Zoological Studies **46**(2): 186-193 (2007)

Zoological Studies

Two Hybrids of Carangid fishes of the Genus *Caranx*, *C. ignobilis* x *C. melampygus* and *C. melampygus* x *C. sexfasciatus*, from the Hawaiian Islands

Kirk Murakami[1], Shelley A. James[2], John E. Randall[3,*], and Arnold Y. Suzumoto[3]

[1]*Waikiki Aquarium, 2777 Kalakaua Ave., Honolulu, HI 96815, USA*

[2]*Bishop Museum, Pacific Center for Molecular Biodiversity, 1525 Bernice St., Honolulu, HI 96817-2704, USA*

[3]*Bishop Museum, Natural Sciences - Ichthyology, 1525 Bernice St., Honolulu, HI 96817-2704, USA*

Contribution 2006-017 to the Hawaii Biological Survey

(Accepted Apirl 18, 2006)

Kirk Murakami, Shelley A. James, John E. Randall, and Arnold Y. Suzumoto (2007) Two hybrids of carangid fishes of the genus *Caranx*, *C. ignobilis* x *C. melampygus* and *C. melampygus* x *C. sexfasciatus*, from the Hawaiian Is. *Zoological Studies* **46**(2): 186-193. The popular game fishes, the giant trevally (*Caranx ignobilis*) and bigeye trevally (*C. sexfasciatus*) are shown to hybridize with the bluefin trevally (*C. melampygus*) at O'ahu, Hawaiian Is. Evidence for the hybrids is provided by color photographs, morphology, and DNA analysis. Future submissions for a record game fish, especially if substantially larger than the existing record, should include a tissue sample of the intended record fish. http://zoolstud.sinica.edu.tw/Journals/46.2/186.pdf

Key words: Hybrid, Carangid fishes, *Caranx*, Hawaiian Is.

The International Game Fish Association (IGFA) keeps the world angling records for game fishes. The all-tackle world record for *Caranx melampygus*, the bluefin trevally (Hawaiian name, '*omilu*) is 26 lb 7 oz (12 kg), a fish caught at Clipperton I. The all-tackle record for *C. ignobilis*, the giant trevally (Hawaiian name, *ulua aukea*) is 145 lb 8 oz (66 kg) from Maui. The largest giant trevally landed, not by angling, weighed 191 lb (86.6 kg), also from Maui.

In Dec. 1987 Dr. Harvey K. Minatoya of Honolulu caught a carangid fish from a shallow landlocked lagoon at Christmas I. (Kiritimati), Line Is. that weighed 96 lb 0 oz (43.54 kg), shown here as figure 1. He believed his fish to be a bluefin trevally and applied to the IGFA for the world record for *Caranx melampygus*.

Dunn-Rankin (1988) published an article for *Hawaii Fishing News* that illustrated Minatoya's "controversial fish". He wrote, "The unique coloration of Dr. Minatoya's trevally makes me suspect that it may be some variation of the giant trevally, perhaps a hybrid. Both the bluefin and the giant trevally travel together in schools during their spawning period." He added, "Perhaps a new species is present at Christmas I.". Dr. Minatoya's record was initially accepted, but later rejected. He informed us that *Caranx melampygus* was also present in the lagoon. We believe his fish was a hybrid of *C. ignobilis* and *C. melampygus*.

In a world review of the literature on hybrids of fishes, Schwartz (1972) found none in the family Carangidae. He recently advised us of the following publication that reported a naturally occurring hybrid of carangid fishes. Berry and Cohen (1974) found three specimens from Madeira that were intermediate to *Trachurus trachurus* (Linnaeus)

*To whom correspondence and reprint requests should be addressed. Tel: 1-800-8484130. E-mail:jackr@hawaii.rr.com

and *T. picturatus* Bowdich; they considered them to be hybrids.

Herein we document 2 naturally occurring hybrids of the genus *Caranx* from Hawaiian waters.

