

## Chapter 4

# Citrus taxonomy

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## 4.1 The genus *Citrus* definition

### 4.1.1 The botanical treatment of the genus *Citrus*

Taxonomists now agree that the *Citrus* L. genus is part of the Sapindales Berchtold and J. Presl, order in the Rutaceae Jussieu family (Stevens, 2017; NCBI, 2017), while it was before included in the Geraniales order (Swingle and Reece, 1967). The family Rutaceae, whose name comes from the genus *Ruta* L., includes herbaceous and woody plants with essential oil glands. According to Swingle and Reece (1967), *Citrus* belongs to the subfamily Aurantioideae, which is divided into two subtribes: the Clauseneae (5 genera) and the Citreae (28 genera). The Clauseneae tribe is considered more primitive than the Citreae tribe. Citreae are divided into three subtribes: Triphasiinae, Balsamocitrinae, and Citrinae. Swingle and Reece (1967) subdivided the Citrinae into three groups including the “true citrus” one composed of six genera: *Citrus*, *Clymenia*, *Eremocitrus*, *Fortunella*, *Microcitrus*, and *Poncirus*. Chloroplast molecular studies (Morton et al., 2003; Bayer et al., 2009) confirmed the monophyly of the Aurantioideae. However, the study by Bayer et al. (2009) based on nine cp-DNA gene regions, suggested nonmonophyly for several subtribes and resulted in proposals for revision of the Swingle and Reece (1967) Aurantioideae classification, even though some clade remained poorly resolved. In the future, whole chloroplast genome sequencing data of Aurantioideae members should better resolve their phylogeny and, therefore, should provide the key to a definitive classification of tribes and subtribes.

### 4.1.2 Phenotypical traits of the true *Citrus*

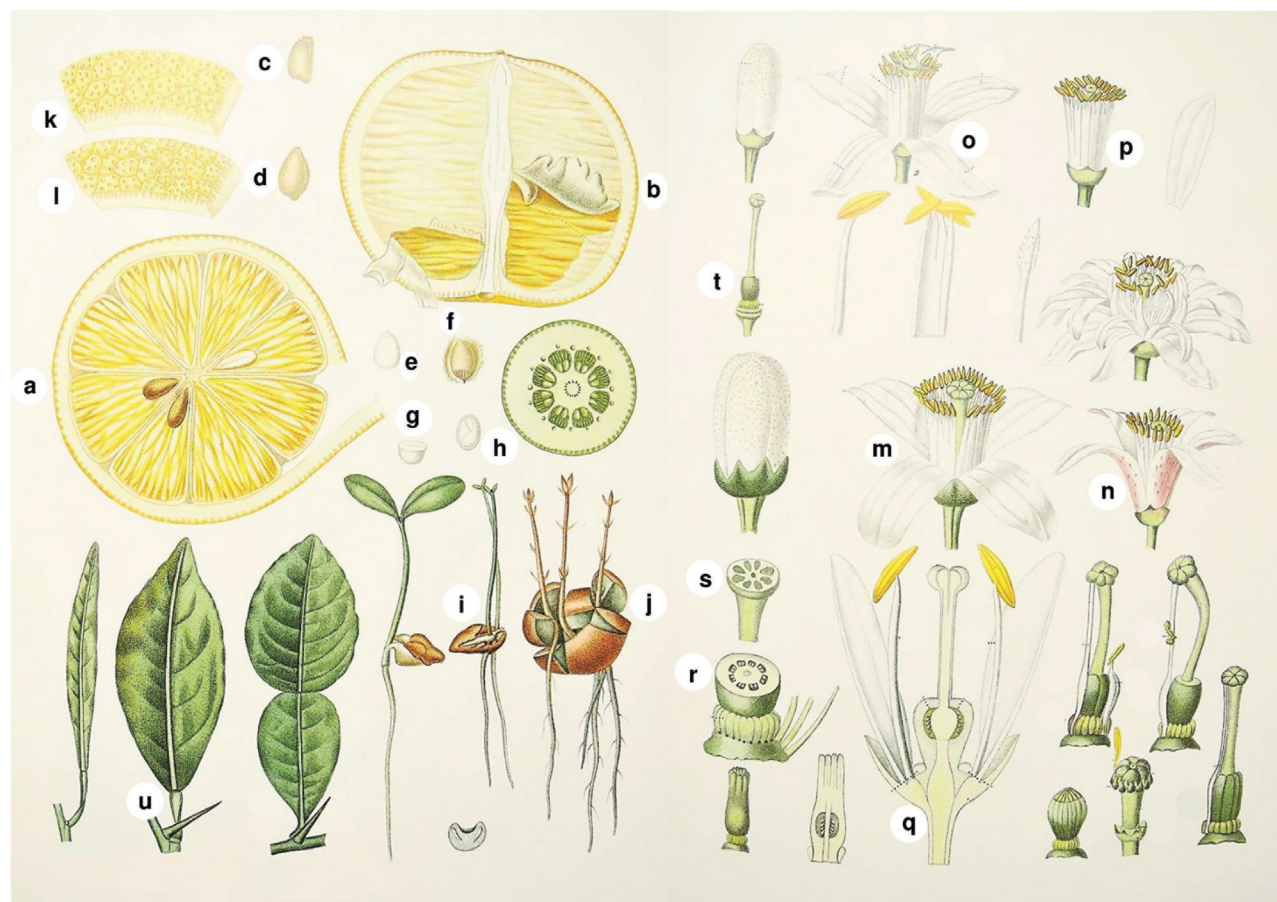
Fruits of *Citrus* are apparently some of the first to be domesticated and exploited by humans. The center of origin and diversity for *Citrus* is southeastern Asia (Chapter 2), particularly northeast India, Myanmar, and southern China (Tolkowsky, 1938; Tanaka, 1954). In those areas, citrus were apparently exploited and consumed during ancient times (Chapter 3), later spreading into the Middle East, Europe, and ultimately the Western Hemisphere (Scora, 1975; Webber, 1943, 1967). Ramon-Laca (2003) and Mabberley (2004) rather interestingly trace the movement of citrus from the center of origin and diversity through the Middle East and into Europe by tracing the etymology of the names of the cultivated citrus. The various peoples and languages through which the several *Citrus* species passed undoubtedly recognized their similarities in the form of fruits and trees. This is supported by the fact that Linnaeus (Linnaeus, 1753) combined the previously named *Aurantium* (orange), *Citreum* (citron), and *Limon* (lemon) into the genus *Citrus*, a name previously applied to an entirely different and unrelated species, *Tetraclinis articulata* (Vahl) Mast. (Mabberley, 2004). This author recognized the importance of Swingle (1943) in making sense of some of the taxonomic confusion within the Aurantioideae, while noting that many taxonomic issues still need clarifications.

#### 4.1.2.1 Morphological characteristics of the genus *Citrus* (sensu Swingle)

The classification proposed by Swingle in (1943) was only slightly modified in Swingle and Reece (1967); henceforth, reference will be made to Swingle (1943) with the understanding that the same information is available in the more easily accessed Swingle and Reece (1967). Thus, we may develop a working botanical description of *Citrus* by studying and interweaving the description of Swingle (1943) and the more recent and shorter one of Zhang and Mabberley (2008).

The fruits of *Citrus* are berries, that is, fleshy, indehiscent, many-seeded fruits containing no hard parts except the seeds (Fig. 4.1A; Chapter 12). More specifically, *Citrus* fruits are hesperidia, in which the fleshy parts of the fruit are divided into segments and are surrounded by a separable skin (Fig. 4.1A and B). Hesperidia are confined to the fruits of Aurantioideae (Webber, 1943). The obovoid or flattened seeds (Fig. 4.1C and D) are attached adaxially (near the central axis or core, Fig. 4.1A), have smooth or ridged seed coats, and contain one to many embryos (Fig. 4.1E–J). The segments are filled with stalked fusiform pulp vesicles, which contain very watery, large-celled tissue (Fig. 4.1A and B); this is the economic part of the fruit. The segments are surrounded by a white endocarp, outside of which is the peel, which contains numerous oil glands (Fig. 4.1A, K, and L). The peel is generally green during the early stages of fruit development and turns yellow or orange at maturity. The fruit arises from the fragrant flowers, which are borne singly or in small racemes in the axils of the leaves. The flowers of *Citrus* are perfect or staminate, the latter condition being due to abortion of the pistil. The calyx is cup shaped with three to five lobes, and is subglabrous. There are four to eight petals (usually five), which are white (Fig. 4.1M) or pink (Fig. 4.1N) outside, imbricate, and thick. There are usually four times as many free or basally coherent stamens as petals (Fig. 4.1M, O, and P), although there may be up to 10 times as many. The disk is annular or short, with nectary glands. The ovary contains 3–18 locules (generally 10–14), each of which contains two to eight ovaries in two collateral rows (Fig. 4.1Q–S). The style is large and cylindrical, expanding abruptly into the subglobose or oblate spheroid stigma (Fig. 4.1T).

Members of *Citrus* are evergreen shrubs or small trees, generally 3–10 m in height. Young branches are often flat and angled, becoming cylindrical with age, usually with solitary (rarely paired) spines at the axils. Leaves are generally unifoliate, with petioles that are usually articulated at the base of the blade and conspicuously winged (Fig. 4.1U). The leaf blade is subleathery to leathery with crenulate (rarely entire) margins, and contains numerous fragrant oil glands.



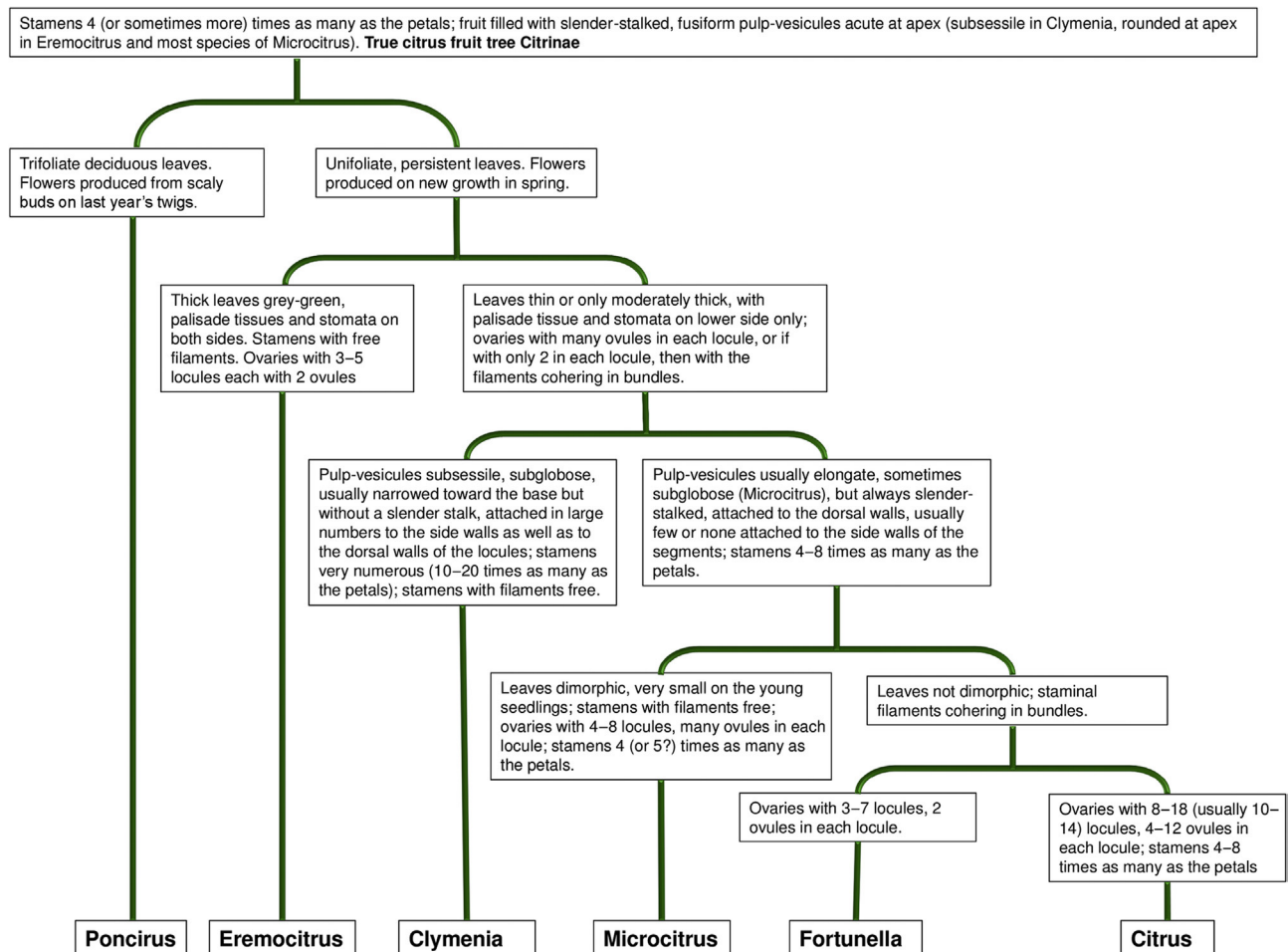
**FIG. 4.1** Botanic traits of the *Citrus* species. A: cross-section through a citrus fruit, B: longitudinal section through a citrus fruit; C: semi-deltoid citrus seed; D: obovoid citrus seed; E: longitudinal section through a citrus seed; F: citrus seed with seed coats, G: cross-section through a citrus seed; H: polyembryonic citrus seed; I and J: polyembryonic citrus seedling; K and L: outside citrus peel section with oil glands; M: open citrus flower; N: open lemon flower; O: open orange flower; P: citrus flower stamens; Q: longitudinal section through a citrus flower; R and S: cross-section through a citrus ovary; T: pistil (ovary, style and stigma) of a citrus flower; U: unifoliate citrus leaves. Modified from “*Histoire Naturelle des orangers*” (Risso A, Poiteau A. *Histoire Naturelle des Orangers*. Paris: Imprimerie de Mme Hérisant Le Doux, Imprimeur ordinaire du Roi et des Musées Royaux; 1818).

