Two new species of rainbowfishes *(Melanotaenia:* Melanotaeniidae), from, western New Guinea (Papua Barat Province, Indonesia)

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Abstract

Two new species of rainbowfishes are described from the southwestern Birds Head region of western New Guinea (Papua Barat Province, Indonesia). *Melanotaenia ammeri* n. sp. is described on the basis of 19 specimens, 46.5-82.2 mm SL, collected near Gusimawa Village, Arguni Bay. It is most closely related to *M. kokasensis* n. sp., also described as new from 25 specimens, 27.2-68.5 mm SL, collected near Kokas Village on the northern Fakfak Peninsula. The two species are easily distinguished on the basis of colour pattern: *M. ammeri* has a distinctive pattern of alternating mauve to greyish blue and yellow stripes and the colour pattern of *M. kokasensis* is dominated by a broad, black midlateral stripe. In addition, males of *M. ammeri* are significantly deeper bodied (maximum body depth as % of standard length 39.3 *versus* 34.5) than those of *M. kokasensis.* Analysis of genetic relationships based on cytochrome *b* sequences indicates a close relationship between the two species and demonstrates that they are part of the unique rainbowfish clade found mainly on the Vogelkop Peninsula and some offshore islands. The new species differed in their mean Kimura 2-parameter genetic divergences by 2.4% from each other. Both were between 5.4 and 5.6% divergent from *M. parva*, the next most closely related species that we obtained genetic data for.

Zusammenfassung

Beschrieben werden zwei neue Regenbogenfisch-Arten aus der südwestlichen Birds-Head-Region des westlichen Neuguinea (Provinz Papua Barat, Indonesien). *Melanotaenia ammeri* n. sp. wird auf der Grundlage von 19 Exemplaren mit 46,5-82,2 mm SL, gesammelt nahe Gusimawa Village, Arguni Bay, beschrieben. Nahe verwandt ist die zweite neue Art *M. kokasensis* n. sp., beschrieben nach 25 Exemplaren mit 27,2-68,5 mm SL, gefangen nahe Kokas Village auf der nördlichen Fakfak-Halbinsel. Nach der Farbgebung lassen sich die beiden Arten leicht unterscheiden. *M. ammeri* zeigt ein auffälliges Muster aus abwechselnden malven- bis graublauen und gelben Streifen, während die Farbe von *M. kokasensis* von einem breiten schwarzen Streifen auf der Flankenmitte beherrscht wird. Außerdem haben die Männchen von *M. ammeri* einen deutlich tieferen Rumpf (mittlere Tiefe in % der Standardlänge 39,3 im Vergleich zu 34,5) als *M. kokasensis.* Die Analyse der genetischen Verwandtschaft auf der Basis der Sequenzen von Cytochrom *b* bestätigt die nahe Verwandtschaft der beiden neuen Arten und zeigt, dass sie zur einzigartigen Regenbogenfisch-Klade gehören, deren Vertreter sich hauptsächlich auf der Vogelkop-Halbinsel und einigen küstennahen Inseln finden. Nach dem mittleren Kimura-2-Parameter genetischer Divergenzen unterscheiden sich die beiden neuen Arten um 2,4% voneinander. Beide unterscheiden sich aber um 5,4 bzw. 5,6% von *M. parva,* der nächstverwandten Art, zu der genetische Daten zur Verfügung standen.

Résumé

Deux nouvelles espèces de poissons arc-en-ciel, du sud-ouest de la péninsule du Vogelkop, Papouasie Occidentale (province de Papua Barat, Indonésie) sont décrites. *Melanotaenia ammeri* n. sp. est décrit sur base de 19 spécimens, de 46,5 à 82,2 mm de LS, collectés près de Gusimawa Village, Arguni Bay. Il est très proche de *M. kokasensis* n. sp., également décrit comme nouvelle espèce, sur base de 25 spécimens, de 27,2 à 68,5 mm de LS, collectés près de Kokas Village, au nord de la péninsule de Fakfak. Les deux espèces se distinguent facilement par leur patron de coloration. *M. ammeri* arbore un patron caractéristique de lignes alternées mauves à bleu grisâtre et jaunes, et le patron de coloration de *M. kokasensis* est dominé par une large bande latérale médiane noire. En outre, les mâles de *M. ammeri* sont nettement plus hauts de corps (hauteur maximale du corps en % par rapport à la longueur standard : 39,3 au lieu de 34,5) que ceux de *M. kokasensis.* L'analyse des relations génétiques basée sur des séquences de cytochrome *b* révèle une parenté étroite entre les deux espèces et prouve qu'elles font partie de l'unique clade de poissons arc-en-ciel trouvé principalement dans la péninsule du Volgelkop et quelques îles au large. Les nouvelles espèces diffèrent, par leurs principales divergences du paramètre génétique Kimura 2, de 2,4% l'une de l'autre. Les deux divergent de 5,4 a 5,6% de *M. parva,* l'autre

espèce la plus proche pour laquelle nous avons obtenu des données génétiques.

Sommario

In questo articolo sono descritte due nuove specie di pesci arcobaleno originari della parte sudoccidentale della regione nota come Birds Head (Provincia di Papua Barat, Nuova Guinea occidentale, Indonesia). *Melanotaenia ammeri* n. sp. è descritta sulla base di 19 esemplari di 46.5-82.2 mm SL, raccolti presso Gusimawa Village, Arguni Bay. Appare strettamente imparentata a *M. kokasensis* n. sp., anch'esso descritta come nuova sulla base di 25 esemplari di 27.2-68.5 mm SL, raccolti presso Kokas Village nella parte settentrionale della penisola di Fakfak. Le due nuove specie sono facilmente distinguibili in base alla colorazione: *M. ammeri* ha una caratteristica livrea costituita da strie alterne le une di un colore che va dal malva al grigio-blu, le altre gialle, mentre la colorazione di *M. kokasensis* è dominata da un'ampia stria nera mediolaterale. In aggiunta, gli individui maschi di *M. ammeri* hanno un corpo significativamente più alto (altezza massima del corpo come % della lunghezza standard 39.3 *versus* 34.5) di quelli di *M. kokasensis*. L'analisi genetica basata sulle sequenze del citocromo *b* indica una parentela stretta tra le due specie e dimostra che entrambe fanno parte di un'unica linea monofiletica diffusa principalmente sulla penisola Vogelkop e in alcune isole al largo di essa. Le nuove specie mostrano una divergenza genetica in base al Kimura 2-parametri del 2.4%. Entrambe sono divergenti per il 5.4% e il 5.6% da *M. parva,* la specie filogeneticamente più vicina e per la quale abbiamo ottenuto dati genetici.

