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Repeated domestication of melon (*Cucumis melo*) in Africa and Asia and a new close relative from India

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PREMISE OF THE STUDY: The domestication history of melon is still unclear. An African or Asian origin has been suggested, but its closest wild relative was recently revealed to be an Australian species. The complicated taxonomic history of melon has resulted in additional confusion, with a high number of misidentified germplasm collections currently used by breeders and in genomics research.

METHODS: Using seven DNA regions sequenced for 90% of the genus and the major cultivar groups, we sort out described names and infer evolutionary origins and domestication centers.

KEY RESULTS: We found that modern melon cultivars go back to two lineages, which diverged ca. 2 million years ago. One is restricted to Asia (*Cucumis melo* subsp. *melo*), and the second, here described as *C. melo* subsp. *meloides*, is restricted to Africa. The Asian lineage has given rise to the widely commercialized cultivar groups and their market types, while the African lineage gave rise to cultivars still grown in the Sudanian region. We show that *C. trigonus*, an overlooked perennial and drought-tolerant species from India is among the closest living relatives of *C. melo*.

CONCLUSIONS: Melon was domesticated at least twice: in Africa and Asia. The African lineage and the Indian *C. trigonus* are exciting new resources for breeding of melons tolerant to climate change.

KEY WORDS crop wild relatives; *Cucumis picrocarpus*; *Cucumis trigonus*; Cucurbitaceae; domestication; melon.

The gourd family, Cucurbitaceae, is among the economically most important plant groups and includes numerous widely cultivated crops such as squash and pumpkin (*Cucurbita* spp.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). Most of the domesticated cucurbit species are susceptible to fungal, bacterial, and viral diseases and insect pests (Whittaker and Davis, 1962). Plant breeders therefore screen landraces and wild relatives of these crops for beneficial traits that can be used in crop improvement programs. For melon, these attempts so far have been complicated by its unclear geographic origin, unknown wild relatives, and conflicting hypotheses for its domestication history.

Truly wild forms of *C. melo* are found in Africa, Asia, and Australia, while the origin of wild melon populations in Madagascar and North America is under debate (Keraudren, 1966; Decker-Walters et al., 2002). The results of the most comprehensive phylogenetic analysis for the genus to date (Sebastian et al., 2010) suggest that the wild ancestor of domesticated melons is from Asia, and the high diversity of landraces in India and East Asia supports the idea of an Asian domestication center (Akashi et al., 2002; Dhillon et al., 2007; Tanaka et al., 2007; Dwivedi et al., 2010). In fact, the earliest melon remains from Asia date to 3000 BC in China (Watson, 1969; Luan et al., 2008), and melon remains from the Indus valley date to 2300–1600 BC (Vishnu-Mittre, 1974). Carbonized melon seeds were also discovered in eastern Iran and dated to ca. 2000 BC (Costantini, 1977). However, on the basis of the high number of wild *Cucumis* species in Africa and their diversity in chromosome numbers, melon may have first been domesticated on the African continent (Whittaker and Davis, 1962; Robinson and Decker-Walters, 1997). The oldest findings of African melon seeds from Lower Egypt are in fact older than the Asian melon remains, dating to 3700–3500 BC (van Zeist and de Roller, 1993; El Hadidi et al., 1996). The discovery of the closest living wild relative of melons in Australia (Sebastian et al., 2010) added a third potential

region of origin to the discussion. Indeed, wild melons are among the traditional medicinal and fruit plants collected by indigenous Australians (O'Connell et al., 1983); however, there is no archaeological evidence for melon domestication in Australia.

Like other crop plants, melon has a long and confusing taxonomic history. The name *Cucumis melo* was introduced by Linnaeus (1753) for domesticated plants cultivated in Uppsala, Sweden. Morphologically similar wild-collected plants were later described from Africa, Asia, and Australia under multiple different names for each continent, but all were subsequently synonymized with and thus included in *C. melo*. Most important among those names are *C. ambigua* Fenzl ex Hook.f. and *C. cognata* Fenzl ex Hook.f. from Sudan; *C. collosus* (Rottl.) Cogn. (widely misspelled as "*C. callosus*"), *C. pubescens* Willd., and *C. trigonus* Roxb. from India; and finally, *C. jucundus* F. Muell., and *C. picrocarpus* F. Muell. from Australia. The first attempt of a comprehensive monograph including an analysis of numerous wild *C. melo* accessions was published more than a century after the first description of Linnaeus by the French botanist Charles Naudin (1859), who maintained a large number of melon accessions in cultivation in the Paris Botanical Gardens. Naudin distinguished his wild melons from the domesticated *C. melo* var. *melo* by the smaller-sized fruits, leaves, and flowers and often bitter or nauseating taste of the fruit pulp (Naudin, 1859). He decided to describe all of them as a single variable taxon, *C. melo* var. *agrestis*, even though he had noticed subtle morphological differences between plants from India, which he called 'melon sauvage de l'Inde' (Fig. 1A, B) and the wild melons from Africa, his 'melon sauvage d'Afrique' (Fig. 1E, F). At the same time, the wild melons in Australia (Fig. 1C, D) were first collected by a European and formally described by Ferdinand von Mueller (1859), but Naudin apparently never saw this Australian material. Naudin was also the first to suggest that domesticated melons are perhaps the result of more than one domestication event. Specifically, he hypothesized that his melon sauvage de l'Inde might be the wild relative of the domesticated melons found in Asia, while the melon cultivars of Northern Africa could be descendants of his melon sauvage d'Afrique, a view that was also supported by Chevalier who discovered large populations of apparently wild *C. melo* while exploring remote areas of today's Egypt, Sudan and Republic of South Sudan and was able to distinguish them easily from cultivated and feral (escaped) melons (Chevalier, 1901).

