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# Pseudojuloides zeus, a new deep-reef wrasse (Perciformes: Labridae) from Micronesia in the western Pacific Ocean 

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#### Abstract

The new species, Pseudojuloides zeus, is described from two specimens obtained from Majuro Atoll in the Marshall Islands and Palau in Micronesia, western Pacific Ocean. The species is distinguished by two prominent jagged blue stripes along the body and a dark spot at the base of the mid-dorsal fin. P. zeus is a rarely seen fish, found only on particularly deep reefs and, thus far, from only two locations. The single paratype from Palau was collected at about 80 m during the 1997 'Twilight Zone' Expedition by the Bishop Museum and, since then, a series of specimens have been collected for the aquarium trade from similarly deep reefs at Majuro. The nearest relative is $P$. mesostigma, from Japan, Philippines, Indonesia, Vanuatu, Tonga, and the Great Barrier Reef, which shares the very slender body and has a dark area on the mid-dorsal fin and body, but does not have the distinctive two blue stripes. The barcode mtDNA COI sequence of the new species is $5.3 \%$ different from the sequence of $P$. mesostigma (minimum interspecific distance, K2P model). A neighbor-joining tree and genetic distance matrix is presented for 11 of the 13 known species in the genus Pseudojuloides.


Key words: new species, taxonomy, systematics, coral reef fishes, twilight zone, Palau, Majuro, DNA barcoding.

## Introduction

The labrid genus Pseudojuloides Fowler was revised by Randall \& Randall (1981), who recognized eight species in the genus, including five new species. Since then, several new species have been described from various locations in the Indo-Pacific Ocean, including P. kaleidos by Kuiter \& Randall (1995) from the Maldives and

Indonesia, P. severnsi by Bellwood \& Randall (2000), from the Maldives to the W. Pacific, and P. edwardi and P. polackorum recently from the south-west Indian Ocean (Victor \& Randall 2014, Connell et al. 2015). 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. We describe here a new species from deep-water collections from the western Pacific Ocean and compare its barcode mtDNA COI sequence to ten of the twelve previously described species in the genus (all except for $P$. argyreogaster and $P$. erythrops).

## Materials and Methods

Specimens have been examined from the Bernice P. Bishop Museum, Honolulu (BPBM). In addition, ethanolpreserved specimens of comparison species were collected for DNA sequencing from Bali, French Polynesia, Cook Islands, New Caledonia, and Hawai‘i in the Pacific Ocean, and South Africa in the Indian Ocean, as well as obtained via the aquarium trade from Philippines, Indonesia, and Vanuatu in the Pacific Ocean and Kenya and Mauritius in the Indian 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 $652-\mathrm{bp}$ segment was amplified from the $5^{\prime}$ region of the mitochondrial COI gene using a variety of primers (Ivanova et al. 2007). PCR amplifications were performed in $12.5 \mu \mathrm{l}$ volume including $6.25 \mu \mathrm{l}$ of $10 \%$ trehalose, $2 \mu \mathrm{l}$ of ultra pure water, $1.25 \mu \mathrm{l}$ of $10 \times \mathrm{PCR}$ buffer $\left(10 \mathrm{mM} \mathrm{KCl}, 10 \mathrm{mM}\left(\mathrm{NH}_{4}\right)_{2} \mathrm{SO}_{4}, 20 \mathrm{mM}\right.$ Tris$\mathrm{HCl}(\mathrm{pH} 8.8), 2 \mathrm{mM} \mathrm{MgSO}_{4}, 0.1 \%$ Triton X-100), $0.625 \mu 1$ of $\mathrm{MgCl}_{2}(50 \mathrm{mM}), 0.125 \mu 1$ of each primer $(0.01 \mathrm{mM})$, $0.0625 \mu \mathrm{l}$ of each dNTP $(10 \mathrm{mM}), 0.0625 \mu \mathrm{l}$ of Taq DNA polymerase (New England Biolabs), and $2 \mu \mathrm{l}$ of template DNA. The PCR conditions consisted of $94^{\circ} \mathrm{C}$ for 2 min ., 35 cycles of $94^{\circ} \mathrm{C}$ for $30 \mathrm{sec} ., 52^{\circ} \mathrm{C}$ for 40 sec ., and $72^{\circ} \mathrm{C}$ for 1 min ., with a final extension at $72^{\circ} \mathrm{C}$ 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 divergences were 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' sequence divergence. Genetic distances were calculated by the BOLD algorithm both as uncorrected p-distances and as K2P distances.

