

SCARDINIUS GENUS IN MOLECULAR STUDIES – A REVIEW

OVIDIU POPESCU¹, DUMITRU COJOCARU¹, MITICĂ CIORPAC^{1*}

Received: 20 November 2015 / Revised: 21 November 2015 / Accepted: 23 November 2015 /

Published: 14 December 2015

Keywords: *Scardinius*, molecular markers, phylogeny, phylogeography.

Abstract. *Scardinius* is a genus of ray-finned fish in the Cyprinidae family commonly called Rudds. The common Rudd (*Scardinius erythrophthalmus*) is a benthopelagic freshwater fish that occurs mainly in nutrient-rich, well vegetated lowland rivers, backwaters, oxbows, ponds and lakes and it is widespread in Europe and middle Asia. It has a medium length of 20-30 cm, but it can reach 50 cm. The classification of cyprinids has always been controversial the morphological traits have an unclear homology this led to the idea that the recognized monophyletic groups are surely misinterpreted. This paper aims to assess the current level of molecular data regarding *Scardinius* genera. Some of the molecular data obtained for *Scardinius* genus is from DNA barcoding studies on fresh water fishes, but studies regarding this genus and Cyprinidae family used mitochondrial genes like cytochrome b (*cyt b*) and cytochrome oxidase (*CO*), but nuclear genes or nuclear microsatellites were also used. We found that molecular data exists for both nuclear and mitochondrial genes, but this genus wasn't studied separately and as many of the researchers suggest more taxonomic studies are required in order to solve the uncertainties within it.

INTRODUCTION

Scardinius is a genus of ray-finned fish in the Cyprinidae family commonly called Rudds. Locally, the name "Rudd" without any further qualifiers is also used for particular species, particularly the common Rudd (*Scardinius erythrophthalmus*). The Rudd can be distinguished from the very similar Roach by way of the Rudd's upturned mouth, allowing the Rudd to pick food items such as aquatic insects from the surface of the water with minimal disturbance (Banarescu and Coad, 1991).

The common Rudd (*Scardinius erythrophthalmus*) is a benthopelagic freshwater fish that occurs mainly in nutrient-rich, well vegetated lowland rivers, backwaters, oxbows, ponds and lakes. It is widespread in Europe and middle Asia: most European rivers north of Pyrenees and Alps, eastward to Ural and Eya drainages, Aral and White Sea basins; Black Sea basin in Europe and northern Asia Minor. Naturally absent from Iberian Peninsula, Adriatic basin, Italy, Greece south of Pinios drainage, Great Britain north of 54° N, Ireland and Scandinavia north of 62° N (Kottelat and Freyhof, 2007). Definitely absent in Siberia. It has been artificially introduced to Spain, Ireland, Norway, USA, Canada, Morocco, Madagascar, Tunisia. In New Zealand it has been illegally introduced in 1960s where they have spread around the country. They have the potential of adapting to unfavorable environmental condition (Kottelat and Freyhof, 2007) and they can irreversibly damage indigenous ecosystems (Chadderton, 2010). The Rudd is often confused with the Roach (*Rutilus rutilus*), morphologically the two species appear very similar (Cihar, 1991). This paper's purpose is to assess the level of molecular data obtained for the *Scardinius* genus until now.

The classification of cyprinids has always been controversial, the number of recognized families ranging from 2 to 12 depending on the author and the number of morphological traits considered (Arai, 1982; Chen et al., 1984; Howes, 1980; Cavender and Coburn, 1992) and even have been elevated to family level, being assigned European and North American leuciscins and phoxinins to the family called Leuciscidae (Chen and Mayden, 2009; Mayden and Chen, 2010). Because the morphological traits are usually subjected to homoplasy (Zardoya and Doadrio, 1999), the systematics based on them sometimes comes in contradiction with molecular data (Zardoya and Doadrio, 1999; Briolay et al., 1998; Gilles et al., 2001; Cunha et al., 2002; Liu et al. 2002; Saitoh et al., 2006; Rüber et al., 2007; Gorgan 2007, 2008, 2013) and also the morphological traits have an unclear homology (Bogutskaya, 1990; Howes, 1991). All these facts lead to the idea that the recognized monophyletic groups are surely misinterpreted (Perea et al., 2010).

