

Full Length Research Paper

Geometric morphometric characterization of species of the *Schistosoma haematobium* group in Central and Northern Côte d'Ivoire

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Identification of species of the *Schistosoma haematobium* group is still mainly based on the morphology of eggs. Indeed, the same snail intermediate host can shed multiple species of cercariae morphologically similar and in addition, these species can hybridize. The aim of this work is to identify the cercariae of *Schistosoma haematobium* and *S. bovis* based on the geometric morphometric characteristics. We have considered two parasites strains of Central and Northern Côte d'Ivoire shed by *Bulinus truncatus* snails: (i) Experimentally infected snails with miracidia coming from urine of children, and (ii) Naturally infected *B. truncatus* collected in waterbodies. The cercariae were stained with a 5% silver nitrate solution on a slide and the ventral and dorsal sensory papillae located on the head were observed under a light microscope and digitized. Geometric morphometric parameters were analysed for 132 cercariae. A difference between the strains of cercariae was observed. No difference was observed within the experimentally obtained cercariae of the Centre and the North of the country. However, the geometric morphometric parameters vary within the naturally-infected strains; the naturally-infective cercariae of Djemitedouo are larger than the naturally-infective cercariae of Korokara. It is likely that the experimentally obtained cercariae were composed of *S. haematobium* species while the naturally obtained strain is either *S. haematobium* or *S. bovis*. The study suggest geometric morphometric parameters of cercariae can be used to identify anthropophilic and zoophilic species of the *S. haematobium* group.

Key words: *Schistosoma haematobium*, *Schistosoma bovis*, *Bulinus truncatus*, Cercariae, Geometric morphometric, characterization.

INTRODUCTION

Schistosomiasis is a chronic waterborne parasitic disease caused by trematode worms of the genus *Schistosoma*.

Human schistosomiasis is a public health problem in developing countries of tropical and subtropical regions

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(WHO, 2019). This neglected tropical disease affects more than 250 million people worldwide (Hotez et al., 2014; McManus et al., 2018). Several species are described and six of them infect humans: *Schistosoma haematobium*, *S. mansoni*, *S. intercalatum*, *S. japonicum*, *S. guineensis* and *S. mekongii* (Gryseels et al., 2006). Some species are known to infect mammals: rodents (*S. rodhaini*) and ruminants, including sheep, goats (*S. curassoni*) and cattle (*S. bovis*) (Gryseels et al., 2006). *S. haematobium*, responsible of human urogenital schistosomiasis infect 112 million people; it is widely distributed mainly in sub-saharan Africa (Gryseels et al., 2006; Ezeh et al., 2015; WHO, 2019). The life cycle of the schistosomes involves an intermediate freshwater snail host and the final vertebrate host (Boissier et al., 2016). People are infected through professional or recreational activities such as fishing, breeding, agriculture and swimming (WHO, 2019). In Côte d'Ivoire, *Schistosoma haematobium* and *S. mansoni* are endemic (Uttinger et al., 2000; N'guessan et al., 2015). *Schistosoma haematobium* species are transmitted respectively by two intermediate host snail species (*Bulinus truncatus* and *B. globosus*) and *S. mansoni* species are transmitted only by *Biomphalaria pfeifferi* (Cecchi et al., 2007; Diakité et al., 2017).

Identification of schistosomes is generally based on the morphology of excreted eggs. However, as the schistosome species can hybridize and some snail intermediate host can shed multiple species of cercariae which are morphologically similar, the identification of these parasite becomes a challenge (Boissier et al., 2015; Berry et al., 2017; Angora et al., 2020). Molecular markers are the only tools for species identification of adult worms and larval stages (miracidium, cercariae) of schistosomes (Huysse et al., 2013; Leger and Webster, 2017). However, these are expensive and not always accessible, especially in endemic settings, thus there is a need of additional tools.

Geometric morphometric can be a cheaper and more accessible approach for the identification and characterization of parasite species. For example, used this approach to characterize populations of the tick *Rhipicephalus (Boophilus) microplus*. This study aims to assess the potential of geometric morphometric techniques to identify cercariae of the *Schistosoma haematobium* group and eventually, infer intra and interspecific differences among species of this group.

MATERIALS AND METHODS

Study sites and populations

This study was conducted between November 2017 and November 2018, in four villages in the Centre (Kongobo and Linguebo) and the North (Djemitedouo and Korokara) of Côte d'Ivoire (Figure 1). Two distinct seasons characterized the study area. The dry season that starts in October and end in January in the Centre and in February in the North, and the rainy season that lasts until

September (<https://fr.tutiempo.net>). There are lakes of dam in each village built respectively on the tributaries of rivers Comoe for Djemitedouo, and Bandama for the other three villages. The villages are endemic for urogenital schistosomiasis and Tian-Bi et al. (2018, 2019) have observed that *S. bovis* infected *Bulinus* snails were predominant in the North while *S. haematobium* and hybrid infecting snails were mainly found in the Centre of Côte d'Ivoire.