The observations of Naudin and Chevalier were subsequently forgotten and the circumscription of the "*C. melo* var. *agrestis*" melons gradually changed from the original concept exclusive to wild types to a morphology-based concept, mixing wild and cultivated melons. *Cucumis melo* was subdivided in two subspecies *C. melo* subsp. *melo* and *C. melo* subsp. *agrestis* (Naudin) Pangalo (Pangalo, 1933; Grebenscikov, 1953; Jeffrey, 1990; Kirkbride, 1993), and all plants with long, spreading hairs on the ovaries were named *C. melo* subsp. *melo*, while plants with short-haired ovaries were named *C. melo* subsp. *agrestis* (Kirkbride, 1993). Since domesticated melons show various pubescence types of their ovaries, the horticultural system of up to 19 cultivar-groups does not match the current subspecies concept (Pitrat, 2013, 2017). Phylogenetic analyses based on DNA data also found no support for the system of Pangalo and Kirkbride but instead showed *C. melo* subsp. *agrestis* to be a polyphyletic taxon, with accessions clustering by geographic origin and not morphology or wild/cultivated origin (Stepansky et al., 1999; Mliki et al., 2001; Nakata et al., 2005; Sebastian et al., 2010; Blanca et al., 2012; Serres-Giardi and Dogimont, 2012; Esteras et al., 2013).

The currently used classification system thus not only ignores most of the observations by Naudin and his colleagues but is also highly unnatural. The idea of a single Asian melon domestication also became more and more unlikely. Our aim here was to build a comprehensive DNA data set for *Cucumis* with an especially dense sampling of wild *C. melo* and its most important cultivar groups. This sampling allows us to search for the links between wild *C. melo* populations in Asia, Africa, and Australia and the domesticated melons. In this way, we aimed to solve the long-standing question of Asian, African, or perhaps Australian origin of *Cucumis melo*, to infer the minimum number and place of its domestication centers, and to provide a more natural classification system for *C. melo* and its wild relatives.

