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Letter to the Editor

One species hypothesis to rule them all: consistency is essential to delimitate species

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Abstract - The newly-desribed *Centropyge cocosensis* has long been considered a variant of the lemonpeel pygmy angelfish *C. flavissima* because of their overall similarity in color patterns. DiBattista and co-authors [J. Hered. 107 (2016) 647–653] recently contested the validity of *C. cocosensis*, based on arguments that are challenged here.

The issues of species definition and species delimitation have been debated for decades. De Queiroz (2007) has proposed a unified species concept where species are defined as independently evolving metapopulation lineages in the global tree of life; and has enumerated the properties of species, those that enable biologists to delimitate species, e.g. morphological distinctness or reproductive isolation. Regardless of the species properties considered, consistency is necessary to delimitate species belonging to a given group of closely related species.

The pygmy angelfish genus *Centropyge* comprises several species complexes and a total of 35 species. These species have been described mainly on the basis of coloration and geographic distribution, as meristics and morphometrics often do not provide diagnoses (Allen et al. 1998, Pyle 2003, Bowen et al. 2006, Gaither et al. 2014, Nelson et al. 2016, Shen et al. 2016). The *C. flavissima* species complex is composed of four valid species, *C. flavissima* (Cuvier 1831), *C. vrolikii* (Bleeker 1853), *C. eibli* Klausewitz 1963, and *C. cocosensis* Shen et al. 2016. The four species maintain distinct color patterns despite cross-species hybridization wherever they occur in sympatry (Pyle and Randall 1994, Allen et al. 2007, Hobbs and Allen 2014). The scarcity of conspecific mates has been suggested as a possible reason for cross-species hybridization. This is the case at Christmas and Cocos (Keeling) Islands where *C. vrolikii* and *C. eibli*, both uncommon there, cross-breed with the more common Cocos lemonpeel pygmy angelfish *C. cocosensis* (Hobbs and Allen 2014). Hybridization results in widespread mitochondrial DNA (mtDNA) introgression, to the extent that mitochondrial lineages in the *C. flavissima* species complex are not aligned with taxonomy but rather with geography (DiBattista et al. 2012). However, the fact that these species remain distinct despite ample opportunity for hybridization is strong evidence for reproductive isolation. DiBattista et al. (2016) recently contested the validity of *C. cocosensis*, based on arguments that are challenged here.

Centropyge cocosensis has long been considered a variant of the lemonpeel pygmy angelfish *C. flavissima* (e.g. DiBattista et al. 2012, 2016) because of their overall similarity in color patterns. DiBattista et al. (2012) found that the three then-recognized species of the *C. flavissima* complex possessed shared mtDNA haplotypes and showed incomplete lineage sorting for three nuclear genes. Following de Queiroz (2007), Di Battista et al. (2012) maintained the validity of *C. flavissima, C. vrolikii*and *C. eibli*, and stated that for"*[...] dilemmas like the one presented here …; use the common element to define the species, and one or more secondary properties as qualifiers to support this designation. In our case, despite the apparent gene flow, these species maintain unique color characteristics and are partitioned into cohesive geographic regions. We therefore suggest that these angelfish remain recognized as taxonomically diagnosable species*". DiBattista et al. (2012)

did not consider the isolated Indian Ocean form of *C. flavissima* (now *C. cocosensis*) as a distinct species. Based on the same mitochondrial marker as DiBattista et al. (2012) but with an extended collection of samples, DiBattista et al. (2016) claimed to have tested "*the validity of [...] the newly named Centropyge cocosensis assigned to the C. flavissima lineage in the Indian Ocean*".They declared that they "*cannot rule out that the putative species in the Indian Ocean is simply a yellow color morph of C. eibli*" as "*the mtDNA presented [by DiBattista et al. 2016] and nuclear DNA data of DiBattista et al. (2012) do not support the distinction between C. cocosensis and C. eibli*". DiBattista et al. (2016) thus challenged the validity of *C. cocosensis* based on the extensive sharing of mtDNA haplotypes with another species of the same species complex. They chose to not mention *C. cocosensis* but "*an Indian morph of C. flavissima*" or "*C. flavissima*" all along their text, table and figures. Doing so, they also refrained to assign it species name "*C. eibli*".

