

# *Polystoma galamensis* (Monogenea): Occurrence and ecological range in Nigeria

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## Abstract

*Hylarana galamensis* collected from three biotopes in Nigeria were investigated for infection with the monogenean *Polystoma galamensis*. Of the 152 specimens examined, 19 (12.5%) were infected with the parasite. Prevalence in the guinea savannah was 21.4%, derived savannah, 15.1% and the freshwater creeks of Ase, 2.27%. The intensity of infection was generally low and this can be principally attributed to the solitary reproductive behaviour of the host. Excessive precipitation and flooding may additionally play limiting roles in the creeks of the Niger Delta. Irrespective of host habitat, parasites recovered from frogs in Nigeria showed close similarity with the type species from Togo in several aspects of their morphometrics. The parasite from Nigeria also had a low haptor/body ratio and the occurrence of prehaptor intestinal anastomoses was infrequent. This close similarity between the parasites from Togo and Nigeria confirms *P. galamensis* as a valid species. This report from Nigeria is the second on the parasite, making Nigeria a new geographical record for the parasite.

**Keywords:** *Hylarana galamensis*; derived savannah; freshwater swamp; guinea savannah; *Polystoma galamensis*; Nigeria.

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## Introduction

*Polystoma galamensis* is a monogenean parasite of the urinary bladder of *Hylarana galamensis* (formerly *Rana galamensis*). To date, there is only one description of this parasite in the literature (Euzet *et al* 1974), based on specimens recovered from frogs collected in Klouto, located in the savannah of Togo. The parasite material from Togo was characterized by a low haptor-body ratio, large-sized hamuli, development of axial caeca and the presence of prehaptor intestinal anastomosis in some specimens.

In three earlier publications on the helminth parasites of anurans from the savannah-mosaic and the guinea savannah of Nigeria, respectively (Aisien *et al* 2003, 2004a,b), we reported the occurrence of this parasite in *H. galamensis* examined from these biotopes. In subsequent investigations of the helminth parasite fauna of anurans from four other locations within the savannah-mosaic of southern Nigeria, we also encountered this polystome in the *H. galamensis* examined. In September and October of 2014, we examined specimens of *H. galamensis* collected from a freshwater creek of the Niger Delta and also recorded infection with *P. galamensis*.

In this paper, we describe the parasites recovered

from frogs examined from three biotopes (derived savannah, guinea savannah and a freshwater creek) in Nigeria, comparing them with the type specimens from Togo.

## Materials and methods

*Hylarana galamensis* were collected from Igarra (7°17'N, 6°06'E), Agenegbode (7°06'N, 6°45'E), Ogbonna (7°07'N, 6°27'E), Agbede (06°50'N, 06°10'E) and Usen (6°45'N; 5°21'E) all in the derived savannah, New Bussa (10°15'N, 4°30'E) in the guinea savannah and Ase (5°17'N; 6°18'E) in the freshwater creeks of the Niger Delta of Nigeria.

The frogs were euthanized by immersion in Benzocaine solution and then dissected. The urinary bladders were removed and placed in Petri dishes containing 0.72% NaCl solution. The isolated parasites were flattened under cover slip pressure on microscope slides and fixed with 5% formol-saline for at least 48 hours. The parasites were washed free of the fixative in changes of tap water and then stained overnight in a dilute solution of acetocarmine. The parasites were washed with tap water to remove excess stain, dehydrated in alcohol series, cleared in xylene and permanent mounts made in Canada balsam (Aisien *et al* 2004a).



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## Results

Of the 152 specimens of *H. galamensis* collected from seven locations in the three biotopes surveyed 19 (12.5%) were infected with *P. galamensis* of which only 16 specimens were mature and egg-producing. The other 15 specimens recovered from Usen and 2 from Agenegbode were sub-adults. Prevalence of infection in the guinea-savannah was 21.4%, derived savannah, 15.1% and the freshwater creek, 2.27%. The prevalence and mean intensity of infection among frogs from the respective locations is presented in Table 1. The morphometric characters of the mature and egg-producing *P. galamensis* from Nigeria and those of the type species from Togo are presented in Table 2 while those of some sub-adults from Usen are presented in Table 3.

