Acanthurus nigros Günther, a Valid Species of Surgeonfish, Distinct from the Hawaiian *A. nigroris* Valenciennes[1]

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Abstract: The Blueline Surgeonfish, *Acanthurus nigroris* Valenciennes, formerly considered as wide-ranging in the central and western Pacific, is restricted to the Hawaiian Islands. *Acanthurus nigros* Günther, type locality Vanuatu, is available for the sister species from the Pitcairn Islands west to the Great Barrier Reef and Caroline Islands. Although these two species are very similar in color, there are fin-ray and gill-raker differences, and the genetic difference (i.e., 4.12% mtDNA cytochrome *b* sequence divergence) alone warrants species recognition.

This study was initiated when differences were noted in the DNA from tissue samples taken of the Blueline Surgeonfish (*Acanthurus nigroris*) from the Hawaiian Islands and from those from other islands of the Pacific. *Acanthurus nigroris* was described by the French ichthyologist Achille Valenciennes in 1835 in the tenth volume of the monumental series *Histoire Naturelle des Poissons* with Georges Cuvier. The brief description was based on a single 161 mm specimen obtained by J. R. C. Quoy and P. Gaimard in the Hawaiian Islands. The description was not diagnostic for any species of *Acanthurus*.

Günther (1861) described *Acanthurus nigros* from three stuffed specimens from the New Hebrides (now Vanuatu). He listed *Acanthurus nigroris* Cuv. & Val. with a question mark below his species heading.

Jordan and Evermann (1903) described a Hawaiian surgeonfish as *Teuthis atrimentatus* from nine specimens obtained in Honolulu. Jordan and Evermann (1905) corrected the name to *Hepatus atramentatus* and illustrated the species. They noted that the species has several times been recorded under the erroneous name of *Acanthurus lineolatus* Valenciennes in Cuvier and Valenciennes.

Herre (1927, 1953) reported *Acanthurus atramentatus* from the Philippines. His specimens from Luzon and Negros were sent on loan from the California Academy of Sciences and reidentified as *A. nigrofuscus* (Forsskål) and *A. mata* (Cuvier).

Fowler (1928), in his *Fishes of Oceania*, used the name *Hepatus lineolatus* for Bishop Museum acanthurid specimens from the Hawaiian Islands, American Samoa, Austral Islands, and Minami Tori Shima (Marcus Island, first reported by Bryan and Herre [1903]). Bauchot and Randall (1996) noted that Valenciennes had only one type specimen of *Acanthurus lineolatus*, adding that it has not been found. The type locality was given only as "la mer des Indes," and the description is not diagnositic for any species of the genus. Neither *A. nigroris* nor *A. nigros* is known from the East Indies.

In a revision of *Acanthurus*, Randall (1956) validated Valenciennes' name *Acanthurus nigroris* for a common surgeonfish of Hawaiian

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reefs from information provided on the holotype in the Muséum National d'Histoire Naturelle in Paris. *Acanthurus nigros* and *Acanthurus atramentatus* were listed as synonyms. He examined surgeonfish material at the U.S. National Museum of Natural History that had been identified as *Acanthurus elongatus* (Lacepède) by Schultz (1943) from the Phoenix Islands and Samoa Islands, and by Schultz and Woods in Schultz et al. (1953) from the Marshall Islands and Guam. The specimens included both *A. nigroris* and *A. nigrofuscus*. The counts of the dorsal and anal soft rays, as well as the gill rakers of *A. nigroris* were recorded as higher in the Hawaiian Islands and Johnston Atoll and the caudal fin slightly more emarginate than in specimens from other islands of Polynesia and Micronesia. Randall et al. (1993) recorded *A. nigroris* from Midway Atoll, Northwestern Hawaiian Islands, as abundant outside the lagoon at depths of less than 2 to over 20 m.

