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

La phylogénie mitochondriale des mulets (Acanthopterygii: Mugilidae) suggère une forte proportion d'espèces cryptiques

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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.

Mots clés :
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RÉSUMÉ

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 mulets (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 avions 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.

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 buchanani* 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 tricuspidens* 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*, *Gracilimugil*, *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://wwwabi.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. crenilabis* 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

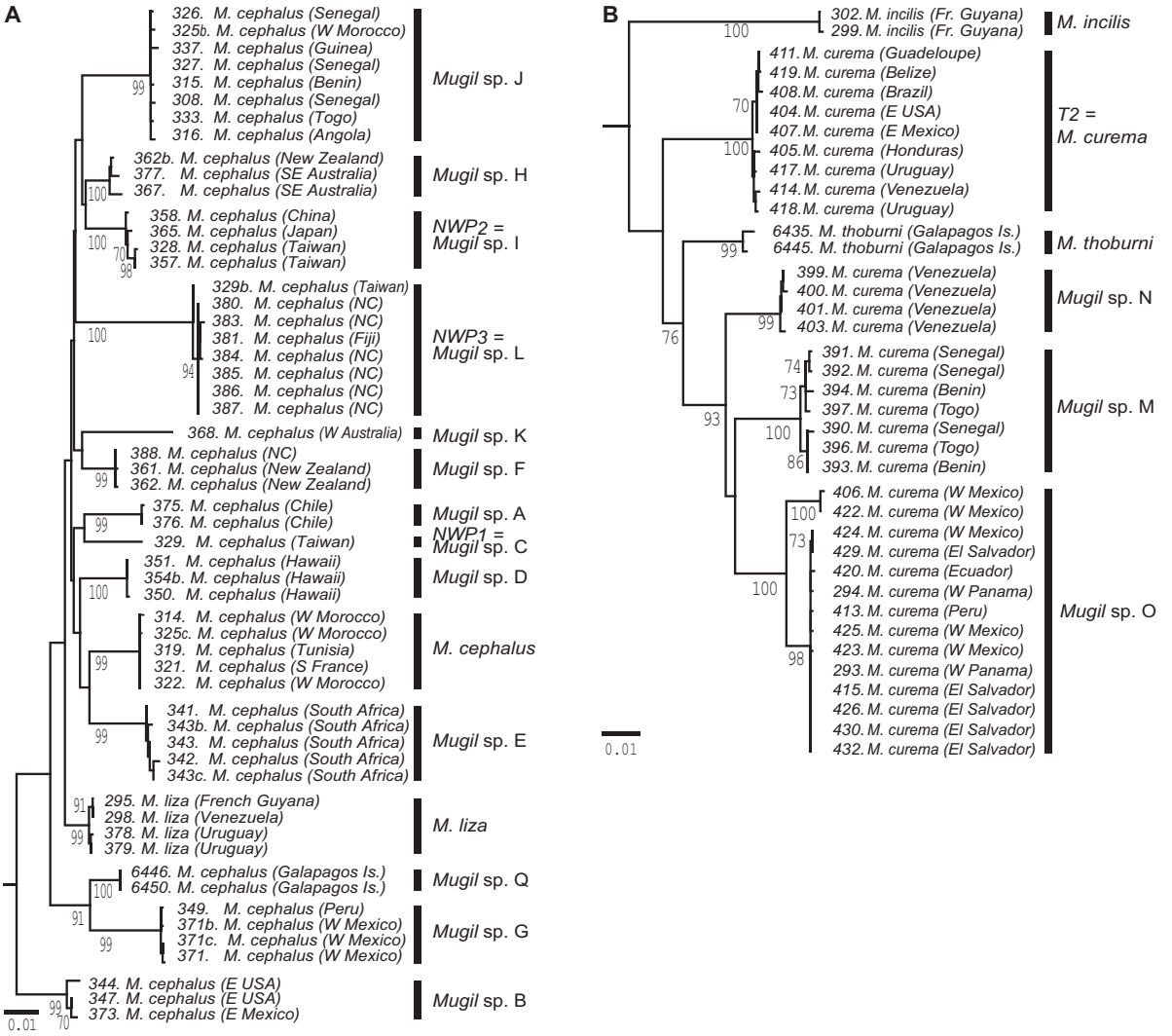

Fig. 2. Phylogenetic trees of species in the genus *Mugil*, constructed from partitioned maximum-likelihood (ML) analysis of 3885 aligned nucleotides from *16S rRNA*, *COI* and *cytb* gene sequences [13]. Bootstrap scores >50% are indicated. Vertical bars on the right of the tree indicate statistically 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

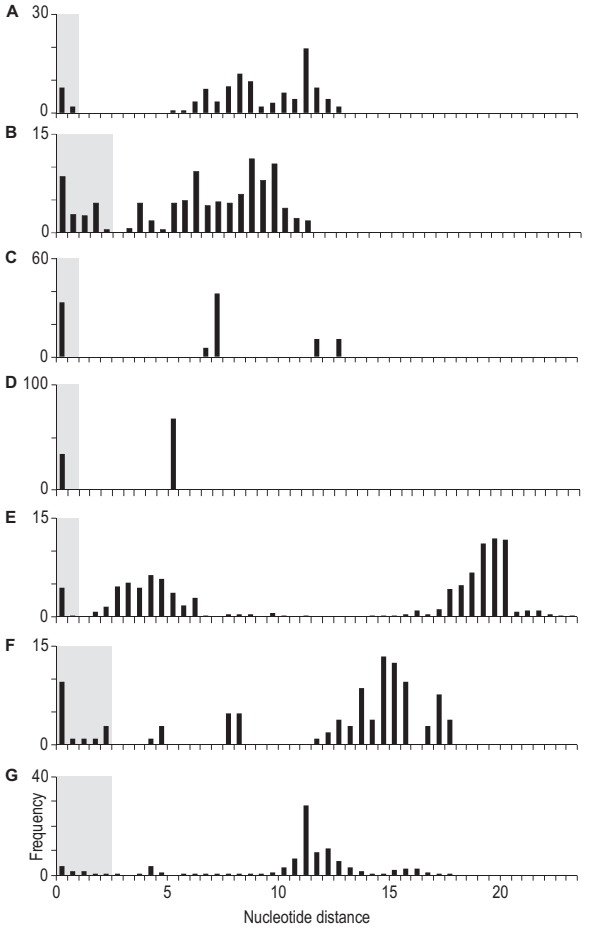

Fig. 3. Frequency distribution of pairwise nucleotide distance estimates (TN93 + G + I model; MEGA5 [20]) among individuals within each of 7 mugilid genera. *Shaded rectangles* highlight pairwise nucleotide distances $\leq$ 1% (in *Chelon*, *Dajaus*, *Ellochelon*, and *Mugil*) or $\leq$2.5% (in *Crenimugil*,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

