Parasitology Research (2019) 118:3359–3370
https://doi.org/10.1007/s00436-019-06532-2

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

A new African species of parasitic *Dero* (Annelida, Clitellata, Naididae) in the urinary tract of reed frogs

U. Sinsch[1] · J.M. Dehling[1] · P. Scheid[1,2] · C. Balczun[2]

Received: 2 August 2019 / Accepted: 28 October 2019 / Published online: 15November 2019

Abstract
A new species of naidid oligochaete, *Dero rwandae*, detected in the bladder and the Wolffian ducts of reed frogs *Hyperolius kivuensis* from Rwanda, is described. Until now, *D. bauchiensis* was the only endoparasitic *Dero* known to infect African frogs infesting the eyes and Harderian glands. To the best of our knowledge, the finding of *D. rwandae* is the first record of an African *Dero* species infecting the urinary tract of anurans. In general morphology, the two African *Dero* parasites resemble each other, but differences in the features of ventral setae morphology exist. Parts of the mitochondrial 16S rRNA locus and the nuclear 18S and 28S rRNA loci were sequenced to assess the phylogenetic relationships to other *Dero* spp. Among those few species, that are barcoded so far, the closest relative of the new taxon is *D. superterrenus*, a free-living South American species. The species groups formerly termed subgenera *Allodero*, *Aulophorus* and *Dero* within the genus *Dero* do not represent distinct evolutionary lineages and the genus is paraphyletic including *Branchiodrilus*.

Keywords Naididae · *Dero* · Molecular phylogeny · *Dero rwandae* · Parasite · *Hyperolius kivuensis*

Introduction

Among Annelida, the few endoparasitic species are restricted to two genera (*Chaetogaster* and *Dero*) in the Naididae (Clitellata; Harman 1971; Gelder 1980). Only species of the genus *Dero* Oken 1815 use vertebrates (frogs and toads) for phoresis (species groups historically coined subgenera *Dero* and *Aulophorus*) or are endoparasites of their urinary tract or eyes (species group/subgenus *Allodero*) (Oken 1815; Stephenson 1930; Lopez et al. 1999). The taxonomic subdivision of *Dero* is still a matter of controversy. Sperber (1948) considers the three species groups as subgenera *Dero*, *Allodero* and *Aulophorus*, whereas Grimm (1988) suggested that morphological differentiation among these three subgenera warrants full genus status. Recent molecular investigation shows that the *Dero* clade includes *Aulophorus* spp. rendering a generic or subgeneric distinction as a monophyletic group invalid, but the status of the putative subgenus/genus *Allodero* remains unresolved (Erséus et al. 2017). Still, free-living *Dero* (*Allodero) hylae* (endoparasites of North American hylids) form a well-developed gut and feed upon free-living food items indicating that a free-living stage is probably a natural component in the life cycle of parasitic *Dero* (Andrews et al. 2015).

Six parasitic *Dero* species are currently assigned to the *Allodero* species group, five found in the host's urinary tract and one exclusively in the eyes (Gelder 1980; Pinder et al. 1998; Andrews et al. 2015; Morais et al. 2017). The association between frog host and worms has been considered either as parasitism (Michaelsen 1926) or commensalism (Harman 1971; Pinder et al. 1998). Recently, Andrews et al. (2015) provided evidence that the worm (*Allodero*)-frog relationship is a case of parasitism because the worms harm their hosts by rupture of the ureter, leading to death in certain cases.

The geographical distribution of parasitic *Dero* covers Africa (*D. bauchiensis* [Stephenson 1930]), the Americas (*D. floridana* Harman 1971; *D. hylae* Goodchild, 1951; *D. lutzi* [Michaelsen 1926]), Asia (*D. malayana* Stephenson 1931) and Australia (*D. litoria* Pinder et al. 1998). *Dero*

Section Editor: Christoph G. Grevelding

✉ U. Sinsch
sinsch@uni-koblenz.de

[1] Institute of Integrated Sciences, Department of Biology, University of Koblenz-Landau, Universitätsstr. 1, 56070 Koblenz, Germany

[2] Laboratory of Medical Parasitology, Department XXI (Med. Microbiology), Central Military Hospital Koblenz, Koblenz, Germany

(*Allodero*) *bauchiensis*, known from Nigeria and Mozambique, is exceptional because it is the only *Dero* infecting eyes and Harderian glands of *Phrynomantis bifasciatus* and *P. microps* (Stephenson 1930).

Here, we present the first report on an African parasitic *Dero* species, found in the urinary tract (bladder and Wolffian ducts) of the Kivu reed frog *Hyperolius kivuensis* Ahl, 1931 in Rwanda. We compare the morphological and molecular features of the specimens collected in Rwanda with those of the *Dero* species known to occur in Africa (Brinkhurst 1966, 1970; Grimm 1987, 1988). Currently, nine free-living species from sub-Saharan Africa are known, but there is no record for Rwanda. Aims of this study are (1) to provide evidence for the undescribed status of the *Dero* species collected from *H. kivuensis*; (2) to provide a formal description of the new species; and (3) to discuss the generic structure with respect to the putative subgenus *Allodero*.

Material and methods

Sampling

A total of 76 male and 13 female Kivu reed frogs (*Hyperolius kivuensis*) were collected from the cultivated wetlands ('marais', 2.60059° S, 29.75964° E, 1645 m a.s.l.) near Huye (Butare), Rwanda (Sinsch et al. 2012). Collecting dates were October 2, 2015 ($n = 18$), January 10, 2017 ($n = 35$) and March 19, 2017 ($n = 26$), all within the rainy period at this locality. At the same locality and dates, we collected 95 male and 5 female common reed frogs (*H. viridiflavus*). All specimens were euthanized immediately after collection by immersion into a 2% solution of tricaine methane-sulfonate (MS 222). Each individual was sexed and snout-vent length (SVL, distance between snout tip and cloaca) measured to the nearest 0.1 mm using a calliper. The body cavity, digestive tract, lungs, kidneys and bladder were subsequently examined macroscopically and light-microscopically for the presence of endoparasites. We used a femur bone of each individual for skeletochronological age determination. Laboratory protocols followed the standard methods of skeletochronology (Sinsch 2015). The samples were embedded in HistoresinTM (JUNG) and stained with 0.5% cresylviolet (Sinsch and Dehling 2017). Diaphysis was cross-sectioned at 12 μm using a JUNG RM2055 rotation microtome. Cross sections were examined light-microscopically for the presence of growth marks at magnifications of × 400 using an OLYMPUS BX 50. We distinguished strongly stained lines of arrested growth (LAGs) in the periosteal bone, separated by faintly stained broad growth zones. The number of LAGs corresponded the years of life (Sinsch and Dehling 2017). Carcasses of frogs were fixed in 10% buffered formalin, transferred to 70% ethanol for long-term storage and deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK; Hk, 102849-102962, Hv, 102963-103062).