The study population was composed of schistosome cercariae, shed by *B. truncatus* snails. These cercariae provided from experimentally infected snails with miracidia, obtained from the urine of children of the villages of Djemitedouo in the North and Kongobo in the Centre or from snails naturally infected, were collected in each study village.

Collection of schistosome eggs and snails

The schistosome eggs were collected from consenting children who reported visual hematuria after written informed consent and oral assent was obtained from each participant's parent or legal guardian. Fifteen children aged 7 to 10 years of Kongobo in the Centre and Djemitedouo in the North provided urine samples. Schistosome eggs were collected using the urine filtration technique (Plouvier et al., 1975) and filters with eggs were stored in physiological saline solution (0.9 wt % NaCl) and transported on ice to the laboratory where they were stored at a fridge temperature before use. Snails were collected using a forceps and/or landing net for a period of 15 min. The first method consisted of collecting snails from all floating supports in order to avoid damaging the shell. The collection with the landing net was used for the points of difficult access. This technique consisted of mowing to a depth of about 50 cm in the submerged vegetation from the shoreline to the middle of the water body.

Obtaining juvenile snails, schistosome miracidia and snail infection

B. truncatus snails collected in the field were tested for schistosome infection once a week over a period of one month, which corresponds to the parasite pre-patent development period in the snail. Cercarial shedding was stimulated by exposure of individual snails to artificial light for four hours (10 to 14 h). This allowed separating infected snails and non-infected ones at the end of the test period.

Then, 10 snails not infected, randomly chosen of each of the villages (generation G0) were isolated in pill boxes containing 10 ml of water for egg laying. After hatching, juvenile (generation G1), were maintained with their parents for about a week. Hence, five G1 juveniles (size 1.8-2.0 mm) were randomly selected from each of the 10 boxes.

These 100 G1 snails of each village were split into two batches of 50 individuals. These snails were used for experimental infection by schistosomes. The snails were individually exposed for 6 hours, in the dark to five schistosome miracidia from villages of Kongobo or Djemitedouo. Surviving snails were tested for cercarial shedding 30 days after exposure to miracidia. Throughout the monitoring of snails in the laboratory, water was renewed two to three times a week and the snail was fed *ad libitum* with boiled lettuce and fish manufactured food.

Revelation of the sensory taste buds and digitizing images of cercariae

The cercariae were stained with a silver nitrate solution to reveal the sensory papillae on the head of cercaria, according to the technique described by N'Goran (1997). Hence, 24 h after staining,

