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Amphiprion barberi, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa

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Abstract

Amphiprion barberi, a new species of anemonefish fish, is described from 46 specimens, 16.3-85.8 mm SL, collected at depths of 2-10 m from coral reefs of Fiji, Tonga, and Samoa. It is closely allied to *A. melanopus*, which is widely distributed in the western Pacific. The two species exhibit significant colour-pattern differences, including a mainly reddish orange body in *A. barberi* and dark brown or blackish body in *A. melanopus*. Adults of the new species also possess fewer spinules (11-19 *versus* 19-26) in the upper-opercular series than *A. melanopus*. Genetic data presented here confirms the separation of these species.

Zusammenfassung

Die neue Art der Riffbarsche: *Amphiprion barberi*, wird auf der Grundlage von 46 Exemplaren mit 16,3 bis 85,8 mm SL beschrieben, die in Tiefen von 2 bis 10 Metern über Korallenriffen von Fidschi, Tonga und Samoa gefangen wurden. Sie ist nahe mit *A. melanopus* verwandt, die im Westpazifik weit verbreitet ist. Die beiden Arten zeigen deutliche Unterschiede im Farbmuster: U.a. ist bei *A. barberi* der Rumpf hauptsächlich rötlich orange, bei *A. melanopus* dunkelbraun oder schwärzlich. Außerdem haben die erwachsenen Tiere der neuen Art auf den Reihen oberhalb vom Kiemendeckel weniger Dörnchen (Spinulae), nämlich 11 bis 19 im Vergleich zu 19 bis 26. Die hier vorgelegten genetischen Daten sprechen ebenfalls für eine Trennung der beiden Arten.

Résumé

Amphiprion barberi, une nouvelle espèce de poisson-clown, est décrit sur base de 46 spécimens, de 16,3 à 25,8 mm de LS, collects à des profondeurs de 2 à 10 m dans des récifs coralliens de Fidji, Tonga et Samoa. L'espèce est très proche d'*A. melanopus* qui connaît une vaste distribution dans le Pacifique ouest. Les deux espèces montrent des différences sensibles dans le patron de coloration, incluant un corps principalement orange-rougeâtre chez *A. barberi* et brun foncé à noirâtre chez *A. melanopus*. Les adultes de la nouvelle espèce présentent aussi moins de spicules (11-19 contre 19-26) dans les rangées supra-operculaires qu'*A. melanopus*. Les données génétiques qui figurent ici confirment la distinction entre les deux espèces.

Sommario

Amphiprion barberi, una nuova specie di pesce pagliaccio è descritto sulla base di 46 esemplari, di 16.3-85.8 mm SL, raccolti a profondità di 2-10 m lungo le barriere coralline di Fiji, Tonga e Samoa. Appare strettamente imparentato con *A. melanopus*, che è ampiamente distribuito nel Pacifico occidentale. Le due specie mostrano significative differenze nella colorazione. In particolare, mentre il corpo di *A. barberi* è principalmente arancio-rossastro quello di *A. melanopus* è bruno scuro o nerastro. Inoltre, gli adulti della nuova specie posseggono un numero inferiore di spinule nella serie opercolare superiore rispetto *A. melanopus* (11-19 verso 19-26). I dati genetici presentati qui confermano la separazione in due specie distinte.

INTRODUCTION

The brightly coloured members of the pomacentrid subfamily Amphiprioninae are well known for their commensal relationship with large sea anemones. Allen (1972, 1980) provided comprehensive reviews of the group and summary of their biology. More recently, these fishes were treated by Fautin & Allen (1992), who recognized 27 species in *Amphiprion* Bloch & Schneider, 1801 and one species in *Premnas* Cuvier, 1816. The present paper describes a new species that was previously considered as a geographic colour variation.

Primarily on the basis of colour-pattern similarities, Allen (1972) included specimens of a reddish orange *Amphiprion* from Fiji, Tonga, and Samoa in his account of *Amphiprion rubrocinctus* Richardson, 1842. However, further investigation (Allen 1980) revealed that the latter species is restricted to northwestern Australia. The same study considered specimens from Fiji, Tonga, and Samoa as geographic colour variants of *A. melanopus* Bleeker, 1852 and suggested this population was perhaps deserving of separate subspecific status.

Subsequent field surveys by the first author throughout the western Pacific, including three visits to Fiji in recent years provided an opportunity for additional observations and collections. In addition, collaboration with Paul Barber of Boston University offered the chance to elucidate relationships by means of DNA analysis. We conclude that the fish from Fiji and western Polynesia are a distinct species, which is described herein.

