Morphological and genomic characterisation of the hybrid schistosome infecting humans in Europe reveals a complex admixture between *Schistosoma haematobium* and *Schistosoma bovis* parasites.

Short-title: Introgression analysis of the European *Schistosoma* parasite hybrid.

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Abstract

Schistosomes cause schistosomiasis, the world's second most important parasitic disease after malaria. A peculiar feature of schistosomes is their ability to produce viable and fertile hybrids. Originally only present in the tropics, schistosomiasis is now also endemic in Europe. Based on two genetic markers the European species had been identified as a hybrid between the ruminant-infective *Schistosoma bovis* and the human-infective *Schistosoma haematobium*.

Here we describe for the first time the genomic composition of the European schistosome hybrid (77% of *S. haematobium* and 23% of S. *bovis* origins), its morphometric parameters and its compatibility with the European vector snail and intermediate host. Compatibility is a key parameter for the parasites life cycle progression. We also show that egg morphology (a classical diagnostic parameter) does not allow for differential diagnosis while genetic tests do so. Additionally, we performed genome assembly improvement and annotation of *S. bovis,* the parental species for which no satisfactory genome assembly was available.

For the first time since the discovery of hybrid schistosomes, these results reveal at the whole genomic level a complex admixture of parental genomes highlighting (i) the high permeability of schistosomes to other species' alleles, and (ii) the importance of hybrid formation for pushing species boundaries not only conceptionally but also geographically.

Keywords: Schistosomiasis, *Schistosoma haematobium*, *Schistosoma bovis*, Hybridization, Genomic Introgression, Whole Genome Next Generation Sequencing.

Author summary

In 2013, schistosomiasis reached Southern Europe. Since then, endemic infections were recurrently identified in 2015 and 2016, clearly indicating that the parasite has settled and established locally. Using two molecular markers, we had previously demonstrated that the parasite is a hybrid between *Schistosoma haematobium* and *S. bovis* that are known to infect humans and livestock, respectively. Nevertheless, this method had very low resolution and did not allow to give clear answers on the origins and the mechanisms of hybrid generation, e.g. if the hybrid had been generated recently on Corsica or if it invasive. The genome-wide sequencing approach used in this work allowed us to reveal a complex admixture between the parental genomes and suggests that hybridization between these two species may be the result of ancient crossing events.

Additionally, wherever in Africa or in Europe, a clear discrepancy exists between the egg shape usually used for species identification and the genomic composition. Therefore, egg shape cannot be used anymore as a good indicator for hybrid detection. Knowing the traits and the genetic features of the hybrid has implications in terms of diagnostic as well as disease management either through vector control strategies or treatment of patients.

Introduction

Schistosomes are parasitic flatworms, responsible for the major tropical disease schistosomiasis, The epidemiological statistics associated with the disease are sobering: 800 million people are at risk in 78 countries, mostly concentrated in sub-Saharan Africa; 230 million are infected and the disease causes more than 200 000 deaths each year as well as between 1.7 and 4.5 million Disability Adjusted Life Years (DALYs) [1]. The most exposed groups are children and young adults that have predominant

activities linked to contaminated freshwater environments. In addition to humans, schistosomiasis severely impacts livestock in Africa and Asia where an estimated 165 million animals are infected [2].

Schistosomes have a complex life cycle that includes passage through a freshwater snail intermediate host (hereafter designed as the parasite vector) and a final vertebrate definitive host. Global changes, both anthropogenic and environmental modifications, may contribute to modifications in the geographical distribution of species and expand their potential ecological niches [3]. Distinct species may thus acquire a new capacity to interact, hybridize and subsequently introgress their genomes by backcrossing with parental species or other hybrids, a phenomenon called “hybrid swarm”. Hybridization between individuals from two previously reproductively isolated species is generally expected to produce offspring less fit than the parents, sometimes infertile or even sterile but in some cases however, hybridization may lead to greater fitness than parental species, also known as hybrid vigour or heterosis [4]. Hybridizations of human parasites are not scarce and are a real concern in terms of parasite transmission, epidemiology and disease [5].

Hybridizations between schistosomes have already been identified: (i) between different human-specific schistosome species, (ii) between different animal-specific schistosome species, (iii) and between human-specific and animal-specific schistosomes species [6]. These latter hybrid forms are particularly alarming because they raise the possibility of the emergence of new zoonotic parasitic strains, introducing an animal reservoir, and therefore greatly hampering our ability to properly control the life cycle.

