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ORIGIN OF INTRODUCED PSEUDORASBORA PARVA POPULATIONS IN ROMANIA, BASED ON GENETIC MARKERS (16S rRNA)

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INTRODUCTION

The place and role of organisms in nature is the result of a long evolutional process, which had as result the integrity of ecosystems. The geographic distribution of species is determined by abiotic (environmental factors, geophysical barriers etc.) and biotic (interspecific relations) factors. In consequence a species will enlarge its area as far as abiotic and biotic factors will allow (Eber&Brandl 1997).

One of the side effects of the socio-economic globalization process is the voluntary or involuntary translocation of organisms by humans and the changing of natural barriers. Although introduction of species is not a new phenomenon, the recognition of the impact of these species on native organisms is just started (Lodge 1993).. The initial lack of interest may be explained by the fact, that most of non-native species do not cause spectacular environmental changes or ecological catastrophes (Williamson & Fitter 1996). However, today invasive species and introduction of species are one of the top issues of the scientific world.

Although, even today the introduction of species is made mainly based on economic reasoning and the study of the mechanisms and the impact of introductions are usually neglected (Pasarin & Stan 1996). Today there is an urgent need of such studies, not only because of ecological but also economical reasoning. In many cases the introduction of species turned out to have beside the expected benefices enormous expenses (Pimentel et al. 2000) as well, because of the invasive specie. Invasive is a species …*that spread, with or without the aid of humans, in natural or seminatural habitats, producing a significant change in composition, structure, or ecosystem processes, or cause severe economic losses to human activities…* (Copp et al. 2005.).

They may have several negative effects on native species, like: trophic competition, reproductive competition, introduction of new parasites and pathogens, predation upon native species etc (Copp et al. 2005.).Invasive species may cause the displacement of native species. Most researchers agree on the fact that invasive species are the fifth major reason of species extinction, and the most important one in case of lakes (Kolar & Lodge 2002).

The vast majority of the papers publicated on this topic deals with the presence of new invasive species in different countries, life history traits of this species, or they try to asses the impact of invasive species on native ecosystems. Based on the life history traits of invasive organisms there is an attempt to draw the profile of potentially invasive species, which may be important prophylactic tool in the near future. These kinds of studies are involving classical investigation methods, like growth-, nutrition-, reproduction-, morphologic studies etc. Even in these studies new, modern techniques may be applied, for example molecular genetics. These techniques are opening new horizons and offering the tools to investigate inaccessible aspects until today.

Exponential developing of molecular biology methods led to a more complex taxonomic and population studies base on molecular markers. Many aspects of species biology can be studied using molecular markers. For example to detect bottlenecks and other demographic events in a population's history, to establish population structures, reproduction systems, dispersal and migration patterns. These population aspects are difficult to determine directly, but can be inferred using molecular biology methods. In our days nuclear and mitochondrial genes are sequenced routinely for taxonomic purposes (phylogenies) and microsatellites are common markers for populations studies (Halliburton, R. 2004).

We try to benefit from these new findings and to apply them in the present study, aiming a top invader, a small size fish species, which accomplished to colonize whole Europe. This is the topmouth gudgeon, *Pseudorasbora parva* (Schlegel 1842).

MATERIALS AND METHODS

The topmouth gudgeon (*Pseudorasbora parva*) – fig.1. - from taxonomical point of view belongs to clasa Osteichthyes, subclasa Actinopterygii, Teleostei, ordo Cypriniformes, familia Cyprinidae, subfamilia Rasborinae.

It is an accidentally introduced species. It was introduced in Romania in 1960 togheter with the fingerlings of such economically valuable species as *Ctenopharyngodon idella, Hypophtalmichthys molitrix, Aristichthys nobilis,* from U-han, the lower region of the Jiang-He River. Romania was one of the first countries were these introductions took place.. A few individuals have escaped from the fisheries from Nucet (Dambovita County) and spread along the Ilfov River. It is assumed that some may escaped from the fisheries from Cega (Banarescu 1964). Today it is common in in Mures, Cris Rivers (Banarescu 1964), but it is also present in Olt Basin Somes Rivers (Falka 2005). Along the Danube it reached other parts of Europe and colonized the whole continent. Now days it can be considered a real cosmopolitan. It is present in Asia, Europe and Africa.

