Molecular Dating applied to biogeography: Did Crypteroniaceae really disperse out-of-India?

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Introduction

Crypteroniaceae (Myrtales) is a **small family of evergreen tropical trees in South East Asia**. It comprises the genera *Crypteronia* (7 species, SE Asia), *Axinandra* (4 species, 1 endemic to Sri Lanka and 3 to SE Asia) and *Dactylocladus* (1 species endemic to Borneo). **The sister clade of Crypteroniaceae** comprises Peneaeceae (23 species, Cape Province), Oliniaceae (8 species, S Africa), *Rhynchocalyx* (1 species, S Africa) and and *Alzatea* (1 species, S America; see Fig. 1).

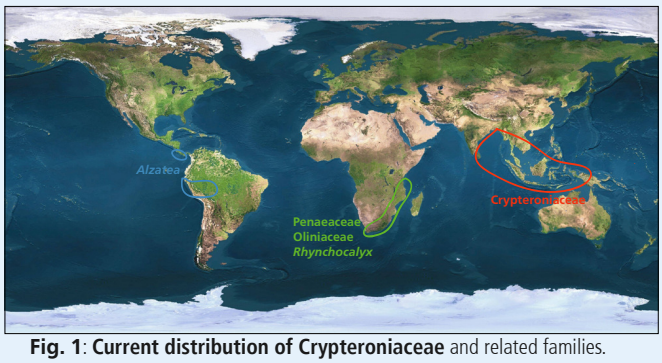

Fig. 1: **Current distribution of Crypteroniaceae** and related families.

According to one biogeographic hypothesis, **all these plant families have their common origin** in the **West Gondwanan** subcontinent about 100 million years (mys) ago. Then the stem lineage of the SE Asian Crypteroniaceae **dispersed from W Gondwana to the Deccan Plate** (comprising India and Madagascar) while it was rafting along the African coast. This happened between 110 and 88 mya (Fig. 2a), when India split from Madagascar and rafted towards Asia. The **rafting Indian island** therefore served as a "Noah's Ark" that **transported Crypteroniaceae to Asia**, from where they dispersed **out-of-India** into SE Asia (Fig. 2b, Conti et al. 2002).

To test this hypothesis, we used different **molecular dating approaches** that allowed us to **estimate the ages of the relevant nodes** in the phylogenetic trees. If the ages of these **phylogenetic splitting events** were congruent with the ages of **geologic events**, we would have some evidence in support of the out-of-India hypothesis.

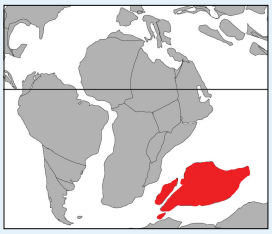

Fig. 2a: India splitting from Gondwana, about 110 mys ago.

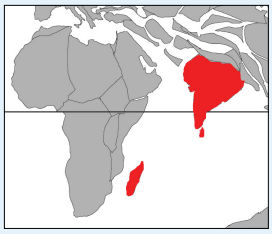

Fig. 2b: India connecting to Asia, about 50 mys ago.

Methods

The chloroplast DNA regions of ***rbc*L**, ***ndh*F**, and the ***rpl*16 intron**, as well as the nuclear ribosomal ***18S*** and ***26S*** gene loci were first amplified and sequenced (48 taxa; 5210 characters). The data was combined and **Bayesian phylogenetic trees** were reconstructed by using MrModeltest 1.1b (Nylander 2002) and MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). Maximum Likelihood (ML) bootstrap support was inferred by using PHYML (Guindon & Gascuel 2003) and BootPHYML v3 (Nylander 2004). For the clock-independent molecular dating, we used **Penalized Likelihood** implemented in r8s v1.6 (Sanderson 2003) and the **Bayesian approach** in PAML/multidivtime (Yang 1997; Thorne et al. 1998), which are both able to account for rate variation among lineages and allow multiple calibration constraints.