Some of the molecular data obtained for *Scardinius* genus is from DNA barcoding studies on fresh water fishes with few exceptions where the species of this genus are treated separately. Another type of studies regarding this genus and Cyprinidae family used mitochondrial genes like cytochrome b (*cyt b*) and cytochrome oxidase (*CO*), but nuclear genes or nuclear microsatellites were also used.

PHYLOGENY RELATIONSHIPS AND LIFE HISTORY

One of the earlier studies regarding European Cyprinids, that used the *cyt b* gene (1140 bp) as marker for establishing the relationships and the radiation of this fishes in Europe, was conducted in 1999 by Zardoya and Doadrio who found that the first major radiation within leuciscins occurred in the mid-Miocene approximately 13.6 mya, and extant lineages such as those leading to, e.g., *Scardinius*, *Rutilus*, *Leuciscus*, and *Alburnus* were originated and that the fossil records of *Leuciscus* on the Iberian Peninsula that dates back to the middle Miocene is supporting these datings. Also the molecular data obtained in this study support the previous biogeographical hypothesis, stated by Bianco in 1990, Doadrio in 1990 and 1994, that points out the importance of the southern Mediterranean area in the evolution of some European cyprinid taxa.

Another study from 2004 conducted by Ketmaier et al., which is centered on the phylogeny of *Telestes* and *Scardinius* from the peri-Mediterranean area, based also on *cyt b* gene (786 bp), shows that in the *Scardinius* phylogeny the Greek species are situated basal on the tree. In the same paper is stated that the genetic data for *S. erythrophthalmus* in Italy shows an altered situation due to man-mediated fish transfer. Also these species populations do not appear to be clustered on monophyletic clade, *S. scardafa* being positioned between them. Regarding the biogeography of the *Scardinius* group the authors suggest that during the glaciations the dropping of the sea level determined confluences of water between rivers flowing into the epicontinental area of the Mediterranean Sea. Thus, this event has led to a major dispersal of this genus through river confluences in low-lands.

An article published in 2005, which used the entire *cyt b* region of 1141 bp, by Freyhof et al., that aimed the introgression of mitochondrial DNA in Dalmatian cyprinids shows a case of a population of *S. dergle* that had mitochondrial DNA originating from *Squalius tenellus* in a clay-pit lake. Their findings are unclear regarding to the introgression hybridization events in the Leuciscini because this type of event could be a localized one or it could be more common than previously assumed. Nevertheless, this type of hybridization is common within close related cyprinids but in this case the *Scardinius* and *Squalius* are phylogenetically separated since the middle Miocene (~10 MYA) and further studies are required.

Scardinius erythrophthalmus species was included in a study published in 2008 regarding the molecular phylogeny of the Romanian cyprinids from the Danube River by Luca et al. In order to achieve this the authors used a fragment from the *COX2* gene (302 bp). Their findings show the positioning of *S. erythrophthalmus* species on the same clade with *Abramis sp.* and *Rutilus sp.* in the *Leuciscinae* group.

In 2010, a complex study was performed by Perea et al. regarding phylogenetic relationships and biogeographical patterns of Leuciscinae subfamily in the Mediterranean area. The research team used both mitochondrial (*cyt b* -1140 bp and *COI* – 646 bp genes) and nuclear (Recombination Activating Gene 1 (*RAG-1*) and Ribosomal Protein Gene S7 (*S7*) genes – 2647 bp) markers. Because the *Scardinius* genus was previously considered a sister group of *Tropidophoxinellus* (Zardoya and Doadrio, 1999; Bianco, 1988) and also the hypothesis that this genus and *Alburnus* genus are closely related (Briolay et al., 1988; Zardoya and Doadrio, 1999), the authors compared their findings considering this two cases. Their results regarding the phylogeny of this group strongly support the first theory. Also the phylogeny based on the nuclear markers showed a low support for the relationship between *Scardinius* and *Rutilus*, a fact already theorized using morphological characters (Cavender and Coburn, 1992). The analysis of *cyt b* gene grouped the lineages of *Scardinius*, *Tropidophoxinellus*-*Pseudophoxinus callensis*-*P. chaizoni*

