

Cucumis bisexualis, Buried in the Taxonomic Wastebasket *C. melo* subsp. *agrestis*, is the Correct Name for Mapao Egg, Hermaphrodite Line Y101, and Weedy Melon UT1, and Helps Focus on Trait Evolution and Domestication of *C. melo*

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This is a continuation of efforts to clarify the taxonomy of *Cucumis*, which despite receiving a lot of attention from plant breeders and molecular-developmental biologists is lacking modern taxonomic treatments (Renner et al., 2007; Sebastian et al., 2010; Schaefer and Renner, 2021). The only complete treatment of the genus, Kirkbride's (1993) monograph, is greatly outdated, covering only 32 of the 65 species currently recognized in *Cucumis* (<https://cucurbit.de/calycophysum/cucumis/>). Five more species are in the process of being described (H. Schaefer, Technical University of Munich, pers. comm. 22 Jan. 2024).

Kirkbride had a rather broad concept of *Cucumis melo*, a taxon name under which he synonymized 522 names published by earlier workers, creating what might be called a taxonomic wastebasket. To gain some control over the mass of variation, he subdivided the thousands of collections into two clusters, subspecies *melo* and subspecies *agrestis*, based on ovary pubescence. As shown with molecular characters, these two clusters are unnatural, with accessions instead clustering by geographic origin and not ovary pubescence or their wild/cultivated origin (Endl et al., 2018).

Among the hundreds of names that Kirkbride (1993) synonymized under subspecies *agrestis* is *Cucumis bisexualis* A.-M. Lu & G.C. Wang published in 1984. The type collection consists of two plants collected in June 1979 by Gui-Chen Wang #01 and labelled as "Introduced from Ningyang County, Shandong Province, now cultivated in vegetable fields in Beijing. Growing alongside fields." The two plants are mounted on two sheets with identical labels in Chinese and with annotation labels indicating that Charles Jeffery identified the material as *Cucumis melo* var. *agrestis* on 3 Apr. 1980 (one of the two annotation slips is in his own handwriting) and that An-Min Lu and Zhi-Yun Zhang identified the material as *Cucumis bisexualis* A.-M. Lu & G.C. Wang on 8 May 1983. The sheets have been digitized by the Beijing herbarium with the unique identifiers PE01178335 and PE01178336. Neither is annotated as the holotype, but

PE01178335 bears a cellophane envelope with two black-and-white photos of fruit cross sections (Fig. 1), which are also visible through the cellophane. This specimen is presumably the holotype.

The original species description is in Latin and states that the species differs from *Cucumis melo* by its consistently bisexual flowers and smaller fruits, 3-3.5 cm long and 2-3 cm in diameter. The first author of the description, Professor Lu An-Min (1939-) from the Beijing Institute of Botany, worked on the Cucurbitaceae for the *Flora of China* (Lu and Zhang, 1986), which therefore includes *C. bisexualis* as a distinct species. In the mid-1980s, Lu visited Charles Jeffrey (1934 - 2022), the best western expert on the cucurbits, then working at the Kew herbarium (Renner and Hind, 2022). Lu and Jeffrey collaborated for many years on the English-language treatment of the Chinese Cucurbitaceae (Lu and Jeffrey, 2011) in which they synonymized *C. bisexualis* under *C. melo* subsp. *melo*, a surprising decision given that Lu himself had described the species as distinct from *C. melo* and given that Jeffrey in 1980 had annotated the Beijing type collection as var. *agrestis*.

Cucumis bisexualis is of interest biologically because of its bisexual flowers and in terms of the light it may shed on the history of domestication of melons. While the two type collections were made in 1979 alongside agricultural fields in Beijing, their labels state that the species was introduced from Ningyang County, which is located about 530 km south of Beijing. The natural range of *C. bisexualis* is not entirely clear but the species is widespread in the Yellow River delta, where its seeds germinate in low-salinity conditions (Zhang et al., 2011). Other studies that have accepted the taxon as a good species have focused on the coumarin-rich fruits, which go by the common name 'mapao egg' or 'muskmelon egg' (Ma et al., 2018, 2020). These studies state that the species is mainly distributed in the eastern Chinese provinces Henan, Shandong, Anhui, and Jiangsu.

Archaeobotanical and molecular evidence suggests that melon domestication occurred independently in Northeast

Africa, in India, and perhaps a third time in the lower Yangtze region of China (Fuller, 2006, 2012; Renner and Schaefer, 2011; Fuller et al., 2014; Endl et al., 2018). The archaeobotanical evidence consist of the seeds, which prove the presences of melons at a particular site and time, and the increase of seed size during the domestication process, since seed size correlates allometrically with fruit size and the seeds themselves might also have been used as a snack or for oil extraction. Figure 2 shows the seeds of modern *C. bisexualis* next to seeds of domesticated *C. melo* and seeds of the wild African *C. melo* subsp. *melooides*, one of the many names misplaced in the wastebasket “*agrestis*.” It is clear that *C. bisexualis* has much smaller seeds than wild “*agrestis*.”