MATERIALS AND METHODS

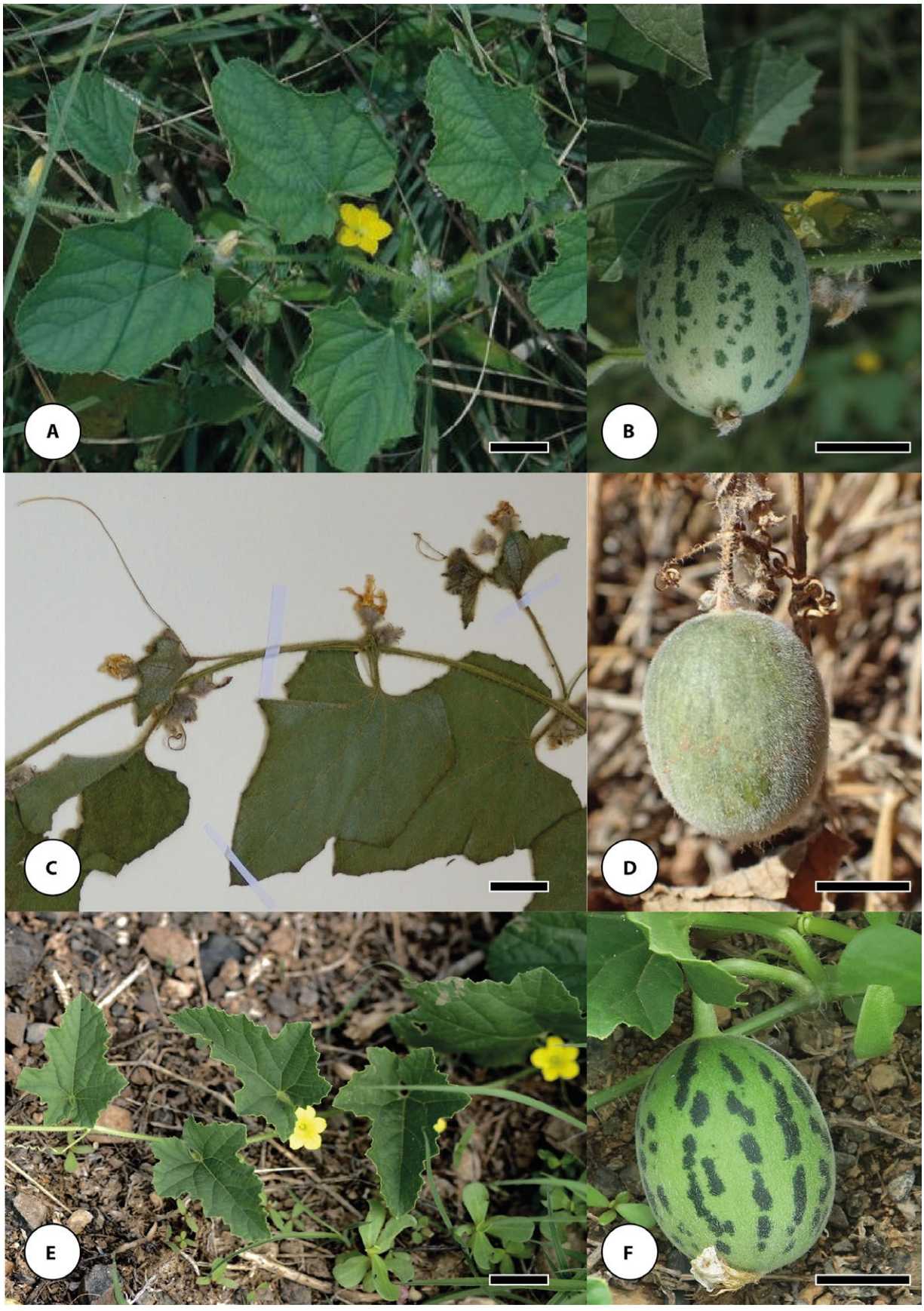
Plant material

DNA was extracted from 88 samples, including herbarium material, seeds, and field-collected plants (silica-dried) from Africa, India, Australia, and Indian Ocean islands. Our sampling includes 90% of the currently accepted species of *Cucumis* (Appendix S1, see the Supplemental Data with this article). The only missing species are the African *C. aetheocarpus* (C.Jeffrey) Ghebret. & Thulin, *C. engleri* (Gilg) Ghebret. & Thulin, *C. jeffreyanus* Thulin, *C. kirkbridei* Ghebret. & Thulin, *C. prolatior* J.H.Kirkbr., and *C. reticulatus* (A.Fern. & R.Fern.) Ghebret. & Thulin. Based on morphology, these six species belong to either section *Cucumella* or section *Aculeatosi*, far from melon (Fig. 2). Materials for the main cultivars and some wild-collected samples were provided by the U. S. Department of Agriculture-Agricultural Research Service (USDA-ARS) seed bank (npgsweb.ars-grin.gov) and from the COMAV-UPV and Melrip collections (Leida et al., 2015). We also used a few store-bought melons and seeds bought from private companies; the latter were planted and grown to a size suitable to make voucher specimens (deposited at TUM herbarium). Appendix S1 provides voucher information and NCBI GenBank accession numbers.

DNA extraction and PCR amplification

Total genomic DNA was isolated either from seeds, silica-dried leaves, or from herbarium specimens using the NucleoSpin Plant II DNA extraction kit (Macherey-Nagel, Germany) following the manufacturer's manual. For PCR amplification of six chloroplast loci (*trnL* intron, *trnL-trnF* spacer, *rpl20-rps12* spacer, *trnS-trnG* spacer, *matK* and *rbcL* genes) and the nuclear ribosomal ITS1-5.8S-ITS2 region, we used the KAPA2G Fast HotStart Ready Mix

