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Pseudojuloides edwardi, n. sp. (Perciformes: Labridae): an example of evolution of male-display phenotype outpacing divergence in mitochondrial genotype

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Abstract

The new species *Pseudojuloides edwardi* is described from aquarium-trade specimens obtained from the African coast near Mombasa, Kenya. The species is distinguished from its two sibling species, *P. severnsi* (from the Philippines, Indonesia, Japan, New Caledonia, and Sri Lanka) and *P. erythrops* (from Mauritius), by a spectacular yellow-on-magenta reticulum on the head and forebody of the terminal-phase male and other details of the markings and color patterns. Despite the arresting color differences, the barcode COI mtDNA sequences for specimens of *P. edwardi* are very close to the *P. severnsi* clade, differing by 3 base pairs out of 652, well within the intraspecific range of variation. The two species likely represent a case of evolution of reproductive isolating mechanisms outpacing the accumulation of neutral mutations in mitochondrial DNA sequences. As in a number of other examples of shared mitochondrial sequences between recently diverged reef fish species, the phenotypic differences are primarily in color patterns on the head, the focus of mating displays for species recognition in many coral reef fishes.

Key words: new species, taxonomy, systematics, phylogeography, wrasses, mtDNA, barcoding, coral reef fishes.

Introduction

The labrid genus *Pseudojuloides* Fowler was revised by Randall and Randall (1981), who recognized eight species in the genus, including five new species. In the subsequent three decades, only two additional species have been described: *Pseudojuloides kaleidos* by Kuiter and Randall (1995) from the Maldives and Indonesia, and *Pseudojuloides severnsi* by Bellwood and Randall (2000), from the Maldives to the W. Pacific. The genus comprises a set of small fast-swimming wrasses, typically found on deeper slopes and in habitats dominated by rubble rather than live coral. They are distinguished morphologically by having chisel-like incisiform side teeth (unusual among the labrids) and torpedo-shaped fusiform bodies with relatively large scales. Their mating system, like many other wrasses, is haremic, with most individuals being drab reddish females that are difficult to distinguish among the congeners (initial phase or IP), dominated by larger males who usually display conspicuous patterns and colors (terminal phase or TP, following the terminology of Warner and Robertson (1978)). Terminal-phase males of all *Pseudojuloides* described to date are very colorful, with the exception of the

all-green *Pseudojuloides argyreogaster* (Günther), which is found in seagrass beds where being green to match the background is evidently more important than being prominently colored. The intensive collection effort by the aquarium fish trade for the most beautiful coral reef fishes has recently resulted in the discovery of a remarkable new species of *Pseudojuloides* from the East African coast that we are privileged to describe here.

The description of the new species includes sequencing of the COI mtDNA gene used in the Fish Barcode of Life project (Ward *et al.* 2009). Most recent studies have found that the majority of marine fish species are characterized by monophyletic mitochondrial lineages well separated from related species, in most cases by more than 2% (Steinke *et al.* 2009, Ward *et al.* 2009). There are, however, exceptions that challenge our definition of species and reveal complex patterns of speciation among coral-reef fishes. In the case of this new species, it appears that the rate of evolutionary change in reproductive displays between congeners may well have outpaced the accumulation of mutations in the mitochondrial genome, providing an interesting case study of incipient speciation among coral reef fishes.

Materials and Methods

Specimens have been examined from the Bernice P. Bishop Museum, Honolulu (BPBM). In addition, ethanol-preserved specimens of comparison species were collected for DNA sequencing from French Polynesia, Cook Islands, New Caledonia, and Hawai'i in the Pacific Ocean and obtained via the aquarium trade from Kenya and Mauritius in the Indian Ocean and the Philippines and Indonesia in the Pacific Ocean (Appendix 1).

DNA extractions were performed with the NucleoSpin96 (Machery-Nagel) kit according to manufacturer specifications under automation with a Biomek NX liquid-handling station (Beckman-Coulter) equipped with a filtration manifold. A 652-bp segment was amplified from the 5′ region of the mitochondrial COI gene using a variety of primers (Ivanova *et al.* 2007). PCR amplifications were performed in 12.5 µl volume including 6.25 µl of 10% trehalose, 2 µl of ultra pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM (NH4)2SO4, 20mM Tris-HCl (pH8.8), 2mM MgSO4, 0.1% Triton X-100), 0.625 µl of MgCl2 (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM), 0.0625 µl of *Taq* DNA polymerase (New England Biolabs), and 2 µl of template DNA. The PCR conditions consisted of 94°C for 2 min., 35 cycles of 94°C for 30 sec., 52°C for 40 sec., and 72°C for 1 min., with a final extension at 72°C for 10 min. Specimen information and barcode sequence data from this study were compiled using the Barcode of Life Data Systems (Ratnasingham & Hebert 2007). The sequence data is publicly accessible on BOLD and GenBank. Sequence divergence was calculated using BOLD with the Kimura 2-parameter (K2P) model generating a mid-point rooted neighbor-joining (NJ) phenogram to provide a graphic representation of the species divergence (pairwise distances are also calculated for comparison).

