THE FREQUENCY OF THE MOLECULAR MARKERS USED IN PHILOGENY AND PHILOGEOGRAPHY STUDIES APPLIED ON INDIVIDUALS BELONGING TO CLASS *PISCES*

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Abstract. The elected project attempts to make a statistical evaluation of the most frequent used molecular markers in studies of fish phylogeny and phylogeography and to detect which orders and families of class *Pisces* are the most studied ones from this point of view. 52 recent studies, published between 1996 and 2006, where analysed. It could be observed that the most used molecular marker is the cytochrome b gene. The majority of studies were made using as a research material species belonging to the order *Perciformes*.

Keywords: molecular markers, phylogeny, phylogeography, Pisces.

Rezumat. Frecvenţa markerilor moleculari utilizaţi în studii de filogenie şi filogeografie la reprezentanţi ai clasei *Pisces*. În lucrarea de faţă ne-am propus sa evaluăm statistic care sunt cei mai frecvent folosiţi markeri moleculari în studiile de filogenie şi filogeografie ale peştilor şi care sunt ordinele şi familiile Clasei *Pisces* cele mai studiate din acest punct de vedere. Au fost luate în calcul 52 lucrări recente, publicate în perioada 1992-2006. Dintre studiile analizate, markerul folosit cu cea mai mare frecvenţă este gena ce codifică informaţia sintezei citocromului b. Cele mai mute cercetări s-au realizat folosind ca material de studiu specii aparţinând Ordinului Perciformes.

Cuvinte cheie: markeri moleculari, filogenie, filogeografie, Pisces.

Introduction

Molecular phylogeny is a new field of interest, which started to be thoroughly studied on an international level just a few years ago. In the past decades, the progress of molecular biology has offered new studying methods on evolution. They are based on the high level of stability of the DNA sequences. Evolutionary modifications in the structure of the DNA sequences are so slow, that they can supply detailed information about the origin and history of their evolution. Phylogeny studies use the mitochondrial DNA, due to the fact that it contains, in its structure, certain areas with a high number of polymorphisms in the nucleotide sequences and more seldom the nuclear DNA.

Results and Discussion

First of all we monitories all species, families and orders of class *Pisces*, used in the 52 studied papers and also all the molecular markers that were taken into account. Each study was included in Table 1, observing the alphabetical order of the authors.

Table 1. The monitoring of studied experiments.

MOLECULAR MARKERS	STUDIED SPECIES	AUTHOR
cyt b	Ord. Gadiformes, Fam Gadidae: Theragra chalcogramma	Aranishi, F., 2006
d-loop	Ord. Perciformes, Fam. Pomacentridae	Bay, L., K., Crozier, R., H., Caley, M., J., 2006
cyt b	Ord. Beryciformes, Fam. Trachichthyidae, Hoplostethus atlanticus, Ord Gadiformes, Fam	Beker, C., S. *et al.*, 1995