and *Alburnus-Anaocypris-Leucaspis-Pseudophoxinus punicus*, but the basal relationships between these lineages could not be solved using *COI* gene, *RAG-1* and *S7* most probably because the different rate of evolution of these genes in comparison with *cyt b* gene. Still, the combined data matrix for all genes retrieved this relationship and inside the *Scardinius* genus the data obtained by the authors support some of the recognized species (Kottelat and Freyhof, 2007). So, according to their findings *S. erythrophthalmus* is a Central and Eastern European species, the specie *S. scardafa* is endemic to Lake Scanno in Central Italy as described by Bianco in 1994 and Ketmaier et al. in 2003 and it is a close relative of *S. hesperedicus* (Bianco, 1994; Bianco et al., 2001). Also the species *S. plotizza* formed an independent and well defined clade.

In the same study, the authors made a biogeographical approach. Their results showed that the Pleistocene glaciations were important in the European distribution of some taxa and that their origin is more recent because the colonization process from glacial refuges (for example Danube basin) happened after. Also the Danube basin probably was not the only refuge and other rivers of the Black Sea basin were refuges (Kotlik et al., 2004). The pattern of the widespread distribution of *S. erythrophthalmus* in Europe can be explained by the homogeneity conferred by the Danube basin refuge (Banarescu, 1992; Durand et al., 1999; Perdices et al., 2003; Culling et al., 2006).

A study made in 2013 regarding the phylogenetic relationship of two populations of *Scardinius* sp. from the Adriatic Sea drainage in Croatia was performed by Valić et al. who used as molecular markers the *cyt b* gene (1140 bp) and non-coding nuclear region *Cyfun P* (Cyprinid formerly unknown nuclear Polymorphism) and also sequences from GeneBank database. Analyzing the mitochondrial marker (*cyt b* gene) the researchers showed that the populations from the north and central Adriatic evolved recently, but that is difficult to analyze the relationship between them because probably they don't reach monophyly yet due to too short time. The phylogeny based on the nuclear marker (*Cyfun P*) was different than the one using the mitochondrial marker. Although this nuclear marker is not adequate to compare the relationships between closely related species the authors found that *S. erythrophthalmus* from Germany, *S. graecus* from Greece, *S. dergle* from Krka River and Vrana Lake group together in one lineage in phylogenetic tree distant from other cyprinid species. Also they concluded that *S. dergle* is a close relative of *S. hesperidicus* but the two are not the same species.

PHYLOGEOGRAPHIC APPROACHES AND GENE DIVERSITY

In 1999, a study that analyzed the allozyme patterns of four common cyprinid species (one of the species was *S. erythrophthalmus*) for a comparison of intraspecific genetic variability was performed by Wolter, the study area being represented by Berlin and its surroundings. The results regarding *S. erythrophthamus* show that this specie had the lowest abundance in the studied area from the species considered in this study. Also the genetic distance between subpopulations is high supported by a high bootstrap value (92%), but this high genetic distance was expected for the rudd because of its type of habitat preferences. This specie relatively high level of specialization and its reduced abundance led to a low capability of dispersion and implicitly to a high genetic distance between subpopulations.

A phylogeographic study of *Scardinius erythrophthalmus* was performed in 2004, by Stefani et al., in order to determine the differentiation of Italian populations in relation to the presence of mountain barriers. Their results were based also on a portion of *cyt b* gene (409 bp) and lead to a plausible scenario of this specie phylogeography in the Italian peninsula that implies a former colonization of Adriatic regions by Balkan populations during glacial periods, and a first

colonization of Thyrrenian basins. This followed by a succession of isolation processes between populations caused divergences supported by restricted gene flows at single watershed scale. Also the authors concluded that because of the presence of a defined and natural phylogenetic structure of *S. erythrophthalmus* in the Italian peninsula no significant anthropogenic influences can be highlighted on its original distribution pattern.