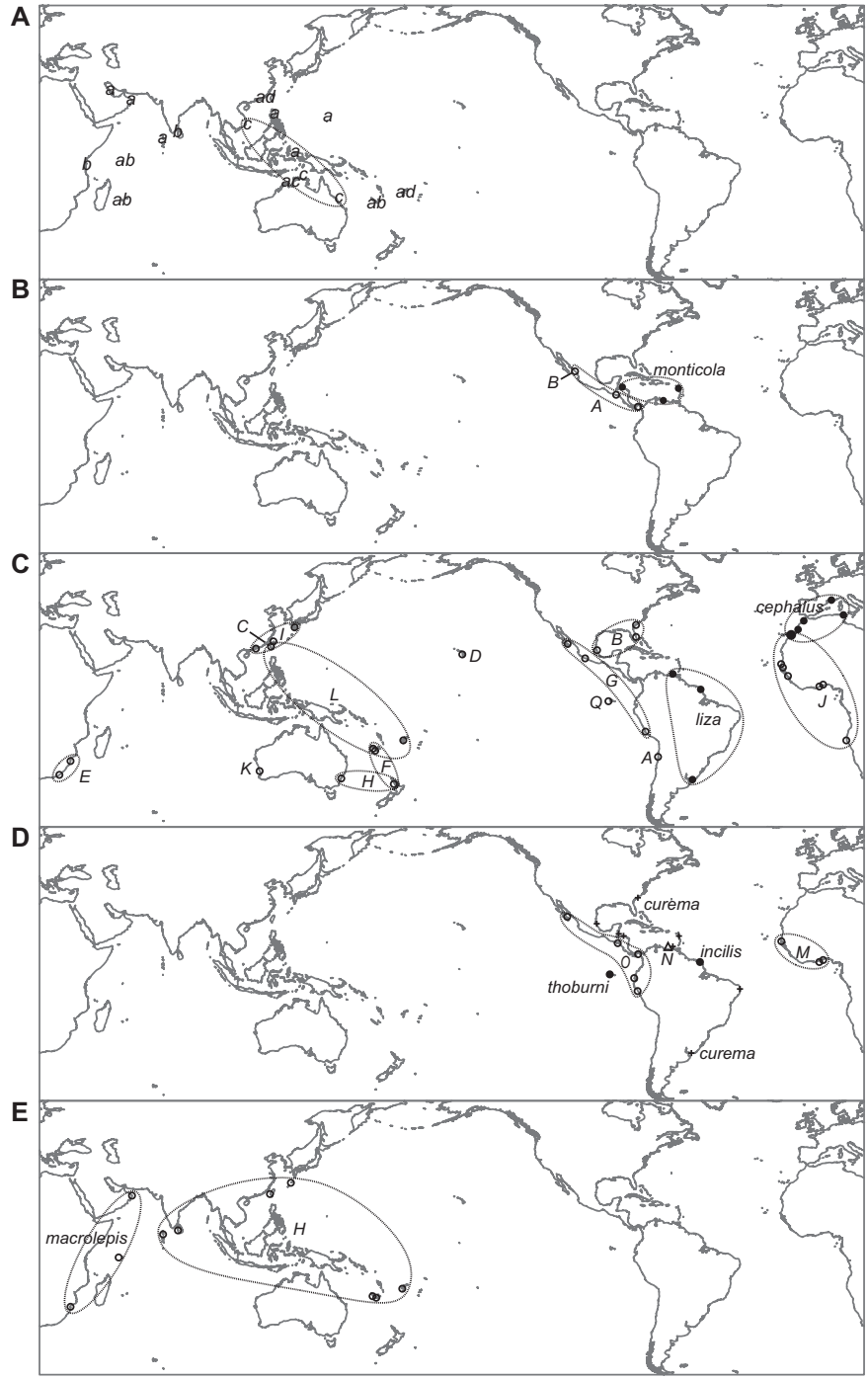

Fig. 4. Geographic distribution of mitochondrial lineages (putative species) in grey mullets, based on the sampling of [13,18] (Figs. 1 and 2). Background map of the Indo-West Pacific was obtained from Digital Vector Maps, San Diego (http://digital-vector-maps.com/). A. Lineages of the *Crenimugil seheli* species complex. *a–c*, putative cryptic species *Crenimugil* spp. A to C, respectively; *doted 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 *16S*	*CO1*	*cytb*
Chelon (JF911706)			
C. auratus	T_{1099} C_{1228} C_{1299}	G_{129} T_{244} A_{247} T_{249} T_{267} A_{579} G_{591} G_{675}	G_{240} T_{279} G_{387} T_{540} T_{580} A_{585} G_{699} C_{783}
C. bandialensis	–	T_{393} C_{582} T_{666}	T_{264} G_{303} T_{321} C_{381} T_{399} C_{426} T_{630} G_{714} C_{774}
C. dumerili	A_{988} T_{1141} G_{1176} A_{1191} G_{1193} T_{1196} C_{1217} G_{1223} C_{1246} C_{1275} [T] G_{1296} [A] A_{1297} C_{1306} C_{1323} T_{1348} T_{1396}	T_{114} G_{237} G_{324} C_{414} C_{609}	C_{136} G_{221} A_{472} G_{498} G_{573} G_{576} T_{621} T_{625} T_{710} T_{717} T_{753} G_{771}
C. labrosus	T_{1096} T_{1237}	C_{132} T_{264} A_{276} T_{327} C_{552} G_{690}	T_{232} T_{396} T_{561} T_{585} T_{597} C_{603} T_{669} G_{681} G_{690}
C. ramado	–	T_{282} T_{336} C_{337} G_{396} T_{444} T_{450} T_{468} T_{630} G_{666} T_{699}	T_{102} A_{108} C_{153} T_{237} C_{243} T_{303} T_{345} G_{471} T_{573} T_{624} G_{645} G_{753} A_{795}
C. richardsonii	T_{1098} T_{1138} G_{1218}	A_{231} A_{279} G_{360} G_{477} A_{624}	T_{444} T_{476} A_{550} G_{609} G_{635} G_{641} T_{698} G_{795}
Chelon sp. B	G_{1021} C_{1066} C_{1095} G_{1181} T_{1183} C_{1192} [ATC] T_{1203} C_{1234} G_{1270} G_{1290} [C]	C_{114} G_{228} A_{258} T_{273} T_{279} C_{285} C_{366} C_{390} G_{456} G_{672} T_{687}	T_{129} A_{136} T_{138} G_{213} T_{216} C_{219} C_{245} A_{447} A_{453} C_{498} A_{501} T_{543} T_{612} T_{660} T_{715} T_{798}
Crenimugil (JF911707)			
C. buchanani	T_{1174} T_{1199} T_{1236}	T_{123} C_{270} C_{337} C_{381} A_{399} T_{447} C_{453} G_{465} G_{471} C_{507} G_{534} C_{555} T_{570} C_{609} T_{618} T_{642}	C_{135} C_{261} A_{306} G_{339} A_{363} T_{420} G_{471} C_{507} T_{564} T_{567} T_{573} C_{580} T_{582} A_{583} T_{654} T_{655} C_{690} T_{693} C_{696} C_{714} G_{753} C_{789} T_{862} T_{873}