MATERIALS AND METHODS

Lengths of specimens are given as standard length (SL) measured from the anterior end of the upper lip to the base of the caudal fin (posterior edge of hypural plate); head length (HL) is measured from the same anterior point to the posterior edge of the opercle flap; body depth is the maximum depth taken vertically between the belly and base of the dorsal spines; body width is the maximum width just posterior to the gill opening; snout length is horizontal distance measured from the anterior end of the upper lip to the anterior edge of the eye; orbit diameter is the horizontal fleshy diameter, and interorbital width the least bony width; upper-jaw length is taken from the front of the upper lip to the posterior end of the maxilla; caudal-peduncle depth is the least depth, and caudal-peduncle length is the horizontal distance between verticals at the rear base of the anal fin and the caudal-fin base; lengths of fin spines and rays are measured to their extreme bases (i.e., not from the point where the ray or spine emerges from the basal scaly sheath); caudal-fin length is the horizontal length from the posterior edge of the hypural plate to a vertical at the tip of the longest ray; pectoral-fin length is the length of the longest ray; pelvic-fin length is measured from the base of the pelvic spine to the filamentous tip of the longest soft ray; pectoral-ray counts include the small splint-like, uppermost rudimentary ray; only the tube-bearing anterior lateral-line scales are counted; a separate count is given for the deeply pitted scales occurring in a continuous series midlaterally on the caudal peduncle; gill-raker counts include all rudiments and are presented as separate counts for the upper and lower limbs as well as a combined count, the raker at the apex of the arch is included in the lower limb count; the last fin ray element of the dorsal and anal fins is usually branched near the base and is counted as a single ray.

Counts and proportions are given for the holotype followed by the range of values (if different than the holotype) for the paratypes in parentheses. Proportional measurements expressed as percentage of the standard length are provided in Table I. Type specimens are deposited at the Bernice P. Bishop Museum, Honolulu (BPBM), National Museum of Natural History, Washington, D.C. (USNM), and the Western Australian Museum, Perth (WAM).

We also conducted a genetic analysis to assess the monophyly of the new species. The addition of genetic data to meristic information allows for a fuller resolution of the evolutionary relationships between species (Schultz & Randall 2006). These additional data are particularly salient when a regional variation is being elevated to specific status, as colour and geography are not always reliable characters. Data were generated from 12 specimens of the new species and 12 specimens of its suspected closest relative, *A. melanopus*, from Papua New Guinea. Genomic DNA was extracted using a 10% Chelex solution (Walsh et al. 1991). A region of the mitochondrial control region was amplified using the primers CrA & CrE (Lee et al. 1995) with the following thermocycler parameters: Initial denature 94 °C for 4 min then 40 cycles of 94 °C (30 s) denaturing 50 °C (30 s) annealing and 72 °C (40 s) followed by a final extension step of 3 min at 72 °C.

We also amplified the nuclear Recombination Activating Gene 2 (RAG2) of a subset of the above samples (4 from Fiji 4 from Papua New Guinea) to provide additional information from an independent nuclear locus. The gene segment was amplified with the primers RAG2F1 and RAG2R3 (Westneat & Alfaro 2005) and using the following thermocycling parameters: 94 °C (4 min) [94 °C (30 s) 55 °C (30 s) 72 (40 s)]x40 72 °C (3 min). Specimens of *Amphiprion clarki* (Bennett, 1830) and *A. perideraion* Bleeker, 1855 were used as respective mtDNA and nDNA outgroups.

Each PCR product was cleaned using a digestion of 5U shrimp alkaline phosphatase and 0.5U exonuclease for 30 min at 37 °C followed by 15 min at 80 °C. The cleaned double-stranded product was then directly sequenced with Big Dye 3.0 terminator chemistry (Applied Biosystems) using the PCR primers and manufacturer protocols. Sequencing products were cleaned via isopropanol precipitation and visualized on an ABI377 automated sequencer or on an ABI3730 (Applied Biosystems). Forward and reverse sequences for each region were reconciled and compiled in Sequencher (Gene Codes) with subsequent alignment by eye.

We used ModelTest v.3.06 (Posada & Crandall 1998) to determine the appropriate model of evolu-

tion. For the mitochondrial DNA, the model of K81uf+I was suggested as the least complicated model of evolution appropriate for our data using The Akaike Information Criterion. This model assumes two transversion parameters while allowing for unequal base frequencies (Kimura 1981). ModelTest suggested the K80+I model for the nuclear DNA (Kimura 1980). The parameters resulting from these models of evolution were subsequently incorporated into a maximum likelihood analysis.

Phylogenetic reconstructions were generated using maximum parsimony and maximum likelihood criteria in PAUP 4.0 (Swofford 2002). Heuristic searches were performed using 100 random taxon addition replicates with tree bisection and reconnection (TBR) branch swapping. Bootstrap support was determined using 100 bootstrap replicates, each using 10 random taxon addition replicates with TBR branch swapping. Searches were performed in parallel on a Beowulf cluster using the clusterpaup program housed at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Amphiprion barberi n. sp.