MATERIALS AND METHODS

Molecular techniques were used to provide confirmation of hybridization of 2 carangid fishes of the genus *Caranx*. The mitochondrial 16S ribosomal DNA and cytochrome c oxidase subunit 1 (COI or *cox*1) genes of the 2 hybrid specimens and 3 parental species (Table 1) were determined. Randomly amplified polymorphic DNA (RAPD) fingerprinting using 6 primers was undertaken to confirm the parental species of the 2 hybrid individuals.

DNA extraction. Genomic DNA was extracted from 25 mg of muscle tissue from each of the specimens (Table 1) and preserved in 95% ethanol using a DNEasy Tissue Kit (Qiagen, Valencia, CA, USA) following the recommended protocol after washing the tissue with phosphate-buffered saline (PBS). Voucher DNA was accessioned into the Pacific Center for Molecular Biodiversity (Table 1).

Sequencing of mtDNA. Polymerase chain reaction (PCR) was used to amplify a 587-589-bp region of the mitochondrial 16S ribosomal DNA

Fig. 1. Presumed hybrid, *Caranx ignobilis* x *C. melampygus*, 43.54 kg, Christmas I., Line Is., with the angler H. Minatoya.

Table 1. Species used in this study and their corresponding voucher numbers in Bishop Museum collections and 16S ribosomal DNA and cytochrome c oxidase (COI) GenBank accession numbers

Species	Collection date d-m-y	Locality	BPBM #	PCMB #	GenBank 16S rDNA	Genbank COI
Caranx melampygus	9-9-2004	Kāne'ohe Bay, O'ahu	39589	414	DQ427053	DQ427059
	14-3-2005	Ka'a'awa, O'ahu	39696	480	DQ427058	DQ427062
Caranx ignobilis	21-9-2004	Kahuku, O'ahu	39590	415	DQ427054	DQ427060
Caranx sexfasciatus	16-8-2004	Kāne'ohe Bay, O'ahu	39581	416	DQ427055	DQ427061
Caranx ignobilis x *melampygus*	1-6-2001	Ka'a'awa, O'ahu	n/a	417	DQ427056	DQ427064
Caranx melampygus x *sexfasciatus*	16-8-2004	Kāne'ohe Bay, O'ahu	39582	418	DQ427057	DQ427063

gene and a 625-bp region of the cytochrome c oxidase subunit I (COI) gene for each specimen. Due to nucleotide differences in the GenBank sequence for *C. melampygus* (GenBank accession no. AP004445) with the specimen collected for this study, a 2nd *C. melampygus* specimen was also sequenced. The GenBank-published *C. melampygus* mitochondrial sequences did not match those of the 2 specimens collected for this study, but instead matched the sequences of the *C. sexfasciatus* specimen, indicating the importance of maintaining voucher specimens. Reactions were performed in 50 μL of a solution containing approximately 10 ng of genomic DNA, 400 μM of each dNTP, 1.5 units *Taq* polymerase (D4545, Sigma Chemical, St. Louis, MO, USA), 2 mM $MgCl_2$, each primer at 1 μM, and 1x PCR buffer. The primers used to amplify the 2 gene regions are as follows (5'-3'): 16S (Palumbi, 1996): forward CGC CTG TTT ATC AAA AAC AT, reverse CCG GTC TGA ACT CAG ATC ACG T; COI (Ward, 2005): forward TCA ACC AAC CAC AAA GAC ATT GGC AC, reverse ACT TCA GGG TGA CCG AAG AAT CAG AA. PCR cycling parameters (PTC 100, MJ Research, Waltham, MA, USA) for the initial double-stranded amplification were 94°C (1 min), 50°C (1 min), and 72°C (1 min), repeated for 45 cycles with a final extension of 72°C (5 min). The PCR product was gel extracted using QIAQuick Gel Extraction Kit or QIAQuick PCR Purification Kit (Qiagen,Valencia CA,USA), and quantified on a 1.5% agarose gel using ethidium bromide. Cycle sequencing of 100 fmol of the double-stranded PCR product was carried out with each primer using a CEQ DTCS Quick Start Kit (Beckman-Coulter, Fullerton CA) following the recommended protocol and the products sequenced on a CEQ8000 genetic analysis system. All sequences have been submitted to GenBank (Table 1).