C. crenilabis	T_{1340}	C_{159} A_{348} G_{423} C_{486} G_{498} C_{654}	A_{177} T_{315} T_{324} A_{483} T_{597} A_{657}
Crenimugil sp. A	C_{1115} G_{1132} T_{1215}	G_{129} C_{267} C_{279} T_{303} C_{366} G_{408} C_{525} T_{699}	T_{273} C_{306} G_{645} C_{747}
Crenimugil sp. B	T_{1135}	C_{246} T_{264} T_{270} G_{543}	C_{369} T_{612} C_{804}
Crenimugil sp. C	C_{1203} C_{1298} T_{1364}	T_{174} A_{177} A_{246} T_{345} T_{384} C_{411} T_{414} T_{438} T_{454} A_{456} G_{678}	C_{138} G_{159} C_{171} T_{180} T_{216} A_{258} T_{267} A_{324} C_{345} A_{429} C_{438} T_{456} C_{477} T_{522} C_{528} A_{627} A_{630} T_{678} A_{693} T_{705} T_{765} G_{771} G_{789} C_{867}
Crenimugil sp. D	T_{988} C_{992} A_{1010} C_{1043} C_{1063} T_{1066} G_{1069} G_{1175} G_{1205} T_{1207} C_{1221} T_{1226} [CA] A_{1298} T_{1315} [A] A_{1359} T_{1369} A_{1480}	T_{111} T_{121} T_{234} T_{288} C_{372} G_{411} C_{433} T_{465} C_{531} T_{576} G_{585} A_{603} A_{612} C_{669} T_{700}	T_{225} T_{378} C_{390} C_{429} C_{453} T_{462} A_{472} A_{558} T_{784} T_{858}
Dajaus (JF911702)			
D. monticola	C_{993} T_{994} G_{1138} G_{1139} T_{1300} T_{1319} G_{1331} T_{1503}	C_{141} C_{162} C_{219} T_{252} C_{264} C_{282} C_{297} C_{306} T_{310} C_{315} T_{336} C_{351} G_{378} A_{408} G_{435} T_{447} C_{450} G_{516} A_{534} G_{558} C_{565} C_{660} C_{687} G_{690} G_{696}	T_{105} C_{108} T_{120} G_{144} T_{165} A_{178} C_{183} T_{204} T_{225} T_{243} C_{288} T_{303} T_{322} C_{345} G_{351} T_{384} T_{429} T_{459} G_{489} T_{522} T_{537} T_{555} G_{576} T_{621} T_{627} T_{669} A_{702} A_{718} A_{750} A_{783} T_{784} A_{798} T_{810} T_{834} C_{837} T_{846} T_{852}
Dajaus sp. A	T_{990} G_{1129} G_{1165} T_{1228} A_{1300}	G_{129} G_{213} A_{222} A_{247} T_{249} C_{336} T_{351} T_{381} T_{435} T_{441} C_{447} C_{483} T_{528} A_{582} C_{591} T_{615} C_{636} T_{643} C_{654} G_{669}	T_{102} G_{108} T_{117} A_{123} T_{138} A_{144} A_{171} T_{288} C_{303} T_{326} G_{343} T_{378} C_{426} G_{457} G_{471} T_{492} A_{498} C_{522} C_{528} C_{594} C_{597} T_{672} T_{715} C_{730} C_{741} T_{774} G_{783} G_{789} T_{795} C_{798} C_{804} G_{807} T_{840} T_{843}
Mugil cephalus species complex (AP002930)			
M. cephalus	–	T_{234} T_{435} C_{693}	T_{183} T_{483} G_{510}
