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Cranial morphometrics and mitochondrial DNA sequences distinguish cryptic species of the longface emperor (*Lethrinus olivaceus*), an emblematic fish of Indo-West Pacific coral reefs

*Deux espèces cryptiques chez le léthrinus olive (*Lethrinus olivaceus*), un poisson emblématique des récifs coralliens de l'Indo-Pacifique, identifiées à partir de la morphométrie du crâne et des séquences de l'ADN mitochondrial*

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ABSTRACT

Range-wide morphometric variability (cranial measurements) and genetic variability (nucleotide sequences of the cytochrome *b* gene) were investigated in the longface emperor, *Lethrinus olivaceus* (Lethrinidae), an emblematic large predatory fish of Indo-West Pacific coral reefs. Two cranial morphotypes were observed, one present from the Indian Ocean to the Coral Triangle and the other one, from the Coral Triangle to the western Central Pacific. The two morphotypes are concordant with reciprocally monophyletic mitochondrial lineages separated by 9.5% net nucleotide distance. These results suggest an old evolutionary history for *L. olivaceus*, that consists of two distinct species (*Lethrinus* sp. A in the Indian Ocean and Coral Triangle, *Lethrinus* sp. B in the western Pacific Ocean), whose distribution ranges meet or overlap in the eastern part of the Coral Triangle, in Taiwan and in West Papua. *Lethrinus* sp. A comprises two distinct mitochondrial lineages separated by 1.7% net nucleotide distance, one exclusive to the populations from the Indian Ocean, the other exclusive to Coral Triangle populations. The latter observation might be explained by vicariance, whereby the two lineages have been isolated from one another on either side of the Sunda Shelf because of low sea level in the Pleistocene. To clarify the nomenclature of this species complex, we recommend sequencing a fragment of the cytochrome *b* gene of the holotypes of *L. olivaceus* and of its first junior synonyms *L. rostratus* and *L. waigiensis*.

Keywords: cytochrome *b*; Lethrinidae; nomenclature; phylogeography; taxonomy

RESUME

La variabilité morphométrique (mensurations du crâne) et la variabilité génétique (séquences nucléotidiques du gène du cytochrome *b*) du léthrinus olive, *Lethrinus olivaceus* (Lethrinidae), un grand poisson prédateur emblématique des récifs coralliens de l'Indo-ouest Pacifique, ont été étudiées à l'échelle de sa distribution géographique. Deux morphotypes crâniens ont été observés, l'un présent de l'Océan Indien au Triangle de Corail, l'autre du Triangle de Corail au Pacifique central ouest. Ces deux morphotypes correspondent à deux lignées mitochondriales réciproquement monophylétiques, séparées l'une de l'autre par une distance nucléotidique nette de 9.5 %. Ces résultats suggèrent une histoire évolutive très ancienne pour *L. olivaceus*, qui comprend ainsi, en fait, deux espèces distinctes (*Lethrinus* sp. A dans l'Océan Indien et le Triangle de Corail, *Lethrinus* sp. B dans l'Océan Pacifique ouest) dont les aires de répartition se jouxtent ou se superposent à Taiwan et en Papouasie Occidentale, soit dans la partie orientale du Triangle de Corail. *Lethrinus* sp. A comprend lui-même deux lignées mitochondriales distinctes, séparées par une distance nucléotidique nette de 1.7 %, l'une exclusive des populations de l'Océan Indien, l'autre exclusive des populations du Triangle de Corail. Ce dernier résultat pourrait être expliqué par un événement de vicariance où les deux lignées sont restées isolées de part et d'autre du plateau de la Sonde du fait de la baisse du niveau marin au Pléistocène. Afin de fixer la nomenclature du complexe d'espèces, nous recommandons d'identifier en priorité les holotypes de *L. olivaceus* et de ses premiers synonymes *L. rostratus* et *L. waigiensis* à partir des séquences partielles du gène du cytochrome *b*.

Mots-clés : cytochrome *b* ; Lethrinidae ; nomenclature ; phylogéographie ; taxinomie

