

## Phylogenetics of *Cucumis* (Cucurbitaceae) as understood in 2008<sup>1</sup>

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### Abstract

2007 saw the publication of two molecular phylogenies of *Cucumis* that relied on combined chloroplast and nuclear gene regions, and broad species sampling. One of these studies also addressed the position of *Cucumis* in the Cucurbitaceae family tree. It was found that the genera *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* are embedded within *Cucumis* and that the closest relative of *Cucumis* is *Muellerargia*, a genus with one species in Australia and Indonesia, the other in Madagascar. Cucumber and its sister species, *C. hystrix*, are nested among Australian, Malesian, and Western Indian species that had traditionally been placed in *Mukia* or *Dicaelospermum*. *Cucumis melo* is sister to this Australian/Asian clade, rather than being close to African species as previously thought. Molecular clock dating suggests Eocene ages for the deepest divergences within *Cucumis*.

### INTRODUCTION

Knowing the closest relatives and natural composition of the genus *Cucumis* L. is important because of ongoing efforts by plant breeders worldwide to improve melon (*C. melo*) and cucumber (*C. sativus*) with traits from wild relatives. The year 2007 saw great progress on both fronts. First, a molecular phylogeny for the entire family (Kocyan et al. 2007) placed the genus *Cucumis* in a solid context based on a sample of 21% of the family's c. 800 species and 95% of its 130 genera (following the classification of Jeffrey 2005; a new classification of the entire family will appear in the *Families and Genera of Flowering Plants* series published by Springer; Schaefer and Renner, submitted).

Following the discovery by Kocyan et al. that *Cucumis* as traditionally circumscribed (Kirkbride 1993) is highly unnatural, two molecular phylogenetic studies re-investigated species relationships in a much more broadly circumscribed *Cucumis* (Renner et al. 2007; Ghebretinsae et al. 2007a). While largely agreeing with each other, these studies found different placements for *C. melo*. Here we summarize what is now known about phylogenetic relationships in *Cucumis*, discuss the points of disagreement between the two *Cucumis* phylogenies, and add new findings made since the publication of Renner et al. (2007).

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## MATERIALS, METHODS, AND RESULTS

The sequencing and analytical approaches used in our work are described in Kocyan et al. (2007), Renner et al. (2007), and Schaefer et al. (2008). An important step in these studies was a broader genus and species sampling in Cucurbitaceae than used in any previous molecular study of *Cucumis* (e.g., Perl-Treves and Galun 1985; Perl-Treves et al. 1985; Garcia-Mas et al. 2004). This led to the discovery that the genera *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* are embedded among species of *Cucumis* (Fig. 1). The results also imply that the Cucumerinae sensu C. Jeffrey (2005) are polyphyletic and need to be dissolved. The genera *Cucumeropsis* Naudin, *Melancium* Naudin, *Melothria* L., *Posadaea* Cogn., and *Zehneria* Endl., which were all included in Cucumerinae, group far from *Cucumis* (Kocyan et al. 2007) This fits with the geographic concentration of three of these genera in the New World, where *Cucumis* and its immediate relatives are absent.

The sister genus to *Cucumis*, *Muellerargia* (Fig. 1), consists of one species in Madagascar (*Muellerargia jeffreyana* Keraudren) and one in Indonesia (Timor) and Queensland (*M. timorensis* Cogn.). Both are herbaceous trailers or climbers with softly spinose fruits. *Muellerargia* has never been recognized as closely related to *Cucumis*, perhaps because it is extremely poorly collected, with but a few specimens even in major herbaria.

The within-*Cucumis* species relationships shown in Fig. 1 differ from those found in earlier studies (e.g., Perl-Treves and Galun 1985; Perl-Treves et al. 1985; Akashi et al. 2002; Garcia-Mas et al. 2004; Chung et al. 2006), partly probably because the earlier studies rooted their trees with *Citrullus lanatus* and/or *Cucurbita pepo*, which are phylogenetically very distant from *Cucumis* (Kocyan et al. 2007). This may have caused erroneous tree topologies due to long-branch attraction. Other possible causes for different species relationships are the different taxon sampling, insufficient signal in some of the earlier molecular data, and misidentified material. Comparison of the ITS sequences of Garcia-Mas et al. (2004) to a new ITS sequence showed that the sequence labeled *Cucumis membranifolius* in GenBank (AJ488223) and *Oreosyce africana* in the published paper do not represent *Oreosyce africana*. The old sequence came from a seed provided by the North Central Regional Plant Introduction Station in Ames, Iowa, and since no voucher material is cited, the identification probably cannot be checked.

