Chromosome numbers, Sudanese wild forms, and classification of the watermelon genus *Citrullus*, with 50 names allocated to seven biological species

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Abstract Watermelons are among the most important vegetable crops worldwide, but targeted breeding is hindered by problems with *Citrullus* taxonomy. Here we clarify nomenclature and species relationships in *Citrullus*, its chromosome numbers, and the likely geographic region of watermelon domestication. We correct an erroneous chromosome count in recent literature, provide a count for an understudied species, and data on chromosome numbers for the entire genus. We also use a nuclear/plastid locus phylogeny to summarize data on *Citrullus* sexual systems, loss of tendrils, life history, and geographic ranges. Key insights from new sequences are that (i) material of *C. vulgaris* var. "*colocynthoides*", collected by Schweinfurth in Egypt in 1882 represents the colocynth, *C. colocynthis*, and (ii) the citron or bitter watermelon, *C. amarus*, is indeed a separate species, not a subspecies of the dessert watermelon. Schweinfurth's varietal name, a nomen nudum, has been widely used for seeds as old as 5400 BP, and it now needs to be investigated whether these seeds are colocynths, watermelon or a hybrid. To help improve *Citrullus* taxonomy and nomenclature, we allocate some 50 scientific names to seven biological species, tabulate chromosome counts, and provide notes on misidentified germplasms traceable by accession numbers. More wild-collected herbarium-verified material from northeast Africa is urgently needed, especially given the discovery of Russian breeders that populations of wild "*cordophanus*" from Sudan are closer to the cultivated watermelon than are any other wild populations so far known.

Keywords chromosome counts; crop plants; herbarium resources; natural geographic ranges; wild relatives

Supplementary Material The Electronic Supplement (Table S1; Fig. S1) is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax); DNA sequence alignments are available from Dryad (http://doi.org/10.5061/dryad.2434f) and from TreeBASE (accession number: S14552)

INTRODUCTION

Between January 2000 and January 2017, 2115 papers have been published on the crop species "watermelon", which they refer to as *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Web of Science, http://apps.webofknowledge.com, accessed 4 Jan 2017). Other cultivated taxa of the genus *Citrullus* Schrad. are the southern African citron, tsamma, or bitter watermelons, used as a water source, for cooking, and as animal fodder and in a domesticated form for jams across the Mediterranean region (Table 1); the egusi watermelon, grown for its tasty seeds that are eaten as a raw snack in West Africa; and the colocynth, cultivated for diverse medical purposes since Ancient Egypt (Wasylikowa & Van der Veen, 2004; Dane & Liu, 2007; Dane & al., 2007; McGregor, 2012; Paris, 2015) (Table 1). The 2000+ papers all use an outdated classification from the 1930s that is partly based on Bailey's (1930) synonymization of Thunberg's *Momordica lanatus* Thunb. and Linnaeus's dessert watermelon *Cucurbita citrullus* L. Nuclear and plastid DNA sequences obtained from Thunberg's specimen, collected in 1773 near Cape Town and described by him as having "fructu lanato" or woolly fruits (Thunberg, 1794: 13), have shown that it is not the same as the dessert watermelon, which Linnaeus named from cultivated material (Chomicki & Renner, 2015). The two species are neither each other's closest relatives nor is one a partial genetic progenitor of the other. This finding would not have surprised *Citrullus* researchers before 1930, all of whom accepted two species, the southern African bitter watermelon (*C. lanatus* (Thunb.) Matsum. & Nakai) and the crop that Linnaeus described. The erroneous merging of the bitter watermelon with the dessert watermelon led to numerous subsequent errors, most importantly the assumption that watermelons were domesticated in southern Africa (Vavilov, 1935; Bates & Robinson, 1995; Dane & Lang, 2004; Wehner, 2008; Meyer & al., 2012; Levi & al., 2013; Khoury & al., 2016).

Sequencing of the watermelon genome showed that the West African egusi watermelon, *Citrullus mucosospermus* (Fursa) Fursa (traditionally treated as *C. lanatus* subsp.

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mucosospermus), is very close to the dessert watermelon (Guo & al., 2013). *Citrullus mucosospermus* is grown for its seeds, which lack a hard seed coat and can be eaten raw; the fruit pulp, which is white, is too bitter for human consumption (Achigan-Dako & al., 2015). Fresh seeds have a gelatinous seed coat (unique in the genus), a trait controlled by a single recessive gene mutation (Gusmini & al., 2004; Prothro & al., 2012). It is noteworthy that Gusmini & al. (2004) found no intermediates between the gelatinous seed coats and the dry seed coats (p. 269: "We never observed any intermediate trait that could have made our evaluation uncertain."), and Prothro & al. (2012) later mapped the seed coat gene to chromosome 2. The seeds of the egusi watermelon further differ from those of the cultivated watermelon in their thin, cream-colored seed coat (versus black in the cultivated watermelon), slightly rough surface (versus smooth and shiny in the dessert watermelon), and winged rim around the entire seed (fig. 1 in Prothro & al., 2012, shows photos of the seeds next to each other). Many further differences are implied by the finding of Guo & al. (2013: fig. 4) that a large region on chromosome 3 (from ~3.4 Mb to ~5.6 Mb) is highly differentiated between accessions labelled *mucosospermus* and those called *lanatus*.

Guo & al.'s (2013) watermelon genome study did not include samples of wild *Citrullus* species or populations from northeast and southern Africa, and it therefore could not test the relationships of the dessert watermelon to all its potential relatives. Especially relevant here are Egyptian and Sudanese wild watermelons (Paris, 2015). These were first drawn attention to by Schweinfurth (1883) who discussed his Egyptian material as *Citrullus vulgaris* var. "*colocynthoides*", a name widely used since then but never validly published (we therefore place it in inverted commas throughout). His Sudanese material, collected in 1869 south of Khartoum on the White Nile, Schweinfurth identified as *C. vulgaris* Schrad. In Schweinfurth's (1883, 1884) view, some population of Upper Egyptian watermelons was the progenitor of the dessert watermelon. Ancient watermelon seeds are known from Libya, dated to 3000 BC (Wasylikowa & Van der Veen, 2004), and Sudan, dated to 1500 BC (Van Zeist, 1983 [lacking illustrations or morphological details]; accepted by Paris, 2015). A large watermelon served on a tray and clearly eaten raw (hence not bitter) is depicted in an Egyptian tomb painting (Paris, 2015). Schweinfurth's (1883) original Egyptian and Sudanese samples are preserved in B, but prior to our study appear not to have been examined with modern methods.

A further problem in our understanding of the species of *Citrullus* is the frequent use of misidentified material. This has affected, for example, conclusions about *C. naudinianus* (Sond.) Hook.f., a species from the Namib-Kalahari region (Jeffrey, 1962; Schaefer & al., 2009; Schaefer & Renner, 2011; Chomicki & Renner, 2015). Germplasm accessions labeled as "*C. naudinianus*" have long been maintained and shared both by the United States Department of Agriculture (USDA) and the Beijing Vegetable Research Center (BVRC; see Results). As we will show here, some of the "*C. naudinianus*" seeds were *Cucumis myriocarpus* Naudin, which explains erroneous inferences in cytogenetic work and re-sequencing studies (e.g., Li & al., 2016; Zhang & al., 2016).

In the present study, we clarify the application of names, based on morphology (as seen in herbarium specimens) or in some cases DNA sequences from type material. Specifically, we (i) bring together chromosome counts for all species of *Citrullus* and provide the first count for *C. ecirrhosus* Cogn. (because of surprising cytogenetic conclusions in Li & al., 2016); (ii) allocate all available names of species, subspecies, and varieties to the seven biological *Citrullus* species; (iii) report the phylogenetic placement of Schweinfurth's 19th century *Citrullus* collections from Egypt and Sudan (from locations 1900 km distant from each other), and (iv) report *Citrullus* ranges and traits, focusing on life history, tubers, tendrils, spines, and sexual systems.

■ MATERIALS AND METHODS

Plant taxon sampling, sequence alignment, and phylogenetic analyses. — Material sequenced for this and our previous study (Chomicki & Renner, 2015) is listed in Appendix 1 (and in Electr. Suppl.: Table S1). We also traced the accession numbers of material labeled as *Citrullus* in the National Plant Germplasm System (NPGS) Germplasm Resources Information Network (GRIN; http://www.ars-grin.gov/npgs) and used in studies since 2004. Since few, if any, germplasms are associated with herbarium voucher specimens, their taxonomic identifications usually cannot be verified unless photos can be obtained from colleagues or curators of germplasm collections or informative DNA sequences can be compared to herbarium-verified material (see Acknowledgements).