M. liza	–	-	G_{580} T_{657} C_{843}
Mugil sp. A	–	G_{309} C_{312} A_{603}	G_{582} A_{627} C_{653} A_{711}
Mugil sp. B	T_{1223}	C_{219} G_{357} G_{462} T_{611} T_{660}	C_{384} C_{537} T_{708} A_{789} G_{831}
Mugil sp. D	–	G_{267} C_{270}	T_{318} T_{627} C_{719} C_{792} G_{849}
Mugil sp. E	–	A_{390} G_{579}	T_{516} T_{612} A_{637}
Mugil sp. F	–	–	T_{492} C_{876}
Mugil sp. G	T_{1208} T_{1244}	A_{438}	G_{219} C_{426} T_{444} T_{465} T_{507} T_{717} G_{810} G_{874}
Mugil sp. H	G_{1216} A_{1218}	–	C_{468}
Mugil sp. I	T_{1216}	A_{611}	C_{300} T_{357} T_{777}
Mugil sp. J	C_{1240} C_{1314}	C_{342}	G_{246} T_{312} T_{322} T_{636}
Mugil sp. L	G_{1106} T_{1189} T_{1205} A_{1238} A_{1294} T_{1311}	C_{210} C_{294} A_{330} T_{337} A_{348} G_{366} G_{393} T_{399} G_{400} A_{480} C_{510} T_{663}	T_{237} C_{288} G_{333} T_{342} G_{522} T_{540} T_{600} T_{756} T_{765} T_{768}
Mugil sp. Q	–	T_{552} C_{555} G_{597}	T_{346} G_{390} A_{426} C_{738}
M. curema species complex (JF911710)			
M. curema	G_{1180} C_{1232} C_{1279}	C_{117} T_{153} A_{216} C_{270} T_{394} T_{411} C_{475}	T_{121} T_{150} G_{219} C_{279} C_{318} T_{324} T_{357} G_{387} C_{426} T_{585} T_{678} C_{685} T_{713} C_{852}
M. incilis	A_{979} T_{985} A_{993} T_{1029} C_{1056} G_{1082} G_{1139} A_{1191} T_{1194} T_{1200} C_{1202} T_{1206} G_{1209} A_{1214} A_{1215} C_{1218} G_{1224} T_{1226} C_{1233} T_{1234} [TATTTT] T_{1297} G_{1298} T_{1312}	C_{108} T_{135} G_{144} G_{246} G_{339} T_{390} A_{429} C_{483} C_{504} C_{525} A_{534} C_{555} G_{606} T_{609} C_{642} A_{690} C_{700}	C_{111} C_{153} C_{207} T_{216} G_{231} C_{286} G_{318} G_{331} A_{369} T_{399} G_{411} C_{429} T_{477} G_{501} T_{504} C_{537} T_{564} C_{567} G_{570} A_{585} A_{591} G_{609} T_{648} G_{681} C_{709} T_{724} T_{750} C_{810} C_{837} T_{862}
Mugil sp. M	C_{1138} T_{1361}	G_{105} G_{264} A_{390} T_{492}	T_{225} T_{684} T_{687} T_{765} T_{846}
Mugil sp. N	–	C_{142} T_{313} A_{420} T_{441} C_{552}	T_{183} G_{234} T_{336} C_{396} A_{522} C_{555} T_{627} T_{819}
Mugil sp. O	T_{1198} A_{1211} T_{1320}	T_{183} G_{477} C_{579} G_{591} T_{604}	T_{138} G_{159} T_{201} C_{204} T_{285} T_{840}
M. thoburni	A_{1174}	C_{174} A_{228} C_{429} G_{639} T_{654}	G_{127} T_{129} T_{258} C_{561} G_{634} T_{663} T_{675} C_{747}

