



Taxonomy/Taxinomie

Mitochondrial phylogeny of grey mullets (Acanthopterygii: Mugilidae) suggests high proportion of cryptic species

*La phylogénie mitochondriale des mullets (Acanthopterygii: Mugilidae) suggère une forte proportion d'espèces cryptiques*Jean-Dominique Durand ^{a,*}, Philippe Borsa ^b^a Institut de recherche pour le développement (IRD), UMR5119 ECOSYM, Montpellier, France^b IRD, UR 227 CoReUs, Nouméa, Montpellier and Denpasar, France

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ABSTRACT

The low level of morphometric variability and the poor phylogenetic information borne by the morpho-anatomical characters used thus far in the systematics of grey mullets (Mugilidae) emphasize the utility of molecular systematics in this family. A recent mitochondrial phylogeny of grey mullets has uncovered multiple deep lineages within several species, flagging putative cryptic species. Here, we considered that several of the deeply divergent lineages represent separate species based on either the tree topology, independent data from nuclear markers, geographic distributions, or a combination of the foregoing. By analogy with these well-documented cases, we considered other deep lineages in seven genera we focused on to represent putative cryptic species. Up to two cryptic species were thus potentially detected in the genus *Chelon*, three in *Crenimugil* (including two within the single *Crenimugil seheli*), two in *Dajaus*, one in *Ellochelon*, 16 in *Mugil* (including 13 within the single *M. cephalus*), two in *Osteomugil*, and 10 in *Planiliza*. Wherever possible, we kept the current species epithets to designate those lineages that unambiguously correspond to the type material, based on type locality, and we assigned arbitrary letters (sp. A, B, etc.) to the other lineages. We present a molecular diagnosis for 24 of the species analysed in this work, as well as for 25 putative cryptic species.

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R É S U M É

Le faible niveau de variabilité morphométrique et la faible information phylogénétique portée par les caractères morpho-anatomiques utilisés à ce jour dans la systématique des mullets (Mugilidae) montrent l'intérêt de la systématique moléculaire dans cette famille. Une phylogénie mitochondriale récente de la famille des Mugilidae a montré de multiples lignées profondes au sein de plusieurs espèces, signalant de possibles espèces cryptiques. Ici, nous avons considéré que plusieurs de ces lignées profondes représentaient des espèces distinctes en nous basant, soit sur la topologie de l'arbre, soit sur des données génétiques nucléaires obtenues indépendamment, soit sur les distributions géographiques. Par analogie avec ces cas bien documentés, nous avons examiné d'autres lignées profondes dans sept genres sur lesquels nous avons concentré notre effort d'échantil-

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lonnage d'espèces. Jusqu'à deux espèces cryptiques présumées ont ainsi détectées dans le genre *Chelon*, trois dans le genre *Crenimugil* (dont deux dans le seul *C. seheli*), deux dans le genre *Dajaus*, une dans le genre *Ellochelon*, 16 dans le genre *Mugil* (dont 13 dans le seul *Mugil cephalus*), deux dans le genre *Osteomugil*, et 10 dans le genre *Planiliza*. Autant que possible, nous avons conservé les épithètes d'espèces actuelles pour désigner les lignées qui correspondent clairement au matériel-type sur la base de la localité-type, et nous avons attribué des lettres arbitraires (sp. A, B, etc.) aux autres lignées. Nous présentons une diagnose moléculaire pour 24 des espèces analysées dans le présent travail, ainsi que pour 25 espèces cryptiques présumées.

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1. Introduction

The determination of species boundaries, one of the main objectives of taxonomy, is important to evolutionary ecology and conservation ecology, because species remain the fundamental units and operational entities in most disciplines in these fields. Species misidentification and species confusion could lead to overestimating genetic diversity, biasing estimates of genetic differentiation between populations, overestimating densities, underestimating risks of local extinction, or producing meaningless estimates of demographic parameters. This in turn may misguide management actions. A common problem is that of cryptic species, undetected using traditional taxonomic approaches.

Cryptic species are defined as distinct evolutionary lineages with a substantial amount of genetic distinctiveness and no apparent morphological differences [1–3]. Highly divergent mitochondrial clades within a nominal species, where within-clade diversity is several times lower than divergence between clades might be caused by either secondary contact, or introgression following interspecific hybridization, or the occurrence of hitherto-unrecognized, “cryptic” species. The barcoding literature shows several examples of deep divergence at the mitochondrial cytochrome-oxidase 1 (*CO1*) locus within fish species, which have been ascribed to cryptic species (e.g., [4–12]). These examples thus illustrate the potential of mitochondrial sequences to flag putative new species in marine fishes.

The low level of morphometric variability and the poor phylogenetic information borne by the morpho-anatomical characters used so far in the systematics of the grey mullets (Actinopterygian fish family Mugilidae) have led to contradictory hence unreliable morphology-based phylogenies (reviewed in [13]). This emphasizes the need for molecular systematics in this family. Molecular phylogenetics has demonstrated the occurrence of distinct, deep, sometimes paraphyletic mitochondrial lineages in a proportion of species in the Mugilidae, pointing to the possible occurrence of cryptic species [13–15]. As a consequence, the species richness of the family Mugilidae is currently underestimated and possibly largely so. The species concept on which the present revision is based is the unified species concept of de Queiroz [16], which views species as separately evolving metapopulation lineages. Reciprocal monophyly and reproductive isolation are two of the relevant properties of species [16] one expects to

observe or infer from molecular population genetic data. These two properties of species will be the focus of the present taxonomic review of the Mugilidae.

Based on the only comprehensive, mitochondrial phylogeny of species in the family Mugilidae available to date [13], the objectives of the present paper are:

- to identify deeply divergent mitochondrial lineages that correspond to putative cryptic species in several mugilid genera;
- to revise the current nomenclature of species by proposing new, provisional names to these lineages;
- to provide molecular diagnoses to species and putative cryptic species.

Addressing these objectives is a necessary step to clarify the nomenclature of species in the Mugilidae, in a taxonomic context where genetic markers are replacing traditional morphological characters.

2. Materials and methods

2.1. Rationale of the present systematic revision

Durand et al.'s [13] mitochondrial phylogeny of the Mugilidae has uncovered a number of deeply divergent lineages within nominal species. Several of the lineages were paraphyletic with other species; other lineages represented reproductively isolated sympatric species, as demonstrated by genotypic frequencies at nuclear loci or inferred from karyotypes. Last, in some instances, deeply divergent sister-lineages characterized geographically separate populations within a species. Thus, there was substantial evidence for cryptic species in Mugilidae, based on the tree topology, on independent data from nuclear markers, and on the geographic distribution of sister lineages. We used W.N. Eschmeyer's fish database [17] as the reference for the current nomenclature. The current nomenclature was maintained for a lineage when its geographic distribution was compatible with the type locality of the species. By analogy with these cases where specific status was documented, we considered other deep lineages in Mugilidae, i.e. lineages whose distance to its nearest neighbour exceeded the gap between infra-specific and inter-specific pairwise distances (see section 2.5), to potentially represent additional cryptic species. We maintained the current nomenclature to designate those lineages that unambiguously correspond to the type

material, based on the type locality, and we arbitrarily assigned capital letters to the other lineages. The other lineages were thus provisionally denominated “sp. A”, “sp. B”, etc.

We emphasize that our approach is not one of DNA barcoding, but one of molecular taxonomy, where molecular diagnoses of species and putative cryptic species are provided. We use gaps in the distributions of pairwise genetic distances as a means to distinguish deep lineages, which is where one may find analogy with barcoding. Nevertheless, the utility of *CO1* barcoding for identifying species in the family Mugilidae will be the topic of a separate paper.