The length of specimens is given as standard length (SL), measured from the median anterior end of the upper lip to the base of the caudal fin (posterior end of the hypural plate); body depth is the greatest depth from the base of the dorsal spines to the ventral edge of the abdomen (correcting for any malformation of preservation); body width is measured just posterior to the gill opening; head length from the front of the upper lip or anterior upper teeth (whichever is most anterior) to the posterior end of the opercular flap; orbit diameter is the greatest fleshy diameter of the orbital rim, and interorbital width the least bony width; snout length is measured from the median anterior point of the upper lip to the nearest fleshy rim of the orbit; caudal-peduncle depth is the least depth, and caudal-peduncle length the horizontal distance between verticals at the rear base of the anal fin and the caudal-fin base; predorsal, prepelvic and preanal lengths are angular measurements; lengths of spines and rays are measured to their extreme bases; caudal-fin and pectoral-fin lengths are the length of the longest ray; pelvic-fin length is measured from the base of the pelvic spine to the tip of the longest soft ray. Morphometric data are presented as percentages of the standard length. Proportional measurements in the text are rounded to the nearest 0.05.

The upper rudimentary pectoral ray is included in the count. Lateral-line scale counts include the last pored scale that overlaps the end of the hypural plate; scales above the lateral line are counted in an oblique row from the first pored scale to the origin of the dorsal fins, and scales below the lateral line in an oblique row from the anal fin origin rearward, not including very small scales that may be present at base of fins; median predorsal scale counts are only approximate counts because these scales are not in a regular series. The count of gill rakers is made on the first gill arch and includes all rudiments. The range of counts and measurements for the paratypes are shown in parentheses following data for the holotype.

Figure 1. *Pseudojuloides edwardi,* BPBM 41172, holotype, 73 mm SL, Mombasa region, Kenya (B.C. Victor).

Pseudojuloides edwardi, n. sp.

Figures 1–4, 6; Table 1.

Holotype. BPBM 41172, 73 mm SL, male, Mombasa region, Kenya, aquarium-trade collectors, July 1, 2013.

Paratypes. BPBM 41173, 2: 70.6 mm SL male, same location data, Dec. 2, 2013 & 63.4 mm SL female, same location data, Jan. 11, 2014.

Diagnosis. Dorsal rays IX,11; anal rays III,12; pectoral rays 13; lateral-line scales 27 (+1 on tail); no scales on head; gill rakers 14; a single pair of large, projecting, and slightly recurved canine teeth anteriorly in each jaw, the upper pair slightly out-flaring, the lowers curving forward and fitting between uppers when mouth closed; a short irregular row of 4–7 chisel-like incisiform teeth on each side of upper and lower jaws, no canine posteriorly at corner of mouth; elongate body, body depth 4.2–4.9 in SL; only slightly compressed, body width 1.4–1.8 in depth; caudal fin truncate in initial phase, with slightly extended upper and lower lobes in terminal-phase male;

Figure 2. *Pseudojuloides edwardi,* BPBM 41172, holotype, 73 mm SL, Mombasa, Kenya (J. Hale), image reversed so color of right side can be compared to that of left side (note color variation ventrally on operculum, on chest, and abdomen).

