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Multiple nuclear and mitochondrial genotyping identifies emperors and large-eye breams (Teleostei: Lethrinidae) from New Caledonia and reveals new large-eye bream species

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ABSTRACT

Species identification is fundamental to address questions about community ecology, biodiversity, conservation and resource management, at any life history stage. Current studies on fish larval ecology of tropical species are hampered by the lack of reliable and effective tools for identifying larvae at the species level. Emperors and large-eye breams comprise fish species from the perciform fish family Lethrinidae. They inhabit coastal and coral-reef habitats of the tropical Indo-Pacific, and they are important fishery resources. Their taxonomy is considered difficult and identification to species is often problematic. Lethrinidae larvae and juveniles can be identified on the basis of meristic counts at the subfamily level, but no further. In this study, we developed a set of polymorphic PCR markers (size polymorphisms at the intron regions from 4/5 nuclear protein-coding genes and single-strand conformation polymorphism of a 205-bp fragment at the mitochondrial 16S rRNA locus), to characterize 341 specimens from 21 Lethrinidae species from New Caledonia (southwestern tropical Pacific). A genetic data-bank was constructed using the genotypes screened from the multiple gene loci of adult or sub-adult specimens used as references for these species. The 16S rRNA gene fragment was able to differentiate species for the genus Lethrinus, but it provided little diagnostic resolution among different species within the genus Gymnocranius. A combination of the 16S rRNA marker and 4 nuclear markers developed herein allowed to sort out species within Gymnocranius spp. from New Caledonia. Using genotype distributions at nuclear loci to test for reproductive isolation, we found that three apparently undescribed large-eye bream species may exist, provisionally referred to as Gymnocranius sp. A, sp. B and sp. C. Subsequent genotyping of 137 Lethrinidae larvae collected from the bays of the Noumea peninsula, New Caledonia, found a total of three species (Lethrinus genivittatus, L. olivaceus and Gymnocranius sp. A).

Keywords: Fish; Indo-Pacific; Cryptic species; Biological species concept; Identification; Larvae; PCR markers

1. Introduction

Studies on biodiversity, community ecology and management of natural resources require species level analysis for the accurate assessment of community structure (Bhadury et al., 2006; Pfenninger et al., 2007; Valentini et al., 2008). Coral reef ecosystems harbour a high diversity of fishes that may include entire families that contain commercially important species. In addition to continued research on adult fishes, it is necessary to investigate the biology and ecology of their larval stages for understanding the environmental determinants of community structure. Yet our ability to access the ecological information relative to larval fish communities is limited given the general helplessness to correctly identify larvae to species using morphological or meristic features as diagnostic characters (Wilson, 1998; Leis and Carson-Ewart, 2000).

Emperors and large-eye breams (Perciformes: Lethrinidae) are a significant component of the fish communities of various types of habitats in the coastal tropical Indo-Pacific (Carpenter and Allen, 1989). Those habitats include coral reefs, where Lethrinidae occur from the back-reef shallow seagrass beds to the reef front at depths down to a few hundred metres. While some Lethrinidae species have a range restricted to a single maritime region, e.g. the western Coral Sea for Gymnocranius audleyi (Carpenter, 2001), other species range from the Red Sea and the East coast of Africa to the Polynesian Islands (e.g. Gnathodentex aureolineatus, Lethrinus olivaceus, Monotaxis grandoculis), or exhibit antitropical distribution (e.g. L. miniatus, L. ravus) (Carpenter and Allen, 1989; Carpenter and Randall, 2003). Thus, Lethrinidae is a model-family to address questions of species diversity and adaptive radiation in tropical marine ecosystems, a well as the biogeography and patterns of lineage diversification in Indo-Pacific fishes. Moreover, since Lethrinidae generally consist of large-size fishes, which are targeted by commercial fisheries and sold on the fish markets throughout the Indo-Pacific (Carpenter and Allen, 1989; Ebisawa and Ozawa, 2009), sound fisheries management at the local or regional scales is also an issue. All these cases emphasize the importance of a reliable and effective tool for unambiguous species identification, since lack or incorrect knowledge of taxonomy will mislead conclusions from any of comparative results.

The family Lethrinidae currently comprises 39 species in five genera grouped asymmetrically into two subfamilies: Lethrininae (a single genus, *Lethrinus*) and Monotaxinae (genera *Gnathodentex*, *Gymnocranius*, *Monotaxis* and *Wattsia*). This taxonomic view is supported by morphology (Carpenter and Allen, 1989) and pro parte by the only molecular phylogeny of Lethrinidae available to date (Lo Galbo

et al., 2002). Traditional characters such as fin-ray and scale counts, dentition, etc. are of little value to the taxonomy of Lethrinidae, which therefore has to rely on body shape, colouration and pigmentation patterns (Carpenter and Allen, 1989). The taxonomy of *Gymnocranius* spp., with six to eight species recognized thus far, is not definitive (Sato, 1986; Carpenter and Allen, 1989): "The identity of the species in this genus has caused considerable confusion. The main reason for this problem is the great similarity in shape and coloration among the species" (Carpenter and Allen, 1989:19). For instance, Sato (1986) has listed 20 nominal species pertaining to that genus, nine of which he was not able to ascribe with certainty to any of the six *Gymnocranius* species he recognized as valid.

Current identification keys for Lethrinidae larvae and juveniles are effective for the sub-family level but not further (Leis and Carson-Ewart, 2000; but see Wilson, 1998). As for adults, meristic characters are of little help for identifying Lethrinidae larvae and juveniles. Moreover, the latter are characterized by great similarity in colouration. Given the richness and abundance of Lethrinidae in all coastal habitats of the tropical indo-Pacific, the development of suitable taxonomic criteria for distinguishing early stages at the specific level is highly desirable. A step towards this endeavour was taken by Wilson (1998) who summarized the colour patterns of greatest value for distinguishing juveniles of 11 *Lethrinus* species from the Great Barrier Reef, and provided the descriptions of the late pre-settlement stages of three species (*L. atkinsoni*, *L. genivittatus* and *L. variegatus*). However, identification to species was not formally validated in Wilson's (1998) study.

Molecular population genetics provides powerful tools for delineating species and geographical populations (e.g. Richardson et al., 1986). Polymorphic nuclear-DNA markers are especially useful to the analysis of reproductive barriers between sympatric species, since they permit to investigate the genotypic composition of samples, which can be tested against the null hypothesis of panmixia (Quignard et al., 1984; She et al., 1987; Creech, 1991; Hoarau and Borsa, 2000; Quattro et al., 2006). The same markers can in turn be used to construct genetic fingerprinting for the identification of individuals to species, especially for identifying morphologically undifferentiated larvae and juveniles.

The aims of the present work were (1) to develop mitochondrial 16S rDNA and intron sizepolymorphic PCR markers for a set of 21 Lethrinidae species, mainly from New Caledonia; (2) to evaluate their performance to diagnose Lethrinidae species; further taxonomic problems in the genus *Gymnocranius* were highlighted by the discovery of new, apparently undescribed species; (3) to establish a reference database consisting of the multiple-locus DNA genotypes specific to each Lethrinidae species sampled as sub-adults or adults in New Caledonia; (4) to identify Lethrinidae larvae collected in the southern lagoon of New Caledonia, using those multiple- locus DNA markers.

2. Materials and methods

The species names used in this article follow Carpenter and Allen (1989) and Carpenter and Randall (2003).

2.1. Sampling

Adult or sub-adult specimens of Lethrinidae were either collected by us or were sampled from the Noumea fish market between 2002 and 2005; they were identified to species using the identification key of Carpenter and Allen (1989) and sub-sampled for genetics by clipping fins. Whole specimens were kept as vouchers in formalin and alcohol and deposited in the ichthyological collection at Museum national d'histoire naturelle, Paris (MNHN) (Table 1). Other specimens sub-sampled were photographed, including *Lethrinus erythracanthus* and *L. lentjan* from the Pasar Lelong fish market (Makassar, Indonesia), and *L. nebulosus* and *Monotaxis grandoculis* from the Kedonganan fish market (Bali, Indonesia).

The list presented in Table 1 includes 18 of 21 valid Lethrinidae species so far reported from New Caledonia (Carpenter, 2001; Béarez, 2003; Carpenter and Randall, 2003; Fricke and Kulbicki, 2007; Froese and Pauly, 2009) and three apparently undescribed *Gymnocranius* species (see Results). The three species reported from New Caledonia and missing in our sample were *Gymnocranius elongatus*, *Lethrinus laticaudis* and *Wattsia mossambica*.

Lethrinidae larvae were collected using light-traps (Aquafish, Lattes, France), which attract latestage larvae, prior to their settlement on benthic habitat. These traps are outlined in Carassou et al. (2009). Light-traps were set at 2.5 m below the surface, and were used in three bays in the southwest lagoon of New Caledonia: Grande Rade, Dumbéa Bay and Sainte-Marie Bay. Light-traps were set every new moon, from January 2002 to June 2003. Details on the sampling design have been given previously (Carassou and Ponton, 2007). Immediately after collection, the larvae were anaethesized in 0.75 g.l⁻¹ benzocaine and fixed in 95% ethanol.

2.2. Identification of larvae to sub-family

An application of the molecular tools here developed to assist Lethrinidae taxonomy is the identification of unknown larvae to species. Prior to this, a total of 137 Lethrinidae larvae sampled with light-traps were sorted into different groups according to morphological, pigmentation and meristic characters (Leis and Carson-Ewart 2000). Fin-ray counts allowed the separation of Monotaxinae from Lethrininae (Table 3). Within Lethrininae, three groups were distinguished according to body shape and pigmentation patterns (LET 1 to LET 3 in Table 3). Sub-family Monotaxinae was represented by a single individual (LET 4 in Table 3).