The precise characterization of the introgression levels of hybrid populations is essential for diagnostics purpose but is also necessary to better understand the parasite life history traits, the disease dynamics and the epidemiology in the field. Next

generation whole-genome sequencing is now the tool of choice for a deeper insight into the genomic composition of natural hybrids.

In this study, we aimed at fully characterize schistosome hybrids that have recently emerged in Europe. We described hybrids based on the morphology of eggs, the disease- and diagnostic-relevant stage, as well as compatibility with potential European snail vectors. We characterized the extent of introgression in natural hybrids at the whole genome scale.

Results

Morphology of the eggs does not allow for differential diagnosis of the hybrid

Morphometric analysis is a classical way to identify species. In Appendix S1 we deliver a precise morphological description of the adults and eggs of the European hybrid. Since eggs are easily accessible in the field, they are commonly used to diagnose the infecting schistosoma species. Therefore, egg morphology was of particular interest to us. A total of 44 eggs collected from hamsters infected with the European hybrid were examined for morphological characterization. The length and width were assessed on all eggs, but the spine length has been measured on a subset of 36 that had a spine distinctive enough to allow for proper estimation of their sizes. The results are presented in Table 1.

Table 1: Morphological measurements results of the Corsican hybrid schistosome eggs (SD = Standard deviation).

	Length (including spine)	Width	Spine length

Nb of eggs	**44**	**44**	**36**
Mean (± SD) in **µm**	126.4 (±22.9)	60.8 (±13.0)	8.2 (±2.1)
Minimum in **µm**	73.9	40.9	3.95
Maximum in **µm**	170.9	92.5	13.6

Most of the eggs displayed a representative elliptical morphotypes and were characterized by a terminal spine, reminiscent of *S. haematobium* infection in humans. Nevertheless, not all eggs had a typical *S. haematobium* morphotype and were in some cases intermediate with *S. bovis*-type eggs (Fig 1). In summary, egg morphology alone cannot differentiate between the hybrid and putative parental species. Nevertheless, this morphological analysis supported the initial conclusion, based on only two genetic markers, that the hybrid originates from a cross between *S. bovis* and *S. haematobium*.

Whole genome sequencing shows introgression of *S. bovis* into *S. haematobium*

To precisely characterize the level of hybridization we anticipated to use Illumina short read massive sequencing and alignment to parental genomes. As the previous *S. bovis* assembly was highly fragmented (111 328 scaffolds, N50 7kb), we resequenced the genome using PacBio long reads. This produced 4,102,584 filtered subreads (48,987,175,429 bases). Genome assembly led to a new genome version of 486 scaffolds (N50 3.1Mb). Gene prediction led to identification of 14,104 protein-coding genes of 18,725 bp average length (5.3% of genome length) (Table 2) which is consistent with the known characteristics of *Schistosoma* genomes [7].

Table 2: Assembly and gene prediction metrics of *S. bovis.*

Proportion of genome that is coding (%)	5.27%
Number of genes	14,104
Average gene length (bp)	18,725 ± 21,625
Average number of exons per gene	7.9
Average exon length (bp)	299
Average intron length (bp)	2,389
Repeat rate (%)	49.5%

For the European hybrid, a total of 289,873,531 reads (76.5%) were mapped against the 681.2 Mb concatenated genomes of *S. haematobium* [7] and *S. bovis* (this work), representing 42X mean coverage (Table 3). We also sequenced F1 males from experimental first generation cross between male *S. haematobium x* female *S. bovis* as a control. A total of 4,910,354 reads (92.3%) were mapped against the concatenate of *S. haematobium* and *S. bovis* genomes.

Table 3: Summary of the introgression level analysis of the experimental F1 hybrids (control) and the Corsican natural hybrid strain.

