Journal of the
Ocean Science Foundation

2015, Volume 14

Cercamia melanogaster, a new species of cardinalfish (Apogonidae) from West Papua, Indonesia

GERALD R. ALLEN
Department of Aquatic Zoology, Western Australian Museum,
Locked Bag 49, Welshpool DC, Perth, Western Australia 6986
E-mail: gerry.tropicalreef@gmail.com

MARK V. ERDMANN
Conservation International Indonesia Marine Program,
Jl. Dr. Muwardi No. 17, Renon, Denpasar 80235 Indonesia
California Academy of Sciences, Golden Gate Park, San Francisco, CA 94118, USA
Email: mverdmann@gmail.com

ANGKA MAHARDINI
Indonesian Biodiversity Research Center, Faculty of Veterinary Medicine,
Udayana University, Denpasar, Bali 80266 Indonesia

Abstract

A new species of apogonid, *Cercamia melanogaster*, is described from 11 specimens, 17.1–28.0 mm SL, collected in 15–42 m in the Bird's Head region of West Papua Province, Indonesia. Diagnostic features include dorsal rays VI-I,9; anal rays II,11–13; pectoral rays 9–11; gill rakers 1 + 10–12; scales cycloid, about 22–23 in lateral series and 9–10 in transverse series; scales present on preopercle and opercle; colour overall transparent pinkish with broad red zone on side of snout to rear margin of head, red on ventralmost part of head, and dark brown (nearly black) abdomen with diagonal pearly white band from upper rear margin of operculum to vent. Genetic evidence is provided to show a high level of divergence in the mtDNA COI marker between the three recognized species in *Cercamia*, including the new taxon, *C. cladara*, and *C. eremia*.

Key words: taxonomy, phylogenetics, coral reef fishes, Indo-Australian Archipelago, western Pacific Ocean.

Introduction

The apogonid genus *Cercamia* Randall & Smith 1988 contains small (< 40 mm SL), mostly transparent, larval-like fishes that inhabit dark crevices of Indo-Pacific coral reefs at depths of about 10–55 m. Previously two species were known, *Cercamia eremia* (Allen 1987), ranging widely from the Red Sea to Samoa, and *Cercamia cladara* Randall & Smith 1988 from the South Pacific, including Rapa, Austral Islands, Cook Islands, Tonga, and the Chesterfield Islands (Coral Sea). Diagnostic generic features include a unique vertebral count for the family of 9 + 15, two pairs of epipleural ribs, no predorsal bones, and hypurals 1–4 fused to form a double fan-shaped structure (Hayashi 1991). The genus was recently classified on the basis of genetic comparisons (Mabuchi *et al*. 2014) in the tribe Gymnapogonini of the subfamily Apogoninae, which also contains three other genera composed of small, transparent Indo-Pacific species: *Gymnapogon* Regan 1905, *Lachneratus* Fraser & Struhsaker 1991, and *Pseudamiops* Smith 1954. The present paper describes a third member of *Cercamia*, which we collected during ichthyological surveys of West Papuan coral reefs in September 2014 and February 2015.

Materials and Methods

Lengths given for specimens are standard length (SL), the straight-line distance from the front of the upper lip to the base of the caudal fin (posterior end of the hypural plate). Head length (HL) is measured from the same median anterior point to the end of the opercular membrane, and snout length from the same point to the fleshy edge of the eye. Body depth is the maximum depth, and body width the greatest width just posterior to the gill opening. Eye diameter is the greatest fleshy diameter of the orbit, and interorbital width the least bony width. Caudal-peduncle depth is the least depth; caudal-peduncle length is measured horizontally from the rear base of the last anal-fin ray to the caudal-fin base. Spines and rays are measured to their extreme base. Gill-raker counts were made on the first gill arch and include developed elements and not the very low, inconspicuous rudiments.

Proportional measurements for the new species are presented in Table 1 as percentages of the standard length. Data in parentheses in the description refer to the range for paratypes if different from that of the holotype. Type specimens are deposited at the Museum Zoologicum Bogoriense, Cibinong, Java, Indonesia (MZB) and the Western Australian Museum, Perth (WAM). We have also included a photograph of a specimen deposited at the Royal Ontario Museum, Toronto, Canada (ROM).

