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**Lemonpeel (*Centropyge flavissima*) and yellow (*C. heraldi*) pygmy angelfishes each consist of two geographically isolated sibling species**

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**Abstract** Genetic variation was examined in two complex cases of Indo-Pacific pygmy angelfishes (genus *Centropyge*, Pomacanthidae). The lemonpeel pygmy angelfish *C. flavissima* (Cuvier and Valenciennes) has a geographically disjunct Indian vs. Pacific distribution and the individuals from these two regions differ by their colour patterns. Previous research on *C. flavissima* has shown mitochondrial introgression from two related species, *C. eibli* in the eastern Indian Ocean and *C. vrolikii* in the Pacific Ocean. Using the 16S rDNA and the *CO1* gene as phylogeographic markers, we found no mitochondrial haplotypes in common between Indian-Ocean *C. flavissima* and *C. eibli*, confirming partial genetic isolation, albeit recent. Also, we found substantial genetic differences between Indian and Pacific *C. flavissima* populations at the nuclear *ETS-2* intron locus. The Indian-Ocean form of *C. flavissima*, thus geographically isolated by > 2000 km distance from its Pacific-Ocean counterpart is described as a new species, *Centropyge cocosensis* sp. nov. *Centropyge cocosensis* sp. nov. differs in appearance from *C. flavissima* in having a conspicuous blue iris and a fainter, bluish eye ring. We also found that the yellow pygmy angelfish *C. heraldi* Woods and Schultz consists of two genetically distinct entities, one distributed widely in the northern tropical Indo-West Pacific, the other one distributed in the southern Pacific Ocean. The name originally given to the blackfin pygmy angelfish, *Centropyge woodheadi* Kuitert is here resurrected to designate the latter.

*Keywords:* Indo-West Pacific; colour patterns; molecular taxonomy; mitochondrial introgression

## Introduction

Molecular genetics offers the tools and concepts to address the problem of cryptic species (Knowlton 2000) and increasingly provides evidence to new species discoveries in fishes (e.g., Borsa et al. 2010, 2013a,b, 2014; Baldwin et al. 2011; Liu et al. 2013; Puckridge et al. 2013; Delrieu-Trottin et al. 2014; Durand and Borsa 2015). Examples of geminate coral reef fish species between the Indian Ocean and the Pacific Ocean have been reported (Randall 1999; Woodland 1990, 1999; Craig et al. 2011; Borsa et al. 2014; Woodland and Anderson 2014). Genetic differentiation and subsequent speciation are thought to have been caused by the Indo-Pacific barrier, which extends from the Sunda Shelf to the Sahul Shelf (Rocha et al. 2007). During glacial periods, connectivity between reef fish populations from either ocean was hampered: coral reef habitat suitable to adults was restricted due to lower sea-level and cool upwelling caused by increased land mass, and conditions suitable to larval life were affected by lower salinity and higher turbidity caused by the discharge of large rivers (Fleminger 1986). These geminate or sister Indian-Pacific fish species are morphologically similar but generally differ subtly by their colour patterns (e.g. Craig et al. 2011; Borsa et al. 2014; Woodland and Anderson 2014). Colour patterns in vertebrates are presumed to be subject to intense sexual selection hence are likely to play an essential role in speciation (Endler et al. 2005). Following initial divergence in allopatry, reproductive isolation may have been reinforced (Butlin et al. 2012) despite secondary gene flow after the populations initially separated by the Indo-Pacific barrier have once again expanded and overlapped. However, geographic segregation of color morphs may not necessarily be accompanied by parallel phylogeographic divergence as inferred from the available genetic markers (Schultz et al. 2007, and references therein).

Pygmy angelfishes (genus *Centropyge*, Pomacanthidae) are generally colourful reef fishes. Species in this genus are mostly distinguished by their colour patterns, as meristics and morphometrics usually fail to provide diagnoses (Pyle 2003). While in some cases among Pomacanthidae, sister species distinguished by colour patterns have proven genetically distinct (Randall and Rocha 2009; Shen et al. 2012), in other cases mitochondrial haplotypes of different species within a species complex did not show genetic segregation (Bowen et al. 2006; Rocha et al. 2007) and remarkably distinct colour morphs within another species possessed the same mitochondrial haplotypes (Schultz et al. 2007).

We examined the genetic structure of two Pacific *Centropyge* species, the lemonpeel pygmy angelfish *C. flavissima* (Cuvier and Valenciennes, 1831) and the herald or yellow pygmy angelfish *C. heraldi* Schultz and Woods, 1953 (Schultz et al. 1953). *C. flavissima* is one of the popular angelfishes in the ornamental fish trading market. It is characterized by blue eye ring and blue-margined gill operculum, spine and fins. Its geographic distribution ranges across the western and central Pacific and in the Cocos (Keeling) and Christmas Islands in the northeastern Indian Ocean (Hobbs et al. 2013; Fig. 1). Individuals of the latter population are similar in colour patterns to Pacific-Ocean *C. flavissima* but they have a distinctive blue iris and a fainter bluish eye ring (Fig. 2A, B). It has been emphasized that the population in the northeastern Indian Ocean is isolated by more than 2000 km from the Pacific Ocean populations (Hobbs et al. 2013; Hobbs and Allen 2014). Based on distinct colour patterns and geographic isolation, Allen et al. (1998) hypothesized that the Indian-Ocean and the Pacific-Ocean populations of lemonpeel pygmy angelfish represent separate species. This differs from the view promoted by DiBattista et al. (2012) who reported that the Indian-Ocean *C. flavissima* is genetically indistinct from the distinctly coloured blacktail angelfish *C. eibli* Klausewitz, 1963 while noting that some Pacific-Ocean *C. flavissima* populations are genetically indistinct from the pearlscale angelfish *C. vrolikii* (Bleeker, 1853), the sister-species of *C. eibli* (Fig. 3; DiBattista et al. 2012). The Indian-Ocean form hybridizes with *C. eibli* while the Pacific Ocean form similarly hybridizes with *C. vrolikii* (DiBattista et al. 2012). Three hypotheses may explain the paraphyly of *C. flavissima* haplotypes with *C. eibli* and *C. vrolikii* in the phylogeny of DiBattista et al. (2012) (Fig. 3): (1) incomplete lineage sorting among recently diverged species, (2)

introgression, or (3) different color morphs within a single species (Gaither et al. 2014). The age of the mitochondrial lineages in the *C. flavissima* species complex (3.5–4.2 MYR; Gaither et al. 2014) tends to exclude hypothesis (1), whereas the observation of *C. flavissima* x *C. eibli* and *C. flavissima* x *C. vrolikii* hybrids (DiBattista et al. 2012; Hobbs and Allen 2014) supports hypothesis (2). However, few haplotypes are shared between Indian-Ocean *C. flavissima* and *C. eibli* (DiBattista et al. 2012: their figures 4c and 5a), indicating partial reproductive isolation. A third lineage in the species complex characterizes *C. flavissima* from Moorea in the geographically remote Society archipelago (Fig. 3). *Centropyge vrolikii* being absent from the Society archipelago (Randall 2005), the distinct *C. flavissima* lineage sampled in Moorea is thought to be a relict of the original lineage (DiBattista et al. 2012; Gaither et al. 2014).

The taxonomy of the Indo-Pacific yellow pygmy angelfish *C. heraldi* has also been subject to dispute. The distinctly coloured *C. woodheadi* Kuitert, 1998 was described recently, but was quickly synonymized with *C. heraldi* (Randall and Carlson 2000; Eschmeyer 2014). *Centropyge heraldi* is distributed in the western and central Pacific Ocean while *C. woodheadi* under its current definition occurs in the southern half of the tropical western Pacific Ocean (Fig. 1 B) (Froese and Pauly 2012). *Centropyge woodheadi* has a black blotch of variable size at the rear end of the dorsal fin, absent in *C. heraldi* (Fig. 2C, D). Whether this colour variant deserves species status remains unclear. In the absence of apparent meristic or morphometric differences with *C. heraldi*, the observation of aquarium-kept *C. woodheadi* specimens losing their black pigmentation on the dorsal fin has been interpreted as a confirmation that the differences in colour patterns were merely phenotypic (Randall and Carlson 2000). However, R.H. Kuitert (pers. comm., December 2013) pointed out that the reverse phenomenon, i.e., full-yellow individuals acquiring a black blotch, has not yet been observed in *C. heraldi* from the northern Pacific Ocean. Also, substantial nucleotide divergence at the *CO1* locus between typical *C. heraldi* from the Philippines and Sri Lanka, and the *woodheadi* form of *C. heraldi* collected from Fiji and Tonga has been reported and viewed as supporting the resurrection of *C. woodheadi* as a valid species (Steinke et al. 2009).

The present study focuses on the genetic relationships of *C. flavissima* from the Indian and Pacific Oceans. It also compares the *woodheadi* form to the typical form of *C. heraldi*, using a range of samples from all over the distribution of the two forms. The results, together with colour pattern differences and considerations on distribution patterns lead us to challenge the current taxonomy of both *C. flavissima* and *C. heraldi*.

## Materials and methods

### Sampling

Specimens of the lemonpeel pygmy angelfish (Fig. 2A, B) were obtained from the Cocos (Keeling) Islands ( $N = 6$ ), New Caledonia ( $N = 7$ ), Vanuatu ( $N = 5$ ), Fiji ( $N = 5$ ), Kiribati ( $N = 5$ ) and French Polynesia, including the Australes, Gambier and Marquesas archipelagos (total  $N = 13$ ) (Table 1). In addition, three presumed *C. flavissima* x *C. vrolikii* hybrids were collected, one from New Caledonia and two from Vanuatu (Table 1). The specimens from Cocos, Vanuatu, Fiji and Kiribati were purchased from a trusted commercial importer of aquarium fish in Taiwan. Specimens from New Caledonia and French Polynesia were collected by rotenone poisoning. We also obtained specimens of *C. eibli* from Bali ( $N = 3$ ) and *C. vrolikii* from Cebu ( $N = 5$ ), from aquarium fish traders.

New specimens of the yellow pygmy angelfish included *C. heraldi* from Taiwan ( $N = 1$ ), the Philippines ( $N = 3$ ), Bali ( $N = 2$ ) and an undisclosed sampling site in Indonesia ( $N = 1$ ); *C. heraldi* (*heraldi* form) from the Great Barrier Reef ( $N = 5$ ) and the Gambier archipelago ( $N = 2$ ); and *C. heraldi* (*woodheadi* form) from the Great Barrier Reef ( $N = 5$ ), Vanuatu ( $N = 6$ ), and Fiji ( $N = 2$ ). All new yellow pygmy angelfish specimens

were either obtained from trusted aquarium fish traders (Taiwan, Philippines, Indonesia, Great Barrier Reef, Vanuatu, Fiji) or collected by rotenone poisoning during a dedicated barcoding campaign (Gambier archipelago). The distribution ranges for the nominal form and the *woodheadi* form of *C. heraldi* species are shown in Fig. 1.

A total of 106 wild-caught *Centropyge* spp. specimens was analysed for this study (Table 1). From these, 74 came from aquarium retailers; this includes 17 specimens from Sri Lanka, Vietnam, Philippines and Tonga barcoded by Steinke et al. (2009). The remainder ( $N = 32$ ) were collected during dedicated barcoding campaigns.

## Genetic analyses

A small piece of muscle tissue was excised from the dorsal part of the body and preserved in 95% ethanol prior to DNA extraction. Genomic DNA was extracted using the DNA purification kit of Bioman (Taipei), preserved in Tris-EDTA buffer, and then quantified and diluted to 1 ng/ $\mu$ l prior to polymerase chain reaction (PCR). Two mitochondrial genes (*16S* and *CO1*) and an intron of a nuclear oncogene (*ETS-2*) were used as genetic markers. Polymerase chain reaction (PCR) followed protocols detailed in Shen et al. (2012). Sequences were analyzed in an automated ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA) at the Taiwan Normal University Sequencing Facility (Taipei).

