 μm) long; maximum width 357 μm (212–443 μm); distance from right follicles to end of body 1626 μm (1112–2300 μm); distance from right follicles to beginning of body on the right 1611 μm (1376–1835 μm); number right follicles (25–11). Left vitelline gland 1144 μm (903–1463 μm) in length; maximum width 366 μm (265–484 μm); distance from left follicles to end of body 1758 μm (1351–2141 μm); distance from left follicles to beginning of body 1462.44 μm (1142–1780 μm); number left follicles (11–29). Excretory vesicle Y-shaped, excretory pore terminal. Eggs elliptical, operculate, brown in colour, 50 μm (46–54 μm) long by 26 μm (21–29 μm) wide.

Taxonomic summary

Type host

Gerbilliscus vicinus (Peters, 1878).

Other host

Acomys spinosissimus Peters, 1852.

Site of predilection

Pancreatic ducts, the dissection of biliary ducts allowed recovery of this worm, folded longitudinally.

Type locality

Maguha, Morogoro Region, eastern-central Tanzania, (S6°17.187'; E37°11.890').

Type specimens

Type specimens were deposited in 'Museu de Ciències Naturals de Barcelona' (Barcelona Natural Science Museum, Catalonia, Spain), codes of holotype MZB 2011-0009, and eight paratypes MZB 2011-0010–0017.

Collection date

December 2009.

Prevalence, intensity of infection and range

Gerbilliscus vicinus 45.5%; 3.2, 2–4; *Acomys spinosissimus* 25%; 2, 2–2.

Etymology

This species is named in honour of Professor Herwig Leirs for his valuable contribution to the understanding of the ecology and biology of small African mammals.

Remarks

Paraconcinnum leirsi n.sp. differs from *P. hylomisci*, the single known species of this genus described by Vassiliadès & Richard (1970) using morphology and metrical characters. In *P. leirsi* n. sp. the testes extend posteriorly to the level of the ventral sucker, unlike in *P. hylomisci* (Fig. 1b) where they never reach posterior margin of ventral sucker. In *P. hylomisci*, the uterine fields only reach the central region of the anterior part of the body, whilst in *P. leirsi* they cover all the anterior part. *Paraconcinnum leirsi* n.sp. shows a higher number of vitelline follicles (10–29 compared with 8–10 μm in *P. hylomisci*). Ratio length/width of vitellogen glands 3:1, being 1:1 in *P. hylomisci* (according to the drawing as measurements of vitelline glands are missing in the description of *P. hylomisci*). Oral sucker longer and wider in *P. leirsi* n.sp. (294–431 μm and 392–484 μm , respectively) than in *P. hylomisci* (250–300 μm and 300–350 μm , respectively).

Phylogenetic relationship

The 18S based estimate of evolutionary divergence between *Paraconcinnum leirsi* n. sp. and other microcoeliids is $1.60 \pm 0.22\%$ (range: $1.14 \pm 0.27\%$ – $2.05 \pm 0.31\%$) base differences per site. The phylogenetic analysis places the new species within the microcoeliids, consistent with the morphological and metrical analysis. The ML tree is shown in Fig. 3. Two major clades were resolved within the microcoeliids: the first clade groups *Brachylecithum lobatum* with *Dicrocoelium* spp. The second clade, in which both members sequenced herein (*Paraconcinnum leirsi* n.sp. and *Corrigia vitta*) belong, is not fully resolved: a cluster grouping *Concinnum ten* with *Eurytrema* spp. is highly supported, but the remaining members form a polytomy. The limited number of available sequences of the 18S rDNA gene, which is the gene the most represented for the microcoeliid family in current public databases, limits further interpretation.

Concluding remarks

Our knowledge of Dicrocoeliidae in Rodentia is far from complete, as indicated by numerous recent descriptions, e.g. in Europe (Hildebrand *et al.* 2007); North America (Lamothe-Argumedo *et al.* 2005) and South America (Gardner & Pérez-Ponce de León 2002; Rivillas *et al.* 2004). In his review of the family Dicrocoeliidae, Pojmańska (2008) found that the great variability of body-shape, size and topography of the internal organs makes it diffi-

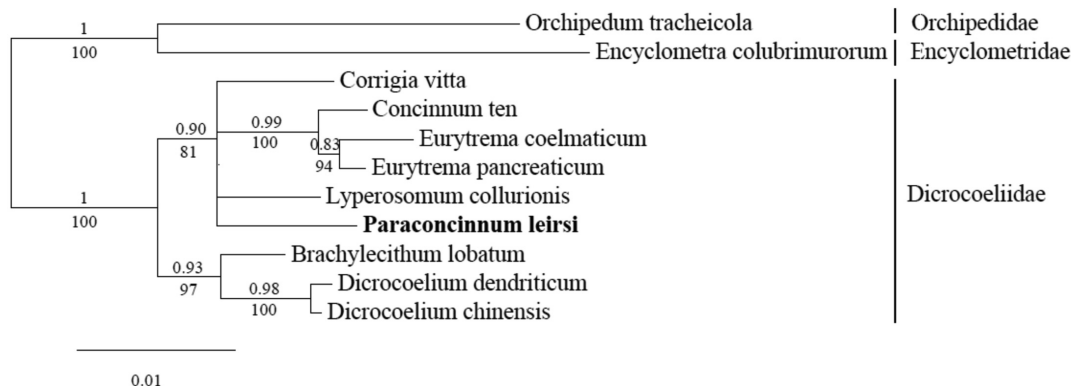


Fig. 3. Phylogenetic analyses of dicrocoeliidae trematodes based on the 18S rDNA showing the position of *Paraconcinnum leirsi* n.sp. The new species is shown in bold. Numbers on the branches represent the aLRT SH-like support and below the branch percentage bootstrap support (1000 replicates). *Encyclometra colubrimurorum* and *Orchipedum tracheicola* were used as outgroups. All sequences are taken from GenBank except *Paraconcinnum leirsi* n.sp. and *Corrigia vitta* sequenced for this study. Scale bar indicates number of substitutions per site. Branches with an aLRT support ≤ 0.75 or with a bootstrap support $< 50\%$ were collapsed.

cult to arrange this family into a clear system from higher taxa to the genus level, which has led different authors to propose several taxonomic arrangements. Molecular analyses, as in the present study, or with additional markers, would likely clarify the phylogenetic positions of the dicrocoeliid genera. *Paraconcinnum leirsi* n. sp. is the second species to have been described in this genus and the first rodent-borne dicrocoeliid described from Africa in 40 years. The first *Paraconcinnum* species, *P. hylomisci*, was reported from a rodent species, *H. stella*, that inhabits rain-forest habitats (Schlitter & Van Der Straeten 2008). In contrast, the new species, *P. leirsi* was found in two rodent species characteristic of more open and dry habitats such as miombo woodlands and grasslands (Verheyen *et al.* 2011). This habitat difference may have played a role in the speciation of the genus *Paraconcinnum*. Additional trematode surveys of rodents across these different habitats may clarify the relative influence of habitats and hosts (intermediate and final) in *Paraconcinnum* speciation.

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