Tissue samples were obtained for the new taxon and the two other recognized species of *Cercamia*. Mitochondrial DNA was extracted using a 10% Chelex solution (Walsh *et al*. 1991). A portion of the cytochrome C oxidase subunit I (COI) region was amplified via PCR using primers jgLCO1490 and jgHCO2198 (Geller *et al*. 2013). The PCR reaction was carried out in 25 µL volumes, using 1 µL of template. Each reaction included 2.5 µL (10x PCR Buffer Red), 2.5 µL (8 mM dNTPs), 2 µL (25 mM $MgCl_2$ solution), 1.25 µL of each primer at 10 µM, 0.125 µL PE AmpliTaq RedTM (5 units/µL) (Applied Biosystems) and 14.5 µL ddH_2O.

The thermocycling profile for COI included an initial denaturation of 94°C for 15s, 38 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 45s, with a final extension of 72°C for 5 min. PCR reactions were checked on 1% agarose gels stained with ethidium bromide. PCR product was sequenced at the University of California Berkeley sequencing facility.

Forward and reverse sequences were proofread in MEGA5 (Tamura *et al*. 2011) then aligned using MUSCLE (Edgar 2004). Three methods were used to generate phylogenetic reconstructions: neighbor joining and maximum likelihood using MEGA5 and Bayesian inference using BEAST 1.7.5 (Drummond & Rambaut 2007). Neighbor joining was used to construct phylogenetic relationship between individuals based on genetic distance. Maximum likelihood analysis was used to assess the model of best fit for the nucleotide substitution. Bayesian Information Criterion (BIC) ranked the Hasegawa Kishino Yano (HKY) model with a discrete Gamma distribution (HKY+G) as having the best fit to the data. This model assumes different rates of transitions and transversions as well as different nucleotide frequencies, and was chosen as the appropriate model of evolution as determined by JModelTest 0.1.1 (Posada 2008). Bootstrap support was determined using 1000 replicates in MEGA5. For the Bayesian analysis we used a Markov Chain Monte Carlo approach with four chains. Analyses were run for 30,000,000 generations. After 30,000,000 generations, the resulting phylogeny was checked for convergence using Tracer v1.5 (Rambaut & Drummond 2009).

Figure 1. *Cercamia melanogaster*, holotype, 27.3 mm SL, underwater photograph, Fak Fak Peninsula, West Papua Province, Indonesia (M.V. Erdmann).

Cercamia melanogaster Allen, Erdmann & Mahardini, n. sp.

Blackbelly Cardinalfish

Figures 1–3, Table 1.

Holotype. MZB 22706, 27.3 mm SL, Sebakor, 03° 17.034' S, 132° 43.995' E, Fak Fak Peninsula, West Papua Province, Indonesia, 15 m, clove oil, M. Erdmann, 8 October 2014.

Paratypes. MZB 22707, 4 specimens, 18.6–26.0 mm SL, large bay near northern tip of Kawe Island, 00° 00.272' S, 130° 07.572' E, Raja Ampat Islands, West Papua Province, Indonesia, 42 m, rotenone, M. Erdmann, 23 February 2015; WAM P.34296-001, 2 specimens, 17.1–17.8 mm SL, collected with holotype; WAM P.34342-001, 4 specimens, 17.6–28.0 mm SL, same collecting data as MZB paratypes.

Diagnosis. Dorsal rays VI-I,9; anal rays II,11–13; pectoral rays 9–11; gill rakers 1 + 10–12; body depth 3.7–4.5 in SL; scales cycloid, deciduous (most scales missing), about 22–23 in lateral series and 9–10 in transverse series; scales present on preopercle and opercle; snout blunt, 4.0–4.4 in HL; caudal peduncle long and slender, its length 3.4–4.3 in SL, its depth 3.4–3.9 in HL; longest dorsal spine 7.4–8.0 in SL; longest dorsal soft ray 4.8–5.5 in SL; longest anal rays 5.2–6.4 in SL; colour overall transparent pinkish with broad red zone on side of snout to rear margin of head, red on ventralmost part of head, and dark brown (nearly black) abdomen with diagonal pearly white band from upper rear margin of operculum to vent.

Description. Dorsal rays VI-I,9; anal rays II,11 (5 paratypes with 11, 4 with 12, and one with 13); pectoral rays 9 on both sides of holotype (2 paratypes with 9 on both sides, 2 with 9 on one side and 10 on the other, 4 with 10 on both sides, one with 10 on one side and 11 on the other, and one with 11 on both sides); principal caudal rays 17; branched caudal rays 15; upper and lower procurrent caudal rays 7 (6–8); most scales missing but scale pockets and myomeres indicate lateral scale count of 22–23; gill rakers 1 + 12 (1 +10–11); pseudobranch filaments 6 (6–7); vertebrae 9 + 15.