2.3. Molecular analyses

Whole genomic DNA of an individual was extracted from fin-clips preserved frozen or in ethanol, using either the classical phenol-chloroform protocol (Sambrook et al., 1989), or the DNeasy Tissue Kit" of Qiagen GmbH (Hilden, Germany) according to the manufacturer's instructions. DNA extracts were conserved in ultrapure water at -20°C. DNA amplification was conducted by polymerase chain reaction (PCR) for each targeted gene locus. PCRs were carried out in 25 µl reaction mixture containing 1.5-2.0 mM MgCl₂, 0.64 mM dNTP mix, 0.1-0.3 µM of each primer and 0.03-0.2 U Taq DNA polymerase (Promega, Madison WI, USA). The primers used to amplify an intron of the metallothionein gene were designed in the flanking exons, from the alignment of homologous metallothionein genes of *Carassius curieri* (GENBANK AY165048), *Dicentrarchus labrax* (GENBANK AF199014), *Takifugu rubripes* (GENBANK CA847265) and *Salmo salar* (GENBANK BG935118). The references for the primers designed to amplify other introns are given in Table 2. PCRs were run in a RoboCycler Gradient 96 thermocycler (Stratagene, Cedar Creek TX, USA), with annealing temperature set to 50°C (*CK*), 51°C (*16S rRNA*) or 52°C (*Aldo-B*, *GnRH-1*, *Met*). The primers for *16S rRNA* locus were designed from the alignment of *Lethrinus ornatus* (GENBANK AF247446), *Lethrinus rubrioperculatus* (GENBANK AF247447) and *Beryx splendens* (GENBANK AY141406) homologous sequences.

Polymorphisms at the intron regions of the targeted nuclear gene loci were scored according to the size of amplified fragments as revealed by vertical electrophoresis in denaturing, 0.4-mm thick polyacrylamide gels [6% acrylamide:bis-acrylamide (29:1) solution, TBE 1X, 7 M urea] at 50 W. Immediately before migration, 6 µl denaturing loading buffer (95%

formamide, 10 mM NaOH, 84‰ bromophenol blue, 5% glycérol) was added to each well of the PCR plate and the mixture was heated for 5 min at 95°C. Amplified 16S rDNA fragments [205 base pairs (bp)] were subjected to single-strand conformation polymorphism (SSCP) analysis (Orita et al., 1989; Desmarais et al., 1995) by electrophoresis of the heat-denatured DNA fragments in vertical, non-denaturing polyacrylamide gels (MDE 1X, FMC corporation, Rockland, USA) overnight at 2 W at ambient temperature (25°C). After electrophoresis, the gel was stained with silver nitrate to visualize DNA bands (Borsa and Coustau, 1996). Each resulting profile in DNA conformation or SSCP phenotype was considered as an individual haplotype. It has been estimated that >90% of single-nucleotide changes in 300-bp to 450-bp fragments can be detected using SSCP screening (Lessa and Applebaum, 1993); that percentage is likely to be higher in shorter fragments such as the 16S rDNA fragment screened here.

2.4. Analysis of data

The gene diversities at a locus were estimated from the haplotype frequencies (x_i) as: $h = n/(n-1)\cdot(1-\Sigma_i x_i^2)$, where n = number of haplotypes sampled (Nei, 1978).

3. Results

3.1. Characteristics of the DNA markers used in this study

Our results showed that the nuclear intron loci exhibited either sample monomorphism or poor polymorphism within species in most of cases, except *Aldo-B slow* in *Lethrinus miniatus* and in *L. nebulosus*, *GnRH-1* in *L. atkinsoni* and in all five *Gymnocranius* species, and *CK-6* in *G. grandoculis* and in *Gymnocranius* sp. C (Table 4, Table 5). The 16S rDNA fragment assayed by SSCP (Fig. 1, Fig. 2) generally exhibited sample monomorphism, or low polymorphism, in any given species from our sample. The exceptions to this general pattern were *Lethrinus rubrioperculatus* and *L. semicinctus* (Table 4), and *Monotaxis grandoculis*, for which 5 haplotypes were scored in a sample of 9 individuals (Table 5). Gene diversity (^*h*) values at the *16S rRNA* locus were comprised between 0 and 0.38, except in *L. rubrioperculatus*, *L. semicinctus* and *M. grandoculis*, where ^*h* = 0.53, 0.67 and 0.87, respectively.

3.2. Evidence of cryptic species

Reproductively isolated groups of individuals within the sample initially identified as *G. grandoculis* were inferred from multiple-locus genotypic data: we observed a lack of heterozygotes at two nuclear loci and a strict correlation between mitochondrial and nuclear genotypes at those loci (Table 5; individual genotypes provided in Supplementary material). Therefore, two biological species could be distinguished a posteriori, namely *G. grandoculis* and a tentative new species coined *Gymnocranius* sp. A. Reproductive isolation was similarly deduced from the distribution of nuclear genotypes within the sample initially identified as *Gymnocranius* sp. sensu Carpenter and Allen (1989), leading to the post-hoc distinction of two species, here provisionally designated as *Gymnocranius* sp. B and *Gymnocranius* sp. C.

3.3. Reference genetic database

Table 4 gives allele frequencies per species in *Lethrinus* spp. Intron locus *Aldo-B fast* was diagnostic for each *L. miniatus* and *L. variegatus*. Intron locus *Aldo-B slow* allowed further, partial separation between species. Intron locus *GnRH-1* was diagnostic for each *L. genivittatus* and *L. variegatus*. Intron locus *Met-1* allowed further distinction between some of the species characterized at this locus. The 205-bp 16S rDNA fragment allowed the distinction of all species from each other, except *L. ravus*, *L. rubrioperculatus* and *L. semicinctus* which partly shared one SSCP phenotype (presumed haplotype) (*K*). Altogether, multiple-locus DNA fingerprints allowed the identification of individuals of all *Lethrinus* species sampled in New Caledonia, except the above three for which the assignment to species of a proportion of individuals was not possible. A key to the identification of Lethrininae from New Caledonia is proposed in Table 6.

Table 5 gives allele frequencies per species in Monotaxinae. Two alleles were present at intron locus *Aldo-B*, one of which was exclusive to *Gymnocranius* sp. A. Intron locus *CK-6* was diagnostic at the genus level and it further allowed the distinction of *G. euanus* against all other four *Gymnocranius* species. Intron locus *GnRH-1* was monomorphic in *Gnathodentex aureolineatus* and *M. grandoculis* and polymorphic in all *Gymnocranius* species tested, but was diagnostic of none. Intron locus *Met* was monomorphic in every species tested. In *Gymnocranius*, *Met* was diagnostic to *Gymnocranius* sp. A and it further allowed the separation of *G. euanus* and *Gymnocranius* sp. C, from *G. grandoculis* and *Gymnocranius* sp. B. The 16S rDNA fragment was diagnostic at the genus level and it further allowed the distinction of *G. grandoculis*

from all other four biological species scored in the present survey. Altogether, the five loci scored here allowed the unambiguous separation of all seven Monotaxinae species, including three apparently undescribed *Gymnocranius* spp., that we sampled in New Caledonia. A key to identifying Monotaxinae from New Caledonia on the basis of molecular markers is here proposed (Table 7).

3.4. Identification of larvae

The Lethrinidae larvae sampled in the New Caledonian lagoon were subsequently identified to species by comparing their multiple-locus genotypes against those of the reference samples using the keys provided in Table 6 ad Table 7. Both LET 1 and LET 2 larvae were thus unambiguously assigned to *L. genivittatus*, so was LET 3 to *L. olivaceus*, and so was LET 4 to *Gymnocranius* sp. A. Drawings made after composite photographs are presented for each of the four morphotypes recognized here (Fig. 3; Fig. 4), all of which were thus formally identified to species using molecular genotyping. The variability in pigmentation patterns among *L. genivittatus* larvae of identical sizes was noticeable (Fig. 1c, d). However, *L. genivittatus* larvae were generally less pigmented than *L. olivaceus* and *Gymnocranius* sp. A. Finally, *L. olivaceus* and *Gymnocranius* sp. A. Finally, *L. olivaceus* and *Gymnocranius* sp. A larvae presented a deeper body form than *L. genivittatus* larvae (Fig. 3; Fig. 4; Table 1).

4. Discussion

4.1. Performance of the DNA markers used in this study

The molecular methods developed in this study allowed an almost complete discrimination of 21 Lethrinidae species that have been reported from New Caledonia. This represents 72% of the total number of Lethrinidae species present in the tropical southwest Pacific (Carpenter, 2001), 62% of those reported from the entire Pacific, and 53% of those reported from the entire Indo-Pacific (Carpenter and Allen, 1989). Given their high discriminating power when used in combination, the markers developed here are useful to species identification at the local and regional scales. However, complementary studies may be necessary to extend this assay to identify Lethrinidae from other regions of the Indo-Pacific. The *16S rRNA* gene has proven an efficient marker for DNA barcoding fish species (e.g. Aoyama et al., 2001; Craig et al., 2002; Akimoto et al., 2005; Papasotiropoulos et al., 2007; Saitoh et al., 2009). Here, single-strand conformation polymorphism of a 205-bp fragment of the *16S rRNA* gene appeared as a relatively efficient barcode in Lethrininae: it allowed the discrimination of all *Lethrinus* species from New Caledonia, except the triplet (*L. raws, L. rubrioperculatus , L. semicinctus*). Insufficient resolution of the genetic differences among those three species may suggest their close genetic relationship and, eventually, a species complex. To test these hypotheses would require several steps. First, sequencing the fragment appearing as SSCP-identical in all three species would help address the hypothesis of their identity in nucleotide sequence. If their nucleotide sequences were also found identical, then a longer or more variable fragment of the mitochondrial DNA should be sequenced. If the hypothesis of shared mitochondrial lineages still could not be rejected, then only a thorough survey of nuclear-DNA variation would help investigating the degree of genetic exchange among the three species and tell whether or not they form a species complex. The 16S rDNA fragment was of little help to discriminate among *Gymnocranius* spp., indicating shallower divergence between species within that genus, compared to the general level of divergence among species within *Lathrinus*.

4.2. Cryptic species in Gymnocranius spp.

Before undertaking this study, four *Gymnocranius* species had been reported from New Caledonia: *G. elongatus, G. enanus, G. grandoculis,* and *Gymnocranius* sp. sensu Carpenter and Allen (1989) (Laboute and Grandperrin, 2000; Carpenter, 2001; Béarez, 2003; Randall, 2005; Fricke and Kulbicki, 2007). In addition, *Gymnocranius audleyi* had been reported from the nearby Chesterfield islands (Fricke and Kulbicki, 2007). *Gymnocranius rivulatus,* mentioned in Kulbicki (1988) and Fricke and Kulbicki (2007) is a junior synonym of *G. grandoculis* (Carpenter and Allen, 1989; Eschmeyer, 1998).