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-fin ray is included in the count. Lateral-line scale counts include the last pored scale that overlaps the end of the hypural plate as +1 ; scales above the lateral line are counted in an oblique row from the pored scales under the mid-spinous dorsal fin, the much smaller scale abutting the base of the fin is counted as 0.5 scales. The count of gill rakers is made on the first gill arch and includes all rudiments. The counts and measurements for the paratype is shown in parentheses following data for the holotype. Proportional morphological measurements are presented in Table 1.


Figure 1. Pseudojuloides zeus, BPBM 41215, male holotype, 60 mm SL, Majuro, Marshall Islands (B.C. Victor).

## Pseudojuloides zeus, n. sp.

## Zeus Pencil Wrasse

Figures 1-3, Table 1.
Holotype. BPBM 41215, 60 mm SL, male, Majuro, Marshall Islands, Micronesia, aquarium-trade collectors, Sep. 15, 2013.

Paratype. BPBM 37701, 67.5 mm SL, male, Blue Holes, Ngemelis Island, Palau, Micronesia, $7.137^{\circ} \mathrm{N}$, $134.221^{\circ}$ E, 79-88m, J.L. Earle, May 11, 1997.

Diagnosis. Dorsal-fin rays IX,11; anal-fin rays III,12; pectoral-fin rays 13-14 (13); lateral-line scales 27 (+1 on tail), 1.5 scales above anterior lateral line to dorsal-fin base; no scales on head; gill rakers 14 ; a single pair of large, forward projecting canine teeth anteriorly in each jaw, the lowers fitting between uppers when mouth closed; few irregular short pointed but flattened teeth on each side of upper and lower jaws, no canine posteriorly at corner of mouth; very elongate body, body depth $5.65-5.75$ in SL; only slightly compressed, body width 1.4-1.55 in depth; caudal fin slightly rounded; terminal-phase male in life with yellow-green head and body, two prominent jagged-edged iridescent blue stripes along sides of body, a yellow spinous dorsal fin, a large black spot at base of mid-dorsal fin, and a mostly black caudal fin.


Figure 2. Pseudojuloides zeus, BPBM 41215, male holotype, 60 mm SL, Majuro, Marshall Islands, image reversed so markings of right side can be compared to that of left side (J. Hale).

Description. Dorsal-fin rays IX, 11; anal-fin rays III,12; all soft dorsal and anal segmented rays branched, last split to base; pectoral-fin rays $13-14$ (13), the first rudimentary, the second unbranched; pelvic rays I,5; principal caudal rays 14 , the upper and lower unbranched, one additional segmented ray and 3-4 visible unsegmented procurrent rays upper and lower; pored lateral-line scales 27 ( +1 on caudal-fin base); gill rakers 14 (14).

