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***Ecsenius springeri*, a new microendemic species of blenny (Teleostei: Blennidae) from the Fakfak Peninsula, West Papua, Indonesia**

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Abstract

The new species *Ecsenius springeri* is described from 6 specimens, 32.7–42.8 mm SL, collected at the Kokas area of the Fakfak Peninsula in the Bird's Head Seascape of western New Guinea, West Papua Province, Indonesia. The new species is a member of the Bicolor Group of *Ecsenius*, which also contains *E. bicolor* and *E. namiyei*. The group is distinguished from congeners in having cirri on both the anterior and posterior rims of the anterior naris (vs. on the posterior rim only) and in commonly having multiple vertical pairs of pores in the lateral line. *Ecsenius springeri* is most closely related to *E. bicolor* and superficially resembles the striped variety of that species. However, it consistently differs in having a narrower black mid-lateral stripe that ends more anteriorly; a more distinct white stripe along the mid-side of the body; a more extensive orange area on the posterior half of the body; and either lacks a pinkish-to-orange diagonal band behind the eye or, if present, it is very faint. The two species diverge by 8.5% in the mtDNA COI barcode marker sequence (mean K2P).

Key words: taxonomy, systematics, ichthyology, coral-reef fishes, endemism, phylogenetics, DNA barcoding, Bird's Head Seascape, conservation.

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Introduction

The genus *Ecsenius* McCulloch, 1923 comprises numerous small and colorful blennioid fishes that are common inhabitants of coral reefs throughout the Indo-West and central Pacific region, from East Africa to the Marquesas Islands. The widespread use of scuba-diving equipment by researchers and concomitant underwater photography have played a major part in the discovery of a plethora of new species. Consequently, the genus is now the largest in the family, containing 53 valid species (Fricke et al. 2019). The majority (38 species) were described by Smithsonian researcher Victor Springer and various coauthors, including 24 species that were described in two major revisions (Springer 1971, 1988). An additional 14 species were described in various publications between 1972 and 2004 (Springer 1972, McKinney & Springer 1976, Springer 1991, Springer & Randall 1999, Springer & Allen 2001, Springer 2002, Springer & Allen 2004). The East Indian region, extending from the Andaman Sea to the Solomon Island and northward to the Philippines, is home to more *Ecsenius* species than any other area, presently with 33 documented species, including this addition (Allen & Erdmann 2012).

We describe here a new species of *Ecsenius* that was collected during a Conservation International (CI) marine biodiversity survey of the Fakfak Peninsula of western New Guinea, West Papua Province, Indonesia, in March 2018. Six specimens were collected at Sariga, a small island about 10.7 km from the mainland town of Kokas. This location lies within the Bird's Head Seascape (Fig. 1), an area that has been intensely surveyed by CI beginning in 2001. Our observations and collections in this region indicate an extraordinary wealth of reef-associated fishes with 1,822 species recorded to date.

The Fakfak region includes some of the least-explored reefs of the Bird's Head Seascape and appears to be a center of microendemism as discussed by Allen et al. (2018). Its coral reefs are isolated from adjacent West Papuan mainland areas by Bintuni Bay in the north and Arguni and Etna Bays in the south. All of these

