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Nematodes of *Chathariniella* (Pharyngodonidae) from freshwater fishes in Senegal, with a key to species

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Summary

Three nematode species of *Chathariniella* (Pharyngodonidae), *C. citharini, C. khalili,* and *C. gonzalesi,* were recorded from the recta of squeaker (Mochokidae: Siluriformes) and citharinid (Citharinidae: Characiformes) fishes from Senegal, West Africa. Morphological characteristics obtained by scanning electron microscopy (form of oral aperture and cephalic papillae, presence of lateral alae, distribution and form of cloacal papillae, simple or paired papillae on tail of males, eggs with numerous long filaments on each pole in females) correspond well to the generic diagnosis and represent species differences. The shape and size of the cephalic papillae and lips were identified as a new determination feature. *C. gonzalesi* is reported for the first time from Senegal and its host, *Paradistichodus dimidiatu*s, represents a new host record. A tentative simple key for differentiating *C. citharini, C. khalili, C. gonzalesi and C. petterae* is provided based upon these results.

Keywords: Nematoda; *Chathariniella*; Siluriformes; Characiformes; fishes; West Africa; key to species

Introduction

The nematode genus *Chathariniella* Khalil, 1964 presently includes four nominal species that parasitize African freshwater fishes only. The type species, *Chathariniella citharini* Khalil, 1964, has been reported from the Sudan (Khalil, 1964), Egypt (Moravec, 1974) and Senegal (Petter *et al.*, 1972). Other species are: *C. khalili* Petter, Vassiliadès et Troncy, 1972, reported from Senegal and Chad (Petter *et al.*, 1972); *C. gonzalesi* Van Waerebeke, Chabaud, Bain et Georges, 1988, from the Central African Republic (Van Waerebeke *et al.*, 1988); and *C. petterae* Khalil, 1974, from Zimbabwe (Khalil & Polling, 1997). Moravec (1994) first time studied the head morphology of *C. citharini* using scanning electron microscopy and stated that species of *Chathariniella* are morphometrically similar and, therefore, the taxonomic revision is necessary.
In this study, *C. citharini*, *C. khalili* and *C. gonzalesi* nematodes were collected from freshwater fishes of Senegal (West Africa) and both light and scanning electron microscopy were used for a morphological and metrical comparison.

Materials and Methods

Fishes were collected at two localities in the Niokolo Koba National Park in West Senegal (the Gambia River and the Mare de Simenti, a large, shallow, temporary oxbow lake that is seasonally connected to the main channel of the Gambia River) using gill and seine nets during November 2005 and March 2006. Nematodes were removed from the digestive tract during dissection, placed in 0.6 % saline solution and fixed in hot 4 % formaldehyde solution. Nematodes were gradually cleared in glycerine before examination under a light microscope equipped with differential interference contrast, a digital image analysis system (analySIS five auto) and a drawing attachment for morphometric analysis.
Five male and five female specimens of each species were washed several times in 0.6 % saline, fixed with hot 4 % formaldehyde solution and then stored at room temperature for examination using scanning electron microscopy (SEM). Prior to examination in a VEGA scanning electron microscope operating at 20 kV, the parasites were dehydrated using ethanol, dried in a CPD 030 critical point drying apparatus (Bal-Tec) using liquid CO_2, mounted on aluminium stubs with double-sided adhesive discs and coated with gold in an SCD 040 sputter coating unit (Balzers).
Reference materials (5 males and 5 females) were depo-

Table 1. Comparison of measurements of *Citharinielle* males (all measurements in µm). (1) According to Khalil (1964), (2) our material, (3) Moravec (1974), (4) Khalil (1974), (5) Petter et al. (1972), (6) Van Waerebeke *et al*. (1988)

