

What is *Citrus*? Taxonomic implications from a study of cp-DNA evolution in the tribe Citreae (Rutaceae subfamily Aurantioideae)**

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Abstract

The taxonomy of *Citrus* is discussed in the light of the phylogeny of *Citrus* and allied genera inferred from the evolution of the segments trnL(UAA)-trnF(GAA) and trnT(UGU)-trnL(UAA) of the cpDNA. Twenty-eight species from twelve genera of subfamily Aurantioideae were sampled. Phylogenies constructed using maximum parsimony and neighbor-joining are well supported by high bootstrap values. The molecular data support a clade constituted by *Citrus*, *Poncirus*, *Fortunella*, and *Microcitrus*, but do not support an hypothesis of monophyly of *Citrus* due to the isolated position of *C. medica*. These results are congruent with an analysis of morphological evolution of diagnostic characters within the tribe Citreae. A more conservative position with a wider definition of *Citrus* to include all the cited relatives is intended to avoid great nomenclatural changes in such an economically important group.

Key words: Rutaceae, Aurantioideae, *Citrus*, phylogeny, taxonomy, cpDNA

Introduction

The taxonomy of *Citrus* L. and allies has been a challenge for botanists. Most of its complexity derives both from the biology of the species and its cultivation history. The species have great ability to cross and to produce hybrids (both intra- and intergeneric). Some of these hybrids are fertile by zygote-derived embryos or can become fertile by spontaneous formation of adventitious nucellar embryos, which contributes to the maintenance of genetic stability and to the perpetuation of the hybrids as apomictic clones (Chapot 1975, Scora 1975). Associated with these biological properties, species of *Citrus* were transferred between areas in the Far East (Chapot 1975, Scora 1975), where crossing between species became possible, blurring the taxonomic limits previously maintained by geographic isolation. This resulted in a swarm of morphological forms whose number ranges in different interpretations from as few as 11 (Engler 1931) to as many as 145 species (Tanaka 1977).

The taxonomic situation is further complicated by shifting boundaries between *Citrus* and allied genera. The history of the subfamily Aurantioideae was marked by the proposal of new genera separated from *Citrus*, such as *Poncirus* Raf. (Swingle & Reece 1967), *Fortunella* Swingle (Swingle 1915a), and *Microcitrus* Swingle (Swingle 1915b). Engler (1896) initially treated the allied species of *Citrus* in just one genus, but later on he accepted this subdivision (Engler 1931). However, there are no strong morphological characters to separate these genera, and many of the supposedly diagnostic characters of one genus can be found in the species of another. Burkill (1931) treated Swingle's genera as subgenera of *Citrus*, but his proposal was largely overlooked. More recently, Mabberly (1998), when revising *Citrus* and related genera in Australia, merged with *Citrus* the genera *Fortunella*, *Eremocitrus* Swingle, and *Microcitrus*.

One of the most usually accepted classifications for the subtribe Citrinae (Swingle & Reece 1967) is clearly

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gradist, with the genera being arranged as if they were in a progressive series: “Primitive Citrus Fruit Trees”, “Near-Citrus Fruit Trees” and “True Citrus Fruit Trees”. In the present work, this problem is treated with an explicit phylogenetic hypothesis for *Citrus* and allied genera, based on the evolution of two segments of cpDNA. A complete analysis of the molecular evolution of the group with discussion of the parental lineages in *Citrus* is being prepared by Araújo and Machado.

Material and methods

Plant material was obtained from the active germplasm banks of the Sylvio Moreira Citrus Centre (CCSM – IAC, Cordeirópolis, São Paulo, Brazil) and the National Centre of Cassava and Tropical Fruits (CNPMP – EMBRAPA, Cruz das Almas, Bahia, Brazil) (Table 1). Total DNA was extracted from lyophilised leaves according to the methodology of Murray & Thompson (1980) modified by Machado et al. (1996).

Table 1. Species included in this study. Taxonomic treatment follows Swingle & Reece (1967).