INTRODUCTION

The colourful rainbowfishes of the family Melanotaeniidae are common freshwater inhabitants throughout New Guinea and northern Australia. Several revisions and reviews were provided by the first author and various colleagues (Allen 1980, 1985, 1989, 1995; Allen & Cross 1982; Allen et al. 2002). A total of 74 species in seven genera are currently recognized. Their greatest diversity occurs on the island of New Guinea where new species are still being discovered, particularly in remote, previously unsampled regions. The present paper describes two new species of *Melanotaenia* Gill, 1862 that were collected during an exploratory cruise by the first author to Arguni Bay and the neighbouring Bomberai Peninsula during January 2008 (Fig. 1).

Melanotaeniids belong to the order Atheriniformes (Nelson 2006) and typically possess a compressed body covered in relatively large scales, two separate dorsal fins (the first with 3-7 spines and the second with a single spine and 6-22 segmented rays), a long-based anal fin, and no lateral line. Sexual dimorphism is often apparent; males tend to be deeper-bodied and more vividly coloured than females.

Rainbowfishes are prized by aquarists for their bright colouration and placid nature. Aquarium spawning has been achieved for many species. Males initiate spawning by 'flashing' vivid colours, often including a bright stripe on the nape, to attract a mate. The pair swims above aquatic vegetation and releases eggs and sperm with a vigorous shudder of the body. The eggs are demersal and have adhesive threads which attach to the foliage. Females typically release a small number of eggs daily. The eggs hatch within 7 to 18 days and most species attain sexual maturity by the end of the first year. In the natural habitat, rainbowfishes often form large shoals near the surface. The diet consists of a variety of insects and their larvae, micro-crustaceans, and some algae.

Allen (1990) reviewed the seven species that were known at that time from the mainland portion of the Birds Head region (Fig. 1) at the extreme western end of New Guinea (Papua Barat Province, Indonesia). The coverage included *Melanotaenia ajamaruensis* Allen & Cross, 1980, *M. angfa* Allen, 1990, *M. arfakensis* Allen, 1990, *M. boesemani* Allen & Cross, 1980, *M. fredericki* (Fowler, 1939), *M. irianjaya* Allen, 1985, and *M. parva* Allen, 1990. Several additional species including *M. catherinae* (De Beaufort, 1919), *M. batanta* Allen & Renyaan, 1996, *M. misoolensis* Allen, 1982, and *M. synergos* Allen & Unmack, 2008 are known from the nearby Raja Ampat Islands (Allen & Unmack 2008). The authors are currently studying genetic relationships within the family using mitochondrial and nuclear DNA sequence analysis. The species inhabiting the Birds Head region are of particular interest and constitute our current research focus. They appear to form a separate clade within the genus *Melanotaenia* that is well differentiated from those found in Australia and the remainder of New Guinea (McGuigan et al. 2000). However, we are yet to clearly establish the origins of the Birds Head species.

MATERIALS AND METHODS

Counts and measurements that appear in parentheses refer to the range for paratypes if different from the holotype. Type specimens are deposited at the Australian Museum, Sydney (AMS), Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia (MZB), United States

National Museum of Natural History, Washington, D.C. (USNM), and the Western Australian Museum, Perth (WAM).

The methods of counting and measuring are as follows: dorsal and anal rays – the last ray of the anal and second dorsal fins is divided at the base and counted as a single ray; lateral scales – number of scales in horizontal row from upper edge of pectoral-fin base to caudal-fin base, excluding the small scales posterior to the hypural junction; transverse scales – number of scales in vertical row between anal fin origin and base of first dorsal fin; predorsal scales – number of scales along midline of nape in front of first dorsal fin; cheek scales – total number of scales covering the suborbital and preoperculum; standard length (SL) – measured from the tip of the upper lip to the caudal-fin base; head length (HL) – measured from the tip of the upper lip to the upper rear edge of the gill opening; caudal peduncle depth is the least depth, and caudal peduncle length is measured between two vertical lines, one passing through the base of the last anal ray and the other through the caudal-fin base; caudal concavity is the horizontal distance between verticals at the tips of the shortest and longest rays.

DNA material: a number of rainbowfish species from the Birds Head region were included for DNA sequence comparison to the new species. This included the following species: *M. ammeri* (from type locality, see below), *M. kokasensis* (from type locality, see below), *M. misoolensis* (Wai Tama River, 1°51.635'S 129°55.401'E, Misool Island, Raja Ampat Islands); *M. fredericki* (Kali Doktur, 1°01.036'S 130°39.943'E, Salawati Island, Raja Ampat Islands), *M. parva* (obtained via aquarium trade, originally collected from Lake Kurumoi,

Fig. 1. Map of the Birds Head (Vogelkop) region, Papua Barat Province, Indonesia with inset map of New Guinea. The approximate collections locations of *Melanotaenia ammeri* and *M. kokasensis* are shown by a star and triangle respectively. The known collection locations for *M. irianjaya* and *M. parva* are indicated by circles and a diamond respectively.

2°09.528'S 134°05.217'E, Papua Barat Province), *M. batanta* (Warmon Creek, 0°49.948'S 130°43.123'E, Batanta Island, Raja Ampat Islands), *M. catherinae* (Waisam Creek, 0°17.415'S 130°57.079'E, Waigeo Island, Raja Ampat Islands), *M. synergos* (Wei Bin Stream, 0°49.774'S 130°45.874'E, Batanta Island, Raja Ampat Islands). Two populations of *M. affinis* from the Gogol River and Sepik River, Papua New Guinea were included as outgroup taxa.