Nucleotide sequences, including those obtained in the present study and homologous sequences retrieved from the GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov/>) were aligned using BIOEDIT (Hall 1999). Individual nucleotide sequences of the *16S* rDNA ( $N = 88$  ingroup sequences), the *CO1* gene ( $N = 101$ ) and the *ETS-2* intron ( $N = 63$ ) (Table 1) were aligned over 597 bp, 647 bp, and 439 bp, respectively. For the *16S* rDNA fragment, the first nucleotide of the alignment started at the nucleotide site homologous to nucleotide site no. 1099 of the *16S* rDNA of flame angelfish *C. loriculus* (GenBank no. NC\_009872); for the *CO1* fragment, the alignment started at homologous site no. 52 of the *CO1* gene in the same species. The numbering of nucleotides of the *ETS-2* intron fragment was the same as on the homologous fragment in *C. nox* previously deposited in GenBank (JQ904576). Prior to phylogenetic analysis, the matrix of *ETS-2* genotypes was transformed into a matrix of haplotypes ( $N = 126$ ) using PHASE v. 2.1 (Stephens et al. 2001) implemented in DNASP v. 5.10 (Librado and Rozas 2009).

## Data analysis

Phylogenetic trees were reconstructed using the maximum-likelihood (ML) and Neighbor-Joining (NJ) algorithms implemented in MEGA6 (Tamura et al. 2013). M. Kimura's 2-parameter model with gamma-distributed rate variation among sites (K2+G) was selected as the best model of nucleotide substitution according to the Bayesian information criterion (MEGA6) for each the *16S* rDNA, the *CO1* gene, and the *ETS-2* intron marker. All sequences obtained in this study were deposited in GenBank (see Table 1). Homologous sequences of sixbar angelfish *P. sexstriatus* and yellowface angelfish *Pomacanthus xanthurus* (GenBank nos. KJ542547, KJ542548, KJ551856-KJ551858, KJ624708, KJ624709, KJ624691-KJ624693) were used as outgroups.

Hierarchical analysis of molecular variance (AMOVA: Excoffier et al. 1992) on each *16S*, *CO1* and *ETS2* sequence datasets for each *C. flavissima* and *C. heraldi* was done under ARLEQUIN v. 3.5 (Excoffier and Lischer 2010) to examine the partitioning of the total variance among regional groups of samples. Two groups of populations, Indian vs. Pacific, were considered in *C. flavissima* based on patterns of geographic distribution (Fig. 1B). Two groups, northern Pacific vs. southern Pacific, were similarly considered in *C. heraldi* (Fig. 1C). Data files were prepared under DNASP. The best substitution model according to the Bayesian information

criterion was determined for each dataset with MEGA6. Significance of  $\Phi$ -statistics and associated variance components was tested by 1000 random permutations (ARLEQUIN).

#### Measurements on type specimens

Morphometric measurements and meristic counts were done on the type material of the Indian-Ocean form of the lemonpeel pygmy angelfish, following Randall and Rocha (2009). Standard length, body depth, caudal-peduncle length, caudal-peduncle depth, pre-dorsal length, pre-anal length, pre-pelvic length, lengths of the fin spines and rays, and caudal fin length were taken from X-ray images of the specimens, using the X-ray imager (XL-100, Laiko Co., Tokyo) of the National Museum of Marine Biology and Aquarium in Pingtung (Shen et al. 2012). The other measurements (Supplementary Table S1) were taken directly on the fish using electronic calipers and were rounded to the nearest 0.1 mm. Dorsal-, anal- and caudal-fin spines and rays were counted from X-ray images (Shen et al. 2012).

## Results

#### Hierarchical analysis of molecular variance

The components of molecular variance according to the hierarchical level of grouping are summarized in Table 2. Here, we are interested in the percentage of genetic variation that can be assigned to differences between groups of populations defined geographically. Fisher's combined probability test (Sokal and Rohlf 1969) on the exact probabilities of the  $\Phi_{CT}$  values allowed the rejection of the null hypothesis ( $\Phi_{CT} = 0$ ; no genetic differentiation between groups) in both *C. flavissima* ( $P = 0.043$ ) and *C. heraldi* ( $P < 0.001$ ). The  $\Phi_{CT}$  values in both cases ( $\Phi_{CT} = 0.438-0.484$  in *C. flavissima*;  $\Phi_{CT} = 0.752-0.900$  in *C. heraldi*) corresponded to high levels of genetic differentiation indicative of separate species (see Fauvelot and Borsa 2011; Borsa et al. 2012; and Borsa et al. 2016 for comparisons of estimates of genetic differentiation across shorefish species, where values  $> 0.400$  are observed between species in species complexes, not among populations within a species).

#### Mitochondrial phylogeny of four yellow Indo-Pacific *Centropyge* spp.

In both *16S* rDNA and *CO1* gene phylogenies (Figs. 4A, 4B), *C. flavissima* mitochondrial haplotypes from Rapa and the Gambier archipelago formed a subclade sister to all the other *C. flavissima*. This subclade also included haplotypes from Moorea as seen from the *CO1* tree (Fig. 4B). Within the main *C. flavissima* subclade, the Marquesas haplotypes represented a distinct lineage. Haplotypes of the Cocos (Keeling) Islands form of the lemonpeel pygmy angelfish belonged to a subclade distinct from Pacific *C. flavissima* (Figs. 4A, 4B). Haplotypes of the Cocos form were closest to *C. eibli* although distinct from the latter, as visible from the *CO1* phylogeny (Fig. 4B) and from analysis of the concatenated sequence dataset (Supplementary material, Figs. S2 and S3); *C. vrolikii* haplotypes were shared with those of western-Pacific Ocean *C. flavissima*, i.e., those sampled in New Caledonia, Vanuatu, Fiji, and Kiribati.

Haplotypes of the *woodheadi* form of *C. heraldi* clustered with all other southern-Pacific Ocean *C. heraldi*, as a subclade sister to northern-Pacific Ocean and Indian-Ocean *C. heraldi* (Figs. 4A, 4B; Supplementary material, Figs. S2 and S3).

#### Nuclear phylogeny

The Cocos (Keeling) Islands and the Pacific forms of lemonpeel pygmy angelfish mostly differed by a single G/C transversion along the 438 bp-long fragment of the *ETS-2* intron. Eight out of 12 *ETS-2* haplotypes sampled from Cocos possessed C (4/12 with G) at nucleotide site 180 of the fragment while all 23 *C. flavissima* individuals sampled from the Pacific Ocean were homozygous for nucleotide G. The presence of C at nucleotide site no. 180 was systematically associated with T at sites nos. 219 and 225 while G at site no. 180 was mostly associated with C at sites nos. 219 and 225, thus defining two major alleles in lemonpeel pygmy angelfish, hereafter coined *CTT* and *GCC*. One individual from the Cocos population of the lemonpeel pygmy angelfish was heterozygous for the possibly recombinant *CCC* allele and another individual was heterozygous for the possibly recombinant *CTC* allele. Allele *CTT* was dominant in frequency in the Cocos population and absent from Pacific *C. flavissima* and *C. eibli* samples. Two alleles, *GCC* and *GTC*, were recorded in *C. vrolikii*. In the nuclear phylogeny (Fig. 4C), haplotypes sampled in the Cocos form of lemonpeel pygmy angelfish generally clustered separately from the other *C. flavissima* haplotypes. The latter were shared or mixed with *C. eibli* and *C. vrolikii* haplotypes and with 2/12 haplotypes from the Cocos lemonpeel pygmy angelfish. In the NJ tree (not shown) all Cocos lemonpeel haplotypes clustered as a single lineage.

All *ETS-2* intron haplotypes of the *woodheadi* form of *C. heraldi* clustered together with all other southern-Pacific *C. heraldi*, separately from northern-Pacific *C. heraldi* (Fig. 4C). Northern-Pacific *C. heraldi* intron haplotypes were characterized by T at nucleotide site no. 90 and C at site no. 187, whereas the *woodheadi* form and southern-Pacific *C. heraldi* possessed A and T, respectively, at these sites.

## Morphology

For the sake of formal taxonomic description, morphological measurements for four lemonpeel pygmy angelfish individuals from the Cocos (Keeling) Islands, including the holotype and the three paratypes, are presented in Supplementary Table S1. The only salient feature that distinguished Indian-Ocean *C. flavissima* from its Pacific counterpart was the colour of the iris (Fig. 2), as noted previously (e.g. DiBattista et al 2012: their figure 2).

## Discussion

Species in the *C. flavissima* species complex (*C. flavissima*, *C. eibli*, and *C. vrolikii*) are represented by deep mitochondrial lineages that characterize geographic region rather than species designation as based on colour patterns. It has been proposed that ancient mitochondrial divergences between species within this complex have been erased by subsequent introgression (DiBattista et al. 2012; Gaither et al. 2014). In the present study, the phylogenetic analyses based on both mitochondrial and nuclear genes showed that the Indian-Ocean form of the lemonpeel pygmy angelfish is genetically different not only from its counterpart *C. flavissima* from the western and central Pacific, but also from the sympatric *C. eibli* and from *C. vrolikii*. This difference in haplotype composition indicates that despite inferred past introgression between the Cocos form of the lemonpeel pygmy angelfish and *C. eibli*, gene flow between the two species has since been low or even, perhaps, absent. Species are separately evolving segments of the global genealogical network of living organisms (de Queiroz 2007; Barberousse and Samadi 2010). To hypothesize the existence of distinct species, researchers practically have access to empirical lines of evidence which include reproductive isolation from other species, the possession of fixed character state differences, and monophyly (de Queiroz 2007). Hampered gene flow in a situation of sympatry implies some degree of reproductive isolation (Mayr 1942), an expected property of separate species. Reproductive isolation, separation of mitochondrial haplotypes, and partial separation of nuclear haplotypes thus provide evidence that the Cocos form of the lemonpeel pygmy

angelfish is an entity genetically independent from the sympatric *C. eibli*. The occurrence of *C. cocosensis* sp. nov. x *C. eibli* hybrids at Cocos (Keeling) Islands (Hobbs and Allen 2014) does not contradict our conclusion. When confronted with an apparent deficit of shared haplotypes between the two species (DiBattista et al. 2012), this observation actually indicates that hybrids have lower survival or fertility than individuals from the parental species. Also, under the hypothesis of a single species, one would expect to observe a gamut of hybrid colour morphs against only a small proportion of pure morphs, something that has not yet been reported (DiBattista et al. 2012; Hobbs and Allen 2014).

The Indian-Ocean form of the lemonpeel pygmy angelfish has a unique blue iris that is absent in *C. flavissima* and it has a much less pronounced blue eye ring than that of *C. flavissima*. The blue iris is also characteristic of the local lemonpeel pygmy angelfish population from Christmas Island (Allen and Erdmann 2012; Froese and Pauly 2012). Colour differences are maintained despite gene flow between *C. cocosensis* and *C. eibli* in the Indian Ocean, as they are between *C. flavissima* and *C. vrolikii* in the Pacific Ocean. The loci involved in colour differentiation are impermeable to gene flow, revealing a reproductive barrier – at least a partial one – which is the biological signature of separate species. Here, we use the conspicuous iris colour as the character that diagnoses the geographically and genetically partly-isolated Indian-Ocean lemonpeel pygmy angelfish as a separate species. The evolutionary mechanisms that have led to the colour differences between the Indian and Pacific forms of the lemonpeel pygmy angelfish may include genetic drift, enhanced by the presumably small effective population size of the Indian-Ocean form, and sexual selection. Sexual selection may also be invoked to explain the dominance of the pure parental morphs over hybrids. Further research is warranted, to test these hypotheses.