Body very elongate, the depth 5.65 (5.75) in SL, and only slightly compressed, the width 1.55 (1.4) in depth; head length 3.15 (3.15) in SL; dorsal profile of head nearly straight on snout, forming low angle of about $20^{\circ}$ to horizontal axis of body, and slightly convex on nape; snout sharply pointed, its length 3.6 (3.5) in head length; orbit diameter 4.7 (4.85) in head length; interorbital space broadly convex, the least bony width 4.55 (5.1) in head length; caudal peduncle short and narrow, the least depth 3.5 (3.6) in head length, caudal-peduncle length 3.15 (2.8) 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 open. Lips moderate. A pair of large, forward projecting canine teeth anteriorly in each jaw, the lower pair fitting between uppers when mouth closed (canines of holotype broken); paratype with two smaller canines behind lower canines, very few teeth present along bony ridges behind canines, one or two on each quadrant, flattened but pointed; 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. 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 6-7 in single series; preopercular pores in a curved series after start of free edge near mandible, numbering 9 or 10 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 with 27 pored scales, nearly following contour of back to $19^{\text {th }}$ pored scale, below base of eighth or ninth dorsal-fin soft ray, where deflected sharply ventrally to straight peduncular portion, single small pore per scale, first portion with short dorsal branch to pore, last pored scale on caudal-fin base (except for no pore on first scale of tail on one side of paratype). One and a half scales between scales of first portion of lateral line and dorsal-fin base.

Origin of dorsal fin above second lateral-line scale; dorsal spines progressively longer, the first 4.7 (5.35) and the ninth 3.25 (3.25) in head; longest dorsal soft ray 3.05 (2.7) in head; origin of anal fin below base of last dorsal spine; first anal-fin spine short, 10.65 (9.3) in head; second anal-fin spine 6.2 (5.95) in head; third anal-fin spine 4.45 (4.65) in head; longest anal soft ray 3.2 (2.9) in head; caudal fin slightly rounded, caudal-fin length 1.85 (1.8) in head; third pectoral-fin ray longest, 1.8 (1.8) in head; pelvic fins short, 2.4 (2.35) in head.

Color in life. Terminal phase male with yellow-green head grading to greenish on rear body. Two broad jagged-edged iridescent blue stripes along the sides of the body, usually two scales wide: the upper stripe wider and starting behind the operculum, the lower with a thin extension onto the cheek and only one scale wide from the pectoral-fin base to midbody. Both stripes widening rearward to mostly cover the narrow caudal peduncle. Spinous dorsal fin bright yellow, soft dorsal fin with yellow band distally; a black, mostly oval spot on the lower half of the fin over the first few dorsal-fin soft rays. Proximal two-thirds of caudal fin prominently black with upper and lower margins with an iridescent blue band. Pelvic and anal fins mostly yellowish to transparent. Iris bright yellow to orange-red. Initial phase females and juveniles are unknown, but based on P. mesostigma and the genus in general, they are reddish orange with a white ventral aspect and yellowish fins (in some live photographs, P. mesostigma females can have whitish bars as well).

Color in alcohol. Color is yellowish brown except for dark markings on fins in formalin-preserved paratype. The ethanol-preserved holotype retains the white ventrum and dark markings, as well as some indication of the iridescent blue stripes.

## TABLE 1

Proportional measurements of type specimens of Pseudojuloides zeus and comparison P. mesostigma as percentages of the standard length

