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Original article

Morphological and genetic characterizations of *Avitellina* tapeworms from domestic ruminants in Senegal: An evidence of specificity among sheep and cattle host

Mallé Ndom[a,*], Gora Diop[a], Tetsuya Yanagida[b], Yann Quilichini[d], Alioune Dieye[e], Bernard Marchand[d], Minoru Nakao[c], Akira Ito[c], Tidiane B.A. Cheikh[a]

[a] Laboratoire de Biologie Évolutive, d'Écologie et de Gestion des Écosystèmes, Faculté des Sciences et Techniques, Université Cheikh Anta DIOP de Dakar, BP 5005 Dakar, Sénégal
[b] Laboratory of Veterinary Parasitology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8515, Japan
[c] Department of Parasitology, Asahikawa Medical University, Asahikawa 078-8510, Japan
[d] CNRS –UMR SPE 6134, Service d'Étude et de Recherche en Microscopie Électronique, Campus Grimaldi, BP 52, 20250 Corte, Université de Corse, Corse, France
[e] Service d'Immunologie FMPO, Université Cheikh Anta DIOP de Dakar, Dakar, Sénégal

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ABSTRACT

Avitellina tapeworms are common intestinal parasites of ruminants with a worldwide distribution. In Senegal, only *Avitellina centripunctata* tapeworm has been reported to date, and genetic diversity was previously confirmed by enzymatic analysis. This study aims to clarify the diversity of *Avitellina* tapeworms isolated from sheep and cattle in Senegal. In total, 613 adult *Avitellina* tapeworms were collected from sheep and cattle. Morphological analysis by the light microscopy and scanning electron microscopy identified three *Avitellina* "morphospecies": *A. centripunctata* and *Avitellina* sp.2 were detected in sheep while *Avitellina* sp.3 was identified in cattle. Molecular phylogenetic analysis based on the complete mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1) sequences revealed that 101 *Avitellina* tapeworms were divided into 54 haplotypes grouped into three clades, of which two were specific to sheep and one specific to cattle. Three morphospecies corresponded to each of three clades and the maximum pairwise divergence among the clades ranged from 9.7 to 18.5% in *cox1*. The present study demonstrates the unexpected diversity of *Avitellina* tapeworms in domestic ruminants, and emphasize the necessity of re-evaluation of the taxonomy of the genus.

1. Introduction

Avitellina tapeworms are the intestinal cestodes of wild and domestic ruminants. Members of the genus have an **indirect life cycle** with **ruminants** as **final hosts,** with oribatid mites or collembolans as intermediate hosts (Narsapur, 1988; Denegri et al., 1998). After ingestion of the intermediate hosts, cysticercoid larvae actively move to small intestine of the definitive host and mature (Soulsby, 1986; Denegri et al., 1998; Ndom et al., 2016).

Avitellina tapeworms have a worldwide distribution, and a great veterinary importance (Woodland, 1935; Schmidt, 1986; Belem et al., 2001; Achi et al., 2003a,b). The prevalence of *Avitellina* tapeworms varies among regions and hosts. Among sheep, prevalence was documented to be around 35% and 8% in Ivory Coast (Achi et al., 2003b) and in Burkina Faso, respectively (Ouattara and Dorchies, 2001). In Senegal, 15 to 38.7% infection was reported (Ba et al., 1994; Ndom et al., 2016). Infections in goats were reported to be 30%, 2.5% and 8% in Burkina Faso (Belem et al., 2005), Mongolia (Sharkhuu, 2001) and Senegal (Ba et al., 1994), respectively. Prevalence of *Avitellina* in cattle was estimated around 4% in Ivory Coast (Achi et al., 2003a) and 7% in Senegal (Ba et al., 1994).

In heavy infestation, *Avitellina* tapeworms may cause serious disorders (Euzeby, 1966a,b), particularly in lambs, kids and calves under 1 year old (Soulsby, 1986). Thus, *Avitellina* tapeworms represent an economic point in animal industry (Narsapur, 1988).

In spite of the importance of these parasites, little is known about their ecology, evolutionary biology or population genetics (Ndom, 2018). Among the members of the genus, 25 species have been described from wild and domestic ruminants in different regions of the world, but only 15 are currently considered valid (http://www.tapewormdb.uconn.edu). In domestic ruminants, three species *A. centripunctata* (Rivolta, 1874) Gough, 1911, *A. chalmersi* Woodland, 1927,

* Corresponding author.
E-mail address: mall.ndom@ucad.edu.sn (M. Ndom).

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and *A. tatia* Bhalerao, 1936 are considered valid (Spasskii, 1951). This species have been described based on their relatively narrow set of morphological features (Ndom, 2018). Thus *A. chalmersi* is morphologically distinct for *A. centripunctata* by his annular thickening on the proglottids margin (Woodland, 1927), while *A. tatia* is characterized by a very long cirrus pouch (3–4 times large than the copulative portion of vagina) (Spasskii, 1951).

In Senegal, *A. centripunctata* has been reported as the only species among domestic ruminants (Vassiliades, 1981; Ba, 1989; Tamssar Missam, 2006). However, a multilocus isoenzyme electrophoresis study reported the existence of cryptic species in *Avitellina centripunctata* in Senegal (Ba et al., 1994). Which are often convergent, causing controversy about the taxonomy of this genus, further questing the value of only using morphological characters in identifying and differentiating *Avitellina* cestode species. In addition, recent studies in Anoplocephalids tapeworms showed the necessity of molecular analysis and re-evaluation of taxonomy and species identification (Diop et al., 2015, Guo, 2015, 2016, 2017, Ndom et al., 2018).

