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RESEARCH PAPER

Molecular phylogeny of Mugilidae fishes revised

Sandra Heras · María Inés Roldán · Mariano González Castro

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Abstract Systematics derived from morphological characters often does not correspond with the evolutionary processes underlying the divergence within a group of organisms. In the family Mugilidae (Teleostei) morphological similarities have resulted in inconsistencies between taxonomy and phylogeny among its species, and particularly for the genera *Mugil, Liza* and *Chelon* where both intrageneric and intergeneric phylogenetic clarifications are needed. To address these issues, the direct sequencing of the mitochondrial region that encodes Phenylalanine (69 bp), 12S rRNA (842 bp), cytochrome *c* oxidase subunit I (651 bp) and cytochrome *b* (702 bp) was carried out. The data reveal that *Mugil platanus* and *Mugil liza* represent a continuum of a single species, closely related to but distinct from *Mugil cephalus* which itself appears to comprise a grouping of multiple and closely related species. This species complex was genetically distinct from *Mugil curema*, which, based on three clearly diverged species identified in this study along the Atlantic coast of the Americas, requires extensive taxonomic revision throughout its world-wide distribution. Unlike the monophyly supported within *Mugil*, relationships within *Liza* are paraphyletic, and a taxonomic revision of the genera *Liza, Chelon* and *Oedalechilus* is needed.

Keywords Phe · 12S rRNA · Cytochrome *b* · COI · Mugilidae phylogeny · Taxonomy

S. Heras
Laboratori d'Ictiologia Genètica, Facultat de Ciències, Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

M. I. Roldán (✉)
Laboratori d'Ictiologia Genètica, Facultat de Ciències, Universitat de Girona, Campus Montilivi, 17071 Girona, Spain
e-mail: marina.roldan@udg.edu

M. G. Castro
Laboratorio de Ictiología, Departamento de Ciencias Marinas, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina

Introduction

Taxonomic ambience

Since the eighteenth century, systematics simply refers to the process of classifying organisms within taxonomic categories hierarchically organized within the widely used Linnaean system. Historically, these similarities and differences among groups were mainly based on morphological characters. Presently, this classification and organization attempts to infer the evolutionary history of the groups within the hierarchy. However, problems can arise when evolutionary processes are estimated through morphological data within long-accepted taxonomic groups (Hey 2001). In recent decades, phylogenetic

trees to reconstruct evolutionary history and relationships based on molecular genetic data have provided independent comparisons to augment those relying on morphological information. Using appropriate markers (Avise 1994; Maddison 1996), such molecular-based information has permitted previously unattainable insights because a species' phylogenetic history is directly impregnated within the DNA molecule. Presently, these capabilities are focused through an international initiative to construct the evolutionary tree of all living organisms (Tree of life project; http://tolweb.org/tree/). However, despite these and similar efforts, many gaps in understanding of phylogenies still exist in teleost fishes.

Background on Mugilidae family

Mugilidae, with 17 genera, is the single family of order Mugiliformes (series Mugilomorpha) (Nelson 2006). Mugilid species, commonly known as mullets, are pelagic-coastal fishes with worldwide distribution (Thomson 1997) and are very important as food fish (FAO 2005) where entire individuals are marketed fresh, dried, salted and frozen. In addition, mullet roe is sold fresh, smoked, and after a press and dry process, represents a very important economic resource in some Mediterranean countries. Mullets also are used in Chinese medicine and are widely cultivated in freshwater ponds in south-eastern Asia (FAO 2005). Mugilid species have a highly conservative morphology, and classification among them using classical morphometry and morphology has proven to be complex and difficult (Menezes 1983; Gilbert 1993; Thomson 1997).

Mugil platanus versus synonymy of *Mugil cephalus*

The representative species of Mugilidae family, *Mugil cephalus* Linnaeus, 1758, commonly known as gray or striped mullet, nominally has a worldwide circumtropical distribution (51°N–42°S) (Harrison and Howes 1991), and according to FAO (2004), this species is included in the region of Rio de la Plata (Argentina). However, Gilbert (1993) and Thomson (1997) have disputed this breadth of distribution while Menezes (1983) and Cousseau et al. (2005) reported *Mugil platanus* Günther, 1880 to inhabit coastal waters from southern Brazil southward towards Viedma (Argentina). Two alternative possibilities are (1) *M. platanus* is synonymous with *M. cephalus* (Thomson 1997), and (2) *M. platanus* is a distinct taxonomic entity, with a distribution from Rio de Janeiro (Brazil) towards Viedma (Argentina) (Menezes 1983; Cousseau et al. 2005).

Uncertainties with *Mugil liza*

The taxonomy of *Mugil liza* Valenciennes, 1836, *M. platanus* and *M. cephalus* is further confounded in Western Atlantic. *M. liza* has been reported as a valid species by Menezes (1983) and Thomson (1997) with a distribution from southern Florida to Rio de Janeiro. A substitution of *M. liza* for *M. platanus* with more southerly latitudes represents a parapatric distribution involving separate but adjacent habitats (Menezes 1983). The western Atlantic distribution of *M. cephalus* is restricted from New England to Campeche Gulf (Mexico) (Gilbert 1993); however, *M. liza* and *M. cephalus* are particularly difficult to distinguish morphologically (Rivas 1980).

Mugil curema overview

Mugil curema Valenciennes, 1836, commonly named white mullet, is distributed in the western Atlantic from Cape Cod to southern Brazil, in the eastern Pacific from California to northern Chile and in the eastern Atlantic from Gambia to the Congo (Menezes 1983; Thomson 1997). Recently, its presence has also been detected in Argentinean waters (37°46′S, 57°27′W) (González Castro et al. 2006; Heras et al. 2006), at more southern latitudes than previously described.

Recent studies (Nirchio et al. 2005, 2007) have identified three *M. curema*-like subgroups based on karyotype and allozyme differences. The chromosome number of *M. curema* from Brazil ($2n = 28$; cytotype 1, Nirchio et al. 2005) is also considered the true *M. curema* karyotype reported by LeGrande and Fitzsimons (1976) in a study in the Gulf of Mexico. A second karyotype ($2n = 24$; cytoptype 2) was found in Venezuela (Nirchio and Cequea 1998; Nirchio et al. 2003). A third karyotype ($2n = 48$; cytotype 3) also found in Venezuela and the Pacific coast of Panama (Nirchio et al. 2003, 2007) has been described as *M. rubrioculus* by Harrison et al. (2007)

who made a morphological diagnosis and supplied a description of this new species. Hence, three genetically different species or types of *M. curema* may coexist in American waters.