We disagree with the views of DiBattista et al. (2016). *Centropyge cocosensis* differs from *C. flavissima* by several fixed color characters. Because of this, and considering that the two forms are quarantined from each other by >2000 km of ocean, the Cocos form had been recognized as an undescribed distinct species by several authorities (Allen 1998; Hobbs and Allen 2014; Shen et al. 2016). DiBattista et al. (2016) rationale is simultaneously based on two antagonistic hypotheses for species delimitation. On the one hand, they kept distinguishing *C. eibli*, *C. flavissima* and *C. vrolikii* on the basis of color patterns and admitted that their mitochondrial similarity in the western Pacific Ocean is a consequence of cross-species introgressive hybridization (Hypothesis 1). On the other hand, they suggested that *C. cocosensis* may be a yellow color morph of *C. eibli*. Distinct mitochondrial lineages would then represent distinct species while color patterns would distinguish different color morphs within a lineage (Hypothesis 2). According to Hypothesis 2, the mitochondrial lineage common to *C. eibli* and *C. cocosensis* in the Indian Ocean ('Lineage 3' of DiBattista et al. 2012, 2016; Fig. 1) would thus represent a single species. This would amount to fusing *C. cocosensis* with *C. eibli* and imply that *C. eibli* would have a second color morph that not only converged with the *C. flavissima* color morph, but is also maintained in the Christmas and Cocos (Keeling) Islands despite hybridization. The corollary of Hypothesis 2 is that in the Central Pacific, *C. flavissima* is a simple color variant of *C. vrolikii* as the mitochondria of the two species belong to the same lineage ('Lineage 1'; Fig. 1). This implies that a second species, *C. vrolikii* has once again a yellow color variant that has by chance the exact same color as *C. flavissima* in French Polynesia. In addition, the *C. eibli* morph would have independently arisen twice, and so would have the *C. vrolikii* morph (Fig. 1). Thus, the species delimitation criteria adopted by DiBattista et al. (2016) lack consistency. Moreover, when applied to the whole species-complex, Hypothesis 2 is not parsimonious because it implies repeated, independent events of convergence in color morph.

Shen et al. (2016) found no shared haplotypes at the *COI* and *16S* loci between *C. eibli* (3 individuals) and *C. cocosensis* (6 individuals), which suggests that despite recent, occasional hybridization the two species remain reproductively isolated. DiBattista et al. (2016) argued that such result may only be due to a sampling effect: this does not constitute an argument against the validity of *C. cocosensis*. DiBattista et al (2016) also acknowledged that "*this also makes it difficult to find "pure" individuals to designate as a C. cocosensis holotype. Centropyge cocosensis may prove to be a valid species, but the genetics do not yet support this designation*". Their concern about the requirement of such a "pure" individual as holotype is another example of the double standards they apply on the taxonomy of the *C. flavissima* species complex. If genetic 'purity' were an issue, one wonders why DiBattista et al. (2016) did not equally challenge the validity of *C. flavissima* which similarly appears to be introgressed by *C. vrolikii* mtDNA. Alternatively, if DiBattista et al. (2012, 2016) consider *C. eibli*, *C. flavissima* and *C. vrolikii* as valid despite introgression, they have no reason to question the validity of *C. cocosensis*. To support the alternative, extraordinary view that such distinct color patterns merely reflect infra-specific variability within *C. eibli* would not only require data other than additional mitochondrial sequences, but also evidence of selection towards a yellow color morph similar to that of *C. flavissima*, with the rapid elimination of all intermediate color morphs between *C. eibli* and *C. cocosensis*. To be consistent, similar directional selection should be demonstrated within Lineage 1 on the other side of the Indo-Pacific barrier (Fig. 1).

Species are hypotheses, and it is part of the taxonomic process to test their validity (Eschmeyer et al. 2010). The rtionale and data of DiBattista et al. (2016) fell short of providing evidence against the validity of *C. cocosensis*. We stress the importance of being consistent when delimitating species within a same species complex. Finally, we concur with Di Battista et al. (2016) that next-generation sequencing data "*may provide better resolution and reveal loci that are linked to natural or sexual selection processes*". This new approach could help spot genes that are linked to pre-zygotic reproductive isolation, for example those encoding specific color patterns, or genes linked to post-zygotic reproductive isolation ("speciation genes"; Mensch et al. 2013).