#### 4.1.2.2 Traits of the related species of the true citrus

Although recent taxonomic treatments, such as [Zhang and Mabberley \(2008\)](#), have synonymized the corresponding genera with *Citrus*, they are described in this section as distinct genera following [Swingle \(1943\)](#). The true citrus fruit trees, including *Citrus*, share certain characteristics but are clearly differentiated according to the morphological taxonomic key of [Swingle \(1943\)](#), as presented in [Fig. 4.2](#). The fruit generally resembles those described above in the botanical description of *Citrus*. The pulp vesicles contain droplets of oil, which are more abundant in *Poncirus*, *Microcitrus*, and the papedas. The vesicles differ from those of other members of Aurantioideae in that they narrow into slender stalks with a point at the apex, except for those in *Clymenia*, which has pyriform vesicles. The fruits of the true citrus fruit trees are segmented and the fruit of the genera other than *Citrus* is smaller than those of *Citrus* itself. *Fortunella* and *Eremocitrus* have ovaries with three to five locules, each of which has only two ovules, whereas *Citrus*, *Microcitrus*, and *Poncirus* have ovaries with six to eight locules, each of which contains many ovules. Members of the true citrus fruit trees are generally cross and graft compatible with other members of the group ([Krueger and Navarro, 2007](#); [Siebert, 2016](#); [Siebert et al., 2015](#)).

*Fortunella* (Kumquat) closely resembles *Citrus* ([Swingle, 1943](#)), but (in addition to the distinctions described above) has a much larger stigma containing a few large oil glands; pale green abaxial leaf surfaces; and small, angular flower buds. The trees, leaves, flowers, and fruits are generally smaller than those of *Citrus*. The flowers are 1.0–1.5 cm diameter. The fruit is 1.5–2.5 cm in diameter, round to ovoid-ellipsoid, with a peel that is orange and sweet at maturity and acidic flesh. Kumquats are adapted to climates that are marginally cool for most other Aurantioideae, they require less heat to achieve fruit maturity and have a certain level of winter dormancy ([Swingle, 1943](#)).

*Eremocitrus* and *Microcitrus* are both endemic to the Oceania region. Both differ from *Citrus* in having dimorphic foliage and free stamens; however, *Microcitrus* has an ovary with four to eight locules, whereas *Eremocitrus* has an ovary with three to five locules. Both have rather small, coriaceous leaves; however, the leaves of *Eremocitrus* are thick and



**FIG. 4.2** Key to the genera of the true citrus fruit trees of the subtribe Citrinae according to [Swingle and Reece \(1967\)](#).



have a thick palisade layer in the cuticle with stomata on the upper and lower leaf surface. The subglobose or obovoid fruit of the monotypic *Eremocitrus* is small ( $0.7\text{--}1.2 \times 0.8\text{--}1.0\text{ cm}$ ) and is yellow at maturity, whereas fruit of *Microcitrus* is larger ( $4\text{--}5 \times 6\text{--}7\text{ cm}$ ), more variable in form (globose-ovoid or cylindrical, sometimes curved), and varies in color at maturity from greenish-yellow to black. Swingle (1943) describes the xerophytic adaptation of *Eremocitrus*, noting the thick cuticle and this genus's ability to withstand prolonged droughts and extremes of heat and cold (as compared to other Aurantioideae). The cold hardiness of *Eremocitrus* stated in Swingle (1943) and Swingle and Reece (1967) is in error; *Eremocitrus* can probably tolerate temperatures to about  $-5.5^\circ\text{C}$ , consistent with the original description of the genus in 1914 (Krueger and Navarro, 2007; Swingle, 1914). *Microcitrus*, on the other hand, is considered semixerophytic and able to withstand prolonged periods of drought (Swingle and Reece, 1967; Swingle, 1943). *Eremocitrus* shows some unusual graft relationships (Siebert, 2016; Siebert et al., 2015).

Trifoliolate orange was for many years considered a mono-typic genus, represented by *Poncirus trifoliata* (Swingle, 1943), with distinctive trifoliolate leaves (unique among the true citrus fruit trees) and deciduous growth habit. The small leaf buds and larger-scale-covered flower buds form in the summer and over winter on leafless terminal twigs, flowering the following spring. This gives to trifoliolate oranges the highest degree of cold hardiness among the true citrus fruit trees, surpassing that of kumquats. *Poncirus* flowers are nearly sessile, with petals that open flat, entirely free stamens, and an ovary with six to eight locules. The fruit is smaller than those of *Citrus* ( $3\text{--}5\text{ cm}$  diameter), densely and finely pubescent, with many oil glands, and is very seedy. The adaptation of *Poncirus* to cold conditions led Swingle (1943) to speculate that the remote ancestor of the true citrus fruit trees originated in a tropical or semitropical climate. While the other genera of the true citrus fruit trees remained in these climates, *Poncirus* (or its ancestors) "migrated" to the temperate climate of Northeastern Asia, during which time it developed the adaptations to colder winters mentioned previously. In addition to cold tolerance, *Poncirus* exhibits many other characteristics that have been and continues to be used in citrus rootstock breeding, notably disease tolerance (including citrus tristeza virus immunity) and dwarfing. For a more complete treatment of *Poncirus*, the reader is referred to Krueger and Navarro (2007). Relatively recently, a new species, *Poncirus polyandra*, was published (Ding et al., 1984; Duan, 1990), which differs from *P. trifoliata* by its larger leaves, some floral differences, and, most notably, being evergreen. Perhaps, this latter characteristic is related to its habitat in Yunnan, the southernmost province in China.

*Clymenia* is a very distinctive member of the other true citrus fruit trees. *Clymenia* was separated from *Citrus* by Swingle (1943) based upon the structure of the pulp vesicles, which are short, plump, blunt, ovoid or subglobose, sessile, or very short stalked, and attached to the side walls of the 14–16 locules. In addition, the leaves of *Clymenia* differ from those of the other true citrus fruit trees, and the flowers have enlarged disks with 10–20 times as many stamens as petals. The fruits resemble sweet limes and are edible.

As we will discuss below, a chloroplastic phylogeny (Bayer et al., 2009) integrated *Oxanthera* into the true citrus phylogenetic cluster and, therefore, we will describe it here. According to Swingle and Reece (1967), *Oxanthera* is a very distinct group in having "glabrous, glaucous leaves bluntly rounded or retuse at the tip and cuneate at the base, borne on spineless twigs. The leaves are thick and coriaceous in all the species except one. All the *Oxanthera* types agree in having large, orange-like flowers, and glabrous, more or less glaucous leaves, which usually are rather blunt at the tip and cuneate at the base. All species are thornless, and apparently all have more or less elongate fruits that are longitudinally ribbed at least when young." Therefore, the *Oxanthera* group is easily differentiated from other taxa even though *Oxanthera* flowers are similar to those of cultivated *Citrus* species. However, this group displays an unusual range of variation for characters having high taxonomic value in other plant species. According to Swingle and Reece (1967): "Three of the four species of *Oxanthera* have unifoliolate leaves with clearly articulated petioles that are usually wingless, but are plainly but narrowly winged in one species. One of the species has a hypomerous ovary with only two locules, another has an isomerous ovary with five or six locules, whereas a third species has a hypermerous ovary with seven locules." *Oxanthera* is a specialized xerophytic group like *Microcitrus* but less so than *Eremocitrus* (Swingle and Reece, 1967).

### 4.1.3 Reproductive biology, cytogenetics and molecular data, and the definition of the genus *Citrus*

#### 4.1.3.1 The genus *Citrus* and the biological concept of species; genus or species

According to the biological species concept (BSC) developed by Mayr (1942), "species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups." This is a popular concept among biologists; however, the debate around the species concepts is still very active and many other concepts have been proposed based, in part, on different biological properties (reviewed in Mayden (1997) and de Queiroz (2007)). For example, the BSC emphasizes the property of reproductive isolation (Mayr, 1942; Dobzhansky, 1970), while the ecological



species concept is based on the occupation of a distinct niche or adaptive zone (Andersson, 1990); one approach of the phylogenetic species concept emphasizes diagnosability (Nixon and Wheeler, 1990), and another, monophyly (Donoghue, 1985; Mishler, 1985).

None of the actual citrus classifications fits with the BSC because all *Citrus* species are sexually compatible producing hybrids with moderate-to-high fertility and should, therefore, be joined in a single species according to the BSC. However, nuclear genome sizes of *Citrus* species display differentiation up to 10% between *C. reticulata* (360 Mb per haploid genome) and *C. medica* (398 Mb) (Ollitrault et al., 2003), the two taxa with the smallest and largest genomes in the genus *Citrus*. The differentiation of nuclear genome sizes agrees with cytogenetic observations of chromosome morphology differentiation between the ancestral taxa of cultivated citrus (Raghuvanshi, 1969; Nair and Randhawa, 1969; Guerra, 1993; Hynniewta et al., 2011). In addition, the intermediate and preferential disomic inheritance observed in some doubled diploids of interspecific origin such as for “Volkamer” lemon (*C. reticulata* × *C. medica* (Dirceu et al., 2016)) or “Mexican” lime (*C. micrantha* × *C. medica* (Rouiss et al., 2018)) and allotetraploid somatic hybrids (Kamiri et al., 2011; Xie et al., 2015) attest to preferential chromosome pairing between chromosomes of the same ancestral taxon. All these results reveal a significant genomic differentiation between the four ancestral taxa of the cultivated *Citrus* (*C. maxima*, *C. medica*, *C. micrantha*, and *C. reticulata*). The monophyly of each ancestral taxon has been demonstrated by nuclear markers analysis (Herrero et al., 1996a,b; Nicolosi et al., 2000; Barkley et al., 2006; Garcia-Lor et al., 2013a) and maternal phylogenetic studies (Bayer et al., 2009; Nicolosi et al., 2000; Yamamoto et al., 1993; Froelicher et al., 2011; Carbonell-Caballero et al., 2015; Curk et al., 2016). Recently, nuclear phylogenomic studies have revealed a huge number of diagnostic (discriminant) single-nucleotide polymorphisms (SNPs) for each of these four ancestral taxa (Wu et al., 2014, 2018; Curk et al., 2015; Oueslati et al., 2017). Moreover, an important part of the phenotypic diversity of the cultivated *Citrus* results from the allopatric evolution of the four ancestral taxa (see Section 4.3 for more details). Therefore, recognition of the four ancestral taxa of most cultivated citrus at species rank is supported by the phylogenetic species concept based on diagnosability and monophyly as well as the ecological species concept, considering the past allopatric evolution of the ancestral taxa under different environmental contexts (see below).

#### 4.1.3.2 Sexual compatibility and phylogenetic relationships with related genera of the true citrus; toward a new definition of the genus *Citrus*?

Two elements disagree with the circumscription of the genus *Citrus* as proposed by Swingle and Reece (1967). The first is the demonstrated sexual compatibility (Fig. 4.3) of the different species of the other “true citrus” genera with the species of *Citrus* as defined by Swingle and Reece (1967). Many fertile hybrids have been produced between *P. trifoliata* and several *Citrus* species and *Poncirus* is a very important genetic resource for rootstock breeding by “intergeneric” hybridization. The so-called citrange, citrumelo, citremon, citradia, and citrandarin result, respectively, from hybridization between sweet orange, grapefruit, lemon, sour orange, and mandarin with *P. trifoliata*. Some of these hybrids were involved in a second round of hybridization producing trigeneric hybrids with *Fortunella* (citrangequat) and *Eremocitrus* (Citrangeremo) or backcrosses in *Citrus* (citrangor) and *Poncirus* (cicitrangle). Several hybrids between *Citrus* and *Fortunella* were also created during the 20th century (mandarinquat, limequat) or identified in the germplasms (calamondin) and involved in trigeneric hybridization with *Microcitrus* (faustrimedina, faustrime) and backcrossed in *Fortunella* (procimequat) and *Citrus* to develop triploid hybrids (Vilorio et al., 2004). Several hybrids have been created between *Citrus* and *Microcitrus* (e.g., “Australian blood” lime). Hybrids between *Citrus* and *Eremocitrus glauca* were also obtained (eremorange and eremolimon, respectively, with sweet orange and lemon) as well as hybrids between *Fortunella* and *Poncirus* (citrumquat). An accession (CRC 4109) derived from the open pollination of a *Clymenia polyandra* × procimequat hybrid was described by the University of California (UCR, 2017a).

The second discordant element is the nonmonophyly of the chloroplast genomes of the Swingle *Citrus* species, revealed first by Bayer et al. (2009) and more recently from whole genome sequencing (WGS) resequencing data by Carbonell-Caballero et al. (2015). Indeed, while *C. maxima*, *C. reticulata*, and species of the subgenus *Papeda* form a well-supported clade, *C. medica* is in a separate well-supported clade with Australian citrus (*Microcitrus* and *Eremocitrus*). The Bayer et al. (2009) study also revealed that *Clymenia* and the New Caledonian citrus *Oxanthera* are part of this last clade. Moreover, *P. trifoliata* and *Fortunella* spp. join the *C. maxima*/*C. reticulata*/*Papeda* clade before the Australian citrus/*C. medica* clade (Bayer et al., 2009; Carbonell-Caballero et al., 2015). The “true citrus” group plus *Oxanthera* form a strongly supported clade, highly differentiated from the other Citreae genera (Bayer et al., 2009).

