

Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia

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Among the fundamental questions regarding cultivated plants is their geographic origin and region of domestication. The genus *Cucumis*, which includes cucumber (*Cucumis sativus*) and melon (*Cucumis melo*), has numerous wild African species, and it has therefore been assumed that melon originated in Africa. For cucumber, this seemed less likely because wild cucumbers exist in India and a closely related species lives in the Eastern Himalayas. Using DNA sequences from plastid and nuclear markers for some 100 *Cucumis* accessions from Africa, Australia, and Asia, we show here that melon and cucumber are of Asian origin and have numerous previously overlooked species-level relatives in Australia and around the Indian Ocean. The wild progenitor of *C. melo* occurs in India, and our data confirm that the Southeast Asian *Cucumis hystrix* is the closest relative of cucumber. Most surprisingly, the closest relative of melon is *Cucumis picrocarpus* from Australia. *C. melo* diverged from this Australian sister species approximately 3 Ma, and both diverged from the remaining Asian/Australian species approximately 10 Ma. The Asian/Australian *Cucumis* clade comprises at least 25 species, nine of them new to science, and diverged from its African relatives in the Miocene, approximately 12 Ma. Range reconstruction under maximum likelihood suggests Asia as the ancestral area for the most recent common ancestor of melon and cucumber, fitting with both having progenitor populations in the Himalayan region and high genetic diversity of *C. melo* landraces in India and China. Future investigations of wild species related to melon and cucumber should concentrate on Asia and Australia.

ancestral areas | crops | economic plants | wild progenitors

Among the most fundamental and debated questions regarding the evolution of cultivated plants is their geographic origin and region of domestication (1). Recent phylogeographic and phylogenetic work on cassava, pumpkin, corn, potato, and rice (2–6) has uncovered the likely places of origin and domestication of these crops. Although many pre-molecular hypotheses about the domestication of particular species still require testing, it is clear that the Indo-Chinese region has produced a particularly long list of crops. These include rice (*Oryza sativa*), millets (*Setaria* spp.), beans (*Vigna mungo*; *Vigna radiata*), angled loofah (*Luffa acutangula*), yams (*Dioscorea* spp.), and taro (*Colocasia esculenta*) (7–9). Archaeological evidence from northern India documents these Neolithic crops from 7,000 BC onward, and by the early second millennium, there is evidence of Western crops arriving in India through trade, such as wheat, barley, lentils, grasspea, and peas (7).

One of the crops domesticated in the Indo-Gangetic plain is cucumber, *Cucumis sativus*. Evidence for this consists in the occurrence there of a wild progenitor, *C. sativus* var. *hardwickii* (10, 11) and in comparative linguistic evidence (7, 8). Fossil seeds of cucumber and melon cannot be reliably distinguished, and archeological reports therefore are of limited value for pinpointing areas of melon or cucumber domestication or identifying the routes by which these crops arrived in a particular region (12). Cucumber and melon today are among the 20 most important vegetable crops worldwide

(13). The first complete genome of cucumber was released last year (14), and the genome of melon is being completed (15).

In contrast to cucumber, the geographic origin and region of domestication of melon (*Cucumis melo*) have remained unclear. Nineteenth-century taxonomists suggested that melon probably originated and was domesticated in Asia (e.g., ref. 11). This idea became discredited as workers began to study *Cucumis* chromosome numbers (16). *C. sativus* has a chromosome number of $2n = 14$, whereas *C. melo* has a chromosome number of $2n = 24$. At least 30 other species of *Cucumis* have had their chromosomes counted, all but one from Africa and all having $2n = 24$ or multiples thereof (17). Based on the impressive species richness in Africa and the identical chromosome number of *C. melo* and African *Cucumis*, modern authors have held that *C. melo* is of African origin (18–22). This view has persisted even in the face of genetic data pointing to greatest genetic diversity in Indian and East Asian landraces of *C. melo* (23–26) and despite numerous failed attempts to produce fertile F1 offspring from crosses of *C. melo* and African species of *Cucumis* (17).

