Molecular Phylogeny of Mugilidae (Teleostei: Perciformes)

D. Aurelle[1], R.-M. Barthelemy[*,2], J.-P. Quignard[3], M. Trabelsi[4] and E. Faure[2]

[1]*UMR 6540 DIMAR, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France*

[2]*LATP, UMR 6632, Evolution Biologique et Modélisation, case 18, Université de Provence, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France*

[3]*Laboratoire d'Ichtyologie, Université Montpellier II, 34095 Montpellier, France*

[4]*Unité de Biologie marine, Faculté des Sciences, Campus Universitaire, 2092 Manar II, Tunis, Tunisie*

Abstract: Molecular phylogenetic relationships among five genera and twelve Mugilidae species were investigated using published mitochondrial *cytochrome b* and *16S rDNA* sequences. These analyses suggested the paraphyly of the genus *Liza* and also that the separation of *Liza, Chelon* and *Oedalechilus* might be unnatural. Moreover, all the species of the genus *Mugil* plus orthologs of *Crenimugil crenilabis* clustered together; however, molecular analyses suggested possible introgressions in *Mugil cephalus* and moreover, that fish identified as *Mugil curema* could correspond to two different species as already shown by karyotypic analyses.

Keywords: Mugilidae, grey mullets, mitochondrial DNA, *Mugil cephalus*, introgression.

INTRODUCTION

The family Mugilidae, commonly referred to as grey mullets, includes several species which have a worldwide distribution; they inhabit marine, estuarine, and freshwater environments at all latitudes except the Polar Regions [1]; a few spend all their lives in freshwater [2]. They are euryhaline species and can often penetrate lagoons and estuaries, migrating back to the sea to spawn. This family is generally included in the order Perciformes; however, recent authors have considered that this family would constitute a separate order: Perciformes (e.g. [3, 4]).

Within the family, several taxonomic revisions have also been made, raised by the conservative morphology and the paucity of useful taxonomic characters. In a most comprehensive and recent systematic review [1], 14 genera are been identified for a total of 64 valid species. Most of these are included in the genera *Mugil* and *Liza*, which have 12 and 23 species respectively. The author of this review recognized 17 nominal taxa in the uncertain condition of *species inquirenda* [1]. Moreover, the morphological classification stayed conflicted [2, 5] and did not provide conclusive answers to phylogenetic questions [6, 7]. Previously, identities of *Mugil* species have been studied by morphological methods [1, 6], protein electrophoresis [8-11], mtDNA analyses ([12, 13] and references therein). Moreover, 17 nominal species of mugilids that have been karyotyped shown differences within both the *Mugil* and *Liza* genera ([14, 15] and references therein). However, most of these studies provided conflicting results and left numerous phylogenetic questions unsolved including, the relationships within the genera *Mugil and Liza*.

*Address correspondence to this author at the LATP, UMR 6632, Evolution Biologique et Modélisation, case 18, Université de Provence, 3 Place Victor Hugo, 13331 Marseille cedex 3, France; Tel: (33) 4 91 10 63 30;
Fax: (33) 4 91 10 62 65; E-mail: roxane.barthelemy@univ-provence.fr

We have focused this study on Mugilid species for which both *cytochrome b* (*cytb)* and *16S rDNA* mtDNA sequences have been already published. Their geographic distributions are briefly presented here. *Oedalechilus labeo* is limited to the Mediterranean Sea and the Moroccan Atlantic coast, whereas, *Liza* and *Chelon* inhabit also the Eastern Atlantic coasts as well as the Indo-Pacific area [2, 16]. *Mugil cephalus* has a worldwide distribution, occurring in several continental waters [17]. On other *Mugil* species: *M. platanus, M. liza* and *M. incilis* live on the Western Atlantic Coasts; whereas, *M. hospes* and *M. curema* are distributed on both sides of the American continent [1]. The last species also occurs on the Eastern Atlantic Coasts, from Gambia to Congo [1, 18].

In this study molecular phylogenies of mugilids have been established. Moreover, this gave us the opportunity to contribute to the understanding of the rare occurrence of hybridization and/or introgression in marine environments.

MATERIALS AND METHODOLOGY

Mugilids *cytb* and *16S rDNA* mtDNA sequences have been extracted from GenBank; when identical sequences have been found, only one sequence has been used in the dataset. The characteristics of these sequences are in Table **1**. The alignments have been made with the BioEdit software [19]. But rare shorter haplotypes, for *cytb* and *16S rDNA*, the alignment lengths were respectively 376 bp and 483 bp (including insertions/deletions).

The methods used for tree reconstructions were:

- Neighbor-Joining algorithm (NJ) applied to the Kimura 2 parameters implemented in the Phylowin software [20].
- Maximum parsimony (MP) with Phylowin.
- Maximum likelihood (ML) using the Guindon and Gascuel's algorithm [21] implemented in the Phyml

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Table 1. Data Concerning the Various Sequences Used for Phylogenetic Analyses (*, Shorter Sequence; N.D., Not Determined)