|  | Pseudojuloides zeus |  | Pseudojuloides mesostigma |  |
| :---: | :---: | :---: | :---: | :---: |
|  | holotype | paratype |  |  |
|  | BPBM | BPBM | BPBM | BPBM |
|  | 41215 | 37701 | 41216 | 41216 |
|  | TP | TP | TP | IP |
| Standard length (mm) | 60.0 | 67.5 | 60.6 | 60.9 |
| Body depth | 17.3 | 17.7 | 17.2 | 16.9 |
| Body width | 12.1 | 11.3 | 12.7 | 12.3 |
| Head length | 31.7 | 32.0 | 32.5 | 32.7 |
| Snout length | 9.0 | 8.8 | 9.2 | 8.4 |
| Orbit diameter | 6.5 | 6.8 | 6.3 | 6.6 |
| Interorbital width | 6.2 | 7.0 | 6.8 | 6.4 |
| Caudal-peduncle depth | 8.9 | 9.2 | 9.4 | 9.9 |
| Caudal-peduncle length | 11.4 | 10.2 | 11.2 | 11.2 |
| Predorsal length | 28.4 | 30.0 | 30.4 | 29.4 |
| Preanal length | 53.0 | 57.5 | 54.3 | 56.2 |
| Prepelvic length | 32.6 | 35.0 | 34.7 | 34.0 |
| Base of dorsal fin | 57.0 | 58.2 | 56.9 | 56.7 |
| First dorsal-fin spine | 5.9 | 6.8 | 5.9 | 5.6 |
| Ninth dorsal-fin spine | 9.8 | 9.8 | 9.2 | 9.9 |
| Longest dorsal-fin ray | 11.9 | 10.5 | 11.4 | 11.5 |
| Base of anal fin | 35.0 | 33.2 | 35.8 | 36.5 |
| First anal-fin spine | 3.4 | 3.0 | 4.0 | 3.9 |
| Second anal-fin spine | 5.3 | 5.2 | 5.6 | 5.6 |
| Third anal-fin spine | 6.8 | 7.2 | 7.4 | 7.4 |
| Longest anal-fin ray | 11.0 | 10.0 | 11.1 | 10.5 |
| Caudal-fin length | 17.8 | 17.3 | 18.5 | 19.0 |
| Pectoral-fin length | 17.5 | 17.7 | 15.3 | 16.1 |
| Pelvic-spine length | 9.3 | 9.0 | 7.6 | 8.2 |
| $\underline{\text { Pelvic-fin length }}$ | 13.6 | 13.3 | 13.2 | 13.0 |



Figure 3. Pseudojuloides zeus, Japanese aquarium trade, note dark spot at base of mid-dorsal fin (unknown photographer).
Etymology. Since the jagged blue stripes of this species resemble lightning bolts, this species is named for the Greek god Zeus, who liked to cast bolts of lightning at unsuspecting mortals, such as the physician Asclepius who became a little too presumptuous. The specific epithet is a noun in apposition.

Distribution and habitat. The new species appears limited to Micronesia, thus far being collected only in Palau and Majuro. The first specimen (paratype) was collected by the 1997 'Twilight Zone' Expedition of the Bishop Museum by John Earle diving with Richard Pyle at 80 to 90 m depth in the famed Blue Holes dive site in Palau. Subsequently, several individuals have been collected for the aquarium trade from similar depths in Majuro (and primarily shipped to Japan). However, the particularly deep habitat is very poorly sampled and surveyed and the species may well have a wider range. Curiously, another deep-water wrasse, Cirrhilabrus earlei Randall \& Pyle 2001, was also discovered on the same dive in Palau as $P$. zeus, and is also collected for the aquarium trade by the same commercial collectors in Majuro.

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. Following the database management recommendation of the BOLD, the sequence of the holotype (GenBank accession number KJ591656) is presented here as well:

CCTTTATCTAGTATTCGGTGCCTGAGCTGGGATGGTGGGCACAGCCCTAAGCCTGCTCATCCGGGC TGAGCTTAGCCAGCCCGGCGCTCTCCTCGGAGACGACCAAATTTATAACGTAATCCCACGCCTTCG TAATAATCTTCTTTATAGTAATACCAATTATAATCGGCGGGTTCGGAAACTGATTAATTCCCTTAATGA TTGGGGCCCCCGACATGGCCTTCCCTCGAATAAATAACATAAGCTTCTGGCTTCTTCCTCCGTCTTT CСTTСТТСТССTCGCCTCGTCAGGCGTAGAAGCAGGGGCTGGCACTGGGTGAACAGTTTACCCTCC CCTAGCTGGTAATCTTGCCCACGCAGGGGCCTCTGTAGACCTCACTATCTTCTCCCTTCACTTGGCA GGTATTTCTTCAATCCTGGGAGCAATCAACTTTATTACTACCATTATTAACATGAAACCCCCTGCTAT TTC TCAGTACCAAACACCTCTCTTTGTATGAGCCGTTTTAATTACAGCAGTCCTCCTCCTTCTCTCG CTGCCCGTTCTTGCTGCCGGCATCACAATGCTTCTAACTGATCGTAACCTCAACACCACCTTCTTTG ACCCTGCTGGCGGAGGTGACCCCATTCTTTATCAACATCTC


Figure 4. Pseudojuloides mesostigma, underwater photograph, Kashiwajima, Kochi, Japan (© K. Nishiyama).

