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Punctuated plastome reduction and host-parasite horizontal gene transfer in the holoparasitic plant genus *Aphyllon*

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Authors

Schneider, Adam C
Chun, Harold
Stefanović, Saša
[et al.](#)

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SUPPLEMENTARY RESULTS

Differences in gene content of Aphyllon sect. Aphyllon plastomes:

- atpE*—This gene codes for ATP synthase subunit c. It is the only ATP synthase gene remaining in *Aphyllon* subsp. *Aphyllon*, and is present in only three species: *A. epigalium* subsp. *notocalifornicum*, *A. “franciscanum”*, and *A. uniflorum*. In *A. epigalium* subsp. *epigalium*, the open reading frame (ORF) has been disrupted by two C to T nonsense mutations.
- petG*—This gene codes for the cytochrome b6-f complex subunit 5. The ORF of this gene is intact in *A. purpureum* and *A. uniflorum*. In *A. epigalium* and *A. “franciscanum”*, the ORF is intact except for a G to T point mutation in the start codon.
- petN*—This gene codes for the cytochrome b6-f complex subunit 8. *Aphyllon epigalium* subsp. *epigalium*, and *A. “franciscanum”* both have 90bp ORFs but high enough amino acid sequence divergence that blastx did not report any similar proteins in the NCBI database. In *A. epigalium* subsp. *notocalifornicum*, a one-base frameshift has been introduced following site six. Additionally, the open reading frame of *petN* appears to be intact in subsp. *epigalium* but not subsp. *notocalifornicum* (one base frameshift after site 6), but with high enough amino acid sequence divergence that blastx did not report any similar proteins in the NCBI database.
- psbM*—This gene codes for a protein in the photosystem II reaction center. This gene has an intact ORF in both samples of *A. “franciscanum”*, but not in any other samples of *A. sect. Aphyllon*.
- psbN*—This gene codes for a protein in the photosystem II reaction center. In *A. fasciculatum* it has a 129-bp ORF, which is only one codon shorter than the ORF in its photosynthetic relatives *Lindenbergia* and *Schwalbea*. However, high sequence divergence at the amino acid level and its absence in other holoparasitic relatives suggest it is likely not functional.
- rpl23*—This gene codes for 50S ribosomal protein L23. This gene is present in *A. epigalium*, and has been lost in *A. fasciculatum*, *A. purpureum*, and *A. uniflorum*. Both samples of *A. “franciscanum”* have an intact ORF; however, there is no apparent stop codon before the downstream rpl2 ORF.
- rps4*—This gene codes for ribosomal protein S4. All species show an intact ORF, however *A. epigalium* subsp. *notocalifornicum* also contains a 189bp in-frame deletion from the middle of the gene, however it otherwise differs from *A. e* subsp. *epigalium* at only four other sites.

Pseudogenization of the xenologous *rbcL* in *Aphyllon epigalium*—The xenologous *rbcL* in both species of *Aphyllon epigalium* is approximately 1400bp long and contains several deletions to the open reading frame, some of them in frame and others frameshifted, and some shared by both subspecies while others limited to individuals of one or the other. Relative to the functional copy in *Galium angustifolium*, about the same amount of evolutionary change has occurred in the *rbcL* pseudogene of each of the subspecies of *A. epigalium*, as autapomorphic (unique derived) states (i.e. two point mutations and one 9bp deletion vs. two point mutations and four deletions, with one of these deletions only present in some populations). Additionally, sites 83-91 have been lost in both subspecies of *A. epigalium* and sites 388-396 in subsp. *notocalifornicum* and two of the four sampled individuals of subsp. *epigalium*, all in frame. However, the frameshift mutations in each subspecies are unique. In *A. epigalium* subsp. *notocalifornicum* a deletion of sites 416-419 results in a premature stop codon at site 485, whereas in *A. e.* subsp. *epigalium* a deletion at sites 630-640 results in a stop codon at site 678. Reversing either of these deletions manually restores the open reading frame (i.e. no downstream nonsense mutations would be present).