	C. citharini				*C. petterae*	*C. khalili*			*C. gonzalesi*			
Distribution	Sudan (1)	Senegal (2)		Egypt (3)	Zimbabwe (4)	Senegal (5)	Senegal (2)		Central African Republic (6)		Senegal (2)	
Measurements	Range	Range	Mean	Range	2 males only		Range	Mean	Range	Mean	Range	Mean
Body length	3270-3710	2306-3722	3031	1470-2240	2270-2930	1550	1550-1970	1799	1700-2020	1900	718-1936	1305
Max. body width	170-200	146-256	208	95-163	170-250	100	95-125	112	139-151	146	97-142	124
Entire oesophagus length	340-400	329-382	354	375-516	665-704	275	339-405	375	463-541	497	300-366	337
Bulb length	90-120	78-104	90	75-105	127-139	-	64-80	71	diameter	85	70-78	74
Bulb width	89-90	84-104	93	84-96	104-127	-	65-84	70			72-78	75
Nerve ring	150-170	137-201	154	105-165	181-193	110	117-184	150	139-151	146	105-121	14
Excretory pore	670-700	441-642	559	504-669	700-860	440	482-595	541	528-639	580	366-467	383
Tail length	670-940	732-882	805	405-450	440-520	260	288-366	323	296-352	325	277-352	300
Pseudosucker - cloaca distance	670-870	449-756	602	-	-	-	-	-	-	-	-	-
Spicule length	170-190	166-190	179	78-102	99-105	85	76-101	89	73-86	81	99-116	107
Capitulum length	30-40	36-41	38	-	-	-	18-26	21	-	-	24-34	29
Max. spicule width	9	8-13	10	-	12	-	8-9	8.5	-	-	10-13	12
Gubernaculum length	30-50	45-63	53	45-60	**35***	25	23-38	31	30-33	32	51-57	55
Cloaca - tail papillae distance	80	71-118	96	69-99	77	60	115-149	121	-	-	28-44	36
Vestibulum length	15-21	19-25	22	-	11	-	13-22	19	-	-	10-12	11
Tail end - tail papillae distance	-	610-778	709	-	363-443	200	173-217	202	-	-	249-285	264

* This figure was erroneously given as 0.35 in Khalil (1974)

sited in the helminthological collection of the Institute of Parasitology of the Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (*C. citharini* - Cat. No. N–18, *C. khalili* - N–906 and *C. gonzalesi* - N–905), and further voucher specimens were deposited in the Department of Botany and Zoology of Masaryk University in Brno, Czech Republic.

Results

Pharyngodonidae Travassos, 1919
Chathariniella citharini Khalil, 1964 (Figs. 1, 4A)

Description
Small whitish nematodes, with fine transverse striation of cuticle in cervical and median part of the body, narrow lateral alae (10 – 12 μm high) initiating posteriorly to level of anterior extremity and ending anteriorly to level of cloaca in males (Fig. 1F) and anus in females (Fig. 1C). Oral aperture triangular and surrounded by six oral lamellae raised above apical surface, 4 being dorsolateral and 2 ventrolateral (Fig. 1A). Four small oval cephalic papillae and pair of lateral amphids present. Ratio of cephalic papilla width and dorsal lip width 1:3 (Fig. 4A). Dorsal lip bears two papillae; ventrolateral lips each bear a single papilla only. Mouth opening leads into a shallow buccal cavity at the bottom of which there are three sclerotised ribbed flaps. Oesophagus cylindrical, uniform in diameter and ending in a single bulb that is separated by a constriction (isthmus) from the end of the corpus and provided with the valvular apparatus. Intestine straight, narrow, its anterior end slightly extended. The excretory pore lies relatively far below the bulb.



Fig. 1. Scanning electron micrographs of *Chathariniella citharini*. A) Cephalic end of female, a - amphid, b - cephalic papilla, c - anterior end of lateral ala; B) Vulva and anus of female; C) Caudal end of female with lateral ala; D) Eggs with filaments; E) Caudal end of male, lateral view; F) Caudal end of male with spicula, single post-cloacal papilla and lateral ala, sub-lateral view; G) Pre-cloacal sucker-like organ of male, lateral view; H) Cloacal region of male, a - four cloacal papillae, b - pair of post-cloacal papillae; I) Detail of cloacal papillae; J) Detail of post-cloacal papilla, ventral view. Scale-bars: A, H, J = 50 μm; B, C = 200 μm; D = 30 μm; E, F, G = 100 μm; I = 20 μm.

Table 2. Comparison of measurements of *Chitariniella* females (all measurements in µm). (1) According to Khalil (1964), (2) Moravec (1974), (3) and (6) Petter et al. (1972), (4) our material, (5) Khalil (1974), (7) Van Waerebeke et al. (1988).