FIGURE 1. The three wild "Agrestis" type melons. (A, B) "Asian Agrestis": *Cucumis melo* subsp. *melo* f. *agrestis* (India), syn.: *C. collosus* (Rottl.) Cogn. or misspelled "*C. callosus*", *C. pubescens* Willd.; (C, D) "Australian Agrestis": *C. melo* subsp. *melo* f. *agrestis* (Australia), syn.: *C. jucundus* F.Muell.; (E, F) "African Agrestis": *C. melo* subsp. *meloides*, Cape Verde (Boavista) syn.: *C. ambigua* Fenzl nom. nud., *C. cognata* Fenzl, nom. nud. Scale bars = 1 cm. *Photo credits:* (A, B) Balkar Singh; (C, D) South Australian Seed Conservation Centre (South Australia Botanical Gardens); (E, F) H. Schaefer.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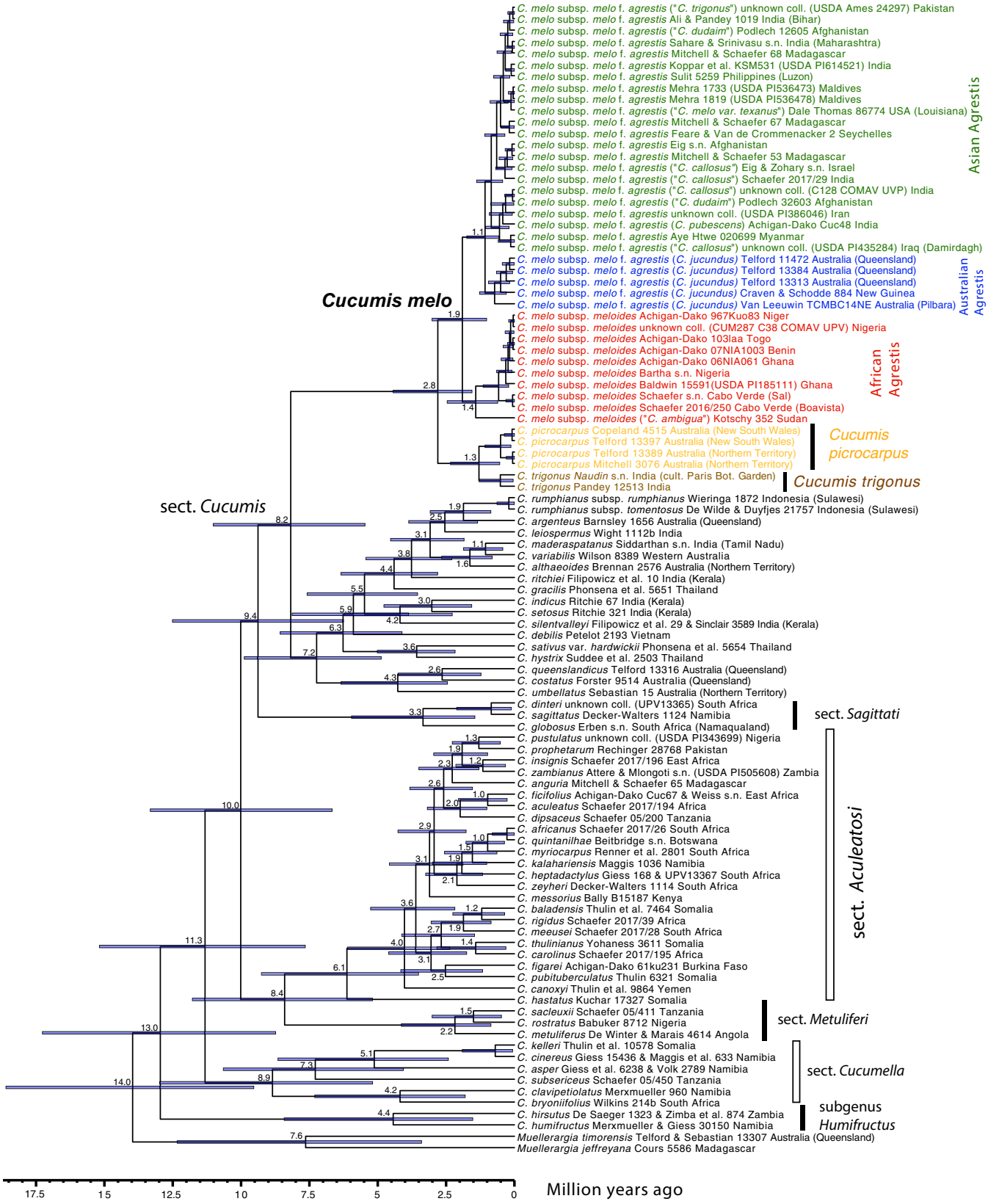

FIGURE 2. Chronogram from Bayesian relaxed molecular clock analysis of *Cucumis* based on the combined nuclear and plastid sequence matrix (5696 nucleotides, wild accessions only). Blue bars show 95% confidence intervals of the divergence time estimates for each split. Letter coding: green, Asian/Malagassy/American melons; blue, Australian/New Guinean accessions; red, African melons; yellow, *C. picrocarpus*; brown, *C. trigonus*.

(KAPA BIOSYSTEMS) and the manufacturer's protocol. The PCR products were enzymatically purified with the EXO-SAP mix (Jena Bioscience, Jena, Germany) according to the manufacturer's protocol. Sequencing reactions consisted of 2.5 μL clean PCR product, 5 μL H_2O and 2.5 μL primer (10 μM). For amplification and sequencing, primers for the ITS1+5.8S+ITS2 regions were described by Balthazar et al. (2000), for the *trnL* intron and *trnL-trnF* spacer by Taberlet et al. (1991), for *trnS-trnG* spacer and *rpl20-rps12* by Hamilton (1999). For *matK*, the primers were AF and 8R and internal primers F1 and R1 of Ooi et al. (1995). For *rbcL*, we used primers 1F and 1460R and internal primers 600F and 800R (Yokoyama et al., 2000). Cycle sequencing was performed by GATC Biotech (Konstanz, Germany) with the BigDye Terminator cycle sequencing kit on an ABI Prism 3100 Avant automated sequencer (Applied Biosystems, Foster City, CA, USA).

Editing, alignment and phylogeny construction

Editing and assembly of reads were done in Geneious R6 version 6.0.6. and version 11 (Biomatters Ltd., Auckland, New Zealand), and for the alignment, we relied on the Geneious alignment algorithm and MUSCLE (Edgar, 2004) as implemented in Geneious. The 425 newly generated sequences were complemented with selected *Cucumis* sequences, mainly from Sebastian et al. (2010), which were downloaded from the GenBank Nucleotide database (https://www.ncbi.nlm.nih.gov/genbank/). Phylogenetic analyses were performed using RAxML v. 8.1.18 (Stamatakis et al., 2008; Stamatakis, 2014) and MrBayes v. 3.3.6-svn(r1040) x64 (Ronquist and Huelsenbeck, 2003) on the CIPRES Science Gateway v.3.3 (Miller et al., 2010). RAxML was run under the GTR-GAMMA model with 20 heuristic searches from distinct random stepwise addition sequence parsimony starting trees, followed by selection of the best-scoring tree. RAxML bootstrap values were calculated using 1000 replicates. For the MrBayes analysis, we executed six runs in parallel with four chains each running for 50 million generations and sampling every 1000 generations. We used Tracer v. 1.6.0 (Rambaut et al., 2014) to check for convergence of the chains. Specifically, we visually inspected the Tracer plots and further checked that all effective sample size (ESS) values were above 200. After exclusion of the first 20% of the sampled trees, we computed a Bayesian majority-rule consensus tree (the allcompat command in MrBayes) of the remaining 240,000 trees. Each DNA region was analyzed separately first, then all plastid regions combined. Comparison of the ML phylogeny estimates for the separate nuclear and plastid data sets revealed incongruent positions for two African accessions, a Senegal landrace and the Sudanese cultivar Fadasi (Appendix S2; for further discussion see below). After removal of the two detected cases of conflict between the plastid and the nuclear ITS phylogeny estimate, all regions for the remaining taxa were concatenated, which resulted in a matrix of 139 ingroup taxa plus two outgroups spanning 6174 aligned nucleotides. We additionally coded the insertions–deletions for each DNA region with the 2matrix perl script (Salinas and Little, 2014) using the simple gap coding method of Simmons and Ochoterena (2000) and added them as separate partitions. The alignments were then run in RAxML under the GTRCAT model for the DNA partition and BINGAMMA for the indel partition. We used the GTR+G model for the DNA partition and a binary model for the indel partition in MrBayes. All trees were rooted on the two known species of the genus *Muellerargia*, which according to the results of the family-wide analysis by Schaefer et al. (2009) is the sister group of *Cucumis*.

