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Research Note

Gastrointestinal helminths in *Amietia* sp. (Anura: Pyxicephalidae) from the Albertine Rift of Central Africa

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Summary

Fourteen *Amietia* sp. (Pyxicephalidae), from the Albertine Rift of Democratic Republic of the Congo were examined for helminths. Five species of Nematoda were found: *Amphibiophilus chabaudi, Aplectana praeputialis, Falcaustra congoensis, Foleyellides duboisi* and *Orneoascaris chrysanthemoides*. *Amphibiophilus chabaudi* was the most numerous nematode (*n* = 40) with the highest prevalence (57 %). Five new host records are reported.
Keywords: Africa; Democratic Republic of Congo; Helminthology; Nematoda

Introduction

River frogs of the genus *Amietia* are medium-sized frogs found near rivers streams, and other aquatic habitats throughout central, eastern and southern Africa.

Of the 16 species currently assigned to the genus, three are known to occur in the Albertine Rift, which is the most biodiverse highland region in continental Africa (Plumptre *et al*., 2007; Frost, 2020). Based on nearly 2500 base pairs from four genes, the phylogenetic study of Larson *et al*. (2016) provided evidence for additional, cryptic species of *Amietia* in the Albertine rift, including a possible new species (*Amietia* sp. 6) that occurs from the poorly known Lendu Plateau at the northern limit of the Rift to the Kichanga River in southwestern Virunga National Park. Based only on similarity of 16S sequences, Channing *et al*. (2016) synonymized *Amietia* sp. 6 with *A. nutti*, a common and widespread species from the eastern side of the Albertine Rift. Because numerous studies have demonstrated that some frogs with minor genetic divergences from congeners can be valid species (e.g. Portillo & Greenbaum, 2014), at least some of the taxonomic changes of Channing *et al*. (2016) are likely premature and require additional scrutiny with more robust data sets.

Herein, while acknowledging the need for a more thorough revision of Central African *Amietia*, we continue to recognize *Amietia* sp. 6 of Larson *et al*. (2016) as a candidate species that is likely distinct from *A. nutti*. There is little information on the helminths infecting this genus; known helminths are listed in Table 1. In view of the paucity of information for African anurans, we conducted a helminthological examination of *Amietia* sp. (*A.* sp. 6 *sensu* Larson *et al*. 2016) from Ituri and North Kivu Provinces, Democratic Republic of the Congo (DRC).

Materials and Methods

Frogs had been previously fixed in 10 % formalin and later stored in 70 % ethanol. Our research complied with all the relevant national regulations and institutional policies for the care and use of animals. Identification of these specimens was confirmed by

* – corresponding author

Table 1. Helminths reported for species assigned to the genus *Amietia*.

Host Species	Distribution	Helminth	Reference
A. angolensis (Bocage, 1866)	Central Africa[1,2]	*Cylindrotaenia jaegerskioeldi*	Jones, 1987
		Amphibiophilus acanathocirratus	Schmidt and Canaris, 1968
		Amphibiophilus chabaudi	Puylaert, 1967
		Amphibiophilus natalensis	Baker, 1981
		Amphibiophilus versterae	Baker, 1981
		Aplectana chamaeleonis	Baker, 1987
		Aplectana macintoshii	Halahan et al., 2013
		Entomelas sylvestris	Halahan et al., 2013
		Falcaustra sp.	Schmidt and Canaris, 1968
A. chapini (Noble, 1924)	Central Africa	No Helminths reported	
A. delalandii (Duméril and Bibron, 1841)	Southeastern Africa	*Amphibiophilus mooiensis*	Svitin and DuPreez, 2018
		Rhabdias picardiae	Svitin et al., 2018
		Amphibiophilus sp. 1	Svitin et al. 2020
		Amphibiophilus sp. 2	Svitin et al. 2020
A. desaegeri (Laurent, 1972)	Eastern DRC and western Rwanda	No Helminths reported	
A. fuscigula (Duméril and Bibron, 1841)	Southern South Africa	No Helminths reported	
A. hymenopus (Boulenger, 1920)	Lesotho and adjacent South Africa	No Helminths reported	
A. inyangae (Poynton,1966)	Eastern Zimbabwe	No Helminths reported	
A. johnstoni (Günther, 1894)	Southern Malawi	No Helminths reported	
A. moyerorum Channing, Dehling, Lötters, and Ernst, 2016	Malawi and Tanzania	No Helminths reported	
A. nutti (Boulenger, 1896)	Central and East Africa from Tanzania to Ethiopia	*Amphibiophilus chabaudi*	Bursey et al., 2018
		Falcaustra congoensis	Bursey et al., 2018
A. poyntoni Channing and Baptista, 2013	Namibia to South Africa	No Helminths reported	
A. ruwenzorica (Laurent, 1972)	Albertine Rift of DRC and Uganda	No Helminths reported	
A. tenuoplicata (Pickersgill, 2007)	Highlands of Kenya and Tanzania	No Helminths reported	
A. vandijki (Visser and Channing, 1997)	Namibia to South Africa	No Helminths reported	
A. vertebralis (Hewitt, 1927)	Lesotho and adjacent South Africa	No Helminths reported	
A. wittei (Angel, 1924)	Western Kenya to northern Tanzania[2]	No Helminths reported	