Taxa	Place of origin	Voucher ^a	GenBank Accession No ^b		
			R1	R2	R3
Tribe Clauseneae					
Subtribe Clauseniinae					
<i>Murraya paniculata</i> (L.) Jack	Malay Peninsula	CV415	AY116525	AY115655	AY115632
Subtribe Merrilliinae					
<i>Merrillia caloxylon</i> (Ridl.) Swingle	Malay Peninsula	CN733	AY116523	AY115653	AY115630
Tribe Citreae					
Subtribe Balsamocitrinae					
<i>Aegle marmelos</i> (L.) Corrêa	India	CV411	AY116508	AY115638	AY115615
<i>Feroniella oblata</i> Swingle	Indo-China	CV410	AY116511	AY115641	AY115618
Subtribe Citrinae					
<i>Atalantia monophylla</i> DC.	India	CRZ01	AY116509	AY115639	AY115616
<i>Citrus</i> subg. <i>Citrus</i>					
<i>Citrus aurantifolia</i> (Christm.) Swingle	East Indian Archipelago	CN323	AY116510	AY115640	AY115617
<i>Citrus aurantium</i> L.	southeastern Asia	CV244	AY116533	AF434803	AF434804
<i>Citrus grandis</i> (L.) Osbeck	southeastern Asia	CV357	AY116512	AY115642	AY115619
<i>Citrus jambhiri</i> Lush.	southeastern Asia	CN695	AY116517	AY115647	AY115624
<i>Citrus latifolia</i> Tanaka	southeastern Asia	CN654	AY116518	AY115648	AY115625
<i>Citrus limettioides</i> Tanaka	southeastern Asia	CN318	AY116519	AY115649	AY115626
<i>Citrus limon</i> (L.) Burm.f.	southeastern Asia	CN644	AY116520	AY115650	AY115627
<i>Citrus limonia</i> Osbeck	southeastern Asia	CN685	AY116521	AY115651	AY115628
<i>Citrus medica</i> L.	China and India	CN689	AY116534	AF434806	AF434807
<i>Citrus paradisi</i> Macf.	?	CN333	AY116526	AY115656	AY115633
<i>Citrus reticulata</i> Blanco	Philippines and southeastern Asia	CN224	AY116527	AY115657	AY115634
<i>Citrus sinensis</i> (L.) Osbeck	China and southeastern Asia	MT02	AY116535	AF434809	AF434810
<i>Citrus tachibana</i> (Mak.) Tanaka	Japan	CN708	AY116528	AY115658	AY115635
<i>Citrus</i> subg. <i>Papeda</i> (Hassk.) Swingle					
<i>Citrus hystrix</i> DC.	Indonesia	CN384	AY116515	AY115645	AY115622
<i>Citrus ichangensis</i> Swingle	China	CV373	AY116516	AY115646	AY115623
<i>Citrus junos</i> Sieb. ex Tanaka	China	CN703	AY116531	AY115661	AY115637
<i>Fortunella hindsii</i> (Champ.) Swingle	southern China	CN729	AY116514	AY115644	AY115621
<i>F. margarita</i> (Lour.) Swingle	southern China	CV423	AY116522	AY115652	AY115629
<i>Hesperethusa crenulata</i> (Roxb.) M. Roem.	India	CV413	AY116513	AY115643	AY115620
<i>Poncirus trifoliata</i> (L.) Raf.	central and northern China	VEL835	AY116529	AY115659	AY115636
<i>Microcitrus</i> sp.	Australia	CV418	AY116524	AY115654	AY115631
<i>Severinia buxifolia</i> (Poir.) Ten.	southern China	CV419	AY116532	AF434800	AF434801
Subtribe Triphasiinae					
<i>Triphasia trifolia</i> (Burm.f.) P.Wils.	southeastern Asia	MT01	AY116530	AY115660	AY059643

^a Samples encoded CN, CV, MT or VEL are deposited at IAC- CCSM, Cordeirópolis, São Paulo, Brazil; samples CRZ at Embrapa-CNPMP, Cruz das Almas, Bahia, Brazil

^b Region of cpDNA: R1 = intergenic spacer trnT-trnL; R2 = intron trnL 5' exon – trnL 3' exon; R3 = intergenic spacer trnL-trnF

Amplification was conducted according to procedures described by Sambrook et al. (1989), using as primers the sequences 5'-CGAAATCGCTAGAGCTACG-3' and 5'-ATTGAACTGGTGACACGAG-3' for the trnL(UAA)-trnF(GAA) segment, and 5'-TCTACCGATTTCGCCATATC-3' and 5'-CATTACAAATGCGATGCTCT-3' for the trnT(UGU)-trnL(UAA) region (Taberlet et al. 1991). The amplification reactions were carried out using a thermal cycler MJ Research model PTC-100™ and later sequenced via dideoxy-terminator on an ABI 377 (Perkin Elmer).

