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Interactive comment on “Comprehensive phylogenetic reconstruction of relationships in *Octocorallia* (*Cnidaria: Anthozoa*) from the Atlantic ocean using *mtMutS* and *nad2* genes tree reconstructions” by K. J. Morris et al.

Anonymous Referee #1

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General Comments

The most comprehensive phylogenetic analysis of the sub-class Octocorallia published to date is that of McFadden et al. (2006), who used two mitochondrial markers (*mtMutS* and *ND2*) to reconstruct the phylogenetic relationships among 115 genera belonging to 46 families representing all recognized orders and sub-ordinal groups of octocorals. Although never explicitly stated, the broader goal of this manuscript appears to be to address weaknesses in the taxonomic sampling in McFadden et al.’s paper by adding 87 species, the majority from Paragorgiidae and Isididae, two families that were not

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represented in the earlier phylogeny. Inexplicably, however, the authors have chosen not to simply add representatives of those taxa to the cosmopolitan dataset of McFadden et al., but rather to limit their phylogenetic reconstruction to species occurring in the Atlantic. They do not justify this decision, other than to state that “there has never been an in-depth study of octocorals from the Atlantic.” What do we stand to learn about phylogenetic relationships among families that have cosmopolitan distributions by focusing only on their Atlantic representatives?

The consequence of the authors’ decision to limit their study geographically is a severe taxonomic bias; although their tree does indeed include more taxa than that of McFadden et al., it is much less comprehensive across higher taxonomic levels. It includes no representatives of one of the three octocoral orders (Helioporacea), only 5 species from the phylogenetically diverse subordinal groups Scleraxonia, Stolonifera, and Alcyoniina (a combined total of 20 families), and representatives of only 4 genera of order Pennatulacea. Well over half of the taxa in the tree belong to just 4 families: Plexauridae, Gorgoniidae, Chrysogorgiidae, and the isidid sub-family Keratoisidinae. As a result of this strong taxonomic bias, the authors actually recover a phylogeny in which the three previously-recognized major clades of octocorals (Holaxonia-Alcyoniina, Calcaxonia-Pennatulacea, and Anthomastus-Corallium) are more strongly supported than in other studies. This outcome can be attributed to the absence of a number of taxa of Scleraxonia, Stolonifera and Alcyoniina that cluster near the base of Octocorallia and do not fall cleanly into any of the three major clades. A more recent, taxonomically comprehensive phylogeny based on both mitochondrial and nuclear markers illustrates this phylogenetic complexity very clearly (see McFadden & Ofwegen, 2012, *Invert Syst*).

The primary conclusions reached by the authors are (1) that there is a fourth major clade of Octocorallia (Pennatulacea + Isididae) that is revealed by mtMutS but not ND2; and (2) that the concatenated dataset of mtMutS + ND2 gives better resolution than either single-gene dataset. The second conclusion is trivial (when congruent gene trees are combined appropriately, the result is almost always better resolution and

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stronger support values), and the first conclusion is simply not supported by either the mtMutS or concatenated trees. As shown in Fig 2, clade D is simply Pennatulacea along with *Viminella* (family Ellisellidae, not Isididae as stated in Table 1), a clade that was recovered by McFadden et al. and has been confirmed in subsequent analyses. There is, however, no support for the relationships among *any* of the clades A, B, C and D shown in the concatenated tree. More inclusive taxon sampling and additional markers would show clades C and D united within a well-supported, monophyletic [Pennatulacea + Calcaxonia] (see McFadden & Ofwegen 2012).

An additional troubling aspect of this manuscript is the number of taxonomic errors apparent within the tree that appear to have gone unnoticed (or at least are not remarked upon) by the authors. Whether these are due to taxonomic misidentifications or sequence contamination is unclear, but some notable outcomes that should have raised red flags include: two specimens of *Acanella* nested in Pennatulacea, while a pennatulacean (*Gyrophyllum*) falls in Keratoisidinae; specimens of *Acanthogorgia* and *Narella* in Chrysogorgiidae; and a *Viminella* clustered with *Acanthogorgia*. Such obvious anomalies should have prompted the authors (none of whom are recognized taxonomic experts in this group) to have their specimen identifications confirmed by an appropriate expert.