Phylogenetic studies on the genus *Cucumis* have been Africa-biased in terms of the included species (27–29). These studies yielded contradictory results regarding the closest relatives of *C. sativus* and *C. melo*. A 2007 study (29) inferred that South African *C. sagittatus* is the sister species of *C. melo*, although this was only observed with nuclear ribosomal internal transcribed spacer (ITS) sequences, not chloroplast sequences. Other studies that also used ITS (27, 28, 30) found *C. melo* isolated from the other included African species and instead sister to a small clade of five Asian and one Australian species. Although not resolving the position of *C. melo*, these studies clarified that the genus *Cucumis* in its traditional circumscription (17) was paraphyletic (28, 29, 31), with species from five other genera nested inside it. [The necessary nomenclatural changes were made previously (32, 33); here we use these up-dated species names.]

Here we investigate the evolutionary relationships and geographic origin of melon and cucumber by sampling *Cucumis* accessions covering the entire natural range of the genus from Africa to Southeast Asia to Australia, the Pacific and the Indian Ocean islands. The geographic origin of the plants sequenced is shown in Fig. 1. We included new collections from fieldwork in Australia and Thailand as well as old material from herbaria, including type material of long synonymized names.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database. For a list of accession numbers, see Table S1.

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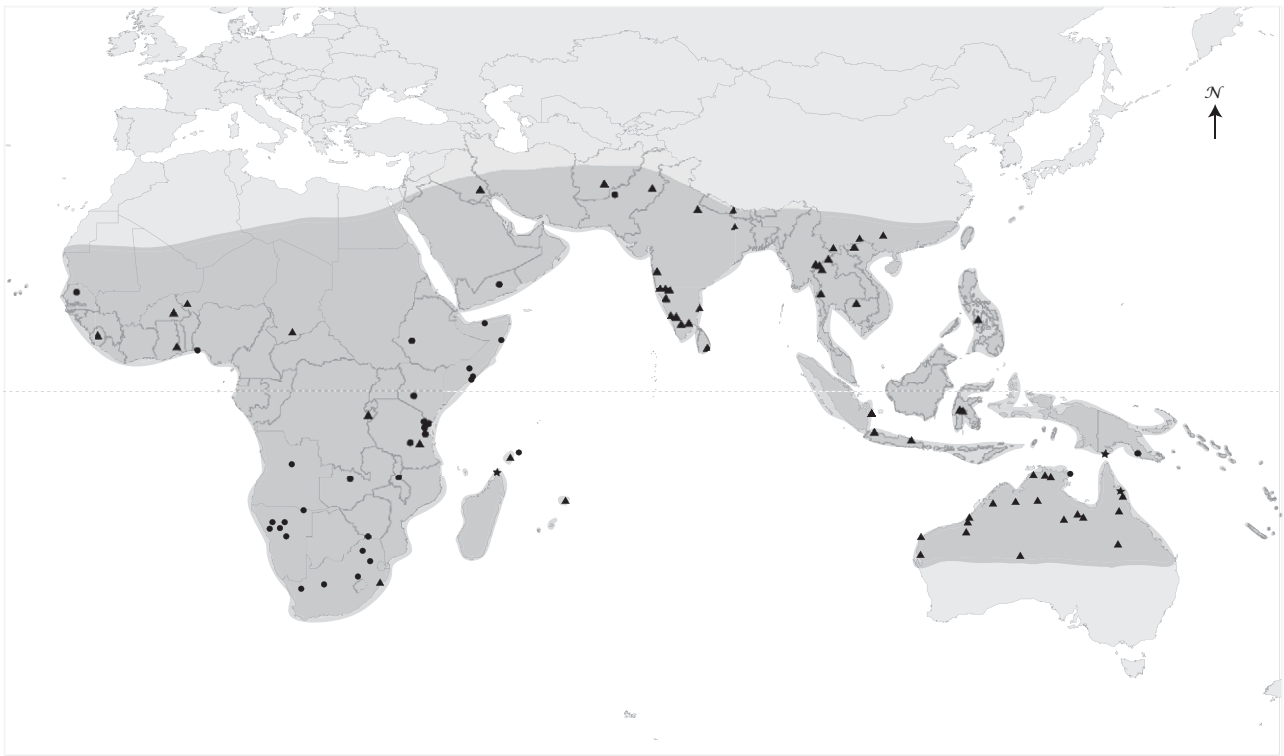


Fig. 1. The natural geographic range of *Cucumis* (shaded) and the geographic origin of the sequenced plant material. Triangles, Asian/Australian clade comprising cucumber and melon; circles, African grade; stars, outgroup.