	Experimental F1 genome (males)	**Corsican hybrid genome**	
		Males	Females
mapped reads (% of total number)	4,910,354 (92.3%)	150,994,161 (80.8%)	138,879,370 (72.2%)
reads mapped against *S. haematobium* genome	2,417,196 (49.2%)	116,052,836 (76.9%)	106,969,327 (77%)

reads mapped against *S. bovis* genome	2,493,158 (50.8%)	34,941,325 (23.1%)	31,910,043 (23%)
reads mapped against *S. haematobium* mitochondrion	168 (1.7%)	50,436 (2.2%)	33,454 (2.2%)
reads mapped against *S. bovis* mitochondrion	9,825 (98.3%)	2,229,096 (97.8%)	1,455,815 (97.8%)

As expected, the mapping of the F1 reads to *S. haematobium* and *S. bovis* genomes gave a proportion that matched the expectations for a first generation hybrid (~50% of each of the parental species genomes; Table 3). This control was crucial to validate our analytical pipeline and thus the results obtained for the Corsican natural European hybrid parasite. Moreover, 98.3% of reads of mitochondrial origin aligned to *S. bovis*, which is consistent with maternal inheritance of the mitochondrial genome.

Interestingly, the mapping of the Corsican hybrid reads against *S. haematobium* and *S. bovis* reference genomes revealed a complex admixture between the parental genomes with a proportion of 76.9% of sequences mapping on *S. haematobium*, and 23.1% mapping on *S. bovis* genomes for both male and female parasites (Table 3). Alignment to the mitochondrial genomes of both parents also showed results concordant with the previous Sanger sequencing data for this marker, with 97.8% of reads being mapped on *S. bovis* mitochondrial genome, and 2.2% on *S. haematobium* mitochondrial genome [8]. To figure out the divergence level between the two pure species genomes, we identified the orthologous regions between *S. haematobium* and *S. bovis* using CACTUS. A total of 234,5 Mb sequences aligned between the parental species, which represents 64% of the *S. haematobium* genome length. The mean similarity of these shared sequences was 95.88%.

Besides being of fundamental interest for the evolutionary biology of the parasite, this finding could also have immediate consequences for parasite control. One of the few phenotypic features that are of relevance for infection success and for which the genetic basis is known is resistance to Oxamniquine (OXA). In *S. mansoni* the mutations that confer resistance occur in the SmSULT-OR gene (Smp_089320), encoding a sulfotransferase that is required for drug activation, are p.E142del and p.C35R [9,10]. In *S. haematobium* the drug is predicted not to be efficient due to a F39 *Sm* > Y54 *Sh* substitution [9]. We used blast searches with Smp_089320 against the *S. haematobium* and the new *S. bovis* genome to identify orthologues. Orthologues exist in both genomes and have no mutations in *Sm* C35 or *Sm* L256 or deletion in *Sm* E142, but both possess the F39 > Y54 which is predicted to negatively impact drug binding. We expect therefore the European hybrid to be genetically resistant to OXA, excluding the drug as treatment option.

We conclude that the European hybrid was generated by an initial cross between *S. haematobium* and *S. bovis* and successive backcrosses with *S. haematobium.*

The European hybrid is compatible with the natural hosts of *S. haematobium*, *Bulinus truncatus* but not with *Planorbarius metidjensis*, host of *S. bovis.*

The presence of 23% of *S. bovis* genetic information in the hybrid genome raised the possibility that the hybrid was capable of infecting the vector snail of *S. bovis.* To test this hypothesis we exposed *Planorbarius metidjensis,* which is a specific host to *S. bovis,* to miracidia of the natural Corsica hybrid The susceptibility of the parental snail hosts was inferred at 35 days after exposition to 5 parasite larvae (miracidia). The prevalence in *Bulinus truncatus* was 24% (9 infected snails out of 37 alive). In *Planorbarius metidjensis* the prevalence was 0% (0 infected snails out of 29 alive), but we cannot exclude the