Species	Gene	Corresponding Name(s) in the Phylogenetic Trees, Number of Identical Sequences and Accession Number(s)	Site of collection
Chelon labrosus Cuvier 1758 thicklip grey mullet	*cytb*	*C. labrosus.1A* (2 identical seq. EF427544, EF427545)	A: Cantabric Sea, Spain
		C. labrosus.3Ad (Z70772)	Ad: Italy, Lagoon of Venice
	16S	*C. labrosus.Ad* (AY169697)	Ad: Italy, Valle Figheri
Crenimugil crenilabis Forsskål 1775 fringelip mullet	*cytb*	*C. crenilabis* (AP002931)	N.D.
	16S	*C. crenilabis* (AP002931)	N.D.
Liza aurata Risso 1810 golden grey mullet	*cytb*	*L. aurata.15A* (EF427572) identical to EF439541	A: Cantabric Sea, Spain M
		L. aurata.1M (9 seq. EU122431) - *L. aurata.2M* (1 seq. EU1224312) - *L. aurata.6M* (1 seq. EU122433) - *L. aurata.7M* (1 seq. EU122434) identical to EF439540 and group with Z70773*	M: Tunisia, Kuriat Islands M Ad: Italy, Lagoon of Venice
	16S	*L. aurata.1M* (AY169698) - *L. aurata.2M* (AY169699)	M: Italy, Orbetello
Liza ramada Thomson 1986 thinlip mullet	*cytb*	*L. ramada.Ad* (Z70779*)	Ad: Italy, Lagoon of Venice
	16S	*L. ramada.1M* (AY169700) - *L. ramada.2M* (AY169701) group with AY141408* (*Liza sp.)*	M: Italy, Orbetello N.D.
Liza saliens Risso 1810 leaping mullet	*cytb*	*L. saliens.1M* (EU122428), *L. saliens.2M* (EU122428) group with Z70774*	M: Lion Gulf, France Ad: Italy, Lagoon of Venice
	16S	*L. saliens.M* (AY169702)	Ad: Italy, Valle Figheri
Mugil cephalus Linnaeus 1758 flathead mullet	*cytb*	*M. cephalus.1M* (4 seq. EU122430) groups with DQ225777*, DQ225778*, DQ225779*	M: Lion Gulf, France M: Spain, Balearic Archipelago
		M. cephalus.5P (EF426419) groups with Z70776*	Pacific, Chile, Pacific, CHI308 Ad and A: Italy, Lagoon of Venice and South Carolina
	16S	*M. cephalus.9* (AP002930)	N.D.
		M. cephalus.1A (AY655501) identical to AP002930	A: USA, Florida, Panama City N.D.
		M. cephalus.3P (DQ307686)	P: East Coast of the Gulf of California, Mexico
		M. cephalus.4I (DQ185446)	I: India, Chennai
		M. cephalus.6P (2 seq. EF397139, EF397140)	P: CHI308 - CHI309
		M. cephalus.8M (2 seq. AY169703, AY169704)	M: Italy, Orbetello
		M. cephalus.10A (EF095582*)	A
Mugil curema Valenciennes 1836 white mullet	*cytb*	haplotype I: *M. curema.1A* (8 seq. from EF426363 to EF426367, EF426370, EF426422, EF426423) - *M. curema.6A* (EF426368) - *M. curema.7A* (EF426369)	A: Brasil and Venezuela
		haplotype II: *M. curema.15A* (8 seq. from EF426371 to EF426376) - *M. curema.21A* (EF426377) - *M. curema.22A* (EF426378)	A: Brasil
		group with DQ225776*,	A: Mar Chiquita, Argentina
		DQ225775*,	A: Mar Chiquita, Argentina and Galveston Bay, USA
		Z70775*	A: USA, South Carolin
		M. curema.11A (DQ225774*)	A: Galveston Bay, USA
	16S	haplotype I: *M. curema.1A* (32 seq. from EF397033 to EF397064) - *M. curema.33A* (EF397065) - *M. curema.34A* (EF397066)	A: Brasil and Venezuela
		haplotype II: *M. curema.35A* (8 seq. from EF397067 to EF397074) - *M. curema.43A* (6 seq. from EF397075 to EF397080) - *M. curema.49A* (EF397081)	A: Brasil
		M.curema.50P (DQ532914)	P
		M. curema.51P (DQ307687)	P: East Coast of the Gulf of California, Mexico
		M. curema.52P (AY947852*)	P: California
Mugil hospes Jordan and Cuvier 1895 hospe mullet	*cytb*	*M. hospes.1A* (8 seq. from EF426354 to EF426359, EF426361, EF426362) - *M. hospes.7A* (EF426360)	A: Brasil
	16S	*M. hospes.1A* (25 seq. from EF397005 to EF397029) - *M. hospes.26A* (EF397030)- *M. hospes.27A* (EF397031) - *M. hospes.28A* (EF397032)	A: Brasil
Mugil incilis Hancock 1830 parassi mullet	*cytb*	*M. incilis.1A* (11 seq. from EF426379 to EF426383, EF426387, EF426388, EF426395, EF426397, EF426399, EF426424) - *M. incilis.6A* (4 seq. from EF426384 to EF426386, EF426390) - *M.incilis. 11A* (EF426389) - *M. incilis.13A* (EF426391) - *M. incilis. 14A* (EF426392) - *M. incilis.15A* (2 seq. EF426393, EF426400) - *M. incilis.16A* (EF426394) - *M. incilis.18A* (EF426396) - *M.incilis.20A* (EF426398)	A: Brasil
	16S	*M. incilis.1A* (26 seq. from EF397082 to EF397107) - *M. incilis.27A* (EF397108)	A: Brasil
Mugil liza Valenciennes 1836 liza	*cytb*	*M. liza.A* (9 seq. from EF426401 to EF426407, EF426420, EF426421)	A: Brasil and Venezuela
	16S	*M. liza.A* (16 seq. from EF397109 to EF397124)	A: Brasil and Venezuela
Mugil platanus Günther 1880	*cytb*	*M. platanus.A* (8 seq. from EF426408 to EF426415) 3 seq. from EF426416 to EF426418 are identical to *M. liza*.A	A: Brazil
	16S	*M. platanus.13A* (EF397137) - *M. platanus.14A* (EF397138) - 12 seq. from EF397125 to EF397138 are identical to *M. liza.A*	A: Brazil
Oedalechilus labeo Cuvier 1829 boxlip mullet	*cytb*	*O. labeo.Ad* (Z70777*)	Ad: Italy, Lecce
	16S	*O. labeo.M* (Y169705)	M: Italy, Livorno
Elassomatidae *Elassoma evergladei* Jordan 1884	*cytb*	*E. evergladei* (AP002950)	N.D.
	16S	*E. evergladei* (AP002950)	N.D.
Atherinomorpha *Oryzias latipes* Temminck and Schlegel 1846	*cytb*	*O. latipes* (AP004421)	N.D.
	16S	*O. latipes* (AP004421)	N.D.
Gasterosteiformes *Gasterosteus aculeatus* Linnaeus 1758	*cytb*	*G. aculeatus* (AP002944)	N.D.
	16S	*G. aculeatus* (AP002944)	N.D.

