Acta Tropica
November 2021, Volume 223, Pages 106075 (16p.)
https://doi.org/10.1016/j.actatropica.2021.106075
https://archimer.ifremer.fr/doc/00711/82268/

First morphogenetic analysis of parasite eggs from Schistosomiasis haematobium infected sub-Saharan migrants in Spain and proposal for a new standardised study methodology

Reguera-Gomez Marta [1], Valero M. Adela [1, *], Oliver-Chiva M. Carmen [1], De Elias-Escribano Alejandra [1], Artigas Patricio [1], Cabeza-Barrera M. Isabel [2], Salas-Coronas Joaquín [2], Boissier Jérôme [3], Mas-Coma Santiago [1], Bargues M. Dolores [1]

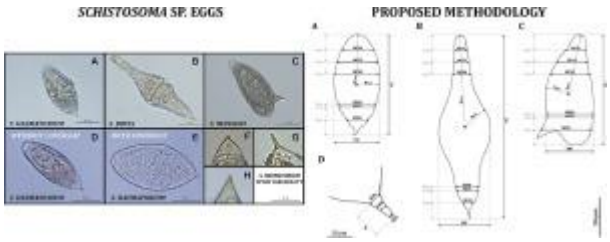
[1] Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain
[2] Unidad de Medicina Tropical. Hospital de Poniente, El Ejido, Almería, Spain
[3] Université de Perpignan Via Domitia, IHPE UMR 5244, CNRS, IFREMER, Univ. Montpellier, F-66860 Perpignan, France

* Corresponding author : M. Adela Valero, email address : madela.valero@uv.es

Abstract :

Schistosomiasis is a Neglected Tropical Disease caused by trematode species of the genus Schistosoma. Both, autochthonous and imported cases of urogenital schistosomiasis have been described in Europe. The present study focuses on eggs, considered pure S. haematobium by genetic characterisation (intergenic ITS region of the rDNA and cox1 mtDNA). A phenotypic characterisation of S. haematobium eggs was made by morphometric comparison with experimental populations of S. bovis and S. mansoni, to help in the diagnosis of S. haematobium populations infecting sub-Saharan migrants in Spain. Analyses were made by Computer Image Analysis System (CIAS) applied on the basis of new standardised measurements and geometric morphometric tools. The principal component analysis (PCA), including seventeen non-redundant measurements, showed three phenotypic patterns in eggs of S. haematobium, S. bovis and S. mansoni. PCA showed that the S. bovis population presented a large egg size range with a pronouncedly larger maximum size. Similarly, S. bovis shows bigger spine values than S. haematobium. Mahalanobis distances between each pair of groups were calculated for each discriminant analysis performed. In general, S. mansoni and S. bovis present larger distances between them than with S. haematobium, i.e. they present the greatest differences. Regarding the spine, S. haematobium and S. mansoni are the most distant species. Results show the usefulness of this methodology for the phenotypic differentiation between eggs from these Schistosoma species, capable of discerning morphologically close eggs, as is the case of the haematobium group. Schistosoma egg phenotyping approaches may be applied to assess not only hybrid forms but also potential influences of a variety of other factors.

Graphical abstract



Highlights

► *S. haematobium* eggs involved in cases from sub-Saharan migrants present in Spain are genetic and phenotypic characterized. ► Eggs are considered pure *S. haematobium* by genetic characterisation (ITS of rDNA and *cox*1 mtDNA). ► *S. haematobium* eggs are phenotypically characterise by morphometric comparison with experimental populations of *S. bovis* and *S. mansoni* eggs. ► The results of principal component analysis (PCA), including seventeen non-redundant measurements, showed three phenotypic patterns corresponding to *S. haematobium, S. bovis* and *S. mansoni eggs.* ► The methodology used for the phenotypic differentiation is capable of discerning morphologically close eggs as it is the case of the *haematobium* group.

Keywords : S. haematobium S. bovis, S. mansoni, eggs, phenotypic analysis, standardized methodology, sub-Saharan migrants, Spain

1. Introduction

Schistosomiasis is a parasitic chronic disease caused by trematodes of the genus *Schistosoma*. This disease is considered a Neglected Tropical Disease (NTD) by WHO (WHO, 2020) in spite of having been ranked second only to malaria in socioeconomic terms and importance for public health. It affects more than 250 million people in 78 countries worldwide, 779 million are at risk of infection, and it is estimated to have caused 1.4 million disability-adjusted life years in 2017 (GBD 2017 DALYs and HALE Collaborators, 2018; Hotez et al., 2014; McManus et al., 2018).

There are six *Schistosoma* species known to affect humans: *Schistosoma haematobium, S. mansoni, S. intercalatum, S. guineensis, S. japonicum, S. mekongi*, some of them such as mainly *S. japonicum* but secondarily also *S. mansoni* being acknowledged to be zoonotic able to infect a broad range of livestock and wildlife animals (Colley and Secor, 2014; Webster et al., 2006; WHO, 2015).

Schistosoma haematobium has been reported in 54 countries, with more than 110 million people in Africa only (Boissier et al., 2016; McManus et al., 2018) and it is the most common species worldwide, i.e. more people are infected with *S. haematobium* than with all the other schistosome species combined (Boissier et al., 2016; Rollinson, 2009). Schistosomiasis is usually found in travellers and migrants in Europe (Lingscheid et al., 2017). Both autochthonous and imported cases of urogenital schistosomiasis have been described in Europe. In this sense, autochthonous cases were described in the French island of Corsica (Berry et al., 2014; Boissier et al., 2015), changing the paradigm of tropical diseases and rising awareness of the globalisation of pathogens. Moreover, molecular studies

identified as aetiological agents of this introduction both pure *S. haematobium* and hybrids of *S. haematobium* and the ruminant parasite *S. bovis.* Furthermore, genetically pure *S. bovis* was also found in a patient of Corsica (Boissier et al., 2016).

On the other hand, *S. haematobium* cases in Spain have increased in the last decade (Salas-Coronas et al., 2020). Yet, studies that characterize genetically and phenotypically the etiological agents in Europe are scarce.

The identification of schistosome species can be done indirectly, using molecular methods, but the direct identification of eggs in faeces or urine, based on their morphology, remains the cheapest, easiest and most viable way. Each trematode species has its own particular egg shape and the length and width of the eggs are generally within a specific range (Valero et al., 2009). These features allow for the aetiological diagnosis of schistosomiasis using easily and non-invasive obtained samples. In schistosomiasis, the usual diagnosis is based on the classification of eggs, found in, faeces and / or urine (WHO, 2020). *Schistosome* eggs have very characteristic shapes and features, with the morphology of the egg being diagnostic for each *Schistosoma* species (Candido et al., 2018), i.e. *S. mansoni* eggs show a prominent lateral spine, while those of *S. haematobium* present a terminal spine. On the other hand, *S. japonicum* eggs present only a rudimentary spine, varying from a small bump on the shell to a complete absence of this feature (Candido et al., 2018; Karl et al., 2013; Leiper, 1911; Woolley and Huffman, 1911). Likewise, different species of schistosomes present eggs that vary in size, not only between different species but within the same species of the *Schistosoma* parasite (Lewis and Tucker, 2014; Southgate and Bray, 2009). Until recent years, the

tropism of egg excretion could be considered as a unique proxy for species identification. Eggs recovered from faeces were supposed to be *S. mansoni*, whereas eggs recovered from urine were supposed to be *S. haematobium*. However, since the discovery of several hybrid forms this proxy was challenged, and eggs with *S. mansoni* shape (*S. mansoni x S. haematobium* hybrids) can be found in urine and eggs with *S. haematobium* shape (*S. bovis x S. haematobium* hybrids) can be found in faeces (Huyse et al., 2009; Le Govic et al., 2019; Webster et al., 2019). The situation becomes much more complex if the parasite is a hybrid between animal and human infecting schistosomes, because egg shapes of animal schistosomes (e.g. *S. bovis* or *S. mattheei*) can be found in human excreta (Boissier et al., 2018; Huyse et al., 2009; Webster et al., 2019).

The *Schistosoma haematobium* group includes species that presents terminal spine eggs and develops in freshwater snails of the genus *Bulinus*. This group contains eight species, some of which are of medical (*S. haematobium*, *S. intercalatum*, *S. guineensis*) and veterinary (*S. bovis*, *S. mattheii* and *S. curassoni*) importance (Leger and Webster, 2017) and comprise a complex of species with a challenging diagnosis. The difficulty of egg species differentiation is further complicated by the presence of intraspecific morphometric variation and potential natural hybridisations, as previously shown experimentally (Taylor, 1970; Wright and Southgate, 1976).

Morphological studies carried out to date with *S. haematobium*, *S haematobium-S. bovis* hybrid eggs have failed to demonstrate morphological differences in shape and size between “pure” and hybrid eggs. Previous studies carried out both in adults and in eggs of flukes of other species between which hybridization takes place have shown that this possibility is feasible. These morphological studies in *F.*

hepatica and F*. gigantica* in humans and animals show that the characterisation of pure standard populations facilitates subsequent comparisons and has made it even possible to identify intermediate morphological forms (Ashrafi et al., 2006; Periago et al., 2006, 2008; Valero et al., 2005, 2009; Afshan et al., 2014; Ahasan et al., 2016). Furthermore, studies in eggs of *Fasciola* species, show the influence of different factors on the morphological phenotype of parasite eggs, such as the geographical location or the host species (Valero et al., 2001, 2002, 2009). However, Fasciolidae eggs have different morphological characteristics than Schistosomatid eggs. Therefore, a search for a new analytical methodology that are useful is necessary for Schistosomatids.

Until now, there is no method available or measurement standardisation to properly analyse the phenotype of Schistosome eggs. Surprisingly, the particular morphology of *Schistosoma* eggs is mainly characterised by only three parameters, i.e. length, width, and the length/width ratio; the latter being highly variable (about 70% of this variation was present within a single patient) (Almeda et al., 1996). In addition, these classical morphometric measurements do not reveal the "spindle" character of some species. Therefore, with the aim of discerning *S. bovis*, *S. haematobium* and *S. mattheei* eggs, the width of the egg at an arbitrary distance of 50 µm from the non-spiked end was chosen (Alves, 1949). Later on, the width of the posterior process at 40 µm from the tip of the spine was introduced to characterise the eggs of the same species (Pitchford, 1965).

In the last years, geometric morphometrics has been proved as a useful tool for the differentiation of eggs of helminths such as the genus *Fasciola* (Valero et al., 2009) or *Trichuris* (García-Sánchez et al., 2020). As previously suggested, the egg shape of *Schistosoma* spp. could be determined more precisely by taking additional

measurements and by using geometric morphometric techniques (Boon et al., 2017).

The objectives of this work are: i) to characterise morphogenetically eggs of *S. haematobium* involved in cases of sub-Saharan migrants present in Spain by means of a morphometric comparison with pure experimental populations of *S. bovis* and *S. mansoni;* and ii) to propose a new analytical methodology. In order to do so, only eggs considered pure *S. haematobium* by genetic characterisation (ITSs of rDNA and *cox*1 mtDNA) have been used.

2. Material and Methods

2.1. Ethics statement

Human samples were obtained at the Tropical Medicine Unit of Poniente Hospital (El Ejido, Almeria, Spain) for diagnostic purposes as part of the standard protocol care for sub-Saharan patients attended at such a unit. Anonymised data were collected retrospectively. This study was approved by the Ethics Committee of the Coordinating Site (protocol Schis-01-UMT-2018). At the end of the study, all participants were informed about their parasitological results and free treatment was offered when found positive for schistosomiasis (single 40 mg/kg dose of Praziquantel).

Animal samples were obtained at the laboratory of the IHPE (Hôtes-Pathogènes-Environnements), CNRS, Université de Perpignan (Perpignan, France) which has permission A66040 from «Ministère de l'Enseignement supérieur de la Recherche et de l'Innovation (France)» for animal experimentation. Experimenters hold the certificate for animal experimentation (authorisation 007083, decree 87-848 and

2012201-0008). Housing, breeding and animal care followed the national and European ethical requirements.

2.2. Material

A total of 174 *Schistosoma* eggs were analysed (**Table 1**):

S. haematobium obtained from urine (humans): 84 eggs from six urine samples of young migrant men (20-27 years old, average 24; 5 from Senegal, 1 from Mauritania) living in Spain (from 1 to 14 months) and diagnosed with urogenital schistosomiasis at the Tropical Medicine Unit of Poniente Hospital (El Ejido, Almeria, Spain) and considered pure *S. haematobium* by genetic characterisation (intergenic ITS region of rDNA and *cox*1 mtDNA). Epidemiological and clinical records were also collected for each patient. Parasite eggs were recovered from urine by using a 40 µm cell strainer (Falcon®) and 0.9% NaCl saline solution to wash the sample through the filter. All eggs trapped on the strainer were collected in a Petri dish containing 20mL of 0.9% saline solution.