Comparisons. Among the Pseudojuloides, P. zeus most closely resembles P. mesostigma in marking patterns and the very slender shape. The body depth is less than $18 \%$ SL for the two species (Table 1) vs. $18 \%$ to $20 \%$ for P. mesostigma and P. erythrops in Randall \& Randall (1981) and $19 \%$ to $23 \%$ for $P$. severnsi and P. edwardi in Victor \& Randall (2014). Other species, including the P. cerasinus complex, have a body depth well over 20\% SL (Randall \& Randall 1981, Connell et al. 2015). In addition, P. zeus and P. mesostigma share broad iridescent blue markings on the side, a dark area on the mid-dorsal fin, a yellowish spinous dorsal fin, and a mostly black caudal fin; however, in P. mesostigma the blue markings are reticulated and not organized into stripes and the black area on the dorsal fin is a large patch extending to the distal fin edge and also extends in a broad patch onto the upper body (Figs. $4 \& 5$; Nishiyama \& Motomura 2012). The two species are apparently allopatric, with $P$. mesostigma reportedly ranging from Japan through the Philippines and Indonesia to New Guinea and the northern GBR in Australia (Kuiter 2010), as well as Tonga (Randall et al. 2003) and Vanuatu (aquarium trade), but not New Caledonia (Fricke et al. 2011). P. zeus is apparently limited to Micronesia, well beyond the continental influence of the Coral Triangle. The habitat of the two species is deep rubble slope areas on reefs, although $P$. mesostigma is found shallower than P. zeus, usually cited as 25 to $45 m$ (Allen \& Erdmann 2012).


Figure 5. Pseudojuloides mesostigma, BPBM 41216, 60.6 mm SL male above, 60.9 mm SL female below, Vanuatu aquarium imports (B.C. Victor).


Figure 6. 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 Leptojulis cyanopleura is used as an outgroup. The location "aqua" indicates an aquarium trade specimen of unknown provenance. GenBank accession numbers and collection data for the sequences in the tree are listed in Appendix 1.

DNA Comparisons. The neighbor-joining phenetic tree based on the COI mtDNA sequences of 11 of the 13 known Pseudojuloides species, following the Kimura two-parameter model (K2P) generated by BOLD (Barcode of Life Database), shows deep divergences between species and relatively small differences within species, except for the $P$. edwardi and $P$. severnsi sequences, which are very close (Fig. 6). 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. It appears that the majority of reef fish species (with many exceptions) differ by more than $2 \%$ from their

## TABLE 2

K2P distances for mtDNA COI sequences of 11 species of Pseudojuloides
Minimum Interspecific and Maximum Intraspecific Distances (\%)

|  | cer | pyr | xan | pol | kal | edw | sev | elo | ata | mes | zeu |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| P. cerasinus | 0.18 |  |  |  |  |  |  |  |  |  |  |
| P. pyrius | 3.50 | NA |  |  |  |  |  |  |  |  |  |
| P. xanthomos | 11.34 | 11.23 | NA |  |  |  |  |  |  |  |  |
| P. polackorum | 9.47 | 9.47 | 7.50 | 0.31 |  |  |  |  |  |  |  |
| P. kaleidos | 10.40 | 10.71 | 4.48 | 8.42 | NA |  |  |  |  |  |  |
| P. edwardi | 16.06 | 15.27 | 15.51 | 14.58 | 15.90 | 0.31 |  |  |  |  |  |
| P. severnsi | 16.34 | 15.61 | 15.45 | 14.34 | 15.83 | 0.46 | 0.93 |  |  |  |  |
| P. elongatus | 18.80 | 20.38 | 19.16 | 18.69 | 19.60 | 15.81 | 16.14 | 0.62 |  |  |  |
| P. atavai | 19.32 | 18.57 | 16.22 | 14.83 | 16.61 | 17.97 | 17.76 | 17.78 | 0 |  |  |
| P. mesostigma | 17.64 | 16.76 | 15.08 | 14.83 | 16.49 | 9.07 | 9.30 | 17.50 | 16.89 | 0.93 |  |
| P. zeus, n. sp. | 17.49 | 17.23 | 16.28 | 17.09 | 17.51 | 9.29 | 8.61 | 17.94 | 18.20 | 5.31 | NA |

