

ITS/cpDNA Phylogenies

When dealing with such a recent and taxonomically challenging group of plants, is this "standard" approach (cpDNA and ITS) the best way to establish credible phylogenetic hypotheses, especially considering the rampant hybridization among these taxa? Also, which molecular markers/approaches would be best to test the author's hypothesis regarding "rapid and contemporary speciation"?

In the cpDNA phylogeny and combined cpDNA and ITS1 phylogeny, the ILD test showed the data partitions were significantly incongruent. Do you agree with the authors reasoning to continue to move forward and combine the data sets, and how does this affect their interpretation of their results?

Is it expected to find more species being recovered as non monophyletic in cpDNA than nDNA when reticulation is present?

The authors were unable to amplify the ITS segment and instead used the sub segment ITS1. Why do primers fail to lead to amplification and how are such problems managed when they occur?

Neighbor-Net Analysis

I understand that the NN analysis groups sequences based on distance, but I don't follow where the rest of the internal structure comes from. Could we discuss this approach to detecting reticulation?

Ramdhani et al. 2011 choose the Neighbor-Net method to construct their phylogenetic network. In previous readings we have seen the software TCS used (Beatty & Provan 2011). Do you think Ramdhani et al. chose the most appropriate network? (See Woolley SM, Posada D, Crandall KA, 2008 A Comparison of Phylogenetic Network Methods Using Computer Simulation. PLoS ONE 3(4): e1913, and Cassens I, Mardulyn P, Milinkovitch MC (2005) Evaluating intraspecific "network" construction methods using simulated sequence data: do existing algorithms outperform the global maximum parsimony approach? Systematic Biology 54: 363–372).

It is not clear for me how to interpret the split graphs of the NN analysis. What do all those lines connecting the sample branches mean?

Taxonomic Problems/Speciation

Treutlein et al (2003) argued that because revising the taxonomy of *Haworthia* and related genera of *Alooiidae* would be complicated nothing should be done. Is a complicated phylogeny/taxonomy a valid scientific reason to do nothing in this situation?

What is the best way to treat groups undergoing contemporary speciation?

The current taxonomy in the Rhamdhani paper seems rather a muddle with species confusion, low support measures, multiple polytomies and quite incongruent phylogenies; as it cries out for a taxonomic makeover, how would you approach what appears to be a daunting task?

They also talk about undergoing hybridization in subgenus *Haworthia*, and some examples are cited. When looking at the phylogenies, some few species appear in different positions on the tree. It looks like in addition to hybrids there is a very small circumscription for actually broader species, as is common in very ornamental groups (if you read Mr. Bayer comments probably you'll agree on that possibility).

Morphology

If morphology is directly related to molecular data, how is it that "the morphological rate and temp of evolution outpace the molecular rate and the sensitivity of molecular markers"?

The authors seem to favour a contemporary speciation scenario and state that assembling the phylogeny of this group under it may be difficult because of the lag of the molecular characters in relation to morphology. Can't this be assessed by a combined analysis of molecular and morphological data?, the morphology of the group seems to be complicated, but a better supported phylogeny can result.

The authors first state "None of these natural hybrids are between *Haworthia* subgenera, suggesting that the floral morphologies or other reproductive barriers are sufficient to ensure isolation." A page later (1010) they state "[pollinator specialization] is unlikely to be important in *Haworthia* owing to the similarity in flower structure among taxa." If the structures are so similar as to allow visitation by common pollinators, how can they be barriers?

The Rhamdhani paper on pages 1008 and 1010 seems to imply that floral morphological evolution precedes molecular evolution. Are they implying a Lamarckian process or am I missing something?

Sampling

Is sampling from herbarium specimens not an option for this 'genus'? Is this generally true for most succulents, or at least members of the Asphodelaceae? Is there even a great deal of material for this group in herbaria?

Conservation Concerns

How would the conservation status of any of the species involved (either *Aloe*, *Haworthia*, or *Astroloba*) be affected if some *Haworthia* are sunk into *aloe* or separated into their own Genus? Would some then be considered of lesser conservation priority?