RAPD analysis. Six ten-mer primers (University of British Columbia) were used in PCR reactions (5'-3'): 212: GCT GCG TGA C; 239: CTG AAG CGG A; 244: CAG CCA ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 347: TTG CTT GGC G. PCR was performed in a volume of 15 μL containing 2 mM $MgCl_2$, 0.24 μM of each dNTP, 15 ng BSA, 0.36 μM primer, 0.3 ng genomic DNA, and 0.6 unit of *Taq* DNA polymerase, well mixed and the surface covered with 20 μL sterile mineral oil. PCR cycling parameters were 94°C (1.5 min) for initial strand separation, then 40 cycles of 38°C (2 min), 72°C (2 min), 91°C (1 min). An additional step of 72°C (5 min) was used for final extension. Amplification products were analyzed by electrophoresis (75 V, 60 min) in 1.5% agarose gels and detected by staining with ethidium bromide. Only bands that were unambiguous, well amplified, and reproducible in replicate tests were scored as present or absent. Data were analyzed by principal components analysis using NTSYSpc version 2.1 (Rohlf 2000).

RESULTS

Caranx ignobilis x *C. melampygus*

In June 2001 KM caught a carangid fish by hook and line off Ka'a'awa, O'ahu that was 735 mm in fork length (FL), 800 mm in total length, and weighed 8.4 kg (Fig. 2 A). It was intermediate in life color to the giant trevally, *Caranx ignobilis* (Fig. 2 B) and the bluefin trevally, *C. melampygus* (Fig. 2 C). KM described the life color as silvery gray-green overall, darker dorsally, grading to pearly white ventrally, with only a few small pale blue spots dorsally and no small black spots as seen on *melampygus*; cheek with faint blue-green markings; median fins silver-gray with a subtle bluish tinge, the anal with a narrow white border. The color darkened as the fish died, turning somewhat bronze overall, and the fins appeared very dark bluish gray. The fish was brought to the Bishop Museum where it was examined by AYS and JER and determined to be a probable hybrid. The 2 species of *Caranx* are distinguished in keys primarily by the fully scaled chest of *melampygus*, compared to the naked ventral part of the chest of *ignobilis*, typically with only a prepelvic patch of scales. The fish was like *ignobilis* with respect to the chest scalation. The dorsal and anal soft rays and the number of gill rakers of these 2 species differ (Smith-Vaniz in Carpenter and Niem 2000); a comparison of these counts can be made with those to the presumed hybrid in table 2. The meristic data from the hybrid slightly favor those of

Table 2. Meristic Data of Species of *Caranx* and Hybrid

	Dorsal rays	Anal rays	Gill rakers
C. ignobilis	18-21	15-17	20-24
Hybrid	22	17	18
C. melampygus	21-24	17-21	17-21

Gill-raker counts are those of the lower-limb of the first gill arch, including the one at the angle.

melampygus. The specimen was not preserved, but a fiberglass reproduction was prepared from a plaster mold.

The 16S ribosomal DNA (rDNA) mitochondrial gene sequence differed by 9 bp for *C. melampygus* and *C. ignobilis* (98.5% similarity), 13 bp for *C. melampygus* and *C. sexfasciatus* (97.8% similarity), and 15 bp for *C. ignobilis* and *C. sexfasciatus* (97.5% similarity). The cytochrome c oxidase subunit 1 (COI) mitochondrial gene sequence differed by 57 bp for *C. melampygus* and *C. ignobilis* (90.9% similarity), 35 bp for *C. melampygus* and *C. sexfasciatus* (94.4%), and 54 bp for *C. ignobilis* and *C. sexfasciatus* (91.4% smilarity). The hybrid specimen PCMB417 (*Caranx ignobilis* x *melampygus*) had only a single nucleotide difference in the