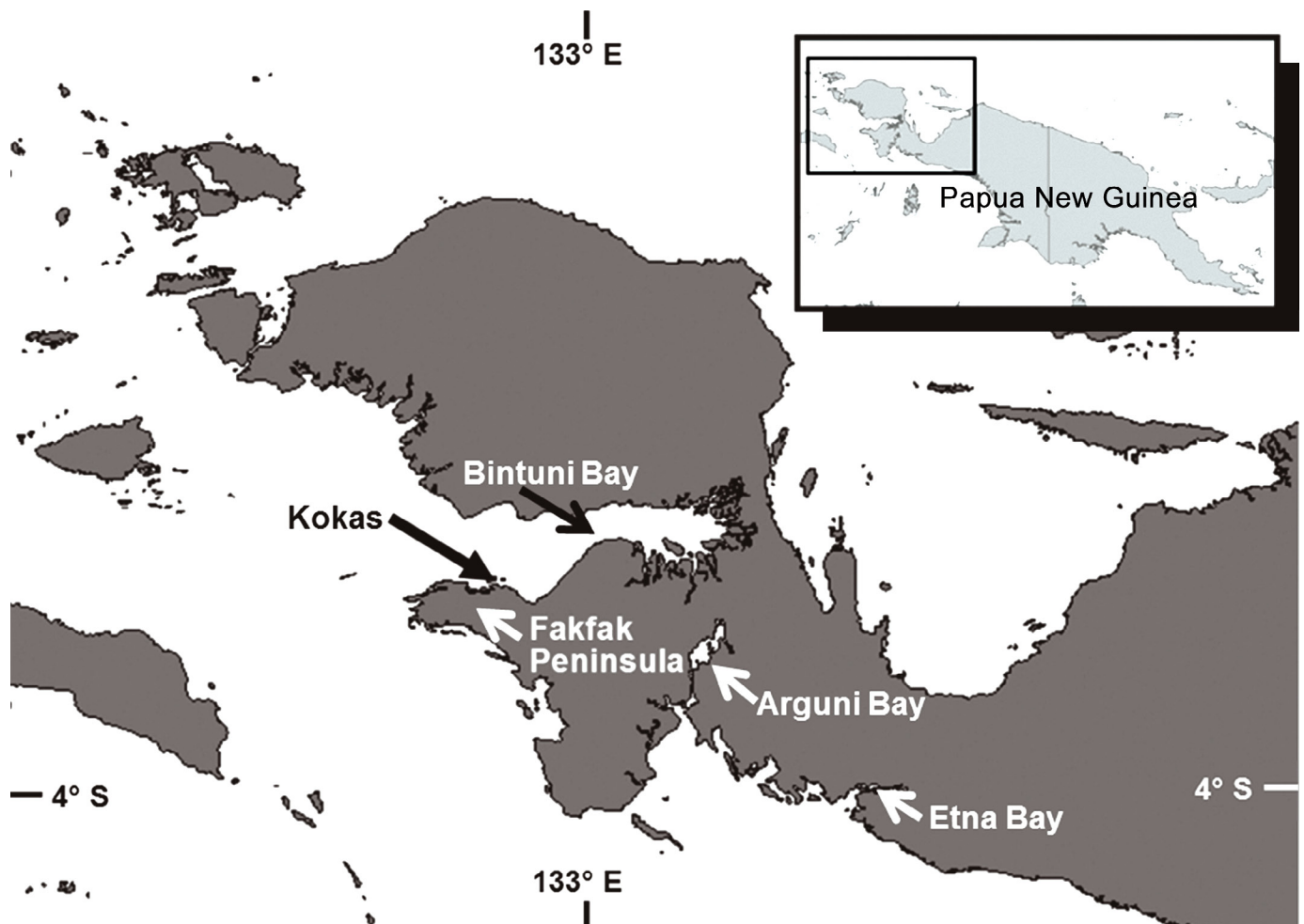


Figure 1. Map of the Bird's Head Seascape of western New Guinea showing the location of the Kokas area, northern Fakfak Peninsula, West Papua Province, Indonesia.

bays are characterized by considerable freshwater runoff and siltation and exceptionally large tidal fluctuations, and hence are generally lacking coral reefs. These conditions may serve as a barrier to planktonic dispersal of some reef organisms, perhaps explaining to some degree the surprising microendemism found in this particular locale. Other microendemic species of fishes described from there in recent years include *Manonichthys jamali* Allen & Erdmann, 2007 (Pseudochromidae); *Chrysiptera giti* Allen & Erdmann, 2008; *Chrysiptera uswanasi* Allen, Erdmann & Cahyani, 2018; *Pomacentrus bellipictus* Allen, Erdmann & Hidayat, 2018; and *Pomacentrus fakfakensis* Allen & Erdmann 2009 (Pomacentridae); *Paracheilinus nursalim* Allen & Erdmann, 2008 (Labridae); and *Eviota gunawanae* Greenfield, Tornabene & Erdmann, 2019 (Gobiidae).

Materials and Methods

Type specimens are deposited at the Museum Zoologicum Bogoriense, Cibinong, Java, Indonesia (MZB) and the Western Australian Museum, Perth, Australia (WAM).

The relatively brief description follows the methodology and format of Springer's previous works on this genus. As explained by Springer (1988), measurements are generally of little use for differentiating species and therefore are not included. They are extremely variable within a given species and are difficult to record accurately due to the vagaries of preservation and handling damage. Also, many measurements, unlike meristic features, change during ontogeny and may further vary due to geography and sex. Consequently, after studying hundreds of specimens, Springer concluded that all species of *Ecsenius* can be distinguished without measurement data, primarily on the basis of color-pattern differences and several key meristic features, including fin-ray and vertebral counts and the number of dentary incisor teeth. Dentary incisor teeth include the total number of teeth on both dentaries, except for the posterior canines. Digital x-rays were utilized for vertebral counts.

Counts are presented for the holotype, followed by a range in parentheses for the paratypes if different from the holotype.