Therefore, it needed to investigate the genetic diversity of *Avitellina* tapeworms in domestic ruminants in Senegal, to establish reliable identification and to reevaluate the taxonomy of the genus. In the present study, morphological examination and genetical characterization based on mitochondrial DNA (mtDNA) of *Avitellina* tapeworms from cattle and sheep in Senegal were carried out.

2. Materials and methods

2.1. Sampling and preparation of worms

Cattle ($n = 99$) and sheep ($n = 462$) were examined for the presence of intestinal tapeworms in the main slaughterhouse of Dakar, Senegal, during June 2013 to May 2014. These animals were two to five years old and bred in different West African localities, mostly from Northern to Eastern part of Senegal. Parasites were kept alive in physiological salt solution from slaughterhouse, and were brought back to nearby laboratory. Light microscopic studies were performed in nearby laboratory. A part of mature proglottids were prepared and fixed in ethanol 70% for anatomy. For molecular analysis, five or six proglottids of each parasite were freshly kept in ethanol 70% until use.

For SEM, living tapeworms were placed into a small amount of saline buffer. Then, scolex, mature and gravid proglottids were fixed overnight in cold 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in a gradual ethanol series and dried using CO_2 in an Emitech K850 critical point dryer. After being mounted on metal stubs, specimens were coated with gold/palladium in a Quorum Technologies SC7640 sputter coater and examined with a Hitachi S-3400 N scanning electron microscope at acceleration voltages between 3 and 20 kV at the “Service d'Etude et de Recherche en Microscopie Electronique de l'Université de Corse”.

2.2. Cestodes identification: anatomy and scanning

Light microscopic studies were performed to identify mature proglottids of fresh tapeworms. The identification was done following different reference keys helminths identification as previously described (Spasskii, 1951; Yamaguti, 1959; Schmidt, 1986; Khalil et al., 1994). For anatomy, the mature proglottids, previously prepared and kept in ethanol 70%, were stained with iron hydrochloric carmine, dehydrated in a gradual ethanol series, cleared with eugenol (clove oil) and finally mounted in Canada balsam. Scanning electron microscopies (SEM) were done to analyse scolex, mature and gravid proglottids of living tapeworms. Samples were processed as previously described (Ndom et al., 2018).

2.3. Molecular analysis

Genomic DNA was extracted from 101 *Avitellina* tapeworms (12 from cattle and 89 from sheep) using DNA Mini Kit (Qiagen), following the tissue extraction protocol. DNA were used to amplify the full mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene as described previously by Ndom et al., 2018. PCR products were cleaned up and Sanger sequenced. Nucleotide sequences were edited using Geneious Pro software ver. 10.2.3 (created by Biomatters, available from http://www.geneious.com) and multiple sequence alignment was done with MAFFT version 7(Katoh and Standley, 2013). Haplotypes were detected using DnaSP v.5 (Librado and Rozas, 2009) and only unique haplotype was used for the phylogenetic analysis. The phylogenetic tree was inferred based on the *cox1* gene sequences using the Maximum Likelihood (ML) method under Tamura-Nei model with Gamma distribution (Nei and Kumar, 2000) in MEGA7 (Kumar et al., 2016). A 1000 bootstrap replicates tested the robustness of the tree. Anoplocephalid cestodes *Moniezia expansa, Moniezia benedeni* and *Thysaniezia ovilla* were use as outgroup.

3. Results

3.1. Prevalence

In this study, 613 adult worms of *Avitellina* spp. were collected from 462 sheep and 99 cattle in the animal's main slaughterhouse (Dakar, Senegal). The prevalence was 38.7% and 7.1% with a mean intensity of 5.9 (range 1–40) and 1.7 (range 1–4) in sheep and cattle respectively.

3.2. Mitochondrial DNA analysis

Nucleotide sequences of *cox1* (1569 bp) were obtained for all the examined specimens. Overall, 54 haplotypes were confirmed in 101 worm and the nucleotide sequences of all the.