Relationships among *Chelon, Liza* and *Mugil*

The Mugilid genera *Mugil, Chelon* and *Liza* represent a situation where modern molecular phylogenetic studies may serve to corroborate or to modify the described classification of the target groups (Caldara et al. 1996; Rossi et al. 1998b, 2004; Papasotiropoulos et al. 2001, 2002, 2007; Turan et al. 2005; Fraga et al. 2007; Semina et al. 2007). Particular attention is warranted on the paraphyletic basis recently suggested for *Liza*.

Summarizing, the disagreements between taxonomy and phylogeny for several members of Mugilidae family provide opportunities for useful scientific investigations that may be augmented with genetic data through modern molecular techniques. Accordingly, the present work probes the genetic distances between several Mugilidae taxa. Four mitochondrial genes, are used to clarify phylogenetical inferences and to resolve existing discrepancies permitting revision of current taxonomy.

Material and methods

Sampling

A total of 137 individuals belonging to genera *Mugil, Liza* and *Chelon* were collected from Mediterranean coasts of Spain, Atlantic waters of Morocco, Atlantic coasts of USA, Brazil, Uruguay and Argentina, and in Caribbean waters (Table 1). Morphological species identification was based on Menezes (1983), Thomson (1997) and Harrison (2002).

DNA extraction, amplification and sequencing

White muscle tissue was excised from frozen-upon-capture fishes and preserved in 95% alcohol. DNA isolation and polymerase chain reaction of phenylalanine transfer RNA (Phe), 12S rRNA, cytochrome *b* (cytb) and cytochrome *c* oxidase subunit I (COI) followed the procedures outlined in Heras et al. (2006). The primers used were DLHR: 5′-CAT CTG GTT CTT ACT TCA GG-3′ reverse of DLH (Tiedemann et al. 1996) and H1358-12S: 5′-CGA CGG CGG TAT ATA GGC-3′ (Miya and Nishida 2000) for Phe and first domain of 12S rRNA amplifications (Phe + 12S rRNA-I); L1091: 5′-CAA ACT GGG ATT AGA TAC CCC ACT AT-3′ and H1478: 5′-TGA CTG CAG AGG GTG ACG GGC GGT GTG T-3′ (Kocher et al. 1989) for second domain of 12S rRNA (12S rRNA-II); L14850-CYB: 5′-GCC TGA TGA AAC TTT GGC TC-3′ and H15560-CYB: 5′-TAG GCA AAT AGG AAG TAT CA-3′ (Miya and Nishida 1999) for cytb and FishF2: 5′-TCG ACT AAT CAT AAA GAT ATC GGC AC-3′ and FishR1: 5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′ (Ward et al. 2005) for COI. DNA sequencing reactions were performed with BigDye Terminator v. 1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. Primers used for sequencing were the same as for PCR except for first domain of 12S rRNA where the primer used was 12SAR-H: 5′-ATA GTG GGG TAT CTA ATC CCA GTT-3′ (Palumbi et al. 1991). Finally, labeled sequences were loaded onto an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) at the Girona laboratory.

Sequence data analysis

The nucleotide sequences obtained were aligned and edited using SeqScape v. 2.5 (Applied Biosystems). Final alignments and edition were optimized with BioEdit v. 7.0.4.1 (Hall 1999) using a GenBank *Mugil cephalus* Phe, 12S rRNA, cytb and COI sequences from Japan as reference (accession no. AP002930). Polymorphic sites and Tamura and Nei (1993) mean distance values between groups were calculated using MEGA v. 3.1 (Kumar et al. 2004). Nucleotide saturation was tested with DAMBE v. 4.5.2 (Xia and Xie 2001). Evidence for lack of substitutional saturation was tested by plotting transitions (s) and transversions (v) accumulation for each pair of haplotypes against Tamura–Nei distances (1993). Substitution is expected to increase with haplotype divergence. On the other hand, saturation is observed if accumulated changes reach a plateau in the plot. The partition homogeneity test ($\alpha = 0.05$) was conducted with PAUP* v. 4.0b10 (Swofford 2002) to combine or not the all genedatasets based on their evolutionary signal. Phylogenetic relationships were

Table 1 Specimens and abbreviation code, location of collection sites, sample size, haplotypes (*in bold*) and frequencies (*in parenthesis*) for each gene

Species	Code	Locality	Number	Haplotypes			
				Phe + 12S rRNA-I	12S rRNA-II	COI	Cytb
M. cephalus	Mcep	Palamós, Spain	3	**1**(3)	**1**(1), **2**(2)	**1**(2), **2**(1)	**1**(1), **2**(2)
		Ter Vell lagoon, Spain	7	**1**(6), **2**(1)	**2**(7)	**1**(6), **3**(1)	**1**(1), **2**(3), **3**(3)
		Galveston Bay, USA	5	**3**(5)	**3**(5)	**4**(2), **5**(1), **6**(1), **7**(1)	**4**(5)
M. platanus	Mpl	Río Grande, Brazil	2	**4**(2)	**4**(1), **5**(1)	**8**(2)	**5**(2)
		Montevideo, Uruguay	6	**4**(6)	**5**(1), **6**(4), **7**(1)	**8**(5), **9**(1)	**6**(3), **7**(1), **8**(1), **9**(1)
		Samborombón Bay, Argentina	11	**4**(10), **5**(1)	**5**(6), **6**(3), **7**(1), **8**(1)	**8**(10), **10**(1)	**6**(9), **10**(1), **11**(1)
		Mar Chiquita lagoon, Argentina	18	**4**(18)	**5**(7), **6**(8), **7**(1), **9**(1), **10**(1)	**8**(17), **11**(1)	**6**(16), **12**(1), **13**(1)
		Viedma, Argentina	6	**4**(6)	**5**(1), **6**(3), **11**(1), **12**(1)	**8**(6)	**6**(4), **14**(1), **15**(1)
		San Lorenzo lagoon, Argentina	16	**4**(15), **6**(1)	**5**(2), **6**(10), **13**(1), **14**(1), **15**(1), **16**(1)	**8**(13), **12**(1), **13**(1), **14**(1)	**6**(14), **16**(1), **17**(1)
M. liza	Mli	Tunas de Zaza, Cuba	6	**4**(6)	**7**(3), **17**(3)	**8**(5), **15**(1)	**10**(2), **13**(1), **18**(1), **19**(1), **20**(1)
M. curema	Mcur	Galveston Bay, USA	4	**7**(3), **8**(1)	**18**(3), **19**(1)	**16**(3), **17**(1)	**21**(3), **22**(1)
		Mar Chiquita lagoon, Argentina	18	**8**(18)	**19**(4), **20**(2), **21**(10), **22**(2)	**17**(4), **18**(3), **19**(3), **20**(7), **21**(1)	**22**(5), **23**(8), **24**(1), **25**(1), **26**(2), **27**(1)
		Mar del Plata, Argentina	14	**8**(14)	**21**(13), **23**(1)	**20**(13), **22**(1)	**22**(11), **28**(1), **29**(1), **30**(1)
		San Salvador de Bahia, Brazil	1	**9**(1)	**24**(1)	**23**(1)	**31**(1)
L. aurata	Lau	Palamós, Spain	3	**10**(2), **11**(1)	**25**(1), **26**(2)	**24**(1), **25**(1), **26**(1)	**32**(1), **33**(2)
L. ramada	Lra	Ter Vell lagoon, Spain	3	**12**(3)	**27**(3)	**27**(3)	**34**(1), **35**(1), **36**(1)
L. saliens	Lsal	Moulay Bousselham lagoon, Morocco	7	**13**(6), **14**(1)	**28**(7)	**28**(4), **29**(2)	**37**(1), **38**(1), **39**(1), **40**(1), **41**(3)
C. labrosus	Chl	Ter Vell lagoon, Spain	7	**15**(7)	**29**(7)	**30**(5), **31**(1), **32**(1)	**42**(5), **43**(2)