We increased the taxon sampling of our earlier alignment of ten gene regions (Chomicki & Renner, 2015), namely the nuclear ITS region (ITS1, 5.8S rDNA, ITS2), and the plastid *trnL-trnF* spacer, *rpl20-rps12* spacer, *trnR-atpA* spacer, *trnG-trnS* spacer, *Ycf9-trnG* spacer, and *Ycf6-PsbM* spacer, and the genes *ndhF*, *rbcL* and *matK*. Total genomic DNA was extracted from ~20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin, Macherey-Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using *Taq* DNA polymerase (New England Biolabs, Ipswich, Massachusetts, U.S.A.) and the same plastid and nuclear primers as Dane & Lang (2004) and Sebastian & al. (2010). PCR products were purified using the ExoSap clean-up kit (Fermentas, St. Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, California, U.S.A.) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher v.5.1 (Gene Codes). All new sequences were BLAST-searched in GenBank. Sequences were added to our previous alignment using Mesquite v.2.75 (Maddison & Maddison, 2011). In the absence of statistically supported topological incongruence (i.e., maximum likelihood bootstrap support values >80%; Electr. Suppl.: Fig. S1), we combined the chloroplast and nuclear data, and the final concatenated matrix had 9212 aligned nucleotides. Maximum likelihood tree inference relied on RAxML v.8 (Stamatakis & al., 2008), under the general time-reversible model of substitution with unequal rates across sites (GTR+Γ). Statistical support relied on bootstrapping, with 100 replicates under the same model.

Molecular clock dating. — Molecular dating analyses relied on BEAST v.2 (Bouckaert & al., 2014). BEAST analyses were performed under both strict and uncorrelated lognormal relaxed clocks. The UCLD standard deviation of 0.601 in the relaxed clock analysis indicated that a relaxed clock fit our data best, and we therefore selected it. We used the GTR+Γ substitution model with four rate categories and a Yule tree prior. Monte Carlo Markov chains (MCMC) were run for 20 million generations, with log parameters sampled every 10,000 generations. We used Tracer v.1.6 (Rambaut & Drummond, 2007) to check that the effective sample size (ESS) of all parameters was >200 and that runs had converged. Trees were annotated in TreeAnnotator (part of the BEAST package) after discarding 10% as burn-in and using a target maximum clade credibility tree with a posterior probability limit of 0.98; the final tree was visualized in FigTree v.1.4 (Rambaut, 2006–2009). To calibrate our tree, we used a secondary constraint from the most comprehensive dated phylogeny of Cucurbitaceae, which used three fossils and one geological calibration, an island with an endemic radiation (Schaefer & al., 2009). Based on this study, we set the root of our tree to 34 million years, assigning it a normal distribution with a mean of 16 and a standard deviation of 2, representing the 95% HPD of Schaefer & al. (2009).

Ancestral state reconstruction. — We used stochastic character mapping to infer life history and sexual system evolution in *Citrullus* and relatives, not only at nodes but also along branches in the phylogenies. Based on literature and online resources, we coded "0" for perennial and "1" for annual species; and for sexual systems, "0" for monoecy and "1" for dioecy. We used the function make.simmap in the phytools package (v.04-60) (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using an equal rate model, and then simulated

Table 1. Uses, domestication, and common names of the four cultivated *Citrullus* Schrad. species.

Species	Common names	Wild range/ Cultivated range	Uses	Trait changes during domestication	Region of domestication	Reference
Citrullus amarus Schrad.	• *Wild form:* citron melon, tsamma or tsama melon (same name is also used for *C. ecirrhosus*), fodder melon, bitter melon • *Cultivated form:* preserving melon, pastèque a confiture, gingérine, citre, jejerine, méréville (French), pateca (Corsican), gila (Spanish and Portuguese)	Southern Africa/ Throughout the Mediterranean region	Fruits require cooking before consumption, used for jam; also as a source of water and as animal fodder	Fruit enlargement, rind thickening, loss of bitterness, acquisition of oblong shape	Unknown	Bailey (1930), Laghetti & Hammer (2007), McGregor (2012)
Citrullus colocynthis (L.) Schrad.	Colocynth	Northern Africa to west India/Initially cultivated in northern Africa; now naturalized in Australia	Fruit used in medicine (purgatory, abortion); Seeds can be used as food, or to produce oil used to make soap	None known	Ancient Libya, Sudan, and Egypt	Wasylikowa & Van der Veen, (2004), Dane & al. (2007), Paris (2015)
Citrullus lanatus (Thunb.) Matsum. & Nakai	Watermelon	Region of domestication controversial /Worldwide	Fruit (seeds of certain cultivars are used as a snack in China)	Loss of bitterness, acquisition of sweetness, lycopene accumulation, and fruit enlargement	Ancient Upper Egypt/Sudan, earliest remains date to 5400 BP in Libya	Van Zeist (1983), Wasylikowa & Van der Veen, (2004), Paris (2015)
Citrullus mucosospermus (Fursa) Fursa	Egusi melon	West Africa/West Africa	Protein and lipid-rich seeds are eaten (they lack a hard seed coat)	Larger seeds, richer in proteins and lipids (fruit pulp is not eaten)	West Africa	Fursa (1983), Prothro & al. (2012)

The name *Citrullus lanatus* (based on *Momordica lanata* Thunb.) is conserved against *C. battich* Forssk., following acceptance of the proposal by Renner & al. (2014) at the XIX IBC in Shenzhen 2017.

1000 character histories on the maximum clade credibility trees form BEAST. We summarized the 1000 simulated character histories using the function densityMap (also in phytools).

Preparation of chromosome spreads. — Seeds of GRIF 16056, originally from Namibia, were received from R. Jarret in September 2013 and grown in a greenhouse of the Munich Botanical Garden, where the resulting plants have been continuously growing and flowering since then. A voucher of a dried plant with flowers and photos of the habit has been deposited in M as *S.S. Renner 2855*. Chromosome counts were obtained from young flower buds, previously selected by size, after observation of meiocytes (cells surrounded by callosec) using a phase contrast microscope. Dissected anthers were fixed in 3 : 1 (v/v) ethanol / glacial acetic acid at room temperature overnight and stored at −20°C until use. Fixed anthers were shortly washed in distilled water, dissected in a drop of 45% acetic acid and squashed. Cover slips were removed after freezing, air-dried at room temperature, and the best slides were stained with DAPI (2 µg/ml). At least 20 cells, corresponding to different phases in meiosis, were analyzed. Images were taken with a Leica DMR microscope equipped with a KAPPA-CCD camera and the KAPPA software. They were optimized for best contrast and brightness using Adobe Photoshop CC 2014.

■ RESULTS

Species relationships and key traits in *Citrullus*. — The phylogeny (Fig. 1A) includes 13 *Citrullus* accessions, including sequences from wild-collected *C. colocynthis* (L.) Schrad. from Afghanistan and from the seeds collected by Schweinfurth in 1882 in Egypt between Asyut and Aswan (annotated by him as *C. vulgaris* var. "*colocynthoides*"). We also sequenced some of the same DNA regions from Schweinfurth's 1869 Sudanese seeds, collected near Khartoum and identified by him as *C. vulgaris* (Appendix 1); those plants appear to have been a wild population of dessert watermelons. The phylogeny implies that the dessert watermelon is closest to *C. mucosospermus*, but this node has no support (Fig. 1) and may change with sampling of wild northeast African populations; Discussion). The citron watermelon (also called bitter, tsamma, or preserving watermelon [cultivated form]), *C. amarus* Schrad., is closest to *C. ecirrhosus*.

Our ancestral state reconstructions imply that the perennial habit is ancestral in *Citrullus*, and that *C. ecirrhosus* probably represents a return from annual to the perennial habit (Fig. 1B). Sexual systems are labile in Cucurbitaceae (even at the population level), and thus it is not possible to securely infer ancestral states. However, our reconstruction suggests that dioecy might have been the ancestral state in *Citrullus*, and a single shift to monoecy occurred after the split of *C. naudinianus* (Fig. 1C).

Figure 2 shows a simplified depiction of the geographic ranges, sexual systems, and key morphological traits in the genus *Citrullus*. The only dioecious species is *C. naudinianus*, while the other six species are monoecious. However, it is not unusual for plants to produce only male flowers for quite a long period of time (months to two years) before producing female flowers (R. Jarret, USDA, pers. comm., 2016). *Citrullus ecirrhosus* from the Namib Desert is also one of the few Cucurbitaceae to have lost tendrils, probably because there are few other plants to support a scrambler in its habitat. In another of the desert *Citrullus* species, *C. naudinianus*, the tendrils are modified into spines.