1. Introduction

The longface emperor, originally described by A. Valenciennes [1] as the “*léthrinus olive*” from a specimen collected in the Sunda Strait by J.R.C. Quoy and P. Gaimard is the largest and the longest-snouted lethrinid [2,3]. This carnivorous fish, whose adult size commonly reaches 70 cm, dwells on the sandy shores, reef slopes and lagoons of the tropical Indo-West Pacific [2-6]. It is a species of prime commercial importance throughout the region [2]. Among the many other *Lethrinus* spp. species described by A. Valenciennes [1], three, namely *L. rostratus*, from the East Indies (“Batavia”), *L. waigiensis* from Waigeo Island, West Papua, and *L. xanthopterus* from Ulea, Caroline Islands, represent the same species and have all been synonymised with *L. olivaceus* [2]. The existing type material for these species actually consists of individuals of small size that have been poorly preserved, except the holotype of *L. olivaceus* ([2]; Fig. 1). Given the circumstances, meaningful morphological differences between these type specimens, if any, arguably may have gone unnoticed. No type specimen is known for *L. xanthopterus* and A. Valenciennes’ short description of this species only refers to “*les riches collections de dessins que MM. de Mertens et Kettlitz ont bien voulu nous communiquer à leur retour du dernier voyage des Russes autour du monde*” [1]. R.L. Playfair and A.C.L.G. Günther later described *L. longirostris* from Zanzibar [7] which was eventually considered as yet another junior synonym of *L. olivaceus* [2,8].

A phylogenetic tree based on nucleotide sequence variation of the mitochondrial control region in several *Lethrinus* species illustrated a deep split between *L. olivaceus* haplotypes from Bali and New Caledonia [9], suggesting the possible occurrence of a cryptic species. In this study, we further investigated morphological variation in *L. olivaceus*. Mitochondrial DNA haplotype (cytochrome *b* gene) sequences for samples from these and from additional locations throughout the Indo-West Pacific were also scored. The combined evidence from morphology and phylogeography allowed us to address taxonomic issues in this species.

2. Materials and Methods

Fifty four *Lethrinus olivaceus* individuals were sampled in 2006-2011 from ten localities in the Indo-West Pacific (Fig. 2) as part as an ongoing program on the multiple-species phylogeography of the Lethrinidae. Identification to species was made on the basis of the distinctive morphological and meristic features given by K.E.C. and G.R. Allen [2], which include the ratio of head length to body depth and the count of scale rows between the lateral line and the basis of the first dorsal fin. Voucher specimens (Fig. 1, Appendix 1) included one individual from Sorong (West Papua) shipped frozen to LIPI-Oceanografi in Ancol (Indonesia), one specimen from Moruroa (French Polynesia) shipped frozen to IRD UR 227 in Nouméa (New Caledonia) and subsequently fixed in formalin and deposited at Museum national d’histoire naturelle, Paris (France) under collection no. MNHN 2009-0016, and the skulls and jawbones of 36 individuals from the Maldives, Bali, West Papua, New Caledonia, and Moruroa, now preserved at IRD-UR

227 in Montpellier (France). Two *L. microdon* individuals were also sampled from the Baa atoll (Maldives) and the Kedonganan fish market (Bali), respectively.

The samples analyzed for morphometric variation consisted of heads or carcasses of *L. olivaceus* purchased from the Male (Maldives) ($N=1$), Kedonganan ($N=4$), Sorong ($N=1$), and Nouméa ($N=22$) fish markets in 2007-2008. To prepare the neurocrania and the jawbones, the specimens were boiled, and the boiled skin and flesh were then separated from the bone using forks, fingers, twizzles, toothpicks, toothbrushes and a wash bottle. The specimens sampled in the Moruroa lagoon, June 2006 ($N=8$) were shipped frozen to Noumea and the heads were spared for similar processing. Neurocrania of individuals from Bali ranged in condylo-basal length from 81.2 mm to 111.9 mm; those from New Caledonia, from 80.9 mm to 102.2 mm; and those from Moruroa, from 86.6 mm to 126.3 mm. The neurocranium of the single specimen examined from the Maldives was 130.8 mm long; that of the single specimen examined from West Papua was 129.8 mm long. Nine morphometric variables (Fig. 3A) were measured on the neurocranium and jawbones. To normalize the variables and standardize them for size, prior to analysis each was log-transformed and divided by the log-transformed condylo-basal length [11,12]. We used principal component analysis (PCA) [13] to determine whether longface emperor populations were craniometrically distinct. Principal component analysis was run using the ADE-4 software [14].

The choice of the neurocranium (instead of, e.g. the whole fish) to study geographic variation in morphology in *L. olivaceus* was dictated by site accessibility and size of specimens. In some of the places where they were sampled (Maldives and New Caledonia), emperors were filleted and the carcasses were sold separately, hampering the provision of entire specimens. Specimens of this species are usually of such large size that preserving them whole and shipping them poses a challenge when operating from remote field sites. Neurocrania, however, have the advantage that once cleaned and dried, they do not necessitate specific preservation conditions nor much storing space.