Knowing the progenitors and closest relatives of melon and cucumber is important because these crops are highly susceptible to drought and pathogens, including powdery mildew and several mosaic viruses (14). Besides the interest in varieties with new taste or fruit shape, there is great interest in introducing resistance genes from wild relatives. In the case of melon, the search for these relatives has so far concentrated on Africa because of the view that *C. melo* is of African origin (18–22).

Results

Closest Relatives of Cucumber and Melon. A maximum likelihood phylogeny from the combined plastid and nuclear data (Fig. 2) shows that *C. sativus* is sister to *Cucumis hystrix*, confirming previous findings (28–30). *Cucumis setosus*, an entity that the most recent monograph of *Cucumis* (17) synonymized under *C. sativus*, instead is closest to *Cucumis silentvalleyi* and *Cucumis indicus* from southern and southwestern India. Sequences of the Himalayan entities *Cucumis trigonus* and *Cucumis callosus* are nearly identical to those of *C. melo* (Fig. 2) and likely represent the wild progenitor of cultivated melon as these forms are fully crossable with *C. melo* (34). The sister species to *C. melo* populations is the Australian *Cucumis picrocarpus*, which had been synonymized under *C. melo* (17), but is genetically and morphologically highly distinct (Fig. 2; Fig. 3 shows a color photo of *C. picrocarpus*).

Cucumber and melon are part of a clade that comprises 25 poorly collected and understudied species-level entities from India, Indochina, Malesia, Australia, Africa, and Indian Ocean islands (Figs. 1 and 2). Species recently transferred to *Cucumis* based on morphology (32, 33) indeed belong in the genus as indicated by chloroplast and nuclear DNA sequences (Fig. 2). Our results, however, reveal another 18 close relatives (labeled in Fig. 2) of cucumber and melon that were neither accepted in the 1993 monograph of the genus by Kirkbride (17) nor included in previous phylogenetic studies (29, 30). Nine (labeled with an upper script N in Fig. 2) represent as yet undescribed species. An important factor in the unexpected Asian/

Australian diversity of *Cucumis* is *Cucumis* (*Mukia*) “*maderaspatanus*.” This polymorphic taxon, revised in 2006 (35), turns out to be a highly unnatural assembly comprising at least nine species-level entities (labeled with an upper script M in Fig. 2) that are not close to each other (Fig. 2 and Table S1). *Cucumis maderaspatanus* was thought to range from Africa across Asia to Australia, but accessions from these three continents do not group together.

Fruit morphology, a key trait in *Cucumis* breeding, differs greatly among the species of the Asian/Australian clade (Fig. 3). Early-diverging species, such as the Australian sister clade of *C. javanicus* and several species from India and the Eastern Himalayas (*C. hystrix*, *C. sativus* var. *hardwickii*, *Cucumis debilis*, *C. setosus*, *C. silentvalleyi*, *C. indicus*), have more or less ellipsoid fruits that stay green or turn yellow-orange at maturity. By contrast, the *Cucumis ritchiei/C. maderaspatanus* clade is characterized by smooth, round fruits that turn red at maturity.

Age of Asian/Australian *Cucumis* Clade and Ancestral Area Reconstruction.

A relaxed molecular clock for the Asian/Australian radiation of *Cucumis*, calibrated with a secondary calibration from a Cucurbitaceae clock study that used three fossil and one geological calibration (36), indicates that the lineage comprising melon and cucumber split from its African ancestor 11.9 ± 2.0 Ma ago (Fig. S1). Ancestral area reconstruction under maximum likelihood yields Asia as the area of the most recent common ancestor of melon and cucumber (Fig. S2). The split between melon and its Australian sister species, *C. picrocarpus*, occurred approximately 2.8 ± 1.0 Ma, and that between cucumber and its sister species *C. hystrix*, approximately 4.6 ± 1.4 Ma.