DNA was isolated with the Genomic DNA extraction kit (Genomics BioScience and Technology, New Taipei City, Taiwan) from muscle tissue preserved in 95% ethanol according to the manufacturer's recommendations. A partial fragment of the mtDNA cytochrome-c-oxidase subunit I (COI) gene and the ribosomal S7 intron were amplified by a polymerase chain reaction (PCR) with FishF2 and FishR2 primers (Ward et al. 2005) and S7RPEX1F and S7RPEX2R primers (Chow & Hazama 1998), respectively. The PCR reaction took place in a Labnet gradient thermocycler over an initial denaturation step at 95 °C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 1 min, and a final extension step at 72°C for 5 min. Each reaction contained 30 ng DNA, 12.5 ul Taq DNA Polymerase 2X Master Mix RED (15 mM MgCl₂, 0.4 mM each dNTPs, 200 nM each of primers, 0.2 unit of Ampliqon Taq DNA polymerase (Ampliqon, Denmark) with a final volume of 25ul.

DNA sequences for the two markers sequenced for the two species in this study were accessioned into GenBank. The mtDNA COI sequences were compared to COI sequences for 8 other species from a variety of other projects in GenBank for an interspecific comparison (accession numbers and sources for all 10 species studied are listed in Appendix 1).

Phylogenetic analyses were used to reveal potential genetic divergences among specimens from different geographic locations, with maximum likelihood (ML) and Bayesian inference assessments being performed on the CIPRES Science Gateway (Miller et al. 2015) and MrBayes (MB) version 3.2.2 (Ronquist et al. 2012), respectively. The latter implemented two parallel runs of 4 simultaneous Markov chains for 10 million generations, sampling every 1000 generations and using default parameters. The first million generations (10%) were discarded as burn-in, based on the stationarity of log-likelihood tree scores. ML analyses were conducted in RAxML version 8.1.24 (Stamatakis 2014) using the HKY substitution model chosen by MEGA 7. Supporting values on the branch were evaluated by non-parametric bootstrapping with 1000 replicates performed with RAxML (ML) and by posterior probabilities (MB).

Ecsenius springeri, n. sp.

Springer's Coral Blenny

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Figures 2, 3 & 7A.

Holotype. MZB 24898, 40.6 mm SL, Indonesia, West Papua Province, Fakfak Peninsula, Kokas District, Sariga Islet, -2.6061°, 132.4124°, 3–6 m, clove oil & handnet, M.V. Erdmann & R. Mambrasar, 8 March 2018.

Paratype. MZB 24899, 2 specimens, 37.0–38.2 mm SL, same data as holotype; WAM P.34825-001, 3 specimens, 32.7–42.8 mm SL, same data as holotype.

Diagnosis. A species of *Ecsenius* in the Bicolor Group, with 28 total dorsal-fin elements; cirri on both anterior and posterior rims of anterior naris; multiple vertical pairs of lateral-line pores; live color pattern consisting of gray to brown dorsally on head and anterior one-third to one-half of body grading to orange at about level of junction of spinous and soft-dorsal fin, a black stripe about pupil width from behind eye to below junction of spinous and soft-dorsal fin, overlying a distinct white stripe along mid-side that is usually narrower than pupil diameter and extends farther posteriorly, to level of posterior dorsal-fin segmented rays; no pinkish-to-orange diagonal band behind eye, or if present, very faint.

Description. Dorsal-fin elements XII,16, notch between spinous and segmented ray portions with depth about half length of first dorsal-fin ray; dorsal-fin origin posterior to vertical from middle (posteriormost) pores of preopercular series; anal-fin elements II,18 (17 in two paratypes); pectoral-fin rays 13 (except 12 on one side of one paratype); segmented caudal fin-rays 14 (13 in two paratypes). Vertebrae 11+22=33 (11+23=34 in two



Figure 2. *Ecsenius springeri*, underwater photographs, approx. 35–42 mm SL (except lower right approx. 25 mm SL), Sariga Islet, Kokas area, West Papua Province, Indonesia (G.R. Allen & M.V. Erdmann).



Figure 3. *Ecsenius springeri*, preserved holotype, MZB 24898, 40.6 mm SL, Sariga Islet, Kokas area, West Papua Province, Indonesia (G.R. Allen).

paratypes). Dentary incisor teeth (including anterior canines) 39 (35–38, mean for all types=37). Lateral line with 4 (4–13) vertical pairs of pores, extending along one-fourth or more of its length; lateral-line extending to below tenth (ninth to twelfth) dorsal-fin spine. Cirri present on both anterior and posterior rims of anterior naris.