Fin clips of a number of samples ($N=28$; Appendix 1) were preserved dry or in 95% ethanol and an aliquot of these was shipped to the Marine Phylogenomics Laboratory, Taipei, for DNA extraction and sequencing analysis. DNA was extracted from subsamples of the fin clips using the Qiagen DNAeasy extraction kit (Qiagen, Valencia CA, U.S.A.) according to the manufacturer's instructions. The concentration of extracted DNA was measured by spectrophotometry using an Eppendorf spectrophotometer (Eppendorf, Hamburg, Germany). Conditions for amplification by polymerase chain reaction (PCR) were as follows: 0.5 U GoTaq® Flexi DNA polymerase (Promega, Madison, U.S.A.), 1x reaction buffer, 2 mM MgCl₂, 200 μ M each dNTP, 0.2 μ M each primer, and 20–50 ng genomic DNA in 25 μ L final reaction volume. Primers *MT14673F* (5'-TAACCAGGACTAATGGCTTGAAA-3') and *Let15856R* (5'-GAGCTACTAGTGCATNGTCAKCC-3') designed by W.-J.C. (this study) were used for amplifying the entire cytochrome *b* gene. Thermocycling conditions for PCR were: initial denaturing step at 95°C for 4 min followed by 35 cycles of 95°C (for 40 s), annealing at 55 °C (for 40 s), and 72°C (for 1.5 min), and a final extension step of 72°C (for 7 min) before thawing at 4°C. The PCR product was purified using AMPure magnetic beads (Agencourt Bioscience Corp., Beverly MA, U.S.A.) and resuspended in 30 μ L sterile water. Nucleotide sequences of the PCR-amplified DNA fragments were then determined using

the sequencing facilities of the National Taiwan University and Genomics Inc. (Taipei, Taiwan) using ABI 3730xl analyzers (Applied Biosystems, Foster City CA, U.S.A.). Nucleotide sequences were edited and managed using the CODONCODE ALIGNER v. 3.0.7 (CodonCode Corporation, Centerville MA) and SE-AL v. 2.0a11 [15] together with sequences AF381265 (*L. microdon*), AF381252 (*L. olivaceus*), AF381253 (*L. reticulatus*), and AF24075 and AF381263 (*L. rubrioperculatus*) retrieved from GENBANK (<http://www.ncbi.nlm.nih.gov>). The final matrix of aligned sequences used for phylogenetic analysis, representing a total of 33 haplotypes, was 1107 base pairs in length. *L. microdon*, *L. reticulatus* and *L. rubrioperculatus* were selected as outgroups because of their close evolutionary affinity to *L. olivaceus* [16]. Phylogenetic analyses were based on a partitioned maximum likelihood (ML) method as implemented in RAxML 7.0.4 [17]. A mixed model analysis was used that allows the independent estimation of individual models of nucleotide substitution for each partition (by codon position). A GTR+G model for each partition was used and an Optimal ML tree search was made with 100 separate runs using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees in each run by comparing likelihood scores under the GTR+G model. To evaluate the robustness of the internal branches of the ML tree, 1,000 bootstrap replications were run for the data set under the GTR+ G model. Ancillary descriptive statistics were computed using the FREE STATISTIC SOFTWARE (CALCULATOR) software [18] and the MEGA5 software [19].

3. Results

Values for cranial morphometric variables in 36 *L. olivaceus* specimens are presented in Appendix 2. Principal component (PC) 1 (not shown) from PCA on cranial morphometric variables was correlated with condylo-basal length (Spearman's rank correlation [18,20]: $\rho=0.838$; $P<0.001$). This suggests a strong and expected effect of growth allometry. The pattern defined by PC 2 and PC 3 (Fig. 3B) segregated the individuals by geographic origin. All individuals from New Caledonia and Moruroa (Pacific Ocean) were grouped into a single cluster, albeit with some statistical differences between the two populations. Individuals sampled in Bali were clustered separately (Fig. 3B). The single specimen from West Papua and the single specimen from the Maldives were positioned close to the Bali cluster along PC 2 (Fig. 3B).

Differences in neurocranial morphology are likely to translate into differences in head shape. The few pictures available (some of which are presented in Fig. 1) suggest that *Lethrinus* sp. B has a more slender snout than *Lethrinus* sp. A.

Cytochrome *b* gene sequences from 27 *L. olivaceus* individuals sampled across the species range allowed the reconstruction of the phylogeny of mitochondrial lineages distribution-wide (Fig. 4). Two major clades were observed: one clade comprised mitochondrial haplotypes sampled from the Pacific Ocean, including two locations from the eastern Coral Triangle (Taiwan and West Papua); the other clade consisted exclusively of haplotypes sampled in the Indian Ocean (forming an exclusive sub-clade) and in the Coral Triangle (forming another sub-clade). The specimen from West Papua that was used for the morphometric analysis (Gutilla 12: Appendices 1-3) was found to belong to the 'Coral Triangle' sub-clade.