Discussion

As per this study, *Cucumis* comprises some 25 Asian and Australian species (Fig. 2) in addition to its approximately 30 African species. The increase in Asian/Australian *Cucumis* species, compared with the 12 known in 2008 (32, 33) or the two known in 1993 (17), implies that *Cucumis* was much less understood than hereto

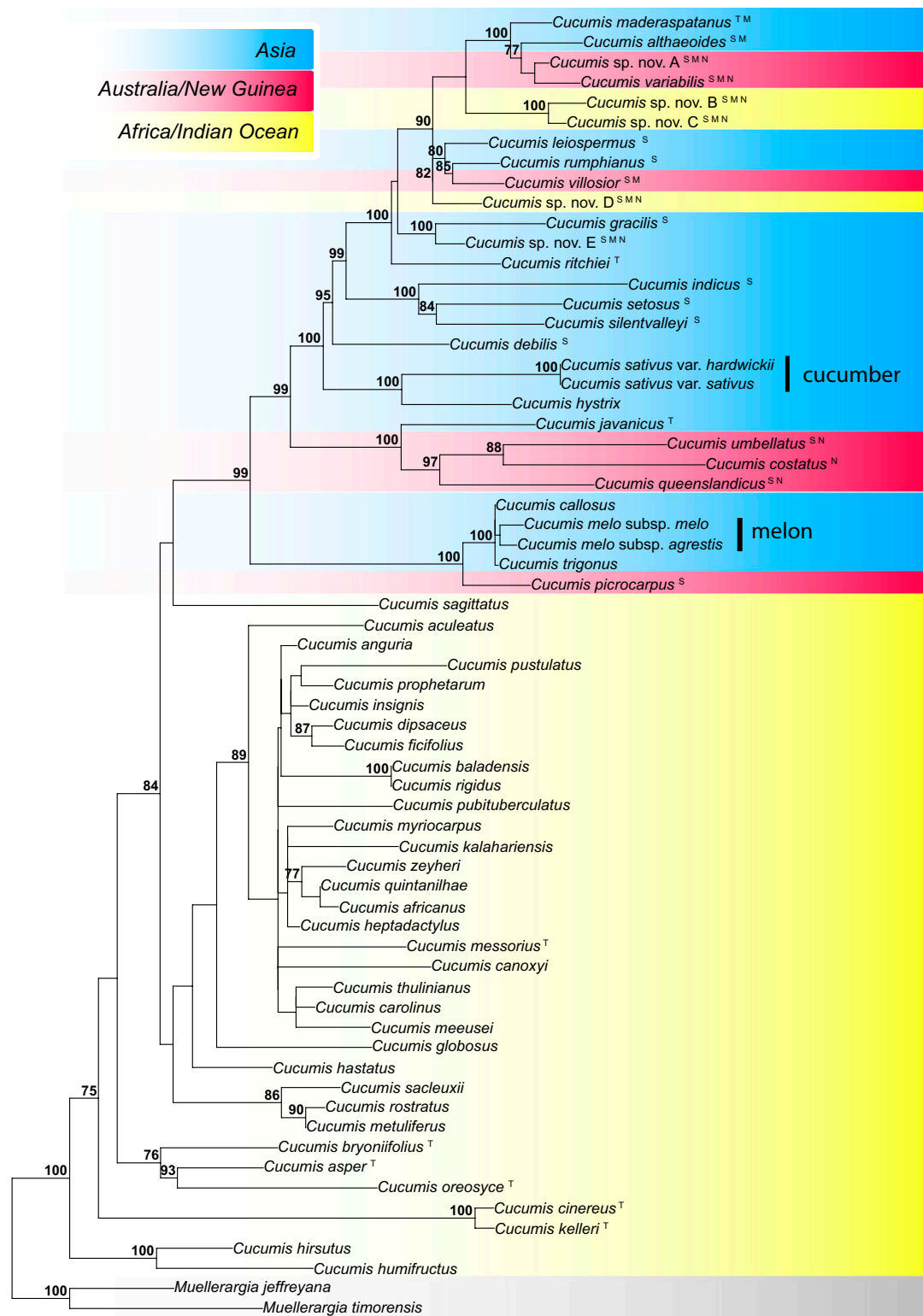


Fig. 2. ML tree for 63 taxa of *Cucumis* (Fig. S3 shows a ML tree of all 113 accessions) based on combined sequences from chloroplast and nuclear data (6,202 aligned nucleotides; Table S1), analyzed under a GTR + Γ model. The tree is rooted on *Muellerargia*. Likelihood bootstrap values of at least 75% are given at the nodes; geographic occurrence of species is color-coded (Inset); superscript letters refer to the following: T, species transferred into *Cucumis* (32, 33) based on DNA sequences; S, species never before sequenced; M, specimens formerly identified as *Mukia maderaspatana* (*Cucumis maderaspatanus*); and N, species new to science. Authors of old and new names appear in Table S1.