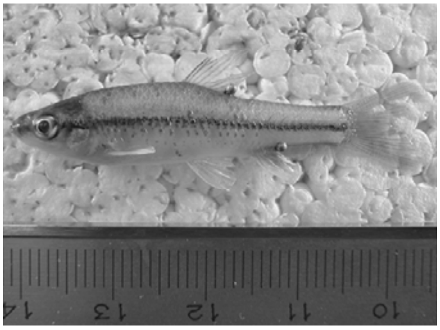

Fig.1. *Pseudorasbora parva*

We proposed ourselves to study the genetic variability of this species molecular marker. **Mitochondrial DNA** was a preferential as molecular marker for demographics studies (population divergence) and the inferring of phylogenetic history and phylogeographic patterns of species, because it evolves faster than nuclear DNA. Most eukaryotic cells contain mitochondria in more copies, occupying as much as 25% of the volume of the cytoplasm. Animal mitochondria contain a circular 16-18 kbp DNA molecule, encoding 13 proteins, 22 transfer RNA and two ribosomal RNA genes (12S rRNA and 16S rRNA) (Ballard, J. W. O. and Whitlock M.C. 2004). The faster evolution of mitochondrial DNA can be explained by the lack of most DNA repair pathways. This also explains the higher mutation rates per nucleotides in mtDNA than it is in nuclear DNA. Moreover it is inherited usually maternally, thus mitochondrial gene trees can be interpreted as maternal phylogenies. On the other hand, because the mitochondrial genes are usually inherited only maternally, there are only one-quarter as many copies of these genes in the population (if we assume equal number of males and females in the population) as there are copies of autosomal genes. Therefore, gene diversity should decay faster and allele frequency variance should increase faster than for autosomal genes (Zhang and Hewitt, 2003, Halliburton, 2004).

The most common mitochondrial markers are coding regions for: cytochrome b, cytochrome oxidase, 12S rRNA, 16S rRNA, etc, or noncoding ones like the control region (Feral 2002).

We consider the use of genetic markers to be an appropriate method because it gives as the chance to track the genetic differences between populations. This way it is possible to determine the genetic relatedness and we can reconstruct a genealogical tree of the populations. Actually is the same method used in phylogeny, but in this case the branches of the tree may be considered as the pathways of invasion and not like evolutional ones (Robert et al. 2005). On the other hand it is possible identify the genetic source of the invasion, the population from which all invasion pathways starts (Robert et all 2005).

We proposed ourselves to study the genetic variability of this species using as molecular marker the gene encoding the 16S rRNA. In order to accomplish in the first step we isolated the genomic DNA with DNeasy® Tissu Kit, Qiagen**.** In the next step the 16S rRNA gene was amplified with 16Sar/16Sbr universal primer pairs. The purified PCR products were sequenced with BigDye® terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems).

Fig. 2. The sampling sites (1 - Crisul Alb, 2 - Tarnava Mica, 3- Olt, 4 – Arges, 5 - Danube)

Based on these date the phylogenetic tree was constructed with MEGA program, version 3.1, the parameters were: Minimum Evolution (Boostrap value 10000 replicates), Kimura-2 algorithm and as out-group we used the *Pungtungia herzi* species.

The examined fishes were caught during electrofishing. The investigated individuals are representing five populations from five river basins: Olt, Crisul Alb, Tarnava Mica, Arges and Danube (fig. 2.).

RESULTS AND DISSCUSIONS

According to the results of the genetic analysis two out of five populations, respectively the population from the Danube and the population from the Tarnava Mica river basin, - R66, R131, R132 samples in in the gene three (fig. 3.)- are identical with the Chinese Yangtze haplotypes.