The detected endoparasites included several species of nematodes, trematodes and cestodes, but in this study, we focus exclusively on the finding of parasitic annelids in the bladder and kidneys of six frogs collected in March at the end of the rainy period. Eight annelid specimens were extracted from the Wolffian ducts of a male reed frog and transferred to an Eppendorf tube with 1.5 ml ethanol for molecular identification. Another 16 worms parasitizing the same frog were fixed in 10% buffered formalin and stored for morphological examination. The same procedure was applied to another 34 annelids collected from the remaining five infected frogs.

Morphological examination of annelid samples

We succeeded in identifying the annelid worms from the urinary tract of *H. kivuensis* as Naididae based on the morphological criteria summarized in Harman (1980). Allocation of specimens to the genus *Dero* (comprising the three species groups) bases on features considered diagnostic for the genus (Sperber 1948; Grimm 1988): presence of dorsal setae from segments IV or VI onwards, bifid or pectinate needle setae and bundles of fan-shaped ventral needle setae. To further assess the taxonomic status of our specimens, we compared the bundles of hair and needle setae with those of the nine free-living *Dero* species known to occur in Africa and all parasitic *Dero* species (Sperber 1948; Grimm 1986). We chose randomly a sample of 13 complete specimens out of 54 preserved individuals for morphometric measurements. We examined whole specimens mounted on an Olympus BX 50 microscope equipped with a high-resolution camera Olympus DP20 to record the following quantitative features of each specimen: (1) body length (mm); (2) maximum body width (mm); (3) total number of segments; (4) length of dorsal hair seta and needle (μm); (5) number of ventral setae per bundle and their length at the anterior segments (II–V); (6) number of ventral setae per bundle and their length at the posterior segments (≥ VI).

Nucleic acid extraction and polymerase chain reactions

Three morphologically identified ethanol-fixed worms were separately incubated in 1.5 ml reaction tubes, with open cap, at 40 °C in a Thermomixer (Eppendorf, Germany) until the ethanol was completely evaporated. The whole-body samples were mixed with 180 μl ATL buffer, 20 μl proteinase K and incubated at 56 °C until the specimens were completely lysed, and then further processed following the protocol for DNA purification from tissues of the QIAamp DNA mini kit (Qiagen, Germany). The extracted DNA was used to amplify

Table 1 Primers used for amplification and sequencing

Gene	Primer	Sequence	Reference
16S	Ann16SF	5′-GCGGTATCCTGACCGTRCWAAGGTA-3′	Sjölin et al. (2005)
	Ann16SR	5′-TCCTAAGCCAACATCGAGGT GCCAA-3′	
18S	TimA	5′-AMCTGGTTGATCCTGCCAG-3′	Norén and Jondelius (1999)
	1100R	5′-GATCGTCTTCGAACCTCTG-3′	
28S	28SCI	5′-ACCCGCTGAATTTAAGCAT-3′	Dayrat et al. (2001)
	28SC2	5′-GAACTCTCTCTTCAAAGTTCTTTTC-3′	

parts of the mitochondrial 16S rRNA locus and the nuclear 18S and 28S rRNA loci (Table 1). Polymerase chain reaction (PCR) was conducted using *Taq* PCR core kit as recommended by the manufacturer (Qiagen). The following programs were used: 40 cycles of 1 min at 94 °C, 1 min at 53 °C and 1 min at 72 °C for 16S; 40 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C for 18S; 40 cycles of 1 min at 94 °C, 1 min at 45 °C and 1 min at 72 °C for 28S. All PCR cycles were initiated with a denaturation step for 3 min at 94 °C, and terminated with an extension step of 72 °C for 7 min. Amplicons were separated on a 1% agarose gel, stained with ethidium bromide and visualised on a UV transilluminator. Prior to sequencing, PCR products were purified using QIAquick Purification Kit (Qiagen).

Sequencing and phylogenetic analysis

For bidirectional sequencing, the same primers as for PCR were applied (Table 1). Assembly of DNA sequence files was conducted with DNA baser (Heracle BioSoft, Romania), and primer sequences were clipped. Sequences of 16S, 18S and 28S locus were deposited in GenBank (see Table 2 for accession numbers). Closest matches of sequences were identified by a BLAST search against GenBank entries (Altschul et al. 1990).

Phylogenetic trees were computed from the alignment of concatenated 16S, 18S and 28S sequences of different Naidinae, and *Trieminentia corderoi* (Opistocystidae) as outgroup (Table 2; Erséus et al. 2017) using the maximum likelihood algorithm with PhyML (Guindon et al. 2010) or Bayesian inference in MrBayes (Huelsenbeck and Ronquist 2001). Positions containing gaps and missing data were eliminated. Branch support was calculated based on 1000 bootstraps and the resulting tree was manually refined using Fig Tree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Uncorrected pairwise distances within sequences were calculated with MEGA 7 (Kumar et al. 2016).

Table 2 Accession numbers of nuclear ribosomal sequences used for phylogenetic reconstruction. For original reference, see Erséus et al. (2017)

	Locus		
Species	16S	18S	28S
Allonais gwaliorensis	KY633311	KY633330	KY633416
Allonais inaequalis	DQ459952	DQ459967	KY633417
Branchiodrilus hortensis CE1819	KY633312	KY633331	KY633419
Branchiodrilus hortensis CE1820	KY633313	KY633332	KY633420
Dero borellii	KY633324	KY633342	KY633428
Dero digitata	DQ459954	DQ459984	KY633425
Dero furcata	KY633325	KY633343	KY633427
Dero sp1	GQ355407	GQ355426	GQ355446
Dero superterrenus	KY633326	KY633355	KY633429
Dero vaga	DQ459953	DQ459966	KY633426
Dero rwandae sp. nov. HK1	MN215405	MN215422	MN215423
Dero rwandae sp. nov. HK1B	MN555814	MN555439	MN555442
Dero rwandae sp. nov. HK1C	MN555815	MN555440	MN555443
Rhyacodrilus coccineus	DQ459931	DQ459969	GU902025
Rhyacodrilus falciformis	DQ459938	DQ459965	KY633464
Trieminentia corderoi	GU002447	GU002448	KY633463

Table 3 Features of frog hosts (*Hyperolius kivuensis*), location and number of *Dero* parasites. Age of host was determined skeletochronologically, for details, see Sinsch and Dehling (2017). SVL = snout-vent length. Carcasses of hosts were deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK)

Hyperolius specimen (field number, collection number)	sex	SVL (mm)	Age (year)	*Dero* specimens in bladder	*Dero* specimens in Wolffian ducts
HK 100, ZFMK 102910	Male	23.7	2	2	9 (left), 11 (right)
HK 101, ZFMK 102911	Male	27.1	< 1	1	–
HK 108, ZFMK 102918	Male	30.6	1	1	4 (left), 4 (right)
HK 114, ZFMK 102924	Male	30.1	1	–	3 (left), 2 (right)
HK 121, ZFMK 102931	Male	30.2	1	–	2 (left), 3 (right)
HK 123, ZFMK 102933	Male	30.1	< 1	–	6 (left), 6 (right)