The general morphological characteristics of egg producing parasite from Nigeria is presented in Figure 1. Variations in intestinal arrangement in the parasites examined are shown in Figures 2A-F. While the lateral and medial diverticula were slender in some specimens

(Figures 2A-D), they were broad and lobed in others (Figures 2E and F).

The overall occurrence of intestinal anastomosis among the specimens examined was 26.7%, but the frequency of occurrence varied among parasites recovered from the different biotopes. The occurrence of anastomoses was highest among the parasite specimens from the guinea savannah (75%), followed by Usen (22.2%) and Agenegbode (20%) in the derived savannah. None of the specimens from Agbede and the single specimen from Ase possessed this feature. The hamuli variation in terms of the separation of handle and guard is shown in Figure 3A-F. In most of the specimens examined, the handle and guard were well separated (Figures 2A-E) while in a few, the separation was not so distinct (Figure 2F). The marginal hook C1 in the Nigerian specimens had a mean size of  $39.95 \pm 1.04 \mu\text{m}$  ( $39\text{-}41.6 \mu\text{m}$ ). Of the 16 egg-laying specimens studied, 11 had one egg each in the uterus, one had three eggs, another one had four eggs while two had six eggs each.

**Table 1.** Prevalence of *Polystoma galamensis* in *Hylarana galamensis* from Nigeria.

Location	No of hosts examined	No infected	Prevalence (%)	No of parasites	Mean intensity
<b>Derived Savannah</b>					
Igarra	2	–	–	–	–
Agenegbode	21	3	14.2	04	1.3
Ogbonna	15	1	6.70	01	1.0
Agbede	41	8	19.5	08	1.0
Usen	15	3	20.0	15	5.0
<b>Guinea Savannah</b>					
New Bussa	14	3	21.4	09	3.0
<b>Freshwater creek</b>					
Ase	44	1	2.27	01	1.0

**Table 2.** Comparison of the morphometric parameters of *Polystoma galamensis* from Nigeria and the specimens from Togo.

Parameters ( $\mu\text{m}$ )	<i>Polystoma galamensis</i> specimens	
	Nigeria	Togo*
Body Length	8332 $\pm$ 927.0(6933-9990)	8000-10000
Greatest width	2885 $\pm$ 551.31 (2200-3730)	–
Width at vaginae	2045 $\pm$ 299.80 (1667-2531)	1900-2200
Haptor length	2013 $\pm$ 297.10(1598-2500)	1700-1900
Haptor width	2848 $\pm$ 330.20 (2397-3463)	2600-3200
Haptor/length ratio	0.24 $\pm$ 0.03 (0.19-0.30)	–
Haptoral sucker diameter	547 $\pm$ 54.19.05 (439-692)	550-650
Oral sucker	540 $\pm$ 77.05 (412-644)	550-600
Lateral diverticula	47 $\pm$ 10.78 (30-62)	–
Medial diverticula	20 $\pm$ 2.76 (15-25)	–
Pharynx length	346 $\pm$ 34.40 (266-412)	320-400
Pharynx width	325 $\pm$ 32.14 (266-376)	300-400
Ovary length	971 $\pm$ 147.83 (738-1275)	1000
Ovary width	357 $\pm$ 88.25 (201-505)	500
Hamulus length	569 $\pm$ 69.24 (490-718)	650-700
Hamulus point	91 $\pm$ 10.15 (67-101)	–

Table 2 cont'd

Genital bulb	93.3±9.90 (67-106)	–
No of genital spine	7-8	–
Length of spine	35±3.26 (33-43)	35
Egg length	226 (206-242)	190-220
Egg diameter	136±32.20 (120-160)	140-150
Marginal hook C1	35.8±0.45 (35-36)	35
Presence of anastomosis	(25%)	20%