Abbreviations: A, Atlantic Ocean; Ad, Adriatic Sea; M, Western Mediterranean Sea; I, Indian Ocean; P, Pacific Ocean.

software. The model of molecular evolution used for reconstruction was chosen with the Modeltest software using the FindModel website at Los Alamos National Laboratory (http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html, accessed 23 July 2008). This allowed us to choose among 28 nucleotide models with the Akaike Information Criteria. The chosen model was a General Time Reversible (GTR) with Gamma distribution of rate variation among sites. The parameter of the Gamma distribution as well as the base frequencies were estimated by the software.

RESULTS

Molecular Phylogeny Using Partial *cytochrome b* Gene

For these analyses, 111 Mugilidae sequences have been extracted from GenBank. Molecular analyses using three reconstruction methods (MP, ML and NJ) give the same general topology. Differences lie in the haplotypes positions inside these groups and in the position of *Liza saliens* haplotypes. Fig. (**1A**) presents the ML tree with bootstraps values (BP) which are generally good for inner nodes thereby supporting these main clades. The numbers of variable and informative sites are respectively 198/77, these values being very high for genes used as markers for intra- and interspecific purposes [22].

Mugilidae constitute a monophyletic group highly supported statistically in ML and NJ analyses (BP, 99 and 80% respectively) but not in MP (57%). They are divided in two groups. The first group (named A; BP, NJ:100%, ML:100%, MP:69%) contains all the *Mugil* sequences plus the ortholog of *Crenimugil crenilabis*, while the second (B) contains the genera *Liza, Chelon* and *Oedalechilus*. Nevertheless this second group is only well supported by NJ (BP: 96%) but not with MP (28%) nor ML (<20%) for which *Liza saliens* are grouped apart from the other sequences. Within the group A, three monophyletic sub-groups can be shown