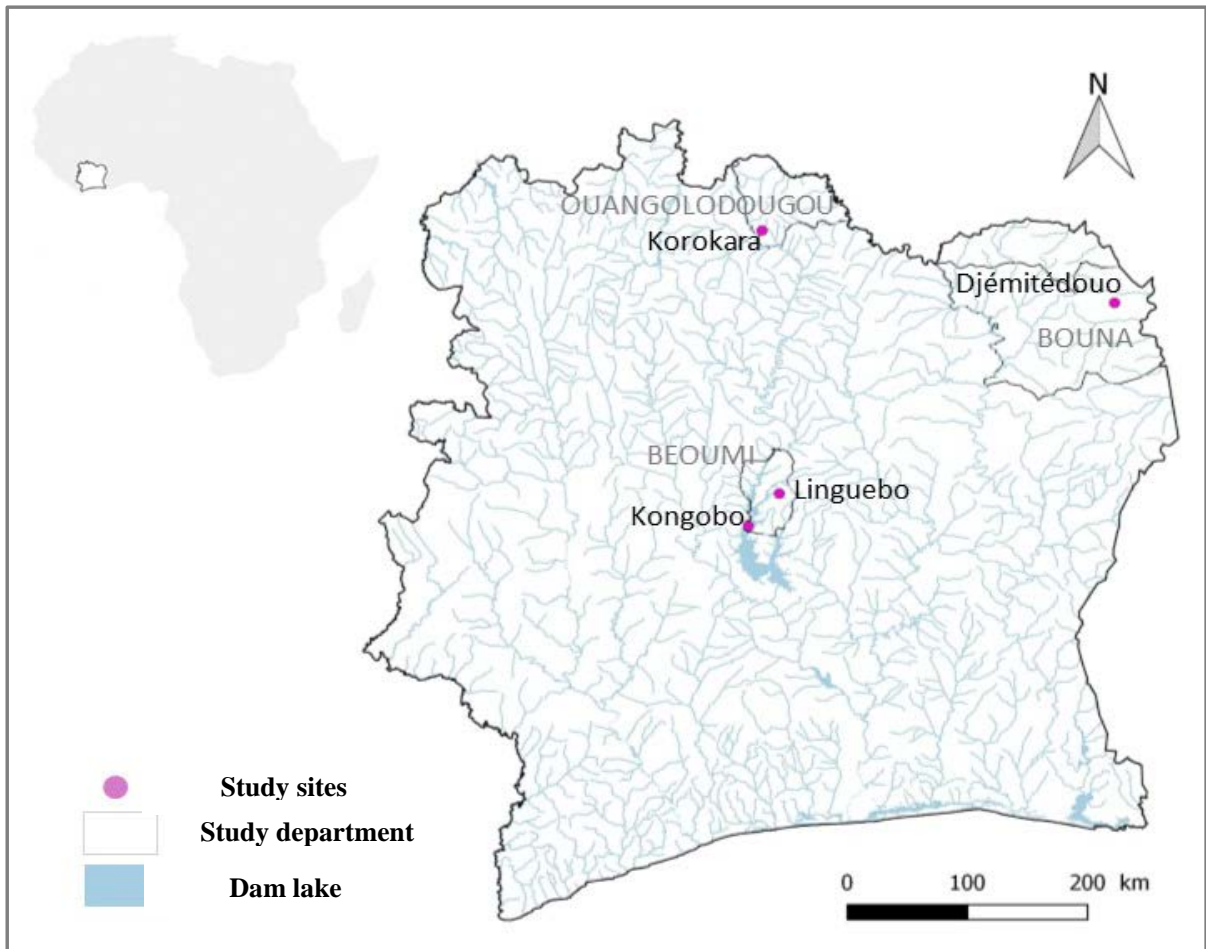


Figure 1. Location of study sites.
Source: Author.

the sensory papillae on the head of each cercaria were observed by microscope (x40), and pictures were taken and recorded through a dispositive adapted to the microscope, composed of a camera (Leica-Microsystems, LAS EZ) connected to a laptop. The lens of the microscope was the same for all the photos and an individual identification code indicating the geographical origin of the parasite and the type of snail infection; natural cercariae (CN) or experimental cercariae (CE) was attributed.

The digitizing of images consisted of identifying on the head of each cercariae eight landmarks of type I (Rohlf and Bookstein, 1990). These landmarks were positioned in the same order by the same operator (from 1 to 8). Digitization was performed using the COO module of the CLIC software version 1998 (<http://mom-clic.com>). The isometric estimator known as centroid size (CS) was used for size comparisons. Centroid size is defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark. This module automatically generates the coordinates (x; y) representing two different variables from each landmark, hence a total of 16 variables in a virtual orthonormal reference frame. Per population, 12 to 40 cercariae were analysed.

Six populations of cercariae, divided in experimental and natural were considered. The experimental cercariae come from Kongobo (EC-KON) and Djémitédouo (EC-DJE); the natural cercariae were from Kongobo (NC-KON), Linguebo (NC-LIN), Djémitédouo (NC-

DJ) and Korokara (NC-KOR).

Ethical considerations

This study is part of a Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project (Tian-Bi et al., 2018) which aimed to interrupt seasonal transmission of *S. haematobium* in northern and central settings of Côte d'Ivoire. The project protocol was approved by the Comité National d'Éthique des Sciences de la Vie et de la Santé (Ref. 055-19/MSHP/CNESVS-KP). In addition, written informed consent and oral assent was obtained from each participant's parent or legal guardian and children respectively. All infected children with schistosome eggs in the urine have received treatment with praziquantel offered by the Programme National de lutte contre la Schistosomiase, les Geohelminthiases et la Filariose Lymphatique (PNL-SGFL, Côte d'Ivoire).

Data proceeding and analysis

Measurement of errors could have introduced an artificial variance between individuals. Errors introduced during data generation, as each individual was not perfectly perpendicular to the focal axis or

Table 1. Centroid sizes of cercariae.

Cercariae provenance	Study region	Study site	N	Centroid size	95% CI
Experimental Cercariae	Centre	Kongobo	40	0.024	0.023-0.026
	North	Djemitedouo	40	0.027	0.025-0.029
Natural Cercariae	Centre	Linguebo	15	0.023	0.021-0.025
		Kongobo	13	0.025	0.022-0.028
	North	Djemitedouo	12	0.046	0.045-0.048
		Korokara	12	0.037	0.036-0.040

N: Total number of cercariae; CI: Confidence Interval.
Source: Author.