[1]Until recently, this species was thought to be widespread in Central Africa, but was restricted to Angola by Channing et al. (2016), and all previous records refer to other species, including possible new species noted by Larson et al. (2016).

[2]**Records** of *Amietia angolensis* and *A. wittei* from the Lendu Plateau, DRC by Mali et al. (2019) are erroneus.

Appendix 1. *Amietia* (Pyxicelphalidae) from Democratic Republic of the Congo (DRC) examined from the herpetology collection of the University of Texas at El Paso Biodiversity Collections (UTEP), El Paso, Texas, USA.

UTEP 22256, UTEP 22252-22253, UTEP 22257, DRC,Ituri Province, Lendu Plateau eastern escarpment, Songa, N01.94724, E30.87836, 1771 m, 29 April 2017
UTEP 22254, UTEP 22261, UTEP 22262, DRC, Ituri Province, Lendu Plateau eastern escarpment, Batisodda, N01.94512, E030.87565, 1795 m, 3 May 2017
UTEP 22258, DRC, Ituri Province, Lendu Plateau eastern escarpment, Yaokpadda, N01. 94408, E30.88128, 1864 m, 27 April 2017
UTEP 22259, DRC, Ituri Province, Lendu Plateau eastern escarpment, Sepeadda. N01.94717, E30.91238. 851 m, 9 May 2017
UTEP 22255, 22260, DRC, Ituri Province, Lendu Plateau eastern escarpment, Rodda, N01.95315, E30.89357, 1385 m, 2 May 2017
UTEP 22263, 22264, DRC, North Kivu Province, Bunyantenge, S00.26315, E28.94348, 1692 m, April 2015 UTEP 22265, DRC, North Kivu Province, Lohulu, S0041257, E29.03162, 1785 m, April 2015

Appendix 2. Helminths from *Amietia* sp. from the Democratic Republic of the Congo deposited in the Harold Manter Laboratory (HWML), University of Nebraska, Lincoln.

Amphibiophilus chabaudi (HWML 112148), *Aplectana praeputialis* (HWML 112149), *Falcaustra congoensis* (HWML 112150), *Foleyellides duboisi* (HWML 112151), *Orneoascaris chrysanthemoides* (HWML 112152).

comparison of newly generated 16S mitochondrial data (EG and AJR, unpubl. data) that were compared to data from Larson *et al*. (2016). Sampling included 14 *Amietia* sp. adult female specimens (Mean SVL = 75.4 mm ± 7.0 SD, range = 63 – 88 mm) housed in the University of Texas at El Paso (UTEP) Biodiversity Collections (Appendix 1). The body cavity of each specimen was opened by a longitudinal incision and the gastrointestinal tract was removed by cutting across the esophagus and rectum. An incision was made using a stainless steel razor blade, pinned with insect pins to remain open, and the contents were examined using a dissecting microscope. Each nematode was removed with jewelers forceps. Nematodes were cleared in lactophenol, examined under a compound microscope, and identified to species utilizing Anderson *et al*. (2009), Gibbons (2010) and by comparisons with the original descriptions. Nematoda were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, USA (Appendix 2). Parasite terminology is in accordance with Bush *et al*. (1997). The number of recorded parasites, their prevalence and range are summarized in Table 2.