Randall (2002, 2007) wrote that the name *A. nigros* Günther, with a type locality of Vanuatu, is available if one were to treat the non-Hawaiian population as a species or a subspecies.

Here we conclusively show that *Acanthurus nigroris* is an endemic species in the Hawaiian Islands and Johnston Atoll, and the name *Acanthurus nigros* is valid for the species elsewhere in Oceania and the Great Barrier Reef.

MATERIALS AND METHODS

Surgeonfish specimens for this study are primarily those deposited in the Bernice P. Bishop Museum, Honolulu (BPBM). Other specimens were examined from the Australian Museum, Sydney (AMS); the California Academy of Sciences, San Francisco (CAS); and the United States National Museum of Natural History, Washington, D.C. (USNM). A photograph of the lectotype of *Acanthurus nigros* was obtained from the Natural History Museum, London (BMNH) and is reproduced here as Figure 1. Lengths of specimens are given as standard length (SL) from the tip of the snout to the base of the caudal fin. Other measurements and methods of counting follow Randall (1956).

As part of a larger study on the phylogeography of Indo-Pacific reef fishes (for examples see Craig et al. 2007, Gaither et al. 2010), a total of 544 tissue samples was collected from individuals of "*Acanthurus nigroris*" at 20 locations throughout the Hawaiian Archipelago (sampling sites: Hawai'i, Lāna'i, O'ahu, Nīhoa, Necker, French Frigate Shoals, Gardner Pinnacles, Maro Reef, Laysan, Lisianski, Pearl and Hermes Atoll, Midway Atoll, and Necker Island), Johnston Atoll, and the central Pacific (sampling sites: Society Islands, Line Islands, American Samoa, Tokelau Islands, and Marshall Islands) between 2004 and 2007. Total genomic DNA was extracted from each sample using a "HotSHOT" protocol (Meeker et al. 2007). A 797 base pair (bp) segment of the mitochondrial (mtDNA) cytochrome *b* (cyt *b*) gene was amplified using a heavy-strand (5′-GTGACTTGAAAAACCACCGTTG-3′ [Song et al. 1998]) and light-strand primer (5′-AATAGGAAGTATCATTCGGGTTTGATG-3′ [Taberlet et al. 1992]). Polymerase chain reaction (PCR) mixes contained BioMix Red (Bioline, Inc., Springfield, New Jersey), 0.26 μM of each primer, and 5–50 ng template DNA in a 15 μl total volume. PCR cycling parameters were used as follows: initial 95°C denaturation (10 min) followed by 35 cycles of 94°C (30 sec), 63°C (45 sec), and 72°C (45 sec). Each reaction ended with a final 10-min extension at 72°C. All PCR products were cleaned using ExoSAP (USB, Cleveland, Ohio) and then sequenced in the forward direction (and reverse direction, where appropriate) using a genetic analyzer (ABI 3130XL, Applied Biosystems, Foster City, California) at the Hawai'i Institute of Marine Biology EPSCoR Sequencing Facility. The sequences were aligned, edited, and trimmed to a common length using Geneious Pro *vers.* 4.8.4 DNA analysis software (Drummond et al. 2009). Representative mtDNA cyt *b* haplotypes were deposited in GenBank (accession numbers HM242298–HM242393). jModelTest *vers.* 1.0.1 (Posada [2008], but see Guindon and Gascuel [2003]) was also used with an Akaike information criterion (AIC) test to determine the best nucleotide substitution model; the Tamura-

FIGURE 1. Lectotype of *Acanthurus nigros* Günther, BMNH 1861.5.31.29, 160 mm SL, Aneiteum (Anotom), Vanuatu (photo by P. Hurst).