The obtained sequences were aligned with the Clustal V programme (Higgins et al. 1992), but for many sequences manual alignment was necessary. *Murraya* L. and *Merrillia* Swingle, both of the tribe Clauseneae, were used as outgroups.

Two different parsimony analyses (MP) were carried out. In the first analysis gaps were treated as a fifth state, yielding 111 (of 889) parsimony informative characters. When the gaps were treated as missing data (second analysis), there were 43 informative characters. These analyses were performed on the aligned sequences using the heuristic search option (excluding uninformative characters) in PAUP 4.0b4a (Swofford 2000), with branch-swapping algorithm set to TBR (Tree-Bisection-Reconnection) and the MULPARS option in effect, keeping only 10 trees per replicate. Neighbor-joining analysis was conducted with the same version of PAUP, with the option criterion set to distance. An HKY85 model (Hasegawa et al. 1985) was employed to estimate the distances between sequences. Five hundred replicates were performed with the option Maxtrees = 5000 to obtain bootstrap support values both for MP and NJ analyses. The aligned matrix can be obtained from the first author.

Evolution of morphological characters putatively apomorphic for the subfamily Aurantioideae (Table 2) was analysed by their optimization on one of the most parsimonious trees obtained from the first analysis using Winclada (Nixon 1999).

Results

We sampled 28 species from 12 different genera of the Aurantioideae (Table 1). The trnL(UAA)-trnF(GAA) and trnT(UGU)-trnL(UAA) regions provided data both

Table 2. Putative apomorphic characters for Rutaceae subfamily Aurantioideae.

Character
1. Spines present
2. Leaves simple or with articulated rachis
3. Palmate trifoliolate leaves
4. Stamens at least 4x the number of petals
5. Hesperidium berry (locules filled with pulp vesicles)
6. Pulp-vesicles with elongate shape
7. Pulp-vesicles slender-stalked
8. Pulp-vesicles attached only to the dorsal walls of the locule
9. Seeds woolly
10. Fruit with rind woody
11. Parietal placentation

from point mutations and segment duplications (Table 3) that were phylogenetically informative for the relationships of *Citrus* and allies. The sequenced regions included 889 characters, 198 (22.3%) were variable, and 111 (12.5%) were parsimony informative. The genetic distance between each pair of genotypes calculated by PAUP ranged from 0 among species of the *Citrus aurantium*-*C. paradisi* clade, the *Citrus reticulata*-*C. jambhiri* clade, and the *Fortunella hindsii*-*F. margarita* clade (Fig. 1) to 0.02364 between *Murraya paniculata* and *Hesperethusa crenulata*.

The phylogeny obtained from point mutation data is highly congruent with the main events of duplications observed in the two segments analysed (Table 3). The evolution of the trnL(UAA)-trnF(GAA) and trnT(UGU)-trnL(UAA) segments within the Citrinae is marked by 11 duplication and 10 insertion/deletion events. In the present study, the duplication events support six clades while only two clades were supported by an indel event (Fig. 1).

Parsimony analysis (MP) produced six most parsimonious trees (213 steps, ci 0.90, ri 0.97) when considering gaps as new states. Bootstrap tests show strong support for the main clades of the trees. One of the most parsimonious trees from the first analysis, identical to the strict consensus tree, is illustrated in Fig. 1. The consideration of gaps as missing states produced three trees with 63 steps, ci 0.94 and ri 0.98. There was less resolu-

Table 3. Events of duplication in the trnL(UAA)-trnF(GAA) and trnT(UGU)-trnL(UAA) regions of the cpDNA of the representatives of subfamily Aurantioideae.