These elements, and the very high synteny and collinearity observed between genetic maps of *Poncirus* and *Citrus* species (Chen et al., 2008; Bernet et al., 2010) and cytogenetic maps (da Costa Silva et al., 2015) strongly support the proposal of Mabberley (1998, 2004) and Zhang and Mabberley (2008) to integrate *Poncirus*, *Fortunella*, *Microcitrus*, *Eremocitrus*,

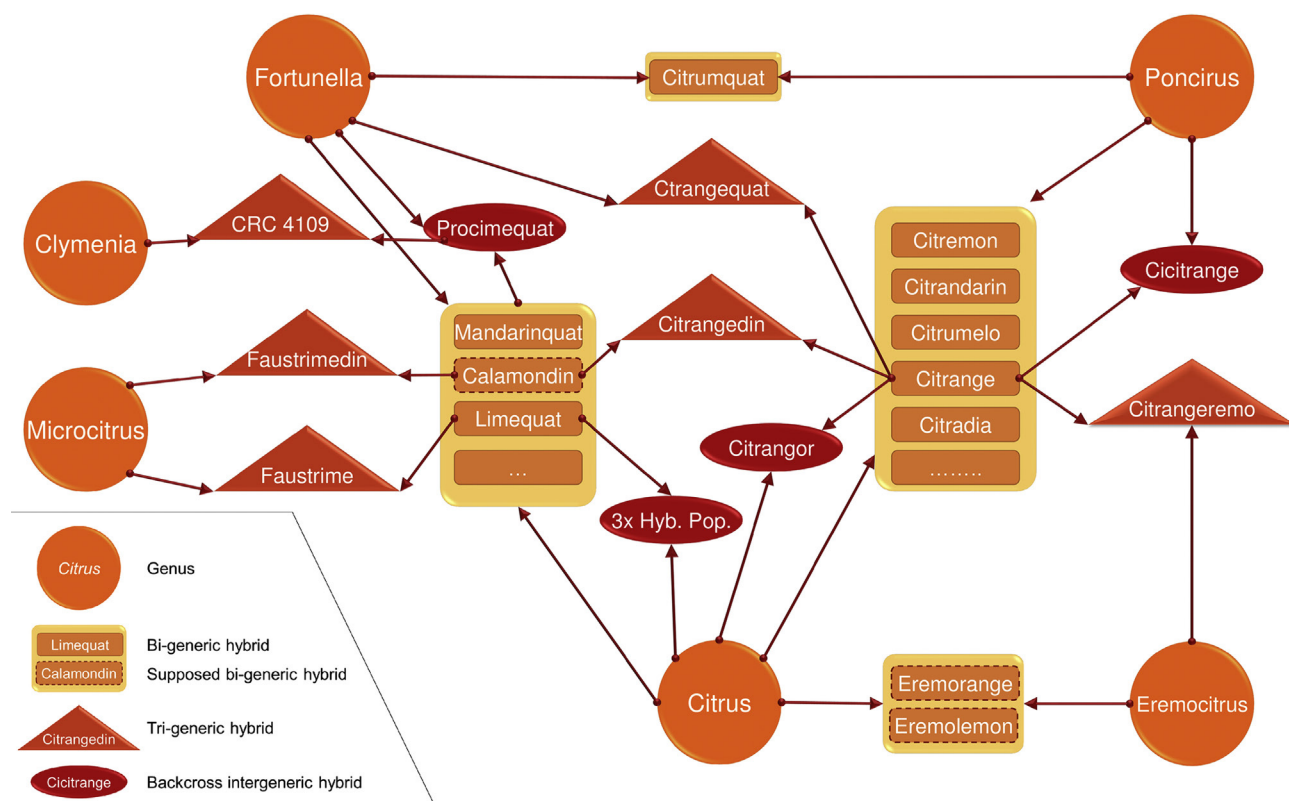


FIG. 4.3 Sexual hybrids obtained between the six genera of Swingle True citrus group.

and *Clymenia* into the genus *Citrus*. According to the results of Bayer et al. (2009), *Oxanthera* may also be integrated into *Citrus*. Some other aspects regarding the specific subdivisions delimitations within the *Citrus* genus and the origin of admixture types proposed by Mabberley (1997, 2004) are not in agreement with recent molecular studies (see below) and its classification system is still incomplete. This could explain why the Swingle and Reece (1967) classification of the true citrus group still remains popular in the citrus scientific community.

## 4.2 The genus *Citrus* classifications; an historical, biological, genetic, and phylogenomic perspective

### 4.2.1 The history of citrus botanical classifications

The extraordinary ability of citrus plants to hybridize with many species and close genera, frequent morphological mutations, and apomixis have complicated matters for early citrus taxonomists, who often did not have the opportunity to observe the plants in their natural environment. We inherited a natural history of citrus rich in folk classifications and in many ancient texts dealing with citrus fruits, the various acidic citrus (lime, lemon, and citron) and the sour and the sweet orange are readily confused.

*The ancient descriptions:* In China, citrus have been grown for >4000 years. The earliest known mention of citrus fruit is in a text from one of the “Shu Jing” books, also called the Book of Documents or Classic of History, revealing that two types of citrus fruits, large ones and smaller ones (probably pummelos and mandarins), were offered to the emperor (23rd-century BC) as high-value fruits (Medhurst, 1846). Other descriptions can be found in the early Mediterranean literature of Virgil (the Georgics, probably before 37 BC) and Dioscorides (*De Materia Medica*, between AD 50 and 70) who described the citron and its traditional uses. The first certified monograph on citrus fruits, the *Ju Lu*, dating from 1178 was written in China by Han Yanzhi. In this book, 27 citrus fruits were described as well as the different stages of their cultivation, from the propagation to harvest (Dioscorides, 2000).

#### 4.2.1.1 The early classifications

Ferrari (1646) was the first to make a citrus classification in his *Hesperides, siue, de malorum aureorum cultura et usu*, a classification followed and elaborated by Johann Christoph Volkamer in his two volumes of *Nurenbergische Hesperides*

published in 1708 and 1714 (Volkamer, 1708, 1714). Ferrari and Volkamer are between what Magnin-Gonze (2009) named the *Descriptive Botany* and the *Biological Botany* periods. Linnaeus, with his new *Fundamenta botanica*, established our current binomial nomenclatural system and organized plants by their reproductive features. In his “*Genera plantarum*” (Linnaeus, 1737), basing his work for *Citrus* on that of de Tournefort (1700), Linnaeus included the genus *Citrus* with three species: *Citrus aurantium* Tournef., *Citrus citreum* Tournef., and *Citrus limon* Tournef. according to flower and leaf descriptions. In “*Species plantarum*” (Linnaeus, 1753), Linnaeus recognized only two species subdivided into several varieties: *C. aurantium* var. *aurantium* (the sour oranges), *C. aurantium* var. *sinensis* (the sweet oranges), *C. aurantium* var. *grandis* (renamed *C. aurantium* var. *decumana* by Linnaeus = *Citrus maxima*; the pummelos), *Citrus medica* var. *limon* (the lemons), and *C. medica* var. *medica* (the citrons). In the second edition, a third species, *Citrus trifoliata* (the trifoliate orange) was added.

Between 1700 and 1800, citrus classification transitioned through the *Classificatory Botany* period to the *Evolutionary Botany* period (Magnin-Gonze, 2009) with illustrious taxonomists such as Burman (1768), de Loureiro (1790), and Blanco (1837). Blanco (1837), using the Linnaean sexual system, described seven *Citrus* species and included the first mention of mandarin (*Citrus reticulata* Blanco). The best illustration of what Magnin-Gonze (2009) called *Evolutionary Botany* is the incredibly modern “*Tableau synoptique du Genre Citrus*” that Galesio (1811) published in 1811, in his *Traité du Citrus*. Another work coming from the *Evolutionary Botany* period is still considered as an important reference: the “*Histoire naturelle des orangers*” of Risso and Poiteau (1818). Many other taxonomists including Blume, Macfadyen, Tenore, Fortune, Oliver, Pasquale, Hooker, Bonavia, Engler, and Bailey tried to organize and classify citrus taxa (Nicolosi, 2012).

#### 4.2.1.2 The 20th-century classifications

In his “*Flore Générale de l’Indo-Chine*” based on morphological traits, Guillaumin (Citrus, 1911) included six *Citrus* species (*Citrus decumana* Murr., *C. aurantium* L., *C. medica* L., *Citrus nobilis* Lour., *Citrus japonica* Thumb., and *C. trifoliata* L.).

During the 20th century, two important, but very different taxonomic systems were established by Tyôzaburô Tanaka (1954, 1961, 1977) in Japan and Walter T. Swingle (Swingle and Reece, 1967; Swingle, 1943) in the United States. Both the Tanaka and Swingle systems are still widely used by the citrus scientific community.

The Tanaka system recognizes 157 species of *Citrus*, including 35 species of mandarins in its first publication (Tanaka, 1954). It was expanded to 162 species in the 1977 version (Tanaka, 1977). With the current knowledge on cultivated citrus interspecific admixture, it is clear that the Tanaka classification has too many species, corresponding essentially to varietal groups of clonal origin (due to facultative apomixis) resulting from different reticulation events.

Swingle (1943) was the first citrus taxonomist to propose the use of biochemical markers (glycosides) for taxonomy. The Swingle classification (Swingle, 1943) and the revised version of Swingle and Reece (1967), based on history, morphological, and biochemical characters, also takes into account the vegetative reproduction and particularly the facultative apomixis present in several citrus taxa. Swingle and Reece were aware of the problem of the species concept in agamic complexes as discussed by Stebbins (1950) and Lawrence (1951), who stated “The perpetuation of apomictic hybrids has resulted in some descriptive taxonomists treating each biotype as a morphologically distinct and seed producing species.” Taking this into account, the Swingle (1943) classification displays a spectacular reduction of species (Krueger and Navarro, 2007) compared with the Tanaka (1954) classification (157 species). The reduction in species number is particularly important for mandarin with three (*C. reticulata*, *C. tachibana*, and *C. indica*) and 36 species in the Swingle and Tanaka classifications, respectively. Swingle recognized two subgenera and sections: (i) subgenus *Papeda*, with two species in section *Papeda* (*C. latipes* and *C. ichangensis*) and four species in section *Papedocitrus* (*C. hystris*, *C. macroptera*, *C. micrantha*, and *C. celebica*); and (ii) subgenus *Citrus* (formerly *Eucitrus*), with 10 species (*C. aurantiifolia*, *C. medica*, *C. limon*, *C. grandis*, *C. paradisi*, *C. aurantium*, *C. sinensis*, *C. reticulata*, *C. tachibana*, and *C. indica*). Modifications of the Swingle (1943) classification include 17 species (Bhattacharya and Dutta, 1956), 36 species (Hodgson, 1961), or 31 species (Singh and Nath, 1969). Despite its strengths, the Swingle system does not recognize the hybrid nature of very important horticultural groups such as sweet orange, sour orange, grapefruit, lemon, and lime classified, respectively, as *C. sinensis*, *C. aurantium*, *C. paradisi*, *C. lemon*, and *C. aurantiifolia*. More recently, Mabberley (1997, 1998, 2004) was the first taxonomist to try to integrate new phylogenetic knowledge into the *Citrus* classification. Mabberley (1997) proposed three main species for commercial fruits of *Citrus*: *C. medica*, *C. reticulata*, and *C. maxima*. He also proposed four hybrids: *C. × aurantium* for sweet oranges, sour oranges, grapefruits, tangelos, and tangors; *C. × jambhiri* for rough lemon; *C. × aurantiifolia* for “Mexican” lime types; and *C. × limon* for lemons (considered by Mabberley (1997) as backcrosses of a lime by a citron). In 2004, Mabberley extended its proposal for the treatment of hybrids. However, several doubts remained on the origin of admixture taxa and some hypotheses made by Mabberley (1997, 2004) are now clearly inappropriate given recent phylogenomic data (Curk et al., 2016; Wu et al., 2018; Oueslati et al., 2017). In his first classification, Mabberley (1997)



listed the following cultivars of *C. reticulata*: “tangerine, mandarin, satsuma, clementine; cultivars include “Clementine,” “Dancy,” “Emperor,” “Fina,” “Imperial,” “Nova,” and “Owari.” However, recent phylogenomic studies (Wu et al., 2014, 2018; Oueslati et al., 2017) revealed that all cited cultivars are not pure *C. reticulata* but are introgressed by *C. maxima*. These introgressions are close to 20% for satsuma and clementine and 30% for Nova tangelo. These varieties should be treated as *C. × aurantium* rather than *C. reticulata*. In 2004, Mabberley treated rough lemon (formerly *Citrus × jambhiri* in Mabberley, 1997) as *C. × taitensis* with two potential origins: *C. reticulata* × *C. medica* or *C. reticulata* × *C. limon*. Recent data (Curk et al., 2016; Wu et al., 2018) agree with the first hypothesis. The origin of *C. × aurantiifolia* (lime) proposed in Mabberley (2004) (*C. ichangensis* × *C. maxima*) is erroneous. As proposed by Nicolosi et al. (2000) and confirmed recently (Curk et al., 2016; Wu et al., 2018), *C. × aurantiifolia* = *C. micrantha* × *C. medica*. Even if Zhang and Mabberley (2008) split *papeda* into two species *C. cavaleriei* and *C. hystrix* in agreement with the Swingle classification of sections *papeda* and *papedocitrus* (Swingle, 1943), the treatment of *papeda* types is still too limited. Therefore, the conceptual framework proposed by Mabberley (1997) for the classification of edible *Citrus* represents a good foundation to which robust phylogenomic data can now be applied.

Mabberley (1998, 2004) and Zhang and Mabberley (2008) proposed to include all the “true citrus” taxa in the genus *Citrus* with the following names: *C. australasica* (finger lime), *C. australis* (Australian lime), *C. cavaleriei* and *C. hystrix* (papeda), *C. glauca* (desert lime), *C. japonica* (kumquat), *C. maxima* (pummelo), *C. medica* (citron), *C. reticulata* (mandarin), and *C. trifoliata* (trifoliate orange). As discussed previously, this is fully justified by sexual compatibility and chloroplastic phylogenetic data. According to the chloroplastic phylogenetic study of Bayer et al. (2009), *Oxanthera* spp. from New Caledonia should also be included in *Citrus*. However, by not providing a subgeneric classification Mabberley (1998, 2004) and Zhang and Mabberley (2008), did not fully convey phylogenetic relationships within *Citrus*. The close relationships between the Australian citrus could be reflected in a sectional classification.