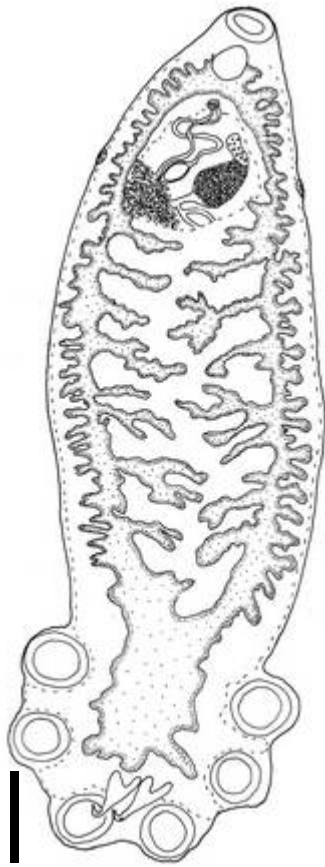
\*Euzet, Bourgat and Salami-Cadoux (1974).

**Table 3.** Morphometric parameters of subadult *Polystoma galamensis* specimens from Usen, Nigeria.

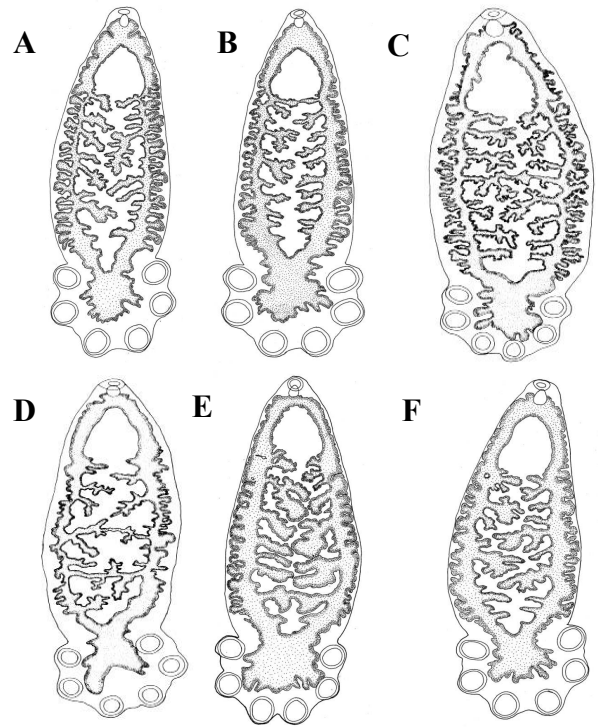
Parameters (µm)	Specimens from Usen, Nigeria
Body Length	6321±803(5594-7026)
Greatest width	2113±229 (1732-2431)
Width at vaginae	1428±125 (1198-1565)
Haptor length	1565±113(1399-1732)
Haptor width	2305±253 (2098-2931)
Haptor/length ratio	0.25±0.02 (0.21-0.27)
Haptoral sucker diameter	439 ±23.72 (399-479)
Oral sucker	334±46.28(292-433)
Lateral diverticula	51±7.49 (38-59)
Medial diverticula	23±1.86 (20-26)
Pharynx length	232±40.77 (186-279)
Pharynx width	222±42.38 (160-279)
Ovary length	653±73.40 (532-758)
Ovary width	286±33.92 (239-335)
Hamulus length	529±43.98 (439-599)
Hamulus point	22±1.63 (19.8-23.1)
Genital bulb	83±16.73 (66.5-106.4)
No of genital spine	7-8
Length of spine	9.73.±3.92 (33.3-42.9)
Egg length	–
Egg diameter	–
Marginal hook C1	?
Presence of anastomosis	(27.3)

