K2. KEYNOTE ADDRESS 2: On the Origins of *Citrus*, Huanglongbing, *Diaphorina citri* and *Trioza erytreae*

Beattie G.A.C. ¹, Holford P. ¹, Mabberley D.J. ¹,², Haigh A.M. ¹ and Broadbent P. ⁴
¹ Centre for Plant and Food Science, University of Western Sydney, Locked Bag 1797, Penrith South DC, New South Wales 1797, Australia; ² Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, United Kingdom; ³ PO Box 46 Mulgoa, NSW 2745, Australia. Email: a.beattie@uws.edu.au

We question widely held assumptions about the origins of huanglongbing (HLB) and the genus *Citrus* [Rutaceae: Aurantioideae: Aurantieae] and propose alternative hypotheses. In doing so, we comment on recent changes in the systematics of the family Rutaceae, particularly the subfamily Aurantioideae.

The widely accepted assumptions are that the genus *Citrus* originated in China and that HLB originated, in *Citrus*, in the same region. We present evidence that suggests: that *Citrus* evolved in Australasia (where HLB and its vectors do not occur naturally); and that ‘*Candidatus* Liberibacter’ forms known to infect *Citrus* and other Rutaceae originated in Africa in association with the African citrus psyllid, *Trioza erytreae* (del Guercio) [Hemiptera: Sternorrhyncha: Triozidae] and one or more species of *Vepris* [Rutaceae: Rutoideae].

Other evidence suggests that the ‘*Ca*. Liberibacter’ forms that cause HLB spread from Africa to India in infected citrus budwood or plants, then from India to Guangdong in China (directly or indirectly) about 1930, then from Guangdong to Taiwan, the Philippines, Indonesia, Malaysia after 1945, and subsequently more widely to other countries.

Our view that HLB did not originate in China is supported by published literature and other documents that have not been cited in major reviews of HLB and its vectors, particularly the Asiatic citrus psyllid, *Diaphorina citri* Kuwayama [Hemiptera: Sternorrhyncha: Psyllidae].

Recent hypothesis about the origins of ‘*Ca*. Liberibacter’ in Gondwana 100-300 million years ago, and the possible role of parasitic plants in natural spread of the bacteria, are considered. Our views are continually being revised as new information, including historical records, becomes available.

The genus *Citrus*

The most widely used taxonomic systems for classifying citrus are those of Walter Swingle (Swingle 1943, Swingle & Reece 1967) and Tyozaibu Tanaka (Tanaka 1977). They recognised 16 and 162 species respectively. Their views have led to widespread confusion in the use of names of cultivar groups, inappropriate species status of hybrids, and the names of true species (Scora 1975, Mabberley 1997), and a profound misunderstanding of generic limits (Mabberley 1998). Confusion and turmoil has been exacerbated by the use of a plethora of species names for apomictic hybrid clones. There has been no consensus on the names of these entities and many dubious synonyms and invalid names are widely used in books, journals and, most recently, in poorly referenced and inconsistent popular and technical internet websites that could perpetuate errors *ad infinitum*. Some papers, including molecular studies, deal with plants for which claimed
taxonomic relationships are invalid or poorly understood and for which verifiable voucher specimens have not been preserved. Many such publications are therefore of limited value and may mislead the unwary. Recent work suggests that the genus Citrus comprises about 25 species (Mabberley 2004). This view is based on (i) recent reunification of Eremocitrus, Fortunella, Microcitrus and Poncirus with Citrus (see Mabberley 1998), (ii) molecular studies by Guerra et al. (2000) and Samuel et al. (2001) that support these reunifications, and (iii) other molecular studies that suggest that the six species of Oxanthera from New Caledonia and the monotypic Clymenia polyandra (Tanaka) Swingle from New Ireland in eastern Papua New Guinea should be reunited with Citrus (see Mabberley 2004, Bayer 2004, Bayer et al. 2009). Australasia is therefore a ‘hot-spot’ for Citrus biodiversity with up to 13 endemic species (about 50% of all extant species) in the region. Most cultivated citrus is derived from the handful of Southeast Asian species (Table 1). The citron, C. medica, the first described species of Citrus, and long considered to be native to India, may have originated in Australasia, possibly in New Guinea. Its closest relative is C. polyandra, a species native to New Ireland, a small island to the north-east of mainland Papua New Guinea (Bayer et al.2009).

Common hybrids are listed in Table 2. Mabberley (1997) suggested that where the history of a particular cultivar is not certain it may be preferable to not use a Linnaean classification, and, for example in the case of the ‘Meyer’ lemon, refer to it as Citrus ‘Meyer’. Where there is certainty, he considered it more informative to use a Linnaean system, where species and hybrid names for citrus crops indicate their presumed relationship with wild plants. The oldest name for the hybrid group involving oranges (pomelo-mandarin crosses) is C. × aurantium L. and that for the wild tangerine (i.e., mandarin) is C. reticulata Blanco, so that a Valencia orange is classified as C. × aurantium L. ‘Valencia’ and ‘Dancy’ tangerine is classified as C. reticulata Blanco ‘Dancy’.

Table 1. Specific and common names of 23 species considered to be true species of the genus Citrus (Rutaceae: Aurantioideae; Aurantiae) and their endemic region(s). Widely cultivated species are in bold. Good photographs of some Australasian species, excluding those from New Caledonia, can be viewed on Mike Saalfeld’s website (http://www.saalfelds.freeserve.co.uk/HobbyCitrusGrowers.htm).

<table>
<thead>
<tr>
<th>Species</th>
<th>Common names</th>
<th>Endemic region</th>
</tr>
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<tbody>
<tr>
<td>C. amblycarpa (Hassk.) Ochse</td>
<td>sambal, djerok leemo, nasranan mandarin, ‘Celebes’ papeda</td>
<td>Malaysia &amp; Indonesia</td>
</tr>
<tr>
<td>C. australis (Mudie) Planch.</td>
<td>Australian round lime, dooja</td>
<td>Australia</td>
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<tr>
<td>C. australasica F. Muell.</td>
<td>Australian finger-lime</td>
<td>Australia</td>
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<tr>
<td>C. cavalieri H. Léveillé ex Cavalerie (C. ichangensis Swingle)</td>
<td>Ichang papeda/lime/lemon</td>
<td>China</td>
</tr>
<tr>
<td>C. garrawayi F. M. Bailey</td>
<td>Mount White lime, Garraway’s Australian wild lime</td>
<td>Australia</td>
</tr>
<tr>
<td>C. glauca (Lindl.) Burkill</td>
<td>Australian desert lime</td>
<td>Australia</td>
</tr>
<tr>
<td>C. gracilis Mabb.</td>
<td>Humpty Doo lime</td>
<td>Australia</td>
</tr>
<tr>
<td>C. halimii BC Stone</td>
<td>sultan lemon, limau kadangsa, limau kedu kera</td>
<td>West Malaysia &amp; Sumatera in Indonesia</td>
</tr>
<tr>
<td>C. hystrix DC. (C. macroptera Montrouz.)</td>
<td>kaffir lime, limau purut, limau hantu</td>
<td>Southeast Asia</td>
</tr>
<tr>
<td>C. inodora F. M. Bailey</td>
<td>Russell River lime, large-leaf Australian wild lime</td>
<td>Australia</td>
</tr>
<tr>
<td>C. japonica Thunb. syn. Fortunella</td>
<td>Kumquat</td>
<td>China</td>
</tr>
<tr>
<td>C. medica L.</td>
<td>citron</td>
<td>Possibly north-east Australia</td>
</tr>
<tr>
<td>C. maxima (Burm.) Merr.</td>
<td>pomelo</td>
<td>Indo-China</td>
</tr>
<tr>
<td>C. neocaledonica Guill.</td>
<td>false orange, large-leaf oxanthera</td>
<td>New Caledonia</td>
</tr>
<tr>
<td>C. oxanthera Beauvaisage</td>
<td>orange flower oxanthera</td>
<td>New Caledonia</td>
</tr>
<tr>
<td>C. polyandra Tanaka syn. Clymenia</td>
<td></td>
<td>New Ireland in Papua New Guinea</td>
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The possible origins and dispersal of Citrus

It is widely considered that Citrus originated in Southeast Asia between India and China and southwards through Malesia (Webber et al. 1967, Dugo & Di Giacomo 2002), but scant attention has been given by citrus horticulturists, pathologists and entomologists to the biogeography of Citrus and its close relatives since the impact of plate tectonics on the geography of the Earth were accepted in the 1960s and 1970s. The current perception of a Southeast Asian origin for Citrus is based on Swingle’s circumcision of the genus (Swingle 1943, Swingle & Reece 1947), and widespread lack of awareness of the origins of both Southeast Asia and Australasia and how the landmasses are related. The eastern-most limit to the distribution of true species of Citrus is New Caledonia, which has been in the same relative position, some 1200 km east of Australia, for 55 mya: about the time that India collided with Asia (Hartley 2001a). The flora of New Caledonia contains many groups of plants that appear to be remnants of the late Cretaceous–early Tertiary (97 to 23 mya).