(Figs 1-4, Table I)

Holotype: WAM P.32261-001, female, 79.2 mm SL, Namena Island, approximately 17°07'S 179° 04'E, Lomaiviti Group, Fiji, 5-12 m depth, spear, G. R. Allen, 17 May 2003.

Paratypes: BPBM 10808, 4 specimens, 44.7-64.3 mm SL, near Suva, Fiji, O. McCausland, October 1970; USNM 204274, 5 specimens, 36.5-85.8 mm SL, Neiafu, approximately 18° 39'00"S 174°00'30"W, Vavau Group, Tonga, 0-16 m depth, rotenone, R. Bolin and others, 28 June 1965; USNM 204275, 79.8 mm SL, Pago Pago, approximately 14°17'S 170°41'W, Tutuila Island, American Samoa, rotenone, R. Bolin and others, 18 June 1965; USNM 238890, 20 specimens, 16.3-64.0 mm SL, Yanutha Islet, approximately 20°37'S 178°40'W, Ono Ilau, Lau Group, Fiji, 0-2 m, rotenone, V. Springer and others, 29 April 1982; USNM 334855, 2 specimens, 54.6-82.7 mm SL, Eua, reef just south of Ohonua Harbour, approximately 21°20'15"S 174°58'14"W, Tonga, 18-27 m, J. T. Williams and others, 2 November 1993; USNM 335157, 6 specimens, 20.1-68.4 mm SL, Uoleva Island, approximately 19°51' 36"S 174°25' 06"W, Ha'apai Group, Tonga, 0-2 m, J. T. Williams and others, 11 November 1993; USNM 338150, 2 specimens, 45.3-57.1 mm SL, Ovaka Island, approximately 18°44' 31"S 174°06'36"W, Vava'u Group, Tonga, 0-10 m depth, J. T. Williams & others, 17 November 1993; WAM P.32332-002, 6 specimens, 57.9-79.2 mm SL, collected with holotype.

Diagnosis: A species of the pomacentrid genus *Amphiprion* with the following combination of characters: dorsal rays X,16-18 (usually X,17); anal rays II,14; pectoral rays 18 (rarely 17); tubed lateral-line scales 36-43; gill rakers 5 + 12-14 (total 17-19); oper-

Fig.1. *Amphiprion barberi*, holotype, 72.5 mm SL, Namena Island, Fiji. Photo by G. R. Allen.

Table I. Proportional measurements of selected type specimens of *Amphiprion barberi* as percentage of the standard length.

	Holotype WAM 32261-001 Fiji	Paratype USNM 204274 Tonga	Paratype USNM 204275 Samoa	Paratype WAM 32261-002 Fiji	Paratype WAM 32261-002 Fiji	Paratype WAM 32261-002 Fiji	Paratype WAM 32261-002 Fiji	Paratype WAM 32261-002 Fiji
Standard length (mm)	72.5	85.8	79.8	71.8	65.7	59.4	58.3	57.9
Body depth	57.9	1.7	1.8	54.6	53.3	54.4	54.4	51.8
Body width	24.8	3.0	3.1	24.1	23.7	22.1	22.8	20.4
Head length	30.3	3.0	3.2	32.0	31.1	30.0	31.0	29.9
Snout length	11.4	2.7	2.7	12.1	11.1	10.1	11.7	10.2
Orbit diameter	7.6	4.3	3.9	8.2	8.4	9.1	8.9	9.3
Interorbital width	10.9	3.1	3.0	10.7	10.4	10.1	9.4	9.7
Caudal-peduncle depth	17.5	1.7	1.8	17.1	16.6	17.3	17.2	17.1
Caudal-peduncle length	12.3	2.7	2.4	13.1	14.5	13.1	11.5	13.6
Upper jaw length	11.2	2.7	2.6	12.0	13.7	11.6	11.7	11.1
Predorsal length	41.1	2.2	2.4	41.2	40.5	40.6	42.9	40.4
Preanal length	64.6	1.5	1.5	63.0	59.4	62.6	62.6	60.1
Prepelvic length	40.7	2.4	2.4	39.7	36.7	39.1	38.9	37.7
Length dorsal-fin base	65.2	1.6	1.6	64.8	63.6	63.1	65.5	62.2
Length anal-fin base	30.9	2.0	2.0	32.6	32.6	32.8	32.2	32.8
Length pectoral fin	31.4	3.2	3.4	29.4	28.8	30.5	31.7	32.5
Length pelvic fin	27.2	3.5	3.4	27.4	28.6	28.6	28.6	30.1
Length pelvic-fin spine	16.1	1.8	1.9	16.0	16.9	18.4	17.3	17.4
Length first dorsal spine	10.1	3.4	2.9	8.1	8.2	8.2	8.7	9.2
Length second dorsal spine	12.1	2.9	2.5	10.4	12.2	10.6	11.0	11.6
Length third dorsal spine	12.8	2.6	2.3	11.7	14.0	12.8	12.3	12.3
Length last dorsal spine	13.0	2.9	2.2	11.6	12.2	13.5	13.9	12.4
Length longest dorsal ray	19.0	1.6	1.6	20.1	18.7	19.5	19.9	19.7
Length first anal spine	5.9	5.9	3.9	6.0	6.4	6.6	5.8	6.7
Length second anal spine	13.0	3.0	2.7	12.3	12.3	12.5	12.3	11.1
Length longest anal ray	17.1	1.8	1.5	18.4	18.4	17.5	17.2	17.4
Length caudal fin	30.6	3.4	3.6	28.1	27.1	29.5	27.3	30.1