2.2. Genus nomenclature

In this paper, genus nomenclature accords with our recent revision [18], where the following changes have been made, relative to the previous nomenclature: *Moolgarda seheli* and *Valamugil buchani* have been placed together with *Crenimugil crenilabis* under *Crenimugil*, and *Moolgarda cunnesius*, *Moolgarda engeli*, *Moolgarda perusii*, and *Valamugil robustus* have been placed under the resurrected genus *Osteomugil*; likewise, *Liza aurata*, *Liza bandialensis*, *Liza dumerili*, *Liza ramada*, *Liza richardsonii*, *Liza saliens*, and *Liza tricuspiciens* have been placed together with *Chelon labrosus* under *Chelon*; likewise, *Chelon macrolepis*, *Chelon melinopterus*, *Chelon subviridis*, *Liza abu*, *Liza affinis*, *Liza alata*, and *Liza haematocheila* have been placed under the resurrected genus *Planiliza*; *C. planiceps* has since then been synonymized with *Liza tade* [17] and placed under *Planiliza*; also, *Sicamugil cascasia*, *Agonostomus monticola*, *Liza argentea*, *Rhinomugil nasutus*, and *Oedalechilus labiosus* have been placed, respectively, under the resurrected genera *Minimugil*, *Dajaus*, *Gracilmugil*, *Squalomugil*, and *Plicomugil* whereas *Xenomugil thoburni* has been placed under *Mugil*; the genus names *Liza*, *Moolgarda*, *Valamugil* and *Xenomugil* have been dismissed; three new genera have been erected: *Neochelon* (for *Liza falcipinnis*), *Parachelon* (for *Liza grandisquamis*), and *Pseudomyxus* (for *Myxus capensis*).

Durand et al. [18] have also synonymized the genus *Paramugil* [19] with *Planiliza*. We must acknowledge that this was an error as explained in the following. We erroneously used as reference specimen for *Planiliza parmatus* individual MNHN-IC-2011-0212, numbered 118 in [18], which had been collected in south Java by S. Kleinertz. On the basis of photographs that he kindly agreed to examine, H. Senou identified this specimen as a *Planiliza* (“*Chelon*”), and not a *Paramugil*. This specimen was subsequently examined by J. Ghasemzadeh who also rejected our identification as *Paramugil* and identified it as *Planiliza* (“*Liza*”) *melinoptera* based on its external morphological features.

2.3. Choice of a reference database

Durand et al.’s comprehensive mitochondrial phylogeny of the Mugilidae [13,18], which is based on the concatenated partial 16S rRNA, *COI* and cytochrome *b* gene sequences (3885 bp long in total) of 257 reference

specimens (including 120 vouchers deposited in museum collections), was used for the present investigation. Zooms on regions of interest in this phylogeny are presented in Figs. 1 and 2.

2.4. Identification of within-genus gaps in nucleotide distance

Pairwise nucleotide distances between haplotypes sampled within each of seven mugilid genera (*Chelon*, *Crenimugil*, *Dajaus*, *Ellochelon*, *Mugil*, *Osteomugil*, *Planiliza*) were estimated under MEGA5 [20] from the concatenated haplotype sequences at loci 16S rRNA, *COI* and *cytb*, which have been published previously [13,18]. Nucleotide distance was estimated according to the model of molecular evolution that, among the list of models proposed by MEGA5, ranked as the most likely after the GTR-related model used to construct the phylogeny of [13], because the GTR model is not proposed by MEGA5 for estimating nucleotide distances. The model thus chosen was the Tamura-Nei (TN93; [21]) with gamma distribution and invariable sites (+G+I) model. Nucleotide distances between lineages estimated according to the Kimura-2 parameter (K2P; [22]) model of molecular evolution were also presented. For each of the seven genera or species complexes we focused on, the resulting phylogenetic tree was examined together with the matrix of pairwise nucleotide distances between haplotypes. Our objective was first to determine the threshold below which distances all were infra-specific and above which they were all inter-specific. We then used this value as a yardstick to determine deep lineages that may represent cryptic species.

Further, alternative analysis of the dataset was done using the automatic gap determination algorithm proposed by N. Puillandre and co-authors to detect putative species from barcode datasets (ABGD; <http://www.wabi.snv.jussieu.fr/public/abgd/>; [23]). The analysis was run on each of the seven sequence datasets representing genera or species complexes, using the default settings of the program. This algorithm detects the gap in the distribution of pairwise nucleotide distances as the first significant gap beyond infra-specific distances and uses it to partition the dataset. Inference of the limit and gap detection are then recursively applied to previously obtained groups to get finer partitions until there is no further partitioning [23].

3. Results and discussion

3.1. Evidence of nucleotide-distance gaps within mugilid genera

Pairwise distributions of nucleotide distances among individuals within each of the seven genera focused on in the present paper are presented Fig. 3. Detailed examination of the distribution in the genus *Mugil* (Fig. 3E) revealed a gap after 1%: it is therefore sensible to consider the values $\leq 1\%$ separately from the rest of the distribution and to ascribe them to genetic variation at the infra-specific level. This 1% threshold value also precisely coincided with the right boundary of the first mode of pairwise nucleotide

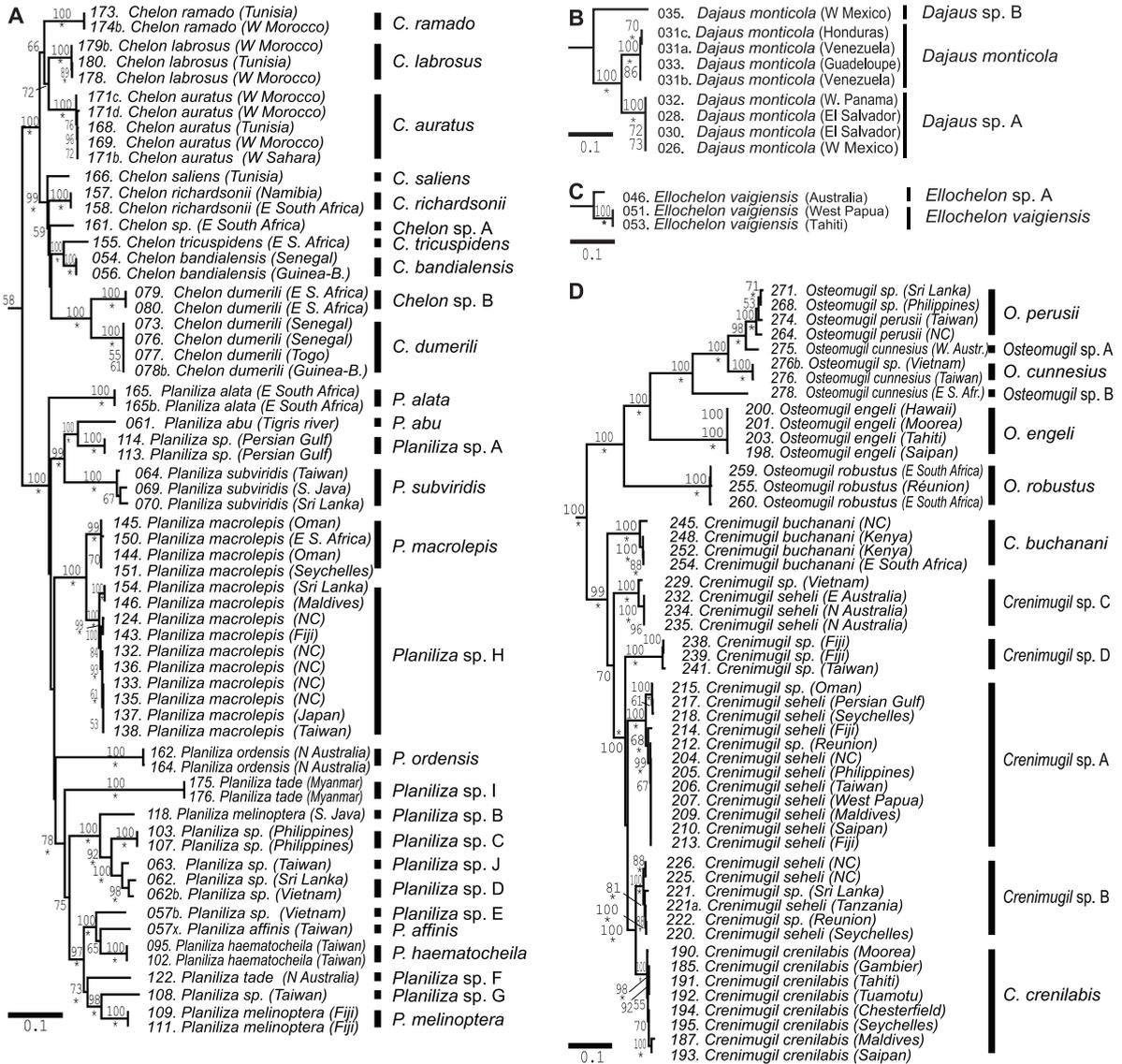


Fig. 1. Phylogenetic trees depicting relationships among mugilid species, constructed from partitioned maximum-likelihood (ML) analysis of 3885 aligned nucleotides from 16S rRNA, COI and *cytb* gene sequences [13]. Vertical bars on the right of the tree indicate well-supported lineages that potentially represent distinct species. NC: New Caledonia. A. Among species within genera *Chelon* and *Planiliza* as redefined by [18]. B. Within genus *Dajaus* as redefined by [18]. C. Within genus *Ellochelon*. C. Among species within genera *Osteomugil* and *Crenimugil* as redefined by [18].

