

## Molecular confirmation of the position of *Gossypium trifurcatum* Vollesen

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### Abstract

Taxonomic understanding is a necessary prerequisite for intelligent germplasm maintenance and evaluation. Here, we use molecular evidence to address the generic position of the poorly known and morphologically unusual taxon *Gossypium trifurcatum* Vollesen. This species possesses dentate leaves, a feature not otherwise found in *Gossypium* L. but one that is common in *Cienfuegosia* Cav., a related genus in the small Malvaceous tribe Gossypieae. *G. trifurcatum* is a rare plant, restricted to deserts of Eastern Somalia and known from only two collections, the last in 1980. Using DNA extracted from an herbarium specimen, we amplified and sequenced the chloroplast gene *ndhF*. Phylogenetic analysis reveals *G. trifurcatum* to be cladistically nested within *Gossypium*. These data diagnose dentate leaves as an autapomorphy within a genetically diverse assemblage of African–Arabian species, which remain the least well-represented cottons in germplasm collections.

### Introduction

Cultivated cotton belongs to *Gossypium*, a diverse genus of 50 species that are cosmopolitan inhabitants of arid to semi-arid environments (Fryxell 1979). Both allotetraploid (*Gossypium barbadense* L., *Gossypium hirsutum* L.) and diploid (*Gossypium arboreum* L., *Gossypium herbaceum* L.) species are cultivated for their fibers, meaning a wide range of species are potential sources of genetic variation for incorporation into cultivated lines. To this end, considerable effort has been directed at understanding natural relationships among *Gossypium* species. These efforts have culminated in morphological (Fryxell 1992) and molecular phylogenetic (Seelanan et al. 1997) syntheses.

African–Arabian members of the genus remain the least well-understood, both with respect to taxonomic diversity and phylogenetic relationships. Many are known only from a few collections, are not represented in germplasm collections, and have been subjected to relatively little taxonomic, cyto-

genetic, and molecular phylogenetic study. Among these African species, *Gossypium trifurcatum* Vollesen remains one of the most elusive, known only from two herbarium sheets and seen last during floristic work conducted during 1980 (Vollesen 1987). The species itself is described by Gillett as being “locally abundant, not seen elsewhere” (specimen Gillett 23071). *Gossypium trifurcatum* is distinguished from other species by several unique morphological characters. It is the sole *Gossypium* species to possess dentate leaves in a genus otherwise characterized by entire or entire lobed leaves (Fryxell 1979). The epicalyx is also distinctive, with each bract being dissected into three delicate, linear lobes with the lateral lobes sometimes bearing an extra tooth near the base. The flowers are also pink, which is not seen elsewhere among the African–Arabian cottons. Noting these distinctive characters, authors have raised the suggestion that *G. trifurcatum* may belong in *Cienfuegosia* rather than *Gossypium* (Fryxell 1992). The holotype (Figure 1) bears annotation from the taxonomist