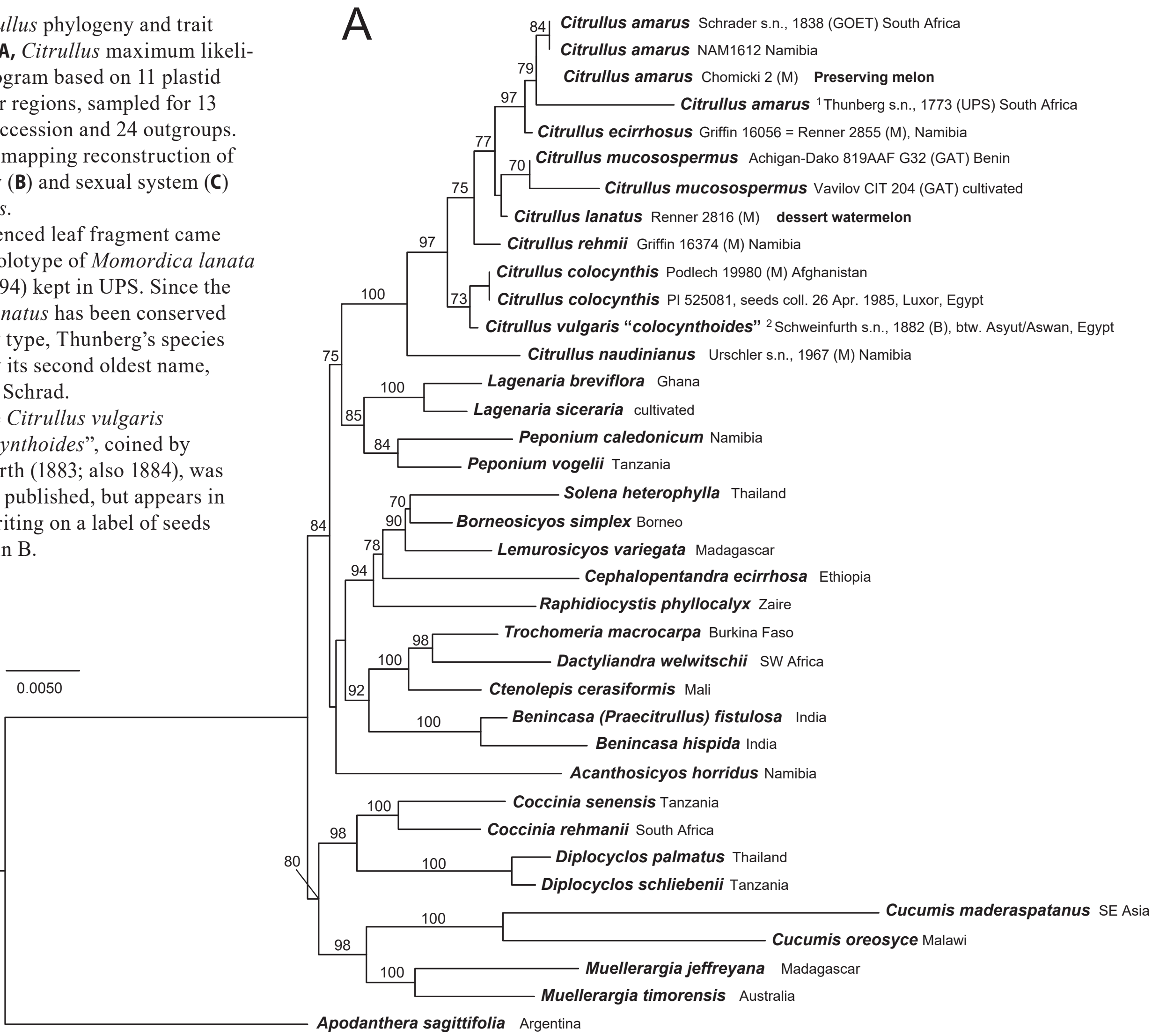
Chromosome numbers in *Citrullus* and widely used misidentified *Citrullus* germplasms. — Table 2 shows chromosome counts for the seven species of *Citrullus*. Only one count is linked to permanent voucher material, enabling verification of the cytogenetically studied plant; this is a new count for *C. ecirrhosus*, illustrated in Fig. 3. A count of $2n = 24$ reported for *C. naudinianus* (Li & al., 2016) came from PI 596690, which, however, was *Cucumis myriocarpus* (as shown by ITS sequences). Details about this and other misidentified germplasms that have caused erroneous conclusions in cytogenetic and phylogenetic work are in the footnotes of Table 2. Besides PI 596690, another example of misidentified germplasm is PI 270144, distributed as the West African endemic *C. mucosospermus*, but instead collected in Greece and really *C. lanatus*. Both seed batches have now been removed from the National Plant Germplasm System (NPGS Web Site, http://www.ars-grin.gov/cgi-bin/npgs/) and are no longer distributed. The so-called core collection of Zhang & al. (2016) also includes germplasms labeled as *Citrullus* (such as PI 671961, PI 618817, and CXG in their figure 3: all labeled *C. naudinianus*), but that are *Cucumis myriocarpus* (see footnotes in our Table 2).

Allocation of 50 species, subspecies, and variety names to seven biological species. — All names published in the genera *Citrullus* and *Colocynthis* Mill. with their taxonomic status based on a combination of genetic sequence data, ongoing work on morphology, and geographic origin are listed in Table 3, along with their allocation to the biological species shown in Fig. 2. The allocation of Schweinfurth's (1883) doubtful name *C. vulgaris* var. "*colocynthoides*" to the synonymy of *C. colocynthis* is based on nuclear and plastid sequences produced for this study from the original Schweinfurth material (consisting only of seeds) preserved in the Berlin herbarium (Appendix 1).

■ DISCUSSION

Relationships of cultivated and domesticated watermelons to wild species. — Excavations in Egypt, Libya, and Sudan have indicated that northeastern Africa is a primary center of watermelon domestication (Schweinfurth, 1883; Van Zeist, 1983; Wasylikowa & Van der Veen, 2004; Paris, 2015), and Greek, Latin and Hebrew writers of the first centuries CE clearly distinguished watermelons, melons, and colocynths (Paris, 2015). People were eating *sweet* dessert watermelons in Israel by the second century and in Italy by the early sixth century (Paris, 2015: 144).

To the Americas, watermelon cultivars were introduced after the second voyage of Columbus and during the time of the slave trade and colonization (Dane & al., 2013; Paris, 2015).