Three tentatively new *Gymnocranius* species, here coined 'sp. A', 'sp. B' and 'sp. C' (Table 1) were sorted out posterior to genetic analyses. Reproductive isolation of each of those three species from any other sympatric *Gymnocranius* species was ascertained by the lack of inter-specific heterozygotes at one or several nuclear-DNA loci. *Gymnocranius* sp. A individuals had been initially identified by us as *G. grandoculis*, as they fell out with that species in the identification keys of both Sato (1986) and Carpenter and Allen (1989). Posterior re-examination of a single voucher specimen of *Gymnocranius* sp. A allowed its separation from *G. grandoculis* (from which we had retained photographs): in *Gymnocranius* sp. A, the ratio of standard length to body depth was higher, the body was more symmetrical dorso-ventrally and the caudal fin was more elongate, than in *G. grandoculis*. Both *Gymnocranius* sp. B and sp. C individuals had been initially identified by us as *Gymnocranius* sp. of Sato (1986) and Carpenter and Allen (1989) on the basis of colour patterns and shape of caudal fin. However, voucher specimens of *Gymnocranius* sp. B differed from the latter by a higher ratio of standard length to body depth and less rounded caudal fin; they were similar to the '*G. griseus*' (non Temminck and Schlegel 1844) photographed by Coleman (1981). The formal descriptions of *Gymnocranius* sp. A and *Gymnocranius* sp. B are currently under consideration. Unfortunately, we retained no voucher specimen of *Gymnocranius* sp. C.

Large-eye breams of the genus *Gymnocranius* are large-size, commercial fishes abundantly distributed throughout the tropical Indo-West Pacific (Carpenter and Allen, 1989; Carpenter, 2001) where they are sold on fish markets; their flesh is prized (Coleman, 1981; Sato, 1986; Carpenter and Allen, 1989). A reason for which apparently undescribed *Gymnocranius* species have remained ignored up to now may be, in part, the difficulties generally encountered for finding diagnostic characters within that genus, as for *Lethrinus* spp. (Carpenter and Allen, 1989). The morphological similarity between species in *Gymnocranius* is in line with their generally close genetic proximity, inferred from the present results. It is also possible that those apparently undescribed species have narrow geographic distributions around New Caledonia, or that they are much rarer in other regions, hence have escaped detection elsewhere.

A revision of the sub-family Monotaxinae, based on complete taxon sampling (including all described *Gymnocranius* species from multiple localities) and preferably incorporating a phylogenetic analysis, is warranted.

4.3. Ecological insights from molecular identification of larvae

Here, for the first time, Lethrinidae larvae were formally identified to species using molecular genotyping. Such reliable identification of larvae at the species level will improve current studies of fish larval ecology and behaviour.

The abundance of *L. genivittatus* larvae in the inshore, shallow waters of the bays of the Noumea peninsula is consistent with this species' known habitats, which primarily are shallow sandy and seagrass areas (Carpenter and Allen, 1989). According to Carpenter and Allen (1989), *L. olivacens* inhabits sandy coastal areas, lagoons and reef slopes, occurring to depths of 185 m, while juveniles are

found in shallow sandy areas. Very little information is available on its reproduction, except that spawning reportedly occurs along the edges of reefs (Carpenter and Allen, 1989). The presence of an *L. olivaceus* larva in the shallow waters of a protected bay within the lagoon may suggest that habitat use varies with life stage in *L. olivaceus*. Alternatively, spawning habitat may not be exclusively the reef edge, but may extend towards the lagoon. The question of whether adults also congregate inside the lagoon for spawning, or whether the larva captured was a strayer carried close to the shore by tide currents remains to be investigated. The same question arises for *Gymnocranius* sp. A.

Barcoding of Lethrinidae larvae, using either the markers developed in this study or other markers like the since-then widely adopted *cox-1* barcode (Ward et al., 2009) will be required for answering the above questions and addressing further questions in larval ecology, in New Caledonia and elsewhere.

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References

Akimoto, S., Sezaki, K., Mitani, I., Watabe, S. 2005. Distribution around the Izu Islands of eggs and larvae of the alfonsino *Beryx splendens* identified by mitochondrial 16S rRNA gene analysis. Nippon Suisan Gakkaishi 71, 205–211.

- Aoyama, J., Ishikawa, S., Otake, T., Mochioka, N., Suzuki, Y., Watanabe, S., Shinoda, A., Inoue, J., Lokman, P.M., Inagaki, T., Oya, M., Hasumoto, H., Kubokawa, K., Lee, T.W., Fricke, H., Tsukamoto, K. 2001. Molecular approach to species identification of eggs with respect to determination of the spawning site of the Japanese eel Anguilla japonica. Fisheries Sci. 67, 761– 763.
- Bhadury, P., Austen, M.C., Bilton, D.T., Lambshead, P.J.D., Rogers, A.D., Smerdon, G.R. 2006. Development and evaluation of a DNAbarcoding approach for the rapid identification of nematodes. Mar. Ecol. Prog. Ser. 320, 1–9.
- Béarez, P. 2003. First record of *Scolopsis taeniopterus* (Nemipteridae) and *Gymnocranius elongatus* (Lethrinidae) from New Caledonia. Cybium 27, 61-62.
- Borsa, P., Collet, A., Durand, J.-D., 2004. Nuclear-DNA markers confirm the presence of two anchovy species in the Mediterranean. C. R. Biol. 327, 1113-1123.
- Borsa, P., Coustau, C. 1996. Single-stranded DNA conformation polymorphism at the *Rdl* locus in *Hypothenemus hampei* (Coleoptera: Scolytidae). Heredity 76, 124-129.
- Carassou, L., Ponton, D. 2007. Spatio-temporal structure of pelagic larval and juvenile fish assemblages in coastal areas of New Caledonia, southwest Pacific. Mar. Biol. 15, 697-711.
- Carassou, L., Mellin, C., Ponton, D. 2009. Assessing the diversity and abundances of larvae and juveniles of coral reef fish: a review of six sampling techniques. Biodiv. Conserv. 18, 355-371.
- Carpenter, K.E. 2001. Lethrinidae Emperors (emperor snappers). Pp. 3004-3050 in Carpenter K.E., Niem V.H. (Eds.) FAO species identification guide for fishery purposes. The living marine resources of the Western central Pacific. Volume 5: Bony fishes part 3 (Menidae to Pomacentridae). FAO, Rome.
- Carpenter, K.E., Allen, G.R. 1989. FAO Species Catalogue. Vol. 9. Emperor fishes and large-eye breams of the world (family Lethrinidae). An annotated and illustrated catalogue of lethrinid species known to date. FAO Species Synopsis 125, 118 pp.
- Carpenter, K.E., Paxton, J.R., 1999. The future of systematic ichthyological research in the tropical Indo-Pacific. In: Séret, B., Sire, J.-Y. (Eds.) Proceedings of the Fifth Indo-Pacific Fish Conference, Nouméa. Soc. Fr. Ichtyol., Paris, pp. 683-693.

Carpenter, K.E., Randall, J.E. 2003. *Lethrinus ravus*, a new species of emperor fish (Perciformes: Lethrinidae) from the western Pacific and eastern Indian oceans. Zootaxa 240, 1-8.

Coleman, N., 1981. Australian sea fishes north of 30°S. Doubleday, Sydney, 297 pp.

- Craig, M.T., Pondella, D.J.2, Franck, J.P.C., Hafner, J.C. 2002. On the status of the serranid fish genus *Epinephelus*: evidence for paraphyly based upon 16S rDNA sequence. Mol. Phyl. Evol. 19, 121-130.
- Creech, S. 1991. An electrophoretic investigation of populations of *Atherina boyeri* Risso 1810 and *A. presbyter* Cuvier, 1829 (Teleostei: Atherinidae): genetic evidence in support of the two species. J. Fish Biol. 39, 807–816.
- Desmarais, E., Vigneron, S., Buresi, C., Roizes, G. 1995. Détection du polymorphisme dans l'ADN, applications en biologie et médecine diagnostique, épidémiologique et pronostique. INSERM, Paris, 185 pp.
- Ebisawa, A., Ozawa, T. 2009. Life-history traits of eight *Lethrinus* species from two local populations in waters off the Ryukyu Islands. Fisheries Sci. 75, 553-566.
- Eschmeyer, W.N. (Ed.) 1998. Catalog of fishes (3 vol.). California Academy of Sciences, San Francisco, 2605 pp.
- Fricke, R., Kulbicki, M. 2007. Checklist of the shore fishes of New Caledonia. Doc. Sci. Techn. (IRD, Nouméa), Sér. II 7, 2nd edn., 357-401.
- Froese, R., Pauly, D. (Eds.) 2009. FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2009).
- Hoarau G., Borsa P. 2000. Extensive gene flow within sibling species in the deep-sea fish *Beryx splendens*. C. R. Acad. Sci., Sér. III 323, 315-325.
- Kulbicki, M. 1988. Correlation between catch from bottom longlines and fish censuses in the SW lagoon of New Caledonia. Proc. 6th Int. Coral Reef Symp., Townsville, 8-12th August 1988 2, 305-312.
- Laboute, P., Grandperrin, R. 2000. Poissons de Nouvelle-Calédonie. Catherine Ledru, Nouméa, 520 pp.
- Leis, J.M. (1991) The pelagic stage of reef Fishes: the larval biology of coral reef Fishes. In: Sale, P.F.(Ed.) The Ecology of Fishes on Coral Reefs. Academic Press, San Diego, pp. 183-230.
- Leis, J.M., Carson-Ewart B.M. (Eds.) 2000. The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae. Brill, Leiden, 850 pp.

- Lessa, E.P., Applebaum, G. 1993. Screening techniques for detecting allelic variation in DNA sequences. Mol. Ecol. 2, 119-139.
- Lo Galbo, A., Carpenter, K.E., Reed, D.L. 2002. Evolution of trophic types in emperor snappers (*Lethrinus*, Lethrinidae, Percoidei) based on cytochrome *b* gene sequence variation. J. Mol. Evol. 54, 754-762.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
- Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., Sekiya, T. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc. Natl. Acad. Sci. U.S.A. 86, 2766–2770.
- Papasotiropoulos, V., Klossa-Kilia, E., Alahiotis, S.N., Kilias, G. 2007. Molecular phylogeny of grey mullets (Teleostei: Mugilidae) in Greece: evidence from sequence analysis of mtDNA segments. Biochem. Genet. 45, 623-636.
- Pfenninger, M., Nowak, C., Kley, C., Steinke, D., Streit, B. 2007. Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. Mol. Ecol. 16, 1957–1968.
- Quattro, J.M., Stoner, D.S., Driggers, W.B., Anderson, C.A., Priede, K.A., Hoppmann, E.C., Campbell, N.H., Duncan, K.M., Grady, J.M. 2006. Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). Mar. Biol. 148, 1143–1155.
- Quignard, J.-P., Pasteur, N., Shehata, S. 1984. Biosystématique du complexe *Solea vulgaris* du golfe du Lion (Poissons, Téléostéens, Soléidés). Rev. Trav. Inst. Pêches Marit. 46, 273–284.
- Randall, J.E. 2005. Reef and shore fishes of the South Pacific. University of Hawai'i Press, Honolulu, 707 pp.
- Richardson, B.J., Baverstock, P.R., Adams, M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney, 410 pp.
- Rohfritsch, A., Borsa, P. 2005. Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and secondary contact in the Central Indo-West Pacific seas. Heredity 95, 315-322.
- Sato, T. 1986. A systematic review of the sparoid fishes of the subfamily Monotaxinae. Pp. 602-612 in Uyeno, T., Arai, R., Taniuchi, T., Matsuura, K. (Eds.), Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes, Ichthyological Society of Japan, Tokyo,.