Region	Event	Code	Sequence
trnL(UAA)-trnF(GAA)	dupl.	<i>a</i>	CTTTT
	dupl.	<i>b</i>	AAAGAAA
	indel	<i>c</i>	GG
	indel	<i>d</i>	TATATAGAC
	dupl.	<i>e</i>	27pb
	dupl.	<i>f</i>	GAAAAA
	dupl.	<i>g</i>	TGTTAT
	dupl.	<i>h</i>	GTTTTTTT
	indel	<i>i</i>	CAAGTT
	dupl.	<i>j</i>	GGATAT
	indel	<i>k</i>	TT
trnT(UGU)-trnL(UAA)	indel	<i>l</i>	ATGAACACC
	dupl.	<i>m</i>	ATTGCT
	dupl.	<i>n</i>	TTAGAT
	indel	<i>o</i>	AT
	indel	<i>p</i>	AA
	indel	<i>q</i>	AT
	dupl.	<i>r</i>	CGAAT
	indel	<i>s</i>	TGGAGAAA
	indel	<i>t</i>	GGG
	dupl.	<i>u</i>	TTTATA

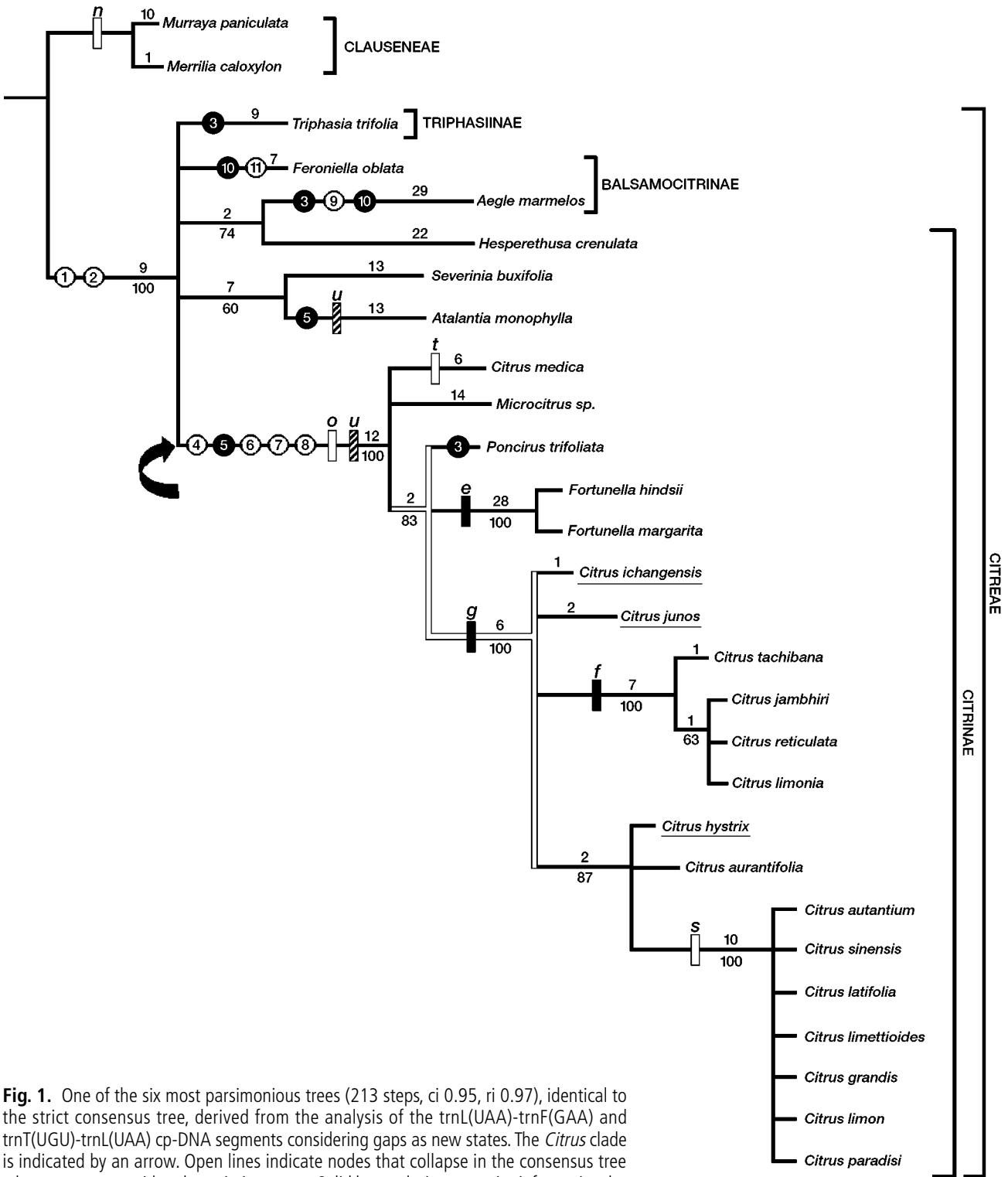


Fig. 1. One of the six most parsimonious trees (213 steps, ci 0.95, ri 0.97), identical to the strict consensus tree, derived from the analysis of the trnL(UAA)-trnF(GAA) and trnT(UGU)-trnL(UAA) cp-DNA segments considering gaps as new states. The *Citrus* clade is indicated by an arrow. Open lines indicate nodes that collapse in the consensus tree when gaps are considered as missing states. Solid boxes designate major informative duplication events in the trnL(UAA)-trnF(GAA) segment (see Table 3 for codes), open boxes indicate major informative duplications in the trnT(UGU)-trnL(UAA) segment, and striped boxes indicate homoplasious duplications in this segment. Morphological characters (Table 2) plotted on the cladogram are represented by numbers inside circles: open circles = non-homoplasious forward transitions, solid circles = homoplasies. Numbers above lines represent the number of nucleotide substitutions, numbers below lines are bootstrap support values (500 replications). The underlined names refer to the species of *Citrus* subgenus *Papeda*. Taxa indicated on the respective right side of the brackets refer to the classification of Swingle & Reece (1967).

tion in the *Citrus* clade (Fig. 1), just retaining the *Fortunella*, the *C. reticulata*-*C. limonia* and the *C. aurantium*-*C. paradisi* clades.