S. bovis obtained from livers (hamster): 47 eggs from infected hamster liver squashes from the laboratory strain (Silva et al., 1977), identified as genetically pure, maintained in the IHPE (Interactions Hôtes-Pathogènes-Environnements), CNRS, Perpignan, France. They were mashed and passed through a column of metal sieves of different pore size to isolate parasite eggs from the debris. All eggs trapped on the last sieve were collected in a Petri dish containing 20mL of 0.9% saline solution.

S. mansoni obtained from livers (mice): 43 eggs from infected mice liver squashes from the laboratory strain (*Sm*BRE strain, Brazil) (Théron et al., 1997), identified as genetically pure, maintained in the IHPE (Interactions Hôtes-Pathogènes-

Environnements), CNRS, Perpignan, France. Parasite eggs were recovered from the livers following the same technique and procedure as described above for *S. bovis*.

This study has its limitations. It must be taken into consideration that there might be differences in the size and shape of eggs depending on their method of sampling as geographical origin. As previously highlighted (Touassem, 1987), the shape of *S. bovis* eggs from the host faeces (the typical ones) and from the distal portion of the worm uterus tended to be homogeneous, whereas those from the host liver were more heterogeneous for two different strains of this species. Previous studies of *Fasciola hepatica* egg size showed characteristic morphometric traits to be related to their definitive host species (Valero et al., 2009). Nevertheless, this phenomenon has not been previously described in the genus *Schistosoma*. *Schistosoma bovis* and *S. mansoni* eggs used in this work were recovered from hamster livers and not from natural host samples, which might have influenced the morphometry outcome. Nevertheless, the high specificity of pure *S. bovis* for ruminant host makes it difficult to plan a study design with samples of human origin (as only sporadic cases of *S. bovis* have been detected in humans, with a very small burden). For this reason and given the need to use genetically characterised material, experimental *S. bovis* eggs from hamsters were used. On the other hand, pure *S. mansoni* eggs, were used in this study as external group, as these eggs have a very different morphology (lateral spine). The egg sample was obtained from mice, a highly validated experimental model.

Furthermore, egg sizes obtained herein experimentally have been verified to agree with previous egg descriptions from humans (see discussion). However, we consider size and shape of these three species to be truly different, so that the influence of host and sample methods can be disregarded.

As fixation is known to affect size and shape of helminth eggs, egg materials used in the present study were analysed without prior fixation, suspended in 0.9% NaCl saline solution, preserved in darkness at 4 °C until required and digitalised in the shortest possible time, within the next fortnight after collection (Valero et al., 2009).

2.3. Molecular analysis of Schistosoma eggs from urine samples

S. haematobium-like eggs obtained from the six human urine samples were molecularly characterised by a mitochondrial marker (partial cytochrome c oxidase subunit I - *cox*1), and a nuclear marker (intergenic ITS region of the rDNA). Genomic DNA was extracted individually from each egg using the InstaGeneTM Matrix kit (Bio-Rad Laboratories® CA, USA) following the manufacturer's instructions, and stored at −20 °C until use.

For mitochondrial *cox*1 profiling, a RD-PCR from each of the 84 eggs was performed using species-specific primers (Boon et al., 2017; Van den Broeck et al., 2011; Webster et al., 2010) to amplify a specific *cox*1 DNA region (differing in length) for *S. bovis* (306 bp), *S. mansoni* (375 bp) and *S. haematobium* (543 bp). The PCR reactions were performed in a final volume of 12.6 µL, comprising 8 µL of DNA template, 2.5 µL of 5X Colorless GoTaq Flexi Buffer (Promega), 0.75 µL of $MgCl_2$ (25 mM), 0.25 µL of dNTP (10 mM), 1 µL of primer mix (1 µM), and 0.1 µL of Go Taq G2 Hot Start Polymerase (Promega). The PCR conditions were 3 min at 95 °C, followed by 35cycles of 30 s at 94 °C, 1 min 30 s at 58 °C and 1 min 30 s at 72 °C followed by a final cycle at 72 °C for 7 min. The *cox*1-PCR products were visualised for electrophoresis on a 2.5% agarose gel stained with GelRed (Biotium) and photographs were taken using the UVP xgel documentation system.

To complement the information provided by mitochondrial DNA profiling, inherited maternally, the complete ITS-1-5.8S-ITS-2 of the rDNA were PCR amplified independently and each PCR product was sequenced from a subsample of 25 eggs (4-6 eggs/ per patient), using BD1 and BD2 primers (Luton et al., 1992). PCR amplifications were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA USA), following PCR conditions previously described (Bargues et al., 2017; Chougar et al., 2020; Valero et al., 2018). PCR products were purified using the Ultra Clean™ PCR Clean-up DNA Purification System (MoBio, Solana Beach, CA, USA) according to the manufacturer's protocol and resuspended in 50 µl of 10 mM TE buffer (pH 7.6). The final DNA concentration was determined by measuring the absorbance at 260 and 280 nm on an Eppendorf BioPhotometer (Hamburg, Germany). Sequencing was performed on both strands by the dideoxy chain-termination method. It was carried out with the Taq dye-terminator chemistry kit on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using PCR primers.

Sequences were edited and assembled using Sequencher v.5.4.6 (Gene Codes Co.) and aligned with MEGA version 7 (Kumar et al., 2016), using default settings. A careful inspection of all nucleotide positions in the raw sequence chromatograms allowing the detection of sequence polymorphisms between *S. haematobium* and *S. bovis*, was checked to identify possible heterogeneity, as previously described (Angora et al., 2020; Savassi et al., 2020; Webster et al., 2013). Homologies were performed using the BLASTN program from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Schistosome egg digitalization and measurements

Schistosome egg measurements were performed on 174 samples [84 eggs from human urine (*Sh*), 47 from a hamster liver (*Sb*), and 43 from a mouse liver (*Sm*)] (**Table 1**). The measurements were taken by means of a Computer Image Analysis System (CIAS), including a computer connected to a digital camera linked to a microscope. Each egg was individualised in a 0.9% NaCl drop on a slide and photographed using a microscope (Leica DMR 72-89663) with 400 magnifications and the high-resolution camera Leica DFC450C controlled by the LAS software. Representative images of the photographed eggs and their spines are shown in **Figure 1.**

All pictures were taken without coverslip for the establishment of a correct standardisation of the measurements. During the workup of the standardisation, the influence of the coverslip on egg shape and size was assessed. The same egg was photographed before (**Figure 1D**) and after (**Figure 1E**) a cover slip was gently lowered on the saline solution drop where it was placed. No pressure was applied. In addition, the lack of coverslip allowed the recovery of individual eggs, and preservation in EtOH 70º, for posterior molecular characterisation. For morphometric characterisation, the software Image Pro Plus v5.1 (Media Cybernetics, USA) was used.

A total of twenty-five measurements were designed to phenotypically characterise the different schistosome eggs. Twenty-five measurements were designed. In order to identify the ones that give some valuable information about the egg morphology and therefore, were suitable for the principal component analysis, an iterative process of measurement combinations was performed. As a result, 17

measurements were considered of interest as they yield high contribution values in the multivariate analysis. These 17 selected measurements are detailed in **Figure 2** and include:

- **Egg Area (EA)**. Area included in the polygon defining the egg outline.
- **Egg Perimeter (EP)**. Length of the egg outline.
- **Radius max (Rmax)**. Maximum distance between egg centroid and outline.
- **Radius min (Rmin)**. Minimum distance between egg centroid and outline.
- **Egg length (EL)**. Feret diameter along major axis of egg.
- **Egg width (EW)**. Feret diameter along minor axis of egg.
- **Egg Roundness (ER)**. Defined as ($perimeter^2/4\pi \cdot Area$). It is a measurement of how circular an object is (the expected perimeter of a circular object divided by the actual perimeter). A circular object will have a roundness of 1.0, while more irregular objects will have larger values (Anonymous, 2001; Ohnuma et al., 2006).
- **Width at 20 µm from blunt end (BE20).**
- **Width at 35 µm from blunt end (BE35).**
- **Width at 50 µm from blunt end (BE50).**
- **Width at 20 µm from spine end (SE20).**
- **Width at 35 µm from spine end (SE35).**
- **Width at 40 µm from spine end (SE40).**
- **Spine length (SL).**
- **Spine base width (SBW).**
- **Spine medium width (SMW).**
- **Spine end width (SEW).**

The above mentioned 17 measurements included size, shape and spine features and were not redundant (EA, EP, Rmax, Rmin, EL, EW, ER, BE20, BE35, BE50, SE20, SE35, SE40, SL, SBW, SMW and SEW).

Furthermore, the following ratios were used:

Egg Shape Ratio (ESR). Defined as ($EL/BE35 \cdot Roundness$). It is a measurement of how spindled an object is, as it relates the width of the egg at 35 µm from the blunt end (where the narrowing is usually found) with its total length and circularity.

- **Elongation Ratio (ERatio).** Defined as (Rmax/Rmin).

2.5. Statistical data and analyses

Statistical analysis was performed using SPSS Statistics 26. Comparison of the average egg measurements from the different populations was carried out using one-way ANOVA and the Bonferroni test. Values were considered statistically significant when $P<0.05$.

The software Ene 3.0 (Servei d'Estadistica Aplicada, Universidad Autonoma de Barcelona, Cerdanyola del Valles, Spain, distributed by GlaxoSmithKline) was used to establish the minimum number of eggs needed to obtain statistically significant results (P: 0.05, power: 80%).

Current statistical techniques in morphometrics make it possible to test the null hypothesis of conspecific populations being simply the allometric extension of each other, provided a common allometric trend is identifiable (Rohlf and Marcus, 1993). Biometric data was examined using multivariate analysis. Morphological differences were quantified by geometrical morphometrics, which includes validated methods for analysing variation in organismal shape (Klingenberg, 2016). These procedures focus on the retention of geometric information throughout a study and provide efficient, statistically powerful analyses that can readily relate abstract, multivariate results to the physical structure of the original specimens (Slice, 2007).

Principal component analysis (PCA) is an estimator of certain parametric structure characteristics of the population. It may also be used as a dimension-reduction technique that identifies orthogonal linear combinations of the original variables that most efficiently reproduce sample variability. The latter use is particularly

important in morphometric research as the number of shape variables to be analysed can be very large and often exceeds reasonable sample sizes (Slice, 2007). For each analysis, we built a two-dimensional morphospace plot based on the first two principal component.

Size-free canonical discriminant analysis was used on the covariance matrix of measurements transformed into logarithms to assess morphometric variation among samples. This technique consists of removing the effect of within-group ontogenetic variation, regressing each character separately on the within group first principal component (a multivariate size estimator) (Periago et al., 2008).

The resulting "allometry-free" (effect of size on morphological variation), or size-free, variables were submitted to a canonical variate analysis (CVA) and Mahalanobis distances were derived (Mahalanobis, 1936). The Mahalanobis distance can be used to measure how distant a point is from the center of a multivariate normal distribution. The degree of similarity between egg populations was assessed through pairwise Mahalanobis distances (García-Sánchez et al., 2020).

PCA was carried out using the natural logarithm of measurements applying the BAC software. PVA and Mahalanobis distances were calculated using PAD software and tested by non-parametric permutation tests with 1,000 iterations each. Values were considered statistically significant when $P<0.05$ (Dujardin, 2002; García-Sánchez et al., 2019). Nevertheless, to avoid matrix singularities, some measures were discarded because of their partial overlap with other measures. The following 17 non-redundant measurement (i.e. one is not included in another one) were considered: EA, EP, Rmax, Rmin, EL, EW, BE20, BE35, BE50, SE20, SE35, SE40, ER, SL, SBW, SMW and SEW.

Linear regression was calculated for the ERatio values and PC1 and PC2 (SPSS Statistics 26). This morphometric trait has been used in egg characterisation of other animal species, proving to be a useful tool of classification (Attard et al., 2018). Results were considered statistically significant when $P<0.05$.

3. Results

3.1. Molecular criteria for species identification

Genetic profiles provided by *cox*1 mtDNA and ITS region of rDNA, allowed defining that the parasites (schistosome terminal-spined eggs from urine samples) were *S. haematobium* and not a hybrid species.

Cox1 rapid diagnostic PCR

The 84 eggs from the urine samples from the six *Schistosoma*-infected patients furnished an *S. haematobium cox*1 profile. No egg/miracidium gave an *S. bovis* or *S. mansoni* profile.

ITS rDNA sequence analysis

ITS rDNA profiles showed that *S. haematobium* were present in all the six human samples selected for this study. No hybrid profiles were detected in the chromatograms. Among the 25 sequences obtained for the nuclear Internal Transcribed Spacers (ITSs), two profiles of ITSs were retrieved; one of them (Sh-1A) showed sequence identity (100%) with *S. haematobium* (consensus sequence retrieved from GenBank = Acc. Nos. GU257398, JQ397400-JQ397414, FJ588861, MT884914 and MT158873) and was obtained in 17 of 25 sequences (68%), and in patients from both, Mauritania and Senegal. The second profile (Sh-2A) presented

a heterozygotic signal (C/T) in position 533 of the ITSs alignment and did not correspond to any discriminative position among other *Schistosoma* species with terminal-spined eggs. This Sh-2A sequence was present in 32% (8/25) of samples, and only in two patients from Senegal, and showed sequence identity (100%) with *S. haematobium* from the Ivory Coast (MG554667).