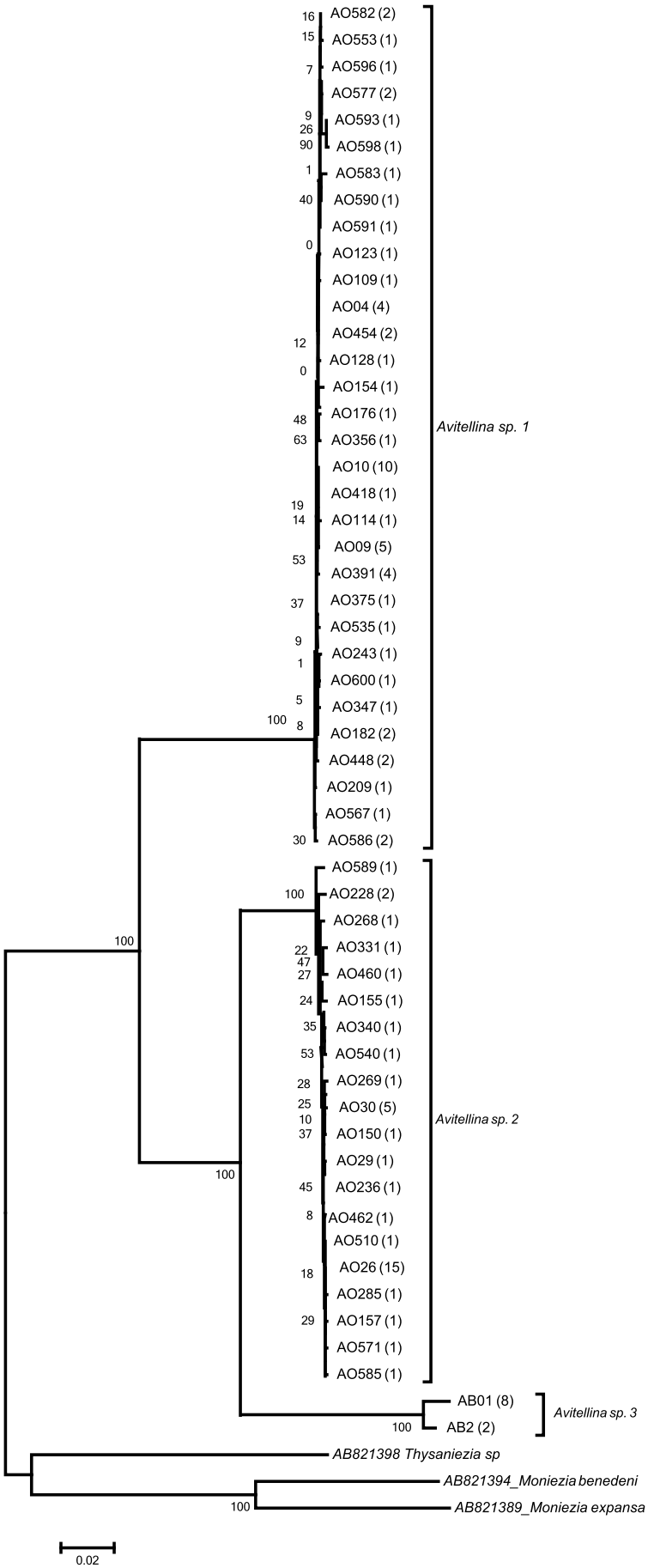
haplotypes were deposited into DDBJ/EMBL/GenBank databases under the accession numbers.

MN398412-MN398465. Among them, 52 haplotypes were obtained from sheep and remaining two were obtained from cattle. Phylogenetic analysis revealed that *Avitellina* tapeworms from ruminants in Senegal split into three clades, genetically distinct (Fig. 1). Analysis revealed that the *Avitellina* tapeworms from sheep and cattle are genetically distinct, with the pairwise divergence ranging from 9.7 to 18.5%. The pairwise genetic divergence ranged from 13.4% to 13.8% between *Avitellina* sp.1 and *Avitellina* sp.2, from 17.7% to 18.5% between *Avitellina* sp.1 and *Avitellina* sp.3 and from 9.7% to 10.8% between *Avitellina* sp.2 and *Avitellina* sp.3. Noteworthy the genetic divergence intraclade was 0.1 to 0.7% in *Avitellina* sp.1 clade, 0.1 to 0.2% *Avitellina* sp.2 clade and 0.1 to 1.4% *Avitellina* sp.3 clades (Table 2).

3.3. Morphology of Avitellina spp.

Morphological observations were conducted for 32, 14 and 6 tapeworms from clades 1, 2 and 3 respectively. At all, the morphology was similar in term of nature and structure of proglottids (Fig. 2). The strobila thin and narrow proglottids were weakly distinguished (Fig. 2a), broader than long,

flat in the proximal portion of the strobila, nearly cylindrical in the posterior portion. Genital pores irregularly alternate. Four distinct sets



(caption on next page)

Fig. 1. Phylogenic tree of *Avitellina* spp. using complete *cox1* sequences isolated from sheep and cattle in Senegal using the maximum likelihood (ML) method. Values of each node are bootstrap percentages. Number of tapeworms possessing each mitochondrial haplotype is shown in the parentheses. Three clades were obtained: *Avitellina centripunctata* and *Avitellina* sp.1 in sheep and *Avitellina* sp.2 in cattle.

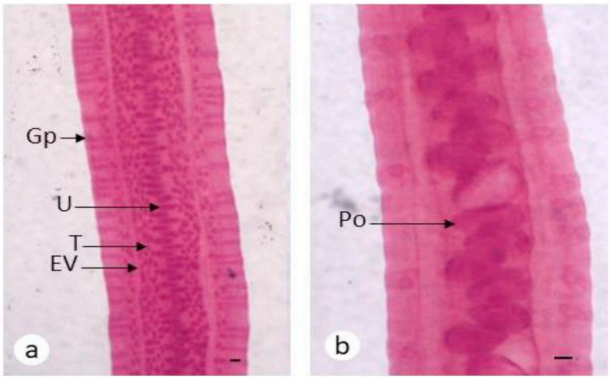


Fig. 2. Staining of *Avitellina* spp. (a) Mature proglottid, (b) Gravid proglottid (Scale bar = 100μm). Ev: Excretory vessel, Po: Paruterines organs, U: Uterine, T: Testes.

of testes in each segment, one right and one left of each longitudinal canal, but no testes in the middle field (Fig. 2). Paruterine organs are single in gravid proglottids (Fig. 2b). However, differences in the size of certain organs such as testes diameter, paruterine organs were observed, revealing three *Avitellina* morphotypes (Table 1).