Table 1 (*Continued*)

Genus/species complex, Lineage	Locus *16S*	*CO1*	*cytb*
Osteomugil (JF911717)			
O. cunnesius	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}
O. engeli	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} $C1_{98}$ 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}
O. perusii	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}
O. robustus	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}
Planiliza (JF911709)			
P. affinis	–	A_{315} G_{585}	G_{114} A_{475} T_{546} G_{714} G_{729} T_{777}
P. alata	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}
P. haematocheila	–	T_{394}	T_{276} G_{426} T_{612} T_{873}
P. macrolepis	–	G_{243} T_{363}	G_{234} T_{823} C_{879}
P. melinoptera	–	T_{147} A_{450} A_{594}	C_{357} T_{420} C_{867}
P. ordensis	A_{1198} G_{1263} C_{1265}	C_{108} G_{132} A_{249} A_{312} A_{543} G_{576} T_{630} G_{672}	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}
Planiliza sp. A	A_{1068}	G_{372} T_{477} A_{513} A_{612} C_{672}	T_{102} T_{303} A_{462} T_{570} A_{579} T_{624}
Planiliza sp. C	T_{1041} C_{1042} C_{1092} G_{1213} G_{1398} A_{1473}	C_{138} G_{169} G_{300} C_{354}	G_{156} T_{384} G_{627} T_{660} C_{729} G_{774} G_{777} T_{582} G_{681}
Planiliza sp. D	–	–	–
Planiliza sp. H	–	T_{267}	–
Planiliza 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_{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}
P. subviridis	T_{1028} T_{1136} T_{1197} A_{1371}	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 (T_{234}, T_{435}, C_{693}) at locus *CO1* and (T_{183}, T_{483}, G_{510}) 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.crvi.2015.01.007.

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