The Neighbor-joining (NJ) analysis gave results similar to those found in MPtrees except for less resolution within the *Citrus* clade (Fig. 2). Both methods recovered a large monophyletic group joining the representatives of the genera *Citrus*, *Microcitrus*, *Fortunella*, and *Poncirus*. MP trees also recovered a monophyletic group including all representatives of the genus *Citrus* except for *C. medica* (Fig. 1). This group was not obtained in the NJ analysis (Fig. 2), but neither method supports monophyly of the genus *Citrus* due to the positioning of *C. medica*. In the NJ tree, *C. medica* appeared in a clade with *Microcitrus* but this group collapsed in the bootstrap consensus tree and its position remains unresolved in both analyses.

MP and NJ analyses support sister-group relationships between *Aegle-Hesperethusa* and *Severinia-Atalantia* but do not provide evidence for the sister-group relationship of the *Citrus* clade.

Discussion

Tribes and subtribes

Murraya and *Merrillia*, the two genera of the tribe Clauseneae included in the analysis, appear as a clade supported by a duplication of 6 bp in the trnT(UGU)-

trnL(UAA) region. This seems to support the traditional division of the subfamily Aurantioideae into two tribes, Clauseneae and Citreae, differentiated mainly by the presence in the tribe Citreae of spines, leaves pinnate or with an articulated rachis (characters 1, 2), and a more elaborate berry. In spite of this, the diagnostic characters of the Clauseneae seem to be plesiomorphies in the Aurantioideae and a recent cpDNA phylogeny has shown that this tribe may not be monophyletic (Samuel et al. 2001). On the other hand, this division into two tribes is justified if *Merrillia* and *Murraya* (except for the species segregated as *Bergera* L.) are transferred to Citreae (Samuel et al. 2001). This narrower concept of the Clauseneae is also supported by the presence of carbazoles and by a limited quantity of heterochromatin per haploid chromosome set (Guerra et al. 2000). Unfortunately, the present study did not include a large enough sampling of the genera of Clauseneae to allow discussion of its circumscription.