3.2. Comparative biometric analysis among Schistosoma species

Biometric features were compared between different species (**Table 2**). As previously known, the longest eggs belong to *S. bovis,* while the widest are those of *S. mansoni*. The overlap in the length range of some *S. haematobium* and *S. bovis* eggs is noteworthy, being inexistent between the latter and *S. mansoni* eggs.

The significant differences obtained by comparing each egg measurement in pairs of *Schistosoma* species using the Bonferroni tests are shown in **Table 3**. *S. haematobium* eggs are significantly different from those of *S. bovis* and *S. mansoni* for Egg Area (EA), Egg Perimeter (EP), Width at 50 µm from Blunt End (BE50), Width at 20 µm from Spine End (SE20), Width at 35 µm from Spine End (SE35), Width at 40 µm from Spine End (SE40), Egg Shape Ratio (ESR), Spine Length (SL), Spine Base Width (SBW), Spine Medium Width (SMW), and Spine End Width (SEW).

Eggs of *S. bovis* are significantly different from those of *S. mansoni* regarding Radius maximum (Rmax), Egg Perimeter (EP), Egg Length (EL), Egg Width (EW), Egg Roundness (ER), Width at 20 µm from Blunt End (BE20), Width at 35 µm from Blunt End (BE35), Width at 50 µm from Blunt End (BE50), Width at 20 µm from Spine End (SE20), Width at 35 µm from Spine End (SE35), Width at 40 µm from Spine End (SE40), Egg Shape Ratio (ESR), Spine Length (SL), Spine Base Width

(SBW), Spine Medium Width (SMW) and Spine End Width (SEW). These results suggest that the morphological traits designed offer detailed and specific values for each schistosome species proving to be useful to characterise and compare them.

3.3. Multivariate analysis among species

First, a principal component analysis was performed. Initially, the 17 non-redundant measurements were analysed all together (EA, EP, Rmax, Rmin, EL, EW, ER, BE20, BE35, BE50, SE20, SE35, SE40, SL, SBW, SMW and SEW), regardless of the nature of each of them (size, shape, spine) (**Figure 3A**). The three species appear well differentiated in the factor map, i.e. three phenotypic patterns could be distinguished in the egg material analysed: *S. haematobium, S. bovis* and *S. mansoni*. PCA showed that the *S. bovis* population presented a large egg size range with a pronouncedly larger maximum size. Contribution of the two principal components was PCI: 57% and PCII: 30%. Moreover, for subsequent analysis, measurements were divided into three groups: size, shape and spine measurements. Size measurements included EA, Rmax, Rmin, EP, EL and EW. *S. haematobium* and *S. mansoni* mostly overlapped as their eggs have a similar size, slightly bigger for *S. mansoni* (**Figure 3B**). Shape measurements included the Width at six different distances from both the Spike and Blunt End of the eggs (BE20, BE35, BE50, SE20, SE35 and SE40) and ER (**Figure 3C**). In this factor map, *S. haematobium* is closer to *S. bovis* although a certain overlap occurs with *S. mansoni*. Spine measurements included SL, SBW, SMW and SEW (**Figure 3D**) and show clear differences between *S. mansoni* and the other two species. Despite the overlap between *S. haematobium* and *S. bovis*, the latter show larger spine values than *S. haematobium* when projected on the X axis.

Second, a multivariate discriminant analysis of the seventeen non-redundant measurements was performed (EA, EP, Rmax, Rmin, EL, EW, ER, BE20, BE35, BE50, SE20, SE35, SE40, SL, SBW, SMW and SEW)(**Figure 4A**). In this case, canonical factor contribution was significant, and the three species appear well differentiated. Therefore, no more analyses were needed. Spine measurements were also included for a detailed description (**Figure 4B**). The contribution of canonical factors increased for this analysis and species appeared differently, appearing closer together (even overlapping) but still well differentiated.

Mahalanobis distances between each pair of groups were calculated for each discriminant analysis performed (**Table 4).** In general (**Table 4A** = 17 measurements: (EA, EP, Rmax, Rmin, EL, EW, ER, BE20, BE35, BE50, SE20, SE35, SE40, SL, SBW, SMW and SEW), *S. mansoni* and *S. bovis* present larger distances between them than with *S. haematobium,* i.e. they present the greatest differences. Regarding the spine, *S. haematobium* and *S. mansoni* are the most distant species (**Table 4B** = spine measurements: SL, SBW, SMW and SEW).

3.4. Linear regression analysis

The relationship between the elongation ratio (ERatio) values and aforementioned PC1 and PC2 scores was represented through a linear regression analysis, one for each combination of measurements.

- **Size, shape and spine measurements** (17 measurements: EA, EP, Rmax, Rmin, EL, EW, ER, BE20, BE35, BE50, SE20, SE35, SE40, SL, SBW, SMW and SEW).). PC1 scores (57%) showed a significant positive correlation with ER values for the eggs of *S. haematobium* (rs=0.0855, P=0.0070), *S. bovis* (rs=0.3374, P<0.0001) and *S. mansoni* (rs=0.1519, P=0.0098) (**Figure 5A**).

PC2 scores (30%) were significantly negatively correlated with ER values for eggs of *S. haematobium* (rs=0.6249, P<0.0001), *S. bovis* (rs=0.2331, P=0.0006) and *S. mansoni* (rs=0.5464, P<0.0001) (**Figure 5B**).

- **Size and shape measurements** (13 measurements: EA, EP, Rmax, Rmin, EL, EW, BE20, BE35, BE50, SE20, SE35, SE40 and ER). PC1 scores (77%) showed a significant positive correlation with ER values for eggs of *S. haematobium* (rs=0.7787, P<0.0001), *S. bovis* (rs=0.6754, P<0.0001) and *S. mansoni* (rs=0.6981, P<0.0001) (**Figure 6A**). PC2 scores (14%) were equally significantly positively correlated with ER values for eggs of *S. bovis* (rs=0.3494, P<0.0001) and *S. mansoni* (rs=0.1507, P=0.0101), whereas no correlation was detected for *S. haematobium* eggs (rs=0.000667, P=0.8156) (**Figure 6B**).
- **Size measurements** (6 measurements: EA, EP, Rmax, Rmin, EL and EW). PC1 scores (70%) showed a significant and strong negative correlation with ER values for eggs of *S. bovis* (rs=0.4154, P<0.0001) and *S. mansoni* (rs=0.184, P = 0.0035), whereas no correlation was found for *S. haematobium* eggs (rs = 0.004829, P = 0.5299) (**Figure 7A**). On the contrary, PC2 scores (27%) were significantly positively correlated with ER values of all species: *S. haematobium* (rs=0.8695, P<0.0001), *S. bovis* (rs=0.8799, P<0.0001) and *S. mansoni* (rs=0.8295, P<0.0001) (**Figure 7B**).
- **Shape measurements** (7 measurements: ER, BE20, BE35, BE50, SE20, SE35 and SE40). PC1 scores (88%) showed a significant and strong positive correlation with ER values for eggs of all species: *S. haematobium* (rs=0.7256, P<0.0001), *S. bovis* (rs = 0.6358, P<0.0001) and *S. mansoni* (rs=0.6425, P<0.0001) (**Figure 8A**). A significant negative correlation was

found for PC2 scores (10%) with ER values for *S. haematobium* eggs (rs=0.08718, P=0.0064), whereas no correlation was found neither for eggs of *S. bovis* (rs=0.002111, P=0.7591) nor *S. mansoni* (rs=0.07454, P=0.0765) (**Figure 8B**).

The results obtained in the lineal analysis show different correlation patterns among the *Schistosoma* species. Although *S. bovis* and *S. mansoni* are the most distant species in terms of taxonomy and morphology, they present a similar behaviour in terms of correlation of their principal components and elongation ratios when size and shape measurements are analysed. However, *S. haematobium* behaves differently than the other two species, showing no correlation when size measurements are involved, while being significantly correlated when only shape measurements are considered.

4. Discussion

4.1. Morphogenetic characteristics of S. haematobium

This is the first morphogenotypic study to characterise *S. haematobium* eggs thoroughly analysis. Genetic profiles provided by the *cox*1 mtDNA and the intergenic ITS region of the rDNA allowed defining that the parasites (schistosome terminal-spined eggs from urine samples) were *S. haematobium* and not a hybrid species.

Morphological traits *of Schistosoma* eggs have been characterised by using, for the first time, 19 parameters, which allow increasing the specific accuracy substantially when compared to classical measurements, i.e. length and width. Furthermore, this technique has been applied in *S. bovis* and *S. mansoni.* The latter alsoplays the role of external group.

S. haematobium and *S. bovis* eggs are both terminal-spined but significant differences are noteworthy. *S. bovis* eggs released in stool are larger (usually between 200 - 300 µm) and spindle-shaped, one bearing a well differentiated spine, the other evenly rounded (Boon et al., 2017; Taylor, 1970; Touassem, 1987). Previous descriptions of *S. bovis* egg showed a wide range in the measurements included (**Table 5**). Despite the fact that the eggs analysed herein come from hamsters, their length range (162.63 - 251.16 µm) fits perfectly with depictions of cattle samples 130 – 300 µm (MacHattie et al., 1933; Savassi et al., 2020), suggesting that differences found between parasite species eggs are minimally influenced by the host species. Due to the spindle shape of *S. bovis*, their maximum width of 40-90 µm (Alves, 1949; Savassi et al., 2020) and 35.65 - 81.21 µm (present study) does not differ significantly from that of *S. haematobium* of 38-95 µm (Savassi et al., 2020; Zeibig, 2012) and 38.17 - 66.85 µm (present study), providing further evidence that this measurement on its own is insufficient for a detailed characterisation. The length/width ratio of the eggs analysed herein (data not shown) fits within the ratios previously described (**Table 5**).

The preceding width measurements proposed at 40 µm and 50 µm (Alves, 1949; Pitchford, 1965) were designed especially for *S. mattheei* phenotyping. 40 µm was longer than the length of the longest spine of *S. mattheei* measured at their laboratory and fell well within that portion of the egg where the shouldering would affect the shape. These two measurements seem sufficient for the characterisation of *S. bovis* and *S. mattheei* although width at a greater distance from blunt and spike ends of *S. bovis* eggs should be considered as differences that can usually be found closer to the halfway point. However, when applying them to *S. haematobium* eggs, relevant information about their shape would be missing, as 50

and 40 µm are too close to the egg midway and, therefore, yield a very similar value to their maximum width. In this study, four shorter measurements have been included, allowing a thorough description of the egg shape. However, when comparing exclusively *S. bovis* populations, width at 70 and 60 µm from blunt and spine end, is highly recommended in order to detect fluctuations that might be present in different strains.

S. haematobium eggs are smaller and rounder than *S. bovis* and are usually found in urine. In previous descriptions, length varied from 83 µm to 170 µm in eggs originating from human urine (**Table 6**) (Alves, 1949; Pitchford, 1965; Savassi et al., 2020). The average size (139.13 µm, range 111.64 - 164.12 µm) of the *S. haematobium* eggs analysed herein falls within this range although our minimum record is slightly larger. The maximum width (66.85 µm) detected in the *S. haematobium* eggs analysed in the present study is also significantly smaller than previously described (95 µm) (Savassi et al., 2020), probably due to the use of the coverslip in similar studies.

Previous descriptions showed a wide range in the measurements included, indicating the necessity of further studies on the influence of the different factors involved such as geographical origin.

Earlier phenotypic characterisations of *S. mansoni* eggs by length, width and spine length show similar values to those yielded in the present study (**Table 7**). Length ranges vary from 112 up to 180 µm (present study: 106.55 - 156.53 µm) and width from to 40 to 73 µm (present study: 43.41 - 77.51 µm) (Candido et al., 2018; CDC, 2019; de Souza et al., 2019; Lenzi et al., 2008). The length/width ratio obtained in *S. haematobium* eggs analysed in this study agrees with other reports (CDC, 2019), but it is slightly smaller than the rest of descriptions (i.e. a smaller difference

between length and width are present). Regarding the spine, *S. haematobium* eggs analysed in this study show a larger average spine (25.06 μm) when compared to previous descriptions but similar to a hybrid strain reported in the Ivory Coast (Depaquit et al., 2019). The statistically significant variability found in three different Brazilian strains is noteworthy, evidencing intraspecific variation, including the influence of the geographical origin on *Schistosoma* species (Euzebio et al., 2012).