distances in the genus *Chelon* (Fig. 3A), and it encompassed the homologous first modes in *Dajaus* (Fig. 3C) and *Ellochelon* (Fig. 3D). Detailed examination of intraspecific distances within *C. crenilabris* and its morphologically distinct sister-species *Crenimugil* sp. B, the two most closely related lineages in the genus *Crenimugil* (Fig. 1D), showed no infra-specific distance greater than 1.5%. Within the other *Crenimugil* lineages (Fig. 1D) the highest pairwise distance was 2.2% while the lowest inter-lineage distance was 3.4%. Similarly, in the genus *Osteomugil* a gap in pairwise distances occurred between 2.1% and 4.3%. In *Planiliza*, a similar, although narrower gap was observed between 2.1%, the highest distance found within *P. subviridis*, and 2.6%. Thus, placing a threshold at 1% allowed the delineation of the first mode of the distribution of pairwise nucleotide distances in four

(*Chelon*, *Dajaus*, *Ellochelon*, and *Mugil*) (Fig. 3A, C–E) of the seven mugilid genera tested. In the three remaining genera (*Crenimugil*, *Osteomugil*, and *Planiliza*) (Fig. 3B, F, G), the threshold should be placed at 2.5% based on the gap in the distribution of pairwise nucleotide distances.

3.2. Mitochondrial lineages that characterize cryptic species

In this section, we review all cases where lineages separated from the closest neighbouring lineage by a nucleotide distance larger than the threshold defined in the preceding section correspond to distinct species. In the mitochondrial phylogeny of the Mugilidae published by [13], *Dajaus monticola* consisted of three deeply rooted lineages (Fig. 1B), the two most recently diverged of which

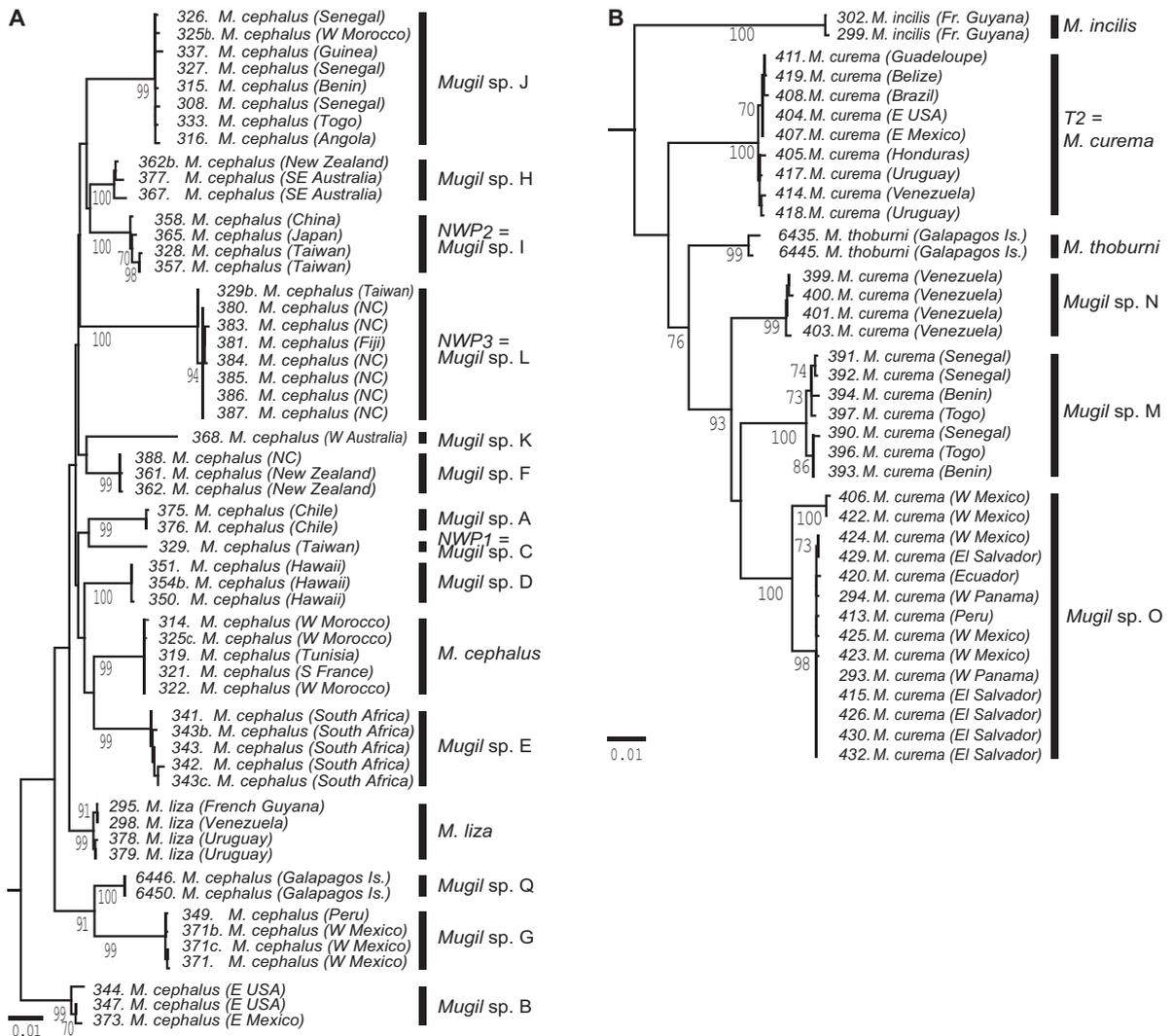


Fig. 2. Phylogenetic trees of species in the genus *Mugil*, constructed from partitioned maximum-likelihood (ML) analysis of 3885 aligned nucleotides from 16S rRNA, *COI* and *cytb* gene sequences [13]. Bootstrap scores >50% are indicated. Vertical bars on the right of the tree indicate statistically supported lineages that potentially represent distinct species [13,18]. A. Tree of individual haplotypes in the *Mugil cephalus* species complex, including *M. cephalus* and *Mugil liza*. NWP1, NWP2, NWP3: lineages characteristic of three respective cryptic species (*Mugil* sp. C, *Mugil* sp. I, *Mugil* sp. L) sampled in the East China Sea [14]; the other lineages were assigned species names *Mugil* spp. A, B, D–J, and Q; the lineage sampled in the northeastern Atlantic and in the Mediterranean is the actual *M. cephalus*. NC: New Caledonia. B. Tree of individual haplotypes in the *Mugil curema* species complex.

were geminate lineages distributed on either side of Central America, separated by 7.9% net nucleotide divergence under the K2P model and 7.0% under the TN93 + G + I model, all three markers combined. These two lineages have likely been geographically isolated from one the other for millions of years, hence are likely to represent separate species. The third lineage, from the eastern Pacific, branches externally to the two latter and is likely to represent another species. The type-locality of *D. monticola* is Jamaica [17]. Therefore, we here maintain the epithet *monticola* for the Atlantic lineage and provisionally designate the two other lineages, both from the eastern Pacific, as *Dajaus* sp. A and sp. B. The geographic distribution of the different lineages within *D. monticola* is presented in Fig. 4B. A subsequent study [15] estimated

the divergence between the two geminate lineages *D. monticola* and *Dajaus* sp. A to be 31.8–11.8 million years old; it also evoked morphological differences between the two lineages from the eastern Pacific, confirming their status as separate species. The same study reported a fourth lineage currently under *D. monticola* from the Mexican rivers of the Gulf of Mexico, that is, geographically separated from what we here consider to be the true *D. monticola* [15].