The second author extracted genomic DNA from muscle tissue from each specimen using the DNeasy Tissue Kit (QIAGEN Inc., Chatsworth CA). The cytochrome *b* (cyt *b*) gene was amplified using two primers (Glu31 5'-GTGACTTGAAAAACCACCGTT-3' and RF.Thr48 5'-GCAGTAGGAGGGAATTTAAC-CTTCG-3') that flanked the entire gene. When this failed to produce sufficient PCR product the gene was amplified in two halves using Glu31 – HD and rainL505 – RF.Thr48. Primer HD (5' GGGTTGTTTGATCCTGTTTCGT 3') is from T. Schmidt as given in Dowling & Naylor (1997) while primers Glu31, RF.Thr48, and rainL505 (5' TCYGTAGATAATGCCACCCT 3') were designed by us. Final concentrations for polymerase chain reaction (PCR) components per 25 µL reaction were as follows: 25 ng template DNA, 0.25 μM of each primer, 0.625 units of Taq DNA polymerase, 0.1 mM of each dNTP, 2.5 μL of 10X reaction buffer and 2.5 mM $MgCl_2$. Amplification parameters were as follows: 95°C for 2 min followed by 35 cycles of 95°C for 30 sec, 48°C for 30 sec, and 72°C for 90 sec, and 72°C for 7 min. The PCR products were examined on a 1.5% agarose gel using SYBR safe DNA gel stain (Invitrogen, Eugene, OR, USA). The PCR products were purified using a Montage PCR 96 plate (Millipore, Billerica, MA, USA). Most sequencing reactions and clean up were performed using a Parallab 350 (Parallabs, Worcester, Massachusetts, USA). Some were also obtained via cycle sequencing with Big Dye 3.0 dye terminator ready reaction kits using 1/16th reaction size (Applied Biosystems, Foster City, CA). Sequencing reactions were run with an annealing temperature of 52°C. Sequenced products were purified by passing reactions through sephadex columns. Sequences were obtained with an Applied Biosystems 3730 XL automated sequencer at the Brigham Young University DNA Sequencing Center.

DNA sequences were edited using Chromas Lite 2.0 (Technelysium, Tewantin, Queensland, Australia) and then imported into BioEdit 7.0.5.2 (Hall, 1999) and aligned by eye. Sequences were checked for unexpected frame shift errors or stop codons in Mega 4.0 (Tamura et al. 2007). Phylogenetic analyses were performed using both parsimony and likelihood approaches using PAUP* 4.0b10 (Swofford 2003). Maximum parsimony (MP) was conducted via a heuristic search with 1,000 random additions and TBR branch swapping. For Maximum likelihood (ML) analysis we identified the best fitting model of molecular evolution using the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada & Crandall 1998). The best model of evolution found by Modeltest was the K81uf+G with the following parameters: Lset Base=(0.2583 0.3097 0.1416) Nst=6 Rmat=(1.0000 11.3616 0.2421 0.2421 11.3616) Rates=gamma Shape=0.2118 Pinvar=0. Robustness of nodes was estimated with PAUP* by bootstrap with 1,000 replicates for MP using a heuristic search with 10 random additions of taxa and TBR branch-swapping, and 1000 replicates for ML via a heuristic search with five random additions of taxa and TBR branch-swapping. All tree lengths reported for MP include both informative and uninformative characters. Among species variation was calculated using Kimura 2-parameter divergences in MEGA 4.0.

Melanotaenia ammeri n. sp.

(Figs 2-3; Tables I, II)

Holotype: MZB 16455, male, 82.2 mm SL, small creek near Gusimawa , 3°02.438'S 133°52.844'E, Arguni Bay, Papua Barat Province, Indonesia, 0-0.5 m, seine net, G. R. Allen and M. Ammer, 12 January 2008.

Paratypes (collected with holotype): AMS I.44640-001, 4 specimens, 46.5-66.5 mm SL; MZB 16456, 4 specimens, 55.9-71.5 mm SL; USNM 391630, 5 specimens, 46.1-71.6 mm SL; WAM P. 33011-001, 5 specimens: 53.8-73.5 mm SL.

Comparative material examined: *Melanotaenia irianjaya* – MZB 4952, 50.0 mm SL (holotype), Fruata, 2°58.980'S 133°31.977'E, Bomberai Peninsula, Papua Barat Province, Indonesia; WAM P. 27863-001, 12 specimens (paratypes), 20.0-51.0 mm SL, Fruata; WAM P.29955-001, 10 specimens, 26.8-64.6 mm SL, Kali Satu, 2°05.913'S

Table I. Proportional measurements of selected type specimens of *Melanotaenia ammeri* expressed as % of the standard length.

	Holotype MZB 16455	Paratype WAM P.33011	Paratype USNM 391630	Paratype USNM 391630	Paratype WAM P.33011	Paratype WAM P.33011	Paratype USNM 391630
Sex	male	female	male	male	female	male	female
Standard length (mm)	82.2	73.5	69.2	62.4	58.0	53.8	46.1
Body depth	39.3	31.7	35.4	34.9	32.4	32.5	31.7
Body width	14.2	13.9	12.9	13.5	12.9	12.3	13.2
Head length	26.5	26.4	26.0	27.9	27.1	28.3	28.9
Snout length	8.4	9.5	9.0	9.5	9.3	9.7	9.8
Maxillary length	9.4	9.4	9.5	10.9	10.2	10.6	11.1
Eye diameter	7.3	6.8	7.7	7.7	8.4	9.1	9.3
Bony interorbital width	9.2	8.6	9.2	9.3	9.5	9.5	9.8
Depth of caudal peduncle	12.9	11.0	12.3	11.7	11.2	11.9	10.6
Length of caudal peduncle	15.5	14.7	16.3	13.8	14.3	17.5	15.8
Predorsal distance	48.3	49.4	49.3	51.1	48.6	48.7	49.9
Preanal distance	49.6	50.3	52.0	51.3	51.6	50.4	51.2
Prepelvic distance	36.1	37.7	38.4	38.8	38.3	37.9	38.8
2nd dorsal fin base	29.2	25.6	24.7	23.9	24.7	23.8	21.5
Anal fin base	42.2	40.0	39.5	39.9	38.1	37.5	35.4
Pectoral fin length	18.4	19.3	19.7	21.6	20.3	19.1	20.6
Pelvic fin length	19.7	17.4	17.8	22.3	18.6	19.3	16.1
Longest ray 1st dorsal fin	16.3	11.8	15.5	15.5	11.6	15.6	13.0
Longest ray 2nd dorsal fin	19.5	9.9	15.9	14.4	13.4	14.3	12.8
Longest anal ray	18.5	14.6	16.8	17.3	15.5	17.8	25.8
Caudal fin length	21.8	23.7	22.8	23.9	24.7	23.2	24.9
Caudal concavity	5.7	5.9	7.7	7.1	7.4	6.5	8.0