197 possibility that other strains of *P. metidjensis* could be compatible with the European
198 schistosome hybrid.
199
200 **Discussion**
201 The emergence of new infectious diseases and pathogens is currently among the great
202 concerns of our changing world and has strong outreach effects for society. Besides the
203 important impacts that global changes (climate changes and anthropogenic activities)
204 may have on the spread and transmission of tropical infectious diseases in higher
205 latitudes, other phenomenon may combine and act as a driving force promoting the
206 emergence of novel disease in unsuspected areas. The importance and the frequency of
207 hybridization in infectious agents are certainly underestimated, and very little attention
208 has been given so far to the role of gene introgression on infectious disease emergence,
209 spread and control [11]. In the genus *Schistosoma,* latest reports have revealed that
210 hybrids are frequent throughout West Africa, and are already a real concern for human
211 health [5,6]. Nevertheless, it is the first time that a hybrid schistosome is involved in a
212 large-scale outbreak in Europe [8,12,13]. Although usually restricted to tropical areas,
213 schistosomiasis is now persisting in Corsica and the hybrid status of the parasite might
214 have increased its invasive and adaptive capacities. The hybrid status of the parasite
215 may indeed have important implication for disease control in term of host spectrum,
216 diagnostic, and treatment.
217 **Implications for host spectrum and parasite distribution**
218 *S. haematobium* and *S. bovis* have different intermediate host specificities. *S*
219 *haematobium* only infect mollusks from *Bulinus* genus while *S. bovis* also infect
220 *Planorbarius* snails (widely present in the Iberian Peninsula). The potential distribution
221 range of the disease may be enhanced if the hybrid is able to infect the intermediate

hosts of both parental species. Interestingly, the natural Corsican hybrid schistosome that we recovered was not able to infect our laboratory strain of *Planorbarius metidjensis*, but displayed high levels of compatibility with *Bulinus truncatus* from Corsica (24% of prevalence) which is consistent with previous compatibility assessments for the hybrid parasite recovered directly from an infected patient in 2014 [8]. This also goes in line with the predominance of *S. haematobium* sequences in this hybrid, and may open the door for vector based control, e.g. by outcompeting these snails. One other fundamental concern is the capacity of such introgressed schistosomes to infect livestock or other reservoir hosts. The zoonotic potential of the hybrids would strongly impact the parasite transmission in the field in and out of endemic areas, and may hamper our capacity to maintain adequate control strategies as schistosomiasis treatment focuses almost exclusively on humans. Nevertheless, despite a recent study showing the presence of pure species (*S. bovis, S. haematobium*, and *S. mansoni*) as well as hybrids between *S. bovis* and *S. haematobium* in rodents (most probable hosts in which hybridization occurs) [14], no other wild animals have been found with hybrid schistosomes. The situation in Corsica also needs to be precisely investigated.

Implications for diagnostics

The hybrid status of the parasite may impair the parasitological, serological and molecular diagnostic. In endemic countries, parasitological diagnostic is the rule whereas serological tests are commonly used for imported schistosomiasis in non-endemic, developed countries. In humans, schistosome eggs that are partly retained in the tissues are the cause for the disease and induced pathology, but are also classical tools for diagnosis and species identification. At first sight, egg morphology and localization in the urine of infected patients in Corsica strongly suggested an *S. haematobium* infection [8]. Indeed *S. haematobium* eggs that are usually voided by the

247 urine have a typical round to oval shape (elliptical or elongated) with a terminal spine.
248 According to previous studies, *S. haematobium* eggs measure between 100-156 µm long
249 and 40-50 µm wide with usual length between 115-135 µm long [15-17]. A previous
250 analysis of this Corsican hybrid schistosome eggs revealed smaller eggs (n=15) with a
251 mean length of 106.5 µm and width of 42.8 µm, and with a spine length of 10.4 µm [13].
252 According to our results the eggs generally show an ovoid shape measuring 126.4 x 60.8
253 µm (Table 1) more similar to *S. haematobium* eggs. This is also consistent with the
254 introgression levels that show a predominance of S. *haematobium*-type sequences
255 (Table 3). Sometimes eggs were intermediate with spindle or diamond shapes, which
256 are characteristic of *S. bovis* eggs (usually bigger and measuring between 170-223.9 µm
257 long and 55-66.0 µm wide) and have an elongated spindle or diamond shapes [18,19]
258 (Fig 1). In addition, whereas *S. haematobium* eggs are found in the urine of infected
259 patients, *S. bovis* eggs are released in the feces. Thus, we can expect that for the hybrid
260 parasite eggs may also be released in part in the feces. This could explain, together with
261 the low parasite intensity, why only 30% of patients infected in Corsica excreted eggs in
262 the urine [20]. The route of excretion associated with egg shape is the gold standard for
263 diagnostic and species determination, nevertheless our results confirm earlier
264 publications showing that it is impossible to detect hybridization in schistosome species
265 using egg morphology alone [21].
266 Concerning the serological diagnostic, the majority of commercial tests, ELISA or IHA
267 (indirect hemagglutination) use *S. mansoni* antigens. A discrepancy between those
268 antigens and the infecting species may induce false negative results [22]. The efficiency
269 of these commercial diagnostic kits need thus to be reevaluated in a context of hybrid
270 infection. Finally, molecular diagnostic for urogenital schistosomiasis using PCR has
271 already been used in urine or serum, targeting a highly repeated sequence (DraI), which

is restricted to the *S. haematobium* group of schistosomes (including both *S. haematobium* and *S. bovis*) [23,24]. We expect that this test would be efficient to detect but not to identify the hybrid status of the parasite.