The net nucleotide distance (Kimura-2 parameter distance [19]) between the ‘Pacific Ocean’ clade and the (‘Indian Ocean’ + ‘Coral Triangle’) clade of *L. olivaceus* was 0.095. The net distance between the ‘Indian-Ocean’ and ‘Coral-Triangle’ subclades was 0.017. Nucleotide sites diagnostic between the ‘Pacific Ocean’ clade vs. the (‘Indian Ocean’ + ‘Coral Triangle’) clade are highlighted in Fig. 5.

4. Discussion

In the only molecular phylogeny of species of the genus *Lethrinus* published to date [16], the nucleotide divergence at the cytochrome *b* locus between sister species at the extremity of the tree was comprised between 7% and 14%. The lowest value in this comparison (7%, between *L. lenjan* and *L. ornatus*) separates lethrinid species which are morphologically distinct by current taxonomic standards [2]. Using this as a yardstick, the deep branching in the phylogenetic tree (Fig. 4) and the associated nucleotide divergence between the ‘Indian Ocean-Coral Triangle’ and ‘Pacific Ocean’ forms of *L. olivaceus* (9.5%; greater than 7%) indicate distinct species. These two species are here provisionally designated as *Lethrinus* sp. A and *Lethrinus* sp. B, respectively. The distinction of two species is further supported by morphometric differences.

The level of divergence between the ‘Indian-Ocean’ and the ‘Coral-Triangle’ forms of *Lethrinus* sp. A (1.7%), although much lower than morphologically recognizable species pairs in Lethrinidae, may nevertheless be high enough to compare with Indo-West Pacific species pairs in other phylogenetically well-documented fish families, e.g. Acanthuridae [21], Chaetodontidae [22], and Siganidae [23,24]. Pending further investigation, we here adopt a conservative approach by considering the latter two forms to represent allopatric or parapatric populations of a single species (namely, *Lethrinus* sp. A). The Indo-West Pacific barrier, which runs from the Indo-Malay Sunda Shelf in the west to the Papuan-Australian Sahul shelf in the east, separates the marine biota of the Indian Ocean and of the Pacific Ocean [25]. Both shelves were exposed to form uninterrupted continental masses west and east of the Indo-Malay-Papua archipelago when the sea level fell during glacial periods, thus restricting the water flow between the two oceans [26]. Pleistocene vicariance on either side of the Indo-West Pacific barrier may account for the genetic differences observed between the ‘Indian Ocean’ lineage and the ‘Coral Triangle’ lineage of *Lethrinus* sp. A. Nuclear genotyping will be helpful to assess the degree of reproductive isolation between these two forms. Further examination based on time-calibrated molecular phylogenetic analysis will certainly be helpful to address more specifically the evolutionary history of this species.

It has been argued here that the two main clades observed within *L. olivaceus* correspond to distinct species, *Lethrinus* sp. A and *Lethrinus* sp. B. It is now necessary to assign to either of the latter the type specimens of *L. olivaceus* (MNHN 0000-5741) and *L. rostratus* (RMNH D443). Depending on the results, it might further be necessary to do so with *L. waigiensis* (MNHN 0000-9065), and it might also be necessary to collect specimens from the Caroline Islands, the type locality of *L. xanthopterus* (a nomen nudum) and assign them similarly. Finally, it might also be necessary to examine the type specimens of *L. longirostris* (BMNH 1865.9.21.6), the last in the list of nominal species synonymised with *L. olivaceus* [2]. To base the

assignment on type locality would be hazardous: the type-localities of both *L. olivaceus* (Sunda Strait, Indonesia) and *L. waigiensis* (Waigeo Island, West Papua) lie within the Coral Triangle where both species may occur, according to our genetic evidence. Also, the type locality of *L. rostratus* has not been clearly indicated by A. Valenciennes, who only mentioned it was “*envoyé de Batavia par MM. Kuhl et van Hasselt*” [1]. Because of this, and because of possible overlap in the geographic distribution of the two species, nucleotide sequencing the type specimens at the cytochrome *b* locus is warranted. In Table 1, we propose a molecular-based strategy to rationalize the actions needed for the purpose of revising the taxonomy and nomenclature of species currently under *L. olivaceus*. Additional nomenclatural problems might arise in case the ‘Coral-Triangle’ form and the ‘Indian Ocean’ form also proved upon further research to be distinct species within *Lethrinus* sp. A.