inferred by different methods of analyses. Maximum likelihood (ML) and maximum parsimony (MP) analyses were performed using PAUP* with an heuristic search and TBR branch-swapping algorithm with ten random sequence addition. Modeltest v. 3.7 (Posada and Crandall 1998) was used to estimate the best-fit model of DNA substitution in ML analyses. Neighbor-joining analyses (NJ) were based on Tamura–Nei model (1993) employing MEGA and on a ML distance matrix employing PAUP*. Robustness of trees was tested using bootstrap analysis (Felsenstein 1985) with 1,000 replicates. Bayesian inference was performed by MrBayes v. 3.1.2 program (Ronquist and Huelsenbeck 2003) using the model of evolution previously selected by MrModeltest v. 2.2 (Nylander 2004). Four Metropolis-coupled Markov Chain Monte Carlo chains with 1×10^6 generations length were sampled every 100th and 2,500 trees were discarded as burn-in. Then, a consensus phylogram of 7,500 recorded trees and posterior probability was calculated. Unclear relationships inside Acanthopterygii family regarding to Mugilidae family were reported before (Miya et al. 2001) but available information indicate that Paracanthopterygii are their common ancestor, consequently, *Gadus morhua* (Paracanthopterygii-GenBank accession no. X99772) was used as outgroup species for all analyses.

Results

Sequence features

Consensus sequence alignments of Phe + 12S rRNA-I (499 bp), 12S rRNA-II (412 bp), COI (651 bp) and cytb (702 bp) were obtained. In addition, 15 (GenBank accession nos. EU715412-EU715426), 29 (GenBank accession nos. EU715427-EU715450, DQ225769-DQ225773), 32 (GenBank accession nos. EU715451-EU715474, DQ441603-DQ441610) and 43 (GenBank accession nos. EU7 15475-EU715510, EU189962, DQ225774-DQ225 779) haplotypes were stated, respectively (Table 1). No association between geographic distribution and haplotypes was evident in *M. platanus* and *M. platanus* and *M. liza* share haplotypes for all molecular markers studied (Table 1). Because of this absence of distinction, these taxa are jointly considered as Mpl/Mli in all subsequent analyses. On the other hand, *M. cephalus* from Mediterranean and Atlantic specimens clearly had a differential haplotype distribution. Although *M. curema* sampled in Argentina shared haplotypes with one individual from the USA, three USA individuals had a unique haplotype, as did a single individual sampled from Brazil. Consequently, three haplotype groups are considered for *M. curema* in subsequent analyses, including (1) the Brazilian sample McurBra, (2) three individuals sampled in Galveston Bay McurUSA, and (3) the remaining 32 individuals from Argentina and one from the USA sharing haplotypes McurArg.

Species divergence

No saturation was detected for each dataset (data not shown). Furthermore, a genetic pairwise distance matrix was generated from Tamura–Nei mean values between created groups (Table 2). Mean genetic distances between *M. platanus* and *M. liza* unique haplotypes for 12S rRNA-II, COI and cytb were 0.0070 ± 0.0034, 0.0033 ± 0.0016 and 0.0050 ± 0.0018, respectively. The data clearly indicate that mean distances detected among *M. cephalus* samples overlapped with values shown between Mpl/Mli and *M. cephalus* samples consistent with close relationships. It is noteworthy that the level of genetic distances presented between McurBra, McurArg and McurUSA are very similar to those of interspecific comparisons between *M. curema*-*M. cephalus* and *M. curema*-Mpl/Mli. Much higher distances were displayed between *Mugil* sp. and *Liza* sp. or *C. labrosus* than between *Liza* sp. and *C. labrous*. In addition, distances among *Liza* sp. haplotypes were very similar to mean distances between *C. labrosus* and *Liza* sp. indicating close genetic relationships among species of *Chelon* and *Liza*.

Phylogenetic relationships

The partition homogeneity test did not reveal incongruence between molecular markers ($P = 0.3810$) allowing their combination for succeeding analyses (Table 3). The different phylogenetic analysis generated similar topologies (Figs. 1, 2). All the trees strongly separated haplotypes into two major clusters corresponding to *Mugil* (Figs. 1a, 2a) and to *Liza* and *Chelon* (Figs. 1b, 2b). Two distinct groupings within *Mugil* represented (1) four phylogroups within

Table 2 Pairwise Tamura–Nei mean genetic distances of Mugilid species and genera sampled in this study, and standard errors for four mitochondrial genes