Implications for treatment

Praziquantel (PZQ) is currently the only available drug used to treat schistosomiasis and the application of mass chemotherapy programs is the prevailing strategy for schistosomiasis control [25]. The treatment is efficient on bovine schistosomiasis but the dose needed (60 mg/kg for >95% deworming in goat [26,27]) cannot be considered in endemic areas were schistosomiasis is first of all a human health concern. Moreover, it has been shown that a dose of 40 mg/kg of PZQ is only 63.5% efficient for mixed infections, compared to 76.7% and 77.1%, for *S. mansoni* and *S. haematobium*, alone,respectively [28]. A lower sensitivity to PZQ of *S. bovis* x *S. haematobium* hybrid schistosomes compared to pure *S. haematobium* parasites has been proposed to be at the origin of the spread of the hybrid form in Senegal [29,30]. Hybridization may also affect Oxamniquine efficiency [31]. Since the genetic basis of Oxamniquine residence is known, our data can be used to propose a hypothesis on the degree of susceptibility. To date, neither experimental nor field trials have tested the sensitivity of hybrid parasites to praziquantel.

This work provides new insight into the schistosome hybrids that emerged in Europe, revealing admixture between *S. haematobium* and *S. bovis* parasites. As the strain we maintain in the lab has been recovered from a single infected patient in 2013, it is now necessary to extend our conclusions to subsequent contamination events in Corsica, and to investigate the dynamics of hybridization in the original endemic areas in Africa and especially in Senegal from where the parasite that have been introduced in Europe may have originated [8]. It is now essential to precisely characterize the impact of

297 hybridization and introgression on the life history traits of the parasites, including
298 sensitivity to current treatments, and assess the molecular mechanisms underlying
299 these phenotypic changes. These hybrids may have the capacities to take over the
300 geographical distribution of the parental species, but also rise out of endemic areas, and
301 specifically in the close future with ongoing climate change, up north and towards
302 Europe, making them an emerging global threat.
303
304 **Materials and Methods**
305 **Ethics approval**
306 Housing, feeding, animal care and experiments were carried out according to the
307 national ethical standards established in the writ of 1 February 2013 (NOR:
308 AGRG1238753A). The Direction Départementale de la Cohésion Sociale et de la
309 Protection des Populations (DDSCPP) provided the permit N°C66-136-01 to our
310 laboratory for experiments on animals. The investigator possesses the certificate for
311 animal experimentation (Decree n° 87–848 du 19 octobre 1987; authorization 007083).
312 **Parasite / snail strains and experimental infections**
313 The Corsican hybrid parasite was recovered from eggs found in the urine of an infected
314 patient and maintained in the laboratory in sympatric snails *Bulinus truncatus* and
315 hamsters *Mesocricetus auratus* [8]. Adult worms produced in hamsters were recovered
316 after portal perfusion and male/female couples were manually separated. Detailed
317 methods were described previously [32]. *Schistosoma bovis* (Spain) and *Schistosoma*
318 *haematobium* (Cameroon) were also maintained in the lab using *Planorbarius*
319 *metidjensis* and *Bulinus truncatus* as intermediate hosts, respectively, and *Mesocricetus*
320 *auratus* hamsters as definitive hosts [33]. F1 hybrids were produced after experimental
321 cross between male *S. haematobium* and female *S. bovis*. For that, we infected molluscs

with a single miracidium of the parental species to obtain male or female clonal cercariae. The latter were sexed using molecular markers efficient on both species that we developed previously [33]. We simultaneously exposed hamsters to 300 cercariae of male *S. haematobium* and 300 cercariae of female *S. bovis*. Three months after infection, hamsters were euthanized and F1 hybrid eggs were used to infect molluscs. The infected molluscs were then used to infest hamsters (pools of 600 cercariae) and adult male worms were collected 3 months after exposition.