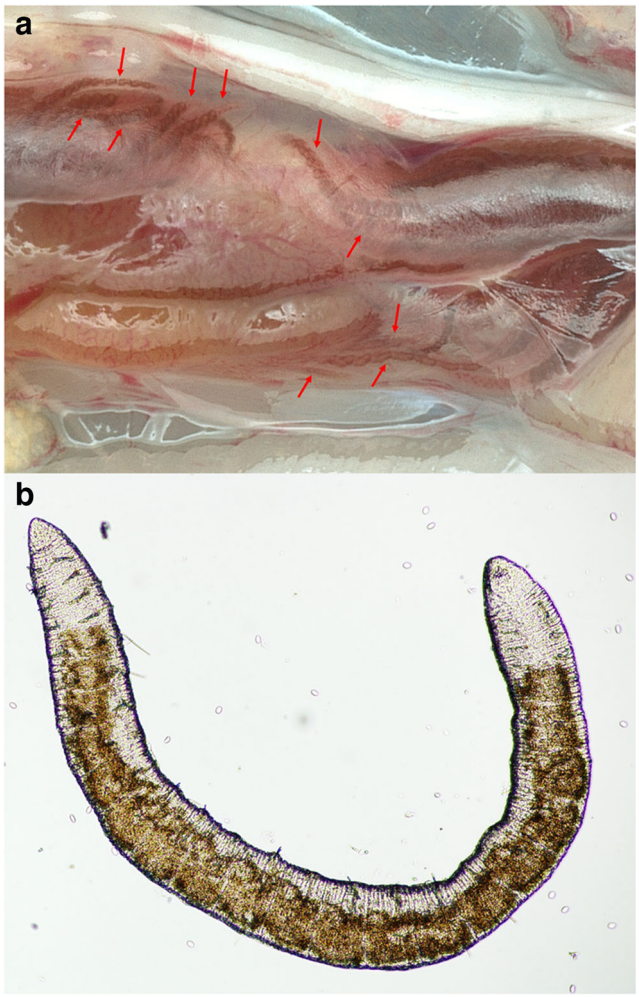

Fig. 1 *Dero rwandae* sp. nov. **a** Ventral view on the kidney of a heavily infected *H. kivuensis* host. *Dero* specimens (marked by red arrows) are partially visible within the Wolffian ducts. **b** Ventro-lateral view on the preserved holotype: mouth, ventral and dorsal setae visible at the anterior end (left), alimentary duct covered with brown chlorogogen tissue and the posterior end with anus and a rudimentary branchial fossa (right). Dimensions of the holotype are given in text

Results

Six male *Hyperolius kivuensis* (prevalence, 5.7%) harboured annelid parasites (mean intensity of infection ± SD, 9.2 ± 7.3) in bladder and Wolffian ducts (Table 3; Fig. 1a). The bladder contained none to a maximum of two worms, whereas up to 11 worms parasitized each of the Wolffian ducts. Infections of the Wolffian ducts affected always both simultaneously. The eldest frog (2 years) carried the highest number of worms (n = 22), whereas younger ones only 1–12. In the bladder of the youngest host specimen, we found only one worm, while its Wolffian ducts were worm-free.

Morphological features

The worms collected from bladder and Wolffian duct exhibited typical morphological characters diagnostic for the putative subgenus *Allodero* by possessing bundles of ventral setae from segment II on and dorsal hair setae and needles from segment IV on (Figs. 1b and 2). Body lengths varied between 2.5 and 4.3 mm, the number of segments between 17 and 38. Maximum body width was 0.2–0.32 mm. Eyes, genital organs and dilatation of oesophagus to a stomach were absent, brown chloragogen tissue was present from segment VI onwards. Dorsal setae consisted of a pair formed by a long hair seta and a short, bifid needle (Table 4; Fig. 3a–c). Proximal and distal teeth of the dorsal needle were equally sized. At the anterior end (segments II–V), ventral bundles included 4–5 bifid setae, from segment VI to the posterior end bundles included 5–6 bifid setae (Table 4; Fig. 3d, e). Proximal and distal teeth of the ventral setae were equally sized. The prostomium was triangle-shaped. At the posterior end of some worms, the postero-dorsal anus resembled a rudimentary branchial fossa, which was absent in others with the last segment recently regenerated (Fig. 4).

The specimens examined differed from all free-living *Dero* with known African distribution by the absence of a stomach and a branchial organ, the presence of dorsal setae in segment IV and the number of short setae in segments II–V (Table 4).

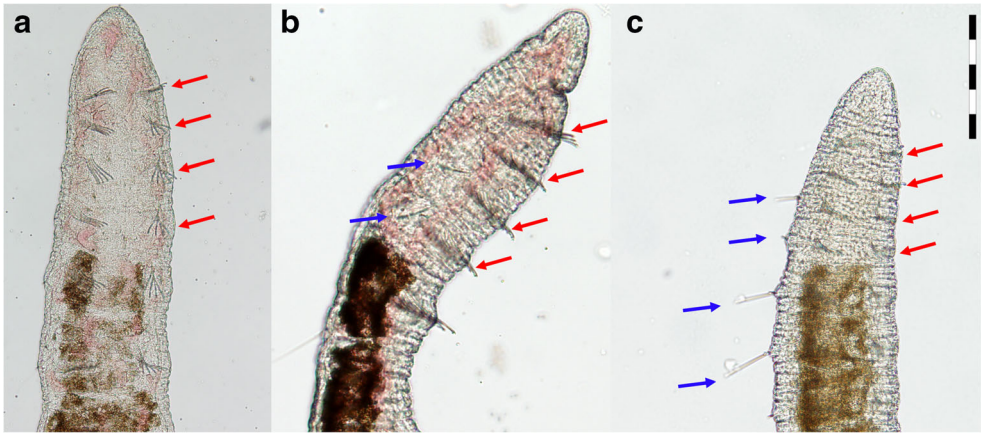

Fig. 2 Ventral (**a**, **c**) and lateral (**b**) views on the prostomium and the anterior segments of a specimen of *Dero rwandae* sp. nov. collected from the Wolffian duct of a *Hyperolius kivuensis* male (HK 114). Red arrows indicate bundles of ventral setae, blue arrows dorsal hair setae and needles. Scale bar represents 200 μm

In contrast, the general morphological features resembled those of other parasitic *Dero* from other parts of the world, but differed in the following characters (Table 5). The anterior ventral setae are shorter than in the Australian and South and North American species, but the length range is widely overlapping with *D. bauchiensis* and *D. malayana*. Distinction from *D. malayana* bases on the smaller number of segments, the presence of up to two needles and two hair setae per dorsal bundle and the free-living life mode. *Dero bauchiensis* from Mozambique shares the many morphological features with the Rwandan specimens except for the shorter posterior ventral setae, whereas *D. bauchiensis* specimens from Nigeria (type locality) are much larger. Still, the feature of the Rwandan specimens that is different from *D. bauchiensis* is the host organ infected, i.e. urinary tract *vs.* eyes and Harderian glands. *Dero* specimens never were found infecting the eyes of *H. kivuensis*. In summary, the Rwandan specimens examined differed in significant morphological aspects from all African and all parasitic *Dero* species that have been described so far.