Disclosure of interest

We have no conflicts of interest concerning this article.

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Table 1

Recommended molecular-based strategy to solve the nomenclatural problem posed by the occurrence of two species within *L. olivaceus* under its current definition [2]. The actions to be done are arranged in a chronological order compatible with page priority regarding species names in [1] and chronological priority otherwise [27]. *MNHN*: Museum national d'histoire naturelle, Paris; *RMNH*: Rijksmuseum van Natuurlijke Historie, Leiden; *BMNH*: Natural History Museum, London.

Step no.	Action, hypothetical result	Conditional taxonomic decision	Next step
1.	Sequence holotype of <i>L. olivaceus</i> (MNHN 0000-5741)		
	1a. if MNHN 0000-5741 = <i>Lethrinus</i> sp. A (1a)	then <i>Lethrinus</i> sp. A = <i>L. olivaceus</i>	go to 2
	1b. if MNHN 0000-5741 = <i>Lethrinus</i> sp. B (1b)	then <i>Lethrinus</i> sp. B = <i>L. olivaceus</i>	go to 2
2.	Sequence holotype of <i>L. rostratus</i> (RMNH D443)		
	2a. if RMNH D443 = <i>Lethrinus</i> sp. A	then <i>Lethrinus</i> sp. A = <i>L. rostratus</i>	if (1a) true then 3; else 4
	2b. if RMNH D443 = <i>Lethrinus</i> sp. B	then <i>Lethrinus</i> sp. B = <i>L. rostratus</i>	if (1b) true then 3; else 4
3.	-	Maintain synonymy of <i>L. rostratus</i> with <i>L. olivaceus</i>	go to 5
4.	-	Resurrect <i>L. rostratus</i>	end
5.	Sequence holotype of <i>L. waigiensis</i> (MNHN 0000-9065)		
	5a. if MNHN 0000-9065 = <i>Lethrinus</i> sp. A	then <i>Lethrinus</i> sp. A = <i>L. waigiensis</i>	if (1a) true then 6; else 7
	5b. if MNHN 0000-9065 = <i>Lethrinus</i> sp. B	then <i>Lethrinus</i> sp. B = <i>L. waigiensis</i>	if (1b) true then 6; else 7
6.	-	Maintain synonymy of <i>L. waigiensis</i> with <i>L. olivaceus</i>	go to 8
7.	-	Resurrect <i>L. waigiensis</i>	end
8.	Sequence specimens from the Caroline Islands		
	8a. if Caroline Islands specimens = <i>Lethrinus</i> sp. A	then <i>Lethrinus</i> sp. A = <i>L. xanthopterus</i>	if (1a) true then 9; else 10
	8b. if Caroline Islands specimens = <i>Lethrinus</i> sp. B	then <i>Lethrinus</i> sp. B = <i>L. xanthopterus</i>	if (1b) true then 9; else 10
9.	-	Maintain synonymy of <i>L. xanthopterus</i> with <i>L. olivaceus</i>	go to 11
10.	Choose neotype for <i>L. xanthopterus</i>	Resurrect <i>L. xanthopterus</i>	end
11.	Sequence holotype of <i>L. longirostris</i> (BMNH 1865.9.21.6)		
	11a. if BMNH 1865.9.21.6 = <i>Lethrinus</i> sp. A	then <i>Lethrinus</i> sp. A = <i>L. longirostris</i>	if (1a) true then 12; else 13
	11b. if BMNH 1865.9.21.6 = <i>Lethrinus</i> sp. B	then <i>Lethrinus</i> sp. B = <i>L. longirostris</i>	if (1b) true then 12; else 13
12.	-	Maintain synonymy of <i>L. longirostris</i> with <i>L. olivaceus</i>	if (1a) true then 14; else 15
13.	-	Resurrect <i>L. longirostris</i>	end
14.	-	<i>Lethrinus</i> sp. B = undescribed species from the Indo-West Pacific	go to 16
15.	-	<i>Lethrinus</i> sp. A = undescribed species from the Indian Ocean and the Coral Triangle	go to 16
16.	-	Describe new species	end