thought. Few of the Asian and Australian species are in cultivation, most have never had their chromosomes counted, and little is known about their ecology and distribution. A likely reason for

the lack of attention paid to Asian and Australian *Cucumis* is the almost dogmatic view among *Cucumis* specialists that the genus comprised just two Asian species-level taxa (*C. hystrix* and

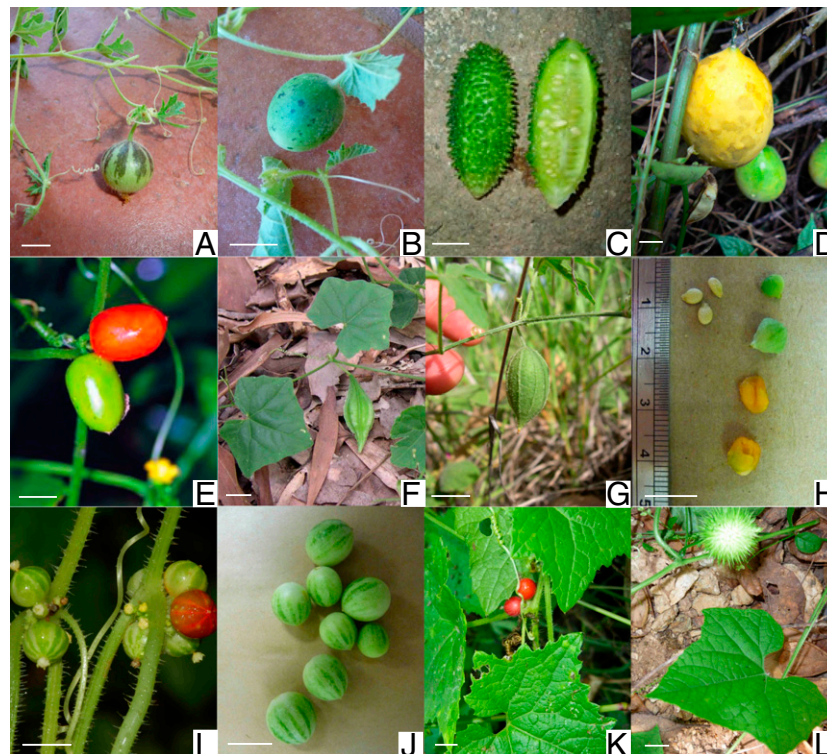


Fig. 3. Habits, fruits, or seeds of (A) *Cucumis picrocarpus*, (B) *C. melo* subsp. *agrestis*, (C) *C. hystrix*, (D) *C. debilis*, (E) *C. javanicus*, (F) *C. costatus*, (G) *C. queenslandicus*, (H) *C. umbellatus*, (I) *C. ritchiei*, (J) *C. villosior*, (K) *C. sp. nov. E*, and (L) *Muellerargia timorensis*. (Scale bar: 1 cm.) Photographs by P. Sebastian (A–C, H, J, K, L) Z. v. Herwijnen (D); B. Wannan (F and G); M. Sardesai (I); and W.J.J.O. de Wilde (reproduced from ref. 35) (E).

C. sativus) and that any Australian *Cucumis*-like plants could safely be called *C. melo* (17) or *C. (Mukia) maderaspatanus* (35).

The newly revealed Asian/Australian *Cucumis* radiation of 25 species completely changes the biogeography of the genus. Molecular-clock dating suggests divergence of the Asian/Australian clade from its African relatives at 11.9 ± 2.0 Ma, i.e., during a Miocene period when the African-Arabian plate joined the Asian plate, leading to a closure of the seaway that had previously separated Africa from Asia (37). The ancestor of the Asian/Australian *Cucumis* clade probably spread to Eurasia via this land bridge, as did numerous vertebrates (38). One species, *C. prophetarum*, still ranges from Africa to India and Pakistan, but based on the tree topology (Fig. 2), it dispersed to Asia independently of the ancestor of the *C. sativus/C. melo* clade. The seven species in Australia arose from four dispersal events into that continent, all from Southeast Asia, but at widely different times (Fig. S1).