Then, a detailed morphological screening was performed using scanning electron microscopy (SEM). The morphological analysis based on specimens of three clades reveals three morphospecies: *A. centripunctata* corresponding to *Avitellina* sp.1 and two other species matching *Avitellina* sp.2 and *Avitellina* sp.3. *A. centripunctata* and *Avitellina* sp.2 were isolated from sheep and *Avitellina* sp.3 from cattle. The morphometric characters of these morphospecies and others *Avitellina* species were summarized in Table 1. The characters were summarized, as follow: *A. centripunctata* presented an unarmed scolex spheric in shape (1160 to 2261 μm in diameter) with four triangular-opening suckers (Fig. 3a, b). The tegument of the worm was covered with acicular and capilliform filitriches (Fig. 3c, d). *Avitellina* sp.2 showed an acraspedote strobila. It presents a small spherical scolex (540 to 840 μm in diameter), with four suckers triangular-opening (Fig. 4a, b). The tegument of the scolex, suckers and strobila were covered with two different microtriches: acicular and capilliform filitriches (Fig. 4c, d). The genital apertures usually opened alternately on the right and left margins of the strobila. Every genital pore opened on distinct annular thickening (Fig. 4e, f). These thickening were covered with capilliform filitriches and had a triangular opening (Fig. 4h). *Avitellina* sp.3 were collected from cattle, showing an acraspedote strobila (Fig. 5e). Its scolex was generally smaller to *A. centripunctata* (850–1100 μm in diameter) and presents suckers with triangular or circular opening (Fig. 5b, c). The genital pores were irregularly alternate. The tegument of the scolex, suckers and strobila were surrounded by two kinds of microtriches: acicular and capilliform filitriches (Fig. 5b–d, f).

4. Discussion

In the present study, we investigated the level of *Avitellina* tapeworms infection in cattle and sheep originating in different localities throughout Senegal. The data showed higher prevalence in sheep, confirming, therefore, that worms infect more small ruminants than cattle. Our work has shown that *Avitellina* spp. were not found in goat (data not mentioned in results) confirming that sheep were more infected in small ruminants (Belem et al., 2001; Achi et al., 2003a,b; Ndom et al., 2016). In other studies, from domestic ruminants, *Avitellina* infections remains one of the most prevalent tapeworms infection, and more in sheep than cattle (Abassa, 1975; Ndom et al., 2016).

To date, more than twenty *Avitellina* species have been described from several hosts, mostly from ruminants. Among them, 14 species are considered valid; 11 of which were described in wild ruminants and three in domestic ruminants (Table 1). However, some species were described with missing data such as scolex morphology, which is a fundamental characteristic of diagnosis in cestodes. Rivolta has firstly recorded *A. centripunctata*, the type species, in Italian sheep in 1874. Since Rivolta's description, this species has been reported from several other hosts, bovinae, caprinae, camelidae (Spasskii, 1951; Euzeby, 1966a,b), then redescribed using the specimens collected from sheep in South Africa by Gough in 1908 (Gough, 1911). In addition, two other species *A. chalmersi* Woodland, 1927 and *A. tatia* Bhalerao, 1936 were reported from domestic ruminants, sheep and goat respectively. *A. chalmersi* was described in Indian sheep (Spasskii, 1951; Schmidt, 1986), it is characterized by presence of annular thickening on the proglottids margin (Woodland, 1927). *A. tatia* is characterized by a very long cirrus pouch (3–4 times large than the copulative portion of vagina) (Spasskii, 1951). Nevertheless, it seems that this feature is not as peculiar because one of the varieties of *A. centripunctata*, *A. woodlandi*, also has long cirrus pouch (Spasskii, 1951). According to Spasskii (1951). *A. tatia* could be considered as a variety of *A. centripunctata*.

In this study, a part of the tapeworms collected from sheep was identified morphologically as *A. centripunctata*. They had a very wide scolex (1160–2261 μm diameter) with four triangular suckers opening, and the strobila was acraspedote with indistinct proglottid demarcations. The genital pores were found to be irregularly alternated. The testes (34–50 μm in diameter) were disposed in the usual four columns. Another part of the tapeworms from sheep (*Avitellina* sp.2) and the worms from cattle (*Avitellina* sp.3) were different from all the other *Avitellina* species recorded and could not be identified at species level. In comparison with *A. centripunctata*, they presented a smaller scolex, immature and mature proglottids. *Avitellina* sp.2 presents testes (54–93 μm diameter) with dimensions very close to those of *A. centripunctata* (34–85 μm diameter). The cirrus pouch, the paruterine organs and the ovaries have dimensions similar to those of *A. centripunctata* (see Table 1). The annular thickening presented by *Avitellina* sp.2, brings it closer to *A. chalmersi*. Nevertheless, *A. chalmersi* presents annular thickening on both sides poral and antiporal of each proglottids mature or gravid, contrary to *Avitellina* sp.2, which presents annular thickening only on the poral side (Fig. 4). Furthermore, *Avitellina* sp.2 seem to be smaller than those *A. chalmersi* described by Woodland (1927), particularly the diameter of the scolex and the suckers and the width of the immature and mature proglottids (Table 1). However, the cirrus pouch and paruterine organs were longer in *Avitellina* sp.2 than *A. chalmersi*.