The differences between the recent-most phylogenies of *Cucumis* concern the amount of sequence used (4998 nucleotides in Renner et al. 2007 vs. 1631 in Ghebretinsae et al. 2007), the number of ingroup species included (23 vs. 40), and the placement of *C. melo* in relation to *C. sagittatus* and *C. metuliferus*. Renner et al. (2007), using nuclear and chloroplast DNA data separately and in combination, found *C. melo* sister to a clade of Asian/Australian species. By contrast, Ghebretinsae et al. (2007a), whose sampling did not include any Australian species, with their nuclear data found *C. melo* sister to *C. sagittatus* while their chloroplast data placed *C. melo* as sister to *C. metuliferus*. Their material of *C. sagittatus* and *C. metuliferus* came from a US germplasm source, and the possibility of this material reflecting hybridization with *C. melo* needs to be investigated.

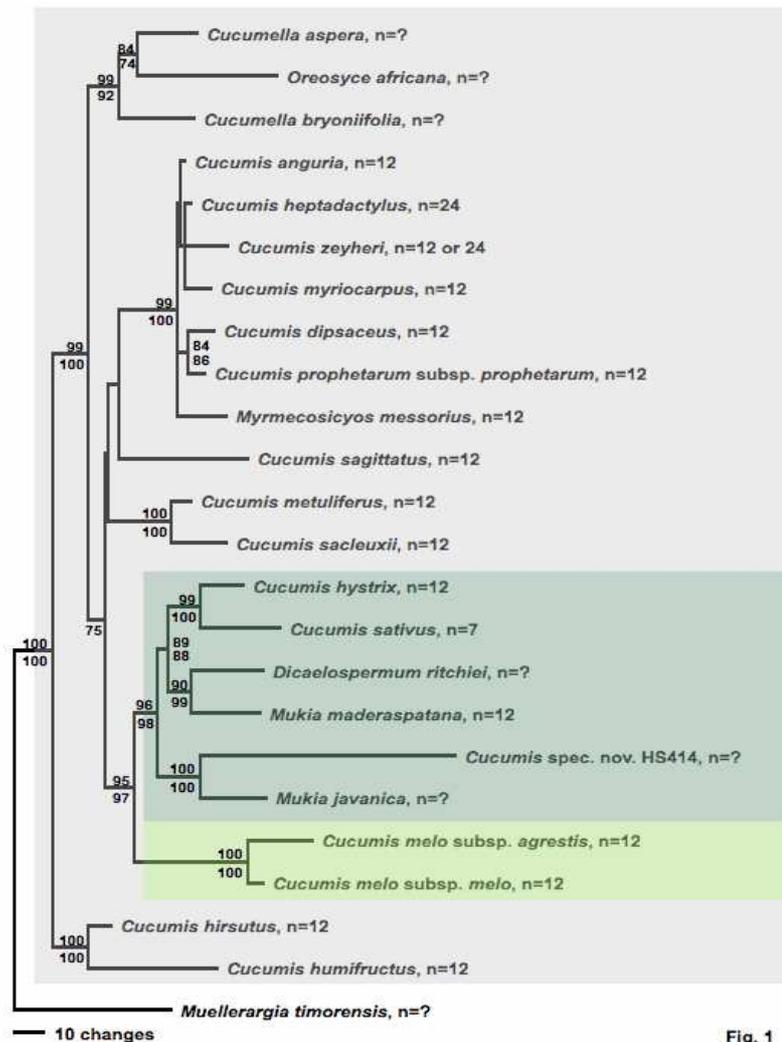


Fig. 1

**Figure 1.** Parsimony tree for *Cucumis* based on combined chloroplast and nuclear DNA sequences (4998 nucleotides) and rooted on *Muellerargia*. Parsimony bootstrap values (> 75%) based on 1000 replicates above branches and maximum likelihood bootstrap values from 100 replicates below branches. Species on pale grey background occur in Africa (*C. prophetarum* extends into India); the clade marked in grey-green occurs in Australia, the Malaysian region, Indochina, China, and India (*Mukia maderaspatana* extends into the Yemen and sub-Saharan Africa); the natural range of melon (*C. melo*) (light green) is unclear. Information on chromosome numbers is from the Index to Plant Chromosome Numbers database available online at the Missouri Botanical Garden's web site.