Fig. 1. *Lethrinus olivaceus* type material and voucher specimens. **A.** Above: holotype of *L. olivaceus* Valenciennes 1830 (specimen MNHN 0000-5741), from Antjer, Sunda Strait, Indonesia [1]; standard length (SL) 175 mm. Below: holotype of *L. waigiensis* Valenciennes 1830 (specimen MNHN 0000-9065), from Waigeo, West Papua [1]; SL 107 mm (PB). **B.** Voucher specimen Gutila 11 (LIPI-P2O, Jakarta, unregistered) from Raja Ampat, West Papua, November 2007; SL 442 mm; of the 'Pacific Ocean' form (or *Lethrinus* sp. B) according to nucleotide sequence at the cytochrome *b* locus (GENBANK KC596047) (PB). **C.** Voucher specimen Gutila 12 (skull preserved at IRD-UR 227, Montpellier) from Raja Ampat, West Papua, November 2007; SL 539 mm; of the 'Coral Triangle' form (or *Lethrinus* sp. A) according to nucleotide sequence at the cytochrome *b* locus (GENBANK KC596051) (PB). **D.** Specimen MNHN 2009-0016 from Moruroa, French Polynesia, June 2006; SL 382 mm; of the 'Pacific Ocean' form (or *Lethrinus* sp. B) according to nucleotide sequence at the cytochrome *b* locus (GENBANK KC596057) (PB)

Fig. 2. Longface emperor, *Lethrinus olivaceus*. Sampling locations of individuals selected for morphometric analysis and/or genetics. Mayotte: Bancs Geyser and Z, Mozambique Channel, April 2011 ($N=2$); Maldives: Baa atoll, June 2008 ($N=4$); Bali: Kedonganan fish market, February 2007 ($N=5$); Taiwan: Kenting, May-June 2011 ($N=4$); West Papua: Sorong, November 2007 ($N=6$); New Ireland: Kavieng, August 2006 ($N=1$); New Caledonia: Northern lagoon, April 2007 ($N=22$) and Chesterfield Islands, Coral Sea, August 2011 ($N=1$); Fiji: T Levu, September 2008 ($N=1$); Moruroa: Moruroa lagoon, June 2006 ($N=8$). Shaded: distribution of the species, as inferred from the point map provided by FISHBASE [10] (accessed 02 November 2012).

Fig. 3. Longface emperor, *Lethrinus olivaceus*. Morphometric analysis of head skeleton. **A.** Measurements on neurocranium, left pre-maxilla (upper jaw) and left lower jaw, made to the nearest 0.1 mm using a Vernier calliper: *cbL*, condylo-basal length; *dO*, orbit diameter; *H*, neurocranium height; *iO*, interorbital width; *w*, neurocranium width; *sw*, width of snout where narrowest; *Lpmx*, length of premaxilla; *pmx*, length of toothed part of premaxilla; *Llj*, length of lower jaw. To normalize the variables and standardize them for size, prior to analysis each was log-transformed and divided by the log-transformed condylo-basal length [11,12]. **B.** Principal component analysis (output from ADE-4 [14]): scores for principal components (PC) 2 (horizontal: 23.6% of total variance) and 3 (vertical: 10.6% of total variance) of individuals from the Indian Ocean (Maldives: *N*=1), the Coral Triangle (Bali: *N*=4; West Papua: *N*=1) and the Pacific Ocean (New Caledonia: *N*=22; Moruroa: *N*=8), characterized by the above-defined morphometric variables. Inset: correlation circle [14]; legend to symbols; graduation on axes.

Fig. 4. Longface emperor, *Lethrinus olivaceus*. Phylogenetic tree of mitochondrial lineages reconstructed using RAXML [17], from the *cyt b* gene sequences of individuals from the following sampling locations: Baa atoll, Maldives ($N=4$); Kedonganan fish market, Bali ($N=4$); Philippines ($N=1$: GENBANK AF381252 [16]); Kenting fish market, Taiwan ($N=4$); Raja Ampat, West Papua ($N=6$); Kavieng, New Ireland ($N=1$); New Caledonia ($N=3$); Tai Levu, Fiji ($N=1$); and Moruroa atoll, French Polynesia ($N=2$). The tree was rooted by *cyt b* sequences of the known closest relatives to *L. olivaceus* [16], namely *L. reticulatus* (GENBANK AF381253), *L. rubrioperculatus* (GENBANK AF240752, AF381263) and *L. microdon* (from Bali and the Maldives, and GENBANK AF381265 from the Philippines). Branch length proportional to number of substitutions under the GTR+G model. Numbers on branches are ML bootstrap values; those below 50% are not shown.

Fig. 5. Variable nucleotide sites in cytochrome *b* gene sequences of longface emperor, *Lethrinus olivaceus*. Nucleotide sites numbered and mutations marked using Lo Galbo et al.'s sequence from the Philippines (GENBANK AF381252) [16] as reference. *Asterisk*: nucleotide site diagnostic between *Lethrinus* sp. A and *Lethrinus* sp. B; *dot*: nucleotide identical to reference; *dash*: no data.