	Phe + 12S rRNA-I	12S rRNA-II	COI	Cytb
Mcep vs Mpl/Mli				
McepMed-McepUSA	0.0174 ± 0.0058	0.0163 ± 0.0060	0.0414 ± 0.0075	0.0586 ± 0.0106
McepMed-McepJapan	0.0216 ± 0.0061	0.0201 ± 0.0070	0.0269 ± 0.0064	0.0574 ± 0.0103
McepUSA-McepJapan	0.0397 ± 0.0089	0.0201 ± 0.0069	0.0420 ± 0.0079	0.0576 ± 0.0105
Mpl/Mli-McepMed	0.0146 ± 0.0049	0.0168 ± 0.0057	0.0229 ± 0.0051	0.0479 ± 0.0092
Mpl/Mli-McepUSA	0.0303 ± 0.0075	0.0253 ± 0.0072	0.0325 ± 0.0065	0.0498 ± 0.0091
Mpl/Mli-McepJapan	0.0369 ± 0.0083	0.0269 ± 0.0078	0.0248 ± 0.0059	0.0406 ± 0.0081
Mcep-Mpl/Mli	0.0241 ± 0.0054	0.0215 ± 0.0058	0.0279 ± 0.0045	0.0468 ± 0.0073
Mcur vs Mcep				
McurUSA-McurArg	0.0553 ± 0.0105	0.0642 ± 0.0127	0.1385 ± 0.0155	0.1485 ± 0.0211
McurUSA-McurBra	0.1103 ± 0.0155	0.1159 ± 0.0180	0.1857 ± 0.0183	0.1693 ± 0.0232
McurArg-McurBra	0.0944 ± 0.0141	0.1244 ± 0.0183	0.1829 ± 0.0183	0.1720 ± 0.0228
McurUSA-McepMed	0.1602 ± 0.0201	0.1151 ± 0.0171	0.1797 ± 0.0192	0.1982 ± 0.0245
McurUSA-McepUSA	0.1694 ± 0.0206	0.1135 ± 0.0173	0.1800 ± 0.0190	0.2142 ± 0.0273
McurUSA-McepJapan	0.1732 ± 0.0217	0.1133 ± 0.0171	0.1866 ± 0.0194	0.2092 ± 0.0259
McurUSA-Mpl/Mli	0.1586 ± 0.0198	0.1211 ± 0.0178	0.1747 ± 0.0186	0.2214 ± 0.0282
McurArg-McepMed	0.1445 ± 0.0188	0.1513 ± 0.0198	0.1812 ± 0.0185	0.1904 ± 0.0247
McurArg-McepUSA	0.1589 ± 0.0200	0.1440 ± 0.0192	0.1839 ± 0.0194	0.2062 ± 0.0270
McurArg-McepJapan	0.1571 ± 0.0204	0.1402 ± 0.0189	0.1783 ± 0.0192	0.2042 ± 0.0265
McurArg-Mpl/Mli	0.1413 ± 0.0183	0.1513 ± 0.0200	0.1755 ± 0.0184	0.1946 ± 0.0257
McurBra-McepMed	0.1586 ± 0.0195	0.1284 ± 0.0191	0.1882 ± 0.0186	0.2014 ± 0.0241
McurBra-McepUSA	0.1705 ± 0.0206	0.1160 ± 0.0177	0.1825 ± 0.0182	0.1851 ± 0.0219
McurBra-McepJapan	0.1716 ± 0.0212	0.1077 ± 0.0175	0.1921 ± 0.0195	0.2151 ± 0.0270
McurBra-Mpl/Mli	0.1554 ± 0.0190	0.1193 ± 0.0175	0.1905 ± 0.0183	0.2091 ± 0.0256
McurUSA-Mcep	0.1649 ± 0.0198	0.1142 ± 0.0168	0.1807 ± 0.0189	0.2036 ± 0.0248
McurArg-Mcep	0.1505 ± 0.0187	0.1467 ± 0.0195	0.1822 ± 0.0188	0.1963 ± 0.0252
McurBra-Mcep	0.1648 ± 0.0197	0.1201 ± 0.0179	0.1859 ± 0.0185	0.2009 ± 0.0235
Liza vs *Chelon*				
Lau-Lra	0.0218 ± 0.0064	0.0292 ± 0.0083	0.0850 ± 0.0115	0.1126 ± 0.0170
Lau-Lsal	0.0228 ± 0.0064	0.0358 ± 0.0097	0.0688 ± 0.0102	0.1004 ± 0.0158
Lra-Lsal	0.0350 ± 0.0085	0.0385 ± 0.0096	0.0949 ± 0.0130	0.1148 ± 0.0165
Lau-Chl	0.0155 ± 0.0056	0.0239 ± 0.0073	0.0649 ± 0.0098	0.0977 ± 0.0157
Lra-Chl	0.0207 ± 0.0065	0.0357 ± 0.0092	0.0866 ± 0.0117	0.1171 ± 0.0177
Lsal-Chl	0.0261 ± 0.0073	0.0228 ± 0.0072	0.0654 ± 0.0099	0.1131 ± 0.0172
Mugil vs *Liza* vs *Chelon*				
Mugil-Liza	0.1794 ± 0.0183	0.1400 ± 0.0173	0.2220 ± 0.0183	0.2583 ± 0.0264
Mugil-Chelon	0.1811 ± 0.0188	0.1315 ± 0.0169	0.2190 ± 0.0192	0.2660 ± 0.0293
Liza-Chelon	0.0208 ± 0.0051	0.0266 ± 0.0060	0.0687 ± 0.0078	0.1112 ± 0.0153

Med Mediterranean, *USA* Galveston bay, *Arg* Argentina, *Bra* Brazil

M. cephalus-like species corresponding to *M. cephalus* from the Mediterranean Sea, *M. cephalus* from the Northwestern Atlantic, *M. cephalus* from Japan and Mpl/Mli group, and (2) each of the three haplogroups noted above for *M. curema*. In the second major cluster a strong association (with ≥99% robustness) between *C. labrosus, L. aurata, L. ramada* and *L. saliens* haplotypes was identified (Figs. 1b, 2b).

Table 3 Specimens and abbreviation code, location of collection sites, sample size, haplotypes (*in bold*) and frequencies (*in parenthesis*) for combined markers with a total of 2,264 bp length

Species	Code	Locality	Number	Haplotypes
M. cephalus	Mcep	Palamós, Spain	3	**1**(1), **2**(1), **3**(1)
		Ter Vell lagoon, Spain	7	**3**(2), **4**(1), **5**(1), **6**(3)
		Galveston Bay, USA	5	**7**(2), **8**(1), **9**(1), **10**(1)
M. platanus	Mpl	Río Grande, Brazil	2	**11**(1), **12**(1)
		Montevideo, Uruguay	6	**13**(3), **14**(1), **15**(1), **16**(1)
		Samborombón Bay, Argentina	11	**13**(1), **17**(1), **18**(4), **19**(1), **20**(1), **21**(1)
		Mar Chiquita lagoon, Argentina	18	**13**(7), **18**(7), **22**(1), **23**(1), **24**(1), **25**(1)
		Viedma, Argentina	6	**13**(2), **18**(1), **26**(1), **27**(1), **28**(1)
		San Lorenzo lagoon, Argentina	16	**13**(6), **18**(1), **29**(1), **30**(1), **31**(1), **32**(1), **33**(1), **34**(1), **35**(1), **36**(1), **37**(1)
M. liza	Mli	Tunas de Zaza, Cuba	6	**17**(1), **38**(1), **39**(1), **40**(1), 41(1), **42**(1)
M. curema	Mcur	Galveston Bay, USA	4	**43**(3), **44**(1)
		Mar Chiquita lagoon, Argentina	18	**45**(2), **46**(2), **47**(1), **48**(1), **49**(4), **50**(1), **51**(1), **52**(2), **53**(1), **54**(1), **55**(1), **56**(1)
		Mar del Plata, Argentina	14	**49**(10), **57**(1), **58**(1), **59**(1), **60**(1),
		San Salvador de Bahia, Brazil	1	**61**(1)
L. aurata	Lau	Palamós, Spain	3	**62**(1), **63**(1), **64**(1)
L. ramada	Lra	Ter Vell lagoon, Spain	3	**65**(1), **66**(1), **67**(1)
L. saliens	Lsal	Moulay Bousselham lagoon, Morocco	7	**68**(1), **69**(2), **70**(1), **71**(1), **72**(1), **73**(1)
C. labrosus	Chl	Ter Vell lagoon, Spain	7	**74**(4), **75**(1), **76**(1), **77**(1)

Discussion

Mugil cephalus: cosmopolitan species or species complex?