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                                 160       170       180       190       200
                        ....|....|....|....|....|....|....|....|....|....|
Yangtze                 ATTGATCTATCCGTGCAGAAGCGGGTATAAACATACAAGACGAGAAGACC
R66-TarnavaMica         ..................................................
R131-Dunarea            ..................................................
R132-Dunarea            ..................................................
Hija                    ...............................T..................
Sue                     ...............................T..................
LakeBiwa-2              ...............................T..................
Tenpaku                 ...............................T..................
Kasumigaura             ...............................T..................
Chikuma-2               ...............................T..................
Pir-Amur-2              ...............................T..................
Pir-Amur-1              ...............................T..................
Chikuma-1               ...............................T..................
R114-Olt                ...............................T..................
R115-Olt                ...............................T..................
R81-Olt                 ...............................T..................
R77-Olt                 ...............................T..................
R78-Olt                 ...............................T..................
R71-Olt                 ...............................T..................
R72-Olt                 ...............................T..................
R4-CrisulAlb            ...............................T..................
R5-CrisulAlb            ...............................T..................
R6-CrisulAlb            ...............................T..................
R7-CrisulAlb            ...............................T..................
R15-CrisulAlb           ...............................T..................
R65-TarnavaMica         ...............................T..................
R67-TarnavaMica         ...............................T..................
R130-Dunarea            ...............................T..................
R133-Dunarea            ...............................T..................
R46-Baneasa             ...............................T..................
R52-Baneasa             ...............................T..................
Miya                    ...............................T..................
Kiso                    ...............................T..................
Nagano                  ..................................................
Naruse                  ...............................T..................
Pungtungia herzi,Minami ..........................G...TA..................
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Fig. 3. Amplified 16S rRNA gene fragment sequences alignment with BioEdit program version 7.0.1. (partial representation). The dots represent the same nucleotide in all sequences.

Three out of five populations, respectively the Olt, the Crisul Alb and the population from the Arges river basin - R46, R133, R130, R67, R65, R15, R7, R6, R5, R4, R72, R71, R78, R77, R81,R115, R114, R52 samples in the gene three (fig.3.)- are are identical with Hija and Chikuma (Japan) haplotypes. Based on these results we were able to create a gene tree (fig. 4.)