Color in life. (Fig. 2) Charcoal gray to dark brown dorsally on head and anterior one-third to one-half of body; ventral head and lower anterior side of body much paler gray (sometimes slightly pinkish on lower head), grading to orange posteriorly with transition to orange at about level of junction of spinous and soft-dorsal fin; a black midlateral stripe, about pupil width or slightly narrower, from upper preopercle to middle of side below junction of spinous and soft-dorsal fin, overlying a distinct white stripe usually narrower than the pupil diameter, tapering posteriorly, and extending farther posteriorly than black stripe, about to level of posterior dorsal-fin segmented rays. Iris dark brown with narrow silvery ring around pupil; narrow pale gray area on rear margin of eye, followed by indistinct blackish spot or short bar; nasal cirri brown. Dorsal fin mainly translucent; anal fin narrowly orange basally and pink on remainder of fin; caudal fin orange basally grading to translucent on remainder of fin; pelvic fins pale grayish to translucent; pectoral fins translucent

Color in alcohol. (Fig. 3) Dark brown dorsally on head and anterior one-third to one-half of body; ventral head and lower anterior side of body lighter brown (sometimes head with tan blotches on cheek), grading to tan posteriorly with transition of color at about level of junction of spinous and soft-dorsal fin; whitish midlateral stripe extending from upper rear corner of opercle to below middle or rear portion of soft dorsal fin; fins mainly translucent except basal portion of dorsal fin and distal portion of anal fin dusky grayish to brownish.

Etymology. The new species is named in honor of Dr. Victor G. Springer, Senior Scientist Emeritus and past curator in the Division of Fishes at the Smithsonian's National Museum of Natural History (USNM), Washington, DC, USA, in recognition of his extensive and excellent research on this group.

Distribution and habitat. The new species is currently known only from a small area in the Kokas District on the northern coast of the Fakfak Peninsula of West Papua Province, Indonesia (Fig. 1). It was relatively common at the type locality, at a depth range of about 2–8 m, in coral-reef habitat, perching on a variety of substrates including live corals, tunicates, sponges, and algae. The type locality (Fig. 4) is at a small, roughly circular islet with a diameter of about 850 m, lying approximately 8 km from the mainland. The surrounding waters have characteristics more typical of estuarine habitats, i.e. with lowered salinity, high turbidity, and sedimentation, and frequently elevated sea-surface temperatures up to about 31°C. Allen et al. (2018) suggested this unusual combination of conditions, and the general geographic isolation of the coral reef areas of the Fakfak Peninsula may account for its suite of microendemic species (Allen et al. 2018).

Comparisons. *Ecsenius springeri* is a member of the Bicolor Group of *Ecsenius* as defined by Springer (1988). This group, which also contains *E. bicolor* and *E. namiyei* (Jordan & Evermann, 1903) is distinguished from other *Ecsenius* by having cirri on both the anterior and posterior rims of the anterior naris (vs. on the posterior rim only) and in commonly having more than one (up to 13) vertical pairs of pores in the lateral line. *Ecsenius namiyei* (Fig. 5) is separable from the other two species in lacking a notch in the dorsal-fin margin between the spinous and segmented portion of the fin, a slightly more anterior dorsal fin origin (at or anterior to a vertical from the middle preopercular pores vs. posterior to the middle pores), and 30–33 (vs. 37–30) total dorsal-fin elements.



Figure 4. Sariga Islet (the type locality) is the most distant island in this photograph of the coastal islands off Kokas, Fakfak Peninsula, West Papua Province, Indonesia (M.V. Erdmann).



Figure 5. *Ecsenius namiyei*, underwater photographs, approx. 45–60 mm, Indonesia: A) Raja Ampat Islands, West Papua Province; B) Lembah Strait, North Sulawesi Province (G.R. Allen).

The new species is apparently most closely related to *E. bicolor*, which ranges widely in the Indo-West Pacific from the Maldives to the Phoenix Islands and Samoa, and from Australia northward to the Ryukyu Islands of Japan, but has a different color pattern and mtDNA sequence (see below). As noted by Springer (1988), *E. bicolor* is one of the more variably pigmented species in the genus, exhibiting three basic patterns (Fig. 6): 1) a uniformly dark head and body; 2) a bicolored pattern with a dark head and anterior half (rarely more) of the body and a pale posterior body; and 3) a striped pattern with a dark head and dorsal portion of the body and a paler ventral body divided by a dark stripe on the side of body, with a pale caudal fin.