	Chitariniella citharini					*C. petterae*	*C. khalili*			*C. gonzalesi*			
Distribution	Sudan (1)	Egypt (2)	Senegal (3)	Senegal (4)		Zimbabwe (5)	Senegal, (6)	Senegal (4)		Central African Republic (7)		Senegal (4)	
Measurements	Range	Range	One female	Range	Mean	Range		Range	Mean	Range	Mean	Range	Mean
Body length	5270-5830	1810-4580	4000	3460-4961	4210	3780-5190	2300	2331-3580	2839	2640-3130	2910	1519-3424	2338
Max. body width	280-300	204-340	-	215-435	291	300-410	160	172-287	227	139-211	169	118-325	193
Vestibulum length	15-21	-	30	17-29	22	15-19	20	15-26	23	-	-	-	-
Entire oesophagus length	410-500	615-707	450	407-461	426	770-950	400	495-559	527	657-720	678	394-534	450
Bulb length	130-160	123-138	-	99-120	106	136-174	-	84-130	109	-	112	86-135	106
Bulb width	100	129-138	-	94-130	118	127-164	-	96-132	115	-	-	80-138	106
Nerve ring	170-200	123-186	180	157-200	180	185-232	150	161-196	175	163-191	176	104-156	133
Excretory pore	960-1150	621-1088	675	601-742	664	980-1100	540	617-825	707	713-815	774	451-811	55
Head-vulva distance	3920-4530	850-3520	-	2278-3585	2961	2360-3290	1440	1444-2228	1746	1810-2260	2000	900-2311	1374
Vulva-anus distance	200-270	210-217	180	109-254	192	442-776	360	338-570	426	-	-	233-664	391
Tail length	1150-1210	750-843	1100	901-1241	1087	978-1124	500	493-789	656	445-563	529	361-765	493
Ovijector length	220-270	-	-	212-305	254	260-290	400	93-143	122	200 by sphincter	400	111-477	298
Ovijector width	50-60	-	-	50-83	67	-	-	40-57	47	-	-	39-82	64
Egg length	122	129-138	110	105-115	109	116-152	110	100-122	112	99-119	-	100-124	106
Egg width	30	42-45	35	26-37	32	38-45	35	33-43	39	33-38	-	24-37	31

Male (10 specimens): Width of the cephalic end 58 – 68 µm (mean 62), corpus length 212 – 251 µm (236), isthmus 12 – 42 µm (27). Single testis extends anteriorly to a level slightly posterior to the excretory pore. Gubernaculum boat-shaped, not strongly sclerotised. Both cloacal lips are provided with a pair of papillae, one placed laterally on each side of the lip (Fig. 1H, I). A single postcloacal large papilla, 14 – 25 µm long (21), situated 661 – 778 µm (709) from the end of the tail tip (Fig. 1E, F, J). Precloacal pseudosucker organ present (Fig. 1G). Other measurements in Table 1.

Female (10 gravid specimens): Width of the cephalic end 72 – 88 µm (79), corpus length 273 – 324 µm (288), isthmus length 20 – 48 µm (33). Vulva without prominent lips, situated a short distance from the anus (Fig. 1B). Two ovaries present, the anterior lying slightly below the bulb and the posterior attaining the vulva. Ovijector muscular. Uterine branches joining into a relatively long, posteriorly directed vagina, uterus coiled and filled with eggs. Tail long, posterior anal lip slightly elevated. Eggs elongate, thin walled, partly embryonated at the time of laying; provided with very long filaments (8 – 10) at each pole (Fig. 1D). Other measurements in Table 2.

Host: *Citharinus citharus* (Geoffroy Saint-Hilaire) (Citharinidae, Characiformes).

Site of infection: rectum.

Prevalence and intensity of infection: 60 % (10 fish examined), 2 – 125 parasites per fish.

Locality: Gambia River, Mare de Simenti temporary oxbow lake, Niokolo Koba National Park, Senegal, West Africa.

Date: November 2005, March 2006

Comments: The type-species of the genus *Citharinella* (*C. citharini*) has been described from *Citharinus citharus* (Citharinidae) (Khalil, 1964), and re-described from the same host and from *Distichodus brevipinnis* Günther, 1864 (Citharinidae) by Petter *et al.* (1972), and from *Synodontis*