In commenting on the origins of Microcitrus, which they considered a primitive genus related to Citrus, Swingle & Reece (1967) opined that ‘these remarkable citrus fruits are extremely interesting, in that they show how evolution has proceeded in regions isolated as Australia and New Guinea have been during the last 20 or 30 my since they were cut off from all other landmasses’. Swingle & Reece 1967 also stated ‘perhaps some of its species are very like the ancestral species from which Citrus developed’ and ‘the evolution of other citrus fruits is not so easily followed, since Citrus, Fortunella, and Poncirus did not originate in regions that were geographically isolated in definitely dated geologic eras.’ They quoted Brough (1933), who in turn quoted Benson (1923), to support his view that ‘Australia is considered to have been joined to the Asiatic mainland at least during the Cretaceous period, but probably a complete separation has existed since the

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Note: The table and the text are largely self-contained, and the references are properly cited within the document. The content is consistent with the extracted text and does not introduce any hallucinations.
‘The migrations of higher plants into Australia are held to have occurred during later Cretaceous times.’ The notion of the distribution of the Aurantioidae from Southeast Asia through Malesia to Australasia during the Neogene or Quaternary (up to 1.6 mya) periods, more recently than the Cretaceous era (146 to 65 mya), was considered likely as recently as the 1990s (Stace et al. 1993) in relation to movement of plants across ‘land-bridges’ (Armstrong 1975, Barlow 1981). However, Australia has never been joined to the Asiatic mainland (Hall 1997, 2001, 2002, van Welzen et al. 2005) and there is no record of species of Citrus being native to Wallacea, the islands between Wallace’s Line in the west and Lydekker’s Line, which runs along the Australasian continental shelf in the east.

Swingle’s view was that the genus Citrus may have originated in the New Guinea-Melanesia region, and that it evolved into fragrant, delicious-flavoured species from a few species with sour and bitter-flavoured, almost inedible fruit, such as C. hystrix and C. cavaleriei (syn. C. ichangensis), that had developed in the East Indian Archipelago, in the Philippines, New Guinea and Melanesia. He regarded this evolutionary path as the culmination of a very long period of progressive evolution that certainly began after Australia was cut off from land connection with New Guinea and Asia, probably more than 20 mya. Swingle (Swingle & Reece 1967) regarded the genera Eremocitrus, Fortunella, Microcitrus and Poncirus as ‘ancestral’ to his circumscription of Citrus. He regarded C. polyandra (syn. Cly. polyandra) as monospecific and probably the most primitive of all the genera within the ‘True Citrus Fruit Trees’, and with an extraordinarily close resemblance of its leaves and petioles of those of Monanthocitrus, represented by the then monospecific Monanthocitrus cornuta (Lauterb.) Tanaka (there are now four described species of Monanthocitrus: see Stone & Jones (1988)). This convinced Swingle that Cly. polyandra was an entirely new type of citrus fruit tree, possibly having descended from a remote ancestral species common also to Monanthocitrus. He considered Oxanthera, which has large, white fragrant flowers very much like those of Citrus, to be a highly specialized, xerophytic genus that possibly developed from the common ancestor of the genera Wenzelia and Monanthocitrus, and that the latter arose from an ancestral form much like that of some species of Wenzelia. He placed Oxanthera, Wenzelia and Monanthocitrus with several other ‘minor citroid’ genera in the subtribe Triphasiinae.

It is now clear that the species of Oxanthera, as species of Citrus (see Bayer et al. 2004, Bayer et al. 2009), belong to the true citrus group of fruit trees within Swingle’s subtribe Citrinae (the Aurantioidae). Moreover, Bayer et al. (in press) have shown that citron, C. medica, the first described species of Citrus, and long considered to be native to India, probably originated in Australasia, possibly in New Guinea. It and other Australasian species belong to a distinct Australasian clade that shares a common progenitor with Asiatic species that belong to an distinct Asiatic clade (Bayer et al. 2009). The closest relative of the citron is C. polyandra, a species native to New Ireland, a small island to the north-east of mainland Papua New Guinea (Bayer et al. 2009). Its next closest relatives are the Citrus species of New Caledonia, then the remaining Australasian species in Australia and New Guinea.

Most of Southeast Asia is derived from a complex agglomeration of terranes now far removed from their Gondwanan origins. North and South China (excluding Hainan and Taiwan), Indochina, Myanmar (Burma), Thailand, Malaysia and western Indonesia rifted from northeast Gondwana in three significant tectonic events starting in the Early Devonian (about 400 mya)

Australasia comprises Australia (including Tasmania), New Guinea, the Bismarck Archipelago Solomon Islands, New Hebrides, Fiji, Lau, Tonga, Kermadec Islands, New Caledonia and New Zealand. Progressive separation of the now western margins of this region from India from 96 mya in the Late Cretaceous to 35.5 mya in the Late Eocene marked the second major phase in the breakup of eastern Gondwana. Separation of Madagascar from India, Australia from Antarctica, and New Zealand and New Caledonia from Australia also began about 96 mya in the Late Cretaceous. Separation of Australia and Antarctica ended when sea formed between Tasmania (then still linked to the Australian landmass) and Antarctica about 35.5 mya (Hartley 2001a, b). India rifted northwards to collide with Asia in the Middle Eocene about 50 to 55 mya. At this point, both the northern margin of the Indian plate and Sundaland (Java, Sumatra, Borneo, western Celebes, Malaya, Shan and peninsular Myanmar, Thailand and Indochina) experienced an ever-wet, equatorial climate (Morley 1998).

Southern New Guinea is part of the Australasian craton whereas the northern sector comprises accretions of terranes of Pacific and Gondwanan origins, the latter including terranes that rifted into the Pacific and then back (e.g., New Britain and New Ireland). The terranes that form Wallacea originated from parts of Australasia, Gondwanic and Pacific accretions, and volcanoes following the collision of Australasia with Sundaland about 20 mya in the Middle Miocene (16 to 11.6 mya). These events over the past 55 million years have been most recently summarised by several authors (Veevers et al. 1991, Metcalfe 1998, Hall 1997, 2001, 2002, Hartley 2001a, b). Animations of events over the past 55 my can be viewed on the internet (SE Asia Research Group 2006: http://searg.rhul.ac.uk/current_research/plate_tectonics/globe_2001_svga.mov).

Other animations can be viewed at:
- http://www.pbs.org/wgbh/nova/eden/media/sttnq.html,

These animations and detailed documentation of reconstructions (Hall 2001a, 2002) suggest that favourable opportunities existed for migration of *Citrus* from eastern Australasia to Asia via island arcs in the Pacific Ocean from the Bartonian (40.4 to 37.2 mya) stage of the Middle Eocene epoch (48.6 to 37.2 mya) until the present age, and likewise, but less likely given the movement of terranes and directions of flow of equatorial currents, for migration from Asia to Australasia.