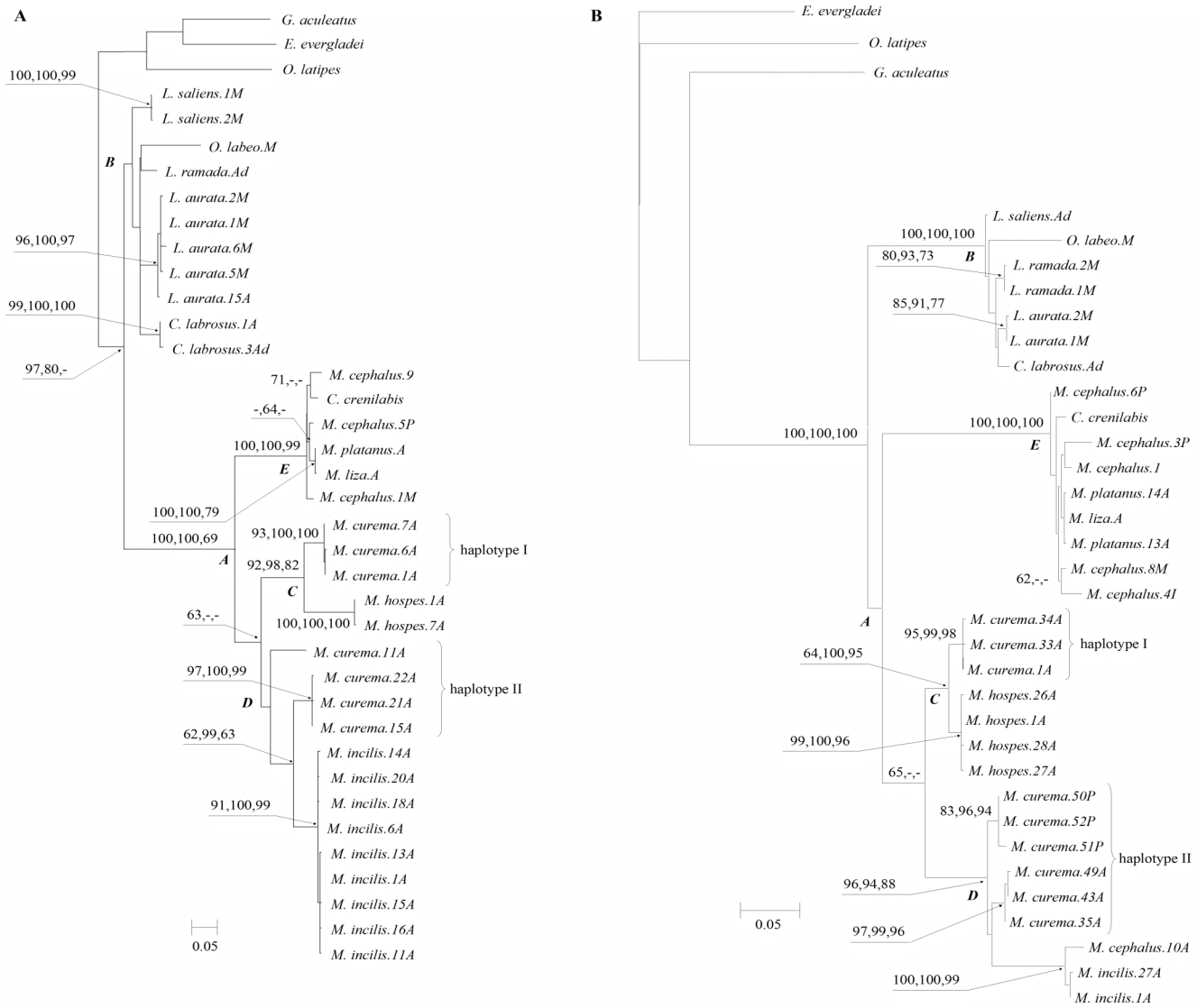

Fig. (1). Phylogenetic trees of Mugilids. A and B, phylogenetic trees using Maximum Likelihood method based on respectively *cytb* and *16S rDNA* partial sequences. Bootstrap values (BP in %), carried out with 1000 iterations, are given for each node only if they exceed 60, ML (left BP), NJ (middle BP) and MP (right BP). Sequences from three non mugilids Smegmamorpha (Elassomatidae: *Elassoma evergladei*; Atherinomorpha: *Oryzias latipes*; Gasterosteiformes: *Gasterosteus aculeatus*) have been used as outgroups.

which are well statistically supported. The *M. curema* haplotype I sequences constitute a monophyletic group (C) with these of *M. hospes* (BP, ML:92%, NJ:98%, MP:82%). The *M. curema* haplotype II sequences form a sub-group (D) with these of *M. incilis* (BP, ML:62%, NJ:99%, MP:63%, if not including the sequence *M. curema* 11). This last sequence which came from a fish caught on the US Atlantic Coasts did not group with the sequences of other *M. curema* caught on this coast. In the sub-group E is constituted by *M. cephalus, M. platanus, M. liza* and *C. crenilabis* sequences (BP≥99%), the *M. platanus* and *M. Liza* sequences group together with high bootstrap values (BP, ML:100%, NJ:100%, MP:79%). Surprisingly, on the portion of 507 nucleotides of the *cytb* gene sequenced, all the *M. Liza* sequences (from Brazil and Venezuela) are identical between them and identical to three of *M. platanus* and exhibit only one nucleotide difference with the other *M. platanus* sequences [13]. Moreover, *M. cephalus* sequences are divided in three sub-groups, an haplotype groups with the ortholog sequence of *C. crenilabis*; another haplotype clusters with *M. liza* and *M. platanus* sequences, whereas the last haplotype is alone [three partial sequences from Balearic archipelago [23] bear this haplotype, data not shown]. However, these groupings are not supported statistically.

The phylogeny of the genera *Liza, Chelon* and *Oedalechilus* remains unresolved; indeed, none of the generic and intra-generic (this concerns only *Liza*) groupings are supported statistically. However, *L. aurata* and *C. labrosus* could constitute a clade but this is not statistically supported.

Molecular Phylogeny Using Partial *16S rDNA* Gene

For these analyses, 157 mugilid sequences have been extracted from GenBank. The numbers of variable and informative sites are respectively 161/117. Interestingly, these analyses globally confirm the previous results using the *cytb* gene, and the trees using three methods (ML, NJ, and MP) gave similar topologies for nodes strongly supported by bootstrap analyses (Fig. **1B**). Whatever the outgroup sequences, the Mugilidae are monophyletic (BP 100% for the three methods). However, if the group B containing the genera *Liza, Chelon* and *Oedalechilus* is supported by high bootstrap values (BP:100%), this is not the case for the group A (*Mugil* and *C. crenilabis* sequences) which is recovered with ML (BP:51%) but appears paraphyletic with NJ and MP, where the group E appears as the sister group of the clade B for NJ, and groups C and D are sister of B for MP; however, this is statistically not supported.

