 

# From a study of genetic biodiversity to a determination of cryptic species. The significance of molecular studies of Nematodes on the example of *Toxascaris* complex

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Studying intraspecific molecular differences of parasites is an important research subject as the results could provide information valuable for systematics, phylogeny, epidemiology and biodiversity. Such research could reveal cryptic species, i. e. morphologically indistinguishable biological groups incapable of interbreeding because of their significant genetic differences. Cryptic species are considered to be relatively common among parasites, and have been described particularly often in Nematodes. This is because of the species richness, similarity of its morphological features and a relatively high prevalence among people and animals.

We realised the importance of examining the intraspecific molecular diversity when studying *Toxascaris leonina* (Ascarididae). It is a parasite of felids and canids, occurring in definitive hosts in all latitudes and morphologically very similar to *Toxocara* spp. We were interested whether there were molecular differences between the nematodes depending on their geographical location and host species. We examined *T. leonina* collected during necropsy of red foxes, *Vulpes vulpes*– 23 individuals of Nematodes from North-Western Poland and 10 from South-Western Poland. Total genomic DNA was extracted from individual specimens using a Genomic Mini kit. Fragments of two mitochondrial genes – cox1 and nad1 as well as ITS1 were amplified and sequenced. Additionally, a fragment of cox1 was used as the DNA-barcode region. The sequences were used to perform phylogenetic analyses by comparing the results obtained in Poland with those deposited in GenBank for different species of hosts and from different latitudes. The results were both interesting and surprising. All the analyzed sequences of *T. leonina* collected from foxes in Poland represented the same genotype. The analyses of individuals collected from different hosts revealed that *T. leonina* clustered into three well-supported clades depending on their host species, i.e. dogs and wolves, wild felids and foxes. We found that *T. leonina* is a complex species and that there is no relationship between a genetic structure of *T. leonina* populations and the geographical region of their origin. We also noticed that some individuals initially recognized as *T. leonina* on the basis of their morphological features, turned out to be *T. canis* when subjected to molecular analyses.

Our conclusions are that molecular analyses of parasites deliver valuable information because: 1) they could reveal the existence of cryptic species, which after adding morphological

 

characteristics, naturally leads to isolating a new species of parasite, 2) they could change a previously determined phylogenetic tree, 3) they show that among polyxenic parasites, genotypes could significantly differ depending on host species but not on a geographical location, 4) they are a reliable tool for species identity (for individuals with no pronounced morphological traits, damaged or in larvae forms), 5) they allow to determine more accurately the prevalence and intensity of the infection on a given area.