Molecular features

Partial DNA sequences of the three loci 16S, 18S and 28S rDNA were obtained. The topology of the maximum likelihood tree based on the concatenated dataset (2259 nucleotides) shows three main clades (Fig. 5a). One contains the *Rhyacodrilus* species, another one contains the *Branchiodrilus* species and the last clade contains the species of *Dero* (including species formerly considered as *Aulophorus*) and *Allonais*. The Rwandan specimens form a cluster with *D. superterrenus*. The sequences of this specimen and *D. superterrenus* differ by an uncorrected pairwise distance of 1.89%. Based on the considerable genetic divergence of *D. superterrenus* from the Rwandan specimen, we recognise the naidid worms collected from the urinary tract of *H. kivuensis* as a new species. The Bayesian tree (Fig. 5b) shows a similar topology. *Rhyacodrilus* separates from the other species (*Dero* and *Allonais*) and the Rwandan specimens cluster with *D. superterrenus*. The genus *Dero* seems to be paraphyletic since *D. vaga* is more closely related to *Branchiodrilus* spp. then to other *Dero* spp, as already noticed by Erséus et al. (2017).

Taxonomic description

Dero rwandae sp. nov.

Holotype: ZMH Ol 15490, deposited at Centrum für Naturkunde (CeNak) - Center of Natural History, Universität Hamburg - Zoologisches Museum. Rwanda, Marais of Huye (Butare); 2.60059° S, 29.75964° E, 1645 m a.s.l. Host *Hyperolius kivuensis* carrying specimen in the urinary tract collected March 19, 2017, by J. M. Dehling and B. Dumbo.

Paratypes: ZMH Ol 15491, deposited at Centrum für Naturkunde (CeNak) - Center of Natural History, Universität Hamburg - Zoologisches Museum. Same locality and date. Five complete specimens, among them a large individual short before fragmentation, and a short individual with a recently regenerated posterior end.

Additional collections: ZMH Ol 15492, deposited at Centrum für Naturkunde (CeNak) - Center of Natural History, Universität Hamburg - Zoologisches Museum. Same locality and date. Twenty complete specimens and fragments.

Description of preserved holotype (Fig. 1b): Body length 3.0 mm, maximum width 0.23 mm. Number of segments 30. Prostomium triangular, posterior end with a rudimentary branchial fossa. Eyes, genital organs, budding zone and dilatation of oesophagus to a stomach absent. Alimentary channel difficult to make out and covered with brownish chloragogen tissue from VI onwards. No gut contents visible. Dorsal setal bundles from IV, 1 hair and 1 bifid needle per bundle. Length of hairs about 270 μm, length of needles 45–54 μm. Note that other specimens may lack hairs in some or all segments. Ventral bifid setae in II–V 4–5 per bundle, in the posterior

Table 4 Morphological features of free-living African *Dero* species including the new parasitic species from Rwanda. *This species is usually referred to as *Aulophorus africanus*. Note that African members of the "tonkinensis" species group (Grimm 1989) are not considered because the needle setae are fan-shaped

	Dero rwandae sp. nov.	*Dero africana** (Michaelsen 1914)	*Dero cooperi* Stephenson 1932	*Dero digitata* (Müller, 1773)	*Dero furcata* (Oken 1815)	*Dero nivea* Aiyer, 1930	*Dero oblongata* Grimm, 1985	*Dero obtusa* d´ Udekem, 1855	*Dero pectinata* Aiyer, 1930	*Dero raviensis* (Stephenson 1914)
Mode of living	Parasite, in urinary tract of *Hyperolius kivuensis*	Free-living	Free-living	Free-living	Free-living	Free-living	Free-living	Free-living	Free-living	Free-living
Body length [mm]	2.5–5.1	6–7	3.4–4.3	6–32	Not mentioned	2.5–10	Not mentioned	5–17	Not mentioned	Ca. 3
Number of segments	17–38	27–64	33–46	20–105	27–64	23–45	31–57	21–35	19–28	13–30
Dorsal setae beginning in segment	IV	V	VI	VI	V	VI	VI	VI	VI	VI
Dorsal setae per bundle	One hair seta and one bifid needle	One hair seta and one bifid, trifid or pectinate needle	One hair seta and one bifid needle	One hair seta and one bifid needle	One hair seta and one bifid needle	One hair seta and one bifid needle	One hair seta and one bifid needle	One hair seta and one bifid needle	One hair seta and one pectinate needle	One hair seta and one bifid needle
Length of dorsal setae [μm]	41–56 (needle) 212–282 (hair)	63–86 (needle) 142–387 (hair)	53–77 (needle) 139–250 (hair)	82–121 (needle) 260–390 (hair)	40–68 (needle) 90–218 (hair)	35–61 (needle) 95–189 (hair)	70–82 (needle) 177–215 (hair)	50–75 (needle) 98–182 (hair)	37–46 (needle) 77–104 (hair)	33–46 (needle) 80–137 (hair)
Ventral setae beginning in segment	II	II	II	II	II	II	II	II	II	II
Ventral setae per bundle	4–5 (II–V) 5–6 (≥ VI)	3–4 (II–V) 3–4 (≥ VI)	3–5 (II–V) 1–4 (≥ VI)	3–6 (II–V) 2–5 (≥ VI)	2–5	3–6	3–6 (II–V) 1–5 (≥ VI)	2–4 (II–V) 3–6 (≥ VI)	4 (II–V) 2–4 (≥ VI)	2–5 (II–V) 1–4 (≥ VI)
Length of ventral setae [μm]	40–59 (II–V) 68–73 (≥ VI)	78–99 (II–V) 56–94 (≥ VI)	90–126 (II–V) 75–90 (≥ VI)	117–167 (II–V) 86–125 (≥ VI)	53–79 (II–V) 47–75 (≥ VI)	65–109 (II–V) 52–70 (≥ VI)	102–116 (II–V) 75–87 (≥ VI)	95–120 (II–V) 57–82 (≥ VI)	70–90 (II–V) 39–51 (≥ VI)	65–90 (II–V) 39–51 (≥ VI)
Dilatation of oesophagus to stomach in segment	Absent	Not mentioned	IX–X	X–XI	Stomach dilatation (VIII) inconspicious	VIII	IX	IX–X	VIII	Stomach dilatation (VII–VIII) inconspicious
African distribution	Rwanda	Ghana, Namibia, South Africa, Sudan, ZAR	Ethopia, South Africa	Ghana, Nigeria, South Africa, Sudan, Tanzania, Zimbabwe	Ghana, South Africa, Tanzania	South Africa	South Africa	South Africa, Tanzania	South Africa, Tanzania	Kenya, Nigeria, Sudan, ZAR, Zimbabwe
References	This study	Michaelsen (1914), Hrabě (1966),	Stephenson (1932), Brinkhurst	Grimm (1988), Brinkhurst (1966)	Oken (1815), Brinkhurst (1966), Hrabě	Aiyer (1929), Brinkhurst (1966),	Grimm (1985a)	Sperber (1948), Brinkhurst (1966),	Aiyer (1929), Sperber (1948),	Grimm (1985b), Stephenson (1914),

