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SNP-SchistoLAMP: Rapid Identification Method to Differentiate Between *Schistosoma* Species Based on a Single Nucleotide Polymorphism Using LOOP-Mediated Isothermal Amplification

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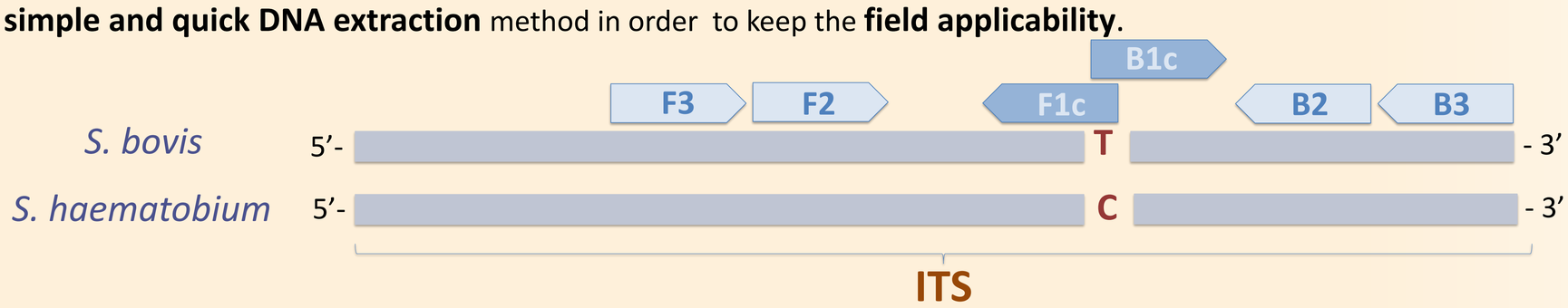
Introduction:

Single nucleotide polymorphisms (SNPs) are single-based mutations in DNA specific location. The identification of SNP polymorphism is widely used in several domains such as medical diagnostic, drug resistance associated marker or species identification. Recently, it has been demonstrated that the use of LOOP-mediated Isothermal Amplification method (LAMP) could be a good alternative of the classical PCR-based method for SNP identification. Interestingly, the LAMP method possess all the characteristics required for easy adaptability to field conditions and ecological applications. LAMP uses low cost products and equipment, and the cold chain is not an obligation.

Objective: Development of a new rapid identification tool enable to distinguish between two parasites species where larval stages are not morphologically distinguishable.

Methods:

The method was developed on ***Schistosoma haematobium*** (human parasite), and ***Schistosoma bovis*** (animal parasite) and the **hybrids** resulting from their hybridization. We focused our development on one of the five **Internal Transcribed Spacer** (ITS) **SNPs** known to differentiate the two species. The **SNP-SchistoLAMP** method was validated using **adults** and all the free parasite larval stages (**miracidium** and **cercariae**) using extracted DNA processed by a **simple and quick DNA extraction** method in order to keep the **field applicability**.



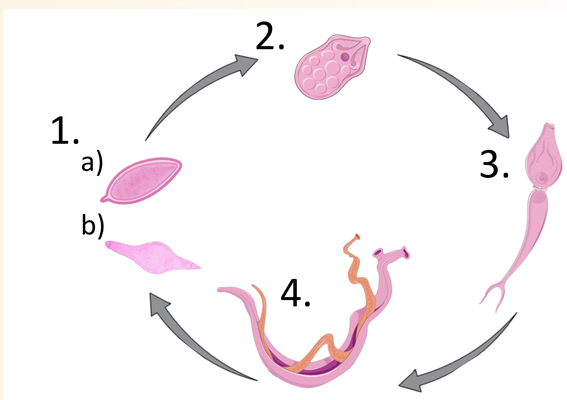
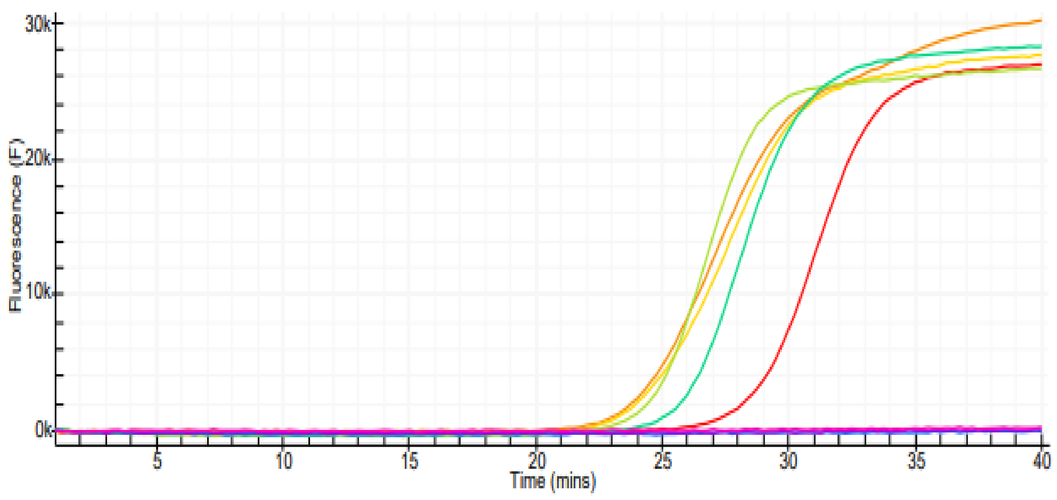