- Saitoh, K., Uehara, S., Tega, T. 2009. Genetic identification of fish eggs collected in Sendai Bay and off Johban, Japan. Ichthyol. Res. 56, 200-203.
- She, J.-X., Autem, M., Kotoulas, G., Pasteur, N., Bonhomme, F. 1987. Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). Biol. J. Linn. Soc. 32, 357-371.
- Valentini, A., Pompanon, F., Taberlet, P. 2008. DNA barcoding for ecologists. Trends Ecol. Evol. 24, 110-117.
- Ward, R.D., Hanner, R., Hebert, P.D.N. 2009. The campaign to DNA barcode all fishes, FISH-BOL. J. Fish Biol. 74, 329-356.
- Wilson, G.G. 1998. A description of the early juvenile colour patterns of eleven *Lethrinus* species (Pisces: Lethrinidae) from the Great Barrier Reef, Australia. Rec. Austr. Mus. 50, 55-83.

Captions to figures

Fig. 1. Single-stranded DNA conformation polymorphism at the *16S rRNA* locus in 14 Lethrininae species from New Caledonia. Composite drawing of 24/25 SSCP phenotypes found in the total sample. Arrow indicates migration of DNA fragments. An individual SSCP pattern usually consisted of two bands, as expected; three- or four-band patterns can be explained by conformation instability (Borsa and Coustau, 1996).

Fig. 2. Single-stranded DNA conformation polymorphism at the *16S rRNA* locus in 7 Monotaxinae species from New Caledonia. Composite drawing of all 11 SSCP phenotypes found in the total sample. Arrow indicates migration of DNA fragments.

Fig. 3. Lethrinidae larvae from the New Caledonian lagoon. **a-c:** LET 1 of a range of sizes (10.7 – 19.8 mm SL), identified as *Lethrinus genivittatus* from multiple-locus genotyping; **d:** LET 2, identified as *L. genivitattus* from multiple-locus genotypes (19.9 mm SL). Scale bar: 2 mm

Fig. 4. Lethrinidae larvae from the New Caledonian lagoon. **A:** LET 3, identified as *Lethrinus olivaceus* from multiple-locus genotyping (19.3 mm SL); **b:** LET 4, identified as *Gymnocranius* sp. A from multiple-locus genotypes (21.8 mm SL). Scale bar: 2 mm

Table 1. List of the Lethrinidae samples analysed by multiple-locus DNA genotyping. All specimens from New Caledonia except *Lethrinus erythracanthus* and a subsample of *L. lentjan*, from Makassar, Sulawesi, and a subsample of each *L. nebulosus* and *Monotaxis grandoculis* from Kedonganan, Bali. *N* sample size ; *IRDN* IRD, Nouméa; *MNHN* Museum national d'histoire naturelle, Paris; *JNC* J.-L. Justine's catalogue, Nouméa

Species	Abbreviation	Ν	Voucher specimens
Reference samples, Lethrininae			
Lethinus atkinsoni	Latk	21	MNHN 2006-1305
Lethinus erythracanthus	Lera	1	photo-voucher
Lethinus genivittatus	Lgen	11	MNHN 2006-1296 to -1299; MNHN 2007-1609
Lethinus harak	Lhar	10	MNHN 2006-1295, -1303, -1310; MNHN 2007-1615
Lethinus lentjan	Llen	17	MNHN 2006-1304, -1312, -1313; MNHN 2007-1616; photo vouchers
Lethinus miniatus	Lmin	57	MNHN 2007-1606
Lethinus nebulosus	Lneb	101	MNHN 2006-1293, 2007-1611; photo voucher
Lethinus obsoletus	Lobs	8	MNHN 2006-1302, -1306, -1307, -1316; MNHN 2007-1617 to -1619
Lethrinus olivaceus	Loli	17	MNHN 2006-1294
Lethrinus ravus	Lrav	16	MNHN 2006-1308, 1309; MNHN 2007-1612 to -1614
Lethrinus rubrioperculatus	Lrub	10	MNHN 2007-1607
Lethrinus semicinctus	Lsem	4	MNHN 2006-1300, -1301, -1314, -1315
Lethrinus variegatus	Lvar	2	MNHN 2007-1608
Lethrinus xanthochilus	Lxan	7	photo-voucher
Reference samples. Monotaxinae			
Gnathodentex aureolineatus	Gaur	10	MNHN 2009-00030004; IRDN z283. z284. z286. z289 to z292
Gymnocranius euanus	Geua	12	MNHN 2007-1610: JNC 1110: photo vouchers
Gymnocranius grandoculis	Ggra	5	photo-vouchers
Gymnocranius sp. A ^a	GspA	6	MNHN 2009-0007
Gymnocranius sp. B	GspB	17	MNHN 2009-0010, -0011; IRDN z179; photo vouchers
Gymnocranius sp. C ^a	GspC	2	-
Monotaxis grandoculis	Mgra	9	MNHN 2006-1311, 2009-0017; photo voucher
Larvae			
Lethrinus genivittatus ^a	Lgen	135	photo-youchers
Lethrinus olivaceus ^a	Loli	1	photo-voucher
Gymnocranius sp. A ^a	GspA	1	photo-voucher
Syntheer annual spirit	00P.1	1	proto countra

^a Distinguished a posteriori by their multiple-locus DNA fingerprints

Table 2. Oligonucleotide primers for the PCR-amplification of one mitochondrial and five nuclear-DNA

markers in Lethrinidae

Locus, primer	Abbreviation	Primer sequences	Reference
Aldolase B intron 1 (2 loci) Aldo B 1.1F Aldo B 1R	Aldo-B fast, slow	5'-GCTCCAGGAAAGGGAATCCTGGC-3' 5'-CCTTTGTCGAAAACCTTGATGCC-3'	Rohfritsch and Borsa (2005) Rohfritsch and Borsa (2005)
Creatine kinase intron 6 CK 6 F CK 7R	СК-6	5'-GACCACCTCCGAGTCATCTC-3' 5'-CAGGTGCTCGTTCCACATGA-3'	Borsa et al. (2004) Borsa et al. (2004)
Gonadotropin-releasing hormon 3 intron 1 GnRH 1 F GnRH 1R	GnRH-1	5'-AATGCACCACATGCTAACAAGGC-3' 5'-CGCACCATCACTCTGCTGTTCGC-3'	Rohfritsch and Borsa (2005) Rohfritsch and Borsa (2005)
Metallothionein intron 1 MetCcSsTr 1 F MetCcSsTr 1 R	Met-1	5'-ATGGAYCCYTGHGACTGCTC-3' 5'-RCAGGATCCWCCGCAGYTGC-3'	Present work Present work
16S rRNA Leth 16S 3' Leth 16S 5'	16S rRNA	5'-GCCCAACCAAAGACATTAGGGCAG-3' 5'-GACCCGTATGAATGGCATAACGAG-3'	Present work Present work

Table 3. Meristic, pigmentation and morphological characters of the different morphotypes of Lethrinidae larvae sampled in the bays around noumea peninsula, New Caledonia. Meristic characters correspond to the number of spines (in roman letters) and soft rays (in arabic letters) on the dorsal (D) and anal (A) fins. Pigmentation and general shape patterns are described for specimens conserved in 70% ethanol. Particular morphological structures (e.g., spinations) are also indicated

Code	Meristic	cs	Pigmentation			Shape		Particular structures	
	D	А	Body	Fins	Head	Body	Head		
LET 1	X, 9	III, 8	Unpigmented when <18 mm SL. Diffuse pigmentation from 19 mm SL on	Unpigmented	Snout and mouth pigmented when \geq 19 mm SL; dark spot above the head at all sizes	Elongate	Pointed	Supra-occipital crest and pre-opercular spine highly visible. Silvery scales under the cheek	
LET 2	X, 9	III, 8	Almost entirely pigmented	Unpigmented	Snout and mouth pigmented; dark spot above head	Elongate	Pointed	Supra-occipital crest and pre-opercular spine highly visible. Silvery scales under the cheek	
LET 3	X, 9	III, 8	Light-brown pigments distributed along discontinuous vertical bands	Spines of the dorsal, anal and pelvic fins pigmented	Snout and mouth with no pigment; dark spot above head	Elongate	Pointed	Supra-occipital crest and pre-opercular spine less developed than for LET 1 and LET 2	
LET 4	X, 10	III, 10	Dark-brown pigments distributed along sharp vertical bands	Dorsal, anal and pelvic fins, and superior and inferior margins of the caudal fin densely pigmented	Starry dark pigments around eye	Elongate but higher than LET 1 to 3	Pointed	As for LET 3	

Table 4. Haplotype frequencies at four nuclear-DNA loci and at mitochondrial locus *16S rRNA* in 14 emperor (Lethrininae) species from New Caledonia. Abbreviations for species in Table 1. *n*, number of haplotypes sampled (in brackets); *nd*, no data