Six nominal species are currently considered synonyms of *C. flavissima* (Eschmeyer 2014). These are *Holacanthus cyanotis* Günther, 1860, *H. luteolus* Cuvier and Valenciennes, 1831, *H. monophthalmus* Kner, 1867, *H. ocellularis* Peters, 1868, *H. sphynx* DeVis, 1864 and *H. uniozellatus* Borodin, 1932. The type localities of the foregoing species are in the Pacific Ocean, except *H. ocellularis* which is from the “*Süidsee*”, a term that designates both the tropical Indian and Pacific Oceans. However, *H. ocellularis* is described as “*ganz gelb*” and has “*um jedes Auge ein blauer schwarz eingefasster Ring und auf dem hinteren Rande des Operculums eine senkrechte, hellblaue schwarz eingefasste Binde*” (Peters 1868). Thus, the description of W.C.H. Peters’ *H. ocellularis* colour patterns is typical of Pacific *C. flavissima*, leaving the Indian-Ocean form of the lemonpeel pygmy angelfish still undescribed, hence clearing the way for its description as a new species (International Commission on Zoological Nomenclature 1999).

*Centropyge heraldi* under its current definition is distributed throughout the central and western Pacific while the blackfin or *woodheadi* form solely occurs in southern tropical western and central Pacific. The two forms co-occur in the north-western part of the Coral Sea (Debelius et al. 2003), in Fiji and in the Society Islands (Randall and Carlson 2000) but not in Samoa where the blackfin form is the sole present (Randall and Carlson 2000). The present study indicates that *C. heraldi* actually consists of two genetically distinct antitropical populations while no genetic character allowed the distinction of the *woodheadi* form from sympatric, southern-Pacific *C. heraldi*. Reciprocal monophyly characterized the southern-Pacific vs. northern-Hemisphere populations. Therefore, the southern-Pacific and the northern-Hemisphere populations of *C. heraldi* should be considered as separate evolutionary units. Epithet *woodheadi*, which is available, should be resurrected for the southern-Pacific population.

## Taxonomy

*Centropyge cocosensis*, new species <http://zoobank.org/4B6BB6D8-9B7D-4128-B7FD-F8AA1D04693E>

Previous references. *Centropyge flavissima* (Allen et al. 1998; Allen et al. 2003; Allen and Erdmann 2012; DiBattista et al. 2012; Gaither et al. 2014; Hobbs and Allen 2014).

*Vouchers.* The type material of *C. cocosensis* sp. nov. was deposited at the National Museum of Marine Biology and Aquarium (NMMB) in Pingtung, Taiwan and at the United States National Museum (USNM) in Washington DC, USA under nos. NMMB-P19870 (paratype), NMMB-P19875-1 (paratype), NMMB-P19875-2 (holotype) and USNM 410766 (paratype). Two additional vouchers were deposited at NMMB (Table 1). All the foregoing specimens were collected from the Cocos (Keeling) Islands.

*Description.* Meristic counts and morphological measurements on holotype and paratypes are presented in Supplementary Table S1. The morphological description and the *16S*, *CO1* and *ETS2* nucleotide sequences of the holotype are also provided in Supplementary Material.

*Comparison with closely related species.* *Centropyge cocosensis* sp. nov. is closely related to the group of pygmy angelfishes which includes *C. eibli*, *C. flavissima* and *C. vrolikii*. The four species cluster as a single clade in the multiple-locus phylogeny of the group (Gaither et al. 2014). Both *C. cocosensis* sp. nov. and *C. flavissima* possess a blue eye ring, but the eye ring is paler, narrower and fainter in the former.

*Diagnosis.* Colour of live specimens lemonpeel yellow with blue margined gill operculum, spine and fins; conspicuous blue iris (iris is golden yellow in *C. flavissima*) and faint blue eye ring surrounding the eye (Fig. 2A); colour in alcohol uniformly yellowish with dark eye and dark posterior dorsal-, anal- and caudal-fin margins. *C. cocosensis* sp. nov. can be separated from *C. flavissima* and the two other species of the *C. flavissima* species complex by nucleotide quasi-synapomorphies at the *CO1* locus. These include G at both nucleotide sites 247 and 366 of the gene.

*Distribution.* Known from the Cocos (Keeling) Islands (present study) and from the nearby Christmas Island in the East Indian Ocean (Allen et al. 1998; DiBattista et al. 2012; Hobbs and Allen 2014).

*Etymology.* Epithet *cocosensis* is the latin derivation of Cocos, from Cocos (Keeling) Islands, the type locality of the new species. As vernacular name, we propose that the new species be called Cocos pygmy angelfish.

*Remarks.* In their original description of *C. flavissima* (“*L’Holacanthé tout-jaune*”) from the drawing of a specimen from Ulea, Cuvier and Valenciennes (1831) did not mention the blue eye-ring and other blue ornaments. It is likely that the blue colour of the specimen had faded after death. The type locality (Ulea) is in the Central Pacific, hence there is no ambiguity that the two authors referred to the Pacific form of *C. flavissima*.

*Centropyge woodheadi* Kuitert, 1998, resurrected species

*Previous references.* *Centropyge woodheadi* (Kuitert 1998; Seeto and Balwin 2010); *C. heraldi* (non Woods and Schultz, 1953) (Randall 1997, 2005; Laboute and Grandperrin 2000; Randall and Carlson 2000; Allen et al. 2003; Steinke et al. 2009).

*Re-description.* *Centropyge woodheadi* is here re-described from nucleotide sequences at the *16S* rDNA, *CO1* gene and *ETS-2* intron. Based on the individual sequences listed in Table 1, *C. woodheadi* possesses (C, T) at nucleotide site positions nos. (1378, 1479) of the *16S* rDNA; (A, G, A, G, C, C, C, G, T, A, G, G, C, T) at nucleotide site positions nos. (147, 168, 255, 342, 444, 453, 495, 540, 621, 630, 645, 678, 687, 690) of the *CO1* gene; and (A, T) at nucleotide site positions nos. (90, 187) of the *ETS-2* fragment. The foregoing molecular characters distinguish it from *C. heraldi* under its present new definition (see next sub-section).

*Distribution.* Western and central South Pacific Ocean, from the Great Barrier Reef to the Gambier archipelago (Table 1; Fig. 1C).

*Suggested vernacular name.* Keeping the vernacular name coined previously for *C. woodheadi* (the “blackfin pygmy angelfish”; Kuitert 1998) could be misleading. This fish is called *paraharaba* in Tahiti (Froese and Pauly 2012). We suggest this name be used to distinguish *C. woodheadi* under its present, new definition, from the yellow pygmy angelfish, *C. heraldi*.

*Centropyge heraldi* Woods and Schultz, 1953

*Re-description.* *Centropyge heraldi* is here re-described from nucleotide sequences at the *16S* rDNA, *CO1* gene and *ETS-2* intron. Based on the sample of individual sequences listed in Table 1, *C. heraldi* possesses (T, C) at nucleotide site positions nos. (1378, 1479) of the *16S* rDNA; (C, A, G, A, T, T, T, A, A, C, A, A, T, C) at nucleotide site positions nos. (147, 168, 255, 342, 444, 453, 495, 540, 621 630, 645, 678, 687, 690) of the *CO1* gene; and (T, C) at nucleotide site positions nos. (90, 187) of the *ETS-2* fragment. The foregoing molecular characters distinguish it from *C. woodheadi* under its present new definition.

*Distribution.* In the Indian Ocean, *C. heraldi* is known from Sri Lanka (Steinke et al. 2009) and Bali (present study) whereas in the Pacific Ocean it has a wide distribution, from the Indo-Malay archipelago to the Marshall Islands (Table 1; Fig. 1C).

## Notice

The present article in portable document (.pdf) format is a published work in the sense of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 2012) or Code and hence the new names contained herein are effectively published under the Code. This published work and the nomenclatural acts it contains have been registered in ZooBank (<http://zoobank.org/>), the online registration system for the International Commission on Zoological Nomenclature. The ZooBank life science identifier (LSID) for this publication is 4B6BB6D8-9B7D-4128-B7FD-F8AA1D04693E. The online version of this work is archived and available from the *Marine Biodiversity* and Hal-IRD repository (<http://www.hal.ird.fr/>) websites.

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## Appendix: Supplementary material

Supplementary material associated with this article can be found, in the online version, at <http://xxx>

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**Table 1** Specimens of pygmy angelfishes (*Centropyge* spp.) of the *C. flavissima* and *C. heraldi* species complexes analyzed for nucleotide sequence variation at the *16S*, *CO1*, and *ETS-2* loci, with sampling details, specimen numbers, and GenBank accession numbers. **BOLD**: Barcode of Life Database (<http://www.barcodinglife.com/>); **MNHN**: Muséum national d'histoire naturelle, Paris; **NMMB**: National Museum of Marine Biology and Aquarium, Pingtung; **USNM**: United States National Museum, Washington DC; *N*: sample size

| Species,<br>Sampling location                          | Sampling date  | <i>N</i> | Individual no.     | Voucher                                     | Photo-<br>graph | Locus  |                     |                    |
|--|----------------|----------|--------------------|---|-----------------|--|---------------------|--------------------|
|  |                |          |                    |   |                 | <i>16S</i>   | <i>CO1</i>          | <i>ETS-2</i>       |
| <i>C. cocosensis</i> sp. nov.                          |                |          |                    |   |                 |  |                     |                    |
| Cocos (Keeling) Is.                                    | Aug. 2010      | 1        | 104                | NMMB-P19870 (paratype)                      | -               | KJ551861   | KJ534314            | KJ624654           |
| Cocos (Keeling) Is.                                    | Oct. 2011      | 1        | 173                | NMMB-P19875-1 (paratype)                    | Fig. 2A         | KJ551862   | KJ534315            | KJ624655           |
| Cocos (Keeling) Is.                                    | Oct. 2011      | 1        | 174                | NMMB-P19875-2 (holotype)                    | -               | KJ551863   | KJ534316            | KJ624656           |
| Cocos (Keeling) Is.                                    | Oct. 2011      | 1        | 318                | USNM 410766 (paratype)                      | -               | KJ643461   | KJ534317            | KJ624659           |
| Cocos (Keeling) Is.                                    | Feb. 2012      | 1        | 359                | NMMB-P20395                                 | -               | KJ551865   | KJ534318            | KJ624658           |
| Cocos (Keeling) Is.                                    | Dec. 2013      | 1        | P4                 | NMMB-P20399                                 | -               | KJ551864   | KJ534319            | KJ624657           |
| <i>C. eibli</i>  |                |          |                    |   |                 |  |                     |                    |
| Vietnam  | Oct. 2006      | 2        | -                  | HLC-15215, -15216                           | <b>BOLD</b>     | -  | FJ5829560, FJ582959 | -                  |
| Bali   | Jun. 2010      | 1        | 48                 | NMMB-P19743                                 | -               | KJ551867   | KJ534321            | -                  |
| Bali   | Sep. 2010      | 1        | 113                | NMMB-P19745                                 | -               | KJ551866   | KJ534320            | KJ624660           |
| Bali   | Dec. 2013      | 1        | P17                | NMMB-P20397                                 | -               | KJ551868   | KJ534322            | KJ624661           |
| <i>C. flavissima</i>                                   |                |          |                    |   |                 |  |                     |                    |
| New Caledonia  | Dec. 2010      | 7        | 45-51              | MNHN IC-2010-1332 to -1334                  | <b>BOLD</b>     | -  | KJ542555-KJ542561   | KJ624695- KJ624697 |
| Vanuatu  | May 2010       | 5        | 73-77              | NMMB-P19868-1 to -5                         | -               | KJ551870, KJ551873-<br>KJ551876                        | KJ534323-KJ534327   | KJ624662-KJ624665  |
| Fiji   | Dec. 2013      | 1        | P5                 | NMMB-P20403-1                               | Fig. 2B         | KJ551878   | KJ534351            | KJ624688           |
| Fiji   | Dec. 2013      | 4        | P6, P11-P13        | NMMB-P20403-2 to -5                         | -               | KJ551880, KJ551882,<br>KJ551886, KJ551888              | KJ534352-KJ534354   | KJ624689-KJ624690  |
| Nuku'alofa, Tonga                                      | Sep. 2005      | 1        | -                  | HLC-10882                                   | <b>BOLD</b>     | -  | FJ582964            | -                  |
| Rapa, Australes Is.                                    | Nov. 2002      | 6        | 18, 20-24          | -   | -               | KJ643441, KJ643443-<br>KJ643447                        | KJ624645-KJ624650   | KJ624702-KJ624705  |
| Moorea, Society Is.                                    | Mar. 2006      | 4        | 556, 557, 558, 559 | MNHN 2008-720, 2008-220, 2008-219, 2008-719 | <b>BOLD</b>     | -  | JQ431556-JQ431559   | -                  |
| Moorea, Society Is.                                    | Fev. 2009      | 1        | 941                | FLMOO 446                                   | <b>BOLD</b>     | -  | KJ967941            | -                  |
| Line Is., Kiribati                                     | Jan. 2014      | 5        | P19-P23            | NMMB-P20404-1 to -5                         | -               | KJ551881, KJ551884,<br>KJ551885, KJ551887,<br>KJ551889 | KJ534328-KJ534332   | KJ624666-KJ624669  |
| Gambier  | Sep.-Oct. 2010 | 2        | F1, F2             | GAM-039, GAM-786                            | -               | KJ643457, KJ643458                                     | KJ624651, KJ624652  | KJ624706           |
| Marquesas Islands                                      | Oct. 2011      | 7        | 28-31, 35-37       | -   | -               | KJ643450-KJ643456                                      | KJ624640-KJ624644   | KJ624698-KJ624701  |
| <i>C. flavissima</i> x <i>C. vrolikii</i> <sup>a</sup> |                |          |                    |   |                 |  |                     |                    |
| New Caledonia  | Dec. 2010      | 1        | NC                 | MNHN IC-2010-1535                           | -               | KM453706   | KM453711            | KM453716           |
| Vanuatu  | Dec. 2009      | 1        | 54                 | NMMB-P20394                                 | -               | KM453707   | KM453712            | KM453718           |
| Vanuatu  | Jul. 2012      | 1        | 336                | NMMB-P19867                                 | -               | KM453708   | KM453713            | KM453717           |