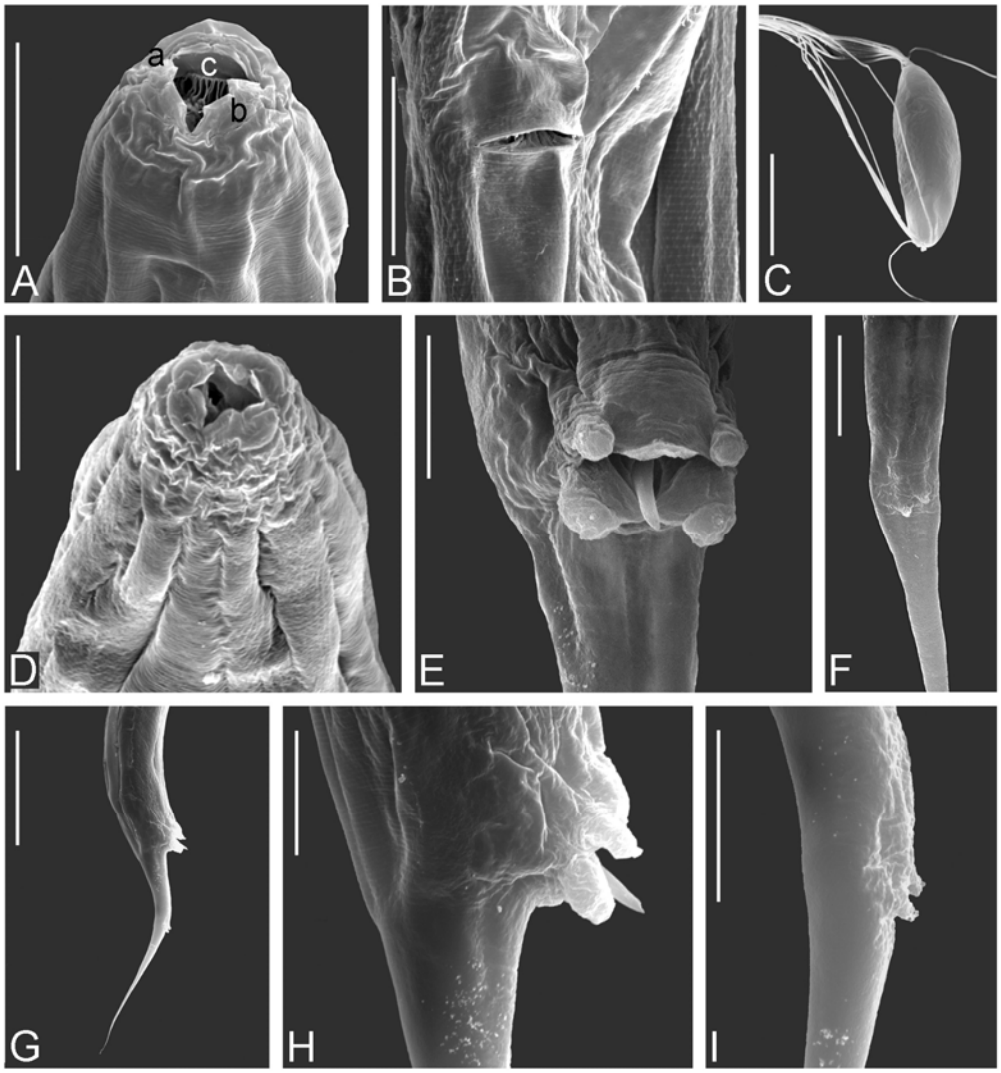


Fig. 2. Scanning electron micrographs of *Citharinella khalili*. A) Cephalic end of female, a - amphid, b - cephalic papilla, c - ribbed flap; B) Vulva of female; C) Egg with filaments; D) Cephalic end of male; E) Cloacal region of male with spicula and cloacal papillae, ventral view; F) Two post-cloacal papillae of male, ventral view; G) Caudal end of male, lateral view; H) Cloacal region of male, lateral view; I) Detail of two post-cloacal papillae, lateral view. Scale-bars: A, B, C = 50 µm, D, E, F, H, I = 20µm, G = 100µm.

schall and *S. serratus* (Mochokidae) by Imam (1971), Moravec (1974, 1994), Fahmy *et al.* (1976), El-Naffar *et al.* (1983) and Imam *et al.* (1991). The presence of a precloacal pseudosucker-like organ on the ventral side of males is typical of this species. This organ was observed in all males of our material. Moravec (1994) described the morphology of the cephalic end in detail using SEM. Despite slight differences from the original description of Khalil (1964) (mainly in the measurements of the body and individual organs), specimens were assigned to the type-species *C. citharini*.

Our measurements of various characteristics of *C. citharin*i from the type host (Tables 1 and 2) confirm a range of interspecific variability within this taxon. We agree with Moravec (1974) that these differences are associated with the age of the nematodes or fish host species. For example, we found the ratio of tail length to total body length in males to be 20.5 – 25.4 %, 23.7 – 31.7 % and 20.1 – 27.6 %, and the ratio of spicule length to total body length 5.12 – 5.20 %, 5.10 – 7.2 % and 4.6 – 5.3 %, in the Sudan, Senegal and Egyptian nematode populations, respectively.