Nei model (Tamura and Nei 1993) with a gamma parameter of 0 was selected for phylogenetic inference. Neighbor-joining distance, maximum-parsimony, and maximum-likelihood tree-building methods were applied using PAUP* *vers*. 4.0 (Swofford 2000), which resulted in identical topologies. *Acanthurus nigrofuscus* sequences (i.e., Genbank accession no. FJ376753) were also used to root the tree (see Figure 2*A*). Although not sister taxa, *A. nigrofuscus* is sometimes mistaken for *A. nigroris* in the field (both have a black spot at the rear base of the dorsal and anal fins), and it represents the closest living relative for which mtDNA sequences are available. Due to the abundance of singleton haplotypes in this data set ($n = 74$ out of 96 total haplotypes), we only considered the most common haplotypes ($n \geq 2$ sequences) for subsequent tree analyses. Support for the tree was evaluated by bootstrapping over 1,000 replicates in all cases (Felsenstein 1985). The maximum parsimony network was also built using these haplotypes with the program TCS *vers*. 2.21 (Clement et al. 2000) to confirm phylogenetic relationships among sampling sites.

SYSTEMATIC RESULTS

Acanthurus nigros Günther
Figure 1; Figure 3*D–F;* Tables 1, 2

Acanthurus nigros Günther, 1861: 332 (type locality: Aneiteum [Anatom], Vanuatu).

DIAGNOSIS. Dorsal rays IX,23–26; anal rays III,22–24; pectoral rays 15 or 16; gill rakers 21–25; teeth incisiform, denticulate, and close-set, the distal end of upper teeth rounded; adults with 12 upper and 14 lower teeth; body depth 1.8–2.0 in SL; caudal-fin spine 2.7–3.5 in head length; caudal-fin concavity 6.7–10.5 in head length; light to dark brown, the body with slightly irregular, longi-

tudinal, dotted blue lines narrower than brown interspaces; suborbital and opercular region with blue lines paralleling snout; a black spot less than half eye diameter at rear base of dorsal and anal fins; a broad whitish bar often present at base of caudal fin; dorsal and anal fins with longitudinal orangish brown to dark brown bands and a narrow blue margin (more distinct on anal fin); posterior margin of caudal fin narrowly white. Largest specimen, 180 mm SL.

GENETICS. The genetic analyses, based on mtDNA cyt *b* sequences from a total of 544 "*Acanthurus nigroris*" samples, revealed a clear separation between individuals from the Hawaiian Archipelago and the rest of the Pacific. All phylogenetic methods converged on a single-tree topology, so only the maximum-likelihood results are presented here (Figure 2*A*). The maximum parsimony haplotype network also supports this separation (Figure 2*B*). There is a significant genetic break (bootstrap values, 99–100), there are no shared haplotypes, and corrected sequences diverge by 4.12% (29 diagnostic mutations). This genetic distance is comparable with or greater than that of other congeneric pairs of most reef fishes (e.g., angelfishes [Bellwood et al. 2004, Randall and Rocha 2009*a*]; butterflyfishes [Fessler and Westneat 2007]; grunts [Rocha et al. 2008]; wrasses [Rocha 2004, Weaver and Rocha 2007, Luiz et al. 2009, Randall and Rocha 2009*b*]). Within each geographic region, on the other hand, sequences diverged by no more than 0.1% in the Hawaiian Islands and 0.6% elsewhere in the Pacific, supporting the conclusion that these distinct clades represent reciprocally monophyletic groups that warrant species-level designation.

MATERIAL OF *Acanthurus nigros* EXAMINED. Minami Tori Shima (Marcus Island), BPBM 2439, 2: 121–125 mm; BPBM 2440, 124 mm; BPBM 8578, 13: 44–53 mm; BPBM 8582, 7: 39–134 mm. Wake Island, BPBM 4276, 3: 40–41 mm. Marshall Islands, Enewetak Atoll, BPBM 6334, 147 mm; Arno Atoll, BPBM 15257, 93 mm. American Samoa, Rose Atoll, BPBM 23963, 3: 111–129 mm. Cook Islands, Pukapuka Atoll, BPBM 39482, 92 mm; Aitutaki, BPBM 10706, 70 mm. Line Islands, Jarvis Island, BPBM 4275, 180 mm; Teraina (Washington Island), BPBM 4272, 2: 125–154 mm; Tabuaeran (Fanning Island), BPBM 4263, 2: 124–129 mm; BPBM 7658, 2: 131–140 mm. Marquesas Islands, Ua Huka, BPBM 12410, 162 mm. Tuamotu Archipelago, Gambier Group, Temoe Atoll, BPBM 13515, 96 mm. Pitcairn Islands, Henderson Island, BPBM 12279, 149 mm.