The mitochondrial phylogeny of the *M. cephalus*/*M. liza* species complex (Fig. 2A) revealed 15 separate lineages, each with deep (>1%) rooting and shallow (<1%) within-lineage diversity. Three of these lineages, which occur in sympatry in Taiwan, belong to genetically distinct forms reproductively isolated from one another as demonstrated

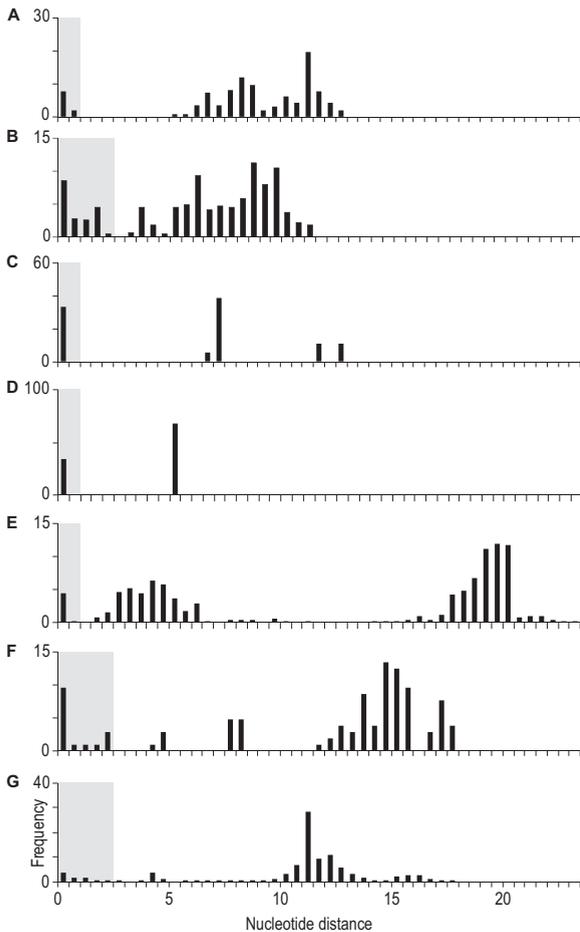


Fig. 3. Frequency distribution of pairwise nucleotide distance estimates (TN93+G+I model; MEGA5 [20]) among individuals within each of 7 mugilid genera. Shaded rectangles highlight pairwise nucleotide distances $\leq 1\%$ (in *Chelon*, *Dajaus*, *Ellochelon*, and *Mugil*) or $\leq 2.5\%$ (in *Crenimugil*, *Osteomugil*, and *Planiliza*) within a deep lineage. A. *Chelon*. B. *Crenimugil*. C. *Dajaus*. D. *Ellochelon*. E. *Mugil*. F. *Osteomugil*. G. *Planiliza*.

by their distinct composition at nuclear loci and by the quasi-absence of hybrids [14]. These lineages, coined NWP1-3 by [14], show 3.2%–4.8% nucleotide divergence under the K2P model, all three markers combined, and 3.3%–5.1% divergence under the TN93+G+I model [20]. The three lineages are here assigned arbitrary species names sp. C, sp. I and sp. L., respectively (Fig. 2A). *Mugil curema* similarly consisted of a complex of species, where deeply rooted lineages were paraphyletic with another species, *Mugil thoburni* [18] (Fig. 2B). The type locality of *M. curema* is Bahia, Brazil [24] where only one lineage, also characterized by a chromosome complement number of $2n=28$, is present [13]. This is the “Type 2” (T2) karyological form of [25]. The topology of the tree (Fig. 2B) shows lineage T2 to root externally to the subclade consisting of *M. thoburni* and the other *M. curema* lineages. We here keep the name *M. curema* exclusively for lineage T2 and we designate the other lineages as *Mugil* sp. M to sp. O. The case of the three *Mugil* sp. M to sp. O lineages will be discussed in the following section.

3.3. Recognizing deeply divergent lineages as putative new species

A number of deeply divergent lineages potentially represent additional cryptic species in the Mugilidae. These cases are examined genus by genus in the following, where each lineage either was assigned a capital letter, or conserved its current name.

In the genus *Chelon* (Fig. 1A), Durand et al. [13] had sampled an unidentified *Chelon* sp. lineage from south-eastern Africa (their specimen no. 161), which is here provisionally designated as *Chelon* sp. A. In the same genus, the haplogroup corresponding to *Chelon dumerili* actually comprised two distinct lineages separated by a net nucleotide distance, all three markers combined, of 7.5% (under the K2P model) or 8.1% (under the TN93+G+I model). One lineage was exclusively sampled in western Africa, including Saint-Louis, Senegal [13], which is the type locality of the species [26]. We maintain epithet *dumerili* for this lineage. The other lineage was sampled exclusively in southeastern Africa and is here provisionally referred to as *Chelon* sp. B. We consider *Chelon* sp. B to be putatively a species distinct from *C. dumerili* based on the disjunction in geographic distributions and the level of nucleotide distance between the two lineages.

In the genus *Crenimugil* (Fig. 1D), three distinct lineages were observed within *C. seheli* under its current definition. These three lineages, which occur sympatrically in the Indo-West Pacific, are separated by a net nucleotide divergence, all three markers combined, of 4.5%–7.8% under the K2P model and 4.8%–8.6% under the TN93+G+I model, whereas intraspecific nucleotide diversity under both models was $\leq 2.2\%$. The three lineages were paraphyletic with *C. crenilabis* and with an undescribed *Crenimugil* species sampled from Taiwan and Fiji, represented by individuals Nos. 238, 239 and 241 of [13]. Therefore, we consider them to characterize putative, distinct species, here designated as *Crenimugil* spp. A–C. The undescribed *Crenimugil* sp. species from Taiwan and Fiji is here designated as *Crenimugil* sp. D. Fig. 4A presents the geographic distribution of all four deep lineages within the *C. seheli*/*C. crenilabis* species complex.

In the genus *Ellochelon* (Fig. 1C), two separate lineages were observed, which diverged by 4.8% net nucleotide distance under the K2P model and 5.1% under the TN93+G+I model, all three markers combined. One lineage included specimens from Waigeo, the type-locality [27] and French Polynesia, and another lineage was represented by a specimen from an unknown location in Australia. Epithet *vaigiensis* is here provisionally retained for the *Ellochelon* lineage sampled in Waigeo while the other lineage is provisionally assigned putative species name *Ellochelon* sp. A.

In the genus *Mugil*, the 13 distinct lineages originally uncovered within *M. cephalus* belonged to the same subclade as *M. liza* [13]. The average \pm SD net nucleotide distance between lineages, from which *M. liza* was excluded was, all three markers combined, $3.6\% \pm 1\%$ under the K2P model and $3.8\% \pm 1\%$ under the TN93+G+I model. Subsequently, a fourteenth lineage comprising haplotypes sampled from the Galapagos Islands was reported [18] (Fig. 2A). Three