Females from our material (Table 2) exhibited some non-significant proportional differences to those of Moravec (1974), e.g., in total oesophagus length (shorter) and tail length (larger). An apparent difference in egg dimensions was observed in comparison to both Khalil (1964) and Moravec (1974). Eggs length of the Egyptian *C. citharini* population (sampled from *Synodontis* spp.) was found to be longest (Moravec, 1974) and those of the Senegalese population (one female only) were shortest (Petter *et al.*, 1972). Specimens from the Sudan were intermediate to those of Egypt and Senegal (Table 2). Differences found in egg measurements can be considered as the effect of variability, fixation, developmental stage of the eggs, or possibly incorrect measuring. The presence of polar filaments has already been mentioned as a remarkable characteristic by Imam (1971), Petter *et al.* (1972) and Moravec (1974), but not by Khalil (1964). Polar filaments are characteristic for all species of the genus *Chathariniella.*

Chathariniella khalili Petter, Vassiliadès et Troncy, 1972 (Figs. 2, 4B)

Description

Cuticle with fine transverse striations. Narrower lateral alae (about 8 – 10 µm high) present. Oral opening triangular, provided with six oral lamellae raised slightly above the apical surface, four of them being dorsolateral and two ventrolateral. Four large cephalic papillae (Fig. 2A, D). Ventrolateral lips each bearing a single papilla. Pair of amphids present. Ratio of cephalic papilla width and dorsal lip width 1:2 (Fig. 4B). Mouth opening with three sclerotised ribbed flaps at bottom (Fig. 2A). Oesophagus cylindrical, uniform in diameter, divided into corpus, isthmus and bulb. Intestine straight, narrow, its anterior end not extended. Excretory pore situated posteriorly to bulb.

Male (10 specimens): Width of the cephalic end 37 – 42 µm (mean 40), vestibulum 13 – 22 µm in length (19), corpus length 253 – 316 µm (286), isthmus length 14 – 25 µm (19), bulb 64 – 80 µm long (71) and 65 – 84 wide (70). Cloacal opening provided with two lips, both with two large papillae, one on each side (Fig. 2E, H). A pair of small post-cloacal papillae situated 173 – 217 µm (202) from the end of the tail (Fig. 2F, G, I). Maximum width of spicule 8 – 9 µm (8.5), capitulum length at the proximal end 18 – 26 µm (21). Sucker-like organ absent. Other measurements in Table 1.

Female (10 gravid specimens): Width of cephalic end 45 – 67 µm (58), corpus length 377 – 420 µm (399), isthmus 11 – 33 µm (20), bulb 84 – 130 µm (109), bulb width 96 – 132 µm (115). Vulva without prominent lips, lying in posterior third of the body (Fig. 2B). Ovijector directed anteriorly, muscular. Uterus didelphic, running in opposite direction, coiled and filled with eggs. Tail long and narrow. Eggs elongate, thin walled, partly embryonated and provided with very long filaments (5 – 7) at each pole (Fig. 2C). Other measurements in Table 2.

Hosts: *Synodontis ocellifer* Boulenger*, S. batensoda* Rüppell*, S. nigrita* Valenciennes*, S. schall* (Bloch & Schneider) (Mochokidae, Siluriformes).

Site of infection: rectum.

Prevalence and intensity of infection: 68 % (22 fish examined; 1 – 45 parasites per fish).

Locality: Gambia River, Mare de Simenti temporary oxbow lake, Niokolo Koba National Park, Senegal, West Africa.

Date: November 2005, March 2006.

Comments: Chathariniella khalili was described by Petter *et al.* (1972) from the type host *Synodontis sorex* Günther from Senegal and another host, *S. gambiensis* Günther, from Chad. Single male and female specimens, respectively, were measured. Our *C. khalili* recorded in two new host species (*S. ocellifer* and *S. batensoda*) correspond morphologically with the original species description (both males and females). The metrical features (nematode population from *S. ocellifer*) answer to the values from the original description (Petter *et al.*, 1972), with small variations representing apparently the natural intraspecific variability of this taxon (Tables 1 and 2). Some unimportant differences in metrical characters (longer oesophagus and distance of excretory pore from anterior end) in males and females are probably associated with nematode age or reflect host variability. Differences in the length of the muscular part of the ovijector in females (Table 2) may be the result of incorrect measuring.