Egg variability associated to geographical origin has previously been described in other trematodes such as *F. hepatica* and *F. gigantica* (Valero et al., 2005, 2009). In the genus *Schistosoma*, intraspecific egg variability has been described in few species. The variability in size and shape of *S. intercalatum* eggs from São Tomé represented an obstacle in its differentiation from *S. haematobium* eggs through microscopical observations in that region. The results showed that this variation is not related to age, sex or intensity of infection (Almeda et al., 1996). Egg variability in size has also been found within two populations of *S. haematobium* from Algeria (Kechemir and Théron, 1997), but studies on this species remain scarce .

Consequently, the variability in size and shape of *Shistosoma* eggs highlights the necessity of having standardised measurements available which enablea detailed morphological analysis.

4.2. Proposal of a new analytical methodology

Most of the scientific work published in the last decade about *Schistosoma* species is based on molecular characterisation. Herein, we propose a wide selection of morphometric traits that may complement molecular tools. Schistosome eggs

have specific morphological features which play a central role in the diagnosis of the disease. Although several studies have been carried out to elucidate morphological characteristics of the eggs, none of them has proposed a standardised methodology capable of discerning morphologically close eggs as is the case inside the *haematobium* group.

Both *S. haematobium* and *S. bovis* belong to the haematobium group complex. Despite the differences found in average size and shape of eggs and spines at first sight, the length and width ranges of these species overlap. Furthermore, crossing experiments between *S. haematobium* and *S. bovis* resulted in eggs with intermediate sizes and shapes (Brémond et al., 1993; Taylor, 1970).

The present study proposes a new standardised approach able to discern clearly among pure species. As a matter of fact, without having previously demonstrated this possibility, the differentiation of similar eggs such as hybrid forms cannot be addressed. In this sense, the results obtained in the present work are expected to lay the groundwork of comparative morphometric studies, making the differentiation of even potential intermediate forms possible.

Additionally, these new morphometric concepts provide appropriate tools to characterise *Schistosoma* egg morphological features in order to evaluate the influence of the different factors on the morphological phenotype of parasite eggs such as the geographical location.

Funding

Studies funded by Health Research Project No. PI16/00520, Subprograma Estatal de Generación de Conocimiento de la Acción Estratégica en Salud (AES) y Fondos FEDER, Plan Estatal de Investigación Científica y Técnica y de Innovación, ISCIII-MINECO, Madrid, Spain; by the Red de Investigación de Centros de Enfermedades Tropicales – RICET (Projects Nos. RD16/0027/0023 and RD16/0027/0013 of the PN de I+D+I, ISCIII-Subdirección General de Redes y Centros de Investigación Cooperativa RETICS), Ministry of Health and Consumption, Madrid; by Project No. 2016/099 of the PROMETEO Program, Programa de Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain; and by Proyecto de Investigación en Salud PI-001-2019, Consejería de Salud de la Junta de Andalucía, Sevilla, Spain.

Technical support provided by the Servicio Central de Secuenciación para la Investigación Experimental (SCSIE) of Universidad de Valencia (Dr. A. Martínez, M.T. Minguez, Dra. P. Gómez, Dr. R. Benito, Dr. E. Navarro).

MRG was supported by a fellowship of the Programa de Ayudas de Formación de Profesorado Universitario 2015, Ministerio de Ciencia, Innovación y Universidades, Spain. ADE was supported by a fellowship of the Programa de Ayudas de la Generalitat Valenciana y Fondo Social Europeo, 2019 (ACIF/2019/182).

Declaration of competing interests

The authors have declared that no competing interests exist. The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CRediT author statement

Marta Reguera-Gomez: Methodology, Investigation, Writing- Original draft preparation. **M. Adela Valero:** Conceptualization, Formal analysis, Data curation, Writing**,** Reviewing and Editing. **M. Carmen Oliver-Chiva**: Methodology, Investigation. **Alejandra de Elias-Escribano:** Methodology, Investigation. **Patricio Artigas**: Methodology, Investigation. **M. Isabel Cabeza-Barrera**: Methodology, Investigation. **Joaquín Salas-Coronas:** Methodology, Investigation, Original draft preparation and Funding. **Jérôme Boissier**: Investigation, Writing- Reviewing, Conceptualization. **Santiago Mas-Coma**: Writing-Reviewing, Conceptualization, Funding. **M. Dolores Bargues:** Conceptualization, Formal analysis, Writing- Reviewing and Funding.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The English stylistic help by Mr. R. Wilk (Valencia, Spain) is greatly acknowledged.

References

Afshan, K., Valero, M.A., Qayyum, M., Peixoto, R.V., Magraner, A., Mas-Coma, S., 2014. Phenotypes of intermediate forms of *Fasciola hepatica* and *F. gigantica* in buffaloes from Central Punjab, Pakistan. J. Helminthol. 88 (4), 417-26. https://doi.org/10.1017/S0022149X13000369

Ahasan, S.A., Valero, M.A., Chowdhury, E.H., Islam, M.T., Islam, M.R., Hussain Mondal, M.M., et al., 2016. CIAS detection of *Fasciola hepatica/F. gigantica*

intermediate forms in bovines from Bangladesh. Acta Parasitol. 61(2):267-77. https://doi.org/ 10.1515/ap-2016-0037

Agrawal, M. C., 2012. Schistosomes and Schistosomiasis in South Asia. Springer India.

Almeda, J., Ascaso, C., Marcal, G.A., Corachan, M., Southgate, V.R., Rollinson, D., 1996. Morphometric variability of *Schistosoma intercalatum* eggs: a diagnostic dilemma. J. Helminthol. 70 (2), 97-102. https://doi.org/10.1017/s0022149x00015224

Alves, W., 1949. The eggs of *Schistosoma bovis, S. mattheei* and *S. haematobium*. J. Helminthol. 23 (3-4), 127-134. https://doi.org/10.1017/s0022149x00032466

Angora, E. K., Allienne, J. F., Rey, O., Menan, H., Toure, A. O., Coulibaly, J. T. et al., 2020. High prevalence of *Schistosoma haematobium* x *Schistosoma bovis* hybrids in schoolchildren in Cote d'Ivoire. Parasitology 147 (3), 287-294. https://doi.org/10.1017/S0031182019001549

Anonymous, 2001. The proven solution for image analysis. Image-Pro1 Plus, Version 4.5 for Windows™, Start-Up Guide. Media Cybernetics, Inc., Silver Spring, MD. Unpublished manuscript.

Ashrafi, K., Valero, M.A., Panova, M., Periago, M.V., Massoud, J., Mas-Coma, S., 2006. Phenotypic analysis of adults of *Fasciola hepatica, Fasciola gigantica* and intermediate forms from the endemic region of Gilan, Iran. Parasitol. Int. 55(4), 249-60. https://doi.org/10.1016/j.parint.2006.06.003.

Attard, M.R.G., Sherratt, E., McDonald, P., Young, I., Vidal-Garcia, M., Wroe, S., 2018. A new, three-dimensional geometric morphometric approach to assess egg shape. PeerJ. 6, e5052. https://doi.org/10.7717/peerj.5052

Bargues, M.D., Gayo, V., Sanchis, J., Artigas, P., Khoubbane, M., Birriel, S., et al., 2017. DNA multigene characterization of *Fasciola hepatica* and *Lymnaea neotropica* and its fascioliasis transmission capacity in Uruguay, with historical correlation, human report review and infection risk analysis. PLoS Negl. Trop. Dis. 11 (2), e0005352. https://doi.org/10.1371/journal.pntd.0005352

Berry, A., Mone, H., Iriart, X., Mouahid, G., Aboo, O., Boissier, J., et al., 2014. Schistosomiasis haematobium, Corsica, France. Emerg. Infec. Dis. 20 (9), 1595-1597. https://doi.org/10.3201/eid2009.140928

Boissier, J., Mone, H., Mitta, G., Bargues, M. D., Molyneux, D., Mas-Coma, S., 2015. Schistosomiasis reaches Europe. Lancet Infec. Dis. 15 (7), 757-758. https://doi.org/10.1016/S1473-3099(15)00084-5

Boissier, J., Grech-Angelini, S., Webster, B.L., Allienne, J.F., Huyse, T., Mas-Coma, S., et al., 2016. Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. Lancet Infec. Dis. 16 (8), 971-979. https://doi.org/10.1016/S1473-3099(16)00175-4

Boon, N.A.M., Fannes, W., Rombouts, S., Polman, K., Volckaert, F.A.M., Huyse, T., 2017. Detecting hybridization in African schistosome species: does egg morphology complement molecular species identification? Parasitology 144 (7), 954-964. https://doi.org/10.1017/S0031182017000087

Brémond, P., Sellin, B., Sellin, E., Nameoua, B., Labbo, R., Theron, A., et al., 1993. [Arguments for the modification of the genome (introgression) of the human parasite *Schistosoma haematobium* by genes from *S. bovis*, in Niger]. C. R. Acad. Sci. III 316(7), 667-670.

Candido, R.R.F., Morassutti, A.L., Graeff-Teixeira, C., St Pierre, T.G., Jones, M.K., 2018. Exploring Structural and Physical Properties of Schistosome Eggs:

Potential Pathways for Novel Diagnostics? Adv. Parasitol. 100, 209-237. https://doi.org/S0065-308X(18)30007-1

CDC, 2019. CDC - DPDx - Diagnostic Procedures. Stool Specimens. https://www.cdc.gov/dpdx/diagnosticprocedures/stool/morphcomp.html (Accessed: Oct 2020)

Chougar, L., Mas-Coma, S., Artigas, P., Harhoura, K., Aissi, M., Agramunt, V.H., et al., 2020. Genetically 'pure' *Fasciola gigantica* discovered in Algeria: DNA multimarker characterization, trans-Saharan introduction from a Sahel origin and spreading risk into north-western Maghreb countries. Transbound. Emerg. Dis. https://doi.org/10.1111/tbed.13572

Colley D.G., Secor W.E., 2014. Immunology of human schistosomiasis. Parasite Immunol. 36 (8), 347-357. https://doi.org/10.1111/pim.12087

de Souza, R.P., Favero, V., Pascoal, V.F., Lindholz, C., Bittencourt, H.R., Graeff-Teixeira, C., 2019. Criteria for identification of *Schistosoma mansoni* eggs in faecal sediments prepared with the Helmintex method and stained by ninhydrin. Mem. Inst. Oswaldo Cruz 114, e180529-02760180529. https://doi.org/S0074-02762019000100409

Depaquit, J., Akhoundi, M., Haouchine, D., Mantelet, S., Izri, A., 2019. No limit in interspecific hybridization in schistosomes: observation from a case report. Parasite 26, 10. https://doi.org/10.1051/parasite/2019010

Dujardin, J.P., 2002. BAC and PAD software [computer software]. Unité de Recherches 062- Unité Mixte de Recherches UMR9926, Institut de Recherches pour le Développement (IRD, France).

Euzebio, A.A., Zuim, N.R., Linhares, A.X., Magalhaes, L.A., Zanotti-Magalhaes, E.M., 2012. Experimental evaluation of the pathogenicity of different strains of

Schistosoma mansoni. Interdiscip. Perspect. Infect. Dis. 894940. https://doi.org/10.1155/2012/894940

Gadgil, R. K., 1963. Human schistosomiasis in India. Indian. J. Med. Res. 51, 244-251.

García-Sánchez, A.M., Rivero, J., Callejón, R., Zurita, A., Reguera-Gomez, M., Valero, M.A., et al., 2019. Differentiation of *Trichuris* species using a morphometric approach. Int. J. Parasitol. Parasites Wildl. 9, 218-223. https://doi.org/10.1016/j.ijppaw.2019.05.012

García-Sánchez, A.M., Reguera-Gomez, M., Valero, M.A., Cutillas, C., 2020. Differentiation of *Trichuris* species eggs from non-human primates by geometric morphometric analysis. Int. J. Parasitol. Parasites Wildl. 12, 214-219. https://doi.org/10.1016/j.ijppaw.2020.07.001

GBD 2017 DALYs and HALE Collaborators, 2018. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392 (10159), 1859-1922. https://doi.org/10.1016/S0140-6736(18)32335-3

Hotez, P. J., Alvarado, M., Basáñez, M., Bolliger, I., Bourne, R., Boussinesq, M., et al., 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl. Trop. Dis. 8 (7), e2865. https://doi.org/10.1371/journal.pntd.0002865

Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T., Polman, K., et al., 2009. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog. 5, e1000571. https://doi.org/10.1371/journal.ppat.1000571

IARC., 2018. Biological Agents. Working Group on the Evaluation of Carcinogenic Risk to Humans. Vol 100B. International Agency for Research on Cancer. ISSBN: 9789-283201526.

Jain, A., Jain, P., 2019. Essentials of Microbiology. Elsevier Health Sciences.