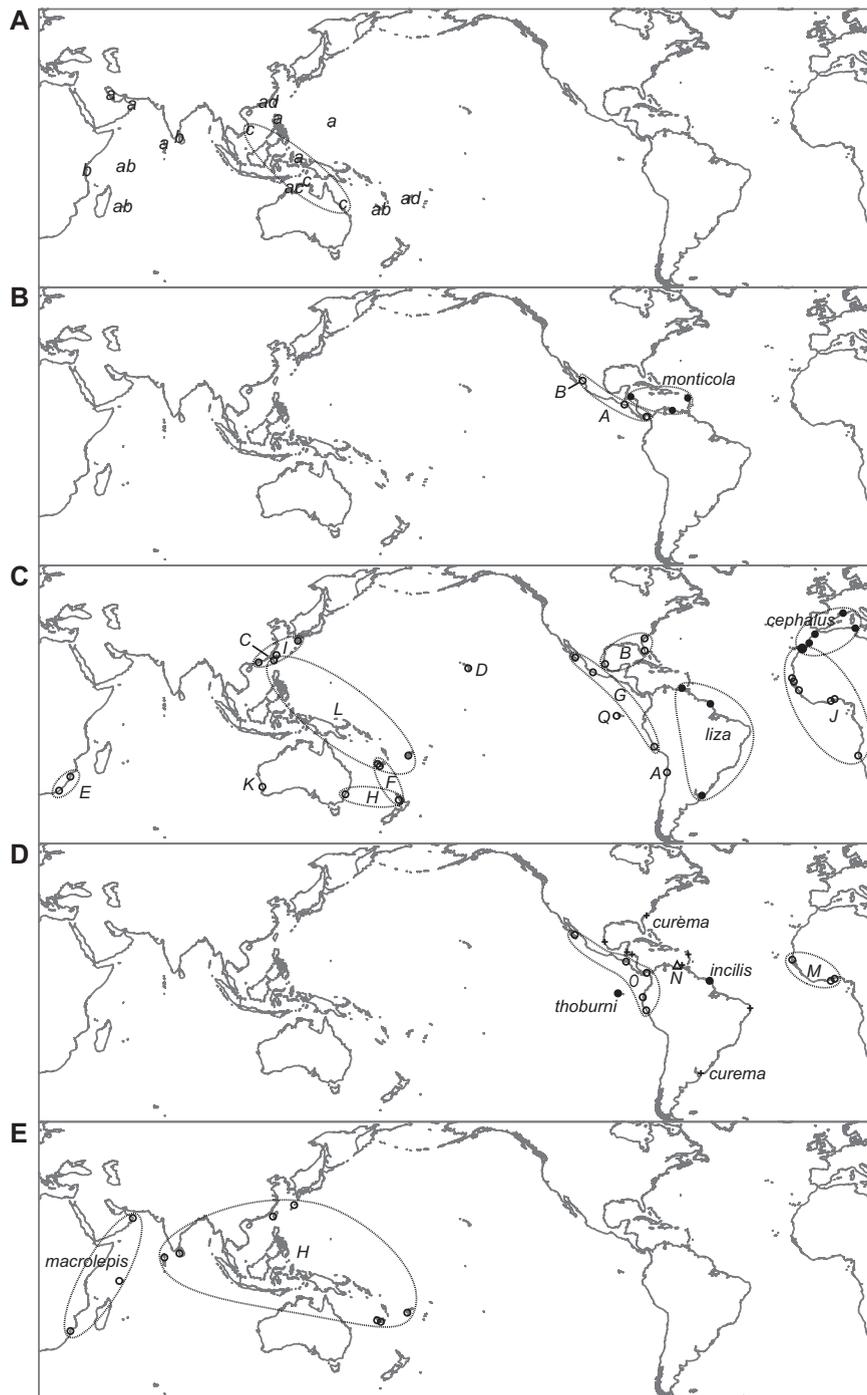


Fig. 4. Geographic distribution of mitochondrial lineages (putative species) in grey mullets, based on the sampling of [13,18] (Figs. 1 and 2). Background map of the Indo-West Pacific was obtained from Digital Vector Maps, San Diego (<http://digital-vector-maps.com/>). A. Lineages of the *Crenimugil seheli* species complex. *a–c*, putative cryptic species *Crenimugil* spp. A to C, respectively; *dotted ellipse* encompasses all known locations where *Crenimugil* sp. C occurs; *d*, *Crenimugil* sp. D. B. Lineages of *Dajaus monticola*. A, B: putative cryptic species *Dajaus* spp. A and B, respectively; all three samples from the Pacific coast of Central America included *Dajaus* sp. A. C. Lineages of the *Mugil cephalus* species complex. A–L, Q: putative cryptic species *Mugil* spp. A to L and Q, respectively. D. Lineages of the *Mugil curema* species complex. Crosses (+): *M. curema*; M–O: putative cryptic species *Mugil* spp. M to O, respectively. E. Lineages of *Planiliza macrolepis*. H, putative cryptic species *Planiliza* sp. H.

of the lineages currently within *M. cephalus* (i.e., sp. C, sp. I and sp. L), which occur in sympatry, belong to genetically distinct forms reproductively isolated from one another (see preceding section). Hence, basing our analogy on similarity in

ratios of inter- to intra-lineage nucleotide distance, and also taking into account the current taxonomic standards that designate *M. liza* as a species separate from *M. cephalus*, we consider all other 11 lineages within *M. cephalus* under its

current definition to be putative, distinct species. One notes that these lineages apparently have allopatric or parapatric distributions (Fig. 4C). The original description of *M. cephalus* [28] geographically refers to a species which “habitat Oceano Europeo”. Accordingly, we can designate without ambiguity the only lineage sampled in the Mediterranean Sea [13,29] as characterizing the actual *M. cephalus*. The 10 remaining lineages are here provisionally assigned putative species names *Mugil* sp. A, sp. B, spp. D–H, sp. J, sp. K and sp. Q. Fig. 4C presents the geographic distribution of the 15 deeply rooted lineages within the *M. cephalus* species complex (i.e., *M. cephalus*, *M. liza*, cryptic species *Mugil* spp. C, I, L, and putative cryptic species *Mugil* spp. A, B, D–H, J, K, Q). Three other *Mugil* spp. lineages within the species initially designated as *M. curema* were uncovered (see preceding section). *Mugil* sp. N and *Mugil* sp. O differ from *M. curema* by their karyotypes (respectively, $2n = 24$ and 48) indicating that they are likely reproductively isolated from the latter as well as from one the other [13]. *Mugil* sp. M, from the Pacific Ocean, is paraphyletic with *Mugil* sp. N and *Mugil* sp. O, both from the Atlantic Ocean (Fig. 2B). These lineages differ by 3.2%–5.4% net nucleotide distance under the K2P model and 3.4%–5.8% under the T93 + G + I model, all three markers combined. In comparison, nucleotide diversities within a lineage ranged from 0.2% to 0.5% (under both models). Hence, we consider the three lineages to represent distinct species. Still in *Mugil*, a lineage originally assigned to *Mugil hospes* by [13] was represented exclusively by haplotypes sampled from the Gulf of Mexico. Because the type-locality of *M. hospes* is Mazatlan in the eastern Pacific [30], it is sensible to provisionally assign the lineage from the Gulf of Mexico to a yet undetermined *Mugil* species, here designated as *Mugil* sp. R. Last, *Mugil rubrioculus* comprises two distinct lineages, one sampled from Venezuela, the type locality of the species [31], and the other one from the eastern Pacific [13]. The lineage from Venezuela retains epithet *rubrioculus* while the eastern Pacific lineage is here provisionally designated *Mugil* sp. P. This lineage may represent the same species as *M. aff. rubrioculus* previously mentioned from the eastern Pacific [31]. Fig. 4D presents the geographic distribution of the 7 deep lineages (i.e., *M. curema*, *Mugil incilis*, *M. thoburni*, and putative cryptic species *Mugil* spp. M–P) within the *M. curema* species complex.

In the genus *Osteomugil* (Fig. 1D), three distinct lineages representing *Osteomugil cunnesius* under its current definition have been found to be paraphyletic with *Osteomugil perusii* [13]. The type locality of *O. cunnesius* is the Moluccas [24]. Hence, the lineage sampled in Taiwan and in Vietnam by [13], geographically closest to the Moluccas, is provisionally retained as the actual *O. cunnesius* while the two other lineages, one from eastern Western Australia, the other one from South Africa, are here assigned provisional names *Osteomugil* sp. A and *Osteomugil* sp. B, respectively.