DISCUSSION

The higher dorsal-ray, anal-ray, and gill-raker counts of the Hawaiian and Johnston Atoll specimens of *Acanthurus nigroris* given by Randall (1956), compared with elsewhere in Oceania, are reproduced here as Tables 1 and 2. His decision to treat the two populations as a single species was based mainly on the similarity in color of Hawaiian to non-Hawaiian individuals. Figure 3*A–C* of *A. nigroris* and Figure 3*D–F* of *A. nigros* provide a comparison.

Our genetic analyses based on samples sequenced at the cyt *b* mtDNA gene also indicate population genetic breaks between the Hawaiian and Johnston Atoll specimens of *Acanthurus nigroris* compared with those sampled elsewhere in Oceania, therefore validating Günther's name *Acanthurus nigros*. Assuming a divergence rate at the cyt *b* gene of 2% per million years (calibrated as per Bowen et al. [2001]), we can infer that fish from those two regions have been reproductively isolated for approximately 2 million years. This provides another example where molecular approaches have proven useful in confirming suspected evolutionary differentiation among regions at species level (also see Randall and Rocha 2009*a*,*b*, Luiz et al. 2009).

Randall (1956:190) selected the adult of the three syntypes of *Acanthurus nigros* Günther, BMNH 1861.5.31.29, 160 mm SL, as the lectotype (Figure 1). It is a dried stuffed specimen and by museum policy could not be sent on loan. The identification was determined by data provided at that time by Alwyne C. Wheeler.

Günther (1861:331) described *Acanthurus bipunctatus* in the same publication as *A. nigros* from two specimens, an adult 6.5 inches (165