Jewsbury, J. M., 1968. Variation in egg shape in *Schistosoma haematobium*. Exp. Parasitol. 22 (1), 50-61. https://doi.org/10.1016/0014-4894(68)90078-7

Jurberg, A. D., Goncalves, T., Costa, T. A., de Mattos, A. C., Pascarelli, B. M., de Manso, P. P., et al.,2009. The embryonic development of *Schistosoma mansoni* eggs: proposal for a new staging system. . Dev. Genes Evol. 219(5), 219-234. https://doi.org/10.1007/s00427-009-0285-9

Karl, S., Gutiérrez, L., Lucyk-Maurer, R., Kerr, R., Candido, R. R., Toh, S.Q. et al., 2013. The iron distribution and magnetic properties of schistosome eggshells: implications for improved diagnostics. PLoS Negl. Trop. Dis*. 7* (5), e2219. https://doi.org/10.1371/journal.pntd.0002219

Kechemir, N., Theron, A., 1997. Intraspecific variation in *Schistosoma haematobium* from Algeria. J. Helminthol. 71 (1), 29-33. https://doi.org/10.1017/s0022149x00000754

Klingenberg, C. P., 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev. Genes Evol. 226 (3), 113-137. https://doi.org/10.1007/s00427-016-0539-2

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33 (7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Leger, E., Webster, J.P., 2017. Hybridizations within the Genus *Schistosoma*: implications for evolution, epidemiology and control. Parasitology 144 (1), 65-80. https://doi.org/10.1017/S0031182016001190

Le Govic, Y., Kincaid-Smith, J., Allienne, J.F., Rey, O., de Gentile, L., Boissier, J., 2019. *Schistosoma haematobium-Schistosoma mansoni* Hybrid Parasite in Migrant Boy, France, 2017. Emerg. Infect. Dis. 25, 365-367. https://doi.org/ 10.3201/eid2502.172028

Leiper, R.T., 1911. Note on the presence of a lateral spine in the eggs of *Schistosoma japonicum*. Trans. Royal Soc. Trop. Med. Hyg. 4 (5), ii-136. https://doi.org/10.1016/S0035-9203(11)90019-4

Lenzi, H.L., Jurberg, A.D., Coelho, P.M.Z., Lenzi, J.A., 2008. Migração e desenvolvimento de *Schistosoma mansoni* no hospedeiro definitivo. In: *Schistosoma mansoni* Esquistossomose: uma visão multidisciplinar. Editora FIOCRUZ.

Lewis, F.A., Tucker, M.S., 2014. Schistosomiasis. Adv. Exp. Med. Biol. 766, 47-75. https://doi: 10.1007/978-1-4939-0915-5_3

Lingscheid, T., Kurth, F., Clerinx, J., Marocco, S., Trevino, B., Schunk, M., et al., 2017. Schistosomiasis in European Travelers and Migrants: Analysis of 14 Years TropNet Surveillance Data. Am. J. Trop. Med. Hyg. 97 (2), 567-574. https://doi.org/10.4269/ajtmh.17-0034

Loker, E. S., 1983. A comparative study of the life-histories of mammalian Schistosomes. Parasitology 87, 343-369. https://doi.org/10.1017/s0031182000052689

LoVerde, P. T., 2019. In Toledo R., Fried B. (Eds.), Schistosomiasis. In: Digenetic Trematodes (2nd ed.). Springer International Publishing.

Luton, K., Walker, D., Blair, D., 1992. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). Mol. Biochem. Parasitol. 56 (2), 323-327. https://doi.org/0166-6851(92)90181-I

MacHattie, C., Mills, E.A., Chadwick, C.R., 1933. Can sheep and cattle act as reservoirs of human schistosomiasis? Trans. Royal Soc. Trop. Med. Hyg. 27(2). https://doi.org/10.1016/S0035-9203(33)90006-1

Mahalanobis, P.C., 1936. On the generalized distance in statistics. Proc. Nat. Inst. Sci. India, 12, 49-55.

MCD International. Schistosomiasis - Microscopy Findings. https://mcdinternational.org/trainings/malaria/english/dpdx5/html/frames/S-Z/Schistosomiasis/body_Schistosomiasis_mic1(Accessed: Oct 2020)

McManus, D.P., Dunne, D.W., Sacko, M., Utzinger, J., Vennervald, B.J., Zhou, X.N., 2018. Schistosomiasis. Nature Rev. Dis. Primers 4 (1), 13-8. https://doi.org/10.1038/s41572-018-0013-8

Mehlhorn, H., 2016. Schistosoma Species. In H. Mehlhorn (Ed.), Encyclopedia of Parasitology (pp. 2449-2462). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-43978-4_2822

Moné, H., Minguez, S., Ibikounlé, M., Allienne, J. F., Massougbodji, A., Mouahid, G., 2012. Natural Interactions between *S. haematobium* and *S. guineensis* in the Republic of Benin. Sci. World J. 793420. https://doi.org/10.1100/2012/793420

Ohnuma, K., Yomo, T., Asashima, M., Kaneko, K., 2006. Sorting of cells of the same size, shape, and cell cycle stage for a single cell level assay without staining. BMC Cell Biol. 7 (1), 25. https://doi.org/10.1186/1471-2121-7-25

Othman, A., El Ridi, R., 2014. Schistosomiasis. In F. Bruschi (Ed.), Helminth Infections and their Impact on Global Public Health (pp. 49-92). Springer Vienna. https://doi.org/10.1007/978-3-7091-1782-8_3

Periago, M.V., Valero, M.A., Panova, M., Mas-Coma, S., 2006. Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). Parasitol. Res. 99, 368-78. https://doi.org/10.1007/s00436-006-0174-3

Periago, M.V., Valero, M.A., El Sayed, M., Ashrafi, K., El Wakeel, A., Mohamed, M.Y., et al., 2008. First phenotypic description of *Fasciola hepatica*/*Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. Infect. Genet. Evol. 8 (1), 51-58. https://doi.org/S1567-1348(07)00141-4

Pitchford, R.J., 1965. Differences in the Egg Morphology and Certain Biological Characteristics of some African and Middle Eastern Schistosomes, Genus *Schistosoma*, with Terminal-Spined Eggs. Bull. World Health Organ. 32, 105-120.

Rohlf, F.J., Marcus, L.F., 1993. A revolution morphometrics. Trends Ecol. Evol. 8 (4), 129-132. https://doi.org/10.1016/0169-5347(93)90024-J

Rollinson, D., 2009. A wake-up call for urinary schistosomiasis: reconciling research effort with public health importance. Parasitology 136 (12), 1593-1610. https://doi.org/10.1017/S0031182009990552

Ross, A. G., Bartley, P. B., Sleigh, A. C., Olds, G. R., Li, Y., Williams, G. M., McManus, D. P., 2002. Schistosomiasis. N. Engl. J. Med. 346(16), 1212-1220. https://doi.org/10.1056/NEJMra012396

Salas-Coronas, J., Vazquez-Villegas, J., Lozano-Serrano, A. B., Soriano-Perez, M. J., Cabeza-Barrera, I., Cabezas-Fernandez, M.T., et al., 2020. Severe complications

of imported schistosomiasis, Spain: A retrospective observational study. Travel Med. Infect. Dis. 35, 101508. https://doi.org/S1477-8939(19)30212-1

Savassi, B., Mouahid, G., Lasica, C., Mahaman, S.K., Garcia, A., Courtin, D., et al., 2020. Cattle as natural host for *Schistosoma haematobiu*m (Bilharz, 1852) Weinland, 1858 x *Schistosoma bovis* Sonsino, 1876 interactions, with new cercarial emergence and genetic patterns. Parasitol. Res. 119 (7), 2189-2205. https://doi.org/10.1007/s00436-020-06709-0

Silva, M.L., Vicente, F. S., Avelino, I.C., Martin, V.R., 1977. Susceptibility of *Planorbarius metidjensis* from Portugal and Spain to *Schistosoma bovis* from Salamanca, Spain. Malacologia 16 (1), 251-254. https://agris.fao.org/agris-search/search.do?recordID=US201303039795

Slice, D.E., 2007. Geometric Morphometrics. Ann. Rev. Anthropology 36 (1), 261-281. https://doi.org/10.1146/annurev.anthro.34.081804.120613

Southgate, V.R., Bray, R.A., 2009. Medical helminthology. *Manson's* Tropical Diseases (pp. 1633-1698). Elsevier.

Taylor, M.G., 1970. Hybridisation experiments on five species of African schistosomes. J. Helminthol. 44 (3), 253-314. https://doi.org/10.1017/s0022149x00021969

The Australian Society for Parasitology Inc. Schistosoma. http://parasite.org.au/para-site/text/schistosoma-text.html (Accessed: Oct 2020)

The Ohio State University. Schistosomiasis. Parasitology Genitourinary. http://www.pathologyoutlines.com/topic/parasitologyschisto.html (Accessed: Oct 2020)

Théron, A., Pages, J.R., Rognon, A., 1997. *Schistosoma mansoni*: distribution patterns of miracidia among *Biomphalaria glabrata* snail as related to host susceptibility and sporocyst regulatory processes. Exp. Parasitol. 85 (1), 1-9. https://doi.org/10.1006/expr.1996.4106.

Touassem, R., 1987. Egg polymorphism of *Schistosoma bovis*. Vet. Parasitol. 23 (3-4), 185-191. https://doi.org/0304-4017(87)90004-5

Valero, M.A., Darce, N.A., Panova, M., Mas-Coma, S., 2001. Relationships between host species and morphometric patterns in *Fasciola hepatica* adults and eggs from the northern Bolivian Altiplano hyperendemic region. Vet. Parasitol. 102(1-2), 85-100. https://doi.org/10.1016/s0304-4017(01)00499-x.

Valero, M.A., Panova, M., Comes, A.M., Fons, R., Mas-Coma, S., 2002. Patterns in size and shedding of *Fasciola hepatica* eggs by naturally and experimentally infected murid rodents. J. Parasitol. 88(2), 308-13. https://doi.org/10.1645/0022-3395(2002)088[0308:PISASO]2.0.CO;2.

Valero, M.A., Panova, M., Mas-Coma, S., 2005. Phenotypic analysis of adults and eggs of *Fasciola hepatica* by Computer Image Analysis System. J. Helminthol. 79 (3), 217-225. https://doi.org/S0022149X05000326

Valero, M.A., Perez-Crespo, I., Periago, M.V., Khoubbane, M., Mas-Coma, S., 2009. Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop. 111 (2), 150-159. https://doi.org/10.1016/j.actatropica.2009.04.005

Van den Broeck, F., Geldof, S., Polman, K., Volckaert, F.A.M., Huyse, T., 2011. Optimal sample storage and extraction procotols for reli-able multilocus genotyping of the human parasite *Schistosoma mansoni.* Infect. Genet. Evol. 11, 1413–1418. https://doi.org/10.1016/j.meegid.2011.05.006

Webster, B.L., Southgate, V.R., Littlewood, D.T., 2006. A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. Int. J. Parasitol. 36(8), 947-955. https://doi.org/S0020-7519(06)00107-X

Webster, B.L., Rollinson, D., Stothard, J.R., Huyse, T., 2010. Rapid diagnostic multiplex PCR (RD-PCR) to discriminate *Schistosoma haematobium* and *S. bovis*. J. Helminthol. 84 (1), 107-114. https://doi.org/10.1017/S0022149X09990447

Webster, B.L., Diaw, O.T., Seye, M.M., Webster, J.P., Rollinson, D., 2013. Introgressive Hybridization of *Schistosoma haematobium* Group Species in Senegal: Species Barrier Break Down between Ruminant and Human Schistosomes. PLoS Negl. Trop. Dis. 7 (4), e2110. https://doi.org/10.1371/journal.pntd.0002110

Webster, B.L., Alharbi, M.H., Kayuni, S., Makaula, P., Halstead, F., Christiansen, et al., 2019. Schistosome Interactions within the *Schistosoma haematobium* Group, Malawi. Emerg. Infect. Dis. 25, https://doi.org/10.1017/1245-1247.10.3201/eid2506.190020

WHO, 2015. Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected tropical diseases 2015. World Health Organization, Geneva, Switzerland.

WHO, 2019. Bench aids for the diagnosis of intestinal parasites (2nd ed. ed.). World Health Organization, Geneva, Switzerland.

WHO, 2020. Schistosomiasis. https://www.who.int/neglected_diseases/diseases/en/ (Accessed: Oct 2020)

Woolley, P.G., Huffman, O.V., 1911. The ova of *Schistosoma japonicum* and the absence of spines. Parasitology 4 (2), 131-132. https://doi.org/10.1017/S0031182000002572

Wright, C.A., Southgate, V.R., 1976. Hybridisation of schistosomes and some of its implications. in: Genetic aspects of host-parasite relationships, 14th Symposium of the British Society for Parasitology (ed. Taylor, A.E.R. and Muller, R.), pp.55–86. Blackwell Scientific Publications. Oxford.

Zeibig, E., 2012. Clinical Parasitology. A Practical Approach (2nd ed.). Saunders, Elsevier.