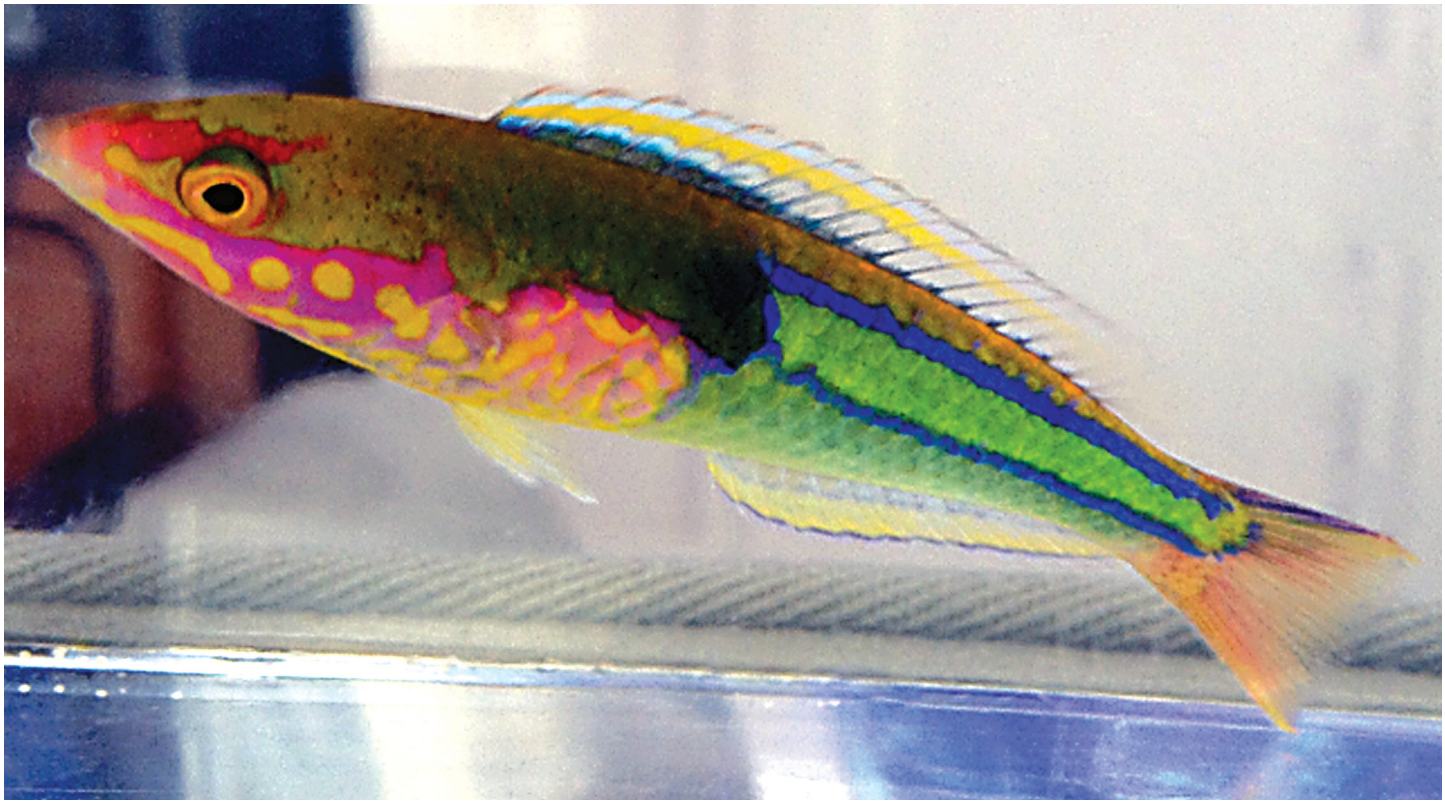

Figure 3. *Pseudojuloides edwardi,* BPBM 41173, paratype, 70.6 mm SL, Mombasa, Kenya (J. Edward).

initial phase reddish orange to pink, often with more yellow tint anteriorly and grading to white ventrally on the head and abdomen; terminal-phase male in life with rear half of body emerald green with two bright blue stripes, lower along lateral midline, upper along mid-upper body, dorsum above upper blue line olive green; upper head and anterior body olive green, becoming darker to black at mid-body; lower half of head and anterior body with prominent yellow reticulum over magenta background; magenta line extends forward below orbit along upper jaw and back across snout to end over the orbit; dorsal and anal fins with bluish base and edge surrounding mid-line thick yellow band; caudal fin blue-edged top and bottom with a short blue extension of the mid-lateral stripe onto the fin.

Description. Dorsal rays IX,ll; anal rays III,12, all soft dorsal and anal segmented rays branched, last split to base; pectoral rays 13, the first rudimentary, the second unbranched; pelvic rays I,5; principal caudal rays 14, the upper and lower unbranched; upper and lower procurrent caudal rays 6 or 7 (usually 6); pored lateral-line scales 27 (+1 on caudal-fin base); scales above lateral line to origin of dorsal fin 4 (3–4); scales below lateral line to origin of anal fin 8; median predorsal scales about 7–9; gill rakers 14.

Body elongate, the depth 4.2 (4.5–4.9) in SL, and only slightly compressed, the width 1.8 (1.4–1.8) in depth; head length 3.0 (2.9–3.0) in SL; dorsal profile of head nearly straight on snout, forming low angle of about 20° to horizontal axis of body, and slightly convex on nape; snout sharply pointed, its length 3.5 (3.1–3.4) in head length; orbit relatively small, diameter 5.0 (4.7–4.9) in head length; interorbital space broadly convex, the least bony width 4.8 (5.1–5.2) in head length; caudal peduncle short and narrow, the least depth 3.6 (3.5–3.6) in head length, caudal-peduncle length 3.5 (3.1–3.6) in head length.

Mouth very small, terminal, the corner of gape with closed jaws well anterior to anterior nostril; end of maxilla buried, even when jaws gape. Lips moderately thick, the upper puffed with striations along the underside, the lower lip with prominent ventral-projecting flap along side of jaw. A pair of large, moderately projecting, and slightly recurved canine teeth anteriorly in each jaw, the upper pair slightly out-flaring, the lowers curving forward and fitting between uppers when mouth closed; a short row of 4–7 irregularly placed chisel-like incisiform teeth along each side of upper and lower jaw; no canine tooth posteriorly on upper jaw. Upper preopercular margin free nearly to level of lower edge of orbit; lower margin free anterior to a vertical through anterior nostril. Gill rakers short, the longest on first arch (at angle) about one-fifth to one-tenth length of longest gill filament.