Based on recent molecular evidence, Bayer et al. (2009) Beattie et al. (2006) hypothesised that the genus evolved in Gondwana. They (Beattie et al. 2008a, b) subsequently, hypothesised that the genus evolved in Australasia, as Australasia rafted northwards and fragmented (Hall 1997, 2001, 2002) after separation from Antarctica.
Fruit of early species, which may have been thick-rinded, buoyant (such as the citron and the pomelo) and salt tolerant species growing in coastal river deltas (such as the pomelo: see Groff 1927), may have dispersed westward in equatorial currents, to Southeast Asia, when such currents existed to the north of what is now New Guinea (Beattie et al. 2008). Dispersal may have also been linked to island terranes that moved 1,000s of kilometres eastward across the same region (Hall 1997, 2001, 2002).

Some dispersal could have been endozoochorous, through migrations of birds and bats. Evidence to support such dispersal is scant, as there have been no detailed studies. Mature fruits of extant native Australasian *Citrus* are relatively small compared to most extant cultivated Asiatic forms. Mature fruit of native Australian rainforest species are green. This suggests that their seeds may be dispersed by palaeotropical, non-echolocating, frugivorous, pteropodid bats; birds are more closely linked to dispersal of brightly coloured red and orange fruit (Hodgkison et al. 2003, Ingle 2003). However, mature fruits of extant citrus relatives native to New Guinea range from green to orange and red: *Mo. cornuta* fruit are small and red, and those of *Wenzelia dolichophylla* (Lauterb. & K. Schum.) Tanaka are red (Swingle & Reece 1967). Such endozoochorous dispersal if it occurred would have been aided by formation, movement and accretion of terranes to the east, north and west of New Guinea over the past 37 my (see Burrett et al. 1991, Hall, 2001a, 2002), particularly terranes that now form the Halmahera Islands and most of the Philippines (see Hall 2001a, b; Morley 2003). Evidence to support possible endozoochorous dispersal of primitive *Citrus* germplasm with relatively small fruit is provided by Hartley (2001a) in his treatise on the taxonomy, origins and biogeography of the Australasian genera *Euodia* and *Melicope*. Evidence of dispersal of sour and sweet oranges by water and animals is provided by Gade (1976) who noted that the distribution of these fruit in Paraguay was related to ‘dissemination of the abundant propagules to uncultivated sites is achieved via the gaudy and buoyant fruit by birds, mammals, water and man’ and that ‘orange tree populations in remote forest areas may be attributed more to zoochory than anthropochory’. Parrots, attracted to brightly-coloured ripe fruit, carry orange pulp in their beaks, dropping pips along the way, and certain macaws, are fruit eating species. Mammalian dispersers of seeds include monkeys, rodents, cattle and pigs (Gade 1976). Gade (1976) also mentioned that ‘birds and mammals may disgorge or excrete viable seeds as their germinability is enhanced by gastric juices acting on the tough and woody seed coat’.
On the origins of huanglongbing
The accepted view, as expressed by Zhao (1981), da Graça (1991), Bové (2006), and in many other publications, is that that HLB\(^4\) originated in China. It is based on three assumptions: (a) that the disease was present in China in the 1800s (Lin 1956), (b) that Reinking (1919), Lee (1921), Tu (1932), and some other authors, described symptoms of the disease, and (c) that HLB evolved with *Citrus*. However, presence of the disease in China in the 1800s was based on interviews with farmers and technicians between 1947 and 1955 (Lin 1956), and the symptoms of maladies described by Reinking (1919) and Lee (1921) do not describe HLB. Furthermore, there is no evidence that HLB evolved with *Citrus*. Wallace (1978), in reviewing leaf-mottle yellows disease in the Philippines, concluded that the ‘mottle leaf’ studied by Lee (1921), was caused by Zn deficiency and the severity of leaf mottle on trees on pomelo was due to the susceptibility of the stock to citrus tristeza virus. Reinking (1921), in his report on ‘Citrus diseases of the Philippines, Southern China, Indo-China and Siam’, did not mention symptoms akin to those of HLB.

Early Indian records of symptoms resembling those of the disease seem to have been ignored. These records suggest that symptoms resembling those of HLB in *Citrus* were reported in the mid 1700s in the central provinces of India by Roghoji, the Bhonsla Raja of Nagpur (Capoor 1963). Other reports suggest that the disease was present in north-western and north-eastern India in the 1800s and early 1900s (Bonavia 1888-1890, Husain & Nath 1927, Pruthi & Mani 1945, Asana 1958, Capoor 1963, Fraser et al. 1966, Chadda et al. 1970, Raychaudhuri et al. 1972).

Husain & Nath (1927), when describing severe damage by *D. citri* populations at Sargodha in the western Punjab between 1915 and 1920, were the first, in a widely overlooked paper, to describe damage resembling that caused by HLB. In part they said: ‘... *D. citri* neither produces galls nor causes any malformation of the plant tissues. The only sign of injury is defoliation and death of the shoots attacked and the drying up of the branches. On badly infested trees the continual feeding of myriads of insects is, in itself, a very great drain on the food-supply of the plant, but most probably some poison is also injected into tissues of the host and this produces the more serious results. This is evident from the fact that the fruit of the infested tree is dry and insipid to taste, and branches other than those actually attacked also dry up. Besides, young shoots are killed, old leaves fall off and in course of time an attacked tree is denuded of leaves altogether ...’ ‘During the second year of attack, if the pest has been allowed to develop unchecked, all the new shoots are destroyed, and most of the branches are left without leaves and the tree begins to dry up. Very little fruit is borne and that too is of small size, insipid and dry.’ They noted that ‘The symptoms of attack by the nymphs of the citrus psylla consists in the malformation of leaves which are badly curled, look sickly and fall off prematurely’.

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\(^4\) Huanglongbing is the official name of a disease of *Citrus* and other Rutaceae caused by ‘*Ca. L. americanus*’, ‘*Ca. africanus*’ and ‘*Ca. L. asiaticus*’ (Moreno et al. 1996; van Vuuren 1996). It should not be called citrus huanglongbing. Recent use of huanglongbing in relation to ‘*Ca. L. psyllaurous*’ (Hansen et al. 2008) in tomato and potato is invalid and misleading.
Adult *D. citri* and eggs on lemon flush growth at the orchard at Palai, Malakand, 17 July 2006, at 47°C (GAC Beattie).

With the benefit of current knowledge, it is clear that the severe symptoms described by Husain & Nath (1927), and later by Pruthi & Mani (1945), were symptoms now known to be caused by ‘*Ca. L. asiaticus*’. Damage caused by *D. citri* is much more benign and does not lead to death of trees.
Fraser (1966) commented on the citrus industry in India before and after partition in 1947 from Pakistan. Most of the industry was confined to a band extending southwest from Assam and West Bengal in the east through central India around Nagpur in Maharashtra State to the states of Madras (now Chennai) in the southeast and Mysore and Kerala in the southwest (Fraser 1966). Fraser (1966 noted that:

- HLB was not present in Assam and West Bengal in the 1940s;
- in Maharashtra, a 48 year-old mandarin planting that she observed must have predated the introduction of HLB into the area;
- sudden wilting that she thought was due to HLB may have occurred in Maharashtra at Poona in 1946;
- the first commercial citrus trees planted at Kalimpong in West Bengal in 1864 lived for 50-60 years; and

Plate I from Husain & Nath (1927), with illustrations of *D. citri* and its impact on citrus in the Punjab.
• that a major extension of plantings in Assam commenced in 1927-1929, but there were no reports of dieback (HLB) before 1947-48, although there was a reference to some chlorosis in 1940-1941 in one area.

The citrus industries of Pakistan and India underwent considerable expansion in the 1930s and 1940s (Fraser et al. 1966). A further expansion in the (eastern) Punjab followed partition, when citrus growers from Pakistan were encouraged to plant large acreages, particularly of sweet oranges, in newly opened irrigation settlements. The spread of dieback (HLB) coincided with this expansion and the use of infected planting material (Fraser 1966).