|                                  |                |   |                    |                                   |         |  |  |                    |
|----------------------------------|----------------|---|--------------------|-----------------------------------|---------|--|--|--------------------|
| <i>C. beraldi</i> <sup>b</sup>   |                |   |                    |                                   |         |  |  |                    |
| Sri Lanka                        | Oct. 2006      | 1 | 966                | HLC-15168                         | -       | -                                      | FJ582966                               | -                  |
| Taiwan                           | Dec 2013       | 1 | P3                 | NMMB-P20400                       | Fig. 2D | KJ551909                               | KJ534345                               | KJ624682           |
| Philippines                      | -              | 4 | 968, 969, 972, 973 | HLC-11144, -11143, -10794, -10793 | BOLD    | -                                      | FJ582968, FJ582969, FJ582972, FJ582973 | -                  |
| Philippines                      | Jul. 2010      | 1 | 116                | NMMB-P19872                       | -       | KJ551906                               | KJ534343                               | KJ624679           |
| Philippines                      | Aug. 2012      | 1 | 156                | NMMB-P19876-1                     | -       | KJ551907                               | KJ534344                               | KJ624680           |
| Philippines                      | Aug. 2012      | 1 | 324                | NMMB-P19876-2                     | -       | KJ551908                               | KM434228                               | KJ624681           |
| Bali                             | Feb. 2014      | 2 | HB1, HB2           | NMMB-P20756 -1, -2                | -       | KM453709, KM453710                     | KM453714, KM453715                     | KM453719, KM453720 |
| Indonesia                        | Apr. 2012      | 1 | 408                | NMMB-P20759                       | -       | KJ551910                               | KM434227                               | KM434230           |
| <i>C. vrolikii</i>               |                |   |                    |                                   |         |  |  |                    |
| Cebu                             | Feb. 2010      | 3 | Cebu3, 4, 5        | NMMB-P20754 -1 to -3              | -       | KJ551871, KJ551872, KJ551877           | KJ542550-KJ542552                      | KJ764882           |
| Cebu                             | Dec. 2013      | 2 | P15, P16           | NMMB-P20396, 20398                | -       | KJ551879, KJ551883                     | KJ542553, KJ542554                     | KJ624694, KJ764883 |
| Cebu                             | Dec. 2009      | 1 | 55                 | NMMB-P20758                       | -       | KJ551869                               | KJ542549                               | KJ764884           |
| Philippines                      | Sep.-Dec. 2005 | 4 | -                  | HLC-11065, -11638, -11639, -11715 | BOLD    | -                                      | FJ583001, FJ582998, FJ582997, FJ583002 | -                  |
| Nuku'alofa, Tonga                | Sep. 2005      | 2 | -                  | HLC-11078, -11079                 | BOLD    | -                                      | FJ582996, FJ582999                     | -                  |
| <i>C. woodbeadi</i>              |                |   |                    |                                   |         |  |  |                    |
| Great Barrier Reef <sup>c</sup>  | Feb. 2011      | 2 | 144,146            | NMMB-P19873-1, -2                 | -       | KJ551890, KJ551891                     | KJ534333, KJ534334                     | KJ624670, KJ624671 |
| Great Barrier Reef <sup>c</sup>  | Dec. 2013      | 2 | P1, P14            | NMMB-P20401-1, -3                 | -       | KJ551903, KJ551911                     | KJ534335, KJ534337                     | KJ624672, KJ624674 |
| Great Barrier Reef <sup>c</sup>  | Dec. 2013      | 1 | P7                 | NMMB-P20401-2                     | Fig. 2C | KJ551898                               | KJ534336                               | KJ624673           |
| Great Barrier Reef <sup>d</sup>  | Dec 2013       | 5 | P2, P8-P10, P18    | NMMB-P20402-1 to -5               | -       | KJ551899, KJ551901, KJ551892, KJ551905 | KJ534338-KJ534342                      | KJ624675-KJ624678  |
| Vanuatu <sup>c</sup>             | Feb. 2011      | 2 | 303, 304           | NMMB-P19874-1, -2                 | -       | KJ551892, KJ551895                     | KJ534347, KJ534348                     | KJ624685, KJ624686 |
| Vanuatu <sup>c</sup>             | Apr. 2011      | 2 | 151,152            | NMMB-P19877-1, -2                 | -       | KJ551893, KJ551894                     | KJ534345, KJ534346                     | KJ624683, KJ624684 |
| Vanuatu <sup>c</sup>             | Feb. 2013      | 1 | I003               | NMMB-P19880                       | -       | KJ551904                               | KJ534349                               | KJ624687           |
| Vanuatu <sup>c</sup>             | Feb. 2014      | 1 | 387                | NMMB-P20760                       | -       | KM434225                               | KM434226                               | KM434229           |
| Fiji <sup>c</sup>                | May 2013       | 2 | I001, I002         | NMMB-P19881-1, -2                 | -       | KJ551896, KJ551897                     | KJ534350                               | KJ764880, KJ764881 |
| Nuku'alofa, Tonga <sup>c</sup>   | Sep. 2005      | 3 | 965, 967, 970      | HLC-15119, -12131, -11027         | BOLD    | -                                      | FJ582965, FJ582967, FJ582970           | -                  |
| Moorea, Society Is. <sup>d</sup> | Mar. 2006      | 2 | 560, 561           | MBIO1259.4, MBIO974.4             | BOLD    | -                                      | JQ431560, JQ431561                     | -                  |
| Gambier <sup>d</sup>             | Sep.-Oct. 2010 | 2 | H1, H2             | GAM-085, GAM-416                  | -       | KJ643459, KJ643460                     | KJ624653                               | KJ624707           |

<sup>a</sup> presumed hybrids

<sup>b</sup> as originally defined (Schultz et al. 1953); the full-yellow *C. beraldi* individuals sampled from the western and central South Pacific were eventually determined as *C. woodbeadi* based on molecular markers (present study)

<sup>c</sup> *woodbeadi* form as originally defined (Kuiter 1998), i.e. possessing a black blotch at the rear end of the dorsal fin

<sup>d</sup> *beraldi* form, i.e., without black blotch on dorsal fin, determined as *C. woodbeadi* from molecular markers (present study)

**Table 2** Analysis of molecular variance (AMOVA; Excoffier et al. 1992) among populations of the *Centropyge flavissima* and *C. heraldi* species complexes based on the geographic partition presented in Fig. 1A (*C. flavissima* vs. *C. cocosensis* sp. nov.) and Fig. 1B (*C. heraldi* vs. *C. woodheadi*); the Sri Lanka and Bali *C. heraldi* samples were arbitrarily grouped with northern tropical Pacific *C. heraldi*

| Species complex,<br>Source of variation,<br>Marker | d.f. | % variation | F-statistic        | P      |
|--|------|-------------|--------------------|--------|
| <i>C. flavissima</i>                               |      |             |                    |        |
| Among groups                                       |      |             |                    |        |
| 16S  | 1    | 44.1        | $\Phi_{CT}=0.440$  | 0.114  |
| CO1  | 1    | 43.8        | $\Phi_{CT}=0.438$  | 0.116  |
| ETS2   | 1    | 48.4        | $\Phi_{CT}=0.484$  | 0.113  |
| Among populations within groups                    |      |             |                    |        |
| 16S  | 6    | 51.1        | $\Phi_{SC}=0.914$  | <0.001 |
| CO1  | 8    | 54.6        | $\Phi_{SC}=0.971$  | <0.001 |
| ETS2   | 6    | -1.1        | $\Phi_{SC}=-0.021$ | 0.024  |
| Among populations relative to total                |      |             |                    |        |
| 16S  | 41   | 4.8         | $\Phi_{ST}=0.952$  | <0.001 |
| CO1  | 34   | 1.6         | $\Phi_{ST}=0.984$  | <0.001 |
| ETS2   | 50   | 52.7        | $\Phi_{ST}=0.473$  | <0.001 |
| <i>C. heraldi</i>                                  |      |             |                    |        |
| Among groups                                       |      |             |                    |        |
| 16S  | 1    | 85.4        | $\Phi_{CT}=0.854$  | 0.030  |
| CO1  | 1    | 90.0        | $\Phi_{CT}=0.900$  | 0.002  |
| ETS2   | 1    | 20.5        | $\Phi_{CT}=0.752$  | 0.029  |
| Among populations within groups                    |      |             |                    |        |
| 16S  | 5    | -1.6        | $\Phi_{SC}=-0.111$ | 0.800  |
| CO1  | 8    | 7.6         | $\Phi_{SC}=-0.005$ | 0.568  |
| ETS2   | 5    | 1.0         | $\Phi_{SC}=-0.077$ | 0.833  |
| Among populations relative to total                |      |             |                    |        |
| 16S  | 20   | 16.2        | $\Phi_{ST}=0.838$  | <0.001 |
| CO1  | 25   | 24.0        | $\Phi_{ST}=0.899$  | <0.001 |
| ETS2   | 43   | 15.4        | $\Phi_{ST}=0.733$  | <0.001 |

**Fig.1** Geographic ranges [grey areas, being the polygons obtained by joining the most peripheral occurrence points reported in FishBase point maps (Froese and Pauly 2012)] of pygmy angelfishes of the *Centropyge flavissima* species complex. Background map of the Indo-West Pacific from Digital Vector Maps, San Diego (<http://digital-vector-maps.com/>). **A.** *C. cocosensis* sp. nov. and *C. flavissima*,. **B.** *C. heraldi*. Grey ellipses: Sri Lanka (Steinke et al. 2009), Bali (present work); dark-grey squares: localities where the black-fin form of *C. heraldi* (*C. woodheadi* sensu Kuitert 1998) has been reported; from West to East: Holmes Reef, Solomon Islands, Vanuatu, Kwajalein, Maloa (type locality), Tonga, American Samoa, Cook Islands, and Huahine (Randall 1997; Kuitert 1998; Randall and Carlson 2000; Steinke et al. 2009). The distribution of *C. heraldi* in the Pacific (as from Froese and Pauly 2012) includes the *woodheadi* form without distinction. **C.** *C. eibli* and *C. vrolikii*.