TABLE 1

Proportional measurements of type specimens of *Pseudojuloides edwardi* and "matched-size" *P. severnsi* as percentages of the standard length

	Pseudojuloides edwardi			*Pseudojuloides severnsi*			
	holotype	paratypes					
	BPBM 41172 TP	BPBM 41173 TP	BPBM 41173 IP	BPBM 41174 TP	BPBM 41174 TP	BPBM 41175 TP	BPBM 41175 IP
Standard length (mm)	73.0	70.6	63.4	79.8	72.3	70.1	55.4
Body depth	23.6	20.5	22.4	23.2	19.5	21.8	19.1
Body width	13.2	14.3	12.8	14.2	12.6	13.8	12.3
Head length	33.8	33.3	34.5	34.8	32.8	35.7	29.2
Snout length	9.6	9.8	11.2	11.0	11.9	11.3	9.0
Orbit diameter	6.7	7.1	7.1	6.0	6.4	6.6	7.2
Interorbital width	7.1	6.5	6.6	7.5	6.1	7.6	6.1
Caudal-peduncle depth	9.3	9.5	9.6	8.5	9.5	9.1	9.9
Caudal-peduncle length	9.7	9.3	11.0	10.2	9.3	9.7	12.3
Predorsal length	32.9	31.4	32.2	31.1	31.4	32.1	28.5
Preanal length	56.7	59.1	60.4	59.5	59.9	58.5	56.7
Prepelvic length	36.4	36.1	37.1	36.7	37.1	38.4	33.6
Base of dorsal fin	59.0	57.6	57.6	59.4	58.9	56.6	57.4
First dorsal spine	5.6	5.2	4.9	5.8	5.8	4.4	6.1
Ninth dorsal spine	10.3	9.9	10.7	9.9	8.4	9.0	9.6
Longest dorsal ray	13.4	10.9	11.8	10.4	11.3	11.0	11.4
Base of anal fin	34.4	33.4	35.5	34.2	35.1	35.7	34.3
First anal spine	2.6	2.5	3.0	2.6	4.0	3.0	2.2
Second anal spine	4.9	5.5	4.9	3.9	6.8	4.3	4.5
Third anal spine	7.8	6.5	7.3	6.1	7.9	6.1	7.2
Longest anal ray	11.5	10.2	11.2	10.0	11.1	10.1	11.2
Caudal-fin length	21.5	18.8	17.8	19.0	19.5	21.0	20.4
Pectoral-fin length	18.4	16.7	16.4	15.0	15.4	16.5	12.3
Pelvic-spine length	10.3	8.9	8.0	8.9	10.2	8.6	9.2
Pelvic-fin length	15.3	15.2	14.4	13.5	15.5	14.4	13.7

Figure 4. *Pseudojuloides edwardi,* BPBM 41173, paratype, 70.6 mm SL, Mombasa region, Kenya (B.C. Victor).

Nostrils small, in front of upper edge of orbit, the anterior in a short membranous tube elevated posteriorly, the posterior in advance of a vertical through front of orbit by a distance slightly less than internarial space. Pores on lower half of head comprise one over rear maxilla, then two anterior to orbit, followed by a curving suborbital series (counting up to rear mid-eye level) numbering 4–7 in single series; preopercular pores in a curved series after start of free edge near mandible, numbering 10 or 11 along free margin of preopercle, plus 1 or 2 more up to rear mid-eye level, in a single series at distal tips of canals.

Scales thin and cycloid; scales on side of thorax less than half as high as largest scales on side of body, becoming still smaller ventroanteriorly; head naked except for small partially embedded scales on nape in irregular rows; median predorsal scales extending forward to slightly posterior to a vertical through upper free end of preopercular margin; fins naked except for several progressively smaller scales on basal region of caudal fin and mid-ventral scale projecting posteriorly from base of pelvic fins. Lateral line continuous, nearly following contour of back to 18th pored scale, below base of eighth dorsal soft ray, where deflected sharply ventrally to straight peduncular portion, single small pore per scale, last pored scale on caudal-fin base. Origin of dorsal fin above anterior edge of second lateral-line scale; dorsal spines progressively longer, the first 6.0 (6.4–7.1) and the ninth 3.3 (3.2–4.0) in head; longest dorsal soft ray 2.5 (2.9–3.1) in head; origin of anal fin below base of last dorsal spine; first anal spine very short, 13.0 (11.5–13.1) in head; second anal spine 6.9 (6.0–7.1) in head; third anal spine 4.3 (4.8–5.1) in head; longest anal soft ray 2.9 (3.1–3.3) in head; caudal fin with slightly extended upper and lower lobes in terminal-phase males, caudal-fin length 1.6 (1.8–1.9) in head; third pectoral-fin ray longest, 1.8 (2.0–2.1) in head; pelvic fins short, 2.2 (2.2–2.4) in head.

Color in life. Terminal phase male with rear half of body emerald green with two bright blue stripes, lower along lateral midline, upper along mid-upper body, dorsum above upper blue line olive green; upper head and anterior body olive green, becoming darker to black at mid-body; lower half of head and anterior body with prominent yellow reticulum over magenta background; magenta line extends forward below orbit along upper jaw and back across snout to end over the orbit; dorsal and anal fins with bluish base and edge surrounding thick central yellow band; caudal fin blue-edged top (and sometimes bottom) with a blue extension of the mid-lateral stripe onto the fin base. Pelvic fins yellowish. Iris in life can be yellow, orange, red or magenta. The initial phase specimen was reported by the aquarium dealer to be reddish orange to pink, with more yellow tint anteriorly and grading to white ventrally on the head and abdomen. Initial-phase *P. edwardi* are likely indistinguishable from initial-phase *P. severnsi* in life, which show a reflective white stripe from the upper jaw running below the orbit, sometimes only a white patch on the mid-upper jaw, and juveniles show a fine overlay of thin lavender stripes, breaking up into lines of small spots and dashes, running the length of the body from behind the eye to the caudal fin (Fig. 5).