Aubert (1990b) considered movement of citrus material as the most efficient way of disseminating HLB and D. citri. His view (Aubert 1990b) was that they had been introduced unwittingly into many countries in Southeast Asia after 1940, particularly by nurserymen, orchardists and home gardeners replacing traditional local varieties.

With the possible exception of China, HLB symptoms were not recorded in Southeast Asia until after 1940. It was first recorded in:
Indonesia in 1948 (Aubert et al. 1985);
Taiwan in 1951, when it was recorded as serious (Su & Huang 1990);
the Philippines in 1957 (Martinez & Wallace 1967);
Thailand in the 1960s (Schwarz et al. 1973a, b, Aubert 1990c); and
Malaysia in the 1970s (Ko 1988, 1991), where its presence was confirmed in 1989 (Lim et al. 1990a).

Human-assisted spread of the disease through the Indonesia archipelago (Tirtawidjaja 1980) to Papua New Guinea (northern Australasia) (Davis et al. 2000, Weinert et al. 2004) took more than 50 years.

Recently observed records from Indonesia indicate that citrus was introduced from China on 2-3 occasions to the Bogor Botanic Gardens in 1945 (Inggit Puji Astuti, Bogor Botanic Gardens, pers. comm., 25 January 2008). This was around the time that the disease became widespread and severe in Guangdong (Lin 1956) and 11 years after Zhou Yuwen recorded D. citri in Guangdong (Hoffmann 1936). The origin of HLB in the Philippines is not known. However, there were many introductions of budwood and small budded trees from mainland China, India, Japan and Taiwan before 1957 (Martinez & Wallace 1969). Information provided by Ko (1991) and Saamin et al. (1991) suggests that the first record of the disease in Peninsular Malaysia may have been related to the introduction of C. reticulata seedlings (in this instance Tanaka’s C. suhuiensis, a mandarin variety from Sihui near Guangzhou in Guangdong) to Terengganu in peninsular Malaysia from China in the 1950s and 1960s.

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5 Extensive irrigation systems were constructed in the western Punjab, of Pakistan, between 1885 and 1947 (Ali 1988)
6 Zhou (Djou) Yuwen became a professor of entomology at Zhongshan University on the original site of Lingnan University. In the 1940s he worked with John Lindsley Gressitt (Bishop Museum, Hawaii) and Stanley Ellsworth Flanders (University of California, Riverside) on parasitoids of red scale, Aonidiella aurantii (Maskell).
7 The symptoms of ‘mottle leaf’ reported in the Philippines by Morada (1930) resemble nutrient deficiencies, and it is clear from his notes on the severity of the malady that it was not HLB. Wallace (1978), in reviewing leaf-mottle yellows disease in the Philippines, concluded that the ‘mottle leaf’ studied by Lee (1921), was caused by zinc deficiency and the severity of leaf mottle on trees on pomelo was due to the susceptibility of the stock to citrus tristeza virus.
It is highly unlikely that the disease was widespread in China before the 1940s or occurred there before 1930:

- the first authentic record of the disease in China appears to have been made by Chen Qibao in 1938 (Chen 1943, Lin 1956), four years after Zhou Yuwen collected D. citri on citrus and other hosts at Lingnan University in Guangzhou (see Hoffmann 1936);
- parasitoids were not associated with these psyllid populations (Hoffmann 1936);
- Condit et al. (1937) clearly, and unequivocally, linked major losses of trees in Guangdong to poorly drained lowland areas;
- Benemerito (1938) in his paper on oranges of Guangdong, in which he notes the dates of introductions of varieties from China to the Philippines, does not mention maladies consistent with HLB-like symptoms;
- reports on citrus cultivation by the Botanical and Forestry Department of Hong Kong from 1853 to 1941\(^8\) do not mention records of D. citri, or any citrus maladies resembling HLB, from 1924 to 1939; and
- Aubert (1990a), in discussing Jiaogan (Tankan) tangor production in the Chaoyan district of Guangdong from 1946 and 1990, noted that the first dramatic HLB epidemic in the region occurred from the late 1950s following use of contaminated trees, and that subsequent epidemics were related to natural spread of the disease by D. citri.

Condit et al. (1937) noted: ‘As stated by Tu (1932) the short life of the trees is undoubtedly largely due to the high water table, poor drainage, shallow roots and the subsequent failure of the root system to renew itself and to function properly. The fact that well drained and deeper soils adjacent to nearly all lowland orchards, citrus trees up to fifty years old are to be found still vigorous and productive in spite of insect borers and bark diseases, leads one to regard the soil moisture content as the principal factor contributing to the early decline of the lowland trees.’ They attributed chlorosis, that considerably alarmed Ch’ao-an growers, to the inability of the leaf to get sufficient iron on sandy soils. They reported that ‘mottle leaf’ of rare occurrence, in contrast to Tu (1932) who considered ‘mottle leaf’ being worst in the Guangzhou delta region. ‘The leaves may be sickly, pale green, or yellow, but seldom distinctly mottled. In fact the deep green color of the foliage in most orchards is remarkable, considering the soil conditions in which the roots are growing.’

Contrary to other reports, Condit et al. (1937) said that they wished ‘to emphasize the fact again that trees of the sweet orange and of loose-skin oranges up to twenty-five or thirty years and of pummelo up to fifty years old are common in well drained soils. For this reason we doubt that bark diseases are so much ‘the limiting factor’ as poor drainage and the consequently restricted root system.’ Condit et al. (1937).

These records suggest eastward movement, directly or indirectly, of the disease and the psyllid from the Indian subcontinent to Guangdong, and then to Taiwan, the Philippines, Thailand, Malaysia and Indonesia. The introduction to China appears to have occurred in the late 1920s or early 1930s and may have involved lemon and pomelo cultivars. In addition to movement of plants by nurserymen, orchardists and home gardeners, such introductions may have been linked to exploration for new species of plants.

Exploration for Citrus species and varieties, and Citrus relatives, in China, Việt Nam, Thailand and the Philippines was initiated by the eminent citrus taxonomist Walter Tennyson Swingle, who visited Lingnan University\(^9\) in 1916 and collaborated with staff there, and in the Philippines.

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\(^8\) Hong Kong Government Reports Online (1853-1941): http://sunzi1.lib.hku.hk/hkgro/index.jsp.

\(^9\) Now the location of Zhongshan Daxue (Zhongshan University): also called Sun Yat-Sen University.
over many years (Groff 1927, McClure 1931, Cooper 1989). These plants appear to have been both grafted and ungrafted. Some plants were introduced to the United States of America, most via the Philippines, so as to reduce the risk of new pests and diseases entering America (Groff 1927). Records are still being obtained, and in the case of those written in Chinese, translated. One record (Wang 1934) mentions ‘Citrus magner ex Hort.’ (sic), a lemon type (C. limonia Osbeck var. nov. wagner), being introduced to Guangdong by George Groff from the garden of Mr Wagner, a French resident of Saigon (now Ho Chi Minh City). There is no evidence that this plant, or other plant material taken from Viêt Nam by Groff, McClure and their colleagues, harboured HLB or D. citri, but there is possibility that such plants, if initially imported from a region where both the disease and vector occurred (e.g., India) may have led to the introduction of both to China. Nevertheless, such plants may have occurred in Viêt Nam, or elsewhere in Indo-China and the Malay Peninsula, but their presence may not have been noticed given that populations of major cities were relatively small. Moreover, citrus cultivation appears to have been low (below 500 ha?) in Viêt Nam, and largely restricted to home gardens in rural areas. Feldwick (1917) does not mention citrus cultivation in the Saigon region. Between 1860 and 1923, the population of Saigon from 6,000 to 100,000. Growth there after was less dramatic: between 1921 and 1947 it increased 1.7 times (Guillaume 1985). Together with the nearby town of Cholon, the total population of Saigon-Cholon in 1917 was about 250,000 (Feldwick 1917).