Fig. 2. *Amphiprion barberi*, underwater photograph of adult pair, about 75.0 mm SL, Namena Island, Fiji, 5 m depth. Photo by G. R. Allen.

cular spinules 11-19; body depth 1.7-1.9 in SL; generally red-orange including fins, grading to brownish on upper back of adults and a single white bar immediately posterior to eye, its greatest width equal to that of eye or greater, narrowing to one-third to one-half of greatest width at dorsal midline.

Description: Dorsal rays X,17 (X,16-18); anal rays II,14; all dorsal and anal soft rays branched except first (second anal ray also unbranched in one paratype and no unbranched dorsal rays in another paratype), the last dorsal and anal rays branched to base; pectoral rays 18 (one paratype with 17), the upper and lowermost pairs unbranched; pelvic rays I,5; principal-caudal rays 15, branched-caudal rays 15 (13-14); scales in longitudinal series 52 (51-58); tubed lateral-line scales 38 (36-43); scales below lateral line to origin of anal fin 18 (18-19); scales above lateral line to middle of dorsal-fin base 4; gill rakers

Fig. 3. *Amphiprion barberi*, underwater photograph of subadult, about 50.0 mm SL, Namena Island, Fiji, 5 m depth. Photo by G. R. Allen.

Fig. 4. *Amphiprion barberi*, underwater photograph of juvenile, about 25.0 mm SL, Namena Island, Fiji, 5 m depth. Photo by G. R. Allen.

on first branchial arch 5 + 14 (5 + 12-14), total rakers 19 (17-19).

Body ovate, the depth 1.7 (1.7-1.9) in SL, and compressed, the width 2.3 (2.5-3.1) in body depth; HL 3.3 (3.0-3.3) in SL; forehead steeply sloped, ventral profile of head gently rounded from snout to pelvic fin origin; snout much longer than orbit, its length 2.7 (2.6-3.0) in HL; orbit diameter 4.0 (3.2-4.3) in HL; interorbital space slightly convex, its width 2.8 (2.4-3.3) in HL; caudal-peduncle depth 1.7 (1.7-1.9) in HL; caudal-peduncle length 2.5 (2.1-3.0) in HL.

Mouth terminal, oblique, jaws forming an angle of about 30° to horizontal axis of head and body; maxilla reaching a vertical at anterior edge of pupil, the upper-jaw length 2.7 (2.3-2.7) in HL; teeth uniserial, consisting of 40 (34-40) in upper jaw and 34 (30-36) in lower jaw; tongue broadly rounded, papillose on dorsal surface; gill rakers moderately elongate, the longest on lower limb near angle about one-half longest gill filaments; nostril (no posterior nostril detected) round with slightly raised rim, about midway between anterior edge of eye and upper lip.

Spinules on posterior margins of interopercle, subopercle, and opercle well developed; interopercle spinules 7 (5-8), subopercle spinules 15 (15-20), and opercle spinules 18 (11-19); preopercle serrations/crenulations weakly developed, about 22 (17-29) on upper limb; suborbital spinules moderately to weakly developed, 12 (9-14).

Scales finely ctenoid; head scaled except lips, snout, preorbital, suborbital, chin, anterior portion of dentary, and lower/posterior margin of preopercle; transverse scale rows on cheek 6 (5-7); a scaly sheath at base of dorsal and anal fins, averaging about one-half eye width at base of spinous portion of dorsal fin and slightly less at base of anal fin; a column of scales on each membrane of dorsal and anal fins, narrowing distally, becoming progressively longer to middle of soft portion of fin where they cover about basal half of fin, then gradually shorter on remainder of fin; small scales covering most of caudal fin, extending about two-thirds to three-fourths distance to posterior margin; small scales on basal one-fourth to one-half of middle pectoral-fin rays; a cluster of several scales forming median process culminating in enlarged scale between bases of pelvic fins, posterior-most scale extending slightly beyond middle of pelvic spine; axillary scale above base of pelvic spine about one-half length of pelvic-fin spine.

