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RESEARCH PAPER

# When do different $C_4$ leaf anatomies indicate independent $C_4$ origins? Parallel evolution of $C_4$ leaf types in Camphorosmeae (Chenopodiaceae)

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## Abstract

**Broad-scale phylogenetic studies give first insights in numbers, relationships, and ages of $C_4$ lineages. They are, however, generally limited to a model that treats the evolution of the complex $C_4$ syndrome in different lineages as a directly comparable process. Here, we use a resolved and well-sampled phylogenetic tree of Camphorosmeae, based on three chloroplast and one nuclear marker and on leaf anatomical traits to infer a more detailed picture of $C_4$ leaf-type evolution in this lineage. Our ancestral character state reconstructions allowed two scenarios: (i) *Sedobassia* is a derived $C_3$/$C_4$ intermediate, implying two independent gains of $C_4$ in *Bassia* and *Camphorosma*; or (ii) *Sedobassia* is a plesiomorphic $C_3$/$C_4$ intermediate, representing a syndrome ancestral to the *Bassia*/*Camphorosma*/*Sedobassia* lineage. In *Bassia*, a kochioid leaf type (*Bassia muricata* and/or *Bassia prostrata* type) is ancestral. At least three independent losses of water-storage tissue occurred, resulting in parallel shifts towards an atriplicoid leaf type. These changes in leaf anatomy are adaptations to different survival strategies in steppic or semi-desert habitats with seasonal rainfall. In contrast, *Camphorosma* shows a fixed $C_4$ anatomy differing from *Bassia* types in its continuous Kranz layer, which indeed points to an independent origin of the full $C_4$ syndrome in *Camphorosma*, either from an independent $C_3$ or from a common $C_3$/$C_4$ intermediate ancestor, perhaps similar to its $C_3$/$C_4$ intermediate sister genus *Sedobassia*. The enlarged bundle sheath cells of *Sedobassia* might represent an important early step in $C_4$ evolution in Camphorosmeae.**

**Key words:** *Bassia*, bundle sheath, $C_4$ photosynthesis, *Camphorosma*, Kranz anatomy, *Sedobassia*, water-storage tissue.

## Introduction

Phylogenetic inference methods present a powerful means of identifying putative shifts between $C_3$ and $C_4$ photosynthesis (Sage *et al.*, 2011, and references therein). If photosynthetic syndrome is treated as a single (functional) character with two states (either $C_3$ or $C_4$), it is straightforward to infer the number and timing of shifts between states in a given clade. On this basis, Christin *et al.* (2008) inferred numerous independent origins of $C_4$ and dated them to the Oligocene decline in atmospheric $CO_2$. However, the $C_4$ syndrome is both complex (comprising multiple individual adaptations) and diverse (the adaptations differ across clades), and may have originated in response to different selective pressures in

Abbreviations: cp, chloroplast; ITS, internal transcribed spacer; ML, maximum-likelihood; mya, million years ago.