The characteristics of *Avitellina* sp.3 has also a very small scolex and suckers compared to *A. centripunctata*. *Avitellina* sp.3 was wider than *A. tatia* but thinner than *A. chalmersi* (Table 1). The cirrus pouch were

Table 1
A comparison of morphometric characters of Avitelina spp.

Avitellina	References	hosts	Localities	length of cestode	Width of immature proglottids (μm)	width of matures proglottids (μm)
Avitellina centripuncta Rivolta, 1874	Spasskii, 1951	*Bos taurus, Bubalus bufellus, Capra hircus, Ovis aries, Camelus sp., Aepicros melappus, Cephalophus grimmia, Hippotragus equknus*	Europe, Africa, Asia	1 m	1800	2500
Avitellina centripuncta (Rivolta, 1874) Gough, 1911	Euzeby, 1966a, 1966b	*Bos taurus, Bubalus bufellus, Capra hircus, Ovis aries, Camelus sp., and wild ruminants*	Europe, Africa, Asia	1 – 2.5 m	1000	4000
Avitellina aegyptica Nagaty, 1929	Nagaty, 1929	*Cephalophus* sp. *Camelus dromedarius*	Northeast Rhodesia, Africa	fragments 20–100 mm		
Avitellina arctica Kolmakov, 1938	Spasskii, 1951	*Rangifer tarandus, Capreolus pygargus*	*USSR (west Siberia)*		275	1209
*Avitellina chalmersi*Woodland, 1927	Spasskii, 1951	*Ovis aries*	North Africa, India	4.572 mm	1720	3000
Avitellina edifontaineus Woodland, 1928	Woodland, 1928	*Taurotragus oryx*	Tanganyika, East Africa		680	2200
Avitellina monardi Fuhrmann, 1933	Spasskii, 1951	*Taurotragus oryx*	Angola (West Africa)			2300
Avitellina pygargi Kholodkovsky, 1902	Spasskii, 1951	*Capreolus pygargus*	Altai (USSR)	1.5 m		4000
Avitellina ricardi Woodlan, 1928	Woodland, 1928	*Kobus* sp.	Zeeland (East Africa)		590–710	1140–1620
*Avitellina sandgroundi*Woodland, 1935	Woodland, 1935	*Hippotragus equinus*	North Katanga, Africa	fragment (40 cm)		3420
Avitellina tatia Bhalerao, 1936	Spasskii, 1951	*Capra hircus*	United provinces, India			2470
Avitellina centripunctata (n = 32)	present study	*Ovis aries*	Senegal	1.2–3.75 m	1478.56 (774–2257)	2719.41 (1116–3995)
Avitellina sp.2 (n = 14)	present study	*Ovis aries*	Senegal	0.75–1.10 m	560 (273–833)	1968.43 (769–2945)
Avitellina sp.3 (n = 6)	present study	*Bos taurus*	Senegal		782 (655–1200)	1886.5 (889–2932)

Avitellina	Scolex diameter (μm)	Sucker diameter (μm)	Nombers of testicular columns	excretory vessels	testes diameter (μm)	Length X width of cirrus pouch (μm)	Length X width ofparuterin organe (μm)
Avitellina centripuncta Rivolta, 1874	1500–2200	500–1000	4	2 pairs	40–70	210–250 × 38–45	560–1000
Avitellina centripuncta (Rivolta, 1874) Gough, 1911	1500–3000	400–500 × 300–400	4	2 pairs	80	60–100 × 30–40	
Avitellina aegyptica Nagaty, 1929			4	2 pairs	90 × 54	83 × 24	245 × 100
Avitellina arctica Kolmakov, 1938			4	2 pairs		122 × 76	
*Avitellina chalmersi*Woodland, 1927	2080–2420	950–1020	4	2 pairs		63–72 × 14–22	425 × 200
Avitellina edifontaineus Woodland, 1928			4	1 pair	80	25–27 × 18	340 × 660
Avitellina monardi Fuhrmann, 1933			2	1 pair		36 × 06	170–200
Avitellina pygargi Kholodkovsky, 1902	500	160	2				
Avitellina ricardi Woodlan, 1928			2	1 pair	44–50 × 18–33	18 × 32	210–240 × 430–490
*Avitellina sandgroundi*Woodland, 1935	2060	880	4		87	117 × 73	
Avitellina tatia Bhalerao, 1936			4	2 pairs		300–393 × 42–50	600 × 340
Avitellina centripunctata (n = 32)	1841.13 (1160–2661)	370 (334–406)	4	2 pairs	51 (34–85)	196 (140–344) X 52 (27–85)	324.6–635 × 106–136
Avitellina sp.2 (n = 14)	671 (540–840)	135 (107–171)	4	2 pairs	75 (54–93)	277 (190–258) X 73 (58–97)	365–1000 × 169–214
Avitellina sp.3 (n = 6)	898 (850–1150)	105 (78–139)	4	2 pairs	41 (33–56)	170 (89–233) X 40 (28–42)	306–463 × 198–311

Table 2
Pairwise genetic divergence (expressed as percentages) of *cox1* gene.