**Table 4.** Size range of hamuli in some African polystomes.

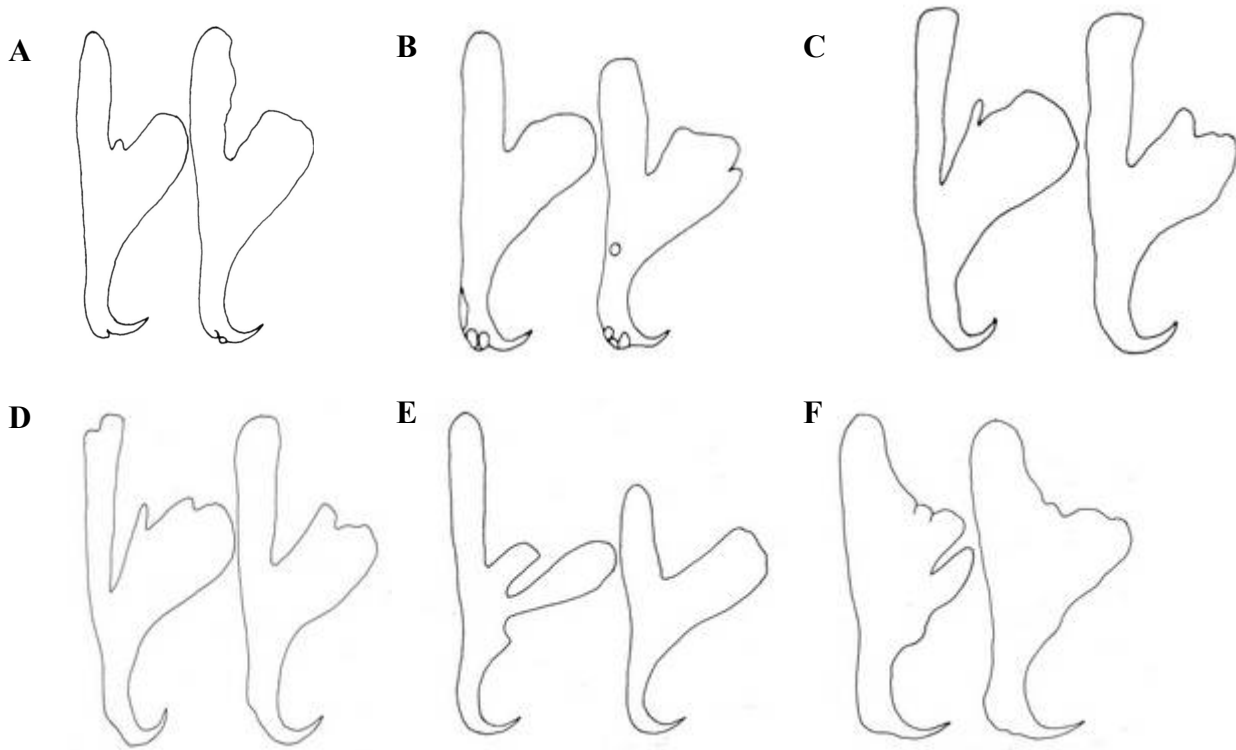
Parasite	Hamuli size (µm)	Authority
<i>P. aeschlimanni</i>	378-495	Bourgat and Murith (1980)
<i>P. africanum</i>	335-452	Aisien and Du Preez (2009)
<i>P. baeri</i>	330-420	Maeder <i>et al</i> (1970)
<i>P. channingi</i>	327-385	Du Preez (2013)
<i>P. dawiekoki</i>	285-485	Du Preez <i>et al</i> (2002)
<i>P. ebriensis</i>	270-404	Dupouy (1978)
<i>P. lamotei</i>	336-395	Bourgat and Murith (1980)
<i>P. makereri</i>	390-425	Tinsley (1973)
<i>P. manganoti</i>	470-530	Maeder <i>et al.</i> (1970)
<i>P. okomuensis</i>	325-456	Aisien <i>et al.</i> (2011)
<i>P. perreti</i>	347-402	Maeder (1973)
<i>P. ragnari</i>	360-400	Maeder <i>et al</i> (1970)
<i>P. sodwanensis</i>	407-442	Du Preez and Kok (1992)
<i>P. testimagna</i>	334-442	Du Preez and Kok (1993)



**Figure 1.** *Polystoma galamensis* infecting *Hylarana galamensis*. Scale bar: 1 mm.



**Figures 2A-F.** Variation in the intestinal arrangement of *P. galamensis* from Nigeria. Parasite without intestinal anastomoses (A); with one intestinal anastomosis (B, C, E); with two intestinal anastomoses (D); with slender lateral and medial diverticula (A-D); with broad lateral and medial diverticula (E, F).