## P-distances (uncorrected pairwise) for mtDNA COI sequences of 11 species of Pseudojuloides

Minimum Interspecific and Maximum Intraspecific Distances (\%)

|  | cer | pyr | xan | pol | kal | edw | sev | elo | ata | mes | zeu |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| P. cerasinus | 0.18 |  |  |  |  |  |  |  |  |  |  |
| P. pyrius | 3.40 | NA |  |  |  |  |  |  |  |  |  |
| P. xanthomos | 10.36 | 10.29 | NA |  |  |  |  |  |  |  |  |
| P. polackorum | 8.74 | 8.76 | 7.07 | 0.31 |  |  |  |  |  |  |  |
| P. kaleidos | 9.55 | 9.83 | 4.30 | 7.83 | NA |  |  |  |  |  |  |
| P. edwardi | 14.12 | 13.55 | 13.71 | 12.93 | 14.02 | 0.31 |  |  |  |  |  |
| P. severnsi | 14.34 | 13.82 | 13.67 | 12.75 | 13.98 | 0.46 | 0.92 |  |  |  |  |
| P. elongatus | 16.41 | 17.51 | 16.74 | 16.28 | 17.05 | 14.17 | 14.44 | 0.61 |  |  |  |
| P. atavai | 16.50 | 15.98 | 14.29 | 13.21 | 14.59 | 15.67 | 15.51 | 15.67 | 0 |  |  |
| P. mesostigma | 15.37 | 14.75 | 13.36 | 13.21 | 14.44 | 8.41 | 8.60 | 15.51 | 14.90 | 0.92 |  |
| P. zeus, n. sp. | 15.20 | 15.05 | 14.29 | 14.90 | 15.21 | 8.57 | 7.99 | 15.82 | 15.82 | 5.07 | NA |

nearest relatives (Steinke et al. 2009, Ward et al. 2009, Victor 2015). Our genetic results agree with the close relationship between $P$. zeus and P. mesostigma based on appearance; the two species share a branch on the neighbor-joining tree and differ by $5.31 \%$ in COI sequence (K2P; $5.07 \%$ uncorrected pairwise). This degree of divergence is similar to that of some other sibling-species pairs in the genus, where, for all but one pair, minimum interspecific distances range from $3.5 \%$ to $20.38 \%$ (K2P distances, vs. $3.4 \%$ to $17.51 \%$ uncorrected pairwise). The maximum intraspecific distances range from 0 to $0.93 \%$ ( 0 to $0.92 \%$ uncorrected pairwise), showing a clear "barcode gap" between species (Table 2). The exception is the species pair of P. edwardi and P. severnsi, which diverge by only $0.46 \%$ (three nucleotides of the $652-\mathrm{bp}$ barcode segment), and may be an example of phenotypic differences outpacing the rate of neutral substitutions in the mitochondrial COI DNA sequence early in the process of speciation (Victor \& Randall 2014, Allan et al. 2015).

Other material of Pseudojuloides examined. P. mesostigma- Vanuatu (aquarium trade), BPBM 41216, 2: 60.660.9 mm, Mar. 15, 2014.