We used **two fossils for calibrating** the molecular clock simultaneously at two nodes:

- **Node E**: fossil Melastomataceae leaves from the Eocene of Washington State (**53 mys**)
- **Node F**: fossil *Myrtaceidites* pollen from the Santonian of Gabon (**86 mya**)

Based on their morphological characters, there is no unique solution how to assign these fossils to specific nodes in the tree (Rutschmann et al., *in press*). Therefore, we performed **two different calibrations** to cover the most probable fossil assignments: an **"early" calibration** (which resulted in the **red time bar**; see Fig. 3), and a **"late" one (purple time bar)**.

Results

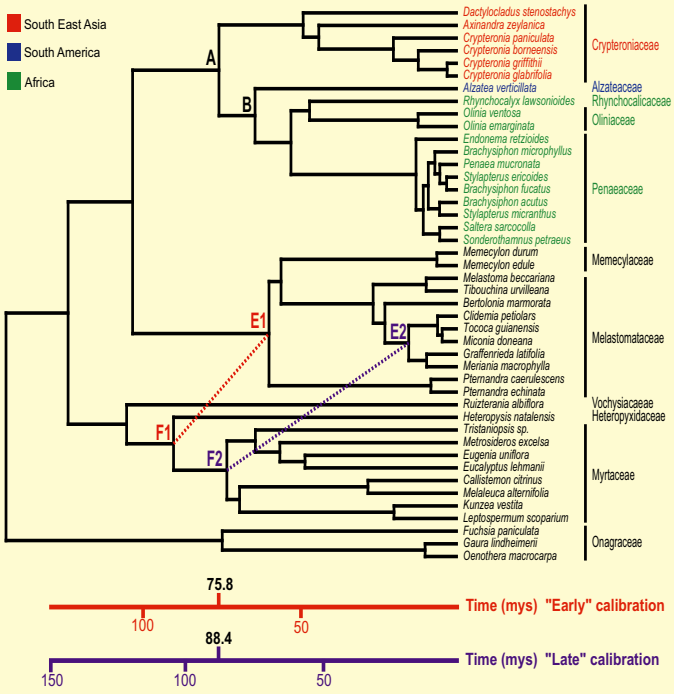

Fig. 3: **Chronogram generated by Bayesian molecular dating** (Thorne et al. 1998) based on Bayesian tree reconstructions and ML branch length estimates using combined *rbc*L, *ndh*F, *rpl*16 intron, n*18S*, and n*26S* DNA sequences. **Node A** represents the splitting of Cryperoniaceae from its sister clade, and **node B** the split between the African and S American taxa. The **red time bar** is the result of the **"early" calibration** by assigning both fossils to the more basal nodes in the tree, and the **purple time bar** was obtained by the **"late" calibration** where the fossils were assigned to the more derived nodes in the tree.

	"early" calibration	"late" calibration
Node A	75.8 CI: 67.3-84.3)	88.4 CI: 78.0-98.8
Node B	64.3 CI:55.6-73.0	75.1 CI: 64.4-85.7

Table 1: **Estimated ages for nodes A and B** in mys, based on the Bayesian chronogram (Fig. 3) obtained with multidivtime (Thorne & Kishino 2002). CI = 95% confidence intervals.

Conclusions

We estimate an **age range of 88 to 76 mys** for the split between the Crypteroniaceae stem lineage and its African-South American sister clade. At this time, the **Deccan plate was sufficiently close to the African coast** to receive biotic elements from Africa. This suggests that the Indian (Deccan) plate likely played a **central role** in promoting the **range expansion of the stem lineage** of Crypteroniaceae from West Gondwana to Asia.

However, **alternative scenarios** are possible, but, given the global evidence available so far, they seem to be **less likely**. Only the retrieval of pre-Tertiary fossils attributable to Crypteroniaceae from Africa, Madagascar, or India would unquestionably support the out-of-India hypothesis.

Crypteronia glabrifolia (Crypteroniaceae)

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