INTRASPECIFIC GENETIC DIVERSITY OF *DIDYMOSULCUS KATSUWONICOLA* PARASITISING BLUEFIN TUNA (*THUNNUS THYNNUS*) GILLS

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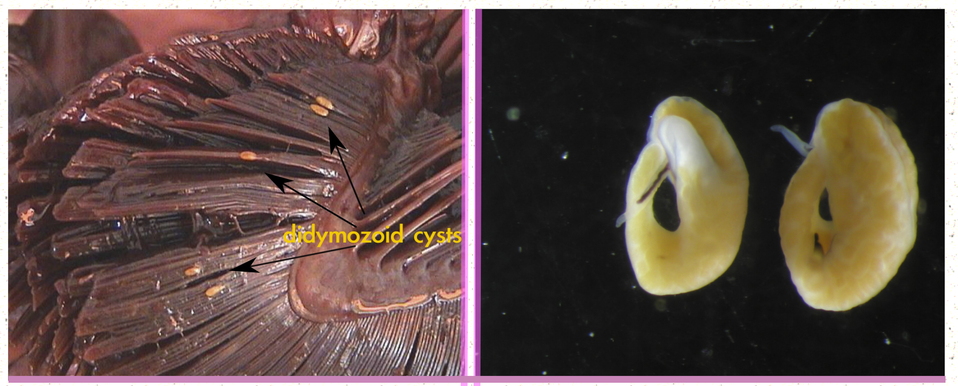
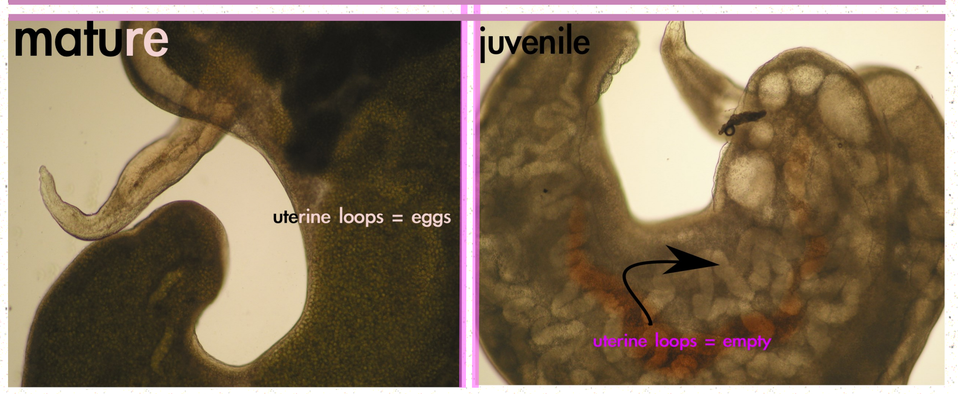
Introduction

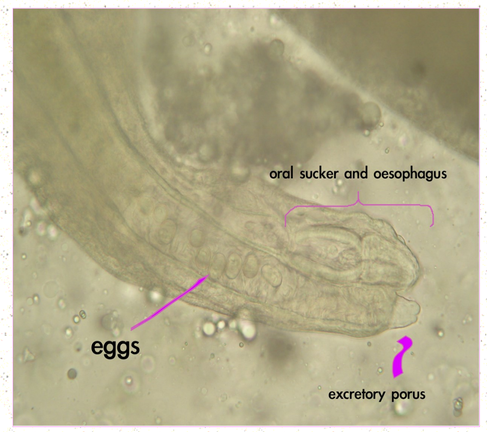
Didymozoids (Didymozoidea) are considered one of the most taxonomically complex digenean families parasitising oceanic pelagic fish, distributed world-wide in tropical to subtropical areas (23 species occurring only in the Mediterranean). Similarly, they have acquired a wide distribution within the host where they have been isolated from almost all tissues. In the Atlantic bluefin tuna (*Thunnus thynnus*, Scombridae) where most didymozoid species are encysted in pairs in connective-tissue capsule, they can occur in skin, muscle and external mucoses, as well as digestive tract, peripheral nervous tissues and kidney. In this study the aim was to investigate in more details the level of differentiation between *Didymosulcus katsuwonicola* population sampled in the Adriatic Sea.