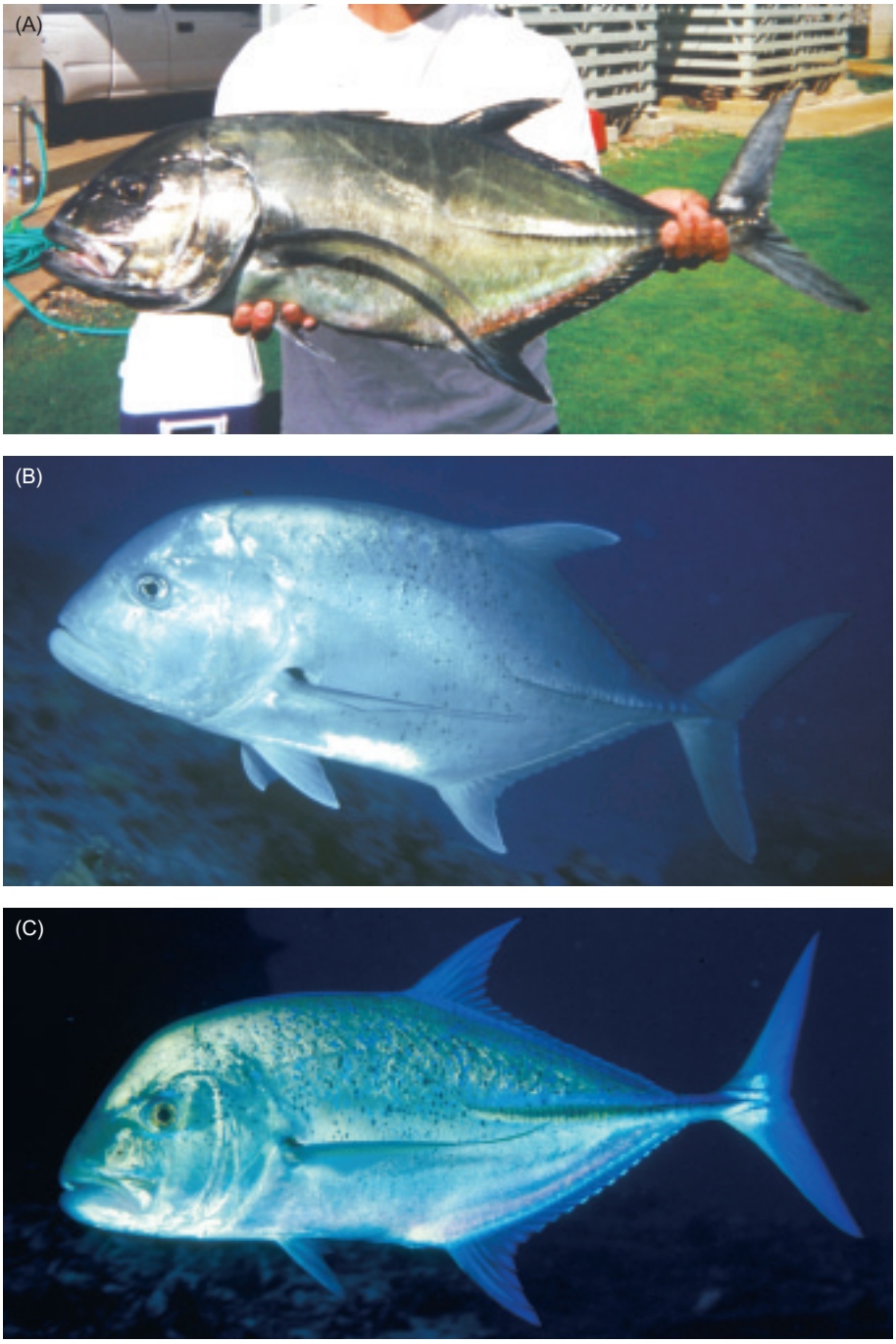

Fig. 2. (A) *Caranx ignobilis* x *C. melampygus*, 735 mm FL, O'ahu. (B) *Caranx ignobilis*, Midway. (C) *Caranx melampygus*, Maui.