Small seed size is the reason that at least some of the many *Cucumis* seeds reported from archaeobotanical sites in China (dating to 6000-4000 BP) have been identified as *Cucumis bisexualis* (Zheng and Chen, 2006). Molecular data are needed to confirm such morphology-based identifications of ancient seeds and also to assess a possible role of *C. bisexualis* in the domestication of *C. melo* in China.

Cucumis bisexualis provides an excellent opportunity to study the control of fruit flesh thickness and stamen and pistil development in *Cucumis* (Fig. 3). Two mapao melon accessions, both classified as var. *agrestis*, are maintained by the National Mid-Term Genebank for Watermelon and Melon, China as ‘x207’ and ‘1114wd’. Liu et al. (2020) re-sequenced their genomes and suggested that mapao melon might be a feral form of cultivated melon, which would imply a dramatic reversal in seed size (Fig. 1) as well as the evolution of bisexual flowers from the monocious condition.

The first reference genome of mapao melon was produced by Lyu et al. (2023), using HiFi long reads and high-throughput chromosome conformation capture (Hi-C) technologies. Lyu and colleagues discovered a super long sequence absence on Chromosome 4 in mapao compared to eight other forms of *C. melo* for which genome assemblies are currently available, including two Chinese germplasms classified as “*agrestis*” (‘HS’ and ‘IVF77’).

Two other studies have focused on the genetic control of the male and female organs in *C. bisexualis* flowers. The first of these, by Wang et al. (2022), applied genome re-sequencing to the hermaphrodite line Y101 obtained from the Laboratory of Cucurbits Germplasm Innovation and Genetic Improvement, Northwest A & F University, Yangling, Shaanxi, China, where unsurprisingly it is classified as “*agrestis*.” The paper’s photos of the flowers, as well as the description of the thin-skinned small fruits, unambiguously identify Y101 as *C. bisexualis*. The second study, by Nashiki et al. (2023), applied re-sequencing to ‘Japanese weedy melon (UT1)’ collected from an island in the Seto Inland Sea, which the authors classify as “*agrestis*.” The plants bear exclusively bisexual

flowers on the main stem and lateral branches, and the paper’s color photo of the plant habit and flowers (Fig. 3b) clearly shows *C. bisexualis*. A polymorphism discovered in UT1 was consistent with that of the bisexual line Y101 (Wang et al., 2022), further confirming the identity of Y101 and UT1.

In conclusion, *Cucumis bisexualis*, described by Lu An-Min in 1984 is the correct, and by chance also informative, name – the species’ flowers are indeed always bisexual – for mapao egg, hermaphrodite line Y101, and weedy melon UT1. Using this name would help bring together insights on trait evolution and domestication of *C. melo*.

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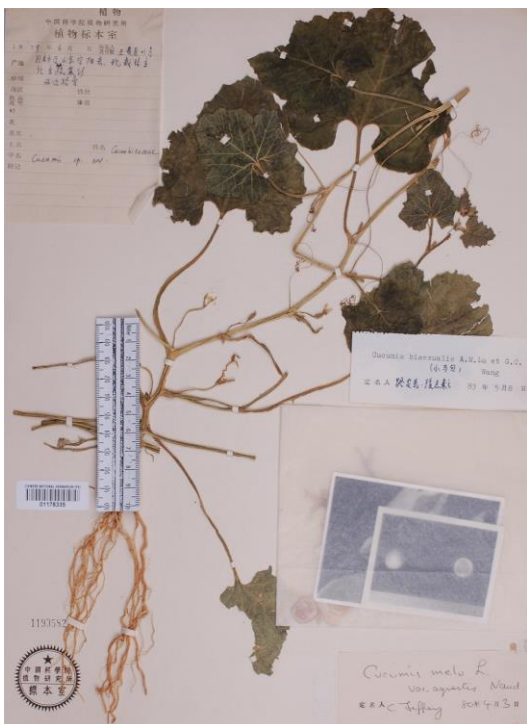


Figure 1. The type specimen of *Cucumis bisexualis* deposited in the Beijing herbarium (acronym PE), China.



Figure 2. Seeds of *Cucumis bisexualis* in cellophane next to seeds of (A) domesticated *Cucumis melo* and (B) *Cucumis melo* subsp. *meloides* from Africa (“agrestis”). Photo credits: A) P. Renner, Jan. 2023; B) H. Schaefer, Feb. 2023



Figure 3. Fruits and habit of *Cucumis bisexualis*. (A) Fruits on the hand of Luo Shi-Xiao; (B) habit and flower of Japanese weedy melo UT1. Photo credits: A) S-X. Luo, 6 Nov. 2022; B) Kindly provided by Yosuke Yoshioka.