133°30.930'E, Bintuni, Papua Barat Province, Indonesia; WAM P.29960-001, 53 specimens, 29.9-78.1 mm SL, Kali Tujuh, 2°05.959'S 133°29.988'E, Bintuni, Papua Barat Province, Indonesia; *Melanotaenia parva* – WAM P.29970-001, 125 specimens (paratypes), 21.2-52.6 mm SL, Lake Kurumoi, 2°09.528'S 134°05.217'E, Papua Barat Province, Indonesia.

Diagnosis: A species of melanotaeniid rainbowfish distinguished by the following combination of characters: dorsal rays IV to VI-I,12 to 15 (usually 13 or 14); anal rays I,20-25 (usually I,21 to 23); pectoral rays 12 to 14 (usually 13 or 14); lateral scales 34-36 (usually 35), predorsal scales 17 to 19 (usually 17 or 18); total gill rakers on first arch 16 to 18 (usually 16); colour in life generally bluish dorsally with series of alternating mauve to blue-grey and pale yellow stripes (one per scale row) on side of body, midlateral blue stripe usually much darker than others; median fins generally yellow, brighter in adult males.

Description: Dorsal rays IV-I,14 (IV to VI,12 to 15); anal rays I,22 (I,20 to 25); pectoral rays 12 (12 to 14); pelvic rays I,5; branched caudal rays 16; lateral scales 35 (34 or 36); transverse scales 11 (10 or 11); predorsal scales 19 (17-19); cheek scales 12 (11-15); total gill rakers on first arch 18 (16-18).

Body depth 2.5 (2.3-3.4) in SL, head length 3.8 (3.4-3.8) in SL; greatest width of body 2.8 (2.3-3.3) in greatest body depth; snout length 3.2 (2.8-3.1) in HL; eye diameter 3.6 (3.1-3.9) in HL; interorbital width 2.9 (2.8-3.2) in HL; depth of caudal peduncle 2.1 (2.1-2.7) in HL; length of caudal peduncle 1.7 (1.6-2.1) in HL.

Jaws about equal, oblique, premaxilla with an abrupt bend between the anterior horizontal portion and lateral part; maxilla ends below anterior edge of eye; maxillary length 2.8 (2.6-2.8) in HL; lips thin; teeth conical with slightly curved tips, extending on to outer surface of lips; teeth of upper jaw in 4-5 irregular rows anteriorly, reduced to a single row posteriorly, where they are exposed when mouth is closed; teeth in lower jaw in about 7-8 irregular rows anteriorly, reduced to 1 or 2 rows posteriorly; narrow row containing several small, conical teeth on vomer and palatines.

Scales of body cycloid, relatively large, and arranged in regular horizontal rows; scale margins weakly crenulate; predorsal scales extending forward to about middle of interorbital space; preopercle with 2-3 scale rows between its posterior angle and eye.

Predorsal length 2.1 (1.9-2.1) in SL; preanal length 2.0 (1.9-2.0) in SL; prepelvic length 2.8 (2.5-2.7) in SL; length of second-dorsal fin base 3.4 (3.8-4.7); length of anal-fin base 2.4 (2.4-2.8).

First dorsal fin origin about level with anal fin origin; longest spines (usually second to fourth) of first dorsal fin 1.6 (1.7-2.3) in HL, its depressed tip reaching spine or first soft ray of second dorsal fin in females and reaching to about base of second or third soft ray in mature males; longest rays (generally anterior ones in females and middle ones in males) of second dorsal fin 1.4 (1.6-2.7) in HL, the depressed posterior rays extending about one half to two-thirds length of caudal peduncle in females and full length of caudal peduncle in mature males; longest (middle rays in males and females) anal rays 1.4 (1.1-2.0) in HL; pelvic fin tips when depressed reaching to base of fourth or fifth soft anal fin ray in mature adults; length of pelvic fins 1.3 (1.2-1.8); length of pectoral fins 1.4 (1.3-1.5) in HL; length of caudal fin 1.2 (1.1-1.2) in HL; caudal fin moderately forked, caudal concavity 4.6 (3.3-5.5) in head length.

Colour of male holotype in life (Fig. 2, upper fish): blue on upper back; eight mauve to blue-grey stripes, corresponding with horizontal scale rows on side of body, alternating with narrower pale yellow stripes; midlateral stripe (at level of

Fig. 2. Aquarium photograph of *Melanotaenia ammeri*, male holotype (upper), 82.2 mm SL, and female paratype (WAM P.33011-001), 73.5 mm SL, Gusimawa, Papua Barat Province, Indonesia. Photo by G. R. Allen.

upper pectoral-fin base) generally darkest with blue stripes below progressively more inconspicuous and forming interrupted dotted lines; broad, horizontally bluish streak immediately above abdomen; upper portion of head blue grayish, lower half silvery white; breast white; dorsal, anal, pelvic and caudal fins pale yellow; pectoral fins translucent with white base. Colour pattern of female similar to that of male except blue and yellow hues of body stripes generally less vivid and median fins mainly translucent, only slightly yellow.