**Fig. 2** Specimens representative of four yellow pygmy angelfish species, *Centropyge* spp. **A.** *C. cocosensis* sp. nov., no. NMMB-P19875-1 (paratype) from the Cocos Islands. **B.** *C. flavissima*, no. NMMB-P20403-1 from Fiji. **C.** *C. woodheadi*, no. NMMB-P20401-2 from the Great Barrier Reef. **D.** *C. heraldi*, no. NMMB-P20400 from Taiwan

**Fig. 3** Phylogenetic tree of mitochondrial DNA cytochrome *b* haplotypes (594 base pairs) for *Centropyge flavissima*, *C. eibli*, and *C. vrolikii*, based on Bayesian inference. Scale bar: 0.4 substitution/site. Modified from DiBattista et al. (2012)

**Fig. 4** Phylogeny of yellow pygmy angelfishes, *Centropyge* spp.: Maximum-likelihood (ML) trees of nucleotide sequences (list in Table 1) reconstructed using MEGA6 (Tamura et al. 2013). Numbers at nodes are bootstrap scores in % (1000 random resampling runs; MEGA6); bootstrap scores below 50% are not shown. *Gam.* Gambier islands; *Kir.* Kiribati; *Mar.* Marquesas islands; *Moo.* Moorea; *NC* New Caledonia; *Phi.* Philippines; *SL* Sri Lanka; *Van.* Vanuatu. <sup>a</sup>Haplotypes designated *C. woodheadi* under its new definition (Table 1). **A.** ML tree (based on K2+G model) of 88 *16S* rDNA sequences aligned over 597 bp, rooted by homologous sequences of *Pomacanthus xanthometopon* (GenBank KJ551856-KJ551858). **B.** ML tree (K2+G model) of 101 *CO1* gene sequences aligned over 647 bp, rooted by homologous sequences of *P. sexstriatus* (GenBank KJ542547, KJ542548) and *P. xanthometopon* (GenBank KJ534356-KJ534358). **C.** ML tree (K2+G model) of 126 intron *ETS-2* haplotype sequences aligned over 439 bp, rooted by homologous sequences of *P. sexstriatus* (GenBank KJ624708, KJ624709) and *P. xanthometopon* (GenBank KJ624691-KJ624693)

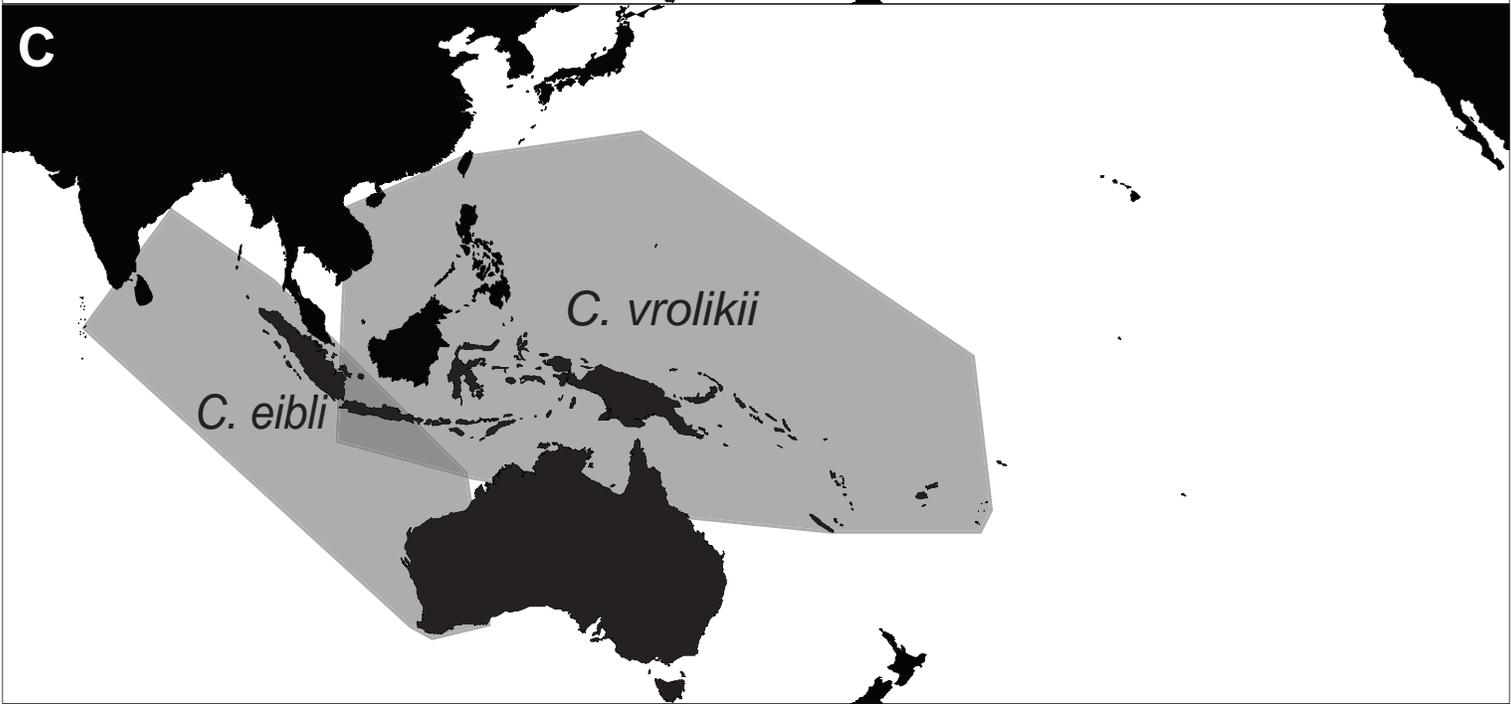
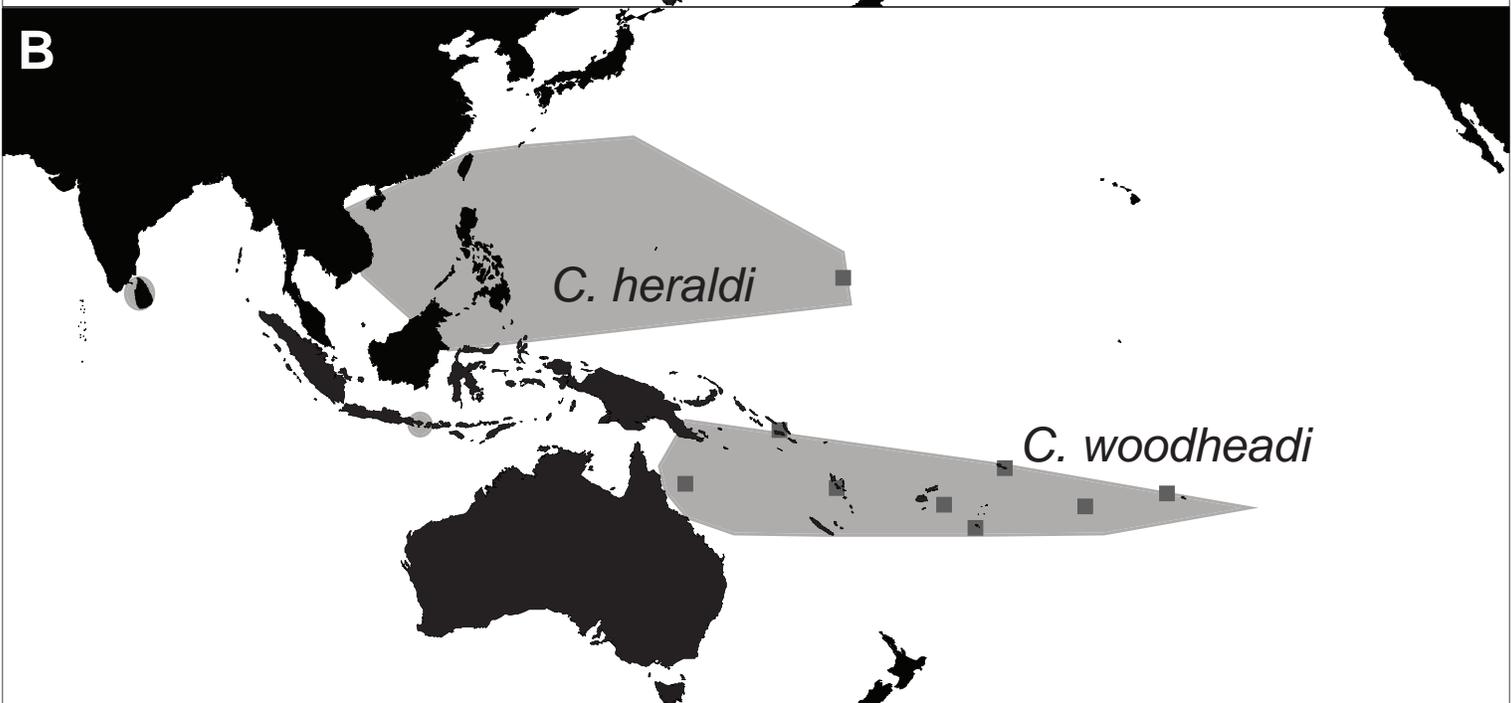
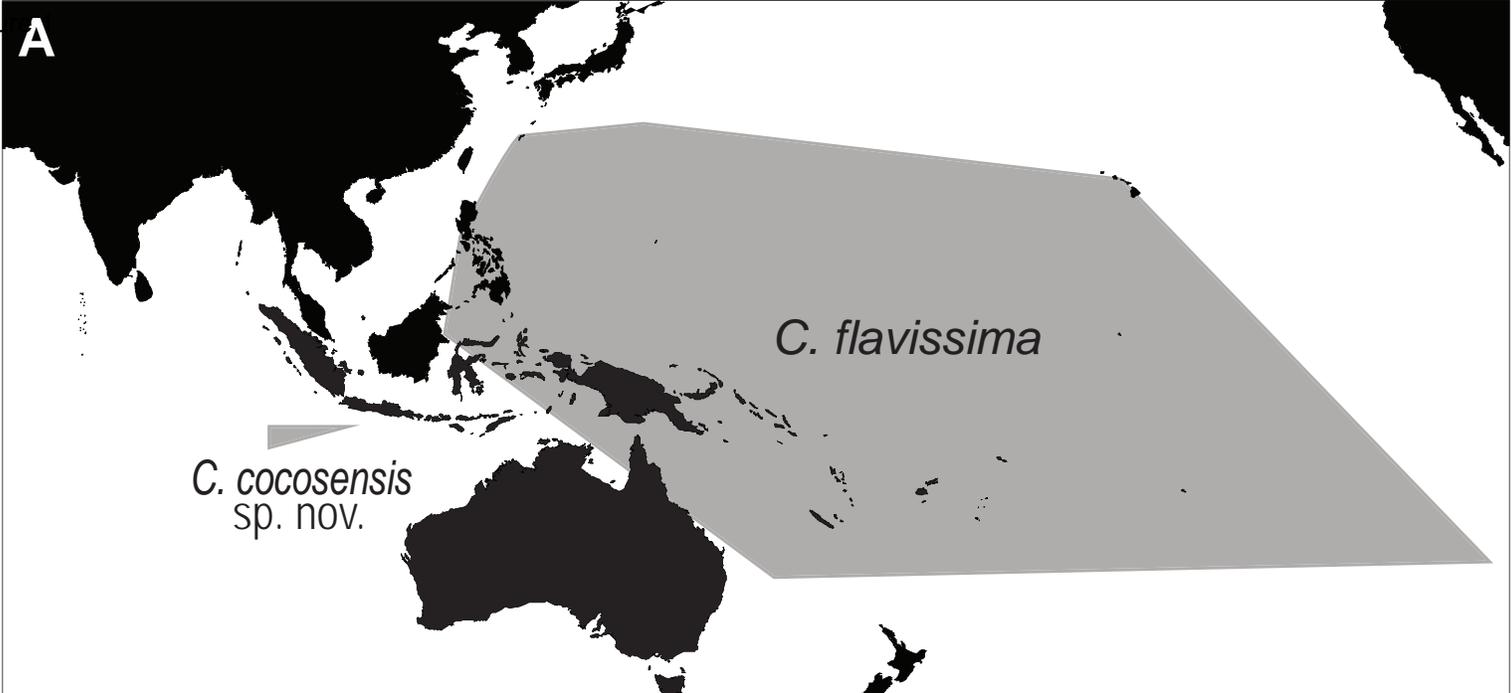