Body depth 3.7 (3.7–4.5) in SL; body width 2.2 (2.2–2.7) in body depth; head length 2.6 (2.6–2.9) in SL; orbit 3.7 (3.0–3.9) in HL; snout bluntly pointed, profile of head nearly straight from snout to nape; interorbital region gently convex, its bony width 4.8 (3.7–4.9) in HL; caudal peduncle long and slender, 4.2 (3.4–4.3) in SL, nearly uniform in depth throughout its length, its depth 3.7 (3.4–3.9) in HL.

Mouth nearly terminal, lower jaw slightly projecting and oblique, forming angle of about 25° with the horizontal axis of head and body; maxilla reaching a vertical through rear margin of pupil; upper jaw length 1.9 (1.9–2.1) in HL; posterior edge of maxilla concave, its corners rounded; upper jaw teeth tiny, in narrow band with edentulous space at symphysis of jaw; similar tiny teeth on dentary in narrow band anteriorly, forming single row on side of jaw; vomer with pair of embedded teeth on each side; palatines toothless; tongue narrow and spoon-shaped.

Longest gill raker about 1.8 in eye of holotype; anterior and posterior nostrils round, anterior slightly larger with low fleshy rim; single opercular spine largely obscured by thin fleshy flap, extending to above pectoral-fin base; thin, relatively broad fleshy membrane at rear of operculum, connected to branchiostegal rays; small spine on posttemporal; pair of short spines at angle of preopercle.

Scales cycloid, strongly deciduous, holotype with only a few scales remaining on anteriormost body (paratypes also with few scales), 2–3 lateral rows just below dorsal fins, and above base of anal fin; three large scales covering most of preopercle, none on upper limb just behind eye, but probably missing; several large scales also remaining on upper half of opercle; presence or absence of lateral-line scales undetermined; scattered sensory papillae on interorbital region more or less arranged in 5–6 transverse rows.

Dorsal-fin origin slightly behind pectoral-fin base; first four interspaces between dorsal spines progressively longer, fifth notably greater than fourth; first dorsal spine 3.3 (2.8–3.9) in HL; second and third dorsal spines subequal; spine of second dorsal fin 2.9 (2.6–2.9) in HL; longest dorsal soft ray 2.0 (1.7–2.0) in HL; second

Figure 2. *Cercamia melanogaster*, underwater photograph, approximately 25 mm SL, Kawe Island, Raja Ampat Islands, West Papua Province, Indonesia (G.R. Allen).

Figure 3. *Cercamia melanogaster*, preserved holotype, 27.3 mm SL, Fak Fak Peninsula, West Papua Province, Indonesia (G.R. Allen).

dorsal fin with concave margin; all dorsal and anal soft rays branched; origin of anal fin below origin of second dorsal fin; first anal spine 2.8 (2.3–3.4) in length of second anal spine; second anal spine 4.3 (3.4–5.0) in HL; first and second anal soft rays longest, 2.2 (1.9–2.4) in HL; anal fin with concave margin, similar in shape to second dorsal fin; caudal fin deeply forked with bluntly pointed lobes; caudal concavity 2.6 (2.7–3.4) in HL; pectoral-fin base narrow, middle pectoral rays longest, 1.6 (1.5–1.7) in HL, reaching to about base of second or third soft anal ray; origin of pelvic fins anterior to pectoral-fin base; pelvic spine 1.6 (1.5–2.0) in length of pelvic fin; pelvic fin relatively short, not reaching anus, its length 2.4 (2.1–2.7) in HL; median pelvic ray joined to abdomen for most of its length.

Color of holotype in life. (Fig. 1). Overall transparent pinkish with broad red zone on side of snout to rear margin of head, red on ventralmost part of head, dark brown (nearly black) abdomen due to dark peritoneum showing through transparent body wall, and diagonal pearly white band (delineating the dorsal surface of the peritoneum) from upper rear margin of operculum to vent; subdermal concentration of melanophores on interorbital region exending posteriorly onto dorsal surface of first 7–8 vertebrae; iris yellowish green and narrow white ring around pupil. Live paratypes (Fig. 2) from Kawe Island, West Papua, differ in having only a few melanophores dorsally on the head and display an extensive network of subdermal reddish pigmentation, including scale margins, dorsal surface of the head (including snout), and red spotting on the dorsal and anal fins.