Because of the unavailability of nuclear markers for *S. erythrophthalmus*, Holmen et al. tested in 2009 a number of 36 microsatellite loci developed for *Pimephales promelas* (Ardren et al., 2002), *Barbonymus gonionotus* (McConnell et al., 2001; Kamonrat et al., 2002), *Cyprinus carpio carpio* (Crooijmans et al., 1997), *Anaocypris hispanica* (Salgueiro et al., 2003) and *Carassius auratus auratus* (Zheng et al., 1995) on this species and *Phoxinus phoxinus*. Their results indicate that a number of 18 species-locus combinations were amplified for the rudd from which 9 are polymorphic and 15 for minnow (*Phoxinus phoxinus*) from which 7 polymorphic. The authors conclude that this positive cross-species amplification could represent an important establishment of nuclear marker sets for population genetics studies on these two species.

In 2011 Triantafyllidis et al. conducted a study in which they analyzed the fish species diversity in four north Greek lakes (three natural eutrophic lakes and one dam-lake) by DNA barcoding. The marker used in this study was *COI* gene (655 bp) and the authors obtained 145 *COI* barcodes for 27 genera, including *Scardinius* genus. The results have shown a hybridization phenomenon between *S. dergle* and *Squalius* in the mitochondrial genome as previously demonstrated by Freyhof et al. in 2005. Also, for *S. erythrophthalmus*, high divergences between individuals in conspecific populations were found, this species having the highest divergence from all the species analyzed. According to the authors this is the first time when an analysis indicates such divergence between populations and more studies are needed as soon as possible.

A study that followed the identification of 89 commercially important fish species found in Turkey using DNA barcoding was conducted by Keskin and Atar in 2013. Although *S. erythrophthalmus* has little economic importance it was included in this study that used as marker *COI* gene (654 bp). The NJ tree obtained by the authors has shown no taxonomic deviation at the species level and they conclude that DNA barcoding is an efficient tool for identifying fish fauna.

A large scale study which followed the barcoding accuracy of freshwater fishes in Mediterranean Biodiversity Hotspot was published in 2014 by Geiger et al. and used *COI* gene as marker with a mean length of 646 bp. The *Scardinius* genus was taken in the study but it is not discussed because the purpose of the study was another. So the results of this study, according to the authors, support the expected divergence levels within the species, but not within genera, mainly because the scale of this study. Yet, the *Scardinius* species are mentioned as closely related but with the need for more research.

CONCLUSIONS

The *Scardinius* species are important freshwater fishes mainly because *S. erythrophthalmus* is widespread across Europe and western Asia, but also because more than half of this genera species have a status from near threatened to critically endangered on IUCN Red List.

Although for the *Scardinius* genera molecular data are available for both nuclear and mitochondrial genes, this genus wasn't studied separately and as many of the researchers suggest more taxonomic studies are required in order to solve the taxonomic, phylogenetic and phylogeographical uncertainties within it.

