Supplementary Material for Schneider *et al*. (2018) — Punctuated plastome reduction and host-parasite horizontal gene transfer in the holoparasitic plant genus *Aphyllon*
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SUPPLEMENTARY METHODS

Aphyllon habit and sampling

Species in the genus *Aphyllon* are obligate root exo-parasites, and attach to a single host root using a specialized organ called a haustorium (Figure 2, inset). These plants may be annual or perennial. During the vegetative phase of growth, the plant is completely subterranean and exists as a mass of tissue attached to the plant root. When reproductive, *Aphyllon* species send up short flowering stalks that emerge from the soil. Given that their hosts are generally perennial herbs or shrubs, the host root system can be extensive, so that the emergence of the parasitic plant flowers is not necessarily near the stem of the host. *Aphyllon* species generally parasitize a suite of closely related species.

Floral tissue was used for DNA extraction, following standard practice with holoparasitic Orobanchaceae. Leaves in this genus are reduced to scale-like bracts, and stems are succulent and starchy. When possible, flowers were sampled before anthesis to avoid pollen contamination from other individuals. Bulk DNA has been observed as traveling through symplastic connections, and sampling the flowers also minimizes the already low chance of host contamination (see Discussion, Horizontal gene transfer of plastid genes and their evolutionary fate).

Illumina sequencing coverage of organellar genomes

Sequencing coverage of the chloroplast genomes ranged between 56x and 503x, depending on the individual (Table 1), in rough proportion to total sequenced reads (see below). The two individuals of *Aphyllon epigalium* had intermediate plastome coverage, on the order of 100x. In contrast, sequencing coverage of the *A. epigalium* mitochondrial contigs that contained the *Galium*-like *rbcL* sequence was 11–25x across several independent *de novo* assemblies, though this increased to 41x and 49x, respectively, when the original trimmed Illumina reads were reference-mapped to the contigs. This is consistent with the sequencing coverage of other *de novo* contigs from these individuasl that contained only mitochondrial genes.

rbcL sequencing

PCR amplifications of *rbcL* were performed using AccuPower PCR PreMix kits (Bioneer, Alameda, California, USA) with the following thermocycling conditions: 92° C, 5 min; 35 or 40 x (94° C, 45 s; 53.5° C, 45 s; 72° C, 1 min); 72° C, 5 min. The forward primer sequence was 5’ ATG TCA CCA CAA ACA GAA AC 3’ and the reverse primer was 5’ CAG CAA CTA GTT CAG GRC TCC 3’. PCR product was purified using ExoSAP (USB Products, Cleveland, Ohio, USA), then both complementary DNA strands were sequenced using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, California, USA).