

# The emergence of hybrid Fascioids within central Vietnam



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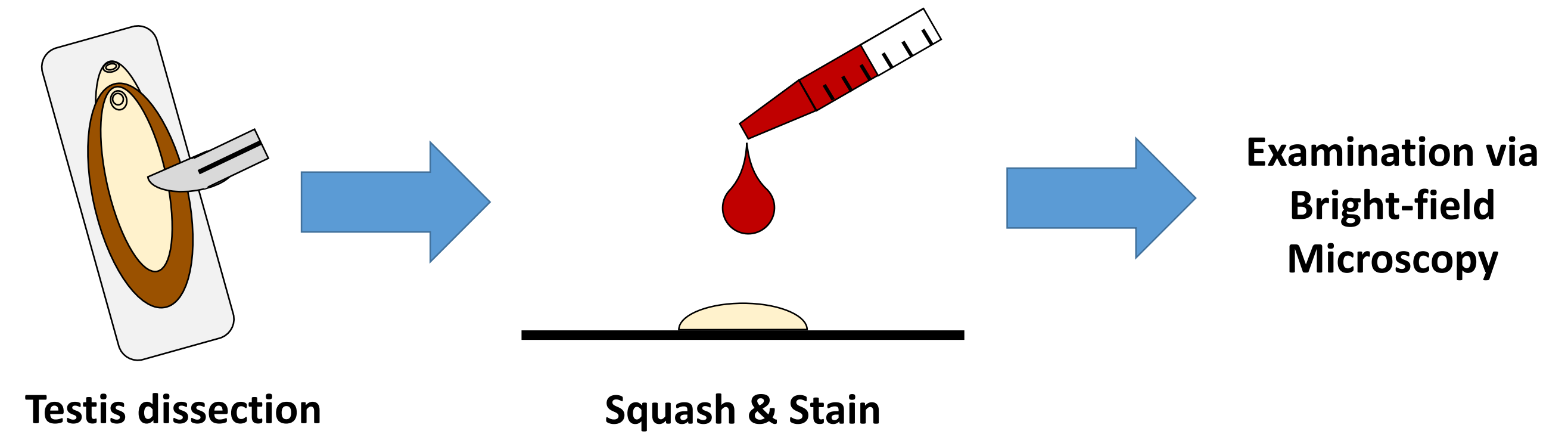
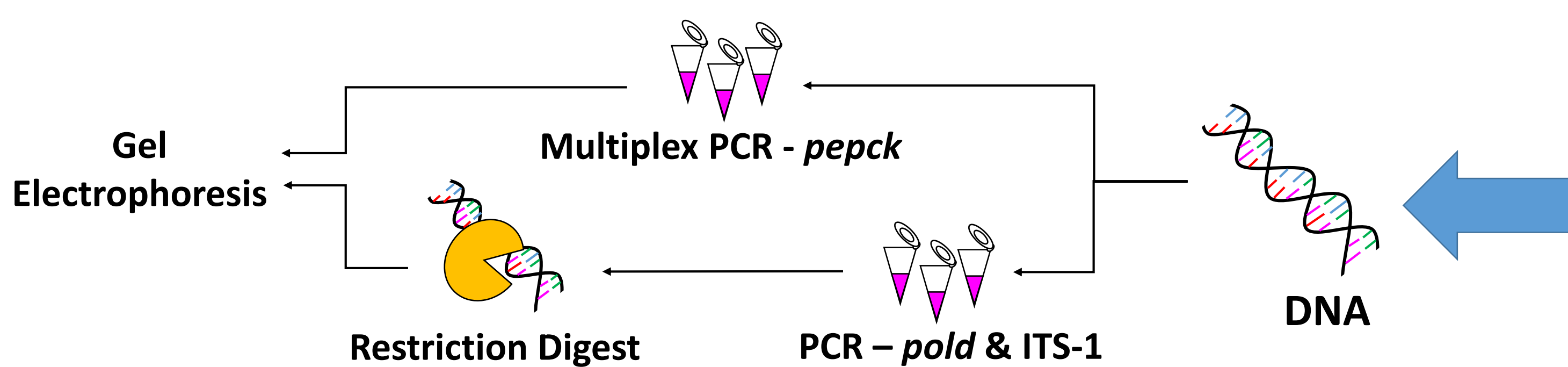
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## Introduction

Fasciolosis, traditionally a foodborne trematodiasis of livestock, is now recognised as an emerging human zoonosis with 2.4 - 17 million people infected worldwide<sup>[1]</sup>. The causative agents, *Fasciola hepatica* and *F. gigantica*, occur within temperate and tropical climates respectively, although hybrid forms have also emerged, notably in Southeast Asia. Whilst endemic in livestock, central Vietnam has emerged as a “hotspot” for human infection with up to ~92% of past national cases reported here<sup>[2]</sup>. Despite this, few studies have molecularly characterised flukes in provinces where infection is highest. Here, we have used molecular and microscopical analyses to determine hybrid prevalence and fluke reproductive strategies in the Phu Yen and Binh Dinh provinces.