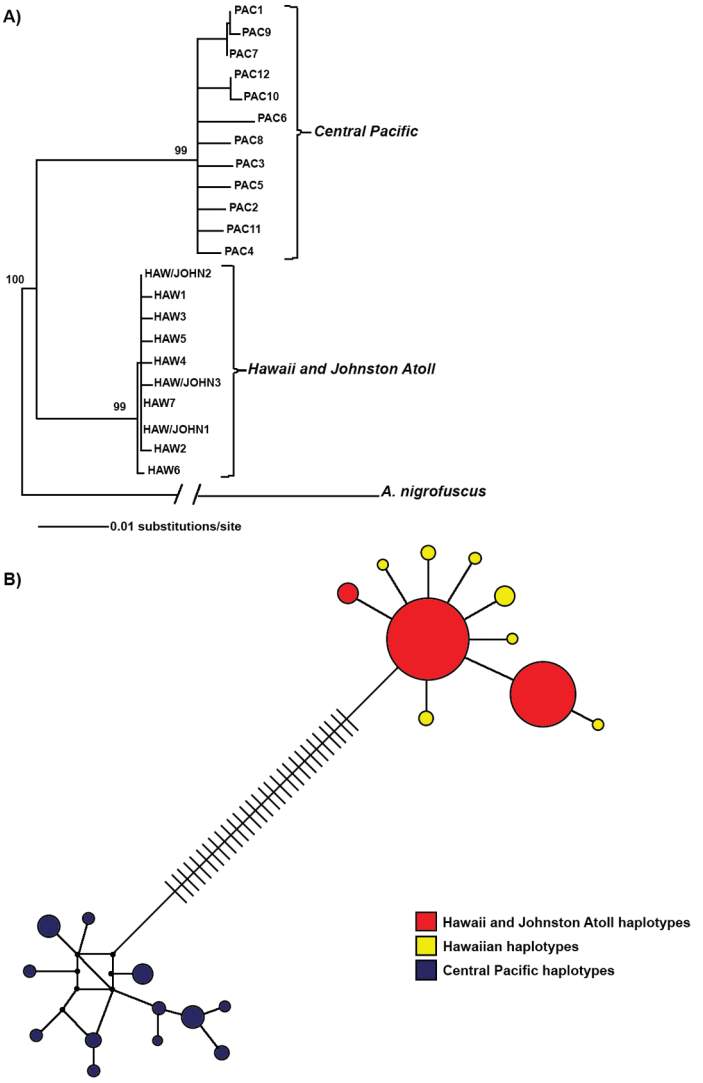

FIGURE 2. (*A*) Maximum-likelihood phylogeny showing relationships among mtDNA, nonsingleton cyt *b* haplotypes ($n = 22$) for "*Acanthurus nigroris*" sampled in the Hawaiian Archipelago (HAW), Johnston Atoll (JOHN), and the central Pacific (PAC). Bootstrap values (>95%) are shown above the nodes ($n = 1{,}000$ reps), and branch lengths are according to indicated scale (note that the branch leading to the outgroup was reduced by 50%). (*B*) Statistical parsimony network (TCS vers. 2.21 [Clement et al. 2000]) in which the size of the circles is proportional to haplotype frequencies. Small, black circles or dashed lines represent intermediate haplotypes either not observed in our survey or not included here (i.e., singleton haplotype).

TABLE 1

Counts of Dorsal and Anal Soft Rays of Species of *Acanthurus*

	Dorsal Soft Rays					Anal Soft Rays			
Taxon	23	24	25	26	27	22	23	24	25
A. nigroris	3	47	44	2		20	62	14	
A. nigros		4	17	35	2	1	11	27	19

mm) in total length from China, and a juvenile from Fiji. Wheeler's data on the specimens enabled Randall (1956:190) to identify the juvenile as *A. nigrofuscus* and the adult as *A. nigroris*. He designated the adult of *A. bipunctatus* as the lectotype and placed the species in the synonymy of *A. nigroris* (now *A. nigros*). Because we know of no specimens of *A. nigros* from China, Taiwan, or Japan, James Maclaine of the Natural History Museum in London was asked if the locality of China (also given by Günther as Sea of China) might be an error. He checked the register for the BMNH collection and found that the locality given there was the Malay Archipelago. That is also an unacceptable locality for *Acanthurus nigros*, so we conclude that there was a locality error for *A. bipunctatus*. As first revisors, we place *A. bipunctatus* Günther in the synonymy of *A. nigros* Günther.

Figure 4 provides the distributions of *Acanthurus nigroris* and *A. nigros*. The following references and observations are given to confirm localities. Günther (1873:112, plate 73*A*) illustrated *A. nigros* as *A. lineolatus* from Raiatea, Society Islands. Jordan and Seale (1906:352) listed it as *Hepatus atramentatus* from Samoa, based on one specimen. Fowler and Ball (1925:18) included the species from Wake Island as *Hepatus elongatus* (reidentified as *A. nigroris* by Lobel and Lobel [2004:76]). Harry (1953:148) reported it as *Acanthurus elongatus* from Raroia Atoll, Tuamotu Archipelago. Randall (1955:185) included it as *A. nigroris* from the atolls of Onotoa and Tarawa in the Gilbert Islands (now Tungaru Islands). Randall (unpubl. data) collected *Acanthurus nigros* at Takaroa Atoll, Tuamotu Archipelago, in 1956, first deposited in the fish collection of Stanford University, but now CAS 98316. The California Academy of Sciences also has specimens from Ifalik Atoll, Kapingamarangi Atoll, and Pohnpei in the Caroline Islands that were transferred from Stanford University. Randall (unpubl. data) observed a large spawning aggregation of *Acanthurus nigros* at 40 m in Teavaraa Pass, Tahiti, at 1100 hours on 26 February 1969.

