



Letter to the Editor

Response to Delrieu-Trottin et al.: Hybrids, Color Variants and the Consistently Devilish Taxonomy of Pygmy Angelfishes

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The question of species delineation continues to be a controversial topic, compounded in the oceans by our limited accessibility and lack of strong vicariant barriers. Long before molecular genetic evaluations, coloration was a basis for many species descriptions, especially for congeneric reef fishes. Most of these designations have held up to scrutiny, but coloration can be a tricky basis for taxonomy in this group (e.g., Gaither et al. 2014).

The controversy addressed by DiBattista et al. (2016) and Delrieu-Trottin et al. (2017) revolves around species' identities for the lemonpeel angelfish *Centropyge flavissima*. This coral reef fish was formerly recognized as a single species with a disjunct distribution, its Pacific Ocean and Indian Ocean forms occupy ranges separated by over 3000 km. Dr Gerry Allen, a taxonomic authority on *Centropyge*, first recorded the Indian Ocean morph of *C. flavissima* more than 40 years ago (Allen et al. 1998), but declined to describe it as a species due to a lack of supporting evidence. Shen et al. (2016) subsequently describe the Indian Ocean *C. flavissima* as a distinct species (*C. cocosensis*) based on subtle differences in color, an isolated distribution and a lack of shared mitochondrial DNA (mtDNA) haplotypes. DiBattista et al. (2012) previously appraised relationships within the entire *C. flavissima* complex (including both forms of *C. flavissima*, Indian Ocean *Centropyge eibli* and western Pacific *Centropyge vrolikii*; also see Figure 1) with mtDNA cytochrome *b* (*cyt b*) and 3 nuclear introns. This study found that the Indian Ocean *C. flavissima* was genetically distinctive from the Pacific Ocean form, but genetically indistinguishable from sympatric *C. eibli*. This species complex sorted into 3 monophyletic lineages well-defined by geography but only imperfectly matching color-based taxonomy. With expanded sampling, DiBattista et al. (2016) again show shared haplotypes between *C. eibli* and "*Centropyge cocosensis*," and therefore question the validity of the latter designation.

Our seemingly conflicting results are due in part to differences in sample availability. Based on $N = 6$ "*C. cocosensis*" and $N = 5$ *C. eibli* 16S rDNA and mtDNA cytochrome oxidase I (COI) samples, Shen et al. (2016) observed a single, nondiagnostic base pair substitution in the former locus and no shared haplotypes in the latter locus. None of the variation was parsimony-informative, indicating no diagnostic difference distinguishing putative "*C. cocosensis*." DiBattista et al. (2016) based their conclusions on $N = 88$ "*C. cocosensis*" and $N = 20$ *C. eibli* at the *cyt b* locus, a faster evolving marker that revealed multiple shared haplotypes. Hence, the much larger data set contradicted the conclusion of genetic differentiation presented by Shen et al. (2016).

Additional lines of evidence cast doubt on the species status of "*C. cocosensis*." Field observations spanning 15 years reveal that throughout their entire geographic range (Christmas and Cocos-Keeling Islands), "*C. cocosensis*" forms stable and persistent harems (i.e., spanning at least 6 consecutive years) with *C. eibli* and *C. vrolikii* (Hobbs and Allen 2014; Hobbs et al. in preparation). The resulting hybrids cohabit and form harems with each other and the parental species, and backcross with "*C. cocosensis*," resulting in a continuum of color morphs between "*C. cocosensis*," *C. eibli* and *C. vrolikii* across the entire range of "*C. cocosensis*," with some individuals possessing color characteristics of all 3 putative species (Figure 1d–l).