[illegible]

Appendix 1

Lethrinus spp. Material examined for nucleotide sequence variation at the cytochrome *b* locus, with sampling details and GENBANK ([http:// www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) sequence accession numbers, and comparative material from GENBANK. IRD number allocated by PB to tissue sample preserved in the tissue collection of IRD-UR 227 (Montpellier, France); NTU number allocated by WJC to aliquot preserved at the Marine Phylogenomics Laboratory of NTU-Institute of Oceanography (Taipei, Taiwan). USNM United States Natural History Museum, Washington DC; LIPI-P2O Lembaga Ilmu Pengetahuan Indonesia-Pusat Penelitian Oseanografi, Jakarta; MNHN Museum national d'histoire naturelle, Paris.

Species, Sampling location	Sampling date	Tissue no.		Voucher	GENBANK
		IRD	NTU		
<i>Lethrinus microdon</i>					
Maldives	June 2008	Lmic 117	Let110	-	KC596059
Bali	January 2005	Z201	Let151	-	KC596060
Philippines	-	-	-	USNM 340523	AF381265
<i>Lethrinus olivaceus</i>					
Mayotte	April 2011	PhylIP, L. olivaceus	Let362	-	KC596033
Mayotte	April 2011	PhylIP, L. olivaceus	Let363	-	KC596034
Maldives	June 2008	20080621 XL	Let049	IRD, unregistered (skull)	KC596035
Maldives	June 2008	Loli 124	Let117	-	KC596036
Maldives	June 2008	20080621 L	Let128	IRD, unregistered (skull)	KC596037
Maldives	June 2008	Loli 83	Let130	-	KC596038
Bali	February 2007	Loli 23	Let047	IRD, unregistered (skull)	KC596039
Bali	February 2007	Loli 24	Let141	IRD, unregistered (skull)	KC596040
Bali	February 2007	Loli 25	Let142	IRD, unregistered (skull)	KC596041
Bali	February 2007	Loli 34	Let150	-	KC596042
Taiwan	May 2011	-	Let382	20110520HC004	KC596043
Taiwan	May 2011	-	Let384	20110506HC006	KC596044
Taiwan	May 2011	-	Let385	20110513HC010	KC596045
Taiwan	June 2011	-	Let431	-	KC596046
Philippines	-	-	-	USNM 345988	AF381252
West Papua: Raja Ampat	November 2007	Gutula 11	Let050, Let164	LIPI-P2O, unregistered	KC596047
West Papua: Raja Ampat	November 2007	Gutula	Let155	-	KC596048
West Papua: Raja Ampat	November 2007	Gutula 3	Let156	-	KC596049
West Papua: Raja Ampat	November 2007	Gutula 9	Let162	-	KC596050
West Papua: Raja Ampat	November 2007	Gutula 12	Let165	IRD, unregistered (skull)	KC596051
New Ireland: Kavieng	August 2006	Loli 15	Let166	-	KC596052
New Caledonia: N lagoon	April 2007	Loli 44	Let171	IRD, unregistered (skull)	KC596053
New Caledonia: N lagoon	April 2007	Loli 48	Let175	IRD, unregistered (skull)	KC596054
New Caledonia: Chesterfield Is.	August 2011	-	Let423	-	KC596055
Fiji: Tai Levu	September 2008	Loli 70	Let060	-	KC596056
Moruroa	July 2006	Loli M	Let048, Let234	MNHN 2009-0016	KC596057
Moruroa	July 2006	Loli 3	Let218	IRD, unregistered (skull)	KC596058
<i>Lethrinus reticulatus</i>					
Philippines	-	-	-	USNM 340558	AF381253
<i>Lethrinus rubrioperculatus</i>					
Western Australia	-	-	-	-	AF240752
Philippines	-	-	-	USNM 346714	AF381263

Appendix 2

Lethrinus olivaceus. Measurements on neurocranium, left pre-maxilla (upper jaw) and left lower jaw (see Fig. 3A), made to the nearest 0.1 mm using a Vernier calliper: *cbL*, condylo-basal length; *dO*, orbit diameter; *H*, neurocranium height; *iO*, interorbital width; *Llj*, length of lower jaw; *Lpmx*, length of premaxilla; *pmx*, length of toothed part of premaxilla; *sw*, width of snout where narrowest; *w*, neurocranium width;. The unit for all measurements is the mm.