Molecular clock analysis

Bayesian molecular clock estimation was performed under a log normal relaxed clock uncorrelated-rates model in BEAST v.1.8.4 (Drummond and Rambaut, 2007) on the CIPRES Science Gateway (Miller et al., 2010) with a combined chloroplast and nuclear data set containing only wild *Cucumis* accessions (no cultivars/landraces or feral melons). The analysis was run with a Yule tree prior and a GTR+G+I model with six gamma categories. Since we do not know of any *Cucumis* fossil records, we chose a secondary calibration approach and calibrated our analysis with four secondary calibration points from an earlier Cucurbitaceae-wide analysis that had used four fossil and geological calibration points (Schaefer et al., 2009). The following constraints were used, each with a normal prior distribution: the root height, the split between *Cucumis* and *Muellerargia*, was set to 16 Ma (SD = 3), the split between the two *Muellerargia* species to 12 Ma (SD = 3), the split between the *C. sagittatus* clade and the remaining species of *Cucumis* to 9 Ma (SD = 3), and the split between *C. sativus* and *C. hystrix* to 3 Ma (SD = 1). We ran four Markov chain Monte Carlo (MCMC) chains for 50 million generations, sampling every 1000th generation. Of the 50,001 posterior trees, we discarded the first 5000 trees as burn-in. Convergence of the MCMC chain was checked with Tracer version 1.6.0 by inspection of the plots and the ESS values (all >200). Trees were summarized using TreeAnnotator version 1.8.0. In total, 45,001 trees were combined into the final consensus chronogram, which was visualized in FigTree version 1.4.2 (Rambaut, 2009).

Networks

The genetic structure of the *C. melo* clade was analyzed in more detail with median-joining networks (Bandelt et al., 1999) based on the ITS data set built using PopART (http://popart.otago.ac.nz).

RESULTS

Wild melons comprise three geographically distinct lineages, and their closest relatives come from Australia and India

The phylogeny estimates of our sampling of wild *C. melo* accessions reveal three genetically distinct melon lineages of different geographic origin (Fig. 2, Appendix S3): clade 1 includes the African accessions, informally named "African Agrestis" by other authors, here formally described as *C. melo* subsp. *meloides* (Appendix S4). Within the African clade, the East and West African lineages split ca. 1.4 Ma, while the Cape Verde population split from the West African mainland plants ca. 0.6 Ma. The wild melon accessions from Australia and New Guinea form the second clade (labelled "Australian Agrestis" in Fig. 2, formally described as *C. jucundus* F. Muell.). The third and largest clade, labelled "Asian Agrestis" in Fig. 2, includes all Asian wild melon accessions plus our samples from Madagascar and the Indian Ocean islands and the sample from the southern United states ("*C. melo* var. *texanus*"). Our molecular dating analysis reveals that the split between the African *C. melo* subsp. *meloides* and the Asian/Australian *C. melo* subsp. *melo* clades dates to ca. 1.9 Ma. (Fig. 2). Already a million years earlier, about 2.8 Ma, the melon lineage had diverged from its closest wild relatives, which are *C. picrocarpus* from Australia (Fig. 3) and *C. trigonus* (Fig. 4), an Indian species, which was described by Roxburgh (1832).

Modern melon cultivars go back to two lineages

When we combine our sampling of wild *C. melo* accessions with all major cultivar groups, we still find the distinct clades of African and Asian wild melons, but the latter now also includes the Australian clade (Appendix S3). The major melon cultivar groups including the 'Inodorus', 'Cantaloupensis', 'Reticulatus', and 'Adzhur' melons as well as cultivars mainly found in India and South East Asia and those from Central and Far East Asia (e.g., 'Chinensis', 'Conomon', 'Makuwa') are all nested in the "Asian Agrestis" clade. A few *C. melo* cultivars of African origin ('Moussa', 'Tirama') also group in the Asian clade. In contrast, the 'Tibish' melon, an African landrace today still grown in Sudan, groups in the African wild melon clade (= *C. melo* subsp. *meloides*).