**Figure 3A-F.** Variation in the hamuli shape of *P. galamensis* from Nigeria. Hamuli with handle well separated from the blade (A-E); hamulus with indistinct separation of handle and blade (F).

## Discussion

*Polystoma galamensis* was first described by Euzet *et al.* (1974) from *Rana galamensis* (now *Hylarana galamensis*) from Klouto, Togo, located in the savannah biotope of the country. Frogs from which this parasite were recovered in Nigeria were mostly collected from the savannah biotope, with Igarra, Agenegbode, Ogbonna, Agbede and Usen located in the derived savannah and New Bussa in the guinea savannah. For this reason this polystome was thought to be a parasite of frogs inhabiting drier environments as obtains in the savannah (Aisien *et al.* 2004b). It was therefore an interesting find when we recovered this parasite, albeit a single specimen from *H. galamensis* collected from the very humid environment of the freshwater creeks of the Niger Delta, where the rainy season spans over nine months of the year. It therefore seems that humidity is not a restricting factor in the distribution of the parasite since it infects hosts in the drier environment of the savannah as well as the humid environment of the Niger Delta.

Except for Igarra, *P. galamensis* was recorded from other locations in the derived savannah. The non-recovery of parasites from frogs collected at Igarra is most likely due to the small number of host specimens examined rather than the absence of *P. galamensis* from this location. While the frogs from Ogbonna and Agenegbode had parasite prevalence values less than 15% (6.70% and 14.20%, respectively), frogs from Agbede and Usen had prevalence values in the range of the 21.3% reported from Togo by Euzet *et al.* (1974). So also was the prevalence recorded in the frogs from the Guinea savannah at New Bussa. The situation was however very different in the creek environment at Ase, where only one (2.27%) of the 44 frogs examined was infected, despite the fact that *H. galamensis* was the dominant frog species encountered at Ase; occurring on land near the water front and several other locations inland. The reason for this low prevalence is unknown and can therefore only be a matter of conjecture. According to Roedel (2000), *H. galamensis* does not form breeding aggregations and in all the locations where we have encountered this frog, we did not observe any amplexant pair, even though the males make advertisement calls. This solitary tendency of individual frogs effectively reduces their chances of transmitting infection to new hosts. This assumption is supported by the prevalence of polystome infection in other frog species from Ase that form aggregations (*Ptychadena bibroni*, 12.5%; *P. mascareniensis*, 9.1% and *P. pumilio*, 40%), (Aisien *et al.* 2017). Another possible explanation for the low prevalence of *P. galamensis* in this location may be the level of precipitation. The rainy season in this region lasts from February to October and is accompanied by run-offs that possibly wash off the eggs of the parasite laid in inland puddles. At the later part of the rainy season (September and October), the area is often inundated and the ensuing flood results in egg/larval dilution that

again limit the chances of oncomiracidia/tadpole interaction. This assumption is supported by the observation that the highest prevalence of infection (21.4%) was recorded in the guinea savannah where the wet season is much shorter and level of precipitation smaller, affording the parasites better reproductive success.

The intensity of infection with *P. galamensis* seems generally low with most frogs harbouring at most two parasites. This may not be unconnected with the generally large sizes of the parasites. The parasites recovered from the frogs examined in Togo ranged from 8 mm to 10 mm in body length while egg-producing specimens from Nigerian frogs ranged from approximately 6.93 mm to 9.99 mm (mean  $8.33 \pm 0.93$  mm). Even in a case from Usen where a frog harboured 11 sub-adult parasites, they were only marginally smaller, measuring 5.59 mm to 7.03 mm (mean  $6.32 \pm 0.80$ ) in total body length (Table 3).