Hybrid parasite compatibility with snail hosts

Planorbarius metidjensis (n=40) and *Bulinus truncatus* (n=40) snails that are the natural hosts of *S. haematobium* and *S. bovis* respectively were individually exposed overnight to 5 miracidiae of the Corsican parasite strain maintained in the lab in well plates. Molluscs were individually checked from 35 days after infection for parasite emission of the cercariae after light stimulation.

Morphological analysis of the Corsican schistosome hybrid eggs

Encysted eggs were collected from hamster livers and washed in 8.5% w/v Tris-NaCl. After whole-mounted on glass slides, eggs were viewed under light microscopy with objective lens magnification set to x10, and photographed on a Wild Heerbrugg M400 ZOOM Makroskop (Leica, Germany) coupled to Nikon digital sight DS – Fi1 digital camera. All measurements were produced with ImageJ version 1.51 [34] and drawings were done by image overlay in Adobe Photoshop CS2 version 9.0.1.

DNA extraction and sequencing for *Schistosoma bovis* assembly

Two hundred clonal adult male worms were used to prepare high molecular weight genomic DNA using CHEF Genomic DNA Plug Kits (BioRad). The DNA was quantified on a FEMTO Pulse®, qubit and nanodrop. A total of 8.1 µg genomic DNA was used to generate a size selected PacBio library. First the DNA was sheared to an average

fragment size of 45 kb by gently passing the DNA sample through a 2" long, 26 gauge needle, 4 times and then concentrated using Ampure PB (Pacific Biosciences 100-265-900) before the library was prepared following the standard PacBio size selected library preparation protocol using BluePippin™ Size-Selection System. The library was size selected at 15kb, and run on 6 SMRT cells on the Sequel platform, generating 47.9 Gb of data.

RNA extraction and sequencing for *Schistosoma bovis* annotation

Pools of 10-12 adult male or female worms were frozen with liquid nitrogen and grounded using Retsch MM400 cryobrush (2 pulses at 300Hz for 15s). Total RNA was extracted using TRIzol Thermo (Fisher Scientific) followed by DNase treatment with Turbo DNA-free kit. RNA was then purified using the RNeasy mini kit (Qiagen). The TruSeq stranded mRNA library construction kit (Illumina) was used on 300 ng of total RNA per condition. Library preparation and sequencing was performed at the McGill University in the Génome Québec Innovation Centre, Montréal, Canada on a Illumina HiSeq 4000 (100 bp paired-end reads).

***Schistosoma bovis* genome assembly and annotation**

A total of 48 Gb PacBio reads were assembled using HGAP4 to generate a 446 Mb assembly in 486 contigs, with an N50 of 3.1 Mb in 38 contigs.

Gene prediction was carried out with AUGUSTUS 3.3.1. No new training was performed for our data but the parameter set "schistosoma2" of the AUGUSTUS distribution was used. RepeatMasker 4.0.7 and RepeatScout 1.0.5 were used for repeat masking. RNAseq data from male and female adult worms was employed as external hints. This RNA-seq data was aligned with STAR version 020201.

DNA extraction and sequencing for the Corsican hybrid strain and experimental *S. haematobium x S. bovis* F1

372 Genomic DNA of the Corsican schistosome hybrids was recovered from one pool of 10
373 adult males and one pool of 40 adult females separately, while DNA of the experimental
374 F1 progeny was recovered from a pool of 10 adult males. DNA was extracted using
375 Qiamp DNA microkit tissue kit (Qiagen) followed by RNase A treatment. Genomic DNA
376 of the Corsican hybrid worms was then sent to Genome Quebec for library construction
377 using the Illumina TruSeq kit starting from 200 ng of genomic DNA (for each sex), and
378 sequencing was performed on a Illumina HiSeq 2000 (100 bp paired-end reads). For the
379 experimental F1 hybrid males, library construction was performed using the Nextera XT
380 kit starting from 1 ng and sequenced on a Illumina NextSeq 550 (150 bp paired-end
381 reads) on the Bio-Environment NGS platform at University of Perpignan.