Matsuura (1982:86) reported *Acanthurus nigros* (as *A. nigroris*) from Palau; however, he did not list *Acanthurus nigrofuscus*. When both species are present at a locality, the latter is usually more common. Keiichi Matsuura (pers. comm.) determined that the Palau specimen is now correctly identified as *A. nigrofuscus*. Myers (1989:246, plate 127*B*) listed the species as occurring throughout Micronesia; he used a photograph he took on O'ahu to illustrate the species. Myers (pers. comm.) has positive records from Guam and Pagan in the Mariana Islands and Kosrae in the Caroline Islands. Gerald R. Allen provided an underwater photograph of a juvenile from Yap (Figure 3*F*); therefore, *A. nigros* might be expected in Palau. Randall et al. (1990:424) reported the species as rare from the Great Barrier Reef. The Australian Museum in Sydney had Great Barrier Reef specimens from Heron Island and One Tree Island of the Capricorn Group, Myrmidon Reef, and Lizard Island identified as *Acanthurus nigroris*, but only one lot (AMS I.32394-001, 100 mm,

TABLE 2

Anterior Gill-Raker Counts of Species of *Acanthurus*

Taxon	21	22	23	24	25	26	27	28	29	30	31
A. nigroris						3	5	6	4	1	1
A. nigros	3	7	10	12	6						