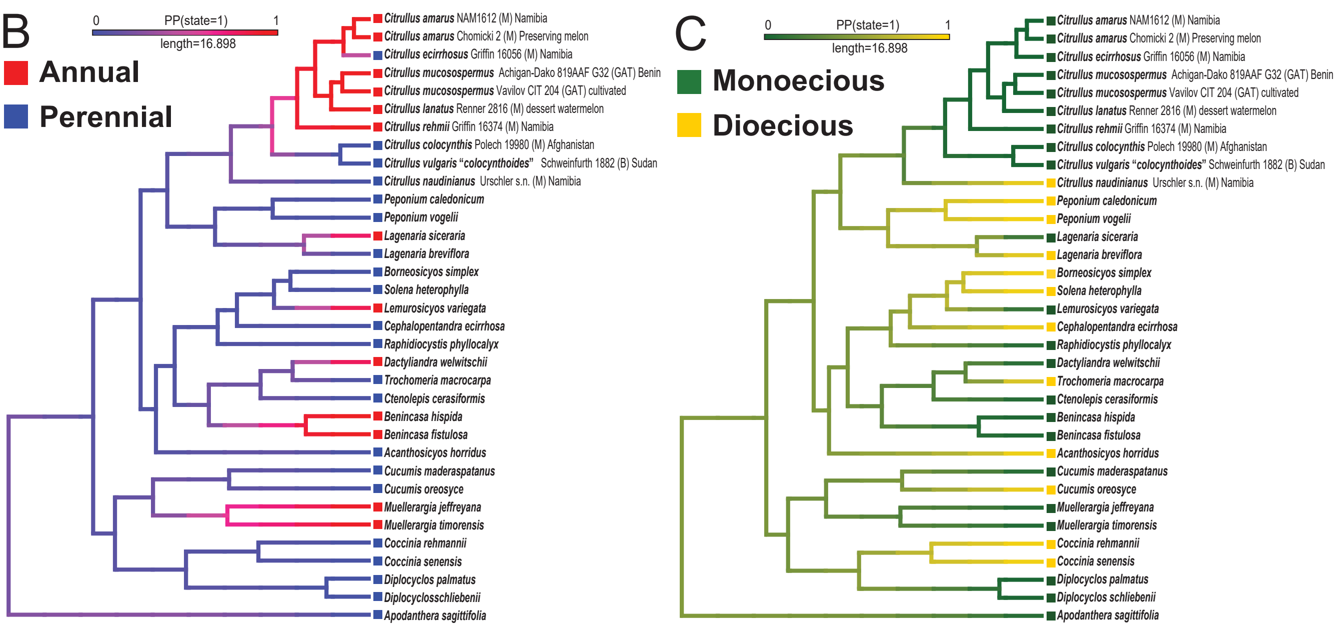


Fig. 1. *Citrullus* phylogeny and trait evolution. **A,** *Citrullus* maximum likelihood phylogram based on 11 plastid and nuclear regions, sampled for 13 *Citrullus* accession and 24 outgroups. Stochastic mapping reconstruction of life history (**B**) and sexual system (**C**) in *Citrullus*.
[1] The sequenced leaf fragment came from the holotype of *Momordica lanata* Thunb. (1794) kept in UPS. Since the name *C. lanatus* has been conserved with a new type, Thunberg's species is going by its second oldest name, *C. amarus* Schrad.
[2] The name *Citrullus vulgaris* var. "*colocynthoides*", coined by Schweinfurth (1883; also 1884), was not validly published, but appears in his handwriting on a label of seeds deposited in B.

 TAXON 66 (6) • December 2017: 1393–1405

Some, but not all, of the breeding history is now understood, especially from the re-sequencing of 20 watermelon accessions meant to represent the cultivated dessert watermelon (referred to as var. *vulgaris*), the West African egusi watermelon (referred to as var. *mucosospermus*), and Thunberg's South African bitter watermelon (var. *lanatus*; Guo & al., 2013). Guo & al. identified gene regions related to selected traits including carbohydrate metabolism, fruit flavor (terpene metabolism), and seed oil content (fatty acid metabolism). This important study, however, did not sample wild material of the southern African *C. ecirrhosus* (the closest relative of Thunberg's African bitter watermelon), eastern and North African wild watermelons and colocynths, and the Namib endemic *C. rehmii* De Winter. The precise origin of the domesticated watermelon therefore needs further study, with the first step being coordinated collecting of germplasm and herbarium material.

The possible importance of *C. colocynthis* as a genetic contributor to the dessert watermelon has long been discussed (Schweinfurth, 1883, 1884; Zamir, 2001; see their partly sympatric geographic ranges in Fig. 2). The archaeobotanist Georg Schweinfurth, who traveled and collected extensively

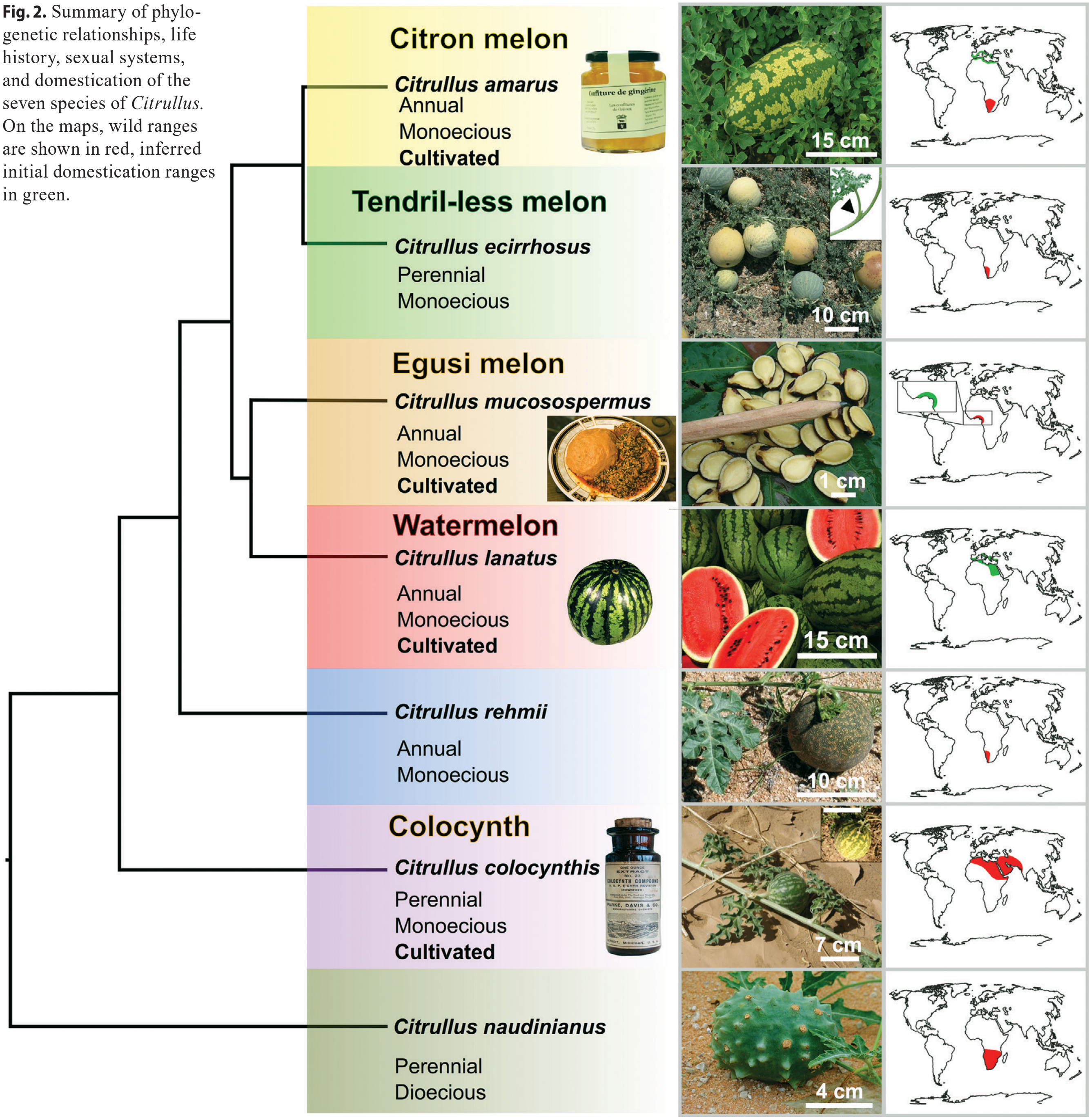


Fig. 2. Summary of phylogenetic relationships, life history, sexual systems, and domestication of the seven species of *Citrullus*. On the maps, wild ranges are shown in red, inferred initial domestication ranges in green.

throughout Egypt, Sudan, Yemen, and Algeria suggested that the small-fruited gurum or gurma watermelons cultivated in Upper Egypt of which he collected seeds in 1882 were the wild form from which watermelon was domesticated. However, our sequences from his 1882 seeds showed that his plants were *C. colocynthis*, not dessert watermelon. Natural and experimental hybrids between *C. colocynthis* and the dessert watermelon produce fertile F1 progeny (Shimotsuma, 1958; Singh, 1978; Fulks & al., 1979; Sain, 2003), causing Wehner (2008: 380) to suggest that, "Selection should be made for good watermelon flavor, independent of sweetness (sugar content). Flavor should include freedom from bitterness, which is controlled by a single dominant gene, and may be introduced in crosses with *C. colocynthis* accessions." Some of the earliest *Citrullus* archeological remains from Libya, dated to 5400 ± 80 BP, have been called var. "*colocynthoides*" (e.g., Wasylikowa & Van der Veen, 2004), using the name Schweinfurth created for his Egyptian material, but failed to validate. The cucurbit taxonomist C. Jeffrey (then working at Kew) to whom the ancient Libyan seeds were sent for confirmation concluded that they "fall within the range of variation of both *C. colocynthis* and *C. lanatus* [by which he meant dessert watermelon], so are not specifically diagnostic" (Wasylikowa & Van der Veen, 2004: 215). Our DNA sequences from Schweinfurth's (1883, 1884) Egyptian and Sudanese seeds, along with his observations on seeds and leaves found in tombs of pharaohs, point to a long co-domestication of both species, the perennial colocynth for the medicinal properties of bitter seed oil, the annual dessert watermelon for its tasty pulp.