Specimen no.	Sampling location	Morphometric variable								
		<i>cbL</i>	<i>H</i>	<i>w</i>	<i>sw</i>	<i>iO</i>	<i>dO</i>	<i>Lpmx</i>	<i>pmx</i>	<i>Llj</i>
Loli 3 ^a	Moruroa	119.1	55.4	50.6	13.8	30.2	34.9	42.0	76.3	56.9
Loli 4	Moruroa	94.3	44.4	41.7	11.8	25.2	29.0	30.2	57.5	43.6
Loli 5	Moruroa	123.7	58.4	54.7	14.5	34.3	38.0	43.8	78.1	61.1
Loli 6	Moruroa	90.0	43.9	40.3	10.9	24.4	28.0	31.0	54.8	42.9
Loli 7	Moruroa	125.3	59.1	55.3	15.9	40.0	38.0	44.4	80.2	60.7
Loli 8	Moruroa	126.3	58.4	55.6	13.0	34.5	38.0	46.7	80.4	62.9
Loli 9	Moruroa	90.5	43.4	40.7	11.6	24.9	28.7	29.4	52.1	42.0
Loli 10	Moruroa	86.6	40.8	37.6	10.1	22.9	28.1	28.6	52.0	41.3
Loli 21	Bali	84.3	38.8	36.1	10.6	27.2	25.9	28.9	54.9	42.0
Loli 23 ^a	Bali	111.9	50.6	46.4	12.1	27.8	33.1	39.6	74.0	57.9
Loli 24 ^a	Bali	90.2	40.9	37.8	10.2	22.1	28.6	31.3	57.3	44.3
Loli 25 ^a	Bali	81.2	36.6	35.8	8.8	19.4	27.1	27.8	50.1	39.3
20080621 L ^a	Maldives	130.8	59.9	47.5	14.3	33.7	39.7	47.8	90.6	69.8
Gutilla 12 ^a	West Papua	129.8	58.6	55.0	13.3	33.6	38.6	56.9	105.5	80.2
Loli 40	New Caledonia	99.1	47.5	44.3	11.4	26.0	29.7	35.3	63.6	47.8
Loli 41	New Caledonia	102.2	49.0	44.6	12.3	27.3	31.0	34.8	64.2	49.3
Loli 42	New Caledonia	92.0	43.4	40.3	11.7	24.1	28.8	30.6	58.0	43.5
Loli 43	New Caledonia	88.6	41.7	38.1	10.9	22.0	28.0	29.6	55.3	41.0
Loli 44 ^a	New Caledonia	91.0	43.5	39.8	11.1	24.0	29.0	30.4	57.0	43.4
Loli 45	New Caledonia	98.8	46.9	44.0	12.2	26.8	30.8	33.6	62.9	48.0
Loli 46	New Caledonia	97.9	47.3	43.8	12.2	25.8	29.5	33.9	61.7	47.7
Loli 47	New Caledonia	99.6	47.9	44.1	12.1	25.8	29.5	34.0	64.3	48.7
Loli 48 ^a	New Caledonia	84.0	40.4	38.0	10.5	22.7	25.6	27.8	51.8	39.6
Loli 49	New Caledonia	100.5	47.4	44.0	12.1	26.2	30.9	34.1	64.5	46.7
Loli 50	New Caledonia	85.1	40.3	37.1	9.3	21.7	28.4	28.7	52.5	41.1
Loli 51	New Caledonia	98.9	47.6	43.0	11.0	25.7	30.1	33.1	60.8	45.9
Loli 52	New Caledonia	95.2	46.1	41.9	10.7	25.2	29.5	31.2	59.5	42.3
Loli 53	New Caledonia	80.9	38.4	36.8	9.7	20.8	26.6	26.9	49.4	38.0
Loli 54	New Caledonia	91.5	44.3	39.7	10.4	24.0	29.5	31.2	58.4	45.1
Loli 55	New Caledonia	89.7	42.9	39.5	10.2	22.7	27.5	30.8	56.8	43.1
Loli 56	New Caledonia	98.8	47.2	43.0	11.3	25.3	30.5	33.4	62.8	46.8
Loli 57	New Caledonia	91.2	43.2	40.0	10.4	24.0	29.0	30.6	57.9	44.8
Loli 58	New Caledonia	90.0	44.1	40.3	9.9	23.0	29.0	29.7	56.3	43.1
Loli 59	New Caledonia	99.4	47.8	41.4	11.5	24.8	29.3	32.0	60.4	45.8
Loli 60	New Caledonia	93.2	44.7	40.9	10.7	23.4	28.7	31.2	57.7	44.8
Loli 61	New Caledonia	95.7	45.3	42.2	10.8	24.8	29.2	32.2	58.0	47.0

^a also sequenced at the cytochrome *b* locus