Origin of dorsal fin over second or third lateral-line scale; predorsal distance 2.4 (2.3-2.5) in SL; preanal distance 1.5 (1.5-1.7) in SL; prepelvic distance 2.5 (2.2-2.7) in SL; bases of soft and spinous portions of dorsal fin about equal in length; dorsal-fin spines gradually increasing in length to third spine, remaining spines about equal; first dorsal spine 3.0 (2.9-5.3) in HL; second dorsal spine 2.5 (2.5-3.4) in HL; third dorsal spine 2.4 (2.2-2.9) in HL; last dorsal spine 2.3

Fig. 5. *Amphiprion melanopus*, underwater photograph of adult, about 75.0 mm SL, Milne Bay, Papua New Guinea, 3 m depth. Photo by G. R. Allen.

(2.2-2.9) in HL; ninth or tenth dorsal soft ray longest, 1.6 (1.5-1.9) in HL; first anal spine 5.1 (3.9-5.9) in HL; second anal spine 2.3 (2.3-3.0) in HL; longest (tenth or eleventh) anal soft ray 1.8 (1.5-2.0) in HL; sixth or seventh pectoral ray longest, 3.2 (3.1-3.6) in SL; pelvic-fin spine 1.9 (1.6-2.2) in HL; pelvic-fin length 3.7 (3.3-3.8) in SL; caudal fin rounded, its length 3.3 (3.3-3.8) in SL.

Colour in alcohol (Fig. 1): brown on snout, forehead, and upper back, grading to tan on lower head and side; grey bar immediately behind eye extending to ventral edge of operculum, its maximum width (slightly exceeding eye diameter) about level with lower edge of eye; fins yellowish tan with narrow dark margin on dorsal and anal fins, and anterior edge of pelvic fins. Most adult and subadult paratypes are similar except subadults are frequently lighter. Three juvenile paratypes (USNM 238890), 16.3-19.6 mm SL, possess a second, narrower pale bar below the middle of the dorsal fin. This feature apparently disappears in slightly larger juveniles; it is not visible in four specimens, 23.6-29.0 mm SL, from the same lot.

Colour when alive (Figs 2-4): reddish brown on forehead and upper back, grading to reddish orange on remainder of body and fins; a brilliant white bar immediately behind eye extending to ventral edge of operculum, its greatest width equal to that of eye or greater, narrowing to one-third to one-half of greatest width at dorsal midline; a narrow black margin on dorsal, anal, and anterior edge of pelvic fins. Small juveniles under about 25 mm SL (Fig. 4) overall dull pinkish orange with brilliant white head bar. The red-orange colouration of the body and fins intensifies with increased growth.

Etymology: This species is named *Amphiprion barberi* in honour of Dr. Paul Barber of Boston University, USA in recognition of his valuable contributions to our understanding of genetic relationships of Indo-Pacific coral reef organisms.

Remarks: *Amphiprion barberi* appears to be most closely related to *A. melanopus* (Fig. 5), which is widely distributed in the western Pacific (Fig. 6). The two species share similar meristic and morphometric proportions, but are generally separable on the basis of colouration and genetic composition. Adults of the new species are mainly reddish orange in life with a single white head bar. In contrast, the typical colouration of *A. melanopus* consists of a reddish-orange head and breast, single white head bar, dark