There is no support for the subdivision of the Citreae into the subtribes Triphasiinae, Citrineae and Balsamocitrineae. Representatives of the three subtribes appear as an unresolved basal polytomy in the Citreae clade (Fig. 1). The type of fruit has been traditionally used to diagnose the subtribes of Citreae. Triphasiinae is characterised by small fruits with thin rind and locules filled with mucilaginous gum. Balsamocitrinae is diagnosed by large fruits with woody rind and locules filled with resinous gum. Citrineae is usually defined by its berries with coria-

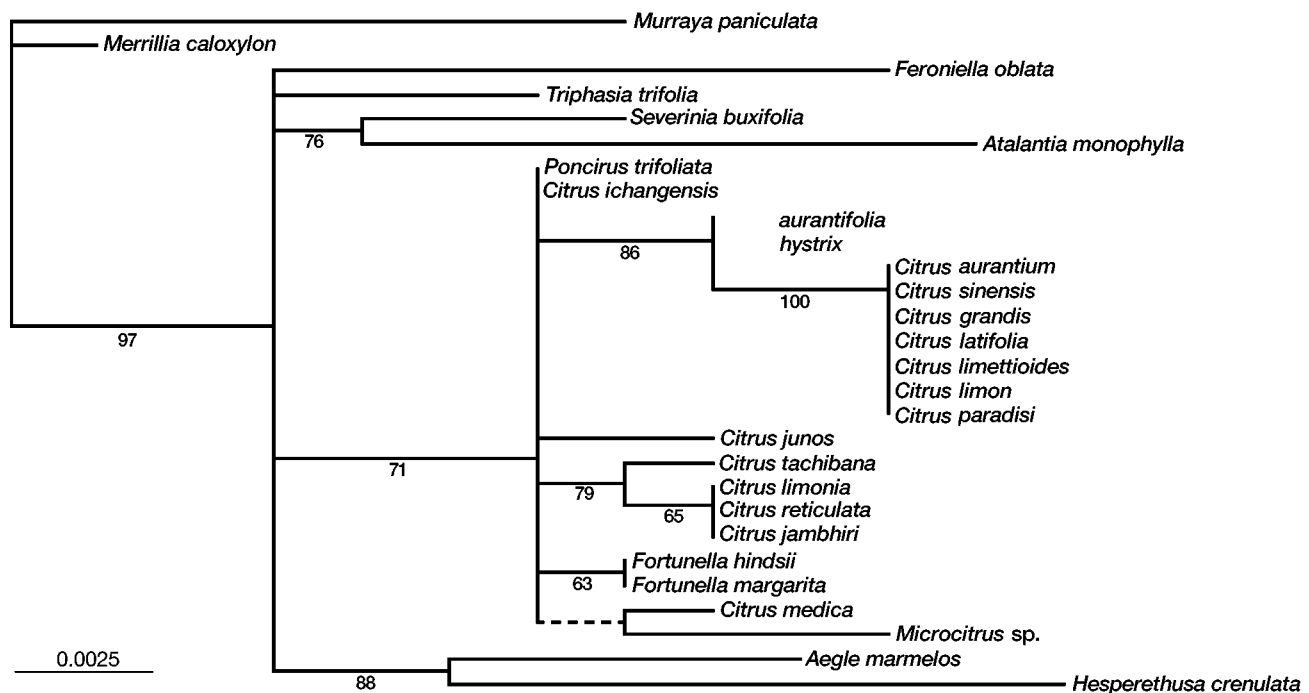


Fig. 2. Tree obtained from neighbor-joining analysis. Numbers below lines are bootstrap support values (500 replications). Broken lines indicate nodes that collapse in the bootstrap consensus tree.

ceous rind and locules filled with pulp vesicles, the hesperidium (Swingle & Reece 1967), although this type of fruit is not universal in this subtribe.