Figure 2

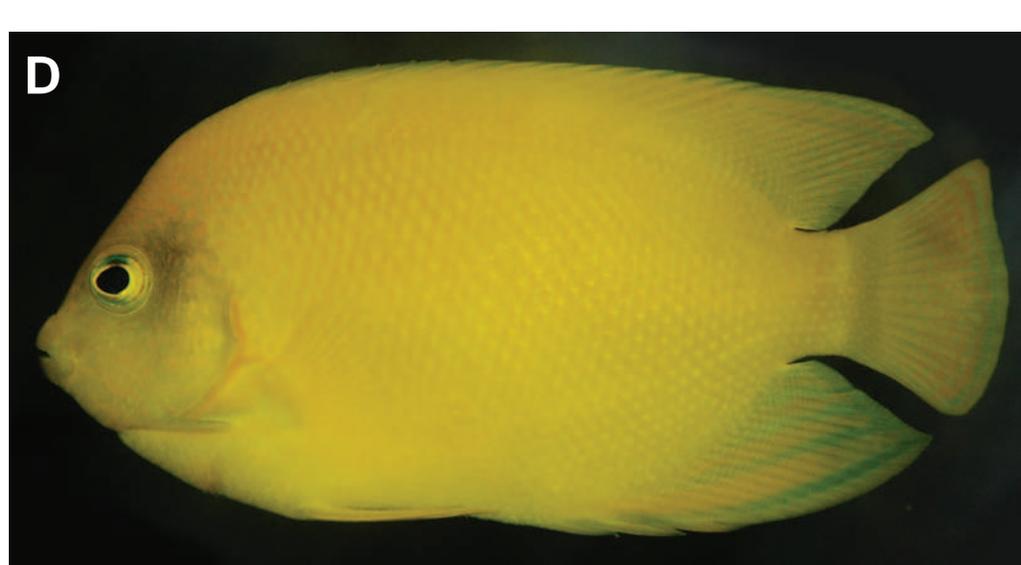
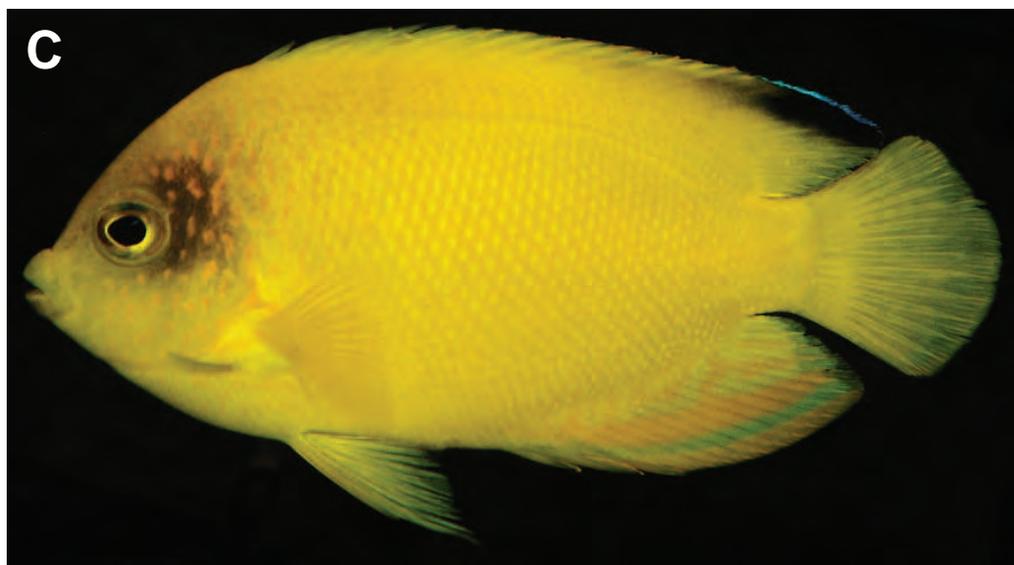


Figure 3

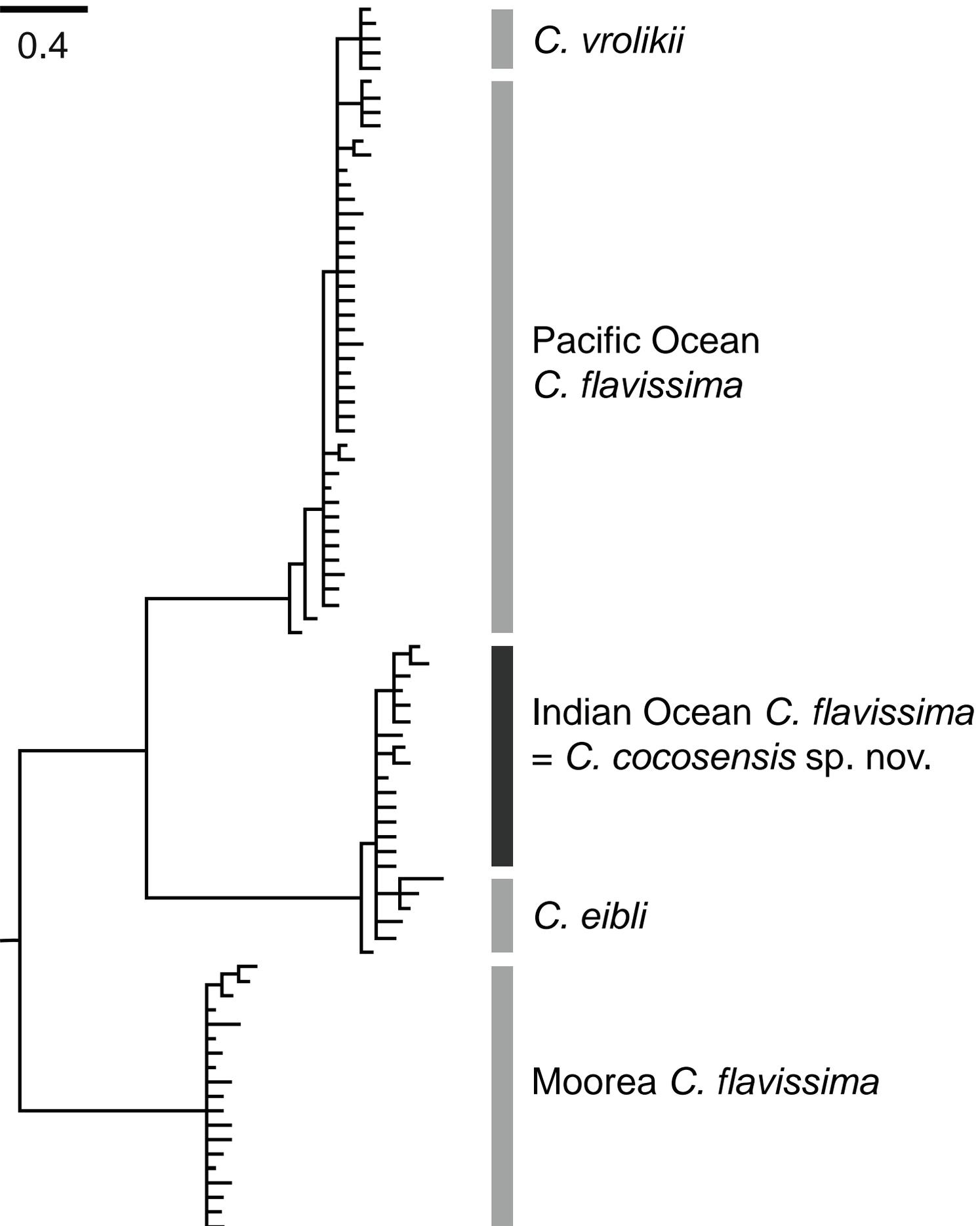
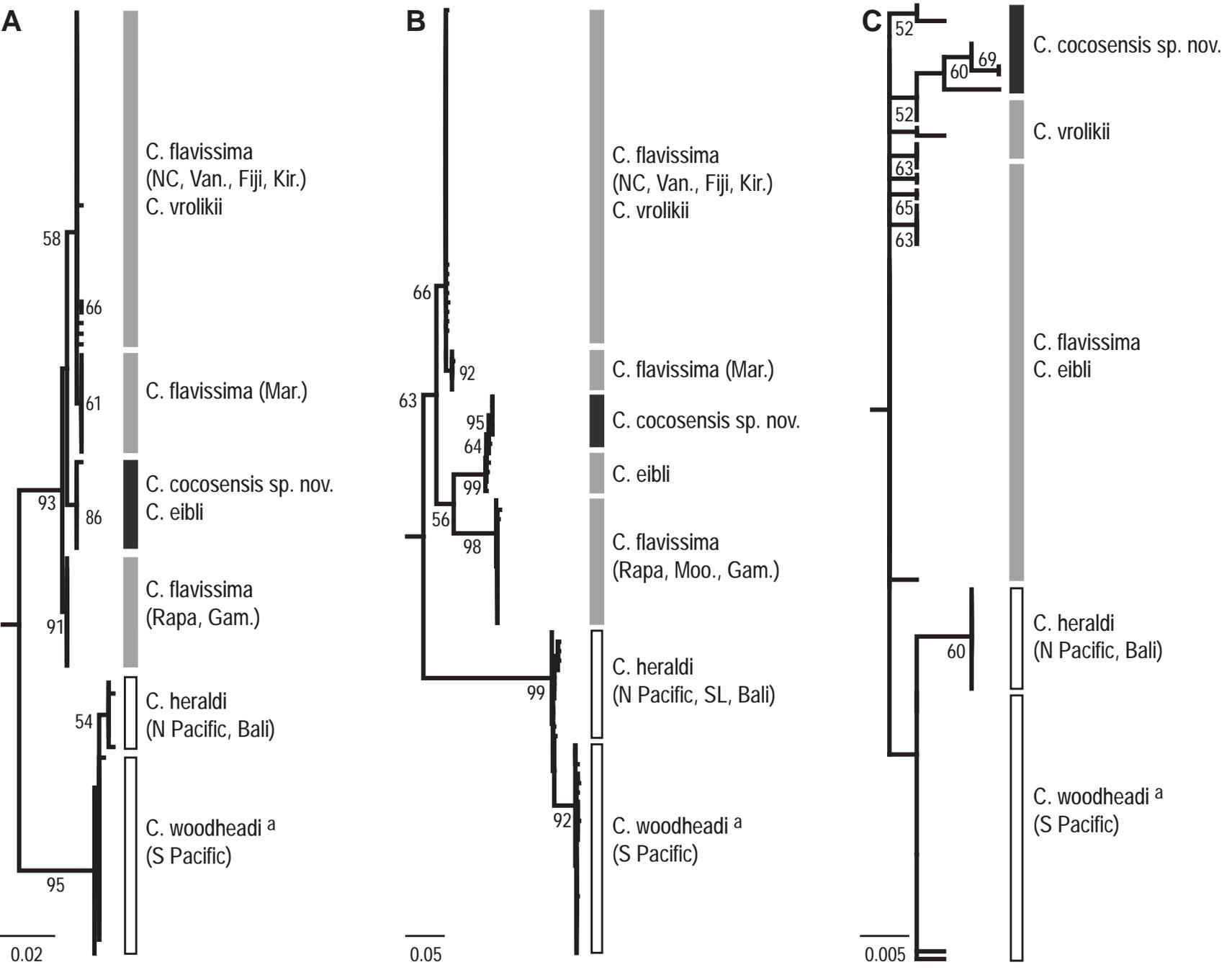