Colour of holotype in alcohol (Fig. 3): overall tan including narrow stripe between each horizontal scale row of body; 2-3 uppermost horizontal scale rows with broad, elongate brown botch covering most of each scale; similar, but much darker (brown to blackish) markings on two midlateral scale rows, these merging to form broad, dark brown stripe on posterior half of body; horizontal scale row immediately below midlateral rows with faint rectangular brown blotch on each scale; ventral third of side tan with very faint indication of brown stripe along middle of each horizontal scale row; upper portion of head grey brown grading to tan on cheek and opercle; pectoral fins translucent; remaining fins variable dusky brown. Paratypes similar in colour to holotype, but intensity of two midlateral stripes highly variable, ranging from intense blackish to barely darker than adjacent scale rows.

Sexual dimorphism: Similar to most *Melanotaenia*, males are generally deeper bodied than females and have a more elongate, pointed shape posteriorly on the soft dorsal fin. The longest soft dorsal-fin rays of males are located in the posteriormost portion of the fin, in contrast to that of females, which are situated in the anterior half of the fin. In addition, the depressed first dorsal fin of adult males extends to the base of the second or third soft ray of the second dorsal fin, compared with the spine or first ray in females. The body depth (as % of SL) of 13 males, 53.8-82.2 mm SL, ranged from 32.5-39.3 with an average of 34.7; that of 6 females, 46.1-73.5 mm SL, was 29.7-32.6 with an average of 31.4.

Comparisons: The distinctive pattern of alternating mauve to blue-grey and yellow stripes is unique among species of *Melanotaenia*. Genetic analysis (see below) revealed that *M. ammeri* and *M. kokasensis* n. sp. (described below) are closely related, forming a subclade with *M. parva* Allen, 1990 (Fig. 4) that is well separated from other members of the genus. Although we presently lack tissue samples for *M. irianjaya* Allen, 1985 (Fig. 5), this species is possibly a member of the same species group, judging from meristic and morphological

Fig. 3. *Melanotaenia ammeri*, preserved male, holotype, 82.2 mm SL, Gusimawa, Papua Barat Province, Indonesia. Photo by G. R. Allen.

Table II. Summary of dorsal, anal, pectoral fin-ray, gill-raker, lateral-scale, and predorsal-scale counts for *Melanotaenia ammeri, M. kokasensis, M. irianjaya, and M. parva.*

	First Dorsal Fin Spines					Soft Dorsal Rays			
	IV	**V**	**VI**	**11**	**12**	**13**	**14**	**15**	**16**
M. ammeri	8	10	1		2	6	8	3	
M. kokasensis	8	17		1	12	12			
M. irianjaya	11	20	1			10	15	7	1
M. parva		24	9	4	13	13	3		

	Soft Anal Rays								
	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
M. ammeri		1	6	6	4	1	1		
M. kokasensis			1	5	17	2			
M. irianjaya			1	5	6	12	7	1	1
M. parva	1	6	13	12		1			

	Pectoral Rays				Gill Rakers					
	12	**13**	**14**	**15**	**14**	**15**	**16**	**17**	**18**	**19**
M. ammeri	6	12	1				13	4	2	
M. kokasensis		3	20	2			5	8	11	1
M. irianjaya		3	23	7		7	14	6	5	
M. parva	1	15	16	1	1	5	10	12	5	

	Lateral Scales						Predorsal Scales					
	33	**34**	**35**	**36**	**37**	**38**	**14**	**15**	**16**	**17**	**18**	**19**
M. ammeri		2	13	4						9	6	4
M. kokasensis			3	20	2				11	12	2	
M. irianjaya			8	20	3	1			6	14	12	
M. parva	1	18	13	1			1	2	6	15	9	

similarities. All four species inhabit the Bomberai/ Birds Neck region of western New Guinea, but exhibit allopatric distributions (Fig. 1).

The members of this species group or subclade are clearly distinguished on the basis of colour pattern (Figs 2, 4, 5, 7) and also exhibit modal differences related to several meristic features (Table II). *Melantoaenia ammeri* and *M. parva* tend to have a lower number of soft anal-fin rays, most often 21 or 22 (mean 22.1 and 21.2 respectively) compared with *M. kokasensis* and *M. irianjaya* that most frequently possess 23 or 24 rays (mean 22.8 and 23.8 respectively). Furthermore, *M. ammeri* has a lower modal number of pectoral-fin rays, usually 12 or 13 rays (mean 12.7), compared with an equal spread of 13 or 14 rays (mean 13.5) in *M. kokasensis*, and a usual count of 14 rays for *M. irianjaya* and *M. parva* (mean 14.0 and 14.1 respectively). *Melanotaenia kokasensis* has a higher mean number of total gill rakers on the first branchial arch (17.3) compared with *M. parva* (16.5), *M. ammeri* (16.4), and *M. irianjaya* (16.3). Additionally, *M. kokasensis* and *M. irianjaya* usually have 36 lateral scales (mean 36.0 and 35.9 respectively) compared with usual counts of 35 for *M. ammeri* (mean 35.1) and 34-35 (mean 34.4) for *M. parva*. Finally, *M. ammeri* tends to have slightly more predorsal scales (mean 17.7) compared to *M. irianjaya* (mean 17.2), *M. parva* (mean 16.9), and *M. kokasensis* (mean 16.6).

Zoogeography and habitat: The new species is currently known only from the type locality (Fig. 6), a small creek flowing into the northern part of Arguni Bay. It no doubt occurs in nearby streams, but the exact limits of distribution remain to be determined. The type locality consists of a narrow (to about 2-3 m wide), relatively shallow (to about 0.5 m) stream with gradual gradients flowing

Fig. 4. Aquarium photograph of *Melanotaenia parva*, males, approximately 50 mm SL, Lake Kurumoi, Papua Barat Province, Indonesia. Photo by N. Khardina.