CAPTION FOR FIGURES

Figure 1. Egg sample of each *Schistosoma* species without coverslip: (A) *S. haematobium*, (B) *S. bovis*, (C) *S. mansoni* and comparison of one *S. haematobium* egg (D) without and (E) with coverslip. (F, G, H) Great variability in the *S. haematobium* spines was observed (optical microscopy, original magnification, A–E = × 400; no stain used). Scale bars: A–E = 50 µm; F–H = 25 µm.

Figure 1. Standardised measurements designed for the morphometric phenotyping of the eggs of *Schistosoma* species.

Figure 2. Principal component analysis factor map of A) all measurements (17), B) size measurements (6), C) shape measurements (7) and D) spine measurements (4) of the three Schistosoma species analysed (*S. haematobium, S. bovis* and *S. mansoni*). Larger symbols represent the group centroid. In brackets, PC contribution.

Figure 3. Discriminant analysis factor map of A) all measurements (17) and B) spine measurements (4) of the three *Schistosoma* species analysed (*S. haematobium, S. bovis* and *S. mansoni*). Larger symbols represent the group centroid. In brackets, canonical factor (CF) contribution.

Figure 4. Relationship between elongation ratio (ER) values (maximum ratio divided by minimum ratio) and A) PC1 scores (57% contribution) and B) PC2 scores (30% contribution) of size, shape and spine measurements. On PC1, increasingly elongated eggs are associated with increasingly significative higher scores for the three *Schistosoma* species. On PC2, increasingly elongated eggs are associated with decreasingly significative lower scores for the three *Schistosoma* species. rs: r square, Y: orthogonal regression line.

Figure 5. Relationship between elongation ratio (ER) values (maximum ratio divided by minimum ratio) and A) PC1 scores (77% contribution) and B) PC2 scores (14% contribution) of size and shape measurements. On PC1, increasingly elongated eggs are

associated with increasingly significative higher scores for the three *Schistosoma* species. On PC2, increasingly elongated eggs are associated with increasingly higher scores for *S. bovis* and *S. mansoni*, while no significant correlation was detected for *S. haematobium*. rs: r square, Y: orthogonal regression line.

Figure 6. Relationship between elongation ratio (ER) values (maximum ratio divided by minimum ratio) and A) PC1 scores (70% contribution) and B) PC2 scores (27% contribution) of size measurements. On PC1, increasingly elongated eggs are associated with increasingly lower scores for *S. bovis* and *S. mansoni*, while no correlation was detected for *S. haematobium.* On PC2, increasingly elongated eggs are associated with increasingly higher scores for all species. rs: r square, Y: orthogonal regression line.

Figure 7. Relationship between elongation ratio (ER) values (maximum ratio divided by minimum ratio) and A) PC1 scores (88% contribution) and B) PC2 scores (10% contribution) of shape measurements. On PC1, increasingly elongated eggs are associated with increasingly higher scores for all species. On PC2, negative significant correlation was only detected for *S. haematobium* eggs. rs: r square, Y: orthogonal regression line.