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## References

Allen, G.R. \& Erdmann, M.V. (2012) Reef Fishes of the East Indies, Volume 2. Tropical Reef Research, Perth, Australia, 425-856 pp.
Allen, G.R., Erdmann, M.V. \& Dailami, M. (2015) Cirrhilabrus marinda, a new species of wrasse (Pisces: Labridae) from eastern Indonesia, Papua New Guinea, and Vanuatu. Journal of the Ocean Science Foundation, 15, 1-13.
Bellwood, D.R. \& Randall, J.E. (2000) Pseudojuloides severnsi, a new species of wrasse from Indonesia and Sri Lanka (Perciformes: Labridae). Journal of South Asian Natural History, 5(1), 1-5.
Connell, A.D., Victor, B.C. \& Randall, J.E. (2015) A new species of Pseudojuloides (Perciformes: Labridae) from the south-western Indian Ocean. Journal of the Ocean Science Foundation, 14, 49-56.
Fricke, R., Kulbicki, M. \& Wantiez, L. (2011) Checklist of the fishes of New Caledonia, and their distribution in the Southwest Pacific Ocean (Pisces). Stuttgarter Beiträge zur Naturkunde A, Neue Serie, 4, 341-463.
Ivanova, N.V., Zemlak, T.S., Hanner, R.H. \& Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes, 7, 544-548.
Kuiter, R.H. (2010) Labridae Fishes: Wrasses, First Edition. Aquatic Photographics, Seaford, Australia, 390 pp.

Kuiter, R.H. \& Randall, J.E. (1995) Four new Indo-Pacific wrasses (Perciformes: Labridae). Revue française d'Aquariologie Herpétologie, 21, 107-118.
Nishiyama, K. \& Motomura, H. (2012) Photographic Guide to Wrasses of Japan. Toho Publishing, Inc., Osaka, Japan [In Japanese], 302 pp.
Randall, J.E. \& Pyle, R.L. (2001) Three new species of labrid fishes of the genus Cirrhilabrus from islands of the tropical Pacific. Aqua, Journal of Ichthyology and Aquatic Biology, 4(3), 89-98.
Randall, J.E. \& Randall, H.A. (1981) A revision of the labrid fish genus Pseudojuloides, with descriptions of five new species. Pacific Science, 35, 51-74.
Randall, J.E., Williams, J.T., Smith, D.G., Kulbicki, M., Tham, G.M., Labrosse, P., Kronen, M., Clua, E. \& Mann, B.S. (2003) Checklist of the shore and epipelagic fishes of Tonga. Atoll Research Bulletin, 502, 1-35.

Ratnasingham, S. \& Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes, 7(3), 355-364.
Steinke, D., Zemlak, T.S., \& Hebert, P.D.N. (2009) Barcoding Nemo: DNA-Based Identifications for the Ornamental Fish Trade. PLoS ONE 4(7) e6300. doi:10.1371/journal.pone. 0006300
Victor, B.C. (2015) How many coral reef fish species are there? Cryptic diversity and the new molecular taxonomy. In: Mora, C. (Ed.), Ecology of Fishes on Coral Reefs: The Functioning of an Ecosystem in a Changing World. Cambridge University Press, Cambridge, United Kingdom, pp. 76-87.
Victor, B.C. \& Randall, J.E. (2014) Pseudojuloides edwardi, n. sp. (Perciformes: Labridae): an example of evolution of male-display phenotype outpacing divergence in mitochondrial genotype. Journal of the Ocean Science Foundation, 11, 1-12.
Ward, R.D., Hanner, R. \& Hebert, P.D.N. (2009) The campaign to DNA barcode all fishes, FISH-BOL. Journal of Fish Biology, 74, 329-356.