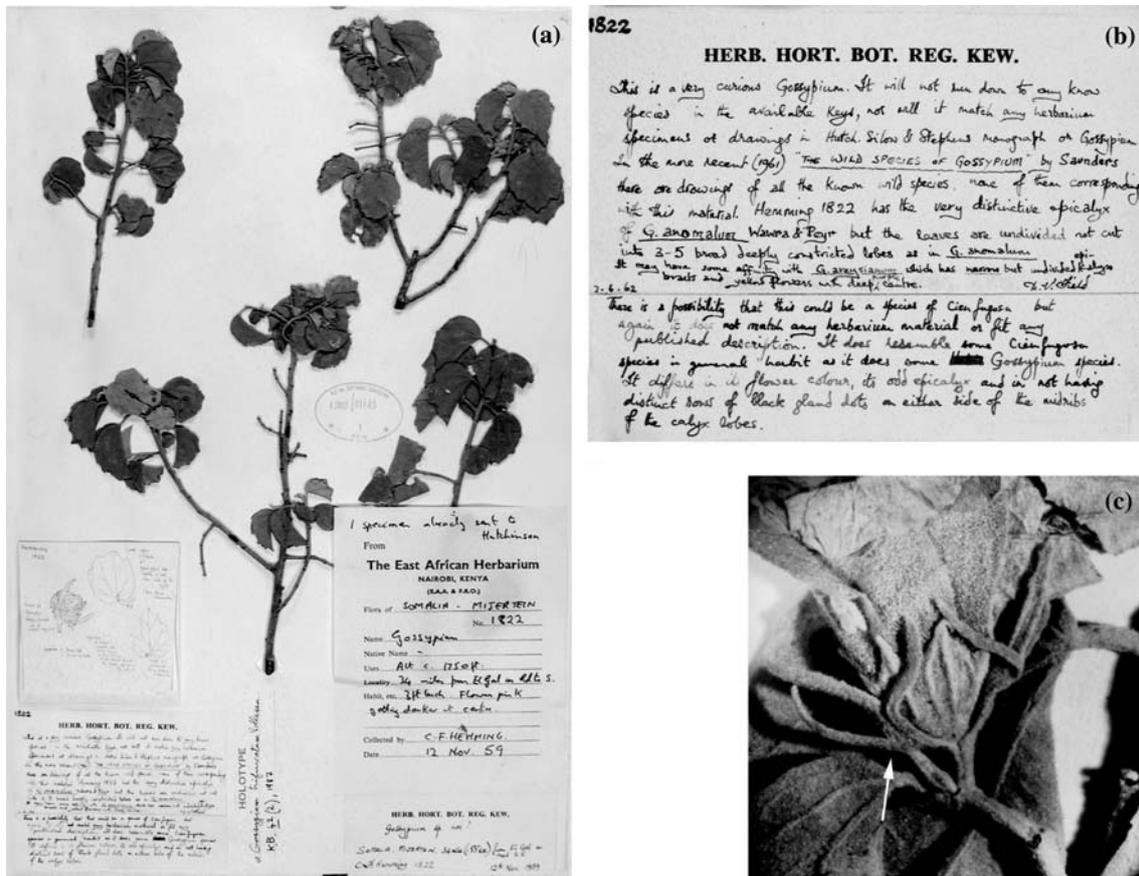


Figure 1. Holotype showing diagnostic morphological characters of *G. trifurcatum*. A. Entire specimen; note dentate leaves. B. Close-up of 1962 commentary by D.V. Field in lower left portion of holotype. C. Epicalyx, illustrating elaborate trifid epicalyx (arrow).

D.V. Field in 1962 suggesting that "There is a possibility that this could be a species of *Cienfugosa* but again it does not match any herbarium material or fit any published description".

When first described by Vollesen (1987), he allied *G. trifurcatum* closely to *G. anomalum* Wawra ex Wawra et Peyritsch, based primarily on the shape of the epicalyx (Figure 1). Fryxell's later revision of the genus erects a monotypic section *Serratta* Fryxell for *G. trifurcatum* after taking into account his and other's observations concerning the dentate leaves (Fryxell 1992).

Notwithstanding our incomplete understanding of diversity among African *Gossypium* species, they presently are partitioned into four "genome groups" (B, E, F, A) based originally on meiotic pairing behavior in interspecific hybrids (Endrizzi et al. 1985). Because of Vollesen's suggestion of an affinity with *G. anomalum*, *G. trifurcatum* has been

tentatively placed in the B-genome group. Here, we use the molecular marker *ndhF* and our prior understanding of the phylogenetics of the *Gossypium* genus to explicitly test this proposed relationship, and if falsified, generate a new hypothesis for a phylogenetic placement of *G. trifurcatum* within the tribe Gossypieae.

#### Materials and methods

To assess the phylogenetic and taxonomic position of *G. trifurcatum*, we amplified an ~1000 bp (base pair) portion of chloroplast locus *ndhF*. This gene is among the most widely employed cpDNA locus for phylogenetic analyses in angiosperms (Soltis et al. 1998). In addition, we earlier generated *ndhF* sequence for exemplar taxa from throughout *Gossypium* as well as seven of the



dentate shape and the epicalyx, which is unusually long and trifold.