Table 4 (continued)

	*Dero africana** (Michaelsen 1914)	*Dero cooperi* Stephenson 1932	*Dero digitata* (Müller, 1773)	*Dero furcata* (Oken 1815)	*Dero nivea* Aiyer, 1930	*Dero oblongata* Grimm, 1985	*Dero obtusa* d' Udekem, 1855	*Dero pectinata* Aiyer, 1930	*Dero raviensis* (Stephenson 1914)
Dero rwandae sp. nov.	Grimm (1985b)	(1966), Grimm (1988)		(1966), Grimm (1989)	Grimm (1988),		Grimm (1988)		Grimm (1988)

segments 5–6. The anterior setae markedly shorter (49–56 μm) than posterior ones (about 70 μm). Distal tooth about the same size of proximal tooth.

Colour in life reddish-brown.

Molecular markers: Partial 16S, 18S and 28S rDNA sequences are deposited in GenBank (Table 2).

Etymology: The epithet rwandae refers to the geographical origin of the specimens collected.

Geographical distribution: Only known from the type locality. Infections of hosts seems to be limited to the end of the rainy season (6 out of 26 frogs, 23%), earlier surveys of hosts (18 frogs in October; 35 in January) at the same locality failed to yield worms in the urinary tract.

Remarks on reproduction: None of the specimens examined showed sexual organs. Reproduction is probably asexual by fragmentation, as in other parasitic *Dero* (Stephenson 1930; Sperber 1948; Harman & Lawler 1975; Pinder et al. 1998). The largest paratype (5.1 mm, 36 segments) showed a mid-body constriction (between XX and XXI) in which chloragogen tissue was absent and seemed to prepare fission. The originally posterior end with still detectable anus was in process of change to a new anterior end. Another paratype is small-sized (3.2 mm, 17 segments) with a disc-like posterior end and intact body wall, but without anus or rudimentary branchial fossa (Fig. 4b). The posterior end probably has been regenerated following recent fragmentation.

Discussion

Naidid endoparasites of amphibians have been placed in the putative subgenus *Allodero* of the genus *Dero* because they share putatively diagnostic features such as branchial organ (if present) without palpes, dorsal setae from IV onwards, ventral setae all similarly shaped and asexual reproduction by fragmentation (Sperber 1948; Grimm 1988). The first segment, at which dorsal setae are present, is certainly not suited to distinguish parasitic and free-living *Dero* spp. because *D. dorsalis* Ferronière 1899 and *D. superterrenus* (Michaelsen 1912) share this feature (Michaelsen 1912; Lee and Jung 2014). The remaining putative diagnostic features are probably phenotypic adaptations to the endoparasitic life mode that can change, if specimens are transferred to a free-living environment (Andrews et al. 2015). Specifically, *D.* (*Allodero*) *hylae* developed a branchial fossa with gills after 2 weeks, *D.* (*Allodero*) *lutzi* a branchial fossa without palps and the two species augmented the number of dorsal setae (A. Lutz in Stephenson 1931; Andrews et al. 2015). When free-living *D. (Allodero) hylae* infects a host, the worm loses its hair setae, most of its dorsal setae, and the gills in the fossa are minimized within 72 h of entering a host (Andrews et al. 2015). Consequently, morphological evidence put forward for the definition of subgenera within *Dero*, structure of

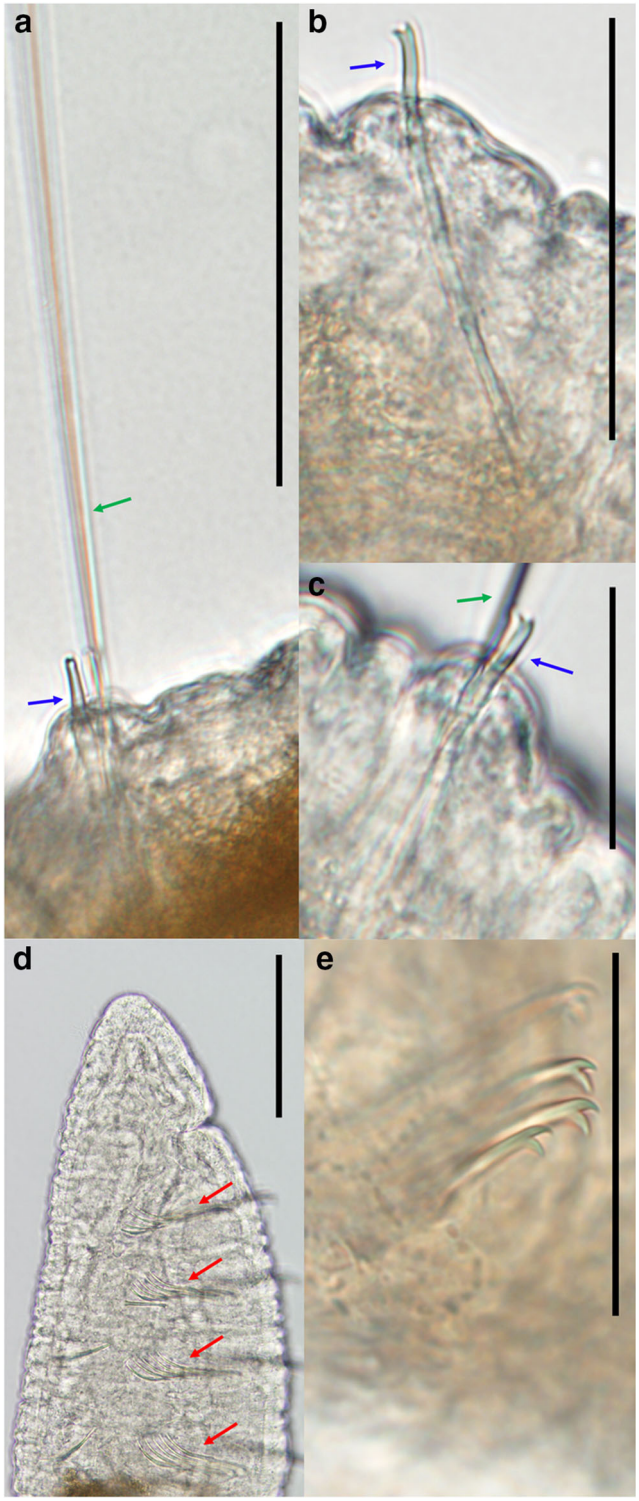

Fig. 3 Dorsal and ventral setae of *Dero rwandae* sp. nov. **a**, **b**, **c** Dorsal needles (blue arrows) and parts of the hair setae (green arrows). **d**, **e** Bundles of ventral setae (red arrows). Scale bar represents 100 μm in **a** and **d**, 50 μm in **b**, **c** and **e**