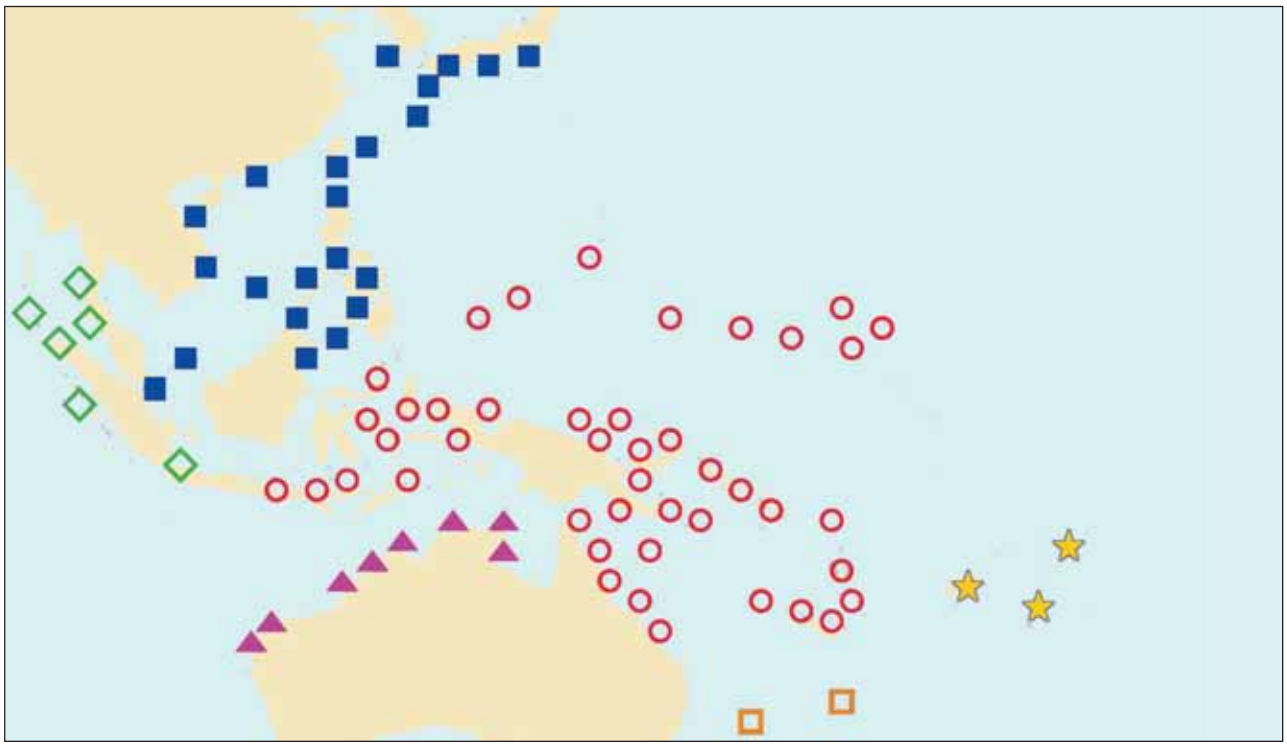

Fig. 6. Map of the western Pacific Ocean with approximate distributions of members of the *Amphiprion ephippium* complex indicated: *A. ephippium* (diamonds), *A. frenatus* (solid squares), *A. rubrocinctus* (triangles), *A. melanopus* (circles), *A. mccullochi* (open squares), and *A. barberi* (stars).

brown to nearly black body, and dark brown pelvic and anal fins. Very small juveniles of both species usually have two pale bars including the head bar and another across the middle of the side. A third bar or small saddle on the caudal-fin base is sometimes evident in *A. melanopus.* The extra bars(s) persist in *A. melanopus* to at least 30.0 mm SL, whereas in *A. barberi* it is no longer visible in fish over 23.0 mm SL. Occasional adults of *A. melanopus* from Vanuatu and New Caledonia sometimes have considerably lighter (reddish orange) colouration on the side of the body, in which case geography provides the best means of separation. However, there is also a strong modal difference in the number of spinules in the upper opercular series for adults in excess of about 55 mm SL: *A. barberi* has 11 to 19 spinules versus 19-26 for *A. melanopus*.

Allen (1972) placed *A. melanopus* and allied species into the *"ephippium* complex" on the basis of their similar morphology, colour patterns, and host anemone preferences. The members of this group include *A. ephippium* (Bloch, 1790), *A. frenatus* Brevoort, 1856, *A. mccullochi* Whitley, 1929, *A. melanopus*, and *A. rubrocinctus*. Their geographic distributions are summarised in Fig. 6. *Amphiprion ephippium* from western Indonesia and the East Andaman Sea differs from all other members of the