Chathariniella gonzalesi Van Waerebeke, Chabaud, Bain et Georges, 1988 (Figs. 3, 4C)

Description

Mouth opening triangular; six small oral lamellae and two laterally situated amphids present. Head cuticle smooth, body cuticle with fine transverse striations. Four large cephalic papillae, two on dorsal lip and one at each ventrolateral lip (Fig. 3A). Head cuticle smooth, body cuticle

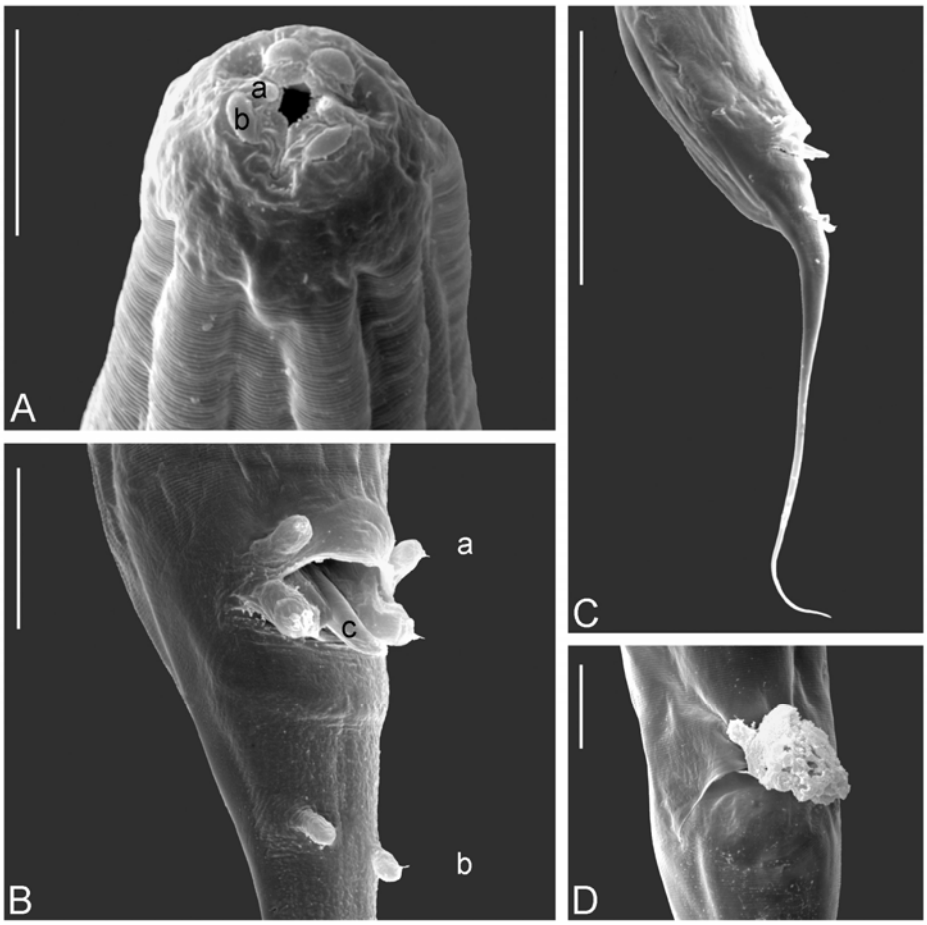


Fig. 3. Scanning electron micrographs of *Chitariniella gonzalesi*. A) Cephalic end of female, a - amphid, b - cephalic papilla; B) Cloacal region of male, lateroventral view, a - four cloacal papillae, b - pair of post-cloacal papillae, c - spicula; C) Caudal end of male, lateral view; D) Anal region of female. Scale-bars: A, B, D = 20 μm; C = 100 μm.

with fine transverse striations. Ratio of cephalic papilla width and dorsal lip width 1:2 (Fig. 4C). Buccal cavity triangular and shallow with three sclerotised, ribbed flaps at bottom. Narrow cuticular lateral ala present. Oesophagus cylindrical, formed by corpus, isthmus and bulb. Bulb with valvular apparatus. Intestine straight, its anterior end slightly extended. Excretory pore situated posteriorly to bulb.

Male (5 specimens): Width of cephalic end 26 – 31 μm (mean 29), corpus length 209 – 271 μm (240) and isthmus length 16 – 22 μm (19). Testis extended anteriorly slightly posterior to excretory pore. Spicule strongly sclerotised. Gubernaculum boat-shaped and slightly sclerotised. Cloacal opening with two lips, which are provided with a pair of papillae, one at each side. A pair of small papillae is situated 249 – 285 μm (264) from the end of tail and 28 – 44 μm (36) from the cloaca (Fig. 3B, C). Maximum width of spicule 10 – 13 μm (12), capitulum at the proximal end 24 – 34 μm (29) in length. Sucker-like organ absent. Other measurements in Table 1.