Figure 4



*Supplementary material to:*

**Lemonpeel (*Centropyge flavissima*) and yellow (*C. heraldi*) pygmy angelfishes each consist of two geographically isolated, sibling species**

Kang-Ning Shen · Chih-Wei Chang · Erwan Delrieu-Trottin · Philippe Borsa

Supplementary Table S1, description of the holotype of *C. cocosensis* sp. nov., and Supplementary Figs. S1-S3 here appended

**Supplementary Table S1** Morphological measurements for the type material of *Centropyge cocosensis* sp. nov. NMMB National Museum of Marine Biology and Aquarium, Pingtung; USNM United States National Museum, Washington DC

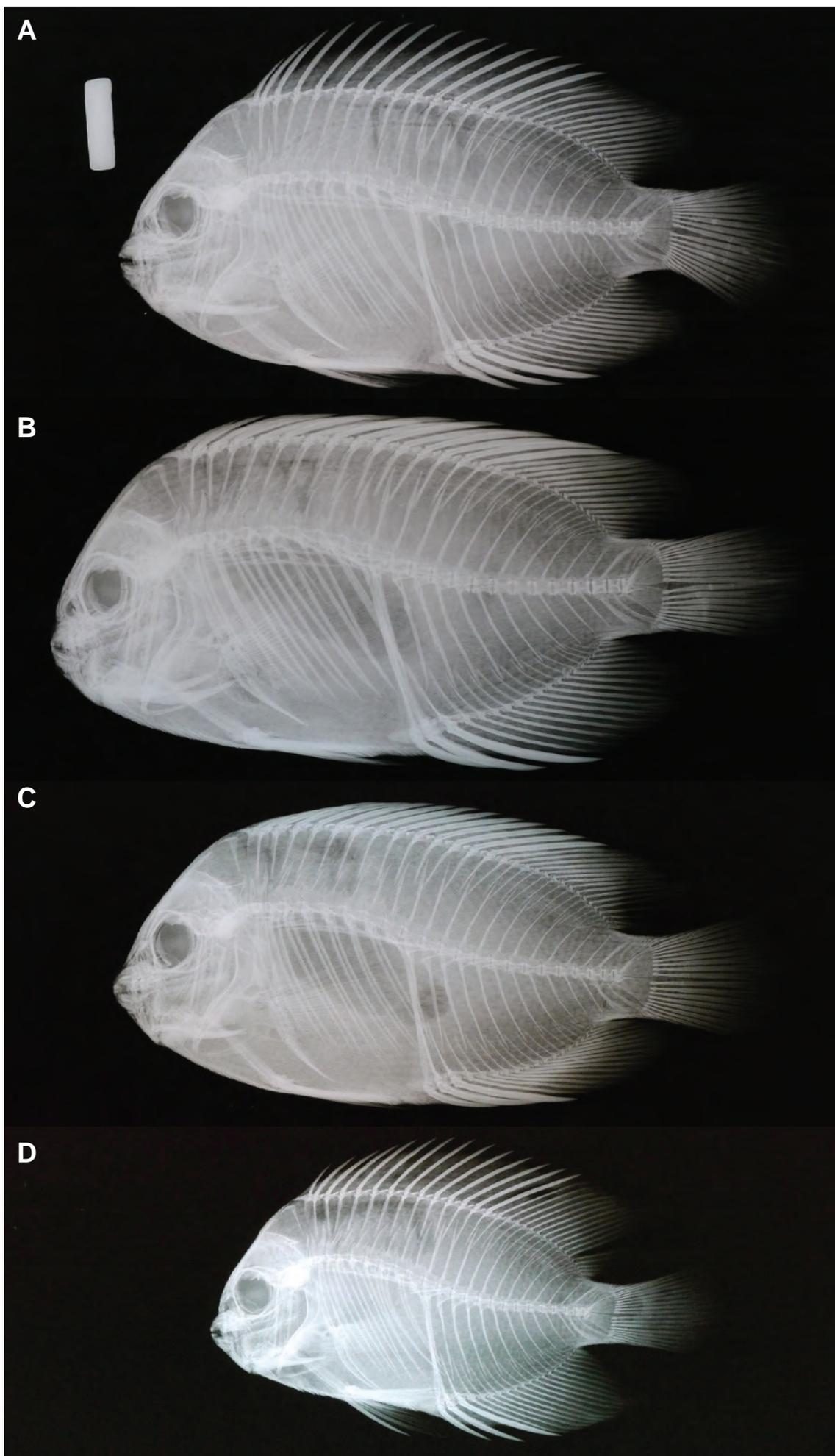
| Measurement                              | Specimen no.              |                             |                             |                           |
|--|---------------------------|-----------------------------|-----------------------------|---------------------------|
|  | NMMB-P19870<br>(paratype) | NMMB-P19875-1<br>(paratype) | NMMB-P19875-2<br>(holotype) | USNM 410766<br>(paratype) |
| Standard length (SL; mm)                 | 73.2                      | 74.1                        | 61.2                        | 46.4                      |
| No. dorsal-fin spines                    | 14                        | 15                          | 14                          | 14                        |
| No. dorsal-fin rays                      | 16                        | 15                          | 16                          | 17                        |
| No. anal-fin rays                        | 3                         | 3                           | 3                           | 3                         |
| No. pectoral-fin rays                    | 16                        | 16                          | 16                          | 17                        |
| Body depth (%SL)                         | 51.5                      | 52.5                        | 51.5                        | 52.1                      |
| Body width (%SL)                         | 18.6                      | 18.4                        | 17.7                        | 17.3                      |
| Head length (%SL)                        | 24.9                      | 23.8                        | 26.5                        | 26.5                      |
| Snout length (%SL)                       | 6.8                       | 5.1                         | 6.5                         | 5.0                       |
| Cheek depth (%SL)                        | 14.2                      | 14.7                        | 13.78                       | 15.2                      |
| Orbital diameter (%SL)                   | 10.3                      | 9.5                         | 10.6                        | 11.8                      |
| Interorbital width (%SL)                 | 8.9                       | 10.0                        | 10.4                        | 10.5                      |
| Length of preopercular spine (%SL)       | 12.9                      | 16.3                        | 12.1                        | 10.1                      |
| Caudal-peduncle depth (%SL)              | 15.4                      | 15.6                        | 15.7                        | 15.6                      |
| Caudal-peduncle length (%SL)             | 9.8                       | 9.8                         | 10.5                        | 8.7                       |
| Predorsal length (%SL)                   | 23.0                      | 21.6                        | 25.1                        | 22.5                      |
| Prepelvic length (%SL)                   | 31.3                      | 31.5                        | 31.6                        | 32.6                      |
| Preal length (%SL)                       | 59.5                      | 59.3                        | 57.6                        | 55.0                      |
| Length of 1st dorsal-fin spine (%SL)     | 9.4                       | 9.6                         | 7.8                         | 9.5                       |
| Length of 2nd dorsal-fin spine (%SL)     | 12.7                      | 13.0                        | 13.8                        | 13.6                      |
| Length of longest dorsal-fin spine (%SL) | 23.4                      | 23.1                        | 22.5                        | 25.5                      |
| Length of longest dorsal-fin ray (%SL)   | 23.0                      | 22.9                        | 21.6                        | 25.9                      |
| Length of 1st anal-fin spine (%SL)       | 13.5                      | 13.4                        | 14.0                        | 15.1                      |
| Length of 2nd anal-fin spine (%SL)       | 19.0                      | 19.1                        | 18.6                        | 19.5                      |
| Length of 3rd anal-fin spine (%SL)       | 23.4                      | 25.6                        | 23.0                        | 25.5                      |
| Length of longest anal-fin ray (%SL)     | 23.1                      | 23.9                        | 22.8                        | 23.9                      |
| Caudal-fin length (%SL)                  | 23.6                      | 24.1                        | 25.9                        | 28.4                      |
| Pectoral-fin length (%SL)                | 27.4                      | 28.3                        | 30.2                        | 32.4                      |
| Pelvic-fin spine length (%SL)            | 20.6                      | 20.6                        | 20.2                        | 21.5                      |
| Pelvic-fin length (%SL)                  | 27.8                      | 28.2                        | 26.3                        | 29.9                      |

**Morphological description of the holotype of *C. cocosensis* sp. nov.**

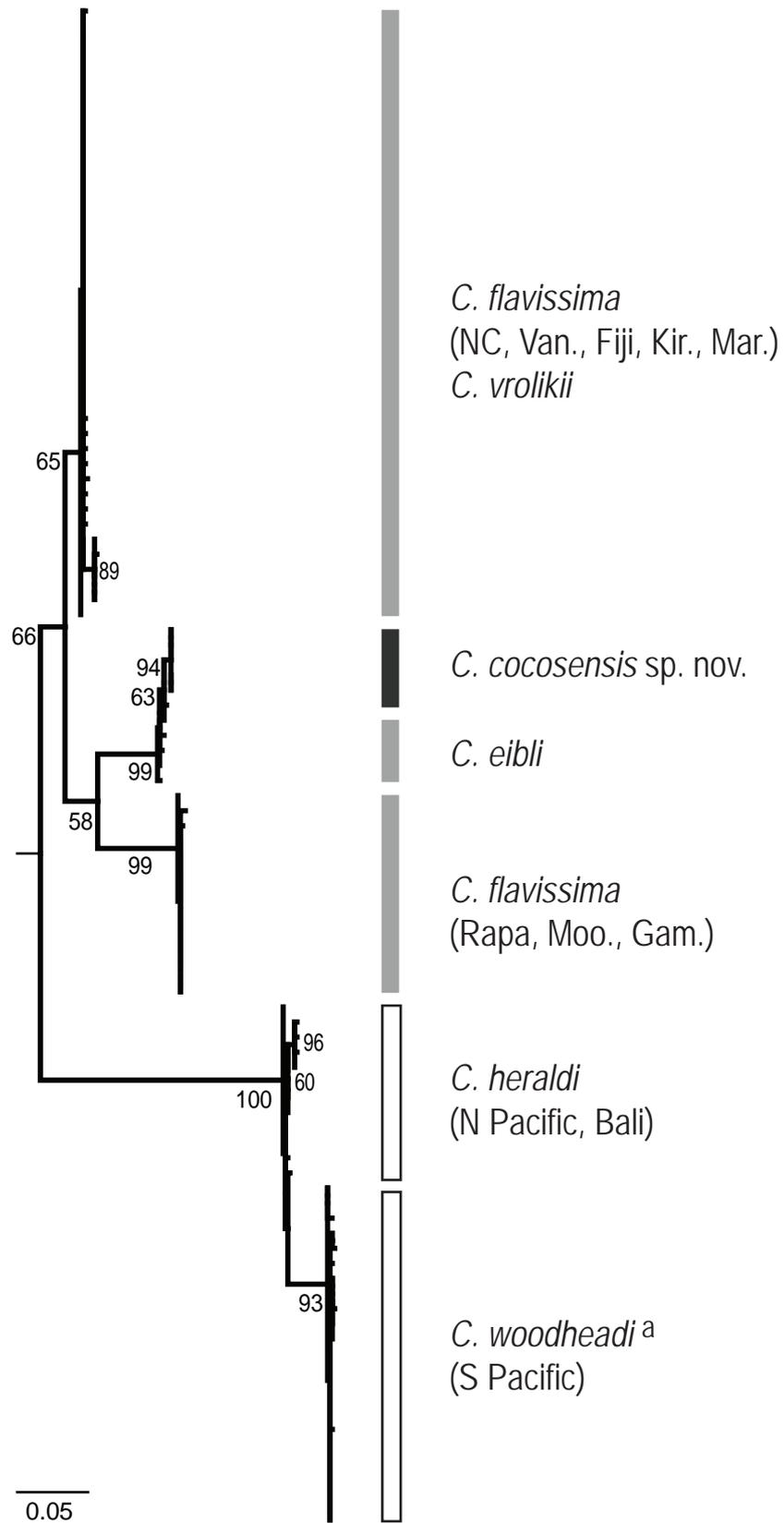
Dorsal fin XIV-XV, 15-17; anal fin III, 16-17; all dorsal- and anal-fin rays branched; pelvic fin I, 5. Body moderately deep, depth 1.9 in standard length (SL), and compressed, width 2.8-3.0 in depth. Head length (HL) 5.4-5.8 in SL; snout short, length 3.7-5.3 in HL; interorbital width 2.5-2.8 in HL; cheek depth 1.6-1.9 in HL; caudal-peduncle depth 1.5-1.7 in HL; caudal-peduncle length 2.4-3.1 in HL. Mouth small and terminal, maxilla reaching below anterior nostril, and strongly oblique when fully closed, lower jaw not projecting; jaws slightly protractible. Strong spine at corner of preopercular, length 6.1-9.9 in SL. Predorsal length 4.0-4.6 in SL; length of first dorsal-fin spine 25-3.4 in HL; length of second dorsal fin-spine 1.8-1.9 in HL; last dorsal-fin spine longest, length 1.0-1.2 in HL; 8th or 9th dorsal-fin ray longest, length 1.0-1.2 in HL; preanal length 1.7-1.8 in SL; length of first anal-fin spine 1.8-1.9 in HL; length of second anal-fin spine 1.2-1.4 in HL; third anal-fin spine longest, its length 0.9-1.2 in HL; 8th or 9th anal-fin ray longest, length 1.0-1.2 in HL; origin of pelvic fins below mid base of pectoral fins, prepelvic length 3.1-3.2 in SL; length of pelvic spine 1.2-1.3 in HL.