Color in alcohol. Color is lost except for dark markings in males, but the pattern of the reticulum is evident as a network of more opaque white lines. Initial-phase fish are uniform pale yellowish.

Etymology. This species is named for Jason Edward, who was instrumental in obtaining the male type specimens and generously supplying them to the authors. The specific epithet is a noun in the genitive case.

Barcode DNA sequence. A 652-nucleotide sequence of the segment of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype and paratypes (Appendix 1). Following the database management recommendation of the BOLD, the sequence of the holotype (GenBank accession number KJ591643) is presented here as well:

CCTTTATCTAGTATTCGGTGCCTGAGCTGGGATGGTAGGCACAGCCCTAAGCCTGCTCATCCGAGCC
GAGCTTAGCCAGCCCGGCGCTCTCCTCGGAGACGACCAAATTTATAACGTAATCGTTACGGCCCACG
CCTTCGTAATAATCTTCTTTATAGTAATGCCAATTATGATTGGCGGATTCGGAAACTGATTAATTCCCT
TAATGATCGGAGCCCCTGACATAGCTTTTCCTCGAATAAACAACATGAGCTTCTGGCTCCTTCCCCC
ATCTTTCCTTCTTCTTCTTGCCTCATCAGGCGTAGAAGCAGGAGCTGGGACTGGTTGAACAGTTTAC
CCCCCTCTAGCTGGTAATCTTGCCCATGCAGGGGCCTCTGTAGACCTTACTATCTTCTCTCTCCACTT
AGCCGGTATCTCTTCAATCCTAGGGGCGATCAACTTTATTACAACTATCATTAATATGAAACCCCCCT
ATTTCTCAGTACCAAACACCCCTCTTTGTCTGAGCCGTTCTAATTACAGCAGTTCTCCTTCTTCTTTC
ACTACCCGTTCTTGCTGCCGGCATCACTATGCTTCTAACTGATCGTAACCTTAACACCACCTTCTTTG
ACCCTGCCGGCGGAGGAGATCCTATTCTTTATCAGCACCTC

Comparisons. The genus *Pseudojuloides* is relatively conservative in morphology and counts, although clearly not in color (Randall & Randall 1981). Most counts and proportions are shared or overlap extensively, although pore patterns on the head can vary to a small degree. *P. edwardi* clearly belongs in a species complex with *P. severnsi* and *P. erythrops*. All three species share the basic color motif in terminal-phase males of an abrupt break in the color pattern midway along the body with a prominent transtion from dark to light (Fig. 6). *P. severnsi* males share many of the color patterns of *P. edwardi*, including the rear body with blue stripes on green and mostly the same patterns on the fins and tail. The primary difference is the head and forebody in *P. severnsi* is dark above and white below divided by a blue, red, or even magenta line that also runs along the upper jaw and extends

Figure 5. *Pseudojuloides severnsi*, juvenile (above) female (below), Japan (K. Nishiyama).