Color of holotype in alcohol. (Fig. 3). Overall plain whitish without pigment except black abdomen and cluster of expanded melanophores on interorbital. The diagonal white band on the upper abdomen mentioned in the previous section is evident on the right side of one of the paratypes.

Distribution and habitat. The new species is currently known only from two locations in the Bird's Head region of West Papua Province, Indonesia. The habitat of the type locality at Sebakor Bay, Fak Fak Peninsula consisted of a dark crevice on a near-vertical wall at a depth of about 15 m. Paratypes at Kawe Island in the Raja Ampat Islands were collected from a metre-high coral formation (in 42 m depth) as they began to emerge at dusk from crevices. Like most apogonids, this species appears to shelter in reef crevices and caves during the day and is active at night. The general habitat at both locations consisted of large, sheltered bays exposed to freshwater influx and significant siltation.

Etymology. The new species is named *melanogaster* (Greek: "black stomach") with reference to the most conspicuous colour pattern feature of this otherwise mainly transparent fish.

Comparisons. Genetic results indicate a sister-species relationship between the new species and *C. cladara*. However, the two species are clearly separable on the basis of developed gill-raker counts (1 + 10–12 versus 2–3 + 14–16 for *C. cladara*). The two species also exhibit similar colour features, particularly the red colouration on

TABLE 1

Proportional measurements of selected specimens of *Cercamia melanogaster* as percentages of the standard length

	holotype	paratypes					
	MZB 22706	WAM P. 34342	MZB 22707	WAM P. 34342	MZB 22707	WAM P. 34296	WAM P. 34296
Standard length	27.3	28.0	25.6	24.3	20.6	17.8	17.1
Body depth	26.9	26.7	25.4	26.9	23.6	22.1	22.7
Body width	12.0	11.6	11.3	11.8	10.1	8.2	9.5
Head length	37.9	38.3	37.3	35.6	36.7	34.5	34.4
Snout length	9.0	9.6	8.4	8.2	8.5	8.4	7.7
Orbit diameter	10.2	9.9	10.4	9.8	9.9	9.6	11.5
Interorbital width	7.8	7.9	8.3	7.2	8.8	9.0	9.3
Upper jaw length	20.0	18.1	18.7	17.9	17.8	18.4	17.4
Caudal peduncle depth	10.2	10.8	9.6	10.3	9.7	9.7	9.6
Caudal peduncle length	23.6	23.3	25.4	25.8	29.6	26.7	25.2
Predorsal length	39.8	39.3	40.4	38.6	40.3	33.4	37.9
Preanal length	55.8	55.1	55.9	58.3	53.9	49.8	51.8
Prepelvic length	36.3	33.8	36.5	33.1	33.8	31.3	34.1
First dorsal spine length	11.3	9.8	damaged	12.1	10.4	damaged	12.4
Second dorsal spine length	13.2	13.0	damaged	12.8	12.5	damaged	13.5
Third dorsal spine length	12.4	13.0	damaged	12.4	12.1	13.4	12.3
Second dorsal fin spine	9.9	9.0	damaged	8.9	9.3	11.5	12.8
Longest dorsal ray	18.7	19.2	19.0	20.7	19.9	19.9	18.0
First anal spine length	3.1	2.4	2.3	2.5	2.9	3.3	4.4
Second anal spine length	8.8	7.6	7.6	damaged	9.8	8.0	10.2
Longest anal ray	17.2	17.7	15.7	16.9	19.1	17.4	17.8
Pectoral fin length	23.5	23.0	21.5	21.0	23.3	23.1	20.8
Pelvic spine length	9.7	8.8	7.6	9.5	9.0	8.8	11.3
Pelvic fin length	15.8	15.1	14.9	15.3	13.7	16.1	16.7
Caudal fin length	27.1	25.9	24.5	25.0	29.8	29.0	29.4
Caudal concavity	14.6	11.9	11.1	11.0	12.3	12.8	11.7

Figure 4. *Cercamia cladara*, ROM 60988, freshly collected specimen, 30.0 mm SL, Moorea, Society Islands (R. Winterbottom).

the head and blackish abdomen. However, the red areas are more diffuse in *C. cladara* (Fig. 4) and the abdomen is not entirely blackish as in *C. melanogaster*. Gill raker counts are essentially the same for *C. melanogaster* and *C. eremia*, but the diagnostic red mask and extensive dark colouration on the abdominal region are lacking in the latter species (Fig. 5).