In the genus *Planiliza* (Fig. 1A), both *Planiliza melinoptera* and *Planiliza tade* were found to be polyphyletic ([13]; present work). Individual 118 of [13], which was initially, erroneously identified as *Planiliza parmata* is now recognized as a cryptic lineage of *Planiliza melinoptera* (see section 2.2). This lineage was separated from the *P. melinoptera* sampled in Fiji (individuals 109 and 111 of

[13]) by 10.3% nucleotide distance (under the K2P model) and 11.4% (under the TN93 + G + I model), all three markers combined. We kept the Fiji specimens under *P. melinoptera*, because of the geographic proximity of Fiji with Vanikoro, the type locality [24] and we assigned provisional species name *Planiliza* sp. B to the lineage sampled in South Java. One of the two *P. tade* lineages concerned specimens sampled in Myanmar; the other lineage was sampled in northern Australia. These two regions being remote from the Red Sea, the type-locality of *P. tade* [32], we here followed a cautious line by designating as *Planiliza* sp. F the lineage from northern Australia, and *Planiliza* sp. I the lineage from Myanmar. Two sister-lineages were observed within *P. macrolepis*, separated by 3.5% (under the K2P model) or 3.7% (under the T93 + G + I model) net nucleotide divergence, all three markers combined: one lineage was exclusive to the western Indian Ocean west of the Seychelles Islands, including the Seychelles Islands and including South Africa, the type-locality [33] while the other lineage had a wide geographic distribution, as it consisted of all haplotypes sampled east of the Seychelles Islands, from the Maldives Islands to Fiji [13]. The latter is here provisionally designated as *Planiliza* sp. H, while its sister lineage retains the epithet *macrolepis*. Fig. 4E presents the geographic distribution of the two deep lineages within *P. macrolepis* (i.e., the actual *P. macrolepis*, and *Planiliza* sp. H). A lineage represented by a single individual from Taiwan (no. 108, “*Planiliza* sp.” of [18]) separated from its sister-lineage, *P. melinoptera*, by 7.2% nucleotide distance (under the K2P model) and 7.8% (under the TN93 + G + I model), all three markers combined, is here provisionally designated as *Planiliza* sp. G. Five other undetermined *Planiliza* species were here assigned provisional species names *Planiliza* sp. A (including individuals nos. 113 and 114 of [13]), *Planiliza* sp. C (nos. 103, 107), *Planiliza* sp. D (nos. 062, 062b), *Planiliza* sp. E (no. 057b), and *Planiliza* sp. J (no. 063). Our distinguishing *Planiliza* sp. J from its sister-lineage *Planiliza* sp. D is justified by the distance between the two lineages (2.6–2.8%, above the 2.5% threshold set for the genus).

Through automatic gap determination using the ABGD algorithm of [23], the present sequence dataset was found to conceal 10 separate lineages in genus *Chelon*, 6 in *Crenimugil*, 3 in *Dajaus*, 15 in the *M. cephalus* species complex, 6 in the *M. curema* species complex, 6 in *Osteomugil*, and 17 in *Planiliza*. The lineages designated by ABGD were all identical to those reported on Figs. 1 and 2, except for one lineage in *Planiliza* (*Planiliza* sp. J) that escaped detection using the default settings of ABGD.

3.4. Molecular diagnoses of species

The present results lead us to propose molecular diagnoses for a number of mugilid species currently considered as valid [17]. We aligned the partial sequences of the specimens characterized at the three loci (i.e., *16S*, *CO1* and *cytb*) used as phylogenetic markers [13,18] in each of 7 cases treated in the present study (i.e., *Chelon* spp., *Crenimugil* spp., *Dajaus* spp., species in the *Mugil cephalus* species complex, species in the *M. curema* species complex, *Osteomugil* spp. and *Planiliza* spp.). *Ellochelon* spp. was

Table 1

Nucleotide diagnostic of a lineage in the genera *Chelon*, *Crenimugil*, *Dajaus*, *Osteomugil* and *Planiliza*, and in the *Mugil cephalus* and *M. curema* species complexes. Based on the individual sequence data presented in [Supplementary material](#), Tables S1–S7 (locus 16S), Tables S8–S14 (locus CO1), and Tables S15–S21 (locus cytb).

Genus/species complex, Lineage	Locus	CO1	cytb
<i>Chelon</i> (JF911706)			
<i>C. auratus</i>	T1099 C1228 C1299	G129 T244 A247 T249 T267 A579 G591 G675	G240 T279 G387 T540 T580 A585 G699 C783
<i>C. bandialensis</i>	–	T393 C582 T666	T264 G303 T321 C381 T399 C426 T630 G714 C774
<i>C. dumerili</i>	A988 T1141 G1176 A1191 G1193 T1196 C1217 G1223 C1246 C1275 [T] G1296 [A] A1297 C1306 C1323 T1348 T1396	T114 G237 G324 C414 C609	C136 G221 A472 G498 G573 G576 T621 T625 T710 T717 T753 G771
<i>C. labrosus</i>	T1096 T1237	C132 T264 A276 T327 C552 G690	T232 T396 T561 T585 T597 C603 T669 G681 G690
<i>C. ramado</i>	–	T282 T336 C337 G396 T444 T450 T468 T630 G666 T699	T102 A108 C153 T237 C243 T303 T345 G471 T573 T624 G645 G753 A795
<i>C. richardsonii</i>	T1098 T1138 G1218	A231 A279 G360 G477 A624	T444 T476 A550 G609 G635 G641 T698 G795
<i>Chelon</i> sp. B	G1021 C1066 C1095 G1181 T1183 C1192 [ATC] T1203 C1234 G1270 G1290 [C]	C114 G228 A258 T273 T279 C285 C366 C390 G456 G672 T687	T129 A136 T138 G213 T216 C219 C245 A447 A453 C498 A501 T543 T612 T660 T715 T798
<i>Crenimugil</i> (JF911707)			
<i>C. buchanani</i>	T1174 T1199 T1236	T123 C270 C337 C381 A399 T447 C453 G465 G471 C507 G534 C555 T570 C609 T618 T642	C135 C261 A306 G339 A363 T420 G471 C507 T564 T567 T573 C580 T582 A583 T654 T655 C690 T693 C696 C714 G753 C789 T862 T873 A177 T315 T324 A483 T597 A657
<i>C. crenilabis</i>	T1340	C159 A348 G423 C486 G498 C654	T273 C306 G645 C747
<i>Crenimugil</i> sp. A	C1115 G1132 T1215	G129 C267 C279 T303 C366 G408 C525 T699	C369 T612 C804
<i>Crenimugil</i> sp. B	T1135	T246 T264 T270 G543	C138 G159 C171 T180 T216 A258 T267 A324
<i>Crenimugil</i> sp. C	C1203 C1298 T1364	T174 A177 A246 T345 T384 C411 T414 T438 T454 A456 G678	C345 A429 C438 T456 C477 T522 C528 A627 A630 T678 A693 T705 T765 G771 G789 C867 T225 T378 C390 C429 C453 T462 A472 A558 T784 T858
<i>Crenimugil</i> sp. D	T988 C992 A1010 C1043 C1063 T1066 G1069 G1175 G1205 T1207 C1221 T1226 [CA] A1298 T1315 [A] A1359 T1369 A1480	T111 T121 T234 T288 C372 G411 C433 T465 C531 T576 G585 A603 A612 C669 T700	T105 C108 T120 G144 T165 A178 C183 T204 T225 T243 C288 T303 T322 C345 G351 T384 T429 T459 G489 T522 T537 T555 G576 T621 T627 T669 A702 A718 A750 A783 T784 A798 T810 T834 C837 T846 T852 T102 G108 T117 A123 T138 A144 A171 T288 C303 T326 G343 T378 C426 G457 G471 T492 A498 C522 C528 C594 C597 T672 T715 C730 C741 T774 G783 G789 T795 C798 C804 G807 T840 T843
<i>Dajaus</i> (JF911702)			
<i>D. monticola</i>	C993 T994 G1138 G1139 T1300 T1319 G1331 T1503	C141 C162 C219 T252 C264 C282 C297 C306 T310 C315 T336 C351 G378 A408 G435 T447 C450 G516 A534 G558 C565 C660 C687 G690 G696	T105 C108 T120 G144 T165 A178 C183 T204 T225 T243 C288 T303 T322 C345 G351 T384 T429 T459 G489 T522 T537 T555 G576 T621 T627 T669 A702 A718 A750 A783 T784 A798 T810 T834 C837 T846 T852
<i>Dajaus</i> sp. A	T990 G1129 G1165 T1228 A1300	G129 G213 A222 A247 T249 C336 T351 T381 T435 T441 C447 C483 T528 A582 C591 T615 C636 T643 C654 G669	T102 G108 T117 A123 T138 A144 A171 T288 C303 T326 G343 T378 C426 G457 G471 T492 A498 C522 C528 C594 C597 T672 T715 C730 C741 T774 G783 G789 T795 C798 C804 G807 T840 T843
<i>Mugil cephalus</i> species complex (AP002930)			
<i>M. cephalus</i>	–	T234 T435 C693	T183 T483 G510
<i>M. liza</i>	–	–	G580 T657 C843
<i>Mugil</i> sp. A	–	G309 C312 A603	G582 A627 C653 A711
<i>Mugil</i> sp. B	T1223	C219 G357 G462 T611 T660	C384 C537 T708 A789 G831
<i>Mugil</i> sp. D	–	G267 C270	T318 T627 C719 C792 G849
<i>Mugil</i> sp. E	–	A390 G579	T516 T612 A637
<i>Mugil</i> sp. F	–	–	T492 C876
<i>Mugil</i> sp. G	T1208 T1244	A438	G219 C426 T444 T465 T507 T717 G810 G874
<i>Mugil</i> sp. H	G1216 A1218	–	C468
<i>Mugil</i> sp. I	T1216	A611	C300 T357 T777
<i>Mugil</i> sp. J	C1240 C1314	C342	G246 T312 T322 T636
<i>Mugil</i> sp. L	G1106 T1189 T1205 A1238 A1294 T1311	C210 C294 A330 T337 A348 G366 G393 T399 G400 A480 C510 T663	T237 C288 G333 T342 G522 T540 T600 T756 T765 T768
<i>Mugil</i> sp. Q	–	T552 C555 G597	T346 G390 A426 C738
<i>M. curema</i> species complex (JF911710)			
<i>M. curema</i>	G1180 C1232 C1279	C117 T153 A216 C270 T394 T411 C475	T121 T150 G219 C279 C318 T324 T357 G387 C426 T585 T678 C685 T713 C852
<i>M. incilis</i>	A979 T985 A993 T1029 C1056 G1082 G1139 A1191 T1194 T1200 C1202 T1206 G1209 A1214 A1215 C1218 G1224 T1226 C1233 T1234 [TATTTT] T1297 G1298 T1312	C108 T135 G144 G246 G339 T390 A429 C483 C504 C525 A534 C555 G606 T609 C642 A690 C700	C111 C153 C207 T216 G231 C286 G318 G331 A369 T399 G411 C429 T477 G501 T504 C537 T564 C567 G570 A585 A591 G609 T648 G681 C709 T724 T750 C810 C837 T862
<i>Mugil</i> sp. M	C1138 T1361	G105 G264 A390 T492	T225 T684 T687 T765 T846
<i>Mugil</i> sp. N	–	C142 T313 A420 T441 C552	T183 G234 T336 C396 A522 C555 T627 T819
<i>Mugil</i> sp. O	T1198 A1211 T1320	T183 G477 C579 G591 T604	T138 G159 T201 C204 T285 T840
<i>M. thoburni</i>	A1174	C174 A228 C429 G639 T654	G127 T129 T258 C561 G634 T663 T675 C747