REFERENCES

- Arai R.**, 1982. *A chromosome study on two Cyprinid Fishes Acrossocheilus labiatus and Pseudorasbora pumila pumila, with notes on Eurasian Cyprinids and their karyotypes*. Bull Natn Sci Mus, Tokyo Ser A 1982, 8:131-152.
- Arden W.R., Miller L.M., Kime J.A., Kvitrud M.A.**, 2002. *Microsatellite loci for fathead minnow (Pimephales promelas)*. Mol Ecol Notes 2002, 2(3):226-227.
- Banarescu P.**, 1992. *Zoogeography of Fresh Waters. Distribution and dispersal of freshwater animals in North America and Europe*. Wiesbaden: Aula Verlag 1992, 2.
- Banarescu P., Coad B.W.**, 1991. *Cyprinidae of Eurasia*. In Cyprinid fishes, Systematics, Biology and Exploitation. Edited by: Winfield IJ, Nelson JE. London: Chapman and Hall; 1 1991:127-155.
- Bianco P.**, 1990. *Potential role of the paleohistory of the Mediterranean and Paratethys basins on the early dispersal of Euro-Mediterranean freshwater fishes*. Ichthyol Explor Freshwaters 1:167-184
- Bianco P.G.**, 1988. *Leuciscus cephalus (Linnaeus), with record of fingerling adult males, Leuciscus pleurobipunctatus (Stephanidis) and their hybrids from western Greece*. J Fish Biol 1988, 32:1-16.
- Bianco P.G.**, 1994. *L'ittiofauna continentale dell'Appennino umbromarchigiano, barriera semi permeabile allo scambio di componenti primarie tra i fl. opposte versanti dell'Italia centrale*. Biogeographia 1994, 17:427-485.
- Bianco P.G., Ketmaier V., Bussato T.**, 2001. *Approccio multidisciplinare all'analisi tassonomica del genere Scardinius (Cyprinidae) en Europa*. Quaderni ETP 2001, 30:115-120.
- Bogutskaya N.G.**, 1990. *Morphological basics in cyprinid fish of Leuciscinae subfamily (Leuciscinae, Cyprinidae)*. Vop Ikhtiol 1990, 30(6):920-933.
- Briolay J., Galtier N., Brito R.M., Bouvet Y.**, 1998. *Molecular Phylogeny of Cyprinidae inferred from cytochrome b DNA sequences*. Mol Phylogenet Evol 1998, 9:100-108.
- Cavender T.M., Coburn M.M.**, 1992. *Phylogenetic relationship of North American Cyprinidae*. In Systematics, historical ecology and North American freshwater fishes. Edited by: Mayden R.L. California: Stanford University Press; 1992:293-327.
- Chadderton W. L.**, 2010. *Management of invasive freshwater fish: striking the right balance!*, Hamilton, NZ: Department of Conservation.
- Chen W.-J., Mayden R.L.**, 2009. *Molecular systematics of the Cyprinoidea (Teleostei: Cypriniformes, the world's largest clade of freshwater fishes: Further evidence from six nuclear genes)*. Mol Phylogenet Evol 2009, 52:544-549.
- Chen X.L., Yue P.Q., Lin R.D.**, 1984. *Major groups within the family Cyprinidae and their phylogenetic relationship*. Acta Zool Sinica 1984, 9:424-440.
- Cihar, J.**, 1991. *Freshwater fish*. Silverdale Books, Leicester.
- Crooijmans R.P.M.A., Poel J.J.Vd., Groenen M.A.M., Bierbooms V.A.F., Komen J.**, 1997. *Microsatellite markers in common carp (Cyprinus carpio L.)*. Animal Genetics 1997, 28(2):129-134.
- Culling M., Janko K., Boron A., Vasil'ev V., Coté I., Hewitt G.**, 2006. *European colonization by the spined loach (Cobitis taenia) from Ponto-Caspian refugia based on mitochondrial DNA variation*. Mol Ecol 2006, 15(1):173-190.
- Cunha C., Mesquita N., Dowling T.E., Gilles A., Coelho M.M.**, 2002. *Phylogenetic relationships of Eurasian and American cyprinids using cytochrome b sequences*. J Fish Biol 2002, 61:929-944.
- Doadrio I.**, 1990. *Phylogenetic relationships and classification of west palaearctic species of the genus Barbus (Osteichthyes, Cyprinidae)*. Aquat Living Resource 3:265-282.
- Doadrio I.**, 1994. *Freshwater fish fauna of North Africa and its biogeography*. Ann Mus R Afr Centr Zool 275:21-34.
- Durand J.D., Persat H., Bouvet Y.**, 1999. *Phylogeography and postglacial dispersion of the chub (Leuciscus cephalus) in Europe*. Mol Ecol 1999, 8(6):989-997.
- Freyhof J., Lieckfeldt D., Pitra C., Ludwig A.**, 2005. *Molecules and morphology: Evidence for introgression of mitochondrial DNA in Dalmatian cyprinids*. Molecular Phylogenetics and Evolution 37 (2005) 347-354.
- Geiger M.F., Herder F., Monaghan M.T., Almada V., Barbieri R., Bariche M., Berrebi P., Bohlén J., Casal-Lopez M., Delmastro G.B., Denys G.P.J., Dettai A., Doadrio I., Kalogianni E., Karst H., Kottelat M., Kovacic M., Laporte M., Lorenzoni M., Marcic Z., Ozulug M., Perdices A., Perea S., Persat H., Porcelotti S., Puzzi C., Robalo J., Sanda R., Schneider M., Slechtova V., Stoumboudi M., Walter S. and Freyhof J.**, 2014. *Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes*. Molecular Ecology Resources (2014).
- Gilles A., Lecointre G., Miquelis A., Loerstcher M., Chappaz R., Brun G.**, 2001. *Partial combination applied to phylogeny of European cyprinids using the mitochondrial control region*. Mol Phylogenet Evol 2001, 19:22-23.