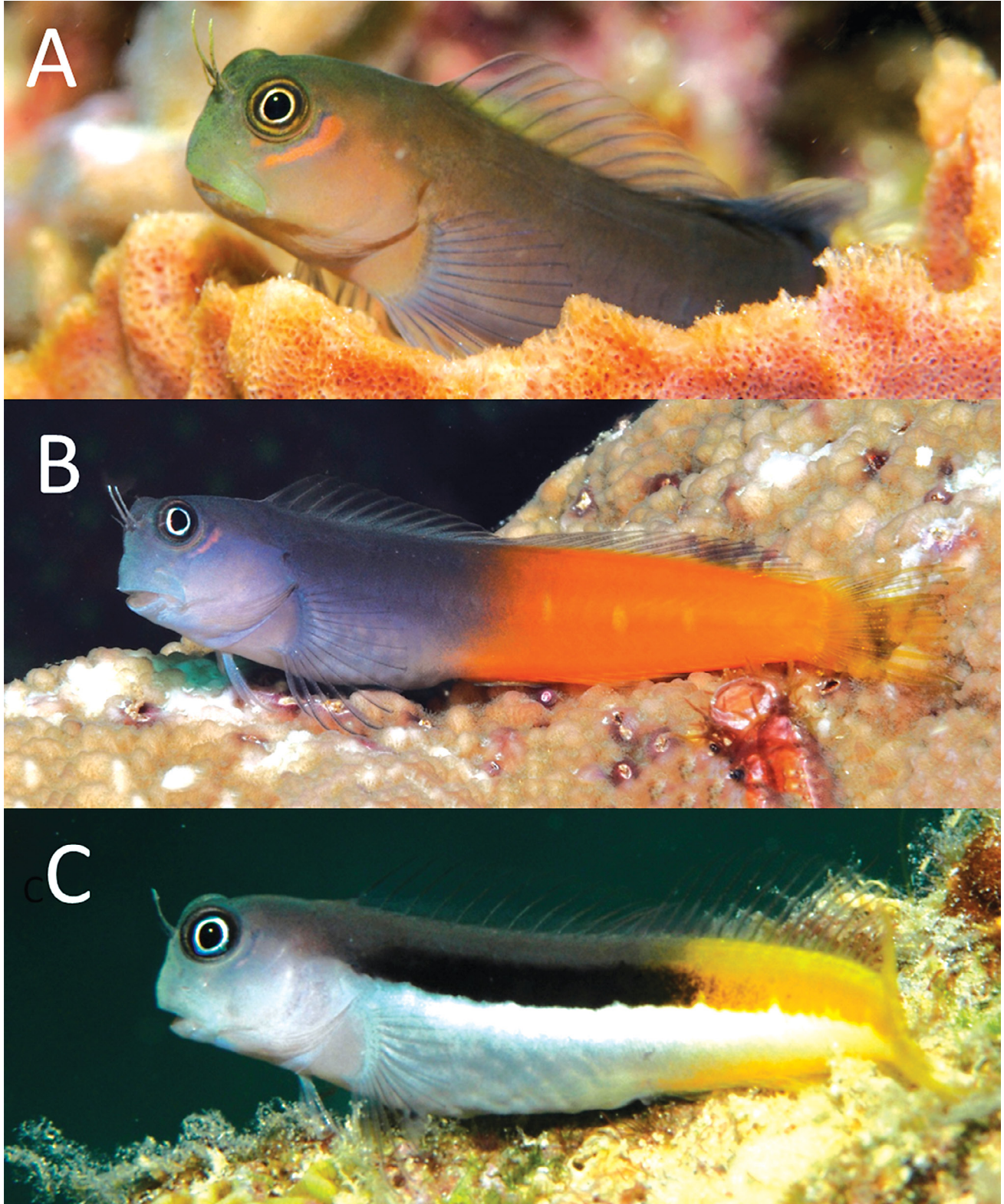


Figure 6. *Ecsenius bicolor*, underwater photographs approx. 45–55 mm: A) uniformly dark variety, Raja Ampat Islands, West Papua, Indonesia; B) bicolor variety, Brunei; and C) striped variety, Christmas Island, Indian Ocean (G.R. Allen).

Although the color pattern of *E. springeri* is similar to the striped variety of *E. bicolor*, it consistently differs in the following 4 features (Fig. 7): 1) the black mid-lateral stripe is narrower (about pupil width) and generally ends below the junction of the spinous and soft-dorsal fin (vs. wider than the pupil and extending beyond the level of the junction); 2) a distinct white stripe on the mid-side that is usually narrower than the pupil diameter (vs. a more diffuse white stripe with a poorly contrasting lower margin); 3) a more extensive orange area on the posterior half of the body that extends ventrally to the anal-fin base (vs. confined to the upper half with white ventrally); and 4) no pinkish-to-orange diagonal band behind the eye, or if present, very faint (vs. usually present and well defined).