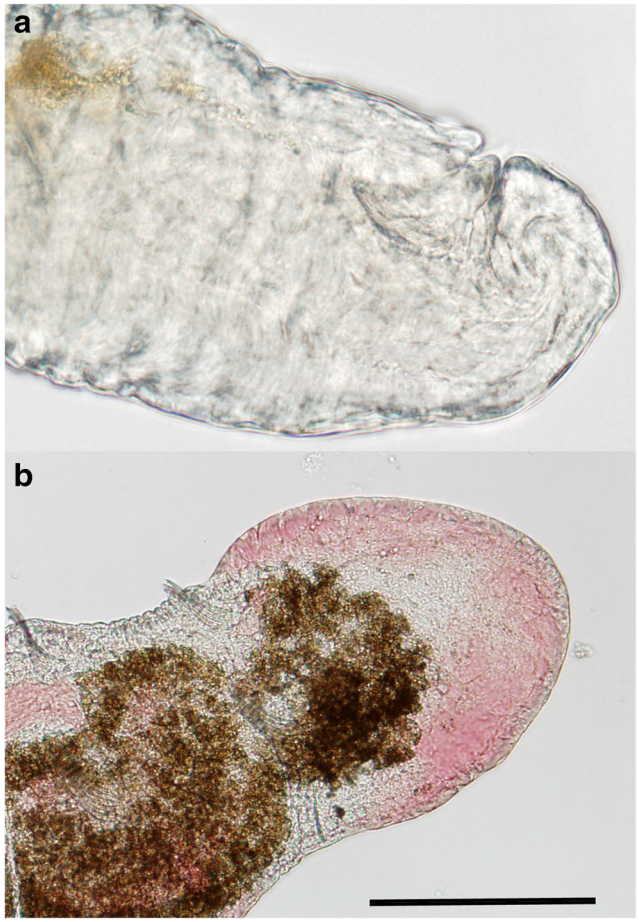

Fig. 4 Posterior end of two specimens of *Dero rwandae* sp. nov. **a** Lateral view on a rudimentary branchial fossa. **b** Recently regenerated end segment with complete body wall. Scale bar represents 200 μm

branchial organs and setae features, strongly depends on ecological context of the worm examined, i.e. on the type of habitat (aquatic or host) and lifestyle (free-living or parasitic).

Molecular evidence suggests that the Rwandan species (morphologically a member of the putative subgenus *Allodero*) is the sister species of the free-living *D. superterrenus*, a member of the putative subgenus *Aulophorus*. Moreover, *Dero digitata*, a member of the putative subgenus *Dero*, is the basal taxon of the *Dero-Branchiodrilus* clade (Erséus et al. 2017; this study) demonstrating that the proposed subgenera *Dero*, *Allodero* and *Aulophorus* do not represent separate evolutionary lineages.

Considering that free-living *D.* (*Allodero*) *hylae* are easily confused with nonparasitic species of *Dero* (Andrews et al. 2015), evidence for the species status of the Rwandan *Dero* specimens requires a thorough comparison with the features of the known African species. We consider that a confusion with the free-living *Dero* of Africa can be excluded because dorsal setae are present from segment IV onwards in the Rwandan specimens, whereas all free-living taxa bear dorsal setae from VI onwards. Since the passage from free-living to parasitic mode of living includes a loss, not an increase of dorsal setae presence, we conclude that a potentially free-

Table 5 Morphological features of *Dero* (*Allodero*) species parasitizing anurans including the new species from Rwanda

	Dero bauchiensis (Stephenson 1930)	*Dero rwandae* sp. nov.	*Dero malayana* (Stephenson 1931)	*Dero lutzi* (Michaelsen 1926)	*Dero hylae* Goodchild, 1951	*Dero floridana* Harman 1971	*Dero litoria* Pinder et al. 1998
Site of parasitism, host	Eyes and Harderian gland of microhylids (*Phrynomantis*)	Urinary tract of *Hyperolius kivuensis*	Free-living (in a treehole near a pond)	Urinary tract of hylids and *Rhinella* spp.	Urinary tract of hylids	Urinary tract of *Anaxyrus terrestris*	Urinary tract of *Ranoidea caerulea*
Body length [mm]	3.5–5 (Mozambique) 7.5–11 (Nigeria)	2.5–5.1	3–5	3.1–7.5 (11.3)	4.1–4.2	5.5–8.3	3.2–6.3
Number of segments	26–39 (Mozambique) 62–93 (Nigeria)	17–38	37–73	34–73	31–40	24–50	49–79
Dorsal setae beginning in segment	IV	IV	IV	IV	IV	IV	IV
Dorsal setae per bundle	One hair seta and one bifid needle each	One hair seta and one bifid needle each	1–2 hair setae and 1–2 bifid needles	If present, one hair seta and one bifid needle	If present, one hair seta and one bifid needle	One hair seta and one bifid or trifid needle	One hair seta and 1–2 bifid needles
Length of dorsal setae (μm)	46–53 (needle) 210–280 (hair)	41–56 (needle) 212–282 (hair)	59–61 (needle) 250 (hair)	59–66 (needle) 330–360 (hair)	38–75 (needle) 68–236 (hair)	35–61 (needle) 130–209 (hair)	55–73 (needle) 95 (hair)
Ventral setae beginning in segment	II	II	II	II	II	II	II
Ventral setae per bundle	4–5 (II–V) 5–7 (≥ VI)	4–5 (II–V) 5–6 (≥ VI)	3–4	3–4	3–5	3–6	4–6 (II–V) 2–5 (≥ VI)
Length of ventral setae (μm)	53–65 (II–V) 60–62 (≥ VI)	40–59 (II–V) 68–73 (≥ VI)	50–67	85–99	53–100	70–79 (II–V) 78–86 (≥ VI)	73–93
Distribution	Africa: Mozambique, Nigeria	Africa: Rwanda	Southeast Asia	South America	North America	North America	Australia
References	Stephenson (1930), Sperber (1948) Brinkhurst (1966)	This study	Stephenson (1931), Sperber (1948)	Michaelsen (1926), Sperber (1948), Rodrigues (1982), Morais et al. (2017)	Goodchild (1951), Harman (1975)	Harman (1971)	Pinder et al. (1998)