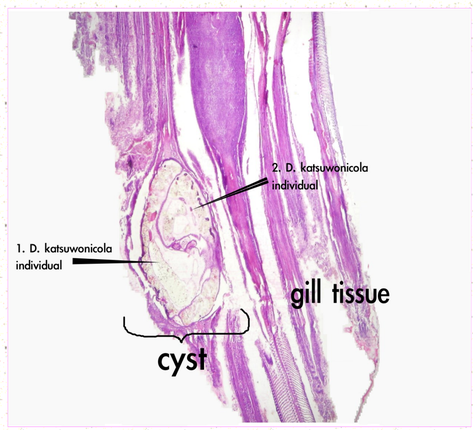


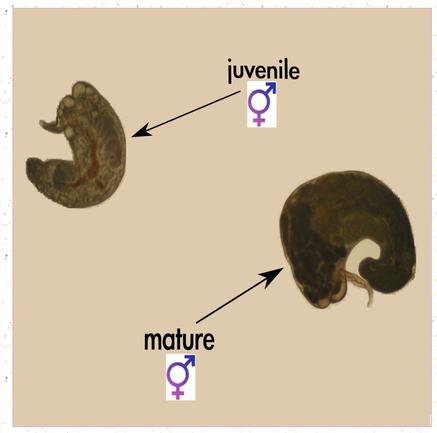


Material and Methods

In total five loci of a nuclear (ITS1, ITS2) and mitochondrial (COX1, COI, ND1) DNA were used to asses intraspecific genetic diversity of a didymozoid digenean species *Didymosulcus katsuwonicola*, parasitising gills of Atlantic bluefin tuna (*Thunnus thynnus*). Nucleotide sequences were compiled and aligned with Clustal X 1.83 and verified by GBlocks. Molecular diversity was measured using Dnasp 5.0 and Arlequin 3.5. To illustrate haplotype distribution within the group, a median-joining network was inferred using COX1 haplotypes and the program NETWORK v 4.5.1.6.







Results

The length of the cox1 in the analysis was 727 bp (N=56). In total, 41 variable sites were observed and 34 haplotypes were detected for the cox1 fragment. Sequence divergence (Tamura and Nei distance) among cox1 haplotypes ranged from 0% to 2.3%, with an average of 0.4%. Among 41 polymorphic sites observed in cox1 fragment, 27 were singleton variable sites and 14 were parsimony informative. Among 34 haplotypes defined, most (27, 79.4%) were unique and represented by a single individual. Only six haplotypes were shared among two designated populations (H1, H4, H10, H25, H27, H34) and H1 was the most abundant haplotype present in two populations. Genetic diversity indices for each population are summarised in Table 1, indicating high levels of haplotype diversity and low nucleotide diversity. As well, cox1 displayed high value of average number of nucleotide differences k.

Population genetic structure

Genetic differentiation among populations was assessed using FST pairwise comparison. Global F_{ST} values were very low (Table 2), showing no significant genetic structure in the investigated populations (F_{ST} (cox1) = -0.01353, P = 0.97165). Same pattern of genetic structure was confirmed by AMOVA, which attributed -1.35% of the genetic variation to variability among population, and 101.35% variation within populations (Table 3). Overall samples, non-differentiation exact P values were not significant (P = 0.79953 ± 0.02792), not rejecting that the population of *D. katsuwonicola* in Adriatic *T. thynnus* is panmictic.

Phylogenetic and network analyses

The topology of trees built from cox1 locus using Bayesian inference (BI) was mostly resolved in its upper part, with well-supported groups that formed small, relatively short-branch but obvious clades. Polytomy was observed in the lower part. The haplotype network showed a star-like phylogeny with most of the unique haplotypes closely related to the common central haplotype (H1). Central haplotype was composed of *D. katsuwonicola* belonging to both populations. If parasite underwent expansion, the central common haplotype was likely the ancestral one, from which one or a few stepwise nucleotide substitutions could explain all other unique haplotypes.

Table 1: Sampling localities, hosts and descriptive statistics of genetic diversity of *D. katsuwonicola*, based on cox1 sequence data.