## Methods

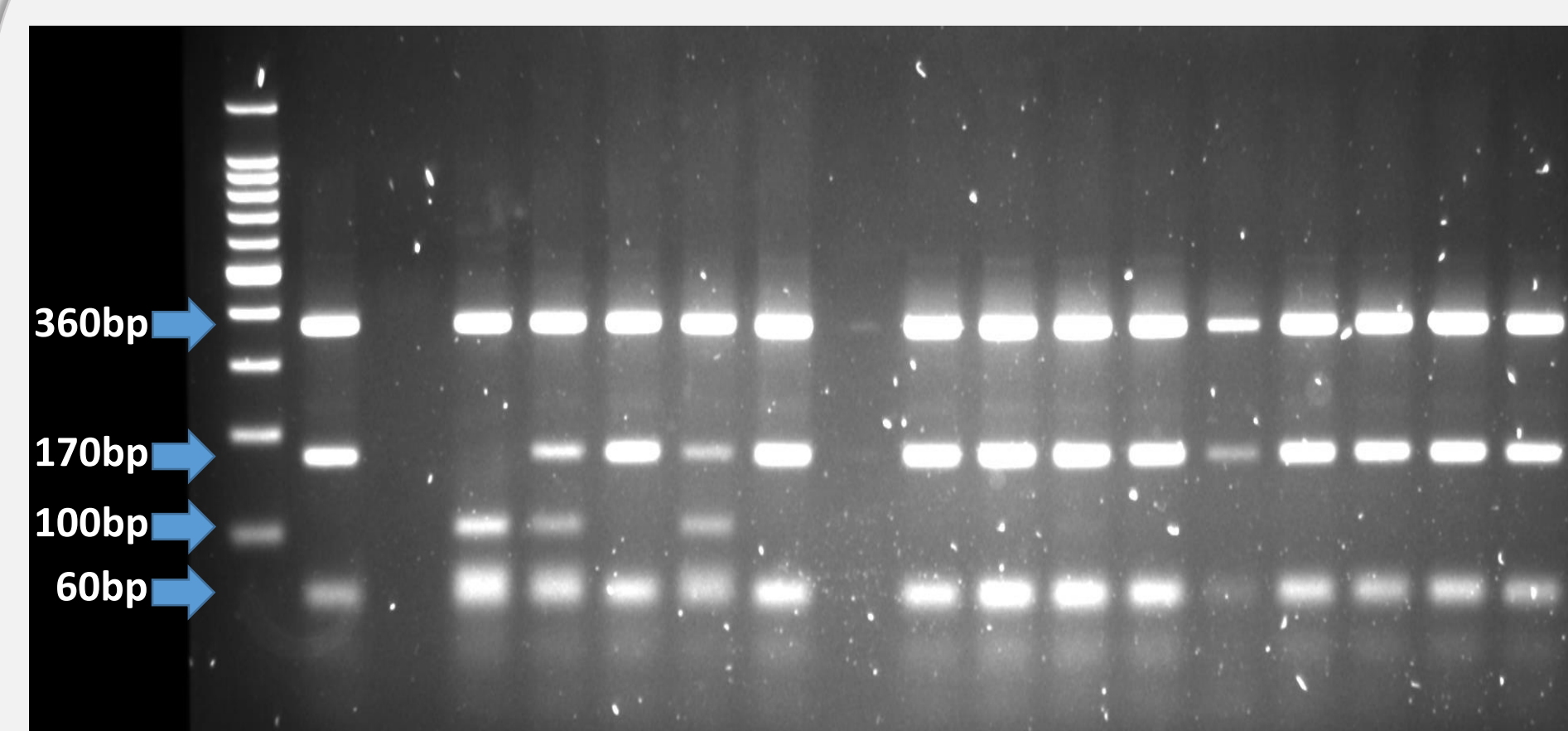
DNA templates were extracted from posterior vitellaria tissues. Multiplex PCR and Restriction Fragment-Length Polymorphism (RFLP) analysis of Phosphoenolpyruvate carboxykinase (*pepck*); Polymerase Delta (*pold*) & Internal Transcribed Spacer Region 1 (ITS-1) respectively enabled speciation.