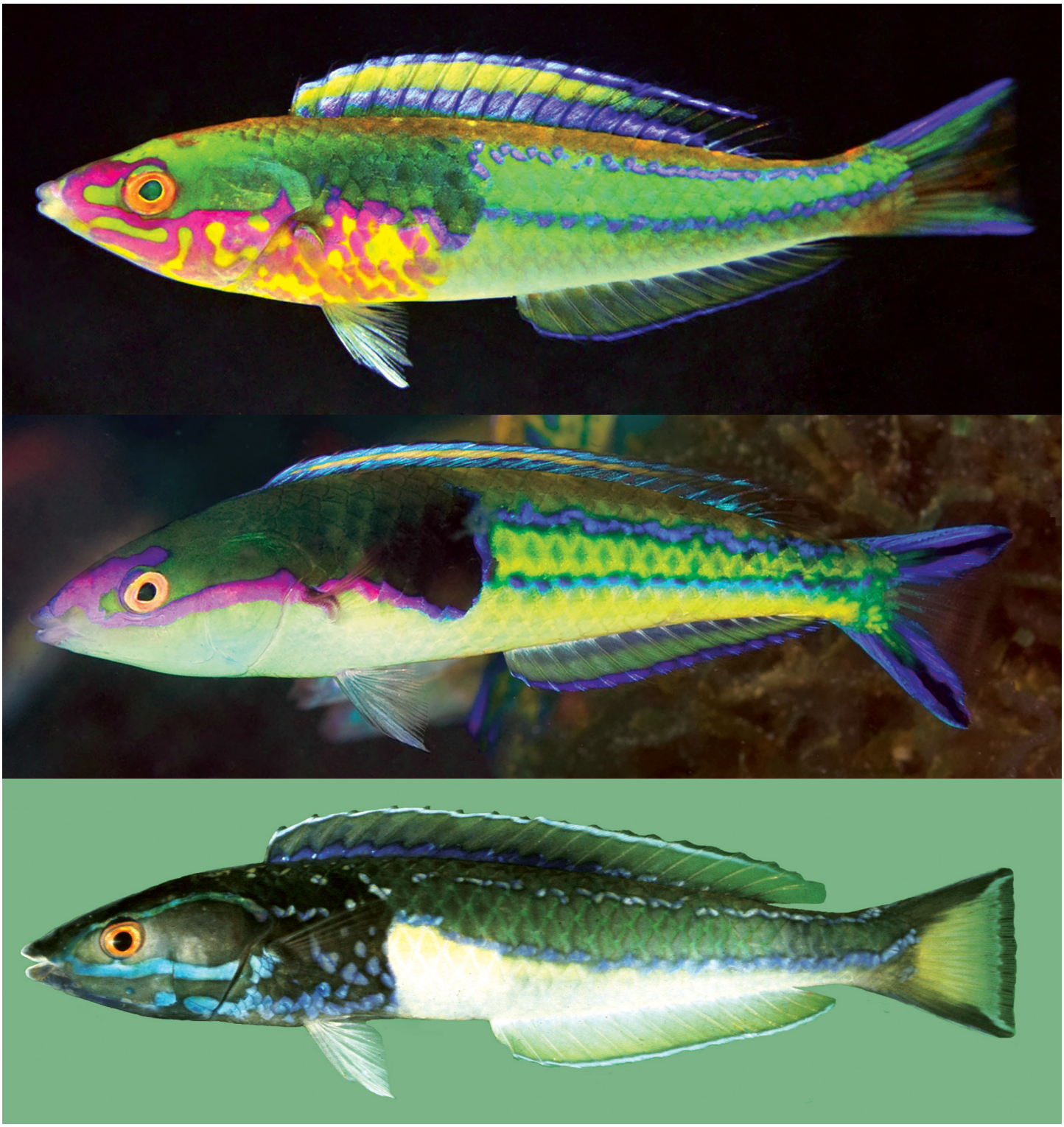

Figure 6. top: *Pseudojuloides edwardi,* BPBM 41172, holotype, 73 mm SL, Mombasa, Kenya, image reversed (V. Altamirano); middle: *P. severnsi*, Japan (K. Nishiyama); bottom: *P. erythrops*, BPBM 24772, holotype, 83.6 mm SL, Mauritius (J.E. Randall).

back over the eye, as in *P. edwardi*, but there is no development of the complex network of contrasting lines and patches characteristic of *P. edwardi*. Interestingly, the terminal male of *P. erythrops* has a pattern of bright blue spots and lines over the lower head and forebody reminiscent of the reticulum of *P. edwardi*, however it is blue against dark green and, instead of the main contrast being between a dark upper head and white or colorful lower head, the prominent contrast in male *P. erythrops* is between a uniformly dark head and forebody and a bright white rear abdomen.

DNA Comparisons. The neighbor-joining phenetic tree based on the COI mtDNA sequences of *Pseudojuloides* species, following the Kimura two-parameter model (K2P) generated by BOLD (Barcode of Life Database), shows deep divergences between species and only minimal differences within species, except for the *P. edwardi* and *P. severnsi* sequences, which cluster together (Fig. 7). No haplotypes are shared between the two species (with the small sample sizes here), but the difference between the two species is well within the range of typical differences within species. As a broad generality, among most reef fishes the minimum interspecific distance between close congeners is often up to an order of magnitude greater than the maximum intraspecific distance, which is precisely what makes the Barcode database particularly useful (Ward *et al.* 2009). The exceptions to the generality are interesting cases and deserve a closer look. Among the *Pseudojuloides* excluding *P. edwardi*, minimum interspecific distances range from 3.5 to 20.38% (K2P distances, vs. 3.4 to 17.52% pairwise) and maximum intraspecific distances from 0 to 0.93% (0 to 0.92% pairwise), showing a clear "barcode gap" between species (Table 2). However, *P. edwardi* sequences differ from *P. severnsi* by only 0.46% (three nucleotides of the 652 bp barcode segment), much less than the intraspecific variation within *P. severnsi* (maximum intraspecific distance of 0.92%). It is possible, if not likely, that additional sequencing would reveal closer or shared haplotypes.