Locus,	Species													
Haplotype	Latk	Lera	Lgen ^a	Lhar	Llen	Lmin	Lneb	Lobs	Loli ^a	Lrav	Lrub	Lsem	Lvar	Lxan
Aldo D fast														
Aluo-D Jusi		nd											1.00	
900 1000	-	nd	-	-	-	-	-	-	-	-	-	-	1.00	-
1000	-	na	-	-	-	1.00	-	-	-	-	-	-	-	-
1007	0.79	na	1.00	1.00	1.00	-	1.00	1.00	0.90	1.00	1.00	1.00	-	1.00
1008	-	na	-	-	-	-	-	-	0.07	-	-	-	-	-
1009	-	nd	-	-	-	-	-	-	0.03	-	-	-	-	-
1017	0.21	nd	-	-	-	-	-	-	-	-	-	-	-	-
<i>(n)</i>	(38)		(32)	(20)	(34)	(108)	(186)	(16)	(30)	(32)	(20)	(8)	(4)	(10)
Aldo-B slow														
930	-	nd	_	_	-	-	0.34	-	-	-	_	-	-	-
937	1.00	nd	1.00	1.00	1.00	0 59	0.66	1.00	1.00	_	-	-	1.00	_
945	-	nd	-	-	-	0.37	-	-	-	1.00	1.00	1.00	-	1.00
(n)	(38)	nu	(30)	(20)	(32)	(108)	(180)	(16)	(30)	(32)	(20)	(8)	(4)	(10)
(n)	(30)		(50)	(20)	(32)	(100)	(100)	(10)	(30)	(32)	(20)	(0)	(4)	(10)
GnRH-1														
976	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-
984	-	1.00	-	0.95	0.12	0.18	1.00	1.00	-	-	-	-	-	-
992	0.33	-	-	-	0.88	-	-	-	-	-	-	-	-	-
1000	0.67	-	-	0.05	-	0.82	-	-	1.00	1.00	1.00	1.00	-	1.00
1016	-	-	_	-	-	-	-	-	-	-	-	-	1.00	-
(n)	(42)	(2)	(292)	(20)	(34)	(114)	(202)	(16)	(36)	(32)	(20)	(8)	(4)	(12)
	()	(-)	(-/-/	()	(2.1)	()	(= • =)	()	(0.0)	(=_)	(-*)	(-)		()
Met-1														
842	nd	nd	-	nd	nd	0.05	-	nd	-	nd	nd	nd	nd	nd
964	nd	nd	-	nd	nd	-	-	nd	-	nd	nd	nd	nd	nd
1000	nd	nd	-	nd	nd	0.95	-	nd	-	nd	nd	nd	nd	nd
1011	nd	nd	1.00	nd	nd	-	-	nd	-	nd	nd	nd	nd	nd
1013	nd	nd	-	nd	nd	-	1.00	nd	-	nd	nd	nd	nd	nd
1014	nd	nd	-	nd	nd	-	-	nd	1.00	nd	nd	nd	nd	nd
<i>(n)</i>			(40)			(114)	(202)		(34)					
IOS RNA	0.05													
D	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-
D2	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-
D3	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
E^*	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-
G	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-
В	-	-	-	-	0.94	-	-	-	-	-	-	-	-	-
B4	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
С	-	-	-	-	-	0.96	-	-	-	-	-	-	-	-
<i>C</i> 2	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-
C4	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-
E'	-	-	-	-	-	-	0.96	-	-	-	-	-	-	-
E'2*	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-
E'3*	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-
E'4*	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-
E''	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
L	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-
Κ	-	-	-	-	-	-	-	-	-	0.88	0.40	0.50	-	-
K'	-	-	-	-	_	-	-	-	-	0.06	-	-	-	-
 K*	-	-	-	-	_	-	-	-	-	0.06	-	-	-	-
K2	-	_	_	_	_	_	_	_	_	-	0.60	_	_	_
F	-	_	_	_	_	_	_	_	_	_	0.00	0.50	_	-
I I	-	-	-	-	-	-	-	-	-	-	-	0.50	-	-
I T	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-
J ID	-	-	-	-	-	-	-	-	-	-	-	-	-	0.71
$J \angle$	-	-	(25)	-	-	-	-	-	-	-	-	-	-	0.29
(n)	(20)	(1)	(25)	(10)	(17)	(57)	(101)	(8)	(18)	(10)	(10)	(4)	(2)	(/)

^a Includes larvae a posteriori identified to species by their multiple-locus DNA genotypes

Table 5. Haplotype frequencies at four nuclear-DNA loci and at mitochondrial-DNA locus 16S rRNA in seven large-eye bream (Monotaxinae) species from New Caledonia. Abbreviations for species as in Table 1. *n*, number of haplotypes sampled (in brackets)

Locus,	Species						
Haplotype	Gaur	Geua	Ggra	GspA ^{a, b}	GspB	GspC ^c	Mgra
Aldo-B fast							
1007	1.00	1.00	1.00	-	1.00	1.00	1.00
1017	-	-	-	1.00	-	-	-
<i>(n)</i>	(18)	(20)	(10)	(12)	(32)	(4)	(14)
СК-6							
140	-	-	-	1.00	-	0.25	-
142	-		0.38	-	1.00	0.75	-
144	-	1.00	-	-	-	-	-
145	-		0.63	-	-	-	-
147	-	-	-	-	-	-	1.00
190	0.20	-	-	-	-	-	-
200	0.80	-	-	-	-	-	-
<i>(n)</i>	(20)	(24)	(8)	(12)	(34)	(4)	(14)
GnRH-1							
968	-	-	0.20	-	0.12	-	-
984	-	-	0.10	-	0.06	-	-
992	-	-	0.10	0.42	0.18	0.50	-
1000	-	0.50	0.50	-	0.26	-	-
1008	1.00	0.50	0.10	0.58	0.38	0.50	1.00
<i>(n)</i>	(20)	(20)	(10)	(12)	(34)	(4)	(14)
Met-1							
926	-	1.00	-	-	-	1.00	-
935	-	-	1.00	-	1.00	-	1.00
949	1.00	-	-	1.00	-	-	-
<i>(n)</i>	(20)	(24)	(10)	(12)	(34)	(4)	(18)
16S RNA							
М	0.80	-	-	-	-	-	-
M2	0.10	-	-	-	-	-	-
М3	0.10	-	-	-	-	-	-
$A^{\prime\prime}$	-	1.00	-	1.00	0.94	1.00	-
Н	-	-	1.00	-	-	-	-
H3	-	-	-	-	0.06	-	-
Α	-	-	-	-	-	-	0.22
A2	-	-	-	-	-	-	0.11
A3	-	-	-	-	-	-	0.22
A4	-	-	-	-	-	-	0.11
A5	-	-	-	-	-	-	0.33
<i>(n)</i>	(10)	(12)	(5)	(6)	(17)	(2)	(9)

 ^a Includes one larva a posteriori identified to species by its multiple-locus DNA genotype
 ^b Individuals of that species distinguished a posteriori from those of *G. grandoculis* by their genotypic composition at loci Aldo-B fast and 16S RNA [°] Distinguished a posteriori from *Gymnocranius* sp. B by its genotypic composition at locus *Met*

Step	Marker locus	Allele	Result	
1	Aldo-B fast	1000 988 else	Lethrinus miniatus Lethrinus variegatus 20 to Step 2	
2	GnRH-1	976 else	Lethrinus genivittatus go to Step 3	
3	16S rRNA	see Table 4	see Table 4	

Table 6. PCR-marker based key to the Lethrininae of New Caledonia.

Step	Marker locus	Allele	Result	
1	СК-6	≥190	Gnathodentex aureolineatus	
		147	Monotaxis grandoculis	
		144	Gymnocranius euanus	
		≤142	Gymnocranius sp. C	
		else	go to Step 2	
2	Aldo-B fast	1017	Gymnocranius sp. A	
	-	1007	go to Step 3	
3	16S rRNA	Н	Gymnocranius grandoculis	
		else	Gymnocranius sp. B	

Table 7. PCR-marker based key to the Monotaxinae sampled in New Caledonia

D D2 D3 E* G E B B4 C C2 C4 E' E'2*E'3*E'4* E" L K K' K2 F I J J2

Fig. 1

Fig. 2

M M2 M3 A" H H3 A A2 A3 A4 A5







Species.	Initial identification			Locus		
Individual no.	from morphology	Aldo B fast	Aldo B slow	GnRH 1	Met-1	16S rRNA
L. atkinsoni						
Z 04	L. atkinsoni	1007	937	1000	-	D
Z 11	L. atkinsoni	1007 1017	937	992	-	D
Z 14	L. atkinsoni	1007	937	1000	—	D2
Z 16	L. atkinsoni	1007	937	1000	—	D
Z 23	L. atkinsoni	1007 1017	937	992	—	D
Z 29	L. atkinsoni	_	-	1000	—	D3
Z 158	L. atkinsoni	1007 1017	937	1000	_	D
Z 159	L. atkinsoni	1007	937	1000 992	-	D2
Z 160	L. atkinsoni	1007 1017	937	1000 992	_	D
Z 161	L. atkinsoni	1007	937	992	-	D
Z 218	L. atkinsoni	1007	937	1000	_	D
Z 219	L. atkinsoni	1007	937	1000 992	_	D
Z 220	L. atkinsoni	1007	937	1000	_	D
Z 221	L. atkinsoni	1007	937	1000	_	D
Z 222	L. atkinsoni	1007	937	1000 992	_	D
Z 223	L. atkinsoni	1007 1017	937	1000 992	_	D
Z 224	L. atkinsoni	1007 1017	937	992	_	D
Z 225	L. atkinsoni	1007 1017	937	1000 992	_	_
Z 226	L. atkinsoni	1007	937	1000	_	D
Z 235	L. atkinsoni	_	_	1000	_	D
Z 308	L. atkinsoni	_	_	_	_	_
Z 348	L. atkinsoni	1007 1017	937	1000	_	D*
L. ervthracanthus						
Z 217	L. erythracanthus	-	-	984	_	E*
I. genivittatus						
Z 26	L. genivittatus	1007	_	976	1011	G
Z 20 7 27	L. genivittatus	1007	_	976	1011	G
734	L. genivittatus	1007	037	976	1011	G
Z 34 7 35	L. genivittatus	1007	931	970	1011	G
Z 33 7 26	L. genivittatus	1007	—	970	1011	G
Z 30 Z 27	L. genivittatus	1007	—	970	-	G
Z 37	L. genivillalus	1007	-	970	1011	G
Z 38	L. genivittatus	1007	937	976	1011	G
Z 39	L. genivittatus	-	937	976	1011	G
Z 40	L. genivittatus	1007	937	976	1011	G
Z 41	L. genivittatus	_	-	976	_	G
Z 42	L. genivittatus	_	-	976	_	G
Zj 01	Lethrininae	_	-	976	_	_
Zj 02	Lethrininae	-	_	976	—	-
Zj 03	Lethrininae	-	-	976	_	-
Zj 04	Lethrininae	-	-	976	-	-
Zj 05	Lethrininae	_	-	976	-	-
Zj 06	Lethrininae	-	-	976	—	-
Zj 07	Lethrininae	_	-	976	—	-
Zj 08	Lethrininae	_	-	976	—	_
Zj 09	Lethrininae	-	-	976	_	-
Zj 10	Lethrininae	-	-	976	-	_
Zj 11	Lethrininae	-	-	976	-	-
Zj 12	Lethrininae	-	-	976	-	-
Zj 13	Lethrininae	_	-	976	-	-
Zj 14	Lethrininae	_	-	976	-	_
Zj 15	Lethrininae	_	-	976	-	_
Zj 16	Lethrininae	-	-	-	_	-

Appendix 1.	Lethrininae of New Caledonia: individual multiple-locus genotypes.

