

Loaches of the genus *Cobitis* are small-sized, bottom dwelling fish represented by about 70 species widely distributed in Eurasia. They are known as model vertebrates in the studies of hybridization and polyploidization processes as for example several European *Cobitis* species are distributed in diploid-polyploid populations. Such populations in Poland are dominated by triploid hybrid *Cobitis* females, which co-exist with few individuals of one parental species, mainly the spined loach *C. taenia* or rarely Danube loach *C. elongatoides* and tetraploid hybrids of both sexes. Genomes of *Cobitis* triploid females depend of hybridizing species and most often contain genome (symbolically given in brackets) of the spined loach *C. taenia* (TT), the Danubian loach *C. elongatoides* (EE) and/or *C. tanaitica* (NN), a species that has not yet been found in Poland. Polyploid hybrids and parental species are very similar morphologically, so genetic methods are required to distinguish them.

Triploid hybrid *Cobitis* females, mainly reproduce unisexually via gynogenesis (=sperm depended parthenogenesis); they lay triploid eggs developing under *C. taenia* spermatozoa stimulation. As tetraploid males are sterile/infertile, *C. taenia* males, representing c. 3% of the population, are the only 'donors of sperm' in reproductive processes of *Cobitis* females in diploid-polyploid populations. Studies conducted by our team showed that: a) 3n hybrid *Cobitis* females laid 3n eggs and under experimental conditions only about 40% of them developed clonally into triploid progeny, while the remaining eggs were fertilized, giving tetraploid progeny, b) ability to clonal (gynogenetic) and sexual reproduction was manifested by each female, regardless of the population, c) the ratio of 3n and 4n individuals varied during ontogenetic development, indicated that tetraploids were significantly less viable than triploids. Thus, 3n *Cobitis* taxa may reproduce in dual modes of unisexual via gynogenesis and sexual reproduction which is known in only several vertebrates.

The aim of the proposal is to study the effectiveness of clonal and sexual reproduction of natural triploid hybrid *Cobitis* females in relation to their genome composition and water temperature, and their allopolyploid progeny viability (hatching success, regularity of embryonic and larval development, ploidy level, karyotypes, *Hox* genes organization, transcriptome profiles). We expect that the reproduction mode of 3n hybrid *Cobitis* females and its effectiveness is conditioned by their genomic composition, the water temperature during fertilization and embryonic development. We also expect that survival of tetraploid *Cobitis* offspring will be linked to the possibility of matching the parental genomes and is associated with the genes responsible for their proper development.

The proposed subject is consistent with trends in world science regarding the evolutionary importance of hybridization and polyploidization as well as asexual (clonal) and sexual processes among vertebrates. The results of the research undertaken will have a special general-biological significance. They will be a brick in learning the basics of the fascinating phenomenon of speciation through hybridization and polyploidization, which generate the processes of asexual reproduction. The project's results will have also an impact on the knowledge of reproductive biology and evolutionary biology of polyploid vertebrates and the coexistence of unisexual and sexual organisms that are closely related.