	1	2	3	4	5	6	7	8
1. AO10 (*Avitellina centripunctata*)								
2. AO109 (*Avitellina centripunctata*)	0.1							
3. AO593 (*Avitellina centripunctata*)	0.6	0.7						
4. AO29 (*Avitellina* sp.2)	13.4	13.4	13.8					
5. AO150 (*Avitellina* sp.2)	13.4	13.4	13.8	0.1				
6. AO236 (*Avitellina* sp.2)	13.4	13.4	13.8	0.2	0.1			
7. AB1 (*Avitellina* sp.3)	18.0	18.0	18.5	10.8	10.8	10.9		
8. AB2 (*Avitellina* sp.3)	17.7	17.8	18.3	9.7	9.8	9.9	1.4	

similar dimensions to those of *A. centripunctata*, but longer than *A. chalmersi* and shorter than *A. tatia* (Table 1). The paruterine organs dimensions were very close to those *A. tatia*. Their testes and the cirrus pouch showed similar size than *A. centripunctata* (Table 1).

Phylogenetic analysis based *cox1* gene sequences clearly showed three distinct clusters supported by very high bootstrap values (100%). As for the 52 specimens used for morphology, phylogenetic clustering supported the morphological identification of three species. The pairwise divergence between these three groups shows very high values (9.7–18.5), that are comparable to that reported between *Moniezia expansa* and *M. benedeni* (12.8 to 13.2%) (Diop et al., 2015) and between *Thysaniezia ovilla* and *T. connochaeti* (9.8 to 12.2%) (Ndom et al., 2018). This result indicates that the morphological and molecular diversity among *Avitellina* tapeworms collected from sheep and cattle in Senegal

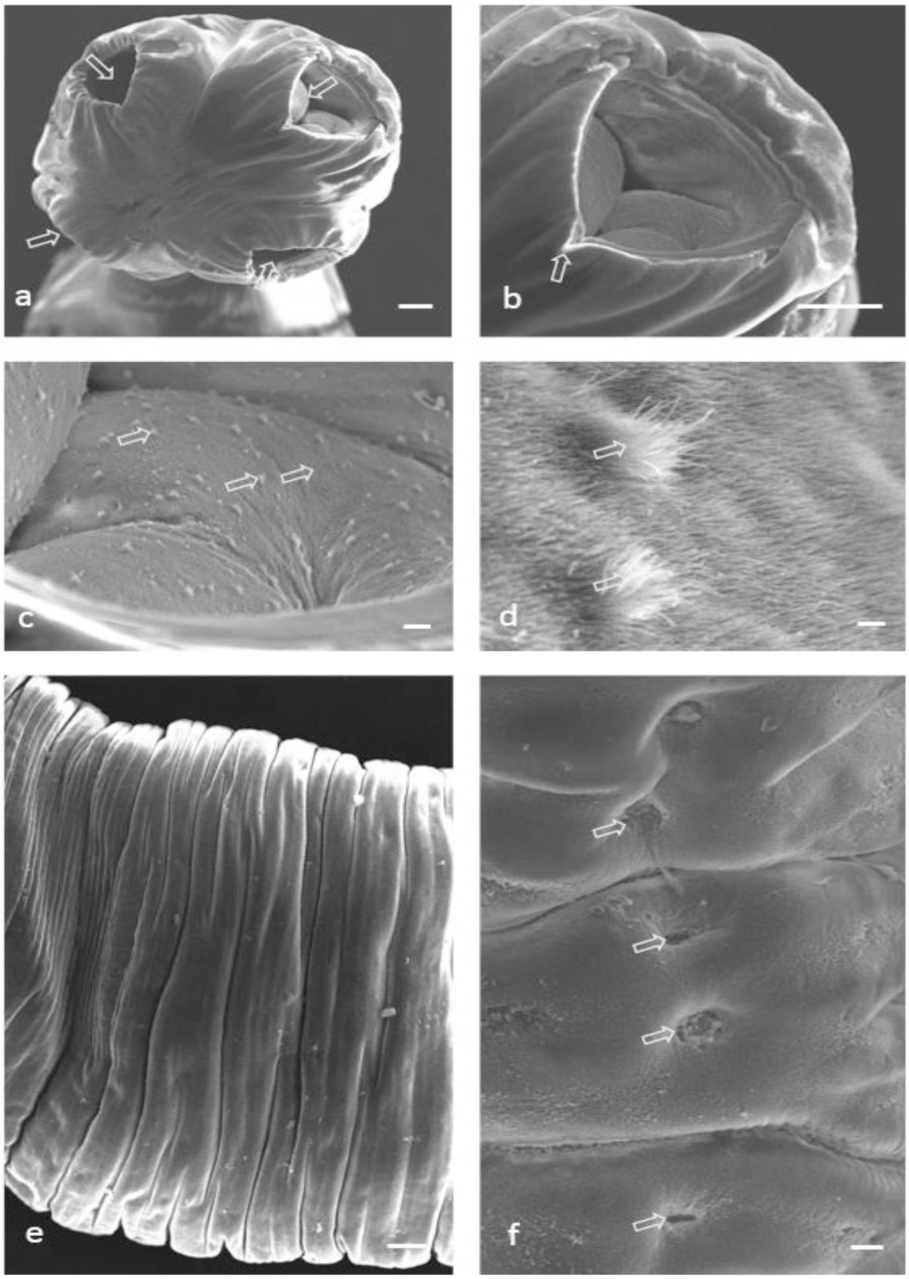


Fig. 3. (a)–(f) Scanning electron micrographs of *Avitellina centripunctata*. (a) General anterior view of scolex, arrows show invaginated suckers. (b) Invaginated sucker, arrow showing triangular opening. (c) Peripheral sucker, arrows show acicular and capilliform filitriches (Scale bar = 10μm). (d) Acicular and capilliform filitriches covering sucker (scale bar = 1μm). (e) Portion of strobila showing acraspedote proglottids. (f) Lateral portion of strobila, arrows show genital pores (Scale bar = 10μm). Scale bar = 100μm, unless stated otherwise.