Table 2. Mahalanobis distances for population pairs and between regions.

	EC-DJE	EC-KON	NC-DJE	NC-KOR	NC-LIN
EC-KON	1.64 (0.035)				
NC-DJE	2.34 (0.001)	2.32 (0.001)			
NC-KOR	2.29 (0.001)	2.48 (0.001)	1.77 (0.049)		
NC-LIN	1.66 (0.054)	1.59 (0.034)	2.44 (0.001)	2.31 (0.001)	
NC-KON	1.63 (0.065)	1.61 (0.034)	2.62 (0.001)	2.37 (0.002)	1.57 (0.050)

EC-DJE: Experimental Cercariae of Djemitedouo; EC-KON: Experimental Cercariae of Kongobo; NC-DJE: Natural Cercariae of Djemitedouo; NC-KOR: Natural Cercariae of Korokara; NC-LIN: Natural Cercariae of Linguebo; NC-KON: Natural Cercariae of Kongobo. The *P* values for each distance in brackets are significant at a risk de 5% if $p < 0.003$.
Source: Author.

due to a differential resolution between images related to the size of each individual were eliminated by correcting the coordinates of the homologous landmarks. That was possible by adjusting the scale or resizing using the TET module of the CLIC software. The same module was subsequently used to combine all the coordinate files into one (concatenation procedure) for the analysis of the homologous landmarks.

The analysis of the average size and conformation of each parasite was based on the generalized Procrustean analysis (Procrustean overlay). It includes the steps of centering by translation of the reference points on a common origin (the centroid), normalization of these reference points by centroid size and rotation. The Procrustean overlay procedure allowed generating the centroid sizes of the individuals of each population. The Procruste analysis was performed using the MOG module of the CLIC software. Mean centroid size and 95% CI were computed for each population. Variation of centroid size was assessed by calculating mahalanobis distance for each population pair. The Mahalanobis distances were generated with the PAD module of the CLIC software. The significance of these distances was evaluated using the Bonferroni test with 1000 permutations. The centroid sizes were plotted to identify groups of individuals and/or populations.

RESULTS

Centroid size variation among cercariae

Total digitized cercariae images was 132. Means centroid size were 0.024 and 0.027 mm, for experimentally infected cercariae of Kongobo (EC-KON) and

Djemitedouo (EC-DJE) respectively. These means were ranged between 0.023 and 0.025 mm in Kongobo and Linguebo (Centre), and, 0.037 and 0.046 mm in Korokara and Djemitedouo (North) for the naturally infected cercariae (Table 1).

Comparison of Mahalanobis distances

The pairwise Mahalanobis distances based on centroid sizes were ranged between 1.57 and 2.62 (Table 2). No difference was detected between experimentally obtained cercariae from Kongobo (EC-KON) (Centre) and those from Djemitedouo (EC-DJE) (North). In the same way, no difference was observed between naturally obtained cercariae from Kongobo (NC-KON) and those from Linguebo (NC-LIN). On the other hand, a significant difference was observed between experimentally obtained cercariae from Kongobo and naturally obtained cercariae from Djemitedouo; and also between naturally obtained cercariae from Djemitedouo and naturally obtained cercariae from Korokara. A significant distance was detected between naturally obtained cercariae (NC) from the Centre and those from the North (Table 2).

