I commend the authors on their hard work. I'm sure that assembling this dataset was an enormous effort! Nice job! There is lots to like in this paper, and I think that it could be an important contribution.

However, to make sure the author's hard work is not wasted, this paper needs major changes to the analysis and how the results are discussed. First of all, the analysis of genetic variation needs to better take sample size into account. As shown below, doing so will change their conclusions significantly. Second, the authors need to let the data "speak". Instead, they are trying to find a way to force the data to support their a priori hypothesis: that the species has spread from Guam to other locations recently. A more balanced analysis of their data is unlikely to find support for this hypothesis, and I explain why below. There is no point in collecting data if you will say that it supports your a priori conclusion no matter what it looks like.

Introduction:

In the introduction, the authors seem to have done a nice job of laying out the background of what is known about this species (though I'm not an expert, so I can't comment on whether they cited the literature appropriately). However, they say very little to introduce or justify the current study:

"Despite gaining an understanding of the mechanisms of T. hoshinota's competitive success, little is known about the genetic diversity of T. hoshinota across its range. Such genetic data would be useful in theorizing how the species has spread, and in potential management strategies."

OK, sounds good! But now I think we need to understand what the possible hypotheses are, and how the data might reject them. I imagine these might include things like: if the species originated in Guam, and then spread explosively in just a few decades, we would expect much higher genetic diversity in Guam than other places; indeed, the diversity might be extremely low in other places. In contrast, if the species originated somewhere else but eventually spread to Guam, the pattern might be the same but with a different diverse population. A pattern with equal diversity across a broader region would seem to support a very different scenario: one in which the species has been endemic over a large area, but has only recently been noticed, or perhaps become much more abundant in places where people dive. Perhaps, as the authors state, the competative balance between sponge and coral has recently changed due to other factors.

With a clear idea of what the different scenarios could be, and how the data might support or reject those different patterns, the data can be viewed more clearly.

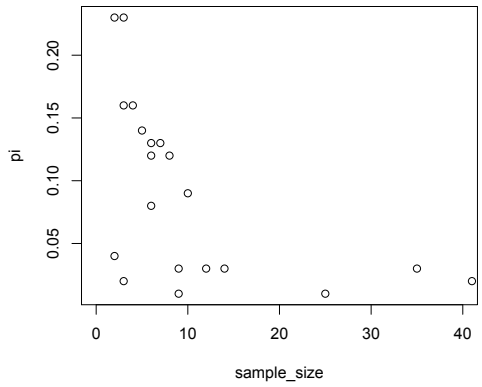
Results:

The way the results are reported needs significant changes.

First: I would suggest using pi and theta, rather than pi and haplotype diversity. Unlike a mitochondrial locus, there can be recombination in the nucleus, so haplotype diversity seems hard to interpret.

Second: you need to think carefully about the implications of sample size. I understand that it was impossible to collect large samples from everywhere, and I certainly think we should try to learn from your data despite this limitation. However, in order to learn from it, we need to make sure we are analyzing it correctly. You mention that you can't calculate diversity when n=1, but can you really calculate it when n=2? n=3? I think there is a decent body of literature suggesting that estimates of pi are very inaccurate without 15 or 20 haplotypes.

As a way to investigate this question, I plotted all the estimates of pi from the table vs. the sample size.



As you can see, all the samples very high diversity have n < 10. This is a concerning pattern and suggests a statistical artifact.

I would suggest trying to get standard deviations on your estimates, and/or testing whether differences in diversity are significantly different. This could be done with a bootstrap resampling scheme: if your difference is more extremely than the bootstrapped values, you have a significant difference. I think you will find that the standard deviation of most of your estimates is very large. Population genetic studies usually try to collect 20 or more samples to get a good estimate of pi. Again, this is no reason not to report your data: but we need to make sure your conclusions don't outstrip their empirical support. At the very least, you will probably need to estimate diversity only for the larger regions, and/or not include samples with n<5. I suspect that in the end, you data will more not support a hypothesis of different diversity among regions...and that is interesting!

I am also very suspicious of some of your numbers. Nucleotide diversity (pi) of 0.2 would mean that any two random sequences from that population differ by 20%! That is extremely high! I don't see where it says how long your sequenced region is (you need to state that), but it looks

like there are only about 11 genetic steps between any two haplotypes in your network. How can pi = 20%? I suspect this is some sort of error relating to extremely small sample sizes. If not, you will need to explain how this locus has such extraordinary levels of diversity.