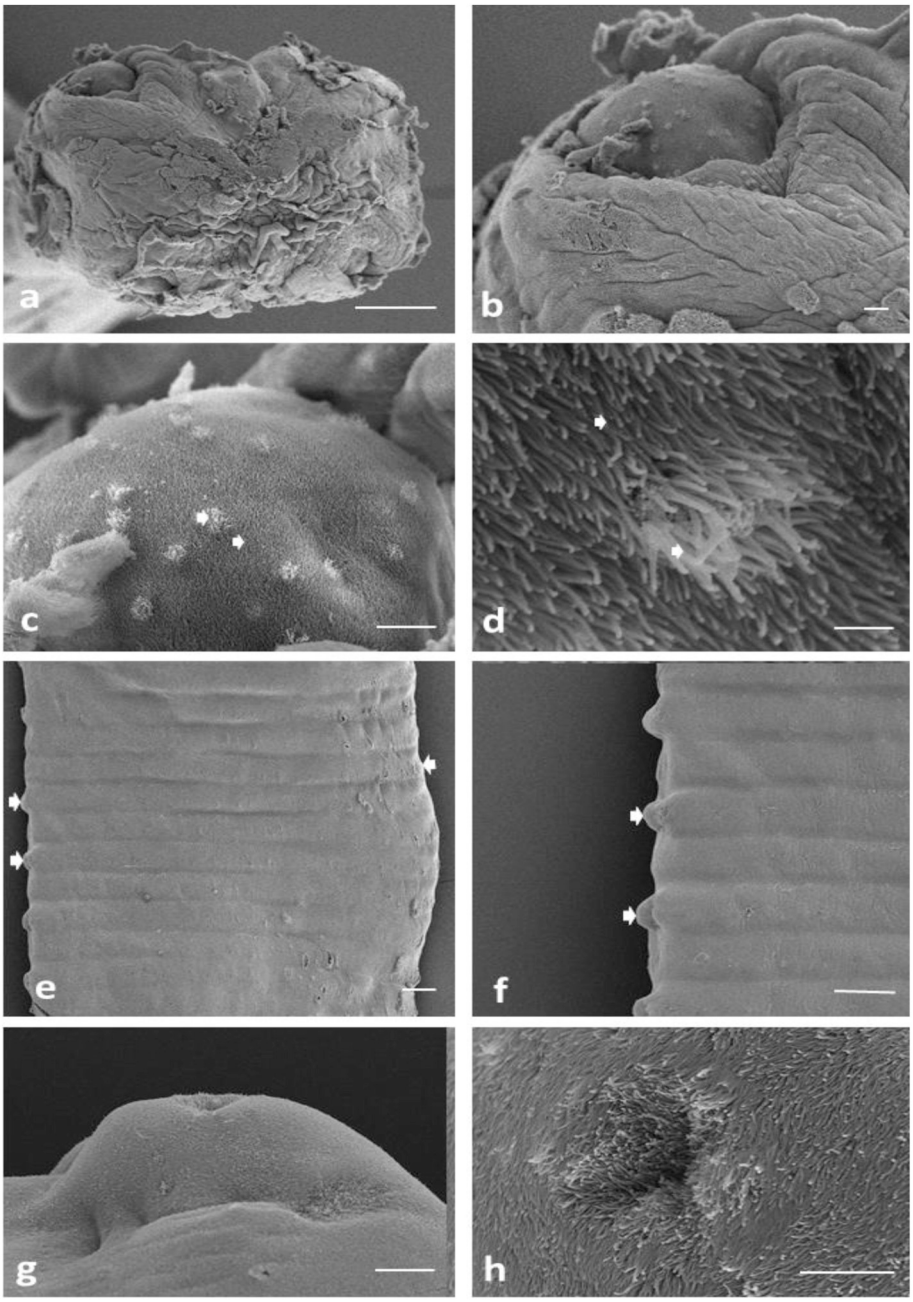


Fig. 4. (a)–(h) Scanning electron micrographs of *Avitellina* sp.2. (a) General anterior view of scolex. (Scale bar = 100 μm).(b) Evaginated sucker, showing triangular opening (Scale bar = 10μm). (c) Peripheral sucker, arrows show acicular and capilliform filitriches (Scale bar = 10μm). (d) Acicular and capilliform filitriches covering sucker (scale bar = 1μm). (e) Portion of strobila showing acraspedote proglottids, arrows show annular thicknening. (f) Lateral portion of strobila, arrows show annular thicknening (Scale bar = 10μm). (g) Annular thicknening (Scale bar = 10μm). (h) Microtriches covered annular thickening, Scale bar = 100μm unless stated otherwise.

is not intraspecific variation but rather shows the presence of several species.