The median-joining network based on our ITS data set including 39 wild accessions and 38 cultivars from Africa, Asia, the Mediterranean and the Australian region (Fig. 5, Appendix S5; see Appendix S6 for network of wild accessions only) confirms the three clades detected in the ML and Bayesian phylogenies: one major haplotype group comprising wild melon and cultivar accessions from Asia and Indian Ocean islands (H1) and a subgroup with the Australian/New Guinean samples (H5-H8). A second group, separated by multiple substitutions from the Asian group, consists exclusively of African wild melons (H9-H14) together with the African landraces Fadasi (H13) and Tibish (H14). The African Moussa melon, a modern cultivar introduced only in the 1950s, the Tirama melon from Burkina Faso and a nonclassified market melon from Zambia belong to the Asian cluster. The very close relationship between these African market melons and Asian cultivars indicates that the modern African cultivars are in fact of Asian origin, most likely imported with the global seed trade during the past few decades. Cultivars from the Near East and the Mediterranean also group with the Asian accessions.

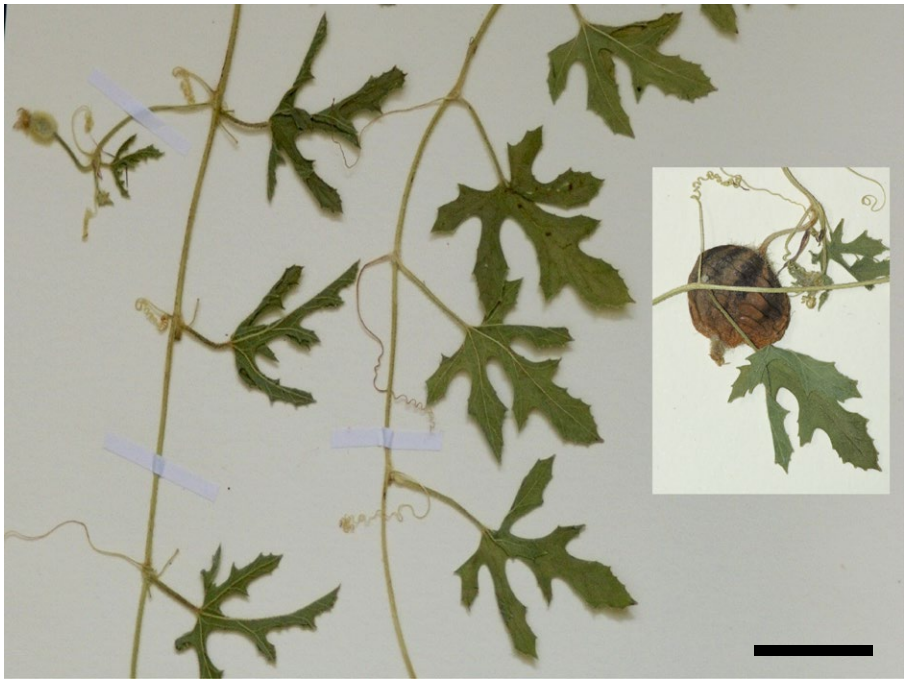

FIGURE 3. The Australian *Cucumis picrocarpus* [here *I.R. Telford 13314* (M)] is one of the two closest living relatives of *C. melo*. Note the globular fruits and the deeply dissected leaves. Scale bars = 1 cm. *Photo credits:* H. Schaefer.

Evidence for hybridization between the wild African and Asian melons

Comparison of the phylogeny estimates for the nuclear ITS region with the results obtained for the combined chloroplast data (Appendix S2) revealed two cases of incongruence pointing to possible hybridization events: (1) the Fadasi melon, a landrace from Sudan and (2) accession PI436534, an unnamed landrace from Senegal obtained from the USDA seed collection. Based on the ITS sequence, the Fadasi melon groups in the "African Agrestis" cluster together with the Sudanese Tibish landrace and a wild accession from Sudan (*Kotschy 352*). In contrast, in the phylogeny estimate based on the combined chloroplast data, it is placed in the "Asian/Australian Agrestis" clade (Appendix S2). For the Senegalese landrace USDA PI436534, we find the opposite situation. Here, the ITS is very similar to Mediterranean cultivars, whereas the plastid regions belong to the African gene pool (Appendix S2).

DISCUSSION

Two closest wild relatives of the melon clade, one from Australia and one from India

We show that all wild and cultivated *C. melo* accessions are the sister group to two wild melon species: the Australian *C. picrocarpus* (Fig. 3) and the Indian *C. trigonus* (Fig. 4). Even though the split between *C. picrocarpus* and *C. trigonus* is dated to only 1.3 Ma (Fig. 2), the perennial *C. trigonus* clearly differs in both DNA and morphology from the annual *C. picrocarpus*, which had been identified as the single closest wild relative of melon in the most recent genus-wide phylogenetic analysis (Sebastian et al., 2010). This additional crop wild relative has been overlooked for decades mainly because of taxonomic confusion. Material originally distributed by North Central Regional Plant Introduction Station (Ames, IA, USA) of the USDA-ARS, as "*Cucumis trigonus* Ames 24297" and widely used in genomic studies, including some of our own work (Sebastian et al., 2010; Díaz et al., 2017), does not belong to *C. trigonus* Roxb. but instead represents the common Asian wild melon form *C. melo* subsp. *melo* f. *agrestis* (Fig. 2, Appendices S2, S7). In contrast, the real *C. trigonus* in the sense of Roxburgh (1832) is available in Indian germplasm collections under the misapplied name "*C. callosus*". As noted before, that name is a misspelled version of the name *C. collosus* (Rottl.) Cogn., the basionym of which is *Bryonia collosa* Rottler (Appendix S8). In the original protologue, Rottler (1803) describes the species as "Br[yonia] collosa" because of its sticky and scabrid leaf surface ("Foliis … colloso-scabris"). There are obvious morphological differences between this *C. collosus* (Appendix S8) and Roxburgh's *C. trigonus* (Fig. 4), especially in fruit and leaf shape and life form (annual vs. perennial). At the sequence level, the Indian material is almost identical to *C. trigonus* herbarium specimens by