MOLECULAR MARKERS	STUDIED SPECIES	AUTHOR
	Merlucciidae: Macruronus novaezelandiae	
d-loop	Ord. Perciformes, Fam. Sparidae: Pagrus auratus	Bernal-Ramírez, J., H. *et al*., 2003
cyt b, 16S ARNr, first intron of S7 ribosomal protein	Ord. Perciformes, Fam. Labridae: Thalassoma sp.	Bernardi G. *et al*., 2004
cyt b, 18S rRNA, 12S rRNA, 16S rRNA	Ord. Acipenseriformes, Fam. Acipenseridae	Birstein, V., J., Hanner, R., DeSalle, R., 1997
cyt b	Ord. Perciformes, Fam. Carangidae: Decapterus macrosoma	Borsa, P., 2003
cyt b	Ord. Beryciformes, Fam. Holocentridae: Myripristis jacobus, Holocentrus ascensionis	Bowen B., W. *et al*., 2006
cyt b	Ord. Gadiformes, Fam Gadoidae	Calo-Mata, Pilar *et al*., 2003
cyt b	Ord. Perciformes	Cantatore, P. *et al*., 1994
cyt b	Ord. Perciformes, Fam. Serranidae: Epinephelus adscensionis and Rypticus saponaceous	Carlin, J.,L. *et al*., 2003
cyt b	Ord. Gasterosteiformes, Fam. Syngnathidae: Urocampus carinirostris	Chenoweth, S., Hughes, J., Connolly, R., 2002
ND 2, nuclear RAG1 gene	Ord. Perciformes, Fam. Cichlidae	Clabaut, Celine, Salzburger, W., Meyer, M., 2005
cyt b, microsatellite loci	Ord Anguilliformes, Fam Anguillidae : Anguilla anguilla	Daemen, E. *et al*. 2001
cyt b	Ord. Perciformes, Fam Cichlidae	Farias, I., P. *et al*., 2001
cyt b	Ord. Perciformes, Fam. Lethrinidae: Lethrinus	Galbo, Alicia, M., Lo. *et al*., 2002
cyt b, 12S rRNA	Ord. Cyprinodontiformes Fam. Cyprinodontidae: Cynolebias sp.	García, Graciela *et al.* 2004
cyt b, ATPase 8, ATPase 6	Ord Scorpaeniformes, Subord. Cottoidei	Grachev, M., A. *et al*., 1992
cyt b	Ord. Perciformes, Fam. Labridae, Xyrichtys larvae	Hare, J. A *et al*., 1994
cyt b	Ord. Perciformes, Fam. Labridae, Xyrichtys larvae	Hare, J., A. *et al*., 1998
cyt b, nuclear creatine kinase introns	Ord. Perciformes, Fam. Gobiidae: Gillichthys mirabilis	Huang, D., Bernardi, G., 2001
cyt b, ND 5	Ord Anguilliformes, Fam. Congridae: Conger myriaster	Inoue, J., G. *et al*., 2001
satellite DNA	Ord. Characiformes, Fam. Anostomidae: Leporinus obtusidens	Koehler, M., R. *et al*., 1997
cyt b	Ord. Cypriniformes, Fam. Cyprinidae, Subfam. Barbinae	Kong, Qing-Peng *et al.*, 2003
cyt b	Ord. Osteoglossiformes, Fam. Mormyridae: Pollimyrus castelnaui	Kramer, B. *et al*., 2003
cyt b, d-loop	Ord. Cyprinodontiformes, Fam. Poecicliidae, Xiphophorus sp.	Lockhart, P., J., Penny, D., Meyer A., 1995
cyt b	Ord. Cypriniformes, Fam. Cyprinidae, Cyprinella ornata	Mayden, R., L., 2002
cyt b, tRNA-proline	Ord. Perciformes, Fam. Chaetodontidae	McMillan, W., O., Palumbi, S., R., 1997
cyt b, d-loop	Ord. Mugiliformes, Fam Mugilidae	Murgia, R. *et al*., 2002
cyt b	Ord. Perciformes, Fam. Pomacentridae, Subfam. Amphiprioninae: Amphiprion ocellaris	Nelson, J., S. *et al*., 2000
cyt b, d-loop, 5 subunits of ND	Ord Salmoniformes, Fam. Salmonidae, Salvelinus malma	Oleinik, Alla, G. *et al.*, 2004
cyt b	Pisces	Parson, W. *et al*., 2000