Appendix 1. Specimen data and GenBank accession numbers for the mtDNA COI barcode sequences used to generate the phenogram in Fig. 6, following the order in the tree. Holotype in bold.

| Genus | species | Collection site | Voucher | GenBank \# | Collector/Source |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Pseudojuloides | zeus, n. sp. | Majuro, Marshall Islands | BPBM 41215 | KJ591656 | J. Edward/aq. trade |
| Pseudojuloides | mesostigma | Vanuatu | BPBM 4121660.6 | KP975989 | J. Edward/aq. trade |
| Pseudojuloides | mesostigma | Vanuatu | BPBM 4121660.9 | KP975968 | J. Edward/aq. trade |
| Pseudojuloides | edwardi | Mombasa, Kenya | BPBM 41172 | KJ591643 | J. Edward/aq. trade |
| Pseudojuloides | edwardi | Mombasa, Kenya | je14pe1 | KP975964 | J. Edward/aq. trade |
| Pseudojuloides | edwardi | Mombasa, Kenya | BPBM 4117363.4 | KJ591642 | A. DeJong/aq. trade |
| Pseudojuloides | edwardi | Mombasa, Kenya | BPBM 4117370.6 | KJ591644 | J. Edward/aq. trade |
| Pseudojuloides | severnsi | Philippines | BPBM 4117472.3 | KJ591652 | J. Edward/aq. trade |
| Pseudojuloides | severnsi | New Caledonia | BPBM 4117555.4 | KJ591651 | A. Teitelbaum |
| Pseudojuloides | severnsi | Philippines | M1496 | JQ839573 | D. Bellwood, JCU |
| Pseudojuloides | severnsi | New Caledonia | BPBM 4117570.1 | KJ591655 | A. Teitelbaum |
| Pseudojuloides | severnsi | Indonesia | je13ps | KJ591653 | J. Edward/aq. trade |
| Pseudojuloides | severnsi | New Caledonia | qm14ps2 | KJ591654 | A. Teitelbaum |
| Pseudojuloides | severnsi | Philippines | BPBM 4117479.8 | JQ839574 | J. Edward/aq. trade |
| Pseudojuloides | kaleidos | aquarium trade | je14pk610 | KP975974 | J. Edward/aq. trade |
| Pseudojuloides | xanthomos | Mauritius | dej13px360 | KJ591657 | A. DeJong/aq. trade |
| Pseudojuloides | polackorum | South Africa | DSFSG592-11 | KF489719 | A. Connell/ SAIAB |
| Pseudojuloides | polackorum | South Africa | DSFSG925-13 | KP975998 | A. Connell/ SAIAB |
| Pseudojuloides | polackorum | Kenya | BPBM 41207 | KP975967 | J. Edward/aq. trade |
| Pseudojuloides | polackorum | Kenya | BPBM 41208 | KP975996 | J. Edward/aq. trade |
| Pseudojuloides | polackorum | South Africa | ac13pc | KP975978 | A. Connell/ SAIAB |
| Pseudojuloides | pyrius | Marquesas Islands | MARQ-424 | KJ591650 | J. Williams/S. Planes |
| 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 | cerasinus | Hawai' ${ }^{\text {i }}$ | h83pc260 | JQ839571 | B. Victor |
| Pseudojuloides | elongatus | New Caledonia | jr14pe3 | KJ591647 | A. Teitelbaum |
| Pseudojuloides | elongatus | New Caledonia | jr14pe2 | KJ591649 | A. Teitelbaum |
| Pseudojuloides | elongatus | New Caledonia | jr14pe1 | KJ591648 | A. Teitelbaum |
| Pseudojuloides | atavai | Rarotonga, Cook Islands | ck98425pa210 | JQ839568 | B. Victor |
| Pseudojuloides | atavai | Moorea, French Polynesia | MBIO1549 | JF435150 | S. Planes/J. Williams |
| Pseudojuloides | atavai | Moorea, French Polynesia | M106 | JQ839569 | D. Bellwood, JCU |
| Pseudojuloides | atavai | Moorea, French Polynesia | MBIO1289 | JF435151 | S. Planes/J. Williams |
| Leptojulis | cyanopleura | Bali, Indonesia | bal11700px 124 | JQ839546 | B. Victor |