Table 1 (Continued)

Genus/species complex, Lineage	Locus 16S	CO1	cytb
<i>Osteomugil</i> (JF911717)			
<i>O. cunnesius</i>	G ₁₂₁₃	G ₁₆₈ G ₃₃₉ T ₃₆₀ C ₃₆₆ T ₅₉₁ C ₆₀₆ G ₆₁₅ T ₆₆₃ G ₆₇₂ G ₆₈₄	G ₁₁₇ C ₁₃₇ C ₂₉₁ T ₃₀₆ C ₃₆₉ G ₄₄₇ T ₄₉₈ A ₅₁₉ T ₅₇₆ G ₅₇₉ T ₆₃₀ T ₆₈₄ A ₈₁₀ G ₈₃₁ T ₈₅₅
<i>O. engeli</i>	T ₉₉₈ C ₁₀₆₉ T ₁₀₈₆ T ₁₁₀₀ T ₁₁₁₉ T ₁₁₄₂ C ₁₁₄₃ G ₁₁₉₈ C ₁₂₀₃ T ₁₂₀₆ T ₁₂₂₀ A ₁₂₃₂ A ₁₂₇₀ G ₁₂₇₂ C ₁₂₇₇ A ₁₃₀₆ A ₁₃₂₆ C ₁₃₄₂ T ₁₃₅₂	C ₁₀₅ T ₁₁₇ G ₁₂₀ G ₁₂₃ C ₁₄₁ A ₁₇₇ C ₂₄₆ C ₃₁₂ T ₃₃₇ C ₃₉₃ C ₄₃₅ T ₄₄₁ T ₄₉₈ T ₅₅₅ G ₅₅₈ A ₅₆₄ A ₅₆₇ C ₆₂₁ G ₆₃₃ A ₆₆₆ T ₆₇₅	G ₁₁₄ A ₁₂₃ A ₁₄₇ G ₁₆₈ C ₁₉₈ T ₂₀₁ A ₂₂₈ G ₂₇₄ G ₃₇₃ C ₃₇₄ A ₃₈₇ C ₄₂₉ G ₄₄₁ G ₄₇₇ C ₅₈₀ T ₆₄₅ T ₆₄₈ T ₆₈₅ C ₇₀₅ T ₇₀₈ A ₇₂₃ T ₇₂₄ A ₇₅₃ T ₇₇₀ A ₈₆₄ T ₈₇₃
<i>O. perusii</i>	T ₁₃₀₇	T ₁₂₆ A ₂₈₅ T ₃₁₈ G ₄₀₈ T ₄₄₇ C ₅₂₅ G ₅₄₀ G ₆₃₀ T ₆₇₈	T ₃₆₃ T ₃₆₄ T ₄₄₅ A ₄₈₃ G ₅₈₂ C ₇₀₀
<i>O. robustus</i>	C ₉₈₄ T ₉₉₀ C ₉₉₃ C ₁₀₇₄ T ₁₀₈₇ C ₁₁₂₀ A ₁₁₄₁ T ₁₁₄₃ C ₁₁₈₂ G ₁₁₈₉ T ₁₂₀₄ G ₁₂₂₀ C ₁₂₂₇ T ₁₂₃₃ C ₁₂₇₄ T ₁₂₉₁ C ₁₃₁₀ G ₁₃₄₅ C ₁₃₅₁ [A] T ₁₄₃₀ A ₁₄₃₈	A ₁₂₃ C ₁₃₅ A ₁₃₆ T ₁₅₀ G ₂₉₁ A ₃₃₃ C ₃₆₀ A ₃₆₉ G ₃₇₈ T ₃₈₇ G ₄₃₅ C ₄₅₃ T ₄₆₂ A ₄₆₈ C ₄₈₀ T ₅₄₃ C ₅₄₉ G ₆₃₉ C ₆₅₄ T ₆₉₀	C ₁₃₆ C ₁₄₇ T ₁₆₂ C ₁₆₈ A ₁₇₄ T ₁₇₈ G ₁₈₀ A ₂₁₃ A ₂₃₄ C ₂₈₈ T ₃₂₆ G ₃₈₇ C ₃₉₀ C ₃₉₉ A ₄₁₇ T ₄₂₉ C ₄₅₈ G ₅₃₁ A ₅₇₄ A ₅₈₃ T ₅₈₈ G ₅₈₉ C ₅₉₁ T ₆₀₇ C ₆₃₅ T ₆₃₆ A ₆₅₅ T ₆₆₀ A ₆₈₈ T ₆₉₇ T ₆₉₈ T ₇₁₂ T ₇₁₃ T ₇₁₉ C ₇₂₃ C ₇₂₄ A ₇₃₆ T ₇₄₂ T ₇₄₇ C ₇₈₉ T ₈₄₁
<i>Planiliza</i> (JF911709)			
<i>P. affinis</i>	–	A ₃₁₅ G ₅₈₅	G ₁₁₄ A ₄₇₅ T ₅₄₆ G ₇₁₄ G ₇₂₉ T ₇₇₇
<i>P. alata</i>	G ₁₃₉₇ T ₁₃₃₆	T ₅₉₅	T ₁₁₅ C ₁₃₇ T ₁₄₄ A ₁₇₈ C ₂₆₅ G ₃₀₀ A ₃₅₅ C ₃₆₇ C ₄₅₈ T ₅₂₆ C ₅₂₈ T ₅₄₃ C ₅₆₂ G ₅₉₂ C ₅₉₆ T ₆₀₆ C ₆₉₃ G ₇₀₀ G ₇₀₉ T ₇₁₉ A ₇₃₈ A ₇₅₁ G ₇₇₈ A ₈₄₆ T ₂₇₆ G ₄₂₆ T ₆₁₂ T ₈₇₃
<i>P. haematocheila</i>	–	T ₃₉₄	G ₂₃₄ T ₈₂₃ C ₈₇₉
<i>P. macrolepis</i>	–	G ₂₄₃ T ₃₆₃	C ₃₅₇ T ₄₂₀ C ₈₆₇
<i>P. melinoptera</i>	–	T ₁₄₇ A ₄₅₀ A ₅₉₄	C ₁₃₆ A ₁₆₈ C ₂₀₇ A ₂₄₀ A ₂₅₈ C ₄₃₈ G ₄₈₉ T ₅₁₀ G ₅₇₇ T ₅₇₈ C ₅₉₅ A ₇₁₂ T ₈₅₈
<i>P. ordensis</i>	A ₁₁₉₈ G ₁₂₆₃ C ₁₂₆₅	C ₁₀₈ G ₁₃₂ A ₂₄₉ A ₃₁₂ A ₅₄₃ G ₅₇₆ T ₆₃₀ G ₆₇₂	T ₁₀₂ T ₃₀₃ A ₄₆₂ T ₅₇₀ A ₅₇₉ T ₆₂₄ G ₁₅₆ T ₃₈₄ G ₆₂₇ T ₆₆₀ C ₇₂₉ G ₇₇₄ G ₇₇₇ T ₅₈₂ G ₆₈₁
<i>Planiliza</i> sp. A	A ₁₀₆₈	G ₃₇₂ T ₄₇₇ A ₅₁₃ A ₆₁₂ C ₆₇₂	–
<i>Planiliza</i> sp. C	T ₁₀₄₁ C ₁₀₄₂ C ₁₀₉₂ G ₁₂₁₃ G ₁₃₉₈ A ₁₄₇₃	C ₁₃₈ G ₁₆₉ G ₃₀₀ C ₃₅₄	–
<i>Planiliza</i> sp. D	–	–	–
<i>Planiliza</i> sp. H	–	T ₂₆₇	–
<i>Planiliza</i> sp. I	C ₉₇₈ A ₉₈₄ C ₉₈₈ T ₉₉₂ G ₁₀₁₈ C ₁₀₄₀ A ₁₀₆₃ G ₁₀₆₉ C ₁₀₇₈ T ₁₀₉₆ C ₁₁₁₅ T ₁₁₃₈ C ₁₁₈₃ C ₁₁₈₆ C ₁₁₈₇ C ₁₁₈₈ [CAA] C ₁₁₉₅ G ₁₁₉₆ A ₁₁₉₉ C ₁₂₀₁ [TCAT] T ₁₂₁₉ C ₁₂₂₃ C ₁₂₃₇ T ₁₂₅₂ A ₁₂₅₈ A ₁₂₆₁ C ₁₂₆₃ G ₁₂₇₁ T ₁₂₇₅ G ₁₂₉₆ [TAC] A ₁₃₀₅ A ₁₃₀₈ A ₁₃₀₉ G ₁₃₃₃ T ₁₀₂₈ T ₁₁₃₆ T ₁₁₉₇ A ₁₃₇₁	T ₁₆₈ T ₁₈₉ T ₂₂₈ A ₃₃₀ A ₃₄₉ C ₃₅₀ A ₃₈₁ A ₃₉₀ T ₃₉₆ G ₄₃₈ A ₄₆₅ C ₄₇₄ T ₅₃₁	T ₁₈₂ A ₂₈₈ G ₃₁₈ G ₄₀₅ A ₄₂₉ T ₆₀₃ T ₆₈₄ T ₇₀₆ A ₇₂₁ G ₇₃₂ A ₇₇₁ T ₈₂₂ T ₈₅₅ T ₈₆₄
<i>P. subviridis</i>	–	T ₁₄₂ G ₁₇₇ T ₃₆₀ G ₄₅₉ G ₄₆₂ C ₄₈₀ T ₅₂₅ G ₆₃₀ G ₆₉₀	T ₁₁₄ T ₁₂₁ C ₅₇₄ T ₇₇₀