Fig. 5. Aquarium photograph of *Melanotaenia irianjaya*, male, approximately 55 mm SL, Fruata, Papua Barat Province, Indonesia. Photo by N. Khardina.

Table III. Proportional measurements of selected type specimens of *Melanotaenia kokasensis* expressed as % of the standard length.

	Holotype MZB 16453	Paratype WAM P.33010	Paratype USNM 391629	Paratype WAM P.33010	Paratype WAM P.33010	Paratype USNM 391629	Paratype WAM P.33010
Sex	male	male	male	female	female	male	female
Standard length (mm)	56.5	68.5	64.0	59.4	52.9	51.8	47.9
Body depth	31.9	32.4	32.2	34.5	31.2	33.0	31.3
Body width	11.7	13.0	11.6	13.3	11.0	10.4	11.3
Head length	26.7	26.4	25.9	27.9	27.0	26.8	28.0
Snout length	9.4	9.6	9.1	9.4	9.5	8.9	9.4
Maxillary length	9.4	10.1	9.8	10.1	9.6	9.3	10.4
Eye diameter	8.1	7.7	8.3	8.4	8.5	8.1	9.0
Bony interorbital width	9.7	9.8	9.8	9.9	10.0	9.7	10.2
Depth of caudal peduncle	11.7	11.5	11.3	11.3	11.2	11.8	11.5
Length of caudal peduncle	17.9	16.6	17.8	16.0	17.4	17.8	17.1
Predorsal distance	49.4	48.8	49.5	49.3	49.1	48.8	50.7
Preanal distance	50.6	50.1	48.4	51.7	49.9	49.6	52.6
Prepelvic distance	37.5	38.4	37.0	38.7	37.2	38.0	37.2
2nd dorsal fin base	22.8	23.1	20.9	25.1	23.4	23.9	22.8
Anal fin base	38.1	36.8	38.1	38.6	38.0	36.9	37.8
Pectoral fin length	19.8	21.5	19.7	20.2	19.3	18.9	19.4
Pelvic fin length	17.5	20.7	18.0	19.0	16.1	17.6	18.0
Longest ray 1st dorsal fin	12.7	13.1	12.7	13.0	12.1	15.8	12.7
Longest ray 2nd dorsal fin	12.9	13.3	13.8	12.6	12.9	13.3	14.4
Longest anal ray	13.1	15.2	14.7	14.3	13.4	15.6	14.0
Caudal fin length	24.8	24.1	22.7	25.1	19.1	24.1	23.6
Caudal concavity	7.3	10.4	damaged	9.1	7.9	8.9	6.7

Fig. 6. Habitat of *Melanotaenia ammeri* at type locality, Gusimawa, Papua Barat Province, Indonesia. Photo by G. R. Allen.

through second growth forest, about one kilometre upstream from the sea. The type specimens were collected over sand and gravel bottoms with substantial leaf litter and dead tree branches.

Etymology: The new species is named *ammeri* to honour Max Ammer of Sorong, Papua Barat Province. His enthusiasm for nature exploration is infectious and he has provided invaluable logistic support, beginning in 1998, enabling the second author to travel and collect extensively around the Birds Head region of western New Guinea.

Melanotaenia kokasensis n. sp.

(Figs 7-8; Tables II, III)

Holotype. MZB 16453, male, 56.5 mm SL, small creek above waterfall (at edge of sea) near Kokas, 2°.185'S 132°25.697'E, northern Fakfak Peninsula, Papua Barat Province, Indonesia, 0-0.5 m, seine net, G. R. Allen and M. Ammer, 16 January 2008.

Paratypes (collected with holotype): MZB 16454, 10 specimens, 27.2-52.9 mm SL; USNM 391629, 7 specimens, 36.2-64.0 mm SL; WAM P. 33010-001, 7 specimens, 40.7-68.5 mm SL.

Diagnosis: A species of melanotaeniid rainbowfish distinguished by the following combination of characters: dorsal rays IV or V-I,11 to 14 (usually 13 or 14); anal rays I,21-24 (usually I,22 or 23); pectoral rays 13 to 15 (usually 14); lateral scales 35-37 (usually 36); predorsal scales 16 to 18 (usually 16 or 17); total gill rakers on first arch 16 to 19 (usually 17 or 18); colour of adult male in life generally pale blue grey with blackish midlateral stripe at level of upper pectoral-fin base (sometimes faint on anterior part of body); narrow orange stripe between each scale row on upper half of body; broad, horizontally bluish streak immediately above abdomen; pelvic and anal fins pale yellowish, remaining fins mainly colourless or slightly grey except narrow white margin on second dorsal fin and narrow blackish dorsal and ventral margins on caudal fin.

Description: Dorsal rays IV-I,13 (IV or V,11 to 14); anal rays I,23 (I,21 to 24); pectoral rays 14 (13

Fig. 7. Aquarium photograph of *Melanotaenia kokasensis*, male (upper) and female paratypes (WAM P. 33010-001), 68.5 mm and 59.4 mm SL, near Kokas, Papua Barat Province, Indonesia. Photo by G. R. Allen.

Table IV. Mean Kimura 2-parameter divergences between rainbowfish species for cytochrome *b*.

	ammeri	*kokas.*	*parva*	*misool.*	*fredericki*	*batanta*	*catherin.*	*synergos*	*affinis*-G
M. ammeri									
M. kokasensis	0.024								
M. parva	0.054	0.056							
M. misoolensis	0.109	0.108	0.105						
M. fredericki	0.105	0.106	0.100	0.018					
M. batanta	0.102	0.103	0.101	0.024	0.015				
M. catherinae	0.130	0.127	0.131	0.119	0.128	0.128			
M. synergos	0.126	0.121	0.121	0.109	0.118	0.123	0.027		
M. affinis Gogol	0.165	0.165	0.160	0.150	0.156	0.155	0.171	0.167	
M. affinis Sepik	0.162	0.160	0.158	0.150	0.158	0.156	0.166	0.163	0.010

to 15); pelvic rays I,5; branched caudal rays 15; lateral scales 37 (35 or 37); transverse scales 11 (10 or 11); predorsal scales 17 (16-18); cheek scales 15 (13-16); total gill rakers on first arch 18 (16-19).