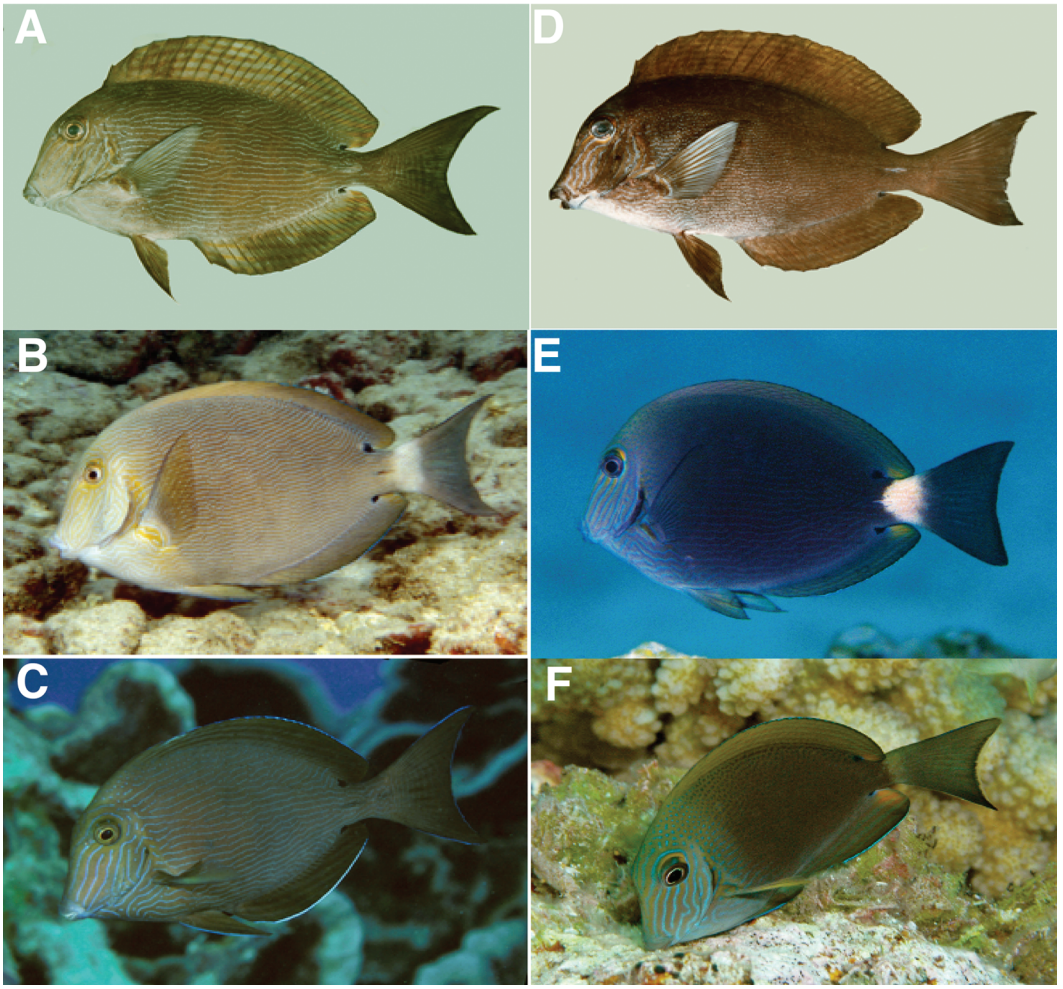

FIGURE 3. (*A*) *Acanthurus nigroris*, BPBM 8940, 122 mm SL, Johnston Atoll (J. E. Randall). (*B*) *Acanthurus nigroris*, O'ahu, Hawaiian Islands (J. E. Randall). (*C*) *Acanthurus nigroris*, subadult, Johnston Island (J. E. Randall). (*D*) *Acanthurus nigros*, BPBM 6334, 147 mm SL, Enewetak Atoll, Marshall Islands (J. E. Randall). (*E*) *Acanthurus nigros*, Tahiti, Society Islands (P. Bacchet). (*F*) *Acanthurus nigros*, juvenile, Yap (G. R. Allen).

from Heron Island) was found. A photograph of the specimen was sent by Amanda Hay and confirmed as *A. nigros*. No specimens of *A. nigros* are present in the Queensland Museum (Jeffrey W. Johnson, pers. comm.) or the Western Australian Museum (Gerald R. Allen, pers. comm.). Randall (1999:28) listed the species from one specimen from Henderson Island of the Pitcairn Group. Randall and Earle (2000:21) included the species in a checklist of the fishes of the Marquesas Islands based on one specimen speared by J.E.R. in Ua Huka. Randall et al. (2004:29) included it in a checklist of the fishes of Tonga. Randall (2005:580, left figure) erred in using an underwater photo taken at Johnston Atoll (hence *Acanthurus nigroris*) rather than a photo from the South Pacific showing *A. nigros*. John L. Earle provided an underwater photograph of a small aggregation of *Acanthurus nigros* taken at Malden Island in the southern Line Islands in 2008.