The above records suggest that ‘Ca. L. asiaticus’ may have originated in India, as also suggested by Halbert & Manjunath (2004). It is possible that this may have occurred in association with asymptomatic forms of M. paniculata, B. koenigii or species of Clausena, but not species of Citrus, as recent studies by Bayer et al. (in press) suggest that no true species of Citrus are native to India. However, the authors of this IMP consider sub-Saharan Africa to be the most likely origin of the ‘Ca. Liberibacter’ forms that cause HLB.

The African form of HLB was first reported (as Transvaal citrus greening) in Rustenberg in Western Transvaal (now Northern Province) in South Africa in about 1928-29 (predating our views on when it first occurred in China) and was spread to other areas with infected planting material (van der Merwe & Andersen 1937, Oberholzer et al. 1965, Moll 1977). The disease is characterised by chlorotic leaf symptoms, as well as poor crops of undersized and poorly coloured fruit of inferior quality. It caused severe crop losses in the Eastern Transvaal (now Mpumalanga) during the periods 1932-36 and 1939-46, but was of little importance elsewhere. From 1958, severe crop losses occurred in the Eastern and Western Transvaal and greening spread elsewhere in South Africa, presumably due to the upsurge of T. erytreae as a serious pest in citrus orchards, particularly in cooler areas > 700 m asl, where humidity rarely falls below 25% and mean monthly maximum temperatures vary between 18°C and 30°C (Oberholzer et al. 1965, Buitendag & von Broembsen 1993). Previously, T. erytreae had been troublesome only in nurseries (Buitendag & von Broembsen 1993). When a few HLB-affected trees (with their origins in the north) were found in the Eastern and Western Cape in the 1960s, they were destroyed and restrictions were placed on the movement of planting material from greening

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10 Other foreign scientists involved in exploration and introductions during this period included George Weidman Groff, Franklin Post Metcalf, Floyd Alonzo McClure and Elmer Drew Merrill.
11 McClure’s field notes, Zhongshan University herbarium, Guangzhou, photographed by GAC Beattie, 2007.
12 In 1991 the total area in Viêt Nam was about 5,000 ha (Whittle 1992).
13 These reports coincide with the first records of T. erytreae being recorded as a pest of citrus in South Africa: albeit with damage caused directly by the pest, not HLB: see, van der Merwe (1923).
infected provinces. In 1995, the disease was detected in an isolated spot in the Eastern Cape and trees were eradicated. At this point it was also recorded at Stellenbosch in the Western Cape, where the disease continues to spread slowly (le Roux 2006). 

Africa is the only region in which a common association between a HLB pathogen and a seemingly asymptomatic and preferred host of a vector appears to occur: in an association involving T. erytreae and V. lanceolata. Moran (1968) suggested that V. lanceolata, Cl. anisata and Z. capense could be the original native hosts of T. erytreae in Africa, but Cl. anisata is not native to Africa (Molino 1994) and Z. capense is a relatively poor host. da Graça (1991) mentioned that efforts (JV da Graça & SP van Vuuren unpublished data) to transmit the African form of the disease to V. lanceolata, Cl. anisata and Z. capense by T. erytreae were unsuccessful, but he did not mention how the tests were done, or whether there was an expectation that the plants would exhibit symptoms of the disease normally observed on Citrus. However, subsequent studies indicate that Cl. anisata and V. lanceolata can harbour the bacterium. van den Berg et al. (1991-1992, 1992) reported detection of the pathogen from psyllid infested Cl. anisata growing in close proximity to a HLB-infected citrus orchard. Detection was based on tests involving grafting of sweet orange indicator plants to the Cl. anisata trees. Simultaneous tests with the native V. lanceolata and Z. capense were negative (van den Berg et al. 1991-1992), but Korsten et al. (1996), using dot blot hybridisation, detected the bacterium in a leaf sample taken from a V. lanceolata tree on which many T. erytreae-induced leaf deformations were observed. van den Berg et al. (1991-1992) recommended removal of Cl. anisata plants growing near citrus orchards and Korsten et al. (1996) concluded that V. lanceolata is a reservoir plant for the pathogen14. van den Berg et al. (1991-1992, 1992) and Korsten et al. (1996) did not mention the presence of HLB symptoms in any of these non-Citrus hosts of T. erytreae, and there are no reports of dieback or death of trees.

It is highly unlikely that HLB originated in Asia or Australasia in association with Citrus or any Citrus relative. There would be a high probability that any such association would lead to rapid extinction of the host plant(s) and the bacterium. Moreover, HLB does not occur naturally in Australasia and no indigenous psyllid species have been recorded feeding on indigenous species of Citrus.

In 2006, we considered it possible that the three currently recognised ‘Candidatus Liberibacter’ species15 (‘Ca. L. asiaticus’, ‘Ca. L. africanus’ and ‘Ca. L. americanus’) and the one ‘subspecies’ (‘Ca. L. africanus subsp. capensis’) that cause HLB (da Graça & Korsten 2004; Teixeira et al. 2005a, b), may represent forms of a single ‘species’ that had adapted to new hosts and environments in the recent past (perhaps < 500 years) (Beattie et al. 2006). Our opinions differed about the validity of the three ‘species’ of ‘Ca. Liberibacter’, and we were concerned that the bacteria may have the capacity to adapt rapidly to new hosts and environments (Beattie et al. 2006). We reasoned that the probability of three forms evolving on more than one continent after

14 Observations reported by Evers & Grisoni (1991), and reports by Temu & Andrew (2008) and Burgess et al (2002), suggest that two other species of Vepris, V. mildbraediana G.M. Schulze (of uncertain taxonomic status: Miziray 1992) and V. morogorensis var subalata (Kokwaro) W. Miziray, may be also be hosts of T. erytreae, and possible ‘Ca. L. africanus’ in Morogoro, Tanzania.

15 ‘It is important to note that the category Candidatus is not covered by the Rules of the Bacteriological Code. Consequently, a name included in the category Candidatus cannot be validly published, and it also cannot be designated sp. nov., gen. nov., etc.’: Euzéby JP. 2008. List of Prokaryotic names with standing in nomenclature. http://www.bacterio.cict.fr/candidatus.html.
the separation of Gondwana, and in an initial association with a single, most probably asymptomatic, host plant species and with a single psylloid vector, would be negligible.

Based on this evidence and movement of plants by humans, we consider it possible that the HLB pathogen was transmitted from *V. lanceolata* to orange or mandarin trees by *T. erytreae* in one of the European colonies on the southeast coast of Africa and then taken to the Indian subcontinent in infected plants or budwood some 300-500 years ago. It could then have been acquired and spread by *D. citri*. Spread could also have occurred through marcotting (air-layering) and grafting, and enhanced by changes in horticultural practices, that through increased use of irrigation and fertilisers within monocultures, would have led to more abundant and frequent growth flushes. The latter would have led to far higher populations of *D. citri* than would have occurred in its original environment. Aubert (1988) commented that although widely distributed throughout the Orient, *D. citri* has a relatively narrow habitat restricted to *Citrus, M. paniculata* and two rare *Clausena* spp., and that *D. citri*, unlike *T. erytreae*, is not able to build up massively on a wide range of alternative rutaceous forest trees or shrubs. He suggested this as a reason why ‘*Ca. L. asiaticus*’ in Asia is generally far more associated to man’s activity in densely populated areas than ‘*Ca. L. africanus*’ in Africa. He (Aubert 1988) considered *D. citri* to be an ecologically opportunistic insect that dispersed ‘along the main communication tracks’ of naturally occurring and cultivated ‘*Citrus and Murraya paniculata*’.