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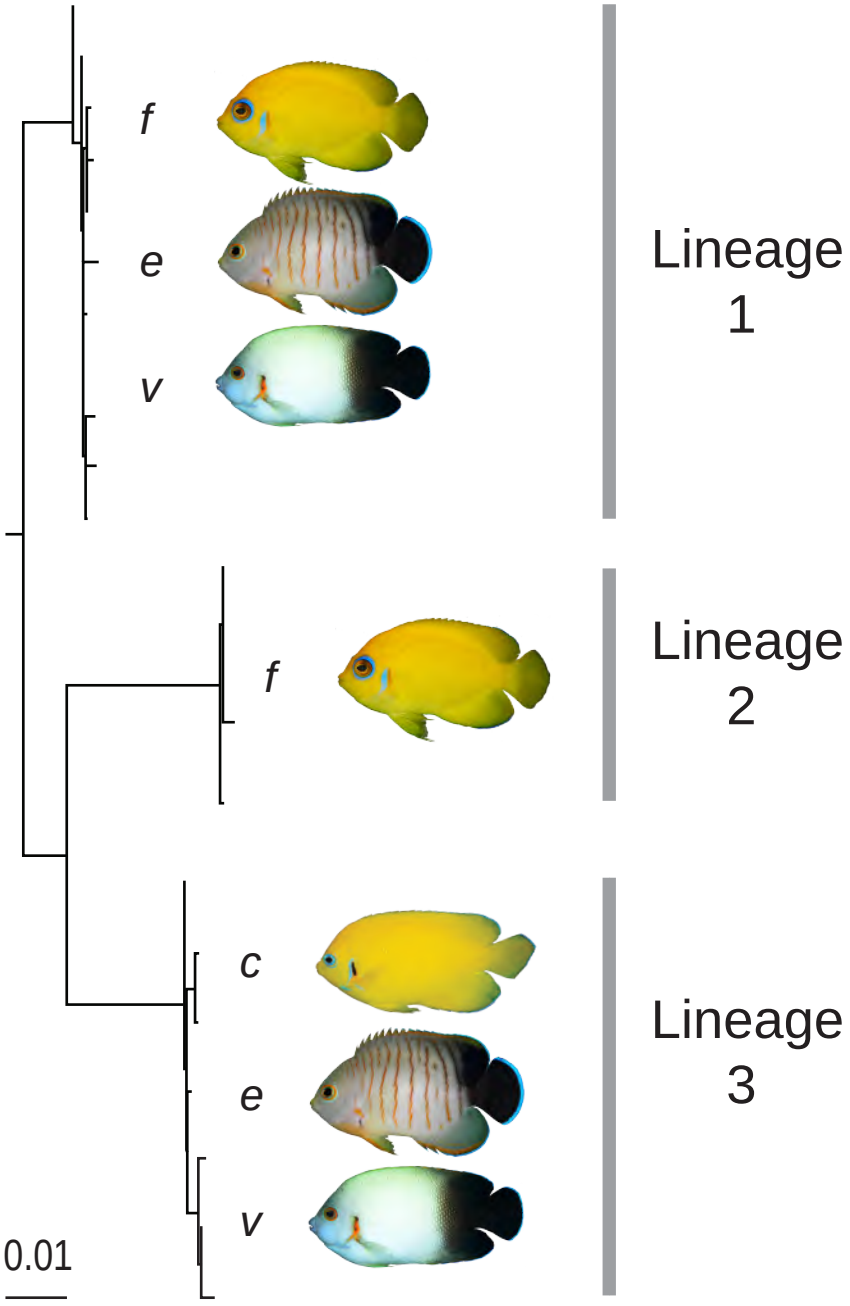
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Fig. 1. Phylogeny of mitochondrial haplotypes in the *Centropyge flavissima* species complex (modified from DiBattista et al. 2016), with indications on the placement of presumed color morphs, for convenience here coined "*c*" (for *cocosensis*), "*e*" (for *eibli*), "*f*" (for *flavissima*) and "*V*" (for *vrolikii*), within each mitochondrial lineage. Under DiBattista et al.'s (2016) mitochondrial-DNA based taxonomic hypothesis (Hypothesis 2), each mitochondrial lineage would be a species and would harbor distinct color morphs previously recognized as separate species, and arisen independently within a lineage. There is yet no evidence to support this unparsimonious hypothesis. Photo credits: Ching-Che Lin (*C. vrolikii*) and Kang-Ning Shen (*C. cocosensis*, *C. eibli,* and *C. flavissima*).


f
e
v
Lineage 1
f
Lineage 2
c
e
v
Lineage 3
0.01