#### 4.2.2 1967–2017, from traditional taxonomy to phylogenomy: 50 years to clarify the genetic organization of the genus *Citrus* and the origin of modern citrus varieties

During the 1970s, numerical taxonomy resulted in a better understanding of citrus domestication and of the relationships between the various cultivated species of *Citrus*. Barrett and Rhodes (1976) were the first to propose, based on morphological descriptors, that three basic taxa (*C. maxima*, *C. medica*, and *C. reticulata*) gave rise to all cultivated *Citrus*. During the 1980s, essential oils and polyphenols were the first molecular markers used for taxonomic purposes. Chemotaxonomic studies revealed four true *Citrus* species (*C. halimii* B.C. Stone, *C. maxima*, *C. medica*, and *C. reticulata*) (Scora, 1988). During the same period, the importance of *C. maxima*, *C. reticulata*, and *C. medica* was also emphasized by total protein analysis (Handa and Ishizawa, 1986). The development of codominant isozyme markers (Herrero et al., 1996a,b; Torres et al., 1982; Hirai et al., 1986) opened the modern era of citrus phylogenetic studies. Indeed, codominant markers allow revealing the high heterozygosity of admixture taxa and their haplotype sharing with the ancestral taxa. Restriction fragment length polymorphisms (RFLPs) (Federici et al., 1998; Fanciullino et al., 2007) significantly increased the number of useful codominant markers; however, RFLP assays are time consuming and labor intensive. Since the second part of the 1990s, the development of polymerase chain reaction (PCR) markers and particularly simple sequence repeats (SSRs) (Barkley et al., 2006; Kijas et al., 1995; Chen et al., 2006; Luro et al., 2008; Froelicher et al., 2008; Ollitrault et al., 2010; Liu et al., 2013a; Biswas et al., 2014; Liang et al., 2015; Ramadugu et al., 2015; Shimizu et al., 2016) strongly reinforced the *Citrus* phylogenetic studies. Mitochondrial (Froelicher et al., 2011) and plastome data (Bayer et al., 2009; Nicolosi et al., 2000; Yamamoto et al., 1993; Carbonell-Caballero et al., 2015) provided important information on *Citrus* maternal phylogeny. During the last 5 years, with the availability of the first complete reference sequences of the citrus genome (Wu et al., 2014; Xu et al., 2013), the era of phylogenomics began. WGS and genotyping by sequencing (GBS) data provided a huge number of SNPs and allowed the identification of discriminant polymorphisms of the different ancestral taxa, covering the whole genome (Wu et al., 2014, 2018; Curk et al., 2015; Oueslati et al., 2017). Efficient SNP genotyping methods have been developed for scalable experiments using competitive allele amplification (KASPar© technology (Curk et al., 2015; Garcia-Lor et al., 2013b; Cuenca et al., 2013)). Cleaved amplified polymorphic sequence approaches were successfully developed in Japan (Shimada et al., 2014; Omura and Shimada, 2016). SNP arrays have been developed for high-throughput studies (Ollitrault et al., 2012; Fujii et al., 2013) and recently, in California, two Affymetrix Axion SNP arrays with about 1.5 million and 56,000 SNPs were developed (Eck et al., 1996). GBS (Oueslati et al., 2017) and its variant DArTseq (Penjor et al., 2014, 2016; Curtolo et al., 2017) approaches were also recently developed in citrus. The diagnostic polymorphisms of the ancestral species were successfully used to identify the origin of admixture (Curk et al., 2015, 2016; Wu et al., 2014, 2018) and to infer the phylogenomic karyotypes all along their genomes (Wu et al., 2014, 2018; Oueslati et al., 2017).

Recent phylogenetic and phylogenomic studies validated most of the hypotheses of interspecific hybrids origin proposed in the important paper of Nicolosi et al. (2000). Curk et al. (2016) proposed clarifications for the lemon and lime horticultural groups on the basis of nuclear and cytoplasmic diagnostic markers of the ancestral taxa. The conclusions of these two papers and the phylogenomic studies by Wu et al. (2014, 2018) from WGS data, by Oueslati et al. (2017) from GBS data for the *C. reticulata*/*C. maxima* complex, and by Penjor et al. (2016) from RAD-Seq for several lime and lemon types are summarized in Fig. 4.4. It provides a good framework to lay the foundation of a classification based on the reticulate evolution of citrus and the resulting phylogenomic structures.

### 4.2.3 The ancestral and admixture taxa

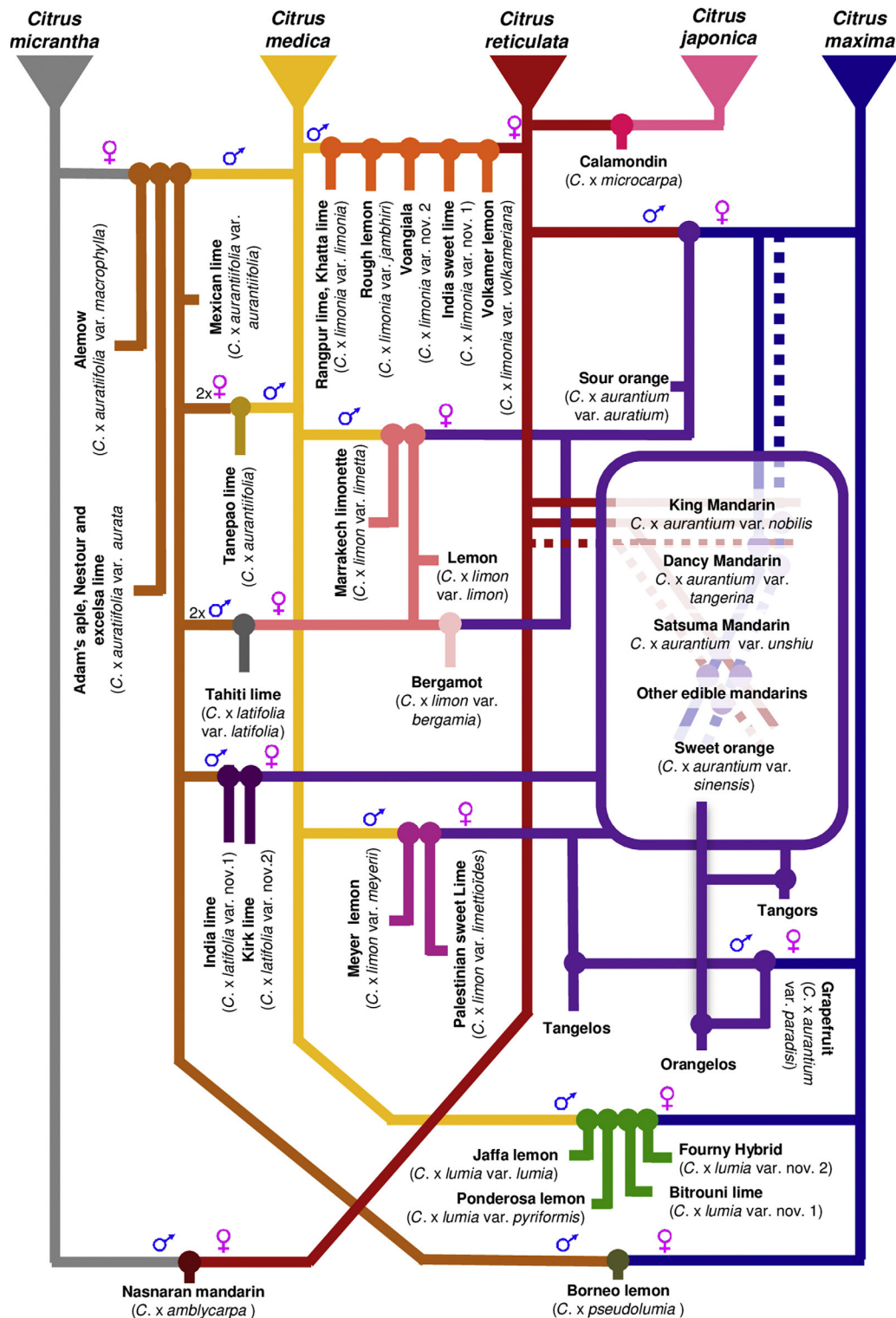
We propose a new trinomial concept for *Citrus* admixture classification. As illustrated in Fig. 4.5 for the admixture types between the four ancestral taxa of the cultivated *Citrus*, the species name is determined by the phylogenomic admixture revealed by recent phylogenetic and phylogenomic data. Three potential admixture combinations implying *C. micrantha* have not been yet revealed by phylogenomic studies. The variety rank corresponds to the groups of modern cultivars diversified, by mutations, transposable element mobilities, or stable epigenetic variations, without further sexual recombination, from each ancestral reticulation events. An example within *C. × aurantium* is given in Fig. 4.6. Sweet oranges and willow leaf mandarins are two *C. reticulata*/*C. maxima* admixtures groups with unknown origins (Wu et al., 2014, 2018) but both deriving from a single hybrid. A little more than one century ago, in Algeria, Father Clement selected Clementine as a chance seedling from ‘Mediterranean’ willow leaf mandarin. Phylogenetic and phylogenomic studies demonstrated that it resulted from *C. reticulata* var. *deliciosa* × *C. × aurantium* var. *sinensis* hybridization (Nicolosi et al., 2000; Wu et al., 2014; Curk et al., 2015; Oueslati et al., 2017; Ollitrault et al., 2012). Numerous cultivars with significant phenotypic diversity were selected from this initial hybrid. They are treated as *C. aurantium* var. *clementina*. In the same way, the first grapefruit resulted from a spontaneous hybridization in the Carribean between *C. maxima* and *C. × aurantium* var. *sinensis* (Wu et al., 2018; Curk et al., 2015; Oueslati et al., 2017; Penjor et al., 2016) followed by an important asexual diversification leading to the actual grapefruit cultivars. They are treated as *C. aurantium* var. *paradisi*. The older is the ancestral reticulation event, the higher is the within-variety diversity, particularly under human selection of phenotypical variants. For sweet oranges, it resulted in a huge amount of phenotypical diversity generally organized in common oranges, navel oranges, blood oranges, and acidless cultivars. They are all treated as *C. × aurantium* var. *sinensis*.

The actual citrons, pummelos, and “small flower” *papeda* are mostly pure representatives of, respectively, *C. medica*, *C. maxima*, and *C. micrantha* (Wu et al., 2018; Curk et al., 2014, 2015). The situation is even more complex for *C. reticulata*. Indeed many of the mandarins included in *C. reticulata* by Swingle and Reece (1967) and Mabberley (1997) display introgressions of *C. maxima* (Wu et al., 2014, 2018; Curk et al., 2014, 2015; Oueslati et al., 2017). Recent phylogenomic studies (Wu et al., 2014, 2018; Oueslati et al., 2017) revealed a continuum of *C. reticulata*/*C. maxima* admixture when including modern mandarins, tangors, tangelos, sweet and sour oranges, orangelos, and grapefruits (Fig. 4.7).

Sweet orange and grapefruit horticultural groups are ideotypes, each arising from a single reticulation event that have been very successful and spread all over the world, but they are fully part of this continuum of *C. reticulata*/*C. maxima* admixture. Moreover, sweet orange genome share a significant proportion of haplotypes with modern mandarins (Wu et al., 2018). We propose to treat all modern varieties of mandarins classified as type 3 by Wu et al. (2018), due to *C. maxima* introgression, as *C. × aurantium*. The species names of the Tanaka (1954, 1961) classification may be used for variety rank when they have taxonomic priority. Indeed, even if the Tanaka classification erroneously gave species rank to hybrids with their structure fixed by apomixis, it had the advantage of recognizing among mandarins many of the different reticulation events. This definition of *C. × aurantium* extends the one proposed by Mabberley (1997) to all *C. reticulata*/*C. maxima* admixtures including tangors, tangelos, and some mandarins. It also retains the variety concept proposed by Linnaeus (1753) for *C. aurantium* var. *sinensis* and *C. aurantium* var. *aurantium* and extends it to all admixture genotypes resulting from independent reticulation events.

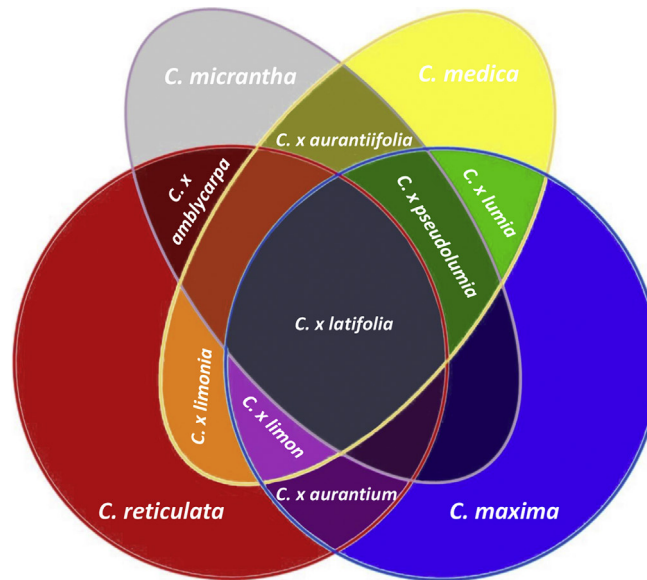
In the same way for other *Citrus* admixture species, when *Citrus* types sharing the same kind of phylogenomic admixture result from independent reticulation events, we propose to use the former Tanaka species names (Tanaka, 1954; Tanaka, 1961; Tanaka, 1977), when appropriate (priority), or the priority name for this type, for variety rank.

From the phylogenetic/phylogenomic data actually available (Nicolosi et al., 2000; Curk et al., 2015, 2016; Wu et al., 2014, 2018; Oueslati et al., 2017; Penjor et al., 2016), a revised classification of citrus based on the identified phylogenomic structures could be as follows. Table 4.1 summarizes the correspondence between the proposed classification and the former most important ones of Tanaka (1961), Swingle and Reece (1967), and Mabberley (2004) revised by Zhang and Mabberley (2008).

