

## **Expanding the scope of leaf symbiosis: *Caballeronia* endophytes in *Empogona* and *Tricalysia* (Coffeae, Rubiaceae)**

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Leaf symbiosis is a remarkable phenomenon observed in Rubiaceae host plants, where they interact with bacterial endophytes residing within their leaves. Although approximately 650 species from 8 genera across 4 tribes exhibit this symbiosis, its full extent within Rubiaceae remains unknown. Our primary objective is to investigate the potential presence of leaf endophytes in the African plant genera *Empogona* and *Tricalysia* and, if confirmed, determine their identity. To accomplish this, we extracted total DNA from the leaves of four Coffeae tribe species (*Empogona congesta*, *Tricalysia hensii*, *T. lasiodelphys*, and *T. semidecidua*) and sequenced it. Subsequently, we filtered out bacterial reads. Through phylogenetic analysis of the endophytes, we were able to ascertain their identity and establish their relationship with known symbiotic organisms. Notably, all four species harboured non-nodulated leaf endophytes, specifically identified as *Caballeronia*. Although these endophytes are distinct from one another, they are related to other nodulated and non-nodulated endophytes. Interestingly, we did not observe any discernible phylogenetic or geographic patterns among the endophytes or their host plants. Our findings demonstrate the presence of *Caballeronia* endophytes in the leaves of *Empogona* and *Tricalysia*, two genera previously not associated with leaf symbiosis. This interaction is likely to have a broader distribution, and further discoveries are inevitable in the future.