Barcoding Acanthocephala. A neglected phyllum of parasitic helminths

García-Salguero, Alejandro[1]; Delgado-Serra, Sofía[1]; Juan-Amengual, Mateo[1]; Miranda, Miguel Ángel[1]; Sola-Sánchez, Jessica[2] & Paredes-Esquivel, Claudia[1]*

[1] University of the Balearic Islands, Spain
[2] *Consorci per a la recuperació de la Fauna de les Illes Balears* (COFIB), Spain
*Corresponding author: claudia.paredes@uib.es

INTRODUCTION

Acanthocephalans (thorny-headed worms) are a small group of arthropod-borne endoparasites that infect the digestive system of vertebrates. These can cause serious damage, which can lead to death. However, their role as pathogens of wildlife is largely unknown. A correct identification of parasites has important medical and epidemiological implications, since some of these are zoonotic.

Two forms of Acanthocephalans were observed in the Balearic hedgehog *Atelerix algirus vagans* (Fig. 1). Adult forms were present in the small intestine and immature larvae were found outside the peritoneal cavity. We sequenced the barcoding region of all specimens (*Cytochrome oxidase I)* to determine if these forms belong to the same or to different species.

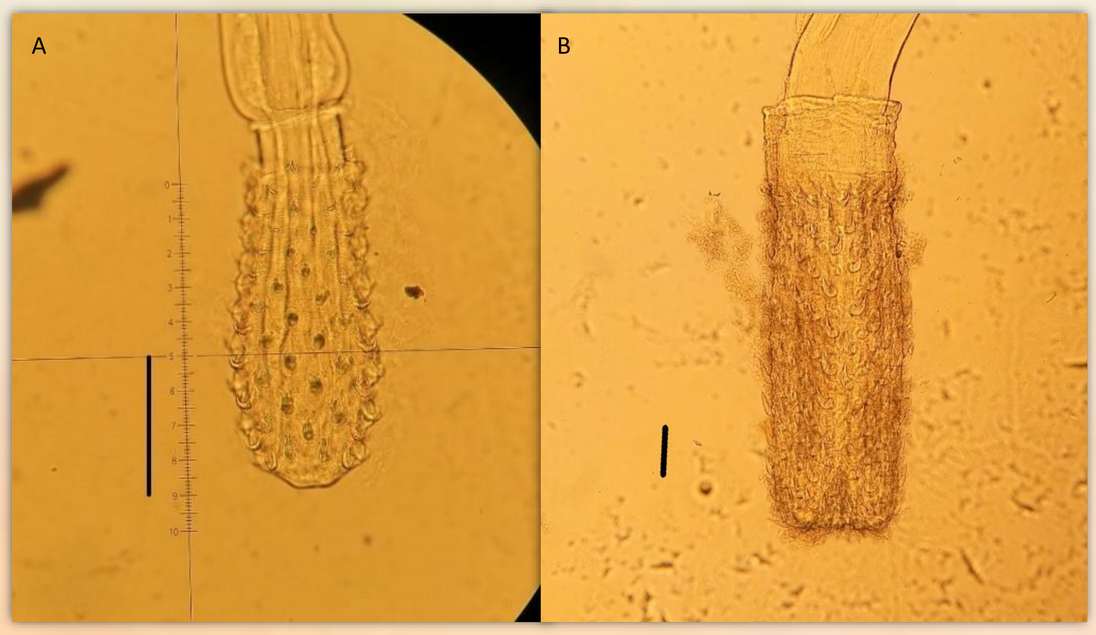

Figure 1. Proboscis of *Moniliformis saudi* (A) and *Plagiorhynchus cylindraceus* (B). Scale bar 100 µm.

MATERIAL AND METHODS

Morphological identification was carried out using keys by Amin *et al*. 2011 and 2016.

We used Qiagen® Dneasy Blood & Tissue Kit to carry out DNA extraction and PCR amplification was performed with primers HCO2198 & LCO1490. PCR product were purified using a QIAquick PCR Purification Kit and specimens were sent for sequencing to Macrogen Spain.

Sequences (563-618 bp) were analyzed with Bioedit, and Nucleotide Blast and BOLD System servers were used for identification purposes. Pairwise genetic distance was calculated using the Kimura 2-P model in MEGA 6.

RESULTS AND DISCUSSION

Adult specimens were morphologically identified as *Moniliformis moniliformis,* although they showed an atypical morphology, with specimens larger than those described in the literature. In the absence of keys for immature stages, larval forms could not be identified morphologically.

The sequence analysis showed that adult specimens were in fact *Moniliformis saudi* (99% identity) which is a parasite species that had not been reported before in *Atelerix algirus.* Interestingly, specimens showed a 30% pairwise difference (Table 1) which is high for intrageneric species.

Larva specimens belonged to species *Plagiorhynchus cylindraceus* (98-99% identity). This report would be a new host and geographic record for this species. Since birds are the definitive host of this acantocephalan, their presence in *Atelerix algirus* open several questions regarding the role of hedghogs in the parasite's life cycle.

Table 1. K2P-corrected pairwise distances

	(Mm)	(Ms)	(Ms*)	(Pc1)	(Pc2)	(Pc3)	(Pc4*)
*M.moniliformis*_AF416998.2 (Mm)							
*M.saudi*_KU206783.1 (Ms)	0,316						
M.saudi (Ms*)	0,319	0,004					
*P.transversus*_NC029767.1 (Pc1)	0,652	0,691	0,686				
*P.transversus*_KT447549.1 (Pc2)	0,652	0,691	0,686	0,000			
*P.cylindraceus*_DQ089714.1 (Pc3)	0,657	0,686	0,682	0,011	0,011		
P. cylindraceus (Pc4*)	0,657	0,701	0,696	0,019	0,019	0,022	
P. cylindraceus *	0,662	0,696	0,691	0,015	0,015	0,019	0,007

Samples marked with (*) are those sequenced in this work.
**P. cylindraceus* and *P. transversus* are synonyms. The first one is currently accepted.

CONCLUSION

This study shows the importance of molecular-based techniques in the identification of poorly known parasites. Particularly those collected as immature stages. Due to the absence of molecular-based studies in this group, it is likely that these parasites might be more widely distributed than previously thought and their identification is crucial to determine their epidemiological importance in wildlife.