Within the group A, the sub-groups D (BP ML:96%, NJ:94%, MP:88%) and E (BP=100%) are highly statistically supported. The sub-groups C and D are grouped together, but this is not statistically supported (BP <66%). Similarly to *cytb* analyses, the sub-group C contains the *M. curema* haplotype I sequences and those of *M. hospes*; however, this is statistically supported only with two methods (BP, ML:64%, NJ:100% and MP:95%). The sub-group D is constituted by *M. curema* haplotype II sequences, *M. incilis* sequences and one sequence from an Atlantic *M. cephalus*; this sequence (AC:EF095582) is strictly identical to all the *M. incilis* orthologs, but one which exibits only one ponctual mutation. In the ML analysis, concerning the sub-group E, a part of the *M. curema* haplotype II sequences has grouped with those of *M. incilis* [and one of *M. cephalus*], whereas the other *M. curema* sequences constitute a sister group of this last grouping; however, this is not statistically supported (BP≤58%). In NJ and MP analyses, all the *M. curema* haplotype II sequences could be constitute a clade (BP, NJ:88% and MP:55%). In the sub-group E, all the various branchings are not statistically supported and even the monophyly of the group *M. liza/M. platanus* is not supported. Moreover, concerning the *16S rDNA* partial genes sequenced, all the *M. liza* sequences are identical between them and strictly identical to 12 onto 14 orthologs of *M. platanus* [13].

The *M. cephalus* sequences do not form an homogeneous group, as some *M. cephalus* haplotypes appear closer to haplotypes from other species. Similarly to *cytb* analyses, a *M. cephalus* haplotype group with with *M. platanus* and *M. liza* samples (BP≤54%), another haplotype has the greatest homology with *C. crenimugil* sequence; whereas the last haplotype contains *M. cephalus* from Mediterraean Sea (two identical sequences) and one caught in the Indian Ocean. Nevertheless, even if the non-monophyly of *M. cephalus* is suggested by all analyses, they do not agree on the branching order of these sequences.

All *16S rDNA* reconstructions indicate the grouping of sequences from *Liza, Chelon* and *Oedalechilus* haplotypes (group B). Nevertheless the relationships inside this group are not well defined. In ML analysis, *L. saliens* sequence could be the sister group of all the other sequences, but this is not supported statistically. Whereas, NJ and MP analyses suggest that *Oedalechilus* could be the sister group of the two other genera (BP, NJ:95%, MP:79%).

Moreover, we have re-analysed two allozyme datasets including: *M. cephalus, O. labeo, C. labrosus, L. saliens, L. aurata* and *L. ramada* [9, 11] (Fig. **2**). However, the UPGMA trees show none branching is statistically supported, and the relationships among these groups are not clear.

DISCUSSION AND CONCLUSION

Phylogenetical Analyses of the Genera *Liza, Chelon* and *Oedalechilus*

To our knowledge, in all the published molecular studies, but one allozyme analysis [10], the monophyly of the genus *Liza* has been never supported. However, in all the articles in which species of the genera *Liza, Chelon* and *Oedalechilus* have been studied, these three genera constituted a monophyletic group. Studies which used mtDNA sequences [13, 24] (respectively, *cytb* and *16S rDNA*, and *cytb* and *12S rDNA*) and the PCR-RFLP of three mtDNA genes [12, 25] have found that *L. saliens* and *C. labrosus* group together. *16S* mtDNA analyses ([9] and present study) suggested a clade *L. aurata/C. labrosus*; however, this grouping was not statistically supported. Our *cytb* mtDNA analyses have also suggested this last grouping with weak support and suggested more surprisingly an *O. Labeo/L. ramada* clade. A recent work using allozymes and our *16S rDNA* analyses, have both shown a *C. labrosus/L. Aurata/L. ramada* clade [11]. Interestingly, these three species have strictly the same distribution of Nucleus Organizing Regions (NORs) and *5S rDNA* sites, which underlined a higher affinity of *Chelon* to the subgenus *Liza (L. Aurata/L. ramada*) [26]. Within the genera *Liza, Chelon* and *Oedalechilus, O. labeo* appeared to

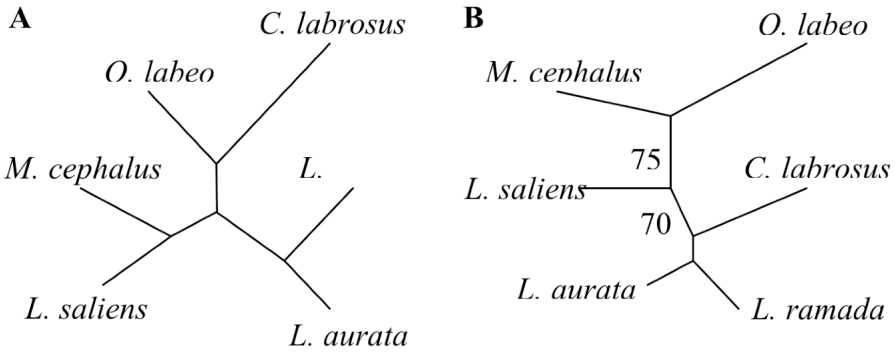

Fig. (2). A and B, reanalysis of the allozyme datasets of respectively Turan *et al.* (2005) [11] and Rossi *et al.* (2004) [9], for six species of Mediterranean mullets (*M. cephalus, Liza ramada, L. aurata, L. saliens, Chelon labrosus, Oedalechilus labeo*) in order to construct an UPGMA dendrogram applied to Cavalli-Sforza's chord measure; robustness of nodes in the dendrogram was analyzed by bootstrapping locus over samples (1000 random permutations of the original data). All calculations were performed using the Phylip software package. In these dendrograms, bootstrap values are given for each node only if they exceed 60.