Because sample sizes differ among sites, I think figure 2 is impossible to interpret. If you plot the number of haplotypes at each location, genetic diversity is confounded with sample size (since a larger sample size should discover more diversity). Instead, you need to plot something like pi or theta...perhaps colors where the shade of the circle is different depending on the genetic diversity at that site.

Discussion: despite the limitations due to sample size, I think you will be able to reject some hypotheses. The most obvious one is that we seem to be able to reject a hypothesis where Guam (or some other population) is highly diverse and diversity of extremely low everywhere else. What would this mean? The standardof evidence is modest, but I'm sure the authors could come up with some new hypothese to test in the future, such as: the sponge has been present broadly, but not common enough to be noticed; the sponge was cryptic in regions with low investigative effort, perhaps in Indonesia, and has spread in a way that has prevented very strong population bottlenecks, etc.

The authors would have to add important caveats:

1. Sample sizes are small, so result is preliminary

2. It is possible they have sampled multiple species and mixed them, since they didn't examine morphology

3. It sounds like the way samples were collected might be very different among sites, and this could be a problem. If some samples were all near each other on one reef, while other locations had samples spaced out or from multiple sub regions, this could create diversity differences.

Another note about diversity:

In the discussion (line 340), the authors state that one population might have had enough time to accumulate diversity since 1993. If the authors mean the accumulation of diversity through mutation, this is incorrect, and they need to go back and look up what is meant by "a long time" in evolutionary biology (diversity might accumulate from multiple introductions on the order of decades, I suppose). The existence of similar genetic diversity throughout much of the species range does NOT support their a priori hypothesis is recent spread from a founder population. Instead it might mean:

-The species (or species complex) is endemic over a broad region, and was just not noticed until recently (perhaps this can be rejected for some regions, using other data?)

-The species (or species complex) is endemic over a broad region, and has suddenly / recently increased in population size in areas where people go (this seems most likely to me)

-The historical story of expansion is correct, but the data don't show it for some reason (see caveats above)

I hope the authors will reanalyze their data and present it in a way that is less prejudiced, as it seems like it would be a great contribution! I know the above seems like a lot more work, after you have already done so much, but it seems very worthwhile, as in the current form, I don't think we can conclude much.

Additional comments:

Title:

The review document has two versions of the title, and one contains the out-of-date taxonomy: Suberitidae: Hadromerida

Abstract:

typo: "Guam Bryan in 1973" missing the "by"

"Results indicate two major haplotypes derived from Guam may play an important role in the spread of this sponge in the Indo-West Pacific region"

-You have no evidence they are "derived" from Guam. They occur there, but they occur other places. How does the data show they are derived from Guam?

"These trends of dominant haplotypes may indicate an advantage for a certain type of T. hoshinota invasion and colonization."

-You would need to supoprt this statment with citations or other evidence. I don't htink you will find it. There is no reason to assume that a pattern of some dominant haplotypes with other rare ones indicates a selective advantage. Indeed, the Grant paper they keep citing (about genetic diversity in fish mitochondria) says this pattern is common and due to demographic history.

"The results of this study indicate that the distribution and occurrence of T. hoshinota is based on the dispersal of haplotypes from Guam to other locations ranging from Japan to southern Indonesia via oceanic current transport"

-I don't think that it does: see above.

The occurrence of unique haplotypes distributed across the region is speculated to be due to such local outbreaks of T. hoshinota.

-I don't think this makes sense. Outbreaks don't create the mutations.

Methods:

I would suggest citing the paper that originally developed your universal primers

How long is the ITS region that you sequenced?

The sequences need to be deposited in Genbank, with accession numbers in the manuscript.

I don't think you cite any papers explaining why you chose ITS as a region to sequence, which would be nice to do.

Results:

FIG2 : what are these arrows and abbreviations? Everything in the figure should be explained inthe legend. What is the star?

Also: please explain in the methods or legend how the map was made, or, if it is borrowed, what the source is.

Also: as noted above, haplotype number is not the right thing to plot, I think, because of varying sample size.

Discussion:

I think the two conclusions here are not supported by the data.

"For some populations (ARA; GI, MDV) there was no genetic diversity": not zero, in fact.

Figure S1 seems to be the same as a figure in paper...?

Table 2 would need some improvement to be readable. Maybe location names on the axes, with colors denoting regions? Again, though, you have a serious sample size issue for some of these locations.