Females (5 gravid specimens): Width of cephalic end 43 – 62 μm (mean 52), length of corpus 290 – 366 μm (325), isthmus 11 – 25 μm (18) and bulb 86 – 135 μm (106), bulb width 80 – 138 μm (105). Vulva without prominent lips, situated 223 – 507 μm (391) anterior to anus. Ovijector directed anteriorly, muscular. Uterus didelphic, running in the opposite direction, coiled and filled with eggs. Tail long and narrow. Eggs elongate, thin-walled, partly embryonated and provided with 5 – 8 (7) very long filaments at each pole. Other measurements in Table 2.

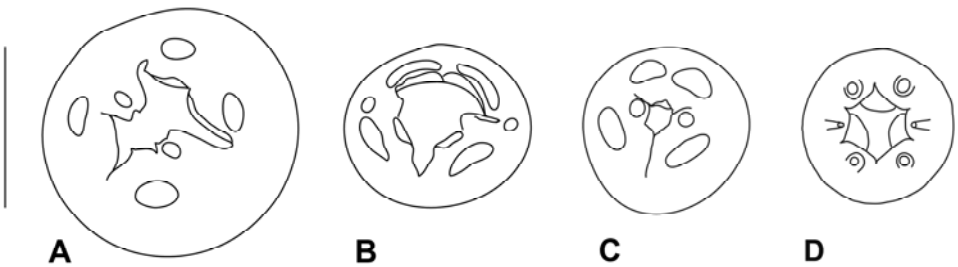


Fig. 4. Line drawings of female cephalic end, apical view; reconstruction. A) *Chitariniella citharini*; B) *Chitariniella khalili*; C) *Chitariniella gonzalesi*. D) *Chitariniella petterae*, according to Khalil (1974). Scale-bar = 50 μm.

Table 3. Ratio of selected characteristics of *Chitharinella* males as a percentage (i.e. oesophagus length x 100 / body length). *C. citharini, C. khalili* and *C. gonzalesi* from our material; *C. petterae* according to Khalil (1974)

	C. citharini		*C. petterae*	*C. khalili*		*C. gonzalesi*	
Ratios	Range	Mean	Range	Range	Mean	Range	Mean
Oesophagus length/body length	10.0-13.5	11.7	24.1-29.3	17.9-24.8	20.9	17.7-47.2*	25.8
Tail length/body length	22.4-31.1	26.6	17.7-19.4	15.2-20.5	18.0	16.8-40.9 **	23.0
Distance of tail papillae from tail end/total tail length	86.3-90.3	88.1	82.5-85.2	58.2-66.7	62.5	86.0-89.9	88
Distance of cloaca from tail papillae/total tail length	7.8-14.2	11.9	9.0-17.5	33.7-41.8	37.5	10.1-13.5	12.0

* High values were obtained from two males with a short body length (718 and 807 µm). When these were removed, the ratio for long-bodied males only (>1200 µm) was 16.8-22.4.
** High values were obtained from two males with a short body length (718 and 807 µm). When these were removed, the ratio for long-bodied males only (>1200 µm) was 17.7-24.2.

Table 4. Ratios of selected measured characteristics of *Chitharinella* females as a percentage (i.e. tail length x 100 / body length); *C. citharini, C. khalili* and *C. gonzalesi* from our material; *C. petterae* according to Khalil (1974)

	C. citharini		*C. petterae*	*C. khalili*		*C. gonzalesi*	
Ratios	Range	Mean	Range	Range	Mean	Range	Mean
Tail length/body length	22.4-28.1	25.8	21.7-25.9	21.2-25.7	23.1	13.6-24.0	19.5
Vulva-anus distance /body length	3.2-5.8	4.7	11.2-15.0	13.7-17.2	15.7	12.3-19.5	16.7
Vulva-anus distance /tail length	10.1-21.4	17.7	45.2-69.0	55.0-77.4	72.0	61.8-89.4	79.3
Oesophagus length/body length	8.9-11.5	10.1	18.3-20.4	14.9-21.4	17.4	15.6-25.9	19.3
Head-vulva distance/body length	59.6-72.3	70.3	62.4-63.4	60.2-64.1	62.6	55.3-69.8	63.9

Host: *Paradistichodus dimidiatus* (Pellegrin) (Distichodontidae, Characiformes).
Site of infection: rectum.
Prevalence and intensity of infection: 60 % (5 fish examined); 1 – 25 parasites per fish.
Locality: Gambia River, Mare de Simenti temporary oxbow lake, Niokolo Koba National Park, Senegal, West Africa.
Date: November 2005, March 2006.