FIGURE 4. Illustration of Indian perennial *Cucumis trigonus* by Roxburgh, who described the species in 1832, but it has been misidentified and confused with other taxa ever since. Together with the Australian *C. picrocarpus*, it forms the clade of the closest crop wild relatives of *C. melo*. Note the perennial rootstock, the globular fruits, and the characteristic deeply lobed leaves. *Photo credits:* A. Pandey; scale bars = 1 cm.

Naudin from the Geneva herbarium, so we conclude that the Indian "*C. callosus*" discussed by John et al. (2013) represents in fact Roxburgh's *C. trigonus*.

Furthermore, our results confirm earlier suggestions that "*C. callosus*" material from European and American (but not Indian) germplasm collections and herbaria is identical to *C. melo* subsp. *melo* f. *agrestis* (Fig. 2; Appendices S2, S7), which confirms Kirkbride's (1993) suggestion to place *C. collosus* in synonymy of *C. melo* subsp. *melo*. A list of reclassified germplasm and herbarium collection material of this confusing complex is given in Table S2.

The discovery of another close wild relative of melon in India helps to explain the strange biogeographic situation detected in the previous large-scale analysis of the genus (Sebastian et al., 2010), where the Australian endemic *C. picrocarpus* was found to be the sister species to all *C. melo* populations. This result was surprising not only because of its geographically isolated Australian range, but also because *C. picrocarpus* is genetically and morphologically rather distinct from *C. melo* (Sebastian et al., 2010). On the basis of this finding, we hypothesize that further overlooked relatives of the melon clade are likely to be discovered in the Southeast Asian region between India and Australia and the biogeographic pattern is just a sampling artefact resulting from the now very dense sampling of *C. melo* available for both India and the Australian continent but not for the area in between. *Cucumis trigonus* is of particular interest for melon breeding not only because of its perennial life form, but also importantly, as shown by John et al. (2013 [as "*C. callosus*"]), because crossing attempts with *C. melo* cultivars were in a few cases successful. Such crosses are rare for the genus *Cucumis* and will make it easy to introduce any beneficial traits of *C. trigonus* into commercial melon germplasm. For *C. picrocarpus*, crossing experiments are still lacking, but it seems likely that it will be possible to cross it at least with some of the melon cultivars in a controlled greenhouse.

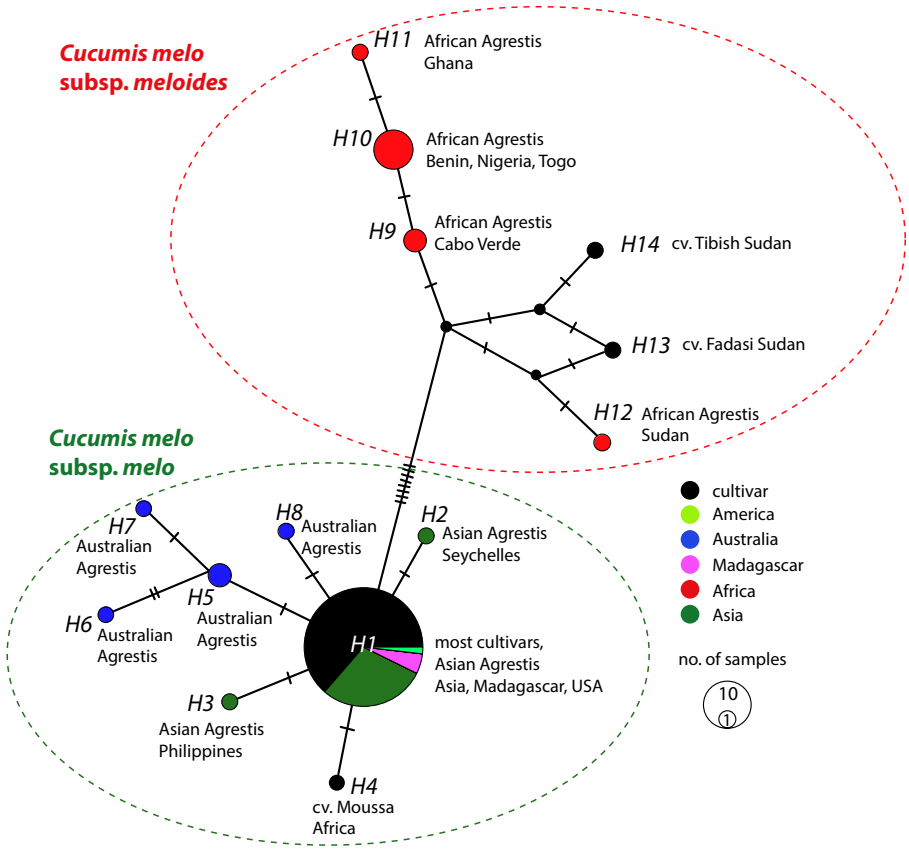

FIGURE 5. Median-joining network of the *Cucumis melo* clade (wild and cultivated material) based on the nuclear ribosomal ITS region built using PopART (http://popart.otago.ac.nz). Each circle represents an ITS haplotype; the diameter indicates number of accessions sampled; color indicates geographic origin. Hatch marks on the lines connecting the different haplotypes indicate unsampled haplotypes.