## Results and discussion

An aligned data matrix of 1016 nucleotide positions was subjected to maximum parsimony and maximum likelihood analysis. Previous molecular work has generated a phylogenetic understanding of the cotton tribe, placing *Cienfuegosia* as the most basal genus, with *Gossypium* being sister to a clade composed of the two genera *Gossypioides* Skovst. ex J. B. Hutch. and *Kokia* Lewton (Seelanan et al. 1997; Wendel et al. 2002). Using *Gossypioides* as an outgroup, parsimony analysis led to the recovery of three equally parsimonious trees and one most likely tree. All inconsistencies between the parsimony trees occurred in genera outside *Gossypium*. There was no topological incongruence between the likelihood and parsimony-inferred trees.

In all phylogenetic analyses, *G. trifurcatum* was embedded within a monophyletic *Gossypium* (Figure 2). Fifteen of the 47 parsimony-informative characters, including 13 base changes and two 6-bp indels, support the placement of *G. trifurcatum* within *Gossypium* and not *Cienfuegosia* (Table 1). In contrast, no characters exclusively united *G. trifurcatum* with *Cienfuegosia*. Thus, based on cpDNA evidence, *G. trifurcatum* is more properly placed within *Gossypium* than *Cienfuegosia*. The dentate leaves are thus diagnosed as derived within *Gossypium*, although this character is common in *Cienfuegosia* and elsewhere in the Malvaceae.

The morphological distinctness of *G. trifurcatum* (pink petal color, dentate leaves, trifold epicalyx), and the fact that it has so rarely been observed, underscores the need for additional exploration and collection of Africa–Arabian cottons, particularly from the Horn of Africa and Arabian Peninsula. This region contains an appreciable portion of the total cladistic and taxonomic diversity in the genus (Fryxell 1979; Seelanan et al. 1997; Wendel et al. 2002), yet it remains the most poorly understood.

### Material examined

Because known representatives of *G. trifurcatum* are limited to two herbarium vouchers, we have

Table 1. Phylogenetic apomorphies of the chloroplast *ndhF* gene that demonstrate the inclusion of *G. trifurcatum* within *Gossypium* and its distinctiveness from *Cienfuegosia*.

Site	Site(s) within the 1017 bp <i>ndhF</i> alignment		
	<i>Gossypium</i> species <sup>a</sup>	<i>G. trifurcatum</i>	<i>Cienfuegosia</i>
241	G	G	T
342	A	A	C
401	A	A	C
473	G	G	A
513	A	A	C
587	C	C	A
603	G	G	A
705	A	A	G
749	G	G	T
857	6 bp deletion	6 bp deletion	–
942	T	T	C
948	6 bp deletion	6 bp deletion	–
960	A	A	G
962	A	A	G
1003	G	G	A

<sup>a</sup> Sequences from 10 species representing all major clades within the genus (Seelanan et al. 1997).

included the information from both labels. Hemming 1822, Holotype, K; From the East African Herbarium, Nairobi, Kenya; The Flora of Somalia- Mijertein; *Gossypium* ? Alt. c. 1750 ft 34 miles from El Gal on road S.; 3 ft bush, flowers pink, getting darker at center; Collected by C.F. Hemming 11/12/1959.

Gillett 23071, K; East African Herbarium, Northern Somalia; Rangelands Resource Management and Research Survey 1980; *Gossypium* ? vel fors. gen. nov. aff.; Shrublet c. 40 cm tall. Dried corolla rose. Fruit a three-locular capsule. Epicalyx of three cuneate bracteoles each divided apically into three narrow lobes, sometimes a 4th lobe on one side near the base; Locally abundant, not seen elsewhere; N.E. Somalia (site 8), 2 km. North of Balli Dhiddin on Iskushuban-Candala Rd., 10°54'N, 50°24'E alt. 570 m; Limestone slopes open *Acacia*–*Commiphora* bushland with *Adenium*, large *Mimusops anjel*, *Acacia ankokib*, and *Bridelia somalensis* in valleys; 7/8/1980 and 7/10/1980.

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