- Gorgan D. L.**, 2007 – Filogenie Moleculară în cadrul genurilor *Cyprinus* și *Carassius*, Editura Universității „Alexandru Ioan Cuza” Iași, 247-260.
- Gorgan D. L., Gherasim S. R.**, 2007 – The study of phylogenetic relationships and genetic diversity for *Carassius* genera individuals from eastern part of Romania, Proceedings, Taal, 12th World Lake Conference, Jaipur, India, p. 578 – 584.
- Gorgan D. L.**, 2008 – Introducere în studiul filogeniei și filogeografiei moleculare, Editura Bioflux, Cluj-Napoca. Online, 96-158.
- Gorgan L. D., Ciorpac M.**, 2013 - Identification of *Carassius gibelio* migration patterns in Europe, AACL Bioflux, 6(2):92-96.
- Howes G.J.**, 1980. *The anatomy, phylogeny, and classification of barilene cyprinid fishes*. Bull Brit Mus Nat Hist Zoology 1980, 37:129-198.
- Howes G.J.**, 1991. *Systematic and Biogeography: an overview*. In *Cyprinid fishes: Systematic, biology and exploitation*. Edited by: Winfield IJ, Nelson JE. London: Chapman and Hall; 1991:1-33.
- Kamonrat W., McConnell S.K.J., Cook D.I.**, 2002. *Polymorphic microsatellite loci from the southeast Asian cyprinid, Barbodes gonionotus (Bleeker)*. Mol Ecol Notes 2002, 2(2):89-90.
- Keskin E. and Atar H.H.**, 2013. *DNA barcoding commercially important fish species of Turkey*. Molecular Ecology Resources (2013) 13, 788–797.
- Ketmaier V., Bianco P. G., Cobolli M., Krivokapic M., Caniglia R., De Matthaeis E.**, 2004. *Molecular phylogeny of two lineages of Leuciscinae cyprinids (Telestes and Scardinius) from the peri- Mediterranean area based on cytochrome b data*. Molecular Phylogenetics and Evolution 32 2004 1061–1071.
- Ketmaier V., Bianco P.G., Cobolli M., De Matthaeis E.**, 2003. *Genetic differentiation and biogeography in southern European populations of the genus Scardinius (Pisces, Cyprinidae) based on allozyme data*. Zool Scr 2003, 32(1):13-22.
- Kotlik P., Bogutskaya N.G., Ekmeckçi F.G.**, 2004. *Circum Black Sea phylogeography of Barbus freshwater fishes: divergence in the Pontic glacial refugium*. Mol Ecol 2004, 13(1):87-95.
- Kottelat, M. and Freyhof J.**, 2007. *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin, 646 pp.
- Liu H., Tzeng C.S., Teng H.Y.**, 2002. *Sequence variation in the mtDNA control region and their implications for the phylogeny of Cypriniformes*. Can J Zool 2002, 81:1938-1946.
- Luca C., Manea A., Luca A., Dinischiotu A., Costache M.**, 2008. *Molecular phylogeny of the Romanian cyprinids from the Danube River*. Roumanian Biotechnological Letters, Vol. 13, No. 5, 2008, pp. 3970-3975.
- Mayden R.L., Chen W.-J.**, 2010. The world's smallest vertebrate species of the genus *Paedocypris*: A new family of freshwater fishes and the sister group of the world's most diverse clade of freshwater fishes (Teleostei: Cypriniformes). Mol Phylogenet Evol 2010.
- McConnell S.K.J., Skibinski D.O.F., Mair G.C.**, 2001. *Microsatellite markers from a commercially important South-east Asian cyprinid, the silver barb (Barbodes gonionotus Bleeker)*. Mol Ecol Notes 2001, 1(3):135-136.