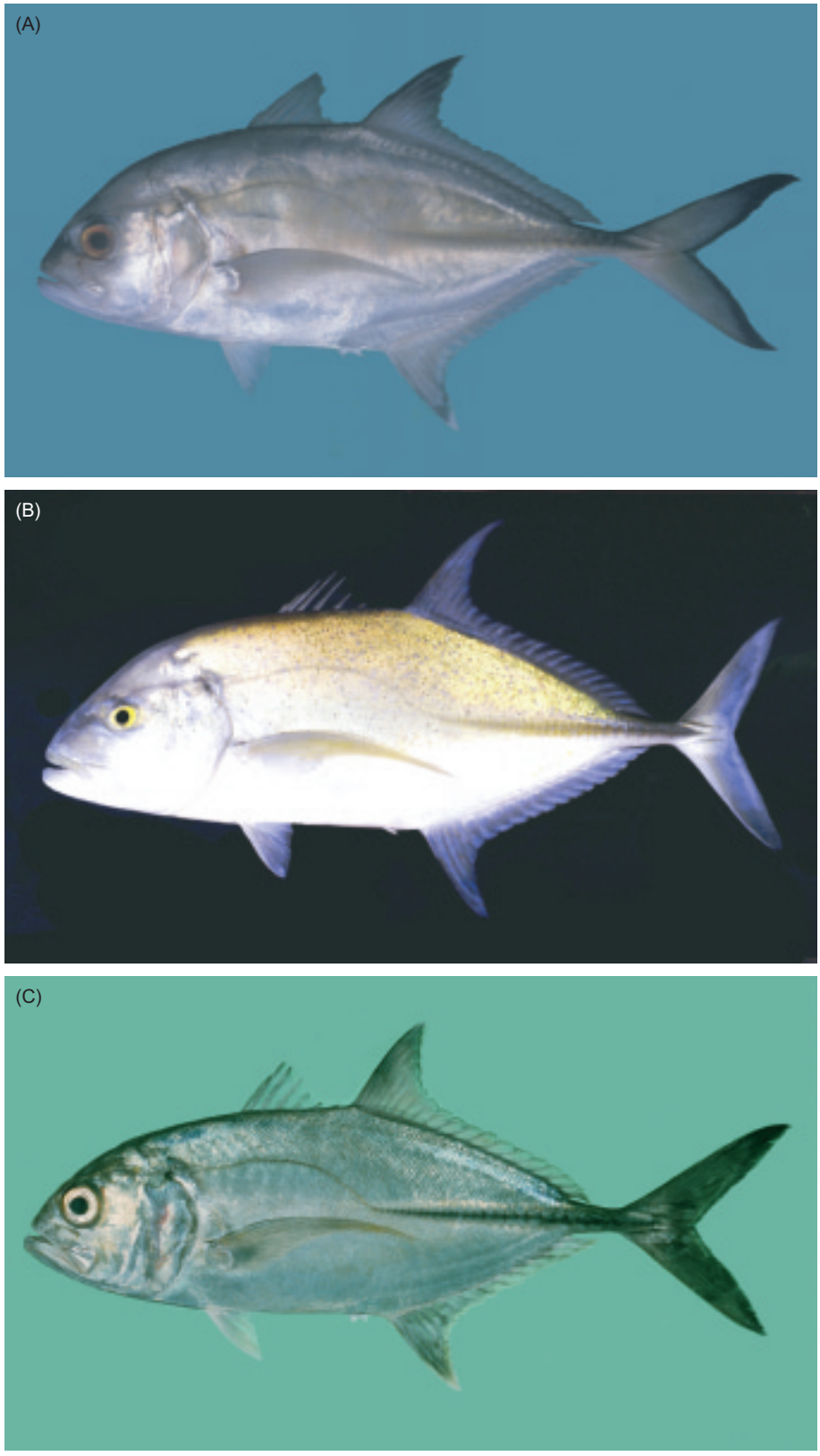

Fig. 3. (A) *Caranx melampygus* x *C. sexfasciatus*, BPBM 39582, 261 mm FL, O'ahu. (B) *Caranx melampygus*, BPBM 39696, 490 mm fork length, O'ahu. (C) *Caranx sexfasciatus*, BPBM 20770, 358 mm FL, Red Sea.

mitochondrial 16S and COI rDNA sequences from the two *C. melampygus* individuals, which is within the range of variation found within fish species for the COI gene (Ward et al. 2005). The 16S and COI sequence data indicated that *C. melampygus* is the maternal parent of the hybrid specimen. Analysis of 76 RAPD bands indicate that PCMB417 (*C. ignobilis* x *C. melampygus*) is intermediate in banding between *C. melampygus* (PCMB414) and *C. ignobilis* (PCMB415) individuals (Fig. 4), therefore confirming the hybrid origin of the specimen.

Caranx melampygus x *Caranx sexfasciatus*

In Aug. 2004 KM caught a carangid in Kāne'ohe Bay, O'ahu near the Coconut I. pier that seemed to be the bigeye trevally, *C. sexfasciatus* at 1st glance, but the black spot at the upper end of the gill opening that is characteristic of this species was very faint, the scutes were not as blackish as they should have been, and the eye seemed too small. Again suspecting a hybrid, the fish was brought