Based on its substantial flaws, I am afraid I cannot recommend publication of this manuscript. Even if the authors were to (a) have their specimen identifications confirmed by an appropriate taxonomic expert; (b) make the specimen vouchers available in a public repository; and (c) increase the taxonomic representation within their phylogeny by adding their sequences to the cosmopolitan McFadden et al. 2006 dataset, I do not think the resulting phylogeny would prove enlightening. The positions of Keratoisidinae and Paragorgiidae within Octocorallia are now well established (see e.g. Pante et al. 2012; Herrera et al. 2010; Brockman and McFadden 2012), and simply adding those groups to the mtMutS+ND2 tree of McFadden et al. will not further resolve the problematic basal relationships within and among the major clades of Octocoral-

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lia. The field has moved beyond the stage at which a single-locus (e.g. mitochondrial) gene tree can be considered adequate for resolution of deep relationships within such a phylogenetically complex and taxonomically problematic group.

Specific Comments and Technical Corrections

general note throughout paper: names of higher taxa (sub-class, orders, sub-ordinal groups, families) should not be italicized

p. 16979: 15, 18: correct spelling to Isididae

p. 16980: 5: insert comma after “soft corals”

15: include Pante et al. 2012, Herrera et al. 2010, McFadden & Ofwegen 2012

17: “with taxonomic revisions occurring frequently”; I would say the problem with Octocorallia is that there have been far too few taxonomic revisions – many more are needed than have been made

19: change “lags” to “lag”

21: not clear what “a monophyly within Octocorallia and Hexacorallia” means; should this read “monophyletic Octocorallia and Hexacorallia”? cite the original source for this work rather than the McFadden et al review

p. 16981: 1: “has been shown to reach twice the amount variation” doesn’t make sense; also, the amount of variation in a gene doesn’t make it more or less suitable than other genes for phylogenetics in general, just for different levels of phylogenetic questions

7: Shearer et al. didn’t work on octocorals. . . .cite original sources for these studies

11: see also Pante et al. 2012 and McFadden & Ofwegen 2012 for comprehensive phylogenies that include other genes

20: correct spelling is Coralliidae

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p. 16982: 2: a larger phylogeny in what context? presumably not a broader sample of genera and families than McFadden 2006

p. 16983: 1: where are specimen vouchers deposited?

13: change “fractionated” to “fractionated”

20: change “Mudd University Institute” to “Harvey Mudd College”

p. 16984: 2, what models of evolution were chosen? did models and congruence of single-gene phylogenies support concatenation?

10: how were gaps treated in maximum parsimony analyses?

16: what does “levels of species identification” mean, and how was it assessed using genetic distance measures?

21: this section is difficult to interpret; standard reporting would give the range of nucleotide sequence lengths (not including indels) followed by the length of the alignment, for example “mtMutS fragments ranged from 477-573 nucleotides (159-191 amino acids) in length for a total alignment length of 709 nucleotides”; for NAD2, presumably the alignment did not vary in length, but the sequences within the alignment did

p. 16985: 8: how can the monophyly of Octocorallia be assessed without inclusion of outgroup taxa?

10: “the first of these clades” – what clades? no clades have yet been introduced

16: the subordinal group is Alcyoniina, not Alcyoniinae

20: what is “New species Ma33”? can’t someone at least put a family and genus on it?

22: Alcyoniinae is not a family; should this be Alcyoniidae?

25: what does “at the top of the clade” mean?

p. 16896: 1: Were any sub-families of Isididae represented other than Keratoisidinae?

other studies (Pante et al. 2012) have shown Isididae to be polyphyletic

8: correct spelling to Pennatulacea

9: these two Acanella samples have to be either misidentified or switched with another sample, perhaps the Gyrophyllum that falls within Isididae?

p. 16988: 12: what does “the nad2 gene order appearing to be conserved” mean, and what does it have to do with sequence variation?

15: what’s a polyphyletic origin of Isididae? does this mean hybrid origin?

24: shouldn’t this be Acanthogorgiidae rather than Chrysogorgiidae?

p. 16989: 3: the France 2007 study is about isidids, so how is that relevant to Paramuricea?

7: because most samples have not been identified to species in this study, there is no way to comment on the suitability of mtMutS for barcoding, a topic that has been addressed well in other studies

Fig, 2: it would be very helpful if on the tree it could be indicated which specimens are new and which are from GenBank

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