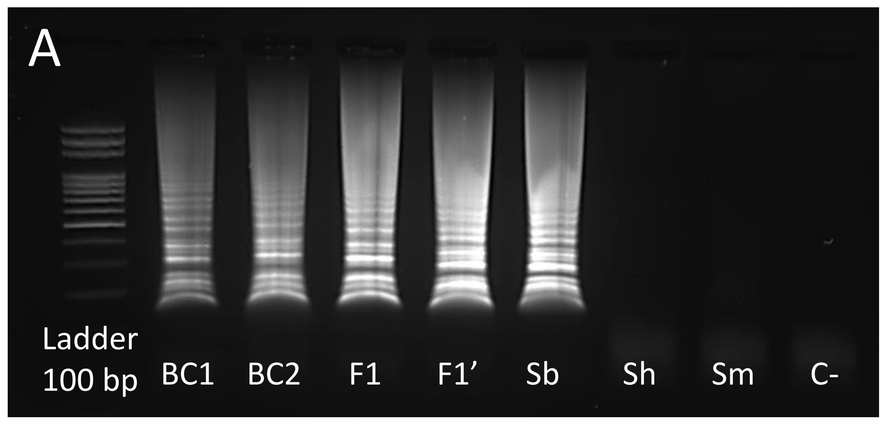
Figure 1. Schistosoma all stages.
1. a) S. haematobium and b) *S. bovis eggs; 2. Miracidium; 3. Cercariae; 4. Adults.*
Only the egg stage allows to differentiate morphologically the two species.

Results: Three revelation methods

	Name	Result
	Backcross *S. bovis x S. haematobium*	+
		+
	S. bovis & *S. haematobium* first generation crossing	+
		+
	S. bovis	+
	S. haematobium	-
	S. mansoni	-
	Negative control	-



First method: Real-time fluorescence method. *The primer set used here was designed to amplify only S. bovis and its hybrids. As expected, S. haematobium and S. mansoni are not amplified. Results were obtained with Genie® III (Optigene).*



End-point revelation methods.
A. Agarose gel. B. Colorimetric change visualization by eyes. DNA samples: BC1 and BC2: Backcross S. bovis x S. haematobium; F1 and F1': S. bovis & S. haematobium first generation crossing; Sb: S. bovis; Sh: S. haematobium; Sm: S. mansoni; C-: Negative control (reaction mix + ultrapure water).

Conclusions:

Because it is **simple** and **affordable**, this method has interesting application **in the field** to **differentiate between human and animal infecting parasites**, and their **hybrids** and thus the **zoonotic risk** of this pathogen. More generally LAMP methods have **great potential in ecology** for SNP-LAMP detection.

Future work:

Develop reverse detection primer sets to amplify *S. haematobium* and its hybrids.

References:

Yongkiettrakul, S. *et al.* Simple detection of single nucleotide polymorphism in Plasmodium falciparum by SNP-LAMP assay combined with lateral flow dipstick. *Parasitol. Int.* **66**, 964–971 (2016).

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