be the most divergent species in several analyses using various markers: *cytb* and *12S rDNA* [24], *cytb* and *16S rDNA* [13], both allozymes and *16S rDNA* [9], but not in our *16S rDNA* analysis using ML method. On the basis of morphological data, it has been suggested a possible paraphyly of *Liza* with respect to *Chelon* [6, 27]. On the contrary, studies based on allozyme data [10, 28] revealed an appreciable genetic differentiation between *Liza* and *Chelon*, verifying, in spite the low number of studied species, the traditional view for the monophyly of genus *Liza*. In addition, in the latest systematic review, *Chelon* has considered as a valid genus, "doubtlessly" derived from *Liza* [1]. All these data contribute to the long systematic debate carried out on these two genera. The difficulty in discriminating *Chelon, Oedalechilus* and *Liza* was already revealed by investigations based on chromosome analysis ([14, 29] and references therein), the three species of *Liza, C. labrosus* and *O. labeo* showing one subtelocentric chromosome pair (chromosome pair 24 in both *Liza* and *Chelon* and chromosome pair 9 in *O. labeo*) among the remaining acrocentric chromosomes [26]. However, the karyotype of *L. saliens* differed from those of both the two other *Liza* species and *C. labrosus* by the locations of additional *5S rDNA* genes (in a location close to the one shown by major ribosomal genes in *M. cephalus*, i.e. the subtelomeric region of chromosomes 1) and variable *18S rDNA* genes. Moreover, *O. labeo* karyotype differed from the four other species by the locations of both constant and variable *18S rDNA* genes [30]. A karyotypic analysis suggested that *L. saliens* (named subgenus *Protomugil* Popov, 1930) can be considered as intermediate between the karyotype of *M. cephalus* which could be the more primitive and those of the other *Liza* (subgenus *Liza*) species and of the genus *Chelon* [26]. According to these authors, *Oedalechilus* might be a derived branch of *Protomugil* which could explain the position of this species in the phylogenetic trees, an hypothesis strengthened by the long branches both in *16S rDNA* and *cytb* analyses. Interestingly, in a recent study using both allozymes and *16S rDNA*, *L. saliens* was the sister group of both the other *Liza* species and of *Chelon* [9], but this was not statistically supported, whereas in our *16S rDNA* analyses using ML method, *L. saliens* could be the sister group of the subgenera *Liza, Chelon* and *Oedalechilus*.

Phylogenetical Analyses of the Genus *Mugil*

Our molecular phylogeny of the genus *Mugil* revealed several interesting features. Although, Mugilidae constitute a monophyletic group highly supported statistically in ML and NJ analyses, but not in MP, three groups can be found: one group contains some of the sequences of *M. curema* and of *M. hospes*, a second one sequences of *M. curema* and of *M. incilis*; and a last one with *M. cephalus, C. crenilabis, M. liza* and *M. platanus* sequences.

The grouping of in one hand *M. liza* and *M. platanus*, and in another hand *M. curema* and *M. incilis* has been already made using morphological analyses [1] but also by traditional Brazilian populations. Indeed in Brazil, mullets are commonly called *tainhas* and *paratis* or *curimãs*; these two last names are synonyms and their use depend on dialect areas [31]. In Brazil, the *Mugil* genus consists of seven species and, according to Brazilian inhabitants, two of which are *tainhas* - *M. liza* and *M. platanus* - and five are *paratis/curimãs* - *M. curema, M. incilis, M. trichodon, M. gaimardianus* and *M. curvidens* [18]. Nor, it is well known that folk taxonomy can perceive biotic units similarly to academic scientists [32]; for example, preliterate peoples of New Guinea had vernacular names for 136 out of the 137 native birds recognized as separate species by academically trained Western zoologists [33].

In both *cytb* and *16S rDNA* analyses, *M. liza* and *M. platanus* constituted a clade always supported by high bootstrap values ([13] and present study). These two species also share numerous morphological characteristics as ultrastructure of the gills ([34, 35] and references therein). According to a recent study, *M. liza* and *M. platanus* should be treated as a single species or even populations of *M. cephalus*, due to their great similarity in their morphological features and to the fact that they bear the same diploid chromosome number (n=48) [13].

In our analyses, *M. cephalus, C. crenilabis, M. liza* and *M. platanus* sequences clustered together. Moreover, in *M. cephalus*, at least three mtDNA haplotypes have been found in *cytb* analyses, and one of them has been found for *M. cephalus* sequences (haplotype I) issued from fish caught in the three oceans and the Mediterranean Sea. The *16S rDNA*

