Respuesta a lo revisores (Response to reviewers):

Revisor 1

Sugerencia de título: Pollen viability and germinability in a population of Bursera hybrids. Y eliminar a lo largo del texto el término "zone" es confuso

R= Estamos agradecidos con el revisor por sus valiosos comentarios que han mejorado la calidad de nuestro trabajo. Con respecto a este comentario, el término hybrid zone es ampliamente usado en la literatura sobre hibridación natural. Sin embargo, dado que aún no contamos con evidencia genética para afirmar que los individuos con características morfológicas intermedias de *B. bipinnata y B. cuneata* son verdaderos híbridos como sugiere el revisor 2, hemos decidido cambiar el título para reflejar este hecho. El nuevo título es "**Pollen viability and germinability of putative *Bursera* hybrids (section *Bullockia*) in Mexico"**

Sustituir por "frequently" ya que no se puede generalizar, pues hay pocos estudios al respecto

R= Hecho

Nombres de especies siempre deben ir con sus respectivos autores, siguiendo las abreviaturas de Brummit

R = Hecho con todos los nombres científicos

Indicar el significado de las siglas que aparecen a lo largo del texto..tal vez al pie de página

R = Se indicaron las abreviaturas desde el inicio.

Mencionar que clasificación se usa para las comunidades vegetales

R= Se añadió la referencia. Ver Ln 84

Eliminar párrafo aquí y pasar a conclusiones:

R= Hecho. Líneas 282-284.

Otros comentarios de estilo y redacción.

R= Se hicieron todos los cambios sugeridos de estilo

Revisor 2

The author(s) have made assumption that the morphologically intermediate individuals are true genetic hybrids and have not considered the limitations of the pollen viability tests, which is problematic for how the results are interpreted and their overall conclusions. There is no genetic data to back up their assumption of hybridity, and no detailed morphological analysis presented in this manuscript to demonstrate their morphological intermediacy, aside from pictures in Figure 1. I agree that the morphologically intermediate individuals do look like hybrids between *Bursera bipinnata* and *B. cuneata*, but the authors should take a more circumspect approach to test this as a hypothesis. It is only safe to say that these are putative or hypothesized hybrids. The authors are encouraged to view the pollen viability as a first test of hybridity. Seen from this light, the results of this study are important but inconclusive in important ways not discussed by the authors

R = We are thankful to the reviewer for the valuable comments provided, which have improved the quality of our work. In relation to this comment, we agree with the reviewer that there is no genetic data to prove that the hybrids are true hybrids of *Bursera bipinnata* and *B. cuneata,* which is indeed the next step to test in our lab. To avoid overinterpretation in the light of the available evidence in this work, we have modified the title, all the text, and figure legends to point that they are putative hybrids. Also, we have modified the discussion to stress the limitations of this work and that further genetic analyses should be performed to confirm the genetic background of putative hybrids. See lines:

Lines 209-212: "Our study represents a first test of hybridity between *B. cuneata* and *B. bipinnata* since no genetic data is available to confirm the hybrid status of individuals with intermediate morphological characteristics. Yet, it contributes with relevant information to understand the role and consequences of hybridization on Mexican *Bursera* species"

Lines 281-286: "Given the frequent anecdotical observation of hybridization among Mexican *Bursera* species, more studies are needed to better understand the mechanisms and consequences of natural hybridization within this group. Specifically, genetic studies are required to confirm the genetic identity of putative hybrids, the maternal and paternal contribution of hybrid origin, the occurrence of diploid or polyploid hybrids and if several generations of hybrids coexist and if genetic introgression has occurred".

The authors do not consider the different types of hybrids in their interpretation of the viability results. The morphologically intermediate individuals may be diploid hybrids or they may be polyploid hybrids. One might suspect allopolyploidy, incorporating *Bursera bipinnata* and *B. cuneata*, but an autopolyploid history cannot be ruled out. Polyploid hybrids frequently have excellent pollen viability as compared to diploid hybrids. Could this be an explanation of their results?

I suggest incorporating side-by-side images of the *Bursera bipinnata* and *B. cuneata* and "hybrid" pollen grains at similar scale. The TTC stained pollen image at 40X could be placed under the photograph of each tree in Figure 1. Polyploid grains are usually larger. This type of comparative image would allow the reader to assess this possibility.

R= We agree with this assertion; the ploidy level could be one reason of the higher viability of hybrids, although genetic analysis would be needed to test this. Regarding to the pollen size, from our initial observations we did not notice a difference in the size of pollen grains or in other apparent morphological characteristics among the 3 groups. This is the reason we did not include an image for each species and the hybrid in the first place. However, we agree that it is relevant to show these images, so the reader can see this data. We have included the side by side images of pollen grains (40x TTC staining) for the 3 groups within Fig. 2

Also, we included these ideas in the discussion:

Lines 235-257: "Consequences of hybridization highly depend on hybrid fitness (Campbell and Wasser, 2007). It is usually expected that spontaneous hybrids present low fertility, but polyploid hybrid plants may have increased fertility and local adaptation respective to their diploid parental species (Alix et al., 2017). Allopolyploids are typically expected since it occurs via hybridization from the combination of divergent species of diploid genomes (Osabe et al., 2012), although autopolyploid hybrids may also occur (Barker et al., 2015). The observed higher pollen germination rate for the putative hybrid relative to at least one parent (*B. bipinnata*) did not follow our initial expectations. This result might be explained by the occurrence of hybrids with different genetic backgrounds, such as the formation of polyploid hybrids. Some authors argue that polyploids hybrids are characterized by a larger pollen size relative to their progenitors (Hossain et al. 1990; Wronska-Pilarek et al. 2013; 2016), while others have found no such relationship (Franssen et al. 2001; Karlsdottir et al. 2008; Lazarevic et al. 2013). For example, Wronska-Pilarek et al., (2013) conducted a pollen morphological comparison among three *Crataegus* species, and they found that natural hybrids had larger pollen grains than the parental species, except for one species whose pollen size was similar to that of hybrids. On the other hand, Franssen et al., (2001) for ten *Amaranthus* species and their interspecific hybrids, observed no differences on pollen size between hybrids and parental species, but marked differences on the number of pollen apertures. From our pollen count observations, we did not notice apparent differences on the size of pollen grains between the putative hybrids and the parent species (Fig. 2C-E), however we cannot rule out such morphological differences as we did not systematically evaluate the morphology of pollen grains. Other possibility is that the putative hybrids are not true hybrids, but only genetic data can confirm their identity".

Given that pollen viability of the "putative hybrid" is not significantly lower than its putative parent species, this leaves multiple possibilities open that should be broached in the discussion. The morphologically intermediate individuals 1) may not be not hybrids, 2) may be fertile diploid hybrids, 3) may be fertile polyploid hybrids. If they are hybrid, the parentage still remains a hypothesis. Determining the true parentage, the directionality of the hybridization events and its frequency would be fascinating next steps for the authors to consider.

R= Related to previous comments. See lines 209-212; 235-257; 282-287.

I included minor corrections for the English language

R= We have corrected all sentences as suggested through the manuscript

Please clarify this phrase as a separate sentence.

R= Corrected as suggested

By “bare” I think you mean smooth. Thus the phrase would be “smooth, grey bark”

R= Yes, corrected.

About how many putative hybrid trees where observed? 10? 100? 1000? Even a rough estimate can help flesh out the scale of the possible introgression between bipinnate and cuneate.

R= During our field sampling we did a rough estimation of species and hybrid frequency in two small forest patches. Our observations suggest that *B. bipinnata, B. cuneata* and their putative hybrid occur with a proportion of 6:2:1 individuals respectively. We added this information in the text, lines 146-148.

State the exact number of individuals sampled for each species and the putative hybrids

R= The total number of sampled trees were added within parenthesis in the sentence. Line 152

What did you test for, generally? It is not clear in this paragraph.

R= Sentence corrected to clarify we are testing for significant differences on the proportion of viable pollen among the 3 groups. Lines 185-186.

What does n equal? Fields of view observed? Number flowers? It is hard to assess the power of the observations without reporting the total number of pollen grains counted. There might have been 4 grains in the field of view, or 400 grains.

R= We added sample size for the number of field observed and the sample size of the total number of pollen grains observed. Lines 193-196 and 201-203.

In which direction? Species MORE viable than hybrid?

R= Yes, *B. pibinnata* more viable than the hybrid and to *B. cuneata*. We changed the sentence. Lines 197-199.

The first two sentences should be saved for the Discussion section – they are not appropriate for the Results.

R= Removed

Again, what is the difference that is significant

R= Same as previous, we corrected the sentence to clarify direction. See lines 205-206.

Does this mean that you did not measure rate, or that you measured it, but didn't note any differences?

R = We removed this sentence as it was confusing.

Unclear what is meant by differential rates

R= Changed to contrasting results; see lines 228-230.

What viability levels are typical for closely related species, or trees in the TDF? Providing a citation would be able to qualify your assertion that <50% is actually low. Maybe <50% is normal for trees in the TDF.

R= We agree with this comment that a reference was needed for comparison. There are no many studies testing pollen viability on TDF trees. We have added two studies on *Protium spruceanum* and *Pachira quinata*, which show 90% and 50% of pollen viability respectively. We modified the assertion of low pollen viability in our study as no enough evidence is available to state this. The sentence was modified as:

Lines 269-279: "Pollen viability studies for TDF tree species are still scarce. Reproductive biology data is highly relevant information with consequences for species conservation and restoration. For instance, for *Pachira quinata* (Jacq.) W.S. Alverson fruit production was related to pollen load size, while half of the pollen grains were able to germinate and develop pollen tubes on the flower stigma (Quesada et al., 2001). Other study in *Protium spruceanum* (Benth.) Engl., a tree species of the Burseraceae family, showed the occurrence of high percentage (90%) of viable pollen (De Almeida-Viera et al., 2010). We found pollen viability rates below 50% for both *Bursera* species and the putative hybrid, which may have consequences on seed production and viability. Germination studies on *Bursera* species highlight low germination rates likely due to high occurrence of empty seeds (Bonfil et al., 2008; Hernández-Téllez, 2015)."

I agree, but I also think genetic tests are necessary first to confirm that the hybrid is actually truly a hybrid. Very quickly, you would be able to determine who is the maternal vs. paternal parent, if the initial crossing event happened once or mulitple times, and if the hybrids are diploid or polyploid.

R= Agree, we have expanded the need of genetic analyses to test such aspects.

Lines 282-287: Specifically, genetic studies are required to confirm the genetic identity of putative hybrids, the maternal and paternal contribution of hybrid origin, the occurrence of diploid or polyploid hybrids, and if several generations of hybrids coexist and if genetic introgression has occurred.