What is conspicuously lacking in current watermelon breeding is collecting in Libya, Upper Egypt, and Sudan, with follow-up genomic studies, to address a possible progenitor

Table 2. Chromosome numbers in *Citrullus* Schrad. with notes on germplasm material that was misidentified at some stage, therefore leading to erroneous conclusions.

Species	Herbarium voucher	Mitotic count	Meiotic count	Reference
C. amarus Schrad. (bitter melon)	None	$2n = 22$	None	Shimotsuma (1963)
C. amarus (bitter melon)[a]	None; germplasm PI 500335[a]	$2n = 22$	None	Li & al. (2016)
C. colocynthis (L.) Schrad.	None	$2n = 22$	$n = 11$	Shimotsuma (1958) Beevy & Kuriachan (1996)
C. colocynthis[b]	None; germplasm PI 386021[b]	$2n = 22$	None	Li & al. (2016)
C. ecirrhosus Cogn.	None	$2n = 22$	None	Shimotsuma (1958)
C. ecirrhosus	*S.S. Renner 2855* (M)	$2n = 22$	$n = 11$	This study
C. ecirrhosus[c]	None; germplasm PI 632751[c]	$2n = 22$	None	Li & al. (2016)
C. lanatus subsp. *vulgaris* (Schrad.) Fursa = Dessert watermelon	None; cultivar 97103[d]	$2n = 22$	None	Li & al. (2016)
C. lanatus subsp. *vulgaris* = Dessert watermelon	None	$2n = 22$	$n = 11$	Whitaker (1930) and subsequent studies
C. mucosospermus (Fursa) Fursa	*T.B. Fursa 1298* (WIR)	$2n = 22$	None	Fursa (1983: 110)
C. "*mucosospermus*", this germplasm is misidentified[e]	None; germplasm PI 270144[e]	$2n = 22$	None	Li & al. (2016)
C. naudinianus (Sond.) Hook.f.	None	$2n = 22$	None	Shimotsuma (1963)
C. "*naudinianus*", this germplasm is misidentified[f]	None; germplasms PI 596690, PI 671961 and Grif 14021[f]	$2n = 24$	None	Li & al. (2016)
C. rehmii De Winter, identity unclear[g,h]	None; germplasms Grif 16135[g] and Grif 16376[h]	$2n = 22$	None	[g]Reddy & al. (2013), [h]Li & al. (2016)

a PI 500335 is from Zambia and cited as *C. lanatus* var. *citroides* (L.H.Bailey) Mansf. in Dane & Liu (2007). Based on the DNA sequences produced by Dane & Liu, and the new classification proposed in this paper, this plant represents *C. amarus*.
b PI 386021 is from Iran. This germplasm was not used in other publications.
c PI 632751 was collected in Namibia as *C. ecirrhosus* by Richard Robinson (e-mail from Bob Jarret, 18 August 2016). It has now been removed from the USDA website: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1557731. Renner received 10 seeds of this from R. Jarret on 18 August 2016: ITS sequences place this between *C. amarus* and *C. ecirrhosus.*
d 97103 is the inbred line sequenced by Guo & al. (2013) for the watermelon draft genome.
e PI 270144 is from Greece and identified as *Citrullus lanatus* in the USDA germplasm databank: https://data.nal.usda.gov/dataset/germplasm-resources-information-network-grin/resource/66d4d8e0-0b59-46a3-b47e-4de430a7dcf6
f PI 596690, PI 671961, and Grif 14021 are *Cucumis myriocarpus*. Herbarium vouchers in M.
g Grif 16135, accession donated on 16 September 2005 by B and T World Seeds. Comment: No longer available as of 13 April 2017: https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1682645
h Grif 16376, accession donated on 23 Jan 2006 by B and T World Seeds, Comment: Purchased by Dr. Robert Jarret in 2002 (https://data.nal.usda.gov/dataset/germplasm-resources-information-network-grin/resource/66d4d8e0-0b59-46a3-b47e-4de430a7dcf6)

role of watermelon populations from northeast Africa (Paris, 2016). The most recent such collecting was carried out by Russian breeders, when David Ter-Avanesyan (1909–1979) between 1959 and 1964 travelled to Kurdufan (Kordofan; Sudan) and brought back seeds from which populations were and are maintained at the Vavilow Institute (Ter-Avanesyan, 1966; Larisa Bagmet, Curator of VIR, pers. comm., 30 Mar 2017; for Ter-Avanesyan life and travels see http://www.rasl.ru/science/14_Exhibitions/ter.php). These Sudanese watermelons (probably the same entity as Schweinfurth's 1869 watermelons from near Khartoum), have non-bitter fruits with white pulp and beige seeds (photos in Ter-Avanesyan, 1966 and Fursa, 1972). Ter-Avanesyan (1966) described them as *C. lanatus* subsp. *cordophanus*, while Fursa down-ranked them to a mere variety (Table 3); both considered these plants to represent the progenitors of cultivated watermelon. Based on immunochemical analysis of seed proteins, Fursa & Gavrilyuk (1990) showed that subspecies *cordophanus* is closer to the dessert watermelon than is *C. mucosospermus*; that the southern African *C. amarus* is closest to *C. ecirrhosus*; and that *C. naudinianus* is sister to the remaining species of *Citrullus*, which is borne out by molecular data (Chomicki & Renner, 2015); the only species

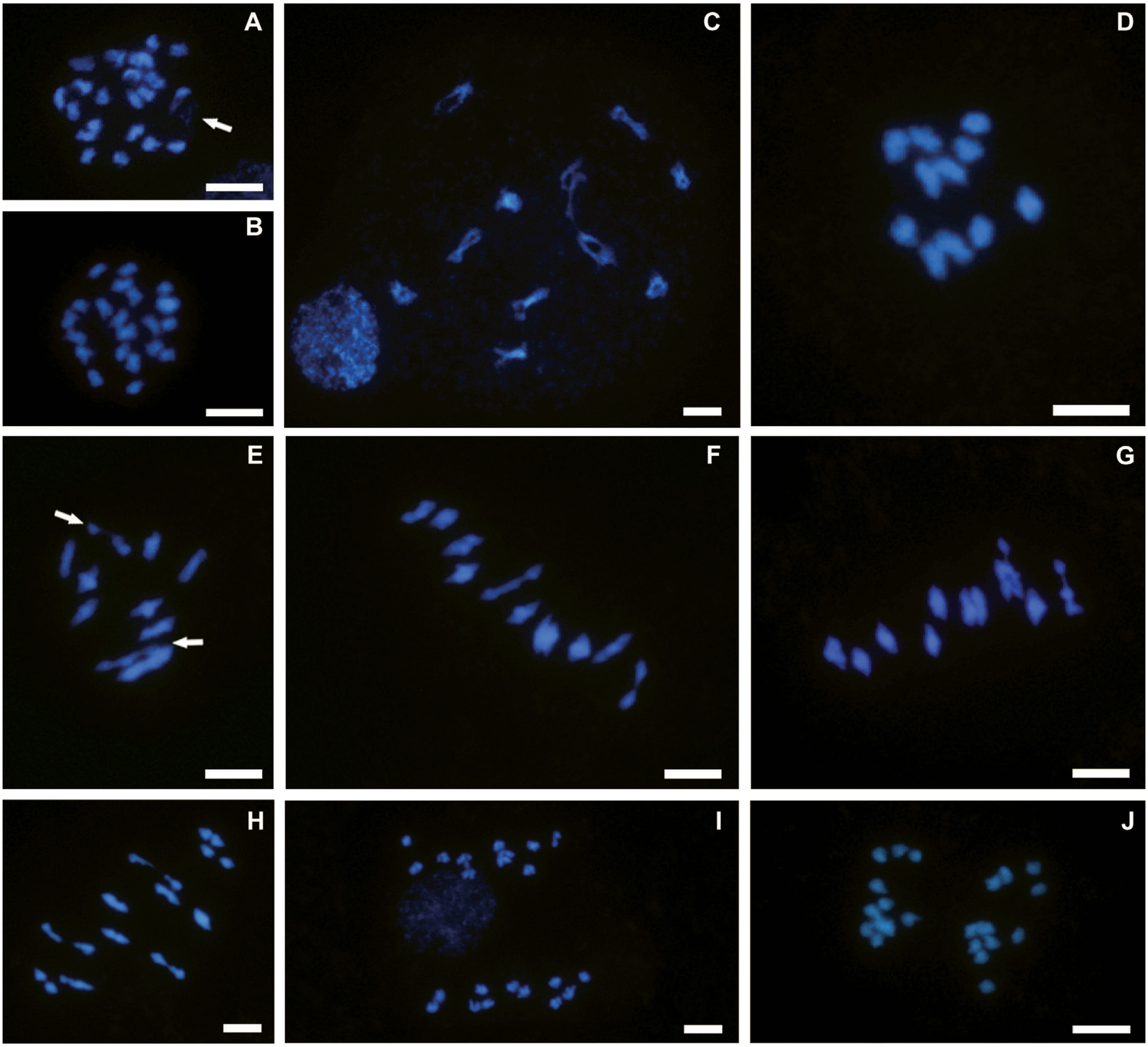


Fig. 3. Mitotic and meiotic nuclei of *Citrullus ecirrhosus*. **A & B,** Mitosis/pre-meiosis showing the $2n = 22$ chromosomes. The arrow in A marks a DAPI-negative small chromosome. **C & D,** Meiosis: Diplotene and diakinesis of prophase I with 11 bivalents. **E–G,** Organization of bivalents in metaphase I. At this stage, microtubules are attached to the centromeres of each homologous chromosome, and their contraction causes chromosomes to assume a more flattened form. The arrows in E mark chromosomes belonging to different bivalents and possessing satellites. **H–J,** Early and late anaphase I. Each nucleus receives a set of 11 chromosomes that will undergo meiosis II. — Bars correspond to 5 μm.