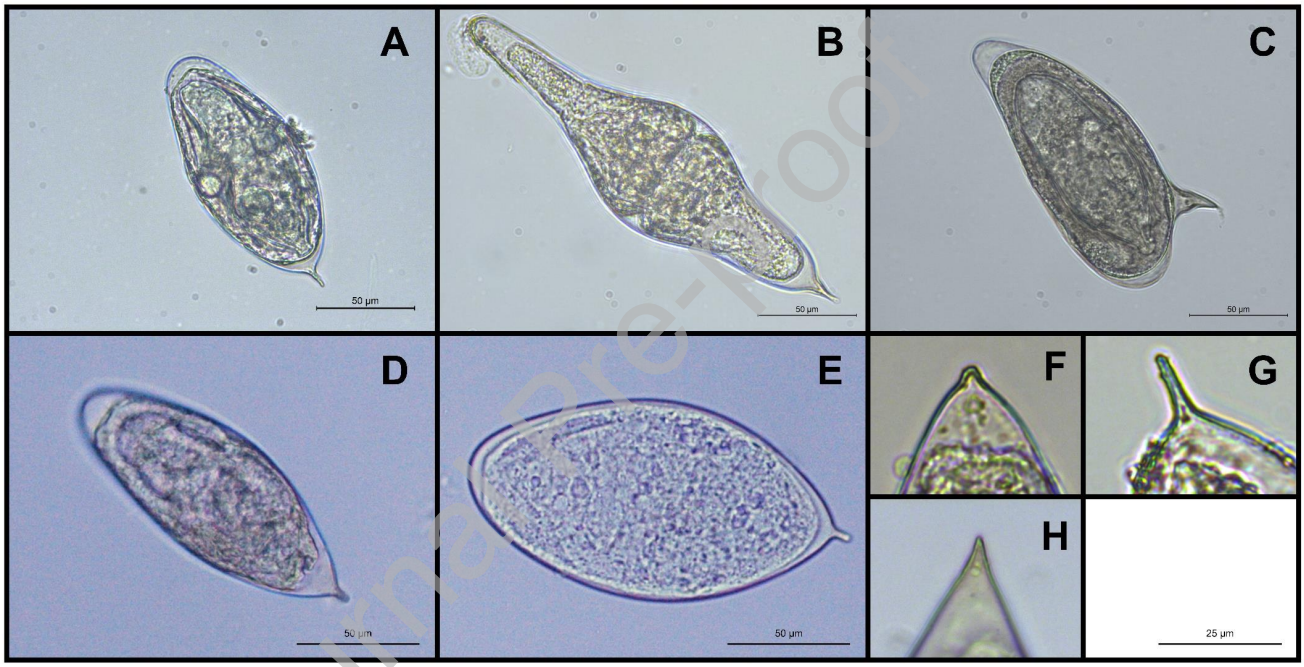


Figure 1

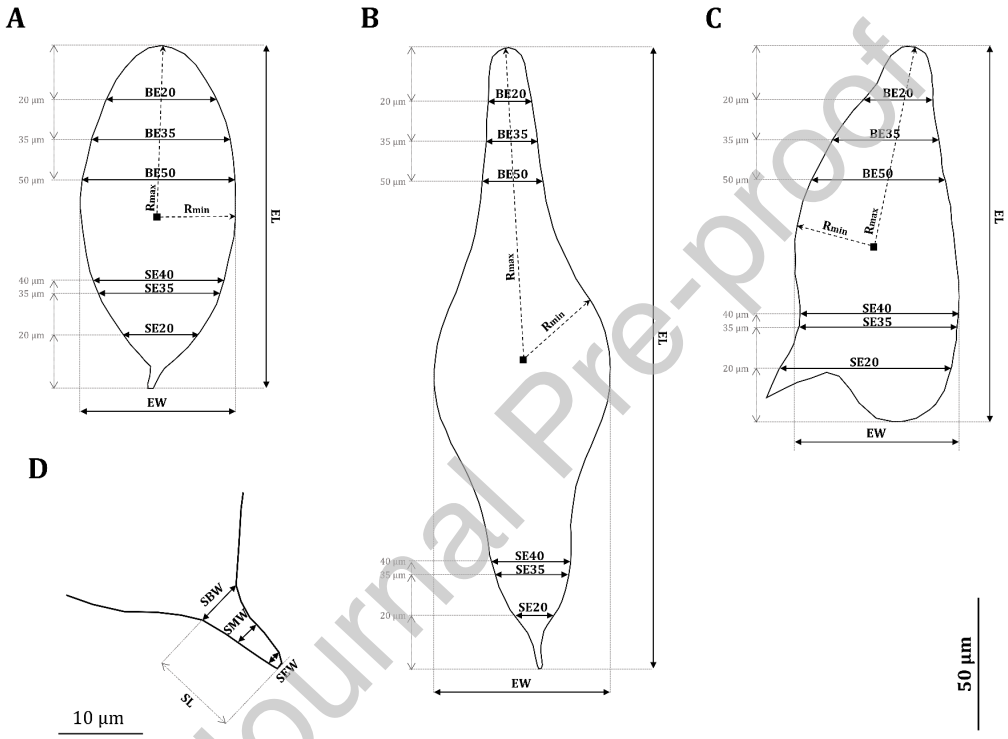


Figure 2

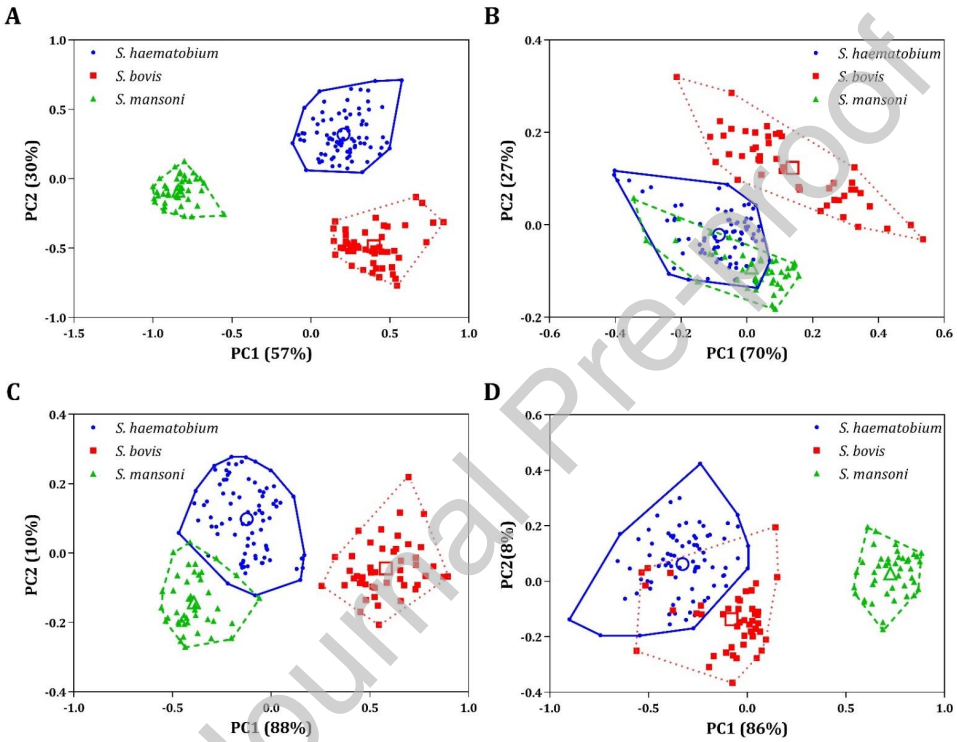


Figure 3

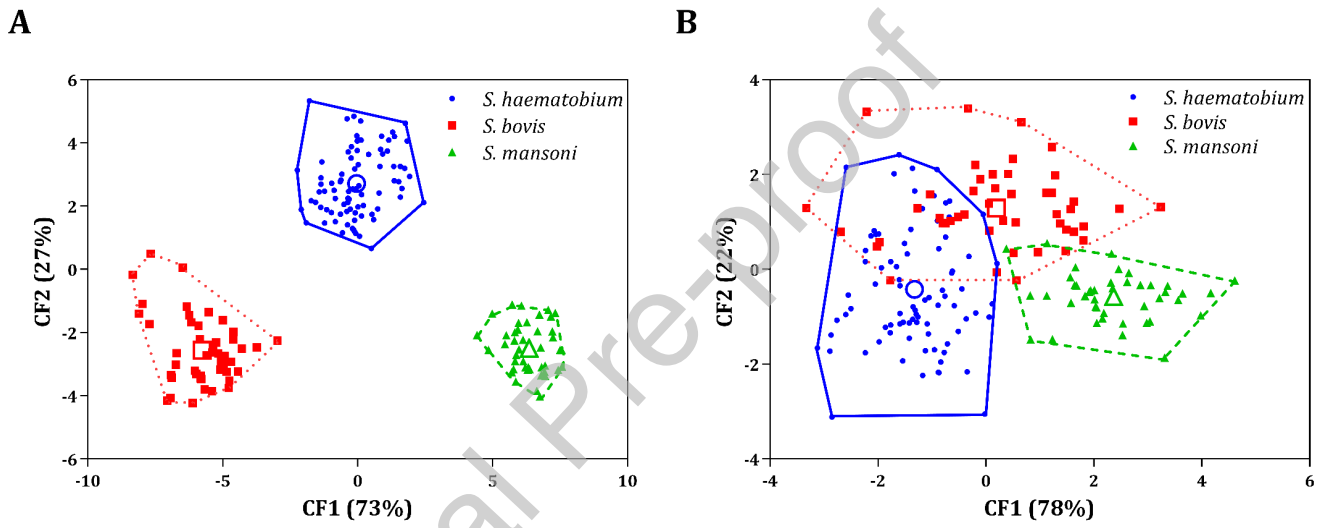


Figure 4

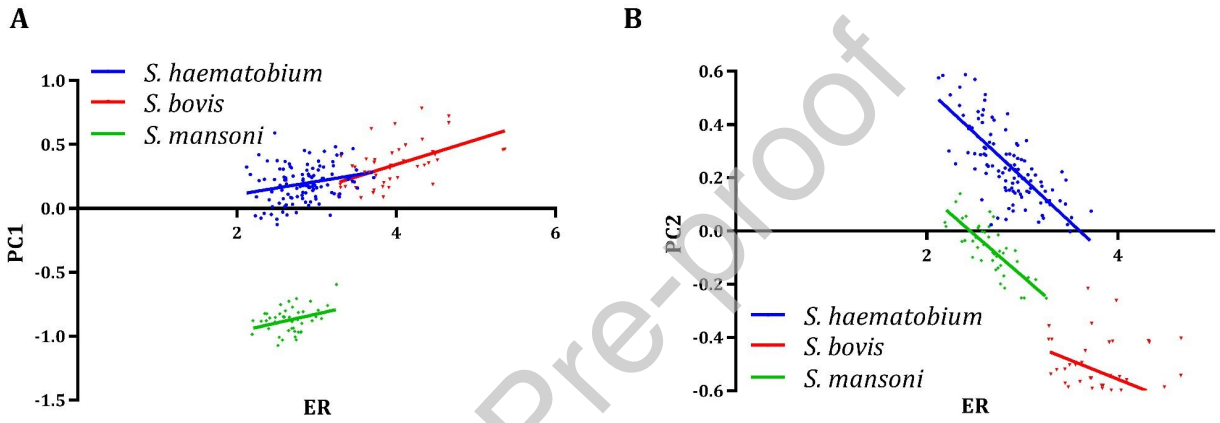


Figure 5

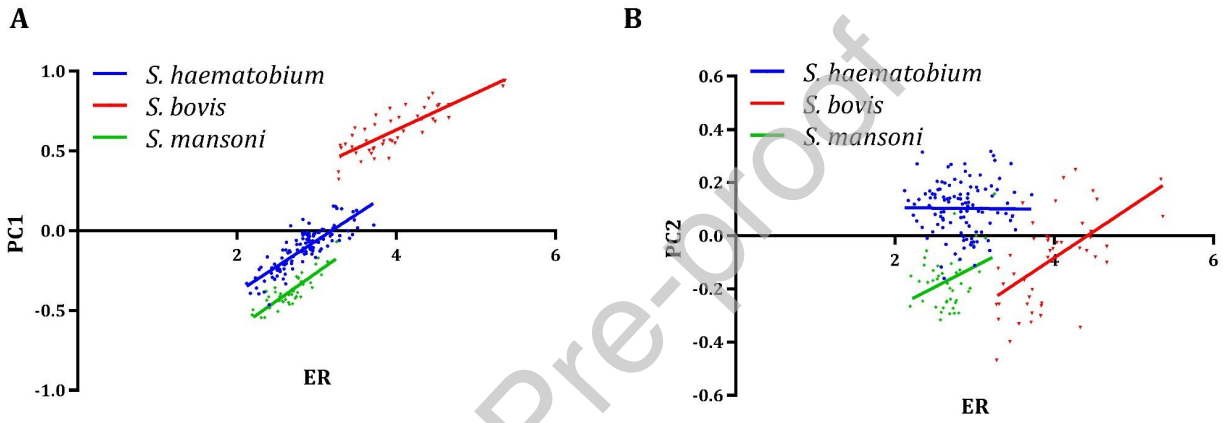


Figure 6

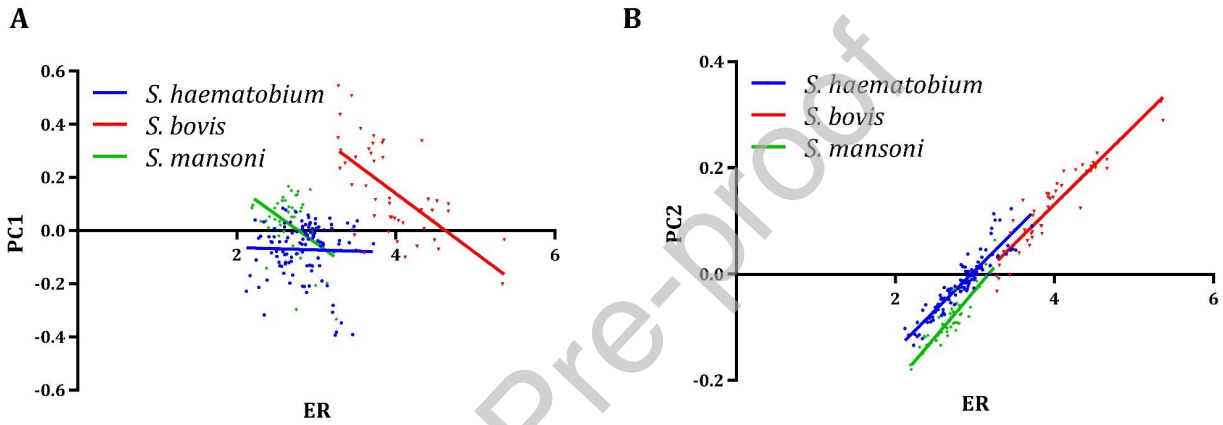


Figure 7

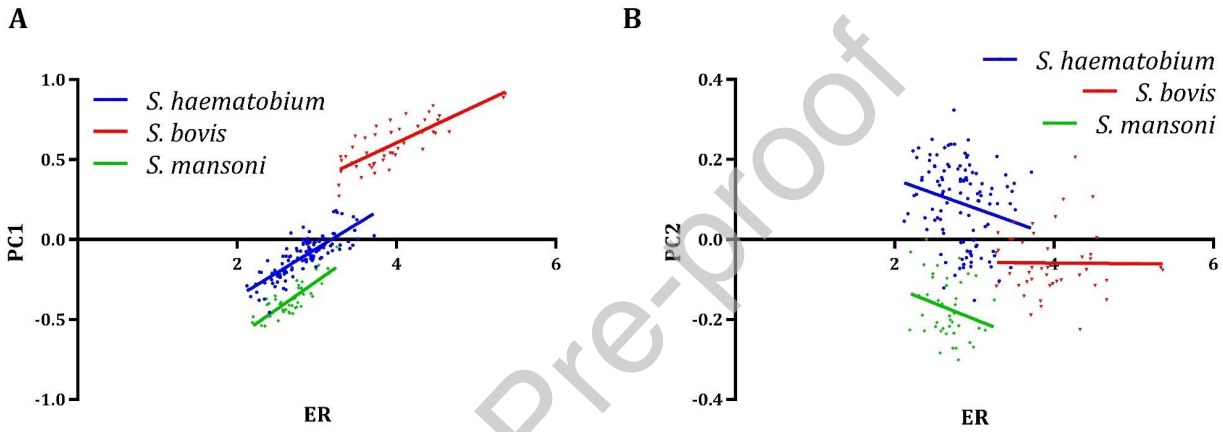


Figure 8

Species	Country	Sample/Host /Patient code	No. eggs phenotyped
***S. haematobium* (*Sh*)**	**MAURITANIA**	Urine/Human/P-15	**14**
	SENEGAL	Urine/Human/P-4	12
		Urine/Human/P-16	30
		Urine/Human/P-18	6
		Urine/Human/P-19	7
		Urine/Human/P-21	15
		Total	**84**
***S. bovis* (*Sb*)**	**SPAIN**	Liver/Hamster/Lab strain *Sb*	**47**
***S. mansoni* (*Sm*)**	**BRAZIL**	Liver/Mouse/Lab. Strain *Sm*	**43**

Table 1. Number of eggs phenotyped of each species and their origin.

	S. haematobium	*S. bovis*	*S. mansoni*
Egg Area (EA)	5027.1 ± 791.1 (2890.27 - 6494.93)	5901.72 ± 1807.79 (2962.75 - 11071.52)	6104.77 ± 1042.86 (3314.58 - 7562.29)
Radius maximum (Rmax, µm)	72.52 ± 6.65 (56.94 - 85.57)	103.22 ± 11.69 (82.88 - 131.61)	73.59 ± 6.41 (57.66 - 83.76)
Radius min (Rmin, µm)	25.89 ± 2.99 (17.23 - 33.12)	26.6 ± 5.21 (16.87 - 40.14)	27.82 ± 3.33 (18.41 - 33.37)
Egg Perimeter (EP, µm)	309.4 ± 27.22 (252.45 - 355.84)	425.95 ± 48.95 (344.49 - 539.46)	349.1 ± 27.46 (266.79 - 392.71)
Egg Length (EL, µm)	139.13 ± 13.48 (111.64 - 164.12)	201.41 ± 22.61 (162.63 - 251.16)	136.69 ± 11.94 (106.55 - 156.53)
Egg Width (EW, µm)	53.95 ± 5.58 (38.17 - 66.85)	55.44 ± 10.4 (35.65 - 81.21)	64.73 ± 6.57 (43.41 - 77.51)
Egg Roundness (ER)	1.54 ± 0.14 (1.25 - 1.82)	2.55 ± 0.3 (2 - 3.42)	1.62 ± 0.11 (1.41 - 1.88)
Width at 20 µm from Blunt End (BE20, µm)	31.18 ± 5.65 (20.2 - 42.53)	15.54 ± 1.79 (11.96 - 18.87)	30.73 ± 3.73 (24.18 - 39.88)
Width at 35 µm from Blunt End (BE35, µm)	39.35 ± 7.26 (24.45 - 53.41)	18.73 ± 2.08 (14.62 - 23.12)	41.87 ± 4.46 (31.63 - 51.28)
Width at 50 µm from Blunt End (BE50, µm)	46.83 ± 7.5 (31.09 - 62.19)	23.31 ± 3.41 (17.01 - 29.5)	50.53 ± 4.69 (39.06 - 59.25)
Width at 20 µm from Spine End (SE20, µm)	24.8 ± 4.62 (16.74 - 38.25)	14.24 ± 2.9 (7.71 - 20.73)	51.09 ± 9.56 (32.41 - 65.09)
Width at 35 µm from Spine End (SE35, µm)	39.18 ± 4.81 (30.56 - 54.75)	22.23 ± 3.81 (14.62 - 31.63)	54.19 ± 5.43 (36.66 - 61.66)
Width at 40 µm from Spine End (SE40, µm)	43.25 ± 4.81 (34 - 57.94)	23.92 ± 4.31 (14.35 - 34)	54.76 ± 5.54 (38.25 - 63.25)
Length/Width Ratio (L/W)	2,6 ± 0,3 (1,99 - 3,31)	3,69 ± 0,42 (3,03 - 4,73)	2,12 ± 0,18 (1,76 - 2,56)
Egg Shape Ratio (ESR)	2.36 ± 0.38 (1.7 - 3.16)	4.28 ± 0.59 (3.19 - 5.77)	2.03 ± 0.18 (1.71 - 2.49)
Elongation Ratio (ERatio, Rmax/Rmin)	2.83 ± 0.36 (2.12 - 3.72)	3.96 ± 0.50 (3.28 – 5.36)	2.67 ± 0.24 (2.19 – 3.25)
Spine Length (SL, µm)	6.55 ± 2.09 (3.19 - 11.32)	12.55 ± 2.95 (5.71 - 18.07)	25.06 ± 3.66 (17.3 - 31.56)
Spine Base Width (SBW, µm)	4.68 ± 1.1 (2.13 - 7.56)	5.89 ± 1.8 (2.66 - 9.03)	20.06 ± 3.36 (13.93 - 29.25)
Spine Medium Width (SMW, µm)	2.8 ± 0.69 (1.19 - 4.45)	3.46 ± 1.18 (1.59 - 6.11)	10.07 ± 1.69 (6.03 - 13.22)
Spine End Width (SEW, µm)	1.54 ± 0.37 (0.59 - 2.67)	1.35 ± 0.41 (0.59 - 2.94)	2.2 ± 0.5 (1.13 - 3.1)

Table 2. Biometric data of the three schistosome species eggs. Mean ± SD (Min – Max).

	S. bovis	*S. mansoni*
S. haematobium	EA, Rmax, EP, EL, L/W, ER, ERatio, BE20, BE35, BE50, SE20, SE35, SE40, ESR, SL, SBW, SMW, SEW.	EA, Rmin, EP, EW, L/W, BE50, SE20, SE35, SE40, ESR, SL, SBW, SMW, SEW.
S. bovis		Rmax, EP, EL, EW, L/W, ER, ERatio, BE20, BE35, BE50, SE20, SE35, SE40, ESR, SL, SBW, SMW, SEW.

Table 3. . Significantly different measurements among the three schistosomes species eggs by Bonferroni tests (P<0.05).

A	*S. haematobium*	*S. bovis*	*S. mansoni*
S. haematobium	0.00		
S. bovis	7.78	0.00	
S. mansoni	8.25	12.12	0.00

B	*S. haematobium*	*S. bovis*	*S. mansoni*
S. haematobium	0.00		
S. bovis	2.29	0.00	
S. mansoni	3.69	2.86	0.00

Table 4. Mahalanobis distances between the three *Schistosoma* species (*S. haematobium, S. bovis* and *S. mansoni*) for the two discriminant analysis performed: (A) all measurements (17) and (B) spine measurements (4). All distances were statistically significant.