We agree with Shen et al. (2016) and Delrieu-Trottin et al. (2017) that *C. flavissima* in the Indian Ocean is not the same as *C. flavissima* in the Pacific Ocean; instead we refer to them as "color forms" and "color morphs" in DiBattista et al. (2016), as well as acknowledge that they are distinct based on mtDNA (DiBattista et al. 2012). In their Figure 1, Delrieu-Trottin et al. (2017) reiterate the most interesting conclusion from DiBattista et al. (2012), that the mtDNA indicates incongruence between molecular data and body

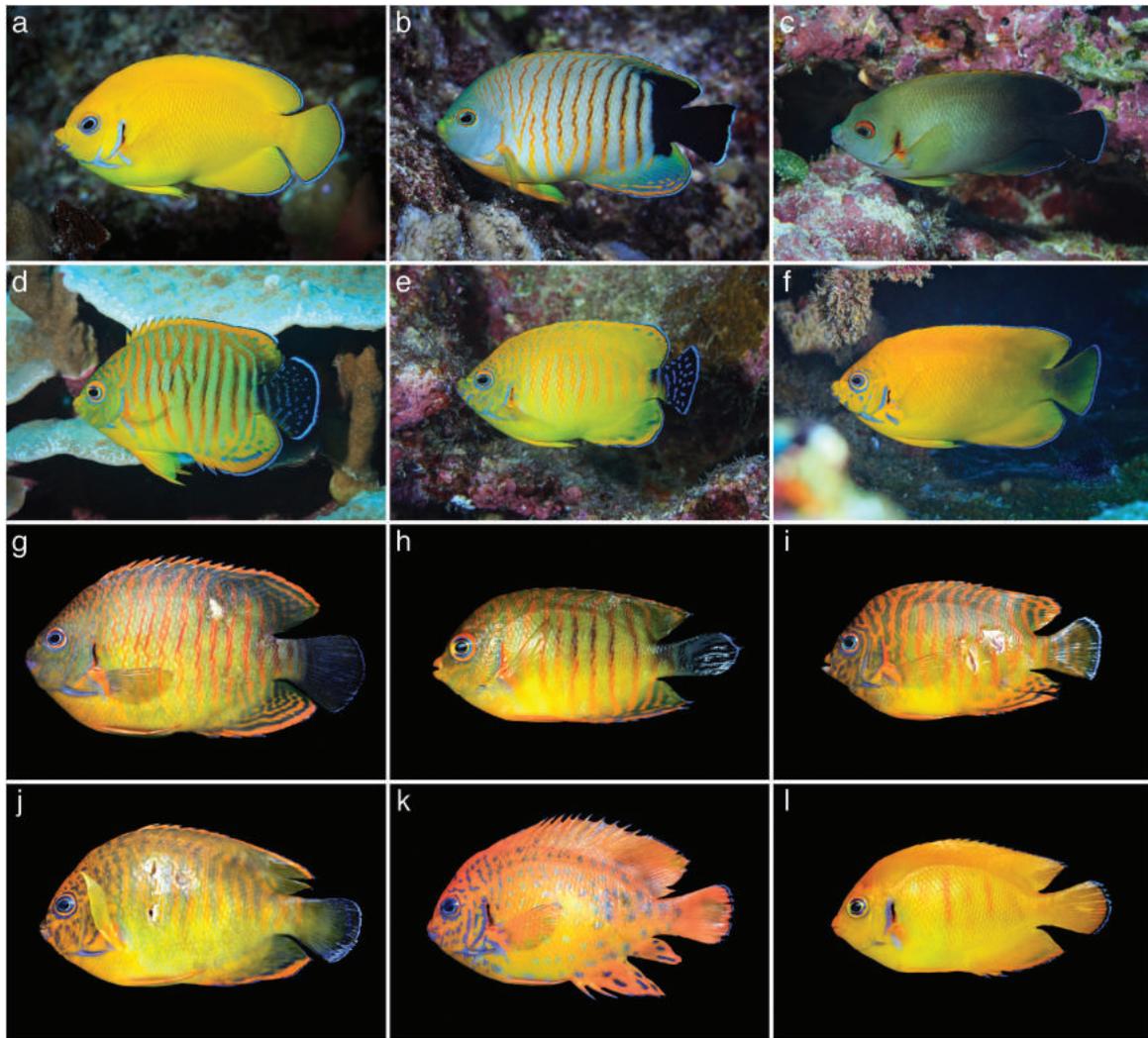


Figure 1. *Centropyge flavissima* (a; Indian Ocean morph), *Centropyge eibli* (b), *Centropyge vrolikii* (c) and the wide range of intermediate color variations (hybrids and backcrosses: d–l) at Christmas Island in the eastern Indian Ocean. Photo credits: Tane Sinclair-Taylor.

color, grouping the yellow color morph at the Cocos-Keeling Islands with *C. eibli* instead of the Pacific Ocean morph of *C. flavissima*. However, based on this figure, as well as our own, there are only 3 mtDNA lineages and not 4, and so this seems an uncertain foundation for describing a new (fourth) species in this complex.