Body depth 3.1 (3.0-3.5) in SL, head length 3.7 (3.6-3.9) in SL; greatest width of body 2.7 (2.5-3.2) in greatest body depth; snout length 2.8 (2.8-3.0) in HL; eye diameter 3.3 (3.1-3.4) in HL; interorbital width 2.7 (2.6-2.9) in HL; depth of caudal peduncle 2.3 (2.3-2.5) in HL; length of caudal peduncle 1.5 (1.5-1.7) in HL.

Jaws about equal, oblique, premaxilla with an abrupt bend between the anterior horizontal portion and lateral part; maxilla ends below anterior edge of eye; maxillary length 2.8 (2.6-2.9) in HL; lips thin; teeth conical with slightly curved tips, extending on to outer surface of lips; teeth of upper jaw in about four irregular rows anteriorly, reduced to a single row posteriorly, where they are exposed when mouth is closed; teeth in lower jaw in about 7-8 irregular rows anteriorly, reduced to 1 or 2 rows posteriorly; narrow row containing several small; several small conical teeth on vomer; palatines edentate.

Scales of body cycloid, relatively large, and arranged in regular horizontal rows; scale margins crenulate; predorsal scales extending forward to about middle of interorbital space; preopercle with 3-4 scale rows between its posterior angle and eye.

Predorsal length 2.0 (2.0-2.1) in SL; preanal length 2.0 (1.9-2.1) in SL; prepelvic length 2.7 (2.6-2.7) in SL; length of second-dorsal fin base 4.4 (4.0-4.8); length of anal-fin base 2.6 (2.6-2.7).

First dorsal fin origin about level with anal fin origin; longest spines (usually second to fourth) of first dorsal fin 2.1 (1.7-2.2) in HL, its depressed tip reaching first or second soft ray of second dorsal fin; longest rays (generally anterior ones in both females and males) of second dorsal fin 2.1 (1.9-2.2) in HL, the depressed posterior rays extending about one-half to two-thirds length of caudal peduncle; longest (middle rays in males and

Fig. 8. *Melanotaenia kokasensis*, preserved male, holotype, 56.5 mm SL, near Kokas, Papua Barat Province, Indonesia. Photo by G. R. Allen.

females) anal rays 2.0 (1.7-2.0) in HL; pelvic fin tips when depressed reaching to base of third or fourth soft anal fin ray; length of pelvic fins 1.5 (1.0-1.7); length of pectoral fins 1.3 (1.2-1.4) in HL; length of caudal fin 1.1 (1.1-1.4) in HL; caudal fin moderately forked, caudal concavity 3.7 (2.5-4.2) in head length.

Colour of male holotype in life (Fig. 7): overall pale blue grey with blackish midlateral stripe at level of upper pectoral-fin base, the stripe more or less solid on rear half of body, but incomplete anteriorly where it is composed of darkened posterior scale margins; narrow orange stripe between each scale row on upper half of body; most of scales on upper two-thirds of side with narrow greyish margins; upper portion of head greyish, lower half white; a poorly defined stripe, about equal to pupil width, from rear edge of eye to immediately above pectoral-fin base where it merges with midlateral body stripe; belly whitish with broad, oblong blue blotch extending from just below pectoral-fin base to above middle of anal fin; dorsal fins mainly greyish blue with narrow white margin on second dorsal fin; caudal fin whitish with slightly dusky grey basal half and faint blackish dorsal and ventral margins; anal fin dusky yellow; pelvic fins bright yellow; pectoral fins translucent with small black spot on upper base and smaller silvery-white spot just above. Colour pattern of females similar to that of holotype except generally less vivid and pelvic fins whitish.

Colour of holotype in alcohol (Fig. 8): upper half of body brown, white on lower half; dark midlateral stripe about 1-1.5 scales wide extending from below level of first dorsal fin origin to base of caudal fin; dorsal surface of head grey brown, grading to white or tan on side and ventral region; a dusky blackish stripe from rear edge of eye to upper rear margin of operculum; pectoral and pelvic fins translucent; median fins dusky pale

Fig. 9. Habitat of *Melanotaenia kokasensis* at type locality near Kokas, Papua Barat Province, Indonesia. Photo by G. R. Allen.