Intriguingly, *Avitellina* sp.2 from sheep is genetically and morphologically closer to *Avitellina* sp.3 from cattle than to *A. centripunctata* from sheep. It is speculated that the speciation among these *Avitellina* spp. has occurred in the wildlife before domestication of ruminants and the coinfection of *A. centripunctata* and *Avitellina* sp.2 in sheep resulted from host-switching. To elucidate the evolutionary history of *Avitellina* spp. and to re-evaluate the taxonomy of the genus, accumulation of molecular data on parasite specimens from wild ruminants is necessary.

5. Conclusion

A. centripunctata coexist with other Anoplocephalid species (*M. expansa, M. benedeni, T. ovilla, Stilesia globipunctata*) in the small intestine of domestic ruminants. Some of which, like *Moniezia* spp. and *Thysaniezia* spp. are complexes (Ba et al., 1993, 1994; Ndom et al., 2018). Our study shows a complexity among *Avitellina* spp., genetically, morphologically and suspected many species. Thus, real questions are possessed; this diversity noted in several anoplocephalidaen species has always existed, or is it the result of an allopatric or sympatric speciation from a host-parasite co-speciation?

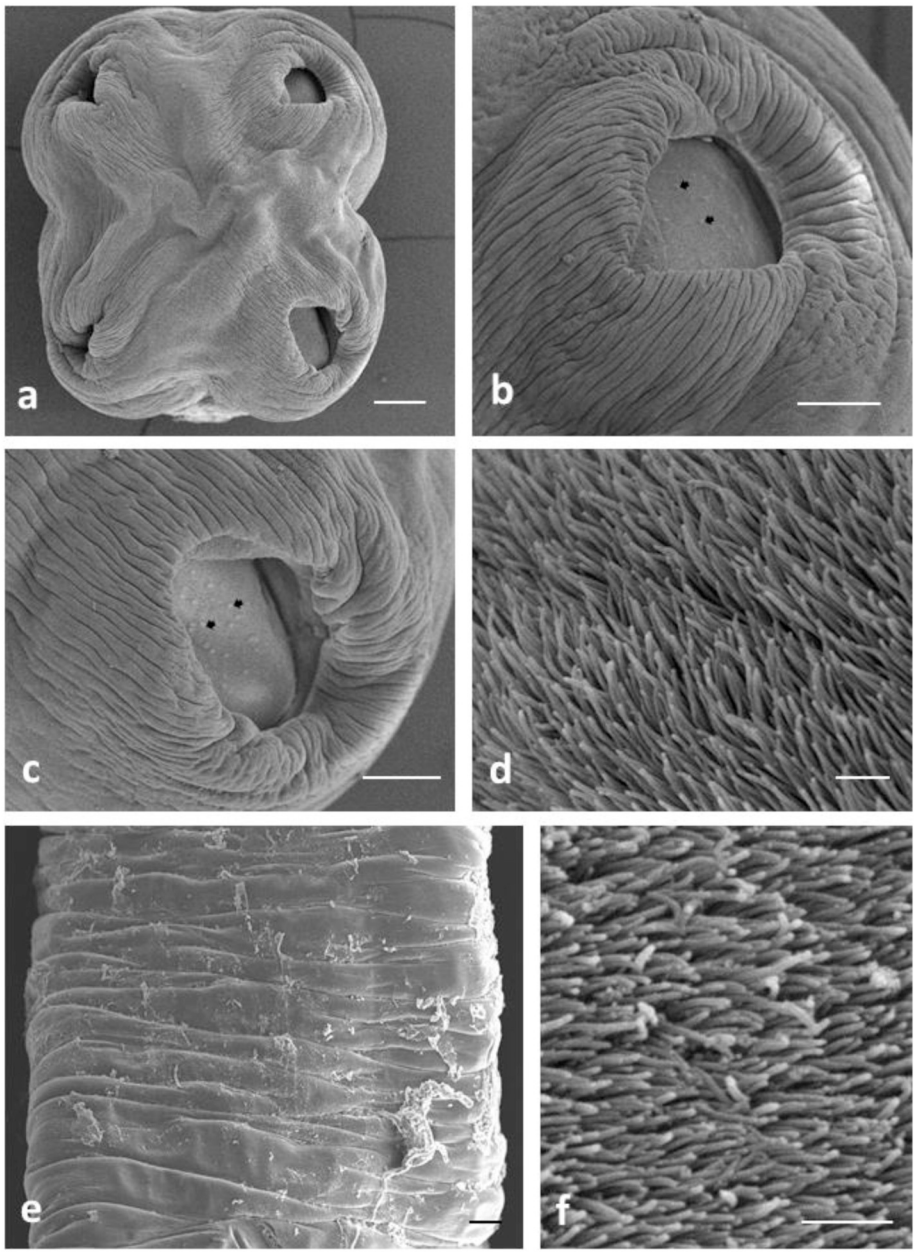


Fig. 5. (a)–(e) Scanning electron micrographs of *Avitellina* sp.3. (a) General anterior view of scolex. (b) Invaginated sucker, showing linear opening (Scale bar = 10μm). (c) Portion of strobila showing acraspedote proglottids. (d) Acicular and capilliform filitriches covering sucker (scale bar = 10μm). (e) Acicular and capilliform (scale bar = 1μm). Scale bar = 100μm unless stated otherwise.

Ethical statement

Approval and clearance for the study was obtained from Ethic comity of University Cheikh Anta DIOP of Dakar (Reference: 0141/2015/CER/UCAD).

Declaration of Competing Interest

All authors declare that there is no conflict of interest.

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