MOLECULAR MARKERS	STUDIED SPECIES	AUTHOR
cyt b	Ord. Salmoniformes, Fam. Salmonidae: Parasalmo (Oncorhynchus) mykiss	Pavloy S., D. *et al.*, 2004
cyt b	Ord. Albuliformes, Fam. Albulidae: Albula sp.	Pfeiler, E. *et al.*, 2002
cyt b	Ord Cypriniformes, Fam. Cyprinidae	Qi, Delin *et al.*, 2006
cyt b, parvalbumin, growth hormon	Ord. Salmoniformes, Fam. Salmonidae	Rehbein, H., 2005
16S rRNA	Ord. Mugiliformes, Fam. Mugilidae	Rossi, Anna, Rita *et al.*, 2004
cyt b	Ord. Perciformes, Fam. Pomacanthidae: Centropyge loriculus	Schultz Jennifer, K. *et al.*, 2006
cyt b	Ord. Perciformes, Fam. Serranidae, Subfam. Epinephelinae	Shaoxiong, Ding *et al.*, 2006
cyt b, d-loop	Ord. Perciformes, fam. Sparidae: Pagellus bogaraveo	Stockley, B. *et al*., 2005
d-loop	Ord. Perciformes, Fam. Gobiidae: Rhinogobius sp.	Takahashi, D., Ohara, K., 2004
cyt b	Ord. Cypriniformes, Fam. Botiidae	Tang, Qiongying *et al*., 2005
d-loop	Ord. Salmoniformes, Fam. Salmonidae: Oncorhynchus mykiss	Thrower, F. *et al.*, 2004
cyt b	Ord Anguilliformes, Fam Anguillidae, Anguilla sp.	Tsukamoto, K., Aoyama, J., 1998
ND 5/6	Ord. Cypriniformes, Fam. Cyprinidae: Hypophthalmichthys molitrix	Wang, Z. *et al*., 2003
d-loop, ND 5/6	Ord. Siluriformes, Fam. Bagridae: Pelteobagrus fulvidraco, Pelteobagrus vachelli	Wang, Z. *et al.*, 2004
cyt b, d-oop, ND 5/6	Ord. Siluriformes, Fam. Bagriidae: Leiocassis longirostris	Wang, Z. *et al.*, 2006
ND 5/6	Ord. Gasterosteiformes, Fam. Gasterosteidae: Gasterosteus aculeatus	Yamada, M., Higuchi, M., Goto, A., 2001
cyt b	Ord. Cypriniformes, Fam Cyprinidae, Subfam. Gobioninae	Yang, Jinquan *et al*., 2006
cyt b	Ord. Cypriniformes, Fam. Cyprinidae	Zardoya, R. *et al.*, 1999
d-loop	Ord. Acipenseriformes, Fam. Acipenseridae: Acipenser sinensis	Zhang, Si-Ming *et al*., 2003
d-loop, ND 5/6	Ord. Cypriniformes, Fam. Cyprinidae: Cyprinus carpio	Zhou, J., F. *et al*., 2003

The following molecular markers have been used in the studied experiments: cyt b gene, d-loop gene, the five ND subunits genes, 12S, 16S, 18S rRNA genes, micro-satellites loci and satellite loci, RAG1 protein gene, first intron of S7 ribosomal protein, ATPase 6 and 8 genes, nuclear creatine kinase introns, tRNA-proline genes, parvalbumin genes and growth hormones genes.

It could be observed that the most frequent used and studied molecular marker was the cytochrome b gene. We could find this gene in 40 from the total of 52 studies, gathering 76.92% (Table 2).

The other molecular markers used in high percentages are: the mithocondrial control region (d-loop) with 23.07% and the encoding gene for nicotinamide dehydrogenasis region with 15.38%. In the same manner the less used molecular markers are the ones belonging to the nuclear DNA. RAG1 protein gene, the first intron of S7 ribosomal protein, nuclear creatine kinase introns, tRNA-proline genes, parvalbumin

genes and growth hormone genes were found to be used in the studied experiments only in 1.92%. There also are some mtDNA regions seldom used in the phylogeny and phylogeography research studies. These are: 18S rRNA and ATPase 6 and 8 genes.

Table 2. The frequency of the molecular markers from all the analyzed papers.

TYPES OF MOLECULAR MARKERS	STUDIES (NUMBER)	% FROM ALL THE STUDIES
cyt b gene	40	76.92
d-loop gene	12	23.07
ND gene	8	15.38
12S rRNA genes	2	3.84
16S rRNA genes	2	3.84
microsatellite loci	2	3.84
18S rRNA genes	1	1.92
nuclear RAG1 gene	1	1.92
first intron of S7 ribosomal protein	1	1.92
ATPase 6 genes	1	1.92
ATPase 8 genes	1	1.92
nuclear creatine kinase introns	1	1.92
tRNA-proline genes	1	1.92
parvalbumin genes	1	1.92
growth hormone genes	1	1.92

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Mitochondrial molecular markers are more often used and with more efficient results than the nuclear markers (Table 3, Fig. 1).

Table 3. The comparative use of nuclear and mitochondrial markers.