Zj 17	Lethrininae	-	-	976	-	-
Zj 18	Lethrininae	_	_	976	_	_
Zi 19	Lethrininae	_	_	976	_	_
Z; 20	Lathrininga			076		
ZJ 20	Leummae	_	-	970	-	-
Zj 21	Lethrininae	-	-	976	-	-
Zj 22	Lethrininae	-	_	976	_	—
Zi 23	Lethrininae	_	_	976	_	_
7i 24	Lethrininae	_	_	976	_	_
ZJ 24 7: 05				076		
Zj 25	Lethrininae	-	_	9/6	-	-
Zj 26	Lethrininae	-	-	976	-	-
Zj 27	Lethrininae	_	-	976	-	_
7i 28	Lethrininae	_	_	976	_	_
Zj 20 Z; 20	Lethrininge			076		
ZJ 29	Leummae	_	_	970	-	_
Zj 30	Lethrininae	-	-	976	-	-
Zj 31	Lethrininae	-	_	976	_	—
Zi 32	Lethrininae	_	_	976	_	_
7i 33	Lethrininae	_	_	976	_	_
ZJ 55				076		
Zj 34	Lethrininae	-	_	9/6	-	-
Zj 35	Lethrininae	-	-	976	-	-
Zj 36	Lethrininae	_	_	976	_	_
7i 37	Lethrininae	_	_	976	_	_
Zj 57 7: 20	Lethrininge			076		
ZJ 38	Leummae	_	-	970	-	_
Zj 39	Lethrininae	-	-	976	-	-
Zj 40	Lethrininae	_	_	976	_	_
Zi 41	Lethrininae	_	_	976	_	_
-j /2 7i /2	Lethrininge	_	_	976	_	_
ZJ 4 2		_	_)70 074	_	_
Zj 43	Lethrininae	_	-	9/6	-	-
Zj 44	Lethrininae	-	-	976	-	-
Zj 45	Lethrininae	_	_	_	_	_
7i 46	Lethrininae	_	_	976	_	_
Zj 40 7: 47	Lethrininge			076		
ZJ 47	Leummae	_	_	970	_	_
Zj 48	Lethrininae	-	-	976	-	-
Zj 49	Lethrininae	-	-	976	-	-
Zi 50	Lethrininae	_	_	976	_	_
7i 51	Lethrininae	_	_	976	_	_
ZJ 51 7: 50	Lethrininge			076		
ZJ 32	Leummae	_	-	970	-	_
Zj 53	Lethrininae	-	-	976	-	-
Zj 54	Lethrininae	-	-	976	_	—
Zi 55	Lethrininae	_	_	976	_	_
7i 56	Lethrininae	_	_	976	_	_
7:57	Lethrinings			076		
ZJ <i>31</i>	Leummae	_	-	970	-	-
Zj 58	Lethrininae	-	-	976	-	-
Zj 59	Lethrininae	-	_	976	_	—
Zi 60	Lethrininae	_	_	976	_	_
7i 62	Lethrininge	_	_	076	_	_
ZJ 02				076		
Zj 63	Lethrininae	-	_	9/6	-	-
Zj 64	Lethrininae	-	-	976	-	-
Zj 65	Lethrininae	_	_	_	_	_
Zi 66	Lethrininae	_	_	976	_	_
-j ••• 7i 67	Lethrininge	_	_	976	_	_
ZJ 07				076		
Zj 68	Lethrininae	-	_	9/6	-	-
Zj 69	Lethrininae	-	-	976	-	-
Zj 70	Lethrininae	_	_	976	_	_
Zi 71	Lethrininae	_	_	976	_	_
7;72	Lethrininge	_	_	976	_	-
j / -				076		_
ZJ / 5	Letnrininae	-	-	9/0	-	-
Zj 74	Lethrininae	_	-	976	_	-
Zj 75	Lethrininae	_	_	976	_	_
Zj 76	Lethrininae	_	_	976	_	_
7:77	Lethrininge	_	_	976	_	-
				076		_
۲J /ð	Leunrininae	-	-	9/0	-	-

Zj 79	Lethrininae	_	_	976	_	_
Zj 80	Lethrininae	_	_	976	_	_
Zj 81	Lethrininae	_	_	_	_	_
Zi 82	Lethrininae	_	_	976	_	_
Zi 83	Lethrininae	_	_	976	_	_
Zi 84	Lethrininae	_	_	976	_	_
Zi 85	Lethrininae	_	_	976	_	_
Zi 86	Lethrininae	_	_	976	_	_
Zj 80 Zi 87	Lethrininae	_	_	976	_	_
Zj 88	Lethrininae	_	_	-	_	_
Zj 80 Zj 80	Lethrininge	_	_	976	_	_
Zj 0) Zj 00	Lethrininge		_	976	_	_
Zj 90	Lethrininge	_	_	976	_	_
ZJ 91 Z: 02	Letthrininge	—	_	970	_	—
ZJ 92 7: 02	Letthrininge	—	_	970	_	—
ZJ 95 7: 04	Lethrininae	-	—	970	_	—
ZJ 94	Lethrininae	-	_	976	_	_
Zj 95	Lethrininae	_	_	976	_	_
Zj 96	Lethrininae	_	_	976	_	_
Zj 9/	Lethrininae	-	-	976	-	-
Zj 98	Lethrininae	-	—	976	_	—
Zj 99	Lethrininae	-	-	976	-	-
Zj 100	Lethrininae	-	-	976	-	-
Zj 101	Lethrininae	-	-	976	-	-
Zj 102	Lethrininae	-	_	976	_	_
Zj 103	Lethrininae	-	-	976	-	-
Zj 104	Lethrininae	-	-	976	-	-
Zj 105	Lethrininae	-	-	976	-	_
Zj 106	Lethrininae	-	-	976	-	_
Zj 107	Lethrininae	-	_	976	_	_
Zj 108	Lethrininae	_	_	976	_	_
Zj 109	Lethrininae	_	_	976	_	_
Zj 110	Lethrininae	_	_	976	_	_
Zj 111	Lethrininae	_	_	976	_	_
Zj 112	Lethrininae	_	_	976	_	_
Zi 113	Lethrininae	_	_	976	_	_
Zi 114	Lethrininae	_	_	976	_	_
Zi 115	Lethrininae	_	_	976	_	_
Zi 116	Lethrininae	_	_	976	_	_
Zi 117	Lethrininae	_	_	976	_	_
Zi 118	Lethrininae	_	_	976	_	_
Zi 119	Lethrininae	_	_	976	_	_
Zi 120	Lethrininge	_	_	976	_	_
Zj 120 Zj 121	Lethrininge	_	_	976	_	_
Zj 121 7; 122	Lethrininge	_	_	976	_	_
Zj 122 7; 122	Lethrininge	_	_	976	_	_
ZJ 123 7: 124	Letthrininge	—	_	970	_	—
ZJ 124	Letininiae	_	_	976	_	—
Zj 125 7: 126	Lethrininae	_	_	976	_	_
ZJ 120	Lethrininae	-	-	976	-	-
LET 1_01	Lethrininae	1007	937	976	1011	G
LET 1_02	Lethrininae	-	-	976	1011	G
LET 1_03	Lethrininae	-	937	976	1011	G
LET 1_04	Lethrininae	-	_	976	1011	G
LET 1_05	Lethrininae	-	937	976	1011	G
LET 1_06	Lethrininae	1007	937	976	1011	G
LET 1_07	Lethrininae	1007	937	976	1011	G
LET 1_08	Lethrininae	1007	937	976	1011	G
LET 1_09	Lethrininae	_	_	976	_	G
LET 1_10	Lethrininae	1007	937	976	1011	G
LET 1_11	Lethrininae		937	976	1011	G
LET 1_12	Lethrininae	1007	937	976	1011	G
LET 1_13	Lethrininae	_	_	976	_	G

LET 2 Zj 61	Lethrininae	1007	937	976	1011	G
LET 2 (new)	Lethrininae	1007	937	976	1011	_
L. harak						
Z 07	L. harak	1007	937	984	-	E
Z 08	L. harak	1007	937	984	-	E
Z 13	L. harak	1007	937	984	-	E
Z 15	L. harak	1007	937	984	-	E
Z 229	L. harak	1007	937	984	-	E
Z 247	L. harak	1007	937	984	-	E
Z 248	L. harak	1007	937	984	-	E
Z 249	L. harak	1007	937	984	_	Е
Z 250	L. harak	1007	937	984	_	Е
Z 309	L. harak	1007	937	1000 984	-	Е
L. lentian						
Z 02	L. lentian	1007	937	992	_	В
Z 195	L. lentian	1007	_	992	_	В
Z 205	L. lentian	1007	937	992 984	_	B2
Z 206	L. lentian	1007	937	992 984	_	B4
Z 200	L. lentian	1007	937	992 984	_	B2*
Z 207	L. lentjan	1007	937	992 984	_	B
Z 210	L. lentjan	1007	937	992	_	B
Z 210 Z 212	L. lenijan I. lentian	1007	937	992	_	B
Z 212 Z 213	L. tentjan I. lentian	1007	937	992		B2
Z 215 Z 215	L. lenijan L. lentian	1007	937	992	_	D2 R
Z 215 Z 216	L. tentjan L. lontian	1007	937	992	_	D
Z 210 Z 251	L. lenijan L. lentian	1007	937	992	—	D
Z 251	L. tentjan L. lontian	1007	937	992	-	D
Z 252	L. tentjan L. lanting	1007	957	992	_	D
Z 255	L. lentjan	1007	937	992	-	В
Z 277	L. lentjan	1007	937	992	-	В
Z 278 Z 279	L. lentjan L. lentjan	1007	937 937	992 992	_	B
I miniatus						
<i>Z</i> . <i>minialus</i> 7.03	I miniatus	1000	937 945	1000 984	1000	C
Z 89	L. miniatus I. miniatus	1000	937 945	1000 904	1000 842	C
Z 0) Z 00	L. miniatus	1000	945	1000	1000 842	C
Z 90 Z 91	L. miniatus I. miniatus	1000	945	1000	1000 842	C
Z 91 Z 92	L. miniatus I. miniatus	1000	037 0/15	1000 984	1000 842	C
7.03	L. miniatus I. miniatus	1000	037	1000 984	1000 842	C
Z 93 Z 94	L. miniatus	1000	037	1000 984	1000 842	C C
Z 94 Z 05	L. miniatus	1000	937	1000 084	1000	C C
Z 95 Z 06	L. miniatus	1000	027	1000 984	1000	C C
Z 90 Z 07	L. miniatus	1000	937	1000	1000	C
Z 97	L. miniaius	1000	957	964	1000	C
Z 98	L. miniaius	1000	957	1000	1000	C
Z 99	L. miniatus	1000	937 945	1000 984	1000	C
Z 100	L. miniatus	1000	937	1000	1000	C
Z 101	L. miniatus	1000	937 945	1000 984	1000	C
Z 102	L. miniatus	1000	937	1000	1000	C
Z 103	L. miniatus	1000	-	1000	1000	C
Z 104	L. miniatus	1000	937 945	1000	1000	C2
Z 105	L. miniatus	1000	937 945	1000 984	1000	C
Z 106	L. miniatus	1000	937 945	1000 984	1000	C
Z 107	L. miniatus	1000	937 945	1000 984	1000	C
Z 108	L. miniatus	1000	937 945	1000	1000	С
Z 109	L. miniatus	1000	937 945	1000 984	1000	С
Z 110	L. miniatus	1000	937 945	1000	1000	С
Z 111	L. miniatus	1000	937 945	1000	1000	С
Z 112	L. miniatus	-	-	1000	1000	С
Z 113	L. miniatus	1000	937	1000	1000	С

