Annals of Parasitology 2021, 67(2), 351–352
doi: 10.17420/ap6702.350

Letter

Comments on "Genetic characterization and phylogenetic analysis of *Fasciola* species based on ITS2 gene sequence, with first molecular evidence of intermediate *Fasciola* from water buffaloes in Aswan, Egypt"

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Dear Editor-in-Chief,

In *Annals of Parasitology* 2021, 67(1), 55–65, a paper entitled "Genetic characterization and phylogenetic analysis of *Fasciola* species based on ITS2 gene sequence, with first molecular evidence of intermediate *Fasciola* from water buffaloes in Aswan, Egypt" was published with great interest [1]. After reading the article carefully and critically, we think some points should be noted.

Fasciola species are meiotically functional diploid, can produce sperm and temporarily and store in the seminal vesicles. This type is named spermic fluke [2]. On the other hand, intermediate *Fasciola* with morphological characteristics intermediates between *F. hepatica* and *F. gigantica* with no sperm or aspermic and no sperm in seminal vesicles. However, this is also seen in older flukes [3–5]. It seems that morphological studies based on spermatogenesis ability were necessary for this study.

Also, this parasite's anthelmintic resistance is due to aspects of biology, and population structure depends on genetic diversity [6]. We question whether there are any documents about and sequences of mitochondrial markers as COX (Cytochrome Oxidase) and NAD (Nicotinamide Adenine Dinucleotide) to analyze intraspecific phylogenetic relationship in addition to nuclear gene?

In Table 3, the pairwise distances between three groups of *Fasciola* spp. from different livestock animals were low, ranging from 0.004 to 0.01 with an overall mean of 0.008. Genetic diversity is described as a tendency of genetic characteristics to vary and serves as a way for the population to adapt to changing hosts and environments [7]. The nature of the nuclear gene (ITS) is instability. It is better to use mitochondrial sequence data to compare diversity.

Also, genetic discrimination grade from infra population to meta population is annotated by *F*st value ranging; 0 to 1. *F*st values between 0–0.05 indicated a low genetic differentiation population [8]. It seems that by calculating *F*st and showing the gene migration based on mitochondrial sequences data of specimens, this study's species population will be obtained. Also, Tajima's D and Fu's F in all loci populations based on GenBank data may show the *Fasciola* haplotypes' population proximity.

Here we recommend, that Omar et al. [1] studies that molecular phylogeny with mitochondrial DNA efectively used for appropriate diferentiation of haplotypes and spermatogenic ability by carmen allium staining helps them find the physiological aspects. Of course, more prominent populations are needed to find intermediate types.

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Received 05 April 2021