Subscript: nucleotide site number; *brackets* indicate the GenBank accession number (<http://www.ncbi.nlm.nih.gov/>) of the sequence of reference chosen for a genus or a species complex; *square brackets* determine inserts unique to a species in the genus or species complex. *Dash* no diagnostic nucleotide.

excluded because it consisted of two main lineages only, one of which was represented by a single individual in our dataset, thus insufficient for a comparison of inter-lineage vs intra-lineage variation. Variable nucleotide sites in each alignment were highlighted (Supplementary material, Tables S1–S21). Nucleotide sites diagnostic of species were determined. This information is summarized in Table 1. For example, *M. cephalus* L. is here diagnosed relative to the other species in the *M. cephalus* species complex by triplets (T₂₃₄, T₄₃₅, C₆₉₃) at locus CO1 and (T₁₈₃, T₄₈₃, G₅₁₀) at locus cytb, where nucleotide sites are numbered from the start of the gene, using the mitochondrial DNA sequence of *Mugil* sp. C (GenBank no. AP002930) as reference. Anonymous lineages designated by alphabetical letters were similarly diagnosed (Table 1). No molecular diagnosis was proposed for those species for which a single specimen was available: *Chelon saliens*, *C. tricuspidens*, *Osteomugil cunnesius*, and *Planiliza abu*. Similarly, no diagnosis was proposed for lineages *Chelon* sp. A, *Dajaus* sp. B, *Mugil* spp. C, K, *Osteomugil* sp. A, and *Planiliza* spp. E–G.

We are aware that future additional samples may lead to restricting the number of diagnostic sites for any given species relative to the other species in a genus. This is most

likely to occur if additional cryptic species are sampled. However, the information in Table 1 may still provide the basis to future identification keys.

4. Conclusion

The morphological features that delineate species in the family Mugilidae [34] are insufficient to describe its actual species diversity. This was documented in *Dajaus monticola*, where two sister lineages are geographically isolated from one the other by a continent [13,15], in *Mugil cephalus* from the South and East China Seas where the three lineages present characterize reproductively isolated species [14], and in *M. curema* where distinct lineages are characterized by distinct karyotypes [13]. The mitochondrial phylogeny of [13,18] reveals an additional proportion of deeply rooted lineages that by analogy with the foregoing, flag as many additional putative cryptic species.

Future population genetic investigations based on nuclear markers are expected to provide clues to the degree of reproductive isolation between the populations harbouring separate mitochondrial lineages, in the cases where populations are sympatric or parapatric. In the case of allopatric lineages, reproductive isolation cannot be

tested directly, hence additional lines of evidence would be necessary to distinguish species (e.g., [10,35]). Pending possible confirmation that the deeply divergent lineages listed in this paper are cryptic species, we anticipate changes to the current species nomenclature of the Mugilidae. Although new species descriptions might eventually be necessary in some cases, it will be first necessary to evaluate the validity of available names formerly assigned to a proportion of the lineages and subsequently considered junior synonyms. Epithets to be considered *a priori* for possible resurrection should be based on geography, i.e. by ensuring that the type-locality lies within the geographic range of the lineage, and on chronological priority [36].

Molecular genetic surveys of species in the Mugilidae may help uncover additional deep lineages. DNA-barcoding surveys potentially represent such opportunities [37]. For this purpose, it will be first necessary to evaluate the ability of the *COI* fragment used as barcode, to identify deep lineages that represent species or potential cryptic species in the Mugilidae.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.crv.2015.01.007>.

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