Z 114	L. miniatus	1000	937 945	1000	1000	С
Z 115	L. miniatus	1000	937	1000	1000	С
Z 116	L. miniatus	1000	937	1000 984	1000	С
Z 117	L. miniatus	1000	945	1000 984	1000	С
Z 118	L. miniatus	1000	937 945	1000	1000	С
Z 119	L. miniatus	_	_	1000	1000	С
Z 120	L. miniatus	_	-	1000	1000	С
Z 121	L. miniatus	1000	945	1000	1000	С
Z 125	L. miniatus	1000	_	1000	1000	С
Z 139	L. miniatus	1000	937	1000	1000	С
Z 140	L. miniatus	1000	937 945	1000	1000	С
Z 141	L. miniatus	1000	945	1000	1000	С
Z 142	L. miniatus	1000	937	1000	1000	С
Z 143	L. miniatus	1000	-	1000	1000	С
Z 144	L. miniatus	1000	937	1000	1000	С
Z 244	L. miniatus	1000	937	1000 984	1000 842	С
Z 332	L. miniatus	1000	937	1000	1000	С
Z 333	L. miniatus	1000	945	1000 984	1000	С
Z 334	L. miniatus	1000	937 945	1000 984	1000	С
Z 335	L. miniatus	1000	937	1000	1000	С
Z 336	L. miniatus	1000	-	1000 984	1000	С
Z 337	L. miniatus	1000	937	1000 984	1000	С
Z 338	L. miniatus	1000	937 945	1000	1000	С
Z 339	L. miniatus	1000	937 945	1000	1000	С
Z 345	L. miniatus	1000	945	1000	1000	С
Z 346	L. miniatus	1000	945	1000 984	1000	С
Z 347	L. miniatus	1000	937 945	1000 984	1000	C4
Z 349	L. miniatus	1000	937	1000	1000	С
Z 350	L. miniatus	1000	-	1000	1000	С
Z 351	L. miniatus	1000	937 945	1000	1000	С
Z 352	L. miniatus	1000	937 945	1000	1000	С
L. nebulosus						
Z 06	L. nebulosus	1007	937	984	1013 965	E'
Z 43	L. nebulosus	1007	937	984	1013 965	E'
Z 44	L. nebulosus	1007	937	984	1013 965	E'
Z 45	L. nebulosus	1007	937	984	1013 965	E'
Z 46	L. nebulosus	1007	937	984	1013 965	E'
Z.47	L. nebulosus	1007	930 937	984	1013 965	E'
7 48	I nebulosus	1007	937	984	1013 965	- F'
Z 40	L. nebulosus	1007	020.027	084	1012 065	
Z 49	L. nebulosus	1007	930 937	984	1013 903	E
Z 50	L. nebulosus	1007	930 937	984	1013 965	E
Z 51	L. nebulosus	1007	930 937	984	1013 965	E'
Z 52	L. nebulosus	1007	930 937	984	1013 965	E'
Z 53	L. nebulosus	1007	930 937	984	1013 965	E'2*
Z 54	L. nebulosus	1007	930 937	984	1013 965	E'
Z 55	L. nebulosus	1007	930 937	984	1013 965	E'
Z 56	L. nebulosus	1007	930 937	984	1013 965	E'
7 57	L. nebulosus	1007	930 937	984	1013 965	E'
759	L. nebulosus	1007	020.027	084	1013 705	
Z 38	L. nebulosus	1007	930 937	984	1013 965	E
2.59	L. nebulosus	1007	930 937	984	1013 965	E'
Z 60	L. nebulosus	1007	930 937	984	1013 965	E'
Z 61	L. nebulosus	1007	930 937	984	1013 965	E'
Z 62	L. nebulosus	1007	930 937	984	1013 965	E'4*
Z 63	L. nebulosus	1007	930 937	984	1013 965	E'
Z 64	L. nebulosus	1007	930 937	984	1013 965	E'
7.65	L nebulosus	1007	937	984	1013 965	- F'
7.66	L. nobulosus	1007	030 037	084	1013 065	L L'
2.00	L. neoulosus	1007	220 221	204	1015 905	E

Z 67	L. nebulosus	1007	930 937	984	1013 965	E'
Z 68	L. nebulosus	_	_	984	1013 965	E'
Z 69	L. nebulosus	1007	930 937	984	1013 965	E'
Z 70	L. nebulosus	1007	930 937	984	1013 965	E'
Z 71	L. nebulosus	1007	930 937	984	1013 965	E'
Z 72	L. nebulosus	1007	930 937	984	1013 965	E'
Z 73	L. nebulosus	1007	930 937	984	1013 965	E'
Z 74	L. nebulosus	1007	930 937	984	1013 965	E'
Z 75	L. nebulosus	1007	930 937	984	1013 965	E'
Z 76	L. nebulosus	1007	930 937	984	1013 965	E'
Z 77	L. nebulosus	1007	930 937	984	1013 965	- E'
Z 78	L. nebulosus	1007	930 937	984	1013 965	- E'
Z 79	L. nebulosus	1007	930 937	984	1013 965	= E'
Z 80	L. nebulosus	1007	930 937	984	1013 965	E'
Z 81	L. nebulosus	1007	937	984	1013 965	E'
7.82	L. nebulosus	1007	930 937	984	1013 965	E'
7.83	L. nebulosus	1007	930 937	08/	1013 965	E'
Z 83	L. nebulosus	1007	030 037	084	1013 965	E'
Z 04	L. nebulosus	1007	027	084	1013 905	E E'
Z 85	L. nebulosus	1007	937	984	1013 903	E E'
Z 122	L. nebulosus	-	—	984	1013 903	E E'2*
Z 123	L. nebulosus	1007	-	984	1013 965	E 2*
Z 124	L. nebulosus	1007	930 937	984	1013 965	E
Z 12/	L. nebulosus	1007	937	984	1013 965	E
Z 128	L. nebulosus	1007	937	984	1013 965	E
Z 129	L. nebulosus	1007	937	984	1013 965	E'
Z 130	L. nebulosus	1007	937	984	1013 965	E'
Z 131	L. nebulosus	1007	937	984	1013 965	E'
Z 132	L. nebulosus	1007	937	984	1013 965	E'
Z 133	L. nebulosus	1007	937	984	1013 965	Ε'
Z 134	L. nebulosus	1007	930 937	984	1013 965	E'
Z 135	L. nebulosus	1007	930 937	984	1013 965	E'
Z 136	L. nebulosus	1007	930 937	984	1013 965	E'
Z 137	L. nebulosus	1007	937	984	1013 965	E'
Z 138	L. nebulosus	1007	930 937	984	1013 965	E'
Z 162	L. nebulosus	1007	930 937	984	1013 965	E'
Z 163	L. nebulosus	1007	930 937	984	1013 965	E'
Z 164	L. nebulosus	1007	930 937	984	1013 965	E'
Z 165	L. nebulosus	1007	930 937	984	1013 965	E'
Z 166	L. nebulosus	1007	930 937	984	1013 965	E'
Z 167	L. nebulosus	1007	-	984	1013 965	E'
Z 168	L. nebulosus	_	-	984	1013 965	E'3*
Z 169	L. nebulosus	_	_	984	1013 965	E'
Z 170	L. nebulosus	_	_	984	1013 965	E'
Z 171	L. nebulosus	1007	930 937	984	1013 965	E'
Z 172	L. nebulosus	1007	930 937	984	1013 965	E'
Z 173	L. nebulosus	_	_	984	1013 965	E'
Z 174	L. nebulosus	_	_	984	1013 965	E'
Z 175	L. nebulosus	1007	930 937	984	1013 965	E'
Z 176	L. nebulosus	_	_	_	1013 965	E'
Z 182	L. nebulosus	1007	930 937	984	1013 965	E'
Z 197	L. nebulosus	1007	930 937	984	1013 965	E'
Z 198	L. nebulosus	1007	930 937	984	1013 965	E'
Z 200	L. nebulosus	1007	930 937	984	1013 965	E'
Z 227	L. nebulosus	1007	937	984	1013 965	E'