## DISCUSSION: IMPLICATIONS FOR THE EVOLUTION AND BIOGEOGRAPHY OF *CUCUMIS*

As a result of the phylogenetic findings in Renner et al. (2007) and Ghebretinsae et al. (2007a), the species of *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* have been transferred into *Cucumis* (Ghebretinsae et al. 2007b; Schaefer 2007). A complete morphological key to all species now included in *Cucumis* is provided in Schaefer (2007). The new names of Ghebretinsae et al. (2007b) have priority over those in Schaefer (2007), but fortunately both publications proposed identical epitheta. One transfer, *C. rumphianus* (Scheff.) H. Schaefer. was only made in the latter study. Furthermore, *Oreosyce africana* was replaced by the name *Cucumis oreosyce* H. Schaefer., but since there are two other apparently validly published names for this taxon, *Cucumis subsericeus* Hook. f. and *C. membranifolius* Hook. f., the correct name remains unclear.

In the discussion below we use the up-to-date nomenclature of the species of the natural (i.e., monophyletic) genus *Cucumis*.

The phylogeny of *Cucumis* resulting from combined nuclear and chloroplast data (Fig. 1) implies that the deepest divergence lies between the common ancestor of *C. hirsutus*/*C. humifructus* and the stem lineage of the remainder of the genus. The area of origin of *Cucumis* cannot be inferred because its sister genus, *Muellerargia*, has one species in Madagascar, the other in tropical Australia and Indonesia. The next closest relatives are in African/Asian clades including the genera *Coccinia*, *Zehneria*, *Neoachmandra*, and *Peponium* but their exact position is still unresolved.

Strict and relaxed molecular clocks suggest that the deepest divergence in *Cucumis* may date back to 48-45 my and that the split between the *C. melo* lineage and its Australian/Asian sister clade is only slightly younger (Renner et al. 2007). The divergence of *C. sativus* from *C. hystrix* may be about 8 my old and that of their common ancestor from the ancestor of *C. ritchiei* (C. B. Clarke) Ghebret. & Thulin (formerly *Dicaelospermum ritchiei*) and *C. maderaspatanus* L. (formerly *Mukia maderaspatana*) about 19 my. The bulk of the African species appears to have evolved more recently. The absence of a fossil constraint within *Cucumis*, however, cautions against over-confidence in the molecular clock estimates.

Based on the tree (Fig. 1), the earliest divergence events in *Cucumis* likely took place in Africa. However, contrary to the traditional classification (Kirbride 1993), which grouped *C. melo* with the African *C. hirsutus*, *C. humifructus*, and *C. sagittatus*, melon instead is closest to an Australian/Asian clade (marked in grey-green in Fig. 1). The closest relatives of melon comprise a new Australian species (I. Telford, N.C.W. Beadle Herbarium, Armidale, Australia, personal communication, 2007), *C. javanicus* (Miquel) Ghebret. & Thulin, *C. maderaspatanus*, and *C. ritchiei* from Western India plus *C. sativus* and *C. hystrix* from India, China, Burma, and Thailand. A few other species that have not yet been sequenced based on morphology also are expected to cluster with *C. maderaspatanus*; they range from Indo-China southeast to Java, Borneo, and the Philippines, and west to India (De Wilde and Duyfjes 2006, 2007). Given the geographic distribution of these extant closest relatives, *C. melo* itself could have originated somewhere in Asia and then reached Africa from there, rather than originating in Africa as traditionally assumed (e.g., Kirkbride 1993; Ghebretinsae et al. 2007a).

## CONCLUSIONS

The evolution of morphological traits relevant for *Cucumis* breeders, for example fruit type, habit, and sexual system, will need to be reinterpreted based on the new composition of the genus. Most of the 52 species of the “new” *Cucumis* are monoecious perennials, and this combination of traits may be the ancestral condition from which an annual habit and dioecy evolved several times. However, the sexual system and habit of key taxa, such as *Dicaelospermum*, *Muellerargia*, and the as yet undescribed species from Australia (Fig. 1) are not reliably known because species are under-collected and have not been studied in the field. The evolution of smooth fruits from spiny fruits, a traditional key character in *Cucumis*, and the mode of fruit opening also are much more plastic than formerly thought (details in Renner et al. 2007).

The new insights about the closest relatives of melon and cucumber have implications for ongoing genomics and breeding efforts. For example, to understand the genome rearrangements that must have taken place during the evolution and domestication of *C. sativus*, with just 7 chromosomes, analyses of co-linearity will be required between the cucumber lineage and its closest relatives *C. ritchiei* (*Dicaelospermum ritchiei*) and species of the former genus *Mukia* (*C. maderaspatanus*, *C. javanicus*). Likewise, the possibility of *C. melo* having evolved in Asia and having reached Africa secondarily needs to be tested. And last not least, the newly discovered close relatives of cucumber and melon need to be collected and brought into cultivation for comparative study.

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