For detecting the presence of sperm, 1mm<sup>3</sup> portions of testis tissue were dissected and prepared using an aceto-orcein squash method. Slide preparations were then examined using bright-field microscopy.

## Results

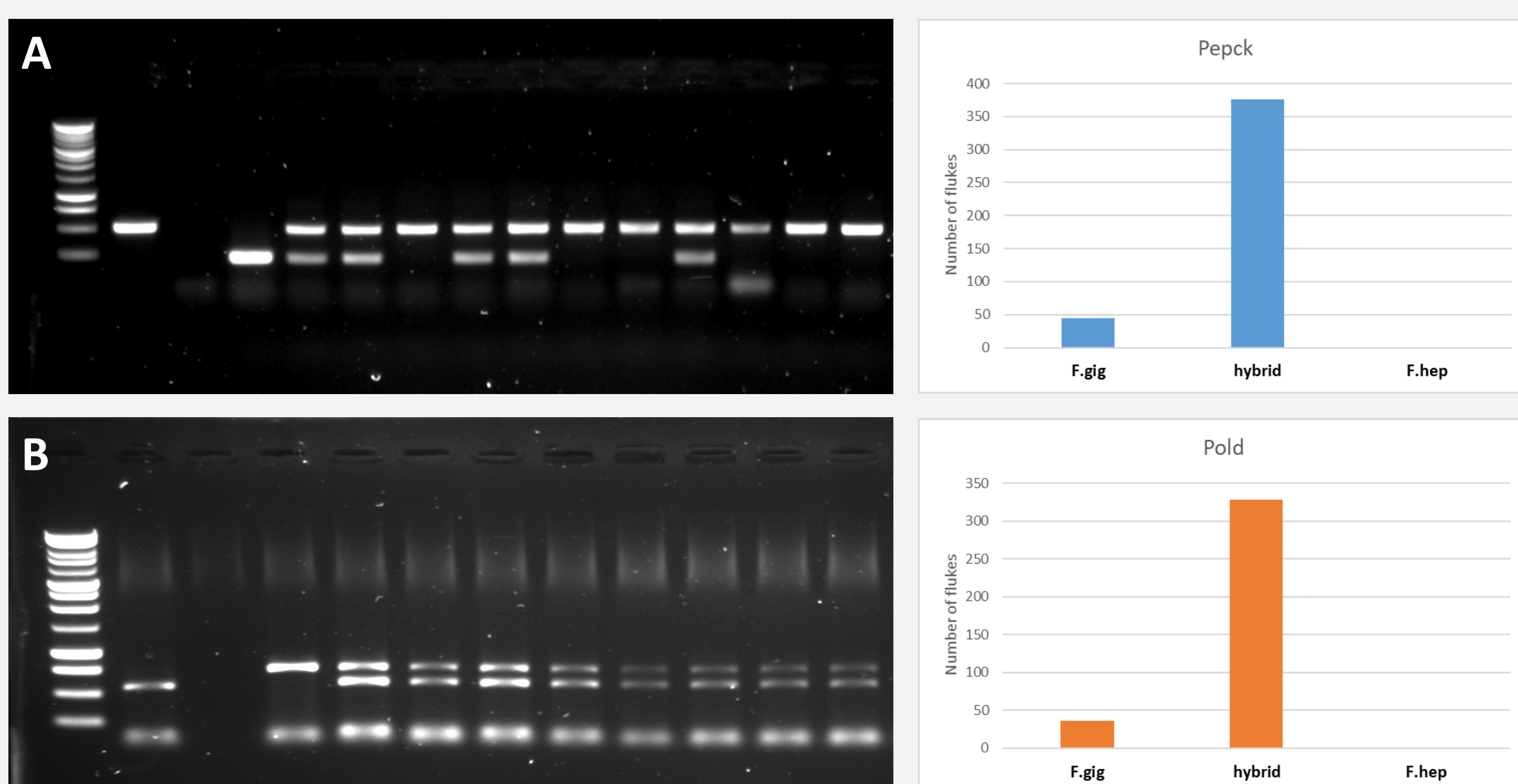
### PCR-RFLP of ITS-1



**Digest Banding Patterns:**  
*F. gigantica*: 360, 170 & 60bp.  
*F. hepatica*: 360, 100 & 60bp.  
 Hybrid: 360, 170, 100, & 60bp.