Z 228	L. nebulosus	1007	937	984	1013 965	E'
Z 230	L. nebulosus	1007	937	984	1013 965	E'
Z 231	L. nebulosus	1007	937	984	1013 965	E'
Z 232	L. nebulosus	1007	937	984	1013 965	E'
Z 233	L. nebulosus	1007	937	984	1013 965	E'
Z 234	L. nebulosus	1007	930 937	984	1013 965	E'
Z 236	L. nebulosus	1007	937	984	1013 965	E'
Z 237	L. nebulosus	1007	937	984	1013 965	E'
Z 238	L. nebulosus	1007	937	984	1013 965	E'
Z 255	L. nebulosus	1007	937	984	1013 965	E'
Z 321	L. nebulosus	1007	930 937	984	1013 965	E'
Z 322	L. nebulosus	1007	937	984	1013 965	E'
Z 323	L. nebulosus	1007	_	984	1013 965	E'
Z 324	L. nebulosus	1007	930 937	984	1013 965	E'
Z 325	L. nebulosus	1007	930 937	984	1013 965	E'
Z 326	L. nebulosus	1007	930 937	984	1013 965	- E'
Z 327	L. nebulosus	1007	930 937	984	1013 965	- E'
Z 328	L. nebulosus	1007	930 937	984	1013 965	
Z 329	L. nebulosus	1007	930 937	984	1013 965	E'
Z 330	L. nebulosus	1007	930 937	984	1013 965	E'
Z 330 7 331	L. nebulosus	1007	930 937	984	1013 965	E F
7 3/4	L. nebulosus	1007	930 937	08/	1013 965	E'
Z 344	L. neoutosus	1007	750 751	704	1015 705	L
L. obsoletus						
Z 12	L. obsoletus	1007	937	984	_	E"
Z 145	L. obsoletus	_	_	-	_	_
Z 146	L. obsoletus	1007	937	984	_	E''
Z 280	L. obsoletus	1007	937	984	_	E"
Z 281	L. obsoletus	1007	937	984	-	E''
Z 282	L. obsoletus	1007	937	984	_	E"
Z 312	L. obsoletus	1007	937	984	_	E"
Z 313	L. obsoletus	1007	937	984	—	E"
Z 314	L. ODSOIETUS	1007	937	984	_	E
L olivaceus						
Z 09	L. olivaceus	1007	937	1000	1014	L
Z 10	L. olivaceus	1007	937	1000	1014	L
Z 86	L. olivaceus	_	_	1000	1014	L
Z 183	L. olivaceus	_	-	1000	1014	L
Z 184	L. olivaceus	-	-	1000	1014	L
Z 196	L. olivaceus	1007	937	1000	1014	L
Z 254	L. olivaceus	1007	937	1000	1014	L
Z 256	L. olivaceus	1007	937	1000	1014	L
Z 257 Z 259	L. olivaceus	1007	937	1000	-	L
Z 238 7 259	L. olivaceus	1007	937	1000	1014	L
Z 260	L. olivaceus	1007	937	1000	1014	L
Z 261	L. olivaceus	1007	937	1000	1014	L
Z 262	L. olivaceus	1007	937	1000	1014	L
Z 263	L. olivaceus	1007 1008	937	1000	1014	L
Z 264	L. olivaceus	1007	937	1000	1014	L
Z 265	L. olivaceus	1007 1009	937	1000	1014	L
LET 3	Lethrininae	1007	937	1000	1014	L
L. ravus	T	1007	0.45	1000		
Z 31 Z 149	L. ravus	1007	945	1000	-	K TZ
Z 148	L. ravus	1007	945	1000	—	K

Z 149	L. ravus	1007	945	1000	_	К
Z 150	L. ravus	1007	945	1000	_	К
Z 151	L. ravus	1007	945	1000	_	К
Z 152	L. ravus	1007	945	1000	_	Κ
Z 153	L. ravus	1007	945	1000	_	Κ
Z 154	L. ravus	1007	945	1000	_	К
Z 155	L. ravus	1007	945	1000	_	К
Z 156	L. ravus	1007	945	1000	_	К
Z 157	L. ravus	1007	945	1000	_	K*
Z 239	L. ravus	1007	945	1000	_	К
Z 240	L. ravus	1007	945	1000	_	К
Z 241	L. ravus	1007	945	1000	_	К
Z 242	L. ravus	1007	945	1000	_	K'
Z 243	L. ravus	1007	945	1000	_	К
L. rubrioperculatus	1					
Z 05	L. rubrioperculatus	1007	945	1000	_	К
Z 19	L. rubrioperculatus	1007	945	1000	_	К
Z 20	L. rubrioperculatus	1007	945	1000	_	K2
Z 21	L. rubrioperculatus	1007	945	1000	_	К
Z 22	L. rubrioperculatus	1007	945	1000	_	K2
Z 30	L. rubrioperculatus	1007	945	1000	_	К
Z 340	L. rubrioperculatus	1007	945	1000	_	К
Z 341	L. rubrioperculatus	1007	945	1000	_	К
Z 342	L. rubrioperculatus	1007	945	1000	_	K2
Z 343	L. rubrioperculatus	1007	945	1000	_	K2
L. semicinctus						
Z 87	L. semicinctus	1007	945	1000	_	F
Z 88	L. semicinctus	1007	945	1000	_	F
Z 310	L. semicinctus	1007	945	1000	_	К
Z 311	L. semicinctus	1007	945	1000	_	Κ
L. variegatus						
Z 28	L. variegatus	988	937	1016	_	Ι
Z 33	L. variegatus	988	937	1016	_	Ι
	0					
L. xanthochilus						
Z 185	L. xanthochilus	1007	945	1000	_	J2
Z 186	L. xanthochilus	_	_	_	_	J
Z 187	L. xanthochilus	1007	945	1000	_	J
Z 188	L. xanthochilus	1007	945	1000	_	J
Z 189	L. xanthochilus	1007	945	1000	_	J
Z 190	L. xanthochilus	1007	945	1000	_	J2
Z 191	L. xanthochilus	_	_	1000	_	J

L. miniatus: to estimate allelic frequencies in the sample, we considered weak band patterns at locus *Aldo-B slow* as heterozygotes 945 937, but we cannot exclude they were null or quasi-null homozygotes

Species,	Initial identification Locus					
Individual no.	from morphology	Aldo B fast	СК-6	GnRH 1	Met-1	16S rRNA
Cu atha dantan ann	alin antun					
Gnainoaeniex aure	Conneanus	1007	200/100	1009	0.40	м
Z 283	G. aureolineatus	1007	200/190	1008	949	M
Z 284	G. aureolineatus	1007	200	1008	949	M
Z 285	G. aureolineatus	1007	200	1008	949	IVI M
Z 280	G. aureolineatus	1007	200/190	1008	949	IVI M
Z 287	G. aureolineatus	1007	200	1008	949	M
Z 288	G. aureolineatus	1007	200/190	1008	949	M
Z 289	G. aureolineatus	-	200	1008	949	M2
Z 290	G. aureolineatus	1007	200	1008	949	M
Z 291	G. aureolineatus	1007	200	1008	949	M3
Z 292	G. aureolineatus	1007	200/190	1008	949	М
Gymnocranius eua	nus					
Z 01	G. euanus	1007	144	1008	926	A''
Z 17	G. euanus	1007	144	1008/1000	926	A''
Z 18	G. euanus	1007	144	1008/1000	926	A''
Z 24	G. euanus	1007	144	1008/1000	926	A''
Z 25	G. euanus	1007	144	1008	926	A''
Z 126	G. euanus	_	144	1000	926	A''
Z 147	G. euanus	1007	144	1008	926	A''
Z 315	G. euanus	_	144	_	926	A''
Z 316	G. euanus	1007	144	1000	926	A"
Z 317	G. euanus	1007	144	1000	926	A"
Z 318	G. euanus	1007	144	1008 1000	926	A"
Z 319	G. euanus	1007	144	_	926	A''
<i>a</i> .	1 1.					
Gymnocranius gra	ndoculis	1007	1.42	1000.070	0.25	
Z 32	G. grandoculis	1007	142	1008 968	935	H
Z 1//	G. grandoculis	1007	145	1000 984	935	H
Z 1/8	G. grandoculis	1007	-	1000 992	935	H
Z 245	G. grandoculis	1007	145	1000 968	935	H
Z 320	G. grandoculis	1007	145/142	1000	935	Н
<i>Gymnocranius</i> , une	described sp. A					
Z 199	G. grandoculis	1017	140	992	949	A''
Z 293	G. grandoculis	1017	140	1008 992	949	A"
Z 294	G. grandoculis	1017	140	1008 992	949	A''
Z 295	G. grandoculis	1017	140	1008	949	A''
Z 296	G. grandoculis	1017	140	1008 992	949	A''
LET 4	Monotaxinae	1017	140	1008	949	A"
Cumpo and in -	desceribed on D					
<i>Cymnocranius</i> , uno Z 179	Gymnocranius sp	1007	142	1000	935	Δ ''
7 180	Gymnoeranius sp.	1007	142	1000	935	Δ"
7.246	Gymnoeranius sp.	1007	142	1000	935	Δ"
7 260	Gymnoeranius sp.	1007	142 142	1008	025	Δ."
7 270	Gymnoeranius sp.	1007	142	1008	935	Δ."
7 271	Gymnoeranius sp.	1007	142	984 968	025	Λ Δ"
	Gymnoeranius sp.	1007	142	704 700	755	п

Appendix 2.	Monotaxinae of Ne	w Caledonia	: individual	l multiple-locu	s genotypes
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Z 273	Gymnocranius sp.	1007	142	1008 968	935	A''
Z 274	Gymnocranius sp.	1007	142	1008 968	935	A''
Z 275	Gymnocranius sp.	1007	142	1000 968	935	A''
Z 276	Gymnocranius sp.	1007	142	1000 992	935	A''
Z 300	Gymnocranius sp.	1007	142	1008 992	935	A''
Z 302	Gymnocranius sp.	_	142	1008 1000	935	H3
Z 303	Gymnocranius sp.	1007	142	1008 992	935	A''
Z 304	Gymnocranius sp.	1007	142	1008 992	935	A''
Z 306	Gymnocranius sp.	1007	142	1008 984	935	A''
Z 307	Gymnocranius sp.	1007	142	1000 992	935	A"
Gymnocranius,	undescribed sp. C					
Z 301	Gymnocranius sp.	1007	142	1008 992	926	A''
Z 305	Gymnocranius sp.	1007	142/140	1008 992	926	A''
Monotaxis grav	ndoculis					
Z 181	M. grandoculis	1007	147	1008	935	А
Z 192	M. grandoculis	1007	147	1008	935	A2
Z 204	M. grandoculis	_	_	1008	935	А
Z 266	M. grandoculis	1007	147	1008	935	A3
Z 267	M. grandoculis	1007	147	1008	935	A4
Z 268	M. grandoculis	1007	147	1008	935	A3
Z 297	M. grandoculis	_	147	1008	935	A5
Z 298	M. grandoculis	1007	_	1008	935	A5
Z 299	M. grandoculis	1007	147	1008	935	A5