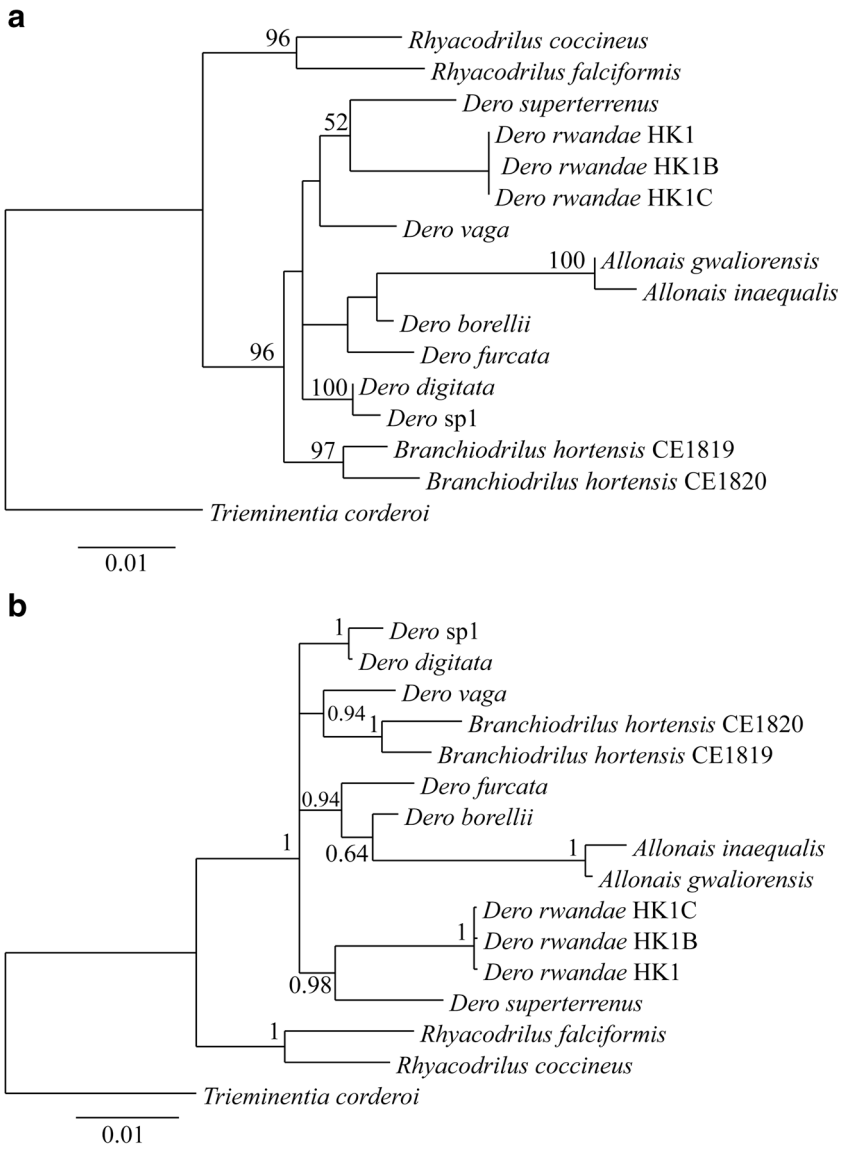

Fig. 5 Maximum likelihood analysis (**a**) and Bayesian tree (**b**) of concatenated 16S, 18S and 28S rDNA loci of selected Naididae species and *Trieminentia corderoi* as outgroup. Support values of maximum likelihood bootstrap analysis (1000 replicates) and posterior probability values are given. Scale bar gives substitutions per site

living form of the taxon from Rwanda has not yet been described. Molecular evidence supports this assumption in case of *D. digitata*, the only African species with published barcoding sequences (Envall et al. 2006; Erséus et al. 2017). Morphological distinction of the Rwandan taxon from *D. bauchiensis*, specifically from the specimens collected in Mozambique, is difficult and exclusively based on the fact that the posterior ventral setae are longer. Yet, we consider that the host organ infected by the worms is a clear indicator that *D. bauchiensis* is not conspecific with the taxon from Rwanda. *Dero bauchiensis* is only known to parasitize the eyes and Harderian glands of microhylid hosts, but not the urinary tract, whereas the taxon from Rwanda was never found in the eyes or Harderian glands (Stephenson 1930), but exclusively in the bladder or the Wolffian ducts of the *Hyperolius* host. To the best of our knowledge, we conclude that *D. rwandae* sp. nov. is not conspecific with any African *Dero* taxon described so far.

We have found the species only in adult males of *Hyperolius kivuensis*. In contrast, none of 100 syntopic *H. viridiflavus* examined by us was infected. If the association with *H. kivuensis* is species-specific remains to be investigated with a taxonomically broader spectrum of samples from possible hosts and experimental infections. The infection pattern of hosts (23% in the March sample) is presumably seasonal. If so, the worms have probably a free-living stage that enters via the cloaca to the urinary tract of the frog host following a long period of rainfall. We cannot exclude that a *Dero* infection is lethal for the host, but rather assume that worms leave the host after a period of asexual reproduction when the frog voids bladder urine (Andrews et al. 2015). The reduced alimentary tract with rich chloragogen tissue indicates resorption of

resources from the host as discussed in Pinder et al. (1998). The presence of constrictions in large worms and that of small worms with regenerated posterior end parallels the observations in *D.* (*Allodero*) *hylae* (Harman and Lawler 1975) and *D.* (*Allodero*) *litoria* (Pinder et al. 1998) and may suggest the use of host resources for worm propagation. We propose that *D. rwandae* uses the frog host to obtain resource for massive asexual reproduction (parasitism) and for dispersal to suitable freshwater habitats (phoresis).

Acknowledgements We are indebted to B. Dumbo for assistance during the collection of *Hyperolius* in the field. Collecting and export permits (no. 2/ORTPN/V.U/09, 08/RDB-T&C/V.U/12, 18/RDB-T&C/V.U/12 and 10/RDB-T&C/V.U/15) of *Hyperolius kivuensis* and *H. viridiflavus* for parasitological screening were issued by the Rwandan Development Board. The manuscript benefitted from the comments of three anonymous referees.