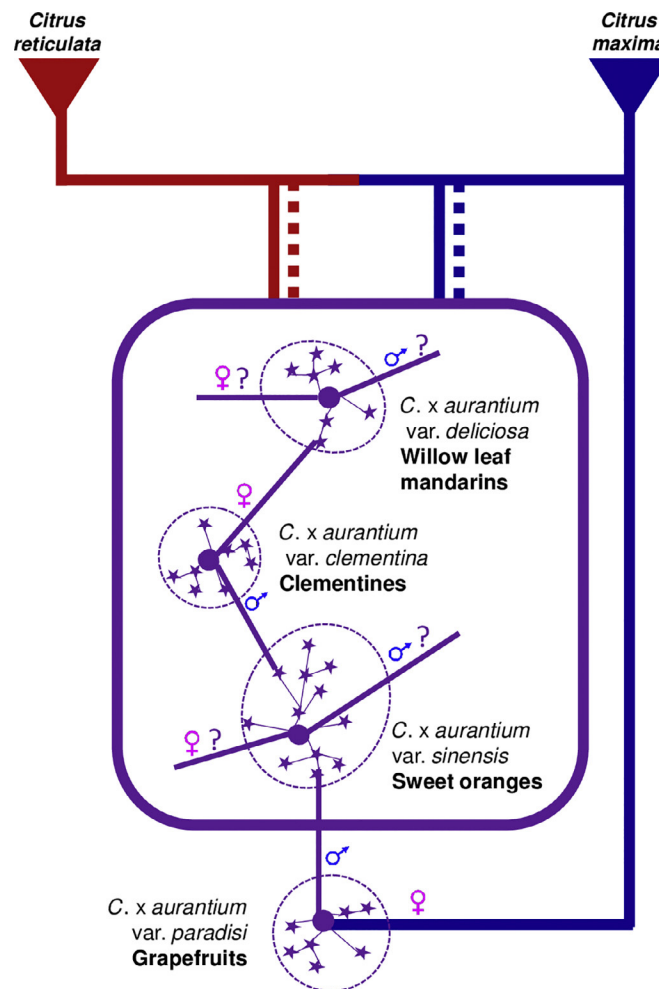


**FIG. 4.4** Verified origins of admixture citrus varieties based on phylogenetics and phylogenomics. Each small circle represents an independent recombination event. Based on Nicolosi E, Deng ZN, Gentile A, Malfa SI, Continella G, Tribulato E. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theor. Appl. Genet.* 2000;100(8):1155–1166, Wu GA, Prochnik S, Jenkins J, Salse J, Hellsten U, Murat F, et al. Sequencing of diverse mandarin, pummelo and orange genomes reveals complex history of admixture during citrus domestication. *Nat. Biotechnol.* 2014;32(7):656–662, Wu GA, Terol J, Ibanez V, Lopez-Garcia A, Perez-Roman E, Carles B, et al. Genomics of the origin, evolution and domestication of citrus. *Nature* 2018., Turk F, Ollitrault F, Garcia-Lor A, Luro F, Navarro L, Ollitrault P. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. *Ann. Bot.* 2016;117(4):565–583, Turk F, Ancillo G, Ollitrault F, Perrier X, Jacquemoud-Collet JP, Garcia-Lor A, et al. Nuclear species-diagnostic SNP markers mined from 454 amplicon sequencing reveal admixture genomic structure of modern citrus varieties. *PLoS One* 2015;10(5):e0125628, Penjor T, Mimura T, Kotoda N, Matsumoto R, Nagano AJ, Honjo MN, et al. RAD-Seq analysis of typical and minor Citrus accessions, including Bhutanese varieties. *Breed. Sci.* 2016;66(5):797–807, and Oueslati A, Salhi-Hannachi A, Luro F, Vignes H, Mournet P, Ollitrault P. Genotyping by sequencing reveals the interspecific *C. maxima*/*C. reticulata* admixture along the genomes of modern citrus varieties of mandarins, tangors, tangelos, orangelos and grapefruits. *PLoS One* 2017;12(10): e0185618.

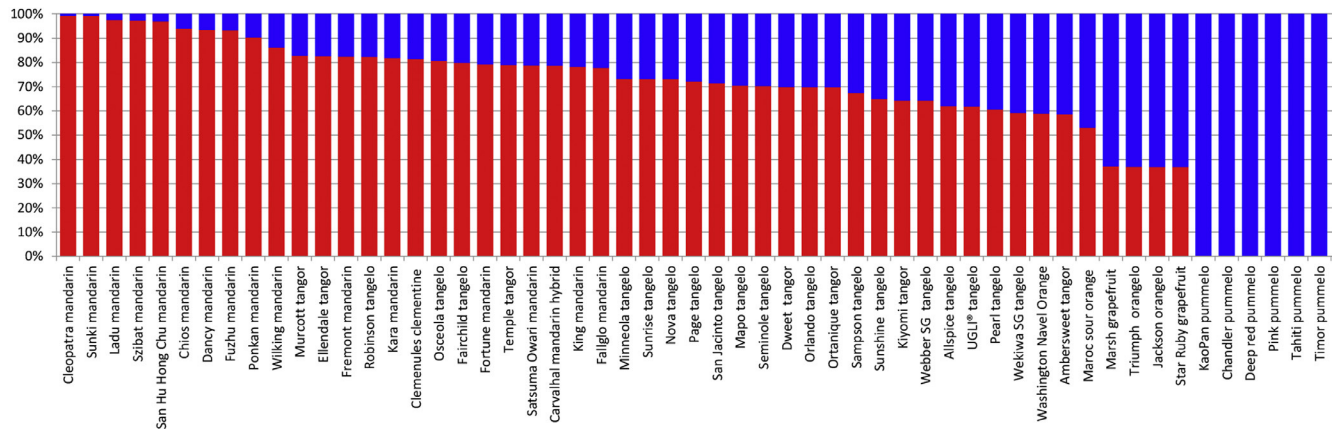




**FIG. 4.5** Proposal for an unambiguous *Citrus* classification based on phylogenomic admixture; for example, from the *C. maxima*/*C. medica*/*C. micrantha*/*C. reticulata* gene pool.



**FIG. 4.6** Illustration of the proposed treatment for variety rank in admixture species; an example in *C. x aurantium*. *Small circle*: ancestral reticulation event, *stars*: modern cultivars diversified, by mutation, transposable element mobility or stable epigenetic variations, from the ancestral hybrid, without further sexual recombination.



**FIG. 4.7** Relative admixtures of *C. reticulata* and *C. maxima* in mandarins, tangors, tangelos, orangelos, grapefruits, and pummelos. Red: *C. reticulata* contribution; blue: *C. maxima* contribution. Modified from Oueslati A, Salhi-Hannachi A, Luro F, Vignes H, Mournet P, Ollitrault P. Genotyping by sequencing reveals the interspecific *C. maxima*/*C. reticulata* admixture along the genomes of modern citrus varieties of mandarins, tangors, tangelos, orangelos and grapefruits. *PLoS One* 2017;12(10): e0185618. GBS data.

#### 4.2.3.1 Pure Citrus species

Given the recent phylogenomic data and biological characteristics (particularly sexual compatibility), we agree with the inclusion of the former genera *Microcitrus*, *Eremocitrus*, *Clymenia*, *Poncirus*, and *Fortunella* in the *Citrus* genus as proposed by Mabblerley (1997, 1998, 2004). We also propose to include the New Caledonian genus *Oxanthera* in *Citrus*. Phylogenetic (Bayer et al., 2009) and phylogenomic studies (Wu et al., 2018) do not validate the subgenera *Citrus* and *Papeda* proposed by Swingle (1943). Indeed, they are not monophyletic. Only a few species classified in subgenus *Papeda* by Swingle (1943) have been deeply characterized by genomic studies (Wu et al., 2018; Wang et al., 2017).

The genomic diversity within trifoliate orange, kumquat, *Microcitrus*, *Eremocitrus*, *Clymenia*, and *Oxanthera* has not yet been established. Therefore, at this point, we do not have a definitive phylogenomic basis to classify the former Swingle species of these genera and the *Papeda* group as all belonging to true species of *Citrus*. Future WGS analysis of the different papedas, trifoliate orange, kumquats, *Microcitrus*, *Eremocitrus*, *Clymenia*, and *Oxanthera* taxa and particularly the clarification of the phylogenetic relationships between the Australian/Oceanian taxa will allow an exhaustive proposal of pure *Citrus* taxa and correspondence with former classifications. As of 2017, the phylogenomically confirmed pure species are as follows:

***C. cavaleriei* H. Lév. ex Cavalerie** (*C. ichangensis* Swingle; *C. latipes* (Swingle.) Tanaka) has, as its natural area of distribution, West-central and Southwestern China. WGS data (Carbonell-Caballero et al., 2015; Wu et al., 2018) reveal a clear differentiation between *C. ichangensis* and *C. micrantha* both for chloroplastic and nuclear phylogenies and a low level of nuclear heterozygosity. These data are coherent with the subdivision in sections *Papeda* and *Papedocitrus* of Swingle classification (Swingle, 1943). It is, therefore, justified to rank *C. cavaleriei* as a species as proposed by Zhang and Mabblerley (2008). It should be a parent of *C. × junos* (Yuzu).

***C. maxima* (Burm.) Merr.** is the species described as *C. grandis* (L.) Osbeck by Swingle (1943) that originated in the Malay Archipelago and Indonesia. It should include all nonintrogressed pummelos. It is involved in several interspecific hybrid taxa: *C. × aurantium*, *C. × lemon*, *C. × latifolia*, and *C. × lumia*.

***C. medica* Linnaeus** fits with the species of the same binomial described by Swingle (1943) that initially evolved in Northeastern India and the nearby region of Burma and China. It should include all nonintrogressed citrons. Swingle and Reece (1967) distinguished the standard *C. medica* type, *C. medica* var. *ethrog* Engl. (= *Citrus limonimeditica* Lush. for Tanaka (1961)) and *C. medica* var. *sarcodactylis* (Hoola van Nooten) Swingle. The molecular analysis done with citrus germplasm of the INRA/CIRAD collection (Corsica) does not justify this subdivision (Curk et al., 2015; Luro et al., 2012). However, a structure analysis based on the SSR markers and including Mediterranean and Chinese *C. medica* accessions revealed a differentiation between the two regions and a substructuring of Chinese accessions in two clusters (Ramadugu et al., 2015). All fingered lemons were in one of these clusters but associated with some nonfingered varieties. Therefore, at this stage, we do not retain the subdivision proposed by (Swingle and Reece (1967)). A deeper phylogenomic analysis of *C. medica* diversity in its main areas of diversification (Yunan, India, Mediterranean Basin) will be necessary to subdivide, or not, this species in different varieties. *C. medica* is involved in several interspecific hybrid taxa: *C. × lemon*, *C. × limonia*, *C. × aurantiifolia*, *C. × latifolia*, *C. × lumia*, and *C. × pseudolumia*.

**TABLE 4.1** Correspondences between the new phylogenomic classification and the former classifications of **Tanaka (1961)**, **Swingle and Reece (1967)**, and **Mabberley (2004)** revised by **Zhang and Mabberley (2008)**.

| Phylogenomic classification                             | Tanaka (1961)                        | Swingle and Reece (1967)                   | Zhang and Mabberley (2008)  | Common names (examples)                                   | Phylo-genomic references   |
|---|--------------------------------------|--|-----------------------------|---|--|
| <i>Citrus cavaleriei</i> H. Lév. ex Cavalerie           | <i>C. ichangensis</i> Swingle        | <i>C. ichangensis</i>                      | <i>C. cavaleriei</i>        | Adsae   | Wu et al. (2018)   |
| <i>C. maxima</i> (Burm.) Merr.                          | <i>C. maxima</i>                     | <i>C. maxima</i>                           | <i>C. maxima</i>            | Pummelos (Pink, Deep Red, Timor, ...)                     | Curk et al. (2015) and Wu et al. (2018)  |
| <i>C. medica</i> L.                                     | <i>C. limonimedica</i> Lush.         | <i>C. medica</i>                           | <i>C. medica</i>            | Etrog citron  | Curk et al. (2015), Curk et al. (2016), and Wu et al. (2018)   |
|   | <i>C. medica</i>                     |  |                             | Citrons (Corsican, Diamante, Buddha's hand, Humpang, ...) | Curk et al. (2015), Curk et al. (2016), and Wu et al. (2018)   |
| <i>C. micrantha</i> Wester                              | <i>C. micrantha</i>                  | <i>C. micrantha</i>                        | <i>C. hystris</i> DC.       | Small-flowered papeda, small-fruited papeda               | Curk et al. (2015), Curk et al. (2016), and Wu et al. (2018)   |
| <i>C. reticulata</i> var. <i>austera</i> Swingle        |                                      | <i>C. reticulata</i> var. <i>austera</i>   | <i>C. reticulata</i> Blanco | Sun-Chu-Sha-Kat mandarin                                  | Wu et al. (2018)   |
| <i>C. reticulata</i> var. <i>tachibana</i> ined.        | <i>C. tachibana</i> (Makino) Tanaka  | <i>C. tachibana</i>                        | <i>C. reticulata</i>        | Tachibana mandarin  | Wu et al. (2018)   |
| <i>C. × amblycarpa</i>                                  | <i>C. amblycarpa</i>                 | <i>C. reticulata</i> hybrid                |                             | Nasnaran mandarin   | Curk et al. (2015)   |
| <i>C. × aurantiifolia</i> var. <i>aurantiifolia</i>     | <i>C. aurantiifolia</i>              | <i>C. aurantiifolia</i>                    | <i>C. × aurantiifolia</i>   | Mexican, Key, West Indies limes...                        | Curk et al. (2016), Wu et al. (2018), and Penjor et al. (2016)   |
| <i>C. × aurantiifolia</i> var. <i>macrophylla</i> ined. | <i>C. macrophylla</i> Wester         | <i>C. aurantiifolia</i> (Christm.) Swingle |                             | Alemow  | Curk et al. (2016)   |
| <i>C. × aurantiifolia</i> var. <i>aurata</i> ined.      | <i>C. aurata</i> Risso               | <i>C. limon</i> (L.) Burm. f.              | <i>C. × aurantium</i> L.    | Adam's apple  | Curk et al. (2016)   |
|   | <i>C. excelsa</i> Wester             | <i>C. aurantiifolia</i>                    |                             | Excelsa and Nestour lime                                  | Curk et al. (2016)   |
| <i>C. × aurantium</i> L. var. <i>aurantium</i>          | <i>C. aurantium</i>                  | <i>C. aurantium</i>                        | <i>C. × aurantium</i>       | Sour orange, Bouquetier                                   | Wu et al. (2014), Curk et al. (2015), Oueslati et al. (2017), Wu et al. (2018), and Penjor et al. (2016) |
|   | <i>C. myrtifolia</i> Raf.            |  |                             | Myrtle-leaf orange, Chinoto                               | Curk et al. (2015)   |
| <i>C. × aurantium</i> var. <i>clementina</i> ined.      | <i>C. clementina</i> hort. ex Tanaka | <i>C. reticulata</i>                       |                             | Clementine  | Wu et al. (2014), Curk et al. (2015), Oueslati et al. (2017), and Wu et al. (2018)                       |
| <i>C. × aurantium</i> var. <i>deliciosa</i> ined.       | <i>C. deliciosa</i> Ten.             | <i>C. reticulata</i>                       | <i>C. reticulata</i>        | Willowleaf, Chios mandarins                               | Wu et al. (2014), Curk et al. (2015), Oueslati et al. (2017), and Wu et al. (2018)                       |
| <i>C. × aurantium</i> var. <i>erythrosa</i> ined.       | <i>C. erythrosa</i> hort. ex Tanaka  | <i>C. tachibana</i>                        | <i>C. reticulata</i>        | Fuzhu and San hu hong chu mandarins                       | Oueslati et al. (2017)   |