Phylogenetics. We resolved relationships between *Cercamia melanogaster* and other *Cercamia* species using a 657-base-pair segment of the mtDNA COI gene from our three collected *Cercamia* individuals. In addition, available GenBank sequences for *Cercamia cladara* and *Pseudamia zonata* (an apogonid outgroup) were added to the analysis (Tables 2 & 3 and http://www.ncbi.nlm.nih.gov).

Figure 5. *Cercamia eremia*, underwater photograph, approximately 35 mm SL, Anilao, Batangas Province, Philippines (G.R. Allen).

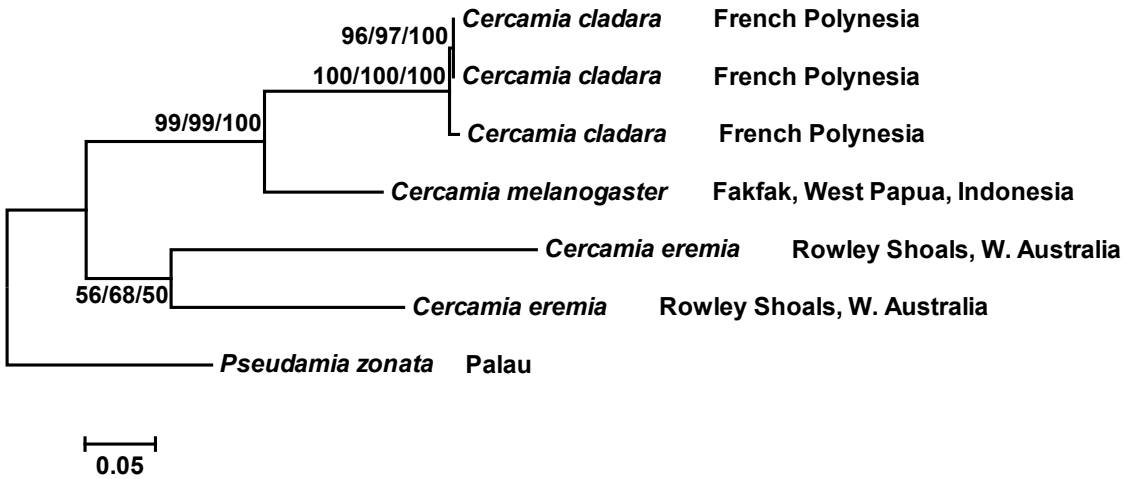

Figure 6. Maximum likelihood (ML) tree (HKY+G) of the mitochondrial COI data for three species of *Cercamia* (including *C. melanogaster*). Numbers above the major nodes indicate bootstrap support for 1000 replicates using neighbor joining, maximum likelihood and Bayesian posterior probability. GenBank accession numbers are listed in Table 3.

In the total *Cercamia* alignment there were 83 parsimony-informative characters. Nucleotide frequencies for the combined *Cercamia* samples were as follows: A = 23.5, C = 29, G = 19.1, T = 28.4. Pairwise distances between the three species of *Cercamia* ranged from 0.151–0.222, with the least distance between *C. melanogaster* and *C. cladara* (Table 2). Our results indicate that *Cercamia melanogaster* and *C. cladara* are sister species, forming a clade with 99% bootstrap support using neighbor joining and maximum likelihood analyses and 100% posterior probability using the Bayesian method (Fig. 6). The pairwise genetic distance between *C. melanogaster* and *C. cladara* is 0.151 (Table 2), and each of the phylogenetic trees produced clearly separate *C. melanogaster* from *C. cladara*. The phylogenetic analyses produced identical trees for most of the taxa, but could not resolve the relative positioning of the *C. melanogaster* and *C. cladara* clade with the *C. eremia* clade (with low bootstrap support <50%).

It is interesting to speculate the possible reason for the sister relationship between the two geographically distant species, *C. melanogaster* and *C. eremia*. Perhaps they are relicts of a former widespread ancestral species. It seems highly likely that *Cercamia* populations will eventually be found at intermediate areas in the South Pacific Ocean, which will provide additional clues on the relationships in this genus. Unfortunately, due to their inconspicuous appearance and cryptic habits, these fishes are seldom collected or observed.

TABLE 2
Interspecific pairwise genetic distance matrix for mtDNA COI sequences.