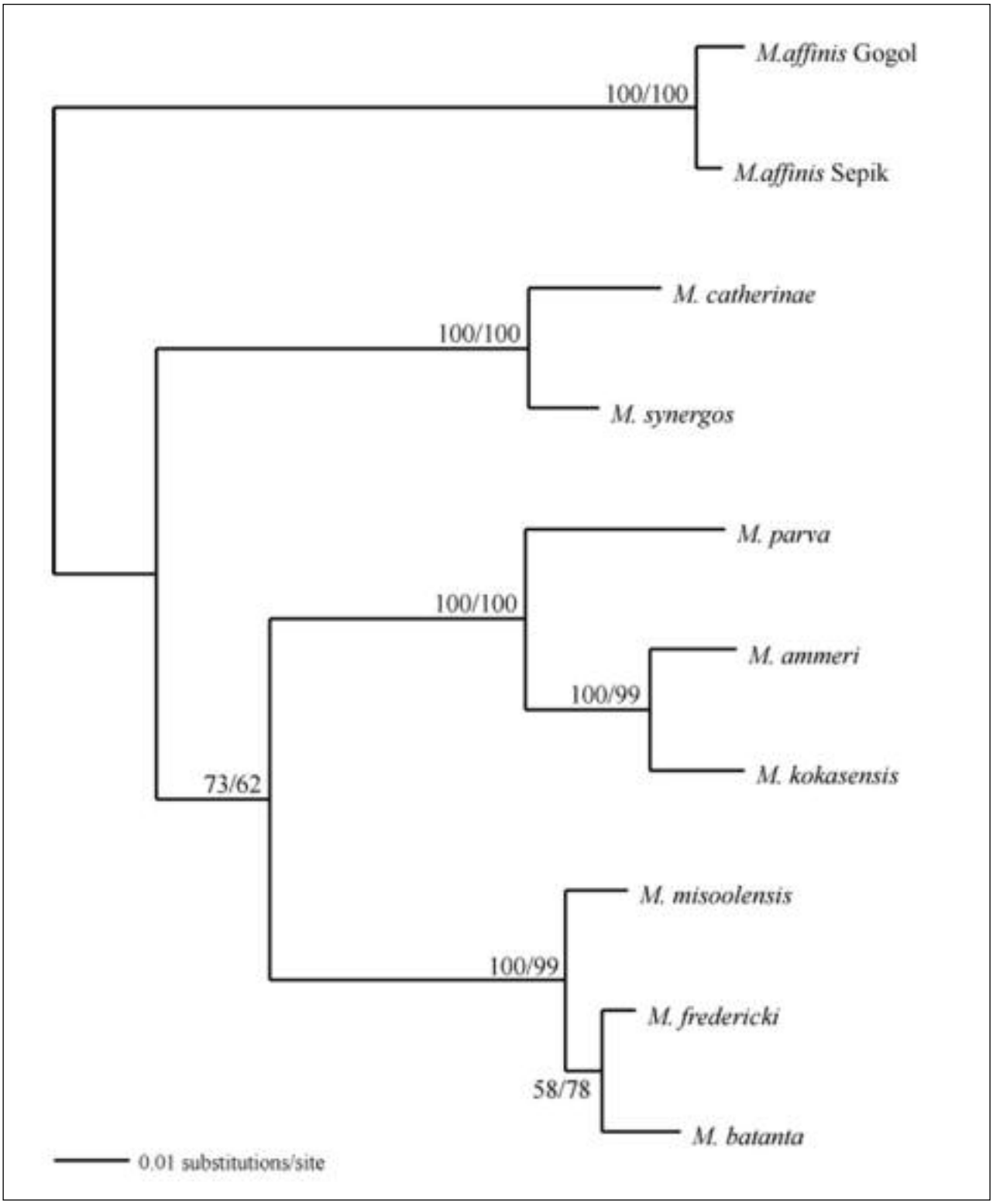

Fig. 10. Maximum likelihood tree for rainbowfish species based on analysis of cytochrome *b* sequences (1,140 bp) sampled from 10 individual fish. Branch lengths were estimated using maximum likelihood assuming the K81uf+G model of evolution. Bootstrap values were obtained from 1,000 replicates from Maximum Parsimony and Maximum Likelihood (MP/ML). The tree was rooted with *Melanotaenia affinis*.

grey; caudal fin with narrow blackish dorsal and ventral margins. Paratypes similar in colour to holotype with variable intensity and extent of dark midlateral stripe (extends forward to edge of upper operculum in some specimens); many specimens have horizontally elongate brown area just above abdomen.

Sexual dimorphism: Unlike most members of *Melanotaenia*, males and females are difficult to differentiate on the basis of body depth or other external features. The body depth (as % of SL) of 19 males, 36.2-68.5 mm SL, ranged from 29.0-33.0 with an average of 32.4; that of four females, 47.9-59.4 mm SL, was 31.2-34.5 with an average of 32.5.

Comparisons: Genetic and morphological data suggest that this species is most closely related *to M. ammeri* (see above comparisons for *M. ammeri).*

Zoogeography and habitat: The new species is currently known only from the type locality (Fig. 9), a small stream flowing along a limestone creek bed through primary forest. The stream plunges down a steep 20 m-high ramp next to the sea into a mangrove-lined inlet near the village of Kokas on the northern Fakfak Peninsula (Fig. 1). The fish was located about one kilometre upstream, in a circular pool with an approximate diameter of 15-20 m and maximum depth of about 0.5 m. This pool was situated only about 20 m downstream from a series of limestone fissures that appear to be the stream's underground origin.

Etymology: The new species is named *kokasensis* in reference to the nearby village of Kokas, which is the major landmark in the area.

DNA ANALYSIS OF *MELANOTAENIA* FROM THE BIRDS HEAD REGION

A total of 17 individuals representing nine species (Table IV) were sequenced for 1,140 bp of cyt*b*, including two samples of each species except for a single sample of *M. parva*. The final analysis consisted of a single individual per species except *M. affinis* as all individuals were identical except for one individual of *M. catherinae* which differed by a single base pair. Of 1,140 bp, 855 were constant, 43 variable characters were parsimony uninformative, and 242 characters were parsimony informative. A heuristic search via Maximum Likelihood (ML) recovered one tree with a -ln score of -3395.27721 (Fig. 10). Maximum Parsimony (MP) analysis with all characters weighted equally recovered two most parsimonious trees with a length of 400 (CI=0. 775, RI=0.806) (Fig. 10). The two MP trees differed slightly in that the positions of *M. batanta* and *M. misoolensis* flipped. In one tree, *M. batanta* is the sister species to *M. misoolensis* and *M. fredericki*, in the other *M. misoolensis* is the sister to *M. batanta* and *M. fredericki* (the latter tree was the same result as that recovered by ML) (Fig. 10). All nodes except two received very high bootstrap support for MP/ML (>99) (Fig. 10). Between species the mean Kimura 2-parameter genetic divergences varied between 1.5 and 17.1% (Table IV). The two new species, *M. ammeri* and *M. kokasensis* were most closely related to each other, differing by 2.4%. The next closest species was, *M. parva*, which differed by 5.4- 5.6% (Table IV). Note that we are not suggesting that *M. parva* is the true sister species to *M. ammeri* and *M. kokasensis* as we are lacking other species from our sequence dataset from this region that may be more closely related (e.g. *M. irianjaya).* These levels of genetic divergence between species are similar to those exhibited by many other rainbowfishes. For example, based on the first 601 bp of cyt*b* most pairwise species comparisons within the *"australis," "maccullochi,"* and *"nigrans"* species groups (clades A-C respectively in McGuigan et al., 2000) differ by only 2-5% (15 species total; Unmack, 2005).

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