The DNA phylogeny (Fig. 2) and ancestral area reconstruction (Fig. S2) rejects Kirkbride's (17) grouping of *C. melo* with the African species *C. hirsutus*, *C. humifructus*, and *C. sagittatus* and instead supports the view of 19th century taxonomists (11, 39) that the wild progenitor of melon would be found in India. The Himalayan entities *C. callosus* and *C. trigonus* (names often synonymized with each other and/or with *C. melo*) (17, 40, 41) produce fertile F1 offspring when crossed with *C. melo* (34) and clearly are the wild progenitor from which melon was domesticated. The melon land races occurring in South and East Asia exhibit high genetic diversity (23–26, 42) and deserve to be sampled more densely in future studies. The surprising finding that an Australian species (*C. picrocarpus*) is the sister of the *C. melo/C. callosus/C. trigonus* complex underscores how little is known of the Australian Cucurbitaceae diversity. Most native Australian cucurbits have close relatives among tropical Asian and even Eurasian lineages (e.g., *Austrobryonia*, *Diplocyclos*, *Neoalsomitra*, *Neoachmandra*, *Trichosanthes*) and reached Australia from the north (43). It is

possible that other relatives of *C. melo* exist in under-collected regions between India and Australia or may be hiding among unidentified or misclassified material in herbaria.

Most of the approximately 66 species of *Cucumis* now known are monoecious annuals, but dioecious mating systems and a perennial habit evolved several times within the genus. We have begun bringing Asian and Australian species into cultivation to study fruit morphology and chromosomes. The evolution of smooth fruits from spiny fruits (Fig. 3), a traditional key character in *Cucumis*, and the mode of fruit opening are much more plastic than formerly thought (see ref. 28). Overall, the loss of spines appears correlated with a round shape and red color at maturity, probably in connection with bird dispersal. This fits with the inferred dispersal from Asia to Australia, Africa, and various Indian Ocean islands of taxa in the *C. ritchiei/C. maderaspatanus* clade (Fig. 2).

Analyses of the synteny between *C. sativus*, *C. melo* (melon), and *Citrullus lanatus* (watermelon) have revealed that five of the seven chromosomes of *C. sativus* arose by fusions of 10 ancestral chromosomes after the split between *C. sativus* and *C. melo* (14). To more fully understand the rearrangements, it will be useful to now study the chromosomes of other species in the newly revealed Asian/Australian cucumber/melon clade. Study of these species' karyotypes (and other biological traits) will be key in the search for new sources of genes for melon and cucumber improvement. The data on the phylogenetic and geographic relationships of melon and cucumber provided here represent a step toward redirected breeding efforts, which should concentrate on *Cucumis* in Asia and Australia, instead of sub-Saharan Africa. Further population sampling of *C. callosus*, *C. melo*, and *C. trigonus* across Asia will be necessary to assess whether melon was domesticated multiple times.

Materials and Methods

Fig. 1 shows the locations of 97 of the 113 accessions included in this study (including outgroups). We sequenced five chloroplast markers (the *trnL* intron, the intergenic spacers *trnL-F*, *rpl20-rps12*, and *trnS-G*; and the genes *rbcL* and *matK*) and the nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2, plus the intervening 5.8 S gene using standard procedures (*SI Materials and Methods*). The dataset comprised 6,202 aligned positions. Sequences were edited with Sequencher (version 4.7; Gene Codes) and aligned by eye by using MacClade version 4.0.8 (44). Maximum likelihood (ML) analyses and ML bootstrap searches were performed using RAxML version 7.0.4 (45) (<http://phylobench.vital-it.ch/raxml-bb/>). Tree searches relied on the GTR + Γ model, with model parameters estimated over the duration of specified runs and 100 bootstrap replicates. We carried out Bayesian time estimation with an uncorrelated-rates model, using BEAST version 1.5.3 (46), with a Yule tree prior and the GTR + Γ model with six rate categories. There

are no *Cucumis* fossils, and we therefore used a secondary calibration from a Cucurbitaceae-wide analysis that used four calibration points (36). Mixing of the Markov chain Monte Carlo (MCMC) chain was checked using Tracer version 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) and convergence with AWTY (47). Final trees were edited in FigTree version 1.2.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). Ancestral areas were inferred under maximum likelihood as implemented in Mesquite version 2.6 (<http://mesquiteproject.org/>), using the Markov k-state one-parameter model, which assumes a single rate for all transitions between character states (geographic regions in this case). Further details on methods are provided in *SI Materials and Methods*.

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