Our study supports the close relationships and possible conspecificity of *M. liza* and *M. platanus* based on shared haplotypes (Table 1; Figs. 1, 2) and justifies our combined consideration of these taxa. This kinship is supported by mtDNA data (Fraga et al. 2007) and morphological similarities including gill rakers (Eiras-Stofella et al. 2001) and overlapping values of lateral series scales (LT) counts (data summarized from Menezes 1983; Gilbert 1993; Cousseau et al. 2005; Heras et al. 2007). Otherwise, LT counts do not overlap for *M. liza* and *M. cephalus* and minimally overlap (only significantly differentiated by mean values; *t*-test = 0.00000*, $P = 0.05$) between *M. platanus* and *M. cephalus* (Heras et al. 2007; González Castro et al. 2008).

M. cephalus, while separated from the Mpl/Mli haplogroup, is itself diverged among Mediterranean, Atlantic, Pacific and Japanese *M. cephalus*' haplogroups (Figs. 1, 2; Table 2; Table 4). The divergence observed by other authors in *M. cephalus* sampled worldwide supports the establishment of a possible speciation (Crosetti et al. 1994; Rossi et al. 1998a, b; Rocha-Olivares et al. 2000). Such distinction is also indicated in the present data where, for instance, significant genetic distances for cytb ranging from 0.0406 to 0.0586 (Table 2) were found in pairwise comparisons among *M. cephalus*-like haplogroups (Figs. 1, 2). This range of cytb mean distances falls within the most frequently found values of the same metric detected in congeneric species of fishes (see Fig. 5 in Johns and Avise 1998).

The observed levels of isolation of *M. cephalus* haplogroups (including Mpl/Mli haplogroup) may represent either genetically isolated populations of the same species or a complex of closely related species as anticipated by Briggs (1960). Cryptic species complexes are not easily differentiated with classical morphology, but are identifiable by diagnostic genetic divergence (Price 1996; Fontdevila and Moya 2003). Therefore, the lack of useful

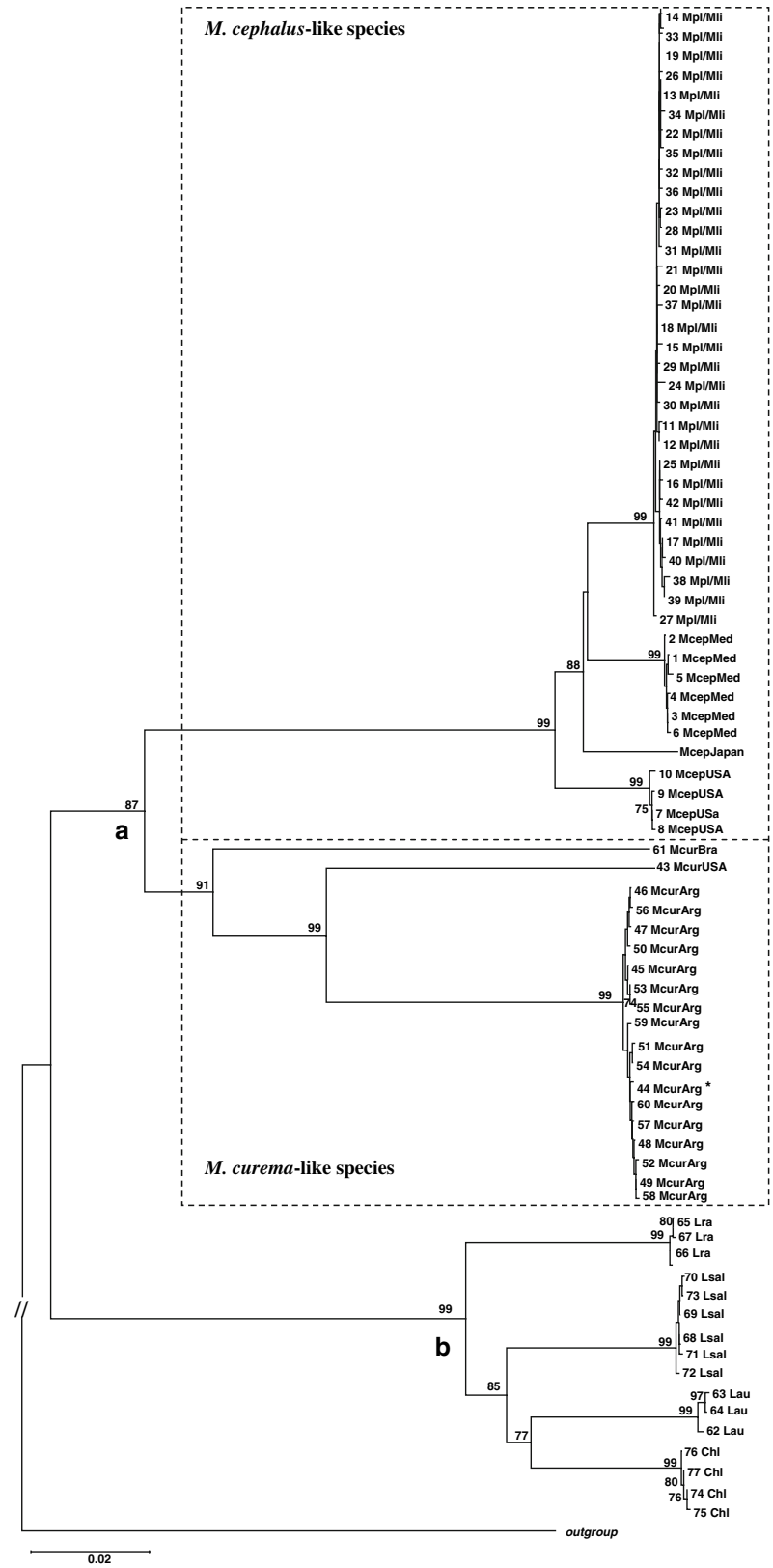

Fig. 1 NJ tree based on combined markers and Tamura–Nei setting model. Bootstrap values (≥70%) are indicated above the branches. Haplotype code as in Table 3. *Individual from Galveston Bay. *Gadus morhua* was used as outgroup species