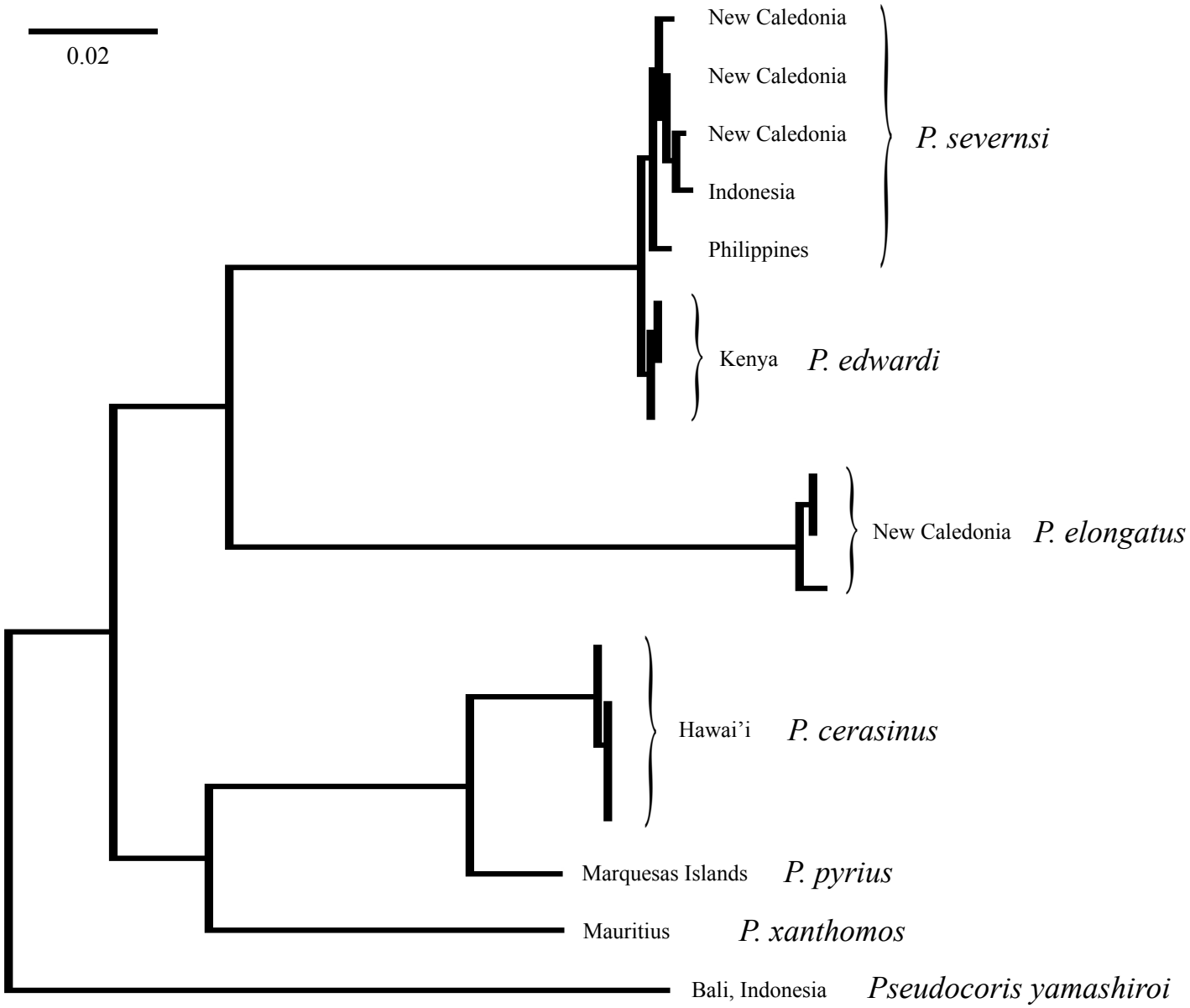

Figure 7. The neighbor-joining phenetic tree of *Pseudojuloides* following the Kimura two-parameter model (K2P) generated by BOLD (Barcode of Life Database). The scale bar at left represents a 2% sequence difference. Collection locations for specimens are indicated, and *Pseudocoris yamashiroi* is used as an outgroup. GenBank accession numbers and collection data for the sequences in the tree are listed in Appendix 1.

Remarks. The development of reproductive isolating mechanisms early in the process of speciation is a key step in the splitting of populations into separate species. Among coral-reef fishes, the color displays of breeding males are typically complex, eye-catching, and clearly species-specific, serving, no doubt, to facilitate species recognition. Indeed, a number of published cases of recent divergence of species pairs and complexes of reef fishes are characterized by development of different colors and markings, especially on the head and among breeding males (e.g. Bowen 2006, Rocha & Bowen 2008, Victor & Randall 2010, Baldwin *et al.* 2011). In some of these cases, the development of these reproductive isolating mechanisms can outpace the divergence in neutral mtDNA sequences, leading to the uncomfortable situation where clear differences in phenotype are present between closely related species that otherwise overlap or even share mtDNA haplotypes. This is likely the case for the new species described here, and, in this example, the color differences that have developed between the African *Pseudojuloides edwardi* and its mainly Pacific Ocean congener, *P. severnsi*, are undeniably spectacular.

An important question is the consistency of color patterns displayed by *P. severnsi*, *P. erythrops,* and *P. edwardi*. The latter two species are known from very few individuals, but the geographic variation can be assessed for *P. severnsi*, which is much better known. Indeed, live specimens and live photographs from widely disparate locations are available, including the entire reported range, from southern Japan (Nishiyama 2012), Philippines, Indonesia (Bellwood & Randall 2000, Allan & Erdmann 2012), New Caledonia, and Sri Lanka (Bellwood & Randall 2000). At all locations, the appearance is the same: the head and anterior body show the same bicolored pattern of dark dorsal half and whitish ventral half with an intervening single line of color and no evidence of the development of any reticulations characteristic of the new species.