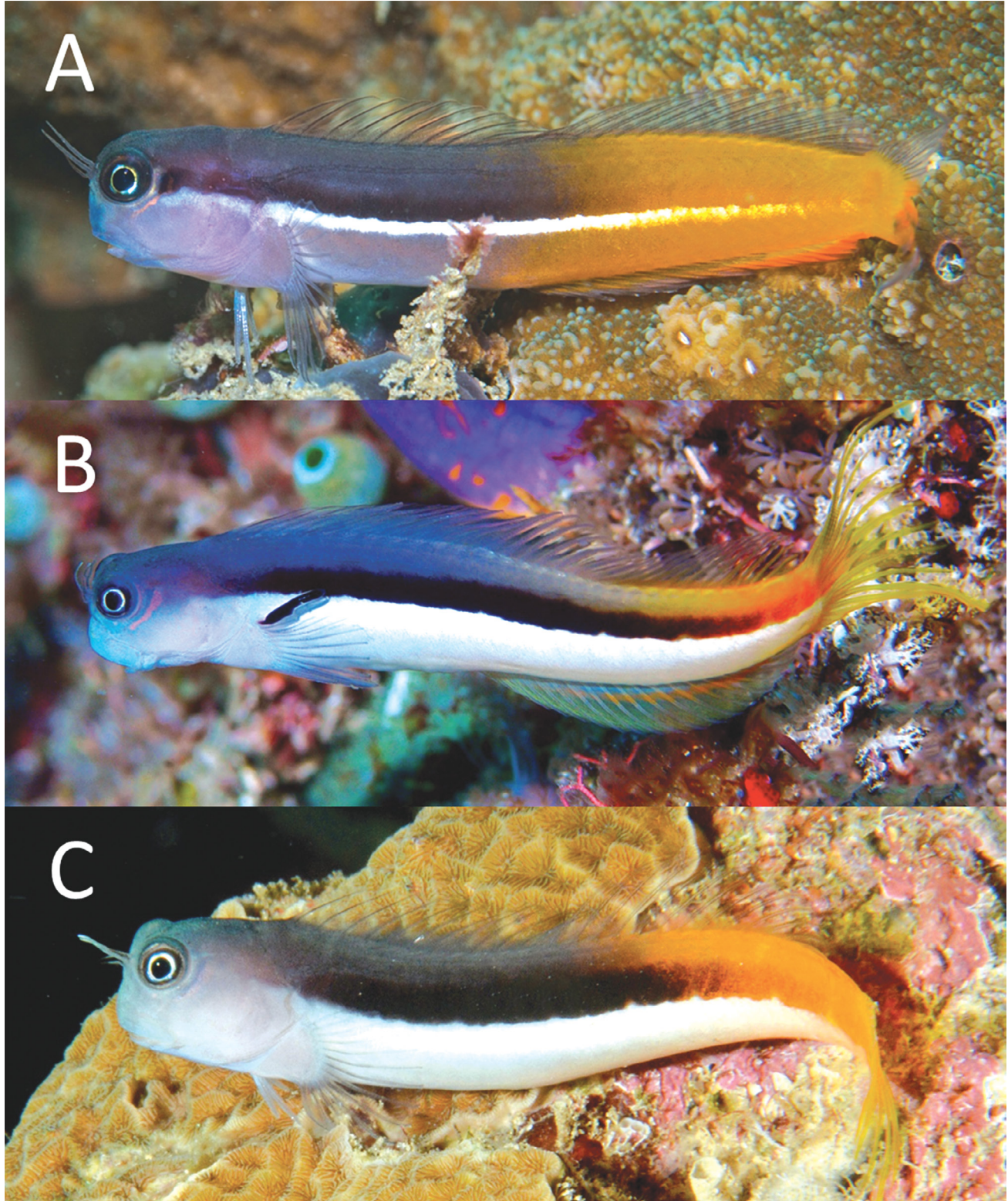


Figure 7. *Ecsenius springeri* vs. striped *E. bicolor*, underwater photographs: A) *E. springeri*, West Papua Province, Indonesia; B) *E. bicolor*, Raja Ampat Islands, Indonesia; and C) *E. bicolor*, Milne Bay Province, Papua New Guinea (G.R. Allen).