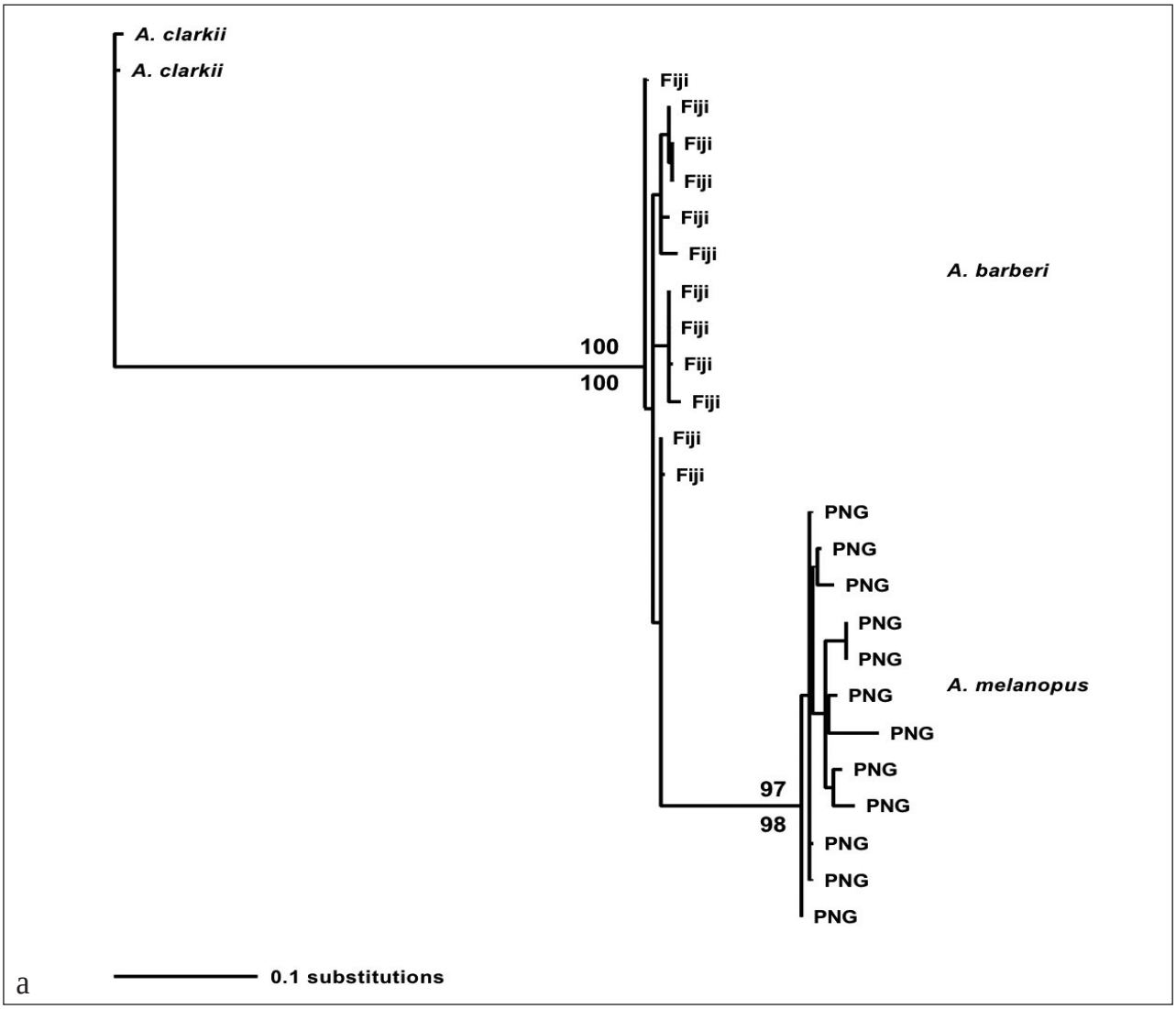

Fig. 7a. Maximum likelihood tree (K81uf+I) of the mitochondrial control region data. ML bootstrap support of 100 replicates are shown above and below respectively. The number of specimens is shown in parentheses. Fiji = *Amphiprion barberi;* PNG (Papua New Guinea) = *A. melanopus.*

complex in lacking a white head bar at all sizes and by its predorsal scales, which extend to the front of the orbits rather than the mid-interorbital level. *Amphiprion mccullochi* from Lord Howe and Norfolk islands is entirely dark brown except for a light grey snout, whitish caudal fin, and incomplete white head bar. It also has a slightly emarginate caudal fin in contrast to the usual rounded shape. *Amphiprion frenatus* from the South China Sea, Taiwan, and Japan is similar to *A. rubrocinctus* of north-western Australia. Adults of these species differ from *A. barberi* in having dark brown sides broadly margined with red, although males of *A. frenatus*, which seldom exceed about 50 mm SL, are entirely red (with white head bar). In addition, *A. frenatus* differs in having pronounced blackish margins on the white head bar.

We were successful in resolving a 387 base pair segment of the control region of which 107 bases were variable and 88 were parsimony informative. Base composition was A: 41.8%, C: 15.2%, T: 31% and G: 11.9%, which is consistent with previous findings of the control region being AT rich (Lee et al. 1995). Pairwise genetic distances ranged from d=0.002 to 0.031 within clades but between clade differences ranged from d=0.079 to 0.116. Both parsimony and likelihood results indicate that the two populations are clearly resolved into two separate clades, with high bootstrap support,