Continued



**TABLE 4.1** Correspondences between the new phylogenomic classification and the former classifications of Tanaka (1961), Swingle and Reece (1967), and Mabberley (2004) revised by Zhang and Mabberley (2008)—cont'd

| Phylogenomic classification                           | Tanaka (1961)                           | Swingle and Reece (1967)    | Zhang and Mabberley (2008) | Common names (examples)                                   | Phylo-genomic references   |
|---|---|-----------------------------|----------------------------|---|--|
| <i>C. × aurantium</i> var. <i>kinokuni</i> ined.      | <i>C. kinokuni</i> hort. ex Tanaka      | <i>C. tachibana</i>         |                            | Kinokuni, Kishu, Huanglingmiao mandarins                  | Oueslati et al. (2017) and Wu et al. (2018)  |
| <i>C. × aurantium</i> var. <i>nobilis</i> ined.       | <i>C. nobilis</i> Lour.                 | <i>C. reticulata</i> hybrid | <i>C. × aurantium</i>      | King mandarin   | Curk et al. (2015), Oueslati et al. (2017), and Wu et al. (2018)                       |
| <i>C. × aurantium</i> var. <i>paradisi</i> ined.      | <i>C. paradisi</i> Macfad.              | <i>C. paradisi</i>          | <i>C. × aurantium</i>      | Star Ruby, Marsh, Duncan, etc.                            | Curk et al. (2015), Oueslati et al. (2017), Wu et al. (2018), and Penjor et al. (2016) |
| <i>C. × aurantium</i> var. <i>paratangerina</i> ined. | <i>C. paratangerina</i> hort. ex Tanaka | <i>C. reticulata</i>        |                            | Ladu Mandarin   | Oueslati et al. (2017)   |
| <i>C. × aurantium</i> var. <i>sinensis</i> L.         | <i>C. sinensis</i> (L.) Osbeck          | <i>C. sinensis</i>          | <i>C. × aurantium</i>      | Sweet oranges (Valencia, Washington Navel, Tarroco, etc.) | Wu et al. (2014), Curk et al. (2015), Oueslati et al. (2017), and Wu et al. (2018)     |
| <i>C. × aurantium</i> var. <i>suhuiensis</i> ined.    | <i>C. suhuiensis</i> hort. ex Tanaka    | <i>C. reticulata</i>        | <i>C. reticulata</i>       | Szibat and Se Hui Gan mandarins                           | Oueslati et al. (2017)   |
| <i>C. × aurantium</i> var. <i>tangerina</i> ined.     | <i>C. tangerina</i> hort. ex Tanaka     | <i>C. reticulata</i>        | <i>C. reticulata</i>       | Dancy, Beauty mandarins                                   | Curk et al. (2015), Oueslati et al. (2017), and Wu et al. (2018)                       |
| <i>C. × aurantium</i> var. <i>temple</i> ined.        | <i>C. temple</i> hort. ex Yu. Tanaka    | <i>C. sinensis</i>          |                            | Temple tangor   | Oueslati et al. (2017)   |
| <i>C. × aurantium</i> var. <i>unshiu</i> ined.        | <i>C. unshiu</i> Marcow.                | <i>C. reticulata</i> clone  | <i>C. reticulata</i>       | Satsuma mandarins   | Curk et al. (2015), Oueslati et al. (2017), Wu et al. (2018), and Penjor et al. (2016) |
| <i>C. × latifolia</i> var. nov. 1                     |   |                             |                            | India lime  | Curk et al. (2016)   |
| <i>C. × latifolia</i> var. nov. 2                     |   |                             |                            | Kirk lime   | Curk et al. (2016)   |
| <i>C. × latifolia</i> var. <i>latifolia</i>           | <i>C. latifolia</i>                     | <i>C. aurantiifolia</i>     | <i>C. × latifolia</i>      | Bears, Tahiti, Persian limes                              | Curk et al. (2016)   |
| <i>C. × limon</i> var. <i>bergamia</i> ined.          | <i>C. bergamia</i> Risso and Poit.      | <i>C. aurantiifolia</i>     | <i>C. × limon</i>          | Fantastico, Femminello, Castagnaro bergamots              | Curk et al. (2016), Penjor et al. (2016)   |
| <i>C. × limon</i> var. <i>meyerii</i> ined.           | <i>C. meyerii</i> Yu. Tanaka            | <i>C. limon</i>             | <i>C. × limon</i>          | Meyer lemon   | Curk et al. (2016)   |
| <i>C. × limon</i> var. <i>limettioides</i> ined.      | <i>C. limettioides</i> Tanaka           | <i>C. aurantiifolia</i>     |                            | Palestinian and Brazil sweet limes and Butnal sweet lemon | Curk et al. (2016), Penjor et al. (2016)   |
| <i>C. × limon</i> var. <i>limetta</i> ined.           | <i>C. limetta</i> Risso                 | <i>C. limon</i>             |                            | Marrakech limonette                                       | Curk et al. (2016)   |

**TABLE 4.1** Correspondences between the new phylogenomic classification and the former classifications of Tanaka (1961), Swingle and Reece (1967), and Mabberley (2004) revised by Zhang and Mabberley (2008)—cont'd

| Phylogenomic classification                           | Tanaka (1961)                   | Swingle and Reece (1967)    | Zhang and Mabberley (2008)  | Common names (examples)                                       | Phylo-genomic references                                       |
|---|---------------------------------|-----------------------------|-----------------------------|---|--|
| <i>C. × limon</i> var. <i>limon</i> (L.) Burm. f.     | <i>C. limon</i> (L.) Burm. f.   | <i>C. limon</i>             | <i>C. × limon</i>           | Lemons (Lisbon, Eureka, Verna, Luminciana, Interdonato, etc.) | Curk et al. (2016), Wu et al. (2018)                           |
| <i>C. × limonia</i> var. nov. 1                       |                                 |                             |                             | India sweet lime, Indian lemon                                | Curk et al. (2016)   |
| <i>C. × limonia</i> var. <i>jambhiri</i> ined.        | <i>C. jambhiri</i> Lush.        | <i>C. limon</i>             | <i>C. × taitensis</i> Risso | Rough lemon   | Curk et al. (2016), Wu et al. (2018), and Penjor et al. (2016) |
| <i>C. × limonia</i> Osbeck var. <i>limonia</i>        | <i>C. limonia</i>               | <i>C. limon</i>             |                             | Rangpur lime  | Curk et al. (2016), and Wu et al. (2018)                       |
|   | <i>C. karna</i> Raf.            |                             |                             | Khatta Kharna lime  | Curk et al. (2016)   |
| <i>C. × limonia</i> var. nov. 2                       |                                 |                             |                             | Voangiala   | Curk et al. (2016)   |
| <i>C. × limonia</i> var. <i>volkameriana</i> Pasquale | <i>C. limonia</i> Osbeck        | <i>C. limon</i>             |                             | Volkamer lemon  | Curk et al. (2016)   |
| <i>C. × lumia</i> var. nov. 1                         |                                 |                             |                             | Bitrouni lime   | Curk et al. (2016)   |
| <i>C. × lumia</i> var. nov. 2                         |                                 |                             |                             | Fourny hybrid   | Curk et al. (2016)   |
| <i>C. × lumia</i> var. <i>lumia</i>                   | <i>C. lumia</i> Risso and Poit. | <i>C. limon</i>             |                             | Jaffa lemon   | Curk et al. (2016)   |
| <i>C. × lumia</i> var. <i>pyriformis</i> ined.        | <i>C. pyriformis</i> Hassk.     | <i>C. limon</i>             | <i>C. maxima</i>            | Ponderosa lemon   | Curk et al. (2016)   |
| <i>C. × microcarpa</i>                                | <i>C. madurensis</i> Lour.      | <i>C. reticulata</i> hybrid | <i>C. × microcarpa</i>      | Calamondin, Calamansi   | Curk et al. (2016)   |
| <i>C. × pseudolumia</i> ined.                         |                                 |                             |                             | Borneo, Barum, Baboon lemons                                  | Curk et al. (2016)   |

***C. micrantha* Wester** originated from the Southern Philippines. According to Swingle and Reece (1967), it should include *C. micrantha* var. *micrantha*, the “small flowered papeda” locally called Biasong and *C. micrantha* var. *microcarpa*, the “small-fruited papeda” with the native name Samuyao. Some chloroplast (Bayer et al., 2009; Nicolosi et al., 2000), mitochondrial (Froelicher et al., 2011), and nuclear phylogenetic studies (Nicolosi et al., 2000; Curk et al., 2015; Ollitrault et al., 2012) suggest that *C. micrantha* and *C. hystrix* are closely related. They may eventually be treated as a single species after deeper genomic analysis of *C. hystrix*. *C. micrantha* is involved in several admixture taxa: *C. × amblycarpa*, *C. × aurantiifolia*, and *C. × latifolia*.

***C. reticulata* Blanco** is proposed to include only nonintrogressed mandarins. According to Wu et al. (2018) WGS data, it includes two mandarins. One is classified by Swingle (1943) and Tanaka (1931) as *C. tachibana* (Mak.) Tanaka and is widespread in southern Taiwan, the Ryukyu Islands, and southern Japan (Tanaka, 1931). The second one is the Sun-Chu-Sha-Kat Chinese mandarin treated as *C. reticulata* var. *austera* by Swingle (1943) but confusingly treated (UCR, 2017b) as *C. erythrosa* by Tanaka (1954). Regarding *C. tachibana*, these conclusions for pure *C. reticulata* concern only the type classified as *C. tachibana* (Mak.) Tanaka by Tanaka; indeed, four other Tanaka species *C. erythrosa* hort. ex Tanaka, *C. kinokuni* hort. ex Tanaka, *C. ponki* hort. ex Tanaka, and *C. oleocarpa* hort. ex Tanaka are included in *C. tachibana* Swingle and

Reece (1967). Among these four species, molecular data suggest that at least *C. kinokuni* (“Nanfeng Miju” mandarin) and *C. erythrosa* (“Fuzhu,” “San hu hong chu” mandarins) are introgressed at low level by *C. maxima* (Curk et al., 2015; Oueslati et al., 2017). WGS data clearly differentiated *C. tachibana* (Mak.) Tanaka from “Sun-Chu-Sha-Kat” (Wu et al., 2018). Moreover, “Sun-Chu-Sha-Kat” displays much more relatedness with other mandarins than Tachibana does (Wu et al., 2018). Therefore, Wu et al. (2018) suggest “it may be more useful to consider *C. tachibana* (Mak.) as a subspecies of *C. reticulata* arising from allopatric isolation.” To keep a similar system for pure and hybrid species, we propose to treat them as *C. reticulata* var. *tachibana* and *C. reticulata* var. *austera*, respectively. The treatment of “Cleopatra” and “Sunki” mandarins (respectively, *C. reshni* and *C. sunki* in Tanaka and *C. reticulata* var. *austera* in Swingle), which have only one minor *C. maxima* putative introgression (Wu et al., 2018; Oueslati et al., 2017) needs deeper analysis. A phylogenomic characterization of *C. daoxianensis* S. W. He and G. F. Liu, a wild Chinese mandarin, found to be pure *C. reticulata* in a discrete diagnostic SNP study (Curk et al., 2015), is also necessary to determine whether it is synonymous with *C. reticulata*. *C. reticulata* is involved in several admixture taxa: *C. × aurantium*, *C. × amblycarpa*, *C. × limonia*, *C. × microcarpa*, *C. × lemon*, and *C. × latifolia*.

#### 4.2.3.2 Admixture types

##### Bispecific admixture

*C. × amblycarpa* should include all admixtures between *C. micrantha* and *C. reticulata* such as “Nasranan,” an Indonesian citrus considered to be a direct *C. micrantha* × *C. reticulata* hybrid (Curk et al., 2015; Ollitrault et al., 2012). Indeed, it shares the *C. micrantha* mitochondrial genome (Froelicher et al., 2011) and displays interspecific heterozygosity (*C. micrantha*/*C. reticulata*) for nuclear markers all along its genome (Curk et al., 2015; Ollitrault et al., 2012).