GROUPS OF MOLECULAR MARKERS	STUDIES (NUMBER)	% FROM ALL THE STUDIES
mitochondrial DNA	51	99.36
nuclear DNA	7	13.46

The phylogeny and phylogeography studies have been used only on a few species of fishes and most of them used especially individuals belonging to the Order *Perciformes*, with Families: *Pomacentridae, Sparidae, Labridae, Carangidae, Serranidae, Cichlidae, Lethrinidae, Labridae, Gobiidae, Chaetodontidae,* and the Order *Cypriniformes*, with Families: *Cyprinidae* and *Botiidae* (Annex 1). We found these two orders in 37.25% (*Perciformes*) and 15.68% (*Cypriniformes*) from the studied papers (Table 4, Fig. 2).

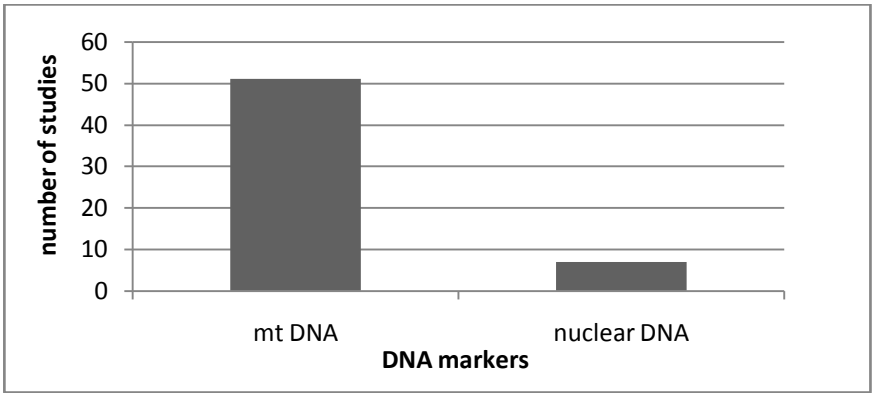

Figure 1. The difference between the values of use for the mitochondrial and nuclear markers.

Table 4. The comparative use of orders belonging to class Pisces.

ORDER	STUDIES (NUMBER)	%FROM ALL THE STUDIES
Ord Perciformes	19	37.25
Ord. Cypriniformes	8	15.68
Ord. Salmoniformes	4	7.84
Ord Anguilliformes	3	5.88
Ord. Gadiformes	3	5.88
Ord. Beryciformes	2	3.92
Ord. Acipenseriformes	2	3.92
Ord. Gasterosteiformes	2	3.92
Ord. Cyprinodontiformes	2	3.92
Ord. Mugiliformes	2	3.92
Ord. Siluriformes	2	3.92
Ord Scorpaeniformes	1	1.96
Ord. Characiformes	1	1.96
Ord. Osteoglossiformes	1	1.96
Ord. Albuliformes	1	1.96

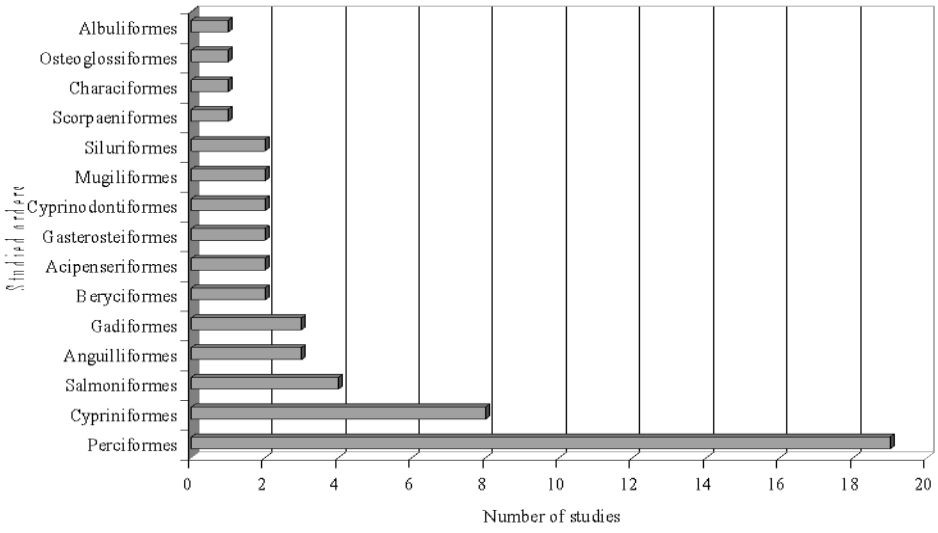

Figure 2. Orders’ utilisation degree values for phylogeny and phylogeography studies.