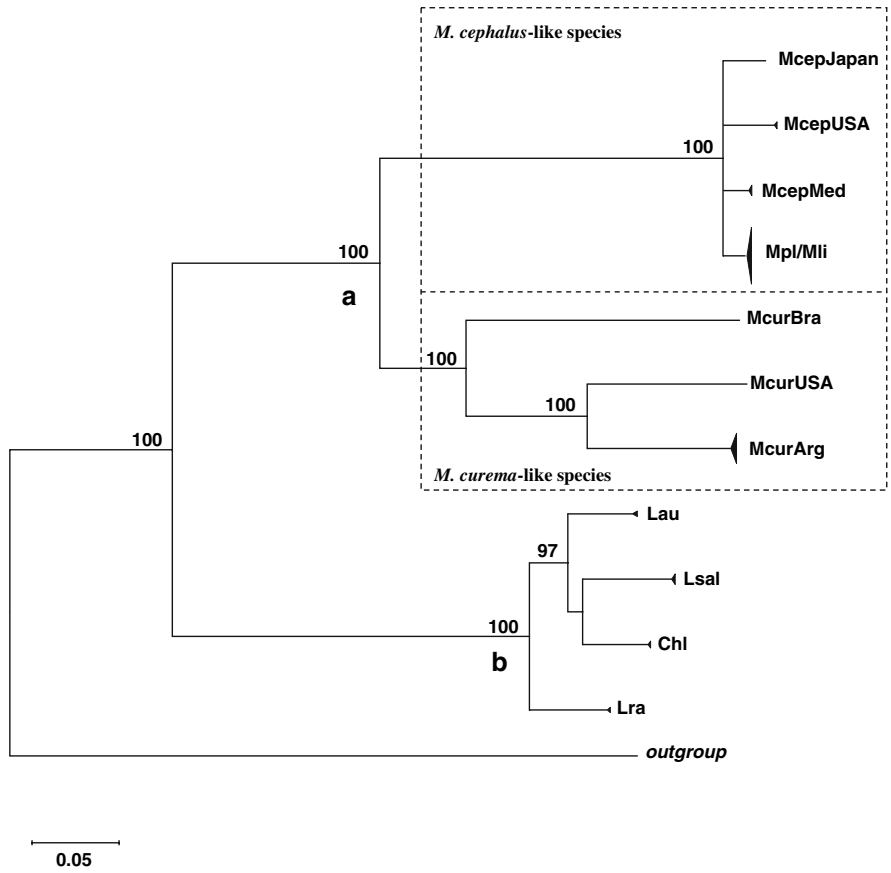

Fig. 2 Bayesian condensed tree based on combined markers. Posterior probability values (≥70%) are indicated above the branches. Size of triangles is proportional to number of haplotypes. *Gadus morhua* was used as outgroup species

morphological traits for distinguishing between closely related species (i.e., morphological stasis, Lefébure 2007) results in an underestimation of species number (Bernardi and Goswami 1997). According to Knowlton (1993) this situation probably exists with many, perhaps most cosmopolitan marine fishes species such as *M. cephalus*. In circumglobal organisms the biological species concept cannot be applied in most cases due to the difficulty in determining the interbreeding level between adjacent species. Because of the allopatry of *M. cephalus* haplogroups (a prelude of species splitting Price 1996) and the absence of shared haplotypes among them (including Mpl/Mli haplogroup), we conclude that *M. cephalus* is a species complex on a global scale (Fig. 4a).

Mugil curema "species" revision

Three clearly differentiated *M. curema* haplogroups detected in our study (Figs. 1, 2; Tables 2, 4) are consistent with each representing congeneric species. Genetic distance in cytb and COI observed between *M. cephalus*-McurArg (0.1963, 0.1822), *M. cephalus*-McurUSA (0.2030, 0.1807) and *M. cephalus*-McurBra (0.2009, 0.1859) fit the values between *M. cephalus* and *M. curema* previously reported by Johns and Avise (1998; ≈0.2000) and by Peregrino-Uriarte et al. (2007; 0.158), respectively.

Recently Fraga et al. (2007) reported two types of *M. curema* using cytb and 16S rRNA collected mainly in Brazilian waters. We constructed phylogenies (Fig. 3a, b) incorporating comparable data from Fraga et al. (2007) with those of our study where (1) *M. curema* type II (from Fraga et al. 2007) is the same as McurArg from this study and that identified in South Carolina, USA by Caldara et al. (1996; Fig. 3b), (2) similarly *M. curema* type I corresponds to McurBra, and (3) haplotypes from Fraga et al. (2007) for *M. hospes* and *M. incilis* are incorporated. Fraga et al. (2007) observed that *M. curema* type I has $2n = 48$ chromosomes and Harrison et al. (2007)

Table 4 Pairwise Tamura–Nei mean genetic distances of Mugilid species and genera from this study ($n = 137$) and Fraga et al. (2007; $n = 41$) and standard errors for cytb

	Cytb
Mcep vs Mpl/Mli	
McepMed-McepUSA	0.0575 ± 0.0123
McepMed-McepJapan	0.0463 ± 0.0109
McepUSA-McepJapan	0.0527 ± 0.0124
McepPacif-McepMed	0.0356 ± 0.0090
McepPacif-McepUSA	0.0363 ± 0.0101
McepPacif-McepJapan	0.0365 ± 0.0101
Mpl/Mli-McepMed	0.0413 ± 0.0101
Mpl/Mli-McepUSA	0.0365 ± 0.0094
Mpl/Mli-McepJapan	0.0418 ± 0.0106
Mpl/Mli-McepPacif	0.0208 ± 0.0069
Mcep-Mpl/Mli	0.0372 ± 0.0072
Mcur vs Mcep	
McurUSA-McurArg	0.1233 ± 0.0182
McurUSA-McurBra	0.1510 ± 0.0218
McurArg-McurBra	0.1810 ± 0.0228
McurUSA-McepMed	0.1626 ± 0.0222
McurUSA-McepUSA	0.1885 ± 0.0259
McurUSA-McepJapan	0.1876 ± 0.0250
McurUSA-McepPacif	0.1849 ± 0.0250
McurUSA-Mpl/Mli	0.1894 ± 0.0255
McurArg-McepMed	0.1652 ± 0.0223
McurArg-McepUSA	0.1721 ± 0.0234
McurArg-McepJapan	0.1701 ± 0.0227
McurArg-McepPacif	0.1663 ± 0.0234
McurArg-Mpl/Mli	0.1672 ± 0.0226
McurBra-McepMed	0.2043 ± 0.0251
McurBra-McepUSA	0.1957 ± 0.0245
McurBra-McepJapan	0.2249 ± 0.0271
McurBra-McepPacif	0.2104 ± 0.0258
McurBra-Mpl/Mli	0.2211 ± 0.0266
McurUSA-Mcep	0.1748 ± 0.0221
McurArg-Mcep	0.1673 ± 0.0217
McurBra-Mcep	0.2073 ± 0.0247
M. incilis vs *M. hospes* vs Mcep vs Mcur	
M. incilis-*M. hospes*	0.2288 ± 0.0304
M. incilis-Mcep	0.1812 ± 0.0230
M. incilis-McurUSA	0.1657 ± 0.0229
M. incilis-McurArg	0.1033 ± 0.0166
M. incilis-McurBra	0.2014 ± 0.0274
M. hospes-Mcep	0.2245 ± 0.0260
M. hospes-McurUSA	0.2312 ± 0.0290
M. hospes-McurArg	0.1960 ± 0.0255
M. hospes-McurBra	0.1469 ± 0.0203