*C. × aurantiifolia* includes all *C. micrantha*/*C. medica* admixtures and particularly direct hybrids between *C. micrantha* and *C. medica* such as *C. × aurantiifolia* var. *aurantiifolia* (“Mexican” lime, “West Indies” lime, and “Thornless” lime) according to Nicolosi et al. (2000), Curk et al. (2016), Penjor et al. (2016), and Wu et al. (2018). According to Curk et al. (2016), *C. × aurantiifolia* var. *aurata* (“Adam’s apple”; “Excelsa” and “Nestour” limes) and *C. × aurantiifolia* var. *macrophylla* are also direct hybrids between *C. micrantha* and *C. medica*. Kaghzi and New Caledonian limes displaying a more complex phylogenomic structure with homozygous areas (Curk et al., 2016) should be classified as *C. × aurantiifolia*. The triploid Tanepao, Ambilobe, Coppenrath, and Mothasseb limes, and Madagascar lemon share a similar *C. micrantha*/*C. medica* structure and probably derive from an interspecific backcross ((*C. micrantha* × *C. medica*) × *C. medica*) involving a diploid ovule of *C. × aurantiifolia* (Curk et al., 2016). They should also be classified as *C. × aurantiifolia*.

*C. × aurantium*, as stated before, includes all *C. reticulata*/*C. maxima* admixtures. If we refer to the demonstrated admixture (Wu et al., 2014, 2018; Curk et al., 2015; Oueslati et al., 2017), it should concern: *C. × aurantium* var. *aurantium* (sour oranges, “Bouquetiers”); *C. × aurantium* var. *sinensis* (sweet oranges), *C. × aurantium* var. *paradisi* (grapefruits); *C. × aurantium* var. *tangerina* (“Dancy,” “Beauty” mandarins), *C. × aurantium* var. *unshiu* (satsuma mandarins), *C. × aurantium* var. *clementina* (clementines), *C. × aurantium* var. *nobilis* (“King” mandarin), *C. × aurantium* var. *temple* (“Temple” mandarin), *C. × aurantium* var. *deliciosa* (“Willowleaf” and “Chios” mandarins), *C. × aurantium* var. *erythrosa* (“Fuzhu” and “San hu hong chu” mandarins), *C. × aurantium* var. *paratangerina* (“Ladu” mandarin), and *C. × aurantium* var. *suhuiensis* (“Szibat” and “Se Hui Gan” mandarins). All others mandarins classified as type 3 by Wu et al. (2018) from WGS data, should be treated as *C. × aurantium*. Among these varieties, sour orange appears to be the only direct hybrid, while the other ones display more complex genomic structure with phylogenetically homozygous fragments (*C. maxima*/*C. maxima* or/and *C. reticulata*/*C. reticulata*) in addition to *C. reticulata*/*C. maxima* heterozygosity. Recent hybrids from breeding programs (mandarin hybrids, tangors—mandarin × sweet orange-, tangelos—mandarin × grapefruit-, orangelos—sweet orange × grapefruit) as well as natural tangors and tangelos should also be classified in *C. × aurantium*.

*C. × limonia* includes all *C. reticulata*/*C. medica* admixture types and particularly according to Curk et al. (2016) and Wu et al. (2018) the direct hybrids between these two species: *C. × limonia* var. *limonia* (“Rangpur,” “Karna,” “Khatta,” “Khatta Karna” limes); *C. × limonia* var. *volkameriana* (“Volkamer” lemon; “Kaghi” lime); *C. × limonia* var. *jambhiri* (“Rough” lemon). Cytogenetic studies also provide evidence for a mandarin × citron origin of “Volkamer” lemon, “Rough” lemon, and “Rangpur” lime (Carvalho et al., 2005), while mitochondrial markers (Froelicher et al., 2011) and chloroplast sequences (Carbonell-Caballero et al., 2015) revealed that the female mandarin parent was close to *C. reticulata* var. *austera*. Unclassified cultivars such as the “Voangiala” lemon on one hand and India lemon and Indian sweet lime on the other hand, represent two other *C. reticulata* × *C. medica* independent reticulation events (Curk et al., 2016).

*C. × lumia* corresponds to a *C. medica*/*C. maxima* admixture. According to Curk et al. (2016), it may include *C. × lumia* var. *lumia* (“Jaffa” lemon), *C. × lumia* var. *pyriformis* (“Ponderosa” lemon), and the previously unclassified “Bitrouni” lime and “Hybride de Fourny” lemon. The “Bitrouni” lime displays a *C. aurantium* var. *aurantium* cytoplasm, while the others



have the *C. maxima* cytoplasm shared with sweet orange (Curk et al., 2016). The ‘Hybride de Fourny’ appears to be a direct hybrid, while the others have more complex structure with *C. maxima* or *C. medica* homozygosity.

*C. × microcarpa* includes all the kumquat/*C. reticulata* admixtures and particularly the calamondin or calamansi *C. × microcarpa* var. *microcarpa* treated as a *C. reticulata* hybrid by Swingle (1943) and *C. × microcarpa* by Mabberley (2004). Indeed, according to Wu et al. (2018), the calamondin is a direct hybrid between kumquat and mandarin with a kumquat cytoplasm (Carbonell-Caballero et al., 2015).

### Complex tri and tetraspecific admixtures

*C. × limon* includes all *C. reticulata*/*C. maxima*/*C. medica* admixtures. Molecular and cytogenetic studies (Nicolosi et al., 2000; Garcia-Lor et al., 2013a; Curk et al., 2016; Ollitrault et al., 2012; Carvalho et al., 2005; Gulsen and Roose, 2001; Ramadugu et al., 2013) suggested that the “yellow lemon” types originated from a *C. × aurantium* var. *aurantium* × *C. medica* hybridization and this was definitively proved by WGS data (Wu et al., 2018). *C. × limon* var. *limon* should include all lemon types derived by mutation (“Lisbon,” “Eureka,” “Vern” or “Berna,” “Fino,” “Santa Theresa,” “Adamopoulos,” “Luminciana,” “Interdonato,” etc.) of the original hybrid. *C. × limon* var. *limetta* (“Marrakech” limonette) had the same parents as *C. × limon* var. *limon* but resulted from an independent reticulation (Curk et al., 2016). *C. × limon* should also include *C. × limon* var. *limettioides* (“Palestinian” and “Brazilian” sweet limes and “Butnal” sweet lemon) and *C. × limon* var. *meyerii* (“Meyer” lemon). These two types probably resulted from hybridization between a *C. × aurantium* female parent different than var. *aurantium* (with *C. maxima* cytoplasm) pollinated by *C. medica* (Curk et al., 2016). The Bergamot is also included in this tri-specific group as *C. × limon* var. *bergamia* (Curk et al., 2016; Penjor et al., 2016). It probably resulted from hybridization between *C. × limon* var. *limon* and *C. × aurantium* var. *aurantium*. Several genotypes, erroneously named citrons, may also be classified as *C. × limon*: the “Damas,” “Mak Nao Si,” and “Rhobs el Arsa” “citrons” that share the *C. aurantium* var. *aurantium* cytoplasm (Curk et al., 2016). The “Milam” lemon and the “Alikioti” lime also display the tri-specific structure of *C. × limon* with a *C. reticulata* cytoplasm (Curk et al., 2016).

*C. × pseudolumia* is proposed for admixtures between *C. maxima*, *C. medica*, and *C. micrantha*. Such constitution was revealed by Curk et al. (2016) for Borneo and Barum lemons of the INRA-CIRAD Corsican collection. The two accessions were identical with about 50% *C. maxima*, 38% *C. medica* and 12% *C. micrantha* nuclear genome contributions and a *C. maxima* cytoplasm shared with *C. × aurantium* var. *sinensis*. These two varieties may result from a *C. maxima* *C. × aurantiifolia* natural cross. Borneo lemon was morphologically described by Chapot (1964) and considered close to the *Lumia* but different of the *Lumia* cultivars previously described, with serious similarities with the Indian “Gulgul” or “Galgul” fruits. It displays profiles of leaf and peel oils very different than other lemons and *lumia* types with high content in linalool/linalyl acetate and α-terpineol and linalool/linalyl acetate, respectively (Lota et al., 2002). Despite its name, Chapot (1964) states that the Borneo lemon was not known in Indonesia but probably originated in India. It was cultivated in North Africa during the 20th century and introduced in the United States under the Baboon lemon name.

*C. × latifolia* includes the genotypes with admixtures of the four ancestors, *C. reticulata*/*C. maxima*/*C. medica*/*C. micrantha*. According to Curk et al. (2016), it may include *C. × latifolia* var. *latifolia* (the triploid “Tahiti,” “Bears,” “Persian” limes) and two diploid limes (Kirk and India) with complex admixture of the four ancestral taxa. Kirk lime and India lime share the *C. micrantha* and the *C. reticulata* var. *austera* cytoplasm, respectively. The triploid *C. × latifolia* var. *latifolia* limes may result from a (*C. × limon* var. *limon*) × (*C. × aurantiifolia* var. *aurantiifolia*) hybridization with a diploid *C. × aurantiifolia* pollen (Rouiss et al., 2018; Curk et al., 2016).

## 4.3 Phenotypic diversity structure strongly reflects evolutionary history

The limitation of gene flow between populations is, with selection, one of the main driving factors for genetic and phenotypic differentiation and can lead to speciation. For *Citrus* s.l. (true citrus plus *Oxanthera*), allopatric evolution has been a clear determinant of the gene pool structure. This is apparent for endemic species of Australia and the Oceanic Islands. There is also evidence in the ancestral Asian species of cultivated *Citrus*, as explained below. Differences in flowering season were also probably a key component for the parapatric differentiation of mandarins, trifoliate oranges, and kumquats in China.

We discuss in this part the phenotypic diversity of the edible Asian citrus classified in the genus *Citrus* by Swingle (1943), not including the kumquats and their hybrids. Indeed, the genomically proven contribution of kumquat to admixtures in citrus germplasm is limited to calamondin (mostly an ornamental type and a condiment in the Philippines cooking) and few studies of phenotypic diversity structure of edible citrus have included kumquat. A description of morphological characteristics of kumquat was provided in Section 4.1.2.2.

### 4.3.1 Reticulate evolution, apomixis, and the correlation between the structures of genetic and phenotypic diversities in the Asian edible *Citrus* species

The differentiation between the four ancestral taxa of Asian edible *Citrus*, which are sexually compatible, can be explained by a founder effect in four geographic zones and by initial allopatric evolution (Swingle and Reece, 1967; Scora, 1975; Webber, 1967; Wu et al., 2018). *C. maxima* originated in the Malay Archipelago and Indonesia, *C. medica* evolved in Northeastern India and the nearby region of Burma and China, *C. reticulata* was originally found over a region including Vietnam, Southern China, and Japan, and *C. micrantha* is native to the southern Philippines, particularly islands of Cebu and Bohol. As described before, the other edible *Citrus* ideotypes resulted from admixture of these taxa. In addition, vegetative propagation occurred immediately or a few generations after the reticulation events owing the facultative apomixis present in most admixture ideotypes. Therefore, the number of interspecific meiosis and recombination events was limited and large parts of the genome of modern citrus remain in interspecific heterozygosity (Wu et al., 2014, 2018; Oueslati et al., 2017). This reticulate evolution coupled with apomixis also led to generalized linkage disequilibrium when considering the global gene pool of the genus *Citrus*, sensu Swingle (Garcia-Lor et al., 2012). As a consequence: (i) an important part of the actual phenotypic diversity of edible citrus should be related to the differentiation between species before reticulation and introgression processes and (ii) the structures of the phenotypic and genetic diversities are closely correlated. Such correlations were observed for morphological and pomological characters (Ollitrault et al., 2003; Barrett and Rhodes, 1976), flavone constitution (Mizuno et al., 1991), peel oil volatile compounds (Liu et al., 2013b), carotenoid contents (Fanciullino et al., 2006), coumarin and furanocoumarin constitution (Dugrand-Judek et al., 2015), and fingerprinting of secondary metabolites (Matsukawa and Nito, 2017). Recently, Wu et al. (2018) found a relationship between the proportion of *C. maxima* genome and fruit size in the *C. maxima*/*C. reticulata*/*C. × aurantium* gene pool.

### 4.3.2 Traits of the four Asian ancestral taxa of the edible *Citrus* (Fig. 4.8)

#### 4.3.2.1 *C. maxima* (Burm.) Merri

*Citrus maxima* is widely distributed and cultivated in Southeastern Asia and the East Indian Archipelago with the English common name of pummelo. It was introduced into the Caribbean during the discovery period of the New World, where it is named shaddock. A natural hybridization with sweet orange occurring in the Caribbean region produced the grapefruit (see more detail below). According to the description made by Swingle and Reece (1967), *C. maxima* has the biggest flowers (with five sepals and petals and 20–25 stamens, with large linear anthers) and produces the biggest fruits in *Citrus*, which are oblate-spheroid or subpyriform with large, thick, wrinkled seeds. The fruit usually has a thick peel and very large pulp vesicles compared with other *Citrus* species. The membranes enclosing the segments are very strong and can easily be peeled. The weakly adherent pulp vesicles can then be separated. *Citrus maxima* presents additional distinctive characteristics compared with other *Citrus* species. Young angular twigs, leaf midribs, and large veins and petioles are often pubescent. Leaves are “large or very large, oval or elliptic-oval, with a blunt point at the tip and a broadly rounded base, often subcordate and even slightly overlapping the winged petiole... the petiole is broadly winged, and more or less cordate” (Swingle and Reece, 1967). *C. maxima* produces a high level of several secondary metabolites such as naringin (Swingle and Reece, 1967) and coumarins and furanocoumarins (Dugrand-Judek et al., 2015). It is a monoembryonic species with a gametophytic self-incompatibility system (Soost, 1968).