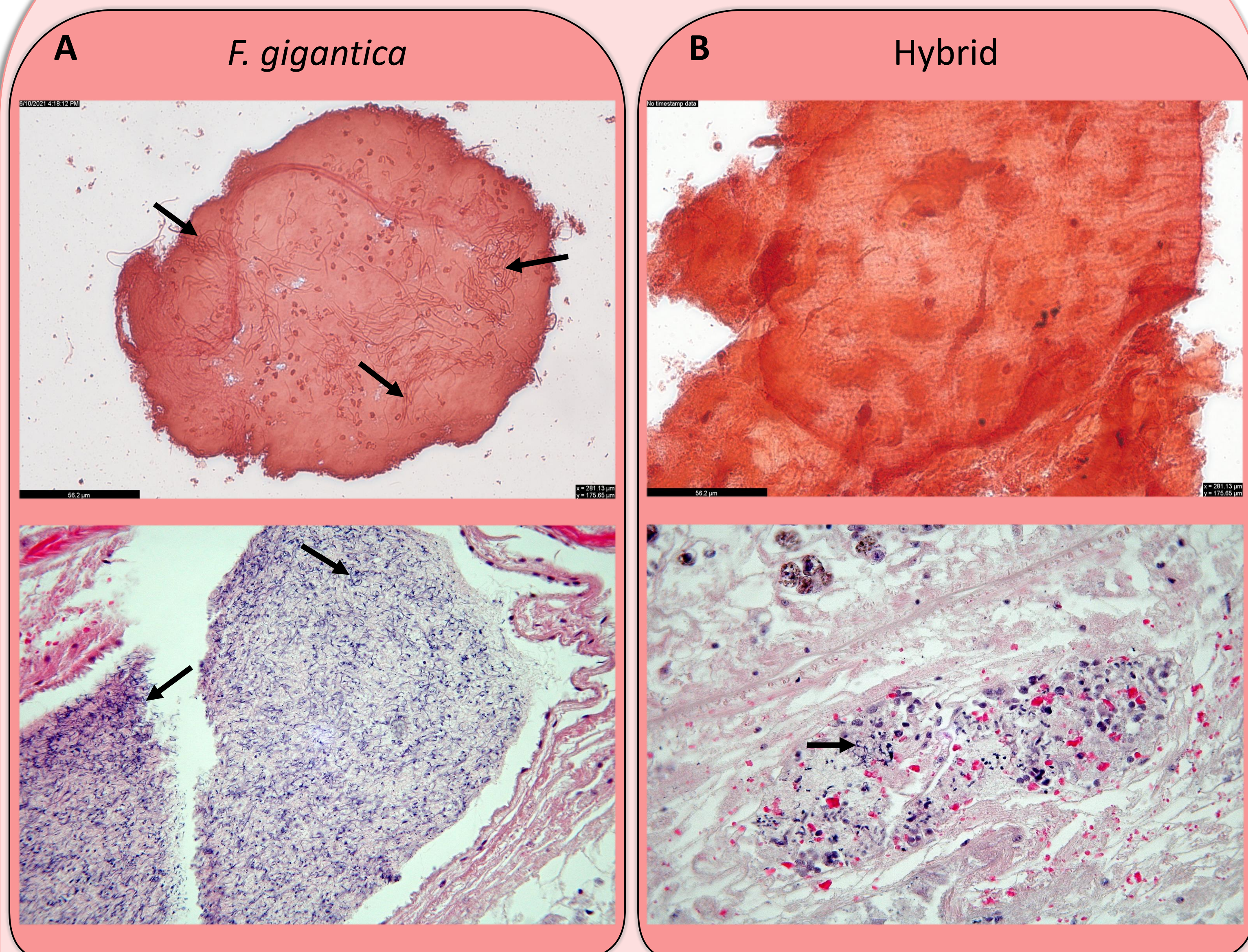
Traditionally, ITS-1 is a gold standard method for speciating between *F. hepatica* and *F. gigantica*. However, PCR bias and the recombinogenic nature of ribosomal DNA (i.e. ITS-1) causes inaccuracies in hybrid speciation.

### Population screening via *pepck* and *pold*



PCR amplification of two nuclear DNA markers; *pepck* (A) and *pold* (B), has provided an accurate large-scale speciation analysis. Indicating hybrid *Fasciola* are the predominant form (89%) with the remaining population comprising of only *F. gigantica* (11%).