Table 3. Names published in the genera *Citrullus* Schrad. and *Colocynthis* Mill. with their taxonomic status based on morphology, genetic sequence data, and geographic origin of type material as applicable. Accepted names are shown in bold.

Proposed name	Accepted name
Citrullus amarus Schrad.	***C. amarus***
Citrullus anguria (Duchesne) H.Hara	*C. lanatus* nom. cons.; [a]Duchesne, in accordance with Linnaeus, placed watermelon in the genus *Cucurbita*, but named it *Cucurbita anguria* (Paris, 2000)
Citrullus aquosus Schur	Probably *C. lanatus* nom. cons.
Citrullus battich Forssk.	*C. lanatus* nom. cons.; oldest valid name for the sweet watermelon (cf. Renner & al., 2014)
Citrullus caffer Schrad.	*C. amarus*
"*Citrullus caffrorum*" Schrad.	Not validly published (*C. amarus*)
Citrullus chodospermus Falc. & Dunal	Protologue not seen, probably *C. lanatus* nom. cons.
Citrullus citrullus H.Karst., nom. illeg.	*C. lanatus* nom. cons.
Citrullus citrullus Small, tautonym	*C. lanatus* nom. cons.
Citrullus colocynthis (L.) Schrad.	***C. colocynthis***
Citrullus colocynthis var. *capensis* Alef.	Not validly published (no material, no clear link to a species) = *Citrullus lanatus* nom. cons. var. *capensis* (Alef.) Fursa (1972: 37)
Citrullus colocynthis var. *insipidus* Pangalo	*C. colocynthis* (fide Fursa, 1972)
Citrullus colocynthis subsp. *insipidus* (Pangalo) Fursa	*C. colocynthis* (fide Fursa, 1972)
Citrullus colocynthis var. *stenotomus* Pangalo	*C. colocynthis* (fide Fursa, 1972)
Citrullus colocynthis subsp. *stenotomus* (Pangalo) Fursa	*C. colocynthis* (fide Fursa, 1972)
Citrullus colocynthoides Pangalo	*C. amarus* (Pangalo, 1930, calls this the "citron forage melon")
Citrullus ecirrhosus Cogn.	***C. ecirrhosus***
Citrullus edulis Spach, nom. illeg.	*C. lanatus* nom. cons.
Citrullus edulis Pangalo, nom. illeg.	Later homonym of *C. edulis* Spach
Citrullus fistulosus Stocks	*Benincasa fistulosa* (Stocks) H.Schaef. & S.S.Renner[b]
Citrullus lanatus (Thunb.) Matsum. & Nakai	***C. lanatus*** nom. cons.
Citrullus lanatus var. *albidus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus f. *amarus* (Schrad.) W.J.de Wilde & Duyfjes	*C. amarus*
Citrullus lanatus var. *caffer* (Schrad.) Mansf.	*C. amarus* (Fursa, 1972: 37 correctly placed this variety here)
Citrullus lanatus var. *citroides* (Bailey) Mansf.	*C. amarus* (Fursa, 1972: 37 correctly placed this variety here)
Citrullus lanatus subsp. *cordophanus* Ter-Avan., no type indicated but neotypified by Fursa (1972: 38)	Based on plants grown from seeds collected by Ter-Avanesyan in Kurdufan (Kordofan), Sudan and initially grown out near the city of Tashkent, later at VIR. For validation of this name and status of this taxon, see next row.
Citrullus lanatus subsp. ***vulgaris*** var. ***cordophanus*** (Ter-Avan.) Fursa	The nominal variety is var. *vulgaris* Fursa. Because of the lack of a type specimen for Ter-Avanesyan's (1966) name (previous row), Tatiana Fursa (1972: 38) choose a neotype, K-4102 (VIR). Type photo received from Irina Gashkova, curator of the *Citrullus* seed collection, and Larisa Bagmet, both at VIR, 29 May 2017. The variety differs from var. *vulgaris* in its non-edible whitish pulp.
"*Citrullus lanatus* var. *fistulosus*" Babu	Not validly published. Babu erred in citing the basionym as "*C. vulgaris* var. *fistulosus* Steward, Punj. Pl. 96 (1869)" (sic, he meant J.L.Stewart). The preceding name itself is a new combination based on *C. vulgaris* Stocks (1851). *Melbourne Code* Art. 41 Ex. 20 applies.
Citrullus lanatus var. ***lanatus***	***C. lanatus*** nom. cons.
Citrullus lanatus var. *minor* (Chakrav.) Maheshw.	Probably *C. lanatus*

Table 3. Continued.

Proposed name	Accepted name
Citrullus lanatus subsp. *mucosospermus* Fursa	***C. mucosospermus***
Citrullus lanatus f. *nigroseminius* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *oblongus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *pulcherrimus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *pumilus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *rotundus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *senegalicus* (Fursa) Fursa	*C. mucosospermus*; Fursa provided a Latin description and cited "*C. vulgaris* var. *senegalicus* Fursa 1962, Bot. Zhurn. 47: 1039" (invalid) as a synonym.
Citrullus lanatus var. *shami* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *variegatus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *virgatus* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus lanatus var. *viridis* (Chakrav.) Maheshw.	Probably *C. lanatus* nom. cons.
Citrullus mucosospermus (Fursa) Fursa	***C. mucosospermus***
Citrullus mucosospermus var. *senegalicus* (Fursa) Fursa	*C. mucosospermus*; see under *C. lanatus* var. *senegalicus* (Fursa) Fursa and *C. vulgaris* var. *senegalicus* Fursa
Citrullus naudinianus (Sond.) Hook.f.	***C. naudinianus***
Citrullus pasteca Sageret	*C. amarus*
Citrullus pseudocolocynthis M.Roem.	*C. colocynthis*
Citrullus rehmii De Winter	***C. rehmii***
Citrullus variegatus Schrad. ex M.Roem.	*Cucurbita pepo* L.
Citrullus vulgaris Schrad.	SSR has photos of all Schrader material, but it is unclear which of many sheets might be the type of this name. Fursa (1972: 37) regarded this as a separate subspecies, *C. lanatus* subsp. *vulgaris* (Schrad.) Fursa.
"*Citrullus vulgaris* var. *caffrorum*" Alef.	Not validly published (no material, no clear link to a species)
"*Citrullus vulgaris* var. *colocynthoides*" Schweinf., nom. nud.	According to Art. 33.1 of the *Melbourne Code* this name is not validly published
Citrullus vulgaris var. *fistulosus* (Stocks) Duthie & Fuller	*Benincasa fistulosa* (Stocks) H.Schaef. & S.S.Renner[b]
Citrullus vulgaris var. *senegalicus* Fursa	*C. mucosospermus*; see under *C. lanatus* var. *senegalicus* and *C. vulgaris* var. *senegalicus*
Citrullus vulgaris var. *sudanicus* Filov & Fursa	*C. lanatus* nom. cons. subsp. *cordophanus* (see Fursa, 1971: 38)
Colocynthis amarissima Schrad.	*C. amarus* (see Fursa, 1972)
Colocynthis citrullus (L.) Kuntze	*C. lanatus* nom. cons.
Colocynthis citrullus Fritsch	*C. lanatus* nom. cons.
Colocynthis cocolobus Schrad.	Illegitimate, no protologue found
Colocynthis ecirrhosus (Cogn.) Chakrav.	*C. ecirrhosus*
Colocynthis naudinianus Kuntze	Illegitimate
Colocynthis officinalis Schrad.	Doubtful name, *C. colocynthis* fide Fursa (1972)
Colocynthis vulgaris Schrad.	*C. colocynthis*