A comparison of the parasites from Nigeria and those from Togo (Euzet *et al.* 1974) showed close similarity in several aspects of their morphometry (Table 2). The characteristics of *P. galamensis* we recovered in Nigeria fit very closely into the description given by Euzet *et al.* (1974), thus confirming *P. galamensis* as a valid species. The values obtained for some of the measured parameters either overlapped or were in the same range for parasites collected in both countries (Table 2). The occurrence of intestinal anastomoses was also infrequent in parasites recovered from frogs in Nigeria although slightly higher (26.6%) than the value recorded in the specimens from Togo (20%). It however needs to be mentioned that parasites from the guinea savannah tended to have a greater frequency of occurrence (75%) of this feature than those from the derived savannah. The hamuli of *P. galamensis* are indeed very large when compared with those of some African polystomes (Table 4), whose upper range seldom exceeded 500  $\mu\text{m}$  in length. In the Nigerian specimens, the hamuli size ranged from 490-718  $\mu\text{m}$  (mean  $569 \pm 69.24$   $\mu\text{m}$ ) while in those from Togo the range was from 650-700  $\mu\text{m}$ .

As was observed in the parasites from Togo, there was also variation in the hamuli shape of parasites recovered from frogs in Nigeria. While most hamuli in the Nigerian materials had handles which were well separated from their guards (Figures 3A-E), the separation in a few of them was not so distinct (Figure 2F). Whereas Euzet *et al.* (1974) reported the size of the C1 marginal hook to be 35  $\mu\text{m}$ , the C1 marginals of *P. galamensis* from Nigeria were consistently longer, with a mean length of  $39.9 \pm 1.04$   $\mu\text{m}$  and ranged from 39.0-41.6  $\mu\text{m}$ . A re-examination of the type specimen may be necessary to clear this point. While the lateral and medial diverticula in the parasites from Togo were all shown to be slender, those from Nigeria did not share this uniformity: some were slender (Figures 2A-D) with highly branched medial diverticula (Figure 2C and D); others were broad and lobed (Figures 2E and F).

In the original description of *P. galamensis*, Euzet *et al* (1974) observed intra-uterine egg development, a phenomenon which had previously been reported in two European *Polystoma* species (*P. integerimum* and *P. pelobatis*) by Combes (1968). Although we did not observe this phenomenon in the parasites we collected from Nigeria, it is important to note that this phenomenon is now known to be widespread among members of the Polystomatidae studied in Africa and elsewhere. For members of the genus *Polystoma*, this reproductive strategy occurs in *P. africanum* (Salami-Cadoux, 1979), *P. grassei* (Dupouy and Combes, 1977; Murith *et al* 1977), *P. mashoni* (Beverley-Burton, 1962), *P. natalensis* (Combes and Channing, 1979), *P. nearcticum* (Paul, 1938), and *P. togoensis* (Bourgat, 1977; Murith, 1981). For *Eupolystoma* spp. intra-uterine development is known to occur in *E. alluaudi* (Combes *et al* 1973; Salami-Cadoux, 1975; Tinsley, 1975), *E. anterorchis* (Tinsley, 1978) and *E. vanasi* (Du Preez *et al* 2003). This process is also known to occur in *Metapolystoma brygoonis* (Euzet and Combes, 1964), *M. cachani* (Murith *et al* 1977), *M. porrosissima* (Du Preez and Kok, 1992), *Kankana manampoka* (Raharivololoniaina *et al* 2011) and *Madapolystoma biritika* (Du Preez *et al* 2010). In summary, we have described here *Polystoma galamensis* recovered from *Hylarana galamensis* in Nigeria. This is the second description of this parasite in Africa. Whereas previous reports until now have confined the parasite to frogs in the drier environment of the savannah, this report has shown that *P. galamensis* also infects frogs occurring in the very humid environment as occurs in the Niger Delta region of Nigeria. Parasites from Nigeria and Togo share many common morphometric characteristics, which confirm *P. galamensis* as a valid species. The low prevalence of the parasite may be generally connected with the solitary reproductive behaviour of the host while in the Niger Delta, excessive precipitation and flooding may additionally play limiting roles.

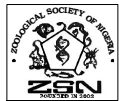
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