### Spermic-typing and Spermatogenesis



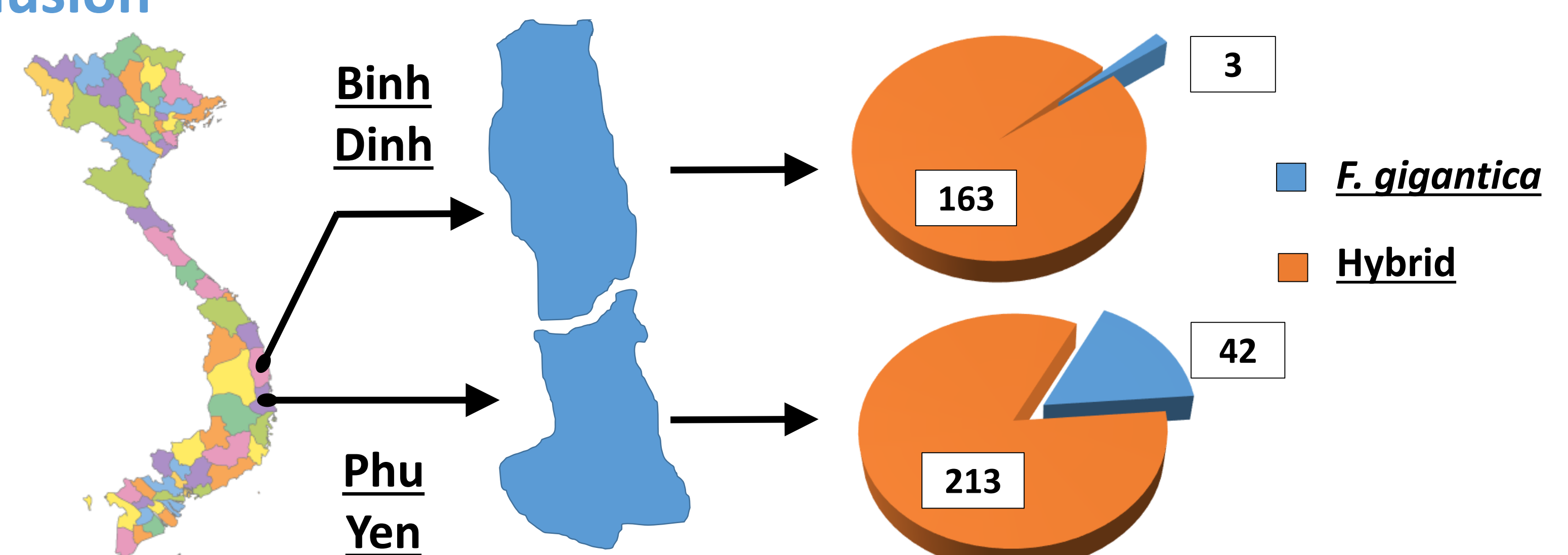
Microscopic and histological examination reveals adult *F. gigantica* (A) are capable of producing abundant mature spermatozoa (arrows), whilst adult hybrids (B) typically either lack or produce low yields of spermatozoa with aberrant morphology (arrow), indicating incomplete spermatogenesis.

Given these hybrid forms lack mature spermatozoa, it is likely that they cannot partake in cross-/self-fertilization. Therefore, their primary method of producing offspring is likely to be parthenogenesis, a form of clonal reproduction.

## Conclusion

It is apparent that aspermic hybrid *Fasciola* are the predominant causative agent of Fasciolosis within Phu Yen and Binh Dinh. Given the absence of *F. hepatica*, it is likely that hybrids were introduced here via movement of infected livestock. Sequencing of mitochondrial markers will provide further information on fluke population structure and the origin of hybrid forms.

Although prevalence is rising, further research is required to determine the intermediate host range, infectivity and virulence of hybrid *Fasciola* sp. compared to the pure *F. hepatica* and *F. gigantica* species.



## References:

- [1] Caravado, M. A., & Cabada, M. M. (2020). Human Fascioliasis: Current Epidemiological Status and Strategies for Diagnosis, Treatment, and Control. *Research and reports in tropical medicine*, 11, 149–158.  
 [2] Nguyen, N. T. et. al., (2017). High prevalence of cattle fascioliasis in coastal areas of Thua Thien Hue province, Vietnam. *The Journal of veterinary medical science*, 79(6), 1035–1042.

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