A version of this table with the year of publication for each name is available from the first author.

a The name *Citrullus lanatus* has been conserved against *C. battich* Forssk. with a new type as proposed by Renner & al. (2014) to preserve current usage of the name as referring to the dessert watermelon.

b *Benincasa fistulosa* (Stocks) H.Schaef. & S.S.Renner (2011) ≡ *Citrullus fistulosus* Stocks in Hooker's J. Bot. Kew Gard. Misc. 3: 74. 1851 ≡ *Praecitrullus fistulosus* (Stocks) Pangalo in Bot. Zhurn. S.S.S.R. 29: 203. 1944.

not included in their study is *C. rehmii*, which was only described in 1990 (De Winter, 1990). Tatjana Borisovna Fursa is now in her 90s and still active at VIR in Saint Petersburg (L. Bagmet, pers. comm., 9 Jun 2017). Watermelon seeds collected by Schweinfurth near Khartoum (B180004823) look exactly like the seeds collected in Kurdufan by Ter-Avanesyan (1966) and like modern seeds from North and South Darfur, close to Kurdufan (Renner & Chomicki, unpub. data). If watermelon breeding started from *cordophanus*-like populations in Northeast Africa, farmers would "only" have had to select for increased sweetness and color of the pulp, while if it started with *mucosospermus*-like populations in West Africa, they would have first needed to get rid of the bitterness of the pulp and secondly switched from selecting on seed traits (mainly oiliness) to selecting for pulp traits.

Conclusions. — The need for an up-dated classification of *Citrullus* has often been stressed, most recently by Zhang & al. (2016), and the present assignment of 50 available names to seven biological species, along with morphological and ecological traits as well as a tabulation of chromosome counts should help towards that goal. The USDA's National Plant Germplasm System maintains a collection of 1800 *Citrullus* germplasms (see http://www.ars-grin.gov/npgs). Similarly, the Beijing Vegetable Research Center (BVRC) maintains 1197 accessions labeled as *Citrullus* (Zhang & al., 2016). The National Botanical Research Institute (NBRI), National Plant Genetic Resources Centre (Namibia) and the Zambia Agriculture Research Institute (ZARI) also store many *Citrullus* seed batches (McGregor, 2012). We believe that collaboration between researchers relying on these collections and herbarium taxonomists familiar with type specimens (which could easily be sequenced for informative loci) would lead to speedier progress in understanding of *Citrullus* evolution and domestication.

DEDICATION

This paper is dedicated to ***Tatjana Borisovna Fursa*** *(12 May 1928–), Saint Petersburg, Russian Federation, for her unparalleled work on species boundaries and relationships in Citrullus.*

Tatjana Borisovna Fursa in Astrakhan, 1977. Photograph provided by her daughter; received via e-mail from Irina Gashkova (VIR) on 21 June 2017.

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Appendix 1. Plant material included in this study (for a tabular version, see Electr. Suppl.: Table S1).

Taxon, authors, vouchers, their geographic origin, and GenBank accession numbers for ITS1+5.8S rRNA+ITS2, *trnL* intron, *trnL-F* spacer, *matK*, *rbcL*, *rpl20-rps12* spacer, *ndhF*, *trnS-trnG* spacer, *trnR-atpA* spacer, *Ycf9-trnG* spacer, *Ycf6-PsbM* spacer. TCN refers to The Cucurbit Network, Miami, Florida, which no longer exists, but gave their herbarium vouchers to FTG. Grif refers to the Plant Genetic Resources Conservation Unit, Griffin, Georgia; numbers preceded by PI refer to the USDA National Plant Germplasm System, see http://www.ars-grin.gov/npgs/; numbers preceded by NAM refer to the collection of the National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Windhoek, Namibia; BG refers to botanical gardens. GenBank numbers beginning with KY68 were newly generated for this study.