The fruits of the species of Triphasiinae and Balsamocitrinae share a plesiomorphic condition in Aurantioideae, locules filled with mucilaginous or resinous gum. The hard-shelled fruits of the Balsamocitrinae (those of *Feroniella* Swingle and *Aegle* Koenig) seem to be an independent development (character 10). This putative parallelism is reinforced by the quite different structure of the fruit in these genera. In *Feroniella*, the fruit results from a fusion of the locules of the ovary into a single cavity with the placentation becoming parietal (character 11). In *Aegle*, the fruit retains the septa dividing the locules in a manner similar to that found in the Triphasiinae, except for its woody rind and larger size. The occurrence of woolly seeds (character 9) appears, in the context of the present study, as an autapomorphy of the genus *Aegle*, although this condition may be found in other genera of Balsamocitrinae such as *Swinglea* Merrill and *Feronia* Corrêa (Swingle & Reece 1967).

Relationships between the genera of the subtribe Citrinae

Within the Citrinae, the alliances recognised by Swingle & Reece (1967) are not supported. These authors grouped *Severinia* Ten. and *Hesperethusa* M. Roem. in their "Primitive Citrus Fruit Trees" because of their fruits with pulp vesicles without definite shape which become oily or resinous at maturity, and *Atalantia* Corrêa in their "Near-Citrus Fruit Trees" because of its fruit with conical pulp vesicles embedded in the locule wall. These two grades share a diplostemonous androecium, a character that seems to be a plesiomorphy within the Citrinae.

The genus *Atalantia* has a puzzling position in the phylogeny of the Citreae. It was grouped with *Severinia* in all MP trees in the first analysis because they share a 6 bp duplication in the trnL(UAA)-trnF(GAA) region and one additional point mutation in the same segment, yielding seven characters to support this relationship. On the other hand, *Atalantia* shares a 6 bp duplication in the trnT(UGU)-trnL(UAA) region with the *Citrus* clade (striped boxes in Fig. 1). The fruit structure of *Atalantia* subgenus *Atalantia* is more similar to that found in the *Citrus* clade because of the presence of pulp-vesicles in the mature fruit, but these vesicles are sessile, broad-based, and present on the lateral walls of the locules. *Atalantia* subgenus *Rissoa* (Arn.) Swingle has a quite different, nearly dry fruit, which may suggest the genus is not monophyletic, a possibility also suggested by isoenzyme data (Herrero et al. 1996). The optimisation of the hesperidium as a character on the gene tree (character 5) favours the hypothesis of parallel acquisition of

this kind of fruit by *Atalantia* and the *Citrus* clade. However, the unique structure of the hesperidium within the angiosperms makes the hypothesis of an independent origin of this kind of fruit in the Rutaceae quite improbable. Therefore, the hypothesis of a group bringing together *Atalantia* and the *Citrus* clade seems to be more congruent with morphological evolution of the fruit, that of *Atalantia* subgenus *Atalantia* with broad-based vesicles immersed in all the locule walls probably being more primitive than the fruit with stalked vesicles found in the *Citrus* clade. A cladistic analysis based on morphological characters could test this hypothesis.

Citrus, *Poncirus*, *Microcitrus* and *Fortunella* constitute a monophyletic group (bootstrap 100). This group (the *Citrus* clade in Fig. 1) is also supported by morphological characters such as numerous stamens (at least 4× the number of the petals; character 4) and elongate, stalked pulp-vesicles attached only on the dorsal wall of the locule (characters 6, 7, 8). More recently, a study based on the atpB/rbcL spacer of cpDNA (Samuel et al. 2001) showed these genera to belong to the same clade. Guerra et al. (2000) showed that this group may be characterised also by strongly banded karyotypes and an increased amount of heterochromatin.

However, in none of the MP or NJ analyses is *C. medica* L. grouped with the other species of *Citrus*. Besides lacking duplication g (Table 3) of the trnL(UAA)-trnF(GAA) segment, it has an indel in the trnT(UGU)-trnL(UAA) region not shared with other *Citrus*. This result seems to corroborate the isolated position of this species within *Citrus*, as previously indicated by cytoplasmic genome data (Handa et al. 1986, Yamamoto et al. 1993, Araújo & Machado 1999). Further data would provide more resolution at the base of the *Citrus* clade, but this set of evidence strongly supports the isolated position of *C. medica* within the genus *Citrus* obtained in this present analysis.