to the Bishop Museum, a photograph was taken (Fig. 3A), and a sample of tissue removed. The specimen measured 261 mm in FL. It was fixed in formalin, preserved in alcohol, and cataloged as BPBM 39582. The chest of *C. sexfasciatus* is fully scaled, which is true for 3 of the 4 species of *Caranx* known from the Hawaiian Is., *lugubris*, *melampygus*, and *sexfasciatus*. The 4th is *ignobilis* with a chest that is largely naked ventrally. *Caranx lugubris* was not considered to be a possible parent species because of its somber hue, steep dorsal head profile, and long anterior lobe of the 2nd dorsal and anal fins. *Caranx melampygus* thus became the prime suspect as a parent of the presumed hybrid. KM caught 2 specimens of this species from O'ahu for tissue samples, one (BPBM 39696, 490 mm FL) was photographed (Fig. 3B). A specimen photograph of *C. sexfasciatus* is provided here as figure 3 C.

The fin-ray and gill-raker counts (Table 3)

Table 3. Meristic Data of Species of *Caranx* and Hybrid

	Dorsal rays	Anal rays	Gill rakers
C. melampygus	21-24	17-21	17-21
Hybrid	22	18	19
C. sexfasciatus	19-22	14-17	15-19

Gill-raker counts are those of the lower-limb of the first gill arch, including the one at the angle.