Groups of cercariae according to centroid size

Three distinct groups were observed (Figure 2): (i) The

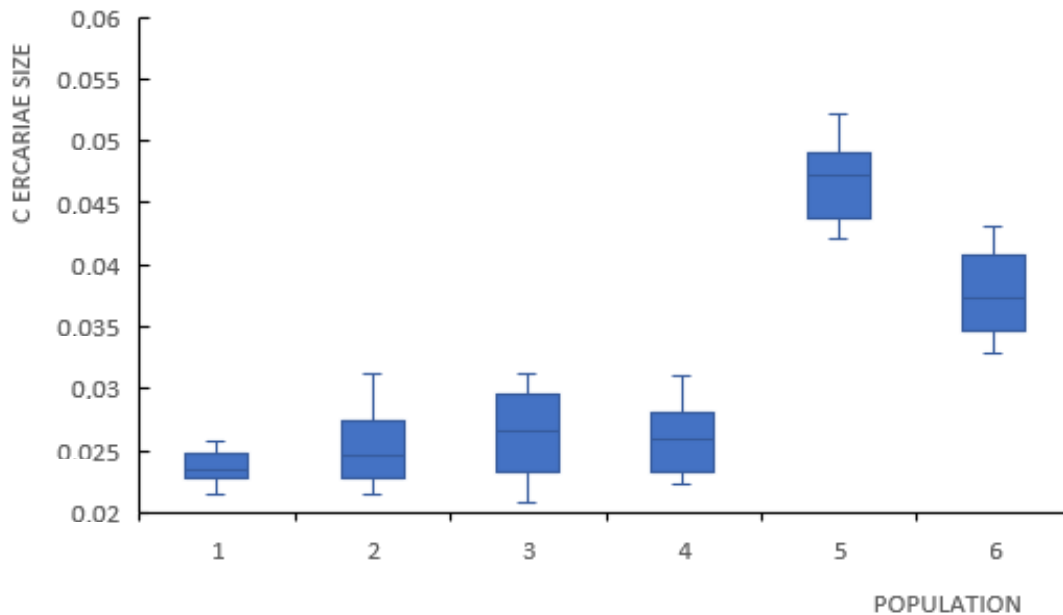


Figure 2. Distribution of cercariae according to the size. The x-axis represents the individual cercarial size by group. The y-axis represents the different groups (six) of cercariae by type of infestation (natural or experimental) and study sites. 1: Experimental Cercariae of Kongobo; 2: Experimental Cercariae of Djemitedouo; 3: Natural Cercariae of Kongobo; 4: Natural Cercariae of Linguebo; 5: Natural Cercariae of Djemitedouo; 6: Natural Cercariae of Korokara. Pop 1-2-3-4: Small cercariae; Pop 5: Large cercariae; Pop 6: Medium cercariae. Source: Author.

small cercariae, with size ranged between 0.022 and 0.032 mm composed of experimentally infected cercariae (EC) from the centre and north, and naturally infected cercariae (NC) from the centre; (ii) The medium cercariae (size: 0.033-0.043 mm) only composed of naturally infected cercariae of Korokara (North); and (iii) the large cercariae (size: 0.044-0.054 mm), which was only composed of naturally infected cercariae from Djemitedouo (North).

DISCUSSION

This study presents, the results of characterization of schistosomes using geometric morphometrics of cercariae stained with a silver nitrate solution.

The results showed that in central Côte d'Ivoire, there was no significant difference in centroid size between experimentally obtained cercariae (EC) and naturally obtained ones (NC). This suggests that the compared cercariae belong to the same specie. However, the centroid size of naturally obtained cercariae of the Centre and the North were higher, suggesting the presence of different species of schistosomiasis. Several studies relative to arthropods or snails have shown a geographical variation in the shape of different species (e.g., the tick *Rhipicephalus sanguineus*: Sanches et al., 2016 and *Bulinus africanus* group: Stothard et al., 2002).

Indeed, the homologous points contain information on the shape and the size of individuals allowing to differentiate them (Villemant et al., 2007; Dujardin, 2011). Variations in the shape and the size among populations within the same species can be related to both environmental and genetic factors (Dujardin et al., 2014; Dupraz et al., 2016). Thus, variation in centroid size, detected in this study, could be explained by the combination of those factors. In the hypothesis of a genetic variation, this might reveal a difference in schistosome species between the Centre and the North of Côte d'Ivoire. Indeed, although *Schistosoma haematobium* and *S. bovis* have been shown to circulate in these regions, considering natural infections in snail hosts, *S. haematobium* is the predominant parasite in the Centre, whereas *S. bovis* is the most prevalent in the North (Tian-Bi et al., 2019; Glitho et al., 2020). Accordingly, the results of this study may suggest that the small cercariae in the Centre (with lower centroid size) may be mainly *S. haematobium* and the large cercariae in the North (with higher centroid size) may be mostly *S. bovis*.

This assumption might be confirmed by the significant difference in centroid size and in Mahalanobis distance between experimental and natural cercariae from the North. Indeed, the experimental cercariae were obtained from experimental infection of snails with miracidia from the urine of infected human hosts. Yet, it is known that humans are predominantly infected with *S. haematobium*

(Angora et al., 2020). Moreover, the naturally obtained cercariae from the North, which come from naturally infected snails, are mostly known to be *S. bovis*.

Conclusion

Use of morphometric geometric approach allowed differentiating groups of cercariae. Thus, in the study area two species of schistosomes, namely the anthropophilic species *Schistosoma haematobium* and the zoophilic species *S. bovis* were identified. Hence, geometric morphometric approach is suggested as a tool to identify larval stages of parasites species such as schistosomes. However, to better evaluate the performance of this tool, there is the need to combine the use with the other identification tool applied on the different stages of the same parasite.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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