Swingle & Reece (1967) divided the genus *Citrus* into the subgenera *Citrus* and *Papeda* based on the presence of acridic oil in the fruit and of broad-winged petioles in subgenus *Papeda*. The present study does not support this proposition. Besides the evident polyphyly of subgenus *Citrus*, due to the position of *C. medica*, there are no synapomorphies linking the species of subgenus *Papeda* (underlined species in Fig. 1), and *C. hystrix* DC. shares two point mutations with the *C. aurantifolia*-*C. paradisi* clade.

Poncirus was segregated from *Citrus* by its deciduous, palmately trifoliolate leaves (character 3) developing from scaly buds. These characters are autapomorphies within the Citrinae and have been interpreted as adaptations to winters conditions in the temperate zone in North and Central China where the genus occurs. Fang & Zhang (1994) have argued that a more recently discovered species, *P. polyandra* S. Q. Ding, X. N.

Zhang & M. Q. Liang, could link this genus with *Citrus*.

Microcitrus is another small genus (ca. 6 species) from Australia and New Guinea. It is closely related to *Citrus* from which it differs mainly by dimorphic leaves in the seedlings. One of the characters used to segregate *Microcitrus* from *Citrus* is the presence of free filaments (Swingle & Reece 1967). However, this character is variable in *Citrus*; most species of the subgenus *Papeda* have free filaments, while some other species of this subgenus and those of the subgenus *Citrus* have filaments cohering in bundles. Mabberley (1998) considered these characters insufficient to justify maintaining *Microcitrus* as an independent genus and proposed sinking it into *Citrus*. He also cited as evidence the formation of hybrids between species of these two genera.

The genus *Fortunella* includes four species of the "kumquats" from eastern Asia (China, Hong Kong, and Malay Peninsula). It is traditionally separated from *Citrus* by quantitative characters, 3–7 (versus 8–18) locules in the ovary with 2 (vs 4–12) ovules per locule, and by smaller fruits. In other vegetative, floral, and fruit characters, *Fortunella* is quite similar to *Citrus*, including the polyadelphous androecium (character 4) with numerous stamens cohering in bundles, a character more commonly found in *Citrus* subgenus *Citrus*.

Eremocitrus (not investigated in the present work) is a monotypic genus from central-eastern Australia. It is closely related to *Citrus* and its main distinctive characters (small leaves with palisade parenchyma on both faces, thick cuticle, and stomata sunken in pit-like depressions) may be interpreted as xerophytic adaptations to semiarid habitats, without great taxonomic value. This position was adopted by Mabberley (1998) who transferred *Eremocitrus glauca* (Lindl.) Swing. to the genus *Citrus*.

Both the present results and the recently accumulated information about Aurantioideae phylogeny support the view of an early diversification of different lineages of *Citrus* and allies. The absence of sharp delimitation of these genera may reflect the spreading of these early lineages over south-eastern Asia, Australia and major archipelagos without great morphological changes and with many parallel developments. The taxonomic assumptions about the boundaries between *Citrus* and related genera have been marked by the recognition of genera diagnosed by inconsistent characters. The present study shows that the *Citrus* clade is a natural group, and the recognition of *Microcitrus*, *Fortunella*, and *Poncirus* as valid genera makes *Citrus* polyphyletic because of the position of *C. medica*. *C. medica* is the type species of the genus *Citrus*, thus a more conservative interpretation with a wider definition of *Citrus* to include all the cited relatives seems preferable, as this avoids great nomenclatural changes in such an economically important group. At the same time, this supports the

proposition of Mabberley (1998) who merged Australian species of *Microcitrus* and *Eremocitrus* in the genus *Citrus*. This position is well supported by both molecular and morphological data, and *Citrus*, so defined, becomes a group with ca. 27–35 species and with a morphological coherence defined by many synapomorphies, especially the polystemonous androecium and the fruit, a hesperidium berry filled with slender-stalked vesicles attached only to the dorsal wall of the locules.

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