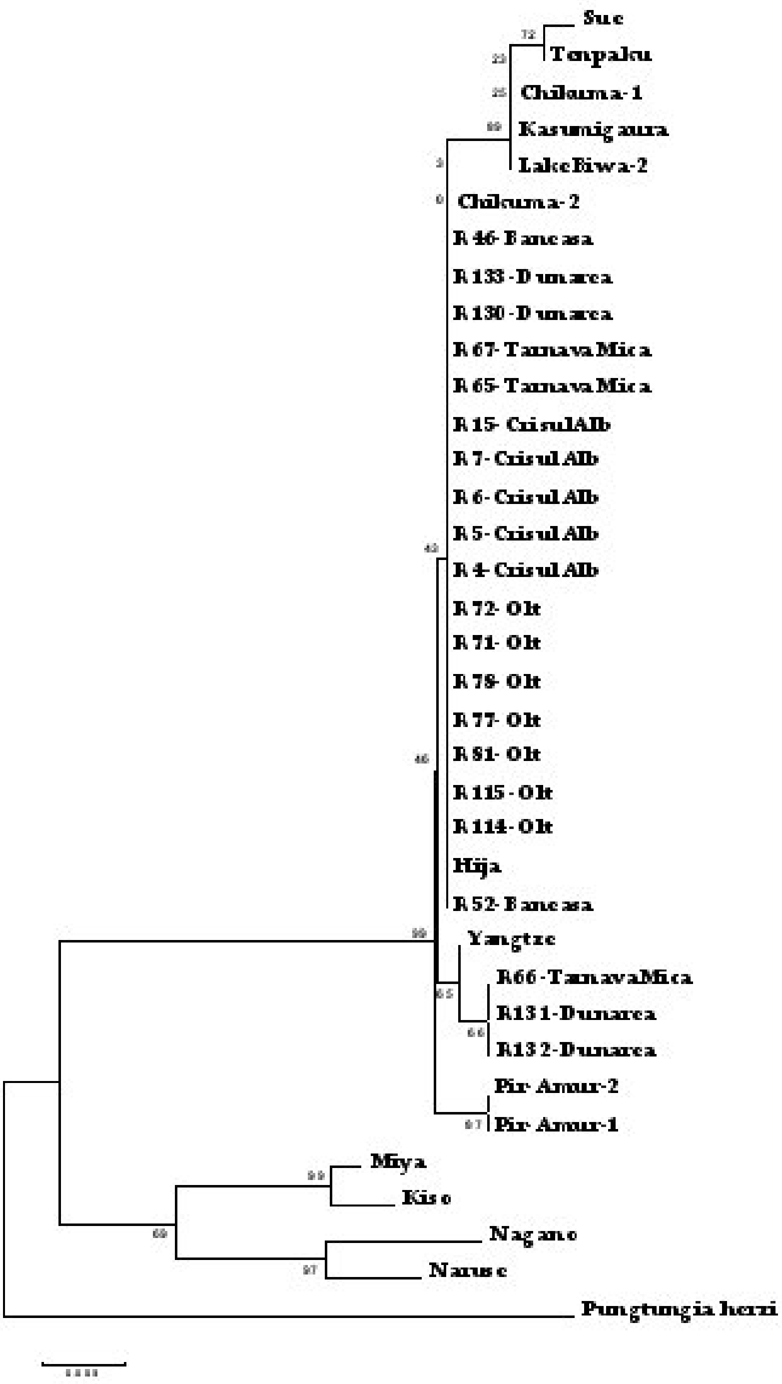

Fig. 4. 16S rRNA gene tree based on *P. parva*, *P. pumila* sequences and as outgoup *Pungtungia herzi*. The tree was contructed with MEGA program, version 3.1, the parameters were: Minimum Evolution (Boostrap value 10000 replicates), Kimura-2 algorithm. The sequences named as Miya, Kiso, Nagano, Naruse, belong to the species *P. pumila*

ABSTRACT

The topmouth gudgeon (*Pseudorasbora parva*) is an invasive fish species, accidentally introduced in the 1960`s among the fingerlings of Chinese carps, imported from China, according to Bănărescu (1964) and others.

We proposed ourselves to study the genetic variability of this species using as molecular marker the gene encoding the 16S rRNA. The genomic DNA was isolated with DNeasy® Tissu Kit, Qiagen**.** The 16S rRNA gene was amplified with 16Sar/16Sbr universal primer pairs. The purified PCR products were sequenced with BigDye® terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). The phylogenetic tree was constructed with MEGA program, version 3.1, the parameters

were: Minimum Evolution (Boostrap value 10000 replicates), Kimura-2 algorithm and as out-group we used the *Pungtungia herzi* specises.

We have found among the studied populations 2 samples out of five which were identical with the Chinese Yangtze haplotypes, thus supporting the initial theory about the origin of the Romanian populations, but we have also found 3 samples out of five identical with Hija and Chikuma (Japan) haplotypes. Thus, based on 16S rRNA gene tree we can propose two sources of origin (Chine and Japan) of the introduced *P. parva* populations in Romania.

CONCLUSIONS

In the light of these results of the genetic analyses it seems that Romanian topmouth gudgeon (*Pseudorasbora parva*) populations may have not one, but two sources of origin. On one hand they were accidentally introduced with the Chinese carps from the Yangtze River basin – in concordance with data from the literature (Banarescu 1964) – but on the other hand we have also identified another possible origin, Hija and Chikuma river basins from Japan.

These facts do not suggest by all means an introduction from Japan. It may be explained by the genetic variability of the species in its native range. It also may be explained by geographical isolation of the Japanese population, followed by a translocation to the continent and there from to Romania together with fingerlings of Chinese carps.

Finally, the genetic similarities shown by the studied Romanian populations of *Pseudorasbora parva* to Japanese ones is a new fact, which opens new questions and requires further studies on this topic.

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