Conclusions

1. This statistical study has taken into account 52 published experiments of fish phylogeny and phylogeography.

2. The most frequent used and studied molecular marker was the cytochrome b gene.

3. The less used molecular markers are the ones belonging to the nuclear DNA and also some mtDNA regions (18S rRNA and ATPase 6 and 8 genes).

4. Most of the studied fish families belonging to *Perciformes* and *Cypriniformes* orders.

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ANNEX 1

ORDER	FAMILY	SPECIES	MOLECULAR MARKER
Ord. Perciformes	Fam. Pomacentridae	-	d-loop
		Amphiprion ocellaris	cyt b
		Centropyge loriculus	cyt b, d-loop
	Fam. Sparidae	Pagrus auratus	d-loop
	Fam. Labridae	Thalassoma sp.	cyt b, 16S ARNr, first intron of S7 ribosomal protein
	Fam. Carangidae	Decapterus macrosoma	cyt b
	Fam. Serranidae	Epinephelus adscensionis	cyt b
		Rypticus saponaceous	
		Subfam. Epinephelinae	
	Fam. Cichlidae	-	cyt b, ND 2, nuclear RAG1 gene
	Fam. Lethrinidae	Lethrinus	cyt b
	Fam. Labridae	Xyrichtys larvae	cyt b
	Fam. Gobiidae	Gillichthys mirabilis	cyt b, nuclear creatine kinase introns
		Rhinogobius sp.	d-loop

ORDER	FAMILY	SPECIES	MOLECULAR MARKER
	Fam. Chaetodontidae	-	cyt b, tRNA-proline
Ord. Cypriniformes	Fam. Cyprinidae	Subfam. Barbinae	cyt b
		Subfam. Gobioninae	
		Cyprinella ornata	
		Hypophthalmichthys molitrix	
		Cyprinus carpio	cyt b, ND 5/6
	Fam. Botiidae	-	cyt b
Ord. Salmoniformes	Fam. Salmonidae	-	cyt b, parvalbumin, growth hormon
		Salvelinus malma	cyt b, d-loop, 5 subunits of ND
		Parasalmo (Oncorhynchus) mykiss	cyt b, d-loop
Ord Anguilliformes	Fam Anguillidae	Anguilla anguilla	cyt b, microsatellite loci
		Anguilla sp.	cyt b
	Fam. Congridae	Conger myriaster	cyt b, ND 5
Ord. Gadiformes	Fam Gadidae	Theragra chalcogramma	cyt b
	Fam Merlucciidae	Macruronus novaezelandiae	cyt b
Ord. Beryciformes	Fam. Trachichthyidae	Hoplostethus atlanticus	cyt b
	Fam. Holocentridae	Myripristis jacobus	cyt b
		Holocentrus ascensionis	
Ord. Acipenseriformes	Fam. Acipenseridae	-	cyt b, 18S rRNA, 12S rRNA, 16S rRNA
		Acipenser sinensis	d-loop
Ord. Gasterosteiformes	Fam. Syngnathidae	Urocampus carinirostris	cyt b
	Fam. Gasterosteidae	Gasterosteus aculeatus	ND 5/6
Ord. Cyprinodontiformes	Fam. Poecicliidae	Xiphophorus sp.	cyt b, d-loop
	Fam. Cyprinodontidae	Cynolebias sp.	cyt b, 12S rRNA
Ord. Mugiliformes	Fam Mugilidae	-	cyt b, d-loop, 16S rRNA
Ord. Siluriformes	Fam. Bagridae	Pelteobagrus fulvidraco	d-loop, ND 5/6
		Pelteobagrus vachelli	
		Leiocassis longirostris	cyt b, d-oop, ND 5/6
Ord Scorpaeniformes, Subord. Cottoidei	-	-	cyt b, ATPase 8, ATPase 6
Ord. Characiformes	Fam. Anostomidae	Leporinus obtusidens	satellite DNA
Ord. Osteoglossiformes	Fam. Mormyridae	Pollimyrus castelnaui	cyt b
Ord. Albuliformes	Fam. Albulidae	Albula sp.	cyt b