Two scenarios may explain the origin of the yellow *Centropyge* in the Indian Ocean: 1) “*C. cocosensis*” started with the arrival of *C. flavissima* from the Pacific Ocean to Christmas and Cocos-Keeling Islands; these first colonizers hybridized with and acquired the mtDNA of local *C. eibli* through introgression, or 2) “*C. cocosensis*” is the result of a founder effect at Christmas and Cocos-Keeling Islands started by a population containing xanthic (yellow) *C. eibli*. We can draw on examples from the literature to support both hypotheses. Indeed, there are other species of reef fish that have a disjunct distribution consisting of Christmas Island, Cocos-Keeling Islands and parts of the South Pacific. As one example, the wrasse *Halichoeres claudia* has a distribution that almost exactly matches the combined distributions of “*C. cocosensis*” in the Indian Ocean and *C. flavissima* in the Pacific Ocean (Randall and Rocha 2009). Alternatively, many species of fish show an occasional xanthic form, which, like melanism or albinism in many vertebrates, may be due to

a simple disruption of pigmentation pathways. For example, Carson (2011) lists 10 families of fish known to host xanthic variants, with some seemingly inherited as single locus traits. Moreover, a xanthic form exists in the congeneric Potter’s angelfish *Centropyge potteri* (<https://reefbuilders.com/2010/12/07/xanthic-angelfish-potters-centropyge/#>). In making taxonomic designation based on coloration, xanthic patterns may therefore be among the least reliable traits.

We agree with Delrieu-Trottin et al. (2017) that “regardless of the species properties considered, consistency is necessary to delimitate species belonging to a given group of closely related species.” This is an important point, as many marine species described with color (including those in the *C. flavissima* complex) are now being revisited with genetic tools, resulting in taxonomic revisions in keystone groups such as corals (e.g., Fukami et al. 2008; Arrigoni et al. 2016a) and the fish that inhabit them (e.g., Randall and DiBattista 2013). However, genetic data do not always delineate species, particularly for groups like *Centropyge* where hybridization is common. Furthermore, we do not agree that DiBattista et al. (2016) apply an inconsistent ‘double standard’ in retaining skepticism about “*C. cocosensis*” as a valid species in the Indian Ocean while endorsing *C. flavissima* in the Pacific Ocean. It is true that

C. flavissima in the Pacific Ocean also hybridizes with congeneric fish, just like “*C. cocosensis*.” However, the 3 recognized species in the *C. flavissima* complex have extensive geographic regions where they do not co-occur (and thus cannot hybridize) with each other, and largely agree with their respective mtDNA lineage. In contrast, “*C. cocosensis*” hybridizes with *C. eibli* and *C. vrolikii* throughout its entire geographic range (Hobbs and Allen 2014), producing an astounding continuum of color variation in the hybrids (Figure 1d–l), and most importantly, is not part of a unique mtDNA lineage. This is the backbone of our skepticism about “*C. cocosensis*.” Furthermore, the species-wide hybrid zone undercuts one of the foundations for species designation, the pure holotype.

One of the conclusions by DiBattista et al. (2016) is that the species proposed by Shen et al. (2016) may yet prove to be valid, but that formal nomenclature is premature. Additional hypothesis testing is mandated for this difficult group, ideally using genome wide techniques. In DiBattista et al. (2016), we urged “caution and taxonomic restraint until the true nature of this species complex can be revealed.” Skipping this important step may contribute to confusion in online databases and field guides, and taxonomic instability may ultimately work to the detriment of conservation initiatives. It is most important to invest the time and effort into fully understanding the evolutionary history of the group, as well as the ecological system in which they live. Clearly, *Centropyge* angelfishes are an enigma, as indicated by the title phrase “devilish taxonomy” of DiBattista et al. (2016), and future studies of this group will undoubtedly offer many valuable insights into the evolution of biodiversity in our oceans.

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