Geographical Origin	Host	Sample	Technique	Size (µm) Mean ± Standard deviation (Minimum -Maximum)	N	Comments	Reference
Iraq	-	-	-	EL: 130 -260	-		(MacHattie et al., 1933)
-	Goat, sheep, cattle	Feces	Measurements were taken with a stage micrometer and a camera lucida, the eggs being drawn and afterwards measured with a rod calibrated against a standard magnification and micrometer	EL: 208 (179 - 232) EW: 55 (40 -73) BE50: 31 (24-43)	500		(Alves, 1949)
Sudan	Cattle	Feces	-	EL: 211 ± 14 (178 x 257) SL: 8.2 ± 3.0 (3.9 x 15.8)	500	70% of spines not measurable	(Pitchford, 1965)
-	-	-	-	EL: 202 EW: 58	-		(Loker, 1983)
Kessounou Village, Ouémé, Benin	Cattle	Feces	-	EL: 247.83 ± 4.03 (205 – 300) EW: 69.58 ± 1.56 (57.50-90) SL: 17.38 ± 0.68 (12.50-22.50) L/W Ratio: 3.59± 0.07 (2.93-4.17)	30 (20 for spine)		(Savassi et al., 2020)

Table 5. *Schistosoma bovis* eggs published descriptions. EL, Egg Length; EW, Egg Width; BE50, Width at 50 µm from blunt end; SL, Spine Length. Dash: unknown data.

Geographical Origin	Host	Sample	Technique	Size (µm) Mean ± Standard deviation (Minimum -Maximum)	N	Comments	Reference
West Africa, the Sudan and Southern Rhodesia	Human	Urine	Measurements were taken with a stage micrometer and a camera lucida, the eggs being drawn and afterwards measured with a rod calibrated against a standard magnification and micrometer	EL: 142 (115-170) EW: 59 (46-80) BE50: 54 (44-74)	500		(Alves, 1949)
Malagasy (Magadascar)	Human	Urine	All microscopic work was done with wet preparations under a supported cover-slip. According to the author, it had been shown that the shape and measurements of eggs are not affected significantly by fixation in formol.	EL: 131±11.6 (102-160) SL: 6.7±2.4 (3.3-13.5)	150		(Pitchford, 1965)
India				EL: 132±11.8 (83-187) SL: 6.3±2.4 (1.6-10.1)	200		
Egypt				EL: 131±11.6 (108-162) SL: 6.4±2.5 (1.9-19.7)	100		
Bechuanaland (Botswana)				EL: 139±13.0 (95-174) SL: 7.3±2.6 (1.9-15.8)	500		
Komatipoort, E. Transvaal (South Africa)				EL: 136±13.6 (83-182) SL: 7.0±2.6 (1.9-19.7)	1944		
E. Transvaal (South Africa)		Feces		Represented in a graph, no exact data			
		Bladder		Represented in a graph, no exact data			
	Rodents (various)			EL: 122±11.8 (85-163) SL: 6.4±2.7 (1.6-16.9)	980		
Ghana	Hamster	Liver and intestinal tissue	Liver and intestinal tissue was digested by the method of Smithers (1960) and the eggs laid by the parasites recovered. They were stored in 0.0% saline containing 125 IU penicillin per ml. Eggs were measured using a crossed micrometer eyepiece, either in utero in temporary mounts of adult females, or as suspensions of digested eggs. All eggs which were distorted, not lying horizontally, or partly obscured were rejected for purposes of measurements.	EL: 107.20 EW: 40.84 Length to max breadth: 60.94 SL: 7.91	116		(Jewsbury, 1968)
Nigeria				EL: 100.94 EW: 45.00 Length to max breadth: 52.37 SL: 5.69	16		
Shambat (Sudan)				EL: 102.54 EW: 39.78 Length to max breadth: 56.48 SL: 5.92	2238		
Cairo (Egypt)				EL: 99.12 EW: 36.73 Length to max breadth: 51.88 SL: 5.74	812		
Kisumu (Kenia)				EL:92.28 EW:36.93 Length to max breadth: 48.31 SL: 4.00	76		
Baghdad (Iraq)				EL:114.29 EW: 44.27 Length to max breadth: 65.17 SL: 6.83	321		
Total				EL: 102.72 EW: 39.49 Length to max breadth: 56.17 SL: 5.98	3579		

Shambat (Sudan)	Mice			EL: 88.21 EW: 34.00 Length to max breadth: 46.14 SL: 4.50	14	
Cabemba (Angola)				EL: 99.69 EW: 40.09 Length to max breadth: 52.70 SL: 5.44	270	
Cairo (Egypt)				EL: 94.30 EW: 37.10 Length to max breadth: 49.90 SL: 6.79	484	
Total				EL: 96.08 EW: 38.09 Length to max breadth: 50.82 SL: 6.27	768	
Shambat (Sudan)	*Arvicanthis*			EL: 91.64 EW: 35.74 Length to max breadth: 47.58 SL: 5.60	352	
	Meriones			EL: 85.12 EW: 33.84 Length to max breadth: 44.38 SL: 4.89	207	
-	*Mastomys*			EL: 86.98 EW: 35.84 Length to max breadth: 44.09 SL: 6.17	58	
Ghana	Human	Urine	Eggs were measured using a crossed micrometer eyepiece, either in utero in temporary mounts of adult females, or as suspensions of digested eggs. All eggs which were distorted, not lying horizontally, or partly obscured were rejected for purposes of measurements.	EL: 135.59 EW: 59.09 Length to max breadth: 72.74 SL: 6.80	46	
Nigeria				EL: 135.02 EW: 58.36 Length to max breadth: 73.20 SL: 6.92	50	
Cairo (Egypt)				EL: 134.34 EW: 54.62 Length to max breadth: 69.21 SL: 7.07	95	
Dirdag (Yemen)				EL: 137.66 EW: 58.34 Length to max breadth: 70.89 SL: 7.09	79	
Said (Yemen)				EL: 122.92 EW: 54.95 Length to max breadth: 62.41 SL: 6.98	63	
Kuwait				EL: 118.15 EW: 45.15 Length to max breadth: 63.00	13	

				SL: 7.31			
Baghdad (Iraq)				EL: 135.38 EW: 59.82 Length to max breadth: 70.26 SL: 7.62	34		
Total				EL: 132.92 EW: 56.62 Length to max breadth: 69.26 SL: 7.06	380		
Nigeria	Hamster	Tissues	-	EL: 144.0±14.2 EW: 58.4 ±6.5	75		(Taylor, 1970)
Iran				EL: 135.4±12.4 EW: 54.8 ±6.5	75		
-	-	-	-	EL: 144 EW: 58	-		(Loker, 1983)
-	-	-	-	EL: 150 EW: 62	-		(Ross et al., 2002)
Doh (Republic of Benin)	Human	Urine	Mounted in saline solution (9‰ NaCl) beneath coverslips on glass slides	EL: 139±3 EW: 62±1	25	Based on morphology, might be hybrids	(Moné et al., 2012)
Dangbo (Republic of Benin)				EL: 146±1 EW: 61 ±1	94		
Pakh, Northern Senegal	Human	Urine	Milli-Q water under a coverslip	EL: 136.5±15.2 (92.4-176.4) EW: 61.8±10.5 (35.7-93.0)	218	Mixed with hybrids	(Boon et al., 2017)
Pakh, Northern Senegal		Stool		EL: 136.9±12.8 (110.4-173.2) EW: 63.9±9.1 (42.0-86.5)	85		
-	-	-	-	EL: 83–187 EW: 60			(Candido et al., 2018)
Kessounou Village, Ouémé, Benin	Cattle	Feces	The eggs were mounted individually at random in a 9‰ NaCl solution beneath glass coverslips on glass slides using a Pasteur pipette. They were photographed and measured by microscopy.	EL: 140 ± 42.43 (110 – 170) EW: 67.50 ± 17.68 (55-80) SL: 17.50 ± 3.54 (15-20) L/W Ratio: 2.06 ± 0.09 (2 -2.13)	2	For the *S. haematobium* morphotype, no major difference was observed between the origins (schoolchildren or cows)	(Savassi et al., 2020)
	Human (schoolchildren)			EL: 138.85± 4.68 (120 – 175) EW: 68.85 ± 3.25 (50-95) SL: 10.31 ± 0.61 (7.50-12.50) L/W Ratio: 2.04 ± 0.07 (1.71 -2.50)	13 (8 for spine)		
		Urine		EL: 132.01 ± 1.66 (117.50 – 170) EW: 68.96 ± 1.62 (50-85) SL: 12.59± 0.48 (10-20) L/W Ratio: 1.94 ± 0.04 (1.63 -2.60)	36 (29 for spine)		
-	-	-	-	EL: 110 -170 EW: E38 – 70	-	-	(Zeibig, 2012)
-	-	-	-	EL: 150 EW: 62	-	-	(Othman & El Ridi, 2014)
-	-	-	-	EL: 144 EW: 58	-	-	(Lewis & Tucker, 2014)
-	-	-	-	EL: 150 EW: 50	-	-	(Mehlhorn, 2016)
-	-	-	-	EL: 140	-	-	(Jain & Jain,

				EW: 40			2019)
-	-	-	-	EL: 140 EW: 60	-	-	(LoVerde, 2019)
-	-	-	-	EL: 143 (112-170) EW: 60 (40-70)	-	-	(CDC, 2019)
-	-	-	-	EL: 110-170 EW: 40-70	-	-	(The Ohio State University,)
-	-	-	-	EL: 110-170 EW: 40-70	-	-	(MCD International,)
-	-	-	-	EL: 110-170 EW: 40-70	-	-	(The Australian Society for Parasitology Inc.,)
-	-	-	-	EL: 110 -170	-	-	(WHO, 2019)
-	-	-	-	EL: 144 EW: 58	-	-	(IARC, 2018)

Table 6. *Schistosoma haematobium* eggs published descriptions. EL, Egg Length; EW, Egg Width; BE50, Width at 50 µm from blunt end; SL, Spine Length. Dash: unknown data.

Geographical Origin	Host	Sample	Technique	Size (µm) Mean ± Standard deviation (Minimum -Maximum)	N	Comments	Reference

-	-	-	-	EL: 142 EW: 60	-		(Loker, 1983)
-	Mice	Intestine	On each day of incubation, eggs laid in vitro were collected and mounted on regular flat slides using solid Vaseline as a spacer. For comparison between egg growth under in vitro and in vivo conditions, isolated eggs from the murine host were also analyzed.		30	The area A of each egg was estimated by considering its longest length (major axis) and its longest width (minor axis), in accordance to the area formula of an ellipse: A= (Π × major axis × minor axis)/4.	(Jurberg et al., 2009)
-	-	-	-	EL: 112–175 EW: 45–70	-		(Candido et al., 2018)
-	-	-	-	EL: 117 – 150 EW: 40-70	-		(de Souza et al., 2019)
Belo Horizonte, Minas Gerais, Brazil (BH strains)	Mice	Feces	Measured using Image Pro Lite	EL: 139.34 EW: 56.89 SL: 21.22	31	The length and width of the SD-strain eggs were significantly longer than the eggs from other strains and the eggs of the BH and SJ strains did not differ in length. Eggs from the BH strain had the shortest width. The size of the spicule was not significantly different among the strains; however, some eggs from the SD strain had spicules with a very curved tip.	(Euzebio et al., 2012)
Vale do Rio Paraíba, São Paulo, Brazil (SJ strains)				EL: 141.11 EW: 59.37 SL: 21.09	31		
Jardim São Domingos, Campinas, São Paulo, Brazil (SD strains)				EL: 155.60 EW: 64.63 SL: 20.85	23		
-	-	-	-	EL: 150 EW: 40	-		(Lenzi et al., 2008)
-	-	-	-	EL: 140 EW: 61	-		(Othman & El Ridi, 2014)
-	-	-	-	EL: 140 EW: 60	-		(Lewis & Tucker, 2014)
-	-	-	-	EL:140 (114–180) EW: 66 (45-73)	-		(CDC, 2019)
-	-	-	-	EL: 150	-		(Michigan State University, 1999)
-	-	-	-	EL: 115-175 EW: 45-70	-		(The Australian Society for Parasitology Inc.,)
Côte d'Ivoire	Human	Urine	Microscopic examination at 100x and 400x magnifications	EL: 150 ± 3.20 (146 - 156) EW: 62.5 ± 1.73 (60–66) SL: 25 ± 1.35 (23.5–27.5)	10		(Depaquit et al., 2019)

Table 7. *Schistosoma mansoni* eggs published descriptions. EL, Egg Length; EW, Egg Width; BE50, Width at 50 µm from blunt end; SL, Spine Length. Dash: unknown data

Graphical abstract