Acanthosicyos horridus Welw. ex Hook.f., *E. van Jaarsveld s.n.* (PRE), S Angola, Namibia, N Cape region, Cult. Kirstenbosch BG, KP036525, KP036536, DQ536630, DQ535778, KM281395, –, KM281387, –, –, –; ***Apodanthera sagittifolia*** (Griseb.) Mart.Crov., *B. Schlumpberger BOS 317/I* (M), Argentina, Cordoba, HQ201962, EU436346, EU436398, EU436374, EU436320, –, –, –, –, –; ***Benincasa fistulosa*** (Stocks) H.Schaef. & S.S.Renner, [a]*D. Decker-Walters 883* (FTG) / [b]PI 540917, [a]India / [b]India or Sri Lanka, –, [a]DQ536851, [a]DQ536719, [a]DQ535837, [a]DQ648185, [b]AY527173, [b]AY693772, [b]AY522507, [b]AY522525, [b]AY507989; ***Benincasa hispida*** (Thunb.) Cogn., [a]*M.A. Ali & A.K. Pandey 1001* (BHAG) / [b]*S.S. Renner & al. 2760* (M), [a]India / [b]Cultivated Mainz BG, [a]GQ183037, [b]DQ536789, [b]DQ536636, [b]DQ535784, [b]DQ536619, –, –, –, –, –; ***Borneosicyos simplex*** W.J.de Wilde, *SAN (Postar & al.) 144251* (L), Borneo, –, DQ535877, DQ536638, DQ535785, DQ536620, –, –, –, –, –; ***Cephalopentandra ecirrhosa*** (Cogn.) C.Jeffrey, [a]*M.G. Gilbert & B.M.G. Jones 112* (K) / [b]*M.W. Chase 929* (K), [a]Ethiopia / [b]Kenya, Kechilu Pass, [a]HQ201965, [b]DQ536794, [b]DQ536645, [b]AF534744, [b]DQ648160, –, –, –, –, –; ***Citrullus amarus*** Schrad. (***Momordica lanata*** Thunb., **holotype**), *C.P. Thunberg s.n.*, year 1773 (UPS), South Africa, –, –, –, –, KM281400, –, KM281391, –, –, –; ***Citrullus amarus***, *G. Chomicki 2* (M), Cult., Elne, France, KP036524, KP036537, –, –, KM281396, –, KM281393, –, –, –; ***Citrullus amarus*** (= ***C. caffer*** Schrad., **isolectotype**), *H.A. Schrader s.n.*, year 1838 (GOET), Namibia (cultivated in Göttingen), KP036526, KP036538, –, –, KM281397, –, KM281388, –, –, –; ***Citrullus amarus***, NAM 1612, Namibia, –, –, –, –, –, AY527190, DQ171962, AY522518, AY522536, AY508000; ***Citrullus amarus***? Unusual, sequences not typical *amarus*, PI 632751 (M), used in Li & al. (2016), Zhang & al. (2016), Namibia, KY613612, KY613618, –, –, KY613621, KY613624, KY613616, –, –, –; ***Citrullus amarus*** cf., *H.S. Paris s.n.* (M), Paqqu'a 12, Israel, near Ramat Yishay, –, –, –, –, –, –, KY613615, –, –, –; ***Citrullus colocynthis*** (L.) Schrad., [a]PI 195927, F. Dane, Auburn University / [b]*H. Schaefer s.n.* (voucher destroyed), [a]Ethiopia / [b]Portugal, [a]KP036527, [a]KP036539, [b]HM850817, [b]HM849900, [a]KM281398, [a]AY527184, –, –, –, –; ***Citrullus colocynthis***, *D. Podlech 19980* (M), Afghanistan, Prov. Nangahar, 8 km from Ghaziabad, KY613613, KY613619, –, –, KY613622, –, –, –, –, –; ***Citrullus colocynthis***, [a]TCN 955 (FTG) / [b]PI 195927, [a]Morocco / [b]Ethiopia, –, –, –, –, –, –, [a]AY693766, [b]AY522509, [b]AY522527, [b]AY507991; ***Citrullus colocynthis***, PI 525081, Egypt, Luxor, –, –, –, –, –, –, –, AY693744 (Dane & al., 2007), –, AY693729 (Dane & al., 2007); ***Citrullus colocynthis***, PI 525082, Egypt, Luxor, –, –, –, –, –, –, –, AY693745 (Dane & Luo, 2004), –, AY693730 (Dane & al., 2007); ***Citrullus colocynthis*** (***C. vulgaris*** var. **'*colocynthoides*'** nom. nud.), *G. Schweinfurth s.n.*, year 1882 (B 180004824), Egypt, btw. Asyut and Assuan, KY681091, KY681096, –, –, KY681095, KY681097, KY681093, –, –, –; ***Citrullus ecirrhosus*** Cogn., [a]Grif 16056 (M) = *S.S. Renner 2855* (M) / [b]NAM 956, [a]Namibia / [b]Namibia, [a]KP036528, [a]KP036540, [a]KP036546, [a]KP036548, [a]KM281394, [b]AY527189, [a]KP036535, [b]AY522515, [b]AY522533, [b]AY507997; ***Citrullus lanatus*** (Thunb.) Matsum. & Nakai, nom. cons., *E.G. Achigan-Dako 06NIA224* (GAT), Mali (Segou), KP036529, KP036541, –, –, KM281401, –, KM281384, –, –, –; ***Citrullus lanatus***, **neotype**, *S.S. Renner 2816* (M), Cult. Saint Louis, Missouri, U.S.A., KP036530, KP036542, DQ536650, –, KM281402, –, KM281389, –, –, –; ***Citrullus lanatus***, *M. Abd-el-Ghani s.n.*, year 1978 (M), no information on fruits, Egypt, Oasis Bahariya, KY613614, KY613620, –, –, KY613623, –, –, –, –, –; ***Citrullus lanatus***, *G. Schweinfurth s.n.*, year 1869 (B 180004823), Sudan, S of Khartoum, White Nile, KY681092, –, –, –, –, –, KY681094, –, –, –; ***Citrullus mucosospermus*** (Fursa) Fursa, *E.G. Achigan-Dako 819AAFG32* (GAT), Benin, KP036531, KP036543, –, –, KM281403, –, KM281390, –, –, –; ***Citrullus mucosospermus***, **holotype**, *N.P. Oltarshevskyi 3833* (VIR), Ghana, 5 Aug 1957, –, –, –, –, –, –, KP058579, –, –, –; ***Citrullus mucosospermus***, Seeds received from Vavilov Research Institute (VIR) and cultivated in Gatersleben as U 1311/93 = CIT 204 (GAT), Benin, KP036532, KP036544, –, –, KM281404, –, KM281386, –, –, –; ***Citrullus naudinianus*** (Sond.) Hook.f. (≡ ***Acanthosicyos naudinianus*** (Sond.) C.Jeffrey), Grif 14031 (M), Namibia, Kalahari, KT757533, –, –, –, –, –, –, –, –, –; ***Citrullus naudinianus***, *I. Urschler s.n.*, year 1967 (M), Namibia, Windhoek, –, EU436347, EU436399, –, EU436321, –, –, –, –, –; ***Citrullus rehmii*** De Winter, [a]Grif 16374 (M) / [b]NAM 1303, [a]Namibia / [b]Namibia, Windhoek, [a]KP036533, [a]KP036545, [a]KP036547, [a]KP036549, [a]KM281405, [b]AY527192, [b]AY693771, [b]AY522523, [b]AY522541, [b]AY508005; ***Coccinia rehmannii*** Cogn., *S.S. Renner & A. Kocyan 2749* (M), Southern Africa, Swaziland, Cult. in Munich BG, HQ608218, DQ536799, DQ536652, –, HQ625508, –, HQ608479, –, –, –; ***Coccinia senensis*** (Klotzsch) Cogn., *K. Vollesen MRC4316* (WAG), Tanzania, Lindi, HQ608219, HQ608425, HQ608267, –, HQ608362, –, HQ608487, –, –, –; ***Ctenolepis cerasiformis*** (Stocks) C.B.Clarke, [a]*E.G. Achigan-Dako 06NIA222* (GAT) / [b]Cult. *M. Wilkins 279*, [a]Mali, Segou, Bla / [b]Zimbabwe, [a]AM981142, [b]DQ536803, [b]DQ536656, [b]DQ535797, [b]DQ648164, –, –, –, –, –; ***Cucumis maderaspatanus*** L., [a]*S. Siddarthan s.n.* (M) / [b]*J. Maxwell 02-434* (CMU), [a]India, Tamil Nadu / [b]Thailand, [a]HM596915, [b]DQ536843, [b]DQ536705, [b]DQ535761, [b]DQ648182, –, [a]HM597085, –, –, –; ***Cucumis oreosyce*** H.Schaef., [a]*H. Schaefer 05/450* (M) / [b]*E. Phillips 2821* (Z) /, [a]Tanzania, Usambara Mts / [b]Malawi, [a]EF093528, [b]DQ536845, [b]DQ536711, [b]DQ535833, [b]DQ536576, –, [b]HM597090, –, –, –; ***Dactyliandra welwitschii*** Hook.f., *W. Giess 3664* (M), SW Africa, HQ201973, DQ536810, DQ536669, DQ535750, DQ536545, –, –, –, –, –; ***Diplocyclos palmatus*** (L.) C.Jeffrey, *J. Maxwell s.n.*, 2 Sep 2002 (CMU), Thailand, Chiang Mai, –, DQ536769, DQ536671, –, DQ536625, –, HQ608495, –, –, –; ***Diplocyclos schliebenii*** (Harms) C.Jeffrey, *H.J. Schlieben 4363* (M), Tanzania, Kilimanjaro, HQ608223, HQ608427, –, –, –, –, HQ608496, –, –, –; ***Lagenaria breviflora*** (Benth.) Roberty, [a]*E.G. Achigan-Dako 06NIA009* (GAT) / [b]*M. Merello & al. 1331* (MO) / [c]TCN 1524 (FTG), [a]Ghana, Winneba, Apam / [b]Ghana / [c]Zimbabwe, [a]AM981086, [b]AY935788, [b]AY935934, [b]AY935747, [b]AY973020, [b]AY968505, –, [c]AY437538, –, –; ***Lagenaria siceraria*** (Molina) Standl., [a]Fujian Academy of Agricultural Sciences H30 / [b]Cult. Missouri BG, 2002 / [c]S. Lewthwaite, Institute for Crop and Food Research, Pukekohe, New Zealand / [d]TCN 1367 (FTG), [a]Unknown / [b]Cult. U.S.A. / [c]Unknown / [d]Chile, [a]FJ951163, [b]DQ536771, [b]DQ536694, [b]DQ535825, [b]DQ536627, –, [c]DQ282111, dAY437553, –, –; ***Muellerargia jeffreyana*** Keraudren, **holotype,** *G. Cours 5586* (P), Madagascar, –, EU436361, EU436411, EU436387, EU436337, –, –, –, –, –; ***Muellerargia timorensis*** Cogn., [a]*D.L. Jones 3666* (NE) / [b]*I.R. Telford & P. Sebastian 13307* (M), Australia, Queensland, [a]EF093525, [a]DQ536842, [a]DQ536704, –, [a]DQ536571, –, [b]HM597130, –, –, –; ***Peponium caledonicum*** (Sond.) Engl., Seeds leg. *M. Wilkins 405* in Namibia, Cult. in Tuscon, Texas, U.S.A., –, DQ536774, DQ536714, DQ535765, DQ536579, –, –, –, –, –; ***Peponium vogelii*** (Hook.f.) Engl., [a]*S.S. Renner 2704* (MO) / [b]*S.S. Renner 2710* (MO) / [c]*S.S. Renner 2722* (LE), [a]Tanzania / [b]Tanzania / [c]Tanzania, [a]KP036534, [b]HQ608428, [b]HQ608272, [c]DQ535835, [b]HQ608367, –, [b]HQ608497, –, –, –; ***Raphidiocystis phyllocalyx*** C.Jeffrey & Keraudren, *M.G. Gilbert 2162* (M), Congo, Stanleyville / (Kisangani), –, DQ536855, –, DQ535839, DQ536583, –, –, –, –, –; ***Solena heterophylla*** Lour., [a]*A. Kocyan & al. AK191* (BKF) / [b]*M.A. Ali & A.K. Pandey 1094* (BHAG), [a]Thailand, Chang Mai / [b]South India, [b]GQ183047, [a]DQ536870, [a]DQ536737, [a]DQ535851, [a]DQ536598, –, –, –, –, –; ***Trochomeria macrocarpa*** (Sond.) Harv., [a]*W. Giess 13286* (M) / [b]*E.G. Achigan-Dako 06NIA158* (GAT), [a]Namibia, Taebis farm / [b]Burkina Faso, Ouagadougou, [b]AM981141, [a]DQ536877, [a]DQ536745, [a]DQ535858, [a]DQ536606, –, –, –, –, –