382 **Estimation of the genomic introgression levels for the hybrid strain**

383 The sequencing reads had PHRED quality scores over 30 with no adapter contamination.
384 All reads were retained for further analysis and aligned to a chimeric concatenate of *S.*
385 *haematobium* and *S. bovis* genomes using Bowtie 2 [35]. We used the SchistoDB *S.*
386 *haematobium* genome [7] and the *S. bovis* SBOS_v1.1 assembly genome produced for this
387 study. To avoid mapping bias due to differences in assembly size between the two
388 genomes, only scaffolds > 1Mb were retained for further analysis. The genome size after
389 concatenation of *S. haematobium* and *S. bovis* was 681.2 Mb. Mapping was thus
390 performed by allowing each read, depending on its origin, to map against the more
391 similar location in one or the other species genome. We then counted the proportion of
392 best location of aligned reads (*S. bovis* or *S. haematobium* genomes) in the SAM files. The
393 same procedure was applied on the mitochondrial genomes using a concatenate of the
394 scaffold 000439F that contained the mitochondrial genome of *S. bovis*, and the *S.*
395 *haematobium* mitochondrial genome from GenBank accession NC_008074.

396 **Similarity analysis between *S. haematobium* and *S. bovis* genomes**

397 The two species genomes were aligned with CACTUS [36]. We then processed the
398 output, identifying all alignments blocks composed of exactly one genome part of *S. bovis*
399 and exactly one genome part of *S. haematobium*.
400

401

402 **Availability of data**

403 The Illumina datasets for the Corsican hybrid strain are available in the SRA repository
404 under submission number SUB4330327 (to be released upon publication). The *S. bovis*
405 PacBio data are available under accession number ERS2549235. The *Schistosoma bovis*
406 assembly SBOS_v1.1 is available at
407 ftp://ftp.sanger.ac.uk/pub/project/pathogens/Schistosoma/bovis/SBOS_v1.1_pilon.fast
408 a. Annotations are available as trackhub at http://ihpe.univ-
409 perp.fr/IHPE_tracks/S.bovis/hub.txt.

410

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415

416 **Authors' contributions**

417 ET and JB conceived and designed the study. JB, AO and SMC procured the parasite and
418 snail strains. JKS, AR, and JB performed the experiments. RA conducted the
419 morphological analysis. NH and JFA prepared the DNA for sequencing. NH coordinated
420 the long read DNA sequencing experiments. JKS, AT, IB, MB and ET conducted the
421 computational data processing and analysis with significant assistance and input from
422 OR, CC and CG. JKS and ET wrote the paper. All authors read and approved the final
423 manuscript.

424

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434
435

Supporting information captions

Appendix S1: Morphological analysis of the European schistosome hybrid eggs and adult worms

Figures

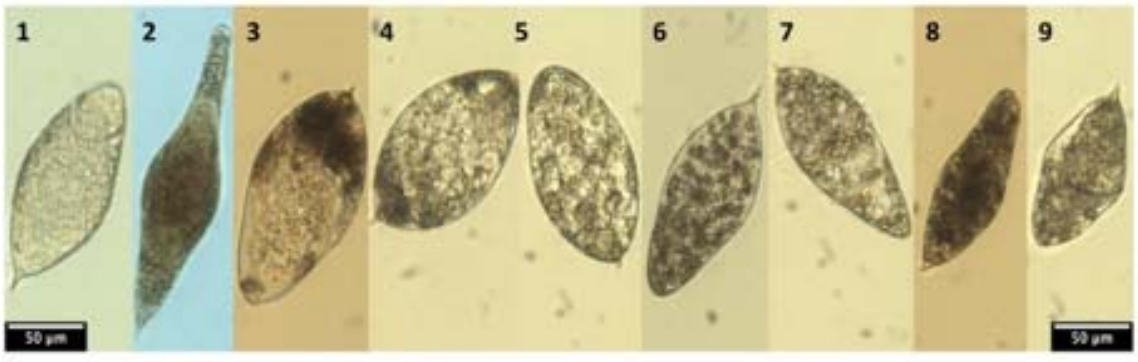

Figure 1: Egg morphologies of the pure parental species and the Corsican *S. haematobium X S. bovis* hybrid. Eggs 1 and 2 show typical morphologies of *S. haematobium* (elliptical with a terminal spine) and *S. bovis* (spindle shape with a terminal spine), respectively. Eggs 3-8 show the egg morphology of the Corsican hybrid schistosome. While most eggs were typical to *S. haematobium* (3-7), a high variability of morphotypes was observed (8-9).

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