No.	Species	1	2	3
1	*Cercamia cladara* (French Polynesia)			
2	*Cercamia eremia* (Rowley Shoals, W. Australia)	0.222		
3	*Cercamia melanogaster* (Fakfak, West Papua)	0.151	0.218	
4	*Pseudamia zonata* (Palau)	0.204	0.206	0.210

TABLE 3
GenBank Accession numbers

Species	IBRC ID	GenBank Acc. no.
Cercamia cladara (Moorea, French Polynesia)		KJ967953–54
Cercamia eremia (Rowley Shoals, W. Australia)	MB134601	KP842561
Cercamia eremia (Rowley Shoals, W. Australia)	MB134701	KP842562
Cercamia melanogaster (Fakfak, West. Papua)	MB134801	KP842563
Pseudamia zonata (Palau)		AB890006

Other material examined. *Cercamia cladara*: WAM P.29655-001 (paratype), 26.6 mm SL, Rapa. *C. eremia*: WAM P.25815-020 (holotype), 37.5 mm SL, South Murion Island, Western Australia; WAM P. 26071-013 (paratype), 26.1 mm SL, Abrolhos Islands, Western Australia; WAM P.27581-001 (paratype), 39.6 mm SL, Abrolhos Islands, Western Australia; WAM P.28024-009 (paratype), 22.0 mm SL, Rowley Shoals, Western Australia; WAM P.27459-001 (paratype), 30.4 mm SL, Escape Reef, Great Barrier Reef, Queensland; WAM P.26085-015 (paratype), 25.5 mm SL, Christmas Island, Indian Ocean; WAM P.26092-023 (paratypes), 2 specimens, 20.0-21.0 mm SL, Christmas Island, Indian Ocean.

Acknowledgements

We are especially grateful to owner Craig Howson, Captain Chad Avenell, and the crew of *True North*, for their gracious hospitality during the 2014 cruise to West Papua and continuing support of our ichthyological explorations. We also thank Ken and Josephine Wiedenhoeft and the crew of the MV *Putiraja* for their support during the February 2015 cruise at the Raja Ampat Islands. Warm appreciation is also due to Ngurah Mahardika, Dita Cahyani and the staff of the Indonesian Biodiversity Research Centre at Udayana University, who provided excellent support for the genetic analysis. Finally, we also thank Renny Hadiaty (MZB) and Glenn Moore and Sue Morrison (WAM), who provided registration numbers and curatorial assistance. Financial support for the genetic analysis was provided by the United States Agency for International Development's "Supporting Universities to Partner across the Pacific" program (Cooperative Agreement No. 497-A-00-10-00008-00). The manuscript was reviewed by Tom Fraser and an anonymous reviewer.

References

Allen, G.R. (1987) New Australian fishes. Part 2. Four new species of Apogonidae. *Memoirs of the Museum of Victoria*, 48 (1), 3–8.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214. doi:10.1186/1471-2148-7-214

Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* 32, 1792–1797. doi:10.1093/nar/gkh340

Geller, J., Meyer, C., Parker, M. & Hawk, H. (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13, 851–61. doi:10.1111/1755-0998.12138

Hayashi, M. (1991) Redescription of *Cercamia eremia* (Perciformes: Apogonidae) from Japan with comments of the osteological characters. *Science Reports of the Yokosuka City Museum*, 39, 35–44.

Mabuchi, K., Fraser, T.H., Song, H., Azuma, Y. & Nishida, M. (2014) Revision of the systematics of the cardinalfishes (Percomorpha: Apogonidae) based on molecular analyses and comparative reevaluation of morphological characters. *Zootaxa*, 3846 (2), 151–203.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253–6. doi:10.1093/molbev/msn083

Rambaut, A. & Drummond, A.J. (2009) Tracer V1.5. Available from http://beast.bio.ed.ac.uk/Tracer.

Randall, J.E. (2005) *Reef and shore fishes of the South Pacific. New Caledonia to Tahiti and the Pitcairn Islands.* University of Hawai'i Press, Honolulu, 707 pp.

Randall, J.E. & Smith, C.L. (1988) Two new species and a new genus of cardinalfishes (Perciformes: Apogonidae) from Rapa, South Pacific Ocean. *American Museum Novitates*, 2926, 1–9.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–9. doi:10.1093/molbev/msr121

Walsh, P.S., Metzger, D.A. & Higuchi, R. (1991) Chelex-100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. *BioTechniques*, 10, 506–513.