Our views on the origins of the nominal genus ‘*Candidatus Liberibacter*’ are being reconsidered in light of recent reports (Liefting 2008a, b) of a fourth ‘species’ of ‘*Ca. Liberibacter*’ being discovered in potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.) [Solanales: Solanaceae]16 and other solanaceous plants in New Zealand (Liefting et al. 2008a,b) in association with the recently introduced potato/tomato psyllid *Bactericera (=Paratrioza cockerelli*) (Sule) [Psyllidae], presumably from North and/or Central America17, and reports of possibly the same pathogen being detected in tomato and potato in California (Hansen et al. 2008). These are the first reports of Liberibacters occurring naturally in plants other than species of Rutaceae, and it is uncertain as to when symptoms caused by the pathogen in the Solanaceae were first noticed in the Americas, prior to the report of Hansen et al. (2008). Hansen (2008) noted that the most similar 16S rRNA sequence to the new ‘*Ca. Liberibacter*’ species is sequence DQ471901 ‘*Ca. L. asiaticus*’ from Brazil.

Eveillard et al. (2008), in an estimation of ‘speciation’ dating, considered ‘*Ca. L. asiaticus*’ and ‘*Ca. L. africanus*’ diverged some 150 million years ago, while speciation of ‘*Ca. L. americanus*’ might have started some 300 million years ago. Doddapaneni et al. (2008b) considered that speciation may have occurred 110-120 mya, before the breakup of Gondwana. Teixeira et al. (2008) estimated that ‘*Ca. L. africanus* and *Ca. L. asiaticus*’ diverged 147 mya and that the splitting between ‘*Ca. L. americanus*’ and the asiaticus/africanus branch would have occurred 309 mya. At the family level, psyllids appear to have colonised relatively few major lineages of plants during the diversification of both angiosperms and psyllids in the Cretaceous (146-65 mya), and subsequently evolved and diversified with their hosts (Hodkinson 1984). Gullan & Martin (2003) noted that the fossil record of the Psylloidea extends back to the Permian (> 270 mya). The extant families probably diversified in the Cretaceous (Gullan & Martin 2003).

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16 Solanales are estimated to be from the mid-Cretaceous (Bremer et al. 2004)

However, there is no evidence that ‘Ca. L. americanus’ occurs naturally in the Americas: the only known hosts are not native to the Americas. Furthermore, there appears to be no logical reason, given the paucity of native species of Rutaceae and psylloids in South America, to support notions that the disease, in any form, evolved in Rutaceae in South America. Both ‘Ca. L. americanus’ and ‘Ca. asiaticus’ were discovered at the same location in two orchards in São Paulo State in Brazil, and are spreading from that point (Silvio Lopes, pers. comm, September 2008, Lopes et al. 2008). Since discovery of the disease in São Paulo State in 2004, ‘Ca. L. americanus’ has been the most prevalent species in citrus (Gottwald et al. 2007), and it appears to have had sufficient time to move freely from citrus to orange jasmine, without any of the limitations provided by removal of symptomatic trees or applications of insecticides, practices currently adopted in the management of HLB in citrus orchards (Lopes et al. 2008, these proceedings). However, in the last 4 years, a disproportional increase in the incidence ‘Ca. L. asiaticus’ has been observed in citrus. It appears that both forms were introduced simultaneously in the mid 1990s to Brazil, where D. citri has been present since about 1940 (Halbert & Núñez 2004).

If the bacteria evolved before the breakup of Gondwana, then their widespread distribution in Africa, Asia and South America can be explained by vicariance. However, the difficulty with this interpretation is the date of origin of plants in which the organisms are known to occur. To accommodate the age estimates of Eveillard et al. (2008) and Doddapaneni et al. (2008b) it will be necessary to identify hosts that may have occurred up to 300 mya, when only Gymnosperms were present, and how the bacteria moved from plant to plant. Currently, the liberibacters are only known to colonise two families of Angiosperms, the Rutaceae, within the subfamilies Rutoideae and Aurantioideae, and the Solanaceae. The putative oldest member of ‘Ca. Liberibacter’, ‘Ca. L. americanus’ has only been detected in two Aurantieae genera (Citrus and Murraya), and no genera within the Aurantiaceae, or in the Clauseneae, the other recognised tribe within the Aurantioidae, are indigenous to the South America. ‘Ca. L. asiaticus’ has only been detected in the Aurantioidae. ‘Ca. L. africanus’ has only been found naturally within African Rutoideae, and the Rutoideae evolved before the Aurantioidae (Bayer et al. in press) and occur naturally in all continents aside from Antarctica.

A brief summary, based on literature referred to above, and some other sources, of our interpretation of events related to the evolution of Rutaceae, Solanaceae, psylloids and liberibacters, and the spread of HLB into Citrus is presented in Table 1.

Table 1. Summary of historical events related to the evolution of Rutaceae, Solanaceae, psylloids and liberibacters, and the spread of HLB into Citrus.

<table>
<thead>
<tr>
<th>Tectonics &amp; Flora</th>
<th>Era</th>
<th>Period</th>
<th>Epoch</th>
<th>Start of each period (mya)</th>
<th>Fauna &amp; bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extant Rutaceae and Solanaceae. Movement of plants by humans. Citrus monocultures from 1800s.</td>
<td>Current</td>
<td></td>
<td></td>
<td>0.002</td>
<td>Three ‘Ca. Liberibacter’ transmitted from their natural asymptomatic hosts to symptomatic Citrus and Citrus relatives, independently on three continents; only one form, ‘Ca. L. africanus’ with a possible natural host and a possible natural vector; ‘Ca. L. asiaticus’ with a possible natural vector but no identified natural asymptomatic host; ‘Ca. L. americanus’ with...</td>
</tr>
</tbody>
</table>
no identified natural host or vector. First encounters of *D. citri* and *T. erytreae* with *Citrus*. No beneficial or detrimental impacts of ‘Ca. Liberibacter’ on vectors demonstrated. Psyllid vectors move from natural hosts to colonise citrus monocultures.

<table>
<thead>
<tr>
<th>Cenozoic</th>
<th>Quarternary</th>
<th>Holocene</th>
<th>0.1</th>
<th>Pleistocene</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing evolution of <em>Citrus</em>?</td>
<td></td>
<td></td>
<td></td>
<td>‘Ca. Liberibacter’ exist in unknown hosts and with no identified insect vector(s)?</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>Pliocene</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miocene</td>
<td>23</td>
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<td></td>
</tr>
<tr>
<td>Oligocene</td>
<td>35</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Eocene</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paleocene</td>
<td>65</td>
<td>‘Ca. Liberibacter’ present in asymptomatic Rutaceae, initially Rutoideae? and/or Solanaceae? Geographic separation of ‘Ca. Liberibacter’ complete?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesozoic</td>
<td>Cretaceous</td>
<td>Late</td>
<td>97</td>
<td>Evolution of psyllids on Rutoideae then Aurantioidae?</td>
<td></td>
</tr>
<tr>
<td>Jurassic</td>
<td></td>
<td>Late</td>
<td>157</td>
<td>Ongoing speciation of ‘Ca. Liberibacter’ Diversification of psyllids (146 mya)</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>146</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>208</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Citrus japonica* (18 mya)
*Citrus trifoliata* (18 mya)
*Citrus glauca* (22 mya)
Speciation of *Citrus* continues in Australasia and in Asia, in the absence of ‘Ca. Liberibacter’ and psyllids that feed on citrus.

*Citrus medica* (?? mya)
*Citrus* progenitor (?? mya).
*Citrus* evolves in Australasia in the absence of ‘Ca. Liberibacter’ and psyllids that feed on citrus.