Med Mediterranean, *USA* Galveston Bay, *Arg* Argentina, *Pacif* Chile

GenBank accession nos. *M. curema* type I EF426363-70, EF6422-23; *M. curema* type II EF426371-78; *M. liza* EF426401-7; EF426420-21; *M. platanus* EF426408-18; *M. cephalus* EF426419; *M. hospes* EF426354; *M. incilis* EF426379

proposed the name *Mugil rubrioculus*. However, the McurUSA haplogroup does not correspond with any other *M. curema* sequence available.

The inclusion of *M. incilis* and *M. hospes* modifies the relationships between the three *M. curema* haplogroups as we expected (Table 4; Fig. 3a, b). In agreement with Fraga et al. (2007), *M. incilis* appears closely related to McurArg/typeII/SC and *M. hospes* with McurBra/type I. We conclude that each of the three haplotypes of this study, two of which conform with described *Mugil* species and the third previously unidentified, represent distinct congeneric species.

Summarizing all genetic data available (Fig. 4b) we can infer that *M. curema* type II has an extended American distribution, with a known range from the coast of South Carolina, USA to Mar del Plata, Argentina. Moreover, McurBra/type I or *M. rubrioculus* was found in Venezuelan and Brazilian waters and, curiously, in the Pacific coast of Panama (Nirchio et al. 2003; Fraga et al. 2007 and this study). We propose identifying the McurUSA haplogroup as *M. curema* type III following the nomenclature used by Fraga et al. 2007; future karyotypic investigation will determine whether $2n = 24$ (Nirchio and Cequea 1998), $2n = 28$ (LeGrande and Fitzsimons 1976), or perhaps another configuration is correct.

These three types of *M. curema* represent independent and, in places sympatric evolutionary lineages. Their existence generates several opportunities for further investigation. For instance, which types occurs in Atlantic African waters? How many types occur in Pacific American waters? Answers to these and other questions will clarify biogeographical aspects which still remain unclear (Fig. 4b), and

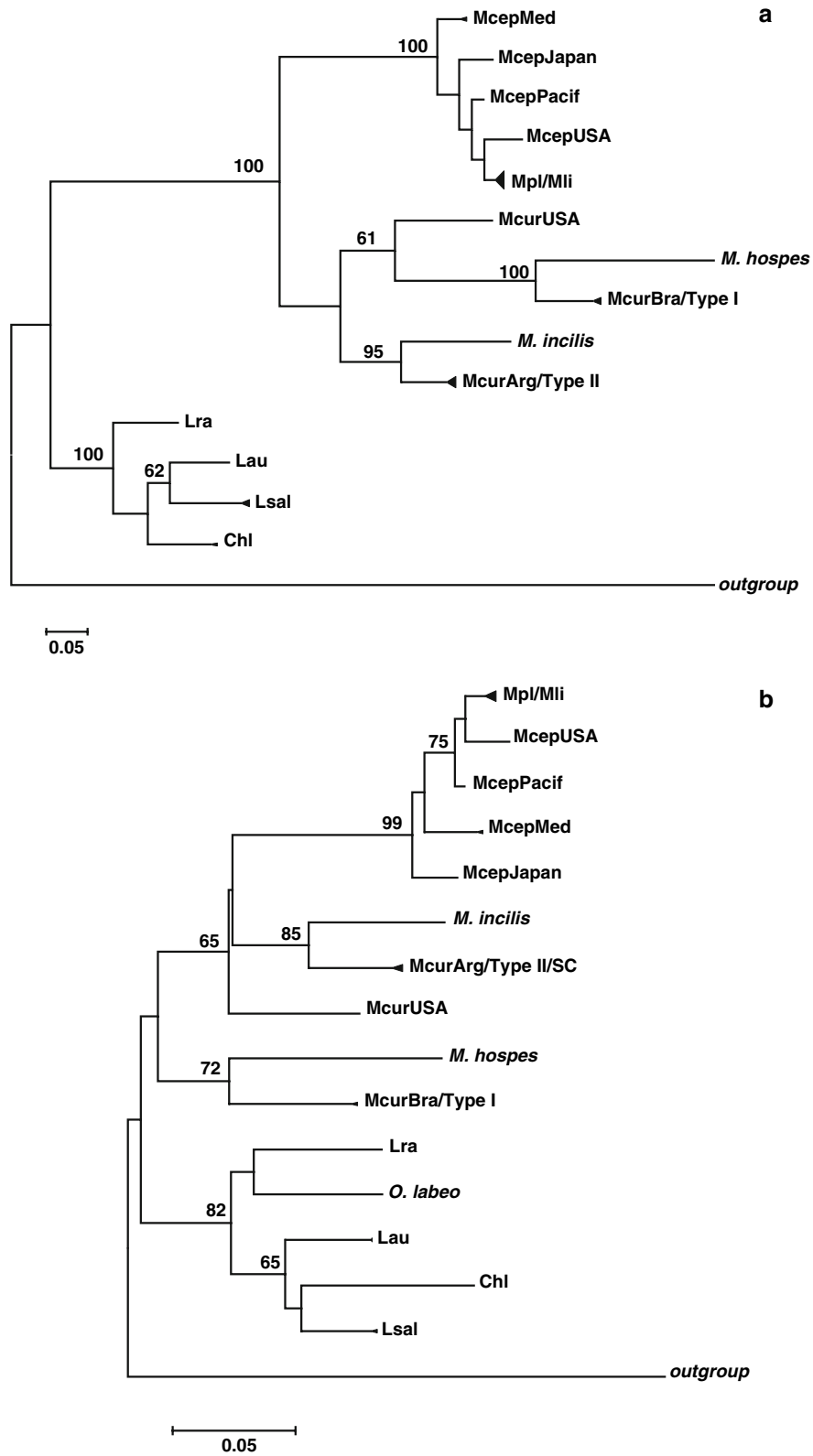

Fig. 3 Phylogenetic hypothesis of Mugilidae based on all cytb data available. **a** Bayesian tree of cytb (402 bp) of 137 sequences from this study and 41 *Mugil* sequences from Fraga et al. (2007). Posterior probability values (≥60%) are indicated above the branches. Size of triangles is proportional to number of haplotypes. **b** NJ tree based on Tamura–Nei genetic distances of cytb (198 bp) with 137 of this study, 41 *Mugil* sequences from Fraga et al. (2007) and seven sequences from Caldara et al. (1996) corresponding to *M. cephalus, M. curema* from South Carolina (SC), *L. aurata, L. ramada, L. saliens, C. labrosus* and *Oedalechilus labeo*, respectively. Numbers above the branches indicate the bootstrap value (≥60%). *Gadus morhua* was used as outgroup species