analyses also suggest strong differences between sequences of this species. Within the Mugilids, *M. cephalus* is the only cosmopolitan species [17]; the variations within the haplotype I could suggest that this species is structured in subpopulations according to a geographic distribution pattern. The clade *M. liza/M. platanus* groups with *M. cephalus* (haplotype II) sequences from fish caught in the Pacific Ocean. Interestingly, the terminal position of NORs on the largest pair of acrocentric chromosomes has been suggested to be the plesiomorphic condition in the genus *Mugil*, a feature shared by *M. cephalus* [36], *M. platanus* [37] and *M. liza* [9, 38], but not in the karyotype of other *Mugil* species analysed to date. Another *M. cephalus* sequence (haplotype III) group with the ortholog of *C. crenilabis*. The maximum levels of divergence estimated among the *M. cephalus* sequences using *cytb* and *16S rDNA* (if exclude the sequence which groups with *M. incilis*) datasets are respectively 4.2% to 3.8%. These levels of nucleotide divergence are in congruence with that proposed by authors of two previous studies among congeneric species [39, 40]. However, these results show that the various haplotypes of *M. cephalus* sequences do not correspond to geographical haplotypes, but more likely to the incorporation into *M. cephalus* of genome(s) or part of genome(s) (in this case mtDNA genome) of another species (i.e., introgressive hybridization [33]). Indeed, the same haplotype is shared by fish caught in various oceans or seas (suggesting high gene flow capacities of *M. cephalus*) and each haplotype, but one, is similar to orthologs of other species. In addition, more commonly, genetic exchanges between hybridizing taxa are not reciprocal but directional, in this case, from another species to *M. cephalus*. This, in addition to the fact that, if exclude the sequence which groups with *M. incilis,* none *M. cephalus* sequence is identical to those of other species putatively implied in introgression events could suggest the following evolutionary history life: introgression(s) followed by long periods with great restriction in gene flow and secondary contacts. However, to our knowledge no other studies have shown introgressions in Mugilidae.

Although, our dataset is very partial, it confirms previous morphological analyses showing that *M. curema, M. hospes* and *M. incilis* belong to the same clade (excluding all the other species of our dataset, but one surprising *M. cephalus 16S rDNA* sequence) [1]. Moreover, morphological analyses also suggest that the two first species are closer relatives than the former [1, 41]. However, for both *cytb* and *16S rDNA* analyses, some of the *M. curema* sequences grouped with those of *M. incilis* and the others with those of *M. hospes*. Indeed, two haplotypes of both *cytb* and *16S rDNA* have been found in *M. curema* species [13]. Their distribution is interesting: in the North and Northeast Brazil the two haplotypes are present in sympatry, in Venezuela only haplotype I has been found, whereas, the haplotype II occurs alone from both the Atlantic (South Brazil, Argentina, USA) and the Pacific (Mexico) Coasts. Moreover, these two different haplotypes have been never obtained from an alone individual. In *M. curema*, these two haplotypes for both *12S rDNA* and *cytochrome oxydase I* sequences have also been shown, as the great molecular proximity of the American and Argentinean individuals, indicating thus that this species does not follow the geographical pattern they expected [23].

Similarly to the two mtDNA haplotypes, two different karyotypes have been found in populations of *M. curema*. Karyotype I (2n = 28) has been found in fish off Louisiana [42] and South of Brazil [14, 29]; while, karyotype II (2n = 24) off Venezuela and North of Brazil [13, 15, 29, 38, 43]. Moreover, the karyotype of South Brazilian *M. curema* differed from those of Venezuela, besides the diploid number, by the constitutive heterochromatin distribution and NORs location [29]. In addition, morphological comparison revealed differences (both in the number of scales in the lateral line and of the number of pectoral fin rays) in specimens from South of Brazil versus those from Venezuela. Based on geographical considerations and on the coastal habits of the species, it is noteworthy that the farthest away populations (Louisiana and South of Brazil) share a similar chromosome complement, which is different from the one found in the population collected in an area (Venezuela and North of Brazil) which is geographically intermediate. As suggested by these authors, if these two morphs definitely belong to the same species, it would be possible to observe individuals in the wild with an intermediate karyotype between karyotype I and karyotype II (i.e. 2n = 26); however, in spite of the analysis of 150 samples, individuals with this intermediate karyotype did not find [29]. The cytogenetic and morphologic differences added to the leak of putative hybrid, lead these authors to suggest that both two karyotypes are not merely related to geographic polytipic variations but could correspond to different cryptic species. Moreover, the karyotype I of *M. curema* is similar to those of *M. incilis* species from Brazil [44, 45], further studies are needed in order to know that of *M. hospes*. With the exception of the *M. curema* (2n = 24 or 28) and *M. incilis* (2n = 28), the other Mugilid species studied to date have a diploid number of 48 and the primitive teleost karyotype is thought to have consisted of 46-48 chromosomes [42]. Thus, the karyotypes of *M. curema* and *M. incilis* suggest a rare occurrence of extensive chromosomal rearrangements among the Mugilids and their derivated karyotypes, are probably originated by centric fusions and pericentric inversions [14].

Supplementary Data on *Mugil cephalus*

In spite a relatively low number of sequences, our molecular analyses could suggest that three or more mtDNA haplotypes are present in the *M. cephalus* sequence dataset and that the geographical distribution of these haplotypes does not generate a phylogeographical signal. More data concerning the life history of this species added to analyses of other loci (and particularly nuclear loci) could help to resolve the pattern and the process of the geographical distribution of the haplotypes. The striped mullet, *M. cephalus* is globally distributed between the latitudes of 51°N and 42°S, although less abundant in tropics [3]. Because of this distribution and the dependency of *M. cephalus* on coastal waters during various phases of its life cycle, questions have been raised regarding levels of genetic divergence and gene flow among transoceanic populations. The notion of cosmopolitan species has been questioned. In general, movement and supposed gene flow for *M. cephalus* appear to be relatively high along contiguous coastlines [46]. To the exception of one mtDNA study [47], other previous mtDNA [17] and allozyme [48, 49] analyses of global populations of *M. cephalus* indicated a pronounced population subdivision for

the species. Indeed, populations of striped mullets from the Mediterranean Sea and the three oceans showed high levels of genetic divergence and extremely reduced inter-ocean gene flow suggesting that some of the *M. cephalus* populations are at a stage of incipient speciation [48].