*Aurantioidae* (71 mya):
*Clauseneae* (to comprise now extant *Bergera, Clausena, Micromelum & Glycosmis* in Asia & Australasia) evolve before *Aurantiaceae* (initially *Murraya sensu stricto*, in South and Southeast Asia, finally *Citrus* in the Oligocene?).
*Rutaceae* (91 mya)

Breakup of East Gondwana begins (110-120 mya)
*Cuscuta* progenitor
*Solanales*

Geographic separation of ‘Ca. Liberibacter’ commences?
Breakup of East and West Gondwana commences 167 mya
Angiosperms (180-140 mya)
We cannot gauge the accuracy of the age estimates of Eveillard et al. (2008) and Doddapaneni et al. (2008b). However, we still wonder whether diversification of the bacteria occurred more recently than their estimates even, as Beattie et al. (2006) hypothesised, in the past 1,000 years? Is it possible that modern agricultural practices may have led to relatively rapid adaptations to new hosts and environments? Geographical variation in ‘Ca. L. asiaticus’ has been reported in regions where the pathogen does not occur naturally (e.g., Garnier et al. 1991, Gao et al. 1993, Bastianel et al. 2005, Teixeira et al. 2005c, Subandiyah et al. 2006, Doddapaneni et al. 2008a, Zhou et al. 2008a), and severe symptoms have been reported in hosts, e.g., limes and pomeloes, that until recently have been considered tolerant (Susan Halbert, Florida Department of Agriculture and Consumer Services, pers. comm, 2008, Tsai et al. 2008). Is it possible, if the phylogenetic relationships derived by Bastianel et al. (2005) and Teixeira et al. (2005c), and the age estimates of Eveillard et al. (2008) and Doddapaneni et al. (2008b), are accurate, that all three forms of ‘Ca. Liberibacter’ that cause HLB originated on one continent, Africa, and that two of the forms have spread anthropogenically from there?

With the possible exception of *V. lanceolata*, no asymptomatic hosts have been identified, nor have insect vector-associations with these plants and plants in which the pathogens cause disease been identified. In the absence of psyllid vectors, the only avenues we can suggest for natural spread from plant to plant are root grafts and transmission by parasitic plants, particularly *Cuscuta* spp [Solanales: Convolvulaceae].

Halbert & Manjunath (2004) considered natural transmission of HLB by *Cuscuta* spp. unlikely. However, there is a possibility that natural and anthropogenic dispersal of species such *Cu. australis* and *Cu. campestris* may have contributed to the spread of several pathogens known to be transmitted by them, including HLB. Dodder is common parasite of a broad range of plants in Asia (Browne 1968, Rajak et al. 1985, Abu-Irmaileh 1987, Abu-Irmaileh & Fucik 1989, Banerjee et al. 1993). Seeds can persist in soil, and are often planted with contaminated seed of other plants (O’Driscoll 2003). Some species of *Cuscuta* have broad host ranges, and hosts can
be cereals, lucerne, clover, herbs, vegetables (e.g., carrot, celery, potato, capsicum and eggplant), flax, linseed, shrubs, and trees (Knorr 1949, Rajak et al. 1985, Abu-Irmaileh 1987, Abu-Irmaileh & Fucik 1989, Banerjee et al. 1993, O'Driscoll 2003, Stefanović et al. 2007).

The genus *Cuscuta* evolved before the breakup of Gondwana and diversified in South and Central America (Stefanović et al. 2007). The genus comprises some 165–175 currently described species. It is nearly cosmopolitan in distribution with species found on every continent (except Antarctica), ranging from 60° N to 47° S (Stefanović et al. 2007). The vast majority of species of *Cuscuta* only occur in the Americas, with Mexico and adjacent regions as a centre of diversity. Despite their wide cosmopolitan distributions, *Cu. australis* and *Cu. campestris* (one of the most successful parasitic weeds) are of North American origin.


HLB and pathogens of citrus other than those that cause HLB can be transmitted by dodder. Species of *Cuscuta* have been used to transfer liberibacters between hosts (Tirtawidjaja 1981, Garnier & Bové 1983, Ke et al. 1988, Olfato et al. 1990, 1991, Subandiyah 1994, Duan et al. 2008, Zhou et al. 2007). ‘Ca. L. asiaticus’ can multiply and spread within infected *Cu. ceanothi* Behr (syn. *Cu. subinclusa* Dur. & Hilg.), *Cu. campestris* (Ghosh et al. 1977) and *Cu. australis* (Su & Huang 1990)21. Ghosh et al. (1977) observed that the pathogen multiplied more favourably in dodder on diseased sweet orange (which they assumed to be natural host of the pathogen) and suggested this as evidence that it could be possible to use dodder as an alternative host of the pathogen. Zhou (2008b) reported that infected *Cu. pentagona* plants did not exhibit symptoms of HLB even when they contained high titres of ‘Ca. L. asiaticus’. Other diseases transmissible by dodder include transmission by *Cu. reflexa* Roxb. in India of citrus yellow corky vein (Reddy & Naidu 1989), a viral ringspot disease of citrus (Byadgi & Ahlawat 1995), and a badnavirus associated with citrus mosaic disease (Ahlawat et al. 1996).

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18 All members of the genus are vines with twining, slender, pale stems, with reduced, scalelike leaves, and no roots. They become attached to stems by haustoria and depend almost entirely on their hosts to supply water and nutrients (Stefanović et al. 2007). Most species have reduced amounts of, or no, chlorophyll (Stefanović et al. 2007). They divert sugars from the host crop, weakening plants and often causing total failure to set fruit.

19 The monophyletic nature of these species, nested within a clade with narrow distributional ranges, led Stefanović et al. (2007) to conclude that that their physiological capability to use hundreds of genera as hosts probably arose once, in their common ancestor. This capability thus allowed them to spread over large geographic areas, either naturally or as consequence of anthropogenic influences. Stefanović et al. (2007) concluded that there was little doubt that the initial jump in dispersal and speciation had to occur from the Old World, most likely Africa, to the New World, with the first major split and subsequent diversification of *Cuscuta* species in the New World most likely occurring between South America and Mexico. They regarded Mexico as the centre of biodiversity for *Cuscuta* and a staging ground for subsequent diversification.

20 Possibly *Cu. australis*, not *Cu. campestris*. See next footnote.

In addition to transmission of HLB by *Cuscuta* spp., transmission by another parasitic plant, dodder laurel (*Cassytha filiformis* L.\(^{22}\) [Magnoliidae: Lauraceae]) may prove possible. This possibility is based on reports of successful transmission of citrus mosaic (Reddy et al. 1985), CTV (Reddy & Murti (1988), citrus yellow corky vein (Reddy & Naidu 1989), and of a mycoplasma-like organism from coconut (*Cocos nucifera* [Arecales: Arecaceae]) to periwinkle (Saskikala et al. 1989).

Dodder (*Cuscuta* sp.) covered citrus seedlings in a citrus nursery at Sargodha in Punjab Province, Pakistan, in July 2006 (GAC Beattie).

Dodder laurel (*Cassytha* sp.) covered Chinese box orange (*Atalantia buxifolia* (Poir.) Oliv.) in a conservation reserve in coastal Hainan in October 2008 (GAC Beattie).

\(^{22}\) *Ca. filiformis* is a leafless, climbing, twining, vine-like, autoparasitic and plant-hyperparasitic phanerogam. It is indigenous to Hawaii and infests a wide variety of plants, mainly woody hosts, including plants of agricultural and economic value, throughout the tropics worldwide. It belongs to a genus with about 20 species, and occurs in Australia. Hosts of economic importance include citrus, and it may cover and parasitise dozens of host species simultaneously (Nelson 2008a, b).
The origins of *D. citri* and *T. erytreae*

Modern psyllids, including the Diaphorinae and Triozinae, probably evolved with the Sapindales in Gondwana (Hollis 1985, 1987, White & Hodkinson 1985). The Diaphorinae have an ecological preference for dry climates (Hollis 1987). The psyllid was first recorded as a serious pest of citrus in India by Husain & Nath (1927) who, in describing the damage it caused, were the first to describe what are now known to be symptoms of huanglongbing. In the early to mid 1930s, the psyllid did not assume such a destructive status in China, the Philippines, Malaya or Indonesia (Clausen 1933, Hoffmann 1936). Hoffmann and Clausen were both aware of the destruction wrought by the psyllid in India (Hoffmann 1936) as described by Husain & Nath (1927).