Site and sampling year	Host	Pop	N	H	S	h	π	k
2013, Adriatic Sea	*T. thynnus*	**A**	29	20	21	0.93842±0.0345	0.063379±0.038881	2.598522±1.432206
2013, Adriatic Sea	*T. thynnus*	**B**	26	20	31	0.93538±0.0427	0.090807±0.052771	3.723077±1.942182
		Total	56	34	41	0.93064±0.027	0.00426	3.11044±1.90955

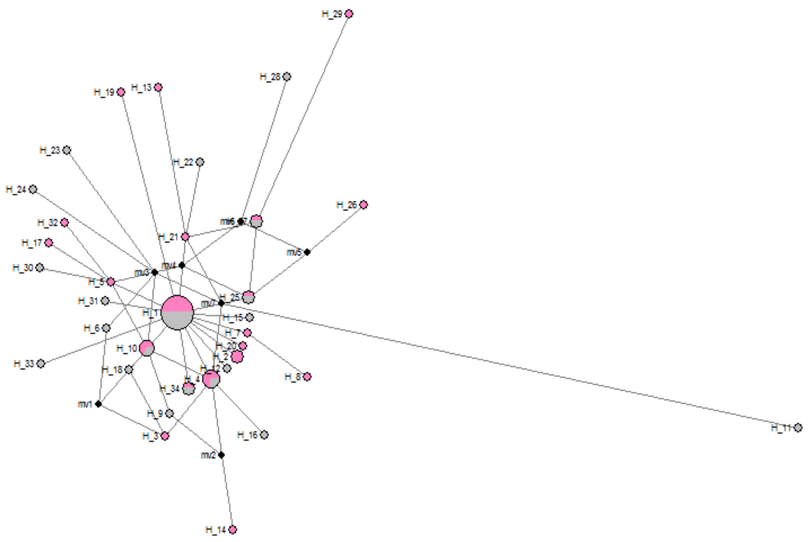
N, sample size; H, number of haplotypes; S, number of polymorphic loci; h, haplotype diversity (±SD); π, nucleotide diversity (±SD); k, mean pairwise difference (±SD).

Table 2: Pairwise F_{ST} (lower diagonal) and F_{ST} P values (upper diagonal, in italic) among populations of *D. katsuwonicola* based on mitochondrial cox1 sequences data.

	A	B
A	0.0000	*0.96396± 0.0142*
B	-0.01353	0.0000

Table 3: Analysis of molecular variance (AMOVA) for *D. katsuwonicola* populations, based on mitochondrial cox1 sequences data.

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Among populations	1	0.297	-0.00625	-1.35	-0.01353	0.97361± 0.00482
Within populations	54	24.830	0.46850	101.35		
Total	55	25.127	0.46224			



Demographic patterns

The demographic history of *D. katsuwonicola* was investigated using mismatch distribution. The goodness of fit test showed that no mismatch distributions for sample localities deviated significantly (P > 0.05) from predicted values under the sudden expansion model of Harpending (1994), thereby providing further evidence for population expansion in the past.

Discussion

Analysing population genetic structure of the digenean *D. katsuwonicola* infecting gills of the Atlantic bluefin tuna in the Adriatic Sea we observed the existence of genetically unstructured populations. We have evidenced that a negligible number of individuals (16.67%) sharing the same cysts belong to the same haplotype (H1, H25, H27, H34), against the general belief that they are all 'twins' resulting from self-fertilisation of the mature hermaphroditic parent. Most paired individuals (83.33%) originate from different parent, belonging to a haplotype different from the haplotype of the second individual within the cyst. Haplotype diversity in Adriatic didymozoid is indicative of a relatively large number of didymozoid individuals that contributed to the offspring hatching and would consequently suggest a non self-fertilisation as the preferred mechanisms in this species or a large effective population size. Adriatic population had a negative value of Tajima's D and Fu tests pointing towards a bias of rare alleles, which is a landmark of recent population expansion and colonisation event in specific areas. Regular observation of two encysted individuals of similar age in respect to only two cases when an individual from the pair was immature (and thus 'younger'), suggests that two individuals of same 'age' encyst, only after meeting each other in the peripheral gill circulation.