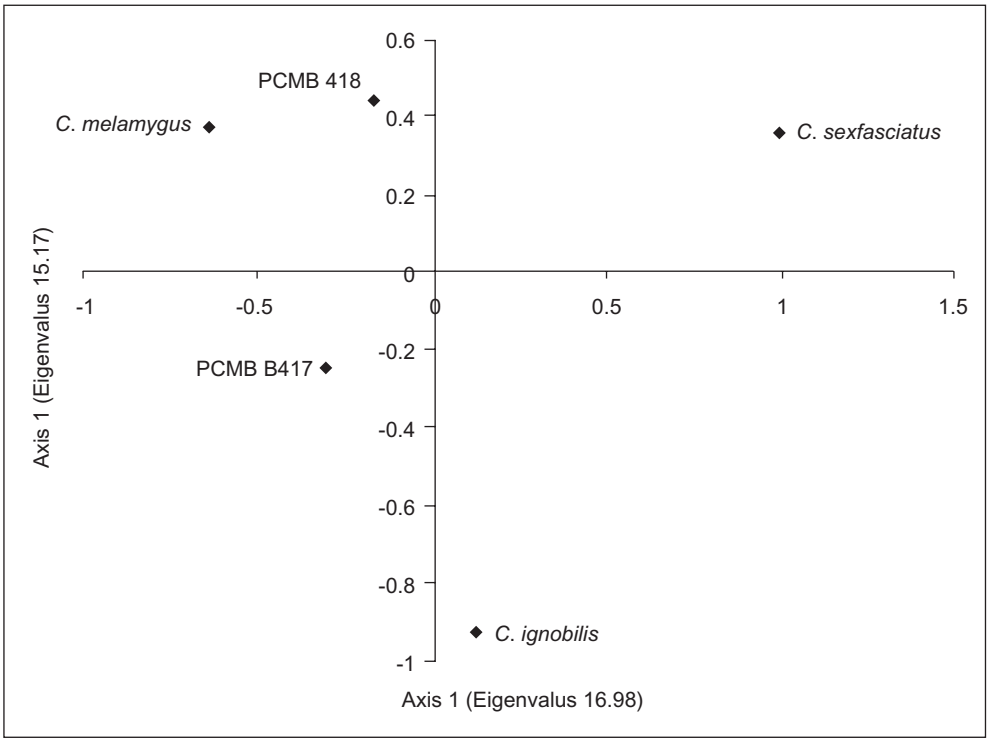

Fig. 4. Principal components analysis of 76 RAPDs bands for the 2 hybrid specimens and the 3 potential parent *Caranx* species. The 1st and 2nd axes respectively explained 38.6% and 35.4% of the variation within the data.

support the *melampygus*-*sexfasciatus* cross. In addition, 3 morphometric characters are intermediate. The snout length of *melampygus* is contained 9.2-13.7 times in the FL; that of *sexfasciatus* is shorter, 13.1-18.4 in the FL (after Smith-Vaniz in Carpenter and Niem, 2000). The value for the hybrid is 12.0, hence slightly favoring *melampygus*. The posterior end of the upper jaw of *melampygus* extends at most to below the front edge of the pupil; in *sexfasciatus* it reaches beyond the posterior edge of the pupil and often to or beyond the posterior edge of the orbit; in the hybrid it extends to below the posterior edge of the pupil. The size of the eye is an obvious morphometric difference separating the small-eyed *melampygus* from the large-eyed *sexfasciatus*, but specimens of nearly the same size should be compared. Bishop Museum specimens of *melampygus* of 279 and 309 mm FL have a bony orbit diameter 4.75-5.0 in the head length. Specimens of *sexfasciatus* 273 and 307 mm FL have a bony eye diameter of 3.2-3.8 in the head length. The bony orbit diameter of the 261 mm hybrid is 4.15 in the head length.

As found for the other hybrid specimen, PCMB418 (*C. melampygus* x *C. sexfasciatus*) had only a single nucleotide difference in the mitochondrial 16S and COI rDNA sequences from the 2 *C. melampygus* individuals, indicating that *C. melampygus* is the maternal parent. RAPDs analysis showed that the hybrid specimen PCMB418 is intermediate in banding between the individuals of *C. melampygus* (PCMB414) and *C. sexfasciatus* (PCMB416) (Fig. 4), thus confirming the hybrid origin of the specimen.

DISCUSSION

Caranx ignobilis and *C. melampygus* are diurnal predaceous fishes that are wide-ranging in the Indo-Pacific region from the Red Sea and east coast of Africa to the Hawaiian Is. and the Pitcairn Is; the latter species has also extended its distribution to the tropical eastern Pacific. As mentioned by Dunn-Rankin (1988), the 2 species may be seen in the same aggregation. A number of authors of papers on hybrids of fishes, such as Randall (1956), have pointed out that hybridization is more apt to take place when 1 parent species is common and the other is rare. The same would apply if both species are rare. The populations of these 2 species of *Caranx* in the Hawaiian Is. have been reduced by overfishing. This is especially true around the island of O'ahu.