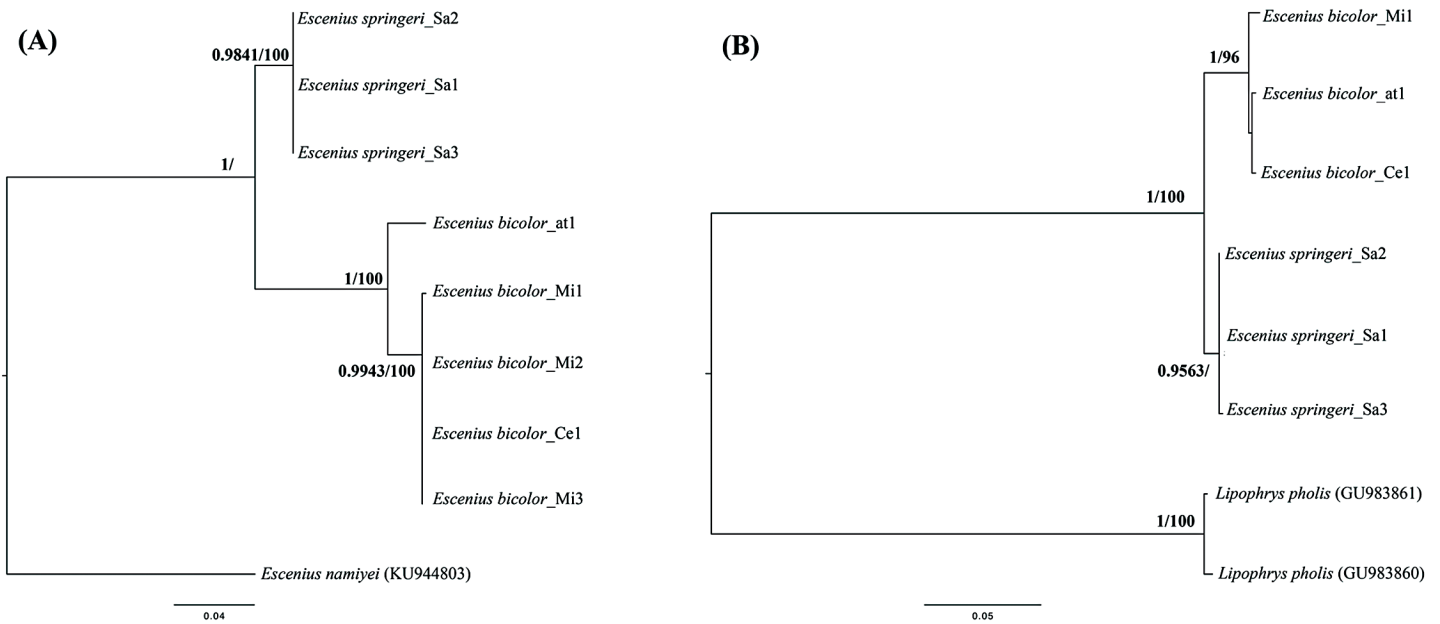


Figure 8. Maximum Likelihood tree based on (A) COI and (B) S7 intron data sets. Nodes are presented only for those with bootstrap scores >95% majority rule for maximum likelihood and >95% majority probabilities for Bayesian probability values (BI/ML).

Phylogenetics. A 551-bp fragment of the mtDNA COI gene and a 666-bp fragment of S7 intron were amplified from 8 samples (3 specimens of *E. springeri* collected from the type locality at Sariga Islet, 3 specimens of *E. bicolor* from Raja Ampat [2 failed for S7], one from Cenderawasih Bay and one from the aquarium trade) (list in Appendix 1). A COI sequence derived from *E. namiyei* and two S7 sequences from *Lipophrys pholis* served as outgroups. Among the COI sequences, 107 variable sites were observed, including 44 parsimony-informative sites. Among the S7 sequences, 181 variable sites were observed, including 171 parsimony-informative sites. The ML trees and BI trees based on both loci showed a similar tree topology with a clear grouping of *E. springeri* and *E. bicolor* with high supporting values at the nodes (Fig. 8). Interspecific divergences in mtDNA COI sequences (K2P) among 10 species of *Ecsenius*, including the new species, ranged from 0.075 to 0.266 and the pairwise distance between *E. springeri* and *E. bicolor* (also from Indonesia) is 0.085 (Table 1). The results of the phylogenetic analyses show that *E. springeri* and *E. bicolor* comprise two distinct and genetically divergent clades.