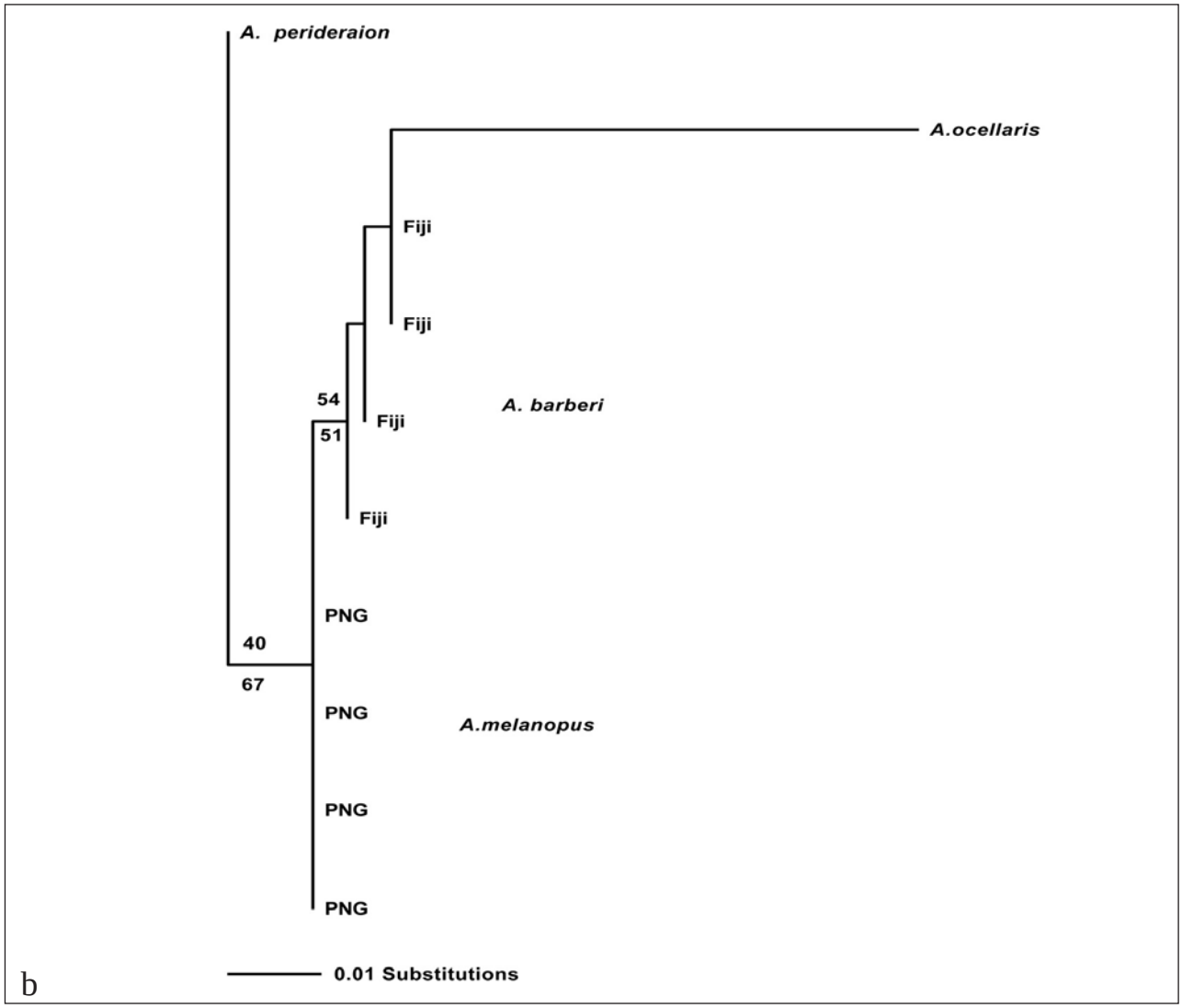

Fig. 7b. Maximum likelihood tree (K80+I) of the nuclear Recombination Activating Gene 2 data. ML and MP bootstrap support of 100 replicates are shown above and below respectively. The number of specimens is shown in parentheses. Fiji = *Amphiprion barberi;* PNG (Papua New Guinea) = *A. melanopus*.

indicative of separate evolutionary trajectories (Fig. 7a and 7b).

A total of 630 base pairs were resolved for the nuclear data, of which 12 bases were variable and 5 were parsimony informative. Base composition was A: 21.7%, C: 27.5%, T: 24.2%, and G: 26.7%. Pairwise genetic distances ranged from d=0.000 to 0.001 to 0.031 within clades while between clade differences ranged from d=0.005 to 0.007. Our results indicate that the Fijian population is a monophyletic clade nested within the samples from Papua New Guinea (Fig 7b). The nuclear data was much less variable than the mitochondrial dataset which is to be expected due to the four fold greater effective population size, which results in a slower rate of evolution (Avise 2000). There was low bootstrap support for the resolved topology because of the relatively fewer phylogenetically informative characters. However, since this topology is similar to that suggested by the more quickly evolving mitochondrial data we feel confident in the monophyly of the Fijian samples, and the recognition of this population as a valid species.

Distribution and habitat: *Amphipron barberi* is reliably known only from Fiji, Tonga, and Samoa. Allen (1972) examined a specimen at the California Academy of Sciences (CAS 7024) that was questionably collected at Tahiti by Zane Grey in 1931, but intensive collecting and observations by J. Randall and the author in the Society Islands failed to find this species. The species is common on Fijian coral reefs at depths ranging from about 2-10 m. It is generally commensal with large sea anemones, either *Entacmaea quadricolor* (Rüppell & Leuckart, 1828) or *Heteractis crispa* (Ehrenberg, 1834). This fish is usually seen in groups that swim a short distance above their host anemones, apparently feeding on zooplankton. Paul Brown, a marine ecologist of the National Park of American Samoa, recently reported (personal communication) that it is frequently encountered on local reefs in about 6-8 m depth, usually in association with *Entacmaea quadricolor*.

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