We could find no report of the spawning by *C. melampygus*. Johannes (1981: 165) observed a very large aggregation of this species in Peleliu, Palau from which he speared individuals with ripe gonads, but he did not observe spawning. Von Westernhagen (1974) reported on the spawning of *C. ignobilis* in the Philippines. In aggregations of sometimes more than 100 fish, individual females were chased by 2 or 3 males (distinguished by the darker dorsal coloration). Eventually, 1 male accompanied the female, and the 2 moved down to a sandy bottom, where they circled, while releasing eggs and sperm. They were easily approached while spawning. The time of day was not given.

Caranx sexfasciatus is also a widely distributed species throughout the Indo-Pacific and the tropical eastern Pacific. A hybrid of this species and *C. melampygus*, however, is not so easily explained because *sexfasciatus* is primarily nocturnal. It tends to form semi-stationary aggregations by day. Randall (2005: 230, lower Fig.) observed courtship at dusk, with pairs swimming together, the male assuming a black coloration. Spawning was not observed but seemed imminent in the fading light. Breder (1951: 170) published a note on the spawning behavior of the horseye jack in an enclosure at the marine laboratory in Bimini, Bahamas. He identified the species as *C. sexfasciatus*; however, it is *C. latus*, a close relative of *sexfasciatus*, and also nocturnal. Breder noticed the pair formation beginning at 17:15 in the late afternoon; he observed the pairs in side-to-side position with the ventral surfaces in contact. He could not see the actual gamete release.

In the future any person submitting a world record of a game fish, particularly one that greatly exceeds the existing record, should be advised to provide a tissue sample.

REFERENCES

Berry FH, L Cohen. 1974. Synopsis of the species of *Trachurus* (Pisces: Carangidae). Quart. J. Florida Acad. Sci. **35:** 177-211.

Breder CM Jr. 1951. A note on the spawning behavior of Caranx sexfasciatus. Copeia **1951:** 170.

Carpenter KE, VH Niem (eds.). 2000. The living marine resources of the western central Pacific, Vol 4, Bony fishes part 2 (Mugilidae to Carangidae). Rome: Food and Agricultural Organization.

Dunn-Rankin P. 1988. Incredible catch is not an'omilu. Hawaii Fishing News **13:** 6-7.

Johannes RE. 1981. Words of the lagoon. Berkeley, CA: Univ.

of California Press.
Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. *In* D.M. Hillis, C. Moritz and B.K. Mable, eds. Molecular Systematics. 2nd ed. Sunderland, MA: Sinauer Associates, pp. 205-247.
Randall JE. 1956. *Acanthurus rackliffei*, a possible hybrid surgeon fish (*A. achilles* x *A. glaucopareius*) from the Phoenix Islands. Copeia **1956:** 21-25.
Randall JE. 2005. Reef and shore fishes of the South Pacific. Honolulu, HI: University of Hawaii Press.
Rohlf FJ. 2000. NTSYSpc Numerical taxonomy and multivariate analysis system. New York: Exeter Software.
Schwartz FJ. 1972. World literature to fish hybrids with an analysis by family, species, and hybrid. Pub. Gulf Coast Res. Lab. **3:** 1-328.
Von Westernhagen H. 1974. Observations on the natural spawning of *Alectis indicus* (Rüppell) and *Caranx ignobilis* (Forsk.) (Carangidae). J. Fish Biol. **6:** 513-516.
Ward RD, TS Zemlak, BH Innes, PR Last, PDN Hebert. 2005. DNA barcoding Australia's fish species. Phil. Trans. Royal Soc. Biol Sci. **360:** 1847-1857.