TABLE 1

Interspecific pairwise K2P mean genetic distance matrix for mtDNA COI sequences for 10 *Ecsenius* species

No.	Species	1	2	3	4	5	6	7	8	9
1	<i>E. bicolor</i>									
2	<i>E. springeri</i>	0.085								
3	<i>E. gravieri</i>	0.213	0.212							
4	<i>E. frontalis</i>	0.225	0.230	0.081						
5	<i>E. dentex</i>	0.184	0.200	0.176	0.162					
6	<i>E. aroni</i>	0.208	0.224	0.110	0.089	0.161				
7	<i>E. lineatus</i>	0.201	0.228	0.157	0.150	0.183	0.144			
8	<i>E. yaeyamaensis</i>	0.204	0.231	0.198	0.187	0.075	0.158	0.191		
9	<i>E. midas</i>	0.189	0.177	0.250	0.242	0.214	0.242	0.266	0.224	
10	<i>E. mandibularis</i>	0.240	0.228	0.218	0.213	0.205	0.196	0.249	0.196	0.234

Comparative material examined: *Ecsenius bicolor* (90 specimens, 17–62 mm SL, all WAM): P.24767-001, 10 specimens, 33–58 mm SL, Lizard Island, Queensland, Australia; P.25111-008, 13 specimens, 45–62 mm SL, Dampier Archipelago, Western Australia; P.26110-011, 19 specimens, 19–48 mm SL, Christmas Island, Indian Ocean; P.28025-032, 16 specimens, 29–55 mm SL, Rowley Shoals, Western Australia; P.29912-016, 10 specimens, 17–38 mm SL, Cocos-Keeling Islands, Indian Ocean; P.30624-014, 52 mm SL, Madang, Papua New Guinea; P.30835-025, 5 specimens, 23–49 mm SL, Scott Reef, Western Australia; P.30962-010, 28 mm SL, Komodo Islands, Indonesia; P.33074-005, 6 specimens, 36–54 mm SL, Mergui Archipelago, Myanmar; P.33260-006, 2 specimens, 43–57 mm SL, Andaman Islands; P.33827-010, 43 mm SL, Raja Ampat Islands, West Papua, Indonesia; P.34647-004, 6 specimens, 28–54 mm SL, Aru Islands, Indonesia.

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APPENDIX 1

Species	GenBank (COI)	GenBank (S7)	Source
<i>Ecsenius aroni</i>	MF123861		Israel: Eilat, IUI reef (N. Kimmerling et al.)
<i>Ecsenius bicolor</i>			
Ce1	MK458595	MK458608	Suandi Island, Cenderawasih Bay, Indonesia
At1	MK458598	MK458603	Aquarium trade
Mi1	MK458599	MK458604	Pulau Lili, SE Misool, Raja Ampat, Indonesia
Mi2	MK458596		Pulau Lili, SE Misool, Raja Ampat, Indonesia
Mi3	MK458597		Pulau Lili, SE Misool, Raja Ampat, Indonesia
<i>Ecsenius dentex</i>	MF123862		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123863		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123864		Israel: Eilat, IUI reef (N. Kimmerling et al.)
<i>Ecsenius frontalis</i>	MF123865		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123866		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123867		Israel: Eilat, IUI reef (N. Kimmerling et al.)
<i>Ecsenius gravieri</i>	MF123868		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123869		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123870		Israel: Eilat, IUI reef (N. Kimmerling et al.)
	MF123871		Israel: Eilat, IUI reef (N. Kimmerling et al.)
<i>Ecsenius lineatus</i>	KU986284		Maldives (B.C. Victor)
	KU986295		Maldives (B.C. Victor)
	KU986301		Maldives (B.C. Victor)
<i>Ecsenius mandibularis</i>	KP194592		Australia: Queensland, Lizard Island (Steinke et al.)
<i>Ecsenius midas</i>	JF493406		Mozambique: Pomene (Connell et al.)
	KF489571		Mozambique: Pomene (Connell et al.)
	KF489572		South Africa: DAR 1 (Connell et al.)
<i>Ecsenius namiyei</i>	KU944803		Taiwan (Shao et al.)
<i>Ecsenius springeri</i>			
Sa1	MK458600	MK458605	Sariga, Kokas, Fakfak, West Papua, Indonesia
Sa2	MK458601	MK458606	Sariga, Kokas, Fakfak, West Papua, Indonesia
Sa3	MK458602	MK458607	Sariga, Kokas, Fakfak, West Papua, Indonesia
<i>Ecsenius yaeyamaensis</i>	KP194220		Australia: Queensland, Lizard Island (Steinke et al.)
	KU986290		Palau: Koror, Malakal, Ngel Channel (B.C. Victor)
<i>Lipophrys pholis</i>		GU983860	Spain: Cadiz (Almada et al.)
		GU983861	Portugal: Estoril (Almada et al.)