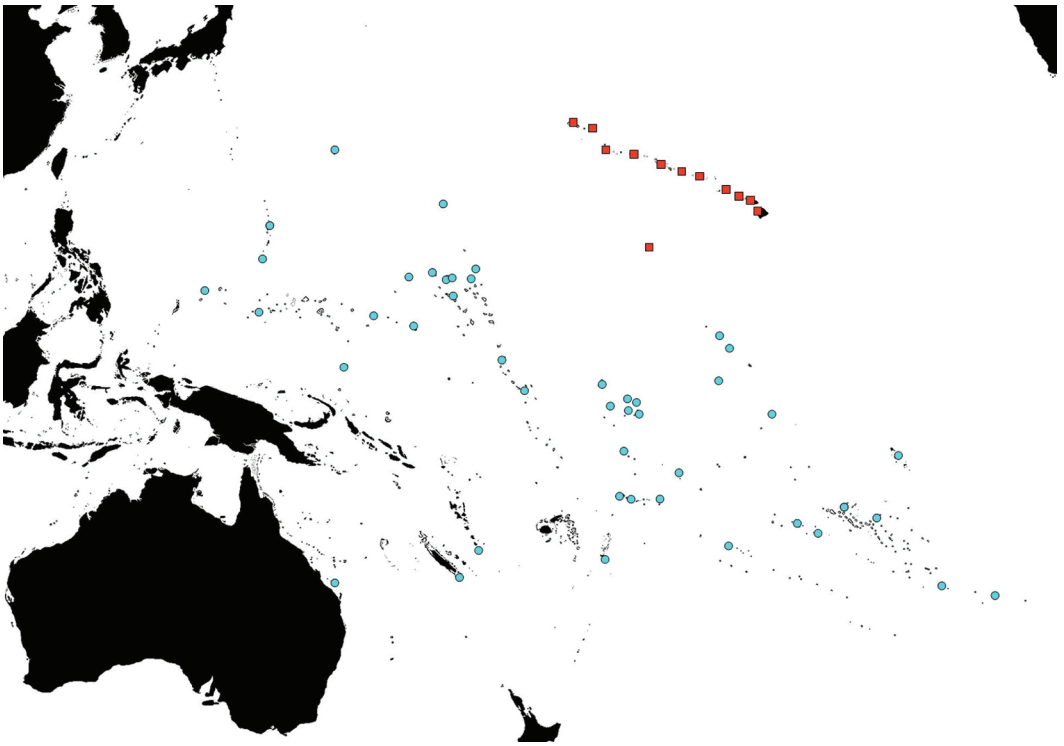

FIGURE 4. Distribution of *Acanthurus nigros* (light-shaded circles) and *A. nigroris* (dark-shaded squares).

We have found no specimens correctly identified as *Acanthurus nigros* from the Indian Ocean, the Indo-Malayan region, Taiwan, or Japan, including the Ryukyu Islands and the Ogasawara Islands. The record of *Acanthurus nigroris* from the Cocos-Keeling Islands by Allen and Smith-Vaniz (1994:17) is based on one specimen in the Academy of Natural Sciences of Philadelphia (ANSP 134683, 110 mm) that was reidentified for us as *A. nigrofuscus* by Mark H. Sabaj Perez. Allen (2000) included *A. nigroris* in a survey of the reef and shore fishes of the Calamian Islands, Philippines, and Allen and Adrim (2003:62) listed it from the Talaud Islands in a checklist of the coral reef fishes of Indonesia. Allen (pers. comm.), however, has informed us that both of these records are misidentifications. At our request, Diane Pitassy examined specimens in the U.S. National Museum of Natural History from localities other than the Hawaiian Islands that had been identified as *Acanthurus nigroris*. With the use of the key from Randall (1956), she confirmed records of *Acanthurus nigros* from the following islands: Moorea and Bora Bora in the Society Islands; Tabuaeran (Fanning Island) in the Line Islands; Swains Island and Rose Atoll in American Samoa; Rawaki, Enderbury, Canton, Hull (Orona), and McKean in the Phoenix Islands; Atafu Atoll in the Tokelau Islands; 'Eua Island, Tonga; Baker Island and Howland Island in the U.S. Outlying Minor Islands; Onotoa Atoll in the Tungaru Islands; the atolls of Bikar, Taka, Rongelap, Rongerik, Bikini, Kwajalein, and Enewetak in the Marshall Islands; and Pohnpei, Caroline Islands. There is one lot (USNM 196290) collected by Wilbert M. Chapman in 1943–1944 from uncertain locality (given as New Caledonia, Solomon Islands, or Vanuatu, but most likely the last mentioned). USNM specimens from the Philippines that had been identified as *Acanthurus nigroris* were reidentified as *A. nigrofuscus*. Indeed, three USNM lots

of juveniles from the Philippines (USNM 332004, 344384, and 347041) were sent on loan to J.E.R. and reidentified as *A. nigrofuscus*.

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