#### 4.3.2.2 *C. medica* L.

*C. medica* is now widespread in northeastern, central and southern India, Bangladesh, Myanmar, Bhutan and Yunnan Province, and China (Swingle and Reece, 1967; Hodgson, 1967; Gmitter and Hu, 1990; Hazarika, 2012). It was the first species introduced to the Mediterranean Basin following the invasion of Persia by Alexander the Great around 325 BC. It is monoembryonic, self-compatible, and mainly cleistogamous, which led to the high homozygosity of modern cultivars owing to endogamy (Curk et al., 2016; Wu et al., 2018; Curk et al., 2015) and observation that probably explains why it is systematically found as the male parent in admixtures (Nicolosi et al., 2000; Curk et al., 2016; Wu et al., 2018). The following description is adapted from Swingle and Reece (1967). Citron trees are shrubs or small. Leaves are glabrous, elliptic-ovate or ovate-lanceolate, bluntly pointed or rounded at the tips, cuneate or rounded at the base with stout, short, single spines in the axils. Their petioles are short, wingless, or narrowly margined. Inflorescences are short with few-flowered racemes. The flowers are large with generally purplish buds with five petals. They are perfect or male with very numerous stamens (Ollitrault et al., 2003; Raghuvanshi, 1969; Nair and Randhawa, 1969; Guerra, 1993; Hynniewta et al., 2011; Dirceu et al., 2016; Rouiss et al., 2018; Kamiri et al., 2011; Xie et al., 2015; Herrero et al., 1996a,b). The ovary has a height of 18 (usually





**FIG. 4.8** Illustrations of morphological traits of the four ancestral taxa of Asian edible *Citrus*. (A) *C. maxima* (©F. Curk-Inra); (B) *C. medica* (© F. Curk-Inra); (C) *C. micrantha* var. *microcarpa* (photo: Courtesy UC Riverside Citrus Variety Collection); and (D) *C. reticulata* var. *austera* Sun Chu Sha Kat' (photo: Courtesy UC Riverside Citrus Variety Collection).

10–14) locules with—four to eight or more ovules in each locule in two collateral rows. Fruits are medium (10 cm) to very large (50 cm) according to [Ramadugu et al. \(2015\)](#), oblong, oval, or fingered with smooth or more often rough and bumpy surface. The fruit is very fragrant and yellow when ripe. The rind is very thick with small segments, filled with pale greenish pulp vesicles. They generally contain few seeds ([Webber, 1943, 1967](#)) with one white embryo.

#### 4.3.2.3 *C. micrantha* Wester

Future WGS studies of the different Tanaka and Swingle papada species could lead to *C. micrantha* and other species being synonymized as discussed above for *C. hystrix* (Combava, kaffir lime). The description provided here is from Wester in [1915](#). *C. micrantha* is cultivated on a small scale as a hair wash in the southern Philippine Islands (Cebu, Bohol, Negros, and Mindanao). It is not eaten and is of no economic importance. [Wester \(1915\)](#) recognized two varieties: *C. micrantha* var. *micrantha* Wester (the small-flowered papada locally named “Biasong”) and *C. micrantha* var. *microcarpa* Wester (small-fruited papada, locally named “Samuyao”). The two types present similar traits but *C. micrantha* var. *microcarpa* displays a global reduction of morphological characters. According to the original description of the species by [Wester \(1915\)](#), the characteristics of both *C. micrantha* var. *micrantha* (and *C. micrantha* var. *microcarpa* in parentheses), are as follows: a tree attaining a height of 7.5–9 (4.5) m; leaves 9–12 (5.5–8) cm long, 27–40 mm broad ([Ding et al., 1984; Duan, 1990; Mayr, 1942; Mayden, 1997; de Queiroz, 2007; Dobzhansky, 1970](#)), broadly elliptical to ovate, petioles 35–60 mm long ([Ding et al., 1984; Duan, 1990; Mayr, 1942; Mayden, 1997; de Queiroz, 2007; Dobzhansky, 1970; Andersson, 1990; Nixon and Wheeler, 1990; Donoghue, 1985; Mishler, 1985; Ollitrault et al., 2003](#)), broadly winged, up to 40 (about 14) mm wide; flowers small,

12–13 mm (Bayer et al., 2009; Tolkowsky, 1938; Tanaka, 1954; Scora, 1975; Webber, 1943) in diameter, white, with a trace of purple on the outside, petals 4 (Swingle and Reece, 1967; Morton et al., 2003; Bayer et al., 2009), stamens 15–18 (Bayer et al., 2009; Tolkowsky, 1938; Tanaka, 1954; Scora, 1975; Webber, 1943, 1967; Ramon-Laca, 2003; Mabberley, 2004; Linnaeus, 1753; Swingle, 1943; Zhang and Mabberley, 2008; Krueger and Navarro, 2007; Siebert, 2016), ovary obovoid with 6–8 locules (Tanaka, 1954; Scora, 1975; Webber, 1943); fruits obovate to oblong-obovate, 5–7 cm long and 3–4 cm in transverse diameter (roundish in outline, 1.5–2 cm in diameter). *C. micrantha* is probably monoembryonic (UCR, 2017c).

#### 4.3.2.4 *C. reticulata* Blanco

Current WGS data provide evidence of only *C. reticulata* var. *tachibana* ined. and *C. reticulata* var. *austera* Swingle as being not introgressed by *C. maxima*. However, few of the primitive mandarin types have been re-sequenced until this moment. *Citrus daoianensis*, a Chinese wild mandarin without evidence of admixture in a discrete molecular marker study (Curk et al., 2015), is an example of potentially pure *C. reticulata*. We provide here a description of *C. reticulata* var. *tachibana* and *C. reticulata* var. *austera* based on Swingle (1943) and Tanaka (1954). Both types produce small, highly seedy, and very acidic fruits characteristic of undomesticated types. Their fruits are orange at maturity. Both are polyembryonic.

*C. reticulata* var. *austera* is frequent in the Swatow region of Kwangtung where it is used as rootstock. It is naturally found in Assam (India), China, and Japan (Wu et al., 2018). It differs from the sweet mandarins by its small, intensely acidic fruits. Swingle and Reece (1967) described it as follows: “Fruits slightly depressed globose, 2.9–3.3 cm long, 3.3–3.6 cm diameter, with smooth, loose peel about 4 mm thick, capucine yellow when ripe; oil glands small, round, far apart, fragrant; segments 9, easily separated; segment walls thin, tender, white; core 6–8 mm diameter, soft; pulp deep chrome yellow, composed of small, short, pulp vesicles, clinging together but irregularly arranged and easily broken; juice reddish yellow, very sour; seeds about 9, rounded at one end, pointed at the other, showing white parallel lines from base to tip; leaves lanceolate–elliptical, blades 6.8×2.5 cm, rather acutely cuneate at the base and narrowed to a blunt apex, with about 10 pairs of lateral veins; petioles nearly wingless.”

*C. reticulata* var. *tachibana* is widespread from southern Taiwan to the southwestern province of the main island of Japan. Swingle and Reece (1967) considered it a “wild species that has persisted since prehistoric times.” Many of its characteristics are close to *C. reticulata* var. *austera*. Tachibana is self-compatible (Yamamoto et al., 2006). A description of this species by Makino, as translated by Katsura, and reported by Swingle and Reece (1967) reads: “Tree stands over 10 feet. Branches and leaves grow thickly. Strongly resistant to frost or snow... Leaves long, ovate-elliptical, subcoriaceous, broadly acuminate, obtuse and incised at the tip, somewhat broad and convex at the base, indistinctly dentate at the margin, midrib slender, straight and distinct beneath, veins almost indistinct, oil glands indistinct; petiole short, small, with linear wings which seem to be on the verge of degeneration. Flowers axillary, solitary, small. Pedicels 2 mm long, slender, glabrous; scales at the base triangular, ciliate at the margin. Calyx 3 mm in diameter; sepals somewhat recurved outward, densely ciliate at the margin, etc. Ovary almost globular, attenuate at the base, about 2×2 mm in size, etc. Fruit somewhat flattened, 2–3 cm lateral diam. Skin smooth, oil glands scattered beneath the skin. Segment cases 6–7; juice bitter and almost inedible. Seeds 1–2 in a segment, and rather large in size, etc. Flowers the same as other *Citrus* plants in time of blooming, shape and color.”

### 4.3.3 Traits of some modern citrus taxa resulting from admixture

With the proposed classification concept based on admixture, the morphological and phenological characteristics within admixture taxa may vary a lot, not only from one ancestor to the other one, but also with transgressive forms. Therefore, an exhaustive description of the pattern of variation is difficult to provide, as new hybrid combinations could produce new transgressive forms. We present here the descriptions of some of the most economically important admixture varieties, synthesized from Swingle (1943) and Zhang and Mabberley (2008).

Sour orange (*C. × aurantium* var. *aurantium*) and sweet orange (*C. × aurantium* var. *sinensis*) are believed to have arisen from the admixture of *C. reticulata* and *C. maxima*. As per the common names, these two taxa have much in common. The mutually coherent pulp vesicles are free from oil droplets and never contain acrid oils; the medium-sized fruits (5–9 cm diameter) have adherent peels and contain numerous segments (Tolkowsky, 1938; Tanaka, 1954; Scora, 1975; Webber, 1943, 1967; Ramon-Laca, 2003; Mabberley, 2004; Linnaeus, 1753; Swingle, 1943; Zhang and Mabberley, 2008); the flowers are large (2.5–4.5 cm in diameter); and the leaves have winged petioles less than half as long as the leaf blade. The chief differences between the sweet and sour oranges are mostly concerned with the fruit, although the petioles of sour oranges are broader and longer than those of sweet oranges. The fruits of the sour orange have a brighter and rougher peel than those of the sweet orange; the oil glands are in sunken areas of the peel, whereas sweet oranges have oil glands in convex areas of the peel; and, of course, they are not as sweet as those of the sweet orange (lower brix:acid ratio).



Fruits of sour orange and its hybrids have a tendency to have hollow cores and a flattened form, although this is not a consistent characteristic. Sweet orange is one of the most important cultivated forms of *Citrus* due to its palatability and nutritional value. Sour orange is cultivated for flavoring, marmalade, and perfumery and historically it was used as a rootstock.

The grapefruit (*C. × aurantium* var. *paradisi*) is believed to have resulted from a further cross of sweet orange with pummelo. In contrast to the origin of its parents, in Southeast Asia, the origin of *C. × aurantium* var. *paradisi* is fairly well established as having occurred in historical times in the Caribbean (Kumamoto et al., 1987; Bowman and Gmitter, 1990). It differs from sweet orange chiefly in having larger (9–13 cm diameter) fruits with large, coherent juice vesicles. The yellow fruits have a lower brix:acid ratio than sweet orange, with a distinctive flavor and sometimes with pinkish or pink-tinged flesh. Grapefruit is cultivated commercially as an edible fruit, although its importance has been decreasing in the recent years.

The lemon, *C. × limon* var. *limon*, has resulted from a cross between *C. × aurantium* var. *aurantium* and *C. medica*. As with sweet and sour oranges, *C. × limon* var. *limon* has mutually coherent pulp vesicles that are free from oil droplets and never contain acrid oils; medium-sized fruits (5–9 cm diameter) having adherent peels and containing numerous segments (Tolkowsky, 1938; Tanaka, 1954; Scora, 1975; Webber, 1943, 1967; Ramon-Laca, 2003; Mabberley, 2004; Linnaeus, 1753; Swingle, 1943; Zhang and Mabberley, 2008); and large flowers (2.5–4.5 cm in diameter), generally with a pink tinge in the common acid types. The fruit shape is more or less oval, with a low apical papilla. The thick peel is yellow when ripe, with fairly prominent oil glands. The lemon is generally an acidic fruit, although low-acid selections occur. It is cultivated for use as fresh fruit and for flavoring.

Small fruited limes, *C. × aurantiifolia* var. *aurantiifolia*, have originated as a cross between *C. micrantha* and *C. medica*. As with the admixtures previously described, *C. × aurantiifolia* var. *aurantiifolia* has mutually coherent pulp vesicles that are free from oil droplets and never contain acrid oils. The fruits are small (4–6 cm diameter), ovoid, or subglobose, often with a small apical papilla, with 9–12 segments. The thin peel is yellow-green when mature and has prominent oil glands. The flowers are small (<2.5 cm diameter) and have a pinkish tinge in the common acid types. Although both acid and low-acid selections exist, the acid types are the more commonly cultivated.

The large-fruited acid limes, *C. × latifolia* var. *latifolia*, were proposed by Curk et al. (2016) to have resulted from admixture of *C. limon* var. *limon* × *C. aurantiifolia* var. *aurantiifolia*. The fruit is similar to those of *C. × aurantiifolia* var. *aurantiifolia*, but is larger (6–9 cm diameter), and seedless, probably due to the triploidy. Their taste and aroma are similar to *C. × aurantiifolia* var. *aurantiifolia* but are not as strong. The trees of *C. × latifolia* are also more vigorous and robust than those of *C. × aurantiifolia* var. *aurantiifolia*. Interestingly, this triploid lime is described to be one of the less susceptible cultivar to Huanglongbing, a very damaging bacterial disease due to *Liberibacter* spp. (Folimonova et al., 2009).

## 4.4 Conclusion

The definition and classification of the *Citrus* genus has long been debated by taxonomists. The reticulate evolution combined with partial apomixis has led to very different classification systems. Recently, phylogenomic data revealed the origins and admixtures of modern cultivars and wild types. Coupled with reproductive biology, phylogenomics supports the inclusion of all true citrus of the Swingle system plus Oxanthera in the genus *Citrus*. Considering biological and genomic data, the recognition at species rank of the four ancestral taxa of most cultivated citrus is supported by the phylogenetic species concept based on diagnosability and monophyly. Taking into account the implication of reticulate evolution, partial apomixis and asexual diversification from ancestral hybrids we propose, for the genus *Citrus*, a trinomial concept of classification. For admixture taxa, the species rank is defined by their phylogenomic constitution. The variety rank is defined by the old independent reticulation events from which groups of cultivars were differentiated by asexual mechanisms. It provides an unambiguous conceptual framework for *Citrus* classification based on the phylogenomic and genetic data. However, today, the available genomic data remain parcellar and further WGS studies are needed to establish a definitive classification of the genus *Citrus*.

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