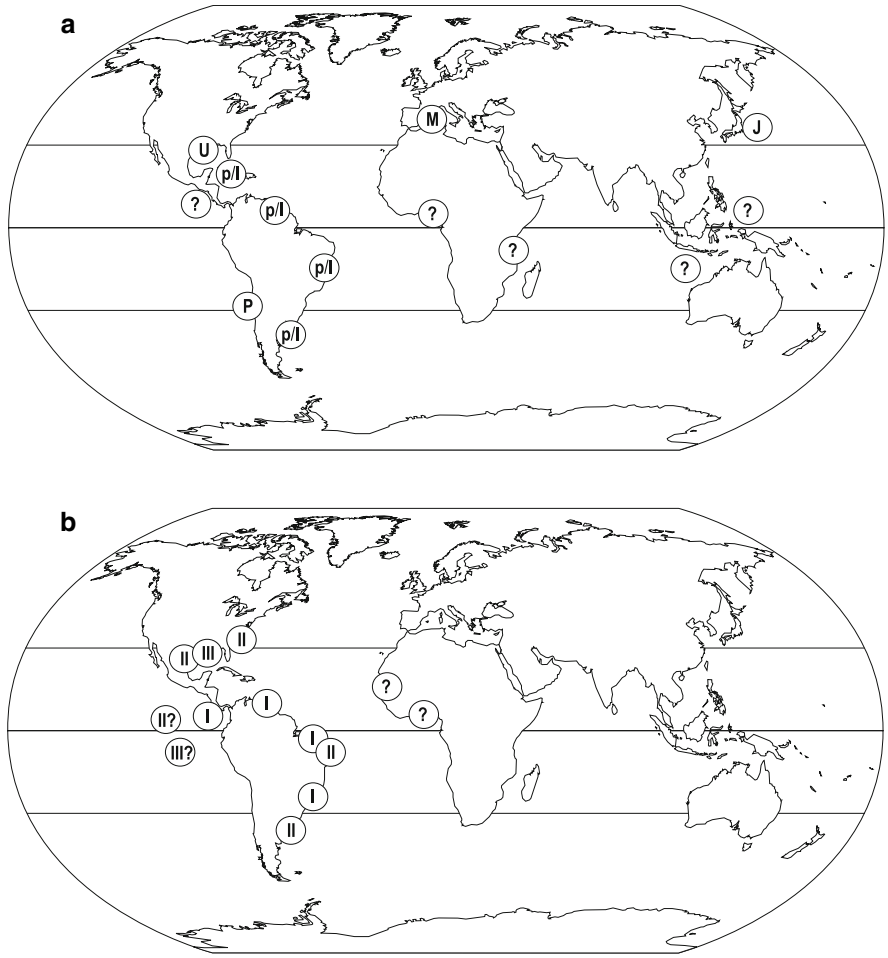

Fig. 4 *M. cephalus* species complex based on mtDNA **a** *J* McepJapan, *M* McepMed, *P* McepPacif, *p/l* Mpl/Mli, *U* McepUSA, ? haplogroups to determine. Current understanding of *M. curema*-like species **b** I *M. curema* type I, II *M. curema* type II, III *M. curema* type III

inevitably raise further questions for additional study. Certainly, more information about their boundary distributions and phylogeography is required for fisheries management policy as was pointed out in Heras et al. (2006).

Insight into genera *Mugil, Liza* and *Chelon*

We identified two distinct intergeneric clades distinguishing *Mugil* species from those of *Liza* and *Chelon* (Figs. 1, 2, 3) which generally agree with complementary molecular genetic data reported in other investigations. A monophyletic basis for *Mugil* was reported by Caldara et al. (1996) analyzing mtDNA in *M. cephalus* and *M. curema*, and by Rossi et al. (1998b) analyzing allozymes in three species, *M. cephalus, M. curema* and *M. gyrans*.

The strong association of *Liza* and *Chelon* in a separate lineage from *Mugil* species with low genetic distances separating *C. labrosus* and *L. aurata* or *L. saliens* (Table 2) agrees with questions of a monophyletic origin of *Liza*, discussed previously by several authors (Caldara et al. 1996; Papasotiropoulos et al. 2001, 2002, 2007; Rossi et al. 2004; Turan et al. 2005; Fraga et al. 2007); only Semina et al. (2007) recommended synonymy for *Chelon* and *Liza*. The monophyly of *Liza* clearly is not supported considering the total relevant biological information for *Liza* and *Chelon* including data from chromosomes, morphology, allozymes, RFLPs and mtDNA sequences. In addition, the inclusion of *O. labeo* in the same lineage as *Liza* (Fig. 3; Turan et al. 2005) as was suggested by Thomson (1997) and Gornung et al. (2001) is warranted. According to the principle of priority (Article 23.3 of the International Commission on Zoological Nomenclature), the genera *Chelon* Artedi 1793; *Liza* Jordan and Swain 1884; and *Oedalechilus* Fowler 1903 should be synonymized

or unified under a new redescribed genus *Chelon* pending subsequent analyses of the remaining species of these three genera.

Concluding remarks and recommendations

1. The close genetic relationships between *M. platanus* and *M. liza* and shared haplotypes indicated a high degree of gene flow and do not support differentiation at species level.
2. The status of *M. cephalus* at a global scale needs to be revised, based on the distinct lineages observed. Accordingly, *Mugil cephalus* haplogroups (including *Mugil platanus/Mugil liza* group) represent a complex of species with close morphological relationships.
3. Detection of three *M. curema*-like species along the Atlantic coast of America indicates the need for a thorough revision of *M. curema* as a unique species.
4. The monophyly of *Mugil* is supported based on a shared common ancestry indicated for all *Mugil* species examined.
5. Based on the present data and all relevant published biological data, we recommend a revision of the taxonomic status of genus *Liza, Chelon* and *Oedalechilus*, including their all constituent species.

Until new phylogenetic groups are fully identified and implemented, the present species' status should be preserved to minimize risks of loss of important components of biodiversity (Agapow et al. 2004), e.g., *M. cephalus*. Currently, the development of a pluralistic system (Hendry et al. 2000) including variation in mtDNA, nuclear DNA and morphological traits, within and among groups above the species level (Avise and Walker 2000) could improve the classical biological classification of Mugilidae. Following Avise and Walker (2000), at the species level, we recommend studies on reproduction and genetics in putative disjunct allopatric haplogroups to estimate interbreeding levels.

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