Ethical Approval and/or Informed Consent

The *Amietia* sp. were collected in accordance with the University of Texas at El Paso approved IACUC protocol #A-2009-02-01. All relevant research and wildlife agencies in Africa issued permits to collect and legally export animals, and US Fish and Wildlife approved form 3-177 to legally import the specimens to the USA.

Results and Discussion

AMPHIBIOPHILIDAE: *Amphibiophilus chabaudi* was originally described from "*Rana angolensis*" (Ranidae) collected in Njoro, Kenya (Puylaert, 1967). This anuran is now in the genus *Amietia*. The greatest number of helminths found (40) and the highest prevalence (57 %) was for *A. chabaudi* (Table 2). *Amphibiophilus chabaudi* was previously reported in *A. nutti* from Democratic Republic of Congo by Bursey *et al*. (2018).
ASCARIDIDAE: *Orneoascaris chrysanthemoides* is a widespread nematode occurring in the stomach and intestine of toads and frogs, lizards, turtles, snakes and a crocodile from east, west and central Africa (Sprent, 1985). *Orneoascaris chrysanthemoides* in *Amietia* is a new host record.
COSMOCERCIDAE: *Aplectana praeputialis* was first described from female worms collected from a host at that time assigned to *Bufo* (current designation unknown, but likely *Sclerophrys*) from East Africa (Baker, 1980). Le Van Hoa (1962) described male and female worms from a host at that time assigned to *Bufo* (current designation unknown, but likely *Sclerophrys*) from the DRC

Table 2. Number of Nematoda (*n*), Prevalence (%), Mean intensity ± SD (X), Range (r) and Collection Site for helminths from 14 *Amietia* sp. from the Democratic Republic of the Congo. * = new host record.

Nematode	*n*	%	X	r	Collection Site
**Amphibiophilus chabaudi*	40	57	5.1 ± 3.8	2-14	small intestine
**Aplectana praeputialis*	2	7	-----	-----	large intestine
**Falcaustra congoensis*	10	29	2.8 ± 2.2	1-6	small intestine
**Foleyellides duboisi*	1	7	-----	-----	stomach
**Orneoascaris chrysanthemoides*	6	7	-----	-----	small intestine

as *Aplectana praeputialis.* Taylor (1924) reported *A. praeputialis* in *Sclerophrys regularis* (reported as *Bufo regularis)* from West Africa. *Aplectana praeputialis* in *Amietia* represents a new host record.

KATHLANIIDAE: *Falcaustra congoensis* was described from *A. nutti* by Bursey *et al*. (2018). Species of *Falcaustra* occur in the digestive tracts of fishes, amphibians and reptiles (Baker, 1987). It should be noted that Schmidt and Canaris (1968) reported *Falcaustra* sp. from *Amietia angolensis* (reported as *Rana angolensis*) collected in Kenya, but this species is now restricted to Angola and adjacent western DRC (Frost, 2020).

ONCHOCERCIDAE: *Foleyellides duboisi* was originally described from an unidentified toad and frog collected in Leopoldville, Belgian Congo (modern-day Kinshasa, DRC) as *Filaria duboisi* by Gedoelst (1916). Yorke and Maplestone (1926) reassigned it to the genus *Foleyella.* Witenberg and Gerichter (1944) redescribed this species and described its life cycle from specimens recovered from the body cavity of *Rana esculenta ridibunda* (currently *Pelophylax ridibundus*) collected in northern Palestine. Esslinger (1986) in his reinstatement of the genus *Foleyellides* Caballero, 1935 reassigned *Foleyella duboisi* to *Foleyellides*. Species of this genus are parasites of subcutaneous connective and muscular tissue of saurians and amphibians; they produce microfilariae, which circulate in blood and utilize mosquitoes as intermediate hosts (Olsen, 1974). Witenberg and Gerichter (1944) described the life cycle of *F. duboisi*. *Foleyellides duboisi* in *Amietia* is a new host record.

Representatives of *Amietia* examined in this study are parasitized by generalist helminths that are also found in other anuran species from Africa. As additional species of *Amietia* are examined, we expect the helminth list for this genus to increase.

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Conflict of Interest

Authors state no conflict of interest.

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