Both mtDNA and allozymic genetic analyses of global populations of *M. cephalus* ([8, 17, 48, 49] and present study) are intriguing for two reasons. Firstly, the genetic heterogeneity found in *M. cephalus* is in conflict with the pattern of general morphological uniformity highlighted along the distribution range of the species [50]. Genetic differentiation among *M. cephalus* samples is conspicuously higher than that usually found in marine fish with transoceanic distributions which may exhibit little or no genetic divergence among geographically distant populations [22, 51]. This lack of major divergence presumably reflects existing or historical levels of gene flow which is reduced in neretic species. Secondly, based on geographical considerations and on the coastal habits of the species, it is noteworthy that very distant populations share a closer relationship than individuals collected in a geographically adjacent regions. Under the neutral theory, the same factors that promote intraspecific polymorphism (neutral mutation and genetic drift) also result in differentiation between species. As that was already suggested [48], it is therefore plausible to imagine that at a certain moment in the past *M. cephalus* included several isolates, with subdivision favouring the fixation of neutral mutations. The current patterns and degrees of mtDNA variation may then be due, at least in part, to random fixation of alleles in a phase when effective population size was small. Moreover, an accelerated rate of mitochondrial evolution in the genus *Mugil* has been suggested [47]. However, the considerable genetic differentiation among *M. cephalus* populations, in conjunction with the extremely reduced, or nonexistent, current gene flow, and the presence of several haplotypes led to the hypothesis that the haplotype(s) originated through several introgression events from at least *M. liza/M. platanus* and *C. crenilabis*. Moreover, if excluded taxonomic misinterpretation is excluded, possible introgression of mtDNA haplotype of *M. incilis* could be also possible, although, the great karyotype differences between the two concerned species [17, 36, 44, 45] probably would render hybridization impossible, and the sympatry of these two species is always discussed.

Our results could suggest introgressions of mtDNA genes in only one direction (from more specialized species to a non specialized species), i.e. from probably more adapted Mugilids species (*M. liza/M. platanus, C. crenimugil* and putatively *M. incilis*) into the genome of a species with a worldwide distribution (*M. cephalus*), which may provide physiological advantages to specific ecosystems including unstable environments, and a high tolerance to changes in salinity could be advantageous. Moreover, the fact that the mtDNA gene flow seem to be unidirectional (from one of its sympatric species to *M. cephalus*), could be a strong argument in favour of this hypothesis. The adaptive role of genetic variability has been hypothesized in many teleosts including Mugilids. In a previous study, authors have suggested that natural selection may play a role in shaping allelic frequency changes during the migratory journey of the similar species *M. cephalus* [52]. Moreover, genetic differences between inland migrating *Liza* populations and the ones returning to the sea were also demonstrated [53].

CONCLUSION

The phylogeny of grey mullets has been challenged, at various systematic levels, using many different morphological characters, but the results were always conflicting and did not provide any conclusive answer. Moreover, allozymic, mtDNA and karyotypic studies on several mullet species did not result in a clear phylogenetic figure. As already suggested by previous studies, our analyses showed that the genera *Chelon* and *Oedalechilus* clustered together within the genus *Liza*. However, the separation of *Liza, Chelon* and *Oedalechilus* might be unnatural, and the monophyletic origin of the genus *Liza* is questionable. Indeed, the genus *Liza* includes more than 20 species, most of which are not yet included in molecular phylogenetic reconstructions; similarly, only one species for both genera *Chelon* and *Oedalechilus* has been analysed. A more extensive genetic survey of representatives of these three genera, including also the non-Mediterranean species of *Liza*, as well as the remaining species of *Chelon* and *Oedalechilus*, is needed to contribute to the systematic debate on whether the two genera on the whole should be synonymised or whether they represent distinct clades. Moreover, to state definitively on the monophyly of the genus *Liza*, needs examination of multiple genetic systems.

More surprisingly, our results do not provide good support for the hypothesis that *Mugil* is a monophyletic group as it includes a *Crenimugil* species; however, as only one mitochondrial genome of this genus has been sequenced to date, other sequences data are necessary for further resolution of intrageneric and intergeneric relationships. In the same manner, further studies are needed to look for the existence of two sibling species in the *M. curema* complex, unless that could be the result of ancient vicariance.

In the future, the analyses will carry on several populations of *M. cephalus* and on the numerous Mugilids sympatric species; this suppose a good morphological identification of these species in order to avoid possible taxonomic misidentifications. In addition, the combination of molecular assays of both nuclear (including microsatellites) and mtDNA will provide the best approach to understand the evolutionary dynamics of these interacting populations. Mating compatibility studies are also needed between *M. cephalus* and the sympatric congeneric species and *Crenimugil* spp.

Considering the many doubts still existing on the taxonomy and ecology of mullet species, we believe that additional data, including increased geographic and species sampling and nuclear molecular investigations, are necessary. Such studies, along with similar investigations in other species groups, could significantly contribute to our understanding of the evolutionary importance of introgressive hybridization in the oceans.

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