Compliance with ethical standards

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

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed, and all procedures performed were in accordance with the ethical standards of the institution at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

References

Aiyer KSP (1929) An account of the Oligochaeta of Travancore. Rec. Indian Museum 3:13–76

Altschul SF, Gish W, Mille W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Andrews JM, Childress JN, Iakovidis TJ, Langford GJ (2015) Elucidating the life history and ecological aspects of *Allodero hylae* (Annelida: Clitellata: Naididae), a parasitic oligochaete of invasive cuban tree frogs in Florida. J Parasitol 101:275–281. https://doi.org/10.1645/14-608.1

Brinkhurst RO (1966) A contribution towards a revision of the aquatic Oligochaeta of Africa. Afr Zool 2:131–166

Brinkhurst RO (1970) A further contribution towards a study of the aquatic Oligochaeta of Africa. Rev Zool Bot Afr 81:101–108

Dayrat B, Tillier A, Lecointre G, Tillier S (2001) New clades of Euthyneuran gastropods (Mollusca) from 28S rRNA sequences. Mol Phylogenet Evol 19:225–235. https://doi.org/10.1006/mpev.2001.0926

Envall I, Kallersjo M, Erséus C (2006) Molecular evidence for the non-monophyletic status of naidinae (Annelida, Clitellata, Tubificidae). Mol Phylogenet Evol 40:570–584. https://doi.org/10.1016/j.ympev.2006.03.021

Erséus C, Envall I, De Wit P, Gustavsson LM (2017) Molecular data reveal a tropical freshwater origin of Naidinae (Annelida, Clitellata, Naididae). Mol Phylogenet Evol 115:115–127. https://doi.org/10.1016/j.ympev.2017.07.016

Ferronière G (1899) Contribution à l'élude de la faune de la Loire Inférieure (Annelides, Oligochètes). Bulletin de la Société des Sciences Naturelles de l'Ouest de la France 9:229–295

Gelder SR (1980) A review of the symbiotic Oligochaeta (Annelida). Zool Anz 204:69–81

Grimm R (1985a) Beitrage zur Systematik der Africanischen Naididae (Oligochaeta). I. Beschreiben von vier neuen Arten. Mitt hamb zool Mus Inst 82:101–108

Grimm R (1985b) Beiträge zur Systematik der afrikanischen Naididae (Oliogochaeta): II. *Dero raviensis* (Stephenson, 1914) und *Aulophorus africanus* Michaelsen, 1914 - zwei verbreitete afrikanischen Arten. Mitt hamb zool Mus Inst 82:109–117

Grimm R (1986) Beiträge zur Systematik der afrikanischen Naididae (Oligochaeta). III. Untersuchungen zur qualitativen und quantitativen Chaetotaxonomie der Naididae. Mitt hamb zool Mus Inst 8:101–115

Grimm R (1987) Contributions towards the taxonomy of the African Naididae (Oligochaeta). IV. Zoogeographical and taxonomical considerations on African Naididae. Hydrobiologia 155:27–37. https://doi.org/10.1007/bf00025628

Grimm R (1988) Beitrage zur Systematik der afrikanischen Naididae (Oligochaeta). VI. Naidinae (Teil 1). Mitt hamb zool Mus Inst 85:73–102

Grimm R (1989) Beitrage zur Systematik der afrikanischen Naididae (Oligochaeta). VII. Naidinae (Teil 2). Mitt hamb zool Mus Inst 86:107–126

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Harman WJ (1971) A review of the subgenus *Allodero* (Oligochaeta: Naididae: *Dero*) with a description of *D. (A.) floridana* n. sp. from *Bufo terrestris*. Trans Am Microsc Soc 90:225–228. https://doi.org/10.2307/3225029e

Harman WJ (1980) Specific and Generic Criteria in Freshwater Oligochaeta, with Special Emphasis on Naididae. In: Brinkhurst RO, Cook DG (eds) Aquatic Oligochaete Biology. Springer US, Boston MA, pp 1–8

Harman WJ, Lawler AR (1975) *Dero (Allodero) hylae*, an Oligochaete Symbiont in hylid frogs in Mississippi. Trans Am Microsc Soc 94:38–42

Hrabě S (1966) On some Naididae from the Volta Lake in the Ghana. Publ Fac Sci Univ Brno 477:373–387

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Lee J, Jung J (2014) Two aquatic oligochaete species, *Dero dorsalis* and *Allonais pectinata* (Annelida: Clitellata: Naididae), New to Korea. Anim Syst Evol Divers 30:119–123

Lopez LCS, Rodrigues PJFP, Rios RI (1999) Frogs and snakes as phoretic dispersal agents of bromeliad ostracods (Limnocytheridae: *Elpidium*) and annelids (Naididae: *Dero*). Biotropica 31:705–708. https://doi.org/10.1111/j.1744-7429.1999.tb00421.x

Michaelsen W (1912) Über einige zentralamerikanische Oligochäten. Arch Naturges 78:112–129

Michaelsen W (1914) Beitrage zur Kenntnis der Land-und Süsswasserfauna Deutsch-Südwestafrikas: Oligochaeta. In: Michaelsen W (ed) Ergebnisse der Hamburger deutsch-südwestafrikanischen Studienreise 1911. Friedrichsen, Hamburg, pp 139–182

Michaelsen W (1926) *Schmardaella lutzi* Mich., oligochaeto endoparasitico de Hylidas sul-americanas. Mem Inst Oswaldo Cruz 19:231–235

Morais DH, Aguiar A, Campião KM, Tavares LER, Almeida WDO, Avila RW, Silva RJD (2017) New Records of *Dero (Allodero) lutzi*, an Oligochaete Parasite of the Urinary Tract of South American Anurans. Herpet Rev 48:739–743

Norén M, Jondelius U (1999) Phylogeny of the Prolecithophora (Platyhelminthes) Inferred from 18S rDNA Sequences. Cladistics 15:103–112. https://doi.org/10.1006/clad.1999.0095

Oken L (1815) Okens Lehrbuch der Naturgeschichte. Dritter Theil, Zoologie, Erste Abtheilung Fleischlose Thiere. August Schmid, Jena

Pinder AM, Hill BD, Green PE (1998) A new species of *Dero* (*Allodero*) (Naididae: Oligochaeta) inhabiting the Wolffian ducts of the green tree frog (*Litoria caerula*) in Queensland. Mem Queensl Mus 42: 559–564

Sinsch U (2015) Review: Skeletochronological assessment of demographic life-history traits in amphibians. Herpetol J 25:5–13

Sinsch U, Dehling JM (2017) Tropical anurans mature early and die young: evidence from eight Afromontane *Hyperolius* species and a meta-analysis. PLOS One 12:e0171666. https://doi.org/10.1371/journal.pone.0171666

Sinsch U, Lümkemann K, Rosar K, Schwarz C, Dehling JM (2012) Acoustic niche partitioning in an anuran community inhabiting an Afromontane wetland (Butare, Rwanda). Afr Zool 47:60–73. https://doi.org/10.3377/004.047.0122

Sjölin E, Erséus C, Källersjö M (2005) Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. Mol Phyl Evol 35:431–441. https://doi.org/10.1016/j.ympev.2004.12.018

Sperber C (1948) The taxonomical study of the Naididae. Zool Bidr Upps 28:1–296

Stephenson J (1914) On a collection of Oligochaeta mainly from Northern India. Rec Ind Mus Calcutta 10:321–366

Stephenson J (1930) XXXIX.—An Oligochæte worm parasitic in frogs of the genus *Phrynomerus*. Ann Mag nat Hist 6:367–377

Stephenson J (1931) Oligochaeta from the Malay Peninsula. J Fed Malay States Mus 16:261–285

Stephenson J (1932) 16. Report on the Oligochæta: Mr. Omer-Cooper's investigation of the Abyssinian fresh waters (Dr. Hugh Scott's Expedition). Proc Zool Soc Lond 102:227–256