All evidence supports the views of Hollis (1987) and Halbert & Manjunath (2004) who suggested, that the psyllid evolved in India in association with a species of *Murraya*\(^{23}\): within the subtribe Clauseneae as circumscribed by Swingle & Reece (1967)\(^{24}\). Which species of *Murraya*, is an issue that needs to be resolved in light of the recent morphological, phytochemical (But et al. 1986) and molecular studies (Samuel et al. 2001, Bayer et al. in press) that are discussed below. Although orange jasmine, *Murraya paniculata* (L.) Jack, is generally regarded as the preferred host of *D. citri*, it was not reported as a host of the psyllid in India until 1975 (Cheema & Kapur 1975), 60, 41 and 13 years respectively after it was reported as a host in Taiwan by (Maki 1915, Kuwayama 1931), in China by He & Zhou (1935), and in the Ryukyu Islands of Japan by (Miyatake 1965). Oddly, *B. koenigii* (cited as *M. koenigii*) was the first *Citrus* relative to be recorded as host of the psyllid in India (Fletcher 1917, 1919, Husain & Nath 1927).

*D. citri* was described from Taiwan (Kuwayama 1908). Crawford (1919) recorded it as being present in the Philippines, Taiwan, Java, Malay Archipelago, Bengal, southern India and southern China, and noted that Frederick Muir collected specimens from Macao and Ambon (Moluccas) in 1906. Clausen (1933) recorded it as being present in China, the Philippine Islands, Taiwan, Malay, Dutch East Indies, Burma, India, and Ceylon. We have not been able to verify the records for Macau and Ambon, and Hoffmann (1936) regarded detection of the psyllid in Guangdong in 1934 as the first record of *D. citri* in China. If it was present in China, it cannot have been widespread: Hoffmann\(^{25}\) commenced his studies on pests of citrus and other plants in Guangdong in 1926 (Jiang\(^{26}\) et al. 1935). Further evidence that the psyllid was not present in China before 1930 is provided by Luh (1936) who, in a paper on factors, including pests and diseases, causing fruit loss in 1935 in Zhejiang, did not mention the presence of *D. citri* or symptoms resembling huanglongbing.

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\(^{23}\) In 1987 the genus *Murraya*, as circumscribed by Swingle & Reece (1967), fell within the tribe Clauseneae. Some species, including orange jasmine (*M. paniculata* (L.) Jack), which is considered to be the favoured host of *D. citri*, have been recently transferred to the Aurantiaceae [Citreeae] (Samuel et al. 2001, Bayer et al. submitted). Others, including the curry plant (*Bergera koenigii* L. = Swingle’s *M. koenigii*) remain within the Clauseneae. In this document the common cultivated ornamental form of orange jasmine is considered to be *Murraya paniculata* (L.) Jack var. *exotica* [sensu Huang] (Huang CC. 1959. Preliminary study on Chinese Rutaceae. Acta Phytotaxonomica Sinica 7: 69-124. 15 plates), unless otherwise stated. The status of the species is complex and resolution of this uncertainty is the objective of a PhD being undertaken at the University of Western Sydney by Nguyen Huy Chung.

\(^{24}\) The assumption of Tsai & Liu (2000) and Tsai et al. (2002) that *D. citri* evolved in the Far East appears to be based on misinterpretation of distribution records cited by Mead (1977).


\(^{26}\) Jiang Zhi was a pseudonym of Ira Judson Condit (1883-1981), who was a visiting professor of horticulture at Lingnan University, Guangzhou (Canton) in 1934-35. He travelled extensively in Hawaii, China (including Taiwan), Philippines and Japan.
Information about two primary parasitoids of *D. citri* also suggests that the psyllid evolved in India. Both parasitoids were first described from India, the ectoparasitoid *Tamarixia radiata* (Waterston) [Hymenoptera: Eulophidae] by Waterston (1922), and the endoparasitoid *Diaphorocyrtus aligarhensis* (Shafee, Alam & Agarwal) [Hymenoptera: Encyrtidae] by Shafee et al. (1975). Most records of *T. radiata*\(^{27}\) in Southeast Asia appear to be related to intentional introductions (Chien et al. 1988, Chiu et al. 1988, Waterhouse 1998) whereas, records of *D. aligarhensis*\(^{28}\) appear to be linked to unintentional movement of parasitised *D. citri* nymphs on live plants to Taiwan (possibly before 1900) and the Philippines, and to natural spread overland with its host. *D. aligarhensis* is not, as assumed by Chien et al. (1988, 1989, 1991), native to Taiwan or the Philippines. No parasitoids were associated with *D. citri* populations when the psyllid was first recorded in Guangzhou (Hoffmann 1936). Tang (1988) mentioned the introduction of *T. radiata* to Fujian in China, but considered it as possibly indigenous, given its wide distribution in Fujian, as recorded in surveys within four years of its introduction. He also considered it possible that the parasitoid occurred in Taiwan before it was released there (Tang 1988) and indigenous in Asia, from Saudi Arabia to China (Tang 1990). This is not supported by other records. An encyrtid with a yellow abdomen (presumably *D. aligarhensis*) was present in the Philippines in 1968, but *T. radiata* was not (Catling 1968): it was introduced in 1988 (Gavarra & Mercado 1988, Gavarra et al. 1990) and the encyrtid with the yellow abdomen was identified as *D. aligarhensis* (Gavarra & Mercado 1988). Both parasitoids were present in Java in Indonesia in 1987 (Nurhadi 1987). Based on comments by Nurhadi (1988) it seems they may have been introduced to Indonesia on psyllid infested plants. In addition to *D. citri*, hosts of *D. aligarhensis* apparently include *Diaphorina cardiae* Crawford), *Diaphorina auberti* Hollis and *Psylla* sp. (Tang & Aubert 1990: *Diaphorina cardiae* is a junior synonym of *Diaphorina aegyptiaca* Puton (Burckhardt 1984).

*T. erytreae* is native to sub-Saharan Africa (Hollis 1984). It is the only species of *Trioza* that is known to feed and develop on Rutaceae (Hollis 1984, Aubert 1987) and belongs to a complex of species that are difficult to define morphologically, but which have discrete host plant preferences (Hollis 1984)\(^{29}\). It has two native rutaceous hosts in Africa on which it can complete its development, *V. lanceolata* and *Z. capense* (Moran 1968, Hollis 1984, Aubert 1987). It also feeds on the native monospecific *Calodendrum capense*, but cannot complete its life cycle on this species (Moran 1968). Synonyms of *V. lanceolata* include *Boscia undulata* Thunb., *Toddalia lanceolata* Lam, *Vepris querimbensis* Klotzsch and *Vepris undulata* (Thunb.) Verdoorn & CA Smith (see Mizyak 1992). *Z. capense* is often cited as *Fagara capensis* Thunb., and sometimes as *F. capense*.). Non-native African hosts on which *T. erytreae* can complete its development in Africa include *Citrus* species and hybrids, *M. paniculata* and the *Cl. anisata* (syn. *Cl. inaequalis* (DC.) Benth.) (Moran 1968, Hollis 1984, Aubert 1987). *Cl. anisatais* often stated as being native to Africa (e.g., Moran 1968, Aubert 1987, OEPP/EPPO 2005b) and is a host of ‘*Ca. L. africanus*’ (Korsten et. al. 1996). It is, however, native to the Western Ghats of India and the northeast part of the Indian subcontinent through to China and has a plethora of synonyms too long to list here (see Molino 1994). Although *Cl. anisata* is the second-most favourable non-citrus host of *T.


\(^{29}\) A second species of *Trioza*, *T. litsea* Bordage (syn. *T. eastopi* Orian) has been recorded feeding occasionally on *Citrus* (Aubert & Quilici 1984, Aubert 1987).
erytreae in Africa (Moran 1968), there does not appear to be any report of *D. citri* feeding or developing on it in Asia. The fact that *V. lanceolata* is the preferred native host of *T. erytreae* in Africa (Moran 1968) suggests that it is the original host of this psyllid.

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