**Nucleotide sequences of the holotype of *C. cocosensis* sp. nov.**

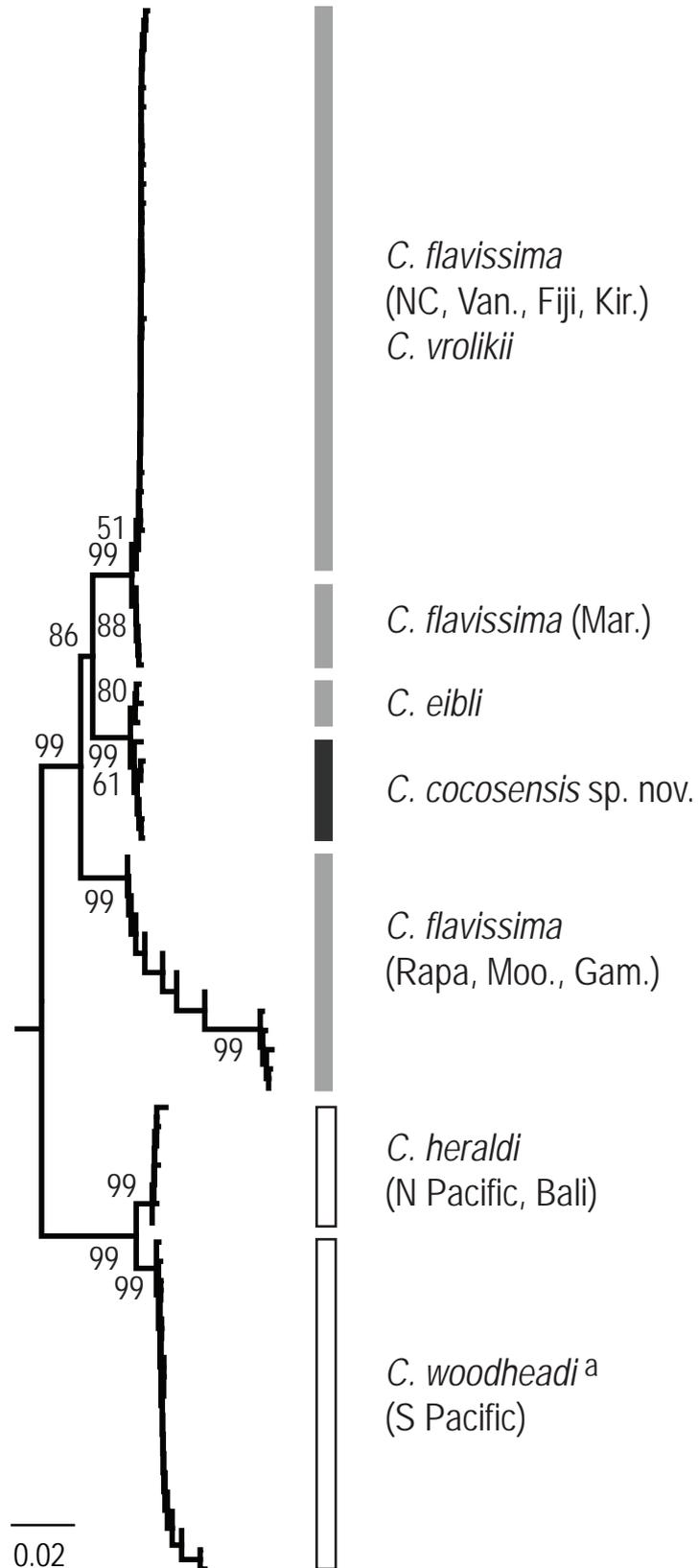
The partial sequence of the *16S* rDNA (GenBank KJ551863) was: 5'-A A A C A T C G C C T C T T G C A A A A C T A A C A A A T A A G A G G T C C C G C C T G C C C T G T G A C T A T A T G T T T A A C G G C C G C G G T A T T T T G A C C G T G C A A A G G T A G C G C A A T C A C T T G T C T T T T A A A T G G A G A C C C G T A T G A A T G G C A T A A C G A G G G C T T A A C T G T C T C C T C T T T C A A G T C A A T G A A A T T G A T C T C C C C G T G C A G A A G C G G G G A T A A G C A C A T A A G A C G A G A A G A C C C T G T G G A G C T T T A G G C A C T A A A G C A G A A T A T G T T A A G T A C C C C A G C T T A A T G A C T A A A A C A A C T T A C A C C C T G C C C T A A T G C C T T C G G T T G G G G C G A C C G C G G G G A A A T A A A A A A C C C C C A T G C A G A A T G G G A G A C A T C T C C T A C A A C T A A G A G C T C C C G C T C T A A T T A A C A G A A C C T C T G A C T A T A C A A G A T C C G G C A A T G C C G A T C A A C G G A C C A A G T T A C C C C A G G G A T A A C A G C G C A A T C C C C T T T T A G A G C C C A T A T C G A C A A G G G G G T T T A C G A C C T C G A T G T T G G A T C A G G A C A T C T A A T G G T G C A G C C G C T A T T A A G G G T T C G T T T G T T C A A C G A T T A A A G T C C T A C G T G A T C T G A G-3'. The partial sequence of the *CO1* gene (GenBank KJ534316) was: 5'- A T A A A G A T A T C G G C A C C C T C T A T T T A C T A T T T G G T G C T T G A G C T G G G A T G G T G G G A A C C G C T T T A A G C C T A C T T A T T C G A G C A G A A T T A A A T C A A C C A G G C A G C C T C T T A G G G G A T G A C C A A A T T T A T A A T G T G A T C G T T A C A G C A C A T G C A T T C G T A A T A A T T T T C T T T A T A G T A A T A C C A G C T A T A T T G G A G G A T T C G G A A A C T G G C T G G T C C C T C T A A T A A T T G G A G C T C C A G A C A T A G C A T T C C C C C G A A T G A A C A A C A T G A G C T T T T G A C T G C T C C C T C C T T C T C T C C T T C T T C T T C T C G C C T C C G C G G G A G T A G A A G C T G G G G C G G G G A C T G G G T G A A C A G T T T A C C C A C C T C T G G C G G G C A A T T T A G C C C A T G C A G G G G C A T C C G T A G A T T T A A C T A T T T T T T C T C T T C A T T T A G C A G G G G T C T C C T C A A T T C T A G G G G C T A T C A A T T T T A T T A C T A C C A T T A T T A A C A T G A A A C C C C C G C C A T T T C C C A A T A C C A A A C A C C A C T G T T T G T T T G A G C A G T A C T A A T C A C T G C T G T T C T T C T T C T T T C A C T A C C A G T C C T T G C T G C A G G A A T T A C G A T A C T C C T C A C A G A C C G A A A T C T A A A T A C T A C T T C T T T G A C C C T G C G G G A G G A G G G G A C C C A A T C C T C T A C C A A C A C T T A T T C T G A T T C T T C G G-3'. The nucleotide sequence of the *ETS-2* intron (GenBank KJ624656) was: 5'- G G C A G T T T C T T C T G G A G C T G C T G A C C G A C A A G T C T T G C C A G T C C T T C A T C A G C T G G A C A G G C A A C G G C T G G G A G T T C A A G C T G T C C G A C C C A G A T G A G G T G A G A G G T C G T G A C C C C G C T G G T C G C C G G C C C A C G T G C T G G G A G G T C C T A A G T A G T T A G C C A G A T G A A C A A G A T G T C T C C T C C C T C T G T T A A A A A A G A A A C A T T T G C A T G C C T T G T T T G G T T C C A T T A A T C T T T T T C G A T G G G T T G A C G T T G G C T G G T T T T G C A T T A G -T G G G G T T T C A T T T C A A A A T G T G G A T G A T T A C A T T T T C A G A T G A G T C A G T T T C T T A A A A G A T G C G G T T T T G A T T T G A A A G T G A A C T A A T T T G T T A C A C A T T T T T T T A G A A A G T C T T T A T G A A T C A T C T T G A C A G A C T T A T T C C C T G C C T T G T T T T A G G T T G C T C G G A G G T G G G G C A A G A G G A A A A C A A G C C C A A G A T G A A C T A C G A G-3'.



**Supplementary Fig. S1** *Centropyge cocosensis* sp. nov. X-ray images of type material. **A** Specimen no. NMMB-P19870 (paratype), SL 73.2 mm. **B** Specimen no. NMMB-P19875-1 (paratype), SL 74.1 mm. **C** Specimen no. NMMB-P19875-2 (holotype), SL 61.2 mm. **D** Specimen no. NMMB-P19875-3 (paratype), SL 46.4 mm.



**Supplementary Fig. S2** Phylogeny of yellow pygmy angelfishes, *Centropyge* spp. Maximum-likelihood tree (K2+G model of nucleotide change; partial deletion; MEGA6) of concatenated *16S* + *CO1* sequences ( $N = 105$ , including 4 outgroups, aligned over 1244 bp). Numbers at nodes are bootstrap scores (500 resampling runs under MEGA6). <sup>a</sup> Haplotypes designated *C. woodheadi* under present study's definition.



**Supplementary Fig. S3** Phylogeny of yellow pygmy angelfishes, *Centropyge* spp. Neighbor-joining tree (K2 distances; partial deletion; MEGA6) of concatenated *16S* + *CO1* + *ETS2* sequences ( $N = 86$ , including 4 outgroups, aligned over 1683 bp). Numbers at nodes are bootstrap scores (500 resampling runs under MEGA6).<sup>a</sup> Haplotypes designated *C. woodheadi* under present study's definition.