Comments: Cithariniella gonzalesi was first described by Van Waerebeke *et al.* (1988) in *S. longirostris* Boulenger from the Central African Republic. Our finding of *C. gonzalesi* is the first record of this parasite in Senegal and *P. dimidiatus* represents a new host record for this parasite. The nematodes collected in this study agree morphologically with the original description, except for the cloacal papillae of males being more prominent (Table 1). This difference could be the result of intraspecific variability, of the fixation and mounting process or of the more detailed observation of the cloacal region using SEM. Small metrical differences in males were observed in oesophagus length, distance of nerve ganglion and excretory pore from anterior end, and of spicule and gubernaculum length (Table 1). A number of metrical differences were observed in females, namely a shorter oesophagus length and a shorter distance of the nerve ring from the anterior end (Table 2).

Discussion

The morphology of all *Cithariniella* species is very similar (Khalil, 1964, 1974; Moravec, 1974, 1994; Petter *et al.*, 1972, Van Waerebeke *et al.*, 1988). In this study, narrow lateral cuticular alae were observed in both sexes of all species studied. The spicule, ending proximaly in the capitulum in males; very long filaments at each pole of the eggs in females are present. Until now, the presence or absence of a sucker-like organ has been used as the key characteristic for species determination, along with the form and number of post-anal papillae on the tail (single or two papillae present) in males and the structure of the cephalic end in females. Metric characteristics were not used.
The use of morphometric characteristics, *Cithariniella* males possessing a single post-anal tail papilla and longer body size (1470 – 3722 µm) were identified as *C. citharini* or *C. petterae*, and species with two post-anal papillae on the tail and a shorter body size (1718 – 2020 µm) as *C. khalili* or *C. gonzalesi*. *C. citharini* and *C. petterae* differ in length of oesophagus, length of spicule and capitulum, in the distance of the post-cloacal papilla from the end of the tail (Table 1). Khalil (1974), in referring to *C. petterae*, stated that "it is not known for certain whether the pair of papillae on the posterior lip of the cloaca are definitely absent or could not be seen in the two available males". The genital cone of *C. khalili* protrudes, its cloacal papillae

Key to species of *Cithariniella*

The present key of four species of Cithariniella does not use the "lateral alae absent" by Anderson and Lim (1996), because narow lateral alae are present in both sexes of *C. citharini, C. khalili* and *C. gonzalesi*, in *C. petterae* has previously been reported by Khalil (1973). Size of cephalic papillae in combination with oesophagus and tail length are a new character to distinguish females.

1. Cephalic papillae smaller, width ratio of papilla and dorsal lip 1 : 3-4. Single papilla on caudal appendage in male 2

- Cephalic papillae bigger, width ratio of papilla and dorsal lip 1 : 2. Two papillae on caudal appendage in male present 3

2. Male: Pre-anal sucker-like organ present. Female: Eggs with 8 – 10 long polar filaments; oesophagus length 407-707 µm, tail length 750-1210 µm..... *C. citharini*

- Male: Pre-anal sucker-like organ absent. Female: Eggs with 5 long polar filaments; oesophagus length 770-950 µm, tail length 987-1124 µm..... *C. petterae*

3. Male: Postcloacal papillae in short finger-like form; ratio distance of cloaca from tail papillae, total tail length (in%) 33- 42 ..... *C. khalili*

- Male: Postcloacal papillae slightly higher and finger-like form, well separated; ratio distance of cloaca from tail papillae, total tail length (in %) 10-14 ..... *C. gonzalesi*

are short and half round and the post-anal papillae are lower, being situated in the middle part of the tail. On the contrary, the genital cone of *C. gonzalesi* is truncated, the cloacal papillae are longer and finger-like, and the post-anal papillae are situated closer to the cloaca (Table 1). Males can be distinguished using the ratio of selected characters (Table 3).

Females of *C. citharini* and *C. petterae* tend to be larger (1810 – 5830 µm) than those of *C. khalili* and *C. gonzalesi* (1519 – 3580 µm), and differ in the length of oesophagus and distance between vulva and anus (Table 2). Metric characteristics cannot be used for differentiation between females as the values overlap considerably (Table 4). Despite this, our SEM study indicates that the head end of female *C. citharini* differs from *C. khalili* and *C. gonzalesi* in the shape and size of the cephalic papillae, which are small and globular in *C. citharini* and oval and longer in *C. khalili* and *C. gonzalesi* (width ratio of cephalic papilla and dorsal lip 1:2).

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