- Perdices A., Doadrio I., Economidis P.S., Bohlen J., P. B.**, 2003. *Pleistocene effects on the European freshwater fish fauna: double origin of the cobitid genus Sabanejewia in the Danube basin (Osteichthyes: Cobitidae)*. Mol Phylogenet Evol 2003, 26(2):289-299.
- Rüber L., Kottelat M., Tan H.H., Ng P.K.L., Britz R.**, 2007. *Evolution of miniaturization and the phylogenetic position of Paedocypris, comprising the world's smallest vertebrate*. BMC Evol Biol 2007, 7(38).
- Saitoh K., Sado T., Mayden R.L., Hanzawa N., Nakamura K., Nishida M., Miya M.**, 2006. *Mitogenic evolution and relationships of the Cypriniformes (Actinopterygii, Ostariophysi): the first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences*. J Mol Evol 2006, 63(6):826-841.
- Salgueiro P., Carvalho G., Collares-Pereira M.J., Coelho M.M.**, 2003. *Microsatellite analysis of genetic population structure of the endangered cyprinid Anaecypris hispanica in Portugal: implications for conservation*. Biol Conserv 2003, 109(1):47-56.
- Stefani F., Galli P., Crosa G., Zaccara S., Calamari D.**, 2004. *Alpine and Apennine barriers determining the differentiation of the rudd (Scardinius erythrophthalmus L.) in the Italian peninsula*. Ecology of Freshwater Fish 2004: 13: 168–175.
- Triantafyllidis A., Bobori D., Koliamitra C., Gbandi E., Mpanti M., Petriki O., & Karaïskou N.**, 2011. *DNA barcoding analysis of fish species diversity in four north Greek lakes*. Mitochondrial DNA, October 2011; 22(S1): 37–42.
- Valić D., Smrzlić I.V., Kapetanović D., Teskeredžić Z., Pleše B., Teskeredžić E.**, 2013. *Identification, phylogenetic relationships and a new maximum size of two rudd populations (Scardinius, Cyprinidae) from the Adriatic Sea drainage, Croatia*. Biologia 68/3: 539—545, 2013.

Wolter C., 1999. *Comparison of intraspecific genetic variability in four common cyprinids, Abramis brama, Abramis bjoerkna, Rutilus rutilus and Scardinius erythrophthalmus, within and between lowland river systems.* Hydrobiologia 364: 163–177, 1999.

Wolter C., 1999. *Comparison of intraspecific genetic variability in four common cyprinids, Abramis brama, Abramis bjoerkna, Rutilus rutilus and Scardinius erythrophthalmus, within and between lowland river systems.* Hydrobiologia 364: 163–177, 1999.

Zardoya R., Doadrio I., 1999. *Molecular evidence of the evolutionary and biogeographical patterns of European cyprinids.* J Mol Evol 1999, 49:227-237.

Zheng W., Stacey N.E., Coffin J., Strobeck C., 1995. *Isolation and characterization of microsatellite loci in the goldfish Carassius auratus.* Mol Ecol 1995, 4(6):791-792.

* mitica.ciorpac@uaic.ro

1 Faculty of Biology, University “Alexandru Ioan Cuza” Iași, Romania, Carol I 20 A, Iasi,

Acknowledgement: This work was co-funded by the European Social Found through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU187/1.5/S/155397, project title “Towards a New Generation of Elite Researchers through Doctoral Scholarships”