TABLE 2

K2P distances for species of *Pseudojuloides*

Minimum Interspecific and Maximum Intraspecific distances (%)

	cer	*pyr*	*xan*	*edw*	*sev*	*elo*
P. cerasinus	0.18					
P. pyrius	3.50	0				
P. xanthomos	11.34	11.23	0			
P. edwardi	16.06	15.27	15.51	0.16		
P. severnsi	16.34	15.61	15.45	0.46	0.93	
P. elongatus	18.80	20.38	19.16	15.81	16.14	0.62

Pairwise distances for species of *Pseudojuloides*

Minimum Interspecific and Maximum Intraspecific distances (%)

	cer	*pyr*	*xan*	*edw*	*sev*	*elo*
P. cerasinus	0.18					
P. pyrius	3.40	0				
P. xanthomos	10.36	10.29	0			
P. edwardi	14.12	13.55	13.71	0.16		
P. severnsi	14.34	13.82	13.67	0.46	0.92	
P. elongatus	16.41	17.51	16.74	14.17	14.44	0.61

The similarity in morphology, counts, and DNA sequences obviously raises the question of species status. There is no clearly defined line in the grey area during speciation where divergent populations become species and even the definition of species is, at present, a hotly contested subject among taxonomists and evolutionary biologists. In general, the definition of species is tailored for the question being discussed (Mallet 2008). There is certainly precedent for color pattern being used as the sole criterion for species distinction among coral reef fishes, in particular among wrasses and parrotfishes, where morphology and counts can be strongly conserved. In some cases, complexes of well-established and distinctly marked species share mitochondrial DNA sequences as well as morphology, as illustrated by the labrid *Thalassoma* species complex of *T. lutescens*, *T. genivittatum*, *T. duperrey*, and *T. grammaticum* (Victor, in prep). Clearly, populations that have split and started their own evolutionary trajectories can be either subject to low levels of introgression or, alternatively, phenotypic changes can outpace the slow accumulation of mutational changes leading to mutually exclusive sets of mitochondrial haplotypes. Large population sizes and recent divergence can exacerbate the problems of incomplete lineage sorting and most likely play a role in the evolution of these reef-fish species complexes.

Other material of *Pseudojuloides* examined. *P. severnsi*- Philippines (aquarium trade) BPBM 41174, 2: 72.3–79.8 mm, Sep. 21, 2013. New Caledonia, BPBM 41175, 2: 55.4–70.1 mm Jan. 14, 2014.

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Appendix 1. Specimen data and GenBank accession numbers for the mtDNA COI barcode sequences used to generate the phenogram in Fig. 7. Holotype in bold.

Genus	species	Collection site	Voucher	GenBank #	Collector/Source
Pseudojuloides	*severnsi*	New Caledonia	BPBM 41175	KJ591651	A. Teitelbaum
Pseudojuloides	*severnsi*	New Caledonia	BPBM 41175	KJ591655	A. Teitelbaum
Pseudojuloides	*severnsi*	New Caledonia	qm14ps2	KJ591654	A. Teitelbaum
Pseudojuloides	*severnsi*	Indonesia	je13ps	KJ591653	J. Edward/aq. trade
Pseudojuloides	*severnsi*	Philippines	BPBM 41174	KJ591652	J. Edward/aq. trade
Pseudojuloides	*edwardi*	Mombasa, Kenya	BPBM 41173	KJ591644	J. Edward/aq. trade
Pseudojuloides	*edwardi*	Mombasa, Kenya	BPBM 41173	KJ591642	A. DeJong/aq. trade
Pseudojuloides	***edwardi***	**Mombasa, Kenya**	**BPBM 41172**	**KJ591643**	**J. Edward/aq. trade**
Pseudojuloides	*elongatus*	New Caledonia	jr14pe2	KJ591649	A. Teitelbaum
Pseudojuloides	*elongatus*	New Caledonia	jr14pe1	KJ591648	A. Teitelbaum
Pseudojuloides	*elongatus*	New Caledonia	jr14pe3	KJ591647	A. Teitelbaum
Pseudojuloides	*cerasinus*	Hawai'i	h83pc260	JQ839571	B. Victor
Pseudojuloides	*cerasinus*	Hawai'i	FLHI398-09	KJ591646	D. Carlon/A. Faucci
Pseudojuloides	*cerasinus*	Hawai'i	h83pc370	JQ839570	B. Victor
Pseudojuloides	*cerasinus*	Hawai'i	FLHI318-09	KJ591645	D. Carlon/A. Faucci
Pseudojuloides	*pyrius*	Marquesas Islands	MARQ-424	KJ591650	J. Williams/S. Planes
Pseudojuloides	*xanthomos*	Mauritius	dej13px360	KJ591657	A. DeJong/aq. trade
Pseudocoris	*yamashiroi*	Bali, Indonesia	bal11800py151	JQ839565	B. Victor