At least two independent domestications of wild melons in Africa and Asia

Besides encompassing Asia and Australia, our sampling covered most of the tropical humid African and Sahel regions (but very little of sub-Saharan Africa) and showed two distinct gene pools of wild melons on the Asian and African continents, each closely related to distinct cultivar groups. A third group is restricted to Australia and New Guinea but does not include any of the analyzed cultivars. However, these groups do not match the currently used subspecies *C. melo* subsp. *agrestis* and *C. melo* subsp. *melo*, which would be part of the Asian clade. Instead, there is an overlooked taxon, which seems to be confined to the Sahel region from western Africa to Sudan, but might turn out to occur also south of the Sahara. This discovery confirms the long-forgotten observations of Naudin (1859) and his colleagues. Our results also suggest that Naudin was right when he suggested that the wild populations of melon in India and in Africa were domesticated independently. Naudin's (1859) "melon sauvage de l'Inde" (our *C. melo* subsp. *melo* f. *agrestis*) groups with the melon cultivars from India and East Asia, while his "melon sauvage d'Afrique" from East Africa (our *C. melo* subsp. *meloides*) groups with the African landraces in our sampling. We thus consider the Asian *C. melo* subsp. *melo* f. *agrestis* as the ancestor of most of our modern market melon cultivars, whereas *C. melo* subsp. *meloides* is the wild progenitor of an economically less important but genetically very diverse and potentially very interesting group of African cultivars, including Tibish, Fadasi and presumably also "Seinat" (not included in our sampling). Other studies analyzing genetic diversity of melons also found particularly high numbers of differential SNPs in the African gene pool (Blanca et al., 2012; Esteras et al., 2013; Leida et al., 2015). The full scale of African landraces deriving from *C. melo* subsp. *meloides* is certainly underestimated in our study because we only had a very limited number of accessions from Africa. Also, some of these samples, including Moussa and Tirama melons from Benin, Mali, and Burkina Faso, clearly belong to the Asian lineage, indicating that they are recent introductions from the international seed market. There is an urgent need for inventory and storage of those traditional African landraces in germplasm collections before they are entirely replaced by modern cultivars of Asian origin.

Two African melon cultigens are very likely the result of hybridization with the Asian lineage

Our phylogenetic analysis of nuclear and plastid data revealed conflicting positions for the African Fadasi melon and a landrace from Senegal (USDA PI436534), which, depending on DNA region analyzed, nest in either the African or the Asian *C. melo* clade. This grouping suggests that both are the result of recent hybridization events between the African and Asian lineages. In the Fadasi cultivar, an ITS type very similar to the Tibish melon is combined with chloroplast regions that seem to be of Asian/Australian origin. The Fadasi melon (or at least our sample) therefore seems to result from introgression of an Asian cultivar into the African Tibish melon. The reverse case is found in the landrace PI436534 from Senegal. Here, nuclear ITS of Asian origin probably was combined with a chloroplast of the African C. *melo* subsp. *meloides*. The Senegal landrace is also intermediate in phenotype; fruits are similar in size to the wild African melons, but its sucrose level is intermediate between wild melons and cultivars (Nakata et al., 2005). Detecting hybridization events between the two lineages in Africa would not be surprising because melons are often cultivated close to wild populations in North and West Africa and can be easily cross-pollinated by bees. Confirmation of these hybrids in future genomewide studies would also demonstrate that even though the two lineages diverged at least 2 Ma, they are still cross-compatible and allow the use of the wild African melon gene pool as a rich complementary source for the improvement of our modern melon cultivars.

CONCLUSIONS

In summary, we show that independent melon domestication took place in Africa and Asia. So far, we are unable to date the onset of these domestication processes and thus cannot say where melon was first domesticated. The archaeological record, however, suggests that African melon domestication started at least 5000–6000 years ago, perhaps earlier than in Asia. Since only few studies have focused so far on African landraces and wild *C. melo* populations on the African continent, a lot of diversity in the African gene pool is probably still undetected. We also suggest a much more detailed exploration of wild melons in Southeast Asia, New Guinea and Australia, where additional phylogenetically close wild relatives can be expected.

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AUTHOR CONTRIBUTIONS

J.E. and H.S. developed the project; E.G.A.D., A.K.P., A.J.M., and B.P. contributed material; J.E. and H.S. performed analyses; J.E. and H.S. drafted the first version of the manuscript; all authors commented on the draft and approved the final version of the manuscript.

DATA ACCESSIBILITY

All sequence data used in our analyses are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Voucher information and accession numbers of the newly produced sequences are given in Appendix S1 of the Supplemental Data.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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